Eukaryotic Gene Expression: Basics and Benefits

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Lecture No. # 24

Gene regulation during Drosophila development

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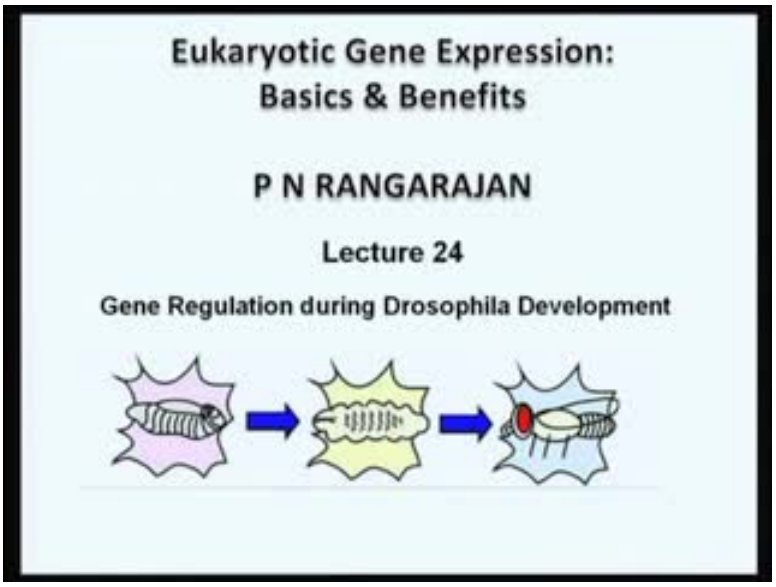

Welcome to this lecture number 24 on this course on eukaryotic gene expression basics and benefits. This lecture number 24, we are going to spend some time to discuss about what is the importance of gene regulation during embryonic development. We are going to start with drosophila melanogaster, the fruit fly and try to understand how from the fertilized egg to the adult fly, how gene expression plays a very important role in various stages of embryonic development. We chose a drosophila is a smaller system to begin these lecture series on the importance of gene regulation embryonic development because drosophila has been an excellent model system to understand embryonic development. It is a versatile and easy to work with compare to other more complicated membrane systems like mouse and so on.

So, a longer fundamental concepts on the importance of genes and the role in earlier embryonic development, how come from drosophila. So, what we will do in the next few

minutes is to understand how from the stage of an egg through larva and finally, metamorphosis into an adult fly. How gene expression plays a very important role and how spatial and temporal expressions of genes at various stages of development play a key role in the conversion or in the development of a fertilized egg into another fly? What is the importance of the gene expression?

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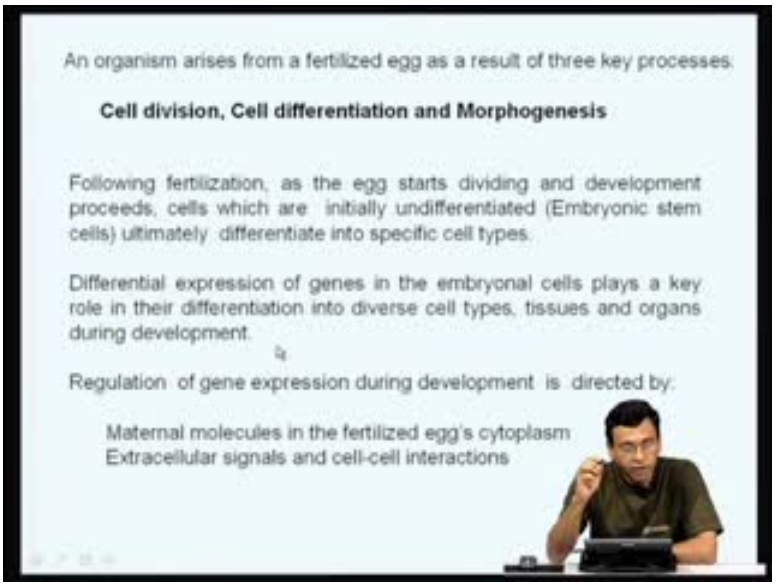

So, we all know that an organism arises from a fertilized egg as a result of three key processes. One is the egg has to divide, so cell division is very important and once a cell division proceeds and after a certain stage of cell division, the cell start undergoing differentiation. So, this is what is called as the cells which are actually totipotent, to begin with. They then become loose the totipotency and they become highly differentiated, so that each cell type now metamorphose or differentiates into distinct tissue types or distinct organs. Finally, morphogenesis takes place ultimately leading the formation of specific organs of tissues and so on, so leading three formation of adult.

So, cell division, cell differentiation and morphogenesis are the three key processes which are involved in embryonic development and in all these processes gene expressions play a very important role. So, regulation of gene expression is very key in the normal and hormone is development of an egg into an adult.

So, following fertilization as the x starts the dividing and development precedes cells which are initially undifferentiated. That is what we now call as embryonic stem cells

which are totipotent. They ultimately differentiate into specific cell types, so this is the crux of embryonic development.

The differential expression of genes in the embryonic cells plays a very important role in their differentiation into diverse cell type tissues and organs during development. So, this is what we are going to try to understand how differential expression of genes plays a very important role in the development of an embryo and the regulation of gene expression during development is directed by two important events. One is maternal molecules which is present in the fertilized eggs cytoplasm and extra-cellular signals as well as cell-cell interactions. So, this was some of the three key players that play a very important role in the regulation of the gene expression embryonic development.

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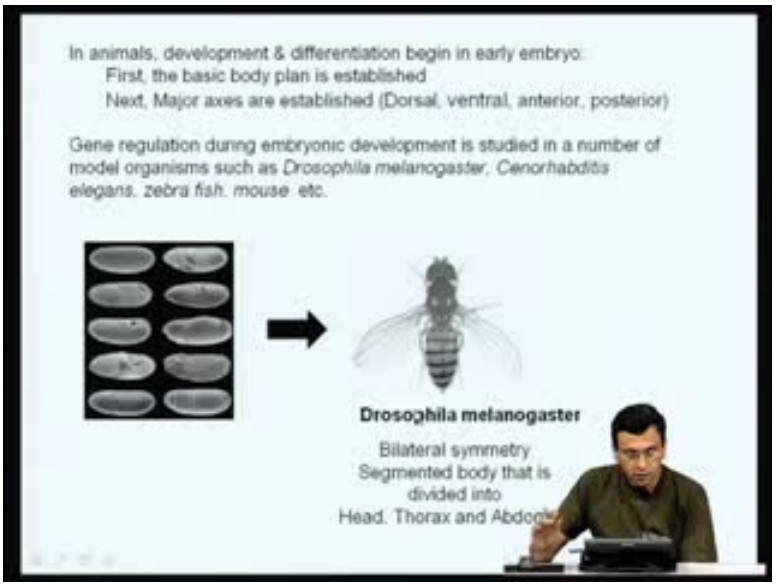

So, let us now try to understand how these processes regulate embryonic development. In animal, developmental differentiation begin early in the embryo, first the basic body plan is established when you say very basic body plan, the anterior, posterior, axis dorsal, ventral so on and so forth.

So, the major axes are established and then gene regulation during embryonic, we studied a number of model organisms which has such as drosophila melanogaster, cenorhabditis, elegans which is a worm, zebra fish, mouse and so on. Now, we can also study in human now. So, among all these organisms I chose drosophila melanogaster to understand the importance of gene regulation expression because a lot of work initially

was done in this organism in its excellent model system for understanding gene regulation. Many of the genes which are discovered in drosophila to be involved in the regulation of gene expression embryonic development for later found to be present in other organisms as well including mouse and humans.

So, drosophila played a very important role in understanding the role of gene expression in understanding in the early embryonic development. So, what I am going to do in the next few minutes to just introduce the key concepts and some very important examples of how differential regulation of genes plays an important role during embryonic development. So, we know that from the fertilized egg, you have a series of events that ultimately leads to a larva and then to a pupa.

Then finally the larva metaphorsis into a fully developed adult fly which contains three segments, the head, thorax and abdomen and a pair of wings which has a bilateral symmetry, a segmented body that is divided into head, thorax and abdomen. So, what we will try to understand in the next few minutes is to what are the key events? How gene expression plays an important role in the development of this egg into an adult fly?

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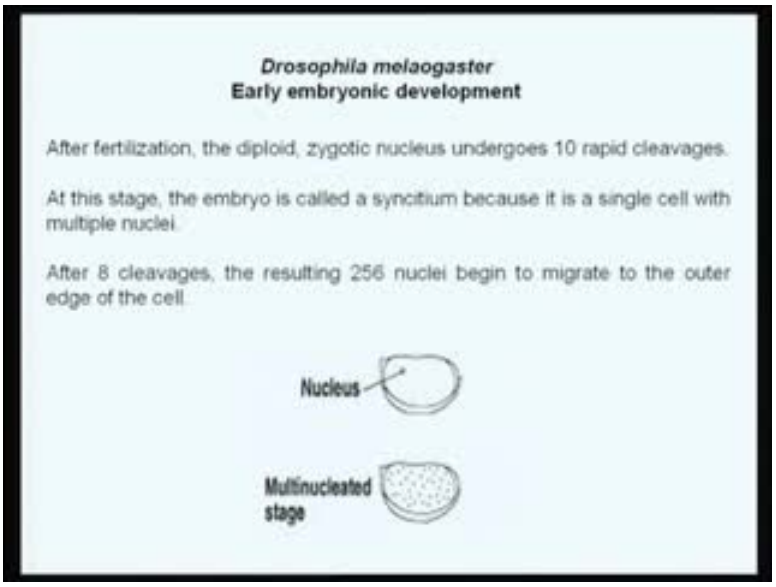

After fertilization, the diploid zygotic nucleus in the case of drosophila, it undergoes the zygotic nucleus undergoes about 10 rapid cleavages. The very important thing of about this cell division is that the (()) kinases or the division of the nucleus is not followed by

cytokinesis. So, this ultimately results in an embryo which is actually called as a syncitium because it is a single cell with multiple nuclei.

So, only the nuclei divides but each nuclei division is not accompanied by cytokinesis and therefore, the nucleus are free floating in the single cell. So, as a result of this, 10 rapid cell divisions you get a multi-nucleated cell after about 8 cleavages but 256 nuclear formed and then slowly this nucleus start migrating towards the periphery of the embryo.

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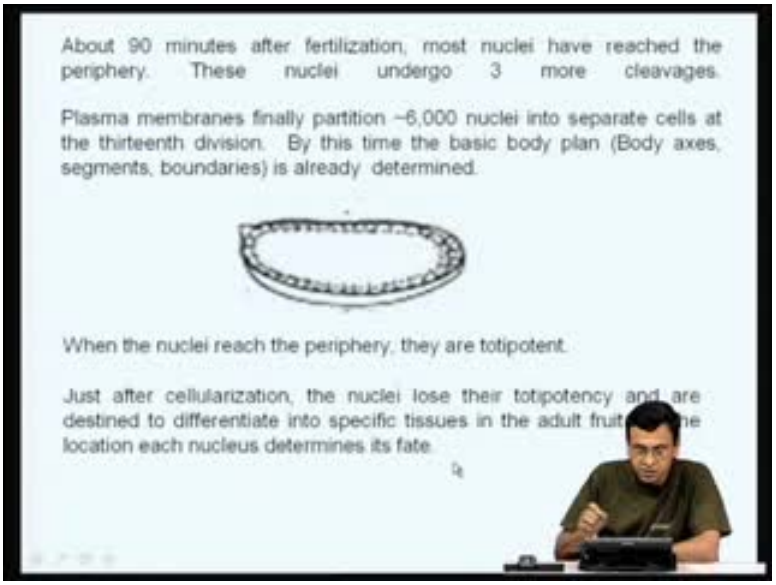

So, you have a nucleus which divides. So, you have a multi-nuclear stage and soon this nucleus start migrating towards periphery of the egg and about 90 minutes after fertilization, most nuclei have reached the periphery. These nuclei then undergo three more cleavages and finally, the plasma membranes partition this about 6000 or nuclei into separate cells at the thirteenth division. So, till the thirteenth divisions, we have multi-nucleated egg and then the cytokinesis takes place.

The cell membrane is plasma membrane is partition the nuclei and this is the time the basic body plan that is which embryo part has to become an anterior and which embryo have become posterior, dorsal, ventral axis as well as segmental identity. All these things get established, so when the nuclei reach which the periphery they are still totipotent but immediately after the cellularisation, the nuclear lose their totipotency and are destined to differentiate into specific tissues in the adult fly and the location of each nucleus embryo determines the fate.

