**Eukaryotic Gene Expression: Basic and Benefits**

**Prof. P N Rangarajan**

**Department of Biochemistry**

**Indian Institute of Science, Bangalore**

**Module No. # 07**

**Lecture No. # 26**

**Homeotic Genes**

Welcome to this lecture series on this eukaryotic gene expression basics and benefits. In the last three classes, we have been discussing how regulation of gene expression is important in regulating the embryonic development. We started discussion about early embryonic development; how, right from the stage of oocyte, the regulation of embryonic development begins. Even at the stage of oocyte development, as the oocyte is the being formed, the nerve cells actually start pushing some messenger RNAs into the oocyte cytoplasm, and these messenger RNAs known as the maternal messenger RNAs get deposited inside the oocyte cytoplasm in different regions– some get distributed in the anterior regions; some get distributed in the posterior region.

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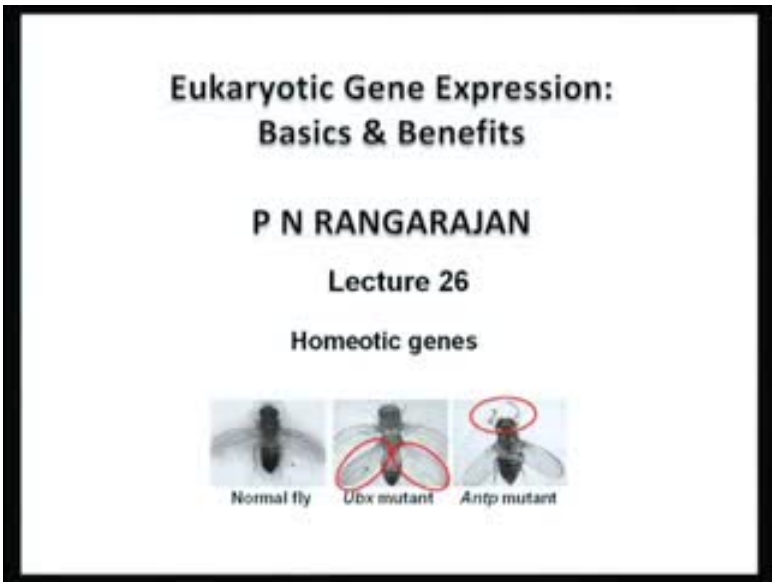


But, they would not get translated, and then after fertilization, these maternal messenger RNAs get translated, and then these proteins, which most of them which are transcription

factors, now induce the expression of what are called as the zygotic genes. And we discussed in the using Drosophila as an example, we discussed about what are called as gap genes, pair-rule genes, segmentation genes, and the take-home message from those early lectures is that spatial and temporal expression of these genes play a very important role in the normal embryonic development.

So, what we will do in this lecture is to just recapitulate some of the observations we made in the earlier two lectures, and then proceed to a very important part of development regulation, namely, homeotic genes, because these homeotic genes are the ones which are, ultimately, responsible for giving identity to the various segments of the Drosophila, and also for the formation of various organs.

So, we initially discussed how homeotic genes are important. What happens when you have mutations in these homeotic genes, not only in Drosophila, but also in vertebrates like mouse and humans? And how important are these homeotic genes? And how the expression of these homeotic genes are actually regulated, and where they are expressed? How they are regulated, and how do they bring about all these various aspects of developmental regulation?

So, this cartoon just shows some of the very popular homeotic mutations, which were originally identified in Drosophila, like if you mutate one of the homeotic genes in Drosophila called as ultrabithorax, you can convert, instead of both the flies having one pair of wings, it will now start having two pairs of wings. Similarly, if you have a mutation in another homeotic gene called antennapedia, instead of antennae coming from the head, you will actually have legs coming from the head, clearly indicating that these genes are master regulators or master control genes, and if you meddle with them, the entire organ identity can be changed.

So, let us now try to understand in detail how these homeotic genes function, and how these homeotic genes are activated, and what are the target genes for these various homeotic genes.

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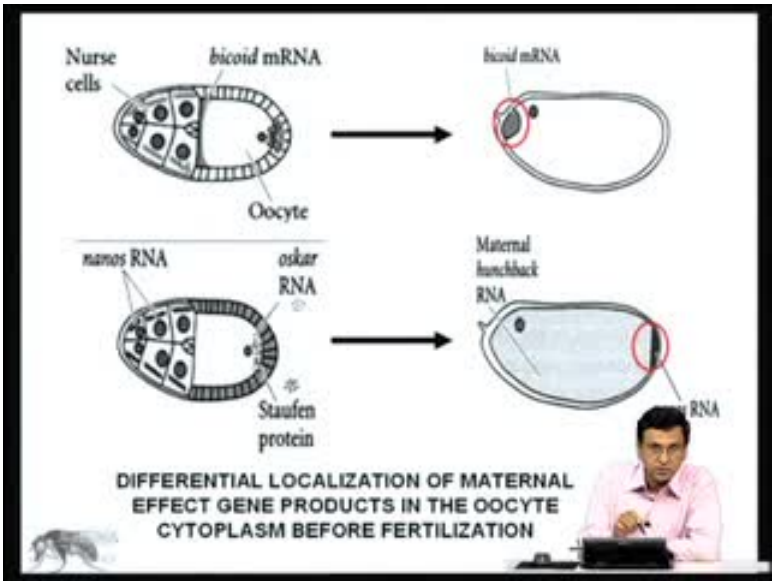


So, this slide just recapitulates some of the very important observations we have made in the last three classes. Like I said in the beginning, the regulation of embryonic development begins right at the stage of oocyte formation or the oocyte development. Even as the oocyte is being formed, the nerve cells in the ovary, through what are called as the cytoplasmic bridges, actually synthesize messenger RNAs and transports these messenger RNAs through these cytoplasmic bridges into the oocyte cytoplasm, and these maternal RNAs known as the maternal RNAs– maternal messenger RNAs– they do not get translated till fertilization, but they get deposited in different regions of the oocyte. For example, an RNA called bicoid mRNA gets deposited in the anterior region of the developing oocyte, whereas another mRNA, called the bicoid mRNA, gets deposited in another mRNA, called the nanos mRNA, gets deposited in the posterior region of the oocyte.

So, after fertilization, when these messenger RNAs get translated, the bicoid protein finally gets deposited in the anterior region, whereas the nanos proteins get deposited in the posterior region, and so, differential localization of this maternal effect gene products in the oocyte cytoplasm, before fertilization, plays a very important role in triggering the expression of some of the early zygotic genes during embryonic development.

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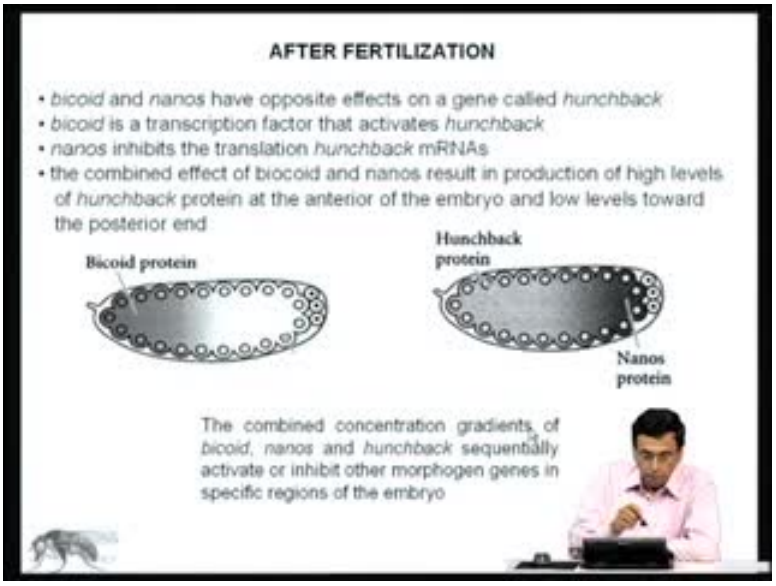


So, the bicoid and nanos, which are just two examples I had mentioned. There are many such maternal effect genes; we will not go into the details. The bicoid and nanos, for example, have opposite effects on a gene called hunchback. Hunchback is a zygotic gene of is an RNA, which gets expressed now by when that bicoid transcription factor goes and bounds the promoter of the hunchback, hunchback genes gets expressed, and the hunchback protein is made.

So bicoid is a transcription factor that are activated hunchback, but since the bicoid is present primarily in the anterior two-thirds of the embryo, the hunchback gets activated. Only in the hunchback protein is made from large amount in the anterior region, but the hunchback protein that is even made in the posterior region does not get translated, because the nanos protein, which is now present in large amounts, goes and binds to the hunchback messenger RNA and prevents its translation. So, as a result, the hunchback protein can act only in the anterior two-thirds of the embryo, whereas the nanos prevents the activity of hunchback in the posterior region.

So, the nanos inhibits the translation of hunchback messenger RNAs. The combined effect of bicoid and nanos results in the production of high levels of hunchback protein at the anterior region of the embryo, and lower towards the posterior. So, these proteins called bicoid, nanos, hunchback– they are called as morphogens, and you can see, they

are all present in a gradient either in the anterior-posterior gradient, or if I take proteins like dorsal and ventral, they are present the dorsal-ventral gradient and so on.

So, by creating these kinds of a morphogen gradients differential gene expression is brought about during early embryonic development. So, the combined concentration gradients of bicoid, nanos, and hunchback sequentially activate or inhibit other morphogens beams in specific regions of the embryo. So, since hunchback is present in the high concentration in the anterior region, it will now activate only those genes in the anterior region of the embryo.

So, the genes which have to be activated, hunchback will not be activated in the posterior region of the embryo, because nano prevents the translation of hunchback, and therefore, hunchback will not be able to activate those genes in the posterior region. So, target genes of hunchback will be activated only in the anterior region of the embryo, but not in the posterior region of embryo, and this will have tremendous implications in the further development of the embryo.

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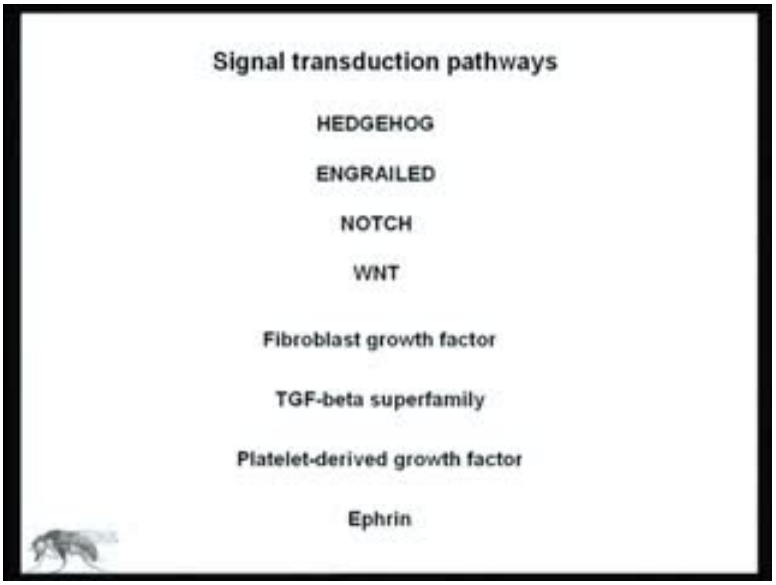


So, in addition to discussing some of these maternal effect genes like nanos, dorsal, bicoid, and so on so forth, we also discussed how these molecules also influence various signal transduction pathways. As I said, this is not a lecture series on development biology; so, we cannot cover the entire developmental programs. But, I gave you a few examples of very important signal transduction pathways that operate during the

embryonic development of Drosophila as well as vertebrate development. Some of them, which will discussed in detail, are what is called the Hedgehog signal transduction pathway, engrailed, Notch, wnt, and so on so forth, and we discussed in detail how, when you have mutations in this signal transduction pathway, it can lead to abnormal embryonic development and even diseases, and many of these pathways are also very important in the adult stages as well, and if you have mutations in some of these pathways, it can lead to genetic disorders, as well as it can lead to cancer, like wnt. Wnt pathway is very important, and if you have mutations in the wnt components of the wnt signaling, you can get colon cancer.

So, all these signal transduction pathways are important not only during embryonic development, but also play a very important role during the adult life as well. So, in addition to some of the pathways that we discussed, there are also many other important signaling pathways that play a very important role in embryonic development; for example, the fibroblast growth factor, the TGF-beta family of proteins, platelet-derived growth factor, ephrin– there are so many signaling molecules which play a very important role in activating the expression of a number of target genes, both in spatially and temporally; that means in space as well as time, and as a result of all these signaling events, the embryonic development proceeds smoothly and from a single cell embryo, you get an adult, finally.

