Introduction to Developmental Biology

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Lecture-16

Cell-cell communication (Part 4 of 4)

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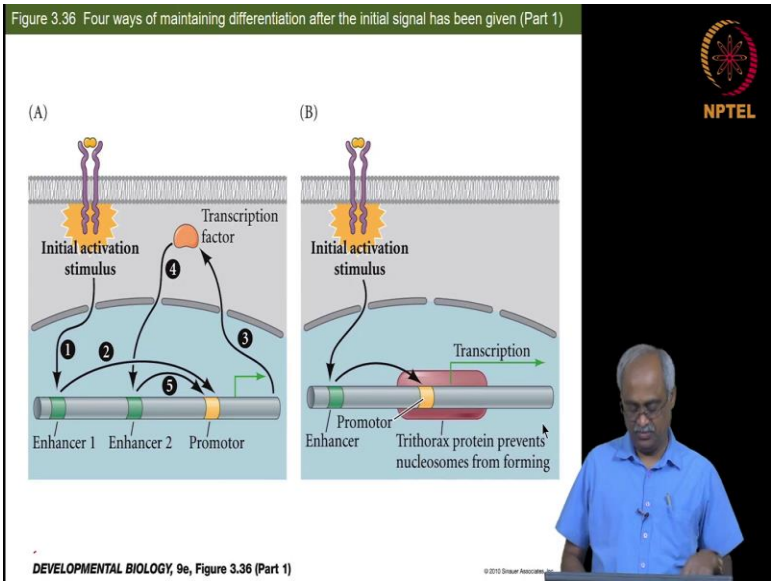

So as a continuation with the cell signaling, let us see the next important step after the signal transduction occurs in a cell. So how is that differentiated state is maintained? So, how do you keep that state of transcription, either on or off. So we will look at it in the next couple of slides. So, there are four different mechanisms by which this happens.

In one situation, once activated, that particular transcription factor may bind to another enhancer and continue to activate the transcription. The product itself is an activator. Here in (A), you see two different enhancers; initially, activation may be via one enhancer, and then it works via the other one once the product is produced. And the second one is a permanent chromatin modification, permanent meaning during that particular organism's lifetime in that particular differentiated tissue; you may have these proteins like Trithorax complex proteins binding and

preventing the nucleosomes from assembling there. So they maintain euchromatin status. So, this is another mechanism.

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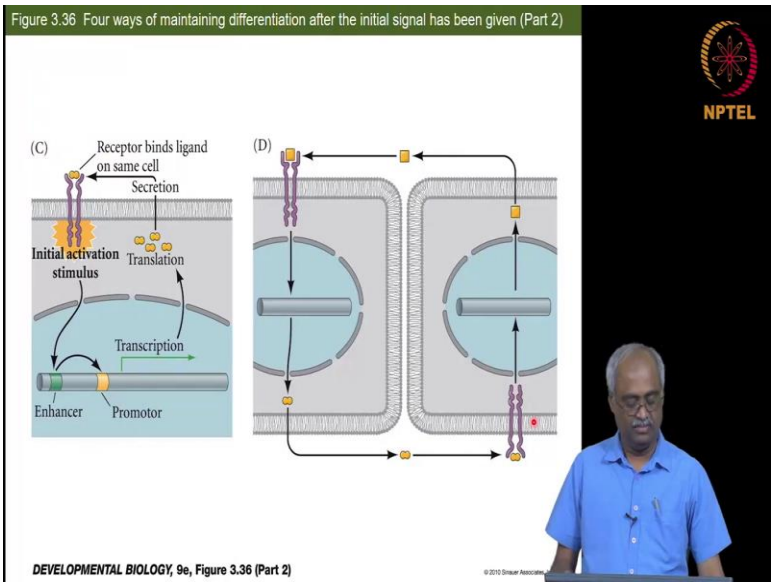

And the third one is one of the downstream activated targets, maybe coding for the ligand that binds the receptor; therefore, it keeps cycling. A small variation of the third one is, the ligand is produced by an adjacent cell and keeps activating the pathway. In the *C. elegans* germ cell niche, the somatic cell produces the Delta ligand that binds to the germ cell that has the Notch receptor, and they need to do this to maintain their mitotic potential continuously. So, there are situations where the ligand produced by the adjacent cell continues, and that is essential. So, these are the four different models to explain how the altered state of transcription is maintained; I am using the word altered because it is not always activation or inactivation.

So, the next, we are going to look at the ECM, extracellular matrix.

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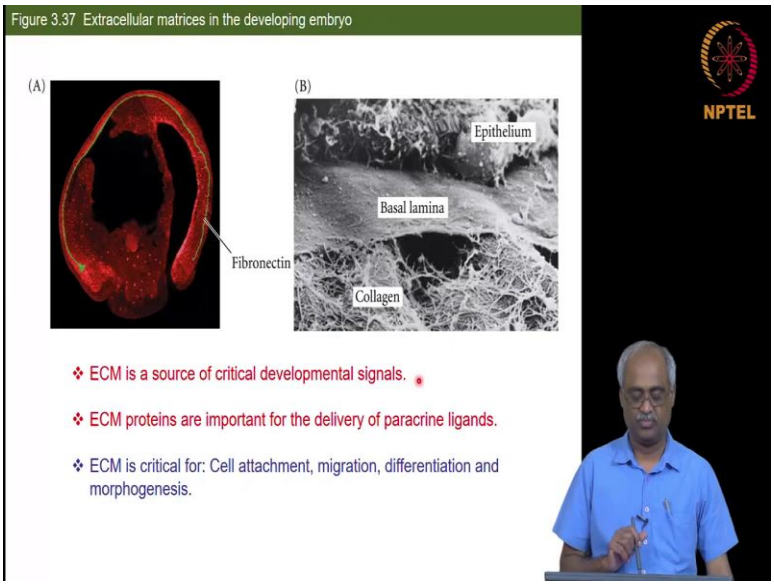

First, let us familiarize with what ECM is and its components and then look at how it affects development. So, figure (A) is an embryo that shows fibronectin expression, a particular component of the ECM, which is essential for embryonic development. So we will see details of that, so this figure (B) shows you the ECM. The top area is the epithelial cells, and below this mesh-like structure is the mesenchymal cell. So the epithelium produces an ECM which is made of a protein called the laminin, and that forms a mat-like mesh, a densely woven sort of mat-like mesh. This sheet that you see where it is labeled the basal lamina is the ECM secreted by the epithelial cells primarily made of laminin. And below that, you have the ECM coming from the below mesenchymal cells; there you have the collagen, fibronectin, then proteoglycans, primarily heparin sulfate and chondroitin sulfate. So these components form the mesh-like structure, and they are the components of the ECM. Collagen is the major protein in ECM, and the other one is the fibronectin. Fibronectin helps in connecting the cytoskeleton to collagen and proteoglycans. These proteoglycans are made of protein and carbohydrate, but the carbohydrate portion is large compared to the protein part. If it is the opposite, you call that as glycoprotein, so they are also present in ECM, but the proteoglycans are the main components. In ECM, collagen or proteoglycans will be bound covalently by fibronectin, that in turn, interact with another protein called integrin, which is a transmembrane protein, and these integrins connect to cytoskeletal components primarily at the periphery of the cell just below the plasma membrane.

