Chapter 8
Cell Signaling Pathways

Cells have an ability to perceive and respond to their microenvironment. External stimuli activate internal signaling pathways that regulate the cell activity. Most signaling pathways function to transfer information from the cell surface to effector systems.

External signals can be transmitted into the cell by several ways (Fig. 8.1):

1. As a hydrophobic molecules directly through the cell membrane;
2. Through ion channels linked receptors;
3. Through receptors associated with G protein;
4. Through receptors which are enzymes or are associated with enzymes;
5. Others (e.g. non-catalytic receptors).

In many cases, an external signal reaches the nucleus and affects gene expression.

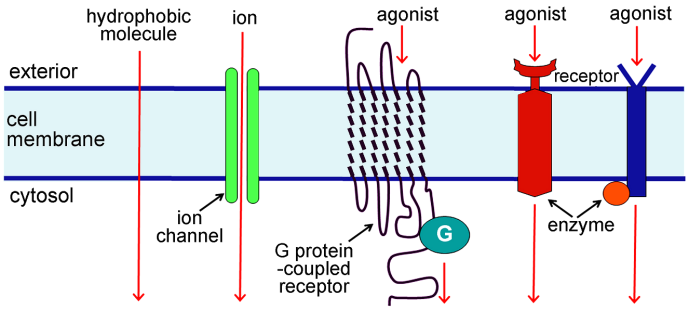

Fig. 8.1 Main pathways of signal transduction into the cell

8.1 Signaling through Hydrophobic Molecules

Hydrophobic molecules, such as nitric oxide (NO), arachidonic acid, or steroids, which play an important role in intracellular signaling, can move

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into or from the cell directly through the cell membrane. In contrast to other signaling cascades within a single cell, NO or arachidonic acid, formed under the influence of external signals, can leave the cell and influence the neighboring cells.

Nitric oxide is formed from L-arginine with the participation of nitric oxide synthase (NOS). In target cells, NO stimulates cytoplasmic guanylate cyclase to produce cyclic GMP (cGMP). Cyclic GMP (Fig. 8.2) is a secondary messenger which regulates the function of many enzymes and ion channels, e.g. it induces smooth muscle relaxation. cGMP is quite rapidly converted to GMP by phosphodiesterase (PDE). Phosphodiesterase inhibitor is a component of a known drug used to treat erectile dysfunction (Viagra).

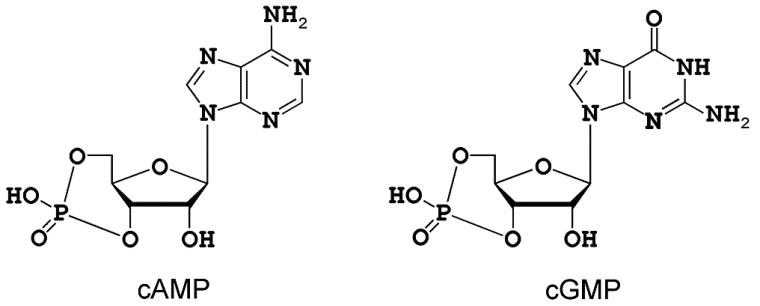

Fig. 8.2 Structure of cyclic nucleotides: cAMP and cGMP, which are among the most important secondary messengers in signaling cascades

Arachidonic acid (AA or ARA) is a polyunsaturated fatty acid that is present in the membrane phospholipids of the body's cells. In addition, it is involved in cellular signaling as a lipid second messenger. It is formed by hydrolysis of phospholipids catalyzed by phospholipase (Fig. 8.3). After reaching the target cell it activates a protein kinase C (PKC), which phosphorylates some molecules in the cell altering their biological activity. Many molecules phosphorylated by PKC are involved in e.g. processes of learning and other processes associated with the activity of neurons. Arachidonic acid can also be converted by other enzymes (cyclooxygenases, lipoxygenases) to biologically active proinflammatory or antiinflammatory compounds.

Steroid hormones (glucocorticoids, mineralocorticoids, androgens, and female sex hormones; general structure shown in Fig. 1.6, in ch. 1.2.2) belong to the superfamily of fat soluble hormones, which also includes thyroid hormones, retinoids (vitamin A derivatives), and vitamin D3. Most of them enter the cell where they bind to their receptors present in the cytoplasm or the nucleus (only some steroid hormones bind to specific protein receptors on the cell membrane). Steroid hormone receptor usually forms a dimeric form after ligand (hormone in this case) binding. The main role of fat soluble hormones is to regulate transcription: if ligand-receptor complex is formed in the

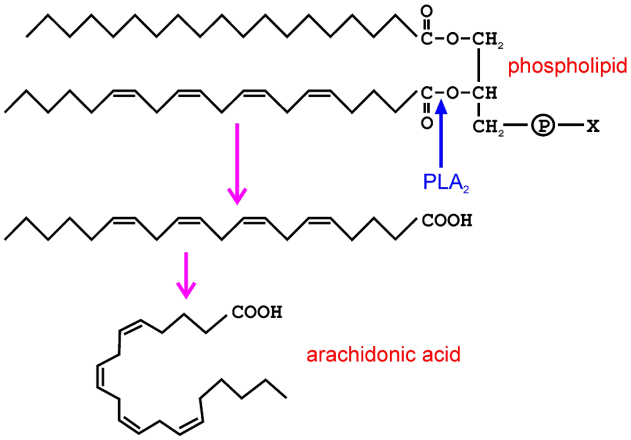

Fig. 8.3 Production of arachidonic acid from phospholipid by phospholipase A_2 (PLA_2)

