**Cellular Biophysics**
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**Department of Biology**
**Indian Institute of Science Education and Research, Pune**
**Lecture 57**
**Turing model**

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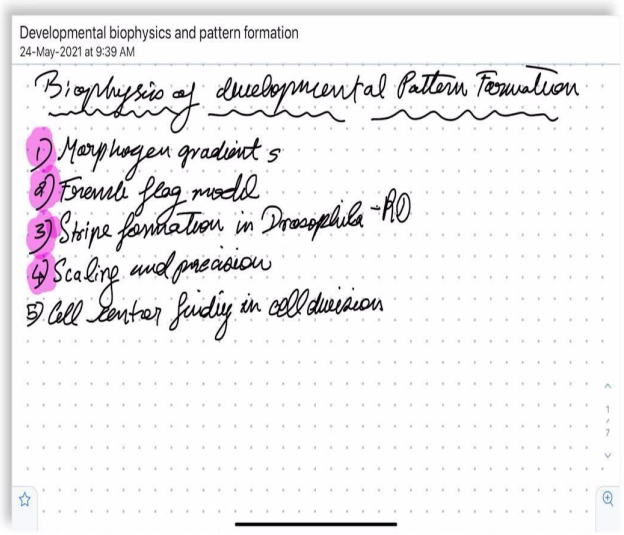


Welcome back. So we have already spoken about the basic concept of morphogen gradients. We discussed very briefly the French Flag model, pardon my English. And we will now talk about stripe formation in Drosophila, using reaction diffusion models and the role of scaling and precision. So let us go to it.

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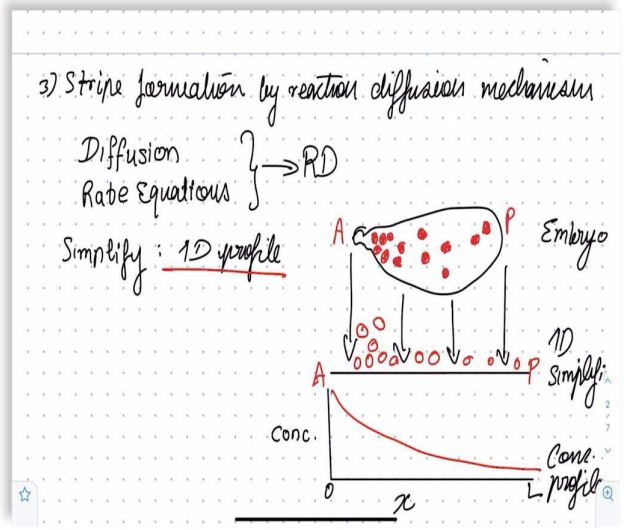

So, stripe formation by this reaction diffusion mechanism is thought to be a combination of the two concepts that we already spoken about. The first part of the semester, we in fact spent quite a bit of time, perhaps too much on diffusion. And the second part, which is rate equations, and this in fact combined to give us a reaction diffusion systems, meaning to say reactions, represented by rate equations and diffusion represented by fix laws.

So for an understanding of what is going on, we going to have to do, make some simplifications. We are going to basically assume from the perspective of experimental measurements, that we consider the profile of concentration to be symmetric, perpendicular to the long axis, but varying along that long axis anterior, posterior.

So this is essentially what will give us one dimensional profile of the concentrations. This is seen over here, which is where we have some molecules, which are formed at higher concentrations at one end, for instance. Remember, we talked about the Bicoid being laid down by the maternal RNA, forming then a gradient of the Bicoid proteins, anterior to posterior.

But these, in fact, if we assume them to be localized in a one dimensional profile, will be more and less along some axis, AP axis which essentially in a geometric sense, we will consider to be the x axis. The 0 to L be the length, and the concentration profile length emerging out of it.

