SIGNAL TRANSDUCTION

6.28.1 Introduction

Key Terms

A **ligand** is an extracellular molecule that binds to the receptor on the plasma membrane of a cell, thereby effecting a change in the cytoplasm.

A **receptor** is a transmembrane protein, located in the plasma membrane, that binds a ligand in a domain on the extracellular side, and as a result has a change in activity of the cytoplasmic domain. (The same term is sometimes used also for the steroid receptors, which are transcription factors that are activated by binding ligands that are steroids or other small molecules.)

A **transporter** is a type of receptor that moves small molecules across the plasma membrane. It binds the molecules on its extracellular surface, and releases them into the cytoplasm.

Internalization is a process through which a ligand-receptor complex is brought into the cell.

Endocytosis is the process whereby cells internalize small molecules and particles from their surroundings. There are several forms of endocytosis, all of which involve the formation of a membranous vesicle from the plasma membrane.

Signal transduction describes the process by which a receptor interacts with a ligand at the surface of the cell and then transmits a signal to trigger a pathway within the cell.

A **second messenger** is a small molecule that is generated when a signal transduction pathway is activated. The classic second messenger is cyclic AMP, which is generated when adenylate cyclase is activated by a G protein (when the G protein itself was activated by a transmembrane receptor).

The ability of a species of kinase to phosphorylate itself is referred to as **autophosphorylation.** Autophosphorylation does not necessarily occur on the same polypeptide chain as the catalytic site; for example, in a dimer, each subunit may phosphorylate the other.

G proteins are guanine nucleotide-binding proteins. Trimeric G proteins are associated with the plasma membrane. When bound by GDP the trimer remains intact and is inert. When the GDP is replaced by GTP, the α subunit is released from the $\beta\gamma$ dimer. Either the α monomer or the $\beta\gamma$ dimer then activates or represses a target protein. Monomeric G proteins are cytosolic and work on the same principle that the form bound to GDP is inactive, but the form bound to GTP is active.

The plasma membrane separates a cell from the surrounding environment. It is permeable only to small lipid-soluble molecules, such as the steroid hormones, which can diffuse through it into the cytoplasm. It is impermeable to water-soluble material, including ions, small inorganic molecules, and polypeptides or proteins.

The response of the cell to hydrophilic material in the environment depends on

interactions that occur on the extracellular side of the plasma membrane. The hydrophilic material binds specifically to the extracellular domain of a protein embedded in the membrane. The extracellular molecule typically is called the **ligand**, and the plasma membrane protein that binds it is called the **receptor**.

Two fundamental types of response to an external stimulatory molecule that cannot penetrate the membrane are reviewed in **Figure 28.1**:

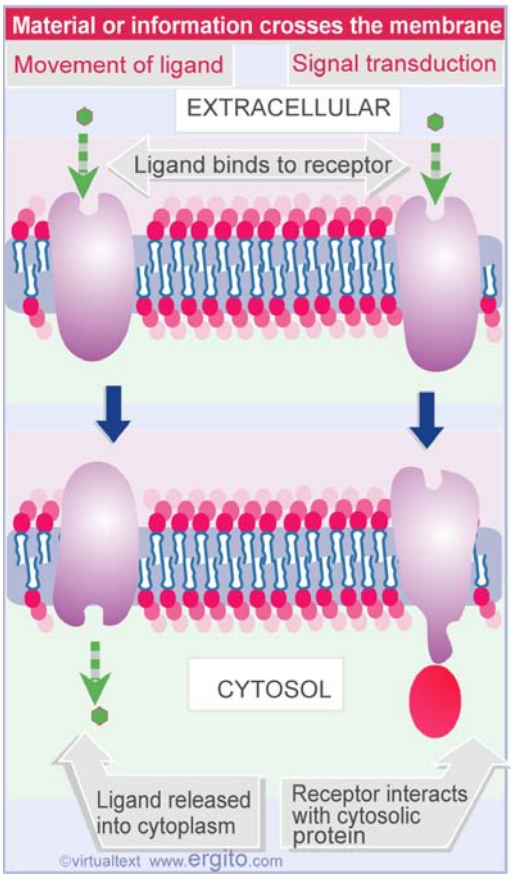

Figure 28.1 Overview: information may be transmitted from the exterior to the interior of the cell by movement of a ligand or by signal transduction.

- *Material* – molecular or macromolecular – is physically transmitted from the outside of the membrane to the inside by transport through a proteinaceous channel in the lipid bilayer.
- *A signal* is transmitted by means of a change in the properties of a membrane protein that activates its cytosolic domain.

The physical transfer of material extends from ions to small molecules such as sugars, and to macromolecules such as proteins. Three major transport routes controlled by plasma membrane proteins are reviewed in **Figure 28.2**:

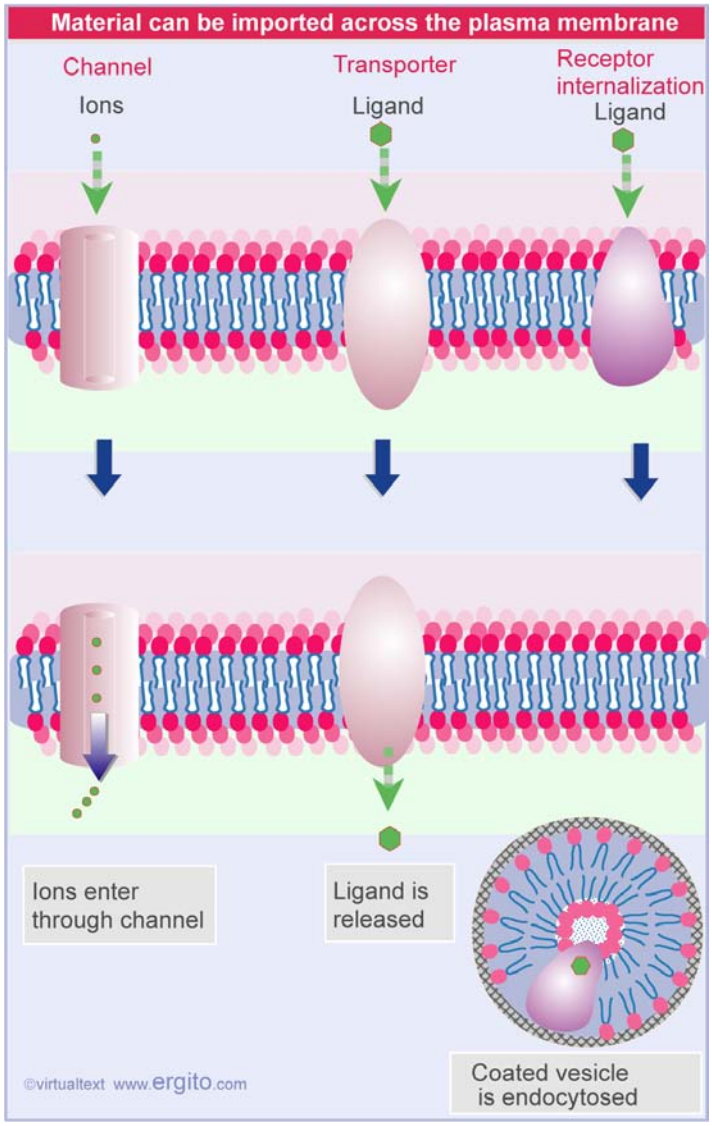

Figure 28.2 Three means for transferring material of various sizes into the cell are provided by ion channels, receptor-mediated ligand transport, and receptor internalization.

- Channels control the passage of ions: different channels exist for potassium, sodium, and calcium ions. By opening and closing in response to appropriate signals, the channels establish ionic levels within the cell (a feature of particular significance for cells of the neural network).

- One means to import small molecules is for a receptor to transport the molecule from one side of the membrane to the other. **Transporters** are responsible for the import of small molecules (such as sugars) across the membrane. The target molecule binds to the receptor on the extracellular side, but then is released on the cytoplasmic side.

- Ligand-binding may trigger the process of **internalization**, in which the receptor-ligand combination is brought into the cell by the process of

endocytosis. In due course, the receptor and ligand are separated; the receptor may be returned to the surface for another cycle, or may be degraded. As described in *Molecular Biology 6.27.15 Receptors recycle via endocytosis*, endocytosis involves the passage of membrane proteins from one surface to another via coated vesicles.

The transmission of a signal involves the interaction of an extracellular ligand with a transmembrane protein that has domains on both sides of the membrane. Binding of ligand converts the receptor from an inactive to an active form. The basic principle of this interaction is that ligand binding on the extracellular side activates the receptor domain on the cytoplasmic side. The process is called **signal transduction**, because a signal has in effect been transduced across the membrane. The amplitude of the cytosolic signal is much greater than the original extracellular signal (the ligand), so signal transduction amplifies the original signal.

Figure 28.3 illustrates the principle of signal transduction. A receptor typically is activated by being caused to dimerize when a ligand binds to its extracellular domain. Its cytosolic domain then interacts with a protein at the plasma membrane. This interaction most often takes one of two forms. It increases the quantity of a small molecule (called a **second messenger**) inside the cell. Or it directly activates a protein whose role is to activate other proteins; one common type of protein that is activated early in such pathways is a monomeric GTP-binding protein.

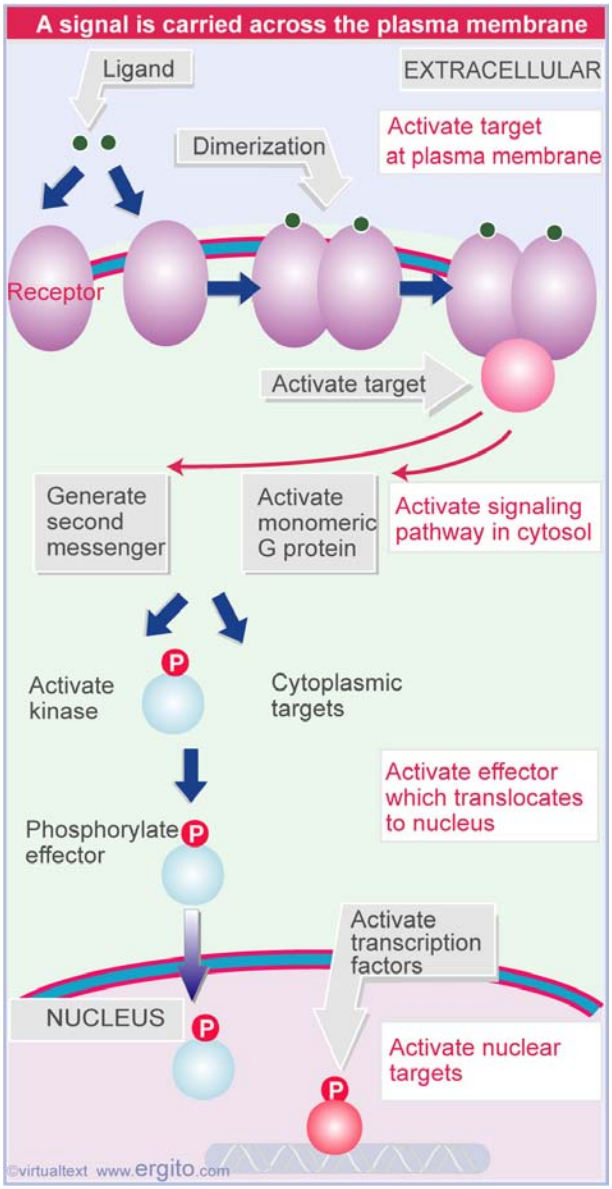

Figure 28.3 A signal transduction pathway carries a signal from the cell surface into the cytoplasm and (sometimes) into the nucleus.

However a signal transduction pathway is initiated, a common means of propagating the pathway through the cytosol is to activate a protein kinase, which activates a series of other protein kinases. Ultimately the signal leads to the activation of effectors, which trigger changes in the cell. Some of the effectors act in the cytosol (for example, to affect the cytoskeleton), but some carry the signal into the nucleus, where the ultimate target is the activation of transcription factors that cause new patterns of gene expression.

The major signal transduction pathways that we discuss in this chapter are extensively conserved throughout animal evolution, and can be found in most or all animal cells. They are essentially absent from plants, which have evolved different

pathways for signal transduction.

Two major types of signal transduction are reviewed in **Figure 28.4**:

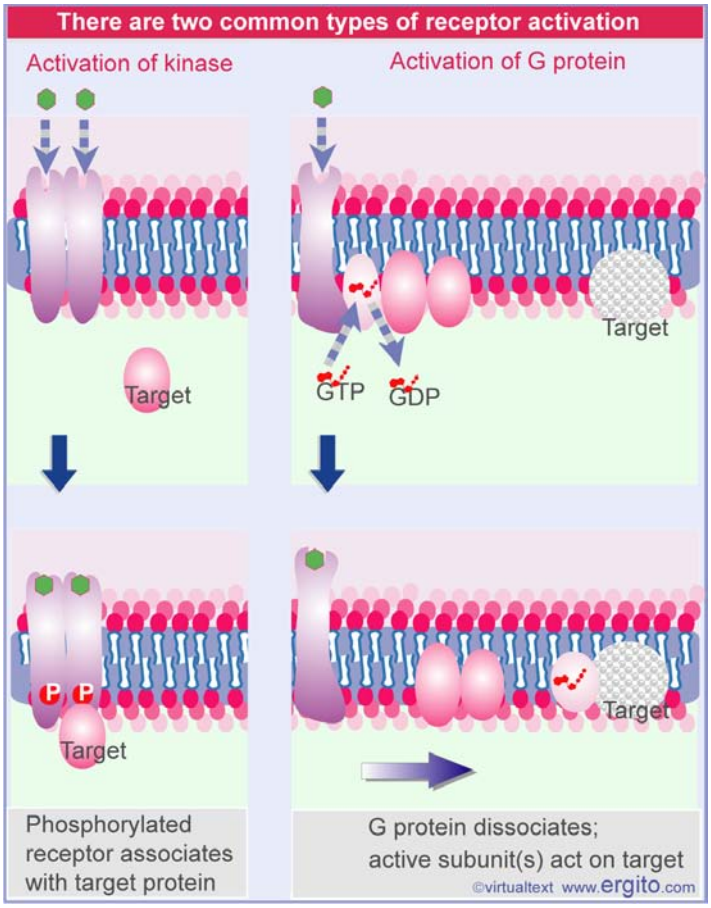

Figure 28.4 A signal may be transduced by activating the kinase activity of the cytoplasmic domain of a transmembrane receptor or by dissociating a G protein into subunits that act on target proteins in the membrane.

- The receptor has a protein kinase activity in its cytosolic domain. The activity of the kinase is activated when ligand binds to the extracellular domain. The kinase phosphorylates its own cytoplasmic domain; this **autophosphorylation** enables the receptor to associate with and activate a target protein, which in turn acts upon new substrates within the cell. The most common kinase receptors are tyrosine kinases, but there are also some serine/threonine kinase receptors. Such pathways most often lead to activation of a GTP-binding protein that activates a cascade of cytosolic kinases.

- The receptor may interact with a trimeric **G protein** that is associated with the cytosolic face of the membrane (for introduction see *Molecular Biology Supplement 32.10 G proteins*). G proteins are named for their ability to bind guanine nucleotides. The inactive form of the G protein is a trimer bound to GDP. Receptor activation causes the GDP to be replaced with GTP; as a result, the G protein dissociates into a single subunit carrying GTP and a dimer of the

two other subunits. Either the monomer or the dimer then acts upon a target protein, often also associated with the membrane, which in turn reacts with a target(s) in the cytoplasm. This chain of events often stimulates the production of second messengers, the classic example being the production of cyclic AMP.

SIGNAL TRANSDUCTION

6.28.2 Carriers and channels form water soluble paths through the membrane

Key Terms

A **concentration gradient** is a change in the concentration of a molecule or ion from one point to another. The gradient might be gradual (as in a solution that is not homogenous) or abrupt (created by a membrane).

An **electrical gradient** is a change in the amount of charge from one point to another.

A change in the concentration of ions from one point to another produces an **electrochemical gradient.** The term indicates that there is a change in the concentration of both electrical charge and of a chemical species.

Passive transport describes movement of molecules along their electrochemical gradient; no energy is required.

Active transport is an energy-consuming process that moves molecules against an electrochemical gradient. Energy for the movement is provided by hydrolysis of ATP.

A **carrier protein** moves directly a solute from one side of the plasma membrane to the other. In the process, the protein undergoes a conformational change.

A **uniporter** is a type of carrier protein that moves only one type of solute across the plasma membrane.

A **symporter** is a type of carrier protein that moves two different solutes across the plasma membrane in the same direction. The two solutes can be transported simultaneously or sequentially.

An **antiporter** is a type of carrier protein that simultaneously moves two different types of solutes in opposite directions across the plasma membrane.

An **ion channel** is a transmembrane protein which selectively allows the passage of one type of ion across the membrane. Ion channels are usually oligomers with a central aqueous pore through which the ion passes.

A channel which only allows passage of its substrate under certain conditions is referred to as "**gated".** Gated channels can exist in at least two conformations, one of which is open and the other closed.

Ligand-gated channels open or close in response to the binding of a specific molecule.

Voltage-gated channels are open or closed depending on the voltage across the membrane.

A **second messenger gated channel** is an ion channel whose activity is controlled by small signaling molecules inside the cell.

Key Concepts

- The electric gradient across the plasma membrane (inside is more negative) favors

entry of cations and opposes entry of anions.

- The concentration gradient depends on the ion, typically with low intracellular levels of Na^+ and Cl^- and high levels of K^+.
- If the overall electrochemical gradient is favorable an ion can enter passively, otherwise it needs to be actively transported against the gradient.
- Carrier proteins that transport solutes across the membrane can be uniporters (one solute), symporters (two solutes) or antiporters (two solutes in opposite directions).
- Ion channels are water-soluble pores in the membrane that may be gated (controlled) by voltage or by ligands.

The impermeability of the plasma membrane to water-soluble compounds enables different aqueous conditions to be maintained on either side. The ionic environment of the cytosol is quite different from the extracellular ionic milieu. Within the cytoplasm, different organelles offer different ionic environments. A striking example is the maintenance of an acid pH in endosomes and lysosomes, with immediate implications for the functions of the proteins that enter them (see *Molecular Biology 6.27.15 Receptors recycle via endocytosis*). Another example is the maintenance of a store of Ca^{2+} in the endoplasmic reticulum. (The nucleus is an exception to the rule that conditions in membrane-bounded organelles usually differ from the cytosol. The nucleoplasm essentially is subjected to the same conditions as the cytosol. This happens because the nuclear pores form relatively large openings in the nuclear envelope, through which ions and other small molecules can diffuse freely.)

A notable feature of the cytosolic environment is that there are more free cations (positively charged) (~150 mM) than anions (~10 mM). The reason is that many cellular constituents are negatively charged – for example, nucleic acids have multiple negative charges for every phosphate group in the phosphodiester backbone. The superfluity of cations therefore establishes electrical neutrality by balancing these fixed charges.

The intracellular concentrations of Na^+ and Cl^- are low (~10 mM) while those outside the cell are high (>100 mM); and the situation for K^+ is reversed. This creates a **concentration gradient** across the membrane for each ion.

The plasma membrane is electrically charged (due to the different phospholipid compositions of the inner and outer leaflets). There is an **electrical gradient** in which the inside is negative compared to the outside. This voltage difference favors the entry of cations and opposes the entry of anions.

Together the concentration gradient and electrical gradient constitute the **electrochemical gradient**, which is characteristic for each solute. A solute whose gradient is favorable can enter the cell when a channel opens; the gradient is sufficient to drive **passive transport** of a solute such as Na^+ or Cl^- into the cell. But a solute that faces an unfavorable gradient requires **active transport** in which energy is used to pump it into the cell against the gradient.

The passage of ions (and other small solutes) through the plasma membrane is mediated by resident transmembrane proteins. A common feature of these proteins is their large size and the presence of multiple membrane-spanning regions, features which together argue that they provide a relatively static feature of the membrane. **Figure 28.5** illustrates two general means of transport across the membrane:

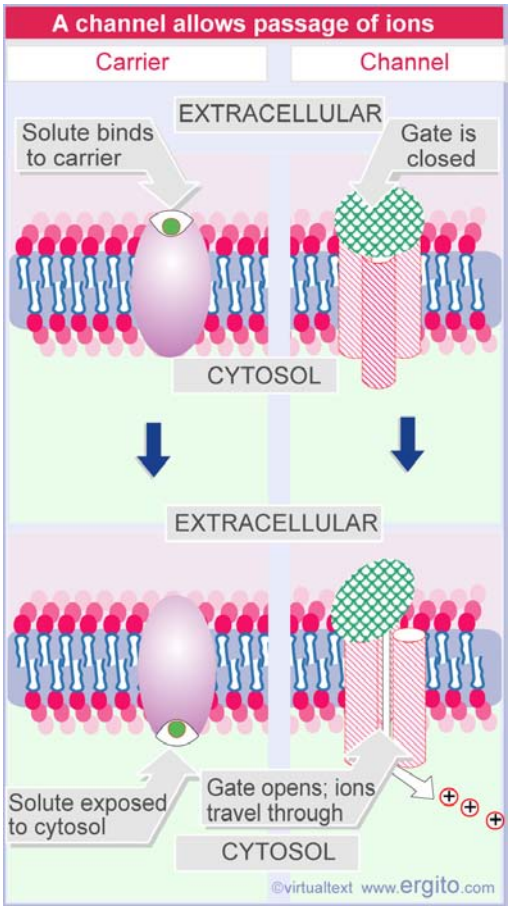

Figure 28.5 A carrier (porter) transports a solute into the cell by a conformational change that brings the solute-binding site from the exterior to the interior, while an ion channel is controlled by the opening of a gate (which might in principle be located on either side of the membrane).

