1 Basics of Cell Signaling

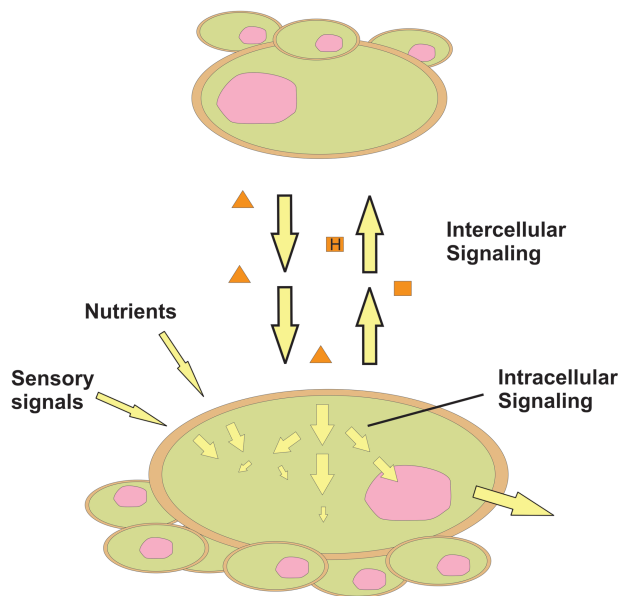## 1.1
Cell Signaling: Why, When and Where?

One characteristic common to all organisms is the dynamic ability to coordinate constantly their 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 adapting the function of an organism to environmental changes in a signal-directed way, other essential features of multicellular organisms require the coordinated control of cellular functions as well.

The formation and maintenance of the specialized tissues of multicellular organisms depend on the coordinated regulation of cell number, cell morphology, cell location and 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 (Fig. 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, 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 programmes.
– Cell motility.
– Cell morphology.

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, many steps are 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.

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, etc., are registered in sensory cells and are transmitted to other cells of the organism via intercellular signaling pathways.

Intercellular signaling controls
– Metabolic fluxes
– Cell division
– Growth
– Differentiation
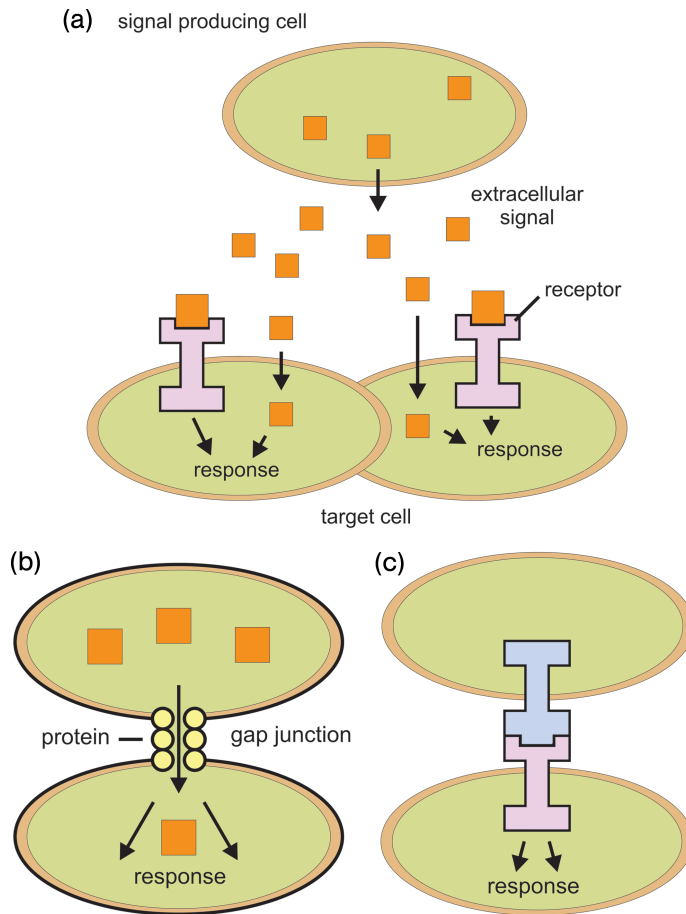– Development
– Processes sensory information

1.2.1
Tools for Intercellular Signaling

We currently know of various forms of communication between cells (Fig. 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.
– *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

Fig. 1.2: Principal mechanisms of intercellular communication. (a) Communication via intercellular messengers. (b) Communication via gap junctions. Gap junctions are direct connections between cells. They are coated by proteins (drawn as circles) that can have a regulatory influence on the transport. (c) Communication via surface proteins.

(a) signal producing cell

extracellular signal

receptor

response

response

target cell

(b)

protein — gap junction

response

(c)

response

■ **Cells communicate via**
– Messenger substances
– Gap junctions
– Surface proteins
– Electrical signals

cells. Communication is then only possible upon direct contact between the target cell with 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 (*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.

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 (Fig. 1.3).

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 like 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 me-
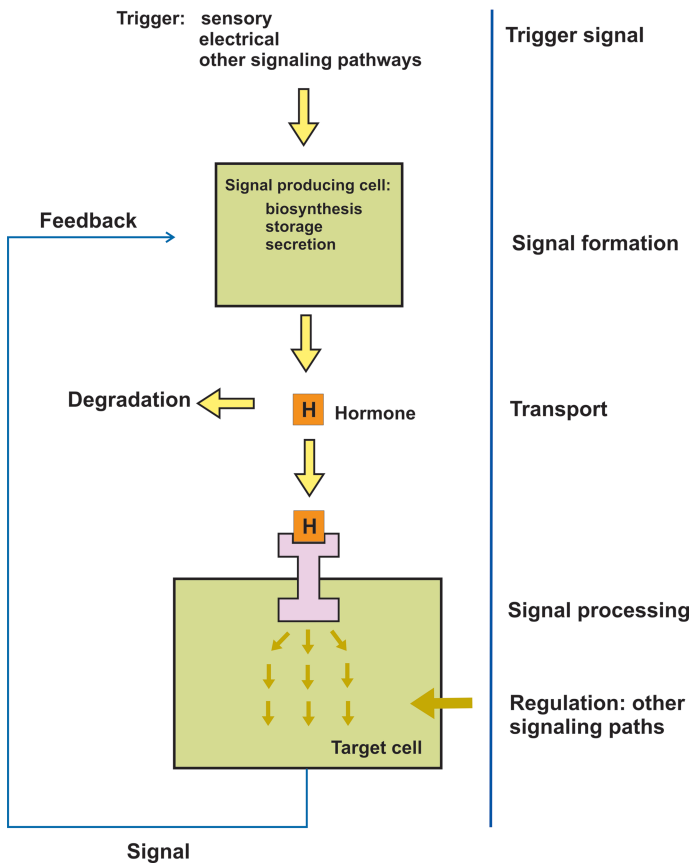
Fig. 1.3: The individual steps of intercellular communication. Upon reception of a triggering stimulus, the signal is transformed into a chemical messenger within the signaling cell. The messenger is secreted and transported to the target cell, where the signal is registered, transmitted further and finally converted into a biochemical reaction. Not shown are processes of termination or regulation of communication which can act at any of the above steps.

chanisms by which the external trigger signals increase the amount of extracellular messenger are diverse, and include stimulation of the biosynthesis of the messenger, increased production of the mature messenger from precursors and release of the messenger from a store form. The latter mechanism is extensively used in the release of hormones of the neural system (neurotransmitters) in response to electrical signals, e.g. at synapses.

■ **Steps of intercellular signaling**
– Trigger signal induces release of stored messenger or stimulates its biosynthesis
– Transport to target cell
– Receipt of signal by the target cell
– Conversion of signal into intracellular signal chain in the target cell

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. This may serve to prevent degradation in the extracellular medium or to provide for docking to specific cells only. Furthermore, processing or metabolization of a messenger during transport may convert it from an inactive form to an active form.

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.

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 a signaling chain 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 a defined biochemical reaction in the target cell. The nature and extent of this reaction 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 (Fig. 1.3):

– *Biosynthesis of the hormone.* The enzymes involved in biosynthesis of a hormone can, for example, be controlled by other signal transduction pathways. There may be feedback mechanisms that couple the activity of the biosynthetic enzymes to the concentration of the circulating enzyme via allosteric mechanisms.
– *Degradation and modification of the hormone.* The active hormone may be inactivated by metabolization 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 are major determinants of the final biochemical reaction in the target cell. We know of several regulatory mechanisms that allow regulation of receptor activity. The receptor may be downregulated in response to the amount of circulating hormone or intracellular signaling paths may control receptor activity from inside the cell (Section 5.2.4).

All of the above steps are subjected to regulation. A precise control of these steps is at the heart of all developmental programs, and we have gained most information on the control of intercellular communication 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 concentrations are mostly based on feedback regulation. Negative and/or positive feedback loops (Section 1.8.1.1) are used to adjust the intercellular communication to the development and function of the whole organism (reviewed in Freeman, 2000). The feedback controls operate mainly at the level of the enzymes involved in hormone biosynthesis, sto-

■ **Hormone signaling is mainly regulated via**
– External trigger signals
– Feedback loops
– Degradation
– Modification
– Amount of receptors
– Activity of receptor

rage or degradation and via the amount of receptor available for conversion of the extracellular signal into an intracellular response. In most cases, multiple, intertwined feedback loops are used to achieve a fine-tuning of the intercellular communication.

1.3
Hormones in Intercellular Signaling

Signaling molecules for the communication between cells are known as *hormones*. Hormones that are proteins and regulate cell proliferation are known as *growth factors*. The hormones are produced in specialized cells (the hormone-producing cells) or they may be introduced into the organism as inactive precursors (e.g. vitamins) that require metabolic activation for generation of 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 the 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.
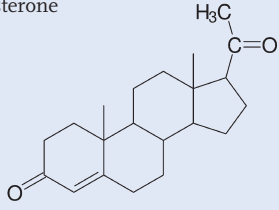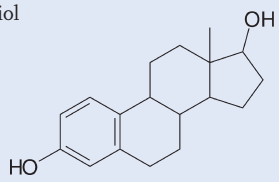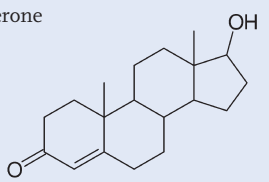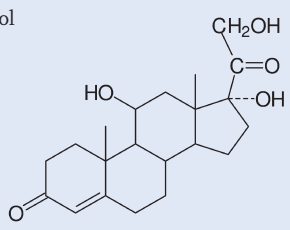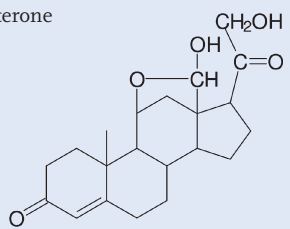
1.3.1
Chemical Nature of Hormones

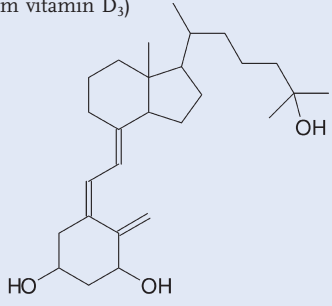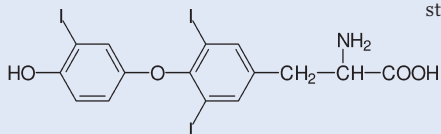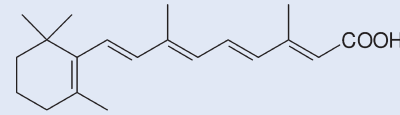The chemical nature of hormones is extremely variable. Hormones can be:
– Proteins.
– Peptides.
– Amino acids and amino acid derivatives.
– Derivatives of fatty acids.
– Nucleotides.
– Steroids.
– Retinoids.
– Small inorganic molecules, such as nitric oxide (NO).
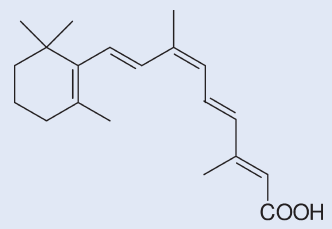
Examples of important hormones are given in Tabs. 1.1–1.3.
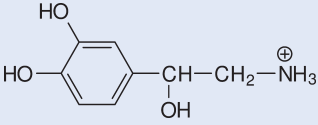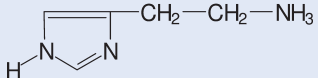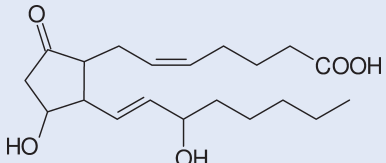
Tab. 1.1: Examples for hormones that bind to nuclear receptors.

Hormone	Biochemical and/or physiological function
Steroids	
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, immunsuppressive, induction of Tyr aminotransferase and Trp cyclooxygenase
Aldosterone	water and ion balance, backresorption of ions in the kidney

Tab. 1.1: Continued.

