

G-protein-coupled receptors.⁷⁹ A defect in the type 2 vasopressin receptor leads to the condition of nephrogenic **diabetes insipidus** in which the body fails to concentrate the urine.^{77,80} Oxytocin acts on smooth muscles of the uterus during childbirth and triggers the release of milk from the mammary glands.⁸¹ The latter response is partially controlled by the suckling of the infant, which induces the nervous system to release oxytocin into the bloodstream.

Hormones related to oxytocin and vasopressin occur in most vertebrates, the compound **vasotocin** shown in Fig. 30-4 being the most common. Substitution of phenylalanine for isoleucine at position 3 gives arginine vasopressin, the vasopressin found in our bodies. Structure of oxytocin and related hormones⁸² are also shown in Fig. 30-4. Like somatostatin, vasopressin and oxytocin may also form antiparallel pleated sheet structures with β turns. The structural requirements for hormone activity have been studied intensively. Both the macrocyclic hexapeptide ring and the tripeptide side chains are necessary for maximal activity.⁸³

The gene for arginine vasopressin is that of a 166-residue precursor protein carrying a 19-residue signal sequence at the N terminus.⁸⁴ This sequence is followed by that of vasopressin, then after a GKR linker by the 95-residue **neurophysin II**. Finally, after one additional arginine there is a 39-residue glycopeptide. Oxytocin originates in a parallel way from its own precursor.⁸⁵

The 93- to 95-residue neurophysins act as carriers for vasopressin and oxytocin, forming specific complexes with them. Neurophysins contain 14 cysteine residues, which form seven disulfide bonds. There is a striking similarity in sequence between the neurophysins, snake venom toxins, a wheat germ lectin (agglutinin), a ragweed pollen allergen, and a small plant protein called hevein. On the basis of the alignment of cysteine residues, Drenth proposed⁸⁶ that all of these proteins have a disulfide-linked core whose structure is shown in Fig. 30-16.

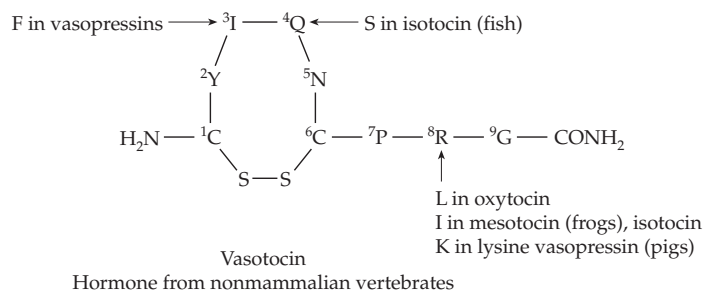


Figure 30-4 Structure of the nonmammalian hormone vasotocin and of related hormones including oxytocin and vasopressin.

Melanocortins. The melanocortin peptides, which are derived from pro-opiomelanocortin as indicated in Fig. 30-2, are formed in varying amounts in the pituitary, in two brain nuclei, and in some peripheral tissues.²⁵ In some animals α -MSH arises primarily in an intermediate part of the pituitary. The hormone has a direct effect on the melanocytes (Box 8-F) causing darkening of the skin. In addition, the various melanocortin peptides (ACTH, α -, β -, and γ -MSH) bind to five different types of receptors. These have been linked to the control of energy homeostasis, appetite, and obesity in both mice and humans.⁸⁷⁻⁸⁹ The sequence His-Phe-Arg-Trp (of the green-shaded sequence in Fig. 30-2) is essential for binding. In keratinocytes α -MSH may form a 1:1 complex with tetrahydrobiopterin,⁹⁰ the coenzyme for tyrosine hydroxylase, and a regulator of tyrosinase, an essential enzyme for melanin formation (Fig. 25-6).

3. Pancreatic and Related Hormones

The functions of the 51-residue insulin (Figs. 7-17 and 7-18) are discussed in Chapter 11. Its actions begin early in life. Mammalian preimplantation blastocysts already show a response to insulin. The glucose transporter GLUT1 is present at the earliest stages; synthesis of GLUT2 and GLUT3 (p. 416) begins at the eight-cell stage. However, the insulin-regulated transporter GLUT4 is not present in the blastocyst. A newly discovered insulin-regulated GLUT8 may function during preimplantation development.⁹¹ Although the secretion of insulin is of primary importance to the regulation of the glucose concentration in mammals, it is still not clearly understood.⁹² The beta cells have insulin receptors and other components of the insulin signaling system such as the insulin receptor substrates IRS-1 and IRS-2 and phosphatidylinositol 3-kinase (PI3-K). The sensing of glucose by the beta cells is also not yet well understood.⁹³ This lack of knowledge has made it difficult to improve the treatment of diabetes. A new approach is to engineer non-beta cells to secrete a steady supply of insulin. Such a possibility has been demonstrated in mice using gut K-cells.⁹⁴

The **insulinlike growth factors** (IGF-I and IGF-II) are produced in many different tissues and promote growth of other cells (see Section 6). **Relaxin**,^{94a} which is produced in the corpus luteum of ovaries during pregnancy, is responsible for inducing widening of the birth canal during the late stages of pregnancy and inhibits contraction of uterine muscle, perhaps by decreasing the activity of the kinase that phosphorylates the 20-kDa light chains of myosin.⁹⁵ Relaxin is found throughout the animal kingdom, even in the protozoan *Tetrahymena*.⁹⁶ Its

structure is apparently identical in pigs, whales, and in a primitive tunicate.⁹⁷ In human males relaxin is apparently produced in the prostate, where it may function as a sperm motility factor.⁹⁷ Relaxin, IGF-I, and IGF-II are all structurally homologous to proinsulin and contain the characteristic 3-disulfide structure of insulin. The IGF-I receptor structure resembles that of the insulin receptor (Fig. 11-11) and also that of the epidermal growth factor (EGF) receptor.⁹⁸

Glucagon belongs to a family that also includes the gastrointestinal hormones **secretin**, **gastrointestinal inhibitory peptide (GIP)**, **vasoactive intestinal peptide (VIP)**, and **glicentin** (Table 30-4). The function of glucagon in regulation of the blood glucose level is considered in Chapter 17, but the hormone may have other effects. A complex processing pathway converts 14- to 16-kDa preproglucagons into the active hormone.^{99,100} Proglucagon is processed to glucagon in the pancreas, but in the endocrine L cells of intestinal mucosa it yields glicentin, a polypeptide containing the entire glucagon sequence, and other products.^{101-102a} Glucagon receptors generate both cAMP and Ca^{2+} as second messengers.¹⁰³

The 27-residue secretin stimulates secretion of bicarbonate into the pancreatic juice and inhibits gastric secretion of acid. The 28-residue VIP is found throughout the gastrointestinal tract of mammals and birds as well as in the brain and the lungs. It is a potent vasodilator and may be the major relaxant of pulmonary smooth muscle.¹⁰⁴ It has been reported totally absent from lungs of asthma patients.¹⁰⁵ The gastrointestinal inhibitory peptide (GIP) is larger than VIP but also has a close homology with glucagon (Table 30-4).¹⁰⁶

The 36-residue **pancreatic polypeptide** is a hormone of uncertain functions. The crystalline polypeptide has at the N terminus an 8-residue collagenlike helix that lies parallel to a C-terminal α helix (Fig. 30-5). The overall shape resembles that of both insulin and glucagon.^{107,108} This PP-fold includes also neuropeptide Y, which is considered in the next section,¹⁰⁹ and neuropeptide YY.¹¹⁰

4. Gastrointestinal and Brain Peptides

The largest endocrine gland in the body is the gastrointestinal tract, which produces a profusion of peptide hormones, many of which are also found in the brain.^{111,112} Indeed, a majority of the known verte-

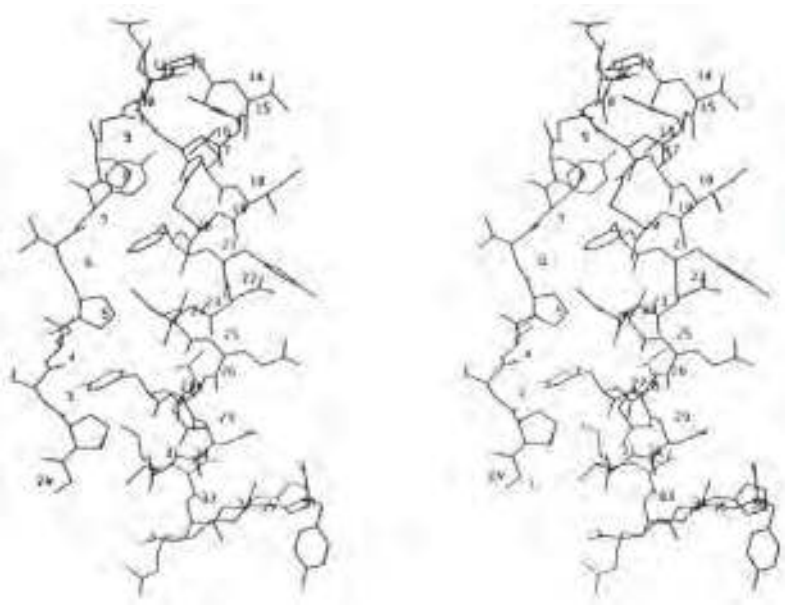


Figure 30-5 Structure of the avian pancreatic polypeptide, a small globular protein. From Blundell *et al.*¹⁰⁷

brate peptide hormones occur in the brain.^{82,112,113} For example, glucagon has been found in the brainstem and hypothalamus.¹¹⁴ Many of these peptides, or closely related ones, are also found in lower invertebrates.¹¹⁵ For example, the 10-residue *Hydra* **head activator** is also present in mammalian brain.^{115,116} The concentrations of these peptides in the brain is very low (10^{-12} – 10^{-15} M).

Gastrin is produced in the lower portion of the stomach and regulates the secretion of acid as well as growth of the gastrointestinal mucosa.^{112,117} It may also function as an Fe^{3+} carrier.^{117a} The shorter gastrin 17 as well as the longer 34-residue gastrin 34 are both active as is a synthetic pentapeptide with the hormone's C-terminal sequence.¹³ The family of **pancreozymin-cholecystokinins (CCK)** are 8- to 58-residue peptides produced in the upper intestinal tract. They have a 4-residue amidated C-terminal sequence in common with gastrin. This tetrapeptide has some biological activity, but eight residues are required for full activity as is conversion of the tyrosine at position seven from the C terminus to an O-sulfate ester. Both gastrin and CCK molecules are partially converted to sulfate esters.^{118,119} Most regions of the brain contain CCK peptides in amounts exceeding those of other neurotransmitters. These arise (in pigs) from a 114-residue preproCCK.¹²⁰⁻¹²² The sulfated insect neuropeptide, **leukosulfakinin** (Table 30-4), is homologous to gastrin and CCK.¹²³ Another hormone **gastrotropin**¹²⁴ is produced by cells of the intestinal mucosa in the distal ileum and stimulates gastric secretion.

Motilin, a 22-residue intestinal neuropeptide, stimulates motor activity of the gastrointestinal tract.^{125,126} **Bombesin** was first isolated from frog skin but probably also functions in both the intestinal tract and the brain. It has a powerful hypothermic effect.^{127,128} A mammalian homolog of bombesin, the 27-residue **gastrin-releasing peptide** (GRP), is found throughout the gastrointestinal and pulmonary tracts as well as the central nervous system.^{120,129} Bombesin-like material, possibly GRP, is produced by some cancers and may serve as an autocrine growth factor.¹³⁰ The 29-residue **galanin** was originally isolated from porcine intestine but is found throughout the central nervous system. It may function as a neurotransmitter or modulator.¹³¹ The 15-residue **guanylin** is an important regulator of epithelial transport in the intestine and probably in other tissues.^{132,133} The active hormone, which is cut from the 99-residue proguanylin, contains two disulfide bonds. Guanylin receptors activate guanylate cyclase with production of cyclic GMP, which functions in the regulation of intestinal fluid and electrolyte absorption. The 18- or 19-residue heat stable enterotoxins of some strains of *E. coli* bind to and activate the guanylin receptors. The resulting overproduction of cGMP causes severe diarrhea (see also Box 11-A).¹³³

Neuropeptides Y (NPY) and YY are 36-residue amidated peptides that are members of the pancreatic polypeptide (PP) family (Fig. 30-5). NPY is produced both in the peripheral nervous system and in the brain,^{110,134} where it is one of the most abundant neuropeptides. Another member of the PP family is **seminalplasmin**, a regulator of calcium ion transport in bovine sperm.¹³⁵ NPY is best known for its stimulation of appetite. It also inhibits anxiety and increases memory retention. It has a vasoconstrictive effect on blood vessels, participating in cardiovascular regulation.^{136,137} Peptide YY is formed in endocrine cells of the intestine, while NPY is formed in neurons of the parasympathetic system.¹³⁸ Both participate in regulation of fluid and electrolyte secretion. Both are found in other vertebrate species.¹³⁹

NPY is one of the most important of several regulators of feeding behavior of animals. PYY₃₋₃₆, another member of the neuropeptide Y family, suppresses appetite by antagonizing the action of NPY.^{139a,b} A large variety of hormonal effects seem to be involved in control of appetite.^{139b,140,141} There are both short-term and long-term mechanisms. For example, when introduced into the gut of rats prior to feeding, CCK and various other gastrointestinal peptides decrease the amount of food eaten.¹⁴⁰

Much attention has been focused on the 146-residue cytokine **leptin**, a hormone produced by adipose tissue.¹⁴¹⁻¹⁴⁴ Leptin, which is sometimes described as the antiobesity hormone, was recognized by mutations of the *obese* gene (OB) or of the OB receptor in

genetically obese mice. When food is scarce, the fat cells shrink and decrease their secretion of leptin. The decrease is sensed by receptors in the hypothalamus, which signal for increased NPY secretion and decreased secretion of α MSH. NPY increases appetite, while α MSH has an opposing role of blocking feelings of hunger.^{144-145a} Nevertheless, there are doubts that leptin's primary role is control of obesity.^{146,147}

The 13-residue **neurotensin** was first isolated from the hypothalamus but is more abundant in cells of the ileum.¹³ It induces gut contraction, lowers blood pressure, and has a variety of other effects.^{127,148}

Substance P (SP; Table 30-4) has been regarded as a possible neurotransmitter for some time¹²⁷ but is also found in the digestive tract. It is the most abundant of a family of five neurokinins (or tachykinins). Others include neurokinin A (substance K), neurokinin B, neuropeptide K, and neuropeptide γ . They have a common C-terminal sequence FXGLM-NH₂.^{149,150} Substance P is thought to be involved in the perception of pain, and mice lacking a substance P receptor appear to have reduced sensitivity to pain.^{151,152} Substance P as well as the related substance K are derived from two large precursor proteins, which appear to arise as a result of alternative modes of splicing of mRNA.²⁹

5. Other Mammalian Peptide Hormones

The action of the 32-residue thyroid hormone **calcitonin**¹⁵³ has been described in Box 22-C. This calcium-regulatory hormone is produced in the thyroid C cells from a precursor having an extra 82 residues at the N terminus and 16 residues at the C terminus. The same gene gives rise in neural tissues to a neuropeptide, possibly a neurotransmitter, called **calcitonin gene-related polypeptide** (CGRPP).^{29,154} The 84-residue **parathyrin** (parathyroid hormone) is present in secretion granules as a 90-residue prohormone containing six extra residues at the N terminus. The primary biosynthetic product **preproparathyrin** contains an additional 25 residues.¹⁵⁵ An N-terminal 34-residue fragment of the hormone, when injected subcutaneously daily, causes an increase in bone density in persons with osteoporosis.¹⁵⁶⁻¹⁵⁷ The hormone acts via a G-protein-coupled receptor in bone and kidney (see Box 22-C).^{158,159} A calcium ion receptor, which binds Ca²⁺ cooperatively, acts as a sensor that regulates release of the parathyroid hormone to regulate the serum Ca²⁺ concentration.¹⁶⁰ A 141-residue **parathyroid hormone-related protein** has an N-terminal sequence homologous with that of parathyroid hormone, eight of the first 13 residues being identical. It is secreted by a variety of cells and serves as a growth factor.¹⁶¹

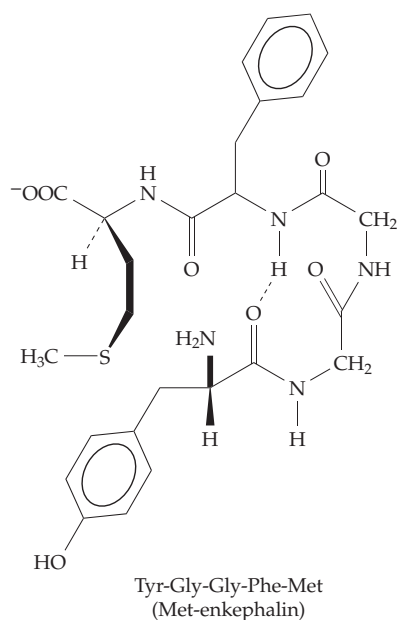
TABLE 30-4
Some Pancreatic and Gastrointestinal Hormones and Neurohormones and Their Sequences

Name and source	No. of residues	Sequence ^a
Glucagon	29	HSQGTFTSDYSKYLDSRRRAQDFVQWLMNT
Secretin (pancreas)	27	HSDGTFTSELSRLRDSARLQRLQLV-NH ₂
Vasoactive intestinal peptide	28	HSDAVFTDNYTRLRKQMARKKYLSILN-NH ₂
Gastrointestinal inhibitory peptide (GIP)	43	YAEGTFISDYIAMDKIRQQDFVNWLLAQ-Q ⁴³
Glicentin	100	A proglucagon containing the entire glucagon sequence in residues 64–92
Pancreatic polypeptide	36	
Neuropeptides Y (NPY) and YY	36	
Gastrin (stomach)		
Gastrin-17	17	pEGPWLEEEEEAYGWMDf-NH ₂ ^b
Cholecystokinin, CCK or pancreozymin (gallbladder, pancreas), many forms exist		
CCK 58	58	
CCK 8	8	DYMGWMDf-NH ₂
Motilin (porcine)	23	FVIFTYGE LQRMQE KERNKGQ
Bombesin	14	pEQRLGNQWAVGHLM-NH ₂
Gastrin-releasing peptide	27	
Galanin	29	
Guanylin	15	PNTCEICAYAACTGC
Neurotensin	13	pELYENKPRRPYIL
Substance P	11	RPKPQQFFGLM-NH ₂
Physaelemin (frog skin)	11	pEADPNKFYGLM
Neurophysins	93 – 95	
β-Endorphin	31	YGGFMTSEKSQTPLVTLFKNAIIKNAHKKGQ
Dynorphin	17	YGGFLRRIRPKLKWDNQ
Met-enkephalin	5	YGGFM
Leu-eukephalin	5	YGGFL
Angiotensin II	8	DRVYIHPF
Bradykinin (BK)	9	RPPGFSPFR
Lys-bradykinin (kallidin)	10	KRPPGFSPFR
Sleep peptide	9	WAGGDASGE
Atrial natriuretic hormone	28	
Chemotactic factors		
for neutrophils	3	f-MLF
for phagocytes	4	TKPR
Speract	10	GFDLNGGGVG

^a Standard one-letter abbreviations; pE, 5-oxoprolyl; f-, formyl; -NH₂, C-terminal carboxamide.

^b Y-12 may be sulfated.

Endogenous opioid peptides. Extensive processing is also involved in formation of analgesic opioid peptides, which are present naturally in the brain (see also Section B). The formation of β -endorphin in the hypothalamus from prepro-opiomelanocortin (Fig. 30-2) has already been mentioned. Prior to the discovery of β -endorphin, the pentapeptides **Met-enkephalin** and **Leu-enkephalin** (Table 30-4) were discovered and were found to compete with opiate drugs for receptors in the brain. The larger β -endorphin, which contains the Met-enkephalin sequence at its N terminus, is a far more potent opiate antagonist than are the enkephalins. Since the Met-enkephalin sequence within β -endorphin is not flanked by basic residues, it apparently is normally not released. Two other recently discovered brain peptides are **endomorphin-1** (YPWF-NH₂) and **endomorphin-2** (YPFF-NH₂). They are also potent agonists for the opioid receptors, especially the μ receptor (see Section B,10).^{161a,161b}



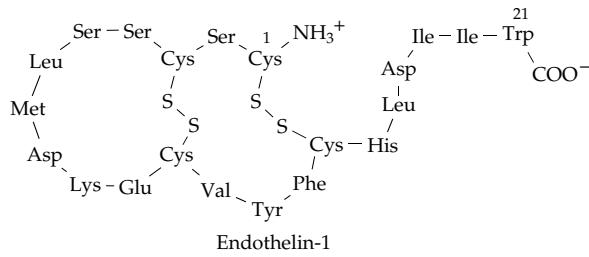
Both Met-enkephalin and Leu-enkephalin have their own pro- and prepro forms.²⁹ Bovine **prepro-enkephalin A** is a 268-residue protein containing a 20-residue signal sequence and four sequences of Met-enkephalin and one of Leu-enkephalin, each flanked by pairs of basic residues. There are also Met-enkephalin-Arg-Gly-Leu (YGGFMRGL) and Met-enkephalin-Arg-Phe sequences. Not all of these are cut out cleanly, and other peptides such as Met-enkephalin-Arg-Arg-Val-NH₂ are also found in brain. **Proenkephalin B** contains three copies of Leu-enkephalin contained within longer peptides. One of these, **β -dynorphin** (Table 30-4), is also a potent opioid compound. The enkephalins are thought to act as neurotransmitters, which are rapidly degraded after their release by two or three membrane-bound

peptidases.^{29,162} Attempts are being made to design inhibitors that might inactivate these enzymes allowing buildup of enkephalin concentrations with a resultant analgesic effect.

ATP, ADP, and adenosine. Usually regarded as a strictly intracellular compound, ATP is also released into extracellular space. There the ATP, as well as ADP and adenosine, have a variety of local hormonal functions. ATP receptors are found in many tissues and are present in some nerve synapses.^{163–166} ATP is one of the substances that induces sensations of pain.^{164,167,168} It may affect secretion of saliva,¹⁶³ signal a full urinary bladder, induce a feeling of warmth,¹⁶⁷ and have functions in the immune system, in platelet clotting,¹⁶⁶ and as a neurotransmitter. Adenosine has been recognized for many years as an extracellular signaling molecule, a local hormone that can arise by breakdown of ATP or by secretion from cells.^{169,170} At least four types of receptor are present in the human body.^{170–174} Adenosine is thought to modulate neural responses in many tissues. It may be involved in sleep,¹⁷² in regulation of serotonin transport,¹⁷¹ and in control of appetite.^{171,174} Extracellular ADP appears to have a role in controlling bone osteoclasts (p. 441).¹⁷⁵

Kinins. These hormones are small peptides that induce contraction of smooth muscles, lower blood pressure (Box 22-D), and increase vascular permeability.¹⁷⁶ They also have a function in contact-activated blood coagulation. The most important human kinins are the nonapeptide **bradykinin**^{177,178} and the related decapeptide **lysine-bradykinin** (Table 30-4). Other forms such as Met-Lys-bradykinin and Ile-Ser-bradykinin (T-kinin) are also known. The precursors to the kinins, the **kininogens**,¹⁷⁶ are cleaved by the protease **kallikrein** (Fig. 12-17) or by kallikreinlike enzymes to form the kinins. Kinins are suspected of being important producers of pain in inflammatory conditions such as arthritis.^{176a}

Endothelins. Endothelial cells of blood vessels produce **endothelins** that cause vascular smooth muscle contraction and a rise in blood pressure.^{179–183} Three human genes code for the closely related endothelins-1, -2, and -3. A 203-residue preproendothelin-1 is processed to form the 39-residue prohormone called **big endothelin-1**. Some of this peptide is secreted and circulates in plasma, where it may have various hormonal functions. Cleavage of the prohormone by a cellular metalloprotease yields endothelin-1, a 21-residue peptide held in a looped configuration by two disulfide bridges. It is homologous to a group of neurotoxins that includes the α -scorpion toxins and ω -conotoxin.¹⁸¹ These toxins act on voltage-dependent ion channels. Endothelin-2 is produced largely in the kidneys and intestine, while endothelin-3 is found in



high concentrations in the brain. Type A endothelin receptors are 7-helix G-protein-coupled proteins, which activate phospholipase C with generation of inositol 1,4,5-trisphosphate and diacylglycerol (Ins- P_3 ; Figs. 11-4, 11-9). The Ins- P_3 causes release of Ca^{2+} , while diacylglycerol mediates mitogenic responses.

Opposing the effects of the endothelins, which act slowly, is a fast-acting endothelium-derived **relaxing factor**, which has been identified as nitric oxide, NO. It is discussed in Chapter 18, Section F, and in Section 7 of this chapter. Also affecting blood pressure is the potent vasorelaxant **atrial natriuretic factor**. This 28-residue peptide, which is discussed in Box 22-D, is produced by the cardiac atria and stimulates the excretion of Na^+ and of water by the kidneys.¹⁸⁴ It also promotes hydrolysis of lipids within human adipocytes.¹⁸⁵

Peptides as attractants. Small peptides as well as larger polypeptides serve to attract cells within the human body and other multicelled organisms. Both unicellular and multicellular organisms also use peptides as pheromones. The human immune system depends upon hormonelike **chemotactic factors**. Neutrophils are attracted by such peptides as formyl-Met-Leu-Phe,^{186–188} which have a bacterial origin, while the basic tetrapeptide Thr-Lys-Pro-Arg activates the phagocytic polymorphonuclear leukocytes and macrophages.¹⁸⁹ Larger 8- to 10-kDa proteins known as **chemokines** (chemotactic cytokines) attract leukocytes to sites of inflammation (Fig. 30-6).^{190–192} Some proteins serve as pheromones. Examples range from the 40-residue mating pheromones of protozoa of the genus *Euplotes*^{193,194} to the 17-kDa sex pheromone of the female hamster.¹⁹⁵ The decapeptide **speract** (Table 30-4) is produced by sea urchin eggs and stimulates the respiration of spermatozoa.¹⁹⁶ Similar factors probably function in fertilization of human ova.

6. Protein Growth Factors and Cytokines

The pituitary growth hormone is only one of a large family of protein growth factors that are secreted by cells and which promote the growth of other cells.²⁰⁰ Many of the growth factors are also described as **cytokines**, local protein hormones that conduct cell-to-cell communication to regulate growth, development,

and differentiation.^{197,201–203} Among the first growth factors to be recognized were the **insulinlike growth factors** (IGF or somatomedins), mitogenic peptides isolated from plasma. They share some of the metabolic effects of insulin but are less active. On the other hand, they are much more active than insulin²⁰⁴ in promoting cell growth and proliferation of cells.²⁰⁵ The abundant IGF-I (somatomedin C), a 70-residue single-chain basic peptide with a sequence and three-dimensional structure homologous to that of proinsulin,^{206,207} is considered a major mediator of the action of the pituitary growth hormone (GH, somatotropin). Studies in cell culture suggest that GH may induce differentiation of cells, and that IGF-I may then cause a rapid proliferation of the newly differentiated cells.²⁰⁸ The homologous 67-residue IGF-II may have a similar function in fetal development.²⁰⁹ The cell surface receptor for IGF-I is similar to the insulin receptor, but IGF-II receptor is structurally different. It is a monomeric 250-kDa protein; and although it is a substrate for a tyrosine kinase, it has no kinase activity of its own.²¹⁰

The 53-residue **epidermal growth factor** (EGF or **urogastrone**) is found in human urine and in very high concentration in the submaxillary salivary glands of male mice. Like the pancreas these glands contain both endocrine and exocrine tissues. EGF is synthesized in mice as a 1217-residue precursor, which contains not only the EGF sequence but also seven other related sequences.²¹¹ Related growth factors include transforming growth factor- α (TGF- α), neuregulins,^{212–214} betacellulin, and epiregulin, all of which promote growth of epithelial cells and are involved in wound healing.²¹⁵ The EGF and related peptide chains are each crosslinked by three disulfide bridges. The three-dimensional structure of EGF, deduced from NMR measurements, contains largely β structure and loops and is organized into two domains in a “mitten shape.”²¹⁶ The receptor for EGF is a 1186-residue transmembrane glycoprotein. Its relationship to cellular oncogene *c-erbB* has been discussed in Chapter 11, Section H. The extracellular glycosylated N-terminal region of the receptor contains the EGF-binding site.^{217–220} It also contains two cysteine-rich repeat sequences homologous to one of those in the insulin receptor A chain (Fig. 11-11). The cytoplasmic C-terminal part of the EGF receptor contains a 250-residue tyrosine-specific protein kinase sequence. Following dimerization the EGF receptor phosphorylates tyrosine residues in various proteins including itself (autophosphorylation).^{212,219,221} The receptor is also phosphorylated on Thr 654 and other residues through the action of the Ca^{2+} - and phospholipid-dependent diacylglycerol-activated **protein kinase C** (Fig. 11-9).²²² Serines 1002, 1046, and 1047 may also become phosphorylated, perhaps resulting in desensitization of the receptor.²²³

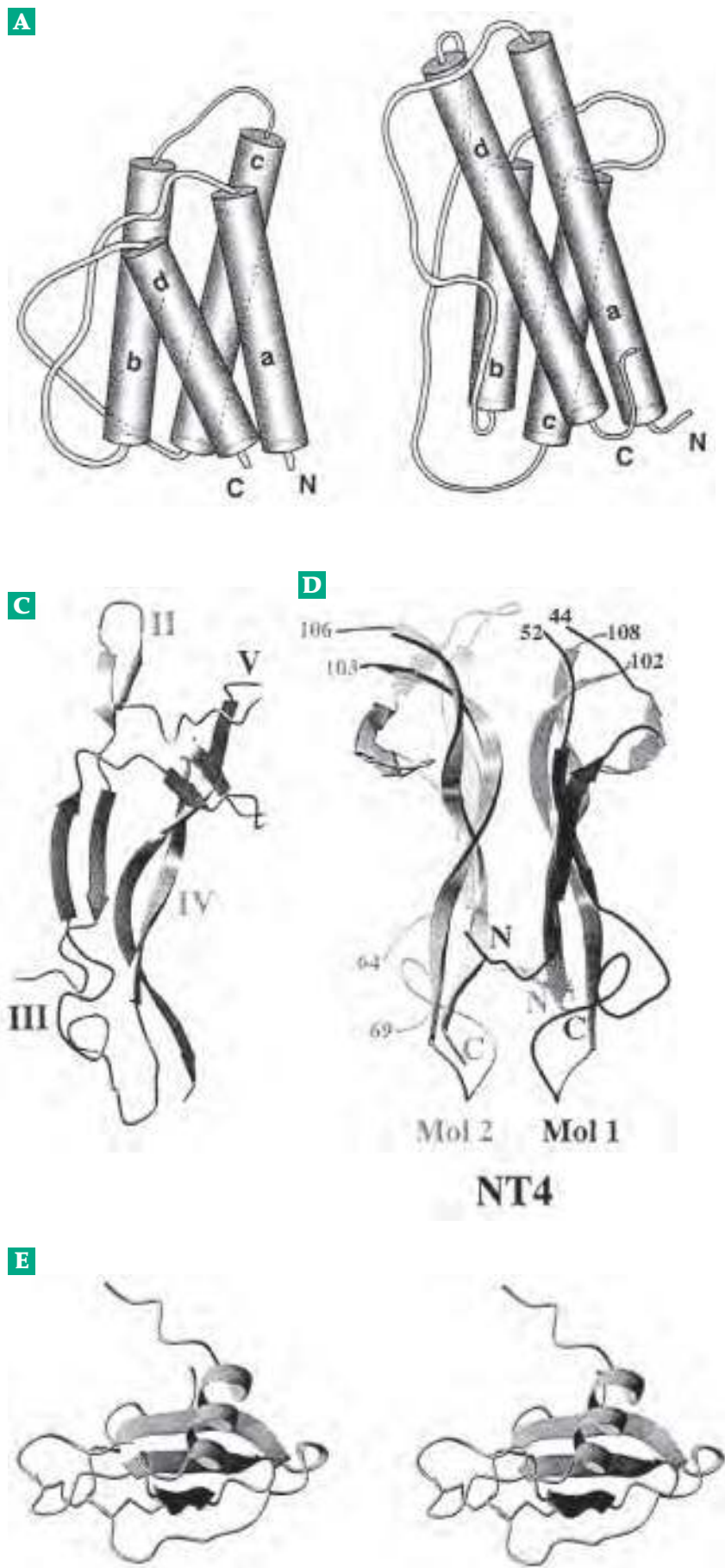


Figure 30-6 Structures of some cytokines and other growth factor proteins. (A) Schematic drawing of representative structures of “short” and “long” helical cytokines. Note the difference in the topology of the connection between helices A and B in the two models. The short cytokines (left) include interleukins-2 and -4. Human growth hormone has a long cytokine structure. Both groups include various colony-stimulating factors. (B) Schematic representation of the disulfide knot topology of cystine knot cytokines. The cysteine residues are numbered I to VI in order of occurrence. See Davies and Wlodawer.¹⁹⁷ (C) Ribbon drawing of a monomer of nerve growth factor (NGF). (D) Ribbon drawing of a dimer of the closely related neurotrophin 4 (NT4). (C) and (D) are from Robinson *et al.*¹⁹⁸ (E) Stereoscopic ribbon drawing of the chemokine **eotaxin-3**. From Ye *et al.*¹⁹⁹

Binding of EGF to its receptor produces within minutes an increased transcription rate for the prolactin gene and other nearby genes.²²¹ The urinary form of EGF, urogastrone, is an inhibitor of ulcer formation. It is found in relatively large amounts in the urine of pregnant women (who tend not to develop ulcers).

Platelet-derived growth factor (PDGF)²²⁴ is released from the α -granules of blood platelets during clot formation and is thought to stimulate the growth and mitosis in fibroblasts that is necessary for wound healing.²²⁵ It consists of two chains, A and B. The 31-kDa precursor of the A chain is encoded by the cellular oncogene *c-sis* (p. 571).²²⁴ The **PDGF receptor** is another transmembrane glycoprotein with a C-terminal tyrosine kinase domain. However, its construction differs from that of the insulin or EGF receptors. The external part of the single-chain receptor appears to contain five immunoglobulinlike domains (see Figs. 2-16 and 12-18).²²⁶ Binding of PDGF to the receptor causes responses within minutes.^{217,226} These include activation of the tyrosine kinase, hydrolysis of phosphatidylinositides, increases in the levels of cAMP and of Ca^{2+} , and increased transcription of a group of genes. The last include the proto-oncogenes *c-myc* and *c-fos*, which encode proteins that regulate transcription. The PDGF receptor is part of a recognized autocrine stimulatory loop in cells infected with a virus carrying the *v-sis* oncogene.²¹⁷ The oncogene product resembles PDGF and binds to the PDGF receptors of the cell producing the *v-sis* product. In this way the cancer cell stimulates its own growth.

Transformation of kidney fibroblasts into cancer-like cells can be induced by the concerted action of PDGF, an analog of EGF, and the **transforming growth factor** (TGF- β).^{225,227,228} The latter is one of 30 or more related growth factors that have numerous functions in normal tissues.²²⁹ Platelets produce a relatively large amount of the 25-kDa TGF- β , and it too may be involved in wound healing. TGF- α is a smaller protein with a structure resembling that of EGF.^{227,228} While TGF- β inhibits epidermal cell growth, TGF- α stimulates growth. It is found in elevated levels in the skin lesions of **psoriasis** (Box 8-F) and may be the cause of the excessive epithelial growth in that disease.²³⁰

There are at least nine **fibroblast growth factors** (FGFs). Originally found in brain, they act on many cells including the endothelial cells that line blood vessels.²³¹ Basic FGF²³² and acidic FGFs²³³ have homologous sequences²³⁴ and are also related to the lymphokine interleukin-1. **Vascular endothelial cell growth factor** (VEGF), which is similar to PDGF, is essential for maintenance of the endothelium. The FGFs and VEGF as well as TFG are potent **angiogenic factors** needed for growth of blood vessels.^{235–238} These proteins are important not only to normal blood vessels but also to invasive tumors that must develop

blood vessels in order to grow. Excessive production of angiogenic factors may also be a factor in eye diseases including the retinal deterioration caused by diabetes.²³⁹ Another protein, **angiogenin**, is a ribonuclease,²⁴⁰ which is discussed on p. 648.

There are four closely related transmembrane FGF receptors and subforms that arise by alternative mRNA splicing.^{241–243} The receptor structures include three external immunoglobulinlike domains and an internal tyrosine kinase domain at the C terminus. Mutations in FGF receptors are associated with a variety of skeletal defects and other hereditary problems.^{241,244} For example, the Gly380Arg substitution in the transmembrane segment of FGF receptor 3 is the major cause of **human dwarfism** (achondroplasia).²⁴⁵ The fibroblast growth factors, as well as other proteins such as the IGFs, HGF, and TGF- β , bind not only to their receptors but also to heparan and heparin. This binding appears to be a major factor in controlling the availability of the growth factors.^{242,246,247}

The **nerve growth factor** (NGF) was identified over 40 years ago by Rita Levi-Montalcini²⁴⁸ on the basis of its activity in promoting the profuse outgrowth of neurites from embryonic neurons (Fig. 30-7). The 118-residue monomer consists largely of three β -hairpin loops, which are held together by three disulfide bridges that form a “cystine knot.” The C15–C80 disulfide passes through a ring formed by the C58–C108 and C68–C110 disulfide bridges (Fig. 30-6B,C).²⁴⁹ A similar folding pattern and disulfide core are found in TGF- β 2 and also in several other **neurotrophins**, growth factors involved in the development and survival of neurons (Fig. 30-6D).^{198,250–251a} NGF may also have a more general function in promoting tissue repair.²⁵² Like EGF nerve growth factor is most abundant in the submaxillary glands of male mice. Larger oligomers containing bound Zn^{2+} are present in mouse submaxillary glands. Two different receptor proteins, one of which is a tyrosine kinase, are present on many cell surfaces.^{198,251,253} The glial cells, which lie between the neurons, have their own growth factors.²⁵⁴

Bone formation and resorption are influenced by several protein factors. For example, IGF-I stimulates formation of bone, but EGF promotes breakdown.²⁵⁶ Additional **bone-derived growth factors** and **morphogenetic factors** also have been described.^{256,257} A **cartilage-inducing** factor has been identified as TGF- β .²⁵⁸

A group of glycoproteins function as hematopoietic growth regulators in the development of blood cells.^{259–264} The 166-residue cytokine **erythropoietin** is the primary regulator of red blood cell formation in mammals.^{260,264,265} At least four glycoprotein **colony-stimulating factors** (CSF) promote proliferation of granulocytes and macrophages.^{259,266,267} The lymphocyte-produced **lymphokines** include the **interleukins** and other proteins. Two species of

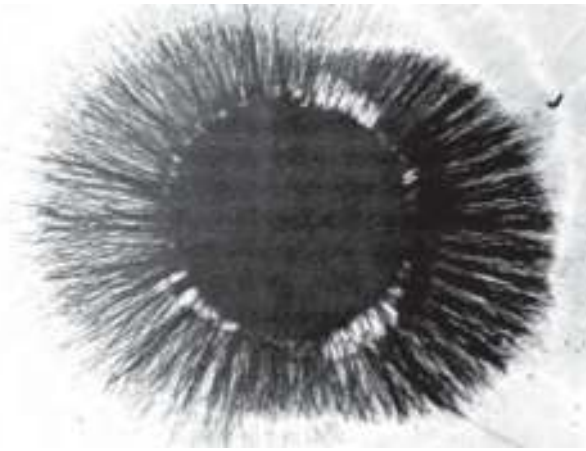


Figure 30-7 Effect of one ng of nerve growth factor in promoting the production of neurites in a chick embryonic sensory ganglion. From Frazier *et al.*²⁵⁵

interleukin-1 (IL-1) serve as mediators of inflammation.^{267,268} They induce proliferation of T lymphocytes and fibroblasts, bone resorption, release of acute phase proteins (Section C), breakdown of cartilage, and fever.

Interleukin-2 (T-cell growth factor; Fig. 30-6A) is secreted by some activated T-lymphocytes. This 133-residue largely helical protein is involved in generation of cytotoxic T-cells, stimulation of interferon release, and of release of a B-cell growth factor.²⁶⁹ Considerable excitement has accompanied the possibility of activating lymphocytes with IL-2 produced from cloned genes in bacteria to increase their ability to kill cancer cells. However, IL-2 is toxic, and this is limiting its use. See also Chapter 31, Section C.

IL-3 is one of the colony-stimulating factors, which stimulates the growth of many types of blood cells.²⁷⁰ Other lymphokines include one derived from T helper cells, which activates resting T lymphocytes thus amplifying an immune response. Others (Chapter 31) are **α -interferon** and the neurotrophic factor (autocrine motility factor) **neuroleukin**.²⁷¹ It acts in monomeric form, but as a dimer it seems to be identical to the enzyme phosphoglucose isomerase.²⁷² While most hormones regulating growth and differentiation seem to be large peptides or proteins, **bursin**, which induces differentiation of lymphocytes, is the amidated tripeptide Lys-His-Gly-NH₂.²⁷³ The corresponding differentiation hormone for T lymphocytes is the 49-residue **thymopoietin**, a hormone of the thymus gland.²⁷⁴

Tumor necrosis factor (TNF, also called cachetin) is a 157-residue hormone secreted by macrophages. It is a mediator of inflammatory responses including fever, shock, and **cachexia**, the wasting of the body during chronic diseases including cancer. TNF was isolated as the causative agent of cachexia and also as

a factor produced in acute bacterial infections, which sometimes caused death of tumor cells and spontaneous recovery from cancer. In the latter case, it is the lipopolysaccharide (Fig. 8-30) and other bacterial endotoxins that induce the release of TNF by macrophages. Its extreme toxicity has prevented immediate harnessing of the tumor-killing potential of TNF. One function of TNF is regulation of transcription factor NF- κ B (Fig. 5-40) in neutrophils and macrophages,²⁷⁵ a key part of the inflammatory response. TNF also mediates programmed cell death (apoptosis)²⁷⁶ and has been linked to obesity-induced insulin resistance.²⁷⁷ The cell surface TNF receptors have a variety of modular structures consisting of various disulfide-linked subdomains.²⁷⁶

This long list of vertebrate peptide growth and regulatory hormones is not complete. The biological actions of these hormones are also complex. Growth factors usually have pleiotropic effects, which may involve many tissues as well as many regulatory systems. Are there any simplifying generalizations? Loret *et al.*²⁷⁸ point out that some growth factors such as IFG-1 and EGF are ubiquitous, affecting virtually all tissues. Others, such as PDGF and thrombin (Fig. 12-17), are more localized in their effects. Some, such as the lymphokines, are more specialized. For one group of hormone receptors the effects are mediated by tyrosine kinases and internalization of the receptors. Another group of receptors activate G proteins and, in turn, adenylate cyclase or phospholipase C. The regulatory domains of the various receptors overlap, a property that allows different tissues to respond differently to hormonal stimuli. The result is the network of interactions that makes the body so sensitive and responsive.

7. Nonpeptide Mammalian Hormones

Most nonpeptide hormones have been considered in other places in the book as indicated in Table 30-2. Because of their importance in the brain adrenaline, noradrenaline, serotonin, and melatonin are also dealt with in Section B,9 of this chapter.

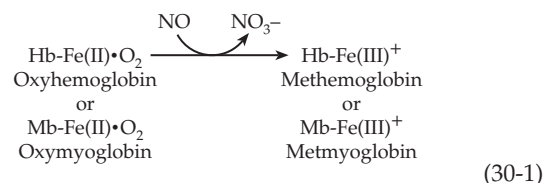
The volatile hormones nitric oxide (NO) and carbon monoxide (CO). The free radical molecule nitric oxide, commonly abbreviated as either \bullet NO or simply NO, is formed by hydroxylation of guanidine groups of arginine (Eq. 18-65). First recognized as the **endothelium-derived relaxing factor**,²⁷⁹ NO has received increasing attention because of its involvement in a broad range of physiological processes. These include regulation of blood pressure through effects on smooth muscles of the vascular endothelium, regulation of several aspects of the innate immune system (Chapter 31), and neurotransmitter functions

both in the brain and in the peripheral nervous system.^{279–281} Roles for NO in bacteria, other microorganisms, and plants have also been discovered.^{281,282} These often involve regulation of transcription.²⁸³ As mentioned in Chapter 18, Section F,2, many of the effects of NO are a result of activation of soluble guanylate cyclase (p. 561).^{283–285a} In the endothelium other hormones, such as the endothelins (p. 1750), atrial natriuretic factor, and bradykinin (Box 22-D), cooperate in the regulation of NO synthase.²⁷⁹ Neuronal NO synthase functions in the brain in olfaction and in formation of memory. In the peripheral system it mediates penile erection^{284,285–286a} and plays a variety of roles in the enteric nervous system.²⁸⁷ Neuronal NO synthase is often localized to synaptic regions by binding to tissue-specific proteins.²⁸⁸ NO may also regulate cellular respiration by inhibition of cytochrome *c* oxidase.²⁸⁵

In high enough concentrations NO is toxic. It is formed of phagocytic cells and utilized in the killing of ingested pathogens.²⁷⁹ It also contributes to the inflammatory response of tissues.^{289,290} Even the firefly's flash is triggered by a pulse of NO.²⁹¹ The dangerous **stonefish**, whose sting causes death within six hours, apparently utilizes NO to kill its victim. A 148-kDa lethal protein (stonustoxin) in its venom induces rapid formation of NO, which causes a fatal drop in blood pressure.^{291a}

Like carbon monoxide, NO binds tightly to many metal centers within a cell.^{292,293} This has added greatly to the problem of understanding the mechanisms of its action. NO also reacts rapidly with thiol groups of proteins and of small molecules such as glutathione.^{294,295} Because of its importance in the regulation of blood pressure, reactions of NO with hemoglobin and the related myoglobin have been studied intensively.^{296–300a} NO binds to hemoglobin 1000 times more tightly than either O₂ or CO, preferentially occupying the hemes of the α subunits.²⁹⁸ Because there is so much hemoglobin in red blood cells, at most one NO per hemoglobin molecule can react. This allows as much as one NO to be carried to tissues along with three O₂ molecules. If the NO could be released in the capillaries, it would activate guanylate cyclase. The resulting cGMP would induce relaxation of smooth muscles and reduce blood pressure.²⁷⁹ However, tight bonding of NO to deoxyhemoglobin would prevent this release. A plausible possibility (with experimental support) is that NO is not bound to Fe but to the SH group of the conserved cysteine 93 of a β subunit of hemoglobin as SNO (*S*-nitrosothiol) hemoglobin. The NO may bind initially to the iron atom of an α subunit, but then be transferred to the nearby β Cys 93 (p. 359) to form the SNO-Hb.²⁹⁶ NO may then move from SNO-Hb to thiol groups in the tissues. Recent evidence suggests that the transfer occurs first to an SH group in the anion exchange **AE1**

(p. 420).²⁹⁹ An alternative explanation, which does not involve SNO-Hb, is that hemoglobin Fe-NO is converted to **nitrite** via oxidation of the iron to form a methemoglobin subunit (Eq. 30-1), and that it is nitrite which serves as the endothelial relaxing agent.³⁰⁰



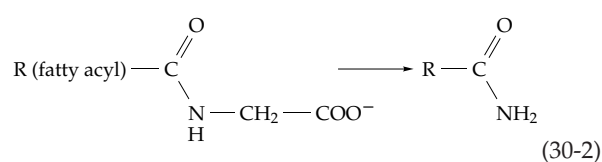
The function of the monomeric myoglobin has often been assumed to be participation in facilitated diffusion of O₂. Although this is an important function under some circumstances, an additional role for myoglobin may be to scavenge NO, via Eq. 30-1, preventing its buildup to dangerous levels. The metmyoglobin produced can be reduced by methemoglobin reductase.^{301,302} A different situation is met by the parasitic nematode *Ascaris*, whose hemoglobin binds O₂ so tightly that it can't serve as an O₂ carrier. It may serve as an **NO-activated deoxygenase**, again using Eq. 30-1 to remove O₂, which can be toxic to the nematode.³⁰³ Free myoglobin can also react with NO to form heme-NO and heme-nitroxyl complexes.^{304,305}

Like NO, CO also binds tightly to heme iron and is able to activate guanylate cyclase.³⁰⁶ CO is formed in the human body by the action of heme oxygenases (Fig. 24-24). Synthesis of heme oxygenase-1 (HO-1) in smooth muscle is induced by a low oxygen tension (hypoxia). The resulting elevated level of CO not only may produce increased vasodilation, but also may inhibit synthesis of vascular smooth muscle cells.³⁰⁷ Heme oxygenase-2 (HO-2) is found in the brain, where it is colocalized with soluble guanylate cyclase.³⁰⁸ Some other organisms have a more active CO metabolism. The CO oxidation system of *Rhodospirillum rubrum* is activated by a CO-sensing heme protein, which acts as a transcriptional regulator. The CO binds to the heme iron, apparently inducing a conformational change that allows the protein to bind to its target DNA sequence.^{309,310}

Hormonal lipids. We have already considered a number of hormones that are not water-soluble but may have to be transported by carrier proteins to their sites of action. These include retinoic acid (Box 22-A), metabolites of vitamin D (Box 22-C), and the platelet-activating factor (Box 8-A). The last functions in the brain³¹¹ as well as in blood. Hormonal lipids also include the prostaglandins (Fig. 21-7), leukotrienes, and lipoxins (Fig. 21-8). These are products of the eicosenoid cascade or network, which is activated by receptors linked to phospholipase C (Fig. 11-9). Ceramide formed by hydrolysis of sphingomyelin initiates

additional responses.^{312,313} Sphingolipids may also be important mediators of apoptosis.³¹⁴

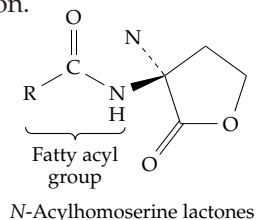
The sleep-inducing oleamide (p. 382) modulates signaling by serotonin-dependent and Gaba-dependent neurons and blocks gap junction signaling in brain glial cells.^{315–318} Oleamide is one of a family of fatty acid amides found in human plasma. One of these, found also in the brain, is **anandamide** (arachidonoyl-ethanolamide). It is an endogenous activator of the brain cannabinoid receptors.^{318–320} The 22-carbon **erucamide** (*cis*-13-docosenamide) stimulates growth of blood vessels.³¹⁶ The fatty acid amides are apparently synthesized from corresponding acylglycines (Eq. 30-2) by the action of the peptidylglycine α -amidating enzyme using the mechanism of Eq. 10-11. See also pp. 1792, 1793.



The fatty acid amides are destroyed by an integral membrane protein, a **fatty acid amide hydrolase**.^{321,322}

8. Nonvertebrate Hormones and Pheromones

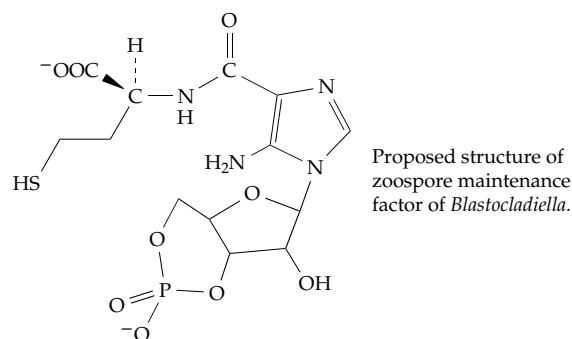
The chemical signals that are passed between bacteria and other microbial cells often resemble hormones of vertebrates. Thus, some bacteria secrete peptide mating pheromones. The sequence of an octapeptide²⁷⁰ of this type from *Streptococcus faecalis* is given in Table 30-5. Many bacteria utilize “quorum signaling.” They do not secrete signaling molecules until they sense that there are enough of them to be effective if they act in unison. Then they all secrete an inducer. The best-known example is the induction of bioluminescence of *Vibrio fischeri* (Eq. 23-49). Long-chain fatty acyl derivatives of L-homoserine lactone act as secreted inducers.^{323,324} A lactonase hydrolytically inactivates the inducer to avoid excessive accumulation.



Depending upon the types of bacteria and the specific response a variety of different fatty acyl groups may be present in the inducer. Other responses include the formation of bacterial film (biofilms) on a surface and release of virulence factors that induce attack on a host.

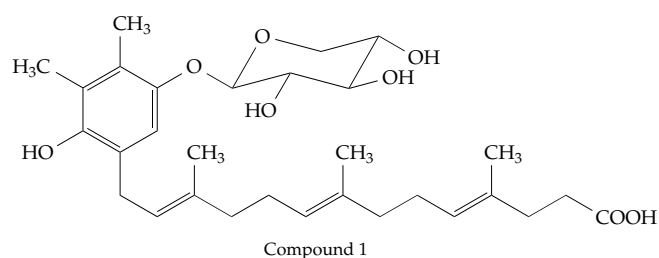
Sexual conjugation in yeast is also induced by pheromones (mating factors).^{325–327} Yeast cells of mating type **a** synthesize the 12-residue mating factor **a** which contains a C-terminal cysteine methyl ester S-alkylated with a *trans,trans*-farnesyl group (Table 30-5). Cells of type α synthesize a 13-residue factor α .^{327a} Cells are attracted to the pheromone produced by cells of the opposite type. The **tremmerogens**, sex hormones of certain basidiomycetes, have related structures (Table 30-5).³²⁸

Peptides are not the only fungal hormones. The water mold *Blastocladiella* releases a **zoospore maintenance factor**, a cyclic phosphate derivative of 5'-phosphoribosyl-5-aminoimidazolecarboxamide.³²⁹ It is similar to the succinocarboxamide, which is an



intermediate in *de novo* synthesis of purines (Fig. 25-15). Substitution of homocysteine for L-aspartate in step g of that sequence could generate a precursor to the zoospore maintenance factor.

Male sperm cells of the alga *Chlamydomonas allensworthii* (Fig. 1-11) are attracted to female gametes by a pentosylated isoprenoid quinone.³³⁰

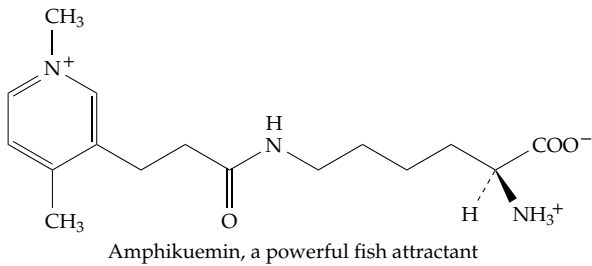


Neurohormones of invertebrate animals. The Cnidaria (coelenterates) have the simplest known nervous system. Simple amidated tetrapeptides, some of which are also found in molluscs, are among their neurotransmitters.³³¹ EGRFamide and L-3-phenyllactyl-LRNamide are found in some sea anemones.³³² When a hydra (Fig. 1-13) is cut into two pieces, one containing a head and one a foot, each piece reforms the missing end. The decapeptide **head activator** (Table 30-5) diffuses upward from the foot end and induces formation of a head. Similarly, a hormone produced by head cells

may induce growth of a new foot end. Glutathione, flowing out from the hydra's prey after wounding by a nematocyst, is the feeding attractant for *Hydra vulgaris*.^{282,333} Termination of the response is dependent upon nitric oxide in this primitive invertebrate.

Certain large anemones enter into a symbiotic relationship with fishes, which recognize chemical signals from the anemones and are also chemically protected from the anemones' stings. One of the fish attractants, **amphikuemin**,³³⁴ is effective at a concentration of 10^{-10} M.

A variety of peptide neurohormones are produced by molluscs. Among these are the sea snail *Aplysia*, which is studied because of its simple nervous system and giant neurons.^{335,336} Proteolytic processing of precursors within single cells often yields neurohormones specific to those cells. The structures of two **small cardioactive peptides** secreted from single neurons³³⁷ are shown in Table 30-5. A 4.4-kDa egg-laying hormone is formed in at least eight processing steps.³³⁸⁻³⁴¹ Among the peptides identified in the freshwater snail *Lymnaea* are many that are characteris-



tic of mammalian pituitary gland, pancreas, brain, and intestinal tract. These include TRH, ACTH, α MSH, arginine vasopressin, oxytocin, calcitonin, gastrin, gastrointestinal peptide, glucagon, insulin, Met-enkephalin, pancreatic poly-peptide, secretin, somatostatin, substance P, and vasoactive intestinal peptide. Also present are FMRF amide and arginine vasotocin.³⁴²

The cardioacceleratory peptide FMRFamide (Table 30-5), which was discovered in 1977, was the first in a large series of related neuropeptides that are found in organisms ranging from the nematode *Caenorhabditis*

TABLE 30-5
Some Microbial and Invertebrate Peptide Hormones

Source name	Number of residues	Sequence ^a
<i>Streptococcus faecalis</i> sex hormone	8	FLVMFLSG
Yeast mating factor a	12	YIIKGV(L)FWD farnesyl ^b PAC–OCH ₃
Tremerogen A-10	10	EHDPSAPGNGYC farnesyl ^b –OCH ₃
<i>Hydra</i> head activator	10	pEPPGGSKVIF
Antho-RF amide (sea anemone)	4	pEGRF–NH ₂
Small cardiovascular peptides (<i>Aplysia</i>)		
SCP-A	11	ARPGYLAFFRM–NH ₂
SCP-B	9	MNYLAFFRM–NH ₂
FMRFamide (coelenterates, molluscs)	4	FMRF–NH ₂
(octopus)	7	YGGFMRF–NH ₂
Shrimp blanching hormone	8	pELNFSPGW–NH ₂
Fidler crab pigment-dispersing hormone	18	NSSELINSILGLPKVMNDA–NH ₂
Proctolin (cockroaches)	5	RYLPT
Myotropic neuropeptide (cockroaches)	11	EQFEDYGHMRF–NH ₂ ↑ sulfate ester in leukosulfakinin
Adipokinetic hormone (locust)	10	PELNFTPNWGT–NH ₂
Crustacean cardioactive peptide	9	EPFCNAFTGC–NH ₂ └─S─S─┘

^a One-letter abbreviations, pE, 5-oxoproline; –NH₂, C-terminal carboxamide.

^b Alkylated on S.

elegans to vertebrate animals. At least 18 genes in *C. elegans* encode 53 distinct FMRFamide-related peptides. Disruption of one of these genes causes hyperactivity, uncoordination, and other behavioral difficulties in the nematodes.³⁴³ Several groups of FMRFamide-related peptides have been found in *Drosophila*³⁴⁴ and other insects.³⁴⁵ One group has the C-terminal FLRFamide and another HMRFamide. Among the latter are sulfate esters such as the cockroach neuropeptide shown in Table 30-5. The sequence of the sea urchin sperm chemoattractant **speract** (sperm attractant peptide-1; SAP-1) is shown in Table 30-4. This is one of a family of egg-associated peptides that stimulate sperm metabolism and mobility. The DNA sequence that codes for speract predicts formation of a 296-residue protein that contains four speract sequences plus six related decapeptide sequences, each separated by a single lysine residue.³⁴⁶ Many other SAP peptides, some containing the unusual amino acid *o*-bromo-L-phenylalanine,³⁴⁷ are formed.

Hormones of insects and crustaceans. Peptide neurohormones of insect brains³⁴⁸ include the pentapeptide **proctolin** (Table 30-5), which was first isolated from the cockroach and has since been found in crustaceans and in mammalian brain. It has been traced to specific insect neurons.³⁴⁹ A nonapeptide neurohormone from the shore crab does not resemble any other known vertebrate or invertebrate hormone.³⁵⁰

The prawn *Pandalus borealis* changes its body color by means of movable pigment granules. The neurosecretory octapeptide **blanching hormone**³⁵¹ (Table 30-5) controls the process. This is a member of a larger family of peptide hormones with related functions such as the 18-residue **pigment-dispersing hormone** of the fiddler crab³⁵² and similar hormones in insects.³⁵³

One of the insect neurohormones, the **activation hormone**, controls the secretion of the corpora allata, paired glands that synthesize the **juvenile hormone** (Fig. 22-4) in insect larvae. While the structure of the juvenile hormone varies somewhat with species, it is usually a polyprenyl ester. A specific binding protein provides the hormone with protection from degradative enzymes. However, in the tobacco hornworm an esterase, able to hydrolyze the protein-bound juvenile hormone, is produced at the start of pupal differentiation.³⁵⁴ The exact mechanism of action of juvenile hormones has been difficult to determine. However, it affects polyamine synthesis.^{355,356}

The **mandibular organ-inhibiting hormone** of the crab *Cancer pagurus* produces two neurohormones that inhibit the secretion of methyl farnesoate, which is thought to function as the juvenile hormone in crustaceans.³⁵⁷

A related role of the insect corpora allata is to store and release the **prothoracicotropic hormone**, a

peptide neurohormone formed in a single neurosecretory cell of the brain.^{358,359} The steroid hormone **ecdysone** (Fig. 22-12) is secreted by the insect's prothoracic gland. Also known as the **molting hormone**, ecdysone is required for the periodic replacement of the exoskeleton of the larvae.^{359a} It induces molting in crayfish and other arthropods and appears to be needed by such members of lower phyla as schistosomes and nematodes. It also controls the biting behavior of mosquitos.³⁶⁰ In addition to α -ecdysone, the 20- and 26-hydroxyecdysones and 20,26-dihydroxyecdysone have been identified in insects.³⁴⁸ It has been suggested that different ecdysones may function at different stages of insect development.

Ecdysone stimulates the synthesis of RNA in tissues. Visual demonstration of the effect is provided by its action on polytene chromosomes of fly larvae (Fig. 26-14).³⁶¹ Fifteen minutes after the application of ecdysone, a puff is induced on one band of the chromosome; a second puff forms at a later time while a preexisting puff diminishes. Thus, like steroid hormones in mammals, ecdysone appears to have a direct controlling effect on transcription. The cuticle-shedding process (**ecdysis**) is initiated by the brain peptide **eclosian**. However, the brain may be responding to the **ecdysis-triggering hormone**, a peptide that is secreted by a series of epitracheal glands located in various segments of the body.³⁶²

Adipokinetic hormones control metabolism of insects during long-distance flight.^{359,363} In the migratory locust these hormones consist of a pair of related octapeptides and a decapeptide (Table 30-5). The hormones stimulate triacylglycerol lipase in the insects' fat bodies, induce release of carbohydrates from body stores, and affect many other aspects of metabolism.³⁶³ Insects also have hormones of the insulin family, proteins consisting of disulfide-linked A and B chains as in insulin. The silkworm *Bombyx mori* has 38 genes for the insulinlike **bombyxins**, which are synthesized in the brain.³⁶⁴

Insects produce many different types of sex attractant pheromones (e.g., see p. 382). By 1995 more than 300 structures had been determined for pheromones from >1600 insect species.³⁶⁵

9. Plant Hormones

Plants possess a kind of circulatory system by which fluids are transported from the roots upward in the xylem and downward from the leaves through the phloem. Many compounds are carried between cells in this manner, while others are transported across cell membranes and against concentration gradients by active transport. A number of compounds that move between cells in either of these two manners have been classified as hormones.³⁶⁶⁻³⁶⁹ The major plant

hormones consist of five compounds or groups of compounds: **auxins** (p. 1446), **gibberellins** (Eq. 22-5), **cytokinins** (Fig. 5-33), **abscisic acid** (Fig. 22-4), and **ethylene** (Fig. 24-16). A number of other plant regulators, some involved in defensive reactions, are sometimes also described as hormone. These include the **brassinosterols** (Fig. 22-9) and related compounds,^{366,367,369,370} **jasmonic acid** (Eq. 21-18), **salicylic acid** (Chapter 25, Section B,7), bacterially produced **lipooligosaccharides** such as the NOD factors (Box 20-E), and polypeptides such as **systemin**. The sugars glucose and sucrose have hormonelike functions as does light, which controls many plant functions.³⁶⁸

Plant hormones have multiple and overlapping functions, which are exerted predominantly by repression of gene expression. This makes it difficult to discuss their functions briefly. Most studied are the auxins, of which the principal member is **indole-3-acetic acid** (Fig. 25-12). This compound, whose biosynthesis is discussed on p. 1446, has been implicated as a controlling agent for cell division and cell elongation. In this capacity auxin influences a great variety of plant processes. Produced principally by growing shoots, auxin diffuses down the stem aided by special **efflux carriers**^{371,371a} and inhibits the growth of lateral buds. However, the hormone stimulates the growth of stems, thus establishing the apical dominance of the tip of a plant. Other hormones also have an influence. Auxin is well established as the controlling agent in phototropism, the tendency of a plant to bend toward the sun.

A sensitive test for auxin, which is dependent on the bending of the coleoptile of *Avena sativa* (the common oat) in response to the hormone, allows detection of as little as three pmol of auxin. Using this assay, it was shown that auxin is transported laterally away from the illuminated side of plants, causing the darker side to elongate more rapidly. Both membrane-associated and soluble binding sites, which may represent natural auxin receptors, have been identified, and auxin response elements have been located in DNA. The membrane-bound receptors may regulate an ATP-dependent proton pump, while the soluble receptors may act to regulate gene transcription.

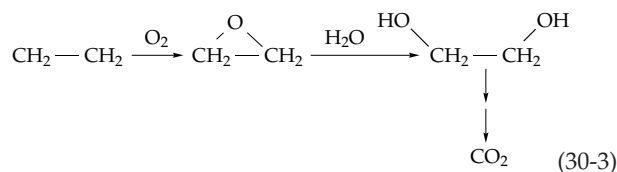
The gibberellins together with brassinosterols are active in helping to determine the *form* of plants. There are 66 different known gibberellins, which are synthesized (Eq. 22-5) in mature leaves and are transported downward. Their very active effect in stimulating RNA synthesis in dwarf varieties of vegetables suggests that gibberellins also serve as gene activators to promote RNA synthesis. A possible function in the **geotropic** response of plant roots is suggested by the presence of higher concentrations of gibberellins in the upper half of horizontal roots than in the lower half.³⁷² On the other hand, auxin has long been known to have a higher concentration on the lower side of the

root,^{371a} and it has been assumed to inhibit elongation (in contrast to stimulation in stems). Because they are structurally and in physical properties somewhat similar to sterols, gibberellins have been assumed to act by a steroid hormonelike mechanism. The brassinosterols, which are true sterols, may also be expected to bind to soluble receptors and to regulate transcription.

The cytokinins are isopentenyladenosine derivatives (Fig. 5-33), which may be hydroxylated or substituted in the 2 position by a methylthio group.^{373,374} Cytokinins are synthesized in roots and translocated upward to other parts of the plant. They may originate in part from degradation of cytokinin-containing tRNA, but there is evidence that they may also be synthesized independently.³⁷⁴⁻³⁷⁶ The N⁶-isopentenyladenine in tRNAs is generated by transfer of the isopentenyl group from Δ^2 -isopentenyl pyrophosphate. The role of cytokinins may be at the level of gene transcription, but it has been difficult to identify signaling sequences.³⁶⁸ Their hormonal influence on plants appears to be independent of their function in tRNA. The most striking effect of cytokinins in solution is on differentiation of plant cells (Chapter 32).

The terpenoid abscisic acid (Fig. 22-4) is synthesized by degradation of a carotenoid precursor. It is formed by plants in response to *stress* from low temperature, high salinity, or drought. Abscisic acid appears to block the growth-promoting effects of hormones such as the gibberellins and cytokinins. It is sometimes regarded as a general gene repressor, which prepares plants for dormancy. Synthesis of abscisic acid occurs in response to the short day and long night pattern of the fall. It often opposes the action of gibberellins. The signaling pathway for abscisic acid apparently involves release of Ca²⁺ ions and formation of cyclic ADP-ribose (cADPR; p. 564). Among other effects this induces the closing of stomata in leaf surfaces.³⁶⁸

Ethylene not only hastens the ripening of fruit but also tends to promote senescence in all parts of plants. Its signaling mechanisms are the best-known for any plant hormone.^{368,369} The synthesis and action of ethylene are discussed in Chapter 24, Section D,4. Ethylene is metabolized slowly in plants by oxidation to ethylene oxide. The latter is hydrolyzed to form ethylene glycol, which is metabolized further to CO₂ (Eq. 30-3).



A postulated flowering hormone, **florigen**, has not been isolated.³⁶⁶ Flowering seems to be controlled by a variety of different hormonal effects.³⁶⁹ Jasmonic

acid (jasmonate) and salicylic acid act as plant defense signals.^{377,378} Salicylic acid activates a large number of transcription factors, which induce resistance to a variety of pathogenic organisms, a response referred to as **systemic acquired resistance**. See also Chapter 31, Section G. Among other compounds synthesized as part of the systemic acquired resistance of plants are proteins known as **systemins**.³⁸² Initially discovered in tomatoes, systemins have been discovered in more than 100 other species of plants. Jasmonic acid emitted from tomato plants acts as a pheromone that attracts wasps to attack caterpillars that feed on the tomato plants.³⁷⁹ In fact, wounding by herbivores may stimulate emission of a variety of volatile compounds that may attract predators to the attacking herbivores.^{380,381}

Many other compounds influence plant growth. Among them are the vitamins, thiamine, pyridoxine, and nicotinic acid, which are synthesized in the leaves and transported downward to the roots. Since they promote growth of roots, they are sometimes referred to as **root growth hormones**. However, they are nutrients universally needed by cells. Various compounds secreted by other organisms can either stimulate or inhibit growth of a given plant. Some are powerful toxins. Others, such as the previously mentioned NOD factors, and evidently also the riboflavin degradation product **lumichrome** (Box 15-B), are beneficial.³⁸³ These **plant growth regulators** may be produced by other plants, by microorganisms, or by fungi.^{384,385} Much use is made in agriculture of synthetic growth regulators.

10. Secretion of Hormones

In Chapter 11 the effects of binding of hormones to cell surface receptors have been emphasized. Equally important are the mechanisms that control the secretion of hormones. The topic of exocytosis has been considered briefly in Chapter 8, Section C.6 and aspects of the Golgi in Fig. 20-8 and associated text. Both hormones and neurotransmitters are secreted by exocytosis of vesicles. Cells have two pathways for secretion.^{386,387} The **constitutive pathway** is utilized for continuous secretion of membrane constituents, enzymes, growth factors, viral proteins, and components of the extracellular matrix. This pathway carries small vesicles that originate in the trans-Golgi network (TGN; Fig. 20-8). The **regulated pathway** is utilized for secretion of hormones and neurotransmitters in response to chemical, electrical, or other stimuli.

Many neurotransmitters are packaged into **small synaptic vesicles** ~50 nm in diameter. These may originate from large endosomes rather than from the Golgi. They are usually recycled and refilled repeatedly.³⁸⁶ Secretion of hormones, and of some neurotrans-

mitters, occurs via **large dense-core vesicles** of ~100 nm diameter. These originate from the TGN and are not recycled. They are prominent in chromaffin cells and other cells that secrete large amounts of a signaling molecule. Secretion of hormones and that of neurotransmitters have several common features. Indeed, hormones of the hypothalamus, neurohypophysis, and the adrenal medulla are secreted by specialized neurons. However, while hormones are often carried in the bloodstream, neurotransmitters are most often secreted into the very small volume of a single synapse. The exocytosis must occur very rapidly from a small number of SSVs.

A common feature, and also a puzzle, of vesicular signaling is the nearly universal response to calcium ions. Exocytosis is usually triggered by a rise in the concentration of Ca^{2+} , and most receptor signaling also leads to an increase in cytosolic Ca^{2+} .³⁸⁸⁻³⁹¹ The puzzle lies in the ability of cells to use a common mechanism for so many specific purposes. This topic is considered further in Section B.8. There are also many other factors that can control exocytosis. Recent evidence suggests that NO may play a role.³⁹²

B. Neurochemistry

The nervous system, a network of neurons in active communication, reaches its ultimate development in the 1.5 kg human brain.^{149,393-396} Many invertebrates, such as leeches,^{396a} crayfish, insects, and snails, have brains containing no more than 10^4 to 10^5 neurons,^{396b,397,398} but the human brain contains $\sim 10^{11}$. Each of these neurons interconnects through **synapses** with hundreds or thousands of other neurons. The number of connections is estimated to be as many as 60,000 with each Purkinje cell of the human cerebellum. There may be many more than 10^{14} synapses in the human brain.^{399,400}

In addition to neurons, the brain contains 5–10 times as many **glial** cells of several types. The neuroglia occupy 40% of the volume of brain and spinal cord in the human. Some glial cells seem to bridge the space between neurons and bloodcarrying capillaries. Others synthesize myelin. Some are very irregular in shape.

1. The Anatomy and Functions of Neurons

Although neurons have many shapes and forms, a common pattern is evident.^{400a} At one end of the elongated cell (Fig. 30-8) is a series of **dendrites**, thin fibers often less than 1 μm in diameter. The ends of the dendrites form synapses with other neurons and act as receivers of incoming messages. Additional messages come into synapses on the **cell body**, while

the **axon** serves as the output end of the cell. The axon, a long fiber of diameter 1–20 μm , is also branched. As a consequence, the nervous system contains both highly branching and highly converging pathways. Many of the axons are wrapped in a myelin sheath (Fig. 30-9; pp. 390 and 1767).

The ends of the fine nerve fibers are thickened to form the **synaptic knobs**, which make synaptic contacts with dendrites on cell bodies of other neurons. In most instances the arrival of a nerve signal at the **presynaptic** end of a neuron causes the release of a transmitter substance (neurohormone). The transmitter passes across the 10–50 nm (typically 20 nm) **synaptic cleft** between the two cells and induces a change in the electrical potential of the **postsynaptic** membrane of the next neuron (Fig. 30-10).^{149,401} Excitatory transmitters usually cause **depolarization** of the membrane. By this we mean that the membrane potential, which in a resting neuron is -50 to -70 mv (Chapter 8), falls to nearly zero often as a consequence of an increased permeability to Na^+ and a resultant inflow of sodium ions. The resulting **postsynaptic**

potential (really a drop in the potential difference) is propagated to the cell body and axon and under appropriate circumstances may initiate an **action potential**. This is a narrow spikelike region of depolarization that travels down the axon at a constant velocity and with undiminished intensity (Fig. 30-11).

A characteristic of many neurons is an *all-or-none response* or firing. An action potential passes down the axon only if there is sufficient depolarization. In general, a stimulus must reach a neuron through *more than one synapse* before the neuron will fire. Furthermore, neurons are often *inhibitory*, releasing transmitters that counter the excitatory synapses and tend to prevent firing. Inhibition is important in damping out small excitations; thus sharpening the response of the nervous system toward strong stimuli. Another characteristic of basic importance to the operation of the brain is that neurons fire at longer or shorter intervals depending upon the strength and duration of the stimulus. The stronger the stimulus to a given neuron, the more rapid the train of spikes that passes down the axon. Thus, the brain functions to a large extent in

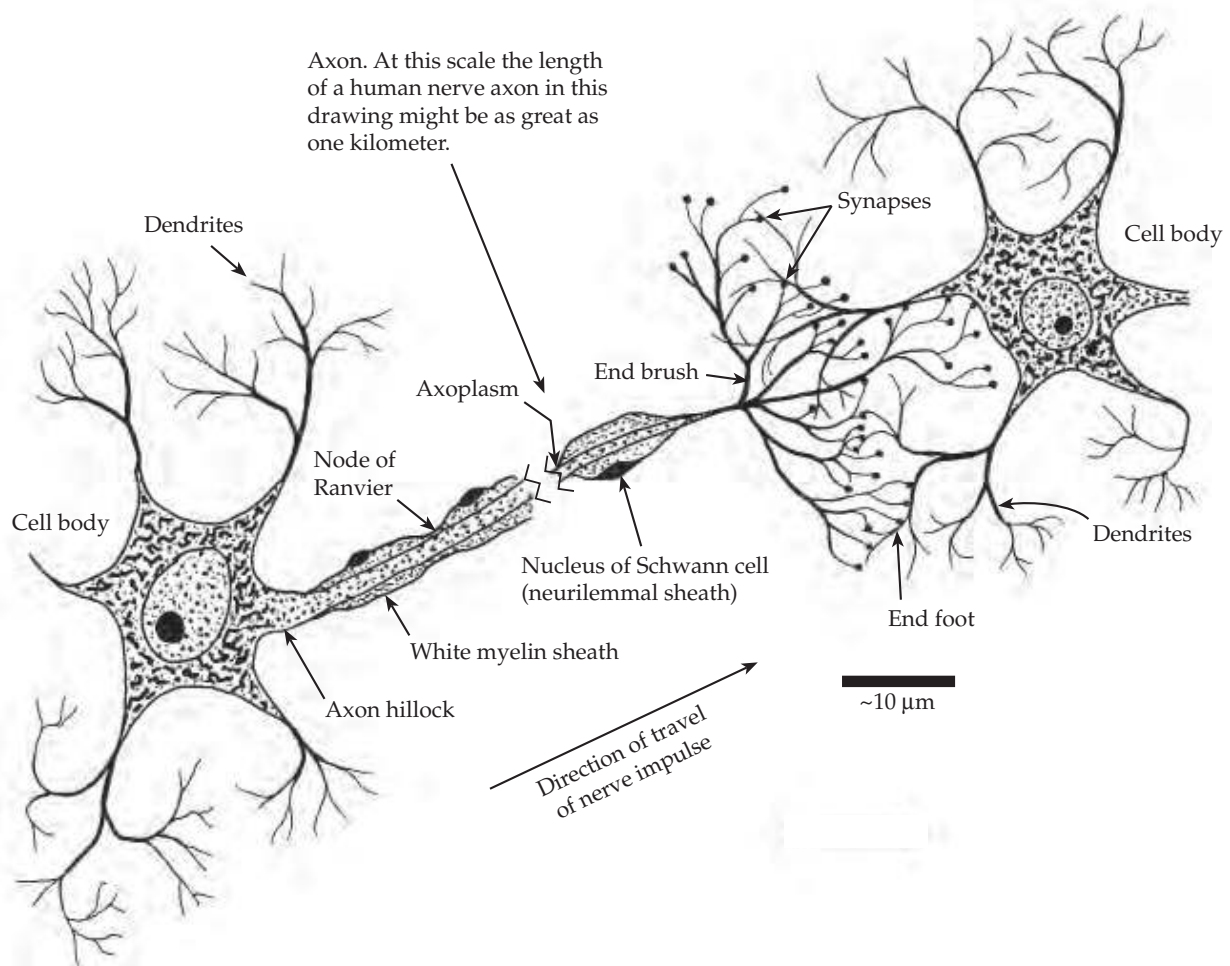


Figure 30-8 Schematic drawing of a neuron (after Brand and Westfall,⁴⁰¹ p. 1192).

Figure 30-9 Micrograph of a section through an axon of a neuron from rat brain. The structure of the myelin sheath can be seen clearly. The growing lip of cytoplasm (X) from a neuroglial cell is advancing around the axon process (NF) and insinuating itself into the space between the plasma membrane of the axon and the membrane that limits the thin layer of cytoplasm (Y) left behind by the growing lip during its previous turn. This cytoplasmic layer disappears as the inner leaflets of its plasma membrane fuse to form the major dense line of the myelin sheath. This process is occurring at the point indicated by the single arrow. The outer leaflet of the plasma membrane surrounding the lip fuses with its own outer leaflet laid down on the previous turn. The two outer leaflets thus give rise to the less dense intermediate line of the sheath (double arrow). The cell body from which the investing cytoplasmic sheet originated cannot be seen in this micrograph, but cytoplasm within the lateral margins of the sheet does appear (X'). The micrograph, by A. Hirano and H. M. Dembitzer, originally appeared in *J. Cell Biol.* **34**, 555 (1967), where a more complete explanation of myelin sheath formation is provided. Figure copied from Porter and Bonneville.⁴⁰² Courtesy of Mary Bonneville.

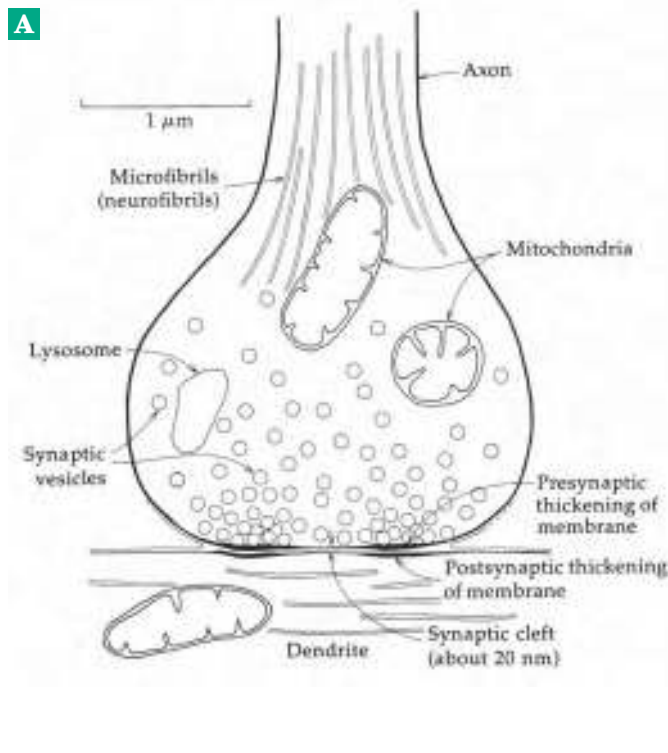
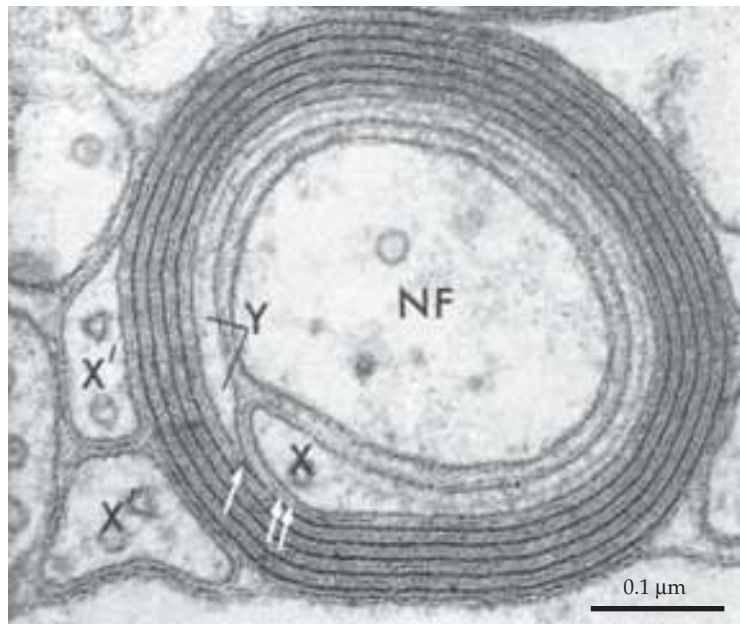


Figure 30-10 (A) Schematic drawing of a synapse. (B) Electron micrograph showing the synaptic junctions in the basal part (pedicle) of a retinal cone cell of a monkey.⁴⁰³ Each pedicle contains synaptic contacts with 12 triads, each made up of processes from a bipolar cell center that carries the principal output signal and processes from two horizontal cells that also synapse with other cones. A ribbon structure within the pedicle is characteristic of these synapses. Note the numerous synaptic vesicles in the pedicle, some arranged around the ribbon, the synaptic clefts, and the characteristic thickening of the membranes surrounding the cleft (below the ribbons). Micrograph courtesy of John Dowling.

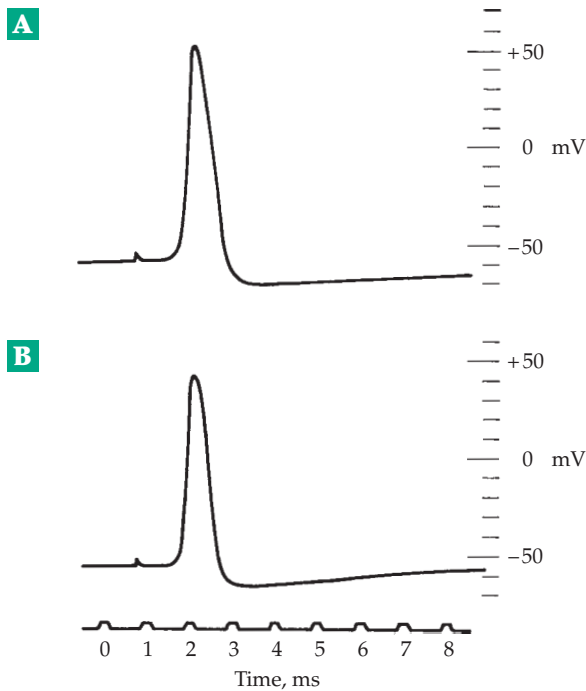


Figure 30-11 (A) Action potential recorded with internal electrode from extruded axon filled with potassium sulfate (16°C). (B) Action potential of an intact axon, with same amplification and time scale (18°C). The voltage scale gives the potential of the internal electrode relative to its potential in the external solution with no correction for junction potential. From A. Hodgkin, *Conduction of Nervous Impulses*, 1964. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.

decoding trains of impulses. The frequency of the impulses from neurons varies from a few per second to a maximum of about 200 s^{-1} in most nerves (up to 1600 s^{-1} in the Renshaw cells of the spinal cord). The maximum frequency is dictated by the refractory period of $\sim 1\text{ ms}$ (Section B,3).

