Introduction to Developmental Biology
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Lecture No- 03
Experimental Embryology

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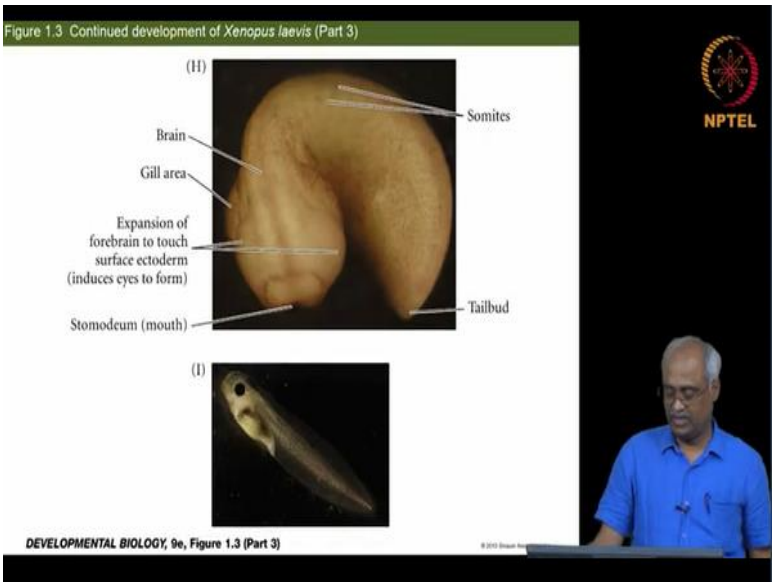

So, we have seen.

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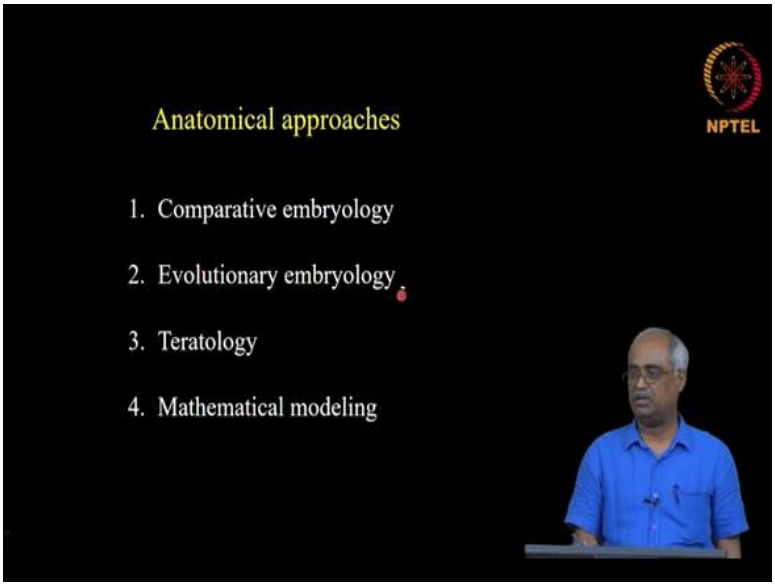

So, we will get back to Von Baer's principle, so we are in this comparative embryology and then transitioning into evolutionary embryology pretty much on the same topic. I am showing this just so that you remember the continuity, so we went through all of this.

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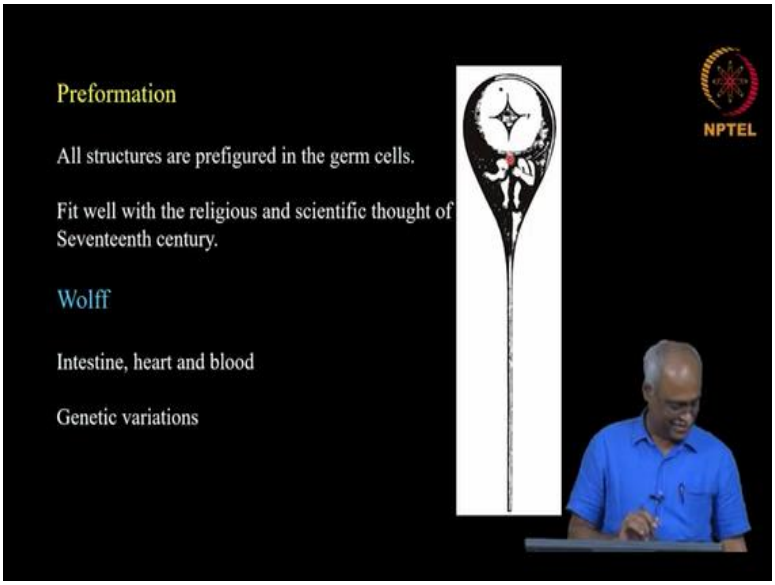

So, you will remember this preformation, the whole structure is in the sperm head, was in the sperm head, and no longer, just to be abundantly clear with facts we went through this.

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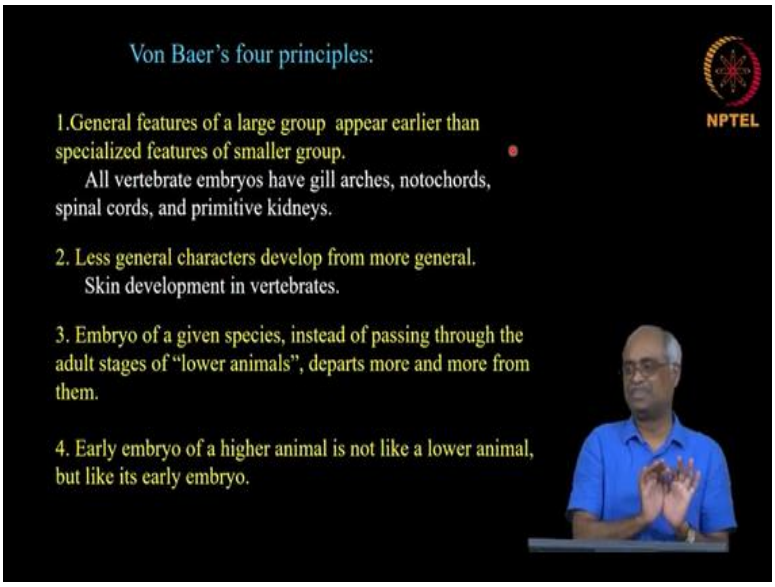

And Von Baer's principle, I felt that I went a little faster through the Von Baer's principle last time, so this time we will go more carefully and I want to make sure you understand it because this is a crucial set of concepts that bridges developmental biology and evolutionary biology. So, therefore, we need to get this clear in our minds. So, all four principles put together the primary point is, if you are going to say a more developed organism comparatively than another one, it does not mean during the embryonic development of this more complex organism you are going through the adult stages of the less complex organism that existed much earlier. Like for example today's birds and reptiles do not go through the adult stages of dinosaurs.

So, that is the main point for right now, having learned a whole lot of molecular biology, evolutionary theory, etc this looks obvious but it was not, actually at the time Von Baer proposed this there was an earlier competing theory which was exactly saying the opposite like the embryos go through the adult-like structures of less evolved organisms. So, that theory was there for long and quite ironically while this theory supported Darwin's theory of evolution, Von Baer spent his entire life opposing Darwin's theory of evolution, while Darwin got very excited how this helps him to find support which we will see in detail because that I want to emphasize because it is an important point.

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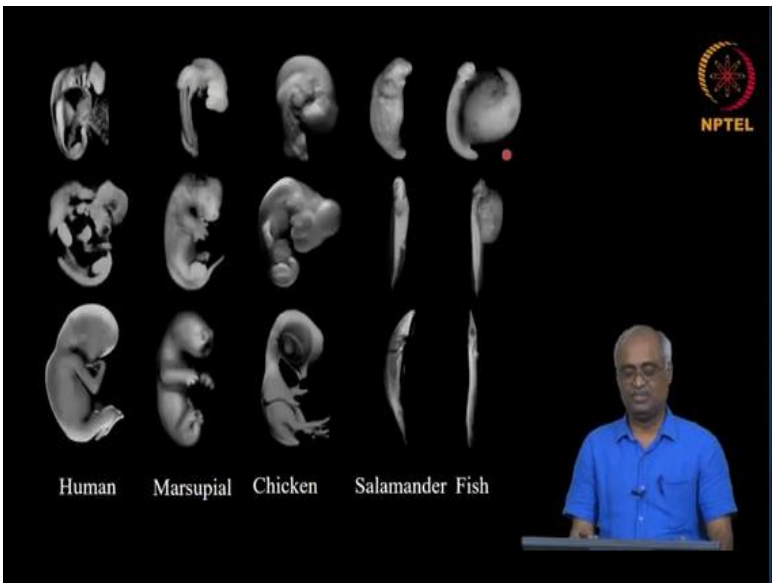

So, this kind of pictorially tells you the same thing that we saw in the previous slide, so if you look at the human, marsupial, chicken, salamander, fish, etc. If you look at the very early embryos they look more or less similar. If you take a closely related two different species of a given genus if someone has not labeled the bottles in which the specimens are you will not be able to distinguish them, very early ones. But as they go through, the species-specific specialized structures become clearer, as the development proceeds.