So what kind of cytoskeletal material are present? A mesh-like network of microfilaments, so that is what integrin connects.

So, we will see that in the next one, but before getting into that, we want to know these three points. So ECM is a source of signaling for development, ECM is critical, and some of the ECM components like, for example, proteoglycans have a very high affinity for these signaling ligands like Wnt and so on. So they bind, and they help get the ligands concentrated for signaling via the plasma membrane-bound receptors. So, the ligand does not directly go to the plasma membrane; in between, you have an ECM, and there are ECM components that help facilitate this ligand-receptor interaction. And sometimes ECM itself is a source of the signal, remember we learned instructive induction and permissive induction; instructive means the inducer tells these are the instructions for a cell to execute, and permissive means a neutral conducive environment, that itself enables the responding cells to develop in a certain way. So, the permissive induction in ECM is one example of that, and I told you already the importance of facilitating the paracrine ligands like Wnt and ECM is critical for cell attachment, migration, etc., they guide the cell in which way to go. We will see an example of how differentiation happens in the presence and absence of ECM.

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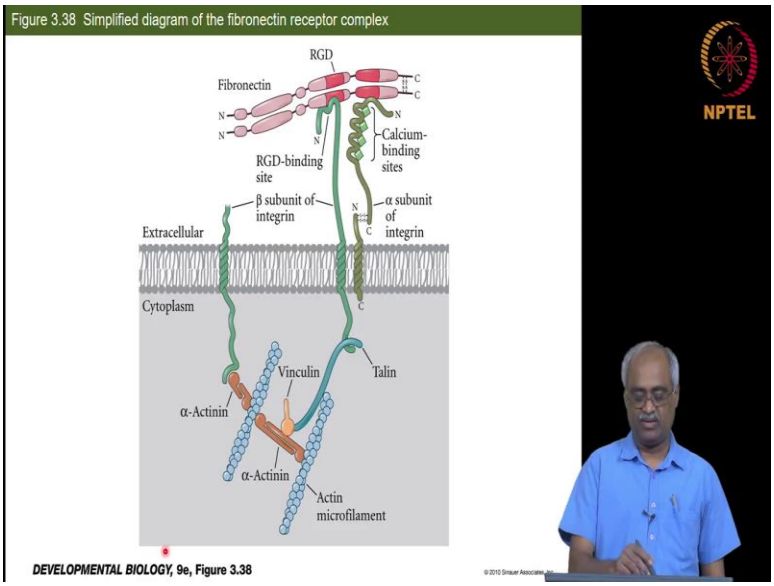

So, in this cartoon, we see fibronectin with its glycine aspartic acid repeats (RGD) that act as a place for these integrins to bind. So, the β-integrin goes as a continuous chain all through the

membrane, and then you have α-integrin, where you have this disulfide bond connecting these two. So this α-integrin and β-integrin attach to fibronectin, and fibronectin, in turn, binds to collagen and proteoglycans, etc. And these integrins inside the cell linked to the actin microfilaments as shown in this cartoon, you see two fiber like thing, but in reality, it is a mesh under the plasma membrane, very much looking like a complex mesh, but below the plasma membrane. So, there again, you have this Vinculin, Talin, and Actinin as intermediates in the interaction between integrins and the actin microfilaments.

So, this is how the structural components are linked to the ECM, which again links it to another cell; you need to imagine this whole thing as another one existing on top here so on the other side. So, that is ECM.

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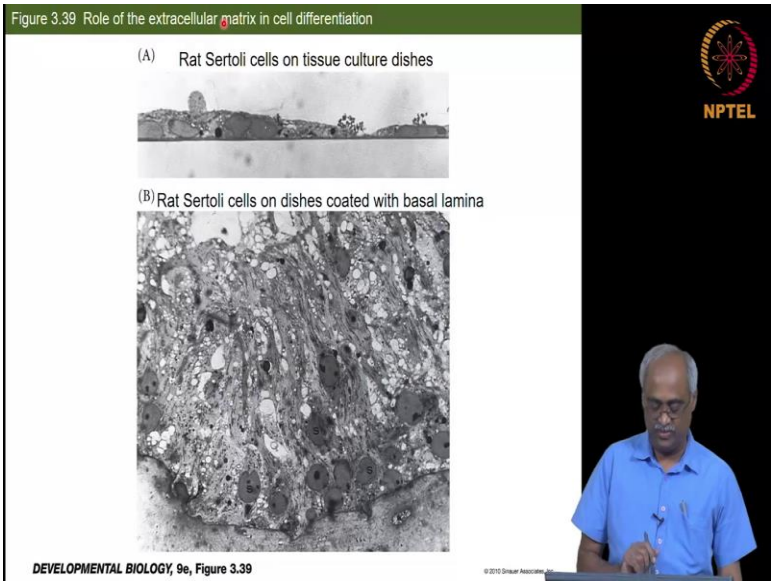

So, now let us look at the developmental consequences of ECM. So here you have this Rat Sertoli cells. So these Sertoli cells are important for germ cells to proliferate in the testis in mammals. So, when you grow them as isolated cells in a tissue culture plate, they grow like a layer (A). But if it is coated with the right ECM, it differentiates like in figure (B). So, they provide a niche where the germ cells differentiate. So without Sertoli cells, you would not have the germ cells in the testis. So, these are grown for the same amount of time and taken under similar magnification. So, this is the impact of the presence and absence of ECM.