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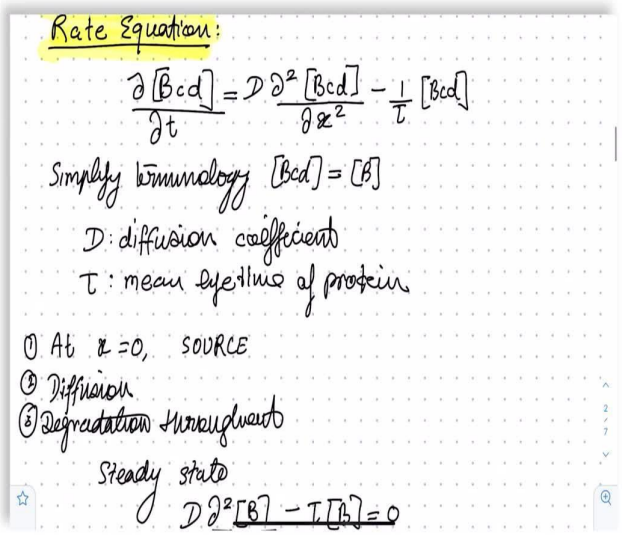

So in a rate equation sense, the equation that we going to be interested in putting down is going to be nothing but a reaction diffusion equation, which looks something like this,

that $\partial\frac{[Bcd]}{\partial}t$, or the partial differential equation of the time evolution of Bcd concentration is the product of the diffusion coefficient times the second derivative of Bcd concentration by the $\partial x^2$, where x is the spatial position minus $1x\frac{[BCD]}{\tau}$.

What is tau? tau(τ) is just simply the mean lifetime of the protein, meaning how long it lasts on an average. And D is ofcoure, diffusion coefficient. We are going to simplify the terminology and simply replace Bcd with B. Square brackets indicate concentrations.

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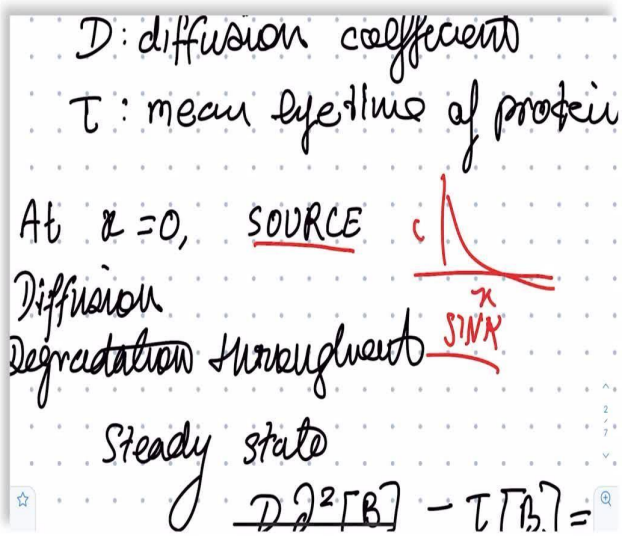


In such a case, then at x is equal to 0, when the, where the source is thought to be, then there will be some maximal value at that point, there will be diffusion connecting that point with every other point. And there will be degradation throughout. This is nothing but a source-sink system, which we had spoken about earlier, will expectedly give us something like an exponential solution, where concentration and x are the y and x, respectively.

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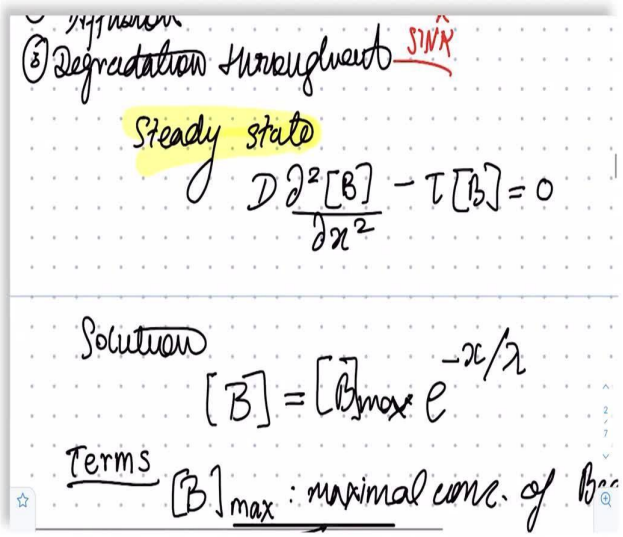


In fact, at steady state, we are going to say that the change in this differential equation should be 0, there should be no change in time. That is why it could be steady state.