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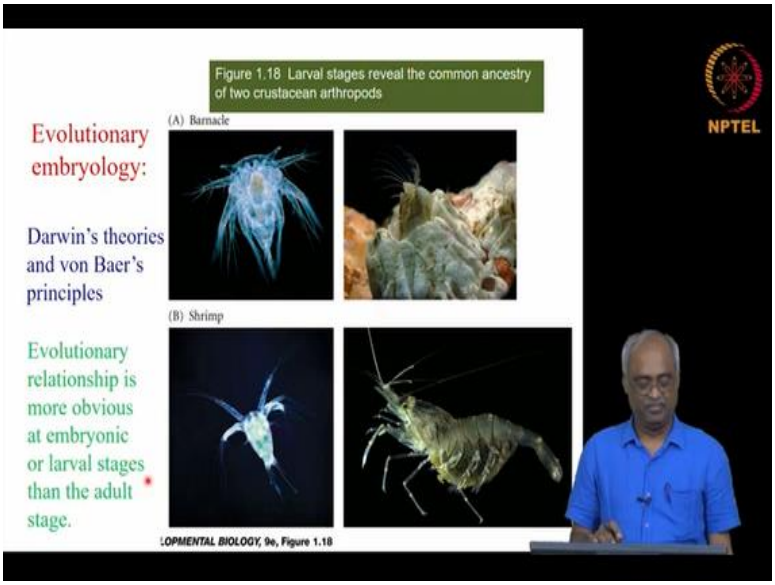

So this was very exciting to Darwin because this gives, two important points: one is that, the very early embryo resembling very early embryos of diverse organisms having similarity in essence; the embryonic similarity supports common origin, descent from a common ancestor, and as the development proceeds specialized structures start to evolve and they become clearer.

As the development progresses that supports adaptation, species-specific adaptation. So, you are seeing two interpretations both consistent with Darwin's theory of natural selection driven evolution, so your starting material is the same but then you are modifying its development, so alterations in development is the point that gives the required variations for natural selection to select.

So that is what this supports and that is where Darwin was very excited about, another point is that the evolutionary relationships become more obvious when you look at the embryonic or larval stage rather than looking at the adult stage and that is very well illustrated in the two organisms shown here if you look at the first-panel barnacle and shrimp both of them are crustacean arthropods. These have joint legs living in the ocean, and during the larval stage, they look significantly similar, one might look a little smaller and less complex but overall, they are very similar compared to the adult. So early taxonomists classified this as a mollusc because its outer structure resembles that of molluscs and this one looks very different.

But while shrimp and barnacle at the larval stage reveal their common ancestry, so this is what Darwin pointed out so we need to look at the embryonic stage for accurate classification

of organisms based on their evolutionary relationship. So, the modern taxonomy, groups organisms based on that, the evolutionary relationship is shown in the way we classify organisms.

So that is one of the main outcomes of Von Baer's theory when looking it from the evolutionary perspective.

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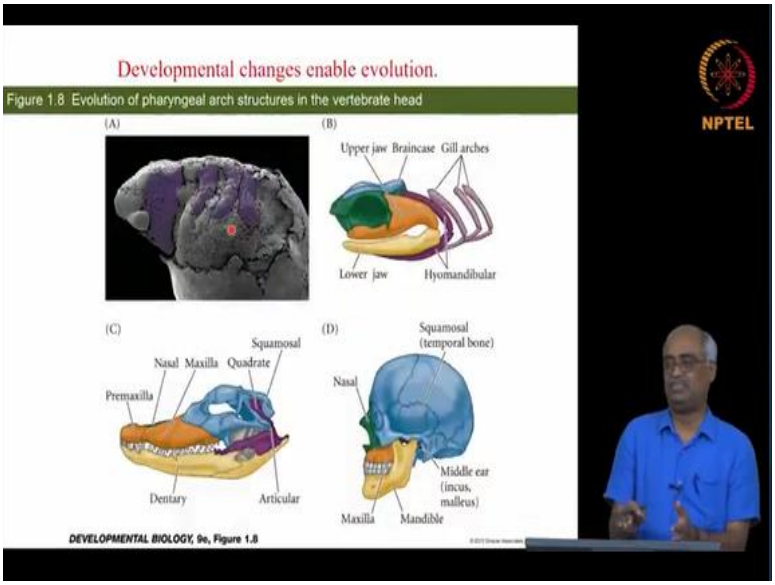

We are going to stay with this theme with the two or three more examples so here is one so this is an embryo of a salamander, the surface is open and the pharyngeal arches highlighted in the purple color. It is a group of cells that form these structures in the early embryo give rise to these gill arches and this hyomandibular in the fishes, these two bones support the two jaws. So they are not supported without this hyomandibular structure in fishes, and the same embryonic structures or the cells, the pharyngeal arches in crocodile for example in reptile, form this quadrate bone of the upper jaw on the articular bone of the lower jaw. So here you see slowly you get two structures that are supporting the two jaws and that is kind of their importance for supporting the jaws is going down from here to here. And when you go to the human they are not there as supporting the upper and lower jaw instead the same group of pharyngeal arch cells forms our incus and malleus the middle ear bones, so this is how a common early embryonic structure has been altered during development to develop species-specific structures that are how adaptations come. So, this is one of the major outcomes of comparative embryology and you can also say evolutionary embryology.

So here you see that these two fields sort of merging we are not seeing as two separate headings, and one more example;

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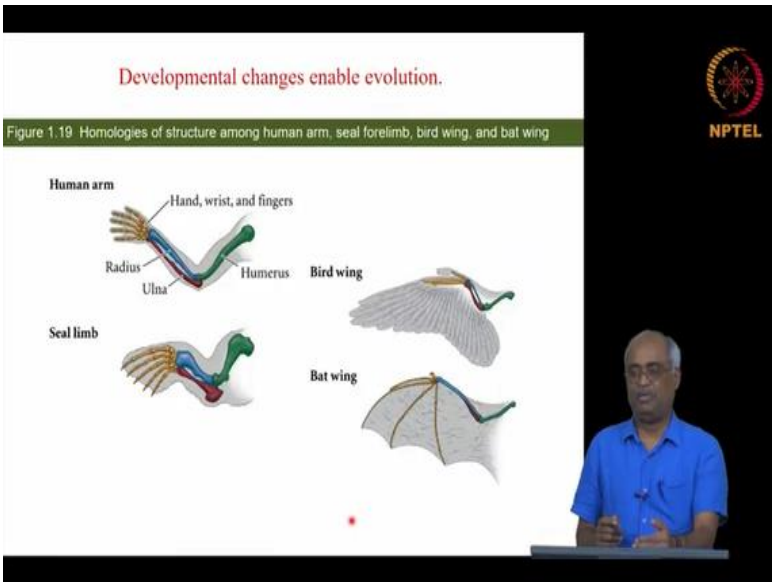

So, before we get into this example couple of important definitions to remember they are homology and analogy, so when we say homology like for example if you take the human arm and seal limb or bird wing or batwing these are all forelimbs modified into hand or fin like structure for swimming or birds wing for flying or bats wing for flying. So the common ancestral structure is called homology. So by that definition, our hand and bat wing and bird wing are homologous organs. But if you look at bird wing and batwing they are analogous, so they are doing the same function both are wings but one wing did not evolve from another wing, batwing did not come from bird wing or vice versa, so as wing these two are analogous organs but as forelimbs, they are homologous organs.