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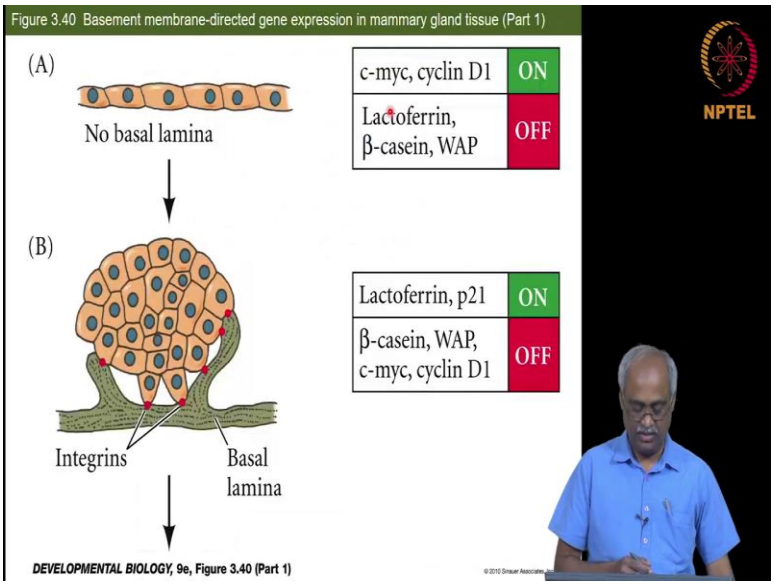

This is a cartoon of the mammary gland. So glands are mostly tubular structures in which cells in the cortex produce and secrete into the tube. Then production from multiple such structures is collected into one particular place. So, mammary gland development happens similarly, so (A) is the epithelium like cells; these are the ones that are going to form the mammary gland tubes finally. But without basal lamina (laminin is secreted by epithelium), they grow and but continue to produce proteins involved in cell proliferation like c-myc and cyclin D1. And do not produce the differentiation-specific ones like the casein, WAP protein. These are the products of differentiated mammary glands, so they are not going to be produced without ECM. But once you provide the ECM and this integrin attaches to the fibronectin. So when that attachment happens, these cells form a lump-like structure instead of a sheet, and then they wrap themselves by drawing this basal lamina.

They produce the basal lamina, and that induces differentiation. And as a mark of that, the lactoferrin and the p21 transcription factor comes on. Still, these are yet to form the glands, but then these proliferation genes are now off. So, the differentiation has set in.

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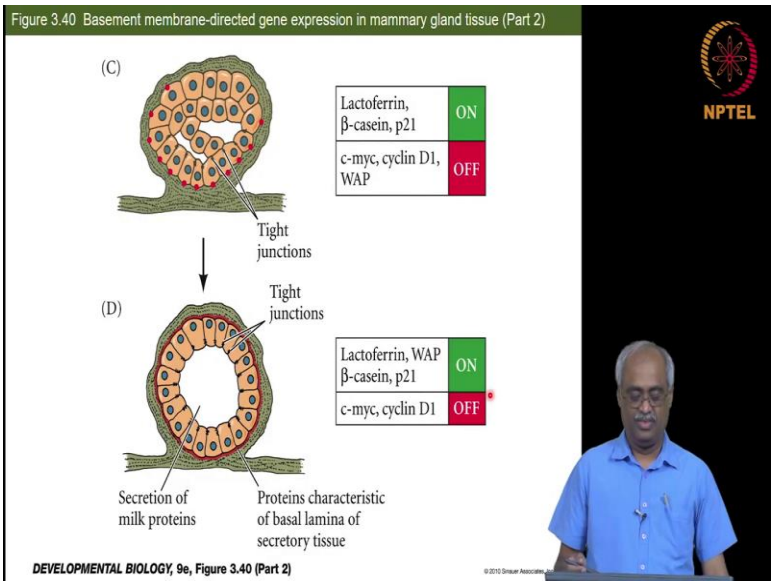

Then they form the basal lamina all around, and the tube is formed, and to the lumen, these differentiated cells will produce the casein, lactoferrin, etc. All the milk components will be produced, and the proliferation is now stopped, and then you form the tight junctions for the luminal content not to leak. So that is the context in which you see the tight junctions. So, another place where you see is the intestinal mucosa. So, there again, you have the tight junctions. Tight junction means you have the lipid bilayer fusion, and there are no protein-based junctions, basically a membrane fusion. This prevents leakage. So, now you have a milk-secreting mammary gland tube. So, you saw the role of the basal lamina. So this differentiation happens only after this attachment, so that is why ECM is critical.

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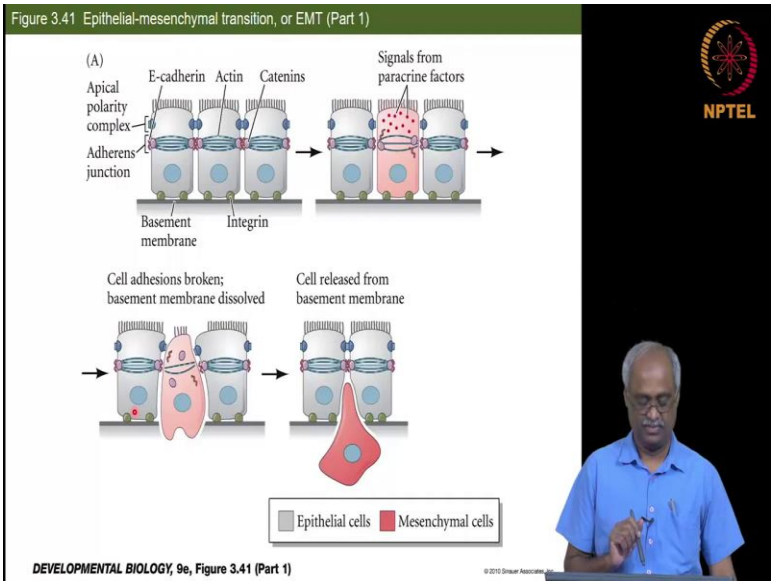

Then we will get into the next one where this epithelium to mesenchymal transition is crucial in different situations but widely popular and appreciated in the context of metastasis. So the epithelial cells have the desmosome, cadherin, catenins, and then actins connections that form adherent junctions. So, these adherent junctions now change when you are going to have epithelial to mesenchymal transition. The first thing is cadherin production is reduced, and then the catenin and cytoskeletal elements like actin rearrange then the basement membrane gets dissolved. Then the cell comes out, and now it has become mesenchyme, then this can go wherever it wants. So, this is how an epithelium based origin cancer becomes metastatic, but this is not like totally an unusual situation; this is a normal process that has gone wrong in the wrong place and time.

