Introduction to Developmental Biology

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Lecture – 17

Genetics of Axis Formation in Drosophila Part 1 of 4

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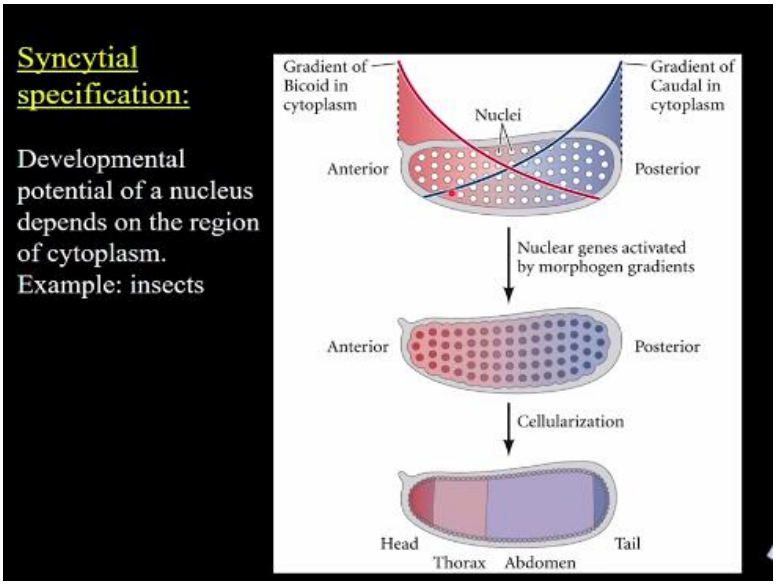

Let us get started. See, in the last class, remember we were discussing different kinds of specifications. Autonomous specification leads to what kind of development? Regulative, and then the last one we discussed was the syncytial specification. So I told you syncytium means having a lot of nuclei in a common cytoplasm. One cell having multiple nuclei, so that is the situation in the Drosophila embryo.

Initially, you have nuclear divisions without cytokinesis generating the kind of structures that you see here in the slide, and there we talked about the specification is based on where the given nucleus is located and why that is important because you have a concentration gradient set up there. Some components are more concentrated in the anterior, and then it slowly decreases as you move towards the posterior and vice versa for some other components. This concentration gradient is going to be the topic today.

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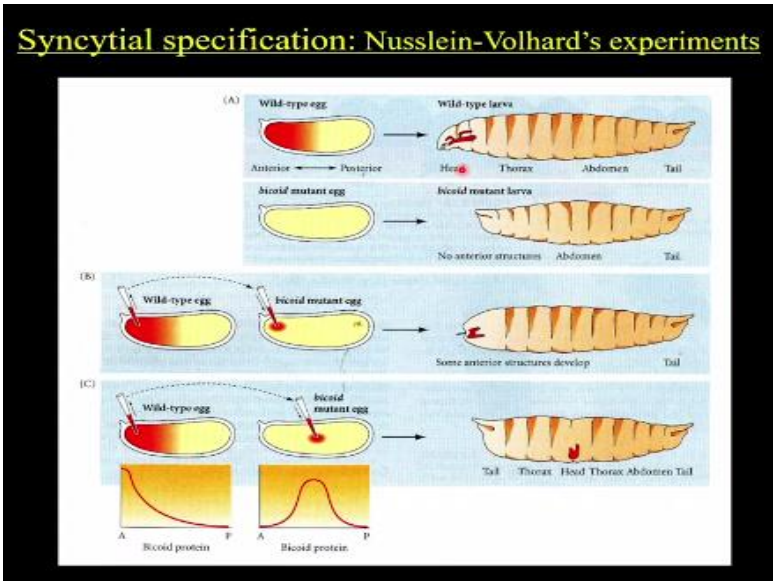

Some molecules function primarily based on their concentration gradient.

