Introduction to Developmental Biology

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Lecture – 19

Genetics of Axis Formation in Drosophila Part 3 of 4

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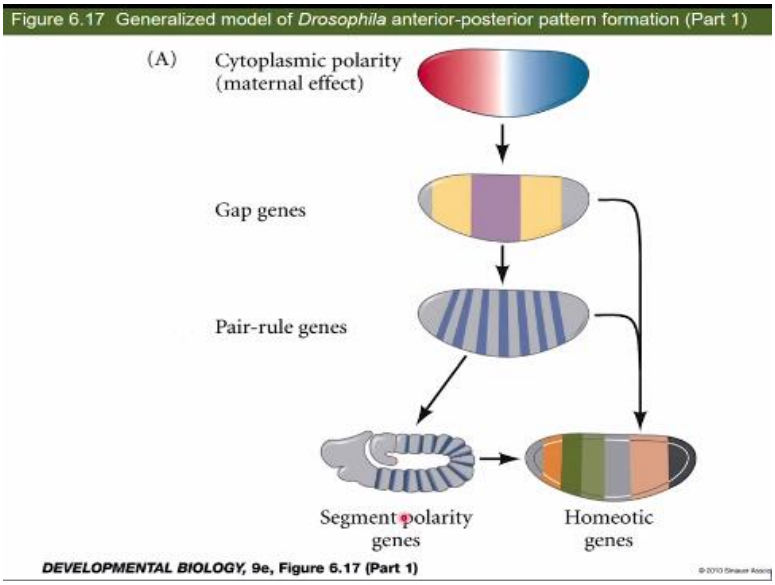

In the last class, we saw the maternal effect, which means a gene product deposited by the mother in the oocyte is sufficient for that oocyte to develop into a normal adult even if it is mutant for that particular gene. Such gene products function during early embryonic development. Generally, the maternal products get degraded late in adulthood; therefore, the phenotype is seen only two generations later: the grandchildren generation. So far, we saw the anterior-posterior and dorsal-ventral axis formation in the oocyte. Now, we will look at pattern formation.

The initial pattern established by Bicoid and Oskar's asymmetric localization in the oocyte is insufficient to make a complex organism. So the asymmetry has to be enlarged by involving various molecules, and that asymmetry has to become localized as well. Only then the embryo can make multiple tissue types that can form the right organs at the right places. So how that happens is best understood in the Drosophila embryogenesis, which is why we are focusing on it. So the first asymmetry starts with the maternal factors that are products of the

maternal germline. They are already deposited either as protein or mRNA into the new embryo. So, these asymmetrically localized factors determine the next set of genes that need to be activated. These maternal mRNAs are asymmetrically localized or asymmetrically translated, which helps in the initial pattern formation. Some of them are translational regulators as well as transcriptional activators or inhibitors. Like for example, Bicoid is a translational inhibitor of Caudal, but at the same time, it is the transcriptional activator of genes that we have not yet discussed; also, it is required for their asymmetric localization. Finally, those gene products will turn on the transcription of downstream genes called Gap genes. They are called Gap genes because their mutations led to large gaps in the embryo that develops.

So today, we will see what the Gap is in terms of the embryo. Gap genes are expressed in a large pattern, as shown in the slide, and this pattern also repeats. So here in the slide, the orange pattern repeats. So this larger band-like expression in the embryo does have overlapping distribution as well. These stripes here are not showing any overlap, but they do overlap like; for example, this orange color may overlap some part of this purple, and purple may overlap the blue, or they might function independently. These are important in activating the next set of genes called the Pair-rule genes. They are called so because they form these stripes like expression patterns, and they usually affect every other segment in the larva that develops. So here, segments mean the segmented body pattern. If you take any insect and look at it, you can see a larger abdomen segment and a thoracic segment where you have the legs and the wings, and then you have the head where you have the antenna, etc. So Drosophila makes fourteen segments.

These pair-rule genes affect alternative segments. So their expression pattern forms these precise stripes, as you see in the slide. So from the gradient pattern, now we have gone to this precise pattern. So the Pair-rule gene products, the Gap gene products, and some of the maternal products altogether coordinate to activate the next set of genes called Segment polarity genes. So these genes specify the expression pattern in each segment.

So by the time these Segment polarity genes get activated, the embryo becomes cellularized. Now the adjacent cells start signaling and influence each other, leading to the activation of homeotic genes.

So today, our goal is to go up to segment polarity genes. Homeotic genes we will see in the next class.