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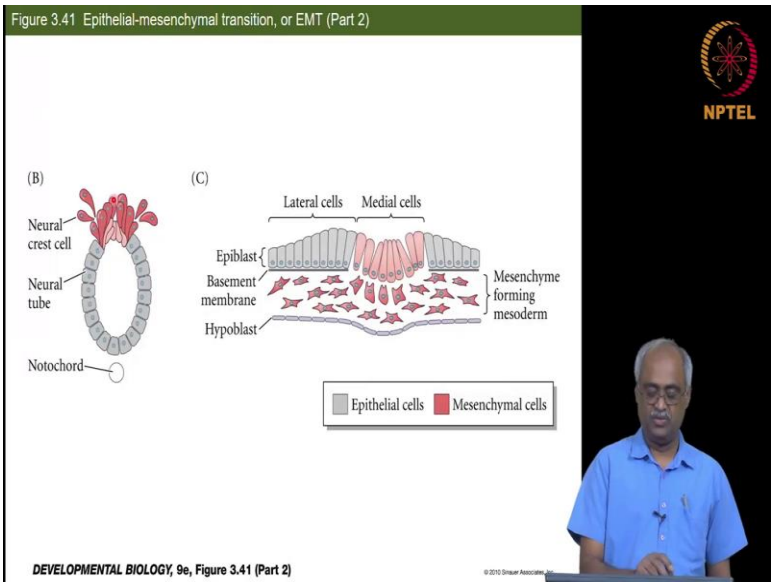

So usually, this EMT is required to make these neural crest cells in (B); these cells come out from that epithelium like structure and migrate to other places to make different kinds of cells. So, this EMT is critical during embryonic development. So, similarly, in (C), this mesenchyme that comes from the epiblast. So essentially, you have sheet-like cells from which other types of cells need to form in the early embryo, and those cells come out to make the mesoderm. So, these are crucial stages in embryonic development where EMT is essential, and when this process happens in the wrong place and time, you get cancer metastasis. So, cancer origin is different from this metastasis. So this is the EMT transition.

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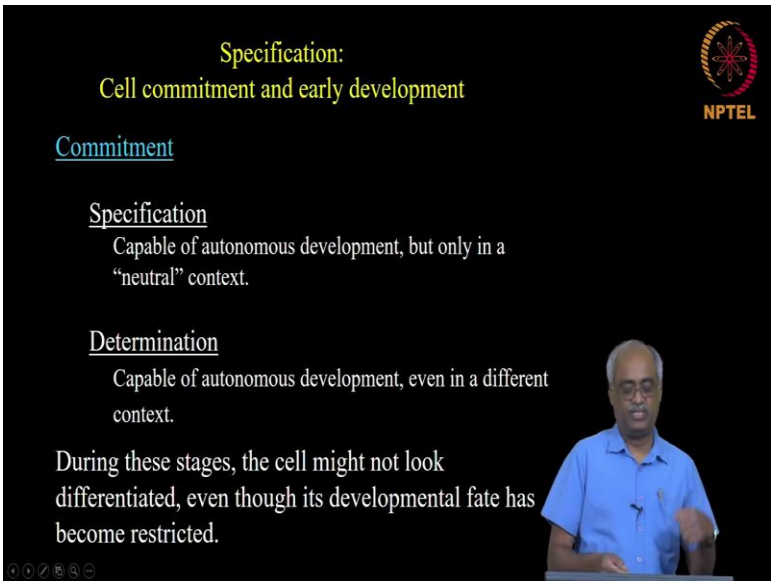

So, now we move on to the next important concept in developmental biology that is cell differentiation. So, here again, we will see some interesting history as we go along. A differentiated cell, like a mammary gland cell, will have its unique biochemistry and morphology, which is not visible.

So, that is what commitment is. So, the commitment itself has different stages, a progressive set of situations. So the easily identifiable states are specification and determination. So, differentiation is the end process of all of this. So, specification means a cell knows what it wants to do and is ready to do as long there is no negative signal. So, if you take a cell and put it in a neutral environment where no one is giving an opposite instruction, it will go on to, let us say, form mammary gland cell. So, that is called specification. The critical thing here is, the required molecular changes have happened for a particular development, but this can be changed. So if you give an opposite signal, you can still reverse this process at a specific stage. And if that cell has gone beyond the stage of coming back, you say that cell is determined. First, it is specified, so at a specified state, it can still go back if the right instructions come, and when it has reached the determined stage, then it is irreversible. So, these two are the subclass of commitment.

So even after determination, the cells might not be a fully differentiated both morphologically and biochemically. There are different types of specifications. So, we are going to look at each one of them and where they play an essential role.

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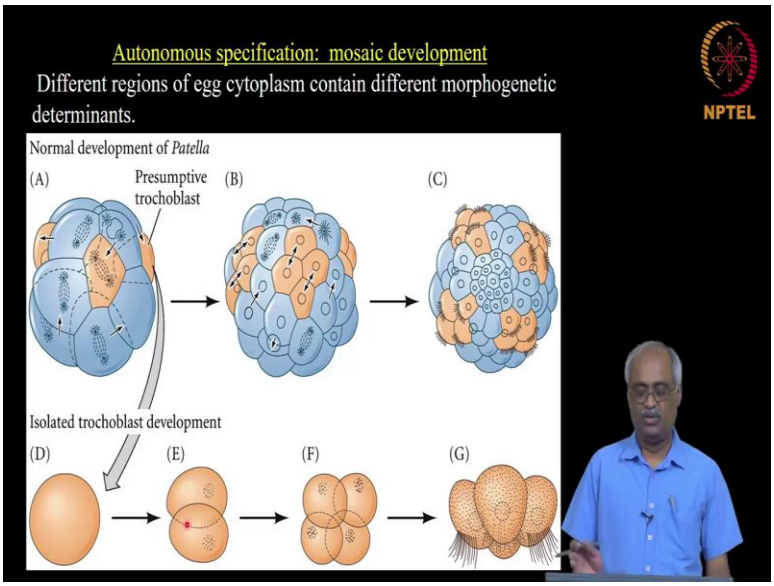

So we go back in history. So, before I get into this, I should show how this organism looks.

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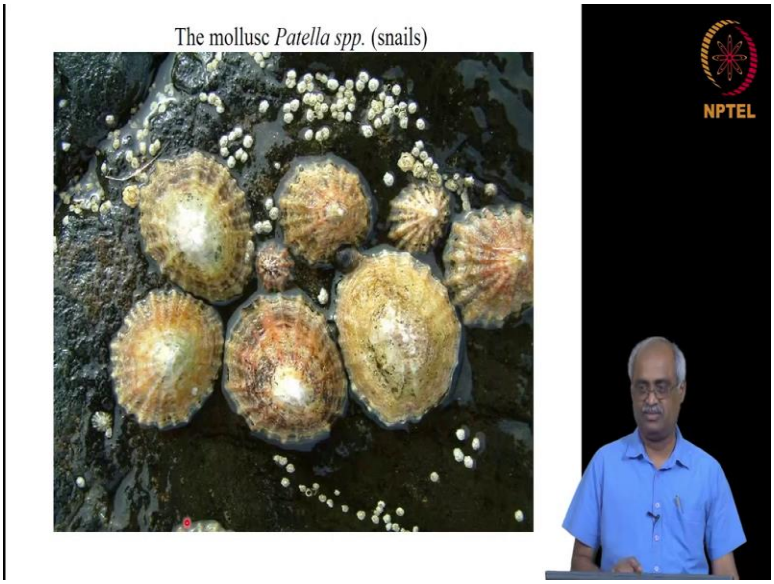

This is how they look like; these are mollusc *Patella*, so now in these molluscs, we will look at how during embryonic development, specification happens. So, in the very early stage, if you isolate the individual cells, essentially you can put them in calcium-free water and shake the cells, they loosen up, and that is why these kinds of organisms are used for experiments. So when you take these individual cells shown in a different color (refer to the previous slide figure (A)), you will see each part made by each cell in that embryo. So, whatever structure this particular cell would have made when it was part of an embryo, eventually, that structure is made even

when isolated. So, the presumptive trochoblast divides into two and then four and then makes these structures whether it is in the embryo or outside. It does not make the other blue color structures.

