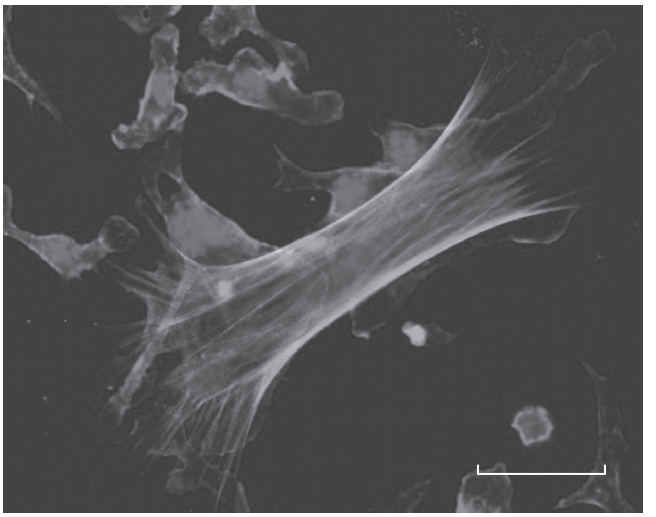
SECTION 2

REGULATORY MECHANISMS OF REGENERATION

5

CELL SIGNALING PATHWAYS AND MECHANISMS



Interaction of bone marrow-derived smooth muscle α actin+ cells with CD11b+ cells in culture. Mouse bone marrow cells were collected and cultured in DMEM with 10% FBS. Smooth muscle α actin+ cells (green) were often found on top of CD11b+ cells (red). When the CD11b+ cells were selectively removed, the number of smooth muscle α actin+ cells was reduced, suggesting that the CD11b+ bone marrow cells play a role in regulating the development of smooth muscle cells from bone marrow progenitor cells. Blue: cell nuclei. Scale: 10 μm. See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

PRINCIPLES OF CELL SIGNALING [5.1]

In a multicellular system, there exist signaling activities at the molecular and cellular levels for cell-to-cell and cell-to-matrix communications, which are essential for the function of cells, tissues, and organs. In order to conduct physiological function, a cell must communicate with other cells to achieve coordinated cellular activities. Often, synchronized molecular and cellular activities are required for tissue and organ functions. A typical example is the control of the contractile activity of skeletal muscle cells in response to an electrical stimulation from a nerve axon. In such a regulatory process, an electrical action potential is generated from a motor neuron center and transmitted through the nerve axon to the muscle cell. At the junction, or synapse, between the nerve axon and the muscle cell, the electrical signal is converted to a chemical signal, which induces the activation of an intracellular signaling cascade in the muscle cells, resulting in cell contraction. Without neuron-to-muscle cell signal transduction, the skeletal muscle cells cannot contract or relax in a controlled manner. Thus, coordinated cell signaling is a fundamental process for accomplishing complicated functional tasks. Here, the principles of cell signaling and common cell signaling pathways are discussed.

Factors Serving as Signals

There are two basic types of factors, which serve as "signals" for regulating molecular and cellular activities as well as cell-to-cell and cell-to-matrix communications: extracellular factors and intracellular factors (Fig. 5.1). *Extracellular facotrs* are present in extracellular space and serve as signals that initiate and control molecular and cellular activities. *Intracellular factors* are present in the cytoplasm, serve as elements for signaling pathways, and regulate cellular activities. In general, an extracellular signal must cooperate with intracellular signals to initiate and control a cellular activity. The extracellular signal may initiate the activation of an intracellular signaling pathway via interacting with a cell membrane or cytoplasmic signaling factor, whereas the intracellular signaling pathway relays the extracellular signal to target subcellular organelles, initiating a specified activity, such as gene expression, cell adhesion, cell proliferation, or cell migration.

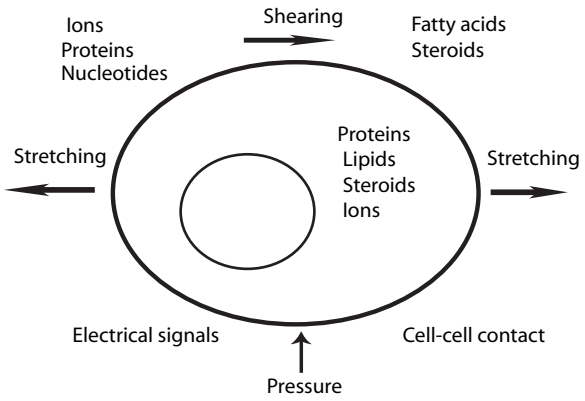


Figure 5.1. Extracellular and intracellular signals that regulate cell activities and functions (based on bibliography 5.1).

Cellular activities are often initiated in response to environmental or extracellular stimulations or cues. Extracellular factors that serve as signaling factors and induce cellular activities may include biochemical molecules and substances, electrical signals, and physical factors. *Biochemical molecules and substances* include cell-secreted factors and extracellular matrix factors, such as ions, proteins, nucleotides, fatty acids, and steroids. Some of these factors, such as proteins (growth factors and extracellular matrix components) and nucleotides, can act on cell membrane receptors, while others, such as steroids, can diffuse through the cell membrane and interact directly with intracellular receptors, inducing activation of signaling pathways. *Electrical signals* are action potentials, or changes in membrane potentials. An electrical signal initiates the activation of nerve and muscular cells. *Physical factors*, such as mechanical forces and deformation, can serve as signaling factors. For instance, mechanical shearing and stretching forces due to bloodflow and pressure, respectively, induce various cellular activities, ranging from cell migration, proliferation to apoptosis, in the vascular system. These biochemical, electrical, and physical signals initiate and control specified cellular activities.

Intracellular signaling factors include proteins, lipids, steroids, and ions. These factors are also referred to as *regulatory factors*. In each cell, there exist a large number of proteins. Most of these protiens serve as signaling molecules. Common types of signaling proteins include cell membrane receptors, ion channels, protein kinases, protein phosphatases, and enzymes. Certain types of structural proteins, such as actin filaments, also play a role in signal transduction. A variety of lipid molecules in the cell membrane and endoplasmic reticulum serve as signaling factors. Ions, especially, calcium, play a critical role in signal transduction and the regulation of cellular activities. The roles of these signaling factors are discussed in the following sections.

Types of Cell Signaling

There are various cell signaling mechanisms. Based on the range of signal transduction, cell signaling events can be divided into long- and short-range events (Fig. 5.2). *Long-range signaling events* involve cells from different systems. These events are mediated by either hormones or synapses. Hormones are biochemical factors, which are synthesized by endocrine gland cells and released into blood. These factors regulate the activities of remote cells. This type of signaling is also known as *endocrine* signaling. Since a hormone is delivered via the bloodflow, it can reach almost all cells in the body. The specificity of hormones is dependent on the receptors on the target cells. *Synapses* are subcellular structures found at the junctions between different neurons and between neurons and muscular cells. In a synapse-related signaling event, signals from a presynaptic neuron are conducted to another neuron or a peripheral muscular cell via the propagation of electrical action potentials, which stimulate the release of neurotransmitters at the terminal of the presynaptic cell. The synapse in turn delivers the neurotransmitters to the postsynaptic target cell, initiating postsynaptic cell activation. Synaptic signaling events influence target cells with great precision. In contrast to long-range signaling, *short-range signaling events* involve cells within a local neighborhood. These events are mediated by regional chemical factors and mechanical forces. Changes in mechanical forces often induce the activation and release of chemical factors, which in turn transmit mechanical signals to the cell. Signaling events mediated by regional chemical factors are often referred to as *paracrine* signaling. Chemical factors for paracrine signaling are usually

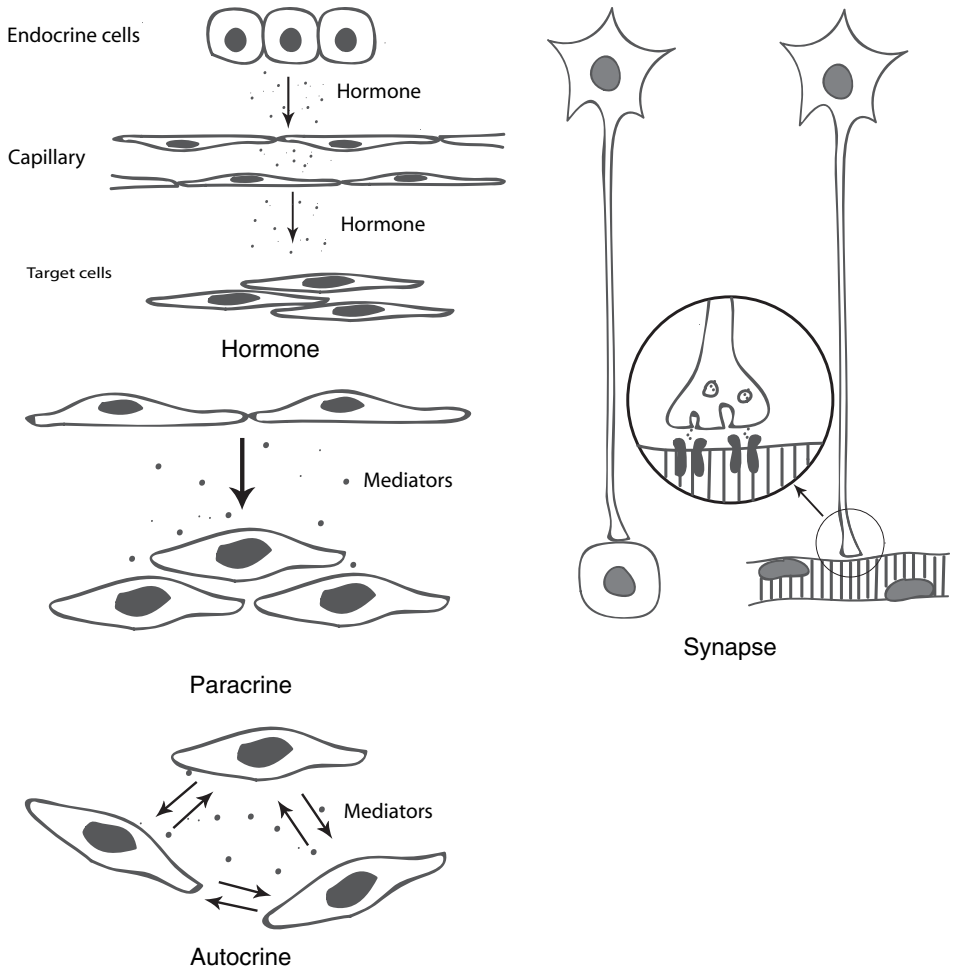


Figure 5.2. Long-range (hormone- and synapse-mediated) and short-range (paracrine and autocrine) cell signaling (based on bibliography 5.1).

endocytosed by neighboring cells or immobilized by extracellular matrix, ensuring a local influence without spreading to remote cells.

General Mechanisms of Cell Signaling

On the stimulation of an extracellular signal, a cell responds by activating its intracellular signaling pathways, leading to specific cellular activities, such as gene expression, cell division, cell migration, cell adhesion, and cell apoptosis. Several steps are involved in the activation of the intracellular signaling pathways: interaction of an extracellular signaling factor with a corresponding receptor or factor in the target cell, transduction of the extracellular signal to the target cell, activation of intracellular biochemical and/or electrical reactions, and termination of intracellular reactions.

A large number of molecules, including proteins, lipids, and ions, can serve as intracellular signaling molecules and participate in signal transduction. Proteins, including receptors, enzymes, and adapters, are among the common signaling molecules. Receptors are distributed in the cell membrane or cytoplasm, and are responsible for receiving extracellular signals and transmitting the signals into intracellular signaling pathways. Enzymes involved in cell signaling primarily include protein kinases, protein phosphatases, and GTPases. Protein kinases and phosphatases are responsible for protein phosphorylation and dephosphorylation, respectively, which are critical processes for cell signaling. GTPases serve as switches for signal transduction to downstream signaling elements. Adapter proteins help to target, recruit, and co-localize proteins. Some adapter proteins serve as scaffolds or docking sites for signaling proteins.

Signaling molecules exist in two modes: inactive and active. In unstimulated cells, signaling molecules are present mostly in the inactive mode. In response to a stimulation, specified signaling molecules can be activated by various mechanisms, including chemical modification, enzymatic activation, conformational changes in signaling molecules, alterations in molecular concentration, and col/localization and clustering of molecules. In most cases, multiple mechanisms are involved for the same or various signaling molecules at a given time and location.

There are several common features for cell signaling. These include signaling specificity, the involvement of signaling cascades, and crosstalk between signaling pathways. In general, each signaling molecule reacts with specific upstream and downstream molecules, ensuring the induction of specific activities. Various signaling pathways are designed and developed for specific cellular activities. For the regulation of each cellular activity, multiple signaling molecules and pathways may be involved. In addition, each signaling molecule may exhibit multiple functions. The abundance and multifunctionality of signaling molecules may be a mechanism that ensures the accomplishment of cellular activities and functions.

Each signaling pathway is composed of a number of signaling molecules, which relay signals from extracellular space to the cytoplasm and nucleus. Such a cascade is also referred to as a *signaling cascade*. In each cell, there exist multiple signaling pathways. These pathways often communicate or crosstalk with each other via branching pathways, forming signaling networks. Through these crosstalk pathways, a signaling molecule can be activated by different upstream signaling molecules and can act on different downstream effectors. With such an approach, various upstream signals can be converged to a single signaling pathway, and one activated signaling molecule can initiate multiple downstream activities. In addition, signals in one pathway can influence signals in other pathways. Thus crosstalk is an effective approach for amplifying and controlling signals and facilitating signal transduction. In the following sections, common signaling pathways in mammalian cells are briefly reviewed.

PROTEIN TYROSINE KINASE-MEDIATED CELL SIGNALING [5.2]

Protein tyrosine kinases belong to the superfamily of protein kinases. *Protein kinases* are enzymes that catalyze the phosphorylation of, or the addition of a phosphate group to, a substrate protein. Protein phosphorylation is a major form of molecular modification, which mediates a variety of molecular activities, including enzymatic activation, cell signaling, gene transcription, activation of ion channels, and reorganization of cytoskeletal

proteins. Protein kinases represent a large family of signaling molecules. The genes encoding protein kinases constitute about 1.7% of total genes in the human. Based on the types of target amino acid, protein kinases are classified into four types: serine/threonine-, tyrosine-, histidine-, and aspartate- (or glutamate-) specific protein kinases. These protein kinases catalyze the phosphorylation of substrate proteins on serine/threonine, tyrosine, histidine, and aspartate (or glutamate), respectively. Among these protein kinases, serine/threonine and tyrosine protein kinases can phosphorylate signaling proteins, play critical roles in signal transduction. Thus, these two types of protein kinase are the focus of this book. The protein tyrosine kinases are covered in this section. The protein serine/threonine kinases are covered later in this chapter.

Structure and Function

Protein tyrosine kinases are enzymes that catalyze the phosphorylation of the tyrosine residues of substrate proteins. A protein tyrosine kinase is often found as an integral part of growth factor receptors in the cell membrane and is referred to as *receptor protein tyrosine kinase*. A receptor with a protein tyrosine kinase is known as *protein tyrosine kinase receptor*. Such a receptor is composed of an extracellular domain for ligand binding, a transmembrane domain for anchoring the receptor to the cell membrane, and an intracellular domain for interaction with cytoplasmic signaling molecules. The receptor protein tyrosine kinase is localized to the cytoplasmic domain of the growth factor receptor. The receptor tyrosine kinase assists the growth factor receptor in transducing signals that induce essential cell activities, such as proliferation, differentiation, and migration. There are also nonreceptor protein tyrosine kinases such as the Src family of protein tyrosine kinases. These kinases are discussed on page 180.

A signaling pathway mediated by a protein tyrosine kinase receptor is composed of several components, including the extracellular ligands, cell membrane receptors, cytoplasmic signaling elements, and transcriptional factors. Extracellular ligands are usually growth factors produced and released by cells. There are a number of growth factors that interact and activate protein tyrosine kinase receptors. These include epidermal growth factor (EGF), platelet-derived growth factors (PDGFs) A and B, nerve growth factor (NGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (ILGF), nerve growth factor (NGF), hepatocyte growth factor (HGF), and ephrins. Based on the type of ligand growth factors, growth factor receptors are classified as EGF receptor, PDGF receptor, NGF receptor, FGF receptor, VEGF receptor, insulin receptor, HGF receptor, and Eph receptor groups, respectively (see Fig. 5.3 for schematic representation of growth factor receptors). The ligand–receptor interaction is highly specific. Each growth factor only interacts with and activates a specific protein tyrosine kinase receptor.

The EGFR group is composed of several receptors, including EGFR (epidermal growth factor receptor), ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 or neuro/glioblastoma-derived oncogene homolog), ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3), and ERBB4 (v-erb-a erythroblastic leukemia viral oncogene homolog 4). These receptors are expressed in various cell types, including epithelial, mesenchymal, and neural cells. The EGFR contains two extracellular cysteine-rich domains, a transmembrane domain, and a cytoplasmic tyrosine kinase domain (Fig. 5.3). This receptor interacts with EGF and similar ligands, regulating cell development, morphogenesis, regeneration, and tumorigenesis.

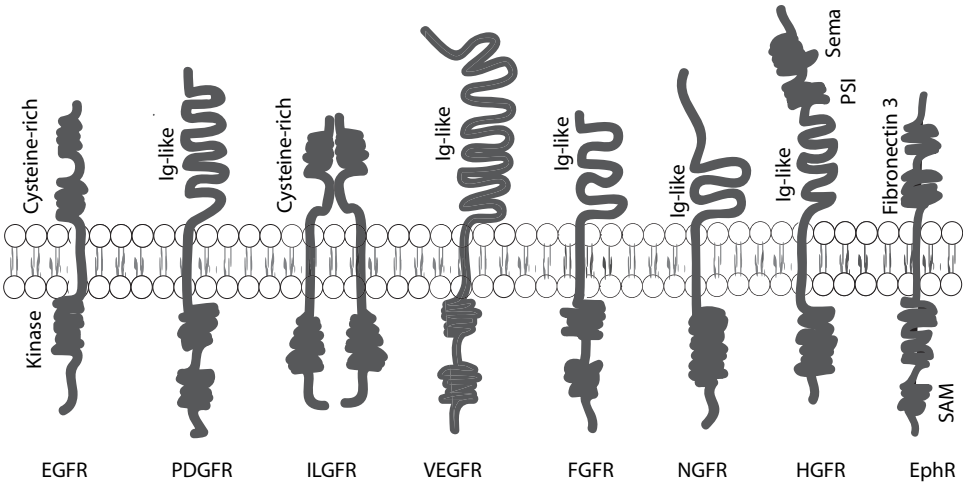


Figure 5.3. Schematic representation of growth factor receptors. Sema: semaphorin domain. Ig: immunoglobulin domain. PSI: plexin, semaphorin, and integrin domain. SAM: sterile alpha motif (based on bibliography 5.2).

The PDGFR group consists of several molecules, including PDGFRα, PDGFRβ, CSF1R, KIT/SCFR, FLK2, and FLT3. Each of these receptors is composed of five IgD-like domains in the extracellular domains (Fig. 5.3). The intrinsic protein tyrosine kinase domain is split into two parts by an intervening segment. These receptors play a critical role in regulating the development of connective tissue cells and vascular smooth muscle cells.

