Introduction to Developmental Biology

Prof. Subramaniam K

Department of Biotechnology

Indian Institute of Technology – Madras

Lecture – 18

Genetics of Axis Formation in Drosophila Part 2 of 4

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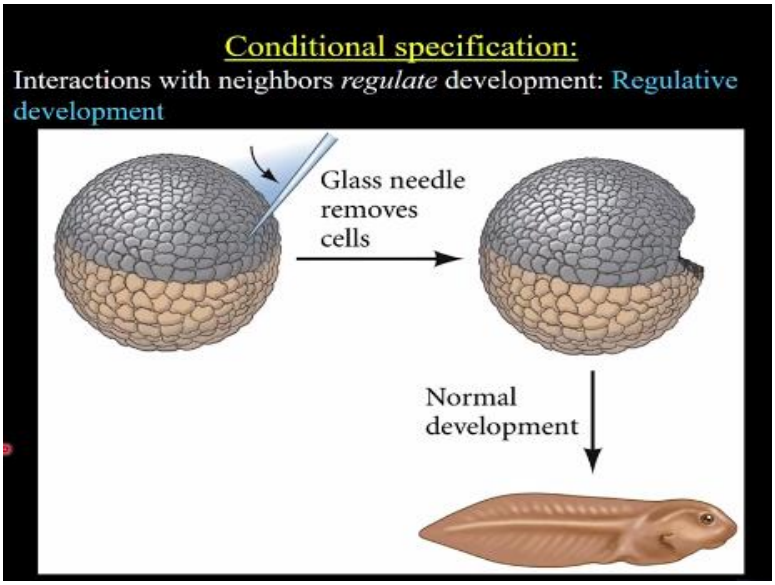

In the last class, while explaining specifications, I mentioned that autonomous specification would lead to mosaic development, and conditional specification would lead to regulative development. We also looked at the syncytial specification. I did not specify one point here, which is all these modes are mutually exclusive. For example, if one organism follows the autonomous specification, is it all through autonomous? or if something follows conditional, is it all through conditional? The answer is NO; it is a mixture of all. For example, in vertebrate development, initially, it is conditional later in specific lineages you will see autonomous specification.

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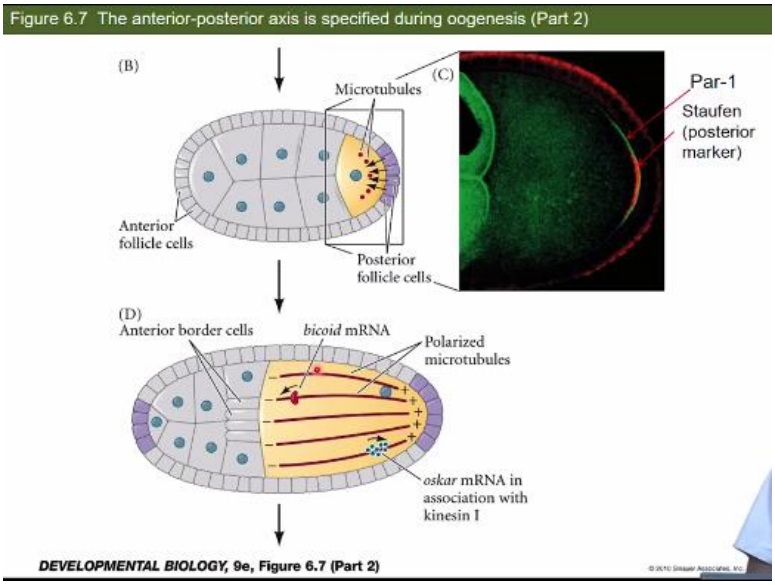

We learned the localization of *gurken* mRNA near the nucleus in the posterior side of the oocyte. So, this Gurken protein binding to this torpedo receptor activates the terminal follicle cell, and then these cells signal back. As a result of that signaling, the polarized microtubule transports Bicoid to the anterior and Oskar to the posterior. One of the key components of this signaling from follicle cells to the oocyte is the localization of Par-1 protein. Once the anterior-posterior polarity is established, with these microtubules, the oocyte nucleus migrates anteriorly on the dorsal side.

