Eukaryotic Gene Expression: Basic and Benefits

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Lecture No. # 25

Signal transduction pathways involved in embryonic development

Welcome to this lecture series on this eukaryotic gene expression, basics and benefits. This is the 25th lecture; in this series, we have been discussing primarily, the role of eukaryotic gene expression or how gene regulation plays a very important role in the regulation of embryonic development.

In the previous class or in the last lecture, we looked at some of the major transcription factors; how, from a fertilized egg, as it is starts dividing, the maternal, maternal effect genes who the messenger (( )), which was stored in the fertilized egg. They get translated and most of these maternal effect gene products or transcription factors, they trigger the expression of zygotic genes and then, these zygotic genes then activate the expression of the fertilized egg, and then the differentiation program continues and the development proceeds.

And the take home point that we really got is that special and temporal expression of these genes play a very important role in the regulation of embryonic development. And we used drosophila as a model system to understand some of the aspects of the early development in drosophila.

Today, the focus of the lecture will be primarily on, how, what kind of signal transduction pathways operate in during embryonic development. In many of our previous lectures, we had actually discussed a number of signal transduction pathways, because we realized, that in order for the genes to be activated in the inside the nucleus, it is, the nucleus has to respond not only to signaling molecule, that are generated inside the cell, but it also has to respond to environmental queues or signaling molecules, which are generated outside the cell. And we discussed about a number of signal transduction pathways, wherein signaling molecules, either interact with specific membrane receptors and as a result of this membrane receptors signaling molecule interaction, a series of

phosphorylation cascades are activated, ultimately communicating either in the phosphorylation or dephosphorylation of transcription factors that in turn, activate or repress the transcription of various genes.

We also looked at signal transduction pathways, where molecules actually diffuse inside the cell, bind to specific intracellular receptors and then, this receptor ligand complex goes and binds to specific sequences, and activate the transcription of target genes. We looked at steroid hormones, as one of the examples.

So, these signal transduction pathways involving both signaling molecules, interact with membrane receptors, as well as, signaling molecules interact in the intracellular receptors also operate during embryonic development. And these signal transduction pathways play a major role in activation or repression or specific target genes, so that specific developmental patterns can be established during development. So, the signal transduction plays a very, very important role during development.

And what we will now study today is that, what kind of signaling molecules are generated during embryonic development and how these signaling molecules interact with either membrane receptors or intracellular receptors, and ultimately activate specific gene expression programs leading to harmonious regulation of development; this will be the focus.

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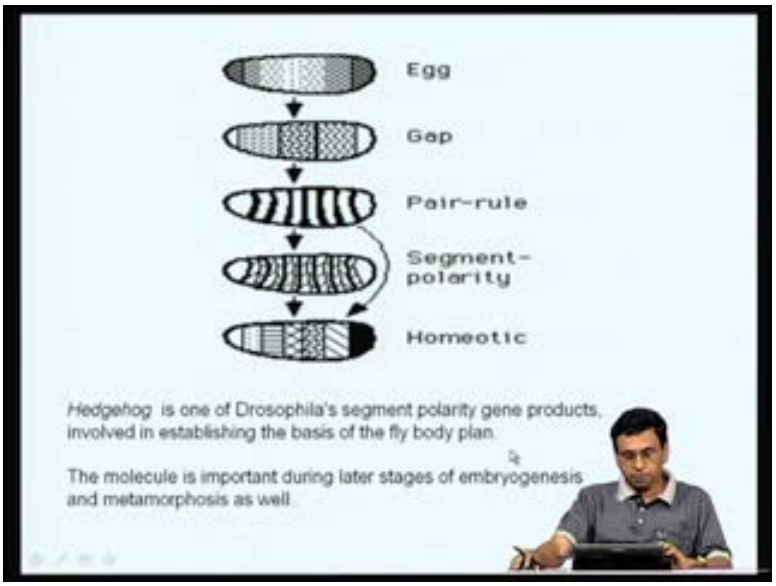

So, what we discussed in the previous class or in the last lecture is how a drosophila egg, to begin with a single nucleus, becomes multi-nucleate, a syncytial sub, since, what is called as syncytium, a multi-nucleate cell, and there, once the membranes are formed and all the nuclei migrate to the periphery of the nucleus and then, the membranes are formed and then the maternal expression, maternal effect genes are expressed, which are the gap gene or pair-rule genes and segment-priority genes, and as a result of expression of all these genes, ultimately the homeotic genes get activated and these homeotic genes ultimately, give an identity to the various segments.

So, we discussed various aspects of how the anterior-posterior polarity, as well as, the darso inter polarities, established during the embryonic development due to the integrated activities of a number of transcription factors, like dorsal, ventral, nanos and so on, and so forth.

The important point that we learnt in the last class is that the localization of RNA molecules play very, very important role. The gradient of transcriptions factors or the gradient of proteins or morphogens plays a very, very important role in the regulation of the embryonic development.

We studied examples, like the dorsal or nanos and how the levels of this transcription or these proteins in the anterior or posterior ends or in the dorsal or ventral ends are responsible for specific activation of target genes. So, the concentration of this proteins in a specific reason of the embryo plays a very, very important role in what kind of genes get activated or what kind of genes get repressed, and so on and so forth.

Now, what we will do today is to discuss in detail one of the segment polarity genes called as Hedgehog and ask the question how does this Hedgehog plays an important role in establishing the basis of the drosophila body plan.

This molecule of the hedgehog is important, not only during the early stages of development, but also during the later stages of development, metamorphosis, as well as, in the adult. So, we will discuss in detail, what is the importance of this Hedgehog signaling pathway and how this signaling pathway results in the activation or repression of transcription of specific genes, ultimately leading to establish (( )) specific embryonic development patterns.

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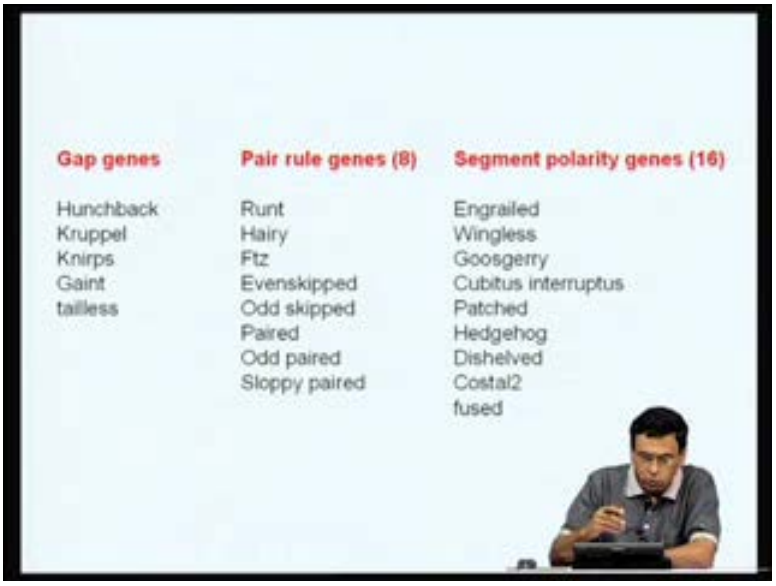

So, this is just a summary of what we discussed in last class. We discussed about, what are called as, the gap genes, which are the 1st genes to be expressed (( )) immediately after fertilization. Most of them are maternal effect genes, their RNAs are made in the (( )) development and deposited. This RNAs are deposited in the fertilized egg in different regions of the embryo and once the fertilization is over, these genes, these RNAs are translated and most of this RNAs code for transcription factors, which then activate the expression of this zygotic genes. We also looked at the pair-rule genes under segment polarity genes and as a result of the activity of all these genes, the segments, the number and the identity of the segments are established. There are about 8 pair rule genes and about 16 segment polarity genes in drosophila.

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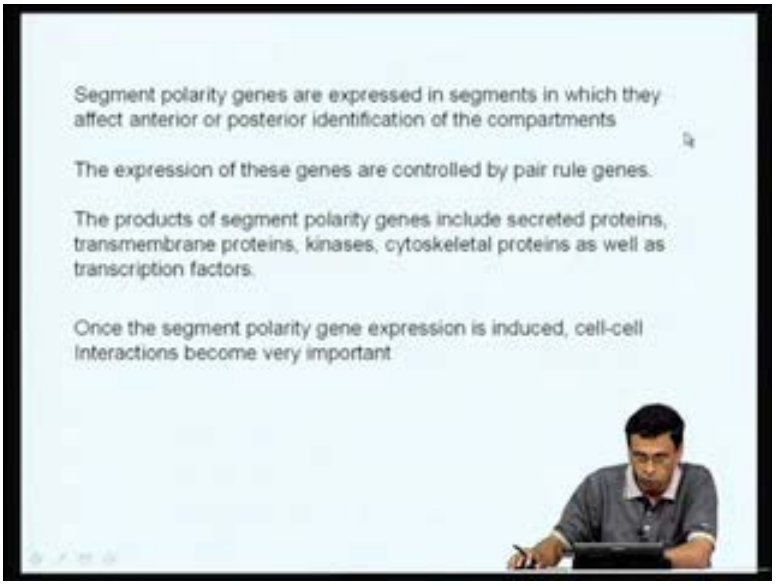

Now, the segment polarity genes are expressed in segments in which they affect the anterior or posterior identification of the various compartments. So, these genes play a very important role determined in the anterior structures and posterior structures in each segment and the expression of these genes are controlled by pair rule genes. So, the gap genes controlled expression of pair rule genes and pair rule genes controlled the expression of the segment polarity genes.

The products of the segment polarity genes include secreted proteins, transmembrane proteins, protein kinases, cytoskeletal proteins, as well as, transcription factors. So, it is not just the transcription factors, which play a very important role in during development, but a number of other kind of proteins, like transmembrane proteins, which are actually involved in the signal transduction pathways protein kinases, which either actually activate or repress the activity of various transcription factors, as well as, cytoskeletal proteins, which govern the movement of this proteins inside the cytoplasm from one end of the embryo to the another end of the embryo. All these things play a very important role during the development and a segment polarity genes code for all these classes of proteins.

Now, once a segment polarity gene expression is induced, cell-cell becomes very, very important. So, what we will discuss in this lecture is we will take 1 or 2 examples of this segment polarity genes and then see, how do they activate repressed transcription of

specific target genes and what is the effect of these gene expression programs on development, and we will also, at the later stage of this lecture, we will realize or we will try to discuss, how cell-cell interactions play a very, very important role in the development of a normal embryo.

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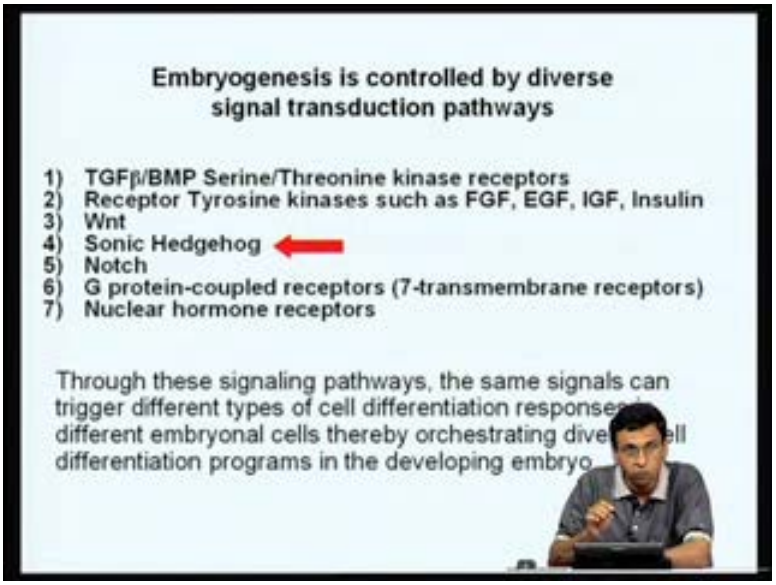

Now, the point you would like to make is that embryogenesis is controlled by diverse and multiple signal transduction pathway and it will be impossible for us to discuss all these signal transduction pathways in this lecture series, because the focus in this lecture series is primary to understand regulation of gene expression.