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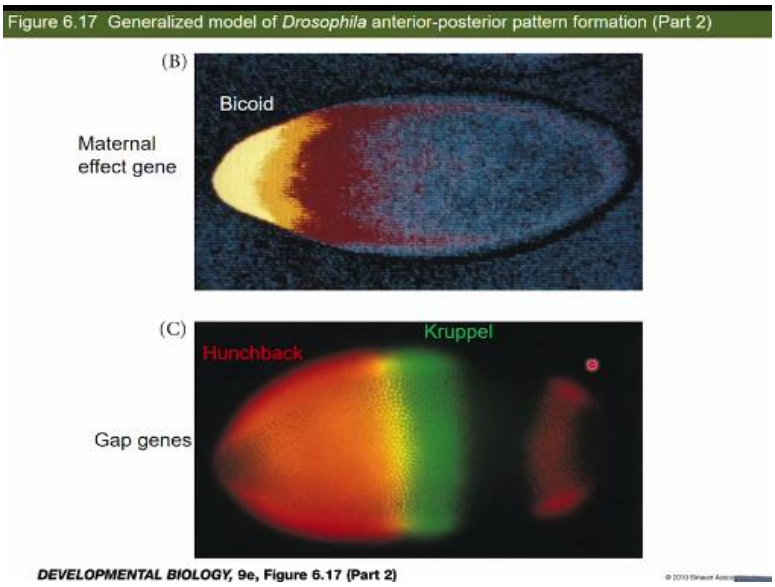

Let us start with maternal effect genes. We are already familiar with Bicoid that gets to the anterior in the oocyte, which gets translated and forms the protein product. While these are happening, the dorso-ventral specification is also simultaneously happening. So the Gurken that has gone to the dorso-anterior suppresses Pipe in the dorsal follicle cells while the ventral follicles cells get primed to transduce the signal via the Toll receptor. That signal transduction is required when Dorsal protein is formed a bit later.

The bicoid mRNA that was localized earlier, now gets expressed, and forms a gradient. I mentioned that the Gurken goes to the dorso-anterior region and inhibits Pipe synthesis in the adjacent follicle cells during the oocyte stage. The signal transduction at the ventral, where Dorsal getting into the ventral nuclei, happens in the embryo stage. So the embryo reaches the blastoderm stage where the nuclear divisions are over, and the nuclei are located cortically. So this is happening continuously while that process is happening. Similarly, this anterior-posterior axis formation is happening too. So it would help if you did not disconnect them; for convenience, we are ignoring all that discussion that we had so far. That does not mean that all these events happen separately. The asymmetric microtubule formation and, as a result, the Bicoid hitching a ride on a motor protein and going to the anterior and the Oskar going to the opposite side, there already the anterior-posterior patterning is forming, and that is at the oocyte stage. But what I am saying is that now leads to further enlargement by including many molecules. Therefore the organism can make more specific structures. The

embryo cannot end up making only two domains like the anterior pole and the posterior pole, and that alone is not enough; it has to make multiple tissue types and structures. Once the asymmetry is initiated, that needs to be made into more localized asymmetries.

Now the highest concentration of Bicoid at the anterior-most activates certain Gap genes. These genes are required for the anterior-most segment development, and there are structures called Acron that form in the anterior segmented part of the body. So the tail-like portion is called the telson. So these structure formation requires the highest concentration of Bicoid and other factors, as we will see later.

The Bicoid will suppress the Caudal expression in the anterior portion, allowing the Hunchback expression to happen there. So Bicoid is a transcription factor as well. So Hunchback activation does not require a high concentration of Bicoid. Gap genes like *empty spiracles, orthodenticle* are the anterior-most, and their activation requires Bicoid, and then you need a slightly lower concentration of Bicoid for activating Hunchback.

These are all determined primarily by the promoter region's affinity and these transcription factors rather than the enhancers. So the bicoid is at the anterior, figure (B) is the actual example of the cartoon we saw in the previous slide, figure (A).

So this particular embryo in figure (B) shows only the protein localized in the anterior region. Figure (C) is an example of three different gap gene expression patterns. So you can see the expression of the Hunchback and the Kruppel, another gap gene.

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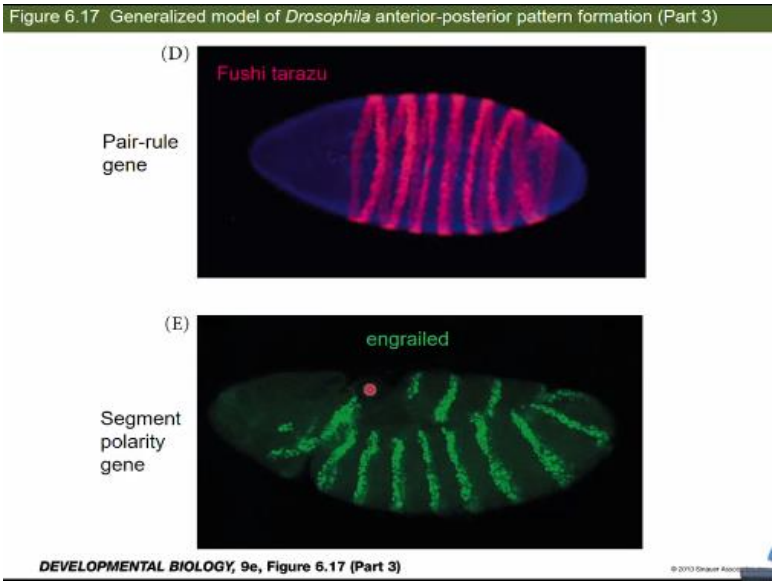

In this slide, figure(D) shows the expression pattern of a Pair-rule gene called Fushi tarazu, which was discovered by a Japanese group. Fushi tarazu means fewer segments. This is a late Pair-rule gene. Pair-rule genes consist of two groups: the early pair-rule gene and the late pair-rule gene; together, there are eight of them, and we will see them in detail. Figure (E) shows the expression pattern of Engrailed, a segment polarity gene. Engrailed is seen only in the anterior part of the segment. If you can count these lines, you will find fourteen of them.

