

## Review of Cell signaling: ligands, receptors, and signaling pathways

Reading: Chp 3: 69-76, 84-98, 101-105

For anyone desiring additional review on cell signaling, the following pages are also useful from Alberts (4<sup>th</sup> ed): 15: 831-852, 853-866, 871-890, 893-900; 17: 1014-1017, 1019-1021, 1023; 19: 1084-1090, 1090-1096, 1103-1106, 1110-1111, 1113-1118; 23: 1333-1340.

Learning goals: Be able to:

1. Describe in general the process by which developmental signals are sent from one cell and received by other cells, and what can happen in the receiving cells as a result.
2. Compare and contrast the major developmental signaling pathways (Wnt, TGF $\beta$ , Hedgehog, RTK).
3. Explain how a mutant component of a signaling pathway can cause a cell to become cancerous.

Differential gene expression and other cell functions in development are often controlled by signals from one cell to another, requiring signaling molecules (ligands), receptors, and machinery for transducing signals through the cell membrane and communicating with the nucleus or cytoskeleton, or both. Some of this machinery should be familiar to you from cell biology. In today's class, we will discuss mechanisms of cell communication used during development.

### Intercellular signaling

Signaling pathways are ancient and highly conserved during evolution, and most of them are found in all phyla. Some have been adapted for special functions, but *many of them seem to play similar developmental roles in all organisms where they have been found*. There are actually fewer than 20 currently recognized intercellular signaling pathways that take care of all known signaling events, and only 5 that are involved in development. This means that each signaling pathway has many different functions during development.

### Classes of transmembrane signaling pathway

Ligands can be small diffusible molecules, large molecules in extracellular matrix, or non-diffusible surface components of neighboring cells. All interact with specific cellular receptors on receiving cells.

For diffusible ligands: Signaling can be classified as autocrine (self-signaling), paracrine, (signaling between nearby cells), and endocrine, (signaling over a long distance, usually via the bloodstream.) Of these, paracrine signaling is most important for embryonic development. Ligands for paracrine signaling are all diffusible between cells, they range from small to quite large, slowly diffusing molecules. Many of the important paracrine ligands are so-called growth factors, described below.

Non-diffusible ligands are involved in juxtacrine signaling, so-called because the signaling and receiving cells must be in contact for the signal to be transmitted. Ligand molecules imbedded in the membrane of the transmitting cell interact with specific receptors on an adjacent receiving cell.

Still other signals can come directly from slowly diffusible components of the extracellular matrix (ECM) between cells. (6.9 shows some examples of juxtacrine, paracrine, and paracrine with ECM)).

### Receptors

Can be grouped into three general classes based on the mechanism by which they transduce signals across the plasma membrane:

Ion-channel-linked receptors function directly to affect ion transport, primarily in neurons; often these channels are ligand-gated, opening or closing in response to a ligand.

G-protein-linked receptors exert their effects indirectly, by activating G-proteins in the membrane when they bind to a ligand. Remember these from previous courses?

Enzyme-linked receptors, the most important group developmentally, are transmembrane proteins with an extracellular ligand binding site that controls the activity of an intracellular effector domain responsible for initiating the ligand-induced response. *All enzyme-linked receptors have in common that binding to ligand on the extracellular domain of the molecule causes a conformational change in the intracellular domain, which can change its ability to bind other proteins or its enzymatic activities.*

These ligand and receptor types are found in various combinations in the pathways we will discuss. Many of the ligands for enzyme-linked receptors are growth factors.

What are growth factors? They were discovered and named as required serum factors in early work on growth of mammalian cells in tissue culture. For example, nerve growth factor (NGF), the first to be discovered, is required for differentiation of neuronal precursor cells in culture. Others are epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF- $\beta$ , insulin-like growth factors (IGFs), and many others; the list is still expanding. Most are polypeptides, similar to the polypeptide hormones. In general, specific growth factors signal committed cells during development either to continue proliferating or to differentiate. Later in life, they are required by many differentiated cell types to prevent apoptosis (programmed cell death), which occurs automatically if growth factor is depleted. All growth factors have specific receptors, most but not all of which are of the RTK class.

