Introduction to Developmental Biology
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Lecture No – 22
Plant Development (Part 12 of 3)

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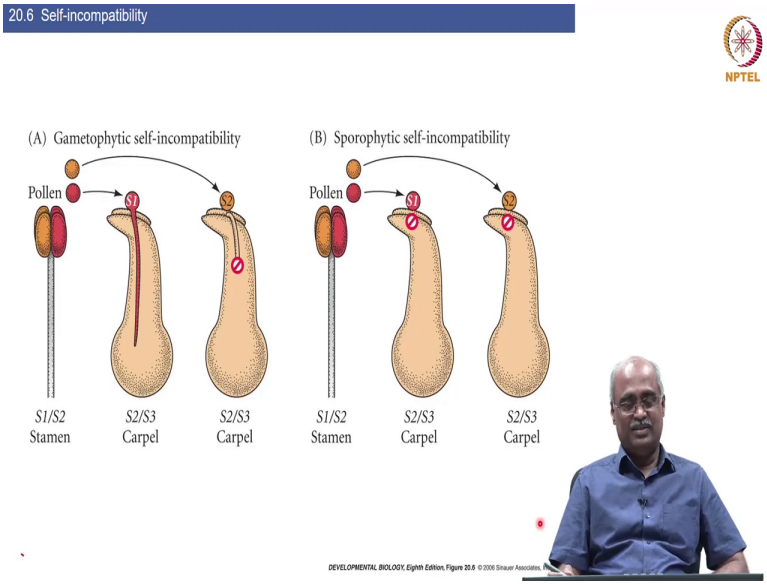

Students welcome back to the developmental biology classes. So in the previous lecture I was, you know, introducing and describing about the structure of the female and male gametophytes and how they form. The main point is that in plants, meiosis does not directly make gametes instead, it makes what are called spores. And these haploid cells of the spores undergo meiosis to finally make them gametes. And we were beginning to understand the process of how the male gamete finds its way to the female gamete and, I told you, that is pollination.

To understand pollination, first, we try to look at the different kinds of flowers we saw in some plants. Both male and female gametes are made on the same plant and we call that monoecious and within that you have a perfect flower where in the same flower both gametes are made. Then we had staminate and carpellate flowers, where the male and female gametes are made respectively. Then we familiarize ourselves with another group of plants called dioecious plants, where one plant makes like or one individual member of that species makes only one type of gamete and not the others. We saw palm trees as an example.

So today, we are going to continue on that theme. um So there is one interesting phenomena that happens in this pollination process and that is avoidance of the pollen grain produced by the

same genotype fusing with the female gamete of the same genotype. So that is called incompatibility. So incompatibility helps in maximizing the, you know, combinations and therefore the genetic diversity generated by the sexual reproduction.

So this is achieved in a number of ways and there are two broad classes. One is called the interspecific incompatibility, where pollen grain of one species does not fertilize the female gamete of another species or a different species. Then you have something called intraspecific self incompatibility where within the species it does not happen. And that is the process that has been well understood and that is what we are going to look at in some detail.

This intraspecific self incompatibility, again, can be classified into two types. One is called this gametophytic self incompatibility and the other one is the sporophytic self incompatibility. So these two cartoons illustrate this process. So first, let us look at the gametophytic self incompatibility which is a lot easier to understand. Like, for example, the genotype of the plant the sporophyte that makes this pollen grain has two alleles of the self incompatibility locus or the S locus.

Let us say it is S1 and S2. These are the two alleles, you know, for a diploid you expect two alleles and these are the two alleles. So you will have two kinds of pollen. You know, let us say this is S1 and this is S2. So now here, when for example, here S1 pollen landing on the stigma of a carpel of a sporophyte having the S2, S3 genotype, so here if you look at these two alleles are shared so this also has one allele of S2 this also has one allele of S2.

So both this gamete, S1, but this one does not have S1 although they share one allele. So purely looking at the allele, of the type of allele present in the gametes, this S1 does not have match with either one of the two types of genotypes possible for the female gametophyte. It will be either S2 or S3 and not S1, so therefore this is a compatible interaction. You have the pollen grain germinating. Remember I was telling about in the previous class while describing the pollen structure.

I said pollen has one cell that we call a tube cell and another cell within this tube cell called the generative cell. So I told you why we call this tube cell will become clearer later. And here you see the tube cell though you know perfectly sphere like, it germinates and elongates forming a tube like structure and this happens when you have compatible interaction. Here, S1 and S2 or S3 are compatible and therefore the germination and therefore pollination is going to be successful. Whereas this pollen with S2 allele for that given self incompatibility locus shares an allele in the carpal and as a result, this is recognized as self and when it is recognized as self this germination does not happen.

So the pollen tube even if it starts, it degenerates and there is no fertilization that is going to happen. So this is called gametophytic self incompatibility, where the gametes sharing the same alleles are recognized. This is S1 and this is on S2, S3 so that these two alleles are completely different from this, but even then it fails to germinate. That is because, I told you, that the pollen grain has an outer layer called the excine and the excine is produced by the sporophytic tissues.

So, sporophyte had S2 and therefore the products of S2 allele will be there in the exchange. And due to that this plant with this carpel having the S2 will be able to recognize that component and as a result it fails to germinate. It is primarily because the sporophytes share a common allele although gametophyte itself does not. And the mechanism for that is because, although this is a gamete having S1 allele its outer cover is derived from the sporophyte and that sphotophyte has products of the S2 allele.And this sporophyte incompatible therefore we call this as such.

So this is the difference between the two intraspecific self incompatibility where you cannot have match between the gametes alleles then its gametophytic self incompatibility and you cannot have match even with the two sporophytes, producing the two gametes and that is the sporophytic incompatibility. So now, let us look at mechanistically in a biochemical sense how these things work.

There are multiple mechanisms. In some species for example the S locus encodes an RNase and the presence of that RNA itself in the pistil is enough to avoid meeting with the same, you know, allele coming from the another plant. There that RNAase is responsible for the, you know, the failure of the pollination and there are other species where f box proteins do the job.

