Introduction to Developmental Biology

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Lecture – 20

Genetics of Axis Formation in Drosophila Part 4 of 4

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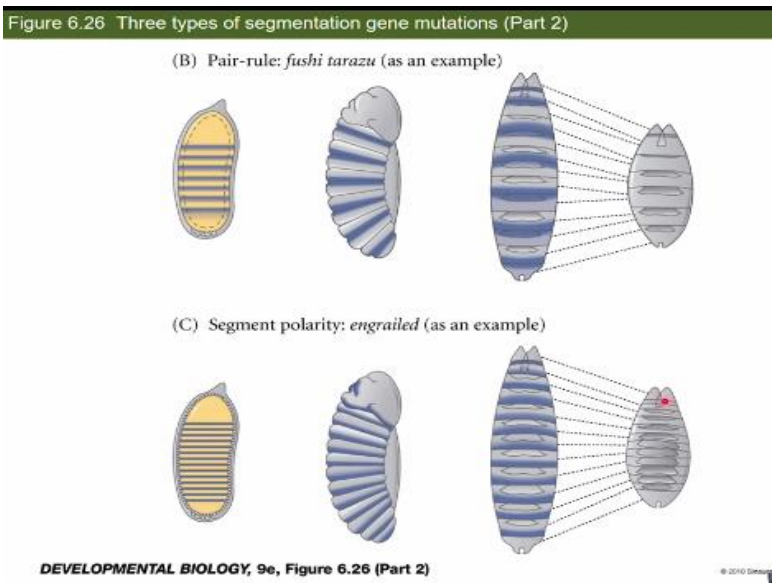

So we are discussing how an animal body plan is established like where to form the head, the tail, etc; also, how to make the head and not make a tail there. This is established in the very early embryonic stage, which is why it is called embryonic patterning. We are using Drosophila as a model to understand where this is well established.

First, we learned dorsal-ventral asymmetry that starts in the oocyte and then continues into the syncytial blastoderm. Then we started with the head to the tail axis that is the anterior-posterior in the previous class. There we learned a set of maternally encoded proteins that act as transcriptional and translational regulators. They make an anterior to posterior and posterior to the anterior gradient. So there is an anterior center and a posterior center of morphogenetic activities. They, in turn, control the expression pattern of genes called gap genes.

Today we are going to start from gap genes and move on. These cartoons in the slide show you the basic expression pattern and the body parts whose formation depends on a particular gene. Figure (B) indicates the expression pattern of a pair-rule gene called *fushi tarazu*. The first image is the early embryonic stage where you see this expression pattern in stripes; one row of nuclei express the gene, but the other one does not, and then the next one expresses and so on. That later corresponds to adjacent sections of two segments in the second image, called parasegment. Later in the third image, which is the pupal stage, you see the corresponding expression pattern. In the mutant, that corresponding domain will be missing.

The segment polarity genes are expressed in even more discreet regions. For example, figure (C) shows the expression pattern of *engrailed*, a segment polarity gene. Its expression pattern corresponds to the parts missing in the mutant.

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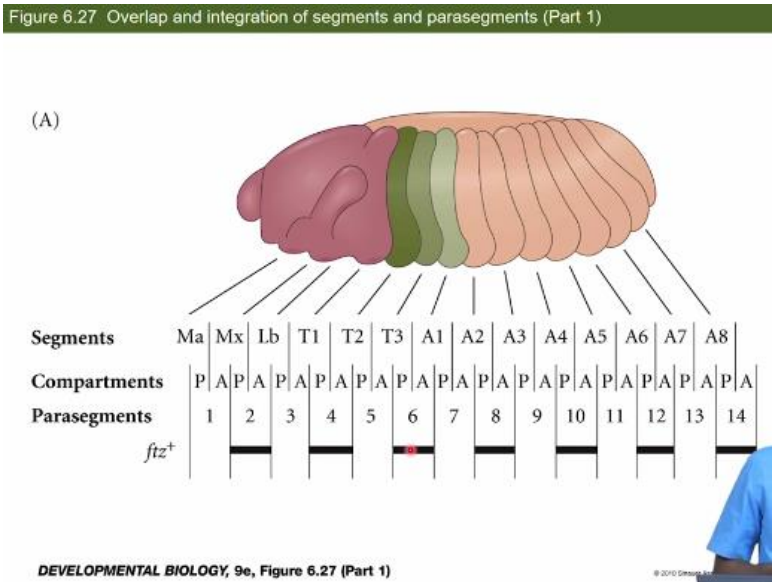

Now we need to understand the relationship between parasegment and segment. The visible morphology is the segments, but internally, when you look at the embryo's molecular constellation, another segmented pattern exists, and that is the parasegments. So the purpose of parasegments will become apparent when we go to the next slide.

First, let us learn what parasegment is; the figure shows various segments like the head segments (Ma, Mx, Lb), the thoracic segments (T1-T3), and the abdominal segments (A1-A8). These are visible body parts, the two adjacent segments, like the posterior part of mandibular(Ma) segments and the anterior of the maxillary(Mx) segment, form one parasegment. Like this, the parasegments 1 to 14 are formed. So the parasegments overlap

the adjacent segments. Here again, to reiterate what parasegments are, they are a pattern of gene expression. For example, gene expression seen in parasegment 6 is distinct from 5 and 7, and that comprises the posterior of T3 and anterior of A1, the abdominal first segment.

