Cell Signaling

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https://open.oregonstate.education/cellbiology/chapter/cell-signaling/

https://www.ncbi.nlm.nih.gov/books/NBK26813/

Introduction

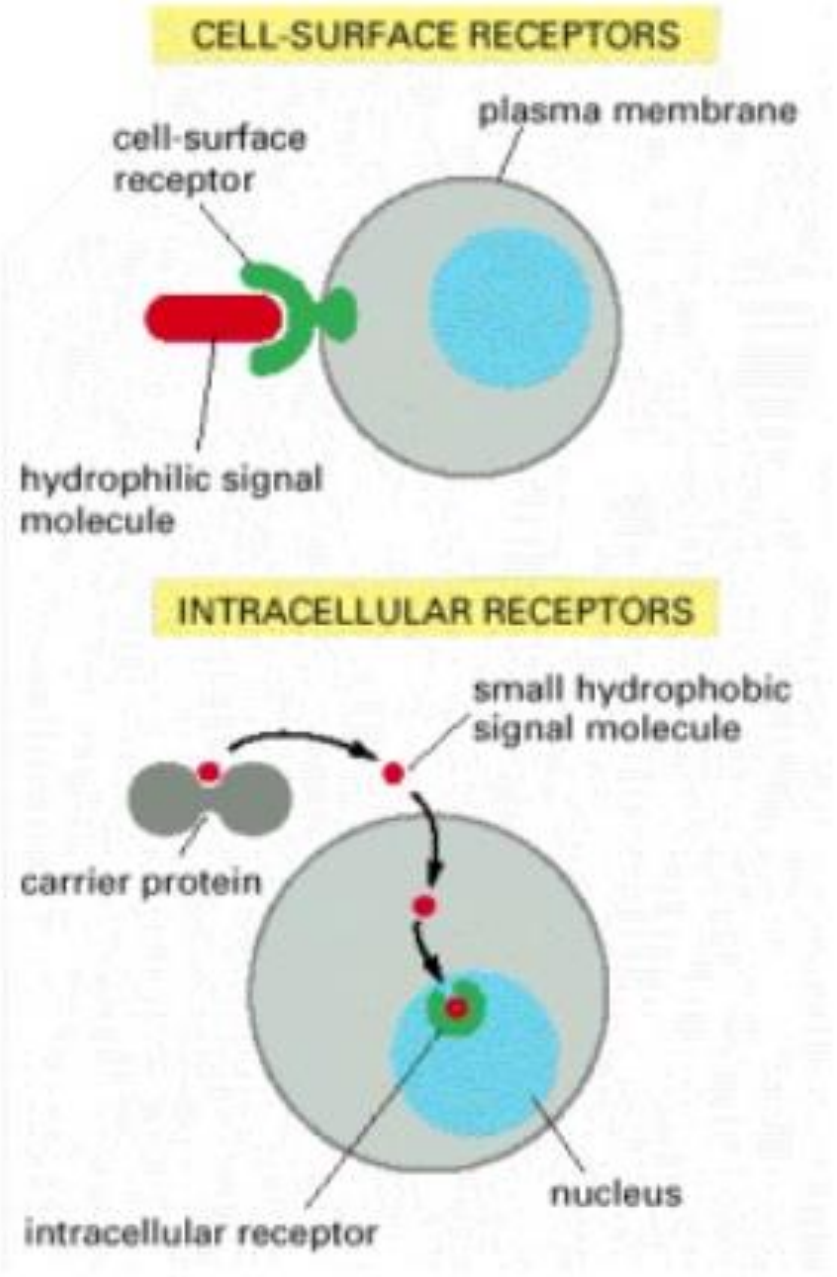
- Mechanisms enabling one cell to influence the behavior of another almost certainly existed in the world of unicellular organisms long before multicellular organisms appeared on Earth.
- Evidence comes from studies of present-day unicellular eucaryotes such as yeasts.
- Although these cells normally lead independent lives, they can communicate and influence one another's behavior in preparation for sexual mating.
- In the budding yeast *Saccharomyces cerevisiae,* for example, when a haploid individual is ready to mate, it secretes a peptide *mating factor* that signals cells of the opposite mating type to stop proliferating and prepare to mate.
- The subsequent fusion of two haploid cells of opposite mating types produces a diploid cell, which can then undergo meiosis and sporulate, generating haploid cells with new assortments of genes.
- Studies of yeast mutants that are unable to mate have identified many proteins that are required in the signaling process.
- These proteins form a signaling network that includes cell-surface receptor proteins, GTP-binding proteins, and protein kinases, each of which has close relatives among the proteins that carry out signaling in animal cells.
- Through gene duplication and divergence, however, the signaling systems in animals have become much more elaborate than those in yeasts.

Cell Signaling

- Cells in higher animals communicate by means of hundreds of kinds of signal molecules.
- These include proteins, small peptides, amino acids, nucleotides, steroids, retinoids, fatty acid derivatives, and even dissolved gases such as nitric oxide and carbon monoxide.
- Most of these signal molecules are secreted from the *signaling cell* into the extracellular space by exocytosis.
- Others are released by diffusion through the plasma membrane, and some are exposed to the extracellular space while remaining tightly bound to the signaling cell's surface.
- Regardless of the nature of the signal, the *target cell* responds by means of a specific protein called a receptor, which specifically binds the signal molecule and then initiates a response in the target cell.
- The extracellular signal molecules often act at very low concentrations (typically ≤ 10^{-8} M), and the receptors that recognize them usually bind them with high affinity.
- In most cases, these receptors are transmembrane proteins on the target cell surface.
- When they bind an extracellular signal molecule *(a ligand),* they become activated and generate a cascade of intracellular signals that alter the behavior of the cell.
- In other cases, the receptors are inside the target cell, and the signal molecule has to enter the cell to activate them: this requires that the signal molecules be sufficiently small and hydrophobic to diffuse across the plasma membrane.

The binding of extracellular signal molecules to either cell-surface receptors or intracellular receptors

- Most signal molecules are hydrophilic and are therefore unable to cross the plasma membrane directly; instead, they bind to cell-surface receptors, which in turn generate one or more signals inside the target cell.
- Some small signal molecules, by contrast, diffuse across the plasma membrane and bind to receptors inside the target cell—either in the cytosol or in the nucleus (as shown here).
- Many of these small signal molecules are hydrophobic and nearly insoluble in aqueous solutions; they are therefore transported in the bloodstream and other extracellular fluids after binding to carrier proteins, from which they dissociate before entering the target cell.



Types of Cell Signaling

- **Extracellular Signal Molecules Can Act Over Either Short or Long Distances**
- Many signal molecules remain bound to the surface of the signaling cell and influence only cells that contact it.
- Such **contact-dependent signaling** is especially important during development and in immune responses. It's also known as juxtacrine signalling.
- In most cases, however, signal molecules are secreted.
- The secreted molecules may be carried far afield to act on distant targets, or they may act as local mediators, affecting only cells in the immediate environment of the signaling cell. This latter process is called **paracrine signaling**.
- For paracrine signals to be delivered only to their proper target cells, the secreted molecules must not be allowed to diffuse too far; for this reason they are often rapidly taken up by neighboring target cells, destroyed by extracellular enzymes, or immobilized by the extracellular matrix.

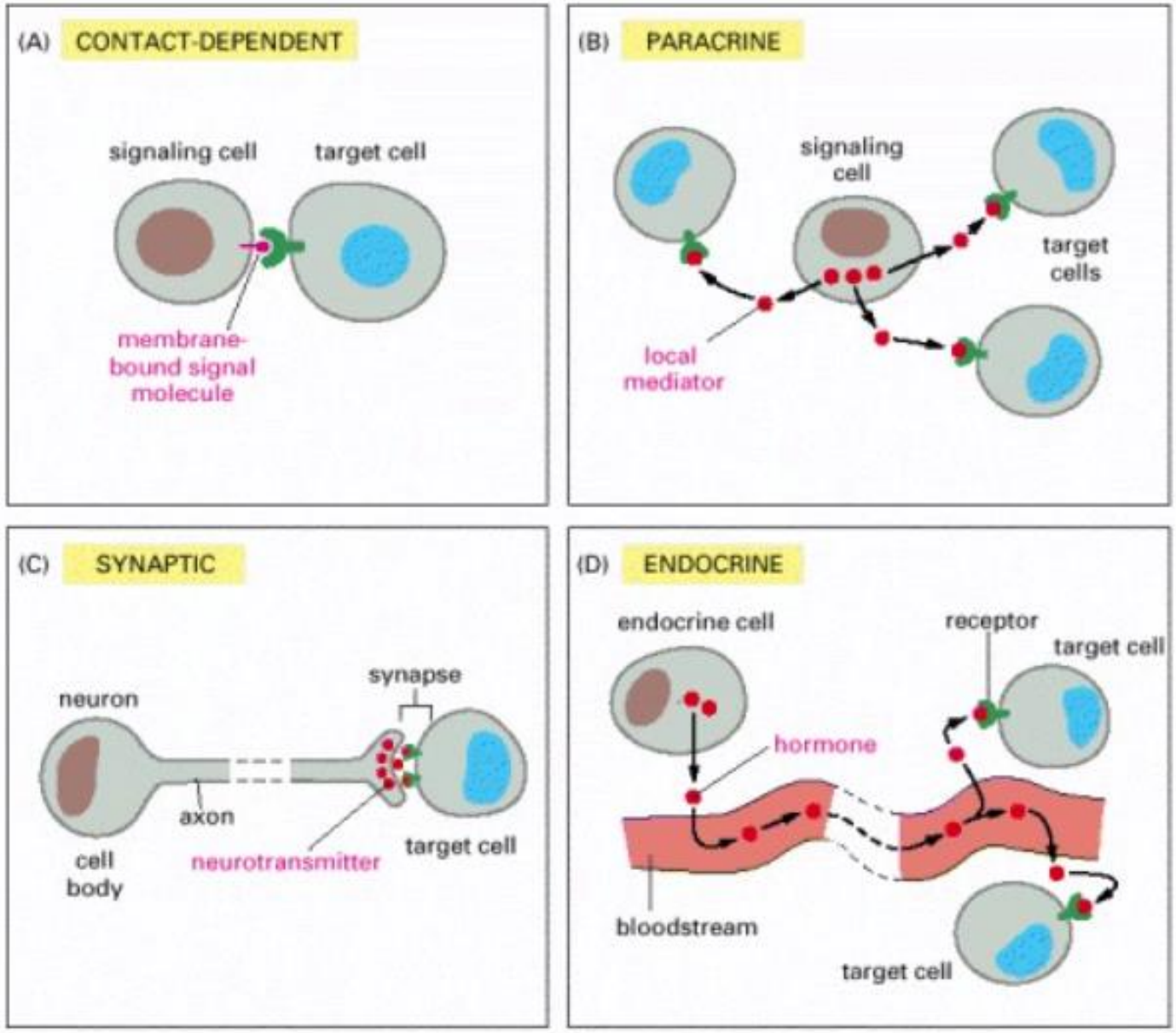
Forms of intercellular signaling

(A) Contact-dependent signaling requires cells to be in direct membrane-membrane contact.

(B) Paracrine signaling depends on signals that are released into the extracellular space and act locally on neighboring cells.

(C) Synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses, which are often located far away from the cell body.

(D) Endocrine signaling depends on endocrine cells, which secrete hormones into the bloodstream that are then distributed widely throughout the body. Many of the same types of signaling molecules are used in paracrine, synaptic, and endocrine signaling; the crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.



Synaptic Signaling

- For a large, complex multicellular organism, short-range signaling is not sufficient on its own to coordinate the behavior of its cells.
- In these organisms, sets of specialized cells have evolved with a specific role in communication between widely separate parts of the body.
- The most sophisticated of these are nerve cells, or neurons, which typically extend long processes (axons) that enable them to contact target cells far away.
- When activated by signals from the environment or from other nerve cells, a neuron sends electrical impulses (action potentials) rapidly along its axon; when such an impulse reaches the end of the axon, it causes the nerve terminals located there to secrete a chemical signal called a neurotransmitter.
- Nerve cells can transmit information over long distances by electrical impulses that travel at rates of up to 100 meters per second; once released from a nerve terminal, a neurotransmitter has to diffuse less than 100 nm to the target cell, a process that takes less than a millisecond.
- Neurotransmitters help the signal move from the neuron to the target cell (like a muscle cell).
- The gap between the nerve cell and the muscle cell is really small, so the signal doesn't have to diffuse very far.
- To increase the chances that the signal will be received as quickly as possible, the nerve cell floods the entire area with the neurotransmitter.
- As such, these receptors are not as sensitive as those used for endocrine signaling…they don't really have to be.
- Thus, we consider the receptors in the synapse to be ***low affinity***.

