Introduction to Developmental Biology

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Lecture-13

Cell-cell communication (Part 1 of 4)

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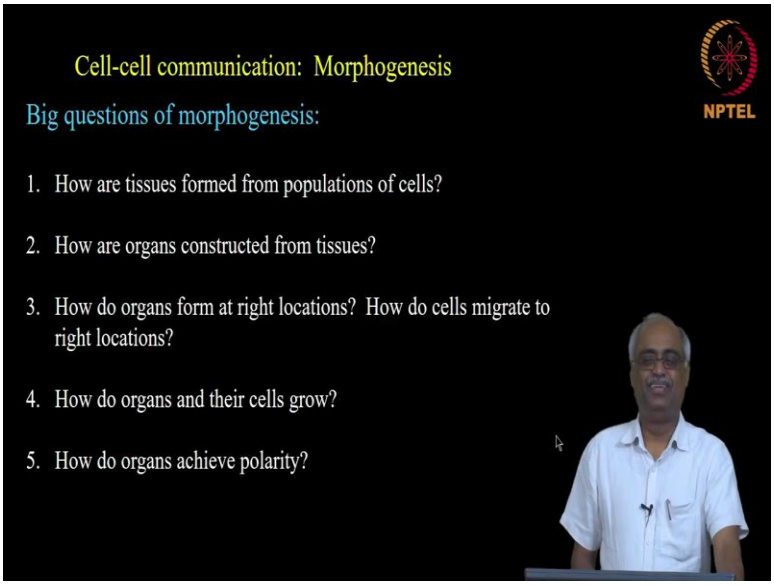

So, now let us get back to developmental biology. So, where did we leave last? Differential gene expression. So before gene expression, we were discussing early embryonic development like pre-formation, epigenesis, etc. So, the next thing we are going to learn is, morphogenesis and in today's lecture, we will primarily focus on cell adhesion, cell shape changes, and differences in their affinity for each cell type. But the underlying thing in all of this is differential gene expression so that is the core of all the changes. So how are tissues formed from a population of cells? So, there are populations of cells but each cell type has to organize into a particular tissue type because organs are made up of multiple tissues. Here the example used in the book is the eye development. The eye has a retina, cornea, lens, and so on; all of them together is an organ, the eye. So, now each one of these things like for example retina is a tissue and that is not made up of only one kind of cell. So a tissue is made up of multiple cell types for example here you

have pigmented retinal cells and neural retinal cells. Sometimes you can have multiple kinds of cells in one tissue. So, you need to understand this hierarchy very well. So a tissue by itself does not make an organ or in other words an organ may not be made up of a single type of tissue so it may have multiple tissues that is why the difference between a tissue and an organ comes into the picture. Then there are organs in the body and within an organ also the tissues have to be organized correctly. So, these questions concern about these aspects, like how the tissue forms correctly.

And then how are organs constructed from tissues? The right tissues have to be in the right place to make the organ. For example, the retina has to be at a certain place regarding cornea to make a functional eye otherwise you would not make the functional eye. You could have the tissue but then if they are not at the right places then it would not form a functional organ. So, how does that happen, and then the organs have to be at the locations; there is no point in a particular cell type differentiating into cornea and retina in your spinal cord or somewhere. It should form in the face at the right place and it should have the right neuronal connections to the right part of the brain. So, how do they all get to the right places and form those structures? And these processes involve cell migration as well. So, how do cells migrate correctly? There are some tissues formed at the right place with no big migration but there are certain other cell types that migrate a lot, like blood cells. For example, the somatic gonad is formed elsewhere compared to where the primordial germ cells (PGCs) are formed during embryonic development and PGCs migrate to the right place. Once they get to the destination they stop migrating any further. So, how that is controlled? Then once let us say the right cell types formed right tissues and tissues in the right location from the organ and organ is at the right place. But it still needs to grow; your eye is not the same as how it looked when you were born. So, how they all grow proportionally? So that is very important. So how does that happen?

And, the organs do have polarity like from a limb bud when hand grows fingers are not found anywhere in the hand, it has be at one end. So, there is a polarity to the structure; every organ has polarity. So how that is achieved?. So, these are all the questions that come under morphogenesis. So, we are going to get a glimpse of this we are not going to get detailed answer

for all of this then there will be no research left for developmental biology. So we are going to get an idea of how these processes happen. So that is today's topic.

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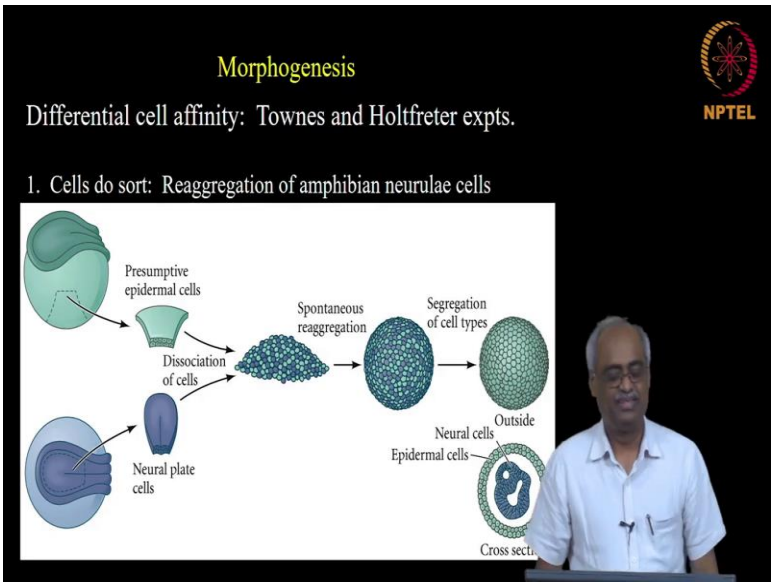

So like any other topic, we start with historical beginnings, like how people started looking at it. So, this is one of the classical experiments. Fortunately, during the frog embryonic stages, the blastomeres, the cells in the early embryo can readily dissociate if you put in an alkaline buffer. So, these scientists took advantage of that, they isolated cells in that way. Like for example, you take two different pigment colored frog embryos, so one is epidermal cells and the other is neural plate cells, and then you heap them together. So now they do not stay like that instead they reorganize. They seem to know where to get like the dark-blue knows that it needs to get with all other dark blue cells and get away from the light green one and they separate in this fashion shown in the picture spontaneously all by themselves.