So, this is where the maternal affect genes or the genes which are expressed during the oocyte. In the during the oocyte development and deposit x cytoplasm play a very important role in the early embryonic development and the fate of the nucleus actually depends on which region that nucleus as migrated and along with the cytoplasm present in that region actually determines what is the fate of this nuclei and what is the destined to become.

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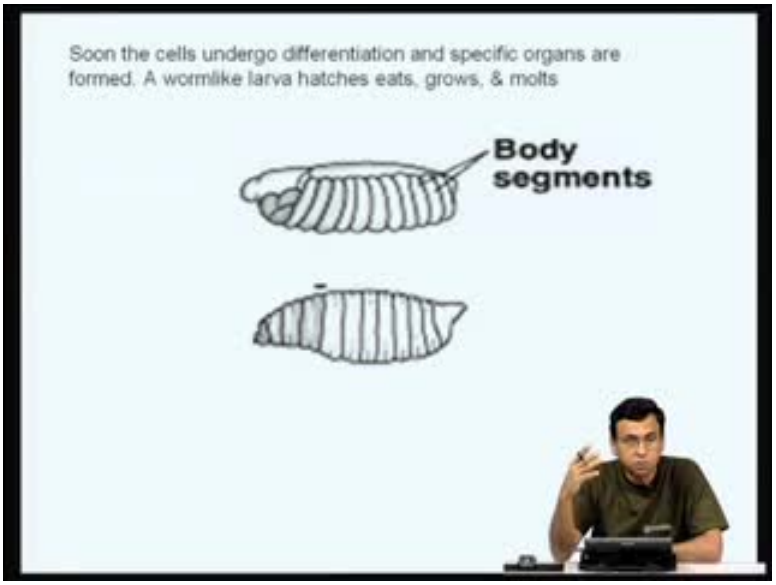

So, to quickly cover what exactly happens, soon the cells undergo differentiation, forms a specific organs and a worm like larva hatches which eats, grows and finally under goes molting and then goes to a pupa stage. Then pupa finally undergoes metamorphoses and an adult fly emerges with head, thorax and abdomen a pair of wings and legs.

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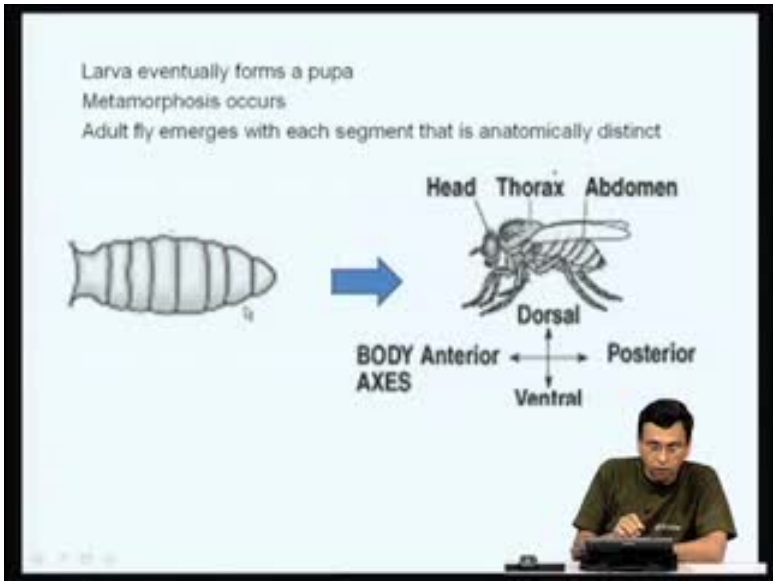

So, what will now do in the next few minutes to understand what are the thin molecular that actually takes place in this entire metamorphoses of a larva into a fly and also during the early embryonic development? How are the dorsal ventral regions determined? How the anterior posterior axes are formed? What are the genes that play a very key role in determining all these structures at the early during the embryonic development? So, what are the molecular events that are involved in the early embryonic development is what we are going to discuss.

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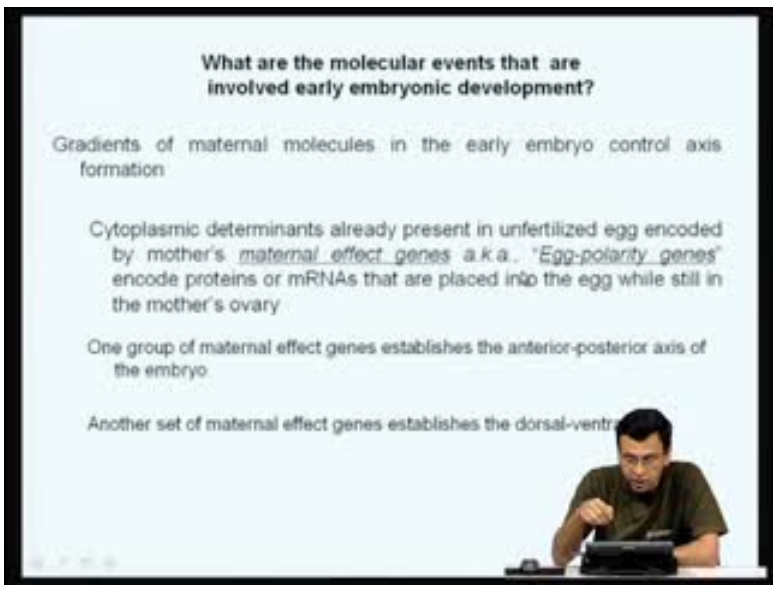

Now, what is important is gradients of maternal molecules in the early embryo control the axis formation. Cytoplasmic determinants which are already present in the unfertilized egg encoded by the mother's maternal effect genes also known as the egg-polarity genes encode proteins or messenger RNA's that are placed into the egg while still in the mother's ovary is a very important aspect. So, these are what are called as the maternal of a genes or egg polarity genes.

These genes are transcribed during the oocyte development in the ovary and the messenger RNA's from these are actually (( )) that in the cytoplasm, some of these messengers RNA's the person anterior, some of the m RNA's deposited in the posterior and sometimes there is an gradient of these messengers along the anterior-posterior axis or the dorsal, ventral axis. This gradient or this specific localization of messenger RNA's in the x cytoplasm plays a very important role in determining the anterior -posterior axis of the dorsal ventral axis.

So, one group of maternal effect genes established anterior-posterior axis of the embryo. Another set of maternal genes establish the dorsal ventral axis. So, the key point that we like to emphasis series is that transcription of genes in the oocyte during development in the ovary, the genes known as the maternal effect genes or egg polarity genes. These are transcribed even in the ovary, in fact by the nurse cells and then these m messenger RNA's are transported into the oocyte through what are called as cytoplasmic bridges and this differential localization of this messenger RNA's during the oocyte development plays a very important role in determining the body plan of the adult fly during development.

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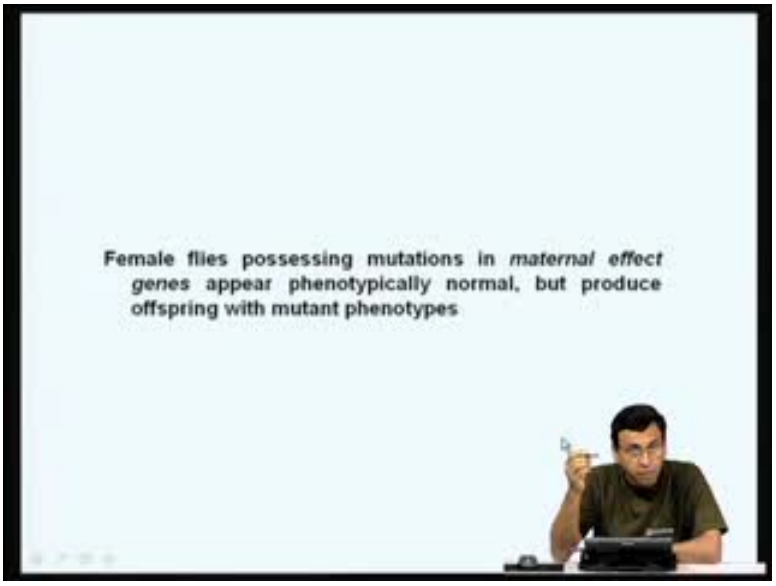

How do you demonstrate that this actually happens? In fact, when you have female flies which are mutations in the maternal effect genes, they appear phenotypically a normal. Nothing happens but their offspring have water of mutant phenotypes indicating that these maternal effect genes play a very important role in the normal development of an embryo.

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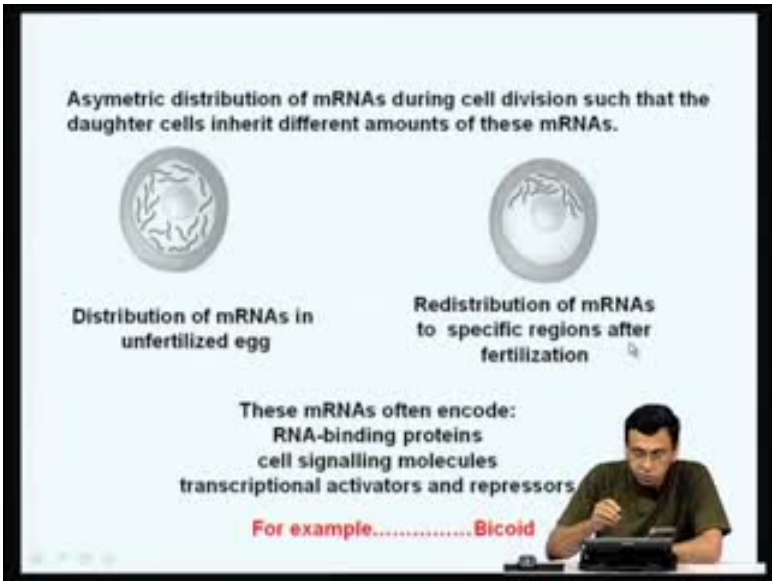

So, asymmetric distribution of messenger RNA's during the cell division such that the daughter cells inherit different amounts of these mRNA play a very important role.

Often, this messenger RNA's which are synthesized during the oocyte stage, they are differentially distributed and this differential distribution of messenger RNA's plays a very important role in the body plan during development. This cartoon just shows you that is many of these messenger RNA's probably or uniformly distributed before fertilization but they undergo redistribution into specific regions immediately after fertilization.

So, repeating from what kind of mRNA's are present in which region of embryo that particular region is the development, then anterior and posterior structures or dorsal ventral structures.

Now, what are these mRNA's? What are this maternal effect genes or what are messenger RNA's code for a maternal effect genes and why are they so important? Why is their distribution so important? These messenger RNA's which comes from the maternal genome of an encode, RNA binding proteins or cell signaling molecules or transcription activators or repressors, that is why their distribution is very important because when they get translated after fertilization, the proteins which are encoded by them or either RNA binding proteins or important cell signaling molecules or they could be transcription activators or repressors. That is why they play very important role in the embryonic development.

All these aspects I am telling of a complicated processes but the way I have organized lecture is not to tell you everything about the drosophila embryonic development but just take a few selected examples which I found very interesting or easy to understand, so that you will appreciate how differential regulation of gene expression plays a very important role during drosophila embryonic development.

To understand importance of this maternal RNA's and the distribution of this messenger inside the egg, let us now take an example of a particular gene known as bicoid. How the messenger RNA and protein coded for this bicoid play a very important role in the embryonic development. Let us try to understand this now.