Endocrine signaling

- A second type of specialized signaling cell that controls the behavior of the organism as a whole is an endocrine cell. These cells secrete their signal molecules, called hormones, into the bloodstream, which carries the signal to target cells distributed widely throughout the body.
- Because endocrine signaling relies on diffusion and blood flow, it is relatively slow.
- The signal must be produced and secreted into the bloodstream, moved throughout the body, and then picked up by another cell that is likely very far from the site of ligand release.
- It can take minutes for the signal to be received, which is quite a long time in the world of cells and signaling.
- On the other hand, since the hormone will become very dilute as it moves through the body and the receptor must be able to find and bind the ligand even in these low-concentration conditions, the receptors for endocrine signals are quite sensitive. In some cases, a single molecule from the bloodstream can be detected.
- A receptor that can be activated by a small, dilute dose of signal is said to have ***high affinity*** for its ligand.
- Examples of endocrine signaling ligands include most hormones (i.e., insulin, adrenaline, estrogen, growth hormones, etc.).

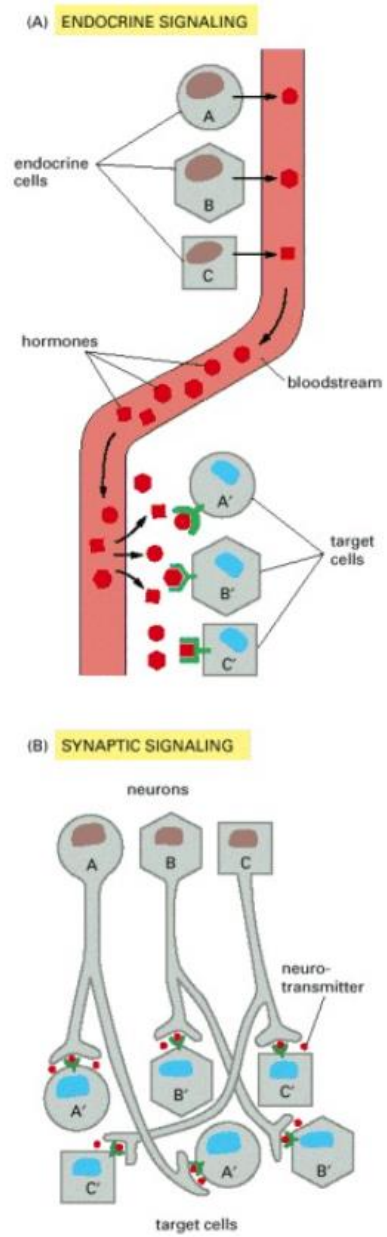


Figure 15-5 The contrast between endocrine and synaptic signaling

Autocrine Signaling Can Coordinate Decisions by Groups of Identical Cells

- All of the forms of signaling discussed so far allow one cell to influence another.
- Often, the signaling cell and target are different cell types.
- Cells, however, can also send signals to other cells of the same type, as well as to themselves.
- In such autocrine signaling, a cell secretes signal molecules that can bind back to its own receptors.
- During development, for example, once a cell has been directed along a particular pathway of differentiation, it may begin to secrete autocrine signals to itself that reinforce this developmental decision.
- Autocrine signaling is most effective when performed simultaneously by neighboring cells of the same type, and it is likely to be used to encourage groups of identical cells to make the same developmental decisions.
- Thus, autocrine signaling is thought to be one possible mechanism underlying the “community effect” that is observed in early development, during which a group of identical cells can respond to a differentiation-inducing signal but a single isolated cell of the same type cannot.
- Unfortunately, cancer cells often use autocrine signaling to overcome the normal controls on cell proliferation and survival that we discuss later.
- By secreting signals that act back on the cell's own receptors, cancer cells can stimulate their own survival and proliferation and thereby survive and proliferate in places where normal cells of the same type could not.

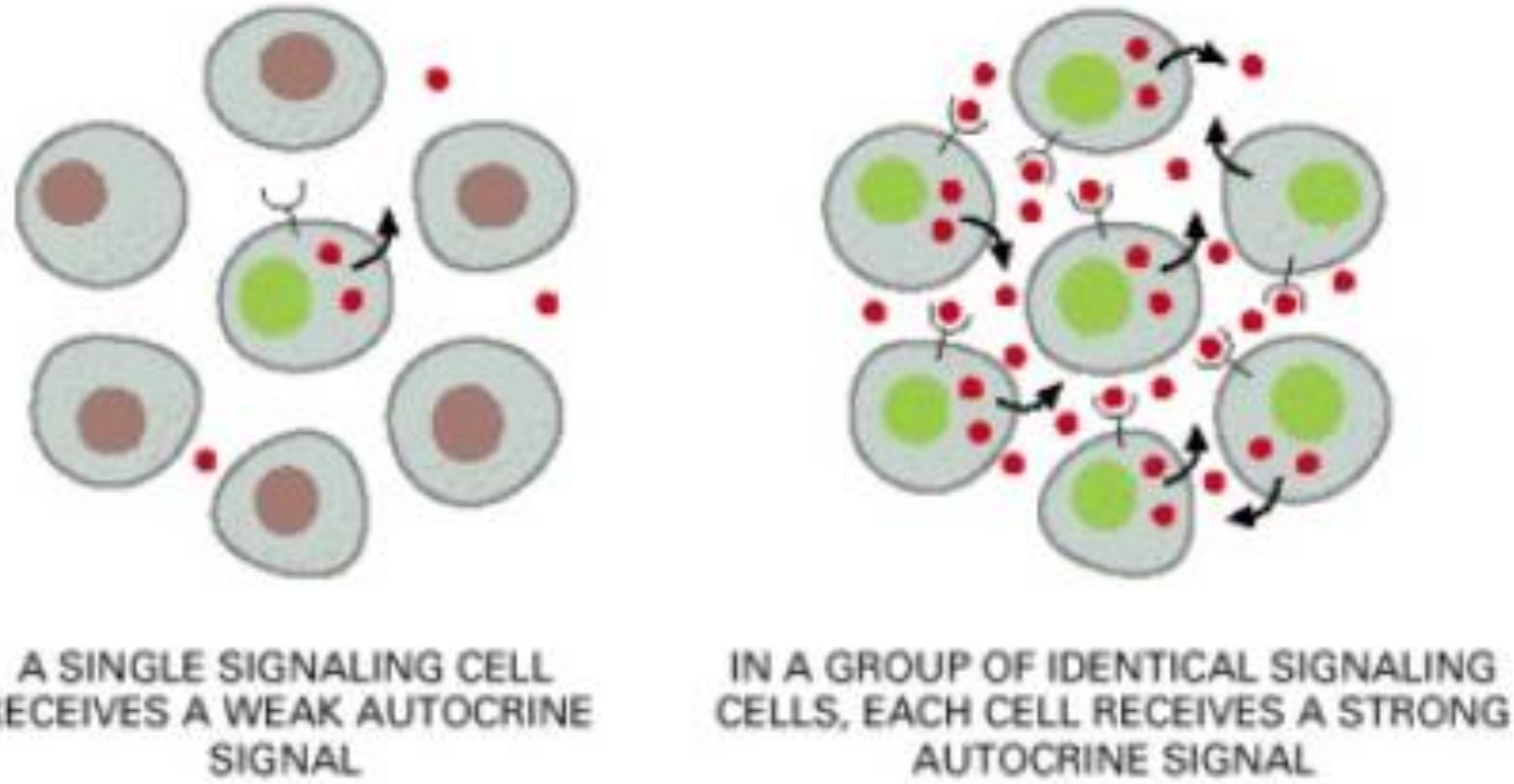
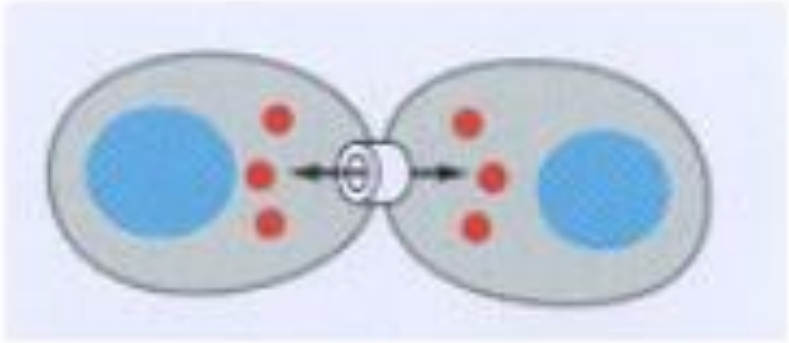


Figure 15-6 Autocrine signaling

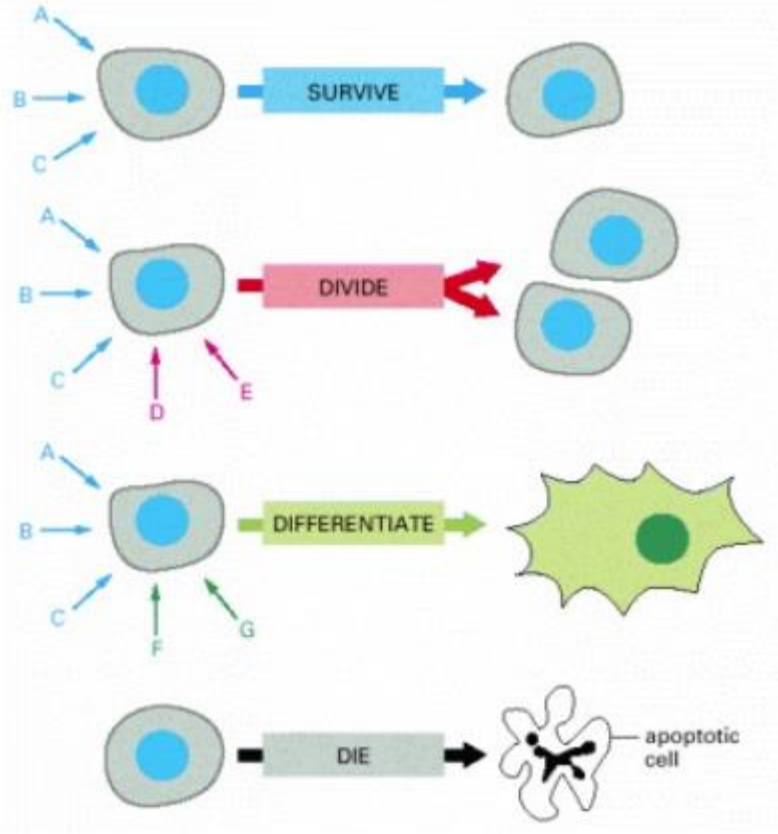
Gap Junctions Allow Signaling Information to Be Shared by Neighboring Cells

- Another way to coordinate the activities of neighboring cells is through gap junctions.
- These are specialized cell-cell junctions that can form between closely apposed plasma membranes and directly connect the cytoplasms of the joined cells via narrow water-filled channels.
- The channels allow the exchange of small intracellular signaling molecules *(intracellular mediators),* such as Ca^{2+} and cyclic AMP (discussed later), but not of macromolecules, such as proteins or nucleic acids.
- Thus, cells connected by gap junctions can communicate with each other directly, without having to surmount the barrier presented by the intervening plasma membranes.
- Like the autocrine signaling described above, gap-junction communication helps adjacent cells of a similar type to coordinate their behavior.
- It is still not known, however, which particular small molecules are important as carriers of signals through gap junctions, and the specific functions of gap-junction communication in animal development remain uncertain.