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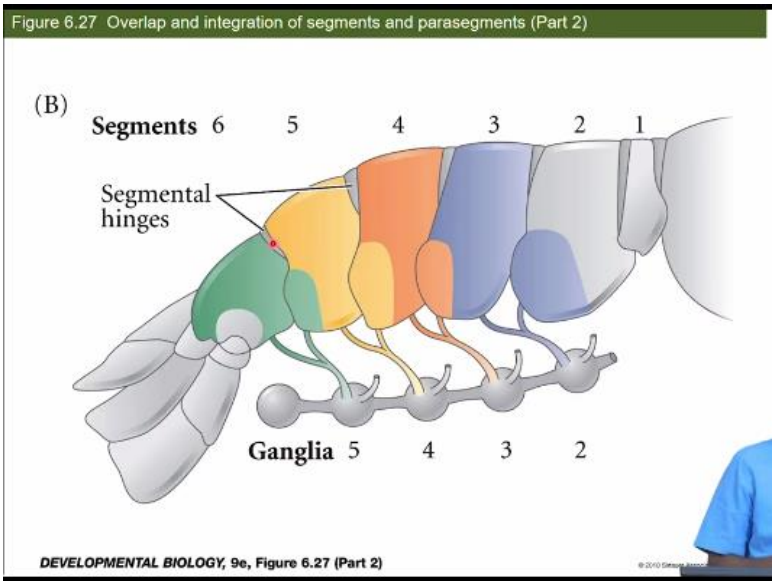

So, the consequence of an expression pattern defining these parasegments distinct from the segments is illustrated in this slide. Parasegments are required to coordinate the different segmental hinges. For example, if you look at the ganglia, the neurons from segments 5 and 6 are innervated by the same coordinate movement at these hinges. So, for the organism to function normally, different segments need to be coordinated, which these parasegments achieve.

So we learned the relationship between segment and parasegment, now we will get back to the genes

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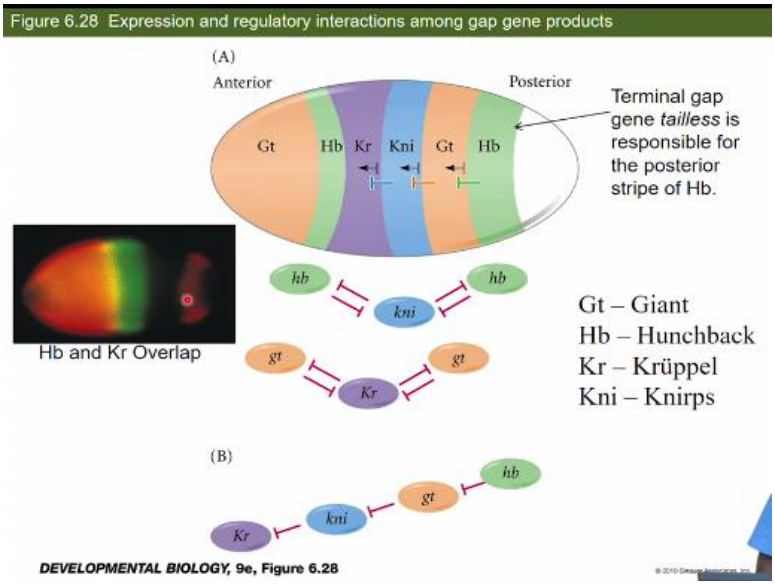

Firstly, let us look at the gap genes. We will continue from the maternal effect genes, so these gap genes are zygotic genes products produced from the zygotic genome. Their expression is controlled by the gradient of the maternal products already established. For example, Bicoid and Nanos regulate the expression of Hunchback and Caudal. Now, they determine the transcriptional activation of these gap genes.

Some of the gap genes are listed here as follows: Gaint, Hunchback, Kruppel, Knirps. These four are the primary gap genes expressed in the segmented part of the body. In the anterior, high concentration of Bicoid and Hunchback activate *gaint* transcription. Slightly away from the anterior where the expression of Bicoid is low, the Hunchback activates Kruppel. Also, Gaint and Hunchback suppress posterior genes such as *kruppel* and *knirps* in the anterior region.

In the posterior, Caudal suppresses the expression of Kruppel and promotes the expression of the Giant. Therefore, the Giant is activated by Bicoid in the anterior and Caudal in the posterior.

I mentioned earlier that Nanos suppresses the maternal *hunchback* mRNA in the posterior region. So the band of Hunchback expression seen in figure (A) at the posterior region is from the zygotic genome activated by tailless. Here the overlaps between Hunchback and tailless are not shown. For example, the in-situ image shows the overlapping expression of Hunchback and Kruppel. Here the green is the Kruppel, and red is the Hunchback; the overlapping region forms the yellow color. These overlaps are essential; for example,

different combinations and different concentrations of the gap gene dictate the formation of different parts of the embryo. So the key thing to remember here is concentration and combination; both are going to vary along the anterior to posterior.