- A **carrier protein** binds a solute on one side of the membrane and then experiences a conformational change that transports the solute to the other side of the membrane. By binding the solute on one side and releasing it on the other, the carrier in effect directly transports the solute across the membrane. Several types of carriers are distinguished by the number of solutes that they transport, and the directions in which they transport them. Carriers that transport a single solute across the membrane are called **uniporters**; carriers that simultaneously or sequentially transport two different solutes are called **symporters**; and carriers that transport one solute in one direction while transporting a different solute in the opposite direction are called **antiporters**. Carrier proteins may be

used for passive transport or linked to an energy source to provide active transport. Energy for active transport is provided by hydrolysis of ATP, the classic example being the Na^+-K^+ pump that functions as an antiporter, pumping sodium out of the cell and potassium into it. Another source of energy is the electrochemical gradient itself; a symporter brings Na^+ into the cell together with some other solute, using the favorable gradient of sodium to overcome the unfavorable gradient of the other solute.

- An **ion channel** comprises a water-soluble pore in the membrane. Its activity is controlled by regulation of the opening and closing of the channel. When it is open, ions can diffuse passively, as driven by the electrochemical gradient. Ion channels allow *only* passive transport. The resting state of an ion channel is closed, and the **gates** that control channel activity usually open only briefly, in response to a specific signal. **Ligand-gated** channels are receptors that respond to binding of particular molecules, amongst which the neurotransmitters acetylcholine, glycine, GABA (γ-amino-butyric acid), and glutamate are prominent examples. **Voltage-gated** channels respond to electric changes, again a prominent feature of the neural system. **Second messenger gated channels** provide yet another means for signal transduction, one interesting example comprising channels that respond to activation of G proteins.

The structures of both carriers and channels present a paradox. They are transmembrane proteins that have multiple membrane-spanning domains, each consisting of a stretch of amino acids of sufficient hydrophobicity to reside in the lipid bilayer. Yet within these hydrophobic regions must be a highly selective, water-filled path that permits ions to travel through the membrane.

One solution to this problem lies in the structure of the transmembrane regions. Instead of comprising unremittingly hydrophobic stretches like those of single membrane-pass proteins, they contain some polar amino acids. They are likely to be organized as illustrated in **Figure 28.6** as amphipathic helices in which the hydrophobic face associates with the lipid bilayer, while the polar faces are aligned with one another to create the channel.

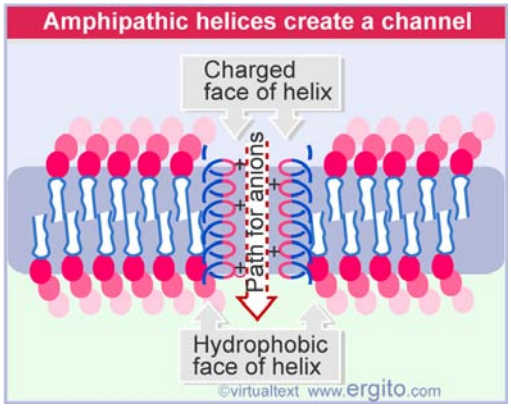

Figure 28.6 A channel may be created by amphipathic helices, which present their hydrophobic faces to the lipid bilayer, while juxtaposing their charged faces away from the bilayer. In this example, the channel is lined with positive charges, which would encourage the passage of anions.

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SIGNAL TRANSDUCTION

6.28.3 Ion channels are selective

Key Terms

Ion selectivity refers to the specificity of an ion channel for a particular type of ion.

Key Concepts

- Channels typically consists of several protein subunits with the water-soluble pore at the axis of symmetry.
- Selectivity is determined by the properties of the pore.
- The gate acts by a mechanism resembling a ball and chain.

The importance of the interior of the channel is indicated by the **ion selectivity**. Different channels permit the passages of different ions or groups of ions. The channels are extremely narrow, so ions must be stripped of their associated water molecules in order to pass through. The channel possesses a "filter" at the entrance to the pore that has specificity for its particular ion, presumably based upon its geometry and electrostatic charge.

The structures of particular ion channels are beginning to reveal their general features. A common feature is that the constituent proteins are large and have several membrane-spanning regions. A channel probably consists of a "ring" of 4, 5, or 6 subunits, organized in a symmetrical or quasi-symmetrical manner. The water-filled pore is found at the central axis of symmetry. The size of the pore generally increases with the number of subunits in the ring. The subunits are always related in structure, and sometimes are identical. They may consist of separate proteins or of related domains in a single large protein (for review see 296; 297).

Voltage-gated sodium channels have a single type of subunit, a protein of 1820 amino acids with a repetitive structure that consists of 4 related domains. Each domain has several membrane-spanning regions. The four domains are probably arranged in the membrane in a pseudo-symmetrical structure. Two smaller subunits are associated with the large protein.

Potassium channels have a smaller subunit, equivalent to one of the domains of the sodium channel; four identical subunits associate to create the channel. Six transmembrane domains are identified in the protein subunit by hydrophobicity analysis; they are numbered S1-S6. The S4 domain has an unusual structure for a transmembrane region: it is highly positively charged, with arginine or lysine residues present at every third or fourth position. The S4 motif is found in voltage-gated K^+, Na^+, and Ca^{2+} channels, so it seems likely that it is involved with a common property, thought to be channel opening. Some potassium channels have only the S5-S6 membrane-spanning domains, and they appear to be basically shorter versions of the protein.

Analysis of the *shaker* potassium channel of the fly has revealed some novel features, illustrated in **Figure 28.7**. The region that forms the pore has been identified by mutations that alter the response to toxins that inhibit channel function. It occupies the region between transmembrane domains S5 and S6, forming two membrane-spanning stretches that are not organized in the usual hydrophobic α-helical structure. The structure could be a rather extended β-hairpin. The state of the channel (open or closed) is controlled by the N-terminal end, which resembles a ball on a chain. The ball is in effect tethered to the channel by a chain, and plugs it on the cytoplasmic side. The length of the chain controls the rate with which the ball can plug the channel after it has been opened.

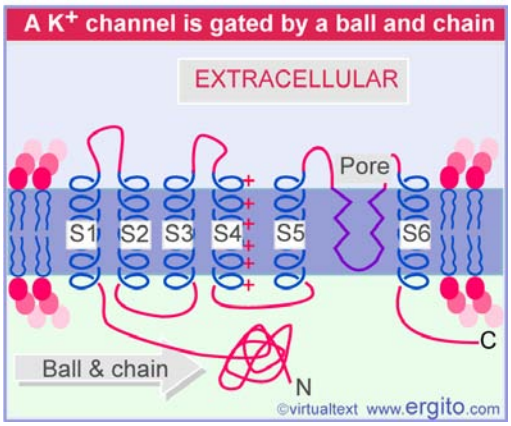

Figure 28.7 A potassium channel has a pore consisting of unusual transmembrane regions, with a gate whose mechanism of action resembles a ball and chain.

A major question about potassium channels is how their selectivity is maintained. K^+ and Na^+ ions are (positively charged) spheres of 1.33 Å and 0.95 Å, respectively. K^+ ions are selected over Na^+ ions by a margin of $10^4\times$, but at the same time, up to 10^8 ions per second can move through the pore, basically close to the diffusion limit. The salient features of the pore of a potassium channel, based on the crystal structure, are summarized in **Figure 28.8**, and shown as a cutaway model in **Figure 28.9**. The pore is ~45 Å long and consists of three regions. It starts inside the cell with a long internal pore, opens out into a central cavity of ~10 Å diameter, and then passes to the extracellular space with a narrow selectivity filter. The lining of the inner pore and central cavity is hydrophobic, providing a relatively inert surface to a diffusing potassium ion. The central cavity is aqueous, and may serve to lower the electrostatic barrier to crossing the membrane (which is at its maximum in the center). The selectivity filter has negative charges and is lined with the polypeptide backbone. When a K^+ ion loses its hydrating water on entering the filter, the contacts that it made with the water will be replaced by contacts with the oxygens of the polypeptide carbonyl groups. The size of the pore may be set so that a smaller sodium ion would not be close enough to make these substitute contacts (822).

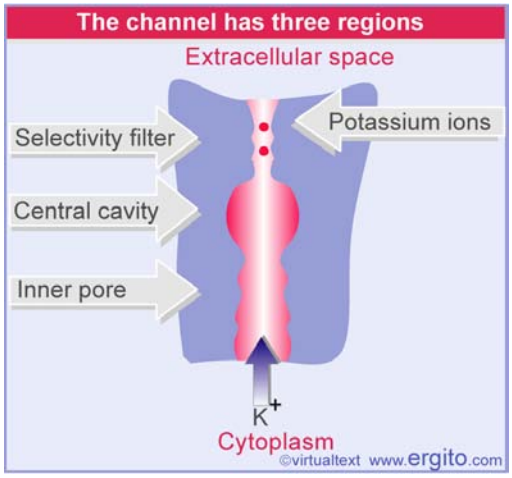

Figure 28.8 The pore of a potassium channel consists of three regions.

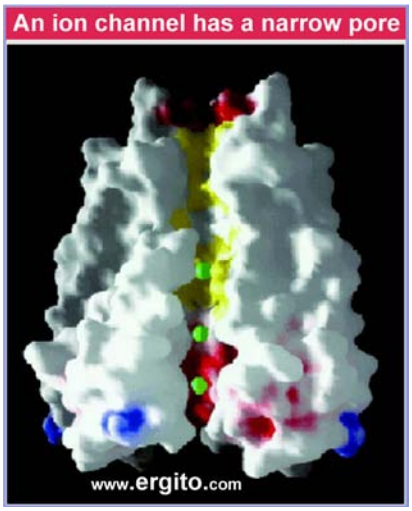

Figure 28.9 A model of the potassium channel pore shows electrostatic charge (blue = positive, white = neutral, red = negative) and hydrophobicity (= yellow). Photograph kindly provided by Rod MacKinnon (see 822).

Reviews

296. Unwin, N. (1989). *The structure of ion channels in membranes of excitable cells*. Neuron 3, 665-676.

297. Miller, C. (1989). *Genetic manipulation of ion channels: a new approach to structure and mechanism*. Neuron 2, 1195-1205.

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822. Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998). *The structure of the potassium channel: molecular basis of K+ conduction and selectivity*. Science 280, 69-77.

SIGNAL TRANSDUCTION

6.28.4 Neurotransmitters control channel activity

Key Concepts

- Neurotransmitter-gated receptors are ion channels that are controlled by neurotransmitters such as acetylcholine, glycine, or GABA.
- The nicotinic acetylcholine receptor is a 5-subunit ion channel that admits several cations but is largely used to control Na^+ uptake by the cell.

Neurotransmitter-gated receptors form a superfamily of related proteins in the 5-member channel class. The nicotinic acetylcholine receptor has been characterized in the most detail, and is a pentamer with the structure α_2 β δ γ. As illustrated in **Figure 28.10**, the bulk of the 5 subunits projects above the plasma membrane into the extracellular space. The openings to the channel narrow from a diameter of ~25 Å until reaching the pore itself. The entrance on the extracellular side is very deep, ~60 Å; the distance on the cellular side is shorter, 20 Å. The pore extends through the 30 Å of the lipid bilayer and is only ~7 Å in diameter.

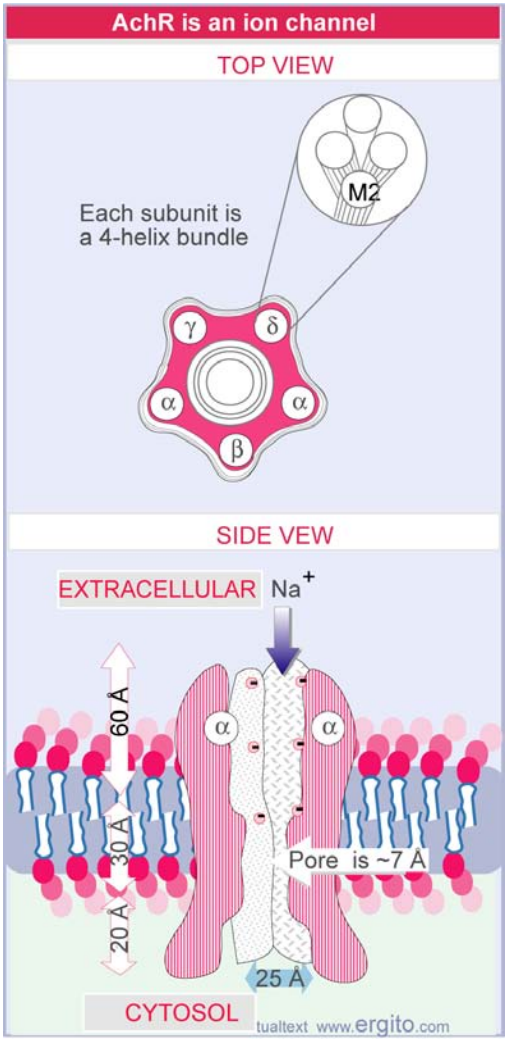

Figure 28.10 The acetylcholine receptor consists of a ring of 5 subunits, protruding into the extracellular space, and narrowing to form an ion channel through the membrane.

Ligand binding occurs on the α subunits. Both α subunits must bind an acetylcholine for the gate to open. Where is the gate? Since the channel is really narrow only in the region within the lipid bilayer, the gate seems likely to be located well within the receptor. Structural changes that occur upon opening seem greatest just by the cytoplasmic side of the lipid bilayer, so it is possible that the gate is located at the level of the phospholipid heads on the cytoplasmic boundary. So the acetylcholine receptor, like many other receptors, must transmit information about ligand binding internally, from the extracellular acetylcholine binding site to the near-cytoplasmic gate.

How does the gate function? It might consist of an electrostatic repulsion, in which positive groups are extruded into the channel to prevent passage of cations. Or it may take the form of a physical impediment to passage, in which a conformational change brings bulky groups to block the pore.

Ion selectivity may be determined by the walls of the wide entry passage. The walls lining the entrances to the pore have negatively charged groups; each subunit carries ~10 negative charges in its extracellular region. These charge clusters could modify the ionic environment at the entrance to the channel, concentrating the desired ions and diluting ions that are selected against. The structure of the acetylcholine receptor allows passage of Na^+, K^+, or Ca^{2+} ions, but because of the prevailing gradients, its main use in practice is to allow the entry of Na^+ into the cell.

The acetylcholine receptor is an example of a superfamily of receptors gated by neurotransmitters. All appear to have the same general organization, consisting of 5 subunits whose structures are related to one another. All the subunits are about the same size (~50 kD), and each is probably organized in the membrane as a bundle of 4 helices (each helix containing a transmembrane domain). In each case, one of the four transmembrane domains (called M2) has an amphipathic structure and seems likely to be involved in lining the walls of the pore itself. The presence of serine and threonine residues, and some paired acid-basic residues, may assist ion passage. The sequences of subunits of the glycine and GABA receptors are related to the acetylcholine receptor subunits. Some changes in the sequences seem likely to reflect the ion selectivity. So the glycine and GABA receptors have positively charged groups in the entrance walls, consistent with their transport of anions such as Cl^-.

SIGNAL TRANSDUCTION

6.28.5 G proteins may activate or inhibit target proteins

Key Terms

A **receptor** is a transmembrane protein, located in the plasma membrane, that binds a ligand in a domain on the extracellular side, and as a result has a change in activity of the cytoplasmic domain. (The same term is sometimes used also for the steroid receptors, which are transcription factors that are activated by binding ligands that are steroids or other small molecules.)

G proteins are guanine nucleotide-binding proteins. Trimeric G proteins are associated with the plasma membrane. When bound by GDP the trimer remains intact and is inert. When the GDP is replaced by GTP, the α subunit is released from the β γ dimer. Either the α monomer or the β γ dimer then activates or represses a target protein. Monomeric G proteins are cytosolic and work on the same principle that the form bound to GDP is inactive, but the form bound to GTP is active.

An **effector** is the target protein for the activated G protein.

A **second messenger** is a small molecule that is generated when a signal transduction pathway is activated. The classic second messenger is cyclic AMP, which is generated when adenylate cyclase is activated by a G protein (when the G protein itself was activated by a transmembrane receptor).

A **serpentine** receptor has 7 transmembrane segments. Typically it activates a trimeric G protein.

Key Concepts

- Ligand binding to a serpentine membrane receptor causes it to activate a G protein.
- The G protein is a trimer bound to GDP in its inactive state.
- The mechanism of activation is that the receptor causes the GDP bound by the G-protein to be replaced with GTP.

G proteins transduce signals from a variety of receptors to a variety of targets. The components of the general pathway can be described as:

- The **receptor** is a resident membrane protein that is activated by an extracellular signal.
- A **G protein** is converted into active form when an interaction with the activated receptor causes its bound GDP to be replaced with GTP.
- An **effector** is the target protein that is activated (or – less often – inhibited) by the G protein; sometimes it is another membrane-associated protein.

- **Second messengers** are small molecules that are released as the result of activation of (certain types) of effectors.

Another terminology that is sometimes used to describe the relationship of the components of the transduction pathway is to say that the receptor is *upstream* of the G protein, while the effector is *downstream*.

The effectors linked to different types of G proteins are summarized in **Figure 28.11**. The important point is that there is a large variety of G proteins, activated by a wide variety of receptors. The activation of an individual G protein may cause it to stimulate or to inhibit a particular effector; and some G proteins act upon multiple effectors (causing the activation in turn of multiple pathways). Two of the classic G proteins are G_s, which stimulates adenylate cyclase (increasing the level of cAMP), and G_t, which stimulates cGMP phosphodiesterase (decreasing the level of cGMP). The cyclic nucleotides are a major class of second messengers; another important group consists of small lipid molecules, such as inositol phosphate or DAG (diacylglycerol; for review see 306).

G proteins have various effectors

G protein	Effector function	Second messenger	Example of receptor
s	Stimulates adenylyl cyclase	↑ cAMP	β-adrenergic
olf	Stimulates adenylyl cyclase	↑ cAMP	Odorant
i	Inhibits adenylate cyclase Opens K^+ channels	↓ cAMP ↑ Membrane potential	Somatostatin "
o	Closes Ca^{2+} channels	↓ Membrane potential	m2 acetylcholine
t (transducin)	Stimulates cGMP phosphodiesterase	↓ cGMP	Rhodopsin
q	Activates phospholipase Cβ	↑ InsP3, DAG	m1 acetylcholine

Figure 28.11 Classes of G proteins are distinguished by their effectors and are activated by a variety of transmembrane receptors.

Although the receptors that couple to G proteins respond to a wide variety of ligands, they have a common type of structure and mode of binding the ligand. They are **serpentine** receptors, with 7 transmembrane regions, and function as monomers. The greatest conservation of sequence is found in hydrophobic transmembrane regions, which in fact are used to classify the serpentine receptors into individual families (for review see 304; 3438).

The binding sites for small hydrophobic ligands lie in the transmembrane domains, so that the ligand becomes bound in the plane of the membrane. The smallest ligands, such as biogenic amines, may be bound by a single transmembrane segment. Larger ligands, such as extended peptides, may have more extensive binding sites in which extracellular domains provide additional points of contact. Large peptide hormones may be bound mainly by the extracellular domains.

When the ligand binds to its site, it triggers a conformational change in the receptor that causes it to interact with a G protein. A well characterized (although not typical)

case is that of rhodopsin, which contains a retinal chromophore covalently linked to an amino acid in a transmembrane domain. Exposure to light converts the retinal from the 11-*cis* to the all *trans* conformation, which triggers a conformational change in rhodopsin that causes its cytoplasmic domain to associate with the G_t protein (transducin).

Reviews

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SIGNAL TRANSDUCTION

6.28.6 G proteins function by dissociation of the trimer

Key Concepts

- When GDP is replaced by GTP, a trimeric G protein dissociates into an α-GTP subunit and a β γ dimer.
- It is most often the α subunit that activates the next component (the effector) in the pathway.
- Less often the β γ activates the effector.