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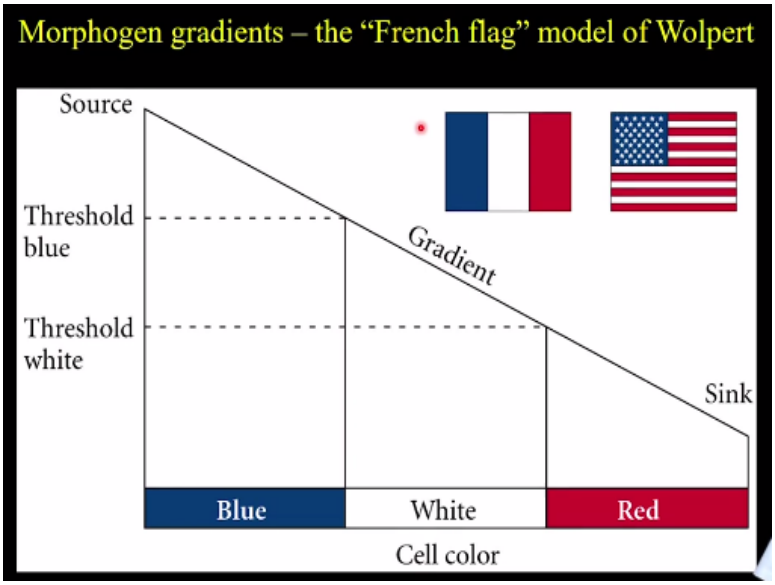

That is what this French flag model by Wolpert tries to explain. So Wolpert has another well-known textbook on developmental biology. So this Scott Gilbert, I feel, is more rather better organized for our purposes, and therefore we follow that, but there is another equally good book that is by Wolpert. So this is the same case as we learned in a different context, where underlying mesenchyme instructs the epidermis above it during skin development. Depending upon the mesenchyme that is being transplanted with an epidermis, you might either form claws or feathers and so on. Then we also saw the Newt and the frog transplantation where the mouthparts being swapped. So if you remember that, you will find this easy to understand. So the keyword, before even we start the class, is this morphogen.

So morphogens are molecules whose activity is directly dependent on their concentration gradient. At different concentrations, they will have a different effect, so that is the hallmark of a morphogen. So morphogen will not have the same effect at different concentrations. So in today's class, let us see the importance of the gradient and the concentration of a morphogen. This French flag model by Wolpert tries to explain the same.

Here in this French flag, you assume that the concentration of a given morphogen is highest near the left end, and then it slowly goes down as you move away in the X-axis from the origin. At different concentrations, it will induce a different fate for those cells.

So please assume that the whole flag is the morphogenetic field and let us say a group of cells that have the highest concentration is going to make the blue pattern. Then the cells in the middle of the field where the concentration is intermediate will make the white pattern of the flag. Then when you have a very low concentration of morphogen like below the white threshold, it is going to make the red pattern. So now, we are going to do a transplantation experiment of the American flag. So let us take this part with the horizontal stripes from the American flag and transplant it to the blue part in the French flag and vice versa. We are merely swapping the parts here.

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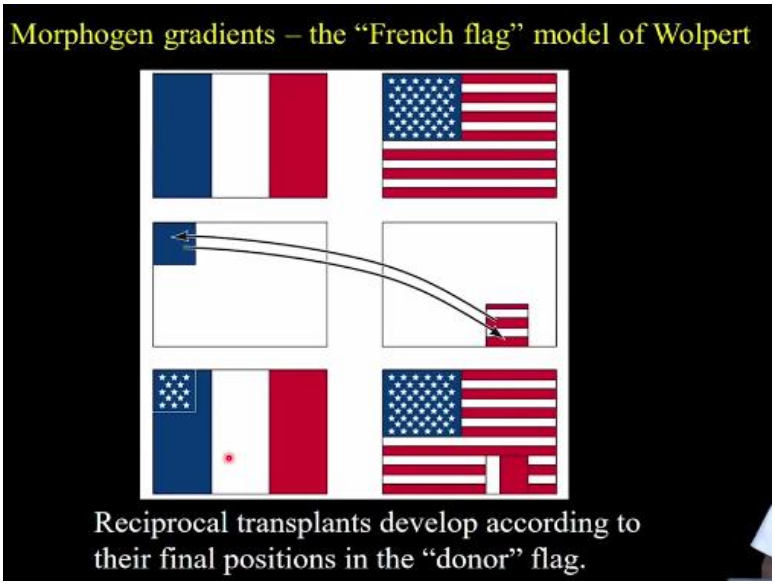

