Introduction to Developmental Biology

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Lecture-14

Cell-cell communication (Part 2 of 4)

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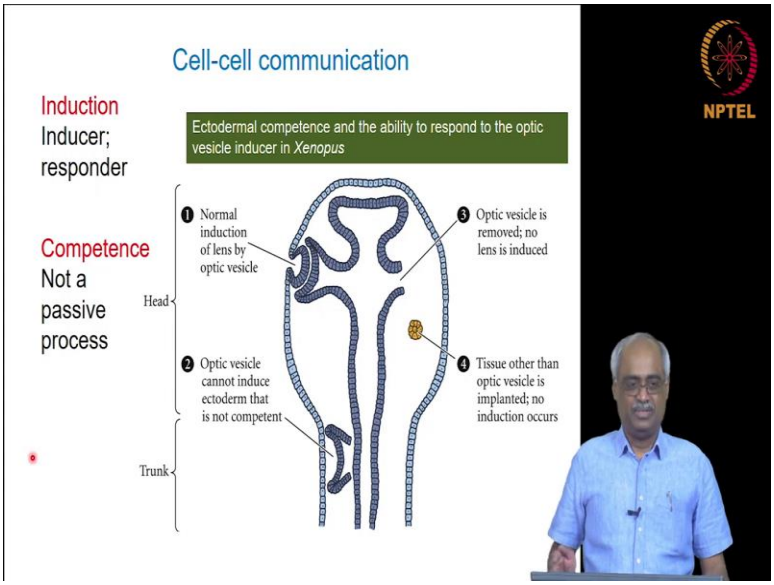

We are continuing on the same theme, which is morphogenesis. So, today we are going to move closer towards cell-cell signaling. So, before getting into the molecular pathway of cell-cell signaling, we need to look at some basic principles that govern the rules of interaction among cells. So, in that there is two important concept defining words, one is induction, and underlying molecules called inducers. Second, is competence. Like for example, I can teach you developmental biology, but unless otherwise you already know high school level education, you will not follow the class. So, you have to be competent to get induced. So that is what competence here is. So, that is why it is defined as a separate thing. So, we are going to learn this using eye development in vertebrates as an example.

So, these are directly from the book. So, what is an inducer? The inducer is a molecule produced by the cells that induce other cells to adopt a specific fate. For example, without having a certain neighboring cell, a given cell may not differentiate in a certain way. So those neighboring cells produce the inducers molecules. And the responder cell will not listen to those inducers unless otherwise, this cell has the competence. For example, a given cell has the receptor for those signals coming from the neighboring cells, and that ability is called competence.

So, if you look at this schematic representation in the slide, this is a cross-section of the early embryonic stage where we are focusing on the eye development, part of the ectoderm and mesoderm where you have the eye development happening. So, you see the head part of the ectoderm, and the rest of the part is the trunk. So that head ectoderm or the anterior ectoderm only has the competence to make the lens. For example, the top left part is taking shape to make the lens. And this shape will not form anywhere in the anterior ectoderm. In the entire eye region, the lens will develop only where the optic vesicle is present. So the anterior ectoderm has the competence to respond to an inductive signal coming from the optic vesicle, and the optic vesicle is the inducer. This can be tested experimentally; for example, if we take the optic vesicle and put it at the lower ectoderm that is not going to induce lens formation there. Similarly, if you do not have the underlying optic vesicle, this ectoderm is not going to form the lens.

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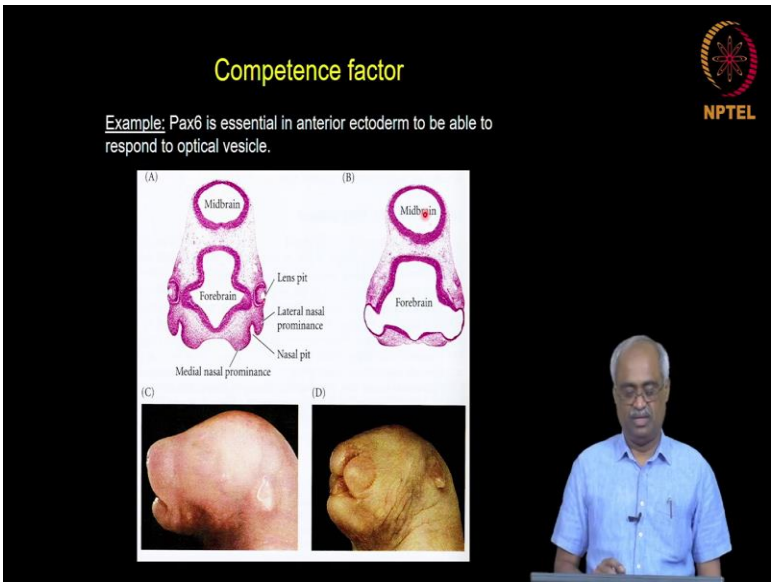

So here is an example, Pax-6 expression in the anterior ectoderm is required for making it competent. So, if you do not have Pax-6 expressed as seen in (B), the lens pit does not form; also, if you have other defects, the mouse ends up not forming the head itself. We already know that Pax-6 is a transcription factor, and multiple modular enhancers control its expression.