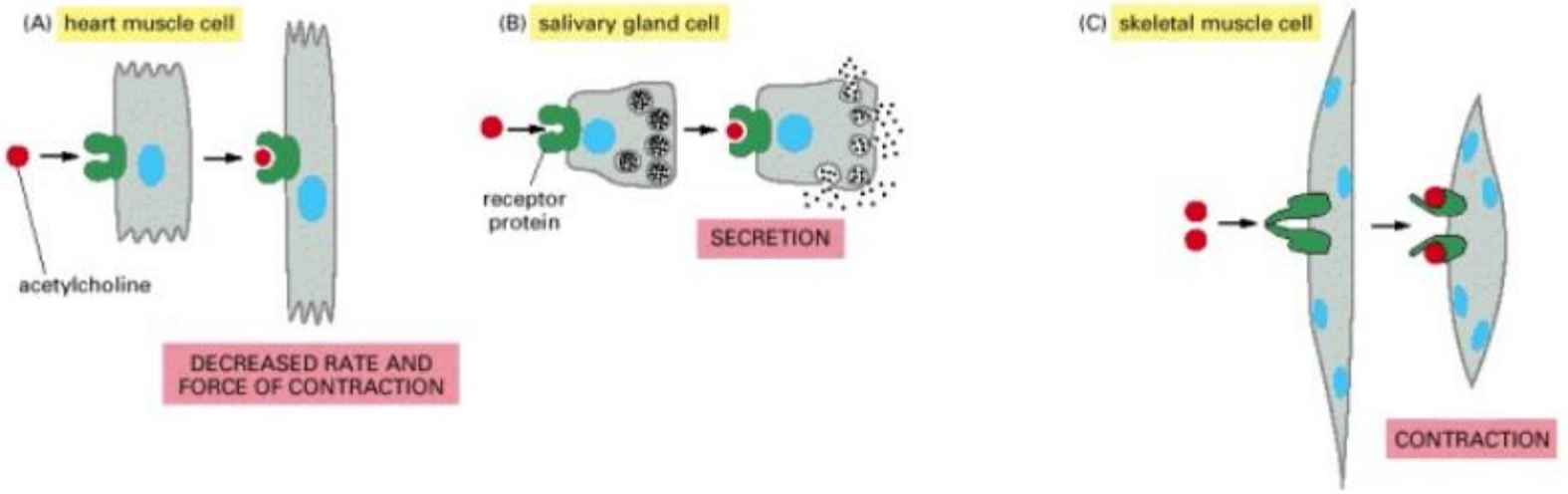
Each Cell Is Programmed to Respond to Specific Combinations of Extracellular Signal Molecules

- Each cell type displays a set of receptors that enables it to respond to a corresponding set of signal molecules produced by other cells.
- These signal molecules work in combinations to regulate the behavior of the cell.
- As shown here, an individual cell requires multiple signals to survive *(blue arrows)* and additional signals to divide *(red arrow)* or differentiate *(green arrows).*
- If deprived of appropriate survival signals, a cell will undergo a form of cell suicide known as programmed cell death, or apoptosis.



Different Cells Can Respond Differently to the Same Extracellular Signal Molecule

- The specific way in which a cell reacts to its environment varies.
- It varies according to the set of receptor proteins the cell possesses, which determines the particular subset of signals it can respond to, and it varies according to the intracellular machinery by which the cell integrates and interprets the signals it receives.
- Thus, a single signal molecule often has different effects on different target cells.
- The neurotransmitter acetylcholine, for example, stimulates the contraction of skeletal muscle cells, but it decreases the rate and force of contraction in heart muscle cells.
- This is because the acetylcholine receptor proteins on skeletal muscle cells are different from those on heart muscle cells.
- But receptor differences are not always the explanation for the different effects.
- In many cases, the same signal molecule binds to identical receptor proteins yet produces very different responses in different types of target cells, reflecting differences in the internal machinery to which the receptors are coupled.



The Stages of Cell Signaling

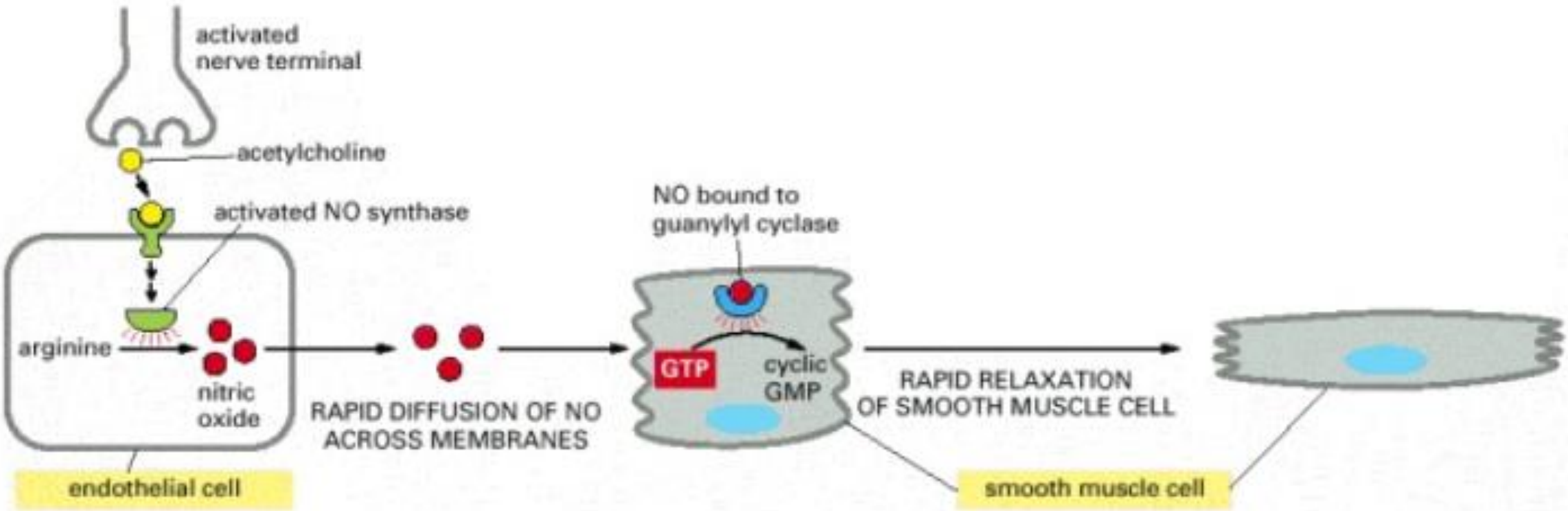
- At the most basic level, signaling happens in the following stages:
- **1. A signal is sent.** There are a number of ways this could happen. A cell could release a molecular clue, or the environmental conditions themselves could provide the molecule that is being detected.
- **2. This signal is received by the cell.** This will require a receptor to recognize the signal and respond to it. Most often the receptor is at the cell surface, since that's where signals would arrive first, but it doesn't have to be. Nitric oxide (NO) is an example of a signaling molecule that can diffuse across cell membranes, so its receptor is in the interior of the cell.
- **3. The signal is "interpreted" by the cell that receives it.** This could involve a number of things, like splitting the signal so that multiple responses are possible; it also likely involves transferring the signal across the plasma membrane by activating specific internal responses. This interpretation step is called transduction.
- **4. Finally, the cell responds to the signal.** A cell could have multiple responses to a signal. Some responses are fast, while others are slow. If the signal is used to start a process, like cell division, apoptosis, or changes in cell identity, then there could be multiple changes in the cell as it prepares for the new behavior.

1. Signaling Molecules

- Signaling events start with a signal that originates from the cell exterior.
- These signals could be something directly from the environment or something that originated from another cell.
- Some examples of signals include the following:
- **Odorants:** Each of the chemicals you taste and smell has a receptor that can bind to it and sends a slightly different signal to your brain so that you can tell the difference between them.
- Animals that are considered to have a better sense of smell will have many (thousands!) more receptors available than those that are not considered to have a good sense of smell.
- **Biological macromolecules:** There are examples of signaling molecules from all four of the biomolecules (lipid, protein, carbohydrate, nucleic acid). Insulin, which helps you regulate blood sugar, is a short peptide. Glucose itself acts as a signal for the release of insulin.
- **Neurotransmitters:** They're usually small chemicals that are synthesized in the cell for eventual release. We've seen examples of these already (acetylcholine, dopamine, epinephrine, serotonin, etc.).
- **Photons:** There are many receptors that are able to respond to light. The photoreceptors in our eyes are a great example.
- Photons are often used to help organisms regulate circadian rhythms, among other things, so that they know when it is time to sleep and when to be awake. Plants use light sensing extensively to decide when and how to grow and when to flower to maximize the change that their pollinators will be available.
- **Mechanical force and other nonchemical signals:** Your skin can respond to a variety of nontraditional signals, like physical pressure and temperature. Your ears respond to sound waves.

1. Nitric oxide gas signal

- Although most extracellular signals are hydrophilic molecules that bind to receptors on the surface of the target cell, some signal molecules are hydrophobic enough and/or small enough to pass readily across the target-cell plasma membrane.
- Once inside, they directly regulate the activity of a specific intracellular protein. These signal molecules include *steroid hormones, thyroid hormones, retinoids,* and *vitamin D.*
- An important and remarkable example is the gas nitric oxide (NO), which acts as a signal molecule in both animals and plants.
- In mammals, one of its functions is to regulate smooth muscle contraction.
- Acetylcholine, for example, is released by autonomic nerves in the walls of a blood vessel, and it causes smooth muscle cells in the vessel wall to relax.
- The acetylcholine acts indirectly by inducing the nearby endothelial cells to make and release NO, which then signals the underlying smooth muscle cells to relax.
- In plants, NO is involved in the defensive responses to injury or infection.
- NO gas is made by the deamination of the amino acid arginine, catalyzed by the enzyme NO synthase.
- Because it passes readily across membranes, dissolved NO rapidly diffuses out of the cell where it is produced and into neighboring cells.
- It acts only locally because it has a short half-life—about 5–10 seconds—in the extracellular space before it is converted to nitrates and nitrites by oxygen and water.
- In many target cells, including endothelial cells, NO binds to iron in the active site of the enzyme guanylyl cyclase, stimulating this enzyme to produce the small intracellular mediator cyclic GMP.
- Carbon monoxide (CO) is another gas that is used as an intercellular signal. It can act in the same way as NO, by stimulating guanylyl cyclase.