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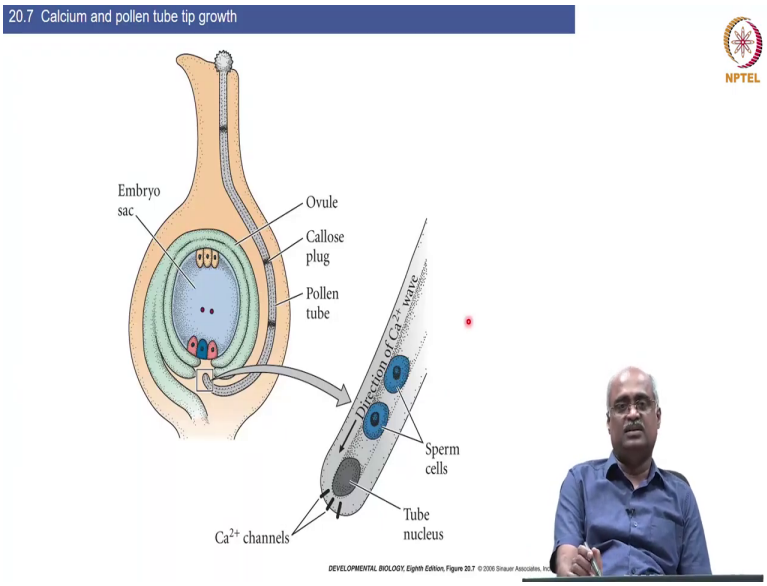

And in a third variety what you have is a calcium wave based rejection. So, when the pollen grain germinates that is, the tube elongates and forms this tube like structure. In this you have a slow moving calcium wave towards the direction of the end of the tube and this calcium wave is important. And you have calcium channels on the tip as well as on the flanks and they are responsible for properly guiding and for the successful pollination, for the successful germination of the pollen grains

And in the incompatible situation it has been observed that this calcium wave is disturbed and when the calcium wave is disturbed again you do not have the successful germination of the pollen grain so this is a third variety.

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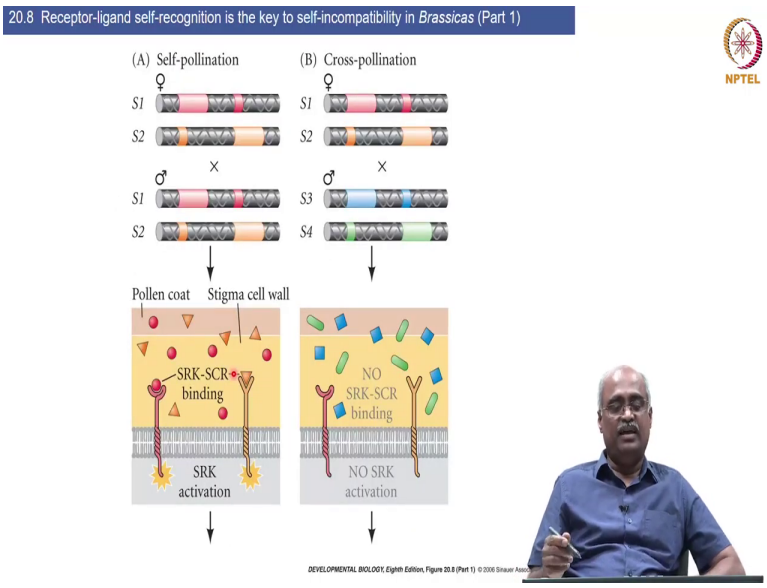

And there is a fourth one, which operates mainly in brassica where it has been extensively studied. There you have a receptor ligand or recognition situation. So, for example , when you have a self pollination between the two plants, one S1 S2 and the other one having the same allele. So what happens is the pollen coat contains the ligands and that binds to the cysteine rich proteins and that is why it is called scr. They bind to receptors which are serine threonine kinase and when you have a compatible look at the shape of the cartoon here in the receptor.

So they are shaped to fit the ligand so therefore it is a compatible interaction - in terms of the ligand receptor binding not in terms of the fertilization itself. And when the ligand fits the receptor, it initiates a reaction and that leads to abrogation of the pollen tube. Due to that pollen grain that pollen is rejected. Whereas when the pollen that lands on the stigma is from a different genotype, like this is S1 S2 and this is S3 S4. So here, you have two shapes to indicate that it is diploid and therefore two kinds of, you know, ligands are possible and similarly you can have two kinds of receptors.

So the same here, so you have the two kinds of ligands coming and then you find that their shape does not fit the receptor present in the stigma. So as a result, there is no activation of the downstream signals that leads to the rejection. So this is a compatible interaction in terms of the successful pollination. So this is another mechanism by which it works. So, in summary there are different kinds of biochemical mechanisms that help in the avoidance of pollen grains landing and successfully pollinating you know the ovules of similar or carrying the same S locus allele.

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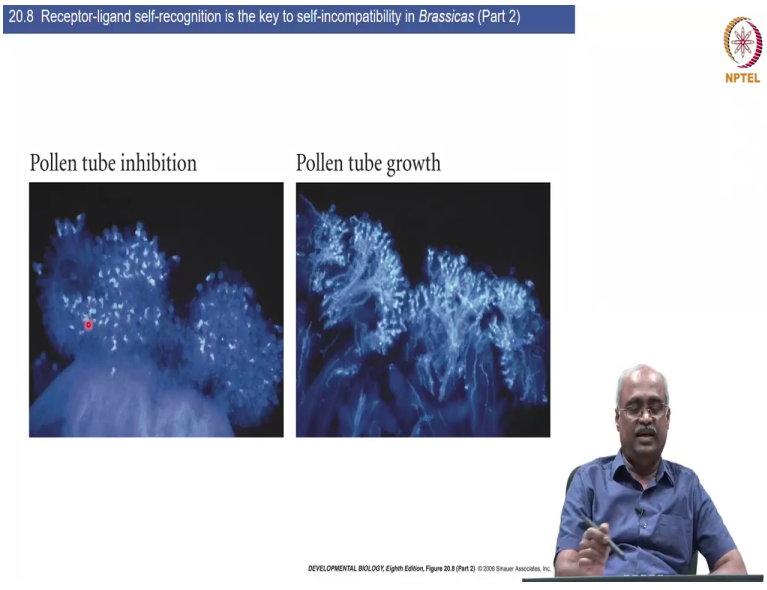

So this is how it looks when you look at the pollen tubes. So, first, let us look at the control you can see these long structures. So this is a tracing the nuclei of the pollen tube and the generative cell within it. So it is stained for the nuclear DNA and with a blue color dye and that visualizes the pollen tube that is coming in all of these. You can see a bundle of this. Whereas you do not see that here, the pollen tube does not even elongate. So this is a successful rejection and this is a successful tube growth initiation.

