Introduction to Complex Biological Systems
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Lecture 27
Pattern formation and growth

Hello everyone, we are in module 6 now, and this is the second lecture of module 6. Here, I am going to discuss pattern formation and growth. During the last lecture, I mostly discussed how different types of cells are established in the body of a multicellular organism, and how different types of cells originate. Today, whatever I am going to discuss is a little bit more complex.

Now, if you see, I already mentioned this during my first lecture in module 1. I mentioned there that although the basic or fundamental understanding about different types of cellular processes, like transcription and translation, is very much conserved in different types of organisms, including plants and animals. But if we go a little bit upward, the upward means here in the level of cells, tissues, and then as a whole, the whole animal or plant body. You will see different types of variations there. So, here, whatever I will explain now, you will see a lot of variation even within a phylum. For example, how birds, amphibians, or mammals develop, how their patterns are actually formed.

So, there are huge variations there. Let us see. So, mostly, I will be discussing these three points here today, the understanding body plan and complex organization, followed by the molecular basis of pattern formation during early development, and finally, understanding the growth of an organism. So, here, if you see, what I already mentioned is that different animals use different mechanisms to establish their primary axis of polarization. So, the major idea here is that when animals are developing, three major axes should be created at the proper timing. These axes should be created in proper timing.

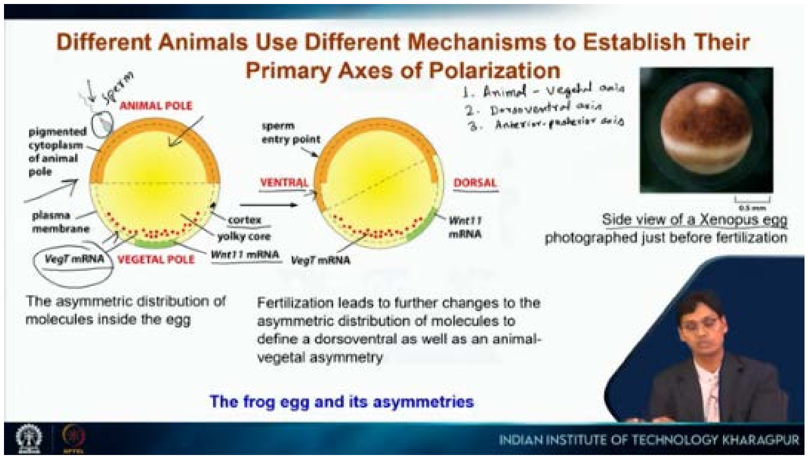
So, the first one is, I would say, number one is the animal-vegetal axis. So, three axes I told you that this one is the animal-vegetal axis and the second one is the dorsal-ventral axis. The dorsal-ventral axis, and number three, number three should be the anterior-posterior axis, the anterior-posterior axis. Now, if you see those three different types of axes. So, the problem here with the naming is the animal-vegetal axis. There is nothing to do with vegetables or anything. This is some kind of weird name. That is why all these things actually determine, I would say, the animal-vegetal axis means that during the development which portion will be internal and which portion should be external. This is just the animal and vegetal axis and, the dorsal-ventral axis, you understand from the name itself. So, that is dorsal, that is the back side, and ventral, the front side. For example, in humans, if you compare, the front side is the ventral part, and the dorsal is the back side, and the third one is the anterior-posterior axis. So, the head and tail part.

So, that is all. These three major axes should be established during development. I would say during the early stage of development itself. So now here, if you see, this is the side view of a general first egg. So now, particularly this egg cell, one important thing is if you see an egg from a frog. A frog is an example of amphibia. So, frog or bird. Their egg size, they're much bigger. But if you see the egg of mammals, particularly the egg cell of a human, it's very small compared to the egg of fish, amphibia, or bird. Now, if you see, there is a lot of information already present in the egg which dictates the future developmental plan. So, here I am just showing.

So, I will not go through all the details here, just the basic outline, the basic features I will discuss. So, here if you see this one is an egg of Xenopus. So, this is a frog egg, and even before fertilization, as you can see, this is the sperm here but even before fertilization, you can see some kind of asymmetric distribution of molecules inside the egg. So, as you can see here, I am mentioning here that VegT mRNA and then here Wnt11 mRNA.