Although the concepts of neuronal function outlined in the preceding paragraphs have been accepted for many years, more recent discoveries require that they be modified somewhat. Dendrites seem to be able to transmit information as well as to receive it. Furthermore, while information is certainly transmitted long distances by spike action potentials, shorter neurons and dendrites may communicate extensively by exchange of chemicals through low resistance gap junctions, also called **electric synapses** (Chapter 1). Small changes in membrane potential transmitted through these junctions may alter the behavior of adjacent neurons. Chemical transmitters do not always have an electrical effect on postsynaptic neurons but may influence metabolism or gene transcription.

2. Organization of the Brain

The anatomy of the brain is quite complex, and only a few terms will be defined here. The **cerebrum**, which is made up of two hemispheres, accounts for the largest part of the brain. The deeply folded outermost layer, the **cerebral cortex**, consists of **gray matter**, a mass of cell bodies, and fine unmyelinated nerve fibers. Beneath this lies a layer of **white matter** made up of myelin-covered axons connecting the cerebral cortex with other parts of the brain. The two cerebral hemispheres are connected by the **corpus callosum**, a band of $\sim 2 \times 10^8$ nerve fibers. Remarkably, these fibers can be completely severed with a relatively minimal disruption of the nervous system. In the past the corpus callosum was sometimes cut to control almost incessant epileptic seizures that could not be prevented by drugs. The “split-brain” patients suffered relatively little disability as long as both eyes functioned normally. Studies of these patients provided some insights into the differing functions of the two hemispheres of the cerebrum.³⁹⁵

Deeper in the cerebrum lie the **basal ganglia**, which include the caudate, lenticular, and amygdaloid nuclei. The lenticular nuclei are further divided into putamen (an outer portion) and the globus pallidus. The putamen and caudate nuclei together are known as the **striatum** (Fig. 30-12). The lower lying subthalamic nuclei and substantia nigra are sometimes also included in the basal ganglia.

The outer parts of the cerebrum, including the basal ganglia, make up the telencephalon. Deep in the center of the brain is the diencephalon consisting of the **thalamus** (actually two thalami), **hypothalamus**, **hypophysis** (Figs. 30-1, 30-13), and other attached regions. A major structure at the back of the brain is the **cerebellum**. Like the cerebrum, its cortex is highly folded. The 30 billion neurons of the cerebellum are organized in a highly regular fashion.^{393,404} The interconnections of the seven types of neurons present in this part of the brain have been worked out in fine detail.

The basal part of the brain or **brain stem** consists of the medulla oblongata and the pons. While the bulk of the tissue consists of myelinated nerve tracts passing into the spinal cord, synaptic regions such as the olivary nucleus are also present.

The brain, which must function in a chemically stable environment, is protected by a tough outer covering, the **arachnoid membrane**, and by the **blood–brain barrier**^{406,407} and the **blood–cerebrospinal barrier**. Both of these barriers consist of tight junctions similar to those seen in Fig. 1-15A. They are formed between the endothelial cells of the cerebral capillaries and between the epithelial cells that surround the capillaries of the **choroid plexus**. The choroid plexus consists of capillary beds around portions

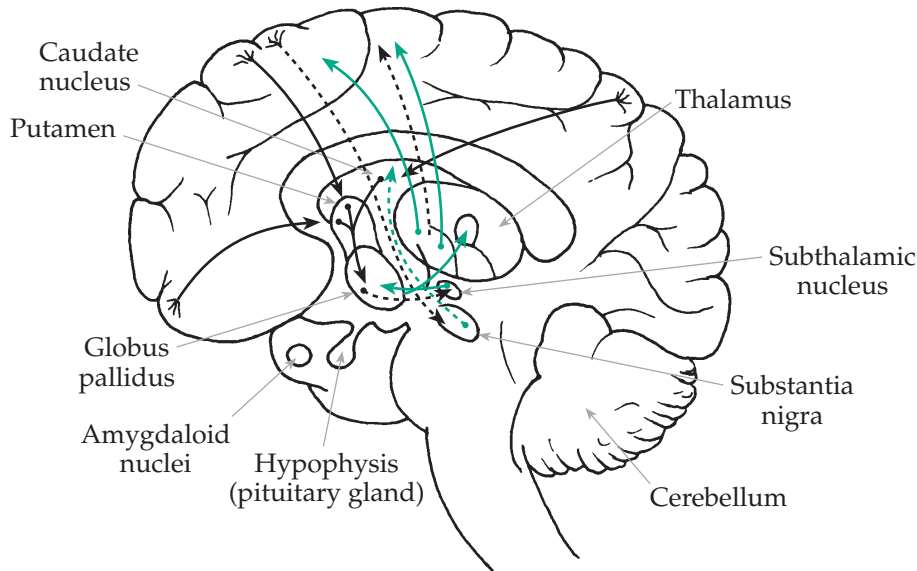


Figure 30-12 Diagram illustrating some of the major interconnections of the “extrapyramidal system” of the brain. Arrows indicate major direction of projections. The nigrostriatal (substantia nigra to striatum) and related neuronal pathways are indicated with dashed lines. After Noback and Demarest,⁴⁰⁵ pp. 182 and 183.

of the fluid-filled **ventricles** deep in the interior of the brain. They serve as a kind of “kidney” for the brain assisting in bringing nutrients in from the blood and helping to keep dangerous compounds out.⁴⁰⁶

3. Neuronal Pathways and Systems

Consider a message originating with a nerve receptor in the skin or in another sense organ. A nerve signal passes via a **sensory neuron (afferent fiber)** upward toward the brain. It may pass through two or more synapses (often through one in the spinal cord and one in the thalamus) finally reaching a spot in the sensory region of the cerebral cortex. From there the signal in modified form spreads through the **inter-neurons** of virtually the entire cortex. In each synapse, as well as in the cortex, the impulse excites inhibitory fibers that dampen impulses flowing through adjacent fibers. Likewise, if a given impulse is not strong enough, it will itself be inhibited before reaching the

cortex. Among the important sensory neurons are those from the seven million cone cells and 100 million rod cells of the eye. The nerve signals pass out of the retina by way of a million axons from retinal ganglion cells reaching, among other parts of the brain, the **visual cortex** (Fig. 30-14).⁴⁰⁸

The neuronal events that occur within the cerebral cortex are extraordinarily complex and little understood.⁴⁰⁹ In what way the brain is able to initiate voluntary movement of muscles is obscure. However, it is established that the signals that travel out of the brain down the **efferent fibers** to the muscles arise from large **motor neurons** of the **motor cortex**,⁴¹⁰ a region that extends in a band across the brain and adjacent to the sensory cortex (Fig. 30-14). The axons of these cells form the **pyramidal tract** that carries impulses downward to synapses in the spinal cord and from there to the **neuromuscular junctions**. These are specialized synapses at which acetylcholine is released, carrying the signal to the muscle fibers themselves. Passing over the cell surface and into the

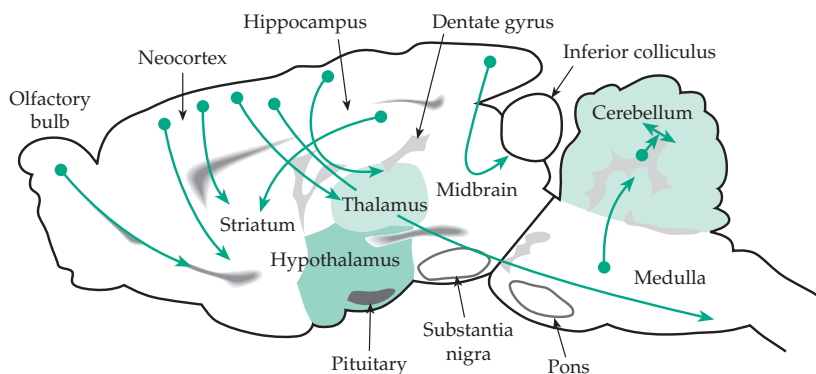


Figure 30-13 Section through a rat brain. This brain, which has been very widely used in neurochemical studies, appears superficially to be quite different from the human brain (Fig. 30-1), which is characterized by its large cerebral cortex. However, basic pathways are the same. Some major pathways for glutamate-secreting (glutamatergic) neurons are marked by arrows. Most of these originate in the neocortex (outer layers of the cerebral cortex) and the hippocampus. From Nicholls.¹⁴⁹ Courtesy of David G. Nicholls.

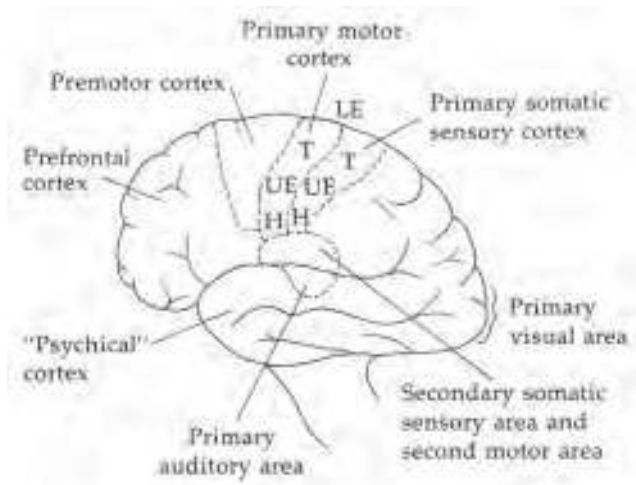


Figure 30-14 The location of several functional areas of the cerebral cortex. The representation of body parts on the primary motor and somatic sensory cortices include the head (H), upper extremity (UE), trunk (T), and lower extremity (LE). After Noback and Demarest,⁴⁰⁵ p. 193.

T tubules (Chapter 19, Section B.4; Fig. 19-21), a wave of depolarization initiates the release of calcium and muscular contraction.

At the same time that the motor neurons send signals to the muscles, branches travel into other parts of the brain including the olivary nuclei, which send neurons into the cerebellum. The cerebellum acts as a kind of computer needed for fine tuning of the impulses to the muscles. Injury to the cerebellum leads to difficulty in finely coordinated motions. Input to the Purkinje cells arises from the climbing fibers, which originate in the inferior olive of the brain stem. Each climbing fiber activates a single Purkinje cell, but the dendrites of each Purkinje cell also form as many as 200,000 different synapses with parallel fibers that run across the cortex of the cerebellum (Fig. 30-15). The parallel fibers receive input from many sources via a complex series of mossy fibers and granule cells and influence the firing of the Purkinje cells. The output from the Purkinje cells is entirely inhibitory. It is transmitted via synapses in the cerebellar nuclei to neurons that lead back to the cerebral cortex, into the thalamus, and down the spinal cord.⁴¹¹ The pathway to the cortex completes an inhibitory feedback loop, of which there are many in the nervous system. For details see Llinás⁴⁰⁴ and Nicholls.¹⁴⁹

In addition to the **somatic motor system** that operates the voluntary (striated) muscles via the pyramidal tract, there is the **autonomic system**, which controls the involuntary (smooth) muscles, glands, heartbeat, blood pressure, and body temperature. This system has its origins in both the cerebral cortex and

hypothalamus. It is subdivided into two systems, the **sympathetic** and **parasympathetic** systems, which are anatomically distinct. The sympathetic system is geared to the fight and fright reactions. Its **postganglionic fibers** (those below the ganglia in the spinal cord) liberate norepinephrine (noradrenaline) and include the adrenal medulla, which consists of specialized neurons, the **chromaffin** cells. The parasympathetic system has to do more with homeostasis and maintenance of body systems. Biochemically it is characterized by the release of acetylcholine as a transmitter substance.

The hypothalamus, a four gram portion of the brain, receives a great deal of biochemical attention because of its function in the autonomic nervous system, in homeostasis, and in endocrine secretion. Its liberation of neurohormones that stimulate the hypophysis has already been considered in Section A.3. The hypothalamus is also involved in the regulation of the body temperature, of water balance, and possibly of glucose concentration.

Two other systems of importance in the brain are the **reticular system** and the **limbic system**. The former is the mediator of the sleep–wake cycle and is responsible for characteristic waves in the electroencephalogram. The limbic system is the mediator of **affect** or mood and of **instincts**. It is anatomically complex with centers in the amygdala, other subcortical nuclei, and the limbic lobe of the cortex. The limbic cortex forms a ring lying largely within the longitudinal fissure between the two hemispheres. It includes the olfactory cortex, the **hippocampus**, a region associated with formation of conscious memories, and other evolutionarily older regions of the cerebral cortex. Within the limbic lobe are the **pleasure centers**. When electrodes are implanted in these regions, animals will repeatedly push levers that are designed to electrically stimulate these centers. There are also **punishing centers**, whose stimulation causes animals to avoid further stimulation.

4. The Propagation of Nerve Impulses

Although the chemical basis of the conduction of nerve impulses via an action potential is not entirely clear, the electrical events have been described with precision. If the permeability of a membrane toward sodium ions is increased in a local region, sodium ions flow through the membrane into the cell neutralizing the negative charge inside and depolarizing the membrane. Such depolarization leads to propagation of an electrical signal of diminishing intensity over the surface of the membrane in a manner analogous to the flow of electrical current along a coaxial cable. It is thought that local increases in Na^+ permeability of the plasma membrane often trigger nerve impulses. Other

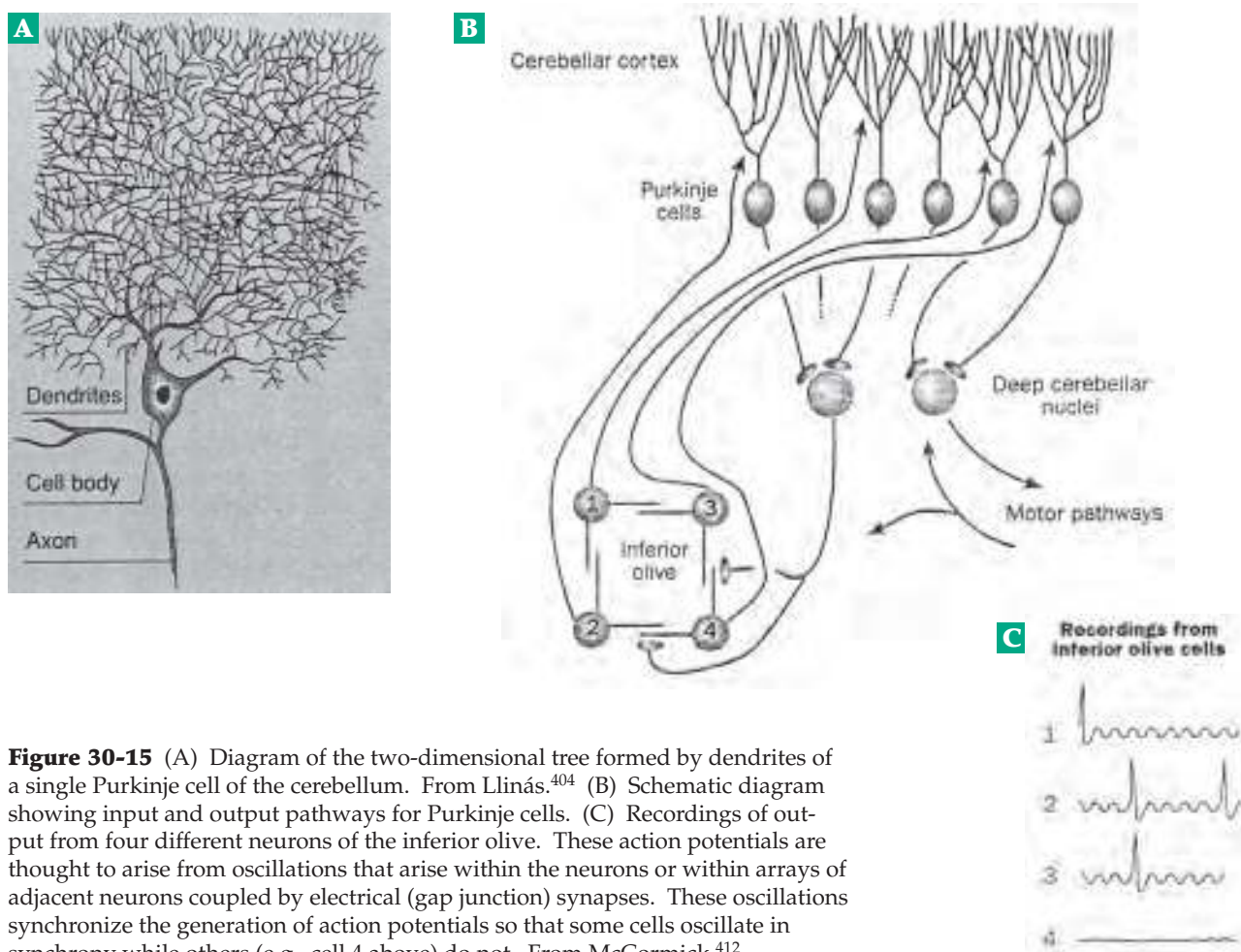


Figure 30-15 (A) Diagram of the two-dimensional tree formed by dendrites of a single Purkinje cell of the cerebellum. From Llinás.⁴⁰⁴ (B) Schematic diagram showing input and output pathways for Purkinje cells. (C) Recordings of output from four different neurons of the inferior olive. These action potentials are thought to arise from oscillations that arise within the neurons or within arrays of adjacent neurons coupled by electrical (gap junction) synapses. These oscillations synchronize the generation of action potentials so that some cells oscillate in synchrony while others (e.g., cell 4 above) do not. From McCormick.⁴¹²

ions such as Ca^{2+} may also play a role. While the kind of passive transmission of electrical signals that results from a local depolarization of the membrane is suitable for very short nerve cells, it cannot be used to send signals for long distances. Most nerve axons employ the more efficient action potential. This is an impulse that passes along the axon and for a short fraction of a second (~ 0.5 ms in mammalian nerves) changes the membrane potential in the characteristic way shown in Fig. 30-11. Initially, the negative potential of 50–70 mV drops rapidly to zero and then becomes positive by as much as 40–50 mV, after which it returns to the resting potential. The remarkable thing about the action potential is that it is propagated down the axons at velocities of 1–100 m/s without loss of intensity.

To establish the chemical basis of the action potential, A. L. Hodgkin and A. F. Huxley in the 1950s devised the **voltage clamp**, a sophisticated device by which the transmembrane current can be measured while using a feedback mechanism to fix the membrane potential at a preselected value.^{413–417} Using the voltage clamp the membrane conductance could be measured as a function of the membrane potential

and of time. It was found that immediately after a decrease in membrane potential was imposed with the voltage clamp, the permeability of the membrane toward sodium ions rose rapidly. Since an increased sodium ion permeability automatically leads to depolarization in an adjacent region of the membrane, a self-propagating wave is established and moves down the axon. The voltage clamp studies also revealed that after a fraction of a millisecond the permeability to potassium ions also increases. At the same time the sodium ion permeability decreases again, and the normal membrane potential is soon reestablished. However, during an **absolute refractory period** of ~ 0.5 ms no other nerve impulse can be passed. The sequence of events during passage of the nerve impulse can be described as the opening of sodium channels followed by the opening of potassium channels, and then by a closing of the channels in the same sequence. The results of these investigations led Hodgkin and Huxley to propose equations that quantitatively describe the action potential and that predict the observed conduction velocities and other features of nerve impulses.

A special feature of nerves that are designed to transmit impulses very rapidly is the presence of the wrapping of **myelin** (Fig. 30-9). As can be seen in this figure, the extracellular surfaces of the consecutive wraps bind tightly together, and the cytoplasm of the cell interior is squeezed out to form the compact myelin sheath.⁴¹⁸ Mutations in the integral membrane proteolipid protein (p. 401) are associated with a variety of defects in myelin formation. Some of these are severe, for example, leading to loosely wrapped myelin.^{419,420} The proteolipid protein is encoded by an X-linked gene. The most abundant protein in peripheral nerve myelin is the integral membrane **peripheral myelin glycoprotein P₀**. It is encoded by an autosomal gene for which 29 known defects account for a variety of human diseases,^{421–422a} including an autoimmune inner ear disease.⁴²³ The extracellular domain of P₀, like many other cell adhesion molecules (p. 407), has a structure related to that of immunoglobulins. Four molecules of P₀, each of which carries a single immunoglobulin domain, associate via these domains in a kind of square donut that protrudes from the outer cell surface. There it can interact with four similar donuts from the apposed cell surface, zipping up the cell–cell interface by a kind of Velcro action.^{422,424,425} Protein P₀ accounts for 50% of the total protein of peripheral myelin, but the **myelin basic protein**, which constitutes 20% of the total protein, is also essential.⁴²⁶ This protein exists as a variety of forms that arise from differential splicing of its mRNA and extensive posttranslational modification. Deimination of arginine side chains to form citrulline residues has been associated with development of the autoimmune disease **multiple sclerosis**.^{427,428} **Peripheral myosin protein 22** is a 160-residue polypeptide with four membrane-spanning helices. It accounts for 2–5% of the myelin protein and is the site of defects that cause the demyelinating **Charcot–Marie–Tooth disease** and other serious human diseases.^{428a,b}

The axon is effectively insulated from the surrounding medium by the myelin sheets except for special regions, the **nodes of Ranvier**, which lie at 1- to 2-mm intervals along the nerve. The nerve impulse in effect jumps from one nerve to the next. This **saltatory conduction** occurs much more rapidly (up to 100 m/s) than conduction in unmyelinated axons. It depends upon Na⁺ and K⁺ channels that are concentrated in the nodes of Ranvier.

5. Ion Conducting Channels

What is known about the channels through which Na⁺ and K⁺ flow during nerve excitation? That the channels for the two ions are separate was shown by the fact that **tetrodotoxin** (found in the puffer fish)^{429,429a} and **saxitoxin** of dinoflagellates, as well as

scorpion toxins (see Fig. 30-16), exert their toxic action by blocking the Na⁺ channels while having no effect upon conductance for K⁺. At the same time the K⁺ channels can be blocked by certain quaternary ammonium salts. Since the binding constants for the toxins are high ($K_f \sim 3 \times 10^8 \text{ M}^{-1}$ for tetrodotoxin), it is possible to titrate the sodium channels. The number is usually quite small, about 10–400 Na⁺ channels / μm^2 of surface⁴³⁰ (the same surface area contains 2×10^6 phospholipid molecules). However, membranes in the nodes of Ranvier of mammalian nerve fibers⁴³¹ contain $\sim 12,000$ channels / μm^2 . Note that the ion channels described here are not the same as those in the ion pump, i.e., the Na⁺,K⁺-ATPase (Fig. 8-25). In some neurons the number of conduction channels for Na⁺ appears to be ten times less than the number of pumping channels, i.e., of Na⁺,K⁺-ATPase.^{432,432a}

Since the number of ion-conducting channels is small, the rate of sodium passage through the open channels must be extremely rapid and has been estimated as $\sim 10^8$ ions / s.⁴³³ This is within an order of magnitude of the diffusion-limited rate (Eq. 9-30). On this basis it is clear that the channels cannot act by means of ionophoric carriers but form pores that can be opened and closed (**gated**) in response to changes in the membrane potential. They are **voltage-sensitive ion channels**.^{433,434} The channels are selective for specific ions and the selectivity parallels that of sites in some cation-exchange resins such as those containing carboxylate groups. This suggested that the inside surface of the channel might contain one or more carboxylate groups from protein side chains as well as other polar groups. A Na⁺ ion approaching the channel entrance might exchange some of its hydration sphere for ligands from the channel surface. The differing affinity of the “ion exchange” sites for various cations could ensure that it is predominately Na⁺ that passes through the channel. Anions could be excluded by electrostatic repulsion. Recent structural studies have allowed these speculations to be replaced with experimental findings as described in the following paragraphs. They have revealed that the selectivity mechanism are similar for Na⁺ and Ca²⁺ channels.

The sodium ion channel of the electric eel.

Making use of the binding of radioactively labeled specific toxins to identify them, the subunits of the sodium channel proteins were purified from several sources including the electrical tissue of the electric eel *Electrophorus electricus*,^{437–439} heart and skeletal muscle, and brain.^{440–441b} In all cases a large ~ 260 -kDa glycoprotein, which may be 30% carbohydrate, is present. The saxitoxin-binding protein from rat brain has two additional 33–36 kDa subunits with a stoichiometry of $\alpha\beta_1\beta_2$. The *Electrophorus* α subunit consists of 1820 residues,⁴³⁷ while rat brain contains α proteins of 2009

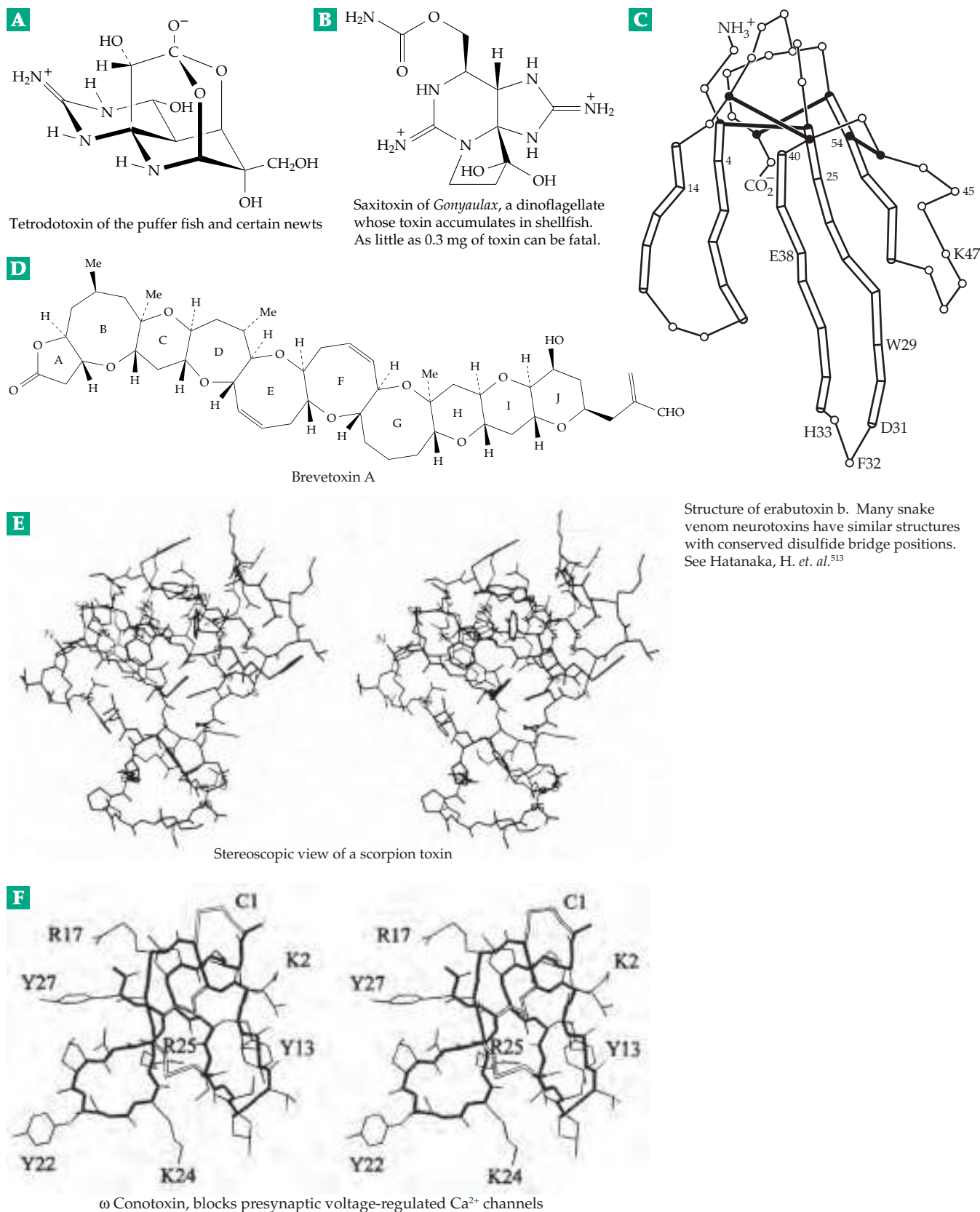


Figure 30-16 Structures of some neurotoxins that affect ion channels. Other neurotoxins include the Na^+ , K^+ -ATPase inhibitor ouabain (Fig. 22-12), batrachotoxin (Fig. 22-12), and picrotoxin (Fig. 22-4). The structure of a scorpion toxin is from Almassy *et al.*,^{494a} that of ω conotoxin is from Pallaghy *et al.*,⁴³⁵ and that of brevetoxin is redrawn after Shimizu *et al.*⁴³⁶

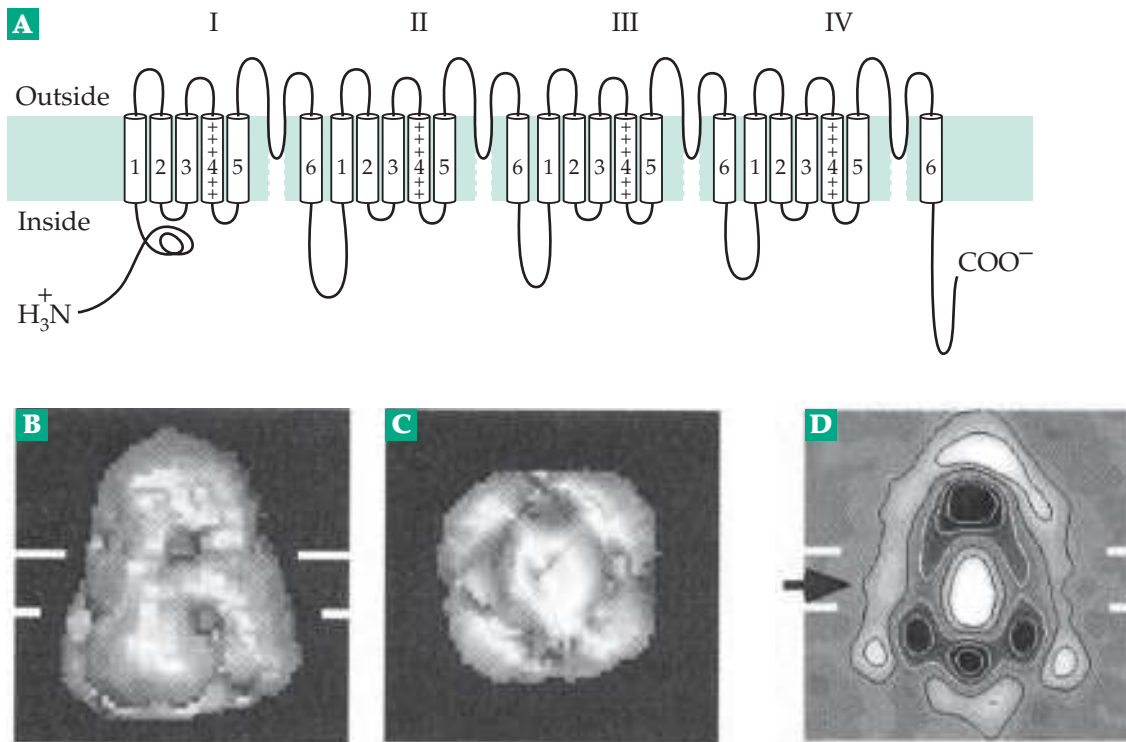


Figure 30-17 (A) Two-dimensional map of the ~260-kDa α subunit of the voltage-gated Na^+ channel from the electric eel *Electrophorus electricus*.^{438,441} (B) Image of the sodium channel protein obtained by cryo-electron microscopy and image analysis at 1.9 nm resolution. In this side view the protein appears to be bell-shaped with a height of ~13.5 nm, a square bottom (cytoplasmic surface) ~10 nm on a side, and a hemispherical top with a diameter of ~6.5 nm. (C) Bottom view of the protein. (D) Axial section which cuts the bottom, as viewed in (C), approximately along a diagonal. From Sato *et al.*⁴³⁸ Notice the cavities (dark) and domain structures (light). The black arrow marks a constriction between upper (extracellular) and lower (cytoplasmic) cavities. White lines indicate approximate position of the lipid bilayer. From Sato *et al.*⁴³⁸ Courtesy of Chikara Sato.

and 2005 residues, respectively, for Na^+ channels designated I and II.⁴⁴¹ In fact, mammals contain ten distinct Na^+ channel genes.⁴⁴² In every case the channel proteins contain four consecutive homologous sequences of about 300 residues apiece. Within these the hydro-pathy plots (see Fig. 2-30) suggest that each homology region forms six membrane-spanning helices as shown in Fig. 30-17A.⁴⁴¹ The four sets may then fold together into a square arrangement that provides a pore somewhat familiar to that of the voltage-gated K^+ channel (see Fig. 30-18). The three-dimensional structure of the sodium channel protein, based on cryo-electron microscopy, appears to be complex. The central channel may resemble that of Fig. 30-18, but there also seem to be smaller peripheral channels (Fig. 30-17).⁴³⁸ Bacteria also contain Na^+ channels but they are tetramers of smaller subunits, resembling in this respect bacterial K^+ channels (Figs. 30-18).^{442a,b}

How do the “gates” to ion channels open? Presumably some part of the channel protein senses the change in potential and undergoes an appropriate alteration in conformation that opens the gate.⁴³⁴ The current carried by the ions flowing out through a small

number or even a single channel can be measured with tiny **patch electrodes** having openings ~1 μm^2 in area. These are pressed against the nerve membrane, where they form a tight seal. With such a small patch of membrane surface the electrical noise level is low, and it is possible to measure the conductance of the pore.^{149,443} From such measurements it was found that a single pore can allow $>10^8$ ions to pass through in one second. Another thing that is apparently measured with patch electrodes is a small **gating current**, which precedes the opening of the channels by ~0.1 ms. This has been interpreted as a flow of ~6 charges across the membrane or the movement of a larger number of dipoles needed to open the gate. One possibility is that a loss of the electrical field from the surface charges on the bilayer induces a rearrangement of charges on protein side chains within the bilayer or induces changes in interactions between two or more dipoles. Such changes could trigger conformation alterations within the proteins, allowing the channel to switch from open to closed.

Recordings with single channels indicate that after a sodium channel is open for a random length of time

it spontaneously closes and passes into a third state, an “inactive” state from which it cannot reopen during the refractory period. After the membrane is repolarized it can function again.^{444a}

Calcium ion channels. Immediately after the Na^+ pores open as a result of membrane depolarization, voltage-sensitive Ca^{2+} channels also open. These allow a rapid influx of Ca^{2+} , which can trigger many processes including the secretion of neurotransmitters within the synapses.^{434,444} There are several types of voltage-sensitive Ca^{2+} channels.^{444a,b} The most abundant type are specifically inhibited by dihydropyridines and are called **dihydropyridine-sensitive** or L-type channels.^{434,445–445b} They are most numerous in the transverse tubular membranes of skeletal muscle where they appear to form a complex with the very large calcium release channels, the **ryanodine receptors** (Fig. 19-21 and associated discussion).^{446,446a} These channels appear to have a structure similar to that of the Na^+ channels.⁴³⁴ Calcium channels are also discussed on p. 422 and on pp. 1114–1115. Calcium ions play a central role in cell signaling and there are a large number of different calcium channels in bacteria, plants, and animals. Many of these are coupled to specific receptors.^{445b,447} Some are involved in controlling intracellular stores.^{447–449} Some release Ca^{2+} in response to mechanical movement and function in feeling, hearing, maintaining balance, and cardiovascular regulation. Plants sense wind and gravity, and microorganisms sense changes in osmotic pressure with the aid of these channel proteins.^{450–452}

Potassium ion channels. Several types of K^+ -selective cation channels have been recognized on the basis of electrophysiological and pharmacological studies.¹⁴⁹ More recently, the cloning of channel genes has permitted the study of the proteins by X-ray crystallography. The first structure determined^{452a} was that of the *Streptococcus lividans* K^+ channel (designated KcsA; Fig. 8-21). There are three large structural families of K^+ channel proteins.^{453–455a} One group consists of voltage-regulated (K_v) channels, such as those involved in the action potential of neurons. Like the *S. lividans* channel, they are tetramers whose predicted structure contains six transmembrane helices per subunit with a pore-forming loop (P region) between helices 5 and 6. This is just what is seen in the *S. lividans* channel and in one-fourth of the much larger Na^+ channel protein (Fig. 30-17A). Furthermore, all known potassium channels, from bacteria to human beings, have the conserved sequence GYGD in the C-terminal half of the P region.⁴⁵³ A great variety of K_v channels are known. There are ~70 genes for these channels in the *Caenorhabditis elegans* genome.⁴⁵⁶ One of the first K_v channel genes to be cloned was from a *Drosophila* mutant known for its

neurological defect as *shaker*. Its structure (Fig. 30-18), which is based in part on modeling from the KcsA channel, has the ion selective filter with the conserved sequence **TVGYG** in the expected location. At the cytoplasmic end of the pore is an additional structure not found in the KcsA channel. This is the **inactivation gate**, so called because it accomplished the rapid self-inactivation of the K^+ channels during passage of the action potential (Fig. 30-18A). This is one of the factors necessary for recovery and repolarization of the axon membrane. The inactivation gate is composed of N-terminal ~130 residue “T1” domains of the α subunits together with parts of the β subunits, which are associated as a tetramer beneath the channel in the cytoplasm (Fig. 30-18B). Various experimental data including mutational analysis suggest that small ball-like domains at the N termini of the β subunits block the channel.^{456–458} Zhou *et al.* propose that the N termini unfold into an extended conformation, passing through “windows” between the T1 domains and the channel and allowing the $-\text{NH}_3^+$ ends to bind into the central cavity in the channel.⁴⁵⁹ The same site can be blocked by well-known quaternary amine inhibitors such as tetraethylammonium, tetrabutylammonium ions, or tetrabutylantimony, an analog used for X-ray crystallography.

The T1 domain of the channel not only participates in control of the ion flux but also stabilizes the pore complex.^{459a} Among the various K^+ , Ca^{2+} , and Na^+ channels the regulatory β subunits are quite variable in their structures and mechanisms of gating.^{459b} Some β subunits have bound NADH. A speculative possibility (p. 737) is that the rapid interconversion of the positively charged thiazolium ion and negatively charged thiolate ion forms of thiamin (Eq. 7-19) plays some role in nerve conduction, e.g., voltage sensing.

Some questions about ion channels have been hard to answer. For example, how are small cations allowed to flow rapidly through a very small opening in a 2–3 nm thick nonpolar core of a membrane?^{460,461} From basic electrostatic principles ΔG for transfer of an ion to the center of a membrane has been estimated as ~160 kJ/mol, a high thermodynamic barrier to transport. A solution to this problem apparently lies partly in the fact that at the center of the lipid bilayer the ion channel contains a cavity large enough (~0.5 nm diameter) to hold about 50 water molecules. Cations tend to enter this cavity, and X-ray studies have shown that the electron-dense Rb^+ does occupy the cavity. A second stabilizing factor is that four helices have their negative (C-terminal) ends pointing toward the cavity. Although the electrostatic effect of these helix dipoles (Fig. 2-20A) might be regarded as negligible, computations indicate that within the low dielectric bilayer the stabilizing effect of the helices becomes significant.^{460,461}

How are the pores in these channels opened and closed? Different channels are gated in different ways.

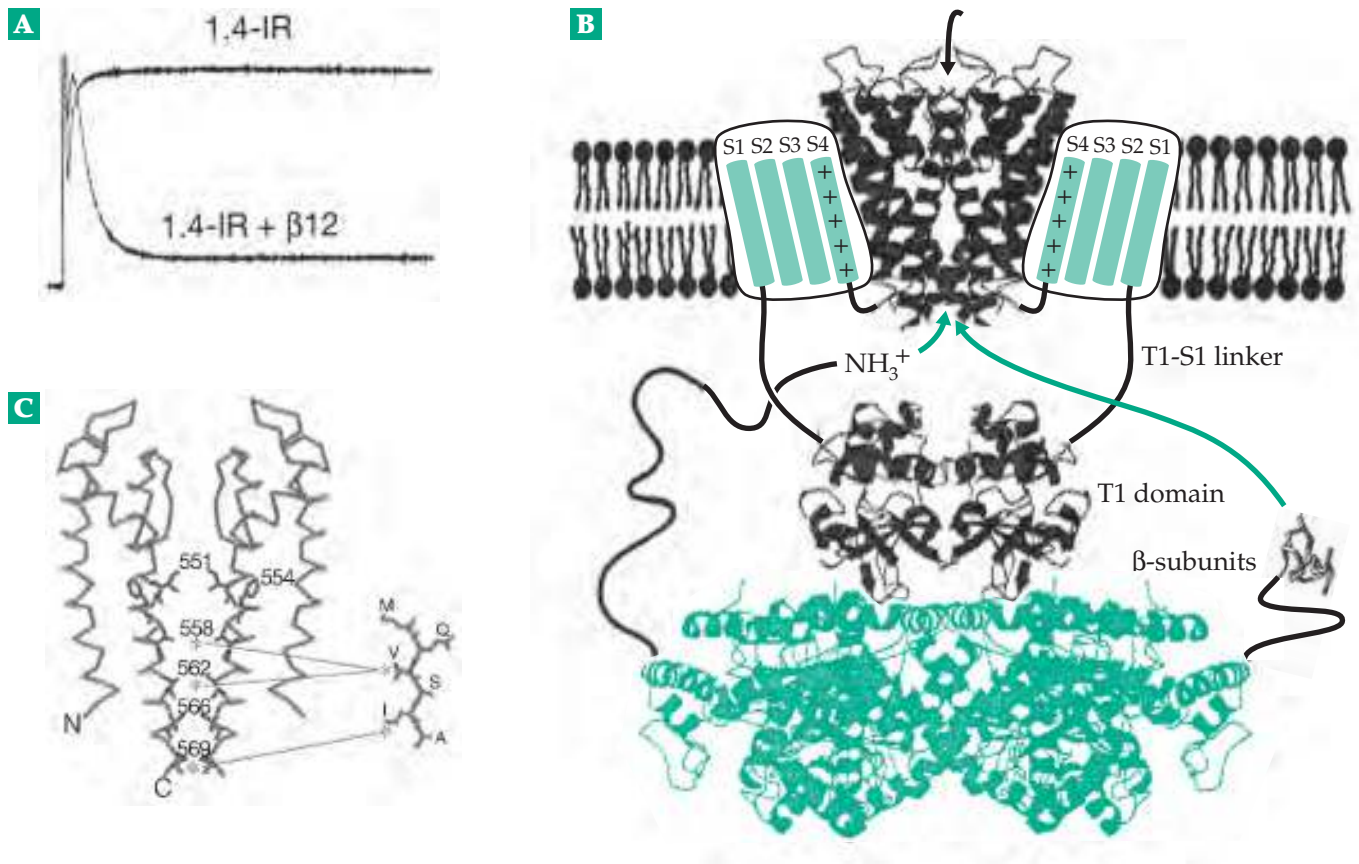


Figure 30-18 (A) K^+ currents recorded from *Xenopus laevis* oocytes carrying cloned genes of *Drosophila* shaker K^+ channels under two-electrode voltage-clamp conditions. Trace 1,4-IR was obtained from a cell expressing channels that lack the inactivation gate. Trace 1,4-IR + β_{12} , obtained from a cell expressing β subunits as well, shows rapid self-inactivation. (From Zhou *et al.*⁴⁵⁹) (B) Composite model of a voltage-dependent K^+ channel. The pore structure in the α subunit is represented by the KcsA channel (Fig. 8-21). The structure of the T1- β complex is from Gulbis *et al.*^{458a} The drawing is modified from that of Zhou.⁴⁵⁹ (C) Ball-and-stick view of the selectivity filter showing positions of four bound K^+ ions. Two of the four TVGYG peptide strands of the conduction pore are shown. Courtesy of Roderick MacKinnon.

The KcsA channel, which is mostly closed at neutral pH, responds by opening at a low external pH.⁴⁶² Using methods of spin labeling and EPR spectroscopy, Perozo *et al.* found small translational and rotational movements of the helices that form the pore (Fig. 30-18). These may alter the diameter of the pore, opening or closing it.⁴⁶³ How do the electrostatic sensors control the process? The details are uncertain, but the sensor is thought to lie in a conserved sequence of arginine and lysine residues interspersed with hydrophobic amino acids in transmembrane helix 4 of the channel protein (Fig. 30-18; see also Fig. 30-17).⁴⁵⁶ How do potassium pores select K^+ over Na^+ or Ca^{2+} ? One factor is that Na^+ is more heavily hydrated than K^+ (p. 311). This allows K^+ to pass through the channel more readily than Na^+ .⁴⁶⁴ Potassium ions travel through the 1.2-nm-long selectivity filter at a rate of $\sim 10^8 \text{ s}^{-1}$ in consecutive steps of dehydration, movement, and rehydration occurring in $\sim 10 \text{ ns}$.^{464a-d} The process is catalyzed by polypeptides and may depend

upon competition between a state in which a ring of four hydrogen-bonded peptide groups is formed and a state in which the four carbonyl groups coordinate a K^+ ion.^{464d}

Belonging to the same structural group as the K_v channels are Ca^{2+} -regulated K^+ channels.^{465,466} Some bacterial channels are controlled by binding of Ca^{2+} ions to a “gating ring” on the intracellular membrane surface.^{466a} A mammalian channel is controlled by a complex of calmodulin with the intracellular end of the α subunits of the channel^{466b} and others.^{453,467} A second large group of K^+ channels, containing seven subfamilies, are the **inward rectifying** (Kir) channels.^{455,468} They are tetramers of 360- to 500-residue polypeptide chains, each chain forming two transmembrane helices with a P region between them.^{453,469} These channels support a large conductance when K^+ ions flow out from a cell but only a small conductance when they flow in.⁴⁷⁰ Kir channels are subject to a variety of controls, which include effects of pH.^{471,472}

Some are inhibited by ATP,^{473–474b} and others by eicosanoids⁴⁷⁵ or inositol hexaphosphate.⁴⁷⁶ Some of the ATP-sensitive channels contain an ABC transporter subunit and are binding sites for sulfonylureas and other drugs. See discussion on p. 421. A number of human disorders in Kir channels have been identified.⁴⁶⁸ The human Kir channels participate in regulation of resting membrane potentials in K⁺ homeostasis, control of heart rate, and hormone secretion.⁴⁶⁸ A third group of K⁺ channels are dimeric, but each subunit contains two tandem P regions and 4–8 transmembrane helices.⁴⁵⁵

Chloride channels and the ionic environment of neurons.

All cells contain voltage-gated chloride channels, which are encoded by the *Clc* genes mentioned on pp. 420, 421.^{477,477a} Recently crystal structures^{477a–c} have revealed chloride channels formed in single polypeptide chains arranged as dimers. The selectivity filter involves stabilization by the positive ends of α -helix dipoles. The importance of the corresponding proteins to the human body is shown by the existence of several specific diseases arising from mutations in their genes (p. 420).^{478,479} A calcium-regulated Cl[−] channel is also present⁴⁸⁰ as is the ATP-gated CFTR channel (Box 26-A).^{480a} In addition, other ligand-gated Cl[−] channels, such as γ -aminobutyrate receptor channels (Section B,9), are found in the central nervous system.⁴⁸¹ A glutamate-gated chloride channel in invertebrate organisms is the site of action of the antihelminthic and insecticidal compound **ivermectin**.^{481a}

The significance of ion channels can be better appreciated by considering the ionic environment of nerve axons.¹⁴⁹ Mammalian neurons have roughly the following millimolar concentrations of ions in the cytosol and in the external medium. (The concentration gradients for the much-studied squid axon are substantially higher.^{149,482}) The membrane potentials that could arise from each one of these concentrations, according to Eq. 8-2, are also given.¹⁴⁹ In a resting

	Cytosol	Extracellular	E_m (mV)
K ⁺	150	5.5	−90
Na ⁺	15	150	+60
Ca ²⁺	10 ^{−4}	1.5	+270
Cl [−]	9	12.5	−70

neuron the K⁺ potential dominates with an observed membrane potential of \sim −80 mV. Some K⁺ channels are open and the K⁺ and Cl[−] concentrations are nearly in Donnan equilibrium across the membrane. The Na⁺ and Ca²⁺ channels are closed, and the sodium and calcium pumps keep the internal concentrations of these ions low.

When an action potential is propagated, a wave of depolarization moves along the axon, changing the membrane potential suddenly to a less negative value. When it reaches \sim 50 mV the Na⁺ channels open, allowing sodium ions to flow into the cell causing further propagation of the wave of depolarization. After \sim 1–2 ms the Na⁺ channels begin to deactivate. At the same time the slower K⁺ channels open allowing potassium ions to flow out and to repolarize the membrane, the membrane potential sometimes transiently reaching more negative values (hyperpolarization) than the \sim 80 mV resting potential. Action of the Na⁺,K⁺-ATPase then restores the original state. The finely tuned properties and sequential opening and closing of the channel proteins are essential to the conduction of nerve impulses.

The existence of voltage-gated ion channels in bilayers are not limited to nerve membranes. They are present to some extent in all cell membranes. Even the paramecium has at least seven kinds of Na⁺, K⁺, and Ca²⁺ channels.⁴⁸³ Channels may also be formed by many peptide antibiotics. Among them are the human defensins (Chapter 31) and the \sim 20-residue **alamethicin**. Six to eleven of the mostly helical monomers of that antibiotic assemble to form a single voltage-dependent channel.^{484,484a} The bacterial toxin colicin E1 (Box 8-D) forms voltage-dependent channels within bacterial membranes.⁴⁸⁵

Receptor-associated ion channels. Many neurotransmitters, including acetylcholine and glutamate, act to open ion channels that are part of the receptor protein or of a tight complex of proteins.^{149,486} Such **ionotropic receptors** are responsible for most rapid neuronal action. For example, binding of acetylcholine to its receptor in the neuromuscular junction causes the release of Ca²⁺ ions from the exterior into the muscle fibers. Binding of glutamate to its ionotropic receptor in a synaptic ending of a dendrite causes an influx of ions into the cytoplasm, initiating an action potential in the dendrite. In most instances the properties of the receptor channel favor the rapid flow of Ca²⁺ ions into the cytoplasm.

Many other receptors are 7-helix transmembrane proteins, which activate guanine nucleotide G proteins (Chapter 11, Section D, 3). The G proteins couple some receptors directly to Ca²⁺ channels; they couple other receptors to adenylate cyclase and cyclic AMP-activated channels and yet others via phospholipase C to K⁺ channels and indirectly to Ca²⁺ channels (Fig. 30-19). All of these G protein coupled receptors are referred to as **metabotropic receptors**. A single synapse often contains both ionotropic receptors and metabotropic receptors. The ionotropic receptors induce a rapid (<1 ms) response, while the metabotropic receptors act more slowly. However, in most cases the final effect is the release of calcium ions into the cytoplasm

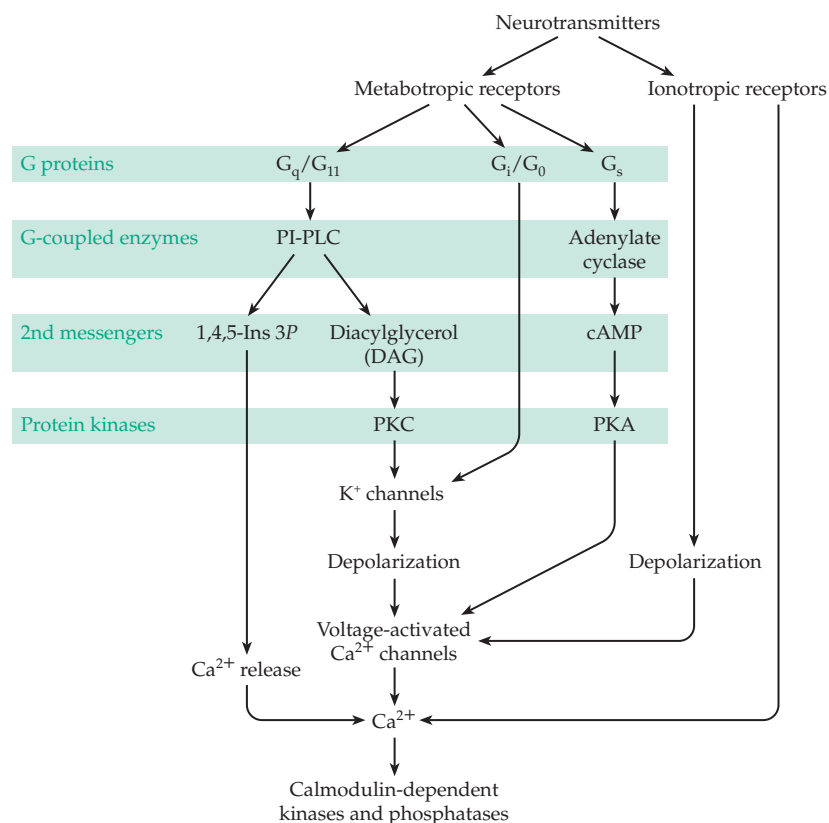


Figure 30-19 Major signaling pathways from metabotropic and ionotropic receptors in neurons. Various G proteins control the signaling from metabotropic receptors using phosphatidylinositol-specific phospholipase C (PI-PLC) and adenylate cyclase or acting directly on K⁺ ion channels. Adapted from Fig. 5.1 of Nicholls' *Proteins, Transmitters, and Synapses*.¹⁴⁹

(Fig. 30-19). The rapid response may be initiation of an action potential, while the slow response may be activation of calmodulin-dependent kinases and phosphatases.¹⁴⁹

6. A Plethora of Neurotoxins

Bacteria, protozoa, and venomous animals synthesize numerous toxins that are used to kill their prey or to defend themselves. Sea anemones, jellyfish, cone snails, insects, spiders, scorpions, and snakes all make potent and highly specific neurotoxins. Plants form a host of alkaloids and other specialized products, some of which are specifically neurotoxic and able to deter predators. More than 500 species of marine cone snails of the genus *Conus* synthesize a vast array of polypeptide toxins (**conotoxins**),^{487–489} some with unusual posttranslational modifications.^{490,491} The slow-moving snails are voracious predators that use their toxins, which they inject with a disposable harpoon-like tooth,⁴⁹² to paralyze fish, molluscs, or worms.⁴⁹³

The targets for natural biological toxins include ion channels and receptors for transmitters. At least four parts of the voltage-gated sodium channels are binding sites for extremely toxic natural products.^{494–499} **Tetrodotoxin** (Fig. 30-16),^{496,497} which is found in the puffer fish, certain newts,^{429a} and venom of the blue-ringed octopus, and also the shellfish poison **saxitoxin** (Fig. 30-16) block the entry of sodium ions into the channels.⁴⁹⁸ **Batrachotoxin** (Fig. 22-12) and related lipophilic compounds such as **veratridine** increase sodium permeability by blocking the channels permanently open. **Pyrethroid insecticides** (p. 1237) prolong the time that the sodium channels stay open after excitation. Some **scorpion toxins** (Fig. 30-16),^{494,499} which all have a hydrophobic core made from a short α helix and a three-strand antiparallel β sheet,^{500–502} and **sea anemone toxins**^{495,503–505} also stabilize the open conformation of the Na⁺ channels. Other smaller ~4-kDa scorpion toxins block K⁺ or Cl[−] channels or other receptors.^{500,506,507} Some are most toxic to insects and others to mammals.⁵⁰⁰ Although their three-dimensional structures resemble those of scorpion toxins, the amino acid sequences of anemone toxins show no homology.⁵⁰⁵ The most potent poison produced by the red tide organism, the dinoflagellate *Gymnodinium breve* (Fig. 1-9), is **brevetoxin A** (Fig. 30-16).^{436,508} It selectively opens one class of sodium channels.⁴⁹⁵

Venoms of **cobras**, **sea snakes**, and pit vipers contain several 6- to 7-kDa proteins that bind to acetylcholine receptors (Fig. 30-23) of the postsynaptic neurons, preventing binding of the neurotransmitter and opening of the ion channels.^{509,510} All of these toxins contain four disulfide bridges and share with certain plant proteins a folding pattern that has been called the **toxin-agglutinin fold**^{511,512} (Fig. 30-16). These toxins include **erabutoxin a** (Fig. 30-16) from a sea snake^{513,514} as well as the 74-residue toxin **bungarotoxin a** (from the banded Krait). This toxin, which has been used to titrate acetylcholine receptors in neuromuscular junctions, is a member of the *long neurotoxin* group, which contains 71–74 residues and five disulfide bonds.⁵¹⁵ Other *short neurotoxins* are 60–62 residues in length with four disulfide bridges.⁵¹⁶ Cobra toxins contain both neurotoxins and **cardiotoxins**, which have somewhat similar structures but quite different modes of action.^{517,518} In contrast, **crototoxin**

from the venom of a South American rattlesnake^{510,510a} and **β -bungarotoxin**⁵¹⁹ consist of 13-kDa phospholipases A₂ complexed with smaller 7.5-kDa proteins. They act at the presynaptic membranes of selected neurons by blocking neurotransmitter release.⁵²⁰

The seven types of **botulinum toxin**^{521–523a} and the **tetanus toxin**⁵²⁴ are the most neurotoxic substances known. Only 10⁸ molecules are sufficient to kill a mouse. Both toxins are zinc proteases, which block presynaptic transmitter release by cleaving specific synaptic vesicle proteins (see p. 1780 and Fig. 30-20).^{522,523,525–528} They bind initially to ganglioside in the neuromuscular junction, one subunit then being internalized as with the diphtheria toxin (Box 29-A). Botulinum toxins specifically enter motor neurons,^{521,528a} while tetanus toxin is taken up via synaptic vesicle endocytosis⁵²⁹ by both peripheral and central neurons. Retrograde axonal transport carries the toxin into the central nervous system and across synaptic clefts into cholinergic interneurons, which are poisoned.

The black widow spider produces the 130-kDa **α -latrotoxin**, which causes massive release of acetylcholine, norepinephrine, dopamine, and GABA from synaptosomal endings.^{530,531} The small **anatoxin-a** or “very fast death factor” (Fig. 30-22), which is synthesized by various cyanobacteria, antagonizes both muscarinic and nicotinic acetylcholine receptors.⁵³² Cone snails synthesize mixtures of the 13- to 17-residue conotoxins (Fig. 30-16).⁴⁹³ They cause rapid paralysis of fish permitting the snails to prey on the much faster fish. They bind to a variety of targets, which include Na⁺, K⁺, and Ca²⁺ channels,^{435,492} and acetylcholine,^{533,534} and glutamate⁴⁹⁰ receptors. One of the toxins is a 17-residue peptide containing five residues of γ -carboxyglutamate and is also notable for the fact that intercerebral injection of less than one microgram of the toxin induces a prolonged sleeplike state in mice.^{490,493} The venom of *Conus geographicus* is so toxic that two-thirds of human stinging cases are fatal.

The most deadly nonproteinaceous toxin known, **palytoxin**, is also the most complex structure ever established without the aid of X-ray crystallography.^{535,536} It is produced by marine zoanthids of the genus *Palythoa* and has the molecular formula C₁₂₉H₂₂₃N₃O₅₄.

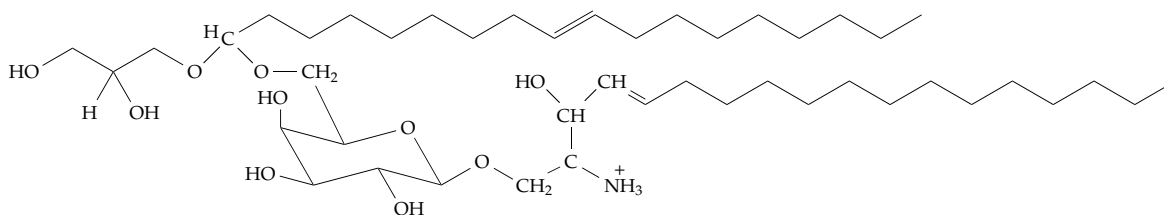
7. Neuronal Metabolism

The brain has a very high rate of metabolism. Although accounting for only 1/50 of the body mass its utilization of energy amounts to 1/5 of the basal metabolism. This is ~20 watts and is nearly constant day and night. It reflects the unusually active metabolism of neurons, a major part of which can be attributed to the sodium–potassium ion pumps in the membranes and to the maintenance of the excitable state.^{536a} The source of energy for these processes is the ATP that is

utilized to drive the ion pumps and thereby to maintain the membrane potential needed to drive the action potentials. The ATP is formed largely by oxidative metabolism of glucose and, to a lesser extent, of acetoacetate. The large surface area of the axons as well as the frequency with which they transmit nerve impulses accounts for the high rate of metabolism.

Another factor peculiar to neurons doubtless contributes also to their rapid metabolism. The nucleus and most of the ribosomes are found in the cell body. Although few ribosomes are seen in axons and dendrites^{536b}, many proteins are needed in high concentrations within the axons and synaptic endings. Among these are enzymes catalyzing synthesis and catabolism of neurotransmitters and membrane proteins. If an axon is cut, the separated synaptic endings soon atrophy, an observation that long ago suggested that essential materials, which may include mRNAs,⁵³⁷ might flow from the cell body. It has now been established experimentally that many materials do move at the rate of 0.3–3 mm / day from the cell body down the axon.⁵³⁸ More remarkable is **fast axonal transport** by which proteins and other materials move at rates of up to 5 μ m / s (0.4 m / day). This transport is specifically blocked by vinblastine (Box 7-D) and batrachotoxin (Fig. 22-12). As has been pointed out in Chapter 20, an ATP-hydrolyzing protein chemically related to the myosin heads functions together with microtubules to provide a kind of miniature railway that moves materials along the microtubules. Transport is sometimes in the opposite direction, i.e., from the synaptic endings to the cell body. This **retrograde axonal transport** may be of importance in altering neuronal properties in response to electrical activity at synaptic endings. It also provides a means of recycling materials originally sent in the other direction.

Brain cells appear to transcribe an unusually large fraction of the genome.^{539–541} About 20% of the DNA of human brain was found to hybridize with mRNA formed by brain cells. In other tissues about half this amount of DNA appears to be transcribed. A related observation that seems surprising is the absence of common electrophoretic variants of enzymes in the brain.⁵³⁹ However, brain cells synthesize specialized isoforms of many proteins, e.g., of the G proteins (p. 558), the cytoskeletal protein 4.1 (Fig. 8-14),⁵⁴² and transglutaminase.⁵⁴³ Unusual lipids, such as the cationic acetal of a galactosylcerebroside shown above,⁵⁴⁴ are also formed. Adult rat brain contains about 30,000 different kinds of polyadenylated messenger RNA,⁵⁴⁰ much of which lacks the poly(A) tail.⁵¹³ Many of these mRNAs contain a specific 82-nucleotide sequence within at least one of their introns. Sutcliffe *et al.* suggest that this is an **identifier sequence** instructing brain cells to express these genes.^{540,545} However, the sequence is also found in genes transcribed in other tissues, and its significance is not clear.^{546,547}



8. Synapses and Gap Junctions

Like the micro-transistors in a computer chip, synapses are the devices by which the brain operates. Synapses process and integrate information from many input channels, send signals on to other neurons, and store information. The information is not stored in digital form, but as chemical alterations in the synapses themselves.^{482,548,549} Synapses are formed when axons, growing in response to a chemical trail, reach their destinations and send out branches, each with a bulbous terminal knob (**bouton**). When these boutons meet receptive regions on dendrites of another axon, synapses are formed.⁵⁵⁰ The synapse is a very firm connection with a thin, tight synaptic cleft through which signaling takes place. It is surrounded in part by astrocytes or other glial cells (Fig. 30-20A,C).

With the advent of electron microscopy, the fine structure of synaptic contacts became evident. The synaptic knobs were often found to contain vesicles of ~30–80 nm diameter, which were later shown by chemical analysis and staining procedures to contain the neurotransmitters (Fig. 30-10). In the case of the acetylcholine-releasing synapses (**cholinergic synapses**) each 80-nm vesicle contains ~40,000 molecules of acetylcholine,⁵⁵¹ the concentration in the vesicle being of the order of 0.5 M. To show that the acetylcholine released at a synapse stimulated the postsynaptic membrane to initiate an impulse, the technique of **electrophoretic injection** or **microiontophoresis** was developed.⁵⁵² By using ultramicrocapillaries a small pulse of current, e.g., 3×10^8 amp for 1 ms, can be used to inject electrically a compound directly into a synaptic cleft. The results may be observed with separate recording electrodes, one of which is inserted into an axon or a muscle fiber. By this means it was shown that amounts of acetylcholine comparable to those released at the large synapses of the neuromuscular junction do cause muscles to contract.

How does the release of neurotransmitter occur? That the release is “quantal,” i.e., involving the entire content of a vesicle, was established from the observation of **miniature end-plate potentials**. These are fluctuations in the postsynaptic potential observed under conditions of weak stimulation of the presynaptic neuron. They reflect the random release of neurotransmitter from individual vesicles.⁵⁵³ Normally, a strong impulse will release on the order of 100–200

quanta of transmitter, enough to initiate an action potential in the postsynaptic neuron.

A synaptic vesicle cycle. The number of synaptic vesicles in a single synapse in the brain varies from fewer than 100 to several hundred. In specialized synapses there may be thousands. However, at any moment only a fraction of the total are in the “active zone,” often aligned along the presynaptic membrane (Fig. 30-20A) or in specialized ribbons such as those in Fig. 30-10B. The vesicles are normally reused repeatedly, undergoing a cycle of filling with neurotransmitter, translocation to the active zone, ATP-dependent priming, exocytosis with release of the neurotransmitter into the synaptic cleft, coating with clathrin, endocytosis, and acidification as outlined in Fig. 30-20B.^{554–557} The entire cycle may be completed within 40–60 s to avoid depletion of active vesicles.^{558,559} A key event in the cycle is the arrival of an action potential at the presynaptic neuron end.

The accompanying depolarization of the membrane at the synaptic ending permits a rapid inflow of calcium ions through a voltage-gated calcium channel.^{444,560} Within less than 0.1 ms the transient increase in intracellular $[Ca^{2+}]$ triggers the release of the contents of the vesicles. About four calcium ions are needed to release one clathrin-coated vesicle (Fig. 30-20A,B). The membrane fusion required for transmitter release involves cytoskeletal proteins of the synaptic endings as well as specialized proteins that are present in the membranes of the synaptic vesicles (Table 30-6). In fact, every step in the cycle depends upon specialized proteins.³⁸⁷

Synaptic vesicles can be isolated in large quantities. Their composition is well known, and the proteins have been studied intensively. Indeed, much of what we know about exocytosis and vesicular transport has been learned from investigation of synaptic vesicles.^{554,561,562} A small synaptic vesicle of 35 nm diameter will contain ~10,000 phospholipid molecules in its membrane and only about 200 protein molecules, at least one of which must be a 13-subunit vacuolar type proton pump (Fig. 18-14). This pump acidifies the vacuole, allowing uptake of a neurotransmitter. Although many different proteins may be found in synaptic membranes, only about 15, which are listed in Table 30-6, are found in all synaptic vesicles and appear essential to function.

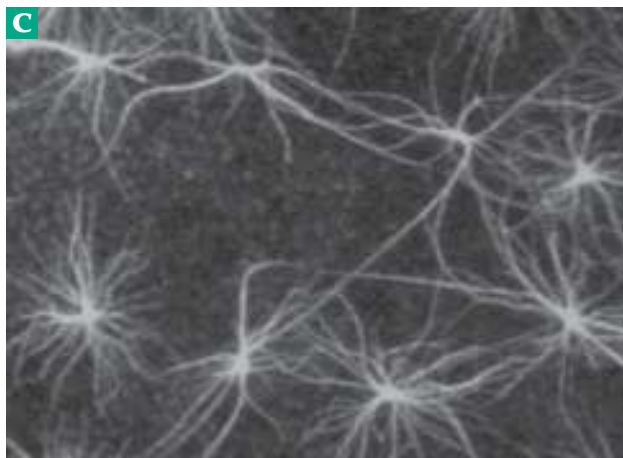
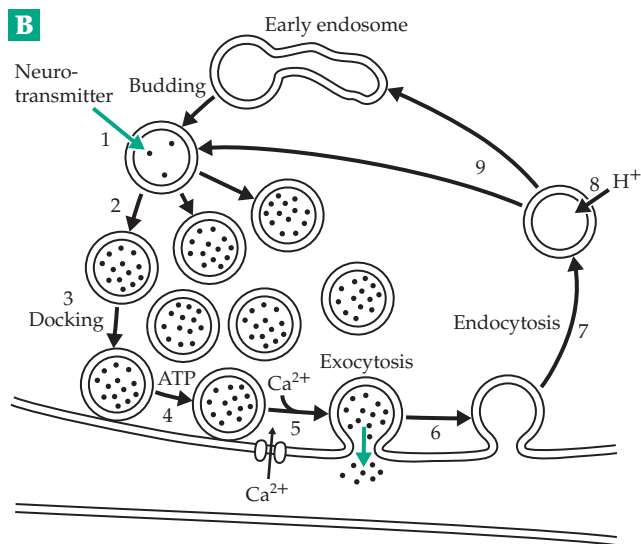
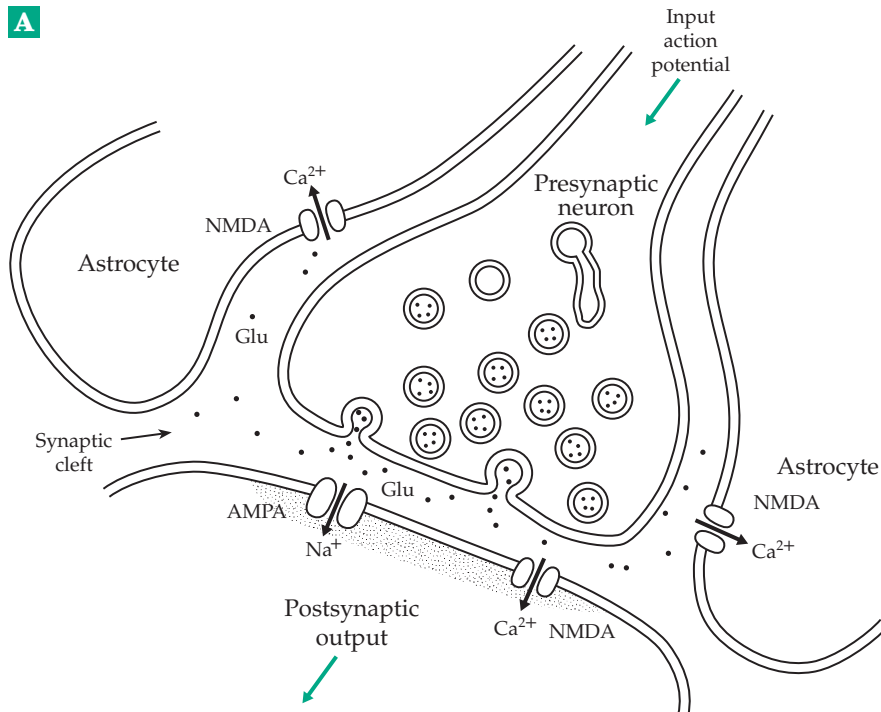
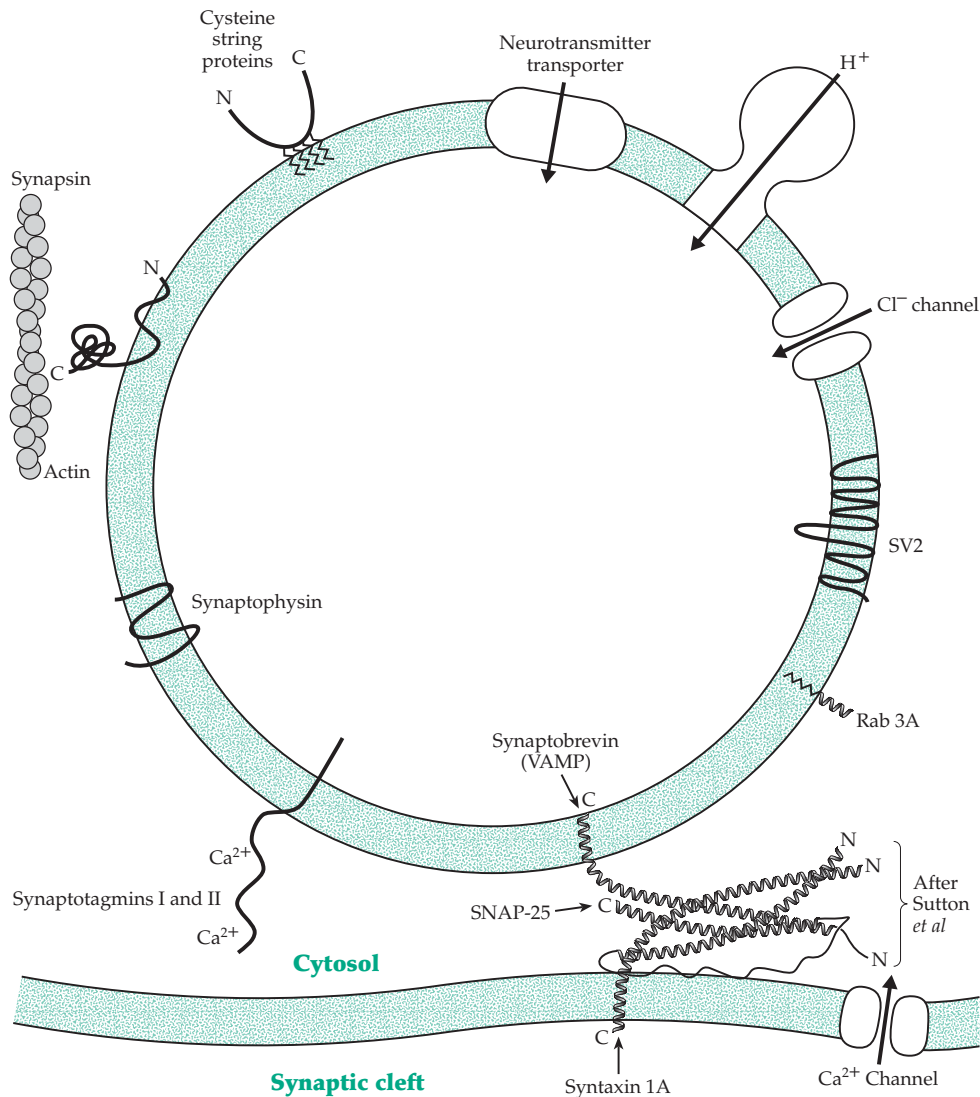


Figure 30-20 (A) Schematic drawing of a fast glutamatergic synapse. An action potential arrives at the synapse, depolarizing the presynaptic membrane and allowing calcium ions to enter the cytoplasm via voltage-gated Ca^{2+} channels. The Ca^{2+} ions induce exocytosis of small synaptic vesicles from the “active zone” near the membrane, releasing glutamate into the synaptic cleft. After diffusing rapidly across the narrow ~50-nm synaptic gap the glutamate binds to its receptors on the ending on a dendrite from a second (postsynaptic) neuron. Glutamatergic synapses usually have two types of receptor, NMDA and AMPA (see Fig. 30-24 and text). Both are ligand-gated ion channels, which release Ca^{2+} and Na^{+} into the cytosol of the postsynaptic ending depolarizing its membrane and possibly initiating an action potential. (B) The synaptic vesicle cycle. The synaptic vesicles, which are formed by budding from an early endosome, are filled with neurotransmitter (1). The filled vesicles are then transported to the active zone near the presynaptic membrane (2), are “docked” on the membrane surface (3), and undergo ATP-dependent priming (4). Binding of four Ca^{2+} ions induces exocytosis and rapid release of the neurotransmitter (5). The empty vesicles receive a clathrin coat (6) and undergo endocytosis (7) and uptake of protons (8) to acidify the content in preparation for a second round of neurotransmitter uptake. Alternatively the vesicle can fuse with an endosome as part of the cycle. After Südhof and Scheller.⁵⁵⁴ (C) Small section of brain stained to reveal the astrocytes whose extensions form synapses not only with neurons, as in (A), but also with capillary blood vessels.¹⁴⁹ From Kimelberg and Norenberg.⁵⁶⁴ Micrograph from Andreas Karschin, Heinz Wässle, and Jutta Schnitzer. (D) Illustration of some proteins essential to the synaptic vesicle cycle. Several are integral membrane proteins. Synaptotagmins contain Ca^{2+} -binding domains and may serve as calcium sensors. The vesicle is portrayed as if docked to the presynaptic membrane by interaction of the SNARE proteins synaptobrevin, syntaxin, and synaptotagmin. The 4-helix bundle is as portrayed by Sutton *et al.*⁵⁶³

D



The synaptic vesicles, which are formed by budding from early endosomes, take up neurotransmitters using one of the transporters (step 1 in Fig. 30-20B). Transmitter uptake is G-protein dependent⁵⁶⁵ and is driven by the proton electrochemical gradient generated by a vacuolar type (V-type) ATPase (Chapter 18).^{149,566} The filled vesicles move into the active zone where they undergo an ATP-dependent priming of uncertain nature.^{555,567} Exocytosis (step 5 in Fig. 30-20B) requires membrane fusion, and it is possible that partial fusion occurs during the priming steps. Priming is also thought to involve interaction between vesicle-associated v-SNAREs and synaptic membrane-associated t-SNAREs (p. 521).^{556,563} A major v-SNARE has been identified as **synaptobrevin**, which is also known as **VAMP** (vesicle-associated membrane protein).^{563,568,568a} The C-terminal-anchored synaptobrevin is inserted into the plasma membrane of neuronal and neuroendocrine cells prior to endocytosis and budding of the synaptic vesicles.⁵⁶⁸ The target

t-SNAREs have been identified as the synaptic plasma membrane proteins **syntaxin**^{568b} and **SNAP-25**.^{569–573} Syntaxin is an integral membrane protein, whereas SNAP-25 is anchored by palmitoylation.⁵⁷¹ These proteins bind together to form a synaptobrevin•syntaxin•SNAP-25 complex, which forms a four-helix bundle as shown in Fig. 30-20. Synaptobrevin and syntaxin each contribute one helix, while SNAP-25 provides two; all four have a mutually parallel orientation.^{563,574,574a} The helix bundle is so tight that it has a high melting temperature and is resistant to proteolytic cleavage. Nevertheless, the helical domains of both synaptobrevin and syntaxin are sites of very specific cleavage by the zinc proteases of tetanus and botulinum toxins.^{527,563,570} Cutting of the protein chains by these toxins prevents proper formation of the four-helix bundle and prevents release of neurotransmitter. It is thought that the complex, which probably forms at several points on the periphery of the docked synaptic vesicle, is essential for membrane fusion.

Other proteins are also needed. All cell fusion processes seem to require regulatory proteins that are essential to neurotransmission in the nematode *C. elegans*. Two of these are encoded by the nematode genes *unc-13* and *unc-18*. The corresponding mammalian proteins **munc-13** and **munc-18** interact with syntaxin and are essential for exocytosis of synaptic vesicles.^{572,575} An ATPase is also needed for correct functioning of the SNARE complex⁵⁷⁴ as are other additional proteins.⁵⁷⁰

Details of the control of exocytosis are also uncertain. **Synaptotagmin I**, which contains two Ca^{2+} -binding domains, is probably the sensor that detects the rapid influx of Ca^{2+} that initiates exocytosis.^{576–578b}

It binds several Ca^{2+} ions via a β -sandwich motif that contains five aspartate side chains at its tip. This motif is conserved in a large family of synaptotagmins. A possibility is that Ca^{2+} -synaptotagmin complexes may self-associate to form a protein ring around the site where the fusion pore forms.⁵⁷⁶ Synaptotagmin I also interacts with both syntaxin and with **neurexins**, proteins related to laminin (Fig. 8-33) and present in numerous variant forms in nerve endings. Neurexins are also targets for the α -lathrotoxin of the black widow spider.^{531,579} Other proteins that may participate in membrane fusion include the unique **cysteine string proteins**, which in *Drosophila* contain 13 cysteine residues, 11 of which are palmitoylated.^{580,581} Nitric

TABLE 30-6
Some Proteins Important to the Formation and Functioning of Synaptic Vesicles^a

1. Synaptic vesicle proteins

Synapsins Ia, Ib, IIa, IIb	Peripheral, abundant
Rab3, rabphilin	Rab 3 has lipid anchor
Cysteine string proteins (CSP)	Ca^{2+} -binding
Synaptotagmins	Single transmembrane helix; Ca^{2+} receptor N terminus in vesicle
Synaptobrevins (VAMPs) ^b	SNARE proteins, C termini in vesicle
Synaptophysins, synaptogyrin	Integral membrane protein
SV2 A, B, C	Integral membrane protein, Cl^- transporter
SCAMPS 1 and 4	Integral membrane protein
SVOP	Integral membrane protein
Vacuolar H^+ pump	13 subunits
Cytochrome 561	H^+ generator
Neurotransmitter transporters	For acetylcholine, glutamate, GABA/glycine, catecholamines, ATP
Ancillary transporters	Zn^{2+} , Cl^-

2. Presynaptic membrane proteins

Syntaxin ^b	t-SNARE
SNAP-25 ^b	t-SNARE
Munc-13	
Ca^{2+} channel	
Agrin	
Neurexin	
Actin and microtubules	In dendrites

3. Postsynaptic specializations

Receptors	e.g., NMDA, AMPA
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^a Based on data of Südhof and Scheller: Südhof, T. C., and Scheller, R. H. (2000) in *Synapses* (Cowan, W. M., Südhof, T. C., and Stevens, C. F., eds), pp. 177–215, Johns Hopkins Univ. Press, Baltimore, Maryland and Südhof, T. C. (1995) *Nature (London)* **375**, 645–653.

^b Targets for clostridial toxins, tetanus, botulinin.

oxide NO may be involved in a late stage of exocytosis,³⁹² and phospholipase D1 may also be required.⁵⁸²

Presynaptic nerve terminals may contain as few as a hundred vesicles, which must be recycled rapidly after exocytosis in order to allow for repetitive firing.^{558,559,583} Several proteins are needed for endocytosis (step 7 in Fig. 30-20). These include **endophilin I**,⁵⁸⁴ the vesicle transport ATPase **NSF**,⁵⁷⁴ GTPases,⁵⁶⁵ and the soluble NSF attachment protein α -SNAP (which is not related to SNAP-25).⁵⁸⁵

Functions of some other abundant proteins of synaptic vesicles have not yet been accurately defined. The **synapsins** are abundant peripheral membrane ATP-binding proteins with multiple phosphorylation sites and variable C-terminal domains that interact with cytoskeletal proteins such as actin microtubules, microfilaments, and spectrin.^{554,561,586,587} Another abundant protein is **synaptophysin**, an integral membrane protein found in all synaptic vesicles.^{554,561,588} Other proteins are discussed by Südhof and Scheller.⁵⁵⁴ The small G protein **rab 3** together with the Ca^{2+} -binding protein **rabphilin** participate in a G-protein cycle that helps to drive exocytosis.⁵⁵⁴ Synaptotagmin, as well as clathrin assembly proteins bind inositol hexaphosphate (InsP_6 ; Fig. 11-9), which undergoes active turnover in synapses. This suggests a role for InsP_6 in the endocytosis steps of the synaptic vesicle cycle.⁵⁸⁹ The brain is rich in zinc ions. Much of the Zn^{2+} is bound into zinc finger domains of transcriptional regulators, but much is also present in a relatively free form within synapses of the hippocampus, cerebral cortex, and other regions.^{590,591} Zinc ions may function as a neuromodulator in glutamatergic synapses.⁵⁹¹

What does a neurotransmitter do at the postsynaptic membrane? In the case of acetylcholine in neuromuscular junctions the principal action appears to be one of opening sodium channels and thereby depolarizing the postsynaptic membrane. If enough nerve impulses arrive, an action potential will be initiated in the postsynaptic neuron. In other cases, the first response may be activation of a protein kinase either directly or by opening a channel for Ca^{2+} , which indirectly regulates protein kinases and phosphatases.⁵⁹² Thus, a complex cascade may be activated. See also Fig. 30-19.

The postsynaptic nerve ending, which is usually the tip of an axonal dendrite, has its own set of proteins, which varies to some extent with the nature of the neurotransmitter. In excitatory cells the plasma membrane of the postsynaptic neuron is thickened to ~30–40 nm to form the “**postsynaptic density**,” a disc-like structure of clustered receptors of two types, which extends ~30 nm into the cytosol.^{593,594} Only single receptor channels are indicated in Fig. 30-20, but many receptors are present in the clusters^{594,595} as are other specialized proteins. One of these, designated

PSD-95, was found to associate with the NMDA receptor using the yeast two-hybrid system (Box 29-F).⁵⁹⁴ Neuronal nitric oxide synthase may also be present.

