

1

Basics of Cell Signaling

1.1

Cell Signaling: Why, When, and Where?

One characteristic common to all organisms is the dynamic ability to coordinate constantly one's activities with environmental changes. The function of communicating with the environment is achieved through a number of pathways that receive and process signals originating from the external environment, from other cells within the organism, and also from different regions within the cell.

In addition to adopting the function of an organism to environmental changes in a signal-directed way, other essential features of multicellular organisms also require the coordinated control of cellular functions.

The formation and maintenance of the specialized tissues of multicellular organisms depend on the coordinated regulation of cell number, cell morphology, cell location, and the expression of differentiated functions. Such coordination results from a complex network of communication between cells in which signals produced affect target cells where they are transduced into intracellular biochemical reactions that dictate the physiological function of the target cell (Figure 1.1). The basis for the coordination of the physiological functions within a multicellular organism is intercellular signaling (or intercellular communication), which allows a single cell to influence the behavior of other cells in a specific manner. As compared to single-cell organisms, where all cells behave similarly within a broad frame, multicellular organisms contain specialized cells forming distinct tissues and organs with specific functions. Therefore, the higher organisms have to coordinate a large number of physiological activities such as:

- Intermediary metabolism
- Response to external signals
- Cell growth
- Cell division activity
- Differentiation and development: coordination of expression programs
- Cell motility
- Cell morphology.

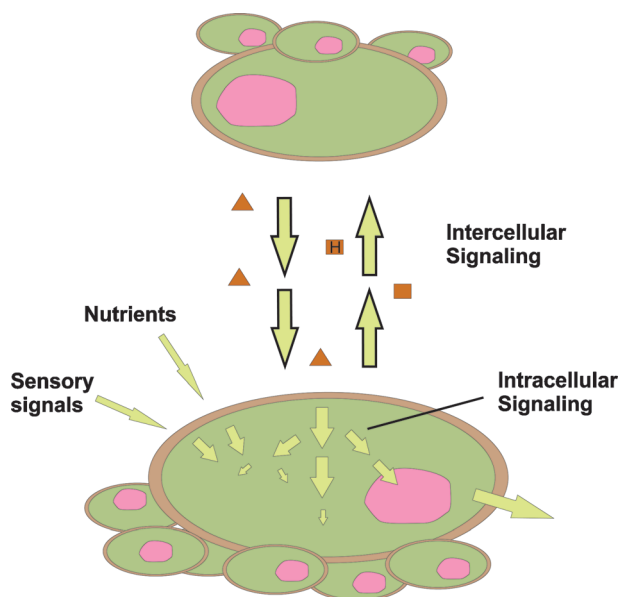


Figure 1.1 Intercellular and intracellular signaling. The major method of intercellular communication employs messenger substances (hormones) that are secreted by signal-producing cells and registered by target cells. All cells produce and receive multiple,

diverse signals. The extracellular signals are transduced into intracellular signaling chains that control many of the biochemical activities of a cell and can also trigger the formation of further extracellular signals.

■ Intercellular signaling:

— Communication between cells.

■ Intracellular signaling:

— Signaling chains within the cell, responding to extracellular and intracellular stimuli.

Signals generated during intercellular communication must be received and processed in the target cells to trigger the many intracellular biochemical reactions that underlie the various physiological functions of an organism. Typically, a large number of steps is involved in the processing of the signal within the cell, which is broadly described as intracellular signaling. Signal transduction within the target cell must be coordinated, fine-tuned and channeled within a network of intracellular signaling paths that finally trigger distinct biochemical reactions and thus determine the specific functions of a cell. Importantly, both intercellular and intracellular signaling are subjected to regulatory mechanism that allow the coordination of cellular functions in a developmental and tissue-specific manner.

1.2

Intercellular Signaling

Intercellular signal transduction influences nearly every physiological reaction. It ensures that all cells of a particular type receive and transform a signal. In this manner, cells of the same type react synchronously to a signal. A further function of intercellular communication is the coordination of metabolite fluxes between cells of various tissues.

In higher organisms, intercellular signaling pathways have the important task of coordinating and regulating cell division. The pathways ensure that cells divide synchronously and, if necessary, arrest cell division and enter a resting state.

■ Intercellular signaling:

— **Processes** sensory information

— **Controls**

- Metabolic fluxes
- Cell division
- Growth
- Differentiation
- Development.

Cellular communication assumes great importance in the differentiation and development of an organism. The development of an organism is based on genetic programs that always utilize inter- and intracellular signaling pathways. Signal molecules produced by one cell influence and change the function and morphology of other cells in the organism.

Intercellular signaling pathways are also critical for the processing of sensory information. External stimuli, such as optical and acoustic signals, stress, gradients of nutrients, and so on, are registered in sensory cells and are transmitted to other cells of the organism via intercellular signaling pathways.

1.2.1

Tools for Intercellular Signaling

Various forms of communication between cells are currently known (Figure 1.2):

- **Extracellular messengers:** Cells send out signals in the form of specific messenger molecules that the target cell transmits into a biochemical reaction. Signaling cells can simultaneously influence many cells by messenger molecules so as to enable a temporally coordinated reaction in an organism.
- **Gap junctions:** Communication between bordering cells is possible via direct contact in the form of “gap junctions.” Gap junctions are channels that connect two neighboring cells to allow a direct exchange of metabolites and signaling molecules between the cells.

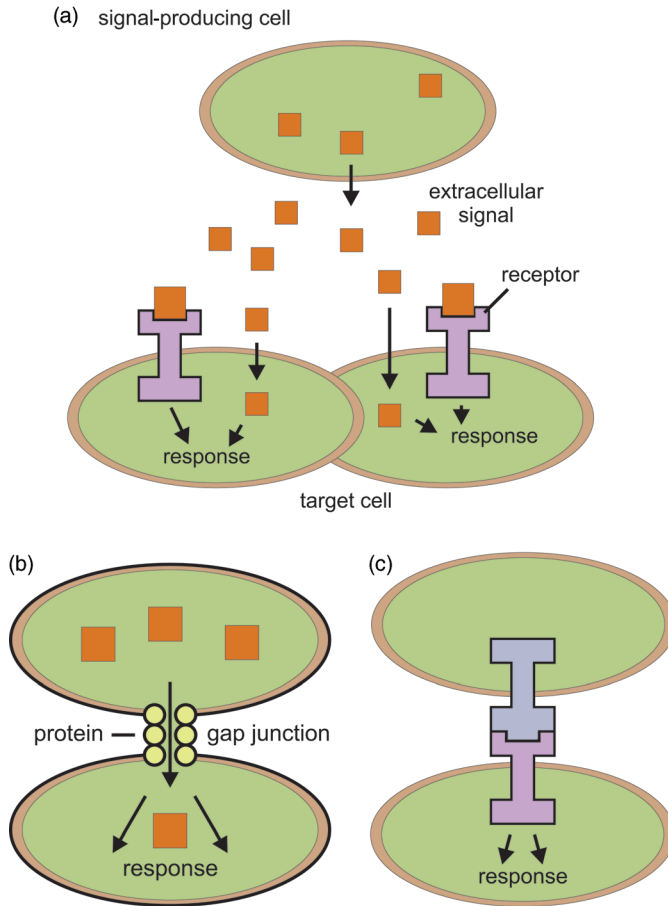


Figure 1.2 The principal mechanisms of intercellular communication.
(a) Communication via intercellular messengers; (b) Communication via gap junctions, which provide direct connections

between cells. Gap junctions are coated by proteins (shown as circles in the figure) that can have a regulatory influence on the transport; (c) Communication via surface proteins.

