3. CELL SIGNALING

Cells respond to extracellular signals produced by other cells or by themselves. This mechanism, called **cell signaling**, allows cell-cell communication and is necessary for the functional regulation and integration of multicellular organisms. Our discussion in this chapter not only provides the basis for understanding normal cell function but serves also as an introduction to the role of abnormal cell signaling in human disease.

Signaling molecules are either **secreted** or **expressed at the cell surface** of one cell. Signaling molecules can bind to receptors on the surface of another cell or the same cell.

Different types of signaling molecules transmit information in multicellular organisms, and their mechanisms of action on their target cells can be diverse. Some signaling molecules can act on the cell surface after binding to cell surface receptors; others can cross the plasma membrane and bind to intracellular receptors in the cytoplasm and nucleus.

When a signaling molecule binds to its receptor, it initiates a cascade of intracellular reactions to regulate critical functions such as **cell proliferation**, **differentiation**, **movement**, **metabolism**, and **behavior**. Because of their critical role in the control of normal cell growth and differentiation, signaling molecules have acquired significant relevance in cancer research.

Cell signaling mechanisms

Five major types of cell-cell signaling are considered (Figure 3-1):

1. **Endocrine cell signaling** involves a signaling molecule, called a **hormone**, secreted by an **endocrine cell and transported through the circulation to act on distant target cells**. An example is the steroid hormone testosterone produced in the testes, that stimulates the development and maintenance of the male reproductive tract.
2. **Paracrine cell signaling** is mediated by a signaling molecule acting **locally** to regulate the behavior of a **nearby cell**. An example is the action of **neurotransmitters** produced by nerve cells and released at a **synapse**. See Box 3-A for a summary of the four major families of paracrine signaling molecules.
3. **Autocrine cell signaling** is defined by **cells responding to signaling molecules that they themselves produce.** A classic example is the response of cells of the immune system to foreign antigens or growth factors that trigger their own proliferation and differentiation. Abnormal autocrine signaling leads to the unregulated growth of cancer cells.
4. **Neurotransmitter cell signaling**, a specific form of paracrine signaling.
5. **Neuroendocrine cell signaling**, a specific form of endocrine signaling.

Mechanisms of action of cell signaling molecules

Cell signaling molecules exert their action after binding to receptors expressed by their target cells. Target cells, in turn, can determine either a **negative** or **positive feedback** action to regulate the release of the targeting hormone (Figure 3-2).

Cell receptors can be expressed on the **cell surface** of the target cells. Some receptors are **intracellular proteins** in the **cytosol** or the **nucleus** of target cells. Intracellular receptors require that the signaling molecules **diffuse across the plasma membrane** (Figure 3-3).

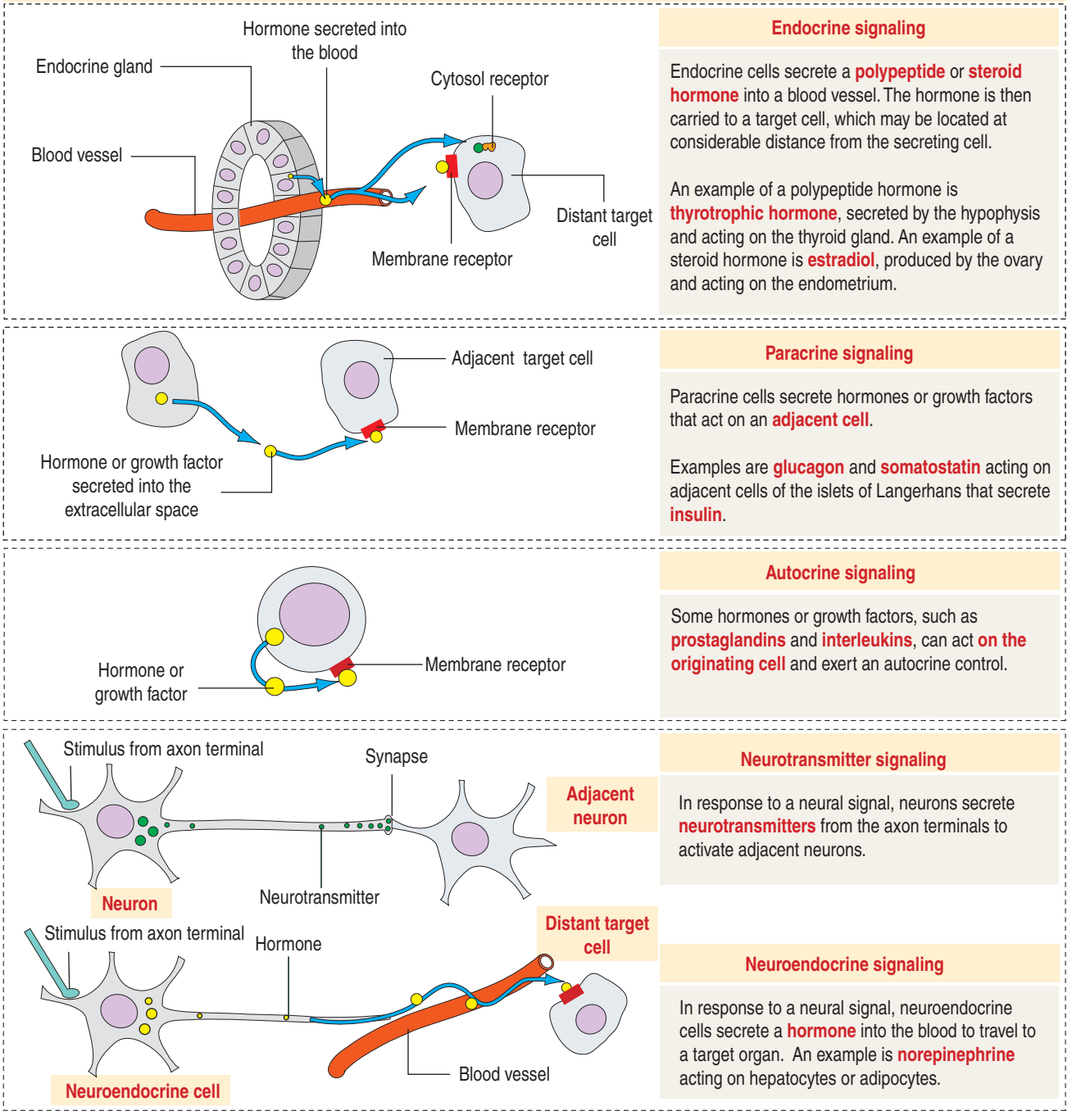
Steroid hormones (Box 3-B) belong to this class of signaling molecules. Steroid hormones are synthesized from **cholesterol** and include **testosterone**, **estrogen**, **progesterone**, and **corticosteroids**.

Testosterone, estrogen, and progesterone are **sex steroids** and are produced

Box 3-A | Paracrine cell signaling

- **Paracrine signaling molecules** include four major families of proteins: 1. The **fibroblast growth factor (FGF) family**. 2. The **Hedgehog family**. 3. The **wingless (Wnt) family**. 4. The **transforming growth factor β (TGF-β) superfamily**.
- Each of these signaling proteins can bind to one or more receptors. Mutations of genes encoding these proteins may lead to abnormal cell-cell interaction.
- The first member of the **Hedgehog family** was isolated in a *Drosophila* mutant with bristles in a naked area in the normal fly. The most widely found hedgehog homolog in vertebrates is **sonic hedgehog (Shh)**. Shh participates in the development of the neural plate and neural tube (see Chapter 8, Nervous Tissue). Shh binds to a transmembrane protein encoded by the ***patched*** gene and suppresses transcription of genes encoding members of the Wnt and TGF-β families and inhibits cell growth. Mutation of the patched homolog in humans (***PTC***) causes the **Gorlin syndrome** (rib abnormalities, cyst of the jaw, and basal cell carcinoma, a form of skin cancer).
- The **Wnt family** of genes is named after the *Drosophila* gene ***wingless***. In vertebrates, *Wnt* genes encode secretory glycoproteins that specify the dorsal-ventral axis and formation of brain, muscle, gonads, and kidneys.
- The **TGF-β superfamily** encodes protein forming **homodimers** and **heterodimers**. Members of this superfamily include the TGF-β family itself, the **bone morphogenetic protein (BMP) family**, the **activin family**, and the **vitellogenin 1 (Vg1) family**. Mutations in a member of the BMP family, **cartilage-derived morphogenetic protein-1 (CDMP1)** causes skeletal abnormalities. Vg1 is a signaling molecule determining the left-right axis in embryos.

Figure 3-1. Mechanisms of hormone action



Box 3-B | Steroid hormones

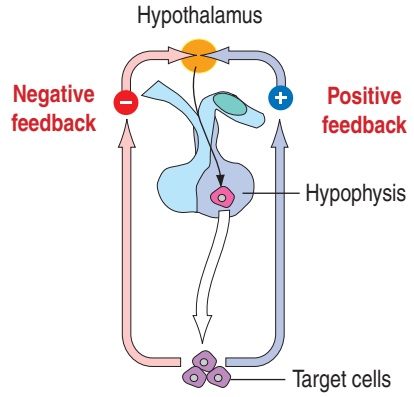
- They derive from **cholesterol**.
- They bind mainly to **intracellular receptors** in the cytosol and nucleus.
- They circulate in blood **bound to a protein**.
- They are **nonpolar** molecules.
- Steroid hormones **are not stored in the producing endocrine cell**.
- Steroid hormones **can be administered orally** and are readily absorbed in the gastrointestinal tract.

by the gonads. Corticosteroids are produced by the cortex of the adrenal gland and include two major classes: **glucocorticoids**, which stimulate the production of glucose, and **mineralocorticoids**, which act on the kidney to regulate water and salt balance.

There are three cell signaling molecules that are structurally and functionally distinct from steroids but act on target cells by binding to intracellular receptors after entering the cell by diffusion across the plasma membrane. They include **thyroid hormone** (produced in the thyroid gland to regulate development and metabolism), **vitamin D_3** (regulates calcium metabolism and bone growth), and **retinoids** (synthesized from vitamin A to regulate development).

Steroid receptors are members of the **steroid receptor superfamily**. They act as transcription factors through their DNA binding domains, which have transcrip-

Figure 3-2. Feedback

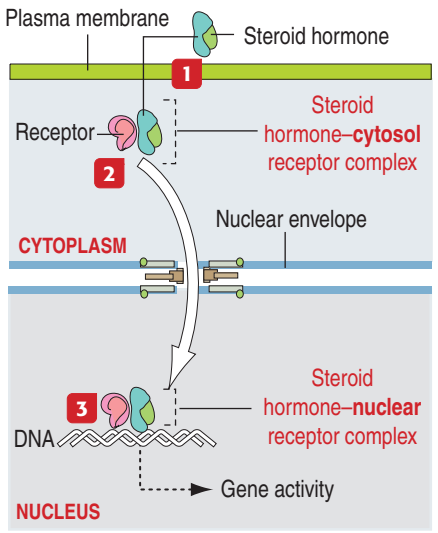


Feedback loops and cell signaling

Various feedback loops coordinate the secretion of hormones. For example, a **negative feedback loop** prevents the unregulated release of a hormone from the hypophysis into the blood circulation when the target cell or tissue may be nonresponsive.

A **positive feedback loop** (more rarely) occurs when the hypophysis senses a decrease in the blood levels of a hormone produced by the target cell or tissue. See Chapter 19, Endocrine System, for additional details.

Figure 3-3. Mechanism of action of steroid hormones



Steroid hormone action

1 Hydrophobic steroid hormone diffuses across the plasma membrane.

2 The steroid hormone binds to a cytosol receptor.

3 The steroid-cytosol receptor complex translocates into the nucleus, binds to DNA and activates—or represses—gene expression.

tion activation or repression functions. Steroid hormones and related molecules can therefore regulate gene expression.

