Introduction to Developmental Biology

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Lecture No – 24

Early Mammalian Development (Part 1 of 2)

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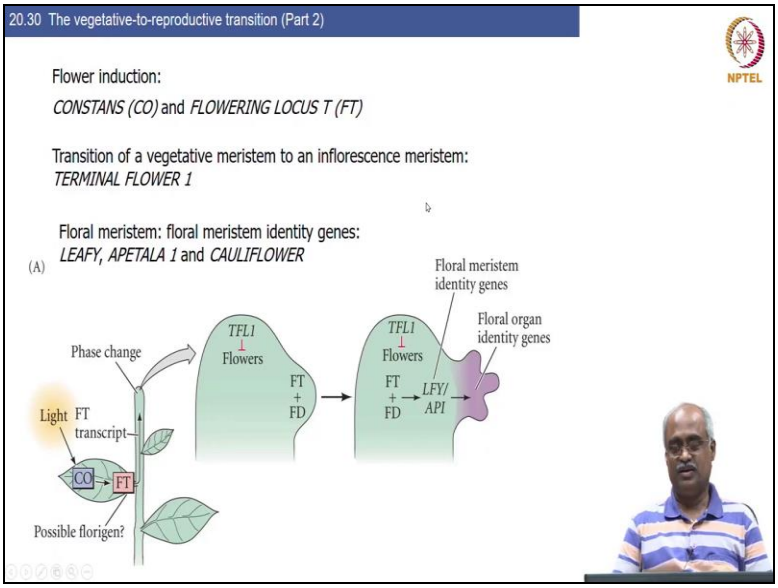

Students welcome back to the developmental biology course. So we will continue from the floral development in angiosperms where we left in the last class. So in the last class, I explained how the different stimuli control the floral flowering formation in angiosperms, that is, the light signal autonomous programs and plant hormones. So all of them play a role in converting the vegetative meristem into inflorescence meristem.

And instead of producing leaves and branches now, the inflorescence meristem will initiate floral meristems. So that is explained in this cartoon which I went through in the last class very briefly. Signals such as light sensed by different plant organs, although in this cartoon it is prominently pointing to the leaves, generates a signal like flowering locus T, which translocate to the tip and associates with transcription factor-like FD and initiates the expression of floral meristem identity genes such as *LEAFY* and *APETALA*. In the meantime, the terminal flower formation

that is normally inhibited now becomes in fluorescence meristem. Now we will go further from the induction of floral meristem identity genes how they turn on the floral organ identity genes.

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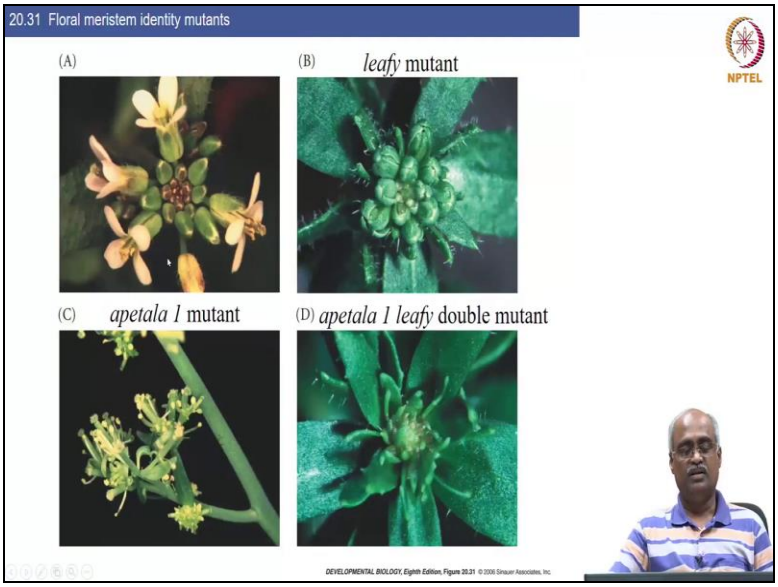

In the last class, we saw that the mutations in floral meristem identity genes such as *LEAFY* and *APETALA* create phenotypes where the floral organs are essentially transformed into leaf or leaf-like structures.

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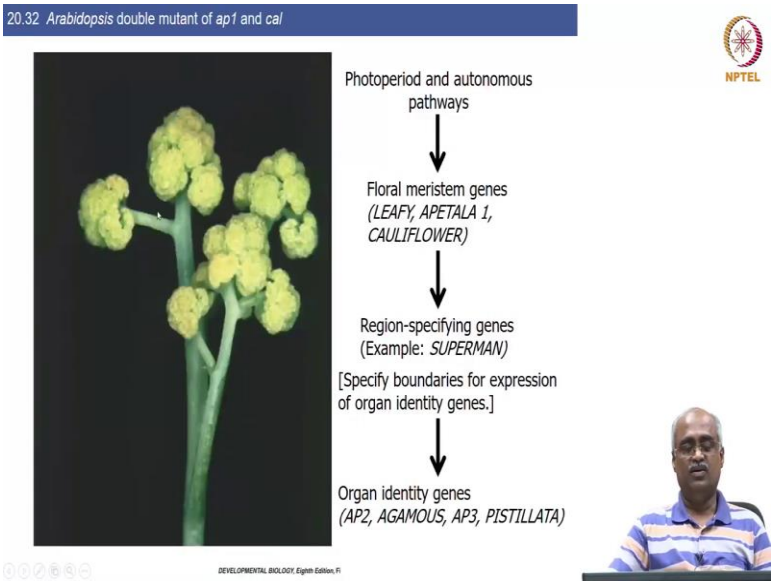

So this is the *APETALA I* and *CAULIFLOWER* double mutant where you have inflorescence meristems repeatedly forming instead of floral meristems, giving a cauliflower-like appearance.

And I also told that the floral meristem genes activate region specifying genes that control the boundaries of expression of the floral organ identity genes. So today, we are going to focus on what are the floral organ identity genes and how do they function. So the floral identity genes belong to three broad categories called A, B, and C groups, and the model to explain how the organs are determined is called the ABC system or the ABC model