So, there is a lot of stuff. So, those two are very well characterized. So, we are mentioning here that some kind of asymmetric distribution, as you can see, this same mRNA is not present on this side, but it is present here on the vegetal pole, but not in the animal pole. So now, what I am trying to explain here is that this information is already present in the egg itself. The zygote is not yet formed, the fertilization is not yet happening, but still, that information is properly organized and now this is the entry point of the sperm, and after sperm entry, you can see there is a little bit of rotation in the egg cortex. So, as you can see here, this is the egg cortex; this is the egg cortex layer here. So, here you can see some kind of rotation in the egg cortex. So, that will lead to further changes in the asymmetric distribution of molecules to define a dorsal-ventral as well as animal and vegetal asymmetry. So, as you can see now, we are also mentioning, in addition to the animal and vegetal pole, we are mentioning the dorsal and ventral side.

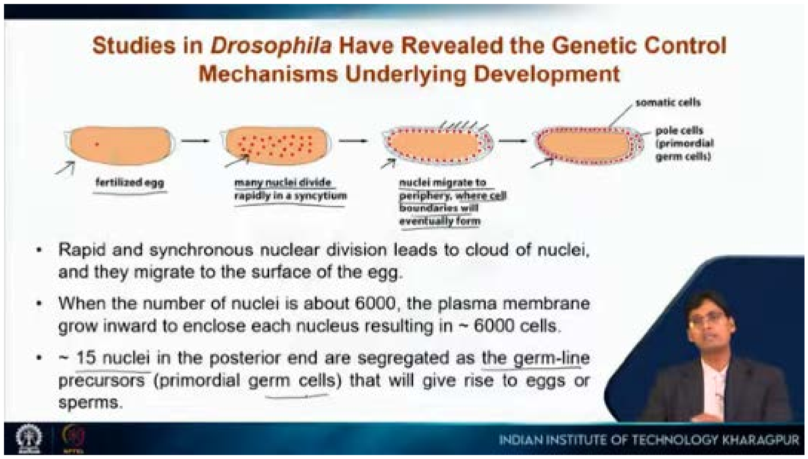
So, still, it is a single cell, but when it will divide into multiple cells, and it is going on by that time, those things are already decided, the dorsoventral axis as well as the animal and vegetal axis. So, this is the whole idea about this slide. The next thing here, so I was discussing the frog egg, but here in this slide, I am trying to explain something else with the Drosophila system, with the Drosophila egg. At the beginning, I mentioned that you will get several types of diversity in this developmental pathway, whether you were taking some pathway from a frog, Drosophila, or human, there will be many changes there. So, here, if you see, studies in Drosophila, particularly, it is a model organism and it revealed the genetic control mechanism underlying development. So, here, this is the fertilized egg of Drosophila. So, the Drosophila egg is not very much spherical; it is an oblongata cell. So, as you can see here, this is a fertilized egg. Now, the beauty here is, just after fertilization, this cell will first not divide; actually, the nucleus will divide very rapidly, and we will get a cloud of nuclei.



So, as you can see, there are many nuclei inside this zygote. I would say it is already a fertilized egg. So, you will get a cloud of nuclei inside and then, over time, these nuclei will migrate to the periphery, where the cell boundaries will eventually form. So, in this figure, as you can see, cells are present in the boundary. So, these are cells.

So, just the cell membranes are getting generated here, not yet fully formed, but it is starting to be generated now. But now, when the number of nuclei is about 6000, the plasma membrane grows inward to enclose its nucleus, resulting in approximately 6000 cells. Although, in this diagram, for example, here you will see this is in 2 dimensions. That is why you can see those cells are present in the boundary, but if you compare it in a 3-dimensional setting, in the entire periphery, they are actually now covered by a layer of cells by these cells, whatever I just mentioned.

Here, another important thing is that at one end, you will get approximately 15 nuclei in the posterior end, and they will finally form the germline precursor, the germ cells will make in the future. So, this is the beginning of Drosophila embryo development. So, at the beginning, how it is getting developed. So, here I just mentioned how initial nuclear division, followed by membrane formation, is overall how it developed from an egg to an embryo. But now we have to understand how this axis is being formed, whatever I mentioned at the beginning, that, for example, the anterior and posterior axis, how the head and tail will be defined.



