Molecular Bioscience 401: Lecture 11.1 Cell Signaling

Slide #1

Cell Communication

- Necessary for multicellular organisms
  - Unlike unicellular organisms, multicellular
    - Organisms require elaborate cell communication
    - Mechanisms
- Depends on production of molecules by
  - One cell that provides instructions
  - To one or more target cells
- Target molecules produce receptor proteins, and the molecules they bind are known as ligands

Audio:
Our last major topic for this course is going to address how cells communicate with one another and your book mentions that life is thought to have existed on the planet for a several of billion years before multicellular organisms arose and the long delay is thought to reflect the complexity involved in coordinating the activity of many cells and generating systems where an individual cell is willing to put the good of the whole organism ahead of their own survival. For the most part, when we discuss signaling, we mean communication that is based on the production of small molecules of some sort by one cell and their detection by another. And these molecules can be steroid or cholesterol based or peptides that are small pieces of proteins and a variety of other things. Therefore this generally does not mean nerve cell signaling which we discussed in the first section of the course. However, neurotransmitters are emitted by nerve synapses and these fit the description of a signaling compound. The reaction of some types of cells to these small molecular is considered to be a cell signaling event. Proteins that bind signaling molecules and receive the signal are known as receptors and the molecules they bind are referred to as ligands.

Slide 2

Cellular Communication: an outline

I. 1. Extracellular signaling molecules
   2. General principles of signaling by extracellular signal molecules
   3. Receptors
      - characteristics; basis for speed of response
   4. Nuclear receptor family
   5. Cell-surface receptors
      - general types, molecular switches, scaffolds
II. 6. G-protein coupled receptors
     - G proteins, targets, second messengers
III. 7. Enzyme linked receptors
   - tyrosine and serine/threonine kinases, histidine kinases
IV. 8. Pathways that require regulated proteolysis

Audio:
Cell signaling is complicated enough that an outline of the next four lectures might be helpful. This lecture will present a broad overview of the general types of signaling mechanisms, and their features, including how long signals last, how far they travel, what kinds of molecules are being used to do the signaling, and so on. We will look broadly at different kinds of proteins involved in detecting signaling molecules, called receptors, and then more specifically at two subclasses of receptors, the first being nuclear receptors and the second being cell surface receptors. In subsequent lectures will then cover three types of signaling events in more detail, including G-protein coupled receptor signaling, enzyme linked receptors signaling and finally signaling systems that are regulated by proteolytic parts.

Slide 3

Methods of Signaling

6 Images:
(1) Contact Dependent
(2) Contact Dependent-Gap Junction
(3) Paracrine
(4) Autocrine
(5) Synaptic
(6) Endocrine

Audio:
So, first some terminology about signaling and the distances that act over them. Signaling over the shortest distance is the signals between two neighboring cells that touch. This is called contact dependent signaling. One example that we have already discussed is signaling through gap junctions. Small molecules and electrical depolarization events can travel between two cells using gap junctions, and this type of signaling plays a major role in some situations. For example, the cells of the heart coordinate their contraction by signaling to each other through calcium signals that travel through gap junctions. Communication through gap junctions has important limitations, however. The molecules must be very small and can’t be very complicated and there is no real control over the direction that a signal is traveling they just basically diffuse across both cells.

Another method is where one cell produces a signaling molecule that is attached to it’s surface. Receptors are produced on other cells and are activated when they bind to that signaling molecule, but they actually have to touch in order for this to happen. Because the signaling molecule is attached to the first cell, the two cells need to actually touch in order for signaling to occur. This style of signaling is used for very local types of
signaling events. For example, sick or diseased cells may signal so called killer cells to kill them. This type of signaling has to be very specific to a single cell, otherwise the killer cell might get confused and go after a sick cells neighbors.

Moderate distances involve a signaling system called paracrine signaling. Here, signaling molecules are secreted by one cell and the secreted molecules are detected by other cells. The distances involved are short and there may be specific mechanism involved to keeping signals located to a small area. For example, the surrounding cells may produce enzymes that degrade a signaling molecule, which will have the effect of limiting its effective range. A lot of developmental events are regulated by paracrine signaling. A special example of paracrine signaling is autocrine signaling, in which a cell produces receptors that are activated by its own signaling molecules that it is also secreting. This type of system is used for both positive and negative feedback loops so that cells can control their own behavior through signaling. Your book mentions that this is an important consideration in cancer biology because some cancer cells signal themselves to grow. Finally, there are two types of long distance signaling, one called synaptic signaling, which involves signaling by neurons, and the other called endocrine signaling. I’ll discuss them in more detail on the next couple of slides.