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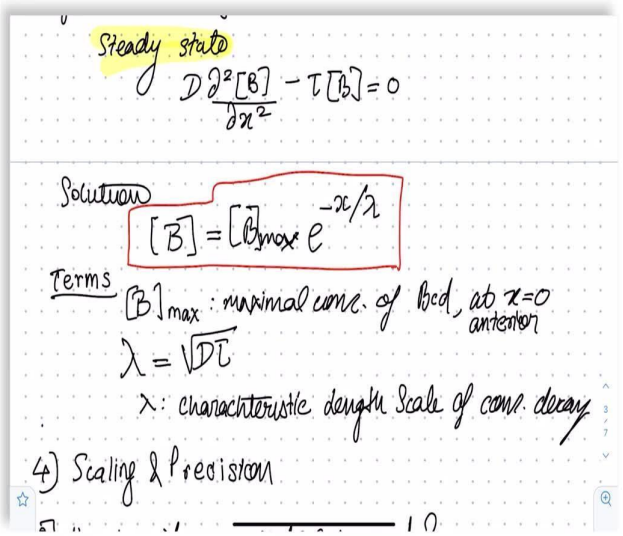


The solution to that, just as we had said earlier, in terms of the source-sink idea is nothing but an exponential decay function where the Bcd concentration, Bicoid concentration is B concentration max times negative exponential x by lambda, B being concentration max is the maximal concentration basically, and lambda is D times $\tau$.

As you can see from the right hand side, D is micron square per second or length square per time, $\tau$ is time. So essentially, you end up with under root length square, which is length. And

this is nothing but the characteristic length scale of the concentration, decay over the spatial domain.

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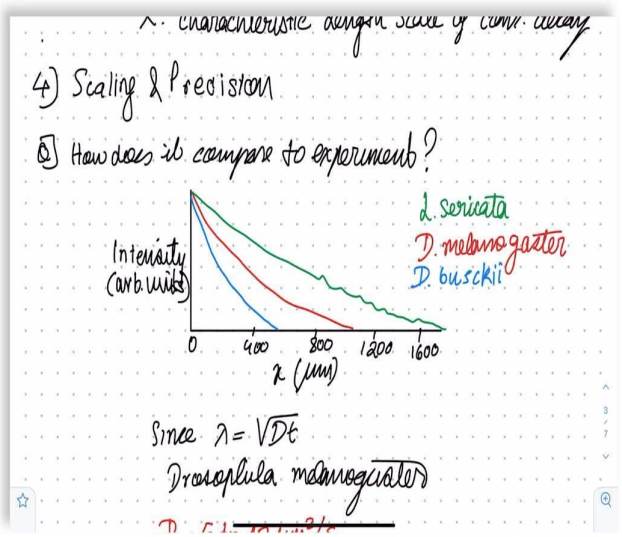


All right, what about scaling and precision, and why do we care? So the question is the following. When we compare this gradient to experiment what do we learn? So I have taken some figures from the text book and from published work of a gentleman called Thomas Gregor, who has written a fantastic set of papers on Bicoid gradient and its evolution.

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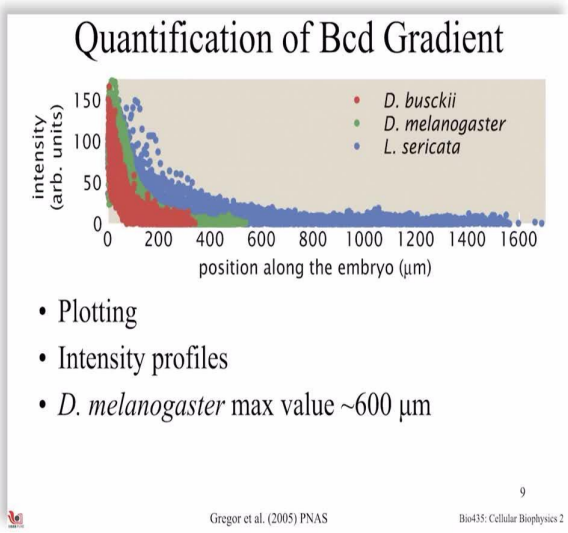


And what you are looking at here is in fact the quantification of the Bicoid gradient using antibody from multiple drosophila embryos. So *Drosophila buskii*, *Drosophila melanogaster*

and *L. sericata*. Now, these are related species, but like some of your relations are good, good sitar players and classical musicians, some of them wonderful long jumpers, not everyone is the same.

