Introduction to Developmental Biology

Prof. Subramaniam K

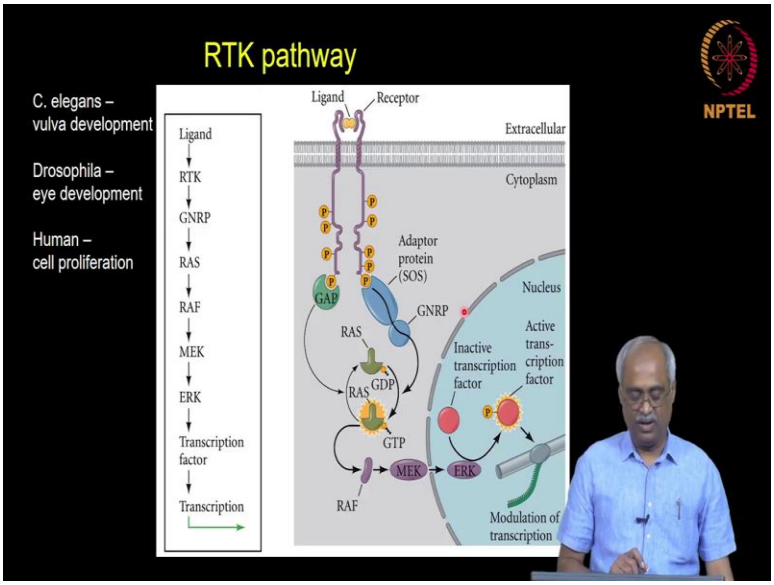
Department of Biotechnology

Indian Institute of Technology - Madras

Lecture-15

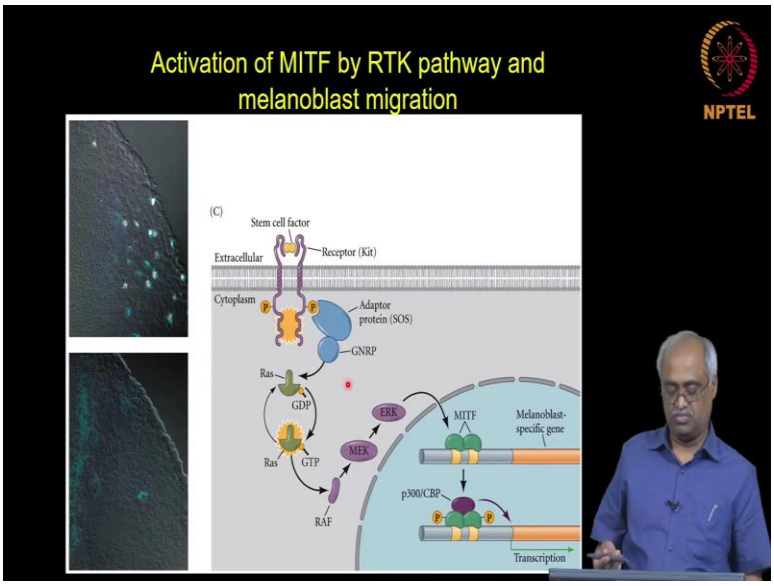
Cell-cell communication (Part 3 of 4)

(Refer Slide Time: 00:14)



So, today we will continue with the cell-cell signaling. Remember, in the last class we began with this RTK. We primarily learned about how the RAS GTPases helps in fine-tuning the signal. As long as the signal comes, you will have the RAS GTP, active form; otherwise, it will automatically become RAS GDP and become inactive because of the GAP and the intrinsic hydrolysis activity. So let us see the other signaling pathways.

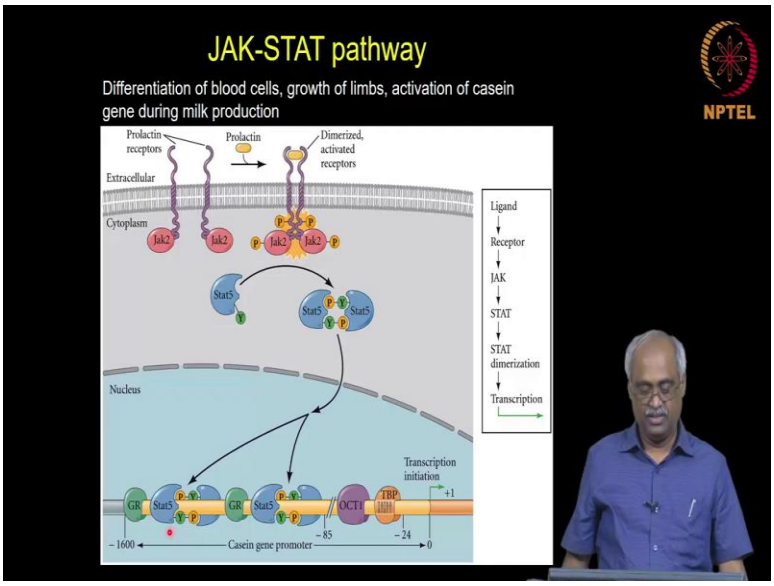
(Refer Slide Time: 01:00)



So, the next one is a small variation in RTK; this is to illustrate that the RTK pathway ligands are diverse. For example, stem cell factor when it binds through a receptor tyrosine kinase; in the case of melanocytes, the receptor is the Kit, which we have already seen as an example earlier. W also saw MITF, a transcription factor.