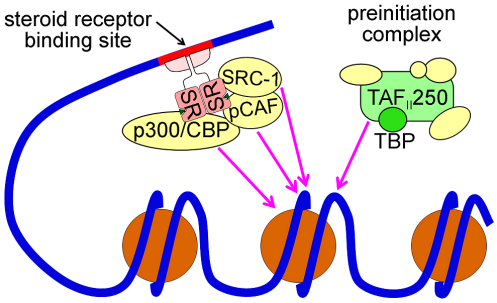

Fig. 8.4 The mechanism of activation of transcription by steroid hormone receptors. Steroid hormone receptor (SR) after joining of ligand (steroid hormone) forms a dimer, which after binding to DNA operates together with steroid receptor coactivators (SRC) and histone acetyltransferases (p300/CBP and p300/CBP-associated factor - pCAF), what stimulates transcription. In the absence of ligand some steroid receptors bind co-repressors (for example SMRT and NCoR) and histone deacetylases, which leads to inhibition of transcription.

cytoplasm, it must be transported to the nucleus, where it acts as a transcription factor. It binds to specific sequences present in regulatory regions of genes and activates or inhibits the transcription (Fig. 8.4).

8.2 Signaling through Ion Channel Linked Receptors

Ion channel linked receptors are ion-channels themselves. It is a large family of multipass transmembrane proteins. They are involved in rapid signaling events. They regulate the flow of ions across the membrane in all cells

(ch. 2.4.2.3). Hydrophilic pore (gate) inside the channel is opened or closed depending on external factors. Ion channels may be classified by gating, i.e. what opens and closes the channels. They are divided into three main groups: voltage-gated ion channels, ligand-gated ion channels, and mechanosensitive ion channels. Due to the selectivity, that is ability to pass specific types of ions, channels are divided into cationic and anionic. When the channels are even more "specialized" - are defined as sodium, potassium, etc. It should be noted that the term e.g. "sodium channel" means only that sodium ions are passed preferentialy. However, other cations could also pass through this channel.

All cells have a resting potential: an electrical charge across the plasma membrane, with the interior of the cell negative with respect to the exterior. Sodium, potassium and chloride ions are the most important for maintaining of the resting potential. Usually the concentration of sodium and chloride ions outside the cell is higher than inside the cell, while the concentration of potassium ions is higher inside the cell. Free diffusion of ions occurs through the cell membrane. Maintaining a constant difference of ions concentration between the interior and exterior of cells is possible thanks to the active (i.e. requiring energy input) transport occurring in the opposite direction than diffusion. A classic example of such active transport mechanism is the **sodium-potassium pump**.

The size of the resting potential varies. It can go for a long period of time without changing significantly. However some cells (excitable cells: neurons, muscle cells, and some secretory cells in glands), in addition to maintaining resting potential, are able to change rapidly and transiently their membrane potential in response to environmental or intracellular stimuli. Such short-lasting event in which the electrical membrane potential of a cell rapidly rises and falls, following a consistent trajectory, is called an **action potential**. It is formed in excitable cell when its membrane potential exceeds a certain limit value. Repeated generation of action potentials, that enables the flow of sodium and potassium ions in the direction consistent with the difference in concentrations, would lead to compensation of extracellular and intracellular concentrations of these ions. In all excitable cells there is mechanism of active transport, pumping ions against concentration differences and thus keeping the concentration of ions at a constant level.

Calcium ions also play an important role in the cell. They are involved in signal transduction pathways, where they act as a second messenger, in neurotransmitter release from neurons, contraction of all muscle cell types, and fertilization. Many enzymes require calcium ions as a cofactor. Extracellular calcium is also important for maintaining the potential difference across excitable cell membranes, as well as proper bone formation.

The intracellular calcium level is kept relatively low with respect to the extracellular fluid. Inside the cell, calcium ions are accumulated in the smooth endoplasmic reticulum and mitochondria. Calcium ions can enter into the cytoplasm either from outside the cell through the cell membrane via

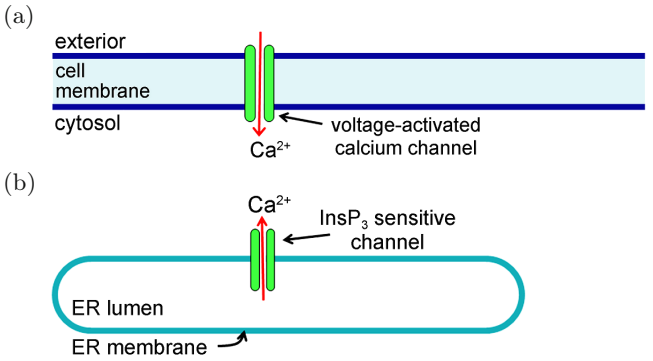

Fig. 8.5 Two types of calcium channels: (a) voltage-gated calcium channel located in the cell membrane; (b) ligand-gated calcium channel located in the membrane of endoplasmic reticulum (ER). $InsP_3$ — triphosphoinositol.

voltage-dependent calcium channels or may be released from the endoplasmic reticulum by a ligand-dependent channels (Fig. 8.5).

8.3 Signal Transduction via Receptors Associated with G Protein

Many signaling molecules bind to specific receptors on the cell membrane (**the molecule does not pass through the membrane**). Such extracellular receptors span the plasma membrane of the cell, with one part of the receptor on the outside of the cell and the other on the inside. A ligand binding to the outside part stimulates a transmition of the signal into the cell, elicting a physiological response. The signal can be amplified. Thus, one signalling molecule can cause many responses.