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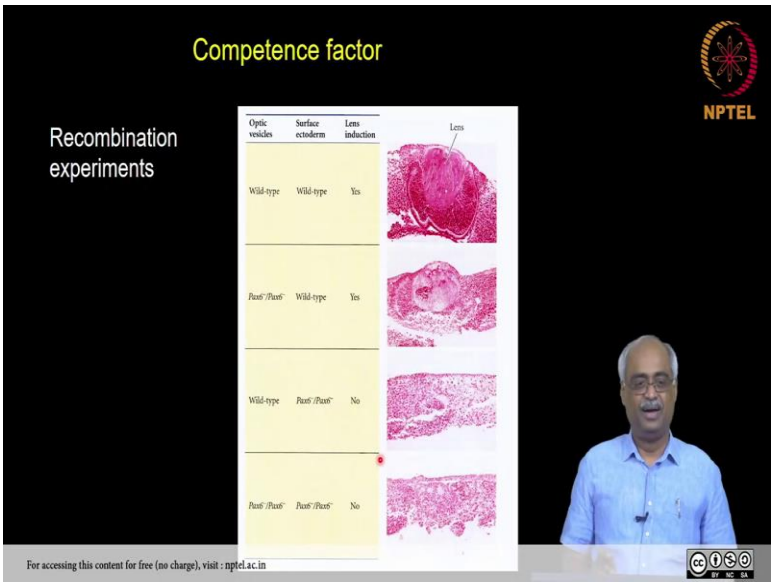

So, this can be tested more thoroughly, as shown in this recombination experiment. So, in the first experiment, both optic vesicle and surface ectoderm are wild-type having the Pax-6 expression; therefore, the lens is formed. In the second experiment, the optic vesicle lacks Pax-6 while the surface ectoderm is wild-type for Pax-6; even then, the lens is formed, indicating that the responding tissue is competent by producing Pax-6. The third experiment is the opposite of the second one; here, the lens is not formed. Even if you have a wild-type inducer, if the responder is mutant, then the lens will not be formed. This optic vesicle has no competency to respond to the inducing signal coming from the other one, and finally, if both are mutant for Pax-6, then the lens is not going to develop. So, this is how you establish competence factors.

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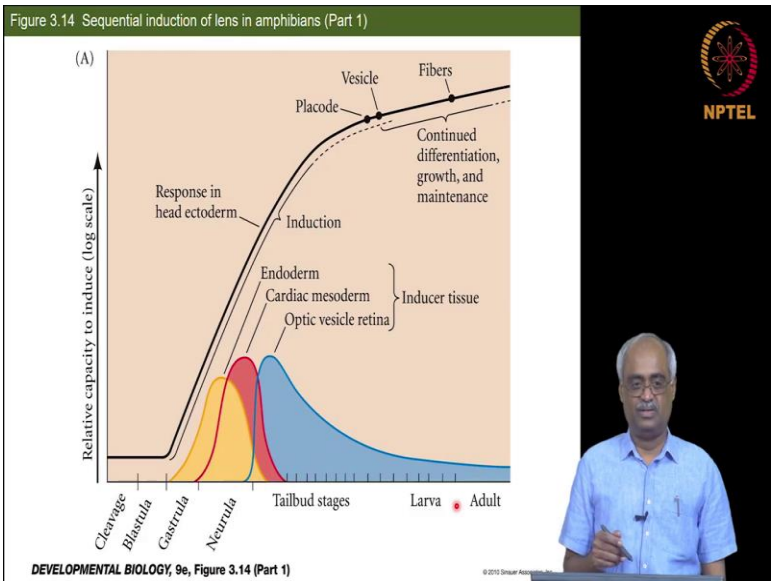

Then we are moving further into the rules that govern induction and competence. So, the main point here is the inducer, and the responder does not remain the same forever, so these are dynamic. An inducer might become a responder, and the responding one might become an inducer. Then these are temporally regulated at different time points. So, the Gilbert book uses the analogy where, in a football match, the one who kicks the ball for the goal is thought to be the one responsible for winning? No, other people in that team are kicking such that the ball reaches the final person. So, that happens in this process too.

For example, in this case, the head ectoderm to become competent to receive the signal from optic vesicles, there were earlier inductive events. Different things have induced it to reach a point where it could become competent, and that is what we are going to see in the next two or three slides. This graph shows the Y-axis having the relative capacity to induce lens formation. The X-axis shows the induction that happens at different stages. For example, gastrula to neurula, the endoderm tissue induces this ectoderm, and then sequentially next is the cardiac mesoderm's turn, then finally the optic vesicle. So, if the endoderm and the cardiac mesoderm did not induce sequentially, then the head ectoderm now will not be competing to respond to the optic vesicle signal. So that is what is illustrated in this graph. So, this is a sequential induction. And about that, we will see in a schematic in the next two slides

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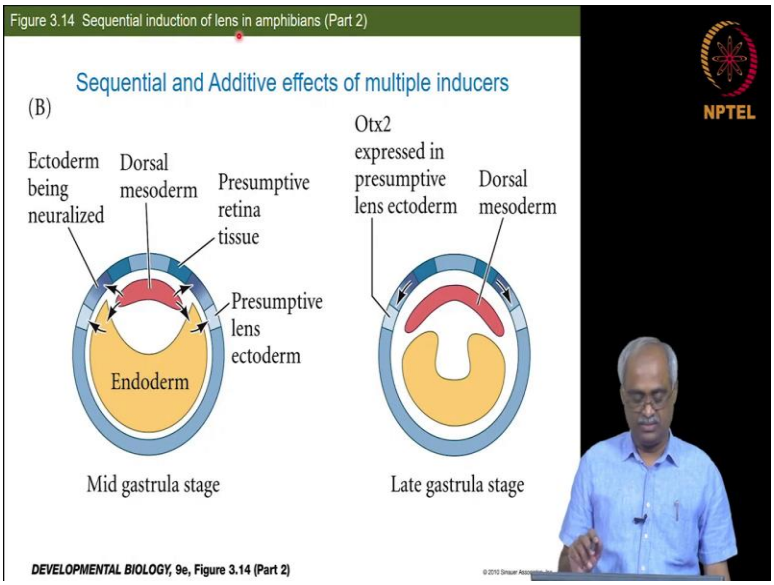

So this is a cartoon showing cross-section of the head ectoderm. This endoderm is the foregut endoderm, which forms the upper part in the gut later. So this endoderm, through an unknown signal, induces the presumptive lens ectoderm. Then the dorsal mesoderm induces the presumptive retina and the endoderm. So these inductions lead to the expression of a transcription factor called Otx2 eventually in the presumptive lens ectoderm that is going to form the lens. So that is how this new transcription factor starts appearing there. There are sequential events, and there is additive, which we will see in a second.