So we will see how each one of these gene's patterns is established in the coming slides.

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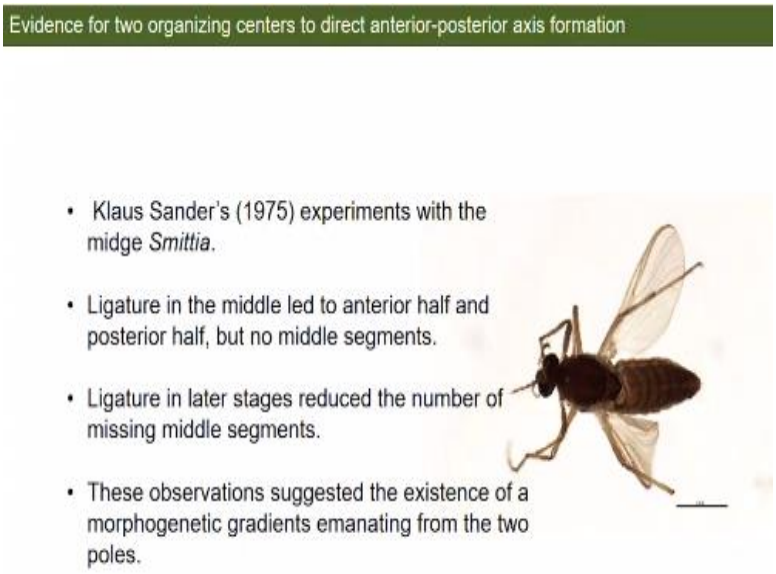

In 1975, Klaus Sanders did a simple experiment, where he took an early embryo of an insect and tied a thread in the middle of it to create a ligature. This will prevent the movement of factors between the anterior and posterior regions. Then he observed that the formation anterior and the posterior structure but not the middle structures. Later he shifted the ligature

timing and found that lesser and lesser of middle segments were missing. So he proposed that there must be two decision points, one at the anterior and one at the posterior, and they probably instruct the rest to form different structures in a gradient fashion. So his observations also suggested the existence of a morphogenetic gradient.

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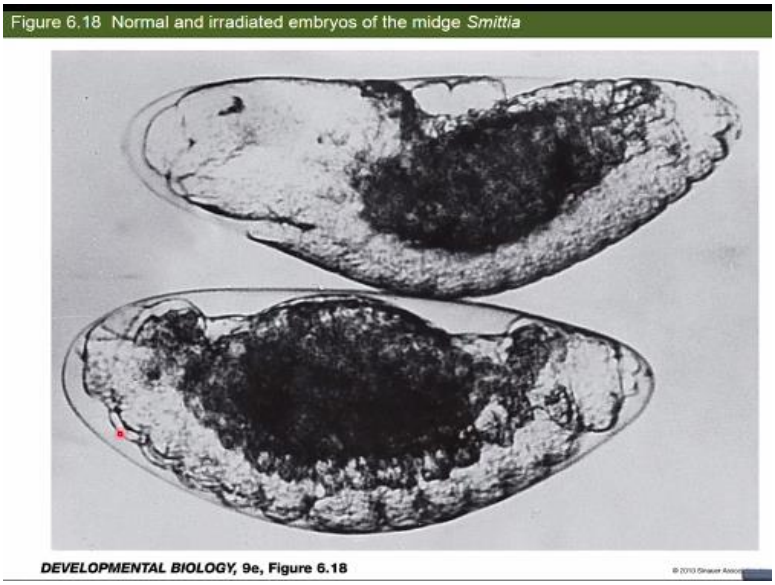

He also did an experiment, where he irradiated the anterior part of the embryo with UV, which will affect the RNA present in the anterior part. So he found that the head structure did not form in that embryo. Instead, it formed two tail-like structures indicating that the RNA behind this morphogen gradient, at least from the anterior part, is affected by UV, as a result that disrupt the morphogen required for the head formation. So this observation led Nusslein Volhard and her group to ask questions like what these morphogens are? How are they forming the gradient? How these structures are made, and how those asymmetries are stabilized? And so on. To answer these questions, they decided to mutagenize and look for mutants where a particular structure was missing. The answers to those questions are the summary we just saw: involvement of the maternal effect gene, the Gap gene, the Pair-rule, and so on.