External signal could be transmitted via G protein-coupled receptors (GPCRs). Although GPCRs are classically thought of working only with G protein, they may signal through G protein-independent mechanisms (Fig. 8.6). And also, heterotrimeric G proteins may play functional roles independent of GPCRs.

8.3.1 G Protein-Coupled Receptors

G protein-coupled receptors (GPCRs) are large family of transmembrane proteins (in human – about 950 proteins) acting as receptors for extracellular molecules that activate intracellular signaling cascade. GPCRs mediate a wide variety of biological processes, ranging from neurotransmission and

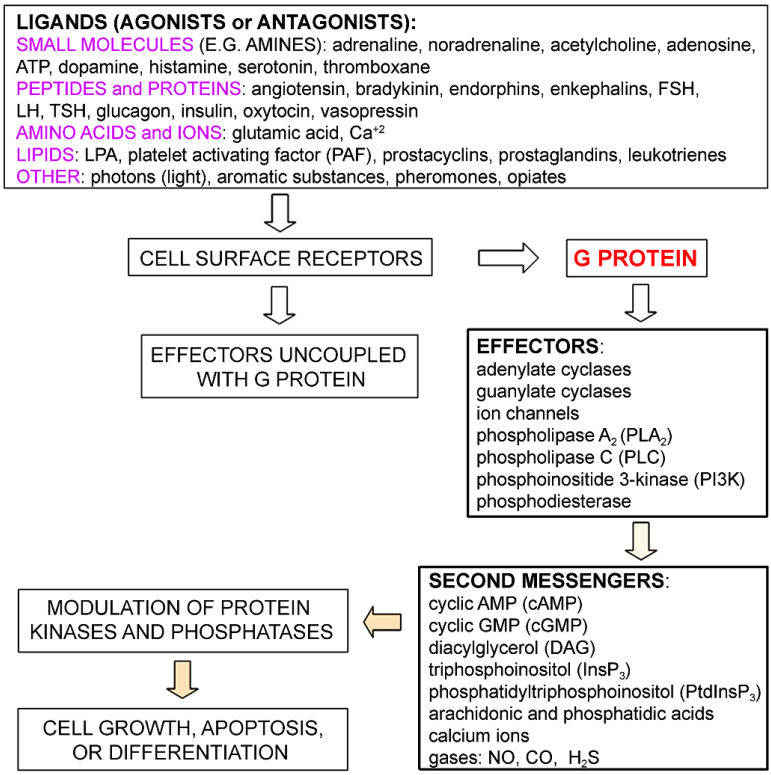

Fig. 8.6 Scheme of signal transduction in cell through the receptors associated with G protein. Activated G protein transfers the signal to one of the effectors what leads to production of the appropriate second messengers.

hormonal control of virtually all physiological responses, to perception of taste, smell, light, and pain. Such receptors are found only in eukaryotes and can bind with a number of ligands listed in the diagram shown in Fig. 8.6 and in ch. 2.4.2.2 (each ligand recognizes its own receptor). Substance (ligand) that binds to the receptor and triggers a response by that cell is called an **agonist**. Whereas an agonist causes an action, an **antagonist** binds to the receptor and blocks it preventing the activation by agonist.

8.3.2 G Proteins

Binding of a ligand to the GPCR causes a conformational change in the receptor. This leads to activation of G protein associated with the receptor, by exchanging its bound GDP for a GTP (Fig. 8.7). G proteins (guanine nucleotide-binding proteins) associated with the GPCRs belong to the family

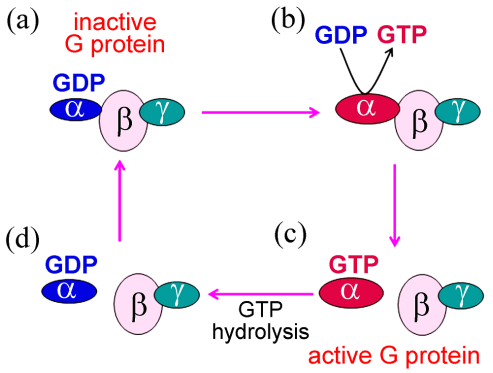

Fig. 8.7 Changes of G protein between inactive and active form: (a) in the inactive form, α subunit is associated with GDP (guanosine diphosphate); (b) interactions between the receptor activated by the agonist and the α subunit lead to the release of GDP. GTP binds in the empty site (its concentration in the cell is much higher than the concentration of GDP); (c) α subunit associated with GTP has small affinity to the $\beta\gamma$ complex, which leads to separation of subunits, so they can activate further signal molecules; (d) α subunit possess GTP-ase activity: it finally hydrolyzes GTP to GDP, what leads to inactivation of the G protein.