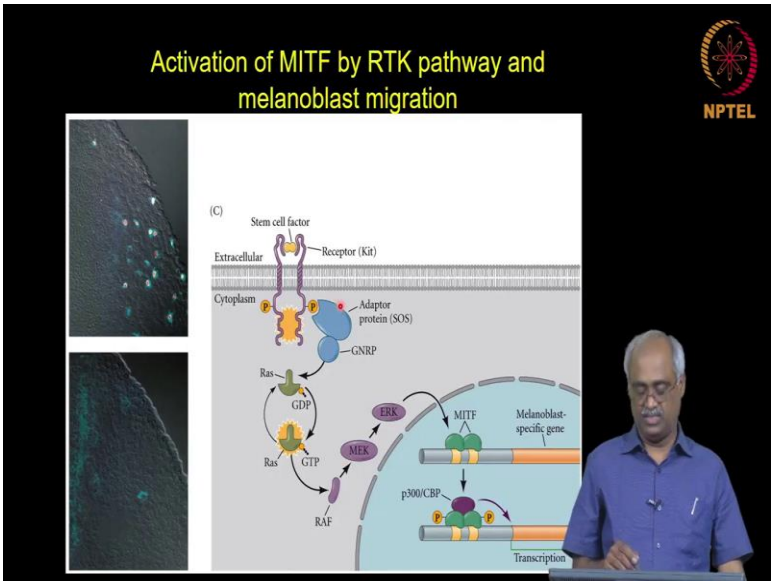
So in the image, you find the colors red and green. So, here in the melanocytes, you see a white shade around the red. So, the red is MITF, and the green is KIT. So when they both are there, they appear whitish. And in the kit knockout, that is absent. So, the migrating melanocytes express these proteins that are required for the specification of the melanoblast, which eventually becomes the pigment-producing cells. So the absence of these causes skin color aberrations if you can remember the forelock of the mother and daughter in that MITF mutant.

(Refer Slide Time: 02:43)



So, a small variation of that same pathway that has a similar receptor, but it is called Janus kinase. The difference here is the way the signal is transduced. So, the transducer is STAT, and it goes into the nucleus to activate transcription.

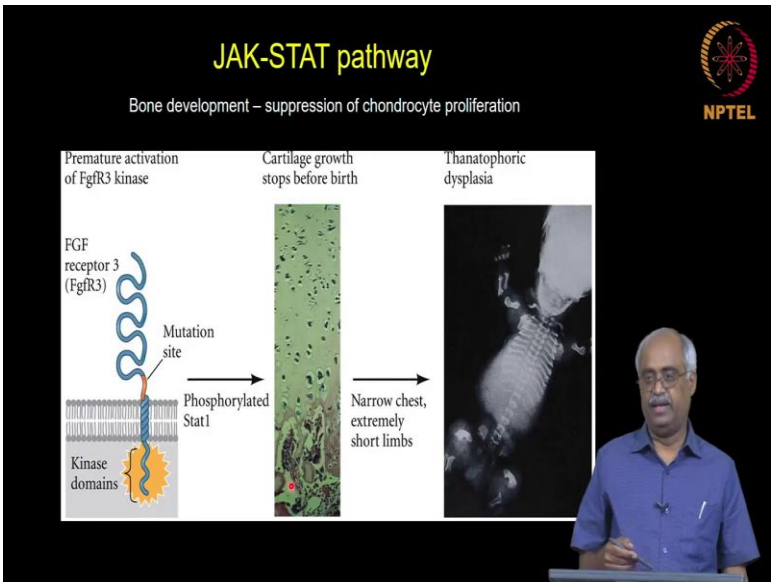
(Refer Slide Time: 03:17)



So like here, the signal transduction happens via a cascade of proteins involving a couple of phosphorylation based regulation, and then a phosphorylated protein gets into the nucleus. So, in the RTK pathway, the transducer, if you take this adapter protein or GNRP, they are not the final effectors; they are not the ones that go inside the nucleus. While in the JAK-STAT pathway, the transducer is the one that directly gets into the nucleus and activates other downstream genes. And as a result, it is called a signal transducer and activator of transcription (STAT). And the

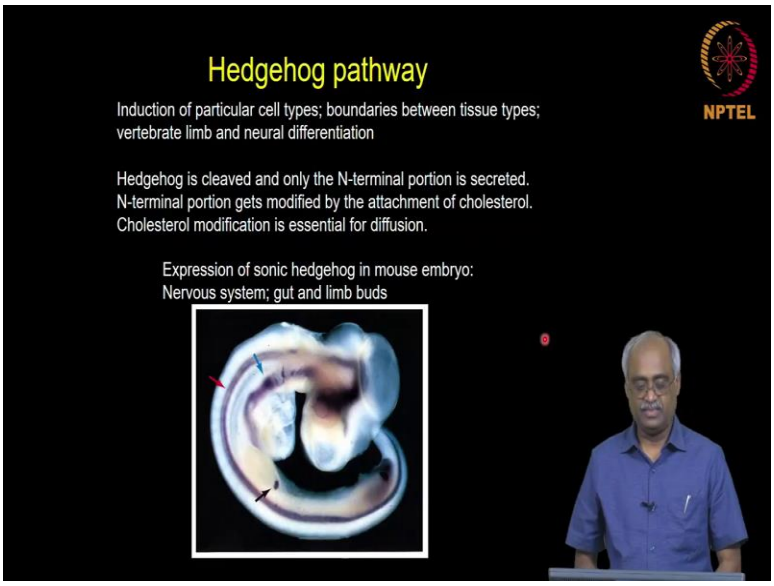
STAT dimerizes and goes inside the nucleus and activates downstream signaling. So, this sort of regulation works in the casein gene during milk production, where the prolactin binds to the receptor, leading to STAT activation.