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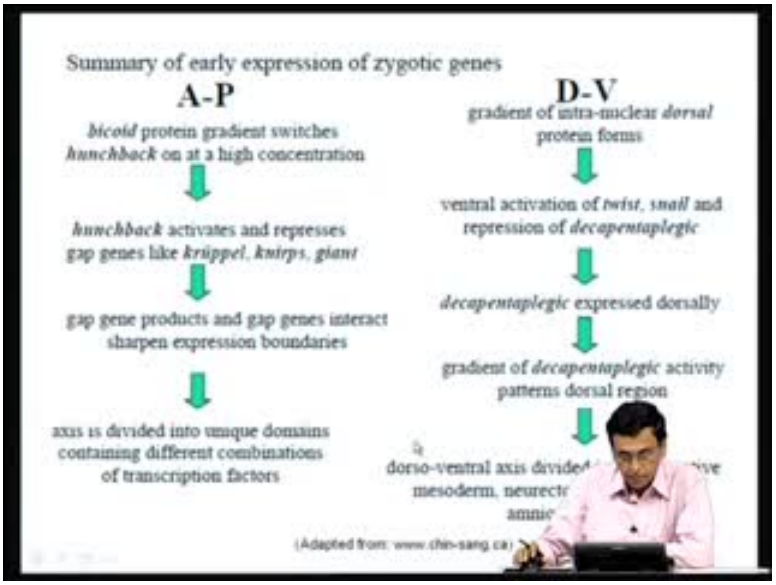

So, just to summarize the key points of the early embryonic development we have studied so far, there is the two things that are important for the early embryonic development is the development of an anterior-posterior axis and a dorsal-ventral axis, that is, anterior posterior has to… The embryo has to know which is the anterior and which is the posterior end; which is the dorsal end; which is the ventral end.

So, as far as the anterior-posterior axis development is concerned, the bicoid protein plays a very important role. So, the bicoid protein gradient switches the hunchback at a very high concentration in the anterior region of the embryo, and hunchback, in turn, now activates or represses gap genes like kruppel, knirps, giant, and so on so forth, and then these gap gene products and gap genes interact and sharpen the expression boundaries, and then the axis now gets divided into unique domains– the unique domains containing different combinations of this transcription factor.

So, the bicoid protein plays a very important role in this anterior-posterior gradient. Similarly, the dorsal-ventral gradient– you need to know which is the dorsal, that is, the back, and which is the belly, which is the ventral region. So, these axis are the specified during embryo early embryonic development; then only you get a normal organism from the embryo.

The dorso-ventral axis, again, the gradient of the intra-nuclear dorsal protein plays a very important role, and the ventral activation of twist, snail, and repression of decapentaplegic are very important. These are all targets of the dorsal protein. The protein called as decapentaplegic gets expressed only dorsally, and a gradient of the decapentaplegic activity patterns the dorsal region, and the dorso-ventral axis divided into prospective mesoderm, neurectoderm, epidermis, and so on so forth, because of the activity of all these maternal genes gap genes and so on so forth.

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So, once the gap gene products are found, the gap genes now activate what are called as the pair-rule genes, and as well as segmentation genes and production of local combinations of the gap gene transmission factors activate what are called as the pair-rule genes in seven transverse stripes along the anterior-posterior axis of the embryo. And the result of this pair-rule gene expression is the formation of what are called as 14 parasegments– each pair-rule gene being expressed in alternate parasegments. So, this is some of the important points that we discussed in the last two lectures.

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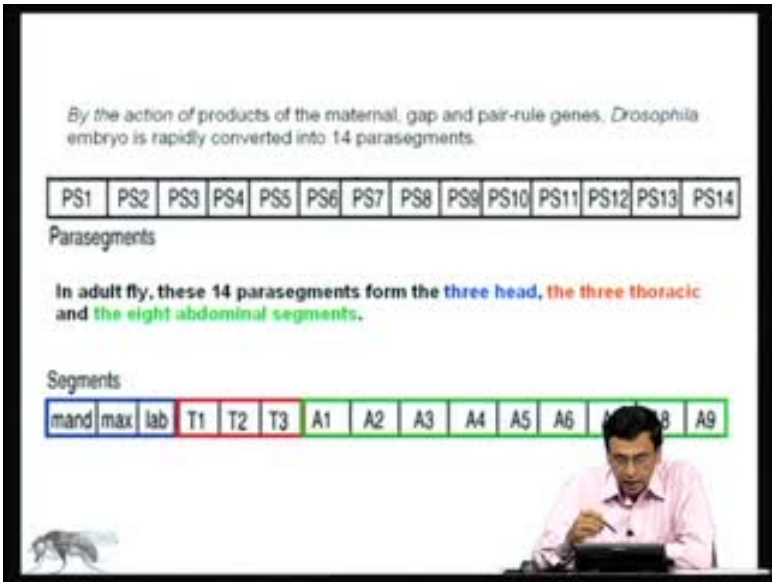

So, by the action of this maternal gap and pair-rule genes, the Drosophila, which was single cell embryo in the beginning, now becomes a multicellular embryo, and it gets divided into about 14 parasegments.

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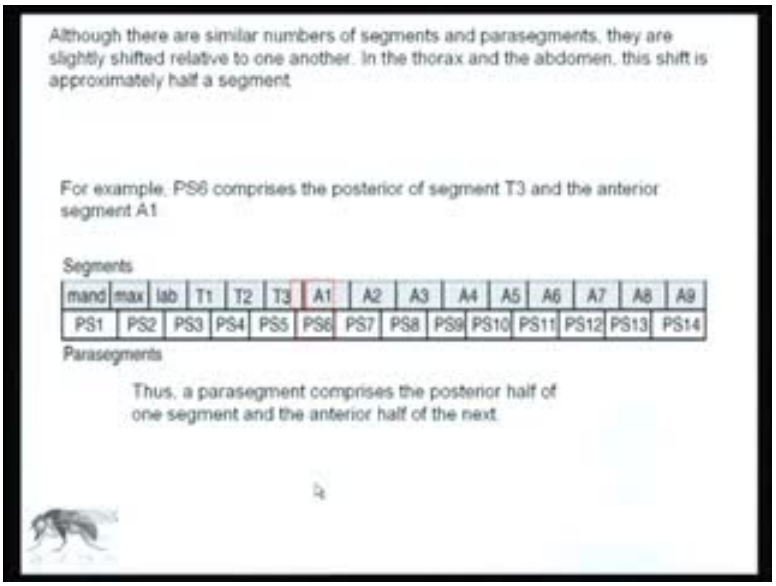


So, these are known as PS1 to PS14. Now, in the adult fly, these 14 parasegments are responsible for the formation of three head, three thoracic, and eight abdominal segments. So, the first three parasegments give rise to the head; the next three give rise to thoracic segments, and the remaining give rise to the abdominal region of the embryo. A very important point– the relationship between the parasegments and the actual segments is that although the number of parasegments and number of segments are number is similar, their arrangement is a little bit peculiar. So, although there are similar number of segments and parasegments, they are slightly shifted relative to one another in the thorax, in the abdomen– the shift is approximately half a segment.

We can see here– if you know, for example, take parasegment 6 as an example. The parasegment 6, here, comprises of the posterior segment of segment T3 and anterior segment of A1; that means, the last parasegment 6 consists of: this is T3 with the thoracic segment 3 and abdominal segment 1, and you can see the parasegment 6 actually comprises of the posterior region of the thoracic segment 3 and anterior region of abdominal segment 1.

This is true for all the parasegments. If you now take, for example, parasegment 9, it consists of posterior regions of the previous segment and anterior region of the next segment. Similarly, parasegment 12 consists of the posterior region of A6 and the anterior region of A7. So, there is a very unique arrangement between the parasegment and the segments during the development. So, a parasegment, therefore, comprises the posterior half of one segment and anterior half of the next segment. This plays a very important role in the normal development of the embryo.

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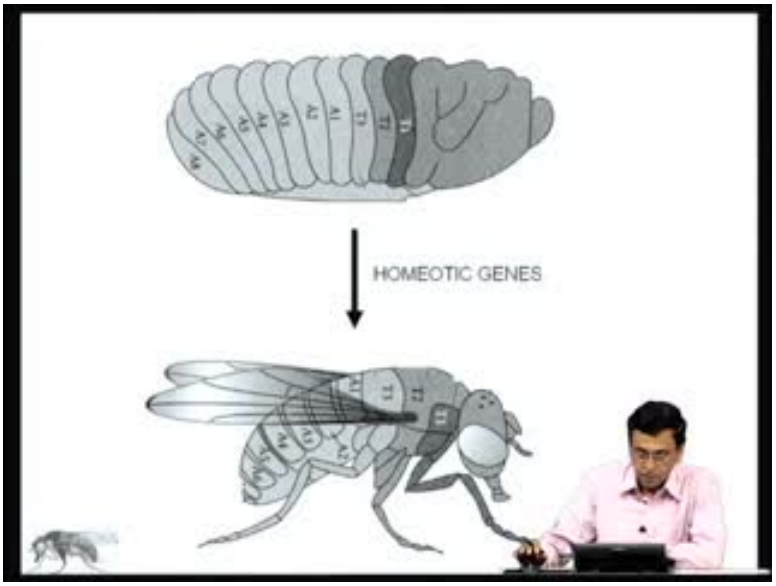


So, now, let us now focus on as the result of all the expression of this gap genes, pair-rule genes, segmentation genes. Now, the embryo has to now become an adult. This is where the homeotic genes play a very important role, because it is the homeotic genes, which gives the identity to each segment. So, let us now try to understand what are these homeotic genes and how do they function.

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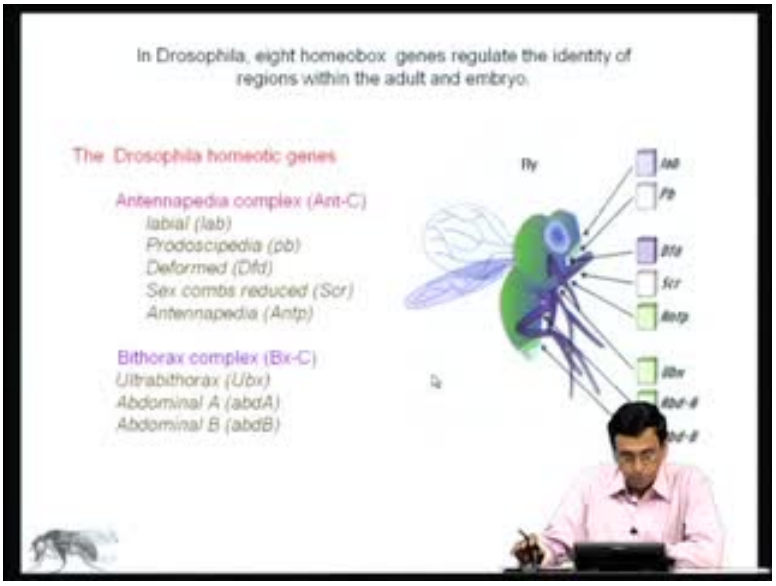


In Drosophila, there are about eight homeotic genes which regulate the identity of the regions within the adult and embryo, and these eight genes– homeotic genes– are clustered in two complexes. One is called as the antennapedia complex; another is called as the bithorax complex. The antennapedia complex consist of homeotic genes known as labial, prodoscipedia, deformed, sex combs reduced, and antennapedia.

These are the major homeotic genes of the antennapedia complex. The bithorax complex of three homeotic genes– the ultrabithorax, abdominal A, and abdominal B, and as you can see in this picture, these homeotic genes are expressed in different regions of the embryo with the bithorax complex is getting expressed in the posterior region, and the antennapedia complex getting expressed in the anterior region and so on so forth.

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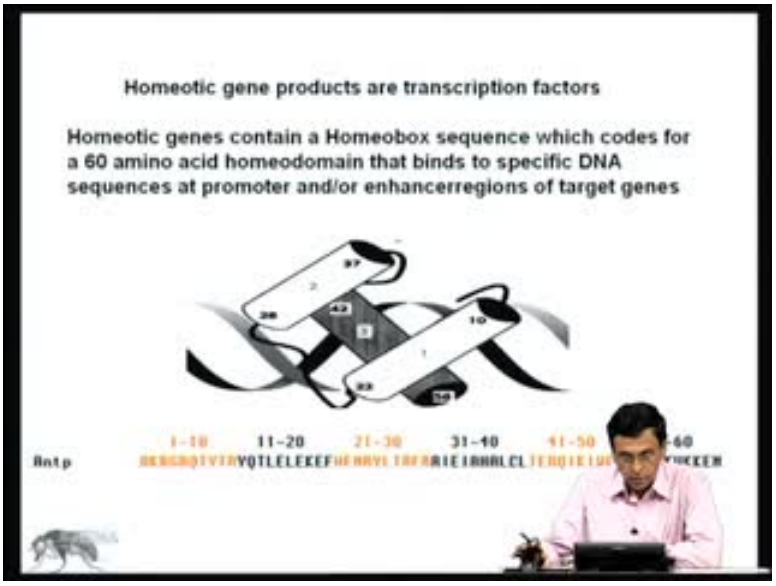


So, these homeotic gene products are nothing but they are transcription factors. All these homeotic genes contain a stretch of DNA sequence known as the homeobox sequence, and on transcription and translation this homeobox sequence codes for a 60 amino acid domain, which is known as the homeo-domain, which is nothing but it can bind to specific DNA sequences. So, it is the sequence-specific DNA binding activity of the homeo-domain proteins is because of the presence of the homeo-domain. We have discussed in detail homeo-domain structure, when we discussed some of the structures of various DNA binding proteins in the previous classes, where we discussed about leucine zipper, zinc finger, helix-turn-helix motif, so on so forth.