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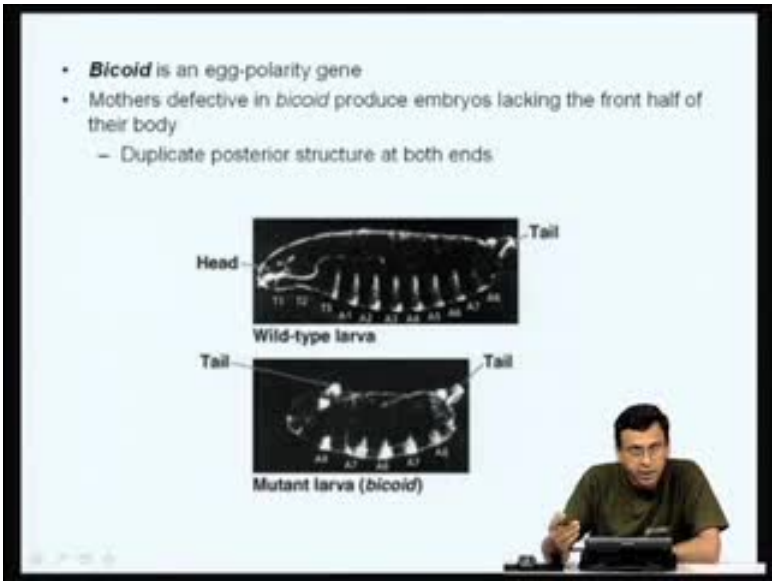

What is bicoid? As I said bicoid is an egg-polarity gene. The muscles defecting in the bicoid produce embryos lacking the front half of their body. So, they do not have anterior structures. So, you can see they have duplicate posterior structure at both ends. So, this is the normal fly. You have the head and tail and you have various segments but if you have a mutations in this bicoid gene, now you have a mutant larva where you see the tail structures in both end and you do not see the head structures are not found clearly indicating that this maternal effect of gene or the egg-polarity gene bicoid plays a very important role in determining the anterior structures of the head structures.

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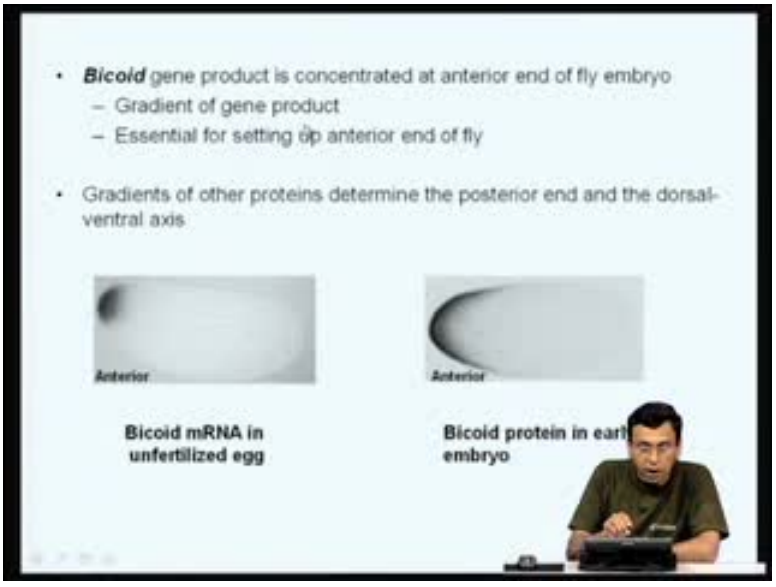

Let us now try to understand, what this bicoid protein is and how it makes such an important difference in the formation of the head structures. The bicoid gene product is concentrated the anterior end of the fly embryo. You can see here this is the localization of the bicoid messenger in the egg before development in the unfertilized egg and this bicoid gene acid tool is very essential for setting up the anterior end of the fly. If have mutation of the bicoid protein the anterior end is not formed and you actually see posterior structures being developed at both ends of the embryo.

The gradients of other protein determine the posterior and the dorsal ventral axis. So, the bicoid is responsible primarily for the anterior structures and there are other proteins like bicoid which actually play an important role in the determination of the other structures. So, after the fertilization this bicoid mRNA is transcribed and there is a gradient of the bicoid protein along the anterior. So, this is the localization of the bicoid protein after fertilization in the early embryo and this is the localization of the bicoid mRNA before fertilization. So, this is primarily localizing the anterior end of the egg.

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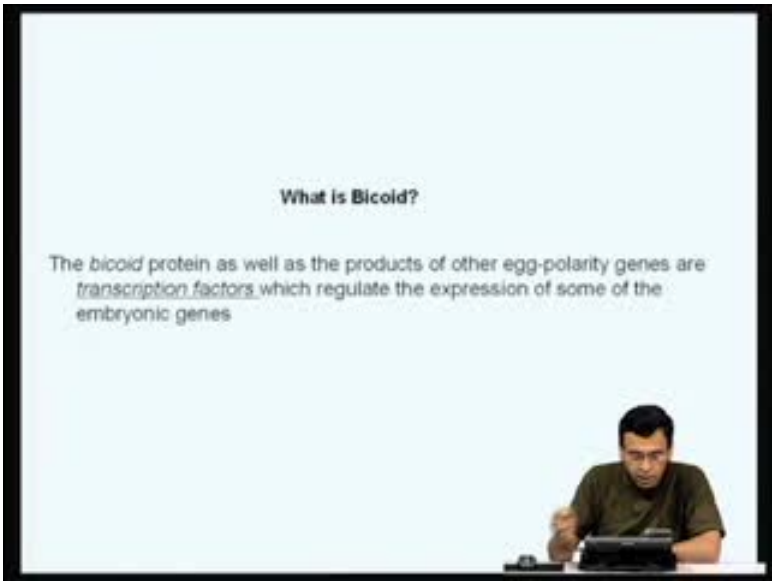

Now, what is bicoid? The bicoid protein as well as many of the genes products of egg-polarity genes, they all are nothing but they are transcription factors and they regulate the expression of many embryonic genes. So, you can see now how transcription factors play a very important role in the regulation of embryonic development.

Many of the genes which are differential expressed either that comes from the maternal genome or which are actually synthesize or transcribing zygotic nucleus. They all encode very potent transcription factors and it is the spatial and temporal expression of these transcription factors are spatial and temporal distribution of this transcription factor which determines whether that particular region should form an anterior structure or posterior structure or the dorsal or ventral region.

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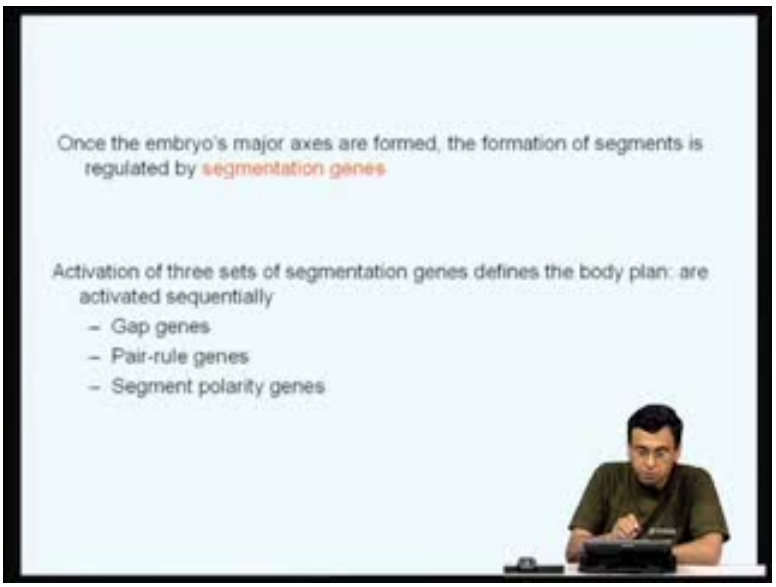

Once the embryo's major axis are formed, so you have a set of genes called as the maternal effect genes. Some of them are present in anterior and posterior and dorsal ventral axis and by the coordinate action of these maternal effect genes this axis of major axis are formed and once these axis are formed, then another set of genes known as the segmentation genes play a very important role.

So, the expression of the segmentation genes follow or the expression is required the maternal effect genes. So, first the maternal effect genes are expressed or the maternal effects gene products are first made and these maternal effect products which are all transcription factors now activate what is called as a zygotic transcription or the expression of the zygotic genes.

The segmentation genes are now transcribed and there are actually about three sets of segmentation genes that play a very important role in determining the body plan and drosophila melanogaster these are called as gap genes, pair-rule genes and segment

polarity genes. Again, these are expressed very sequentially during the embryonic development. So, first you have the maternal effect genes. This messenger RNA's are synthesized by oocytes and deposit at different regions of the embryo and the expression of this or the products of these maternal genes. Most of them are transcription factors. They now prolong the expression of the segmentation genes. There are about three different types of segmentation genes known as gap genes pair-rule genes and segment polarity genes which then take over the further development of the embryo.

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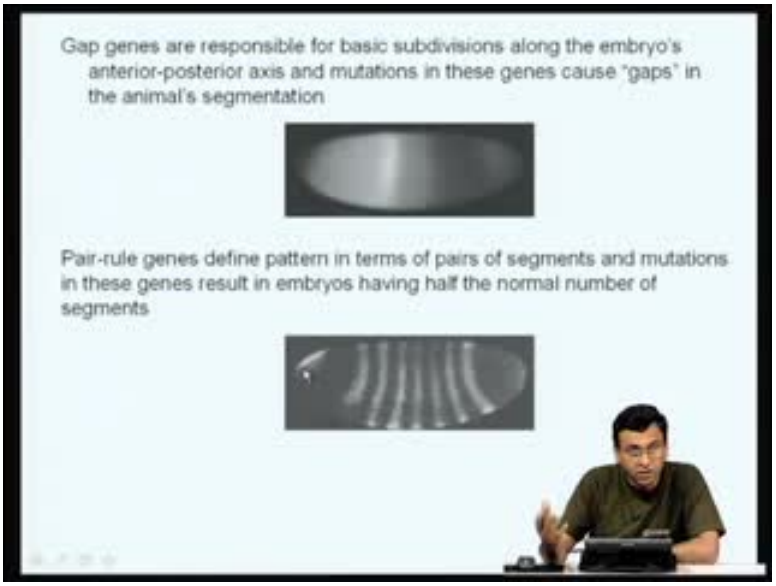

Now, what are these genes? Gap genes are actually responsible for basic sub-divisions along the embryos anterior-posterior axis and mutations in the gene cause gap in the animal segmentation. So, that is why I called them gap genes, whereas I am just given the expression of some of these gap genes and normal embryo. Similarly, the pair-rule genes define the pattern in terms of pairs of segments and mutations in these genes result in the embryos having half the normal number of segments.

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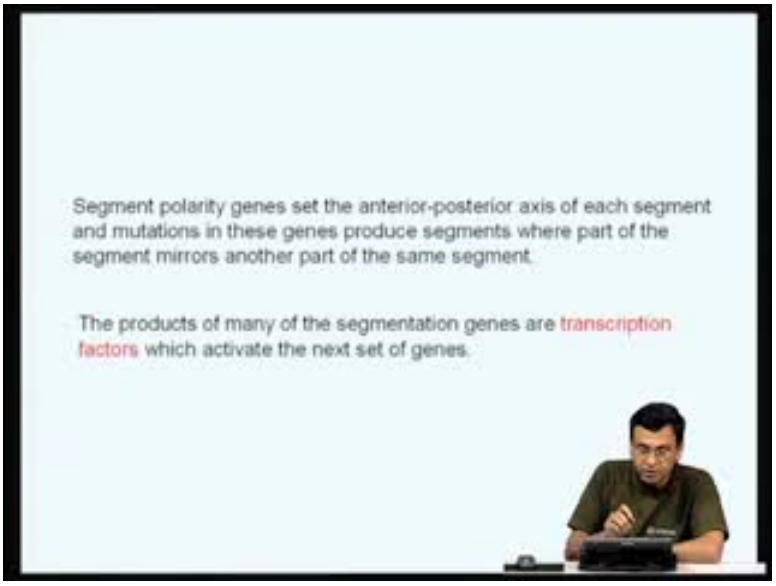

So, the pair-rule genes actually determine the number of segments. How many pairs of segments have to be formed and if you have mutations in the pair-rule genes, the number of segment is dramatically altered and you probably end up only with half the number of segments. The segment polarity genes that then set the anterior-posterior axis of each of segment and mutations in these genes produce segments where part of the segment mirrors in another part of the same segment. So, the identity of the segment is determined by the segment polarity genes.

So, you have gap genes, pair-rule genes and segment polarity genes which play a very important role in the development. The products of many other segmentation genes again are transcription factors which then activate next set of genes and then again the products of these next set of genes can either be transcription factors or can code for some of this structural proteins. So, what I am trying to say is here is that the entire embryonic development ultimately depends on sequential turning on and turning off of transcription factors.

Either this transcription factors may be made for maternal RNA's which are stored in the cytoplasm which then activates the genome of this zygotic nucleus and again they go for transcription factors. This sequential activation or repression of transcription factors play very important role in the normal development of the drosophila embryo.