So, what I will do is to just take 1 or 2 examples of this various signal transduction pathways and discuss, how these signal transduction pathways influence gene expression and how these results in the, how it does affect the developmental programs?

For example, there can be a number of signal transduction pathways, which are essential for normal development. It could be mediated by the transforming growth factor beta, which involves serine/threonine kinase receptors, it can involve the receptor tyrosine kinases, such as fiberglass growth factor, epidemic growth factor, insulin growth factor, as well as, insulin.

It can involve the Wnt signaling; it can involve, what is called as, sonic hedgehog; it can involve a Notch signaling, several G protein-coupled receptors containing the 7-

transmembrane receptors, may play a very, very important role during development and ultimately, finally, the nuclear hormone receptors, especially morphogens, like thyroid hormone, retinoic acid play a very, very important role in the regulation of embryonic development.

So, in this class, we will try to focus only on 1 or 2 of these pathways, primarily I would like to discuss the sonic hedgehog, Wnt and Notch signaling as examples of how signal transduction pathways activation of these signal transduction pathways lead to activation or repression of specific transcription factors, and how these, in turn, regulate the expression of specific target genes involved in the embryonic development.

The important point, that we would like to (( )) emphasize in this discussion is that although these signaling pathways would appear to be diverse, each one of these signaling pathways may respond differently to signals emanating at different stages of development or in different cell types.

So, through these signaling pathways, the same signals can trigger different cell types, different types of cell differentiation responses in different embryonal cells, thereby orchestrating diverse cell differentiation programs in the developing embryo. So, the particular signaling pathway, for example, we take Wnt signaling pathway, it may activate a set of genes in one particular cell type of the embryo, but the same Wnt signaling pathway may activate a different set of genes in different cell types of embryo. So, a combination of signaling events ultimately decide, what kind of gene expression programs are actually activated by each one of the signaling pathways, and the same signaling pathway may be involved in the activation of multiple sets of genes in different cell types of the developing embryo.

So, let us now begin our discussion with discussing, what is this Sonic hedgehog and what kind of signal transduction pathway, is involved in this by a, involves this sonic hedgehog.

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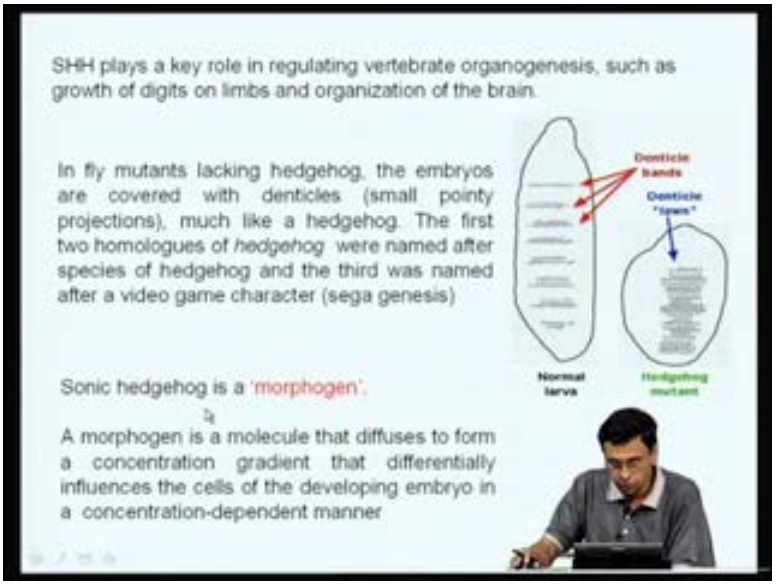

The hedgehog family actually consists of (( )) sonic hedgehog, now abbreviated as SHH, desert hedgehog or DHH and Indian hedgehog or IHH and they are all belong to the same family members. Among these, the sonic hedgehog or SHH plays a very important role in regulating vertebrate organogenesis, such as growth of digits or limbs, as well as, organization of the brain.

Now, in the drosophila if the SHH is mutant, if the hedgehog is mutated or if fly mutants, they lack hedgehog. The embryos are covered with denticles, which are nothing but small pointed projections, much like a hedgehog.

Now, you know, hedgehog has these projections of the surface of the body. If you have mutations in this hedgehog gene, you get this kind of denticles, you can say the difference here is a normal larva in which the hedgehog is normal, but have a mutant in which the hedgehog is mutated and you can see, this denticle, long kind of a thing, so small projections on this wing and that is why, the name hedgehog was for this particular mutant phenotype. So, the fly mutants lacking the hedgehog, the embryos are covered with the denticles, which are nothing but small pointer projections, much like a hedgehog.

The 1st of the 2 homologues of hedgehog were named after the species of hedgehog and the 3rd was named after a video game character. Therefore, these are all, many times when you study some of these developmental regulation and many of the genes have many crazy or fancy names. So, we do (( )) worry, sometimes there is a rational and sometimes there is just, you, it is named after some, for example, a video game character or a particular phenotype, and so on and so forth. So, let us not worry about the naming here.

Now, sonic hedgehog is a morphogen, what is meant by morphogen? A morphogen is a molecule that diffuses to form a concentration gradient, that differentially influences that the cells of the developing embryo in a concentration-dependent manner.

We have seen many of these morphogen in the previous lecture, for example, the dorsal or nanos these are all called as morphogen because they are made in one particular place and then, they slowly diffuse into the other regions of the embryo, and depending upon there is a concentration, the physiological process can be different, different genes may be activated or different developmental patterns may be established, and so on and so forth.

So, a morphogen is a molecule, that diffuses from the place of its synthesis to form a concentration gradient across the embryo and this concentration gradient differentially influences the developing embryo by activating or depressing different target genes in a

concentration dependent manner; that is the important thing. So, sonic hedgehog is also one such morphogen.

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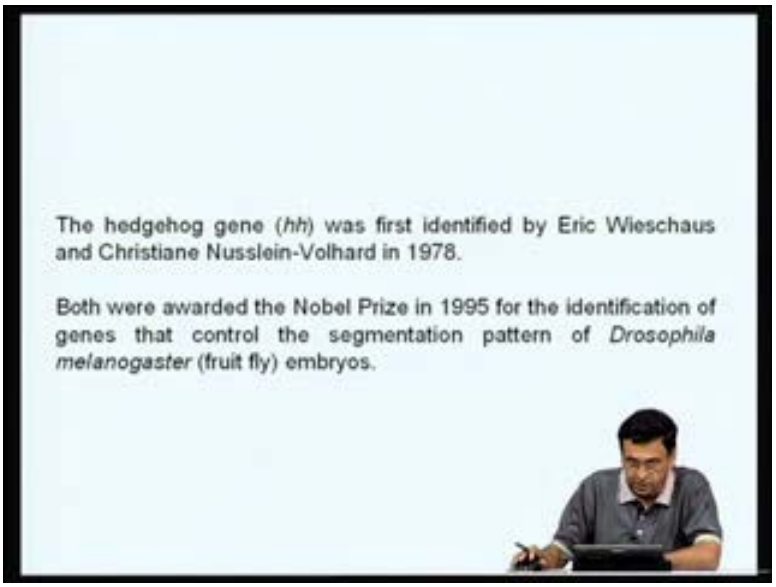

Now, the hedgehog gene was first identified by Eric Wieschaus and Nusslein-Volhard and for this way back in 1978, and they were actually doing a, doing a number of mutagenesis (( )) to identify mutations, which actually affect various developmental programs and one such mutant they identified was the hedgehog. Now, for their work on the developmental regulation or identification of genes, that controlled a segmentation pattern of drosophila, they were awarded Nobel Prize in the year 1995.

So, identification of genes that controlled the developmental pattern drosophila has won a number of Nobel Prizes, including the one that is stated here.

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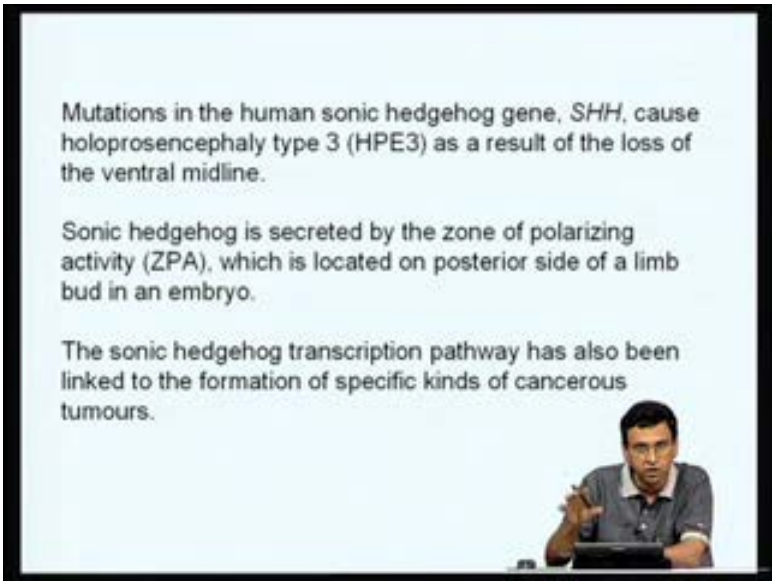

Now, the mutations in the human sonic hedgehog gene, SHH, cause a particular genetic disorder called holoprosencephaly type 3 as a result of the loss of the ventral midline, so it can manifest the loss. When you have mutations in the sonic hedgehog, it can manifest as specific diseases.

Now, the sonic hedgehog is secreted by what is called the zone of polarizing activity or ZPA. We will discuss this later when we start discussing about the mechanism by which a retinoic acid influences gene expression. Right now, just suffice to know, that the sonic hedgehog is secreted by the zone of polarizing activity, which is actually a region located on the posterior side of a limb bud in the embryo.

The sonic hedgehog transcription pathway has also been linked to the formation of specific kinds of cancerous tumors.

Many of these signaling pathways, that we are going to discuss today, play a very important role, not only in the development, but they also play a very, very important role in the adult as well. And when you have mutations in these, in even in these signaling pathways in the adult, it can result a number of diseases, including cancer. So, all these transcription factors and all these signaling pathway, that we are going to discuss in development regulation also play very, very important role with the normal adult life, and if we have problems in these or mutations, it can lead to disease like cancer.

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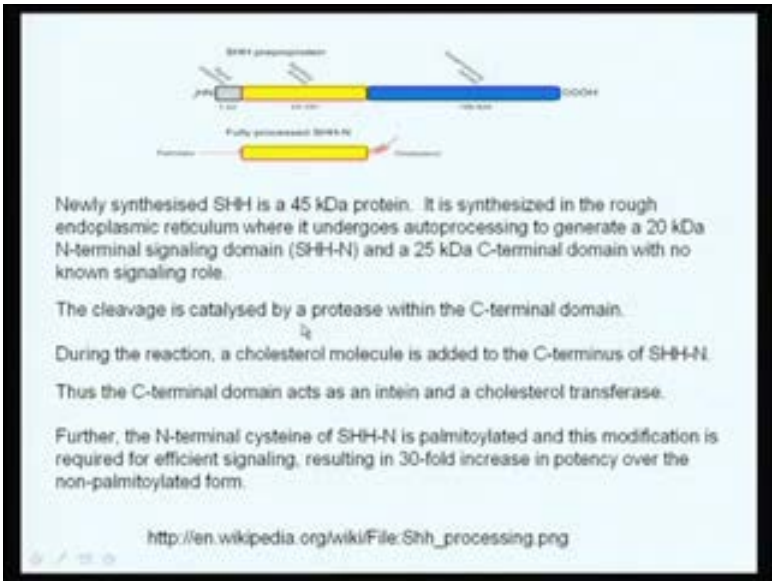

Now, let us now try to understand, what is this protein? What is this sonic hedgehog and what kind of a protein it is and where it is synthesized? Now, here is the schematic diagram of the sonic hedgehog protein this (( )) synthesize is a pre-protein, which consists of the (( )) responsible for a synthesis is shown in the yellow and blue here, and the newly synthesized, the sonic hedgehog protein is a 45 kilo dalton protein. It consists about 424 amino acids and it (( )) it is a secretary protein and as all secretary proteins are synthesized in the rough endoplasmic reticulum. As most of you are aware, all this messenger RNA, that are code for the signaling secretary protein, they are synthesized, they contain, what is called as, a signal peptide sequence in the amino terminus, it is about 20/23 amino acids sequence.