So cells do sort, that is the main observation from this experiment. Then when they tried with the different germ layers and then observed what happens.

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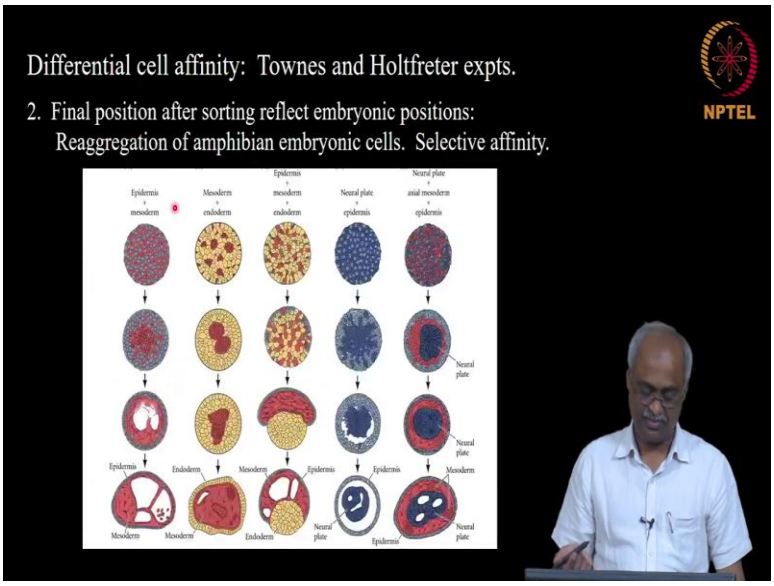

So, this above picture is a summary of those experiments. So, the summary here, the first one is the cells do sort they do not remain randomly. Final position after sorting reflect embryonic positions, in the natural embryo where a particular kind of cells will go and that is where they go even in this in-vitro condition when they are allowed to sort themselves. For example when you take epidermal cells the blue colored ones, mesodermal cells the red colored ones and then mix them and wait for some time, then they migrate and then you will see the epidermis is outside mesoderm is inside.

So the same happens in normal embryos too, the ectoderm will be outside and the mesoderm will be inside. And then you do the same experiment with mesoderm and endoderm and here you find they separate but the endoderm is outside mesoderm goes inside when you have these two alone which is not the embryonic localization but when you mix all three, then you get the same as what happens in the embryo. So endoderm, mesoderm, and then you have epidermis that is the ectoderm. So, they sort that way.

So few more examples, if you see here, the neural plate versus epidermis. So within a single germ layer like from the ectoderm, you get neural cells and then the epidermal cells. If you could remember the pigmented skin surface cells come from the same progenitor as the neurons, so they again separate. And when you have more varieties and mix them, they again separate the way they would normally do in an embryo.

So this suggested to Holtfreter that there is selective affinity, so each cell knows with what it wants to interact and with what it wants to avoid interacting. Like for example, the mesoderm and the epidermis, they seem to interact and the epidermis seems to always avoid the endoderm and so on. So, there he proposed this idea that cells have a selective affinity. So now we are going to look at what is the basis of selective affinity. How do they determine that a particular cell has to be outside and another cell has to be inside?

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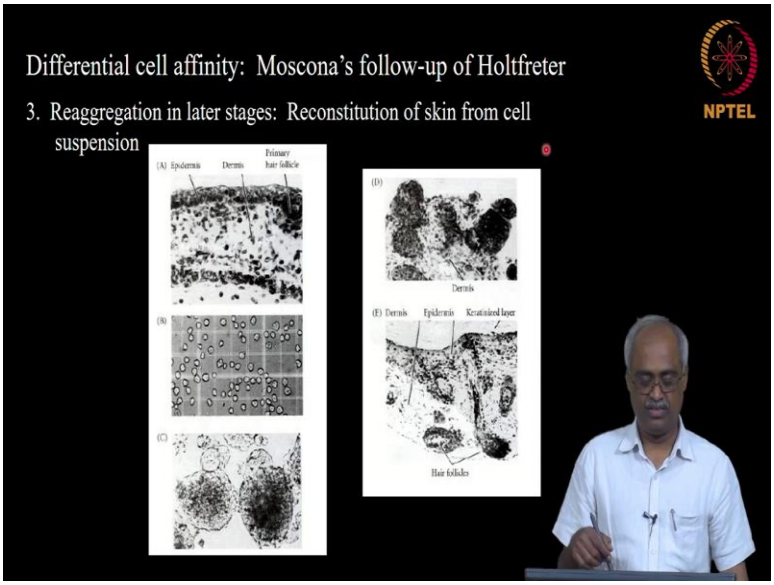

So this is not true just in the embryo, it is also true in the adult body as well. Like for example earlier alkaline solution was easy and they could isolate the frog embryonic cells but now we know how to separate cells even from adult tissues, for example, trypsinization and so on. So when you take skin and separate the cells into a pool of cells and allow them to organize and they finally organize back into the original organization like the epidermis on the top, dermis below, and hair follicles within that. So, they organize themselves.