These gap genes have an interesting suppression pattern where they antagonize the genes in the nonadjacent segments. For example, in the figure (A) that shows the expression pattern of these gap genes in stripes, the Giant is adjacent to the Hunchback in the posterior segment, but the Knirps is nonadjacent to the Hunchback, so the Hunchback suppresses *knirps* expression there. Similarly, the Giant suppresses *kruppel* expression, which is nonadjacent. You see that here in the slide, where the Hunchback and Knirps have a mutually antagonistic relationship. Kruppel and Giant also show a similar antagonism. So this ensures that their expression pattern gets stabilized.

So this is how the gap gene expression gets established asymmetrically. Then you see additional arrows and bars in the image that shows the stripes, that indicates an asymmetry in the way the suppression happens. For example, Hunchback's effect on the Giant is higher towards the anterior part of the *giant* expressing domain; there is an anterior tilt in the expression pattern. The giant will be more concentrated in the stripe's anterior region than in the stripe's posterior region. A similar pattern exists for other genes as well. Therefore there is an anterior tilt in the expression pattern, and this is how the gap genes are established.

The in-situ image of the embryo in the blastoderm stage, where it is still a syncytium, shows a particular band of nuclei expressing certain gap genes. So the band of nuclei present in the yellow region expresses some amount of Hunchback and some amount of Kruppel. That is not the case with the adjacent ones, the red part expresses only the Hunchback, and the green part expresses only the Kruppel. Such combinations and concentrations determine what pair-rule genes will be expressed in a given region of the embryo in terms of the anterior to the posterior axis.

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Figure 6.29 Messenger RNA expression patterns of two pair-rule genes, *even-skipped* (red) and *fushi tarazu* (black) in the *Drosophila* blastoderm

Primary pair-rule genes: hairy; even-skipped; runt
Totally, 8 genes show this sort of expression pattern.

Now let us move to the pair-rule genes. The critical fact about the pair-rule genes is their enhancers. Gap genes are regulated by mutual antagonism among nonadjacent pairs of genes; on the other hand, pair-rule genes are regulated by their enhancer modules.

The enhancers of the pair-rule genes have modular arrangements; each module is responsible for expressing a particular domain and can respond to different concentrations and combinations of the transcription factors activated by the gap genes. In earlier classes, while discussing enhancers, I had mentioned two aspects of the enhancers, one, for each tissue to be turned on, there is one domain of the enhancers; therefore, enhancers come in multiple modules. Second, one transcription factor might not activate a particular module; combinations of transcription factors might bind and activate it. So while the cis-element is modular, the trans factors act in combinations.

A similar thing happens here; for example, if you take the expression pattern of Even-skipped, it is expressed in a narrow band of nuclei (red). So it is expressed in this band but not in the adjacent band because here you will have a different concentration and combination of the gap genes compared to the red band. Among the pair-rule genes, there are early ones and late ones; the early pair-rule genes ensure that the late pair-rule genes form this striped pattern of expression. So the primary ones are hairy, even-skipped, and runt. The figure in the slide shows the in-situ of even-skipped. There are a total of eight stripes, and therefore they divide the embryo into that many domains.

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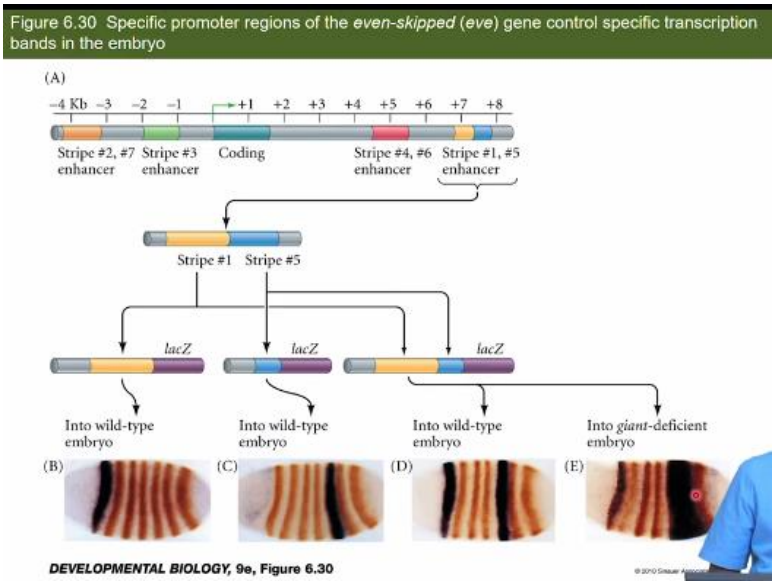

Now let us focus on the enhancers of *even-skipped*. Figure (A) the region upstream of the coding sequence comprises the enhancer module for each strip. For example, the orange one is the enhancer for stripe#2 and#7; the green one shows the enhancer for stripe#3 followed by a coding sequence. Then you see the enhancer for stripe#4 and 6 etc. So these regulations happen in the syncytial blastoderm. This has been experimentally shown here.