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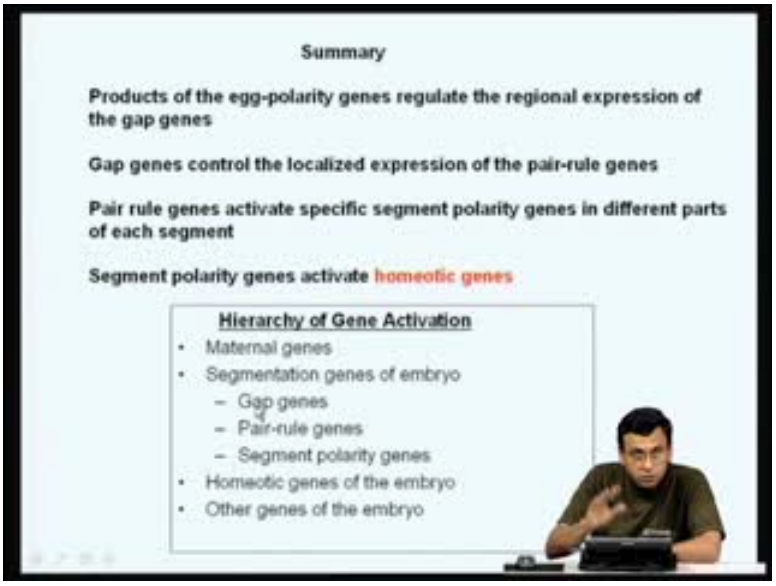

So, just to summarize this part, what we have studied so far the products of egg-polarity genes regulate the regional expression of the gap genes. The gap genes control the localized expression of pair-rule genes and the pair-rule genes activates specific segment polarity genes in different parts of each segment. Segment polarity genes then activate what are known as the homeotic gene which we will discuss in the next couple of minutes.

So, we have the egg polarity genes which now activate the gap genes. Gap genes then control the expression of pair-rule genes. Pair-rule genes, then activates the segment polarity genes and the segment polarity genes then activates what are called as the homeotic genes. So, this sequential expression of these genes plays a very important role in the early embryonic development.

So, what is this there is what is called as a hierarchy of gene expression or gene activation. First, the maternal genes are activated, then the segmentation genes of embryo are activated especially the gap genes are activated first followed by the pair-rule genes and the segment polarity genes. These segment polarity genes then activate the homeotic genes and these homeotic genes then activate other genes of the embryo. So, this hierarchy of gene activation plays a very important role in the development and differentiation of the embryo.

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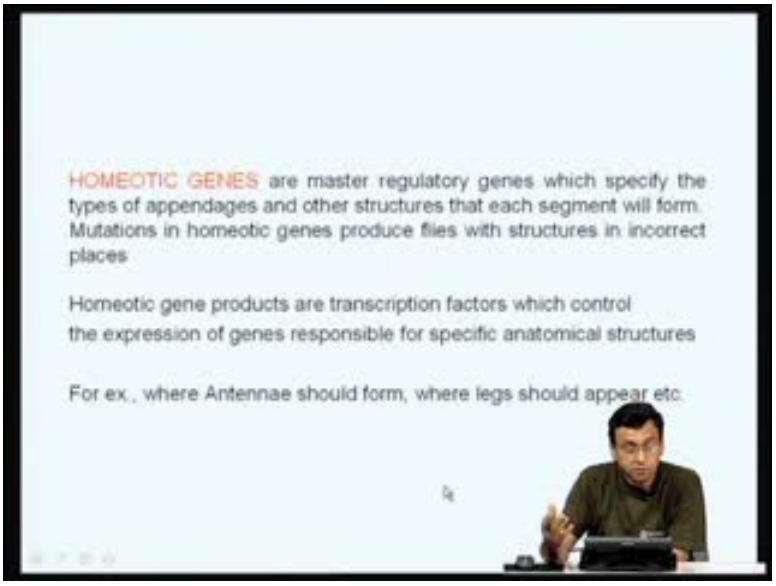

What are homeotic genes? We have discussed some of these homeotic genes in our early lectures when we discussed about some of these structures of the DNA binding domains of various transcription factors and how a helix-turn-helix motif called as a homeobox plays a very important role in the development. At that time, we had actually said will discuss more about this homeotic gene during development when we talk about early embryonic development. So, these homeotic genes are nothing but they are master regulatory genes which specify the types of appendages and other structures that each segment will form.

Mutations and homeotic genes produce flies with structure in incorrect places. For example, if you have mutation in one homeotic gene, for example instead of one pair of wings the fly may develop two pairs of x or if you have mutation another homeotic genes instead of antennae proctoring from the head you may have legs proctoring from the head. So, these genes are actually are master regulators which control the entire differentiation program of the particular segment.

So, if we have mutations in these genes, the entire organ identity can be transformed. So, homeotic gene products are nothing but they are transcription factors which control the expression of the genes responsible for specific anatomical structures. So, that is sub-homeotic genes which control the wing formation or others control the leg formation or antennae formation and so on and so forth. So, some of the examples, for example

homeotic genes control where antennae should form or where leg should come out and so on and so forth.

So, homeotic genes are very important and they are the final players in the sequential activation of genes. A segment polarity genes which are expressed, they are the ones then activates homeotic genes and then the homeotic genes take over and then determined which organs or which anatomical structure should be formed in which segment.

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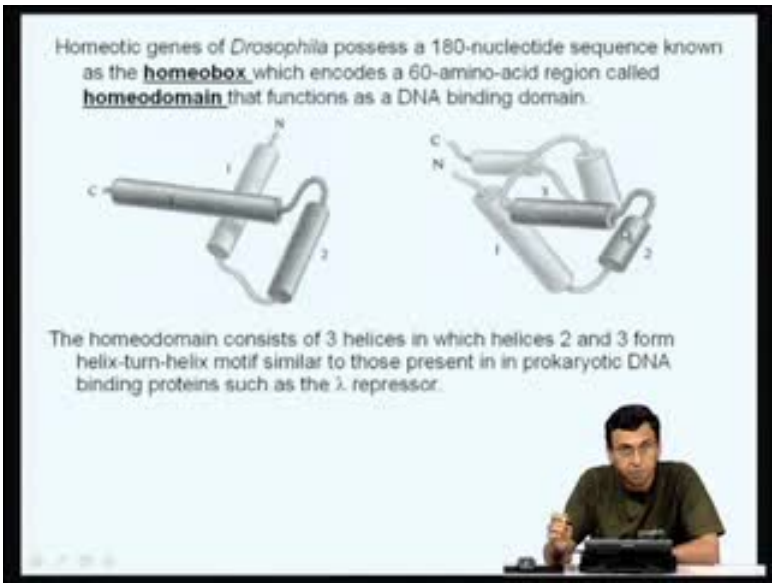

So, homeotic genes of this drosophila possess a 180-nucleotide sequence known as the homeotic homeobox and these homeobox encodes a 60-amino-acid region called as the homeodomain that functions as a DNA binding domain. Again, as I said earlier we had discussed about this homeodomain in some of our earlier lectures when we discussed about the DNA binding domains of various transcription factors like the helix-turn-helix, Xing fingers, lucien zippers and so on and so forth. This helix-turn-helix is actually resembles a helix-turn-helix of a prokaryotic repressors.

The helix-turn-helix basically have the homeodomain, actually consists of 3 alpha helices in which the helix 2 and helix 3 actually form a helix-turn-helix motif which is similar to those present in the prokaryotic DNA binding proteins such as the lambda repressors. So, among the various DNA binding motif that we had discussed earlier, if the helix-turn-helix motif is also present in some other prokaryotic repressors whereas

other DNA binding motif like Xing fingers, Lucien zippers are present only in the eukaryotes. You do not see this motif in any of the prokaryotic transcription proteins.

So, these homeotic genes, the products of the homeotic genes are transcription factors and these transcription factors are characterized by the presence of a very unique domain called as a homeo-domain which is nothing but a 60-amino-acid region that contains 3 alpha helices, helix 1 to 1-3. It is this of the homeo-domain which actually interacts with specific DNA sequences and these homeotic proteins are nothing but sequence specific DNA binding proteins and transcription factors.

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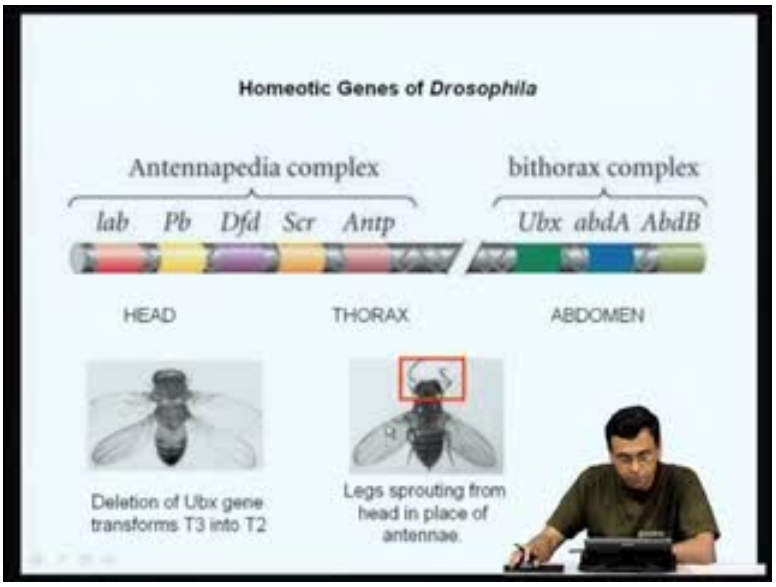

So, there are whole numbers of homeotic genes in the drosophila and in broadly, they are categorized in two different complexes. One is called as an antennapedia complex; another is called as a bithorax complex and each complex again consist of a series of genes which are labeled which are named differentially. We will not go to the details of these. We will discuss some of these homeotic genes later stages of this talk.

Basically, what I would like to say at this time is that these homeotic genes ultimately determine whether that segment should give rise. Which segment should give rise to head structures, which segment should form the thorax and which segment should form the abdomen?

So, the geneses of a head, thorax and abdomen from the segments of after the segment polarity genes or from the segments and the number of segments have been determined which segment has to form which structure is actually determined by the homeotic genes.

As I said, these homeotic genes are very important if you have mutations in these structures, abnormal structures are formed. For example, if you have mutations in the ultra-bithorax or Ubx, you can say the T3 is transformed into T2. Normally, the wings actually comes from the T2 but now if you have mutation ultra-bithorax, the T3 also behaves likes a T2. Therefore, you see two sets of wings rather than one set of wings.

Similarly, if you have for example, mutations in another homeotic gene, legs come out of the head instead of antennae. So, what kind of organs have to be formed, where they have been formed is governed by the homeotic genes and that is why I called as homeotic because these all called as homeotic transformation. If you have mutations in these homeotic genes, it results in homeotic transformation, one organ coming out in place of another organ. So, homeotic genes are very important.

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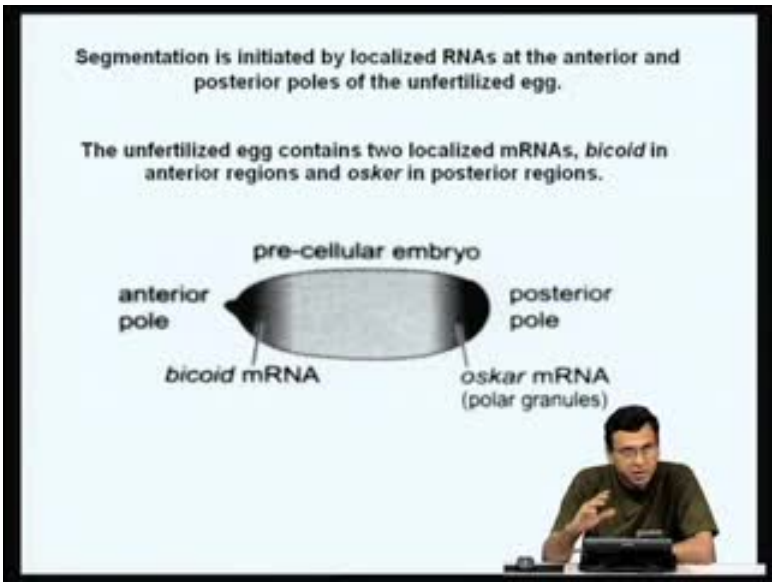

So, we will discuss about these specific homeotic genes and how they regulate gene expression at later stages of this lecture series. What I would like to now focus is how the deposition of RNA in different regions of the embryo plays a very important role during

the early embryonic development. As I said earlier, segmentation is initiated by localized RNA's at the anterior and posterior poles of the unfertilized egg.