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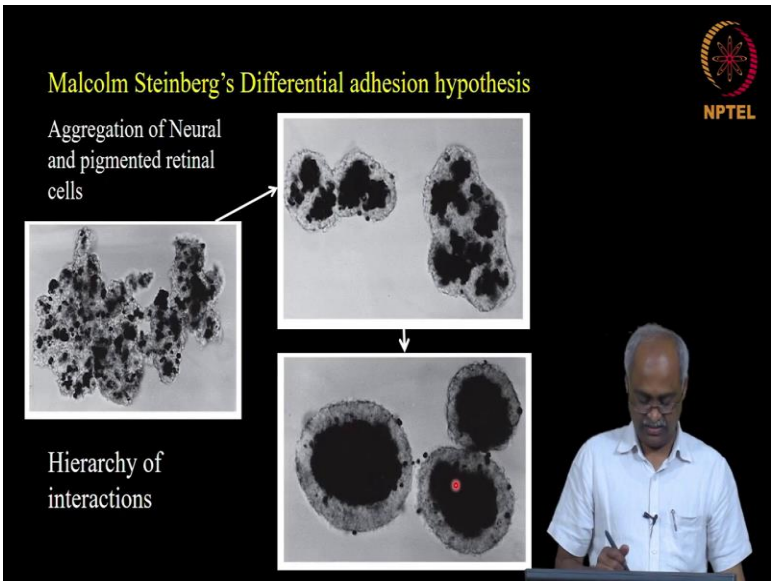

So, the next idea is how do the cells know where to go and for that Malcolm Steinberg proposed a theory called the differential adhesion hypothesis. So, they are still working on it; he proposed this differential adhesion hypothesis in 1964. It is quite simple, he proposed that the surface tension of different kinds of cells differs. And when they are mixed they reorganize themselves such that the overall surface energy is minimized. The embryonic arrangement is an equilibrium of these forces. So, the cells organize such that the final structure is thermodynamically more stable. So, cells having higher surface tension or force of attraction among themselves, go to the central part of the embryo. So, that is the differential adhesion hypothesis is. So like for example here, the neural and pigmented retinal cells; the retina is a tissue having two types of cells if you remember our very first point we discussed in the first slide. So, when you dissociate and mix them then you find whatever Holtfreter observed. Similarly here you have the pigmented cells go inside and the neural cell stays outside. And people in his group tried to measure the surface tension of individual cells and they found proof for his original proposal and that is what is shown in the next slide.

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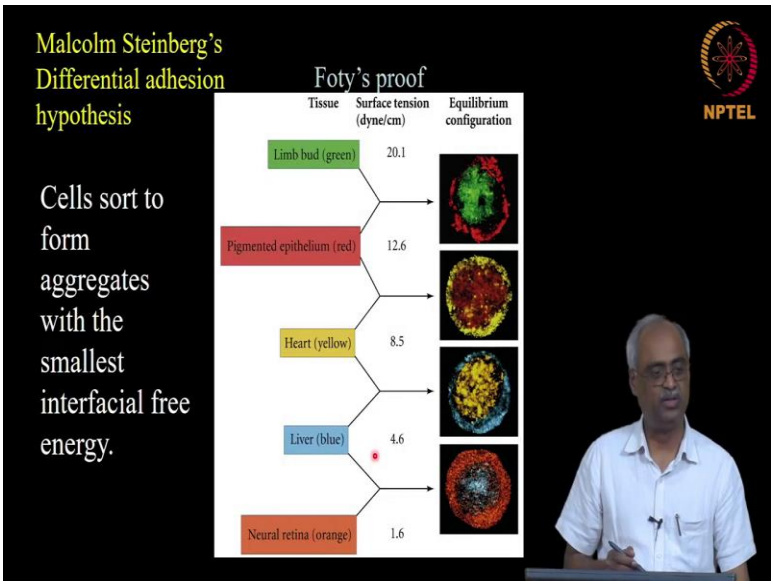

So, here you have the two kinds of cells that are being mixed in two different colors like here green and red; red and yellow; yellow and blue; blue and orange. So, in each pair, if you see, the green has higher surface tension than this red one. So, the green goes inside. In the second one between red and yellow, yellow has lower surface tension. So as a result that goes outside and so on to the next two pairs as well.

So, cells sort to form aggregates with the smallest interfacial free energy. So, one of his students Foty, measured, and showed this is what happens. So next, what is the molecular basis of this? how do the surface tensions change? So initially people thought cell membranes are simply a lipid bilayer and therefore all cell surfaces were the same. Eventually, they found that there may be molecules on the cell membrane and that is different from each cell.

So the molecular basis for this is extremely simple. So, only one group of molecules seems to be sufficient for this and they are the cadherins.

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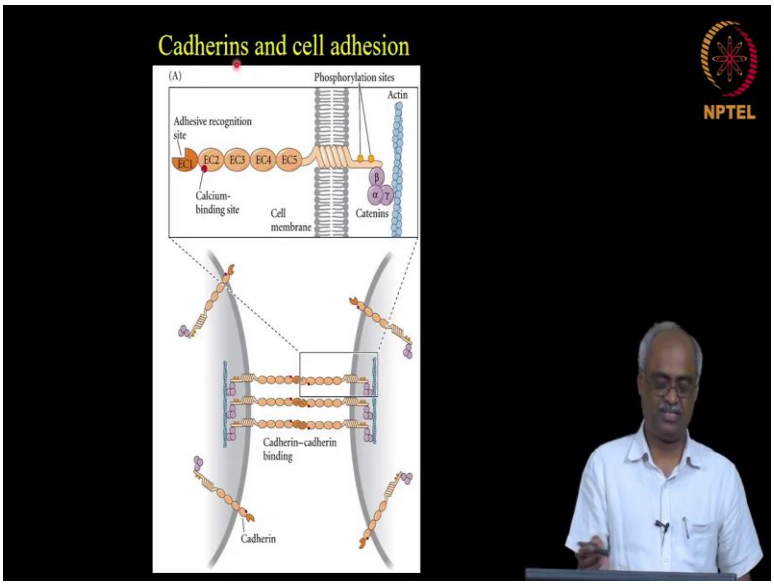

Calcium-dependent cell adhesion so that is what this word stands for. So, it is a calcium-dependent adhesion mediating molecule. So, these are the really important molecules for cell adhesion for the rest of the class we are going to talk only about cadherins, different experiments involving cadherins.