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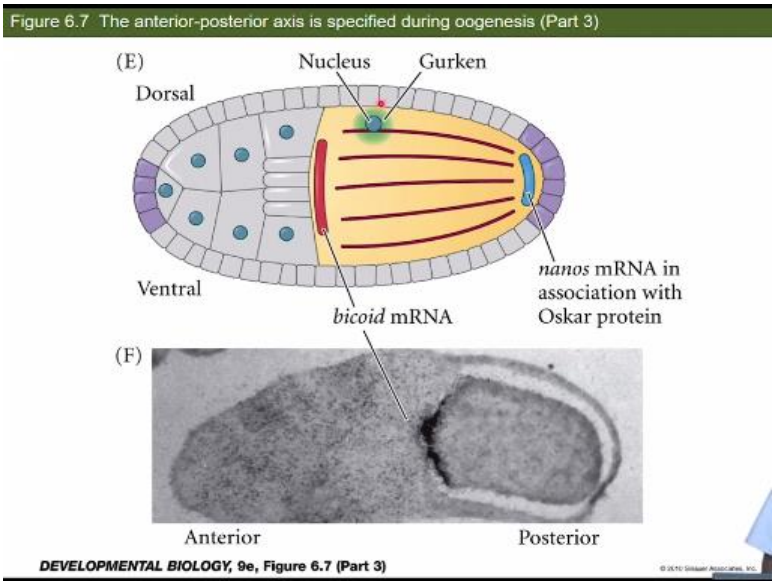

So, along with the nucleus, Gurken also gets transported and gets localized to the anterio-dorsal side of the oocyte, as you see in the cartoon (E). Figure (F) shows the in-situ hybridization of *bicoid* mRNA. So, the *nanos* mRNA gets localized through multiple mechanisms, like the one that gets localized is protected from degradation, and the one that is

not localized gets degraded. Also, only the localized one gets translated. So, these things we will learn later. So now, let us focus on the Gurken some more.

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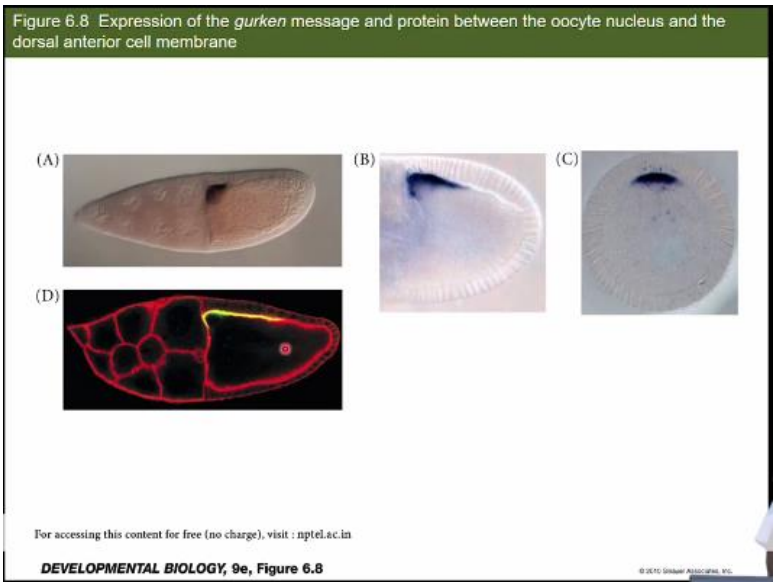

So, in this slide, figure (A) and (D) shows the whole egg chamber. The bigger cell is the oocyte, and the rest are the nurse cells. The purple color in figure (B) indicates the localization of the *gurken* mRNA in the dorsal side. Figure (C) is the cross-section of the same. Figure (D) is immunostaining of the egg chamber that shows the Gurken protein in yellow color, and actin in the red color. So, actin is present in the inner periphery of the cell membrane, and it makes the boundary.

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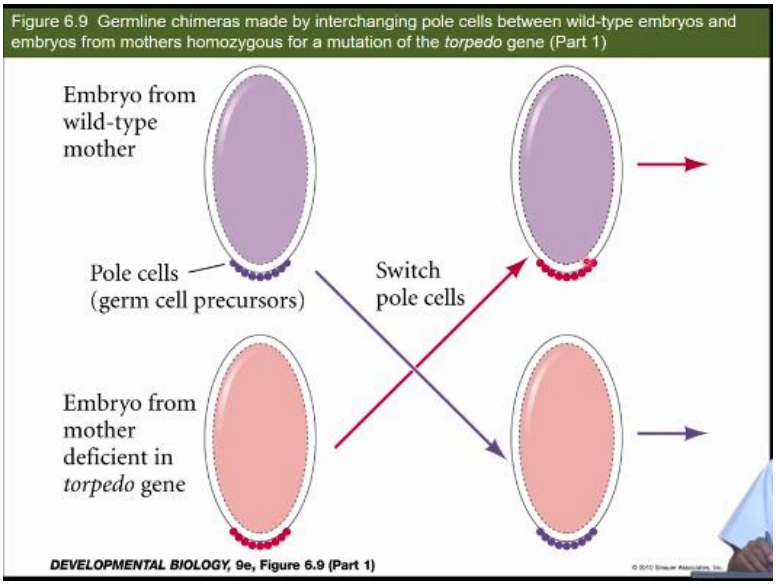

So here is an experiment that helped understand where the torpedo is produced. So, which one should contain the genetic information for Torpedo protein-making, the follicle nuclei, or the oocyte nucleus?

