Physics of Biological Systems
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Lecture - 57
Spatial pattern in biology

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So, what I will do for today and the next day is this topic of pattern formation, will be sort of the last topic that I cover in this course. So, what I will try to do today is to talk a little bit about how patterns form in during development. In particular what I will be talking about this is the fruit fly, the drosophila and some generic reaction diffusion systems, and then I will do the formal mathematical theory the next to next class on Tuesday.

So, one of the generic things that you often see in biology is that regardless of this complexity of constituents and stochasticity, this reaction kinetics and so on, you will find very reliably

that nature produces this very beautiful patterns and across it produces patterns across the length, across length scales.

So, for example, this is cells in the inside of a year, think of a mouse which has patterns on the order of some 5 microns. These are cells on in the eye the rod and the phone cells of the fruit fly, drosophila which is a border of again some around 10 microns.

Then if you look at flowers you will often see very beautiful spots and patterns basically that are occurring and these are you know of the scales of a flower centimetres and so on. Or you could go to even larger scales and for example, it the stripes of a tiger or a zebra on the order of a meters where you again you have this reliable. So, here you could think of fattening as fattening of these pigments that produce the CLO versus this black fur, right.

So, this was a problem that originally turing thought about a long time back in the 1940s and 50s. And he said that given that I see that generically nature forms these sort of patterns across scales, across multiple length scales. Can one come up with a mechanism which will explain the process by which different constituents chemical constituents, beat pigments, beat proteins and so on they can interact with each other and give rise to these sort of patterns, ok. So, that is the this is sort of background with which he started thinking.

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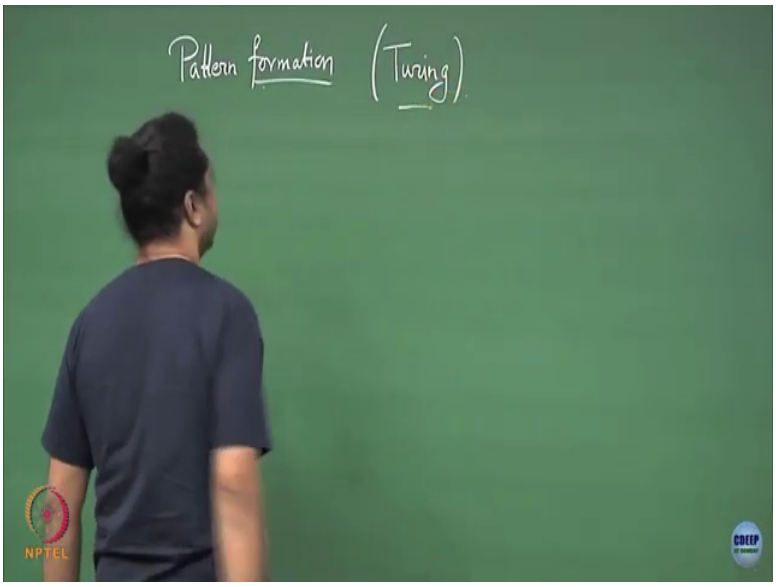

As it turned out the theories, so the generic theory is due to Turing himself, pattern formation of reaction diffusion systems. It was not very applicable in the context that he was thinking of, but it has taught us a lot of how to think of systems like this. So, I will expand a little more as I go long.

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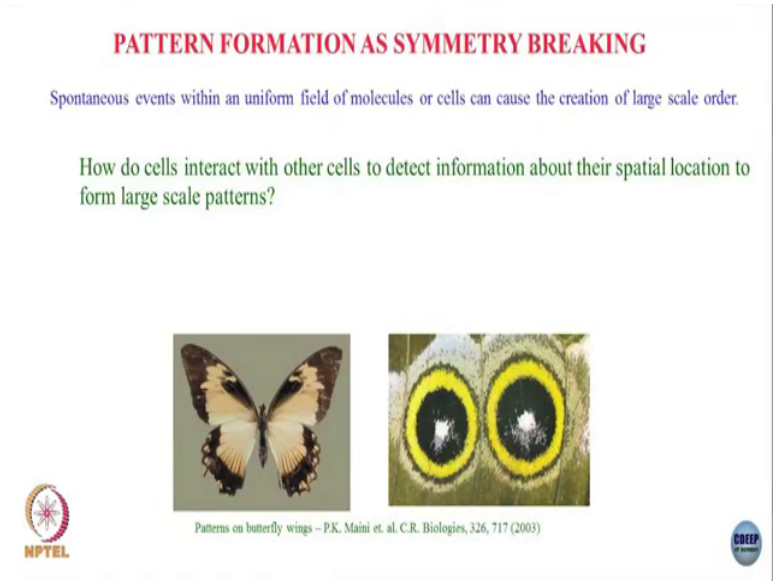

So, I will talk mostly in the first a with regard to a development. So, for example, when I have an embryo. So, all life starts with a single cell embryo.