(Refer Slide Time: 04:26)



So here is an example of a mutant condition where the mutation is in this extracellular domain of the FGF. So in the first figure, the entire thing is called the fibroblast growth factor receptor (FGF), we saw a similar kind in the RTK pathway and now we see STAT as a small variation in the same group. So, when you have a mutation like this, this kinase domain remains constitutively active even when the signaling is not there. So, the receptor is constitutively active, and as a result, these chondrocytes in the second figure prematurely differentiate without making enough of the starting material. Due to that, the bones become short and results in a condition called the thanatophoric dysplasia. So, this highlights the importance of the JAK-STAT pathway in limb development, and this is lethal. So in this condition, the lungs cannot expand, and breathing is impossible, so this baby will not survive.

(Refer Slide Time: 05:42)

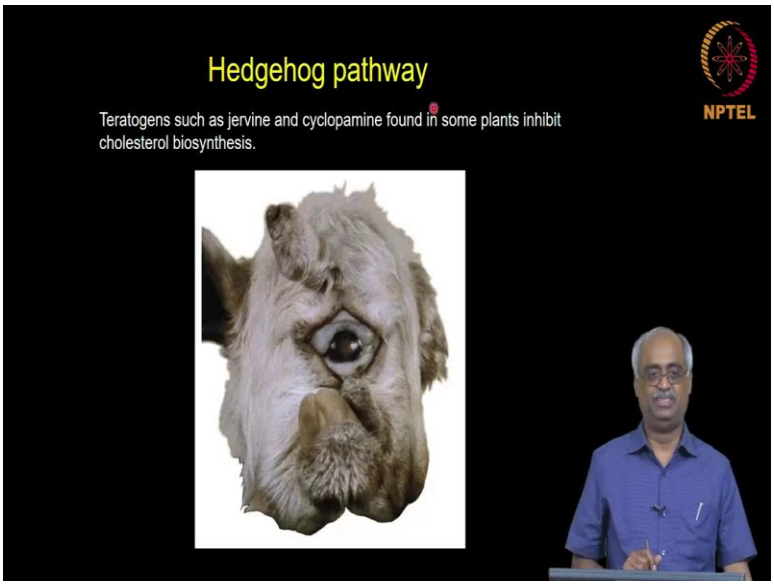


So, the next one is the Hedgehog pathway. So, each pathway has unique characteristics in terms of how the signal is transduced and what kinds of molecules are involved. So, therefore it is not that hard to remember them. And there are only four pathways. So the uniqueness of Hedgehog is it is a protein signal that gets cleaved, and only the N-terminal portion is secreted out and that N-terminal portion is cholesterol modified.

So we have encountered many post-translational modifications, particularly phosphorylation, methylation, acetylation. So, this is an example where cholesterol modification happens. So, this cholesterol modified N-terminal portion of the protein is the signal here. So, the word Hedgehog is based on the Drosophila phenotype, where this was initially found. And in Mouse, the ortholog is called Sonic Hedgehog.

So in the slide, this is a whole-mount embryo in-situ showing expressions of sonic hedgehog three tissues. So, it is expressed in the nervous system, limb buds, and the gut region, so these arrows are showing where they are expressed. So, these are usually important for tissue boundary formation, the boundaries are defined primarily using this signaling, and it is required for limb development and neural differentiation.

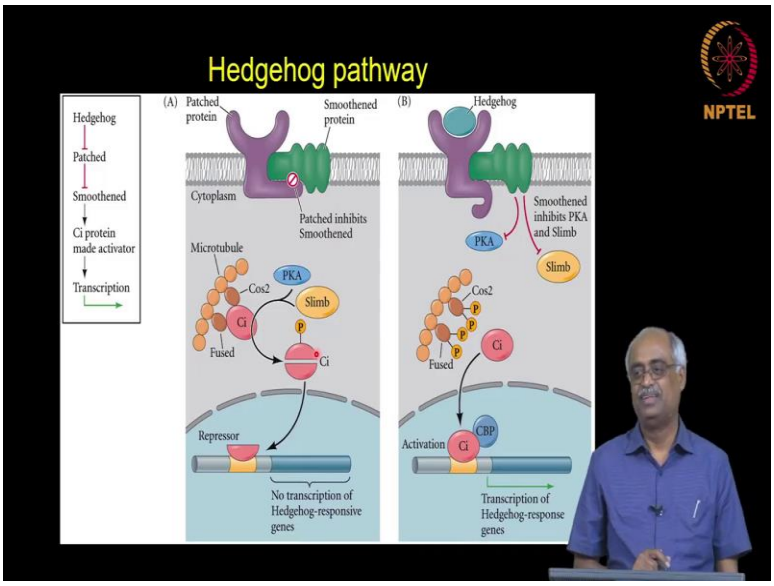
(Refer Slide Time: 08:08)



So, I will tell you something interesting, and then we will come back to the pathway. So if you look at this picture, this is called a cyclops. So some plants that grow in certain parts of the world like in Arizona or California etc., produce certain molecules called an alkaloid, namely Jervine or opamines like cyclopamine. If the sheep eats these molecules, then their offspring will have developmental defects.

So these alkaloids inhibit cholesterol biosynthesis as a result, enough cholesterol is not produced during the embryonic development, and due to that you have craniofacial development problem. The three parts of the brain do not form the separation and they form a few structure due to that only one eye develops and it is called cyclops. So, this illustrates the developmental importance of cholesterol.