Here you have the pole cells; the pole cells are the germ cell precursors like primordial germ cells in *C. elegans*. During cellularization, pole cells are the first set of cells made by the syncytial embryo. And these are the ones that eventually make oocytes.

So, let us take the pole cells from a wild-type mother and then transplant it to an embryo deficient in the *torpedo* gene. So here, the embryo will not produce Torpedo, but the germ cells will develop normally. Similarly, transplant the torpedo mutant germ cells into the wild-type embryo, allowing this embryo to develop into an adult fly.

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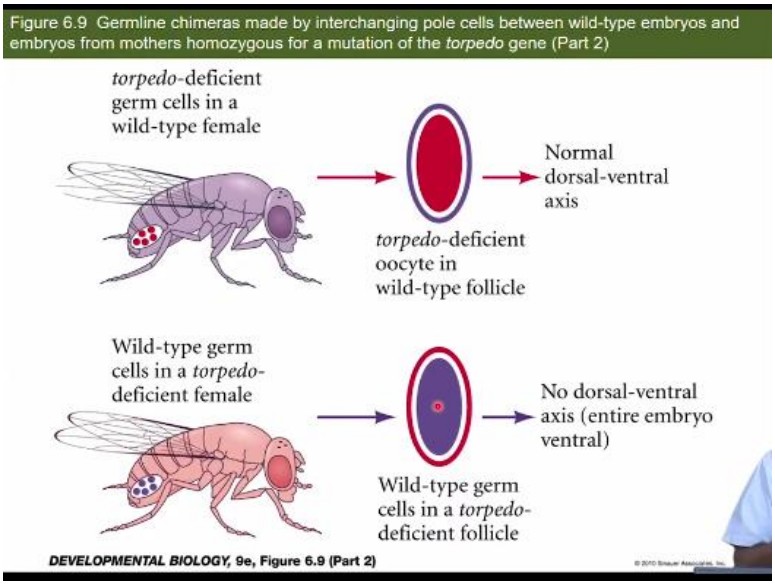

So here, the torpedo-deficient germ cells in a wild-type female will develop and produce oocytes that are torpedo-deficient and follicle cells that are wild-type for the torpedo. These oocytes make normal dorsal-ventral axis even though the oocytes do not have torpedo. The torpedo-deficient female with wild-type germ cells will make follicle cells that will not make Torpedo protein and pole cells, or the oocyte that can make Torpedo. As a result, no dorsal-ventral axis formation happens. These results show that Torpedo from the follicles is required for this dorsal-central axis specification. These experiments are done with germline chimera, meaning; the somatic part belongs to one genotype, and the germline belongs to another genotype. So, these kinds of transplantation experiments proved this model.

So, taking out the pole cells does not affect this embryo because these have come out of the embryos. These are isolatable cells that do not affect the embryo developing into a normal fly, just that the fly will be sterile because it will have no germ cells. So, you take those cells and transplant to an embryo that is developing where the mother is a torpedo mutant. Here, the embryo will develop into an adult; the somatic part of the adult will not have the torpedo. So, if you open its ovariole and go to the germarium and egg chamber where follicle cells are developing, there will be no torpedo because all of them are somatic cells that come from a mother mutant for the torpedo. But the germ cells are of wild-type origin and placed when it was an embryo, and they are the ones that are migrating to the somatic gonad and establish the germline there.

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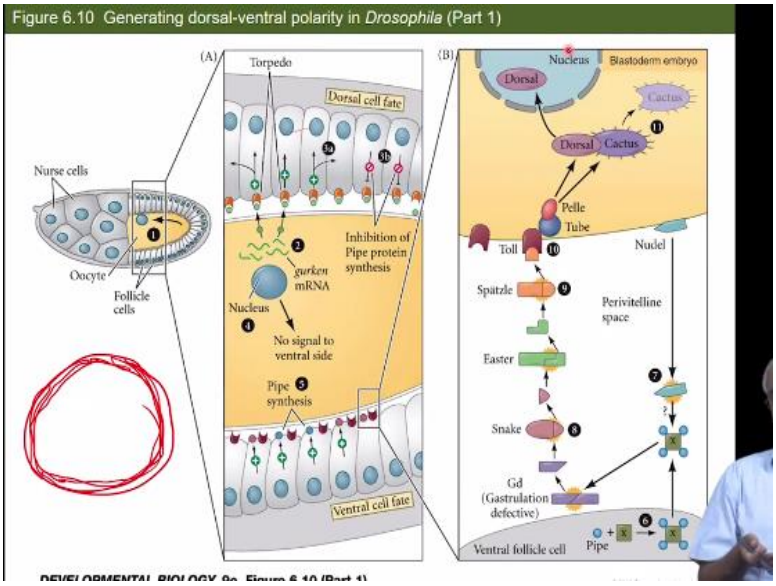

So, this dorsal-ventral polarity generation will be more complicated, but I will take you through very slowly. So, remember, our goal of this series of lectures is to understand how do we make front-back and the top-bottom asymmetry in an embryo, which starts as a symmetrical zygote. So, the example we took is Drosophila, where this asymmetry happens in the oocyte itself. So far, we saw the anterior-posterior asymmetry in the oocyte. Now we will look at top-bottom that is dorsal-ventral; top-bottom does not mean head and tail in us, so your back is top or dorsal, and your front is bottom or ventral.