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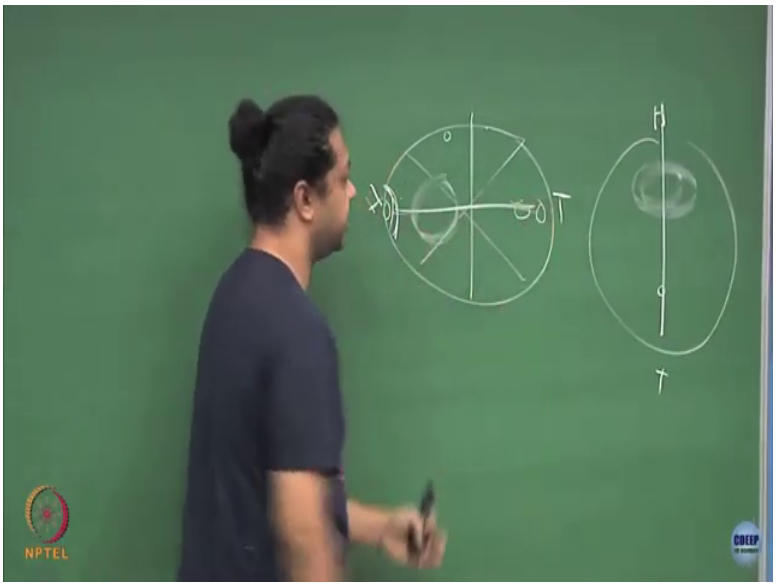

So, I form embryo like this, it is homogeneous, it is a single cell. Then slowly this embryo will start to divide and you will form multiple cells, right; you will form multiple cells. And then what started off as this homogeneous ball of cells we will develops spontaneously some sort of an order, right. You will have a head side and a tail side. Let see you will have a head and we will have a tail, right, so there will be an axis that is going to be formed. Then different cells in different parts of this axis or along this axis, they are going to develop differentially and then ultimately you will get a full-fledged organism, right.

So, the question often is that how do I start off with this homogeneous mixture of cells and how do I spontaneously then generate order. So, one way that one could think of is that there are some spontaneous events within an uniform field, within an uniform homogeneous field

and this somehow breaks the symmetry that is there in this original embryo and that symmetry breaking then cascades downstream and create some sort of a large scale order, ok.

So, there is some spontaneous symmetry breaking with respect to this embryo, that sets your let us say the head tail axis or whatever axis and then things it is a complicated process. So, I am just waving hands and saying, but that is a rough idea that you have some sort of spontaneous symmetry breaking that helps you at least get started on this development process.

Or another way you could think of is that there is it is not as homogeneous as it looks. There is some sort of external signal at some point let see on the head side that introduces and built in a symmetry and that asymmetry then propagates and tells the organism how to develop. So, that is another possible mechanism. Yes?

Student: (Refer Time: 04:56).

So, what it means is that you have some homogeneous ball. Now, let us say the concentration of one species some species mixed uniformly and homogeneously inside this. Let us say the concentration of one, so there are fluctuations in these concentrations because you know whatever stochastic processes are going on. So, this fluctuation let us say beyond a certain strength can sort of generate such that you have more of this molecule on this side less on this side, that happens spontaneously.

So, in some cells let us say this half gets more, so this becomes your head that becomes your tail. In other cell maybe there is a high concentration fluctuation here and high low concentration there. So, that becomes your head tail axis in that sense.

Student: (Refer Time: 05:49).

So, another way to frame this question is that how in this in this system of different cells how do cells interact with other cells to detect information about their spatial location in order to

form these large scale patterns. That is how does the cell over here know that it has to form the head how do the cell over here know it has to form the tail or a something over here knows so to form the hands and so on. So, it in to talk to other cells in order to sort of determine where its relative position is in that drive and then have an appropriate sort of developmental cascade.

So, Turing was this is been this sort of reaction diffusion systems has been used to sort of study various patterns for example, patterns on butterfly wings is a relatively recent paper where through the use of these systems that we look at the reaction equations that we look at today you can sort of explain these sort of macroscopic patterns.

So, then I use explain in a very lose way as I will expand upon, ok. So, let me start before I go into the generic theory, let me start off by taking this specific example of development of drosophila, ok.

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The video, I will play the video separately may be.

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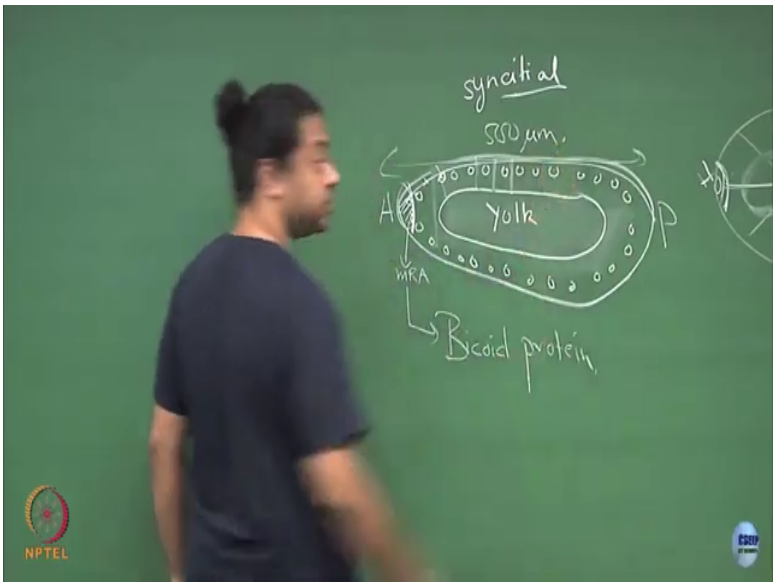