So Otx2 alone is not going to make the lens; there are further signaling from the adjacent ectoderm, that eventually forms retina.

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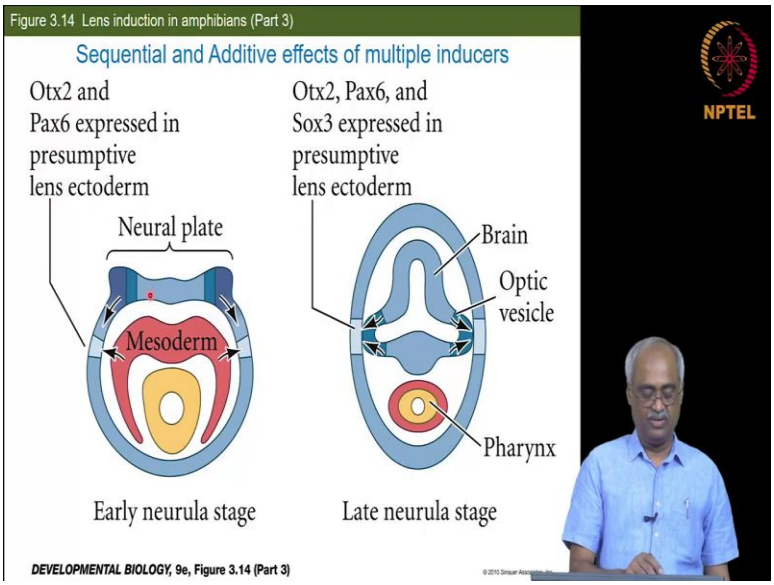

The presumptive retina and mesoderm induce the ectoderm to express Pax-6, which will eventually form the lens. Expression of Pax-6 protein constitutes the competence of this surface ectoderm to respond to the optic vesicle later during development. So at a later stage, when the optic vesicle signal comes, which people believe is a BMP (Bone Morphogenetic Factor), induces the Sox3 expression in lens ectoderm. So, now multiple transcription factors are activated here, and all of them together only leads to the formation of lens placode finally in that place. So, there are sequential and then the additive effect of multiple inducers. Here it is sequential because initially, the endoderm induces, which is followed by mesoderm and then the adjacent ectoderm, then finally, the optic vesicle. So they are activated in a sequence. After all their activity becomes additive finally, a particular tissue gets specified. So, this is a common theme, and this is found in all other organ development.

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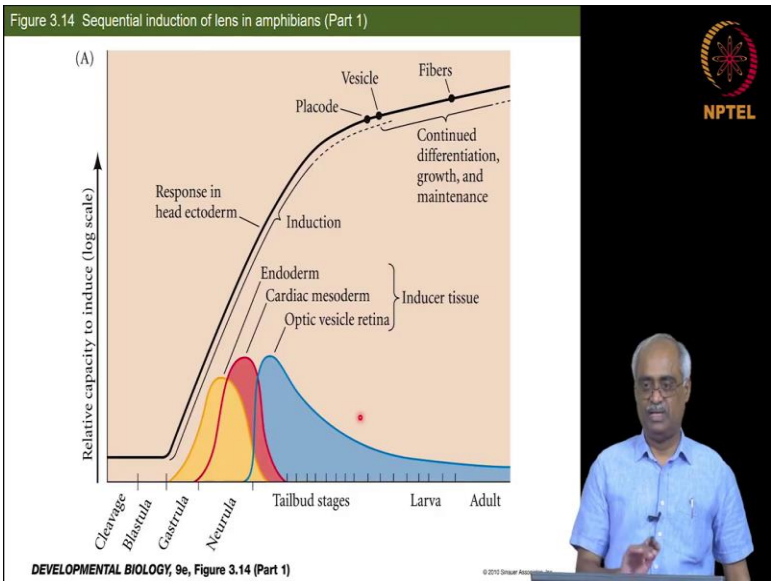

So, purely based on tissue transplantation and recombination experiments, people know that these arrows in the cartoon are accurate, but the underlying molecules are unknown. So, the one molecule that they finally found is the Otx2. So during the early neurula, the neural plate differentiates as well. So those neural cells are the ones that eventually induce the lens ectoderm, so when the optic vesicle finally induces, Sox3 gets expressed. So this gets clearer in the upcoming slides.

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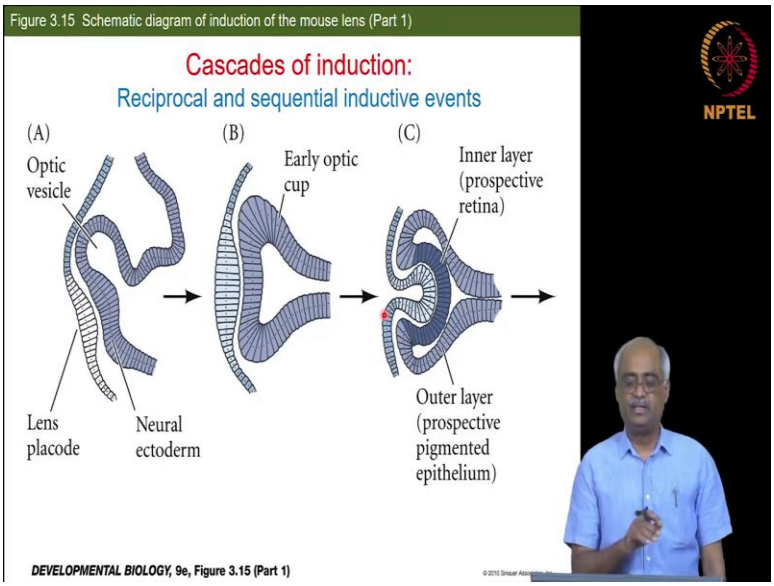

So, these inductions happen in a cascade and a reciprocal manner as well. So, the reciprocal is the new thing that is coming from this slide and the next one. So an inducer becomes competent in the next step. For example, here, the optic vesicle initially induces the head ectoderm to form

the lens placode. At a later step, this developing lens induces the optic vesicle to make an optic cup. And then, they differentiate into two kinds of cells, one is going to be the neural part of the retina, and the other one will be the pigmented part of the retina. So, that differentiation is induced by this developing lens. So the responding one has become an inducer, and the inducer has become the responding, so that is reciprocal. And these go in sequential order, so it is all summarized in a graph in the next slide.

