Cellular Biophysics
Doctor Chaitanya A. Athale
Department of Biology
Indian Institute of Science, Educaton and Research, Pune
Lecture 56
Developmental Pattern Formation

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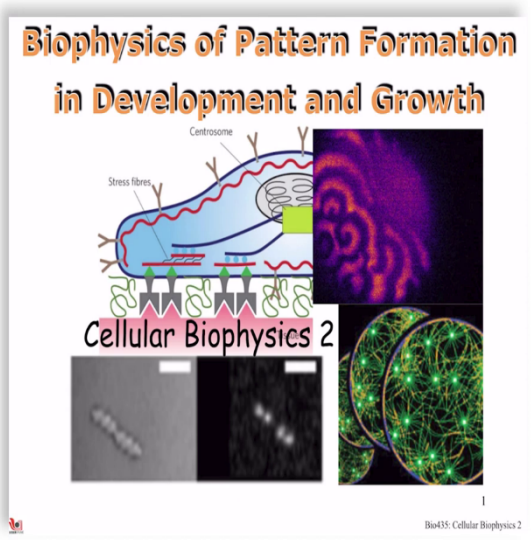

Hi, welcome back. This is the last section of this course, and it is on the most exciting topic in my view, which culminates into, which culminate a lot of aspects of this course, and it is broadly on the biophysics of pattern formation and development and growth. And guest lecture last week was indeed a prelude to this, an introduction to some of the very exciting research problems that are being tackled using biophysical methods, ranging from microscopy, hardware development, image analysis techniques, basically programming and object detection, all the way to mechanical models of cell and tissue development.

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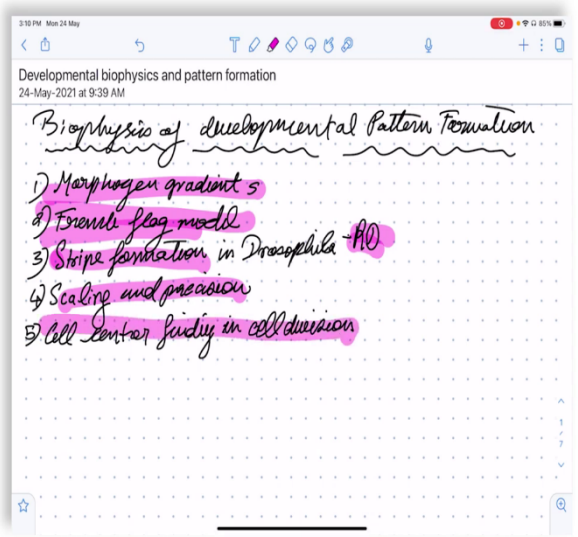

So, for today, I am going to broadly discuss a few aspects which relate to the biophysics of development in terms of morphogen gradients, move on to describe, discuss a very famous model called the French flag model, flinch, French flag model, I am sorry, stripe formation in drosophila, and the role of reaction division models, scaling and precision, and cell centre finding and cell division These are the topics for, that I hope to cover today. So let us get to it.

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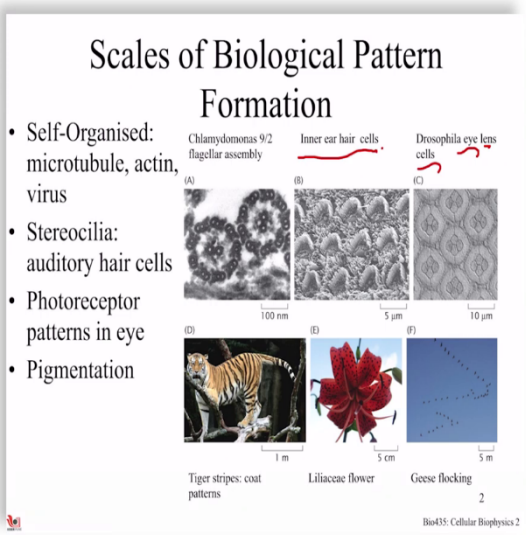

Biological pattern formation has been seen at multiple scales, and what we have talked about so far has been mostly focused on self-organized pattern formation in the sense of microtubule formation, actin formation, in fact what we did not speak much about virus self-assembly, and many other structures that can spontaneously form, lipids is another

example which we talked about last semester. But there are also higher-level structures that can form from these subunits or components, like cytoskeletal elements, such as stereocilia which are found in auditory hair cells, this is sort of in the figure b here.