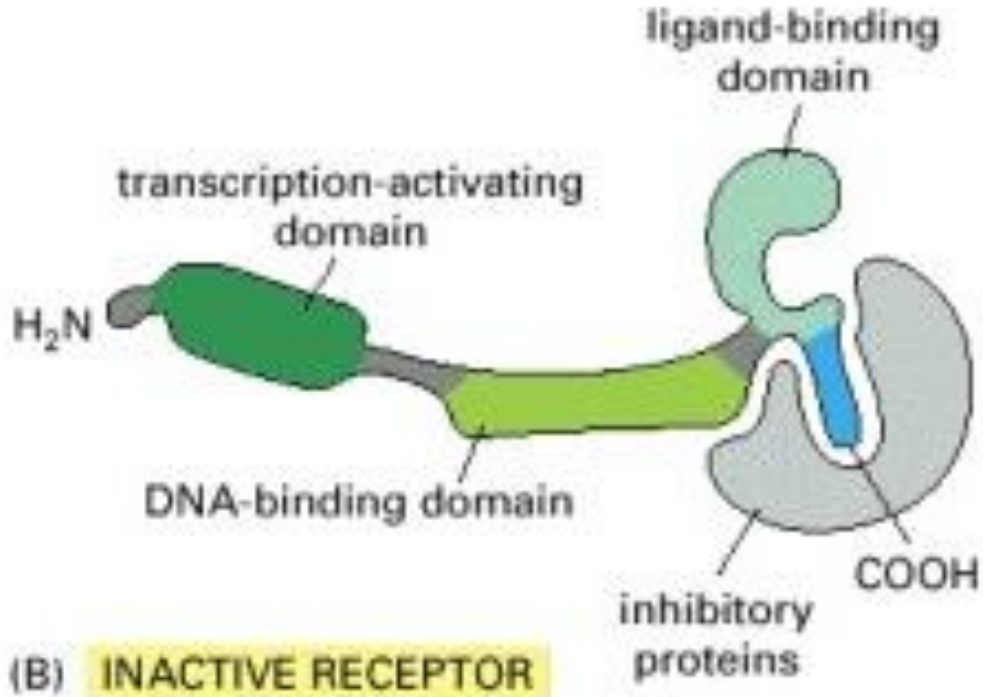

activated
nerve terminal
acetylcholine
activated NO synthase
arginine
nitric
oxide
endothelial cell
RAPID DIFFUSION OF NO
ACROSS MEMBRANES
NO bound to
guanylyl cyclase
GTP
cyclic
GMP
smooth muscle cell
RAPID RELAXATION
OF SMOOTH MUSCLE CELL

2. Receptors

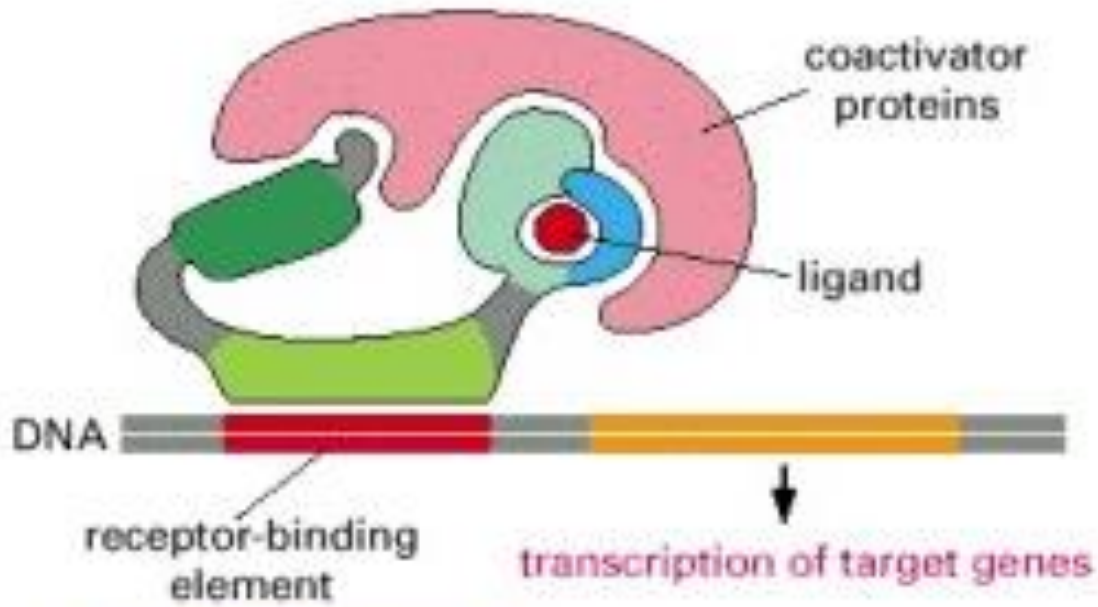
- A cell's first point of contact with the outside world is when one of its receptors binds to a ligand and a signaling pathway is activated.
- As mentioned above, since the ligand is coming from the exterior of the cell, it is quite common for the receptor to be on the cell surface, with the ligand-binding domain facing outward.
- However, if the ligand is able to diffuse across membranes, then the receptor could be somewhere inside the cell.

Nuclear Receptors Are Ligand-activated Gene Regulatory Proteins

- A number of small hydrophobic signal molecules diffuse directly across the plasma membrane of target cells and bind to intracellular receptor proteins.
- These signal molecules include *steroid hormones, thyroid hormones, retinoids,* and *vitamin D.*
- When these signal molecules bind to their receptor proteins, they activate the receptors, which bind to DNA to regulate the transcription of specific genes.
- The receptors are all structurally related, being part of the nuclear receptor superfamily.
- The intracellular receptors for the steroid and thyroid hormones, retinoids, and vitamin D all bind to specific DNA sequences adjacent to the genes the ligand regulates.
- The inactive receptors are bound to inhibitory protein complexes, and ligand binding alters the conformation of the receptor protein, causing the inhibitory complex to dissociate.
- The ligand binding also causes the receptor to bind to coactivator proteins that induce gene transcription.
- The transcriptional response usually takes place in successive steps:
  - the direct activation of a small number of specific genes occurs within about 30 minutes and constitutes the *primary response;*
  - the protein products of these genes in turn activate other genes to produce a delayed, *secondary response;* and so on.
- In this way, a simple hormonal trigger can cause a very complex change in the pattern of gene expression.



(B) INACTIVE RECEPTOR



(C) ACTIVE RECEPTOR

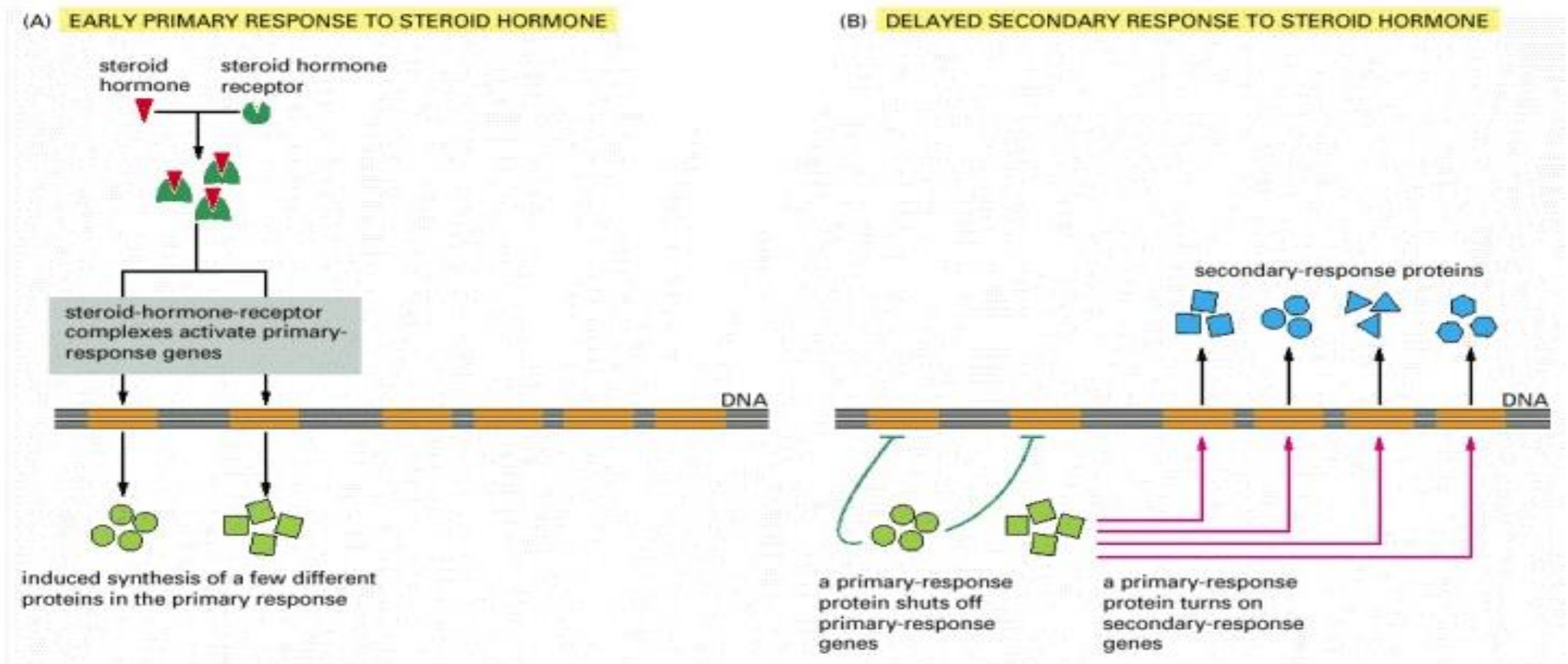


Figure 15-14 Responses induced by the activation of a nuclear hormone receptor

Cellular responses that require protein synthesis will always be slower than responses that rely on preexisting proteins, which can be simply altered.

Water-soluble signal molecules usually mediate responses of short duration, whereas water-insoluble ones tend to mediate responses that are longer lasting, depending upon the length of time they persist in the bloodstream or tissue fluids.

Cell-Surface Receptor (Signal Received)

- All water-soluble signal molecules (including neurotransmitters and all signal proteins) bind to specific receptor proteins on the surface of the target cells that they influence.
- These cell-surface receptor proteins act as *signal transducers.*
- They convert an extracellular ligand-binding event into intracellular signals that alter the behavior of the target cell.
- Most cell-surface receptor proteins belong to one of three classes, defined by the transduction mechanism they use:
  - Ion-channel-linked receptors
  - G-protein-linked receptors
  - enzyme-linked receptors