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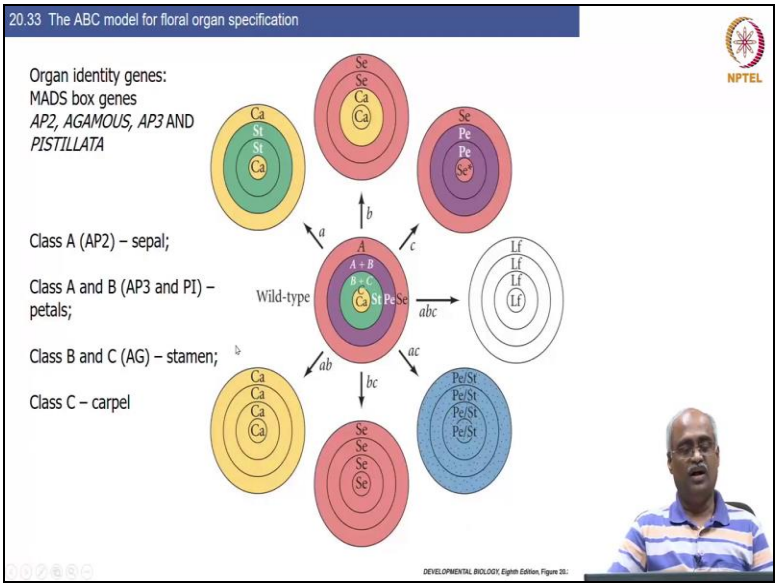

So it is quite simple if we look at it. So you have class A genes in a certain region within the flower primordium; if class A alone is expressed, it will become a sepal-like, for example, the outer whorl, the concentric circle in which the different organs are specified. The outermost forms the sepals or the calyx, and then you have the corolla, which forms the petals, then you have the stamen, and then you have the carpel. So these are the four concentric circles or whorl. So if A alone expressed, then it will become sepal or like the outer one and if AB are expressed like the next concentric circle, it will form petals. And if B and C are expressed, it will be stamen, and if C alone, it will be calyx.

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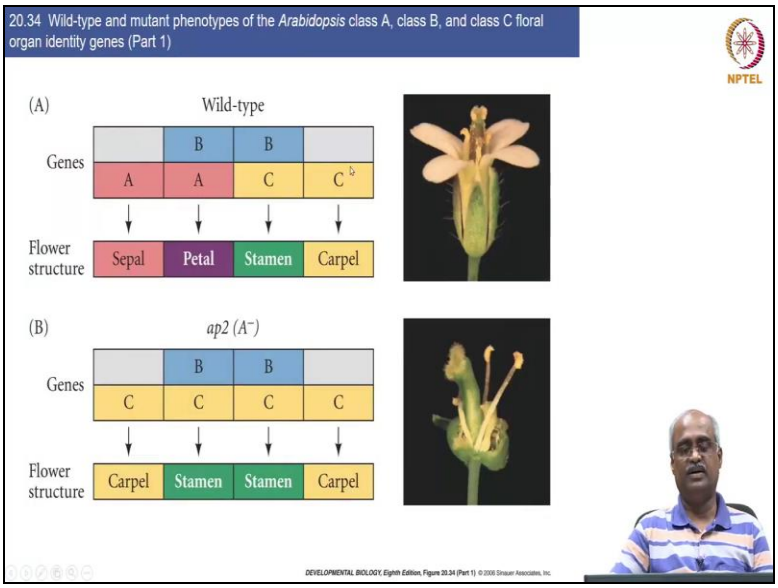

So this is how such an overlap could be there, and it becomes obvious when we consider this sort of a cartoon. So you have A alone, AB, then BC, and then C, so this is wild type forming sepal, petal, stamen, and carpal. So now, if you have a mutation like *APETALA II* where you do not have A expressed. Now C extends into all the layers. Now we know C alone means carpal, and that is what ends up happening in the outermost, the carpal forms where you should get sepals. In the calyx whorl, you have the carpal, and then BC will be stamen like generally in the third one whatever happens now it happens in the second and third is normal, and the fourth is also normal in this.

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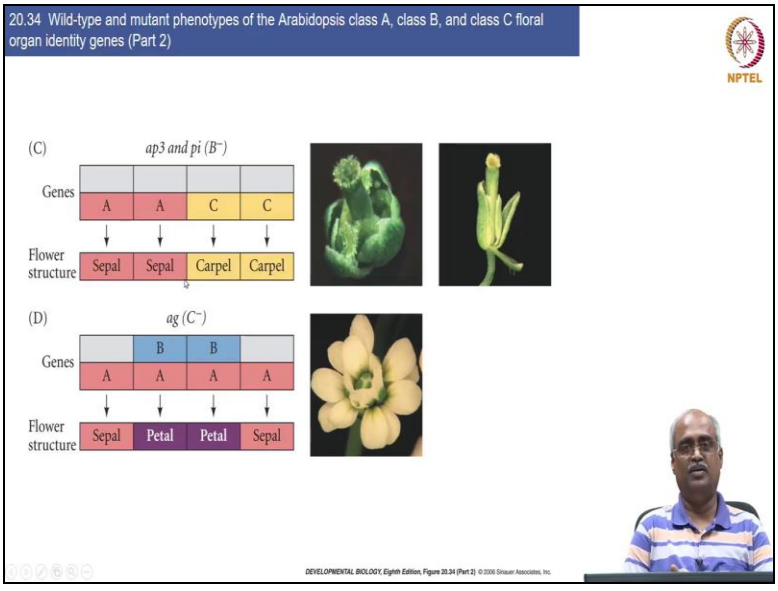

And the same you have in B mutants when you do not have B group gene function, you have sepal-sepal, carpal-carpal because you do not have the BA to make the petals or BC to make the stamen. Instead, you have sepal- sepal carpal, as seen in these two images. And if you do not have C, then you make sepal petal petal sepal. Inside, you can see the green calyx forming and then have the petal petal in both where you should normally get petal and stamen.

So this is the ABC model that explains how the organ identities in the four whorls are determined. So if A alone to reiterate, it is sepal AB you will get petal BC carpal and C carpal.

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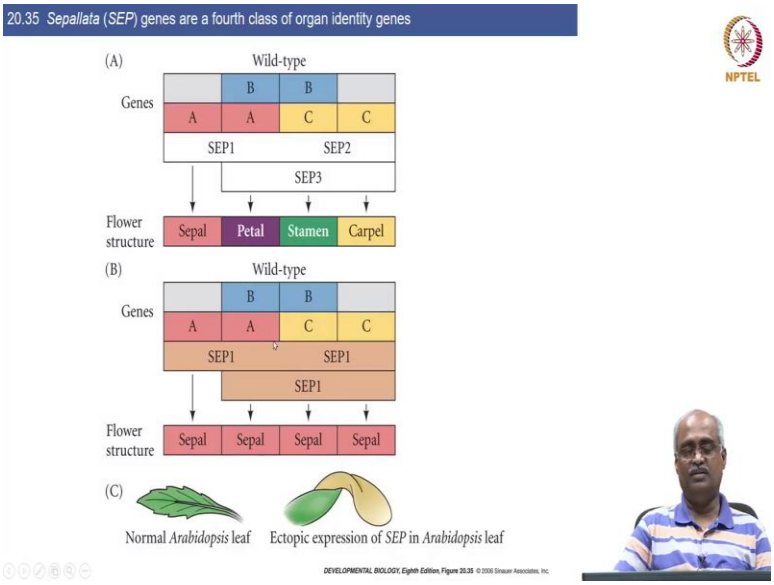

So although this model is very simple and explains to a great extent, it does not explain everything. For example, if you express all the three groups of genes in the leaf, the leaf does not get converted into a flower, so obviously, there are other genes involved, and one such group is called the *SEPALETA* genes. There are three paralogs, *SEPALETA I*, *SEPALETA II*, and *SEPALETA III* and if you remove all of them, what happens is you get sepal in all of them.