This signal peptide recognized were protein, called as signal recognition particle and then, this nascent polypeptide along with the RNA is now recognized by the SRP and then, this SRP takes this RNA along with the nascent protein to the ribosomes present on the rough endoplasmic reticulum. And then, the RNA gets translated the (( )) inside into the lumen of the rough endoplasmic reticulum and thus, all the secretary proteins in this takes place on the rough endoplasmic reticulum.

Hedgehog is also a secretary protein and therefore, it also contains a scenic signal peptide at the amino terminus and signals peptide is ultimately, cleaved near the rough endoplasmic reticulum.

So, it is synthesized, as a rough, in the rough endoplasmic reticulum, where it undergoes auto processing to generate a 20 kilo Dalton N-terminal signaling domain, is called as a SHH-N, shown in the yellow here, as well as a 25 kilo Dalton C-terminal domain, which so far does not have any signaling role. So, the SHH protein is synthesized, is a pre-protein, it is first synthesized in the rough endoplasmic reticulum. The N-terminal pair signal (( )) gets cleaved and then the remaining protein consists of 2 different domains. It has an autoproteolytic activity and it cleaves somewhere in the middle, so that you get a N-terminal region of about 25 kilo Dalton protein, and it is this internal region, which is very, very important for the signaling transduction properties of this protein.

The cleavage is catalyzed by a protease within the C-terminal domain, and during this reaction, a cholesterol molecule is added to the C-terminus of the SHH-N. So, the protein gets polycleaved in somewhere in the middle here and the blue region is removed and to the carboxyl terminal amino acid, a cholesterol biotin is attached during the processing of this protein in the endoplasmic reticulum.

The C-terminal domain therefore, acts as an intein, as well as, cholesterol transferase. So, it has a catalytic activity. The N-terminal cysteine of SHH-N also undergoes, what is called, a palmitoylation and this palmitoylation plays a very, very important role in the signaling of the properties of this protein. And if you compare the activity or the activity of this protein in signal transduction, the palmitoyleted protein has at least 30 times more activity than the non-palmitoylteted prom.

So, the sonic hedgehog protein is synthesized in the rough endoplasmic reticulum, following a synthesis, it undergoes an autocatalytic proteolytic cleavage, the C-terminal domain is also removed, a cholesterol moit is added to the C-terminal end and a palmitoyl group is added to the amino terminal end, and then, this protein comes out into the extracellular region.

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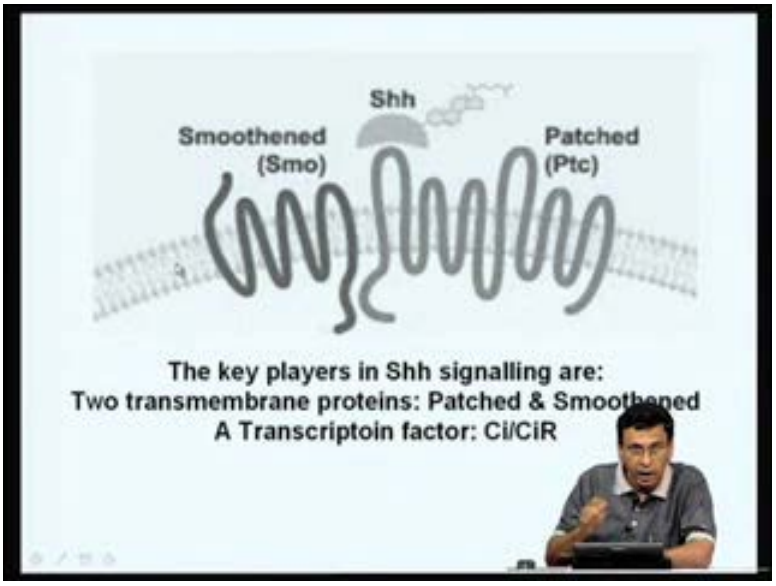

Now, what happens, once the sonic hedgehog comes out of the cell, what does it do? It goes and binds to a transmembrane protein called as patched. There are at least 3 important proteins, play a very important role in the trans-signal transduction pathway, mediated by sonic hedgehog, one of them is called as transmembrane, called as patched, another is called smoothened and a transcription factor called Ci or CiR. So, these are the 3 major players that are involved in the signal transduction by sonic hedgehog.

So, the sonic hedgehog first binds to the patched transmembrane receptor. And let us now try to see, what happens when the sonic hedgehog binds to the patched receptor and what are the function of the other transmembrane protein?

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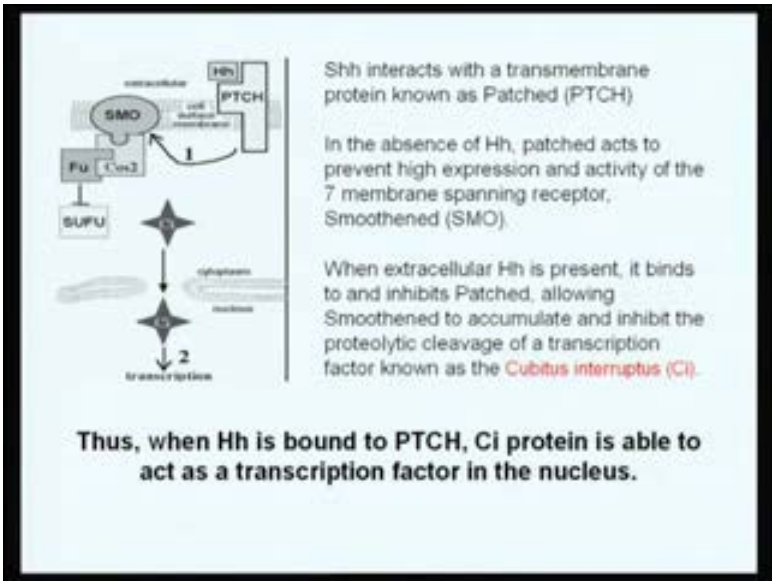

Now, once the sonic hedgehog binds to this patched receptor or the transmembrane protein, in the absence of…, Let us now first see, what happens when there is no sonic hedgehog xydinal transduction.

Now, let us assume, when sonic hedgehog is not there, in the absence of the sonic hedgehog, this receptor, the PTCH are the patched receptor, acts to prevent high expression activity of the 7 membrane spanning receptor, called the assemble and therefore, in the absence of the sonic hedgehog, the patched actually prevents the expression and activity of the SNO. So, the SMO levels would be very, very low.

And when extracellular hedgehog is present, it binds to and inhibits the activity of Patched and therefore, the levels of the smoothened goes up in the cell and this move now accumulates and inhibits the proteolytic cleavage of a transcription factor, known as Cubitus Interrupts or Ci.

So, when SMO is present in high levels, it inhibits the proteolytic cleavage of a transcription factor called Ci, therefore the Ci can now go inside the (( )) nucleus and can activate the transcription of specific target genes. Whereas, when hedgehog is absent, the levels of SMO is very, very low and therefore, it cannot prevent the proteolytic cleavage of the Ci transcription factor. Therefore, the Ci gets degraded in the cytoplasm and therefore, the target genes for Ci cannot be activated.

So, this is how the sonic hedgehog controls gene expression during the development. So, when Hh or the hedgehog is bound to the PTCH or the patched receptor, the Ci protein is now able to act as a transcription factor inside the nucleus.

So, in the absence of Ci hedgehog, no transcription takes place in the presence of hedgehog because the proteolytic cleavages of Ci is inhibited, Ci can go inside the nucleus and activate the transcription of target genes.

So, when the sonic hedgehog comes and binds the patched receptor, it results in the accumulation of synthesis or accumulation of the SMO protein. This SMO protein now inhibits the proteolytic cleavage of the Ci transcription factor. Therefore, Ci can now go inside the nucleus and activate the expression of various target genes.

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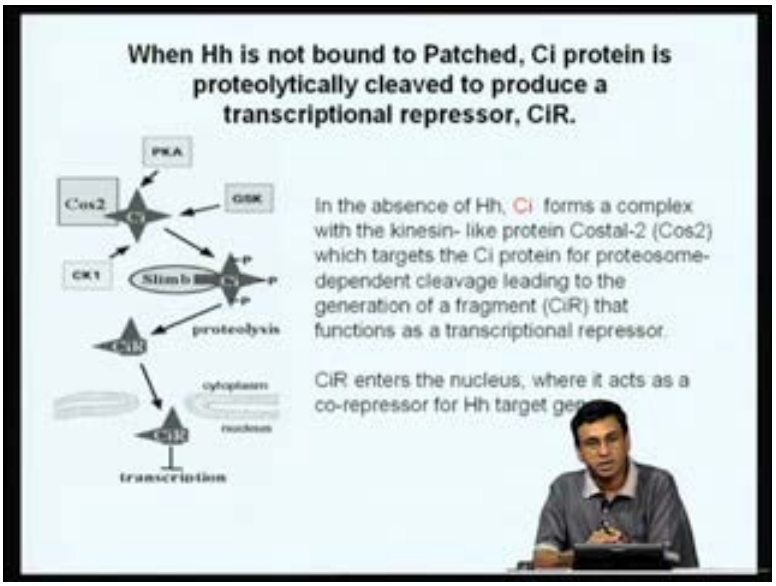

Now, when hedgehog is not bound to patched, the Ci protein is proteolytically cleaved to produce a transcriptional repressor, called CiR.

The various place in the proteolytics of the CiR shown here, we will not go into the details, but suffice to know, that in the absence of the hedgehog, the Ci forms a complex with the kinesin like protein, called Costal-2, which targets the Ci protein for a proteosome dependent cleavage leading to the generation of a fragment called as CiR, and this fragment of the Ci protein functions as a transcriptional repressor. So, this CiR

now goes inside the nucleus, where it acts as a co-repressor for Hh target genes or the hedgehog target genes.

So, there are 2 things, that is happening, when hedgehog is present the Ci protein function as a transcription activator. It goes inside the nucleus and activates the transcription of its target genes.

When the hedgehog is not present, the Ci activator is actually proteolytically cleaved and a repressor is generated from the Ci protein. Now, this repressor goes and then binds to the acts as a co-repressor and suppresses the activation of various target genes of the hedgehog signaling pathway.