In the **androgen insensitivity syndrome** (also known as the **testicular feminization syndrome** [**Tfm**]), there is a mutation in the gene expressing the **testosterone receptor** such that the receptor cannot bind the hormone, and hence the cells do not respond to the hormone. Although genetically male, the individual develops the secondary sexual characteristics of a female. We discuss the androgen insensitivity syndrome in Chapter 21, Sperm Transport and Maturation.

Nitric oxide

Nitric oxide is a signaling molecule. It is a simple gas synthesized from the amino acid **arginine** by the enzyme **nitric oxide synthase**. It acts as a paracrine signaling molecule in the nervous, immune, and circulatory systems. Like steroid hormones, nitric oxide can diffuse across the plasma membrane of its target cells. Unlike steroids, nitric oxide does not bind to an intracellular receptor to regulate transcription. Instead, **it regulates the activity of intracellular target enzymes**.

The following are relevant characteristics of nitric oxide:

1. It is an unstable molecule with a limited half-life (seconds).
2. It has local effects.
3. A well-defined function of nitric oxide signaling is the **dilation of blood vessels**. For example, the release of the neurotransmitter acetylcholine from nerve cell endings in the blood vessel muscle cell wall stimulates the release of nitric oxide from endothelial cells.

Nitric oxide increases the activity of the second messenger cyclic guanosine monophosphate (cGMP; see later in this section) in smooth muscle cells, which then causes cell muscle relaxation and blood vessel dilation. **Nitroglycerin, a pharmacologic agent used in the treatment of heart disease, is converted to nitric oxide, which increases heart blood flow by dilation of the coronary blood vessels.**

Box 3-C | **Peptide hormones**

- They are synthesized as **precursor molecules** (prohormones).
- They are stored in **membrane-bound secretory vesicles**.
- They are generally **water soluble** (polar).
- They circulate in blood as **unbound molecules**.
- Peptide hormones **cannot be administered orally**.
- They usually bind to **cell surface receptors**.

Cell signaling molecules bind to cell surface receptors

A large variety of signaling molecules bind to cell surface receptors. Several groups are recognized:

1. **Peptides** (Box 3-C): This group includes **peptide hormones** (insulin, glucagon, and hormones secreted by the hypophysis), **neuropeptides**, secreted by neurons (**enkephalins** and **endorphins**, which decrease pain responses in the central nervous system), and **growth factors**, which control cell growth and

Box 3-D | Eicosanoids

1. They **derive from polyunsaturated fatty acids** with 18, 20, and 22 carbons.
2. **Arachidonic acid** is the main precursor.
3. This group includes **prostaglandins**, **leukotrienes**, **thromboxanes**, and **prostacyclin**.
4. They have primary **autocrine** and **paracrine** actions.
5. The **synthesis of eicosanoids is regulated by hormones**.
6. They usually bind to **cell surface receptors**.

differentiation (**nerve growth factor** [NGF]; **epidermal growth factor** [EGF]; **platelet-derived growth factor** [PDGF]; and **cytokines**).

NGF is a member of a family of peptides called **neurotrophins**, which regulate the development and viability of neurons. EGF stimulates cell proliferation and is essential during embryonic development and in the adult. PDGF is stored in blood platelets and released during clotting.

2. **Neurotransmitters**: These cell signaling molecules are released by neurons and act on cell surface receptors present in neurons or other type of target cells (such as muscle cells). This group includes **acetylcholine**, **dopamine**, **epinephrine** (adrenaline), **serotonin**, **histamine**, **glutamate**, and **γ-aminobutyric acid** (GABA). The release of neurotransmitters from neurons is triggered by an **action potential**. Released neurotransmitters diffuse across the **synaptic cleft** and bind to surface receptors on the target cells.

There are differences that distinguish the **mechanism of action of neurotransmitters**. For example, **acetylcholine is a ligand-gated ion channel**. It induces a change in conformation of ion channels to control ion flow across the plasma membrane in target cells.

As we will see soon, neurotransmitter receptors can be associated to G proteins, a class of signaling molecules linking cell surface receptors to intracellular responses.

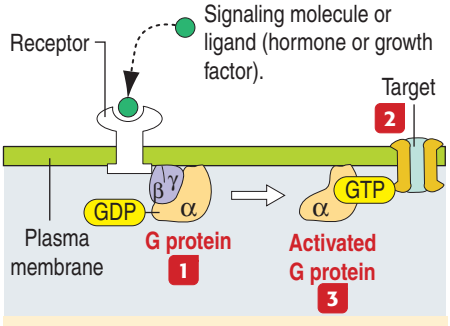
Some neurotransmitters have a **dual function**. For example, epinephrine (produced in the medulla of the adrenal gland) can act as a neurotransmitter and as a hormone to induce the breakdown of glycogen in muscle cells.

3. **Eicosanoids and leukotrienes**: These are lipid-containing cell-signaling molecules that, **in contrast to steroids**, **bind to cell surface receptors** (Box 3-D).

Prostaglandins, **prostacyclin**, **thromboxanes**, and **leukotrienes** are members of this group of molecules. They stimulate blood platelet aggregation, inflammatory responses, and smooth muscle contraction.

Eicosanoids are synthesized from **arachidonic acid**. During the synthesis of prostaglandins, arachidonic acid is converted to **prostaglandin H_2** by the enzyme **prostaglandin synthase**. This enzyme is inhibited by **aspirin** and **anti-inflammatory drugs**. **Inhibition of prostaglandin synthase by aspirin reduces pain, inflammation, platelet aggregation, and blood clotting** (prevention of strokes).

Figure 3-4. G protein–coupled receptors



G protein

1 G protein consists of three subunits (α, β, and γ). The α subunit regulates G protein activity. In the resting state, guanosine diphosphate (GDP) is bound to the α subunit in a complex with β and γ subunits.

2 G protein transmits a cell surface signal to an adjacent **target molecule (adenylyl cyclase** or **ion channel)**.

3 Hormone binding stimulates the release of GDP and its exchange for guanosine triphosphate (GTP).

The activated GTP-bound α subunit dissociates from β and γ and interacts with a target to induce a response.

Pathways of intracellular signaling by cell surface receptors

When a cell-signaling molecule binds to a specific receptor, it activates a series of **intracellular targets located downstream of the receptor**. Several molecules associated with receptors have been identified:

1. **G protein–coupled receptors** (guanine nucleotide–binding proteins): Members of a large family of **G proteins** (more than 1000 proteins) are present at the inner leaflet of the plasma membrane (Figure 3-4).

When a signaling molecule or **receptor ligand** binds to the extracellular portion of a cell surface receptor, its cytosolic domain undergoes a conformational change that enables binding of the receptor to a G protein. This contact activates the G protein, which then dissociates from the receptor and triggers an intracellular signal to an enzyme or ion channel. We return to the G protein when we discuss the cyclic adenosine monophosphate (cAMP) pathway.

2. **Tyrosine kinases as receptor proteins** (Figure 3-5): These surface receptors are themselves enzymes that phosphorylate substrate proteins on **tyrosine** residues. **EGF, NGF, PDGF, insulin, and several growth factors are receptor protein tyrosine kinases**. Most of the receptor protein tyrosine kinases consist of single polypeptides, although the insulin receptor and other growth factors consist of a pair of polypeptide chains.

Binding of a ligand (a growth factor) to the extracellular domain of these receptors induces **receptor dimerization** that results in **receptor autophosphorylation** (the two polypeptide chains phosphorylate one another). The autophosphoryla-

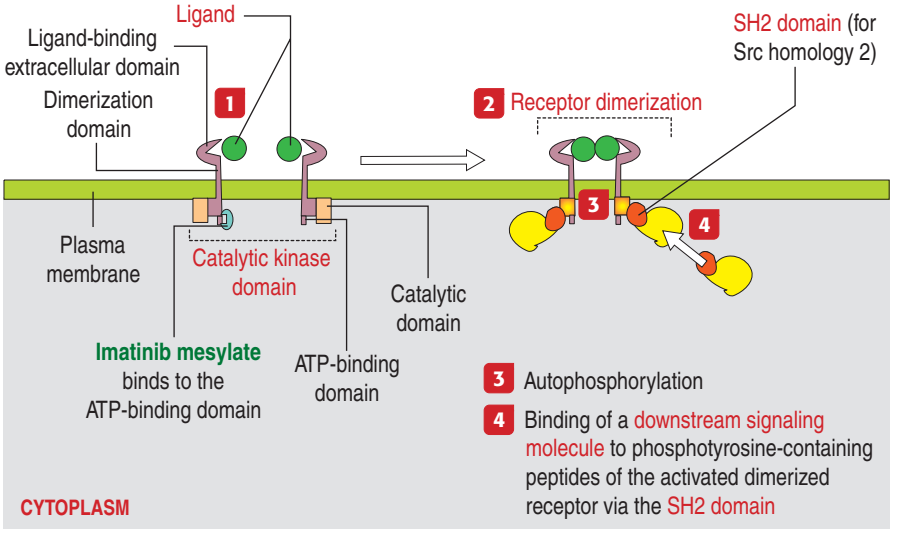
Figure 3-5. Tyrosine kinases

Tyrosine kinase receptor

Binding of a signaling molecule (for example, a growth factor) triggers **receptor dimerization and autophosphorylation** (the two polypeptide chains phosphorylate each other).

Downstream signaling molecules, with an **SH2 domain**, bind to phosphotyrosine-containing peptides of the activated receptor.

Imatinib mesylate binds to the adenosine triphosphate (ATP)-binding domain and prevents downstream signaling. Imatinib is used in the treatment of hematologic malignancies associated with tyrosine kinase dysregulation.



tion of the receptors determines the binding of the tyrosine kinase domain to downstream signaling molecules. Downstream signaling molecules bind to phosphotyrosine residues through domains called **SH2 domains** (for Src homology 2). ***Src*** (for **sarcoma**) is a gene present in the tumor-producing Rous sarcoma virus and encodes a protein that functions as a protein tyrosine kinase.

3. **Cytokine receptors**: This family of receptors stimulates **intracellular protein tyrosine kinases**, which **are not intrinsic components of the receptor**. A growth factor ligand induces the dimerization and cross-phosphorylation of the associated tyrosine kinases. Activated kinases phosphorylate the receptors, providing binding sites for downstream signaling molecules that contain the SH2 domain.

The cytokine receptor–associated tyrosine kinases belong to two families: the ***Src* family** and the **Janus kinase family** (**JAK**).

4. **Receptors linked to other enzymes** (**protein tyrosine phosphatases** and **protein serine and threonine kinases**): Some receptors associate with protein tyrosine phosphatases to remove phosphate groups from phosphotyrosine residues. Therefore, **they regulate the effect of protein tyrosine kinases by arresting signals initiated by protein tyrosine phosphorylation**.

Members of the **transforming growth factor-β** (**TGF-β**) family are protein kinases that phosphorylate serine and threonine residues (rather than tyrosine). TGF-β inhibits the proliferation of their target cells. Like tyrosine kinase and cytokine receptors, binding of ligand to the TGF-β receptor induces receptor dimerization and the cytosolic protein serine or threonine kinase domain cross-phosphorylates the polypeptide chains of the receptor.