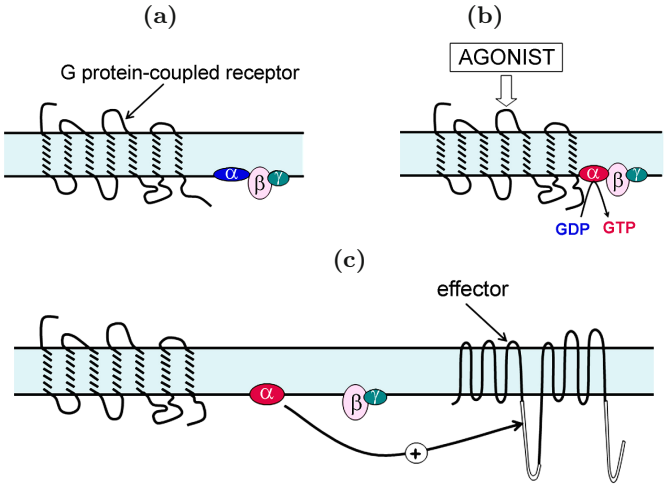

Fig. 8.8 Transmission of signal from the receptor associated with G protein to effector: (a) before the agonist binds to the receptor, three subunits of the G protein form a complex; (b) after binding of agonist, the receptor affecs G protein, changing the GDP in the α subunit for the GTP, so the subunit becomes active; (c) α subunit and $\gamma\beta$ complex are separated and can interact with effector proteins (this figure shows the interaction with adenylyl cyclase responsible for cAMP production). Both, α subunit and $\gamma\beta$ complex, can interact with different effectors (listed in Fig. 8.6).

of heterotrimeric G proteins (composed of α subunit, and β and γ subunits forming the stable complex). They are associated with the inside surface of the cell membrane and serve as a "dashboard" on the way between receptor and effector (Fig. 8.6 and 8.8). G proteins are highly differentiated: over a dozen genes encoding α subunit have been cloned so far (Table 8.1). Also β and γ subunits may exist in different variants. They behave differently in the recognition of the effector, but share a similar mechanism of activation.

Table 8.1 Mammalian G protein α subunits and their effectors. Intracellular communication via G proteins could be affected by bacterial toxins. After binding of the cholera toxin, GTP associated with Gα can not be hydrolyzed to GDP and the G protein remains all the time in the active form. By contrast, pertussis toxin prevents GDP release from the α subunit and G protein is blocked in the inactive state.

Class	Gene variant	Effectors	Sensitivity to toxins
$G\alpha_s$	$\alpha_{s(s)}$ $\alpha_{s(L)}$	(+) adenylate cyclase (+) calcium channel (-) sodium channel	cholera
	α_{olf}	(+) adenylate cyclase	cholera
$G\alpha_{i/o}$	α_{i1} α_{i2} α_{i3}	(-) adenylate cyclase (-) calcium channel (+) sodium channel	pertussis
	α_{oa} α_{ob}	(-) calcium channel (+) phospholipase C (+) phospholipase A_2	pertussis
	α_{t-r} α_{t-c}	(+) cGMP phosphodiesterase	cholera and pertussis
	α_g	(+) phospholipase C	pertussis
	α_z	(-) adenylate cyclase	
$G\alpha_{q/11}$	α_q α_{11} α_{14} $\alpha_{15/16}$	(+) phospholipase C	
$G\alpha_{12/13}$	α_{12} α_{13}	(+) small G protein	

Notes: (+) activation; (-) inhibition

G proteins were discovered by Alfred G. Gilman and Martin Rodbell when they investigated stimulation of cells by adrenaline. For this discovery, they won the 1994 Nobel Prize in Physiology or Medicine.

8.3.3 Effectors and Secondary Messengers

Both activated α subunit and released $\beta\gamma$ subunits of the G protein can activate different signaling cascades and effector proteins (listed in the diagram

in Fig. 8.6). The main effectors on which α subunit acts are listed in table 8.1 ($\gamma\beta$ complex also can act on the same effectors). Adenylate cyclase, a membrane protein, catalyzes the conversion of ATP to cyclic AMP, one of the most important secondary messengers (Fig. 8.2; ch. 8.5.1). Whereas guanylate cyclases (membrane-bound or soluble forms) catalyze the formation of cGMP. Phospholipase A2 (PLA_2) cleaves phospholipids generating the formation of arachidonic acid (Fig. 8.3). Phospholipase C (PLC) also cleaves phospholipids, but in a different position than PLA_2, leading to the formation of diacylglycerol (DAG) (Fig. 8.9). Among the variety of PLC (β, γ, δ, and others), PLC-β is activated by G protein.

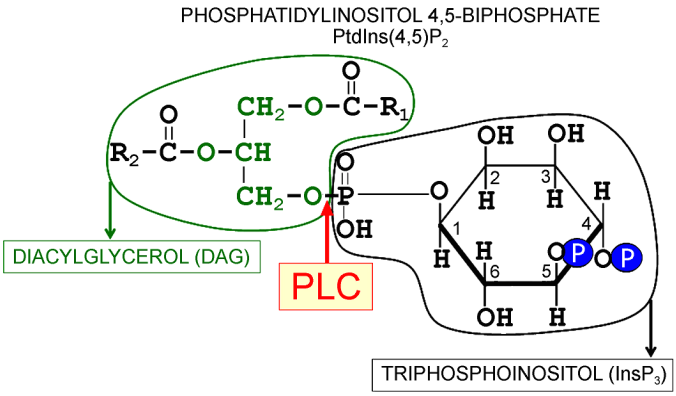

Fig. 8.9 An example of the reaction catalyzed by phospholipase C (PLC): hydrolysis of phosphatidylinositol diphosphate generates the formation of secondary transmitters: diacylglycerol (DAG) and triphosphoinositol ($InsP_3$, IP_3). DAG remains in the membrane and activates protein kinase C (PKC)

Second messengers produced by effectors relay signals to target molecules inside the cell, in the cytoplasm or nucleus. They greatly amplify the strength of the signal.

There are three basic types of secondary messenger molecules:

- hydrophilic (i.e. water-soluble), like cAMP, cGMP, $InsP_3$, and calcium ions, that are located within the cytosol;
- hydrophobic (i.e. water-insoluble), like diacylglycerol, and phosphatidylinositols, which are membrane-associated and diffuse from the plasma membrane into the intermembrane space where they can reach and regulate membrane-associated effector proteins;
- gases: nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H_2S) which can diffuse both through cytosol, and across cellular membranes.