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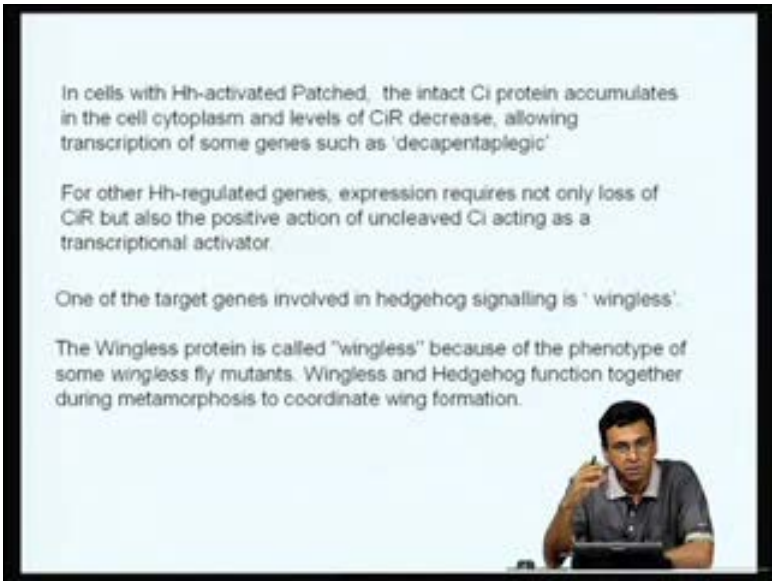

So, in cells with hedgehog activated patched, the intact Ci protein accumulates in the cell cytoplasm and levels of the CiR decrease, allowing transcription of the hedgehog specific genes, such as decapentaplegic, and so on and so forth. For other the, or for many other hedgehog regulator genes, expression requires not only the loss of the CiR or the Ci repressor, but also the positive action of the uncleaved Ci acting as a transcriptional activator.

So, for activation of some of these hedgehog target genes, the removal of repressor is good enough, but for many other genes, the synthesis or the nuclear localization of the activator is also very essential.

So, one of the target genes, which are involved in hedgehog signaling is called wingless. That is a very, very important protein, so we are going to spend some time to understand, what is this interplay between wingless and the hedgehog?

The wingless protein is so called, because the phenotype of some of the wingless, wingless fly mutants, they do not, basically do not develop wings. So, the wingless and hedgehog function together during metamorphosis to form a, to form the wings of the drosophila fly.

So, one of the important target genes or the hedgehog signaling pathway is called as wingless and both wingless and hedgehog work together to the formation of the wings during drosophila development.

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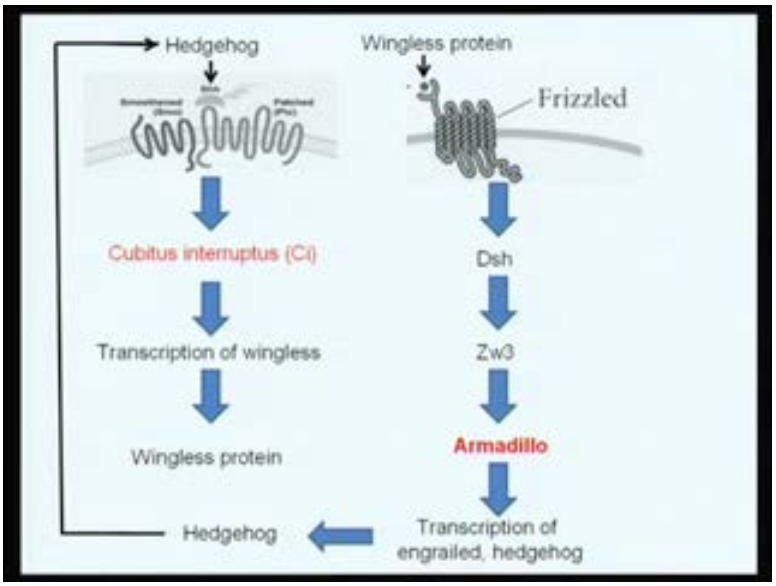

Let us now try to understand, what exactly happens in the hedgehog signaling pathway. As I told you, when hedgehog binds to the patched receptor, it results in the activation of cubitus interruptus transcription factor

One of the target genes of this cubitus interrupts transcription factor is wingless. Now, the wingless protein, that so synthesized now is secreted out, now. It goes and binds to another receptor called as frizzled of the adjacent cells. The binding of this wingless protein to the receptor called as Frizzled now triggers the activation of a protein called as

Dsh, which in turn activates another protein called as Zw3. These ultimately results in the activation of a transcription factor called Armadillo.

The transcription factors in the entire pathway are shown in red; in one case, the hedgehog signaling results in the activation of a transcription factor called Ci; the wingless protein signaling results in the activation of transcription factor armadillo and the armadillo goes inside the nucleus and activates the transcription of proteins called engrailed and hedgehog. And you can see now, the hedgehog again now goes out and then again goes and binds to SSH, and then keeps the wingless levels high.

So, you can see this interplay of the hedgehog and wingless signaling pathways here. The binding of hedgehog to the receptor results in the synthesis of wingless protein and when wingless goes and binds to a cell surface receptor, that results in the synthesis of the hedgehog protein, which again goes and binds and then the wingless is maintained. So, this is how the hedgehog and wingless signal see through an auto regulatory group, keep maintaining each other several constant in the adjacent cells.

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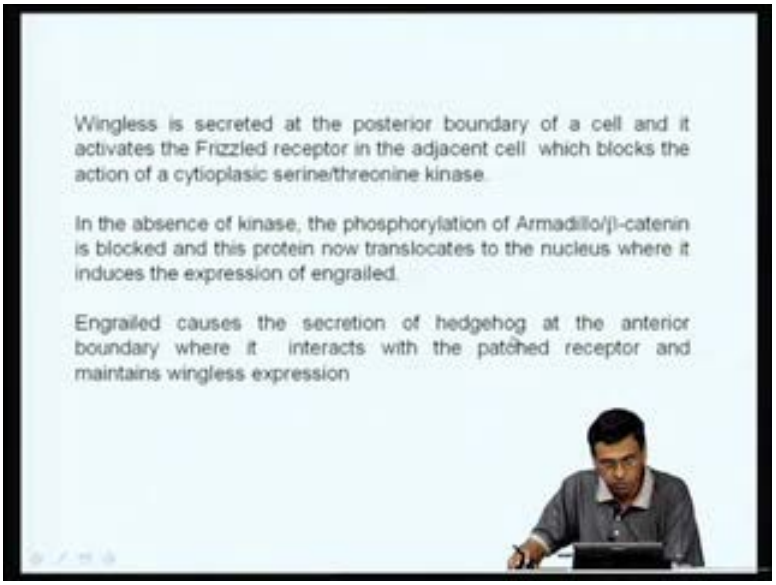

So, the wingless is secreted at the posterior boundary of a cell and it activates the Frizzled receptor in the adjacent cell, which blocks the action of a cytioplasic serine threonine kinase.

In the absence of a kinase, the phosphorylation of the Armadillo in drosophila, this protein is called as Armadillo in (( )) cells, it is called as beta-catenin, both are more or less same, they have similar functions, so in the phosphorylation of this, proteins is blocked. This protein now translocates into the nucleus where it acts as transcription factor and induces the expression of the engrailed, and so on and so forth.

So, basically what happens, when wingless interacts with the wave f t c receptor, a phosphorylation of a transcription factor is blocked and as a result, this can facilitate the nuclear nuclise on the transcription factor and this is in the activation of the Engrailed.

Now, the engrailed protein, once it is made, it causes the secretion of the hedgehog at the anterior boundary, where again it interacts the patched receptor and again activates the expression of wingless and then, wingless goes and activates, make sure that hedgehog levels are maintained.

So, you can see, how nicely 2 molecules, 2 secretary molecules, one case wingless another case hedgehog, both of them interact with their respective membrane receptors and when hedgehog interacts with this cell membrane receptors, wingless is synthesized and when wingless interacts with its membrane receptor, hedgehog is synthesized and this kind of an auto regulatory loop continues.

So, very nice example to see how 2 molecules activating 2 signal transduction pathway, kind of, help each other, so that their protein synthesis can continue during the embryonic development.

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Now, what happens when you have mutations in this sonic hedgehog? As I told you, many of these are very, very important signaling molecules and when you have mutations, you get very, very abnormal development and here is an example, what happens when you have mutation in this sonic hedgehog. And although, we are studying many of this drosophila as an example, all this pathways are also conserved in mammals as well as humans.

Now, in the case of mammals the sonic hedgehog is involved in the separation of the single eye field into 2 bilateral fields. So, till then, you have only 1 univision and once the sonic hedgehog is resem to be expressed, you get a bilateral vision.

And Shh actually produced from the prechordal plate and suppresses a protein, known as Pax6, and this result in the division of the eye field into 2 during embryonic development. And if you have mutations in the Shh gene, it results in cyclopia, a single eye in the center of the face and here is the phenotype.

So, it is having 2 eyes, you have 1 eye in the middle of the head. So, the sonic hedgehog plays a very, very important role in the eye development and there is a very nice paper in nature, which actually, discusses the various phenotypes, that you get when you have mutation in sonic hedgehog, named as cyclopia and defective axial patterning in mice lacking sonic hedgehog gene function. Now, they actually targeted deletion of the sonic hedgehog and what is the effect of this deletion of this sonic hedgehog in the various

phenotypes. So, you can have very drastic phenotypes when some of these proteins are mutated.

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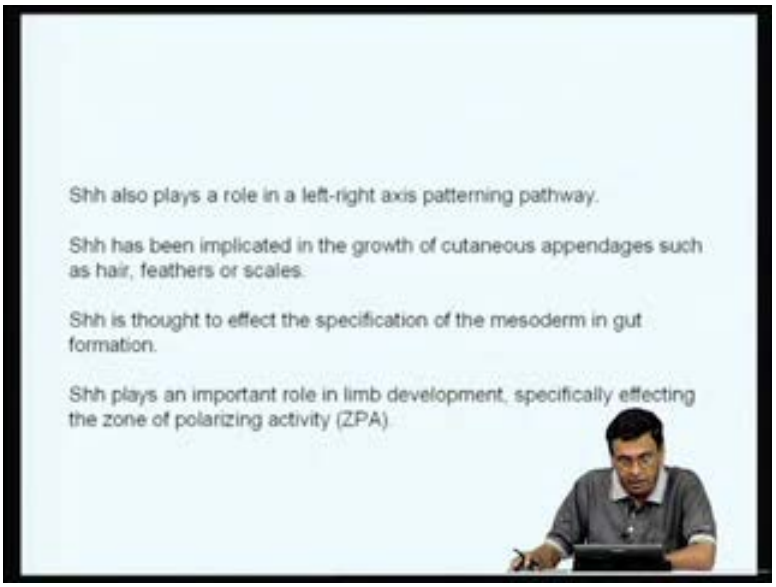

In addition to this cyclops kind of a phenotype, the sonic hedgehog also plays a very important role, a number of other developmental processes as well. For example, the sonic hedgehog plays a very important role in the left-right axis patterning, determine the left or right, which organs have to be in the left or which has to be in the right. The sonic hedgehog also has been implicated in the growth of cutaneous appendages, such as hair, feathers and scales; we will discuss little bit more in detail later. The sonic hedgehog also affects the specification of the mesoderm in the gut formation and it also plays a very important role in the limb development, specifically affecting the zone of polarizing activity.

Let us now first see, how the sonic hedgehog affects the limb development. So, we have just examined, how mutation in sonic hedgehog effects the eye development, we have only 1 eye of 2 eyes, when we have mutations in this protein and let us see, how does it affect the limb development.