So this slide, this is how a cadherins structure looks like. So they are embedded in the membrane. They are transmembrane proteins, outside you have multiple distinct domains that bind calcium. So, there are calcium-binding domains, in this particular one there are five domains and the very end of it has the perfect shape for interacting with another cadherins molecule from an adjacent cell for example as shown in the next one the lower picture. And if you look into the cell inside the plasma membrane right side here. So, there are sites where they can be phosphorylated. So that is one way of regulation and then you have these catenins binding there and what is not shown is the involvement of small GTPases called Rho GTPases, that we will learn as we go through. Then these connect to the actin cytoskeleton which is on the inner periphery of cells.

So this is how the internal cytoskeleton is connected to the extra molecule that goes and interacts with another cell via integrin. So the cadherins of adjacent cells interact and that is how the cells are kept it together and these cadherins are not simply anchored on the membrane they extend into the cytoplasm where they interact with the actin cytoskeleton itself.

These can be regulated by the small molecules there, for example, the phosphorylation can cause the cadherins to dissociate from the beta-catenin or these catenins may be brought here or removed from here. Actin polymerization and depolymerization also can affect this. Therefore changes that happen inside can affect the external adhesion and opposite as well. So, those are going to become clearer as we go through the rest of the slides.

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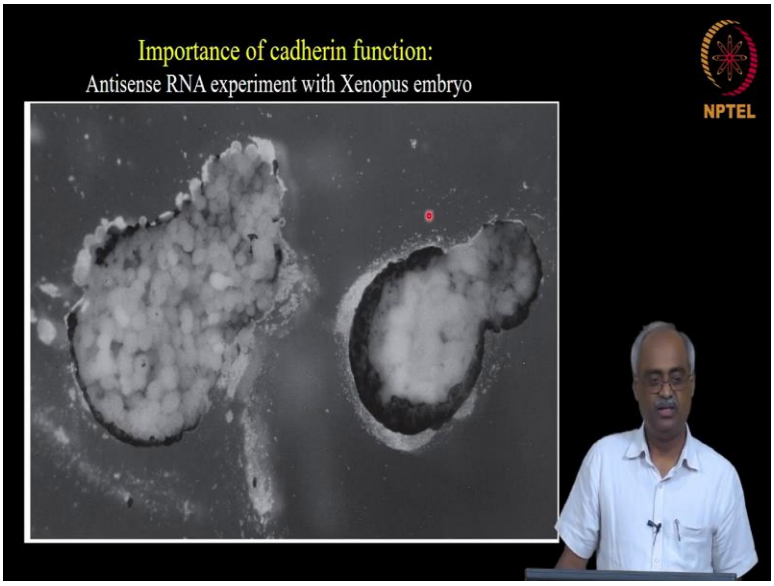

So I said cadherins are important how do we know that cadherins are important? So this is an experiment for that. So, this is a frog embryo where you have anti-sense RNA injected against cadherins and when you open the animal cap these cells fall apart loosely but in the wild-type, they remain indicating that the cells adhere normally and that adherence is gone when you have stopped producing cadherin proteins. So this is one experiment to show the importance of cadherins during embryonic development.

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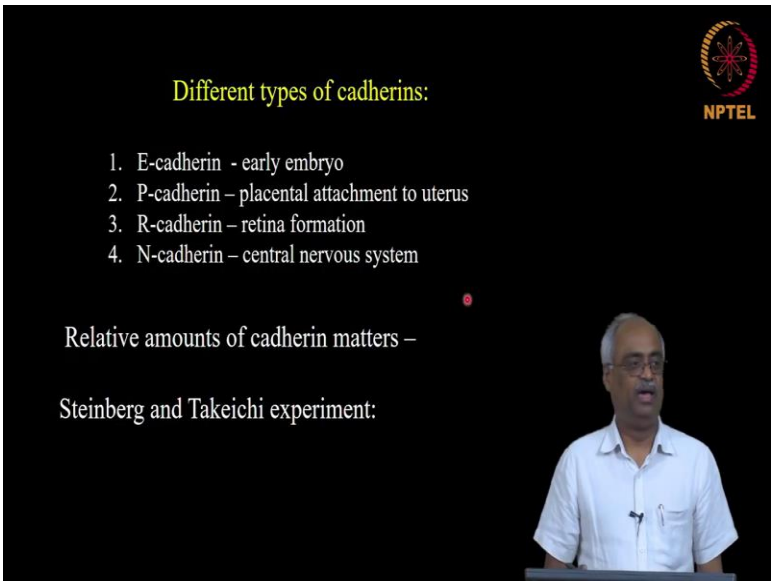

Since they are important there are variations in them as well. So, there are multiple types of cadherins like embryonic cadherins which is slightly different from the placental cadherins which is important for attachment to the uterus. Like dynamic changes in cadherin production is very important when the fertilized oocyte comes to the uterine walls, there it has to get anchored otherwise it is not going to survive and will be lost. So that requires cadherin's attachment of the embryo to the uterine wall.

Then there is a retina-specific one called R-cadherins, the nervous system-specific one called N-cadherins, and so on. And the relative amounts of cadherins matters. So the different surface tensions are primarily based on the concentration of cadherins on the cell membrane. More cadherin, then higher the surface tension therefore more central location in the embryo. So, that is what we are going to see from the subsequent experiments here.

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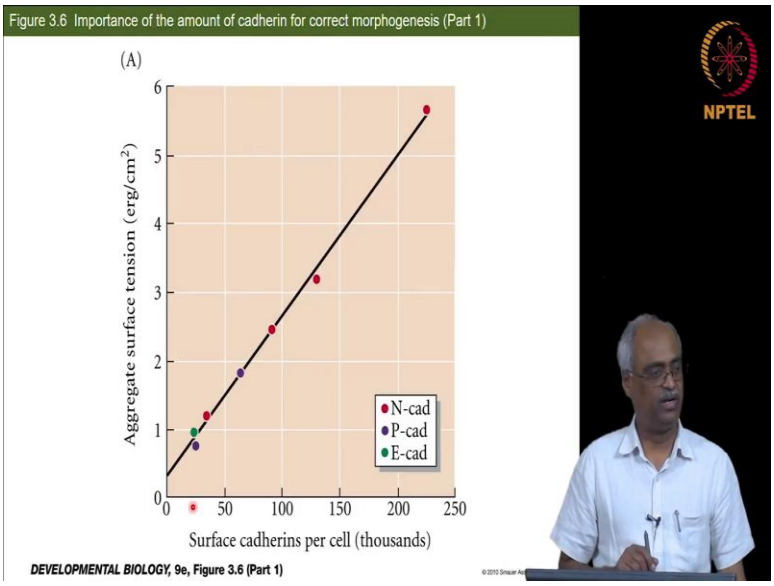

So they did a series of experiments, and we are going to look at one of them. So, here they took genotypically and developmentally identical cells, and expressed different amounts of given cadherins and found the aggregate surface tension linearly increasing. They also observed its consequence.