### **Paracrine signaling pathways of developmental importance**

The important developmental pathways described below provide direct inputs to the nucleus, to the cytoskeleton, or to both (as opposed to the more indirect methods of G-protein linked signaling). These inputs often act in determination of cell fates, and thus in controlling embryonic development.

#### **Wnt receptor pathways (6.20)**

Ligands: Members of the Wnt family of large, secreted, slowly diffusing glycoproteins. Signal many important developmental events, including controls on spindle orientation, cell polarity, and probably cadherin-mediated cell adhesion (which also involves  $\beta$ -catenin; see below). Especially important in early embryonic development.

Receptors: *Drosophila frizzled* gene product and its homologs in other organisms.

Downstream components: The Frizzled receptor acts through a series of negative controls to maintain  $\beta$ -catenin as an active transcription cofactor, which goes to the nucleus and activates transcription. In the absence of ligand, the  $\beta$ -catenin is phosphorylated and degraded, and the transcription factor is deactivated (6.20 A). Different Wnt pathways are primarily used later in development during morphogenesis and establishment of cell polarities (6.20 B and C).

#### **Receptor serine/threonine kinase pathways (TGF- $\beta$ receptor pathways) (6.23)**

Ligands: A large class of growth factors belonging to the transforming growth factor- $\beta$  superfamily. Important in a variety of developmentally important signaling events.

Receptors: Heterodimeric transmembrane protein kinases, which dimerize in the presence of ligand. Dimerization activates their intracellular domains, which phosphorylate serine and threonine residues on target proteins.

Downstream components: These so-called Smad proteins include several Smad transcription factors that become activated and move to the nucleus when they are phosphorylated.

#### **Hedgehog receptor pathways (6.17)**

Ligands: The Hedgehog family of proteins, which appear to act as morphogens (diffusible factors whose concentration determines different cellular responses) in several important aspects of embryonic

pattern formation (establishment of the body plan, limb structures, etc.). They are secreted proteins whose C-terminal domains have autoproteolytic activity. After secretion, the protein cleaves itself in a novel cholesterol-mediated reaction to produce a small N-terminal fragment, which is the active ligand.

Receptors: A large transmembrane receptor with multiple transmembrane domains (Patched), which appears to act through an adjacent transmembrane protein (Smoothened). Note that unlike the first two pathways, binding Hedgehog negatively regulates, i.e. de-activates, the Patched receptor. This in turn activates Smoothened.

Downstream components: Smoothened deactivates two protein kinases. When these are inactive, the transcription factor Ci is allowed to go to the nucleus and turn on target genes; when they are active (absence of Hedgehog), they cause cleavage of Ci into a form that prevents turning on these genes

### **Receptor tyrosine kinase (RTK) pathways (6.10, 6.12, 6.13)**

Ligands: Usually dimers of the growth factors described above (FGF, EGF, etc.). Important in many developmental controls involving growth control and determination of cell fates.

Receptors: Transmembrane receptor tyrosine kinases (RTK's). Ligand binding causes receptor dimerization, which activates the kinase.

Downstream components: RTKs act via small G-proteins such as Ras to regulate the activity of transcription factors and cytoskeletal components by phosphorylation. The phosphorylated intracellular domains activate the specialized G-protein *ras*, a kinase that, with the help of accessory proteins, can cycle between an inactive GDP-binding and an active GTP-binding form. This in turn activates a cascade of additional protein kinases, which ultimately regulate the activity of either transcription factors in the nucleus or cytoskeletal components involved in cell shape changes and cell movement. These pathways are often multiplexed, to integrate input from several pathways. Because the RTK pathways are critical for controlling cell division, they are often implicated in cancers, either through increased activation of the stimulators, or decreased inhibition of the inhibitors.

### **Juxtacrine signaling**

#### **Notch/Delta receptor pathways (6.26)**

Ligands: The Delta family of cell surface proteins.