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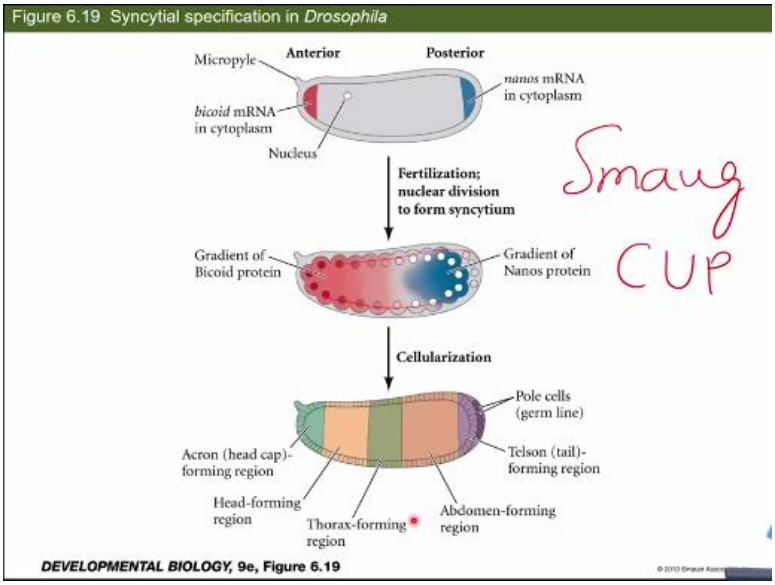

Let us get into the details of each of these gene types. So the first one is maternal effect genes; we learned earlier that Bicoid is present in the anterior, and Nanos is present in the posterior.

So *nanos* mRNA is produced by the nurse cells, and they are deposited into the embryo. Initially, in the egg chamber, the nurse cells are present in the anterior part. So the *nanos* mRNA produced by them diffuses in the cytoplasm; they are not carried like bicoid by the motor proteins. So in the posterior, Oskar binds and protects the *nanos* mRNA. Oskar also helps in removing the translation inhibitors and allows the Nanos translation in the posterior. Generally, *nanos 3'UTR* is bound by Smaug and Cup proteins in the cytoplasm, which prevents its translation. Therefore, Nanos is produced only in the posterior.

Now, this Bicoid and Nanos form this concentration gradient that is shown in the slide. This gradient exists in the syncytium, where the nuclei are nicely arranged in the cortical region. Upon cellularization, various genes get activated as a downstream product.

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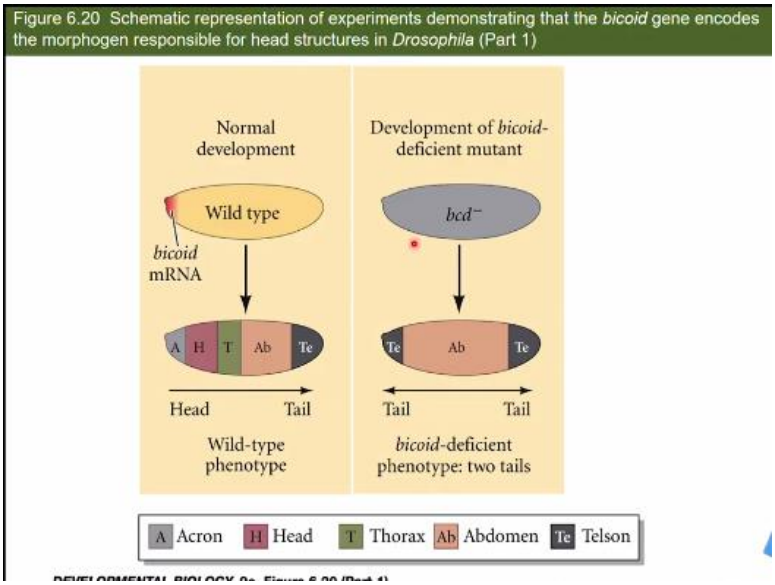

So this is the same experiment that we saw earlier. This is just that it is having more specific labels like acron, telson, etc. In the wild-type embryo, the acron, head, thoracic and abdominal segments are formed. But in the *bicoid* mutant, you see telson, then the abdomen, and again the telson indicating that the anterior structures like this acron, head, and thoracic formation requires Bicoid.