Hormone	Biochemical and/or physiological function
Steroid-related hormones 1,25-Dihydroxycholecalciferol (from vitamin D_3)	metabolism of Ca^{2+} and phosphate, bone mineralization, resorption of Ca^{2+} and phosphate in the intestine

Other hormones 3,5,3′-Triiodothyronine (T_3 hormone)	increased oxygen consumption and increased heat formation, stimulation of glycolysis and protein biosynthesis

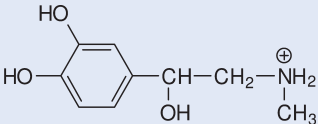|	
Retinoids All-*trans*-retinoic acid	formed from all-*trans*-retinal, broad effect on differentiation and morphogenesis

9-*cis*-retinoic acid

Tab. 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

Tab. 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

Tab. 1.3: Continued.

Hormone	Biochemical and/or physiological function
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.

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 receptor and suppress signaling
Hormone agonists
– Bind to receptor and trigger physiological response

– *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. Such high-affinity antagonists require very low dosages in therapeutic applications. A few important antagonists and agonists of adrenaline are shown in Fig. 1.4. Propranolol is an example of a medically important hormone antagonist. Propranolol binds with an affinity three orders of magnitude greater than its physiological counterpart, adrenaline, on the β-adrenergic receptor. In this manner a very effective blockage of the adrenaline receptor is possible.
– *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*. Application in research and medicine is found especially for those agonists which posses a higher affinity for a receptor than the underivatized hormone.

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. In some tissues, tamoxifen functions as an agonist of the estrogen receptor (ER), whereas in other tissues it behaves as an antagonist of the ER (Section 4.1).
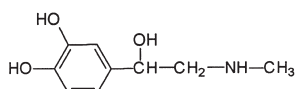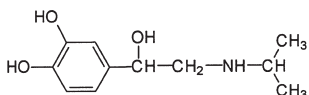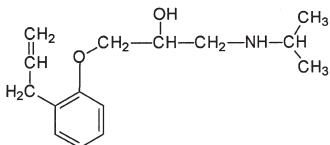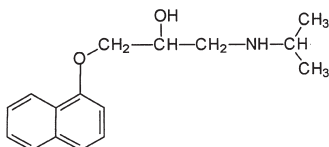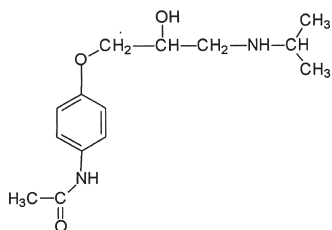
1.3.3
Endocrine, Paracrine and Autocrine Signaling

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

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 elicit a hormonal response that contain the appropriate receptor for the hormone.

Fig. 1.5: Endocrinal, paracrinal and autocrinal signal transduction. (a) Endocrinal signal transduction: the hormone is formed in the specialized endocrinal tissue, released into the extracellular medium and transported via the circulatory system to the target cells. (b) Paracrinal signal transduction: the hormone reaches the target cell, which is found in close juxtaposition to the hormone producing cell, via diffusion. (c) Autocrinal signal transduction: the hormone acts on the same cell type as the one in which it is produced.
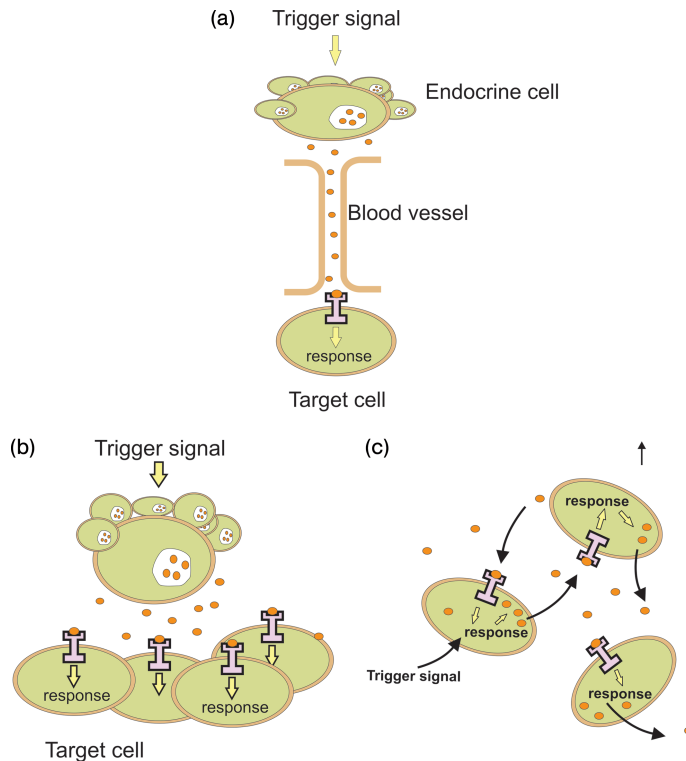
- **Endocrine signaling**
  - Production of hormone in endocrine cells, transport of hormone to target cell via circulation
- **Paracrine signaling**
  - Hormone reaches target cell by diffusion, close neighborhood of signaling cell and target cell
- **Autocrine signaling**
  - Hormone-producing cell and target cell are of the same cell type

Paracrine Signaling

Paracrine signal transduction occurs over the 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*. A special case of paracrine signal transduction is synaptic neurotransmission in which a nerve cell communicates with either another nerve cell or with a muscle cell.

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 occurs in the cells. 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 NO (Section 6.10). NO is synthesized in a cell in response to an external signal and is delivered to the extracellular fluid. Either by diffusion or in a protein-bound form, the NO reaches neighboring cells and modification of target enzymes ensues, resulting 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 or sensory 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 programme.

1.4.1
Reception of External Signals

Cells use two principal ways for transducing external signals into intracellular signaling paths. In one way, the signal receipt and signal transduction occurs at the cell membrane by *transmembrane (TM) receptors* that register the signal at the cell membrane. In the other way, the messenger passes the cell membrane and binds to the *receptor* that is *localized in the cytosol* or in the nucleus (Section 1.5). Upon receiving a signal, the receptor becomes activated to transmit the signal further. The *activated receptor* passes the signal onto components, usually proteins, further downstream in the intracellular signaling pathway, which then become activated themselves for further signal transmission. Depending on the nature of the external stimulus, distinct signaling paths are activated. Finally, specific biochemical processes are triggered in the cell, which represent the endpoints of the signaling pathway.

1.4.2
Activation and Deactivation of Signaling Proteins

■ **Receipt of external signals occurs by**
– TM receptors
– Cytosolic or nuclear localized receptors

Intracellular signal transduction usually comprises many components often acting in a sequential manner where one component passes the signal on to the next component. 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 the signal, the signal transducers exist in an inactive or less-active ground state. Upon receipt of the signal, the signal transducers become activated and transit into the active state. Only if in the active state is further transmission of the signal to the next signaling component possible. The active state is then terminated after some time by deactivation processes and the signal transducer transits back into the inactive state from which it may start another round of activation and deactivation (Fig. 1.6). A multitude of mechanisms are used to activate the signaling proteins. The major mechanisms are:
– Binding of other signaling molecules.
– Conformational transitions.
– Covalent modifications.
– Membrane targeting.
– Compartmentalization.

■ **Mechanisms for activation of signaling proteins**
– Binding of activators, e.g. hormones
– Signal-induced conformational transitions
– Covalent modifications
– Membrane association
– Removal of inhibitors

Fig. 1.6: Activation and deactivation of signaling proteins. Activating signals trigger the 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 return into the ground state.
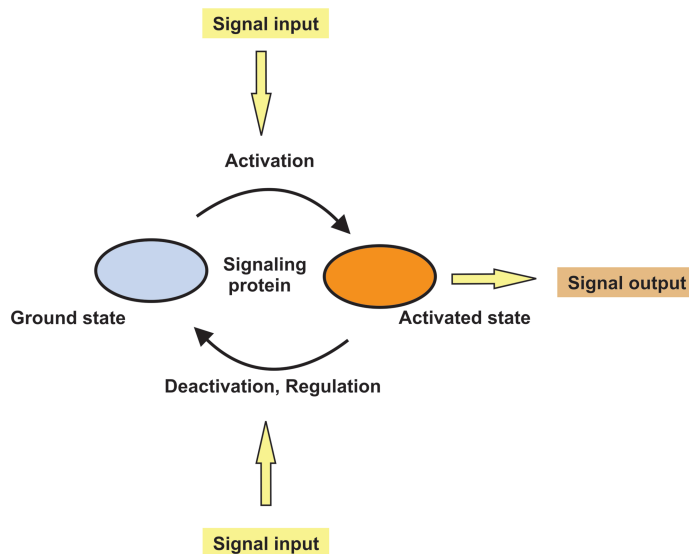
Following activation, the signaling protein must be deactivated in order to terminate or attenuate signaling. By restraining the lifetime of the activated state with the help of specific deactivation mechanisms, the signal flow can be controlled and fine-tuned, and it can be coordinated with signaling through other signaling paths. The mechanisms for deactivation are variable. The deactivation mechanism may be intrinsic to the signaling protein and may be enhanced by specific accessory proteins (see GTPases, Chapters 5 and 9). Other deactivation mechanisms use signal-directed inhibitory modifications of the signaling protein such as phosphorylation. The removal of activating modifications by specific enzyme systems is another way for terminating signaling. The many ways of activating and inactivating signaling proteins are illustrated best by the example of the protein kinases (Chapter 7).

■ **Mechanisms for inactivation of signaling proteins**
– Binding of inhibitors
– Inhibitory modifications
– Removal of activating modifications

1.4.3
Processing of Multiple Signals

A signaling protein often may need to 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 signaling proteins are composed of several signaling domains, each of which may recognize a different signal (Section 1.8). This property allows for the processing of different signals and for the fine-tuning and regulation of signaling.

The main components of intracellular signaling will be discussed in the following; distinct signaling paths will be presented in more detail in later chapters.

1.4.4
Variability of Signaling Proteins

A 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 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.

■ **Isoforms of signaling proteins**
– Increase variability of signaling
– Have similar, but not identical signaling properties
– Are encoded by specific genes
– Arise by alternative splicing

1.5
Molecular Tools for Intracellular Signaling

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

Fig. 1.7: Components of intracellular signal transduction. The reception of an extracellular signal by a membrane receptor, shown here as 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. Adaptor proteins may be involved in the pathways between effector proteins. The transduction of signal from the receptor to its downstream effector is usually a membrane-associated process. The example shown in the diagram above 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. There are signal transduction pathways that are much simpler than the one represented here, and others that involve many more components and are much more complicated.
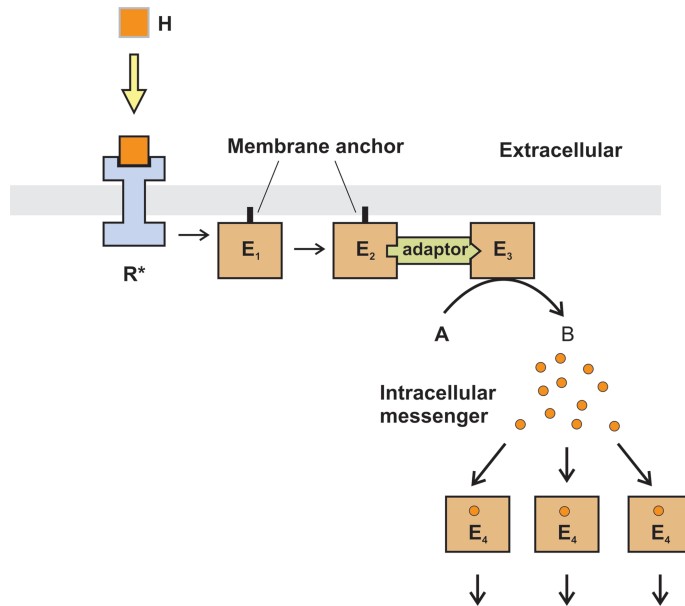
1.5.1
Receptors
Receptors Receive External Signals and Trigger Intracellular Signaling
The first step in processing external signals involves receptors that specifically recognize the signal and initiate intracellular signaling cascades. Signals in the form of hormones are usually produced by specialized cells and initiate a reaction in only a certain cell type. Only those cells that possess a cognate protein, i.e. 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 whose end lies a defined biochemical response.