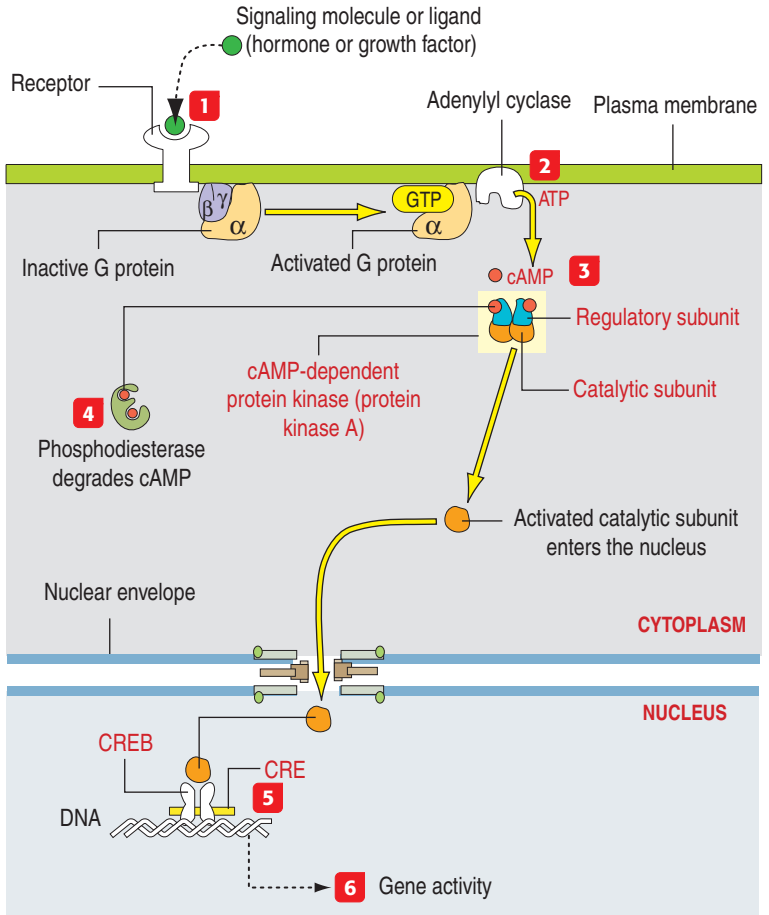
Clinical significance: Tyrosine kinases, targets for therapeutic agents

There are two main classes of tyrosine kinases: (1) **receptor tyrosine kinases** are transmembrane proteins with a ligand-binding extracellular domain and a catalyic intracellular kinase domain (see Figure 3-5), and (2) **nonreceptor tyrosine kinases** found in the cytosol, nucleus, and inner side of the plasma membrane.

The transmembrane receptor kinase subfamily belongs to the PDGF family, which includes c-kit. The subfamily of nonreceptor tyrosine kinases includes the **Src family**, the **Fujinami poultry sarcoma/feline sarcoma** (Fps/Fes), and **Fes-related** (Fer) subfamily.

In the absence of a ligand, receptor tyrosine kinases are unphosphorylated and monomeric. The nonreceptor tyrosine kinase is maintained in an inactive state by cellular inhibitor proteins. Activation occurs when the inhibitors are dissociated or by recruitment to transmembrane receptors that trigger autophosphorylation.

Figure 3-6. Cyclic adenosine monophosphate (cAMP) pathway



cAMP signaling pathway

1 A ligand binds to a cell receptor.

2 **Adenylyl cyclase**, activated by the guanosine triphosphate (GTP)–bound G protein subunit α, forms cAMP from ATP.

3 cAMP, the second messenger, binds to the regulatory subunits of **cAMP-dependent protein kinase (protein kinase A)** and releases the catalytic subunits.

4 cAMP is degraded by a **cAMP-dependent phosphodiesterase**.

5 The activated catalytic subunit translocates into the nucleus and phosphorylates the transcription factor **CREB (CRE-binding protein)** bound to the **cAMP response element (CRE)**.

6 Specific gene expression of inducible genes occurs.

Tyrosine kinase activity terminates when tyrosine phosphatases hydrolyze tyrosyl phosphates and by induction of inhibitory molecules.

The activity of tyrosine kinases in cancer cells can be disrupted by a protein that determines unregulated autophosphorylation in the absence of a ligand, by disrupting autoregulation of the tyrosine kinase, or by overexpression of receptor tyrosine kinase and/or its ligand. Abnormal activation of tyrosine kinases can stimulate the proliferation and anticancer drug resistance of malignant cells.

Tyrosine kinase activity can be inhibited by **imatinib mesylate**, a molecule that binds to the adenosine triphosphate (ATP)–binding domain of the tyrosine kinase catalytic domain. Imatinib can induce hematologic remission in patients with chronic myeloid leukemia and in tumors caused by activated receptor tyrosine kinase PDGF receptor (chronic myelomonocytic leukemia) and c-kit (systemic mastocytosis and mast cell leukemias). Imatinib has been successfully used in the treatment of gastrointestinal solid tumors.

Major pathways of intracellular cell signaling

Upon ligand binding, most cell surface receptors stimulate intracellular target enzymes to **transmit and amplify a signal**. An amplified signal can be propagated to the nucleus to regulate gene expression in response to an external cell stimulus.

The major intracellular signaling pathways include the cAMP and cGMP pathways, the phospholipase C–Ca^{2+} pathway, the **NF-κB** (for nuclear factor involved in the transcription of the κ light chain gene in B lymphocytes) transcription factor pathway, the Ca^{2+}-calmodulin pathway, the **MAP** (for mitogen-activated protein) kinase pathway, and the **JAK-STAT** (for signal transducers and activators of transcription) pathway.

The cAMP pathway

The intracellular signaling pathway mediated by **cAMP** was discovered in 1958 by Earl Sutherland while studying the action of **epinephrine**, a hormone that breaks down glycogen into glucose before muscle contraction.

When epinephrine binds to its receptor, there is an increase in the intracellular concentration of cAMP. cAMP is formed from adenosine triphosphate (ATP) by the action of the enzyme **adenylyl cyclase** and degraded to adenosine monophosphate (AMP) by the enzyme **cAMP phosphodiesterase**. This mechanism led to the concept of a **first messenger** (epinephrine) mediating a cell-signaling effect by a **second messenger**, cAMP. The epinephrine receptor is linked to adenylyl cyclase by G protein, which stimulates cyclase activity upon epinephrine binding.

The intracellular signaling effects of cAMP (Figure 3-6) are mediated by the enzyme **cAMP-dependent protein kinase** (or **protein kinase A**). **In its inactive form, protein kinase A is a tetramer composed of two regulatory subunits** (to which cAMP binds) **and two catalytic subunits**. Binding of cAMP results in the **dissociation of the catalytic subunits**. Free catalytic subunits can phosphorylate **serine residues** on target proteins.

In the epinephrine-dependent regulation of glycogen metabolism, protein kinase A phosphorylates two enzymes:

1. **Phosphorylase kinase**, which in turn phosphorylates glycogen phosphorylase to break down glycogen into glucose-1-phosphate.
2. **Glycogen synthase**, which is involved in the synthesis of glycogen. Phosphorylation of glycogen synthase prevents the synthesis of glycogen.

Note that an elevation of cAMP results in two distinct events: the breakdown of glycogen and, at the same time, a blockage of further glycogen synthesis. Also note that the binding of epinephrine to a single receptor leads to a signal amplification mechanism during intracellular signaling mediated by many molecules of cAMP. cAMP signal amplification is further enhanced by the phosphorylation of many molecules of phosphorylase kinase and glycogen synthase by the catalytic subunits dissociated from protein kinase A. It is important to realize that protein phosphorylation can be rapidly reversed by **protein phosphatases** present in the cytosol and as transmembrane proteins. These protein phosphatases can terminate responses initiated by the activation of kinases by removing phosphorylated residues.

cAMP also has an effect on the transcription of specific target genes that contain a regulatory sequence called the **cAMP response element** (**CRE**). Catalytic subunits of protein kinase A enter the nucleus after dissociation from the regulatory subunits. Within the nucleus, catalytic subunits phosphorylate a transcription factor called **CRE-binding protein** (**CREB**), which activates cAMP-inducible genes.

Finally, cAMP effects can be direct, independent of protein phosphorylation. An example is the direct regulation of **ion channels in the olfactory epithelium**. **Odorant receptors** in sensory neurons of the nose are linked to G protein, which stimulates adenylyl cyclase to increase intracellular cAMP.

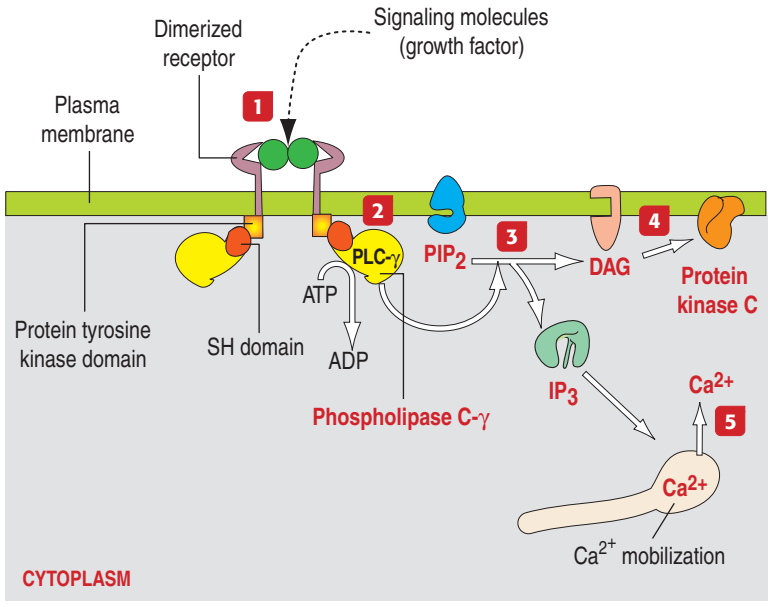
cAMP does not stimulate protein kinase A in sensory neurons but acts directly to open Na^+ channels in the plasma membrane to initiate membrane depolarization and nerve impulses.

The cGMP pathway

cGMP is also a second messenger. It is produced from guanosine triphosphate (GTP) by guanylate cyclase and degraded to GMP by a phosphodiesterase. Guanylate cyclases are activated by nitric oxide and peptide signaling molecules.

The best characterized role of cGMP is in photoreceptor rod cells of the retina, where it converts light signals to nerve impulses. Chapter 9, Sensory Organs: Vision and Hearing, in the eye section, provides a detailed description of this cell signaling process.

Figure 3-7. Phospholipase–protein kinase C–Ca^{2+} pathway



Phospholipase-Ca^{2+} pathway

1. A signaling molecule binds and activates the protein kinase domains of a dimerized receptor.
2. **Phospholipase C-γ (PLC-γ)** contains an **SH domain** that mediates its association with activated receptor protein tyrosine kinases.
3. PLC-γ catalyzes the hydrolysis of **PIP_2** to produce **diacylglycerol (DAG)** and **IP_3**.
4. DAG activates **protein kinase C.**
5. IP_3 signals the **release of Ca^{2+}** from intracellular storage sites.

Phospholipase C–Ca^{2+} pathway

Another second messenger involved in intracellular signaling derives from the phospholipid **phosphatidylinositol 4,5-bisphosphate** (PIP_2) present in the inner leaflet of the plasma membrane (Figure 3-7).

The hydrolysis of PIP_2 by the enzyme **phospholipase C (PLC)**—stimulated by a number of hormones and growth factors—produces two second messengers: **diacylglycerol** and **inositol 1,4,5-trisphosphate** (IP_3).

These two messengers stimulate two downstream signaling pathway cascades: **protein kinase C** and **Ca^{2+} mobilization**.

Two forms of PLC exist: **PLC-β** and **PLC-γ**. PLC-β is activated by G protein. PLC-γ contains SH2 domains that enable association with receptor protein tyrosine kinases. Tyrosine phosphorylation increases PLC-γ activity, which in turn stimulates the breakdown of PIP_2.

Diacylglycerol, derived from PIP_2 hydrolysis, activates members of the **protein kinase C** family (**protein serine and threonine kinases**).

Phorbol esters are tumor growth–promoting agents acting, like diacylglycerol, by stimulation of protein kinase C activities. Protein kinase C activates other intracellular targets such as protein kinases of the **MAP kinase pathway** to produce the phosphorylation of transcription factors leading to changes in gene expression and cell proliferation.

