Chapter 15
Cell Signaling and Communication

Introduction

- Survival of life depends on appropriate responses to the environment, both internal and external.
- Just as it is important for humans to respond appropriately in their daily lives, cells must respond appropriately to stay alive.
- Cells are like tiny computers processing literally millions of inputs and generating millions of outputs daily.
- Both prokaryotic and eukaryotic cells must process information from their environment, and calculate appropriate responses.
  - Signals might be chemical molecules or physical stimuli like light or heat.
  - Cells must be *set up* to interpret signals - not all cells can interpret all signals.
  - Cells have specific receptor proteins that interact with specific signals.
  - Signal transduction is the conversion of a signal from one form to another.

I Signals

- Signals originate from the general environment or from other cells, local or distant.
- Light, sound, and chemicals come from the environment.

A. Cells receive signals from the physical environment and from other cells

- Multicellular organisms' internal cells are exposed to extracellular fluids and other cells, from which they receive information.
- A few of the many types of signals are hormones, neurotransmitters, chemical messages from the immune system, CO_2, H^+, O_2, and changes in osmotic pressure.
- In large animals, signals reach targets via diffusion, when the target is close, or circulation. *(Figure 15.1)*
- Autocrine signals, for example, are signals generated by the same cells they act upon.
- Paracrine signals diffuse to and affect nearby cells.
- Signals and their interpretation create the order we call life.
  - Signals are used every moment of life from the period of earliest embryonic development.
  - Signals and their interpretation provide information to the cells of multicellular organisms on their location within a tissue, organ and organism.
  - Signals are needed for wound healing, cell replacement, and programmed cell death.

    - Moment by moment signals provide information essential for the maintenance of appropriate concentrations of nutrients.
    - Signals and their processing provide information from light and sound.
    - Signals are involved in literally multitudes of other cellular and manifestly organismal activities.
    - Signals are involved in directing which genes a cell will express.

B. Signaling involves a receptor, transduction, and effects

- Cells must be able to detect the availability of nutrients.
- Cells must maintain homeostasis.
- When scientists discuss cell signaling, they tend to describe it as a series of events that occur.
    - Generally many of the events described in the pathway are actually happening at the same time.
    - For example, some signals will just be arriving at the cell when the effects of previous signals are still being processed.
- Just as it can be explained how, when you are talking to someone on the phone who might be in another country, your voice is first converted to electrical impulses, then digital information, then electrical impulses, and then back into sound; we can describe what happens to cell signals as they are received, interpreted by cells and transformed into appropriate outcomes.
- A sample prokaryotic signal pathway: *(See Figure 15.3)*
    - In *E. coli*, *env Z* is a transmembrane protein.
    - It is found in the bacterium's plasma membrane.
    - The bacteria, E. coli, have two membranes, a plasma membrane, which is innermost, and an outer membrane.
    - The part of the *env Z* protein faces the space between plasma and outer membrane. (Part is transmembrane; part faces the inside of the cell.)
    - Increases in the ion concentration in the space between the outer and plasma membrane causes the *env Z* protein to change shape.
    - The shape change includes regions on both sides of the membrane.
    - The *env Z* becomes an active enzyme, a protein kinase, due to the shape change. The enzyme activity is found on the cytosolic-facing side of the protein.
        - Protein kinases are common intermediaries in signal transduction. They add a phosphate to a certain amino acid(s) of proteins they are specific for.
    - The *env Z* protein first phosphorylates its *own* histidine residues. (This is called autophosphorylation.)
    - This again causes conformation change.
    - Now, *ompR*, another protein, can bind to *envZ.*
    - The *envZ* transfers its phosphate (“phosphorylates”) *ompR.*

    - The *ompR*, in a sense, de-phosphorylates the *envZ*, resetting *env Z* back to its original state. Restoration of initial states is an important characteristic of signal transduction pathways.
    - The *ompR*, which is a DNA binding protein, binds the *E. coli's* DNA, initiating transcription of the *ompF* gene.
    - The protein made by the *ompF* gene, the *ompF* protein, inserts into the outer membrane, preventing solutes from entry, restoring the appropriate osmotic condition.