So each cell seems to have instruction to do what it is supposed to do, and it will do it regardless of the neighbors and that we call as autonomous specification. So, the cell is autonomous now, and as a result, the final embryo is viewed as a mosaic, you have the different structures all put together with no connections to each other. So, that is why we call this as mosaic development. So, organisms, where you have the autonomous specification, the development is going to be mosaic.

So the opposite also will be true in this embryo; if you take this trochoblast out, the rest of the embryo will make the final structure. So, that is why it is a mosaic.

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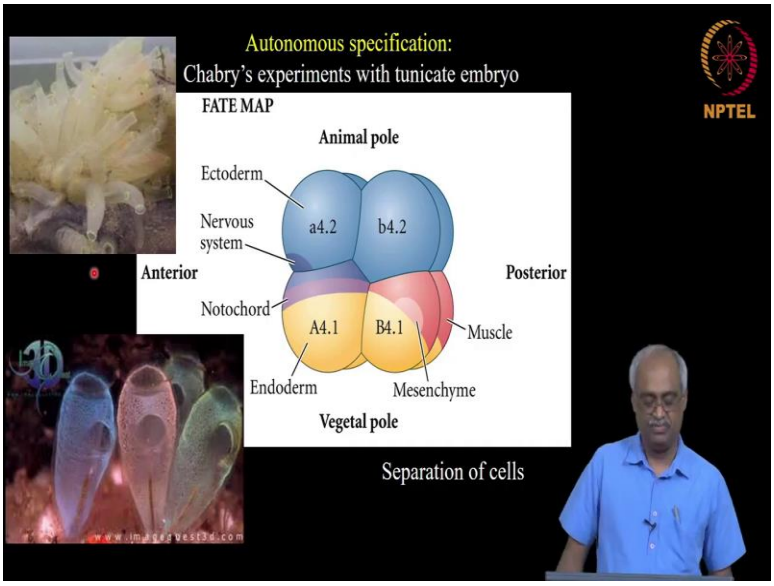

So, this is an autonomous specification. So, Chabry did an exciting experiment with these kinds of organisms, so this is tunicates.

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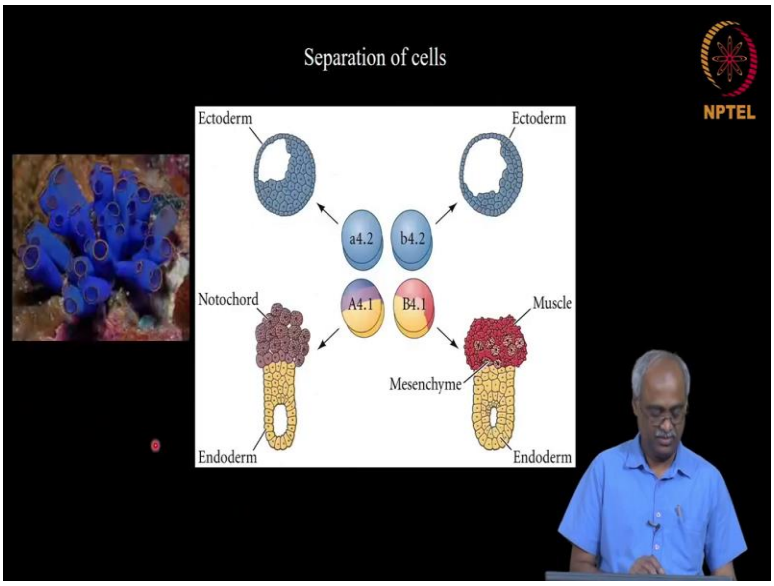

So, these are filter feeders; they allow water to come inside them and filter out the water, and the food stays back; so that is how they survive. So, the blastomeres in these embryos are easy to separate; you can do the fate mapping. Here, the color shows the descendants of a particular cell, so here b4.2 will make the ectoderm. If you separate these cells, they make the respective parts, as we just understood in the autonomous specification. So the different parts are made separately, and if you put them all together, you have a complete embryo. So, Chabry did not stop there; he tried other experiments with them.

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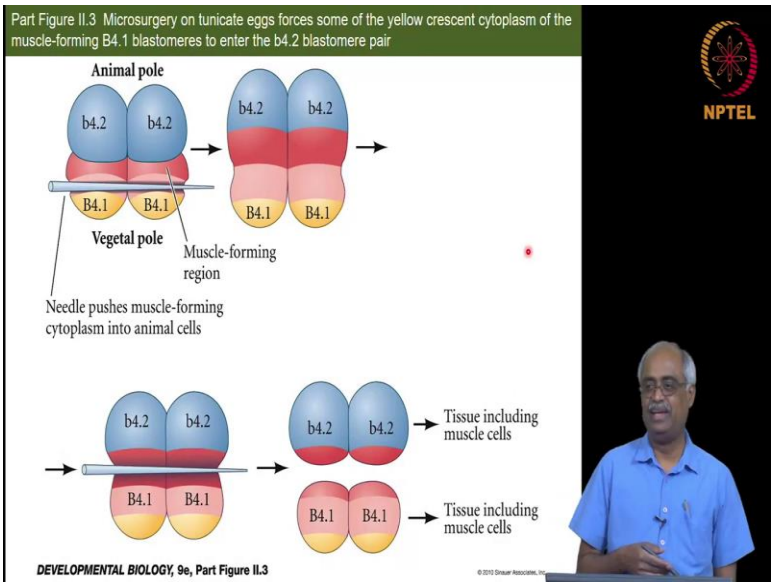

So, what he did is he pressed these two ventral cells with needles such that some amount of the cytoplasm when the spindle forms get into this animal pole derived cells, so they end up

becoming like this (top image). Now from these descendants, you have muscle forming cells coming out. This shows each cell has a set of instructions, and if we alter that, we get a new structure

So, this is an experiment that is consistent with the idea that you have determinants all coming from the oocyte, and now whichever cell inherits a specific instruction that will make that structure.