If you take the enhancer module responsible for stripe one and fuse it with the lacz reporter, it expresses only in stripe#1 and not in the other ones, and then you take the enhancer for stripe#5 and fuse it with the lacz reporter then it is expressed only in the 5th stripe. Both the enhancers together fused with lacz, results in the expression of both the stripes. If this reporter is introduced into a giant mutant embryo, here giant is one of the gap genes, regulating the even-skipped negatively. In the absence of Gaint, the Eve expression continues and extends beyond its boundary. So this is how these stripes are established.

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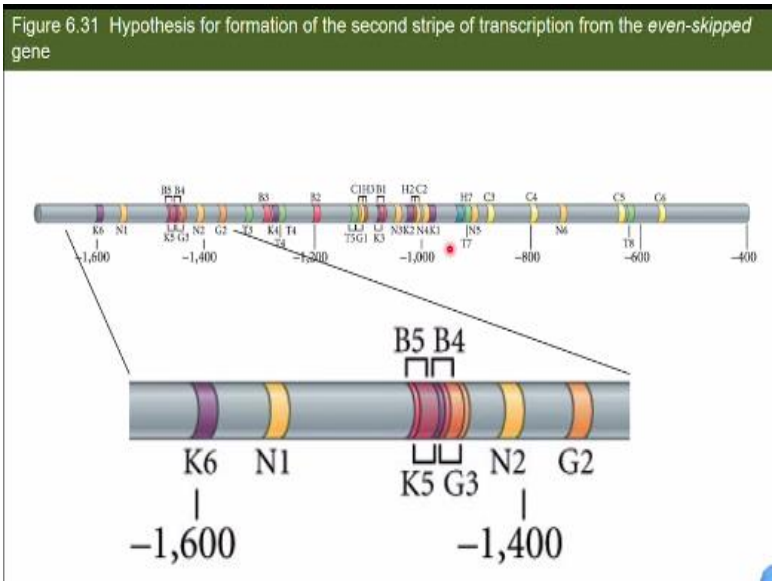

Here we are closely looking at the second stripe. In the slide, these bands in this horizontal bar represent the enhancers where the different proteins bind. Suppose you look at the top ones in the bar, they are the activators like Bicoid(B), Caudal(C), and Hunchback(H). If you look at the bottom of the bar, you see kruppel(K), knirps(N), again kruppel(K), Giant(G), and so on. So these are the suppressors.

Here Bicoid and Caudal are maternal factors, but the others are all gap genes themselves. The main point to focus here is, if you look at the enlarged portion, wherever the activators bind, the suppressors border them. Therefore, to bind to the B5-B4 region, there is a competition between the activators and suppressers, that's why these proteins' concentrations become important. Suppose you have a higher concentration of the activator to the suppressor ratio. In that case, the activator will win over and activate, and if the concentration is the reverse, it will be suppressed. This is seen at every one of the domains where the activators bind. This ensures every narrow domain of expression. So this is about the pair-rule genes.

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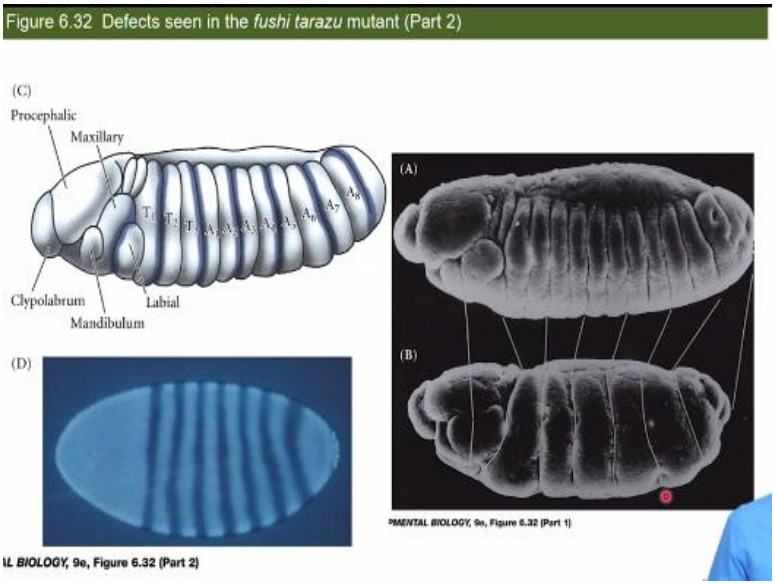

Some of these pair-rule genes activate the late pair-rule genes. If you could remember, out of the eight pair-rule genes that we talked about earlier, three are the early ones, and five are activated later, which are the late pair-rule genes. So one of them is the Fushi tarazu, and that is what we see in the slide. Initially, *fushi tarazu* is transcribed evenly throughout the embryo, but once the early pair-rule genes come into effect, they bind to enhancers in the *fushi tarazu* region and suppress its expression. As a result, these stripes (figure D) are generated. The expression corresponds to the region adjacent to T1, T2, and so on. The absence of this expression gives you the defect that you see in the mutant in figure(B).