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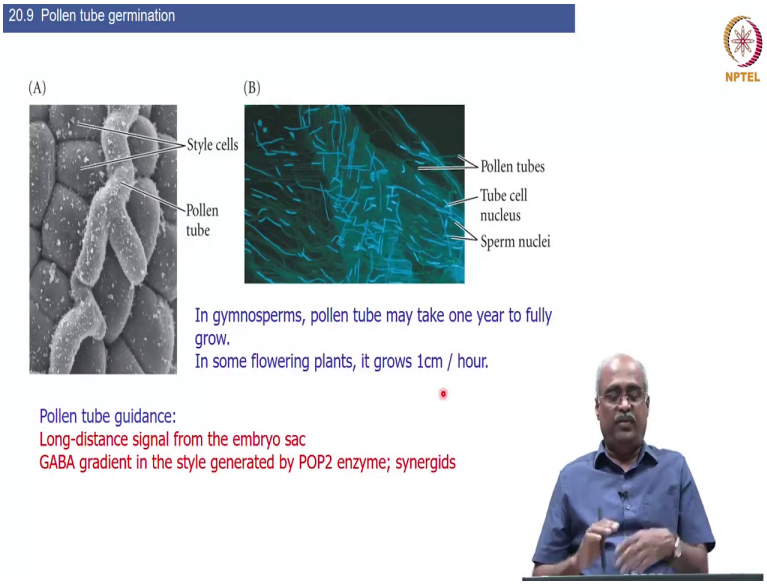

So, that is our discussion on the incompatibility. Now let us continue with the pollination itself. Now once it is compatible and the tube germinates, it starts to grow in some species very rapidly you know as fast as one centimeter per hour. And like in gymnosperms, it's very slow and takes longer. Like sometimes it can be as long as a year for it to fully grow. So this is a scanning electron micrograph showing you know the style cells here and this is the pollen tube that is coming down.

And so here you have the nuclei nicely stained with the DNA binding dye DAPI and you can see the pollen tube cells here. And you know the elongated structures of the sperm nuclei you can see here. So therefore they grow, you know in angiosperm particularly, they grow very rapidly and during this process within the tube cell you do have the generative cell migrating down. So this is only one known exception where a plant cell migrates but not intercellularly, it is intracellularly you have the generative cell migrating.

And during this process generative cells would have undergone mitosis and generated two, you know, haploid nuclei. You know it starts with haploid so therefore not surprising you will have two nuclei there. And it will become clearer a little later why there are two nuclei here how they are going to fertilize two sperm nuclei. So this tube generation and you know its migration all the

way going down to the micropyle, you know as shown in this image, obviously is an elaborate process. So therefore there will be proper guidance, repelling and direction to the right place all of that will be involved.

And experiments so far have shown that the embryo sac itself signals the direction of this pollen tube growth. And you know, experiments have been done where you when you have defect in the embryo sac but not in the surroundings for sporophytic cells amd still there had been problem in proper pollen grain germination in the tube elongation, clearly indicating that the embryos sac does signal the process of tube growth. In addition, the sporophytic cells of the style itself is also required for example, it builds up a gradient of gaba you know which is a neurotransmitter in animals.

And this gaba gradient also matters, you know, there is an enzyme called POP2 that degrades gaba and as a result it generates a gradient from the source where gaba is produced to the sink where it is being degraded. And the gaba gradient in the style has also been shown to be important for the successful pollen tube growth. And in addition, these two cells, the synergids, they are also responsible for guiding the pollen tube to successfully go to the egg cell.

And once it goes there and delivers, it delivers two sperm nuclei. One is going to fuse with this egg cell nucleus and that is the normal fertilization generating a zygote. And the other one is going to fuse with these two nuclei of the you know this endosperm, you know, precursor cell. This is going to become the endosperm. This multinucleate cell finally is going to make the food reserve for the developing embryo which, we call as the seed.

So in the seed that you normally see, only a small part is where the embryo is going to be. The bulk of it is actually the endosperm storing the food. That is what we get from the grains. You know, when you eat rice or wheat you are actually eating the endosperm.

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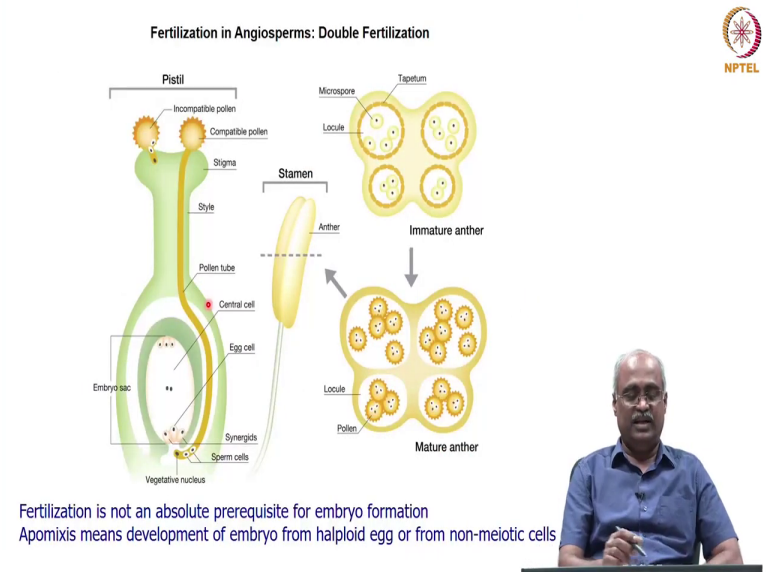

So that is sort of summarized in another cartoon. So these things we have already gone through so again have, you know, this is the microsporangium having multiple pollen grains developing. So that is shown in this diagram, you know multiples of them in that. While the megasporangium generates only one, so it is again recapitulated in this cartoon. And you have the two sperm nuclei here and these are the ones that are going to fuse with this, another one is going to fuse with this and as a result fertilization in angiosperms is called a double fertilization.