In the same way, physical stimuli such as light or pressure can be registered only by those cells that possess the appropriate receptors,
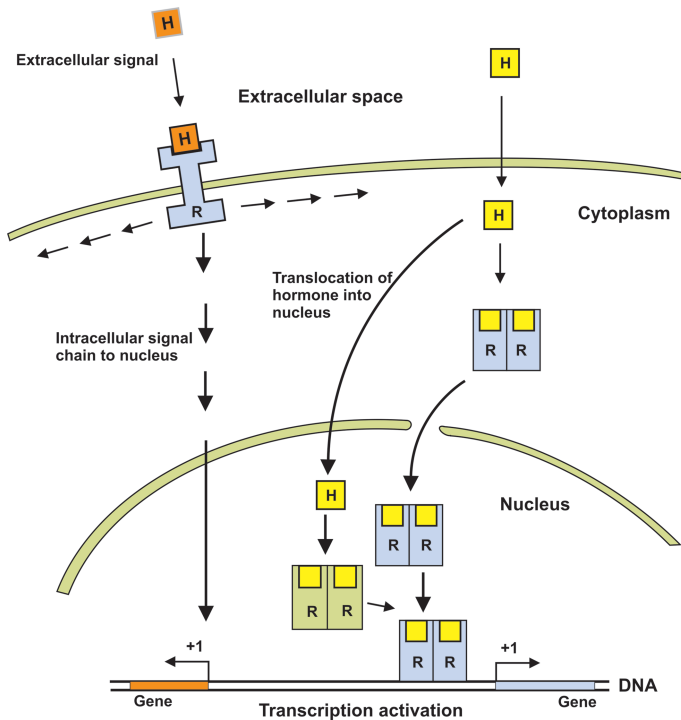
Fig. 1.8: Principles of signal transduction by TM receptors and nuclear receptors. (a) TM receptors receive the signal on the cell surface and convert it into an intracellular signal that can be passed on until it reaches the nucleus. (b) In signal transduction via nuclear receptors the hormone enters the cell and binds the receptor either in the cytosol or nucleus. Nuclear receptors act as nuclear transcription factors that bind specific DNA elements (HRE = hormone-responsive element) found in the promotor region of regulated genes to control their transcription rate.

e.g. rhodopsin in the vision process. Here, 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: the membrane-bound receptors and the soluble cytoplasmic or nuclear localized receptors (Fig. 1.8).

Membrane-bound Receptors

These types of receptors represent the largest class of receptors. The membrane-bound receptors are actually *TM proteins*; they display an extracellular domain linked to an intracellular domain by a TM domain. Many TM receptors are found as *oligomers* (dimers or higher oligomers) composed of identical or different subunits. 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 5, 8 and 11. A 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.

TM receptors
– Receive signals at the extracellular side and transmit the signal into the cytosol

Structural parts
– Extracellular domain
– TM domain
– Cytosolic domain

Intracellular Receptors

The most prominent class of the intracellular or cytoplasmic receptors comprises the nuclear receptors that are found in the cytosol and/or in the nucleus (Chapter 4). To activate the nuclear receptors, the hormone must penetrate the target cell by passive diffusion. The nuclear receptors can be classified as ligand-controlled transcription activators. The hormone acts as the activating ligand; the activated receptor stimulates the transcriptional activity of genes which carry DNA elements specific for the receptor.

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. The 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, i.e. hydrogen bonds, electrostatic interactions (including dipole–dipole interactions), van der Waals interactions and hydrophobic interactions. Signaling molecules bind their cognate receptors 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:

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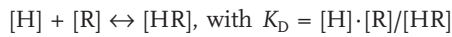
where [H] is the concentration of free hormone, [R] is the concentration of the free receptor and [HR] is the concentration of hormone–receptor complex. The value for the equilibrium constant of dissociation, K_D, usually lies in the range of 10^{-6} to 10^{-12} M. This simple formalism is only applicable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

Decisive for the intensity of the signal transmission is the concentration of the hormone–receptor complex, since the activation of the signal pathway requires this complex to be formed. The concentration of the hormone–receptor 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, whose change 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 results in an increased activation of subsequent reactions in the cell. A major switch for the activation of an intracellular signal-

Intracellular receptor
– Nuclear and/or cytoplasmic localized
– Function as ligand-controlled transcriptional activators

Hormone–receptor interaction
– Reversible complex formation due to noncovalent interactions
– Regulatory input signal is the concentration of hormone

ing pathway is therefore a signal-directed increase in the concentration of the freely circulating hormone ligand.

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. 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. These processes affect the intensity of the signal transduction on a long timescale. The regulatory receptor modifications are mostly found as phosphorylations that are introduced in response to signals originating from the same or other signaling pathways.

■ **Receptor signaling depends on**
– Hormone concentration
– Receptor concentration
– Receptor activity and modification

1.5.2
Signaling Enzymes

Signaling enzymes are at the heart of intracellular signaling. Enzymes can be regulated by allosteric transitions in response to 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, making enzymes the ideal instrument for the receipt and transmission of signals. The most prominent signaling enzymes are the protein kinases and protein phosphatases, the enzymes involved in synthesis and degradation of second messengers and the regulatory GTPases. We know of different ways by which enzymes participate in signaling:

– *Signaling enzymes modify other enzymes or proteins to carry the signal on or to terminate signaling.* The most frequently used tool for signal transmission in a cell is the reversible modification of proteins by phosphorylation that serves to activate or inactivate signaling proteins. The phosphorylation status of a protein is controlled by the activity of protein kinases and protein phosphatases (Chapter 7). 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 (more than 500) of different protein kinases encoded in the human genome. Further examples of regulatory protein modifications will be presented in Section 1.6.1.

■ **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

– *Signaling enzymes can 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 (Chapter 6), 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 (Chapter 5) 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
Adaptors and Scaffolding Proteins

■ **Adaptor proteins**
– Do not carry enzyme activity
– Provide docking sites for other signaling proteins
– Help to organize multiprotein signaling complexes
– Carry regulatory modifications

Adaptor proteins (Chapter 8) do not harbor enzyme activities. Rather, adaptor proteins mediate the signal transmission between proteins of a signaling chain by bringing these proteins together. They function as clamps to colocalize proteins for an effective and specific signaling. Furthermore, adaptor proteins help to target signaling proteins to specific subcellular sites and to recruit signaling molecules into multiprotein signaling complexes.

In the latter case, the adaptor proteins may function as a scaffold or docking site for assembly of 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, adaptor proteins are often subjected to regulatory modifications, e.g. phosphorylations, that provide signal-directed docking sites for signaling proteins.

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 are also termed "second messengers" (Chapter 6). The second messenger molecules activate and recruit cognate enzymes for the 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, the enzymes involved in 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 the 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 they are removed by storage or transport into the extracellular medium (Section 6.5). As for the messenger-producing enzymes, the messenger degrading enzymes can be part of signaling paths and are regulated by distinct signals.

– *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 amplification of signals.* The enzymatic production of large amounts of a messenger makes an important contribution to the amplification of signals. Typically, the enzymes involved in the metabolism of the second messengers are activated by upstream signals. During the lifetime of the activated state, the enzymes may produce large amounts of the second messenger allowing for the activation of a large number of further downstream messenger targets.

Contrary to what is suggested by the term "diffusible intracellular messengers", these signaling molecules normally do not diffuse across the whole cytoplasmic space. Rather, the second messengers are often used to create signals that are limited in time and in space. The second messengers can be formed as well as inactivated in specific compartments and at specific sites of the cell membrane resulting in locally and timely restricted reactions (see Ca^{2+} signaling, Chapter 6). We know of two types of second messengers: the *cytosolic messengers* and the *membrane-associated messengers*. 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 mes-

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

sengers 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 are recruited to the membrane upon binding the second messengers.

1.6
Basic Mechanisms of Intracellular Signaling

1.6.1
Regulatory Modifications

Posttranslational protein modification by covalent attachment of chemical groups to the side-chains of amino acids is a major mechanism by which protein function is regulated in eukaryotes. In cell signaling, regulatory modifications of signaling proteins play a key role in creating, transducing and fine-tuning signals. In addition, protein modification is at the heart of the transcription programme of the cell. Most eukaryotic proteins are modified posttranslationally in one or another form and over 200 protein modifications have been identified (Mann and Jensen, 2003).

We know of stable modifications, e.g. disulfide formation, glycosylation, lipidation and biotinylation, that are essential for vital functions of mature proteins such as compartmentalization, transport and secretion. Many other covalent modifications are transient and are introduced into proteins for regulatory purposes. The reversible modifications may be considered as signals that are transduced by the modifying enzymes to a signaling protein. Such modification signals control the activity, macromolecular assembly and location of signaling proteins, and as such are major tools for shaping signaling pathways.

Based on their function, the regulatory modifications may be divided into two categories:

Regulatory modifications of signaling proteins
– Are introduced in a signal-controlled way
– May induce activity changes by allosteric mechanisms
– May provide attachments points for target proteins

– Modifications can serve to modulate and regulate the activity of the signaling protein by *conformational and allosteric mechanisms*. As an example, the phosphorylation of protein kinases in the activation loop increases their activity by stabilizing an active conformation (Chapter 7).
– Posttranslational protein modification is used to create *attachment points* for the binding of upstream or downstream effectors in signaling pathways and for the assembly of larger protein complexes. The modifications are recognized by interaction modules on proteins that show specificity of the cognate modification. By this strategy, the posttranslational modifications

serve to increase the specificity of interacting proteins during the formation of larger signaling complexes.

An important aspect of regulatory protein modifications is their reversibility. To serve as a regulatory tool, the modifications must be introduced in a signal-directed way and must be removed upon demand. Typically, formation and removal of the adducts is catalyzed by specific enzymes that are activated in a signal-directed way. The enzymes responsible for introducing and removing the modifications are therefore also essential elements of signaling paths.

Examples of Regulatory Protein Modifications
The most important regulatory protein modifications are:
– Ser/Thr phosphorylation (Chapter 7).
– Tyr phosphorylation (Chapters 7 and 8).
– Lysine acetylation (Section 3.5.2).
– Lysine and arginine methylation (Section 3.5.3).
– Lysine ubiquitination (Section 3.5.5).
– Cysteine oxidation (Section 3.4.6).
– Cysteine nitrosylation (Section 6.10).

The modifications may be of small size, e.g. phosphate or methyl groups, or they may comprise complete small proteins such as ubiquitin. In most cases, specific modifying and demodifying enzymes exist that introduce or remove the modification in a regulated manner. Some modifications, e.g. nitrosylation or cysteine oxidation, do not require specific enzymes for the transfer of these groups to the target enzyme. Here, the intrinsic chemical reactivity of the modifying group is often the major determinant for formation of the covalent adduct. However, specific accessory proteins may be necessary to direct the modification to distinct target sites in these cases.

1.6.2
Recognition of Protein Modifications by Modification-specific Protein Modules

A major function of protein modifications is to provide attachment points for upstream or downstream effector proteins in signaling pathways or to guide the assembly of large protein complexes. The protein partners recognize the posttranslational modifications via *interaction modules* that are able to specifically detect and bind a particular modification. Thus, most of the protein modifications with functions in cell signaling have cognate interaction domains on partner proteins that recognize the chemical nature of the modification. In addition to the modification itself, amino acids C- or N-terminal to

Modification-specific interaction modules
– Are often found as independently folding domains
– Exist as multiple subtypes
– Bind to distinct protein modifications

Fig. 1.9: Structural basis of modular protein interaction domain function. Domain-peptide binding on the example of the SH3 domain of the protein kinase Csk. The folding of the SH3 domain shows a close proximity of the C- and N-terminus of the module. The binding site for the peptide ligand Pep-PEST is exposed on the surface of the module. Pep-PEST is derived from the C-terminal tail of the tyrosine phosphatase PEP. From Pawson and Nash (2003).
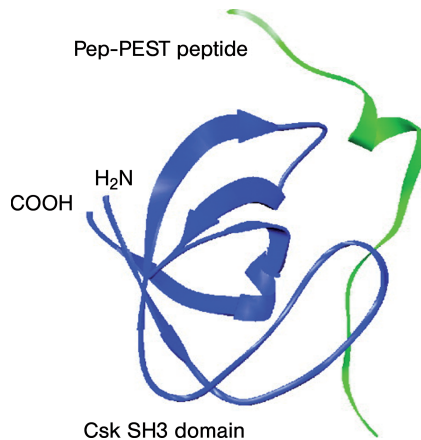
the modification are often used by the interactions domains to select a modification for binding. Importantly, subtypes exist for most interaction domains that recognize the same chemical modification, but differ in the requirements for the neighboring amino acids – a property that serves to create a high diversity of interaction domains. Several interaction domains are present in hundreds of copies in the human proteome and families of interaction domains can be identified that require the same chemical modification but recognize different neighboring sequences. For example, about 115 SH2 domains recognizing phosphotyrosine residues are encoded by the human genome, each of them differing in the details of the sequence requirements of the neighboring amino acids.

Isolated interaction domains can usually fold independently, with their N- and C- termini juxtaposed in space (Fig. 1.9) and are readily incorporated into larger polypeptides in a manner that leaves their ligand-binding surface available. Typical signaling proteins harbor several interaction modules and are thus able to use different protein modifications as attachment points. A more detailed discussion of interaction domains is given below in Section 1.7.