- **Cell–cell interaction via cell-surface proteins:** Another form of direct communication between cells occurs with the help of surface proteins. In this process, a cell-surface protein of one cell binds a specific complementary protein on another cell. As a consequence of the complex formation, an intracellular signal chain is activated which initiates specific biochemical reactions in the participating cells. Communication is then only possible upon direct contact between the target cell and the surface protein of the partner cell.
- **Electrical signaling:** A further intercellular communication mechanism relies on electrical processes. The conduction of electrical impulses by nerve cells is based on changes in the membrane potential. The nerve cell uses these changes to

communicate with other cells at specialized nerve endings, the synapses. It is central to this type of intercellular communication that electrical signals can be transformed into chemical signals. This type of communication will not be discussed in this book.

■ **Cells communicate via:**

- Messenger substances
- Gap junctions
- Surface proteins
- Electrical signals.

In the following, the main emphasis will be on the intercellular communication via extracellular messengers, the hormones.

1.2.2

Steps of Intercellular Signaling

In the communication between cells of an organism, the signals (messengers such as hormones) are produced in specialized cells. The signal-producing function of these cells is itself regulated, so that the signal is only produced upon a particular stimulus. In this way, signaling pathways can be coupled to one another and coordinated.

The following steps are involved in intercellular communication (Figure 1.3).

1.2.2.1 Formation of a Signal in the Signal-Producing Cell as a Result of an External Trigger

Most extracellular messengers are produced in response to external triggers and are released by exocytosis. Physical stimuli such as electrical signals, changes in ion concentration or, most frequently, other extracellular signaling molecules, serve as a trigger to increase the amount of the messenger available for extracellular communication. The mechanisms by which the external trigger signals increase the amount of extracellular messenger are diverse, and include stimulation of the biosynthesis of the messenger, an increased production of the mature messenger from precursors, and the release of the messenger from a stored form. The latter mechanism is used extensively in the release of hormones of the neural system (neurotransmitters) in response to electrical signals for example, at synapses.

■ **Steps of intercellular signaling:**

- 1) Trigger signal induces release of stored messenger or stimulates its biosynthesis
- 2) Transport to target cell
- 3) Receipt of signal by the target cell
- 4) Conversion of signal into intracellular signal chain in the target cell.

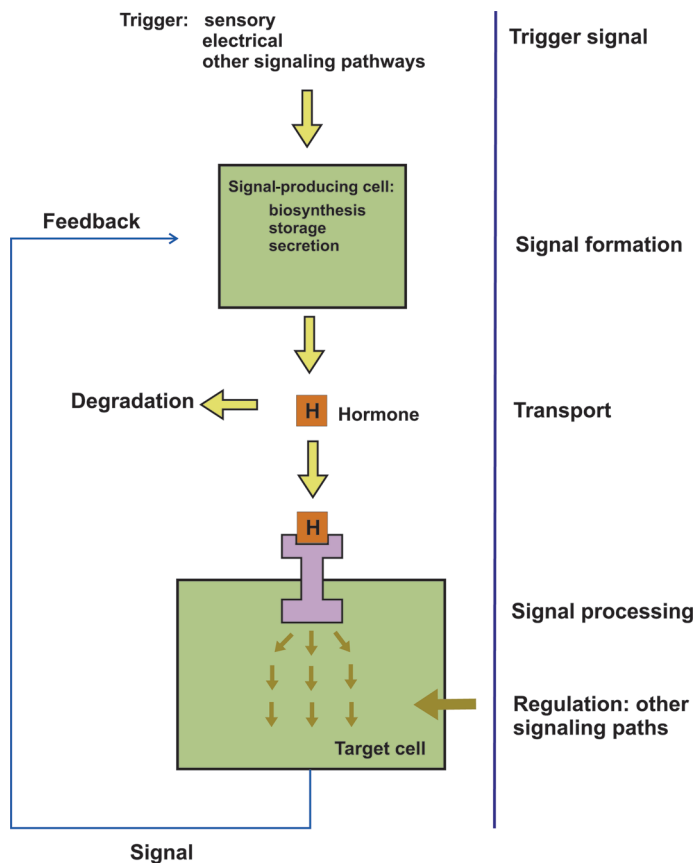


Figure 1.3 The individual steps of intercellular communication. On receipt of a triggering stimulus, the signal is transformed into a chemical messenger within the signaling cell. The messenger is secreted and transported to

the target cell, where the signal is registered, transmitted further, and finally converted into a biochemical reaction. Processes of termination or the regulation of communication, which can act at any of the above steps, are not shown.

1.2.2.2 Transport of the Signal to the Target Cell

The extracellular signal produced may be distributed via the circulation, or it may reach the target cell simply by diffusion. In long-range signaling via the circulation, the extracellular messenger is often bound to specific carrier proteins or incorporated into larger protein complexes. Furthermore, the processing or metabolism of a messenger during transport may convert it from an inactive form to an active form.

1.2.2.3 Registration of the Signal in the Target Cell

A target cell that receives a signal within the framework of intercellular communication transmits the signal in *intracellular* pathways that trigger distinct

biochemical activities in a cell type-specific manner and determine the response of the target cell. Specialized proteins, termed receptors, are utilized for the reception of signals in the target cell. Only those cells that carry the appropriate receptor will be activated for further transduction of the signal into the interior of the cell. The reception of the signals by the receptor is equivalent to the binding of messenger substance on the receptor or the transmission of physical stimuli into a structural change in the receptor which activates the receptor for further signal transduction.