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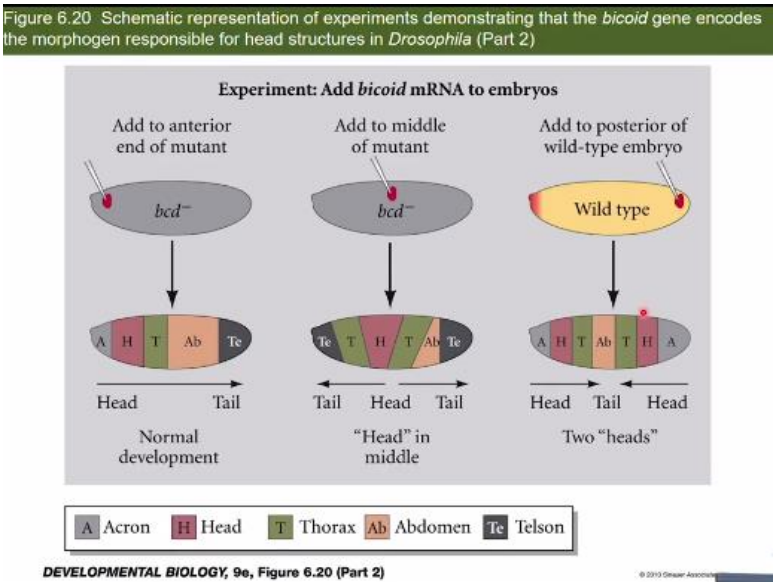

We are familiar with the experiment shown in this slide, where injecting *bicoid* mRNA into various regions of the embryo causes anterior structure formation in that region. Here in the *bicoid* mutant, if we inject *bicoid* mRNA in the anterior end, it forms a normal embryo. If *bicoid* mRNA is injected into the middle of the embryo, acron is not formed, but it forms a head with thorax on both sides, behaving like the morphogen gradient. When injected in the

posterior of a wild-type embryo where Bicoid is already present in the anterior region, the posterior also forms the head structures. We end up making a double-headed insect.

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So this shows an in-situ hybridization for *bicoid* mRNA, and then you see the gradient here.

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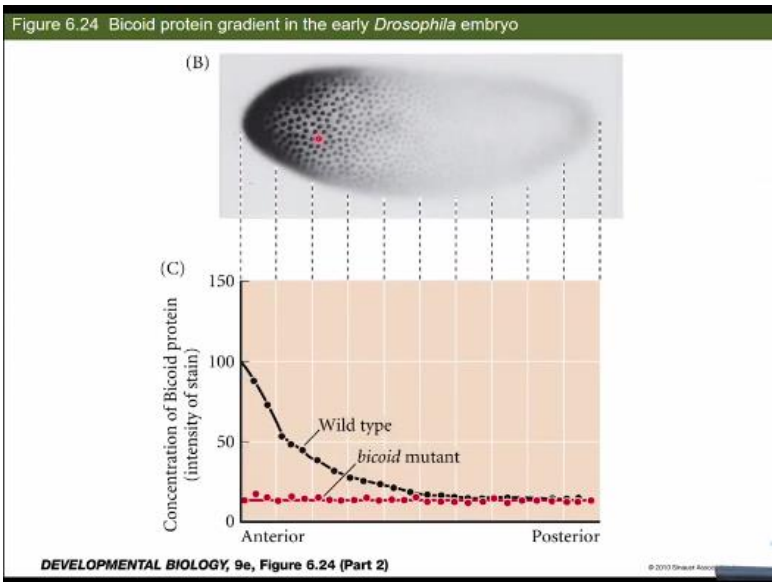

In this slide, figure (B) shows the Bicoid protein gradient in the early drosophila embryo. This forms a shallow gradient that people believe is because some RNA is present little away from the anterior region even they are poised for translation. Therefore, in a very short time, you get this gradient of the Bicoid formed. Figure(C) shows the concentrations of the Bicoid protein in wild-type and the mutant

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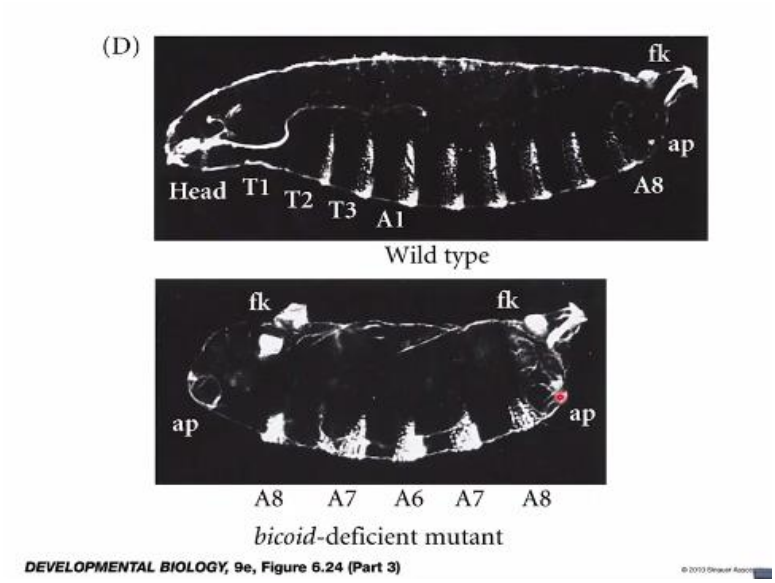

This is how the surface cuticle looks in wild-type and in the *bicoid* mutant, which is missing some of the head structures.