What you are looking at on the right-hand side are now even more complex structures in the eye of the drosophila formed by lens cells, which form these beautiful geometric patterns. They are almost like something that someone would have drawn or made as a pattern on the wall of a historical structure, for instance. And in fact it goes on further, with the pigmentation patterns that you see in natural animals. I mean, this is in fact the tiger's stripes.

The very symmetric and colourful patterns of flowers, which in fact is what makes them very attractive, perhaps for us from a neurobiology perspective, but also acts often as guidance cues for bees and pollinators. And finally at even larger scale structures multiple organisms flocking together like these geese or schooling fish.

So pattern formation is almost inherent in biology, but this slide is only going to remind you that this happens at many different scales, all the way from hundreds of nanometres, in the case of the flagellar assembly of Chlamydomonas to the multicellular structures formed in drosophila eye lenses, and inner ear cells, inner ear hair cells, all the way to coat formation, and floral patterns, and swarming which happens over tens of meters at a time.

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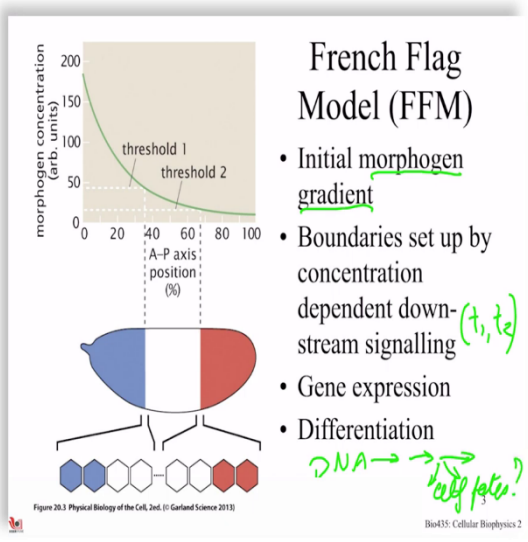

So, we are going to focus on the most fundamental model for the pattern formation at a cellular tissue level, and for that we are going to call up a model, a conceptual model initially which is proposed by Lewis Wolpert, who has just recently passed away, called the French

Flag model or what I am going to refer to now as the FFM. The idea of the French Flag model is the following that there is an initial morphogen gradient, that is the green line here. This leads to boundaries being set up due to the concentration differences, and these concentration differences result in downstream signalling at some thresholds t_1, t_2, leading to gene expression, and differentiation.

And this in essence is the key to this fundamental biological question that when the DNA of every cell that is dividing is the same, then how come cell fates differ. And this is something that every biologist knows as a, as an important question, but certainly from a biophysical perspective, what is often missing is understanding the how. How do these processes happen? A geneticist will try to find a network, a biochemist will try to find interactions, and a biophysicist will try to find models or patterns. And that is what we are going to try and focus on.

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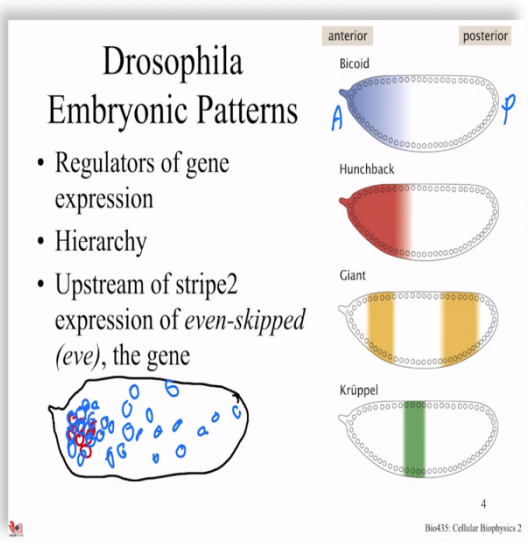

So, we are going to talk a little bit about this drosophila system, which I am sure you have gone, come through, gone through already in the developmental biology course. Because it, there is so much known already about it, it is a modern organism. It is modern organism because we studied it, we have got the genome, we got the proteome, we got the interactive, we have got the metabolome, we have got all the omes, and now we just need to make sense of it, and we cannot, or not completely.