1.6.3
Multisite Protein Modification

Signaling proteins in higher eukaryotes only rarely carry just a single modification. Many proteins involved in cellular regulation are modified at multiple sites – a phenomenon referred to as *multisite modification*. The multiplicity of modification sites on a protein often correlates with its biological importance and the complexity of the corresponding organism. Many proteins perform complex functions in

cell signaling by receiving and transducing different signals. Such multifunctional proteins have several interaction partners and use multiple modifications to produce specific signaling outputs in a dynamic and fine-tuned way. Examples of such proteins include the CDC25 phosphatases (Section 13.7), receptor tyrosine kinases (RTKs; Chapter 8), protein kinase C (PKC; Section 7.5) and the histones (Section 3.3.6). Furthermore, many transcriptional regulators of vertebrates are subjected to multisite modification. The complexity of multisite modifications is illustrated best by the example of the tumor-suppressor protein p53 that is phosphorylated, acetylated, sumoylated and ubiquitinated on many sites (Section 14.6.3).

The following characteristics of multisite modification are important for intracellular signaling:

– *The same modification may occur on several sites of a signaling protein* (e.g. 10 Ser/Thr phosphorylation sites on p53). Each of the sites may be modified by distinct members of an enzyme class (e.g. distinct Ser/Thr-specific protein kinases) and each modification may serve a different function. The neighboring amino acids then specify the functional role of a modification by binding to isoforms of the cognate modification-specific interaction module.

– *The same amino acid can be subject to different modifications.* For example, lysine residues can be modified by acetylation, methylation (Section 3.5), ubiquitination, neddylation and sumoylation (Section 2.5). Lysine methylation leads to the addition of up to three methyl groups to the ε-amino group and, importantly, different methylation stages have different biological consequences (Section 3.5.3). The different lysine modifications may function in a competitive way, one modification excluding the other.

– *The presence of multiple modifications of the same or different type can be considered as a "bar code"* that specifies a distinct function of the signaling protein. The "bar code" controls the catalytic activity of the signaling protein as well as the association of interaction modules, and the code forms the basis of a regulatory programme for the qualitative and quantitative control of its signaling function. An important aspect of multisite modification is the reversibility and the dynamic nature of the modifications. The modification patterns formed change with time and subcellular location which provides a control of function in time and space.

– *Multiple modifications on a protein often show combinatorial characteristics.* The effect of a given modification may be context dependent, e.g. the presence of one modification prevents the modification at another site. Multisite modification events frequently interplay with each other and a cooperative effect of modification events may be observed. In simple scenarios, modifications at two sites can be independent of each other with

Multisite modification of signaling proteins
– Same modification can occur on several sites
– Different modifications can occur on the same protein
– Same amino acid can be differently modified
– Can be considered a "bar code"
– Can show combinatorial characteristics

each being sufficient to achieve the maximal output. Multiple modifications of the same type could also have additive effects, thereby producing a linear output. In complex scenarios, however, the modifications at different sites can synergize with each other to generate an exponential output, thereby functioning as a combined switch. Such systems can function as coincidence detectors that require multiple modification signals to create a distinct biological output. An example of a nonlinear response to multisite modification is the protein kinase inhibitor Sic1. This inhibitor harbors at least nine Thr phosphorylation sites of which six must be phosphorylated before it is recognized by the CDC4 protein which is a component of the SCF ubiquitination complex (Nash et al., 2001). The F-box protein CDC4 captures the phosphorylated forms of Sic1 for ubiquitination in late G_1 phase, an event necessary for the onset of DNA replication. Interestingly, structural analysis of CDC4 in complex with phosphopeptides suggests that CDC4 contains only one strong phospho (p)-Thr binding site that is surrounded by suboptimal p-Thr binding sites. The mechanistic basis for the cooperative behavior is not well understood. According to one hypothesis (Orlicky et al., 2003), phosphorylation of multiple Thr sites on Sic1 increases the local concentration of sites around CDC4 once the first p-Thr site is bound, to the point where diffusion-limited escape from CDC4 is overwhelmed by the probability of rebinding of any one p-Thr site. In a sense, Sic1 becomes kinetically trapped in close proximity to CDC4. This example illustrates how multiple weak, spatially separated ligand sites can be used to cooperatively interact with a single receptor site. For more examples on multisite modification, see Yang (2005).

1.6.4
Protein Interaction Domains

Most signaling proteins are constructed in a cassette-like fashion from domains that mediate molecular interactions or have enzymatic activity (reviewed in Pawson and Nash, 2003). The molecular interactions mediated by interaction domains provide a fundamental means for organizing signaling pathways and signaling networks. Interaction domains can target proteins to a specific subcellular location, providing a tool for recognition of protein modifications or chemical second messengers.

Furthermore, interactions domains are used to assemble multiprotein signaling complexes, and they control the conformation, activity and substrate specificity of enzymes. Typical interaction domains fold independently and recognize exposed sites on their protein partners or they bind to the charged headgroups of phospholipids in

Protein interaction domains
- Fold independently
- Exist as multiple subtypes
- Assemble signaling complexes
- Control enzyme activity

membranes. Some domains serve specific functions by binding to distinct protein modifications. SH2 domains, for example, generally require phosphotyrosine sites in their primary ligands and are therefore dedicated to tyrosine kinase signaling (Chapter 8). Other domains can bind sequence motifs found in a broader set of proteins and display a wider range of biological activities. SH3 domains, for example, recognize Pro-containing motifs and thereby regulate a diversity of processes such as signal transduction, protein trafficking, cell polarization and organelle biosynthesis. The cell therefore uses a limited set of interaction domains, which are joined together in diverse combinations, to direct the actions of regulatory systems.

Binding Properties of Interaction Domains

Most interaction domains can be grouped into classes by sequence comparison. Complexity is, however, introduced (i) by the ability of a particular domain class to recognize distinct motifs, (ii) by the presence of separate ligand-binding sites within an individual domain and (iii) by the importance of ligand conformation in domain recognition. Often, the same class of interaction domain occurs twice in a signaling protein. As examples, the signaling protein Ras–GAP (Section 9.5.6) harbors two SH2 domains and the protein phosphatase SHP2 contains two SH3 domains. Interaction domains can also be assembled from repeated copies (up to 50) of small peptide motifs, yielding a large interaction surface with multifaceted binding properties. Such repeats include ankyrin repeats, Armadillo repeats, leucine-rich repeats, among others.

Interaction domains are remarkably versatile in their binding properties. An individual domain can engage several distinct ligands, either simultaneously or at successive stages of signaling. For example, the MH2 domain of the SMAD proteins (Chapter 12) harbors an extended binding surface that is able to interact with p-Ser motifs, with the scaffolding protein Sara and with components of the transcription apparatus.

Ligands and Types of Interaction Domains

The interaction domains of signaling proteins bind modified amino acid side-chains, peptides or proteins. The interaction domains may be classified by the characteristics of their ligands (Fig. 1.10). Interaction domains may recognize:

– *Protein modifications* (Fig. 1.10a). As outlined above (Section 1.5), a large number of posttranslational modifications of signaling proteins exist that serve as attachment points for partner proteins during formation of signaling complexes. Posttranslational modifications frequently complete binding sites for interaction domains in protein–protein assemblies and make a major contribution to
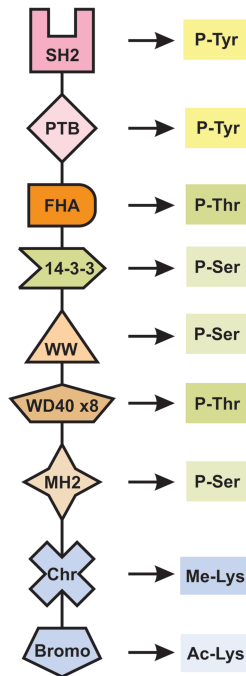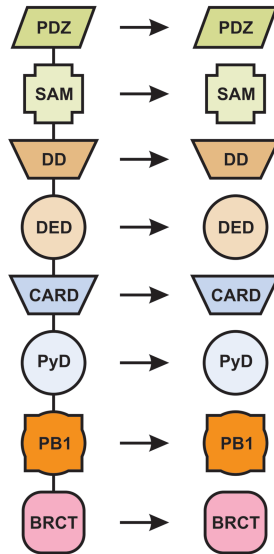
the specificity of the interaction. Thus, the interaction domains serve as detectors of posttranslational protein modifications formed in the course of signaling events.

Protein interaction domains bind to
– Distinct protein modifications
– Sequence motifs
– Other protein domains
– Membrane-bound phosphatidylinositides

– *Peptide motifs* (Fig. 1.10b). This class of interaction domains binds to short peptide motifs that are exposed on the ligand surface. As an example, the SH3 domain (Section 8.2.2) binds to proline-rich motifs of protein ligands and by this property regulates many cellular functions The specificity of binding may be quite low and the biological activities that are regulated by this type of interaction are diverse.

– *Protein domains* (Fig. 1.10c). A number of modular domains undergo homo- or heterotypic domain–domain interactions rather than binding short peptide motifs. Such domains frequently identify proteins involved in a common signaling process and then direct their coassembly into functional oligomeric complexes. Components of apoptotic or inflammatory signaling pathways are characterized by death domains or close structural relatives thereof that form heteromeric structures required for caspase dimerization and activation (Chapter 15). The distinction between domains that bind peptide motifs and those that interact with other folded domain structures is by no means absolute. PDZ domains (Section 8.2.5), for example, generally recognize short peptide motifs of around four residues at the extreme C-termini of their binding partners, but they can also mediate specific heterotypic PDZ–PDZ domain interactions.

– *Phospholipids* (Fig. 1.10d). Many signaling processes are intimately linked to the cell membrane and the recruitment of signaling proteins to the cell membrane is frequently an essential step in signaling (see also Section 2.6). One mechanism for the attachment of signaling proteins to the cell membrane uses membrane phospholipids that are recognized by phospholipid-binding interaction modules on signaling proteins. The specificity of the phospholipid-binding domains is not well characterized and appears to be rather broad. Some phospholipid-binding domains have been reported to bind to peptide motifs too. More details on the function and properties of the phospholipid-binding domains will be found in Section 8.2.3.
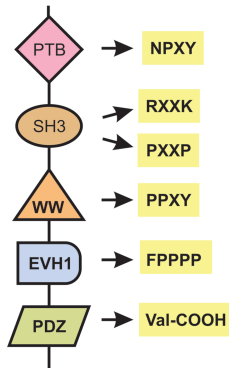
(a) Modified peptides as targets of signaling domains

SH2 → P-Tyr
PTB → P-Tyr
FHA → P-Thr
14-3-3 → P-Ser
WW → P-Ser
WD40 x8 → P-Thr
MH2 → P-Ser
Chr → Me-Lys
Bromo → Ac-Lys

(b) Peptides as targets of signaling domains

PTB → NPXY
SH3 → RXXK, PXXP
WW → PPXY
EVH1 → FPPPP
PDZ → Val-COOH

(c) Domain-domain interactions in signaling complexes

PDZ → PDZ
SAM → SAM
DD → DD
DED → DED
CARD → CARD
PyD → PyD
PB1 → PB1
BRCT → BRCT

(d) Pospholipids as targets of signaling domains

PH → PI(3,4,5)P$_3$ PI(4,5)P$_2$
FYVE → PI(3)P
C1 → DAG
FERM → PI(4,5)P$_2$
C2 → PA/PS
ENTH → PI(3,4,5)P$_3$ PI(4,5)P$_2$
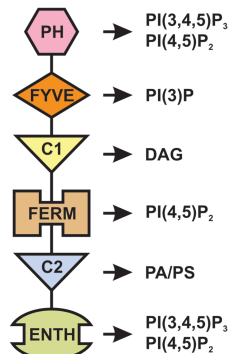
Fig. 1.10: Classification of interaction domains by the nature of the binding substrates. Interaction domains bind modified amino acid side-chains, short peptide sequences, other protein domains or phospholipids. (a) Domain-modified peptide interactions. For SH2, PTB, FHA, 14-3-3, WW domains, see Section 8.2. For MH2, see Section 12.1.3. For Chr (Chromo), Bromo domains, see Section 3.5.7. (b) Domain–peptide interactions. For PDZ, see Section 8.2.5. EVH1 = Ena-Vasp homology 1. (c) Domain–domain interactions. For PDZ, see Section 8.2.5. For DD, DED, CARD, PyD, see Chapter 15; For BRCT, see Section 8.2. SAM = sterile α motif; PB1 = phox and Bem1p domain. (d) For C1, see Section 7.5. For PH, FYVE, C2, see Section 8.2. FERM = four point one, ezrin, radixin, moesin; ENTH = epsin N-terminal homology.