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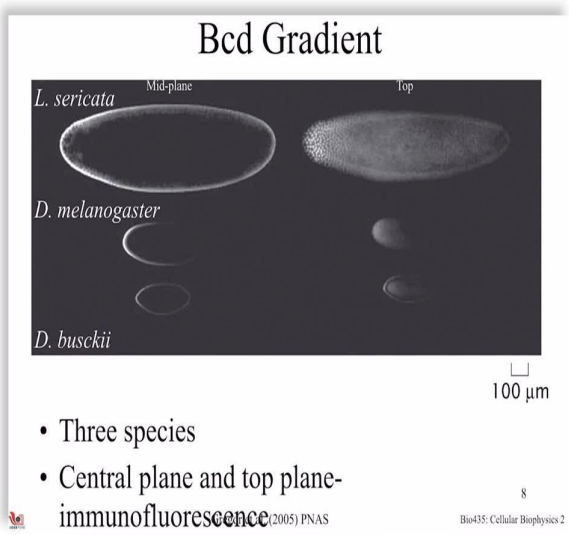


And if you remember, I had shown you this slide earlier, which is that the *L. sericata* embryo is almost in the range of 500 microns to 1 millimeter in diameter, in length, as compared to about 200 micrometers, 200 to 300 micrometers for the *Drosophila melanogaster* embryo, and *D. buskii* is even smaller.

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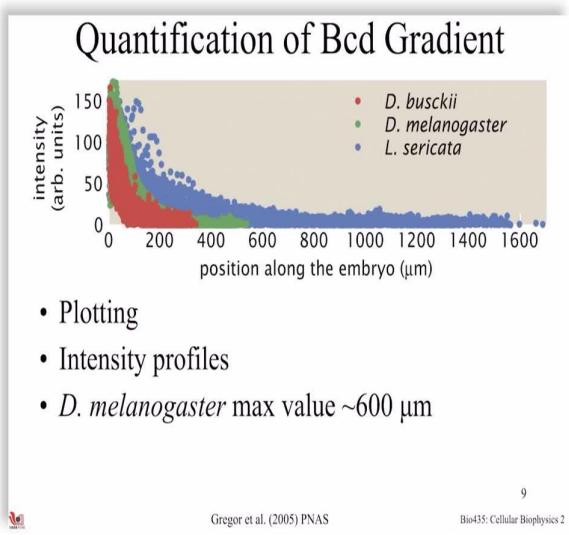

So, this would suggest that the gradient rule will also somehow change. How does it change is an interesting question and it is what we have been talking about earlier. If you remember, last semester, we talked about scaling of horse stride with shoulder length, we talked then about chromosome packing and chromosome size. We have talked more recently about, and in the very, very beginning, we talked a bit about size scaling of molecules, and their entities.

So in this case, we are now asking about more complex process and active process in a way the Bcd gradient and it scaling with embryonic size, effectively an evolutionary coefficient, but from a biophysical perspective. So this is fascinating, right? So when you do that, you find that the intensity profiles of the *Drosophila melanogaster* can reach a maximum value, reach a maximum length of about 600 microns. And its half maximum is somewhere in the middle, closer to the left, because it is an exponential decay. And, and we are going to try and see if what we have calculated earlier, has any connection to this.

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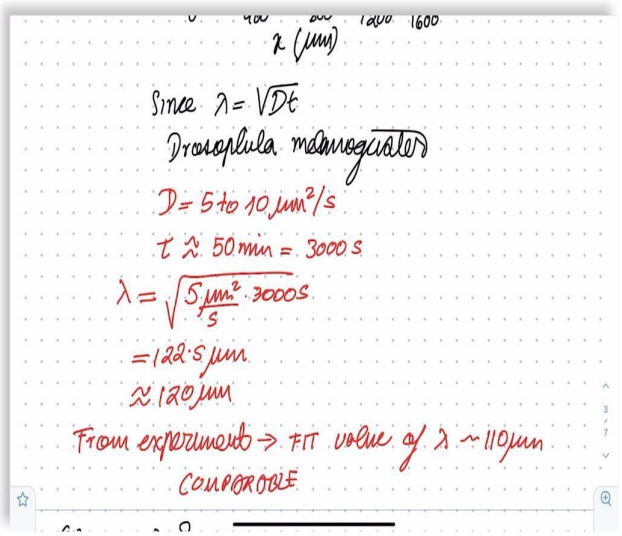


