General Principles of Cell Signaling

Chapter 15, Alberts et al.
A simple intracellular signaling pathway activated by an extracellular signal molecule
Budding yeast cells responding to mating factor
The binding of extracellular signal molecules to either cell-surface receptors or intracellular receptors.
Forms of intercellular signaling

(A) CONTACT-DEPENDENT
- Signaling cell
- Target cell
- Membrane-bound signal molecule

(B) PARACRINE
- Signaling cell
- Target cells
- Local mediator

(C) SYNAPTIC
- Neuron
- Synapse
- Neurotransmitter

(D) ENDOCRINE
- Endocrine cell
- Hormone
- Receptor
- Target cell
The contrast between endocrine and synaptic signaling
Autocrine signaling

A SINGLE SIGNALING CELL RECEIVES A WEAK AUTOCRINE SIGNAL

IN A GROUP OF IDENTICAL SIGNALING CELLS, EACH CELL RECEIVES A STRONG AUTOCRINE SIGNAL
Signaling via gap junctions
An animal cell’s dependence on multiple extracellular signals
Various responses induced by the neurotransmitter acetylcholine
The role of nitric oxide (NO) in smooth muscle relaxation in a blood vessel wall
Some signaling molecules that bind to nuclear receptors

- cortisol
- estradiol
- testosterone
- thyroxine
- vitamin D₃
- retinoic acid
The nuclear receptor superfamily

[Diagram showing the structure of nuclear receptors and their interactions with DNA and ligands.]
Responses induced by the activation of a nuclear hormone receptor

(A) EARLY PRIMARY RESPONSE TO STEROID HORMONE

steroid hormone steroid hormone receptor

steroid-hormone-receptor complexes activate primary-response genes

induced synthesis of a few different proteins in the primary response

(B) DELAYED SECONDARY RESPONSE TO STEROID HORMONE

DNA

secondary-response proteins

a primary-response protein turns on secondary-response genes

a primary-response protein shuts off primary-response genes
Three classes of cell-surface receptors

(A) ION-CHANNEL-LINKED RECEPTORS

(B) G-PROTEIN-LINKED RECEPTORS

(C) ENZYME-LINKED RECEPTORS

signal molecule in form of a dimer

inactive catalytic domain

active catalytic domain

activated enzyme
Different kinds of intracellular signaling proteins along a signaling pathway from a cell-surface receptor to the nucleus
Two types of intracellular signaling proteins that act as molecular switches
Signal integration
Two types of intracellular complexes
A hypothetical signaling pathway using modular binding domains

<table>
<thead>
<tr>
<th>BINDING DOMAIN</th>
<th>MOTIF RECOGNIZED</th>
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<tbody>
<tr>
<td>PH = Pleckstrin homology domain</td>
<td>$\mathbf{P}$ = phosphorylated inositol phospholipid</td>
</tr>
<tr>
<td>PTB = phosphotyrosine-binding domain</td>
<td>$\mathbf{Y}$ = phosphotyrosine</td>
</tr>
<tr>
<td>SH2 = Src homology 2 domain</td>
<td>$\mathbf{P}$ = phosphorylated protein</td>
</tr>
<tr>
<td>SH3 = Src homology 3 domain</td>
<td>$\mathbf{PPP}$ = proline-rich motif</td>
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Primary response of chick oviduct cells to the steroid sex hormone estradiol
Activation curves as a function of signal-molecule concentration
One type of signaling mechanism expected to show a step threshold-like response.
An accelerating positive feedback mechanisms
Five ways in which target cells can become desensitized to a signal molecule.
A G-protein-linked receptor
The structure of an inactive G protein
The disassembly of an activated G-protein into two signaling components
The switching off of the G-protein alpha subunit by the hydrolysis of its bound GTP
An increase in cyclic AMP in response to an extracellular signal
The synthesis and degradation of cAMP
The activation of cAMP-dependent protein kinase A
How gene transcription is activated by a rise in cAMP concentration
Three types of inositol phospholipids