One fertilization is the normal reproductive fertilization the other one is only going to generate the food reserve. But interestingly, this fertilization is not really important for embryo formation. There are situations where this haploid cell or even sporophytic cells need not even be egg cell or need not be capable of generating an embryo and that kind of an embryogenesis is called apomixis, meaning no mixing. No mixing of two different genotypes to make a zygote.

And that is agriculturally important, and you can read more about it but from a developmental perspective it is beyond the scope of this introductory course so I am not going to get into it. So all you need to know is that in plants fertilization is not essential for embryogenesis in all situations. There are contexts where the haploid cell can actually make an embryo or even diploid sporophytic cells can also make an embryo. So the ploidy does not seem to be a crucial thing for embryogenesis.

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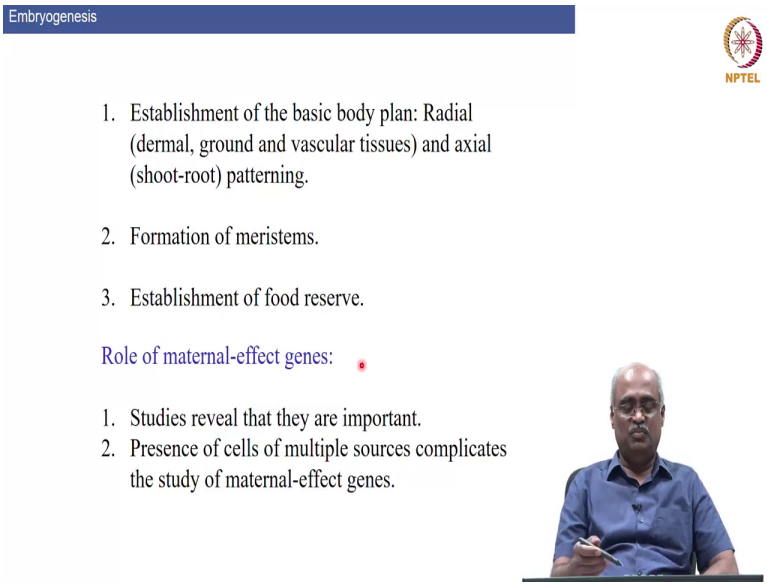

So that is about fertilization. So now, let us continue on that to the next step. Obviously the next step is embryogenesis, how embryo develops. So we first saw how the two gametes are formed and how they fuse. So the next after they are fused, how they are fused and how the zygote itself develops. So, the embryonic development has three clear you know purposes that are achieved and one of them is establishing the body plan. So we saw that in the Drosophila as well. Dorsal-ventral axis and axis anterior-posterior axis formation, how that is set up first.

Here the body plan involves a radial body plan. Like if you take a cross section from the center to the periphery. So that is the radial plant, so from the periphery you have the dermal the outer layer of plant tissues and the cortex that inner but towards the outer part of the inner side, that ground tissue. Then the innermost tissue, which are the vascular tissues. So this forms the bulk of the, you know, stem or root. Then you have the xylem and phloem, the transporting cells and that is the vascular tissue. So these are three different kinds of embryonic cells that are originally set apart.

You know something like the ectoderm endoderm and mesoderm. That is a radial patterning. Then you have top bottom instead of left right here we talk about top bottom because plants are rooted in the soil and they grow, you know, away from the soil and the root goes into the soil right. So therefore there is an axial patterning as well that is shoot root. So, here you have three types of tissues and here you have two types one is the shoot and the other is the root. So these two patterning need to be established, so that is the basic body plan.

And then another important aspect of embryonic development is setting apart meristems. So meristems, we will see elaborately a little later where essentially they are stem cells - undifferentiated cells - from which different kinds of organs can form. They are set apart initially. Most of the plant structures, for example, leaves branches flowers everything comes

from the one of the meristems called the shoot apical meristem and the root, root cap etc., form from root apical meristem.

So and those two meristems have to be established. And the third, which is unique to plants, is you know building up a lot of food. You know, they are not going to have like mammals continuous contact with the mother during the course of embryonic development. Rather, once, you know, the fertilization is over they are going to get disconnected from the mother because they have to, you know, be dispersed to different locations. The plants do not walk around so their embryos have to be broadcast over a big area which means that the product of fertilization here, I want to start using the word, seed has to disperse.

So that means wherever it is going to land, to start off its life it needs to have stored food resources. Therefore building the food reserve is an important goal of embryogenesis, unlike what we saw in other situations. And so that is the third important purpose achieved during embryonic development. So let us go into each one of them in some detail.

So, before we go further since we learnt so much about maternal effect genes in drosophila to keep the continuity with that we will briefly think about maternal effect genes as well. So, in drosophila we saw maternal effect genes are key so they set up the initial morphogen gradient within gradients within the zygote and that drives the rest of the, you know, asymmetries all the way leading to segmentation of the body plan. So here are there maternal effect genes? Do they have that sort of a central contribution? So that is an obvious question. So the answer is yes, they do play a role but here the situation is a lot more complicated. You just saw in our discussion on incompatibility in our aspect, the pollen grain has maternal components right around it.

So similarly the embryo sac is deeply inside layers of maternal tissue. Like, for example, if you see this, you know, this whole thing is maternal tissue. So this is where fertilization happens the integrands which are going to form the seed cover so this is maternal tissue having diploid, you know, cells. So due to this intimate association of the maternal tissues it has been very difficult to really delineate on the exact contribution of maternal effect genes. Nevertheless, some of the mutants isolated in arabidopsis and maize show that the maternal genes do matter. Particularly, the maternal genes contributing to the embryo sac development. So they have been shown to be important.