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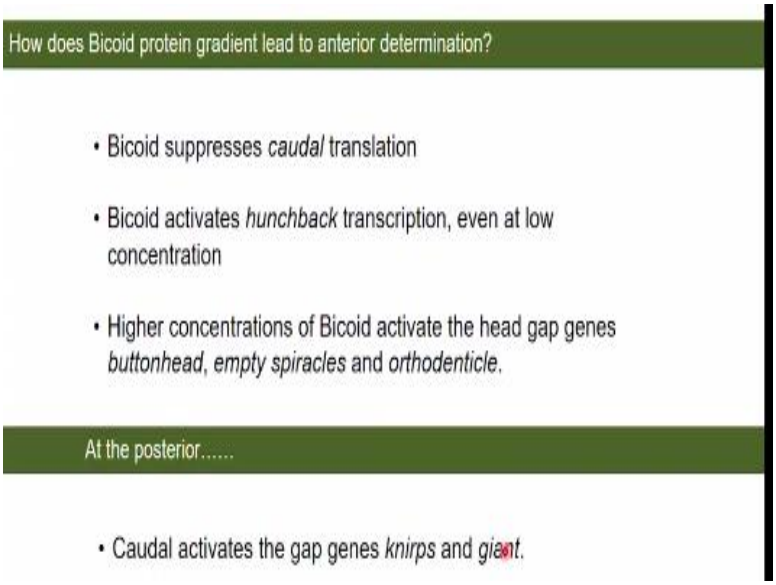

So the Bicoid gradient in the anterior portion suppresses *caudal* mRNA, and it activates hunchback transcription. So here, Bicoid acts as a transcriptional factor to activate zygotic hunchback expression in the nuclei. I want to point out that Bicoid is a maternal factor, but the hunchback is both maternal and zygotic. So this zygotic hunchback activates some of the gap genes.

So the highest concentration of the Bicoid in the anterior activates the *button head*, *empty spiracle*, and *orthodenticle*. Caudal, which is a transcription factor in the posterior, activates the posterior gap genes *knirps* and *giant*. So this is the summary of the anterior and posterior

set up by the maternal effect product gradients. Now let us see about the Nanos in the posterior.

So in the posterior, Nanos binds to the *hunchback 3'UTR* and along with Pumilio. These proteins bind to *hunchback 3'UTR* and suppress Hunchback translation in the posterior. If you can remember, Hunchback comes as a maternal product, so the maternal product distributed throughout the cytoplasm needs to be inhibited in the posterior. Therefore it will not activate the thoracic segment related genes in the posterior.

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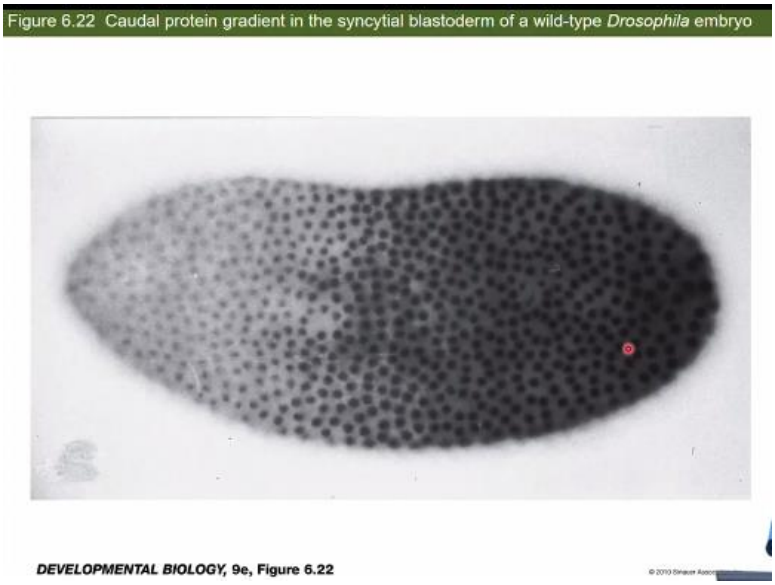

So this is the Caudal protein gradient. You see, it is a mirror image of the Bicoid expression; again, it is in the nuclei. So to remind you what is happening developmentally, these are syncytium, not cellularized. So there are a lot of nuclei in that common cytoplasm. The only thing is the nuclei are on the cortex. Here you see the surface; therefore, you readily see the cortex.