Steps in this Signal Transduction Pathway:

- Receptor changes conformation (shape) upon binding its specific signal molecule.
- Conformational change exposes a protein kinase.
- Phosphorylation alters the functioning of a protein.
- Transcription factors are activated.
- Altered synthesis of specific proteins occurs.
- Protein action alters cell activity.

II Receptors

A. Receptors have specific binding sites for their signals

- Generally receptors have specific binding sites for their signals.
- A ***ligand*** is the signaling molecule that binds the receptor. *(See Figure 15.4)*
- Receptors change shape when bound.
- Ligands are often only signaling molecules, not metabolites.
- Receptors bind ligands according to chemistry's law of mass action *(Chapter 2)*.
- Therefor, binding is reversible.
- The concentration necessary to saturate receptors depends on the affinity of the receptor for the ligand.
    - Some have high affinity, which means low concentration can have large effects.
    - Some receptors bind inhibitors as well as signals.
    - Inhibitors that bind the receptor's ligand binding site are called competitive inhibitors.
    - Inhibitors that bind at sites other than the ligand-binding site are called non-competitive inhibitors.

B. There are several types of receptors

The Major Classes of Signaling Molecules:

- Nonpolar signaling molecules can pass through the plasma membrane. *(Figure 15.2)*
    - Steroids are examples of non-polar signaling molecules.

  - Estrogen for example can easily diffuse across the plasma membrane.
  - Steroids bind receptors that are located in the cytoplasm
  - Only cells expressing the genes that code for a receptor of a certain steroid can be affected by that steroid.
  - There are also other signal molecules that are not steroids, which can pass directly through the membrane.
- Large or polar signals must interact with receptors found facing the outside of the cell's membrane.
- These receptors are generally transmembrane proteins.
  - Insulin is a protein hormone that interacts with a receptor that is transmembrane. *(See Figure 15.5)*

Functionally Different Receptors:

- Some ion channel proteins are signal receptors.
  - Channel proteins can open to let in or out or close to restrict certain ions' movement. *(See Figure 15.6)*
  - What determines the open or closed state of these receptor channels?
    - A signal must be received to direct the change in state.
    - The channel proteins work like molecular switches.
    - The signal could be chemical, light, sound, pressure or voltage.
  - The cell must be "set-up" to respond to the specific stimulus, which means there must be the appropriate receptor proteins present.
  - Also, all other required components for response must be present.
- Cells have the genetic information for all of the components, but often produce certain ones in specific cells only due to the differential gene expression.
  - For example, it is appropriate for liver cells to produce liver enzymes and the cells of a hair follicle to produce hair fibers; luckily liver cells do not grow hair.
  - Cells are set up differently because of signals received previously.
- Protein Kinases:
  - Protein kinase involvement in cell signaling is widely varied and common.
  - Some receptor proteins become kinases when activated.
  - A phosphate is transferred from ATP to a certain amino acid (residue) in a protein, which switches its state on to off, or off to on.
  - Sometimes the protein kinase phosphorylates itself. This is called autophosphorylation.
  - In eukaryotic cells, the usual target for phosphorylation is tyrosine, serine, or threonine (the terminal end of these amino acids' R group).
  - Insulin receptors are an example. *(See Figure 15.7)*
    - Insulin, the signal molecule, is a ligand for two different but necessary receptor subunits.
    - Each of the two subunits recognize part of the insulin molecule.