Slide 4

Signaling Speed, Distance, Concentration

- Speed-relies on diffusion and blood flow
- Precision-effective concentration is approximately 10^-8 M
- Image of endocrine signaling

Audio:
Most of signaling events involved endocrine signaling. Signaling by insulin, testosterone and estrogen and growth hormones and many other things, all use this mechanism. Endocrine signaling uses the circulatory system to carry signals and there the signals are distributed throughout the body nonspecifically. There are some unique features of endocrine signals to keep in mind. First, because signals are spread out through the entire body, their effective concentration is low because the individual contents are going to be diluted by the entire contents of the organism. Consequently, receptors on the receiving end of the signal have to be able to detect small amounts of the signaling compound that are very highly diluted. The molecule will need to be bound to the receptor when it is present at a very low concentration, which means the molecule needs to be bound to the receptor very tightly, also referred to as binding with high affinity. Another issue is that target cells have to detect the appropriate molecule tin the presence of signals that are intended for other cells, so the receptors have to be very specific and selective. Finally, distribution by blood flow takes some time, so endocrine signaling actually takes a fair amount of time, so it’s slow. However, this is not a problem for events that rely on endocrine signaling for their function, such as digestions, growth or the development of gender specific features, these are all slow things so that endocrine signaling is appropriate for regulating these events.
Slide 5

Synaptic Signaling

-Speed-electrical impulse @ 100 meter/second; synapse < 100 nm distance; < diffusion time across synapse
-Precision-effective concentration ~10^-4 M; affects only post synaptic target cell

Audio:
The alternative long distance signaling system is synaptic signaling. The initial signal is a nerve impulse, which travels the distance of the neuron, which can be inches to feet in length. At the nerve synapse, neurotransmitters are released which bind to a receptor on a target cell on the other side of the synapse. The binding of neurotransmitters to receptors is much like endocrine signaling event. However, the signaling molecules only need to ravel a short distance, and as a result they are not diluted by having to spread through the entire body. Therefore receptors for neurotransmitters do not have to bind the signaling molecule known as the ligand as tightly as do endocrine receptors. The signaling process is also selective itself, because a target cell may be attached to only one type of neuron. Finally, nerve impulses and the diffusion of neurotransmitter across the synaptic space occurs much more quickly than does endocrine signaling. Synaptic signaling is involved in events that need to occur with moderate speed, such as changed in the diameter of blood vessels.

Slide 6

Response Speed
Image of Altered Cytoplasmic Machinery with Fast and Slow Sections
Fast: cell movement, secretion, enzyme activity
Slow: cell growth, division, differentiation

Audio:
So we just talked about as signaling mechanism occur over different time frames, the response of target cells can also occur over different time frames. Some responses may be rapid, such as the phosphorylation of target proteins involved in cell motility or secretion events. The events can occur within minutes of receiving a signal. In contrast, other responses take longer periods of time. Such events are likely to include things like changes in gene expression, cell growth or changes in differentiation. These events obviously take much longer and as a result are not going to necessarily going to require signals to propagate very quickly. Occasionally, a single signal can generate both kinds of responses.

Slide 7

Target Cell
Each target cell receives hundreds of signals and is programmed to respond to a distinct manner to each signal. This is seen in 4 steps:
1. Survive
2. Divide
3. Differentiate
4. Die

Audio:
Well, here’s where things start to get more complicated. Target cells don’t usually express just one type of receptor; they often produce several or even dozens of them. The illustration on this slide shows just a few of the various possibilities. The top image shows that it often takes more than a single signal to tell a cell that it needs to survive. In the absence of these signals, the cell may die, as shown at the bottom of the slide. On top of the minimum signals needed to survive are other signals when present may tell a cell to grow and divide or to stop dividing and differentiation into some other type of cell. And so cells are constantly sorting through a variety of signals to decide what they are supposed to be doing.