So this is one of the best examples to explain homology and analogy in developmental systems and the second point we are going to talk about is a continuation from the previous one that is alterations in development helps in evolutions. Convergent means completely two different evolutionary origins but given the environmental context where they have to fit in, independently they have all the same structure, so that is what is convergent evolution. So if you look at the five digits or the five fingers the same bones here you can see it is just that they have divided and grown more rapidly, so they are longer and the wedges that existed between the digits disappeared here but did not disappear here, so only those two alterations were required to make a batwing, the fingers had to rapidly expand, grow with more divisions and even there is an asymmetry like one of them is long and the other one is short but this

web did not disappear. While in our case originally there was webbing that helped in the asymmetry of the five fingers, but for the fingers to function independent of each other the web had to disappear by cell death, so we will later learn programmed cell death this is an example, So essentially you are sculpting a structure, you are removing things from what already exists to create a structure. So developmental alteration enable different adaptations.

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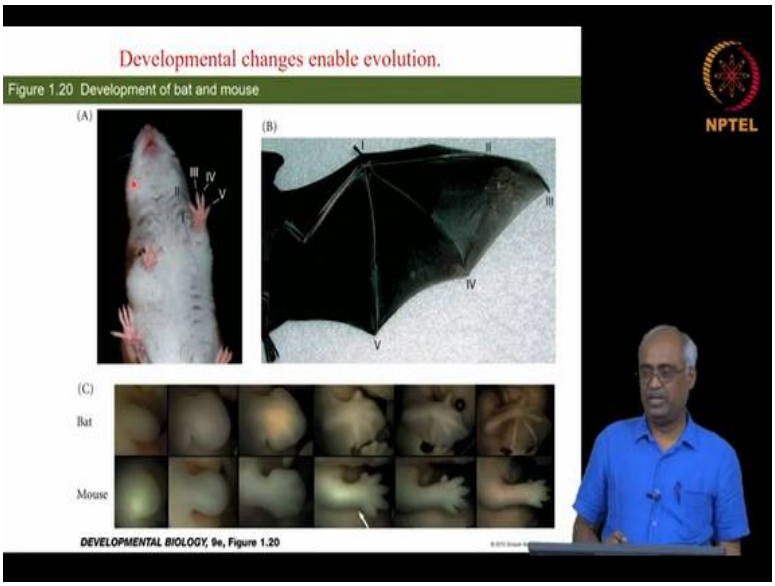

So, here is another example it is pretty much the same theme but instead of human we are looking at the embryonic development of another mammal where we are looking at the mouse, so the panel A shows, in one of them a fully developed five digits and the corresponding ones are so smaller, the little finger is the one here goes very long and if you look at the embryonic development in the lower panel you see that very initially they both are like bud coming out of the embryo. And as you go like particularly you pay attention to this where there is a white arrow, so in the bat, you have the web and it is disappearing in the mouse, but if you go to the previous one the web exists in both, so in one it had to disappear so that the fingers can move independently and you can grab things more readily while this one wants to have the web but to use it as a wing to fly. So, alterations in development help in evolutionary adaptation.

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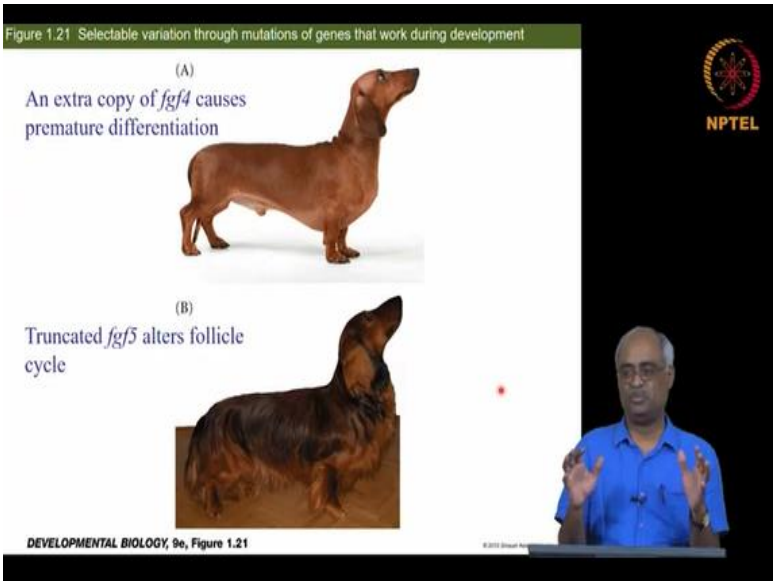

So, the next point is a slight variation in the theme essentially, we are stating the same differently, here the developmental variations or variations during development or rather changes in development provide the required variations for natural selection. So here it is not natural selection it is artificial selection, so you need to imagine the socio-political context of the world in which Darwin published his book, Origin of Species. So he did not want to immediately in the first sentence say that species change, he did not want to say that so he built a long case about the possibility that developmental structures, organism structures do change and to make that point, he gave a lot of examples of artificial selection, how by breeding, selective breeding we made quite a few plant varieties for our crops and similar animals and particularly he goes in great length about dogs.

So here we are looking at a dog which has short legs, that is because the hunters who wanted to hunt a small animal called badger that goes into narrow tunnels, they wanted a dog that could go through the tunnel and catch those badgers and for that they preferred the ones having shorter leg, so they selectively bred every time looking for the one in the offspring having a shorter leg than the parents. So that is where there is a direction, and then they eventually find a shorter one. So, now as developmental biologists, we look at how could that happen? So all you needed is an extra copy or overexpression of a certain factor that stimulates differentiation so when you have too much of that before you make enough of those cells you differentiate, so, therefore, you make a shorter organ, had you made more cells and then differentiated you would have had a longer leg.

So one gene change could provide that variation that was required for the selection and you look at the other one, again a single gene was enough, this is not to mention that single gene changes are always enough for changes these are because most of the time we believe how can this big a change happen, so that is why to highlight the point, examples of single-gene mutations were taken. So here if you look at another gene fgf-5, its alterations in expression lead to follicle development where it produces a lot of long hairs. Selectable variations through mutation of genes work during development. So it is already telling you that genes control development, that is a theme we will get to later and it is not that it works only in dogs.

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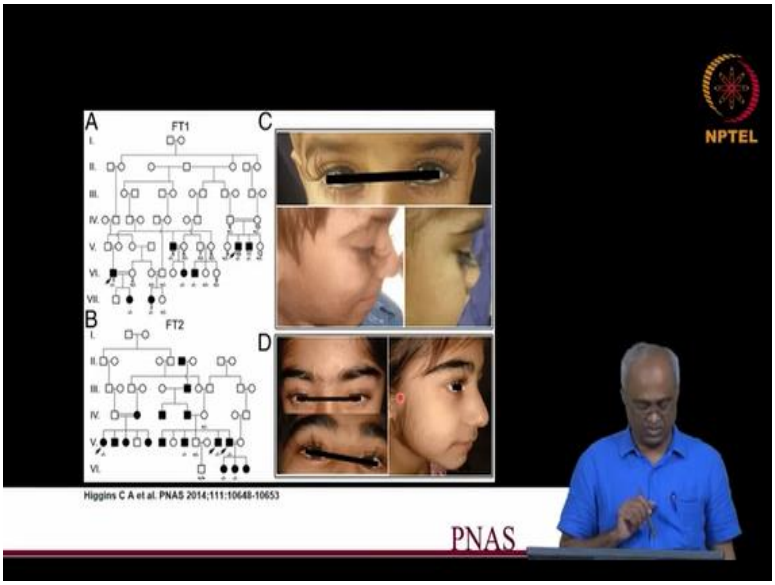

And, so look at here, so this is an fgf-5 mutation in patients leading to long eyelashes and a lot of hair in the forehead and cheek, so this again is coming from the same mutation. So this we are going to touch upon a little later as animal models for studying developmental abnormalities in humans. So for example, if you were to see this first in humans and you want to understand the basics and if you can make this in a dog then you can work out the mechanism here more readily than in human patients. So the main point is developmental changes or alterations of development can generate the variations on which natural selection can work, so that is the summary of this main point.