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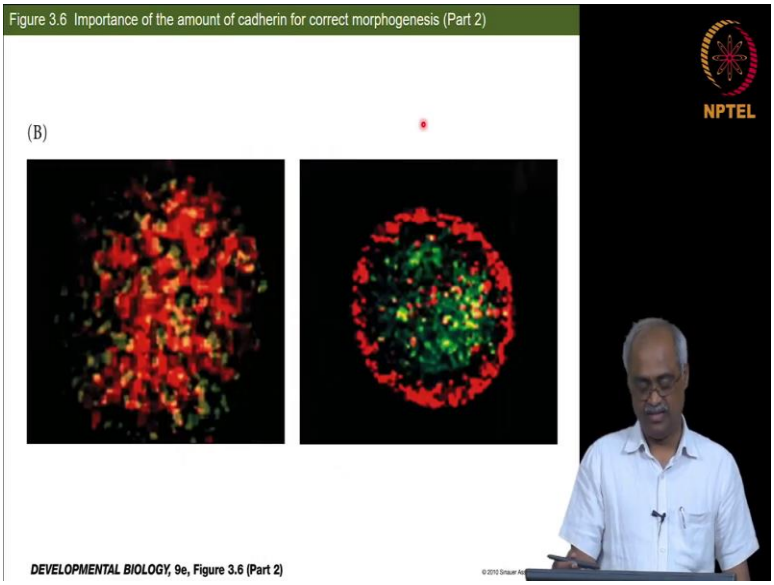

So here there are two cells, the green and red cells, which are identical; just that the green cells express more cadherin than the red cells so as a result, the green one goes inside. So, they did this experiment with a variety of cells with different cadherins and a varying amount of cadherins, and they convinced themselves that the amount of cadherin present on the cell surface matters for where a given cell is going to be present in the embryo.

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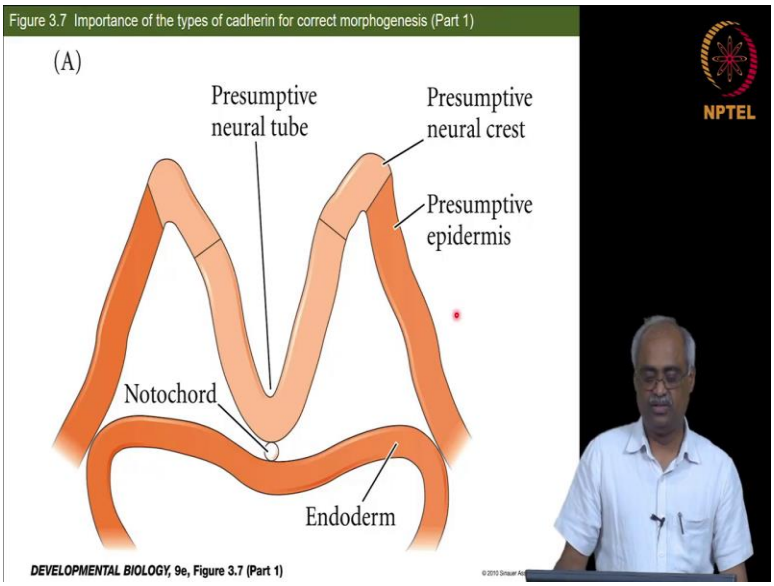

So now, we are going to look at some of the specific cadherins and their role. So, this is a cartoon of a mouse neural tube. So, in the slide, you see the neural crest, so the neural folds are going to come together and form the neural tube. So notochord is seen below the fold, and the endoderm is found below the notochord. So, the epidermis and the neural tube cells come from the ectoderm. Now we are going to focus on how these two separate folds, the neural crest come together to form the neural tube.

While these epidermis cells are going to separate away, they are not going to fold into a tube instead they are going to form a sheet. So this difference is brought by the neural crest cells which start to express the called N-cadherin and that cadherin is not expressed by the epidermis cells. Both neural and epidermis express E-cadherin because they are still embryonic structures. But neural crest cells beginning to express N-cadherin is crucial here.

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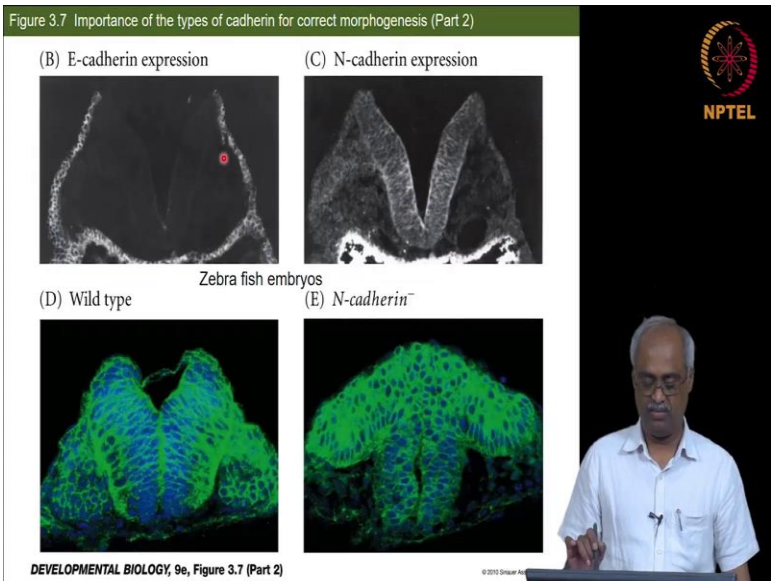