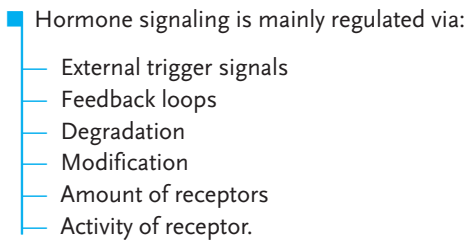
There are two principal ways by which target cells can process incoming signals:

- Cell-surface receptors receive the signal (e.g., a messenger substance) at the outside of the cell, become activated, and initiate signaling events in the interior of the cell. In such signaling pathways, the membrane-bound receptor transduces the signal at the cell membrane so that it is not necessary for the signal to actually enter the cell.
- The messenger enters into the target cell and binds and activates the receptor localized in the cytosol or nucleus.

1.2.3

Regulation of Intercellular Signaling

The result of communication between the signaling and receiving cells is the triggering of multiple biochemical reactions in the target cell. The nature and extent of these reactions depends on many individual reactions that participate either directly or indirectly in signal transduction.



Beginning with the hormone-producing cell, the following processes are all contributing factors for hormonal signal transduction in higher organisms (Figure 1.3):

- **Biosynthesis of the hormone:** The enzymes involved in biosynthesis of a hormone can, for example, be controlled by other signal transduction pathways. Often, feedback mechanisms exist that couple the activity of the biosynthetic enzymes to the concentration of the circulating hormone.
- **Degradation and modification of the hormone:** The active hormone may be inactivated by the metabolism, or inactive hormone precursors may be converted into the active hormone by enzymatic transformation.

- **Storage and secretion of the hormone:** There are signals (electrical signals, Ca^{2+} -signals) to trigger the secretion of stored hormones.
- **Transport of the hormone to the target cell:** The distribution of a hormone via the circulation contributes to the accessibility of that hormone at a particular location of an organism.
- **Reception of the signal by the hormone receptor:** The hormone receptors are primarily responsible for the registration of the signal and the further transduction of the signal in intracellular signaling paths. Therefore, the amount, specificity, and activity of receptors at a target cell is subjected to multiple regulatory mechanisms. For example, the receptor may be down-regulated in response to the amount of circulating hormone, or intracellular signaling paths may control receptor activity from inside the cell (see Section 6.2.4).

All of the above steps are subjected to regulation. A precise control of these steps is at the heart of all developmental programs, and most of the information available on the control of intercellular communication has been gained from developmental studies and from the failure of the control mechanisms, either artificially induced or inborn. The mechanisms for the control of hormone and receptor concentration are mostly based on feedback regulation. Negative and/or positive feedback loops (see Section 3.3.2) are used to adjust the intercellular communication to the development and function of the whole organism. The feedback controls operate mainly at the level of the enzymes involved in hormone biosynthesis, storage or degradation, and via the amount of receptor available for conversion of the extracellular signal into an intracellular response.

1.3

Hormones in Intercellular Signaling

Signaling molecules for the communication between cells are known as hormones, while hormones that are proteins and regulate cell proliferation are known as growth factors. Hormones are either produced in specialized hormone-producing cells, or they may be introduced into the organism as inactive precursors (e.g., vitamins) that require metabolic activation to generate the active form. Examples of the latter type include vitamin D and retinoic acid. Typically, the hormone-producing cells contain biosynthetic pathways that are responsible for production of the hormone; furthermore, hormones may be specifically inactivated by modifying enzymes. Details on the metabolism of hormones are beyond the scope of this book.

1.3.1

The Chemical Nature of Hormones

The chemical nature of hormones is extremely variable.

Hormones can be:

Amino acids and amino acid derivatives

Peptides

Proteins

Nucleotides

Derivatives of fatty acids

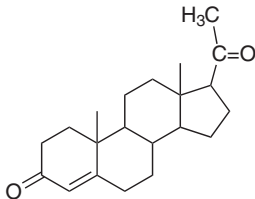
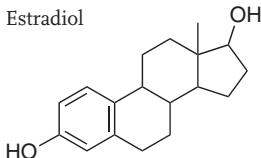
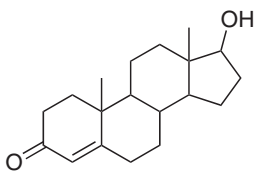
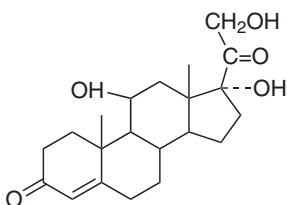
Steroids

Retinoids

Small inorganic molecules, for example, NO

Examples of important hormones are listed in Tables 1.1–1.3.

Table 1.1 Examples for hormones that bind to nuclear receptors.

Hormone	Biochemical and/or physiological function
<i>Steroids</i>	
Progesterone	preparation of the uterus for implantation of the embryo, maintenance of early pregnancy
	
Estradiol	preparation of the uterus to receive the blastocyst, control of uterine contraction, generation of secretory system of breasts during pregnancy
	
Testosterone	differentiation and growth of the male reproductive tract, stimulation of male secondary sex characteristics, skeletal muscle growth
	
Cortisol	metabolism of carbohydrates, lipids and proteins, antiinflammatory, immunosuppressive, induction of Tyr aminotransferase and Trp cyclooxygenase
	

(continued)

Table 1.1 (Continued)

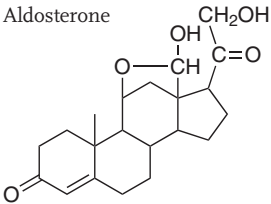
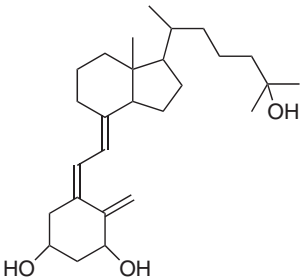
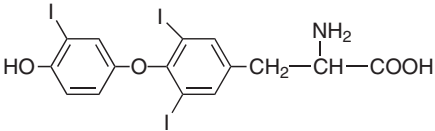
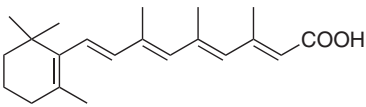
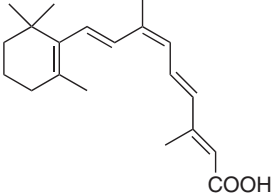
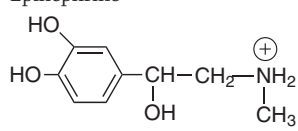
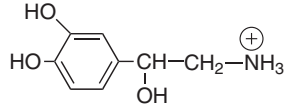
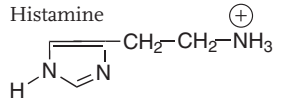
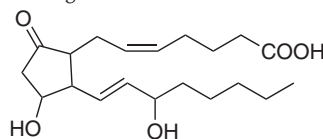
Hormone	Biochemical and/or physiological function
<p>Aldosterone</p> 	water and ion balance, backresorption of ions in the kidney
<p><i>Steroid-related hormones</i> 1,25-Dihydroxycholecalciferol (from vitamin D₃)</p> 	metabolism of Ca ²⁺ and phosphate, bone mineralization, resorption of Ca ²⁺ and phosphate in the intestine
<p><i>Other hormones</i> 3,5,3'-Triiodothyronine (T₃ hormone)</p> 	increased oxygen consumption and increased heat formation, stimulation of glycolysis and protein biosynthesis
<p><i>Retinoids</i> All-<i>trans</i>-retinoic acid</p> 	formed from all- <i>trans</i> -retinal, broad effect on differentiation and morphogenesis
<p>9-<i>cis</i>-retinoic acid</p> 	

Table 1.2 Examples of hormones that bind to TM receptors.