So now we will look at dorsal-ventral axis formation. This specification starts in the oocyte but ends up with an embryo. So, it is continuously developing like when it begins here in (A), it is in the oocyte, but when reaches (B), it is going to be a syncytial embryo. During this

process, the oocyte gets fertilized and divides to form a syncytium. Among many nuclei made, this nucleus shown in (B) is one of them which is on the ventral side.

So, please pay attention to the label here in (B); it is written correctly. I have edited here; you can see that color difference. I could not match this color accurately; the book says oocyte there. So do not get distracted by that; this is a blastoderm embryo.

As I mentioned earlier, the oocyte nucleus moves to the anterior on the dorsal side along with Gurken. The *gurken* mRNA localized here in the dorso-anterior region will bind to the Torpedo receptor on the follicle cells in that region. In the earlier stage, the torpedo is present all over, but only the terminal follicle cells receive the Gurken signal. Now the Torpedo on these dorsal follicle cells receive the signal, and that signal stops the production of a protein called Pipe. So, the Pipe is not produced by the dorsal follicles due to Gurken signaling throughout Torpedo receptors on the dorsal cells.

In contrast, that does not happen in the posterior because you do not have Gurken here. So, posterior follicle cells do not have the Torpedo signaling; therefore, they do not inhibit Pipe synthesis. So, they will make Pipe. Now let us look at the enlarged region of (A). This is shown in (B), where you see the oocyte cytoplasm and the perivitelline space between the two vitelline membranes and then the follicle cells. So, in this enlarged one here, the only thing I have not corrected is this toll receptor, which is drawn on the follicle cells in (A), and here in (B), it is drawn on the oocyte, and that is the correct one.

So here in the ventral side, the Pipe is going to associate with the protein that is still not identified and comes out of that place. The way our blood clotting system gets activated in a cascade of proteolytic cleavage similar thing is going to happen here. So, this Pipe associates with the Nudel protease, and that gets activated and cleaves this Gastrulation defective protein. The activated Gastrulation-defective protease cleaves Snake; the activated Snake protease cleaves Easter; the activated Easter protease cleaves Spatzle. Finally, the Spatzle, which is not a protease, will bind to Toll, and the Toll will transduce a signal.

So, all of this happens in the small perivitelline space; by the time all of this happens, the oocyte is fertilized, and it has gone to the syncytial blastoderm stage. Syncytial blastoderm means the fertilized zygotic nucleus will divide synchronously thirteen times and generate a

lot of nuclei. Initially, the nuclei are going to be everywhere, and later they are localized to the cortex. That stage is what we are looking at here, the syncytial blastoderm, where the nuclei have migrated to the cortex. So, the cortex is the area just below the cytoplasm. In biology, the area outside of the cytoplasm but inside some boundary is the cortex.

So, in the oocyte, let us say the dorso-ventral axis is primarily due to asymmetric activation of the Torpedo, so the dorsal follicle cells do not make Pipe while ventral follicles make the Pipe. So, this is how at the oocyte level, the dorso-ventral axis happens. Now, this molecular asymmetry where the Pipe is produced in the ventral follicle cells becomes consequential when you reach the blastoderm embryonic stage, because this is happening in about ninety minutes. So, by the time this asymmetry has a consequence on development, this nucleus has divided multiple times; thirteen cycles generated a lot of nuclei, and they arranged on the cortex, and now the ventrally located nuclei are getting influenced by the asymmetry caused during oocyte.

So, the consequence of the Toll signaling leads to the activation of a kinase called Pelle, which probably requires the Tube. We do not exactly know how that influences. So Pelle will phosphorylate a protein called Cactus, and that Cactus is going to be degraded.

So dorsal is a protein produced from the maternally encoded mRNA a little later during embryonic development and not in the oocyte stage, but in the zygote, it gets translated into protein, and that protein is present throughout the cytoplasm. So, the syncytial cytoplasm has the dorsal all over, but the Cactus protein binds to it and prevents it from migrating into the nucleus. But in the ventral side, because of this signaling triggered by the Pipe in the ventral follicle cells, the Cactus is going to be degraded, and as a result, dorsal is free. Now, the dorsal concentration is high in the ventral cytoplasm, so it enters into the ventral side in nuclei, and that triggers a chain of transcriptional events. As a result, a new set of genes gets activated or inhibited, and that will specify those nuclei to become ventral nuclei.