NF-κB transcription factor pathway

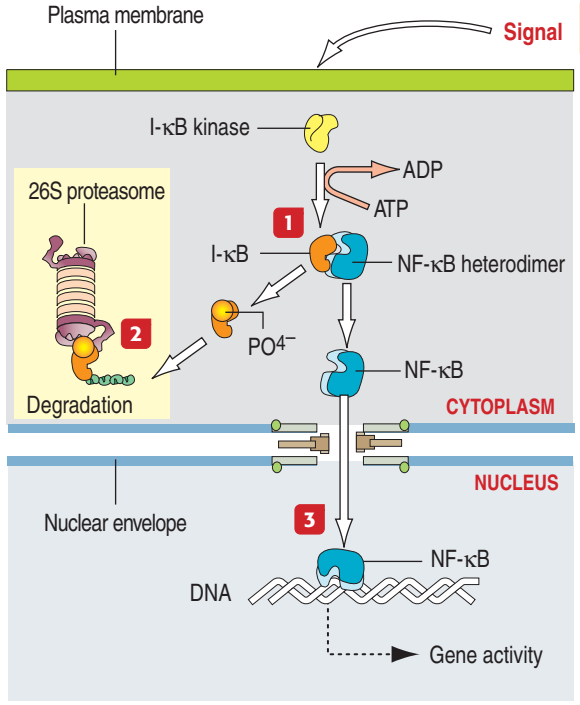
NF-κB is a transcription factor involved in immune responses in several cells and is stimulated by protein kinase C (Figure 3-8).

In its **inactive state**, the NF-κB protein heterodimer is bound to the **inhibitory subunit** I-κB and the complex is retained in the cytoplasm. The phosphorylation of I-κB—triggered by I-κB kinase—leads to the destruction of I-κB by the 26S proteasome and the release of NF-κB. The free NF-κB heterodimer translocates into the nucleus to activate gene transcription in response to immunologic and inflammatory signaling.

Ca^{2+}–calmodulin pathway

Although the second messenger diacylglycerol remains associated with the plasma membrane, the other second messenger IP_3, derived from PIP_2, is released into

Figure 3-8. NF-κB transcription factor pathway

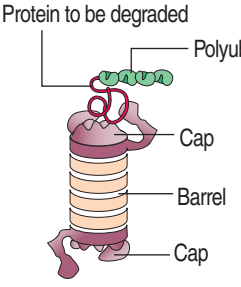


Activation of NF-κB

1 NF-κB is a protein heterodimer that, when associated with the **inhibitory subunit** I-κB, forms an **inactive complex** present in the cytoplasm.

2 When protein kinase C is stimulated, I-κB is phosphorylated and undergoes **phosphorylation-dependent degradation**—after ubiquitinization—by the 26S proteasome (see Figure 3-14 for additional data).

3 Removal of I-κB uncovers the nuclear localization sites of the NF-κB heterodimer that translocates into the nucleus, binds to specific DNA sequences, and regulates gene expression.



The **26S proteasome** is a giant multimeric protease found in the cytoplasm and nucleus of many cells. It consists of a barrel-shaped core—where proteins are degraded—and two caps that recognize proteins with attached **ubiquitin**. Ubiquitinized proteins are taken up by the 26S proteasome and degraded in the **chamber** of the barrel component.

the cytosol to activate ion pumps and free Ca^{2+} from intracellular storage sites. High cytosolic Ca^{2+} concentrations (from a basal level of 0.1 μM to an increased 1.0 μM concentration after cytosolic release) activate several Ca^{2+}-dependent protein kinases and phosphatases.

Calmodulin is a Ca^{2+}-dependent protein that is activated when the Ca^{2+} concentration increases to 0.5 μM. Ca^{2+}-calmodulin complexes bind to a number of cytosolic target proteins to regulate cell responses. Note that **Ca^{2+} is an important second messenger** and that its intracellular concentration can be increased not only by its release from intracellular storage sites but also by increasing the entry of Ca^{2+} into the cell from the extracellular space.

MAP kinase pathway

This pathway involves evolutionarily conserved protein kinases (yeast to humans) with roles in cell growth and differentiation. **MAP kinases** are protein serine and threonine kinases activated by growth factors and other signaling molecules (Figure 3-9).

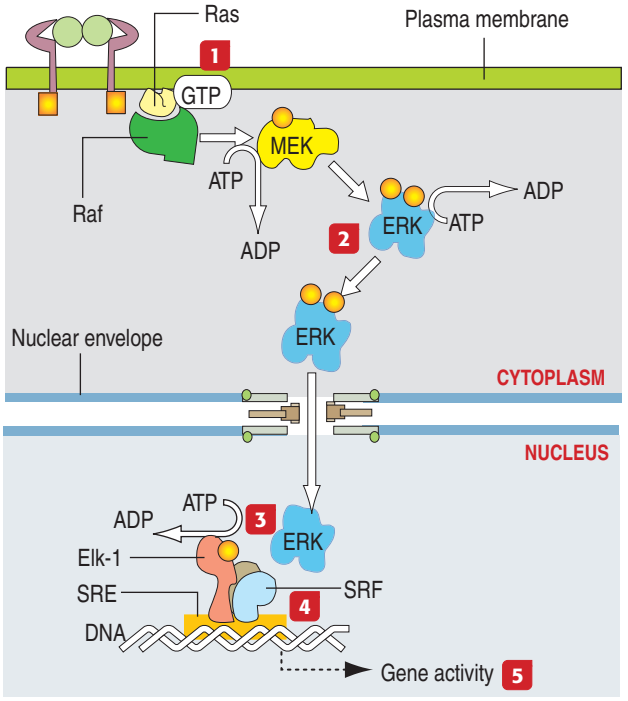
A well-characterized form of MAP kinase is the ERK family. Members of the **ERK** (for extracellular signal–regulated kinase) family act **through either protein tyrosine kinase or G protein–associated receptors**. Both cAMP and Ca^{2+}-dependent pathways can stimulate or inhibit the ERK pathway in different cell types.

The activation of ERK is mediated by two protein kinases: **Raf**, a protein serine or threonine kinase, which, in turn, activates a second kinase called **MEK** (for MAP kinase or ERK kinase). Stimulation of a growth factor receptor leads to the activation of the GTP-binding protein **Ras** (for rat sarcoma virus), which interacts with Raf. Raf phosphorylates and activates MEK, which then activates ERK by phosphorylation of serine and threonine residues. ERK then phosphorylates nuclear and cytosolic target proteins.

In the nucleus, activated ERK phosphorylates the transcription factors **Elk-1** (for E-26-like protein 1) and **serum response factor** (**SRF**), which recognize the regulatory sequence called **serum response element** (**SRE**).

In addition to ERK, mammalian cells contain two other MAP kinases called **JNK** and **p38 MAP kinases**. Cytokines and ultraviolet irradiation stimulate JNK

Figure 3-9. ERK-MAP kinase pathway



Activation of ERK-MAP kinase

1 Ligand binding to a growth factor receptor activates the small GTP-binding protein **Ras** (rat sarcoma virus) which interacts with **Raf** protein kinase.

2 Raf phosphorylates and activates **MEK (MAP kinase** or **ERK kinase)** which then activates **ERK (extracellular signal–regulated kinase)** by phosphorylation of tyrosine and threonine residues.

3 Activated ERK translocates into the nucleus where it phosphorylates the transcription factor **Elk-1**.

4 Activated Elk-1 binds to **SRE (serum response element)** forming a complex with **SRF (serum response factor)**.

5 Gene induction occurs.

and p38 MAP kinase activation mediated by small GTP-binding proteins different from Ras. These kinases are not activated by MEK but by a distinct dual kinase called **MKK** (for MAP kinase kinase).

A key element in the ERK pathway are the **Ras proteins**, a group of oncogenic proteins of tumor viruses that cause sarcomas in rats. Mutations in the Ras gene have been linked to human cancer. **Ras proteins are guanine nucleotide–binding protein with functional properties similar to the G protein α subunits** (activated by **GTP** and inactivated by guanosine diphosphate [**GDP**]).

A difference with G protein is that Ras proteins do not associate with βγ subunits. Ras is activated by **guanine nucleotide exchange factors** to facilitate the release of GDP in exchange for GTP. The activity of the Ras-GTP complex is terminated by GTP hydrolysis, which is stimulated by **GTPase-activating proteins**.

In human cancers, mutation of *Ras* genes results in a breakdown failure of GTP and, therefore, the mutated Ras protein remains continuously in the active GTP-bound form.

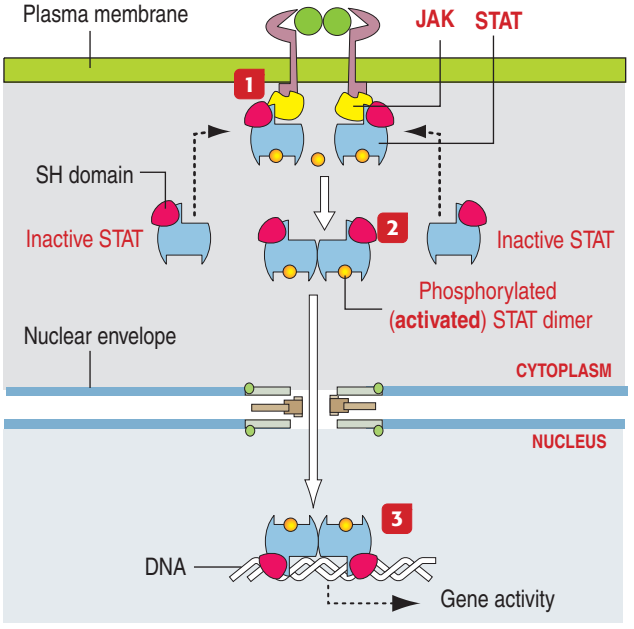
JAK-STAT pathway

The preceding MAP kinase pathway links the cell surface to the nucleus signaling mediated by a protein kinase cascade leading to the phosphorylation of transcription factors.

The **JAK-STAT pathway** provides a close connection between protein tyrosine kinases and transcription factors by directly affecting transcription factors (Figure 3-10).

STAT (for **signal transducers and activators of transcription**) **proteins** are transcription factors with an SH2 domain and are present in the **cytoplasm** in an inactive state. Stimulation of a receptor by ligand binding recruits STAT proteins, which bind to the cytoplasmic portion of receptor-associated **JAK protein tyrosine kinase** through their SH2 domain and become phosphorylated. Phosphorylated STAT proteins then dimerize and translocate into the nucleus, where they activate the transcription of target genes.

Figure 3-10. JAK-phosphorylated STAT dimer pathway



The JAK-STAT pathway

1 Ligand binding to a cytokine receptor leads to the attachment of the inactive transcription factor STAT to the receptor-associated **JAK protein tyrosine kinase** via their **SH2 domains**.

2 Phosphorylated **STAT dimerizes**.

3 The phosphorylated STAT dimer translocates to the nucleus where it activates transcription of target genes.

Transcription factor genes: *SOX9*

Genes encoding proteins that turn on (activate) or turn off (repress) other genes are called transcription factors. Many transcription factors have common DNA-binding domains and can also activate or repress a single target gene as well as other genes (a cascade effect). Therefore, mutations affecting genes encoding transcription factor have **pleiotropic effects** (Greek *pleion,* more; *trope*, a turning toward).

Examples of transcription factor genes include homeobox-containing genes, high mobility group (HMG)-box–containing genes, and the T-box family.