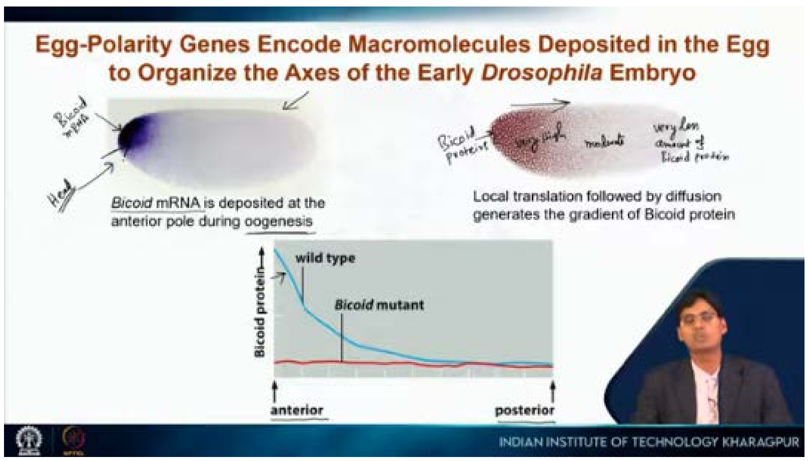
So, let us see. Some egg polarity genes encode macromolecules. These are deposited in the egg and organize the axis of the early Drosophila embryo. So, I will explain this. So, again, this is a single cell.

You can consider that this bicoid mRNA is deposited at the anterior pole during oogenesis, which means this egg is not yet fertilized; this is even before fertilization. You can see at one end here. So, something is getting deposited. So, a lot of research on this topic has actually established that whatever is getting deposited is some mRNA called bicoid mRNA. So, this is bicoid mRNA.

Somehow, this bicoid mRNA is restricted to one pole. So, if this mRNA is restricted to that pole, the mRNA is getting translated. Therefore, we will get some kind of concentration gradient of protein. So, here, because the mRNA is present, we will get more translation there and, after translation, those proteins, the bicoid proteins, will slowly diffuse through the cell inside the egg.

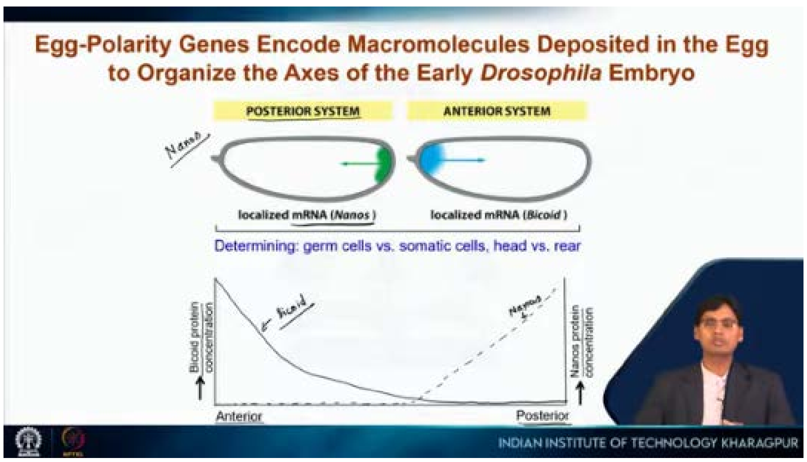
So, as a result of that, you can see here these are bicoid protein molecules, but this local translation followed by diffusion generates the gradient of bicoid protein. So, as a result of that, here you have a very small amount of bicoid protein, here I would say a moderate amount, and here a very high amount of bicoid protein present. So, it will create a gradient, and that will actually dictate the anterior and posterior axis in Drosophila.

Particularly, bicoid proteins or bicoid mRNA, whatever they are, are concentrated in one pole, which should be the head of Drosophila. So, this end will be the anterior side, or it will finally be the head part after so many rounds of cell division going on and on after that. Now, in this curve, we are trying to explain to you that this is the anterior side, and this is the posterior side. Now, if you see the bicoid protein concentration, as I already mentioned, this blue color curve here, this is the bicoid protein concentration at the anterior end, which means this side has more bicoid protein. So, you can see that gradually, as we go towards the posterior side, the amount of bicoid protein is getting lower. But the rate curve here, as you can see, has no changes because this Drosophila is a bicoid mutant, which means they are not making the bicoid protein. So, as a result of that, the anterior side will not be determined. So, this is just for studying this developmental pathway; scientists generated this mutant.