    - The insulin molecule, by binding both subunits, brings the subunits, which are normally floating separately, together.
    - On the cytoplasmic side, the two receptor molecules, because of how they are being held outside the cell by the insulin molecule, can cross phosphorylate each other.
    - The insulin receptors can then phosphorylate other cytoplasmic proteins.
    - This switches cellular activities.
    - Insulin and its receptors are part of the family of enzyme-linked transmembrane receptor systems.
    - There are other signaling pathways that work similarly.
- G-Proteins, a general description:
  - What defines a "G-protein"?
    - G-proteins have a binding site for a nucleotide called GTP.
    - GTP and GDP are like ATP and ADP; they are ribonucleotides. Whereas ATP is a common energy molecule and also a building block for RNA, GTP is used in some signaling pathways and also as a building block for RNA.
    - G- proteins have GTPase activity as well. This means G-proteins hydrolyze the terminal phosphate from GTP to form GDP.
    - When a G-protein hydrolyzes a GTP, and as a result, the binding site is then occupied by GDP, the G-protein changes conformation, which is switching states.
    - When the G-protein exchanges the GDP for a new GTP, the state switches back. Therefor, G-proteins are molecular binary or on/off switches.
  - G-proteins are important in many cell signaling pathways. (G-proteins are also important constituents of the cell's cytoskeleton.)
- G-Proteins and G-Protein Linked Receptors:
  - Another common signaling pathway involves a modular-type system of information transfer across the membrane.
  - Signal from the outside changes the shape of the receptor.
  - The changed shape in the internal domain of the receptor causes it to affect a membrane associated G-protein.
  - Initially, the G-protein, which is composed of subunits, and the internal region of the transmembrane receptor, are associated with each other.
  - When a signal molecule binds the receptor on the outer face of the plasma membrane, a change in conformation to the receptor's cytosolic side causes a subunit of the G-protein to release its GDP and acquire a GTP.
    - The GTP switches the G-protein subunit to on.
    - The subunit separates from the other subunits.

    - It then moves along the plane of the internal plasma membrane.
  - The G-protein subunit interacts with an effector molecule. *(See Figure 15.8)*
  - After the interaction, the G-protein by hydrolyzing its GTP, switches its own activity back to the initial off state.
    - To complete the cycle, this G-protein subunit must find another G-protein receptor, minus the subunit, and associate with it.
    - When a signal causes the subunit to again release its GDP, the cycle begins again.
  - What events do the activated and mobile G-protein subunits trigger?
    - Depending on the particular system, some interact with channel proteins to open or close them.
    - Some activate or inhibit enzymes.
    - An example is epinephrine (adrenaline).
      - The heart is set-up to respond to epinephrine as a signal.
        - The heart muscles express receptors for the hormone.
      - The receptors bind the hormone.
      - The associated G-protein gets activated in response to the change in receptor shape.
      - The G-protein subunit swaps GDP for GTP.
      - The G-protein subunit now slides away.
      - It switches on another enzyme found associated with the cytosolic face, adenylyl cyclase.
        - Adenylyl cyclase activates and using ATP as a substrate, makes a molecule called cAMP (*cyclic* adenosine monophosphate).
        - More on this is discussed later in the chapter.
    - Some G-proteins cause inhibition of adenylyl cyclase.
      - Epinephrine and its associated G-protein have an *inhibitory* effect on the smooth muscle cells of blood vessels.
      - The same events occur as described above, except the GTP/GDP binding-G-protein subunit *inhibits* the activity of adenylyl cyclase instead of stimulating it.
      - This causes relaxation of the muscle cell fibers.
    - This example demonstrates that it depends how the cell is "set-up" to respond to the signal that determines if and how a signaling molecule affects a cell.
- Cytoplasmic Receptors:
  - Not all signal receptors are associated with membrane.
  - Some signaling molecules get through the plasma membrane and into the cytosol. *(See Figure 15.9)*
    - Steroids are an example.
    - Steroids enter all cells of the body.

        - Some cells have cytosolic receptors for certain steroids.
        - When the intracellular receptor binds the steroid, a change in the shape of the receptor causes it or some event related component to bind DNA and influence gene expression.