The large neuromuscular junctions, which contain clusters of acetylcholine receptors, have wider synaptic clefts (> 40 nm), which contain basal lamina, a dense network of collagen fibrils together with the heparan sulfate proteoglycan **agrin** (p. 437). Agrin activates a muscle-specific kinase MusK, which phosphorylates the acetylcholine receptors inducing clustering of the receptors together with other proteins embedded in the plasma membrane and binding to the cytosolic protein **rapsyn** (see Fig. 30-23B).^{596,597} Agrin is also a component of **immunological synapses**, which are important in lymphocyte development (Chapter 31).^{596,598,599} The neuromuscular junction is formed between two cell types, a neuron and a muscle myotube. Both contribute proteins, which include a muscle-specific laminin.⁶⁰⁰

Astrocytes and other glia. Although the glial cells greatly outnumber neurons, they were long regarded simply as glue, as implied by the name glia. We know now that the several types of glial cells have functions in many different aspects of brain chemistry.^{149,564,601–605} The oligodendrocytes generate myelin sheaths around many brain neurons. Macrophages that invade the brain differentiate into **microglia** that serve as part of the innate immune system (Chapter 31). **Bergmann glia** of the cerebellum help guide axons during brain development. The astrocytes have many processes, which not only contact synapses directly (Fig. 30-20A,D) but also form contacts with capillary blood vessels. They often contain receptor ion channels of the same types as are found in postsynaptic membranes (see Fig. 30-20A) and respond to Ca^{2+} influx as do neurons.^{602–603a} Glia often take up neurotransmitters and ions from synapses in order to prepare for consecutive nerve impulses. Glia may also control the number of synapses formed,^{604–604b} and they may have other roles in brain development. For example, an iodothyronine deiodinase (Eq. 15-60) is expressed primarily in neonatal brain, where it supplies thyroid hormone essential to brain development.⁶⁰⁵

Gap junctions in synapses. Not all neurons communicate via chemical synapses. Gap junctions, which are found in both neurons, astrocytes, and other cells, serve as **electrical synapses**. Thus, heart cells are all electrically coupled together by gap junctions.^{606–608} Gap junctions are formed with the aid of hexameric **connexons**, which are present in each of the opposed membranes and are aligned one with the other (Fig. 1-15F,G).^{607,609,610} There may be thousands of connexons in a single gap junction, which resemble ion channels in appearance but contain pores ~1.5 nm in diameter. They are formed from 26- to 43- kDa

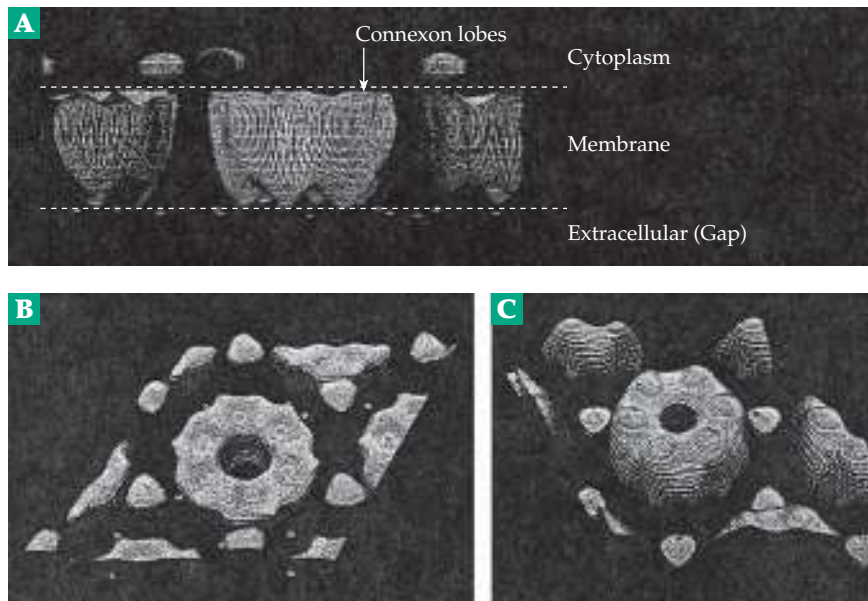


Figure 30-21 Images of gap junction connexins obtained by electron crystallographic methods at a resolution of 1.6 nm. (A) Cross-section. The thickness of the (43 x 6) kDa hexameric connexin is 5.0 nm. (B) View of the connexin from the cytoplasmic side. (C) View from the extracellular side. From Perkins, Goodenough, and Sosinsky.⁶⁰⁹ Courtesy of Guy Perkins.

protein subunits of the multigene family of **connexins**.^{610–611a} Each gap junction consists of a pair of hexameric rings of connexins (Fig. 30-21), one ring from each of the two juxtaposed membrane surfaces.⁶⁰⁹ Defects in connexins cause inherited deafness, neuropathy, malignancy, and cataract formation.^{612–613a} The connexin subunits each contain four transmembrane helices and are related structurally to the peripheral myosin protein 22, the myelin proteolipid (p. 1767), and the protein stargazin (p. 1901), which is involved in synapse formation in the brain.^{428a}

Another type of channel has been recognized quite recently. An ion channel, which regulates Mg^{2+} ion transport in kidney tubules, forms within the tight junctions that seal the extracellular space between cells (Fig. 1-15B). A protein **paracellin** forms channels through the tight junction protein complexes that surround the cells.^{614,615}

9. Neurotransmitters

Studies of neuromuscular junctions of the autonomic nervous system as early as 1904 led to the suggestion that adrenaline might be released at the nerve endings. Later it was shown that, while adrenaline does serve as a transmitter at neuromuscular junctions in amphibians, it is primarily a hormone in mammals. Nevertheless, it was through this proposal that the concept of chemical communication in synapses was formulated. By 1921, it was shown that acetylcholine is released at nerve endings of the parasympathetic system, and it later became clear the motor nerve endings of the somatic system also release acetylcholine.

Acetylcholine is an established neurotransmitter

because it meets five important criteria: (1) a synthetic mechanism exists within the presynaptic neuron; (2) a mechanism of storage (in vesicles) is evident; (3) the transmitter is released in proportion to the strength of the stimulus (frequency of firing); (4) postsynaptic action of the transmitter has been demonstrated directly by microiontophoresis; and (5) an efficient means for inactivation of the transmitter is present. The same five criteria must be met by other compounds if they are to be considered as transmitters.

At present, in addition to acetylcholine, glutamate, and γ -aminobutyrate (GABA), glycine, norepinephrine (norepinephrine), and dopamine and 5-hydroxytryptamine (serotonin) are regarded as established transmitters. Other probable (**putative**) or possible **candidate transmitters** are also known. Aspartate, taurine, and a large number of peptides (Tables 30-1, 30-4) are under consideration.

Some transmitters, including noradrenaline, dopamine, serotonin, and various neuropeptides, are sometimes called **neuromodulators** rather than neurotransmitters. These compounds may not initiate a nerve impulse but may act on adenylate cyclase to increase or decrease cAMP levels and protein kinase activity. They may also diffuse through the extracellular space to influence a region of the brain greater than a single synaptic cleft. However, the distinction between transmitters and modulators is not exact.

For many years it was assumed that a single neuron released only a single transmitter. We know now that this is incorrect.⁶¹⁶ For example, enzymes in neuromuscular junctions synthesize not only acetylcholine but also catecholamines, taurine, and GABA.⁶¹⁷ Some synapses in the central nervous system release both glycine and GABA.⁶¹⁸

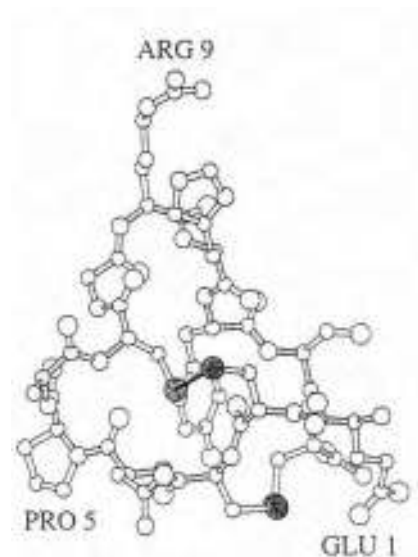
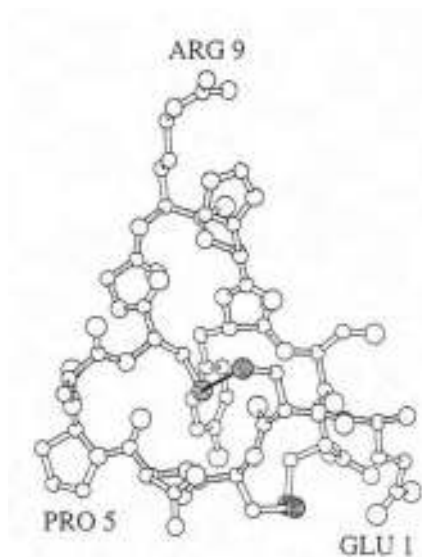
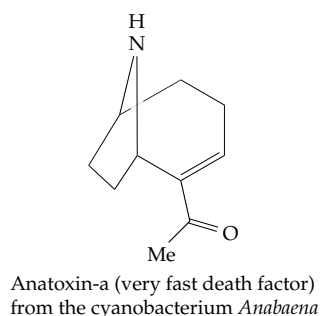
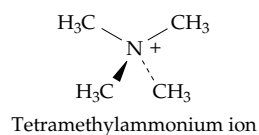
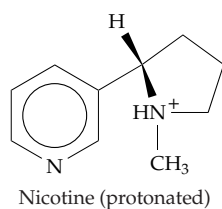
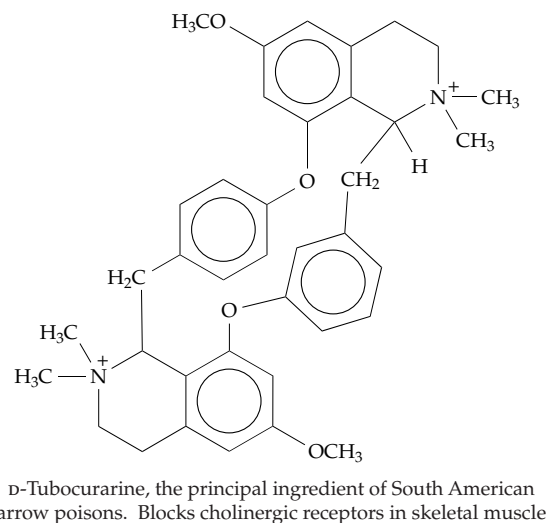
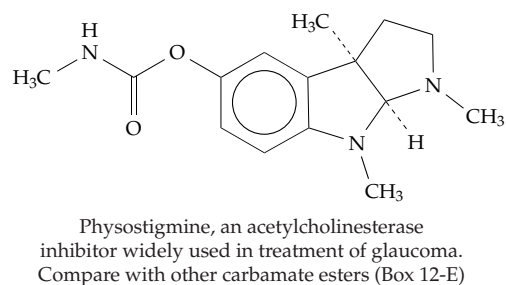
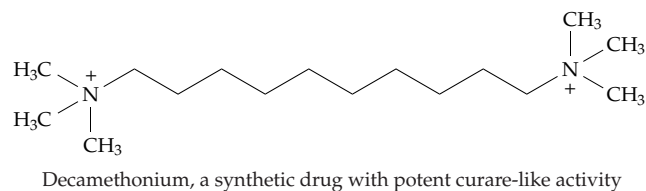
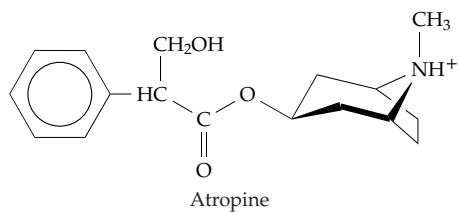
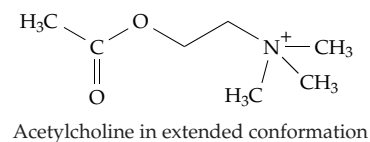
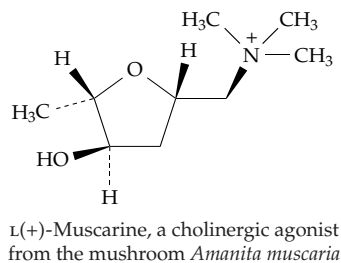
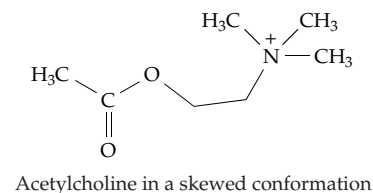


Figure 30-22 Some inhibitors of cholinergic synapses. The structure of conotoxin GI is from Guddat *et al.*⁵³³ Courtesy of A. B. Admundson.

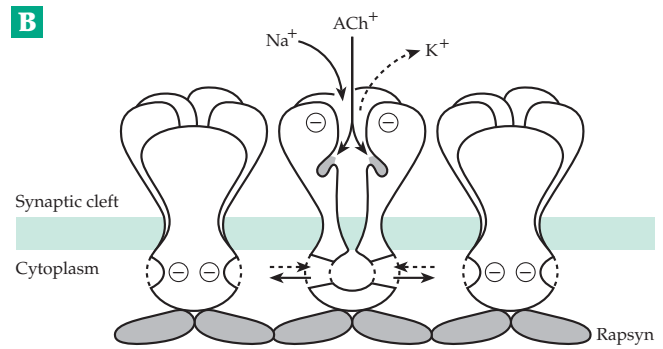
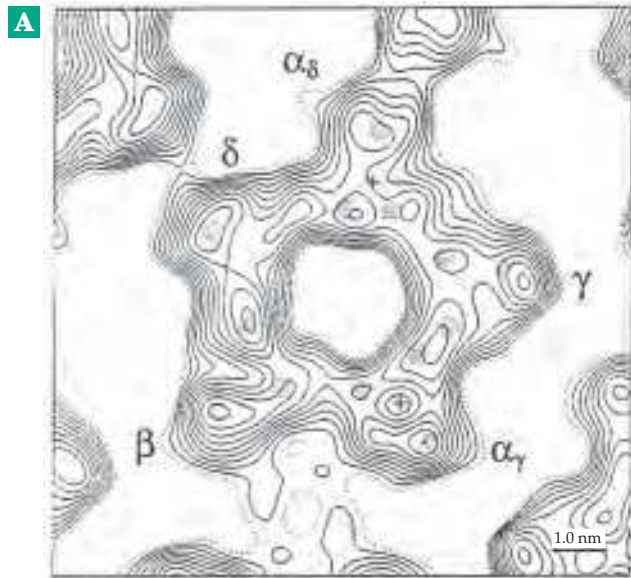


Figure 30-23 The nicotinic acetylcholine receptor from the *Torpedo* ray. (A) The mouth of the receptor channel viewed from the synaptic cleft based on reconstruction from cryo-EM images. Addition of acetylcholine, which binds to the two α subunits, induces small rotations in the five subunits of the $\alpha_2\beta\gamma\delta$ complex causing the channel to open. From Unwin.⁶⁴⁰ (B) Architecture of the subsynaptic membrane and the acetylcholine receptor. The binding of acetylcholine and the

movement of cations through the open channel is illustrated. Cations that leave the cytoplasm may be filtered through narrow openings that lead into the central channel, which is formed by transmembrane helices. Negatively charged amino acid residues may help exclude anions from the region of the pore. From Miyazawa *et al.*⁶²⁴ (A) and (B) Courtesy of Nigel Unwin. (C) Stereoscopic ribbon drawing of one subunit of a pentameric acetylcholine-binding protein, which mimics the receptor structure. Disulfide bonds are shown in a ball-and-stick form. The N terminus in a receptor would point toward the synaptic cleft and the C terminus would continue at the bottom into the transmembrane helix. Courtesy of Brejc *et al.*⁶²⁷

Cholinergic receptors and their agonists and antagonists. Among the acetylcholine-releasing (cholinergic) neurons are the motor neurons that form synapses at neuromuscular junctions, the preganglionic neurons of the entire autonomic system, and the postganglionic neurons of the parasympathetic system. There are also many cholinergic synapses within the brain. In contrast, in insects neuromuscular transmission is mediated by glutamate while acetylcholine is the principal neurotransmitter in the central nervous system.⁶¹⁹

Important in the study of neurotransmitters is the identification of specific agonists, which mimic the action of a transmitter, and of antagonists, which block the action of the transmitter. Two groups of compounds influence acetylcholine-secreting neurons, leading

to the classification of these neurons either as **muscarinic** (activated by muscarine; Fig. 30-22) or **nicotinic** (stimulated by nicotine). The muscarinic receptors, which are found in many autonomic neurons, are specifically inhibited by **atropine** and **decamethonium** (Fig. 30-22). The nicotinic synapses occur in ganglia and skeletal muscle. They are inhibited by curare and its active ingredient **D-tubocurarine** (Fig. 30-22) and by the protein snake venom **α -bungarotoxin**. This toxin has been used to titrate the number of acetylcholine receptors in the motor end plate of the rat diaphragm. About 4×10^7 receptors per end plate (or $13,000/\mu\text{m}^2$) were found.⁶²⁰

Nicotinic receptors (nAChRs; Fig. 30-23) of the type found in neuromuscular junctions are most frequently isolated from the electric organs of the electric

eel *Electrophorus* or from electric fish of the genus *Torpedo*. They have been studied more intensively than any other receptor.^{621–626a} They contain four kinds of subunit with a stoichiometry $\alpha_2\beta\gamma\delta$ and molecular masses of 39, 48, 58, and 64 kDa respectively. The amino acid sequences of the four proteins contain homologous regions, some of which are thought to represent membrane-spanning segments of the peptides. These receptors are ligand-gated ion channels and are closely similar to GABA_A and GABA_C receptors, to glycine receptors, and to 5-hydroxytryptamine (serotonin) receptors of the 5-HT₃ type. Parts of their amino acid sequences are also homologous to those of both the voltage-gated Na⁺ channels and gap junctions,^{433,627} suggesting that the transmembrane domain may resemble that of Fig. 30-18.⁶²⁸ However, notice the difference in symmetry. Acetylcholine binds to the two α subunits (Fig. 30-23). Neurotoxins may bind at several sites.⁶²⁹ Some indication of the possible function of the various subunits comes from studies of the neuromuscular junction in which the different subunits are degraded at different rates with half-lives of from one to ten days. During development fetal ϵ subunits are replaced by adult γ subunits. Perhaps more rapid changes in receptor composition are sometimes needed.⁶³⁰

Similar nAChRs are also found in the brain.^{621,631,632} However, they are not identical but have at least 17 differing amino acid sequences ($\alpha 1$ – $\alpha 10$, $\beta 1$ – $\beta 4$, γ , δ , and ϵ). The neuromuscular junction receptor (muscle type) from fish is described as $(\alpha 1)_2 \bullet \beta 1 \bullet \gamma / \epsilon \bullet \delta$.⁶²⁶ The brain contains homopentamers of subunits $\alpha 7$, $\alpha 8$, and $\alpha 9$ as well as various heteropentamers. The various forms possess different affinities for acetylcholine and for antagonists such as nicotine.^{633,634} In the brain the highest affinity for nicotine is shown by an $\alpha 4\beta 2$ form, which represents over 80% of the nAChR in mammalian brain.^{634,635} Knockout mice in which the $\beta 2$ subunit gene has been deleted lose their sensitivity to nicotine.

Conductance measurements showed that the nicotinic receptors contain channels permeable to Na⁺ and other cations and that they are acetylcholine-gated ion channels. Construction of a three-dimensional image from electron micrographs at various angles of tilt shows a tube with approximate pentagonal symmetry and a narrow channel through the center (Fig. 30-23).^{622,624,636} Acetylcholine binds to sites on the two α subunits ~3 nm away from the ion channel. An allosteric change opens the channel, allowing cations (largely Na⁺) to flow out, depolarizing the membrane. There are at least four structural states in the channel opening-and-closing cycle.^{637,638} The three-dimensional structure has been modeled using an acetylcholine-binding protein of known structure from a snail^{626,627,639} as a mimic of the cytoplasmic nicotine-binding domain of the receptor. The structure

of one subunit of the binding protein is shown in Fig. 30-23C. This protein, which is secreted into synapses by glial cells, may provide a buffering action by binding the acetylcholine. Although the most rapid effect of acetylcholine binding to the nicotinic receptor is depolarization of the postsynaptic membrane, other slower effects follow. Thus, protein kinases are activated and phosphorylate the receptor as well as other proteins.⁶⁴¹

After a pulse of transmitter is released, it must be removed or inactivated quickly to prepare the synapse for arrival of a new nerve impulse. This is accomplished in two ways in cholinergic synapses. The first is via hydrolytic destruction by acetylcholinesterase^{642–645} (pp. 634–637; Eq. 12-25). This esterase and the related butyrylcholinesterase⁶⁴⁶ are present in the synaptic membrane itself. The second mechanism is energy-dependent transport of acetylcholine into the neuron for reuse. Since much of the transmitter is hydrolyzed, new acetylcholine is synthesized by transfer of an acetyl group of acetyl-CoA to choline.⁶⁴⁷

In the central nervous system muscarinic acetylcholine receptors are more abundant than nicotinic receptors. They consist of single-chain proteins of mass ~70 kDa. They are not ion channels but are 7-helix receptors homologous in sequence with β -adrenergic receptors (Fig. 11-6) and with rhodopsin.⁶⁴⁸ Five different subtypes (M1–M5) have been characterized. The M1, M3, and M5 receptors are coupled to the G_q/G₁₁ family of G proteins (pp. 557–558), and M2 and M4 are coupled to G_i/G_o proteins.^{649–651} Their effects are slower and of longer duration than those of the nicotinic receptors. It has been difficult to assign functions to the individual types. Most regions of the brain contain more than one type, but they are thought to be involved in locomotion, learning, memory, thermoregulation, and cardiac and pulmonary functions. Many drugs, some of which are used in treatment of Parkinson and Alzheimer diseases, epilepsy, and asthma, affect muscarinic receptors. The M2 receptors predominate in the heart where they help to regulate the beating frequency and atrial contractility. Sudden infant death may sometimes result from a defect in muscarinic receptors.⁶⁵² Knockout mice lacking M2 receptors also have problems with movement control, body temperature, and pain responses.⁶⁵¹ Mice lacking M3 receptors are lean with very low levels of serum leptin and insulin.⁶⁵³ Many of the muscarinic receptors activate adenylate cyclase, while others are coupled to the phosphoinositide cascade. Some indirectly activate K⁺ channels.⁶⁵⁴ Muscarinic receptors are also studied in insects, but it is difficult to correlate the insect and mammalian receptors.⁶⁵⁵

Amino acids as neurotransmitters. The concentrations of **glutamate** and of its decarboxylation product **γ -aminobutyrate** (GABA) are high in all regions

of the brain. The two compounds are generated sequentially in the γ -aminobutyrate shunt, a pathway that accounts for a quantitatively significant part of the total metabolism of the brain (Fig. 17-5). Because they are present in all parts of the brain in high concentrations, there was initially reluctance to accept glutamate and GABA as neurotransmitters. However, it is now accepted that L-glutamate is the major excitatory transmitter in the central nervous system.^{656–658} It seems to be responsible for nearly all of the very fast acting nerve impulses in the brain. At the same time GABA is recognized as the most important inhibitory transmitter. The role of glutamate as an excitatory transmitter was first established for the neuromuscular junction of arthropods.⁶⁵⁹ Although it is a constituent of all animal tissues, the concentration of glutamate is much higher in brain than in other tissues, and it is higher in neurons than in glia. Microiontophoretic application of either glutamate or aspartate to the brain cortex leads to very strong excitatory responses.

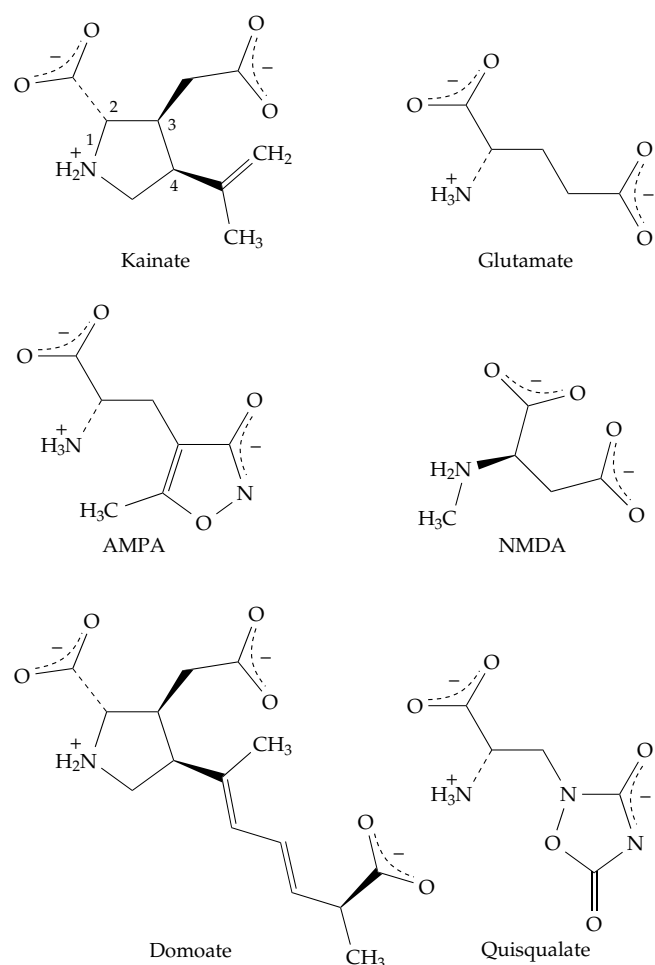


Figure 30-24 Chemical structures of some agonists of ionotropic glutamate receptors (iGluR).

Three subtypes of ionotropic glutamate receptors (iGluR) are named for the specific agonists **α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid** (AMPA), **N-methyl-D-aspartate** (NMDA), and **kainate**. The receptors resemble the acetylcholine receptor in containing a cation channel.^{149,660–662} In addition, there are 7-helix **metabotropic glutamate receptors**, which are coupled to G proteins.^{663,664} The AMPA receptors were in the recent past called **quisqualate** receptors, because they are also activated by the agonist with that name. The toxic domoate (Fig. 30-24) also binds to kainate receptors. Both domoic acid and kainic acid are terrible convulsant toxins. They are formed by two different red algae. Domoic acid accumulates in contaminated mussels and causes shellfish poisoning. The ionotropic glutamate receptors, which may be stimulated by either glutamate or aspartate, are directly linked to the opening of cation channels. Their activation may also induce the inositol phosphate cascade and slower Ca^{2+} -dependent changes. A peculiarity of the high-conductance NMDA channels is that they are blocked by Mg^{2+} in a voltage-dependent manner. They do not open unless the frequency of nerve impulses is high or some other factor causes membrane depolarization.⁶⁵⁶

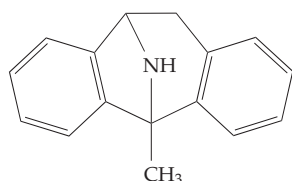
The AMPA receptors, which are thought to be the predominant mediators of fast excitatory transmission in the brain,⁶⁶⁵ are oligomers (probably tetramers^{666,666a}) of 950- to 1500-residue protein subunits. These subunits have large N-terminal domains in the synaptic cleft. There are probably three transmembrane helices and a membrane-associated loop similar to those depicted in Fig. 30-17A. A long C-terminal tail protrudes into the cytosol, while a large loop between transmembrane regions extends from the outer membrane surface, joining with the N-terminal domain to form the ligand-binding site, the structure of which resembles those of bacterial periplasmic binding proteins.^{661,665,667} Four related AMP receptors, designated GluR1, 2, 3, and 4, have been identified. Related kainate receptors, whose properties overlap those of AMPA receptors, are designated GluR5, 6, and 7.⁶⁶² Although AMPA receptors are essential for fast signal transmission they lose sensitivity rapidly (on a millisecond time scale) as a result of conformational alterations.^{667a} Many factors, including inhibition by polyamines,^{667b} affect these receptors. However, brief high-frequency activation of some AMP receptors leads to a long-lasting increase in efficiency, termed LTP, which is important to learning (see p. 1801).^{666a}

The NMDA receptors are heterooligomers with two type of subunits. The NR1 (or ζ) subunits exist as a series of at least eight splice variants. The NR2A, B, C, and D (ϵ series) are encoded by four different genes.^{668,669} NR1 is regarded as the principal subunit and NR2 as a regulatory subunit. As with the AMPA receptors⁶⁷⁰ the oligomeric NMDA receptors are

anchored at appropriate locations in the postsynaptic membrane by scaffolding proteins containing PDZ domains (Table 7-3).⁶⁷¹ The C-terminal domains of the ϵ subunits are unusually long and participate in anchoring. NMDA receptors are found not only in neurons but also in astrocytes (Fig. 30-20), where they are thought to have important signaling functions.^{672,673} These include regulation of Ca^{2+} flow, in part via gap junctions.^{603a}

The N-terminal domain of the NR1 subunit of the NMDA receptor contains a glycine-binding site.⁶⁷⁴ Full activity of the receptor requires a **coagonist** bound in this site. Surprisingly, **D-serine** seems to be the normal coagonist, at least in some sites.^{675,676} This newly recognized neurotransmitter is synthesized from L-serine by a pyridoxal phosphate-dependent recemase and is destroyed by the flavoprotein D-amino acid oxidase. Associated with NMDA receptors are clusters of **ephrin receptors**, proteins that bind the glycosylphosphatidylinositol (GPI)-anchored proteins known as ephrins in presynaptic membranes. Binding of ephrins to their postsynaptic receptors activates tyrosine kinases and enhances the influx of Ca^{2+} ions.^{676a,b}

Specific inhibitors of NMDA channels include a 27-residue "spasmodic" conotoxin,⁴⁹⁰ 2-amino-4-phosphonobutyrate, related longer chain aminophosphonates, and the following potent anticonvulsant drug, which is able to penetrate the blood-brain barrier.⁶⁷⁷



(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine

Metabotropic glutamate receptors have been classified into eight types (mGluRs1–8).^{678–680a} Group I (mGluRs1–5) are selectively activated by 3,5-dihydroxyphenylglycine; Group II (mGluR2 and mGluR3) are activated by L-2-(carboxycyclopropyl)glycine; and Group III (mGluR4 and mGluR 6–8) are activated by L-2-aminophosphonobutyrate. They are all 7-helix G-protein-coupled receptors with external ligand-binding domains that resemble those of bacterial periplasmic binding proteins.⁶⁸⁰ Splice variants for at least mGluR1 are known.⁶⁷⁸ Metabotropic glutamate receptors are neuromodulatory but nevertheless play essential roles in the cerebellum and other parts of the brain. For example, mice deficient in the mGluR1 protein have severe problems with motor coordination and learning.^{681,682} Metabotropic glutamate receptors may participate in calcium sensing and signaling.^{683,684}

Synaptosomal particles have a high-affinity proton-

dependent uptake system for glutamate.⁶⁸⁵ Glutamate and aspartate may also be taken up from the synaptic cleft by neurons or by glial cells, which then transfer the glutamate into neurons for reuse.^{686,687} Five distinct mammalian transporter genes have been cloned.⁶⁸⁸ They are driven by concentration gradients of Na^+ and K^+ across the membrane.^{689,690} However, some serve as glutamate-gated chloride ion channels.^{691,691a}

Excitotoxicity. As essential as glutamate is for brain function it is toxic in excess. Excessive stimulation of the NMDA receptors, which occurs during convulsions, strokes, or traumatic injury and which can accompany anoxia or hypoglycemia, causes neuronal death.^{660,692–694} Blocking these receptors with the above-mentioned anticonvulsant drug or aminophosphonates has a remarkable protective effect against the neurotoxicity of the accumulating glutamate.^{658,677} Vitamin E and **tocotrienols** (Fig. 15-24) may also be protective.⁶⁹⁵

The inhibitory neurotransmitter gamma-aminobutyrate (GABA). Glutamate, aspartate, and cysteic acid are all potent exciters, but their decarboxylation products γ -aminobutyrate (**GABA**), β -alanine, and taurine are inhibitors as is also glycine. Of these GABA is the most important.⁶⁹⁶ Its concentration in the brain is high and varies at least threefold in different parts of the brain. It is hardly present elsewhere in the body. GABA and GABA-binding sites are found in 30–50% of the nerve endings. The function as an inhibitory transmitter has also been demonstrated in inhibitory neurons present in the peripheral nervous system of arthropods. Virtually every neuron in the brain is to some extent subject to inhibition by GABA.^{697,698} Glial cells also have GABA receptors.

The receptors for GABA are divided into type A, which are blocked by **bicuculline**,⁶⁹⁹ and type B, which are stimulated by **baclofen** (Fig. 30-25).⁶⁹⁸ The GABA_A receptors are the major sites of fast synaptic inhibition in the central nervous system.⁷⁰⁰ They are structurally related to the nicotinic acetylcholine, glycine, and serotonin type 3 (5-HT₃) receptors. Cloning has revealed 16 different mammalian subunits: $\alpha 1$ – $\alpha 4$, $\beta 1$ – $\beta 3$, $\gamma 1$ – $\gamma 3$, δ , ϵ , π , and Φ .^{701–704a} The oligomeric receptors are ligand-gated chloride ion channels^{481,705} as are also glycine receptors. These receptors are clustered in synaptic membranes, apparently anchored in part by their β subunits⁷⁰⁶ and scaffold proteins such as the microtubule-binding **gephyrin** (from the Greek word for bridge)^{701,707} and a small ~14-kDa GABA receptor-associated protein.⁷⁰⁸ A novel serine protein kinase is also associated with GABA receptors.⁷⁰³

Whereas excitatory transmitters lead to depolarization of the postsynaptic membrane, inhibitory transmitters cause **hyperpolarization**, apparently by increasing the conductance of K^+ and Cl^- . The result is

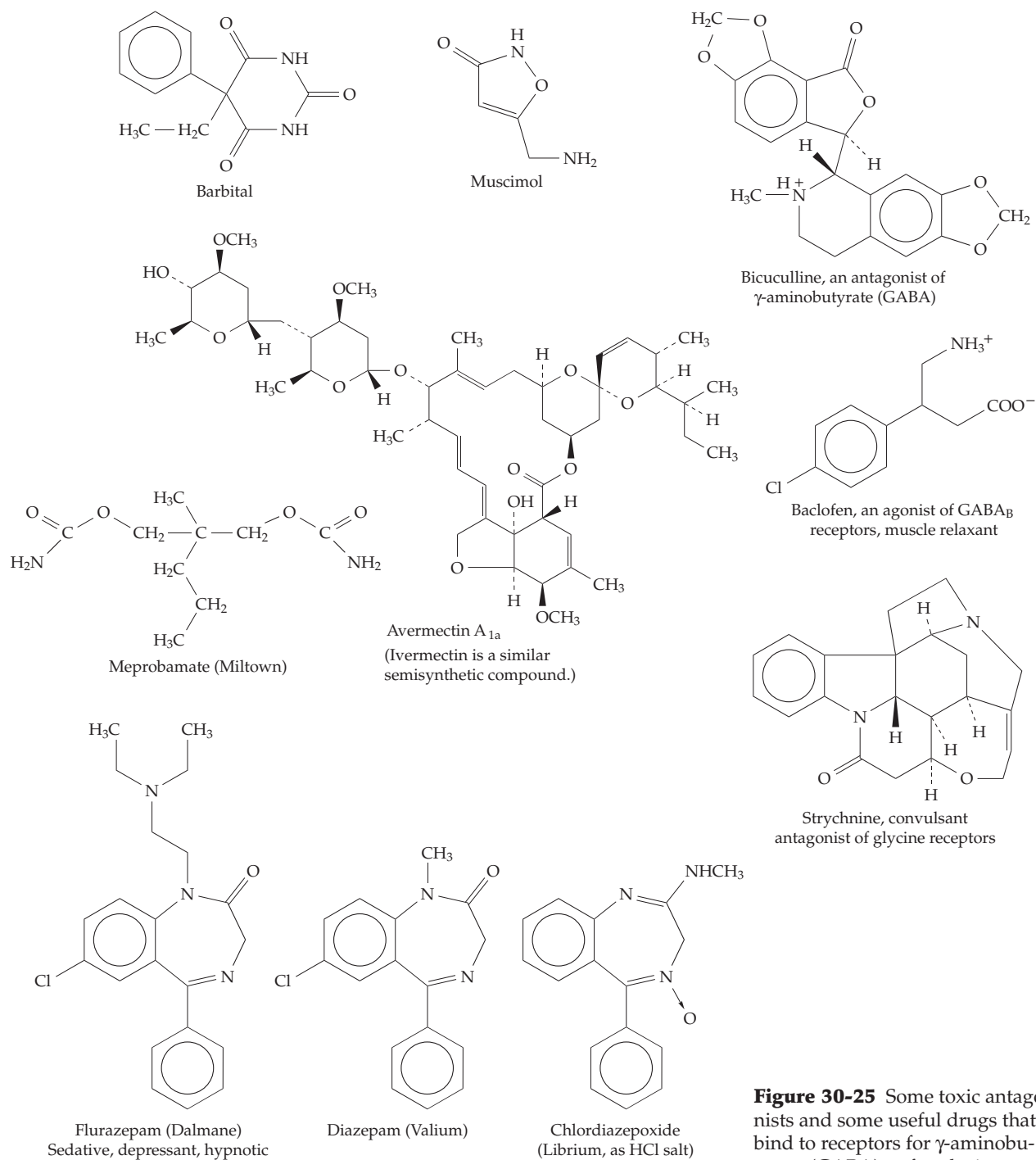


Figure 30-25 Some toxic antagonists and some useful drugs that bind to receptors for γ -aminobutyrate (GABA) or for glycine.

that it is more difficult to excite the postsynaptic membrane in the presence of, than in the absence of, these transmitters. GABA-dependent interneurons also contain the calcium-binding **parvalbumin** (Fig. 6-7), which suggests that a Ca^{2+} -dependent process is involved.⁷⁰⁹

The GABA_B receptors resemble metabotropic glutamate receptors.^{710,711} They are 7-helix G-protein coupled proteins, which activate adenylate cyclase.

They tend to dimerize, and maximum activity is observed for heterodimers of GABA_B1 and GABA_B2 receptors.^{712,713} They are often coupled to inward rectifying K^+ channels.⁷¹⁴

The GABA receptors provide binding sites for a great variety of toxins and drugs.⁴⁸¹ These include barbiturates, anesthetics, antianxiety drugs, and the insecticides such as toxaphene, cyclodienes, and pyrethroids.⁴⁸¹ **Diazepam, chlordiazepoxide, and**

flurazepam^{700,702,715–717} (Fig. 30-25) are antianxiety drugs and muscle relaxants, which, during the 1970s, were the most frequently prescribed drugs in the United States.⁷¹⁶ Binding of benzodiazepines to GABA receptor-chloride channels enhances the effect of GABA. The drugs induce relaxation but can interfere with memory, reduce concentration, and cause physical clumsiness. They may also intensify the effects of alcohol and can be addictive.⁷¹⁸

Specific antagonists for GABA_A receptors include the alkaloid convulsants bicuculline (Fig. 30-25)⁶⁹⁹ and **picROTOXIN** (Fig. 22-4) and the convulsant terpenoid compound **thujone** (Fig. 22-3), which is present in the wormwood plant *Artemisia absinthium*. Thujone is present in the liqueur absinthe, which was the national drink of France in the late 19th century but, because of its toxicity, has been illegal in most countries since ~1915.⁷¹⁹

GABA enters synaptic vesicles via a vesicular GABA transporter, an integral membrane protein whose gene has been found in *Caenorhabditis elegans*.⁷²⁰ Termination of GABA neurotransmission is accomplished by rapid Na⁺-dependent uptake into neurons for reuse and uptake into glial cells.^{721,722} Excess GABA is continuously oxidized to succinic semialdehyde by GABA aminotransferase⁷²³ in the GABA cycle of Fig. 17-4. Notice the manner in which this cycle incorporates synthesis of both of the neurotransmitters glutamate and GABA. Glutamine also functions in neurons, perhaps serving as a buffer for glutamate.

The hereditary triple-repeat disease Huntington's chorea (**Huntington disease**), with an incidence of 5–10 per 100,000 persons, affects principally persons of age over 40 and is associated with a deficiency of GABA in basal ganglia.⁷²⁴ The cortex is also affected. Severe neurologic symptoms arise as a result of premature death of neurons in the basal ganglia. Convulsions may also arise because of a deficiency of GABA in the brain.

Glycine. Glycine appears to be the most important neuroinhibitor in the spinal cord and brainstem. It is present at concentration of 3–5 mM in the spinal cord and in the medulla but is low in the cerebral cortex. **Strychnine** (Fig. 30-25) is a specific antagonist of glycine receptors in spinal synapses.⁷²⁵ Ivermectin (Fig. 30-25) also blocks glycine Cl[−] channels.⁷²⁶ A mutant mouse called *spastic* is deficient in glycine receptor function. A small dose of strychnine produces an effect on a normal mouse that resembles the effect of this mutation.^{727,728} A similar disorder affects some Hereford calves.⁷²⁹ Strychnine-binding studies have suggested a deficit of glycine receptors in human spasticity and in the loss of motor control associated with **Parkinson disease** and **amyotrophic lateral sclerosis**.⁷²⁵ A human **startle disease**, which causes an exaggerated muscular response to unexpected

stimuli, also results from reduced glycinergic neurotransmission.⁷³⁰

Most glycine receptors are Cl[−] ion channels that open in response to transmitter binding.⁷²⁵ The strychnine-binding subunit shows significant homology with the nAChR proteins,⁷²⁵ and the overall structures resemble those of GABA receptors and of nAChRs.^{731,732,732a} Human α1–α4 and β subunits have been identified.^{733,734} Two integral membrane glycine transporters are known.^{735–737}

Anesthetics. Several types of neurotransmitter receptors provide binding sites for anesthetics. Some anesthetics are molecules of moderate size, e.g., **barbiturate** derivatives, while others, such as **diethyl ether** or **halothane** (CF₃CHClBr), are very small. The latter is one of the most widely used inhalation anesthetics. Both Mg²⁺ and Mn²⁺ are also powerful CNS depressants and can cause general anesthesia. It has often been proposed that the effectiveness of anesthetics is related to solubility in lipids, but it has been difficult to pinpoint a site of action. Now it is clear that specific synaptic proteins often provide the binding sites for anesthetics. Important among these are the glycine receptors.^{715,738,739} GABA receptors^{740,740a} and kainate glutamate receptors may also bind anesthetics.⁷⁴¹

Adrenergic synapses: the catecholamines. The three closely related tyrosine metabolites, **dopamine**, **noradrenaline**, and **adrenaline**, known collectively as catecholamines, are important products of neuronal metabolism.^{149,393} Dopamine and noradrenaline serve as neurotransmitters. Catecholamine-containing neurons are found throughout the brain, including the cortex and cerebellum regions. Very large dopamine-containing neurons are present in the brains of gastropod molluscs.⁷⁴² In the human brain a prominent series of dopamine neurons run from the substantia nigra to the caudate nuclei and putamen of the striatum, the **nigrostriatal** pathway (Fig. 30-12).^{149,743,743a} In many invertebrates **octopamine**,^{744–746} which is synthesized via tyramine (Fig. 30-26), apparently functions in place of noradrenaline. Note the precursor–product relationship between dopamine, noradrenaline, and adrenaline. The synthetic pathways to these neurotransmitters involve decarboxylation and hydroxylation, types of reaction important in formation of other transmitters as well. The most important process for terminating the action of released catecholamine transmitters is reuptake by the neurons. High-affinity uptake systems transport the catecholamine molecules back into the neurons and then into the synaptic vesicles. The uptake is specifically blocked by the drug **reserpine** (Fig. 25-12).^{746a} The dopamine transporter is a major binding site for cocaine (see Fig. 30-28).^{747–751} Catecholamine transmitters are catabolized by two enzymes. One is the

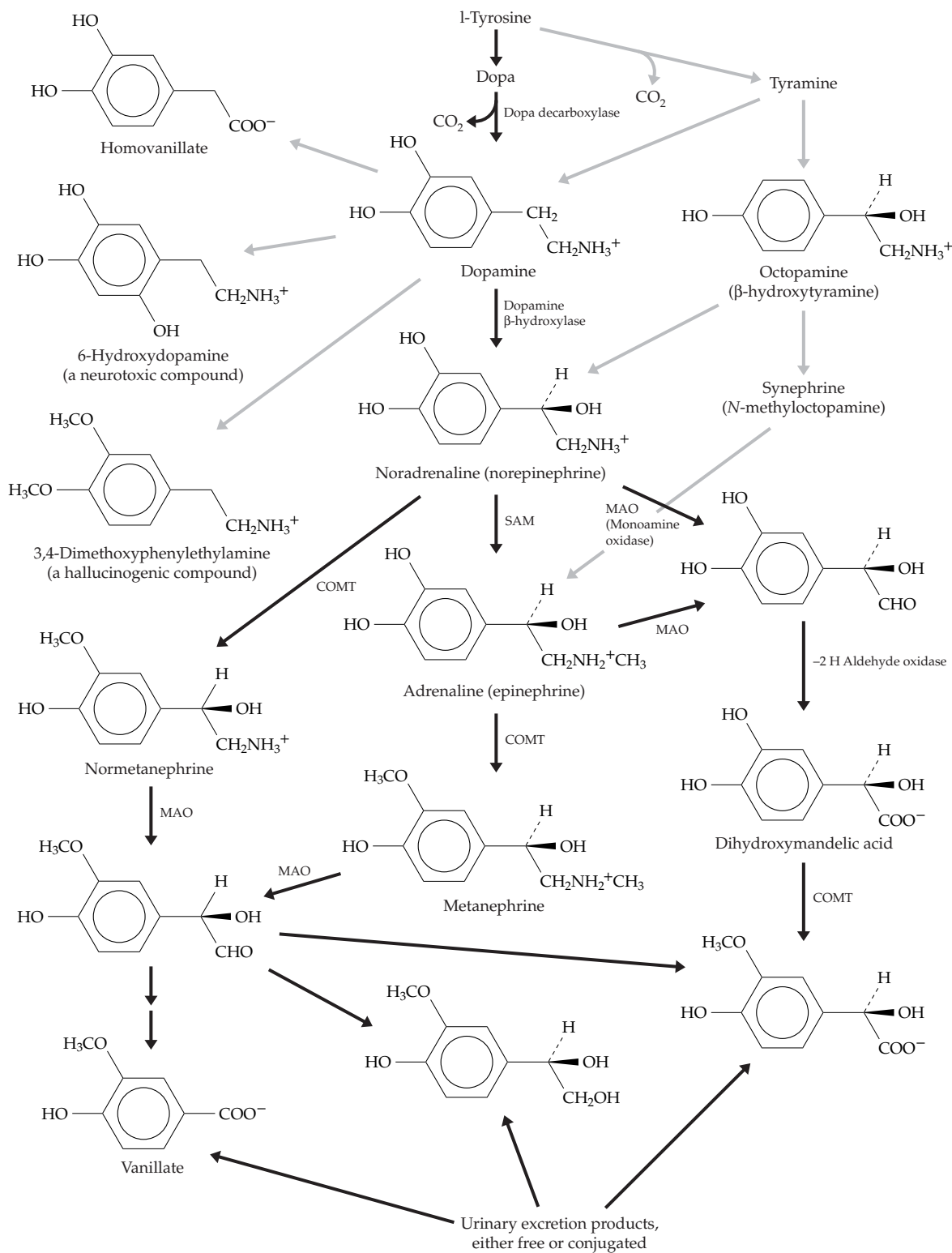


Figure 30-26 Some pathways of metabolism of the catecholamines. See also Fig. 25-5.

flavoprotein **monoamine oxidase (MAO;** Chapter 16), an enzyme present within the mitochondria of neurons as well as in other cells in all parts of the body.⁷⁵² The second enzyme is **catechol-O-methyltransferase**

(**COMT**; Eq. 12-3), which is found in postsynaptic membranes as well as in liver, kidney, and other tissues. It apparently provides the principal means of inactivating circulating catecholamines. In a process

that occurs in all organisms sulfo groups are transferred from PAPS (Eq. 17-38) onto hydroxyl groups of catecholamines, steroid compounds, and proteins (p. 659). Sulfation of catecholamines is relatively specific to humans.^{752a,b}

Both adrenaline and noradrenaline stimulate smooth muscles throughout the body and have a hypertensive effect. Their postsynaptic receptors are 7-helix transmembrane proteins (Fig. 11-6). A comparison of the effects of various analogs led to the classification of these receptors into classes α , α_2 , β , and β_2 , which are discussed briefly on pp. 553–555. The α receptors, which are structurally closely related to rhodopsin,^{753,754} are coupled via Gq / 11 proteins to a phosphoinositide-activated phospholipase C (Figs. 11-9, 30-19).⁷⁵⁵ They usually provoke an excitatory response. However, in intestinal smooth muscles they are inhibitory. Adrenaline is usually more active at α receptors than is noradrenaline. A specific antagonist

is **dibenzamine** (Fig. 30-27). The β receptors usually induce muscular relaxation but cause myocardial stimulation. Noradrenaline is usually more active than adrenaline. In most cases the β receptors of the postsynaptic membrane respond to the neurotransmitter by causing a hyperpolarization of the cell membrane and inhibition of nerve impulses. A specific antagonist is **propranolol** (Fig. 30-27). The β receptors are coupled via proteins of the G_s family (pp. 557, 558). The β_2 receptors have received special attention because of their importance to heart and pulmonary functions. Both heart failure and asthma are associated with poor β_2 receptor function.^{756,757} The β_2 receptors affect many other processes including insulin action.⁷⁵⁸ Intense efforts are being made to understand them at the structural level.^{757,759,760} Of special interest are the mechanisms by which receptors are desensitized after passage of impulses, a process that often involves multiple phosphorylation reactions⁷⁶¹

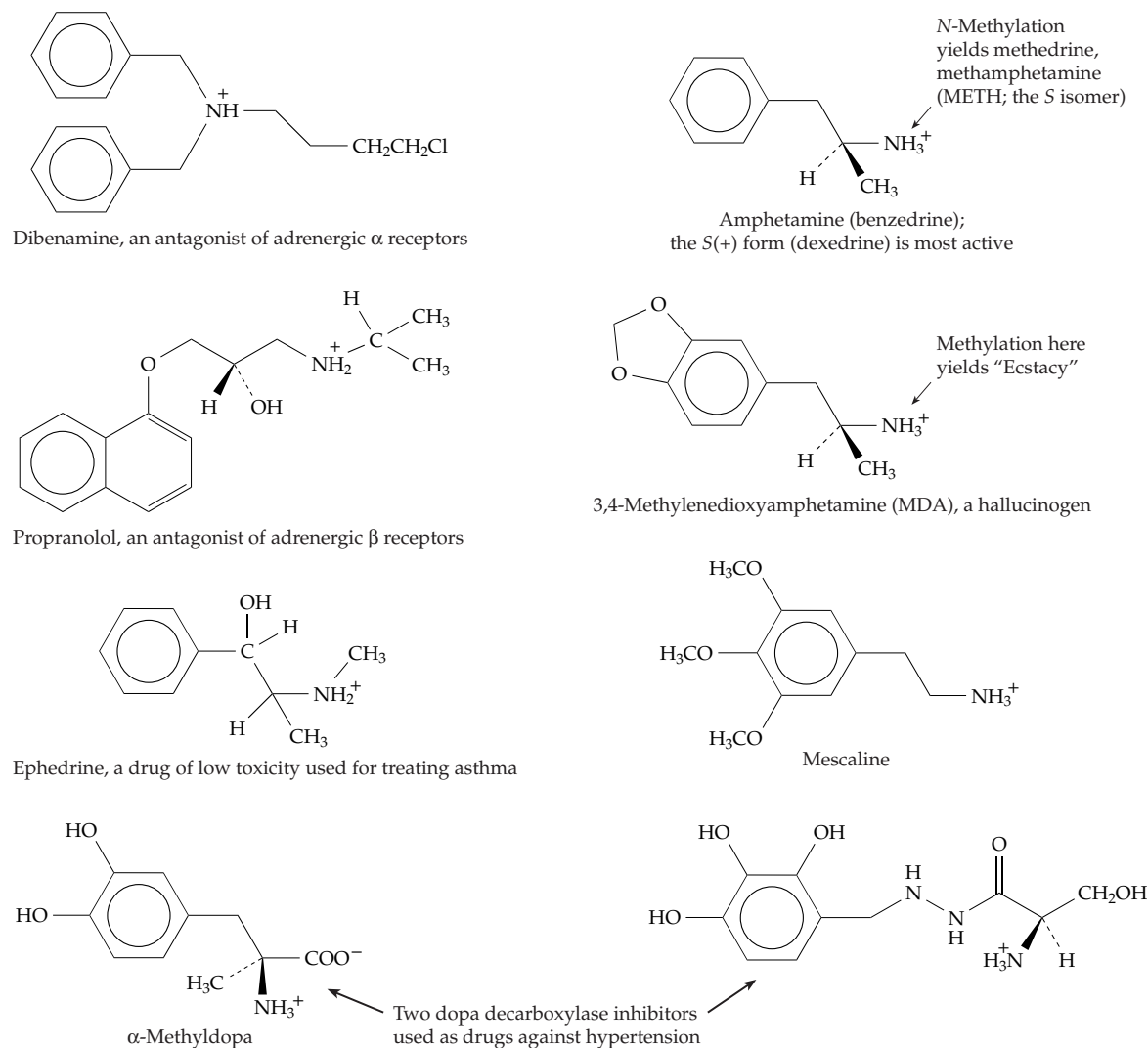


Figure 30-27 Some agonists and antagonists of adrenergic synapses (shown as cations in most cases).

as well as interaction with **arrestin** (Fig. 23-43) and receptor internalization.⁷⁶²

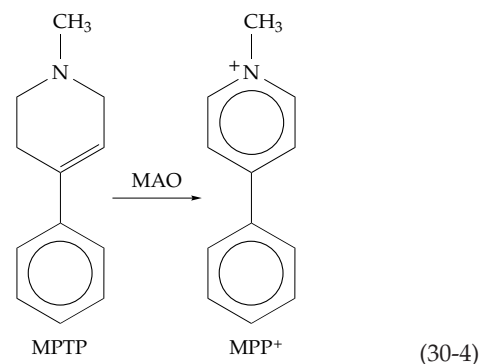
Attention has been focused on dopamine because of its relationship to neurological diseases and to addiction (discussed in Section 10). Dopamine receptors constitute a large family, which are classified into two main subfamilies. The D_1 subfamily consists of D_{1A} and D_{1B} (D_5) receptors and the D_2 subfamily of D_2 , D_3 , and D_4 receptors.^{763,764} The D_1 receptors, which are prominent in the prefrontal cortex and also in the striatum, are more abundant than the D_2 receptors, which are also present in the striatum and the pituitary and are targets for antipsychotic drugs such as **haloperidol** (Fig. 30-33).⁷⁶⁵ The recently discovered and less numerous D_3 receptors are present in only a few regions of the brain. However, a deficiency of D_3 receptors may also be involved in addiction, schizophrenia, and Parkinson disease.^{766,767}

The role of the catecholamines as transmitters in the sympathetic nervous system and in the peripheral ganglia has been well established, but the function in the central nervous system is less clear. Catecholamines are present in varying quantities throughout the brain, and histochemical techniques^{149,768} have made it possible to visualize both dopamine and noradrenaline-containing neurons by the green fluorescence produced from reaction with formaldehyde or glyoxylate.⁷⁶⁹ The reactions are presumably analogous to those in Fig. 25-10. Another method for tracing dopamine receptors in the central nervous system is through labeling with specific antibodies to dopamine- β -hydroxylase (Eq. 18-53), the enzyme that converts dopamine to noradrenaline, to tyrosine hydroxylase, or to other specific neuronal enzymes.⁷⁷⁰

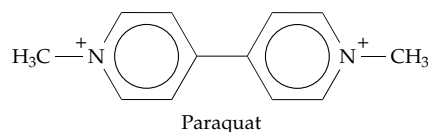
Parkinson disease. Neurons of the nigrostriatal pathway degenerate in Parkinson disease, a condition accompanied by severe tremors and rigidity. The significance of dopamine was illustrated by the finding that the precursor amino acid **L-dopa** caused dramatic improvement in many persons with Parkinson disease.⁷⁷¹ Dopamine and other catecholamines do not cross the blood-brain barrier but L-dopa does. This leads to an increase in the dopamine level in the basal ganglia of the brain, which apparently compensates for the deficiency resulting from the neuronal degeneration.

In 1982 a number of young people in California injected themselves with an illegally manufactured opiate drug that was subsequently found contaminated with *N*-methyl-4-phenyltetrahydropyridine (MPTP). Within a few days they developed irreversible symptoms of Parkinson disease. Subsequent investigation revealed that MPTP itself is not toxic but that it is oxidized by monoamine oxidase B (MAO-B) to the corresponding pyridinium derivative MPP⁺ (Eq. 30-4). It is this pyridinium derivative, or perhaps

related free radicals, that is toxic.⁷⁷² MPP⁺ is readily taken up by mitochondria and is apparently concentrated in the mitochondria of the nigrostriatal cells to a toxic level.⁷⁷³ The MAO inhibitor pargyline (Fig. 30-33) interferes with the oxidation of Eq. 30-4 and prevents development of Parkinson disease in squirrel monkeys exposed to MPTP.⁷⁷⁴ These results suggested



possible environmental causes for Parkinson disease and also a new approach to treatment.^{775,776} MPP⁺ has been marketed as a herbicide, and it has a close structural relationship to another herbicide, **paraquat**.



Many food constituents including peppermint, spearmint, and tea contain 4-phenylpyridine, another close relative.⁷⁷⁵ While administration of L-dopa to replace the deficit in the basal ganglia seemed the ideal treatment for Parkinson disease, mental deterioration is not stopped, and for some patients the drug loses its effectiveness in about three years. Based on the new information about MPTP, treatment with extra vitamin E as an antioxidant along with an MAO inhibitor is being tested as a way to prevent further damage from environmental toxins.⁷⁷⁶

Serotonin and melatonin. The indolealkyl amine serotonin (5-hydroxytryptamine, 5-HT; Fig. 30-28), is found in all mammalian brains and in invertebrates as well. Its distribution in the brain is limited, serotonin-containing neurons being found in the raphe nuclei of the brainstem from which they ascend into the brain and down the spinal cord. Serotonin-containing neurons have been traced within brains of snails using ³H-labeled serotonin.⁷⁷⁷ Studies with these simpler brains have revealed both inhibitory and excitatory responses to these neurons. Serotonin-accumulating neurons are also found in the retina⁷⁷⁸ and are widely distributed in the peripheral nervous

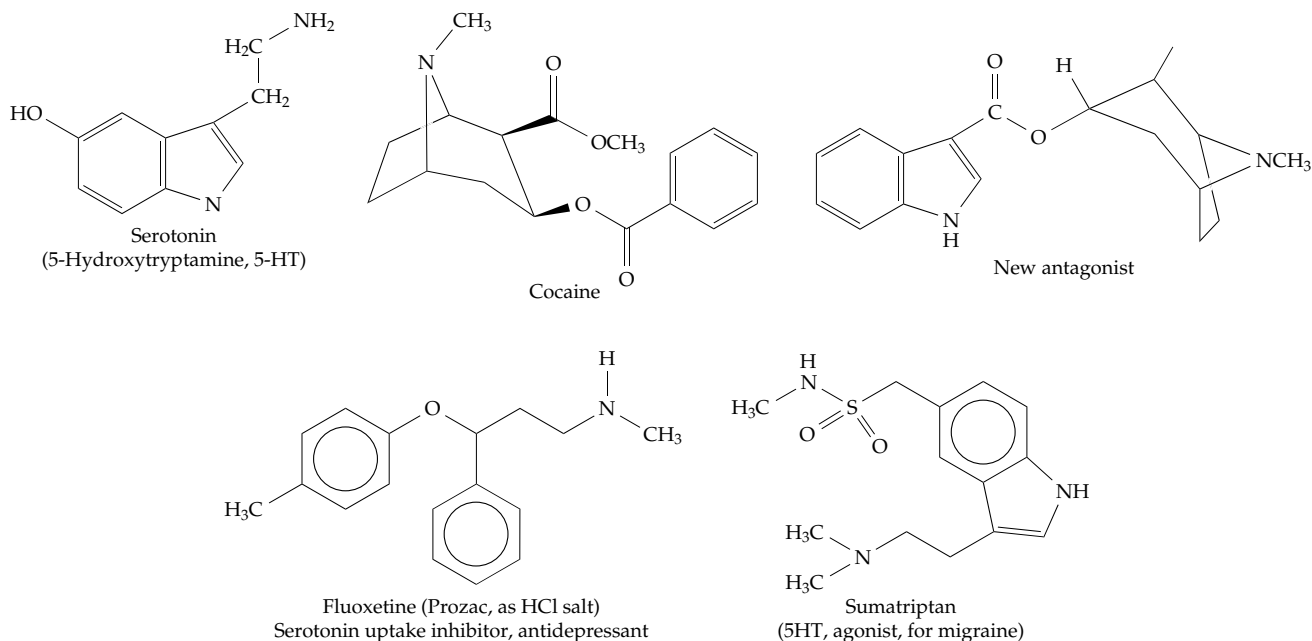


Figure 30-28 Serotonin (5-hydroxytryptamine) and some drugs that affect receptors and transporters.

system.⁷⁷⁹ Serotonin-containing granules are present in blood platelets.⁷⁸⁰

Serotonin appears to be involved in activation of pain fibers, when tissues are injured. **Cocaine** (Fig. 30-28) is a powerful pain killer and a weak antagonist of responses to serotonin, a fact that has led to the synthesis of new antagonists, such as the one in Fig. 30-28 whose structure encompasses that of both cocaine and serotonin.^{779,781} It is active at a concentration as low as 10^{-14} M and is among the most potent known drugs of any type.

Serotonin is synthesized via tryptophan and 5-hydroxytryptophan with decarboxylation of the latter (Fig. 25-12). Within the **pineal body** of the brain and in the retina, serotonin is acetylated to *N*-acetylserotonin,^{782,783} which is then *O*-methylated to **melatonin**, the pineal hormone (Fig. 25-12). A specific inhibitor of serotonin synthesis is *p*-chlorophenylalanine, and studies with this and other inhibitors suggest that serotonin is required for sleep.⁷⁸⁴

At least 14 distinct types of serotonin receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, etc.) have been identified.^{785,786} They are present in the heart, in gastrointestinal tissues, adrenal and other glands,⁷⁸⁷ and bone⁷⁸⁸ as well as in the brain. Drugs, such as **sumatriptan** (Fig. 30-28), which activate serotonin receptors are important in the treatment of **migraine**. This common disorder of serotonin metabolism is characterized by severe or moderately severe headache and a variety of other symptoms, which are frequently preceded by a visual aura.⁷⁸⁹ Serotonin is removed from synapses via a

transporter, which also contains the binding site of the widely used antidepressant **Prozac** (Fig. 30-28) and related drugs.^{790-791b}

Serotonin and melatonin are evidently involved in maintenance of the 24-h circadian rhythm of the body (see Section 13).^{792,792a} Melatonin regulates the sexual cycle in photoperiodic animals and influences the onset of puberty.⁷⁹²⁻⁷⁹⁴ The serotonin content of the brain is influenced by the diet, being higher after a meal rich in carbohydrates. Serotonin may serve as a chemical message sent from one set of neurons to the rest of the brain, reporting on the nature of dietary intake.⁷⁸⁴ Melatonin, which can readily form free radicals, may function as part of the body's antioxidant system.^{795,796}

Other neurotransmitters. The abundant glutamate, GABA, and glycine are major neurotransmitters. Do other amino acids also function in the brain? Roles for *L*-aspartate and *D*-serine (p. 1785) have been identified, but it is very difficult either to discover or to disprove a neurotransmitter function for other amino acids. It is even more difficult for small amounts of various amines and small peptides that are present in the brain. **Taurine** (Fig. 24-25) is one of the most abundant free amino acids in animals and meets several criteria for consideration as both an inhibitory and an excitatory transmitter.^{797,798} However, its function is still uncertain (see Chapter 24). **Homocysteic acid**, formed by oxidation of homocysteine, is a powerful neuroexcitatory substance, but its concentration in the brain is very low.¹⁴⁹ *D*-Aspartate is also present

at high concentrations in the cerebellum, pituitary, pineal gland, and adrenal chromaffin cells. It appears to be a modulator of melatonin synthesis.^{799,800}

Receptors for **histamine**, which probably acts as a neuromodulator,⁸⁰¹ occur in the brain.⁸⁰² Histamine is formed by decarboxylation of histidine (p. 745)⁸⁰³ and is inactivated by histidine *N*-methyltransferase. Histamine is best known for its presence in mast cells,⁸⁰⁴ components of the immune system that release histamine during inflammatory and allergic reactions (Chapter 31). However, histaminergic neurons of the hypothalamus extend throughout the whole forebrain,⁸⁰⁵ and specific receptors have been found both in the brain and in peripheral tissues.⁸⁰⁶ Several other amines that are formed by decarboxylation of amino acids are present in trace amounts but may have im-

portant functions, some of which may be related to psychiatric disorders. These include tyramine (from tyrosine), β -phenylethylamine (from phenylalanine), and tryptamine (from tryptophan). As previously mentioned, octopamine is also present in trace amounts in mammalian brains.⁸⁰⁷

ATP, ADP, and adenosine are among the purines that are present in some synapses and activate a variety of receptors. Adenosine receptors are blocked specifically by methylated xanthines such as caffeine (Fig. 25-18) and theophylline.^{808–808b} A drug almost 10^5 times as potent as theophylline is 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine.⁸⁰⁹ Adenosine receptors, which are present in large numbers in the hippocampus,¹⁴⁹ form functional complexes with metabotropic glutamate receptors.⁶⁷⁸ Adenosine

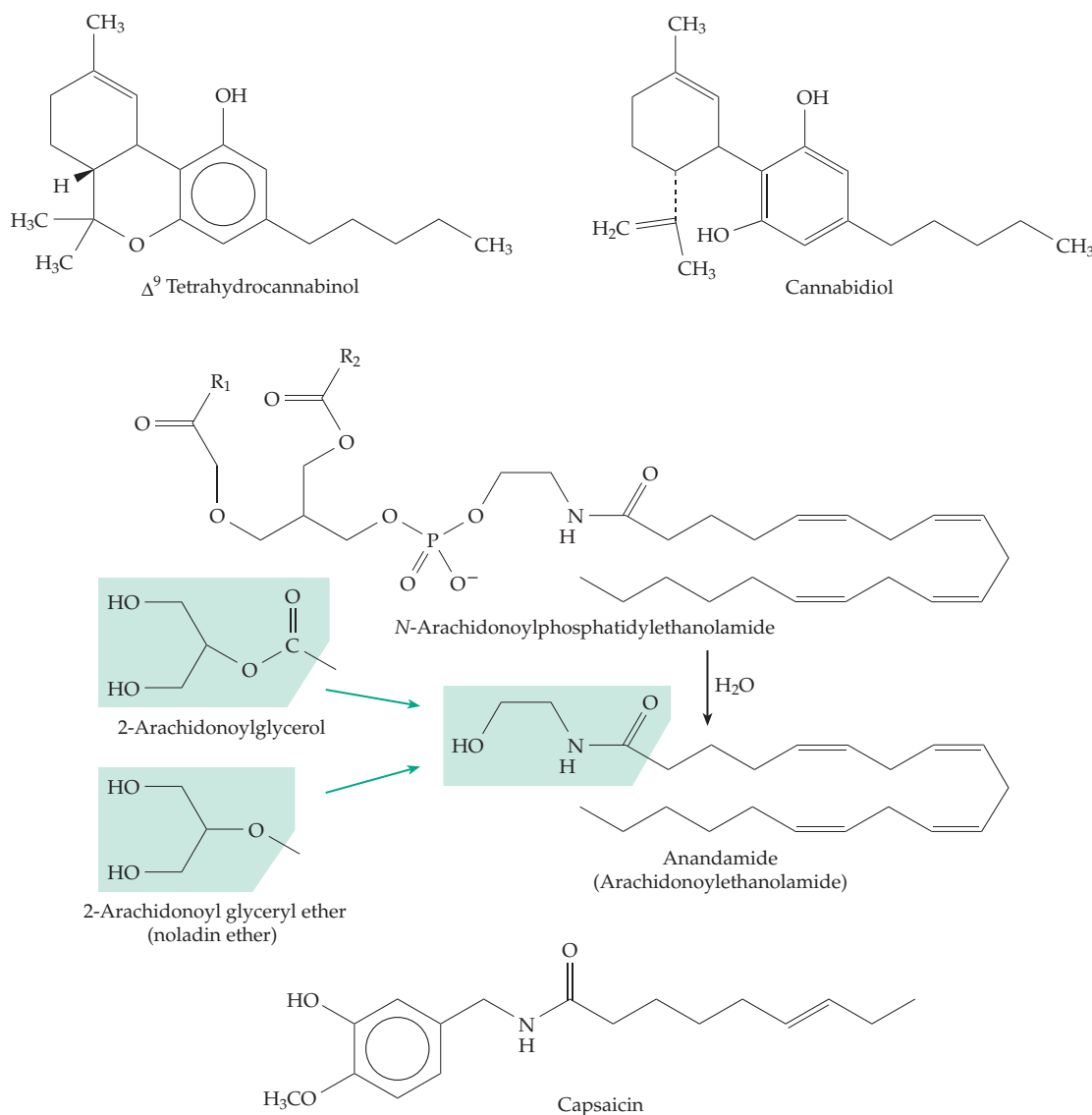


Figure 30-29 Structures of the active components of cannabis, tetrahydrocannabinol, and cannabidiol, and structures of endogenous cannabinoids and of the vanilloid lipid capsaicin.

usually has a depressive effect. Craving for chocolate is often attributed to the methylxanthines present, but it may be a result of anandamide and related compounds.⁸¹⁰

The occurrence of a variety of **neuropeptides** in the brain has been discussed in Section A. The first of these to be discovered⁸¹¹ was the 11-residue **substance P** (Table 30-4), which was isolated in 1931. Like other neuropeptides it may function either as a transmitter or neuromodulator or perhaps both. Substance P, as well as many other neuropeptides, has been localized to specific neurons. Along with somatostatin, CCK, and enkephalins, it is found in high concentrations in the basal ganglia. Enkephalin and substance P are also found in specific neural elements in the visual system of lobsters.⁸¹² In some cases a neuron contains both synaptic vesicles containing a major neurotransmitter and also vesicles containing a peptide or other cotransmitter. The peptide pituitary hormones ACTH, MSH, and vasopressin as well as the hypothalamic neurohormones may have effects on learning and behavior.⁸¹³

Lipid mediators in the brain. The brain is rich in phospholipids, glycolipids, and long-chain unsaturated fatty acids. Many signaling functions seem likely, and some are discussed in Section A,7. Prostaglandin D₂ is a major prostanoid in the brain, which induces both hypothermia and sleep.⁸¹⁴ As mentioned in Section A,7, **oleamide** also induces sleep, perhaps by modulating the effects of 5-HT receptors.^{815,816} **Anandamide** is a lipid derived by hydrolysis of the unusual phospholipid *N*-arachidonoylphosphatidylethanolamide. This is one of a recently discovered series of amides, esters, and ethers derived from arachidonic acid (Fig. 30-29).^{816a,b} They have been identified as endogenous ligands of the abundant **cannabinoid receptors**.⁸¹⁶ The latter were identified as binding sites of Δ^9 -tetrahydrocannabinol and cannabidiol (Fig. 30-29), both of which are constituents of **marijuana**. Anandamide was the first of the endogenous cannabinoids to be isolated.⁸¹⁷ However, the monoglyceride **2-arachidonoylglycerol** (Fig. 30-29) is much more abundant in brain and also activates cannabinoid receptors.⁸¹⁸ It arises by hydrolysis of a diglyceride.^{819,820} Recently 2-arachidonoyl glyceryl ether (**noladin ether**; Fig. 30-29) has been identified as another endogenous agonist of the CB₁ cannabinoid receptors.⁸²¹ A possible alternative pathway for anandamide synthesis is via an energy-dependent coupling of arachidonic acid with ethanolamine.^{822,823} The two known types of cannabinoid receptors are both 7-helix proteins coupled by G_i or G_o proteins to adenylate cyclase and to Ca²⁺ and K⁺ channels.^{824,825} The CB₁ receptors are found largely in the brain and are responsible for the psychoactive effects of cannabis, while the CB₂ receptors are more widely distributed. They seem to have a special role in cells of the immune

system, e.g., in macrophages and B cells.^{818,820,825–828} Palmitoylethanolamide has been proposed as an additional endogenous ligand for CB₂ receptors.^{820,829} Cannabinoid receptors of invertebrate immune system cells and of human monocytes have been found coupled to NO release.⁸³⁰

Cannabinoid receptors are present at extremely high levels in the basal ganglia of the brain,^{831,832} but they do not appear to be essential. Knockout mice lacking the CB₁ receptors appear normal in most respects. However, they do not respond to cannabinoid drugs and, curiously, do not become addicted to morphine as normal mice and have less severe withdrawal symptoms than normal after morphine addiction.⁸²⁶ The CB₁ receptors in the basal ganglia modulate GABA neurons that have outputs to the substantia nigra and the globus pallidus (Fig. 30-30B). The nigrostriatal neurons also secrete substance P and dynorphin, while those extending to the globus pallidus generally contain enkephalin as a cotransmitter.⁸³² These interconnections affect the dopaminergic neurons. Cannabinoids also have pain suppressing and neuro-protective effects. They may have many possible medicinal uses, which are being explored.^{833–837}

The endogenous cannabinoid compounds are lipids and are not stored in synaptic vesicles but are presumably released by enzymatic action following passage of a nerve impulse. Recent evidence suggests that the endocannabinoids are released at a postsynaptic membrane and then diffuse back to a presynaptic surface and outward to other cell surfaces where they affect signaling.^{838–840} This **retrograde signaling** in synapses of the hippocampus is thought to be involved in **long-term potentiation (LTP)**, the changes in synaptic properties that occur during learning and in the formation of memories (Section 12). A monoglyceride lipase participates in inactivation of endocannabinoids.^{840a} Anandamide is also a substrate for cyclooxygenase-2 (Eq. 21-16), whose action may lead to formation of additional immunomodulatory compounds.^{841,842} Long-chain relatives of arachidonic acid such as docosahexaenoic acid (DHA; Box 21-B) are especially high in brain lipids.^{843,843a}

Nitric oxide and carbon monoxide. The gaseous molecules NO and CO have both been found in the brain, and neuronal NO synthase (nNOS or NOS I) has been studied intensively.^{844–847} NO synthases and the functions of NO and CO are discussed in Section A7 and in Chapter 18. Complexity in understanding the role of NO in the brain arises from the fact that different isoenzyme forms of NO synthase occur in three different types of cell: nNOS in neurons, iNOS from microglial immune system cells, and eNOS from endothelial cells of capillary blood vessels.⁸⁴⁶ All three types of cells are so tightly intermingled in the brain that it is hard to interpret observed experimental

effects. Elevated Ca^{2+} concentrations that can arise from stimulation of NMDA receptors in the hippocampus seem particularly effective in activating the calmodulin-dependent nNOS. This suggests that, like the endogenous cannabinoids, NO may be a retrograde messenger in LTP.¹⁴⁹ The possibility that CO may function in a similar way also remains uncertain, as does any pathway for metabolism of CO. Certainly NO and CO generated in the brain will have some effects that arise from their very tight binding to heme groups. An example is the observed inhibition of dopamine β -hydroxylase by N_2O_3 with a resulting decrease in noradrenaline synthesis.⁸⁴⁸

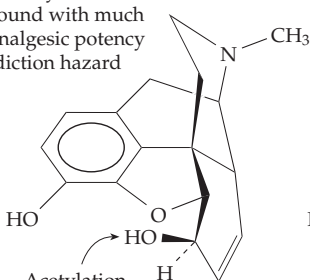
10. Some Addictive, Psychotropic, and Toxic Drugs

Humans have a long history of use of stimulant and mind-altering substances. Tea, coffee, alcohol, tobacco, opium, cocaine, marijuana, and a host of

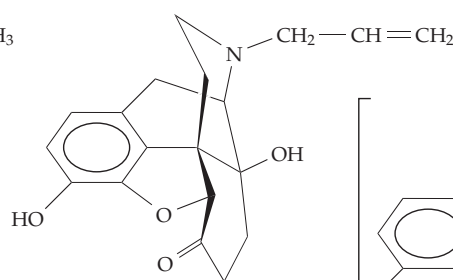
modern synthetic compounds have been used as stimulants, as medications, and for pleasurable experiences.^{849–851} Many are also addictive and sometimes lethal. Stimulant drugs such as nicotine, cocaine, methamphetamine (METH), and other amphetamines (Fig. 30-27)^{849,852–854b} can give users feelings of increased energy, well-being, and self-confidence. Nicotine enhances fast excitatory transmission⁸⁵⁵ and may sharpen memory.⁸⁵⁶ However, all are acutely toxic and are highly addictive. Amphetamines and cocaine act directly to increase the brain dopamine level causing euphoria. However, in response the dopamine receptors rapidly decrease their sensitivity. This leads to mental depression and the desire for more drug. Nicotine appears to indirectly affect the same dopamine neurons.⁸⁵⁷ The wisdom and ethics of giving hypoactive children the addictive stimulant **methylphenidate** (Ritalin; see Fig. 30-33) have been questioned.^{858,859} The depressive drugs, including **morphine** and other narcotics (Fig. 30-30), barbiturates (Fig. 30-22), and ethanol, are all strongly addic-

A

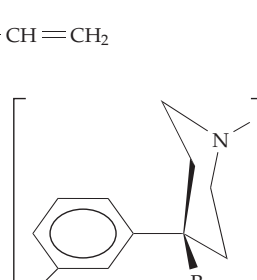
Methylation yields codeine a compound with much lower analgesic potency and addiction hazard



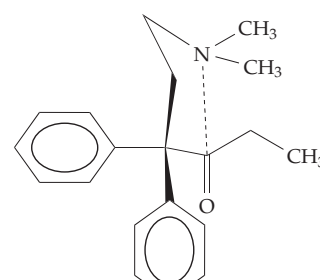
Acetylation yields heroin
Morphine (see also Fig. 25-10)



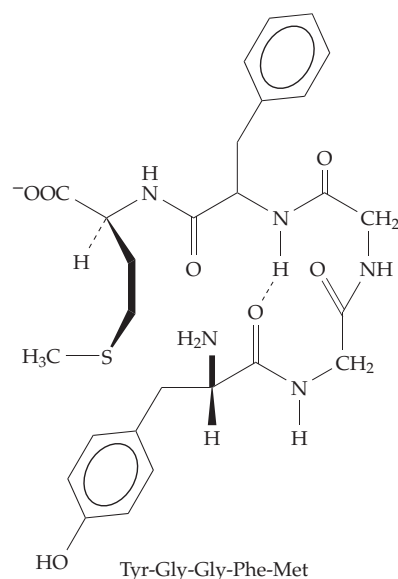
Naloxone



Structure common to many narcotic drugs



Methadone



B

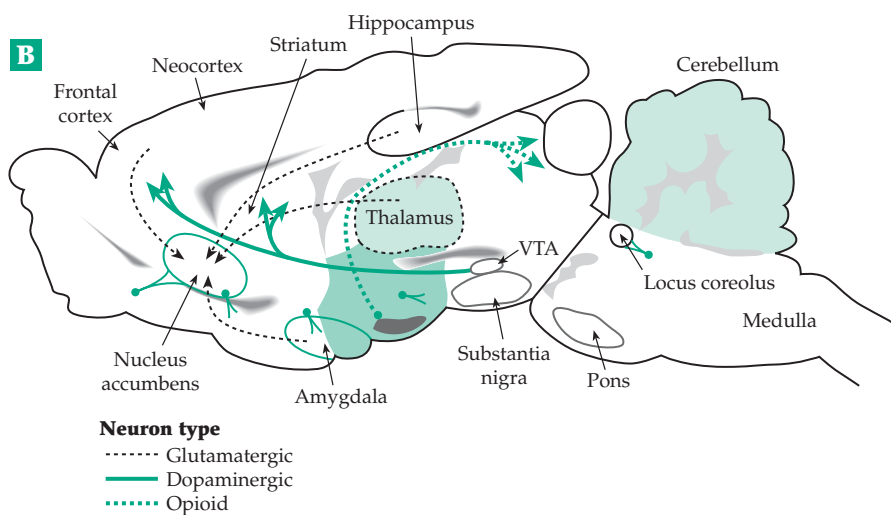


Figure 30-30 (A) The structures of morphine and of some analogs including the brain peptide Met-enkephalin. Also shown is a structure common to many narcotic drugs. (B) Diagram of a rat brain as shown in Fig. 30-13 with some aspects of the mesolimbic dopamine system emphasized. See Shulzeis and Koob.⁸⁶⁹

tive for susceptible individuals. The phenomenon is most striking in the case of the opiates. Addiction leads to physical dependence, a situation in which painful withdrawal symptoms occur in the absence of the drug. At the same time a striking tolerance to the drug is developed. The addicted individual can survive what would otherwise be a fatal dose without ill effect. Aside from the pathological hunger for the drug, an addict can function normally in almost every respect.⁸⁶⁰ Dependence develops only from frequent doses of drug over a long period of time and is not observed with cocaine or amphetamines.⁸⁶¹ Marijuana is only mildly addictive, according to some data about the same as caffeine.⁸⁶² However, this conclusion is controversial.

Opioid receptors. Direct binding of highly radioactive opiates has permitted localization of specific opiate receptors of several types.^{863–866} The three major types (μ , δ , κ) are all 7-helix receptors coupled to adenylate cyclase, K^+ and Ca^{2+} channels, and the MAP kinase cascade.⁸⁶⁶ The μ receptors bind morphine most tightly.^{867,867a} These receptors are found in various cortical and subcortical regions of the brain. Most narcotics are polycyclic in nature and share the grouping indicated in Fig. 30-30. However, the flexible molecule **methadone** binds to the same receptors.⁸⁶⁸ Among antagonists that block the euphoric effects of opiates the most effective is **naloxone** (Fig. 30-30).

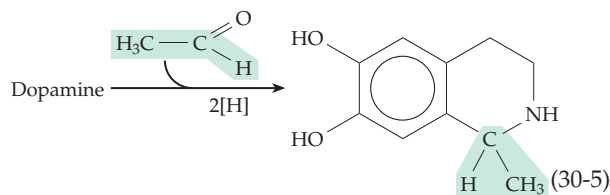
What is the natural function of opiate receptors? Opiates are the most powerful **analgesic agents** known. The existence of the **enkephalins**, **endorphins**, and **endomorphins** (Section A,5; Table 30-4) in the brain suggests that opiate drugs mimic the normal action of these peptides, which may function in controlling pain. Although opiates are powerful drugs, their efficiency in diminishing pain is directly related to their addiction potential. To date, it has not been possible to design a nonaddictive analgesic drug of the potency of morphine.

Addiction seems to follow compensatory changes in the receptor–agonist system that result from the occupation of the receptor sites by the drug. For example, studies of opiate receptors indicate that morphine acts in an inhibitory fashion, lowering the internal level of cAMP.^{861,870} The neuron then compensates by increasing the number or activity of adenylate cyclase molecules restoring the internal cAMP level. This leads to dependence upon morphine because in its absence the cAMP level rises too high. The increased number of adenylate cyclase molecules and associated receptors also accounts for the observed tolerance. It is now clear that this adaptation is complex. The properties of many synapses in various parts of the brain are altered by phosphorylation or dephosphorylation or other reactions of receptors and other synaptic proteins. Some changes are rapid, but

others are slower and involve alterations in transcriptional patterns within neurons. These changes occur in three different neuronal systems: (1) physical control systems, in which changes lead to physical dependence; (2) motivational control systems; and (3) associative memory systems.⁸⁶¹

The **mesolimbic dopamine system** is thought to be involved either directly or indirectly in addiction to many drugs. The dopaminergic neurons of this system have cell bodies in the **ventral tegmental area** (VTA) of the brain (Fig. 30-30B) and extend into the **nucleus accumbens**, a region at the base of the striatum that is thought to provide the “rewarding effects,” i.e., pleasure from drugs such as cocaine or amphetamines. There is direct experimental support for this conclusion.⁸⁷¹ Less certain is the proposal that opiates and other depressive drugs indirectly cause a similar effect in the nucleus accumbens.^{869,870,872} A more recent view is to regard addiction as an aberrant form of learning.^{861,871,873} This concept is applicable also to “behavioral addictions.”^{873a}

Ethanol. As with morphine addiction, tolerance to alcohol is developed, and a lack of ethanol produces withdrawal symptoms. The principal route of metabolism of ethanol (both ingested and the small amount of endogenous alcohol) is believed to be oxidation in the liver to the chemically reactive acetaldehyde (p. 774),^{874,875} which is further oxidized to acetate. Some theories of alcoholism assume that addiction, and possibly also the euphoric feeling experienced by some drinkers, results from a metabolite of ethanol in the brain. For example, acetaldehyde could form alkaloids (Eq. 30-5).⁸⁷⁶

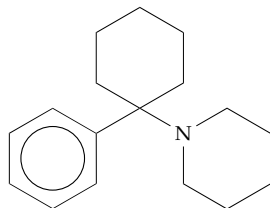


In fact, small amounts of morphine, 6-acetylmorphine, codeine, and thebaine, all opiate compounds, have been found in mammalian brain^{877,878} and have presumably arisen by the same pathway observed in plants (Fig. 25-10). However, there is no cross reactivity between morphine and alcohol in addicted mice,⁸⁷⁹ and acetaldehyde is probably not the addictive agent. Acetaldehyde is very reactive and may be responsible for much of the damage caused by ethanol.⁸⁸⁰ At a blood ethanol concentration of 20 mm a person is legally intoxicated, and large amounts of acetaldehyde may be formed and react with many amines, nucleotides, proteins, etc. Ethanol blocks glutamatergic NMDA receptors and

activates GABA receptors.^{740a} These effects may be involved in the neurodegeneration of fetal alcohol syndrome.^{881,882} Ecitotoxicity may also be a factor in alcohol damage.⁶⁹² Alcoholic liver disease may involve malnutrition as well as direct damage.⁸⁸³

Experiments with mice and rats have established a genetic propensity toward addiction to alcohol. Animals from some strains shun alcohol and become addicted only if force-fed for prolonged periods. Others, which may have low levels of neuropeptide Y in the brain,⁸⁸⁴ accept the alcohol readily and become addicted quickly. That a similar situation holds for humans is quite possible. However, a specific “alcoholism gene” has not been found.