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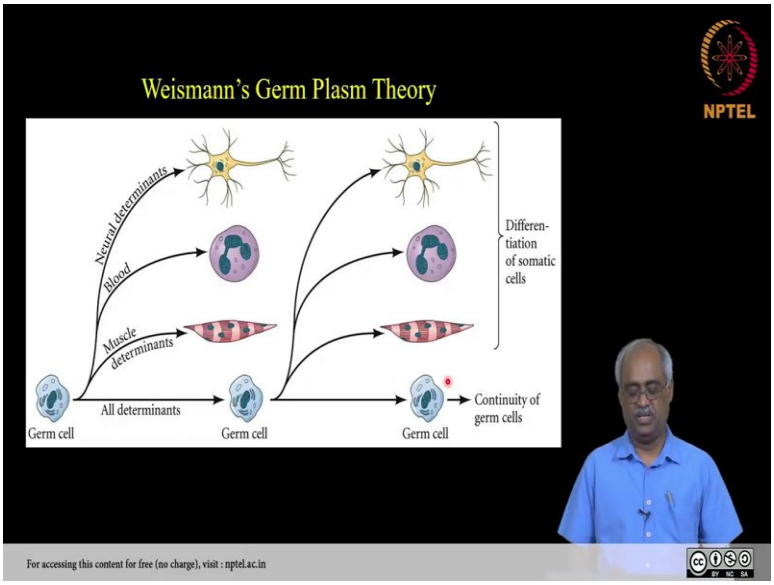

As a continuation of this, we will revisit this Weismann's Germ Plasm theory; if you could remember, we have already learned this genome equivalence. The difference here is that August Weismann proposed everything comes from the chromosome of germ lineage. But the other cells that come they get parts of this chromosome; instead of imagining the cytoplasmic component here, you are assuming chromosome, each one receives one different part of the chromosome; therefore, you have differentiation.

And as an extension of this, he proposed that if you take a frog embryo and separate the animal pole and vegetal pole, they will form only half of the embryo.

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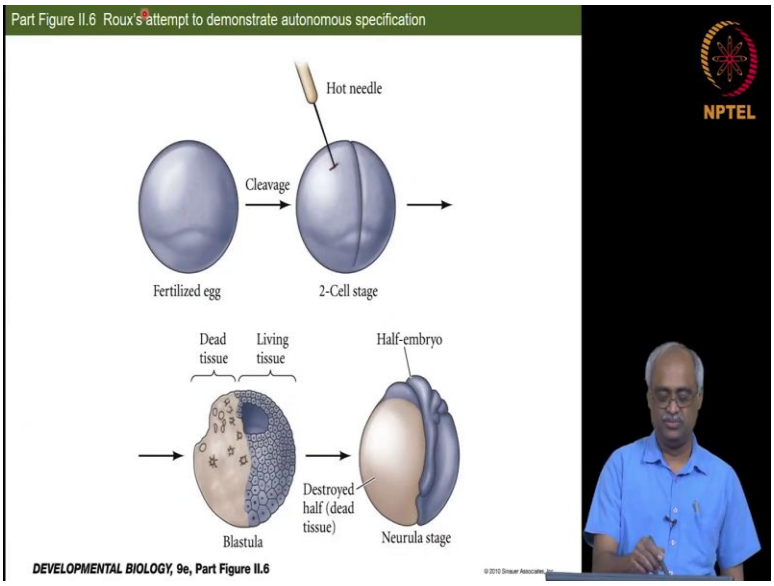

So, Roux wanted to test this out. He experimented with killing half of the embryo with a hot needle, so the half embryo is dead. So, when it completed embryonic development, he saw a half-developed embryo, and the other half was missing, so it was a perfect autonomous specification a mosaic development. So, the previous people are all correct, Chabry and August Weismann.

They are only partly correct, not completely. So, there is a defect in this experiment. Can you figure out what the defect is ? So, we will revisit this experiment a little later after doing some other experiment.

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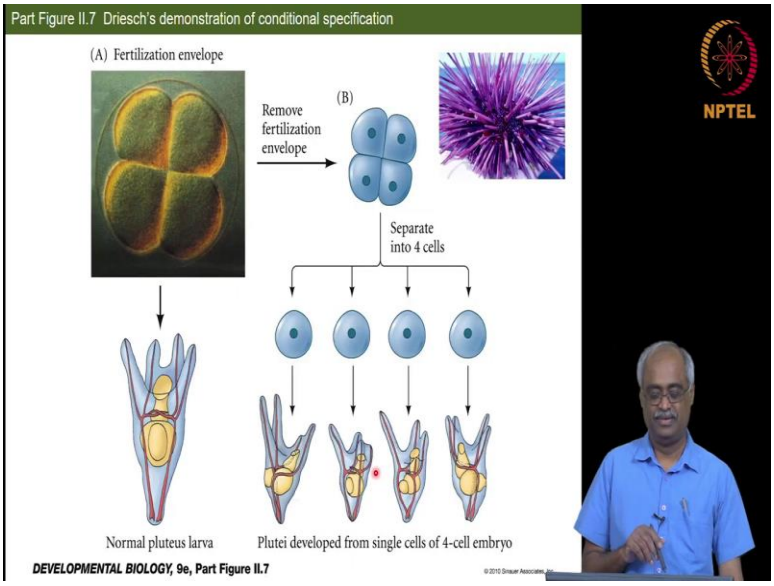

So Driesch, what he did is he took the sea urchin embryos and then separated them into individual cells. To his surprise, he ended up finding each one of them developing into a complete larva as if each one had the information to make the other missing parts. So this is already shaking the autonomous specification. So, he could have stopped at this level, but he went ahead to do other experiment that changed his life.

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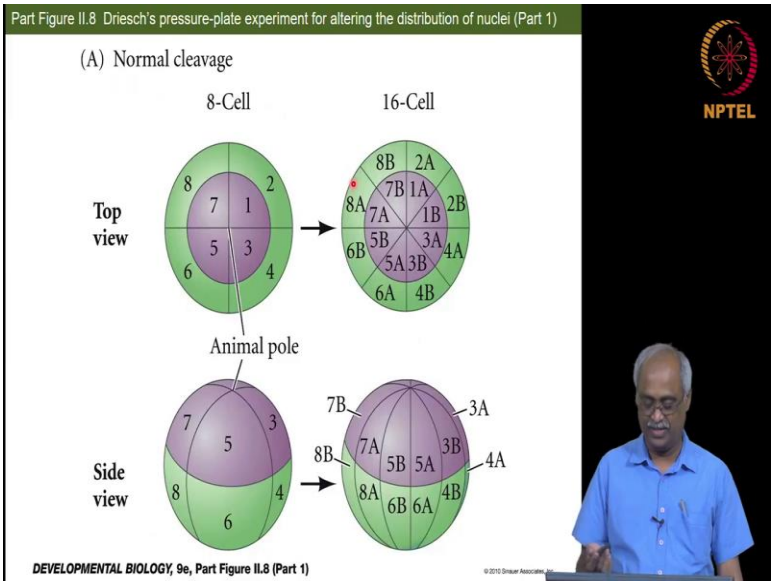

This is how a normal cleavage pattern looks like in (A), so you are seeing the top view, the cross-section, and the side view. These are the cell division patterns; the number tells you what came what cell. So, Driesch did something like what Chabry did.