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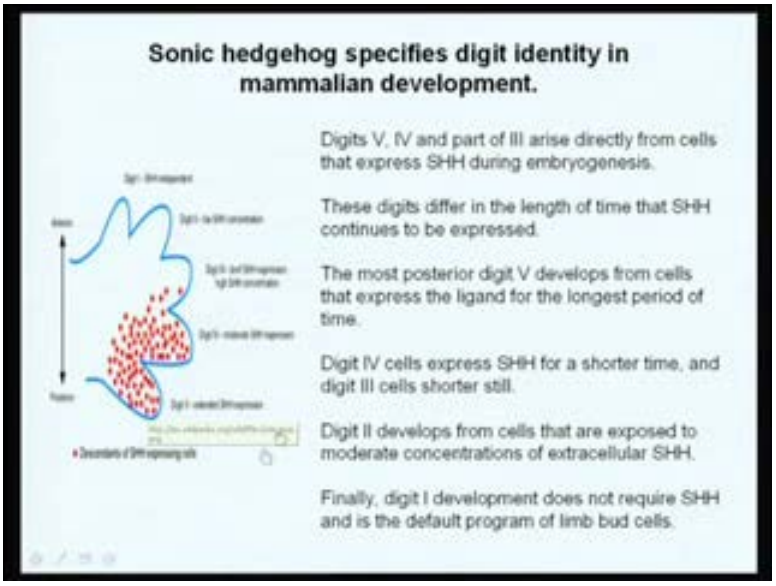

And we all know that we have 5 digits and sonic hedgehog actually, specifies the digit identity in mammalian development. And you can see here, the levels of sonic hedgehog in a developing limb is shown as red dots here; the 5 limbs are shown here and you can see, the digits 5, 4 and 3 arise directly from cells, that express sonic hedgehog during embryogenesis.

So, of the 5 digits that we have, digits 5 4 and 3 arise from regions where the sonic hedgehog is present in higher levels. These digits defer in the length of time, that sonic hedgehog continues to be expressed. So, the level, as well as, the length of time, the sonic hedgehog expresses in these 3 digits, varies.

The most posterior digit 5 develops from the cells that expressed the ligand for the longest period of the time, that is, this is digit 5, so it has, in this digit 5, the sonic hedgehog is expressed in the longest time and that becomes digit 5. The digit 4 cells express sonic hedgehog for a shorter time and the digit 3 cells express still shorter. The digit 2 develops from cells, that are expressed to very moderate concentrations of extracellular sonic hedgehog, whereas the digit 1 development does not require sonic hedgehog and therefore, is a default program of limb bud cells.

So, here is an example, you can see, how the levels and duration of expression of the hedgehog determines what kind of limbs are generated. So, the limb 5, 4, 3 require hedgehog. The limb 1 does not require limb hedgehog at all, whereas the limb 2 requires

very, very small concentrations of hedgehog. So, the levels, as well as, the duration of expression of hedgehog play a very important role in the development of the various limbs.

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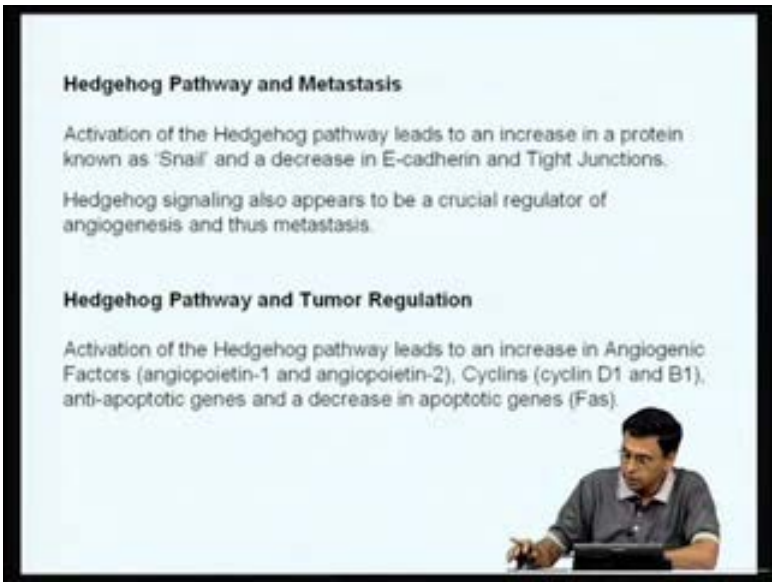

Now, in addition to its role in development, the hedgehog pathway also plays a very, very important role in the adult as well. And if you have mutations in the hedgehog pathway, it can result in cancer. For example, activation of hedgehog pathway leads to an increase in a protein, known as Snail and a decrease in a protein called as a E-cadherin and as well as, Tight Junctions. And as a result of these, these regulator genes, when you have mutation, the hedgehog pathway, it can play a very important role in cancer because the hedgehog signaling pathway plays a very important role in angiogenesis, as well as, metastasis. Both are very important for the tumor formation, as well as, migration. Angiogenesis is very important for tumor cells to get the nutrients and metastasis is very important for the cancer cells to move from one place to another. So, hedgehog pathway plays a very, very important role in cancer metastasis.

The activation of hedgehog pathway leads to an increase in the Angiogenic factors, known as angiopoietin-1 and angiopoietin-2, as well as, Cyclins, which are involved in cell cycled regulations, especially cyclin D1 and cyclin B1, as well as, anti-apoptotic genes, as well as, decrease in the apoptotic genes, such as Fas.

So, the take home message is that the hedgehog pathway not only plays a very, very important role in the embryonic development, but it also plays a very important role in the adults. And if they have mutations in the hedgehog pathway, it can develop into cancer and promote metastasis.

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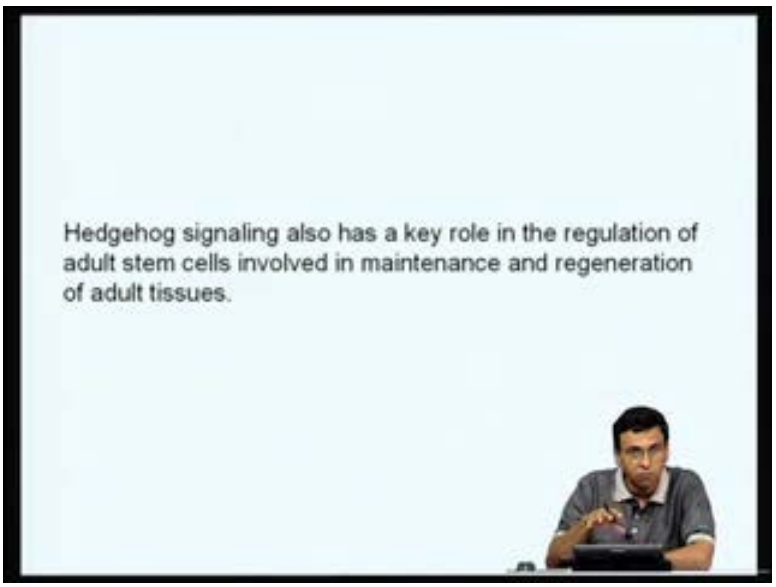

So, the hedgehog signaling also plays a very important role in the regulation of adult stem cells involved in the maintenance and regeneration of the adult tissues. As you can see, not only cell proliferation and cell differentiation is required in development, but it also requires during the adult. For example, in the bone marrow, the bone marrow stem cells are continuously well generated and they have to differentiate into various blood cell types. Similarly, if you take hair for example, there is in the dermis and epidermis, the hair fall, it is continuously being generated and it has to be differentiated and become a hair follicle.

So, there are many such places, where you require continuous proliferation and cell differentiation programs. Many of these, which play a very important in development, also play important role in these kinds of process during the adult life.

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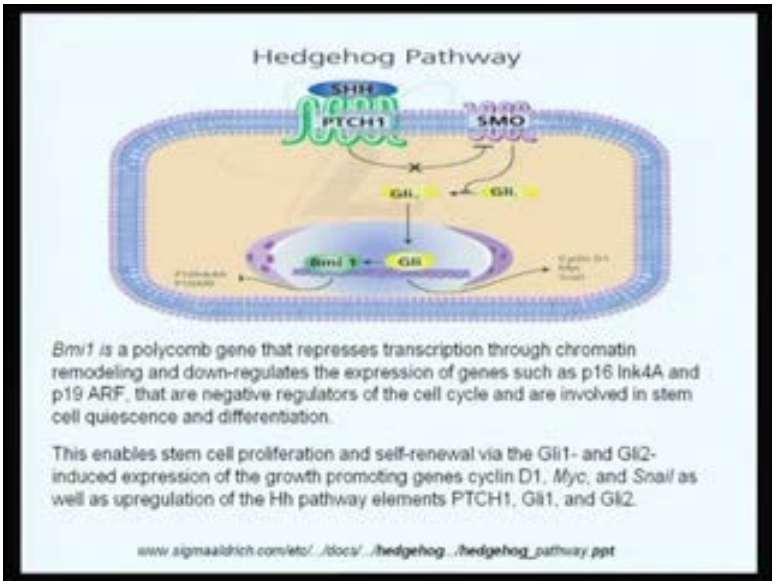

For example, this is a cartoon to show, how a protein called as Bmi 1, which is nothing but a polycomb gene, which actually represses transcription through chromatin remodeling and down regulates the expression of genes, such as p16 Ink4A and p19 ARF, which actually (( )) negative regulators of the cell cycle and are involved in stem cell quiescence and differentiation. And how these kinds of important proteins, which are involved in the stem cells differentiation play or activated through the sonic hedgehog pathway.

So, the activation of the sonic hedgehog pathway results in the activation of this Bmi protein, which is nothing but a chromatin remodeling protein. So, these kinds of processes enable the stem cell proliferation and renewal or via proteins called as Gli 1 and Gli 2 and when these proteins induce the expression of the growth promoting gene, such as cyclin D 1, Myc, as well as Snail, as well as upregulation of the Hh pathway elements, like PTCH1, Gli1 and Gli2.

So, basically, trying to say hedgehog pathway also involved, is involved, in the activation of genes, which encode chromatic remodeling proteins and they, in turn, affect the functions of stem cells, as well as, during the cell cycle regulation.

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So, the hedgehog pathway has been implicated in the development of many cancers and therefore, many pharmaceutical companies are actually involved in developing anti-cancer drugs that specifically target hedgehog signaling pathway, so that you can block this hedgehog signaling pathway and see, whether can prevent the progression of the cancer cells.

Either number of papers, for example, as recently as 2009, there is the paper, which says, hedgehog signaling as target in cancer stem cells in current clinical translation on oncology, and also it is very nice paper in current opening investing drugs about how hedgehog pathway can be used as a drug target in cancer therapy. So, we will not go into the details of how hedgehog pathway, although originally identified as an important pathway required for development, is also now being targeted for, as a drug target for treatment of various cancers.

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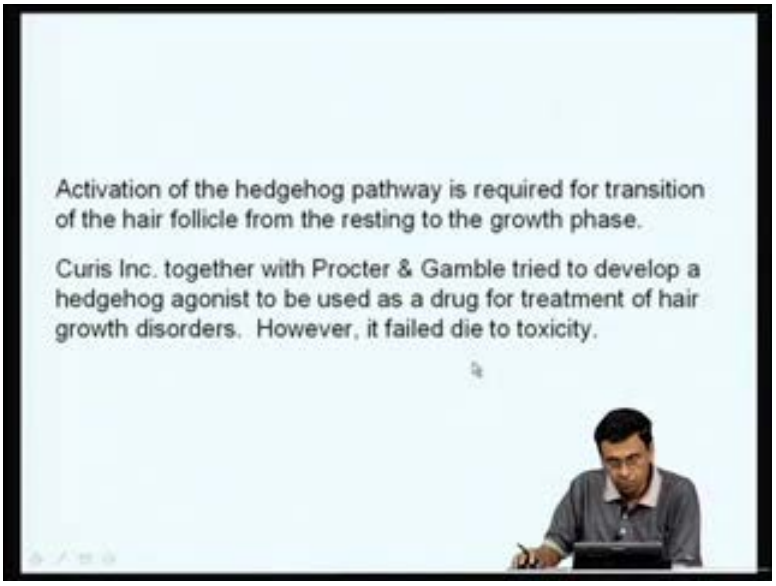

The activation of the hedgehog pathway is required for the transition of the hair follicle from the resting to the growing phase and companies like Curis as well as Procter and Gamble actually tried to develop a hedgehog agonist to be used as a drug for the hair growth disorders, you know, like baldness and so on, and so forth.