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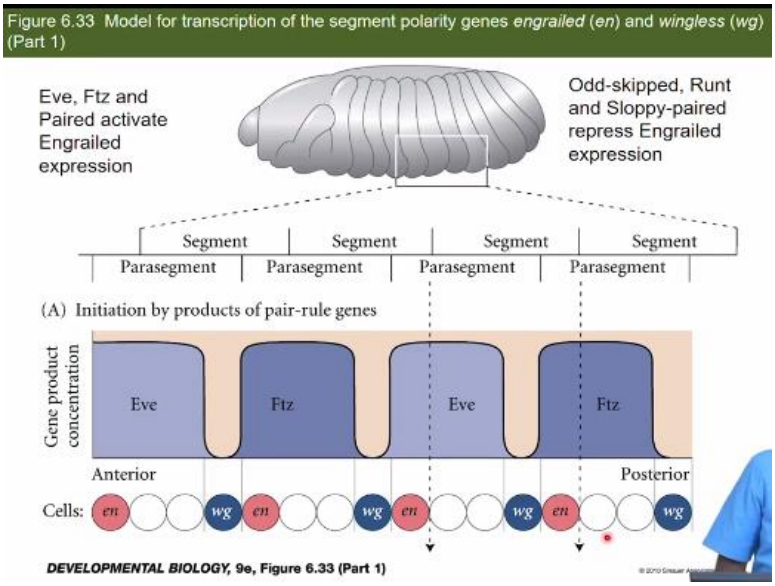

Next, we will see the segment polarity genes. We are looking at the enlarged portion in the slide, which shows the segments and parasegments in the developing embryo. Let us focus on one of the parasegment; in figure (A), the second parasegment shows very high expression of

Ftz, and the two adjacent regions have very little Ftz or the other pair-rule gene Even-skipped(Eve). At this stage, the syncytial blastoderm has progressed into cellular blastoderm where cells can produce different receptors and ligands and influence each other. So you see a single row of cells expressing the segment polarity genes *engrailed* (*en*) and *wingless* (*wg*).

The regions with high Ftz or even-skipped express Engrailed; for example, Engrailed is expressed where Ftz, Eve, and Paired expressions are high. Paired is another gene that activates *engrailed*. If anyone of these three is high, they are going to activate *engrailed*. In the adjacent region where you have low expression of these genes and if other genes like odd-skipped, runt, or sloppy-paired are high, they will repress *engrailed*, but in turn, they activate *wingless*. As a result, one row of cells in each parasegment express Engrailed, and another row of cells express Wingless. This pattern gets established throughout these cells.

Once these gap and pair-rule genes activate the segment polarity genes in this fashion, their expression gets stabilized in the following way.

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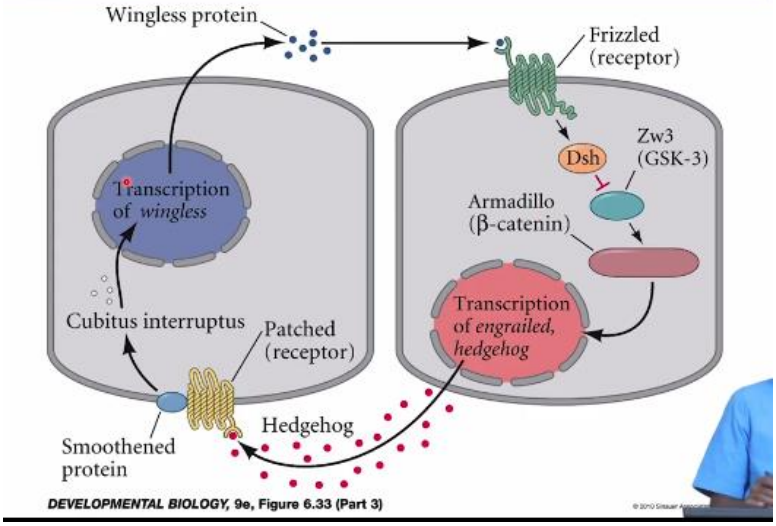
Figure 6.33 Model for transcription of the segment polarity genes *engrailed* (*en*) and *wingless* (*wg*) (Part 3)



So this is what happens in the adjacent cells. These are the cells that were shown to express wingless and engrailed in the previous slide. Wingless is activated by the absence of Ftz or even-skipped and presence of odd-skipped or other genes. This Wingless is a secreted signaling molecule that binds to the adjacent cell, expressing *engrailed*. The *engrailed* expressing cell produces the frizzled receptor; therefore, this *wingless* signal is transduced, and results in the activation of two genes: one is *engrained* itself; consequently, this cell remains as *engrailed* producing cells. In addition, it also produces Hedgehog, which is a signaling molecule involved in paracrine signaling. This secreted Hedgehog binds to the

patched receptor present in the *wingless* producing cells. Only the *wingless* producing cells make the patched receptor, and only the *engrailed* producing cells make the frizzled receptor. This signaling ensures this cell produces Wingless and the other cell producing only the Hedgehog.