The result is shown here. So, we take cells from the American flag and put it in the corner in the French flag, where it is going to sense the morphogen at its highest concentration. So the cells will not make the blue part of the French flag; instead, whatever instruction it already has based on that, it will make the structure as you see in the cartoon. If you consider this flag

as an embryo, in that part, it would have made the stars and blue color background, and that is what it is going to do here. Similarly, when you take the cells from the French flag and put it here in the American flag, it is not going to make the horizontal portion; instead, it senses that the gradient is lower, and it makes the vertical strips of the French flag. So this is the French flag model that explains how morphogen gradients work.

So again, you can compare this with the fate specification during vulva development. So the P6.p cell that gets the highest amount of RTK signaling acquires the primary fate. If the same P6.p cell gets slightly less signal when placed adjacent to the P4.p position, it will acquire the secondary fate, and further away, it will acquire tertiary fate. If the tertiary fate cells were brought to the highest concentration of signaling from the anchor cell, then it is going to acquire the primary fate.

So that is what you see here, so what it means is all these cells have a certain developmental potential, on receiving the right instruction, they could make this blue color tissue or the white color tissue or the red color tissue. So that instruction is determined by the gradient of this morphogen. So the highest morphogen means the same cells that make blue when transplanted to another place with the lowest concentration gradient will make the red. This red and blue pattern making instruction is there in the French flag embryo, but that is not there in the American flag embryo, which also responds to the same morphogen gradient. Still, its genetic programming that has happened in earlier steps instructs what it is supposed to make. Reciprocal transplant develops according to their final positions in the donor flag, so this is the idea of morphogen and morphogen gradient. It is essential because it plays a lot of roles in developmental biology.

So the critical point to remember is, morphogens are molecules whose activity depends on their concentration; at different concentrations, they will have different developmental outcomes. The fate they specify will be different at different concentrations. Morphogen means concentration-specific developmental specification.

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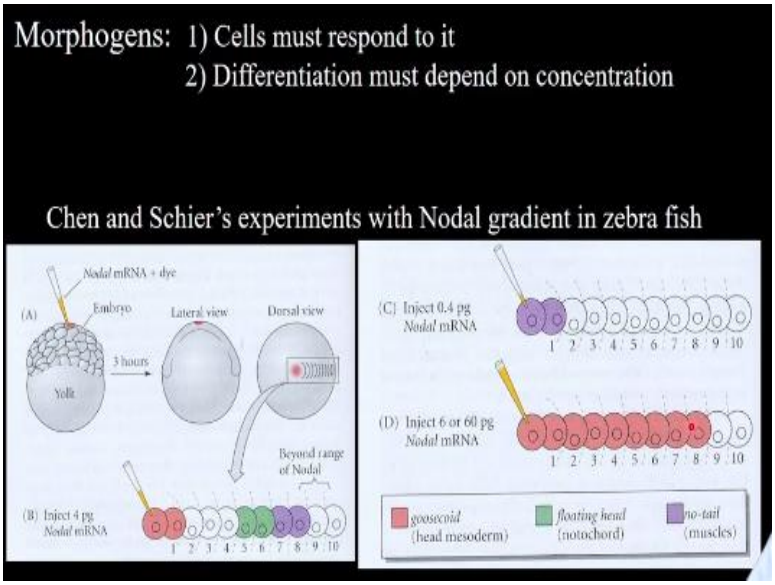

So here is an experiment to show how the morphogen gradient works. So this is done on the Zebrafish embryo. So in the cartoon (A), in the surface ectodermal cells, you inject the nodal mRNA. So this is one of the morphogens here, so nodal protein's gradient is essential. In the lateral view, it is seen on the top, and in the dorsal view, you see it on the surface. So from the center to one end, there is a concentration gradient of nodal.