Psychotropic or mind-changing drugs. Hallucinogenic compounds have long been a source of special fascination to many people. The presence of the indole ring in the powerful hallucinogen **lysergic acid diethylamide** (LSD; Fig. 25-12) suggests that this compound may mimic the action of serotonin. However, other experiments suggest antagonism of dopamine receptors in the striatum.⁸⁸⁵ Other hallucinogens include 3,4-methylenedioxyamphetamine (MDA; Fig. 30-27),⁸⁸⁶ a compound that damages serotonergic neurons, its derivative “ecstasy” (Fig. 30-27),^{886a} mescaline (Fig. 30-27), and phencyclidine (angel dust), a compound introduced in the late 1950s as a general anesthetic. Unfortunately, it produces a long-lasting condition resembling schizophrenia.⁸⁸⁷ A common site of action for a large variety of hallucinogens has been suggested.⁸⁸⁸



Phencyclidine (Angel dust)
a discredited anesthetic

Neurotoxins produced by the body. Some normal body constituents are neurotoxic in excess. These include **quinolinic acid** (Fig. 25-11),⁸⁸⁹ **3-hydroxykynurenine** (Fig. 25-11; p. 1444),⁸⁹⁰ and homocysteine.⁸⁹¹ Elevated levels of homocysteine are also associated with vascular disease and stroke (Chapter 24). 3-Hydroxykynurenine is a precursor to ommochrome pigments of insects and an intermediate in conversion of tryptophan into the nicotinamide ring of NAD in humans (Fig. 25-11). 6-Hydroxydopamine (Fig. 30-26), which may be formed in the body, is severely toxic to catecholaminergic neurons.⁸⁹²

Other neurotoxins can be formed from environmental pollutants. The solvent 1,4-butanediol is converted to **γ -hydroxybutyrate**, which is also a drug of

abuse.⁸⁹³ Many compounds in commercial use have not been adequately tested as neurotoxins.⁸⁹⁴

11. The Senses: Sight, Smell, Taste, Hearing, Touch, and Others

Our brains receive a continuous stream of impulses from receptors that sense light, taste and odor molecules, sound waves, touch, pain, gravitational pull, etc. Of these receptors those of vision, which are discussed in Chapter 23, may be the best known. The photoreceptors consist of rhodopsin and related 7-helix proteins embedded in membranes of the rod and cone cells (Fig. 23-40). A complex series of control mechanisms, some of which are outlined in Fig. 23-43, permit enormous amplification of the initial signal generated by a G protein and a cGMP-gated ion channel. The array of rods and cones in the retina send messages via the optic nerve to the **visual cortex**, an area of $\sim 15 \text{ cm}^2$ on the cerebral cortex surface at the back of the brain.^{149,895} The visual cortex is divided into two halves, but curiously, the right eye sends its signals to the left brain and vice versa. The image viewed by the retina can be mapped to the visual cortex. There it may reside in the form of chemical alterations in the $\sim 40,000$ neurons thought to be present in the visual cortex^{409,896–898} for a short time until it is stored in short-term working memory locations.

Receptors for the other senses, like those for sight, also consist of clusters, often in regular arrays, of 7-helix receptors. Most of these are also G protein-coupled ion channels that are controlled by cAMP or cGMP.⁸⁹⁹

Odor. Even bacteria possess something akin to our ability to taste and smell. As is discussed in Chapter 19, Section A, many bacteria are attracted to L-serine or D-ribose and are repelled by phenol. Receptor proteins in the plasma membrane are involved in sensing these compounds and in allowing bacteria to move toward food and away from danger. Many other examples of chemotaxis are known among the lower invertebrates such as *Euglena*. Chemoreceptors in *Hydra* sense glutathione that flows from the broken tissue of their prey and control the animal's feeding behavior. Related organisms respond to proline. Asparagine induces the bending of the tentacles of the sea anemone *Anthopleura*, while glutathione induces swallowing.⁹⁰⁰ Salmon return to their home streams using a memory of specific odors.⁹⁰¹

Throughout the animal kingdom the sense of smell is essential for survival. Perhaps it is not surprising that from the nematode *C. elegans* to human beings there is a largely conserved mechanism for sensing odors.⁹⁰² A large array of 7-helix G protein-coupled olfactory receptors embedded in an epithelial membrane carry signals directly into the nervous system.

In *C. elegans*, which has only 302 neurons, there are 32 chemosensory neurons and more than 100 genes for 7-helix receptors that are expressed in these neurons.^{903–905} The fruit fly *Drosophila melanogaster* has at least 59 genes for olfactory receptors.^{906,906a} Zebrafish and catfish have ~100.^{905,907} Mice and rats have ~1000 olfactory receptor genes and human beings at least 500, which account for about 1–4 % of the genome.^{905,908,909} In higher animals most receptors are coupled via G proteins, adenylate cyclase, and cAMP to ion channels in the membrane.^{905,908} Insects utilize both cAMP and Ins3-*P* in their chemosensory receptors.⁹⁰⁶ The signaling pathways parallel those of the visual receptors (Figs. 23-40, 23-43), which, however, utilize cGMP. Each gene is thought to give rise to a receptor of a specific **type** able to respond to specific structural features in an odor molecule.

Human olfactory cells are located in the **olfactory epithelium** on the upper surface of the back portion of the nasal cavity. They are neurons with chemosensory cilia similar to the rods and cones of the retina (Fig. 23-40). The cilia, which can be detached and isolated from the olfactory epithelium, contain the odorant-stimulated G-protein-dependent adenylate cyclase.^{910,911} There are ~10 million receptor cells of at least 500–1000 different types. The 10 million axons form bundles of ~5000 axons each and pass through small perforations in the skull directly into the **olfactory bulb** (at the front of the brain before the pituitary, Fig. 30-13), a distance of 3–4 cm. The cortex of the olfactory bulb is lined with ~1800 **glomeruli**. Each glomerulus is a bundle, ~0.1–0.2 mm in diameter, of synaptic endings of the neurosensory nerves coming from the olfactory epithelium with dendrites of neurons that run to the **olfactory cortex** and other regions of the brain.⁹⁰⁹ Each sensory receptor sends signals to a single glomerulus, but the glomerulus receives signals from 500 or more sensory neurons, which are not all of the same types. The glomerular cortex of the mouse is divided into four zones, each of which contains only some of the types of receptor. It seems that the cortex contains a crude “map” that relates position to the type of smell.⁹¹² The neural processing involved in the discrimination of odors is not yet clear.^{912,913} Interneurons of the olfactory bulb are unusual, being continuously discarded and replaced by new neurons that arise from neural stem cells.^{908,914} This process seems to be essential for odor discrimination but not for the sensitivity of odor detection.

Most mammals have a second olfactory apparatus, the **vomerinal organ** (VNO) or “sexual nose,” which is located on the lower surface of the nasal cavity. It is a fluid-filled cavity containing chemosensory receptors through which nasal fluid is literally pumped, when the animal seeks to maximize the sensitivity of detection.^{908,915} The VNO is especially

important to reproduction, defense, and food-seeking. A specialized set of olfactory sensory neurons that project to atypical glomeruli in the olfactory bulb utilize cGMP signaling and may also function in reproductive behavior.⁹¹⁶

The olfactory epithelia are bathed in an aqueous mucus through which odorant molecules must pass. A number of specialized proteins, including **odorant-binding proteins**, are secreted in this fluid.^{917–919} Many odorant-binding proteins are **lipocalins** (Box 21-A) and presumably assist in transporting lipophilic odorant molecules to the olfactory receptors. They tend to have a low specificity for the odorant and a weak binding affinity, properties that are consistent with this function. Pheromone-binding lipocalins encoded by ~30 genes are also found in rodent urine,⁹²⁰ where they play a similar role. In contrast, the pheromone-binding proteins of some male moths are largely α helical.^{920a} Although pheromones are not as important to human physiology, axillary odors from both males and females do apparently carry chemical signals. One well-established effect is the synchronization of menstrual cycles of women living in the same house or dormitory. Alipoprotein D apparently serves as a binding protein that carries odorant precursors that are acted on by bacteria to produce the pheromones.⁹²¹

Virtually all people lack the ability to detect some specific odors. A striking example of such an **anosmia** is the inability to smell the volatile steroid **androstenone** (5 α -androst-16-en-3-one), a constituent of perspiration, of some pork products, truffles, and celery.⁹²²

Taste. Less is known about the biochemistry of taste. The taste that we perceive is affected by odor, temperature, and physical contact. However, five primary tastes are recognized.^{923,923a}

Salty: apparently perceived by an ion-channel-linked receptor

Sour: apparently linked to an H⁺ channel

Bitter: perceived by bitter-sweet G protein-coupled receptors

Sweet: also perceived by bitter-sweet receptors

Umami: a recently recognized taste, that of glutamate

An experimental difficulty lies in the fact that there are only a few thousand taste buds in the tongue, with only 50–100 cells in a bud. They age rapidly, having a lifespan of only about ten days.⁹²⁴ There may be only 30,000–50,000 hard-to-isolate taste receptor cells on the tongue's surface.⁹²³ However, very recently published reports describe a large family of bitter and sweet receptors in mice and humans^{924–928} and in *Drosophila*.^{929,930} The sweet-sour receptors are thought to activate a G protein called **gustducin**,^{931,932} which plays a role similar to that of transducin in vision and

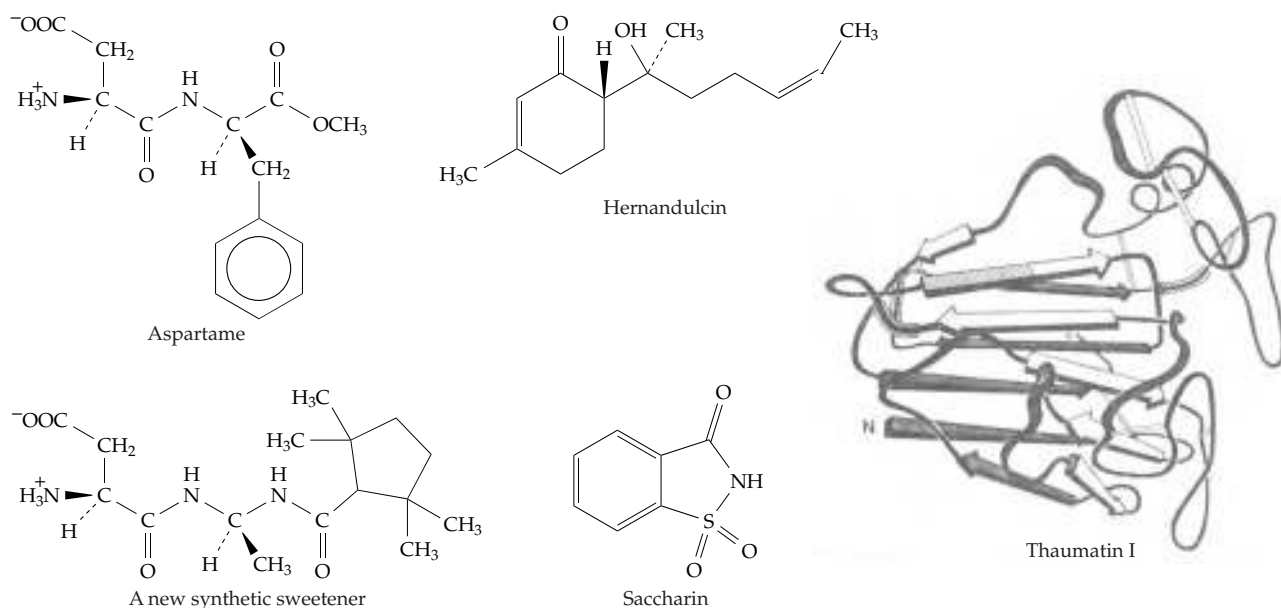


Figure 30-31 Structures of some very sweet compounds. The backbone structure of the protein thaumatin I is included. The main body of this structure consists of two β sheets forming a flattened β barrel. β Strands in the top sheet are shaded light, and those in the bottom sheet are darker. Open bars represent disulfide bonds, and the regions with sequences homologous to monellin are indicated by the hatched marks. From de Vos *et al.*⁹⁴⁰

also activates ion channels.⁹³³ Like the odor receptors, taste buds are also bathed in a special fluid. The **von Ebner's glands** in the tongue contain binding proteins,^{934,935} at least some of which are lipocalins.

The relationship between structures and sweet taste in humans has been investigated intensively, but no simple rules have been discovered (see Robyt⁹³⁶ for a discussion). Sucrose is usually perceived as very sweet. D-Fructose and D-xylose are nearly twice as sweet, but D-glucose is less sweet than sucrose. D-Galactose is usually perceived as not sweet and D-mannose as bitter. Many sucrose derivatives, in which hydroxyl groups have been replaced with Cl or other halogen, are very sweet. One tetrachloro derivative of this type is 7000 times sweeter than sucrose.⁹³⁶ Some especially sweet materials are depicted in Fig. 30-31. These include peptide derivatives^{937,938} such as Asp-Phe-OCH₃ (**aspartame**), the sesquiterpene **hernandulcin**,⁹³⁹ and **chemostimulatory proteins**. Among these are some of the sweetest substances known, the 207-residue **thaumatin**s^{940,941} and **monellin**^{942,943} present in certain tropical berries. Thaumatin is ~3000 times sweeter than sucrose on a weight basis and 10⁵ times sweeter on a molar basis. Thus, sucrose tastes sweet at a concentration of 10⁻³ M or higher but thaumatin⁹⁴⁴ at 3 × 10⁻⁸ M.

The proteins **miraculin** and **circulin** from tropical fruits modify taste. Acids taste sweet rather than sour after the tongue has been treated with either protein.^{945,946} Exposure of the tongue to artichokes often

makes water taste sweet.⁹⁴⁷ Thus, the response of taste receptors can be temporarily altered by binding of other substances, perhaps at adjacent sites on a receptor.

Pain. Receptors for pain (**nociceptors**) are spread over the body in nerve endings found in the skin, muscle, joints, and internal organs. There are several types of receptors, most of which are present in excitatory glutamatergic neurons.⁹⁴⁸ Some release substance P. Some activate tyrosine kinases and others ATP-gated ion channels. Some pain receptors are also activated by intense heat or pressure or by irritant compounds. Among the latter is capsaicin (Fig. 30-29), the active ingredient in chili peppers, and an ultra-potent compound, **resiniferatoxin**. Both capsaicin, which is 10,000 times more potent than jalapeño peppers,⁹⁴⁹ and resiniferatoxin, which is 20-fold more potent than capsaicin, bind to **vanilloid receptors**. These are ligand-gated ion channels related to the Shaker K⁺ channel (Fig. 30-18). They are nonselective but with a high permeability to Ca²⁺ and are members of the **transient receptor potential (TRP)** family.^{948,950–954} Pain seems to stimulate an increase in anandamide (Fig. 30-29), which has an analgesic effect. Nevertheless, anandamide and N-vanillyloleamide activate capsaicin receptors.⁹⁵⁵ Because the activated receptors become desensitized rapidly, capsaicin has been used in a paradoxical manner as an analgesic agent.⁹⁵¹ Sensing of temperature changes also depends upon TRP channels.^{955a,b}

Mechanoreceptors. The transduction of mechanical force into a chemical signal provides the basis for the senses of touch and hearing. Plants detect wind and gravitational force,⁹⁵⁶ and many organisms, even bacteria, respond to changes in osmotic pressure using mechanoreceptors.⁹⁵⁷ One of the best known mechanoreceptors is from *Mycobacterium tuberculosis*. It is a homopentamer whose three-dimensional structure^{450–452,958} resembles that of the nicotinic acetylcholine receptor (Fig. 30-23). A second type of mechanoreceptor is found in the inner membranes of *E. coli* and in plasma membranes of many other bacteria, archaea, and some eukaryotes.^{957a} These receptors, which are also sensitive to voltage changes, are heptamers of a 282-residue protein that forms a symmetric ion channel in the center. There is also a large cytoplasmic domain consisting largely of β structure.^{957b} How do such receptors sense mechanical stress? In bacteria they respond to stretch in the membrane induced by an increase in osmotic pressure. One suggestion is that the membrane expansion pulls apart the radially symmetric ion channel in the receptor.^{958,958a} In higher organisms transmembrane adhesion receptors and their linkage to the internal cytoskeleton provide a framework for detection of mechanical forces and linkage to mechanoreceptors.^{956,957,959}

Hearing. Movement of the **stereocilia** of the hair cells of the inner ear activates mechanoreceptors. Each stereocilium contains a core of crosslinked actin filaments, and tens to hundreds of these cilia are connected in hair bundles, which move in response to arrival of sound waves of appropriate frequencies. The movement of the stereocilia induces the opening of receptor ion channels in the hair cell membrane allowing K^+ and other ions to flow inward.⁹⁶⁰ The matter is much more complex than this because of the tuning and amplification mechanisms in the cochlea of the inner ear.^{960–962} These mechanisms allow receptors in hair cells to respond to very weak vibrations of specific frequencies. Both mechanical and biochemical mechanisms are involved. A number of specific proteins participate. Among these is a motor protein called **prestin**, which seems to be involved in the rapid changes in length and stiffness of some hair cells in the cochlea.^{962,963}

Other sets of hair cells are formed in specialized parts of the inner ear.⁹⁶⁴ The three semicircular canals detect angular acceleration in three directions, while the sac-like utricle and saccule detect linear acceleration including gravitational attraction. These two organs each contain a patch of hair cells whose tips project into a gelatinous layer, which is overlain by a field of small crystals of calcium carbonate. These little stones (**otoliths**) provide an inertial mass, which resists movement causing the hair cell tips to bend and activate mechanoreceptors to send information about balance and orientation to the brain.

While discussing vibrations we may ask whether 60 cycle electromagnetic field fluctuations caused by electrical power transmission can affect the human body? Considerable effort has been expended in addressing this question. The tentative conclusion is that such effects, if they exist, are extremely difficult to detect. However, the possibility has not been disproven.⁹⁶⁵

12. The Chemistry of Learning, Memory, and Thinking

What is known about the chemistry underlying memory, thinking, and the generation of the stream of consciousness within the brain? Nerve impulses originating in sensory receptors are sent to several regions of the brain, among which are the sensory regions of the cerebral cortex (Fig. 30-14). Memory also depends upon other regions of the brain including the hippocampus, amygdala, and cerebellum (Fig. 30-1). Learning, remembering, and thinking all require transfers of information between various neurons and between different parts of the brain. These transfers may perhaps be coordinated via endogenous electrical rhythms (brain waves).

Memory systems. Memories exist in several forms and are found in various regions that are reached by several pathways.^{966–967a} Two major forms of memory are:

Explicit (declarative, episodic):

Conscious recall of facts and events involving people, places, and things

Implicit (associative):

Nonconscious recall of motor skills, conditioned responses, etc.

Explicit memory depends upon the **temporal lobe** of the midbrain, an area that includes the hippocampus and the nearby subiculum and entorhinal cortex.^{966,968–971} Implicit associative learning and memory involve the cerebellum, amygdala, and other regions.^{972,972a}

Both types of memory possess both **short-term** and **long-term** components. Short-term memory lasts only minutes to hours, but long-term memory lasts days, weeks, and sometimes a lifetime. The difference between the two is clearly seen in individuals who have damage to the hippocampus and impairment of short-term memory. A blow to the head may cause total loss of short-term memory of associations (**amnesia**).^{969,973,974} Some persons with damage to the hippocampus may never regain their temporary memory, but long-term memories are intact, and new long-term memories may still be formed. An increasingly important tool for study of memory is brain imaging using

PET or **fMRI** (Box 30-A). These tools have become rapid and sensitive with the ability to observe regions of the brain that become activated by visual, auditory, or other stimuli.^{897,898}

The brain often needs to store information for a short period of time. For example, one can recall many details of a visual image after closing one's eyes or shifting one's gaze. The sensory images may be stored in **working memory**.^{896,975} Similarly, if one mentally multiplies two 2-digit numbers the partial product obtained by multiplying the two right most digits is temporarily stored in working memory until the next arithmetic operation is completed, etc. PET and fMRI tomography indicates that regions in the prefrontal cortex may be involved.^{976,977}

Some short-term memory appears to be stored by neurons that continue to fire after a stimulus has stopped. It has been proposed that such memory consists of reverberations of electrical activity in loops of coupled axons.^{978,979}

Implicit memory can be studied in animals. Much has been learned from the large marine snails *Aplysia* and *Hermisenda* whose simple nervous systems and large neurons have been investigated for over 40 years.^{967a,980-984} The basic chemical mechanism associated with learning in these creatures seems to be similar to those in our own brain. Olfactory memory can be studied in *Drosophila*, even though the organization of the fly's brain differs from ours.^{985-987b}

To be useful for more than a few minutes stored information must be transferred from the temporary to more permanent forms. We know that even temporary memory depends upon chemical changes in synapses. Long-term memory involves both stable chemical changes and also changes in the physical connections between neurons. Before discussing these

changes let us consider briefly the waves of nerve impulses that drive the necessary alterations.

Brain rhythms. A live brain displays characteristic oscillatory activity. Using electrodes placed on the scalp of an awake but relaxed individual, a rhythmic change in the recorded voltage with a frequency of ~ 10 Hz (**alpha waves**) can be detected. Such **electroencephalograms** (EEGs) contain other rhythms at ~ 5 – 6 (theta), ~ 40 (gamma), and ~ 200 (high frequency) Hz.^{978,988,989} More recent studies employ microelectrodes placed on individual neurons. Some cells generate spikes at frequencies as high as 800 Hz. However, the significance is uncertain.⁹⁹⁰ The 40-Hz frequency, which is prominent in the hippocampus, has aroused the most interest⁹⁹¹⁻⁹⁹⁵ because of its probable relationship to learning and memory. Psychophysical experiments have suggested that humans can store only 7 ± 2 items, such as digits in a telephone number. To remember more digits usually requires a conscious effort to place them in longer-term memory. One proposal is that the seven items in temporary memory are stored as 40-Hz oscillations and that ~ 7 such items can be stored within a single 5-Hz theta oscillation.^{978,979} Thinking rates have also been estimated as 7 ± 2 thoughts per second. This is also the same as the syllable rate in speech. This allows us to speak at the same rate that we think.⁹⁹⁶ Stuttering may be a result of lack of synchronization of thinking and speaking.

Individual cells or groups of cells are able to initiate rhythms.⁹⁹⁷ Examples are provided by the slower Ca^{2+} oscillations shown in Box 6-D,⁹⁹⁸⁻¹⁰⁰¹ by the periodic release (at ~ 155 intervals) of cAMP by cells of *Dictyostelium* (p. 20), and by the 24-h circadian cycle observed for virtually all living cells (Section 13). In simple invertebrates the source of neural rhythms appears to reside in **pacemaker neurons** that fire spontaneously at regular intervals. Their cell membranes apparently undergo a cyclic series of changes in ionic permeabilities sufficient to initiate action potentials. Three types of pacemaker output from molluscan neurons¹⁰⁰² are illustrated in Fig. 30-32. In lobsters three-neuron **pacemaker groups** provide a pyloric rhythm. In these groups the oscillation period of a pacemaker neuron is adjusted from its intrinsic value by feedback through inhibitory and electrical connections to the other cells.¹⁰⁰³ Electrical coupling seems to be basic to oscillatory cell networks.¹⁰⁰⁴ Individual neurons or small groups of neurons in our own bodies act as pacemakers for the heartbeat rhythm.⁷⁰⁵ The slow 3- to 8-Hz rhythm observed in the EEG apparently originates in pacemaker bursts from the basal ganglia.⁹⁹³ Rhythms from these endogenous pacemakers may combine with pulses from sensory neurons to evoke **conscious thought**. However, the basis of consciousness is still poorly understood.^{1005,1006}

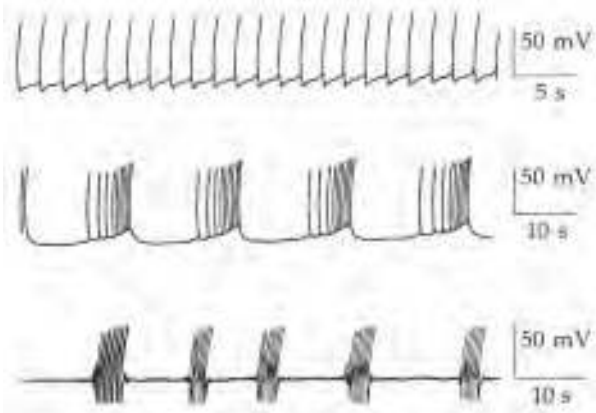


Figure 30-32 Intracellular recordings from isolated neurons of the mollusc *Aplysia*: (A) beating pacemaker, (B) bursting pacemaker, and (C) oscillating pacemaker. From Chen *et al.*¹⁰⁰²

As can be seen from Fig. 30-32, neurons send "trains" of spikes down their axons. These form synapses with dendrites, usually on dendritic spikes, of a postsynaptic cell.^{593,1007-1009} However, each such cell typically receives input from thousands of other neurons. At any moment most of these are probably "silent," but others are sending trains of impulses. Among the important questions are "How does the postsynaptic neuron know whether to fire or not?" and "What kinds of information, if any, are encoded in the trains of impulses both in the presynaptic inputs and in the output of the postsynaptic neuron?"^{1010,1011} Part of the answer to the first question is probably that firing occurs if two or more input impulses arrive synchronously,^{1010,1012-1014} and if there are not too many inhibitory impulses that damp the response. In the hippocampus a network of neurons electrically coupled via gap junctions may be synchronized to the theta and gamma brain rhythms by high-frequency (150–200 Hz) oscillations.⁹⁸⁸ See also Fig. 30-15.

Chemical changes in synapses. It has long been recognized that the synapses are the probable sites of alterations that lead to memory, whether long-term or short-term. Study of individual synapses has demonstrated the phenomena of **potentiation** (facilitation) and **depression** (habituation). Potentiation refers to the fact that a second impulse will often be transmitted through a synapse more effectively than the first, while depression refers to a decreased response to repeated stimuli. Memory may consist of potentiation and depression at specific synapses. The underlying chemical changes in the synapses are referred to collectively as **synaptic plasticity**. Chemical changes associated with short-term memory are often transient. Those associated with long-term memory are described as **long-term potentiation (LTP)** and **long-term depression (LTD)**.

Many experimental results have confirmed the chemical basis of memory. For example, learning is facilitated by administration to animals of small doses of strychnine.¹⁰¹⁵ Puromycin and other inhibitors of protein synthesis disrupt the transfer of information into long-term memory. They are especially effective during the first hour after the initial learning event.¹⁰¹⁶ Increased synthesis both of mRNA and of proteins within the cell bodies of neurons is observed.

Short-term memory is not affected by inhibitors of protein synthesis, but alteration of synaptic proteins and membranes may be induced by covalent modification of existing macromolecules.¹⁰¹⁶ One way in which this happens has been described for *Aplysia*. As the snail learns a simple gill-withdrawal reflex, the duration of the action potentials in sensory neurons is increased, and there is a greater release of transmitters. This change comes about because stimulation of the sensory neuron causes simultaneous activation of

interneurons that synapse with the sensory neurons. The interneurons release the neuromodulator serotonin, which binds to receptors in the membrane of the sensory neurons. This activates adenylate cyclase, which in turn activates a protein kinase that phosphorylates a class of open K⁺ channels. Phosphorylation causes the channels to close with a consequent strengthening of the action potential. Thus serotonin brings about presynaptic facilitation.¹⁰¹⁷ The peptide FMRF amide (Table 30-5) has the opposite effect. It causes hyperpolarization and a decrease in the duration of the action potential. It also binds to a receptor on the neuronal membrane, and presumably via a different second messenger than cAMP causes the K⁺ channels to stay open a longer fraction of the time.

Evidence that LTP is essential to learning in rats was provided by the observation that the antagonist 2-aminophosphonovalerate, which blocks the NMDA class of glutamate receptors (Fig. 30-20A), impairs both LTP and learning.¹⁰¹⁸ The potentiation is thought to result, in part, from Ca²⁺ influx through the nonselective NMDA cation channels. The increased intracellular calcium may then induce phosphorylation of various proteins with associated long-lasting changes in the postsynaptic endings.¹⁰¹⁹ A large amount of evidence favors this interpretation of LTP.¹⁰²⁰ However, it is a great oversimplification. Most studies of LTP in mammals (usually rodents) have focused on the CA1 region of the hippocampus and nearby brain regions. The excitatory axons in this organ are largely glutamatergic, and, as shown in Fig. 30-20A, the postsynaptic (dendritic) membranes contain both fast AMPA receptors and the slower NMDA receptors. Both are ionotropic. The AMPA receptor channels allow mainly K⁺ and Na⁺ to pass and are responsible for most of the nerve transmission. However, the NMDA receptors have an important controlling influence.

A generally accepted theory is that no LTP arises unless both the presynaptic and postsynaptic neurons are activated. This can happen if a presynaptic action potential activates many AMPA receptors in a synapse allowing enough flow of Na⁺ + K⁺ to depolarize the postsynaptic membrane and possibly to initiate an action potential in the postsynaptic neuron. (However, many factors, probably including influences from neighboring neurons,¹⁰²¹ will affect this outcome.) The NMDA receptors are usually blocked by extracellular Mg²⁺ ions, and their ion channels remain closed. However, when the postsynaptic membrane becomes depolarized, the Mg²⁺ dissociates, and if the NMDA receptors are also occupied by glutamate, their channels will open, permitting Ca²⁺ to enter the neuron (Fig. 30-20A). This not only enhances the probability of developing a postsynaptic action potential but is also the trigger for LTP.¹⁰²² Ca²⁺ ions have a variety of effects, one of which is to bind to calmodulin. This

activates a calcium–calmodulin-dependent protein kinase, which phosphorylates postsynaptic structural and signaling proteins to increase the synapse strength.^{641,1023–1025}

Modifications in existing proteins, such as are induced by Ca^{2+} and calmodulin, can provide LTP for a few hours, but other mechanisms must provide for longer-term effects. These require transcription of genes and protein synthesis, processes that occur in the cell bodies of neurons and may depend upon axonal transport mechanisms.^{1026,1027} Among the experimentally observed results of LTP are ultrastructural changes in synapses and in dendrites.^{1009,1028} Long-term memory is also thought to involve changes in the neocortex. Again, NMDA receptor activation seems to be involved.^{1029,1030}

LTP has been demonstrated experimentally, but does it really influence memory? Evidence that it does has been provided by clever experiments with transgenic mice. Using the Cre recombinase (Chapter 27) the NR2B subunit of the NMDA receptor was overexpressed in the hippocampus¹⁰³¹ and in the forebrain of mice.¹⁰³² This was expected to provide better synaptic strengthening than for receptors with the similar NR2A subunit. It was found experimentally that these transgenic mice were more intelligent than normal mice.

LTP is also thought to affect the presynaptic as well as the postsynaptic neuron. One way in which this may happen is for a **retrograde messenger** to pass across the synapse and induce alterations in the presynaptic cell. One proposed retrograde messenger is nitric oxide, **NO**.^{1033,1034} Neuronal NO synthase (nNOS; NOS1) contains a calmodulin binding site and is activated by Ca^{2+} . However, other substances as simple as K^+ might also be the messenger.

Long-term depression (LTD) is the *loss* of synaptic strength after passage of an impulse. There is evidence that during brain activity, including that in the hippocampus, both LTP and LTD are essential.^{1035,1036} LTD may depend upon cyclic ADP-ribose (p. 564).¹⁰³⁷ LTD, like LTP, may also spread via retrograde signaling.¹⁰³⁸

Does learning affect a few specific neurons or a large number of neurons? The rate of glucose utilization in different parts of the brain can be estimated from the rate at which labeled 2-deoxyglucose is taken up (see Box 30-A). From changes in this rate (obtained using ^{14}C -labeling) in brains of split-brain cats performing visual tasks it was estimated that from 10^{10} to 10^{11} neurons are activated.¹⁰³⁹ This supports the idea that memory is distributed over a large area of the brain, just as information about an image is stored in all parts of a hologram.

An alternative to the idea that synaptic potentiation and depression provide the chemical basis for learning is **molecular coding**. Thus, it was reported

that a 15-amino acid peptide isolated from rats trained to avoid the dark carries behavioral information. When this peptide was injected into brains of untrained rats, they also avoided the dark.¹⁰⁴⁰ This was one of several reports of transfer of learned behavior through chemical substances extracted from the brain. These ideas are hard to accept in the light of our present knowledge of the brain. However, in view of the large number of different neuropeptides known (Table 30-4) the possibility that some aspects of long-term memory may be associated with transcription of specific amino acid sequences within specific neurons should perhaps still be considered.

The complexity of the brain. A major obstacle to our understanding of the human brain is its enormous complexity.⁴⁰⁰ This problem can be appreciated if we consider the small nematode *Caenorhabditis elegans*. All of the synaptic connections among its 302 neurons had been mapped by 1986.^{400,1041,1042} There are 5000 chemical synapses and 600 electrical (gap junction) connections. There are 80 different types of K^+ -selective channels, 90 types of ligand-gated receptors, and ~1000 G-protein-linked receptors. Twenty-six of the neurons are GABAergic and are involved in three distinct behavioral motions that involve muscular contractions. Despite intensive efforts the system has been hard to understand. The brain of the macaque monkey has been described in great detail.⁴⁰⁹ Over half of its cerebral cortex is devoted to vision, and this can be subdivided into 20 functional areas. However, the human brain, with its extremely large cerebral cortex, cannot be compared accurately with the monkey brain. Whereas anatomical studies are done on postmortem human tissues, *in vivo* studies rely largely on fMRI and PET imaging (Box 30-A). The resolution of these images is now less than 1 mm, but 1 mm³ of human visual cortex contains more than 40,000 neurons!⁴⁰⁹ The microcircuits in the neocortex are still largely unknown and the tissue is of “apparently impenetrable complexity.” There may be several hundred different classes of neocortical neurons.¹⁰⁴³ The tissue is rich in GABAergic interneurons.^{1044,1045} Some fast-spiking GABAergic neurons are also connected by electrical synapses and may be involved in detecting and promoting synchronous activity.¹⁰⁴⁶

Intelligence. We must all agree that there is such a thing as intelligence, but can it be measured? In 1904, Spearman proposed the existence of a general intelligence factor *g* that could be measured as the IQ (intelligence quotient). Since then various tests have been devised that attempt to measure IQ.^{1047,1048} Most recently use of PET scan data has indicated that various types of analytical analysis lead to brain activity in the lateral frontal cortex in one or both cerebral hemispheres¹⁰⁴⁹ suggesting that this is a region important to

IQ. A question that has been raised is whether analytical intelligence, creative intelligence, and practical intelligence are correlated?¹⁰⁴⁸

Is intelligence hereditary? Both logic and observation say that heredity must be a major factor. However, it is hard to know how to measure the hereditary component.^{1050–1052} Also hard to understand is why IQ scores have been increasing about one standard deviation unit per generation.¹⁰⁴⁷ Is this really true?⁸⁴³ Does environment also influence IQ? The fact that new hippocampal nerve cells are formed continuously provides one mechanism by which learning, nutrition, and other influences may alter intelligence.

A difficult-to-explain aspect of the brain is the existence of rare **savants**, persons with amazing mental abilities in music, art, or computation but who are unable to communicate (autistic) and mentally retarded.^{1053–1055} One boy at age four could play Mozart piano sonatas flawlessly after a single hearing. A three-year old girl drew horses with lifelike perspective from memory but was unable to communicate. Some mathematical savants can instantly state the day of the week for any arbitrary date such as June 12, 1929; others rapidly identify prime numbers. They evidently use the same strategies as mathematically trained persons. Do we all have these abilities but can't have access to them? How can we explain the fact that rarely a blow to the head will convert a person into a savant?

Behavior. It may seem impossible to interpret complex behavioral patterns at the molecular level. However, the genetics of behavior is a well recognized field of investigation, and some behavioral traits have been linked to single genes. If a gene can be located cloning, sequencing, and biochemical studies may follow quickly. The behavioral genetics of lower organisms, e.g., of *Drosophila*, have provided many insights.¹⁰⁵⁶ Recently, however, the mouse has become a major object of behavioral studies.¹⁰⁵⁷ Its genome is well known, and a very large number of mutations have been mapped. The ability to prepare **knockout mice** (p. 1501) and to carry out gene transfer experiments on such animals makes them very attractive for study.

Some behavioral traits are based on simple alterations, often defects, in motor skills. For example, the following traits in mutant mice have been traced to specific brain structures and often to specific biochemical alterations.

<i>Staggerer</i>	Purkinje cell defect
<i>Vibrator</i>	Phosphatidylinositol transfer protein gene
<i>Tottering</i>	Mutation in voltage-gated Ca^{2+} channel
<i>Lurcher</i>	Abnormality in cerebellum
<i>Weaver</i>	Gly \rightarrow Ser mutation in K^+ channel

Knockout mice lacking oxytocin or vasopressin have altered social behavior toward other mice. Those lacking galanin seem less intelligent than normal mice, as if they had Alzheimer disease. Mice lacking neuronal NO synthase became aggressive.¹⁰⁵⁷ Human personality,¹⁰⁵⁸ language abilities,¹⁰⁵⁹ and sexual behavior all have a genetic component. However, claims that a "gay gene" has been found are not generally accepted.¹⁰⁶⁰

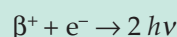
13. Circadian Cycles and Sleep

In mammals an approximately 24-h (**circadian**) rhythm controls behavior and affects many physiological functions. As previously mentioned (p. 1800), the brain has its own rhythms, which originate with pacemaker neurons. The heart beats with another neurally established rhythm. The circadian rhythm has a much longer period and, therefore, seems more mysterious. It is observable, even with single cells and for virtually all organisms.^{1061–1062a} In most instances the cycle becomes synchronized with the daily light–dark cycle with the aid of suitable light-absorbing pigments often cryptochromes (see Chapter 23, Section I,1). However, the cycle can be observed in various ways under conditions of constant light intensity and temperature. For example, the unicellular marine alga *Gonyaulax* undergoes dramatic circadian changes in the intensity of its bioluminescence. Over one 10-day period the luminescence peaked every 22.99 ± 0.01 hours.^{1063,1064} It is more difficult to measure the period for human beings (see Czeisler *et al.*¹⁰⁶⁵ for a discussion), but under suitable conditions during which time cues were missing a precise period of 24.18 hours was observed for the level of melatonin in the blood, the body temperature, and other quantities.¹⁰⁶⁵ From cyanobacteria,^{1066,1067} fungi (*Neurospora*),¹⁰⁶⁸ insects (*Drosophila*),^{1069–1071b} and frogs¹⁰⁷² to mice and people,^{1073,1074} the circadian cycle affects the organism's chemistry and behavior. Green plants likewise observe a circadian cycle.¹⁰⁷⁵

The cycle is thought to originate in feedback loops that control transcription of a small set of genes. In *Drosophila* the set includes seven genes: *period* (*per*), *timeless* (*tim*), *clock* (*clk*), *cycle* (*cyc*), *double-time* (*dbt*), *vri*, and *cryptochrome* (*cry*).¹⁰⁷⁰ Many corresponding genes have been found in mammals. For example, the mouse NPAS2 is a close relative of the *Drosophila* CLOCK protein, and the period proteins PER1 and PER2 and the cryptochromes CRY1 and CRY2 are also related to the *Drosophila* proteins.^{1076,1077} In *Drosophila* the heterodimers PER•TIM and CYC•CLK are thought to serve as DNA-binding transcription factors that repress transcription of their own genes when they reach a high enough concentration in the nucleus.^{1073,1076,1077} Because some time is required for transcription and

BOX 30-A POSITRON EMISSION TOMOGRAPHY (PET), FUNCTIONAL MAGNETIC RESONANCE (FMRI), AND OTHER IMAGING TECHNIQUES

In the widely used technique of transmission computerized tomography (CT) an image of a slice through the body of a patient is obtained using X-rays. An X-ray source moves in a ring around the patient while detectors measure the intensity of the transmitted radiation and send it in digital form to a computer, which generates the desired image. A chemically more sophisticated view of the body can be obtained by positron emission tomography (PET). This technique makes use of a metabolite or drug labeled with a short-lived radioisotope that decays by emission of positrons (antielectrons). Among these are ^{11}C , ^{13}N , ^{15}O , and ^{18}F with half-lives of 20 min, 10 min, 2 min, and 110 min, respectively. The isotopes are produced in a cyclotron, and are rapidly introduced into suitable compounds, which can be injected into a bloodstream.^{a-d} An emitted positron travels only a few millimeters before undergoing annihilation with an electron to produce two high-energy (50 keV) photons (γ -rays) that travel in opposite directions and are detected by an array of scintillation detectors.



Present-day PET technology allows images to

be formed in a few seconds, and in some cases in a fraction of a second. Among the useful compounds for PET imaging is [^{18}F]2-fluoro-2-deoxy-D-glucose (see figure). This compound, which contains the longer-lived ^{18}F , is phosphorylated by hexokinase, and the resulting phosphate ester is effectively trapped in the brain. 3-Deoxy-3-fluoro-D-glucose is another useful tracer.^e One of the most useful PET measurements has been blood-flow monitored by ^{15}O -containing H_2O , which is administered into a vein in the arm.^b The ^{15}O has a half-life of only two minutes and is almost completely gone in ten minutes. However, very low doses of radioactivity are used, and several images can be obtained before the radioactivity has decayed. A common practice is to subtract images obtained after the isotope has decayed from those obtained at various times while it was still present. The technique is also useful for study of the binding and transport of hormones,^f other metabolites, drugs, and other inhibitors.^c

The NMR technique **magnetic resonance imaging (MRI)**,^{g-i} so called to avoid the word nuclear, is rapidly displacing many applications of PET scanning. MRI uses proton NMR spectroscopy to generate very sharp images based largely on the water present in tissues. These images can be made



Left: PET image of a human brain obtained using 2-[^{18}F]fluoro-2-deoxyglucose. This tomographic brain slice at the level of the basal ganglia shows the cortical gray matter and subcortical white matter. As marked on the drawing on the right: V, ventricles; CN, caudate nucleus; P, putamen; T, thalamus; PI, pineal gland; CER, cerebellum. From Rottenberg and Cooper.^p Right: fMRI image illustrating modulation of neural activity in the ventral striatum, an area of the brain associated with reward, when eye contact was made with an attractive face. The activation map shown is derived in a complex manner and is based on recorded brain activity of persons viewing images of a series of faces. It portrays the differences in neural activity when viewing images of attractive faces of either sex with the eye gaze directed at the subject and with the eyes averted. See the report of Kampe *et al.* for details.^q

BOX 30-A (continued)

to depend upon variations in T_1 and T_2 (Chapter 3) as well as upon differences in the water content. The first MRI scans required 20 minutes, but the use of more powerful magnets and more sensitive instruments has reduced the acquisition times in ultrafast MRI to ~ 0.1 s. The decay of the NMR signal from a single RF pulse is observed at several different times.^h The dynamics of blood flow and neural activity can be followed. Every technique has disadvantages as well as advantages. MRI does not use radioisotopes, but overheating of the brain must be carefully avoided. In addition, patients may suffer from uncomfortably loud noises generated by rapidly changing magnetic gradients.^h As with PET scans isotopic tracers may be used. However, most MRI scanning is done with ^1H from the solvent water. As with PET, MRI is often used to measure blood flow but with an indirect method. The Fe of deoxyhemoglobin (Hb) is paramagnetic, but upon oxygenation to HbO_2 it becomes diamagnetic (pp. 850–851), and the ^1H signal of the solvent H_2O becomes sharper. In metabolically active regions of the brain the demand for oxygenated blood is greatly increased. Perhaps surprisingly, the ratio $[\text{HbO}_2]/[\text{Hb}]$ is greater in these areas than in less active areas where a greater fraction of the hemoglobin remains unoxygenated.^g However, the exact interpretation of the ultrafast MRI images is uncertain. In **functional MRI (fMRI)**, differences in images acquired after some physiological change are recorded. For example, after a visual or other sensory stimulus a change in the MRI image of some region of the cortex will be observed (see figure).^{i–l} The technique is allowing many deductions about learning, memory, and communication pathways in the brain^{j,m} and is being used to investigate many aspects of brain disease.

Another brain imaging technique is **magnetoencephalography (MEG)**.^{c,n} It has been uniquely valuable in mapping the sensory regions of the human cerebral cortex. Looking ahead, optical methods, which include use of infrared radiation, are also under development.^o They may not be adequate for study of the human brain but can be used for smaller animals, for studies of embryonic development, etc.

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- ^q Kampe, K. K. W., Frith, C. D., Dolan, R. J., and Frith, U. (2001) *Nature (London)* **413**, 589

protein synthesis, this inhibitory feedback can lead to oscillations in the concentrations of the circadian clock proteins. Proteosomal degradation of the TIM protein may also be a factor.¹⁰⁷¹ The need for proteins encoded by other genes indicates that the matter is more complex. Individual cells or individual tissues, e.g., mammalian retinas,^{1074,1078} may independently set up circadian cycles. However, these normally become **entrained** by the daylight cycle and are reset daily as discussed in Chapter 23, Section I,1. Other factors such as temperature, activity, and food may also affect the resetting. One factor, which may be influenced by food, is the NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ ratios within cells.¹⁰⁷⁷ The circadian cycles for mam-

malian tissues are synchronized by a **master clock** that originates in neural tissues^{1062a} and specifically in a region of the hypothalamus containing the **suprachiasmatic nuclei**.^{1079–1080b}

The pineal gland appears also to play a role in maintaining the mammalian circadian cycle.^{1081–1083} The concentration of the pineal hormone melatonin (Fig. 27-11) as well as its precursor *N*-acetylserotonin and the enzyme serotonin *N*-acetyltransferase (Eq. 30-4) all fluctuate far more than do the concentrations of other metabolites during the 24-h cycle. These metabolites increase over 10-fold concentration at night and decrease by day. During the daytime the serotonin *N*-acetyltransferase, which forms the precursor, is rapidly

and apparently irreversibly inactivated, perhaps through a disulfide exchange reaction.¹⁰⁸¹ Bright light will reset the circadian cycle¹⁰⁸⁴ keeping it approximately (circa) daily. The effect of light is apparently a result of signals sent to the hypothalamus from the optic nerves. In chickens and in lower vertebrates the pineal gland may directly sense light passing through the skull.

The circadian cycle is not the only timing device used by animals. A short-term interval timer helps male doves to know how long to sit on a nest¹⁰⁸⁵ and helps all of us in timing everyday tasks.

We spend a third of our lives asleep, but our understanding of sleep from a molecular viewpoint is minimal. Sleep is essential for the life of mammals, which die if completely deprived of sleep. It has been shown that during prolonged sleep deprivation sleep-inducing material accumulates in the brain. One such substance, isolated from human urine, appears to be a peptide containing glutamate, alanine, diaminopimelic acid, and muramic acid.¹⁰⁸⁶ Thus, it resembles a fragment of bacterial peptidoglycan. Prostaglandin D₂ also induces sleep.¹⁰⁸⁷ Hayashi proposed that a balance between this substance and prostaglandin E₂, which induces wakefulness, is in part responsible for the sleep–wake cycle.¹⁰⁸⁷ More recently oleic acid amide (**oleamide**; p. 382) was identified as a sleep-inducing compound. A fact observed by everyone is that the longer one is awake the higher the probability of going to sleep. The accumulation of sleep inducers is part of a homeostatic mechanism. On the other hand, the circadian cycle probably provides the signal to awake and tends to consolidate our sleep into the characteristic 8-hour period.¹⁰⁸⁸ The melatonin level, which drops in daylight, plays a role.¹⁰⁸⁹ Release of adrenocorticotropin (ACTH) one hour before waking may also be important.¹⁰⁹⁰

During much of the night's sleep the EEG is characterized by the slow 5- to 6-Hz waves.^{989,993} However, after ~90 min there is an ~10-min period of **rapid eye movement (REM) sleep** during which the EEG resembles that of an awake person and dreaming occurs. The closed eyes move rapidly in unison, breathing is irregular, and the heart rate increases. Motor neurons are inhibited allowing only minimal body movement. Three more periods of slow-wave sleep, each shorter than the preceding one, are followed by REM sleep. The REM sleep periods become successively longer. The fourth period lasts 20–30 min and is followed by awakening. All placental and marsupial mammals follow a similar sleep pattern and all dream.⁹⁸⁹ The importance of dreaming is not obvious^{1088a} but is often thought of as a reprocessing of memory, a means of ridding the mind of unneeded memories, a process of **unlearning**. However, this is uncertain as is the relationship of sleep to learning and memory.^{1088a,b}

A number of disorders of sleep are known. Among these is **narcolepsy**, uncontrollable, sudden daytime sleepiness. It affects 1 in 2000 individuals.¹⁰⁸⁸ The same occurs in dogs.¹⁰⁹¹ After a 10-year effort at great expense the narcolepsy gene of dogs (*canarc-1*) was located by positional cloning.^{1088,1092} The corresponding human (and rat) gene was independently discovered by other investigators. It encodes a receptor for neuropeptides produced by the hypothalamus and named **hypocretins** or **orexins** for their stimulation of appetite. It seems probable that the hypocretin / orexin neuropeptides are involved in promoting wakefulness. Another sleep disorder is **familial advanced sleep phase syndrome**. Persons with this trait are “morning larks” who tend to fall asleep at ~7:30 p.m. and awake suddenly at ~4:30 a.m., about four hours in advance of a typical sleep period. A missense Ser → Gly mutation in the human period gene (*hper2*) has been found.¹⁰⁹³

Some mammals hibernate. Special blood proteins that induce hibernation apparently control the process.¹⁰⁹⁴

14. Mental Illness

Whereas many metabolic defects affect only a small number of individuals, emotional illnesses including depression, **schizophrenia**, and other **affective disorders** at one time or another afflict a large fraction of the population. Autism affects thousands of children.¹⁰⁵⁵ Parkinson disease and **Alzheimer disease** are just two of a number of degenerative neural diseases attacking older people. Less commonly, young persons contract **multiple sclerosis** and **muscular dystrophy**, which is often a disease of neuromuscular junctions.

Depression. Depression is our most common mental problem. One in four women and one in ten men will have a major depression during their lifetime.¹⁰⁹⁵ More than 15 million people in the United States are affected by severe depression in any given year and more than 30,000 may commit suicide.^{1096,1097} Worldwide psychiatric problems, mostly depression, account for 28% of all disabilities.¹⁰⁹⁸ The **biogenic amine hypothesis** states that depression results from the depletion of neurotransmitters in the areas of the brain involved in sleep, arousal, appetite, sex drive, and psychomotor activity. An excess of transmitters is proposed to give rise to the manic phase of the bipolar (manic–depressive) cycle that is sometimes observed. In support of this hypothesis is the observation that administration of reserpine precipitates depression, which may be serious in 15–20% of hypertensive patients receiving the drug. Similar effects are observed with the dopa decarboxylase inhibitor **α-methyl dopa**

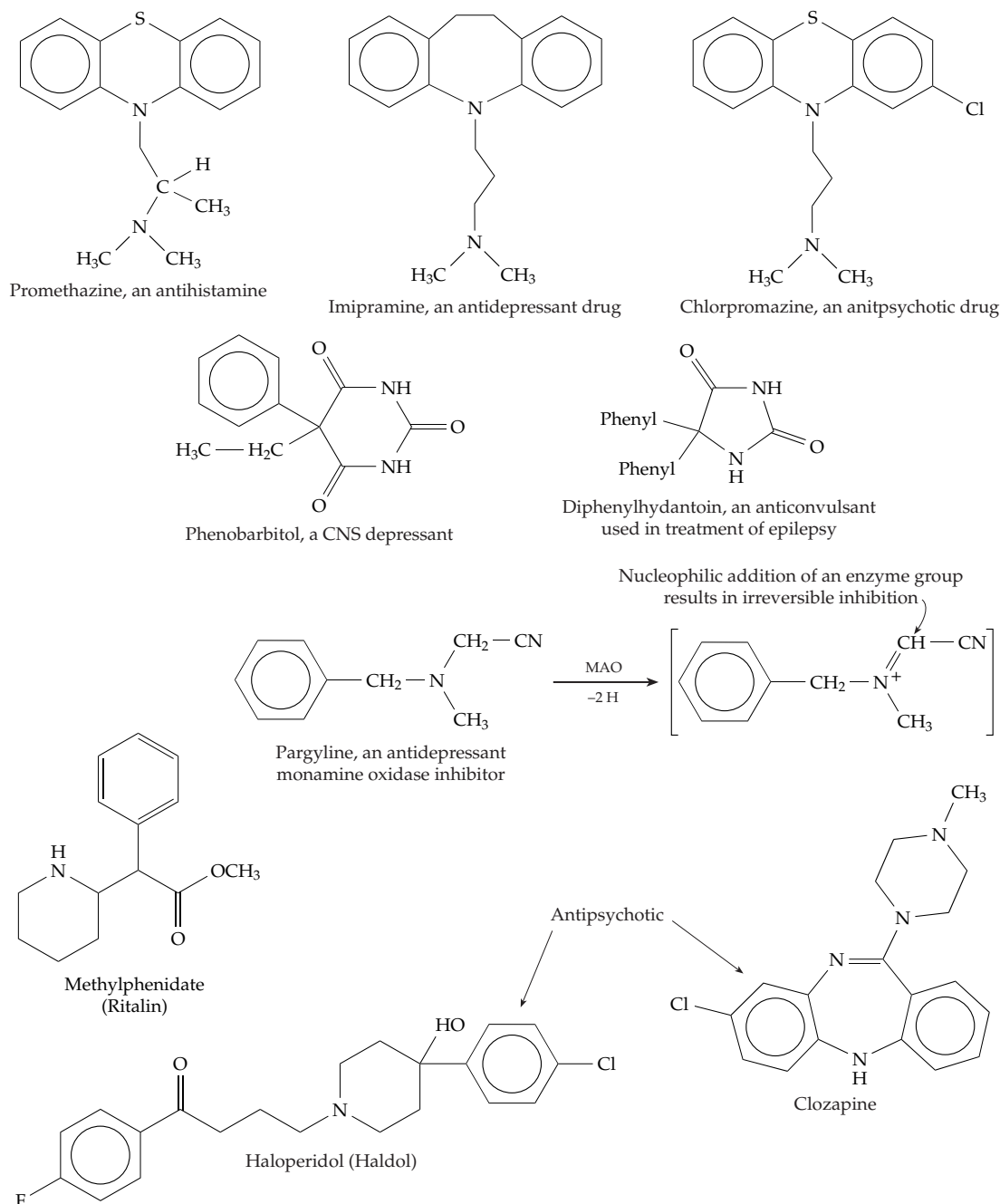


Figure 30-33 Some drugs used to treat psychiatric disorders. See also Figs. 30-25 and 30-28.

(Fig. 30-27). The fact that L-tryptophan has some antidepressant activity, but L-dopa does not, was one clue that a low concentration of serotonin (5-hydroxytryptamine) might be responsible for depression. Excessive formation of histamine¹⁰⁹⁹ and decreased formation of tyramine and octopamine¹¹⁰⁰ have also been suggested as causes of depression.

Strong support for the biogenic amine theory of depression is provided by the powerful antidepressant effect of inhibitors of monoamine oxidase. An example is **pargyline** (Fig. 30-33), which forms a covalent

adduct with the flavin of MAO.¹¹⁰¹ Although effective, this drug is somewhat dangerous. Because their monoamine oxidase activity is so low, patients taking pargyline have been killed by ingesting compounds such as tyramine, which occurs in cheese. Less easy to understand but clinically more important are tricyclic antidepressants such as **imipramine** (Fig. 30-33),^{746a} whose antidepressant action was discovered accidentally. Notice the close similarity to chlorpromazine but the greater flexibility of the central ring.¹¹⁰² Imipramine was found to block transporters of both

noradrenaline and serotonin. In 1986, the less toxic serotonin reuptake inhibitor fluoxetine (Prozac; Fig. 30-28) was introduced and is now used by many millions of people.^{1103,1104} Nevertheless, its mode of action is not entirely clear. For example, it blocks nicotinic acetylcholine receptors and may have many other effects. Interestingly, depression sometimes responds to a placebo just as well as to an antidepressant drug.¹¹⁰⁵ In addition to newer drugs related to Prozac, antagonists of substance P are also effective antidepressants.¹¹⁰⁶ MRI images of brains of depressed patients show that hippocampal volume has decreased and suggest that formation of new neurons is inhibited.^{1106a} Antidepressants seem to stimulate growth of new cells as does exercise, which also has an antidepressant effect.¹¹⁰⁷ Dietary treatment can also help.⁸⁴³ Among older people depression may be caused by deficiency of vitamin B₁₂ and can be treated by injection of the vitamin.¹¹⁰⁸ An **anxiety peptide** that may be the natural ligand for benzodiazepine receptors has been reported.¹¹⁰⁹

Another recognized type of depression is **seasonal affective disorder (SAD)**. People in far northern or southern latitudes develop this condition in the winter, apparently from lack of sunshine needed to lower the melatonin level in the morning (see Section 13). Light therapy is beneficial.¹¹¹⁰ Persons with the SAD syndrome also tend to crave carbohydrates and to stay in bed for 9–10 hours.

An effective treatment for **bipolar disorder** (manic–depressive illness) is the administration of lithium salts.^{445,1111–1113a} Inhibition of the hydrolysis of inositol phosphate by Li⁺ (Fig. 11-9) may be related to its therapeutic effect. Reduced phosphatidylinositol turnover may dampen responses to neurotransmitters.¹¹¹⁴ Li⁺ may affect gene expression in neuropeptide-secreting neurons.¹¹¹⁵ Bipolar disorder apparently has more than one cause. There are strong indications of genetic susceptibility,¹¹¹⁶ and genes that increase susceptibility have been located on chromosomes 4, 12, 13, 18, 21, and X.¹¹¹⁷

Schizophrenia. Among the most baffling of mental illnesses are the group of diseases known as schizophrenia. They involve thought disorder, disturbance of the affect, and withdrawal from interactions with other people. Hallucinations and paranoid feelings are common.⁸¹³ In some cases a striking loss of gray matter in some areas of the brain is revealed by MRI scans.^{1117a,b} The schizophrenias are of varying degrees of severity and shade continuously into the affective or mood disorders, which include manic-depression and depression. As many as one person in a hundred is affected by schizophrenia.^{1118–1119a} There is a complex genetic susceptibility.^{1119,1120} One theory about the persistence of the genes favoring schizophrenia is that they are also associated with creativity.¹¹²¹

A revolution in the treatment of the schizophrenias, as well as in thinking about mental illnesses, took place following the synthesis, in 1950, of the antipsychotic drug **chlorpromazine** (Fig. 30-33). At about the same time the effect of the *Rauwolfia* alkaloid reserpine (Fig. 25-12) in calming mentally disturbed persons was rediscovered. The Indian plant *Rauwolfia* had been used for centuries in Hindu medicine for the same purpose. The tricyclic phenothiazines such as promethazine (Fig. 30-33) earlier had been found to have powerful **antihistamine** activity. It was the search for better antihistamine drugs that led to the synthesis of chlorpromazine.¹¹²² As many as 250 million people throughout the world were treated with chlorpromazine and related drugs in the 20 years following its discovery before newer and safer drugs (e.g., **clozapine**; Fig. 30-33) were developed.¹¹²³ What does chlorpromazine do? A possible clue comes from the fact that it sometimes induces serious “extrapyramidal” side effects including tremors and other symptoms of Parkinson disease. This suggested that chlorpromazine may block dopamine receptors in the corpus striatum, thereby precipitating a functional deficiency of dopamine.¹¹²⁴ If so, it is possible that schizophrenia may result from an overactivity of dopamine neurons, perhaps including some of the same neurons that are hypoactive in Parkinson disease. Supporting this view is the observation that amphetamines (Fig. 30-27), which may substitute for dopamine, worsen the symptoms of schizophrenia and in very high doses induce striking schizophrenialike symptoms in normal individuals.

A stereotyped compulsive behavior is induced both in humans and in laboratory animals by amphetamines. This provided the basis for a method that has been used to measure the action of drugs on amphetamine-sensitive centers of the brain. A lesion in the nigrostriatal bundle on one side of a rat brain was made by injection of a neurotoxic compound such as 6-hydroxydopamine. This caused degeneration of dopamine-containing neurons on one side of the brain. When rats that had been injured in this way were given amphetamines, they developed a compulsive rotational behavior. Administration of chlorpromazine and several other antipsychotic drugs neutralized this behavior and in direct proportion to the efficacy in clinical use, an observation that also supports the theory that schizophrenia involves overactivity of dopamine neurons.

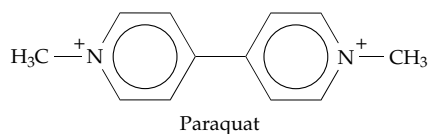
If schizophrenia results from an elevated dopamine content of the brain, the fault may lie with either an oversupply or a reduced rate of metabolism of dopamine. The possibilities of reduced activity of monoamine oxidase or of dopamine β-hydroxylase have both been suggested. The plasma level of the dopamine metabolite **homovanillic acid** (Fig. 30-26) is elevated in schizophrenia and is correlated with the

severity of the illness,¹¹²⁵ suggesting the hypothesis of a decreased rate of metabolism. Possible defects in dopamine receptors may be at fault.¹¹²⁶

Chlorpromazine may also act on brain cholinergic neurons.¹¹²⁷ Blockage of muscarinic acetylcholine receptors in the brain by belladonna alkaloids such as atropine (Fig. 30-22) has often been used in treatment of Parkinson disease. Apparently antagonizing acetylcholine action is to some extent functionally equivalent to increasing dopamine concentrations. There is evidence that suggests a role for cholecystikinin (CCK) in development of schizophrenia. CCK-containing neurons interact with dopaminergic neurons in the mid-brain.¹¹²⁸ GABA neurons in the prefrontal cortex may be faulty.¹¹²⁹ Excessive glutamate may also induce schizophrenia. The schizophrenia-like symptoms induced by phenylcyclidine (p. 1796) are eased by antagonists of metabotropic glutamate receptors. This suggests another possible therapy.¹¹³⁰ Among other suggested causes of schizophrenia are dysregulation by **retinoids**¹¹³¹ and action of retroviruses.¹¹³² Demyelination in portions of the prefrontal cortex may disrupt neural connectivity.^{1132a} Recent genetic evidence points to a possible defect in proline dehydrogenase which reduces Δ^1 -pyrroline-5-carboxylate to L-proline (Fig. 24-9).^{1132b}

Numerous theories of mental illness have embodied proposals that a toxic metabolite is produced in abnormal quantities. An example is **6-hydroxy-dopamine** (Fig. 30-26), which is known to damage dopamine-containing neurons.¹¹³³ Overactive methylation of catecholamines has also been suggested as a cause of mental disorders.¹¹³⁴ The hallucinogen **3,4-dimethoxyphenylethylamine** (Fig. 30-26) has been identified in urine during acute schizophrenic attacks, but the variability is so high that no definite conclusion has been reached. N-Methylation of serotonin yields **bufotenin** (N-methylserotonin) and **N-dimethylserotonin**, known hallucinogenic agents. Enzymatic synthesis of the latter by human brain and other tissues has been demonstrated,¹¹³⁵ and administration of tryptophan and methionine to schizophrenic patients exacerbates their illness.

Another theory of mental illness postulates endogenous alkaloid formation. Aldehydes formed by oxidation of catecholamines as well as formaldehyde and acetaldehyde are present in tissues in small amounts. Condensation with amines could generate Schiff bases and alkaloids as in Fig. 25-10. This "plant chemistry" is spontaneous and can apparently take place in the brain, where it may have a potent effect.



Incubation of tryptamine derivatives with 5-methyl-tetrahydrofolic acid and an enzyme preparation from brain gives **tryptolines**. Dopamine and its derivatives form related tetrahydroisoquinolines such as the product that arises from reaction with acetaldehyde (see Eq. 30-5). This product has been found in elevated amounts in alcoholics (who synthesize excess acetaldehyde), in phenylketonurics, and in L-dopa-treated patients with Parkinson disease.¹¹³⁶

Epilepsy. The brain disorders known as epilepsies affect 1–2 % of the population worldwide. Characteristic of epilepsies are recurrent **seizures**, sudden brief changes in behavior caused by the simultaneous, disordered firing of large numbers of neurons in the brain. Many seizures are thought to be initiated in specific areas of the cerebral cortex. For example, seizure-induced firing of neurons in the thumb area of the right motor cortex will be accompanied by rhythmic jerking in the left thumb. More than 40 different types of epilepsy are known.^{1137–1138a}

GABA is the principal inhibitor neurotransmitter, and one cause of epilepsy may be a deficiency in GABA formation from glutamate. The brain contains two isoforms of glutamate decarboxylase, designated GAD65 and GAD67, in accordance with their molecular masses in kDa. They are encoded by separate genes.^{1139,1140} GAD67 is formed mainly in cell bodies of neurons, binds its cofactor PLP tightly, and is essential to survival of young mice. GAD65 is associated mainly with nerve termini, where it is anchored, apparently by association with other proteins to the membranes of synaptic vesicles.¹¹⁴¹ It binds PLP weakly. Some convulsive agents such as 1,1-dimethylhydrazine are thought to act by interfering with PLP-dependent enzymes (Box 14-C) among which is GAD.^{1140,1142,1143} Convulsions are one of the most striking symptoms of a severe vitamin B₆ deficiency. A zinc deficiency can also cause convulsions, apparently because pyridoxine kinase is a Zn•ATP-requiring protein and the rate of synthesis of PLP is too slow to supply apo-GAD with the PLP needed for GABA synthesis. The PLP in GAD65 undergoes rapid substrate-dependent transamination to pyridoxamine phosphate (see Chapter 14), which must be replaced by new PLP.^{1143,1144}

Epilepsy may arise also from defects in a GABA transporter¹¹⁴⁵ or receptor.¹¹⁴⁶ One form of epilepsy is a triple-repeat disease of cystatin B (Table 26-4). Mutation in potassium channels,¹¹⁴⁷ glutamate receptors,¹¹⁴⁸ absence of neuropeptide Y,¹¹⁴⁹ and absence of L-isoaspartyl / D-aspartyl O-methyltransferase (Box 12-A)¹¹⁵⁰ have all been associated with epilepsy.

Neurodegenerative diseases. As many as 5% of persons of age 65 and 20% of those of age 80 are afflicted with the progressive senile dementia known

as **Alzheimer disease**. The condition is characterized by a gradual loss of memory and of the abilities to speak, think, or take care of one's self. Histologically Alzheimer disease is marked by the accumulation within neurons of **paired helical filaments**. These filaments, of 10 nm diameter, twist about each other to form a helix with an 80-nm pitch. The helices aggregate to create **neurofibrillary tangles**. The tangles are composed largely of a highly phosphorylated form of the microtubule-associated protein **tau** (p. 372)^{1151–1155a,b} together with phosphorylated neurofilaments, apolipoprotein E (p. 1183), and other materials. The tangles are found in the cell bodies, axons, and dendrites of neurons in the hippocampus, amygdala, cerebral cortex, and other areas of the brain. Tangles may also be present in Parkinson disease, in the nearly extinct **Guam disease**,^{1156,1157} and in some types of prion disease.¹¹⁵⁸ Outside of the diseased neurons are numerous, spherical **amyloid plaques**. Their principal component is a 40- to 43-residue fragment called **amyloid β -protein (A β)**, which appears to be toxic to neurons. A β is cut from a larger **amyloid precursor protein (APP)**.^{1154,1159–1160a} The APP gene is a member of a family of 16 related genes found in many organisms including nematodes, flies, and mammals. In humans the APP gene is found on chromosome 21, the chromosome that is present in three copies in **Down syndrome**. People with Down syndrome who live into their late thirties or beyond develop Alzheimer disease,¹¹⁵³ presumably from excessive synthesis of APP. Both APP and its cleavage product A β are formed by nonneuronal cells throughout the body. However, the A β plaques form only in the brain, and the APP gene is essential for life. The rare **familial British dementia** resembles Alzheimer disease in producing amyloid plaques and neurofibrillary tangles. They appear principally in the cerebellum and arise from a different precursor protein.^{1161,1162}

There are many other neurodegenerative diseases, some with a high incidence, and others rare. They include **Parkinson disease** (p. 1790), **Huntington disease** (Table 26-4), **spinal muscular atrophy (SMA)**; a leading hereditary cause of infant mortality),^{1162a,b} amyotrophic lateral sclerosis (**ALS**), prion diseases (Box 29-E), **ataxias**, and other diseases caused by triple-repeat DNA sequences (Table 26-4) and X-linked adrenoleukodystrophy (ALD; p. 945).¹¹⁶³ In the last, membrane function is disrupted. Although these diseases arise from a variety of causes many of them have in common amyloidosis, the deposition of insoluble proteins in or around neurons.^{1163a}

Parkinson disease, some cases of Alzheimer disease, and some types of prion disease are accompanied by the presence of **Lewy bodies** within the cytoplasm of neurons and also in nearby glia. These deposits consist largely of a dense core of fibrils of **α -synuclein**, a small 140-residue protein abundant in various parts

of the brain.^{1164,1164a,b} Mutations in the α -synuclein gene are associated with autosomal-dominant inheritance of early-onset Parkinson disease.^{1165,1166} Just as tau tends to be associated with microtubules, α -synuclein may function in cooperation with microfilaments.¹¹⁵⁸ Studies of an autosomal-recessive form of inherited juvenile Parkinson disease led to mutations in a large (>1 Mbp) gene on chromosome 6. It encodes the 465-residue **Parkin**.^{1167,1168} Parkin is an E3 ubiquitin ligase (p. 524), which ubiquitinates α -synuclein.^{1168,1169} This finding suggests that abnormally slow degradation of synuclein may be an important cause of Parkinson disease.

One of the triple-repeat polyglutamine diseases discussed in Chapter 26 (Table 26-4) is Huntington disease. The defective **Huntingtin** is a cytosolic protein that normally protects neurons but fails when the polyglutamine sequence becomes too long.^{1170,1171} Neurons of the cerebral cortex and striatum die, apparently by apoptosis.¹¹⁷² Huntingtin interacts with p53, with a CREB-binding protein, and with an EGF receptor suggesting that it functions in regulation of transcription.^{1172–1173a} One of the genes whose transcription is regulated is that of the neurotrophin known as **brain-derived neurotrophic factor (BDNF)**.¹¹⁷⁰

Many approaches have been taken in therapy of Parkinson disease. As mentioned on p. 1790 enhancing dopamine production by administration of L-dopa or by use of MAO inhibitors is a standard treatment. Experimental gene therapy with a glial cell line-derived neurotrophic factor also appears promising.^{1174,1175}

Another aspect of neurodegeneration involves oxidative damage. A clue comes from amyotrophic lateral sclerosis (**ALS**), which struck down the New York Yankees baseball player Lou Gehrig, after he had started 2130 consecutive games over a 15-year period. ALS (Lou Gehrig disease) is the most prevalent of more than 70 diseases that cause loss of motor neurons.¹¹⁷⁶ As pointed out on p. 1075, the cause of a rare hereditary form of ALS is a defect in superoxide dismutase, which appears to promote excessive formation of free radicals.¹¹⁷⁷ However, this interpretation is uncertain.¹¹⁷⁸ Parkinson disease induced by the compound MPTP (Eq. 30-4) may also arise as a result of free radical damage.¹¹⁷⁹ Among possible effects, MPTP may induce apoptosis.¹¹⁸⁰ Both oxidative damage and apoptosis may be factors in Alzheimer and other neurodegenerative diseases as well.^{1181,1182}

In every disease in which an abnormal protein is found there must be pathways of processing the protein to generate its functional form and pathways for degradation. These pathways are being investigated for all of the neurodegenerative diseases and none more intensively than for Alzheimer disease. The amyloid precursor protein APP is an integral

membrane glycoprotein with a large ~687-residue extracellular N-terminal portion, which resembles a cell surface receptor. It contains both a protease inhibitor-like domain and a zinc-binding region, which can be phosphorylated. It binds heparin and collagen as well as other proteins.^{1183,1184} Rare mutations that cause early-onset familial Alzheimer disease are found in the APP gene. Some of these mutations alter the regulation of pre-mRNA splicing. Splicing generates eight different APP isoforms varying in length from 677 to 770 residues. The properties of the isoforms vary. For example, if the 18 residues of exon 15 are spliced out a new motif for posttranslational modification is created by fusion of exons 14 and 16. The newly created sequence ENEGSG is recognized by a xylosyltransferase, which initiates formation of the terminal unit for glycosaminoglycan formation. The resulting proteoglycan is known as **appican** (p. 1154).¹¹⁸⁵ The precursor protein APP is transported down axons to the nerve endings and is proteolytically cleaved to form the insoluble amyloid deposits. Alzheimer disease may occur when there is excessively rapid proteolysis of the precursor or if there is a failure to metabolize the amyloid protein.¹¹⁸⁶ The folding and glycosylation reactions of APP occur in the ER and the Golgi, but the major problem in Alzheimer disease appears to be in subsequent proteolytic processing, some of which may occur as the APP is being transported through the Golgi to the cytoplasmic membrane. The protein may be cleaved at three sites by enzymes known as α -, β -, and γ -secretases as is indicated in Fig. 30-34, in which the protein is represented as an unfolded "stick."^{1154,1187-1189a} Most of the mutations in APP that cause Alzheimer disease are near these three cleavage sites. As indicated in Fig. 30-34, cutting at the α site liberates into the extracellular space the large N-terminal portion as a soluble protein called APPs α .^{1190,1191} It is thought to have a protective effect on neurons. If this cleavage occurs the protein is not cut at the β site and fragment A β is not formed. However, if cleavage by β -secretase (beta-site APP-cleaving

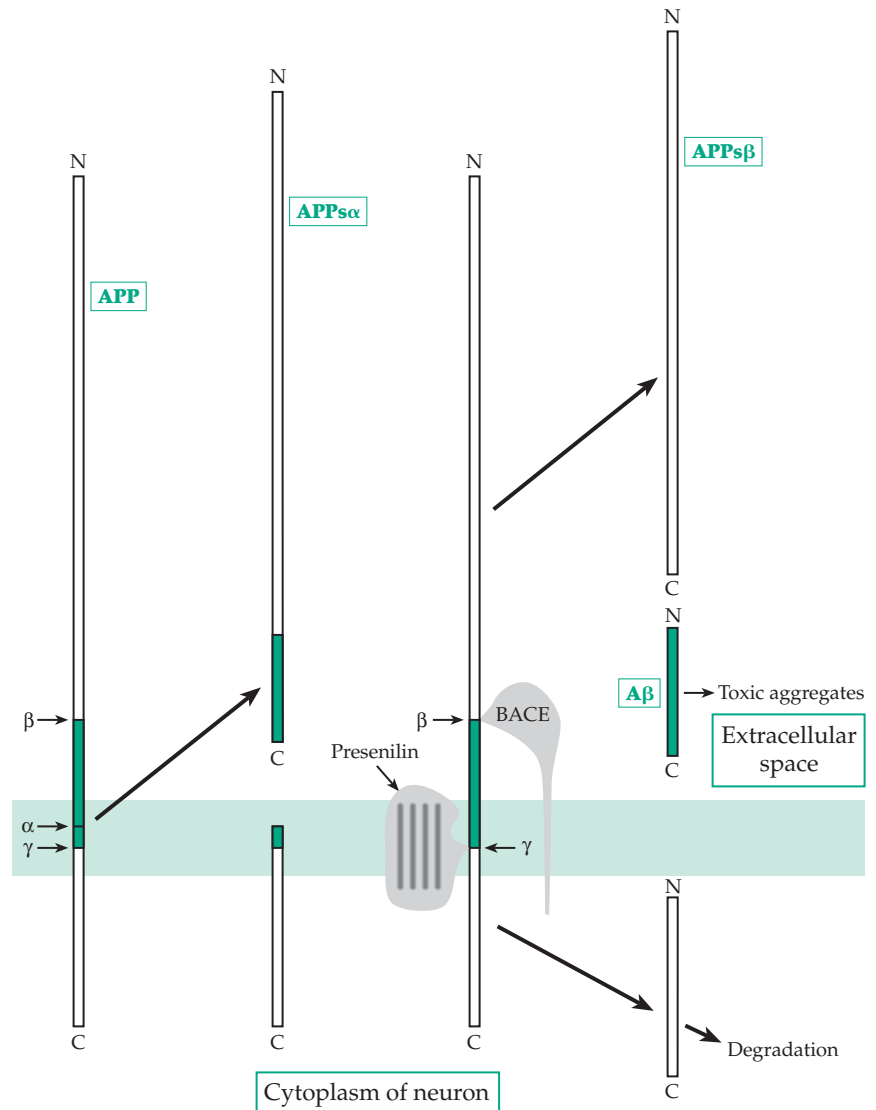


Figure 30-34 Cleavage of the amyloid precursor protein APP with liberation of amyloid A β protein. The proteins are represented as sticks (not to scale) but in reality contain both intracellular and extracellular globular domains.

enzyme or BACE) occurs first and is accompanied by or followed by cleavage at the γ -site, A β is liberated (Fig. 30-34). The β -secretase is an integral membrane protein, which carries a pepsinlike domain in its luminal (or extracellular) part.¹¹⁹²⁻¹¹⁹⁴ Since the A β peptide in an aggregated form appears to be toxic to neurons, a logical therapy for Alzheimer disease may be to block either the β - or γ -secretase.^{1195,1196}

The γ -secretase has been difficult to locate but has been identified as a result of other rare familial forms of Alzheimer disease. These are caused by mutations in genes for proteins known as **presenilin-1** (on chromosome 14) and **presenilin-2** (on chromosome 1).^{1188,1197,1198} The presenilins are integral membrane proteins with multiple transmembrane helices.

They have been regarded as regulators of γ -secretase, but there is much evidence that the presenilin molecules may be cleaved proteolytically and that the C- and N-terminal domains formed in this way may associate to form an unusual aspartyl protease. It, too, is a target for inhibitors.¹¹⁹⁸ The picture is made more complex by the fact that presenilins form complexes with other proteins. These include a newly discovered protein **nicastatin**,^{1199–1200a} a large 709-residue trans-membrane glycoprotein. Nicastrin not only seems to modulate presenilin action but also participates in an important developmental process via the highly conserved **Notch pathway** (Chapter 32).¹²⁰⁰ Many other proteins are found in the amyloid plaques of Alzheimer disease. Among them are acetylcholinesterase,¹²⁰¹ proteoglycans,¹²⁰² hydroxyacyl-CoA dehydrogenase,¹²⁰³ GM1 ganglioside,¹²⁰⁴ apolipoprotein A-1,¹¹⁸⁷ and lithostatine.¹²⁰⁵

What are the possible adverse consequences of accumulation of the A β protein? It may cause inflammation by activation of **microglia**,¹¹⁵⁷ which may cause damage by release of NO.¹²⁰⁶ A β may induce death of neurons by apoptosis.^{1201,1207–1209} A defect in proteosomal degradation may be a factor.¹²⁰⁸ Both A β and the prion protein may promote oxidative damage. The brain derives most of its energy from oxidative metabolism, a major source of damaging radicals. Mitochondria are found in dendrites as well as cell bodies.¹²¹⁰ Methionine residues in glycine-rich parts of the A β and prion proteins are suspected as centers of free radical formation.^{1202,1211}

Both amyloid plaques and the tangles of protein tau-containing paired helical filaments are typically present in Alzheimer disease. Which comes first? Some hereditary neurodegenerative diseases are known in which tau filaments are present in neurons and sometimes also in glia.^{1203,1212,1213} Since mutations in tau don't lead to Alzheimer disease whereas mutations affecting APP do, it is often assumed that the primary defect in the disease is with APP and that accumulating A β induces the observed changes in tau. However, this is by no means certain. Six different isoforms of tau (the longest with 441 residues) are created by alternative splicing of the mRNA. During its normal functioning tau is phosphorylated and carries an average of 2–3 phospho groups. In Alzheimer disease the level of tau is greatly increased (4- to 8-fold) and the molecules carry 5–9 phospho groups.^{1204,1213a} It is this hyperphosphorylated tau that forms the paired helical filaments and tau tangles, which appear to clog the slender neurons.

What does tau do normally? Although it has been studied for many years, its exact functions are elusive. However, the role of the microtubules in axonal transport is well established. The tau isoforms may play a functional role in this process. The hyperphosphorylated tau of Alzheimer disease doesn't promote proper assembly of microtubules and may interfere with axonal transport of materials along the microtubules (see p. 1119).^{1214,1215} Alzheimer disease may reflect an imbalance between the phosphorylation and dephosphorylation processes. Another possible problem with tau may be slow isomerization of prolyl linkages because of a deficiency of a prolyl *cis-trans* isomerase (Box 9-F).¹²¹⁶

Until 1993 **apolipoprotein E** was best known for its central role in plasma lipoproteins and cholesterol transport (Fig. 21-1). However, one of the three common alleles of the apoE gene confers a significant risk of development of Alzheimer disease.^{1217,1218} A high blood cholesterol level is also correlated with increased risk.^{1219,1220} Membrane abnormalities in mitochondria have been associated with Alzheimer disease.¹²²¹ Also related to membranes and lipid metabolism, **vitamin E** appears to combat Alzheimer disease.^{843,1218}

Environmental and nutritional factors may also affect the development of Alzheimer disease and other mental illness. Aluminum frequently accumulates in the neurons containing neurofibrillary tangles.^{1206,1222} Copper and zinc ions can cause the amyloid A β to aggregate. However, Zn²⁺ may actually protect against neurotoxicity.¹²²³ The amino acid β -N-methylamino-L-alanine, a constituent of the toxic seeds of a type of palm (*Cycas circinalis* L.), may have induced both ALS and Guam disease, a condition resembling Parkinson disease, in a population in Guam that traditionally used these seeds as food.¹¹⁵⁷

Can neurodegenerative diseases be prevented or delayed? Much evidence suggests that the answer is yes. Rare early onset forms pose a special problem, but for most of us maintaining an active life style, using our minds, and choosing a good diet with adequate amounts of vitamins and essential ω 3 fatty acids (Box 21-B) may be very helpful.⁸⁴³ New methods of treatment are being tested. Antiinflammatory drugs are helpful,^{1218,1218a} and even vaccination against A β and other amyloid proteins appears possible.¹²²⁴ Is it possible that antibodies and phagocytic cells can clear the cobwebs from our brains?

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Study Questions

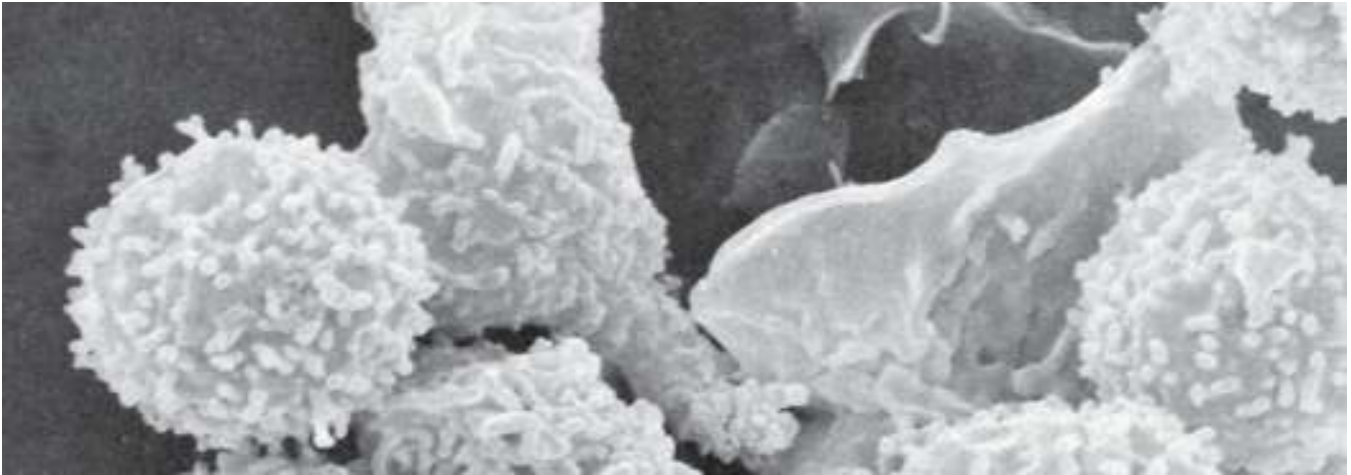
1. Compare the sensing of signals and responses to signals in liver, muscle, or other tissue with signaling in the brain.
2. Compare signaling by ionotropic receptors, metabotropic receptors and gap junctions.
3. List major neurotransmitters in the brain. In what other locations do these compounds function?
4. Compare addiction to gambling with addiction to cocaine. Are they similar on a biochemical basis? What about addiction to Internet games, chatting, pornography, compulsive overeating, etc.^{7873a}
5. Can extracts of leaves of *Ginkgo biloba* counteract age-related neurological disorders?¹²²⁵



The large flat cell, a portion of which is seen here, is a macrophage which has ingested bacterial proteins and is displaying peptide fragments on its surface. Some of the small, spherical T lymphocytes (T cells) seen here, interact with the macrophage, recognize an antigen, and respond by becoming helper T cells. They can then stimulate B lymphocytes (B cells) to multiply and produce antibodies. See Fig. 31-11 for an enlarged view. Scanning electron micrograph courtesy of Morton H. Nielsen and Ole Werdelin, University of Copenhagen.