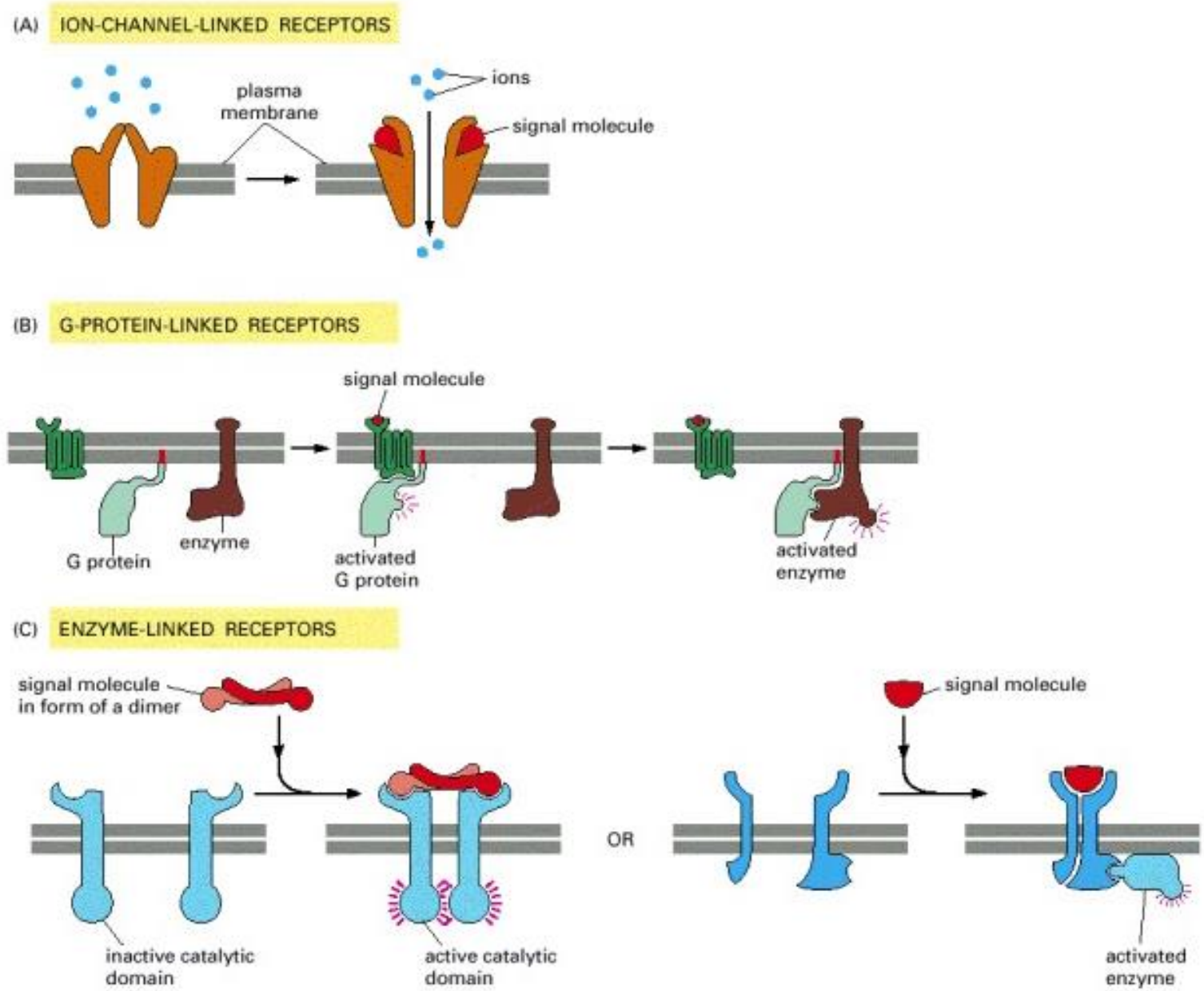


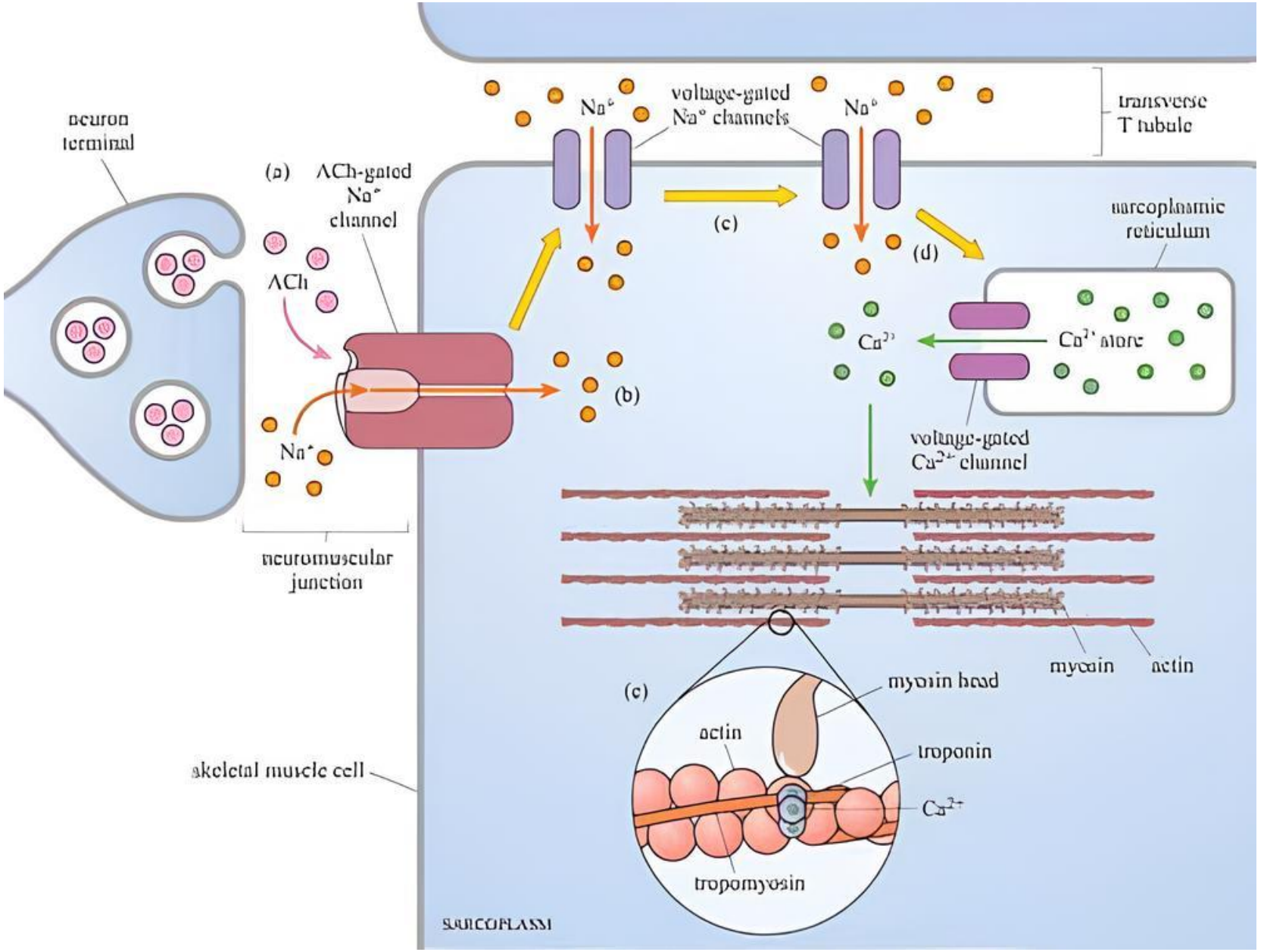
Figure 15-15 Three classes of cell-surface receptors

Ion-channel receptors

- **Ion-channel-linked receptors**, also known as *transmitter-gated ion channels* or *ionotropic receptors,* are involved in rapid synaptic signaling between electrically excitable cells.
- This type of signaling is mediated by a small number of neurotransmitters that transiently open or close an ion channel formed by the protein to which they bind, briefly changing the ion permeability of the plasma membrane and thereby the excitability of the postsynaptic cell.
- The ion-channel-linked receptors belong to a large family of homologous, multipass transmembrane proteins.
- Nicotinic cholinergic receptors are probably the best studied of all receptors.
- Nicotinic receptors are composed of five subunits (two α subunits together with one each of the β, γ and δ subunits), which assemble to form a pore in the membrane.

…Ion-channel receptors

- The pore can switch between an open and a closed state on binding of two molecules of acetylcholine to the two α subunits at sites within the channel.
- Although the channel alternates between an open and a closed state, binding of acetylcholine increases the probability of the channel being in its open state.
- When the channel is open, sodium ions flow into the muscle cell, using concentration and voltage gradients.
- The influx of positive charge due to the Na+ions inside the cell tends to locally neutralize the negative charge inside the cell (called ‘depolarization’).
- Depolarization is propagated in the muscle cell (yellow arrows) by voltage-gated Na^+ channels, which allow further Na^+ ion entry.
- This more general depolarization triggers the very rapid release of Ca^{2+}ions into the sarcoplasm (muscle cytoplasm) through voltage-gated Ca^{2+} channels from stores in the sarcoplasmic reticulum; the Ca^{2+} ions spread through the muscle cell.
- The increase of Ca^{2+} concentration throughout the sarcoplasm enables the rapid and synchronous contraction of the muscle filaments.
- Ca^{2+} achieves this by binding to an inhibitory protein complex of tropomyosin and troponin, which under resting conditions prevents actin and myosin filaments from interacting.

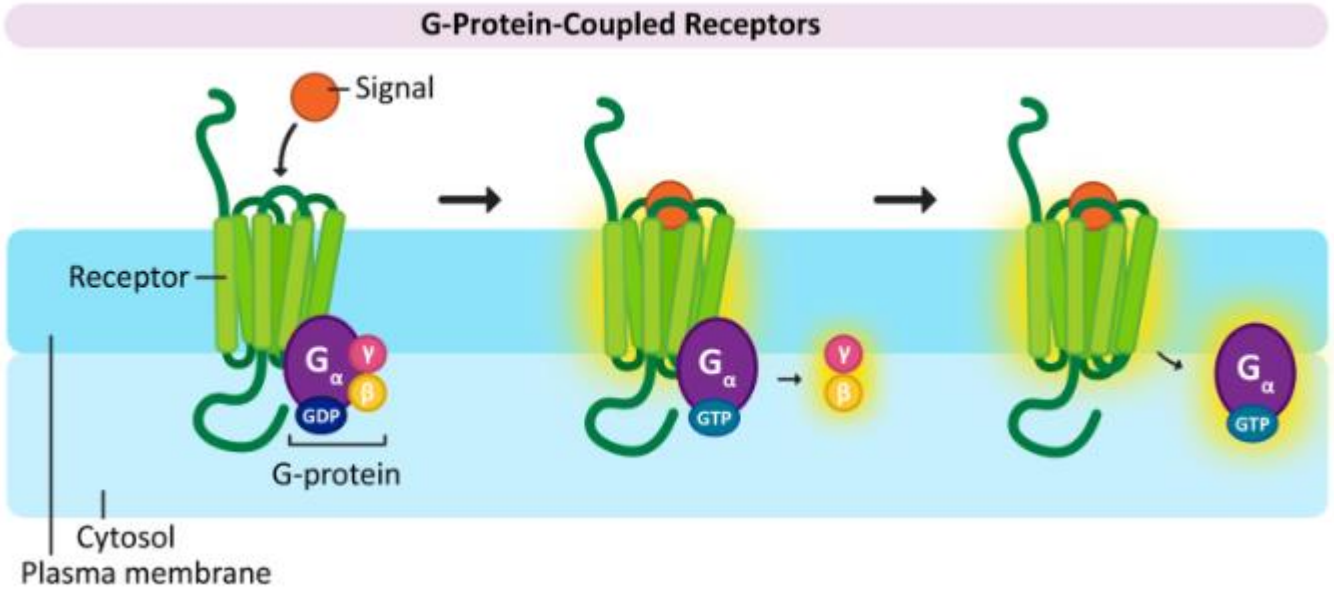

neuron terminal
(a) ACh-gated Na+ channel
ACh
Na+
neuromuscular junction
skeletal muscle cell
Na+
voltage-gated Na+ channels
(b)
(c)
Na+
transverse T tubule
(d)
sarcoplasmic reticulum
Ca2+
Ca2+ store
voltage-gated Ca2+ channel
myosin
actin
(e)
myosin head
actin
troponin
Ca2+
tropomyosin
SARCOPLASM

G-protein-linked receptors

- *G-protein-linked receptors* act indirectly to regulate the activity of a separate plasma-membrane-bound target protein, which can be either an enzyme or an ion channel.
- The interaction between the receptor and this target protein is mediated by a third protein, called a *trimeric GTP-binding protein (G protein)* (Figure 15-15B). The activation of the target protein can change the concentration of one or more intracellular mediators (if the target protein is an enzyme), or it can change the ion permeability of the plasma membrane (if the target protein is an ion channel).
- The intracellular mediators affected act in turn to alter the behavior of yet other signaling proteins in the cell.
- All of the G-protein-linked receptors belong to a large family of homologous, seven-pass transmembrane proteins.

Despite variation in the G-protein family, they also have many similarities:

- Each G-protein complex contains three subunits: the alpha (α), beta (β), and gamma (γ) subunits.
- The three subunits can all bind together in a trimeric complex, or G-alpha can separate from the other two. G-beta and G-gamma almost always stay together as a unit.
- The alpha-subunit is able to bind to GDP or GTP. Both the alpha- and the gamma-subunits have covalently linked lipid tails, which allow them to remain associated with the membrane.
- The beta unit does not have a lipid tail, but since it remains attached to the gamma-subunit, it also remains attached to the cell membrane.



The activation of G-proteins. When a signal molecule binds, this activates the G-protein-coupled receptor. The activated receptor swaps out GDP for GTP on Gα. When bound to GTP, the Gα separates from Gβγ. These two molecules stimulate further downstream signaling.

How GPCR activation works?