So when you inject about 4 picograms (pg) of nodal mRNA into this cell and the nodal protein produced diffuses across. When it reaches a certain gradient, it is going to induce a certain downstream target like the floating head, which is going to be induced and become the notochord. The adjacent cells to the right get much less nodal protein; thus, they start expressing no-tail, which will become the muscles. So no-tail is the name of a protein. So, the one that receives the highest concentration is going to express goosecoid, and that will make the head mesoderm. If these cells were swapped, then according to the concentration of the nodal protein, their fates will be swapped as well. So that is experimentally demonstrated in the subsequent sections here. If you have far less nodal mRNA injected in these cells, they directly activate no-tail.

These are not difficult to understand; if you want to think chemically; think about the affinity between the protein and the DNA sequence. Suppose an enhancer-binding happens only at a higher concentration, and if the affinity is low, then those enhancers will not be active at low concentration. The relative affinities can explain this; you do not need to invoke more complex chemical principles here. If you inject a whole lot, whether absolute value or relative value, here you are giving a really large amount (D). Like six or sixty does not matter, this

ten times difference does not matter, and all of them will activate goosecoid. At a high concentration, they are all going to respond to it.

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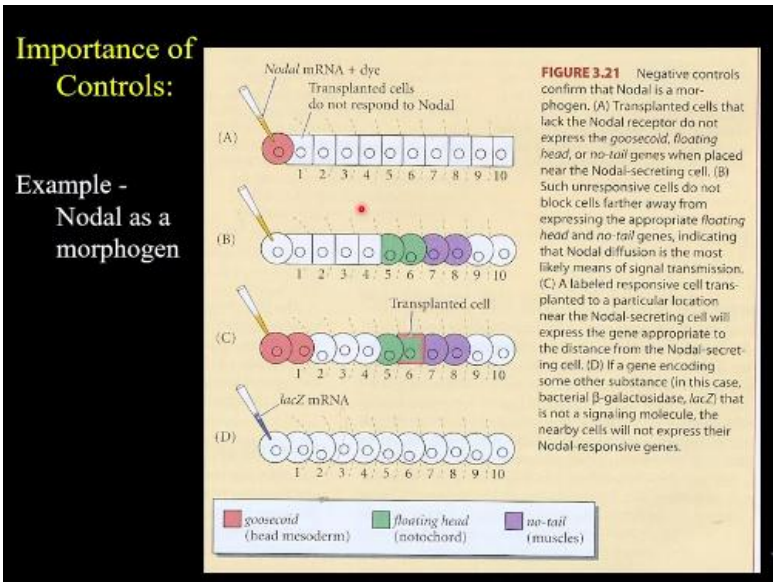

So now we are going to do more testing, like controls to ensure, whatever we are interpreting on those experimental results are correct.

So, one thing to remember is induction and competence. So, not any cell is going to respond to the nodal protein. If you have a different set of cells that are not going to express the nodal receptor, they are unlikely to respond to this concentration gradient (A). They lack competence. So only the diffusion of the morphogen is required and not adjacent cells; like, for example, in (A), the cell that underwent some developmental alteration and, as a result, existed as different kinds of cells will not respond to the signal. So they are not interfering with the nodal diffusion. These competent cells in (B), when the concentration comes to the right range, become green and purple cells.

So the diffusion matters; the intervening cells, whatever they are, it does not matter. If we take a different cell with the appropriate receptor (C) and put it at any position in this field of the nodal gradient, then appropriately, it should respond, and that is what you see with this transplanted cell becoming green. To confirm that this is all nodal dependent not because you pricked a cell with a needle, you add lacz mRNA, and nothing happens. So the effect is only with the nodal. This is how you do control experiments.

In (B), the neighboring cells are not the cause for the activation of the floating head in those cells. It is the nodal that diffuses and induces those cells to express the floating head. This is an important concept in developmental biology—morphogen and morphogen gradient and how they activate a different set of genes and, therefore different fate specifications.