The HMG domain of Sox proteins can bend DNA, and facilitate the interaction of enhancers with a distantly located promoter region of a target gene. Several *SOX* genes act in different developmental pathways. For example, Sox9 protein is expressed in the gonadal ridges of both sexes but is upregulated in males and downregulated in females before gonadal differentiation. Sox9 also regulates chondrogenesis and the expression of type II collagen (see Chapter 4, Connective Tissue). Mutations of the *SOX9* gene cause skeletal defects (campomelic dysplasia), and sex reversal (XY females).

Stem cells, a multipotent cell population

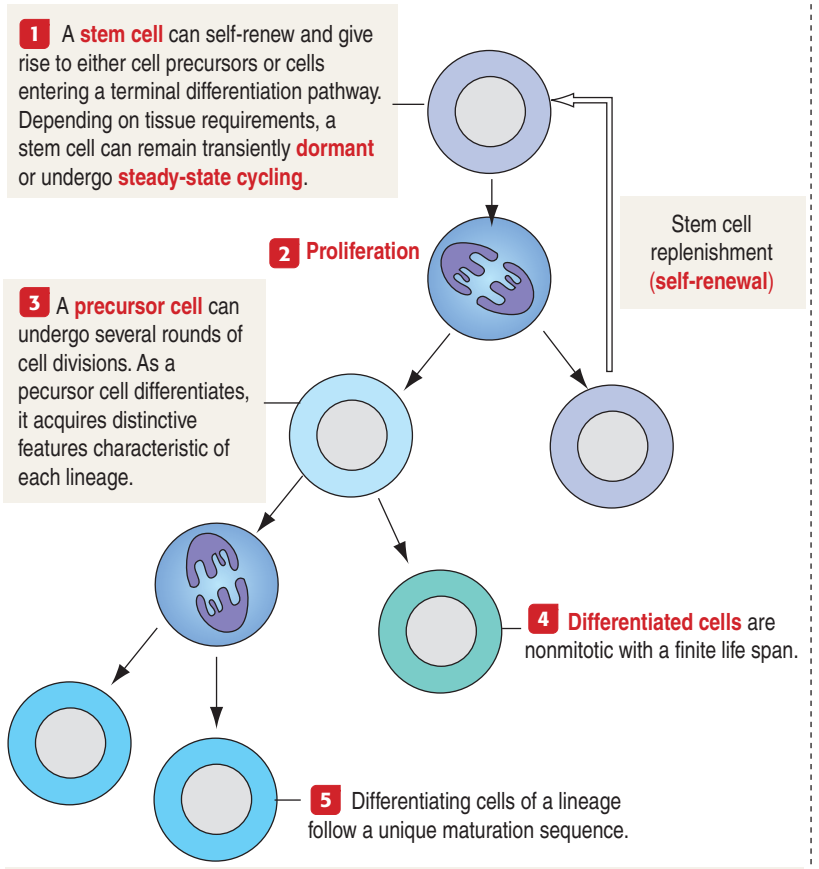
Cells in the body show a remarkable range in ability to divide and grow. Some cells (for example, nerve cells and erythrocytes) reach a mature, differentiated state and usually do not divide. Such cells are referred to as **postmitotic cells**. Other cells, called **stem cells**, show continuous division throughout life (for example, epithelial cells lining the intestine and stem cells that give rise to the various blood cell types).

Many other cells are intermediate between these two extremes and remain quiescent most of the time but can be triggered to divide by appropriate signals. Liver cells are an example. If the liver is damaged, cell division can be triggered to compensate for the lost cells.

Stem cells have three properties: **self-renewal**, **proliferation**, and **differentiation**.

Stem cells have the potential to generate a large number of mature cells continuously throughout life. When stem cells divide by mitosis, some of the progeny differentiates into a specific cell type. Other progeny remains as stem cells (Figure

Figure 3-11. Properties of stem cells



Stem cells have three characteristics: **self-renewal**, **proliferation**, and **differentiation** into mature cells.

Stem cells of the embryo can give rise to cell precursors that generate all the tissues of the body. This property defines stem cells as **multipotent**.

Stem cells are difficult to identify morphologically. Their identification is based on specific **cell surface markers** (cell surface antigens recognized by specific monoclonal antibodies) and on the lineage they generate following **transplantation**.

Four typical examples are the stem cells of **bone marrow**, **stomach, intestine**, and **testis**.

3-11). The intestinal epithelium, the epidermis of the skin, the hematopoietic system, and spermatogenic cells of the seminiferous epithelium share this property. We discuss in detail the significance of stem cells in each of these tissues in the appropriate chapters. Following stress and injury, other tissues, such as liver, muscle, and the nervous system, can regenerate mature cells.

For example, it has been shown that bone marrow stem cells can produce muscle tissue as well as hematopoietic tissue in an appropriate host system (see Chapter 7, Muscle Tissue). Cultured stem cells of the central nervous system are capable of hematopoiesis in transplanted irradiated mouse recipients.

Recall that embryonic stem cells, forming the **inner cell mass** (**embryoblast**) of the early embryo (the blastocyst), give rise to all the tissues and organs except the placenta. Embryonic stem cells provide an experimental source of medically useful differentiating tissues such as pancreatic islets for the treatment of diabetes, skin for the treatment of burns and wounds, regenerating cartilage for the treatment of arthritis, and endothelial cells for the repair of blood vessels affected by arteriosclerosis. A potential complication is that embryonic stem cells injected into mature mice develop an embryonic tumor called a **teratoma**.

In vitro cell proliferation, senescence, and telomerase

Cell culture techniques have been a powerful tool for examining the factors that regulate cell growth and for comparing the properties of normal and cancer cells.

Many cells grow in tissue culture, but some are much easier to grow than others. Culture medium contains **salts**, **amino acids**, **vitamins**, and a source of energy such as **glucose**. In addition, most cells require a number of **hormones** or **growth factors** for sustained culture and cell division. These factors are usually provided by addition of **serum** to the culture medium.

For some cell types the components supplied by serum have been identified, and these cells can be grown in **serum-free, hormone and growth factor–supplemented medium**. Some of these factors are hormones, such as insulin. A number of growth factors have been identified, for example, EGF, fibroblast growth factor (FGF), and PDGF.

When normal cells are placed in culture in the presence of adequate nutrients and growth factors, they will grow until they cover the bottom of the culture dish, forming a monolayer. Further cell division then ceases. This is called **density-dependent inhibition of growth**. The cells become quiescent but can be triggered to enter the cell cycle and divide again by an additional dose of growth factor or by replating at a lower cell density.

Cells cultured from a tissue can be kept growing and dividing by regularly replating the cells at lower density once they become confluent. After about 50 cell divisions, however, the cells begin to stop dividing and the cultures become **senescent**. The number of divisions at which this occurs depends on the age of the individual from which the initial cells were taken. Cells from an embryo will thus keep growing longer than cells taken from an adult.

In our discussion of mitosis (see Figure 1-51 in Chapter 1, Epithelium), we call attention to the role of **telomerase**, an enzyme that maintains the ends of chromosomes, or **telomeres**.

In normal cells, insufficient telomerase activity limits the number of mitotic divisions and forces the cell into **senescence**, defined as the finite capacity for cell division. **Telomere shortening and the limited life span of a cell are regarded as potent tumor suppressor mechanisms**. Most human tumors express **human telomerase reverse transcriptase** (**hTERT**). The ectopic expression of hTERT in primary human cells confers endless growth in culture. The use of telomerase inhibitors in cancer patients is currently being pursued.

Occasionally cells that would normally stop growing become altered and appear to become **immortal**. Such cells are called a **cell line**. Cell lines are very useful experimentally and still show most of the phenotype and growth characteristics of the original cells.

An additional change known as **transformation** is associated with the potential for **malignant growth**. Transformed cells no longer show normal growth control and have many alterations, such as **anchorage-independent growth**. Normal cells can grow when anchored to a solid substrate.

Cells in culture can be transformed by **chemical carcinogens** or by **infection with certain viruses** (tumor viruses). Tumor viruses will also cause tumors in certain host animals, but in different species they may cause ordinary infections. Cancer cells cultured from tumors also show the characteristics of transformation. We will discuss at the end of this chapter the role of retroviruses in carcinogenesis.

Apoptosis or programmed cell death

Cell death occurs by necrosis or apoptosis. Under normal physiologic conditions, cells deprived of survival factors, damaged, or senescent commit suicide through an orderly regulated cell death program called **apoptosis** (Greek *apo*, off; *ptosis*, fall).

Apoptosis (Figure 3-12) is different from **necrosis**. Necrosis is a nonphysiologic

Figure 3-12. Programmed cell death or apoptosis

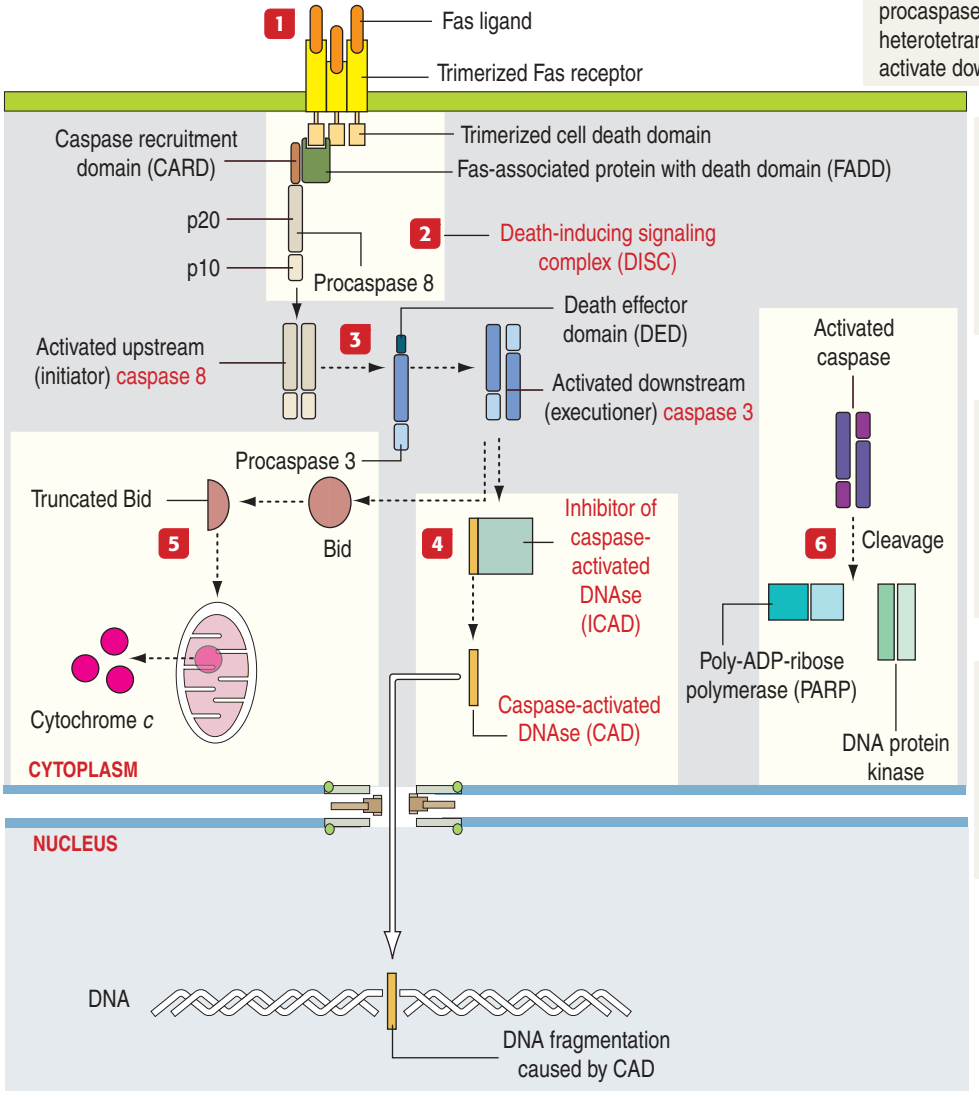
1 Fas ligand binding to Fas receptor causes its trimerization. The trimerized intracellular cell death domain recruits Fas-associated protein with death domain (FADD) adaptor that recruits procaspase 8 through its caspase recruitment domain (CARD).