For discovery of second messengers, Earl Wilbur Sutherland, Jr. won the 1971 Nobel Prize in Physiology or Medicine.

8.4 Signal Transduction through Enzyme Linked Receptors

Enzyme-linked receptors are either enzymes themselves, or are directly associated with the enzymes that they activate. These are usually single-pass transmembrane receptors, with the enzymatic portion of the receptor being intracellular. The majority of enzyme-linked receptors are protein kinases, or associate with protein kinases, mainly tyrosine or serine/threonine kinases. These kinases phosphorylate respectively tyrosine and serine or threonine in target proteins. They are capable of autophosphorylation as well as phosphorylation of other substrates. Protein phosphorylation leads to changes in its enzymatic activity, intracellular localization, or in interactions with other proteins. Protein kinases are also found in the cytosol in the form of enzymes not associated with the receptors.

Abnormal activity of receptor kinases is a common cause of diseases, especially carcinogenesis. Signaling pathways affected by such kinases could serve as targets for cancer therapy. Some drugs that are inhibitors of protein kinases are already approved for treatment, other drugs are in clinical trials stage.

8.4.1 Receptor Tyrosine Kinases

Of the 91 unique tyrosine kinases identified so far, 59 are receptor tyrosine kinases (RTKs). They are transmembrane proteins: the domain with tyrosine kinase activity is located on the cytoplasmic side (together with regulatory domain). Extracellular region contains ligand binding domain. It can be a separate unit connected with the rest of the receptor by disulfide bond. Receptor tyrosine kinases are involved in transmitting signals into the cell, and their activity is extremely important in the regulation of cell division, differentiation, and morphogenesis. The majority of them are receptors for growth factors and hormones like epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin, nerve growth factor (NGF), vascular endothelial growth factor (VEGF), macrophage colony-stimulating factor (M-CSF), etc.

Binding of ligand to the receptor leads to oligomerization (usually dimerization) of monomeric receptor kinase or stabilization of already existing loosely associated dimers. Thereafter *trans*-autophosphorylation of kinases (i.e. phosphorylation of neighboring kinase in dimer) occurs. As a result of phosphorylation, changes in the spatial structure of the receptor are generated, causing the opening of kinase domain and binding of ATP in catalytic center (Fig. 8.10). Activated tyrosine kinase phosphorylates specific target proteins - often they are also enzymes, such as cytoplasmic tyrosine kinases.

This starts the kinases cascade, in which further types of kinases are phosphorylated and activated. As a result, transcription factors coud be activated or inactivated leading to changes in the expression of specific genes.

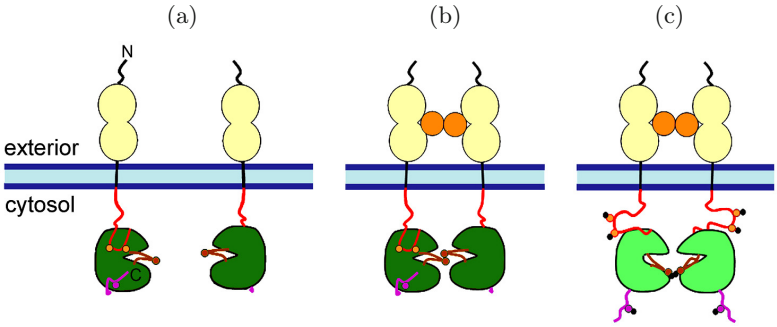

Fig. 8.10 General model of regulation of the activity of receptor tyrosine kinases: (a) in the absence of ligand the tyrosine kinase domain (dark green) of a receptor maintains the basal, low activity due to inhibitory interactions with the region adjacent to the membrane (red) and/or the carboxyterminal tail (violet). In addition, the activation segment (brown) has a structure that blocks an access to the catalytic center; (b) after binding of ligand (orange) and dimerization of the extracellular domains (yellow), the cytoplasmic domains are juxtaposed, which facilitates the trans-autophosphorylation of tyrosine residues (shown as circles) in the region adjacent to the membrane, in the activation segment and in the carboxy-terminal tail; (c) after phosphorylation (black dots) and reconfiguration of the inhibitory segments, the kinase domains become fully active (light green). Then phosphotyrosines interact with proteins containing SH2 (Src Homology 2) domains or phosphotyrosine binding domains. This initiates a series of events which eventually result in altered patterns of gene expression or other cellular responses.
Altered from: `http://www.nature.com/nrm/journal/v5/n6/fig_tab/nrm1399_F1.html`
Hubbard SR (2004) Juxtamembrane autoinhibition in receptor tyrosine kinases. Nature Reviews Molecular Cell Biology 5, 464-471.

Many ligands of receptor tyrosine kinases are multivalent. Also some tyrosine receptor kinases may form heterodimers with similar but not identical kinases. It is the reason why the response to the extracellular signal can be highly varied.

Cell must have a possibility to stop the signal response. Constant activity of receptors, e.g. growth factors, would lead to the uncontrolled division and consequently to carcinogenesis. Ligand-receptor complex, in case of receptor tyrosine kinases, is absorbed and destroyed through receptor endocytosis.

A class of tyrosine kinases recruited to the receptor just after connection of ligand to it is involved in signaling cascades activated among others by cytokines. One of such kinases is JAK kinase (Janus kinase) regulating activity of STAT proteins (ch. 8.5.3). **Cytokines** are protein molecules that affect

growth, proliferation and stimulation of hematopoietic cells and cells involved in immune response.