So, now I just mentioned this bicoid, and this protein is getting deposited, I would say, making some kind of gradient, and that makes the anterior side of Drosophila. But now, similarly in the posterior system, on the posterior side, on the back side, you will see another mRNA which is getting concentrated. That is called nanos and it will make some kind of nanos protein also, exactly like bicoid, but this is opposite. This protein will be more on the posterior side, and it will gradually go low and low towards the anterior side. So, as a result of that, if I try to understand it a little bit in more detail. So, here, I am just mentioning the x-axis.

So, here on the left side, this is the anterior side, and on the right side, this is the posterior side and, on this y-axis, if we are showing here the bicoid protein concentration, and on this right-hand y-axis, the nanos protein concentration, then how it looks like. So, it is very simple, as I already explained. So, in the case of bicoid protein concentration, I would say it will be something like this in normal Drosophila, no mutant, nothing. This is the concentration of bicoid protein. Similarly, nanos protein will be something like this, just opposite. Here, I am just mentioning in a dotted line for understanding purposes, something like this. So, this will be bicoid and this is nanos concentration. So, there are a lot of things and many other things are also involved. I just wanted to explain a few of them. So, that you will get some kind of flavor of how those things are happening, how these complex processes are happening during developmental processes. Now, we just mentioned at the beginning about frog embryo, like we started with the egg of some amphibian, and then next we discussed the Drosophila embryo, and then here we are going into some examples with something like a mammal. So, early developmental events in the mouse embryo, this is, if you see the whole slide, it is very complex.



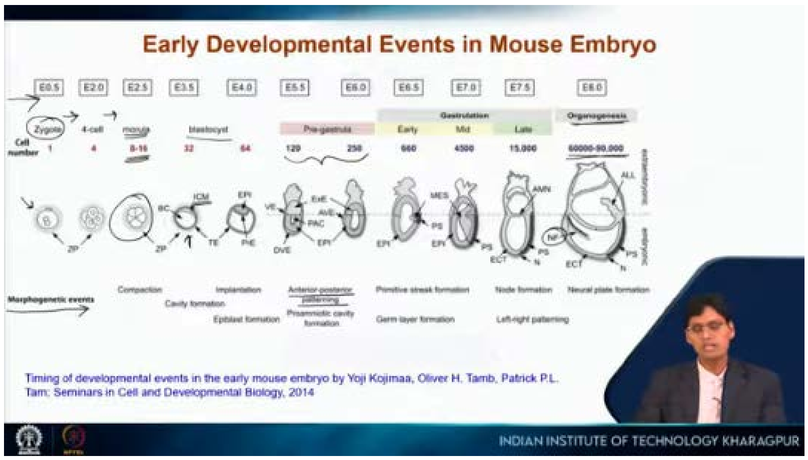
I will only like to mention some major points, like in context to whatever I have already mentioned. So, as you can see in the case of frog embryo, particularly I would say frog egg, as well as in the case of Drosophila egg, it has already been determined some of their axes because of their asymmetric distribution of substances like mRNA as well as proteins. But here, if you see, like early developmental events. So, here at the very top, whatever we are showing here, that E 0.5, E 2, E 2.5, this is nothing but embryonic edge, and then there at the beginning, we are showing that this is zygote, that means one thing we have to understand whatever I mentioned about frog egg as well as Drosophila egg. So, in that case, the female frog or female Drosophila, they just lay eggs outside externally, but in the case of mammals, everything happens inside the body. So, as a result of that, after fertilization of the egg that should develop into an embryo, and it will be implanted inside the uterus.

So, there are huge differences between frogs, Drosophila, and some examples from mammals, for example, mouse, human. So, as you can see here, the zygote. So, zygote means still one cell, but already fertilized, the egg and sperm, the fertilization already happened. Here in the lower panel, we are trying to show you that morphogenetic event, what is happening here. So, this is the zygote, and how many cells are present there, just one cell, just fertilization happened and then it is going like this, some cell division happens, then 4 cells, then 8 to 16 cells, and it continues like this. But remember, in the case of Drosophila, I mentioned that initially, just the nucleus divides, but in this case, no normal cell division is happening. So, as a result of that, you can see, I would say, at around the 8 to 16 cell stage here, which is called the morula. So, in this stage, all cells have the potential to make everything. In the case of the Drosophila egg, I mentioned that even before fertilization, the bicoid and nanos concentrations dictate which side will be the head and which side will be the tail, but in this case, as you can see at this level, the morula.