2 The death-inducing cell signaling complex (DISC) consists of Fas receptor, FADD, and procaspase 8.

Within DISC, procaspase 8 becomes active caspase 8.

3 Procaspases consist of two subunits (p10 and p20) and an N-terminal recruitment domain.

Caspases can be upstream initiators with a long N-terminal prodomain called CARD (such as procaspase 8) or downstream executioners with a short N-terminal prodomain called DED (such as procaspase 3). Activated caspases are heterotetramers. Upstream caspases can activate downstream executioner caspases.



4 Activated caspase 8 can cleave ICAD to become CAD, a caspase-activated DNAse. CAD migrates to the nucleus and induces DNA fragmentation.

5 Activated caspase 8 can cleave Bid, a member of the Bcl-2 family of proteins.

Truncated Bid facilitates the leakage of mitochondrial cytochrome *c* into the cytoplasm.

6 Activated caspases cleave two DNA repair enzymes (PARP and DNA protein kinase). DNA fragmentation proceeds undisturbed.

process that occurs after acute injury (for example, in an ischemic stroke). Necrotic cells lyse and release cytoplasmic and nuclear contents into the environment, thus triggering an inflammatory reaction.

Cells undergoing apoptosis lose intercellular adhesion, fragment the chromatin, and break down into small blebs called **apoptotic bodies**. Apoptotic bodies are phagocytosed by macrophages and inflammation does not occur.

Apoptotic cell death is observed during fetal development. For example, the formation of fingers and toes of the fetus requires the elimination by apoptosis of the tissue between them. During fetal development of the central nervous system, an excess of neurons, eliminated later by apoptosis, is required to establish appropriate connections or synapses between them (see Chapter 8, Nervous Tissue). Mature granulocytes in peripheral blood have a life span of 1 to 2 days before

undergoing apoptosis. The clonal selection of T cells in the thymus (to eliminate self-reactive lymphocytes to prevent autoimmune diseases; see Chapter 10, Immune-Lymphatic System) and cellular immune responses involve apoptosis.

What a nematode worm told us about apoptosis

The genetic and molecular mechanisms of apoptosis emerged from studies of the nematode worm *Caenorhabditis elegans*, in which 131 cells are precisely killed and 959 remain. In this worm, four genes are required for the orderly cell death program: *ced-3* (for cell death defective-3), *ced-4*, *egl-1* (for egg laying-1), and *ced-9*. The products of the first three genes mediate cell death. The gene *ced-9* is an inhibitor of apoptosis.

The proteins encoded by these four genes in the worm are found in vertebrates. Protein ced-3 is homologous to **caspases**, ced-4 corresponds to **Apaf-1** (for **apoptotic protease activating factor-1**), ced-9 to **Bcl-2** (for B-cell leukemia-2), and egl-1 is homologous to Bcl-2 homology region 3 (BH3)-only proteins.

External signals trigger apoptosis: Fas receptor/Fas ligand

External and internal signals determine cell apoptosis. External signals bind to cell surface receptors (for example, tumor necrosis factor-α and Fas ligand). Internal signals (for example, the release of cytochrome *c* from mitochondria) can trigger cell death.

Fas receptor (also known as APO-1 or CD95) is a cell membrane protein that belongs to the **tumor necrosis factor** (**TNF**) **receptor family**. Fas receptor has an intracellular cell death domain. **Fas ligand** binds to Fas receptor and causes its **trimerization**. Fas ligand initiates programmed cell death by binding to the **Fas receptor** and triggers a cell signaling cascade consisting of the sequential activation of **procaspases** into active **caspases**. The trimerized cell death domain recruits procaspase 8 through the **FADD** (for **Fas-associated protein with death domain**) adaptor and forms a **DISC** (for **death-inducing signaling complex**). DISC consists of Fas receptor, FADD, and procaspase 8.

Procaspase 8 autoactivated at DISC becomes active caspase 8. Active caspase 8 can do two things:

1. It can process procaspase 3 to active caspase 3, which can cleave several cellular proteins, including **ICAD** (for **inhibitor of CAD**) giving rise to CAD. **CAD** (for **caspase-activated DNAse**) is released from ICAD, translocates to the cell nucleus, and breaks down chromosomal DNA.
2. Caspase 8 can cleave **Bid**, a proapoptotic member of the Bcl-2 family. The truncated Bid translocates to mitochondria to release cytochrome *c* into the cytoplasm.

As we will discuss in Chapter 10, Immune-Lymphatic System, a cytotoxic T cell destroys a target cell (for example, a virus-infected cell) by first binding to the target cell and then releasing Fas ligand. Fas ligand binds to Fas receptor on the surface of the target cell and triggers the cell death cascade.

Caspases, initiators and executioners of cell death

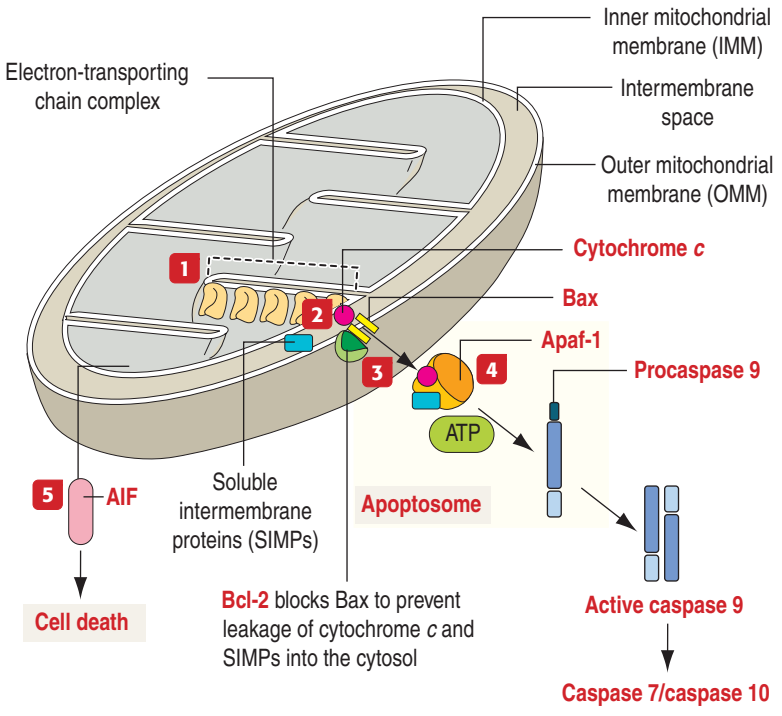
Caspases (for **cysteine aspartic acid–specific proteases**) exist as inactive precursors (procaspases), which are activated to produce directly or indirectly cellular morphologic changes during apoptosis.

Procaspases consist of two subunits (**p10** and **p20**) and an N-terminal recruitment domain (see Figure 3-12). Activated caspases are heterotetramers consisting of two p10 subunits and two p20 subunits derived from two procaspases.

Caspases can be **upstream initiators** and **downstream executioners**. Upstream initiators are activated by the cell-death signal (for example, Fas ligand or TNF-α). Upstream initiator caspases activate downstream caspases, which directly mediate cell destruction.

Completion of the cell death process occurs when executioner caspases activate

Figure 3-13. Role of mitochondria in apoptosis



Cytochrome *c* in apoptosis

1 Cytochrome c shuttles electrons between respiratory chain complexes III and IV. If cytochrome *c* is not present, electron flow stops and ATP synthesis does not occur.
2 Cytochrome *c* is located between the IMM and OMM.
3 **Antiapoptotic Bcl-2** blocks **Bax** which facilitates the release of **cytochrome *c*** and **SIMPs**.
4 During apoptosis, **cytochrome *c* and SIMPs are released across the OMM and interact with apoptosis protease activating factor-1 (Apaf-1)** to form the **apoptosome** (together with **ATP** and **procascapse 9**).
Apaf-1 activates procaspase 9. Caspase 9 activates caspase 7 and caspase 10 leading to the proteolytic destruction of the cell.
5 **Apoptosis-inducing factor (AIF)** is a mitochondrial protein that can be released into the cytoplasm, migrate to the cell nucleus, bind to DNA and trigger DNA fragmentation in the absence of caspases.

the DNA degradation machinery. Caspases cleave two DNA repair enzymes (**poly-ADP–ribose polymerase** [**PARP**], and **DNA protein kinase**), and unrestricted fragmentation of chromatin occurs.

As you realize, the key event in caspase-mediated cell death is the regulation of the activation of **initiator caspases.**

Upstream (initiator) procaspases include procaspases 8, 9, and 10 with a **long** N-terminal prodomain called **CARD** (for **caspase-recruiting domain**). Downstream (executioner) procaspases comprise procaspases 3, 6, and 7 with a **short** N-terminal prodomain called **DED** (for **death-effector domain**).

Caspase activation takes place when a caspase-specific regulatory molecule (for example, FADD) binds to the CARD/DED domain. Caspase activation may become out of control and destroy the cell. To prevent this uncontrolled event, inhibitors of apoptosis are available to interact with modulators of cell death, thus preventing unregulated caspase activation.

Bcl-2 regulates the release of mitochondrial cytochrome *c* through Bax

Cytochrome *c* is a component of the mitochondria electron-transporting chain involved in the production of ATP, and also a trigger of the caspase cascade.

The cell death pathway can be activated when cytochrome *c* is released from the mitochondria into the cytoplasm. How does cytochrome *c* leave mitochondria? To answer this question, we need to consider aspects of members of the **Bcl-2 family**.

Bcl-2 family members can have **proapoptotic** or **antiapoptotic** activities. **Bcl-2 and Bcl-xL have antiapoptotic activity. Bax, Bak, Bid, and Bad are proapoptotic proteins**. Bcl-2 is associated with the outer mitochondrial membrane of viable cells and prevents Bax from punching holes in the outer mitochondrial membrane, causing cytochrome *c* to leak out. As you see, a balance between proapoptotic Bax and antiapoptotic Bcl-2 proteins controls the release of cytochrome *c*.

In the cytoplasm, leaking cytochrome *c*, in the presence of ATP, **soluble internal membrane proteins** (**SIMPs**), and procaspase 9, binds to Apaf-1 to form a

complex called an **apoptosome**.

The apoptosome determines the activation of **caspase 9**, an upstream initiator of apoptosis (Figure 3-13). Caspase 9 activates caspase 3 and caspase 7, leading to cell death.

You can gather from this discussion that external activators such as Fas ligand and TNF-α, and the internal release of cytochrome *c* are two key triggers of apoptosis. However, **AIF** (for **apoptosis-inducing factor**) is a protein of the intermitochondrial membrane space that can be released into the cytoplasm, migrate to the nucleus, bind to DNA, and trigger cell destruction without participation of caspases.

Clinical significance of apoptosis: Apoptosis in the immune system

Mutations in the *Fas receptor*, *Fas ligand*, or *caspase 10* genes can cause **autoimmune lymphoproliferative syndrome** (**ALPS**). ALPS is characterized by the accumulation of mature lymphocytes in lymph nodes and spleen causing **lymphoadenopathy** (enlargement of lymph nodes) and **splenomegaly** (enlargement of the spleen), and the existence of autoreactive lymphocyte clones producing autoimmune conditions such as **hemolytic anemia** (caused by destruction of red blood cells) and **thrombocytopenia** (reduced number of platelets).