So since we can say that λ is equal to $\sqrt{Dt}$, from the characteristic length scale, let us plug in some numbers. We will take for *Drosophila melanogaster* diffusion coefficient of proteins of between 5 and 10 micrometers square per second, we will take tau, the lifetime to be about 50 minutes, which is 3000 seconds, and we ended up with about 122.5 micrometers, which is approximately 120 micrometers.

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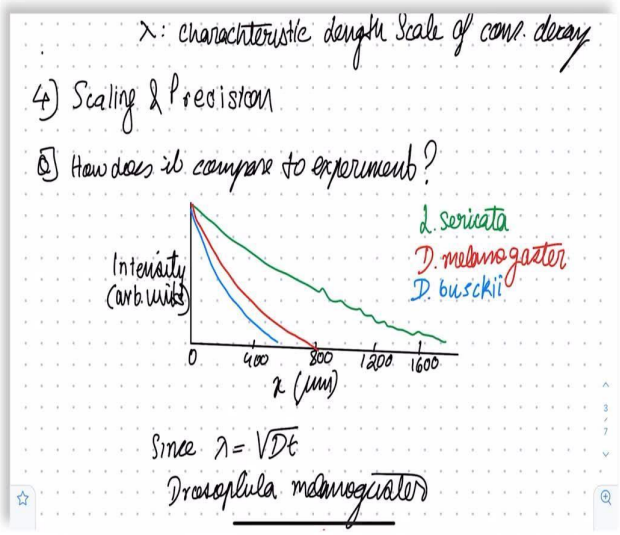


So looking at this graph with, maybe I should correct this guys a little bit, the drosophila line should in fact, be a little shorter based on what I just told you. And this would essentially need a little bit of an editing. That is exactly why I guess I do not draw these figures too often myself. Something like that. These are approximate. Please take that into account.

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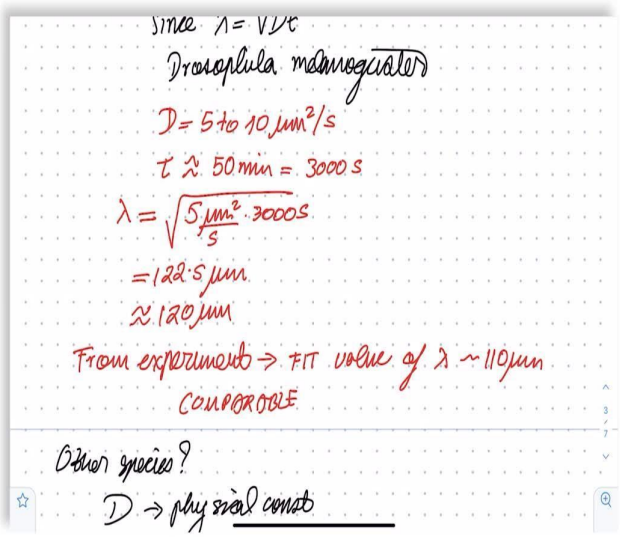


Having said that, if this is now the drosophila gradient of about 600 micrometers total length, then $\tau$ is about 120 microns. When experimental data was fit to obtain the value of lambda, the value found was about a 100 microns. So between 100 and 120 microns, we can safely say that the values are reasonably comparable to each other in terms of measurements.