Hormone	Biochemical and/or physiological function
Epinephrine 	raises blood pressure, contraction of smooth muscles, glycogen breakdown in liver, lipid breakdown in adipose tissue
Norepinephrine 	contraction of arteries
Histamine 	relaxation of blood vessels
Derivatives of arachidonic acid Prostaglandin E2 	contraction of smooth muscles

1.3.2

Hormone Analogs: Agonists and Antagonists

The modification of hormones can lead to compounds that are known as agonists or antagonists.

■ **Hormone antagonists:**

— Bind to a receptor and suppress signaling.

■ **Hormone agonists:**

— Bind to a receptor and trigger a physiological response.

1.3.2.1 **Antagonists**

Hormone derivatives that bind to a receptor but suppress signal transduction are termed *antagonists*. Hormone antagonists find broad pharmaceutical and medical application since they specifically interfere with certain signal transduction pathways in the case of hormonal dysregulation. Antagonists with a much higher affinity for a receptor than the unmodified hormone are medically very interesting.

Table 1.3 Peptide hormones and protein hormones.

Hormone	Biochemical and/or physiological function
Glucagon (polypeptide: 29 aa)	glycogenolysis in liver, release of fatty acids from triglycerides in adipose tissue
Insulin (polypeptide, α -chain 21 aa; β -chain 30 aa)	stimulation of glucose uptake in muscle and adipose tissue, catabolism of carbohydrates, storage of triglycerides in adipose tissue, protein synthesis, cell proliferation; inhibition of glycogenolysis
Gastrin (polypeptide: 17 aa)	secretion of HCl and pepsin in stomach
Secretin (polypeptide: 27 aa)	stimulation of secretion of pancreatic proteases
Adrenocorticotropin (polypeptide: 39 aa)	biosynthesis in anterior pituitary, stimulation of formation of corticosteroids in adrenal cortex, release of fatty acids from adipose tissue
Follicle-stimulating hormone (FSH) (polypeptide: α -chain 92 aa; β -chain 118 aa)	stimulation of growth of oocytes and follicle
Thyrotropic hormone (TSH) (polypeptide: α -chain 92 aa; β -chain 112 aa)	release of thyroxine (T_4 hormone) and of T_3 in thyroid gland
TSH releasing hormone (peptide: 3 aa)	formation in hypothalamus, stimulates synthesis and release of TSH in anterior pituitary
Vasopressin (peptide: 9 aa)	formation in posterior pituitary, backresorption of water in the kidney, contraction of small blood vessels
Parathyroid hormone (polypeptide: 84 aa)	Formation in parathyroid gland, increase of Ca^{2+} in the blood, mobilization of Ca^{2+} from the bone

aa = amino acids.

Such high-affinity antagonists require very low dosages in therapeutic applications. A few important antagonists and agonists of adrenaline are shown in Figure 1.4. Propranolol, which is an example of a medically important hormone antagonist, binds with an affinity that is three orders of magnitude greater than its physiological counterpart, adrenaline, on the β -adrenergic receptor, such that very effective blockade of the adrenaline receptor is possible. Some antagonists have been classified as “neutral” (see Section 7.3.2.2); these have no effect on signaling activity but can prevent other ligands from binding to the receptor.

1.3.2.2 Agonists

Hormone analogs that bind specifically to a receptor and initiate the signal transduction pathway in the same manner as the genuine hormone are termed *agonists*. By their influence on the receptor, agonists have been classified further into subcategories (see also Section 7.3.2.2):

- *Full agonists* are capable of maximal receptor stimulation.
- *Partial agonists* are unable to elicit full activity even at saturating concentrations.

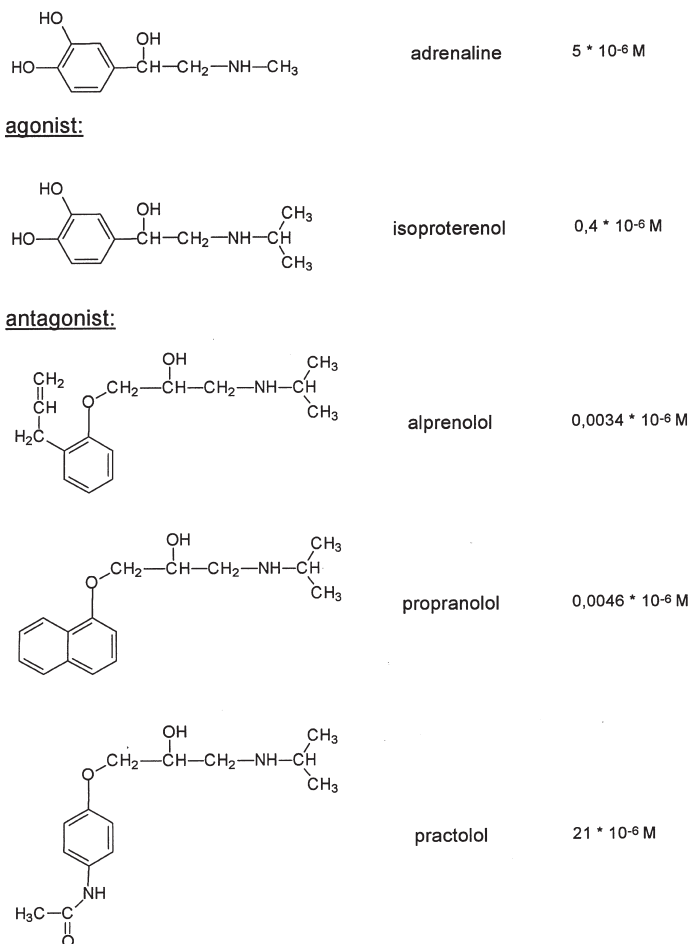


Figure 1.4 Structure of important agonists and antagonists of adrenaline, and their affinity for the β -adrenergic receptor.

- *Inverse agonists* reduce the level of basal or constitutive activity below that of the unliganded receptor.