So these genes function in conjunction with these ABC genes to determine the wild type. So if you remove *SEPALETA* genes, you do not get petal stamen although you have B A or the B C combination correctly. So, in addition to that, you need the *SEPALETA* group genes to form the normal organs. If ectopically expressed, the *SEPALETA* genes can convert a leaf into a sepal, so it is called *SEPALETA*. This completes our discussion on plant development.

We have seen here the way genetic mutants in simple organisms like C elegans and drosophila helped us identify the master regulators and get a framework for thinking about and investigating development in animals. Similarly, in plants, genetic mutants isolated in Arabidopsis helped us understand some of the basic pro basic genes and a framework for thinking about the development of plant organs. So with this now, we switch to our early development in mammals, which is our next topic.

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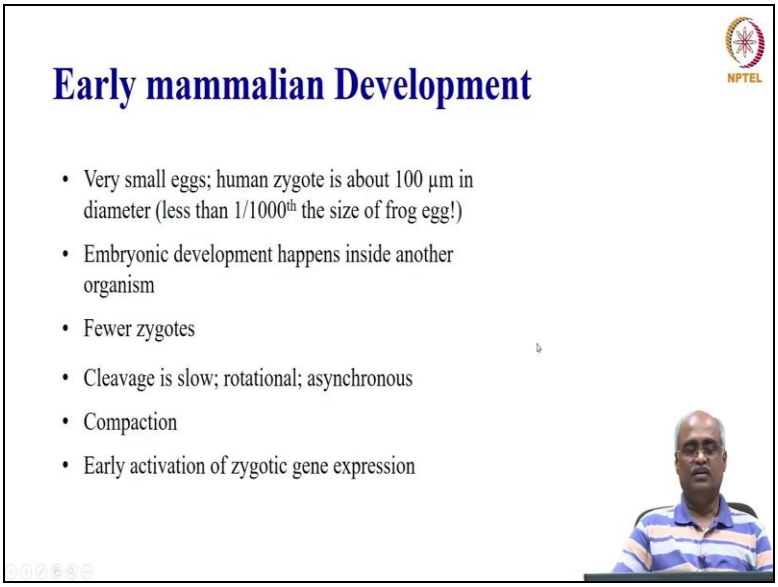

So unlike the embryonic development that we saw in drosophila, it is more complex in mammalian development. The primary reasons are listed here; one of them is the zygote is extremely small; like a human zygote is about 1/1000 the size of a frog egg. Another major complication comes from the fact that embryonic development happens inside another organism. So the embryo develops deep within the tissue layers of tissues in the mother's body, making it harder to access the embryo, unlike the embryos laid out in the case of drosophila or external fertilization in some of the animals such as fish or amphibian.

So here, the material is not readily accessible. This is primarily because mammalian embryonic development draws extensive amounts of nutrients and other supports from the mother's body. The embryo can be significantly sophisticated in terms of its functions. So a sophisticated development of complex structures is limited by stored resources. Therefore, continuous

availability of resources from the mother helps the embryo become more sophisticated and adapt to different selection pressures.

And that is the primary thing about mammalian development that we are going to keep in mind. So there is a lot of continuous supply of nutrients from the mother, and therefore a lot of anatomical and morphological adaptations suit this unique situation. And mammals do not produce a lot of embryos in one go. They produce a few zygotes. So, maximum a dozen and no more than that if you take human beings, it is almost always one at a time.

And coming to the embryo, the cleavage is slow. Each division takes a lot longer than other organisms and the cleavage plane changes from one cell division cycle to the next one; and therefore, we call this is rotational cleavage. So we will see a cartoon representation of that in the next slide, and it is asynchronous. It is not like two becomes four becomes eight becomes 16, and so on. So when one blastomere is in metaphase, another one may still be in the previous interface.

So they are asynchronous; not all cells of the embryo divide at the same time. Another unique feature of the mammalian embryo is compaction. So initially, the blastomeres are loosely adhering to each other within the eggshell equivalent called zona pellucida. Still, later they come together and form cell junctions like cadherin-based junctions and outermost forming tight junctions, which is called compaction. Lastly, unlike invertebrate embryonic development and in the case of amphibians, the maternal products that are the proteins and mRNA are produced and deposited in the oocyte by the mother play a significant role in embryonic development. But in mammals, the zygotic transcriptional activation happens very early, and they play a prominent role in embryonic development. So we will see them in some detail as we move along.

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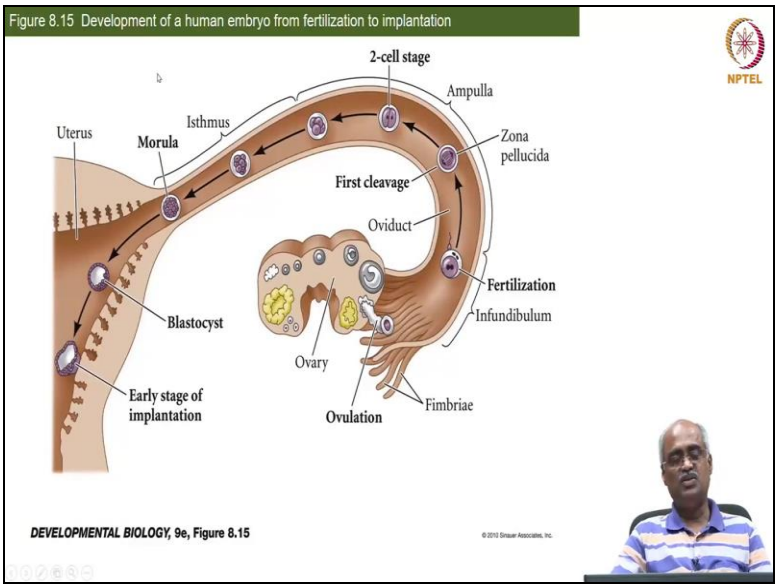

This image gives you an orientation to the anatomy where it all happens. You see the ovary and the oviduct. This oviduct modification into a large body called the uterus is one of those anatomical morphological adaptations required for the extensive association between the developing embryo and the mother. So, upon ovulation, the fimbriae help the oocytes coming out of the ovary to migrate via the oviduct. This region closer to this ovary is called the ampulla, and that is where fertilization happens, as you see in this cartoon here.