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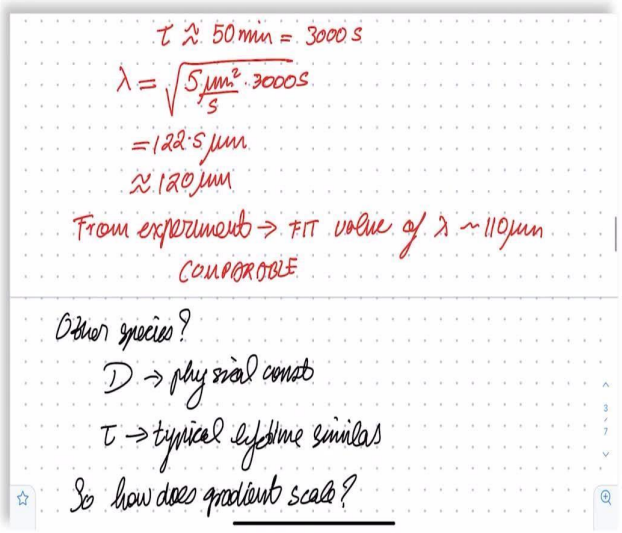


So what about the other species? So Drosophila works, so this is kind of interesting. Do the diffusion coefficients of protein change its species? Now, there is some work going on in my lab, which suggests maybe that might be the case. But turns out in these species, it is not easy to measure it. What about typical lifetimes? It turns out that these are mostly similar.

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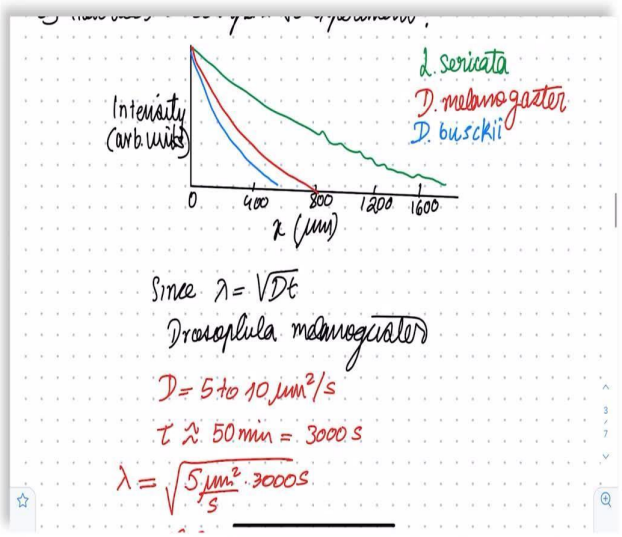


So if they are similar, then just the dependence of lambda on D and τ does not make sense because it suggests that the length scale of the gradient will be the same. There is no effect. It does not matter whether you produce more Bicoid, initially, your maximum value is higher, it does not matter. Your length scale is the same.

So maybe experimental data comparison is very useful over here for a model which would otherwise mathematically be, there is nothing wrong with what we have done, but incorrect in terms of the biology. And this is a big lesson in developmental biophysics, which is, and cellular biology and biophysics, which is that only on comparison to experiment, can we ever tell whether a model is really useful or interesting?

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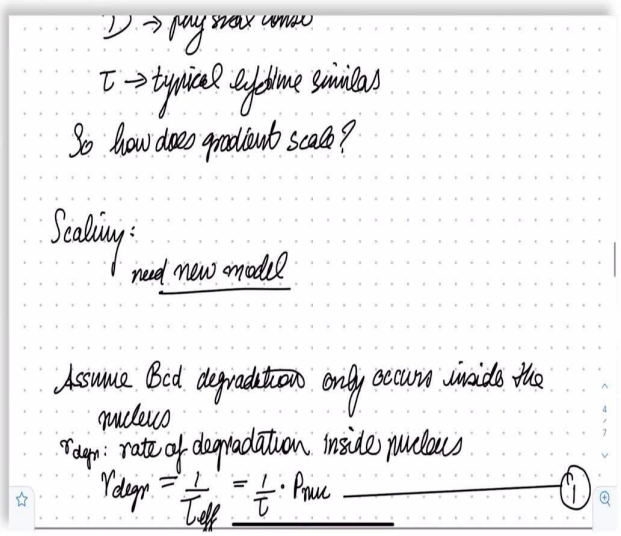