So, hedgehog signaling is involved in the growth and differentiation of the proliferation of the hair follicles and people want to understood, that sonic hedgehog is involved in the hair growth and differentiation. People thought, can we, actually, people who do not develop hair, who have baldness can, now if they had agonist of hedgehog, can you now stimulate the hedgehog pathway and promote hair growth and hair differentiation. These for all, some of the company, but problem is when you use such agonist of which against the sonic hedgehog, since sonic hedgehog plays an important role, not only in hair differentiation and hair follicle proliferation, but also in other possesses, it actually found to be toxic and as a result, the whole program have to be abandoned. So, although, this agonist work, but because of the toxic side effects, they have to be abandon this project.

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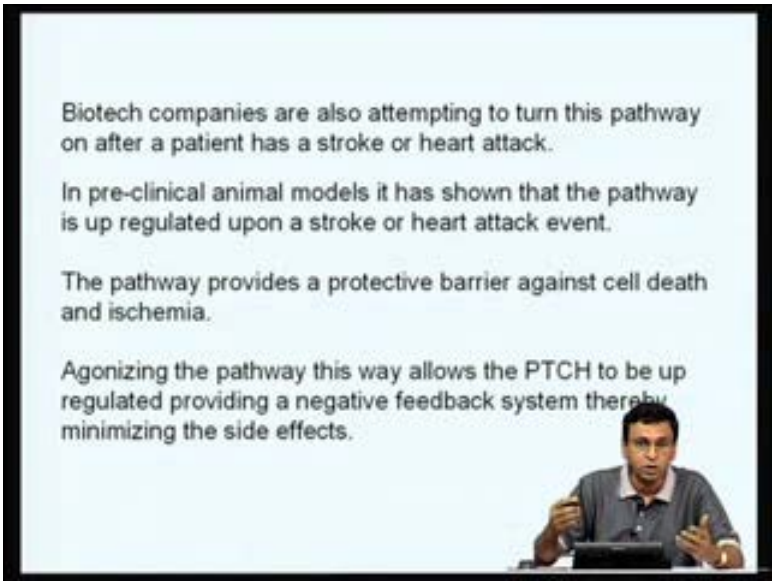

So, biotech companies are also attempting to turn this hedgehog pathway on after a patient, has a, has a stroke or heart attack. So, you can see, this hedgehog pathway seems to have a number of pleiotropic functions: it is involved in development regulation; it is involved angiogenesis; it is involved in cancer; it is involved in hair follicle proliferation, differentiation, and so on, and so forth.

So, in preclinical animal models, it has been shown, that the pathway is up regulated whenever somebody suffers a stroke or a heart attack, and this pathway actually provides a protective barrier against cell death and ischemia, myocardial ischemia. So, agonizing the pathway, this way, allows the PTCH or the patch receptor to be up regulated providing a negative feedback system, thereby minimizing the side effects. So, you can see, understanding the function of the sonic hedgehog not only during development, but also in the adult tissues, can lead to the development of drug molecules and which can be used either as agonies or antagonist, so that you can have multiple therapeutic benefits.

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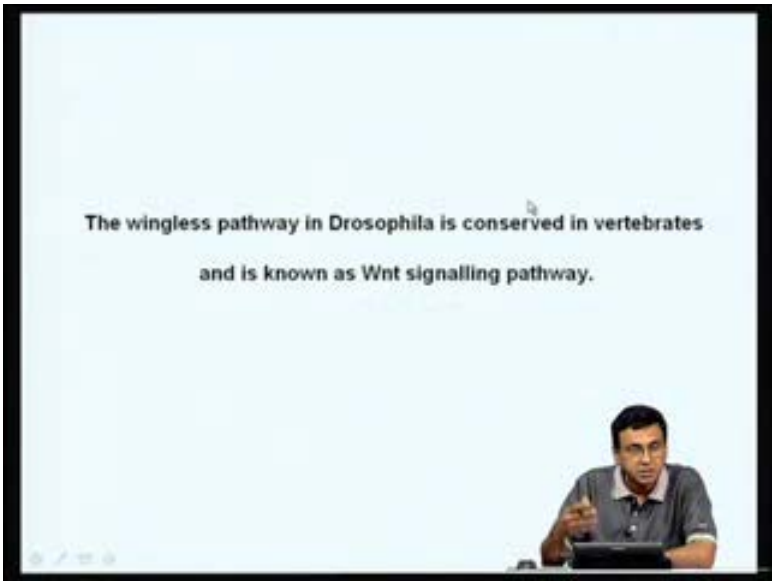

Now, we discuss, that how hedgehog, activate the hedgehog signaling pathway results in the synthesis of wingless and how wingless again binds to its frizzle receptor and again result synthesis of hedgehog, and how this loop is maintained? Now, the wingless pathway, which we discussed so far, the drosophila is actually, which conserved, not only in drosophila, but also in the vertebrates as well, and in the vertebrates, the wingless pathway is actually known as the Wnt signaling pathway. So, let us now try to understand, what is this wind signaling pathway in the vertebrates?

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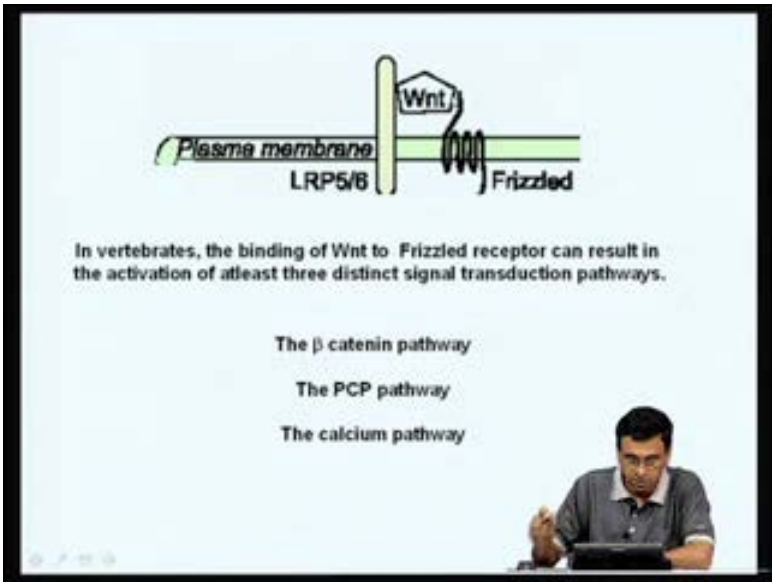

Now, in the vertebrates, it is much more complicated and binding of Wnt. So, Wnt is nothing but wingless, is in the drosophila. The binding of the Wnt to the frizzled receptor can result in the activation of at least 3 distinct signal transduction pathways while in the drosophila, we have shown in only 1, which involves the beta-catenin pathway. Now, in the case of vertebrates, there are at least 3 pathways, which are activated when Wnt binds to frizzled receptors.

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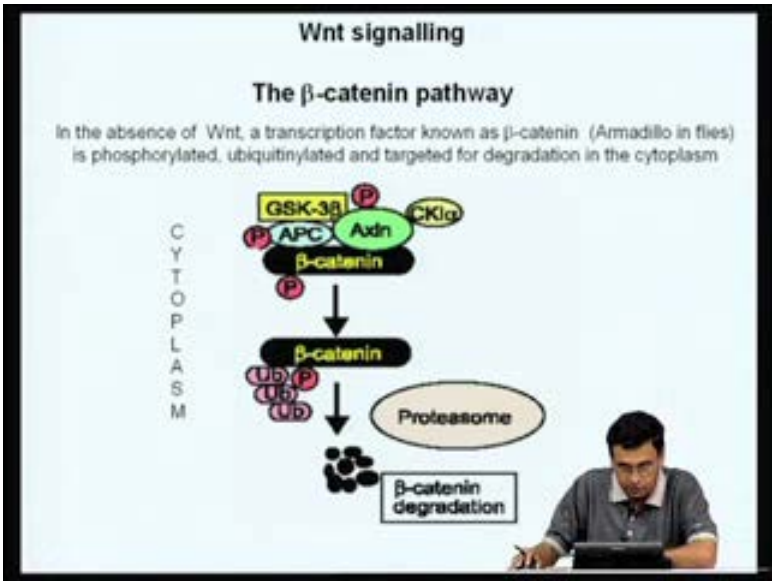

Now, let us see the 1st one. What is the beta-catenin pathway? The beta-catenin is nothing but the same as an armadillo, which we discussed, in the, just a few minutes ago in the drosophila. So, what is known as armadillo? As you know, when the phosphorylation of the armadillo is blocked by the activation of the wingless pathway (( )) armadillo goes inside the nucleus and then, activates the transcription of the engrailed, leading to the synthesis of the hedgehog protein.

Whereas, in the case of vertebrates, the armadillo homolog is called as the beta-catenin and you can see, when there is no Wnt signaling, when the Wnt does not bind to the frizzle receptor, now this beta catenin, which is nothing but a transcription factor is present in a multi-protein complex and phosphorylation of these proteins associate a beta-catenin results in the form for inactive complex and these phosphorylated form of this beta-catenin associated proteins, these targets the beta-catenin to a ubiquited

degradation by (( )) proteasome pathway and therefore, beta-catenin undergoes degradation.

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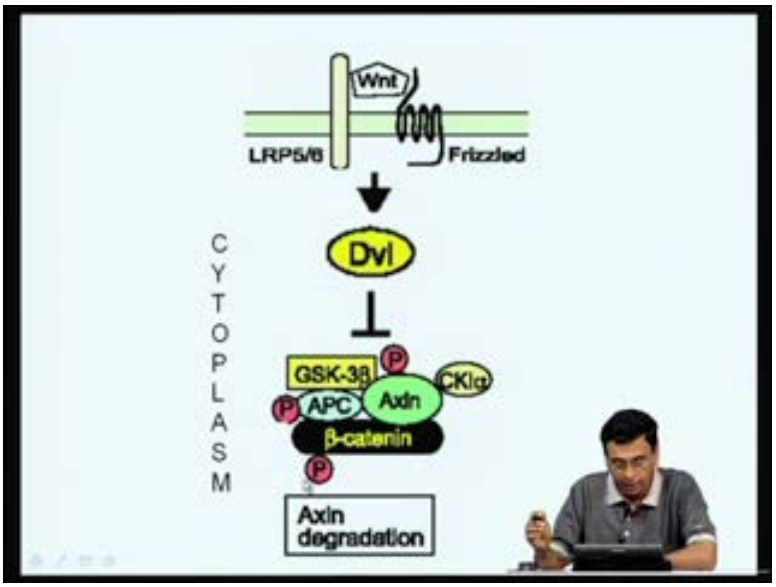

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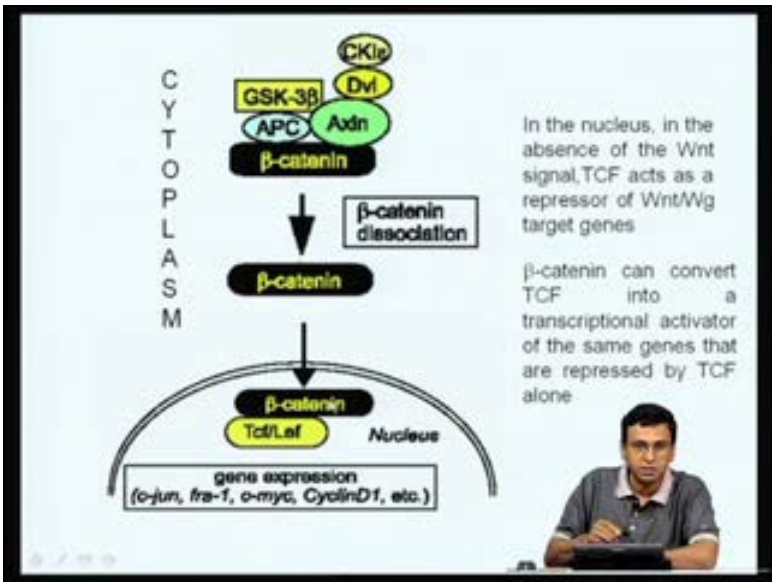

So, what this tells you is that when Wnt protein does not bind to the frizzle receptor, there will be no beta-catenin inside the cell because that beta-catenin gets rapidly degrading. So, in the absence of Wnt, there is no beta-catenin protein present in the cells. Now, when Wnt binds the frizzle receptor, it results in the activation of protein called Dvl and this Dvl inhibits the phosphorylation of these proteins, and when the

phosphorylation of these proteins is prevented, this can no longer be degraded by the protein by the proteasome pathway. Therefore, the beta-catenin dissociates from this multi-protein complex. And now, the beta-catenin is active and can now go inside the nucleus and then, in combination of the protein called TCF and Lef, can activate the transcription of a number of genes involved in cell proliferation. So, in the nucleus, in the absence of the Wnt signals, the TCF access as a repressor of Wnt wing target genes.