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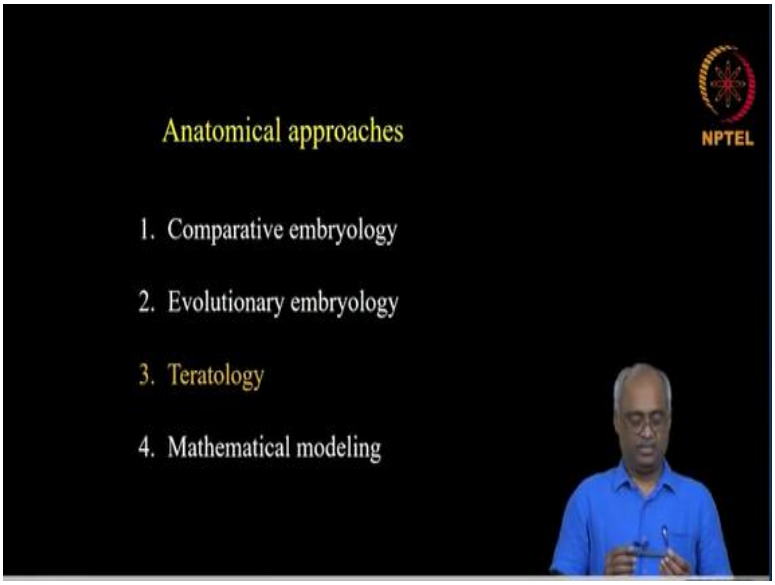

So these sort of variations being observed in animals is okay, can we see developmental abnormalities in humans? we do see there are birth defects and there is something called teratology, so we are going to look at the teratology.

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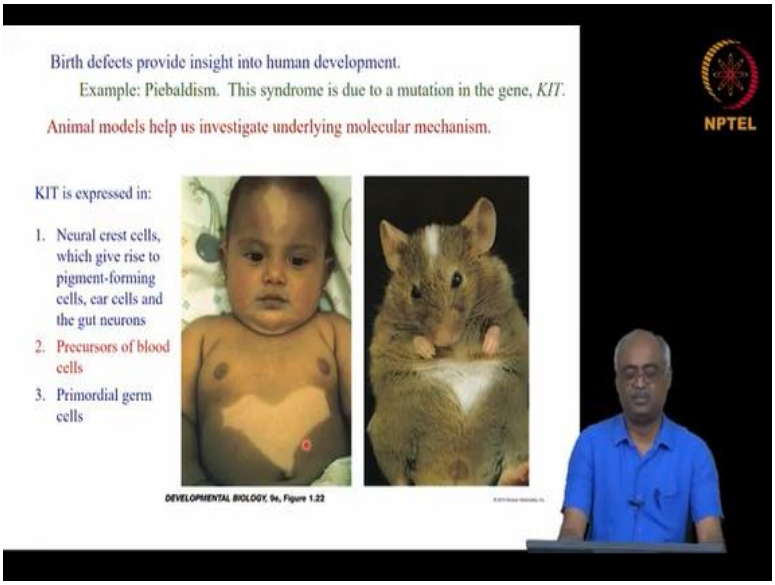

So here is an example a mutation in a gene called KIT, which is a transcription factor causes these defects, the visible defect that you are seeing here is the lack of pigmentation in the forehead and on the abdomen, on the belly of this kid, and this KIT is expressed in neural crest cells from which as I told you earlier the melanocytes, the pigment-producing cells come. And also it is expressed in cells that form the ear cells and gut neurons and as a result, this kid has problem hearing as well as malformation of its intestine and this is also expressed in precursors of blood cells so blood cells do not form and it is also expressed in primordial germ cells and as a result germ cells do not form and this kid is sterile. This kid is not going

to make it up to the adult stage but if it were to then it will be sterile. And you can have a similar defect in an animal where you mutate KIT you develop similar defects, so now this becomes an animal model to study human disease because you can recapitulate the disease symptoms of human disease in an animal, so the main point here is the underlying mechanism is probably the same for the normal development normally what KIT does for development you are unlikely to be able to study in human. But if the defects are the same in an animal then you will be able to do it in that animal and the mechanisms you are going to unravel there are going to apply to humans as well, so that is what we call as an animal model. So animal models help us investigate underlying molecular mechanisms. So this piebaldism is a good example of that.

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Then, so that is a birth defect so this is a good example of actually this is the one for which the teratology title should refer to. So teratology is usually when something toxic happens to embryonic development, so you like for example poison that kills us or causes problems but there are drugs or environmental conditions that can affect embryonic development. A classical example is what happened in the early 1960s, where pregnant women were prescribed a drug called thalidomide it is a mild painkiller to avoid morning sickness. So here the point is that this one episode changed the way we test new drugs for safety. So before that people look at whether there is any problem with adults or animals, so and when there is no problem then you say that is safe. But then some drugs can affect embryonic development, so thalidomide is a good example. So the problem with the thalidomide is this affects the long bone development of limbs and that is why you see this man's hands and legs were extremely short and by talking to the women whom this was prescribed, when they took it how long

they took it and matching with their gestation period they came up with this graph. So if you take 34 days after the last menstruation, let us say 34th day within 34 days of conception then you have problems with ear development and so depending on when she took, the embryo developed these problems but after that, it is safe there is no problems. And now actually thalidomide is coming back because it is useful for treating other things except that you cannot prescribe to pregnant women. So sample size I think it is about 400 individuals who are born with this, so this is not an experiment that was done, so here based on the outcome you go back and look at what happened. So, this is what people often do to study many human diseases and that is why the medical record-keeping and family tree are very helpful to find genes that are linked, so this is a teratogen. So thalidomide is a teratogen because it affects embryonic development so these are helpful to understand development.

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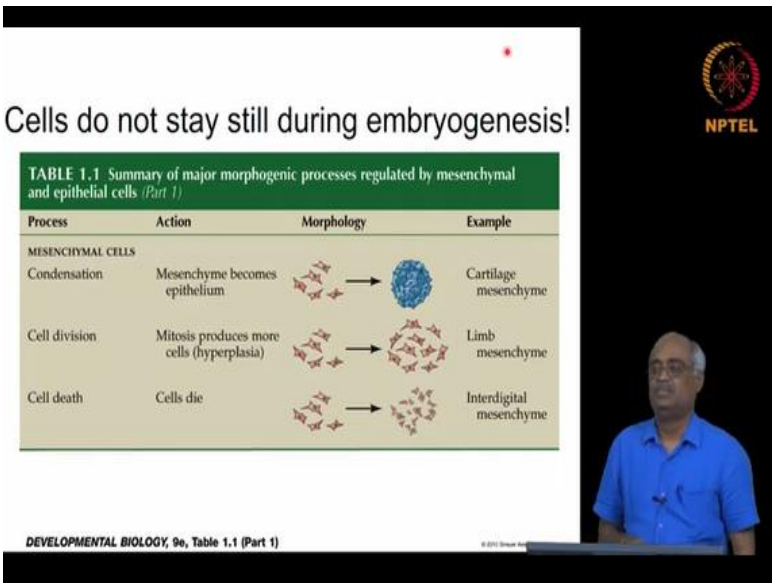

So let us move on to our next topics like going slowly towards morphogenesis. So, now in the embryo the cells are not static they are extremely dynamic, they do a lot of different things they divide and they divide at different rates, at different parts, they migrate, they secrete, they change shape, they stay together as a group or they mix with other groups, all those changes happen. And those are listed in this table which goes into four slides. I am not going to read out all of them, but the main points are we have two major kinds of cells one is mesenchymal where they are not attached to anything and they readily migrate and so within that if you look at it, you have condensation to make a particular kind of structure so it is a morphogenic event. Then you have division they make a lot of cells like limb mesenchyme as an example, cell death; cells do die like interdigital mesenchyme we already saw in human digit development.