The insulin receptor group includes three members: insulin receptor, insulin growth factor (IGF)1 receptor, and IRR, which are present in the form of dimers linked by disulfide bonds (Fig. 5.3). These receptors regulate processes related to cell survival.

The VEGFR group includes three members, including VEGFR1, VEGFR2, and VEGFR3, which are characterized by the presence of seven Ig-like domains in the extracellular domain (Fig. 5.3). These receptors are expressed primarily in vascular endothelial cells and regulate the development of endothelial cells, as well as angiogenesis and vasculogenesis.

The FGFR group consists of FGFR1, FGFR2, FGFR3, and FGFR4. The extracellular region contains three Ig-like domains (Fig. 5.3). These receptors mediate the development and morphogenesis of a variety of cell types in connective tissues and the cardiovascular system.

The nerve growth factor receptor group is composed of three members, including TrkA, TrkB, and TrkC. The extracellular domain of these receptors contains a LRD domain and two Ig-like domains, and the intracellular region contains a single protein tyrosine kinase domain (Fig. 5.3). These receptors are expressed in neurons and neural glial cells and play important roles in regulating the development and morphogenesis of nerve cells.

The HGFR group is composed of two members: Met and Ron. These receptors are expressed primarily in hepatocytes and also in other epithelial cells. The extracellular domain of the receptor is composed of a Sema (semaphorin) domain, a PSI (plexin, semaphorin, and integrin) domain, and four Ig-like domains and the intracellular domain contains a single-protein tyrosine kinase domain (Fig. 5.3). HGF receptors regulate the development and regeneration of the liver and other epithelial tissues.

The Eph receptor group includes at least 14 members, EPHA1 (8 members) and EPHB1 (6 members), and interacts with various types of ephrin. A typical Eph receptor contains two fibronectin 3 domains in the extracellular region and a tyrosine kinase domain in the cytoplasmic region. The cytoplasmic region also contains a SAM (sterile α motif) (Fig. 5.3). These receptors are expressed primarily in nerve cells and vascular endothelial cells, and play a critical role in the regulation of cell migration and morphogenesis.

Most receptors listed above interact with intracellular signaling molecules, including Src homology 2 domain-containing molecules and adapter proteins, leading to activation of a mitogenic signaling cascade composed of mitogen-activated protein kinase kinase kinases (MAPKKKs), mitogen-activated protein kinase kinases (MAPKKs), and mitogen-activated protein kinases (MAPKs). These molecules regulate cell proliferation, differentiation, and migration.

Signaling Mechanisms

The activation of protein tyrosine kinase receptor signaling pathways begins with the binding of extracellular ligands to the protein tyrosine kinase receptors (Fig. 5.4). These receptors undergo a dimerization process on the interaction with ligands, a common mechanism for activating the protein tyrosine kinase receptor signaling pathways. For various groups of protein tyrosine kinase receptors, there exist different forms of dimerization. For example, PDGF receptors are dimerized into a symmetric structure on the binding of a disulfide-bonded PDGF dimeric complex (Fig. 5.4). In contrast, EGF receptors undergo conformational changes in response to EGF binding, forming receptor–receptor complexes. The dimerization of receptors brings the intracellular domains of the two receptors together, a critical process for initiating autophosphorylation of the protein tyrosine kinase domains on tyrosine residues.

The autophosphorylation of a receptor protein tyrosine kinase activates the tyrosine kinase domain, leading to the activation of downstream signaling molecules, sucs as Src homology (SH)2 domain-containing protein tyrosine kinases and adapter proteins.

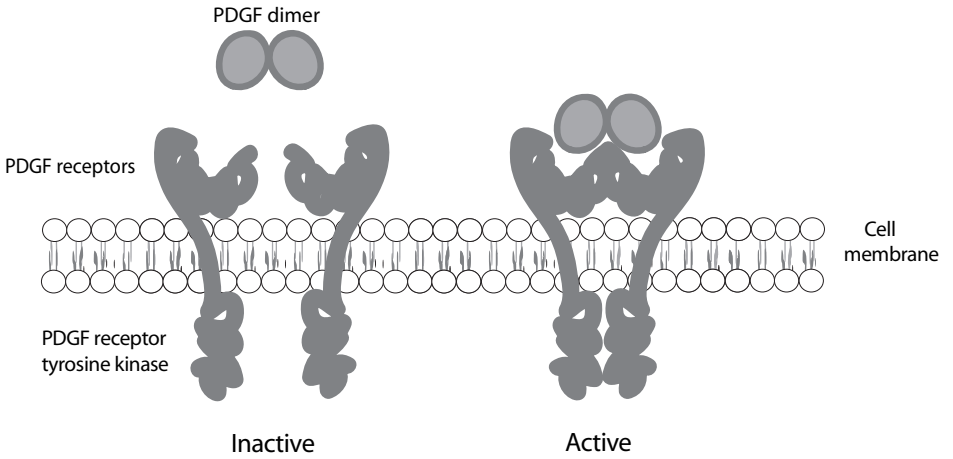


Figure 5.4. Schematic representation of the interaction of platelet-derived growth factor (PDGF) with PDGF receptor. A PDGF molecule forms a dimer with another PDGF molecule (e.g., PDGF-AA and PDGF-BB). A PDGF dimer can interact with two PDGF receptors, causing crosslink between the two PDGF receptors and autophosphorylation of the receptor protein tyrosine kinase in the cytoplasmic domain (based on bibliography 5.2).

A typical example of SH2 domain-containing kinases is the Src protein tyrosine kinase. The molecule possesses intrinsic enzymatic kinase activities and can induce phosphorylation on substrate proteins. *Adapter proteins* are proteins that serve as linkers between receptor protein kinases and downstream signaling molecules. Typical examples of adapter proteins include Grb2/sos, Crk, Nck, and Shc (these and other examples are listed in Table 5.1). These molecules do not possess intrinsic enzymatic kinase activities.

The interaction of an autophosphorylated receptor tyrosine kinase with a SH2 domain-containing kinase or an adapter protein is a critical process for signal transduction. A phosphorylated tyrosine residue in the receptor tyrosine kinase can serve as a docking site for the SH2 domain of signaling molecules, which is organized into a pocket-like structure specific for interaction with a phosphorylated tyrosine. Each receptor tyrosine kinase is capable of interacting with multiple SH2 domain-containing molecules at various tyrosine sites. For example, the PDGF receptor tyrosine kinase contains about 12 tyrosine residues that can be phosphorylated within the intracellular domain (11 are outside and 1 is within the tyrosine kinase). Some of these phosphorylated tyrosine residues serve as docking sites for SH2 domain-containing tyrosine kinases, such as Src, GAP, SHP2, and PLCγ, and others serve as docking sites for adapter signaling proteins, such as Shc, Nck, and Grb2/Sos. The phosphorylated tyrosine at site 581 (pY581) can interact with Src and Shc, pY740 can interact with Shc and PI3-kinase, pY751 can interact with PI3-kinase and Nck, and pY771 can interact with GAP and Shc. The interaction of the tyrosine kinase domain with downstream SH2 domain-containing molecules activates downstream signaling molecules, leading to activation of mitogenic cellular activities, such as cell proliferation, differentiation, and migration. (See Table 5.2.)

Although different tyrosine kinase receptors are present in the cell membrane and responsible for transducing distinct extracellular signals into the cell, there are common mechanisms of action. Here, the PDGF receptor is used as an example to demonstrate how the protein tyrosine kinase transduces PDGF signals into intracellular signaling pathways (Fig. 5.5). On the binding of PDGF ligands, PDGF receptors undergo dimerization, inducing autophosphorylation of the PDGF receptor tyrosine kinase domain. The activated tyrosine kinase domain recruits the adapter protein complex Grb2/Sos to the pY 716 site of the protein tyrosine kinase. Grb2/Sos activates the Ras protein by stimulating the substitution of GTP for GDP in the Ras protein. Activated Ras induces the activation of at least two cascades of signaling molecules, including the extracellular signal-regulated protein kinase (ERK)1/2 cascade and the c-Jun *N*-terminal kinase/stress-activated protein kinase (JNK/SAPK) cascade. Both pathways are collectively known as the *mitogen-activated protein kinase* (MAPK) pathways.

For the ERK1/2 pathway, Ras activates several protein kinases including Raf-1, A-Raf, and B-Raf, members of the mitogen-activated protein kinase kinase kinase (MAPKKK) family. The Raf kinases phosphorylate MAPK/ERK kinase (MEK)1/2, members of the MAPKK family. MEK1/2 in turn phosphorylates the tyrosine and threonine residues of ERK1/2, which is a protein complex belonging to the MAPK family. Activated ERK1/2 can translocate from the cytoplasm to the nucleus, where it activates transcriptional factors such as c-Fos, cAMP response element binding protein (CREB), early growth response (Egr)1, and Elk1, initiating the expression of mitogenic genes.

For the JNK/SAPK pathway, Ras can activate MEK kinase (MEKK) 1, 2, and 3, which are members of the MAPKKK family, possibly via the mediation of Rac/Cdc42 and p21-activated protein kinase (PAK). Activated MEKK 1, 2, and 3 phosphorylate MEK4, a member of the MAPKK family. MEK4 in turn phosphorylates JNK/SAPKs, which belong

TABLE 5.1. Characteristics of Selected Signaling Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Src	Avian sarcoma virus protein (ASV), p60-Src, tyrosine kinase pp60c-src, tyrosine protein kinase SRC-1, protooncogene SRC	536	60	Ubiquitous	A protein tyrosine kinase that regulates cell survival, proliferation, migration, and cell–cell communication
Grb2	Growth factor receptor-bound protein 2, abundant Src homology protein	217	25	Ubiquitous	Containing a SH2 domain and two SH3 domains[a] interacting with growth factor receptors, serving as an adaptor protein, and mediating the transduction of mitogenic signals, such as growth factors
Sos	"Son of sevenless" *Drosophila* homolog 1, SOS1 guanine nucleotide exchange factor	1333	152	Ubiquitous	A guanine nucleotide exchange factor that binds to Grb2 and activates the Ras protein
Crk	v-crk sarcoma virus CT10 oncogene homolog, oncogene Crk	304	34	Lung, kidney	An adapter protein that binds to tyrosine-phosphorylated proteins via interaction with phosphotyrosine residues, and mediating intracellular signal transduction
Nck	Noncatalytic region of tyrosine kinase, NCK1, NCK α, NCK adaptor protein 1, cytoplasmic protein NCK1, SH2/SH3 adaptor protein NCK α	377	43	Ubiquitous	An adaptor protein that transduces signals from receptor tyrosine kinases to downstream signal recipients, such as the Ras protein
Shc	p66, Src homology 2 domain containing (SHC) transforming protein 1	583	63	Ubiquitous	Serving as an adaptor protein that mediates the activation of Ras proteins in response to stimulation of mitogenic factors

[a]Note that the SH2 domain binds tyrosine-phosphorylated sequences and the two SH3 domains bind to proline-rich regions of substrate proteins.
*Based on bibliography 5.2.

TABLE 5.2. Characteristics of Selected Signaling Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
GAP	GTPase-activating protein (GAP), guanosine triphosphatase-activating protein, Ras p21 protein activator 1 (RASA1), p120GAP, RasGAP	1047	116	Ubiquitous	A GTPase-activating protein that activates the GTPase of the p21 Ras protein, induces conversion of GTP to GDP on Ras protein, and thus suppresses Ras activity, resulting in inhibition of cell mitogenic activities
SHP2	SH2 containing protein tyrosine phosphatase 2, protein tyrosine phosphatase nonreceptor type 11 (PTPN11), protein tyrosine phosphatase 2C (PTP2C), tyrosine phosphatase SHP2, PTP-1D, SHPTP2	593	68	Ubiquitous	A molecule of protein tyrosine phosphatase family, regulating cell proliferation and migration
Phospholipase Cγ	PLC γ1, PLC1, PLCG1, PLC148	1290	149	Ubiquitous	Catalyzing the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate, an important process for G-protein receptor- and Ras-mediated cell signaling

TABLE 5.2. *Continued*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Phosphatidylinositol 3-kinase catalytic subunit α	PI3 kinase α, PI3K α, PIK3CA, p110 α, PI3 kinase p110 subunit α	1068	124	Ubiquitous	Serving as catalytic subunit of PI3 kinase, which is composed of a regulatory subunit and a catalytic subunit, inducing phosphorylation of phosphatidylinositol (PtdIns), PtdIns4P, and PtdIns(4,5)P2 on the 3-hydroxyl group of the inositol ring, regulating cell proliferation and differentiation, and contributing to development of cancers
Phosphatidylinositol 3-kinase catalytic subunit β	PI3K β, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit β isoform, phosphoinositide 3-kinase catalytic β polypeptide, phosphatidylinositol 3-kinase catalytic 110-kDa β, p110-β, PI3-kinase p110 subunit β	1070	123	Ubiquitous	Same as PI3 kinase α
Phosphatidylinositol 3-kinase catalytic subunit γ	PI3K γ, phosphatidylinositol 3 kinase catalytic subunit γ isoform, phosphatidylinositol 3 kinase catalytic 110kDa γ, p110 γ, PI3 kinase p110 subunit γ	1102	126	Heart, liver, skeletal muscle, pancreas, leukocytes	In addition to functions as described for PI3 kinase α, PI3 kinase γ regulates inflammatory reactions

*Based on bibliography 5.2.

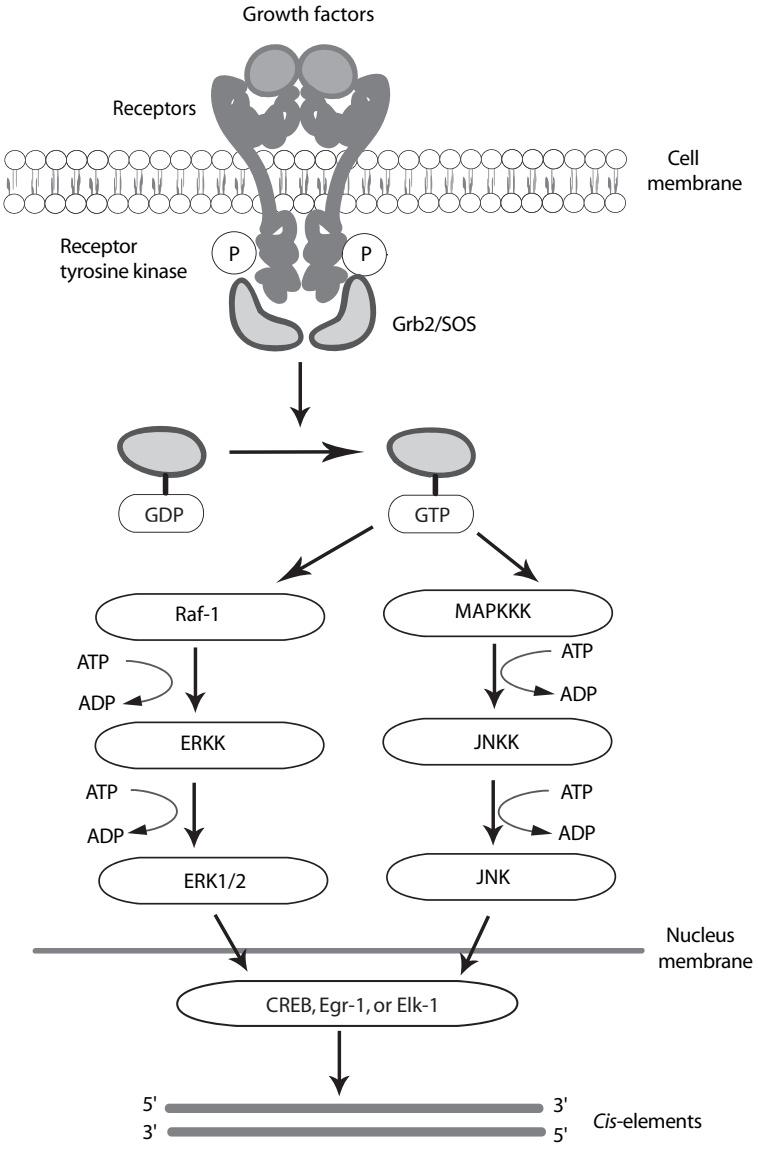


Figure 5.5. Schematic representation of protein tyrosine kinase receptor-mediated cell signaling (based on bibliography 5.2).

to the MAPK family. Activated JNK/SAPKs can translocate from the cytoplasm to the cell nucleus and activate transcriptional factors c-Jun, activating transcription factor (ATF)2, and Elk1. These transcriptional factors interact with corresponding cis-elements in target genes, initiating mitogenic mRNA transcription.