G proteins are trimers whose function depends on the ability to dissociate into an α monomer and a β γ dimer. The dissociation is triggered by the activation of an associated receptor. In its inactive state, the α subunit of the G protein is bound to GDP. **Figure 28.12** shows that the activated receptor causes the GDP to be replaced by GTP. This causes the G protein to dissociate into a free α-GTP subunit and a free β γ dimer. (The basic interactions of G proteins are reviewed in *Molecular Biology Supplement 32.10 G proteins*). They are found in all classes of eukaryotes.

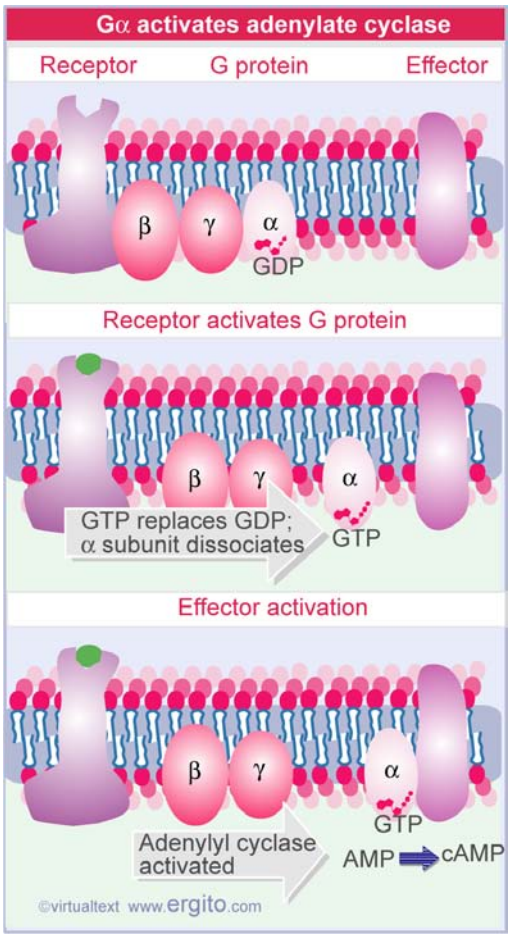

Figure 28.12 Activation of G_s causes the α subunit to activate adenylate cyclase.

The interaction between receptor and G protein is catalytic. After a G protein has dissociated from an activated receptor, the receptor binds another (inactive) trimer, and the cycle starts again. So one ligand-receptor complex can activate many G protein molecules in a short period, amplifying the original signal.

The most common action for the next stage in the pathway calls for the activated α subunit to interact with the effector. In the case of G_s, the α_s subunit activates adenylate cyclase; in the case of G_t, the α_t subunit activates cGMP phosphodiesterase. In other cases, however, it is the β γ dimer that interacts with the effector protein. In some cases, *both* the α subunit and the β γ dimer interact with effectors (for review see 295; 301; 312).

Consistent with the idea that it is more often the α subunits that interact with effectors, there are more varieties of α subunits (16 known in mammals) than of β (5) or γ subunits (11). However, irrespective of whether the α or β γ subunits carry the signal, *the common feature in all of these reactions is that a G protein usually acts upon an effector enzyme that in turn changes the concentration of some small molecule(s) in the cell*. (There are some other pathways in which G proteins behave by activating a kinase.)

In either the intact or dissociated state, G proteins are associated with the cytoplasmic face of the plasma membrane. But the individual subunits are quite hydrophilic, and none of them appears to have a transmembrane domain. The $\beta\ \gamma$ dimer has an intrinsic affinity for the membrane because the γ subunit is prenylated. The α_i and α_o types of subunit are myristoylated, which explains their ability to remain associated with the membrane after release from the $\beta\ \gamma$ dimer. The α_s subunit is palmitoylated.

Because several receptors can activate the same G proteins, and since (at least in some cases) a given G protein has more than one effector, we must ask how specificity is controlled. The most common model is to suppose that receptors, G proteins, and effectors all are free to diffuse in the plane of the membrane. In this case, the concentrations of the components of the pathway, and their relative affinities for one another, are the important parameters that regulate its activity. We might imagine that an activated α-GTP subunit scurries along the cytoplasmic face of the membrane from receptor to effector. But it is also possible that the membrane constrains the locations of the proteins, possibly in a way that restricts interactions to local areas. Such compartmentation could allow localized responses to occur (for review see 315).

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SIGNAL TRANSDUCTION

6.28.7 Protein kinases are important players in signal transduction

Key Terms

A **protein kinase** is a protein which transfers the terminal phosphate group from ATP onto another protein.

A **protein serine/threonine kinase** phosphorylates cytosolic proteins on either their serine or threonine residues.

A **protein tyrosine kinase** is a kinase enzyme whose target is a tyrosine amino acid in a protein.

A **dual specificity kinase** is a protein kinase that can phosphorylate tyrosine or threonine or serine amino acids.

RTK is an abbreviation for "receptor tyrosine kinase". These kinases are membrane-bound proteins with large cytoplasmic and extracellular domains. Specific binding of a ligand, such as a growth factor, to the extracellular domain causes the cytoplasmic domain to phosphorylate other proteins on tyrosine residues.

Key Concepts

- Protein kinases fall into groups that phosphorylate Ser/Thr or Tyr on target proteins.
- Receptor protein kinases are most often protein tyrosine kinases.
- Cytosolic protein kinases are most often protein Ser/Thr kinases.

There are many types of **protein kinases** involved in signal transduction. They all have the same basic catalytic activity: they add a phosphate group to an amino acid in a target protein. The phosphate is provided by hydrolyzing ATP to ADP. A protein kinase has an ATP-binding site and a catalytic center that can bind to the target amino acid (for review see 293; 294). The phosphorylation of the target protein changes its properties so that it in turn acts to carry the signal transduction pathway to the next stage.

Protein kinases can be classified both by the types of amino acids that they phosphorylate in the protein target and by their location in the cell.

Three groups of protein kinases are distinguished by the types of amino acid targets:

- **Protein serine/threonine kinases** are responsible for the vast majority of phosphorylation events in the cell. As their name indicates, they phosphorylate either serine or threonine in the target protein.

- **Protein tyrosine kinases** phosphorylate tyrosine in the target protein.
- **Dual specificity kinases** are less common and can phosphorylate target proteins on either tyrosine or serine/threonine.

Protein kinases are found in two types of location:

- *Cytosolic protein kinases* are most often Ser/Thr protein kinases. They are responsible for the vast majority of phosphorylation events in the cell. One particularly important class are the cdk (cyclin-dependent kinase) enzymes that control the cell cycle (see *Molecular Biology 6.29 Cell cycle and growth regulation*). Dual specificity kinases are found in the MAP kinase signal transduction pathway (see *Molecular Biology 6.28.16 A MAP kinase pathway is a cascade*). The products of some oncogenes, for which *src* is the paradigm, are protein tyrosine kinases (see *Molecular Biology 6.30.16 Src is the prototype for the proto-oncogenic cytoplasmic tyrosine kinases*).
- *Receptor protein kinases* are found in the plasma membrane. They have a domain on the exterior of the cell that binds a ligand, and a catalytic domain within the cell that can act on a target protein (for review see 2899; 2266). Most receptors with protein kinase activity are protein tyrosine kinases (abbreviated as **RTK** for receptor tyrosine kinase), although there are also some receptors of the Ser/Thr kinase class.

All kinases have an active site that binds ATP and a short sequence of the target protein that includes the amino acid to be phosphorylated. The sequence bound at the active site usually conforms to a consensus, typically 3-4 amino acids long. Recognition of the target protein also depends on interactions involving other regions of both the kinase and the target.

Figure 28.13 illustrates the structure of a dual specificity kinase of the MAP kinase family, based on its crystal structure (2900; 2901). The active site consists of a short loop of the enzyme that forms a deep cleft. The sequence of the catalytic loop is generally conserved. ATP binds at the bottom of the cleft. Adjacent to the catalytic loop, on the surface of the enzyme, is a sequence called the phosphorylation lip; many kinases in this group have amino acids in this sequence whose phosphorylation activates the enzyme activity. The phosphorylation lip contacts the amino acid on the N-terminal side of the amino acid that is phosphorylated.

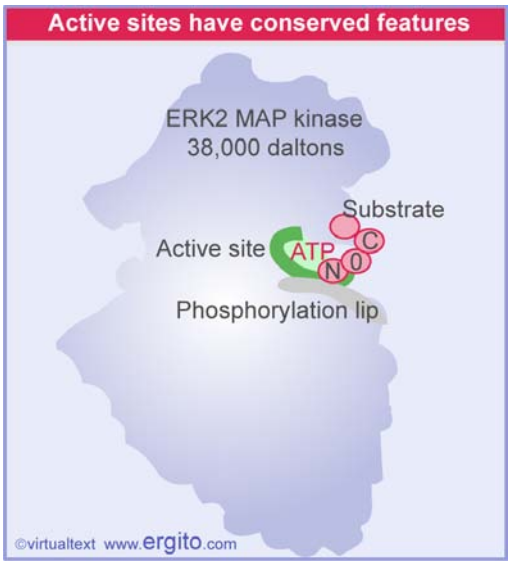

Figure 28.13 The active site of a cytosolic kinase is identified by the catalytic loop (green). The target sequence for phosphorylation binds to the active site. Amino acid 0 is phosphorylated. The amino acid on the N-terminal side (indicated by N) is immediately adjacent to the phosphorylation lip, which is a sequence in the kinase that is often itself phosphorylated.

Receptor tyrosine kinases have some common features. The extracellular domain often has characteristic repeating motifs. It contains a ligand-binding site. The transmembrane region is a single short membrane-spanning alpha helix. The catalytic domain is large (~250 amino acids), and often occupies the bulk of the cytoplasmic region. Certain conserved features are characteristic of all kinase catalytic domains. Sometimes the catalytic domain is broken into two parts by an interruption of some other sequence (which may have an important function in selecting the substrate).

Figure 28.14 illustrates the features of a receptor tyrosine kinase. Because the receptors are embedded in membranes, we do not yet have crystal structures of intact proteins. However, several extracellular and cytoplasmic domains have been independently crystallized (for review see 2266). The extracellular and cytoplasmic domains of the RTK group both show large variations in size (for review see 2899). The receptors are usually activated by binding a polypeptide ligand, which can be a significant size relative to the extracellular domain (the example in the figure shows the scale for an FGF ligand binding to its receptor; see 2902). The cytoplasmic domain of the RTK is large, and contains many sites involved in signaling, as well as the kinase catalytic domain. Crystal structures have identified the features of the active site (2903; 2904). At the active site, the catalytic loop is adjacent to an activation loop, which contains 2-3 tyrosines. When these tyrosines are phosphorylated, the activation loop swings away from the catalytic loop, freeing it to bind the substrate (for review see 2899).

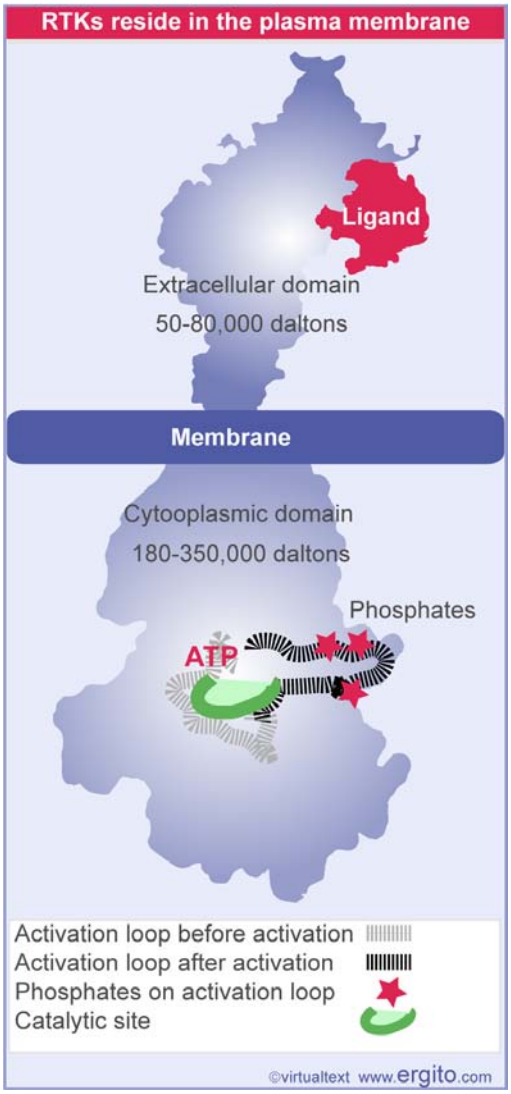

Figure 28.14 A receptor tyrosine protein kinase has an extracellular domain that binds a ligand, which is usually a polypeptide, and a cytoplasmic domain that includes a kinase region. ATP binds adjacent to the catalytic site. An important feature is the activation loop, which contains tyrosine residues whose phosphorylation activates the kinase activity. The activation loop containing these tyrosines changes its position when they are phosphoryated.

Phosphorylation usually activates the target protein, but this is not a golden rule – there are some cases in which phosphorylation inhibits the activity of the target. One way to reverse the effects of a phosphorylation event is for a phosphatase (typically a cytosolic phosphatase) to remove the phosphate that was added by a protein kinase (for review see 310).There are phosphatases with specificity for the appropriate amino acids to match each type of kinase. Most phosphatases are cytosolic, although there are some receptor phosphatases.

Last updated on 9-6-2002

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SIGNAL TRANSDUCTION

6.28.8 Growth factor receptors are protein kinases

Key Terms

A **growth factor** is a ligand, usually a small polypeptide, that activates a receptor in the plasma membrane to stimulate growth of the target cell. Growth factors were originally isolated as the components of serum that enabled cells to grow in culture.

A **cytokine** is a small polypeptide that affects the growth of particular types of cells.

Key Concepts

- Binding of a ligand to the extracellular domain of a growth factor receptor activates the kinase activity of the cytoplasmic domain.
- The receptor may activate a second messenger or may activate a cascade of kinases.

Growth factor receptors take their names from the nature of their ligands, which usually are small polypeptides (casually called **growth factors**, more properly called **cytokines**) that stimulate the growth of particular classes of cells. The factors have a variety of effects, including changes in the uptake of small molecules, initiation or stimulation of the cell cycle, and ultimately cell division. The ligands most usually are secreted from one cell to act upon the receptor of another cell. Examples of secreted cytokines are EGF (epidermal growth factor), PDGF (platelet-derived growth factor), and insulin. In some cases, ligands instead take the form of components of the extracellular matrix, or membrane proteins on the surface of another cell (these are sometimes called counter-receptors).

The receptors share a general characteristic structure: they are group I integral membrane proteins, spanning the membrane once, with an N-terminal protein domain on the extracellular side of the membrane, and the C-terminal domain on the cytoplasmic side. Most receptors, such as those for EGF or PDGF, consist of single polypeptide chains. An exception is provided by receptors of the insulin family, which are disulfide-bonded dimers (each dimer being a group I protein).

The effector pathways that are activated by receptor tyrosine kinases (*RTKs*) fall into two groups:

- *An enzymatic activity is activated that leads to the production of a small molecule second messenger*. The second messenger may be the immediate product of an enzyme that is activated directly by the receptor, or may be produced later in the pathway. Lipids are common second messengers in these pathways. The enzymes include phospholipases (which cleave lipids from larger substrates) and kinases that phosphorylate lipid substrates. Some common pathways are summarized in **Figure 28.15**. The second messengers that are

released in each pathway act in the usual way to activate or inactivate target proteins.

- *The effector pathway is a cascade that involves a series of interactions between macromolecular components*. The most common components of such pathways are protein kinases; each kinase activates the next kinase in the pathway by phosphorylating it, and the ultimate kinases in the pathway typically act on proteins such as transcription factors that may have wide-ranging effects upon the cell phenotype.

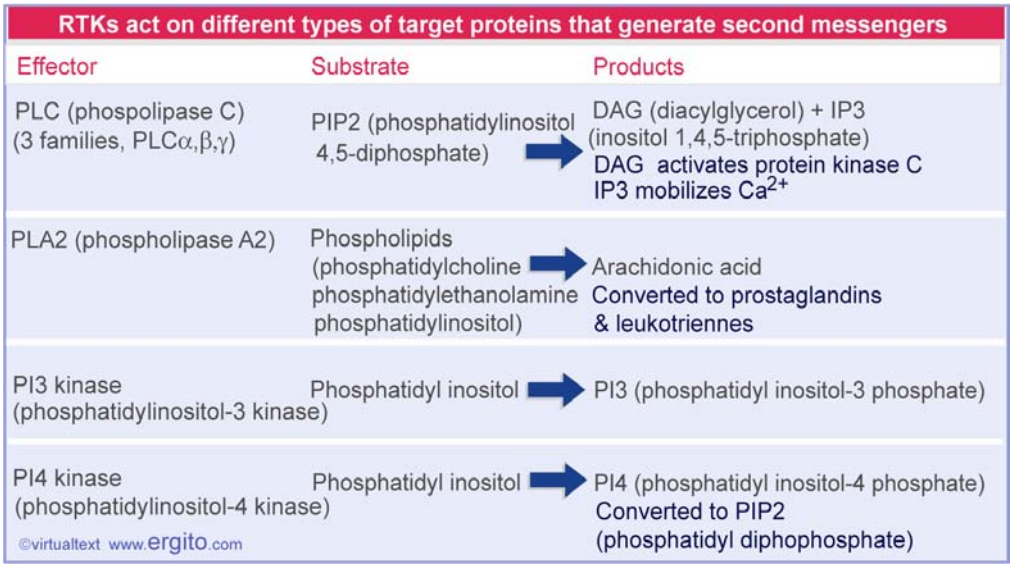

Figure 28.15 Effectors for receptor tyrosine kinases include phospholipases and kinases that act on lipids to generate second messengers.

The basic principle underlying the function of all types of effector pathway is that the signal is amplified as it passes from one component of the pathway to the next. When some components have multiple targets, the pathway branches, thus creating further diversity in the response to the original stimulus.

When a ligand binds to the extracellular domain of a growth factor receptor, the catalytic activity of the cytoplasmic domain is activated. *Phosphorylation of tyrosine is identified as the key event by which the growth factor receptors function because mutants in the tyrosine kinase domain are biologically inactive, although they continue to be able to bind ligand.*

SIGNAL TRANSDUCTION

6.28.9 Receptors are activated by dimerization

Key Terms

The ability of a species of kinase to phosphorylate itself is referred to as **autophosphorylation.** Autophosphorylation does not necessarily occur on the same polypeptide chain as the catalytic site; for example, in a dimer, each subunit may phosphorylate the other.

Key Concepts

- Ligand binding to receptor monomers causes them to dimerize by interactions between the extracellular domains.
- Dimerization is made possible by the ability of membrane proteins to move laterally within the membrane bilayer.
- Dimerization activates the cytoplasmic domains by an autophosphorylation in which the kinase activity of each monomer phosphorylates the other monomer.

A key question in the concept of how a signal is transduced across a membrane is how binding of the ligand to the extracellular domain activates the catalytic domain in the cytoplasm. *The general principle is that a conformational change is induced that affects the overall organization of the receptor.* An important factor in this interaction is that membrane proteins have a restricted ability to diffuse laterally (in contrast with the continuous motion of the lipids in the bilayer). This enables their state of aggregation to be controlled by external events.

Lateral movement plays a key role in transmitting information from one side of the membrane to the other. **Figure 28.16** shows that binding of ligand induces a conformation change in the N-terminal region of a group I receptor that causes the extracellular domains to dimerize. This causes the transmembrane domains to diffuse laterally, bringing the cytoplasmic domains into juxtaposition. The stabilization of contacts between the C-terminal cytosolic domains causes a change in conformation that activates the kinase activity. In some cases, phosphorylation also causes the receptor to interact with proteins present on the cytoplasmic surface of a coated pit, leading to endocytosis of the receptor. An extreme case of lateral diffusion is seen in certain cases of receptor internalization, when receptors of a given type aggregate into a "cap" in response to an extracellular stimulus.

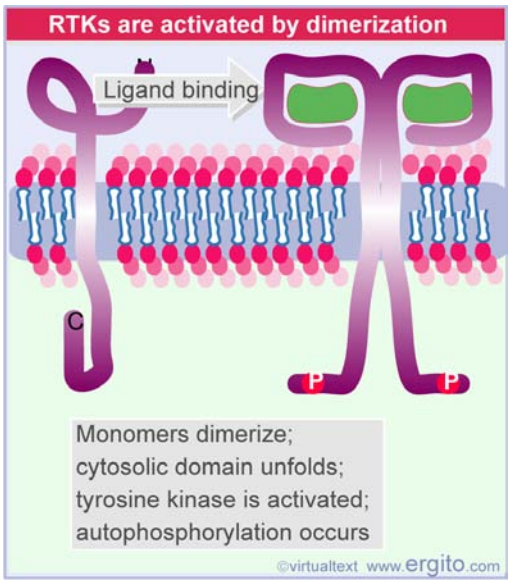

Figure 28.16 The principle underlying signal transduction by a tyrosine kinase receptor is that ligand binding to the extracellular domain triggers dimerization; this causes a conformational change in the cytoplasmic domain that activates the tyrosine kinase catalytic activity.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.6.28.9 to view properly.