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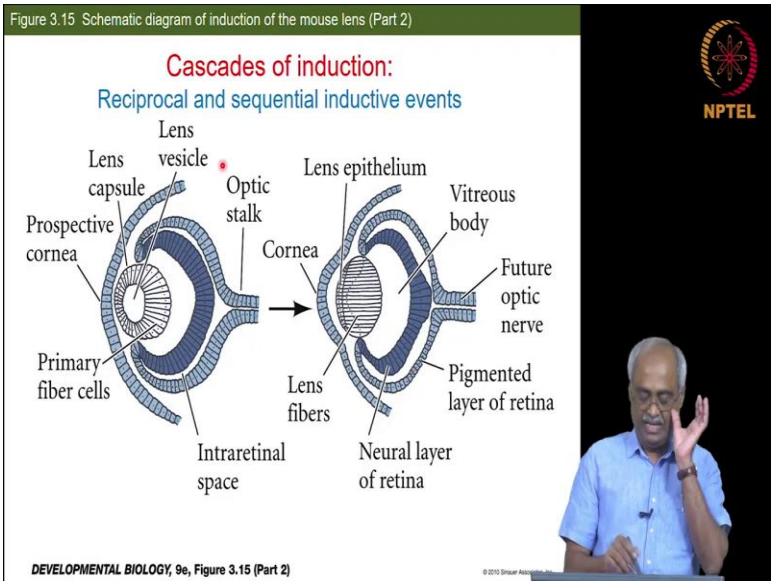

So, here the lens vesicle that formed further induces the adjacent tissues to make the optic stalk, primarily the differentiation of these neural retinal cells and the pigmented ones. So, the neural retinal cells are the ones that are going to form the rods and cones. So, they will convert the light signal into an electrical signal which goes through the optic nerve to the visual cortex in the brain. This induction does not stop only one direction; it also induces the overlying ectoderm to form cornea. So, these overlying ectoderm cells become columnar, if you see, the tissue shape changes, then cell shape is also going to change to make the cornea, but that is not shown here. So, that is the transparent outer covering for the lens. So it goes in this fashion where one induces the other one to acquire a particular fate, and then that, in turn, induces the original inducer as well as additional tissue.

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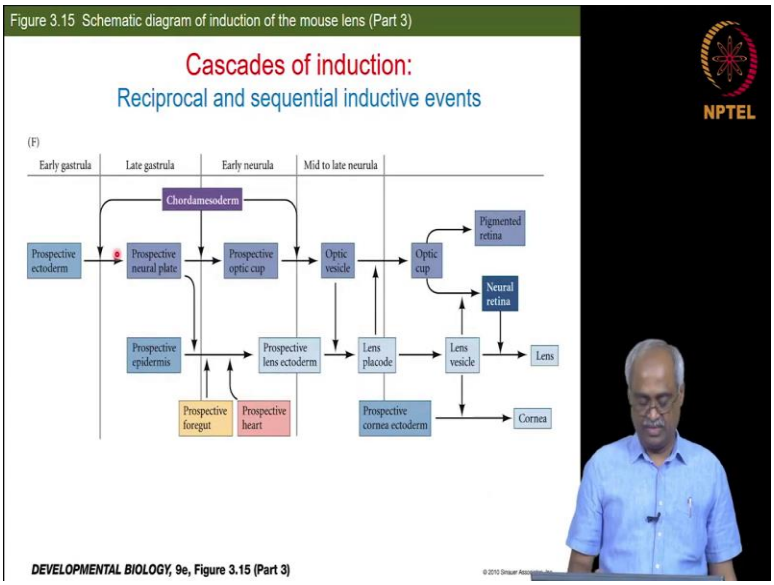

So this is the summary, so this we will go slowly. This happens at various stages such as early gastrula, late gastrula, the early neurula where the neural fold and neural tube formation occur, and then mid to late neurula. So, initially, the prospective ectoderm gets induced by the mesoderm to make this neural plate and prospective epidermis. And then the neural plate, the foregut endoderm, and then the dorsal mesoderm that is going to form the heart, induce the epidermis to make the lens ectoderm. The neural plate gets differentiated into the optic cup, which gets induced by this mesoderm to form the optic vesicle. And this optic vesicle induces the lens ectoderm to form the lens placode, which induces the optic vesicle to form the optic cup. And now, the lens placode becomes the lens vesicle, which induces the optic cup to make the neural retina, and the pigmented retina also induces cornea ectoderm to become cornea. The lens vesicle eventually becomes the lens. So, all of this happened reciprocally and sequentially. So, that is how the tissues finally take shape.

So here one main point that we should not miss is, at every stage of development, these tissues were functional. So, here in the process of making the lens, these tissues do other functions too. These developing tissues during these transient states themselves were specific functional tissues; without that, the whole development is not put together.