It is important to note that, in addition to the stimulatory effect on transcriptional factors as described above, c-Fos activated by ERK1/2 and c-Jun activated by JNK/SAPKs

TABLE 5.3. Characteristics of Selected Signaling Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
PDGF receptor β	Platelet-derived growth factor receptor β, PDGFRB	1106	124	Blood vessel, kidney, pancreas, bone marrow	A cell membrane tyrosine kinase receptor that interacts with platelet-derived growth factors and regulates cell survival, proliferation, differentiation, and migration
H-Ras	Harvey murine sarcoma virus oncogene, HRAS, RASH1, HRSP, C-H-RAS	189	21	Lung, intestine, stomach, and thyroid gland	A protein homologous to the product of transforming gene of Harvey murine sarcoma virus, mediating mitogenic signal transduction, and contributing to carcinogenesis when overexpressed
K-Ras	Kirsten murine sarcoma virus 2, RASK2, oncogene KRAS2, V KI RAS2 Kirsten rat sarcoma 2 viral oncogene homolog, CK RAS, cK Ras protein, K Ras p21 protein, c-Kirsten Ras protein, transforming protein p21	189	21	Lung, liver, intestine, leukocytes	A protein homologous to product of transforming gene of Kirsten murine sarcoma virus, mediating mitogenic signal transduction, and contributing to carcinogenesis when mutated
Raf-1	Oncogene RAF1, c-raf, v-raf-1 murine leukemia viral oncogene homolog 1	648	73	Widely expressed	A MAP kinase kinase kinase phosphorylating MEK1 and MEK2, which in turn phosphorylates the serine/threonine-specific protein kinases ERK1/2 and regulates cell survival, proliferation, and differentiation

MEK1	MAPK/ERK kinase 1, mitogen-activated protein kinase kinase 1 (MAPKK1, MAP2K1, MKK1, MAP kinase kinase 1), dual-specificity mitogen-activated protein kinase kinase 1, ERK activator kinase 1, mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (ERK) kinase 1, MAPK/ERK kinase 1 (MEK1)	393	43	Brain, heart, lung, blood vessel, liver, kidney, spleen, intestine, thymus, skeletal muscle	A serine/threonine kinase serving as an element for the ERK1/2 signaling pathway, activating mitogen-activated protein kinases including ERK1/2, and regulating cell survival, development, proliferation, and differentiation
MEK2	MAPK/ERK kinase 2, MAP kinase/Erk kinase 2, MAP2K2, MKK2, MAPKK2, MAPK/ERK kinase 2, ERK activator kinase 2, mitogen-activated protein kinase kinase 2	400	44	Neutrophils	Similar to function of MEK1
MEK3	MAPK/ERK kinase 3, mitogen-activated protein kinase kinase 3, MAPKK3, MAP2K3, MKK3, and protein kinase mitogen-activated kinase 3	352	40	Heart, blood vessel, lung, liver, kidney, spleen, pancreas. Intestine, thymus, prostate gland, ovary	Similar to function of MEK1, and activating MAPK14/p38-MAPK
MEK4	MAPK/ERK kinase 4, mitogen-activated protein kinase kinase 4, SAPK/ERK kinase 1, MAPKK4, MAP2K4, MKK4, JNK-activated kinase 1, and JNKK1	399	44	Skeletal muscle	Similar to the function of MEK1, activating MAPK8/JNK1, MAPK9/JNK2, and MAPK14/p38
Extracellular signal-regulated kinasel	ERK1/2, mitogen-activated protein 3 (MAPK3), p44ERK1, p44MAPK	379	44	Ubiquitous	A serine, threonine, and tyrosine kinase that forms a complex with ERK2 and regulates cell survival, proliferation, and differentiation

TABLE 5.3. *Continued*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Extracellular signal-regulated kinase 2	ERK2, mitogen-activated protein kinase 1, mitogen-activated protein kinase 2 (MAPK2), protein kinase mitogen-activated 1, protein kinase mitogen-activated 2, protein tyrosine kinase ERK2, p42MAPK, p41MAPK	360	42	Ubiquitous	A serine, threonine, and tyrosine kinase that forms a complex with ERK1 and regulates cell survival, proliferation, and differentiation
c-Fos	Oncogene FOS, FOS, and v-fos FBJ murine osteosarcoma viral oncogene homolog	380	41	Ubiquitous	Forming heterodimeric transcriptional factor complexes, known as activating protein (AP)-1, with proteins of the c-Jun family, and regulating cell survival, development, proliferation, differentiation, and transformation
cAMP response element-binding protein	CREB, CREB1, cAMP response element-binding protein 1, transactivator protein	341	37	Brain, heart, thymus, adrenal gland	A transcriptional factor belonging to the leucine zipper family of DNA-binding proteins, binding to the cAMP-responsive element of target genes, regulating cell survival, proliferation, and differentiation, and regulating hepatic gluconeogenesis
early growth response 1	Egr-1, nerve growth factor-induced protein A, zinc finger protein 225	543	58	Nervous system, cartilage, blood vessel, liver, stomach, leukocytes	Serving as a transcriptional factor that binds to mitogenic genes, regulating cell proliferation, differentiation, and apoptosis
Elk-1	Transforming protein elk-1	428	45	Ubiquitous	Acting as a transcriptional factor that is a target of ERK1/2 in the cell nucleus, and binding to and activating the serum response element in the promoter region of the c-fos gene

MEK kinase 1	MEKK1				
MEK kinase 2	MEKK2, mitogen-activated protein kinase kinase kinase 2 (MAPKKK2), MAPK/ERK kinase kinase 2, MEK kinase 2	620	70	Ubiquitous	Acting as a serine/threonine protein kinase, phosphorylating MAPK7 and MAP2K4, and regulating cell proliferation, differentiation, and apoptosis
MEK kinase 3	MEKK3, mitogen-activated kinase kinase kinase 3, MAP/ERK kinase kinase 3, MAPKKK3, and MAP3K3	626	71	Ubiquitous	Activating the stress-activated protein kinase (SAPK) and extracellular signal-regulated protein kinase (ERK) pathways; also regulating cell development and growth
Rac1	Ras-related C3 botulinum toxin substrate 1, ρ family small GTP-binding protein Rac1, p21-Rac1	192	21	Ubiquitous	Acting as a Ras superfamily GTPase, and regulating cell proliferation, differentiation, and cytoskeletal reorganization
Cdc42	Cell division cycle 42, GTP-binding protein, 25 kDa, G25K	191	21	Ubiquitous	A small GTPase of the ρ subfamily, regulating cell development, morphology, polarity, migration, transformation, and endocytosis
p21-activated protein kinase	PAK, p21/CDC42/RAC1-activated kinase 1, p21-activated kinase 1, serine/threonine kinase PAK 1, P65 PAK, and α PAK	553	62	Ubiquitous	Serving as a target for the small GTP-binding proteins Cdc42 and Rac; also regulating cytoskeleton reorganization and cell motility

TABLE 5.3. *Continued*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
JNK1/SAPK	c-Jun kinase 1, c-Jun *N*-terminal kinase 1, JNK1 α protein kinase, protein kinase JNK1, mitogen-activated protein kinase 8 (MAPK8), stress-activated protein kinase (SAPK)	427	48	Ubiquitous	Acting as a serine/threonine kinase and key element for MAP kinase signaling pathways, mediating the expression of immediate–early genes in response to cell stimulation, regulating cell development, proliferation, differentiation, migration, and transcription; also regulating UV radiation- and TNF α-induced apoptosis
c-Jun	Jun, protooncogene c Jun, v-jun avian sarcoma virus 17 oncogene homolog	331	36	Ubiquitous	Forming heterodimeric transcriptional factor complexes, known as activating protein (AP)-1, with proteins of the c-Fos family; also regulating cell survival, development, proliferation, differentiation, and apoptosis
Activating transcription factor 2	ATF2, cAMP response element-binding protein 2 (CREB2), cyclic AMP-dependent transcription factor ATF 2	505	55	Brain, uterus, lymphocytes	Acting as a transcriptional factor, forming a homodimer or heterodimer with c-Jun, binding to the cAMP-responsive element (CRE) of target genes, stimulating CRE-dependent transcription, and serving as a histone acetyltransferase (HAT) that acetylates histones H2B and H4 and activating transcription by modulating chromatin components

*Based on bibliography 5.2.

can form heterodimers and homodimers, known as activating proteins (AP)1. AP1 serves as a transcriptional factor, which interacts with AP1-specific *cis* elements and regulates the expression of mitogen genes. In addition to the ERK1/2 and JNK/SAPK pathways, there are several other pathways, which transduce signals from protein tyrosine kinase receptors. These include the p38 MAPK, ERK3, ERK5, and ERK6 pathways. Although different signaling molecules are involved, these pathways follow hierarchical orders similar to the ERK1/2 and JNK/SAPK pathways. (See Table 5.3.)

After extracellular ligand signals are transduced into the cell via corresponding protein tyrosine kinase receptors, the ligand–receptor complexes are clustered and internalized via endocytosis, resulting in the formation of endosomes. Within the endosomes, the ligands are dissociated from the receptors. The dimeric receptors are also split into monomers. The receptor tyrosine kinases are dephosphorylated by phosphatases. Monomeric receptors are recycled back to the cell membrane for further use.

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NONRECEPTOR TYROSINE KINASE-MEDIATED CELL SIGNALING

Structure and Function [5.3]

Nonreceptor tyrosine kinases belong to a group of cytoplasmic tyrosine kinases that are attached to the cell membrane but are not intrinsic kinases of any cell membrane receptors. The Src family protein kinases are typical nonreceptor tyrosine kinases that have been well characterized. The Src protein was originally discovered in the Rous sarcoma retrovirus by Dr. Peyton Rous in 1911, for which Dr. Rous won the Nobel Prize in 1966. The viral Src is referred to as *v-Src*, which is responsible for the induction of mesodermal cancers. Further work has demonstrated that a gene similar to the v-Src gene exists in chickens and mammals. The protein encoded by this gene is defined as *c-Src* (cellular Src) in chicken and mammalian cells. In normal cells, the c-Src protein has been shown to regulate cell proliferation and differentiation, contributing to the control of morphogenesis during development. Investigations with molecular approaches have revealed a number of protein tyrosine kinases that are similar to Src in structure and function. These include Yes, Fgr, Lck, Fyn, Yrk, Hck, Lyn, and Blk, which are defined as members of the Src family. Among these proteins, Src, Fyn, Yes, and Yrk are expressed in most cell types, whereas others are expressed primarily in hematopoietic cells. (See list in Table 5.4.)

The Src family proteins are characterized by the presence of several distinct domains, including an *N*-terminal domain with one or more acylation sites, a Src homology (SH)3 domain, a SH2 domain, a catalytic kinase domain, and a *C*-terminal regulatory domain with a tyrosine at location 527 (Tyr527). The *N*-terminal acylation sites anchor the protein to the cell membrane via a myristoyl group (Fig. 5.6), the SH3 and SH2 domains are capable of binding to praline-rich peptides and phosphorylated tyrosine residues, respectively, and the *C*-terminal tyrosine residue regulates the activity of the Src kinases.

Signaling Mechanisms [5.4]

In the quiescent state, the activity of the Src family tyrosine kinases is suppressed mostly as a result of phosphorylation of the *C*-terminal tyrosine residue Tyr527. The phosphorylation of Tyr527 is induced by the Csk kinase. The phosphorylated tyrosine (pTyr527) interacts with the SH2 domain of the same molecule. This interaction renders the SH2 domain of the Src kinase incapable of binding to a phosphorylated tyrosine from a stimulatory factor (Fig. 5.6).

A Src tyrosine kinase can be activated under several conditions. First, dephosphorylation of the *C*-terminal tyrosine (Tyr527) by a protein tyrosine phosphatase prevents the interaction of the *C*-terminal tyrosine with the SH2 domain and allows the access of a stimulatory factor to the SH2 domain of the Src tyrosine kinase, thus inducing Src activation. However, protein tyrosine phosphatases specific to the *C*-terminal tyrosine residue have not been identified. Second, the presence of proteins with phosphorylated tyrosine residues may competitively bind the SH2 domain of the Src tyrosine kinase, thus facilitating Src activation. A number of growth factor receptors, such insulin-like growth factor receptor, platelet-derived growth factor receptor, and epidermal growth factor receptor, contain tyrosine residues in their intracellular domains. Once activated by the binding of growth factors, these tyrosine residues can be autophosphorylated and can serve as docking sites for SH2 domain-containing Src kinases, recruiting the Src kinases to the phosphorylated tyrosine residues. The recruitment process activates Src kinases (Fig. 5.7).

TABLE 5.4. Characteristics of Selected Members of the Src Family*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Src	Avian sarcoma virus protein (ASV), p60-Src, tyrosine kinase pp60c-src, tyrosine protein kinase SRC-1, protooncogene SRC	536	60	Ubiquitous	A tyrosine kinase (the cellular homolog of the avian sarcoma virus protein) that regulates cell survival, proliferation, migration, and cell–cell communication
Yes	cellular yes-1 protein, Yamaguchi sarcoma oncogene, v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1, protooncogene tyrosine-protein kinase YES, and P61-YES	543	61	Nervous system, lung, liver, intestine, kidney, skeletal muscle, skin	Acting as a tyrosine kinase that is cellular homolog of Yamaguchi sarcoma virus oncogene product, and regulating cell survival, proliferation, and differentiation
Fgr	c-fgr, p55c-fgr, Gardner–Rasheed feline sarcoma viral oncogene homolog, oncogene FGR, and SRC2	529	59	Brain, lung, liver, kidney, leukocytes	Regulating cell survival, proliferation, and differentiation
Lck	T-cell-specific protein tyrosine kinase, lymphocyte-specific protein tyrosine kinase, P56-LCK, and oncogene LCK	509	58	Lymphocytes, lung, thymus, intestine, bone marrow	Regulating lymphocyte development and proliferation, mediating immune reactions, and contributing to development of lymphomas
Fyn	Src-like kinase, c-syn protooncogene, src/yes-related novel gene, and protooncogene tyrosine-protein kinase fyn	537	61	Nervous system, skin, lymphocytes	Binding to p85 subunit of phosphatidylinositol 3-kinase, mediating axon outgrowth, and regulating cell development, proliferation, and differentiation
Hck	Hemopoietic cell kinase, tyrosine protein kinase HCK	526	60	Bone marrow, blood cells, lung	A hematopoietic cell protein tyrosine kinase that regulates blood cell development, survival, proliferation, and apoptosis, and mediates leukocyte activation and migration
Lyn	v-yes-1 Yamaguchi sarcoma viral-related oncogene homolog, and oncogene LYN	512	59	Bone marrow, leukocytes, platelet, macrophage	Regulating cytoskeletal organization and remodeling in platelets, mediating lymphocyte proliferation and activation, and regulating immune reactions
Blk	B-lymphocyte tyrosine kinase, p55-BLK, and BLK nonreceptor tyrosine kinase	505	58	B lymphocytes, thymus, liver	Regulating development and activity of B lymphocytes

*Based on bibliography 5.3.

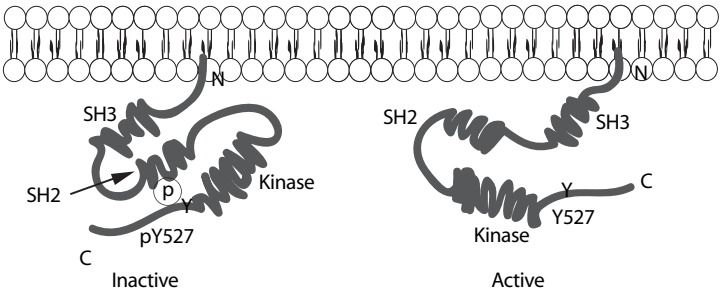

Figure 5.6. Schematic representation of the Src protein tyrosine kinase anchored to the cytoplasmic side of the cell membrane (based on bibliography 5.4).

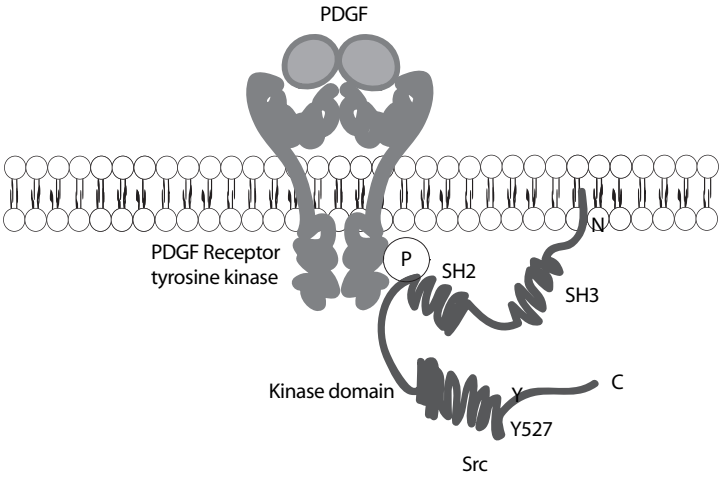

Figure 5.7. Activation of the Src protein tyrosine kinase (based on bibliography 5.4).

Previous investigations have shown that a treatment of quiescent fibroblasts with PDGF induces the activation of the Src tyrosine kinase, suggesting that the PDGF receptor may stimulate the phosphorylation of the Src kinase. Further investigations have demonstrated that several growth factor receptors, including the PDGF receptor, EGF receptor, basic FGF receptor, and colony-stimulating factor 1 receptor, are capable of interacting with the Src family tyrosine kinases, including Src, Fyn, and Yes. A Src kinase can interact with a growth factor receptor at specific sites of phosphotyrosine residues. For example, activated PDGF receptor can recruit Fyn to phosphotyrosines 579 and 581, which are located in the juxtamembrane region of the receptor. It is now clear that the formation of receptor–Src kinase complexes is a critical process for the activation of PDGF-induced cellular activities. Phosphorylated Src tyrosine kinases can activate a number of substrate proteins, including GTPase activating protein, focal adhesion kinase (FAK) (Table 5.5), and the adaptor protein Shc. These Src target proteins play critical roles in the regulation of mitogenic cellular activities.

The Src tyrosine kinases also play a role in signal transduction initiated from the extracellular matrix. Cells can interact with extracellular matrix components via integrins, a family of heterodimeric transmembrane receptors. Integrins can cluster with a number

TABLE 5.5. Characteristics of FAK*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Focal adhesion kinase	FAK, focal adhesion kinase 1 (FAK1), protein tyrosine kinase 2, and pp125FAK	1074	122	Ubiquitous	Found at cell focal adhesion contacts, interacting with membrane integrins, mediating cell adhesion to extracellular matrix, and regulating cell polarity, motility, and proliferation

*Based on bibliography 5.4.

of molecules, including talin, vinculin, actinin, paxillin, and focal adhesion kinase, forming a cell membrane structure known as the *focal adhesion contact*. Among the focal contact molecules, focal adhesion kinase plays a key role in regulating cell adhesion, migration, and proliferation. On interaction of integrins with extracellular matrix, focal adhesion kinase becomes autophosphorylated on a tyrosine residue, establishing a docking site for SH2 domain-containing proteins. Src and Fyn, which contain a SH2 domain, can bind to the phosphorylated tyrosine residue of focal adhesion kinase. Bound Src kinases can phosphorylate additional tyrosine residues in the focal adhesion kinase molecule, further activating focal adhesion kinase and creating docking sites for other SH2 domain-containing molecules. An important molecule that binds to focal adhesion kinase is the Grb2 adaptor protein. This molecule forms a complex with Sos, which activates the Ras–MAPK signaling pathway (see page 151). Thus, the Src tyrosine kinase signaling pathway is linked to the MAPK signaling pathway via focal adhesion kinase. These observations demonstrate that, through interactions with growth factor receptors and integrins, the Src family tyrosine kinases participate in the regulation of cell proliferation, adhesion, and migration.

SERINE/THREONINE KINASE-MEDIATED CELL SIGNALING

Serine/threonine protein kinases are enzymes that catalyze the transfer of the γ-phosphate of an ATP molecule to the OH group of the serine and threonine residues of substrate proteins. Protein serine/threonine kinases exist in inactive and active forms. In unstimulated cells, most protein kinases are inactive. On stimulation by specific signals, protein kinases can be activated by several mechanisms. These include activator binding, phosphorylation of activation sites, and dephosphorylation of inhibitory phosphates. There are a large number of serine/threonine protein kinases, among which receptor serine/threonine kinases, protein kinase A (cAMP-dependent protein kinase or PKA), and protein kinase C (Ca^{2+}-dependent protein kinase or PKC) have been extensively studied and well characterized. These kinases are used as examples in this section to demonstrate the mechanisms of serine/threonine kinase-mediated cell signaling.

Serine/Threonine Kinase Receptors [5.5]

Serine/threonine kinase receptors are transmembrane receptors that contain an intrinsic serine/threonine-specific kinase, which is located in the cytoplasmic domain of the receptors. These receptors can interact with several growth factors, including transforming growth factor (TGF)β, bone morphogenetic proteins, and activins. A typical serine/threonine receptor contains two subunits: receptor type I and type II. Both subunits are required for the activation of the receptor. An activated receptor serine/threonine kinase can lead to activation of a cascade of intracellular signaling molecules, including Smad 2, 3, and 4, and adaptor proteins. Smads are a family of proteins homologous to the products of the *Drosophila* gene *mothers against decapentaplegic* (*Mad*) and the C. elegans gene *Sma*. These proteins form complexes and serve as transcriptional factors and regulate the expression of target genes.