I told you these are called as the maternal effect genes. These are transcribed during the oocyte development and these are actually transcribed by the nurse cells and RNA's are actually transported into the oocyte through what are called as a cytoplasmic bridges and where this RNA's are localized. The unfertilized egg plays a very important role in the early embryonic development. For example, the unfertilized egg contains two localized RNA's, one is called as bicoid in the anterior regions and other called as the oskar in the posterior regions.

So, there are two maternal effect messenger RNA's called Bicoid and Oskar. The bicoid mRNA is present in the anterior region of the embryo whereas the oskar messenger RNA is present at the posterior region of the unfertilized egg.

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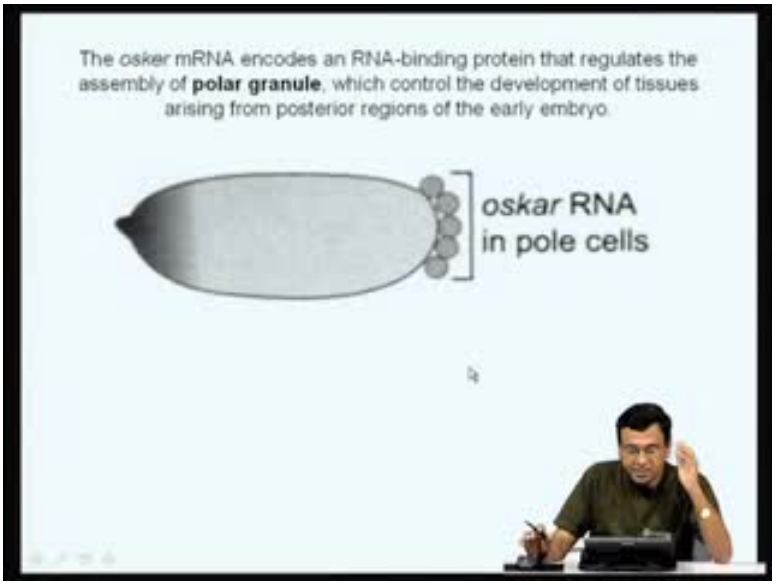

The oskar mRNA is nothing but it encodes an RNA-binding protein that regulates the assembly of what is called as polar granule which actually controls the development of tissues arising from the posterior regions of the early embryo. So, you can see the localization of this RNA plays a very important role whether what kind of structure should be formed, the anterior end or posterior end of the embryo.

The oskar mRNA is primarily localizing the posterior region and this is responsible for the assembly of what are called as a polar granules that ultimately controls the development of tissues arising the posterior region of the embryo.

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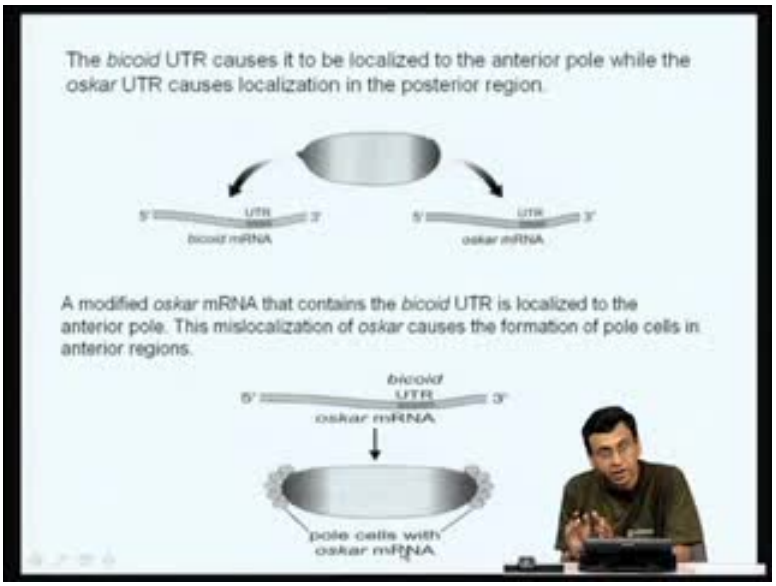

Very elegant experiments have been done to understand the mechanism by these messengers like bicoid and oskar actually function. It turns out the bicoid messenger RNA's contains an untranslated region and this untranslated region is actually responsible for its localization in the anterior pole whereas the untranslated region of the oskar messenger RNA is responsible for its localization in the posterior region.

So, both these messengers RNA's bicoid as well as oskar contains specific untranslated region sequences and these untranslated regions are actually responsible for their specific localizations eggs in the anterior end in the case of bicoid or posterior end, in the case of the oskar. How do you demonstrate that? It is these untranslated regions which are responsible for the differential localization of this messenger RNA's in the early embryo.

Very interesting experiments have been done. What you do is you now take the bicoid untranslated region and replace the oskar messenger and untranslated region with that of bicoid messenger RNA. So, you have an RNA oskar messenger RNA which consist of bicoid untranslated region instead of the oskar untranslated region.

When you have such an mRNA is expressed, you actually develop posterior structures in both ends clearly indicating that these untranslated regions of these two mRNA's are very distinct and one is responsible for formation of the anterior structure. Another is responsible for the posterior structure and if you now replace the oskar messenger RNA untranslated that of the bicoid messenger RNA, you actually see the posterior structure in both. So, a modified oskar messenger that contains bicoid untranslated region is localized to the anterior pole and this mis-localization of the oskar causes the formation of poles in the anterior region.

So, the oskar mRNA should normally be present in the posterior region that is because it has its own untranslated region but if now replace that untranslated during that of bicoid and a bicoid untranslated region actually is responsible for localization of the mRNA anterior region. So, instead of localization in anterior region, now the posterior region, the oskar messenger RNA's now also localized in the anterior region. As a result the posterior structures are formed both in the anterior ends and posterior ends.

So, these kinds of experiments clearly demonstrated that a specific region in these messenger RNA's play a very important role in the differential localization of these messengers in the embryo.

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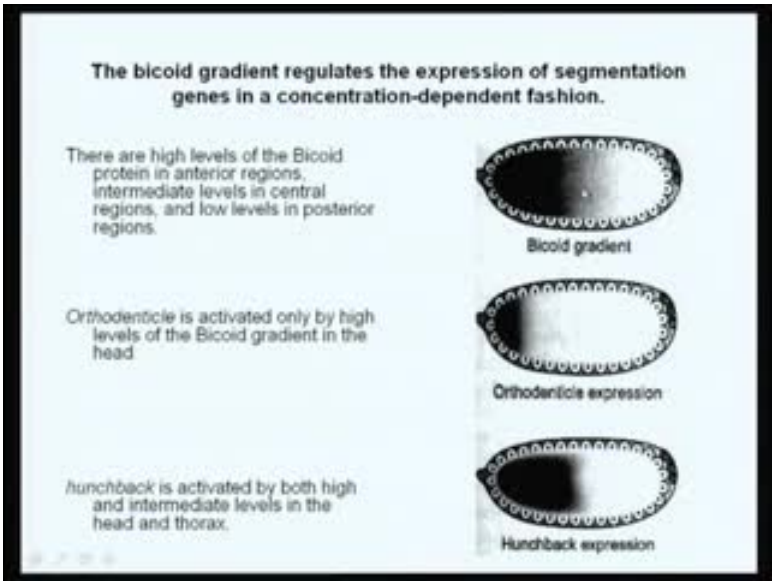

So, the bicoid gradient, so once the bicoid messenger RNA gets translated, it results in the formation of the bicoid gradient and these bicoid gradient which is present in the

anterior-posterior axis that actually regulates the expression of segmentation genes in a concentration dependent manner. So, you can see how the levels of the transcription factor or the concentration gradient of the transcription factors such as bicoid in embryo actually determines what kind of segmentation genes to be expressed in the anterior-posterior axis.

For example, there are very high levels of bicoid protein in the anterior regions and intermediate levels in the central region and very low levels towards the posterior regions. The mRNA you can see in the one-third of the posterior half, you do not see bicoid. So, the bicoid gradient is present in the two-thirds of the embryo with the highest being in the anterior region and little bit less in or intermediate levels in the middle region and very low levels in the posterior region.

Depending upon the levels of this bicoid protein, different kinds of genes are expressed in these different regions. Now, a protein for example a gene course for a protein called orthodenticle is activated only when the bicoid get bicoid levels are very high. So, these orthodenticle therefore is expressed only in the anterior region because this is the region where the bicoid concentration is very high. Why is this so? This is because the enhancer elements to which is bicoid proteins binds or rather low affinity binding sides of for orthodenticle. Therefore, in outer for this binding should be occupied bicoid, we require very high concentration of the bicoid.

So, depending on the affinity of the DNA sequence to the bicoid protein whether the gene is turned on a turned off is determined. So, the orthodenticle which these names are all little bit funny but so you no need to worry. What is important for us to remember is the conceptual concepts that are more important rather than the names. What I am trying to develop, what I am trying to drive at here is that the concentration of the transcription factor along the anterior-posterior axis of the embryo plays a very important role in what kind of genes should be activated in which region.

In the anterior region where the bicoid transcription factors present in a very high level because of these high levels this protein called as orthodenticle, the gene coding for a protein called orthodenticle is activated whereas another protein called the hunchback is activated both by high as well as intermediate levels of the bicoid protein. Therefore, the hunchback is expressed not only in the anterior region but also in the thorax region.

So, you can see when the bicoid protein is present a very high concentration, a gene called orthodenticle expressed only in the head region because this gene expression requires very high levels of bicoid protein. Whereas, another gene called as hunchback can be expressed either when you have high levels or intermediate levels of the bicoid protein. Therefore, this gene is expressed both in the head region as well as the thorax region. So, you can see how the levels of a transcription factor can actually determine which genes have to be turned on and which regions of the embryo.

That is the point I am trying to develop, do not want to worry about these names but what is important is this to understand how levels of a transcription factor can differentially regulate the expression of genes and these genes known as hunchback is also regulate at the level of translation.

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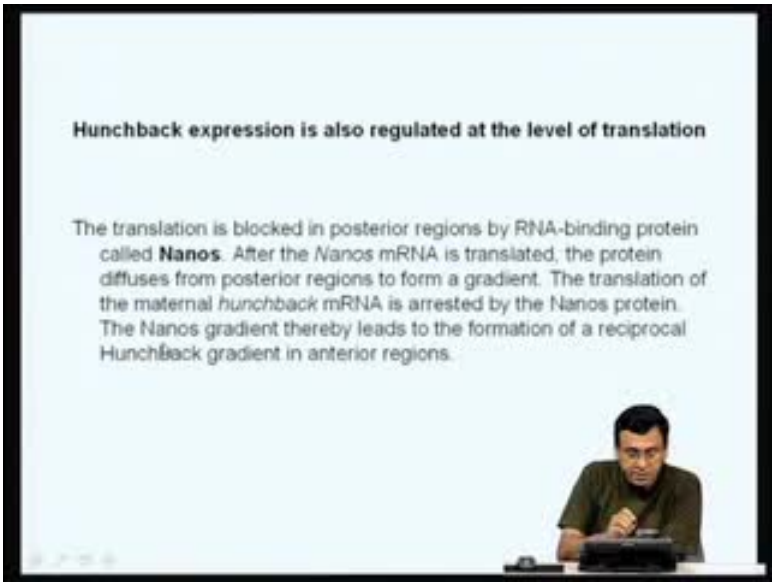

Now, although we are talking primarily in this lecture series, we are going to talk primarily about regulation of gene expression at the level of transcription. Remember, there are other levels of regulation also very important. There is what is called as post transcription regulation of gene expression, there is translation regulation of gene expression, post translation regulation and also regulation of there is nucleoside transport and so on and so forth.

So, although in this lecture series we focused primarily on the regulation of at the level of transcription expression, the other events are also equally important. So, I am just

giving you one example here. As I told in the previous slide, the expression of hunchback requires a protein called as a bicoid protein and the hunchback is expressed in the dorsal in the anterior as well as the head region as well as thorax region because it can be activated by both high as well as intermediate concentrations of the bicoid transcription factor.