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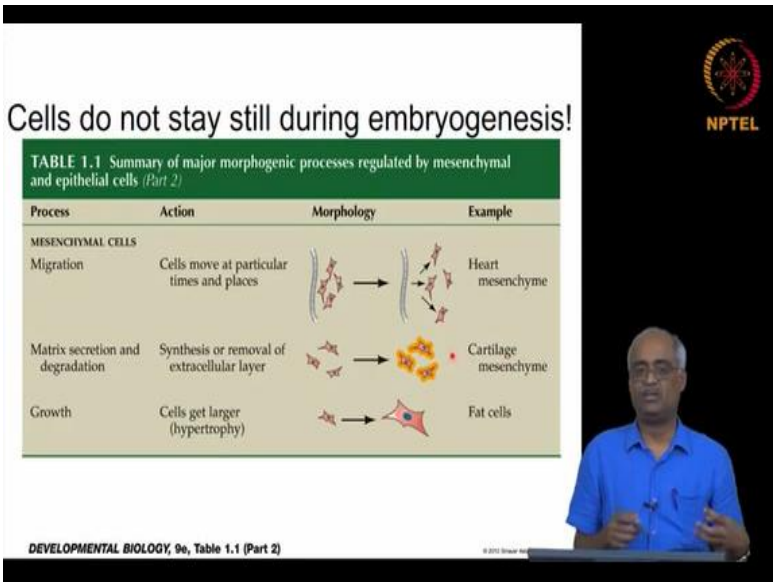

Then you have more migrations: heart cells migrate, germ cells migrate, germ cells migrate over a long distance to get to the somatic gonad then secretion of matrix and also sometimes degrading the matrix, both are essential for development. One good example is cartilage, so the cartilage is secreted as part of this and the ECM, the extracellular matrix is essentially secreted from cells, and sometimes getting rid of them is important if migration is what is required for those cells in the next stage in development. Then growth, some cells simply without dividing their size enlarge, good examples of adipose tissue cells that is the fat cells.

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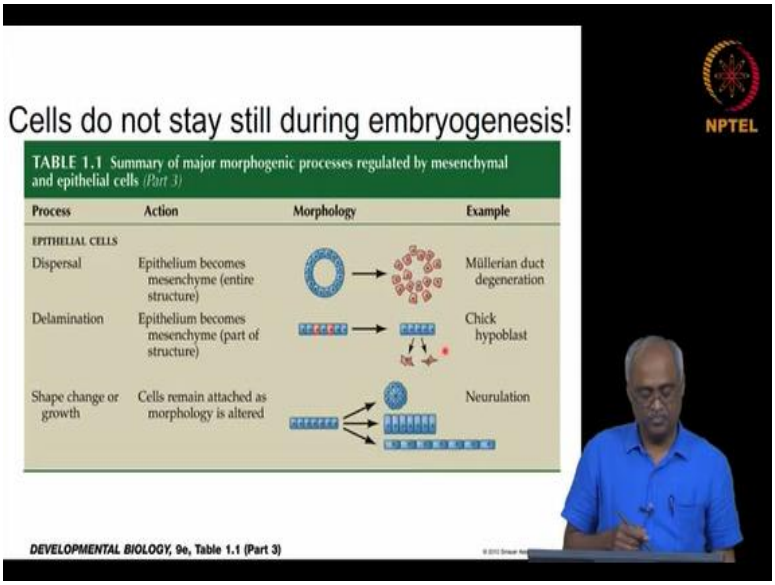

Then another kind of cells are the epithelial cells, where they are attached, they may be attached to a basement membrane as well as they are attached to each other and these are the ones that make sheets and tubes, here is a cross-section of a tube and these cells often, for

example during cancer, these become mesenchymal and that is how metastasis happens. So they call that as EMT, epithelial to mesenchymal transition, and understanding how that happens is an active area of research. Delamination is when part of the epithelium becomes mesenchyme. Then you have shape changes they remain attached but then they make different shapes like for example the lung formation happens in that way, so these are the different things that happen.

So the summary is that cells are very dynamic in the embryo, so if you want to follow what structures come from what cells and what those cells had to undergo in terms of this dynamics you need to trace them, you need to follow them and that is called a lineage tracing and when you have trace lineage of let us say a very early embryo having very few cells then based on what structures formed from those cells you can go back to the early embryo and map different regions of it from this region and that is called fate mapping.

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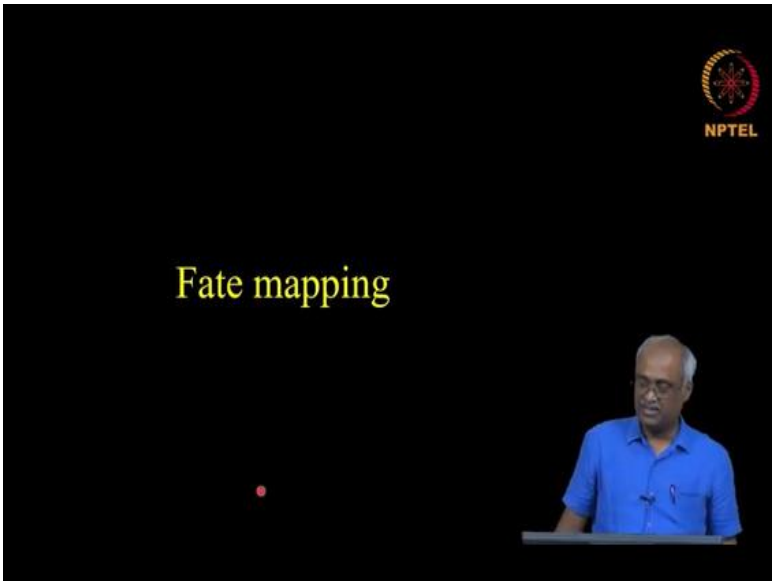

So that is our next major topic probably this is the only topic we will do today for the rest of the time. So, I will explain as we go.

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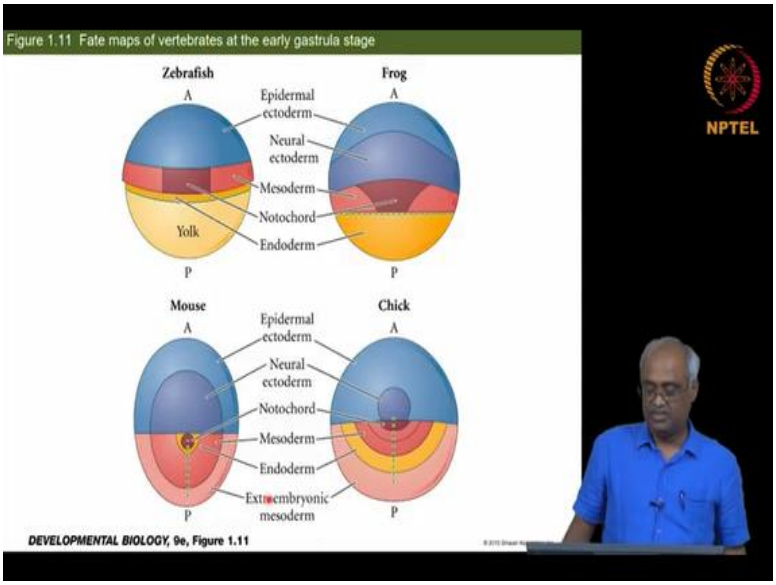

So this is looking at the very early embryo of four different organisms. So all vertebrates, we are looking at an early stage and these colors tell you that this part is going to form the epidermis of the skin and then like it is not visible in this but here this is the neural ectoderm going to form the central nervous system and this is going to form the notochord and this is endoderm, so that is what is mapped. What it means is cells in this region eventually give rise to endoderm; this is what is fate mapping. So you are mapping and you have an early embryo, what kind of structures will later come from that part is what is a fate map and when you look at it some conserved features are obvious like I will just point out one if you look at the notochord in all of them it is kind of in the center of it. So we are looking on from the top okay we are looking at the back, not the ventral side, so it is on the top center, it is where you have the notochord forming and that is right adjacent to the neural ectoderm from which you have the neural tube formation that we saw in the frog life cycle so this structure gain tells you the early similarity among these vertebrates, so now we look at how do we do this mapping? What are the various techniques that allow us to trace the cells?