The signaling mechanisms of serine/threonine kinase receptors are similar among transforming growth factor (TGF)β, bone morphogenetic proteins, and activins. Here, the signal transduction pathway for TGFβ is used to demonstrate the signaling mechanisms (Fig. 5.8). TGFβ is capable of binding to the TGFβ receptor type II (serine/threonine

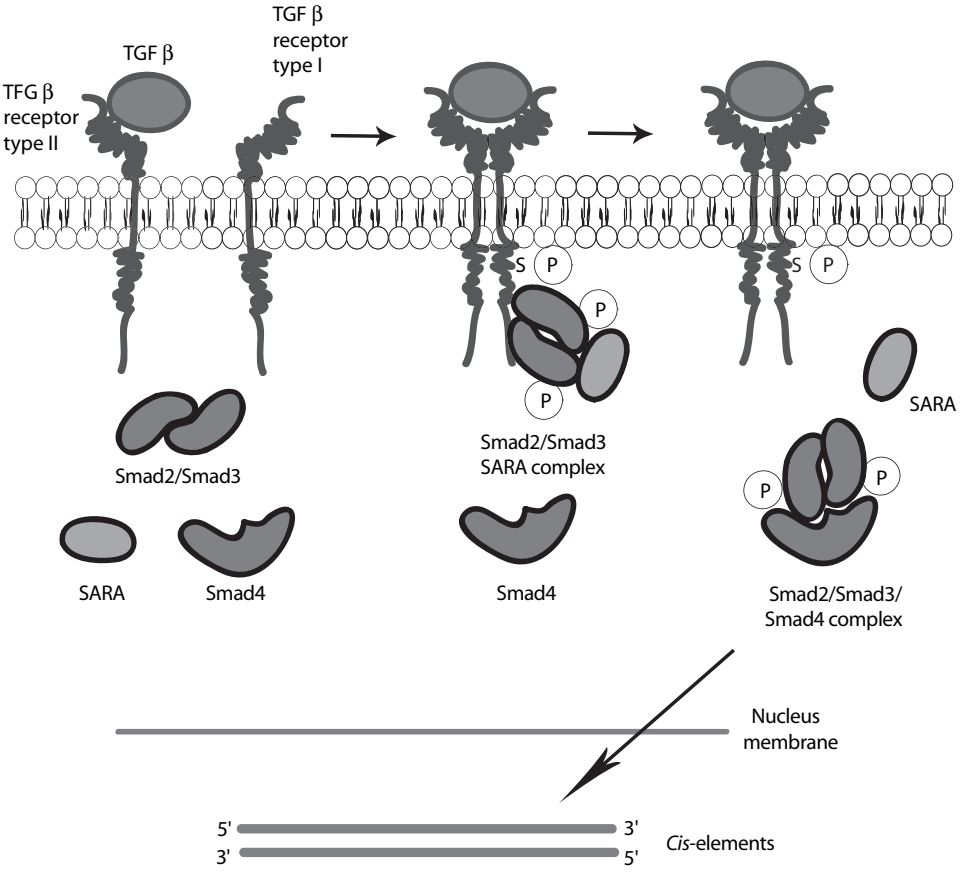

Figure 5.8. Schematic representation of transforming growth factor (TGF)-β-induced cell signaling (based on bibliography 5.5).

kinase receptor type II). The binding of TGFβ induces the association of the TGFβ receptor type I (serine/threonine kinase receptor type I) with the type II receptor, allowing the phosphorylation of the type I receptor by the type II receptor kinase. A complex of Smads, including Smad2 and Smad3, is activated in response to the activation of the TGFβ receptors and initiate interaction with an adapter protein, known as *SMAD anchor for receptor activation* (*SARA*), forming a protein complex. The complex of Smad2, Smad3, and SARA is recruited to TGFβ receptor type I, which in turn phosphorylates the Smad complex. Phosphorylated Smad complex then dissociates from the type I receptor and SARA, and binds to another Smad molecule, known as *Smad4*, which is a critical molecule mediating the translocation of the Smad complex. The Smad2/Smad3/Smad4 complex serves as a transcriptional factor, translocates from the cytoplasm to the cell nucleus, interacts with target genes, and induces gene expression. The TGFβ-activated Smad signaling pathways (see list in Table 5.6) negatively regulate cell proliferation and differentiation by activating inhibitors of cyclin-dependent kinases, in resulting cell cycle arrest in the G1 phase. Activation of the Smad signaling pathways also induces cell apoptosis.

Protein Kinase A [5.6]

Protein kinase A (PKA) (see list of isoforms in Table 5.7) is the primary target of cAMP and is involved in the regulation of sugar and lipid metabolism, ion channel activities, and nerve synaptic transduction. In unstimulated cells, protein kinase A exists in the form of tetramer, composed of two regulatory R subunits and two catalytic C subunits. The catalytic C subunits are masked by the R subunits. In response to stimulation, activated cAMP can bind to the R subunits, resulting in the dissociation of the tetramer into an R—R dimer, which is bound to four cAMP molecules, and two free C monomers. The C monomers are catalytically active and can phosphorylate transcription factors, such as CREB (cyclic AMP response element-binding protein). Phosphorylated transcriptional factors can translocate from the cytoplasm to the cell nucleus, bind to target gene promoters, and stimulate the transcription of target genes (Fig. 5.9).

In mammalian cells, there are four types of R-subunit isoforms, including RIα, RIβ, RIIα, and RIIβ, and three types of C isoforms, including Cα, Cβ, and Cγ. These isoforms mediate distinct biochemical activities, contributing to cell- and tissue-specific functions. The C isoforms contain serine/threonine phosphorylation sites. These sites can be autophosphorylated on stimulation. For all protein kinase A isoforms, there exist a consensus sequence, RRXSX, which catalyzes the phosphorylation of substrate proteins.

The activity of protein kinase A is regulated by several mechanisms. These include alterations in cAMP concentration, phosphorylation on the serine/threonine residues of protein kinase A, and binding of inhibitor proteins. It is important to note that the cAMP concentration is a primary factor that controls the activity of protein kinase A. An increase in cAMP concentration induces activation of protein kinase A. Phosphorylation of protein kinase A enhances the activity of the kinase, whereas the binding of inhibitor proteins exerts an opposite effect.

Protein Kinase C [5.7]

Protein kinase C (calcium-dependent protein kinase or PKC) (see list of isoforms in Table 5.8) is a critical signaling molecule, which is involved in the regulation of cellular activities, including cell proliferation, migration, apoptosis, and secretion. Protein kinase C can

TABLE 5.6. Characteristics of Selected Molecules for the TGFβ Receptor–Smad Signaling Pathway*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
TGF β	TGFB1, transforming growth factor β1	390	44	Lung, kidney, liver	Regulating cell proliferation, differentiation, transformation, and apoptosis; mediating inflammatory reactions, and acting as a negative regulator for certain cell types including vascular smooth muscle cells
TGF β receptor type I	TGFBR1, TGF β receptor 1, TGF-β type I receptor, activin receptor-like kinase 5, serine-threonine protein kinase receptor R4	503	56	Ubiquitous	Forming a heterogenic complex with TGF β receptor type II in response to the binding of TGF β, relaying the TGF β signal from cell surface to cytoplasm, and regulating cell proliferation, differentiation, and apoptosis
TGF β receptor type II	TGF β receptor 2, TGFBR2, and TGFR-2	567	65	Ubiquitous	Forming a heterogenic complex with TGF β receptor type I in response to TGF β binding, relaying the TGF β signal from cell surface to cytoplasm, and regulating cell proliferation, differentiation, and apoptosis
Smad2	SMAD mothers against decapentaplegic homolog 2 (*Drosophila*)	467	52	Fetus, bone, pancreas, ovary, intestine, prostate gland, and skin	Forming complexes with Smad3 and Smad4, serving as a transcriptional factor, mediating signal transduction initiated by TGF β, activin, and bone morphogenetic factors, and regulating cell proliferation, differentiation, and apoptosis
Smad3	SMAD mothers against decapentaplegic homolog 3 (*Drosophila*), SMA- and MAD-related protein 3	425	48	Fetus, bone, pancreas, ovary, intestine, prostate gland, skin	Similar to function of Smad2
Smad4	SMAD mothers against decapentaplegic homolog 4 (*Drosophila*), SMA- and MAD-related protein 4	552	60	Fetus, bone, pancreas, ovary, intestine, prostate gland, skin	Similar to function of Smad2

*Based on bibliography 5.5.

TABLE 5.7. Characteristics of Selected Protein Kinase A Isoforms*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Protein kinase A RIα	cAMP-dependent protein kinase regulatory type I α, CAMP-dependent protein kinase type I-α regulatory chain, and tissue-specific extinguisher 1	381	43	Heart, liver, lymphocytes	Forming a tetramer with other protein kinase A subunits, transducing the cAMP signal to target proteins by phosphorylation, and regulating cell metabolism and activities
Protein kinase A RIβ	cAMP-dependent protein kinase regulatory type I β, cAMP-dependent protein kinase type I β regulatory chain	381	43	T lymphocytes	Similar to functions of protein kinase A RIα̃
Protein kinase A RIIα	cAMP-dependent protein kinase regulatory type II α	404	46	T lymphocytes, testis	Similar to functions of protein kinase A RIα
Protein kinase A RIIβ	cAMP-dependent protein kinase regulatory type II β, RIIβ	418	46	T lymphocytes, testis	Similar to functions of protein kinase A RIα
Protein kinase A Cα	cAMP-dependent protein kinase α catalytic subunit, PKA C α, PKACA, and A kinase α	351	41	T lymphocytes, testis	Forming a tetramer with other protein kinase A subunits, transducing cAMP signal to target proteins by phosphorylation, and regulating cell metabolism and activities
Protein kinase A Cβ	cAMP-dependent protein kinase β catalytic subunit, PKA Cβ	398	46	Brain	Similar to functions of protein kinase A Cα
Protein kinase A Cγ	cAMP-dependent protein kinase γ catalytic subunit, PKA Cγ	351	40	Testis	Similar to functions of protein kinase A Cα

*Based on bibliography 5.6.

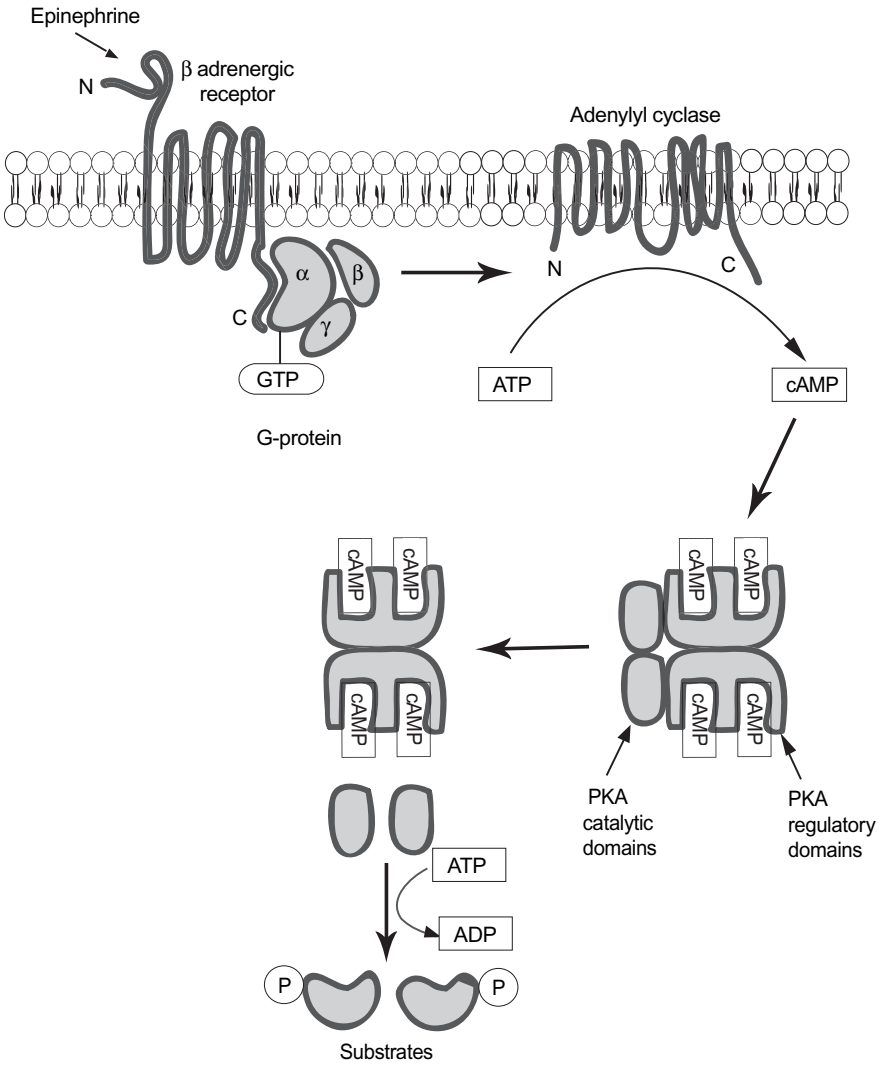

Figure 5.9. Schematic representation of activation of protein kinase A (PKA). Based on bibliography 5.6.

be activated by signals from the receptor protein tyrosine kinase pathways and the G-protein-linked receptor pathways. Protein kinase C represents a family of at least 12 subtypes of protein kinase, including PKCα, β_I, β_{II}, γ, δ, ε, η, θ, ζ, ι, μ, and ν. These subtypes exhibit different distributions in tissues. The α, δ, and ζ subtypes are widely distributed among almost all tissues, whereas others are found in specialized tissues.

Various subtypes of protein kinase C exhibit different characteristics in regulatory mechanisms. Some subtypes, such as PKCα, β_I, β_{II}, and γ, can be activated by diacylglycerol and Ca^{2+}, whereas others such as PKCζ and PKCι cannot be activated by these factors. A common feature for most PKC subtypes is the affinity and responsiveness to phorbol esters, which induce PKC activation. It is thought that tumor promoting esters, such as tetradecanoyl phorbol acetate (TPA), stimulate cell proliferation via the activation of PKC.

TABLE 5.8. Characteristics of Selected Protein Kinase C Isoforms*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Protein kinase Cα	PKCα	672	77	Pancreas, intestine, skeletal muscle	A serine/threonine-specific protein kinase that can be activated by calcium and diacylglycerol, inducing phosphorylation of target proteins, regulating cell proliferation, migration, and transformation; also mediating cardiac contractility
Protein kinase Cβ	PKCβ, PKCB, PKC beta	673	77	Widely expressed	Similar to functions of PKCα
Protein kinase Cγ	PKCγ	697	78	Nervous system	Similar to functions of PKCα

*Based on bibliography 5.7.

The activity of PKC is mediated by several mechanisms, including phosphorylation, cell membrane association, and Ca^{2+} and diacylglycerol binding (see page 217). Protein kinase C can be phosphorylated on the serine/threonine and tyrosine residues, a critical process inducing PKC activation. Such a process can be catalyzed by phosphoinositide-dependent protein kinases. PKC association with cell membrane is another approach for inducing PKC activation. Such a process is mediated by Ca^{2+} and diacylglycerol. Binding of Ca^{2+} and diacylglycerol to PKC promotes PKC association to cell membrane and thus activates PKC.

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PROTEIN PHOSPHATASE-MEDIATED CELL SIGNALING

Cellular activities are often regulated by counterbalanced stimulatory and inhibitory mechanisms. Protein phosphorylation and dephosphorylation are two typical mechanisms that control the activation and suppression of signaling molecules. These two processes are regulated by protein kinases and protein phosphatases, respectively. As discussed on page 151–190, the phosphorylation of mitogenic signaling proteins by protein kinases initiates and enhances cellular activities such as proliferation and migration. In contrast, the dephosphorylation of mitogenic signaling proteins by protein phosphatases elicits inhibitory effects on cellular activities. The stimulatory effects mediated by protein kinases and the inhibitory effect mediated by protein phosphatases coordinately control the mitogenic activities of cells.

Protein phosphatases are classified into three groups based on the type and specificity of substrates: protein serine/threonine phosphatases, protein tyrosine phosphatases, and dual-specificity protein phosphatases. All these enzymes reverse the action of protein kinases. *Protein serine/threonine phosphatases* induce hydrolysis of phosphate esters or dephosphorylation on the serine/threonine residues of substrate proteins. *Protein tyrosine phosphatases* hydrolyze phosphate esters or dephosphorylation on tyrosine residues. The *dual-specificity phosphatases* catalyze phosphate ester hydrolysis on the serine/threonine as well as tyrosine residues. These protein phosphatases are briefly discussed here.

Protein Serine/Threonine Phosphatase-Mediated Cell Signaling [5.8]

Structure and Function. *Protein serine/threonine phosphatases* (see Table 5.9) are enzymes that remove the phosphate esters from the serine and threonine residues of substrate proteins via hydrolysis. Several families of protein serine/threonine phosphatases have been identified in mammalian cells. Among these families, four have been extensively studied and well characterized. These include protein phosphatases (PP) 1, 2A, 2B, and 2C. In structure, a typical protein serine/threonine phosphatase is composed of one or more regulatory domains and a catalytic domain. The phosphatase 1, 2A, and 2B families possess a similar structure in the catalytic domain, which contains several unique motifs, including the -GDxHG-, -GDxVDRG-, and -GNHE- motifs, in the *N*-terminal half of the molecule. The activity of these phosphatases is dependent on two metal ions: Fe^{2+} and Zn^{2+}. The unique motifs listed above play a critical role in the binding of these

TABLE 5.9. Characteristics of Selected Protein Serine/Threonine Phosphatases*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Protein phosphatase 1 catalytic subunit α	Protein phosphatase 1 α, serine/threonine protein phosphatase PP1-α catalytic subunit, PP-1A	341	39	Liver, heart, brain	A catalytic subunit for protein serine/threonine phosphatase 1, which dephosphorylates target proteins on serine/threonine residues and regulates cell proliferation and differentiation, glycogen metabolism, muscle contractility, and learning and memory
Protein phosphatase 1 catalytic subunit β	Protein phosphatase 1 β, PP 1B, serine/threonine protein phosphatase PP1 β catalytic subunit	327	37	Widely expressed, mostly in skeletal muscle	Similar to functions of protein phosphatase 1 catalytic subunit α
Protein phosphatase 1 catalytic subunit γ	PP-1G, protein phosphatase 1 γ subunit, protein phosphatase 1C catalytic subunit, serine/threonine protein phosphatase PP1-γ catalytic subunit	323	37	Widely expressed	Similar to functions of protein phosphatase 1 catalytic subunit α
Protein phosphatase 2A catalytic subunit α isoform	PP2CA, PP2A-α, serine/threonine protein phosphatase 2A catalytic subunit α isoform	309	36	Brain, heart, lung, liver, intestine, pancreas, kidney	Forming a complex with one or more regulatory subunits, dephosphorylating target proteins, and negatively controlling cell proliferation and differentiation
Protein phosphatase 2A catalytic subunit β isoform	Serine/threonine protein phosphatase 2A catalytic subunit β isoform, PP2A β	309	36	Heart	Similar to functions of protein phosphatase 2A catalytic subunit α isoform
Protein phosphatase 2A regulatory subunit A α isoform	Protein phosphatase 2A regulatory subunit A α, PP2A subunit A R1-α isoform, PP2A subunit A PR65α isoform	589	65	Kidney, lung, intestine	Forming a complex with a catalytic subunit, regulating dephosphorylation of target proteins, and negatively regulating cell proliferation and differentiation
Protein phosphatase 2A regulatory subunit B		358	441	Heart, retina, placenta, leukocytes	Similar to functions of protein phosphatase 2A regulatory subunit A α isoform

*Based on bibliography 5.8.

metal ions. The PP2C family is characterized by the presence of several motifs, including the ED- and DG-rich motifs. The action of this phosphatase family is dependent on the metal ions Mg^{2+} and Mn^{2+}.