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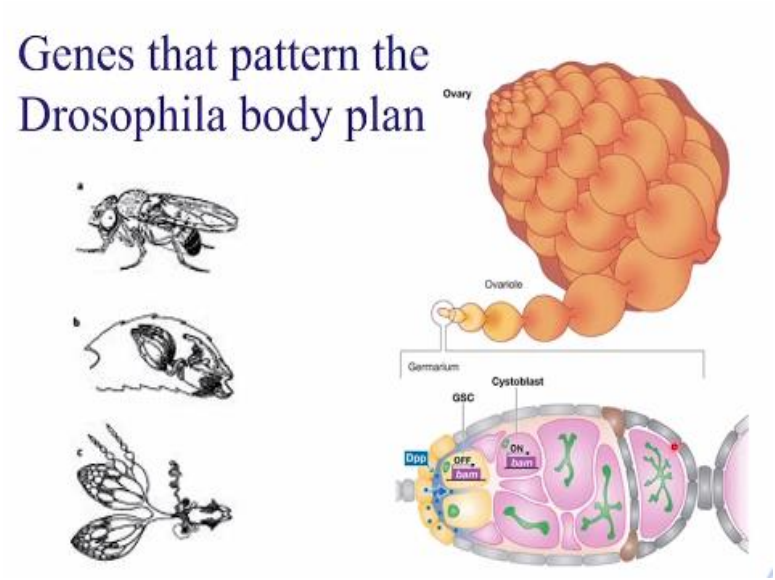

So far, we have covered the very basic concepts and principles, now let us understand the development of a particular system. Drosophila embryonic body patterning is one of the well-understood topics in developmental biology. So over the next two or three classes, we will learn in detail about it.

To begin with, let us understand the organism. So we are going to start with the fly. So all of us have seen insects, not particularly fruit fly but other insects like a beetle, etc. They all have very similar body plans like the head, the thoracic and abdominal regions. They also have six legs and four wings etc. So Drosophila has two wings (figure a), and therefore this is a dipteran insect.

So today, we are going to look at the abdomen region of Drosophila. The abdomen consist of two structures called the ovaries (figure b), and that is our topic interest today. So if you take them out, they look like the one in figure c in the slide. So each ovary will have these kinds of structures called ovariole, as shown in the slide (one of the ovarioles is peeled out from the ovary). Many ovarioles together form the ovary, and one of them is shown here in good detail. If you go to one end of an ovariole, you will see a structure shown in the slide (right side bottom one).

In that, you have these somatic cells and inside that lies two germline stem cells GSCs. These GSCs divide asymmetrically; one cell will be closer to this area called the germline stem cell niche. The other cell is going to be away from it, and that is enough to start the asymmetry. So the one that is staying near the niche receives the niche signaling to remain as a stem cell, and the other that moved away differentiates to become a gamete.

So if you remember, Dpp is a member of the BMP family involved in TGF-β signaling in Drosophila. So the cells that receive Dpp will be GSC, and the one that does not receive Dpp will differentiate to make the oocyte. So that starting cell we call as cystoblast. This cystoblast will undergo four mitotic divisions to form sixteen cells with incomplete cytokinesis; thus, they are linked. So these sixteen interconnected cells together we call a cyst. So the developing cyst moves away and forms an egg chamber. So the place where the GSCs divide and cystoblast formation happens is a germarium.

So this is the following order: ovary, ovariole, germarium, and from germarium, you get these cysts. As I mentioned earlier, cysts are one of the daughters of the germline stem cell; after that initial division, this cell undergoes four successive mitotic divisions with incomplete cytokinesis, and that is shown in the next slide.