The homeo-domain is nothing but a helix-turn-helix motif. It consists of three alpha helices, and this is the sequence of the homeo-domain region of a homeobox protein known as the antennapedia, and this homeobox sequence is very important, because it is this homeobox, which now binds to specific sequences of the promoter regions of the target genes, and therefore, are directly responsible for activation of various target genes during embryonic development.

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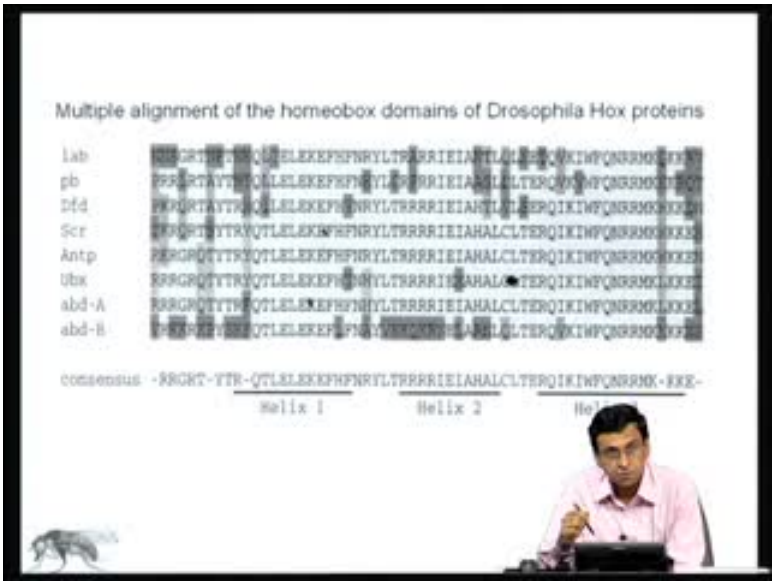


This just shows you the multiple alignment of the various homeo-domain regions of all the homeotic genes of Drosophila, just to tell you that this homeobox region has many conserved amino acids, especially the helix 1, helix 2, and helix 3 region, which is involved in DNA binding, is highly conserved, because they formed the helix-turn-helix motif of all these homeobox transcription factors, and therefore, is responsible for sequence-specific DNA binding. Therefore, it is highly conserved in all the homeotic gene products of the Drosophila.

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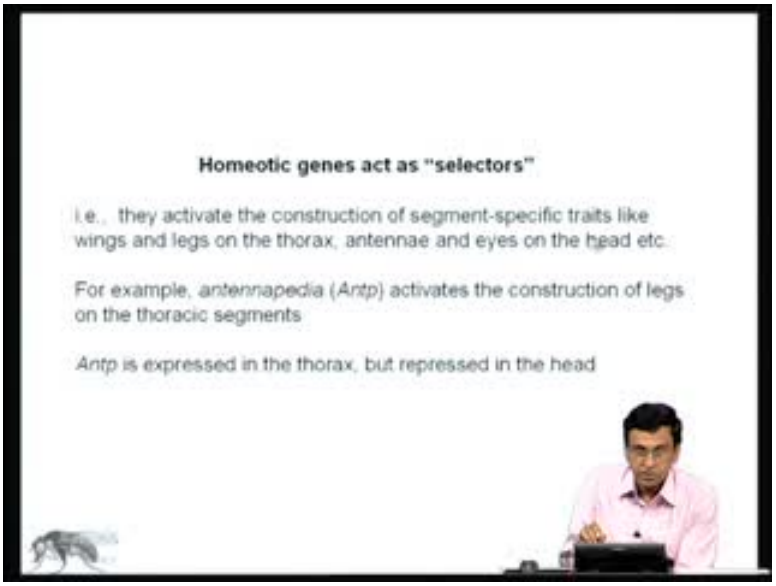

So, what are these homeotic genes? What do they do? Homeotic genes are nothing but they act as selectors; that is, they activate the construction of segment-specific traits like wings, legs, etcetera. So, the homeotic genes, which are expressed in the thoracic region, tells the segment that it is from these segments the wings have to come or legs have to come, and the homeotic genes which are expressed in the head region are responsible for the development of antenna and eyes in the head region and so on so forth.

So, basically, the development of segment-specific traits like the development of eyes or antenna from the head, or development of legs and wings from the thoracic regions, is all because of the expression of specific homeotic genes in these regions of the embryo. We said that there are two major homeotic gene clusters in the Drosophila. The antennapedia, for example, activates the construction of the legs on the thoracic segments, and if antennapedia is a…, therefore, expressed in thorax, but it is not expressed in the head. That is why, if you now expressed, so in a normal development, the job of antennapedia homeotic gene is to form legs from the thoracic segments. Therefore, the antennapedia is expressed only in the thorax, but not in the head.

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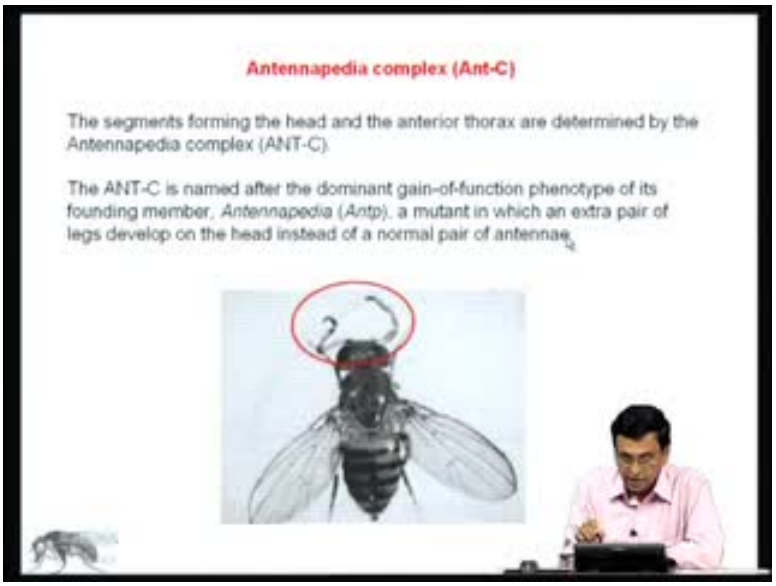


Now, what happens when I express the antennapedia gene in the head? This is what happens. Now, instead of antenna coming from the head, legs starts protruding from the head. So, you can see, segment-specific expression of these homeotic genes are very important for the normal development.

The segments forming the head and anterior thorax are determined by the antennapedia complex, and antennapedia complex is named after the dominant gain-of function phenotype of its founding member–Antennapedia– a mutant in which an extra pair of legs develop on the head, instead of normal pair of antennae. So, these are very important genes, and probably so, that is known as the master regulators of development.

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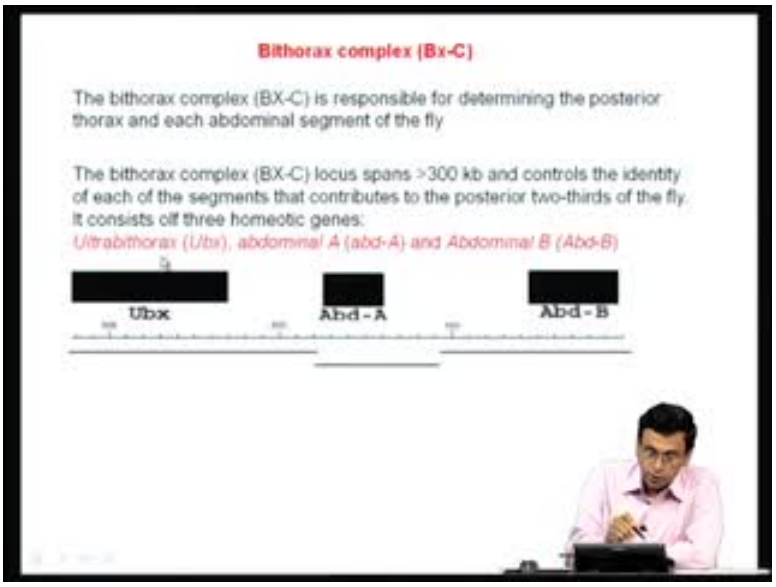


Similarly, the bithorax complex– the bithorax complex is responsible for determining the posterior thorax and abdominal segment of each fly. The antennapedia is primarily responsible for the head region, and bithorax complex responsible for the thorax and abdominal segment of the flies. The bithorax complex, basically, the locus is a huge locus, consists of 300 kilo base long DNA, and it controls the identity of each of the segments that contributes to the posterior two-thirds of the fly, and it consists of three homeotic genes.

So, the development of thorax and abdomen of the fly is regulated by the bithorax complex, which consists of three genes, namely, the ultrabithorax, abdominal A, and abdominal B genes, and the entire region spans to almost 300 kilo base long DNA, and the regulatory regions– the thin lines– indicated here, actually, have been shown to be regulatory regions, which are responsible for the expression of the ultrabithorax, abdominal A, and abdominal B. For example, if you have mutations or helices in this

region, the expression of the ultrabithorax may be affected. Similarly, if you have mutations in this region, the expression of the abdominal B may be affected.

So, the dark boxes here represent the coding region, which contain the exons and introns of each of these three genes, and the thin line encompasses not only coding region, but also the non-coding regulatory regions, which are essential for the expression of these three abdominal, the bithorax complex genes.

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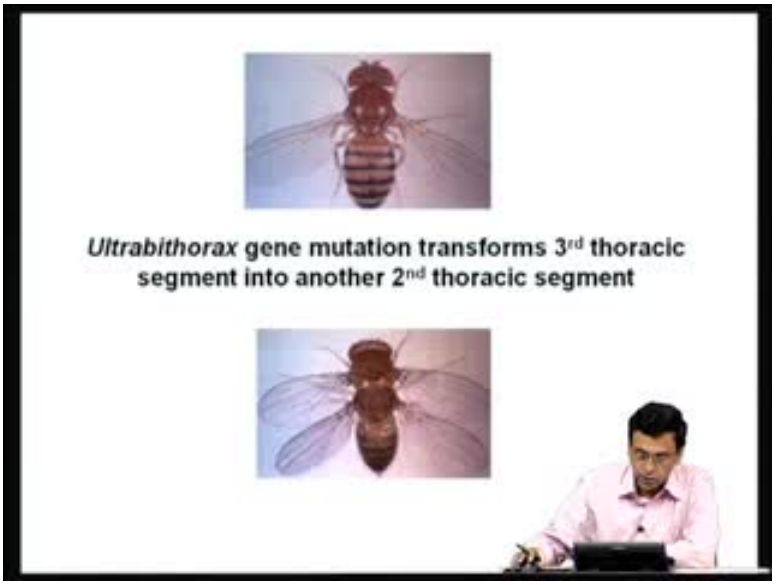


So, just like when you had mutations in antennapedia, the antenna got transformed to legs. Similarly, when you have mutations in the ultrabithorax gene, again, instead of halteres coming from segment, a wings develop. So, the ultrabithorax– it is actually responsible for converting an haltere into a wing.

So, if you now express, if you have mutation in the ultrabithorax gene, it transforms the third thoracic segment into a second thoracic segment. So, normally, only the halteres present in the second thoracic segments should give rise to wings, but if you do not have ultrabithorax, the third segment also, the halteres now develop into wings. So, you can see, this is why the gene– homeotic– these are called as homeotic transformations, where one organ can be transformed to another, and that is why the term homeotic genes have been given to this kind of master regulatory genes.