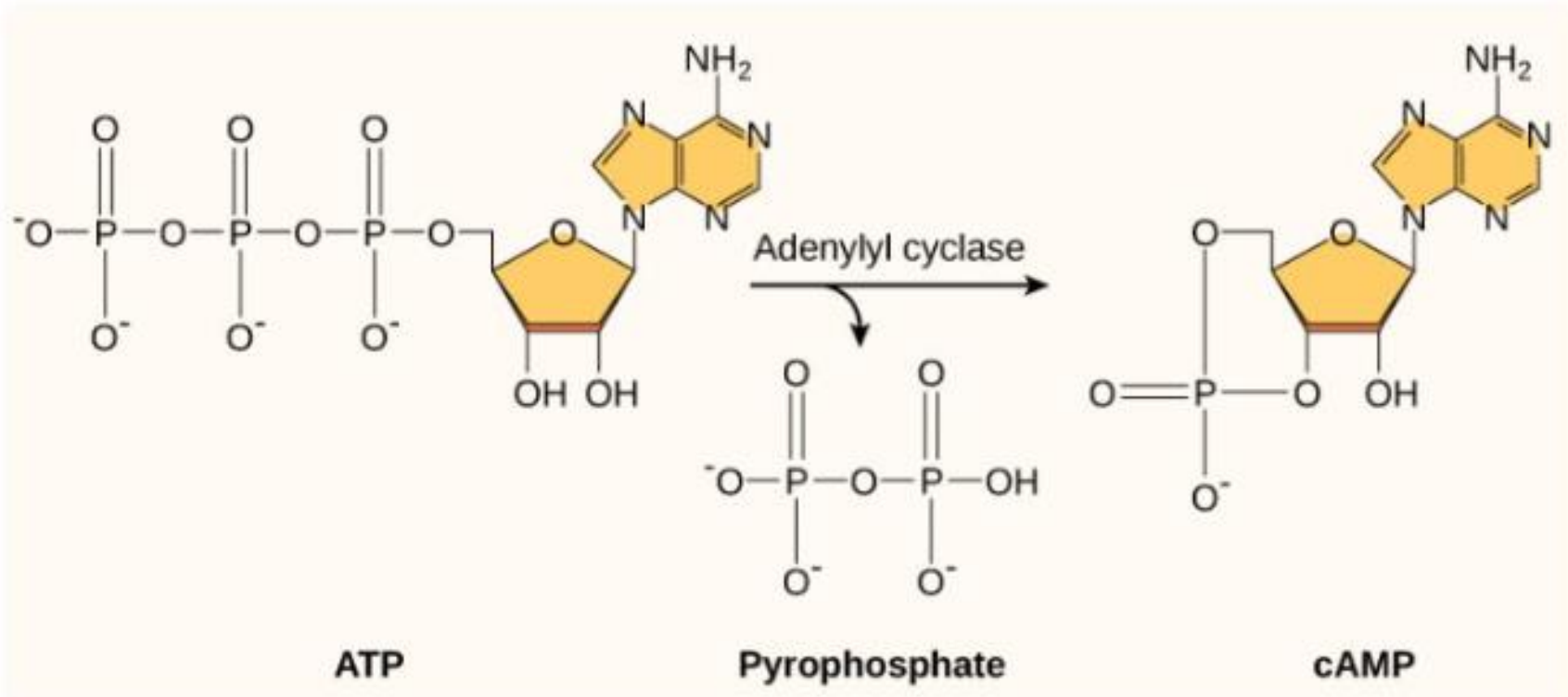
- When the receptor binds to its ligand, it undergoes a conformation change and binds to the α/β/γ complex. This causes the GDP to be released from the α-subunit and get replaced by GTP.
- The binding of the GTP molecule causes a conformation change in the G-protein complex, and the β/γ subunits are released from Gα.
- The separated G-protein subunits (Gα and Gβγ) are now considered active and activate their appropriate downstream targets.

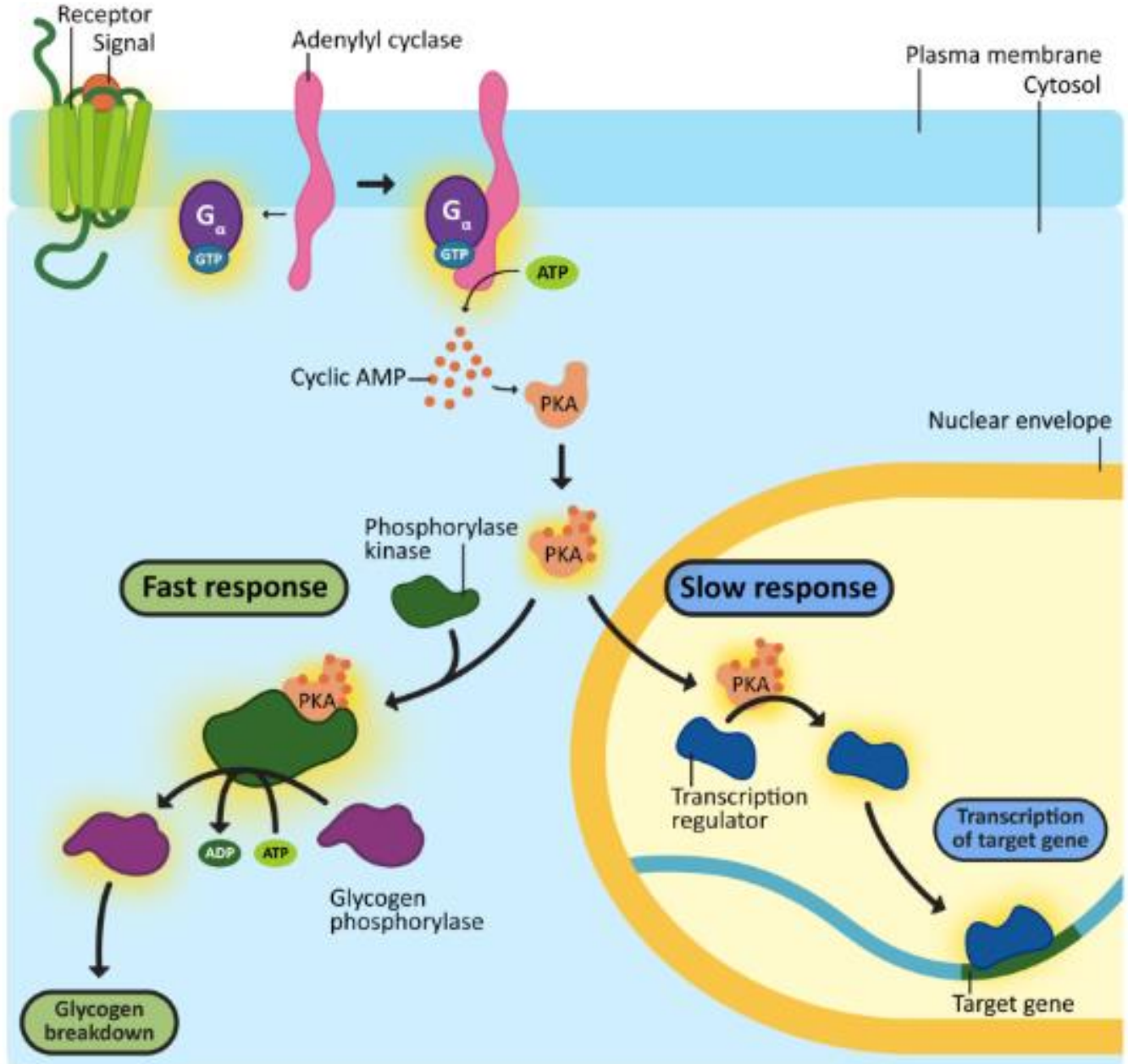
- It's worthwhile to note that as the G-proteins are activated and move away from the receptor, the lipid tails keep them associated with the interior surface of the plasma membrane.
- Eventually the G-proteins will deactivate themselves. The Gα subunit will slowly hydrolyze the GTP so that it is converted back into GDP (and a phosphate molecule, which is released). This inactivates the Gα subunit, which causes all three G-proteins to return to their inactive state.

- Because the activation of the G-proteins is dependent on the presence of GTP in the Gα subunit, this provides a built-in system for shutting down the response.
- The GTP is short lived, so the response lasts only as long as the GTP does.
- The two most common second messengers that are activated by G-proteins: cyclic AMP and IP_3/DAG.

…*G-protein-linked receptors*

- Cyclic AMP is produced when a plasma membrane–bound enzyme, adenylyl cyclase, is activated by the Gα subunit of the activated G-protein.
- Cyclic AMP is then capable of moving throughout the cell and interacting with any enzymes that have binding sites for it.
- cAMP is a short-lived molecule—its half-life in the cell is less than 1.5 minutes.
- An enzyme known as phosphodiesterase breaks it down so that the cellular response is kept short.
- One of the most important enzymes activated by cAMP is a cytosolic protein called protein kinase A (PKA).
- When activated, PKA can have a number of different effects.
- One of which is a “fast” response, illustrated by the breakdown of glycogen in the liver.
- The other is a “slow” response—PKA can also enter the nucleus and phosphorylate transcription factors, which will activate these transcription factors, resulting in gene expression.


NH2
Adenylyl cyclase
ATP
Pyrophosphate
cAMP


Receptor
Signal
Adenylyl cyclase
Plasma membrane
Cytosol
G_α
GTP
ATP
Cyclic AMP
PKA
Nuclear envelope
Phosphorylase kinase
Fast response
Slow response
ADP
ATP
Glycogen phosphorylase
Transcription regulator
Transcription of target gene
Glycogen breakdown
Target gene

Enzyme-linked receptors

- *Enzyme-linked receptors,* when activated, either function directly as enzymes or are directly associated with enzymes that they activate.
- They are formed by single-pass transmembrane proteins that have their ligand-binding site outside the cell and their catalytic or enzyme-binding site inside.
- Enzyme-linked receptors are heterogeneous in structure compared with the other two classes.
- The great majority, however, are protein kinases, or are associated with protein kinases, and ligand binding to them causes the phosphorylation of specific sets of proteins in the target cell.
- Six classes of enzyme-linked receptors have thus far been identified:
- 1. **Receptor tyrosine kinases** phosphorylate specific tyrosines on a small set of intracellular signaling proteins.
- 2. **Tyrosine-kinase-associated receptors** associate with intracellular proteins that have tyrosine kinase activity.
- 3. **Receptor like tyrosine phosphatases** remove phosphate groups from tyrosines of specific intracellular signaling proteins. (They are called “receptorlike” because the presumptive ligands have not yet been identified, and so their receptor function has not been directly demonstrated.)
- 4. **Receptor serine/threonine kinase**s phosphorylate specific serines or threonines on associated latent gene regulatory proteins.
- 5. **Receptor guanylyl cyclases** directly catalyze the production of cyclic GMP in the cytosol.
- 6. **Histidine-kinase-associated receptors** activate a “two-component” signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphate to a second intracellular signaling protein.

Receptor tyrosine kinases

- There are 58 RTKs known to exist within the human genome and they respond to different extracellular signals.
- However, all RTKs possess common structural domains: an extracellular ligand binding domain, a transmembrane helix, a juxtamembrane region, and a tyrosine kinase (TK) domain.
- RTKs are integral proteins that extend through the plasma membrane (via the transmembrane domain which is a single helix).
- The N-terminal region of the receptor is located in the extracellular region, while the C-terminal portion is situated in the cytoplasm.
- The N-terminal region of the RTK contains the extracellular ligand binding site, which is the location of where an external signal will engage with the protein.
- This extracellular region is highly variable between different RTKs, and may include Cys-rich regions, Leu-rich segments, or immunoglobulin-like motifs. This helps maintain diversity for recognition of different external biochemical signals.
- The C-terminal region exists within the interior of the cell (the cytosol) and includes the conserved tyrosine kinase domain and juxtamembrane region.
- This kinase domain carries out the phosphorylation activity of the RTK whereas the juxtamembrane is highly flexible and important for regulatory roles, often leading to auto-inhibition via contacts with kinase domain.
- In all cases, the binding of a signal protein to the ligand-binding domain on the outside of the cell activates the intracellular tyrosine kinase domain.
- Once activated, the kinase domain transfers a phosphate group from ATP to selected tyrosine side chains, both on the receptor proteins themselves and on intracellular signaling proteins that subsequently bind to the phosphorylated receptors.