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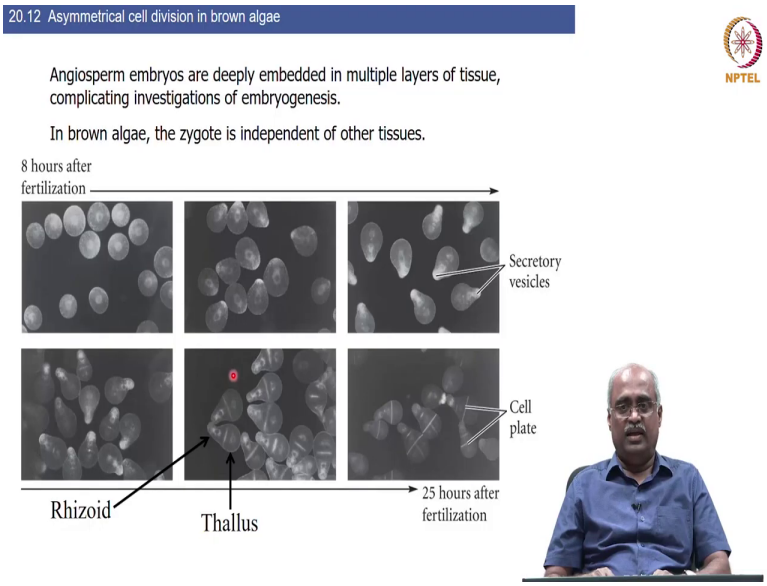

So again, the same issue that the embryo develops are inside several layers, you know, covered by several layers of the sporophytic tissue studying embryogenesis has not been easy. You know Drosophila lays eggs, C elegans lays eggs and so on but you do not have that sort of a thing with the angiosperm. And therefore it is not easy to look at them. So sometimes, what scientists do is they look at other plants like primitive ones, like brown algae where the zygote is independent of the other tissues.

So there is external fertilization of the zygote and it is not, you know, covered in layers of the mother's tissue. So people have looked at the embryogenesis in in brown algae in some detail and one interesting information that comes from is the role of the secretory vesicles and how the environmental cues impacts the polarity the anterior- posterior polarity or, you know, dorsal ventral-polarity that we learnt when we discussed about drosophila. So here, again polarity the important polarity here is the top to bottom, you know, the vertical axis is important you know shoot root.

And there, while like in many animals the point of sperm entry initially sets up the polarity. Then that initial polarity cue provided by the sperm entry can be overridden by light. Because there is no point if the sperm entry point ends up facing up and lying on the soil and if you start developing root upwards it is of no use. So therefore, the environmental cues can override the initial polarity cue provided by the sperm entry. So that is an interesting fact of plant reproduction - embryonic development - that came from studying the embryogenesis of brown algae.

So here, let us look at these series of images. So this is eight hours after fertilization immunostained for a protein that is specific to secretory vesicles. That is what first you start to see and the secretory vesicles then start uh kind of moving towards one end and that is initially

set by the point of sperm entry. That is where the root kind of tissue is going to form which is called the rhizoid. So see, so in this you can see that asymmetry. So this is forming a structure which we call rhizoid. And that starts to form and you can see secretory vesicles, the white color thing, really asymmetrically moving towards this rhizoid end not to the other side.

And these secretory vesicles carry a cell wall component that is distinct and unique to the rhizoid. And that is how the rhizoids forming cell is distinguished from the other one - shoot equivalent called the talus. And the first cleavage is perpendicular to this axis, you can see here the cell plate forming, and so that is how the asymmetry is established. So in summary, the sperm entry site marks the place where rhizoid is going to form and then you have an asymmetric movement of the secretory vesicles towards the rhizoid and there they deposit the specific cell wall components that distinguish the root from the shoot.

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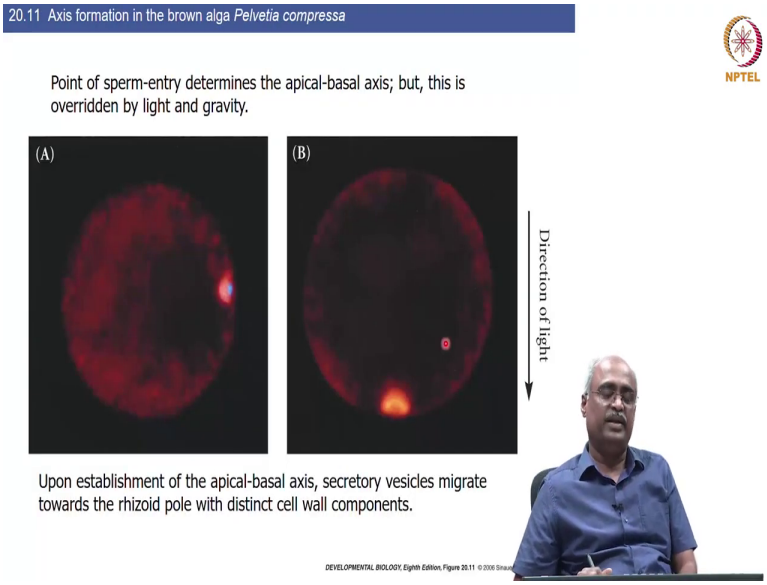

But this axis formation can be, as I said, totally overridden by the direction of light. So here, for example, this blue color is the DNA of the sperm so this is the sperm entry point. So initially F-actin, you know, which is important for the cytoskeletal rearrangement gets deposited here as if this is where rhizoid is going to form and this is where secretory vesicles are going to come and deposit the cell wall components. But that can be overridden if you shine light in this direction, as shown by this arrow.

So now, that sperm entry point is forgotten and instead F-actin forms a foci, focus at the bottom, away from the light at the darkest point.Now, the secretory vesicles are going to move here and deposit the, you know, the rhizoid specific cell wall components. So this is how the apical basal axis instead of anterior posterior here we call, apical basal top bottom axis. And as I said, you know the distinct cell wall component seems to be key for this.