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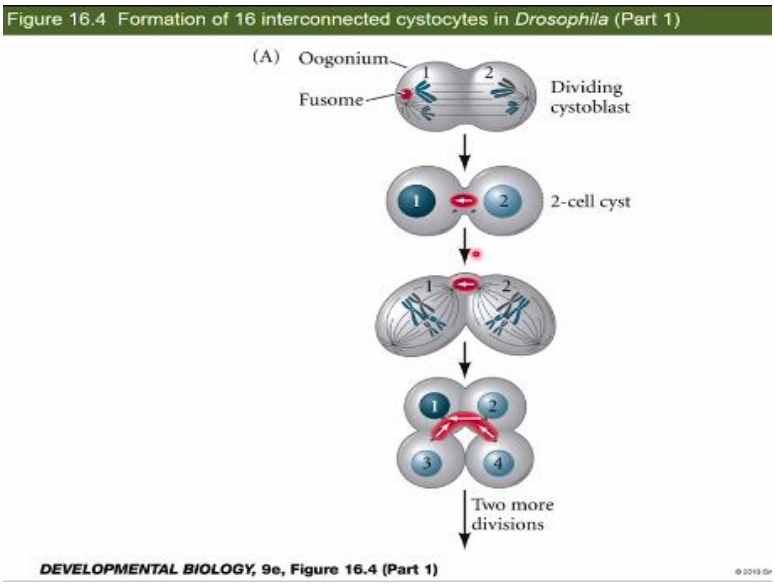

After the first and second division, cells 1 and 2 stay connected. Then these cells divide to form cells 3 and 4. Since cell 3 came from cell 1 and cell 4 came from cell 2; they remain

connected. Since, the cells 3 and 4 came from other cells, they are not connected. So this pattern continues till they form the sixteen cells.

So there is this red color present in these dividing cells in figure (A), I will come back to it; let us first finish the cell division.

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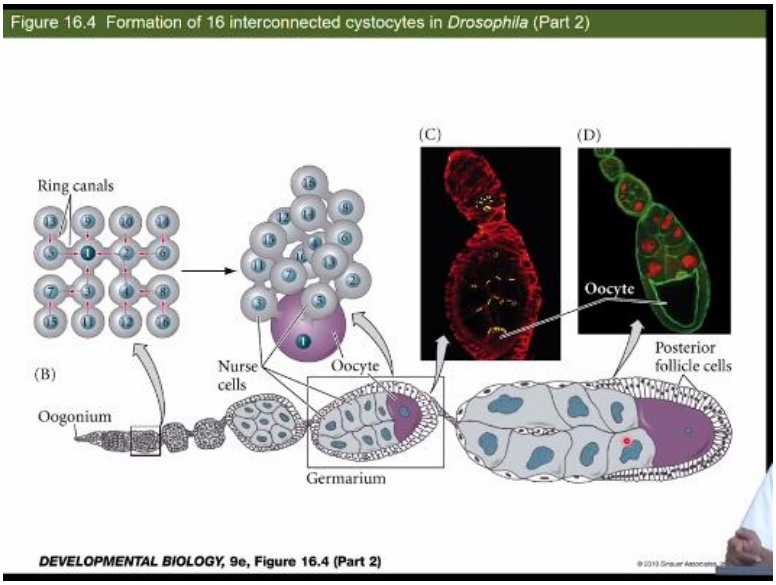

As they keep dividing, they end up forming an interconnected set of sixteen cells where if you see the two cells 1 & 2 have the maximum connection. They are connected to four more cells while the others are connected to either one or two cells. So, the one that has the four connections will get the maximum cytoplasmic output from the other cells. Imagine the transcription and protein production that happens from the nuclei of other cells; all will be moving towards these cells. Finally, either one the two becomes the big cell (figure B), which is the oocyte; the rest of the fifteen cells will become the nurse cells.

So let us go back to the germarium. The cell that will become the cystoblast is also called the oogonium (figure B). So, that oogonium undergoes the cell divisions and becomes bigger and bigger. The cytoplasmic output from all these fifteen other nuclei are all connected through these things called the ring canals. They all deposit the material in the oocyte, and the oocyte eventually grows larger. So I feel this understanding is essential to understand the patterning of the oocyte, embryo, etc.