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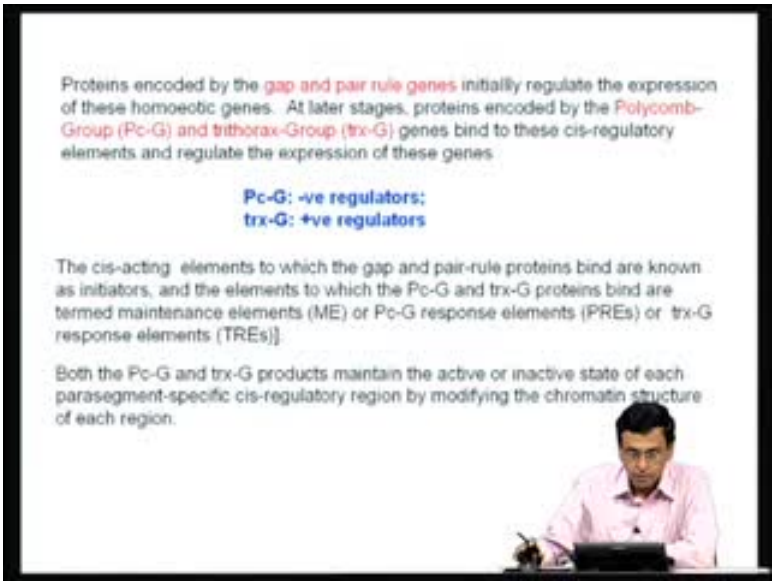


Now, let us now spend some time to understand how is this hox genes or homeobox gene expression is regulated. Now, proteins encoded by the gap and pair-rule genes initially regulate the expression of this homeotic gene. So, as I told, you have the maternal effect genes, which activate the gap, pair-rule genes, and it is these gap and pair-rule gene products, which are our transcription factors, and they go and bind to the promoter regions of this antennapedia or the bithorax complex genes and activate the expression of these homeotic genes.

So, the initial expression of the homeotic genes requires the transcription factors encoded by the gap and pair-rule genes of the developing embryo, but then, these gap and pair-rule genes are expressed only during the early day embryonic development. They are expressed in the later stages, so then how do you make sure that these homeotic genes are expressed only in those places where they have been initiated? They should not get expressed in other regions where the gap and pair-rule genes did not activate them– this is done by two other important groups of transcription factors. So, at later stages, protein coded by… proteins encoded by polycomb-group and trithoracic group genes bind to these cis-regulatory elements and regulates the expression of these genes.

So, there are two important points here– the initial transcription activation of the homeotic genes is carried out, or is because of the binding of transcription factors encoded by the gap and pair-rule genes, but gap and pair-rule genes are expressed only

during the early embryonic development. So, in order to sustain the expression of these homeotic genes in those regions where they got activated by the gap and pair-rule genes, two other groups of transcription factors take over the function of gap and pair-rule genes during later stages of embryonic development. These are called as the polycomb-group proteins and trithorax proteins.

What is important about these two proteins? The polycomb-group, basically, act as negative regulators. Their job is to make sure that the homeotic genes, which they are not expressed in the regions in those regions of embryo by the absence of the gap and pair-rule genes, will remain repressed. The trithorax gene are positive regulators and their job is to make sure that the regions, in which the homeotic gene transcription got activated by the gap and pair-rule genes, continue to get expressed.

So, the polycomb group of proteins act as transcription repressors. They make sure that homeotic genes are not turned on in those regions of embryo where the gap and pair-rule genes did not expressed, whereas the poly the trithorax genes make sure that in those gene the regions of embryo where the gap and pair-rule gene product expressed, therefore, activated homeotic genes continue to remain expressed in those regions.

So, because of these two distinctions, the cis-acting elements to which the gap and pair-rule proteins bind are known as initiators, whereas the elements to which the polycomb group and the trithorax proteins bind to are known as the maintenance elements of the… or polycomb response elements, and trithorax response elements of the homeotic genes.

So, there are two important points here: to initiate transcription of the homeotic genes, you require the gap and pair-rules. They go and bind to cis-acting elements of does DNA elements in promoter regions known as the initiator elements. Once the transcription is initiated during a certain period of the early embryonic development, and once the gap and pair-rule gene products decrease or disappear, to continue to maintain their expression in those regions, the polycomb and the trithorax group of proteins take over the function of the gap and pair-rule genes to sustain. Therefore, to maintain the expression of the homeotic genes in these regions, these two proteins play very important role.

So, therefore, the elements to which the polycomb and the trithorax gene proteins bind are called as maintenance elements, whereas those to be towards the gap and pair-rule

gene products are called as initiator elements, and we have two kinds of maintenance elements: one is called as the polycomb-response elements to which the polycomb proteins bind, and if the polycomb group of proteins bind to these PREs, they repress the expression of homeotic genes, whereas if the trithorax proteins bind to the trithorax response elements, that results in the activation of the homeotic genes. So, I think this is the broad mechanism by which not only expression of the homeotic genes is initiated in specific regions of the embryo, but also make sure that once the gap and pair-rule gene products disappear or get degraded, they continue to express in the same place by these two classes of transcription factors, namely, the polycomb group of proteins and the trithorax group of proteins.

So, both the polycomb group and the trithorax group of products maintain the active and inactive states of each parasegment-specific cis-regulatory regions by modifying chromatin structure of these regions. Lot of literature is available on these proteins, especially the polycomb group of proteins, or known as the chromatin modifiers. So, they have histone methylase or histone deacetylase activity because they are involved in transcription repression, whereas the trithorax proteins are primarily histone modifiers, leading to activation or transcription; so, these are all chromatin modifying proteins.

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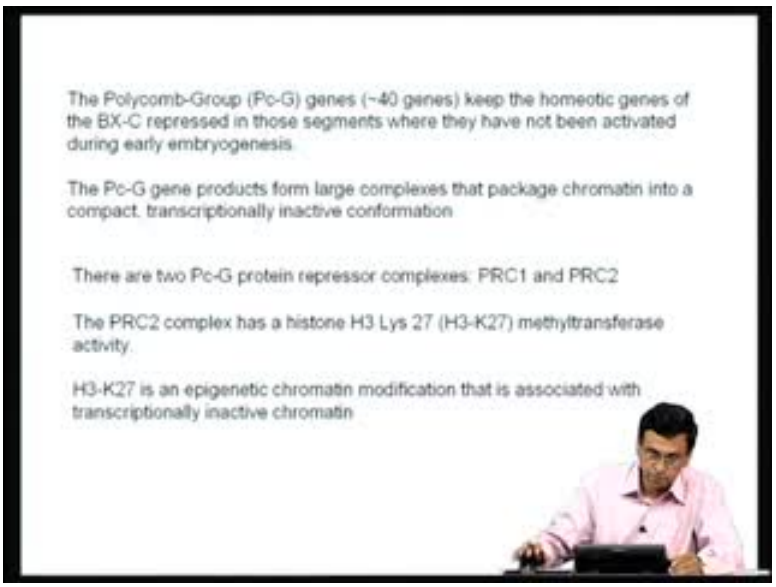


So, the polycomb-group of proteins consists about 40 different genes. They keep the homeotic genes of bithorax complex repressed in those segments where they have not

been activated during early embryonic genesis. I think, these are very important points that one has to understand, if you have to appreciate how beautifully the regulation of development takes place in the developing embryo. So, you can see, the homeotic gene expression is initiated by the products of gap and pair-rule genes in specific regions of the embryo, but once their job is over, their function is taken over by the polycomb and the trithorax group of transcription factors.

So, the job of the polycomb group of proteins is to make sure that in those genes regions of the embryo where the gap and pair-rule genes did not get expressed, in those regions, homeotic genes will not get activated. Therefore, they keep the chromatin under repressed state by modifying histones, so that these homeotic genes will not get activated in those regions.

The polycomb gene products form large complexes that package chromatin into a compact transcription inactive conformation. So, basically, they heterochromatinize those regions, so that these genes– homeotic genes– will not get expressed in those regions. There are basically two polycomb protein repressor complexes, known as PRC1 and PRC2, and the PRC2 complex, actually, has a histone lysine 27 methyltransferase activity, and from our earlier studies, when we start looked at the role of chromatin gene recognition, we discussed how histone methylation and histone de-acetylation play very important roles in transcription repression.

So, one of the mechanisms by which at least one of the polycomb-group of proteins repress the transcription of homeotic genes in those segments where they are not expressed is by methylating the histones, especially at the lysine 27 of the H3, and this histone methylation leads to heterochromatinzation, and therefore, homeotic genes are not expressed in this region. The H3-K27, in fact, is an epigenetic chromatin modification that is associated with transcriptionally inactive chromatin. So, you can see, by methylating the lysine residue of this H3, it converts the chromatin in that region to heterochromatin, and therefore, the homeotic genes in that region will not get expressed.

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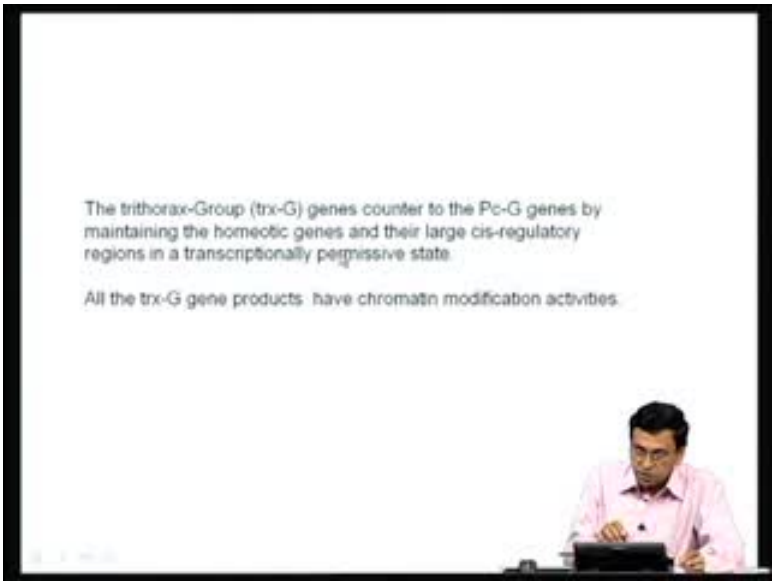


Similarly, the trithorax group of genes counteract the activity of the polycomb group of genes by maintaining the homeotic genes and their large cis-regulatory regions in a transcriptionally permissive state, and all the time, the trithorax group of gene products also have chromatin modification activities, and we can easily guess, since the job of trithorax group of proteins is to activate the expression of homeotic genes, they should, invariably, have chromatin remodeling activities that are known to be associated with transcription activation– it could be either histone acetylation, or it could be inhibition of histone deacetylation, or it could be other chromatin remodeling protein activities that, ultimately, pave the way for transcription activation.

So, the polycomb group of transcription factors or chromatin modifying proteins make sure that the homeotic genes are not expressed in segments where they should not be expressed, whereas the trithorax group of transcription factors make sure that the homeotic genes are expressed only in those places where their transcription was initiated by the gap and pair-rule gene products.

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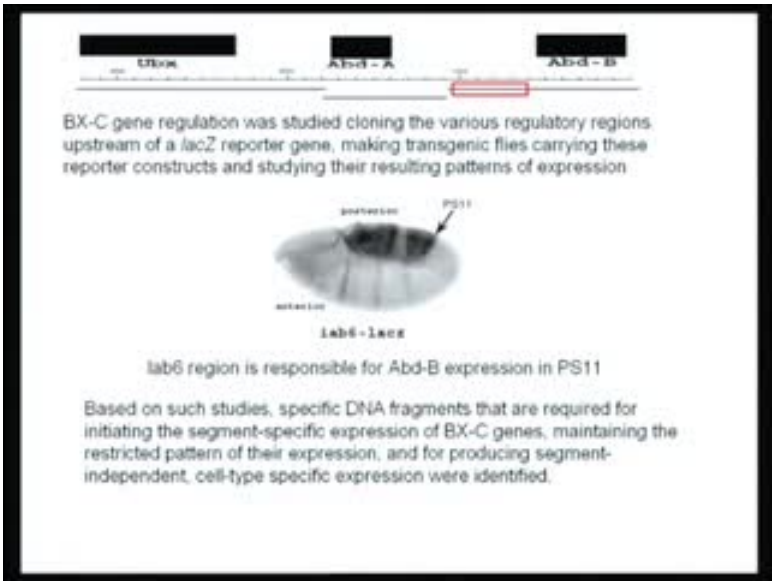


Now, how do we study this? How do we demonstrate that, or how do you map this cis-acting elements of either the trithorax group of proteins or polycomb group of proteins or so on so forth? Here is an example; for example, take the bithorax complex. I told you, the dark boxes here represents the actual coding regions of these three homeotic genes, whereas the thin lines, actually, are the cis-acting elements, which are required for the expression of each of these three genes, and the bithorax gene regulation was studied using by studyin, cloning the various regulatory regions upstream of a lac-Z reporter gene, and making transgenic flies carrying these reporter gene constructs and studying the resulting patterns of expression.