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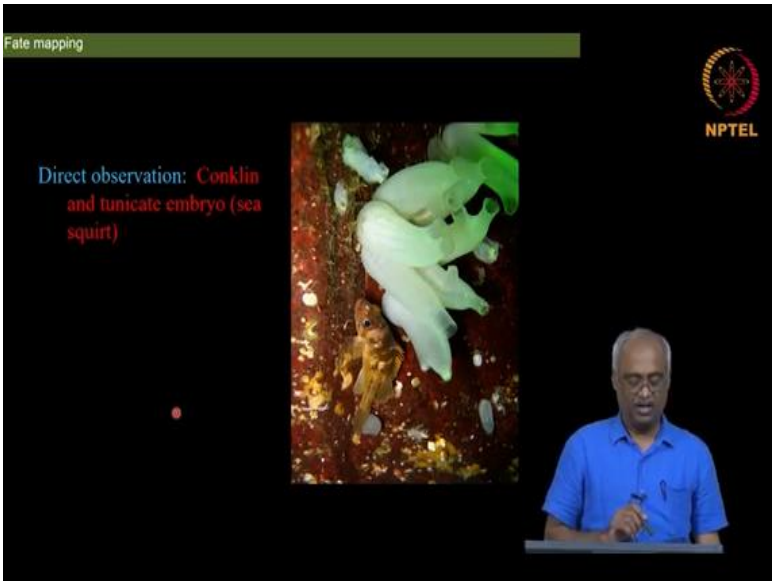

We are going to again look at it historically so that we understand how it all began. So Conklin took an organism called sea squirt that is because their early embryos have fairly large cells and different cells but basically blastomeres have different colored pigments and therefore you can observe where those pigments go as cell division proceeds and therefore he started with those so it is direct observation. So you can do it in many organisms direct observation for example *C. elegans* you can do that direct observation means not with the eye but you need a dissecting microscope to look at and observe them.

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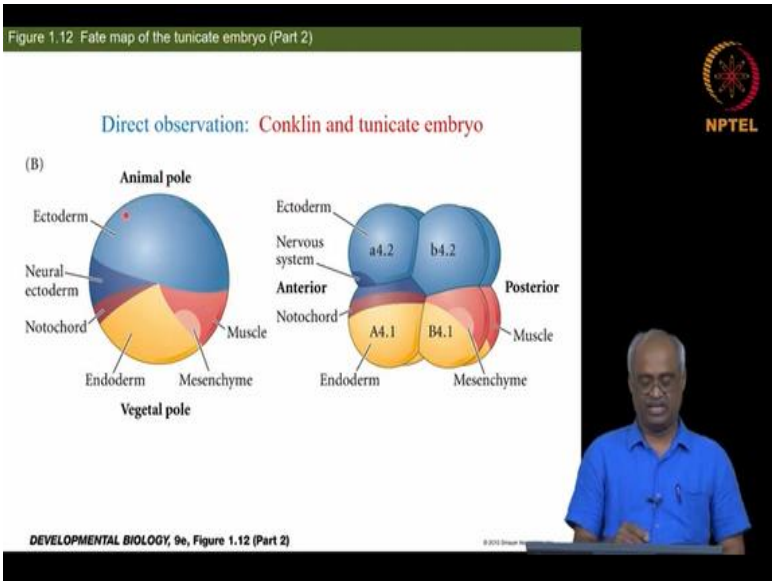

So this is the map that he finally arrived at in a very early stage, this is the animal pole and vegetal pole and he was able to map the future ectoderm to the animal pole and endoderm to the vegetal pole and then the other structures as labeled here and this is like 16 cell stage I think or 8 cell stage. So where you have individual cells already having like this is going to

make only the epidermal ectoderm. And then the nervous system will come from a4.2 and then endoderm so this is not going to make the only endoderm from this cell you are going to get cells that form the notochord as well as part of the nervous system also. So muscles come from these and muscles do not come from these so if you take it out nothing happens to muscle development.

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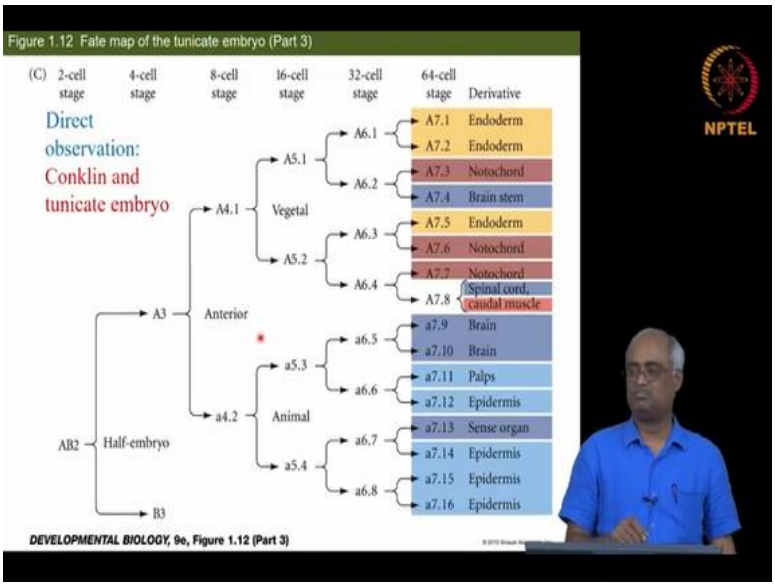

So this is a complex set, here this vertical line is each division and the horizontal line is the two and usually, this is not drawn to scale, this horizontal length indicates the time taken for that cell division. So this is how they draw a lineage map. So far we have complete lineage for only one organism, one species anyone knows that? people got Nobel Prize to that, *C. elegans*, Drosophila is way too complex you do not want to do it in the near future, its brain as million cells. So, *C. elegans*, adult *C. elegans* hermaphrodite has 959 cells, so we know exactly these 959 cells, from where they come from the zygote to the 959 cells and that is the process, this lineaging of an organism in an attempt to find the entire lineage of an organism. They thought that once we know the whole set of cells and their origins it will be easier to tease out its development. And in the process, they ended up finding a cell, for example, if I were to use this *C. elegans* lineage. Let us say AB2 divides into A3 and B3 and then this divides in this and this divides in this and then this divides this, embryo after embryo you see the same pattern and in one first embryo when you find A6.1 divides for example A7.1 and that died. Then the scientists thought, maybe I messed up something while doing it and then you go and redo it again for n number of times and every time you can predict A7.1 is going to die and that is when they realized it is not dying by accident or disease or injury, it is programmed it to die. So that is how the web disappeared here to make the fingers

independent, so that is what we call programmed cell death. So this is an example of unintended discoveries that happen when you focus on basic science research so that is a point away from the developmental biology here but it is a good example, so this is how the initial fate mapping and lineage tracing was done.

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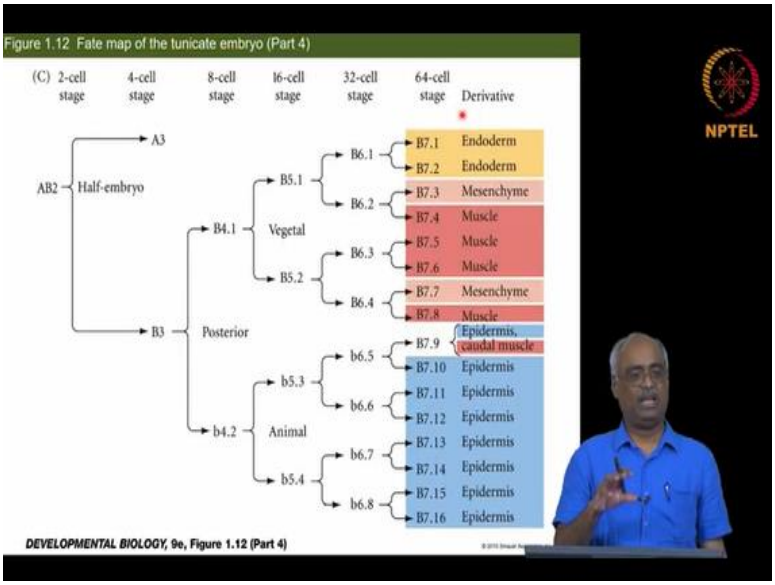

And this works readily where you have large blastomeres and each blastomere has distinguishable pigments and the cell division is invariant. What I mean by invariant is, the pattern of cleavage and position of cells, and the number of cells is constant from one individual embryo to another individual embryo during development. That is not the case in a complex organism like humans, we are of different sizes, right? So, not necessarily the number of cells is identical or the cleavage is invariant during our embryogenesis.