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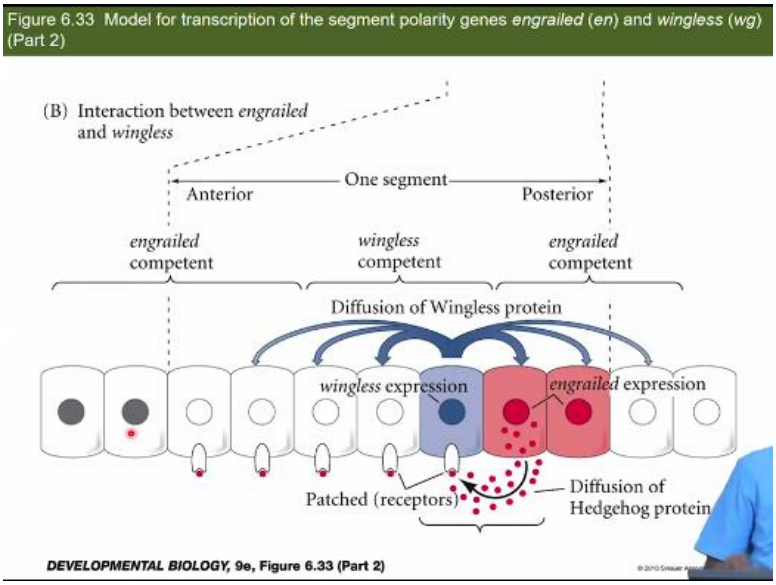

These signaling molecules, Wingless and Hedgehog, are morphogens. As a result, they will generate a gradient. So one side of the cells where *wingless* is expressed will have a diffusing gradient of Wingless; similarly, in the other cells where engrailed is expressed will have a hedgehog gradient.

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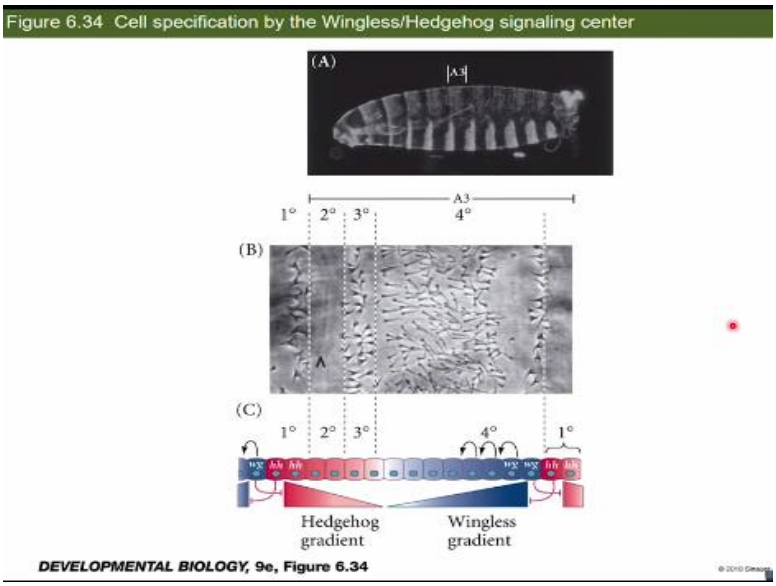

Let us see the consequence of the expression pattern that we saw in the previous slide.

Here we can think of a situation like primary, secondary, tertiary fates for the cells, and that is what happens that is shown in the slide. Suppose you look at the top surface of this embryo in figure (A) and take the abdominal segment 3 (A3) and look closely. In that case, you find the ones producing a high amount of either of the two (*wg* or *hh*) have a single row of hair structure formed on the dorsal epithelium. And where there is no wingless, but less of the Hedgehog, form a smooth surface. The next one, the tertiary fate cells make thick hair-like projections, and then the other ones in the quaternary fate make the fine hairs. So this pattern repeats. Now you have a visible morphology. So from patterning of molecules to asymmetry resulting in the morphology. This is how the segment polarity functions.

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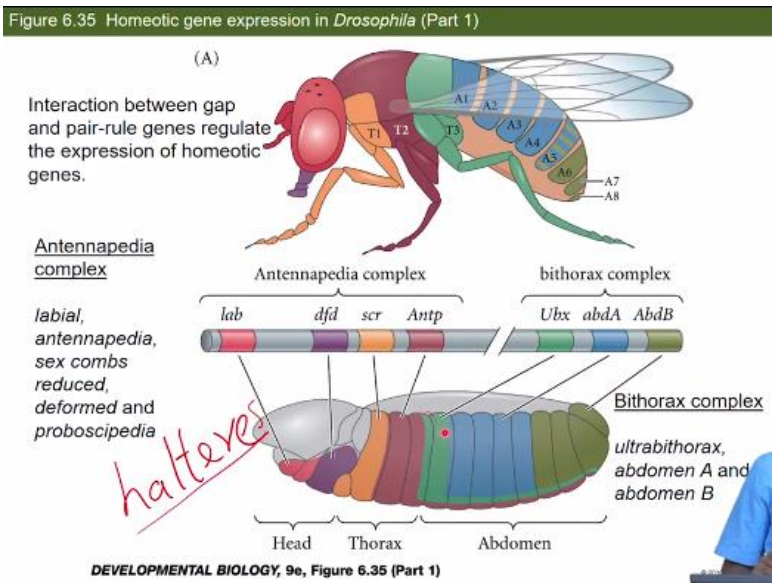

So now we will get to the most fascinating of all genes, the homeotic genes. Their effect on the body plan is dramatic, starting from a little earlier than arthropods and up to us. So their impact on nematodes is not so much. If you see the evolutionary timescale, the earliest organisms have the body plan like our body plan. So our body plan is not very different from Drosophila. Like us, they have the head, the abdomen like our chest, etc. If you look at their mouth, anus, and the rest of the body, it is almost the same as ours. Scientists think this tube-like body pattern started with the nematodes, but there could be organisms earlier than that. In those very early organisms itself, these homeotic genes are present.