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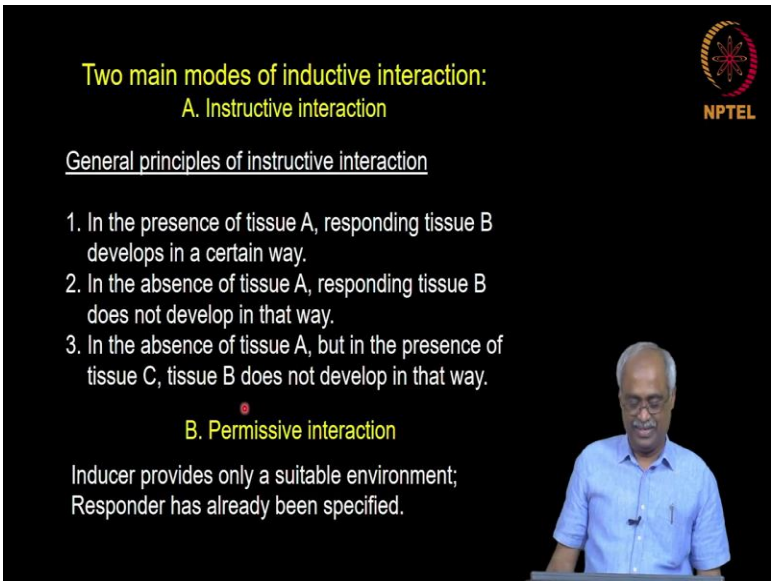

So, this slide is a summary of what we saw. So, in the presence of tissue A, responding tissue B develops in a certain way. For example, when the optic vesicle is underlying the anterior ectoderm, it forms a lens placode. In the absence of tissue A, the responding tissue B does not develop that way; it does not make the lens. And in the absence of A but in the presence of C, B does not develop that way because it needs that specific inducing signal. So, these are the general principles of instructive interaction. So, the inducer instructs what to do. The other one is the permissive interaction.

Some of the cells know exactly what they want to do; it is just that they have to be in the right environment. For example, the cells need to have a substratum on which they can anchor themselves; if that is there, they will develop the ECM. If the cells are in the right environment, they will develop on their own, they do not need any induction, and those kinds of things are called permissive interaction. A good example is in tissue culture plates; the adherent cells need adherence; otherwise, they do not grow the way they usually grow**.**

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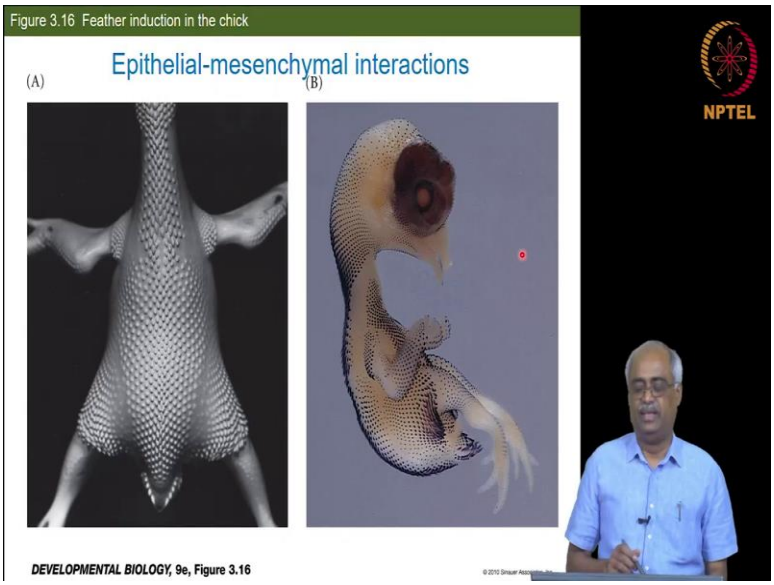

So, the next step that we are moving forward in this induction is two different specificities. One is called a regional specificity; another one is called genetic specificity. Before that, almost all organs have these two cell types: epithelial and mesenchymal. And this epithelial-mesenchymal interaction is very critical for most of the development.

So, the feather development in the chick is one good example. Here in figure (A), you see rows of these primordia from which feathers will develop, and if you observe their arrangement, they are present in between the adjacent rows. So, if you take a closer look at one of them, then you will see in the adjacent one there is a gap on both sides, so they alternate. And if you do an in-situ for a signaling molecule here, it is for sonic hedgehog, its expression is seen precisely in those primordia. So that is where the specification is taking place. So, this is being done by the mesoderm that is underlying this epidermis. So, we will see how that mesoderm will pattern the skin in different parts of the body differently. So, that is where regional specificity comes. So, the induction response between epithelial and mesenchymal cells is very critical. So that is the main point I want to mention here.

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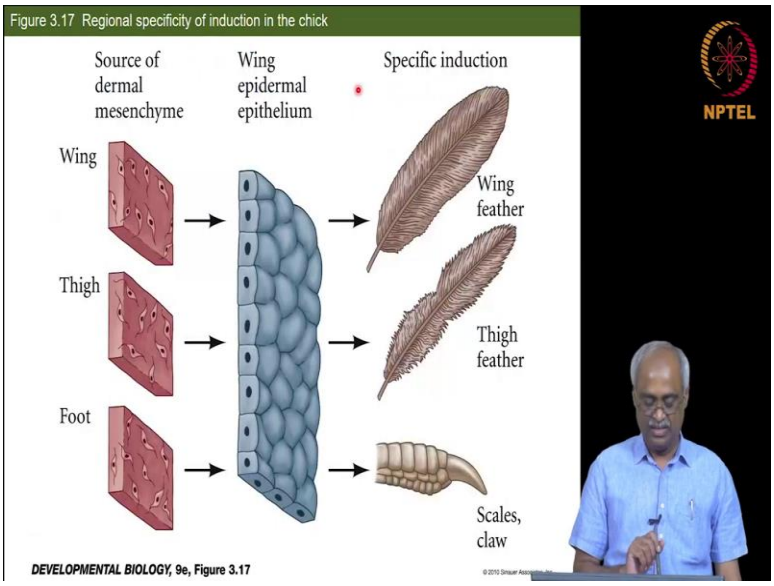

So let us see regional specificity in the chick. So here you are taking a constant epidermis, say here the scientist has taken the wing epidermis. Now, if you place the mesenchyme that was taken from different parts of the body, place it adjacent to this wing epidermis, it develops into various structures. So when you have the wing mesenchyme, the epidermis makes this wing feather, and if it is from the thigh region, it will make the thigh feather. And if it is from the foot, it is going to make the claw. So same surface epidermis produces different structures based on who is instructing it. So, this is what they call regional specificity. A different region of the mesoderm already has a different inducing ability on the epidermis.