- **Phosphatidylinositol (PI)**
- **PI 4-phosphate ([PI(4)P])**
- **PI 4,5-bisphosphate ([PI(4,5)P2])**
Some G proteins activate the inositol phospholipid signaling pathway by activating phospholipase C-beta.
The two branches of the inositol phospholipid pathway
Fertilization of an egg by a sperm triggering an increase in cytosolic Ca
The main ways eucaryotic cells maintain a very low concentration of free Ca
Seven subfamilies of receptor tyrosine kinases
Activated receptor tyrosine kinases phosphorylate themselves
Three ways in which signaling proteins can cross-link receptor chains

(A) PDGF receptors

(B) FGF receptors

(C) Eph receptors
Inhibition of signaling through normal receptor tyrosine kinases by an excess of mutant receptors
The docking of intracellular signaling proteins on an activated receptor tyrosine kinase
• 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 ions and small molecules across a membrane. It binds the molecules on one side of the membrane, and releases them on the other side.

• **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 $\alpha$ subunit is released from the $\beta\gamma$ dimer. Either the $\alpha$ 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.
Carriers and channels form water soluble paths through the membrane

- 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 difference in the concentration of ions across a cell membrane produces an **electrochemical gradient**. The term indicates that there is a difference in the concentrations of both electrical charge and chemical species across a lipid bilayer.
- A **carrier protein** moves directly a solute from one side of a membrane to the other. In the process, the protein undergoes a conformational change. Carrier proteins can be divided into two groups: transporters and pumps.
- A **uniporter** is a type of carrier protein that moves only one type of solute across a membrane.
- A **symporter (cotransporter)** is a type of carrier protein that moves two different solutes across a membrane in the same direction. The two solutes can be transported simultaneously or sequentially.
- An **antiporter (exchanger)** is a type of carrier protein that simultaneously moves two different types of solutes in opposite directions across a membrane.
- An **ion channel** is a transmembrane protein that selectively allows the passage of one type of ion across the membrane. Ions pass through a central aqueous pore of the channel.
- 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.
Carriers and channels form water soluble paths through the membrane

• 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.
**Ion channels are selective**

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

- 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.
Neurotransmitters control channel activity

- 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.
G proteins may activate or inhibit target proteins

- 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.)

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- 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.
G proteins may activate or inhibit target proteins

- 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 function by dissociation of the trimer

• When GDP is replaced by GTP, a trimeric G protein dissociates into an α-GTP subunit and a βγ dimer.
• It is most often the α subunit that activates the next component (the effector) in the pathway.
• Less often the βγ activates the effector.
Protein kinases are important players in signal transduction

- 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.
Growth factor receptors are protein kinases

- 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.

- 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.

### RTKs act on different types of target proteins that generate second messengers

<table>
<thead>
<tr>
<th>Effector</th>
<th>Substrate</th>
<th>Products</th>
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<tbody>
<tr>
<td>PLC (phospholipase C) (3 families, PLCα, β, γ)</td>
<td>PIP2 (phosphatidylinositol 4,5-diphosphate)</td>
<td>DAG (diacylglycerol) + IP3 (inositol 1,4,5-triphosphate)</td>
</tr>
<tr>
<td>PLA2 (phospholipase A2)</td>
<td>Phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol)</td>
<td>Arachidonic acid Converted to prostaglandins &amp; leukotrienes</td>
</tr>
<tr>
<td>PI3 kinase (phosphatidylinositol-3 kinase)</td>
<td>Phosphatidylinositol</td>
<td>PI3 (phosphatidylinositol-3 phosphate)</td>
</tr>
<tr>
<td>PI4 kinase (phosphatidylinositol-4 kinase)</td>
<td>Phosphatidylinositol</td>
<td>PI4 (phosphatidylinositol-4 phosphate) Converted to PIP2 (phosphatidyl diphosphate)</td>
</tr>
</tbody>
</table>
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.
**Signaling pathways often involve protein-protein interactions**

- An **SH2** domain (named originally as the Src Homology 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.
Signal pathways often involve protein-protein interactions

- 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.
Phosphotyrosine is the critical feature in binding to an SH2 domain

• 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.
Prolines are important determinants in recognition sites

• 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.
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.

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.
What determines specificity in signaling?

- 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.
What determines specificity in signaling?

- 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.