The point is, the Dorsal activation in the ventral nuclei is going to activate genes required for the formation of ectoderm structures and also inhibit genes required for the formation of other structures that will be on the dorsal side. So, it is going to do two functions: activation and inhibition. Activation to make the ventral side, inactivation of the genes required to make the dorsal side

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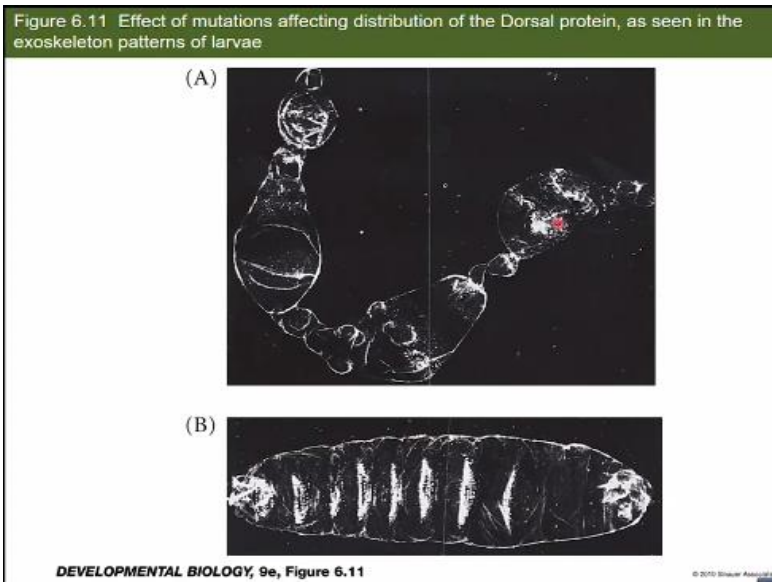

So, this is experimental evidence, here the dorsal mutant does not make the exoskeleton; instead, it is all messed up.

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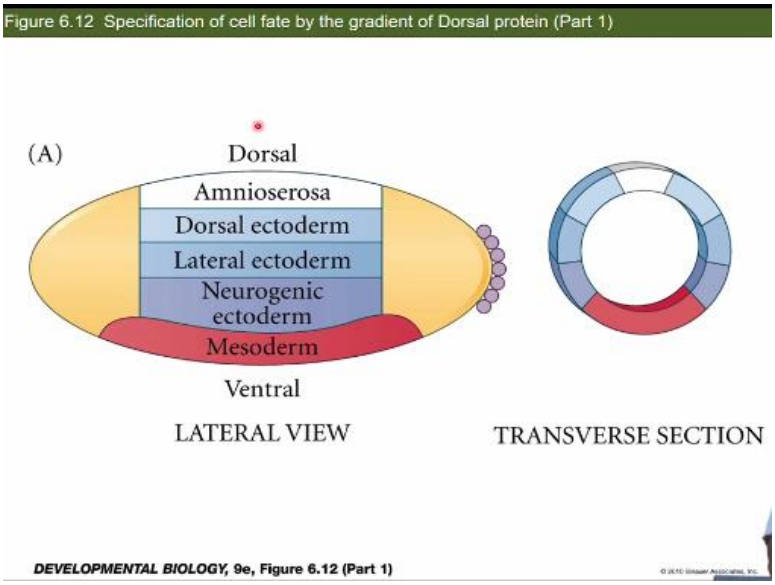

Figure (A) is a summary of what we saw earlier. So, the ventral nuclei that received the maximum of dorsal will become the mesoderm. Then slightly lower is going to become ectoderm, and the lowest is going to form the amnioserosa. Therefore, the dorsal-ventral specification is made by the dorsal protein gradient. So why is the name Dorsal for a protein that makes ventral? That is because the mutant for this protein is dorsalized; therefore, it is called Dorsal. So, the names are based on the phenotypes. So Dorsal protein is required to make the ventral, so in its absence, the embryo gets dorsalized.