So that is shown in the actual embryos here. So, Figure (B) is a cross-section of an embryo where E-cadherin is on the epidermal cells so, this is the same as the cartoon shown in the previous slide. So, you see the E-cadherin expression but this neural tube part starting from the crest does not express E-cadherin instead they have the N-cadherin there (Figure C). And that is what allows them to separate from the other ectodermal cells; the epidermal cells and form the fold (Figure D). And when you prevent the N-cadherin expression then there is a heap of cells (Figure E), they do not separate from the epidermis. Although the epidermis and neural tube have become different, they do not separate. Therefore for this separation differential regulation of the cadherin genes becomes critical.

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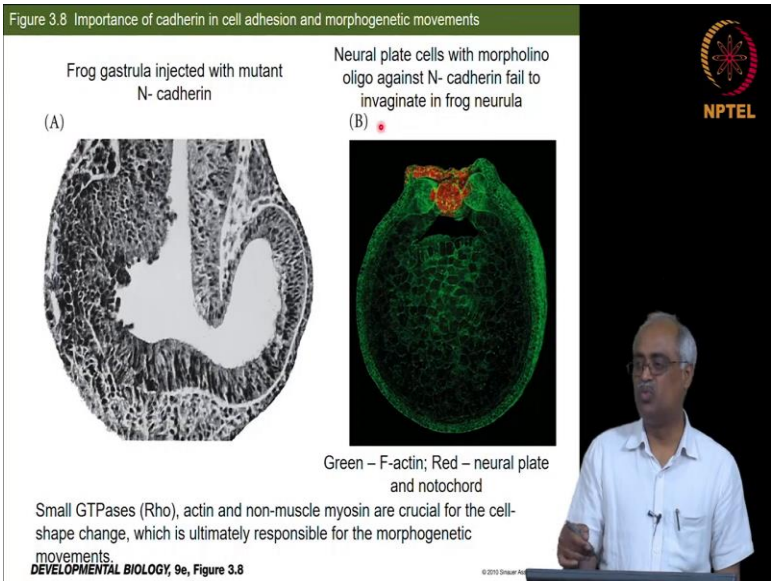

So, we are going to see more examples of the same. So, in the slide, figure (A) is a frog gastrula, earlier stage than the mouse cross-section that we saw in the last slide. So, here the expression of the N-cadherin in the left half of the embryo is prevented, so as a result the epidermal and neural cells do not separate and they remain mixed while in the other half they separate neatly where the epidermis goes outside.

So figure (B) is a later stage of an embryo, the Neurula stage where the neural tube is forming. The top sheet of cells, shown in red color is notochord and the neural crest cells, so they should invaginate and they do not do it when you block the N-cadherin. So, the green-colored are F-actin. So, one of the important steps where cadherin's differential regulation is crucial is when the cells need to migrate to form a new shape. So one of them is this neural fold which makes the neural tube. Let us see other examples as well. So in Drosophila, there is a ventral furrow formation where mesodermal cells migrate inward. So, in the frog gastrula example, it is the ectodermal cells that are migrating. So, in these kinds of situations, these migrations are brought about by cell shape changes which are primarily done by differentially regulating cadherin expression which is again connected to these proteins small GTPases and non-muscle myosin and their interaction with actin polymerization.

So these small GTPases expression and their interaction can be regulated differentially and that is what eventually leads to the proper formation of tissues. So, we will see that when we get to cartoons that explain that. So, the primary point here is the morphogenetic movements like invagination, in this particular case is primarily brought about by changing the cytoskeletal organization as well as cadherin expression and in this process, the molecules like rho GTPases and non-muscle myosin play a very important role.

So essentially they reorganize the cytoskeleton and that in turn changes the cadherin adhesion. So, these are the ones that are very critical for morphogenetic movements.

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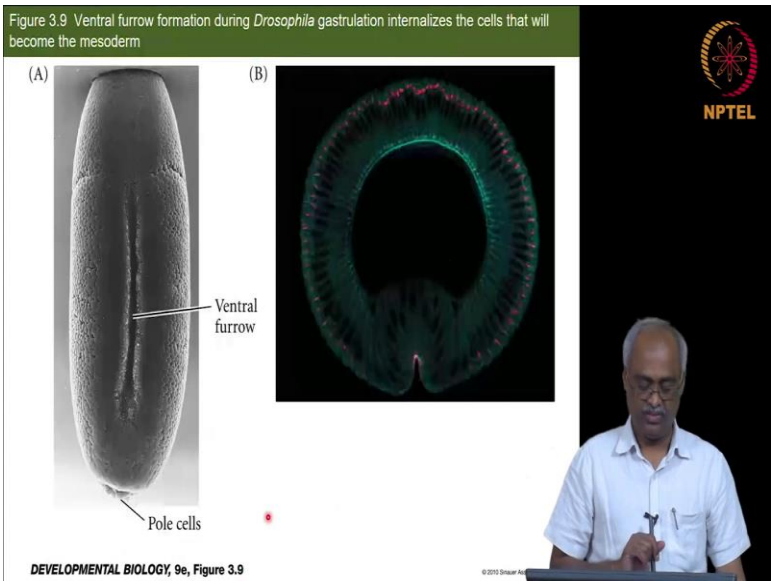

So we will see another example and then we will get to a cartoon where this becomes abundantly clear. So, figure (A) is an SEM image of the Drosophila embryo showing the ventral furrow which is at the base of the embryo-like on your front belly side. So, the ventral mesodermal cells start to migrate inward and that is how these furrow forms and this migration is made possible by a change in the shape of the cells here. So, figure (B) is the cross-section of the embryo. So in this figure, you see a protein that is differentially expressed; the expression is faint in the inner region called the basal where the basement membrane is present, instead the protein is seen in the outer region which is apical. This particular protein gets localized in the central region as well. So, this becomes very clear when we get into a cartoon which is in the next slide.