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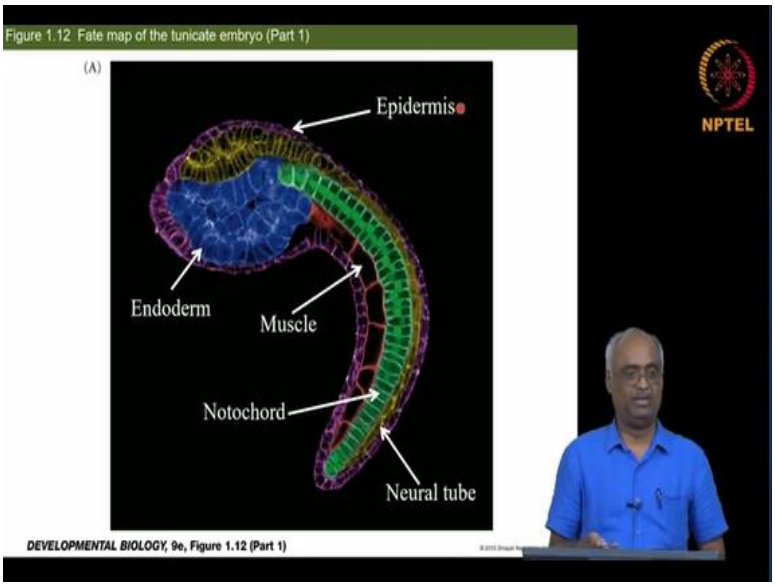

So this is the final, so it is the fully developed sea squirt embryo where they have done a dissection and photographed it using a confocal microscope and the different structures labeled here, have been distinctly colored and this could be achieved through what we just saw through the lineage tracing or the fate mapping so that is how we know this structure is the notochord in the embryo and this structure is what is going to form the neural tube and so on.

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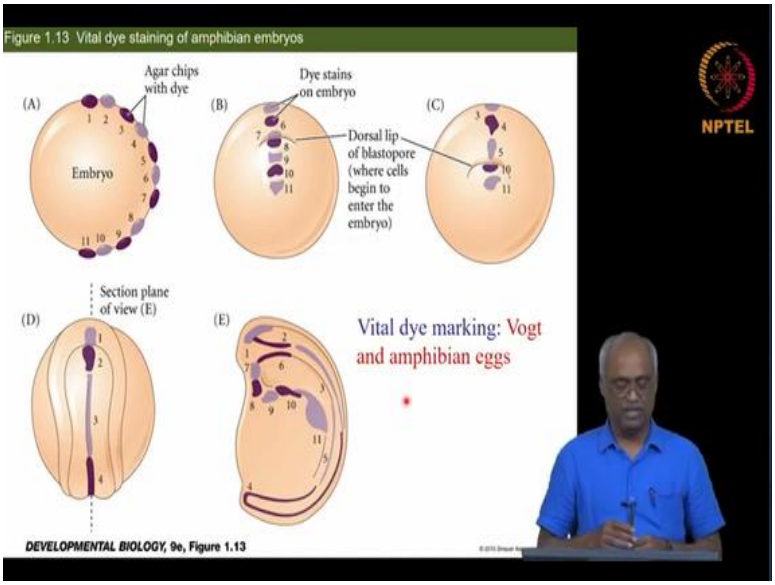

Alright, so that is okay for tunicate embryo where pigments helped, what about other organisms. So this is an attempt by Vogt, what he did is he mixed agar with different dyes and these dyes are vital dyes, so what vital dye means is it is a dye that is taken up by a living cell and does not harm the cell division or the developmental of the organism, so essentially you can label certain parts of the living organism by providing this dye. So they do not kill the organism. So one common example that we do in many labs is looking for apoptotic cells, so we incubate cells or feed the organism with a vital dye, called acridine orange or syto-12 and only the programmed cell death cells are going to take it up, and then you will be able to see that these are the cells that are undergoing apoptosis. So that is a vital dye, vital dye labels living cells, that is the main point so he mixed the vital dyes with agar and allowed it to dry and made small chips of this agar which is impregnated with the dye and gently placed on the embryo. This is a frog embryo, amphibian, different parts have different colors and once the cells took up the dye he removed the agar and watched what happens to the cells or group of cells that he has stained with these dyes.

And you see this is the dorsal lip where the cells are going to go in and then you see them as the cells multiply and migrate you follow with the dyes. So this is a dorsal view of the neural

tube, these are the two neural crest, and then when you take a cross-section and if you cut it you see the other side lateral view and then you see where they have migrated, so cells in this region migrated in this manner. So this is another way of lineage tracing. So one problem with this is as the cells multiply these dyes get diluted and eventually it becomes difficult to see, so as the modification of this is to use fluorescent dyes which are lot more intense and therefore last a little longer.

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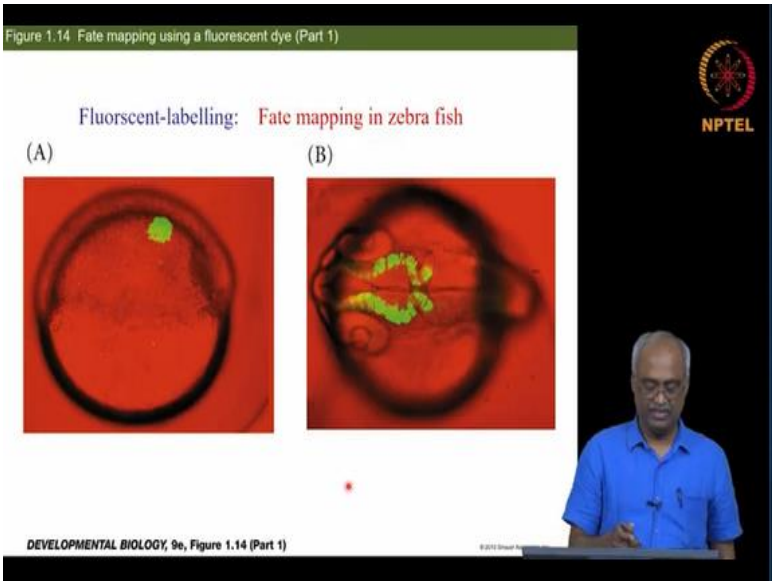

And, that is shown in this zebrafish example. So here a group of cells, in this region what you see in the right panel, you have green color, this is a fluorescent molecule that is injected into the cells there and then you see what happens and later you see here it is into the midbrain and forebrain area of the neural tube, so that is where these cells migrated to and using different color dyes and labeling different parts of the embryo.

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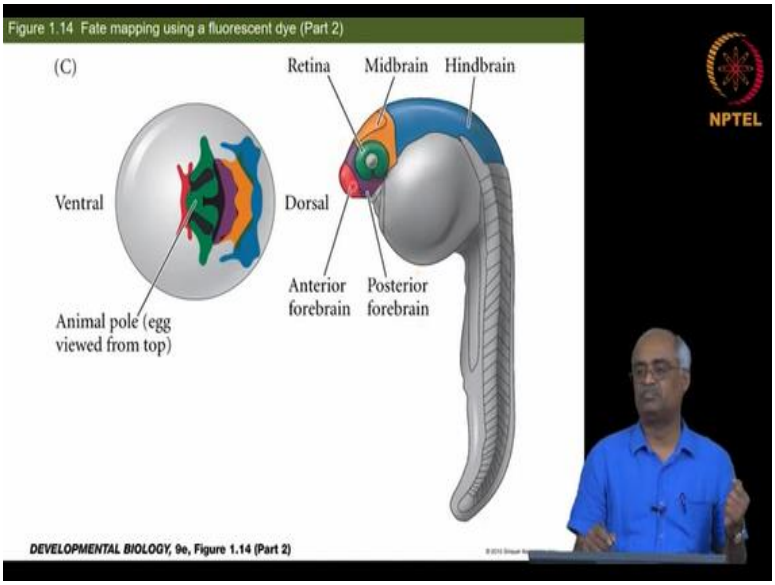

They can map in this manner. So these regions of the early embryo develop into these structures later on. So this fluorescent labeling is advanced a little bit more than vital dyes, but then they did not do a very extensive and sophisticated mapping with this. So these are like initial observations with embryos like frogs, but then quickly fluorescent dyes and right now you use more sophisticated genetic methods. So we will see that as you go on.