The ability of hormone derivatives to function as an agonist or antagonist may depend on the cell type under investigation. A notable example is the synthetic estrogen analog tamoxifen, which in some tissues functions as an agonist and in other tissues as an antagonist of the estrogen receptor (see Section 6.4.1).

1.3.3

Endocrine, Paracrine, and Autocrine Signaling

Various forms of intercellular communication by hormones can be discerned based on the range of the signal transmission (Figure 1.5).

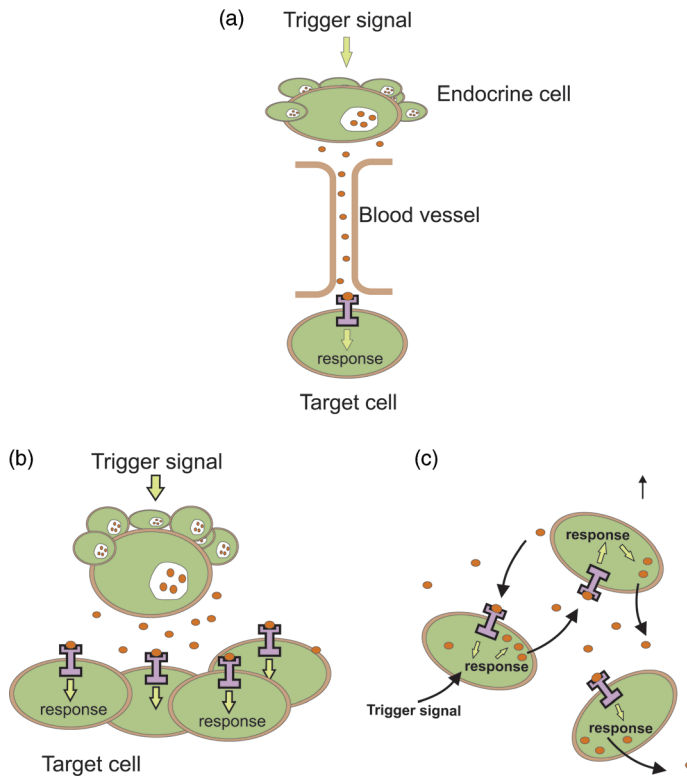


Figure 1.5 (a) Endocrine signal transduction, in which the hormone is formed in the specialized endocrine tissue, released into the extracellular medium, and transported via the circulatory system to the target cells; (b) Paracrine signal transduction, in which the

hormone reaches the target cell, which is found in close juxtaposition to the hormone-producing cell, via diffusion; (c) Autocrine signal transduction, in which the hormone acts on the same cell type as that in which it is produced.

1.3.3.1 Endocrine Signaling

In endocrine signaling, the hormone messenger is synthesized in specific signaling (or endocrine) cells and exported via exocytosis into the extracellular medium (e.g., blood or lymphatic fluid in animals). The hormone is then distributed throughout the entire body via the circulatory system so that remote regions of an organism can be reached. Only those cells or tissues that contain the appropriate receptor for the hormone elicit a hormonal response.

■ Endocrine signaling:

- Production of hormone in endocrine cells
- Transport of hormone to target cell via circulation.

Paracrine signaling:

- Hormone reaches target cell by diffusion
- Close neighborhood of signaling cell and target cell.

Autocrine signaling:

- Hormone-producing cell and target cell are of the same cell type.

1.3.3.2 Paracrine Signaling

Paracrine signal transduction occurs over medium range. The hormone reaches the target cells from the hormone-producing cell by passive diffusion. The producing cell must be found in the vicinity of the receiving cells for this type of communication. The signaling is rather local, and the participating signaling molecules are sometimes termed *tissue hormones* or *local mediators*. One special case of paracrine signal transduction is that of synaptic neurotransmission, in which a nerve cell communicates with either another nerve cell or with a muscle cell.

1.3.3.3 Autocrine Signaling

In autocrine signaling, cells of the same type communicate with one another. The hormone produced by the signaling cell affects a cell of the same type by binding to receptors on these cells, initiating an intracellular signal cascade. If an autocrine hormone is secreted simultaneously by many cells, then a strong response is triggered. Autocrine mechanisms are of particular importance in the immune response.

1.3.4**Direct Protein Modification by Signaling Molecules**

A special case of intercellular signaling is represented by a class of small, reactive signaling molecules, such as nitric oxide (NO; see Section 8.10). NO is synthesized in a cell in response to an external signal and is delivered to the extracellular fluid. NO reaches neighboring cells either by diffusion or in a protein-bound form, and a modification of the target enzymes ensues which results in a change in the activity of these enzymes. NO is characterized as a mediator that lacks a receptor in the classical sense.

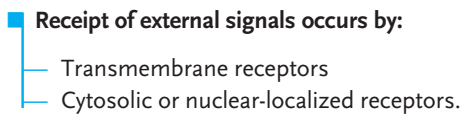
1.4**Intracellular Signaling: Basics**

External signals such as hormones, sensory signals or electrical signals are specifically recognized by receptors that transduce the external signal into an intracellular signaling chain. The intracellular signaling paths control all functions of the cell such as intermediary metabolism, cell division activity, morphology, and the transcription program.

1.4.1

Reception of External Signals

Cells employ two principal methods of transducing external signals into intracellular signaling paths. In the first method, signal receipt and signal transduction occur at the cell membrane by *transmembrane receptors* that register the signal at the cell membrane. In a second method, the messenger passes the cell membrane and binds to the receptor that is *localized in the cytosol* or in the *nucleus* (see Section 1.5.1). Upon receiving a signal, the receptor becomes activated to transmit the signal further. The *activated receptor* passes the signal on to components (usually proteins) further downstream in the intracellular signaling pathway; these components then become activated themselves for further signal transmission. Depending on the nature of the external stimulus, distinct signaling paths are activated and a multitude of biochemical processes are triggered in the cell.



1.4.2

Activation and Deactivation of Signaling Proteins

Intracellular signal transduction occurs in network involving many signaling components that communicate with each other. The key functions in intracellular signaling are performed by proteins that have the ability to specifically recognize, process, and transduce signals. The major signal transducers are:

- Receptors
- Signaling enzymes
- Regulatory GTPases.

In the absence of a signal, the signal transducers exist in an inactive or less-active ground state, but upon receipt of the signal the signal transducers become activated and transit into the active state. Only if they are in the active state is transmission of the signal further to the next signaling component possible. The active state is then terminated after some time by deactivation processes, and the signal transducer transited back into the inactive state from which it may start another round of activation and deactivation (Figure 1.6). A multitude of mechanisms are used to activate the signaling proteins, such as:

- Binding of other signaling molecules
- Conformational transitions
- Covalent modifications
- Membrane targeting
- Compartmentalization.