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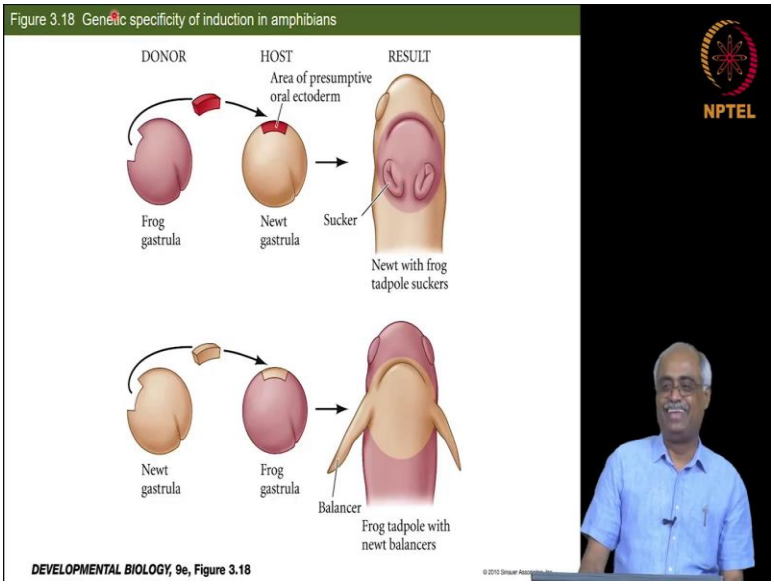

Here you are not transplanting within the same species; in the previous one was from the same individual. Here we are going to do cross-species experiments.

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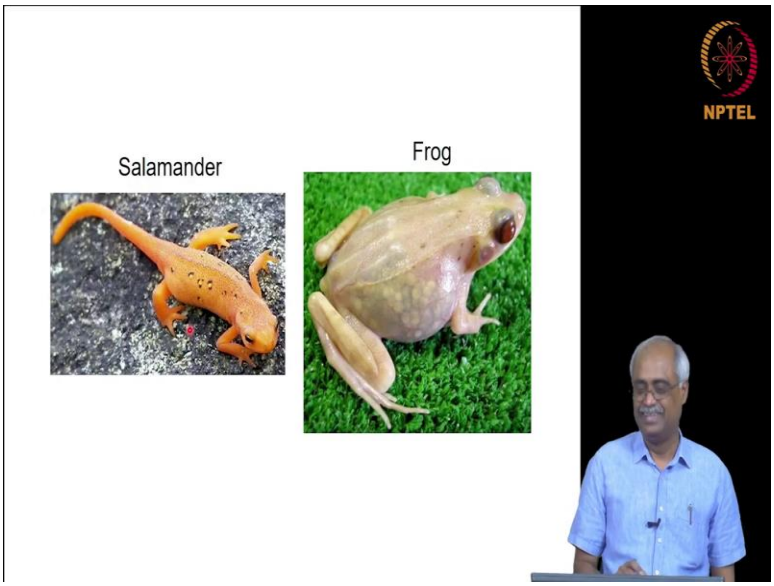

The left one is a salamander called newt; the right one is a frog; both are amphibians. So, they are very closely related species. So we are taking some cells from the frog gastrula and transplanting it to the presumptive oral ectoderm part of newt, the salamander. So here we are doing cross-species recombination of mesoderm and ectoderm. Here in this transplantation, the underlying mesoderm is newt's, but the ectoderm is from the frog. So this mesoderm instructs the ectoderm to make the oral part of a newt, but this ectoderm knows only to make the oral part of a frog, and it makes the suckers of the frog tadpole. And if we do the reverse experiment where the frog mesoderm would usually instruct the ectoderm to make oral organs of a frog, this ectoderm makes these balancers seen in the newt.

So the frog tadpoles have newt balancers. So, this tells you the genetic specificity. So, this epidermis knows to listen to the instruction from the mesoderm, but it has a genetic constellation, such that it can only make particular structures and not other structures, because it has already undergone earlier instructions and already there is differential gene expression that has kicked in, it is only waiting for the go-ahead signal.

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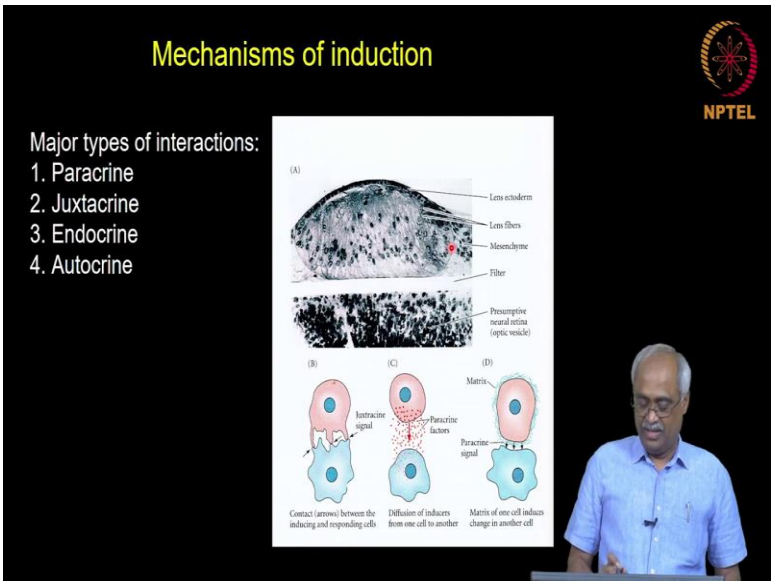