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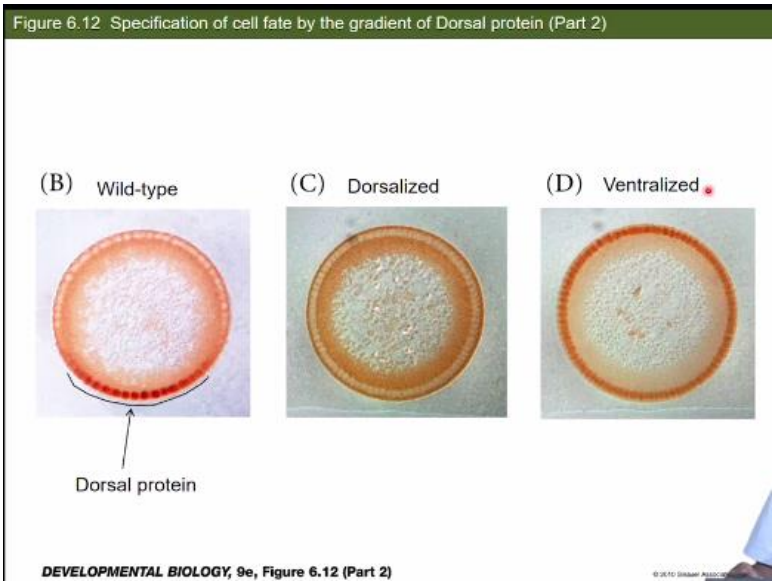

In this slide, figure (B) is a wild-type blastoderm embryo, and the white round structures that are present all over is the cortical arrangement of the nuclei. So here, the dorsal protein is present in the ventral side. In a mutant (figure C), the dorsal does not get into the nucleus, and as a result, the ventral specification does not occur, and the whole embryo is dorsalized. In another mutant where you have dorsal protein everywhere, then that embryo is ventralized.

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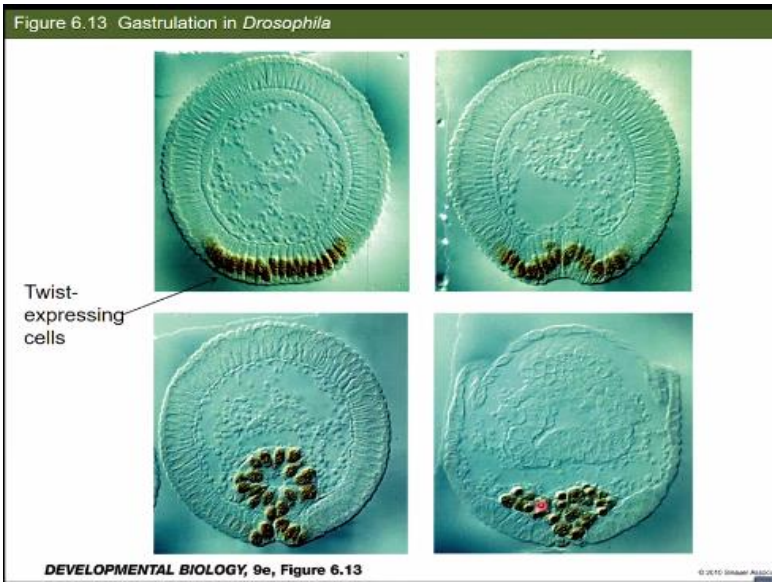

So now, what is the consequence of getting ventralized? So, remember the ventral furrow formation that we learned when we were learning about morphogenesis, so that is coming back here. So, this is just a box item we will get to our main story right away, but one of the events is, a particular transcription factor called twist is expressed only in those cells that will rearrange to migrate inside and form a tube-like structure.

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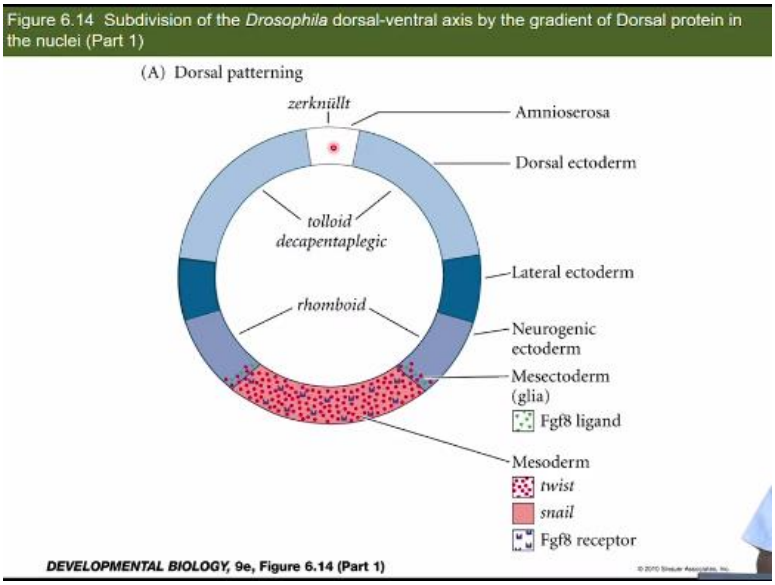

That is explained in this, and the hierarchy is in the next slide. So here, the highest amount of dorsal is in the ventral most area that is going to activate mesoderm-specific genes like Twist, Snail, FGF-8 receptor, not the FGF-8 ligand but the receptor. The ligand is going to be produced in the adjacent cells. So, the highest concentration of dorsal is required to activate Snail transcription; I am specifically focusing on Snail because that is necessary for us to understand the next layer. Slightly dorsal most ventral cells are the ones that are going to make the ectoderm. So, you can imagine the affinity of the enhancers in the Snail for dorsal is probably low. As a result, you need a very high concentration of Dorsal, so when you move slightly away from where you still have a significant amount of Dorsal that is not good enough to activate Snail, but that activates Rhomboid. So Rhomboid is not produced in the ventral most cells because Snail inhibits Rhomboid production.