Clinical significance of apoptosis: Neurodegenerative diseases

Neurologic diseases are examples of the mechanism of cell death. For example, an **ischemic stroke** can cause an **acute neurologic disease** in which necrosis and activation of caspase 1 are observed. Necrotic cell death occurs in the center of the infarction, where the damage is severe. Apoptosis may be observed at the periphery of the infarction, because the damage is not severe due to collateral blood circulation. Pharmacologic treatment with caspase inhibitors can reduce tissue damage leading to neurologic improvement.

Caspase activation is associated with the fatal progression of **chronic neurodegenerative diseases**. **Amyotrophic lateral sclerosis** (**ALS**) and **Huntington's disease** are two examples.

ALS consists in the progressive loss of motor neurons in brain, brainstem, and spinal cord. A mutation in the gene encoding **superoxide dismutase 1** (***SID1***) has been identified in patients with familial ALS. Activated caspase 1 and caspase 3 have been found in spinal cord samples of patients with ALS. Motor neurons and axons die and reactive microglia and astrocytes are present. We come back to ALS in Chapter 9, Nervous Tissue.

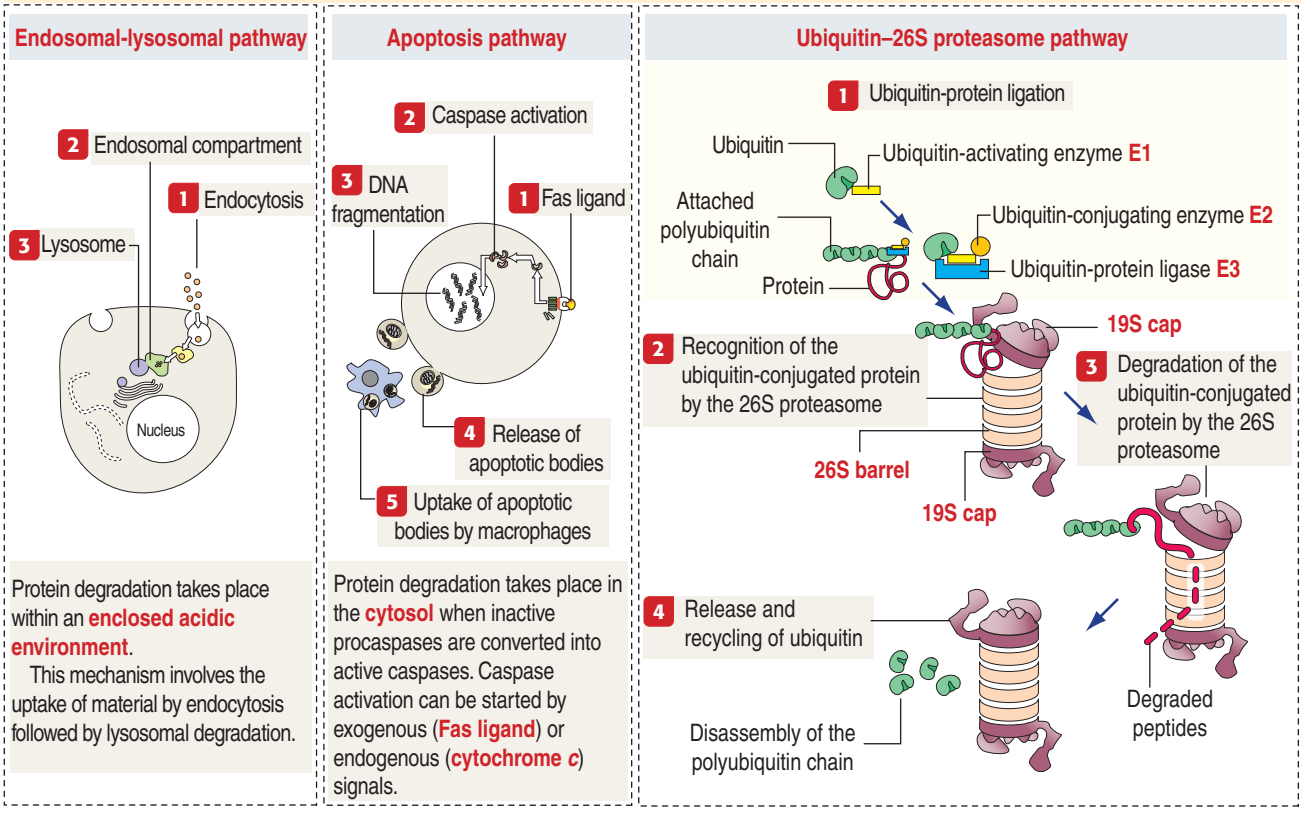
Huntington's disease is an autosomal dominant neurodegenerative disease characterized by a movement disorder (**Huntington's chorea**). The disease is caused by a mutation in the protein **huntingtin**. Huntingtin protein fragments accumulate and aggregate in the neuronal nucleus and transcription of the *caspase 1* gene is upregulated. Caspase 1 activates caspase 3 and both caspases cleave the allelic wild-type form of huntingtin, which becomes depleted. As the disease progresses, Bid is activated and releases mitochondrial cytochrome *c*. Apoptosomes are assembled and further caspase activation leads to neuronal death.

Three major cellular mechanisms are involved in proteolysis

In addition to the **procaspase-caspase pathway** activated by Fas ligand (see Figure 3-12), the intracellular degradation of residual or misfolded proteins (**proteolysis**) can occur by the classic **endosomal-lysosomal pathway** (see Figure 2-19), the apoptosis pathway (see Figure 3-12), and the **ubiquitin-proteasome pathway** (Figure 3-14). We have already seen that the endosomal-lysosomal mechanism operates within a membrane-bound acidic compartment. In contrast, the procaspase-caspase pathway and the ubiquitin-proteasome pathway carry out proteolysis in the cytosol.

The ubiquitin–26S proteasome pathway involves four successive regulated steps:

Figure 3-14. Three proteolytic mechanisms



1. The attachment of a chain of ubiquitin molecules to a protein substrate by an enzymatic cascade. First, **E1**, the ubiquitin-activating enzyme, activates ubiquitin in the presence of ATP to form a thioester bond. **E2**, the ubiquitin-conjugating enzyme, uses the thioester bond to conjugate activated ubiquitin to the target protein. E2 transfers the activated ubiquitin to a lysine residue of the substrate with the help of **E3**, a specific ubiquitin-protein ligase. This process is repeated several times to generate a long polyubiquitin chain attached to the substrate protein destined for degradation in the **26S proteasome**.

2. Recognition of the ubiquitin-conjugated protein by the 26S proteasome. A protein subunit (designated S5a) in the 19S cap of the proteasome acts as a receptor for the polyubiquitin chain.

3. Degradation of the ubiquitin-conjugated protein into oligopeptides in the 26S barrel, the inner proteolytic chamber of the proteasome, in the presence of ATP.

4. The release and recycling of ubiquitin.

The 26S proteasome is a giant (~2000 kd) multimeric protease present in the nucleus and cytoplasm. Structurally, the 26S proteasome consists of a barrel-shaped core capped by two structures that recognize ubiquitinated proteins. Protein degradation occurs within a chamber of the barrel-shaped core. Proteins degraded by the 26S proteasome include molecules involved in the regulation of the cell cycle (cyclins), transcription factors, and the processing of antigens involved in the activation of inflammatory and immune responses.

Proto-oncogenes and oncogenes

Genes that cause cancer are called **oncogenes** (Greek *onkos*, bulk, mass; *genos*, birth). Most oncogenes originate from **proto-oncogenes** (Greek *prōtos*, first). Proto-oncogenes (Box 3-E) are involved in the **four basic regulatory mechanisms of cell growth** by expressing **growth factors**, **growth factor receptors**, **signal transduction molecules**, and **nuclear transcription factors**.

Box 3-E | Proto-oncogenes and oncogenes

A **proto-oncogene** is a normal gene encoding a regulatory protein of the cell cycle, cell differentiation, or a cell-signaling pathway. Proto-oncogenic proteins mimic growth factors, hormone receptors, G proteins, intracellular enzymes, and transcription factors.

An **oncogene** is a **mutated proto-oncogene** that encodes an **oncoprotein** able to disrupt the normal cell cycle and to cause cancer.

Proto-oncogenes and oncogenes are designated by an **italicized three-letter** name. An oncogene present in a virus has the prefix **v**. A proto-oncogene present in a cell has the prefix **c**.

A protein encoded by a proto-oncogene or oncogene has the same three-letter designation as the proto-oncogene or oncogene. However, the letters are not italicized and the first letter is capitalized.

Antioncogenes are also called **tumor suppressor genes**. A loss of activity of a tumor suppressor gene product results in constitutive activation of cell growth.

An oncogene results from the mutation of a proto-oncogene. Oncogenes express constantly active products leading to unregulated cell growth and differentiation. A cell becomes **transformed** when it changes from regulated to unregulated growth.

Although most animal viruses destroy the cells they infect, several types of viruses are able to establish a long-term infection, in which the cell is not killed. This stable virus–host cell interaction perpetuates the viral information in the cell, usually by direct insertion into cellular DNA.

The first oncogenes to be identified came from the study of **retroviruses**. All vertebrate animals, including humans, inherit genes related to retroviral genes and transmit them to their progeny. These are called **endogenous proviruses**, whereas those that infect a cell are called **exogenous proviruses**.

Cancer viruses isolated from every type of vertebrate animal induce a wide variety of tumors and belong to several virus types: **RNA-containing tumor viruses**, called **retroviruses**, and **DNA-containing tumor viruses**, including the **polyomaviruses**, the **papillomaviruses**, the **adenoviruses**, and the **herpesviruses**.

RNA-containing retroviruses have a distinct cell cycle. In the initial stages of infection, the **viral RNA** is **copied into DNA** by the viral enzyme **reverse transcriptase**. Once synthesized, the viral DNA molecule is transported into the nucleus and inserted randomly as a **provirus** at any one of the available sites of host chromosomal DNA. Proviruses contain signals for the regulation of their own viral genes, but such signals can be transmitted to the proto-oncogene, forcing it to produce larger than normal amounts of RNA and a protein.

Retroviruses and polyomaviruses have received the most attention because they carry one or two genes that have specific cancer-inducing properties: so-called **viral oncogenes**. Retroviruses and polyomaviruses like cellular genes, are subject to mutations. A group of such mutants of **Rous sarcoma virus** (**RSV**; species of origin: chicken) has proved useful for determining the role of the **viral** gene **v-*src***. The *src*-like sequences in normal cells constitute a **cellular** gene called **c-*src***, a **proto-oncogene**.

The **viral *src*** derives directly from the **cellular *src***. A precursor of RSV seems to have acquired a copy of c-*src* during infection of a chicken cell. **c-src is harmless but its close relative v-src causes tumors and transform cells after RSV infection**. A chicken fibroblast produces about 50 times more src RNA and protein than an uninfected fibroblast containing only the c-*src* gene. The c-*src* gene assumed great significance when it was recognized that many other retroviruses carry oncogenes, often different from v-*src*. Each of these genes is also derived from a distinct, normal cellular precursor.

The classification of genes as proto-oncogenes is based on the understanding that mutant forms of these genes participate in the development of cancer (see Box 3-F). However, proto-oncogenes serve different biochemical functions in the control of normal growth and development. They can also undergo a variety of mutations that convert them to dominant genes capable of inducing cancers in the absence of viruses.

RSV-infected cells produce a 60-kd protein. This protein was identified as the product that the **v-*src* gene** uses to transform cells. It was designated **p60$^{v\text{-}src}$**. This protein can function as a **protein kinase** and, within a living cell, many proteins can be phosphorylated by **Src kinase** activity. The target for phosphorylation is **tyrosine** residues.

Cell transformation by the v-*src* oncogene causes a tenfold increase in total cellular phosphotyrosine in cellular target proteins restricted to the inner side of the **cell membrane**. Many other proteins encoded by proto-oncogenes or involved in control of cell growth function like the Src protein, such as protein kinases, are often specific for tyrosine.