Protein phosphatases exert inhibitory effects on cellular activities induced by protein kinases. When a protein kinase initiates a mitogenic stimulatory effect, which promotes cell proliferation and migration, the activation of a corresponding protein phosphatase results in the suppression of the mitogenic effect. On the other hand, when protein phosphorylation elicits an inhibitory effect on a cellular activity, dephosphorylation by a protein phosphatase exerts an opposite effect. Thus, protein phosphatases and protein kinases coordinately regulate cellular activities, ensuring appropriate initiation and termination of cellular activities.

Signaling Mechanisms. The activity of protein serine/threonine phosphatases is generally regulated via several processes, including phosphorylation, activator binding, and inhibitor binding. The phosphorylation of phosphatases is a major mechanism of phosphatase activation. The regulatory and catalytic domains of a phosphatase can be phosphorylated by specific protein kinases. Such a process induces changes in the localization and catalytic activity of the phosphatase. The binding of activators to a phosphatase induces phosphatase activation, whereas the binding of inhibitors elicits an opposite effect. The phosphorylation and binding of activators and inhibitors are common regulatory mechanisms for protein serine/threonine phosphatases. Each phosphatase may possess distinct features in action and regulation.

Protein Tyrosine Phosphatase-Mediated Cell Signaling

Structure and Function [5.9]. *Protein tyrosine phosphatases* (PTPs) (see Table 5.10) are enzymes that catalyze dephosphorylation on the tyrosine residues of substrate proteins. The effect of PTPs counterbalances that of protein tyrosine kinases, which induces tyrosine phosphorylation. Tyrosine phosphorylation and dephosphorylation are two critical processes that coordinately regulate cell survival, proliferation, differentiation, migration, and adhesion. In human cells, there exists a family of about 100 protein tyrosine phosphatases. These phosphatases are characterized by the presence of a signature motif, HCxxGxxR[S/T], where H, C, G, R, S, and T are histidine, cysteine, glycine, arginine, serine, and threonine, respectively, and x represents any amino acids. This signature motif constitutes the center of the catalytic domain of PTPs. The cysteine residues play a critical role in the catalytic activity of PTPs. The arginine residues are responsible for interaction with phosphate groups. Both cysteine and arginine residues are well preserved among PTPs and are essential for the function of the enzymes. In addition to these two amino acids, there is another invariant amino acid, aspartic acid, which is located in a conformationally flexible loop and plays a critical role in regulating the catalytic activity of PTPs.

Protein tyrosine phosphatases possess distinct molecular structures and can act on a variety of substrate proteins. Based on target amino acid residues, PTPs can be classified into two subfamilies: *classical tyrosine-specific PTPs*, which recognize and act on phosphotyrosine residues in substrate proteins, and *dual-specificity phosphatases* (DSPs), which recognize and act on phosphotyrosine as well as phosphoserine and phosphothreonine residues.

TABLE 5.10. Characteristics of Selected Protein Tyrosine Phosphatases and Related Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Protein tyrosine phosphatase receptor type α	Protein tyrosine phosphatase α, tyrosine phosphatase α, leukocyte common antigen-related peptide	802	91	Brain, heart, blood vessel, liver, skeletal muscle, kidney, placenta	Dephosphorylating and activating the Src family tyrosine kinases; also regulating cell adhesion, proliferation, and migration
Protein tyrosine phosphatase nonreceptor type 1	Protein tyrosine phosphatase 1B, PTP1B, PTPN1	435	50	Lymphocytes, placenta, and skeletal muscle	Dephosphorylating a variety of protein tyrosine kinases, including insulin receptor kinase, epidermal growth factor receptor kinase, JAK2 and TYK2 kinases, and negatively regulating cell proliferation, differentiation, and migration
Dual-specificity phosphatase 1	Protein tyrosine phosphatase nonreceptor type10 (PTPN10), MAP kinase phosphatase 1 (MKP 1)	367	39	Brain, skin	Inactivating mitogen-activated protein (MAP) kinase by dephosphorylating the phosphothreonine and phosphotyrosine residues, and negatively regulating cell proliferation and differentiation

SH2 containing protein tyrosine phosphatase 1	SHP-1, SHPTP1, protein tyrosine phosphatase 1C (PTP1C), protein tyrosine phosphatase nonreceptor type 6, tyrosine phosphatase SHP1, hematopoietic cell phosphatase (HCP)	595	68	Myeloid cells, bone marrow	Inactivating a variety of protein tyrosine kinases by dephosphorylating the phosphotyrosine residues, and negatively regulating cell adhesion, proliferation, and differentiation
SH2 containing protein tyrosine phosphatase 2	SHP-2, protein tyrosine phosphatase nonreceptor type 11 (PTPN11), protein tyrosine phosphatase 2C (PTP2C), tyrosine phosphatase SHP2, PTP-1D, SHPTP2	593	68	Ubiquitous	Regulating cell mitogenic activation, metabolism, and migration
Signal regulatory protein α	Signal regulatory protein α type 1, protein tyrosine phosphatase nonreceptor type substrate 1, SHP substrate 1 (SHPS1), tyrosine phosphatase SHP substrate 1, inhibitory receptor SHPS1	504	55	Brain, heart, blood vessel, lung, liver, kidney, spleen, thymus, bone marrow, leukocytes	A receptor-type transmembrane glycoprotein belonging to the immunoglobulin superfamily, recruiting SHP1, serving as a substrate of SHP1, activating SHP1 when SHP1 is recruited, negatively regulating receptor tyrosine kinase-mediated signaling events, and suppressing cell adhesion, proliferation, and migration

*Based on bibliography 5.9.

The subfamily of *classical tyrosine-specific PTPs* is composed of 17 known members. Within this subfamily, 9 PTPs are present in the cytoplasm, which are defined as nontransmembrane PTP subtypes, whereas the remaining 8 PTPs are transmembrane molecules that are similar in structure to cell membrane receptors. Most classical nontransmembrane PTPs contain the signature motif as described above within the catalytic domain located near the *C*-terminus, whereas two PTP subtypes, including PTP1B and BDP1, possess the signature motif in the catalytic domain near the *N*-terminus. In addition to the signature motif, each subtype of PTP contains a characteristic domain. For instance, SH2 domain-containing protein tyrosine phosphatase-1 (SHP1) and SH2 domain-containing protein tyrosine phosphatase-2 (SHP2) contain two Src homology 2 domains. The type 9 nonreceptor protein tyrosine phosphatase (MEG2) contains a cellular retinaldehyde binding protein-like domain. For the transmembrane receptor-like PTPs, the catalytic domain is located near the *C*-terminus on the intracellular side. The extracellular region of the transmembrane PTPs contain various domains such as fibronectin III-like repeats, carbonic anhydrase-like domains, RGDS adhesion recognition motifs, and glycosylated domains, depending on the subtypes of PTPs.

All nontransmembrane and transmembrane PTPs can specifically recognize phosphotyrosine residues in substrate proteins. The specificity of PTPs is determined by the structure and conformation of the active-site cleft and the signature motif of the enzyme. Tyrosine-specific PTPs possess a ~9X deep active-site cleft. Such a structure allows only a substrate phosphotyrosine to reach the cysteine nucleophile at the base of the active-site cleft of a PTP, initiating dephosphorylation of the substrate phosphotyrosine, while phosphoserine/phosphothreonine cannot reach the cysteine nucleophile because of a structural mismatch and thus cannot be dephosphorylated. These observations demonstrate how PTPs selectively dephosphorylate substrate proteins.

The subfamily of *dual-specificity phosphatases* is composed of a large number of members, which exhibit a greater level of structural diversity compared with the classical PTPs. While the signature motif is similar between the two subfamilies of PTPs, other structures are significantly different. In particular, the active-site cleft of the dual-specificity phosphatases is more widely open and shallower than that of the classical PTPS, rendering dual-specificity phosphatases accessible by not only phosphotyrosine but also phosphserine/threonine residues. This feature suggests a mechanism for the selection of both phosphserine/threonine and phosphotyrosine by dual-specificity phosphatases. According to their molecular structure, dual-specificity phosphatases can be divided into three groups: VH1-like dual-specificity phosphatases, myotubularins and cdc25 phosphatases. These phosphatases recognize specific target proteins. For instance, mitogen-activated protein kinase phosphatases (MKPs), which belong to the group of VH1-like dual-specificity phosphatases, dephosphorylate mitogen-activated protein kinases, which are critical signaling molecules regulating cell survival, proliferation, and migration. The cdc25 phosphatases induce dephosphorylation of cyclin-dependent kinases, which regulate cell mitosis.

As discussed above, PTPs exhibit high specificity to substrate proteins. A "substrate trapping" approach has been developed and used for identifying individual substrate proteins for PTPs. A mutant form of a PTP can be generated to suppress the catalytic activity of the enzyme, but keep the substrate-binding domain functional. When expressed in the cell, the mutant PTP can still bind to a specific phosphotyrosine-containing substrate, but cannot initiate dephosphorylation. The complex of the mutant PTP and substrate can be immunoprecipitated, and the associated substrate can be identified by immunoblotting or

amino acid sequence analysis. The activity of the substrate can be assessed by immunoblotting with an antiphosphotyrosine antibody. A number of PTP substrates have been identified by the "substrate trapping" approach. Examples include p130cas and VCP (p97/CDC48) as substrates for PTP-PEST and PTPH1, respectively.

Signaling Mechanisms [5.10]. Structural studies have suggested potential mechanisms for the action of PTPs. Once a PTP is engaged with a protein substrate, the cysteine residue (serving as a nucleophile) in the active site of the enzyme interacts with the phosphate group of a substrate phosphotyrosine, forming an intermediate complex of cysteine and phosphate. The ester bond between the phosphate group and the substrate tyrosine residue is cleaved and an aspartic acid residue donates a proton (H) to the cleaved substrate tyrosine residue, resulting in tyrosine dephosphorylation. At the same time, the aspartic acid residue in the conformationally flexible loop, together with a glutamine residue, activates a water molecule and initiates hydrolysis of the cysteine–phosphate intermediate, resulting in the dissociation of the phosphate group from the enzyme. This is a general mechanism of catalytic action for most members of the PTP family.

The catalytic activity of PTPs is regulated by a variety of factors, depending on the structure of PTPs and signaling context. *Transmembrane PTPs*, which are similar to cell membrane receptors in structure, may be directly activated by extracellular ligand binding. For example, soluble pleiotrophin can interact with and activate the transmembrane protein tyrosine phosphatase PTPζ/β, and heparan sulfate proteoglycans can activate PTPσ. Some of the transmembrane PTPs are similar in structure to cell membrane adhesion molecules, suggesting that these PTPs may be activated via cell–cell interactions. In contrast to transmembrane PTPs, *nontransmembrane PTPs* are activated through the mediation of cell membrane receptors and intracellular signaling molecules. In general, there are three known mechanisms for the regulation of PTP activities: (1) phosphorylation of PTPs, (2) conformational changes in the three-dimensional (3D) structure of PTPs, and (3) oxidation of the catalytic cysteine residue of PTPs. These mechanisms are briefly described here.

Phosphorylation is an essential process that induces PTP activation. A typical example is the activation of the protein tyrosine phosphatase Src homology (SH)2 domain-containing tyrosine phosphatase (SHP)1 (Fig. 5.10). SHP1 can interact with the inhibitory receptor signal regulatory protein (SIRP)α, also classified as Src homology 2 domain-containing tyrosine phosphatase substrate (SHPS)1, which is expressed primarily in myeloid cells. SIRP α is a transmembrane glycoprotein that transmits inhibitory signals through tyrosine phosphorylation of its intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM). The phosphorylation of the ITIM, on ligand binding to SIRPα, initiates the recruitment of SHP1 to SIRPα, which is known as a *substrate* of SHP1. The recruitment of SHP1 induces phosphorylation of SHP1, which in turn dephosphorylates protein kinases, possibly including receptor tyrosine kinases, Src family protein tyrosine kinases, phosphatidylinositol 3-kinase, and the Janus family tyrosine kinases. These activities potentially suppress inflammatory and mitogenic cellular activities.

A conformational change in the 3D structure of PTP molecule is another effective mechanism that regulates the activity of PTPs. Here, SHP2 is used to demonstrate this mechanism (Fig. 5.11). A SHP2 molecule contains two SH2 domains. The *N*-terminal SH2 domain serves as a switch. In the absence of a substrate protein with phosphotyrosine residues, the *N*-terminal SH2 domain blocks the active site of SHP2, inhibiting the activity of the enzyme. The binding of SHP2 to a substrate protein induces a conformational

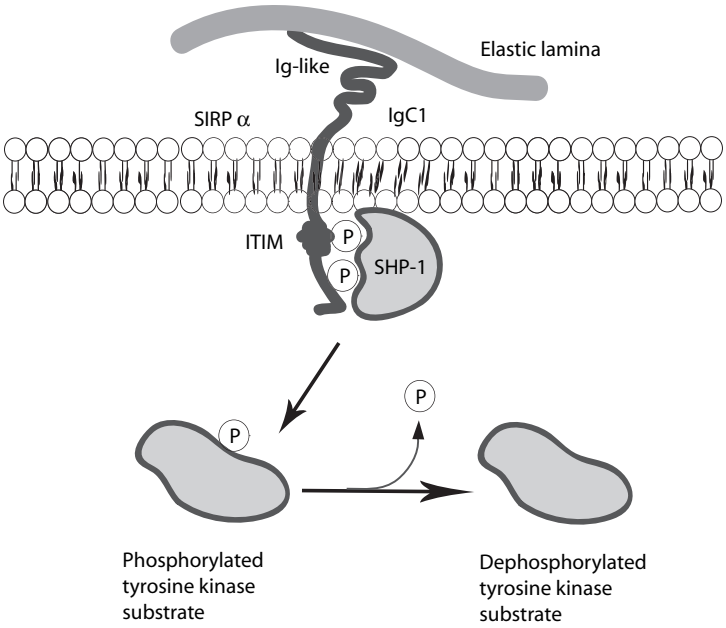

Figure 5.10. Signal regulatory protein (SIRP) α-mediated activation of SH2 domain-containing protein tyrosine phosphatase (SHP)-1 (based on bibliography 5.10).

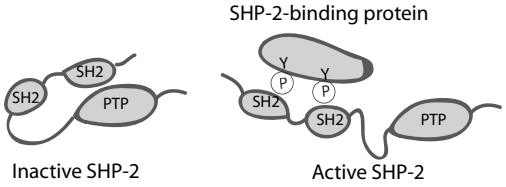

Figure 5.11. Mechanisms of the activation of SH2 domain-containing protein tyrosine phosphatase (SHP)-2. (Reprinted from Hof P et al: Crystal structure of the tyrosine phosphatase, *Cell* 92:441–50, copyright 1998, with permission from Elsevier.)

change, which removes the inhibitory effect of the *N*-terminal SH2 domain. This mechanism also applies to SHP1.

Oxidation of cysteine is a mechanism involved in the inhibition of PTPs. The activity of PTPs is rapidly suppressed when the mission of the PTPs is accomplished. As discussed above, the invariant cysteine residue at the catalytic site of PTPs is critical for the catalytic activity. Cysteine is present as a thiolate anion, which renders the residue susceptible to oxidation, and can be oxidized to a sulfenic acid form (Cys-SOH) or a disulfide form (Cys-S—S—), which inhibit the catalytic activity of PTPs. This process is reversible and is an effective mechanism for temporarily inhibiting the activity of PTPs. Oxidation of PTPs can be initiated in response to the binding of growth factors and hormones, such as epidermal growth factor and insulin, to corresponding receptors, which activate tyrosine phosphorylation-dependent signaling pathways. The suppression of PTP activities further enhances the activation of protein tyrosine kinases. These observations suggest that the activity of PTPs is controlled in coordination with the activity of protein tyrosine kinases.

CYTOKINE-JAK-STAT-MEDIATED CELL SIGNALING [5.11]

Structure and Function

The JAK–STAT, or Janus tyrosine kinase–signal transducers and activators of transduction, signaling pathways are molecular cascades that transduce cytokine signals from the extracellular space to the cell nucleus. This type of signaling pathway is composed of cell membrane and intracellular components, including cytokine receptors, JAKs, and STATs. Cytokines are small proteins (15–30 kDa), including interleukins, interferons (α, β, and γ), erythropoietin, thrombopoietin, leukemia inhibitor factor, cardiotrophin, oncostatin, granulocyte colony-stimulating factors, granulocyte macrophage colony-stimulating factors, and macrophage colony-stimulating factors. Cytokines are produced and secreted primarily by leukocytes. These molecules participate in the regulation of blood cell differentiation and proliferation as well as immune reactions. A cytokine molecule can interact with a specific cytokine receptor, inducing the activation of the JAK-STAT signaling pathways.

Cytokine receptors are classified into three types based on molecular structure: (1) cytokine receptors containing a glycoprotein (gp) 130, such as the interleukin (IL) 6 receptor; (2) cytokine receptors containing a β subunit, such as the receptor for granulocyte-macrophage colony-stimulating factor; and (3) cytokine receptors containing a γ subunit, such as the IL2 receptor. There are four common features for cytokine receptors:

1. The extracellular region is similar for most cytokine receptors.
2. There are no intracellular catalytic domains.
3. Most receptors are arranged into complexes from dimers to tetramers.
4. The intracellular region of a cytokine receptor is associated with JAKs, which relay signals from cytokine receptors and elicit catalytic activities.

The binding of cytokine to cytokine receptor induces the activation of JAKs, which in turn phosphorylate STATs. Phosphorylated STATs serve as transcriptional factors and can translocate to the nucleus, inducing gene expression.

There are four types of JAKs in mammalian cells, including JAK1, JAK2, JAK3, and Tyk2. The molecular weights of these molecules range from 120 to 140-kDa. Each JAK molecule contains 7 functional regions, defined as JAK homology (JH) domains 1–7 starting from the *C*-terminus. The first JAK homology domain, JH1, is a kinase and is able to phosphorylate substrates. JH2 is located upstream to JH1. Although JH2 is similar to a kinase in structure, it does not possess catalytic function because of the lack of several critical amino acid residues. JH2 is thus referred to as a pseudo-kinase domain. JH3 and JH4, located upstream to JH2, are similar in structure to the Src homology (SH) 2 domain, which can bind to phosphotyrosine residues. Thus, these domains are defined as SH2 domains. All remaining domains, including the N-terminal part of the JH4 domain and domains JH5 to JH7, are referred to as four-point-one, ezrin, radixin, moesin homology (FERM) domains. These domains mediate the attachment of JAKs to cytokine receptors in the cell membrane.