8.4.2 Receptor Serine/Threonine Kinases

At least 125 of more than 500 human protein kinases are serine-threonine kinases (STKs). Their activity is regulated by cAMP/cGMP, diacylglycerol (DAG), Ca^{2+}/calmodulin, or DNA damage. STKs select specific residues to phosphorylate on the basis of residues that flank the phosphoacceptor site, which together comprise the consensus sequence.

Receptor serine/threonine kinases transmit mainly signals induced by growth factors belonging to the family of TGFβ (transforming growth factor β). They exist as heterodimers consisting of type I and type II receptor. Ligand binding domain is present in type II receptor. Type II receptor binds ligand and then recruits and phosphorylates type I receptor. Activated type I receptor phosphorylates specifically SMAD proteins, which are transported to the nucleus where regulate gene expression.

8.5 Intracellular Signaling Pathways

There are a large number of intracellular signaling pathways responsible for transmitting information within the cell. The majority respond to external stimuli, which are received by receptors embedded in the plasma membrane. These receptors then transfer information across the membrane using a variety of transducers and amplifiers that engage a diverse repertoire of intracellular signaling pathways. The other categories are the pathways that are activated by signals generated from within the cell. All of these signaling pathways generate an internal messenger that is responsible for relaying information to the sensors that then engage the effectors that activate cellular responses. Signaling pathways often interact with each other. Interactions (positive and negative) of second messengers systems (so called cross-talk) decide on the final activity of different pathways and biological response. Signaling pathways are dynamic and can proceed differently in different cell types. Several examples of the better known cell signaling pathways are outlined below.

8.5.1 cAMP-dependent Signaling Pathway

Cyclic AMP is involved in the activation of protein kinases and regulates the effects of adrenaline and glucagon. It also binds to and regulates the function of ion channels dependent on cyclic nucleotides and other factors. Protein kinase A (PKA) is the main target of cAMP. PKA is present in the cytoplasm in an inactive form as tetramer (R2C2), composed of two catalytic subunits (C) and two regulatory subunits (R). Usually, it is located in the perinuclear

space (thanks to the AKAP protein - membrane-associated archoring protein). Cyclic AMP binds to regulatory subunits, which leads to the release of PKA catalytic subunits. Active PKA can phosphorylate among others: enzymes associated with the synthesis of neurotransmitters and metabolism of cyclic nucleotides, neurotransmitter receptors, ion channel proteins, proteins involved in regulation of transcription and translation, or cytoskeletal proteins. Processes dependent on cAMP/PKA (as well as cGMP/PKG) are mostly short-term processes, because rapid dephosphorylation of these proteins occurs. A more prolonged cellular responses are associated with the influence of cAMP on gene expression (Fig. 8.11). Released PKA catalytic subunits migrate to the nucleus (by passive diffusion), where they phosphorylate CREB protein (cAMP response element-binding protein). This leads to transcription of genes containing CRE sequence (cAMP response element) in the promoters (ch. 5.3.1).

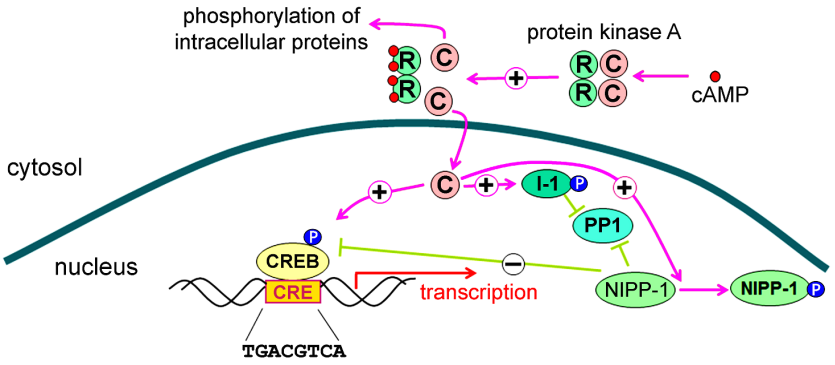

Fig. 8.11 Example of the complexity of processes dependent on cAMP/PKA. cAMP binds to two sites in each of the regulatory subunits (R) of PKA (protein kinase dependent on cAMP). Released catalytic subunits (C) phosphorylate serines and threonines in the target proteins. Long-term PKA activity is associated with effects on the gene expression through CREB. CREB activity can also be regulated by other enzymes: calmodulin, nerve growth factor NGF, and phosphatases. PKA phosphorylates at the same time CREB, I-1 protein and NIPP-1 protein. I-1 in phosphorylated form is an inhibitor of protein phosphatase PP1, while NIPP-1 in dephosphorylated form is an inhibitor of PP1, thus in phosphorylated form has activity opposite to the phosphorylated I-1. PP1 activity is a function of activities of phosphorylated proteins I-1 and NIPP-1. If PP1 is active, it dephosphorylates proteins phosphorylated by PKA.

8.5.2 NF-kappaB Signaling Pathway

NF-κB transcription factor can be activated by many signals, including TNF-α (tumor necrosis factor α), interleukin 1 (IL-1), compounds triggering mitosis (mitogens) in T and B cells, bacterial lipopolysaccharides (LPS), viral

proteins, and others. It controls the expression of genes involved in immune response, apoptosis, and cell cycle regulation. Therefore, the abnormal regulation of NF-κB may cause an inflammation, autoimmune diseases, viral infections, and carcinogenesis. In mammals, the family of NF-κB includes five proteins: NF-κB1 (or p50), NF-κB2 (or p52), RelA (or p65), RelB, and c-Rel. All of them possess conserved in evolution Rel domain, responsible for dimerization, binding to DNA, and for binding of IκB (inhibitor of NF-κB). NF-κB works only as a dimer. The most common active form of NF-κB contains subunits p50 or p52, and p65.