So, here is the idea that I drosophila is a common fruit fly. So, here is my drosophila embryo it looks something, like this like an oval. The drosophila embryo is part of a class of organisms which are called syncitial which are called syncitial in that when it is see initially it is a uniform mixture, then slowly what happens is that you get cells as you have nuclei studded throughout the bulk of this embryo and then at some point this nuclei all move to the surface. So, they all move over to the surface. And you have these nuclei coming over here. So, these are all the (Refer Time: 07:58) nuclei. They all move to the surface. The interior, the yolk sort of face separates out and it goes to the inside.

So, this is the nutrient from which these embryos will sort of take sustenance. Then as time progresses these embryos will divides, so you will get more and more embryos. But it is slightly peculiar in the sense that you do not have proper cells until sometime later. So, these nuclei are not divided up by cell walls in that sense. So, that is what it is meant by syncitial.

The cell walls form after a certain number of divisions are taken to this. Initially it is like this continuous domain where you have these nuclei sort of multiply, ok. So, this is what this movie shows. You can you see if I can play this. Can you see? Yeah.

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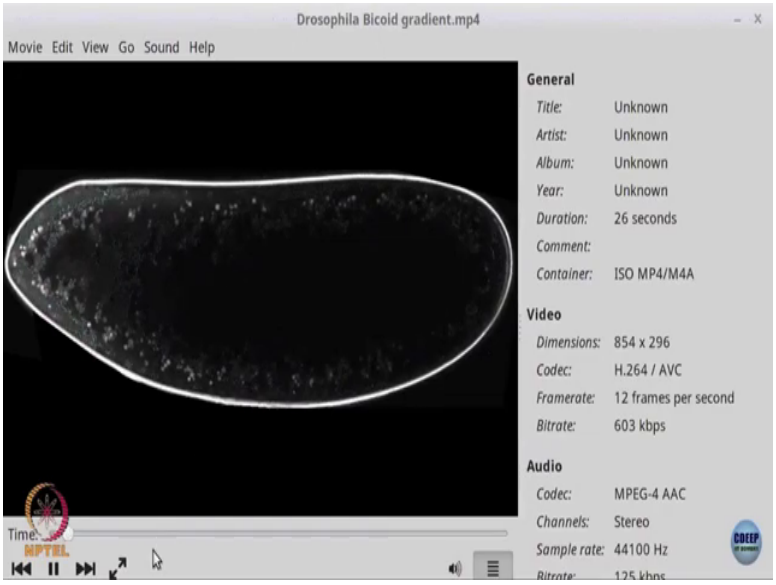

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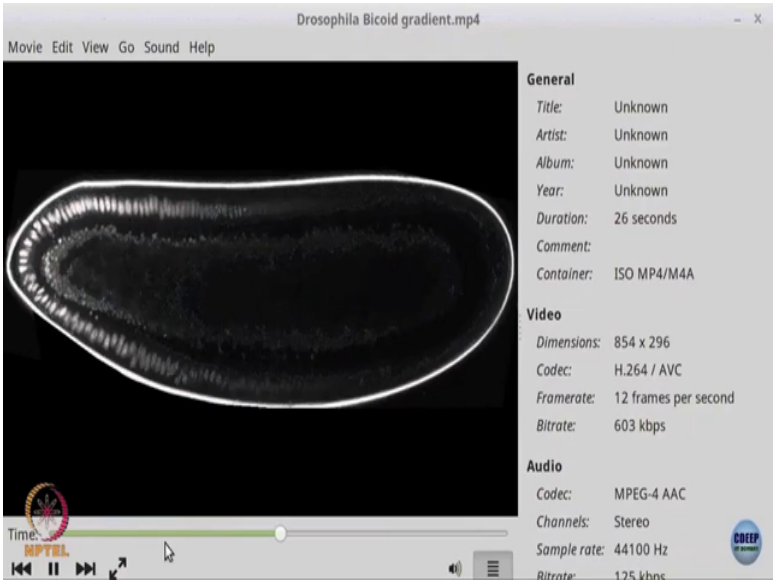

So, this is the drosophila embryo. These white spots the fluorescent spots over here are these nuclei. And you will see this spots will get, as this divide these spots will get denser, so this is when the division is happening. So, let me here I use some fluorescent intensity. So, let me display it from the front.