Slide 8

Decreased Rate and Force of Contraction

-Specific cell response depends on receptors present and internal machinery by which cell integrates signal.
-Acetylcholine binds to same receptor but elicits distinct responses in heart muscle vs. salivary gland cells.

Audio:
In some cases, a single signaling molecule binds to a receptor that is produced by two different types of cells, and each cell responds differently even though the receptor is the same, an example of this is shown by the response of salivary glands and cardiac muscles to the neurotransmitter acetylcholine. This different is caused because each cell has different internal signaling pathways. The trigger or the receptor is the same but the response systems are different and takes different pathways.

Slide 9

Uncommitted vs. Committed cells

-Specific cell response also can depend on the concentration of the signal (morphogen) that reaches the cell

Audio:
One last example of how complex signaling gets before we start looking at specific examples: in some cases, the concentration of signaling molecule can produce entirely different results on target cells, even though the signaling molecule and receptor proteins
remain the same. This type of differential response in response to a certain concentration is commonly seen in embryogenesis, where the distance away from the source of a signaling molecule determine the fate of the target cell. The signaling molecules involved in embryogenesis are often called morphogens, because they alter morphogenesis or shape of the tissues or cells they are affecting.

### Slide 10

**Intracellular receptors vs. Cell-surface receptors**

-2 images of cells with a hydrophobic or hydrophilic component

**Audio:**
The rest of this lecture will concentrate on the general behavior of receptors. There are two basic types of receptors: intracellular and cell surface receptors. Intracellular receptors bind to ligands that can enter a cell with no assistance. Therefore, these ligands must be able to cross the cell membrane and they are typically small hydrophobic molecules like cholesterol and sterols. Because they are hydrophobic, they are often transported to a target cell using some sort of carrier system to prevent interaction with the signaling molecule and the aqueous intracellular environment. The necessity of the ligand to cross the cell membrane severely constrains the types of molecules that can be used for this type of signaling process. Most intracellular receptors are transcription factors that alter gene regulation in the nucleus of target cells. In contrast, cell surface receptors are generally transmembrane proteins with extracellular domains. Often the ligand can bind never needs to enter the cell in order for the receptor to function. As a result, cell surface receptors are much more adaptable and variable than intracellular receptors and there are cell surface receptors for a huge variety of molecules. Cell surface receptors can also alter gene expression, but they generally do this indirectly by activating intracellular proteins and activating them that then enter the nucleus and we’ll see lots of examples of this in future lectures. Cell surface receptors are also involved in many other types of signaling responses.

### Slide 11

**Nuclear Receptor Family**

-Image of an inactive vs. an active receptor
-Image of vitamin D₃, retinoic acid, thyroxine
-Images of different receptors (ex: cortisol, estrogen, etc)

**Audio:** We have already discussed nuclear receptor family when we covered the ran system of nuclear import in the first section of this course. And so here, I’m going to provide a few more details. All members of the nuclear receptor family are intracellular
receptors which directly bind to DNA so their transcription factors. They share a conserved DNA binding motif. The ligands bound by nuclear receptors are small hydrophobic molecules, which look a lot like cholesterol molecules. These include the sex hormones testosterone and estrogen, as well as vitamins like vitamin D, retinonic acid which is derived from it. The mechanism of activation of a nuclear receptor is shown at the top of the slide. Nuclear receptors are present in an inactive state in complex with an inhibitory protein. The binding of the ligand displaces the inhibitory proteins, and allows interaction with coactivator proteins. This also reveals a DNA binding domain which allows the receptor to bind to target gene promoters and recruit additional factors needed for gene transcription.

**Slide 12**

**Activation of Nuclear Receptors**

*Image of Primary Response to Steroid Hormone vs. Secondary Response*

**Audio:** This slide illustrates that the activation of nuclear receptors can have immediate early responses as well as longer term secondary responses. The immediate or early responses are generated by the synthesis of proteins which are under the direct control of genes activated by nuclear receptors. However, many of those gene can also be transcription factors that can alter additional gene expression patterns later on and those would be the later effects.