As a result, Rhomboid is produced in cells adjacent to the ventral most cells. And the Snail is restricted to the ventral most cells and that leads to the production of FGF-8 that will be the ligand to transduce the signal from the FGF-8 receptor. So, these interactions are going to specify the adjacent cells as glial cells. Then slightly away, you have neurogenic and then lateral ectoderm. Then you have these decapentaplegic, tolloid, and zerknullt required for dorsal specifications like dorsal ectoderm and the amnioserosa. These three are inhibited in the central cells by Dorsal; if they are not inhibited, they will dorsalize the whole embryo. Twist, snail, and FGF-8 receptors will activate the ventral region, and they are inhibited on the other side. So, this is an embryonic structure.

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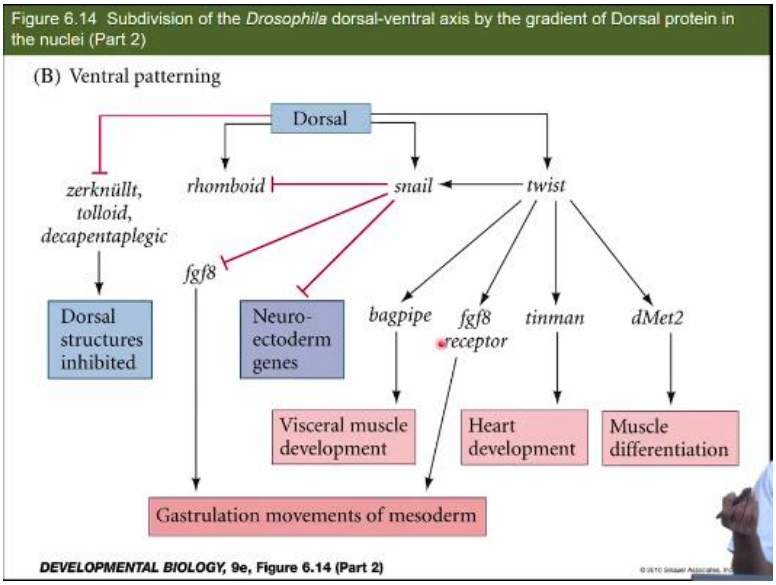

The hierarchy is given here; on the ventral side, the Dorsal will activate rhomboid, snail, twist, and inhibit zerknullt, tolloid, decapentaplegic, which we just saw in that cartoon. Here you see the Snail is inhibiting the Rhomboid, and Dorsal activates it. So dorsal does not activate Snail in the cells adjacent to the ventral most cells because you need a high concentration of Dorsal to activate snail, so this is how the dorsal-ventral patterning happens.

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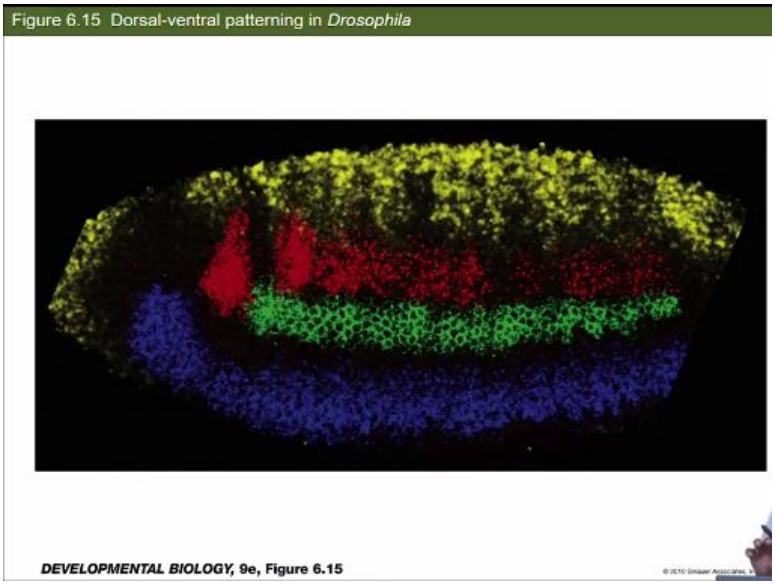