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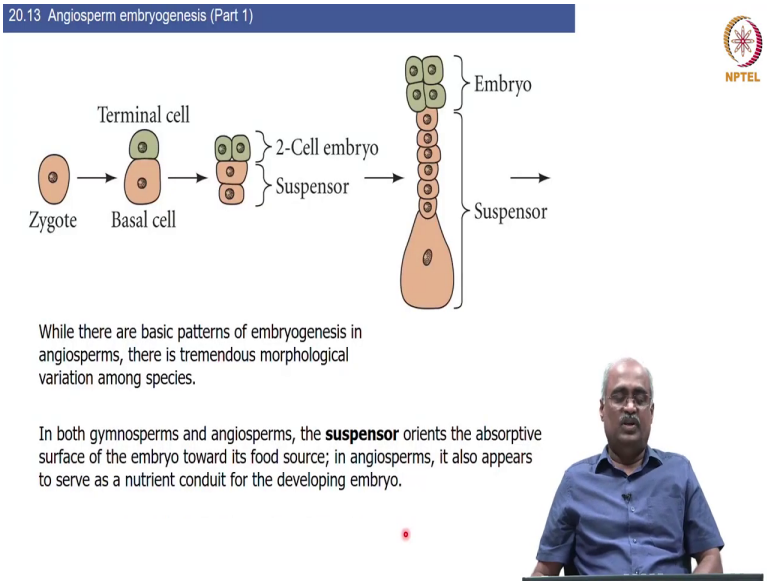

So this is about the asymmetry of the first division we understood by studying brown alga. A similar thing happens in angiosperms as well. So the initial cell division is asymmetric generating two cells , the small cell is called the terminal cell; this is the one from which all of the embryo is going to form. The other cell called the basal cell is not going to contribute to the embryo at all. So this is going to divide and form a structure, a temporary transient structure, called the suspensor.

So this is the basic pattern. You have an asymmetric division producing one cell at the top which is going to form the embryo proper called terminal cell, and then another one called the basal cell which divides to produce a structure called suspensor. So this basic pattern is common in the embryogenesis of angiosperms but then among the different flowering plants you have tremendous morphological, that is shape, variations on this basic theme. So that is something we need to remember that that is why all plants do not look the same.

But if you look at mammals if you go to the very, very early embryo as we have learnt during one earlier last discussion there is great similarity. So what is the role of the suspensor? So what the suspensor does is in both flowering and non-flowering plants like gymnosperm and angiosperm, it orients the absorptive surface of the embryo towards the food source. Therefore, they kind of facilitate the embryo to uptake nutrients from the food source.

And in flowering plants, it actually acts as a conduit as well for the food to the developing embryo so that is the role of suspensor and eventually the suspensor is going to degenerate and the rest of the embryo is going to form from this. So the body plan that we talked about the radial dermal, ground and vascular tissue and the shoot and root you know apical meristem setting up they are all going to happen from these cells - embryo proper.

So before that, let us look a little about what we have understood about suspensors themselves based on the genetic mutants identified in Arabidopsis.

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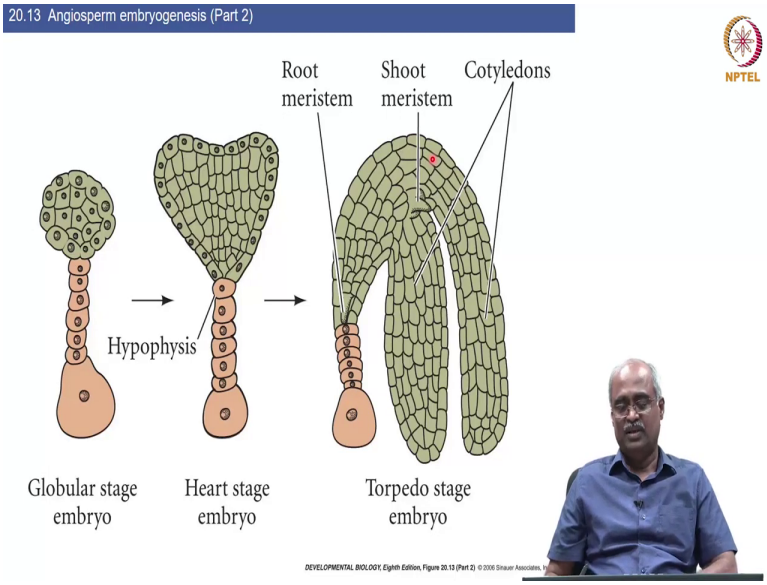

So that is going to be shown in a couple of slides. So this is an overall summary continuing from this step onwards. So the cells here divide again. I want a reiterate there are no cell migrations there is not anything like gastrulation as a result the plane of division the longitudinal and latitude division saw planes of cell division is really critical in generating the body plan and after a few rounds of division, you form a structure called globular stage embryo.

And in the globular stage itself, as we will see in a couple of minutes, the radial patterning gets established. From this, when the cotyledons, the seed leaves start to form. So these projections, you know, these mound like thing forming this globular then ends up changing shape into heart shape. So this is globular embryo and this is heart embryo. In some cases where this develops really elaborately and to be fit within the seed cover remember the integrand is going to form the seed cover to package it when it bends like this it forms a torpedo like shape.

And therefore it is called a torpedo stage. And during this stage clearly the apical and basal polarity becomes very apparent, once these mounds of cells, that is, these two cartilages start to form. This is in dicots. In monocots you have only one cotyledon developing. So, here you have the root meristem and here is the shoot meristem and these are the two cotyledons. And this is an overview so we will get into some you know at some detail each of one of these.

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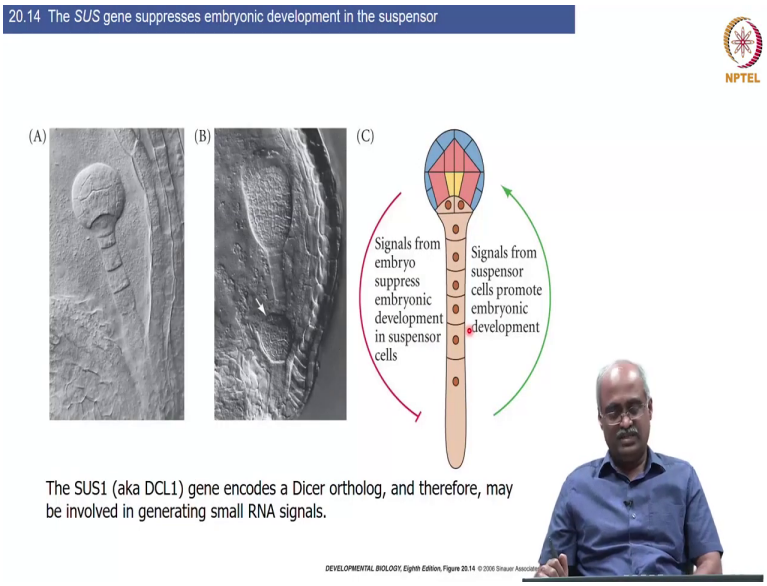

So first let us look at the suspensors. So this is the micrograph of a wild type embryo. So this is the suspensor attached via the placenta to the sporophytic tissue and this is the embryo developing. So in a mutant, in a gene called sus1 which is called dicer-like01 because of its similarity to the dicer. You know dicer you may have heard or in discussions on RNAi, so dicer is a protein involved in cleaving RNA into small signaling RNA molecules so called siRNA's they are involved in even in mi RNA generation.