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Our bodies are under constant attack by viruses, bacteria, protozoa, and metazoan parasites. Persons born without an immune system adequate to fight off these invaders die very quickly unless heroic measures are taken. We have learned to cooperate with our immune systems by immunizing ourselves against some bacteria and viruses. At other times we may fight a stubborn battle with our own defense systems against allergic reactions and a variety of autoimmune responses.¹

A. Locations and Organization of the Immune System

The immune system has many components, many of which are dispersed throughout the human body (Fig. 31-1). Lymphocytes, which are a foundation of the immune system, constitute little more than 1% of the body's mass. However, this represents $\sim 10^{12}$ cells of several types, about 10 times more than there are neurons in the brain. These 10^{12} cells make antibodies and T-cell receptors, both of which are thought to have $\sim 10^{15}$ different peptide sequences.² And this is only the beginning of the complexities.³⁻⁸

Immune responses have often been described in terms of **humoral** and **cellular** components. The humoral response involves the small circulating **B lymphocytes** (B cells), the **antibodies** (immunoglobulins), and proteins of the **complement** system. The cellular response is mediated by another group of small lymphocytes, the **T lymphocytes** (T cells). They resemble B cells in appearance but have quite different functions. However, newer knowledge has provided a somewhat different description of the body's defense

systems, which can be classified into three levels. (1) The skin and internal mucous membranes, which are resistant to infection and have antibacterial properties, provide the first level of defense.^{2,9} (2) A fast-acting **innate immune system** can respond within a few minutes to breaches in the barriers provided by the tough outer skin and the glycoproteins of mucous surfaces and provides a second level.^{2,10-14} (3) A slower **adaptive** (acquired) part of the immune system leads to synthesis of antibodies and to long-term immunity, providing the third level (Table 31-1). Both B and T lymphocytes together with **antigen-presenting cells** (**APCs**) are necessary for the selection and development of immunoglobulin structures appropriate for attack on an invading organism.

The innate immune system utilizes **phagocytic cells** including neutrophils, monocytes, and macrophages¹⁵ to ingest and kill invading organisms. Basophils, mast cells,¹⁶ eosinophils, and other cells release inflammatory mediators, which attract additional lymphocytes and affect their development.¹⁷ Specialized T lymphocytes called **natural killer (NK) cells**¹⁸ may also attack foreign cells (Table 31-2). The innate system is ancient and has apparently evolved to recognize molecular structures that are foreign to the host but are characteristic of pathogens. These structures, which are described as **pathogen-associated molecular patterns (PAMPs)**, include those of lipopolysaccharides of bacterial cell walls (Fig. 8-30), mannans (p. 175),¹⁹ other carbohydrates of surface layers,²⁰ oxidized phosphatidylcholines,^{20a} bacterial flagellins,²¹ various posttranscriptionally modified proteins, teichoic acids (p. 431), etc. However, they do not include patterns characteristic of host cells. Those have been avoided during evolution of the system. Although

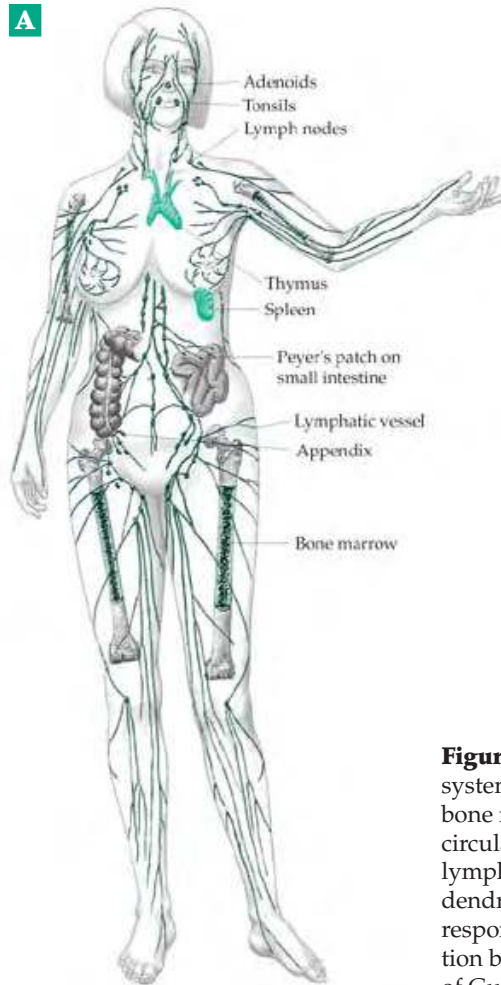
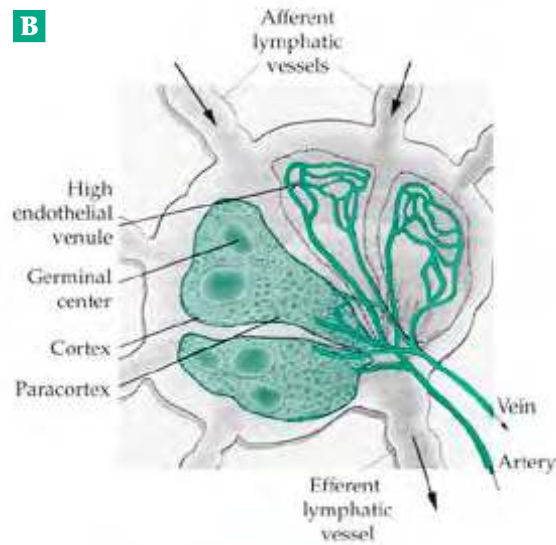
A**B**

Figure 31-1 (A) Locations of the primary and secondary tissues of the immune system. The primary lymphoid organs are the thymus, which makes T cells, and the bone marrow, which forms B cells. After moving from these organs into the blood circulation the cells reach one of the secondary lymphoid organs, which include lymph nodes, spleen, tonsils, and Peyer's patches on the small intestine. Immature dendritic cells are found in body tissues including skin and mucous membranes and respond to foreign proteins by inducing attack by T lymphocytes and antibody formation by B cells. (B) Schematic drawing of a lymph node. From Nossal.¹ Courtesy of Gustav J. V. Nossal.

T lymphocytes are major mediators of the innate immune response they are under control of the **dendritic cells (DCs)**, which are found in "immature" forms in tissues throughout the body.^{9,22–26a}

The immature DCs are phagocytic cells that act as "immunological sensors." They recognize various PAMPs, which act as **danger signals**,^{11b} using what are known as **toll-like receptors (TLRs)**.^{23,24,27,27a,b} They are also the most active APCs. Their proteasomes cleave proteins, both of the host and of invading organisms, into short peptides. These peptide fragments are displayed on the APC surfaces for recognition by T lymphocytes and for activation of adaptive immune responses. Some "autoreactive" B cells are also part of the innate immune system^{28,29} as are the IgA antibodies present in mucous membranes.³⁰ The innate immune system of insects resembles that of vertebrates. The toll-like receptors of the latter are named for their resemblance to the Toll receptors of *Drosophila*, which are utilized in resistance to fungi. In both mammals and insects the innate immune system activates responses via the transcription factor NF- κ B. However, many details of the signaling pathways differ.^{30a–c}

The innate system also provides for synthesis of small antibiotic peptides called **defensins**^{12,31–33} as well as larger proteins. Some of these proteins constitute the **complement system**, while others are described as **acute-phase reactants**. Some defensins are also **cytokines**, which attract lymphocytes.

The innate system is of special importance during early infancy. Prior to birth and for at least 4–12 months after birth a child's immune system is poorly developed. It may not become fully competent until age ~5.^{34,35} During the prenatal period maternal antibodies are transferred to the child. IgG crosses the placenta and enters the fetal circulation. Breast milk provides IgA, which remains largely in the child's gut, as well as other protective proteins. UNICEF and the World Health Organization recommend breast-feeding to two years or beyond.³⁴

While the innate immune system provides for immediate and direct attack on invaders, it also provides information to the slower adaptive system. Genes both for immunoglobulins and for the T-cell receptors of the adaptive system undergo extensive rearrangement during development of an individual.

TABLE 31-1
The Two Major Branches of the Immune System

	Innate (natural)	Adaptive (acquired)
Cells	Dendritic cells Phagocytic cells (neutrophils, monocytes, macrophages) Cells that release inflammatory mediators (basophils, mast cells, eosinophils) Natural killer (NK) cells	Dendritic cells B lymphocytes (B cells) T lymphocytes (T cells) Other antigen-presenting cells, e.g., macrophages
Molecular components	Antibacterial peptides (defensins, complement, acute-phase proteins)	Immunoglobulins
Receptor genes	Fixed in genome	Complement proteins Encoded in gene segments; rearrangement necessary
Recognition	Conserved molecular patterns	Small molecular groups (epitopes)
Immunogenic memory	Absent	Present
Self–nonself discrimination	Perfect	Imperfect
Action time	Immediate	Delayed

This provides potential defensive proteins directed at almost every imaginable invader. It also ensures that every individual has a set of proteins that labels its own cells as “self,” and that virtually every individual on earth has cell surface proteins different from those of every other person. In both the innate and adaptive responses the immune system must carefully distinguish “self” from “nonself.”^{36,37} In the innate system this discrimination developed during evolution of the host and its pathogens. In the adaptive system it depends upon interaction of the T cells with surface molecules, primarily those of the **major histocompatibility complex** (MHC).

Another basic characteristic of immune responses is the development of **immunologic memory**.^{38–40} This is exemplified by the fact that vaccination can sometimes impart immunity for a person’s lifetime. If a foreign protein is injected into an animal, after a lag period of 2–5 days the animal will synthesize antibodies against this foreign antigen. This is called a **primary adaptive immune response**. If after a few days or weeks a second injection of the same protein is made, a much more rapid synthesis of additional antibodies occurs. This **secondary immune response** may take place within hours and will last longer than

the primary response. It is a manifestation of immunologic memory.

1. Development of Lymphocytes and Other Specialized Cells

Both the B cells and T cells arise in the fetal liver or bone marrow (Fig. 31-1) from pluripotent stem cells. In birds the B cells develop in a special organ, the bursa of Fabricius. Mammalian B cells complete their differentiation into mature **lymphocytes** within the bone marrow. However, the T cells must travel to the **thymus**, where they complete their maturation. The T lymphocytes include the previously mentioned NK cells as well as the somewhat similar **cytolytic T cells** and **immunoregulatory T cells**. The latter are further characterized as **helper T cells**⁴¹ or **suppressor T cells**. The adaptive response requires cooperation of helper T cells in many instances. The mature B and T cells leave the bone marrow and thymus, which are known as the **primary lymphoid tissues**, and enter the blood circulation. Following “homing” signals⁴² they take up residence in a variety of locations

in the lymph nodes, spleen, adenoids, tonsils, and Peyer's patches. The last are small clusters of lymphoid cells in the wall of the intestine. All of these tissues, which are referred to as **secondary lymphoid tissues**, are the sites in which the adaptive immune system is developed.

TABLE 31-2
Cells of the Immune System^a

Type	Functions
B lymphocytes	
Plasma cells	Antibody synthesis
Memory B cells	Immunologic memory
T lymphocytes	
Cytolytic	Destroy infected and malignant cells
Helper cells	
Type 1 (T _H 1)	Participate in activation of B cells
Type 2 (T _H 2)	
Memory T cells	Immunologic memory
Natural killer (NK) cells	Destroy infected and malignant cells with pore-forming protein perforin and cytotoxic granules
Dendritic cells	
Interdigitating	Antigen recognition and processing
Follicular	Antigen presentation
Microglia	Defensive network in brain ^b
Cells that release inflammatory mediators	
Mast cells and basophils	Possess high-affinity receptors for IgE May secrete histamine, prostaglandins, leukotrienes Important in allergies
Eosinophils	Weakly phagocytic, secrete cationic proteins, reactive reduced oxygen species, leukotrienes, prostaglandins, cytokinins
Phagocytic cells	
Neutrophils	Acute inflammatory response
Monocytes	
Macrophages	Carry receptors for carbohydrates not normally exposed on surfaces of cells in vertebrates e.g., mannose; kill engulfed organisms with •O ₂ ⁻ , HOCl, NO, cationic proteins and peptides, lysozyme Antigen processing and presentation

^a General reference: Delves, P. J., and Roitt, I. M. (2000) *N. Engl. J. Med.* **343**, 37–49.

^b Streit, W. J., and Kincaid-Colton, C. A. (1995) *Sci. Am.* **273** (Nov), 54–61.

An important component of the immune system that was neglected until recently is located in the mucous membranes and the skin.^{9,30,43,43a} The mucosal surfaces of airways and the gastrointestinal tract provide the point of entry for many diseases. Both internal and external body surfaces are protected by

dendritic cells, whose immature forms in skin are called Langerhans cells. See figure in Box 8-F.^{22,26a}

Eight different types of cells of the immune system (Table 31-2) develop by differentiation of pluripotent stem cells^{44–48} as indicated in Fig. 31-2. Dendritic cells^{26a} (which are not shown in this figure) may be formed from monocytes but may also arise by other routes.²² The development of the various cells takes place under the influence of a number of **hemopoietic regulators**. Among these are the protein hormone **erythropoietin** and various interleukins and colony-stimulating factors.^{44,45,49}

2. Triggering an Immune Response

When a foreign antigen enters the body the B cells, with receptors of appropriate specificity and present in the lymph nodes, are stimulated to divide repeatedly and to produce a large clone of **plasma cells**. These contain a highly developed ER and actively synthesize and secrete immunoglobulins. One activated B cell may produce 10 million antibody molecules per hour.¹ This B-cell response occurs within a network of **follicular dendritic cells** in the **germinal centers** of the lymph nodes (Fig. 31-1B).^{2,50,51} The antibodies are “adaptor” molecules, which bind to antigenic proteins often on surfaces of invading microorganisms. Another part of the antibody binds to one of several **effectors** systems. These immobilize microorganisms, induce phagocytosis, activate the complement system, carry antibodies across placental membranes, etc.^{52,53}

Induction of a T-cell response is more complex and is very demanding.^{22,47,54} Antigenic peptide frag-

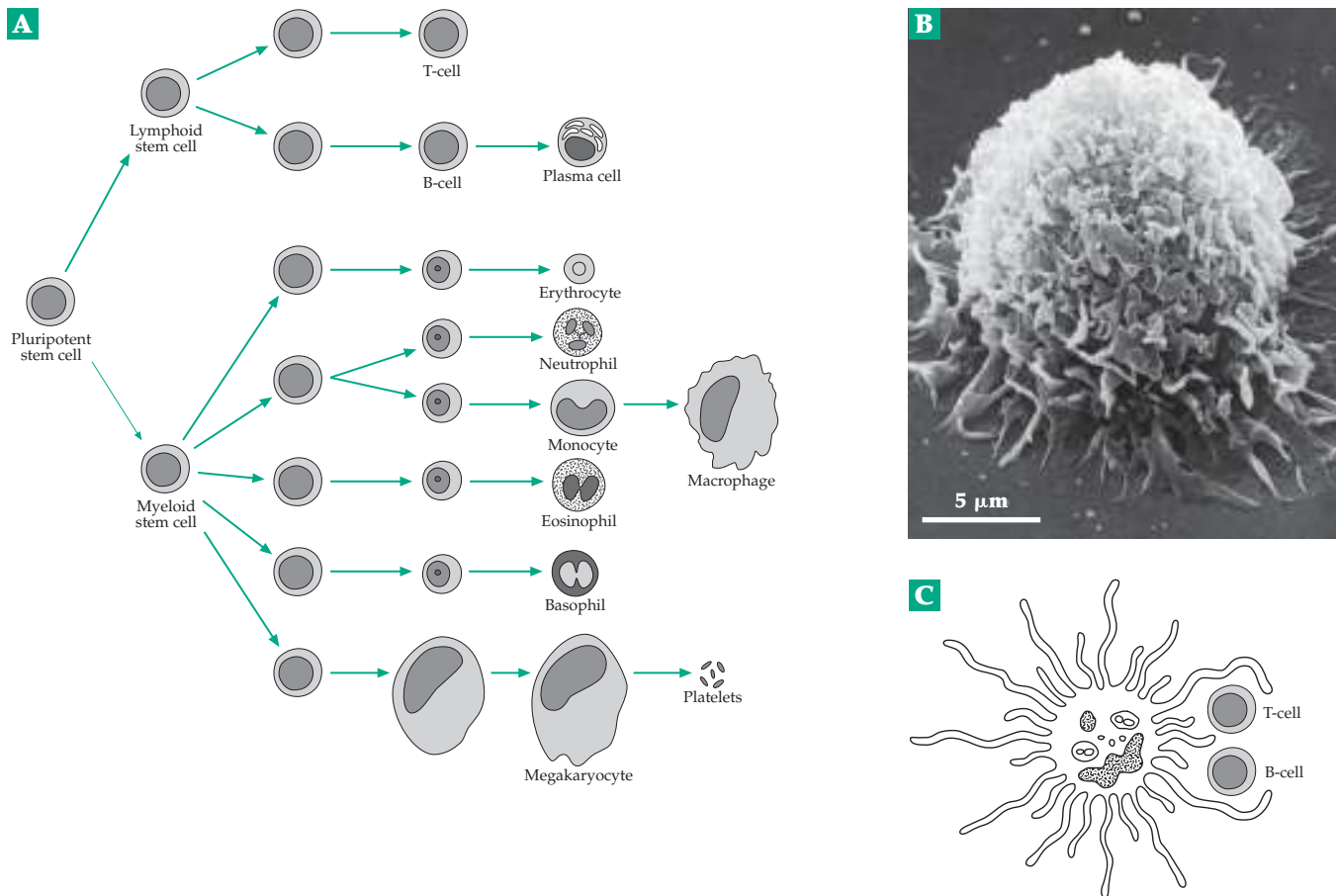


Figure 31-2 (A) Development of eight types of blood cells including those of the immune system from pluripotent (multipotential) stem cells. The cells develop under the influence of a variety of protein growth factors. Some steps, e.g., maturation of B cells, involve complex rearrangements in the DNA of the cell. (B) Scanning electron micrograph of a macrophage, a large motile cell that plays a key role in the immune system. It moves by means of its surface “ruffles.” It actively phagocytoses both pathogens and waste materials and is also one of the cells that releases the hormones known as colony-stimulating factors. Micrograph courtesy of Shirley G. Quam. Drawings courtesy of David W. Golde and Judith C. Gasson.⁴⁴ (C) Schematic drawing of a dendritic cell. Redrawn from Banchereau and Steinman.²² Both macrophages and dendritic cells present antigens for recognition by T cells and synthesize cytokines, which affect lymphocyte development.

ments from infected or malignant cells anywhere in the body must be recognized by an appropriate receptor on a T cell that is circulating in the blood. Only a few T cells with receptors for any given antigen specificity exist.^{54,55} The foreign antigen fragment must bind to a protein of the **major histocompatibility complex (MHC)** while present within a dendritic cell or other APC. The resulting MHC•antigen complexes pass through the ER and the Golgi to the outer cell surface in a rather complex process. Recognition of the antigen that is “presented” in this manner on the APC surface is accomplished with the aid of $\sim 10^{15}$ different receptor proteins (**T-cell receptors**) on the T-cell surfaces. When a T-cell receptor is occupied by an HMC•antigen complex of appropriate specificity the T cell is activated to participate in adaptive immunity. However, some T cells, notably $\gamma\delta$ T cells, like antibodies bind to antigen directly.^{56,56a,b} The recogni-

tion process occurs in an **immunological synapse**, which has elements of similarity to neurological synapses.^{54,57–58a}

B. The Immunoglobulins (Antibodies)

1. Molecular Structures

There are five classes of antibodies or immunoglobulins.^{59,60} The first three, IgG, IgM, and IgA, are quantitatively the most significant, but IgD and IgE are also important. For example, the content of IgE is elevated in allergic persons. The basic structure of all of the immunoglobulins is that of a quasi-symmetric dimer composed of a pair of light chains and a pair of heavy chains whose lengths vary among the different

Symbol	Mass (kDa)	Formula
IgG	150	$\kappa_2\gamma_2$ or $\lambda_2\gamma_2$
IgM	950	$(\kappa_2\mu_2)_{2,5}$ or $(\lambda_2\mu_2)_{2,5}$
IgA	320	$(\kappa_2\alpha_2)_n$ or $(\lambda_2\alpha_2)_n$
IgD	180	$\kappa_2\delta_2$ or $\lambda_2\delta_2$
IgE	190	$\kappa_2\varepsilon_2$ or $\lambda_2\varepsilon_2$

classes of immunoglobulins. Two classes of **light chains**, κ and λ , are found in human antibodies. The **heavy chains** are designated γ , μ , α , δ , and ε (see accompanying tabulation). Both IgM and IgA contain an additional J chain.

Treatment with mercaptoethanol splits the disulfide linkages holding the chains together, permitting preparation of monomeric light and heavy chains. When peptide chains of the immunoglobulins were hydrolyzed enzymatically, the resulting peptide fragments were found to be extremely heterogeneous. They were mixtures of many different kinds of peptides. The result was not unexpected, for it had long been recognized that the body contains millions of different antibodies, with binding sites specific for different antigenic determinants. It had been unclear how different binding sites could be formed, but the heterogeneity in amino acid sequence suggested the correct answer: Each antibody has its own sequence.

Progress toward understanding of the detailed structure of antibodies came when it was recognized that patients with tumors of the lymphatic system, e.g., the bone marrow tumors **multiple myeloma**, produced tremendous quantities of homogeneous immunoglobulins or parts thereof. Similar tumors were soon discovered in mice and provide a ready source of experimental material. The **Bence-Jones proteins** that are secreted in the urine of myeloma patients were found to be light chains of immunoglobulins. Sequence determinations showed that each Bence-Jones protein was homogeneous, even though no two patients secreted the same protein.^{61,62} Later, intact myeloma globulins and macroglobulins (IgM) of a homogeneous kind were also obtained.

The first complete amino acid sequence of an IgG molecule was announced in 1969.⁶³ The protein contained 446 amino acids in each heavy chain and 214 in each light chain. The longer heavy chains of IgM molecules contain 576 amino acids.⁶⁴ In all of the immunoglobulins the heavy and light chains are held together by disulfide linkages, and the chains are folded into loops to form compact domains. The IgM molecule is polymerized through additional disulfide linkages to form a pentamer readily visible with the electron microscope (Fig. 31-3). The heavy chains also carry oligosaccharide units. In IgM there are five of these, as indicated in Fig. 31-4A. They contain mannose and *N*-acetylglucosamine units linked to asparagine. Other immunoglobulins (IgA, IgE, and IgG)

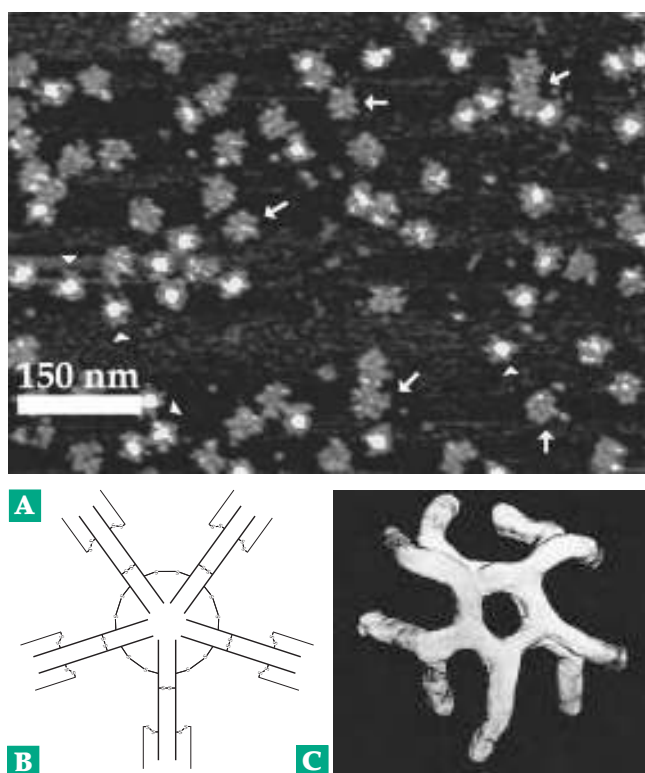


Figure 31-3 (A) Cryo atomic force (AFM) micrograph of molecules of the human immunoglobulin IgM. Courtesy of Zhifeng Shao, University of Virginia. (B) Schematic diagram. One-fifth of this structure is shown in greater detail in Fig. 31-4A. (C) Model based on earlier electron microscopic images. From Feinstein and Munn.^{64c}

contain fucose, galactose, and *N*-acetylneuraminic acid as well. In fact, almost all of the most important macromolecules that participate in innate and adaptive immune responses are glycoproteins.^{64b}

Digestion of an intact molecule of IgG with papain cleaves both heavy chains in the hinge region near the interchain disulfide bridge. This splits the molecule into three parts; two **Fab** (antibody-binding) **fragments**, each containing the N-terminal end of a heavy chain together with a linked light chain, and an **Fc fragment**. Even before it was known that IgG could be split into two Fab fragments, the antibody was known to be divalent, i.e., capable of binding with two different antigens (Fig. 31-4). The shape and overall structure of IgG molecules have been verified by electron microscopy and numerous X-ray diffraction studies.

Sequence determinations showed that in some regions of immunoglobulin molecules there is extreme variation in the amino acid sequence between one homogeneous antibody and the next; other regions have a constant sequence. The molecule can also be divided into domains. The **variable regions**, which occupy the N-terminal ends of the chains, are designated

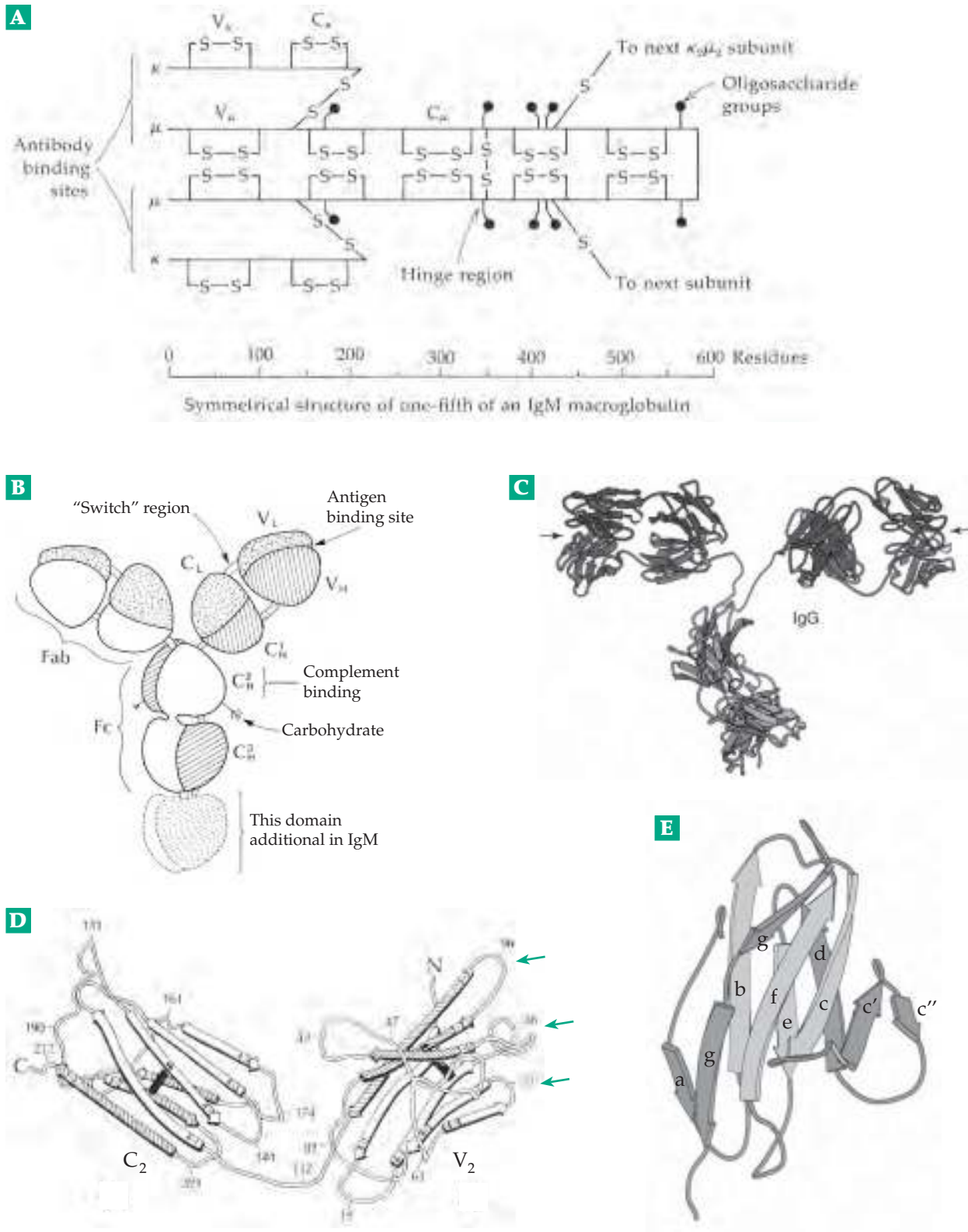


Figure 31-4 Schematic structure of one-fifth of an IgM molecule. From Putnam *et al.*⁶⁴ (A) Covalent structure. (B) Schematic three-dimensional representation. (C) Ribbon diagram of an IgG molecule. From Cochran *et al.*^{64a} (D) Folding patterns of one chain in a constant and a variable domain of a Bence-Jones protein. From Schiffer *et al.*⁶⁶ Green arrows indicate hypervariable regions. (E) MolScript drawing of the common core structure of Ig-like domains. The lighter shaded strands (b, c, e, f) form the core common to all Ig-like domains, which is surrounded by structurally more varied additional strands (darker). The front sheet has up to five strands (a, f, c, e, c'') and the back sheet up to four (a, b, e, d). Strand c'' is very flexible and is not always a part of the β sheet. From Bork, Holm, and Sander.⁶⁵ See also Fig. 2-16.

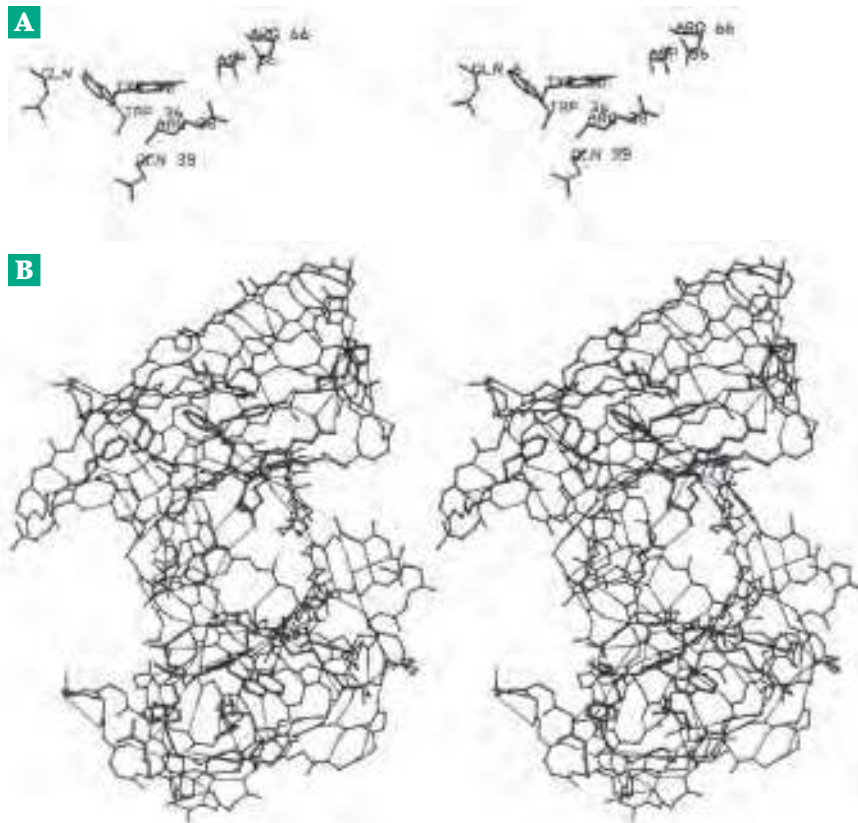


Figure 31-5 The extensive conserved hydrogen-bonding pattern in an immunoglobulin variable domain provided by polar residues buried inside the V_L and V_H domains. (A) To facilitate orientation, prominent side chains are displayed here and identified by names and numbers in the same orientation as in (B). (B) Polypeptide chain backbones of both domains are denoted by heavy lines and hydrogen bonds by light lines. In addition to the regular interbackbone hydrogen-bonding network characteristic of antiparallel β -sheets, there are hydrogen bonds provided by side-chain atoms. Note the two hydrogen bonds of Gln-38 (V_L) and Gln-39 (V_H) that span the domain–domain interface. From Novotny and Haber.⁷⁵

V_L and V_H for the light and heavy chains, respectively. The **constant regions** are C_L and C_H . Examination of the C_H region showed that much of the sequence is repeated after ~ 110 residues. In the IgG molecule the constant region of the heavy chains is made up of three such homologous domains (C_{H1} , C_{H2} , and C_{H3}). A fourth C_H domain is present in IgM. These facts suggest that duplication of a smaller gene coding for about 110 amino acids took place in the evolution of the immunoglobulins. Within the variable regions of immunoglobulin chains are **hypervariable regions** that form the antigen binding sites. These regions are located at the ends of the Fab fragments and involve both the light and heavy chains.^{66a}

Within all of the domains each of the two peptide chains is folded in a similar way. Seven extended lengths of chain form two mostly antiparallel sheets between which hydrophobic side chains are packed. The overall size of the unit is $\sim 4.0 \times 2.5 \times 2.5$ nm. An S–S bridge links the two sheets in the center of each domain. The folding patterns in the variable domains are somewhat more complex. Different domains are linked by segments of extended peptide chain known as hinge or switch regions.^{67–69} These impart a segmental mobility, which seems to be important for functioning of the molecules.⁷⁰

The exact mode of binding to Fab fragments has been established for several specific **haptens**. Haptens are small molecules having the binding properties

of antigenic determinants but unable by themselves to induce formation of antibodies when injected into animals. Binding of the hapten phosphocholine to one Fab fragment and of vitamin K to another⁶⁷ involves the hypervariable regions of both the heavy and light chains. The same is true for the binding of lysozyme^{71–73} and a bacterial oligosaccharide.⁷⁴ The binding sites for the haptens or for the antigenic determinants on larger antigens are largely within the nine-stranded elliptical barrel or β sandwich formed from the two β sheets (Fig. 31-5).^{69,75} Four strands come from the V_L domain and five from the V_H domain. The barrel forms the bottom and sides of the antibody-binding site, which can also be viewed as consisting of six separate loops of peptide chain.⁷⁶ IgA molecules are similar to those of IgG but have structurally different hinge regions as well as an extra 18-residue tail-piece at the C terminus of each heavy chain.⁷⁷

As shown in Fig. 31-5, which provides a three-dimensional view of the variable domain of a Fab fragment of an immunoglobulin, there is a conserved hydrogen-bonded network even in this region. There are also “framework residues,” which are highly conserved.⁷⁸ The antibody-binding site is provided largely by the three hypervariable regions present in each of the V_L and V_H domains. These are usually referred to in current literature as **complementarity-determining regions (CDRs)**.^{78–81} Each pair of heavy and light chains is held together by a conserved

disulfide bridge.⁸² Three-dimensional structures of a substantial number of different Fab fragments have provided precise knowledge about the antibody-binding cavities and about the forces involved in binding.^{78,79,81,83} Among the established structures are those of Fab fragments specific for the following antigens: the haptens *p*-azophenylarsonate⁸⁴ and phencyclidine (p. 1798),⁸⁵ a sweet-tasting hapten,⁸⁶ triple-stranded DNA,⁸⁷ a DNA photoproduct,⁸⁸ creatine kinase,⁸⁹ staphylococcal nuclease,⁹⁰ an HIV capsid protein,⁹¹ and an EGF receptor.⁹² Structures are also known for single-domain antibodies from camels and llamas. These antibodies are naturally lacking in heavy chains but have single chains that fold back to mimic two-chain Fab fragments.^{93,94} Similar single-chain antibody domains have also been created artificially.⁹⁵

Not all proteins bind to antibodies in the usual binding cavity. **Protein G**, a cell surface protein from *Streptococcus* bonds to IgG molecules from many different species. Its binding site is on the outer surface of the heavy chain C_H1 domain.⁹⁶

2. Antigenicity

Antibodies often bind haptens or complete antigens very tightly. The association constants K_f observed for monoclonal antibodies (Box 31-A) range from 10^6 to 10^{12} M⁻¹.⁹⁷ However, most natural antibodies have a lower affinity for their antigens. When protein antigens are denatured, the binding constants often decrease by 10^{-4} to 10^{-5} . This suggested that only antigenic determinants of relatively rigid structures serve as good antigens. However, when the reaction of antibodies with proteins of well-established three-dimensional structure were studied, it was found that the best antigenic determinants are those with some segmental mobility.^{98,99} Furthermore, while some small peptides are good antigens, peptides are most highly antigenic when they can readily fold into a bend or other definite conformation.¹⁰⁰ Good antigenicity apparently requires some segmental flexibility as well as a definite conformation for the antigenic determinant.

3. Responses to Antibody Binding

Both B cells and T cells circulate throughout the body, spending only about 30 min during each cycle. They may meet and bind to an antigen in one of several different places.⁵⁰ Lymphocytes, which encounter blood-borne pathogens, usually initiate an immune response in the spleen. Responses to microorganisms in tissues are usually generated in lymph nodes. Ingested pathogens activate lymphocytes in specialized epithelial **microfold (M) cells** from which the antigen

is transported to the Peyer's patches. Responses to inhaled or intranasal pathogens arise in the tonsils and adenoids. In every case one major aspect of the immune response results from binding of antibodies to antigens.

Antibodies by themselves do not destroy bacteria or viruses, but they induce responses that do. One immediate effect of antibodies is to remove offending materials or cells from circulation. When multivalent antibodies each combine with two different cells **agglutination** occurs. The agglutinated cells or multicellular organisms can then be destroyed by phagocytes. The coating of a cell surface by IgG is one form of a process called **opsonization**, a process that marks the cell as foreign and a target for phagocytosis.^{3,53} Antibody-antigen interactions trigger several other responses as well. One of these results from the binding of protein **C1q**, a component of complement. Complement consists of a series of blood proteins that is poised to respond and to *complement* the action of antibodies in a variety of ways that are described in Section C.2 (see also Figs. 31-8 and 31-9). It has been established that it is the C_H2 domain of the Fc region of IgG that binds to C1q.¹⁰¹ The binding occurs only after antigen (but not a small hapten) binds to the immunoglobulin.

Complement C1q is only one of several types of **Fc receptor**.^{53,102,103} Others are involved in antigenic stimulation of B and T lymphocytes, macrophages, polymorphonuclear lymphocytes, and mast cells. Binding of the antibody-antigen complex to the receptors on phagocytic cells induces phagocytosis and release of oxygen metabolites, leukotrienes, prostaglandins, and other mediators of inflammation. The Fc domain mediates the uptake of antibodies from the mother's milk by young rats.^{104,105} It also is the binding site of antibodies to **protein A**, a constituent of the cell wall of *Staphylococcus aureus*,¹⁰⁶ which is also widely used as a tool in immunological studies (Box 31-C). The neonatal Fc receptor, which is related structurally to Class I MHC antigens (Section D.5), is one of three major types of Fc receptor. The other two are the receptors for Fc γ (of IgG) and Fc ϵ (of IgE). They (like their ligands) are members of the immunoglobulin superfamily. An exception is Fc ϵ R2 (also called CD23), which resembles a C-type lectin. Some Fc receptors, e.g., Fc γ R1 (CD64) and Fc ϵ R1, have a high affinity for their ligands with $K_d \sim 10^{-8}$ to 10^{-10} M. Others, such as Fc γ R2 (CD32) and Fc γ R3 (CD16), have lower affinities with $K_d \sim 10^{-5}$ to 10^{-7} M.¹⁰³ Three-dimensional structures of several Fc receptor fragments, some in complexes with Fc fragments (Fig. 31-6), are known.^{53,103,107-109} These include both IgG and IgE receptors.

It may be worthwhile to recall that many quite different proteins are members of the immunoglobulin structural family (Fig. 2-16). These include proteins

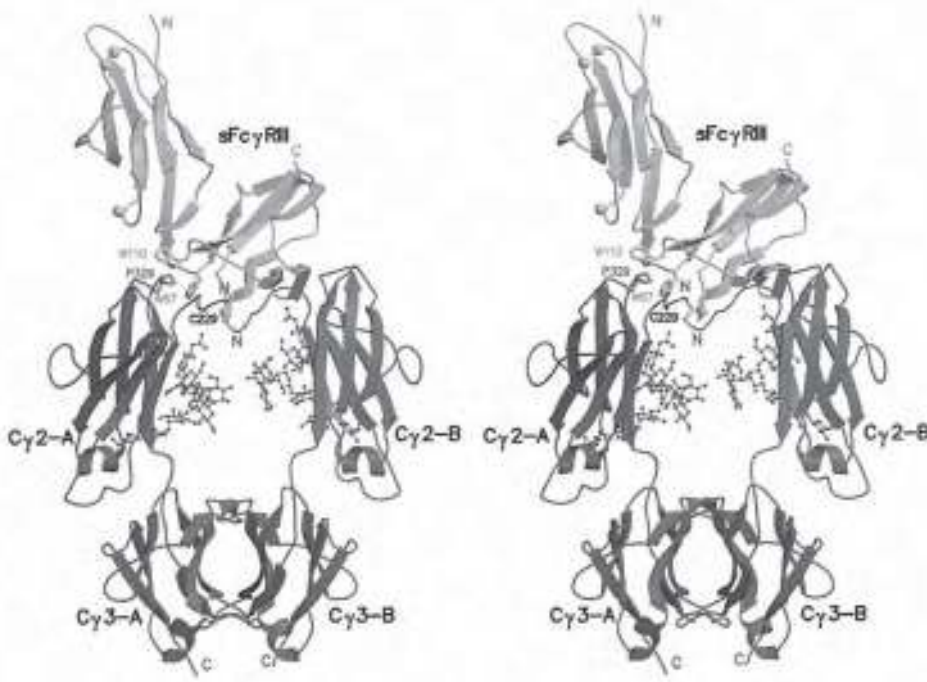


Figure 31-6 Three-dimensional ribbon representation of the structure of a complex of a soluble Fc fragment of a human IgG1 molecule. Pro 329 of the IgG and Trp 87 and Trp 110 of the Fc-receptor fragment form a “proline sandwich,” which is shown in ball-and-stick form. The oligosaccharide attached to the Fc fragment of the antibody and the disulfide bridge between the two Cys 229 residues (at the N termini of the C2 domains of the heavy γ chains) are also shown. The small spheres on the Fc receptor fragment are potential sites for N-glycosylation. From Sondermann *et al.*¹⁰⁷ Courtesy of Uwe Jacob.

encoded by 64 genes of *E. elegans*, an organism that doesn’t form antibodies.¹¹⁰

4. Clonal Expansion of B Cells; Plasma Cells

The immunoglobulins are synthesized both by plasma cells and by their precursors, the B cells. Each B lymphocyte makes antibodies of specific sequence in two forms, secreted and membrane-bound or **antigen receptor**. Mature “virgin” B cells, which are responsible for the primary immune response, make largely monomeric IgM and some IgD.¹¹¹ It isn’t clear why IgD should be the predominant surface immunoglobulin on most B cells.¹¹² The secreted and membrane-bound (receptor) antibodies differ in their C-terminal sequences but are otherwise the same. A B cell responds to the binding of an antigen with a shape complementary to that of its IgM antigen receptor by multiplying and differentiating. Some of the progeny B cells start to divide and begin to differentiate into clones of plasma cells that secrete IgG or into cells of the gut that secrete IgA. Some of the B cells give rise to **memory cells**, long-lived lymphocytes that can be triggered into rapid proliferation many years later if the same antigen is encountered. The B cells also undergo a shift to secretion of pentameric IgM rather than to synthesis of membrane-bound antibody.

5. Help from T Cells

The maturation of B cells is a complex process that requires the cooperation of helper T cells. The B cells

must process some antigen and present the peptide fragments for recognition by the MHC complex and T-cell receptors as described in Section D. If the antigen is recognized as foreign, the differentiation goes forward with the activated T cells secreting lymphokines that promote B-cell growth.

Before activated lymphocytes start to divide, interesting surface phenomena occur. If fluorescent antigens are allowed to bind to a lymphocyte, the cell surface is seen to be relatively evenly covered with the antibody–antigen complexes. Then after a short time the antibodies aggregate to form “patches” and begin to migrate to one side of the cell, where they eventually form a “cap.” At still longer times the cap material is engulfed by the lymphocytes. Perhaps this phenomenon simply reflects the oriented flow of liquid within membranes (Chapter 8). On the other hand, the membrane-bound immunoglobulins, like other cell surface receptors, are integral membrane proteins whose cytoplasmic C termini may be attached on the inside of the cell to the cytoskeleton, which may control the capping process. The binding of lectins sometimes triggers lymphocytes into antibody synthesis, but it is not clear how the binding of a lectin to a carbohydrate receptor can have the same effect as binding of an antigen to a surface IgM.¹¹³

C. Some Specialized Proteins of the Immune System

The immune response depends not only upon recognition of foreign antigens but also upon an extensive signaling network and upon a series of specialized

BOX 31-A MONOCLONAL ANTIBODIES

A mouse may make over 10 million different antibodies. Because of this heterogeneity it was impossible to learn antibody structures until the discovery of the myeloma proteins (Fig. 31-4D). These were produced in the bone marrow by clones of specific immunoglobulin-forming malignant cells. However, it was still not possible to obtain homogeneous antibodies to any desired antigenic determinant. The discovery of a method of forming such **monoclonal antibodies** by Milstein and Köhler^{a-c} in 1975 provided a new tool with many biochemical and medical applications.^{d-f} What Milstein and Köhler did was to immunize mice against an antigen of interest. They then fused B cells from the spleen of the immunized mouse with cultured myeloma cells. The resulting **hybridomas** grow vigorously and produce antibodies of the type dictated by the B cells. Since each hybridoma cell is derived from a single B cell, it makes a single kind of antibody. By plating out and selecting clones of hybridoma cells it is often possible to find a monoclonal antibody that binds well to a specific antigenic determinant. The hybridoma can be cultured indefinitely, producing its monoclonal antibody in any desired quantity.

A major application of monoclonal antibodies is in clinical assays for drugs, bacterial and viral products, tumor antigens, hormones, and other circulating proteins. Their use in conjunction with immunoassays (Box 31-C) has provided increased specificity and sensitivity. Another major application is to observe binding of antibodies to specific proteins by electron microscopy. The location of specific receptor proteins can be established^{g-j} as can the locations of ribosomal proteins and many other cellular components (Fig. 29-1). Monoclonal antibodies to acetylcholine receptors have been shown to induce symptoms of myasthenia gravis (Box 31-D), supporting the autoimmune origin of that disease.^h Monoclonal antibodies specific for such a small hapten as mercuric ion have been isolated.^k

Several problems have limited the wider use of monoclonal antibodies created by the hybridoma method. The antibodies are those of a mouse and are antigenic to humans.^{f,l-n} This long prevented many medicinal uses. Years of effort have gone into

attempts to “humanize” the antibodies. One approach is to introduce human immunoglobulin genes into mice. Another is to use recombinant DNA techniques to clone genes for immunoglobulin fragments and to introduce these into cells of *E. coli* in which additional genetic diversity in the antibodies arises.^{l,o} Selection of antibody fragments is often accomplished using bacteriophage display systems (Fig. 3-16).^{f,l} After selection gene fragments can be reassembled into a final form. Recently, using cloning of large pieces of the several Mbp of human immunoglobulin genes into yeast artificial chromosomes (p. 1497), it has been possible to prepare purely human monoclonal antibodies.^{f,n}

Many attempts have been made to link monoclonal antibodies specific for antigenic determinants on cancer cells to protein toxins such as ricin (Box 29-A). It is hoped that this may provide an effective way of carrying toxins into cancer cells.^{f,p-r} Therapeutic human monoclonal antibodies are already in use as antirejection drugs for kidney transplantation, for treatment of rheumatoid arthritis, Crohn disease, and for some types of cancer.^f

^a Milstein, C. (1980) *Sci. Am.* **243**(Oct), 66–74

^b Milstein, C. (1986) *Science* **231**, 1261–1268

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ⁱ Harlow, E., and Lane, D. (1999) *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Lab. Press, Cold Spring Harbor, New York

^j Goldman, R. D. (2000) *Trends Biochem. Sci.* **25**, 593–595

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^p Collier, R. J., and Kaplan, D. A. (1984) *Sci. Am.* **251**(Jul), 56–64

^q Pastan, I., and FitzGerald, D. (1991) *Science* **254**, 1173–1177

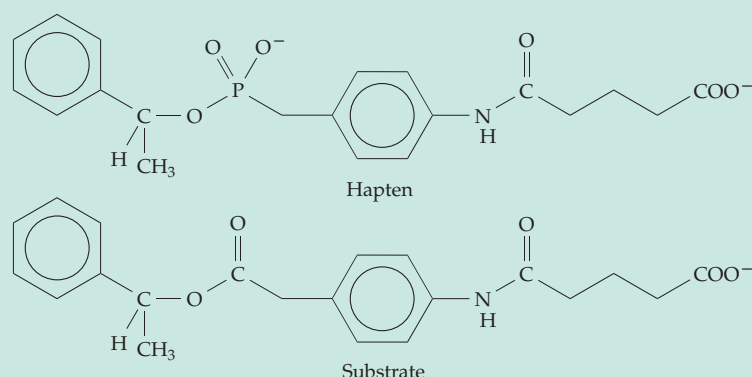
^r Oeltmann, T. N., and Frankel, A. E. (1991) *Trends Biochem. Sci.* **5**, 2334–2337

antibiotics, toxins, and hormones. Some of these, such as the defensins, are major, rapid-acting components of the innate system. Both the innate and adaptive systems utilize the complement proteins, and both

employ numerous cytokines and other signaling proteins. Plants, and perhaps also other organisms, employ gene silencing by small RNA molecules as part of their defense against viruses.^{113a,b}

BOX 31-B CATALYTIC ANTIBODIES

Both enzymes and antibodies are proteins. Antibodies consist of subunits with multiple domains, just as do some enzymes. Both enzymes and antibodies have binding sites for small molecules between domains or subunits. In view of such similarities it isn't surprising that some antibodies have catalytic properties. The possibility was suggested in 1969 by Jencks.^a He also proposed that injection of a mouse with a hapten, that resembled a transition state for an enzyme, might induce formation of antibodies complementary to the transition-state structure. These might be catalytic. By the early 1980s such antibodies were discovered.^{b–d} Some of the first catalytic antibodies (also dubbed **abzymes**) had esterase activity. The haptens used to induce antibody formation were phosphonates such as the following.^{e,f}



Using the transition-state analog shown on p. 485 a catalytic antibody with chorismate mutase activity was isolated.^g Many antibodies catalyzing additional reactions have also been found. Although they are usually less active than natural enzymes, in some cases they approach enzymatic rates. Furthermore, they may catalyze reactions for which no known enzymes exist.^h

Catalytic antibodies, like enzymes, must be isolated and purified to homogeneity before they can be studied. Initially this was done by using the hybridoma technique for isolation of monoclonal antibodies (Box 31-A). After induction of antibody formation by injecting a selected hapten into a mouse, large numbers of monoclonal antibodies had to be tested for catalytic activity. Even if several thousand different monoclonal antibodies were tested, only a few with catalytic properties could be found.ⁱ Newer methods have incorporated recombinant DNA techniques (Box 31-A) and use of combinatorial libraries and phage display.^{j–m} Incorporation of acidic or basic groups into the haptens used to induce antibody formation may yield antibodies capable of mimicking the acid–base catalysis employed by natural enzymes.^{n,o}

A sample of the types of reaction for which catalytic antibodies have been discovered or designed include the following: ester hydrolysis,^e transesterification,^p amide hydrolysis,^q serine protease-like hydrolysis,^r elimination,^{h,s} aldol cleavage,^t decarboxylation,^{u,v} deiodination by a selenium-containing antibody,^w pericyclic rearrangements,^{g,x} and the Diels–Alder reaction.^{y,z} Like natural enzymes catalytic enzymes can be mutated and engineered and can be used to study fundamental aspects of catalysis.^{aa} Fluorescent probes incorporate near active sites may provide information about mechanisms or may signal information of diagnostic significance.^{bb}

In science we must always expect the unexpected. Do antibodies all catalyze the reaction of singlet molecular oxygen $^1\text{O}_2^*$ with H_2O to form H_2O_3 and H_2O_2 ? How?^{cc}

1. Defensins and Other Antibacterial Polypeptides

Only higher vertebrates have an adaptive immune system with circulating antibodies. However, from bacteria to higher plants and human beings all of us utilize defensive polypeptides for protection. More than 500 have been identified.¹¹⁴ Many have a broad specificity, attacking both bacteria and other pathogens. Among these peptides are more than 200 bacterially produced antibiotics such as gramicidin,

tyrocidines, and colicins (Boxes 20-G, 8-D). More recently discovered are the 37- to 59-residue **bacteriocins**, formed by lactic acid bacteria.¹¹⁵ Like colicin E1 (Box 8-D) and alamethicin (p. 1774) they disrupt cytoplasmic membranes of some other groups of bacteria.

Helicobacter pylori, which is associated with stomach ulcers, forms a 38-residue antibiotic that may help protect infected persons from other bacteria.¹¹⁶ This peptide forms a simple two-helix structure and is one of a large number of simple helical antimicrobial polypeptides 40 residues or less in length. Among them

BOX 31-B (continued)

- ^a Jencks, W. P. (1969) *Catalysis in Chemistry and Enzymology*, McGraw-Hill, New York (p. 288)
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- ⁿ Kemp, D. S. (1995) *Nature (London)* **373**, 196–197
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- ^p Wirsching, P., Ashley, J. A., Benkovic, S. J., Janda, K. D., and Lerner, R. A. (1991) *Science* **252**, 680–685
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- ^r Zhou, G. W., Guo, J., Huang, W., Fletterick, R. J., and Scanlan, T. S. (1994) *Science* **265**, 1059–1064
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- ^u Hotta, K., Lange, H., Tantillo, D. J., Houk, K. N., Hilvert, D., and Wilson, I. A. (2000) *J. Mol. Biol.* **302**, 1213–1225
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- ^y Romesberg, F. E., Spiller, B., Schultz, P. G., and Stevens, R. C. (1998) *Science* **279**, 1929–1933
- ^z Heine, A., Stura, E. A., Yli-Kauhaluoma, J. T., Gao, C., Deng, Q., Beno, B. R., Houk, K. N., Janda, K. D., and Wilson, I. A. (1998) *Science* **279**, 1934–1940
- ^{aa} Romesberg, F. E., Santarsiero, B. D., Spiller, B., Yin, J., Barnes, D., Schultz, P. G., and Stevens, R. C. (1998) *Biochemistry* **37**, 14404–14409
- ^{bb} Simeonov, A., and 14 other authors. (2000) *Science* **290**, 307–313
- ^{cc} Wentworth, P., Jr., Jones, L. H., Wentworth, A. D., Zhu, X., Larsen, N. A., Wilson, I. A., Xu, X., Goddard, W. A., III, Janda, K. D., Eschenmoser, A., and Lerner, R. A. (2001) *Science* **293**, 1806–1811

are the **cecropins** of insects and **magainins** and **buforins** of amphibians.^{117,117a} Many of these kill by disrupting membranes or by forming pores in membranes. However, others enter bacteria and disrupt functions of nucleic acids, enzymes, etc.^{117,118} Many antibacterial peptides have been isolated from insects,^{12,119} scorpions,¹²⁰ spiders and horseshoe crabs,^{121,122} and amphibians.¹²³ All of these organisms lack adaptive immunity but have strong innate immunity.

The human body is protected by two groups of defensins formed in the skin, in mucous membranes, in secretions of neutrophils, and other phagocytic cells. The α -defensins (Fig. 31-7) are 29–35 residues in length and are active against both gram-positive and gram-negative bacteria as well as fungi and enveloped viruses including HIV.^{12,124–125a} The β -defensins are mainly active against gram-negative bacteria and yeast. They also possess immunostimulatory activity that is important in activating the adaptive immune response.^{32,125,126} Various tissue-specific defensins have been discovered.¹²⁷ Peptides of the **trefoil family** protect the gastrointestinal tract.^{128–130} Eosinophils,¹³¹ leukocytes, and neutrophils make additional

protective proteins. One leukocyte defensin is a macrocyclic peptide, whose gene may have arisen by fusion of two segments encoding nonapeptide segments of α -defensins.¹³² Neutrophils form, in addition to defensins, α -helical peptides called **cathelicidins**, which protect skin from invasive bacterial infection.^{133,133a} Their synthesis is greatly increased after wounding. They may be among the proteins whose absence after severe burning is likely to be fatal.

Both α and β -defensins consist largely of β strands (Fig. 31-7) and are linked by three disulfide bridges. Some scorpion and insect defensins resemble scorpion toxins (Fig. 30-16) and have four S–S bridges. Fungi and green plants^{135,136} also form antimicrobial peptides. A 30-residue fungal protein is highly knotted and contains four S–S bridges.¹³⁷ Some polypeptides from the oleander and related plants are 29- to 31-residue macrocyclic structures with two S–S bridges in a **cysteine knot** structure^{138–140} (Fig. 31-7). They are exceptionally stable and protease-resistant and may have defensive activity against insects. Defensins are small polypeptides, but larger proteins are also part of the innate defense system. For example, a

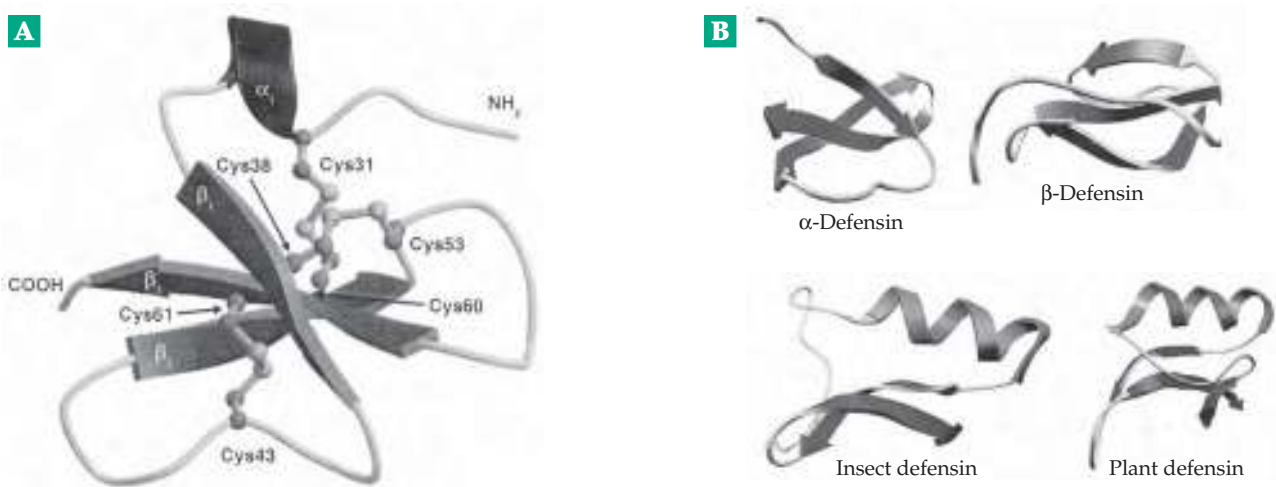
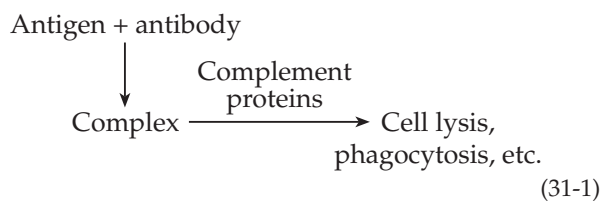


Figure 31-7 Ribbon structures of some defensins. (A) Structure of a human β -defensin showing the three disulfide bonds. From Bauer *et al.*¹³⁴ Courtesy of Heinrich Sticht. (B) Comparison of the folding patterns of four types of defensins. Mammalian α - and β -defensins are all β sheets with somewhat different arrangements of disulfide bridges. Insect and plant defensins have an α helix joined to the β sheet. Mammalian and insect defensins have three disulfide bridges, while plant defensins have four. From Hoffmann *et al.*¹² Courtesy of Jules A. Hoffmann.

93-residue protein from onion seeds resembles plant in lipid-transfer proteins.¹³⁵ Some frog skins contain a 60-residue trypsin inhibitor.¹⁴¹ Ribosome-inactivating proteins are well known (Box 29-A).

2. Complement

Complement is a group of more than 30 proteins found in blood serum, which are activated in a cascade mechanism when antibody and antigen combine^{10,111,142–148} (Eq. 31-1). This **classical pathway** for activation of complement is outlined in Fig. 31-8. The proteins involved in the cascade are designated C1 to C9. Many of them undergo proteolytic cleavage, the



cleavage products being designated by a or b, e.g., C3a and C3b. The b fragment is usually the larger of the two. There is also an **alternative pathway** that is part of the innate system. It is activated by such foreign surfaces as lipopolysaccharides of bacterial cell walls. Its special proteins are called **factors**, e.g., factor B, factor D. A third pathway, the **lectin pathway**, is activated by microbial surface mannans, which bind to a serum **mannan-binding lectin (MBL)**. This protein, a so-called defense collagen, resembles protein C1q (next paragraph).¹⁴⁹ It activates two associated

serine proteases (MASP1 and MASP2), which are able to cause cleavage of proteins C4 and C2 and possibly C3 in the classical pathway (Fig. 31-8). The ultimate effects of the action of complement include destruction of cells by lysis and activation of leukocytes, which engulf foreign cells by phagocytosis. Complement also induces the release of **chemotactic factors** that attract polymorphonuclear leukocytes and monocytes to the site involved.¹⁵⁰

The classical pathway begins with the **recognition component C1** of complement. This is a complex of three proteins, C1q, C1r, and C1s. Proteins C1r and C1s form a mixed tetramer C1r₂s₂, while C1q binds to the C_H² domain of “activated” antibodies, that is, with immunoglobulins that have combined with an antigen. It takes at least a dimer or larger aggregate of IgG to activate C1q, whereas a single molecule of the naturally pentameric IgM suffices. The mechanism by which this activation occurs is uncertain. Perhaps a change of conformation within the immunoglobulin accompanies antibody binding and is responsible for generation of a binding site for C1q. It may seem strange that haptens cannot cause complement binding, and that they do not cause detectable conformational alterations in Fab. Only multivalent antigens able to bind to more than one antibody induce complement binding. However, as we have learned in recent years, many biological responses involve transient assembly of large aggregates of different protein components. In this context, the requirement for two or more antibody molecules doesn’t seem so strange.

The 400-kDa C1q consists of a central portion of diameter 3–6 nm and length 10–12 nm to which are attached six very thin connecting strands. These are

~14.5 nm long and ~1.5 nm in diameter and terminate ~135-residue globular ends of ~6 nm diameter,^{151–153} which are thought to be the sites of combination with the immunoglobulins. The thin connecting strands have, for most of their length, a collagenlike structure with a high content of hydroxyproline and hydroxylysine. The latter is glycosylated by glucosylgalactosyl disaccharides as in collagen itself (pp. 181, 432, 433). The reason for this unusual structure is not obvious. We do know that the binding of antigens activates the complement-binding regions of antibodies, and that the activated antibodies then bind C1q. This binding in some manner activates C1q, which in turn activates C1r subunits of the C1r₂s₂ tetramer.¹⁵⁴ The latter is thought to bind at the center of C1q, while the antibodies bind at the outer ends. We don't know how the

activation message is carried from the outer arms to the center. C1q is a member of a group of collagen-like proteins that includes protein MBL (also designated MBP) and surfactant protein A (SP-A; p. 436).¹⁵⁵

Activated C1r (often designated $\overline{\text{C1r}}$ but here and in Fig. 31-8 as **C1r**) is one of five different serine proteases involved in activation of complement.¹⁵⁶ The substrate for the trypsinlike **C1r** is C1s, a proenzyme which is converted by the action of **C1r** into another trypsinlike serine protease **C1s**.^{157–160} Through a rather elaborate cascade mechanism, depicted in Fig. 31-8, the important proenzyme C2 is activated.¹⁶¹ Its active form **C2a** is a serine protease, which cleaves proteins C3 and C5 to the active forms C3b and C5b. Protein C4 is also cleaved to C4b by activated C1. C4 and C3 are also activated, and protein C5 is cleaved

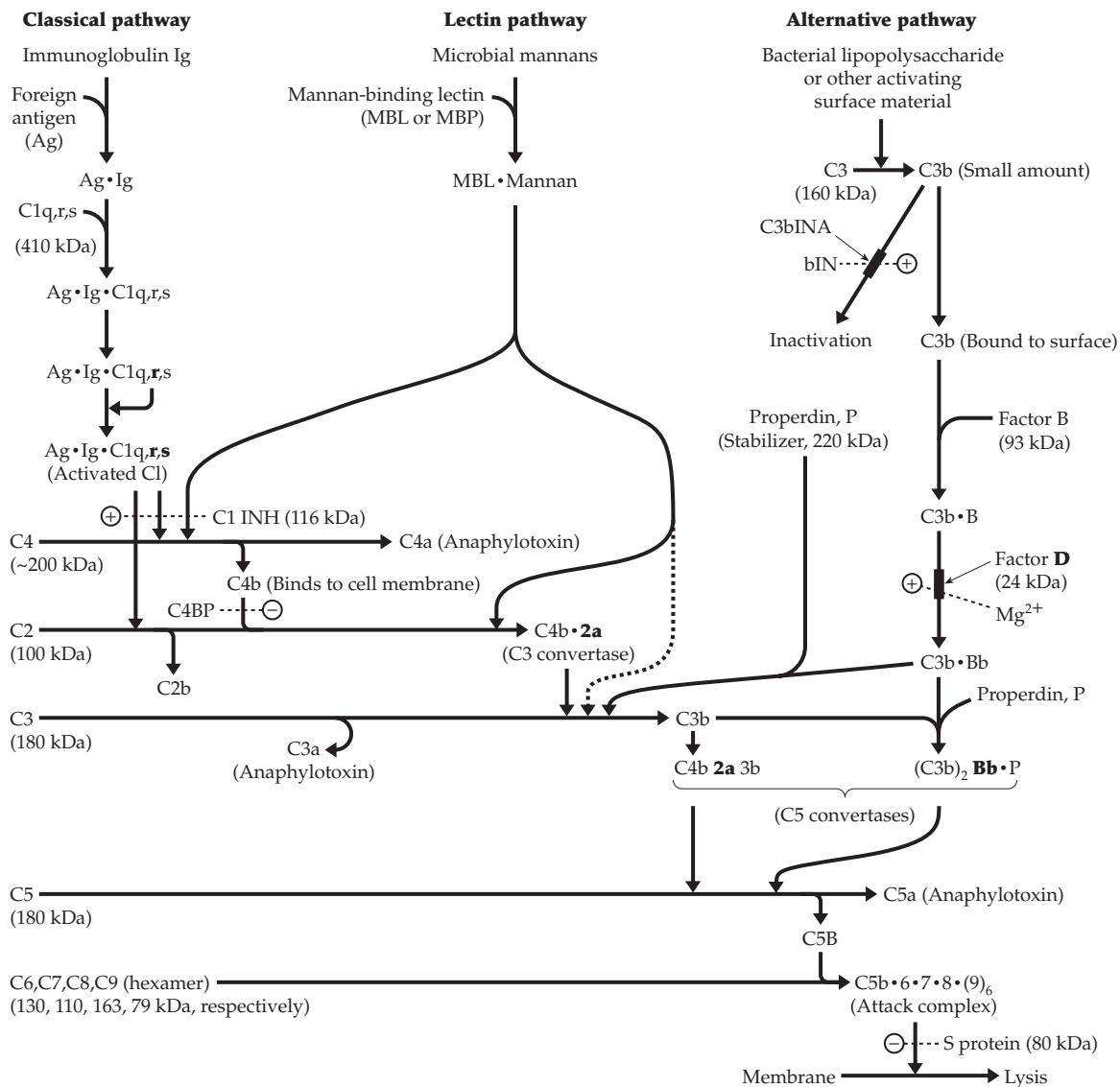


Figure 31-8 Pathways for activation of the complement system. Active proteases are designated by abbreviations in boldface.

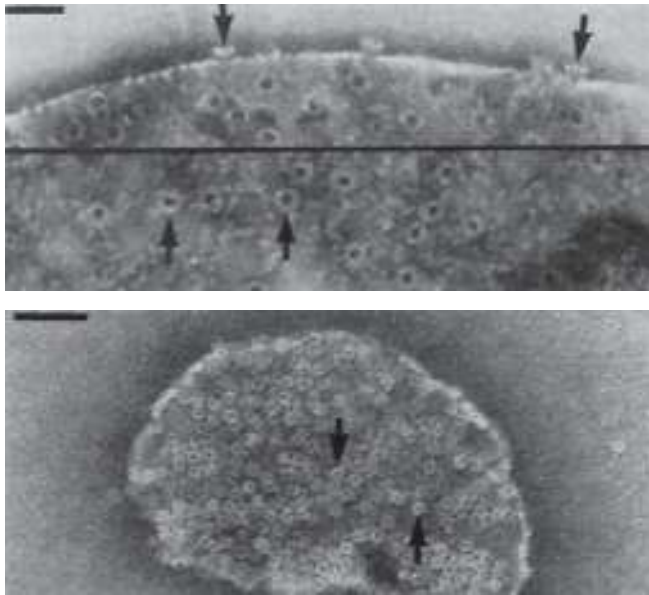


Figure 31-9 Electron micrograph of a negatively stained sheep erythrocyte lysed with human complement. The cylindrical “attack complex” embedded in the membrane is seen in the upper left frame in side projection and in the lower frames in axial projection. The top views are of a proteolytically “stripped” ghost, the lower view from a freshly lysed ghost. The inner diameter of the cylinders is 10 nm; scale bars 50 nm. From Bhakdi and Trandum-Jensen.¹⁶⁹

to C5b. The 200-kDa C4 consists of three chains, all derived by proteolytic cleavage of a precursor. It is also glycosylated and sulfated. Both C4 and C3 contain internal thiol ester linkages and act as “molecular mousetraps” (Box 12-D).¹⁶² They react to fix the proteins covalently to the assembling complement complex.¹⁶³ Protein C5b interacts with C6, C7, C8; and six molecules of C9 to generate an “attack complex,” which inserts donutlike rings into the cell membrane being attacked (Fig. 31-9).^{164,164a} Although there has been some uncertainty about the mechanism of lysis, it seems likely that it is at least partly a result of loss of ions through the holes in the donut.

In the alternative pathway of activation a small amount of C3b is formed and becomes bound to the cell surface. This binds another proenzyme factor B,¹⁶⁵ which is converted by protease factor D¹⁶⁶ to active protease **Bb**. The latter in its complex with C3b is the enzyme that cleaves C3 in large amounts and permits a rapid formation of more **Bb** and also of the complex (C3b)₂ **Bb**•P, which attacks C5 (Fig. 31-8). These complexes are stabilized by the abundant serum protein **properdin** (P).¹⁶⁷

Other components of complement are the plasma C1 protease inhibitor,¹⁶⁸ which prevents accidental activation of the system, and protease C3bINA, which

inactivates C3b. The latter depends upon accessory protein bIN. Another component, serum carboxypeptidase B (SCPB), inactivates anaphylotoxins C3a, C4a, and C5a. These small ~80-residue pieces have a variety of powerful biological activities.^{170–174} They are chemotactic factors for leukocytes and induce release of histamine from mast cells. In excess, they can cause anaphylaxis; hence their rapid degradation is essential. An excess of C5a may be present both in asthma and in rheumatoid arthritis.

The ninth component of complement, C9, is a 70-kDa 537-residue water-soluble glycoprotein, which contains a hydrophobic domain that aggregates to form the ion conducting channels.^{175,176} Proteins that closely resemble C9 and are called **perforins** or **cytolytins** are found in cytoplasmic granules of cytotoxic T-lymphocytes and natural killer cells. These ~66-kDa proteins are assembled into rings similar to those formed by C9 and may be involved in the killing action of these cells (Fig. 31-9).^{177–179} Certain pathogenic amebas, which may cause a fatal infection, also utilize a similar pore-forming protein.¹⁸⁰

Every regulatory system in the body must be prevented from overactivity or activity that is unnecessarily prolonged. This can help us understand that, just as with blood clotting (Fig. 12-17), a network of regulatory factors controls the complement system. Among these are an inhibitory C4b-binding protein (C4BP),¹⁸¹ which acts to prevent excessive formation of the C4b•C2a complex (Fig. 31-8). Complement **cofactor I** is a serine protease that cleaves both C3b and C4b into smaller pieces in the presence of **cofactor H**^{181a} or of C4BP. Its absence leads to excessive consumption of C3 and recurrent pyogenic infections.¹⁸² The **membrane cofactor protein** (MCP) stimulates this action of cofactor I in inhibiting attacks of complement on the cells that carry MCP.¹⁸³ Acting in the opposite direction is **complement receptor 2** (CR2 or CD21), which acts as a receptor for proteolytic fragment C3d. This fragment binds CR2-bearing cells to the B cell receptor, amplifying the B cell response to foreign antigens.¹⁸⁴

Complement is involved not only in attacking foreign cells but in inflammation. Unfortunately, this is sometimes accompanied by serious problems. Human diseases in which complement is thought to be involved include glomerulonephritis, rheumatoid arthritis, myasthenia gravis, and lupus erythematosus.

3. Cytokines, Interferons, and the Acute-Phase Response

The body responds in many ways to infection, injury, or cancer. These include the secretion of cytokines, interferons, and proteins of the acute phase response. These proteins, many of which are quite

small, are involved in communication with other cells, often with specialized cells of the immune system.

Cytokines, some of which are considered in Chapter 30 (Section A,6; Fig. 30-6), are small hormonelike molecules. They may stimulate, inhibit, or exhibit other effects on cells of the immune system. They often have pleiotropic effects, not acting in the same way on all types of cells.¹⁸⁵ The cytokines known as **interleukins** (IL-1, etc.) are produced by leukocytes.

Lymphokines are formed by lymphocytes and **monokines** by monocytes. Based on their functions there are four categories of cytokines.¹¹¹

- (1) Mediation of natural immunity: type I interferons, IL-1, IL-6, and more than 40 **chemokines** (small highly basic chemotactic proteins).
- (2) Regulation of lymphocytes, activation, growth, and differentiation of B and T cells: IL-2, IL-4, IL-21, TGF- β .
- (3) Regulation of immune-mediated inflammation: Interferon- γ , tumor necrosis factor (TNF), IL-5, IL-10, IL-12, and migration inhibition factor (MIF).
- (4) Stimulation of hematopoiesis (IL-3, IL-7), colony-stimulating factors (CSF; see also Chapter 32).

Cytokines all function using a group of transmembrane receptors embedded in the plasma membranes of target cells. The receptors have no tyrosine kinase activity but associate with and activate kinases known as **Janus kinases (JAKs)**. These kinases phosphorylate tyrosine side chains in their receptors, and the phosphorylated receptors activate transcription factors of the **STAT** (signal transducer–activators of transcription) group.^{186–195} The specificity of cytokine action results from a combination of receptor recognition and recognition of the various STAT molecules by different JAKs.¹¹¹ Cytokines have a variety of structures. Many are helix bundles or have β sheet structures (Fig. 30-6).

Interferons. The interferons (IFNs),^{196,197} which were discovered in 1957, are proteins secreted by leukocytes, fibroblasts, and activated lymphocytes. They inhibit replication of viruses as well as the growth of host cells and also have antitumor activity. Interferons are classified as α (from leukocytes), β (from fibroblasts), and γ (from lymphocytes). According to their affinities for the two types of known interferon receptors, interferons IFN- α , IFN- β , and the less well known IFN- ω and IFN- τ are

designated type I,^{198–201} while interferon γ (IFN- γ) is type II. At least 15 homologous 166-residue human α interferons are known.

The binding of interferons to their receptors induces a rapid increase in the transcription of particular genes and synthesis of corresponding proteins.^{196,202} One of the proteins induced is a **double-stranded RNA-activated 2'-5' A synthase**, which polymerizes ATP to a series of 2'-5' linked oligonucleotides containing triphosphates at the 5' termini.^{202–204} Double-stranded RNA is uncommon except in replicating viruses, and it is thought that the activation by dsRNA is related to establishment of an antiviral state. Another interferon-induced enzyme is the small subunit of eukaryotic protein synthesis initiation factor eIF-2. This is converted to an inactive phosphorylated form by a dsRNA-dependent protein kinase²⁰⁵ (Fig. 31-10). The protein kinase also appears to be an interferon-induced protein²⁰⁶ as is the oligo(2'-5' A)-activated RNase indicated in Fig. 31-10.²⁰⁷ Interferons have effects other than inducing the antiviral state. Thus, human IFN- β_2 is identical to a B-cell differentiation factor.²⁰⁸ Both IFN- α and IFN- β have antigrowth activity and are currently in use for treatment of some forms of cancer as well as for viral infections.²⁰⁹

Interleukin-1 (IL-1) plays a key role in the body's response to microbes and to tissue injury.^{210,211} It actually consists of three similar proteins, **IL-1 α** , **IL-1 β** , and **IL-1 receptor antagonist**. The first two are the active cytokines with a wide range of effects among

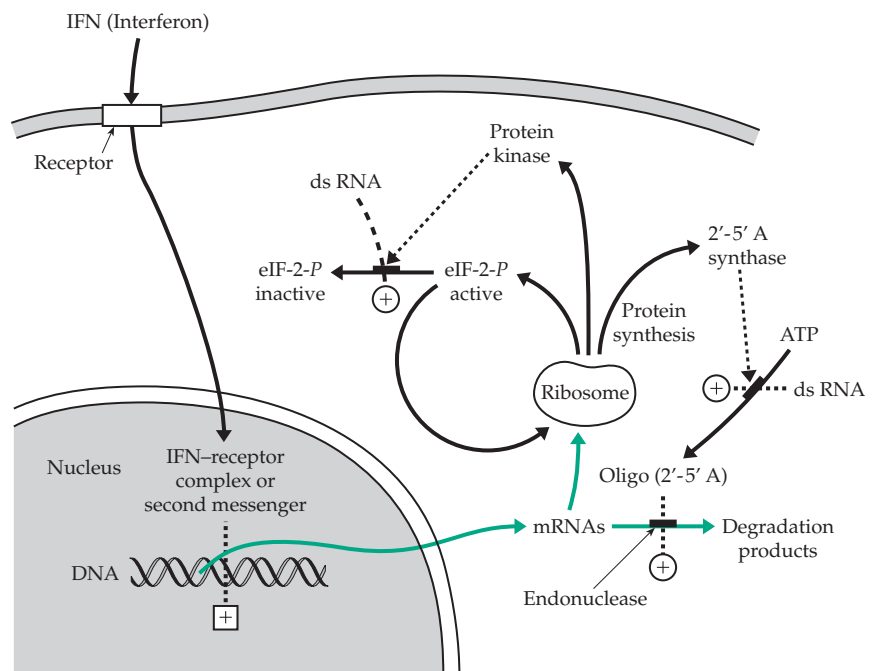


Figure 31-10 Some responses to the binding of an interferon to a cell surface receptor.

which are induction of inflammation and pain.²¹² Il-1 β is thought to be most active in promoting inflammation but only after it is cleaved by **interleukin-1 β -converting enzyme** (see p. 619).²¹³ Blocking of Il-1 receptors provides a potential new method for control of pain.²¹⁴ **Interleukin-6** (IL-6) is also needed for an optimal immune system. Its effects overlap those of

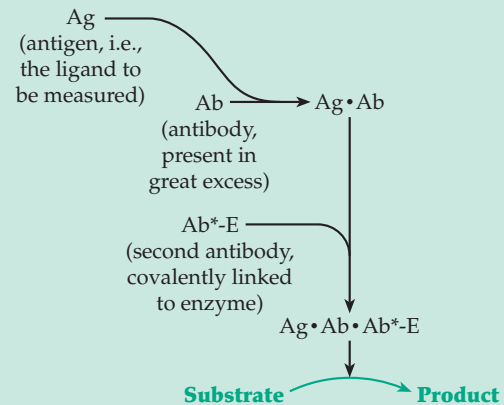
Il-1, and it has a potent activity in inducing the acute-phase response.²¹⁵ Like Il-1, it is a four-helix cytokine.

Also required by the immune response are the numerous **chemokines**. Chemoattractant molecules provide concentration gradients that direct the movement of B and T cells and other leukocytes.^{216–219} Chemokines bind to seven-helix receptors, often

BOX 31-C IMMUNOASSAYS

Among the important techniques that have permitted rapid progress in studies of hormone action is the use of specific antibodies formed against hormones, hormone-protein conjugates, or other molecules.^{a–c} The first of these techniques to come into general use was the **radioimmunoassay** (RIA),^{d–f} which was devised by Yalow and Berson.^d In one form of RIA various amounts of a sample containing an unknown quantity of hormone, e.g., insulin, are placed in a series of tubes. Additional tubes containing known amounts of the hormone are also prepared. Then a standard quantity of radiolabeled hormone (often iodinated with a γ emitter such as ¹²⁵I) is added to each tube together with a standard quantity of the specific antibody to the hormone. The solution is incubated for minutes or hours to obtain equilibrium between hormone (the antigen) and antibody-hormone complex. The antibody-hormone complex is then separated, e.g., by gel filtration or ammonium sulfate precipitation, and the radioactivity of the complex is measured. In the tubes containing higher concentrations of hormone, the labeled hormone has been diluted more, and the amount bound to antibody is less than in tubes with lower concentrations of hormone. The tubes of known concentrations are used to construct a standard curve from which the unknown concentrations can be read. As little as a femtomole of hormone (i.e., the amount present in 1 ml of a 10^{–12} M solution can be detected).^f Methods are available for virtually every pure hormone.^c

The RIA methods were made more convenient by adsorbing either the antibody or antigen to the plastic surface of a tube or depression plate. This facilitates separation of the antibody-ligand complex and washing. A variety of other immunoassays techniques have been devised. For example, in **enzyme-linked immunoabsorbent assays** (ELISA),^c the amount of adsorbed antibody-ligand complex is measured by treating the washed surface with a second antibody, which is directed against the first. The second antibody is linked covalently to an enzyme, whose activity can then be measured by a suitable colorimetric procedure. The reactions involved are as follows.^{g,h}



Variations, which avoid the use of radioisotopes, are replacing RIA. Some utilize stable isotopes. However, ¹⁴C at such low levels that there is no radioactive waste can be coupled with accelerator mass spectrometry to provide very sensitive immunoassays.ⁱ A great variety of other procedures are available. Some involve coupling to antibodies that carry fluorescent labels. Many are now automated. Often protein A from *Staphylococcus aureus* is utilized in various ways that take advantage of its ability to bind to the Fc portion of IgG from virtually all mammals. For example, it may fix antibodies to a surface or to a label.^j

^a Price, C. P., and Newman, D. J., eds. (1991) *Principles and Practice of Immunoassay*, Stockton Press, New York

^b Lindbladh, C., Mosbach, K., and Bülow, L. (1993) *Trends Biochem. Sci.* **18**, 279–283

^c Crowther, J. R. (1995) *ELISA: Theory and Practice*, Humana Press, Totowa, New Jersey

^d Yalow, R. S. (1978) *Science* **200**, 1236–1245

^e Brooker, B., Terasake, W. L., and Price, M. G. (1976) *Science* **194**, 270–276

^f Jaffe, B. M., and Behrmann, H. R., eds. (1974) *Methods of Hormone Radioimmunoassay*, Academic Press, New York

^g van Vunakis, H. and Langone, J. J. (1980) *Methods Enzymol.* **70**, entire volume

^h Langone, J. J., and van Vunakis, H., eds. (1983) *Methods of Enzymology* **92**, entire volume

ⁱ Shan, G., Huang, W., Gee, S. J., Buchholz, B. A., Vogel, J. S., and Hammock, B. D. (2000) *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2445–2449

^j Surolia, A., Pain, D., and Khan, M. I. (1982) *Trends Biochem. Sci.* **7**, 74–76

without rigid specificities. However, some such as **eotaxin** (Fig. 30-6E) are more specific. Eotaxin attracts primarily eosinophils and basophils during allergic reactions.²²⁰ Interleukin-8 (IL-8) is a proinflammatory cytokine and a powerful attractant for neutrophils. Neutrophils are attracted into affected tissues, where they undergo a respiratory burst and generate toxic compounds from O₂ (pp. 1072–1074).^{221–222c}

The second group of cytokines regulate B and T lymphocytes. Among them interleukin-2 (IL-2) stands out as the major promoter of growth and differentiation of T cells. It was the first hormone of the immune system to be recognized.²²³ Both IL-2 and IL-4 have short four-helix structures (Fig. 30-6A). IL-2 is synthesized by activated T cells and binds to a multisubunit receptor complex. The latter associates with tyrosine kinases of both the Src family (p. 572) and with Janus kinases and also activates phosphatidylinositol 3-kinase.^{224–227} IL-2 promotes growth and differentiation, and clonal expansion of T cells, a key aspect of the cellular immune system. It also acts as an immunomodulator of B cells, macrophages, and NK cells. Considerable excitement has accompanied the possibility of activating lymphocytes with IL-2 produced from cloned genes in bacteria to increase their ability to kill cancer cells. However, IL-2 is toxic, and this is limiting its use.