Signaling Mechanisms

JAKs are constitutively associated with cytokine receptors. These molecules are arranged in different configurations between an inactive (unliganded) state and active (liganded)

state. As shown by crystallography, the receptor of the cytokine erythropoietin is composed of two identical molecules, each of which is associated with a JAK2 molecule. In an unliganded state, the two erythropoietin receptors of each homodimer are separated by approximately 70 Å. In response to the binding of an erythropoietin molecule, the two receptors undergo a conformational change, reducing the gap between the two receptors from 70 to ~30 Å. This change also brings together the two JAK2 molecules associated with the erythropoietin receptor homodimer, allowing reciprocal phosphorylation between the two JAK2 molecules. Phosphorylated JAK2 further activates STATs, leading to transcriptional activities.

STATs are a group of molecules that serve as transcriptional (*trans*-acting) factors for the JAK-STAT signaling pathways. Each STAT molecule contains several domains, including a *C*-terminal transcriptional activation domain, a SH2 domain, a linker domain, a DNA-binding domain, a coiled-coil domain, and an *N*-terminal domain. The SH2 domain can be recruited to the phosphotyrosine docking site of a phosphorylated JAK and can be phosphorylated by the JAK on a tyrosine residue, inducing STAT activation.

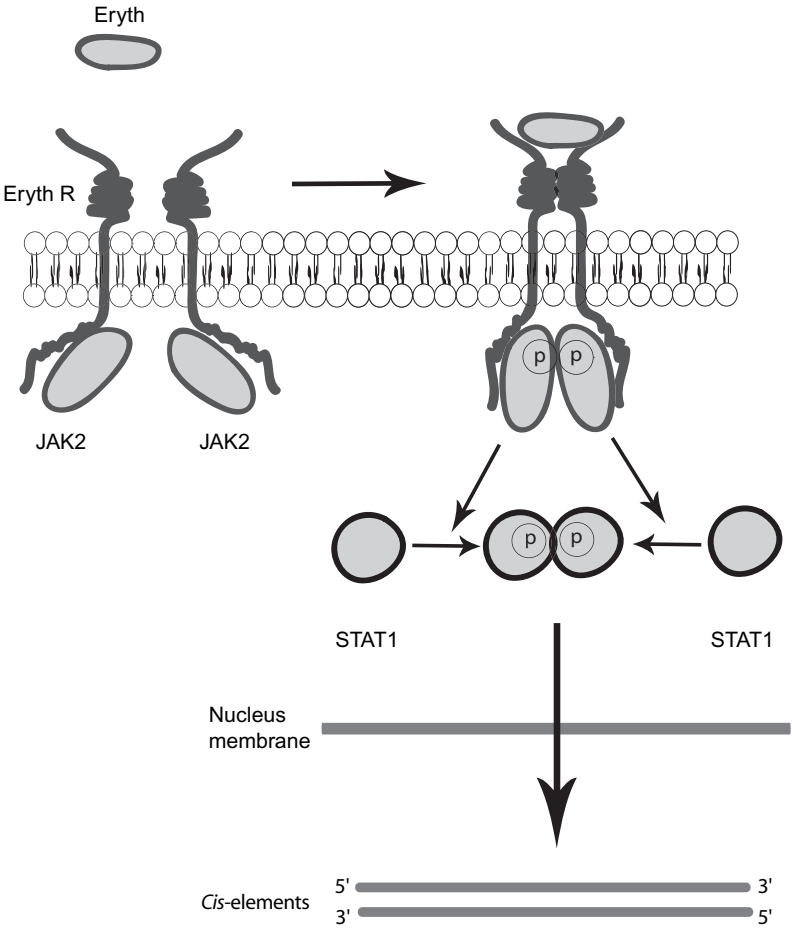

Figure 5.12. Schematic representation of the erythropoietin-mediated JAK-STAT signaling pathway (based on bibliography 5.11).

TABLE 5.11. Characteristics of Selected Molecules for the JAK-STAT Signaling Pathway*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Janus kinase 1	JAK1	1142	132	Ubiquitous	Regulating signaling activities induced by cytokines, such as interferon α, β, γ, and activating transcription factors known as signal transducers and activators of transcription (STATs)
Janus kinase 2	JAK2	1132	131	Ubiquitous	Similar to functions of JAK1
Janus kinase 2					Regulating cytokine-induced signal transduction, activating STATs, and especially regulating the development and activity of lymphocytes and the immune system
Tyk2	Protein tyrosine kinase 2, nonreceptor tyrosine protein kinase TYK2	1187	134	Leukocytes, bone marrow, macrophages, liver, thymus, skin	Regulating cytokine-induced signal transduction and activating STATs
STAT1	Signal transducer and activator of transcription 1	750	87	Ubiquitous	Forming homodimers or heterodimers with other STAT proteins, and serving as a downstream transcription factor for various cytokine ligands, such as interferon-α, interferon-γ, PDGF, and IL6
STAT2	Signal transducer and activator of transcription 2, and Interferon α-induced transcriptional activator	851	98	Thymus, skin	Forming a complex with STAT1 and interferon regulatory factor p48, acting as a coactivator without the capability of binding to DNA directly, and mediating STAT1 activity

*Based on bibliography 5.11.

Two activated STATs then interact between each other at the phosphotyrosine sites of the SH2 domains, inducing the formation of a STAT dimer, which serves as a *trans*-acting factor and translocates to the cell nucleus. A STAT dimer can bind to the promoter region of a target gene together with other transcription–regulatory factors, initiating gene transcription. An example of STAT activation is shown in Fig. 5.12.

Following gene transcription, the JAK-STAT signaling pathways are deactivated by several types of protein, including protein tyrosine phosphatases, suppressors of cytokine signaling family proteins, and inhibitors of STAT family proteins. *Protein tyrosine phosphatases* induce dephosphorylation of JAKs, diminishing their activities. A typical example of this class is the SH2 domain-containing protein tyrosine phosphatase (SHP)1, which can bind and dephosphorylate JAK2. In addition, protein tyrosine phosphatases may directly act on STATs, inducing STAT dephosphorylation and deactivation. The *suppressors of cytokine signaling family proteins* are able to bind to JAKs and block ATP binding to the kinases, inhibiting kinase activities. Some proteins of this family, such as cytokine-inducible SH2 domain-containing proteins, can directly bind to phosphorylated tyrosine residues of JAKs, inhibiting the recruitment and phosphorylation of STATs. The third type of JAK-STAT inhibitors, *inhibitors of STAT family proteins*, can bind to phosphorylated STATs and block their interaction with gene promoters, thereby inhibiting STAT-induced gene expression. These inhibitory mechanisms counterbalance the cytokine-induced activation of the JAK-STAT signaling pathways, ensuring rapid deactivation of these pathways on the accomplishment of the signaling events. (See Table 5.11.)

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G-PROTEIN RECEPTOR-MEDIATED CELL SIGNALING

Structure and Function [5.12]

Cell signaling events regulated by G proteins and G-protein-coupled receptors are referred to as *G-protein receptor-mediated cell signaling* (Table 5.12). *G-proteins* are guanine nucleotide-binding proteins, each composed of three subunits: α, β, and γ (see below). G proteins are localized to the cytoplasmic side of the cell membrane and coupled to a class of cell membrane receptors, defined as *G-protein-coupled receptors*. These receptors pass through the cell membrane back and forth for 7 times, and are thereby called *7-pass transmembrane receptors* (Fig. 5.13). Several types of extracellular ligands, including hormones, growth-related factors, small peptides, and neurotransmitters, can bind to G-protein receptors, initiating activation of the receptors as well as G proteins. In addition, photons and odorants can activate G-protein receptors in vision and olfactory cells, respectively.

G proteins exist in two states, inactive and active, and serve as switches for the regulation of several intracellular signaling pathways. Among the three subunits of the G protein,

TABLE 5.12. Characteristics of Selected Molecules for G-Protein Receptor-Mediated Signaling Pathways*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Guanine nucleotide binding protein G(S), α subunit	Guanine nucleotide-binding protein, α-stimulating activity polypeptide 1, Gs α subunit, adenylate cyclase stimulatory protein α subunit, adenylate cyclase-stimulating G α protein, stimulatory G protein, and GNAS	909	98	Ubiquitous	Activating adenylyl cyclase; also regulating metabolic processes and cell proliferation and differentiation
Guanine nucleotide-binding protein G(q) α subunit	G α q and guanine nucleotide-binding protein Q polypeptide	359	42	Ubiquitous	Inducing activation of phospholipase Cβ, regulating cell proliferation and differentiation, and controlling contractile activity of smooth muscle cells
Guanine nucleotide-binding protein β1 subunit	G protein β1 subunit, GNB1	340	37	Heart, red blood cells	Forming complexes with G protein α, γ subunits, and regulating the activity of α subunit

Guanine nucleotide-binding protein γ3 subunit	G protein γ3	75	8	Nervous system	Forming complexes with G protein α, β subunits, and regulating the activity of α subunit
Adrenergic receptor β1	ADRB1	477	51	Heart, adipocyte	Mediating the action of epinephrine and norepinephrine, and enhancing cardiac contractility
Adenylate cyclase 1	Adenylate cyclase type I	1119	123	Brian, heart, lung, liver, kidney, pancreas, spleen, ovary, skeletal muscle	Catalyzing the formation of cAMP
Phospholipase Cβ1	Phospholipase C β1, 1 phosphatidylinositol 4,5-bisphosphate phosphodiesterase β1, phosphoinositide-specific phospholipase C β1, PLC β1, PLC1	1216	139	Brain, lung, blood vessel	Catalyzing the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate, and participating in regulation of cellular activities such as cell proliferation and migration

*Based on bibliography 5.12.

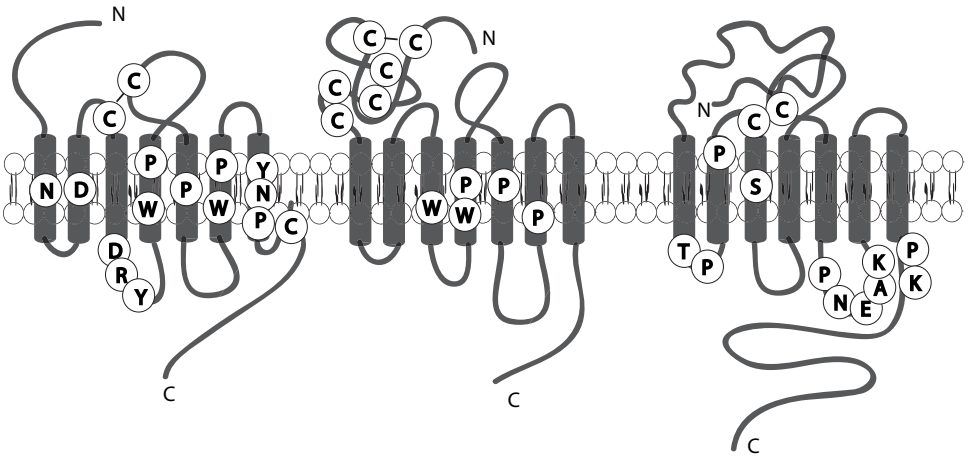

Figure 5.13. Schematic representation of the types of G-protein-coupled receptors. (Based on Gether U: *Endocr Rev* 21:90–113, 2000.)

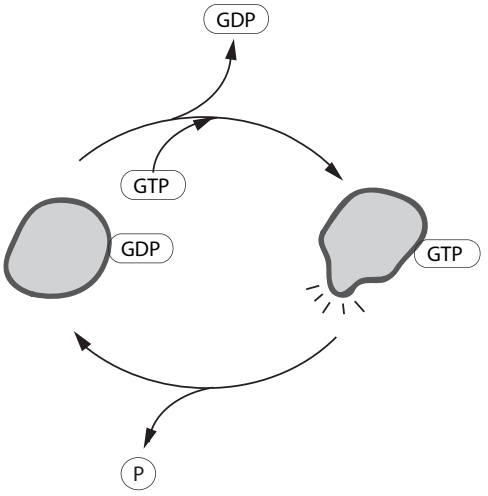

Figure 5.14. Schematic representation of G-protein activation and deactivation. (Based on bibliography 5.12).

the α subunit is responsible primarily for regulating the activity of the G-protein. Each α subunit is composed of a GTPase, which hydrolyses GTP, and a helical domain, which holds a guanine nucleotide, also known as a *guanine nucleotide pocket*. In an inactive state, a GDP molecule is bound to the guanine nucleotide pocket of the α subunit. When a G-protein is activated in response to the binding of a ligand to the G-protein receptor, the GDP molecule is released, and a GTP is bound to the α subunit (Fig. 5.14). Simultaneously, the GTP-bound α subunit is dissociated from the trimeric G protein, forming two distinct modules: the GTP-bound α subunit and βγ complex. These modules are able to activate downstream signaling pathways. On the completion of signal transduction, the GTP molecule with the α subunit is rapidly hydrolyzed by the GTPase associated with the α subunit, diminishing the activity of signaling pathways regulated by G proteins.

There exist various types of α, β, and γ subunits. To date, about 20 α, 5 β, and 12 γ subunits have been found. These subunits can be assembled into a variety of G-protein isoforms. Based on the structure of the α subunits, G proteins have been classified into four groups: G_s, G_q, $G_{i/o}$, and $G_{12/13}$. Among these G proteins, the first three types have been well studied and characterized. The G_s group includes the G_s-proteins and olfactory specific G_{olf} proteins. These G proteins are referred to as *stimulatory G proteins* for their role in the activation of adenylyl cyclase. The G_q group is composed of G_q, G_{11}, G_{14}, and G_{15} proteins. These proteins activate phospholipase Cβ, a key enzyme that induces the activation of protein kinase C (PKC) and phosphatidyl inositol triphosphate signaling pathways (described later in this section). The $G_{i/o}$ group includes a number of members, including G_{i1}, G_{i2}, G_{i3}, G_{oA}, G_{oB}, G_z, $G_{t\text{-}rod}$, $G_{t\text{-}cone}$, and G_{gust}. These $G_{i/o}$ members elicit different effects. Activated G_{i1}, G_{i2}, G_{i3}, G_{oA}, G_{oB}, and G_z inhibit the activity of adenylyl cyclase. For such a function, these G proteins are referred to as *inhibitory G proteins*. However, G_{i1}, G_{i2}, G_{i3} can stimulate the activation of phosphatidylinositol 3-OH kinase γ (PI3Kγ). $G_{t\text{-}rod}$ and $G_{t\text{-}cone}$ can activate cGMP-phosphodiesterase (PDE) and are responsible for regulating the function of rod and cone photoreceptors, respectively, in retinal vision cells. G_{gust} can activate cGMP-phosphodiesterase and regulate the function of gustatory cells. $G_{i/o}$ proteins are usually sensitive to pertussis toxin, a substance possessing the activity of ADP-ribosyl transferase and inducing ADP-ribosylation in $G_{i/o}$ proteins. Such a process suppresses the activity of the $G_{i/o}$ proteins.

Signaling Mechanisms [5.13]

The discussion above indicates that each type of G protein mediates distinct signaling pathway(s). While it is beyond the scope of this book to cover all G-protein-related mechanisms, examples from three major G-protein signaling pathways, including the G_s, G_i, and G_q pathways, are discussed briefly.

G_s proteins are often found in cells undergoing metabolic processes for the breakdown of energy-storing molecules, such as triglyceride and glycogen. A typical example is the G_s protein coupled to the β-adrenergic receptor (Fig. 5.9). The binding of ligands, such as epinephrine and norepinephrine, to the β-adrenergic receptor induces the ejection of the bound GDP molecule from the G_s α subunit and the recruitment of a GTP molecule to the guanine nucleotide pocket, while the α subunit dissociates from the βγ subunits. The free α subunit–GTP complex binds to and activates adenylyl cyclase. Activated adenylyl cyclase acts on an ATP molecule, inducing the formation cyclic AMP or cAMP. cAMP serves as a second messenger and interacts with cAMP-dependent protein kinase, or PKA, inducing the activation of this kinase. PKA is a serine/threonine kinase and can transfer the terminal phosphate group from an ATP molecule to serine and threonine residues of substrate proteins, a process known as *serine/threonine phosphorylation*. A typical substrate of PKA is phosphorylase kinase. Phosphorylated phosphorylase kinase induces the phosphorylation of glycogen phosphorylase, which catalyzes the breakdown of glycogen into glucose 1-phosphates, a process referred to as *glycolysis*. At the same time, activated PKA phosphorylates another enzyme called *glycogen synthase*, which catalyzes the synthesis of glycogen from glucose. The phosphorylation of glycogen synthase negatively regulates the activity of the enzyme, inducing suppression of glycogen synthesis. Thus, the activation of G_s-protein signaling pathway leads to the release of glucose, increasing the blood concentration of glucose and enhancing energy production.

In contrast to the G_s proteins, the *G_i proteins* elicit an inhibitory effect on the activity of adenylyl cyclase. G_i proteins are coupled to α_2-adrenergic receptors. Among the three subunits of a G protein, the β and γ subunits are identical between the G_i and G_s proteins, but the α subunit is different, which determines the distinct functions of the G_i protein. The binding of epinephrine or norepinephrine to α_2-adrenergic receptors activates the G_i proteins, resulting in the substitution of GTP for GDP in the α subunit and the dissociation of the α subunit from the $\beta\gamma$ subunits. The free α subunit acts to suppress the activity of adenylyl cyclase, resulting in inhibition of glucose metabolism. This signaling mechanism counterbalances that of the G_s proteins.

The *G_q-proteins* belong to another group of stimulatory signaling pathways. These G proteins regulate the transport of Ca^{2+} and the activity of several intracellular molecules via the mediation of cell membrane lipids. G_q proteins are coupled to cell membrane receptors, which interact with several extracellular ligands, including vasopressin, acetylcholine, thrombin, and angiotensins I and II. The binding of a ligand to a G_q-protein-coupled receptor stimulates the G_q protein, which activates phospholipase Cβ, a phosphoinositol-specific enzyme. Activated phospholipase Cβ hydrolyzes phosphatidylinositol 4,5-biphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol. These lipid molecules excert distinct functions. The IP_3 molecule can diffuse through the cytoplasm and acts on Ca^{2+} channels in the endoplasmic reticulum (ER), resulting in the opening of these channels and Ca^{2+} release from the ER to the cytoplasm (Fig. 5.15). Ca^{2+} mediates many cellular processes, including actin–myosin interaction in muscular cells, secretion of neurotransmitters, and signal transduction. The other molecule, diacylglycerol, can activate Ca^{2+}-dependent protein kinase, or protein kinase C (PKC), a serine/threonine protein kinase. Activated PKC can phosphorylate two known substrate proteins: mitogen-activated protein kinase (MAPK) and IκB. The phosphorylation of MAPK initiates a cascade of mitogenic activities, which is discussed on page 151. IκB forms a complex with NFκB, a *trans*-acting factor that regulates the expression of mitogenic genes. The phosphorylation of IκB induces the release of NFκB, which translocates to the cell nucleus and induces gene expression.

The G-protein signaling pathways can communicate by crosstalk with other signaling pathways, resulting in diverse biological consequences. For example, the G_q protein α subunit can interact with various molecules of the Ras-MAPK signaling pathways, activating mitogenic cellular activities. Recent studies has suggest that both $G_{\alpha i}$ and $G_{\alpha s}$ may be able to interact with and activate Src, which in turn activate STAT3, a key transcription factor that can be activated by cytokines (see page 207). Thus, the G-protein signaling pathways can impose effects on diverse cellular activities via interactions with various signaling pathways.