So, there are very few then as a device there are more nuclei, it divides once more there are more nuclei and so on. It has already established a sort of axis in that there are more nuclei on this side than on this side, right. This side will ultimately be it is called anterior side. It will ultimately become the head of the fruit fly this side is the tail of the fruit fly. So, it is called the anterior posterior axis. What is marked is a particular; so, what is fluorescently labeled in these movies is a particular protein called bicoid which I will talk about little bit.

It is a one of these precursor proteins that will set this A-P axis basically. And on the inside what you see over here this dark region is olio. There are no nuclei over here in this bulk. And this process will continue. So, here right now there are no cell walls after some time the cell walls will form and you get proper cellularization and the development will continue.

On this side there are actually very few nuclei. So, well there are nuclei, but yes the density is less, ultimately it will get of course filled out. There is another thing over here. So, what is labeled like I said is this bicoid protein, ok. So, it is correlated with the nuclei because these proteins get sort of localized inside this nucleus to a higher degree inside the nucleus which is why you can see this spherical sort of objects as nuclei.

The fact that you cannot see them on this posterior side means two things; one is that initially the nuclear density is low here and secondly, the bicoid also actually as I will tell the bicoid is actually produced over here. So, it needs to go over there and it has not managed to do that yet. So, that is why I have not seen any density inside.

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If you allow, if you let this process sort of play on, here is what the fly looks like at some later time. So, those where within 1 hour or so, worth 1 to 2 hours.

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These are much later I think this is an hours, so 5 hours and so on, so this sped up. And you see that various sort of patterns I have started forming the thorax and so on of the fly have started appearing. So, this is the dorsal view from the top, this is the ventral view from the bottom and these nuclei sort of separate segregate out into different regions and then you can see the sort of abdominal, the mark sort of coming clearly over here.

So, what starts over in this simple from the single single celled embryo? Simple single celled embryo, it goes to these nuclei divisions and then ultimately as time goes on you have this whole organism emerging. Also, if you look at there is another interesting thing since I am showing let me show.

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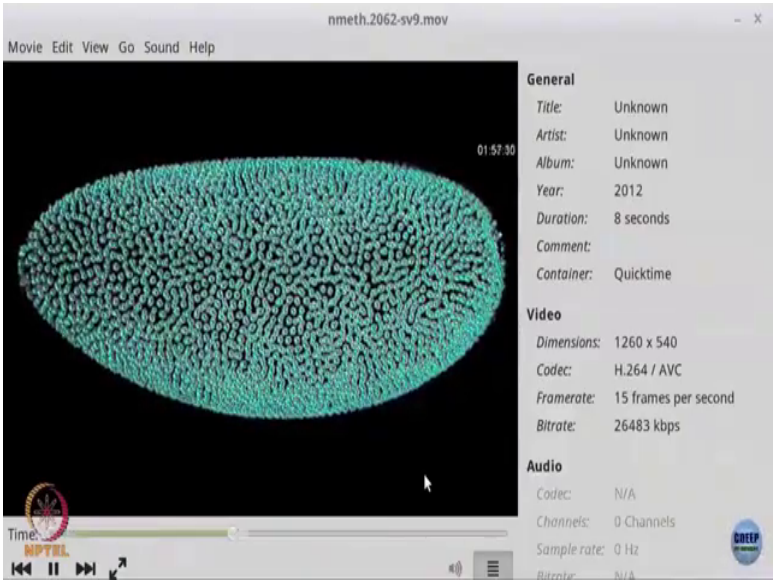

So, this is that initial phase when the cell division, cell compartments of not formed. The nuclei are just dividing. You will see that the way that the nuclei divide is actually very nice, it forms as this the way that is colored is that when the cells are resting, they are not dividing, they are colored in this sign and then when the cells are dividing its colored in this magenta or purple, ok.

So, the cells sort of divide in a wave it starts off at this anterior pole, then this these divide, then these divides and these divide and this wave sort of propagates from the anterior pole to the posterior pole and then again the next division cycle the wave propagates from here to here. So, not only there is sort of pattern that emerges, there is also sort of travelling waves that emerges.

The question is if I think of this simple sort of a system how do I get this sort of an emergence of order, how do I get these different divisions that form starting with the very simple system like this?

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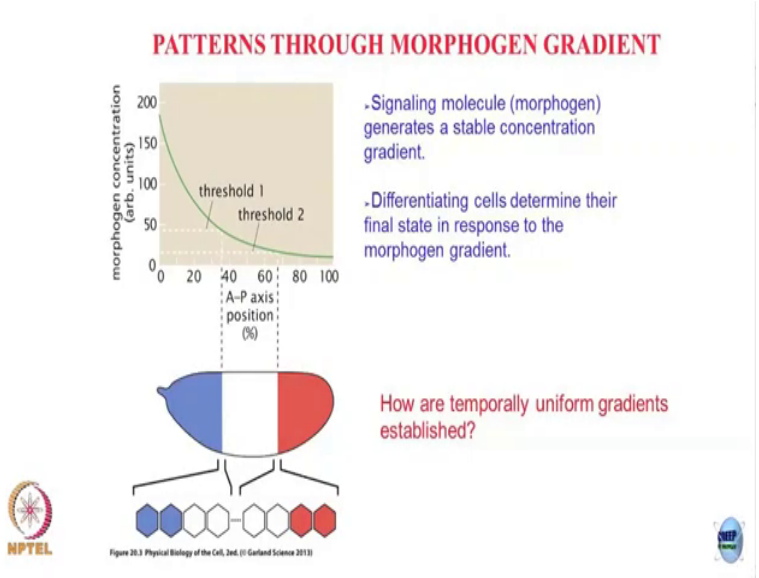