Now, in addition to this transcriptional regulation, the express of hunchback is also regulated the level of translation. Now, let see how it is? Now, the translation of this hunchback RNA is blocked in the posterior regions by RNA-binding proteins called as Nanos. As I told you the hunchback is expressed only in the head and thorax region but it is not expressed in the abdominal region. Why is this protein not expressed in the abdominal region? That is because the translation of this hunchback messenger RNA is prevented by a protein called Nanos which is a RNA binding protein.

So, after the Nanos mRNA is translated the protein diffuses from the posterior regions to form a gradient and the translation of the maternal hunchback RNA is arrested by the Nanos protein. The Nanos gradient therefore leads the formation of a reciprocal hunchback gradient in the anterior region.

So, the Nanos which is present in the posterior region that now binds into the hunchback messenger RNA and as a result, the Nanos is very high the posterior region but as we come towards the anterior region, the Nanos concentration comes down. So, this is the reverse of the bicoid.

So, the bicoid concentration is very high in the head region and comes down towards the posterior region or the Nanos is present in a very high posterior but slowly the levels are low towards the anterior region. When this Nanos is present in high concentration these Nanos binds to the messenger RNA of hunchback and prevents its translation. Let see how it happens.

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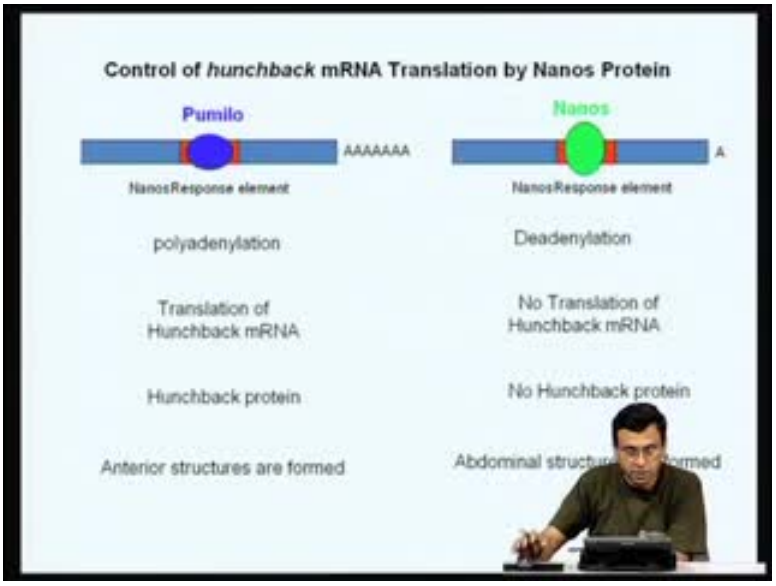

It turns out this hunchback messenger RNA contains a sequence called as a Nanos response element. Now, when Nanos is not there a protein called as pumilo binds to this Nanos response element and as a result this hunchback messenger RNA undergoes polyadenylation and once it is polyadenylated, it is very efficiently translated into a hunchback messenger RNA and then the hunchback protein is formed.

So, the gene coding for hunchback contains a Nanos response elements and in the RNA's of nanos, this is bound by pumilo. Therefore, this RNA can now be polyadenylated and therefore results in the translation of hunchback leading into the formation of the hunchback protein but when Nanos is present and when hunchback is protein, it promotes the formation of anterior structures like head and thorax.

In the posterior regions, since the Nanos is expressed only at the posterior regions. When the Nanos is expressed, the abdominal regions of the posterior regions of the embryo, now Nanos goes and binds to this Nanos response element and binding of this Nanos prevents polyadenylation of the Nanos messenger RNA. Therefore, de-adenylation of messenger RNA therefore is hunchback RNA is not translated and therefore hunchback proteins not made and as a result in the abdominal region the abdominal structures are formed.

So, we can see how a gene called hunchback is regulated both at the level of transcription as well as at the level of translation. The transcription regulation is brought

about by a transmission factor called bicoid. The bicoid levels ensures that the hunchback is transcribed or is transcribed only at the head and thorax regions because the hunchback transcription requires both high as well as intermediate levels of the bicoid mRNA and because the bicoid is present in a gradient with high concentration at the head region, low in the thorax region. This hunchback is expression is activated both in the head and thorax region. Therefore, it results in the formation of the anterior structures.

Why hunchback is not made protein is not made in the abdominal region that is because a protein called as Nanos which is present in high concentration in the posterior regions prevents the polyadenylation of the hunchback messenger RNA and as a result, hunchback protein is not expressed in the abdominal region. Therefore, its anterior structures are not formed here and therefore that region now gets abdominal structures are formed in that particular region.

So, you can see both the transcription as well translational regulation ultimately determine how a hunchback protein can form head and thorax structures in the anterior region and abdominal region in the posterior region.

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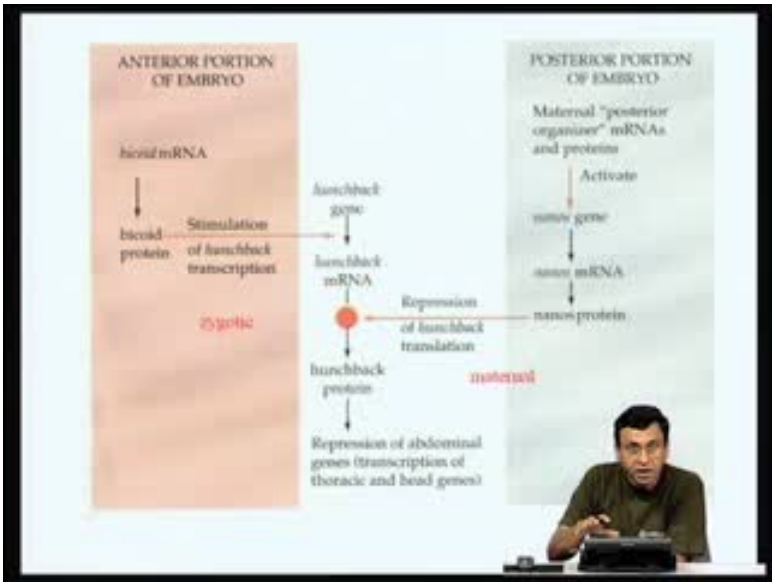

So, this is just the summary of what I told you. The anterior portion of embryo actually is determined by the bicoid messenger RNA which is the maternal effect gene. It is actually made during the oocyte and is deposited in the oocyte before fertilization and is present

in a gradient with very high concentration in the anterior region and the bicoid mRNA's are translated into the bicoid protein immediately after fertilization. Again the bicoid protein is present in a kind of gradient with high levels in the anterior region and slowly the levels keep coming down towards the posterior region.

Now, these bicoid proteins now stimulate hunchback transcription pan transcription because a transcription factor, so this hunchback genome makes hunchback RNA. Hunchback messenger RNA is translated into hunchback protein and this hunchback protein now represses the expression of genes required for abdominal structure formation. Therefore, the thoracic and head genes are transcribed. Therefore, this region their hunchback is expressed or hunchback protein is made now gets transformed leads a formation of head and thorax.

Now, the posterior portion of the embryo, there are maternal posterior RNA's messenger. RNA's and proteins, they activate the Nanos gene. Nanos gene now makes Nanos messenger RNA and these Nanos protein now binds to the hunchback messenger RNA and prevents a translation of the hunchback messenger RNA. Therefore, in the posterior region head and thorax cannot be formed and their development of abdominal structures.

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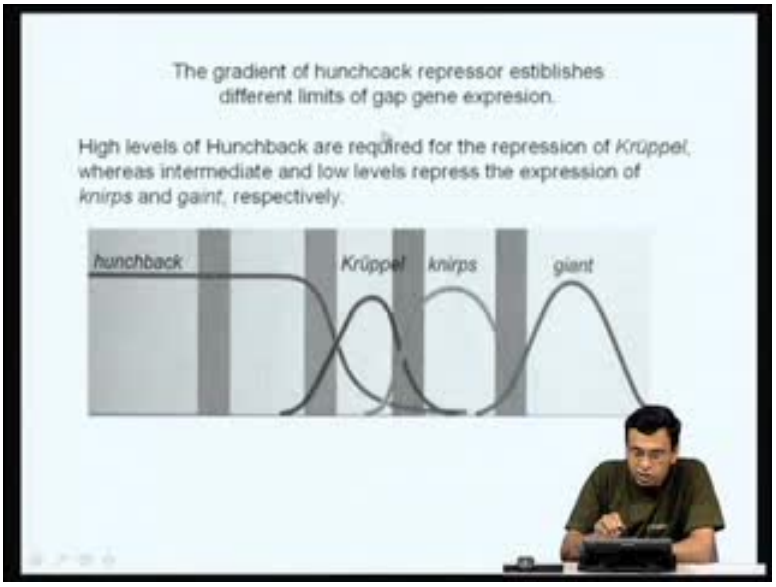

So, emphasizing this to just tell you how beautifully the differential activation of genes have transcription factors and there the level of binding and the affinity of the transcription factors to their response elements as well as couple to a translation

regulation ultimately determines whether the which region of the embryo has to develop the head and thorax and which region has to develop the abdomen. So, the gradient of the hunchback repressor establishes different limits of the gap gene expression.

So, because the hunch back actually now goes and they activate the gap of genes. High levels of hunchbacks are required for the repression of another protein called transmitter kruppel whereas intermediate and low level represses the expression of knirps and giant. So, hunchback is nothing but a transcriptional repressor. So, bicoid is a transcription factor which results in the activation of hunchback.

Now hunchback is again is a transcription repressor that again regulates the expression of proteins like kruppel, knirps and giant and again the expression of these genes regarding depends upon the levels of the hunchback. For example, high levels of hunchback are required for the repression of kruppel and we know the hunchback is present in the very high level is the head. Therefore, kruppel is not expressed in the head region and low levels can repress the expression of the knirps and giant because therefore, as is if in the anterior posterior axis you can see depending upon the levels of hunchback, the expression of kruppel, knirps and giant is determined.

So, the levels of bicoid protein determines the expression of hunchback and the levels of hunchback determines the levels of expression of proteins like a genes like kruppel, knirps and giant.

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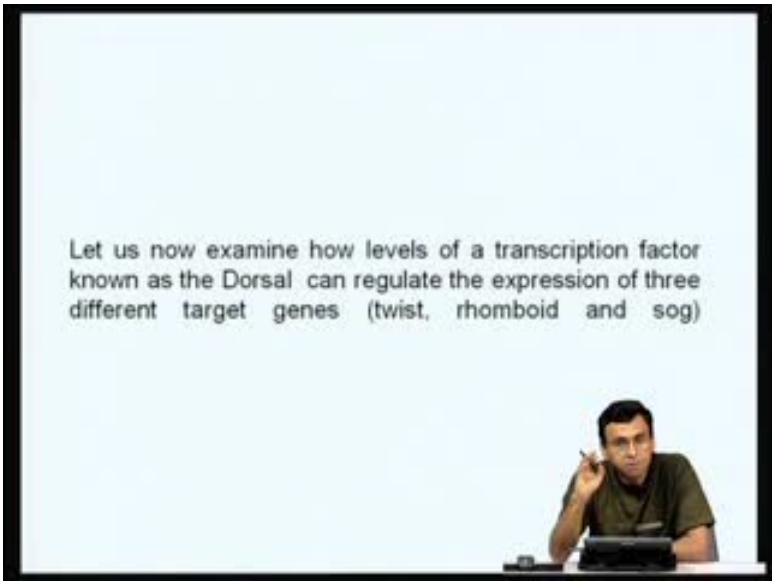

So, what I am trying to say so far is that it is the spatial temporal expression of transcription factors or even RNA binding proteins and their sequential expression that ultimately determines the early embryonic development of the drosophila.

Which region has to form the anterior structure, which region has to form posterior structure and a similar the factors are also involved in the formation of the dorsal elemental regions? Now, I will give one more example as to how levels of a transcription factor known as a dorsal can regulate the expression of three other target genes called twist, rhomboid and sog.

So far we talked about anterior-posterior regions. Now, let us take an example of again how another transcription factor called as dorsal. How that can regulate the expression of three different genes called as twist, rhomboid and sog?