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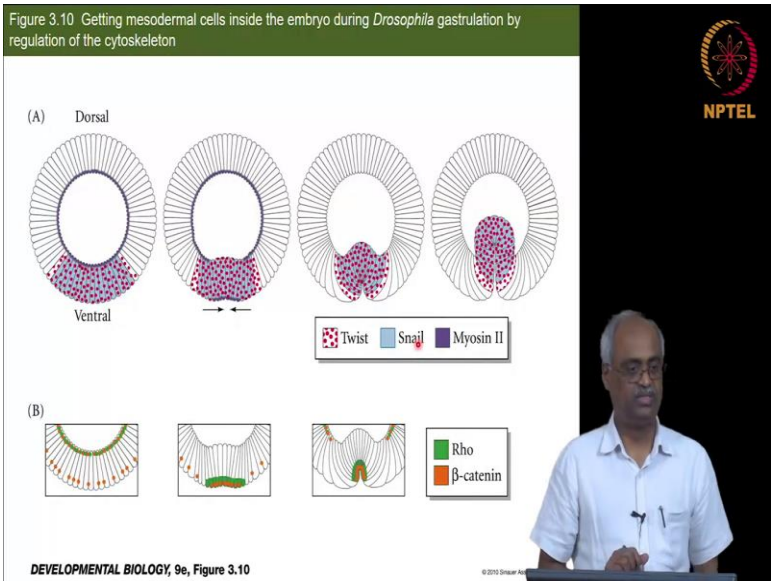

So, this slide gives you a good idea of how these changes happen. So, the first figure in (A) is the same cross-section that we saw in the previous slide but an earlier stage than that where invagination becomes visible. So, here the non-muscle myosin equivalent in fly Myosin II is present on the basal surface. So, basal is where the cell is attached and the other side, the exposed side is apical. Like in our intestine, inside the intestine would be the basal and what is facing the lumen would be apical. So here these transcription factors twist and snail are activated only in the ventral side that eventually leads to this non-muscle myosin migrating to the apical side and as a result, the ventral region is going constrict and when it is constricting it is going to open up. Like if you take a small cloth purse kind of thing where you have the string and you pull the string then that becomes coming closer. So, that sort of a thing happening here makes these cells open up and they move inwards. So, simply cell shape change makes them take finally this shape of the tissue itself.

So, the molecular changes are explained here in the lower panel. As I mentioned earlier, the small GTPases and non-muscle myosin and their interaction with actin are crucial for this movement. So here the Rho and the beta-catenin(critical for attaching cadherins to actin) are localized on the basal. So, in the early stage, the beta-catenin is distributed all over as you see in the figure but Rho is primarily seen in the ventral region. And with the twist, snail expression they now migrate in the ventral cells only. So the subsequent downstream activation of genes lead to the relocalization of the Rho and beta-catenin to the apical surface on the ventral cells only, not in the rest of the embryo and now by interacting with the actin, they try to pull the actin cytoskeleton closer and that in turn out affects the cadherins present outside as well and that changes their interaction such that they start to constrict in the apical side and the basal side opens up internally.

So, this is how cytoskeleton changes and varying cadherin expression is crucial for morphogenetic movements, and the small GTPases variety called the Rho GTPases to play an important role in the reorganization of these molecules.

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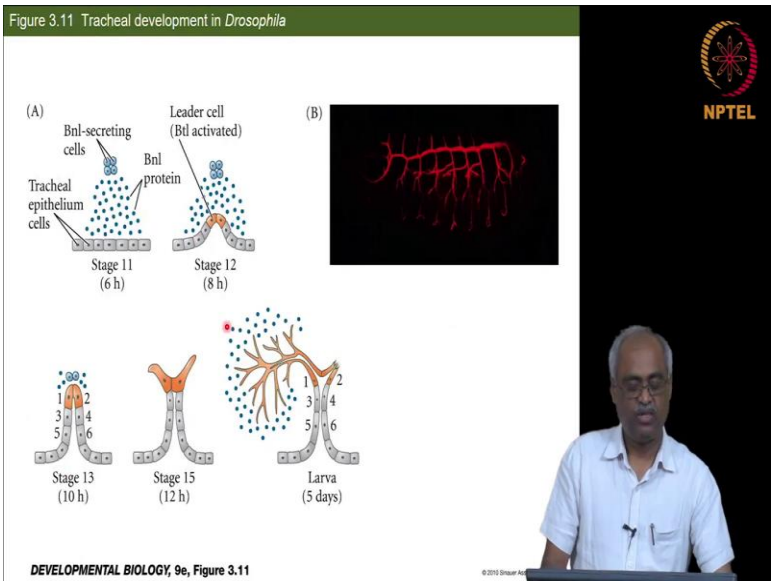

In the previous example, the changes were happening within the cell because the snail, twist expression is within those ventral cells but these morphogenetic movements can happen even for an external cue and that is shown by this Trachea development of Drosophila. So, the Bnl-secreting cells are called branchless, because the mutant does not make branches so therefore the gene is called branchless. So the wild-type gene will be required for branch formation so that is how usually genes are named. So, the branchless is secreted by these Bnl cells and that attracts these sack-like structures called the tracheal epithelium. So these secreted molecules will make the epithelium to move or one leading cell will start to move towards the cells secreting the branchless. And that branchless is what leads to this reorganization as you see in the figure. So the cells continue to migrate and other cells simply through their adhesion via the right type of cadherin of the right quantity follow the leader two cells (orange-colored). And then these leader cells change their shape as well, if you see the tube in stage 15 is formed by the migration of these leader cells. So the next branches are going to happen by the same leader cell they form a unicellular structure. So a simple change in the cell shape can make these branches. So these structures are not made by multiple cells. A single-cell branch out like this by reorganizing its actin cytoskeleton and the cadherin adhesion. So, this is a view of the tracheal system of the Drosophila. So, either through differential gene expression within these cells or through external cues the cell's cytoskeletal and cadherin reorganization leads to migration as well as shape changes. So this is how some of the morphogenesis happen.