This is a very well-known method of studying the promoter function. So, for example, if you want to study what is the cis-acting region that is involved in the regulation of abdominal B homeotic gene, then you take this region of the regulatory region of corresponding to the abdominal B gene linked to the lac-Z gene. Now, you make a transgenic fly which is expressing this particular gene, and now, you… when you was stain this flies with the beta galactosidase based…, look for beta galactosidase activity, you can see lac-Z expression is only in those places where normally abdominal B is actually expressed, that is, near the parasegment 11, clearly indicating that this region, known as the Iab6 region, is actually responsible for abdominal B expression in the parasegment 11.

So, by using these kinds of transgenic flies, expressing different regions of the cis-acting regulatory elements of this homeotic gene cluster linked to lac-Z gene, the exact cis-acting elements that control the expression of these various homeotic genes have actually been mapped, and based on this, we can, actually, precisely identify these are the regulatory element that are responsible for segment-specific expression of these homeotic genes.

So, based on these studies, specific DNA fragments that are required for initiating segment-specific expression of the bithorax complex genes, as well as maintaining the restriction pattern of expression and for producing segment-independent cell-type specific expression have been identified.

So, all the cis-acting elements, which are essential for segment-specific expression or cell-type specific expression of these homeotic genes, have been identified by making these kinds of transgenic flies, in which a reporter gene is linked to different regions of the regulatory regions of this huge 300 Kb region, and by these kinds of study, you can precisely map what are the regulatory regions, which are essential for expression of these genes in a segment or cell-type specific manner.

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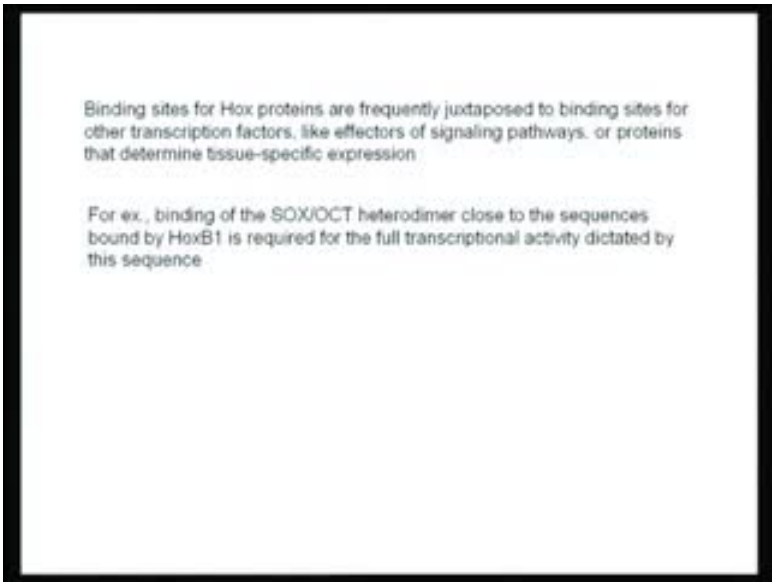


So, having discussed how the homeotic gene expression is regulated, initiated by the gap and pair-rule genes, and then maintained by the polycomb group of proteins and trithorax group of proteins, let us now try to very briefly understand how these homeotic genes

regulate the target genes, and how do they bring about the segment identity. How do they ensure that a leg comes out of thoracic segment, or an eye comes in the head, or an antenna pops out of the head?

Now, the binding sites– again, all these homeo-domain proteins have transcription factors, so the binding sites for these hox proteins are frequently juxtaposed to binding sites of other transcription factors, like effectors of signaling pathways or proteins that determine tissue-specific expression.

Now, the developmental-specific gene expression is pretty complicated. Now, let us not think that just the homeotic gene products alone are responsible for many of these products. Many times, the combined action of the homeotic gene products with other transcription factors are responsible for many of these homeotic transformations, because the promoter regions of many of the target genes that are involved in many of this organ development contain binding sites not only for the homeotic gene products, but also for other transcription factors, which are, actually, activated by other signaling pathways.

For example, the binding of SOX or OCT heterodimer close to the sequences bound to the hox B1 is required for the full transcription activity dictated by the sequence. So, what this tells you is that it is not just homeobox proteins alone which can do the job; they also, many times, have to be combined with other transcription factors, and together by the synergistic action of homeobox proteins and other transcription factors which are activated by other signaling pathways, are responsible for many of the phenotypes that are responsible for this, and that are attributed to these hox genes.

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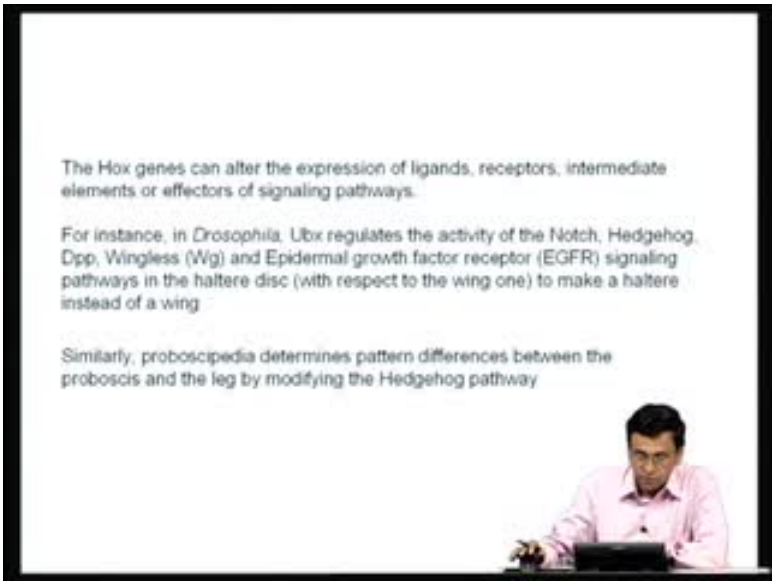


The hox genes can alter the expression of ligands, receptors, intermediate elements, or effectors of various signaling pathways. The functions– the hox genes play a very multi, very diverse roles. They can lead to the expression of ligands, which, in turn, can act as signaling molecules that can, in turn, activate expression of various transcription factors. Some of the target gene products of homeotic genes can be receptors– membrane receptors– to which specific ligands bind, and they activate signaling pathways, or they could be effectors of many signaling pathways. Just to give an example, in Drosophila, the ultrabithorax regulates the activity of Notch, Hedgehog, Dpp, Wingless, as well as epidermal growth factor receptor signaling pathways in the haltere disc to make the haltere instead of a wing.

I told you, ultrabithorax actually responds for the development of… for the halteres, and if you have mutations in the ultrabithorax, the haltere will develop into a wing. To prevent this haltere becoming a wing, the ultrabithorax has to activate a number of signaling pathways. So, the ultrabithorax transcription factor that is responsible for the activation of Notch, Hedgehog, Dpp, Wingless, epidermal growth factor receptor. By activating all these signaling pathways, the ultrabithorax ensures that the haltere remains as a haltere and does not transform into a wing.

Similarly, the proboscipedia homeotic gene determines the pattern differences between proboscis and the leg by modifying the Hedgehog pathway. So, this is the reason why, in

the previous class, we studied many of the signaling pathways like the wnt, Hedgehog, Notch, and so on so forth.

So, ultimately, many of this reeling pathways are actually involved in the mediation of hox gene activities. So, many of the hox gene products– many of the homeotic gene products– actually activate many of the signaling pathways, and that is how they act as transcription factors, and that is why they bring about specific phenotypic effects.

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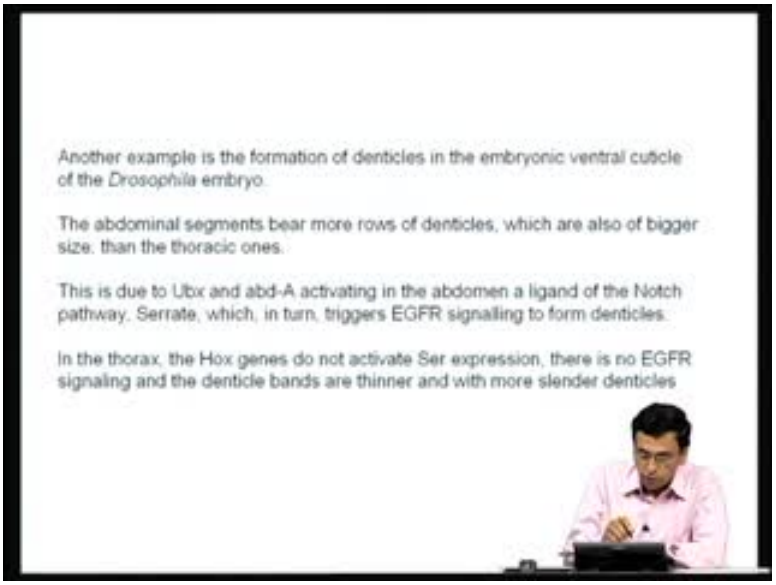


Another example of this– the formation of denticles in the embryonic ventral cuticle of the Drosophila embryo. The abdominal segments bear more rows of denticles, which are also bigger size than the thoracic ones, the denticles are present both in thorax and abdomen, but the denticles row up in the abdomen are much bigger than that of the thoracic ones. This difference in the denticle size and expression is because the ultrabithorax and abdominal A activating the abdomen A ligand for the Notch pathway called as serrate, which, in turn, triggers EGF receptor signaling to form denticles.

So, the formation of denticles in the thoracic region and abdominal region is controlled by the ultrabithorax homeotic gene. How does it do it? The ultrabithorax controls the formation of these denticles in the thorax and abdomen by actually activating the expression of gene, which coats for the ligand for a Notch signaling pathway called as serrate, which, in turn, activates the epidermal growth factors set of pathways. So, in the thorax, the hox genes do not activate the serrate gene expression. Therefore, no EGF

receptor signaling is formed, and therefore, the denticle bands are thinner and more slender.

So, you can see, when I say the denticles, which are nothing but small projections, the denticles are thinner and smaller in the thorax, but bigger in the abdominal region. That is because the hox genes do not activate– the ultrabithorax hox gene does not activate the serrate expression in the thorax. It activates the serrate expression only in the abdomen region, and as a result, the serrate now goes and binds to the Notch receptor and activates signal transduction pathway, leading to the production of epidermal growth factor receptor, which, in turn, is responsible for the production of the denticles on the abdominal region.

So, the non-production of the serrate ligand, which is a ligand for Notch in the thoracic region, is responsible for the smaller denticles in the thorax, whereas the activation of the Notch signaling pathway by the production of serrate by ultrabithorax is responsible for the production of denticles– large denticles– in the abdominal region. So, this is how the ultrabithorax controls differential gene expression in the thorax and the abdomen.

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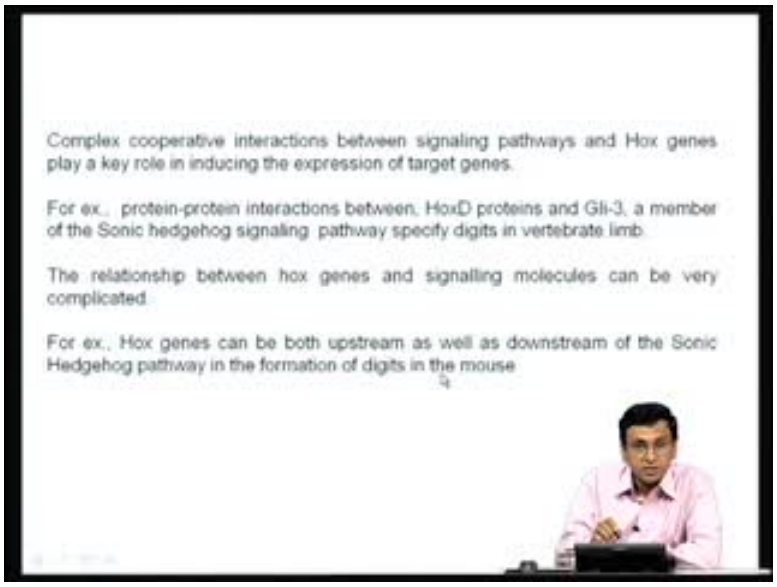


Complex cooperative interactions between signaling pathways and homeobox genes play a very key role in the expression of target genes. For example, protein-protein interactions between the HoxD proteins and Gli-3, a member of this Sonic Hedgehog signaling pathway, specify digits in the vertebrate limb.

We will discuss some of these things in detail, as we go along. The formation of digits, for example, how a limb is formed or how digits are formed, is again controlled by a homeobox group of proteins called as HoxD, which along with another protein called Gli-3, which is a member of the Sonic Hedgehog signaling pathway; we have discussed this in the previous class.