**Slide 13**

**Types of Cell Surface Receptors**

*Image of ion channel-coupled receptors, G-protein coupled receptor, Enzyme coupled receptors*

**Audio:** Cell surface receptors are much more diverse than intracellular receptors, but they can still be broken down into three major subcategories. The first are ion channel coupled receptors. These are receptors that open or close pores in the cell membrane in response to the ligand binding. The opening of such pores usually generates rapid changes in ion concentration within the cell such as calcium, sodium, or potassium. A second major category are the G protein coupled receptors. This is a huge class of proteins that work by activating another set of membrane associated proteins which is a complex with three proteins which use GTP as a secondary signaling mechanism. Finally, there are things called enzyme coupled receptors. Here the receptor has an enzymatic function of it’s own that becomes functionally active when a ligand binds to them.
Slide 14

A General Signaling Pathway

-Image of: Primary transduction: (1) relay (2) transduce and amplify (3) integrate (4) spread (5) anchor (6) modulate

Audio: This diagram illustrates the general design of a signaling pathway. Although it seems complicated, the design makes sense if you consider the tasks that a signaling pathway needs to perform. Imagine that you have a cell which needs to respond to an endocrine signal in order to differentiate. The signal is going to reach the cell at a very low level, activating perhaps only a very small number of receptors on the cell surface. Therefore, the binding of the ligand to its receptor must be converted into something that the inside of the cell can use a control mechanisms and it must be amplified. The binding of the single ligand needs to control many different components of the cell together in order to get the cell to differentiate, so there will be branches in the signaling pathway. In some cases, control mechanisms will need to take into consideration several signaling pathways. For example it makes no sense to activate differentiation pathways if the minimal signals needed for survival are not also present. It may also be important to localize proteins to unique places in cells, and that role is performed by anchoring proteins. We will consider each of these events in more detail when we discuss signaling pathways. Finally, the signal needs to be converted into actions, such as cytoskeletal assembly or disassembly or changes in gene expression. And so there are effector proteins that are going to be used to accomplish these tasks.

Slide 15

Types of Scaffolds

-Image of performed signaling complex on a scaffold protein
-Image of assembly of signaling complex on an activated receptor
-Image of assembly of signaling complex on phosphoinositide docking sites

Audio: The first steps involved in detecting the signal produced by binding a ligand to its receptor involve converting the signal into an activation of intracellular response proteins. In order to make this process fast and efficient, many early signaling events are physically tied together using a system that is called a scaffold. This slide illustrates three different types of scaffolding systems. In the first instance, a very large scaffold protein with multiple binding domains which interact with the activated receptor as well as several downstream signaling proteins that will convert the activation of the receptor into an intracellular signal. In the second example, the receptor itself has a very large domain that will act as a scaffold and has several binding domains. A third mechanism involves the activation of phosphorylated lipids called phosphoinositides in the adjacent membrane. If you remember our discussion of lipid domains from section one of the course you will recognize that these lipids can be localized to restricted regions or patch of a membrane. These can then form a localized area where downstream signaling molecules are first recruited then interact with each other. So as we go through specific
examples of signaling pathways in the next few lectures keep these three general mechanisms for localizing signaling pathways in mind.

**Slide 16**

**Mechanisms of Signal Transduction**

-Image of signaling by phosphorylation
-Image of downstream signals
-Steps: (1) Relay (2) Transduce and Amplify (3) Integrate (4) Spread (5) Anchor (6) Modulate