Some Signaling Proteins That Act Via Receptor Tyrosine Kinases

SIGNALING LIGAND	RECEPTORS	SOME RESPONSES
Epidermal growth factor (EGF)	EGF receptor	stimulates proliferation of various cell types
Insulin	insulin receptor	stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factors (IGF-1 and IGF-2)	IGF receptor-1	stimulate cell growth and survival
Nerve growth factor (NGF)	Trk A	stimulates survival and growth of some neurons
Platelet-derived growth factors (PDGF AA, BB, AB)	PDGF receptors (α and β)	stimulate survival, growth, and proliferation of various cell types
Macrophage-colony-stimulating (M-CSF)	M-CSF receptor factor	stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factors (FGF-1 to FGF-24)	FGF receptors (FGF-R1-FGF- R4, plus multiple isoforms of each)	stimulate proliferation of various cell types; inhibit differentiation of some precursor cells; inductive signals in development
Vascular endothelial growth factor (VEGF)	VEGF receptor	stimulates angiogenesis
Ephrins (A and B types)	Eph receptors (A and B types)	stimulate angiogenesis; guide cell and axon migration

- Ligand binding induced rearrangement in cytosolic tails of the receptors initiates the intracellular signaling process.
- For receptor tyrosine kinases, the rearrangement enables the neighboring kinase domains of the receptor chains to cross-phosphorylate each other on multiple tyrosines, a process referred to as **autophosphorylation**.
- To activate a receptor tyrosine kinase the ligand usually has to bind simultaneously to two adjacent receptor chains.
- PDGF, for example, is a dimer, which cross-links two receptors together (Figure 15-50A).
- Even some monomeric ligands, such as EGF, bind to two receptors simultaneously and cross-link them directly.
- By contrast, FGFs, which are also monomers, first form multimers by binding to heparan sulfate proteoglycans, either on the target cell surface or in the extracellular matrix.
- In this way, they are able to cross-link adjacent receptors (Figure 15-50B).
- In contact-dependent signaling, the ligands form clusters in the plasma membrane of the signaling cell and can thereby cross-link the receptors on the target cell (Figure15-50C); thus, whereas membrane-bound ephrins activate Eph receptors, soluble ephrins will do so only if they are aggregated.

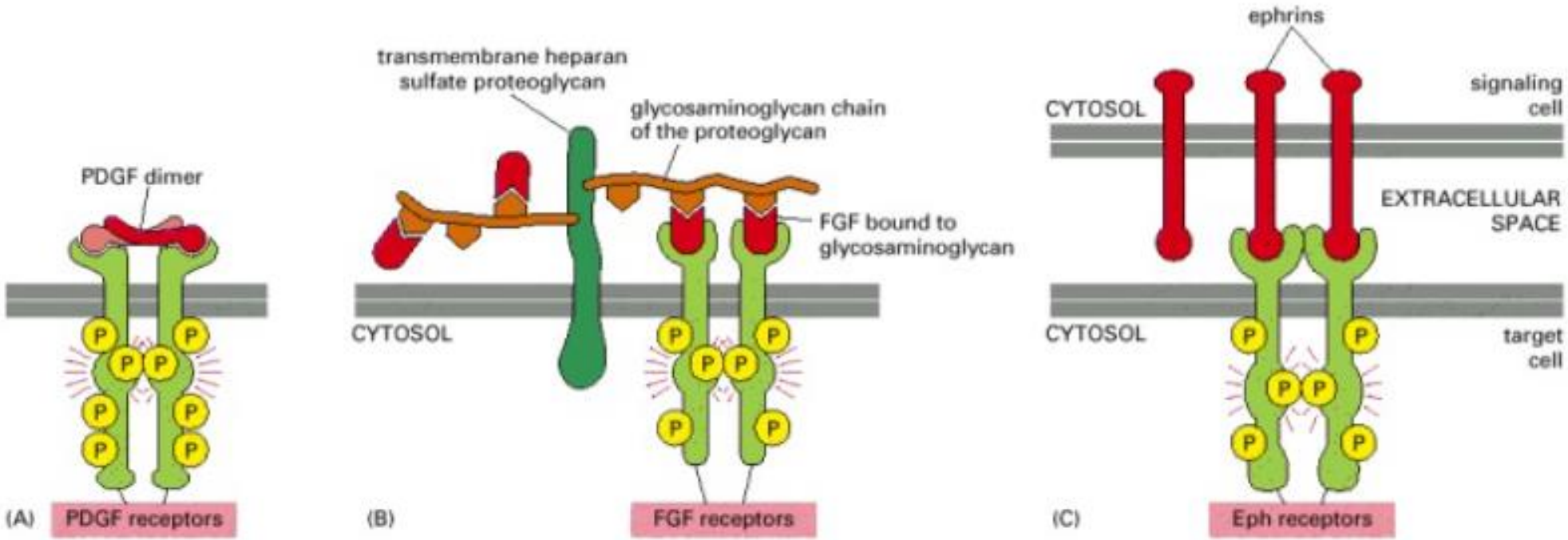
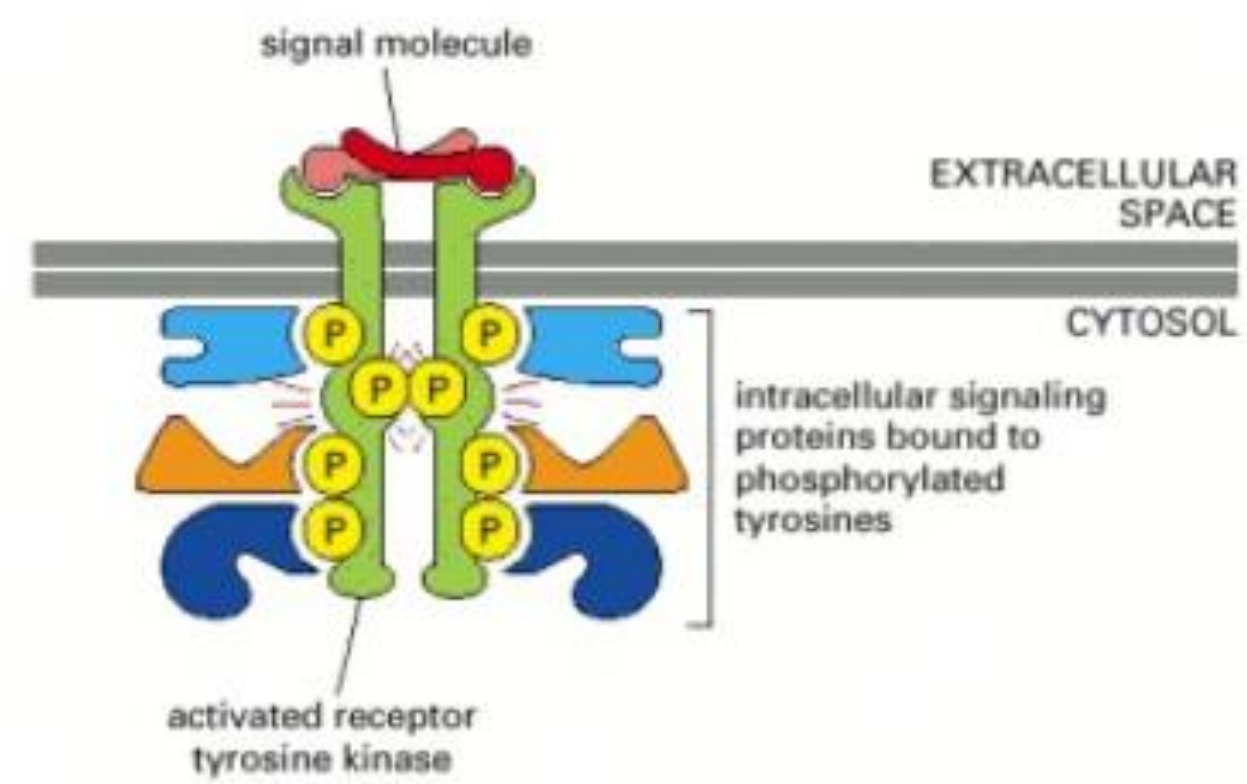


Figure 15-50 Three ways in which signaling proteins can cross-link receptor chains

- Autophosphorylation of the cytosolic tail of receptor tyrosine kinases contributes to the activation process in two ways.
- First, phosphorylation of tyrosines within the kinase domain increases the kinase activity of the enzyme.
- Second, phosphorylation of tyrosines outside the kinase domain creates high-affinity docking sites for the binding of a number of intracellular signaling proteins in the target cell.
- Once bound to the activated kinase, the signaling protein may itself become phosphorylated on tyrosines and thereby activated; alternatively, the binding alone may be sufficient to activate the docked signaling protein.
- In summary, autophosphorylation serves as a switch to trigger the transient assembly of a large intracellular signaling complex, which then broadcasts signals along multiple routes to many destinations in the cell.
- Because different receptor tyrosine kinases bind different combinations of these signaling proteins, they activate different responses.
- A whole menagerie of intracellular signaling proteins can bind to the phosphotyrosines on activated receptor tyrosine kinases to help to relay the signal onward.
- In the case of RTKs, the first commonly activated molecule after receptor activation is a protein called Ras.

The docking of intracellular signaling proteins on an activated receptor tyrosine kinase



Tyrosine-Kinase-Associated Receptors

- These receptors **lack intrinsic kinase activity** but are **associated with cytoplasmic tyrosine kinases** such as **Janus kinases (JAKs)** or **Src family kinases**.
- **Mechanism:**
- Ligand binding induces receptor dimerization.
- The associated kinase (e.g., JAK) becomes activated and phosphorylates both the receptor and intracellular signaling proteins.
- This leads to activation of downstream signaling cascades such as the **JAK-STAT pathway**.

Receptor-Like Tyrosine Phosphatases (RPTPs)

- These receptors **remove phosphate groups** from tyrosine residues on specific intracellular signaling proteins.
- **Mechanism:**
- Unlike kinases that add phosphate groups, RPTPs **dephosphorylate** them, acting as **negative regulators** of signaling.
- The **ligands for many RPTPs remain unidentified**, so their receptor functions are not fully confirmed—hence the term *"receptor-like."*
- **Examples:**
- CD45 (important in T-cell and B-cell receptor signaling).
- Maintains balance in phosphorylation states and regulates immune signaling and neuronal development.

Receptor Serine/Threonine Kinases

- These receptors phosphorylate **serine or threonine residues** on target proteins instead of tyrosine.
- Ligand binding (e.g., transforming growth factor-β or TGF-β) activates receptor complexes.
- The **type II receptor** phosphorylates and activates the **type I receptor**, which then phosphorylates **latent gene regulatory proteins (Smads)**.
- The phosphorylated Smads form complexes that translocate to the nucleus to regulate gene expression.
- **Examples:** TGF-β receptor, Bone Morphogenetic Protein (BMP) receptor
- Regulates embryonic development, cell differentiation, and tissue repair.

Receptor Guanylyl Cyclases

- These receptors directly **catalyze the conversion of GTP to cyclic GMP (cGMP)** in the cytosol.
- Ligand binding (e.g., atrial natriuretic peptide, ANP) activates the guanylyl cyclase domain.
- Increased cGMP acts as a **second messenger**, activating **protein kinase G (PKG)** and regulating ion channels.
- **Examples:** Retinal photoreceptor guanylyl cyclases
- Regulates vascular tone, kidney function, and phototransduction.

Class	Catalytic Activity	Target Residue	Key Examples	Primary Function
1. Receptor Tyrosine Kinase	Intrinsic tyrosine kinase	Tyrosine	EGFR, Insulin receptor	Cell growth, metabolism
2. Tyrosine-Kinase-Associated	Associated cytoplasmic kinase	Tyrosine	JAK-STAT receptors	Immune regulation
3. Receptor-like Tyrosine Phosphatase	Intrinsic phosphatase	Tyrosine	CD45	Signal deactivation
4. Receptor Serine/Threonine Kinase	Intrinsic kinase	Serine/Threonine	TGF-β receptor	Development, differentiation
5. Receptor Guanylyl Cyclase	Intrinsic cyclase	—	ANP receptor	cGMP signaling
6. Histidine-Kinase-Associated	Histidine kinase	Histidine/Aspartate	Bacterial sensors	Environmental response

Histidine-Kinase-Associated Receptors

- These receptors operate through a **two-component signaling system**.
- Commonly found in **bacteria, fungi, and plants**, and rarely in animals.
- **Mechanism:**
- Ligand binding activates a **histidine kinase**, which **autophosphorylates** a specific histidine residue.
- The phosphate group is then **transferred to an aspartate residue** on a response regulator protein.
- The activated response regulator then mediates cellular responses, often by regulating gene expression.
- **Examples:** Bacterial chemotaxis receptors, Plant ethylene receptors
- Enables cells to adapt to environmental changes (e.g., nutrient levels, osmotic pressure).