(Refer Slide Time: 09:39)

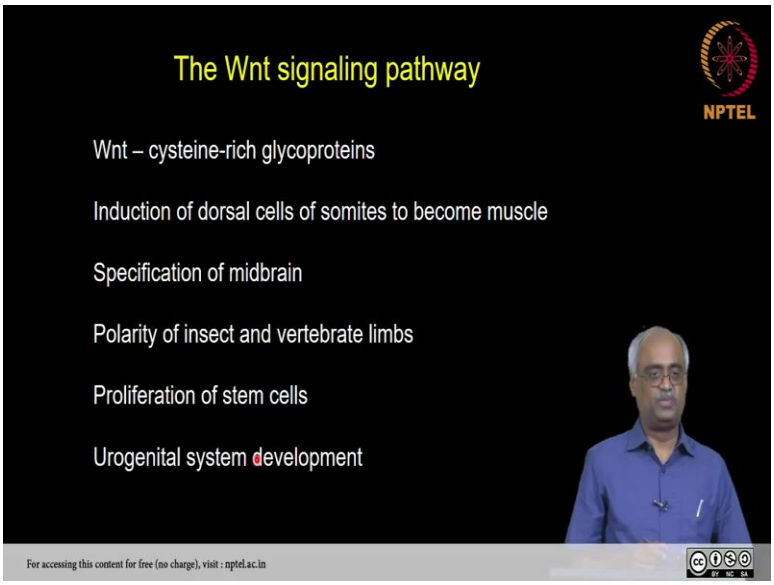


This pathway again works like the accelerator pedal in the car. So, usually, this Cubitus interruptus(Ci) which is attached to microtubules via these Cos2 and fused. Now PKA and Slimb would cleave this Ci, and the cleaved part of Ci goes into the nucleus and acts as a repressor. This is what usually happens.

Hedgehog binds to this transmembrane receptor called patched. Otherwise, patched keeps the other membrane-bound protein smoothened in a repressed condition. So, these are again based on the Drosophila embryonic phenotype. So, when Hedgehog binds patched, the inhibition is released, and due to that, the smoothened inhibits these two proteins, PKA and Slimb. So, we still do not know the exact molecular mechanisms involved in it. So the end result is these two proteins are inhibited and the smoothened by phosphorylating these two proteins Cos2 and fused releases Ci from the microtubules. Now the Ci is not cleaved, and it translocates into the nucleus, and then it recruits a protein called CBP. Now it is no longer a repressor; instead, it becomes an activator. So, this is how the hedgehog response genes are activated, and this is how this pathway works.

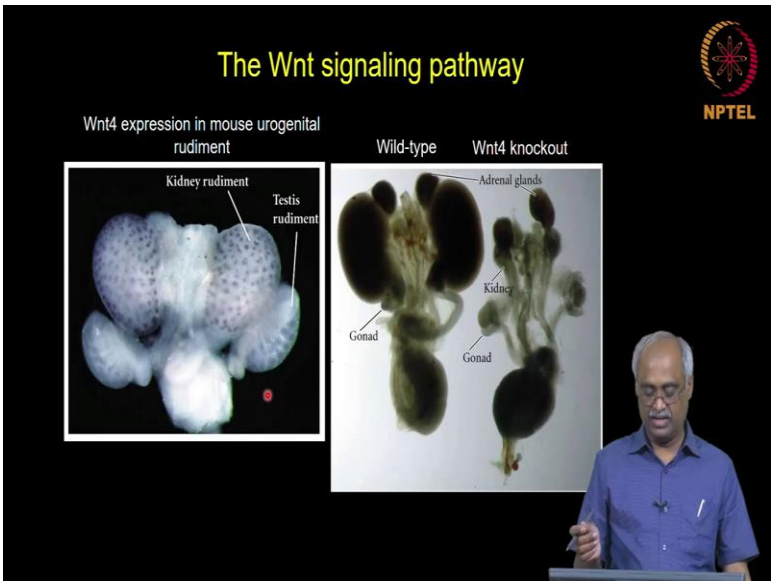
Here, the uniqueness is these molecules are specific to this pathway and then you have this cholesterol modification.

(Refer Slide Time: 11:48)



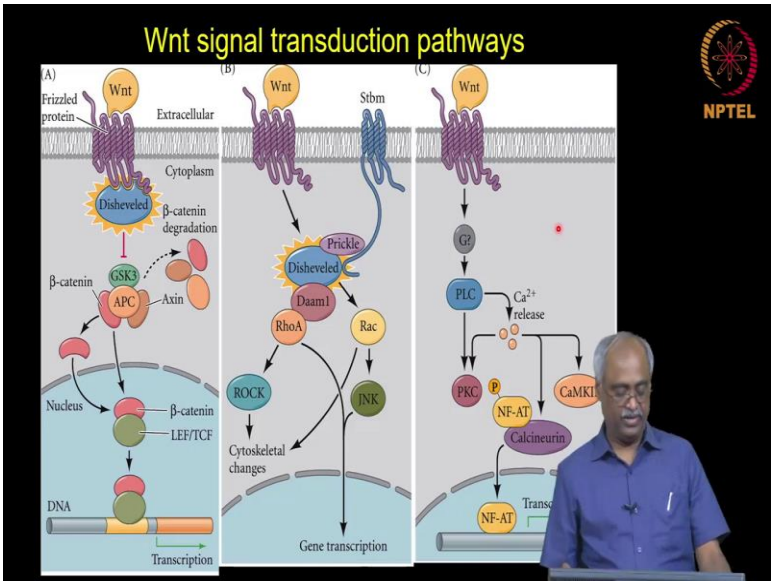
So, the next one is Wnt signaling. So, Wnt, the word comes from wingless, which is a phenotype of a Drosophila mutant, and the integrated is a vertebrate ortholog, so both together, it is called Wnt. The vertebrate ortholog was first found in Xenopus. So, these are glycoproteins that are rich in cysteine. So, they are important for the dorsal somites to become muscle. So, the ventral one becomes cartilage through the action of Hedgehog, and the dorsal ones become muscle through the action of Wnt. And it is required for the specification of the midbrain. So, this is also involved in limb development like the other ones like JAK-STAT. Then it is involved in the proliferation of stem cells in certain contexts and more dramatically for the urogenital system. It is also required for female sex determination. So, let us look at how this works. So, this belongs to a family of proteins; there are several Wnt like molecules and, similarly, several molecules for its receptor, frizzled. And which one of the members' activates depends on which one is expressed in a given tissue. And due to that, there is a divergence like there are three different modes of Wnt signaling.