So miRNA are usually transcribed as tandem repeats and then each of the repeat has to be cut into the monomers and that is done by dicer. So it is basically an RNA cleaving enzyme. SUS1 bears similarity to that and as a result it is also called DCL1. Mutations in that protein result in embryo-like structures forming out of the suspensor cells, indicating that in the wild type SUS1 is important to prevent embryo-like development. Which further indicates that suspensor cells have the ability to form embryo.

And based on this and other mutant studies, the current model of how the embryo suspensor polarity is maintained is explained in this model. So you have signals coming from the embryo telling the suspensor not to form an embryo. So it is a suppressor, so suppressor is usually indicated by a line like this, this sort of structure is suppression in developmental biology and this arrow is an activation. So signal from the embryo suppresses embryonic development in suspensor cells and signals from the suspensor cells promote embryogenesis in the embryo.

So this is what currently, you know, scientists think the way signaling happens between these two to ensure the suspensor remains a suspensor and allows the embryo to develop normally.

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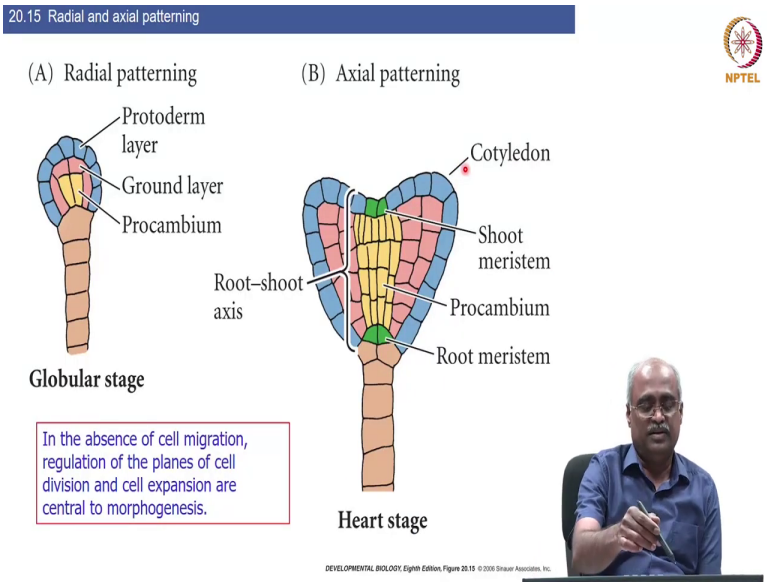

So now let us continue with the establishment of body patterning. I told you in the globular stage itself the radial patterning becomes obvious, that is indicated in this, in a color coded carton here. So the outer is the protoderm. So this is the one that is going to make the dermal tissue, which is going to be the outer cover of the plant organs, and then you have the inner tissue from which cortex, pith etc are going to form. The central portion of the, not the very central, but between the the you know the vascular tissue giving cells under the outer periphery that is the dermal tissue the in between layer that is the ground layer and that is this ground tissue.

Then you have the procambium which gives rise to the vascular tissues, that is xylem and phloem, you know, for conducting water and food respectively. So this becomes clearer at globular stage itself. And once the cotyledons are going to form, cotyledons do not form only from the protoderm. It is derived from the general embryonic cells. And when that is going to form, you get to the heart shape and I just told you that by the time the apical basal polarity becomes obvious and that is indicated in this cartoon by showing where the apical, shoot apical, meristem is set apart and then the root apical meristem is set up.

So these two give you that by this stage the apical basal polarity has been already established. And in this process, the plant hormone auxin plays an important role. Again, I want to highlight before I get into auxin in the options of cell migration regulation of the planes of cell division you know longitudinal and latitude divisions are really critical along with cell expansion for the proper morphogenesis during embryonic development in plants.

So there are no cell migrations, so therefore planes of cell division and cell expansion are important. And only by changing these two all the different shapes are generated. You know, that thought itself is quite fascinating 0 without migration simply by changing shape or sorry,

changing the direction of expansion and plane of cell division you can create a variety of shapes. So now let us move to the auxin discussion.

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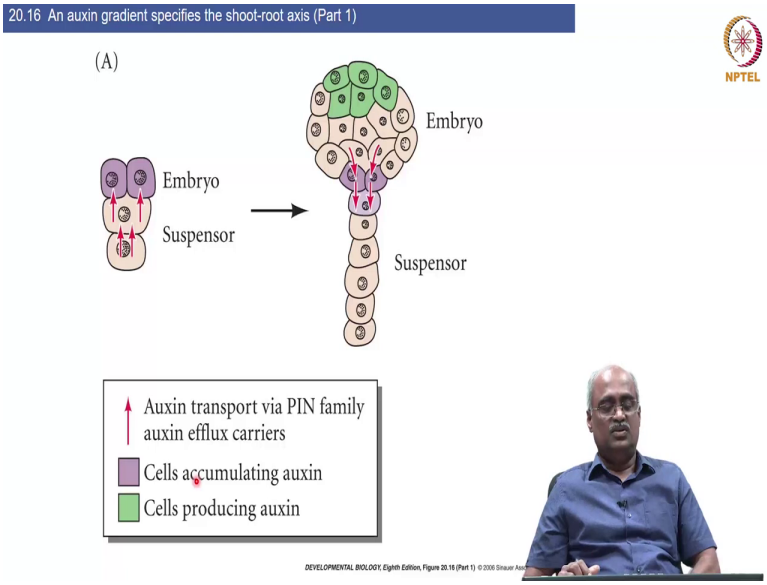

So initially auxin moves like, for example, in this 4 cell stage it moves apically and then later it moves towards the hypophysis, you know that is the junction between the embryo proper and the suspensor. So that is where the root meristem is going to be set up part and it starts moving towards this. And for this, the key protein that is required is this PIN family Auxin efflux carrier. So these are important for carrying the auxins in the direction shown.