In the cytoplasm, NF-κB is inhibited by IκB. Activation signal transmitted from receptor (e.g. after binding of TNF-α to its receptor) leads to phosphorylation of the inhibitor by the IKK kinase (IκB kinase). This results in ubiquitination and degradation of IκB in the proteasome. Released NF-κB migrates to the nucleus, where it activates transcription of various genes, including its inhibitor, which within one hour reappears in the cell (Fig. 8.12).

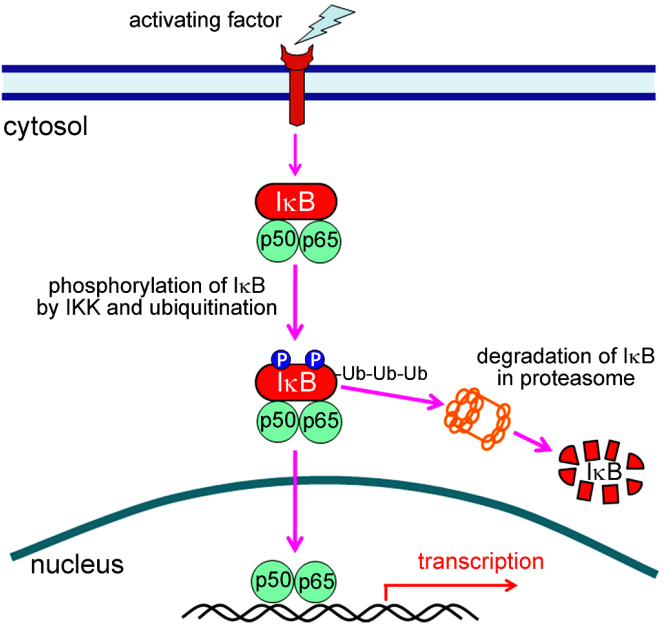

Fig. 8.12 General model of NF-κB activation

8.5.3 JAK-STAT Signaling Pathway

The JAK-STAT system consists of three main components: a receptor, JAK, and STAT. The receptors do not possess catalytic kinase activity, thus they

rely on the JAK family of tyrosine kinases. JAK is short for Janus Kinase. STAT proteins (Signal Transducer and Activator of Transcription) (family of seven proteins) are proteins both transmitting signal and activating transcription. The inactive form is a monomer and is in constant motion between the cytoplasm and the nucleus awaiting for activating signal. The JAK-STAT system is a major signaling alternative to the second messenger system.

Signal activating the JAK-STAT pathway comes from the membrane receptors. They can be activated by interferon, interleukin, growth factors, or other chemical messengers. The receptors exist as paired polypeptides and JAKs are associated with intracellular domains. Ligand binding enables a conformational change of the receptor and then JAK kinase autophosphorylates itself. The STAT protein then binds to the receptor. STAT is phosphorylated, forms a dimer (through the SH2 domain) and translocates into the cell nucleus, where it binds to DNA and promotes transcription of genes responsive to STAT (Fig. 8.13). STAT protein is dephosphorylated and inactivated by nuclear phosphatases, and then transported to the cytoplasm.

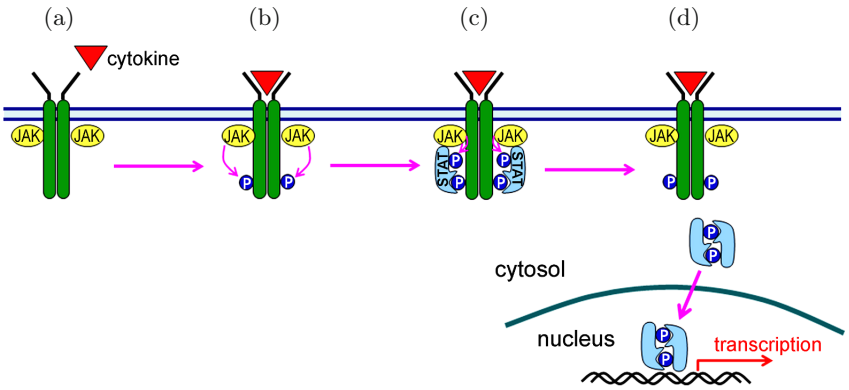

Fig. 8.13 General model of STAT protein activation

8.5.4 MAPK Signaling Pathway

Mitogen-Activated Protein Kinases (MAPKs) are serine/threonine-specific protein kinases. They respond to variety of external signals (mitogens, osmotic stress, heat shock, and proinflammatory cytokines), resulting in either cell growth, differentiation, inflammation, or apoptosis. Generally, the signal is transmitted as follows ("MAPK cascade"):

stimulus $\rightarrow$ **MAPKKK** $\rightarrow$ **MAPKK** $\rightarrow$ **MAPK** $\rightarrow$ **response**,

where MAPKK is MAPK kinase, and MAPKKK - MAPKK kinase.

Ligands that activate MAPK's cascade bind receptor tyrosine kinases. Next, signal is transmitted by small monomeric G proteins (sometimes by calcium ions or certain heterotrimeric G proteins) to MAPKKK. MAPK regulates the activities of several transcription factors (e.g. MYC, FOS), what leads to altered transcription of genes that are important for the cell cycle. Another effect of MAPK activation is to alter the translation of mRNA to proteins.