So now, let us see about the red color, which is shown here as yellow (figure C). So this is a microtubule kind of material called the fusome. This fusome expands through the ring canal; the arrow indicates from which cell it is coming. Figure C is a cross-section stained with actin

to mark the outside red color, and this yellow is the fusome. So this fusome helps in the transport of materials to the oocyte. It has a protein called spectrin, so the fusome structure is made up of these proteins called spectrin.

Now let us focus on what happens to this oocyte. In most organisms, the front-back, top-bottom asymmetry takes place in the zygote. For example, in *C. elegans*, it does not happen in the oocyte. So oocyte is symmetrical when the sperm enters, the site of sperm entry marks the posterior (same happens in humans), leading to a cytoplasmic rearrangement in the embryo. When the embryo divides into AB and P1, P1 gets the posterior cytoplasm, and AB gets the anterior cytoplasm; therefore, their fates are determined accordingly. So, the asymmetric distribution of components within the cytoplasm starts after fertilization in many organisms, but in Drosophila, this asymmetry starts even before fertilization.

In Drosophila, it starts earlier in the oocyte itself. So even before the sperm arrives, the oocyte is already preparing itself for the next generation. So, therefore, we are going to look at how this oocyte forms its top-bottom and front-back.

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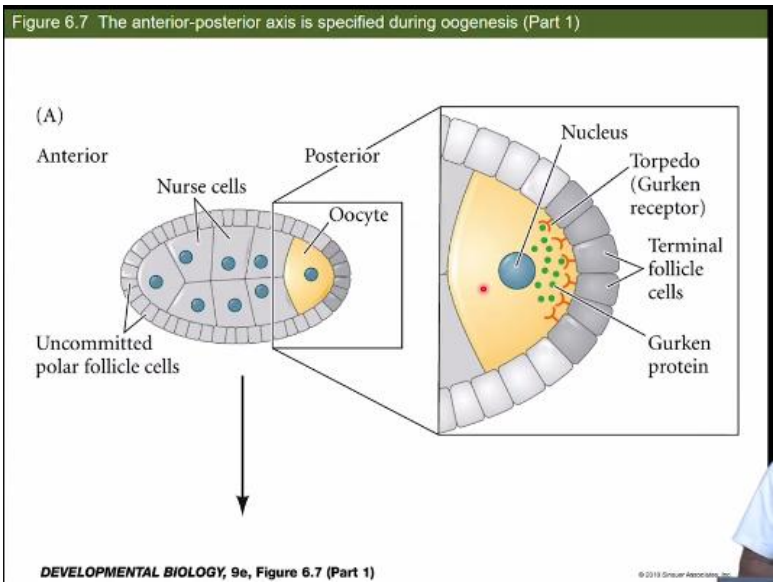

We have an understanding of this structure (A); now, I am going to introduce a set of somatic cells that surrounds the entire egg chamber called the follicle cells. These cells are present throughout the oocyte development, starting from the germarium. So please do not confuse them with nurse cells. Nurse cells are of GSC origin, which helps in nursing the developing oocyte. These follicle cells are not committed to any particular kind of follicle cell.

So now, let us see how the oocyte makes its top-bottom and its front-back. These nurse cells transport many proteins and mRNA into the oocyte and one of them is a protein called Gurken. So the *gurken* mRNA that gets transported into the oocyte ends up localizing between the oocyte nucleus and these follicle cells.

So in this particular stage, the oocyte nucleus ends up being closer to the follicle cells than the nurse cells. The *gurken* mRNA that comes between these follicle cells and this nucleus through, yet unknown mechanism, gets translated only here. The green color dots are the Gurken protein. So that is the starting of everything, this Gurken being made between the oocyte nucleus and these follicle cells.

So during these cell divisions, due to the orientation of the spindle, the nucleus ends up being closer to the posterior. So the Gurken protein made here is received by a receptor on these follicles called the torpedo receptors. That signaling ends up making them as terminal follicle cells, and now these cells are committed. Before this Gurken-Torpedo signaling, all these follicle cells are equal, but after the signal, these are now specified into terminal follicle cells. Now, these follicle cells will send signals back, as we saw in reciprocal induction and competence.