Once fertilization is complete, then meiosis ensues, and meiosis completes then starts the first cleavage. So as it is migrating, it is going through the cleavages. So during all these phases, the outer layer called the zona pellucida, which plays a critical role in fertilization for the sperm entry, protects the embryo from implanting on the oviduct. So the implantation is a new word here implantation meaning attachment of the embryo to the mother's body and sets up a conduit for nutritional flow. That happens only here in the uterus between the outer layers of the embryo and the epithelial lining of the uterus called the endometrium. So we will see that in detail later, and only here should this adherent happen. And having it happen in the fallopian tube region does not permit embryonic development because this is anatomically a narrow region and leads to hemorrhage, leading to the mother's death. So, if this happens, it is called a tubal pregnancy, and that is often fatal. And that is prevented by this zona pellucida layer and as it moves, the first cleavage then second and so on and then finally it comes to the uterus and where it gets implanted. So this is an anatomical overview, we will get into the details and the molecules involved as we proceed further.

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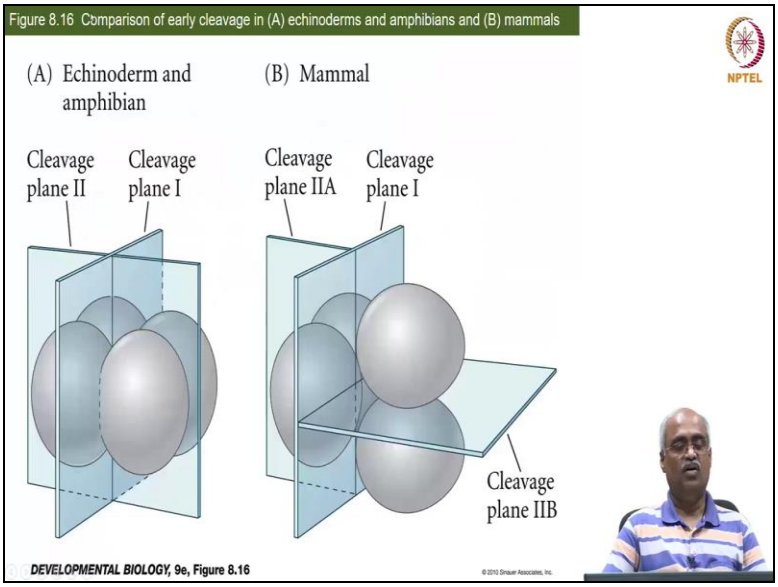

So this tells you about the rotational cleavage. So in amphibian, what you see is we did not get into the amphibian embryonic development for want of time, so we skipped that and we directly moved into the mammalian development. In amphibian the first division as well as second division both as shown by these two plate like structures happen longitudinally that is in this particular cartoon top to bottom axis. Whereas in mammals the first cleavage is like that but the second cleavage for one blastomere it is the same longitudinal or meridional cleavage. And the second one happens horizontal or equatorial cleavage. So that is called rotational cleavage. First meridional, then second one of the blastomeres that is 2A meridional while the other one equatorial. So this is the rotational cleavage. Although this pattern happens, this is not really critical for embryonic development because the later embryo comes from any of these cells. There is no cytoplasmic partitioning of maternal determinants to any specific blastomere.

So none of the four are determined to become the future germ line like the cells that form the gametes and at the same time any of the four can actually become. So you have regulative development happening here which we learnt earlier and as a result these cleavage patterns although they happen and this is characteristic of mammals they are not critical for embryonic development.

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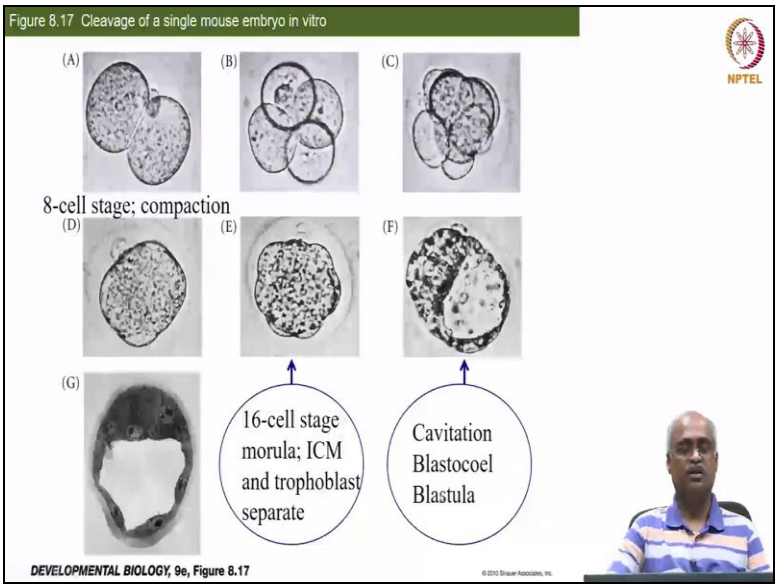

So here we have the microscopic images of the actual embryo. As the figure goes, this is the first cleavage, second cleavage, and in the third, you have eight cells. So at this stage the blastomeres are loosely packed within zona pellucida. So from here, they go through a process of compaction where the cells come together and attach to each other and the outer layer of cells form tight junctions that are membrane fusions. The 16 cell stage is called a morula, where the cells are sealed off from the outside.

So membrane-membrane fusion tight junction means you cannot have any liquid flow from outside to inside across the tight junction. And the cells inside form gap junctions allow the transport of ions among the inner cells, which is how we get to the 16 cell stage called the morula. And a later stage of this we have separation of two cell layers. This is the first sort of differentiation that happens; the inner cells form inner cell mass, and the outer layer forms what is called trophoblast. And this trophoblast is the one that is going to be crucial for this attachment here implantation.

And the inner cell mass is the one from which we get the entire embryo. So this is how this separation happens. So the trophoblast is an anatomical specialization from the embryo's part to establish the mother fetal connection. This is for the nutrient to flow from the mother to the fetus and remove the fetus's waste products through the mother system. So the trophoblast is going to produce differentiated cells and finally contribute to the structure called the chorion.

And the chorion undergoes extensive morphological changes and becomes the embryonic part of a structure called placenta. And mother also contributes to the placenta part and that part is called the decidua. So decidua from the endometrial lining here and the trophoblast derived cells forming the chorion, the decidua and chorion together form the placenta and establish the connection between the two. So the inner cell mass is the one that will produce all the rest of the cell types forming the complete embryo.

So before this stage, any of the cells could have become trophoblast or inner cell mass; therefore, these blastomers are called totipotent because they can generate all cell types of the embryo and the trophoblast as well. While the inner cell must buried within this while giving rise to all other cell types of the embryo it does not produce trophoblast as a result they are not called a totipotent instead they are called pluripotent because they have the ability to make multiple cell types. Until then, there is no fluid-filled space among the cells and that starts to form in the next process called cavitation, where the cells start producing ion channels that will secrete sodium into interstitial space. And due to that to balance the osmotic pressure, the water flows in and leads to the cavity filled with the fluid called the blastocoel.