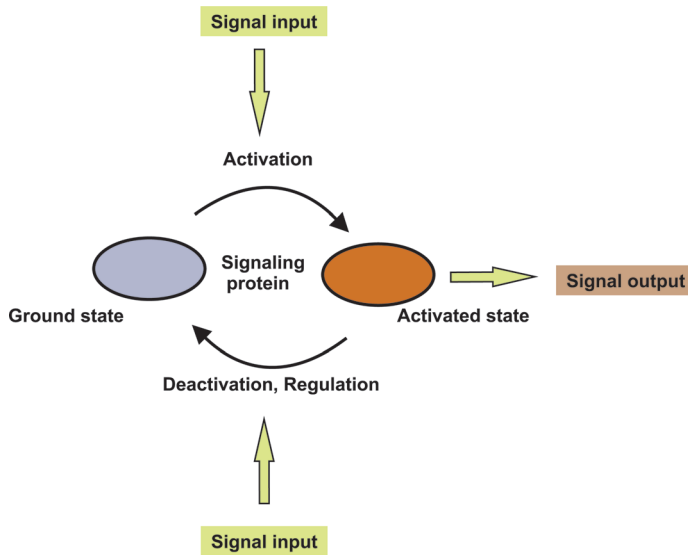


Figure 1.6 Activation and deactivation of signaling proteins. Activating signals trigger a transition from the inactive ground state into the active state, from which signals are passed

on to the next signaling component. Deactivating or regulatory signals limit the lifetime of the activated state and induce a return to the ground state.

These regulatory mechanisms and their functions in signaling organization and networking will be presented in more detail in Chapters 2 and 3.

Following activation, the signaling protein must be deactivated in order to terminate or attenuate signaling. By restraining the lifetime of the activated state with the help of specific deactivation mechanisms, the signal flow can be controlled and fine-tuned, and it can also be coordinated with signaling through other signaling paths. The mechanisms for deactivation are variable.

■ Mechanisms for the activation of signaling proteins:

- Binding of activators (e.g., hormones)
- Signal-induced conformational transitions
- Covalent modifications
- Membrane association
- Removal of inhibitors.

■ Mechanisms for the inactivation of signaling proteins:

- Binding of inhibitors
- Inhibitory modifications
- Removal of activating modifications.

The deactivation mechanism may be intrinsic to the signaling protein and may be enhanced by specific accessory proteins (see GTPases; Chapters 7 and 11). Other deactivation mechanisms use signal-directed inhibitory modifications of the signaling protein, such as phosphorylation. The removal of activating modifications by specific enzyme systems represents another means of terminating signaling. The many methods of activating and inactivating signaling proteins are best illustrated with the example of protein kinases (see Chapter 9).

1.4.3

Processing of Multiple Signals

A signaling protein often must receive several signals simultaneously in order to become fully activated. The ability to process multiple input signals at the same time is based on the modular structure of signaling proteins, many of which are composed of several signaling domains each capable of recognizing a different signal (see Section 2.1). This property allows for the processing of different signals, the fine-tuning and regulation of signaling, and for formation of large interaction networks (see Chapter 3).

1.4.4

Variability of Signaling Proteins

■ Isoforms of signaling proteins:

- Increase variability of signaling
- Have similar, but not identical, signaling properties
- Are encoded by specific genes
- Arise by alternative splicing.

One striking feature of the signaling paths in higher vertebrates is their variability and multiplicity. Different cell types may harbor variants of signaling pathways that control different biochemical reactions. This variability is to a large part due to the existence of subtypes or *isoforms of signaling proteins*. Families of signaling proteins exist whose members have in common a core activity but differ in the details of substrate recognition and regulation. For nearly all signaling proteins, genes encoding the isoforms of a particular signaling protein are found in the genome. In addition, alternative splicing contributes a great deal to the occurrence of multiple forms of signaling proteins (see Section 5.1.2).

1.5

Molecular Tools for Intracellular Signaling

The main tools for intracellular signal transduction comprise the receptors, signaling enzymes, second messengers, and adapter or scaffolding proteins (Figure 1.7). The various signaling components cooperate to trigger specific biochemical activities that underlie the many physiological functions of an

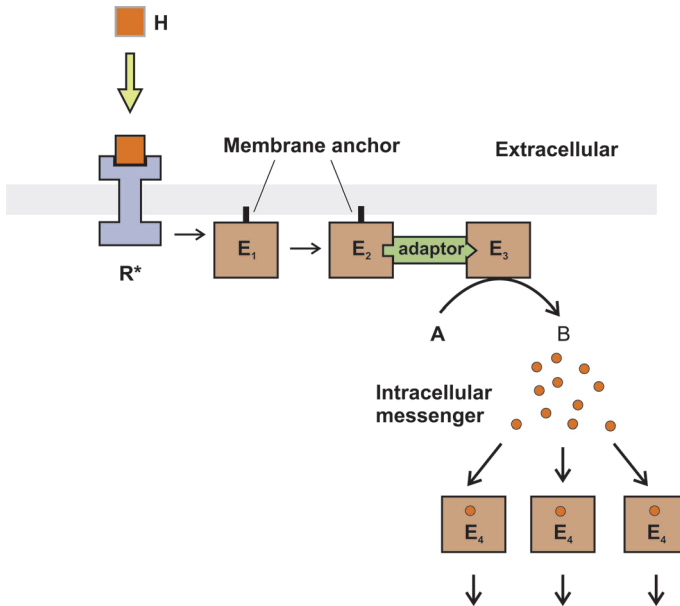


Figure 1.7 Components of intracellular signal transduction. The receipt of an extracellular signal by a membrane receptor (shown here as the binding of a hormone to its receptor) activates the receptor for further signal transduction. The activated receptor R^* passes the signal onto downstream effector proteins, E . Adapter proteins may be involved in the pathways between effector proteins. The transduction of a signal from the receptor to its

downstream effector is usually a membrane-associated process. The example shown in this figure is only to be construed as an example for the composition of a generic signaling pathway. The structure of the intracellular signaling pathways of a cell are highly variable. Some signal transduction pathways are much simpler than that represented in the figure, but others involve many more components and are much more complicated.

organism. In the following subsections, only the basic properties of signaling components will be discussed. The cooperation of signaling molecules and the formation of signaling networks will be detailed in Chapters 2 and 3.

1.5.1

Receptors

1.5.1.1 Receptors Receive External Signals and Trigger Intracellular Signaling

The first step in processing external signals involves receptors that specifically recognize the signal and initiate intracellular signaling. Signals in the form of hormones are usually produced by specialized cells and initiate a reaction in only a certain cell type. Only those cells that possess a cognate protein – the receptor of the hormone – can act as target cells. Hormone receptors specifically recognize and bind the cognate hormone based on their chemical nature. The binding of the hormone to the receptor in the target cell induces an intracellular cascade of reactions at the end of which lies a defined biochemical response.

