In this tutorial, I will cover the basic idea of a morphogen and the role it plays in patterning the developing embryo. Pattern. There are different patterns all around us. You probably see them every day. Yet how are these different patterns created? Perhaps with the right combination of chemicals and minerals, glacial impacts, erosion, pressure and time, the patterns of our landscape can be explained. A seemingly infinite array of different patterns can be seen in all the life around us. The necessity of such patterns shaped through adaptation to selection pressures over the course of many generations. But how? How is pattern created in the developing embryo?

Throughout embryonic development, the common and very important problem of how to pattern different cells and tissues correctly toward functional morphogenesis of the embryo remains one of the most fundamental questions in the field of developmental biology. Whether it's the determination of embryonic axes, setting up different neuronal cell types in the spinal cord, generating the timed formation of segments, or building the different digits of our hands and feet, these structures began with a community of unspecified cells that were somehow instructed to organize into a functional pattern.

What I would like to do in this tutorial is provide a conceptual framework for one of the mechanisms, developmental mechanisms, that regulate pattern formation in the embryo. That of morphogen signaling, in which a diffusible signal presented in a gradient can influence the development of different cell fates at different threshold concentrations. Before we can talk about morphogens, I need to make sure that you understand a little bit about cell-to-cell communication. In fact, what we're doing right now is very similar to what the cell does and how it communicates. I'm talking to you right now. And you are, in fact, receiving my signal. Received through your ability to hear through specifically specialized receptors in the hair cells of your ear.

There are two major forms of cell-to-cell communication I'd like to briefly cover. The first is called juxtacrine signaling and the second is paracrine signaling. Juxtacrine signaling is defined by close interactions between cells or between cells and their environment. Whereas, paracrine signaling refers to the secretion of a signal that is capable of

diffusing to interact with cells at longer distances away. How do cells carry this out on the molecular level? Juxtacrine signaling is usually carried out by receptor-to-receptor binding. This communication could be between two identical receptors in a homophilic binding interaction or heterodimerization could occur between two different receptors. Alternatively, juxtacrine signaling can also use receptor interactions with components in the extracellular matrix seen here surrounding the cell.

Similarly, in paracrine signaling, for a cell to be competent to respond to a given signal, it must express the appropriate receptor. However, the difference with paracrine signaling and juxtacrine signaling really lies in the fact that there is a clear signaling cell, one that is secreting some factor that will serve as a ligand to communicate information to any cell in its local environment, whether adjacent or many cell distances away. OK, so when one is communicating, a signal is being received. and one would hope that there would be a response to this signal. Sort of just like, I'm communicating these amazing things about developmental biology, your likely response would be to, well, become a developmental biologist.

A cell does something very similar. It receives that signal, but the big question is, how does it respond? What are those responses and how are those carried out? Here are two cells, one producing a ligand, packaged into a vesicle, and the receiving cell equipped with the correct transmembrane receptors. This cell also has a variety of other proteins within the cytoplasm, some of which link to the inner leaflet of the membrane. The nucleus here is represented with the genome simplistically illustrated by these three genes. The ligand is exocytosed and secreted into its environment. The ligand will diffuse until it comes in contact with its receptor binding partner. Successful binding and interaction of all necessary receptor parts yields an activated receptor conformation.

This means that the receptor actually changes shape, and does so in a way that facilitates its ability to interact with and activate other intracellular signaling partners. In some cases binding of these intracellular factors to the receptor then triggers the receptor to induce a change in those binding partners, resulting in their activation. This

is illustrated here as a color change from red to orange. And in this way through a cascade of conformational changes, the signal is transduced from one factor to another until the ultimate goal for cellular response, which in this case is the activation of a transcription factor complex that regulates the expression of specific gene targets. What if a lot of a particular signaling protein is being secreted? The concentration of a signaling protein can have a profound effect on the development of an embryo.

For instance, if I was to all of a sudden do this tutorial with a very loud voice, you would likely respond differently than if I were to present this tutorial with a very low voice. In a similar way, the cell responds differently to the way a signal is presented. Here a cell is secreting multiple ligands at once, leading to many receptors becoming activated. This, in turn, leads to the activation of a greater number of intracellular factors and thus increased transcriptional activation which in this example, results in the regulation of three different genes instead of just one or two. Regulation of the combination of genes AB and C will ultimately trigger a different cellular response, perhaps even cause this receiving cell to differentiate into an alternative cell fate.