So, at the 8 to 16 cell stage, but at that time also, everything is very much similar; they have the whole potential here. Now, if we go further forward towards the developmental side, then you will find the blastocyst. So, now, from this point, you can see here some inner cell mass, the ICM, the inner cell mass is getting generated and then slowly, you will see a lot of stuff will be getting established. For example, since I mentioned the anterior and posterior pattern.

So, you can see here at this stage, when we have around 120 to 250 cells in this stage. So, their anterior-posterior patterning is happening in the case of the mouse. So, that is the difference between Drosophila embryo development and here, in the case of the mouse, and slowly it will go towards further developmental stages, and you can see when the cell numbers are very high here. So, almost 60,000 to 90,000 cells and from that point of time the organogenesis will start.

So, as you can see here, the nodal fold is being prepared. So, that is the way it is going on. So, this is, I would say, some kind of a very brief idea about the differences between the embryo or the patterning formation in the case of a frog, drosophila, as well as in a mouse. Now, whatever I mentioned at the beginning, the 8 to 16 cells stage has the potential to make anything, but in the case of drosophila, that is not possible because those are already defined in the egg itself before fertilization.

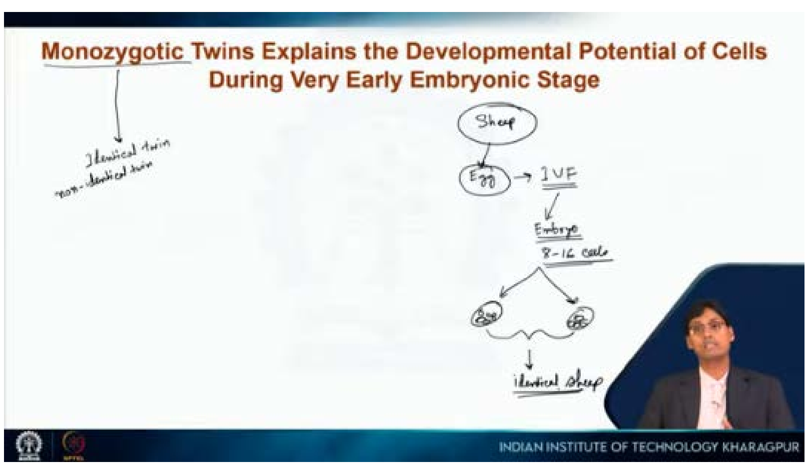


So now, in order to corroborate whatever, I explained in the last slide, I will give you some examples here, some real-life examples. For example, the monozygotic twin explains the developmental potential of cells during the very early embryonic stage. So, for example, in the human population, you see this monozygotic twin, which means there can be two different types of twins that we see in our natural population and one is the monozygotic twin. So, that means they are identical twins, both of them originating from the same zygote, which is the monozygotic twin. So, I am just writing here that this is identical twins, and the other one is dizygotic twins. So, that is something else. During the same time, actually, two egg cells get matured, and both of them are fertilized by two different sperm. So, as a result of that, those two zygotes are different from the beginning, and that will lead to non-identical twins. So, that is nothing but two different zygotes formed at the same time, and both of them get matured, and that means they are forming two babies, that is all.

But now, how does this monozygotic twin actually develop? That means after fertilization, when the zygote forms, and this zygote, when it is getting divided at a very early stage, during around the 8 to 16 cells stage, it can happen accidentally as well as naturally. So, if that, an early embryo gets split, and it is not yet implanted inside the uterus. So, as a result of that, when this split embryo, which will be implanted inside the uterus, it will develop into identical twins.

So, similarly, the same idea that I told you, this is a natural observation, but if you see that has been experimentally demonstrated and also documented. So, what people did? The scientists used sheep, and they took an egg from a sheep, and then it underwent IVF, in vitro fertilization, and then the embryo was formed inside the lab in an in vitro system. So, when this is just at the 8 to 16 cell stage, during this time, if they surgically split this embryo into two.

So, this is a split embryo, but this is surgically conducted. This is a split embryo, and now, if this embryo is implanted in a surrogate mother, in this case, then it will give rise to identical sets, just like our monozygotic twins. So, that suggests that at the very beginning of this mammalian developmental stage, when only a few cells are present, they have the full potential to develop everything. That is why they are being normal even after the splitting, the anterior-posterior system, the dorsal-ventral system, everything is still going normally. So, that is a huge difference between frogs, drosophila, and the mammalian system and their developmental processes. Now, in this part, I will be discussing mostly about growth.