And at this stage the embryo is called a blastula. When the inner cell mass moves to one portion of this area, it is called blastocyst so till then; it is called a blastula. When the inner cell mass moves to one part of this fluid-filled trophoblast surrounded structure, we call the blastocyst. So this is a cross section of a blastocyst where the inner cell mass is not distributed equally; instead, it is one place. And this is a vestigial left over the way an embryo develops on top of the yolk sac, for example, in birds and amphibians.

Because birds and mammals arose from reptilian ancestors, in reptiles, this yolk sac is quite prominent and the nutrition from the yolk is important. It does not have a placental connection, and therefore, the embryo develops on top of it. And a very similar thing the mammalian embryo also goes through is that it does not store a whole lot as the yolk sac. So this is a developmental or a historical process rather than an actual requirement for the mammalian embryonic development. So once it reaches this stage (Fig G), this layer should be removed. So essentially, the layer does not get removed; a hole instead is poked, and the embryo hatches, which is shown here.

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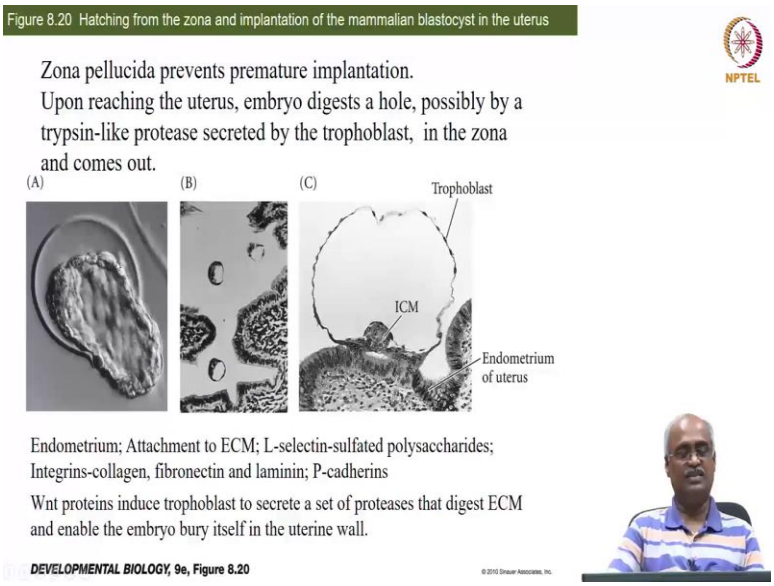

The embryo secretes proteases, probably the trophoblast to digest a hole in the zona pellucida, which prevents premature implantation that I explained earlier, and the embryo comes out. So this is a cross section showing such blastocysts in the uterus, it is coming there, and here it is in a monkey where you see implantation the early stage of implantation.

So figure C shows trophoblast and the fluid-filled blastocoel and the inner cell mass, and there you see the adherence. And this adherence is quite extensive and complex and a sequential process. So in this, both the trophoblast cells and the endometrial cells play a major role, and there is a conversation between the two. So initially, the corpus luteum left after the egg comes out of the follicle. The hormones produced by the corpus luteum induce the endometrium to produce sulphated polysaccharides. The L-selectin is a lectin kind of protein present on the trophoblast that attaches to these sulphated polysaccharides.

So, L-selectin and sulphated polysaccharide forms a more stable attachment. The initial attachment is not strong, which happens between the interactions between the ECM of the two. But a more stable interaction ensures L-selectin sulphated polysaccharides followed by the production of integrin by the trophoblasts, which interact with collagen in the ECM of the endometrium. These integrins interact with fibronectin as well as laminin. And this leads to the next step of interaction where you have P-cadherins which we learned a long time ago when we learned about morphogenesis, and these cadherins play a role as well. And once this establishes a

stable interaction, then wnt proteins produced by both tissues induce trophoblasts to produce proteases that will digest the ECM of that is the extracellular matrix of the endometrium allowing the embryo to burrow into the uterine wall, and that is the implantation.

So embryo gets buried into the uterine wall, and that is where it will develop. So, the embryo develops not in the uterus lumen.

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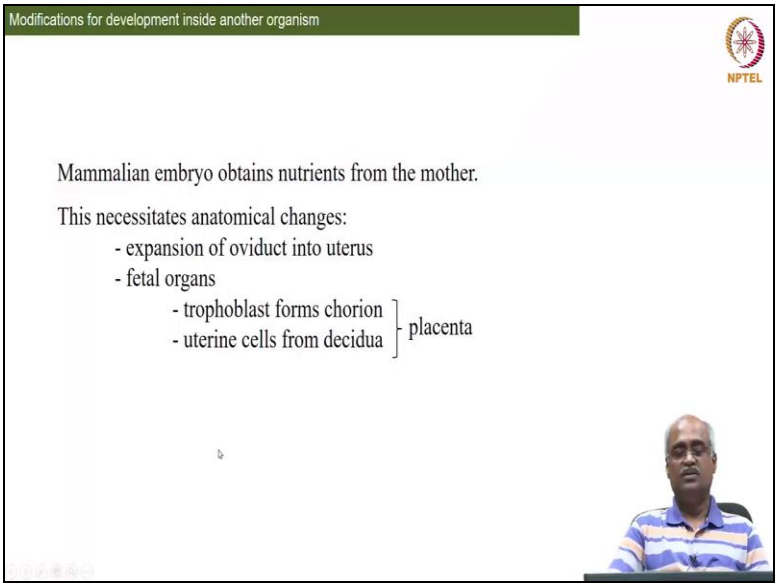

So now we will focus on this nutritional supply part. As I told you I am reiterating the same again in this slide. Mammalian embryo obtains nutrients from the mother, requiring these anatomical changes; expansion of oviduct into the uterus. So that is the change from the mother's part and fetus the formation of chorion and the uterine cells derivative decidua. These two have no other role except to support fetal development and that is why we call them fetal organs; they are organs specific during fetal development. So these are the anatomical changes required for this process.