So, here is one of the proposals. So, the idea is this that let us say there is some signaling molecule which is called let us say a morphogen and that has some sort of a great concentration gradient along this A-P axis. So, mostly I will talk about this is the anterior pole, this is the posterior pole. So, if I go like this, this line constitutes my A-P axis. So, let us say somehow I have formed a morphogen gradient that goes from the anterior pole to the posterior pole like this. So, there is a high concentration at the anterior pole. There is a low concentration at the posterior pole, ok.

Then the cells or the nuclei in this case they might determine their final state in response to the concentration gradient of this morphogen, ok. So, if this morphogen is let us say there is some threshold. So, cells with c this concentration above this threshold will become differentiate to become the head let us say, cells with c concentrations below this threshold maybe will differentiate to become something else, cells in the middle will become something else and so on, ok.

So, there is some sort of a controlling concentration morphogen which sets up a gradient and other cells, so the further division or differentiation of these cells follows is a response to this morphogen gradient. But you could then ask that well is in the case of drosophila if this is a sort of picture I have in my mind, then what is this morphogen gradient, what is this basic morphogen gradient and how is that established.

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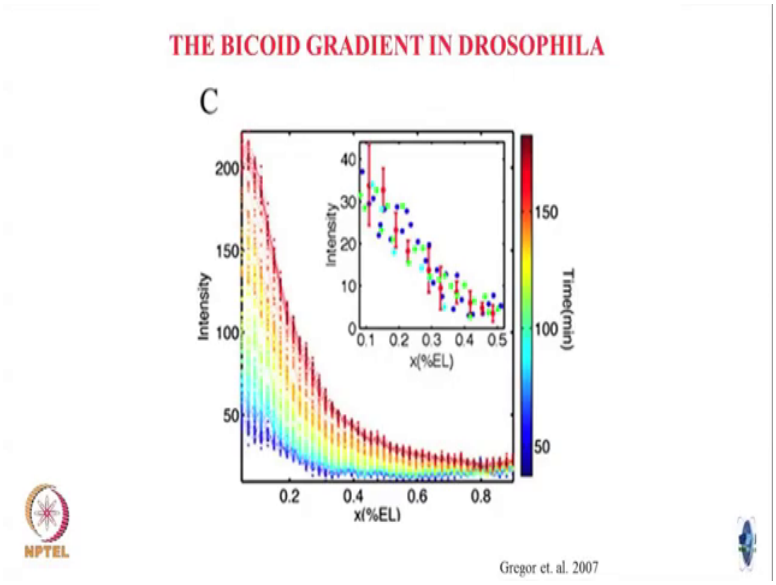

So, the basic gradient as it turns out in drosophila there is a unique answer to that it is this bicoid protein, it is this bicoid protein that I talk about. So, what happens is that when this embryo is formed the maternals the mother sort of deposits some MRNA in this part near the anterior pole. And this MRNA. So, there is some MRNA over here and this MRNA codes for the bicoid protein, ok. So, what that means is that there is a reservoir of bicoid proteins or continuous source of bicoid proteins because this MRNA continuously gets red and bicoid proteins are produced at the anterior pole.

And you can see that by; so, this is experimental plots again off this bicoid intensity as a function of this x, x being the length along this A-P axis, as a fraction of the embryo length, so it goes from 0 to 1. Just to give a sense of the numbers this drosophila embryos are roughly let us say around 500, 600 microns, ok. So, 0.2 means 0.2 of 500 microns or so.

So, this is the bicoid intensity that has been plotted at different time points, ok. So, at very early times it is this blue line then as time goes on it rises, it rises, it rises at very late time, so this is in minutes. So, this is after two and half hours or so, you get this sort of a red line. So, you form a concentration gradient initially there is nothing.

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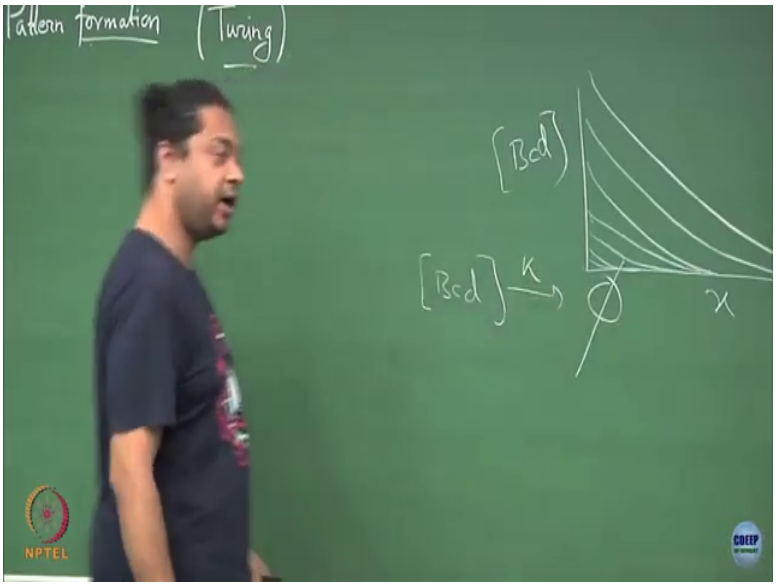