So, inside the nucleus, when there is no beta-catenin, this TCF-Lef complex acts as a repressor for all these genes, which are activated by beta-catenin. Whereas, as beta-catenin is present, beta-catenin can convert the TCF into a transcriptional activator and therefore, these genes can now be transcription, of, can be activated.

So, when Wnt does not bind to the surface receptor, the beta-catenin gets degraded and therefore, the TCF-Lef acts as a repressor. Therefore, these genes cannot be activated, whereas when Wnt binds to the frizzle receptor, the phosphorylation of the protein complex is prevented. Therefore, the proteasome degradation pathway is inhibited, now the beta-catenin dissociates from this protein complex, comes inside the nucleus and now functions an activator, leading to the activation of transcription of the various target genes.

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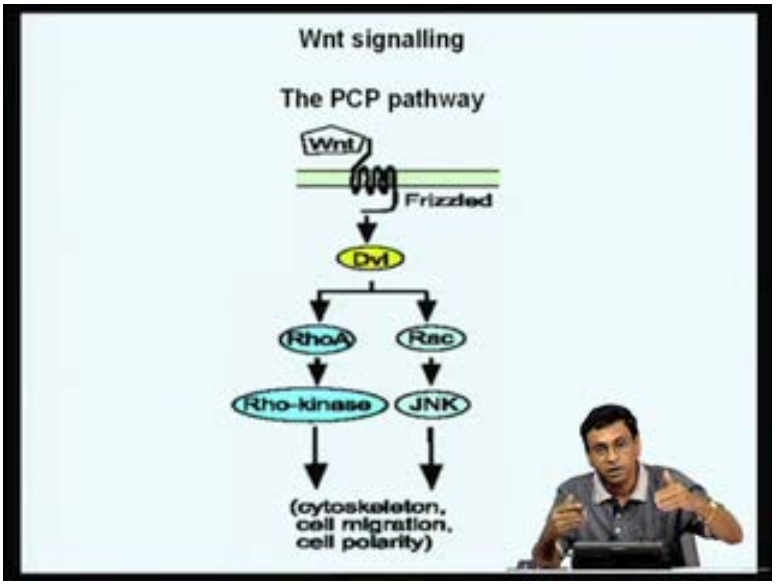

The other pathway by which the Wnt operates in the vertebrates involves, what is called, the PCP pathway where the Dvl, which has been activated in certain cell types instead of

preventing the phosphorylation of the beta-catenin and associated proteins and activated beta-catenin, can also activate the Rho or Rac GTPSAs and as a result, specific protein kinases can be activated like the Jun internal kinase or the Rho-kinase, which in turn go and phosphorylate the number of proteins and these kind of signaling pathway plays a very, very important role in the development of cytoskeleton, cell migration, cell polarity during development.

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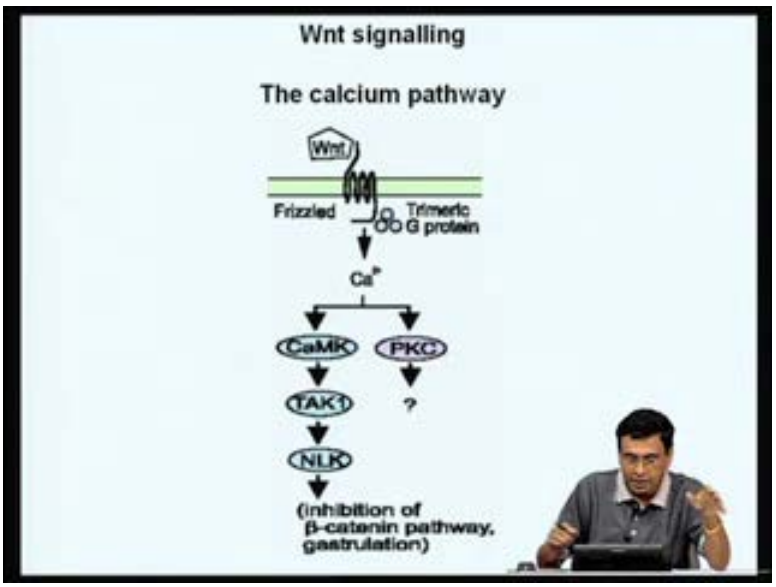

Like I see, the same Wnt protein can trigger different signal transcription pathways in different cell types and finally have, what is called, calcium pathway. Again, by the interaction of the Wnt through the cell surfaces receptor can result in the, you increase the intracellular calcium and this calcium, through the calcium, CAL modeling kinase and other kinases, can now actually act as a inhibiter of the beta-catenin pathway.

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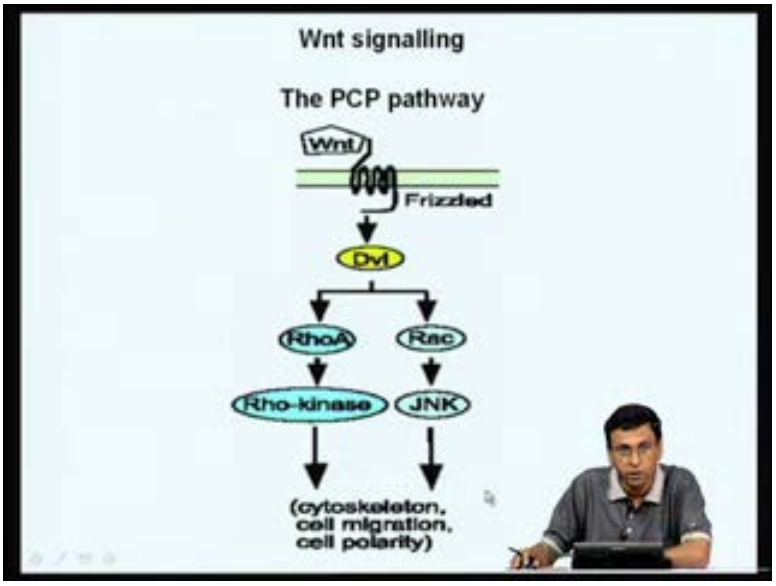

So, when Wnt pathway results in the activation of a, results in the increase in the intracellular calcium. This can actually inhibit the beta-catenin pathway and this kind of a calcium mediated Wnt signaling pathway plays a very, very important role during gastrulation of embryonic development. So, you can see, the Wnt protein interaction of frizzle receptor can have 3 different pathways, either involving beta-catenin or calcium or the Rho or Rac GTPSAs and all these things can have different physiologic concess and activate different set of target genes during embryonic development.

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So, as I told for the hedgehog, the Wnt pathway also plays a very, very important role, not only in development, but also during the adult life, and Wnt pathway is very closely associated with colon cancer. Now, in colon cancer, mutations in a protein, called as APC, which is nothing but adenomatous poluposis coli is very, very common. Now, when you have these mutations in this particular protein called APC, APC actually binds to beta-catenin, which is the transcription factor activated by the Wnt pathway and destabilizes it. Whereas, the mutant protein APCs present in the colon cancer cells allows the levels of beta-catenin to increase. So, when you have (( )) APC protein in the colon cells in the intestine, the beta-catenin is destabilized and therefore, the Wnt pathway is not activated. But when we have mutations in the APC, the mutant protein allows the levels of beta-catenin to increase and therefore, there is constitutive activation of the target genes of beta-catenin and this results in colon cancer.

So, the same Wnt signaling protein, which plays an important role in development, when, can also play a very, very important role when it gets constitutively activated in colon cells, it can lead to colon cancer.

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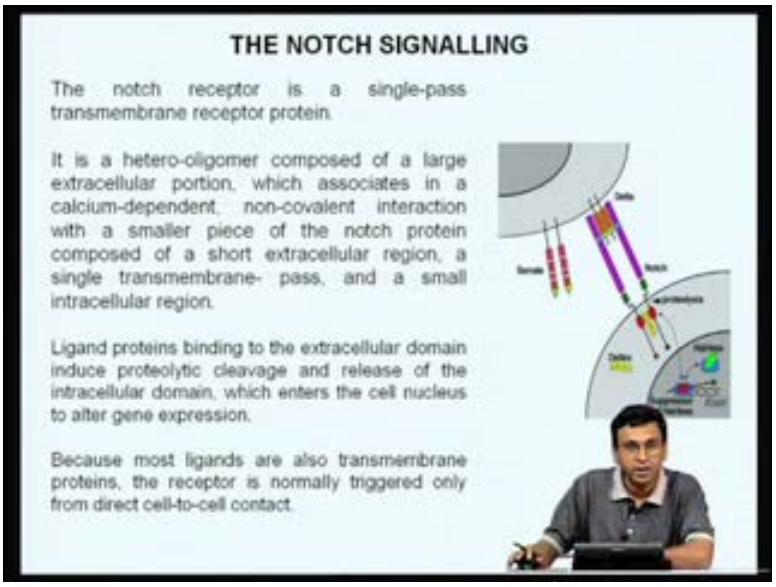

Now, so far we have discussed 2 signal transcription pathways involving hedgehog, the Wnt signaling and how these signaling pathways play a very important role, not only in the embryonic development, but also in the adult life. Now, let us study, now one more

example of a very interesting signaling transaction pathway, called as the Notch signaling.

Now, what is this notch signaling? This is slightly different from what we have discussed so far, both in just a few minutes ago, as far in the previous classes, so I would like to go as a very important example for the signal transduction pathways operate during development.

Now, the notch receptor is a single pass transmembrane protein, unlike the 7 transmembrane protein, it has only 1 membrane domain passing through the traverse synthesis cell membrane. Now, notch is a membrane receptor, it is a hetero-oligomer, composed of a large extracellular portion, which associates in a calcium-dependent non-covalent interaction with a smaller piece of a notch protein composed of a short extracellular region. A single transmembrane pass in the small intracellular region. The notch is schematically shown here, this is the extracellular domain and is intracellular domain of the notch protein.

Now, the ligand proteins, they are binding to the extracellular domain induce proteolytic cleavage and release of the intracellular domain, which now enters the nucleus and alter the gene expression. So, the ligands, which interact with the notch protein, they induce the proteolysis here and as a result, the intracellular domain of the notch protein is now released. Now, that goes and activates the transcription of the target genes.

Now, the most important thing about the notch signaling is that what kinds of ligands interact with notch signal? It turns out, the ligand which interacts the notch receptor are also transmembrane proteins. So, I have 2 cells, 1 cell expressing the ligand for notch receptor, another cell expressing the notch receptor and when these 2 cells come in contact with each other and then this ligand interacts with the receptor, now that triggers the notch receptor. Now, signaling pathway, the notch receptor gets cleaved proteolytically and then the intracellular domain of notch receptor, now goes inside the nucleus and then brings about activation and repression of target genes.

So, this Notch signaling is unique in the sense, that both the ligand as well as the receptor of transmembrane proteins. So, it is a very unique signaling pathway operating during development.