Interleukin-4 (IL-4), cooperating with IL-21,^{227a} stimulates growth of activated B cells, T lymphocytes, and mast cells, induces formation of cytotoxic CD8⁺ T cells, and enhances formation of IgG.^{228,229} The **transforming growth factor- β** (TGF- β) is another cytokine that modulates the development of the immune system. It affects a very broad range of tissues and is discussed in Chapter 32.

Inflammatory influences. Inflammation (p. 1211), which usually accompanies infection and can also arise from allergic responses, is affected by many substances.^{229a–e} These include chemotactic factors that attract neutrophils and monocytes^{222b,229d} and the adhesion molecules that assist in the movement of lymphocytes.^{229e,f} Some epithelial tissues, such as the mucosal surfaces of the gastrointestinal tract, are maintained in a continuous very low level of inflammation. This reflects the balance between activation of the immune system and inhibition of the system by signals from microorganisms both pathogenic and commensal.^{229g,h}

The third group of cytokines^{229b,c} are among the molecules that regulate inflammation. One of these is **interferon- γ** (IFN- γ). Like the type I interferons it induces an antiviral state. However, its most important biological function lies in modulation of the immune system. It induces synthesis of both class I and class II (HLA-DR) antigens (see Section D), activates macrophages, and regulates synthesis and activity

of other lymphokines.^{230–231b} One mechanism of immunomodulation may involve induction of an aminopeptidase that participates in “trimming” of antigenic peptides that participate in antigen presentation (Section D,6). This may directly alter the T-cell response.²³² IFN- γ has a major effect on skin cell-mediated immune responses.²³³ IFN- γ exists in solution as a symmetric dimer, which binds to two molecules of its major surface receptor.^{231,234} The antiviral activity of IFN- γ is largely a result of induction of large amounts of **guanylate-binding proteins**, large 60- to 100-kDa GTPases.^{235,236}

Occasionally a well-established cancer regresses and disappears spontaneously. In the late 1800s it was observed that this sometimes happened, when a person had a concurrent severe bacterial infection. W. B. Coley pursued this lead for many years, treating cancer patients with extracts of killed bacteria, which, although highly toxic were safer than live bacteria. In the laboratory filtrates from cultures of gram-negative bacteria were shown to kill some experimental mouse tumors. The active ingredient was identified as a highly toxic and pyrogenic lipopolysaccharide (Fig. 8-28; Chapter 20, Section E).^{237,238} This lipopolysaccharide has a powerful activating effect on macrophages. More recently it was found that the activated macrophages produce a protein known as **tumor necrosis factor** (TNF- α) that can destroy tumor cells and also acts together with interferons in inducing resistance to viruses.^{239,240} A similar **lymphotoxin** (TNF- β) is secreted by lymphocytes.²⁴¹ Although highly toxic there has been hope of obtaining engineered forms of these proteins more specifically toxic to tumors.

TNF- α is identical to **cachetin**, a protein that suppresses completely the lipoprotein lipase of adipose tissue and is believed to be responsible for **cachexia**, a condition of general ill health, malnutrition, weight loss, and wasting of muscle that accompanies cancer and other chronic diseases. Nevertheless, TNF- α may be overproduced in obesity as well. It has been suggested that abnormal production of TNF- α may induce cachexia while abnormal action of the cytokine may cause obesity.²³³ Some TNF receptors have “death domains” and trigger apoptosis, while other receptors promote proliferation and differentiation via transcription factor NF- κ B.²⁴²

Other cytokines with lymphocyte-regulatory functions are IL-5, IL-10, IL-12, and the **macrophage migration-inhibition factor** (MIF). IL-10 is secreted by B cells, T cells, keratinocytes, monocytes, and macrophages. It suppresses synthesis of many cytokines but stimulates growth and activity of activated B cells.²⁴³ IL-12 is formed by monocytes, macrophages, neutrophils, and dendritic cells. It activates T cells and NK cells, is a very potent stimulator of IFN- γ formation, and also inhibits angiogenesis in tumor cells. It stimulates defenses against a wide range of infectious

diseases caused by bacteria, fungi, protozoa, and worms.^{244,245} The 115-residue MIF is formed not only by the immune system but also by many other tissues. The first lymphokine to be discovered, MIF, inhibits migration of macrophages and is also a mediator of toxic shock.^{246,247} MIF is also an enzyme, a **phenyl-pyruvate tautomerase** (p. 692).²⁴⁷

The fourth group of cytokines are involved in hematopoiesis and control the developmental steps portrayed in Fig. 31-2. They are discussed in Chapter 32.

The **acute-phase response** consists of increased production of a group of plasma proteins in response to tissue injury or inflammation.^{229c} Important acute-phase reactants are the **C-reactive protein**,^{229a,248,249} **serum amyloid A**,^{250,251} **haptoglobin**, **hemopexin**, **α 1-acid glycoprotein**,²⁵² and **α 2-macroglobulin**.²⁵³ The C-reactive protein precipitates pneumococcal polysaccharides in the presence of Ca^{2+} . It is present in primitive invertebrates and may serve as a rudimentary immunoglobulin.²⁴⁸ Serum amyloid A is one of the apolipoproteins associated with high-density lipoproteins (Chapter 21, Section A). Its concentration may increase as much as 1000-fold during the acute-phase response, and during prolonged stress it may precipitate as extracellular amyloid fibers. This secondary amyloidosis is sometimes a severe pathological problem. The level of the general protease inhibitor α 2-macroglobulin can increase several hundredfold.

D. Organizing the Immune Response

A person's immune system must be able to respond to a large variety of foreign antigens without reacting against the individual's own tissues. The huge variety of antibodies that can be formed arise from the existence of B cells with millions of different sequences in their antibody genes. When an immune response occurs only a few B cells are stimulated to proliferate, and it is these selected clones that provide the needed specific antibodies and memory cells. However, it is not immediately obvious how we avoid a disastrous attack of the immune system triggered by the many antigenic determinants (**epitopes**) present in our own cell surfaces and macromolecules. Part of the answer is that the immune system "learns" early in life what is self and what is nonself. Thus, while foreign tissues cannot usually be grafted without rejection, cells of two immunologically incompatible embryos can be mixed at a very early stage of development, and an animal tolerant to both types of cell will develop.

A full understanding of self-discrimination is not yet available.^{253a} The adaptive system, as generally understood, is outlined in the following pages. A current view of the innate system is presented by Medzhitov and Janeway.^{11a} However, an alternative

description, the **Danger model**, is being developed by Matzinger. Her view is that the immune system is designed not so much to recognize *nonself* as to send *alarm signals* from injured tissues.^{11b} Most of the basic mechanisms of the adaptive immune system are not in dispute, but many hard-to-explain phenomena remain uncertain.

1. Coreceptors and the B-Cell Response

Early in life most B cells that would produce antibodies directed against a person's own tissues (auto-reactive B cells) are eliminated or altered to reduce their reactivity.²⁵⁴ When functional B-cell receptors do bind an antigen, the B cell will not be activated unless **coreceptors** also bind to the antigen-bearing particle. The transmembrane glycoproteins known as CD22, CD21, CD72, and $\text{Fc}\gamma\text{RIIb}$ are among the many coreceptor molecules. Coreceptors often induce tyrosine phosphorylation of internal receptor domains and attract other molecules to form a signaling complex that may release cytokines.^{255–256} The coreceptors ensure that an immune response doesn't take place without at least two signals. They also help to localize the immune response.

Among the most important factors in B-cell activation are the effects of T cells. B cells can independently mount an attack using IgMs against surface antigens. However, B-cell responses to many antigens, e.g., those present on flagella or inserted into membranes, are also dependent upon assistance from helper T cells (T_H cells).²⁵⁷ These cells also have a major role in determining the longer term fate of B cells. Upon activation B cells may survive or die via apoptosis. They may proliferate (clonal expansion) and differentiate into plasma cells or may become unreactive (**anergy**). They may become long-lived memory cells.

2. The Leukocyte Differentiation Antigens

Before discussing T-cell responses it seems appropriate to mention the nomenclature of molecules (largely glycoproteins) that have been recognized as antigens present on leukocyte surfaces. These same molecules are found on other cells, but the designation of the antigens by a **cluster of differentiation** number, such as CD1, CD4, or CD8, has provided a convenient way of distinguishing different types of leukocytes.^{258–261} For example, helper T_H cells are usually CD4^+ , carrying predominantly CD4. Cytotoxic T cells are predominantly CD8^+ .^{262,263} Both CD4 and CD8 consist largely of Ig-like domains. CD4 is a 55-kDa transmembrane protein with tyrosine kinase activity.²⁶⁴ It is a monomer containing four Ig-like domains, but CD8 is a disulfide-linked $\alpha\beta$ dimer.²⁶³

Not all CD molecules are related to IgG. Proteins are often designated by a specific name followed by a CD number, e.g., Fc γ RII / CD32, ICAM-1 (CD54).

3. Functions of T Cells

T cells carry the responsibility of identifying antigens as foreign or as belonging to self. They do this in immunological synapses (Fig. 31-11) in conjunction with the major histocompatibility complex MHC (Section 5). T cells circulate through the body searching for antigens that indicate danger to the body. To avoid being swept through the bloodstream too rapidly and to be able to enter the lymphoid organs lymphocytes form tethers with adhesion molecules such as the **selectins** (p. 188).²⁶⁶ They then roll more slowly to their destination. Within the lymphoid tissues the T cells may form synapses with activated B cells, dendritic

cells, and macrophages. Within these cells proteosomes generate a stream of peptide fragments, some of which arise from phagocytosed pathogens. These foreign peptide fragments are displayed on the cell surfaces as complexes with type II proteins of the major histocompatibility complex (MHC; see Fig. 31-13). The complexes are checked by CD4⁺ T cells, some of whose **T-cell receptors** (TCRs) will probably be complementary to the surfaces of the complex of the class II MHC protein and the foreign peptide. The T cell will recognize two things about this complex: the MHC protein is of *self* origin but the antigen is *foreign*. The CD4 on the T cell must also bind to the MHC on the surface of the antigen-presenting cell. Other costimulatory interactions may be needed as well.^{50,267} Both CD4⁺ and CD8⁺ T cells tend to bind to oligomeric **activation clusters** of receptors within the immunological synapses.^{267a} Other proteins also participate in assembly of these activation complexes.^{267b} Of

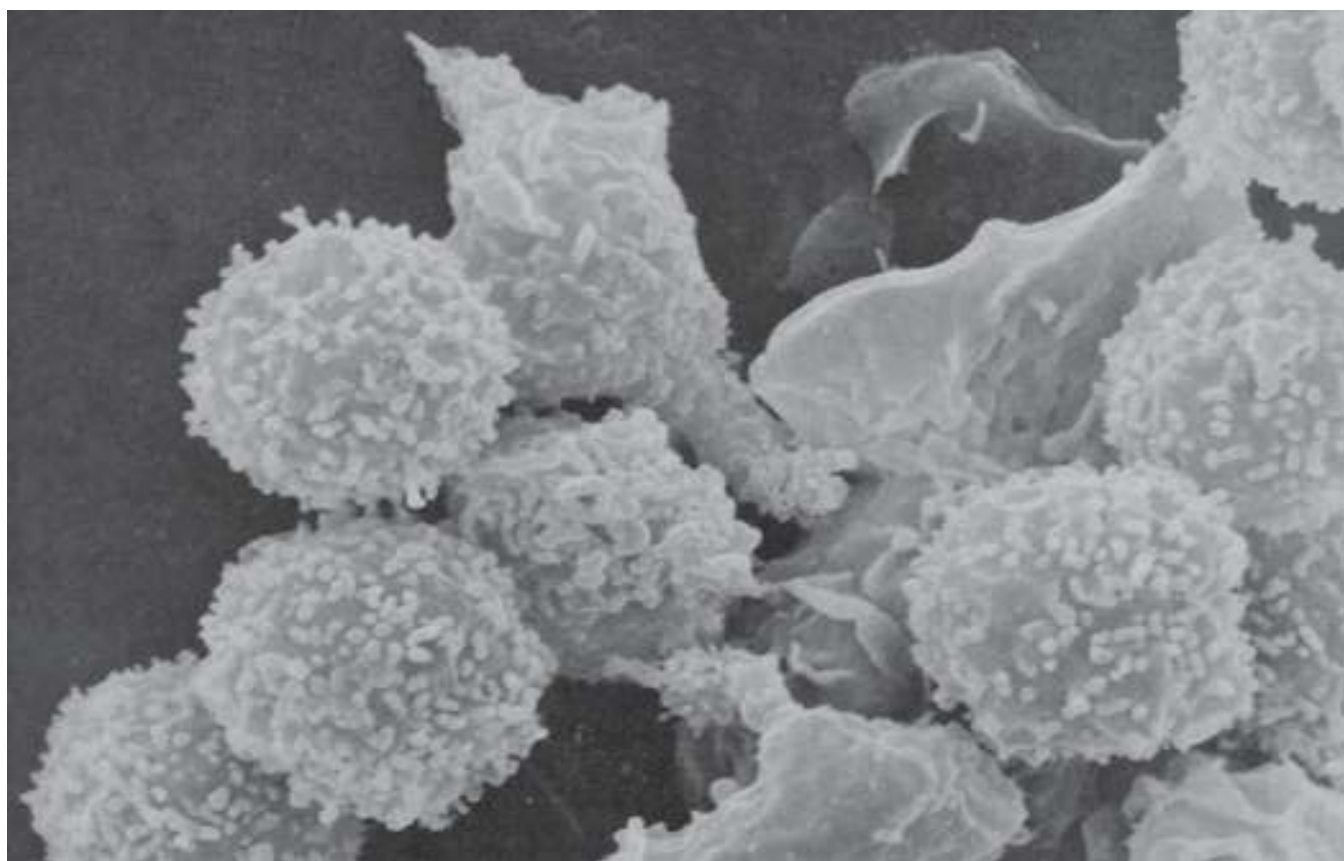


Figure 31-11 The T lymphocytes seen here are forming synapses with the large flat macrophage in the center. The macrophage is displaying antigenic peptide fragments bound to molecules of the major histocompatibility complex (MHC). Most T cells carry their own specific type of receptor. If it is complementary to a displayed antigen fragment it will bind, and the T cell will respond. Depending upon what other coreceptors are activated, it may become a T_H helper cell, or a cytotoxic T cell, or it may become inactive. Micrograph from Grey *et al.*²⁶⁵ Scanning electron micrograph courtesy of Morton H. Nielsen and Ole Werdelin, University of Copenhagen.

particular interest is the recognition of an antigenic peptide produced by a B cell. The B cell has probably already recognized and phagocytized a foreign protein and is displaying peptides from that protein on its MHC I molecules. Recognition of this peptide complex by a CD4⁺ T cell will stimulate the cell to become a T_H helper cell, which will in turn stimulate the B cell to proliferate and differentiate into a clone of as many antibody-forming plasma cells. The essential nature of the costimulation by CD4 is emphasized by the fact that infection by HIV-1, which is mediated by CD4, leads to loss of CD4 from plasma membranes and to the weakening of the immune response toward various pathogens that is characteristic of AIDS.^{268–270}

In a similar manner CD8⁺ T cells recognize peptide fragments displayed on MHC class I molecules. These fragments arise via a somewhat different pathway that forms fragments of viral proteins or proteins of other intracellular pathogens. Recognition by a CD8⁺ T cell usually converts it into a **cytotoxic (killer) T cell**, which will kill the infected cell.^{270a,b} This type of immune reaction was first recognized by the phenomena of **delayed hypersensitivity** and of **transplantation immunity**, i.e., the rejection of transplanted tissues. Both phenomena are caused by cytotoxic T cells. In delayed hypersensitivity they appear to be confused and to attack host cells.

Some very hydrophobic antigens are presented by neither a class I nor a class II MHC molecule but by members of the CD1 family, leukocyte surface proteins that are not encoded in the MHC gene region.^{266,271,272}

4. Natural Killer Cells

An additional line of defense is provided by **natural killer cells** (NK cells), a type of circulating lymphoid cell able to kill cancer cells, to participate in antiviral defenses, and to help control immune responses.^{273–276} NK cells, which utilize their own signaling pathways, are also able to use MHC class I molecules to recognize and to spare the lives of normal, healthy cells.^{277,277a,b} Partial deprivation of a night's sleep can reduce NK cell activity, damaging the cellular immune response.²⁷⁸

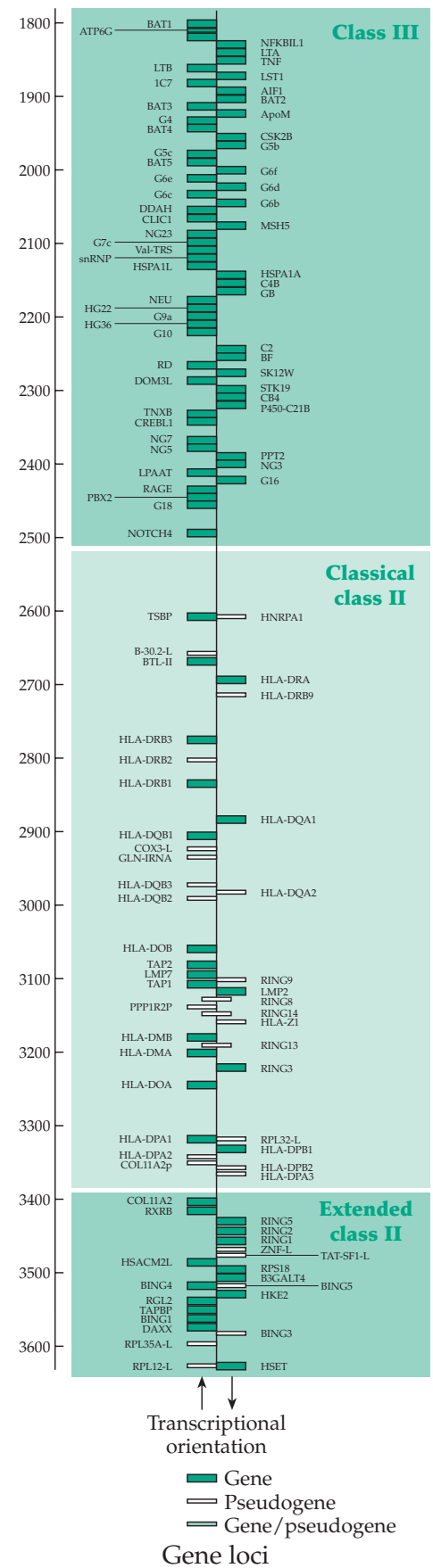
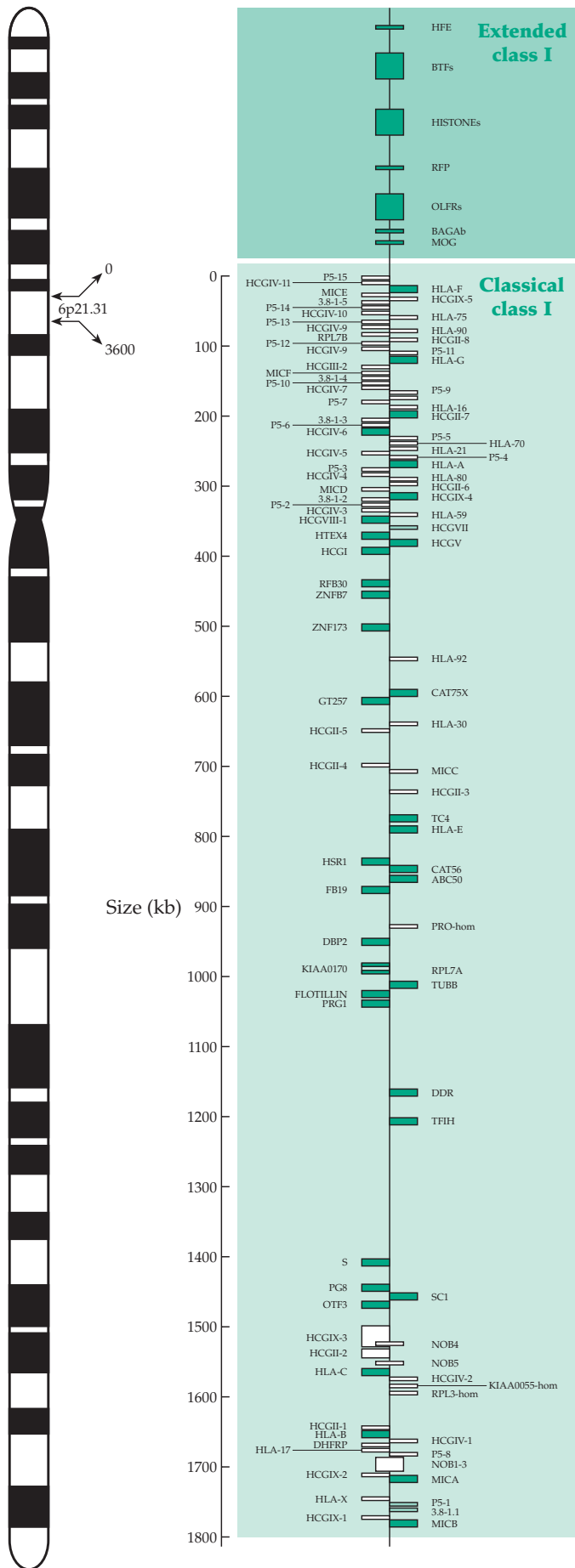
5. Identifying Self: The Major Histocompatibility Complex

Proteins encoded by a single cluster of genes are known as the **major histocompatibility complex** (MHC).²⁷⁹ These proteins, which are essential to T-cell function, were first recognized as the primary determinants of the compatibility of grafted tissues with the host's immune system.²⁸⁰ A lack of histocompatibility can be disastrous. Not only are grafted tissues rejected but T lymphocytes from the grafted tissues sometimes proliferate, attack, and kill the host. The MHC of mice is usually referred to as the **H-2 complex**^{281,282} and that of humans as **leukocyte locus A (HLA)**.^{283,284} Although the MHC is the most important determinant of histocompatibility, differences in other genes may also lead to a slow rejection of transplanted tissues. Since there are many different MHC genes, transplantation is successful only within inbred lines.

Some of the MHC genes have a large number (50–100) of alleles. So great is this genetic polymorphism that it is extremely unlikely that two individuals will have an identical set of histocompatibility genes. The MHC (HLA) genes are located in a 2-centimorgan (~3.6 kb) region of the short arm of human chromosome 6 (Fig. 31-12)^{284,285} and on chromosome 17 of mice. These genes are of at least three classes. **Class I genes** (called HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G in humans; see Fig. 31-12) encode the major transplantation antigens, which are found on the surfaces of nearly all cells of the body. **Class II genes** encode proteins found largely on the membranes of B lymphocytes, macrophages, and dendritic cells. They are designated HLA-DP, HLA-DN, HLA-DM, HLA-DQ, HLA-DR, and HLA-DO.²⁸⁶ **Class III genes** encode several components of the complement system. Many other genes and pseudogenes are interspersed with those of the MHC.

All type I MHC molecules are integral membrane glycoproteins each of which is composed of a 45-kDa heavy chain of about 350 residues together with a noncovalently linked 17-kDa light chain. The genetic variation occurs in the heavy chain between residues 43 and 195 in the human proteins. This chain appears

Figure 31-12 (Opposite page) Arrangement of genes of the human major histocompatibility complex (MHC). Left: Banding pattern of a stained chromosome 6 with the MHC region marked. Center and right: locations of all genes and pseudogenes in this region. The MHC molecules can be divided into three classes on the basis of their structure and function. The class I antigens constitute a single class structurally but fall into two functional groups. The first of these contains the “classical” class I antigens, first discovered as the transplantation antigens and now known to function as target antigens in the recognition and destruction of virus-infected cells by cytotoxic T lymphocytes. They are expressed on virtually all somatic cells. The class II antigens are expressed largely on B lymphocytes and macrophages of the immune system and are essential for presenting antigen to the helper and suppressor T cells that regulate the immune response. Many class III products are components of the complement system. These maps are based on serological and biochemical data, together with complete sequences. From the MHC sequencing consortium.²⁸⁴



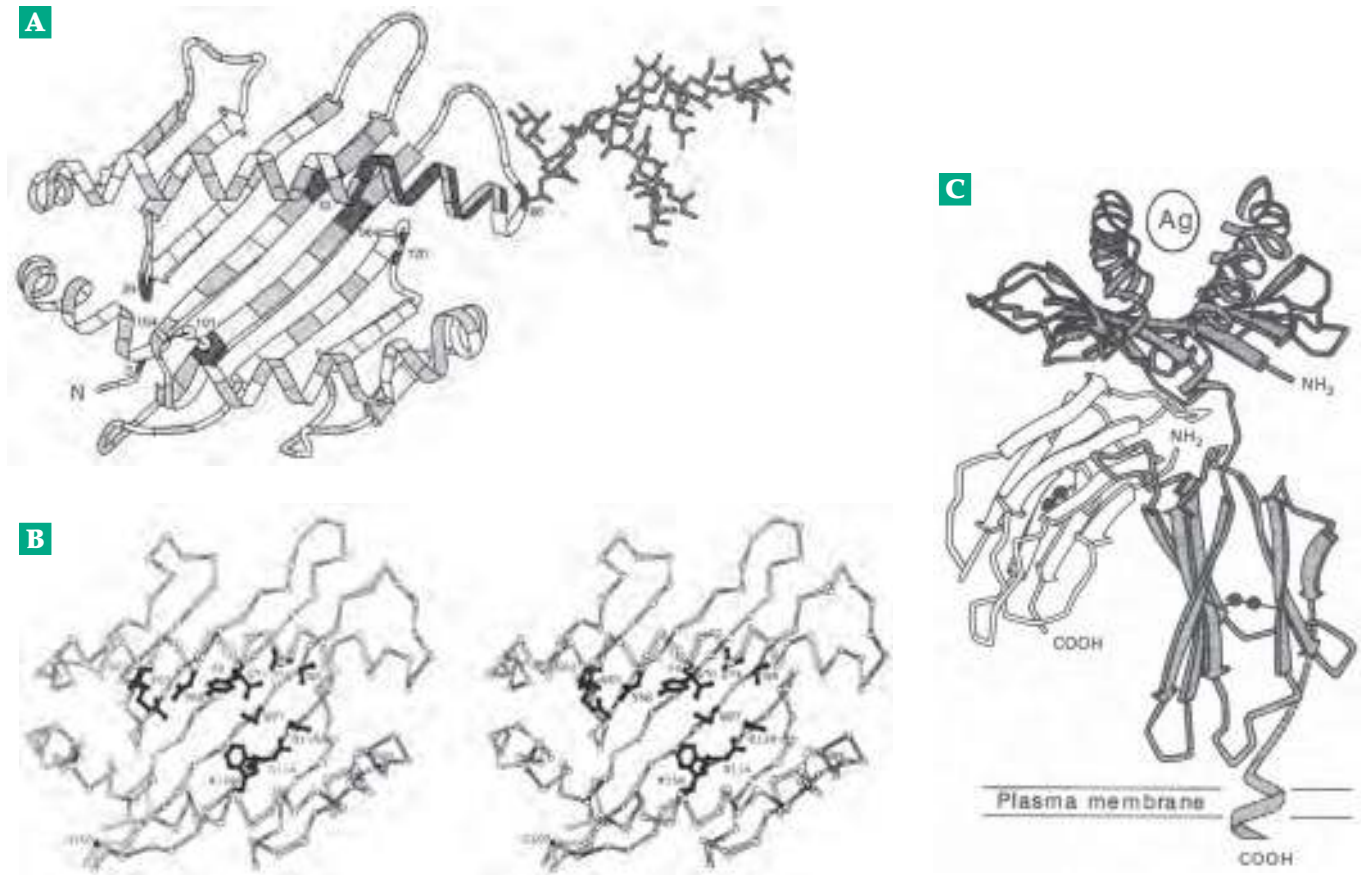


Figure 31-13 The structure of Class I MHC molecules. (A) The specificity pocket in the N-terminal part of the ~360-residue α chain. The numbered residues are invariant in all of the ~20 different Class I molecules. An oligosaccharide is shown on the invariant Asn 86. (B) A stereoscopic view of a similar MHC molecule showing some of the polar residues that protrude into the peptide-binding groove and may form hydrogen bonds with the peptide. From Garrett *et al.*²⁹¹ Courtesy of Don C. Wiley. (C) Side view of a complete MHC molecule with an antigenic peptide (Ag) bound into the peptide-binding groove. The C terminus of the long ~360-residue α chain is in the cytoplasm of the displaying cell. The small 99-residue β chain (unshaded) is a molecule of β microglobulin, which is also a constituent of blood plasma.²⁹² Courtesy of Peter Parham.

to consist of three ~90-residue domains protruding from the outside of the cell, about 25 residues embedded in the membrane, and a short C-terminal tail in the cytoplasm (Fig. 31-13).²⁸⁷ The light chain has an invariant composition and is identical to the plasma protein β_2 -microglobulin, whose gene is located on a different chromosome. Its structure closely resembles that of a single immunoglobulin domain.²⁸⁸ The MHC Class II antigens (Fig. 31-14) are also $\alpha\beta$ dimers, the α chains being 34-kDa glycoproteins and the 28-kDa β chains being larger than in the type I antigen.^{283,289} While the MHC of humans and mice have been studied the most, all vertebrates possess similar self-identification systems. Although both the sequences and the folding patterns of the MHC antigens (Figs. 31-13, 31-14) are somewhat similar to those of immunoglobulins, there are many differences. Furthermore, the cause and significance of the polymorphism is quite different in the two cases. Each individual has millions of antibodies with differ-

ent variable regions but only one set of HLA antigens, which are largely the same on germ, embryonic cells, and adult cells.

Serological tests allow tissue types to be defined by the HLA genes.²⁹⁰ Thus the commonest HLA type in Caucasian populations is HLA-A1 / B8 / Dw3, whereas A1 / B17 is common among Asian Indians. In every case subtypes can be defined, and this fact together with the polymorphism in other genes leads to a unique HLA type for nearly every individual. As is indicated in Fig. 31-12, complete nucleotide sequences are known for typical alleles of all of these genes.²⁸⁴ It is of medical interest that the susceptibility of an individual to many degenerative diseases is determined in part by the HLA type.^{289,295} Thus among patients with a kind of arthritis, **ankylosing spondylitis** that affects 1 or 2 per 1000 men of Caucasian origin, 96% have the HLA-B27 antigen. Of patients with **celiac disease**, a type of intolerance to gluten, 60% have the HLA-B8 antigen. Persons with

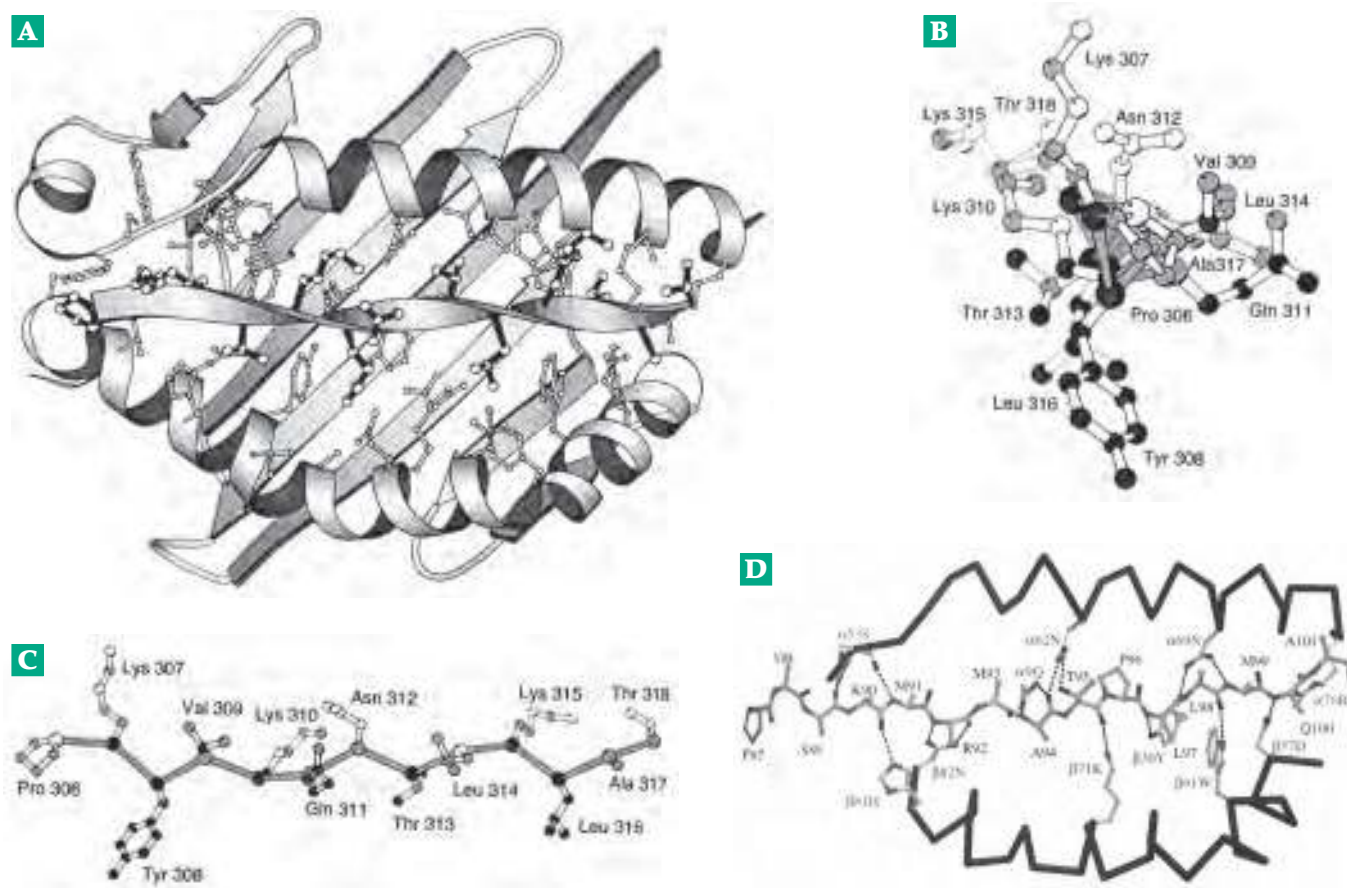


Figure 31-14 Illustration of the binding of a short peptide fragment (central ribbon) into the antigen-binding groove of an MHC type II molecule. (A) The binding groove of molecule HLA-DR1 with a bound peptide (HA) derived from an influenza virus. (B) End view of the bound peptide. (C) Side view of the same peptide. From Stern *et al.*²⁹³ (D) A similar HLA molecule (HLA-DR3) with the peptide CLIP (class II associated invariant chain peptide) bound into the antigen-binding groove. The binding is almost identical to that in (A). Notice the specific hydrogen bonding to side chains of the HLA molecule. From Ghosh *et al.*²⁹⁴ Courtesy of Don C. Wiley.

HLA-Bw17 and B13 have an increased susceptibility to **psoriasis** and those with HLA-DRw4 an increased tendency to develop **rheumatoid arthritis**. Susceptibility to **autoimmune insulin-dependent (Type I) diabetes** is strongly correlated with the presence of the neutral residues Ala, Val, or Ser at position 57 of the HLA-DQ β chain.^{296,297} However, aspartate in position 57 protects against the disease. It may prevent development of a dangerous autoantibody to this cell surface protein. The presence in populations of both humans and apes of a balanced polymorphism among the residues Ala, Val, Ser, and Asp at this position suggests an essential evolutionary origin to this disease susceptibility.²⁹⁷ HLA-B53 protects against severe malaria in Africa. Other diseases with a strong association with HLA type include multiple sclerosis, **Crohn disease** (inflammatory bowel disease),²⁹⁸ and several diseases induced by infections with viruses, bacteria, trypanosomes, etc. For example, arthritis can follow infection by *Salmonella*. This suggests that the killer T cells can be confused

when stimulated by foreign antigens, which are too closely related to the HLA antigens of the host.

6. Antigen Presentation and MHC Restriction

T cells usually do not respond to intact antigens on cell surfaces but only to partially degraded antigens. Antigen-presenting cells (APCs) of various types must process the antigen through endocytosis and partial digestion before the foreign antigen can bind as an MHC complex to a T-cell receptor. Apparently the processed foreign antigen must lie in the binding site of an MHC Class I or Class II chain (Figs. 31-13C, 31-14) before the T cell will recognize the complex and respond.

Antigen processing begins with cytosolic proteasomes that are present in all cells (Box 7-A; Chapter 29, Section D,8). They cleave proteins of the cell and of intracellular parasites into short peptide

fragments,^{55,299–301} which may need to be trimmed to shorter 8- to 11-residue peptides suitable for binding into the groove in an MHC Class I molecule.^{302,303} The peptides are carried into the ER with the aid of the **TAP** (transporter associated with antigen processing) complex,^{304–305a} which is discussed briefly in Chapter 29, Section D.3. Chaperones, such as hsp70, may also participate in the transport. In contrast, MHC Class II proteins receive their antigenic peptides via an endosomal–lysosomal pathway. Proteins from phagocytized pathogens are cleaved by proteases in an endosome or lysosome into fragments that tend to be longer (13–25 residues) than the 9- to 11-residue peptides generated by proteasomes.^{299,306}

Peptides bind into the groove in a Class I MHC molecule in a manner similar to that illustrated in Fig. 31-14 for a Class II MHC molecule. However, in the Class II complex the longer peptides extend from the two ends of the binding groove. The peptide, which assumes a polyproline II helical conformation, is held by hydrogen bonds from the Class II MHC molecule to the peptide backbone.^{307–311} A single peptide may shift and bind in a different register with the possibility of being recognized by a different T cell receptor, when it is displayed.³⁰⁸ In contrast, MHC Class I molecules bind best to 8- or 9-residue peptides, which are held by an array of hydrogen bonds to the $-\text{NH}_3^+$ and $-\text{COO}^-$ termini (not shown in Fig. 31-13, which displays the empty binding groove). The central part of this groove contains a deep pocket, which together with smaller pockets near the ends provides specificity.^{312–318} However, there is a puzzle. Because of the great genetic variability in the MHC genes there will be great differences (polymorphism) in the shapes of the binding pockets among different people. However, an individual has at most six different kinds of MHC molecules. Yet, a single MHC molecule has been estimated to be able to bind more than 10,000 different peptides.³¹⁴ Essential to this process is a final trimming of the peptides at their N termini to provide better fits.^{318a}

Peptides are loaded onto Class I MHC molecules, while they are together in the ER. They move as tightly bound complexes through the Golgi and into the external plasma membrane, where they remain tethered via the MHC molecule (Fig. 31-13C). In contrast, binding to Class II MHC molecules occurs in the endosomes or lysosomes. The process is somewhat complex. Class II MHC molecules are chaperoned from the cytosol into late endosomal/lysosomal organelles with their antigen-binding grooves occupied by a peptide fragment known as the Class II-associated invariant chain peptide (CLIP; Fig. 31-14D). This is cut from the end of an invariant chain known as Ii.^{319–321}

The MHC proteins HLA-DO and HLA-DM (Fig. 31-12) are resident in the lysosome-like organelles and chaperone the class II molecules, until they are ready for loading with peptides. HLA-DM assists in removal

of the CLIP peptide when loading occurs.³²² An asparaginyl endopeptidase may also be required³²³ as well as a disulfide reductase.³²⁴ After loading the Class II MHC•peptide complexes, like the Class I MHC•peptide complexes, are exported to the plasma membrane. There they may be recognized by a T cell, which utilizes its T-cell receptor to recognize an antigen and its CD4 or CD8 proteins to distinguish Class I from Class II MHC complexes. The binding of CD4 and CD8 to their cognate MHC molecules has also been described at the molecular level.^{310,325} As mentioned previously, some hydrophobic antigens are presented by CD1 molecules. They also have an MHC-like fold with a large hydrophobic binding groove.²⁷²

An interesting approach to the treatment of autoimmune diseases is design of peptide mimics that bind into the antigen-binding groove of specific MHC proteins. For example, a protease-resistant pyrrolinone–peptide hybrid has been designed to bind to the rheumatoid-arthritis-associated HLA-DR1.³²⁶

An important distinction between B- and T-cell responses is that T cells recognize a foreign antigen only when associated with an MHC antigen of the same type as is carried by the T cell. This “MHC restriction” limits the actions of T lymphocytes. The function of cytotoxic T cells appears to be primarily one of killing virus-infected cells and perhaps cancer cells. MHC restriction ensures that the T cell is attached by its receptor to a Class I MHC molecule belonging to self. The dual recognition ensures that the cell probably contains a foreign antigen and should be killed. The Class I MHC antigens are found on almost all body cells. Therefore, cytotoxic CD8⁺ T cells can attack infected cells of all types. They may kill their target cells by injecting their cell membranes with the complement C9-like cytolytins.

Regulatory CD4⁺ T cells recognize the Class II MHC molecules, which are found primarily on lymphocytes. Thus, the attention of regulatory T cells is directed towards other lymphocytes. In this case, too, MHC restriction enables helper T cells to recognize B lymphocytes as self. If foreign antigen is present so that B lymphocytes have been activated by the binding of a foreign antigen, they will be stimulated by the T_H cells to proliferate and make and release immunoglobulins. This is accomplished in part by secretion of the lymphokine interleukin-2 (Fig. 30-6) and B-cell growth factors. Some T cells become suppressor T cells.

7. T-Cell Receptors

T cells both mediate the recognition of self and also participate in the immunologic response to foreign antigens. Their surface receptors function much like the immunoglobulins that are attached to the surfaces of B cells. The T-cell receptors are $\alpha\beta$ disulfide-linked

heterodimeric glycoproteins (Fig. 31-15) consisting of 40- to 45-kDa α subunits and 42- to 44-kDa β subunits.^{267,327-330a} They are associated in the T-cell membrane with a larger complex called CD3, which contains additional 26- to 28-kDa γ , δ , ϵ , and ζ chains.^{267,331,332} The polypeptides of the CD3 complex have C-terminal cytoplasmic tails that contain tyrosine residues within several immune system tyrosine-based activation motifs (ITAMs; Fig. 31-15). As is illustrated in this figure, the activating antigen is cradled in the binding groove of an MHC molecule attached to the APC (top) with some side-chain and backbone atoms of the peptide available for bonding to the T-cell receptor.^{333,334} Notice that in the synapse the T-cell receptor also makes direct contact with the MHC molecule (Fig. 31-15).

Signaling by an activated T-cell receptor is quite complex. The ITAMs are sites of tyrosine phosphorylation by kinases of the Src family.^{335,336} Another tyrosine kinase, Zap-70 (Fig. 31-15), associates with the C-terminal tails of the disulfide-linked dimer of subunits ζ . It recognizes the phosphotyrosines groups via its SH2 domains (see Fig. 7-30). Zap-70 appears to act

synergistically with the Src kinases.^{332,337,338} The nature of the APC is also of importance. For example, some dendritic cells secrete Il-12, which favors formation of T_H1 helper cells. A second type of dendritic cell induces formation of T_H2 helper cells.^{338a} Dendritic cells may also control growth and proliferation of T cells by regulating the availability of cysteine, which is a nutritional essential for lymphocytes.^{338b} Other effects may result from endocytosis by T cells of occupied T-cell receptor•MHC complexes.^{338c}

A second type of T-cell receptor, the $\gamma\delta$ receptor, is carried by a small subgroup of T cells. It may have a distinct role in generating an immune response to certain microorganisms including *Mycobacterium tuberculosis*.^{339-342a} Like immunoglobulins, T-cell receptors have a great variety of amino acid sequences. They have C-terminal constant domains and N-terminal variable and hypervariable regions. Thus, T cells can bind to a variety of foreign antigens. However, except as a result of autoimmune diseases, they do not attack cells recognized as self unless these cells are infected with a virus or for some other reason carry foreign surface antigens.

Certain bacterial immunostimulatory molecules known as **superantigens** are able to stimulate MHC Class II molecules to activate large numbers of T cells without any assistance of an antigenic peptide, sometimes with disastrous results.^{343,344} Superantigens are a group of related proteins that includes enterotoxins from species of *Staphylococcus*³⁴⁴⁻³⁴⁶ and *Streptococcus*,³⁴⁷ a staphylococcal exfoliative toxin,³⁴⁸ and **toxic shock syndrome** toxins.³⁴⁹ Superantigen molecules don't occupy the peptide-binding groove in the MHC molecule but bind as intact proteins at an external site. They also bind to the variable region of the TCR in the MHC-TCR complex. Attempts are being made to design decoy molecules that prevent binding of a particular superantigen.³⁵⁰

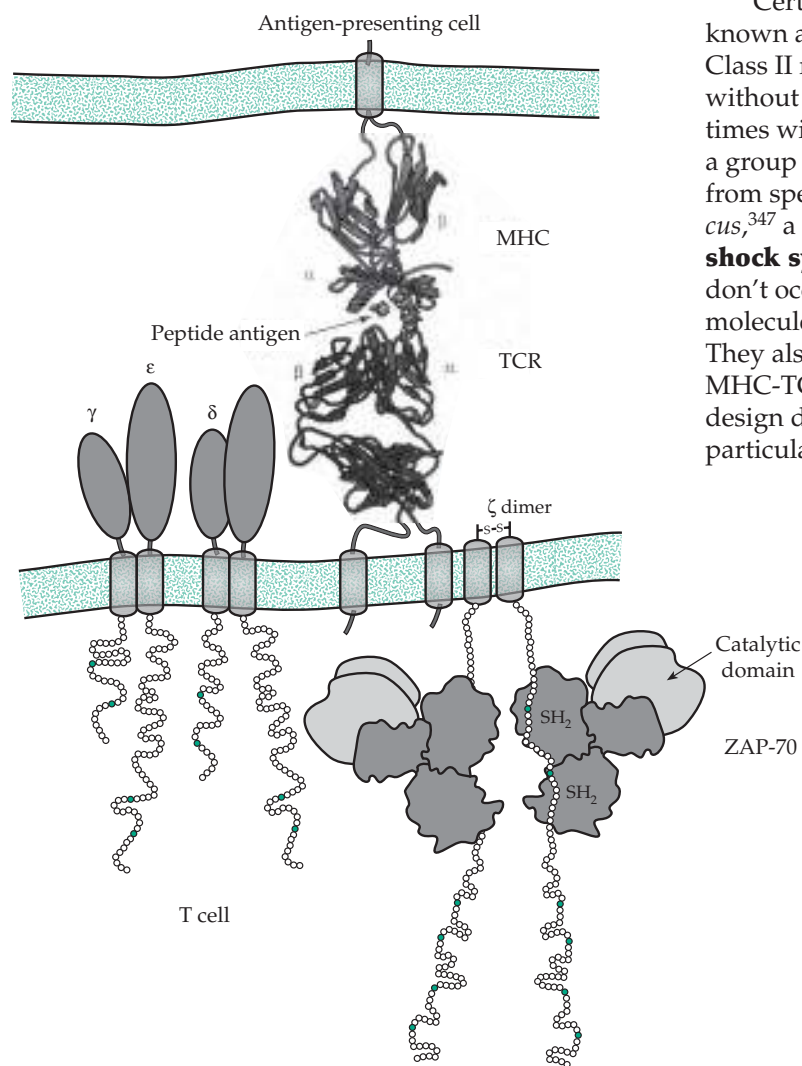


Figure 31-15 Interaction between an MHC•peptide complex on an antigen-presenting cell (APC) with a T-cell antigen receptor (TCR) that is attached to the plasma membrane of a T cell. Structures of the α - and β -subunits of the MHC molecule and of the T-cell receptor are based on crystallographic data. The detailed structures of the disulfide-linked $\sigma\epsilon$ and $\gamma\epsilon$ modules of the T-cell receptor are not shown. The dimeric ζ_2 subunit has large cytoplasmic domains that are thought to be involved in signaling the protein ZAP-70 (zeta-associated protein of M_r 70,000). The σ , ϵ , and γ subunits may also undergo phosphorylation of their tyrosyl groups (gray spheres), which are found in immune system tyrosine-based activation motifs (ITAMs). Drawing modified from those of Cochran *et al.*²⁶⁷ and Hatada *et al.*³³²

8. Self-Tolerance

The immune system is flexible enough and powerful enough to protect us from a great variety of dangers, even from viruses and organisms that may be entirely new. However, it is hard to understand how the immune system completely avoids fatal damage to our own bodies. The answer is complex. It has baffled generations of investigators^{350a,b} and is still not fully understood.

Since the discovery of vaccination in 1796 immunology has claimed the attention of many scientists. However, it was not until 1891 that the German bacteriologist Emil von Behring proposed the term antibody for the protective materials in blood.³⁵¹ By about 1900 Paul Ehrlich and Svante Tiselius, who wrote the first immunochemistry book,³⁵² initiated serious investigations. Ehrlich proposed that binding of an antigen to a surface receptor would induce the cell to make additional identical surface receptors, which would be released to become antibodies.^{351,353} The concept was correct, but it would be many years before knowledge of the structures and biosynthesis of antibodies became available.

Nevertheless, immunological tolerance interested Ehrlich and other immunologists.^{353a} One proposal, offered by Niels Jerne in 1974, was that self-tolerance depends upon **immunological networks**.^{354–356} Consider a lymphocyte bearing a bound immunoglobulin receptor or a bound T cell receptor. It will be specific for some epitope E. The receptors are shown in Fig. 31-16 as having V-shaped antigen-binding sites. Jerne pointed out that the variable region of this receptor will itself carry epitopes that can be recognized by other appropriate antibodies. These epitopes on the

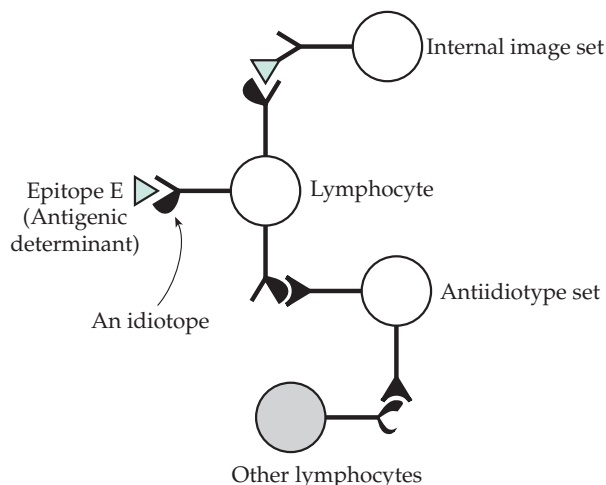


Figure 31-16 Schematic depiction of lymphocyte receptors forming anti-idiotypic and internal image sets as proposed by Jerne.

receptor are called **idiotopes** and as a group define the **idiotype** of that receptor or immunoglobulin.^{356a} There will usually be other lymphocytes with receptors that recognize the idiotype of the first lymphocyte (see Fig. 31-16). These constitute an **antiidiotype set**. In addition there will be lymphocytes, whose idiotopes resemble those of epitope E and which will therefore be recognized by the first lymphocyte as foreign. These lymphocytes constitute an **internal image set**. There will be other sets of lymphocytes that recognize the lymphocytes of the antiidiotype set or of the internal image set. Thus, there will be an elaborate network of clones of interacting lymphocytes. When an immune response occurs many members of this network will respond. A B lymphocyte will recognize a particular antigen and gives rise to a clone of plasma cells making antibodies against that antigen. The body will then make new antibodies against the first antibodies formed, etc. A whole segment of the network will respond in this fashion. Jerne suggested that the overall effect would be to limit and suppress the immune response. Anti-idiotypic antibodies as well as anti-anti-idiotypic antibodies have been prepared³⁵⁶ and have been used in studies of receptors.³⁵⁷ However, Jerne's theory is generally regarded as incorrect or at least a great oversimplification.³⁵⁸

A process of **clonal selection**³⁵⁹ is now thought to be basic to self-tolerance. Credit for the theory, developed in the 1950s, is usually given to F. M. Burnet. However, Ehrlich, Jerne, and David Talmage were also prominent contributors.^{37,351,359,360} An essential postulate of the clonal selection theory is that each B lymphocyte is predetermined to make antibodies of only a single specificity. The mechanism of **allelic exclusion**,³⁵⁹ which makes this possible, is described in Section E. Clonal selection can occur because the B cells carry their antibodies as surface receptors. Binding of an antigen provides a signal for clonal expansion. However, during development in the thymus the progenitor B cells carrying self-reactive antibodies are killed by apoptosis. Later, peripheral B lymphocytes also undergo selection by a complex network of signaling and apoptosis.^{361–363} In a similar manner excess lymphocytes that build up during an immune response must be removed.³⁶⁴

The learning of self by T lymphocytes also happens in the thymus early in development, during the first three weeks of life in mice.^{365,366} This again involves selection against potentially autoreactive lymphocytes carrying idiotypes that are also present on the body's own tissues and which have a high affinity for self peptide•MHC complexes. However, T cells with a weaker affinity for a self MHC molecule but a high potential affinity for a nonself peptide are allowed to develop.^{366,367} Only about 1% of the lymphocytes that develop in the thymus emerge as mature T

cells.^{368,369} Others appear to be killed (clonal deletion) or to become unreactive toward antigen.^{353a} The latter enter a state referred to as **anergy**.^{370–373}

As with every aspect of metabolism, homeostasis is essential to the immune system, which must be able to both grow and shrink rapidly.^{373a–c} Antigens, cytokines, apoptosis-inducing signals, immune inhibitor receptors,^{229h} and receptor tyrosine kinases^{373d} all participate in preserving the delicate balance that is required.

9. Immunologic Memory and Vaccination

In 1796, Edward Jenner carried out the first human vaccination. Attempting to protect a teenaged boy from disfigurement and possible death from smallpox, he vaccinated him with material from a cowpox lesion on the hand of a milkmaid. (She had contracted the disease from a cow named Blossom, whose hide hangs in St. George's Hospital in London.)^{353,374,375} Six weeks later he inoculated the boy with virulent smallpox. Fortunately, the boy didn't contract the disease. Today vaccination is in use for more than 70 bacteria, viruses, parasites, and fungi, and the results have been impressive.³⁷⁶ Poliomyelitis has been almost eliminated.³⁷⁷ Smallpox has not been seen for many years, and the decreases in diphtheria, measles, mumps, whooping cough, and rubella have been impressive.

A nagging question is "How long will vaccination last?" One unplanned experiment resulted from two epidemics of measles in the remote Faroe Islands. The first outbreak was in 1781 after which the islands remained free of measles for 65 years. The second outbreak in 1846 affected 75–95 % of the population, but according to a physician who investigated the epidemic not a single one of the many aged people living who had had measles in 1781 contracted the disease a second time.³⁸ This bit of history confirms that immunological memory is sometimes very long-lived (although it doesn't prove that the smallpox vaccination older people received is still good!).^{377a}

Vaccines have been prepared traditionally by use of viruses or organisms killed by compounds such as formaldehyde or by attenuated viruses or live organisms. These are selected for a low degree of virulence after repeated passages through live animals or cell cultures.³⁷⁶ Newer methods utilize purified viral proteins, bacterial capsular polysaccharides, or DNA.^{378–379a} In the future edible vaccines may be produced in plants.³⁸⁰ Nevertheless, it is often very difficult to devise effective vaccines. In spite of 80 years of effort better vaccines against tuberculosis are needed.^{381,382} All efforts to produce an AIDS vaccine have failed.^{375,379} A satisfactory vaccine must activate both B cells and T cells. Activation of the latter may be especially difficult. Continuous development of new strains of bacteria is a problem for vaccination against

tuberculosis and has been an insurmountable barrier to vaccination against AIDS. However, in the latter case it may be possible to use vaccination to prevent an HIV infection from progressing to AIDS with complete destruction of CD4⁺ T cells.³⁷⁹ As the immune system and also diseases become better understood, it is possible that new strategies for induction of specific cytolytic T cells can be devised,³⁸³ e.g., for AIDS³⁸⁴ and even for cancer.³⁸⁵ A current obstacle to development of new vaccines is that pharmaceutical companies view vaccines as unprofitable.^{385a}

We need a better understanding of how memory B and T cells are formed and selected for long-term survival.³⁸⁶ After differentiation and selection in the bone marrow, B cells move to the spleen. These **transitional B cells** within germinal centers undergo further selection to become mature B cells and B memory cells.^{256,387,388} Interactions with cytokines and with coreceptors play important roles. A subset of B lymphocytes may remain in the germinal centers, serving as a kind of stem cell providing new memory B cells continuously.³⁹ **Naive T cells**, which have not yet encountered antigen, travel throughout the body but apparently don't enter nonlymphoid tissues. However, after being presented with antigen by dendritic cells within lymph nodes, some T cells move to the skin and other peripheral locations.^{389,390} After a pathogen is destroyed most of these T cells die, but a few remain as long-lived memory cells. These are able to respond to a second encounter with a pathogen.^{40,383,391} Apparently continuous new exposure to antigen is not needed for long-term immunity, but the slowly dividing CD8⁺ memory T cells may require continuous stimulation by IL-15 to counteract inhibition by IL-2.³⁹²

E. The Rearranging Genes for Immunoglobulins and T-Cell Receptors

An impressive example of the kind of permanent changes that can occur in the genome of specialized cells is provided by the genes of immunoglobulins and T-cell receptors. B lymphocytes make tens of millions of antibodies of differing sequence, and T lymphocytes make a similar number of different T-cell receptors. This diversity is established in major part in the DNA but also by alternative splicing and editing of RNA.

1. Rearrangements of Germline DNA

Each ~110-residue domain of an immunoglobulin is encoded by a single exon, but the exon for the amino-terminal or variable domain is assembled from two or three small genes (or segments) selected from a large family of such genes present in the germ cells and in the lymphoid progenitor cells.^{393–396} Within the

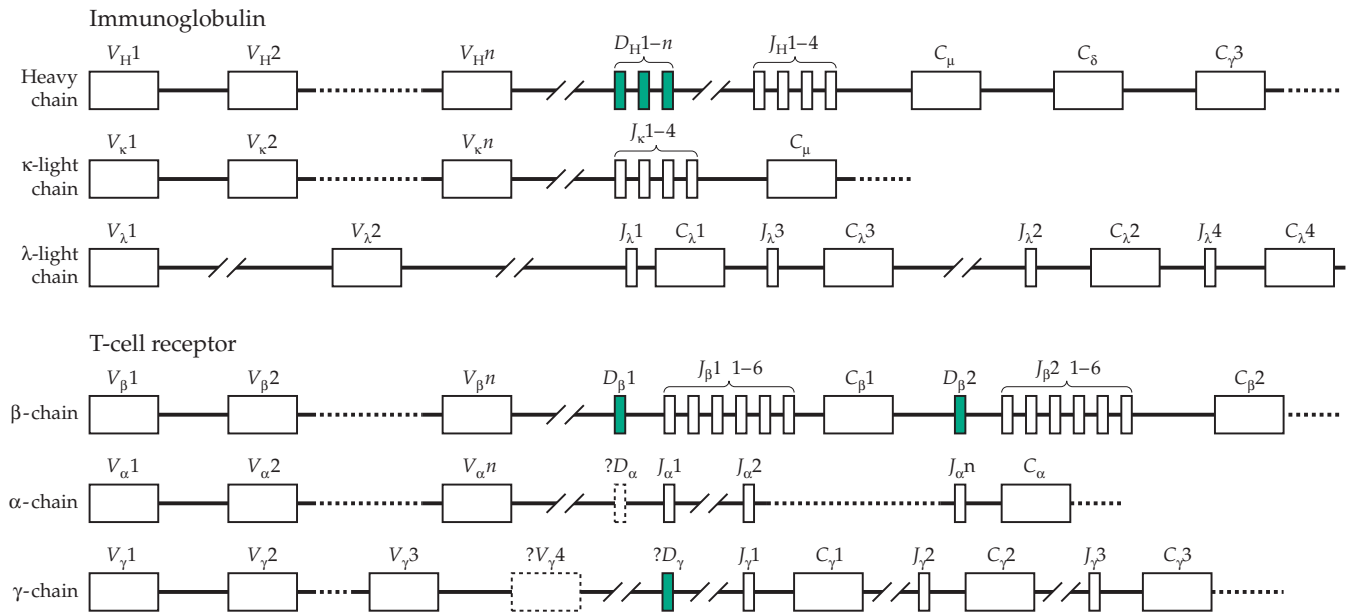


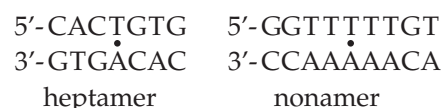
Figure 31-17 Organization of the immunoglobulin and T-cell receptor gene families of the mouse. The human γ -gene pool is larger as is the λ light-chain immunoglobulin gene pool. All six gene pools contain separate gene segments encoding the variable and constant regions of the antigen receptors of lymphocytes. In the course of lymphocyte development, one of the V segments is juxtaposed by chromosomal rearrangement with one of the J segments and, where applicable, a D segment to form a complete variable-region gene. Each V segment has two regions of hypervariability, which are known in the case of the immunoglobulins to contribute to the antigen-binding site in the folded molecule. A third hypervariable region, which also contributes to the antigen-binding site, is generated by the junction of the V segment with the J or the D and J segment(s) at recombination. There is more germline diversity in the T-cell receptor than in the immunoglobulin gene pools in the J segments. The β -gene pool is organized in a way that also allows more combinatorial diversity. The β -gene pool contains fewer J regions than the gene pool, but it has two distinctive features that allow for exceptional diversification during somatic rearrangement. First, the 'rules' for recombination allow in principle the joining of both D to D segments and V segments directly to J segments, neither of which is possible in the immunoglobulin heavy-chain pool. The D segments of the β genes can be read in any of the three possible reading frames, so that varying the site of the V–D junction alone can make a substantial contribution to the diversity of the third hypervariable region. From Robertson.³⁹⁷

V region the three short hypervariable segments alternate with four framework segments that have a more nearly constant structure. The V region of the light-chain genes (of either the κ or λ type) is put together in part from a V gene that encodes an approximately 95-residue sequence making up the first three framework regions plus two hypervariable regions and part of the third hypervariable segment. There are ~100 different V_κ genes and ~30 V_λ genes^{398–400} in the light-chain family. The similar arrangement of genes in the mouse is indicated in Fig. 31-17. These V genes are spaced at intervals of 14–30 kb within the DNA.

The rest of the third hypervariable region and the fourth framework region of the κ and λ chains are encoded by short **J (joining) genes** that specify ~15 residues. There are about five J_κ and three J_λ genes in the mouse. The first mechanism for creating antibody diversity lies in the large number of V genes, which are especially diverse in their hypervariable regions. The second mechanism is the joining of any one of these V genes with any one of the J genes, the joining taking place within the third hypervariable region.

There are also at least six different constant (C_λ) genes in the human genome.

The heavy-chain genes are more complex. There are over 200 V_H genes located at 14- to 16-kbp intervals, which are followed by ten ~15–17 bp **D (diversity) genes** and 4–6 J_H genes. During the differentiation of the lymphoid stem cells pre-pro-B cells can be identified in which the V, D, and J genes are still separate. Later there are pro-B cells containing joined DJ_H segments, then pre-B cells with joined VDJ_H segments, then B cells with a VJ_L (either κ or λ) segment also joined. The overall process of gene rearrangement, mRNA splicing, and immunoglobulin synthesis is outlined in Fig. 31-18. The joining of the gene segments occurs by recombinational mechanisms that involve 7-bp and 9-bp recognition sequences known as **recombination signal sequences (RSSs)**^{401–402b}:



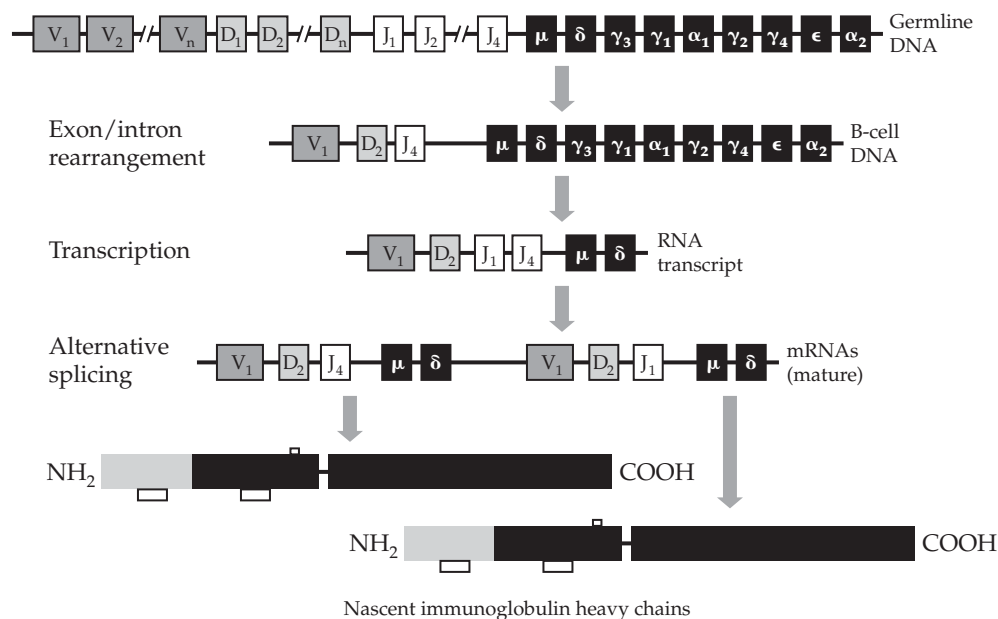


Figure 31-18 Human immunoglobulin heavy-chain gene structure and gene processing. Exons of the heavy-chain genes that encode the variable regions of the immunoglobulin molecule are labeled V_1, V_2, \dots, V_n . Selection from these V exons during embryonic development produces the unique sequences of each B-cell clone. The germline genes for the immunoglobulin heavy chains also contain diversity exons, labeled D_1, D_2, \dots, D_n . Recombination between the V and D regions occurs more frequently than that between the V and J exons in the light-chain exons. Introns between the V and D and between the D and J exons contain signal sequences that regulate synthesis of the Rag1/Rag2 recombinase. This enzyme is responsible for the efficient recombination that gives rise to the epitope-specific B-cell clones with their individual Ig genes. The heavy-chain genes contain exons that encode all of the isotype heavy chains. Class switching, i.e., the change in chain expression that occurs during antibody synthesis after B-cell activation, results from alternative splicing between the J exons and the exons for the various heavy-chain isotypes. Redrawn from Bhagavan.⁴⁰³ Courtesy of N. V. Bhagavan.

The heptamers have twofold rotational symmetry. The recognition sequences adjacent to one or both sides of the coding segments are separated by 12- or 23-bp spacers, and an empirical rule states that joining can occur only when one pair of recognition sequences are separated by 12 bp and the other by 23. Recombination involves cleavage and rejoining of the DNA, a process also used for reorganization of the T-cell receptor genes. The cleavage is catalyzed by a complex of two proteins RAG1 and RAG2, which are encoded by the adjacent **recombination activating genes** *RAG1* and *RAG2*.^{402–406} Cleavage is assisted by HMG chromatin proteins (Chapter 27, Section A.4) and by the level of histone acetylation in an associated enhancer.⁴⁰⁷ In the presence of a divalent metal such as Mg^{2+} or Mn^{2+} a single-stranded nick is made between the 3'-end of the DNA coding region and the heptamer sequence. The released 3'-OH group then attacks the phosphodiester bond on the opposite strand, cleaving it in a transesterification reaction. This leaves the coding sequences capped by a hairpin end and a blunt cut end on the RSS.⁴⁰⁸ The cut ends are apparently held until synapsis with a nonamer RSS further in the 3' direction and rejoining can occur.⁴⁰⁹ Rejoining requires DNA ligase IV.⁴¹⁰ The process resembles that

of transposition (Chapter 27, Section D.4).^{411,412} The details are still uncertain.^{408,413,413a}

The recombinational events are not entirely regular.^{401,404,414,415} There are excisions of pieces of DNA, and additional nucleotides may be inserted randomly through the action of **terminal deoxynucleotidyl transferase**.^{416,417} All of these mechanisms lead to additional diversity. After the rearrangements are completed, the fused VDJ and VJ gene segments are close enough to suitable constant-region (C) sequences that when the genes are transcribed the intervening sequences in the RNA are spliced out to yield the mature mRNAs for light κ and λ chains and H chains.

2. Somatic Hypermutation and Affinity Maturation

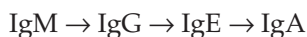
Yet another factor that introduces immunoglobulin diversity is the occurrence of somatic mutation at unusually high rates (**hypermutation**) in the hyper-variable regions. Hypermutation consists largely of point mutations in the V-region gene segment and occurs at a rate 10^5 - to 10^6 -fold higher than for the rest of the genome.^{51,418–423} It occurs after B cells have been

presented with antigen by dendritic cells in the germinal centers in a process called affinity maturation. The result is generation of an enlarged repertoire of B cells, some of which synthesize antibodies with an increased affinity for the antigen. These will be selected for clonal expansion.⁵¹ Further genetic alteration occurs by **gene conversion** (see p. 1566), which involves copying from a homologous segment of DNA, perhaps from a nearby pseudogene.^{423a,b} Some experiments suggested that RNA editing (p. 1642) may also contribute to antibody diversity.^{29,424,424a} However, it appears that the observed deamination of cytosine to uracil rings occurs at the DNA level and is initiated by an **activation-induced cytosine deaminase**.^{424b} The generation of uracil, which may be removed from the DNA by uracil-DNA glycosylase (p. 1579), apparently triggers both somatic hypermutation and gene conversion. Both processes also depend upon DNA repair via homologous recombination or nonhomologous end joining (p. 1581). The error-prone DNA polymerase τ is also needed.^{424c} Class-switching recombination, discussed in the next section, is apparently also initiated by the activation-induced deaminase.^{424d} These are affected by **RNA editing** (Chapter 28).^{29,424} **Receptor editing**, gene rearrangements that occur in the peripheral immune system, also contributes to affinity maturation.^{424,425}

It is important that the genes as finally assembled maintain correct reading frames so that a potentially useful antibody can be made.⁴²⁶ Although lymphocytes are diploid and therefore contain two sets of immunoglobulin genes, a single cell produces only one kind of immunoglobulin (**allelic exclusion**). When the genetic rearrangements produce a light chain able to combine with a heavy chain to form a functional immunoglobulin, a signal may be sent that stops the rearrangements in the other chromosome.⁴²⁷ However, recent findings suggest that one allele may be marked for inactivation early in development, just as one X chromosome becomes inactive in females (Chapter 32).⁴²⁸

3. Immunoglobulin Class (Isotype) Switching

A newly matured B cell produces initially IgM bound to its surfaces. The difference between the C_H domains of bound and secreted antibodies of a given type seems to lie in alternative splicing of the mRNA. More mysterious are the consecutive switches from IgM to other types in the following sequence:



This is the same sequence in which the C_H genes lie (Fig. 31-18). However, the C_δ gene, which codes for IgD, is not utilized in this sequence. A newly matured

B cell transcribes the C_μ gene to give IgM. Later, the class switch occurs, apparently by a looping-out recombination mechanism, allowing a C_γ gene (for IgG) to be expressed.^{429–433} The switches are mediated by tandemly repeated DNA sequences in 1- to 10-kbp switch regions and are controlled in part by cytokines. Other complexities are involved in synthesis of the J chain of IgM⁴³⁴ and in synthesis of IgD. Although the latter is a major surface immunoglobulin on B lymphocytes, its exact functions have been hard to understand. The δ exon of its heavy chain gene is joined to a J exon by alternative splicing of the mRNA (Fig. 31-18).¹¹² In a similar manner, the difference in the surface-bound and soluble forms of IgM arises by alternative mRNA splicing.⁴⁰³

The T-cell receptor gene families are also indicated in Fig. 31-17. Their development is remarkably similar to that of the immunoglobulin genes and involves most of the same mechanism of diversification^{365,397,435} with the exception of somatic hypermutation. The same recombinase may cut the DNA to initiate rearrangements of all of these gene families.⁴³⁶ A single T-cell precursor may give rise to 1000 or more clones with unique β -chain sequences.⁴³⁷ With a total of $\sim 10^6$ different β chains there are potentially $\sim 10^{15}$ unique T-cell receptor structures that could arise from the 42V and 61J segments of the α -chain gene and the 47V, 2D, and 13J segments of the human β -chain gene.⁴³⁸ Allelic exclusion is observed, as with the immunoglobulins.⁴³⁹

F. Disorders of the Immune System

Many things can go wrong with a system as complex as the human immune system. In immunodeficiency disease some component is missing or has been inactivated. In autoimmune diseases the immune system attacks some component of the body. Of the known problems none is more common than **allergy**,^{440–442} which may be described as the inappropriate activation of the immune system by environmental antigens (**allergens**).

1. Allergy

One in 10 persons, ~ 22 million people, in the United States have allergies. Ten million of these suffer from the nasal discomfort of “hay fever” and six million from the more serious **asthma**. Substantial numbers of people in the United States die of allergic reactions to insect stings (more than 30 per year) or to injections of penicillin (300 per year in 1970). Foods, drugs, pollens, mold spores, mites in house dust, and even heat or cold can evoke serious allergic reactions. Among these **eczema** (atopic dermatitis) is very common. A major cause of allergic reactions has been

traced to molecules of immunoglobulins IgE, which bind to the **basophils** in the blood and to the related **mast cells** of tissues. Binding of an antigen to these IgE molecules activates them. These activated antibodies bind (as in Fig. 31-6) to the α subunits of the $F_{c\epsilon}RI$, a transmembrane receptor on basophil or mast cell surfaces.^{108,109,443} If two or more IgE molecules bind to a mast cell, they may aggregate and activate the mast cell to release its histamine-containing granules.^{444,445} The granules also release cytokines and arachidonate, which is converted primarily into prostaglandin D_2 (Fig. 21-7) and into products of the 5-lipoxygenase pathway (Fig. 21-8). The products include the chemotactic leukotriene B_4 and leukotrienes C_4 and D_4 . The latter two constitute the slow-reacting substance of anaphylaxis (Fig. 21-8). The result is a rapid inflammatory response with dilation of blood vessels, increased vascular permeability, infiltration of leukocytes, and destruction of tissues.

What is the normal function of IgE and the mast cells? These cells are located at places where parasites might enter tissues. IgE is involved in killing of schistosomes, and elevated IgE levels are seen in patients infected with various parasites. The killing of schistosomes seems to be mediated by blood platelets as well as by neutrophils and eosinophils with the help of mast cells.^{446,447} Allergic persons often have an IgE level over ten times normal. This high level makes the individual especially sensitive to IgE-mediated reactions, a condition called **atopy** (meaning "strange disease").^{448,448a} Allergies may also be accompanied by increased B cell levels.⁴⁴² This can sometimes be responsible for the sudden and sometimes fatal systemic reaction of **anaphylaxis**. T-cell responses may also cause anaphylaxis.

Most allergy-inducing antigens are proteins, but proteins vary widely in their antigenicity. Only a few natural proteins are major allergens. Many of these are relatively small, with molecular masses of 5–50 kDa. Most are soluble, and some are glycoproteins.⁴⁴⁹ Mites are the closest animals to human life and carry allergens that are among the most important causes of asthma and allergic dermatitis. The allergens are 125- to 129-kDa proteins crosslinked by three disulfide bridges.^{450,451} Cockroaches^{449,452} and other insects also form many allergens. Among them are the hemoglobins of small flies of the chironomid family.⁴⁵³ Studies of the latter suggest that antigenicity may arise both from flexible regions and also from the presence of a preponderance of amino acids with polar side chains.

Proteins of cat saliva dry and flake off as dander, which contains major indoor allergens linked to asthma. Dogs, horses, cattle, and other animals also provide several allergens,⁴⁵⁴ some of which are lipocalins (Box 21-A).⁴⁵⁵ Other close-to-home allergens are provided by fungi that live on skin or nails.⁴⁵⁶

Plants provide a host of allergens. Major allergens

are found in pollen of rye-grass,⁴⁵⁷ of many other grasses, of ragweed,⁴⁵⁸ and of olive trees.⁴⁵⁹ Natural rubber latex would appear to be a harmless high polymer, but it contains antigenic proteins, which have been blamed for 1100 anaphylactic attacks with at least 15 deaths between 1988 and 1992.^{460,461}

Food is a major source of allergens, which are often overlooked. Food allergies may be hard to diagnose and symptoms such as headache, diarrhea, itching, and asthma may be attributed to other causes. However, the occasional rapid death from anaphylactic shock, e.g., from exposure to peanuts,^{461a} is a reminder that unrecognized food allergies exist. About 100–200 persons die annually of food allergies. About 90% of recognized food allergies involve milk, eggs, fish, crustacea, peanuts, tree nuts, soybeans, and wheat.⁴⁶² There are usually only a few allergenic proteins in any one food. Many of these are resistant to digestion in the stomach. Some but not all are compact proteins with multiple disulfide crosslinkages. However, no structural generalization can be applied to all food allergens.

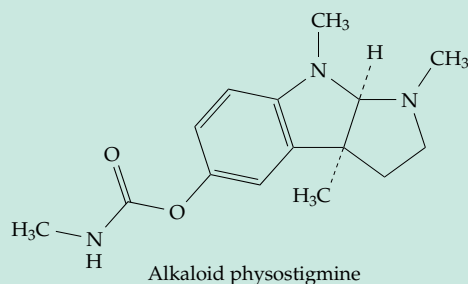
The increasing use of genetic engineering of foods poses both risks and hopes. The accidental incorporation of an allergenic protein into a plant or animal product can make a food previously safe for a person deadly. For this reason, attempts are now made to identify likely allergens and to avoid transferring their genes. However, this process can't be completely reliable, partly because we each have our own personal immune system. This is one reason for requiring accurate labeling of foods. On the positive side recognized major allergens can probably be eliminated by genetic engineering. The muscle protein tropomyosin (Fig. 19-9) is a well-known allergen whose allergenicity varies among different sources. Tropomyosin from beef, pork, and chicken is usually not highly allergenic, but that from shrimp often is.⁴⁶² Perhaps safer shrimp can be created.

Allergies are treated in various ways, often with antihistamines or corticosteroid ointments. Injection of adrenaline is an emergency treatment of anaphylaxis or asthma. Also important is **specific immunotherapy**, better known as "allergy shots."⁴⁴¹ Small amounts of allergen in increasing amounts are injected subcutaneously at intervals to desensitize the patient. Use of purer antigens, which may be engineered to decrease their antigenicity, may provide advances in this technique.⁴⁶³

Asthma is one of the most common chronic diseases in industrialized countries, affecting 10% or more of young children in some countries. An atopic disease with high IgE levels, asthma is induced by small particles of antigen, which are able to penetrate deep into the lungs.⁴⁶⁴ About 80% of asthmatic children are allergic to house mites.⁴⁵⁰ Animal danders are another major cause. The incidence of asthma appears to be increasing in many modern societies, but

BOX 31-D MYASTHENIA GRAVIS

One of the best-understood autoimmune diseases is myasthenia gravis, a condition associated with a decrease in the number of functional postsynaptic nicotinic acetylcholine receptors (Fig. 30-23) in neuromuscular junctions.^{a-e} The resulting extreme muscular weakness can be fatal. Myasthenia gravis is not rare and affects about one in 10,000 people.^c An interesting treatment consists of the administration of physostigmine, diisopropylphosphofluoridate (Chapter 12, Section C,1), or other acetylcholinesterase inhibitors (Box 12-E). These very toxic compounds, when administered in controlled amounts, permit accumulation of higher acetylcholine concentration with a resultant activation of muscular contraction. The same compounds



the reasons are unclear,^{465,466} and the increase doesn't appear to be linked to air pollution. A chronic atopic condition usually precedes an acute attack of asthma. In addition to IgE and eosinophils there are excessive numbers of neutrophils with high-affinity IgE receptors in the airway tissues.⁴⁶⁷ Prostaglandin D₂ released from mast cells may play a role in triggering an attack.⁴⁶⁸ Cytokines,⁴⁶⁹ nitric oxide,⁴⁴⁷ and nerve growth factor⁴⁷⁰ may also participate in the response. The presence of a high concentration of glutathione and of glutathione peroxidase, whose concentration increases in asthmatic lungs, may reflect the action of the antioxidant system in combatting inflammation.⁴⁷¹ The surfactant proteins SP-A and SP-D (Box 8-B) are Ca²⁺-dependent lectins, which serve as regulators of the innate immune response. Their concentrations also increase in asthma.^{472,473}

Treatment of asthma has depended upon inhaled glucocorticoids, quick-acting bronchodilators usually β -adrenergic antagonists, and long-acting beta agonists such as theophyllins and leukotriene antagonists.⁴⁶⁴

2. Autoimmune Diseases

There are numerous **autoimmune diseases** in which the body makes antibodies against its own cells

are widely used in the treatment of glaucoma.

More than 90% of patients with myasthenia gravis have circulating antibodies directed against a subunit of the acetylcholine receptor.^f Immunosuppressive drugs and steroids help to cut down on these autoantibodies, and many patients are benefited by removal of the thymus. Newer approaches involve specific immunotherapy aimed at increasing tolerance to either T cells or to B cells.^{c,d} For example, oral ingestion of purified acetylcholine receptors to desensitize the body's response or inhibition of production of IL-2.

A possible cause for the production of the damaging antibodies may be the sharing of common antigenic determinants between the receptor protein and surface proteins of bacteria such as *E. coli*.^f

^a Fuchs, S. (1980) *Trends Biochem. Sci.* **5**, 259–262

^b Tzartos, S. J. (1984) *Trends Biochem. Sci.* **9**, 63–67

^c Drachman, D. B. (1994) *N. Engl. J. Med.* **330**, 1797–1808

^d Steinman, L., and Mantegazza, R. (1990) *FASEB J.* **4**, 2726–2731

^e Barnes, D. M. (1986) *Science* **232**, 160–161

^f Stefansson, K., Dieperink, M. E., Richman, D. P., Gomez, C. M., and Marton, L. S. (1985) *N. Engl. J. Med.* **312**, 221–225

(Table 31-3).^{363,474–476} In **myasthenia gravis** (Box 31-D) antibodies attack the acetylcholine receptors in postsynaptic membranes.⁴⁷⁷ In **Graves disease** aberrant antibodies are directed against receptors for thyrotropin. They have a stimulatory rather than an inhibitory effect and cause hyperthyroidism.⁴⁷⁸

Childhood onset (Type I) diabetes results from destruction of insulin-secreting cells by an autoimmune reaction triggered by environmental factors in genetically susceptible persons (Box 17-G).^{479,480} The principal autoantigen appears to be the 65-kDa form of glutamate decarboxylase (GAD).^{481–483} While GAD has an essential function in the formation of γ -aminobutyrate in the brain, its role in the pancreatic islets is not clear. What is established is the presence of specific GAD65-stimulated T cells in diabetic individuals. The stimulating autoantibodies, which may appear in the blood years before diabetes is evident, carry HLA-DR4 type surface MHC antigens.^{484,485} After the activated T cells kill enough of the pancreatic β cells, diabetes appears.⁴⁸⁶

Myasthenia gravis, Graves disease, and type I diabetes are organ-specific autoimmune diseases. Another group of autoimmune diseases are systemic, affecting many tissues. For example, in the severe **systemic lupus erythematosus** there are often antibodies against the victim's own DNA.^{487–488a} The

antibodies may then attack any tissue, e.g., the red blood cells. Antibodies against a variety of other nuclear constituents such as histones,²⁰⁷ ribosomal protein L7,⁴⁸⁹ ubiquitin,⁴⁹⁰ enzymes, cardiolipin,⁴⁹¹ and small nuclear RNAs⁴⁹² are also made. The primary defect appears to be an intolerance to chromatin and in particular to nucleosomes.⁴⁹³ Antibodies to nucleolar and other components of nuclei are also present in progressive systemic sclerosis (**scleroderma**).^{494,495} In **rheumatoid arthritis**, a chronic inflammation of joints, the serum and joint fluids contain abnormal complexes, which appear to consist entirely of immunoglobulins. They may be antibody-antiidiotype antibody complexes.^{496,496a} Immunization of animals with type II collagen induces a very similar arthritis,^{496,497} but this collagen probably doesn't supply the offending human antigen. Susceptibility to rheumatoid arthritis is linked to HLA-DR4 class II MHC genes. Molecules of the class II DR4 subtypes may associate with antigenic peptides of uncertain origin to induce a T-cell response. CD4⁺ T cells are thought to drive inflammation in the disease.⁴⁹⁷ Monocytes are attracted from the blood and become inflammatory macrophages.⁴⁹⁸

Another autoimmune disease in which antibodies

attack collagen is **Goodpasture disease**. It is mediated by B cells, which form antibodies directed at the N-terminal domain of the $\alpha 3$ chain of collagen IV (pp. 435–438).^{499–501} The antibodies attack the glomerular basement membranes causing a rapidly progressing glomerulonephritis and also lung hemorrhages. Primary **glomerulonephritis**, a major kidney disease, may be caused by a cross-reaction between the membrane of streptococci and the glomerular basement membranes.