So, here is my whatever plot of bicoid let me say bicoid concentration as a function of x. Initially, there is nothing. Then as this MRNA gets transcribed and the protein gets produced the protein sort of diffuses slowly, ok, it covers out this whole cell and ultimately you get a profile like this.

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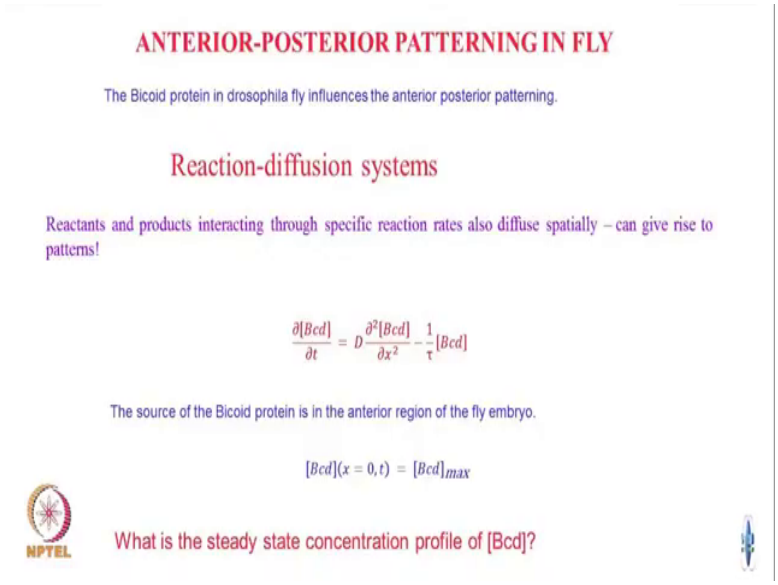

And this you have seen in your I think mid-sem thing. So, I give this drosophila a problem this bicoid. So, this is the context for that basically. So, this is a very simple example of reaction diffusion system, a very very simple example in that sense.

So, in general reaction diffusion system is something where you have some sort of a chemical reaction. So, we have reactants and products, reacting interacting specific reactions, but they are also diffusing and that can give rise to patterns in general. So, but before I go to this sort of generic thing you can think of this bicoid as a very simple case of this reaction diffusion system in some sense. The reaction being that this bicoid protein sort of diffuse is sort of degrades.

So, it degrades with some rate let us say I do not know some rate kappa, ok. It is produced at the anterior pole, so it has a source and it diffuses, ok. So, it is produced over here, it diffuses and it degrades which is why it is called the synthesis diffusion degradation model.

So, the source is at this anterior pole which I say is x equal to 0 and let us say it is some concentration at this interior pole scale everything by the concentration in the anterior pole which I call some maximum bicoid. So, then what this tells me is how this concentration of bicoid evolves in time. So, the rate of change of this bicoid concentration comes because of the diffusion of the bicoid molecules along this A-P axis and the degradation of the bicoid.

So, I have written tau in terms of tau, so kappa is basically 1 by tau. So, tau being the lifetime, ok. So, it is a first order decay process and a diffusion with the constant, with the source at this anterior pole.

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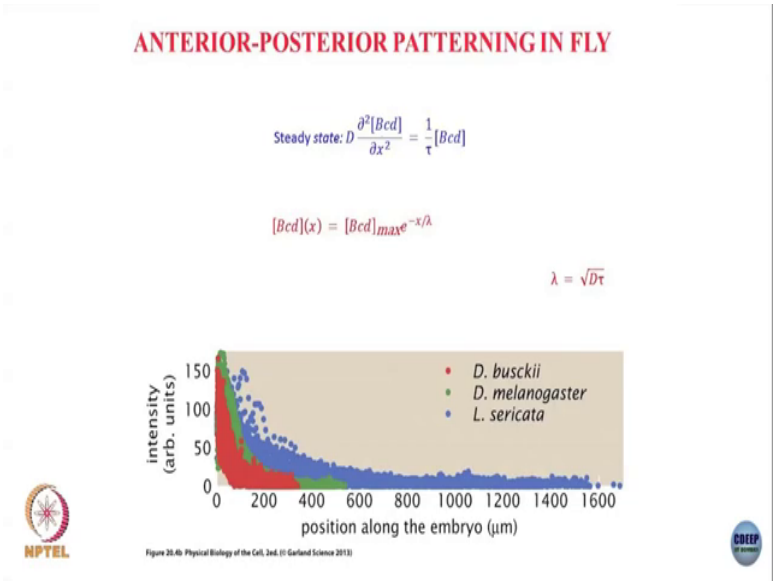

And then as you have solved this the steady state concentration of this is an exponential profiles it is like e to the power of minus x by lambda, right, where this lambda the characteristic length scale is square root D into tau or D by kappa whichever way you write.