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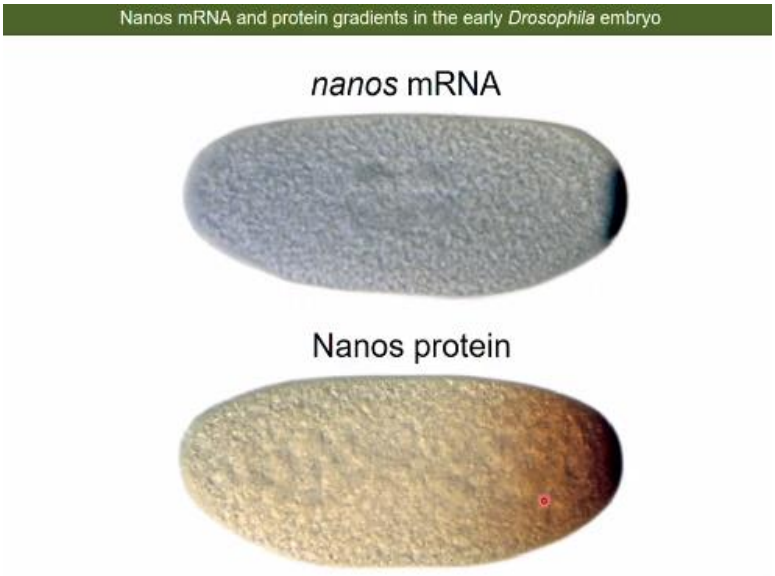

In the posterior, *nanos* mRNA is stabilized by Oskar; otherwise, the Smaug and Cup will bind to the *nanos* mRNA and suppress it by removing the poly-A tail; as a result, *nanos* mRNA gets degraded. Since *nanos* mRNA is primarily present in the posterior region, it gets translated and forms a shallow gradient of protein in the posterior, as you see in the slide. So this Nanos protein produced here collaborates with Pumilio to bind the *hunchback* mRNA and suppress the translation of Hunchback. This will prevent the anterior structures forming in the posterior and allows abdominal structures to form instead.

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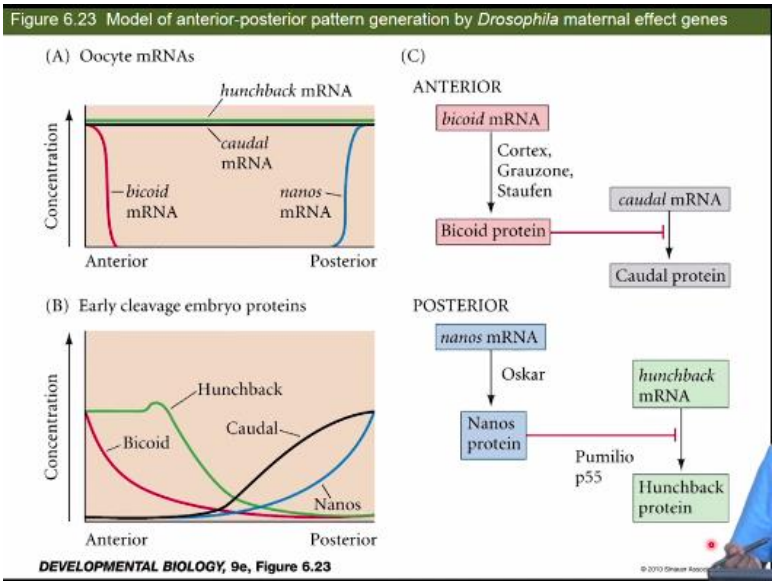

This is a summary of anterior-posterior pattern formation by the maternal effect genes. So *hunchback* mRNA and *caudal* mRNA are distributed throughout the oocyte. But *bicoid* and *nanos* mRNA are restricted to the anterior and posterior region. During the early embryonic cleavage, Bicoid promotes the translation of Hunchback in the anterior and suppresses

Caudal translation. Similarly, in the posterior, Nanos promotes the translation of Caudal and suppresses the translation of Hunchback. These regulations result in a shallow gradient of protein expression. In the anterior, Bicoid translation is promoted by proteins mentioned in figure(C). In the posterior, Nanos translation is enabled by Oskar. Then the Nanos protein, along with Pumilio and p55, suppresses *hunchback* mRNA.

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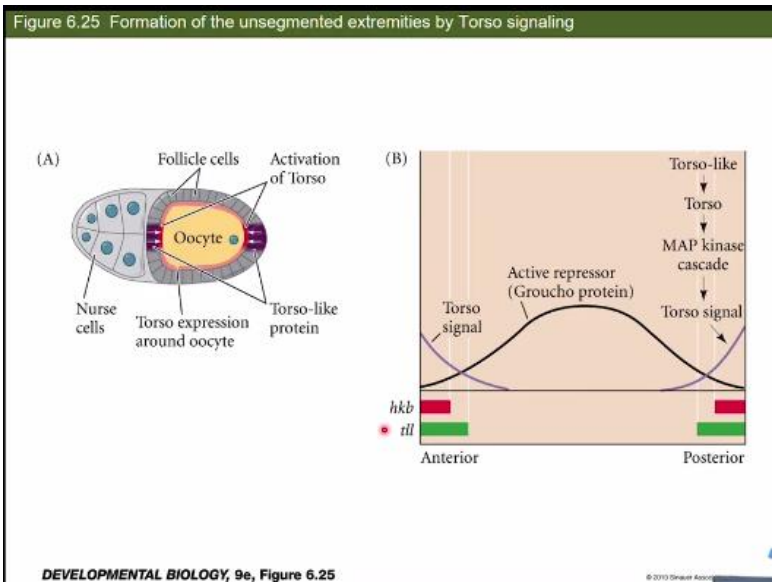

Now let us understand how these extremities such as acron and telson are formed. So this happens by RTK signaling. The anterior and posterior follicle cells produce a protein called torso-like. This protein signals another protein called torso, which further activates the MAP kinase pathway. Even though the torso and RTK receptor are expressed throughout the oocyte membrane, this MAPK signaling occurs only in the extremes due to these follicles cells that produce the ligand torso-like protein.