(Refer Slide Time: 13:39)



We will see that two slides later so before that, again, like the cyclop, here is an exciting experiment that was done. So, this is again an embryonic kidney, testis part that is stained using in-situ hybridisation for the Wnt4 RNA. The purple color in the first figure shows the expression pattern of Wnt4, so it is expressed in the kidney rudiment strongly. In the second figure, these are the wild-type and Wnt4 mutant that are age-matched with the same magnification; kidneys and adrenal gland are on the top, the gonad is at the bottom. In the knockout, the adrenal gland and the gonad are intact but the kidney has become too small. So, the kidney does not form so the nephrons do not develop. So, Wnt signaling is required for this.

(Refer Slide Time: 14:35)



So, there are three different ways by which Wnt works; the first one is called canonical, which is like in the textbook, a classic example. So, the first defined pathway, therefore, is called the canonical, and the ones discovered later are called non-canonical. The first discovered pathway has this sequence where the metabolic enzyme glycogen synthase kinase3(GSK3) is usually associated with the APC. So APC is a tumor suppressor involved in colorectal cancer.

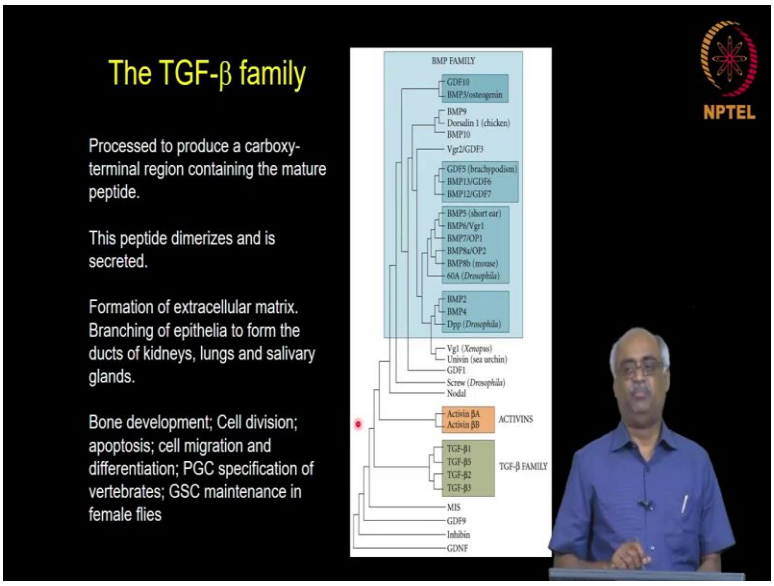
So this GSK3 and APC target beta-catenin for degradation, and as a result, you do not have beta-catenin going in and activating the genes inside the nucleus. But when you have Wnt binding to the frizzled receptor, a transmembrane receptor and the transducer disheveled gets activated and, in turn, suppresses the GSK3. Now, beta-catenin is not going to be degraded, and it will migrate into the nucleus and associate with this factor LEF/TCF and activate transcription. So that is how this works.

So again, normally, the pathway is negatively regulated. This is to fine-tune; only when the signal is there, the pathway gets activated, and the moment the signal is off, the pathway is turned off.

Second is a small variation of (A); here in the (B), the signal is transduced by the disheveled protein tethered to the plasma membrane via this Prickle protein. Then it is going to activate these Rho GTPases. Similarly another protein gets activated called Rac, which is also a small GTPases. So, when these are activated, they are going to alter the cytoskeleton. So without the gene expression change, the cytoskeleton can be altered when it is transduced in this way.

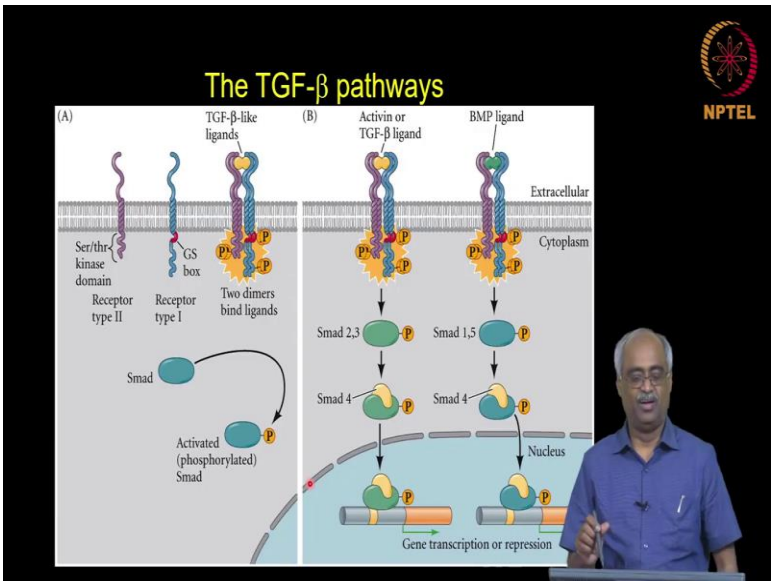
In the third version, an unknown transducer activates a phospholipase-C (PLC), and that in turn causes the calcium release from ER. So transcription activation based on calcium mediated signal transduction takes place. So, these are three different versions by which Wnt signaling works.