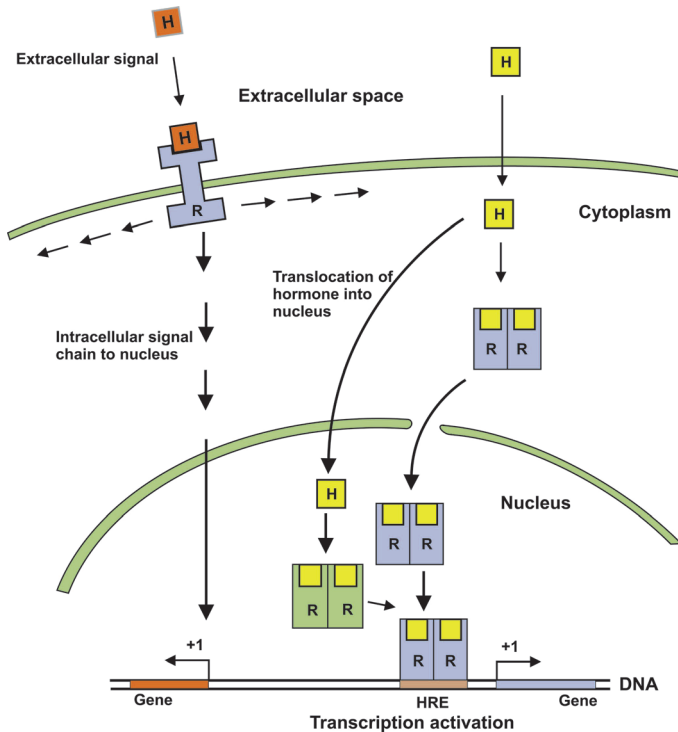


Figure 1.8 Principles of signal transduction by transmembrane receptors and nuclear receptors. Upper part: Transmembrane receptors receive the signal on the cell surface and convert it into an intracellular signal that can be passed on until it reaches the nucleus. Lower part: In signal transduction via nuclear receptors, the hormone enters the cell and

binds the receptor either in the cytosol (R) or nucleus (R'). Nuclear receptors act as nuclear transcription factors that bind specific DNA elements (e.g., HRE: hormone-responsive element) found in the promoter region of regulated genes to control their transcription rate.

In the same way, physical stimuli such as light or pressure can be registered only by those cells that possess the appropriate receptors. An example is rhodopsin in the vision process, where excitation of the receptor by the physical stimulus triggers a conformational change in the receptor that is used for further signal transduction.

The receptors of the target cell can be divided into two classes: (i) membrane-bound receptors; and (ii) soluble cytoplasmic or nuclear-localized receptors (Figure 1.8).

1.5.1.2 Membrane-Bound Receptors

Membrane-bound receptors represent the largest receptor class, and are actually *transmembrane proteins*, in that they display an extracellular domain linked to an intracellular domain by a transmembrane domain. All transmembrane receptors function as *oligomers* (dimers or higher oligomers) composed of identical or different subunits. The binding of a hormone to the extracellular side of the

receptor induces a specific reaction on the cytosolic domain, which then triggers further reactions in the target cell. The mechanisms of signal transmission over the membrane are diverse and will be discussed in more detail in Chapters 7, 10, 13, and 14. One characteristic of signal transduction via membrane-bound receptors is that the signaling molecule does not need to enter the target cell to activate the intracellular signal chain.

■ Transmembrane receptors:

- Receive signals at the extracellular side and transmit the signal into the cytosol
- Structural parts:
 - Extracellular domain
 - Transmembrane domain
 - Cytosolic domain.

1.5.1.3 Intracellular Receptors

The most prominent class of intracellular or cytoplasmic receptors comprises the nuclear receptors that are found in the cytosol and/or in the nucleus (see Chapter 6). In order to activate the nuclear receptors, the hormone must penetrate the target cell by passive diffusion.

■ Intracellular receptors:

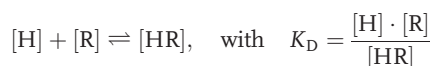
- Are nuclear- and/or cytoplasm-localized
- Function as ligand-controlled transcriptional activators.

The nuclear receptors can be classified as ligand-controlled transcription activators. The hormone acts as the activating ligand, while the activated receptor stimulates the transcriptional activity of genes which carry DNA elements specific for the receptor.

1.5.1.4 The Interaction Between Hormone and Receptor

Receptors are the specific binding partners for signaling molecules; the former are able to recognize and specifically bind the latter based on their chemical structure. Such binding and recognition are governed by the same principles and the same noncovalent interactions as those for the binding of a substrate to an enzyme, namely H-bonds, electrostatic interactions (including dipole–dipole interactions), van der Waals interactions, and hydrophobic interactions. In most cases, receptors bind their cognate signaling molecule with an affinity greater than that usually observed for an enzyme and substrate.

The binding of a hormone to a receptor can in most cases be described by the simple reaction scheme:



where $[H]$ is the concentration of free hormone, $[R]$ is the concentration of the free receptor, and $[HR]$ is the concentration of hormone–receptor complex. The value for the equilibrium constant of dissociation, K_D , usually lies in the range of 10^{-6} to 10^{-12} M. This simple formalism is applicable only to cytoplasmic receptors; for membrane-bound receptors a quantitative treatment of the binding equilibrium is much more difficult.

■ **Hormone–receptor interaction:**

- Reversible complex formation due to noncovalent interactions
- Regulatory input signal is the concentration of hormone.

A decisive factor for the intensity of signal transmission is the concentration of the hormone–receptor complex, as activation of the signal pathway requires this complex to be formed. The concentration of the complex depends on the concentration of the available hormone, the affinity of the hormone for the receptor, and the concentration of the receptor. All three parameters represent – at least in principle – control points for signal transduction pathways. The variable signal – a change in which is registered to thereby activate a signal transmission – is in most cases the concentration of the freely circulating hormone. An increase in the concentration of freely circulating hormone, triggered by an external signal, leads to an increase in the concentration of the hormone–receptor complex, which in turn results in an increased activation of subsequent reactions in the cell. A major switch for the activation of an intracellular signaling pathway is therefore a signal-directed increase in the concentration of the freely circulating hormone ligand.

■ **Receptor signaling depends on:**

- Hormone concentration
- Receptor concentration
- Receptor activity and modification.