So now, who are these inducers? In what shape they come? And how far can they go? And do they need to be adjacent? We are going to deal with all those questions. Here is an experiment where the neural retina and the lens are separated with a filter paper. So, now, if the filter paper is porous, whatever the inducer that comes from top to bottom seems to induce this ectoderm to form the lens, indicating that this produces molecules that can diffuse some distance through a filter paper. But when you put a barrier like an impermeable one, then the lens formation does not happen. So, these experiments finally led to the understandings shown here. So, some signals require cell-cell contact; a good example is the primary fate in vulva inducing the two adjacent Pn.p cells to acquire the secondary fate; that is called lateral signaling among equivalent group cells. Otherwise, those Pn.ps are equally potent in terms of development. So, this is called Juxtacrine signaling (B), where the cells have to be in physical contact like one cell membrane should be in touch with the adjacent cell membrane, the inducer, and the responder. And there is another situation where the molecules produced by the inducer can diffuse in the vicinity, and the distance it diffuses, vary significantly. And these are called paracrine signaling. So most of the cell-cell signaling involved in development, is of this nature. So, figure (C) is paracrine signaling. And then, you have a situation wherein the paracrine signal, the extracellular matrix, produces the signal. So, now in the next few minutes and in the next class, maybe even another class, we will be discussing these paracrine and juxtacrine signaling, the different cell-cell signaling mechanisms.

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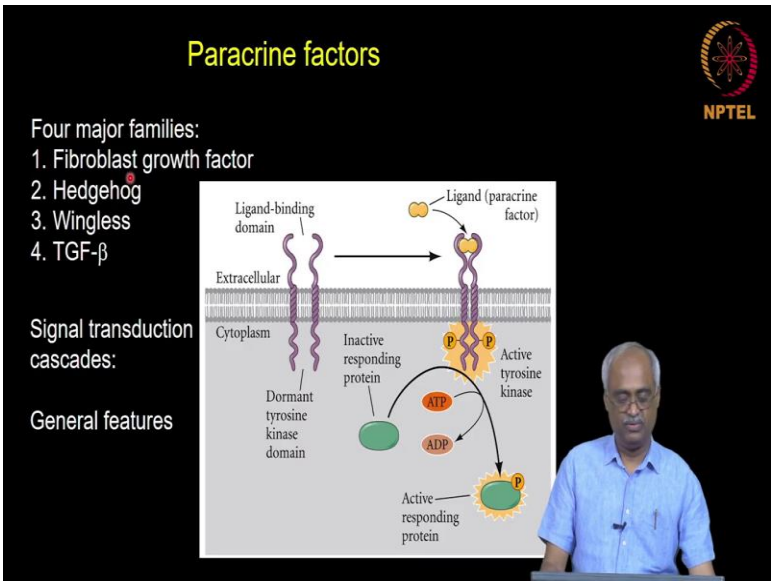

So before we get into a summary of all kinds of signaling, one crucial point exists. To make the complex tissues, our body does not have a variety of tools; it is pretty much how you use a screwdriver and wrench. So, by using a screwdriver, you can put together many things or dismantle many different things. So you can use a screwdriver in a plane, or your bicycle, etc., so that is the kind of logic nature has played around. The toolkit used to make vulva in the nematode, 700 million years ago, is the same toolkit being used in making vertebrates structures as well. Like for example, to induce bone formation or to induce cell proliferation, it uses the same toolkit. And many of these toolkits were initially identified through genetic experiments focused on Drosophila embryo development. We do not have like 100,000 signaling pathways to memorize. We have only four, just four. Imagine with four different cell signaling; we can make all the organs using simple rules. So, we need to learn these four types like what each one is and in which organ development they are involved.

So, before we go to the details, there is a generalized scheme that underlies all four varieties. So, essentially you have a receptor, a transmembrane protein having a cytoplasmic domain and an extracellular domain. Then the inducer from the inducing tissue comes and binds the receptor, which leads to conformation changes in the receptor molecule, and that conformation is transmitted into the cytoplasmic region. For example, in this case in the slide, binding of ligand leads to the phosphorylation of the receptor itself. This is due to a latent kinase activity that gets activated through this conformational change induced by the ligand. And this phosphorylated

version is an active kinase that, in turn, phosphorylates other intracellular molecules. Finally, a factor goes into the nucleus to activate or inhibit or modulate the transcription of downstream genes. So, that is how it works. So here, for example, there is a dimerization where the two polypeptides come together.

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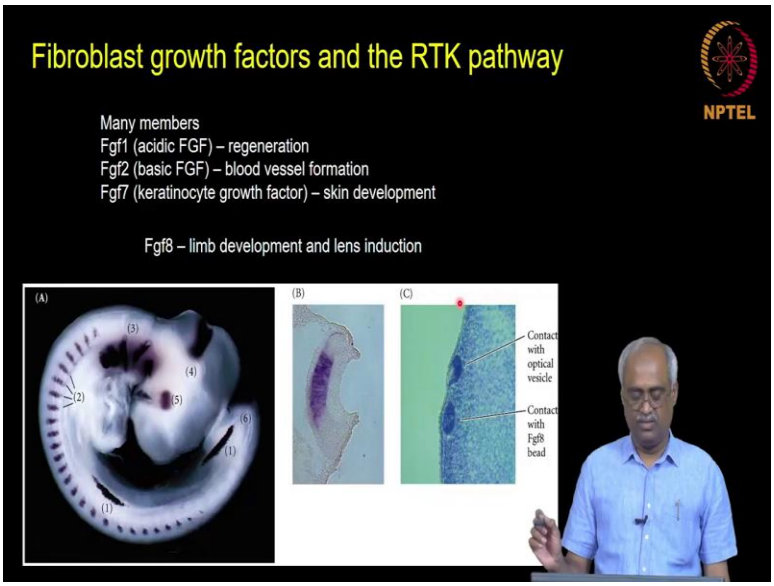

So, that is the general feature, so now we will get into the first one called RTK receptor tyrosine kinase pathway or based on the ligands, fibroblast growth factor, or Fgf. And in vertebrates, there are multiples Fgfs, like Fgf 1, 2, 4, 7, 8. So we have encountered Fgf earlier. So, these are required for limb development and lens development. So that is the example that we are going to look at here. So, figure (A) is a whole-mount embryo in-situ hybridization of the mouse embryo. So this shows where Fgf8 is expressed, like in limb primordium, in the hind limb, forelimb, tail, the lens where it is going to form the pharyngeal arch, somites along the vertebral column, and then between the two brain-like midbrain and hindbrain. So, these are the place where it is expressed, and that is where its function is important, and since we were talking about optic vesicle and lens, that example is shown here in an experimental way.