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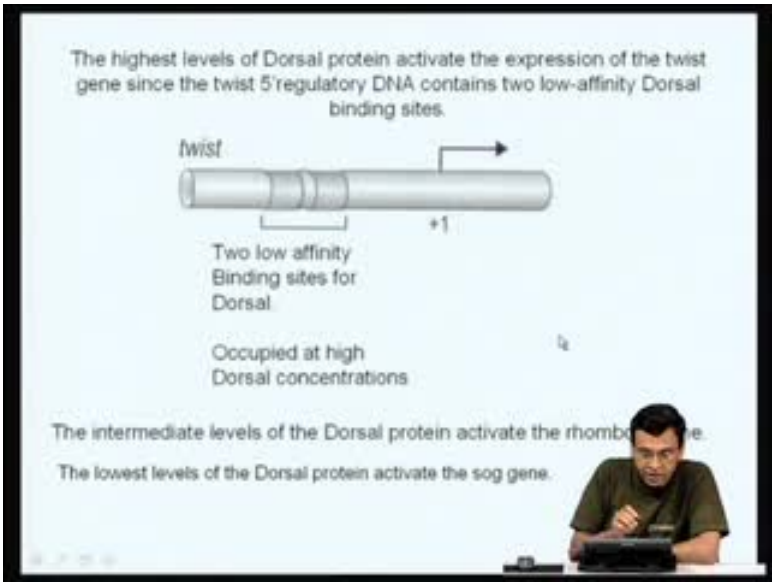

Again, the same way the expression of the twist gene requires the highest levels of dorsal protein and because the 5 prime regulatory DNA or the 5 prime promoter region of the twist gene consist of two low-affinity dorsal binding sites.

So, in order for the twist gene should be activated, you required very high levels of dorsal protein because the affinity of this binding sites for dorsal is very weak. Therefore, this gene will be activated only when you have very high concentration of the dorsal.

On the contrary, the rhomboid and the sog genes can be activated either by intermediate levels or low levels of the dorsal protein. Therefore, depending upon the concentration of the dorsal along the dorsal of ventral axis if you have very concentration, the twist gene is activated. Intermediate concentration of the dorsal will activate the rhomboid gene and low levels of dorsal protein anterior the sog gene.

So, just as we have the bicoid transcription factor levels in the anterior-posterior axis, the levels of the dorsal protein along the dorsal ventral axis govern which kind of genes are activated on the dorsal ventral axis. So, you can see how the gradients of transcription factors in the early embryo determine the subsequent expression or the sequential expression of genes as development proceeds.

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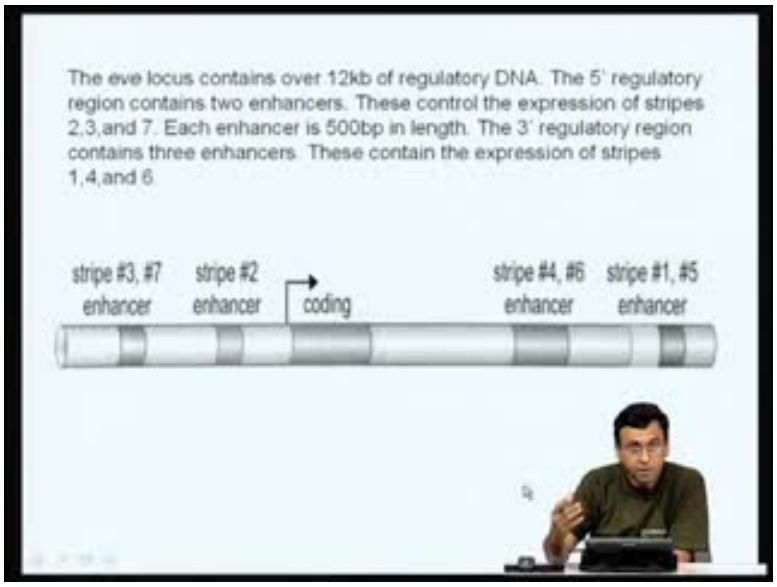

Similarly, there is another gene called as eve. Now, the eve locus which actually is responsible for the formation of various segments which contains about 12kb of regulatory DNA and the 5 prime regulatory genes contains two enhancers and these controls the expressions of what are called as stripes 2, 3 and 7. Now, each enhances about 500 base per in length and 3 prime regulator region contains 3 enhances and these control the expression of stripes 1, 4 and 6.

So, is a huge enhancer region and this enhancer ultimately control the formation of what are called as stripes. Each of these 500 base per enhancer contains a total of twelve

binding sites for a variety of transcription factors like bicoid, hunchback as well as transcription repressors, kruppel and giant proteins.

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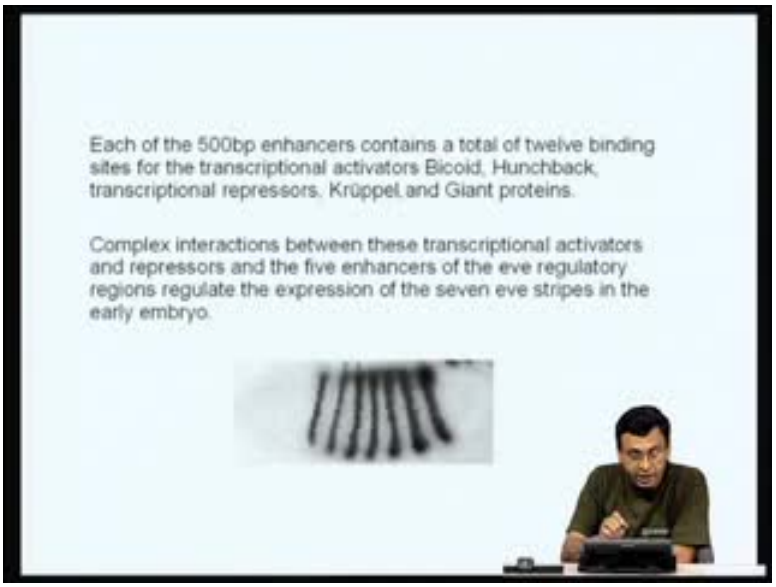

We just discussed how these proteins are expressed. You know bicoid is the maternal effect gene; bicoid controls the expression of hunchback. Hunchback then controls the expression of other proteins and then how a dorsal protein controls the expression of kruppel and gaint so on and so forth.

Now, it turns out these enhancers of this eve protein, eve gene contains binding stripes for all these transcription factors, both transcription activators as well as transcription repressors. Bicoid and hunchback are transcription activators. Kruppel and giant are transcription repressors.

Now, I am not going to the details because the regulation of the eve locus is a very complex pattern. Now, what I am going to details of how a complex interplay or the binding of the transcription activity repressor govern the expression of these gene but suppose to understand at this stage that complex interaction between transcription activators and repressors and the five enhancers of the eve regulator region regulate the expression of the seven eve stripes in the early embryo.

So, how these seven different types are formed? In the drosophila embryo depends on the complex interplay between the binding of bicoid, hunchback, kruppel and giant to the

regulatory regions of the eve gene. I am not going to the details because we do not have time to discuss how these interactions takes place and how these interactions or the mutually exclusive binding of these activities and repressors govern the expression of this eve gene along the embryo.

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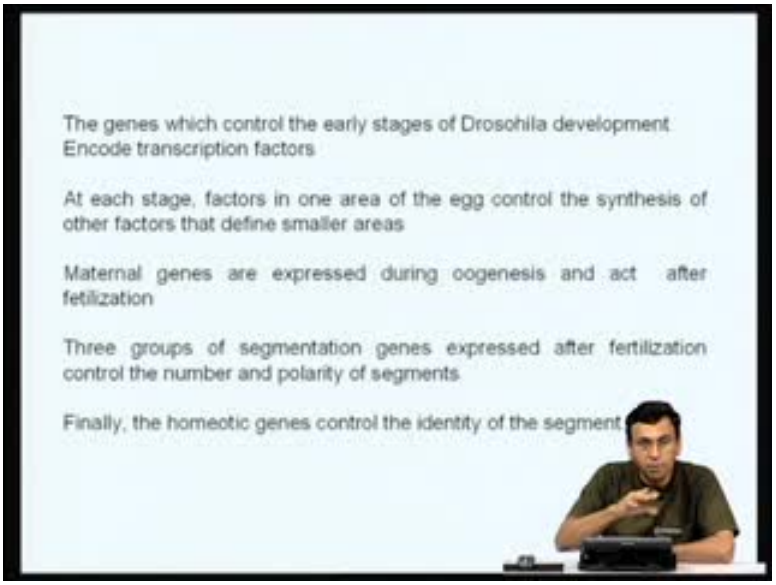

So, what I am trying to say so far is that the genes which control the early stages of the drosophila development encode transcription factors. I gave 2 or 3 examples like how one of the examples has bicoid protein which is involved in the formation of the anterior-posterior axis. I gave an example of like dorsal which is for the dorsal ventral gradient and that is responsible expression of whose concentration is responsible expression of other genes.

So, the concentration of proteins like dorsal and bicoid determines what kind of genes are activated in which regions of the embryo and depending upon the their expression either anterior structures like head and thorax are formed or posterior structures like an abdominal form. Similarly, the dorsal ventral structures are formed. So, at each stage factors in one area of the egg control the synthesis of other factors that in turn defines smaller areas.

This is the driving principles that I wanted to understand. First the maternal genes are expressed during the oogenesis and they act after fertilization. So, these maternal effect genes are actually translated what are called as nurse cells which provide nutrition to the

developing oocyte. These RNA's which are translated by nurse cells through what are called as the cytoplasmic bridges are depositor into the oocyte in either anterior posterior or dorsal ventral gradient.

These maternal genes they are expressed after the fertilization and these maternal RNA's are again represented in a proteins coded by these maternal also form a gradient and it is depending on their levels, the next sets of genes are called as segmentation genes are expressed and these segmentation genes control the number as well as the polarity of the genes. So, once the number and polarity of the genes are established due to the expression of the maternal genes and the segmentation genes that which segment should become what. The identity of the segment is then determined by what are called as the homeotic genes.

So, you can see the number and polarity of the segments is determined by the maternal effect genes or segmentation genes whereas the identity of the segment is determined by the homeotic genes. Hence, a sequential expression of these genes first the maternal genes expressed, they in turn regulate the expression of the segment polarity genes and these segmentation genes in turn regulate the expression of the homeotic genes. Finally, homeotic gene determines which segment has to become head, which segment has to become thorax and what kind of structures have to come out of these.

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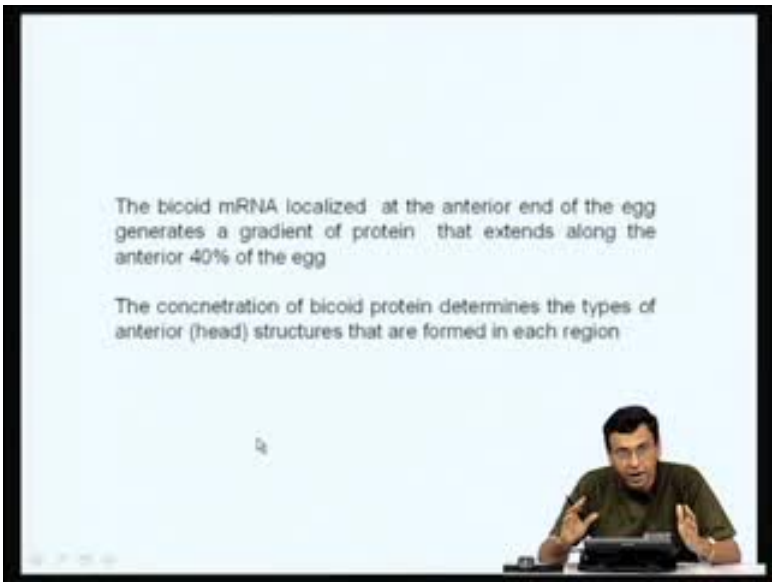

So, the bicoid mRNA is which is a maternal mRNA is actually localised in the anterior end of the egg. It generates the gradient of protein that extends along the anterior 40 percent of the egg. Bicoid proteins are not present in the posterior region. It is highly concentrated in the anterior region and then this distributed slowly there is a gradient of bicoid messenger RNA with very high levels in head region and intermediate levels in the thorax region but its storing nothing in the abdominal region. The concentration of the bicoid protein determines the types of head structure that are formed in each region.

This is what we discussed in detail because how hunchback for example which is required for the formation of the head and thorax structures, how the bicoid protein what high an intermediate also bicoid protein are required for the hunchback expression. Hunchback expression is prevented in the abdominal region because this hunchback messenger RNA cannot be translated in the abdominal region by the presence of proteins like Nanos.