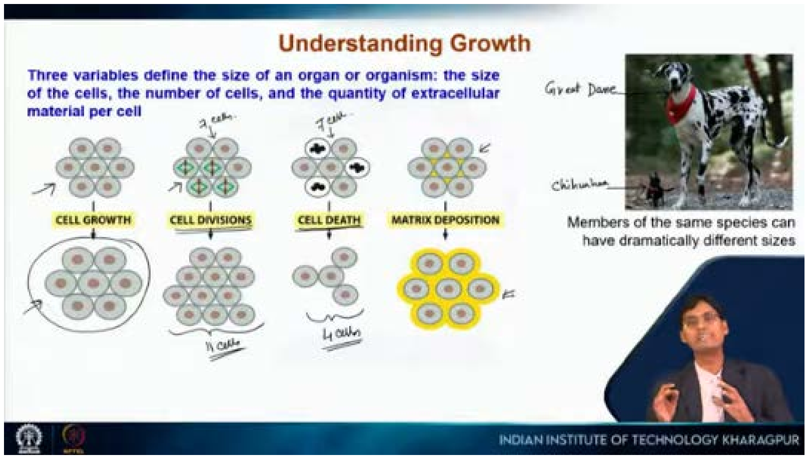
So, how is growth controlled? So, this is very interesting, as you can see sometimes if I compare a human with an elephant. So, obviously, an elephant is huge compared to a human being. So, an elephant might also have so many cells. But sometimes we see even in the same species there is a drastic difference.

For example, here, this dog, some of you may know that this is a Great Dane, it is a huge dog and here you can see a small dog present in black color, as you can see, the Chihuahua, this is just a very small dog actually, you will like it for its size, and both are dogs, they are the same species, but as you can see, they have a massive difference in terms of their size. I would say almost 35 to 40 times the difference in terms of their weight, this Chihuahua and Great Dane, Chihuahua is approximately 3 to 4 kg in weight, it is a very small dog.

Now, here, if you see, like there are multiple variables that define the size of an organ or organism. The size of cells is one, whether cells are small or, you know, bigger, and the number of cells and the quantity of extracellular material per cell. So, as you can see here and here, the number of cells is the same, but somehow if cells grow in size, then the overall volume is increasing here. So, this is also a kind of cell growth.

Now, cell division in this, the second case here, as you can see, you have a total of how many? You have a total of 7 cells present here. Now, out of those 7 cells 4 of them are undergoing division here. So, as a result of that, 4 plus 4 is 8, and 8 plus 3. So, total now you have 11 cells here. It is also increasing, but this is the number of cells increasing because of the cell division and differences in size mean it can grow, or sometimes, because of some reason, some cells may also die so that is cell death. So we have some mechanism called cell death mechanism, programmed cell death, or apoptosis and because of cell death you can see here also we have 7 cells and 3 of them are undergoing apoptosis or cell death. So, as a result of that, now you have 4 cells present here and finally, matrix deposition means here the number of cells and here the number of cells are the same, but somehow in between the cells, the space in between the cells is growing because of matrix deposition. That will also increase the overall increase in volume in size.

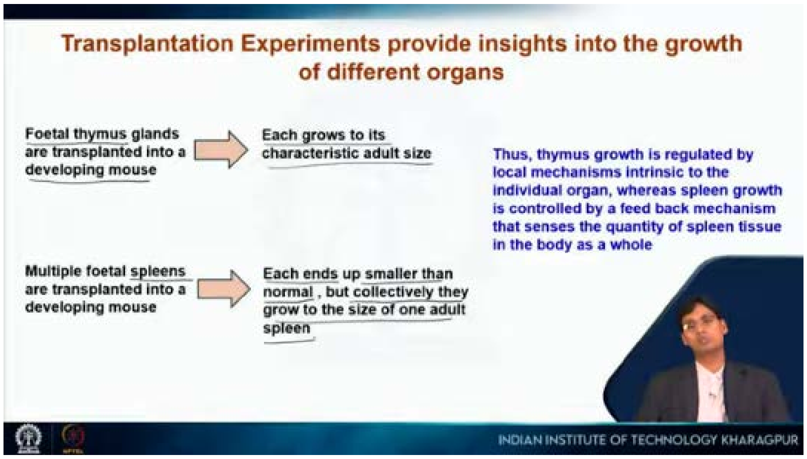
So now in order to understand more about this growth of an organism or of an organ scientists carried out a beautiful experiment. So here I am going to explain one of those experiments. So the transplantation experiments provide insights into the growth of different organs. So you can see here the fetal thymus. So the thymus gland is a part of our immune system. This is some primary lymphoid organ present, our upper part of our chest, behind our sternum, the main bone here close to the heart, actually, there is one organ present called the thymus.