In **Sjögren syndrome** autoantibodies are directed against α fodrin (p. 405).⁵⁰² In primary **biliary cirrhosis** they are directed at mitochondria and specifically to a pyruvate dehydrogenase subunit (Fig. 15-14).⁵⁰³ In the inflammatory muscle disease **polymyositis** autoantibodies are often directed against cytoplasmic proteins including aminoacyl-tRNA synthetases.⁵⁰⁴ In the rare **paroxysmal cold hemoglobinuria** autoantibodies attack red blood cell membranes only when the temperature of an extremity is lowered. **Paroxysmal nocturnal hemoglobinuria**, a serious complement-mediated condition, results from deficiency in the complement decay accelerating factor. This is a result of a defect in the PGI tail on this factor.⁵⁰⁵

Celiac disease (celiac sprue) is an allergic inflammatory condition caused by poorly digested proline-rich sequences of wheat gluten and related proteins (p. 74). The disease is usually not recognized, but it may occur in 3% or more of the United States population. A T-cell response that causes destruction of the smaller intestinal mucosa, celiac disease is characterized by malabsorption and diarrhea.^{505a–c} It can cause death by starvation. A primary target of the autoantibodies is a transglutaminase.^{505c,d}

Most cells of the immune system are ordinarily kept apart from those of the nervous system by means of the blood-brain barrier. However, allergic encephalomyelitis, in which T cells attack the myelin sheath of brain neurons, can easily be induced in mice.⁵⁰⁶ A similar autoimmune process is thought to be involved in human **multiple sclerosis** (see Chapter 30, pp. 1769, 1808, and Fig. 30-9).^{507,508} High levels of circulating IgM are found in some demyelinating diseases of peripheral neurons.⁵⁰⁸ In **Rasmussen's encephalitis**, which causes brain inflammation and epilepsy, serum antibodies attack a glutamate receptor subunit **GluR3**.⁵⁰⁹

The causes of autoimmune disease doubtless lie largely in the difficulty of developing a repertoire of immunoglobulin-forming B cells and of T-cell receptors that will always reliably distinguish self from a foreign antigen. The problem can lie either with B-cell recognition or with the T-cell receptors. Extensive medical use is made of **immunosuppressants** in treatment of persistent allergic reactions, autoimmune problems, and rejection of transplanted tissues. Among these compounds are the steroidal

TABLE 31-3
Some Autoimmune Diseases

Addison disease	Adrenal glands
Ankylosing spondylitis	
Celiac disease	Upper intestines
Crohn disease	Intestines
Diabetes, type I	Pancreatic islets
Glomerulonephritis	Kidney
Goodpasture disease	Kidney
Graves disease	Thyroid gland
Guillain – Barré syndrome	Gangliosides
Multiple sclerosis (MS)	Peripheral myelin
Myasthenia gravis	
Paroxysmal cold hemoglobinuria	Red blood cells
Primary biliary cirrhosis	
Psoriasis	Skin
Polymyositis	
Rasmussen encephalomyelitis	Cerebral cortex
Rheumatoid arthritis	Joints
Scleroderma	Skin
Sjögren syndrome	
Systemic lupus erythematosus	Many tissues
Thyroiditis	Thyroid gland
Ureitis	

BOX 31-E EVADING THE IMMUNE SYSTEM

Parasitic species always have a problem with the antibodies and killer T cells of their hosts, and the chemical makeup of the external coats of parasites tends to reflect this fact.^a An example is provided by **trypanosomes**, which cause sleeping sickness and which make much of Africa unsuitable for cattle grazing.^{b–d} Trypanosomes in the bloodstream evade the immune system by covering the outer surface of their plasma membrane, flagella and all, with a dense 12- to 15-mm thick monolayer of an ~60-kDa **variable surface glycoprotein**.^{e,f} The glycoprotein molecules are anchored in the cell membrane by C-terminal glycosylphosphatidylinositol (GPI) anchors (Fig. 8-13).^g The glycoprotein layer protects the parasite but is soon attacked by the host's immune system. However, the parasite has perhaps 1000 different genes for the variable surface protein, and every ten days or so new clones of trypanosomes appear with new coats that the immune system is not prepared to attack. To accomplish this cells occasionally copy one of the previously unused variable surface glycoprotein genes and place it into a new location in the genome, where it is expressed.^{h–j}

Parasitic **nematodes** shed the outer layers of their external cuticle and like trypanosomes reveal a new layer with different antigenic proteins.^k *Giardia* protects itself in a similar fashion.^l **Schistosomes**, tiny parasitic flatworms, evade a host's immune system by shedding complex glycoproteins from specialized double outer membranes.^m Antigenic determinants including MHC antigens characteristic of the mouse have been identified in the membrane of schistosomes from infected mice. Thus, one aspect of the parasite's defense may be to hide behind surface recognition markers stolen from its host.^{n,o} Schistosomes also secrete the peptide Thr-Lys-Pro, which inhibits macrophages, as well as a small molecule that inhibits T lymphocytes.^o

The malaria parasite *Plasmodium* has a complex life cycle with several forms and spends much of its life hiding within red blood cells.^p It may also suppress the immune system. The unicellular sporozoites, which are injected into the bloodstream by mosquitos, are protected by an external coat protein that is unusual in containing many short repeated sequences. For example, that of *P. falciparum*, which causes the most deadly form of malaria, contains the sequence Asn-Ala-Asn-Pro repeated 37 times.^q These coat proteins undergo unusually rapid evolution, which makes the preparation of vaccines difficult.^r

Trypanosomes, schistosomes, and malaria parasites still represent major health problems.

Malaria kills two to four million persons a year and endangers almost a third of the world's population. It has been impossible to produce suitable vaccines for any of these parasites. However, the cloning of genes for individual parasite proteins has given hope that effective vaccines can be devised.^{e,o–v} One problem is the lack of interest in financing the effort.^w

Many other protozoan parasites and bacteria invade cells and take up residence in macrophages.^x These include species of *Salmonella*, *Legionella*, and *Mycobacterium*.^y Bacteria often employ structural mimicry to gain access to a cell,^z e.g., by mimicking the type III secretion system (p. 520).^{aa} Some bacteria have developed defenses against reactive oxygen species, allowing them to evade the action of phagocytes.^{bb} *Borrelia burgdorferi*, the Lyme disease spirochete, synthesizes an unusual single-layer β -sheet outer surface protein,^{cc} which becomes coated with complement protein H. This may protect the bacteria and allow them to live for a long time within cells.^{dd}

Even the lowly **influenza virus** finds a way around our immunity so that it can strike us repeatedly. As this virus matures, it acquires a lipid membrane by budding from the host cell. Two virally encoded proteins are present in the membrane. One is a trimeric hemagglutinin, which forms small 7.6-nm spikes that protrude from the virus surface.^{ee,ff} The hemagglutinin monomer is a 550-residue peptide containing four antigenic regions. The RNA genome of the virus undergoes rapid mutation (Chapter 28, Section E,2). At least one amino acid substitution was found in each antigenic region, when hemagglutinins from influenza viruses causing epidemics in 1972 and 1975 were compared with the strain that caused a worldwide epidemic in 1968. Recently the type A influenza virus that caused the 1918–1919 pandemic, the greatest acute plague of the 20th century, has been “resurrected” and investigated using viral RNA from three victims.^{gg–ii} The globular part of the hemagglutinin appears to have come from a pig and the “stalk” from a human lineage. The virus takes advantage of the pool of virus in swine, humans, and birds to vary its structure and create new strains. The reason for the deadly nature of the 1918–1919 strain, which killed 20–40 million people, an unusually large number of whom were young, previously healthy adults, is not clear.

Viruses use a large variety of mechanisms to evade cellular defense mechanisms. Almost every aspect of the innate or adaptive immune systems provides some opportunity for evasion.^{jj} The rapid

BOX 31-E (continued)

mutation rate in a population of virus particles contributes greatly to this ability, allowing chronic infections such as those of hepatitis C^{kk} or delayed catastrophic infections such as those of HIV.

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anti-inflammatory agents such as prednisone and the folate antagonist methotrexate. A new vision of the possibilities for immunosuppression came, however, with the discovery of **cyclosporin A** (Box 9-F). This fungal metabolite inhibits lymphokine formation by helper T cells. It alleviates rejection of grafted tissues and prevents graft-versus-host disease. The use of cyclosporin and FK506 (Box 9-F) has permitted organ transplantation to the extent that by 1987 surgeons had transplanted in one year 1200 livers, 1500 hearts, and 9000 kidneys with one-year survival rates of 80% for hearts and over 90% for kidneys.⁵¹⁰ As mentioned in Box 9-F, cyclosporins bind very tightly to **cyclophilins**,⁵¹¹ which have peptidylprolyl *cis*–*trans* isomerase activity and perhaps other independent functions.⁵¹²

3. Immunodeficiencies

More than 95 different problems of impaired im-

munity have been identified. They affect about 1 in 10,000 persons born. The defects may involve T cells, B cells, NK cells, or phagocytic cells.^{513,514} There may be problems in lymphocyte development.⁵¹⁵ Complement proteins may be lacking,^{161,513,516} or their regulation may be faulty.⁵¹⁷ The immune system has specific “blind spots” and fails to recognize all dangerous foreign antigens.⁵¹⁸ In the fatal **X-linked immunoproliferative syndrome**,^{519,520} the immune system of susceptible males does not respond to the Epstein–Barr virus-induced mononucleosis by killing the persistently lymphoblastoid cells characteristic of that disease. Some individuals are born with **severe combined immunodeficiency** disease. This condition was made well known to the public by the plight of David, the “bubble boy,” who lived 12 years in protective sterile rooms and a plastic bubble-like “space suit.”⁵²¹ The condition is often caused by a defect in the interleukin-2 receptor, but there are a variety of other causes. About 15% of cases arise from a defect

BOX 31-F AN INSECTICIDAL PROTEIN

During sporulation the bacterium *Bacillus thuringiensis* forms within its own cells large protein crystals, which are highly toxic to some insect larvae. The crystals account for 20–30% of the dry weight of the bacterial spores and contain more than one toxin^a and, curiously, a 20-kbp piece of DNA.^b Dusting of garden plants with dried spores from these bacteria has become a popular and effective way of combating cabbage worms and other insects. The toxic protein from one strain of bacteria is encoded by a 4222-bp gene.^c The corresponding 133-kDa 1176-residue polypeptide protoxin undergoes glycosylation and perhaps other modifications, presumably prior to crystallization. After ingestion by susceptible insect larvae (largely Lepidoptera) the protein is cleaved to form a smaller ~65-kDa protease-resistant core, which is the active toxin. Other strains of bacteria produce toxins specific for Diptera or Coleoptera.^{d–f}

X-ray crystallography of the 65-kDa form reveal a three-domain structure. The central domain varies among different strains and is probably involved in recognition and in binding to cell surface receptors.^{e–h} The toxin binds to a receptor, apparently an aminopeptidase N,ⁱ after which the toxin is rapidly inserted into the membrane forming a 1- to 2-nm diameter pore. This leads to cell death.^j

Because the toxins appear to be harmless to human beings and higher animals the toxin genes have been transferred into various other bacteria, which are symbiotic with plants and into plants themselves. Toxin genes in suitably modified form (Chapter 27) were first transferred into bacteria that live naturally in association with roots of *Zea mays* and into tobacco and tomato plants. The new host organisms expressed the toxin genes and protected the plants from damage by caterpillars.^{k,l} Since then the toxin genes have been transferred into many crop plants, which are widely planted.

Two problems must be considered. Insects do develop resistance to the Bt toxin.^m This problem

can be combated by protein engineeringⁿ and by location of new sources of toxins.^{o,p} A second problem deals with the environmental impact.^q Will Bt toxin kill desirable insects? Will the gene be transferred in nature to other species and into the environment? The latter may seem unlikely, but as the toxins are applied to fight soil organisms such as nematodes, transfer into organisms of the largely unstudied soil ecosphere may pose problems.

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in adenosine deaminase, an enzyme of the purine salvage pathway (Fig. 25-17). As mentioned in Chapter 25, genetic therapy for this condition is being used. However, the most reliable treatment for these immunodeficiencies seems to be bone marrow transplantation. By 2000 more than 375 patients worldwide had received this treatment with up to 95% chance of survival.⁵¹³ Virus-induced immunodeficiency is the prime characteristic of HIV infection and **AIDS**.^{522,523} Both the amounts of autoantibodies and of amyloid

deposits increase with age, and immune complex disease is suspected of being a cause of aging.

4. Cancers of the Immune System

A major function of the immune system is thought to be destruction of cancer cells. In this case altered cell surface carbohydrates or proteins elicit an antibody response with destruction of the offending cells.

That this process works imperfectly may explain why the incidence of cancer increases with age and also why the concentration of autoantibodies increases. The immune system is also susceptible to cancers, which include multiple myeloma, leukemias, and lymphomas. Some of these, such as Burkitt's lymphoma, involve rearrangement of chromosome segments that carry immunoglobulin genes.^{524,525} These may result from errors in the gene rearrangements involved in the development of lymphocytes.

G. Defense Mechanisms of Plants

Plants make many compounds that repel or poison animals that eat them. Among such compounds are alkaloids, terpenes, calcium oxalate, fluoroacetate, cyanogenic glycosides, and phenolic compounds.⁵²⁶ The chewing of insects or other wounding of plant tissues releases phenolic glycosides and other reactive compounds from vesicles. Some of these compounds, which are often referred to as **phytoalexins** (see also Box 20-E),⁵²⁷ are repellent to predators, have antimicrobial activity, and /or participate in chemical cross-linking and strengthening of the plant cell wall.^{526,528} Some are protease inhibitors that interfere with a predator's nutrition.⁵²⁹ Some released compounds attract insects that may assist in defense by feeding on predator eggs or by attracting wasps that deposit eggs in predator larvae.⁵³⁰ These can all be regarded as part of an innate defense system that in some respects resembles our own innate immune system. For example, plant defensins (Fig. 31-7), most of which are directed against fungi,^{531,532} resemble those in our tissues.

A system of **receptor-mediated surveillance**, part of the innate system, triggers both immediate **local responses** and secondary immunity throughout the plant.^{530,533,534} Immediate responses include **programmed cell death** (called the **hypersensitive response**⁵³⁵), tissue reinforcement, and production of antimicrobial metabolites. Secondary responses, known as **systemic acquired resistance**, develop immunity throughout the plant. The surveillance system of some plants consists of a series of receptors known as **resistance (R) proteins**, which recognize signaling molecules produced by pathogens.^{535a,b} The R proteins are thought of as being paired with **avirulence (Avr) proteins** of the pathogen. If the resistance protein is missing, the plant will be susceptible

to attack by the pathogen. The pathogen's Avr protein is thought to be part of the chemical attack on the plant, apparently assisting in the invasion. However, if the Avr gene has been lost or is mutated, the R protein won't detect the invasion, and the pathogen may have increased virulence. What are the characteristics of the Avr proteins? They are often small and may be crosslinked by S-S bridges. They may be taken up by plant cells via receptors that resemble the type III translocation system of bacteria (p. 520).⁵³⁴ Similar small protein **elicitors** are released directly by wounding even in plants that do not have paired R-Avr genes.^{529,536}

The R proteins, which act as receptors for Avr, and other elicitor proteins, are usually leucine-rich-repeat proteins with a characteristic nucleotide binding site attached (NB-LRR proteins).^{534,537} Like other cell surface receptors they participate in signaling and utilize both ion channels and Ser/Thr protein kinases.⁵³⁸ The *Arabidopsis* genome contains ~150 sequences that may represent NB-LRR receptors.⁵³⁰

What do these receptors do? Like other cell membrane receptors they may induce both rapid and slower responses. The rapid responses may result from transmembrane flow of ions, just as in neurotransmitter action (Figs. 30-19, 30-20). The first response observed is an oxidative burst, which within minutes generates reduced oxygen intermediates (ROIs; pp. 1072–1074).^{533,539–543} These compounds may participate in crosslinking and lignification of cell walls. Together with nitric oxide (NO) and endogenous salicylic acid (Chapter 25, Section B,7),^{533,544,545} they promote transcription of defense-related genes and participate in the hypersensitive response. A second pathway, utilized against some pathogens especially those that kill plants to obtain nutrients,⁵³³ involves production of jasmonic acid (Eq. 21-18 and associated text)^{545a} and ethylene (Fig. 24-16).^{529,546}

Plants also have mechanisms for minimizing the damage from the over 500 known viruses. These don't often kill plants but can cause great damage. There are interferon-like responses⁵⁴⁷ and gene-silencing mechanisms.⁵⁴⁸ The latter often involve synthesis of dsRNA, cleavage by the enzyme Dicer, and interference with transcription as described on p. 1640.^{113a,b,549,550} This defensive reaction can spread between cells and throughout a plant, apparently by transport of RNA through plasmodesmata and the phloem.⁵⁵⁰

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Study Questions

1. Describe major aspects of the vertebrate innate and adaptive immune system. In what ways do they cooperate?
2. Describe briefly the functions of each of the following: antibodies, defensins, cytokines, complement, MHC proteins, B cells, T cells, dendritic cells, monocytes, macrophages, and neutrophils.
3. Why do antibodies produced using a native protein tend to bind only weakly to the corresponding denatured protein?
4. Discuss the topic of self-identity.
5. What are autoimmune diseases? How does the body avoid most autoimmune diseases?
6. List some methods by which viruses, bacteria, protozoa, and pathogenic fungi gain access to cells or to tissues.



Single cells develop into an astonishing variety of different species, all of which find their niches in the ecosystem. Whether a rectangular bacterium, a plant, a frog, or a human being, the size, shape, the body construction and metabolic pathways are established by the sequential expression of the organism's genes. Recent investigations have confirmed many similarities among major families of proteins from virtually all species. The same studies also emphasize the profound genetic differences between species. Understanding these differences, as well as the interrelationships among species, provides a continuing challenge to biochemists and biologists. Such understanding may even be essential to the survival of the human species in a changing environment.

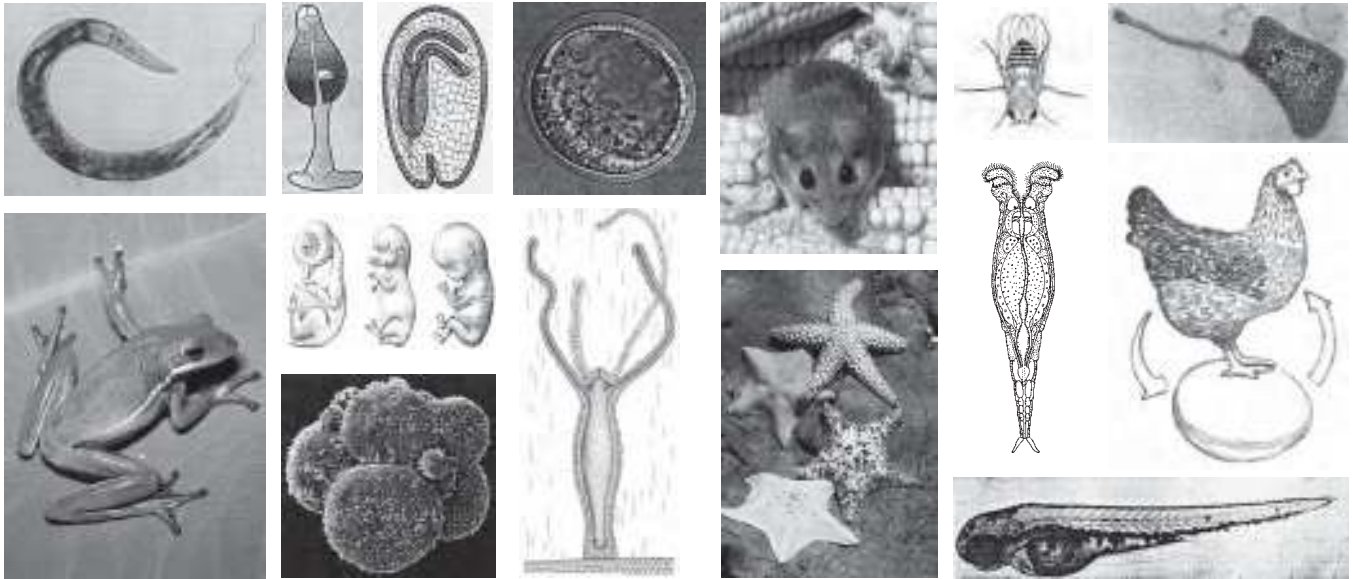
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Growth and Development

32



One of the most fascinating of all biological phenomena is the development of an animal from a fertilized egg. From the early embryonic cells, which appear to be much alike, there arise during the course of a very few cell divisions differentiated organs and tissues such as liver, brain, kidney, muscle, skin, and red blood cells. The biochemical properties of differentiated cells are often highly specialized. Red blood cells make hemoglobin, while muscle cells make large amounts of myosin and actin. The endocrine cells of the pancreas make insulin or glucagon, while the exocrine cells form the digestive enzymes that are secreted into the intestinal tract.

Looking more broadly at the biological world we see many additional specialized features at every level of observation. Developmental patterns differ for every organism. Specialized organs abound. The structures of proteins, lipids, carbohydrates, and nucleic acids all vary, and every species has its own metabolic peculiarities. Even among bacteria we find extreme variation. Furthermore, many unicellular eukaryotic organisms undergo complex development within a single cell. The topics of this chapter are too complex for any detailed discussion. We will examine some aspects of growth and development for a few organisms and will ask whether there are simplifying generalizations. Comprehensive textbooks are available.^{1,2}

Enough has been learned about development to make it clear that the DNA contains genetically coded **developmental programs**, which are followed by cells.³ However, both transcription and translation are

controlled by many chemical signals, which influence the execution of the genetic program. Such signals may arise from within a single cell, from the external environment, and from adjacent cells. The tight control is reflected by the fact that in most human tissues at any stage of development no more than about 10% of the total genes are transcribed at any one time. Chemical analysis makes it clear that most specialized cells contain a full complement of DNA but 90% of the genes are turned off.

A. Basic Concepts and Molecular Essentials

Listed in Table 32-1 are some essential aspects of growth and differentiation. Some of them are obvious. **Cohesion** between molecules provides the basis of specificity. **Receptors**, whether they be enzymes, hormone receptors, or receptors for chemotaxis, are essential. They are usually activated by a **conformational change** that accompanies binding. A cell must have receptors that can respond to a variety of **signals**, which may come from within the cell, from the external medium, or from neighboring cells. The receptor-signal pairs are essential to **local control**, which provides the basis for all of development.⁴

To have any kind of spatial differentiation a cell must develop **directionality** (polarity).⁵ This permits **asymmetric cell division**⁵ and development of poles in a developing cell or embryo. **Adhesion** molecules hold cells together, allowing a cell to have a fixed

position relative to other cells. Development of directionality and of developmental **patterns**^{6–8} are dependent upon **gradients** of concentration, of foods, heat, light, gravitation, etc., that can be detected by receptors. Gradients of compounds called **morphogens**^{9,10} help to provide a **positional identity** to cells.^{10–12}

Movement of molecules of organelles and of intact cells is also essential. In multicellular organisms cells often migrate to new locations by following chemotactic signals.

Growth of individual cells enlarges them and often leads to **cell division**. The **cell cycle** describes this process with emphasis on replication of DNA.

Homeostasis encompasses adaptation to altered nutrient or other environmental variables and to all

processes which influence a cell to change. It provides for defensive reactions to many types of stress.^{12a,b}

1. DNA and Developmental Programs

The genetic developmental program of an organism is encoded in the DNA. The expression of the program is implemented initially at the transcriptional level by a host of transcription factors that act at appropriate times and in appropriate places. Their action usually requires the presence of many preformed compounds. **Alternative developmental programs** may be used by unicellular organisms to adapt to new environmental conditions or by multicellular organisms to differentiate tissues.⁶

Programmed alterations and rearrangements of DNA. Part of the developmental program may involve a temporary or permanent change in the DNA. One of the simplest of these changes makes use of the **transposable recombinational switch** in which a small piece of DNA is present in either of the two possible orientations. (See Chapter 27, Section D.3.) An example, illustrated by Eqs. 27-15 and 27-16, is the variation in “phase” of the flagella produced by *Salmonella*. A somewhat different example is provided by the unicellular yeast *Saccharomyces cerevisiae*, which changes the mating type of its haploid forms in a highly regulated pattern.^{13,14} The **a** mating type is expressed constitutively, but this is frequently switched to the **α** type, which produces a different mating pheromone (see Table 30-5) and responds to a pheromone from **a**-type cells. The change occurs through the transposition of different “cassettes” of DNA from “silent sites” into an expression site.^{14a,b} The cassettes contain several genes, which are copied into the expression site called MAT. This site always contains genes of either **a** or **α** type. However, both **a** and **α** genes are present in other storage locations. When the mating type is switched, a copy is made of one of the stored cassettes and is placed into the MAT locus replacing the cassette already present. The MAT **α** genes encode two regulatory proteins, **α1** and **α2**. Protein **α1** is a positive regulator of the **α**-cell-specific genes, while protein **α2** is a repressor of the **a**-cell-specific genes.^{15–17} A similar mechanism appears to be employed by trypanosomes in changing their variable cell surface proteins (Box 31-E).¹⁸

Inactivation of genes and imprinting. Under some circumstances a chromosome or part of a chromosome is permanently inactivated but remains within the cell as compactly folded heterochromatin. Heterochromatin often consists of reiterated sequences of unknown function, but it may also contain groups of inactivated genes. The most impressive case is the

TABLE 32-1
Some Essentials for Growth and Differentiation

Cohesion of molecules, utilizing specific, hydrogen-bonding, and complementary surface shapes

Recognition, and conformational changes

- **Receptors** and **signals**
- **Local controls**

Polarity (directionality)

- Asymmetric cell division
- Poles

Gradients that can be sensed by receptors

- Food, physical qualities, morphogens

Adhesion between cells is required to hold an organism together and also, together with morphogen gradients, to provide a **positional identity**

Movement of molecules, organelles, and cells

Growth to enlarge cell size and numbers of cells

- **A cell cycle** for **replication of DNA** and **cell division**

Homeostasis to permit adaptation to changes in nutrient concentrations, stress

A developmental program, which is encoded in the genome

- Implementing this program usually requires many preformed compounds
- **Alternative developmental programs** often provide flexibility to an organism

Stem cells of **totipotent**, **pluripotent**, or multipotent nature supply new germ cells and other cells for multicellular organisms when needed

Programmed cell death (apoptosis) is part of many developmental programs and provides for removal of unneeded cells without inflammation

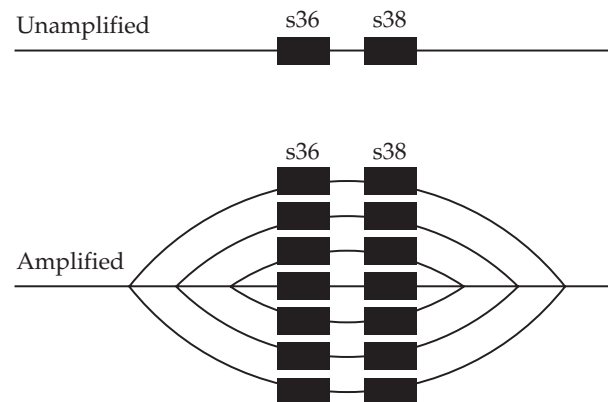
total inactivation of one of the two X chromosomes in cells of female mammals.^{19–21} The entire chromosome appears as heterochromatin. The inactivation occurs early in embryonic development and is random with respect to the two X chromosomes. In some cells the maternal X chromosome, in others the paternal X chromosome, becomes inactive. However, upon further cell divisions the same chromosome in each clone remains inactive. As a consequence of the random inactivation, the female body is a mosaic with respect to genes in the X chromosomes. Formation of heterochromatin in X chromosomes is described in Section C,1. It depends upon **epigenetic markers**, which consist, at least in part, of methylated cytosines in 5'-CpG-3' sequences. These CG pairs are palindromic and can be methylated on both strands (see Eq. 27-2 and Fig. 32-3).

Selective inactivation of genes by methylation accounts for **genomic imprinting**, which occurs in mammals and which marks a gene of either maternal or paternal origin for silencing.^{22–24} About 50 imprinted genes have been identified in mice and in humans.^{23,25} Imprinting, known as **epigenetic inheritance**, also appears to arise in part by methylation of cytosines in CpG sequences. Four m⁵C-methyltransferase genes have been identified in the human genome.²⁶ One of these presumably provides the initial methylation of the cytosine in one strand of the DNA. A second methyltransferase (apparently encoded by gene *Dnmt1*)²⁷ methylates the second strand and serves as the **maintenance methylase** that preserves the methylation pattern upon DNA replication (see Eq. 27-2). The faithful maintenance of imprinting has been demonstrated experimentally for genes of mice formed by nuclear transplantation, i.e., mice grown from somatic cell nuclei transplanted into ova.²⁸ Imprinted genes usually occur in clusters, which also contain imprinted genes for noncoding RNA molecules. The transcription of those genes often is correlated with repression (**silencing**) of protein-coding genes on the same chromosome. This observation is consistent with evidence that genes may be silenced by the binding of small RNA molecules to complementary sequences in the DNA or of mRNAs. The mechanism may be limited to **imprint control regions** or silencer units in the DNA, while the genes to be controlled are set off by **insulator elements** (see also pp. 1882, 1894 and Chapter 28, Section C,1). The significance of genomic imprinting is not clear.^{29,30} However, it is very important to nuclear transplantation because the methylation state of imprinted genes is normally reset (**epigenetic reprogramming**) before embryonic development begins.^{23,25,27,31–33} See Section 5. Methylation of DNA is not essential to the life of filamentous fungi. Nevertheless, *Neurospora* does have a DNA methyltransferase, which methylates about 1.5% of the cytosines present.

Loss of DNA. While some genes are selectively inactivated, others may be irreversibly lost during development. The extreme case is that of the human red blood cell from which the entire nucleus is expelled. Loss of DNA may result from recombinational events. For example, crossing-over between sister chromatids during mitosis has been demonstrated for some cells and chromosomes. This does not alter the genetics of the progeny cells if equal amounts of genetic material are exchanged. However, if two or more similar base sequences occur in tandem in a DNA molecule, unequal crossing-over (Chapter 27, Section D,2) can occur with the loss of genetic material from a chromosome of one of the progeny cells. This may be a deliberately programmed route of differentiation for some cells.

Alternatively, loss of DNA from a chromosome may occur through a **looping-out excision** mechanism. Like the excision of a prophage from the chromosome of *E. coli* (Chapter 27, Section D,3) this loss of genes occurs at specific sites in the DNA. The best known case is the joining of gene segments during the differentiation of lymphocytes (Fig. 31-18). The extent to which similar changes occur during terminal differentiation of other tissues or in nonmammalian species is uncertain.

Amplification of DNA of chromosomes. During formation of oocytes parts of the DNA are “amplified” by repeated replication. This provides a way for the ovum to accumulate ribosomal RNA and various proteins in large amounts. Similarly, genes for two abundant proteins of the egg shell or **chorion** of insects are amplified. Bidirectional replication initiated at discrete positions yields an “onion skin” structure containing many copies of an ~90-kb sequence containing the two genes. The polyploidy observed in some highly specialized cells such as the Purkinje cells



of the cerebellum and of many cells of Diptera larva (Chapter 26, Section F,3) represent another way of amplifying genes. Polyploid cells of animals generally represent a terminal stage of differentiation and do not divide. They tend to contain their full complement of genes in each copy of their DNA, but most genes are not expressed.

2. Receptors and Signals

Receptors and their ligands are numerous, varied, and essential to all forms of life. Cell-surface receptors on bacteria detect feeding attractants as well as dangerous molecules. From bacteria to humans seven-helix receptors function to detect light, odors, hormones, and other molecules. The numbers of different receptors are impressive. For example, the tiny nematode *C. elegans* has 650 seven-helix transmembrane receptors and 411 protein kinases, many of which may be associated with receptors.³⁴ Our bodies have thousands.

Every ligand that binds to a receptor is a signal of some kind. How many signaling molecules are there, and what are their structures? The number of proteins and small peptides affecting growth and development of cells may be enormous. Many of these have been considered in Chapter 30. In addition, the catecholamines, serotonin, histamine, and even bicarbonate ions may act as local hormones.³⁵ For example, HCO_3^- determines whether a thin-walled sporangium or a thick-walled heat-resistant sporangium will appear in the phycomycete water mold *Blastocladiella*. We know that a very large number of different proteins and small peptides are secreted from cells, many in very small amounts.³⁶ Some protein morphogens act at concentrations so low, e.g., 50 pM, that it has been hard to detect them.¹⁰ T-cell receptors respond to extremely small amounts of short peptides that stream outward from many cells bound to surface MHC molecules. Do some of these same peptides act as signals between other cells during development?

Small RNA molecules are now being found to function in many ways within cells,^{37–39} They may also act as messengers within cells and between cells. The small 22 nt RNAs transcribed from genes *lin-4* and *let-7* of the nematode *C. elegans* control key developmental decisions.^{38b,d} In green plants small RNAs move between cells and throughout the plants to trigger defensive responses (p. 1869).^{38c} Much of the RNA transcribed from genes of any eukaryotic cell lacks any known function. Since evolution tends to act on all of the molecular constituents of a cell, this vast amount of RNA may have acquired vital roles in the control of metabolism and growth.^{38e} A poorly understood intracellular structure, the **ribonucleoprotein vault** also remains a biochemical mystery. Differenti-

ated cells may contain 10^4 and embryonic cells over 10^7 of these $\sim 42 \times 75$ nm hollow objects. Vaults have internal cavities large enough to hold two intact ribosomes. Some vaults appear to be empty, but others contain materials, a fact that suggests some role in transport or storage.^{38f}

Homeostasis. A large fraction of the receptors and signaling system of cells is devoted to the maintenance of a constant internal environment. This homeostasis is essential if a cell is to respond to changes in external conditions without disastrous consequences.^{12a,b} Some special aspects of these processes are discussed in Chapters 11, 17, and 28. Within mammalian cells the hypothalamus, pituitary, and adrenals have a primary responsibility for maintaining homeostasis.⁴⁰

Transcriptional control in differentiation.

Development of an organism depends upon an orderly sequence of transcription of genes. Some genes are transcribed in germ cells, others within cells of an early embryo, and others later. As the embryo develops sequential waves of synthesis of the needed protein are observed.^{3,41–43} These are controlled by the actions of numerous transcription factors that act at a variety of **cis-regulatory modules** (CRMs) associated with promoter regions^{42,44–46} and also by controls on translation of mRNAs,⁴⁷ and by negative feedback loops.⁴⁸ The latter may involve hormones, morphogenic proteins, coregulatory proteins of various types, small RNAs, etc.⁴⁹ More than 2000 transcription factors are encoded in the human genome. Most are positive-acting, i.e., they *promote* transcription. Several families are specifically involved in regulation of development (see Table 28-2). These include the Zn^{2+} -containing GATA-1 (p. 1634), which regulates globin synthesis, embryonic factors of *Drosophila* (e.g., bicoid), vertebrate homeotic genes (Hox clusters), Pit1, the muscle-specific helix-loop-helix proteins MyoD and Myf5, and several forkhead (winged helix) proteins.³ A single CRM can bind many different regulatory molecules and single regulators can bind to a variety of CRMs. During development of the simple embryo of the sea urchin *Strongylocentrotus purpuratus* a network of 40 highly regulated genes is needed to coordinate growth and development with the production of the proteins needed at each step.^{41,43}

Part of the control of differentiation lies in the interaction of proteins that regulate transcription with metabolites and hormones. For example, substrate depletion not only decreases growth rate of bacteria but also alters gene transcription. This occurs in *E. coli* as a result of a rise in internal cAMP concentration. The presence of an alternative energy source such as lactose induces changes in gene transcription (Chapter 28, Section A,1). Such **physiological modulation** of a

developmental pattern can also be seen in higher organisms.

More striking is the fact that environmental signals can trigger a cell to switch to an alternative developmental program by which enough new genes are activated to rebuild the cell into a new form. An example is spore formation, a process that occurs with some bacteria when external conditions become unfavorable for vegetative growth (Section B,1). Alternative developmental programs are also evident in eukaryotic organisms that undergo metamorphosis, and they may be important to development. Perhaps persistent states of repression of groups of genes can be passed through several generations of cells until a specific chemical signal triggers the unwrapping of the appropriate nucleosomes and transcription of formerly inactive genes.

3. Adhesion, Cell–Cell Recognition, and Cell Migration

Development of multicellular organisms depends upon both adhesion and on recognition of a correct interaction. Like enzyme–substrate, receptor–hormone, and antibody–antigen binding these interactions of macromolecules on cell surfaces often show a high degree of specificity. They may be accompanied by conformational changes and may trigger signaling cascades. We have already discussed some of these interactions, for example, the binding of a molecule of IgG attached to a surface antigen to protein C1q of the complement system (Fig. 31-8) and the binding of an MHC–antigen complex to a T-cell receptor (Fig. 31-15).

There are many other cell–surface **adhesins**, several of which have been discussed on pp. 402–409. Among them are proteins that contain immunoglobulin-like domains and numerous **glycoproteins**. An example of the latter is the binding of a type of pili found in pathogenic strains of *E. coli* to epithelial cells of the urinary tract. The pilin subunits (Fig. 7-9), like lectins (Box 4-C), bind specifically to the disaccharide group Gal α 1 \rightarrow 4Gal. A lectinlike protein specific for N-acetylglucosamine rings is involved in invasion of erythrocytes by the malaria parasite *Plasmodium*. The unicellular alga *Chlamydomonas* (Fig. 1-11) produces sexual gametes of two mating types. When mixed together, gametes of opposite mating types, prior to fusion, adhere to each other via **agglutinins** present on their flagella. The agglutinins are glycoproteins rich in hydroxyproline, serine, glycine, arabinose, and galactose.⁵⁰ As mentioned on p. 29, colored cells of different strains of the marine sponge *Microciona prolifera* find others of the same strain using highly specific proteoglycan-like aggregation factors.^{51–53} These compounds are highly polymorphic, and it has been suggested that they are part of a primitive immune

system. The aggregation reaction requires calcium ions. In our own bodies Ca²⁺-dependent lectins, the **selectins** (p. 187, 188), bind leukocytes and help to guide them to their sites of action.

Other adhesins include the **integrins**,^{53a} cellular adhesion molecules (**CAMs**), **cadherins**,^{53a–c} and **fibronectin** (Fig. 8-19). These are also discussed on pp. 402–409. The CAMs (Fig. 8-18A),^{54,55} which are members of the immunoglobulin-like protein family, are glycoproteins bearing large 2,8-linked sialic acid polymers.^{56–58} They promote Ca²⁺-dependent aggregation. However, the effect of NCAM, which is widely distributed in a developmentally regulated fashion, can be antiadhesive if long chains of sialic acid are present. NCAM appears to play a role in remodeling and repair of tissues. Adhesion of molecules within cell membranes and the binding of substances to membrane surfaces provides another driving force in development. Within membranes molecules spontaneously sort themselves into lipid **microdomains**, often called **lipid rafts**.^{58a} Related to lipid rafts are caveolae (p. 426). These little craters arise in cholesterol-rich microdomains. They often contain the protein **caveolin** as well as glycosphingolipids and GPI-tailed proteins (Fig. 8-13).^{58b} ATP-dependent linking reactions may also occur to provide more permanent bonding. Membrane-associated molecules, in turn, become centers for attachment of cytoskeletal proteins and other protein complexes. As with the cytosol and extracellular fluids homeostatic mechanisms act to provide a relatively constant membrane–lipid environment.^{58c}

Several types of cell junctions are associated with adhesion and participate in intercellular communication (Fig. 1-15).⁵⁹ The cadherins are transmembrane proteins with large extracellular domains (Fig. 8-18B). They are prominent components of adherens junctions^{59–61a} in which they join the exterior surfaces of pairs of cells in a zipper-like manner. Another protein, **β -catenin**, links the short C-terminal tails of cadherin through α -catenin subunits to the actin cytoskeleton.⁶⁰ In desmosomal junctions other specialized proteins including desmoglein have functions similar to that of cadherins.⁵⁹ Tight junctions, from zebrafish to humans, depend upon a complex of several proteins including those of the claudin family.⁶² Significantly, the cohesive powers of some adhesins, e.g., of cadherin, are altered during development. Cadherin E is nonadhesive in a four-cell mouse embryo but becomes adhesive after the eight-cell stage.⁵⁹ It is obvious that many other changes in intercellular adhesion must also occur during growth and development.

The integrins (see also p. 405) comprise a large family of adhesive receptors that are found in animals from sponges to humans.^{63–65} They have both adhesive and signaling functions. Both subunits of their $\alpha\beta$ heterodimeric structures⁶⁴ have single transmembrane

helices and short C-terminal cytoplasmic tails. The $\beta 1$ subunit tails interact with cytoplasmic proteins. The distribution of integrins varies among cell types. Human leukocytes contain alpha subunits of types αd , αl , αm , and αx with molecular masses of 150–180 kDa. Two ~95-kDa beta subunits ($\beta 1$ and $\beta 2$) are present. However, T lymphocytes express $\beta 1$, $\beta 2$, and $\beta 7$ integrins. Other patterns are observed for other leukocytes,⁶⁶ in skin,⁶⁷ and in other tissues.⁶⁸ Integrin molecules tend to aggregate into clusters, which are found together with other proteins, at the ends of actin stress fibers (p. 370).⁶³ The largest of these clusters are known as focal adhesions. Signals may be sent through integrins in either of the two directions.⁶³ The extracellular domains of integrins interact with a variety of proteins of the extracellular matrix. These include fibronectin, fibrinogen, vitronectin, collagen, and entactin.^{63,69} Other large cell surface adhesins include laminin and osteopontin (Chapter 8), thrombospondin, von Willebrand factor, and related proteins.⁷⁰ These adhesins appear to depend upon the sequence Arg-Gly-Asp (RGD), which binds noncovalently to integrins, which act as cell-surface receptors.^{71,71a} See also Chapter 12, Section C.9.

The functioning of the complex network of integrins, adhesins, and other components of the extracellular matrix is not understood in detail. One fundamental question is how the strength of adhesion can vary with time and stage of development. Roseman postulated an association of an oligosaccharide chain of a glycoprotein attached to one cell with a specific glycosyltransferase of another cell.⁷² The specific interaction would hold cells together, but addition of another glycosyl unit to the oligosaccharide by the transferase would alter the surface properties of the cell carrying the glycoprotein. This, in turn, could cause disaggregation of the cells. Glycosyltransferases can be found on the outer surfaces of cell membranes, and Roseman's proposal may correctly describe one aspect of cell adhesion.

Other molecules that are abundant on cell surfaces include heparan sulfate proteoglycans. Although they have often been regarded as providing a nonspecific "extracellular fly paper," recent evidence from studies of development in *Drosophila* suggest specific and important functions in signaling and in developmental patterning.⁷³ Both hyaluronan and chitin also have been proposed to play an important role in vertebrate development.^{74,75} Proteoglycans of plant cell surfaces, as well as the hydroxyproline-rich proteins of cell walls, may function in plant development.⁷⁶

Movement of cells from one location to another is essential to embryonic development as well as to wound repair and to the immune response. Many brain structures are composed exclusively of immigrant cells.⁷⁷ These **cell migrations** depend upon the cytoskeletal actin filaments, integrins, and focal

adhesions.^{78,79} Chemotactic signals are also required.⁸⁰ A great complexity of underlying chemistry is being elucidated.^{79,81} See also Chapter 19, Section C.

4. Polarity, Asymmetric Cell Division, and Morphogens

Cells of *E. coli* usually divide exactly in the center to form two seemingly identical cells.^{82–84a} However, under the right conditions some bacteria, e.g., *Caulobacter crescentus* and *Bacillus subtilis*, undergo asymmetric division to form two different types of cells (Fig. 32-1).⁸³ There is clearly an axial polarity. This polarity is evident even in *E. coli*, which has flagella streaming out at one end and its chemoreceptor-bearing "nose" at the other (Fig. 32-1).⁸⁵ Axial polarity is also obvious in other bacteria (Fig. 19-1).

Polarity is evident in eukaryotic cells from protists to higher organisms.^{85a,b} Cells of the yeast *S. cerevisiae*, whether haploid or diploid, divide in an asymmetric way by budding.^{5,7} Among body cells of higher animals those of the epithelium are among the most polarized (Fig. 32-2; see also Fig. 1-6, Box 8-F). Polarity is always present in ova of eukaryotes, but the ova may initially be radially symmetric.^{86–88} The **anterior-posterior** axis, which is formed first, establishes a head-to-tail direction.⁸⁹ In bacteria this major axis is determined as perpendicular to the division plane. In the tiny worm *C. elegans* the anterior-posterior axis of an ovum is determined by the position of entrance of the sperm. This marks the posterior end.⁹⁰ In higher animals the axis, which is also known as the **animal-vegetal axis**, is established by uneven distribution of materials that include mRNAs and proteins in the unfertilized ovum. During embryonic development of bilateral species two other axes, the **dorsal-ventral** and **right-left** axes, are also developed and help to establish the body plan. Throughout development polarized movements allow cells to intercalate between one another to help shape the body.^{90a}

Early studies of simple organisms such as *Hydra* (Fig. 1-13) and planaria (flatworms, Fig. 1-14A) showed that distinct chemical differences can be detected along the anterior-posterior axis. These organisms can be cut into pieces, many of which can regenerate complete individuals.^{91,92} Regions near the head regenerate most readily. These and other observations led to the concept of gradients of diffusible **morphogens**, or form-giving molecules.^{9,10} In *Drosophila* eggs an mRNA specified by the gene *bicoid* is localized at the anterior pole. The translation product, the bicoid protein, diffuses through the embryo, which in *Drosophila* lacks cell walls at this stage (see also Section C.4).^{9,93–95} Bicoid is a transcription factor and also one of a number of established morphogens. Many other morphogens are members of the **TGF- β**

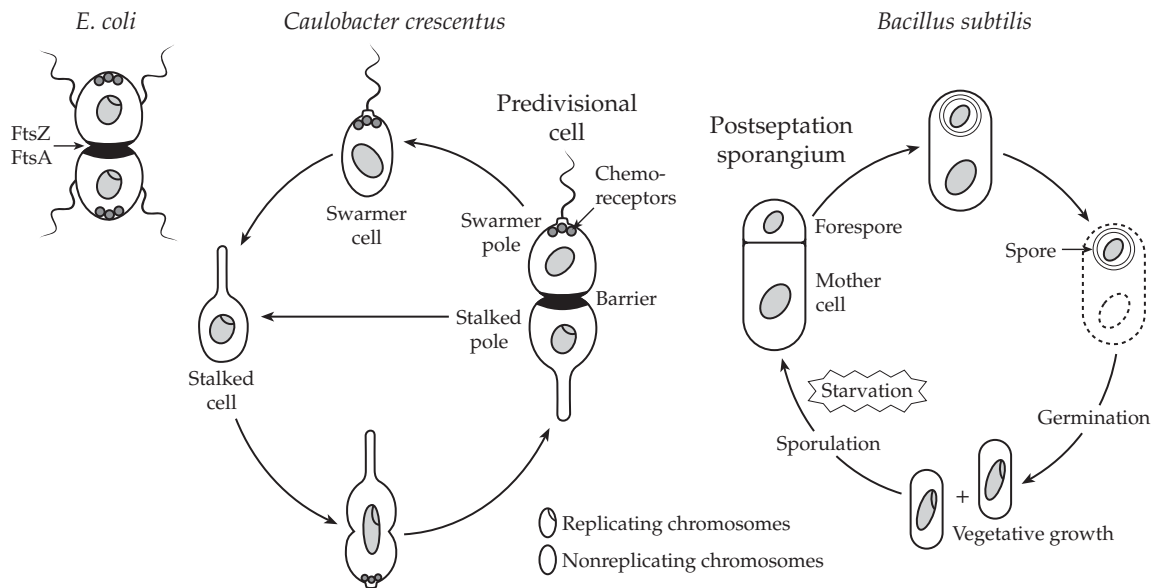


Figure 32-1 Comparison of cell division in three species of bacteria. *Escherichia coli* divides symmetrically after forming a septum in a plane marked by a ring of FtsZ (tubulin-like) and other cell division proteins. *Caulobacter crescentus* divides asymmetrically to give one flagellated swarmer cell and one stalked cell. *Bacillus subtilis*, under starvation conditions, divide to form a mother cell and a forespore. The latter is engulfed by the mother cell, which promotes its conversion to a resistant spore. From Shapiro and Losick.⁸³ Courtesy of L. Shapiro.

(transforming growth factor beta) family. Among them are proteins that establish the dorsal-ventral axis (Section C.4)^{86,96-98} and also bone morphogenic proteins (p. 443). Retinoids also appear to act as morphogens.

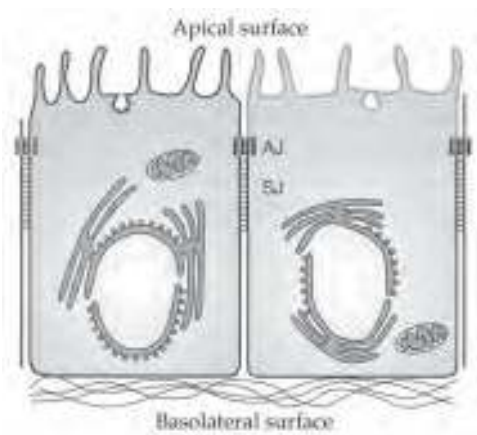


Figure 32-2 A pair of epithelial cells of *Drosophila*. The apical surface (top), e.g., of epithelial cells of the gut, faces the external surface, while the basolateral surface (bottom) binds to a basal membrane. Adheren junctions (AJ) and septate junctions (SJ) are shown between the cells. From Peifer and Tepass.⁸⁴ Drawing by S. Whitfield.

5. Totipotency and Stem Cells

The cambium layer of plant stems (Fig. 1-16) differentiates continuously to form phloem on the outside of the cambium and xylem on the inside. At the same time, cambium cells are retained. Thus, at each cell division one daughter cell becomes a differentiated cell, while another remains the less differentiated cambium. This pattern of continuous differentiation from a line of **stem cells** with constant properties is found in animals as well as in plants. In the differentiation of cambium it appears that chemical signals obtained from the surrounding cells on either the inside or the outside of the cambium layer determine whether the differentiated cell becomes phloem or xylem. Sucrose, auxin, and cytokinins are all involved.

Cloning. Asexual propagation (cloning) of plants ordinarily occurs by virtue of the ability of embryonic meristematic tissue to differentiate into roots and shoots. If isolated phloem cells or other more differentiated cells are cultured, the result is often the formation of a **callus**, a dedifferentiated mass of cells somewhat reminiscent of embryonic cells. Under proper conditions, e.g., in a coconut milk culture and in the presence of the correct auxin-to-cytokinin ratio, some carrot root phloem cells revert to embryonic cells and develop into intact plants.⁹⁹ This experiment provided proof that the differentiated carrot phloem cells

contained a complete genome for the plant. Its nucleus is **totipotent**, able to generate all cell types. However, the experiment cannot be done easily with most plants, and dedifferentiation is not always automatic. It does occur often enough to establish the totipotency of the nucleus of many differentiated cells.

Similar considerations apply to animal cells. In the earliest stages differentiation is readily reversible. Later it becomes difficult to convert a differentiated animal cell into one resembling an embryonic cell. However, Gurdon demonstrated that this is sometimes possible. Nuclei from cells of intestinal epithelia and other tissues were substituted, by transplantation, for the nuclei of egg cells. The process is called **nuclear transplantation**^{100–101a} or nuclear transfer cloning.¹⁰² The resulting eggs in some cases grew into adult toads. Thus, the full genetic information of the toad was present in the differentiated cells.^{103,104} However, it was not possible to accomplish this result with nuclei of neurons, which may have undergone irreversible differentiation. More recently mammalian nuclei have been utilized in the same way to create the famous sheep Dolly as well as mice, calves, pigs, and kittens.^{105–108} These animals are commonly said to have been **cloned**, a term that has long been used to denote asexual propagation, e.g., in a colony of dividing cells or in propagation of plants by grafting.

In nuclear transplantation it is the DNA that is hoped to be the same in every individual in a clone. However, the ovum used for the transplantation contains mitochondria. Some mitochondria may also accompany the nucleus during the transfer. If the donors of the ovum and of the nucleus are different individuals the offspring will be mitochondrial hybrids.¹⁰⁵ In addition, there are questions about the methylation state of DNA in the donated nucleus and about the age and health of the donated mitochondria. That these questions are significant is emphasized by a bit of 3000-year-old knowledge from mule breeders: a mare crossed with a donkey yields a mule but a stallion crossed with a donkey yields a hinny, which has shorter ears, a thicker mane and tail, and stronger legs than does a mule.¹⁰⁹ There are worries because Dolly and many other animals produced by nuclear transplantation have not been completely healthy.^{107b,110} Is something missing from the transplanted DNA or does it carry something extra, such as methyl groups? Recently it has been recognized that incorrect epigenetic marking of cytosine in CpG pairs that control maternally imprinted genes, especially those on chromosomes 11 or 15, may cause death of embryos or devastating human diseases.^{25,111} An important related question for those wishing to clone an animal by nuclear transplantation is “Should the cell that donates the nucleus be in the G_1 state of the cell cycle (Fig. 11-15) or the G_0 or paused state that precedes G_1 ?”¹⁰⁵ See also Chapter 27, Section B,6.

Stem cells. For many years it has been appreciated that, as shown in Fig. 31-2, both erythrocytes and other blood cells arise throughout life from self-renewing stem cells in the bone marrow.^{13,112} Stem cells are also needed for renewal of bones, muscle, skin, neurons, etc. Stem cells appear to be present only in small numbers and in well-protected special **niches** in the body.^{53c,113–115} They are able to live throughout an individual’s lifetime, dividing quite rarely and always producing one or more highly differentiated cells as well as a new stem cell.¹¹³ A fertilized egg (zygote) is totipotent, able to generate all the cells of an animal including those of the placenta and other tissues that are not part of the embryo. However, the most capable stem cells are **pluripotent**, able to form more than one type of specialized cell.^{113,116} Mammalian pluripotent stem cells include tumor cells, **embryonic stem cells**, derived from preimplantation embryos, and **embryonic germ cells**, derived from the primordial germ cells of the postimplantation embryo.¹¹⁷ These germ cells are not only totipotent but, with good luck, may be immortal.¹¹⁸

Recent results indicate that adult-derived somatic cell nuclei may still retain full pluripotency.^{107a,117a} Some confusion has arisen because of the discovery that stem cells may sometimes fuse with differentiated cells.^{118a,b} It is only recently that it has been possible to locate and to cultivate human stem cells. These cells, which may be recovered from both embryonic and mature tissues, include the blood-cell-forming **hematopoietic** stem cells, fetal **neuronal** stem cells, **melanocyte** stem cells, and **mesenchymal** stem cells (or marrow stromal cells). The last give rise to muscle, bone, cartilage, and tendons.^{119–121a} Most stem cells may arise late in development and function principally in tissue renewal.¹²² Among the most abundant are those of epithelial tissues, whose cells provide 60% of differentiated tissue types in the mammalian body.^{123,124} Epidermal stem cells must provide for regular replacement of the outer skin surface (Box 8-F) but must also provide cells for rapid repair of wounds.¹²⁵ The exact locations of epidermal skin cells have been difficult to find. The cells appear to be well-protected in areas deep in the skin. Some are located in hair follicles.^{120a,125} Stem cells of plants are present in specialized structures called **meristems**. A seedling typically has two meristems, at the tips of the shoot and root, respectively.¹²⁶ See Fig. 32-8B.

Cloning of human stem cells is of great medical interest because of the possibility of replacing defective cells or tissues. Tissue engineering may supply urgently needed differentiated cells for replacement purposes^{107,127–128a} and may eventually lead to replacement organs.^{129–131} These efforts must be pursued with caution, but most researchers see a bright future for cloning of tissue cells.^{128,132–134} At the same time there is nearly universal agreement that nuclear trans-

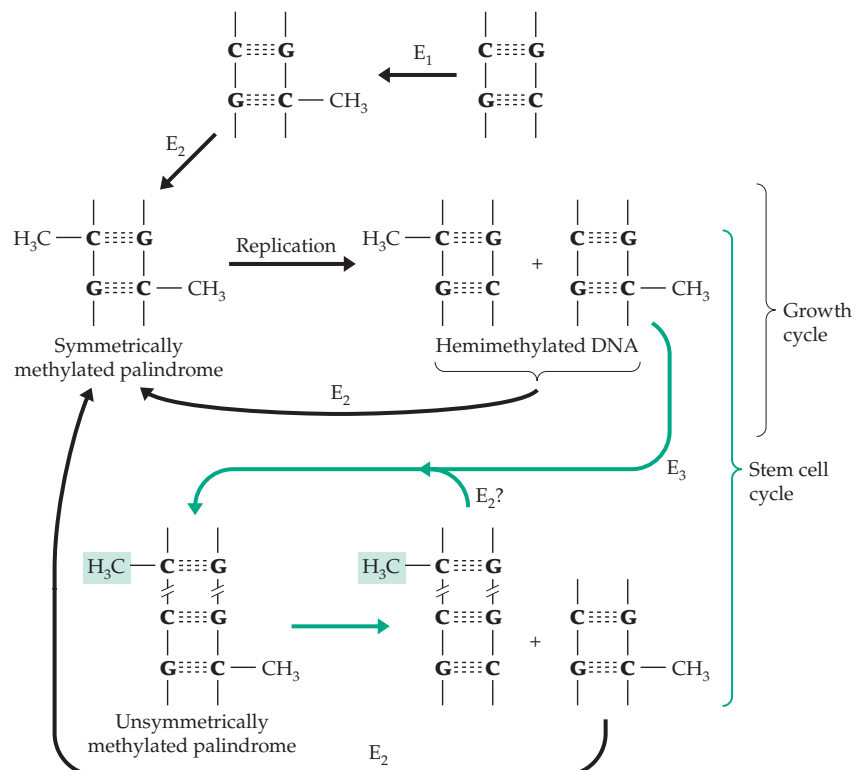
plantation cloning of human beings should not be attempted.^{132,135} One key objection is the near certainty that many seriously defective human embryos would be created.

How can we explain a pattern in which one daughter cell resulting from division of a stem cell undergoes differentiation while the other remains a stem cell? A hypothetical way in which this might happen is illustrated in Fig. 32-3. It depends upon methylation of CpG sequences in DNA (Eq. 27-2). A methyltransferase, E_1 in Fig. 32-3, would modify a site in the DNA that controls differentiation by methylating a base in one of the two strands of a palindromic sequence such as a CpG pair (Eq. 27-2). The maintenance methylase E_2 would further methylate the corresponding cytosine in the palindrome in the second strand. During tissue growth replication would produce a hemimethylated CpG in each daughter cell. These would also be methylated by E_2 (upper part of Fig. 32-3) to complete the replication process. In contrast, a stem cell would need to mark one DNA strand, e.g., by action of a third methyltransferase (E_3) or some other type of DNA-modifying enzyme. As depicted in Fig. 32-3, E_3 would add a methyl group to DNA on some location outside of the palindromic region. Replication in this case would yield one hemimethylated palindrome, which would allow one daughter cell to follow the normal growth and replication cycle. However, the other daughter cell would carry the mark

designating it as a stem cell. The presence of both E_1 and E_2 in cells would lead to the continuous differentiation of the modified cells from unmodified ones, the situation found in stem line cells. A “maintenance methylase” with the properties of E_2 has been identified (Section A,1).

One question that has been asked is whether the 200 cell types of the vertebrate body all arise as a result of chemical interactions between cells and hormones and other external signals? Alternatively, does a **developmental clock** count the number of cell divisions and at the appropriate time turn off one set of genes and turn on another?¹³⁶ Methylases as well as other enzymes might modify DNA at specific times during development. For example, a hydrolase might deaminate the adenine in an AT pair to inosine. Upon replication and cell division one daughter cell would receive an unaltered DNA molecule, but the other would contain in place of the AT base pair an IC pair. Following a second replication, a GC pair would be formed (Eq. 27-20) resulting in AT to GC mutation at a specific site in the DNA of some of the daughter cells. Such a simple change, occurring in response to an enzyme formed at a certain stage of development, could alter the expression of genes in a cell. Schemes involving palindromic sites and modification enzymes that could turn off specific genes after a given number of cell replications have been suggested.¹³⁶ In fact, there seems to be little support for such mechanisms.

Figure 32-3 Hypothetical way of controlling stem cell replication by methylation or other marking system. Methyltransferases E_1 and E_2 methylate the cytosine in a 5'-CpG-3' or other palindromic sequence. In freely replicating cells these two enzymes keep the CG sequences methylated on both DNA strands. In stem cells another enzyme, perhaps a third methylase (E_3), marks a location outside the palindromic DNA on one strand (■). Replication leaves the mark in the duplex, which is retained in a stem cell. The other strand will yield a hemimethylated duplex allowing the cell to follow the normal growth and replication pattern. Based on proposals of Holliday and Pugh.¹³⁶



Transcriptional controls (Section 2) and the sensing of a cell's location may provide adequate control.

If methylation and possibly other covalent modifications of DNA occur, how can one explain the totipotency observed for some nuclei of differentiated cells? During development of the ovum and the sperm there appears to be a “resetting” of the developmental clocks that led to differentiation. At this time all of the methyl groups on the CpG pairs of imprinted genes are removed (Section C,2).^{25,137} The mechanism is uncertain. Perhaps marking of newly replicated DNA stops. For example, in the case of the methylated DNA of Fig. 32-3 if E₁ and E₂ were absent in the cytoplasm of the ovum, no further methylation would occur during subsequent cell divisions. However, there may be active enzyme-catalyzed demethylation (see p.1541). Other mechanisms of gene silencing are also known (pp. 1881,1894).¹³⁸

6. Apoptosis or How the Tadpole Eats Its Tail

Observers have long been fascinated by the rapid resorption of a tadpole's tail as it turns into a frog or toad. The process, designated by the Greek word apoptosis (whose “pop” is pronounced),¹³⁹ or as **programmed cell death**, plays a major role in many aspects of development in nearly all organisms.^{140–142} For example, during human development about one-half of all the neurons generated die.^{143,144} Unneeded lymphocytes, some of which produce antibodies or T-cell receptors directed against a person's self, are also killed. Cells may die accidentally from injuries. In many cases the resulting death occurs by **necrosis** rather than apoptosis. Necrotic death is accompanied by swelling and bursting of the cell and a subsequent inflammatory response.^{145,146} In contrast, cells dying by apoptosis shrink, break into fragments, and are rapidly eaten by surrounding cells.^{146a,b} There is no inflammation. Because of this it has been difficult to determine the extent to which apoptosis contributes to normal development. Apoptosis is also distinguished from **autophagy**, which is intracellular turnover under starvation conditions. Cells may need to scavenge unneeded proteins and organelles, recycling them within the cell.¹⁴⁷

Cells damaged by disease, e.g., dopaminergic neurons in Parkinson disease, may die by apoptosis.^{147a,b} A second form of self-destruction occurs when an axon is cut.^{147b} Failure of the elaborate network of mechanism for repair of DNA and maintenance of the genome normally leads to apoptosis. In cancer essential steps in the apoptosis pathway are often inactivated.^{147c}

Our view of apoptosis (outlined in Fig. 32-4) changed with the tracing of the origins and fates of all of the ~1000 cells of the nematode *C. elegans*.¹³ During development of the adult worm just 131 specific cells

die by apoptosis. Studies of mutant worms revealed mutations in several cell-death (*ced*) genes. Three proteins, encoded by genes *ced-3*, *ced-4*, and *egl-1*, are essential for apoptosis.^{140,148} Somewhat surprisingly worms with defective Ced proteins are apparently healthy, even though they have 131 extra cells. On the other hand, in the fruit fly *Drosophila* mutations in similar death genes are sometimes fatal.¹⁴⁰

The nucleotide sequence of the *ced-3* gene revealed that the Ced3 protein is closely related to the **interleukin-converting enzyme ICE**,^{149–152} which is discussed on p. 619. ICE is a member of the **caspase family** of thiol proteases (p. 619). At least 14 different caspases are found in the human body. Some of them

TABLE 32-2
Some Components of Apoptotic Systems

Apaf1	Mammalian homolog of Ced4; component of apoptosome
Apoptosome	Cytosolic complex: Apaf1•caspase-9•cytochrome c
Bcl2	Mammalian homolog of Ced9 protein of <i>C. elegans</i> ; inhibitor of apoptosis
Bcl-2 family	Group of regulators of apoptosis, both inhibitory and stimulatory (Bad, Bax, Bik, etc.)
CARD	
CD95 (AP-1, Fas)	One of the most studied death receptors
Ced3	<i>C. elegans</i> thiol protease, related to mammalian caspase9
Ced4	Activator of Ced3, related to mammalian Apaf1
Ced9	Inhibitor of Ced3 and related caspases
DD	Death domain of a death receptor
DED	Death effector domain
DISC	Death-inducing signaling complex, formed in plasma membrane
FADD	Fas-associated death domain, an adapter protein
ICE	Interleukin-converting enzyme, structurally related to Ced 3
TNF	Tumor necrosis factor (a family of cytokines secreted by macrophages)
TNFR	Receptors for a TNF family member

function in apoptosis^{152a} and others in maturation of pro-inflammatory cytokines.^{152–155} Most exist as pro-enzymes, which must be activated by proteolysis.¹⁵⁶ The mammalian homology of Ced3 is caspase 9.^{153,157} The Ced4 protein of *C. elegans* is an activator for Ced3. Its mammalian counterpart is called **Apaf1** (apoptotic protease-activating factor 1).^{153,158} Protein **Ced9** is an inhibitor of apoptosis, which probably protects the worm from erroneous deaths.¹⁵⁹ Its mammalian equivalents are proteins of the *Bcl-2* gene family.^{142,152a}

It is well established that caspases participate in the final stages of apoptosis (Fig. 32-4), but what initiates the process? There appear to be many ways in which apoptosis can be triggered. If every cell has a proper location in the body, which is determined by signals from adjacent cells, what will happen if the cell becomes detached? There is evidence that such detachment with the loss of survival signals causes apoptosis.^{53a,152b} Cell damage is also a major trigger. In other cases the cell is “instructed” to die. An example is the death of unneeded lymphocytes, one of many cellular processes induced by cytokines of the tumor necrosis factor (TNF) family. To allow for this process cells have surface receptors of the TNF

receptor (TNFR) superfamily.^{159a} Some TNFRs are **death receptors**, which are called by many names.^{142,160–163} One of the best known is CD95^{164a} (also called Fas¹⁶⁴ or Apo1). CD95 is involved in death of mature T lymphocytes at the end of an immune response and also in the killing of virus-infected cells and cancer cells by cytotoxic T cells or NK cells.

Members of the TNF family that activate CD95 (CD95 ligands or CD95Ls) are trimers. They bind to the cysteine-rich external domains of the transmembrane CD95 molecules inducing them to aggregate (Fig. 32-4). The cytosolic portion of each of these death receptors contain a **death domain** (DD). The bundle of aggregated receptors also bind to an adapter protein such as the Fas-associated death domain (FADD).¹⁵⁶ It is one of many proteins involved in apoptosis whose structures are known.^{157,163} The FADD molecule contains a **death effector domain** (DED), which associates with a similar domain in the proenzyme procaspase 8. A rather large membrane-associated molecular complex, the **death-inducing signaling complex** (DISC; Fig. 32-4), is assembled in this way.^{161,165} Oligomerization of the procaspase domain causes activation via self-cleavage to give active

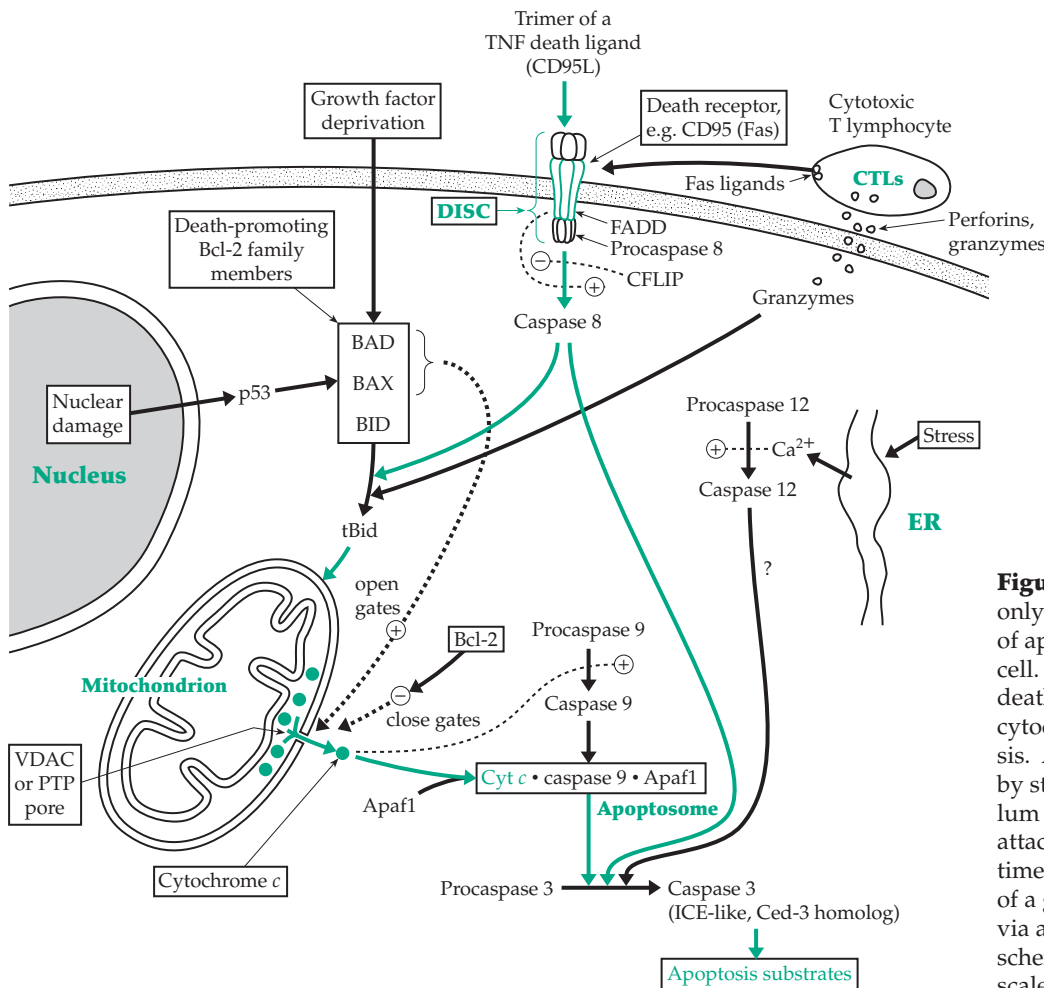


Figure 32-4 Sketch illustrating only a few of the many aspects of apoptosis in a mammalian cell. Emphasis here is on the death receptor pathways and cytochrome *c*-activated apoptosis. A third pathway is initiated by stress in endoplasmic reticulum membranes. In addition, attack by cytolytic T cells sometimes causes apoptosis by action of a granzyme on protein Bid or via a death receptor. Objects in scheme are not drawn to a single scale.

caspase 8, which initiates the apoptotic response. Other “upstream” caspases (caspases 2, 9, and 10) also participate in initiation of apoptosis. In contrast, caspases 3, 6, and 7, the “executioner caspases,” are thought to participate directly in demolition of the cell.¹⁵⁴ A caspase-activated DNase also participates by degrading DNA.^{166,167}

A second major pathway of activation of apoptosis depends upon mitochondria. Various stresses such as lack of needed growth factors, exposure to ultraviolet light, or other apoptosis-inducing signals apparently open pores or gates in mitochondrial membranes allowing materials that would promote apoptosis to flow out into the cytoplasm and stimulate the effector caspases.^{167a} This possibility was supported by the discovery that cytochrome *c* stimulates apoptosis.¹⁶⁸ Cytochrome *c* is a small protein, which is present in inner mitochondrial membranes in a 1:1 ratio with other electron carriers. It is more mobile and less tightly bound than the other components. It carries electrons from complex III to cytochrome *c* oxidase within the intermembrane space (Fig. 18-5). As a result of apoptotic stimuli cytochrome *c* rapidly flows out of the intermembrane space into the cytoplasm both interfering with respiration and triggering other changes in the cell.^{169–172} The outflow of cytochrome *c* may occur via the mitochondrial porin VDAC (p. 1047) or under some circumstances via the mitochondrial permeability transition pore (PTP; p. 1049).¹⁷³ Within the cytosol the escaped cytochrome *c*, together with caspase 9 and Apaf1, forms a large multimeric complex (cyt *c* • caspase 9 • Apaf1) called an **apoptosome**. The apoptosome catalyzes activation of caspase 3, initiating the caspase cascade.

Control of the gates or pores by which cytochrome *c* escapes from mitochondria is poorly understood.^{171,171a,173} Whereas in *C. elegans* a single protein Ced9 has been identified as an inhibitor of apoptosis, vertebrate animals have a large family of proteins that are related to the Ced9 homolog Bcl-2.^{157,174} Of these Bcl-2 and Bcl-*x_L* inhibit the flow of cytochrome *c* out of mitochondria, but several other members of the family, e.g., Bad, Bid, Bik, and Bax, promote apoptosis.^{152a,175,176} Bad carries a signal that indicates a lack of growth factor stimulation. Bid carries a death message from QD95R and other death receptors. Bax carries a signal from p53 (Fig. 11-15) indicating unacceptable DNA damage. However, a truncated form of Bax may prevent apoptosis of neurons.¹⁷⁷ In every one of these pathways there are many complexities. In one of the best known pathways Bid is cleaved by caspase 8 to form a 15-kDa fragment t-Bid that becomes an integral membrane protein in the outer membrane of mitochondria. There it promotes the release of cytochrome *c* (Fig. 32-4).¹⁷⁸

A quite different source of apoptotic signals are ER membranes, which respond to stress by releasing Ca²⁺

ions that activate caspase 12 (Fig. 32-4).¹⁷⁹ Yet another type of apoptosis is sometimes induced by granzyme B (p. 610), which is released from cytolytic T cells.^{180–182}

B. Differentiation in Prokaryotic Cells and in Simple Eukaryotes

Every species undergoes developmental changes. Only a few of these will be considered here briefly.

1. Bacteria

Although they are usually regarded as unicellular, some bacteria develop more than one type of cell,⁸³ and some even form colonies with filamentous growth^{183,184} or other distinct morphology.¹⁸⁵ Many bacteria alter their development in response to changes in environment. For example, unfavorable conditions lead bacteria such as *Bacillus subtilis* to form compact endospores inside the vegetative cells.^{83,184} Many other bacteria including *E. coli* divide symmetrically. This fact also poses a question. How does a cell locate its center and divide? The answer is only partially understood. In all kinds of bacteria a protein known as **FtsZ** (filamentation temperature-sensitive protein Z), a GTP-binding protein homologous to eukaryotic tubulins (Fig. 7-33), is essential. Prior to division FtsZ accumulates as a **septal ring** at the center of the *E. coli* cell. Contraction of the ring is thought to be an essential step in cell division.^{186–188} The FtsZ ring nucleates a growing complex of eight additional proteins known as FtsA, T, K, L, N, Q, W, and ZipA. While ZipA is not highly conserved among bacteria, in *E. coli* it is the first protein to add to the FtsZ ring.^{187,189} ZipA is somewhat related to eukaryotic actin. Another group of proteins is also needed for location of the midcell plane. These are known as MinC, MinD, and MinE. A MinC•MinD complex inhibits potential binding sites for FtsZ. MinD is an ATPase, which structurally resembles the Fe protein of nitrogenase (Fig. 24-2) and appears to propel the MinC•MinD complex in an oscillatory fashion from pole-to-pole.^{190–193} This behavior is not understood, but in some manner the 10-kDa MinE is able to overcome the inhibition and bind to FtsZ initiating division. Division in *E. coli* follows DNA replication by a constant time period (20 min at 37°C). The timing apparently depends upon diadenosine 5'-tetraphosphate (Ap₄A), which acts as a signal to couple division to replication.¹⁹⁴

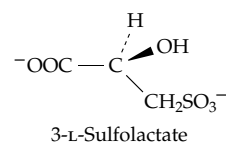
Bacteria with stalks. *Caulobacter crescentis* spreads to new areas while retaining a presence at home. As is illustrated in Fig. 32-1, asymmetric division produces two distinctly different cells. One, like

the maternal cell, has a stalk, while the other has a rotary flagellum with which it travels. In time the motile “swarmer” cell sheds its flagellum and undergoes metamorphoses into a stalked cell. What controls this process? There is apparently a two-component system similar to the one that controls flagellar movement (Fig. 19-5). Two sensor histidine kinases phosphorylate a central response regulator **CtrA**.^{195,195a,b} This represses initiation of replication in the swarmer cells as well as transcription of the cell division gene *ftsZ*. As mentioned on p.1094 changes in DNA methylation may also occur. Flagellar biosynthesis and other steps of differentiation then occur in the swarmer cells but not in the stalked cells in which normal growth and replication take place.^{196–201} Action of proteases is also essential.²⁰²

Sporulation. Bacteria of the genera *Bacillus* and *Clostridium* form metabolically inert spores when deprived of adequate nutrients (Fig. 32-1).^{83,203,204} Bacterial spores are remarkably resistant to heat and can survive boiling water for prolonged periods. Their metabolic rate is essentially zero, but they can be revived and can grow even after many years. For example, bacteria have been grown from a 118-year old can of meat. Some data suggest that spores can survive for no more than ~1000 years, but recent reports, not yet fully verified, say that spores have survived when embedded in salt crystals for 250 million years.^{203,205} At the onset of sporulation the synthesis of ribosomal RNA is turned off completely, and new classes of mRNA are made. More than 50 genetic loci are affected by mutations that cause spore formation. As was mentioned in Chapter 28, one or more specific forms of the 70-kDa σ subunit of RNA polymerase (Chapter 28, Section A,2) are produced and direct the initiation of the new mRNA molecules encoding new proteins.^{204,206–209} Prior to asymmetric cell division the first of the new σ factors, σ_F , is formed together with two regulatory proteins, Spo0A and Spo0B. Spo0B, a protein kinase, phosphorylates Spo0A, inactivating it.²¹⁰ SpoAB also forms an inactive complex with σ_F . After asymmetric cell division σ_F remains inactive in the mother cell but is released in the prespore by action of Spo0E. This is a membrane-bound phosphatase, which dephosphorylates Spo0A-P, allowing it to form a complex with Spo0B with release of σ_F . Another protein, SpoIIIE, appears to direct one copy of the replicated DNA into the forespore.²¹¹ The σ_F factor then directs the transcription of genes in the forespore. In contrast, σ_E is produced only in the mother cell.

One of the most striking metabolic changes in metabolism during sporulation is the accumulation of large amounts of dipicolinic acid (Fig. 24-14). This requires the appearance of at least one new enzyme. In addition, as the spores develop the bacteria take up

large amounts of Ca^{2+} and substantial concentrations of Mn^{2+} and other metal ions. In many bacteria 3-L-sulfolactic acid is also formed.



These components account for the following percentages of the total dry weight of spores of *B. subtilis*: dipicolinic acid, 10%; sulfolactic acid, 3–6%; Ca^{2+} , 3%; and Mn^{2+} , 0.3%. It is often suggested that the dipicolinic acid and other ions protect the proteins from denaturation. However, the heat resistance may arise from the maintenance of the core of the spore in a highly dehydrated state.²¹² When conditions become appropriate for growth again, the spore germinates, and the bacterium again follows the cell growth and division program.

More complex alternative developmental programs are followed by colonial forms of bacteria such as the myxobacteria. The life cycle involves aggregation of cells and formation of fruiting bodies as well as sporulation.¹⁸⁵

Signaling among bacteria. Even bacteria respond to signals from other bacteria. Individuals of a single species often react by secreting pheromones called **autoinducers** using a process called **quorum sensing**. Among the responses are swarming of cells, emission of light by luminous bacteria, synthesis of antibiotics, and formation of biofilms. As mentioned on p.1758, autoinducers used by gram-negative bacteria are often *N*-acetylhomoserine lactones.^{213–215} A furanoyl borate diester (see Box 11-F) may be a more nearly universal autoinducer.²¹⁶ Programmed cell death can also be observed among bacterial populations.²¹⁷

2. Yeasts

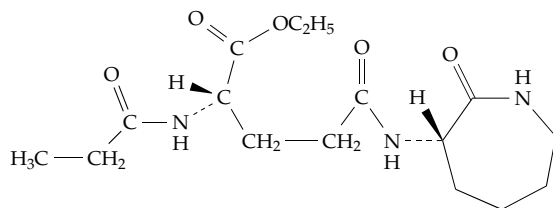
The budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* are the best known fungi. Although they usually grow as individual cells, they can grow with a filamentous form under some conditions.²¹⁸ Other yeast, notably *Candida albicans*, are important pathogens and can also grow in either yeast or pseudohyphal filamentous forms.^{219,220} Like *E. coli*, *S. pombe* undergoes symmetric cell division. However, the strong $\beta(1\rightarrow3)$ linked glycans with their $\beta(1\rightarrow6)$ crosslinkages, mannose polysaccharides, and chitin provide a cell wall very different from those of bacteria.²²¹ Cells of *S. pombe* grow mainly at their tips and begin early in mitosis to form a ring of actomyosin and other proteins at the center. This

corresponds to the mammalian contractile ring. At the end of anaphase the ring contracts, and the septum that separates the two cells develops.^{222–223a} In both types of yeast the septum is rich in chitin, which is secreted from the cell membrane or from vesicles known as chitosomes. After the septum is fully formed and thickened, a chitinase partially hydrolyzes the chitin releasing the cells. In *S. cerevisiae* the cell division is asymmetric.^{5,221} The position of the bud seems to be directed by the actin cytoskeleton.

All fungi form spores (gametes) during their haploid stage, which follows meiosis (Fig. 1-10). The transcriptional program for *S. cerevisiae* involves at least four sets of genes, which are transcribed consecutively. During spore formation the mRNA levels of more than 1000 of the ~6200 protein-encoding genes are changed. About 50% are elevated and ~50% are depressed.²²⁴ The mating type changes in the haploid state have been mentioned on p. 1574. Similar mating and sporulation pathways are observed for *S. pombe*.²²⁵

3. The Cellular Slime Molds

The life cycle of *Dictyostelium discoideum* is described briefly in Box 11-C. About 10^5 individual amebas aggregate to form a moving “slug” in response to the chemoattractant cAMP. Some other species of *Dictyostelium* are attracted to a folic acid derivative or to the ethyl ester of *N*-propionyl- γ -L-glutamyl-L-ornithine- δ -lactam.²²⁶



N-Propionyl- γ -L-glutamyl-L-ornithine- δ -lactam

In all cases the cells also utilize cAMP as an internal second messenger. For *D. discoideum* the components of the chemotactic-aggregation system include a 41-kDa cAMP receptor on the outside, adenylate cyclase, an extracellular diesterase that specifically hydrolyzes the cAMP to AMP, and a diesterase inhibitor protein.^{35,227–230} The inhibitor keeps the phosphodiesterase largely inactive initially, but when cAMP concentrations build up synthesis of the inhibitor is repressed and the cAMP is hydrolyzed, a necessary condition for retaining sensitivity of the receptors for the arriving pulses of cAMP.