(Refer Slide Time: 19:27)



The next one is a super family having multiple families inside; this is called TGF-beta. Here the ligand is the carboxy-terminal region containing the mature peptide. The peptide dimerizes and is secreted out. So, it is required for extracellular matrix, then branching of epithelia to form ducts of kidneys, lungs, salivary glands like wherever you need to have epithelium to tube formation. It is required for bone development; one of the family members, the bone morphogenetic factor BMP, was originally discovered for their ability to promote bone formation. This DPP is one of the BMP members in Drosophila required for germline stem cell maintenance without that; they would not remain as stem cells; instead, they enter into meiosis. So, there will be no stem cells without that; they are required for cell division, apoptosis regulation in many vertebral contexts and cell migration as well. They are involved in many different processes.

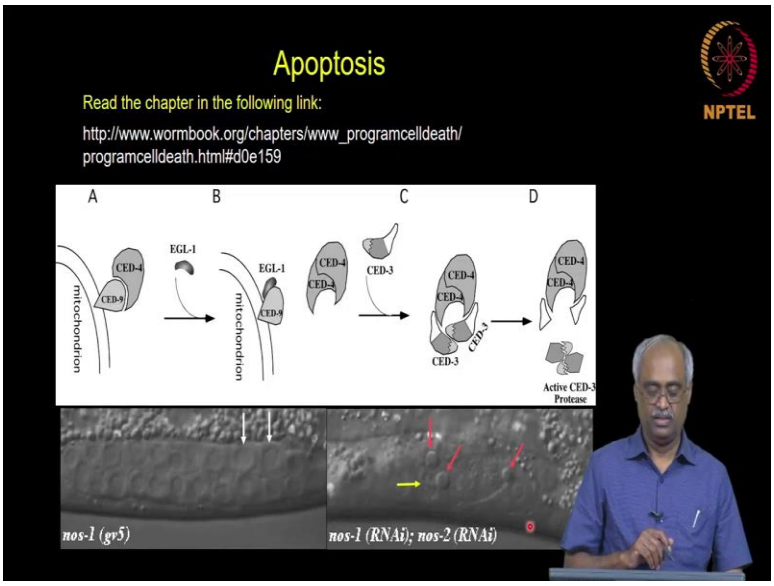
(Refer Slide Time: 21:07)



So, they work in this manner; here, we are looking at two different ligands. So, there are type II and type I two receptors; they dimerize when they bind to the ligand. Type II gets auto phosphorylated and then phosphorylates type I. Now the phosphorylated type I and type II are the active version. So this active form phosphorylates the Smad proteins. So, if activin binds, the Smad2 and 3 get phosphorylated, and that associates with Smad4, and that complex goes in and activates transcription.

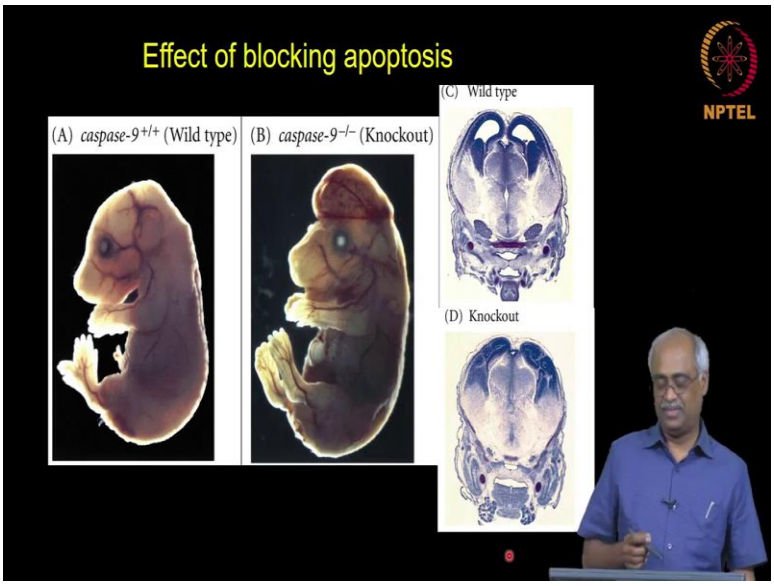
Instead, if BMP binds, then Smad1 and 5 associate with Smad4 and go and activate the transcription. So this is how TGF-beta works. So, they are really critical molecules in almost all organismal developments.

(Refer Slide Time: 23:01)



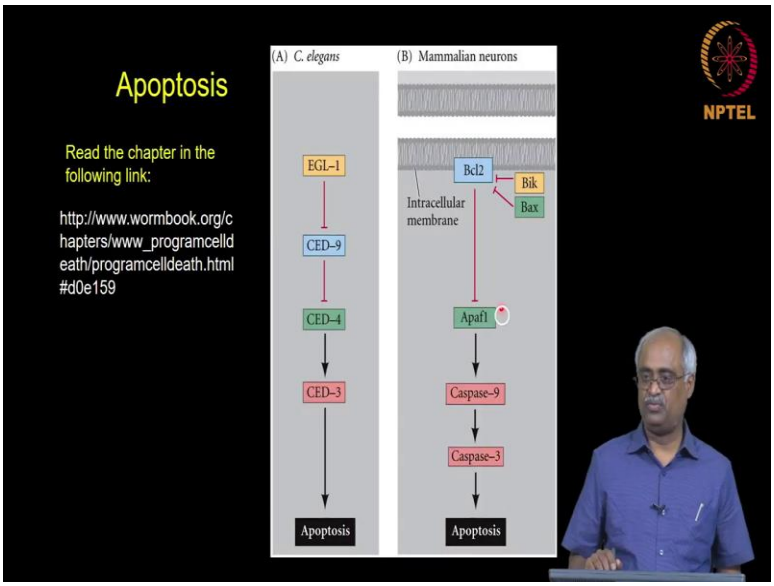
So this is the last one which is not among those four main pathways. So this is again a critical pathway; this is called apoptosis. So, it is a relatively simple pathway, so you already know that in cell lineage tracing, in *C. elegans*, you can trace the entire lineage from P0, and in that process, people discovered that 131 cells die always. And this invariant lineage tracing of those 131 is what convinced people that there must be an intrinsic developmental program that makes a cell to die. So, now we know in many organisms that the default state for all cells is to die, and some factors instruct cells that you are not going to die, and that is how they live. So, that is how it works; for example, here, if CED-9 is not holding on to CED-4, then CED-4 will dimerize and becomes active and associate with this protease CED-3, which is the executioner of cell death. So, CED-3 will be cleaved to form an active protease, which will further chew up all the proteins and DNA and kill that cell. So this will happen only if CED-9 is not holding CED-4. So usually, in a cell that is not destined to die, CED-9 holds on to CED-4; and anytime you get the signal from EGL-1 (egg-laying defective), it is going to compete with CED-4 to bind CED-9; therefore, CED-4 is released, and that goes on to activate CED-3. So, this(bottom figures) is how it looks in *C. elegans.* It is easy to find apoptotic cells, so this is very early larval germline where you have two rows of germ cells, and this is how the normal nuclei will look (white arrow), and when they are going to commit suicide, they become button shaped like this.