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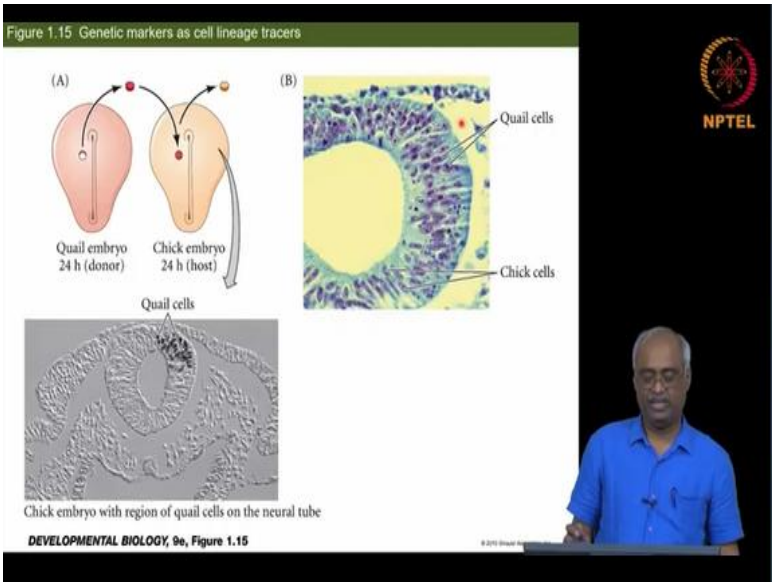

So here they take advantage of the similarity in closely related species, so here you take two birds one is quail and another one is a chick, so at a more or less similar embryonic stage you take quail cells from this region and put it on the similar region in the chick embryo and watch what happens. So here you can follow primarily because of two things illustrated here: one is you have an antibody that recognizes only the quail cells and not chicken cells and you see that. So this is the neural tube here, so the neural crest has fused, here the fold has fused

and you see quail cells put there. And another thing is looking at its cellular morphological differences the quail cells have little larger nucleolus and that helps in identifying quail cells from chicken cells and you can extend further like you can use radioactive labeling.

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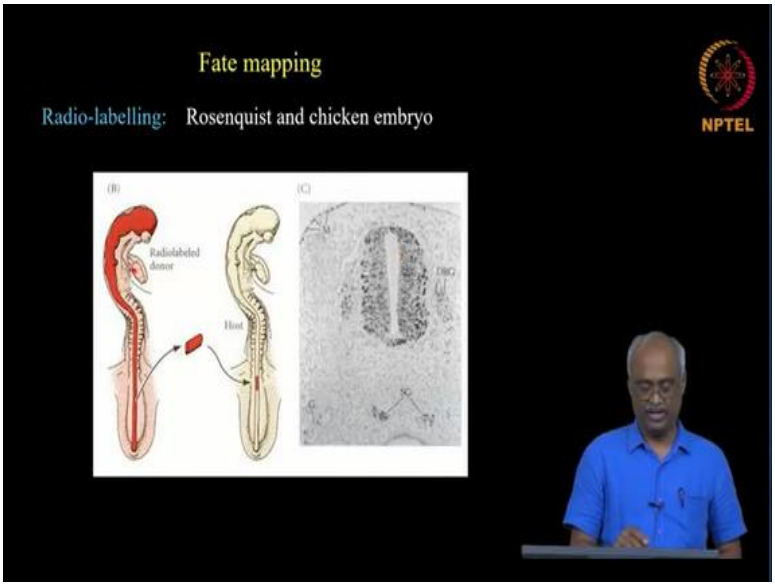

And here, one embryo is grown in radioactive amino acid containing medium and the other one is without radioactivity and then you take and transplant, similar structures at a similar stage, and then you see where it migrates and that is how they found the melanocytes. These epidermis cells in the epidermis that make the pigment actually come from the neural crest and then you have other cells like somatic gonadal cells, glial cells these come from the neural crest and this also shows cells migrate extensively.

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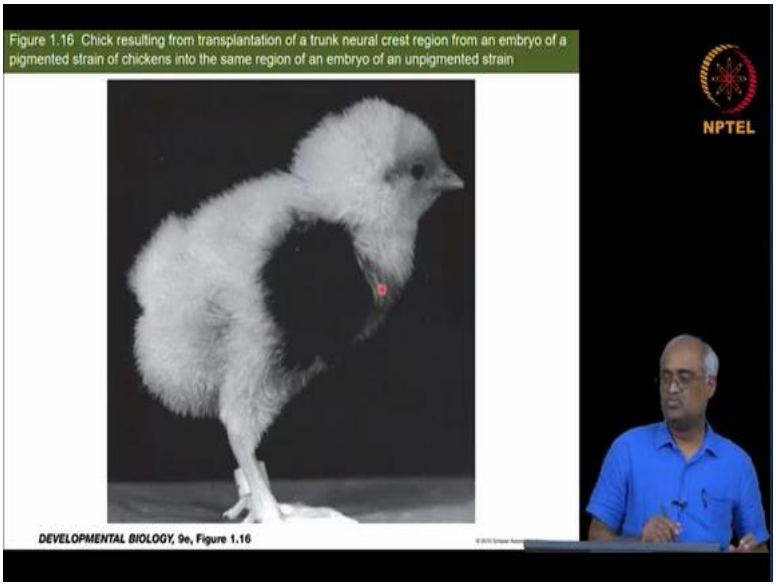

So a little bit more advanced here one chick which has pigmentation all through its part has been taken and transplanted into the other one which did not have any pigmentation and now you wait and watch when it comes out and you see this part of the body where you have the pigmented cells. So you can get up to this stage where you started and what cells contribute to this. So the last example of this is the genetic way of doing it.

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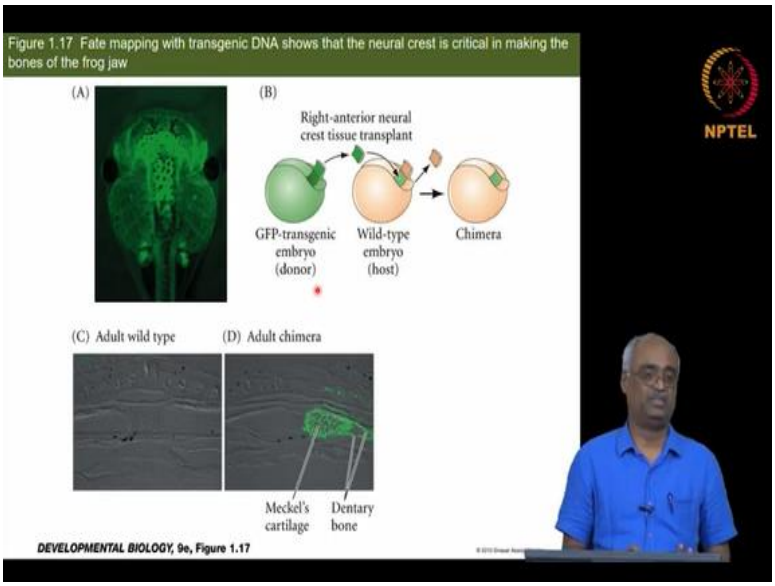

So in really difficult situations this is what helps and this is one difficult situation like for example tadpole, if you remember metamorphosis that we learned it does not have any bony structures, instead it as flexible cartilage. But then the frog has bone it has jaws and it has a skull. So what is the embryonic origin of the skull cells and for that what they did is, they expressed a transgene using a promoter that expresses in all cells and made a transgenic frog. This is a tadpole stage where it is expressed in all cells then you take those cells and transplant into another frog. So here you are not looking at closely related species or anything it is the same species you can even have an Isogenic situation and you transplant it and watch through the tadpole and the adult frog and then you look at the chimera, chimera meaning you are taking cells from one and put in the other one. And then you see this bone developing here this cartilage as well as this lower jaw came from cells that they took from the neural crest area. So even it is very hard to trace, cell lineages can be done using these genetic methods, so here this gene this is a fluorescent protein that is not an endogenous meaning not part of it is the genome, it is from another genome and you are introducing it here and such manipulations or such genes we call as transgenes from another organism, but it is worth the effort then finally what comes from where and then you will be able to trace and then understand the mechanisms involved in development. Cut paste is a surgery, so you need to

take open up the embryo and you cut and do it, so this is one thing I have not done at all because I worked with invertebrates where you do genetic modifications without really doing surgery but people work with the frog they do this, so I think we will stop here today with the fate mapping. So in the next class, we will continue to understand how development is controlled.