So they are produced asymmetrically and that asymmetrical expression is key for the direction of auxin movement initially to apical portion and later to this hypophysis site and the PIN family localization rather their expression itself is controlled by a protein kinase called the PID 1. That is very critical for the directionality of auxin movement and mutations that affect proper setting up of the root meristem. So we will see that as we move on. So here, for example, in this wild type so PIN based on this kinase PID kinase sorry I said PID 1 so it is actually PIN 1 and it is just simply PID.

So the PID protein kinase is responsible for the normal PIN 1 localization. So based on the difference in expression of these two, PIN 1 gets highly concentrated here and as a result auxin flows to this and that auxin is responsible for setting up the place where shoot is going to develop. For the shoot apical meristem formation or flow of auxin towards this place is important and that is made possible by the high concentration of PIN 1 in this region.

So now, if you over express speed one as PID in the apical part now, PIN 1 does not get localized to here. Its expression is not concentrated here and as a result, auxin does not flow towards this.

So what is the consequence> So if you look at the wild type, due to PIN 1 localization here auxin flowing there induces the auxin induced gene expression in these cells. That is critical for the root development on the other hand, when you do not have that PIN 1 concentration, auxin flows everywhere and as a result these cells start expressing auxin target genes, that is auxin induced gene expression. Due to that you have everywhere root like structure forming and that abbreviates the normal embryo development.

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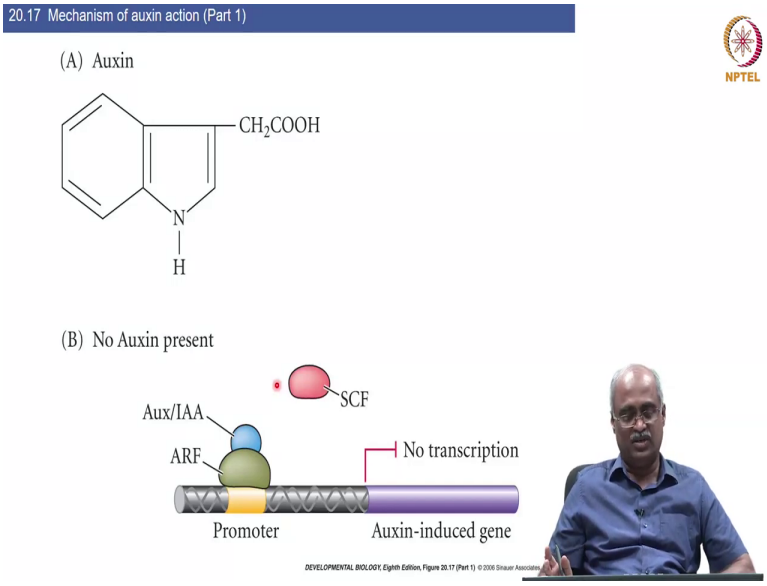

So the last part in the next two minutes, I will just explain how auxin works. So the understanding of how auxin works really helped in connecting plant hormones to the downstream gene expression and the actual cellular events leading to development. So auxin is you know, indole acetic acid. You know, you can see here if you remember tryptophan that has the same side chain so this acetic acid has indole so it is called indole acetic acid or IAA. So that is why this abbreviation IAA comes.

So normally in the promoter region of auxin target genes you have this auxin responsive factor ARF bound and this does not bind all by itself it also has another protein bound to it which is called Aux protein or IAA protein. When they are bound like this, ARF is kept inactive and the downstream gene expression is not activated. But if auxin is present, auxin binds to this SCF which is actually an ubiquitin adding enzyme and that ubiquitinylates this - which is shown in the next step.

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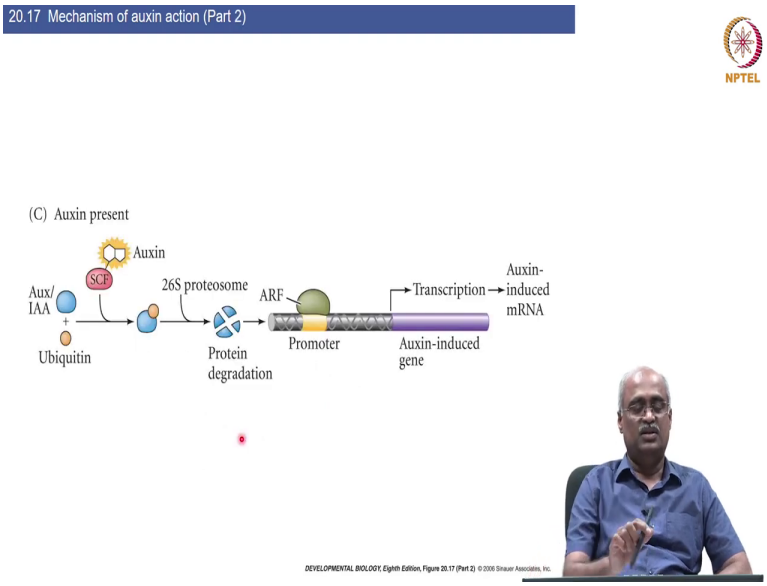

So in the presence of auxin, auxin binds to SCF and that activates it and that ubiquitinates this Aux protein and once ubiquitin related it is targeted to proteasome and gets degraded. And freed by this auxin, now ARF becomes active and that induces the downstream target genes. So this is how the auxin signaling works. So essentially normally the targets are kept suppressed by a suppressor binding to the auxin response factor and in the presence of auxin that suppressor gets degraded and therefore the suppression is removed and the gene expression is activated.

So this is the auxin expression, auxin induced gene expression is activated. And many mutant analysis have shown that this process is key for the normal formation of the root structures here. So with this we will stop today and then we will continue with the embryogenesis, the next steps that is you know setting up of the apical and basal, the two meristems like shoot apical meristem and root apical meristem and food resource in the next class.