Box 3-F | **Proto-oncogenes and tumor suppressor proteins in human cancers**

Chronic myelogenous leukemia: The **c-*abl*** proto-oncogene translocated from chromosome 9 to chromosome 22 (called the Philadelphia chromosome) encodes a fusion protein with constitutive active tyrosine kinase activity.

Burkitt's lymphoma: The **c-*myc*** proto-oncogene is translocated from chromosome 8 to chromosome 14. This translocation places c-*myc* under the control of an active immunoglobulin locus (immunoglobulin heavy-chain gene, Cm) and detached from its normal regulatory elements. Burkitt's lymphoma is endemic in some parts of Africa and affects mainly children or young adults. It generally involves the maxilla or mandible. It responds to chemotherapy.

p53: Inactivation of this **tumor suppressor protein**, a transcription factor expressed in response to DNA damage (see Figure 1-52), is associated with 50% to 60% of human cancers. Inactive p53 enables the progression of cells containing damaged DNA through the cell cycle.

• Cell signaling is the mechanism by which cells respond to chemical signals. Signaling molecules are either secreted or expressed on the cell surface of cells. When a signaling molecule binds to its receptor, it initiates intracellular reactions to regulate cell proliferation, differentiation, cell movements, metabolism, and behavior.

• There are several cell signaling mechanisms. (1) Endocrine signaling involves a hormone secreted by an endocrine cell and transported through blood circulation to act on a distant target. (2) Paracrine signaling is mediated by molecules acting locally to regulate the function of a neighboring cell. (3) Autocrine signaling consists in cells responding to signaling molecules that are produced by themselves. (4) Neurotransmitter signaling is a specific form of paracrine signaling involving neurons and neurotransmitter molecules released at a synapse. (5) Neuroendocrine signaling consists in a neuroendocrine cell releasing a hormone into the bloodstream in response to a stimulus released from an axon terminal.

• Hormones can be protein hormones (for example, insulin, neuropeptides secreted by neurons, and growth factors) or steroid hormones (for example, cholesterol-derived testosterone, estrogen, progesterone, and corticosteroids). Protein hormones bind to a cell surface receptor. Steroid hormones bind to cytosol and nuclear receptors. Nonsteroid signaling molecules, such as thyroid hormone, vitamin D_3, and retinoids (vitamin A), bind to intracellular receptors.

Several specific signaling molecules exist. (1) Epinephrine can be a neurotransmitter and also a hormone released into the bloodstream. (2) Eicosanoids and leukotrienes (derived from arachidonic acid) are lipid-containing signaling molecules which bind to cell surface receptors.

• Nitric oxide is a signaling molecule of very short half-life (seconds). Nitric oxide is synthesized from arginine by the enzyme nitric oxide synthase. Nitric oxide can diffuse across the plasma membrane but it does not bind to a receptor. Its major function is the regulation of the activity of intracellular enzymes. One of the relevant functions of nitric oxide is the dilation of blood vessels. Nitroglycerin, an agent used in the treatment of heart disease, is converted to nitric oxide, which increases heart blood flow by dilation of the coronary artery.

• After binding to a receptor, hormones activate intracellular targets downstream of the receptor.

1. G protein–coupled receptor consists of three subunits (α, β, and γ) forming a complex. The α subunit binds GDP (guanosine diphosphate) and regulates G protein activity. When a signaling molecule binds to its receptor, the α subunit of the associated G protein dissociates, releases GDP, and binds GTP (guanosine triphosphate) to activate an adjacent target molecule.

2. Tyrosine kinases can be a transmembrane protein or present in the cytosol. The first form is called tyrosine kinase receptor; the second form is known as nonreceptor tyrosine kinase. Binding of a ligand to tyrosine kinase receptor produces its dimerization resulting in autophosphorylation of the intracellular domain. Downstream molecules with SH2 (Src homology 2) domains bind to the catalytic kinase domain of tyrosine kinase receptor. The activity of tyrosine kinase receptor can be disrupted by inducing unregulated autophosphorylation in the absence of a ligand. Tyrosine kinase activity can be inhibited by imatinib mesylate, a molecule with binding affinity to the adenosine triphosphate (ATP)-binding domain of the catalytic domain. Imatinib is used in the treatment of **chronic myeloid leukemia, chronic myelomonocytic leukemia, systemic mastocytosis, and mast cell leukemias.**

3. Cytokine receptors are a family of receptors that stimulate intracellular protein tyrosine kinases, which are not intrinsic components of the receptor. Ligand binding to cytokine receptors triggers receptor dimerization and cross-phosphorylation of the associated tyrosine kinases. Members of the cytokine receptor–associated tyrosine kinase family are the Src family and the Janus kinase family (JAK).

4. Receptors can be linked to enzymes such as protein tyrosine phosphatases and protein serine and threonine kinases. Tyrosine phosphatases remove tyrosine phosphate groups from phosphotyrosine and arrest signaling started by tyrosine phosphorylation. Members of the transforming growth factor-β (TGF-β) family are protein kinases that phosphorylate serine and threonine residues. Ligand binding to TGF-β induces receptor dimerization and the serine- or threonine-containing intracellular domain of the receptor cross-phosphorylates the polypeptide chains of the receptor.

• Following ligand binding, most receptors activate intracellular enzymes to transmit and amplify a signal.

1. The cAMP (cyclic adenosine monophosphate) pathway results from the formation of cAMP (known as a second messenger) from ATP by the enzyme adenylyl cyclase. The intracellular effects of cAMP are mediated by cAMP-dependent protein kinase (also known as protein kinase A). Inactive cAMP-dependent protein kinase is a tetramer composed of two regulatory subunits (the binding site of cAMP) and two catalytic subunits. The enzyme phosphodiesterase degrades cAMP. Upon cAMP binding, the catalytic subunits dissociate and each catalytic subunit phosphorylates serine residues on target proteins or migrates to the cell nucleus.

In the cell nucleus, the catalytic subunit phosphorylates the transcription factor CREB (CRE-binding protein) bound to CRE (the cAMP response element), and specific gene activity is induced.

2. The cGMP (cyclic guanosine monophosphate) pathway utilizes guanylate cyclase to produce cGMP which is degraded by a cGMP-dependent phosphodiesterase. Photoreceptors of the retina utilize cGMP to convert light signals to nerve impulses.

3. The phospholipase C–Ca^{2+} pathway consists in the production of second messengers from the phospholipid phosphatidylinositol 4.5-bisphosphate (PIP_2). Hydrolysis of PIP_2 by phospholipase C (PLC) produces two second messengers: diacylglycerol and inositol 1,4,5-triphosphate (IP_3). Diacylglycerol and IP_3 stimulate protein kinase C (protein serine and threonine kinases) and the mobilization of Ca^{2+}. Protein kinase C activates protein kinases of the MAP (mitogen activated protein) kinase pathway to phosphorylate transcription factors.

4. The NF-κB (for nuclear factor involved in the transcription of the κ light chain gene in B lymphocytes) transcription factor pathway is stimulated by protein kinase C and is involved in immune responses. When inactive, the NF-κB heterodimer is bound to the inhibitory subunit I-κB and remains in the cytoplasm. Phosphorylation of I-κB, triggered by I-κB kinase, results in the destruction of I-κB by the 26S proteasome and the nuclear translocation of the NF-κB heterodimer to activate gene transcription.

5. The Ca^{2+}-calmodulin pathway consists in the activation of calmodulin, a Ca^{2+}-dependent protein, when Ca^{2+} concentration increases and binds to calmodulin. You should note that the phospholipase C–Ca^{2+} and Ca^{2+}-calmodulin pathway regulates Ca^{2+} concentration by Ca^{2+} release from intracellular storage as well as entry into the cell from the extracellular space.

6. The MAP kinase pathway involves serine and threonine MAP kinases. The extracellular signal–regulated kinase (ERK)

family is a MAP kinase acting through either tyrosine kinase or G protein–associated receptors. The activation of ERK is mediated by two protein kinases: Raf and MEK (MAP kinase or ERK kinase). Raf interacts with rat sarcoma virus (Ras) protein, a key element of the group of oncogenic proteins.

Raf phosphorylates MEK which activates ERK, and then phosphorylated ERK activates nuclear (Elk-1) and cytosolic target proteins. Two other MAP kinases are JNK and p38 MAP kinases.

7. The JAK-STAT pathway regulates transcription factors. Signal transducer and activators of transcription (STAT) proteins are transcription factors with an SH2 domain and present in the cytoplasm in an inactive state. Ligand binding to a cytokine receptor determines the attachment of STAT to the receptor associated Janus kinase (JAK), a tyrosine kinase, through their SH2 domain. Phosphorylated STAT dimerizes and translocates to the cell nucleus to activate gene transcription.

• Transcription factors activate and inactivate genes. Sox9 is a transcription factor that regulates chondrogenesis (cartilage growth). Mutations of the *Sox9* gene cause campomelic dysplasia (skeletal defects) and sex reversal (XY females).

• Stem cells have three properties: self-renewal, proliferation, and differentiation. Stem cells can give rise to cell precursors that generate tissues of the body. Stem cells are present in the intestinal epithelium, the epidermis of the skin, the hematopoietic tissue, and spermatogenic cells. Stem cells are recognized by the expression of cell surface markers and by the cells they produce following culture or transplantation.

• Cell culture procedures demonstrate that: (1) cells stop growing when they cover entirely the surface of a culture dish. This is called density-dependent inhibition of growth. (2) Cultured cells can continue growing until they stop dividing. The cells have become senescent. Telomerases maintain the end of the chromosomes, the telomeres. Insufficient telomerase activity forces cells into senescence.

Telomere shortening is a potent tumor suppressor mechanism. Most tumors express human telomerase reverse transcriptase (hTERT) and growth in culture is endless. Cells become immortal. Such cells can establish a cell line. (3) Transformed cells have a malignant growth potential and exhibit anchorage-independent growth. In contrast, normal cells grow attached to a substrate.

• Apoptosis or programmed cell death can be determined by external and internal signals. An external signal is the Fas ligand which binds to the Fas receptor. An internal signal is the leakage of cytochrome *c* from mitochondria. The end point is the activation of procaspases to caspases, the initiators and executors of cell death.

A defect in the activity of Fas receptor, Fas ligand, and caspases can cause the **autoimmune lymphoproliferative syndrome (ALPS)**, characterized by the abnormal and excessive accumulation of lymphocytes in lymph nodes and spleen.

Aberrant activation of caspases is associated with neurodegenerative disease, such as **amyotrophic lateral sclerosis (ALS)** and **Huntington's disease.**

• The proteolysis of residual and misfolded proteins can occur by the classic endosomal-lysosomal pathway, the apoptosis pathway, and the ubiquitin–26S proteasome pathway. The first pathway takes place within a membrane-bound acidic compartment. The last two occur predominantly in the cytosol. The apoptosis pathway involves caspases; the ubiquitin–26S proteasome pathway requires the attachment of a polyubiquitin chain to proteins marked for degradation by the 26S proteasome.

• Proto-oncogenes express growth factors, growth factor receptors, signal transduction molecules, and nuclear transcription factors. An oncogene results from the mutation of a proto-oncogene. Oncogenes determine unregulated cell growth and a cell then becomes transformed. The first oncogenes to be identified were the retroviruses (RNA-containing viruses) with cancer-inducing properties (viral oncogenes). DNA-containing viruses (polyomaviruses, the papillomaviruses, the adenoviruses, and the herpesviruses) can induce tumors. The chicken cell Rous sarcoma virus (RSV) includes the viral gene *v-src*. The proto-oncogene equivalent in normal cells is *c-src*. The *v-src* gene encodes the protein $p60^{v\text{-}src}$, which functions as a tyrosine protein kinase. Cell transformation by the *v-src* oncogene results in a significant increase in total cell phosphotyrosine.