It is important to mention that there are many branches to a signal transduction pathway that may result in other responses. Such as the regulation of the cytoskeleton causing cell shape changes or the ability to migrate. Or perhaps the regulation of a variety of biochemical processes to influence cell function. For the purposes of this tutorial I would like to explore the role that paracrine signaling plays in regulating cell fate patterning during embryonic development. I am referring to the developmental concept of morphogen signaling, which serves as a mechanism to create a pattern of different cell fates over a large area of cells.

I like to compare this to the analogy of differential responses to the presence of bubbles. There are few things that kids like more than bubbles. The more bubbles, the happier they are! In this way, bubbles can elicit different responses in kids. Here you see four kids located at increasingly farther positions relative to where the bubbles are being generated. The kids closest to these bubbles are showing, well, a happier more active

response, as compared to those kids located farther away. Morphogen signaling during embryonic development functions in very much the same way. Imagine this to be a plane of unspecified cells in some region of the developing embryo. Perhaps due to other mechanisms such as the asymmetric positioning of maternal contributions, some portion of these cells have matured enough to exhibit some differences in who they are and how they function such as these green cells here. These green cells start to express a signaling protein that functions, in this case, as a morphogen. At this point the other cells are still generally unspecified. But they are competent to respond to this morphogen as they express the appropriate receptor. As the morphogen is secreted, the cells closest to the source experience this signal first, but over time the morphogen diffuses across this plane, and over time becomes displayed in a concentration gradient. Such that those cells closest to the morphogen source experience more ligand-receptor binding and for a longer duration of time as compared to cells located farther away.

Over the course of this morphogenic signaling, cells show a differential response relative to the duration and concentration they are experiencing. As a result, these cells mature along different paths of cell specification. The outcome of all of this is the development of different cell fates along the morphogen path, such as in this example with skin cells developing farthest away, followed by neurons, muscle, and then blood cell types closest to the morphogen source. So now, knowing this mechanism is in place, this mechanism of morphogen signaling, how might you change it in order to create a different pattern.

Let's look at the butterfly wing again. Here is a butterfly that has four eyespots on each wing. Each eye spot is a different size and has differently positioned colors. These eye spots are in part created through morphogen signaling emanating from the central foci. How? What can be done to change morphogen signaling in subtle ways to establish different patterns? Whether it's eye spots in the wing or the number of digits in your hand. Let's return to our example of a population of cells with a signaling source and a plane of competent responding cells. The morphogen is secreted and a concentration

gradient of ligand-receptor binding is seen across this plane. This morphogen gradient yields a pattern of differentially specified cells. Can we change this concentration gradient in any way? Think about it. How would you change or influence the secretion of this protein to alter the gradient?

What if you place obstacles in the path of diffusion? As seen here, these branches greatly impede the flow of bubbles. In the cellular context, these obstacles would be related to the stickiness of the extracellular matrix. Here the matrix does not particularly restrict diffusion. However, should the composition of the ECM be altered in any way, that does obstruct efficient secretion, then it could profoundly reduce the distance a morphogen diffuses as well as sharpen the shape of the gradient. OK, so altering the ECM is one way to change a gradient. Can you think of any others? Let's harken back to our bubbles. Is there anything you can think of that perhaps bubbles don't like? Maybe wind. Wind blowing directly against a flow of bubbles will dramatically impact the direction those bubbles move.

Similarly, antagonists at many levels of pattern formation exist that specifically attenuate or alter the shape of a morphogen. Here is our example of a morphogen gradient. An antagonistic signal could be secreted from the opposing direction to the morphogen, illustrated here as yellow secreting cells. This particular antagonist physically binds to the morphogen and functions as a competitive inhibitor. This inhibitor is also secreted and therefore presented as an opposing concentration gradient, which results in reducing the number of receptors that receive bound morphogen. As a result, the pattern of cell specification across this plane of cells changes.

Morphogen signaling is truly one of the most significant developmental mechanisms that enables the patterning of an array of different cell types throughout embryogenesis. Amazingly, the complexity of pattern that exists in a multicellular organism is achieved with a relatively modest number of different morphogens. To name just a few morphogens like Wnt, Hedgehogs, BMPs, Fgfs. These have all been co-opted to be expressed in different tissues at different times, and by simply changing how these

morphogens are secreted, displayed, and interpreted has provided a seemingly infinite way to establish much of the remarkable diversity in form.