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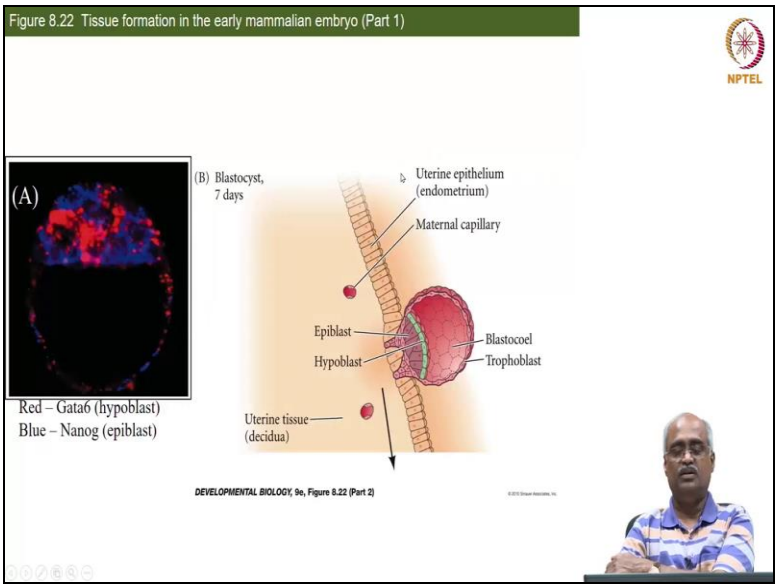

So we will see the implantation along with the embryonic development because both happen simultaneously. So at an early stage, if you look at a seven-day-old blastocyst, this is the inner cell mass blastocoel and trophoblast here. So you have two kinds of cells here in the inner cell mass one is hypoblast and the other one is epiblast. We will see what these two are going to form as we move along. As you see in the two color-coded staining's, this Gata6 is a transcription factor specific to hypoblast. Nanog is a transcription factor specific to epiblast and the both kinds of cells are intermingled here they are not separated into for example two layers so they are mixed. But later they are going to get separated into two layers as you see in this cartoon. The one that abounds the blastocoel will be the hypoblast, and the one on the opposite side is the epiblast. So the entire embryo develops from the epiblast. So we saw setting apart the trophoblast. Then I said the inner cell mass is the one from which the embryo will develop; now, the inner cell mass has separated into epiblast and hypoblast. Now epiblast is the one from which embryo is going to form. Hypoblast is going to form a lining here forming what is called extra embryonic endoderm that we will see as we go along.

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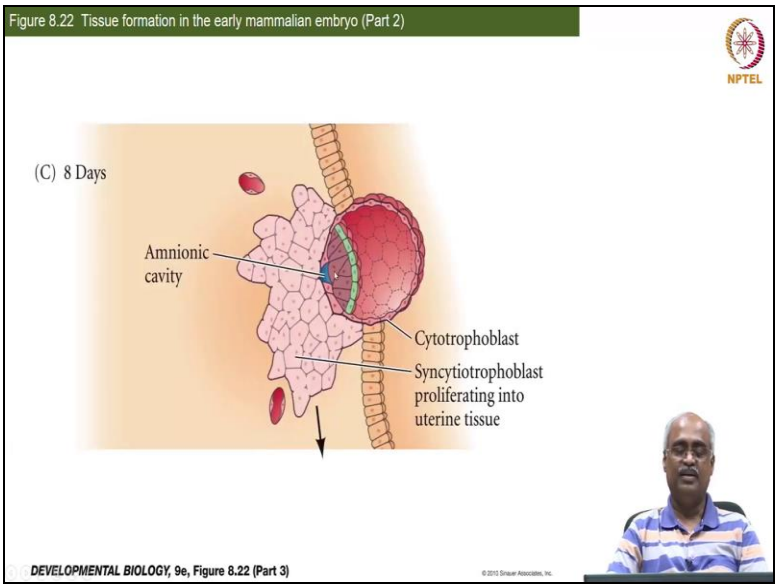

Then, this will again separate into two layers; one will line a cavity that is going to form here. Remember this is blastocoel; this is also a fluid-filled cavity, but for embryonic development, later on, this cavity that will form an amnionic cavity is going to be important. This amnionic cavity is going to enlarge and is going to become a fluid-filled sac called the amnion. And the amnionic fluid acts as a shock absorber for the developing fetus, and will be lined by cells coming from this epiblast and is called the amnionic ectoderm.

And embryonic epiblast the other portion will be the one from which embryo is going to form. So we had ECM becoming epiblast and hypoblast and epiblast will become amniotic ectoderm and embryonic epiblast. In the meantime, the trophoblast will differentiate into cytotrophoblast, which will produce another type of cells called the syncytiotrophoblast. So this syncytiotrophoblast is the one that establishes extensive contact with the uterine wall and finally going to draws the blood vessels, mother's blood supply to the embryo and forms a connection which we will see later.

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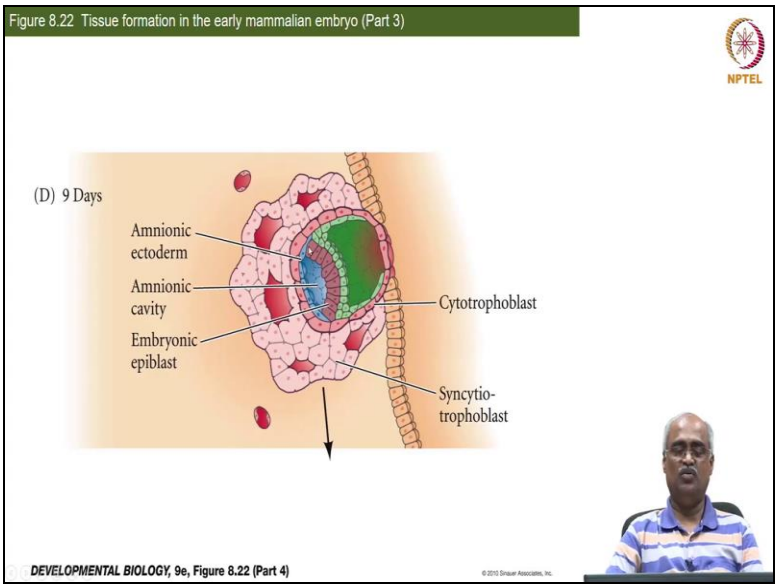

So these structures are now more obvious here. The hypoblast delaminated from this layer (green-colored cells) and now has migrated, forming a lining of the yolk sac (green-colored space), and this will be the yolk sac although yolk is not a critical food supply for embryonic development. So that will happen through this, forming the chorionic structure. And this is the amniotic ectoderm that arises from this epiblast. And this is the embryonic epiblast.

These structures (red-colored spaces) are important to connect with the mother's uterus; these will produce molecules that signal the development of growth of the mother's blood vessels towards this structure. Finally, they will form a structure, the placenta, and the different layers we will learn in the next class. We would not have time to complete them today.

Today, we will focus on the molecular events that happen in setting apart inner cell mass and trophoblasts. The first important differentiation event is the blood vessels merging into the syncytiotrophoblast layer and the extraembryonic endoderm developed from this hypoblast. Then you start seeing extraembryonic mesoderm that also comes from this embryonic epiblast. So, the mesoderm starts to form, and this will be crucial to form the fetal blood vessel connection in establishing the placenta.