1.7
Modular Structure of Signaling Proteins and Signaling Complexes

A characteristic feature of signaling proteins and signaling complexes is their modular construction. Signaling proteins are typically composed of distinct signaling domains or modules, subtypes of which are found in many different signaling proteins. Signaling complexes are frequently also of a modular structure. The signaling proteins assembled in a large signaling complex may be exchanged in a regulated manner and subtypes of signaling proteins may associate depending on the cell type and regulatory signals.

■ **Typical domains of signaling proteins**
– Protein interaction domains
– Catalytic domains
– Regulatory domains
– Membrane-targeting domains

N

```
┌──────────────┐
│  Regulation  │
└──────────────┘
       │
┌──────────────┐
│   Membrane   │
│   targeting  │
└──────────────┘
       │
┌──────────────┐
│    Enzyme    │
│   activity   │
└──────────────┘
       │
┌──────────────┐
│   Protein    │
│  interaction │
└──────────────┘
```
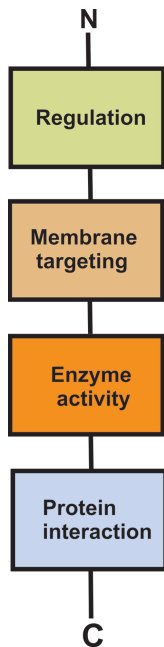
C

Fig. 1.11: Typical domains of signaling proteins. The order of sequence of the domains is variable.

1.7.1
Modules in Signaling Proteins

Typical signaling proteins harbor sites for registration of signals, for transduction of the signal to the next signaling component, for the receipt of controlling signals and for subcellular localization. The multifunctionality of signaling proteins is based on their construction from multiple protein domains that may act independently or in cooperation, and serve distinct functions in signaling (Fig. 1.11). Many signaling proteins harbor interaction domains, sites for post-translational modification and catalytic domains, and these domains may be used to assemble functional signaling complexes, to compartmentalize molecular components and to direct enzymes to their targets. For example, the Tyr modification sites of RTKs serve as attachment points for interaction modules of downstream effector proteins, that in turn may be phosphorylated by the kinase activity of the receptor for further signal transduction and for the association of further signaling proteins (Chapter 8 and Fig. 8.11)

The modular construction endows signaling proteins with the following characteristics:

– *Multivalency*. The presence of multiple modules makes signaling proteins multivalent with respect to interaction partners, regulatory inputs and subcellular localization, and it allows for the association of large signaling complexes. For instance, the platelet-derived growth factor receptor (PDGFR) harbors multiple Tyr-phosphorylation sites that direct the attachment of distinct downstream effectors (Chapter 8 and Fig. 8.11).

– *Differential use of modules*. Modules in signaling proteins may be used simultaneously, in sequential order or in distinct subcellular locations only, allowing for a high versatility and flexibility in signaling. Often, signaling proteins go through cycles of function. In doing this, the modules of the signaling protein may be used in a differential way and the use of one module or modification may

influence the use of other modules or modifications within the same protein. Furthermore, modules may be engaged in a cell-type- and tissue-specific manner, a feature that is central to cell-type- and tissue-specific signaling.

– *Multiple inputs, regulatory influences and outputs.* The construction from multiple modules allows signaling proteins to receive multiple signals and to respond to multiple controlling influences. These inputs may be integrated and converted into differential outputs, depending on the cellular environment.

1.7.2
Modular Signaling Complexes

Interaction domains and protein modifications mediate the association of signaling proteins with upstream and downstream signaling partners, often leading to the formation of large protein complexes in the cytoplasm and nucleus. The organization of these complexes is dynamic and the complexes are often assembled in response to signal input. A large number of proteins may participate in the dynamic formation of the signaling complexes. For example, the *N*-methyl-D-aspartic acid) (NMDA)-type glutamate receptor has been reported to associate with more than 50 different signaling proteins. By the reiterated use of interaction domains, complex machines are built that regulate, targeted proteolysis, endocytosis, protein- and vesicle trafficking, cell polarity, cell division and gene expression, etc. The large complexes allow an efficient and rapid transmission of signals from one signaling component to the other. The interactions involved are illustrated in Fig. 1.12 using the example of the insulin receptor signaling complex.
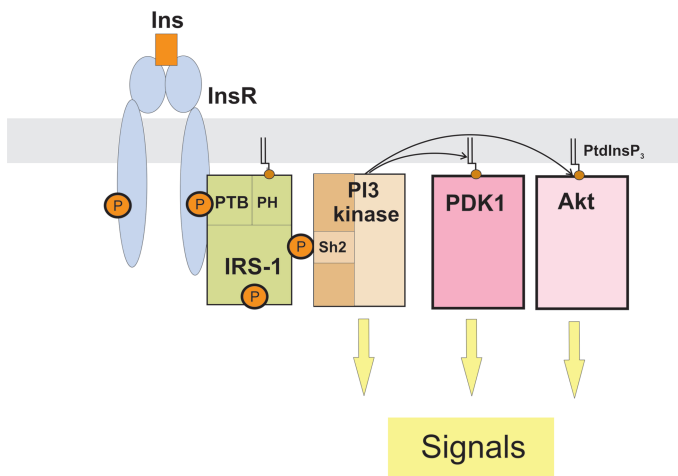
■ **Modules in signaling proteins**
 – Can be differentially used in a time- and location-specific manner
 – Allow for multiple inputs
 – Regulatory influences and outputs
 – Provide for multivalency

■ **Signaling complexes**
 – Harbor multiple signaling proteins
 – Regulate major cellular functions
 – Are of modular structure
 – Allow for specific signaling
 – Allow for coordination of multiple regulatory influences

Fig. 1.12: Insulin signaling complexes. Binding of insulin to the extracellular subunit of the insulin receptor triggers auto-phosphorylation at tyrosine residues on the cytoplasmic part of the receptor. The p-Tyr residues serve as attachment points for the phosphotyrosine binding (PTB) domain of the adaptor protein IRS1 that becomes Tyr phosphorylated too. The p-Tyr residues on IRS1 serve to assemble the PI3K into the signaling complex. PI3K becomes activated and synthesizes the second messenger phosphatidylinositol-3,4,5-trisphosphate (PtdInsP$_3$) that mediates the membrane recruitment and activation of two further protein kinases – Akt kinase and PDK1 (see also Section 7.4).

The organization of signaling proteins in signaling complexes has several distinct advantages:

– *Specificity*. Within a signaling complex, signals can be transduced from one component to the other in a highly efficient way leading to the generation of robust signals. Signal transduction within a complex is rapid because it does not require diffusion of the reactants. The assembly of several components of a signaling path into a multiprotein complex ensures a tight and specific coupling of the various reactions and prevents unwanted dissipation of the signal and side-reactions.

– *Variability*. Signaling components of a signaling complex may be replaced by isoforms that differ in the details of regulation and activity. Such an exchange of signaling components can lead to distinct changes in the output signal. The exchange of components in regulatory complexes appears to be used intensively in gene regulatory complexes (e.g. mediator complexes, see Section 3.2.9) that may associate distinct coactivators, corepressors or chromatin remodeling enzymes depending on the input signals to the system.

– *Regulation*. The components of signaling complexes are often themselves of a modular structure, which allows the receipt of multiple input and regulatory signals in a sequential order or at the same time. Due to the close proximity of the signaling components, regulatory signals can become effective in a rapid and efficient way. For example, we know of signaling complexes that contain both protein kinase and protein phosphatase activity. The presence of two opposing enzyme activities within the same complex is an important tool for the downregulation and termination of signaling events.

1.8
Organization of Signaling

Typically, a large number of signaling components participate in the transduction of an extracellular signal into intracellular biochemical reactions that define the endpoint of signal transduction. To characterize and describe a signal transduction pathway, the number and type of signaling components involved as well as their linkages have traditionally been used. However, it is increasingly recognized that the multivalency and modular structure of signaling proteins, multisite modifications, and the existence of subtypes of signaling proteins allow different signals to enter and to be processed in the same type of signaling path leading to variable outcomes. The description of signaling pathways in terms of their structural organization has therefore proven to become more and more difficult. Signal-

ing pathways have been formerly described as being linearly organized. Now, the features of branching and crosstalk of signaling have made it necessary to include a large number of possible linkages within a signaling path and between different signaling paths. Cell signaling should now be described in terms of signaling networks that endow signaling processes with the properties of plasticity, robustness and variability.

1.8.1
Linear Signaling Pathways, Branching and Crosstalk

The organization of signaling paths in terms of linearity, branching and crosstalk is illustrated in Fig. 1.13(a).

1.8.1.1 Linear Pathways

The classical view of signaling pathways has been that of a sequential transmission of signals in a linear signaling chain. Thereby, a signal is registered by an upstream component of a signaling chain and is then transmitted to the downstream component that then passes the signal on to the next protein in sequence and so on. One component activates the next component in sequence for signal transmission, and signaling is controlled by deactivation mechanisms that are intrinsic to the system or use specific deactivation enzymes. The description of signaling in terms of linear pathways originates form experiments where signaling is initiated by strong signals produced by the overexpression or mutation of signaling proteins or by the exposure of cells to artificially high external signals such as administration of hormones. High signal intensity tends to drive signal transmission through distinct routes, activating biochemical reactions that do not always correspond to the biological response obtained in the *in vivo* situation. It is well known that the routing of signals may depend on signal intensity (amplitude) and also on the duration of the signal (frequency). Nevertheless, the description of signaling in terms of linear pathways is useful for the illustration of the main reaction steps in a signaling cascade and the focus on the main steps serves to outline the logic of a signaling pathway. In many cases, the importance of alternative reactions or of branching reactions is not well established experimentally. Therefore, many of the reaction pathways described in the following chapters of this book are assumed to transmit signals along linear tracts resulting in regulation of discrete biochemical reactions and cellular functions.

Linear signaling pathways
- Signal flows in a linear manner through hierarchically organized signaling components
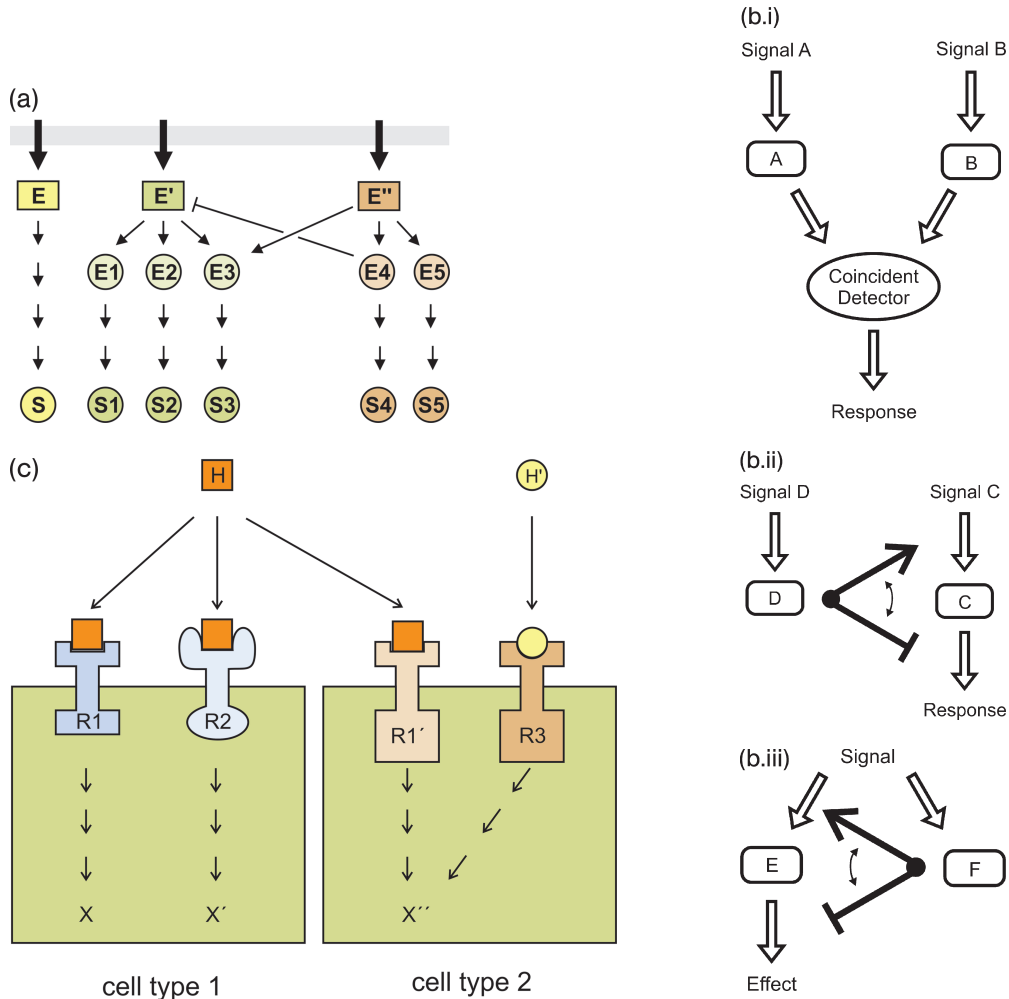- No branching and crosstalk, therefore oversimplification

Fig. 1.13: (a) Linearity, branching and crosstalk in intracellular signaling. Crosstalk refers to a situation where a signaling enzyme from one pathway inhibits (E4) or activates (E'') signaling components involved in signal transduction of a different pathway. (b) Mechanisms by which signaling pathways may interact. (i) Two distinct pathways converge on a coincidence detector, which generates a response that is unique and different from that generated by each individual pathway. (ii) A primary pathway that evokes a biological response is modulated by a second pathway through a gate, resulting in either inhibition or stimulation of the primary pathway. (iii) A single initial signal flows through multiple pathways, with one pathway regulating the other. (c) Variability of receptor systems and signaling pathways. (i) For one receptor of a given binding specificity (binding to hormone H) there can be different subtypes in the same cell (R1, R2) or in other cell types (R1'). (ii) The hormone H can induce different reactions (X1, X2) upon binding the different receptor types (R1, R2). The receptor types R1 and R2 can be found simultaneous in one cell. (iii) The binding of two different hormones (H, H') to different receptors (R1', R3) can induce the same intracellular reaction. The characteristics (i) and (ii) contribute to a high degree to the diversity and variability of hormonal signal transduction. Point (iii) illustrates the principle that important cellular metabolites or reactions can be controlled by different signal transduction pathways.