So, you get a profile that looks basically like this, right. You get a profile that looks like this. So, that is the basic idea that we have that the mother deposits some MRNA which acts as a source of bicoid. So, there is already something that sort of breaks the symmetry, then as this bicoid MRNA gets expressed into bicoid proteins that protein diffuses out and it degrades, and because of this diffusion and degradation it forms an exponential profile, ok. So, that is the first thing that happens in this whole cascade of in this developmental cascade.

And you can do this; so, you can check this for different sort of drosophila species. And you will see that, so these have different embryo lengths. For example, this the common fruit fly

which is melanogaster has something around this 500, 600 microns, this one is smaller, that one is larger, but you get exponential profiles in all of these cases, ok. Yes, yes.

Student: That (Refer Time: 19:48) that what time we (Refer Time: 19:50).

Until this point, it is still being continuously produced, ok.

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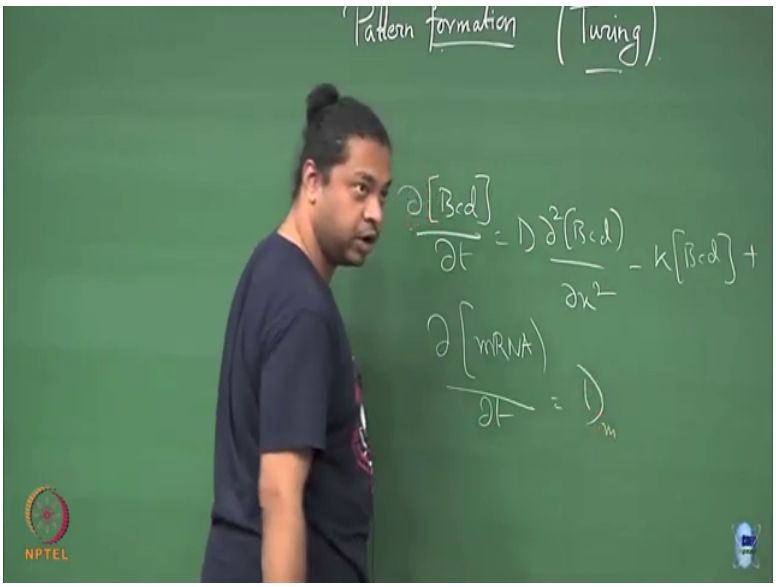

So, another way to write this equation is to say that what was I writing del del bicoid del t is D del 2 bicoid, del x 2 minus kappa bicoid plus a source term. So, let me write the source term as something like a source strength, which happens only at the origins. So, delta x and which happens for all time t greater than 0. So, I put a heavy side theta function. So, for any t greater than 0, t equal to 0 being fertilization, you are continuously producing this bicoid protein at x equal to 0, ok.

In reality, it happens for a long enough time that this exponential profile has had a chance to set in. So, as long as the MRNA is there, the MRNA itself does not get degraded it will keep producing bicoid proteins and the MRNA like time is large enough that you have enough time to form this exponential timescale, ok. So, as far as this graph goes even at these times, I think at least still 3-4 hours definitely it is known the biocid protein is being produced continuously. Student: (Refer Time: 21:29).

The MRNA was to refuse, yes.

Student: Definitely if bicoid protein.

Yes.

Student: That (Refer Time: 21:37).

Yes.

Student. So, that will also (Refer Time: 21:40).

Somewhat different. So, you will have, so you have to write some equation for the MRNA, right which will have some diffusion coefficient of its own. It will it will have some degradation of its own and so on.

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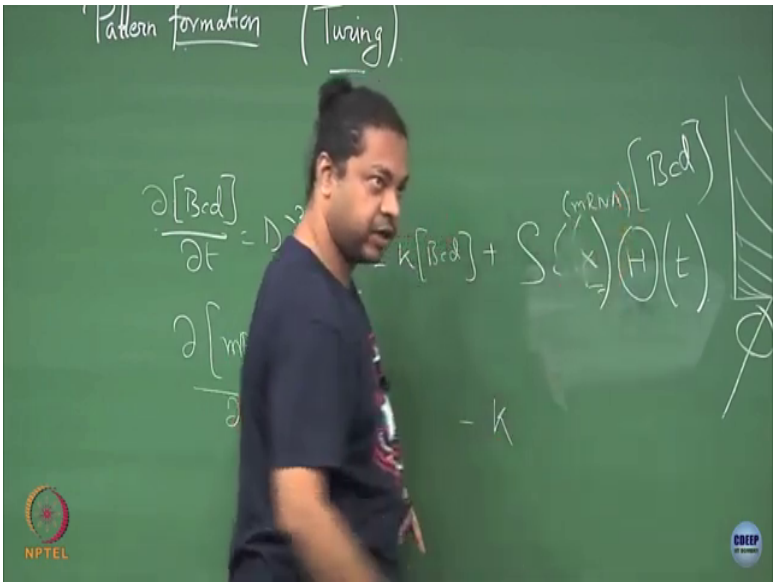