So now, let us see what these genes are. This middle bar in the slide shows the part of chromosome 3 of Drosophila. It comprises two groups of genes: the antennapedia complex, which has four genes: labial, deformed, antennapedia, and sex combs reduced. Then the other complex is the bithorax complex that has ultrabithorax, abdomen A, and abdomen B. These

homeotic genes are turned on by the interaction between the gap genes and pair-rule genes. Different concentrations of these genes in different regions activate these homeotic genes.

The fascinating thing about these genes is, if you look at the color code in the figure, the left to right arrangement of these genes corresponds to the anterior to the posterior domain of expression and the anterior to posterior structure formation. The leftmost gene is required for the anterior-most body part, and the rightmost gene is responsible for the posterior-most body part formation

So, there is collinearity between the location on the chromosome and the body structure. This collinearity and why that is conserved have not yet been understood fully, but that is how these genes are arranged. Now let us see what happens if these genes are not expressed

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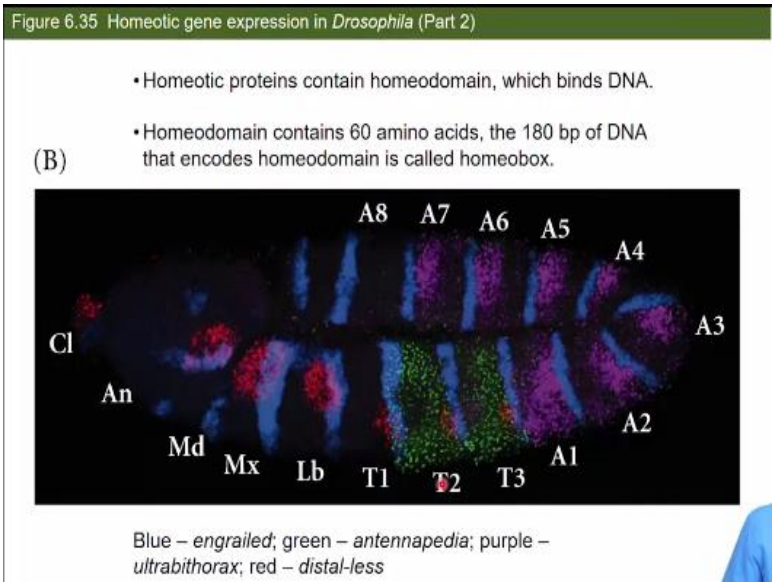
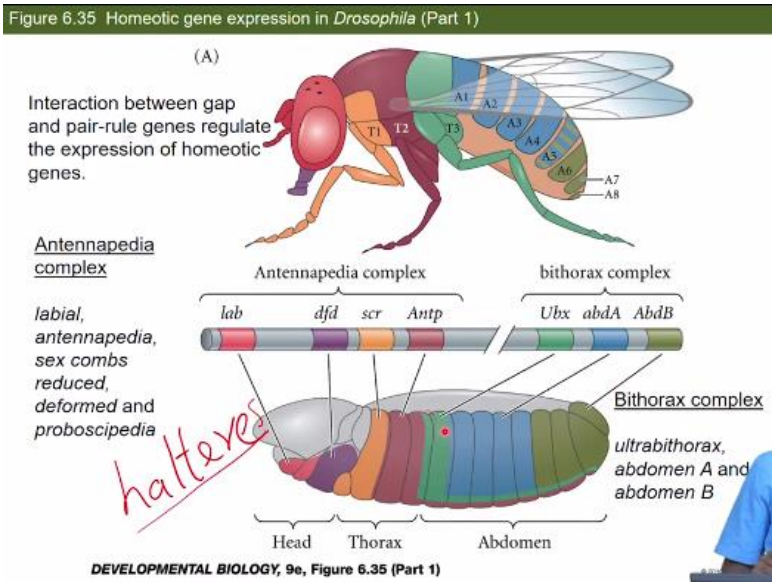


First, let us look at the expression pattern of these homeotic genes. The homeotic proteins contain a domain called homeodomain, which consists of 60 amino acids that are conserved and binds DNA. The 180bp of the DNA sequence that encodes homeodomain is called homeobox. The name homeo primarily comes from the mutants where the same structure as an existing structure is formed in the organism. For example, in the place of the antenna, a leg is formed, which looks similar to the actual leg; therefore, it is called homeo. They are also called hox genes.

Figure (B) in the slide shows the expression pattern of *engrailed* (Blue), *antennapedia* (green), *ultrabithorax* (purple), and *distal-less* (red). So *antennapedia* is expressed only in the thoracic segment where it functions.



So in this slide, which we just saw, this labial is required for the head segments, then for the first thoracic segments, sex combs reduced is required, and then *antennapedia* is required for the second thoracic segment. The *ultrabithorax* is required for the first abdomen segment and the posterior of the last thoracic segment.