(Refer Slide Time: 26:13)



So *C. elegans*, CED-3 homozygous mutants are viable; there is no problem with those few extra cells, but in your case, you would not be born. This (A) is how a wild-type Mouse head will look like; this (B) is a *caspase-9* knockout. So in the absence of caspase-9 (B), many neurons will be produced, and they will stay there. And also, the digit separation in hands will not happen, and eventually, the embryo will be dead. So (C) is a cross-section of the brain area, and you see the ventricle space and the wall in the wild-type, but in the caspase-9 mutant, the wall is thickened, and you do not even see that space. So, it is lethal in vertebrates.

(Refer Slide Time: 26:57)

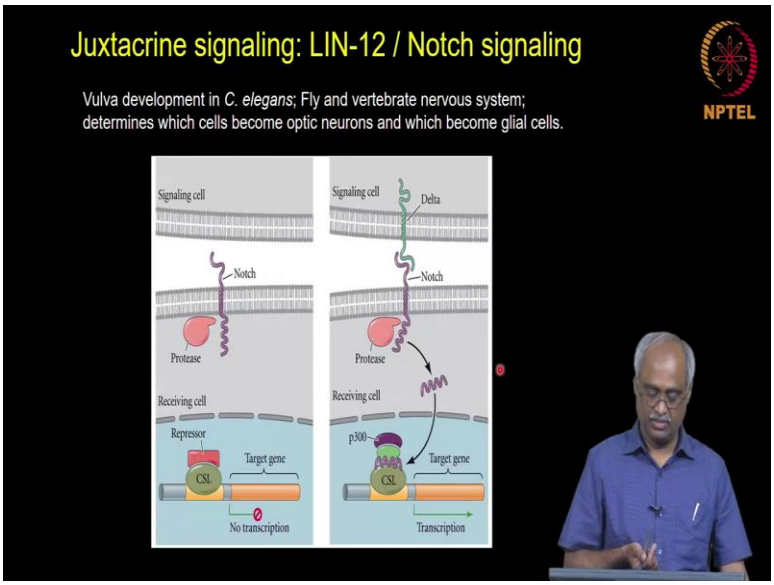


And this is the pathway in the two different contexts and the orthologues are colored similarly. This CED-4 and Apaf-1 are interchangeable you can get rid of CED-4 and put the human Apaf-1

in *C. elegans*, and it will rescue the phenotype in *C. elegans*. So, these are conserved pathways over a few 100 million years. Like EGL-1 equivalent is the Bik and Bax. Bcl-2 is the CED-9, and Apaf-1 is the CED-4 and caspase-9, and 3 are the CED-3 equivalent.

So (A) is seen in *C. elegans*, and (B) is seen in mammalian neurons, and mutations in this pathway will cause cancer. Like for example, cells that are not adhering to a surface where they should normally adhere are activated to commit suicide by apoptosis, and if that fails, then those cells will not die; instead, they will become metastatic cancer. So, many cancer situations occur when there is a mutation in genes involved in this pathway.

(Refer Slide Time: 28:46)



So this is the last signaling pathway. So, these are in addition to those four, only a couple of them, one is apoptosis, a straightforward pathway, you have CED-9, CED-4, CED-3, EGL-1, that is all you need to remember.

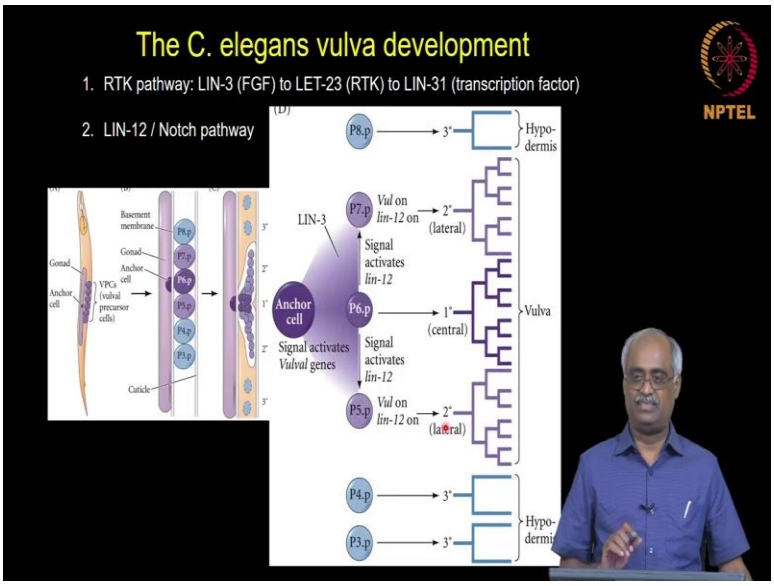
So the next one we are going to look at is not paracrine. So the four main pathways are paracrine. So, once the ligand is secreted out and they bind to the receptor on cells at some distance not very far away like the way endocrine system works. But the next one that we are going to see is called juxtacrine where the ligand and receptor are membrane bound and therefore the cells have to be physically adjacent to each other.