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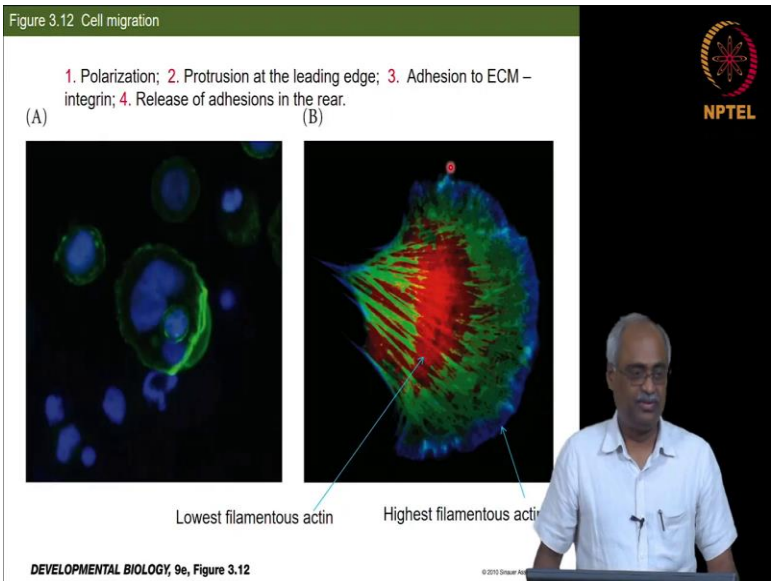

So far we saw a set of movements that is primarily of epithelial cells and all that is from a sheet of cells. So, cell migration happens readily in mesenchymal cells as well but the mesenchymal cell migration is somewhat different and here you have four steps. First, the cell will have to polarize, and second, there is the leading edge, the direction in which the cell is going to migrate is where the cell shape changes as if it is going to make a protrusion or forms a pseudopod kind of structure. And third that edge attaches to a surface that is the adhesion to the extracellular matrix in that direction then the other side is the posterior; once polarization starts the cell has the anterior and posterior. Finally, the direction of migration is anterior and from the other side, the cell needs to get rid of the adhesions. So, these are the four steps and these four steps are brought about primarily by reorganizing the actin cytoskeleton. So in figure (A), the green color shows the polarized arrangement of actin, and the other side does not have much of it. So the organization of actin into microfilaments makes the one side of the cell polarized and that is the migrating direction. So that is anterior and then the actin eventually pushes the plasma membrane such that it why takes a shape.

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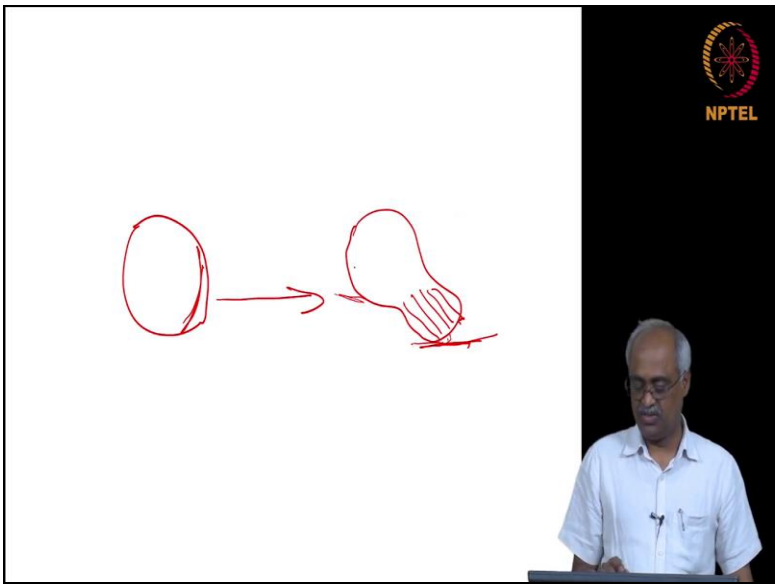

So if you see the slide, the actin forming in one end of the cell pushes the plasma membrane such that the cell forms a protrusion. So, this is because of the way the actin microfilaments get organized in the edge. And now this edge produces cadherins and other molecules such that it attaches to the ECM here, and that is what you are seeing in the slide. So, in this particular case in figure (B) in the previous slide, what they have made is one large structure called lamellipodia and sometimes it could be a single filament like thing called as filopodia. And in this figure (B), what you are seeing is the different concentration of the filamentous actin. So actin is a globular protein when multiple globular actins joined together they form filaments so they could either be a filamentous or single molecule. So blue is the highest of the filamentous and red is the lowest. So, the image is color-coded. So the filamentous actin is more in the migrating side in the lamellipodia part and this leads to adhesion to the ECM and then the fourth step is on the other side where the attachment is detached by producing proteases that would cleave the attachments and then the cell is released. So this is how the cell migrates. It is kind of a clumsy migration but it is good enough for cell migrations to happen in the timescales of development.

So this is how cells migrate normally. So these migrations as well as what we saw in this particular context, the Rho GTPases and actin these are crucial for morphogenetic moments. So, the main message is these movements do not involve a variety of molecules simply by changing the expression levels of different cadherins you can have one cell type as we saw in the epidermis and neural plate cells. So by having two kinds of cadherins and by changing their

levels, you make the separation and sorting of cells as well as cell migration. And these are crucial in different structure formation at different time points. So, these expressions are dynamically regulated. For example, the placental cadherin expression is required when the embryo comes out and for it to attach in the uterine wall. So in this particular example, other molecules are also important; you have to change the ECM concentration as well and all of that put together helps that adhesion. So, the type of cadherins, their localization, and their concentration are spatially and temporarily regulated.

So, I stop here because today I wanted to focus only on a morphogenetic moment and the role of cadherins.