Figure 28.17 shows that dimerization can take several forms (for review see 2898). The most common is that a ligand binds to one or to both monomers to induce them to dimerize (2905). A variation is that a dimeric ligand binds to two monomers to bring them together (2902). In the case of the insulin receptor family, the ligand binds to a dimeric receptor (which is stabilized by extracellular disulfide bridges) to cause an intramolecular change of conformation. The major consequence of dimerization is to allow transmission of a conformational change from the extracellular domain to the cytoplasmic domain without requiring a change in the structure of the transmembrane region (793; for review see 307; 2266).

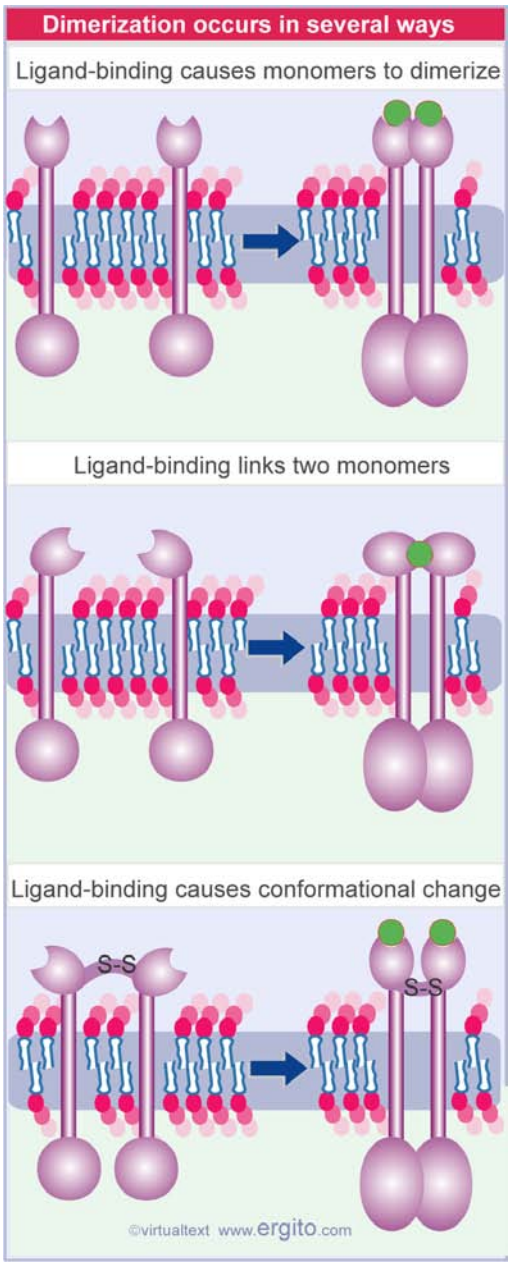

Figure 28.17 Binding of ligand to the extracellular domain can induce aggregation in several ways. The common feature is that this causes new contacts to form between the cytoplasmic domains.

Dimerization initiates the signaling pathway by triggering an **autophosphorylation** in the cytoplasmic domains of the receptor. When the two cytoplasmic domains are brought together in the dimer, each phosphorylates the other. It is necessary for *both* subunits to have kinase activity for the receptor to be activated; if one subunit is defective in kinase activity, the dimer cannot be activated.

Autophosphorylation has two consequences. Phosphorylation of tyrosines in the kinase domain causes the "activation loop" to swing away from the catalytic center,

thus activating the ability of the kinase to bind its substrate (see **Figure 28.14**). Phosphorylation of tyrosines at other regions of the cytoplasmic domain provides the means by which substrate proteins are enabled to bind to the receptor. The existence of these phosphorylated tyrosine(s) in specific signaling motifs causes the cytoplasmic domain to associate with its target proteins (see *Molecular Biology 6.28.10 Receptor kinases activate signal transduction pathways*; for review see 298; 305).

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SIGNAL TRANSDUCTION

6.28.10 Receptor kinases activate signal transduction pathways

Key Terms

Oncogenes are genes whose products have the ability to transform eukaryotic cells so that they grow in a manner analogous to tumor cells. Oncogenes carried by retroviruses have names of the form *v-onc*.

Key Concepts

- Receptor activation causes phosphorylation of Tyr at several short sequence motifs in the cytoplasmic domain.
- Different substrate proteins bind to particular motifs.
- The substrate proteins may be docking proteins that bind other proteins, or signaling proteins that have an enzymatic activities that are activated by associating with the receptor.

Figure 28.18 shows that we can distinguish several types of proteins with which the activated receptor may interact:

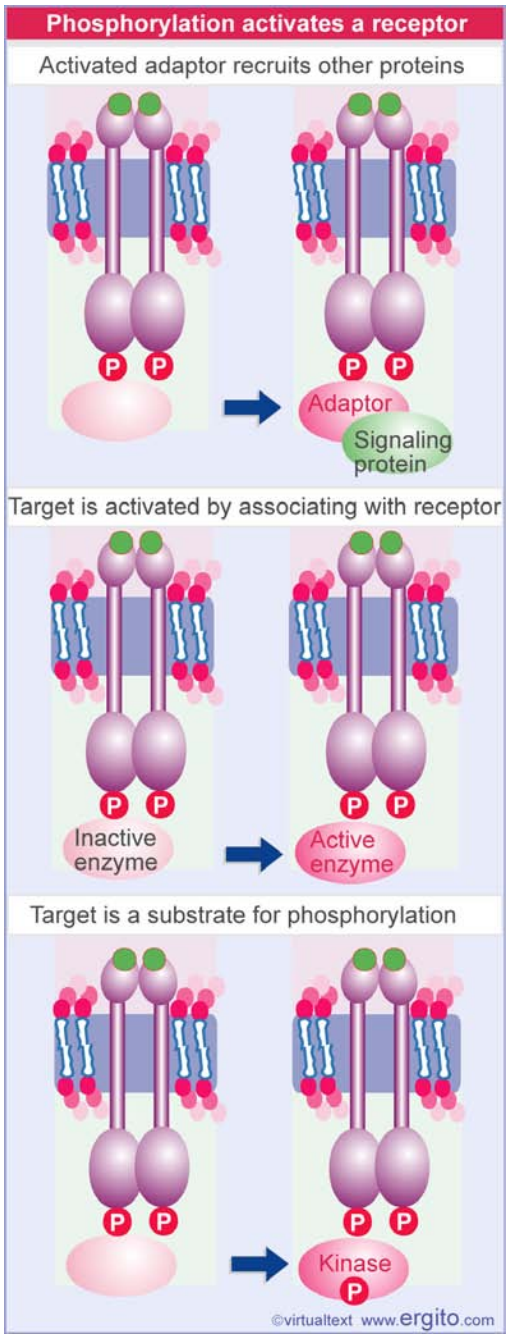

Figure 28.18 Phosphorylation of the intracellular domain of a receptor creates sites that bind cytosolic proteins. Three types of proteins that propagate signaling pathways are adaptors that bind other proteins, enzymes that are activated by associating with the receptor, and substrates, especially kinases, that are activated by being phosphorylated. Other targets can include structural proteins (not shown).

- The protein may be an intermediary that has no catalytic activity of its own, but serves merely to bring other proteins to the receptor. "Docking proteins" or

"adaptors" bind to an activated receptor, and then other proteins(s) bind to them, and may therefore become substrates for the receptor. By assembling complexes via such intermediaries, receptors can extend their range (for review see 2908).

- The protein may be a *target* that is activated by its association with the receptor, but which is not itself phosphorylated. For example, some enzymes are activated by binding to a receptor, such as PI3 kinase (see **Figure 28.15**).

- If the protein is a *substrate* for the enzyme, it becomes phosphorylated. If the substrate is itself an enzyme, it may be activated by the phosphorylation (example: c-Src or PLC γ ; see **Figure 28.15**). Sometimes the substrate is a kinase, and the pathway is continued by a cascade of kinases that successively activate one another.

- Some substrates may be end-targets, such as cytoskeletal proteins, whose phosphorylation changes their properties, and causes assembly of a new structure.

A receptor tyrosine kinase can initiate a signaling cascade at the membrane. However, in many cases, the activation of the kinase is followed by its internalization, that is, it is removed from the membrane and transported to the interior of the cell by endocytosis of a vesicle carrying a patch of plasma membrane. The relationship between kinase activity and endocytosis is unclear. Phosphorylation at particular residues may be needed for endocytosis; whether the kinase activity as such is needed may differ for various receptors. It is possible that endocytosis of receptor kinases serves principally to clear receptor (and ligand) from the surface following the response to ligand binding (thus terminating the response). However, in some cases, movement of receptors to coated pits followed by internalization could be necessary for them to act on the target proteins.

Because growth factor receptors generate signals that lead to cell division, their activation in the wrong circumstances is potentially damaging to an organism, and can lead to uncontrolled growth of cells. Many of the growth factor receptor genes are represented in the **oncogenes**, a class of mutant genes active in cancers. The mutant genes are derived by changes in cellular genes; often the mutant protein is truncated in either or both of its N-terminal or C-terminal regions. The mutant protein usually displays two properties: the tyrosine kinase has been activated; and there is no longer any response to the usual ligand. As a result, the tyrosine kinase activity of the receptor is either increased or directed against new targets (see *Molecular Biology 6.30.15 Growth factor receptor kinases can be mutated to oncogenes*).

Last updated on 9-10-2002

Reviews

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SIGNAL TRANSDUCTION

6.28.11 Signaling pathways often involve protein-protein interactions

Key Terms

An **SH2** domain (named originally as the *S*rc *H*omology domain because it was identified in the Src product of the Rous sarcoma virus) is a region of ~100 amino acids that is bound by the SH2-binding domain of the protein upstream in a signal transduction cascade.

An **SH3** domain is used by some proteins that contain SH2 domains to enable them to bind to the next component downstream in a signal transduction cascade.

Key Concepts

- An SH2-binding site has a phospho-Tyrosine residue that is recognized by an SH2 domain.
- A receptor may have several SH2-binding sites, which are recognized by the SH2 domains of different signaling proteins.
- The signaling protein may have an SH3 domain that recognizes the next protein in the pathway.

A common means for propagating a signal transduction pathway is for a protein specifically to recognize the next protein in the pathway by means of a physical interaction (for review see 2908). (This contrasts with the generation of a small molecule [second messenger] that interacts with the next protein in the pathway.) The usual mechanism for a protein-protein interaction in a signal pathway is for a domain in one protein to recognize a rather short motif in a second protein. The salient feature of the target motif may be its sequence or its structure. Phospho-Tyr residues are often components of such motifs, allowing the motif to be made active by phosphorylation or made inactive by dephosphorylation (for review see 3430). Another common feature of target motifs is the amino acid proline, which causes a characteristic turn in a polypeptide chain (for review see 2918).

Two motifs found in a variety of cytoplasmic proteins that are involved in signal transduction are used to connect proteins to the components that are upstream and downstream of them in a signaling pathway. The domains are named **SH2** and **SH3**, for *S*rc *h*omology, because they were originally described in the c-Src cytosolic tyrosine kinase (see *Molecular Biology 6.30.16 Src is the prototype for the proto-oncogenic cytoplasmic tyrosine kinases*; for review see 299; 302).

The presence of SH2 and SH3 domains in various proteins is summarized in **Figure 28.19**. The cytoplasmic tyrosine kinases comprise one group of proteins that have these domains; other prominent members are phospholipase C γ and the regulatory subunit (p85) of PI3 kinase (both targets for activation by receptor tyrosine kinases; see **Figure 28.15**). The extreme example of a protein with these domains is

Grb2/sem5, which consists *solely* of an SH2 domain flanked by two SH3 domains (see later).

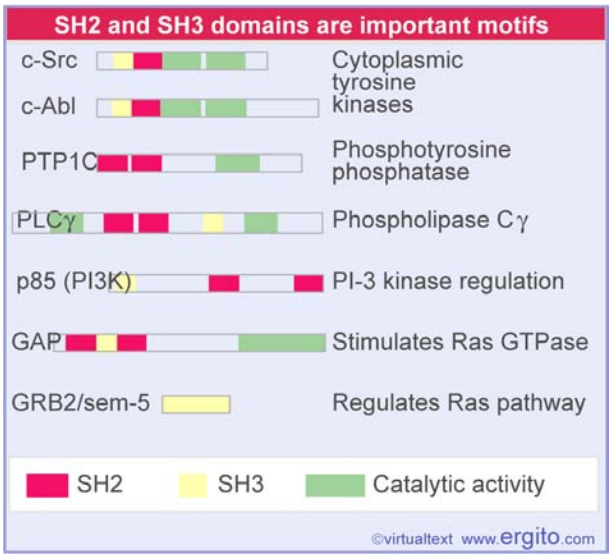

Figure 28.19 Several types of proteins involved in signaling have SH2 and SH3 domains.

Some proteins contain multiple SH2 domains, which increases their affinity for binding to phosphoproteins or confers the ability to bind to different phosphoproteins. A receptor may contain different SH2-binding sites, enabling it to activate a variety of target proteins. **Figure 28.20** summarizes the organization of the cytoplasmic domain of the PDGF receptor, which has ~10 distinct SH2-binding sites, each created by a different phosphorylation event. Different pathways may be triggered by the proteins that bind to the various phosphorylated residues (794).

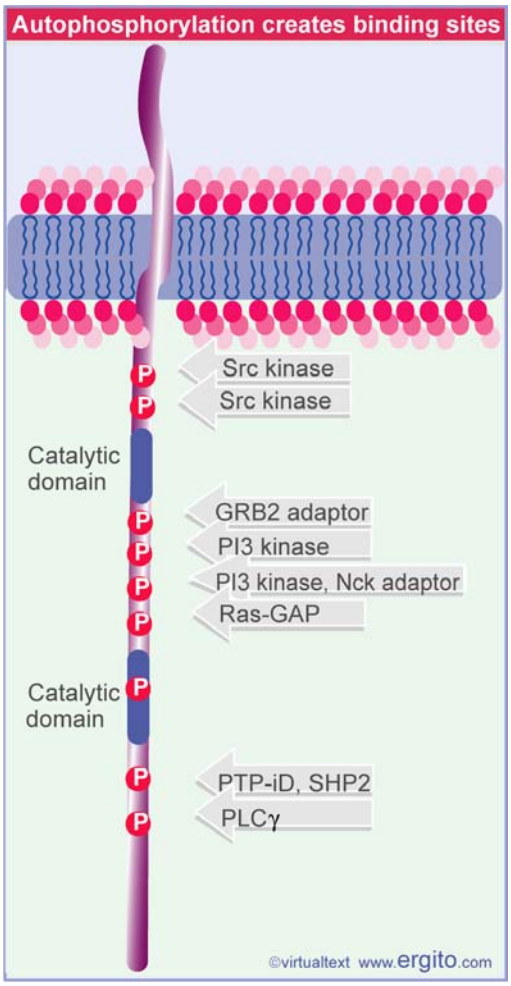

Figure 28.20 Autophosphorylation of the cytosolic domain of the PDGF receptor creates SH2-binding sites for several proteins. Some sites can bind more than one type of SH2 domain. Some SH2-containing proteins can bind to more than one site. The kinase domain consists of two separated regions (shown in blue), and is activated by the phosphorylation site in it.

A protein that contains an SH2 domain is activated when it binds to an SH2-binding site. The activation may involve the SH2-containing protein directly (when it itself has an enzymatic activity) or may be indirect. The enzymatic activities that are regulated directly are most commonly kinases, phosphatases, or phospholipases. An example of a protein containing an SH2 domain that does not have a catalytic activity is provided by p85, the regulatory subunit of PI3 kinase; when p85 binds to a receptor, it is the associated PI3K catalytic subunit that is activated.

Figure 28.21 shows that the **SH3** domain provides the effector function by which some SH2-containing proteins bind to a downstream component. The case of the "adaptor" Grb2 strengthens this idea; consisting only of SH2 and SH3 domains, it uses the SH2 domain to contact the component upstream in the pathway, and the SH3 domain to contact the component downstream. SH3 binds the motif PXXP in a

sequence-specific manner (see *Molecular Biology 6.28.13 Prolines are important determinants in recognition sites*). When an activated receptor binds Grb2, the SH3 domain of Grb2 binds to a target protein that contains the PXXP motif (805).

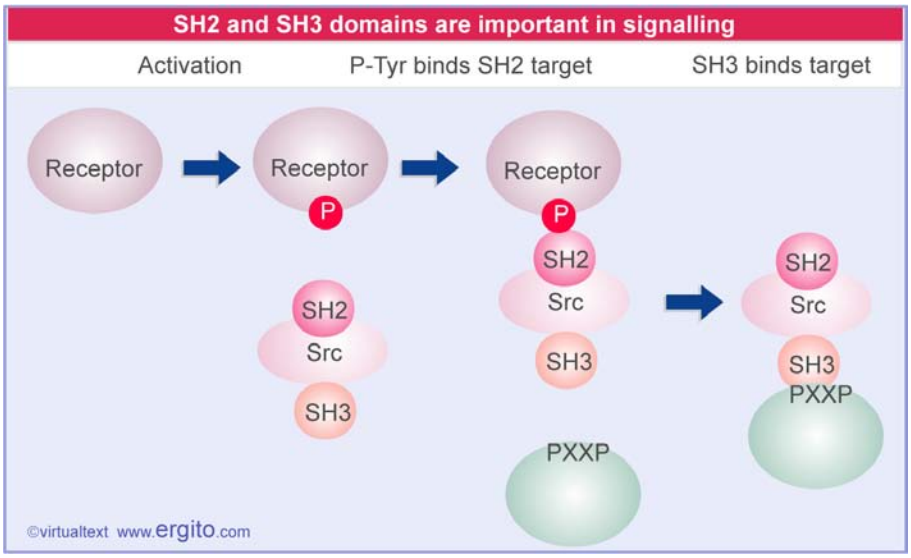

Figure 28.21 SH2 and SH3 domains are used for protein-protein interactions in signal transduction cascades. Typically a phosphorylated receptor recognizes an SH2 target in its substrate, and an SH3 domain in the substrate then recognizes the next protein in the cascade.

SH3 domains often provide connections to small GTP-binding proteins (of which Ras is the paradigm). Another role that has been proposed for SH3 domains (and in particular for the SH3 domain of c-Src) is the ability to interact with proteins of the cytoskeleton, thus triggering changes in cell structure.

Last updated on 9-10-2002

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SIGNAL TRANSDUCTION

6.28.12 Phosphotyrosine is the critical feature in binding to an SH2 domain

Key Terms

An **SH2** domain (named originally as the *S*rc *H*omology domain because it was identified in the Src product of the Rous sarcoma virus) is a region of ~100 amino acids that is bound by the SH2-binding domain of the protein upstream in a signal transduction cascade.

An **SH2-binding site** is an area on a protein that interacts with the SH2 domain of another protein.

Key Concepts

- An SH2-binding site consists of phospho-Tyrosine and <5 amino acids on its C-terminal side.
- An SH2 domain forms a globular structure with a pocket that binds the phospho-Tyrosine of the SH2-binding site of the target protein.

The **SH2** domain is a region of ~100 amino acids that interacts with a target site in other proteins. The target site is called an **SH2-binding site**. **Figure 28.22** shows an example of a reaction in which SH2 domains are involved. Activation of a tyrosine kinase receptor causes autophosphorylation of a site in the cytosolic tail. Phosphorylation converts the site into an SH2-binding site. So a protein with a corresponding SH2 domain binds to the receptor only when the receptor is phosphorylated.

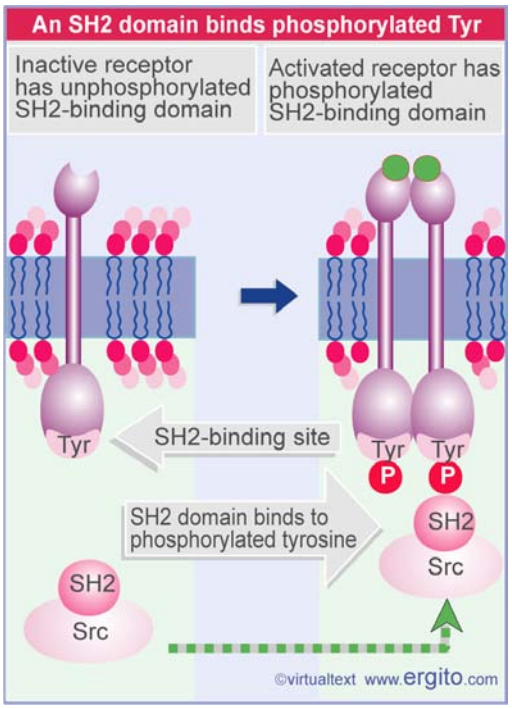

Figure 28.22 Phosphorylation of tyrosine in an SH2-binding domain creates a binding site for a protein that has an SH2 domain.