1.8.1.2 Branching and Crosstalk

Branching

We know of many signaling proteins that have multiple downstream reaction partners they can activate for further signal transduction. This property leads to a branching of signaling pathways and provides for multiple outputs originating from the same type of signaling protein. The conditions for the transmission of the signal to alternative downstream reaction partners are in most cases not well established. Often signal distribution to alternative routs depends on cell type, and may be variable in time and subcellular localization. In further chapters, the dissipation of signals and the distribution to alternative reaction partners is only discussed for those cases where alternative reaction partners have been clearly identified.

■ **Branching**
– Signaling protein has multiple downstream partners

Crosstalk

Cells have to process a large number of signals at the same time and mostly these signals are routed through different signaling pathways. The flow of signals through the various pathways must be coordinated and properly balanced which requires linkages between different pathways. This interdependence of signaling is also called crosstalk. The multivalency of signaling proteins and multisite modification allows components of a signaling path to influence, regulate and modulate the signaling in other pathways. Many examples of crosstalk will be found in the following chapters. Noteworthy is the regulation of the Raf kinase, a main component of the Ras–mitogen-activated protein kinase (MAPK) pathway (Section 9.6) by protein kinases that are part of other signaling pathways.

■ **Crosstalk**
– Linkage of different pathways
– Allows for coordination of pathways

1.8.1.3 Interactions between Signaling Paths

Three major mechanisms have been recognized by which different signaling paths interact, i.e. coincidence detection, gating and feedback (Fig. 1.13b).

Coincidence Detection

Coincidence detection is based on two distinct signaling pathways, A and B, converging on a single functional unit composed of one or more proteins known as the coincidence detector. The coincidence detector is able to recognize when the two converging pathways are activated within a given time window. Both signals are equally important. The detector then produces a unique response different from what is observed when either pathway is activated individually. Thus, the coincident response can be functionally distinct or can be synergistic, i.e. greater than the sum of the responses to A and B. A coincidence detector enables the cell to produce a unique response

only when specific pathways have been activated simultaneously or within a specific amount of time.

Gating
In a gated system, signal flow through the first pathway, C, is regulated by activation of a second pathway. Thus, two different signals interact in a hierarchical fashion. The response elicited is thus only modified, but not distinct from that which is evoked when pathway C is exclusively activated. Gating, therefore, gives a cell the ability to regulate responses based on the state of the cell, which, in turn, may be dependent on the signaling pathways that are activated. Both coincidence detection and gating are similar in that they allow two separate signals to activate different signaling pathways.

Feedback
Feedback is a modified gating mechanism, which is unique in that it is dependent on only one initial signal. This signal can then modulate multiple pathways or activate a single pathway that regulates two or more downstream effectors. In such a system, one effector produces the biological effect and the other regulates the signal flow to the effector that produces this effect. This configuration enables a signaling system to adjust its sensitivity to the environment, preventing or potentiating signaling flow to the endpoint response system.

1.8.2
Signaling Networks

Signaling networks
– Arise from branching and crosstalk

Each signaling protein of a signaling chain is subjected to regulatory influences from the same pathway or from different pathways placing it into a network of interactions and regulatory influences. Real cell signaling should therefore be described in terms of signaling networks that result from interconnections between pathways. In such a network, the same signaling protein is capable of receiving signals from many inputs. The networking may occur within similar classes of signaling pathways, such as between the Rho and Ras pathway (Section 9.10), and between different pathways, such as between the $G_{s,\alpha}$/cAMP and the MAPK pathway (Sections 9.6 and 10.2). The major mechanisms of interactions between pathways have been described already above in terms of coincidence detection, gating and feedback.

1.8.2.1 Complexity of Signaling Networks

The following experimental observations serve to illustrate the complexity of intracellular signaling and signaling networks (Fig. 1.13c):

– For a given hormone, different receptors can exist on the same or on different cells. These receptors can route the signal to different pathways triggering very distinct reactions in different tissues or even within the same cell. An example of such a phenomenon is adrenaline, which can initiate, on the one hand, a cAMP-mediated signal transduction and, on the other hand, an inositol triphosphate-mediated reaction (Chapter 6).

– For a given receptor, signaling enzyme or adaptor protein, subtypes are found which differ in their responsiveness to the incoming signal, in the nature and intensity of the reaction triggered in the cell, and in their capacity for regulation.

– The same secondary reaction can be triggered by different hormone–receptor systems and signaling pathways. This is exemplified by the release of Ca^{2+}, which can be regulated via different signaling pathways (Chapters 5–7).

– Regulatory interaction modules or protein modifications can be engaged depending on time and subcellular location.

– The function of a distinct modification or interaction may depend on the simultaneous presence of another modification or protein–protein interaction. This may result in cooperativity and ultrasensitivity of signaling.

– The signal output in a given signaling system may depend on the amplitude of the incoming signal. Furthermore, the duration and frequency of an incoming signal can modulate the output of the system. An example for the latter is the regulation of calmodulin-dependent protein kinase (CaMK) II by Ca^{2+} (Section 7.6).

1.8.2.2 Properties of Signaling Networks

The construction of signaling proteins from distinct protein domains and the assembly of signaling proteins into larger signaling complexes results in multifunctionality, variability and interconnection of cellular signaling systems. It has been proposed that cells contain a general signaling network that may be operationally divided into large signaling modules or types of signaling paths. The signaling modules contain characteristic core components and are defined by functional input–output characteristics. Examples of such modules are the epidermal growth factor (EGF) receptor–Grb2–mSos–Ras module, the MAPK module and the G-protein-coupled receptor (GPCR)–$G_{\beta\gamma}$–G_α–adenylyl cyclase–cAMP protein kinase A (PKA) module (Fig. 1.14a). The molecular identity of the components of the signaling modules and their interacting partners may be cell-

Signaling networks are based on
– Modular structure of signaling proteins
– Modular structure of signaling complexes

(a)
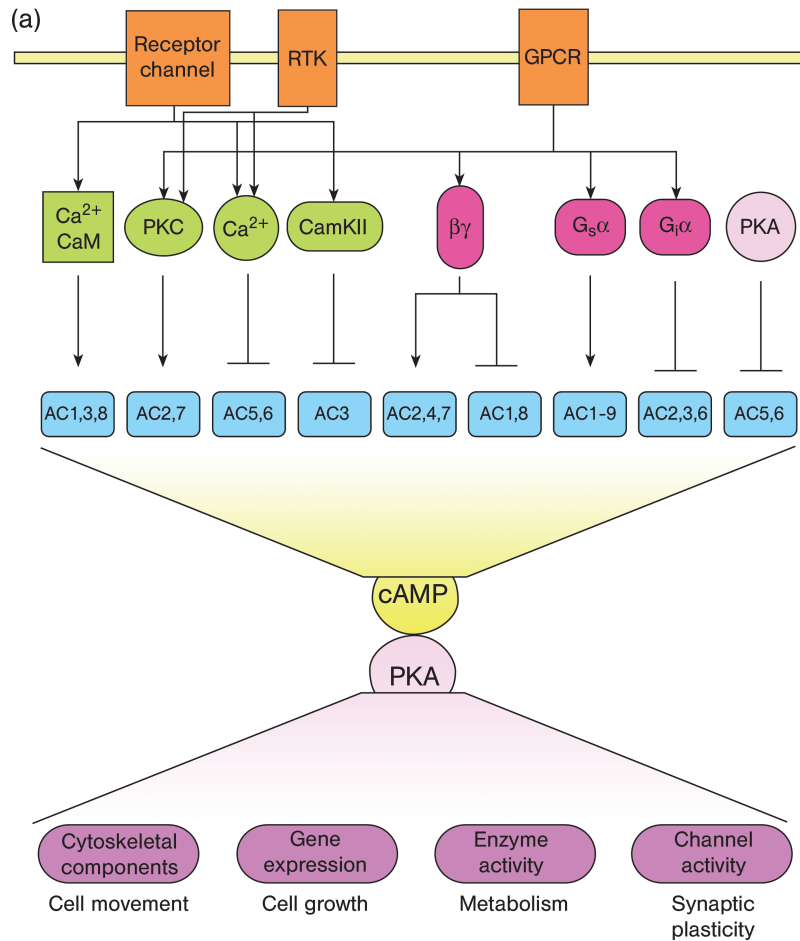
type-specific, but the overall function of these components and the logic of the circuitry is preserved from cell type to cell type. The central signaling system is connected to cellular mechanisms such as the transcriptional, translational, motility and secretory machinery that are responsible for phenotypic functions. The central network that connects the various machine networks also receives and processes signals from extracellular entities such as hormones or neurotransmitters and ions.

Signal transduction through signaling networks depends on the types of interconnection of its components and the properties of the components itself. The large number of possible connections and the multiplicity of possible input and output signals at each component makes the description of signaling networks extremely complex. Nevertheless, one would like to understand how precise
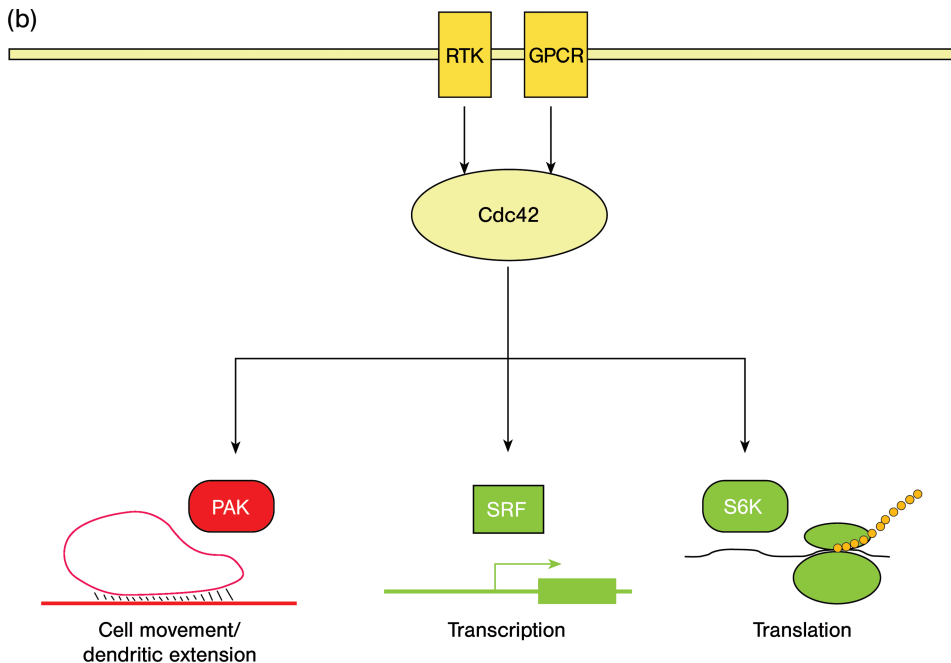
(b)

Fig. 1.14: (a) Adenylyl cyclases as examples of a junction in signaling networks. The signal receiving capabilities of the various adenylyl cyclase isoforms and the capability of the cAMP-dependent protein kinase (PKA) to regulate various physiological functions are shown. Receptor channel, ligand-gated channel (e.g. NMDA receptor); Stimulatory signals are shown as arrows and inhibitory signals as plungers. The various cellular components or processes regulated by PKA are shown in the red ovals and the resultant physiological functions are given below. (b) CDC42, a member of the Rho family of GTPases, as an example of a node. CDC42 may be stimulated both by RTKs as well as GPCRs and in turn regulate different cellular functions by regulating the distinct downstream kinases Pak (p21-activated protein kinase), S6K (p70^{S6} kinase, see Section 3.6.3.4) or the serum response factor (SRF, a transcription factor).

signals leading to defined biochemical reactions can be produced in the cell, how signals can be organized in time and in space, how irreversible switches are generated, and how signals are dissipated and downregulated.