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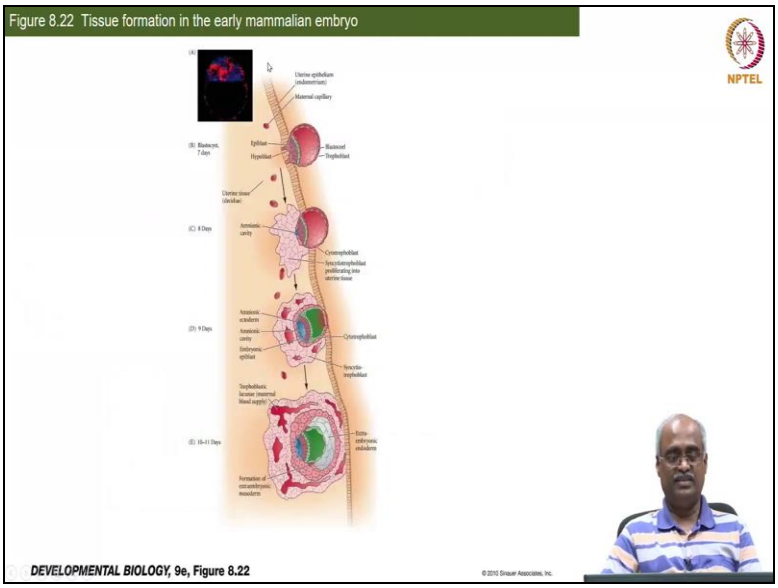

So this summarizes all the four steps that we just went through.

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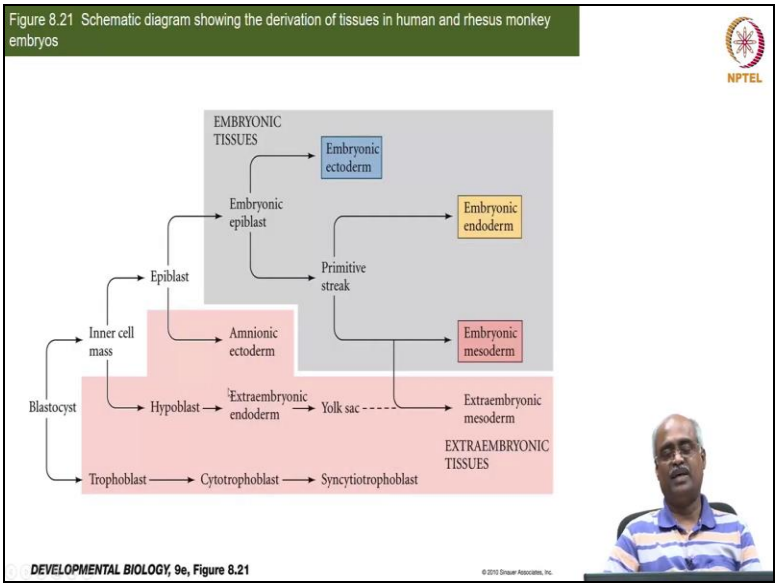

Now, this summarizes the lineage of whatever we have learned so far from the blastocyst. So blastula is when that cavity has formed the blastocoel, and when the inner cell mass moves towards one end of that, we call blastocyst. From that, you have the trophoblast, that is, the outer cells. Then the inner cell mass will give rise to hypoblast and epiblast. So just now, we saw in the cartoon how hypoblast gives rise to the extraembryonic endoderm and yolk sac, and then epiblast forming the amnionic ectoderm and embryonic epiblast.

Here, this color coding shows you the extra embryonic tissues, meaning they do not contribute to the embryo. None of the body parts of yours came from these tissues when you were an embryo. So all of you come from this part, so that's why it is embryonic tissue. So, embryonic epiblast gives rise to embryonic ectoderm. We have not yet gone into this, so we will see this later when we get into gastrulation.

Today we would not get into gastrulation. Instead, we will focus on the molecular events that lead to the separation of these two.

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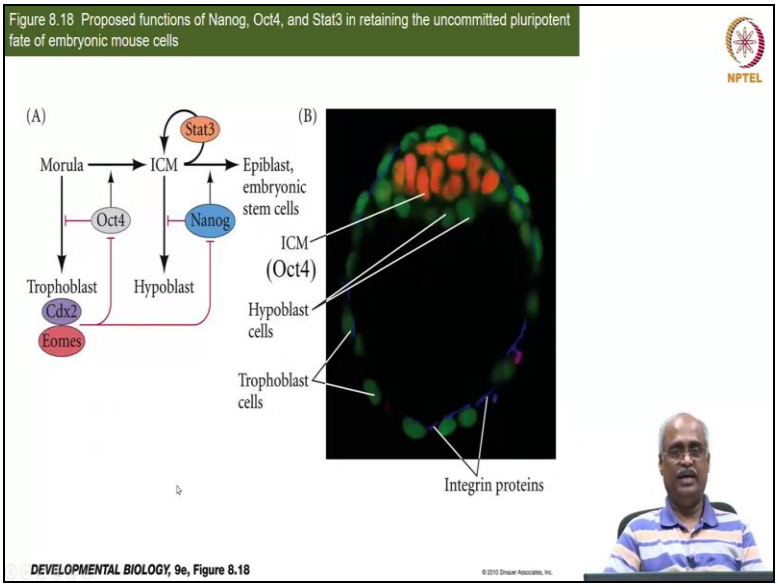

So, that is summarized in the next couple of slides. Initially, at the morula stage, all cells express the Cdx2 transcription factor but later, when trophoblast ECM separates, Cdx2 is expressed only in the trophoblast along with eomesodermin, another transcription factor. They are not expressed in cells that will become ICM. This inner cell mass and trophoblast separation of this gene expression pattern are explained in this slide and the subsequent slides.

So, before we find out what stops Cdx2 expression in cells that will become ICM, let us see what these molecules are drawn here do. Cdx2 expressed in Rho4 blast inhibits Oct4 expression. Oct4 is expressed by the cells that will become the ICM which will eventually produce the Stat 3. Stat3 is required for the self-renewal of these ICM cells. In the next step, Nanog, another transcription factor, is suppressed by Cdx2. Neither of these two (Oct4 and Nanog) will be expressed in the trophoblast, and Nanog will induce these ICM cells to become epiblasts. Nanog

will not allow hypoblast formation. In the diagram, the arrow indicates the induction, and the red line indicates the suppression.

Oct 4, Nanog and Stat3 all three together are essential for the stemness of the embryonic stem cells in the epiblast. Suppose these stem cells are removed from the embryo and cultured by maintaining the expression of these three. In that case, they are embryonic stem cells that can differentiate into different kinds of tissues and differentiated structures.

So these ES cells come from the epiblast, maintaining the expression of these three transcription factors. So here is the structure of an embryo shown. ICM is shown with Oct4 expression, and these green ones are the hypoblast cells, and here you have the integrin protein expression indicated by the blue, and these are the trophoblast cells.