An SH2 domain specifically binds to a particular SH2-binding site. The specificity of each SH2 domain is different (except for a group of kinases related to Src, which seem to share the same specificity). The typical SH2-binding site is only 3-5 amino acids long, consisting of a phosphotyrosine and the amino acids on its C-terminal side. SH2 binding is a high-affinity interaction, as much as $10^3\times$ tighter than a typical kinase-substrate binding reaction (807).

The SH2 domain has a globular structure in which its N-terminal and C-terminal ends are close together, so that its structure is relatively independent of the rest of the protein. The phosphotyrosine in the SH2-binding site binds to a pocket in the SH2 domain, as illustrated in **Figure 28.23**.

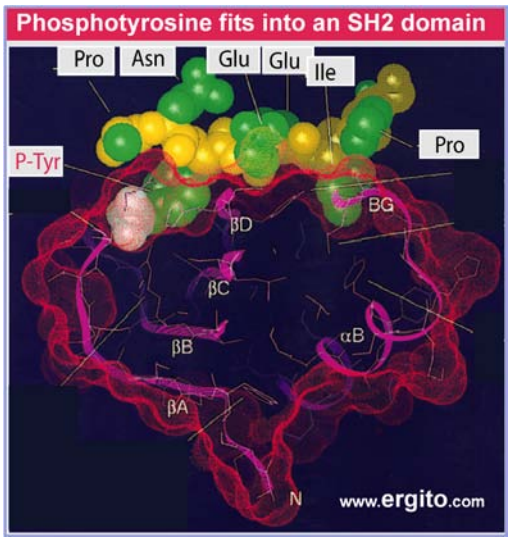

Figure 28.23 The crystal structure of an SH2 domain (purple strands) bound to a peptide containing phosphotyrosine shows that the P-Tyr (white) fits into the SH2 domain, and the 4 C-terminal amino acids in the peptide (backbone yellow, side chains green) also make contact. Photograph kindly provided by John Kuriyan.

Last updated on 9-10-2002

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SIGNAL TRANSDUCTION

6.28.13 Prolines are important determinants in recognition sites

Key Concepts

- An SH3 domain binds to the structure created by a PXXP amino acid sequence.
- Docking proteins often have a PTB domain that binds to the motif NPXpY in the receptor.
- A β-sheet in a PDZ domain binds a C-terminal β-strand in a target sequence.
- A WW domain recognizes a proline-rich target sequence.

Figure 28.24 shows the interaction between an SH3 domain and a PXXP target sequence. The surface of the SH3 domain is hydrophobic and has three shallow grooves where the target sequence binds. The turns in the polypeptide chain at the proline residues create a helix (called the PPII helix), which seen in cross-section resembles a triangle with the two prolines on the base, and the other residues sticking up. The prolines fit into two of the grooves on the SH3 domain surface (for review see 2919). The PPII helix is quite similar when viewed from either the N-terminus or the C-terminus, and the ligands for SH3 domains are classified as class I or class II depending on which orientation is bound. Binding is influenced by hydrophobic and other residues in and adjacent to the core PXXP sequence.

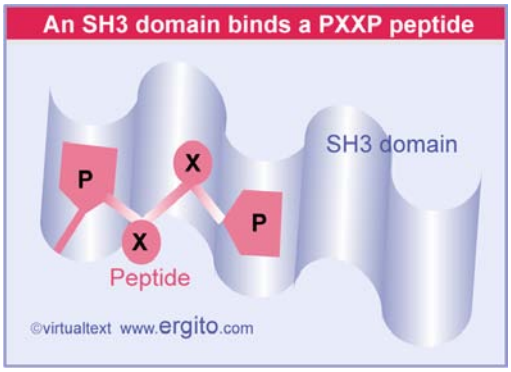

Figure 28.24 A PXXP sequence forms a helical structure in which the two flat prolines form a base and the intervening residues stick up out of the plane. The prolines bind to shallow grooves on the surface of the SH3 domain.

A domain that is often found in the targets of receptor kinases is the PTB (phosphotyrosine-binding motif). The PTB binds to the receptor at a motif that consists of a phosphotyrosine preceded by residues that form a β-turn, usually with the consensus Asn-Pro-X-phosphoY (2906). It functions somewhat differently from SH2 or SH3 in being used principally to bind "docking proteins". A docking protein

is an intermediary that recruits other proteins to the activated receptor. **Figure 28.25** shows that the docking protein uses its PTB to bind to a phosphorylated site on the receptor, and then other components of the signaling pathway in turn bind to other sites on it. The specificity with which the motif is recognized is determined by hydrophilic amino acids located a few amino acids on the N-terminal side of the phosphorylated Tyr in the receptor. The recognition reaction depends on peptide-peptide interactions, when the phosphopeptide forms an antiparallel β-strand juxtaposed to a β-sheet in the PTB (2907). The fact that it does not depend exclusively on the phosphorylation event is emphasized by the fact that some PTB domains can bind to nonphosphorylated motifs.

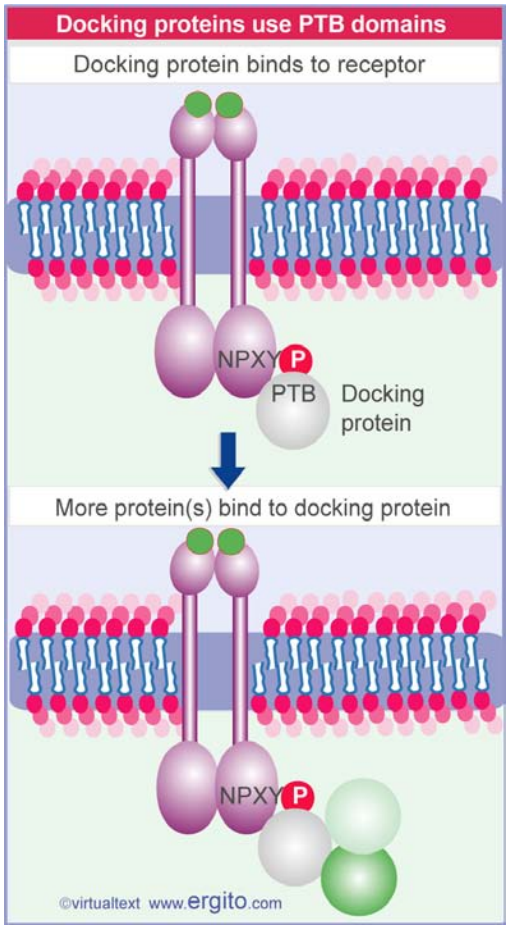

Figure 28.25 A docking protein is an adaptor that connects an activated receptor to a signaling protein(s). It may have a PTB domain that recognizes the motif NPXpY in the receptor.

The use of antiparallel β-strand interactions in protein recognition is a common theme. **Figure 28.26** shows the example of PDZ domain recognition. A PDZ domain is a 90-100 amino acid region, often represented in multiple repeats in a protein. It is particularly important in the clustering of membrane proteins and in binding signaling proteins to membrane complexes. (It is named after three of the proteins in which these repeats are found.) It typically binds the last four amino acids at the C-terminus of a target protein. The consensus sequence for recognition is

X-Thr/Ser-X-Val-COOH. In the example shown in the figure, the target sequence binds between a β-strand and an α-helix of the PDZ domain, and its terminal -COOH group is bound by the carboxy-binding loop (2916). Basically, the β-sheet of the PDZ domain is extended by adding the target peptide as an additional β-strand (for review see 2933).

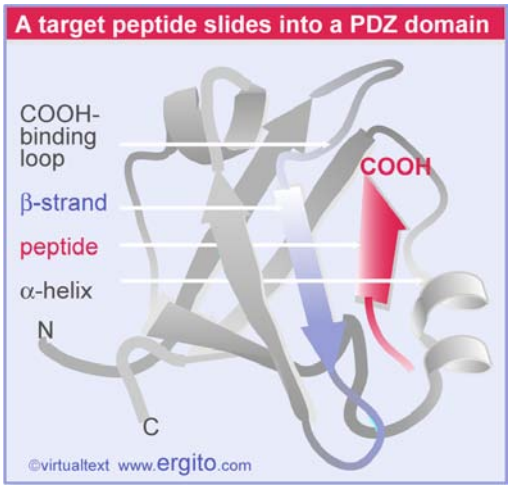

Figure 28.26 A peptide binds to a PDZ domain by inserting as an additional strand in an anti-parallel β -sheet. The strands of the β -sheet are shown as ribbons, with directionality indicated by the arrowheads. The peptide binds between one of the β -strands and an α -helix in the PDZ domain. Its C-terminal end makes contacts with the COOH-binding loop.

SH3-binding sites and PDZ binding-sites have the opposite response to phosphorylation from SH2 binding-sites. Phosphorylation of a serine in the site prevents its recognition by the SH3 domain or PDZ domain, respectively.

The WW domain is another case in which a β-sheet interacts with a target peptide. The domain is ~38 amino acids, and has a high concentration of hydrophobic, aromatic, and proline residues. It binds target proline-rich target peptides. **Figure 28.27** shows an example in which the WW domain forms a β-sheet that interacts with the target consensus sequence PPXY (Pro-Pro-X-Tyr) (2917).

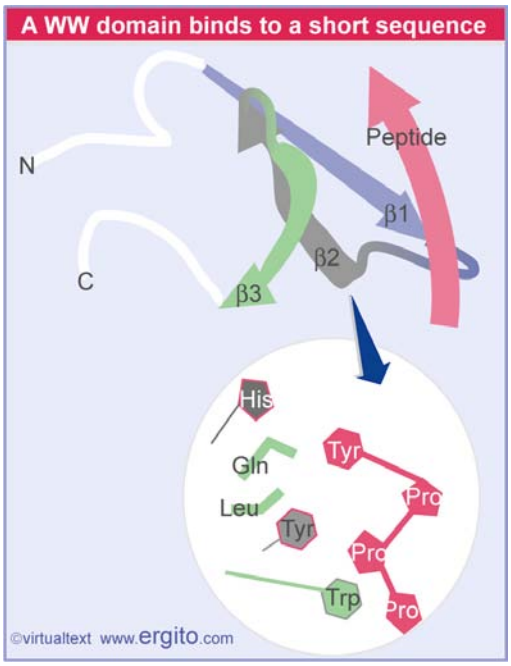

Figure 28.27 A WW domain has a β -sheet consisting of three β -strands that interacts with a target peptide. The insertion shows how amino acids from two of the β -strands specifically interact with the target peptide. The characteristic feature is the insertion of the Trp from the WW domain between the two Pro residues in the target.

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SIGNAL TRANSDUCTION

6.28.14 The Ras/MAPK pathway is widely conserved

Key Terms

A **MAP kinase (MAPK)** is a Ser/Thr protein kinase named for its original identification as a mitogen-activated kinase. There is a large group of cytosolic Thr/Ser protein kinases that form several signaling pathways. The name reflects their original isolation as mitogen-activated protein kinases.

Key Concepts

- The Ras/MAPK pathway starts with activation of the monomeric G protein Ras and then continues by a cascade in which a series of kinases activate one another.

The best characterized pathway that is initiated by receptor tyrosine kinases passes through the activation of a monomeric G protein to activate a cascade of cytosolic kinases. Although there are still some gaps in the pathway to fill in, and branches that have not yet been identified, the broad outline is clear, as illustrated in **Figure 28.28**.

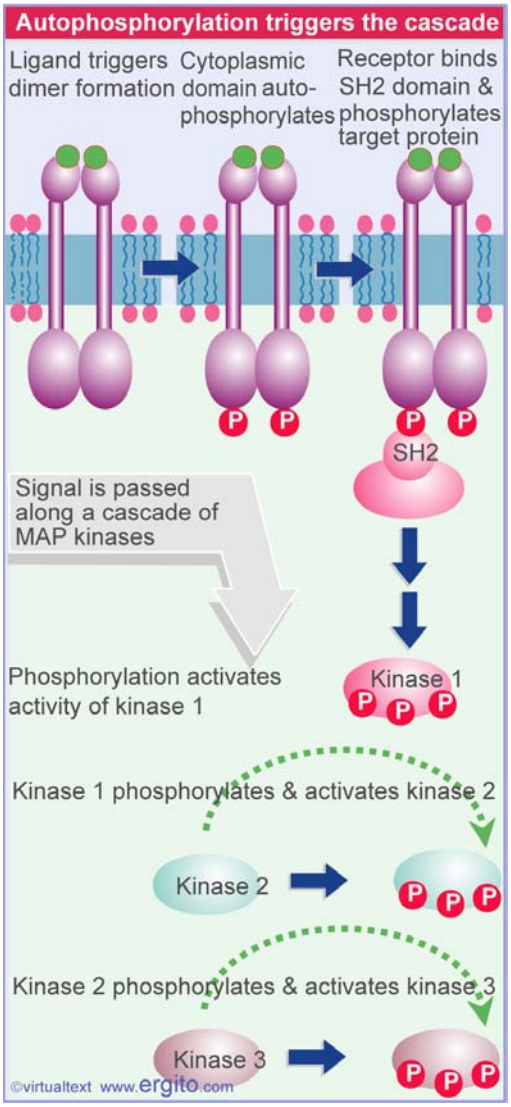

Figure 28.28 Autophosphorylation triggers the kinase activity of the cytoplasmic domain of a receptor. The target protein may be recognized by an SH2 domain. The signal may subsequently be passed along a cascade of kinases.

In mammalian cells, the cascade is often initiated by activation of a tyrosine kinase receptor, such as the EGF or PDGF receptors. The receptor activates the Ras pathway by means of an "adaptor" protein. The activation of Ras leads to the activation of the Raf Ser/Thr kinase, which in turn activates the kinase MEK (formerly known as MAP kinase kinase); its name reflects the fact that it is the kinase that phosphorylates, and thereby activates, a **MAP kinase**.

The name of the family of MAP kinases reflects their identification as *mitogen-activated protein kinases*. One MAPK family has also been called ERKs, for extracellular signal-regulated. Some major effects of Ras are conveyed via this pathway, but there is also a branch at Ras, which involves the activation of other monomeric G proteins.

The cascade from MEK to the end products is sometimes known as the MAP kinase pathway. Each kinase in this part of the cascade phosphorylates its target kinase, and the phosphorylation event activates the kinase activity of the target enzyme, as illustrated in **Figure 28.29**. The cascade of phosphorylation events leads ultimately to the phosphorylation of transcription factors that trigger changes in cell phenotype varying from growth to differentiation, depending on the cell type. Other targets for the kinases include cytoskeletal proteins that may directly influence cell structure.

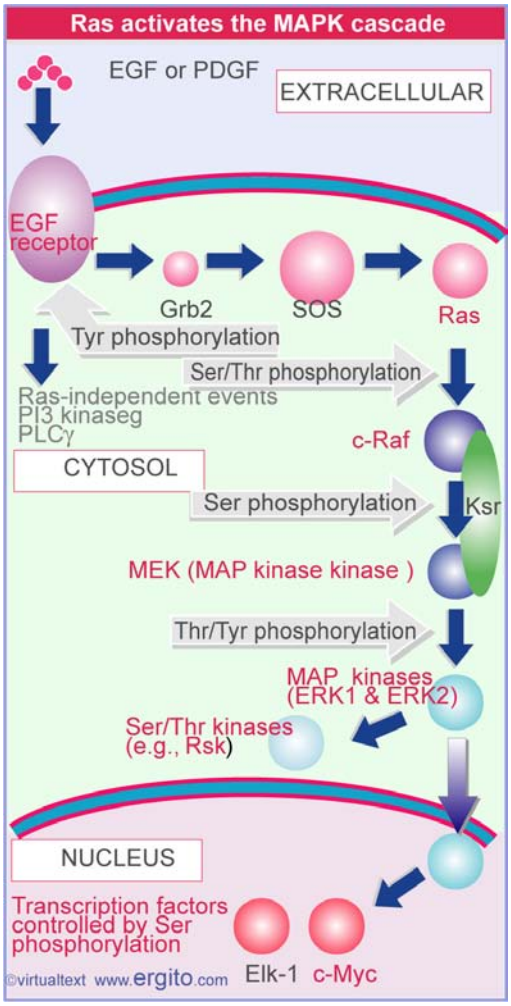

Figure 28.29 A common signal transduction cascade passes from a receptor tyrosine kinase through an adaptor to activate Ras, which triggers a series of Ser/Thr phosphorylation events. Finally, activated MAP kinases enter the nucleus and phosphorylate transcription factors.

The relationship between components of the pathway can be tested by investigating the effects of one component upon the action of another. For example, a mutation that inactivates one component should make it impossible for the pathway to be activated by any components that act earlier. Using such tests allows components to be ordered in a pathway, and to determine whether one component is upstream or downstream of another.

The pathway has been characterized in several situations (see **Figure 28.38**): in terms of biochemical components responsible for growth of mammalian cultured cells, as the pathway involved in eye development in the fly *D. melanogaster*, as the pathway of vulval development in the worm *C. elegans*, and as the response to mating in the yeast *S. cerevisiae*.

The striking feature is that the pathway is activated by different means in each case (appropriate to the individual system), and it has different end effects in each system, but many of the intermediate components can be recognized as playing analogous roles. It is much as though Nature has developed a signal transduction cascade that can be employed wholesale by means of connecting the beginning to an appropriate stimulus and the end to an appropriate effector. The total pathway is sometimes known as the Ras pathway (named after one of the earlier components) or the Ras/MAPK pathway. Several of the components of this pathway in mammals are related to oncogenes, which suggests that the aberrant activation of this pathway at any one of various stages has a powerful potential to cause tumors.

Figure 28.30 shows how the events initiating the cascade occur at the plasma membrane. The activated receptor tyrosine kinase associates with the SH2 domain of the adaptor protein Grb2, which binds to the receptor but is not phosphorylated. The SH3 domain of Grb2 then binds to the protein SOS, which then activates Ras. The sole role of Grb2 in activating SOS appears to be fulfilled by binding to it. The binding reaction brings SOS to the membrane, and thus into the vicinity of Ras (801). SOS causes the GDP on Ras to be replaced by GTP, which is sufficient to activate Ras. [Grb2 is not the only adaptor that can activate Ras; an alternative pathway is provided by the adaptor SHC. Which adaptor is used depends on the cell type (797; 798; 799).]

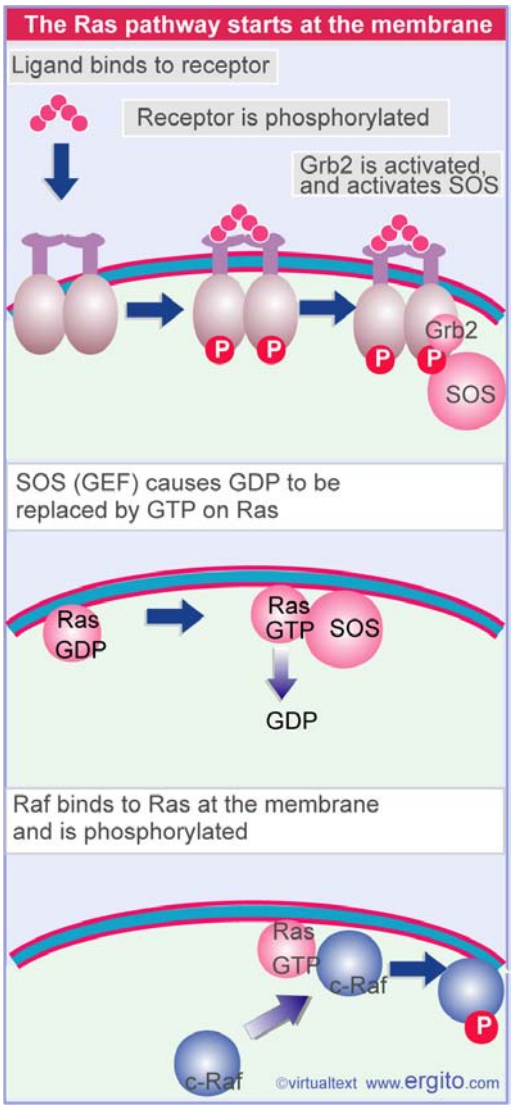

Figure 28.30 The Ras cascade is initiated by a series of activation events that occur on the cytoplasmic face of the plasma membrane.

When a tyrosine kinase receptor is activated, its intracellular domain may be phosphorylated at more than one site, and each site may trigger a different pathway (see **Figure 28.20**). The most common consequence of a phosphorylation is to activate a signal transduction pathway, but in some cases it may have a negative effect, providing a feedback loop to limit the action of the pathway. These effects may be direct or indirect. **Figure 28.31** illustrates an example of a system in which two phosphorylations counteract each other. Torso is a receptor tyrosine kinase that activates the Ras pathway during *Drosophila* embryogenesis. Two sites become phosphorylated when it is activated. Phosphorylation of Y630 is required to activate the downstream pathway. Phosphorylation of Y918 provides negative regulation.