So this signaling translationally suppresses a protein called Groucho. Therefore Groucho forms a reciprocal gradient to the torso signaling. Groucho prevents the production of Huckebein and Tailless proteins, which are required for these extremities to form. In the absence of Groucho, Huckebein and Tailless are produced in the ends. These proteins are required for these extremities to form, but the acron-telson asymmetry is dictated by the Bicoid present in the anterior. If Bicoid is present in the anterior, head structures are formed, and its absence leads to tail structures' formation.

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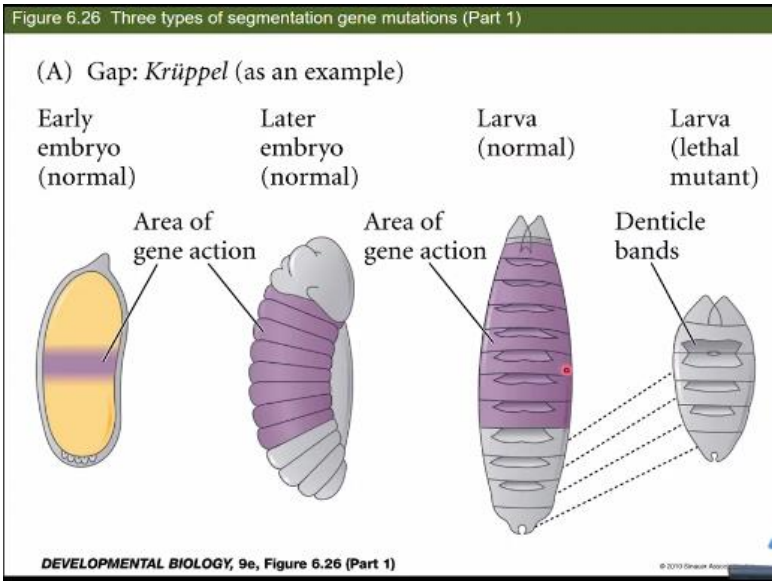

So far, we saw the maternal effect proteins forming the anterior-posterior embryonic patterning. Here patterning means the distribution pattern of the molecules. So now, let us look at the consequences of these gradient setups. So the cartoon in the slide shows the expression pattern of one of the gap genes called the Kruppel in each stage. It has a broader expression pattern in the late embryo. So the *kruppel* mutant larva does not form certain body parts; the missing parts correspond to the proteins' expression patterns.

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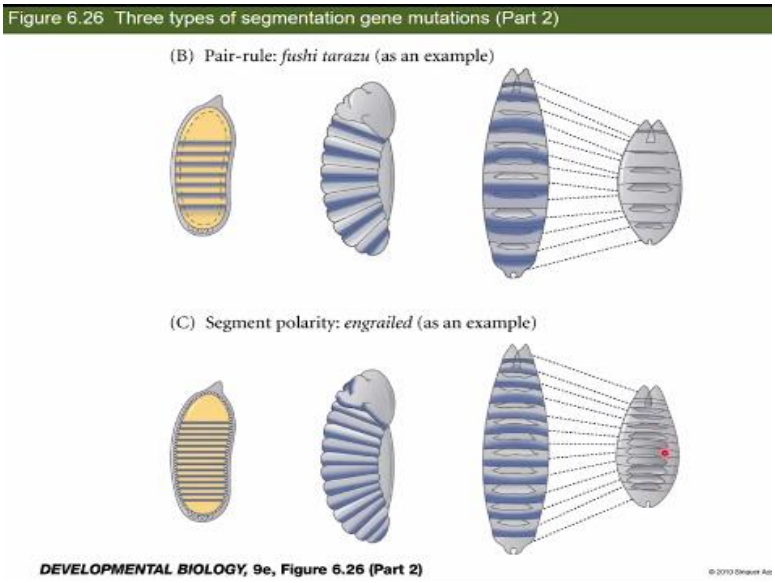

So this slide show a pair-rule gene called Fushi tarazu. Its expression is seen in the very early syncytial embryo. So you see the expression pattern in the late embryo as well. So it is expressed in the posterior region of one segment and anterior region of the adjacent segment. This region is called parasegment, which will be discussed in the next class. So in the

mutant, the anterior part of one segment and posterior part of the adjacent segment will be fused. So wherever this protein is expressed, that parts will be missing in the larva.

Segment polarity genes, on the other hand, form many stripes. So here, the Engrailed forms fourteen stripes in the posterior of each of these segments. Therefore those parts will be missing in the mutant larva.