Theoretical and experimental studies on cellular signaling networks have revealed features of cellular networks that help to explain some essential properties of both intracellular signaling and of intercellular signaling.

1.8.2.3 Nodes and Junctions

The interconnections in signaling networks may be operationally divided into two classes: *junctions*, which are signal integrators, and *nodes*, which split the signal. This classification is, however, by no

means absolute, because we know of quite a number of signaling proteins that can receive and distribute multiple signals.

Figure 1.14(b) illustrates the node function of the Rho-GTPase CDC42. This small regulatory GTPase (Section 9.9) can receive signals from several TM receptors and can deliver signals to different protein kinases and to transcription factors.

A well studied example of a system that contains both junctions and a node is the cAMP/PKA system (Fig. 1.14a and Chapter 7). Here, the various subtypes of adenylyl cyclase function as signal integrators or signaling junctions that receive signals from various TM receptors or ion channels. The adenylyl cyclases transduce the signal to PKA, of which only few isoforms exist. The PKA functions as a node through which the signal is distributed to various downstream partners. Signal distribution by PKA is achieved by the differential use of isoforms of the adaptor proteins [A-kinase anchoring proteins (AKAPs)] that specify the nature and location of the substrate of the protein kinase.

Networks also contain nodes where signals may be split and routed through several different pathways to regulate distinct cellular functions. Like junctions, nodes may also be upstream or downstream in the network. One of the best upstream examples of a node is the RTKs, which can route growth factor signals through many different pathways. Although such routing can result in regulation of multiple independent cellular functions (e.g. growth factors such as PDGF can regulate vascular smooth muscle cell migration and proliferation), signal routing through multiple pathways can produce combinatorial signal specificity at the level of gene expression (Fambrough et al., 1999; Schlessinger, 2000). Such combinatorial specificity may be used as a mechanism to establish hierarchy amongst the regulated cellular processes.

1.8.2.4 Feedback Loops

Feedback is well known from metabolic pathways in bacteria where the product of a metabolic chain regulates reactions that led to its production. Frequently, the purpose of such feedback mechanisms is to adjust the production to the demand. In general terms, feedback can be defined as the ability of a system to adjust its output in response to monitoring itself. In biological systems, feedback is a general regulatory principle both in intracellular and extracellular signaling, e.g. during development. Typically, feedback is organized in loops that can have a negative or positive effect on signaling leading to inhibition or enhancement of signaling. Depending on the details of the interconnections, feedback mechanisms can also serve to convert transient signals into permanent, irreversible responses. Of spe-

cific importance are feedback loops in developmental programs, e.g. in pattern formation (reviewed in Freeman, 2000).

Negative Feedback

Negative feedback occurs when, for example, a signal induces its own inhibitor – it serves to dampen/or limit signaling. The most obvious use of feedback signaling is to limit the duration of a signal. An example of this is the control of cytokine signaling through the Janus kinase (JAK)–signal transducers and activators of transcription (STAT) signaling pathway (Fig. 1.15). JAKs are soluble tyrosine kinases that bind to cytokine receptors and transduce signals by the STAT proteins (Chapter 11). Cytokine signaling is negatively regulated by proteins termed suppressors of cytokine signaling (SOCS). These proteins participate in negative-feedback loops. The physiological significance of the negative feedback is exemplified by SOCS1, which is induced by and inhibits interferon (IFN)-γ signaling; when the gene for SOCS1 is knocked out, mice die as neonates with defects associated with excess IFN-γ signaling.

■ **Negative feedback loops dampen and limit signaling**

Double-negative Feedback and Bistability

This type of feedback control is ascribed a specific role in converting graded or transient inputs into switch-like, irreversible responses. The structure of a simple double-negative feedback circuit is illustrated in Fig. 1.16. In this circuit, protein A inhibits or represses protein B and protein B inhibits or represses protein A. Such a circuit shows

■ **Double-negative feedback provides for**
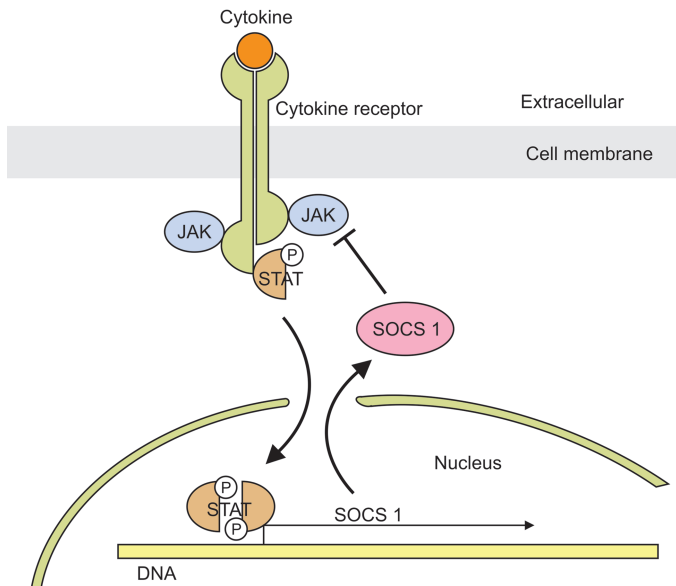– Ultrasensitivity
– Bistability

Fig. 1.15: Negative-feedback loop in cytokine receptor signaling. Ligand-bound cytokine receptors (Chapter 11) recruit protein kinases, JAKs, which in turn phosphorylate the transcriptional activator STAT proteins. The STATs activate the expression of SOCS proteins (Chapter 11) which are inhibitors of cytokine receptors. As a consequence, JAK–STAT signaling is inhibited. After Freeman (2000).

Fig. 1.16: A double-negative feedback loop. In this circuit, protein A (blue) inhibits or represses B (red), and protein B inhibits or represses A. Thus there could be a stable steady state with A on and B off, or one with B on and A off, but there cannot be a stable steady with both A and B on or both A and B off. Such a circuit could toggle between an A-on state and a B-on state in response to trigger stimuli that impinge upon the feedback circuit. After Ferrell (2002).
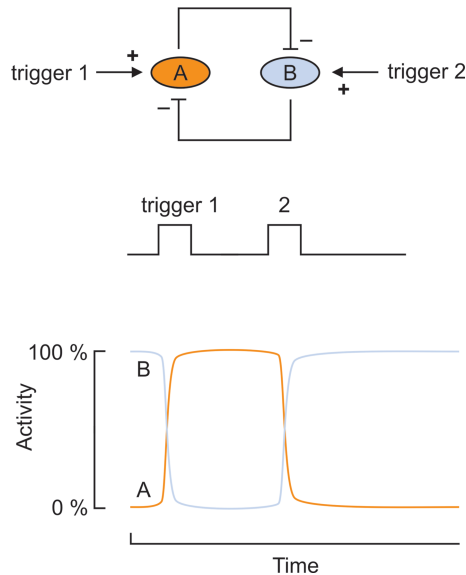
the characteristics of *bistability*: Of the two alternative states, only one is populated. The system cannot rest in intermediate states, i.e. A and B cannot exist at the same time. Upon exposure of a transient stimulus, the system goes from state A to state B in a nonlinear cooperative manner and may stay in state B although the stimulus has died away (reviewed in Ferrell, 2002). The system shows *ultrasensitivity* and has the characteristics of a cooperative transition. Furthermore, bistable systems can have switch-like properties (reviewed in Slepchenko and Terasaki, 2004). Upon a specific stimulus, the system makes a transition from one stable state to another stable state in a nearly irreversible manner. Double-negative feedback loops have been identified in several biological systems and in key signaling pathways such as in MAPK (Chapter 10) cascades (for details, see Ferrell, 2002).

Positive Feedback
Positive feedback occurs when a signal induces more of itself, or of another molecule that amplifies the initial signal, and this serves to stabilize, amplify or prolong signaling. A generalized positive feedback loop is depicted in Fig. 1.17(a). Such a system has the interesting property that the system stays activated even after the initial signal disappeared. We know of many examples of positive feedback signaling. As an example, Fig. 1.17(b) shows an autocrine positive-feedback loop in the *Drosophila* oocyte that ensures an all-or-nothing cell fate decision. Other examples include the autoregulation of

■ **Positive feedback amplifies, stabilizes and prolongs signals**

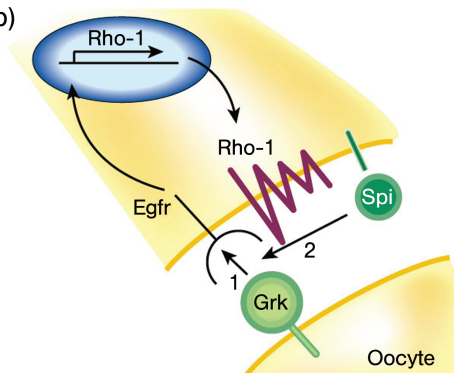**(a) Generalized positive feedback loop**
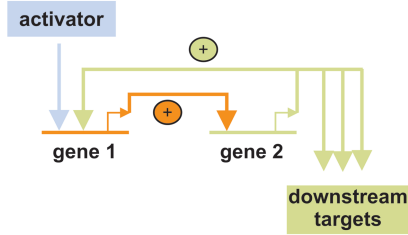
(b)

Fig. 1.17: Circuit diagram of a simple positive-feedback loop. (a) Once turned on by an activator, gene 1 (shown in red) activates gene 2 (shown in green). In addition to acting on downstream targets, gene 2 activates gene 1, forming a positive feedback loop. When the initial activator signal fades, these genes will remain active. After Howard and Davidson (2004). (b) Autocrine amplification by positive feedback in the *Drosophila* oocyte. The extracellular signaling protein, Gurken (Grk), expressed in the oocyte, binds to and activates the EGF receptor (Egfr) in the overlying follicle cells. This activates the expression of Rhomboid-1 (Rho-1), which in turn activates another EGF receptor ligand, Spitz (Spi). Spitz then amplifies and prolongs EGF receptor signaling in the follicle cells.

homeotic genes and chromatin modifications (Schreiber and Bernstein, 2002).

Bistability can be an important consequence of positive feedback loops as shown schematically in Fig. 1.18. In the positive feedback circuit shown, state A is activated by state B and state B is activated by state A. As a result, there could be a stable steady state with both A and B off or A on and B on, but not with A off and B on and *vice versa*.

Many signaling systems contain several intertwined feedback loops leading to a complex regulation that is difficult to describe quantitatively. One example is the protein (cyclin-dependent) kinase CDK1 that undergoes an abrupt activation at the G_2/M transition of the cell cycle (Chapter 13). Here, positive- and negative-feedback loops are involved.

Fig. 1.18: Bistability in a positive-feedback loop. In this circuit, A activates B and B activates A. As a result, there could be a stable steady state with both A and B off, or one with both A and B on, but not one with A on and B off or *vice versa*. Bistable circuits could exhibit persistent, self-perpetuating responses long after the stimulus is removed.
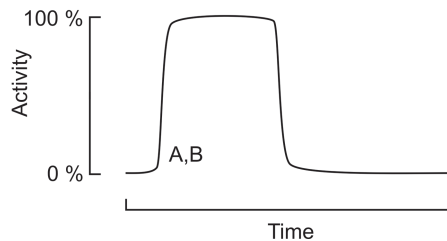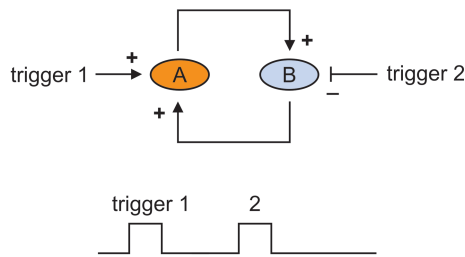
1.8.3
Redundancy and Specificity of Signaling
Redundancy

We know of many cases where multiple signaling pathways converge on the same target conferring stability and robustness on a signal. Such a redundancy of signaling has its parallel in the multiple modifications of signaling proteins, e.g. multiple phosphorylation (Section 1.6.3). Redundancy in signaling may be considered as a safeguard against the failure of a signaling component. When one component fails, its function may be taken over by another protein with similar activity. An illustrative example for redundancy in signaling is provided by the protein kinases of the cell cycle. Here, knockout experiments in mice have shown that CDK2 and cyclin E, long thought to be essential components of the progression through the G_1 phase of the cell cycle (Chapter 13), are largely dispensable for the development of mice. Apparently other CDK–cyclin pairs can replace CDK2–cyclin E in its function during G_1.