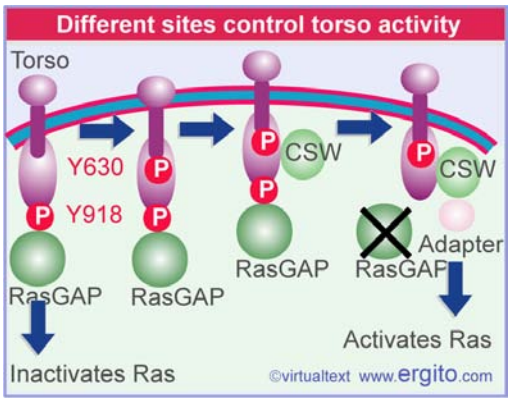

Figure 28.31 Phosphorylation at different sites on a receptor tyrosine kinase may either activate or inactivate the signal transduction pathway.

The receptor binds the regulator RasGAP to the phosphorylated site Y918. This keeps RasGAP it in an activated state in which it prevents Ras from functioning (see *Molecular Biology 6.28.15 The activation of Ras is controlled by GTP*). Y918 is phosphorylated constitutively (or when Torso is activated at a low level). Under these circumstances, the pathway is turned off.

High activation of Torso results in phosphorylation of Y630. This creates a binding site for the cytosolic phosphatase corkscrew (CSW). Corkscrew then dephosphorylates Y918. The result is to release RasGAP, which becomes ineffective, allowing Ras to function. So corkscrew is required for Torso to activate Ras.

Corkscrew may have a second role in the pathway, which is to recruit the adaptor that in turn binds to SOS, which activates Ras.

We see from this example that phosphorylated sites may influence the signaling pathway positively or negatively. The state of one phosphorylated site may in fact control the state of another site. An activating site may act indirectly (to inactivate an inhibitory site) as well as directly to recruit components of the signaling pathway.

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SIGNAL TRANSDUCTION

6.28.15 The activation of Ras is controlled by GTP

Key Concepts

- Ras is a monomeric G protein that is active when bound to GTP and inactive when bound to GDP.
- When GTP is hydrolyzed, the conformation of Ras changes.
- Constitutively active forms of Ras have mutations that affect GTP binding.
- SOS is the Ras-GEF that activates Ras by causing GDP to be replaced with GTP. SOS is activated by the adaptor Grb2, which is activated by a receptor.
- Ras-GAP is the protein that triggers the GTPase activity and deactivates Ras.

We turn now to the events involved in activating Ras. Ras is an example of a monomeric G protein (see *Molecular Biology Supplement 32.10 G proteins*). Other examples are found in protein trafficking (such as the Rabs) or in protein synthesis (such as EF-Tu). (The general principles by which such proteins are controlled are illustrated in **Figure S 33**.) The activity of the G protein depends on whether it is bound to GTP (active state) or bound to GDP (inactive state). Like trimeric G proteins, a monomeric G protein possesses an intrinsic GTPase activity that converts it from the active state to the inactive state.

Figure 28.32 shows that two proteins control the conversion between the active and inactive states of Ras. Ras-GAP is the GAP (GTPase activating protein) that triggers the GTPase activity and thereby inactivates Ras in mammalian cultured cells. SOS is the Ras-GEF (guanine nucleotide exchange factor) that causes GDP to be replaced by GTP, and thereby activates Ras. [SOS is activated when phosphorylation of a receptor tyrosine kinase causes Grb2 to recruit it to the plasma membrane (see **Figure 28.30**) (796; for review see 300).]

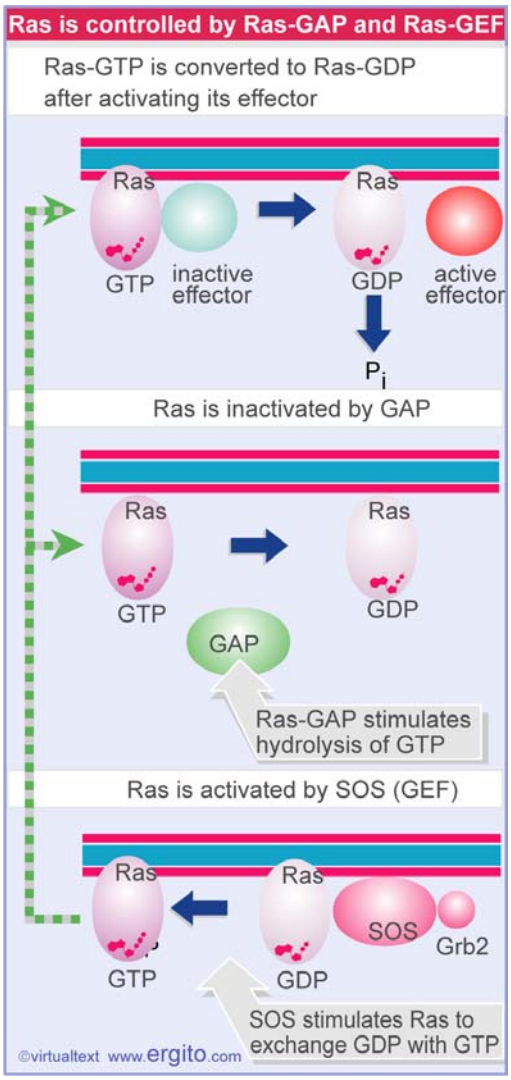

Figure 28.32 The relative amounts of Ras-GTP and Ras-GDP are controlled by two proteins. Ras-GAP inactivates Ras by stimulating hydrolysis of GTP. SOS (GEF) activates Ras by stimulating replacement of GDP by GTP, and is responsible for recycling of Ras after it has been inactivated.

The general structure of mammalian Ras proteins is illustrated in **Figure 28.33**. Three groups of regions are responsible for the characteristic activities of Ras:

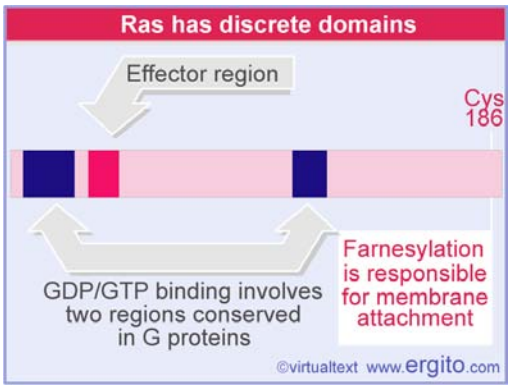

Figure 28.33 Discrete domains of Ras proteins are responsible for guanine nucleotide binding, effector function, and membrane attachment.

- The regions between residues 5-22 and 109-120 are implicated in guanine nucleotide binding by their homology with other G-binding proteins.
- Ras is attached to the cytoplasmic face of the membrane by farnesylation close to the C-terminus. Mutations that prevent the modification abolish oncogenicity, showing that membrane location is important for Ras function. After the farnesylation, the three C-terminal amino acids are cleaved from the protein, and the carboxyl group of the (now C-terminal) Cys^{186} is methylated; also, other Cys residues in the vicinity are reversibly palmitoylated. These changes further increase affinity for the membrane.
- The effector domain (residues 30-40) is the region that reacts with the target molecule when Ras has been activated. This region is required for the oncogenic activity of Ras proteins that have been activated by mutation at position 12. The same region is required for the interaction with Ras-GAP.

The crystal structure of Ras protein is illustrated schematically in **Figure 28.34**. The regions close to the guanine nucleotide include the domains that are conserved in other GTP-binding proteins. The potential effector loop is located near the phosphates; it consists of hydrophilic residues, and is potentially exposed in the cytoplasm.

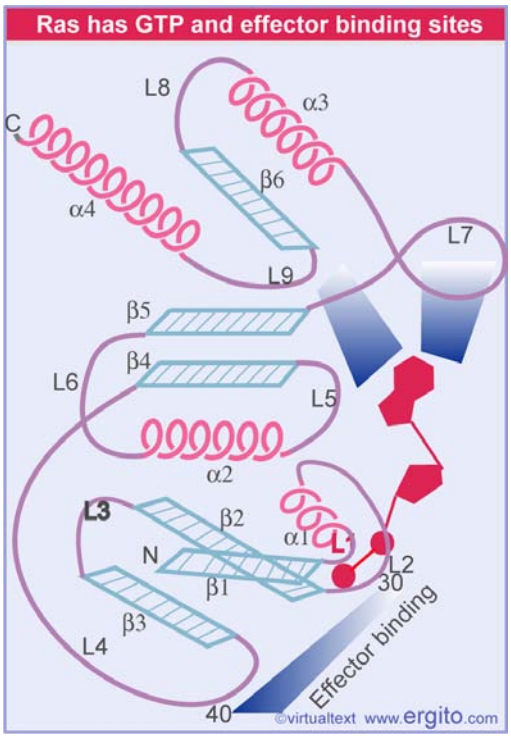

Figure 28.34 The crystal structure of Ras protein has 6 β strands, 4 α helices, and 9 connecting loops. The GTP is bound by a pocket generated by loops L9, L7, L2, and L1.

When GTP is hydrolyzed, there is a switch in the conformation of Ras protein. The change involves L4, which includes position 61, at which some oncogenic mutations occur. Mutations that activate Ras constitutively (these are oncogenic as discussed in *Molecular Biology 6.30.10 Ras proto-oncogenes can be activated by mutation at specific positions*) occur at position 12 in loop 1, and directly affect binding to GTP. The changes between the wild-type and oncogenic forms are restricted to these regions, and impede the ability of the mutant Ras to make the conformational switch when GTP is hydrolyzed. The primary basis for the oncogenic property, therefore, lies in the reduced ability to hydrolyze GTP.

When mitogenesis is triggered by activation of a growth factor, or when a cell is transformed into the tumorigenic state (see *Molecular Biology 6.30 Oncogenes and cancer*), there is a series of coordinated events, including changes in transcription and changes in cell structure. Activation of the Ras/MAPK pathway activates transcription factors that are responsible for one important set of changes. Other changes are triggered by the activation of a group of monomeric G proteins (Rac, Rho, and Cdc42). Each member of this group is responsible for particular types of structural change, as summarized in **Figure 28.35** (for review see 993).

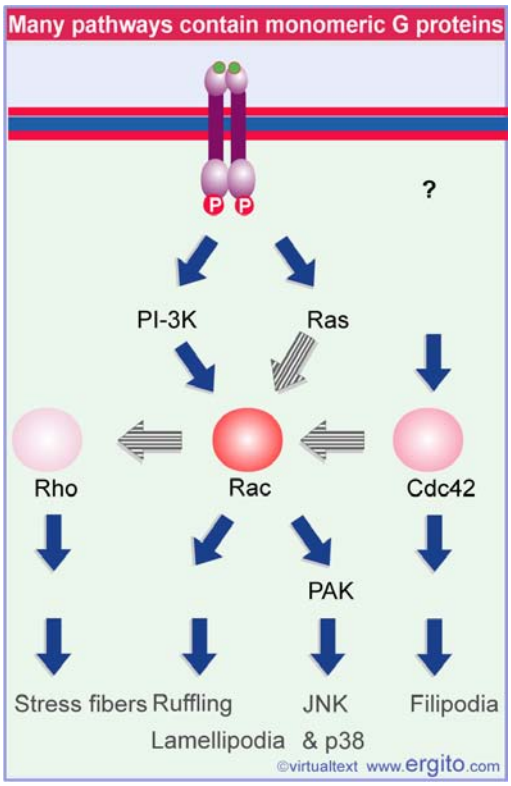

Figure 28.35 Changes in cell structure that occur during growth or transformation are mediated via monomeric G proteins.

The complete set of relationships that activates these factors is not known, but these structural changes generally can occur independently of the activation of Ras, suggesting that there are other pathways from growth factor receptors to the other monomeric G proteins.

Activation of Rho triggers the formation of actin stress fibers and their connection to the plasma membrane at sites called focal adhesions. Rho can be activated in response to addition of the lipid LPA (a component of serum), through activation of growth factors (818).

Rac can be activated by the activation of PI3 kinase (a kinase that phosphorylates a small lipid messenger) in response to (for example) activation of PDGF receptor. It stimulates membrane ruffling, formation of lamellipodia (transient structures that are driven by actin polymerization/depolymerization at the leading edge of the membrane), and progression into the G1 phase of the cell cycle. By an independent pathway it activates the stress kinases JNK and p38 (see *Molecular Biology 6.28.17 What determines specificity in signaling?*). [The two pathways can be distinguished by mutations in Rac that fail to activate one but not the other (819)].

Cdc42 activates the formation of filopodia (transient protrusions from the membrane that depend on actin polymerization), although we do not yet know in detail the pathway by which it is itself activated.

There is some crosstalk between these pathways, both laterally and vertically, as shown by the (grey) arrows in **Figure 28.35**. Rac can be activated by Ras. And Cdc42 can activate Rac, which in turn can activate Rho. This may help the coordination of the events they control; for example, lamellipodia often form along the membrane between two filopodia (making a web-like structure). All of these events are necessary for the full response to mitogenic stimulation, implying that the activation of multiple monomeric G proteins is required (820; 821).

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SIGNAL TRANSDUCTION

6.28.16 A MAP kinase pathway is a cascade

Key Concepts

- There are at least three families of MAP kinases.
- Ras activates the Ser/Thr kinase Raf, which activates the kinase MEK, which activates the ERK MAP kinase.
- An alternative pathway is for a G protein to activate MEKK, which activates MEK.
- Similar Ras/MAP kinase pathways are found in all eukaryotes.
- The end result of activating a MAP kinase pathway is to activate transcription by phosphorylating nuclear proteins.

One of the important features of signal transduction pathways is that they both diverge and converge, thus allowing different but overlapping responses to be triggered in different circumstances. Divergence may start with the initiating event. Activation of a receptor tyrosine kinase may itself trigger multiple pathways: for example, activation of EGF receptor activates the Ras pathway and also Ras-independent pathways involving second messengers (see **Figure 28.29**). There may also be "branches" later in a pathway (816).

Convergence of pathways is illustrated by the ability of different types of initiating signal to lead to the activation of MAP kinases. The original paradigm and best characterized example of a pathway leading to MAP kinase involves the activation of Ras, as summarized in **Figure 28.28**. Returning to the early events in this pathway, the next component after Ras is the Ser/Thr (cytosolic) kinase, Raf. The relationship between Ras and Raf has been puzzling. We know that Ras and Raf are on the same pathway, because both of them are required for the phosphorylation of the proteins later in the pathway (such as MAP kinase). Ras must be upstream of Raf because it is required for the activation of Raf in response to extracellular ligands. Similarly, Raf must be downstream of Ras because the pathway triggered by Ras can be suppressed by expression of a dominant-negative (kinase deficient) mutant of Raf. Ras is localized on the cytoplasmic side of the plasma membrane, and its activation results in binding of Raf, which as a result is itself brought to the vicinity of the plasma membrane. However, the events that then activate Raf, and in particular the kinase that phosphorylates it, are not yet known. The present model is that Ras activates Raf indirectly, perhaps because some kinase associated with the membrane is constitutively active (see **Figure 28.30**). The importance of localization of enzymatic activities is emphasized by the abilities of components both upstream and downstream of Ras (that is, SOS and Raf) to exercise their activating functions as a consequence of being brought from the cytosol to the plasma membrane (800; 802).

Raf activity leads to the activation of MEK. Raf directly phosphorylates MEK, which is activated by phosphorylation on two serine residues. MEK is an unusual enzyme with dual specificity, which can phosphorylate both threonine and tyrosine. Its target

is the ERK MAP kinase (869).

Both types of phosphorylation are necessary to convert a MAP kinase into the active state. There are at least 3 MAP kinase families, and they provide important switching points in their pathways. They are activated in response to a wide variety of stimuli, including stimulation of cell growth, differentiation, etc., and appear to play central roles in controlling changes in cell phenotype. The MAP kinases are serine/threonine kinases. After this point in the pathway, all the activating events take the form of serine/threonine phosphorylations.

The ultimate effect of the MAP kinase pathway is a change in the pattern of transcription. So the initiating event occurs at the cell surface, but the final readout occurs in the nucleus, where transcription factors are activated (or inactivated). This type of response requires a nuclear localization step. General possibilities for this step are illustrated in **Figure 28.36**. In the classic MAP kinase pathway, it is accomplished by the movement of a MAP kinase itself to the nucleus, where it phosphorylates target transcription factors. An alternative pathway is to phosphorylate a cytoplasmic factor; this may be a transcription factor that then moves to the nucleus or a protein that regulates a transcription factor (for example, by releasing it to go to the nucleus).

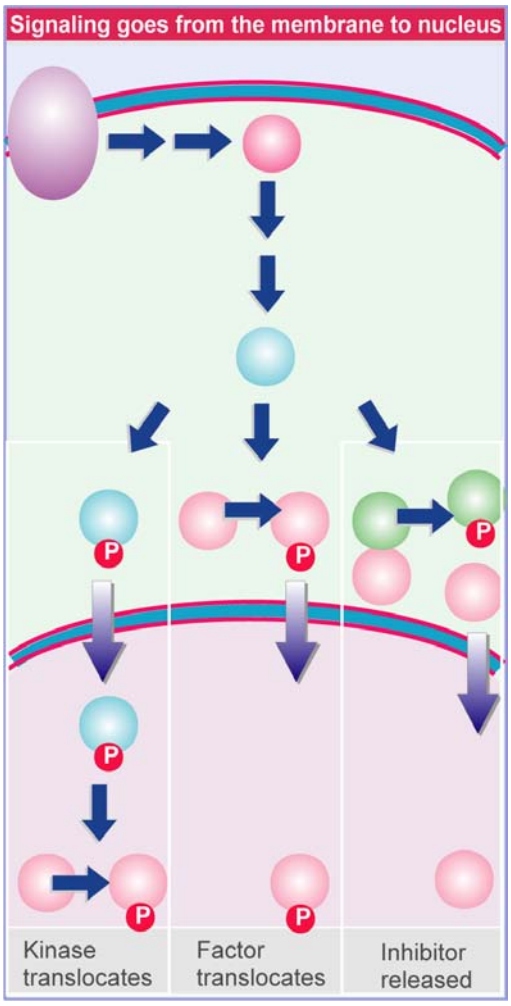

Figure 28.36 A signal transduction cascade passes to the nucleus by translocation of a component of the pathway or of a transcription factor. The factor may translocate directly as a result of phosphorylation or may be released when an inhibitor is phosphorylated.

The MAP kinases have several targets, including other kinases, such as Rsk, which extend the cascade along various branches. The ability of some MAP kinases to translocate into the nucleus after activation extends the range of substrates. In the classic pathway, ERK1 and ERK2 are the targets of MEK, and ERK2 translocates into the nucleus after phosphorylation. The direct end of one branch of the cascade is provided by the phosphorylation of transcription factors, including, c-Myc and Elk-1 (which cooperates with SRF [serum response factor]). This enables the cascade to regulate the activity of a wide variety of genes. [The important transcription factor c-Jun is phosphorylated by another MAPK, called JNK; see *Molecular Biology 6.28.17 What determines specificity in signaling?* (813; for review see 309)].

In the MAP kinase pathway, MEK provides a convergence point. Ras activates Raf, which in turn activates MEK. Another kinase that can activate MEK is MEKK (MEK kinase), which is activated by G proteins, as illustrated in **Figure 28.37**. (We have not identified the component(s) that link the activated G protein to the MEKK.)

So two principal types of stimulus at the cell surface – activation of receptor tyrosine kinases or of trimeric G proteins – both can activate the MAP kinase cascade. Formally, Raf and MEKK provide analogous functions in parallel pathways.

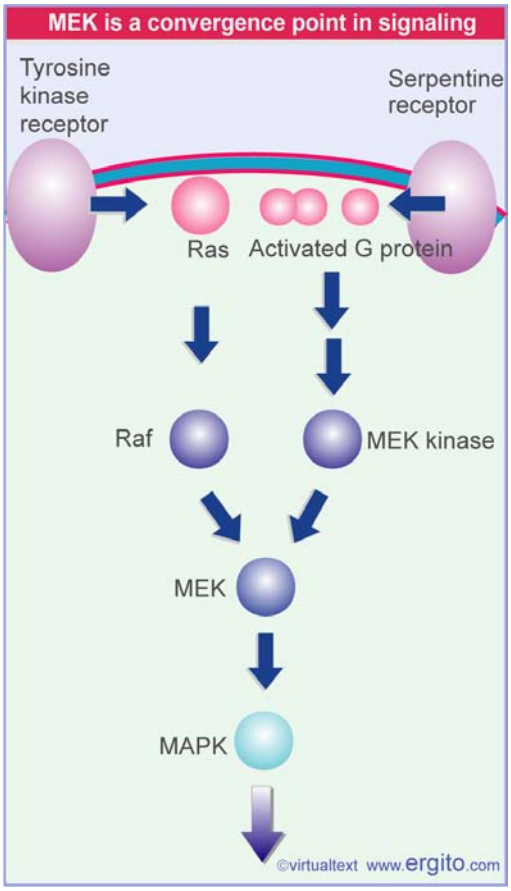

Figure 28.37 Pathways activated by receptor tyrosine kinases and by serpentine receptors converge upon MEK.