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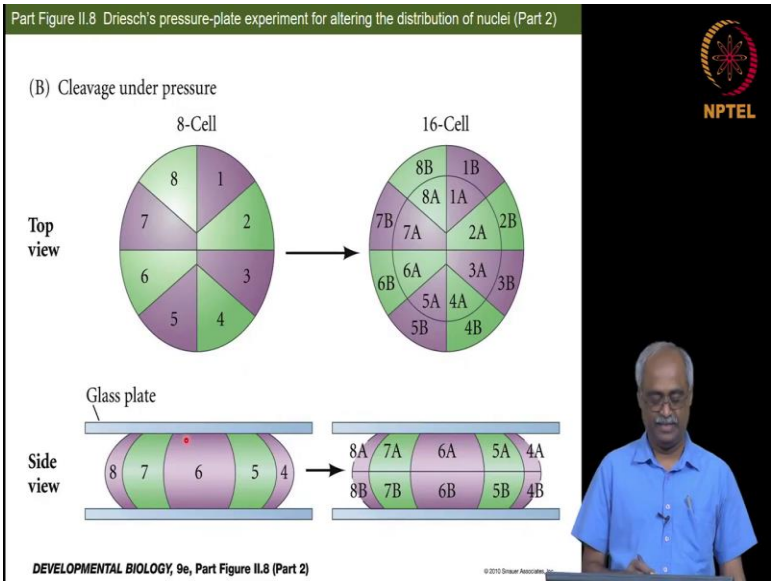

He tried to mix the division planes by pressing them between two glass plates, and when he did that, the spindle orientation changed. So, now he expected mixed structures, probably the tail on the head and eye at the posterior part, and so on, but it did not happen; it formed a normal embryo. So those made him come to the following conclusions.

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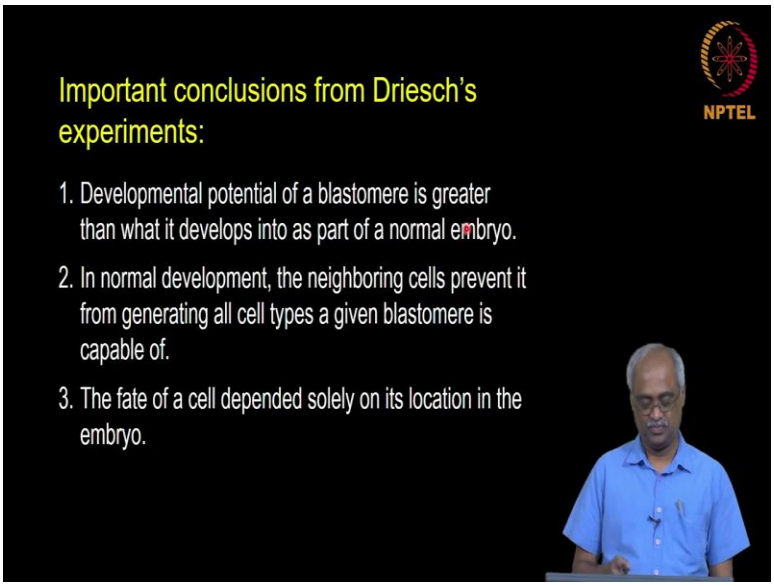

So a particular blastomere, whatever the structure it makes in the embryo that is not only its capacity, it has additional capacities too. If you go back to the vulva, any one of the six cells can be tertiary or secondary, or even primary, but those that do not get those instructions automatically become tertiary. But if you remove the negative signaling, then they will all become primary too. So, that is the conclusion he came to, a blastomere's developmental potential is greater than what it normally does as part of the embryo. And if that is so, why does it not make all the embryonic structures in the embryo? So for that, he concludes that the neighboring cells must be telling it to make only a specific type. And the third one is, in the cell, whatever structure it makes, why does it make that structure and not other structures? He concludes it depends on where in the embryo is and what instruction it is getting.

These are the three conclusions he came to, so obviously, you see genomic equivalence here otherwise, how a cell had all the potential at the early embryonic stage. And at the same time, cells having the same genetic material how they can interact, perform the cell-cell induction and response. So the genomic equivalence made him think that this is not in the realm of science.

So he was sad and depressed in life and quit science and moved on to become a saint or something like that. So, his experiment had such strong consequences to embryology as well as personal life. So, these three, you need to remember the developmental potential of a given blastomere is a lot more than what it does in an embryonic context normal location. This means the neighbors must be telling it not to make the other structures, which means whatever structure it makes is probably due to the position on the embryo.

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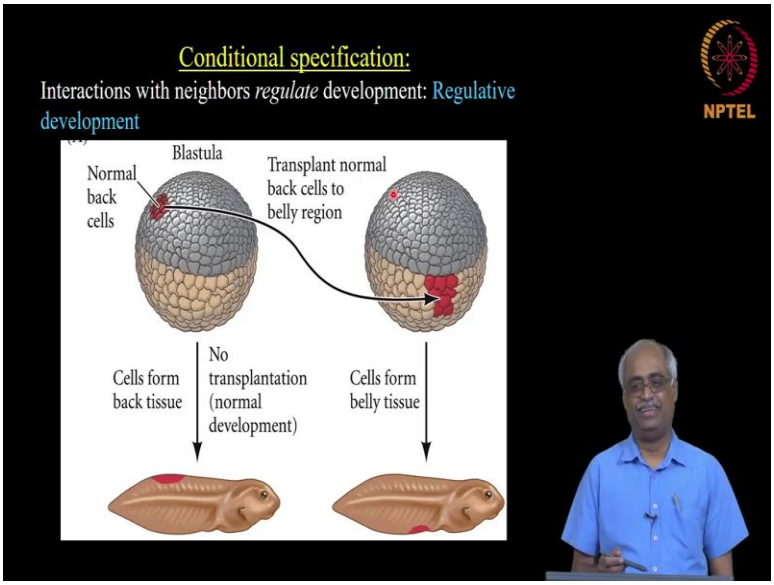

Now we will revisit the Roux mistakes. So, he destroyed the cells, but he did not do the remove or isolate experiments, and that is done here. So, here they are taking the normal back cells and transplant it into the belly region. The back cells end up, making only the belly tissue in that belly region.

So this we call as conditional specification, which leads to regulative development. The neighboring cells regulate the condition, and that is the conditional specification. So the conditional specification leads to regulative development; autonomous specification leads to mosaic development.