Here, T cells get matured. So, I will discuss that in detail during the immunology discussion. So, this thymus gland, when transplanted into a developing mouse, what happened? Multiple thymus glands were actually transplanted into some developing mouse and each of those grows to its characteristic adult size. That means when they transferred, they saw that these thymus glands are growing into normal size. The multiple thymus glands are getting developed. On the other hand, another organ, this is also part of our immune system, the spleen. This is a secondary lymphoid organ. Here, many immune cells get stored in our body.

So, now multiple fetal spleens are transplanted into a developing mouse. The same experiment, but instead of the thymus, here we are transplanting the spleen. However, the result is completely different. As you can see, each of those ends up smaller than the normal spleen size, but collectively they grow to the size of one adult spleen. So, this is very interesting. All those spleens did not grow; they are very miniature in size. But if you add up all those spleens together, you will find their total mass, their total size, should be equivalent to a single spleen of a control mouse. So, as a result of that, what we would like to conclude is that the thymus growth is regulated by a local mechanism Intrinsic to the individual organ. So, as a result of that, although we have transplanted multiple thymus glands, all of them grow to normal size. However, the spleen growth is controlled by a feedback mechanism that senses the quantity of spleen tissue in the body as a whole.

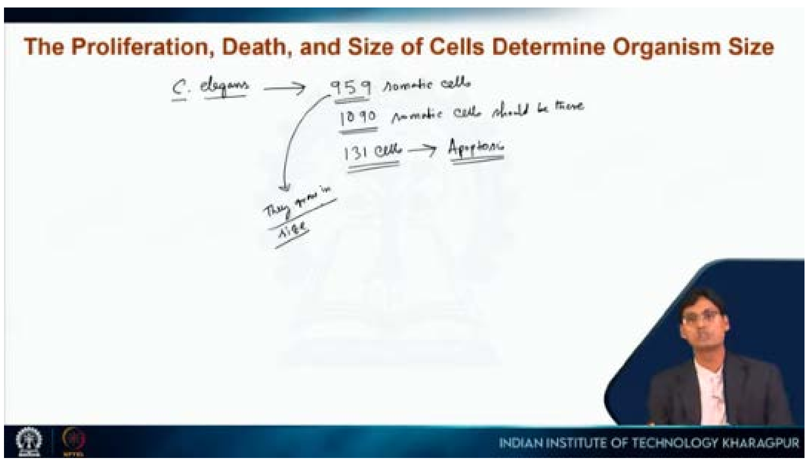
So, as a result of that, you can understand it is a very complex process. In the same individual, in the case of the mouse here, the spleen growth and the thymus growth are controlled by different mechanisms. Now I would like to give you some other examples as well. So, another example here is C. elegans. So, this is again a model organism, C. elegans, which is a worm.



So, now in C. elegans, this is very interesting because this is a model organism for studying developmental biology that follows an astonishingly precise and predictable developmental program. So, we know a lot about C. elegans, and it is also known that in C. elegans, they have 959 somatic cells, the number is so precise, so fixed meaning that the germ cells are not included here, just the body cells. But based on the cell division, because it is a model organism and scientists know a lot about C. elegans, the actual number of somatic cells should be 1090 somatic cells should be there, but what I am telling now that 959 cells are present because 131 cells, 131 cells, they get deleted by the method called apoptosis. I will discuss this in more detail in some other classes. So, as a result of that, now if you add up 959 and 131, then you will get this 1090 somatic cells in C. elegans. But the point here is when this nematode, C. elegans, attains sexual maturity, the number of germ cells might be different, but their somatic cell number is fixed at 959 cells when they attain sexual maturity.

After that, I would say, like one or two weeks later, if you see the size of the C. elegans, the size of this worm gets doubled. But the number of somatic cells remains the same. Then, what is the reason? The number of somatic cells remains the same, but the size of this organism is doubling. That is because these cells, only 959 cells, but they grow in size. As I explained, there are multiple mechanisms in this case. Cells are growing in size, but why? Why are they growing in size? Because of some more complexity. One explanation here is that although the cells are not getting divided, although they just have 959 cells, but the nuclear division, the DNA synthesis is happening. Not the nuclear division, I would say, the DNA synthesis is happening. So, as a result of that, those cells are now polyploid.