The slug of aggregated amebas continues to move and to undergo differentiation into two cell types: about 80% of the cells become pre-spores and the remaining 20%, which are at the “head” of the slug,

become pre-stalk cells. The front-to-back gradient of cAMP within the moving aggregate seems to be involved in differentiation. However, another “differentiation-inducing factor” as well as NH_3 may be involved in the formation of stalk.²³⁰ As the aggregate forms, the cells become cohesive, an 80-kDa surface glycoprotein being involved.²³¹ Later other adhesive **discoidins**, 24- to 27-kDa RGD-containing galactose-binding lectins,²³² also participate in holding the colony together. Some cells begin to produce cellulose. Trehalose is also formed and is stored in the spores. New enzymes have to be made to synthesize these materials. An alternative developmental pattern for some strains of *Dictyostelium* is formation of macrocysts between cells of two different mating types. A diffusible 12-kDa inducing factor appears to be released by cells of one strain.²³³

4. The Hydra

A well-fed hydra (Fig. 1-13) appears immortal. Its body cells are sloughed off and replaced at a steady rate so that within a month or so its body has been completely renewed.³⁵ The hydra contains only ten cell types. These include two kinds of stem cells that give rise to the ectodermal and endodermal cells of the body wall as well as small **interstitial stem cells** (Fig. 1-13) that differentiate nerve cells, germ cells, and the nematocytes or stinging cells. Of the $\sim 10^5$ cells in a hydra about 3600 are interstitial stem cells. Each day they generate 400 nerve cells and 1800 nematocyte precursor cells as well as 3500 new interstitial cells. The nematocyte precursors move up the body of the hydra and take up residence in the tentacles. Their movement is thought to be guided by chemotaxis. The head activator peptide (Table 30-5), which was identified following isolation from 3×10^6 hydras (3 kg),²³⁴ diffuses from the foot end of the animal forming a gradient. A foot activator may diffuse from the opposite end. The interstitial stem cells of hydra also give rise to clones that develop into the gametes. Female hydra always develop female gametes, but stem cells of male hydra give rise to both male and female gametes.²³⁵ This sex switching is reminiscent of the mating type variation of yeast.

5. Cell-Constant Animals

While the hydra is almost immortal as a result of the continuous differentiation of its stem cell lines, other small invertebrates follow a very different course of development. Both the rotifers and the annelid worms (Fig. 1-14) tend to have a constant number of cells in the adult body. The entire developmental program is specified genetically in strict detail.

The one millimeter long adult nematode *Caenorhabditis elegans* contains only 959 somatic cells. The lineal descent of all of these has been traced.^{236–240} The development follows an almost exactly defined pathway with 113 programmed cell deaths during formation of the 558-cell newly hatched larva. In addition, each adult worm contains 302 neurons that make about 8000 synapses. This little nematode also has an alternative developmental pathway. The larvae shed their cuticles in four consecutive molts. If the food supply is inadequate, they enter a persistent nonfeeding state in which they may survive for months and are able to resume development when conditions are appropriate.²⁴¹

C. Development of Animal Embryos

The shapes and body plans of animals vary enormously. Consequently, the study of embryonic development of sea urchins, insects, frogs, chickens, mice, and humans might appear to lead to quite unrelated conclusions. However, there are many similarities as well as variety.

1. Germ Cells and Gametes

Throughout the animal kingdom from protozoa to human beings sexual reproduction predominates. It is true that there are about 1000 species that reproduce asexually.^{242,243} Among them are ~350 species of all-female rotifers²⁴² and even a species of tiny mites, all of which are haploid females.²⁴⁴ Nevertheless, sex seems to have conferred some advantage on most species. There are two theories that may explain this: (1) Sex brings different combinations of genes together, allowing especially favorable combinations to survive, when changing conditions make life difficult.²⁴² (2) Sex helps to remove deleterious mutations from a population.²⁴³ A large fraction of human fetuses (at least 10–25%) contain an “incorrect” number of chromosomes and as many as 20% of oocytes are defective. In contrast only 3–4% of sperm are chromosomally abnormal. Female meiosis I appears to be highly error-prone.^{243a} Abnormal fertilized eggs or embryos are eliminated later in development.

Sex determination. The sex of an individual is determined by the chromosomes. In humans and other mammals presence or absence of a Y chromosome determines the sex. However, in many organisms including *C. elegans* and *Drosophila* this is not true. Although *Drosophila* males like human males are XY, it is the ratio of the number of X chromosomes to the number of sets of autosome (A) that determines the sex. This is also true for *C. elegans*, which has no

Y chromosomes.^{244,245} Apparently because of the differing ratios of X:A in the two sexes, organisms utilize a variety of **dosage compensation** methods. In cells of human females only one X chromosome is active. In *Drosophila* the rate of expression of genes from the X chromosome is roughly doubled in males.^{245–248} In *C. elegans* the expression from both X chromosomes of the (hermaphroditic) female is roughly halved.^{245,246,249,250} The biochemistry underlying these processes is quite complex.

The mammalian Y chromosome. The basic plan of the gonads prior to differentiation is female. However, if a Y chromosome is present (or if genes from a Y chromosome have been translocated to other locations) testes develop and begin to secrete androgen as early as the 60th day of gestation. A male-specific DNA sequence, **SRY** (sex determining region Y), constituting the gene for the **testes-determining factor**, is located in the small arm of the Y chromosome (Fig. 32-5).^{251–253} A small pseudoautosomal segment at the end of the short arm of the Y chromosome carries other genes and undergoes crossing-over during meiosis.^{254,255} The SRY gene lies between this and the centromere. The SRY protein is a member of the HMGA subgroup of **HMG** DNA-binding proteins (p. 1535).^{256,257} It binds tightly to the sequence AACAA(A/T)(G/C) broadening the minor groove of the B-DNA and bending the DNA by more than 70°.^{252,258,259}

Both SRY and the related SOX proteins are critical developmental regulators.²⁶⁰ In early fetal life the mammalian embryo contains an indifferent gonad, able to differentiate into either a testis or ovary. Adjacent to the gonad are two simple ducts, the Müllerian (female) and Wolffian (male).²⁶¹ In the male SRY acts in the developing gonads to induce differentiation into the Sertoli cells of the testis. In the mouse the *Sry* gene is active for only a brief period about ten days after fertilization. During that period cells of the genital ridge start to differentiate. In the absence of protein SRY they develop into the female follicle (granulosa) cells but in the presence of SRY into Sertoli cells.^{252,262} This is, in part, a result of production of the **Müllerian inhibitory substance** (MIS), which induces regression of the Müllerian duct, and later production of testosterone. MIS is a glycoprotein of the TGF- β family. Binding of SRY to a site in the *Mis* gene promoter appears to be involved in activation of the *Mis* gene.²⁵² Recent evidence points to a role for both SRY and SOX proteins in pre-mRNA splicing.²⁶⁰ At least 25 other genes are also involved in spermatogenesis in the mouse.²⁶³ Many of these testis-specific genes have completely unmethylated CpG sequences.^{264,265} For example, a cAMP-responsive element present in a promoter sequence for a testis-specific subunit of pyruvate dehydrogenase must be demethylated for

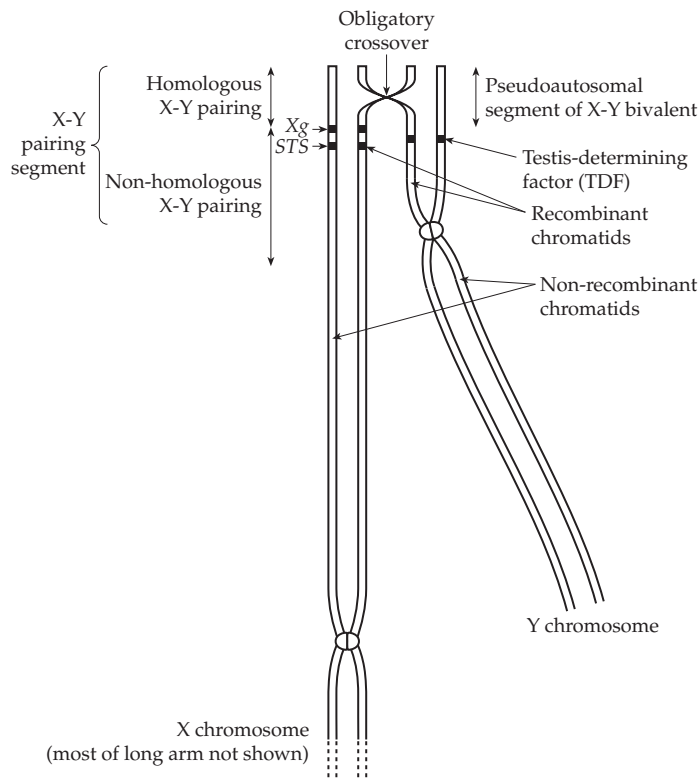


Figure 32-5 Schematic diagram showing crossing-over between the human X and Y chromosome. The pseudoautosomal segment is that part of the X-Y bivalent where there can be X-Y exchange by crossing over. X-Y homology in this segment is maintained by, and may be necessary for, this crossing over. There is always one “obligatory” X-Y crossover, whose position varies. The length of the X-Y pairing segment varies with meiotic stage and can extend well beyond the pseudoautosomal segment into the Y long arm. Much (perhaps all) of the synaptonemal complex formed outside the pseudoautosomal segment represents non-homologous pairing. From Burgoyne.²⁵⁴

transcription to occur. The developing germ cells interact with the surrounding Sertoli cells at every stage both through direct cell-cell contacts and via secreted signals.²⁶⁶ Interstitial cells of the testis differentiate into Leydig cells, which secrete testosterone, promoting development of the Wolffian duct.²⁶¹ A small population of germ-line stem cells provide for continuing spermatogenesis. In *Drosophila* their self-renewal depends upon signals from special hub cells.^{115,267} Other proteins needed for normal male development include the X-linked androgen receptor, whose absence causes testicular feminization, and dihydrotestosterone reductase (Chapter 22).

The development of spermatozoa is unlike that of somatic cells. Extensive reorganization of chromatin occurs under the direction of cis-regulatory elements that are controlled by cells of the testis.^{267a} Among specialized proteins that are synthesized is a testis-specific polyadenylate polymerase.^{267b} During the remodeling histones are replaced by arginine- and cysteine-rich protamines.^{267a-c} In mammals this occurs in two stages. Small intermediate proteins (TP1 and TP2) replace histones in the first stage and are displaced by protamines in the second.^{267a,d} Sulfolipids, which are also present in myelin, are essential to spermatogenesis.^{267e} Both the sphingolipid 3-sulfogalactosylceramide and **seminolipid**, a sulfate ester of monogalactosylalkylacylglycerol (structure on p. 387) are present in large amounts. However, their functions are not clear.

Selenium plays a special role in development and protection of spermatozoa (Chapter 15). The selenoprotein **phospholipid hydroperoxide glutathione peroxidase** (PHGPx; Eq. 15-58, Table 15-4) has a high activity in the testis and in spermatids. However, in mature spermatozoa it forms an enzymatically inactive oxidatively crosslinked capsular material around the midpiece of the cell perhaps providing mechanical stability.²⁶⁸ A similar 34-kDa selenoprotein is present in sperm nuclei and may be essential for condensation of DNA.²⁶⁹ Sperm tails contain specialized cytoskeletal proteins which form “outer dense fibers.”²⁷⁰ In contrast to mammalian spermatozoa, nematode sperm move by ameboid motility that depends upon a specialized actin-like molecule.²⁷¹ Sperm cells are unusually rich in polyamines, most of which are bound to RNA and DNA (Chapter 24).

The X chromosomes. The phenomenon of X chromosome inactivation in mammalian female cells is closely related to imprinting, which has been discussed in Section 1. The inactivation process is quite complex. It involves methylation of 5'-CpG-3' sequences of DNA, as is described in Chapter 27, Section B.6. It also depends upon an **inactivation center**, the *Xist* gene, which is expressed only from the inactivated X_i chromosome, whose *Xist* DNA is unmethylated. On the X_a chromosome this DNA is methylated, and the gene is silent.²⁷² The *Xist* transcript is a long RNA that may bind to and coat much of the X_i

DNA.^{19,21,273–275} The associated chromatin is enriched in a variant of histone H2A and is underacetylated on the tails of histones H2A, H3, and H4 (Fig. 27-4). Also noteworthy is the fact that not all genes on X_i are inactivated. As many as 19% escape this control.²⁷⁶ Another gene *Tsix*, which is adjacent to *Xist* in the DNA, is also involved. *Tsix* encodes an RNA that is *antisense* to the *Xist* transcript^{21,275,277} but is transcribed from the active X chromosome X_a . One hypothesis is that the *Xist* transcript causes X inactivation and that the *Tsix* transcript acts in an opposite way to favor activation of the chromosome. A transcription factor known as **CTCF** has been identified as a possible regulator of the inactivation process.^{21,277} This is a trans-acting factor that is encoded on a chromosome other than X or Y. The process also depends upon methylation of histone H3.²⁷⁸ Methylation of H3 may also be a factor in gene silencing in other organisms.²⁷⁹ CTCF also regulates a number of other genes, e.g., those of the globin gene cluster (Fig. 27-10). It binds to 60-bp sequences, perhaps in enhancer elements.

2. Development of the Ovum

In the early mammalian female embryo the absence of the Müllerian inhibitory substance MIS permits continuing development of the Müllerian duct, while the absence of testosterone permits the Wolffian duct to degenerate. However, positive developmental signals are also required. Among these is the protein **Wnt-4**, a member of a large family of locally acting signal molecules (Section 4). Wnt-4 may be needed both for oocyte development and for further suppression of male development.²⁶¹

The earliest studies of oocyte development were done with sea urchins (often *S. purpuratus*) and with amphibians (often the South African clawed toad *Xenopus laevis*) whose eggs are as much as 1000 times larger than those of mammals.²⁸⁰ However, despite the differences in size, modes of fertilization, and ovary development, oocytes of nematodes, sea urchins, frogs, insects, and mammals have much in common. Oocytes of *C. elegans* and of most other animals undergo a temporary arrest in development at the prophase stage of the first meiotic division (Fig. 26-12).^{13,281–283a} At this stage oocytes transcribe many genes. In some species chromosomes may develop a “lampbrush” appearance (Fig. 27-6) as a result of the transcriptional activity. Many mRNA molecules are stored in the expanding cytoplasm. Proteins are also synthesized and stored.²⁸⁴ Among these are specialized proteins of yolk granules and proteins used to construct an outer egg coat. Surrounding **follicle cells** also contribute nutrients to the oocyte.^{284a} In insects, whose early embryonic development has some special characteristics, 16 surrounding **nurse cells** are connected to the

oocyte by cytoplasmic bridges.^{285,286}

Oocytes may remain in arrest at the beginning of meiosis for prolonged periods before continuing through the **maturation** stage to form an ovum (egg). Women and other female mammals are born with thousands of oocytes, but only a few at a time develop into eggs. Maturation is often delayed until sexual maturity, when it is stimulated by hormones.¹³ In *X. laevis* progesterone stimulates maturation.^{282,287} In *C. elegans* and many other animals a signal from a sperm cell is needed to induce maturation.^{281,283} Maturation of the oocyte is often arrested again, this time at metaphase of the second meiotic division. Transcription is halted, and protein synthesis is slowed. Fertilization then induces rapid completion of meiosis. Penetration of the sperm leads to “activation” of the egg and completion of meiosis. In lower organisms activation can often be carried out by chemical or physical treatment in the absence of a sperm cell, with formation of parthenogenetic offspring.

3. Fertilization

Fertilization of the egg is a biochemically complex process.^{35,288} It involves recognition of sperm and egg, often in a species-specific manner.²⁸⁹ The jelly layer around sea urchin eggs contains peptides such as the **sperm-activating peptide** (speract; Table 30-4), which stimulates increased respiration and motility of the sperm cells.²⁹⁰ Additional chemotactic peptides may also be released from the jelly layer of invertebrate eggs. Chemoattractants for vertebrate eggs are less well known, but a 21-kDa sperm attractant protein from *X. laevis* egg jelly has been characterized.²⁹¹ Both in sea urchins and in mammals the jelly layer, which is called the **zona pellucida**, contains sperm cell receptors.^{288,292–295} These are glycoproteins that interact with proteins (spermadhesins)²⁹⁶ of the sperm cell membrane. One of these is the integrin-associated CD9, an integral membrane protein.^{297,298} Penetration of the sperm through the zona pellucida often involves a large specialized secretory vesicle, the **acrosome**, as well as the enzyme hyaluronidase.^{298a} In some species the acrosome releases a large amount of monomeric G actin, which polymerizes suddenly into a tube of polymeric F actin, which in some way assists the penetration of sperm.³⁵ In the horseshoe crab *Limulus polyphemus* the acrosome in an unactivated sperm cell contains a twisted bundle of as many as 120 cross-linked actin filaments. When the sperm is activated by contact with the jelly coat of the egg, the acrosome straightens into a 50- μ m-long crystalline bundle, which is driven into the egg coat.²⁹⁹ Of importance to all types of sperm cells are proteases and other materials that are also released from the acrosome and which help to etch a hole that allows the sperm to enter the

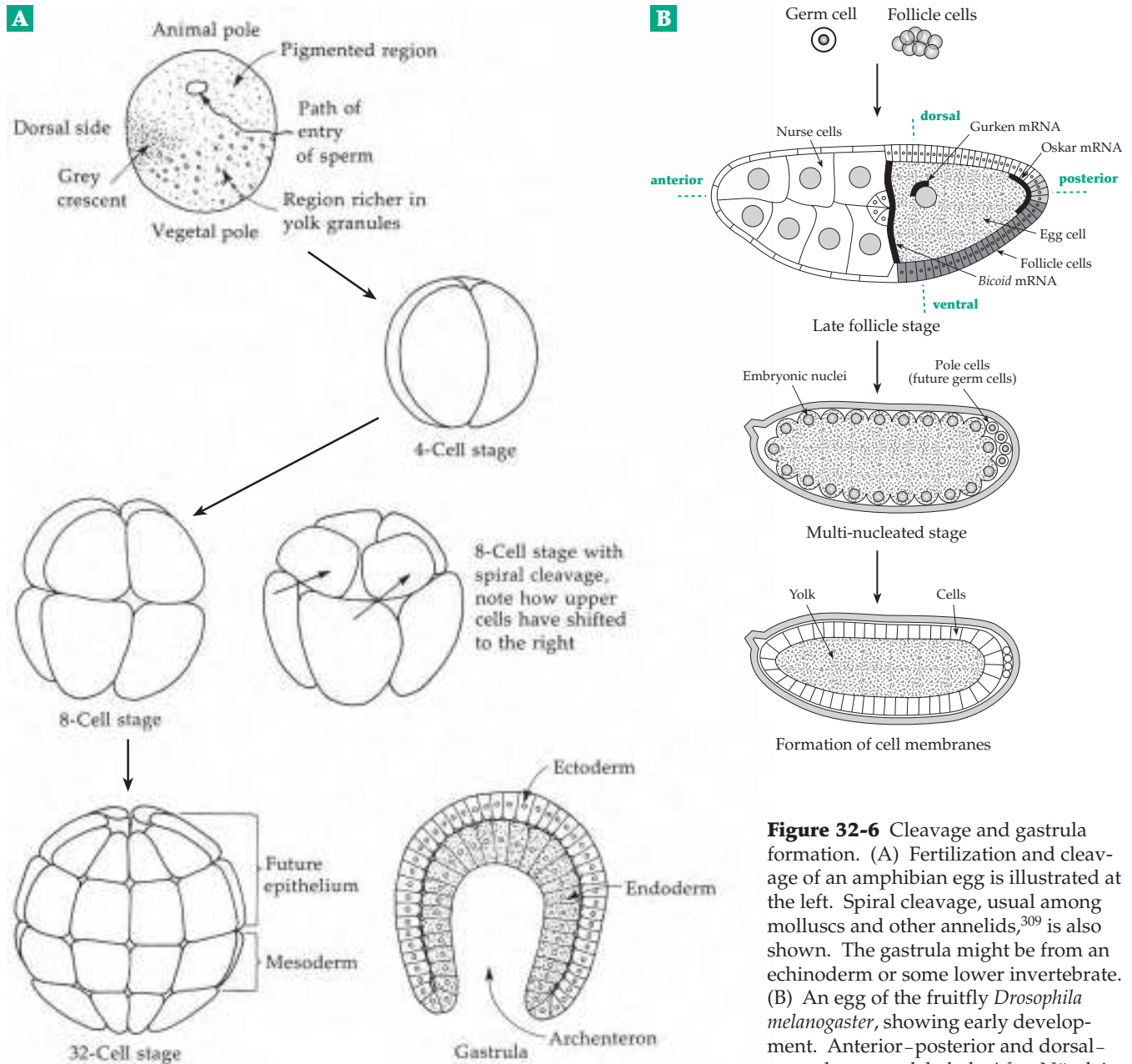


Figure 32-6 Cleavage and gastrula formation. (A) Fertilization and cleavage of an amphibian egg is illustrated at the left. Spiral cleavage, usual among molluscs and other annelids,³⁰⁹ is also shown. The gastrula might be from an echinoderm or some lower invertebrate. (B) An egg of the fruitfly *Drosophila melanogaster*, showing early development. Anterior–posterior and dorsal–ventral axes are labeled. After Nüsslein-Volhard (after a drawing by Laurie Grace).⁹

ovum.^{300,301} The acrosome reaction also activates the egg, a process that may depend upon NO.³⁰² In the sea urchin the acrosome contains a large amount of the protein **bindin**, which mediates a species-specific adhesion of the gametes and presumably fusion with the egg membrane.^{302a}

Fusion of the sperm membrane with that of the ovum causes a rapid depolarization of the membrane of the ovum and an influx of calcium ions.^{303,304} This causes an immediate block to the entrance of any other sperm cells in most species. It also causes the fusion

of the membranes of **cortical granules** (several thousand in a mouse ovum) with the cell membrane and release of their contents.³⁰⁵ The material released to the outside of the ovum includes various proteins and enzymes such as a peroxidase that catalyzes crosslinking of tyrosine side chains (Chapter 25) and hardens the material immediately around the ovum into a tough **fertilization membrane**. Within the ovum a respiratory burst resembling that of activated neutrophils (p. 1074) provides H₂O₂ to the peroxidase.

Fertilization also induces completion of meiosis

and formation of a one-cell embryo containing a maternal pronucleus contributed by the egg and a paternal pronucleus derived from the sperm. Each pronucleus undergoes DNA replication and then enters the first mitosis, which yields a two-cell embryo containing one diploid zygotic nucleus per cell.^{283,306} Under the influence of the cyclin-dependent kinase *cdc2* (see Eq. 26-3) and a hyperphosphorylated form of the protein **nucleoplasmin**, the chromatin of the compact sperm nucleus undergoes decondensation. The sperm basic proteins that coated the DNA are replaced by histones H₂A and H₂B.³⁰⁷

Although an amphibian egg is nearly spherical, there is already a strong polarity. The nucleus lies nearer to the **animal pole** than to the **vegetal pole**, which in many eggs is rich in yolk granules. In eggs of amphibians the animal pole is highly pigmented, but the vegetal pole is less so. On one side above the equator, there is a gray crescent. In some animals this marking appears on the opposite side of the egg from the point of sperm cell entry (Fig. 32-6). The gray crescent marks the future back (dorsal) side of the organism and the opposite part of the cell, the future ventral side.²⁸⁰ The point of sperm entry also marks the ventral side for the mouse, a fact that suggests that the plan of development of mammalian embryos may be basically the same as that of frogs.³⁰⁸ However, it is the internal components of the cell that actually determine the cell's axes. The cytoplasm of the mature ovum contains an unequal distribution of many materials with a well-developed bilateral symmetry. That this distribution is important is seen from the fact that centrifugation of eggs prior to fertilization often leads to formation of abnormal embryos because of

displacement of preformed ribosomes and other materials. It is probably gradients in the concentrations of dormant mRNAs²⁸⁴ and other metabolites that lead to uneven growth of cells and to the indentation of cells at the vegetal pole, a process that initiates the formation of the endodermal layer of the gastrula (Fig. 32-6). In insects the polarity of the developing ovum (the oocyte) is established by the cytoplasmic bridges from surrounding nurse cells, which are asymmetrically arranged (Fig. 32-6B).²⁸⁵

4. Embryonic Development

The fertilized (activated) ovum rapidly undergoes several mitotic divisions, known as **cleavage**, during which no overall growth occurs. The number of cells increases and the DNA replicates at each division, but the overall size of the resulting cell cluster is the same as that of the original ovum (Fig. 32-6). Further development leads quickly to a stage in which a layer of cells (called blastomeres at this stage) surrounds an internal cavity forming a **blastula**. In the sea urchin the blastula, which is released from its protective fertilization membrane ~11h after fertilization,^{310a} consists of a single layer of cells. In frogs and many other organisms there are two or more layers. In *X. laevis* about 4000 cells are formed in eight hours.^{307,310} In mammals a solid cell mass (**morula**) forms first and is later transformed to a **blastocyst**, a hollow ball with an internal cavity.

Early mammalian development has been hard to study because of two facts: the ovum is very small, and a first priority is development of the placenta and of the layers of tissues that surround the embryo.^{308,311,312} This occurs in humans within the first week after fertilization. Both the trophoblast and cells of the inner cell mass (Fig. 32-7) contribute to the extra embryonic tissues.

Development of a mouse beyond the one-cell stage is dependent upon a regulatory gene of the *Oct* family (see p. 1631). The **Oct-3/4** protein, which binds specifically to the DNA motif 5'-ATTTGGAT, consists of two domains, both of which are essential for tight binding to this sequence. One domain is a 75-residue **POU domain** that consists of a helix-turn-helix motif with an amino acid sequence that is highly conserved among mammalian **Pit** and **Oct** regulatory proteins as well as some **Unc** proteins of *C. elegans*.³¹³ The second domain is a 60-residue homeodomain (see p. 1900).^{313,314} Oct-3/4 appears to be essential not only for cleavage of a one-cell egg but also for progression from a two-cell to a four-cell egg (Fig. 32-7) and also in other embryonic cells.³¹⁵ Up to the two-cell stage very little transcription of zygotic genes is observed but further development requires zygotic genes and by the 8-cell stage protein synthesis from maternal mRNAs

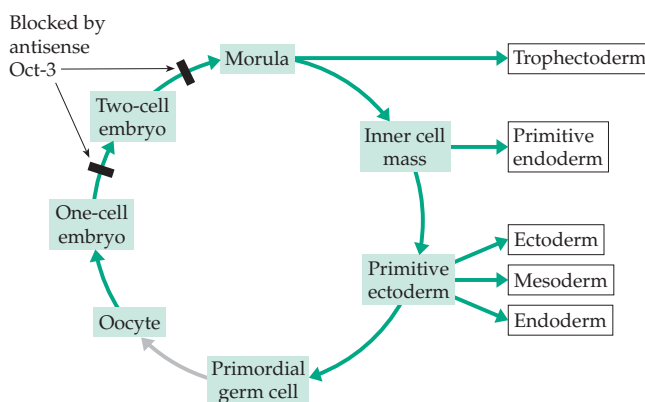


Figure 32-7 Expression pattern of Oct-3 mRNA during mouse development. The green boxes indicate those stages in which Oct-3 is expressed, while the white boxes at the right indicate all types in which little or no Oct-3 mRNA is formed. After Rosner *et al.*³¹⁴

ends.²⁸³ Oct-3/4 continues to be synthesized in the embryo and is necessary for establishment of pluripotent stem cells in the embryo.³¹⁵

Development in *Drosophila* and other insects follows a somewhat different pathway, as is indicated in Fig. 32-6B. The egg, which is surrounded by follicle cells and 16 nurse cells, does not divide. However, its nucleus divides repeatedly, about once every nine minutes, to form ~6000 nuclei. Only then do separating membranes form to give individual cells.^{9,285,316,317} During the first two hours the nuclei form a **syncytium**, in which they are embedded in a common cytoplasm, that allows free diffusion of signaling compounds. At first the nuclei are in the center, but later most migrate to the periphery and form a single layer of cells comparable to the blastoderm of amphibian cells. A few nuclei remain in the central cavity to become yolk cells, and some at the posterior pole become separated into pole cells.

The next stage in embryo formation, which occurs universally, is the invagination of the blastula at the vegetal pole to form a **gastrula**. At this stage the embryo has distinct ectoderm and endoderm cell layers. The cavity, formed in the gastrulation process and connecting to the outside, is referred to as the **archenteron** and is the forerunner of the gastrointestinal tract or enteron. Gastrula formation is more complex in the frog embryo and still more so in the human embryo. In all but the most primitive of animals a third layer of **mesosomal** cells is formed between the endoderm and ectoderm. These three **germ layers** differentiate further as follows. The **ectoderm** yields the skin and nervous system; the **mesoderm** the skeleton, muscles, connective tissues, and circulatory system; and the **endoderm** the digestive tract, lungs, and other internal organs and germ cells.

Organ development occurs largely by infoldings of cells from the endoderm and ectoderm. These infoldings appear to be induced by chemical substances secreted by cells of an adjacent germ layer. Thus, ectodermal cells form the **neural plate**, the prospective brain, and spinal cord in response to induction by mesodermal cells lying beneath the neural plate area. The mammary glands also arise from interactions of mesodermal and ectodermal cells, while the formation of the pancreas, liver, and lungs depends upon interactions of groups of cells from endoderm and mesoderm. Because of their transparency zebrafish are especially useful for study of organ formation.³¹⁸⁻³²¹

During the early stages of embryo growth, development seems to be directed largely by the polarity and gradients of the large amounts of mRNA, yolk and other constituents, which form a prepattern in the ovum.^{321a} However, even at very early stages signaling from the nucleus of the ovum to surrounding cells is a necessary part of establishing the cell axes and developmental pattern.

The anterior-posterior axis. Only recently has it become possible to identify some of the specific mRNAs, the signaling molecules, and receptors that are involved in establishing the principal axes of the ovum (Fig. 32-6). Even for this aspect of development the genetic program is very complex, there are many uncertainties, and the details go far beyond the scope of this book. Much of the most important work has been done with *Drosophila* for which numerous mutants have been identified and characterized. Many names of genes and of proteins are derived from a description of the phenotype of a mutant fly. These same names are often used for the corresponding genes or proteins in other organisms. However, a protein may be known by more than one name, depending upon the species.

A *Drosophila* mother deposits mRNA for ~80% of all of her genes in the egg, but not all of the encoded information is used. Because genetic experiments can be done so readily with *Drosophila*, it is possible to ask what **maternal effects** do come from this mRNA and what effects come from the genes of the zygote.^{9,88,316} For example, the *bicoid* mRNA that accumulates at the anterior end of the *Drosophila* egg is produced by nurse cells and is transported into the ovum. If the mother has defective *bicoid* genes the eggs die. A normal *bicoid* gene present in the father does not prevent death. Another maternal effect protein is encoded by *nanos*, whose mRNA accumulates at the posterior pole of the ovum. The maternal *torso* gene, which acts on follicle cells, is also needed for the anterior-posterior axis formation.³¹⁶ About 30 of these maternal effect genes are active in organizing the pattern of the embryo.

The *Drosophila bicoid* mRNA forms an anterior-posterior (A-P) concentration gradient, which controls early development along the axis. Another protein called **Staufen**, which forms a complex with the *bicoid* mRNA, assists in moving the RNA along on microtubules to form the gradient.^{321b} The microtubules, together with associated transport proteins, are also essential to the prepatterning of the ovum.^{9,88,285,322,323} Bicoid protein is a transcription factor that is synthesized at the sites of its mRNA accumulation. It is absent from the eggs when they are laid but soon appears.³¹⁶ Bicoid binds to the CRMs of target genes and activates them. Cooperative binding of multiple copies of the transcription factor may be necessary to provide the observed sensitivity to concentration. A transcription factor may bind at many sites on the DNA of the zygote or of nurse cells. For example, a gene called *even-skipped* (*eve*) is expressed in seven stripes in the blastoderm embryo. The enhancer that controls *eve*'s second stripe contains at least five binding sites for Bicoid as well as others for the Hunchback, Giant, and Krüppel proteins.⁴⁴

A different gradient along the A-P axis is formed by the **Nanos** protein, whose mRNA localizes in the

cytoplasm of the posterior part of the ovum. Like the *bicoid* mRNA, *nanos* mRNA forms a complex with the Staufen protein. Messenger RNA transcribed from a gene called *oskar* is also necessary for development of the posterior region of the ovum. An additional gene, called *gurken*, is also involved in establishing the A-P axis. The nucleus of the ovum secretes *gurken* mRNA (Fig. 32-6), which is translated to the protein **Gurken**, a TGF- α -like protein that carries signals to follicle cells. They, in turn, help to organize the microtubules in the ovum. The nucleus of the ovum moves, sending the Gurken signal first to the posterior pole cells, then to a position that marks the dorsal side of the ovum. Thus, it participates in establishing both the A-P and dorsal-ventral axes.³²⁴

The animal-vegetal axis of sea urchin eggs is established during oogenesis, but the mechanisms are still unclear. After fertilization a distinct pattern along the axis is established by the 60-cell stage. Signaling from the vegetal pole appears to play an important role.⁸⁹ In the presence of lithium chloride, which is known to affect inositol triphosphate (InsP₃) metabolism (Fig. 11-9), embryos develop an excessive fraction of endoderm and mesoderm (tissues of the vegetal half) at the expense of ectoderm (tissues of the animal half).^{89,325} Some data suggest that this effect implicates the **Wnt signaling pathway** and the protein **β -catenin**. This is the same protein that links C-terminal tails of cadherin to the actin cytoskeleton.⁶⁰ Its cytoskeletal and signaling functions seem to be controlled separately.

In the frog *Xenopus* the ovum accumulates an RNA (Vg1 mRNA) that encodes a growth factor of the TGF- β family (Chapter 30, Section A,6).²⁸⁰ Initially present throughout the ovum, it moves and forms a gradient of concentration that is highest at the vegetal pole.

The dorsal-ventral (D-V) and right-left axes.

Establishment of the D-V axis in *Drosophila* requires the participation of several genes. As mentioned above, *gurken* affects follicle cells. These cells cooperate with the ovum to set up a concentration gradient of the maternal gene **Dorsal**, the protein encoded by the *dorsal* gene.^{9,280,326–328} Dorsal is a transcription factor related to NF- κ B (Fig. 5-40). In the early embryo of the fly it forms a gradient in which it remains largely in the cytoplasm on the dorsal side but is mostly taken up into the nuclei on the ventral side. There it binds to a series of complex enhancers, each 300–1000 kbp in length, which act along the D-V axis. The enhancers interpret the concentration of Dorsal at five different threshold levels.³²⁸

The establishment of a second axis in vertebrate embryos is a complex process, which depends upon prior formation of a mesoderm layer. Development of mesodermal cells (not shown in the more primitive gastrula illustrated in Fig. 32-6) is induced by diffusion

of a growth factor from the vegetal pole.^{329–331}

Activin, a member of the TGF- β family, has been proposed as the natural inducer of mesoderm. More recently **nodal**, a different TGF- β -like protein, has been suggested.³³² The fact that induction can occur through thin (20 μ m) filters without any cell-cell contact indicates that specific chemical agents are responsible. Induction of the mesodermal layer in *X. laevis* appears to be an effect of an epidermal growth-factor-like protein. Additional factors are also needed to establish the D-V axis in vertebrates³²⁶ in which the dorsal side is homologous to the ventral region in *Drosophila*.

Expression of another set of genes establishes the **right-left** axis and characteristic asymmetries of the body.^{333–336} In the chick activin 2 β , also a member of the TGF- β family, as well as Nodal and **Sonic hedgehog** (Shh) participate in control. The gene *Pitx2* is a downstream transcription target for this signaling cascade in vertebrates.

Spemann's organizer. In 1924, the German physiologist Hans Spemann with Hilde Mangold transplanted a small piece of tissue from the dorsal lip of a newt blastula to a site on the ventral side of an early blastula of a differently colored species. The embryo developed a small secondary embryo, most of the tissues of which came from the host, not from the transplanted piece. It was concluded from this famous experiment that the transplanted vegetal tissues had supplied a diffusible inducer.^{13,14,337–339} This morphogen caused the cells of the ventral surface of the blastula to secrete other morphogens. The signaling center in this part of the blastula surface is known as **Spemann's organizer**, or simply the organizer. It utilizes more than one inducer and a complex set of signaling interactions.³⁴⁰ The cascade that induces formation of the organizer involves the Wnt- β -catenin and TGF- β pathways and the transcription factor **Smad4**.^{41,341,342} The organizer secretes protein such as **noggin**, **folistatin**, and **chordin**, as well as nodal and other members of the TGF- β family.^{332,343,344} They establish the D-V axis and also direct the development of the head and the initial patterning of the central nervous system. Noggin seems to be a neural inducer.³³⁸

Patterns, signaling pathways, and homeotic genes. While gradients in *Drosophila* eggs establish the anterior-posterior axis, products of other genes specify the developmental fates of cells of specific lineages and of cells found in particular spatial domains. Many *Drosophila* genes are needed to establish patterns, e.g., spacings of similar elements such as hairs, components of compound eyes, and whorls of plants.³⁴⁵ Insects are organized into a pattern of consecutive segments from head to tail along the A-P

axis.^{13,14} In *Drosophila* there are typically 17 of these segments, some carrying appendages such as antenna, legs, and wings. Each segment develops under a different set of influences from neighboring cells. Development is controlled by ~30 **segmentation genes**, which determine the number of segments and their internal organization. Of these genes one set of at least six **gap genes**, among them *hunchback* (Hb) and *Krüppel*, are expressed first. Both of these genes encode zinc-finger transcription factors (see Fig. 5-38). The Hunchback protein acts mainly on head parts and upper thorax, while Krüppel influences development of the thorax. Also among the segmentation genes are eight “pair-rule” genes and at least 16 segment polarity genes. The interactions of the products of these genes creates a prepattern that provides positional information and guides further development.³⁴⁶ Somewhat similar to the segmentation of the insect body is the development of skeletal muscle from a series of mammalian embryonic blocks known as somites.^{13,347,348} In *Drosophila* signaling pathways involving the secreted proteins EGF, Decapentaplegic (Dpp),³⁴⁹ Wingless (Wg), Hedgehog (Hh),^{349,350} and Notch are used repeatedly to provide positional information. The names of these proteins describe effects of mutations on the limbs of the insect, but the proteins have a much broader significance. They represent an evolutionarily conserved set of intercellular signaling pathways.³⁴⁶ Wingless is the first member of the previously mentioned Wnt family. Proteins of the Hh family, including the vertebrate Shh, control a large variety of processes that include development both of limbs and of the nervous system.^{351–355} The Hh and Shh proteins all carry a molecule of cholesterol covalently bound in ester linkage to the C termini of the biologically active N-terminal domains of these proteins.^{350,353} Defects in Shh signaling causes some human birth defects, and it is possible that drugs that inhibit cholesterol synthesis may have adverse effects on embryonic development.^{352,355}

The *Drosophila* Notch 1 is a 300-kDa integral membrane protein that contains 36 EGF-like repeats. Its activation by proteolysis produces a 200-kDa N-terminal portion and a 120-kDa C-terminal fragment, which contains a transmembrane domain. The small intracellular domain of this fragment is then released by protease action and travels to the nucleus where it activates several target genes.^{356–358} The Notch signaling pathway is conserved in all metazoans and influences many interactions that control cell fate during development.³⁵⁹ The proteolytic cleavages of the Notch protein parallel those of both the ErbB-4 growth factor receptor (see Table 11-3) and the amyloid precursor protein APP that is pictured in Fig. 30-34. The same type of protease (γ -secretase or presenilin in the human brain) cleaves all three of these proteins.^{359–361} Actions of Notch are modulated by posttranslational

alteration, e.g., glycosylation by a fucosyltransferase encoded by gene *fringe*.^{362,363} A homolog of *fringe*, called *lunatic fringe*, encodes an essential component of somite formation in the mouse.^{348,364}

Functioning together with the signaling pathways are **selector genes** that determine which specific pathway is to be influenced. For example, eyes, antennae, legs, or wings of a fly may be selected.³⁴⁶ *Antennapedia* (*Antp*) is one of these genes. Several of the *Drosophila* selector genes as well as some pair-rule genes³⁶⁵ are also known as **homeotic genes** (see also Chapter 28, Section C,6). Homeotic genes were first recognized by the fact that mutation causes conversion of one segment of an insect’s body into the homologous tissues of another segment.^{366,367} For example, a mutation in *antennapedia* (*ante*) changes the antenna into a leg. Similar genes are also active in vertebrates, e.g., in the development of the chick embryo limb bud, the very tip (the last 20 cell diameters of length) contains cells that differentiate into the various elements of the limb in a relatively autonomous manner. If this *progress zone* from one limb bud is grafted onto the end of another limb bud, the bones and cartilaginous elements of the limb are repeated. Both the number and morphology of fingers and toes are determined by homeotic genes^{367a} as is the formation of sphincters in the developing gut.^{367b} Homeotic genes (also known as *Hox* genes) contain a conserved sequence of 180 bp that specifies a 60-residue protein **homeodomain** (also known as a homeobox). The homeodomain folds into a helix–turn–helix motif characteristic of many transcriptional regulators (Figs. 5-35, 28-3, p. 1631).^{365,367–371} *Hox* genes are found among all forms of life. Hundreds have been described.³⁷² They include genes for the previously mentioned transcription factors of the Oct and Pit families as well as for the yeast mating type proteins MATa1 and MAT α 2 (p. 1880).¹⁷ The *Drosophila* genome contains eight *Hox* genes, while the human genome has ~40, which are organized into *Hox* clusters.³⁷³ *Hox* genes are also abundant in plants.^{345,372,374}

Despite intense interest the role of homeodomain proteins in development is not well understood. The highly conserved motif binds to DNA at many places in the genome. Current thinking is that homeodomain proteins interact with other regulatory proteins, and that various combinations of these proteins provide the information needed to direct development.³⁷⁵

D. Specialized Tissues and Organs

Here are a few details about development of mammalian tissues. We’ll begin with the blood and connective tissues, which arise from embryonic mesodermal cells.

1. Blood Cells and the Circulatory System

Every second of life a human must produce about 2.5 million red blood cells, about 2 million granulocytes, and many lymphocytes as well as other less numerous leukocytes. All of these arise from **multi-potential stem cells** found in the bone marrow.³⁷⁶⁻³⁷⁹ Each of these stem cells divides to form one daughter stem cell and one **progenitor cell**.³⁸⁰ The progenitor cells are also stem cells but have differentiated into **myeloid**,³⁸¹ **erythroid**, and **lymphoid**^{382,383} cells. These differentiate further as is indicated in Fig. 31-2. Mature blood cells of most types have short lifespans and must be regenerated from stem cells continuously.

At all stages the differentiation process is regulated by the microenvironment which is rich in specific protein growth factors, several of which have been discussed in Chapter 30. Among the 20 interleukins, three stimulate growth of both multipotential progenitor cells and erythroid progenitor cells.^{380,384} The acetylated tetrapeptide Ac-Ser-Asp-Lys-Pro inhibits stem cell proliferation. Granulocyte-macrophage colony-stimulating factors stimulate proliferation of both granulocytes and macrophages. The kidney cytokine **erythropoietin**, a 30.4-kDa glycoprotein,³⁸⁵ is a primary regulator of red blood cell formation. Its action on a differentiated stem cell initiates massive hemoglobin synthesis and terminal differentiation of the erythrocyte. **Thrombopoietin** promotes formation of megakaryocytes and also their maturation and release of platelets to the blood.³⁸⁶ **Thymopoietin** promotes early T-cell differentiation. Activated macrophages secrete interleukin-1, which stimulates maturation and proliferation of B lymphocytes. Interleukin-2 (T-cell growth factor; Fig. 30-8) is produced by activated T lymphocytes and is needed by T lymphocytes for long-term helper and cytotoxic functions. Differentiation of the stem cells into the erythroid lineage requires transcription factor GATA-1, development into erythrocytes requires GATA-2, while development into T lymphocytes requires GATA-3.³⁸⁷

Globin genes. The genes that encode the human globins from which hemoglobin is formed are found in two clusters, the α -like genes on chromosome 16 and the β -like on chromosome 11. They are developmentally regulated, different genes in the clusters being active at different stages of development. Mammalian hemoglobins (Chapter 7) each contain two α chains or two related ζ chains and two other chains, β , γ , δ , or ϵ . Adult hemoglobin is mainly $\alpha_2\beta_2$ but contains small amounts of $\alpha_2\delta_2$. In early embryos the hemoglobin is $\zeta_2\epsilon_2$, but during the second to sixth weeks of embryonic life the two fetal hemoglobin chains γ^G and γ^A replace the ϵ chains. The switch from fetal to adult hemoglobin begins a few weeks before birth and is complete by about ten weeks after birth. The β -like

gene cluster contains five genes encoding globins ϵ , γ^G , γ^A , δ and β (Fig. 27-10). Each gene consists of three exons separated by two introns and has rather similar control signals. These include CACCC at \sim -100, CACA at \sim -92, CCAAT at \sim -75, and ATAAAA (TATA sequence) at \sim -30, as well as AATAAA (cleavage and polyadenylation). The CCAAT sequence appears twice in the γ^A promoter. A variety of transcription factors and chromatin modifiers influence the expression of these genes.^{388,389}

Only a small amount of fetal hemoglobin ($\alpha_2\gamma_2$) is produced after infancy. There are two genes, γ^G and γ^A , for the β -like chains of fetal hemoglobin. A few adults make large amounts of fetal hemoglobin and this hereditary **persistence of fetal hemoglobin** has survival value for persons carrying thalassemia genes. This condition may result from a single base change in the CCAAT sequence found upstream (\sim 75 bp) of the globin genes. Many other genes are also preceded by the same sequence, which in extended form is often TTGGPyCAAT. In one individual with persistence of fetal hemoglobin the first G in this sequence was replaced by A in one of the two CCAAT sequences present in the γ^A gene.

Thalassemias. In these important hemoglobin diseases the α or β chain either is absent or is present in far less than stoichiometric amounts.^{14,390} About 40 point mutations in the β globin gene have been described among patients with β thalassemia, in which β chains are missing (β^0 thalassemia) or are present in reduced amounts (β^+ thalassemia). These mutations sometimes occur in control regions at the 5' end of the gene. For example, a change of the TATA sequence from ATAAAA to ATACAA causes decreased transcriptional efficiency and β^+ thalassemia. Other mutations result in abnormal splicing or in instability of the β globin. Deletions may result in the complete absence of the gene or in a frameshift that results in nonfunctional globin and β^0 thalassemia.

How do embryonic cells choose to transcribe only the embryonic globin genes? The decisions to switch from embryonic to fetal and from fetal to adult at appropriate times appear to be controlled by interactions with appropriately expressed transcription factors. Especially impressive is the total and permanent cessation of transcription of the embryonic globin ϵ gene at about the seventh week of gestation. Transcription of the β globin gene cluster is controlled by a powerful enhancer, the **locus control region** \sim 6-22 kbp upstream of the promoter.³⁹¹ In addition, \sim 270 bp upstream of the ϵ gene promoter is a **silencer**, a DNA sequence to which inhibitory proteins may bind and, in cooperation with the enhancer, may completely silence the ϵ gene while allowing transcription of other genes in the cluster.^{392,393} The ζ gene, in the α globin cluster, is silenced by the binding of an NF- κ B transcription factor to a 108-bp

segment of DNA located 1.2 kbp to the 3' end of the gene.³⁹⁴ The globin genes are shielded from action of nearby enhancers by **insulators**, DNA sequences that often contain CpG islands.³⁹⁵

Blood vessels: vasculogenesis and angiogenesis. Early in development of an embryo a network of blood vessels is formed from mesenchymal progenitor cells (vasculogenesis).^{396–398} Later, in either the embryo or the adult, new capillaries are formed (angiogenesis). As the organism develops these new vessels are “pruned,” and the vascular bed is remodeled to a tree-like form with vessels of both large and small diameter.^{399,400} A key activator of angiogenesis is the vascular endothelial growth factor **VEGF**.^{401,401a} However, a number of other proteins including **endoglin**, a TGF- β binding protein,⁴⁰² the clotting factor thrombin,^{400,403} and the ribonuclease **angiogenin** (p. 648) exert their influences.⁴⁰⁴ There are diseases that result from defective angiogenesis. However, a major interest in this process arises because of the essential role of angiogenesis in the growth of cancer⁴⁰⁵ and as a complication of inflammatory processes. Angiogenesis in the retina is a major cause of blindness resulting from diabetes mellitus or from macular degeneration.^{405,406} There are natural antagonists of angiogenesis,^{407–409} and efforts are being made to utilize them in therapy. A related project is development of completely tissue-engineered blood vessels for surgical use.⁴¹⁰

2. Cartilage, Tendons, Bone, Muscle, and Fat

Mesenchymal cells differentiate into cartilage, bone, muscle, adipose, and other connective tissues.⁴¹¹ **Chondrocytes** synthesize the variety of collagens (pp. 431–426) that are needed for synthesis of cartilage and other connective tissues. The 32 or more genes encoding the polypeptides needed for synthesis of the 19 types of vertebrate collagens (Table 8-4) are developmentally regulated in a complex manner.⁴¹¹ Their promoters contain TATA and CCAAT sequences as well as other presumed regulatory codes. The first intron of several collagen genes has also been identified as a control region containing enhancers.⁴¹² The elastic fibers (p. 436) owe their properties in part to elastin. The control region of elastin genes lacks the TATA sequence but has SP1 binding sites. As with many other mammalian genes, a diversity of protein products, many in small amounts, are made by alternative splicing.

Collagen fibrils provide the scaffolding for formation of bone, whose composition is considered on pp. 440–443. Bone develops under the influence of **bone morphogenic proteins** BMP-2 to BMP-7.^{413–415} Most of these are cytokines of the TGF- β family. Noggin

(p. 1899) antagonizes the action of the BMPs.³⁴⁴ A characteristic of bone is rapid remodeling (p. 441) by which ten per cent of skeletal bone is replaced every year. The balance between action of the bone-forming osteoblasts and the bone-resorbing osteoclasts is regulated by surface proteins responding to **c-Fos** and to **interferon- β** .⁴¹⁶

Muscle, whose structure and function are discussed in Chapter 19, develops in response to four members of the myoD family. These include myoD, **myogenin**, **myf5**, and **MRF4**.^{417–419} All are muscle-specific transcription factors of the basic helix-loop-helix class. An unusual aspect of muscle development is formation of multinucleate **myotubes** (muscle fibers; p. 1096).⁴²⁰ Apoptosis plays an important role in muscle development and can present significant complications in damaged cardiac muscle.⁴²¹ Defects in several developmental control genes are responsible for congenital heart diseases.⁴²²

3. Epithelia

Epithelial tissues, which line both internal and external surfaces, arise from all three cell layers of the blastula. The epidermis (Box 8-F) arises from ectoderm, while the lining of the digestive tract is formed by endodermal cells. Mesoderm provides the linings of blood vessels. About 60% of differentiated tissue types in the mammalian body are epithelia.¹²³ Stem cells or progenitor cells are present and provide for renewal.^{123,124} While epidermal stem cells are located in deep layers of the skin, **keratinocytes** are readily cultured *in vitro* and can give rise to fully differentiated multilayered skin.⁴²³ Development appears to require transcription factors related to **Oct-2**⁴²⁴ as well as **p63**, a homolog of the tumor suppressor p53.^{425,426} Mice deficient in the aspartyl protease **presenilin 1** (which is defective in some forms of Alzheimer disease, Chapter 30) develop characteristic epidermal skin tumors. The β -catenin–Wnt signaling pathway (p. 1899) seems to be involved.⁴²⁷ The gastrointestinal endoderm develops its highly convoluted surface under some control by the Notch signaling pathway.^{428,428a} Endothelial progenitor cells (**angioblasts**) are responsive to many signaling molecules⁴²⁹ including thrombin.⁴³⁰ The *Drosophila* eye develops from the epithelium, again through signaling via Notch and other morphogens.^{430a} Of outstanding importance to epithelial cells in general is their ability to form complex communicating junctions.^{430b}

4. The Nervous System

Development of the vertebrate central nervous systems is initiated during gastrulation through an

interaction between the dorsal ectoderm and an infolding of the dorsal mesoderm. Several different diffusible inducers are involved. These include noggin,³⁴⁴ follistatin, and other members of the TGF- β family as well as thyroid hormones, basic fibroblast growth factor (bFGF), and sonic hedgehog.³²⁹ The nervous system develops over a period of a few days with differentiation of precise numbers of neurons, astrocytes, and oligodendrites in successive waves. The order in which various cell types arise is determined by the order in which transcription factors such as Hunchback, Krüppel, and others are expressed.⁴³¹ Multipotential neural stem cells provide the new cells that are required.^{432,433} Neural tissue from a region called the **neural plate** develops into a neural fold. The latter is closed to form the **neural tube**,⁴³⁴ within which the notochord, the precursor to the spine, as well as the neurons, glia, and other cells grow. The **neural crest** forms as an outgrowth from the dorsal surface of the neural tube under the influence of inducers of the Wnt and BMP families.^{434a} Cells migrate from this crest to form the peripheral nervous system, melanocytes, and cranial cartilage.^{434b} The pituitary, a central component of the neuroendocrine system, develops from tissues from the midline part of the anterior neural ridge.⁴³⁵

The **floor plate**, which develops along the midline of the ventral surface of the neural tube, is the source of Sonic hedgehog,^{436,437} **netrin-1**, and other secreted molecules.⁴³⁶ Some of these participate in **axon guidance** by which the growing tips (**growth cones**) of axons are able to connect to the correct "targets."^{437a} For example, every visual receptor cell in the retina must send its signal to the correct locations in the visual cortex of the brain.⁴³⁸ How can this be accomplished? Over a century ago Ramon y Cajal proposed that chemoattraction, analogous to chemotaxis of bacteria, might be involved.^{439,440} A hundred years later Tessier-Lavigne and coworkers isolated the first of these attractants, **netrin-1** and **netrin-2**, from 25,000 pulverized chick brains.^{441,442} Of these two closely related 75- and 78-kDa molecules netrin-1 is produced only in the floor-plate cells. Like the less well understood nerve growth factor (Fig. 30-7), netrins induce outgrowths of neurites and also are chemoattractants for nerve growth cones.⁴⁴³ The netrin receptor is known as **DCC** (Deleted in Colorectal Cancer).⁴⁴⁴ A nematode protein UNC-6 is related to the netrins.⁴⁴⁵

Growth cones are subject to both chemoattractant and chemorepellent effects of guidance molecules and also to attraction or repulsion resulting from cell-cell contacts. To complicate the picture further, the netrins and also the brain-derived neurotrophic factor BDNF (see Fig. 30-6D) may first attract, and then after a period of adaptation or desensitization, repel a growth cone.^{443,444,446-448} Consecutive phases of desensitization and resensitization may result in a zig-zag path of

growth. The netrins were recognized first by observing growth of **commissural neurons**. These neurons originate within the spinal cord on one side or the other or the midline. They grow down toward the floor plate attracted by the netrin-1 or **BDNF** produced there. The neurons then cross the midline before turning and growing toward the brain. After crossing the midline the growth cones become insensitive to netrin but are repelled by a molecule (first recognized in *Drosophila*) called **slit**. Its receptor is appropriately named roundabout (**Robo**).⁴⁴⁹ A similar receptor in zebrafish (called **astray**) is required for retinal axon guidance.⁴⁴⁹ An important aspect of neuron guidance is apoptosis induced by misdirected growth.⁴⁵⁰ The Notch receptor apparently participates in this decision in the mammalian CNS.³⁵⁷ Positive signals for axon growth often involve the MAP kinase pathways, while inhibition may involve INS-2P-Ca²⁺ signaling.⁴⁵¹

Chemoattractants that function in development of the cerebral cortex include several **semaphorins**.^{445,452,453} A separate family of attractant and repellent compounds, the semaphorins have been identified in insects, chickens, and mammals. They play a role in regulation of communication between neurons. Because of the complexity of the brain, the study of growth of neurons with the brain is difficult. The >10¹² neurons each contact, on the average, 100 different cells. Some insight comes from mutant mice with names such as *reeler*, *scrambler*, *stargazer*, and *Yotari* (Japanese for tottering).⁴⁵⁴ The single defective gene in these mice can be identified and studied. For example, *reeler* mice are defective in **reelin**, a large glycoprotein of the extracellular matrix (ECM).^{366,379,455,456} The *reeler* phenotype can also result from mutation of the gene *disabled-1*, which encodes a cytosolic tyrosine kinase. Other mutations in mice implicate **VLDL** and **apoE** receptors (Chapter 21) in these developmental abnormalities.^{379,455}

The *stargazer* mutant mouse is ataxic and epileptic. It lacks functional **AMPA receptors** (Fig. 30-1), which apparently are not delivered successfully to the synapses in the cerebellum in which they function.^{380,386} Mutation of a transmembrane protein **stargazin**, which may interact with the AMP receptor, causes the symptoms.^{457,458} **NMDA receptors** (Fig. 30-20) are involved in synapse formation in the brain. Filopodial extensions on dendrites, triggered by electrical activity, are essential for synapse formation,⁴⁵⁹ which occurs rapidly.^{459a} Activation of NMDA receptors is apparently also necessary.^{379,460} Without this stimulation the excitatory glutamatergic neurons of the developing brain undergo apoptosis.

Why do neurons grow in the embryo but not in most parts of the adult CNS? Two proteins called **Nogo** were isolated from bovine brain. Their sequences were utilized in identifying the *Nogo* gene and three human

isoforms of the protein.^{389,461} The large 250-kDa Nogo-A is present both in myelin and in the endoplasmic reticulum. Both Nogo-A and the diffusible 35-kDa Nogo-B are inhibitory of neurite outgrowth.⁴⁶² This effect, as well as the crowding of regenerating neurons by the chondroitin and other matrix components,³⁹⁶ may provide obstacles to nerve regeneration.⁴⁶³

E. Development of Green Plants

Green plants may have diverged from a common ancestor with animals ~1.6 billion (1.6×10^9) years ago. How do the genomes of present-day plants and animals compare? There are many similarities in basic metabolism. These arise from the intrinsic chemical properties and reactivities of cellular components and from the coevolution of plants and animals. Plants and animals also utilize similar structures and similar control of chromatin. However, in the control of development there are great differences.⁴⁶⁴ For example, the *Arabidopsis* genome contains no relative of the *Drosophila* Gurken, no receptor tyrosine kinases, no relatives of transcription factor NF- κ B. However, there are similarities in parallel pathways utilized by plants and animals.

The structures and life cycle of angiosperms⁴⁶⁵ are described briefly on pp. 29–30. The alternating haploid (n , **gametophytic**) and diploid ($2n$, **sporophytic**) phases of the life cycle^{466,467} are diagrammed in Fig. 32-8A. Following flowering a diploid **mother cell** within the **ovule** undergoes meiosis to form four haploid **megaspores**. After mitosis a single egg cell is formed. Within pollen sacs in the anthers of each mother undergoes meiosis to yield four haploid **microspores**. Following mitosis these develop into pollen grains each of which contains two sperm cells as well as a vegetative nucleus. After falling upon the stigma surface and growth of the pollen tube, one of the sperm cells fuses with the egg to give the diploid zygote. The other sperm unites with the specialized diploid **central cell** in the ovule to form a triploid ($3n$) **endosperm nucleus**, which develops into the **endosperm**, the food storage tissue of the seed. Endosperm contains two tissues, a starchy inner layer and a protein- and oil-rich outer layer.⁴⁶⁸

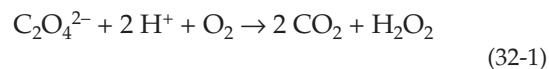
Variations of the life cycle occur. For example, a process called **apomixis** leads to asexual formation of seed.⁴⁶⁹ In many plants, including maize, separate flowers form the ovule and the pollen. This is one mechanism for avoiding inbreeding.⁴⁷⁰ In many plants systems of **self-incompatibility** have evolved.⁴⁷¹ In some, e.g., *Arabidopsis* and other crucifers, pollen germination is disrupted unless it falls on a stigma possessing a different allele-specific receptor. In other cases development of the pollen tube is disrupted at a later stage. In maize and in more than 150 other

species a mitochondrial and therefore maternally inherited trait prevents formation of a functional pollen.^{472,473} Male-sterile plants, which carry this trait, are very useful in plant breeding. However, a near disaster occurred in the United States in 1970 when the fungal disease Southern corn leaf blight attacked the male-sterile maize that had been used for production of 85% of commercial hybrid maize. The mitochondrial defect in pollen formation also resulted in an increased sensitivity to the fungal toxin. The target of the toxin is a 115-residue pore-forming polypeptide in the inner mitochondrial membrane.⁴⁷⁴ The male sterility can be reversed if the plant carries two nuclear **restorer genes**.⁴⁷² One of these encodes an aldehyde dehydrogenase, but its mechanism of action is unclear.

The plant embryo is a juvenile form, the seedling. In *Arabidopsis* the zygote, which is surrounded by maternal diploid tissue, divides asymmetrically. The resulting apical and basal cells (Fig. 32-8B) differ in several ways. The small cytoplasm-rich apical cell is partitioned into eight proembryo cells by two rounds of vertical division and one horizontal division. The larger basal cell contains a vacuole and divides repeatedly horizontally to give 7–9 aligned cells. Only the uppermost of these becomes a part of the embryo. The others form an extra-embryonic suspensor (Fig. 32-8B).⁴⁷⁵ The apical part of the embryo develops the shoot meristem and the central part the radial pattern of tissue layers characteristic of plants. The root meristem develops from the basal portion of the embryo. Movements of proteins that provide positional cues are involved in the development of the embryo.^{476,477} Early embryonic and endosperm development is largely under maternal control. Most paternal genes may be initially silent.⁴⁷⁸

Many angiosperms develop **fruit** from tissues of the ovary (Fig. 32-8A). The development and ripening of fruit is also complex and highly regulated.^{479,480}

Formation of seeds is a slow process. For example, in wheat the mature embryo, which consists of $\sim 10^5$ cells, develops over a seven week period. Seeds may live from a few years to 1000 years or more.⁴⁸¹ Subsequent germination of the seed into a seedling requires only two days.⁴⁸² The very dry embryo is converted into a highly hydrated plant whose further growth requires uptake of very large amounts of water. Many plants also synthesize large amounts of oxalic acid. This may arise from ascorbate (p. 1135) or via oxidation of glycine (Fig. 24-20). One of the earliest mRNAs to appear during seed germination encodes a 125-kDa glycoprotein called **germin**. This protein, which exists as multiple isoforms, is a copper-dependent oxalate oxidase (Eq. 32-1) which generates hydrogen peroxide. The latter is probably needed to



crosslink cell-wall polymers. Germin may also be useful to plants in defense against oxalate-forming fungi.⁴⁸²

The rapid vegetative growth, which includes development of shoots, leaves, and flowers, is controlled by a variety of transcription factors.⁴⁸³ Among these are homeodomain proteins that control differentiation of meristem cells.^{484–486} The induction of flowering is

especially complex, involved day length, light quality, and effects of gibberellins.⁴⁸⁶ At the ends of their lives plant cells die slowly from **senescence**. In this process many materials are recycled for use by new cells. Other plant cells die via the **hypersensitive response**, a form of programmed cell death.^{486a}

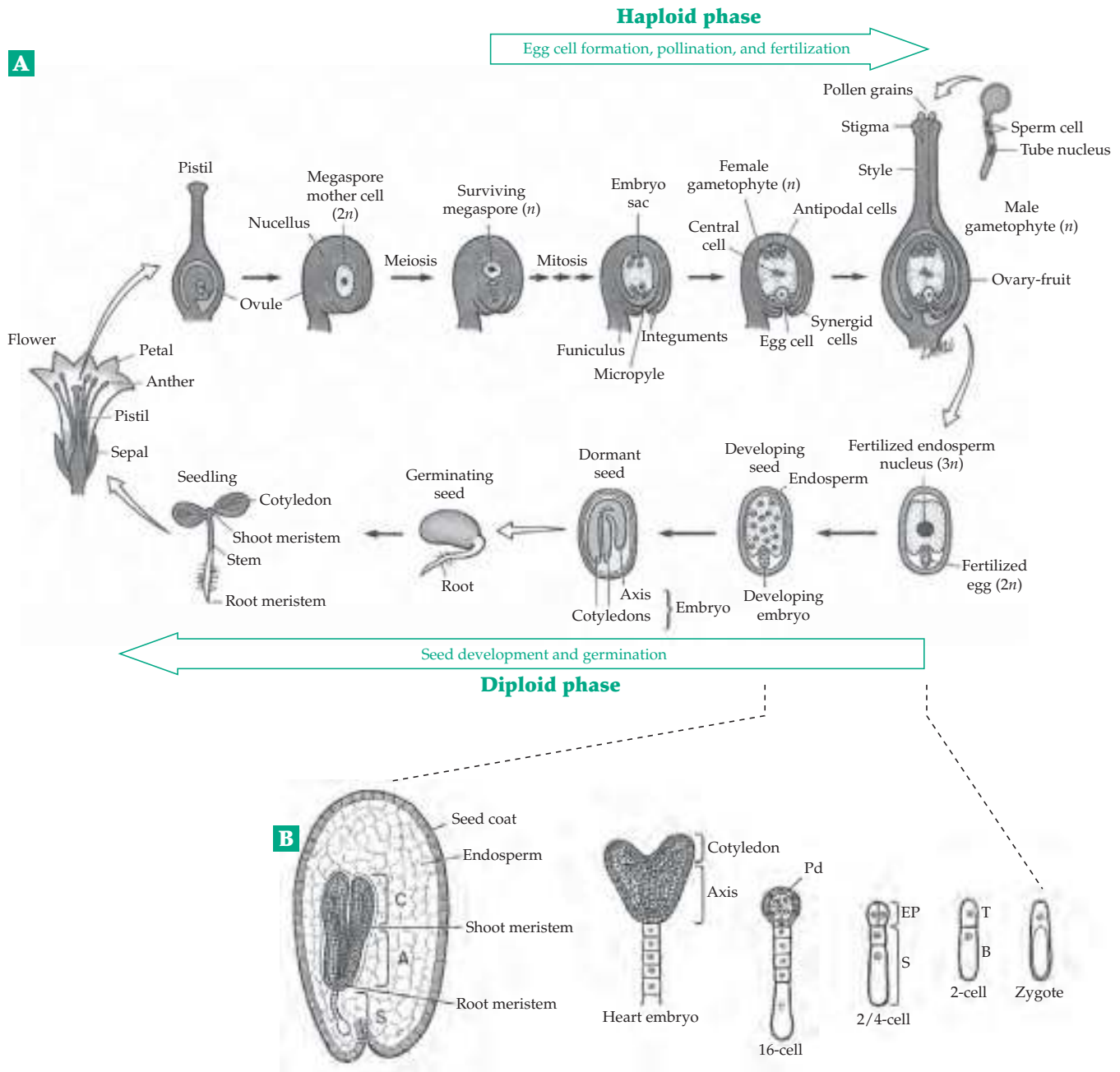


Figure 32-8 (A) The life cycle of a flowering plant with emphasis on egg-cell formation and seed development. (B) Some further details of embryo development. T, terminal cell; B, basal cell; C, cotyledon; A, axis; SC, seed coat; En, endosperm; EP, embryo proper; S, suspensor; SM, shoot meristem; Pd, protoderm; RM, root meristem. From Goldberg *et al.*⁴⁶⁶ with modification.

F. Aging

Why do we age? This question is often asked but the answers are not simple. Do our tissues deteriorate with age as a result of damage to proteins? From an accumulation of mutations in our DNA? From attacks by free radicals? From loss of hormone receptors? From misregulation of mitosis?⁴⁸⁷ From loss of telomeres on the ends of replicating DNA (Chapter 27, Section C,8)?^{488,489} From an internal genetic program that dictates our life span? All of these possibilities may be partially correct. The simple answer is that “we just wear out.” However, different parts wear out at different rates and in different ways.

Perhaps we should be amazed that the human body can live for an average of 75–80 years.^{490,491} If we all avoided accidents and could cure all recognized diseases we might live an average of ~90 years.⁴⁹² A few very healthy people live for 100 years or more, ~20 years longer than average.⁴⁹³ The maximum lifespan at present seems to be ~114 years. Long life tends to run in families, indicating a genetic component that can be identified.⁴⁹³ However, this component is relatively small.⁴⁹⁴

Why do small rodents live only 2–3 years while we often live nearly 100 years?^{494,495} Is it because their rate of metabolism is high? But bats have a comparable metabolic rate to mice, yet live ten times longer.⁴⁹⁶ Nematodes live only ~20 days and fruitflies ~10 days. At the other extreme fish and some reptiles continue to grow throughout their lifetimes, surviving even longer than mammals.⁴⁹⁵ Except for their germ cells nematodes, rotifers, and many insects have no dividing cells in their adult bodies. Their lifespan is presumably determined by the loss of cells through injury or death. In contrast, some simple animals, such as *Hydra*, other coelenterates, and flatworms maintain a pool of pluripotent stem cells that, except for accidental death, seems to make them immortal.⁴⁹⁵

Considerations such as these have helped move contemporary thinking toward an evolutionary view.^{488,489,495–499} If the mortality of an animal in the wild (**extrinsic mortality**) is high it will evolve to have rapid development, good reproductive ability, and a short lifetime. If the extrinsic mortality is low the lifetime will be long. Such animals will require development of good protective functions including a highly developed brain.

Many factors must affect aging. It is generally agreed that one of these is the deleterious effects of free radicals derived from oxygen^{500–503} (see pp. 1074, 1075). The lowered turnover rate of aging tissues may allow the damage to become lethal. According to this theory we might anticipate that free radical scavengers such as vitamin E could prolong life as might a restriction in food consumption.⁵⁰⁴ For example, decreased fat intake might cut down on production of

malondialdehyde (p. 1205) and lipid peroxides that may be especially damaging to cell membranes. The life of rodents can be prolonged substantially by a semi-starvation diet. Although there are uncertainties, a convincing case can be made for humans to keep food intake to a minimum and to eat foods rich in antioxidants and other nutrients.^{491,504a}

For many years after techniques of cell culture had been developed it was commonly believed that cells in tissue culture were potentially immortal.⁵⁰⁵ Challenging this idea, Medvedev⁵⁰⁶ and others proposed that cells are internally programmed for a certain lifetime. This might explain why we have short-lived mammals as well as long-lived mammals. Support for the idea was supplied by Hayflick,^{507,508} who observed that animal cells in culture have a limited potential for doubling. For example, normal human diploid embryonic fibroblasts grow in culture and double their number approximately 50 ± 10 times. Regardless of cultural conditions, the cells die after this number of doublings. Cells taken from older humans undergo a smaller number of doublings before dying, as do those taken from shorter lived animals such as the mouse (14–28 doublings).⁵⁰⁸ These experiments suggested that there is an internal program by which cells are scheduled to die from **replicative senescence**.^{509–511} Malignant transformation overrides this program and transformed cells appear “immortal.”⁵¹² However, unlike fibroblasts some glial stem cells have been identified as possibly having unlimited proliferative capacity. These include cultured rat oligodendrocyte⁵⁰⁹ and Schwann cells.⁵¹³ These results suggest that replicative senescence may not be inevitable.

As mentioned in Chapter 27 (p. 1568), “erosion” of the telomere ends on chromosomes is thought to be a major cause of cell senescence. Old cells have little or no telomerase. However, most human cancer cells, as well as those from immortalized cell cultures, do synthesize telomerase and maintain telomeres of adequate length.^{514,515} Inhibition of telomerase activity in immortalized cells causes telomere shortening and cell death.⁵¹⁶ A second pathway for telomere maintenance is based upon homologous recombination.⁵¹⁷ Experimental elongation of telomeres extends the lifespan of cells in culture.⁵¹⁸ Furthermore, apparently healthy calves have been produced by nuclear transfer cloning from senescent fibroblast cells for which four or fewer cell doublings were expected to be possible. The cells of the cloned animals had the capacity for 90 or more cell doublings.⁵¹⁹

What information about aging can we obtain by study of the “model” organisms *S. cerevisiae*, *C. elegans*, *D. melanogaster*, and the mouse? In every case a variety of mutations may shorten or lengthen the lifespan. In every case dietary energy restriction can lengthen life. Yeast cells grown on 0.5% glucose instead of 2%

glucose may undergo ~25% more cell doublings before a culture becomes senescent. However, mutant strains with a defect in the *SIR2* gene have a shortened life-span, which is not increased by caloric restriction.^{520–522} *SIR2* encodes Sir2p, an NAD⁺-dependent histone deacetylase (p. 1626).^{522,523} It is likely that caloric restriction causes the yeast to switch from anaerobic fermentation to oxidative metabolism. The resulting increase in the [NAD⁺] / [NADH] ratio activates Sir2p, thereby altering chromatin and silencing a group of genes. Mechanisms by which this shift in metabolism decreases deleterious mutations, even though respiration is increased, are probably complex.⁵²³

Nematodes (*C. elegans*) usually live about three weeks but the simultaneous presence of life-extending mutations in two different groups of genes lengthen the lifespan as much as fivefold.⁵²⁴ One of the genes is the maternal-effect Clock gene *Clk-1*. It has been found to code for a hydroxylase involved in the final step of synthesis of ubiquinone-9 (p. 1429, Fig. 25-4).⁵²⁵ The lifetime of wild-type nematodes is lengthened by ~60% by elimination of ubiquinone from the diet.⁵²⁶ The second group of genes that affect longevity regulate an insulin-like signaling system.^{527–529} In every case metabolism is slowed, an effect which may reduce the rate of harmful mutations. However, mutant animals may not be healthy. Some of these mutations induce formation of long-lived “dauer” larvae, providing a means for the larvae to survive for up to two months during periods of starvation.^{527,530} Others affect sensory cilia.⁵³¹ Mutation of a cytosolic catalase gene *reduces* the lifespan.⁵³⁰ The nematode’s lifetime is also affected negatively by its own germ cells, perhaps via a steroid hormone.⁵³² The heat shock proteins (p. 1630), by chaperoning newly synthesized proteins and preventing aggregation, also increase life span both in *C. elegans* and *Drosophila*.^{532a,b}

Some mutants of *D. melanogaster* with extended lifespans have a defective insulin / IGF signaling pathway.^{526,533} The **methuselah** mutant, whose lifespan is 35% greater than average, appears to involve a G-protein-coupled transmembrane receptor.⁵³⁴ Mutation of an insulin receptor homolog extends lifespan, apparently by causing a juvenile hormone deficiency.⁵³⁵ *Drosophila* lifespan is also lengthened by mutation of a transmembrane dicarboxylate transporter⁵³⁶ or by overexpression of a protein repair carboxyl methyltransferase (p. 594).⁵³⁷

Some mutant mice have extended lifespans. The Ames dwarf mouse has a mutation in *p66^{shc}*, a cell-surface protein that contains both Src-homology and collagen-homology domains. It lives almost one-third longer than do wild-type mice.⁵³⁸ Mice deficient in methionine sulfoxide reductase have a reduced lifespan⁵³⁹ but fruit flies with overexpressed activity of the enzyme are more resistant than wild-type flies to oxidative damage.⁵⁴⁰

In humans 100 or more years in age some mitochondrial mutations are associated with good health and longevity.⁵⁴¹ Dietary factors doubtless play a role. For example, supplementation of rats’ diet with lipoic acid improved mitochondrial function and increased the metabolic rate of old animals.⁵⁴²

A number of genetic **progeroid diseases** result in premature aging.^{543,544} Several of these arise from deficiencies in repair of DNA (Box 27-A). Among them are some types of cancer, and **Werner syndrome**, which arises from a defect in a 1432-residue protein with a central domain homologous to the RecQ family of DNA helicases (p. 1550).⁵⁴⁵ Defects in other RecQ homologs cause **Hutchinson-Gilford progeria** as well as the Bloom syndrome (see Box 27-A).⁵⁴⁶ Yet another DNA helicase, a subunit of transcription factor II (TFIIh, p. 1628), is defective in trichothiodystrophy (TTD, see Box 27-A).^{547,548} Another gene which helps to prevent aging is *KLOTHO*. First identified in mice, it encodes a transmembrane protein that has sequence similarities to β -glucosidases.^{549,550} Some mice with mutations in the tumor suppressor **p53** (Box 11-D) have enhanced resistance to tumors but age rapidly.^{551,552}

Aging seems to be inevitably linked to an increase in the incidence of cancer. This uncontrolled growth of cells appears to be allowed by the stepwise accumulation of mutations that affect growth, differentiation, and survival.⁵⁵³ Several aspects of cancer are discussed in other chapters of this book (see Box 11-D). However, the topic is so complex and research so active that it is hard to give even a thumbnail sketch of more recent discoveries.

Much effort is being dedicated to identifying the many signaling pathways that control growth, the mechanisms that cells employ to recognize problems in the control of growth, and the means by which cells can correct the problems or undergo apoptosis and avoid cancer.^{553–558} Some of the complexity arises because of the large number of signaling pathways in which mutations may produce activated proto-onco-genes or faulty tumor suppressors. A large network of these suppressors is present in human cells.^{553,559} Among the relevant signaling pathways are the following:

RAS – RAF – ERK (Fig. 11-12)^{553–555}
 p53^{556–558}
 the PtdIns 3-kinase – PKB/Akt pathway (Fig 11-9), which is opposed by PTEN^{560,561}
 EFG receptor (EFGR) signaling
 Wnt-Catenin signaling^{559,562}
 E. Cadherin^{559,563}

The importance of oncogenes and tumor suppressors has been demonstrated by conversion of human cells in culture into tumor cells in vitro.⁵⁶⁴ Introduction of

an activated *ras* gene, an SV40 viral protein that inhibits formation of both p53 and the Rb gene (Fig. 11-15), and an active telomerase gene sufficed. However, there is some doubt about the relevance of this work to human cancer.

Most cancerous cells have extra chromosomes. The karyotype (p. 1472) is rarely normal.⁵⁶⁵ This and other evidence suggest that **genomic instability** may be the major cause of cancer.^{566,567} In healthy cells stalled RNA polymerase is removed by transcription-coupled repair and lesions in DNA are either repaired (Chapter 27) or the cell undergoes apoptosis. Telomere dysfunction is also a factor.⁵⁶⁷ The two breast cancer susceptibility genes *BRCA1* and *BRCA2* are apparently responsible for about half of all hereditary breast and ovarian cancers.^{568,569} Protein *BRCA1* is an 1863-residue nuclear protein, which is thought to function in transcription. However, recent evidence indicates that *BRCA2* is directly involved in repair of double-strand breaks in DNA by homologous recombination.^{569,570} Other data implicate the Neu-Ras pathway, proto-oncogenes *c-myc* and *Wnt-1*, and cyclin D1 in breast cancer.⁵⁷¹

Yet another aspect of cancer is the **aberrant glycosylation** observed for many proteins.⁵⁷² The state of glycosylation of cell-surface proteins is one of many factors that affect metastasis, which is critical to growth of tumors.⁵⁷³⁻⁵⁷⁵ The recognition that causes of cancer are numerous has led to a new large-scale project to identify as many cancer-associated mutations as possible within the entire human genome. One early success from this effort is identification of mutations in the gene *BRAF*, one of the three human *RAF* genes. These mutations are present in 15% of human malignant melanomas.^{555,576}

G. Ecological Matters (Author's Personal Postscript)

The final section of this chapter deals with interactions among different species. As humans, beset by problems arising from our inability to communicate with other humans, we may feel that ecological relationships are relatively unimportant. However, any careful look at what can be regarded as an extension of metabolic cycles into the biosphere should convince us of the significance of this aspect of biochemistry.

Recall that the original development of eukaryotic creatures may have started with a symbiotic relationship between two prokaryotes and that symbiosis between algae and nonphotosynthetic organisms may have led to development of higher plants. Associations between species are still important today. For example, the bacteria in the protozoa of the digestive tract of ruminant animals are essential to production of meat. Our own bodies play host to bacteria, fungi,

and other organisms with whom we have to try to maintain friendly relations. We depend upon antibiotics produced by bacteria or by fungi to fight our bacterial infections. Plants provide both essential nutrients and oxygen. Our environment has been created in large part by other living forms that coexist with us and which are subject to ecological checks and balances. It is therefore important that we learn more about the effects of one group of organisms on another and also about the effects of human activities on plants and animals of all degrees of complexity. This includes the poorly understood world of soil microorganisms. The consequences of environmental pollution, of depletion of atmospheric ozone or other alterations that affect the radiant energy reaching us, and of the availability to humans of excessive amounts of energy must all be considered. Just as a steady state within cells is often essential to the life of organisms, maintenance of a steady state in the chemical cycles of the biosphere may also be a necessity.

Biochemists and molecular biologists are being called upon to play an increasing role in medicine, agriculture, and industry. As such, they must be prepared to help in the making of decisions that may affect the future of life on earth. Biochemical approaches will be required to cope with many important problems. Among these are the long-term effects of the growing number of synthetic compounds in the environment, problems of antibiotic resistance, and effects of bioengineering of plants, fishes, and other organisms in the biosphere. Some of these scientific and ethical questions have been discussed in Chapter 26, and more are considered in the Study Questions that follow in this chapter.

Despite attempts to ignore it, we cannot avoid facing the war problem. The possibility of virtually total destruction of the more complex forms of life by genetic damage from radiation is real. That we have lived with nuclear weapons as long as we have is encouraging but continuing threats to use them as a last resort may bring eventual catastrophe. A race to put weapons into space might result in having computers decide to fight a war in which all people could be destroyed, but one computer might win! Perhaps biochemists, who understand the technical problems of radiation damage and mutation, have a special obligation to point out the hazard to others.

Just as threatening is the possibility of biochemical warfare, e.g., the use of artificial viruses. Biological weapons have been little used because of their lack of discrimination between friend and foe. However, our increasing knowledge of molecular biology makes possible insidious attacks on a population of unvaccinated persons. Since biochemical work does not require elaborate facilities, the development of biological weapons can be carried on by small groups in a clandestine manner. The recent assembly of a viable

polio virus from oligonucleotides purchased from a commercial supplier emphasizes the ease with which virus warfare might be launched. Finding a way to protect ourselves may be more difficult.

Should we really worry about such matters? Since biochemistry is unable to ascribe any purpose to life, shouldn't we scientists stick to science? Science is amoral, isn't it? And besides, won't society do just what it wants to regardless of our opinions? Questions like these will always be with us, but most of the best scientists in the world seem to act with a great deal of responsibility. Not only do they want the pleasure and excitement of discovery and recognition for their work, but also they want a world for their children and grandchildren. They tend to feel compassion for other human beings. Many of them will give as a principal motivation for becoming biochemists the desire to contribute to the understanding of living things for the purpose of improving health, medical care, nutrition, etc. Most of them would not like to see the evolution of human beings ended through a disaster with nuclear or biological weapons or by irreversible pollution of land and sea. It will be a strange irony if we use our marvelous inquisitive, ingenious, inventive, and compassionate brains, the pinnacle of biological evolution, to destroy our environment and ourselves.

At a conference in Berkeley in 1971,⁵⁷⁷ Joshua Lederberg, discoverer of genetic recombination in

bacteria, talked about these matters. Lederberg asked if fairness and objectivity are possible outside the laboratory. He thought so. He pointed out that the nations of the world agreed to stop production of biological weapons and that genuine steps had been taken to decrease some of the hazards facing us. Nevertheless, progress is slow. Some insist on inspection for violation of agreements. But how can one inspect thoroughly enough? Lederberg suggested that the only possible form of control is now evolving. It must come from scientists themselves who must step out of their roles as "pure" scientists and accept the responsibility of preventing foolish uses of new biological discoveries. It may seem impossible that there could be a scientific community which could be counted on always to act in a responsible way, but it may be the only way that the human beings can survive for long on this planet. Lederberg believes it possible (and so do I).

If this book has helped to bring to the reader some awareness of the knowledge and power of molecular biology, I hope that these final words may lead the reader to heed the advice of Professor Lederberg. I sincerely hope that all the young people now studying biochemistry and modern biology will commit themselves to using the fantastic new knowledge available to us for the betterment of mankind and to proceeding with caution and responsibility as they move into positions of influence in the scientific community.

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Study Questions

1. Discuss the roles of the following proteins in development: receptors, transcription factors, protein kinases, histones, DNA methylases, adhesion molecules, ubiquitin. How do small RNA molecules participate in development?
2. Are all body cells totipotent?
3. Discuss the roles of apoptosis in various groups of organisms.
4. Compare signaling between bacteria and other unicellular organisms with signaling in higher eukaryotes.
5. Are human beings the most highly developed organisms? If so, in what ways? Has evolution of humans stopped or will it continue? Will it be upward?
6. Is it important for the world to achieve a sustainable state in which the population is constant and the environment stable?⁵⁷⁸ How will the world support a projected increase in population from the present 6 billion to 9 billion in 50 years?^{579,580}
7. How seriously is the earth's ecosystem dominated by human activity?^{581,582} Human activities have greatly reduced the amount of area available to wild species. Will the ensuing extinction of many organisms impoverish future diversity?^{583–585} Can the world's fisheries become sustainable?^{586,587}
8. Is science losing its objectivity because of an emphasis on monetary gain rather than on meeting social needs?⁵⁸⁸

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