8.6 Apoptosis

Apoptosis, also known as programmed cell death (PCD), is strictly regulated, active process of self-destruction of an individual cell that may occur in multicellular organisms. It is an important cellular process that starts by specific signaling pathways in response to some external and internal stimuli. Morphologically, apoptosis is characterized by chromatin condensation and shrinkage of cell, and then by fragmentation of the nucleus and cytoplasm. As a result, apoptotic bodies surrounded by the cell membrane are formed, which are absorbed by phagocytes. In contrast to apoptosis, cells that die by necrosis swell and disintegrate, releasing the contents, which induces inflammation.

Apoptosis is an important process in development of organisms. Redundant cells are removed by apoptosis (e.g. in human embryo, during digit formation cells between fingers are removed and lack of apoptosis can lead to webbed fingers called syndactyly). Apoptosis is also important in the aging process and in many diseases. Excessive apoptosis occurs in many autoimmune and autodegenerative diseases, while not sufficiently efficient apoptosis is often one of the causes of the carcinogenesis.

A characteristic molecular feature of cells undergoing apoptosis is activation of specific cysteine proteases called **caspases**. Until now more than 10 different caspases have been identified. Caspases are involved in the signaling cascade, in which consecutive enzymes are activated as a result of limited proteolysis. Some of them, called initiator (apical, e.g. caspase-2, -8, -9, and -10), are activated by specific stimulus and start a cascade. Others, called effector (executioner, e.g. caspase-3, -6, and -7), are activated by initiator caspases and catalyze the proteolysis of other proteins essential for cellular functions. Signaling pathway associated with caspases includes:

1. Activation of apical caspases by specific initiatory signals, e.g. changes in membrane receptors status.
2. Activation of effector caspases by initiatory caspases, which cut inactive caspases (procaspases) at specific sites.
3. Specific proteolysis of important cellular proteins by effector caspases. One of the substrate for effector caspases is inhibitor (DFF45/ICAD protein) of apoptotic nuclease (DFF40/CAD). The cleavage and inactivation of

the inhibitor allows nuclease to enter the nucleus and fragment the DNA, which is an irreversible stage of apoptotic cell death.

Signals inducing apoptosis can reach the cell from the outside. Death receptor (extrinsic) pathway of apoptosis is then triggered (Fig. 8.14). So called death ligands (e.g. FasL/CD95L, TRAIL, APO-3L, or TNF) bind with cell membrane specific death receptors (Fas/CD95, DR3, DR4, DR5, TNFR) possessing the death domain (DD). Binding of ligand to receptor induces its trimerization and attachment of adaptor proteins, also containing death domains (FADD, TRADD, RAIDD, DAXX, and others). As a result, initiatory caspases-8 or -10 are activated.

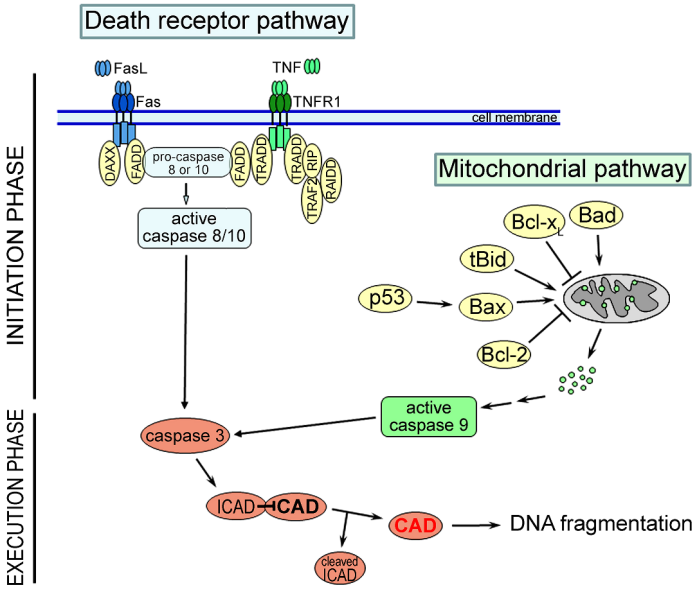

Fig. 8.14 Simplified diagram of transmitting signals leading to apoptosis

Apoptosis can also be caused by intracellular factors, mainly by signals from the mitochondria (mitochondrial or intrinsic pathway of apoptosis). Factors leading to increase of mitochondrial membrane permeability may influence release of proapoptotic factors (such as cytochrome c) into the cytosol. Released cytochrome c, together with APAF1 protein activates the initiator caspase-9, which then activates executioner caspase-3.

In cells undergoing apoptosis the process of cytochrome c (and other apoptotic proteins) release from mitochondria is regulated by a number of supporting proteins from Bcl-2 family. This family consists of three subfamilies. Two of them are formed by proteins present in the outer mitochondrial membrane: pro-apoptotic Bax and Bak proteins, and anti-apoptotic Bcl-2 and

Bcl-xL proteins. These proteins in mitochondrial membrane can form homo- or heterodimers. The third subfamily, including Bid, Bik, and Bim, includes pro-apoptotic proteins present in the cytoplasm. They can interact with membrane proteins from Bcl-2 family and modify their activity. It is believed that membrane proteins from Bcl-2 family can form membrane pores enabling outflow of cytochrome c to the cytoplasm or can interact with proteins forming ion channel VDAC (voltage-dependent anion channel) and modulate its permeability to cytochrome c.

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