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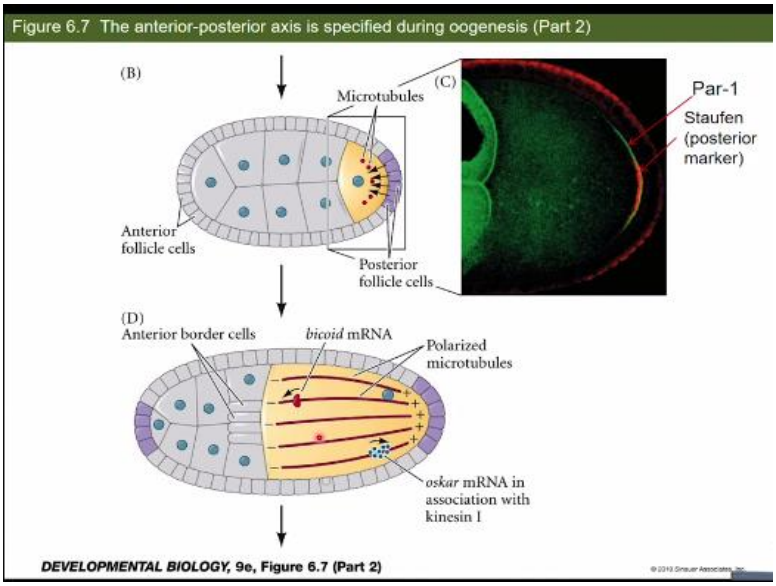

And that signal is unknown, but as a consequence, the microtubules organizes itself in a particular orientation. That unknown signaling from this terminal follicle cells brings this protein Par-1, partitioning defect protein in the *C. elegans*, its ortholog in Drosophila.

Remember, we have learned this Par-1; I did explain this. So we learned this while we were learning about mutagenesis and mapping. In that context, I said one of the ways to confirm the final ORF is by injecting the wild-type copy DNA or by injecting antisense RNA. While mapping one particular gene, they could not rescue the mutant with the wild-type DNA; instead, when they gave antisense, and as the control, the sense, and both affected. And the gene that they were mapping is *par-1*, partitioning defective.

So this Par-1 gets localized due to this terminal follicle cells signaling to the posterior in the oocyte (figure C). That reinforces this microtubule orientation, plus end to the one side and minus-end to the other side. So now, we could start calling the ends as the posterior and anterior. So you are going to have a minus-end at the anterior and a plus-end at the posterior. Now these microtubules that look like rail or road can be used for transporting mRNA and proteins. So the motor proteins kinesin and dynein latch onto it; for example, the *oskar* mRNA move towards the plus end in association with kinesin I, and dynein moves to the anterior carrying the *bicoid* mRNA.

And the Oskar that gets to the posterior gets translated there into Oskar protein, then that again starts setting up the posterior cytoplasm called pole plasm. And this Bicoid that goes to the anterior end determines the anterior specification. During all these events, the size of the oocyte changes because nurse cells are continuously providing the required material, and as a result, the oocyte cytoplasm grows and enlarges (D). In the oocyte, now the Bicoid will have a concentration gradient from anterior to posterior. Similarly, Oskar will have the opposite gradient. So this is how the front and back of the oocyte gets formed.

So it all starts with *gurken* being translated between the nucleus and this Gurken signaling the follicle cells. As a result, they become terminal follicle cells, and only these terminal follicle cells produce the signal that organizes these microtubules. These microtubules are responsible for this Bicoid and Oskar asymmetric localization. One thing that reinforces this microtubule formation and the terminal follicle signaling is the Par-1 localizing to the posterior. That Par-1 localization to the posterior again reinforces this microtubule orientation. That microtubule orientation is critical for Bicoid going to the anterior and Oskar going to the posterior and the rest of the anterior-posterior is a consequence of these two.

So in tomorrow’s class, we will see how the top and bottom are determined. So we have seen the front and back, so we need to know the asymmetry between the top and bottom.