So what we are looking at here is the anterior posterior gradient in the drosophila embryo, which forms these classical embryonic patterns and regulates gene expression. So, there is a

hierarchy, as you can see, from top to bottom. The Bicoid is the most upstream of them all. It is also called a maternal gradient, because the mother, the mother cell that laid, the mother fly, I am sorry, that laid this egg, laid down molecules of Bicoid at the anterior end.

And these molecules are, were laid in the form of mRNA. So let us see if I can draw the embryo, so like the nice image here. And these mRNA molecules, then go on to produce protein molecules of Bicoid, which of course will be more wherever the mRNA was laid down. And if they are transported or diffused through the embryo, then fewer and fewer as we go backwards. This is what leads to this apparent gradient of concentration from the anterior to the posterior, in terms of the Bicoid gradient.

Now, it turns out that the regulatory effects of Bicoid are such, that it leads to a subsequent gradient in the expression of a gene called Hunchback. The expression pattern of the gene Hunchback in turn leads to pattern in the formation of striped pattern of a gene called Giant, that in turn leads to the expression of a gene called Krueppel in a certain band, and that leads to a stripe2 protein expression called evenskipped or the gene eve.

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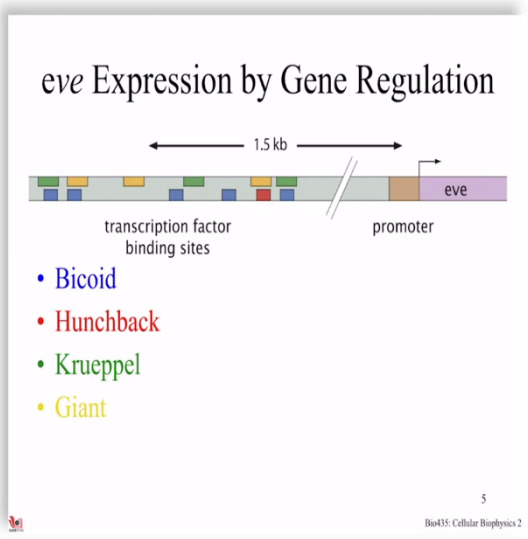

The expression of eve by gene regulation is very complex, and this if 1.5 kb upstream region of the gene coding sequence of eve, that is even skipped, demonstrates that Giant, the yellow box, Krueppel the green box, Hunchback the red box, and we call it the blue boxes, all upstream, act upstream of this gene to regulate its expression, producing something that looks like this.

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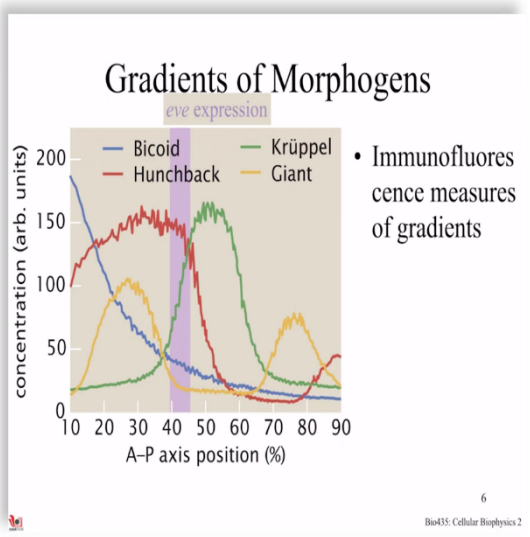

Bicoid, remember is the most primitive or in time, in developmental time, it is the first gradient that forms. That is the blue line. And downstream of it, Hunchback forms. Beyond Hunchback, we get as we had discussed, Giant. And after Giant, we get Krueppel. Now, it turns out that the expression of even-skipped, eve, in the purple band is a very narrow band, in this for 39 to 45 percentage of the anterior posterior axis position. All these data incidentally is taken from immunofluorescence measures of protein stained by antibodies.