Ploidy number means the number of genomes present per cell. So, ploidy number increases, the chromosome number increases in each of these somatic cells. So, as a result of that, those cells are getting huge. They are getting, they are making more and more products, but the number of cells remains fixed. So, this is another way cells can grow in size, and as a result of that, the whole organism here, in this case, the nematode, C. elegans, is getting doubled in size although they are maintaining their total number of somatic cells.



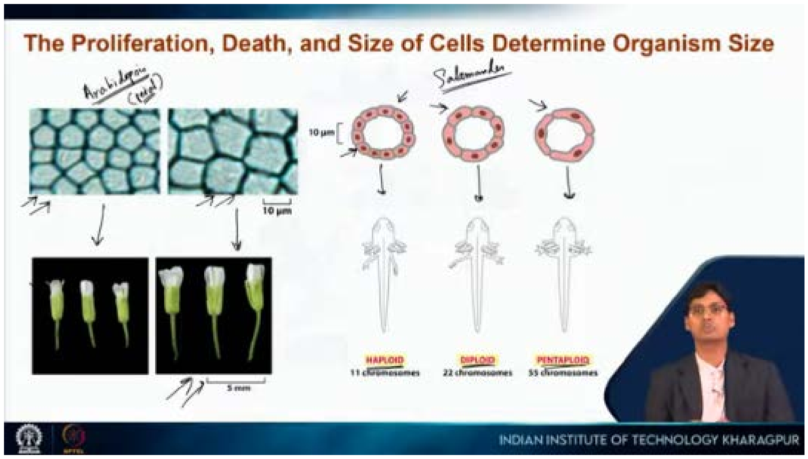
This is the last slide here. I would like to mention that the proliferation, death, and size of cells determine the organism's size. So, I just mentioned in the previous slide how the size of cells actually helps the nematode grow. In this case, this idea of increasing ploidy numbers and, in terms of size, is growing, which is very important in the context of our agriculture. Because many of the vegetables we are eating nowadays have an increased ploidy number just to make bigger vegetables and more productive ones. That is all. So, as you can see here, this is a plant, Arabidopsis, which is again a model organism.

So, today I was discussing frogs, then Drosophila, then mice, humans, and so many other organisms with different features. So, Arabidopsis, for example. So, now in this panel, as you can see here, the cells are much smaller, but here the cells are much bigger because here the ploidy number is more, meaning the genome number per cell is more there. So, as a result, the cells are getting bigger. But finally, what will happen because of that? If you see, these cells, whatever I am showing here, are from the petals. Petal means, you know, the parts from their flower. So, this is the petal.

So, as a result of that, now you see the flower size. This is the normal Arabidopsis plant, and their flower size is much smaller. As a result of that, when this ploidy number is more, the cells are much bigger. You can see here; this Arabidopsis flower is much bigger compared to the normal Arabidopsis flower. This is in the case of Arabidopsis, but now if I give you another example, you will see something different. So, here, whatever this hollow tube you can see, this is part of the kidney tubule in the case of a salamander. This is a salamander. Again, this is an amphibian, just like a frog. So, this is a salamander. If you see their kidney, the kidney tubule.

So, here in this tubule, you will see multiple cells are present, and here the cell numbers are going low here; only a few cells are present, but still, they are forming the tube. But why is this happening? As you can see in this case, if you observe. So, if you see the gross anatomy of the whole body of this salamander during the early stages, you will notice that their overall size and volume are the same. But if you see their renal tube, that is also the same in terms of their size and volume, but the number of cells is getting low when the ploidy number is more. As you can see here this is haploid, this is diploid, and this is pentaploid. So, whatever we observe in the case of the plant Arabidopsis, this is not happening here in the case of the salamander.

Here, the increasing size is getting compensated by lowering the number of cells. So, they can control the overall volume of this, I would say, salamander, the overall size of this salamander, and everything is happening properly. So, as you can see, there are a lot of detailed mechanisms about this growth control mechanism; I just mentioned a few of them, a few interesting aspects of them.



If you want to understand in more detail, you can follow any good developmental biology textbook or, for example, this book, particularly I am referring in this case to 'The Molecular Biology of the Cell' by Alberts. That is all.