1.5.1.5 Regulation of Receptor Activity

The activity of receptors is tightly regulated in order to adapt signaling to the intensity and duration of the extracellular signals. In addition, regulatory mechanisms initiated by intracellular signaling pathways modulate the flow of information through the receptors. The modulation and regulation of signaling through receptors is achieved by multiple mechanisms. The major receptor controls operate at the level of receptor concentration and by receptor modification with concomitant changes in receptor affinity. The amount of receptor present on the cell surface may be regulated via receptor expression, by targeted degradation, and by internalization, all of which processes affect the intensity of the signal transduction on a long time scale. The regulatory receptor modifications are mostly found as phosphorylations introduced in response to signals originating from the same or other signaling pathways.

1.5.2

Signaling Enzymes

Signaling enzymes are at the heart of intracellular signaling. By modifying other enzymes or proteins, signaling enzymes will either carry the signal on or terminate signaling. Much like classical enzymes, signaling enzymes can be regulated by allosteric transitions in response to the binding of effector molecules, by covalent modifications such as phosphorylation, or by membrane targeting. These mechanisms serve to induce the transition of enzymes from an inactive or low active state into the active state, which makes enzymes the ideal instrument for the receipt and transmission of signals. The most prominent signaling enzymes are the protein kinases and protein phosphatases (Chapter 9) involved in the synthesis and degradation of second messengers (Chapter 8), and the regulatory GTPases (Chapters 7 and 11). The most important tool for control signaling processes is the covalent modification of signaling proteins. Nearly all signaling proteins of higher eukaryotes are modified by posttranslational modifications (PTMs), of which many are known (see Section 2.4). The covalent modification of signaling proteins regulates cellular signaling mainly in the following ways:

- PTMs can directly regulate of the activity and function of a signaling protein.
- PTMs often provide attachment points for interaction modules located on partner proteins; in this way, multiprotein signaling complexes may form.
- PTMs may serve to regulate the subcellular distribution of signaling proteins.

The most frequently used tool for signal transmission in a cell is the reversible modification of proteins by phosphorylation or acetylation that serves to either activate or inactivate signaling proteins. The modification status of a protein is controlled by the activity of modifying enzymes (e.g., protein kinases) and demodifying enzymes (e.g., protein phosphatases) (see Chapter 9). Both classes of enzymes are elementary components of signaling pathways, and their activity is subject to manifold regulation. The importance of the protein kinases for cellular functions is illustrated by the large number (almost 500) of these enzymes encoded in the human genome (Chapter 9). An alternative and frequently used modification of signaling enzymes is ubiquitination, which serves primarily to reduce the amount of enzyme available (see Section 2.8). Details of the multitude of regulatory protein modifications, and their functions, are presented in Section 2.4.

■ Signaling enzymes:

- Activate or inactivate other signaling proteins
- Receive and transmit signals
- Produce low-molecular-weight messengers substances, the second messengers
- Switch between active and inactive states.

The following general functions can be attributed to signaling enzymes:

- **Signaling enzymes catalyze the covalent modification of signaling proteins to regulate their activity and subcellular location.**
- **Signaling enzymes catalyze the formation, degradation, or release of small molecule effectors, the second messengers.** The enzymes involved in the formation or degradation of second messengers, such as the phospholipases or the adenylyl cyclases, are major components of signaling pathways (see Chapter 8) and are reversibly activated and inactivated during signal transduction.
- **Regulatory GTPases switch between active and inactive conformations, depending on the binding of GDP or GTP.** The regulatory GTPases (see Chapters 7 and 11) function as switches that can exist in an active, GTP-bound state or the inactive, GDP-bound state. In the active state, the GTPases can transmit signals to downstream components in the signaling chain. In the inactive state, signal transmission is repressed and an activating upstream signal in the form of exchange of bound GDP by GTP is required in order to activate the GTPase for further signal transmission.

1.5.3

Scaffolding Proteins

Scaffolding proteins do not harbor enzyme activities; rather, adapter proteins mediate signal transmission between the proteins of a signaling chain by bringing these proteins together, where they function as clamps to colocalize proteins for effective and specific signaling.

■ Adapter proteins:

- Do not carry enzyme activity
- Provide docking sites for other signaling proteins
- Help to organize multiprotein signaling complexes
- Carry regulatory modifications.

Furthermore, adapter proteins help to target signaling proteins to specific subcellular sites and to recruit signaling molecules into multiprotein signaling complexes.

In the latter case, the adapter proteins may function as a scaffold or docking site for assembling different signaling molecules at distinct sites; the proteins are then also termed docking or scaffolding proteins. Typically, scaffolding proteins contain several binding domains with distinct binding specificities for complementary sites on the target proteins. Furthermore, adapter proteins are often subjected to regulatory modifications, such as phosphorylations, that provide signal-directed docking sites for signaling proteins. The multiple functions of adapter proteins are presented in more detail in Section 3.1.

1.5.4

Diffusible Intracellular Messengers: Second Messengers

The intracellular activation of enzymes in a signaling chain can lead to the formation of diffusible small signaling molecules in the cell. These intracellular signaling molecules, termed “second messengers” (see Chapter 8), activate and recruit cognate enzymes for further signal transduction.

The following properties are important for the function of diffusible intracellular messengers:

- **Second messengers may be rapidly formed from precursors by enzymatic reactions.** Typically, enzymes involved in the formation of second messengers are parts of signaling pathways and are activated during signaling to produce the second messenger in a regulated manner. Often, these enzymes have high turnover numbers and can form a large amount of second messenger, leading to high local concentrations.
- **Second messengers may be rapidly released from intracellular stores.** For example, the second messenger Ca^{2+} is stored in specific compartments and is released from storage upon a regulatory signal. This mechanism provides for the fast and locally controlled production of the second messenger.
- **Second messengers may be rapidly inactivated or stored in specific compartments.** To allow for a termination of the second messenger function, the messengers are degraded by specific enzymes, or are removed by storage or transport into the extracellular medium (see Section 8.5).
- **Second messengers may activate different effector proteins.** Binding sites for a particular second messenger (Ca^{2+} , cAMP) may occur on different signaling proteins. This property allows a given second messenger to regulate multiple target proteins, which leads to a diversification and variability of second messenger signaling.
- **Second messengers allow the amplification of signals.** The enzymatic production of large amounts of a messenger makes an important contribution to the amplification of signals.

■ **Second messengers:**

- May be formed and inactivated by enzymatic reactions
- May be released from stores
- Are cytosolic or membrane-localized
- Activate signaling enzymes
- Allow signal amplification
- Are produced and become active in a timely and locally controlled way.

Currently, two types of second messengers are known:

- *Cytosolic messengers* bind to target proteins in the course of signal transduction functioning as an effector that activates or modulates signaling through the

target protein. The most frequent targets of second messengers are the protein kinases.

- *Membrane-associated messengers* interact with their target protein at the inner side of the cell membrane; in this case the target proteins may also be membrane-associated, or the targets may be recruited to the membrane on binding the second messengers.