The figure in the slide shows the expression pattern of different proteins; these proteins are not expressed everywhere. The blue portion is the ectoderm. The green portion is the muscle cells, and then the yellow is the dorsal cells. So, you see this pattern neatly formed. So, this is how you start with the symmetrical oocyte and end up with a neatly arranged embryonic patterning. So here your patterning the molecular asymmetry, like which molecule is going to

be where, so this is how the differential gene expression is playing out during embryonic development

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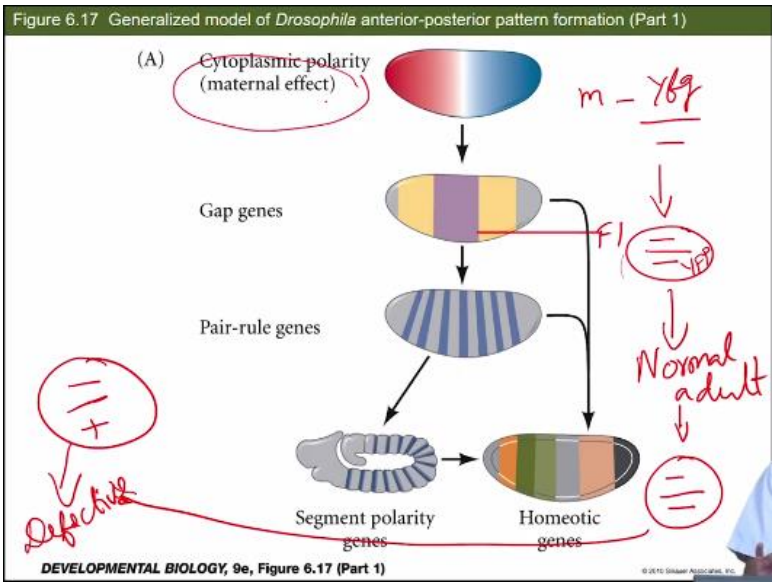

Here we are going to look at a set of genes in a hierarchy. It starts with maternally produced molecules. So, let us learn a keyword called the maternal effect, which means mutation of a particular gene will not show the phenotype in that embryo itself. If the mother were mutant, generally, you would see the phenotype in embryonic development. But here you will see the mutant effect only in the grandchild embryo.

Let us say you are a zygote; both the maternal and paternal alleles are mutant for a particular gene. So, you are having a loss of function phenotype of that particular gene. For example, *yfg* is the null allele for YFP you have, but your mother is heterozygous, and she produces enough of YFP, let us say. She will be depositing a lot of that YFP or the *yfg* mRNA in your zygotic cytoplasm. If YFP functions only during embryonic development, then that is good enough, then you are going to take shape to become a normal adult. The only thing is in the next generation because your genome is not going to make the YFP, you will not make a functional embryo. Such mutants are called maternal effect mutants.

So, the mother is *yfg/-*, now in F1 1/4th of the progeny will be -/-. Let us assume this *yfg/-* mother will make an oocyte that will be negative for this YFP, and let us say this oocyte is fertilized by the sperm that is also negative for YFP; sperm comes from a heterozygous father (*yfp/-*) now you will end up getting -/- embryo. But all of this is not happening in isolation; it is all happening inside the mother. The mother is producing plenty of YFP or *yfg* mRNA and

depositing in the oocyte cytoplasm. Now let us say this protein is not required for the final neuronal connection in the adult brain, but it is necessary for anterior-posterior polarity in the embryonic stage; after that, it does not play any role later in development. The YFP produced by mother's mRNA or YFP deposited by the mother will be enough to take care of that embryonic development.

So, you will have YFP protein, and this is going to be a normal development. Now, if you look at its germ cells, they are -/- for YFP, now even if it mates with a heterozygous father, it will end up producing -/- embryo only and this is not going to develop normally because this YFP protein needed to be made by the mother and deposited is absent. Assume that the zygotic gene expression does not get turned on in time. So, this will end up as a defective embryo or maybe embryonic lethal and such mutations we call a maternal effect.

If a mutant phenotype is visible as a maternal effect, meaning, only after two generations, you see a phenotype and not in the immediate generation. For example, if the homozygous mutant embryo develops normally from a heterozygous mother; then, you call it the maternal effect. Instead, if the homozygous mutant embryo dies, it is embryonic lethal, and you do not add the word maternal. Therefore, you have two kinds of genes; one is the maternal-effect embryonic lethal another one is the maternal effect sterile, meaning the somatic things will all develop normally but the gametes will not form right, and they are maternal effect as well. Some of the maternally produced proteins remember the pole cells that form first; they inherit these proteins, and they are required during early development. So, these are the meanings of those two terms, maternal-effect sterile and maternal-effect lethal. So, I guess we stop with this definition of what is a maternal effect. So, we will continue this in the next class.