So, the relationship between hox genes and signaling molecules is often very complicated. I just gave you two or three examples of how the hox genes cooperate with other signaling pathways, and it is these complex interactions that, ultimately, is responsible for the development of various structures during the embryonic development. And other important thing is the hox genes can be both upstream as well as downstream of some of the signaling pathways and formation of digits; that means, the hox genes can either activate the components of signaling pathway, or the components of the effector molecules arising out of signaling pathway can go and activate hox genes. So, the hox genes can be either upstream or downstream of some of the signaling pathways.

The hox gene products may activate Sonics signaling pathways like the Hedgehog pathway, or the products of Hedgehog signaling pathway can go and activate the homeotic gene expression. So, basically, what I am trying to tell you is that the regulation of homeotic genes, as well as the mechanism by which these homeotic genes activate the expression of various target genes is very complicated. It varies from segment to segment, region to region, as well as in space as well as time. So, by complex interactions involving various signaling molecules, various signaling pathways, other transcription factors and hox is responsible for the ultimate development of the various embryonic structure during the embryonic development.

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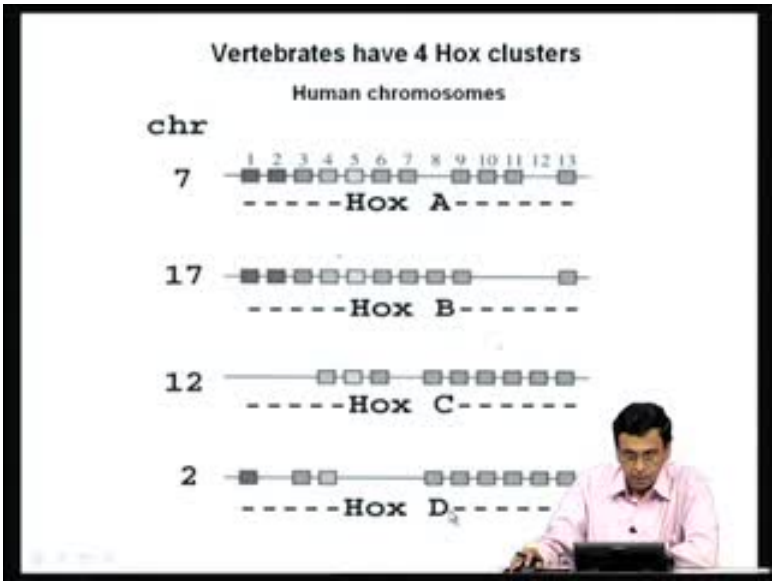


So, in the Drosophila, we told there are two major homo block clusters. Now, in the ultrabithorax in the antennapedia complex. When you go to vertebrates, it becomes more complicated. Many of these homeotic genes got duplicated, and therefore, they vertebrate there about four hox clusters were localized on chromosome 7, chromosome 17, chromosome 12, and chromosome 2, and these are the various hox clusters. There are about 38 to 40 different homeotic genes in the mouse, and these are called as hox A cluster, hox B, hox C, and hox D.

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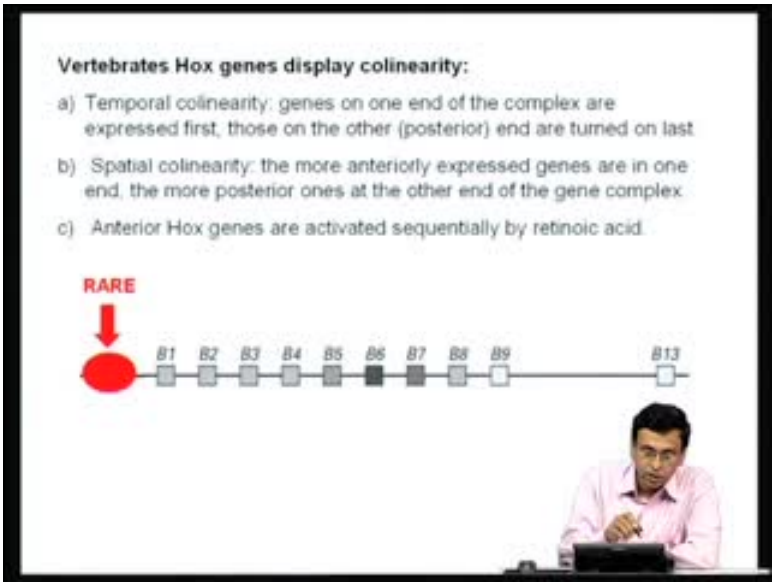

The vertebrate hox genes also display colinearity, just as in the Drosophila. We know that they are arranged in a linear fashion from heterotail fashion; the anterior genes are expressed in the anterior regions, and the posterior genes are expressed in the posterior regions. So, the chromosomal localization is very well collinear with the region where they express in embryonic development. The same way, in the case of vertebrate hox genes, there is what is called as a temporal colinearity. Genes on one end of the complex are expressed first, and those on the other end are turned last.

So, on a chromosome, the genes which are at the 5 prime end are expressed first during development, and genes which are obtained the 3 prime end are expressed in the last. So, this is called as a temporal expression. That means, in time, they are different. Similarly, in space also, they are different. The genes, which are the anteriorly expressed, are more expressed, much higher levels than genes, which are, expressed the posterior end.

The reason why some of these things is that many of the anterior hox genes are activated by morphogens such as retinoic acid. For example, if you take the hox B cluster, there is a nice retinoic acid response element the 5 prime of the gene. Therefore, during embryonic development, some cells start producing the retinoic acid, which is a morphogen. Now, retinoic acid binds to retinoic acid receptor; that now goes and binds to retinoic acid response element and activate the expression of this hox gene cluster, and initially, the anterior genes get activated first at high levels, and slowly, the retinoic acid gradient also is slower to a posterior region. Therefore, the posterior genes are less retinoic acid responsive compared to the anterior genes.

So, molecules like retinoic acid play very important role. We will discuss some of this during the later stage of this talk, and many of these molecules like retinoic acid regulate embryonic development by actually activating the expression of some of the important transcription factors, that as the homeotic genes.

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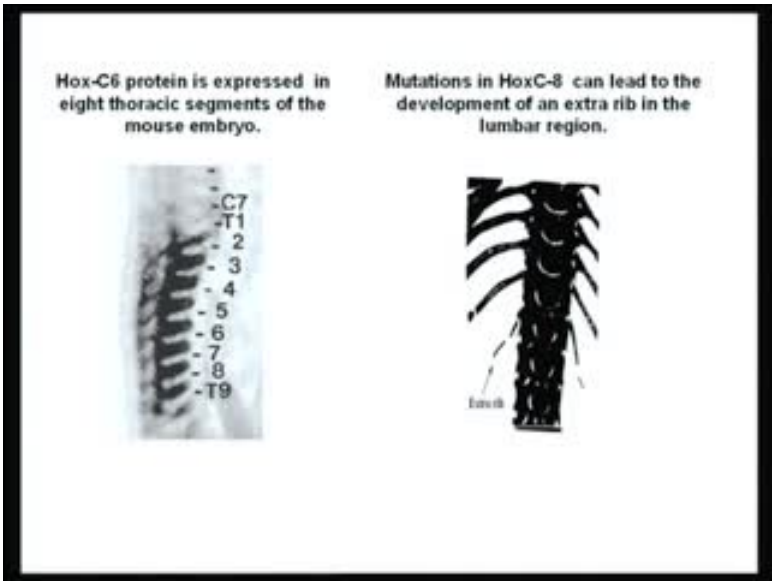


For example, I will just give you a few examples to just tell you how the hox gene expression is very important. For example, if you have a mutation in the hox-C6 protein, we have abnormal development of the vertebral column, because the hox-C6 protein is actually expressed in specific thoracic segments during embryonic development, indicating that they are responsible for the formation of the thoracic vertebrae, and in fact, we have mutations, someone or some of the hox C clusters, for example, we have mutations in hox-C8, it can lead to development of an extra rib in the lumbar region, where, normally, you do not see a rib in the lumbar region, but we have mutations in the hox-C8; it develops the extra rib.

So, the function of the hox-C8 is to make sure or repress the formation of rib during the in the lumbar region, so we have mutations. Now, this repression is not there; therefore, we start forming ribs in the lumbar region as well, just to tell you that these hox genes play very important role in the formation of various (( )) structures.

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Similarly, if you have mutations in specific homeotic genes, it can lead to abnormal limb development. These are just some of the pictures that show you, by depending upon which mutation had worked about hox gene, you can have various kinds of malformations in the limb development.

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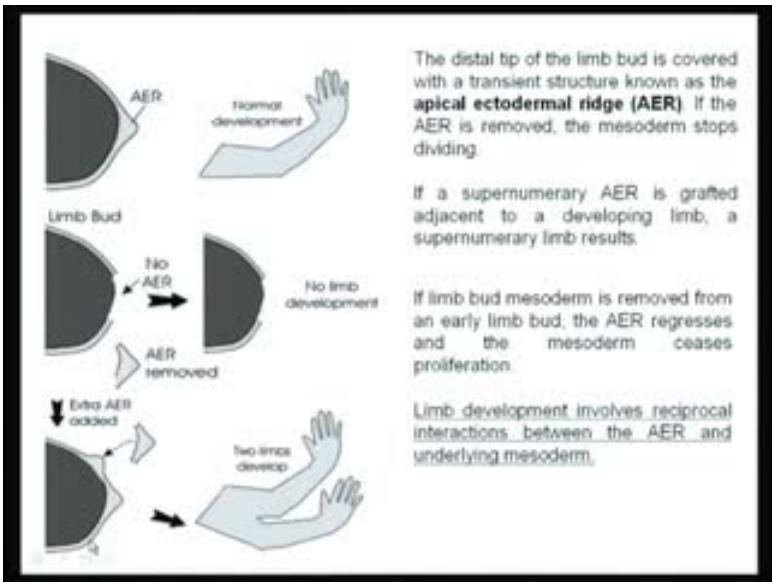


We will now take, maybe, limb development or eye development as an example, just to tell you how nicely the expression of transcription factors play important role in the regulation of embryonic development. Let us take, for example, how the limb is formed.

We know we have a limb which contains of five digits; let us see how these digits are produced during embryonic development. Now, the first thing that happens during the limb development is the formation of what is called as a limb bud.

Mesodermal somites, during the embryonic development, they actually migrate to a region, either in the anterior or the posterior regions, to form legs or the hands, and the first thing that is important for the formation of this limbs where the anterior or posterior limbs is the formation of a limb bud. Now, in the limb bud, there is a very important region that distal tip of the limb bud you consists of a very important structure known as the Apical Ectodermal Ridge or the AER. See, now why is this important? If you cut off this Apical Ectodermal Region or AER in a developing embryo, you do not get limbs, clearly indicating that this apical ectodermal region is the one that, ultimately, is responsible for formation of our limbs, including digits.

Very interestingly, instead of removing the apical ectodermal region, if you now put an extra, if you now cut off this AER from one embryo and put it next to the apical ectodermal region on another embryo, instead of one hand you will now develop two limbs, clearly indicating that this apical ectodermal region plays a very important role in limb development. If you remove these, normal limbs are not formed; if you add an extra AER, you get two limbs instead of one.

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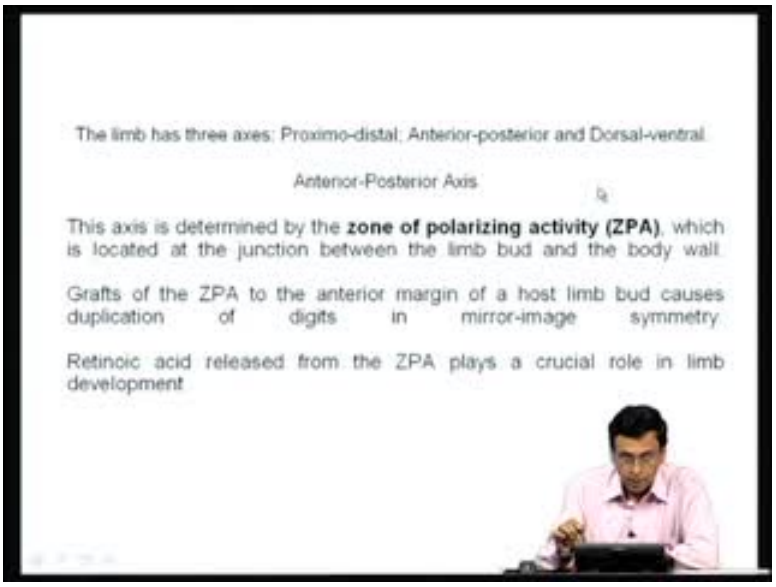

So, limb development, it turns out, it is not just the apical ectodermal region, but the black region shown here, which is nothing but the mesoderm which is lying beneath the apical ectodermal region. This mesoderm also is very important. Instead of apical ectodermal region, if you remove the mesoderm from this early limb bud, then the formation of the apical ectodermal region as well as limb development is suppressed, indicating that limb development involves reciprocal interactions between the apical ectodermal region and the underlying mesoderm.