**Audio:** Although cells need to respond to highly diverse variety of signaling events, the mechanisms that are used to transmit these various signals inside of cells have many similarities. Almost all signals will involve phosphorylation of some proteins on one of three amino acids. In order of frequency, these are serine, tyrosine and threonine. Phosphate groups are added using a phosphate provided by ATP, and this reaction is catalyzed by an enzyme called a kinase. Phosphate groups are removed by enzymes called phosphatases. The activity of a protein can be either enhanced or decreased by phosphorylation, depending on the type of protein. Phosphate groups are also removed to turn off signaling pathways by enzymes called phosphatases. The activity of a specific protein can either be increased or decreased by phosphorylation depending on the type of protein. We already discussed situations like this when we covered mitosis: phosphorylation activated things that destabilized microtubules, but at the same time deactivated proteins that normally stabilized them. Another common response element are G-proteins, and we have already explored the use of such proteins when we covered the RAN system which drives movement of proteins across the nuclear envelope. The addition of GTP to a protein, which replaces GDP, is stimulated by Guanine nucleotide exchange factors, or GEFs, and the reverse reaction is catalyzed by GTPase activating proteins or GAPs. Both systems may contain proteins that integrate information from more than one signaling element, as shown in the diagram on the far right of this slide. Many simply have multiple sites for modification and the response of the integrating protein is either proportional to the input or an all or none response in which all sites need to be activated before any response is generated. If this discussion seems general, we’ll cover more specific examples in the next few lectures that should make these points more clear. Since signaling mechanisms are common to many responses, you may wonder how the response of individual cells can be so different from one another. The simple answer is that there are many hundreds of different kinds of kinases, phosphatases and G-proteins each with different effects on gene expression or cell behavior. Different cell types produce a small number of the possible kinds of signaling molecules and effector proteins that are present in our genomes.

**Slide 17**

**Positive Feedback Systems**
Audio: When I introduced autocrine signaling, I briefly introduced the concept of feedback loops. Feedback systems play important roles in shaping the response of cells to signals. For example, in the absence of feedback loops, the signal generated by receptor activation would be simply reflection of the number of ligands bound to receptors. That type of simple relationship between ligand binding and response is illustrated by the blue line in the graph at the top of this slide, and by the top row of colored symbols at the top right. In contrast, a positive feedback system might generate the type of all or none response shown by the red curve in the graph and the lower row of symbols at the top right. Interestingly, although the bottom row shows an all or none response for individual cells, the population as a whole may still show a graded response to the signal, simply because not all cells are triggered at any one time. For an example of this type of response, you can think about the secretion of insulin by pancreas in response to the a meal. It might be appropriate to have a graded response in which something other than the maximum amount of insulin is secreted into the blood stream. However, individual cells may be able to decide only whether or not to secrete the contents of their secretory granules as a whole. Positive feedback can also prolong the response of a cell to a transient signal, as shown by the curves at the bottom left of the screen. Examples of this type of system included developmental signals, where a transient signal to differentiate creates a very long term change in the developmental fate of a cell. The lower right hand side of the slide shows a simply example of a possible mechanism of a positive feedback system, in which a receptor catalyzes the production of activated molecules that then bind to and reactivate the receptor at another site.

Slide 18

Negative Feedback Systems

Audio: Of course, just as there are positive feedback systems, there are also negative feedback systems. This slide illustrates just one possible negative feedback system and some possible outcomes. Here, a signaling kinase, labeled “S” is converting an inactive downstream effector kinase, labeled “E”, into an active one. Among the targets of the active E kinase is a phosphatase, which becomes active when it is phosphorylated. The phosphatase is able to remove the phosphate group on the active E kinase, which has the effect of dampening the activation of the system as a whole. Because the proteins are not all activate instantaneously at the same time, some interesting effects can be produced by a negative feedback system. The graphs show how a simple feedback system has the
effect of dampening, or lowering the overall activity of a response system if there is relatively little or no delay in the response of the various parts of the system. However, if the activated E kinase takes a long time to turn on the negative feedback loop, you can get the oscillations shown in the bottom graph.

**Slide 19**

**Desensitizing Systems**

- Image of a 5 step process
  (1) Receptor Sequestration
  (2) Receptor Down-regulation
  (3) Receptor inactivation
  (4) Inactivation of signaling protein
  (5) Production of Inhibitory protein

**Audio:** A final thing to consider as we begin discussing specific signaling systems is the desensitization of cell surface receptors. There are many examples of this. For example, the sense of smell is rapidly desensitized, in order to allow the system to respond to change in odors. This is beneficial because it allows an animal to detect new smells even in the presence of another odor. There are many ways to desensitize a receptor system, some of which are shown here. These include sequestering the receptor in an endosome, which can allow the receptor to be recycled to the cell surface, or alternatively degradation of the receptor and destroy it. Receptors can also be inactivated by downstream signaling elements much like the negative feedback loop I discussed on the previous slide. In addition to inhibiting the receptor, the function of other elements of the signaling system can also be inhibited.