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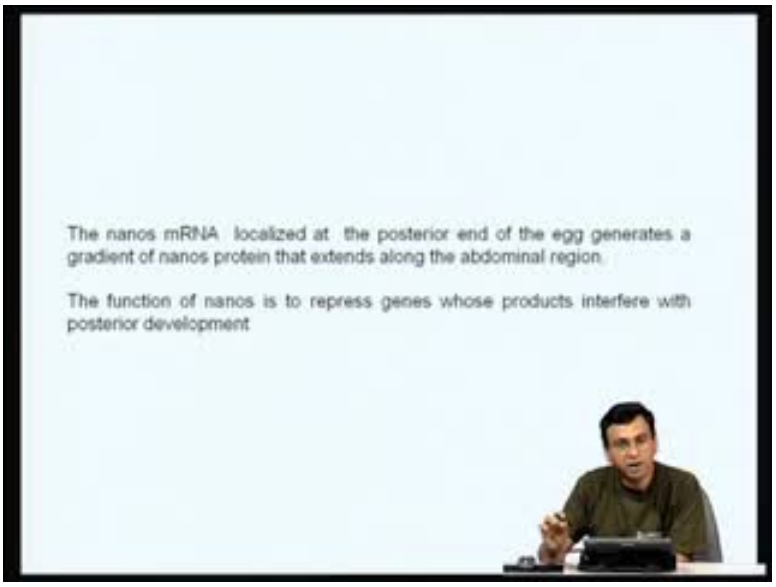

The Nanos messengers RNA localize in the posterior end of the egg generates a gradient of Nanos proteins that extends along the abdominal region and the functional of Nanos is to repress genes whose products interferes the posterior development.

One of the candidate genes which is repressed by Nanos is hunchback. So, when hunchback is expressed, the anterior structures like head and thorax are formed whereas even if hunchback messenger RNA's present in the abdominal region, its translation is

prevented because the Nanos goes and binds to the untranslated region of the hunchback messenger and prevents it polyadenylation. Therefore, hunchback RNA cannot be transcribed and by default, now the posterior structures are developed and abdominal regions are formed.

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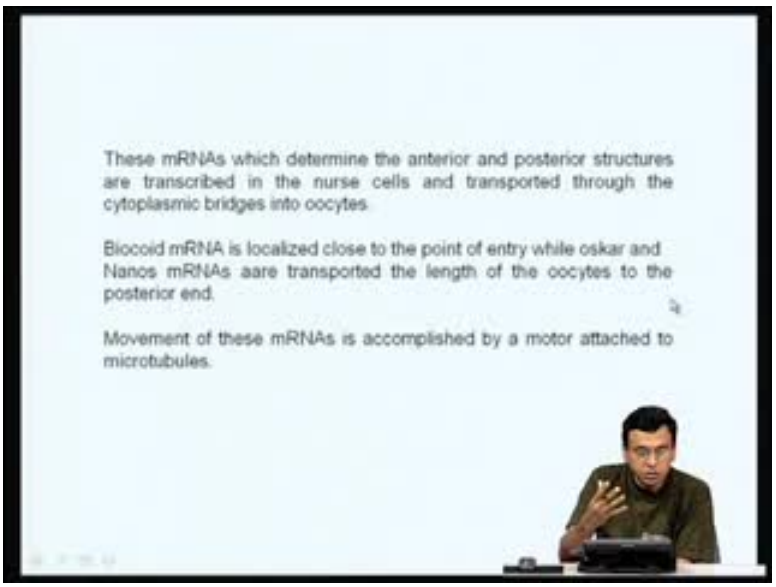

So, these messenger RNA's like dorsal, hunchback, nanos all these messenger RNA's ultimately determines the anterior and posterior structures are transcribed the nurse cells and transported through the cytoplasmic bridges into the oocytes. That is why they called as the maternal effect genes.

So, genes like Nanos and bicoid, they are all maternal effect genes because they are transcribed in the maternal genome by the maternal genome by the nurse cell and then transported into the oocytes before the fertilization are deposited in different regions of the embryo. The bicoid messenger RNA is localized close to the point of entry while the oskar and Nanos messenger RNA's are transported to along the length of the oocytes towards the posterior end.

In fact, how this messenger RNA's are transported into the oocyte from the nurse cells itself is a very interesting topic. In fact, the movement of this messenger RNA's in the oocyte because some are deposited in the anterior end, some of them traverse all through the embryo and depositing in the posterior end and how these messengers are transported and deposited in different regions of the embryo, this is actually accomplished by a

motor attached to microtubules. This itself is a very interesting story of how these maternal mRNA's are transported along the oocyte and some are deposited on the anterior end and some are taken all the way some traverse all the way through embryo deposited on the posterior end. The molecular motors are involved and these RNA's are binding to specific proteins and proteins like a bioscene and actin are involved in the transport of the RNA's are involved in the embryo.

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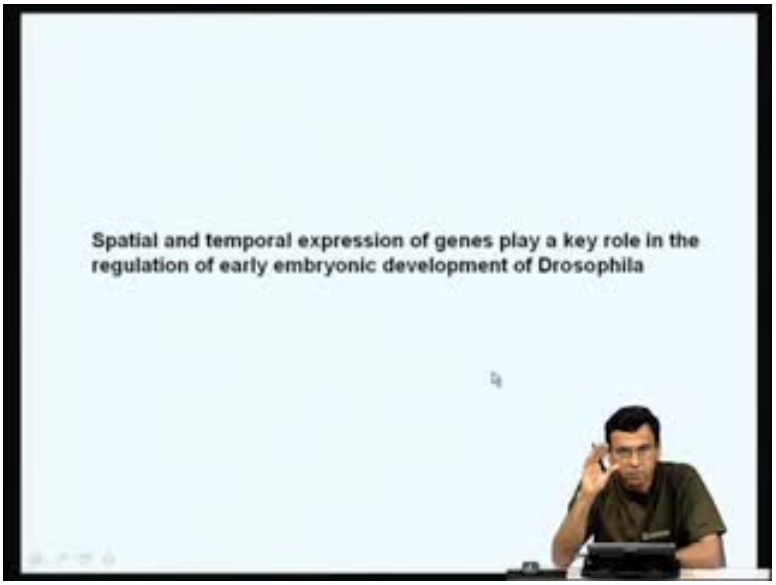

So, the point I try to emphasize in this lecture series is I gave you some very simple examples of how protein transcription like bicoid and hunchback are responsible for the formation of the head and thorax structures and how proteins like Nanos play a very important role in the expression of the posterior structures. Similarly, transcription factors like dorsal play a very important role in the dorsal ventral structures and homeotic genes which encode again homeotic transcription factor contain the homeobox and then work on these various segments and then transform them into specific head, thorax or abdominal segments.

So, to take home message is spatial and temporal expression of genes play a very important role in the regulation of early embryonic development in drosophila. As I said, I have highly simplifies the early embryonic development of drosophila if one can spend one entire lecture just discussing how bicoid protein regulates the expression of hunchbacks or how Nanos regulates the expression or prevents the expression of

hunchbacks and how the bicoid and Nanos proteins are transported along the embryo in the oocyte along the oocyte before fertilization and how these molecular motors ensure that bicoid is deposited high levels in the anterior region whereas, nanos is deposited high levels in the posterior region. Each one itself can be a lecture by itself and these are all wonderful topics to discuss but the purpose of this lecture is to actually introduce to you some of this fascinating aspects of development regulation of gene expression, so that you can now go and refer some of the literature that I am going to site now and read little bit more about and then try to understand the exact mechanism by which these transcription factors work and then bring about early embryonic development.

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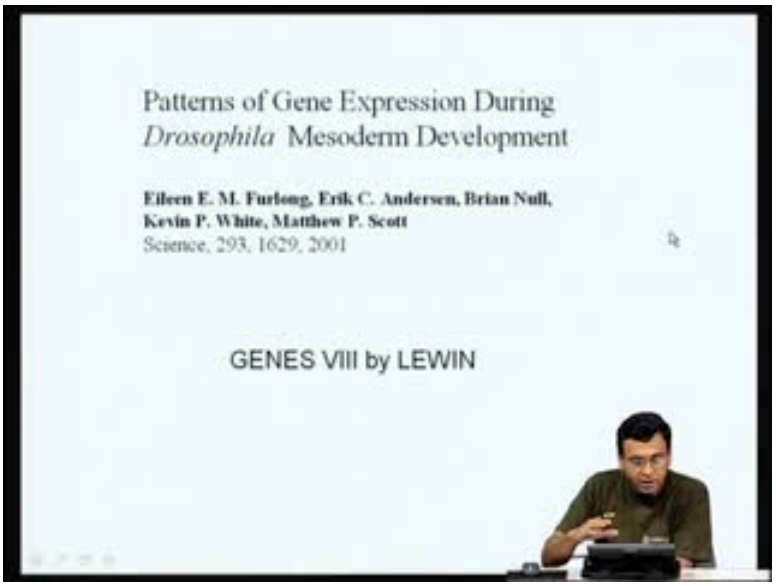

So, what I now gave you is some weights some specific references that you can read and then develop your knowledge further on these topics. There is a very nice review in science in 2000 patterns of gene expression during the drosophila mesoderm development.

The standard text book you can read genes 9, 8 or even genes 9 by Benjamin Lewin. Almost the last but one chapter is devoted for embryonic regulation of gene expression development and with very nice pictorial cartoons and figures. A very nice way the entire development regulation is explained here and there are also a number of websites which you can refer where fantastic information is available including videos. Some of them I have listed here.

There also nice books like the making of a fly by Peter Lawrence, the development of drosophila melanogaster, fly pushing- the theory and practice in drosophila genetics. All give wonderful accounts of how differential regulation of gene expression plays a very important role in earlier embryonic development.

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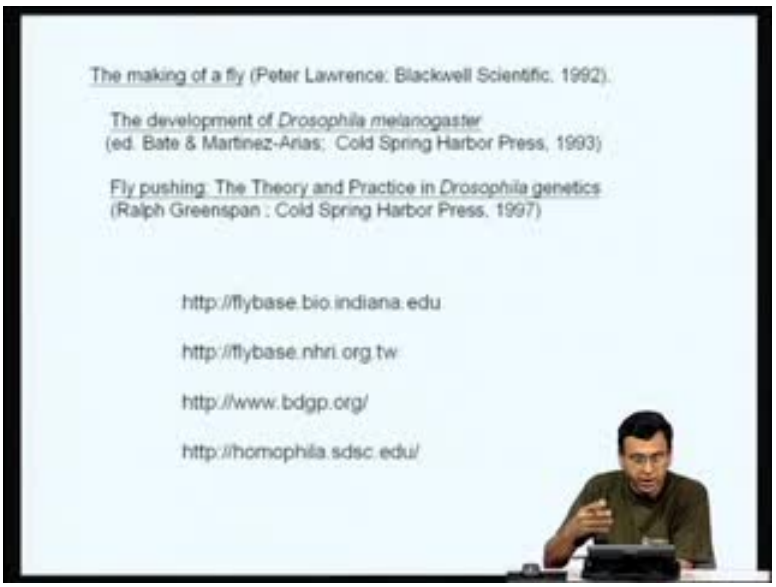

So, I think I will stop here. I try to convey you a very broad guidelines of how a sequential expression of maternal genes segmentation genes and then homeotic genes, these sequential expression as well as spatial and temporal expression of these genes play very important role in the embryo and the regulation of the embryonic development in drosophila.

What will do in the next few classes is instead of as I said that the purpose of this next 2-3 classes not to tell you the entire nuts and bolts of embryonic development but I am going to take select examples and just to tell you how nicely some of these transcription factors work in conjunction with others and how they harmonious or the interplay among the various transcription factors play a very important role in the development of the embryonic development. In addition to what I have discussed now, we basically discussed about the expression and localization of transcription factors and transcription pressures in addition to these cell-cell contacts, cell-cell interactions also play a very important role.

We also then discussed how signal transaction pathways, how molecules which are secreted by one cell of the embryo go and interact with specific membrane receptors of the adjacent cells that leads in the signal transcription pathways leading to the activation or repression of target genes. This again plays a very important role in organogenesis and morphogenesis and so on and so forth.

So, molecules diffusing from one cell, one particular region of embryo would activate a transcription of genes in other cells of the embryo and how this kind of signal transaction pathways play a very important role in the embryonic development. So, we will spend may be another 2-3 hours discussing some of the fascinating aspects of embryonic development and how differential gene expression plays a very important role. I think I will stop here.