III Transducers

- Signaling is a process of transducing.
- An example of transducing is the conversion of electrical impulses to sound in common loudspeakers.
- For cells, transducers convert signals from one form to another.
- Direct transduction is the receptor itself changing shape in response to a signal. *(See Figure 15.10)*
- Indirect transduction involves additional interactions with other cellular components. These can cause signals to branch, converge or amplify.

A. Protein kinase cascades amplify a response to a receptor binding

- *Cascade* is defined as a series of steep waterfalls, or as a connected series, as of amplifiers, for increasing an output.
- A protein kinase cascade is a series of protein phosphorylation reactions: protein kinases phosphorylating other protein kinases in a series of reactions.
- Details of a certain protein kinase cascade were discovered from the investigation into the cause of a certain kind of cancer. *(See Figure 15.11)*
    - *Ras* protein activates a protein kinase and is a membrane associated G-protein.
        - It is a G-protein because exchanging GDP for GTP switches on the protein kinase activity; and, normally it has GTPase activity.
        - The *ras* protein in these cancer cells is always bound with GTP because the GTPase activity is inoperative.
        - Therefor, the *ras* protein causes constant phosphorylation.
        - The result is continuous cell division, cancer.
    - *Ras* is part of a protein kinase cascade that influences cell division.
        - The pathway was called a kinase cascade because one kinase in the pathway phosphorylates the next.
        - Amplification of the signals occurs and a small input becomes a large output signal.
    - *Ras*, in normal cells, is usually activated by a transmembrane receptor protein.

B. Cyclic AMP - a Common Second Messenger

- Scientists investigating the effects of epinephrine on liver cells discovered cAMP as a second messenger.

- Studying the sequence of events by breaking apart cells, it was learned that to activate an enzyme called phosphorylase, plasma membrane and signal were necessary.
  - It failed to matter if the membrane and signal were removed from the medium prior to adding phosphorylase.
  - Therefore, the signal and plasma membrane must have generated a soluble messenger.
  - This was found to be cAMP. *(See Figure 15.12)*

Cyclic AMP - a Cyclic Nucleotide:

  - The cAMP molecule is a small cyclic nucleotide generated from ATP.
  - Called a second messenger, cAMP controls many different cellular activities.
  - Some cells are set up to respond to cAMP uniquely.
  - Certain cell types, like the follicular cells of the ovary, have unique receptors, which when bound by their ligand, cause a rise in cytosolic cAMP.
  - Downstream, this influences steroid synthesis.
  - The enzyme adenylyl cyclase produces cAMP using ATP as a substrate. Adenylyl cyclase is activated to produce cAMP by an activated G-protein subunit.
  - One important target for cAMP is a cAMP-dependent protein kinase called a-kinase.
  - Other targets include ion channels.

C. Two second messengers are derived from lipids

- Lipids are the molecules that make up cell membranes.
- Certain lipids get hydrolyzed by an enzyme called phospholipase C. *(See Figure 15.13)*
- The two parts generated by the hydrolysis each become second messengers.
- What activates the phospholipase C?
  - A signal at the outside of the cell attaches to the ligand-binding site of the receptor protein.
  - This is a G-protein associated receptor protein.
  - The G-protein subunit swaps GDP for GTP and becomes active.
  - This particular G-protein subunit is specific for phospholipase C.
  - It slides around the internal face of the plasma membrane until it encounters a phospholipase C molecule.
- Phospholipase C gets activated.
- Phospholipase C hydrolyses the phospholipid called phosphatidyl inositol into inositol triphosphate (IP_3) and diacylglycerol (the two fatty acid tails of the lipid, abbreviated DAG).
- These second messengers trigger many cellular events.
- The events described thus far are a simplification.