The multiple phosphorylation of RTKs is another example for redundancy in signaling as shown for PDGFR. In response to mitogenic extracellular signals, PDGFR uses multiple p-Tyr docking sites (Fig. 8.11) for the engagement of distinct signaling proteins such as phosphatidylinositol-3-kinase (PI3K), phospholipase Cγ and protein phosphatase SHP2. One specific response to mitogenic signals is the induction of a set of immediate–early genes by PDGFR. Remarkably, when the various modifiable tyrosines of PDGFR were replaced with nonmodifiable phenylalanines (Fam-

■ **Redundancy in signaling**
– Same reaction is controlled by several signaling proteins or signaling pathways
– Safeguard against failure of one component

brough et al., 1999), nearly all of the immediate–early genes could still be induced, although with somewhat lower amplitude. Hence, the immediate–early genes depend on no single pathway, but rather receive input from every pathway. The alternative strategy, that distinct modules of immediate early genes are induced by specific pathways, is not used by the PDGFR to communicate the mitogenic signal.

Specificity

Another important aspect of cell signaling is the specificity of signal transmission in signaling pathways and networks (Pawson, 2004). Specificity of signaling can be considered in terms of "which, when and where" interactions with signaling partners are formed. Considering the "which", i.e. the selection of binding partners, it is important to ask how specific are the protein–protein interactions that are involved in formation of signaling complexes?

In signaling pathways and networks, there is no optimal affinity for protein–protein interactions, but rather a wide range of dissociation constants that are tailored for distinct forms of biological regulation. Tight protein–protein interactions yield a high degree of specificity and are relevant for many biological functions. Strong interactions are long lived, and this can be advantageous, as in the tethering of inactive PKA to the scaffolding protein AKAP in readiness for a cAMP signal. However, tight interactions cannot always provide the flexibility that a cell needs to respond dynamically to changing external conditions or internal programs. Indeed, protein–protein interactions that are dependent on posttranslational modifications must by definition have relatively modest affinities since much of the binding energy must come from the modified residue itself. However, affinities in the micromolar range do not necessarily mean an absence of specificity. Many signaling complexes utilize multiple contacts, each of low affinity, to ensure fidelity. Often, the contacts are used in a cooperative way to achieve specificity in interaction. Thus, a general picture of interactions in signaling pathways and networks emerges, where individual domains of the signaling components are combined in a single polypeptide to enhance binding specificity and to generate allosteric control of signaling proteins and multiprotein complexes.

1.8.4
Regulation of Signaling Pathways

To achieve an appropriate biological response in the target cell, the extracellular signals originating from the environment or from within the organism must be processed by the intracellular signaling

pathways or networks. Thereby, the intracellular network must place a value on the incoming signal such that it is either converted into a biochemical event and subsequently a biological response or safely dissipated within the network. Furthermore, mechanisms must be available that limit the duration of the intracellular response and adapt it to the overall needs of the organism. As an example, the long-lasting exposure to a hormone often leads to a weakening of the intracellular response. Such an attenuation, downregulation or desensitization of signaling can occur at many points of a signaling chain or network. The main attack points for control of intracellular signaling are:

Major regulators attack points of signaling paths
- Receptors
- Signaling enzymes
- Second messengers

- *Receptors.* The amount, activity and specificity of receptors is a main determinant for the transduction of extracellular signals into an intracellular response. Receptor regulation can occur at multiple levels such as: ubiquitination and internalization (Sections 2.5.6.2 and 8.1.5), posttranslational modification: phosphorylation (Section 5.3.5), binding of antagonistic ligands and gene expression.
- *Signaling enzymes.* The signal transducers operating downstream of the receptors may be controlled by deactivating components that limit the life time of the activated state of the signaling enzyme. Examples are the regulatory GTPases that are controlled by specific deactivators, the GTPase-activating proteins (GAPs, see Chapter 5). The protein kinases are another example of tight control of enzymatic activity. Many protein kinases are activated by phosphorylation (Chapter 7) and this modification can be reversed by phosphatases that clip off the activating phosphate modification. Furthermore, we know of protein kinase inhibitors (Chapter 13) that are subjected to regulatory modification.
- *Second messengers.* Chapter 6 presents many examples of second messengers whose production and degradation by specific enzymes is under tight control.

The regulation of the signaling components themselves is always part of a larger signaling network that ensures the generation of physiologically appropriate signals. How important the internal safeguard and control mechanisms are becomes evident when the control mechanism fail. The chapter on tumor formation (Chapter 14) gives many examples where the failure of control mechanisms in signaling pathways leads to inappropriate cell signaling that is characteristic of many tumor cells. Persistent activation of signaling components due to the malfunction of deactivating mechanisms is found in many tumors.

1.8.5
Spatial Organization of Signaling Pathways

A major contribution to the specificity of signaling comes from the localization of signaling events to distinct subcellular sites such as membrane compartments or the cytoskeleton. By restricting signal production to specific sites only, tight spatial and temporal control over signaling is possible allowing for a fast and effective production and downregulation of signals. Most locally controlled signals are produced and processed in multicomponent signaling complexes assembled at the inner side of the cell membrane. Membrane localization of signaling complexes is of outstanding importance in cellular signaling because the vast majority of external signals are received at the cell membrane by membrane receptors that transduce the signal into the cell interior. Furthermore, electric signals, resulting in the influx of, for example, Ca^{2+} ions via ion channels, become active at the cytoplasmic side of the cell membrane. Often, the steps following membrane receptor activation occur in tight association with the inner side of the cell membrane whereby large signaling complexes form in a dynamic way.

The cell uses the following tools for membrane localization of signaling proteins (Fig. 1.19):
– *Adaptor or scaffolding proteins.* We know of many scaffolding proteins that serve to assemble signaling complexes at the membrane or at the cytoskeleton. Examples include the AKAP proteins (Section 7.3.3), the receptor for activated C-kinase (RACK) proteins (Section 7.5.4) and the PDZ-containing proteins (Section 8.2.5). A general theme that emerges from the study of these scaffolding proteins is that they possess a bidirectional specificity. At one end they specifically recognize one or a group of signaling components

Membrane-localization of signaling paths is based on
– Membrane-localized adaptor proteins
– Binding to TM receptors
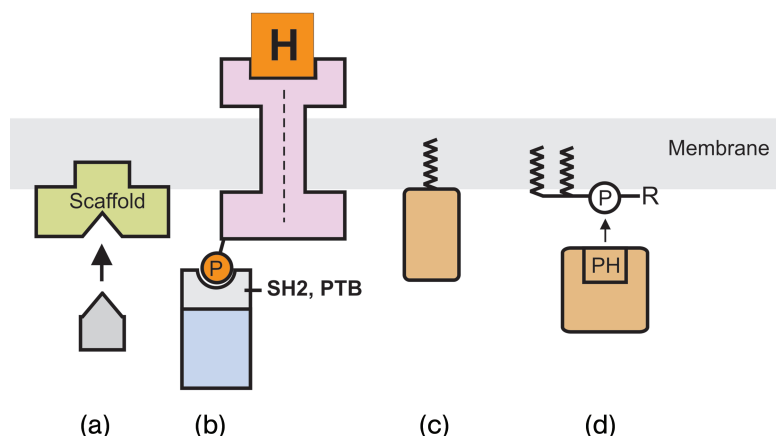– Lipid anchors

Fig. 1.19: Main mechanisms for membrane targeting of signaling proteins. (a) Binding to membrane-associated adaptor proteins. (b) Binding to phospho-amino acids of TM receptors via SH2 or PTB domains. (c) Membrane targeting via lipid anchors. (d) Binding to membrane-bound phospholipids via PH domains.

and at the other end a location within the cell, thus providing the molecular basis for spatial organization of signaling pathways. By organizing signaling enzymes with opposing activities in the same signaling complex with the help of the scaffold, a precise and locally controlled production of signals can be achieved. An example for that strategy is provided by the AKAP proteins (Section 7.3.3).

– *Binding to TM proteins.* This strategy is extensively used by the RTKs. Here, phosphotyrosine residues on the activated receptor are used as docking sites for bringing the next signaling protein in sequence to the receptor and to the cell membrane. This protein then can use protein interaction domains to recruit another signaling component into the signaling complex.

– *Membrane anchors.* The posttranslational modification of signaling components by lipidation provides a means for recruiting signaling proteins to the inner side of the cell membrane and thus into the vicinity of receptors or other signaling components. The lipid anchors insert into the lipid bilayer and thus increase the affinity of signaling proteins to the membrane. For details on the membrane anchors, see Section 2.6.

– *Binding to membrane-localized second messengers.* Some second messengers (e.g. diacylglycerol and phosphatidylinositol trisphosphate, see Chapter 6) are membrane-bound and serve as membrane attachment points of signaling proteins. The hydrophobic messengers are recognized by interaction domains on the signaling protein [e.g. pleckstrin homology (PH) domains for binding of phosphatidylinositol trisphosphate]. Importantly, the trigger for membrane association is the presence of the second messengers which are formed in a signal-directed way.

1.8.6
Compartmentalization and Transport

Compartmentalization
– Signaling proteins shuttle between subcellular compartments

Many signaling proteins shuttle between distinct compartments of the cell to perform specific functions. These proteins carry sequence signals or posttranslational modifications that direct them to distinct subcellular sites. Such a signal-directed translocation of signaling proteins is an important means for the generation of location specific signals. Examples of signaling proteins with variable subcellular localization include PKC (Section 7.5), the Abl tyrosine kinase (Section 8.3.3) and some nuclear receptors (Chapter 4). Furthermore, the shuttling of proteins kinases and transcription factors between the cytoplasm and the nucleus is a frequently observed phenomenon. As outlined in Section 3.4.5, the signal-directed phosphorylation of transcription factors is an important tool for controlling their nuclear localization and thus their transcription activating function.

1.8.7
Evolution of Signaling Pathways

The reiterated use of interaction domains may have developed in part to facilitate the evolution of new cellular functions, because domains may be readily joined in new combinations to create novel connections and pathways within the cell (Pawson, 2004). For example, coupling of protein phosphorylation to ubiquitination could have been achieved by simply linking an interaction module that recognizes p-Ser-p-Thr or p-Tyr sites to a RING domain that binds components of the ubiquitination machinery. The Cbl protein which functions as a E3 ligase for ubiquitination of RTKs is an example for such a strategy. Cbl carries a SH2 domain for binding to p-Tyr sites on the receptor and a RING domain for assembly of E2 enzyme (Section 2.5.6.2).

When going from invertebrates and lower vertebrates to higher vertebrates, an increased use of interaction modules is evident.

Conventional tyrosine kinases and SH2 domains are absent from yeast, but make a coordinate appearance with the development of multicellular animals. An SH2 domain, by its design, can be inserted into preexisting proteins and thereby provide a common means of coupling entirely different proteins to tyrosine kinase signals. Clearly this does not exclude the subsequent elaboration of more sophisticated levels of control within signaling complexes. The joining of separate domains can also create a new composite entity with more complex properties than either domain alone.

Interaction domains and motifs therefore provide a way to increase the connectivity of existing proteins, and thus to endow these proteins with new functions. This is likely one of several reasons that the apparent complexity of organisms can increase so markedly without a corresponding increase in gene number. An attribute of proteins encoded by the human genome is that they have a richer assembly of domains than do their counterparts in invertebrates or yeast, and indeed the assortment of domains into novel combinations is likely an important aspect of genome divergence.

1.9
Variability and Cell-type Specificity of Signaling

The general principles and the types of interconnections in the signaling pathways and networks are largely the same in most cells. However, the details of the interaction modules and interconnections as well as the availability of signaling components differ from cell type to cell type. Thus, cells are able to process signals and activate biochemical events in a cell-type-specific way.

■ **Cell-type specific signaling**
– Based on cell-type-specific availability, modification and activity of signaling proteins

A major contribution to the cell type specificity of signaling pathways and networks comes from the amounts and properties of the signaling components and this depends on the following points:

– Cell-type-specific expression of the gene for the signaling component.
– Cell-type-specific splicing.
– Cell-type-specific stability.
– Cell-type-specific posttranslational modification.
– Cell-type-specific subcellular localization.

Most important for the variability of signaling pathways and networks is the existence of subtypes of signaling proteins. Differential transcription and splicing leads to the presence of subtypes of signaling proteins that differ in the details of signal registration, signal transmission and regulation. Examples of signaling proteins for which many subtypes are found:

– α and βγ subunits of the heterotrimeric G-proteins (Chapter 5).
– Adenylyl cyclase (Chapter 6).
– PKC (Section 7.5).
– PKA (Section 7.3).
– GAP proteins (Section 9.2).
– Protein kinases of the MAPK signaling pathways (Chapter 10).
– JAK and STAT proteins (Chapter 11).
– Scaffold protein AKAP (Section 7.3).

1.10
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