So, the Maf induction is used as a marker for lens development; Maf is a transcription factor. So, in figure (C), whether you have an optic vesicle or just the Fgf8 coated bead, the presence of either one of the two causes the lens forming ectoderm to express Maf. So, the Maf marked here

with a blue color. So this shows that the Fgf8 is the molecule produced by the optic vesicle to induce the lens. So this is how we are moving from cell type to the molecules.

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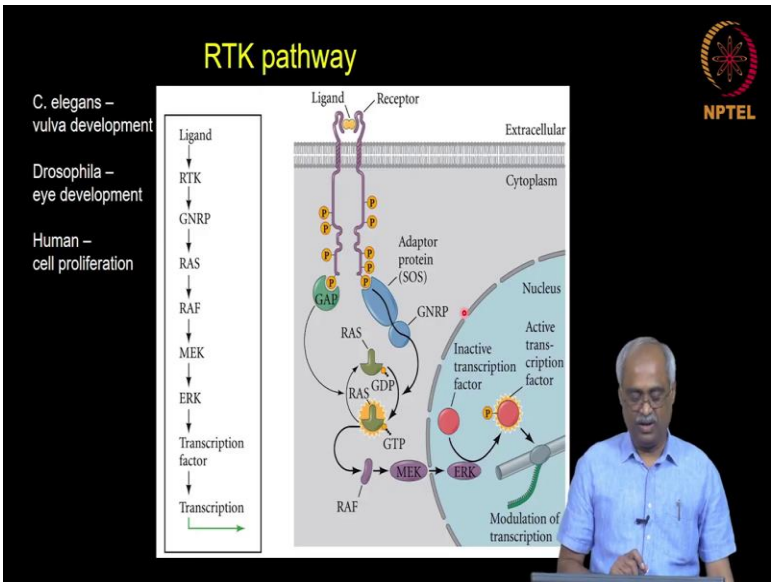

The pathway will look like this in the slide, so we are already familiar with the LET-60, a RAS. So, this pathway, for example, in *C. elegans* induces vulva development; in Drosophila, it is required for compound eye development; in humans for cell proliferation. So essentially, this pathway controls the amount of proliferation. In the previous example, we saw the follicle development in the leg length, so if you make enough of cells before differentiation, then a long leg is developed. And if you prematurely activate differentiation with a smaller amount of cells, then only a shorter leg develops. So, that is how the regulation of this pathway happens when we talk about proliferation. So, let us see the factors involved in this pathway. These factors were identified through epistasis based on pure genetic experiments with no understanding of the molecule.

So, you have a ligand that binds mostly Fgf or stem cell factor; there are varieties of ligands that bind to this pathway. So, we will see an example; when the ligand binds, the two receptors dimerize and gets phosphorylated by themselves. A latent protein kinase activity gets activated in them, and they phosphorylate tyrosine residues, which is why it is called the receptor tyrosine kinase. It is a receptor that is a tyrosine kinase when activated. And that activated version binds to an adapter protein (SOS). This adapter protein in the cytoplasm activates a protein attached to

it called the guanylate nucleotide releasing protein(GNRP). This GNRP releases this guanine nucleotide from RAS protein. So, this typically has a GDP version bound to it, and this GNRP would remove that, and as a result, this RAS will bind a GTP. And the GTP bound RAS is the active version. So, this is how the signal is transduced into the cytoplasm. And this RAS in this pathway activates RAF, and that is going to phosphorylate called MEK, which is a MAP kinase activating kinase. And that is going to phosphorylate another kinase called ERK. So, ERK (Extracellular signal-regulated kinase) now gets into the nucleus. So without that phosphorylation, it is not going to go in. It goes in, and then it binds to the transcription factor, which is inactive, and now transcription factors become active, and that is going to modulate that transcription of downstream target genes.

So the starting point is ligand binding to RTK, and the end is ERK getting phosphorylated and translocating into the nucleus. This is the active part of the pathway, but once a ligand binds, and this activation happens, will this receptor be active forever? No, it works more like the way you regulate automobiles. For example, when we wanted a light to be on, we turn on a switch, and it is on continuously; I am not holding the switch anymore. But why don't we do that with the car? Once we get into the car and start the engine, it keeps running continuously, and the car keeps moving. So, that regulation is not enough; there you need far more refined control. There you have an accelerator pedal, as long as you keep pressing it, the petrol or diesel will be used, and the engine will run. The moment you relax your foot a little bit, the accelerator will stop. So that is the kind of regulation that happens in this pathway.

So, whenever you need such fine tight control, that accelerator is this RAS. So, the RAS has an intrinsic GTP hydrolysis activity that gets activated by a protein called the GAP. So, this Gap stimulates a latent GTP hydrolysis activity of RAS. So, the GTP gets hydrolyzed and becomes GDP, the inactive form.

So as long as the ligand binds and signaling happens, the RAS will be active, but once there is no ligand, then this becomes inactive. So this acts as a negative control; otherwise, the continuous signal will lead to uncontrolled signaling, which is not good for the cell. So the GAP protein gets activated by this receptor kinase itself. So, the signaling activates both GAP and GNRP. That is

why there is a fine control at the level of RAS. So these RAS belongs to a group of molecules that we already learned called the small GTPases. So what is the small GTPase that we learned last? Rho, so it is another GTPase. So Rho is involved in actin cytoskeleton rearrangement, but RAS participate in this pathway, but otherwise, they are coming from a common ancestor, small GTPases. I will stop here and let us see further signaling pathways in the next class.