Now let us see why Cdx2 does not express here in the cells that will become ICM. Those are the cells present inside, and these are the cells present outside this inside-outside difference that seem to be the cue for the differential gene expression here.

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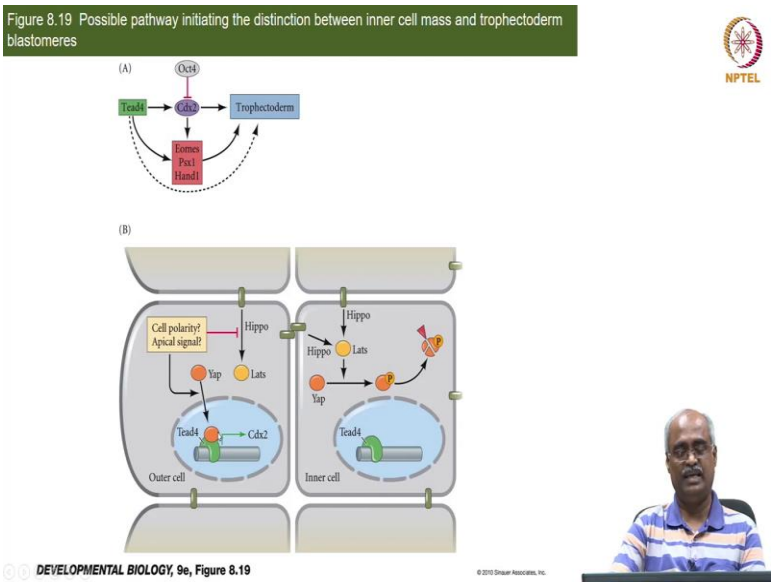

So that is shown in this summary cartoon but we will see in some detail in the expanded version of the same. So we already know Oct4 suppresses Cdx 2, so here we saw that.

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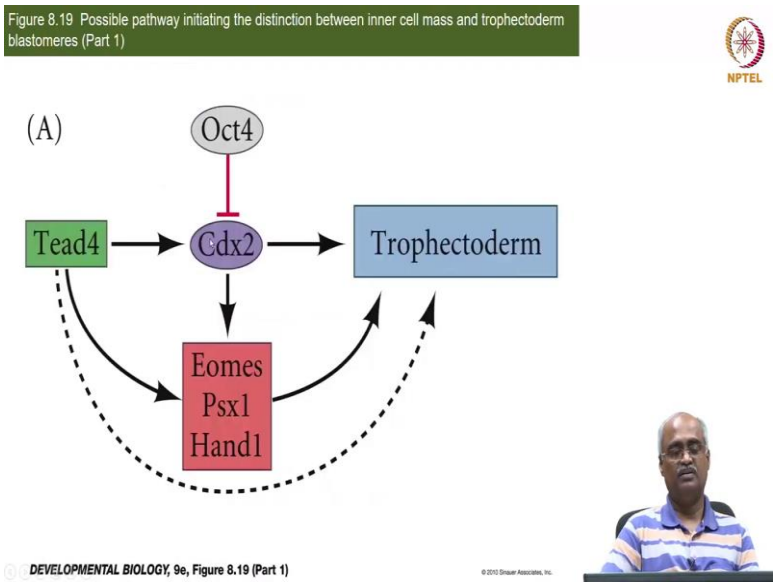

Cdx2 expression is induced by a transcription factor called Tead4. Tead4 is expressed in all cells in the morula stage, and it is located in the nucleus bound to the promoter of Cdx2 but does not activate Cdx2 expression in the ICM cells. It activates only in the trophoblast, and it also induces the other transcription factors.

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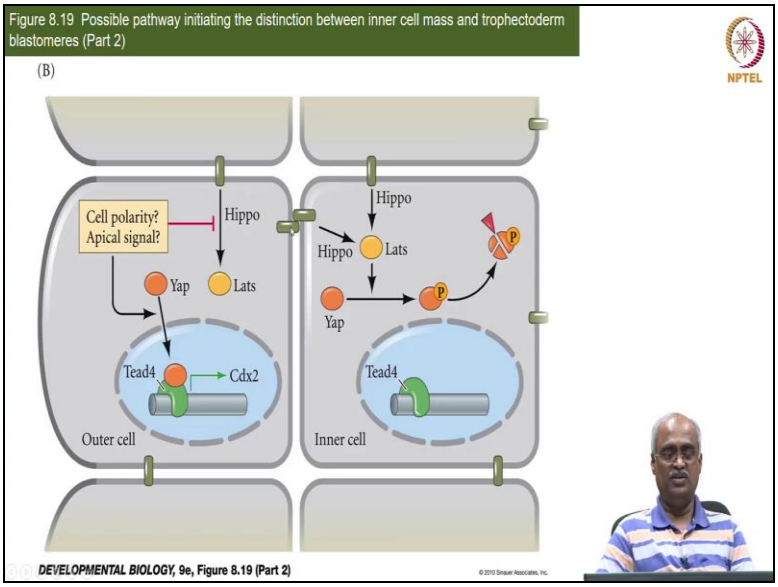

The ICM cells have gap junctions on all sides, which is sensed by the hippo pathway, and that pathway activates a protein called the Lats. Lats ubiquitinates another protein called Yap and promotes its degradation. As a result, the Yap does not go into the nucleus and associates with the Tead4. It has been seen that the Yap is localized to the nucleus only in the trophoblast cells.

In the trophoblast, the hippo signaling is not active, presumably due to cell polarity. Here, the other side is not connected to another layer of cells, as you see here. Here you have an apical, basal polarity in this cell, which presumably inhibits this hippo pathway. As a result, Lats does not get activated and phosphorylate Yap. Only phosphorylated Yap gets degraded, and the unphosphorylated one is not targeted for degradation.

And therefore, it is available to migrate into the nucleus, where it associates with Tead4. This is why Tead4 requires transcription factors acting combinatorically. Here is another example of that, so Yap and Tead4, when both are together, the downstream targets get transcribed. So here, without the Yap, Tead4 does not activate. This is why Cdx2 is expressed only in the trophoblast, and the rest are consequences of that.

Now, Cdx2 is not expressed in these cells, and as a result, they activate Oct4, and Oct4 will suppress Cdx2. This mutual antagonism between these two reinforces the suppression of the trophoblast and ICM remain separate. So that is how the inner cell mass trophoblast separation is controlled by sensing this apical-basal polarity cue that is formed by what we saw here.

So the outer cells that formed tight junctions and the inner cell mass that formed gap junctions remained inside. This is sensed and translated into a differential expression of transcription factors, and that differential expression of transcription factors drives all these changes that we see here. So this completes our description of early embryonic development up to gastrulation. So tomorrow, we will continue on the mammalian embryonic development starting from gastrula.