And then this source instead of being a delta x will respond to this MRNA concentration, right. So, it is a valid model, people have done this. And they have seen how this how the length scale changes once you take into account that the MRNA itself can be diffused. In the 0th order model, what has been found experimentally is that the diffusion constant of the MRNA is relatively much smaller compared to the diffusion constant of the bicoid which is why to first approximation people treat it as a strictly localized source. But people have relaxed that approximation and seen what it gives. It gives something that is slightly better than this model. So, it is good in that sense, ok, right.

So, you can if you look at this if you look at this exponential profiles you can find out what is the length scale of these profiles and it turns out for example, in Drosophila Melanogaster the common fruit fly, the length scale comes out to be a 100 microns.

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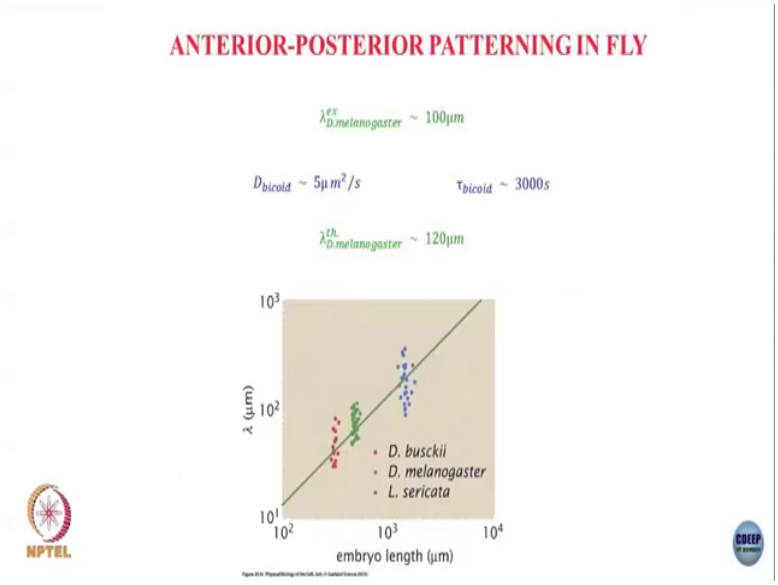

And if you put in measurements of these diffusion constant of the bicoid and the lifetime of the bicoid you get a number which is very close to this experimentally observe value. So, these particular numbers give you 120 microns.

This is some sort of cheating because over the last 15, 20 years, there have been a bunch of measurements of this diffusion constant and people have given values from 0.1to 10. So, it is like a huge variation. Depending on which number you put in you would get a different length scale. You put in the number that gives you the best length scale in some sense.

These measurements are very difficult to do. So, people do frapp, like we discussed if you remember in the earlier this is not very good people do some fluorescence correlation spectroscopy, FCS and so on. But these numbers experimentally, so inside the cell it is somewhat difficult to sort of do it in a control sense because if you do it against the

background of all these other nuclei and all other cytoskeletal filament proteins everything. So, there is some debate about what is the right diffusion constant use, but the general agreement is of the order of micron square per second.

Nevertheless, the point I want to make here is that what this sort of a theory tells you. So, you can immediately see what are the shortcomings of a theory like this. So, what it says is that this characteristic length scale that I get is D by kappa or D into tau, right. It does not matter to me what the length of the embryo is, what is this L, ok. So, regardless of what L I put, I would get a lamp if my diffusion coefficient and the degradation rate were the same, I would get the same lambda irrespective of embryo length, ok. And as you saw in these earlier pictures that is definitely not true. If you do different species for example, which have roughly different average length you see with the lambda scales more or less with embryo lengths.

So, for an embryo which is larger you will have a larger lambda, for an embryo which is smaller you have smaller lambda. So, simple this 0th order model like this. This synthesis degradation diffusion model. It explains one thing which is that you get an exponential profile, which is observed. So, that is good.

On the other hand, it cannot explain a few things. So, for example, it cannot explain how this lambda scales with the length of embryo that is something it fails to do. And it is in that context that various of these other models have been proposed that what happens if it is not steady state for example, if I take the time dependent solution of this equation, then I will get some lambda as a function of t does that match. Or if I take this MRNA that the fact that the MRNA itself diffuses, so the source is no longer a point source, but an extended source, then does the length scale of that source for example, come into this lambda and how does that affect and so on.

So, people have tried a bunch of models which are variations of these this sort of an SDD model. As far as I know no model has satisfactorily answered this question of how to how do these flies regulate the length scale, so that this length scale is lambda is sort of proportional to

the length of embryo itself. Various models are there, but no model completely answers this question.