So, as the limb bud is formed and starts developing to a full-fledged limb, complex interplay takes place between the apical ectodermal region as well as the mesoderm region just below it. Let us try to understand what is these interactions that takes place that ensures normal limb development.

Turns out, for a proper limb to be formed– your hand or legs to be formed properly– there are three axes that are required: the proximo-distal axis, anterior-posterior axis, dorsal-ventral axis. Now, let us just concentrate on one axis; how is an anterior-posterior axis formed? This anterior-posterior axis determined by what is called as a zone of a polarizing activity or ZPA, which is located at the junction between the limb bud and the body wall. Now, if you now take these grafts of ZPA or this zone of polarizing anterior to the anterior margin of host limb bud, it causes duplications of digits in a mirror-image symmetry.

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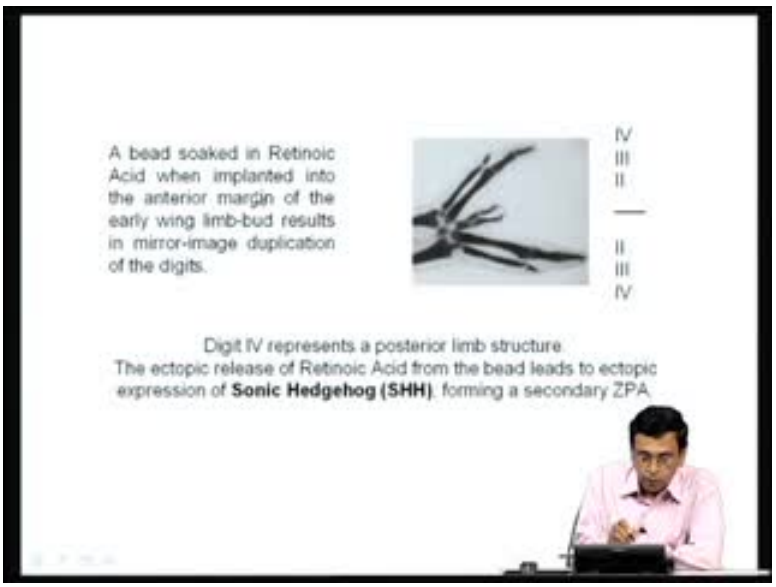

What is this zone of polarizing activity? The zone of polarizing activity is nothing but these are the cells, which actually produce the retinoic acid. Now, the picture, when the picture, when I show next, it becomes very clear; we can see here, if you take this zone of polarizing activity and then duplicate it, you get extra pair of limbs, which are actually mirror-image of each other, and then people actually found out this zone of polarizing activities able to cause this kind of a limb duplication, primarily because this zone of polarizing activity actually produce– cells in this region actually produce– retinoic acid.

So, to prove this point, what they do– you simply take a bead, which is soaked in retinoic acid, and if you now implanted the anterior margin of the early wing bud, now, you can see, a mirror image duplication actually takes place. So, you have 2, 3, 4, and if you now put a retinoic acid bud here, again, 2, 3, 4 is formed, clearly indicating that retinoic acid acts as a powerful morphogen, and is actually responsible for a developmental program involved in the formation of these three digits. Normally, you get only this if you now duplicate another region of retinoic acid producing region, instead of forming one set of these three digits, another set of threes are formed here.

It turns out, the mechanism by which this retinoic acid brings about this kind of a limb duplication is, actually, by activating the expression of the Sonic Hedgehog signaling pathway. So, retinoic acid not only acts the… activates homeotic gene expression, but also activates other signaling pathways. That is why, I told you, embryonic development is a complex interplay of transcription factors, signal molecules produced from diverse signaling pathways.

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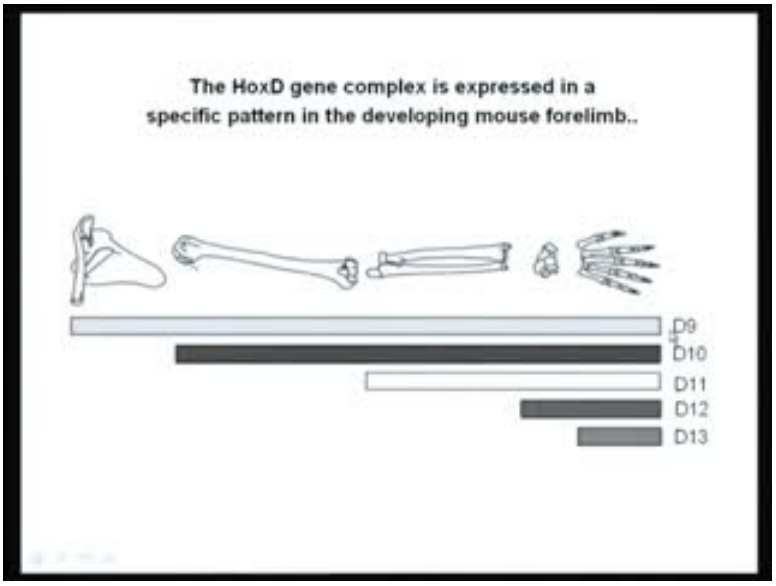


This slide again shows you how the hox gene expression plays a very important role during development– one of the hox clusters in mouse, called as the hox D gene, plays a very important role in the limb development, and very interestingly, this hox gene is also expressed in a different regions of the developing limb.

For example, the hox-D9 homeotic gene is expressed throughout the limb from top to the bottom, whereas the D10 homeotic gene is suspected only in this region, whereas D11 is expressed only in this region D12, here, and D13 expressed only in the distal part, at least, only in the digits of the developing embryo. So, this is what is called as the regulation of spatial expression of the homeotic genes; that means, in space, the expression is different– some are expressed throughout; some are suspected only in specific regions of developing embryo. This called a regulation of spatial expression of genes during development; not only that, their temporal expression is also regulated.

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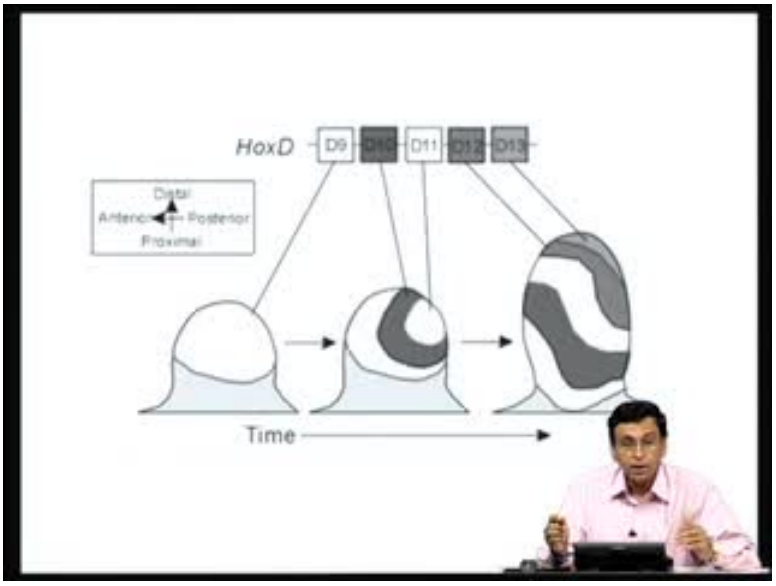


For example, the D9 gene is expressed very early during embryonic development all over, whereas the D13 is expressed at the later stages of embryonic development, that too, only at the distal region. So, the expression of hox gene is regulated both in space as well as time; so, this just tells you the D9 is expressed all over; the D10 expressed only in this region; D11 in this region; D12, here, and D13 only in the digits, and many of this hox gene expression are regulated by morphogens like retinoic acid.

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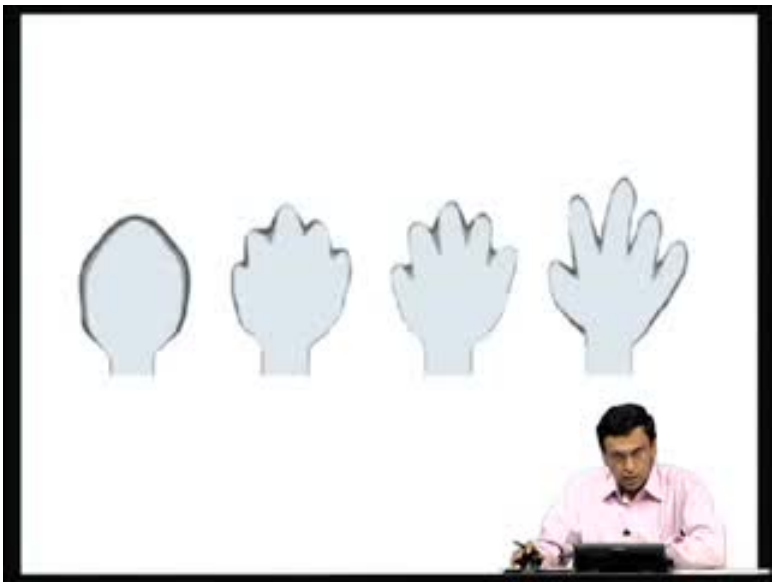

The other important thing that we have to remember in the development of limbs is that when you develop limbs, as cells are growing to form these various digits, the cells, which are in-between digits, have to be degraded or have to be destroyed. So, a very important regulation– again, activation and repression of transcription factors make sure that cells in between the digits undergo apoptosis, so that you get proper digits and you do not have a web. In fact, if you now take bat, for example, this apoptosis does not occur in the back, and that is why you have a web in the back limbs, because this apoptosis does not takes place there, whereas, in higher vertebrates, because cells in between digits undergo apoptosis, you do not have the web, and therefore, you get digits. So, very beautiful regulation of gene expression takes place to make sure some of this embryonic development takes place normally.

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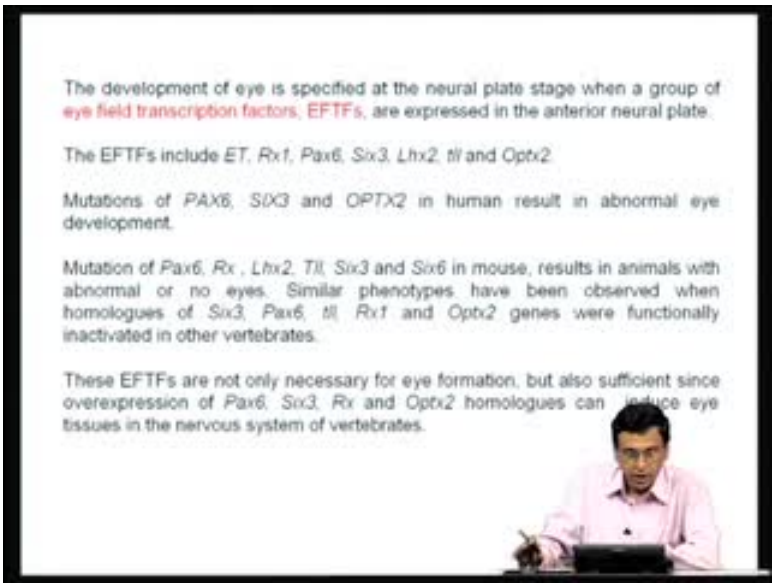


I will just give you one more example about eye development. Again, a whole bunch of transcription factors called as eye field transcription factors or EFTFs play very important role in the normal development of eye. These eye field transcription factors include ET, Rx1, Pax6, Six3, Lhx2, tll, optics– Optx2– and so on and so forth.

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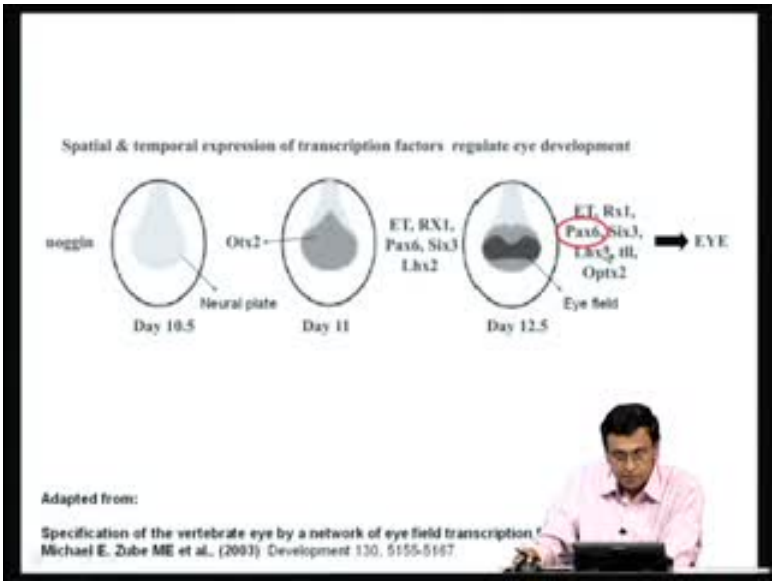


If you have mutations in any of these things, it results in the abnormal eye formation, just to give one example. And this just tells you that again, spatial and temporal expression of all these transcription factors involved in eye development plays a very important role. During day 10.5 of the mouse embryonic development, you have specific expression of the certain factors like noggin, which results in the formation of neural plate; then, another transcription factor called Otx2 is expressed, and the around day 11, this, again, this expression of other transcription factors, and ultimately, an eye field is formed, and ultimately, it results in the expression of eye.