- One thing DAG does is activate a membrane bound enzyme, a kinase, called protein kinase C (PKC).
- The IP_3, the other messenger molecule, diffuses throughout the cytoplasm until it contacts certain Ca^{2+} channels found in the endoplasmic reticulum (ER).
- Calcium levels are normally low in the cytosol and calcium levels are high in the ER. (Calcium levels are also higher outside the cell.)
- So far we have seen that one signaling event outside the cell diverges into two with the generation of two different second messenger molecules.
- This is one point in the signaling pathway that converges.
    - To be activated, protein kinase C needs DAG as already stated, but also elevated calcium, which is caused by the IP_3, the other of the two second messengers.
    - A number of other events that are not convergent also occur.

D. Calcium ions are involved in many transduction pathways

- Calcium is an ion that is also a second messenger.
- The intracellular Ca^{2+} concentration is usually approximately 0.1μM.
- The concentration is kept low via active transport.
- Calcium ions are pumped into and sequestered in the ER.
- Many different signals cause Ca^{2+} channels to open, including IP_3.
- Two other examples of signals include electrical depolarization in muscle cells and the fusion of the egg by a sperm.
- Once a signal triggers Ca^{2+} channels to open, Ca^{2+} concentration rapidly rises to approximately 100 fold resting concentration.
- The calcium ions then affect the activities of cellular proteins.
    - One example is protein kinase C as discussed before.
- Ca^{2+} also binds to Ca^{2+} channel proteins, triggering additional releases of Ca^{2+}, which can be described as a positive feedback loop.
- Calcium ions bind to calcium binding proteins called calmodulin.
    - The calmodulin can activate certain proteins.
        - Calmodulin has four calcium binding sites.
        - At low Ca^{2+} concentrations, the chance that all four Ca^{2+} binding sites are occupied is low.
        - At high Ca^{2+}, the probability is sufficient and some of the calmodulin molecules become active via a change in conformation.
        - Calmodulin then can activate target molecules.
    - One target in muscle cells is a myosin specific protein kinase.

E. Nitric oxide: A second messenger that is a gas.

- Nitric oxide is a gas common in air pollution. It was certainly surprising when it was found to be a second messenger.

- The gas, NO, was found to be a second messenger by scientists studying the effects of acetylcholine.
- Acetylcholine causes the relaxation of smooth muscles of the blood vessels. *(See Figure 15.15)*
  - The sequence of events understood then was:
    1. Acetylcholine molecules bind G-protein-linked receptors.
    2. The activated G-protein subunits trigger phospholipase C to hydrolyze lipid.
    3. The previously discussed series of events would follow with the uniqueness being a downstream target, and the involvement of a cyclic nucleotide similar to cAMP called cGMP.
    4. The downstream target was a tissue specific protein kinase, which via phosphorylation causes muscle relaxation.
  - This was determined from intact blood vessels.
  - Strips of blood vessels failed to respond, though.
  - After difficult work, it was discovered that NO was needed.
  - NO was produced by cells closely associated with the smooth muscle cells, regional epithelial cells.
  - Acetylcholine caused increased Ca^{2+} levels in these epithelial cells. In these cells, the elevated calcium caused, among other things, the activation of NO synthase, the enzyme that makes NO.
  - NO diffuses from the epithelial cells to the smooth muscle cells.
  - In the smooth muscle cells of blood vessels, NO stimulates the formation of cGMP.
  - The discovery of NO as a second messenger provided the beginnings of an understanding on the mechanism of how the drug (and explosive) nitroglycerin actually worked (as a drug).
  - Even today, some drugs are used to treat patients because they work, but no one knows how or why they are effective.

F. Signal transduction is highly regulated

- Often, cells must return to previous states.
- Responsiveness of cells to stimuli depend on returning quickly.
- NO molecules, for example, breakdown quickly, being unstable.
- Ca^{2+} concentrations are returned by additional events that close the Ca^{2+} channels and active transport systems that pump Ca^{2+} ions out of the cytosol.
- Protein kinase cascades are reversed by protein phosphatases that remove the added phosphates.
  - Some phosphatases are activated by the kinase that is the very one whose phosphate additions they reverse.
- Both cAMP and cGMP are converted to AMP and GMP by their specific phosphodiesterases.