NFκB-MEDIATED CELL SIGNALING

Structure and Function [5.14]

Nuclear factor κB (NFκB) belongs to a family of *trans*-acting factors, which stimulate the expression of genes encoding proteins for regulating cell survival and proliferation as well as inflammatory and immune processes. There are five known members of the NFκB family in mammalian cells, including RelA (p65), RelB, c-Rel, NFκB1 (p50), and NFκB2 (p52). These proteins possess a highly conserved domain near the *N*-terminus, known as

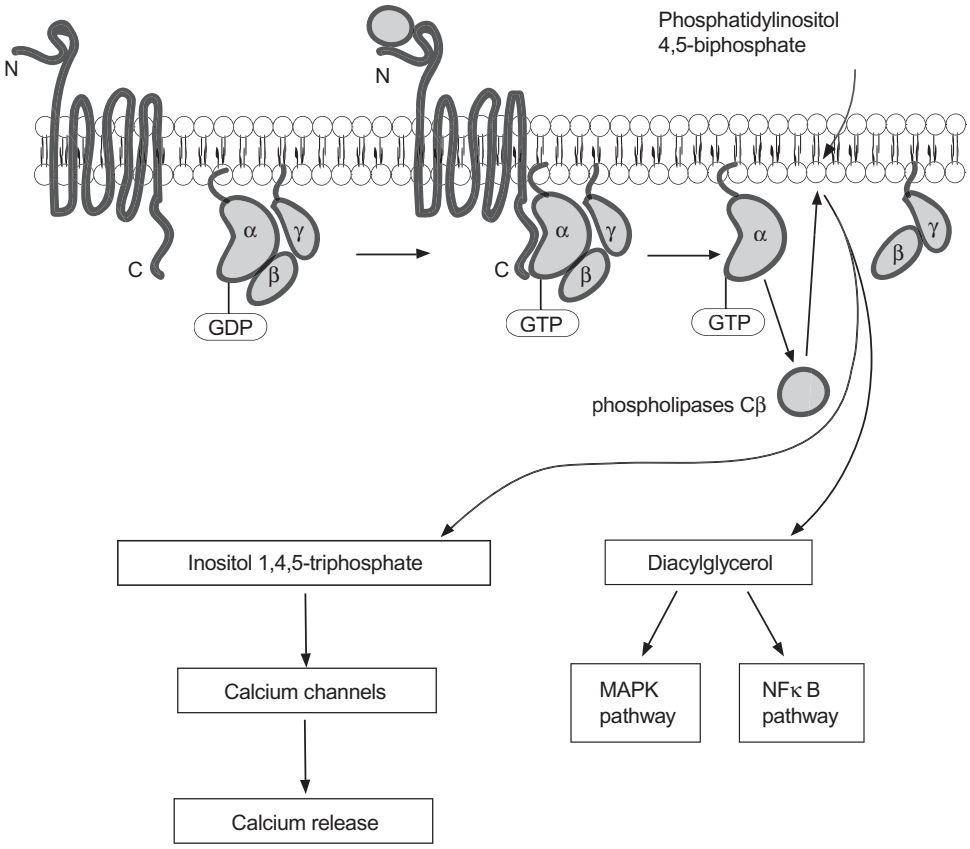

Figure 5.15. Schematic representation of the mechanisms of Gq-protein signaling (based on bibliography 5.13).

the *Rel homology domain* (RHD). This domain is responsible for NFκB binding to target DNA, dimerization, and association with inhibitory κB (IκB), which binds to NFκB and inhibits the activity of NFκB in unstimulated cells. The NFκB family proteins usually exist in the form of homodimer or heterodimer, such as NFκB1/RelA, NFκB1/ NFκB1, and NFκB2/ NFκB2. These NFκB dimeric complexes may impose apparently different effects on target genes. For instance, the NFκB1/RelA heterodimer induces the expression of target genes, whereas the NFκB1/ NFκB1 and NFκB2/ NFκB2 homodimers elicit an opposite effect.

In unstimulated cells, the NFκB family proteins, existing as heterodimers or homodimers, are associated with a protein of the IκB family, which is composed of seven members, including IκBα, IκBβ, IκBε, IκBγ, Bcl3, NFκB1 precursor (p105), and NFκB2 precursor (p100). These IκB family members can bind to and mask the nuclear localization signals of a NFκB molecule. This action renders the NFκB molecule incapable of binding to and interacting with target DNA, thus inhibiting the transcriptional activity of NFκB. The NFκB family proteins can only be activated when the inhibitory IκB protein is degraded or removed. (See Table 5.13.)

TABLE 5.13. Characteristics of Selected Molecules of NFκB-Mediated Signaling Pathways*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Nuclear factor κB subunit 1	Nuclear factor of κ light polypeptide gene enhancer in B cells 1, NFκB, NFKB1, transcription factor NFKB1, NFKB p105	968	105	Ubiquitous	Forming the protein complex of nuclear factor κB (NFκB) and regulating the activity of NFκB
Nuclear factor κB subunit 2	Nuclear factor of κ light-chain gene enhancer in B cells 2, transcription factor NFKB2, NFKB p52/p100 subunit	933	100	Ubiquitous	Forming the protein complex of nuclear factor κB (NFκB) and regulating the activity of NFκB
IκBα	Nuclear factor κB inhibitor, IKBA, nuclear factor of κ light-chain gene enhancer in B cells inhibitor α, nuclear factor of κ light-chain gene enhancer in B cells inhibitor (NFKBI), inhibitor of κ light-chain gene enhancer in B cells α	317	36	Leukocytes	Forming complex proteins with NFκB1 or NFκB2 and inhibiting the activity of these NFκB subunits
IκBβ	NFκB inhibitor β, IKB β, nuclear factor of κ light polypeptide gene enhancer in B-cell inhibitor β, Inhibitor of κ light-chain gene enhancer in B cells β, IKBB	356	38	Ubiquitous	Forming complex proteins with NFκB1 or NFκB2 and inhibiting the activity of these NFκB subunits

IκB kinase α	I κB kinase α, IKKA, I κB kinase 1, IKK1	745	85	Ubiquitous	A serine/threonine protein kinase that dephosphorylates IκB, inducing degradation of IκB via ubiquitination and activation of NFκB
IκB kinase β	Nuclear factor of κ light-chain gene enhancer in B-cell inhibitor kinase β, NFKBIKB, I-κB kinase β, IKKB, I-κB kinase 2, IKK2, inhibitor of nuclear factor κB kinase β subunit, nuclear factor NFκB inhibitor kinase β	756	87	Ubiquitous	A serine/threonine protein kinase that dephosphorylates IκB, inducing degradation of IκB via ubiquitination and activation of NFκB
IκB kinase γ	Inhibitor of κ light polypeptide gene enhancer in B cells, kinase γ, inhibitor of nuclear factor κB kinase γ subunit, I-κB kinase γ, IkB kinase γ subunit, IKKγ	419	48	Ubiquitous	A serine/threonine protein kinase that dephosphorylates IκB, inducing degradation of IκB via ubiquitination and activation of NFκB
Atk	Agammaglobulinemia tyrosine kinase, Bruton's tyrosine kinase, tyrosine-protein kinase BTK, bruton agammaglobulinemia tyrosine kinase, B-cell progenitor kinase	659	76	Lymphocytes, bone marrow	Regulating B-cell development and causing agammaglobulinemia[a] when detected

[a] Agammaglobulinemia is an X-linked immunodeficiency with impaired generation of mature B lymphocytes and failure of Ig heavy-chain rearrangement.
*Based on bibliography 5.14.

Signaling Mechanisms [5.15]

The NFκB signaling pathway can be activated by several factors, including physical stress, oxidative stress, chemical toxins, and bacterial and viral infection. These factors can interact with cells and activate NFκB, which in turn stimulates the expression of many inflammatory cytokines, chemokines, immune receptors, and cell surface adhesion molecules. Thus, NFκB has been considered as a central mediator for immune responses as well as stress responses induced by physical stress, oxidative stress, and chemical substances. For instance, NFκB can be activated in response to the stimulation of IL1. The binding of IL1 to the IL1 receptor activates a molecule known as *NFκB-inducing kinase* (NIK or serine/threonine protein kinase NIK), which in turn phosphorylates the IκB kinase. Activated IκB kinase can phosphorylate the IκB module of the IκB-NFκB complex, inducing the separation of NFκB from IκB. NFκB is a complex of p50 and p65 proetins, which serves as a transcriptional factor and regulates gene transcription. Figure 5.16 shows the mechanisms of NFκB activation in response to the stimulation of cytokines.

In addition, extracellular mitogens and growth factors can activate NFκB, which in turn regulates cell survival and proliferation. The activation of NFκB is mediated by a protein kinase known *as IκB kinase* (IKK), which is composed of at least three subunits: two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ). Extracellular mitogens and growth factors can activate IKK via various signaling pathways. For instance, protein kinases MEKK1, MEKK2, and MEKK3, which are activated through receptor protein tyrosine kinase signaling pathways, are capable of phosphorylating IKK. Activated IKK in turn phosphorylates two specific serine residuals on IκB. The phosphorylation of IκB leads to the ubiquitination and degradation of IκB by proteasome. The degradation of IκB liberates NFκB, which translocates to the cell nucleus, binds to target genes, and regulates gene transcription, resulting in the activation of inflammatory and mitogenic activities.

In addition to the degradation and removal of IκB, serine phosphorylation of NFκB may be required for certain NFκB family members for efficient binding to transcriptional activators and interaction with target genes. For instance, the catalytic domain of protein kinase A can bind to an inactive NFκB p65 protein in unstimulated cells. On IκB degradation, protein kinase A phosphorylates p65 on serine 276, resulting in a conformational change in the p65 protein and consequent interaction with a transcriptional activator, namely CBP (CREB-binding protein or cAMP response element-binding protein-binding protein), which increases the transcriptional activity of NFκB. Another example involves the activation of protein kinases PI3K and Atk (agammaglobulinemia tyrosine kinase), which mediate IL1- and TNFα-initiated NFκB activation. In cultured cells, IL1 and TNFα induce the activation of PI3K and Atk. These protein kinases in turn phosphorylate NFκB p65, enhancing the DNA-binding capacity of NFκB.

UBIQUITIN AND PROTEASOME-MEDIATED CELL SIGNALING

Structure and Function [5.16]

Ubiquitin (see Table 5.14) and *proteasome* are two critical protein structures of a protease system that recognizes and degrades damaged, unfolded, and nonfunctional proteins. Ubiquitin is a 76-amino acid protein that can attach to a target protein via an isopeptide bond linking the terminal carboxyl group of the ubiquitin molecule to a ε-amino group

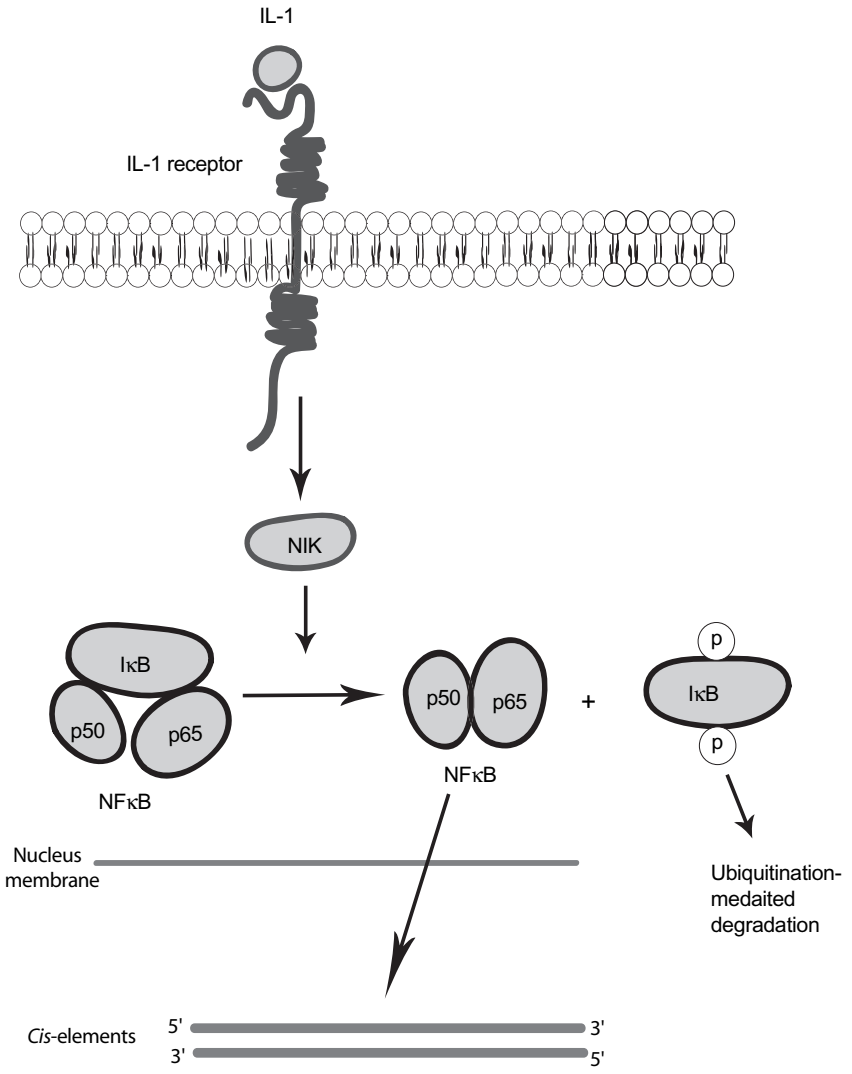

Figure 5.16. Mechanisms of nuclear factor (NF)κB activation in response to the stimulation of cytokines IL6 and tumor necrosis factor (TNF)α (based on bibliography 5.15).

of a lysine of the target protein. Additional ubiquitins can be added to the linked ubiquitin, forming a polyubiquitin chain. Such a process is known as *ubiquitination*. The polyubiquitin chain serves as a tag for the recognition of the targeted protein by a proteasome, a multicatalytic protease that destroys the ubiquitin-tagged protein. Ubiquitination and proteasome activation are an effective means for the destruction of useless proteins, an important process for protein metabolism and recycling.

In addition to ubiquitins and proteasomes, several mediating factors are required for ubiquitination. These include an ubiquitin-activating enzyme, an ubiquitin-conjugating enzyme, and an ubiquitin/protein ligase. The *ubiquitin-activating enzyme* can bind to and activate ubiquitin via a thiolester bond, a process that requires ATP. The *ubiquitin-*

TABLE 5.14. Characteristics of Selected Ubiquitin Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Ubiquitin B	Polyubiquitin B, UBA52	229	26	Brain, dependent pancreas	Regulating ATP-degradation of abnormal and nonfunctional proteins; also mediating gente expression by binding to histone H2A (note that ubiquitins do not cause histone H2A degradation)
Ubiquitin C	Polyubiquitin, PolyUB	685	77	Kidney	Similar to functions of ubiquitin B

*Based on bibliography 5.16.

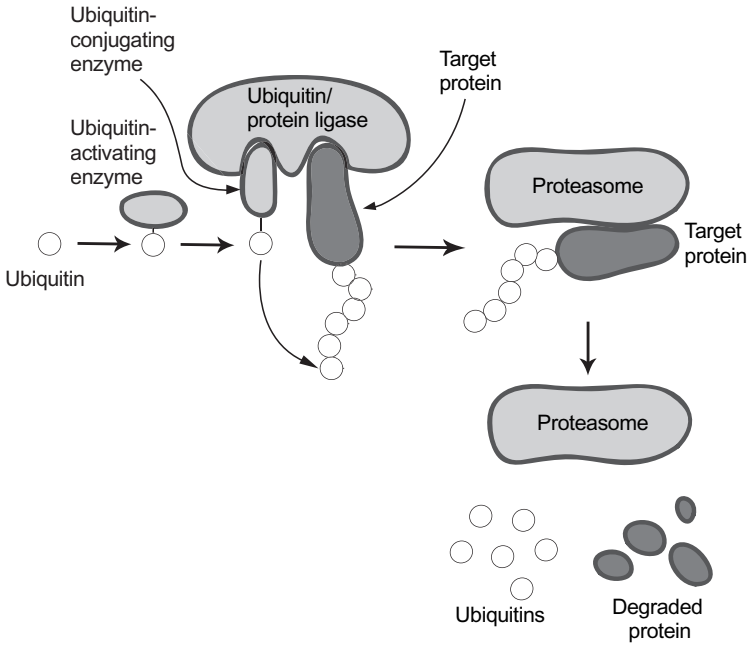

Figure 5.17. Schematic representation of the components of the ubiquitination system. (From Nakayama KI et al: Regulation of the cell cycle at the G1-S transition by proteolysis of cyclin E and p27Kip1, *Biochem Biophys Res Commun* 282:853–60, copyright 2001, with permission from Elsevier.)

conjugating enzyme can transfer an activated ubiquitin to a target protein with the help of the *ubiquitin/protein ligase*, which recognizes target proteins and promotes ubiquitin transfer and ubiquitination (Fig. 5.17). Thus, these factors play a critical role in the initiation and regulation of ubiquitination.

Signaling Mechanisms [5.17]

Protein ubiquitination is regulated at the level of substrate proteins. Two processes are often involved in the regulation of ubiquitination: substrate phosphorylation and hydroxylation. *Substrate phosphorylation* may induce activation or inhibition of ubiquitination, depending on the nature of substrate proteins and ubiquitin ligases, whereas *substrate hydroxylation* activates ubiquitination. Several examples are given here, to demonstrate these regulatory mechanisms.

Stimulation of Ubiquitination by Substrate Phosphorylation. Substrate phosphorylation is required for the activation of several ubiquitin ligases, including S-phase kinase-associated protein 1 (Skp1), Cdc53, and F-box protein, which are designated as SCF ubiquitin ligases. These ubiquitin ligases form complexes with an identical Skp1 and Cdc53 protein, but a varying F-box protein. A specified F-box protein is responsible for the recognition of a specific substrate protein and the affinity of the ligase complex to the substrate. The phosphorylation of a substrate protein can activate a SCF ligase complex, inducing ubiquitination and destruction of the substrate. Several transcriptional factors, including IκB and β-catenin, are known substrates for the SCF ligase complex.

IκB is an inhibitory molecule that binds to and sequesters NFκB in unstimulated cells. On the stimulation of NFκB-related signaling pathways, the IκB molecule becomes phosphorylated at two serine residues, which stimulate the activation of a SCF ubiquitin ligase complex, namely, $SCF^{\beta\text{-TrCP}}$, where β-TrCP is a specific F-box protein. Activated $SCF^{\beta\text{-TrCP}}$ induces ubiquitination and degradation of IκB, a necessary step for the release and activation of NFκB. Activated NFκB serves as a transcriptional factor, migrates to target genes, and initiates gene transcription. In this case, ubiquitination is not only responsible for the degradation of IκB, but also contributes to the activation of NFκB.

β-Catenin is a coactivator of transcriptional factors, participating in the regulation of gene transcription. β-Catenin can be phosphorylated by glycogen synthase kinase 3β. The phosphorylation of β-catenin activates $SCF^{\beta\text{-TrCP}}$, which in turn induces β-catenin ubiquitination and destruction. This is an effective approach for reducing transcriptional activities mediated by β-catenin. (See Table 5.15.)