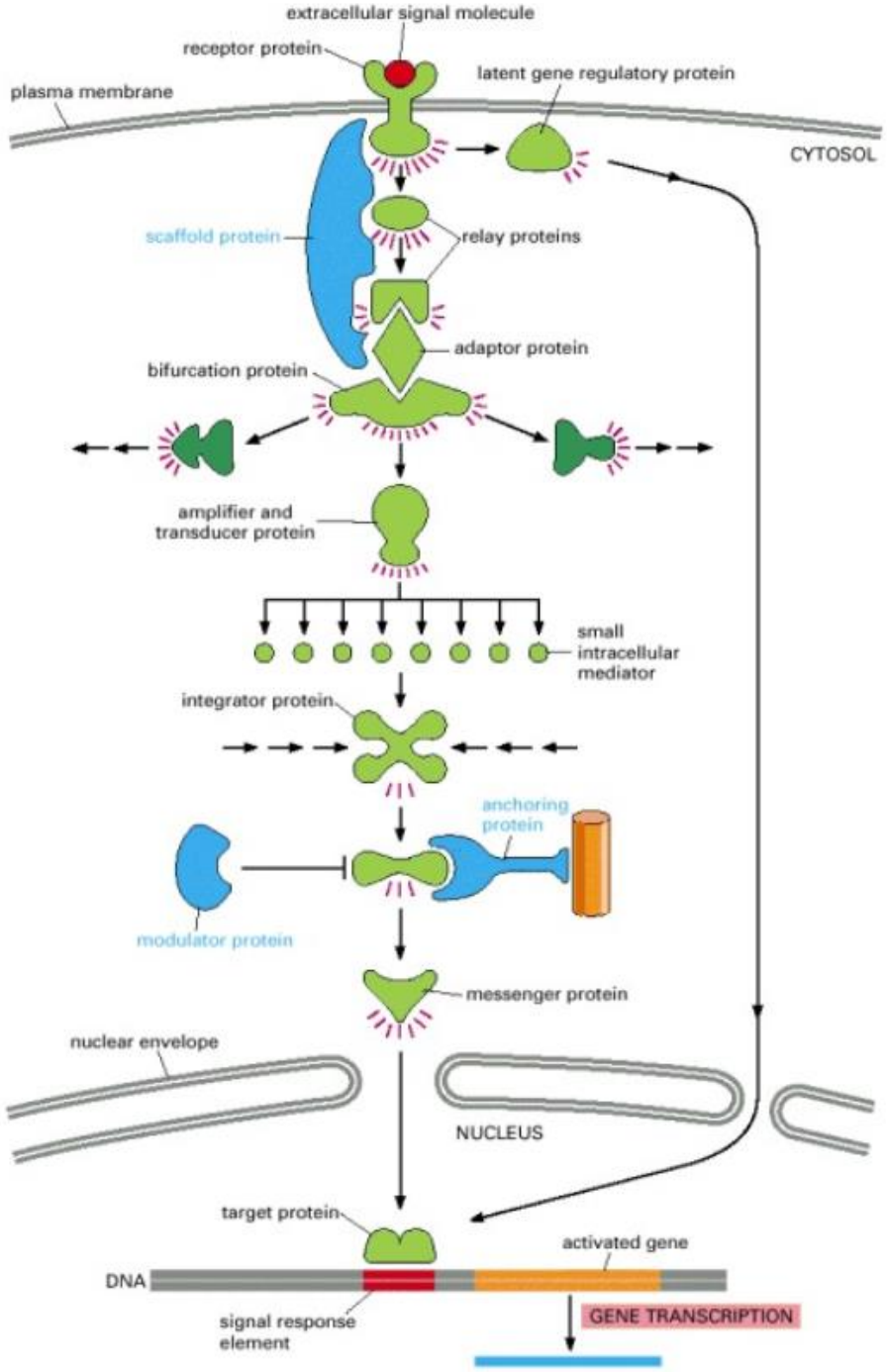
3. Most Activated Cell-Surface Receptors Relay Signals Via Small Molecules and a Network of Intracellular Signaling Proteins (*Signal interpretation*)

- Signals received at the surface of a cell by either G-protein-linked or enzyme-linked receptors are relayed into the cell interior by a combination of small and large intracellular signaling molecules.
- The resulting chain of intracellular signaling events ultimately alters target proteins, and these altered target proteins are responsible for modifying the behavior of the cell.
- The small intracellular signaling molecules are called **small intracellular mediators, or second messengers** (the "first messengers" being the extracellular signals).
- They are generated in large numbers in response to receptor activation and rapidly diffuse away from their source, broadcasting the signal to other parts of the cell.
- Some, such as cyclic AMP and Ca^{2+} , are water-soluble and diffuse in the cytosol, while others, such as diacylglycerol, are lipid-soluble and diffuse in the plane of the plasma membrane.
- In either case, they pass the signal on by binding to and altering the behavior of selected signaling proteins or target proteins.
- The **large intracellular signaling molecules** are intracellular signaling proteins.
- Many of these relay the signal into the cell by either activating the next signaling protein in the chain or generating small intracellular mediators.
- These proteins can be classified according to their particular function, although many fall into more than one category:

large intracellular signaling molecules

- 1. Relay proteins simply pass the message to the next signaling component in the chain.
- 2. Messenger proteins carry the signal from one part of the cell to another, such as from the cytosol to the nucleus.
- 3. Adaptor proteins link one signaling protein to another, without themselves conveying a signal.
- 4. Amplifier proteins, which are usually either enzymes or ion channels, greatly increase the signal they receive, either by producing large amounts of small intracellular mediators or by activating large numbers of downstream intracellular signaling proteins. When there are multiple amplification steps in a relay chain, the chain is often referred to as a signaling cascade.
- 5. Transducer proteins convert the signal into a different form. The enzyme that makes cyclic AMP is an example: it both converts the signal and amplifies it, thus acting as both a transducer and an amplifier.
- 6. Bifurcation proteins spread the signal from one signaling pathway to another.
- 7. Integrator proteins receive signals from two or more signaling pathways and integrate them before relaying a signal onward.
- 8. Latent gene regulatory proteins are activated at the cell surface by activated receptors and then migrate to the nucleus to stimulate gene transcription.
- 9. Modulator proteins modify the activity of intracellular signaling proteins and thereby regulate the strength of signaling along the pathway.
- 10. Anchoring proteins maintain specific signaling proteins at a precise location in the cell by tethering them to a membrane or the cytoskeleton.
- 11. Scaffold proteins are adaptor and/or anchoring proteins that bind multiple signaling proteins together in a functional complex and often hold them at a specific location.

Different kinds of intracellular signaling proteins along a signaling pathway from a cell-surface receptor to the nucleus



4. The cell response to the signal

- The cellular responses at the end of the signaling cascade usually fall into two categories:
  - fast responses and
  - slow responses.
- ***Cellular responses that require protein synthesis will always be slower than responses that rely on preexisting proteins, which can be simply altered.***

Slow Responses Come from Changes in Gene Expression

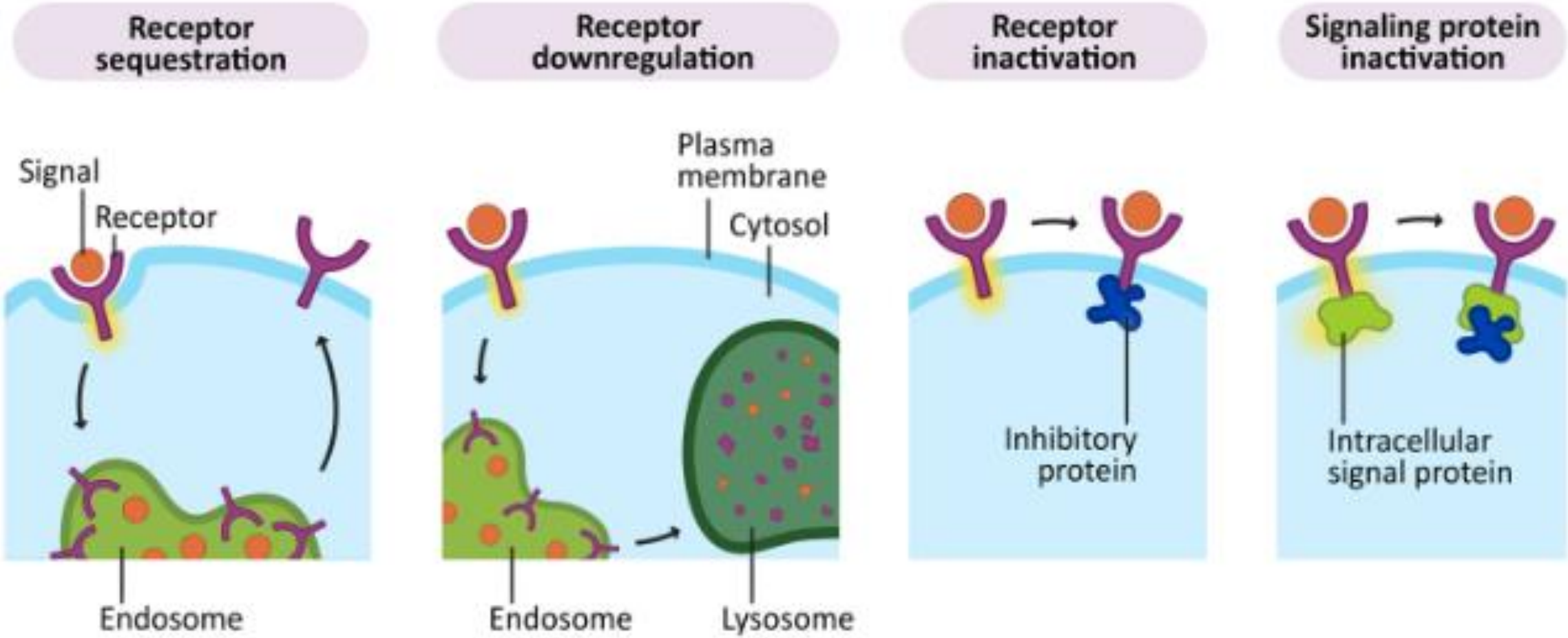
- Often, a signaling event will result in an overall change in cellular identity.
- Changing the identity of a cell will require a number of changes in gene expression: some proteins will need to be synthesized, while others will likely need to be removed from the cell.
- For example, most of the signaling events involved in development will cause changes in gene expression, as cell differentiation is a key step in embryonic development.
- As a secondary example, the immune system also uses alterations in gene expression so that antibodies can be produced in response to a pathogen or vaccine.
- In order to change gene expression, signaling cascades cause the activation or deactivation of transcription factors or proteins that affect chromatin structure (i.e., histone or chromatin-modifying enzymes).
- Proteins that influence histones and/or chromatin will impact the accessibility of the DNA, thus impacting the expression of genes in that area.
- The end result will be changes in the protein composition in the affected cell.

Fast Responses Come from Changes in Preexisting Proteins

- Extracellular signals can stimulate signaling cascades that result in shorter-term changes in cellular function by altering preexisting proteins.
- A very good example is mitosis.
- When mitosis is activated, the cell is vulnerable, so this process must be completed quickly.
- Most of the proteins involved in rearranging the cytosol for mitosis already exist before mitosis starts.
- They are present in the cytosol, waiting for the signal to initiate cell division.
- Once the signal is perceived, these preexisting proteins are modified as a result of the signaling cascade.
- This causes a rapid shift in cellular physiology, and the cells progress through mitosis as efficiently as possible.

Deactivation of a Signaling Cascade

- In some cases, this is the end of the story, as the signal never needs to be turned off. An example apoptosis
- On the other hand, a great many signaling events need to stop eventually so that the cell can go back to “normal.” Mitosis is a good example.
- Common mechanisms to deactivate signaling cascades:
- In receptor sequestration, the receptor is brought from the membrane into an internal compartment (usually an endosome) where it can’t interact with the signal.
- Downregulation of a receptor means that it ends up in the lysosome and is thus destroyed.
- Alternatively, the receptor can be inactivated, usually through the binding of an inhibitory protein, so that the signal can no longer be relayed.
- Finally, it may not be the receptor itself that is inhibited but a downstream protein, which has more or less the same effect of stopping the signaling cascade.


Receptor sequestration
Receptor downregulation
Receptor inactivation
Signaling protein inactivation
Signal
Receptor
Endosome
Plasma membrane
Cytosol
Endosome
Lysosome
Inhibitory protein
Intracellular signal protein