- Some that involve Ca^{2+} intersect with those involving cAMP. Some pathways diverge, others converge and some do both.
- The signaling pathways are like switches in sophisticated electrical systems. Complex cellular behaviors can result from the interactions of many simple switching systems.

IV Effects

A. Membrane channels are opened

- Sense of smell is an example of a sensory system.
- Some mammals have approximately 1000 genes that code for odorant receptors.
- Considering current estimates for the total number of genes in a human range from around 50,000 to 100,000 genes. The possibility that a species of mammal has 1/60th of all their genes dedicated for the detection of odor is astounding.
- We have discovered just recently that each of the thousands of nerve cells present in the nose express just one of these 1000 types of receptors.
  - When an odorant molecule binds to its receptor, a G-protein becomes activated. *(See Figure 15.16)*
  - The activated G-protein subunit causes adenylyl cyclase to make cAMP.
  - The cAMP binds to ion channels causing then to let in Na^+.
  - This is nerve stimulation or an action potential.
  - The signal is transferred to the brain.
- More than 1000 odors can be detected because some odor molecules bind to more than one type of odorant receptor. This provides a means to detect and distinguish enormous numbers of different odors.

B. Enzyme activities are changed

- An example are the effects of epinephrine on liver cells. *(See Figure 15.17)*
  - Phosphorylation can trigger glycogen metabolism.
  - Epinephrine prevents glucose from being stored as glycogen by the liver.
  - Epinephrine also triggers events that stimulate the breakdown of glycogen into glucose.

C. Different genes are transcribed

- *Ras* signaling pathways ends in the nucleus (Figure 15.11).
  - The final protein kinase phosphorylates a DNA binding protein called AP-1.
  - This activates AP-1; it now can bind to DNA and influence gene transcription.
  - Next, a number of genes involved with cell division are transcribed.

- Lipid soluble steroids bind to receptors, which then influence gene transcription.
- In plants, light activates phytochrome, which then bind to regulatory proteins found in the cytoplasm.
- These can then move to the nucleus, bind DNA and influence gene transcription.

V Direct *Intercellular* Communication

- Some cells send signals from their interior to the interior of adjacent cells.
- This transfer is because the two cytoplasms are coupled together.

A. Animal cells communicate by gap junctions

- Gap junctions couple together animal cells. *(See Figure 15.18)*
  - Gap junctions are complexes of proteins that generate pores in membranes. These protein complexes couple the plasma membrane of one cell with adjacent cells that also have these proteins.
  - The pores can be gated and are either open or closed.
  - Each gap junction is made up of numerous channel-generating proteins called a connexon.
  - Each connexon is made up from six small snap together subunits called connexins.
  - The pore is small relative to macromolecules, but large enough for Ca^{2+} and cAMP size molecules to pass.
    - In the developing mammalian egg, for example, the surrounding granulosa cells form gap junctional complexes with the egg.
    - Some evidence exists that cAMP levels maintained by the granulosa control the state of development of the egg.

B. Plant cells communicate by plasmodesmata

- In plant cells, communication directly from the cytoplasm of one cell to another is through plasmodesmata. *(See Figure 15.19)*
  - These are membrane-lined bridges.
  - Generation of plasmodesmata involve fusion of adjacent plasma membranes to make the pore or connection.
  - The pore is large compared to gap junctions.
  - However, usually the space for molecular traffic is about the same as found in gap junctions, approximately 1.5 nm.
  - Therefore, just small molecules generally move through it.
  - Plasmodesmata are important to C_4 plants helping them in the system to control photoxidization.
  - Plasmodesmata pore size can be regulated. Some very large proteins have been found to move through them.