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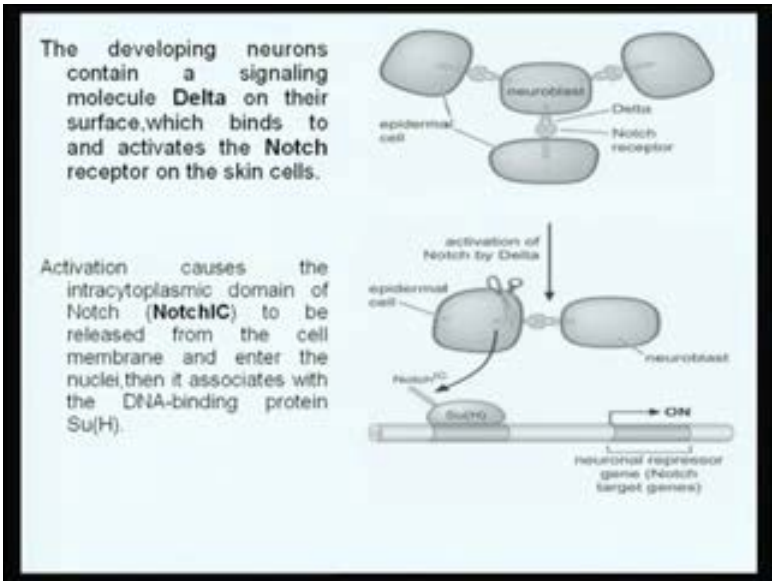

Let us take 1 example. For example, where the notch signaling became important during development of the nervous system, the developing neurons contain a signaling molecules called delta and you can see that the delta is actually a transmembrane protein.

Now, this transmembrane protein delta binds to and activates the notch receptor present on the skin cells. So, the skin cells contain the notch receptor and some of these developing neurons contain the delta protein, and when these 2 cells come in contact with each other, the delta receptor interacts with the, with the notch receptor and this triggers the signal transduction cascade.

Now, what happens? Activation; so, when the delta membrane receptor membrane protein binds to the notch receptor on the other cell, activation causes the intracytoplasmic domain of notch, known as the notch IC or notch, intracellular, intracytoplasmic domain is released. This is shown schematically here. Then, these 2 cells come in contact with each other when the delta protein docks with the notch receptor. The intracellular domain of the notch protein is proteolytically cleaved and this now goes inside the nucleus and activates, associates the DNA-binding protein called Su and these set as the activation of expression of specific target genes.

So, it is a very unique signal transduction pathway and this signal transduction pathway is unique because it involves the contact between 2 different cell types. So, when 2 cell types come in contact with each other, the membrane protein present on one cell interacts

with the receptor present of other cell, and as a result, the intracellular domain. Now, the receptor is cleaved, now this intracellular portion of the receptor goes inside the nucleus and act with the expression of the target genes.

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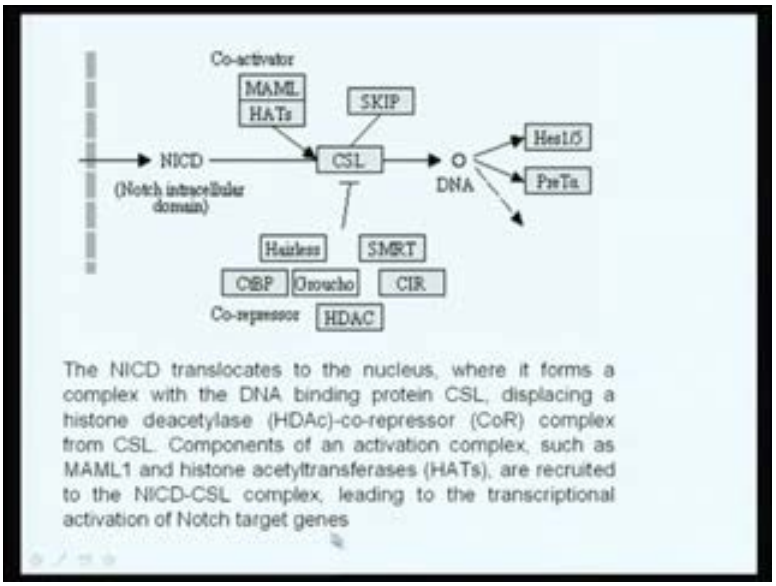

So, this kind of signaling molecule is, this, these are some of the details, what happens when the intracellular domain of the notch goes inside the nucleus? The notch intracellular domain now translocates to the nucleus, where it forms the complex with the DNA binding protein, called CSL, displacing a histone deacetylase co-repressor complex from CSL and components of an activation complex, such as MAML and histone acetyltransferases are recruited to the NICD-CSL complex, leading to the transcriptional activation of the notch target genes.

Since the mechanism by which the intracellular domain of the notch activation transcription, so when the notch intracellular domain is not in the nucleus, a transcription factor called CSL is associated with the histone deacetylase complex shown here. Whereas, in the notch, intracellular domain comes entire nucleus, it displaces this histone deacetylase complex and recluse the histone acetylene transferase complexes and therefore, now this results in the activation of transcription, result in the transcription of the target genes.

So by displacing the histone deacetylasers and recruiting the histone acetylases, target genes for the notch intracellular domain are activated. So, this is the mechanism by which the notch transition signal transition pathway works.

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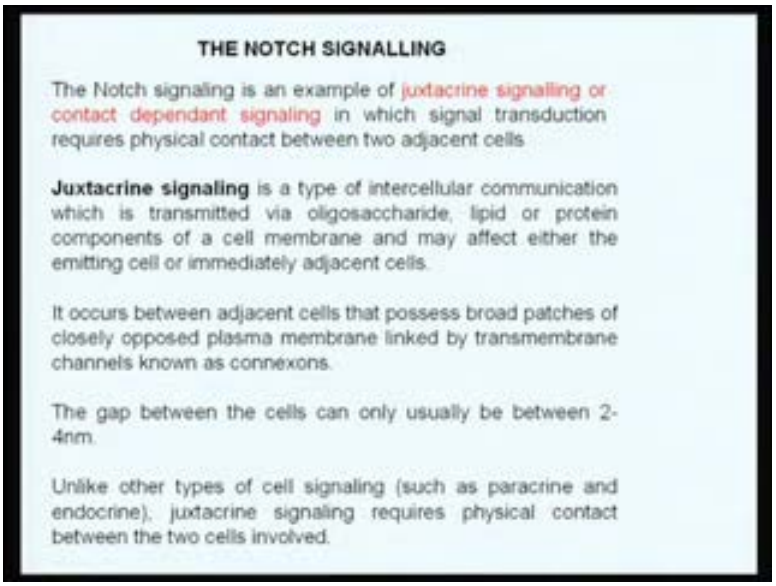

So, this Notch signaling, as I told you is very unique because Notch signaling is an example of, what is called as, juxtacrine signaling or contact dependent signaling in which signal transduction requires physical contact between 2 adjacent cells. So, there are 2 cells during development as the (( )) goes when these 2 cells come in contact with each other. The membrane protein present on the one cell now interacts with the receptor present on the other cell and as a result of the cell-cell contact and membrane protein receptor interaction, the intracellular domain of the receptor is cleaved, which now goes inside the nucleus and activates transcription of target genes.

And juxtacrine signaling is a type of, intracellular communication, intercellular communication, which is transmitted via oligosaccharide, lipid or protein components of a cell membrane and may affect either the emitting cell or immediately the adjacent cells. So, this kind of signaling mechanism occurs between adjacent cells that possess broad patches of closely opposed plasma membrane linked transmembrane channels, known as connexons. And usually, the gap between the cells can be just about 2 to 4 nanometers and then only this kind of a juxtacrine signaling pathways are activated. So, unlike the

other types of the cell signaling, such as paracrine and endocrine signaling, the juxtacrine signaling requires physical contact between 2 cells is involved.

So, unlike the hedgehog, the hedgehog signaling and Wnt signaling, we have actually seen molecules being secreted and these secretary molecules go and bind to the specific membrane receptors and then trigger the signaling pathway. Whereas, the Notch signaling is a very, kind of, different form the Wnt and the hedgehog signaling because here, both the ligand or the signaling molecule, as well as, the receptor, both are membrane bound.

So, only when 2 cells come in contact with each other, there is a possibility, that the membrane, a protein present on one cell can actually come in contact with receptor and on the other cell; that is how the signaling cascading is triggered.

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So, the hedgehog signaling plays a very, very important role not only in development and in cancer, that is what we discussed in detail, the, all these signaling pathways, the hedgehog, Wnt, as well as, the Notch signaling, what I have discussed so far, I have discussed very, very briefly, it is much more complicated.

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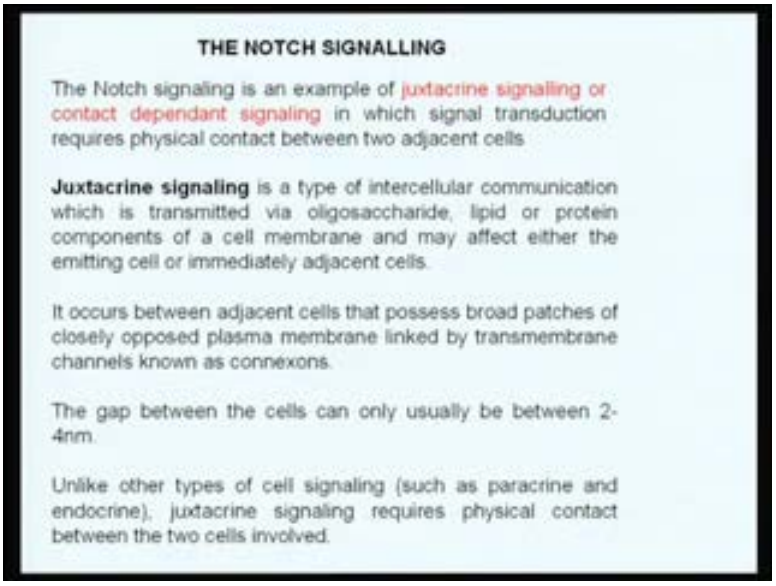

For example, if you now take the Notch (( )) receptor, it actually contains, what are called as, number of EGF receptor like domains in multiple repeats. It also undergoes a number of co-translation of modifications, this is heavily glycosylated and this glycosylation plays a very important role. So, I will not go in the details of these, each one of these signaling cascades, but the either (( )) 3 different signal transduction pathways as an example, to illustrate the point, how signaling molecules interact in specific membrane receptors, can influence specific gene expression programs and when you have mutations in these signaling molecules, it can result in abnormal development, as well as, in the adult it can lead to cancer.

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So, this is a very nice review about the hedgehog signaling, hedgehog signaling, in development cancer, as early as 2008. So, for details of the how the hedgehog signaling plays a very important role in cancer and development, one can read this particular article.

So, I will stop here. So, what we discussed so far is that how signaling transaction pathways play very, very important role during the embryonic development? There are number of signal transaction pathways and the mechanism is similar to what we discussed in the previous classes. Signaling molecules interacting with specific membrane receptors and trigger phosphorylation cascades or prevents degradation of transcription factors, and then these transcription factors now go inside the nucleus and activator repressor transcription of target genes. And as, although there are many such signal transduction pathways, which operate embryonic development, I have taken 3 signal transaction pathways as an example to just to give you an idea about the mechanism by these pathways operate. I gave an example of hedgehog, I gave an example of Wnt, as well as, finally the Notch signaling pathway, and how the Notch signaling is very, very unique from the other 2 signaling pathways.

So, we will now continue our discussion in the next class. We will discuss about how homeotic genes regulate gene expression and the homeobox containing proteins, and how they play a very important role in the conferring the identity of various segments

and how legs, (( )), Wnts, etcetera can be formed when these homeotic genes get activated; that will be the topic of the next class. I will stop here.