So the drosophila names have stuck in the literature more popularly, so the receptor is Notch, and the ligand is Delta. So, officially it is called lin-12/Notch signaling because lin-12 was the first Notch like receptor discovered. So, did we encounter lin-12? Yes, we did in vulva development and we will refresh our memory once more. So what does lin-12 do in vulva development? When the primary fate cell wants to suppress the primary fate on the two adjacent cells, it signals through the Notch-Delta pathway. It is required for retinal development in fly. In the vertebrate nervous system, to tell a cell, not become a neuron instead, to become a glial cell is done by the Notch signaling.

So, here the interaction leads to cleavage of the Notch. So, usually there are three cleavages. It is made as a single polypeptide but on its route to the membrane via ER and Golgi, in Golgi it gets cleaved and it becomes two polypeptides but attached to each other non-covalently; therefore, it is like a heterodimer. And that is what is embedded in the membrane, which is S1 cleavage.

Then the S2 cleavage happens extracellularly only upon Delta interaction and that cleavage triggers an intra membrane cleavage by a protein called Presenilin or Sel-10 or Sel-12 in *C. elegans*. So, that Presenilin cleavage releases the cytoplasmic domain, and that is the active Notch, which is the transducer that goes inside and associates with the CSL. So that association with that transcription factor activates the downstream targets. So, that is how Notch-Delta transduces the signal. So, essentially here the exciting thing about this molecule is the receptor undergoes three proteolytic cleavages—one before going to the membrane, then one extracellular cleavage, and finally, intramembrane cleavage. Then the active molecule translocates into the nucleus. So this is how it works.

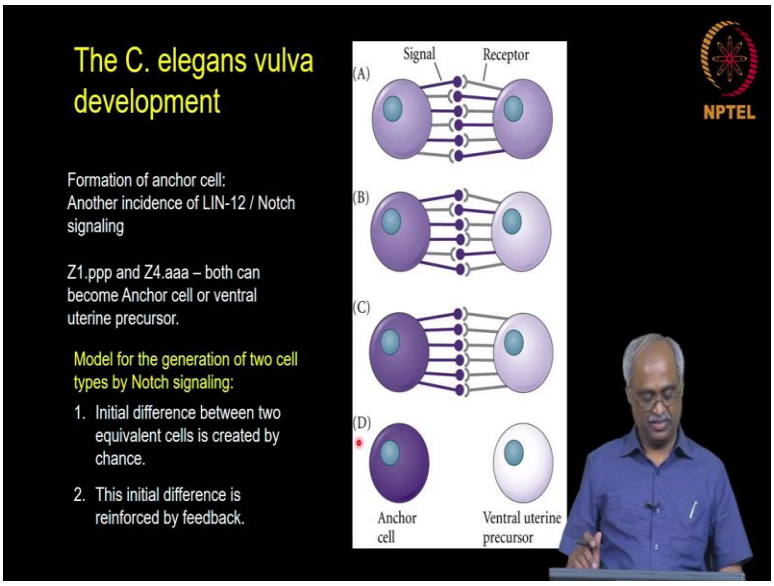
(Refer Slide Time: 33:36)



So, the signaling is easy, but we have a complex model to explain how this finally contributes to development. So, there we are going back to the vulva development for a short time. So, we already know the RTK pathway, the anchor cell through the RTK pathway is going to make P6.p cell to become primary fate, and now P6.p is going to prevent the two adjacent cells, this P5.p and P7.p are equal in terms of their positioning. So they are called equivalence group that is another keyword you need to remember. The equivalence group is cells that have equivalent potential in terms of developmental possibilities. So, these are equivalents group cells, and the lin-12 signaling between P6.p and P7.p and P6.p and P5.p instructs them not to acquire the primary fate; as a result, they acquire the secondary fate. And the ones that are farther away do not get any of this signal and therefore, they go to the default state to become hypodermis, which is due to the negative signaling by lin-15. So, as a result they become hypodermis. So let us see the anchor cell formation. So, there again, the anchor cell and its sister, they both are equivalence groups they are called Z1.AAA and Z4.PPP.

So AAA is the anterior anterior anterior great-granddaughter of Z1; similarly the PPP is the posterior posterior posterior, great-granddaughter of Z4 on the posterior side. So, those two cells are like this P7.p and P5.p, they are in identical situation.

(Refer Slide Time: 35:50)



Here one of them will become ventral uterine precursor cells that make the uterus, and the other will become the anchor cell. So, either one can acquire either one of the two fates; both can become anchor cell and ventral uterine precursor. So the student and mentor who figured out this did a lot of ablations, mosaic analysis. They found out the ligand and receptor both are produced by the both the cells. And finally they put together this model, that is, totally by random chance if one of them ends up producing more ligand, it ends up telling the other one to produce more receptors and produce less ligand.

So the initial difference happens purely by chance, then that difference is reinforced by feedback. So, here you see in the cartoon, both produce the receptor and ligand. If one is producing more of the receptor and the other one is producing more of the ligand, then this ligand producing cell is going to tell the receptor cell not to produce ligands instead produce receptor and that ends up in this difference.

Finally, one becomes anchor cell another becomes ventral uterine precursor. So, this is the model proposed to explain how fate determination might work among equivalence group cells via the Notch-Delta pathway in different context. This is probably how one cell becomes neuron another becomes glial if you think in vertebrate context. So, this brings us to the end of signaling pathways.