The counterparts for the components of the pathways in several organisms are summarized in **Figure 28.38**. And although signaling pathways are generally different in plants from animals, the MAPK cascade is triggered by the plant systems for defense against pathogenic infection (see *Molecular Biology 5.25.21 Innate immunity utilizes conserved signaling pathways*).

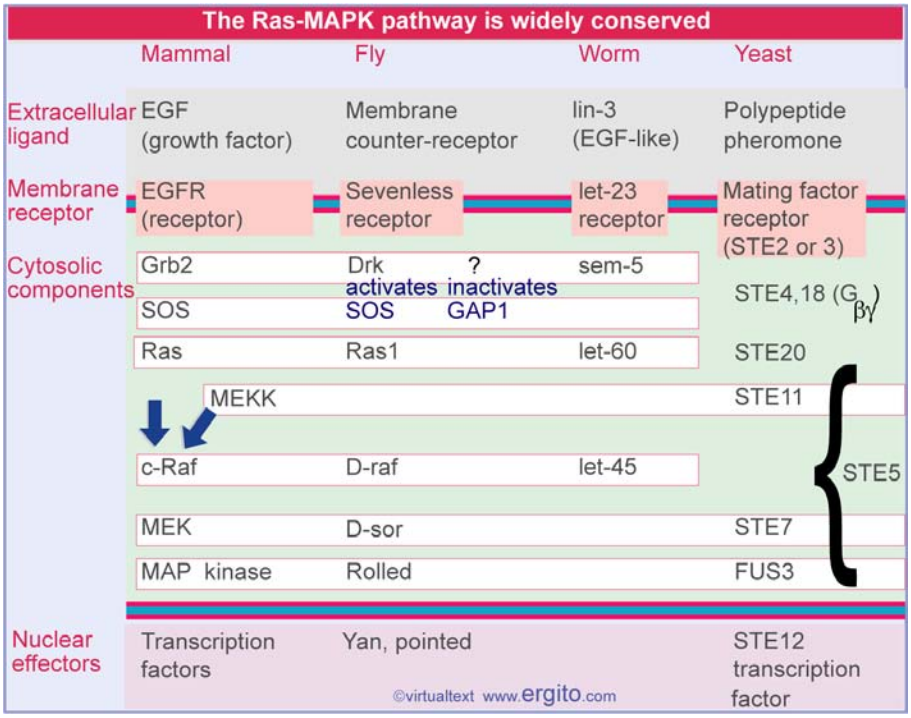

Figure 28.38 Homologous proteins are found in signal transduction cascades in a wide variety of organisms.

In mammals, fly, and worm, it starts by the activation of a receptor tyrosine kinase; in mammals and worms the ligand is a polypeptide growth factor, and in *D. melanogaster* retina it is a surface transmembrane protein on an adjacent cell (a "counter-receptor"). The pathway continues through Grb2 in mammals, and through close homologues in the worm and fly. At the next stage, a homologue of SOS functions in the fly in the same way as in mammals. The pathway continues through Ras-like proteins (that is, monomeric guanine nucleotide-binding proteins) in all three higher eukaryotes. Mutations in a homologue of GAP also may influence the pathway in *D. melanogaster,* suggesting that there are alternative regulatory circuits, at least in flies. An interesting feature is that, although the Ras-dependent pathway is utilized in a variety of cells, the mutations in the SOS and GAP functions in *Drosophila* are specific for eye development; this implies that a common pathway may be regulated by components that are tissue-specific. There is a high degree of conservation of function; for example, Grb2 can substitute for Sem-5 in worms (792; 795).

In yeast, the initiating event consists of the interaction of a polypeptide mating factor with a trimeric G protein, whose β γ dimer (STE2,3) activates the kinase STE20, which activates the MEKK, STE11. We do not know whether there are other components in addition to STE20 between G β γ and STE11, but the yeast pathway at present provides the best characterization of the route from a G protein to the MAP kinase cascade. The pathway then continues through components all of which have direct counterparts in yeast and mammals. *STE7* is homologous to MEK, and *FUS3* and *KSS1* code for kinases that share with MAP kinase the requirement for activation by phosphorylation on both threonine and tyrosine. Their targets in turn directly execute the consequences of the cascade.

The MAP kinase cascade shown in **Figure 28.38** is the best characterized, but there are also other, parallel cascades with related components. In yeast, in addition to the mating response pathway, cascades containing kinases homologous to MEKK, MEK, and MAPK respond to signaling initiated by changes in osmolarity, or activation of PKC (protein kinase C) in *S. cerevisiae* (for review see 308).

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SIGNAL TRANSDUCTION

6.28.17 What determines specificity in signaling?

Key Terms

The **CD region (Common docking)** is a C-terminal region in a MAP kinase (separate from the active site) that is involved in binding to a target protein.

The **docking groove** is a region near to, but distinct from,the active site of a MAP kinase that is involved in binding to a target protein.

The **docking site (D domain)** is a region in a target protein that used by a MAP kinase to bind to it. The docking site has a high concentration of hydrophobic residues separated from two basic residues.

Key Concepts

- A MAP kinase has regions distinct from the active site that are involved in recognizing a substrate.
- Specificity in a MAP kinase pathway may be achieved by a scaffolding protein that binds several kinases that act successively.

The presence of multiple MAPK signaling pathways with analogous components is common. **Figure 28.39** summarizes the mammalian pathways. Each pathway functions in a linear manner, as indicated previously, but in addition there may be "crosstalk" between the pathways, when a component in one pathway can activate the subsequent component in other pathways as well as its own. Usually these "lateral" signals are weaker than those propagating down the pathway. At the very start of the pathway, there is also signaling from Ras to Rac. The strengths of these lateral signals, as well as the extent of activation of an individual pathway, may be important in determining the biological response.

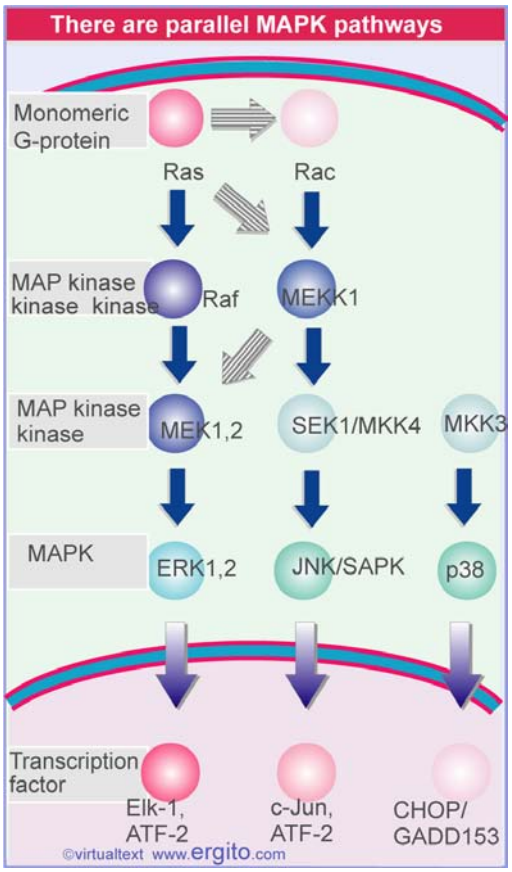

Figure 28.39 Three MAP kinase pathways have analogous components. Cross-talk between the pathways is shown by grey arrows.

The kinases in the MAPK pathways all share a similar mode of action. Each kinase has an active site that binds and phosphorylates a short target sequence containing serine or threonine followed by a proline. The activity of the enzyme is controlled by its state of phosphorylation at a short sequence (Thr-X-Tyr), where it can be activated by a kinase or deactivated by a phosphatase. Given the very short sequences that are involved, these reactions cannot in themselves provide much specificity for target selection. However, each MAP kinase belongs in one (or sometimes more) specific pathways and has a narrow specificity that is appropriate for that pathway (for review see 2841). For example, the substrate specificity of each MEK is quite narrow, and it is able to phosphorylate only one or a very few of the many MAP kinases.

Recognition of an appropriate substrate by a MAP kinase depends on two types of interactions: the catalytic site binds the substrate target sequence; and separate sites bind "docking" motifs in the target protein. **Figure 28.40** shows an example (characteristic of the ERK and p38 MAP kinases) in which two regions of the enzyme are involved in substrate recognition (2843; 2842; 2844). One region is the C-terminal **CD region** (an abbreviation for common docking). The other is the **docking groove**, which is nearby but distinct from, the catalytic active site. The regions that are recognized in the target proteins are called the **docking sites**. The best characterized type of docking site is called the D domain, and is found in many

of the target proteins for MAP kinases. It is characterized by a cluster of hydrophobic residues separated from two basic residues.

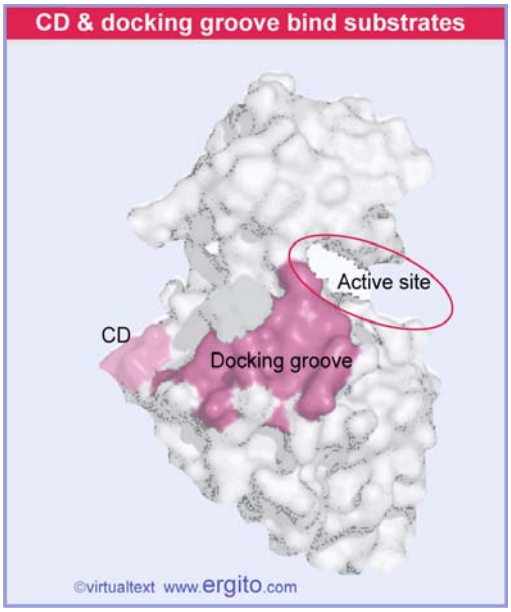

Figure 28.40 A MAP kinase has an active site, a CD domain (pink), and a docking groove (red).

Another mechanism that is used to prevent a component of one MAP kinase cascade from activating a substrate in a parallel pathway is to localize the components. The first example of such a mechanism was provided by the yeast mating pathway. *STE5* in yeast is implicated in the cascade between *STE2,3* and *FUS3*, *KSS1*, but cannot be placed in a single position in the pathway. **Figure 28.41** shows that STE5 binds to three of the kinases, STE11 (MEKK), STE7 (MEK), and FUS3 (MAPK), suggesting that this complex has to form before each kinase can activate the next kinase in the pathway. Each of the kinases binds to a different region on STE5, which provides a scaffold. If the kinases can only function in the context of the STE5 scaffold, they may be prevented from acting upon kinases in other pathways (808). Similarly, Ksr is a scaffolding protein that holds Raf and MEK together, so as to direct Raf to phosphorylate MEK (see **Figure 28.29**) (2374).

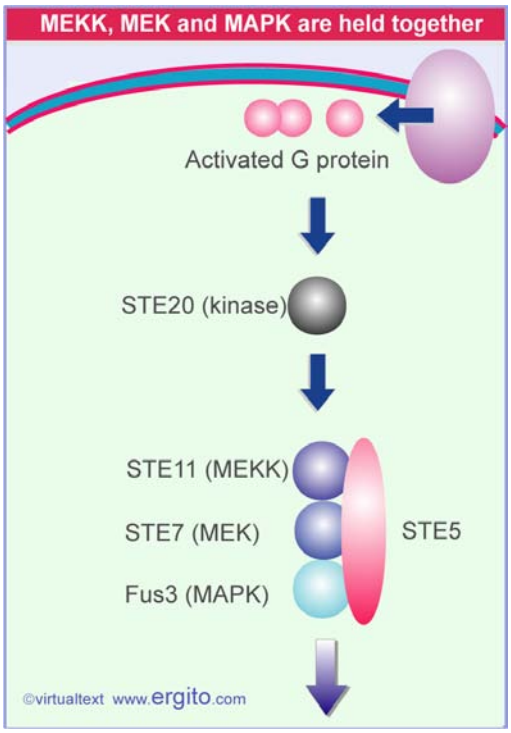

Figure 28.41 STE5 provides a scaffold that is necessary for MEKK, MEK, and MAPK to assemble into an active complex. The complex is activated by STE20, which is localized at the plasma membrane by binding to Cdc42p, and activated by the G β γ dimer.

Localization is important for holding the group of kinases together on the scaffold, and also for making them available to the upstream factors that activate the pathway. The monomeric G protein Cdc42p is localized on the inner side of the plasma membrane at the junction of the mother cell and the growing bud. It binds the kinase STE20 and localizes it to the cell cortex. Then the interaction of pheromone with its receptor causes the release of the G β γ dimer, which also binds to STE20, activating the kinase. When STE20 phosphorylates STE11, the cascade begins (for review see 3006).

The use of scaffolds enables the same components to be employed in different pathways. **Figure 28.42** compares two of the pathways. One is the pheromone-induced activation of the kinases bound by STE5. The second is the response to osmotic pressure, when an activated receptor directly binds the scaffold protein. A difference in the construction of the osmotic pathway is that the scaffolding protein is itself one of the kinases in the cascade. The striking feature is that the first kinase in the osmoadaptation cascade is STE11, the same enzyme that is used in the same position in the pheromone pathway. The function of the osmotic pathway is exactly analogous to the pheromone pathway: STE20, bound to Cdc42p, acts on STE11 that is brought to it as part of a kinase complex. The difference is that STE11 is linked to Pbs2p and Hog1p in the osmoadaptation pathway, so that the ultimate kinase in the cascade is different, and therefore a different set of responses is produced.

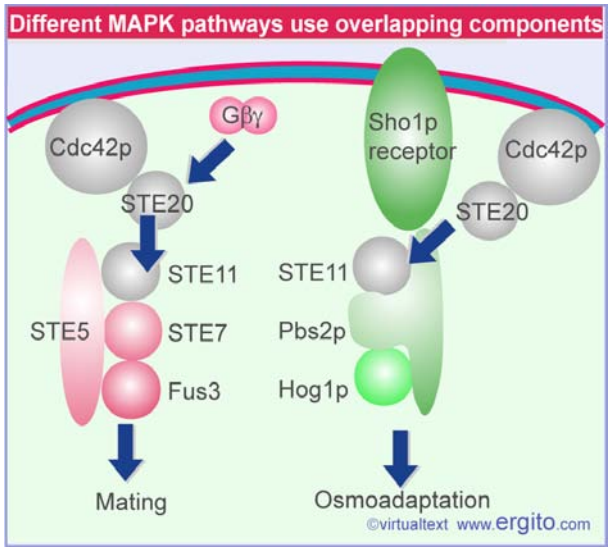

Figure 28.42 MAPK pathways differ in the first and last components, but may have common intermediate components. The components that are unique to the mating pathway are red, those that are unique to osmoadaptation are green, and those that are common to both pathways are gray.

Another example of a pathway that proceeds through a MAP kinase is provided by the activation of the transcription factor Jun in response to stress signals. **Figure 28.43** shows that activation of the kinase JNK involves both convergence and divergence. JNK is regulated by two classes of extracellular signals: UV light (typical of a stress response); and also as a consequence of activation of Ras (by an unidentified branch of the Ras pathway). JNK is a (distant) relative of MAP kinases such as the ERKs, showing the classic features of being activated by phosphorylation of Thr and Tyr, and phosphorylating its targets on Ser (817). The proteins JIP1,2 provide a scaffold that may ensure the integrity of the pathway leading to JNK activation (2236).

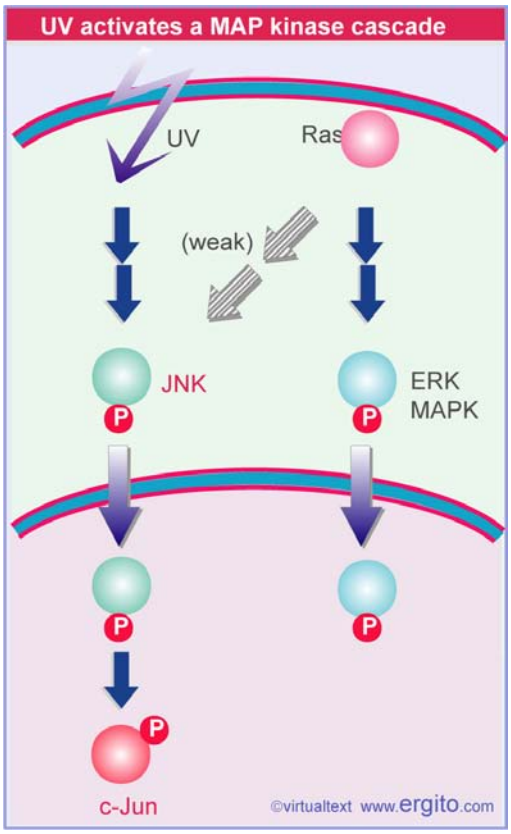

Figure 28.43 JNK is a MAP-like kinase that can be activated by UV light or via Ras.

Last updated on 2-21-2003

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SIGNAL TRANSDUCTION

6.28.18 Activation of a pathway can produce different results

Key Concepts

- Transient activation of a MAP kinase may stimulate cell proliferation, whereas continued activation may trigger differentiation.

Signal transduction generates differential responses to stimuli that vary qualitatively (by activating different pathways) or quantitatively (by activating pathways with different intensities or for different durations). An individual stimulus may activate one or more pathways. The strength of activation of any particular pathway may influence the response, since there are cases in which more intense or long-term stimulation of a single pathway gives a different response from less intense or short-term stimulation. One of our major aims is to understand how differences in such stimuli are transduced into the typical cellular responses.

What degree of amplification is achieved through the Ras/MAPK pathway? Typically an ~10× amplification of signal can be achieved at each stage of a kinase cascade, allowing an overall amplification of $>10^4$ through the pathway. However, the combination of the last three kinases into one complex would presumably restrict amplification at these stages. In mammalian cells, the pathway can be fully activated by very weak signals; for example, the ERK1,2 MAP kinases are fully activated when <5% of the Raf protein molecules bind to Ras.

A puzzling feature of the Ras/MAPK pathway is that activation of the same pathway under different circumstances can cause different outcomes. When PC12 cells are treated with the growth factor NGF, they differentiate (by becoming neuronal-like) and stop dividing. When they are treated with EGF, however, they receive a signal for continued proliferation. In both cases, the principal signal transduction event is the activation of the ERK MAP kinase pathway. The differences in outcome might be explained, of course, by other (unidentified) pathways that are activated by the respective receptors. However, the major difference in the two situations is that NGF stimulation causes prolonged elevation of Ras-GTP, whereas EGF stimulation produces only a transient effect. (One reason for this difference is that EGF receptor is more susceptible to feedback mechanisms that reverse its activation.)

The idea that duration of the stimulus to the ERK MAPK pathway may be the critical parameter is supported by results showing that a variety of conditions that cause persistent activation of ERK MAP kinase all cause differentiation. By contrast, all conditions in which activation is transient lead instead to proliferation. More direct proof of the role of the ERK MAPK pathway is provided by showing that mutations constitutively activating MEK cause differentiation of PC12 cells. So activation of the ERK MAPK pathway is sufficient to trigger the differentiation response. Another point is made by the fact that the same MEK mutation has different effects in a different host cell; in fibroblasts, it stimulates proliferation. This is another example of the ability of a cell to connect the same signal transduction pathway to different

readouts (for review see 311).

How might the duration of the signal determine the type of outcome? The concentration of some active component in the pathway could increase with the duration of activation, and at some point would exceed a threshold at which it triggers a new response. One model for such an action is suggested by *Drosophila* development, in which increasing concentrations of a transcription factor activate different target genes, as the result of combinatorial associations with other factors that depend upon relative concentrations (see *Molecular Biology 6.31 Gradients, cascades, and signaling pathways*). Another possibility is suggested by the fact that prolonged activation is required before ERK2 translocates to the nucleus. The mechanism is unknown, but could mean that transient stimulation does not support the phosphorylation and activation of nuclear transcription factors, so the expression of new functions (such as those needed for differentiation) could depend upon the stimulus lasting long enough to cause translocation of ERK2 (809).

Last updated on 3-16-2002

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SIGNAL TRANSDUCTION

6.28.19 Cyclic AMP and activation of CREB

Key Concepts

- Cyclic AMP is produced when a G protein activates adenylate cyclase at the plasma membrane.
- Cyclic AMP binds to the regulatory subunit of PKA (protein kinase A), releasing the catalytic subunit, which moves to the nucleus.
- One of the major nuclear targets for PKA is the transcription factor CREB, which is activated by phosphorylation.

Cyclic AMP is the classic second messenger, and its connection to transcription is by the activation of CREB (cAMP response element binding protein). **Figure 28.44** shows how the pathway proceeds through the Ser/Thr kinase, PKA.