Inhibition of Ubiquitination by Substrate Phosphorylation. The phosphorylation of certain proteins may reduce the activity of ubiquitin ligases, thus suppressing ubiquitination of the substrate proteins. A typical example is p53, a tumor suppressor protein (see Table 5.18, later in this chapter for p53). Toxic stress can lead to p53 phosphorylation. Phosphorylated p53 in turn induces cell arrest during cell mitosis. Excessive p53 is usually degraded by ubiquitination, which is mediated by an ubiquitin ligase mdm2. Phosphorylation of p53 on the serine residues has been shown to prevent interaction of p53 with the ubiquitin ligase mdm2, suppressing ubiquitination. Such a process stabilizes p53 and enhances the function of p53.

Stimulation of Ubiquitination by Substrate Hydroxylation. In addition to substrate phosphorylation, substrate hydroxylation plays a role in regulating substrate ubiquitination. An example is the ubiquitination of the hypoxic response transcriptional regulator hypoxia inducible factor 1 α (HIF1α) (see Table 5.16). This factor is stabilized and activated under a hypoxic condition but degraded under a normoxic condition. Ubiquitination of HIF1α is a critical step in the degradation of the HIF1α protein. Following recovery from a

TABLE 5.15. Characteristics of S-Phase Kinase-Associated Protein 1A and β-Catenin*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
S-phase kinase-associated protein 1A	SKP1, SKP1A	163	19	Ubiquitous	Serving as a substrate recognition component of the SCF ubiquitin ligase complex, binding to regulatory proteins involved in ubiquitin proteolysis (e.g., cyclin F and S-phase kinase-associated protein 2); also serving as an RNA polymerase II elongation factor
β-Catenin	Catenin β 1, cadherin-associated protein β	781	86	Ubiquitous	Serving as an adherens junction protein, mediating cell–cell adhesion and interaction, regulating cell attachment to matrix, cell proliferation, and differentiation during embryogenesis, wound healing, and tumor cell metastasis; also serving as a coactivator of transcriptional factors

*Based on bibliography 5.17.

TABLE 5.16. Characteristics of Hypoxia Inducible Factor 1α Subunit*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Hypoxia inducible factor 1α subunit	HIF1α	826	93	Ubiquitous	Acting as a transcriptional factor, regulating cellular responses to reduced oxygen concentration or hypoxia, mediating inflammatory reactions, enhancing angiogenesis, and mediating neural development

*Based on bibliography 5.17.

hypoxic condition, increased oxygen concentration stimulates the activation of a proline hydroxylase, which catalyzes the hydroxylation of a proline residue in the HIF1α protein. The hydroxylated proline mediates the interaction of HIF1α with an ubiquitin ligase complex known as the *VBC complex* (Von Hippel–lindau protein–elongin B–Elongin C), which is similar to the SCF complex in assembly and function. Activated VBC complex in turn induces HIF1α ubiquitination and degradation.

NUCLEAR RECEPTOR-MEDIATED CELL SIGNALING

Structure and Function [5.18]

Nuclear receptors are a family of intracellular proteins that interact with steroid, thyroid, and retinoid hormones, which are lipid-soluble molecules and can diffuse through the cell membrane. These receptors are found in the cytoplasm and cell nucleus. Among the nuclear receptors, the *steroid hormone receptor subfamily* has been studied extensively. Common nuclear receptors include estrogen receptor (ER) α and β, glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), androgen receptor (AR), and vitamin D receptor (VDR). These receptors interact with corresponding steroid hormones (Fig. 5.18). In addition, there are several nuclear receptors that are similar to estrogen receptors in function. These are defined as estrogen-related receptors α, β, and γ, which are also referred to as "orphan" nuclear receptors.

A typical nuclear receptor is composed of several functional domains, including a DNA-binding domain (DBD), a ligand-binding domain (LBD), an activation function 1 (AF1) domain, and an activation function 2 (AF2) domain. The *DNA-binding domain* is located in the central region of the receptor and composed of two zinc finger motifs that are responsible for protein–DNA interaction. The DNA-binding domain is well reserved among different nuclear receptors. The *ligand-binding domain* is located at the *C*-terminus and is composed of sequences responsible for ligand interactions, activation of nuclear transcription, binding to chaperone proteins, and dimerization with other

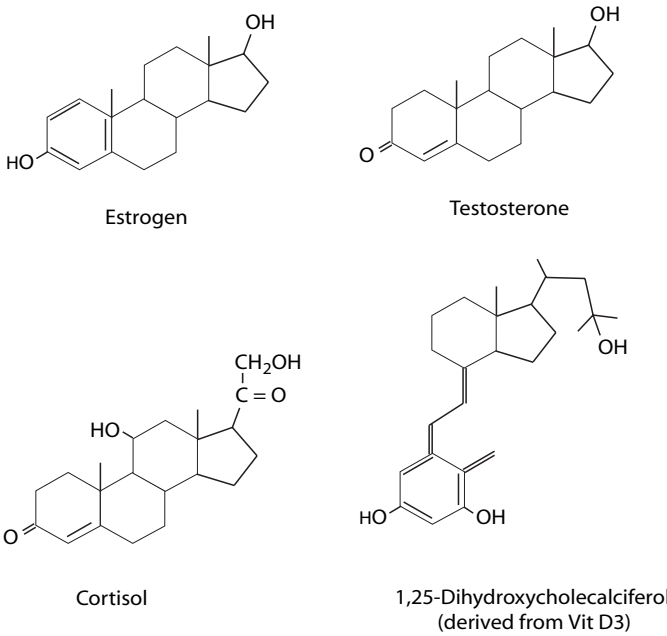

Figure 5.18. Steroid hormones for nuclear receptors. (Based on bibliography 5.18).

receptors. The ligand-binding domain is moderately conserved compared with the DNA-binding domain. The AF1 and AF2 *domains* regulate the activation of the receptor. The difference between these two domains is that the activity of the AF1 domain is independent of ligand binding, whereas the activity of the AF2 domain is dependent on ligand binding. On the interaction with hormone ligands, the hormone–receptor complexes are activated, serve as transcriptional factors, and directly bind to corresponding *cis* elements in target genes, initiating gene transcription. The nuclear receptors participate in the regulation of several physiological processes, including salt balance, glucose metabolism, reproduction, and responses to environmental stress impacts. (See Table 5.17.)

Signaling Mechanisms [5.18]

In unstimulated cells, nuclear receptors are associated with "chaperones," which suppress the activity of the receptors. On the binding of ligands to the nuclear receptors, the chaperones are dissociated from the receptors, resulting in receptor conformational changes and the exposure of the nuclear localization signals. The ligand–receptor complexes often form homodimers or heterodimers and are translocated from the cytoplasm to the nucleus, initiating gene transcription (Fig. 5.19). The activation of the nuclear receptor transcriptional factors is regulated to a large degree by the AF1 and AF2 domains of the nuclear receptor. These domains recruit and activate nuclear receptor cofactors, which are enzymes including acetylases, deacetylases, methylases, kinases, and ubiquitinases. These cofactors play a critical role in regulating the formation of the transcription-initiating complexes, the conformation of target DNA *cis*-acting elements, and the degradation and recycling of the nuclear receptors.

TABLE 5.17. Characteristics of Selected Molecules for Nuclear Receptor-Mediated Signaling Pathways*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Estrogen receptor α	Estrogen receptor 1, ESR, ER, oestrogen receptor α	595	66	Uterus, ovary, blood vessel, kidney, bone	Acting as a transcriptional factor, regulating gene expression and mediating the development of female sex characters, promoting fertilization and implantation, and mediating the formation of bone matrix
Glucocorticoid receptor	GCR, GCCR, GR	777	86	Ubiquitous	Serving as a transcriptional factor, forming a homodimer or heterodimer with another protein (e.g., retinoid receptor and a heatshock protein), regulating the expression of glucocorticoid-responsive genes, promoting gluconeogenesis and elevation of blood glucose concentration, and inhibiting inflammatory and immune responses

TABLE 5.17. *Continued*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Mineralocorticoid receptor	MR, aldosterone receptor	984	107	Kidney, brain, heart, liver, intestine	Acting as a transcriptional factor, stimulating the expression of mineralocorticoid responsive genes, and mediating electrolyte and water balance via controlling ion transport in the renal tubular system, resulting in retention of sodium and water and loss of potassium
Progesterone receptor	PR	933	99	Uterus, ovary, placenta, brain, blood vessel, leukocytes	Acting as a transcriptional factor in response to binding of progesterone; also regulating the establishment and maintenance of pregnancy
Androgen receptor	AR, dihydrotestosterone receptor (DHTR)	920	99	Prostate, adrenal gland, brain	Serving as a transcriptional factor, inducing transcription of androgen responsive genes, and regulating masculinization or the development of male sex characteristics
Vitamin D receptor	1,25-Dihydroxyvitamin D_3 receptor, VDR	427	48	Ubiquitous	Serving as a transcriptional factor and regulating calcium metabolism and the formation of bone matrix

*Based on bibliography 5.18.

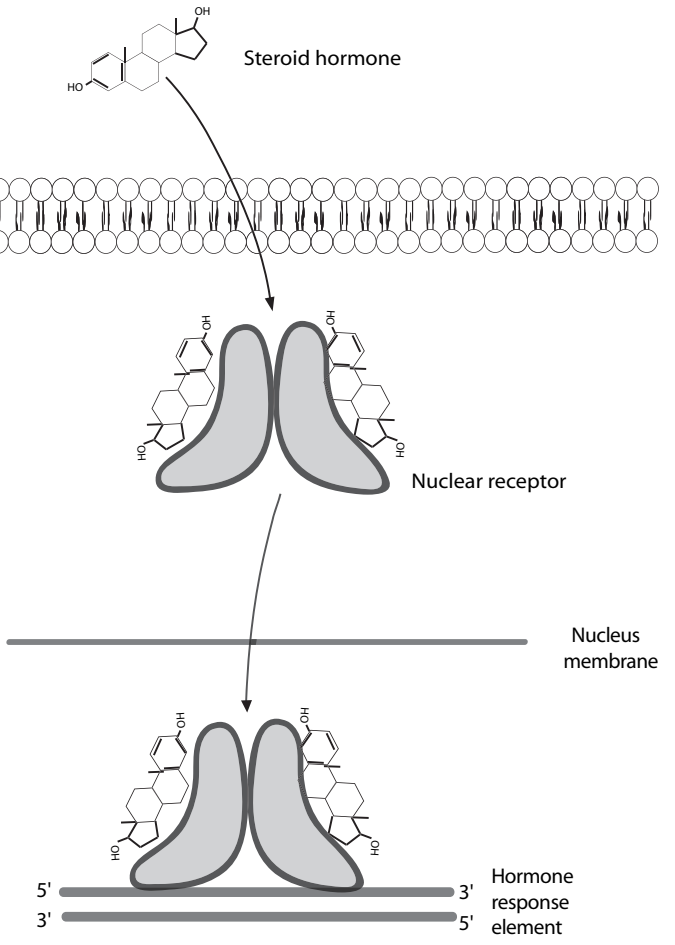

Figure 5.19. Mechanisms of nuclear receptor activation (based on bibliography 5.18).

When activated, the nuclear receptor transcriptional factors interact with specific DNA *cis*-acting elements defined as *hormone response elements* (HREs) with the assistance of cofactors. The HREs are located within the promoter and enhancer regions of target genes and are composed of unique sequences for different steroid ligands. For instance, the hormone response element for estrogen receptors and estrogen-related receptors contains repeating AGGTCA sequences, whereas that for glucocorticoid receptor, mineralocorticoid receptor, progesterone receptor, and androgen receptor contains AGAACA. Thus, the structure of these *cis* elements determines the specificity of the binding of nuclear receptor transcriptional factors to DNA. These transcriptional factors bind DNA in the form of homodimers or heterodimer.

In addition to steroid hormones, other types of molecule can activate nuclear receptors. A typical example is the activation of nuclear receptors by protein kinases, including protein kinase A, mitogen-activated protein kinases, cyclin-dependent kinases, and glycogen synthase kinases. While the exact mechanisms remain poorly understood, it appears that growth factor-induced activation of intracellular protein kinases play a role in the

activation of nuclear receptors. Several ligands, including epidermal growth factor and insulin growth factor, can induce activation of protein kinases, leading to the phosphorylation of serine residues within the AF1 domain of the nuclear receptors. Such an action facilitates the recruitment of coactivators to nuclear receptors, enhancing the activity of the nuclear receptor. These activities have been demonstrated in estrogen receptor-mediated signaling events.

p53-MEDIATED CELL SIGNALING

Structure and Function [5.19]

p53 (Table 5.18) is a 393-amino acid protein that serves as a transcriptional factor. The activation of p53 leads to cell-cycle arrest, growth inhibition, and cell apoptosis. During the period of cell arrest, p53 often repairs impaired genes. These functions are implemented by regulating the expression of specific genes. These genes encode proteins that control cell cycle progression and cell apoptosis. Because of its inhibitory effect on cell growth and stimulatory effect on cell apoptosis, p53 is considered a tumor suppressor protein.

Based on the amino acid sequence and function, the p53 protein is divided into several domains, including the *N*-terminal domain from amino acids 1–101, a central DNA-binding domain from 102 to 292, and a *C*-terminal domain from 293 to 393. The *N*-terminal domain is capable of interacting with regulatory proteins, which activate or suppress the activity of the p53 protein. Several proteins, including TFIID, TFIIH, TAFs, PCAF, and the MDM2 ubiquitin ligase, have been shown to interact with the *N*-terminal domain. In this region, amino acid residues 1–31 and 80–101 are highly reserved among mammals. The central domain of the p53 protein contains DNA-specific binding sites. A consensus DNA-binding site includes two identical segments, each composed of a sequence of RRRCWWGYYY. The *C*-terminal domain contains several sequences, including a nuclear localization signal, a tetramerization sequence, and DNA-binding sequence. Tetramerization of the p53 protein is required for the activation of the protein as a transcriptional factor.

TABLE 5.18. Characteristics of p53*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
p53	Tumor protein p53, transformation-related protein 53, TRP53	393	44	Ubiquitous	A transcriptional factor that binds to target genes, regulates gene transcription, inhibits cell proliferation and differentiation, and suppresses tumor growth

*Based on bibliography 5.19.

Signaling Mechanisms [5.20]

The p53 protein exists in a latent form and does not induce gene expression in unstimulated cells. It can be activated in response to stimulations induced by ionizing radiation, UV light, chemicals, hypoxia, ribonucleotide depletion, microtubule disruption, and oncogene activation. The activation of p53 requires two conditions: a critical concentration of the p53 protein and posttranscriptional modification.

The level of p53 is determined by the relative activity of protein production and degradation. In unstimulated cells, p53 degradation is more predominant than p53 production, resulting in a relatively low level of stable p53. Thus, the p53 activity is suppressed in unstimulated cells. p53 can be rapidly degraded by the ubiquitin–proteasome system (see page 226). Several mechanisms have been discovered for the ubiquitination and degradation of the p53 protein. In the G_0 phase of a cell cycle, the c-JUN *N*-terminal kinase can bind to p53, forming a complex. This complex serves as a target for an ubiquitin ligase, which induces p53 ubiquitination. Similarly, the COP9 signalosome can binds to p53, inducing p53 ubiquitination and degradation. The p53 protein can also be degraded directly by ubiquitination. One of the ubiquitin ligases, MDM2, can bind to p53 and induce p53 ubiquitination. In response to various stimulations as outlined above, the p53 production system can be activated, resulting in an increase in the level of p53. To activate p53, it is necessary to suppress p53 ubiquitination. The expression and activation of p53 are regulated by several processes as discussed below.

Posttranscriptional modification is a process that modulates the structure and function of a protein after mRNA translation. Several types of modification have been found for p53, including p53 phosphorylation and acetylation. These modifications are required for p53 activation. p53 phosphorylation occurs on the serine and threonine residues, whereas acetylation occurs on the lysine residues. Stimulating factors, such ionizing radiation, UV light, chemicals, hypoxia, microtubule disruption, and oncogene activation, may induce p53 phosphorylation and/or acetylation, although each factor may activate distinct signaling pathways. Here, the mechanisms of p53 activation in response to the stimulation of several common factors are briefly discussed.

Stimulatory factors for p53 can be divided into two groups, based on the influence on gene expression: genotoxic and nongenotoxic. Typical genotoxic factors include ionizing radiation, UV light, anticancer drugs (e.g., adriamycin, camptothecin, actinomycin D, and mitomycin C), and toxic chemicals (e.g., arsenite, cadmium, and chromate). These factors often induce gene damage. Nongenotoxic factors include hypoxia, ribonucleotide depletion, microtubule disruption, oncogene activation, and senescence. These factors may not induce significant gene damage.

A treatment with ionizing radiation induces DNA disruption and activation of protein kinase ATM (ataxia–telangiectasia M), a member of the phosphatidylinositol-3-kinase (PI3K) family. Activated ATM can phosphorylate p53 on serine 15 and activates other protein kinases such as Chk1 and Chk2, which further phosphorylate p53. Other protein kinases, such as PKA, PKC, and CDK, can also phosphorylate p53. Phosphorylated p53 serves as a transcriptional factor, stimulates the expression of selected genes, and induces apoptosis and the arrest of cell cycle (Fig. 5.20). Although DNA disruption is considered a factor for activating the protein kinases that phosphorylate p53, the exact mechanisms of protein kinase activation remains poorly understood.

A treatment with UV light induces DNA damage. Damaged DNA triggers the binding of a protein kinase ATR (ataxia–telangiectasia Rad3-related) to the damaged site, leading

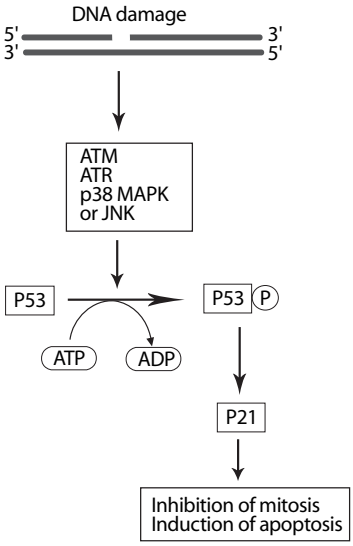

Figure 5.20. Mechanisms of p53 activation (based on bibliography 5.20).

to the activation of ATR. Activated ATR in turn phosphorylates p53 on serine 15 and serine 37. UV exposure can also activate other protein kinases, such as p38 MAPK, HIPK2, JNK, and cdc2/Cdk2 via DNA damage. These protein kinases are capable of phosphorylating p53 on multiple serine residues, leading to cell cycle arrest and apoptosis.

Both ionizing radiation and UV light can induce p53 acetylation on multiple lysine residues at the *C*-terminus, including lysines 320, 373, and 382. It is interesting to note that the *C*-terminal lysine acetylation is enhanced by *N*-terminal serine/threonine phosphorylation. p53 acetylation enhances the stability of the molecule, activates p53, and facilitates p53 binding to target genes, thus enhancing apoptosis and cell cycle arrest.

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5.19. Structure and Function of p53-Related Signaling Molecules

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5.20. Mechanisms of p53-Mediated Signaling

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