So we will focus on the thoracic segment because it makes dramatic structures. So the first thoracic segment makes a pair of legs, the second one also makes a pair of legs, but it also makes a pair of wings as you see here in the fly. The third one makes another pair of legs. Essentially all three thoracic segments make a pair of legs each. The concentration of the factors is such that only the T2 makes wings but not the adjacent ones. Here in T3, the wings do not form primarily because the Ultrabithorax expression converts the wing-like structure into haltere.

Haltere is a short rudimentary wing-like structure that helps in balancing the flight. Drosophila is a dipteran insect, meaning two-winged insects. Many other insects have four wings, so haltere is a vestigial small wing-like structure, not a fully formed wing. Evolutionarily it is a wing-like structure that has become vestigial in the dipteran insects,

primarily because of the *ultrabithorax* expression there, which does not allow the wing to form there. This is how the thoracic segments form various structures.

Now, let us see what happens to the third thoracic segment; suppose *ultrabithorax* expression is absent, and instead, *antennapedia* is expressed fully. In that case, according to the expression pattern, the third thoracic segment will be like the second thoracic segment, which will form a pair of wings and a pair of legs. So that is what happens in the mutants shown in the upcoming slide.

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So this is one example, let us look at one more.

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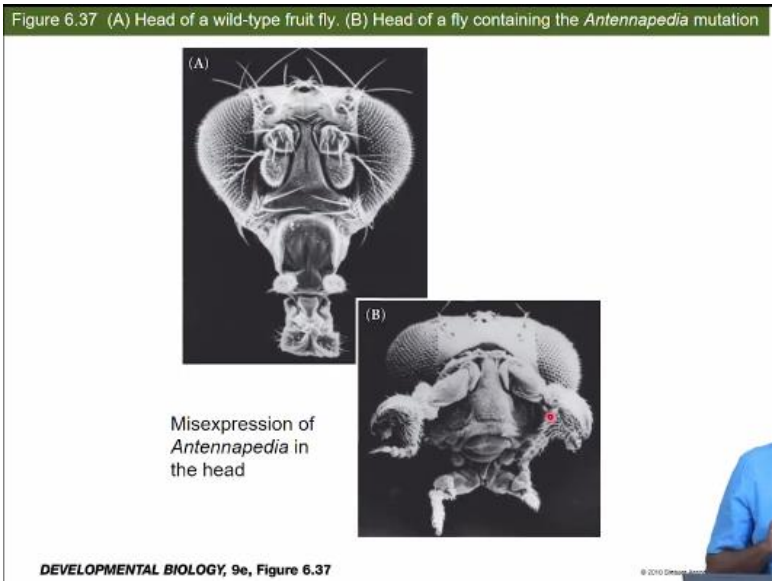

Here misexpression of *antennapedia* in the head causes the formation of legs in the place of antenna (figure B). This is due to the *antennapedia* suppressing the genes required to make the eye and antenna and in addition, promoting the formation of the leg.

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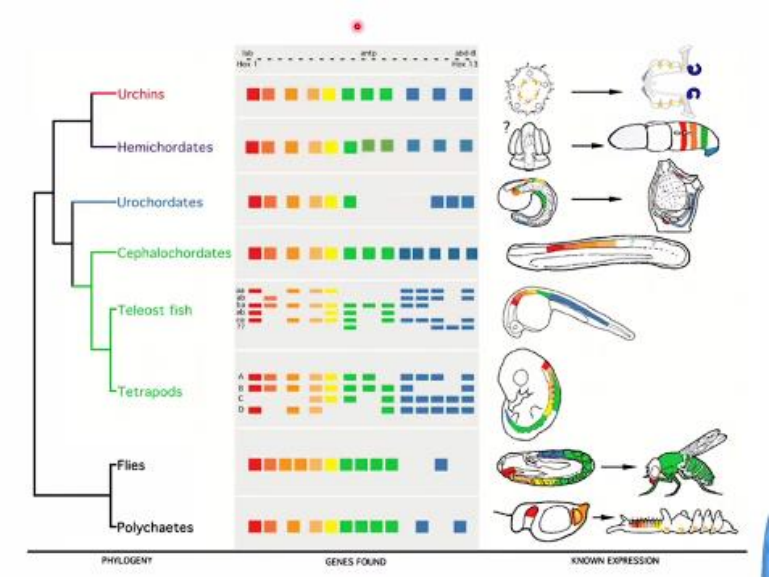

I want to finish today's class with this phylogenetic tree. Here you see the hox gene arrangement, which starts with a common ancestor. So we are looking at the flies where we saw the collinearity in the head to tail expression pattern, and similarly, in the body part formation. This pattern is seen in flies, polychaetes and goes on to tetrapods. So we are from tetrapods, the four-legged animals. Then this pattern continues to fishes and then the cephalochordates. Chordates mean anything that makes notochord, so notochord is the most general structure, including humans, fish, everything. And if you go all the way to urochordate, hemichordate, urchins, all of them have this pattern. So this collinearity of the genes' arrangement on the chromosome and the body parts formation has not been altered for about 500 million years.

I will stop here; in the next class, we will see homeotic genes in further detail.