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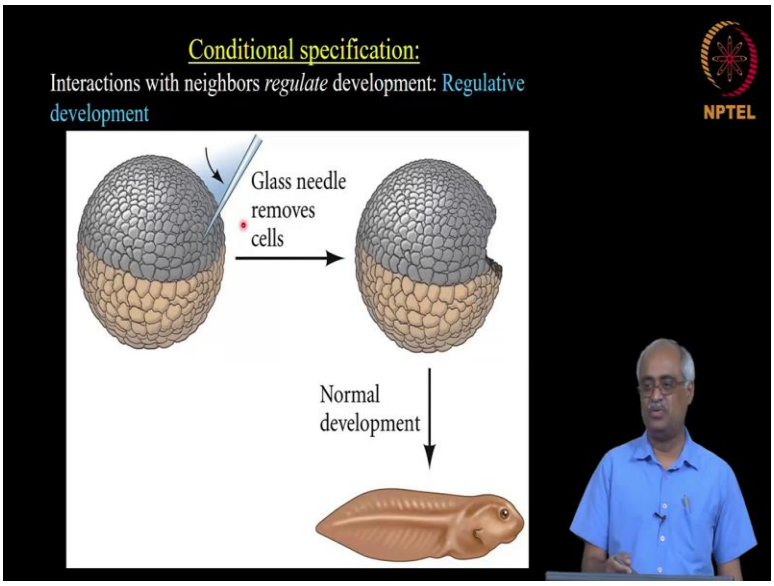

So here, if you remove some cells, it is not going to miss that part, unlike what we saw in mollusc, here it is going to form a fully functional larva.

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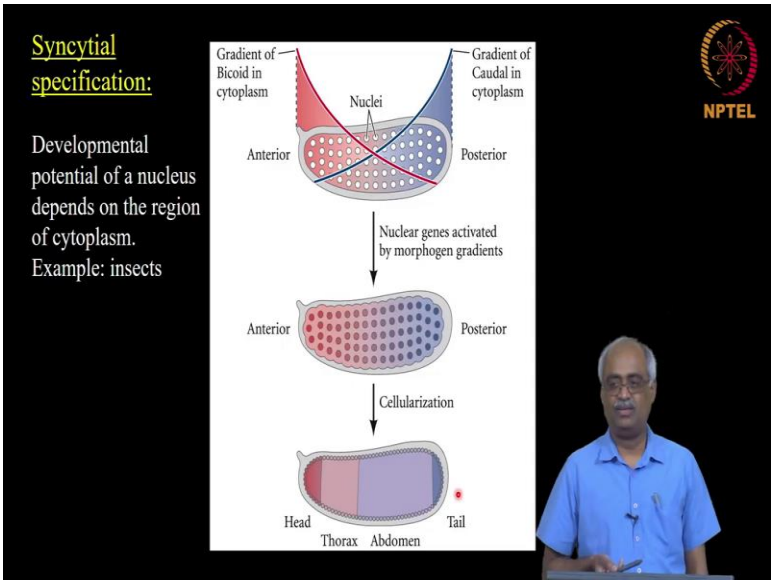

The last thing is syncytial development; this is not very common; it is seen only in some insect species. Here the cartoon is from Drosophila that is the insect that is most studied. So, here during the early embryonic development, nuclear divisions take place without cytoplasm being separated, no cytokinesis. So, the embryo becomes a sac containing a lot of nuclei, but the cytoplasm is asymmetric, meaning the components of the cytoplasm is different in different parts.

So color-coded here in the cartoon, you see the red in the anterior, meaning some component is present in the anterior, not in the posterior and vice-versa. A particular nucleus development depends on the concentration gradient of the asymmetrically distributed molecules. And this we call as syncytial development.

If the embryo is a syncytium, it means multiple nuclei in a single plasma membrane-bound cytoplasm. So, the early embryo of Drosophila is a syncytium. So, you have rapid synchronous nuclear cleavage divisions with no cytoplasm being separated and the position of the nucleus in that cytoplasm determines what it is going to become. So, here is an example of an anterior determinant called bicoid.

It is a transcription and a translation factor. So the anterior specifying molecules are induced by bicoid. Since caudal mRNA is translationally repressed by bicoid, therefore, in anterior caudal is not produced. And this, we will see in detail when we are going to learn about Drosophila embryogenesis. Since many signaling pathways and a paradigm for embryonic development comes from studying the Drosophila embryonic development, we will go through that in detail later, not right now.

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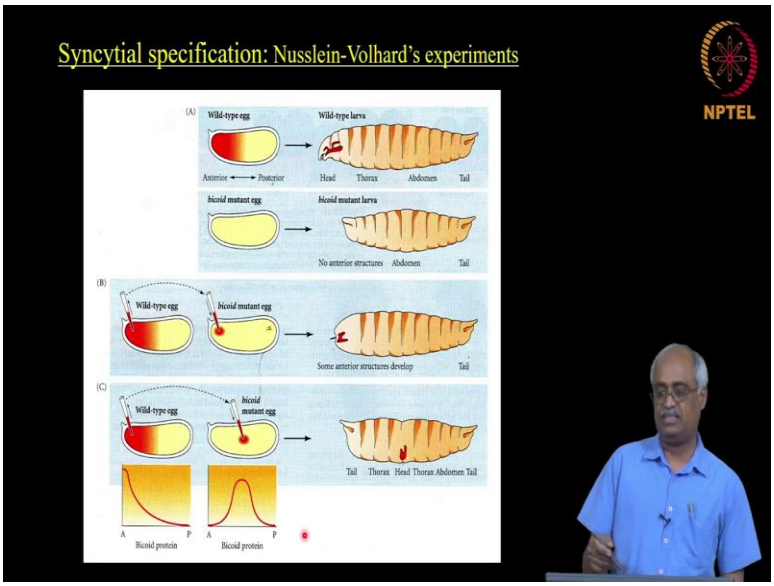

So this is a key experiment done by Nusslein-Volhard. She mutagenized and screened for mutants where the embryos are dead. And then she looked at the dead embryo. So, one of the

mutants that she got did not make the head structure. So this is the wild-type (figure A). This is a syncytial state, where you have the normal development of the head, the tail, and the thoracic segments like the chest part and the abdomen.

The anterior structure is absent in the bicoid mutants; instead, a tail-like structure is formed. So, she just took some cytoplasm in the anterior region of the wild-type egg and added it into the bicoid mutant, and the embryo formed the anterior structure. When it was added in the center, it formed the head in the middle with two tails, so she proposed bicoid probably makes a concentration gradient, and that determines the anterior and posterior structures. The highest concentration forms head, lowest forms tail. By doing similar analysis using genetics is how we learned all those pathways that we were learning and not surprisingly, she got Nobel Prize eventually for this embryonic development, thank you.