Receptors: The Notch family of proteins. Delta-Notch signaling is the most important of the juxtacrine pathways, important in mediating many processes of determination and tissue differentiation throughout development. Both Delta-like ligands and Notch-like receptors are characterized by an extracellular domain containing tandem repeats of an EGF-like motif.

Downstream components: Delta interaction with Notch at the cell surface causes proteolytic cleavage of Notch and release of a fragment of the Notch protein, which now acts as a transcription factor, displacing an inhibitor of another transcription factor called CSL. CSL then activates (or represses) transcription of target genes.

Notch/Delta signaling is primarily involved in early decisions of cell fate determination, accomplishing this through a principle called lateral inhibition. One cell in a field of equivalent cells randomly begins to produce more Delta than the other cells in the cluster. The cells contacting this cell bind Delta via the Notch receptor, which initiates an intracellular cascade in which Dl ultimately stops being produced. Thus, the cells that have their Notch receptor bound by Delta are prevented from taking on the fate of the adjacent cell that expressed a higher level of Delta. In this way, certain cells can be singled out from a field of equivalent cells to take on a different fate.

### **Other pathways we may touch upon**

*Nuclear hormone receptor (NHR) pathways.*

**Ligands:** Steroid hormones and other fat-soluble (lipophilic) small molecules. Most of these hormones are involved in physiological endocrine signaling, but some are important in early development.

**Receptors and downstream components:** Specialized Zn-finger class proteins, with a steroid binding site. In these pathways, the receptor *is* the transcription factor. In the absence of ligand, it is bound to an inhibitor in the cytoplasm and therefore inactive. The steroid ligands are lipophilic and can therefore cross the cell membrane by diffusion (though there is recent evidence for cell surface proteins that may mediate uptake). When a ligand binds to the receptor, the inhibitory protein dissociates, and the active transcription factor dimerizes and goes to the nucleus where it regulates target genes. Besides the steroid receptors, there are similar receptors for a few other lipophilic molecules (e.g. thyroid hormones, retinoids). There are also many other members of this family whose ligands are still unknown.

### ***Cell death (apoptosis) pathways (6.24)***

During development, and throughout the life of an animal, many cells undergo apoptosis, also called programmed cell death. Many different extracellular inputs (the absence of growth factors, for example), as well as some intracellular events like DNA damage, can activate the apoptosis pathway. Many cells in all organisms are programmed to die unless this pathway is kept inactive by growth factor signaling. Control of cell death is important not only during normal development, but also for prevention of tumor formation. In fact, many of the pathways just discussed are used later in an organism's life to control cell division or apoptosis, and thus have implications for cancer.

### **Signaling pathways and oncogenes**

**Oncogenes** and **tumor suppressor genes**, as you probably know from previous courses, are mutant viral or cellular genes whose expression can transform normal cells into cancer cells. They are of interest for developmental biologists because they cause breakdown of normal growth controls – hence they were originally suspected to code for altered forms of components in the growth control system regulated by growth factors. This has turned out to be true – most oncogenes are mutant forms of genes that encode proteins in signaling pathways: ligands, receptors, and "downstream" components of these pathways. Generally the oncogenic forms cause the pathway to be turned on constitutively, whether or not the normal upstream signal is present.

### ***Take home lessons***

- 1) Both differential gene regulation and cytoskeletal functions are controlled during development by communication between cells, in which a ligand interacts with receptors on target cells to activate a "downstream" signaling pathway.
- 2) Of the several modes of intercellular communication in animals, the most important during embryonic development are paracrine signaling, involving diffusible ligands produced by transmitting cells, and juxtacrine signaling, involving membrane-bound ligands on transmitting cells.
- 3) The regulation of gene expression by signaling in embryos is mediated primarily by five major signaling pathways, named for their ligands and/or receptors the Wnt, TGF- $\beta$ , Hedgehog, RTK, and Delta-Notch pathways.
- 4) Different cells in an embryo will respond differently to the same ligand, according to the principle of combinatorial control.