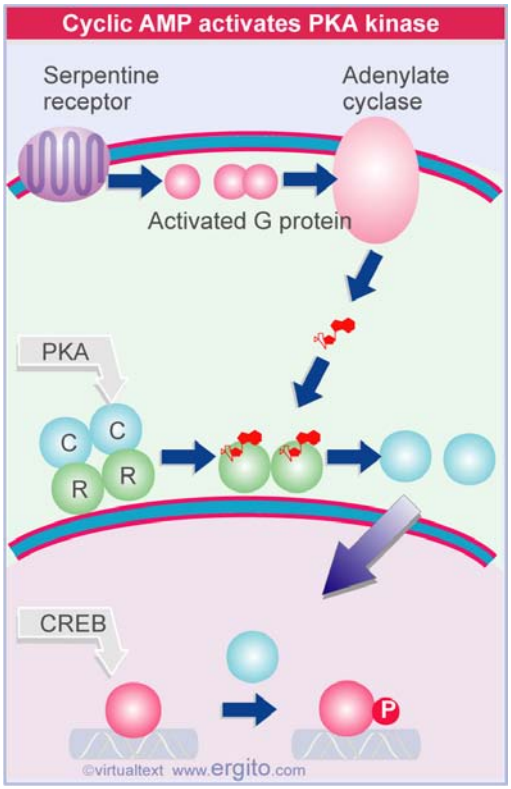

Figure 28.44 When cyclic AMP binds to the R subunit of PKA, the C subunit is released; some C subunits diffuse to the nucleus, where they phosphorylate CREB.

The initial step in the pathway is activation of adenylate cyclase at the plasma

membrane by an activated G protein (see **Figure 28.11**). cAMP binds to the regulatory R subunit of PKA, which is anchored to membranes in the perinuclear region. This causes the R subunit to release the catalytic (C) subunit of PKA, which then becomes free to translocate to the nucleus. Translocation occurs by passive diffusion, and involves only a proportion of the released C subunits. In fact, the free C subunits phosphorylate targets in both the cytosol and nucleus.

The circuitry also has some feedback loops. The end-targets for PKA are also substrates for the phosphatase PPase I, which in effect reverses the action of PKA. However, PKA also has as a target a protein whose phosphorylation converts it into an inhibitor of PPase I, thus preventing the reversal of phosphorylation.

The transcription factor CREB is one of the major nuclear substrates for PKA. Phosphorylation at a single Ser residue greatly increases the activity of CREB bound to the response element CRE, which is found in genes whose transcription is induced by cAMP. The rate of transcription of these genes is directly proportional to the concentration of phosphorylated CREB in the nucleus. The kinetics of the response are limited by the relatively slow rate at which the free C subunit diffuses into the nucleus. Typically the phosphorylated C subunit reaches a maximum level in the nucleus after ~30 min, and then is slowly dephosphorylated (over several hours). Several circuits may be involved in the dephosphorylation, including direct control of phosphatases and indirect control by the entry into the nucleus of the protein PKI, which binds to the C subunit and causes it to be re-exported to the cytoplasm. The kinetics of activating PKA in the nucleus may be important in several situations, including learning, in which a weak stimulus of cAMP has only short-term effects, whereas a strong stimulus is required for long-term effects, including changes in transcription. This parallels the different consequences of short-term and long-term stimulation of the MAPK pathway (see *Molecular Biology 6.28.18 Activation of a pathway can produce different results*) (814; 815).

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SIGNAL TRANSDUCTION

6.28.20 The JAK-STAT pathway

Key Concepts

- Some cytokine (growth factor) receptors activate JAK kinases.
- The JAK kinases phosphorylate STAT transcription factors.
- The activation of JAK and its activation of STAT occurs in a complex at the nuclear membrane.
- The phosphorylated STAT migrates to the nucleus where it activates transcription.

Some signal transduction pathways have large numbers of components (permitting a high degree of amplification) and many feedback circuits (permitting sensitive control of the duration and strength of the signal). The JAK-STAT pathway is much simpler, and consists of three components that function as illustrated in **Figure 28.45**.

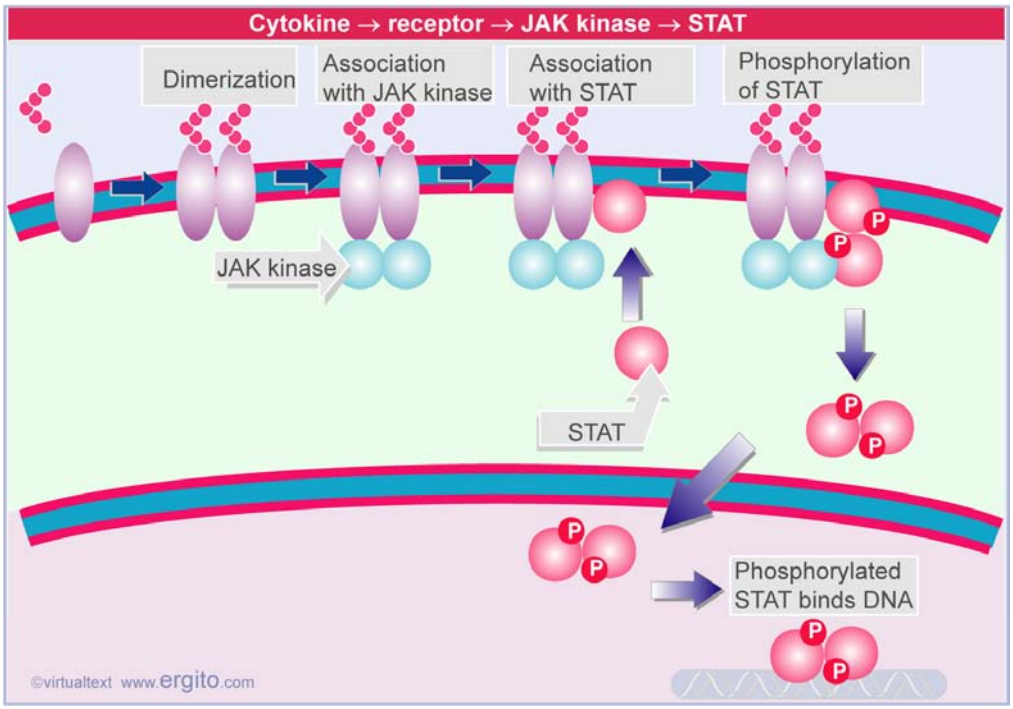

Figure 28.45 Cytokine receptors associate with and activate JAK kinases. STATs bind to the complex and are phosphorylated. They dimerize and translocate to the nucleus. The complex binds to DNA and activates transcription.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.6.28.20 to view properly.

JAK-STAT pathways are activated by several cytokine receptors. These receptors do

not possess intrinsic kinase activities. However, binding of a cytokine causes its receptor to dimerize, which provides the signal to associate with and activate a JAK kinase. The JAK kinases take their name (originally Janus kinases) from the characteristic presence of two kinase domains in each molecule. Several members of the family are known (JAK1,2,3, etc.); each associates with a specific set of cytokine receptors. The interaction between the activated (dimeric) cytokine receptor and JAK kinase(s) in effect produces the same result as the ligand-induced dimerization of a tyrosine kinase receptor: the difference is that the receptor and kinase activities are provided by different proteins instead of by the same protein.

The JAK kinases are tyrosine kinases whose major substrates are transcription factors called STATs. There are >7 STATs; each STAT is phosphorylated by a particular set of JAK kinases. The phosphorylation occurs while the JAK is associated with the receptor at the plasma membrane. A pair of JAK kinases associates with an activated receptor, and both may be necessary for the pathway to function. An example is that stimulation by the interferon IFN γ requires both JAK1 and JAK2 (803; 804; for review see 303).

STAT phosphorylation leads to the formation of both homodimers and heterodimers. The basis for dimerization is a reciprocal interaction between an SH2 domain in one subunit and a phosphorylated Tyr in the other subunit (810).

The STAT dimers translocate to the nucleus, and in some cases associate with other proteins. They bind to specific recognition elements in target genes, whose transcription is activated (for review see 313).

Given a multiplicity of related cytokine receptors, JAK kinases, and STAT transcription factors, how is specificity achieved? The question is sharpened by the fact that many receptors can activate the same JAKs, but activate different STATs. Control of specificity lies with formation of a multipartite complex containing the receptor, JAKs, and STATs. The STATs interact directly with the receptor as well as with the JAKs, and an SH2 domain in a particular STAT recognizes a binding site in a particular receptor. So the major control of specificity lies with the STAT.

Stimulation of a JAK-STAT pathway is only transient. Its activation may be terminated by the action of a phosphatase. An example is the pathway activated by binding of erythropoietin (red blood cell hormone) to its receptor. This activates JAK2 kinase. Recruitment of another component terminates the reaction; the phosphatase SH-PTP1 binds via its SH2 domain to a phosphotyrosine site in the erythropoietin receptor. This site in the receptor is probably phosphorylated by JAK2. The phosphatase then dephosphorylates JAK2 and terminates the activation of the corresponding STATs. This creates a simple feedback circuit: erythropoietin receptor activates JAK2, JAK2 acts on a site in the receptor, and this site is recognized by the phosphatase that in turn acts on JAK2. This again emphasizes the way in which formation of a multicomponent complex may be used to ensure specificity in controlling the pathway (811).

Reviews

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SIGNAL TRANSDUCTION

6.28.21 TGF β signals through Smads

Key Concepts

- TGF β activates the heterodimeric type II receptor.
- The activated type II receptor phosphorylates the heterodimeric type I receptor.
- As part of the tetrameric complex, the type I receptor phosphorylates a cytosolic Smad protein.
- The Smad forms a dimer with a related protein (Smad4) which moves to the nucleus and activates transcription.

Another pathway in which phosphorylation at the membrane triggers migration of a transcription factor to the nucleus is provided by TGF β signaling (for review see 3650). The TGF β family contains many related polypeptide ligands. They bind to receptors that consist of two types of subunits, as illustrated in **Figure 28.46**. Both subunits have serine/threonine kinase activity. (Actually all serine/threonine receptor kinases are members of the TGF β receptor family.)

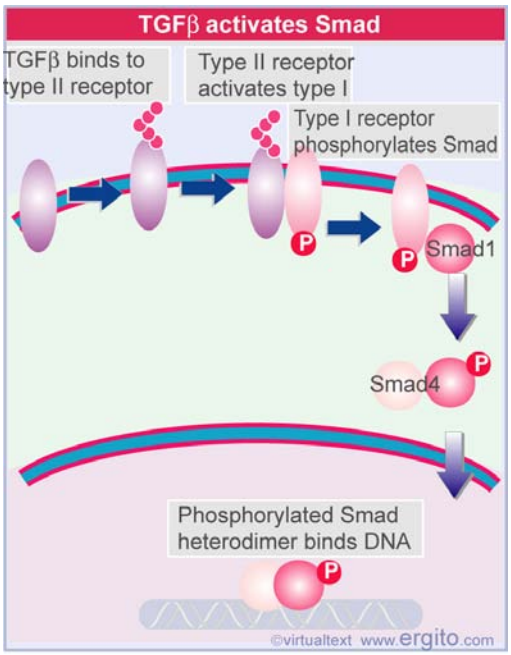

Figure 28.46 Activation of TGF β receptors causes phosphorylation of a Smad, which is imported into the nucleus to activate transcription.

The ligand binds to the type II receptor, creating a receptor-ligand combination that has high affinity for the type I receptor. A tetrameric complex is formed in which the

type II receptor phosphorylates the type I receptor. (A variation occurs in a subset of these receptors that bind BMPs – bone morphogenetic proteins – which are members of the TGF β family. In this case, both type I and type II subunits have low affinity for the ligand, but the combination of subunits has high affinity.)

Once the active complex has formed, the type I receptor phosphorylates a member of the cytosolic Smads family. Typically a Smad activator is phosphorylated at the motif SSXS at the C-terminus. This causes it to form a dimer with the common partner Smad4. The heterodimer is imported into the nucleus, where it binds to DNA and activates transcription (812; for review see 314; 316).

The 9 Smad proteins fall into three functional categories. The pathway-specific activators are Smad2,3 (which mediate TGF β/activin signaling) and Smad1,5 (which activate BMP signaling). Smad4 is a universal partner which can dimerize with all of the pathway-specific Smads. Inhibitory Smads act as competitive inhibitors of the activator Smads, providing another level of complexity to the pathway. Each ligand in the TGF β superfamily activates a particular receptor that signals through a characteristic combination of Smads proteins. Various other proteins bind to the Smads dimers and influence their capacity to act on transcription.

Signaling systems of this type are important in early embryonic development, where they are part of the pathways that lead to development of specific tissues (typically bone formation and the development of mesoderm). Also, because TGF β is a powerful growth inhibitor, this pathway is involved in tumor suppression. The TGF β type II receptor is usually inactivated in hereditary nonpolyposis colorectal cancers, and mutations in Smad4 occur in 50% of human pancreatic cancers.

One striking feature of the JAK-STAT and TGF β pathways is the simplicity of their organization, compared (for example) with the Ras-MAPK pathway. The specificity of these pathways depends on variation of the components that assemble at the membrane – different combinations of JAK-STATs in the first case, different Smad proteins in the second. One the pathway has been triggered, it functions in a direct linear manner. The component that is phosphorylated at the plasma membrane (STAT in the JAK-STAT pathway, Smad in the TGF β pathway) itself provides the unit that translocates to the nucleus to activate transcription – perhaps the ultimate demonstration of the role of localization

Reviews

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SIGNAL TRANSDUCTION

6.28.22 Structural subunits can be messengers

It is a common need to carry a signal from an event at the cell surface to the nucleus. Activation of a receptor in the plasma membrane can stimulate a signaling pathway in many ways, but a common feature is that some component of the pathway either itself translocates to the nucleus or causes the translocation of another protein. The component that translocates can be distant in the pathway (as in the case of MAP kinases) or a protein that actually itself interacts with the receptor (as in the case of the TGF β or JAK-STAT pathways. A more extreme case is presented by some pathways in which the translocating component may be actually a structural subunit of the complex at the plasma membrane.

Figure 28.47 shows a generic model for such a pathway. When a multisubunit complex is activated at the plasma membrane, one of its subunits is released. The subunit translocates to the nucleus, where (directly or indirectly) it activates transcription. A typical pattern is for the translocating subunit to activate a transcription factor that is already in the nucleus.

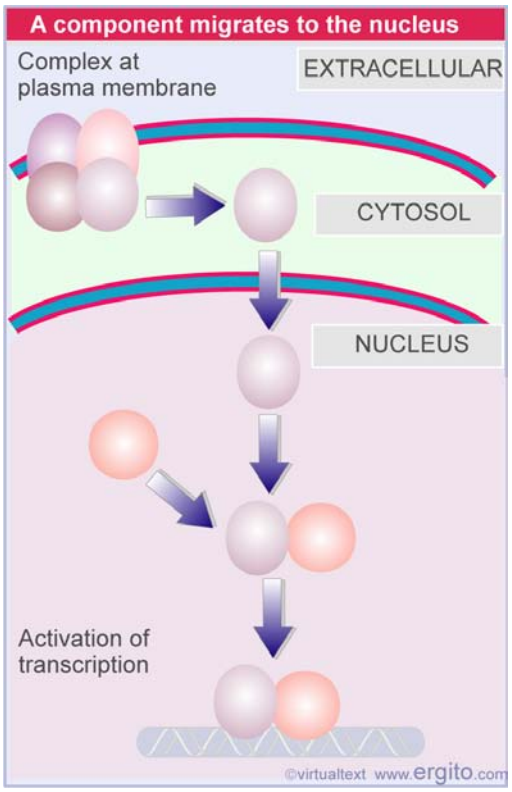

Figure 28.47 Activation of a complex at the plasma membrane triggers release of a subunit that migrates to the nucleus to activate transcription.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.6.28.22 to view properly.

A pathway that follows this general model signals from the synapse (the connection between neurons in the nervous system). One of the multiprotein complexes that assembles at the synapse has cell surface proteins bound to a group of cytosolic proteins that include a MAGUK (membrane-associated guanylate kinase). The MAGUK has PDZ and SH3 domains (typically involved in protein-protein interactions), a protein kinase-like domain, and a guanylate kinase-like domain – all the hallmarks of a "scaffold" protein involved in assembling the components of the signaling complex. One example is the protein CASK in the nuclei of embryonic neurons. The guanylate kinase domain of CASK interacts with the nuclear transcription factor Tbr (955). This raises the possibility that CASK is a bifunctional protein: one function being in assembly of the cell surface complex, the other being in activating transcription in the nucleus.

Last updated on 10-31-2001

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SIGNAL TRANSDUCTION

6.28.23 Summary

Lipids may cross the plasma membrane, but specific transport mechanisms are required to promote the passage of hydrophilic molecules. Integral proteins of the plasma membrane offer several means for communication between the extracellular milieu and the cytoplasm. They include ion channels, transporters, and receptors. All such proteins reside in the plasma membrane by means of hydrophobic domains.

Ions may be transported by carrier proteins, which may utilize passive diffusion or may be connected to energy sources to undertake active diffusion. The detailed mechanism of movement via a carrier is not clear, but is presumed to involve conformational changes in the carrier protein that directly or indirectly allow a substrate to move from one side of the membrane to the other. Ion channels can be used for passive diffusion (driven by the gradient). They may be gated (controlled) by voltage, extracellular ligands, or cytoplasmic second messengers. Channels typically have multiple subunits, each with several transmembrane domains; hydrophilic residues within the transmembrane domains face inward so as to create a hydrophilic path through the membrane.

Receptors typically are group I proteins, with a single transmembrane domain, consisting exclusively of uncharged amino acids, connecting the extracellular and cytosolic domains. Many receptors for growth factors are protein tyrosine kinases. Such receptors have a binding site for their ligand in the extracellular domain, and a kinase activity in their cytoplasmic domain. When a ligand binds to the receptor, it causes the extracellular domain to dimerize; most often the product is a homodimer, but there are some cases where heterodimers are formed. The dimerization of the extracellular domains causes the transmembrane domains to diffuse laterally within the membrane, bringing the cytoplasmic domains into contact. This results in an autophosphorylation in which each monomeric subunit phosphorylates the other.

The phosphorylation creates a binding site for the SH2 motif of a target protein. Specificity in the SH2-binding site typically is determined by the phosphotyrosine in conjunction with the 4-5 neighboring amino acids on its C-terminal side. The next active component in the pathway may be activated indirectly or directly. Some target proteins are adaptors that are activated by binding to the phosphorylated receptor, and they in turn activate other proteins. An adaptor typically uses its SH2 domain to bind the receptor and uses an SH3 domain to bind the next component in the pathway. Other target proteins are substrates for phosphorylation, and are activated by the acquisition of the phosphate group.

One group of effectors consists of enzymes that generate second messengers, most typically phospholipases and kinases that generate or phosphorylate small lipids. Another type of pathway consists of the activation of a kinase cascade, in which a series of kinases successively activate one another, leading ultimately to the phosphorylation and activation of transcription factors in the nucleus. The MAP kinase pathway is the paradigm for this type of response.

The connection from receptor tyrosine kinases to the MAP kinase pathway passes

through Ras. An adaptor (Grb2 in mammalian cells) is activated by binding to the phosphorylated receptor. Grb2 binds to SOS, and SOS causes GDP to be replaced by GTP on Ras. Ras is anchored to the cytoplasmic face of the membrane. The activated Ras binds the Ser/Thr kinase Raf, thus bringing Raf to the membrane, which causes Raf to be activated, probably because it is phosphorylated by a kinase associated with the membrane. Raf phosphorylates MEK, which is a dual-specificity kinase that phosphorylates ERK MAP kinases on both tyrosine and threonine. ERK MAP kinases activate other kinases; ERK2 MAP kinase also translocates to the nucleus, where it phosphorylates transcription factors that trigger pathways required for cell growth (in mammalian cells) or differentiation (in fly retina, worm vulva, or yeast mating).

An alternative connection to the MAP kinase cascade exists from serpentine receptors. Activation of a trimeric G protein causes MEKK to be activated. One component in the pathway between G β γ and MEKK in *S. cerevisiae* is the kinase STE20. The MEKK (STE11), MEK (STE7), and MAPK (Fus3) form a complex with the scaffold protein STE5 that may be necessary for the kinases to function. The use of scaffolding proteins allows the same kinases to participate in different pathways, but to signal to the downstream components only of the pathway that activates them.

The cyclic AMP pathway for activating transcription proceeds by releasing the catalytic subunit of PKA in the cytosol. It diffuses to the nucleus, where it phosphorylates the transcription factor CREB. The activity of this factor is responsible for activating cAMP-inducible genes. The response is down regulated by phosphatases that dephosphorylate CREB and by an inhibitor that exports the C subunit back to the cytosol.

JAK-STAT pathways are activated by cytokine receptors. The activated receptor associates with a JAK kinase and activates it. The target for the kinase is a STAT(s); STATs associate with a receptor-JAK kinase complex, are phosphorylated by the JAK kinase, dimerize, translocate to the nucleus, and form a DNA-binding complex that activates transcription at a set of target genes. In an analogous manner, TGF β ligands activate type II/type I receptor systems that phosphorylate Smad proteins, which then are imported into the nucleus to activate transcription.