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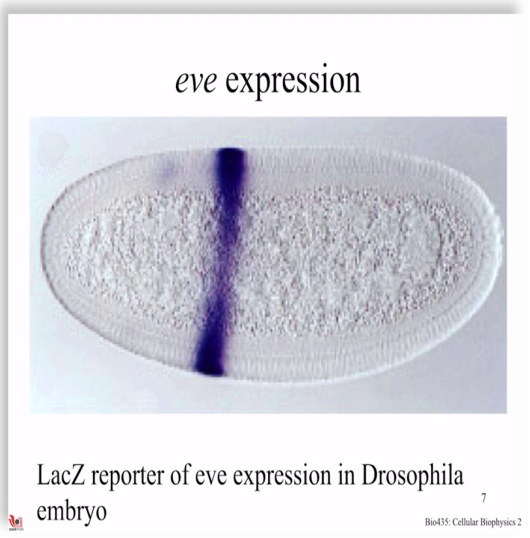

And in such a case, the even-skipped expression which if detected by a LacZ fusion promoter, a fusion reporter, shows this very nice narrow banding pattern, that you can see this bluish dye that is formed as a substrate.

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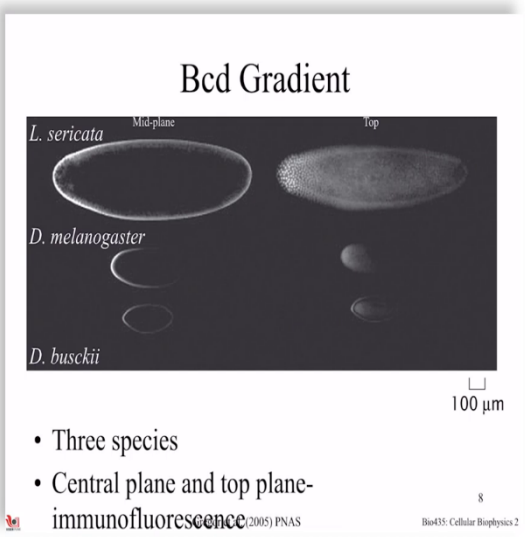

But turns out there is something more going on. So, if we now look at the Bicoid gradient, so suffice to say, I guess we are going to ask the question that not most geneticists do not ask, which is most genetecists as well you will find, try for very obvious reasons to find the mechanism. How does Bcd Bicoid express Hunchback, Krupple, and Giant, and how does that then combine to affect the localization of even-skipped, because we are trying to ask, how does even Bcd form a gradient, what is the basis of the formation.

And asking such simple, seemingly simple questions, surprisingly gives very interesting answers. So, what you are looking at here are mid plane and top views, that is to say the embryo is a 3D object. So you are looking at the cigar from the centre, and you are looking at it from the top. And what you look at it from the top, when you look at it from the top, what you see are these punctate loci, these yellow, bright gray spots of fluorescence. This by the way is nothing, but nuclei, these are cellular nuclei. And in these three species, what you also notice that is L. sericata, Drosophila melanogaster, Drosophila busckii, that the sizes of the embryos are also different.

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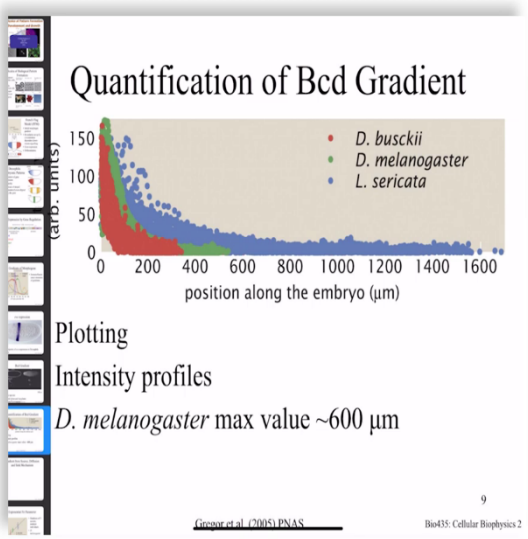

So, for the remainder of this class, I am going to discuss the mechanism by which the Bcd gradient is formed, the models that are used to describe it, and whether the experimental data and the models even can be reconciled to one another.