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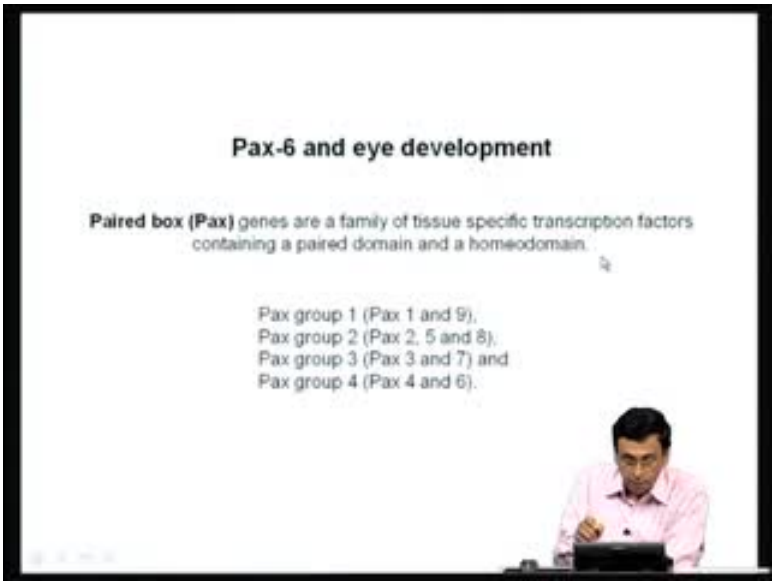

Just to give you one example of how these transcription factors are very important, if you now take the example of one of these transcription factors called pax 6, pax 6 consists of a family of tissue specific transcription factors; they are called as paired box genes.

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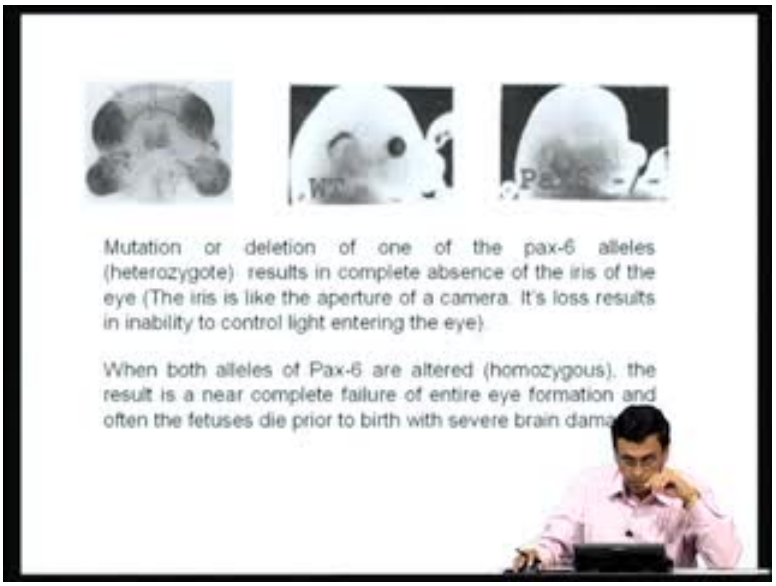


They go for a number of transcription factors, of which let us just look at what does pax 6 do. If you now duplicate the expression of the pax 6, if you now, normally, pax 6 is expressed in the head to form eyes in the head of the Drosophila. If you now express it in the mandible region, instead of mandibles, instead of mouth parts, you get an extra pair of house in the eyes in the mouth, or if you go to the vertebrate like the mouse, if you have mutations in the pax gene, you see an embryo without eye, clearly indicating that these are all master regulators. Pax6 is one of the master regulators that controls the entire development of eye.

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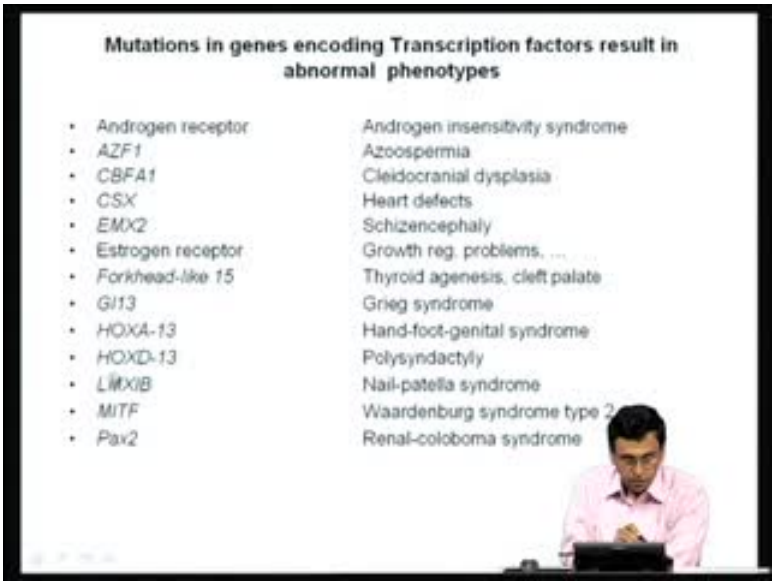


So, I can go on and on like this. The regulation of gene expression in embryonic development is a fascinating area of modern biology, and the last three decades, especially after the advent of molecular biology and novel cell biology tools, excellent research has been done to understand how spatial and temporal expression of various transcription factors is responsible for the normal development of a embryo, and using model organisms such as Drosophila, mouse, Caenorhabditis elegans, zebra fish, a number of research groups have dissected the various molecular mechanisms by which organogenesis and embryonic difference, cell differentiation and development takes place, and in fact, we have… we have fairly good knowledge about how various embryonic structures are formed and what are the various transcription factors, which are involved in the regulation of embryonic development.

So, I just give you, here, a list of transcription factors, and if you have mutations in this transcription factor, what kind of abnormalities or what kind of disease phenotypes will develop. Androgen receptor leads to androgen insensitivity syndrome; if you have mutation a transcription factor called AZF 1 results in azoospermia; if you have mutations in forkhead-like, it results in a cleft palate as well as thyroid agenesis. Similarly, if you have mutations in the hox D13, you get polysyndactyly, that is, limb deformities; if you have mutations in the homeo box A13 homeotic gene hand-foot-genital syndrome is formed, and so on so forth. As I said, pax 2, again, affects eye development, pax 3 different times pax 6. We just discussed the pax 6. In fact, I forgot to

mention if you have, actually, mutation in pax 6, it leads to a very important disease called as aniridia. That means the iris– the eye– the center of that we have an iris, which is nothing but it acts like an aperture of a camera. Now, depending upon the intensity of the light, the aperture either opens up or closes; if you very bright light, the aperture shrinks, and if we have very low light, the aperture becomes large. This development of eye iris– this actually developed controlled by this pax 6 gene– so if you mutations in pax 6, especially heterozygotes condition, the iris is not formed. So, you have humans without iris. But, if you have both alleles of pax 6 mutated, then it is lethal– those embryos do not survive at all– they die, so clearly indicating that these transcription factors– pax 6– play a very important role during the eye development.

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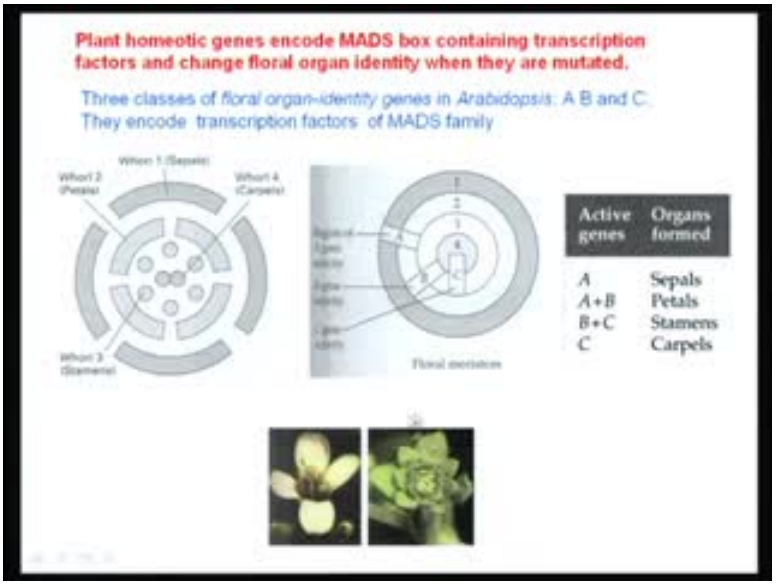


What I have not discussed in this class is another very fascinating area of embryonic development, especially in plant. Just as we discussed homeotic genes in animals, there are also homeotic genes, which regulate floral development in plants. So, the plant homeotic genes contain what is called as MADS box, which is a binding domain of these transcription factors, and they are responsible for floral identity. You know, the flower of plant consists of sepals, petals, the stamen, and the carpels and stamen and the pistil– all these four different, four organs, their expressions is controlled by homeotic genes.

I would like to not go into details of it, because we do not have time. Again, if you know, for example, there are about three major homeotic genes that are responsible for the

floral development in plants that are called as A, B, and C genes. They are all nothing but transcription factors; for example, the A homeotic gene is expressed in both sepals. During the floral development, they are expressed in both these two first and second whorl– the B G– is expressed in the second and third whorl– the C G– is expressed in the third and fourth whorl, and if you now look at what is the importance of these genes, the A homeotic gene is required for the formation of sepals; A and B genes are required for the formation of petals; B and C is required for formation of stamens; and C alone is required for the formation of carpels.

So, again, just like we had the homeotic genes required for the various organ formation in the animals, we have homeotic genes in plants which are required for the formation of various floral organs, and if, in fact, have mutations in the homeotic genes, it can convert one whorl into another– for example, you can convert carpels into petals into sepals, sepals into petals. Again, homeotic transformations– just as we saw, this is just a picture that shows how, for example, you have mutations in some of these homeotic genes, the entire flower looks like green, because everything has been converted into sepals.

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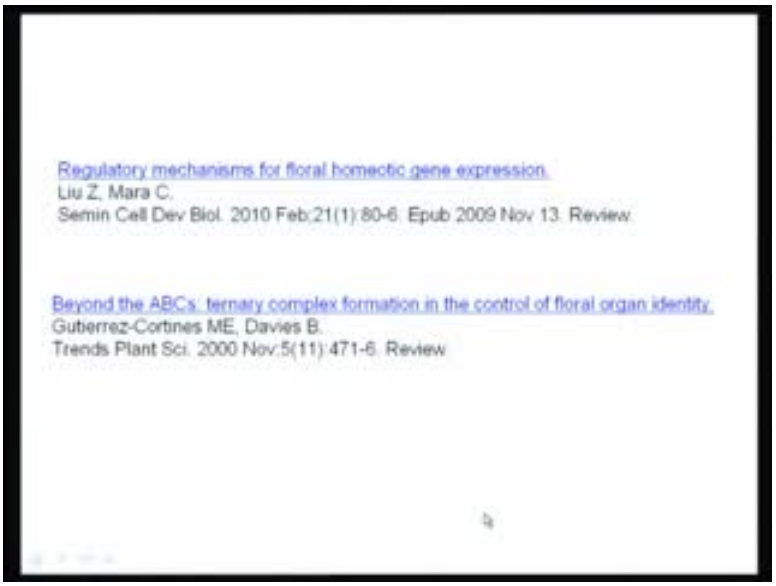

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I just given some of the references for you to go and then read little bit more about how homeotic gene expression is regulated during plant development. These references can be of help. Now, I will also giving you some of the key papers; for example, this paper in 1990 by Huey Lewis, who actually got the Nobel Prize for dissecting the Drosophila embryonic development as well as homeotic genes. It is a very classic paper; one must read this paper to really understand. There are couple of reviews, which gives a very important details about how differential gene expressions regulates embryonic development.

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A few of the references are listed here, and one can go through some of these things to understand how differential gene regulation plays an important role during embryonic development. I think I will stop here. I think, with this, now, you have more or less completed how differential gene expression takes place during embryonic development.

Thank you.