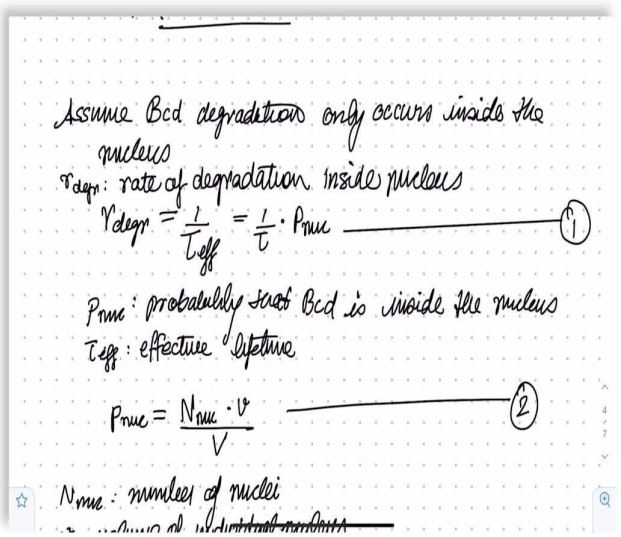


So indeed, in the case of this system, in order to explain the scaling, we need a new model. So to do that, we assume that Bcd degradation only occurs inside the nucleus, which gives us a rate of degradation inside the nucleus, which we say is 1 by τ effective which we say is the effective time interval and can open it out as 1 by τ times the probability of the presence of

Bcd inside the nucleus. This we can write in terms of the number of nuclei per unit volume of the embryo, V capital, all multiplied by the volume of the individual nucleus.

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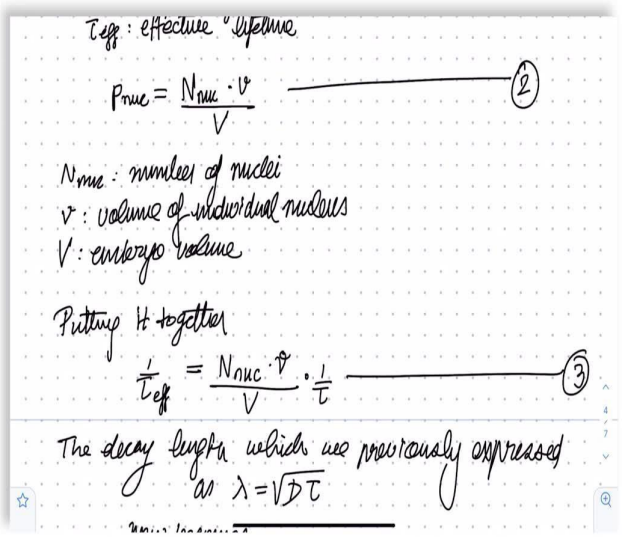


So, these are the terms. $N_{nuc}$ is the number of nuclei, v is, v small is the volume of individual nuclei and $V_{cap}$ is the embryo volume. So, putting it all together, we end up with 1 upon τ effective is equal to the number of nuclei times the volume of the nucleus upon capital, the volume of the embryo into 1 by τ.

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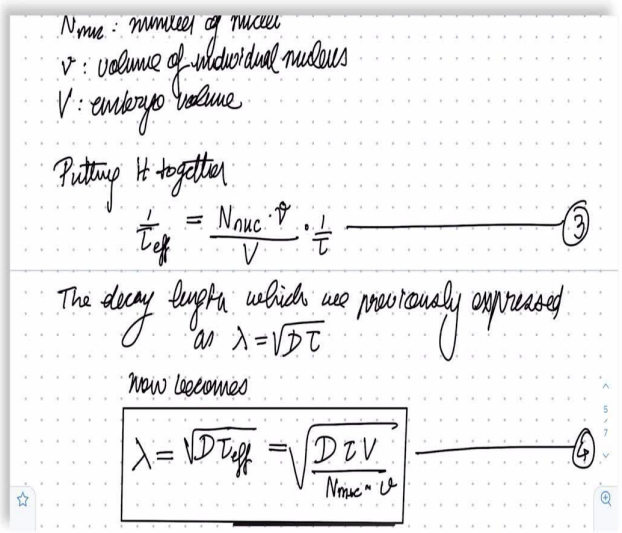

The decay length therefore, while which we previously expressed as λ by, λ times, λ is equal to $\sqrt{D\tau}$, sorry, then of course becomes $D\tau_{eff}$ under root which when we replace the value of τ gives us this expression

$$\sqrt{\frac{D\tau V}{N_{nuc} v}}$$

under root $D\tau V$ upon $N_{nuc}$ times the volume of the nucleus.

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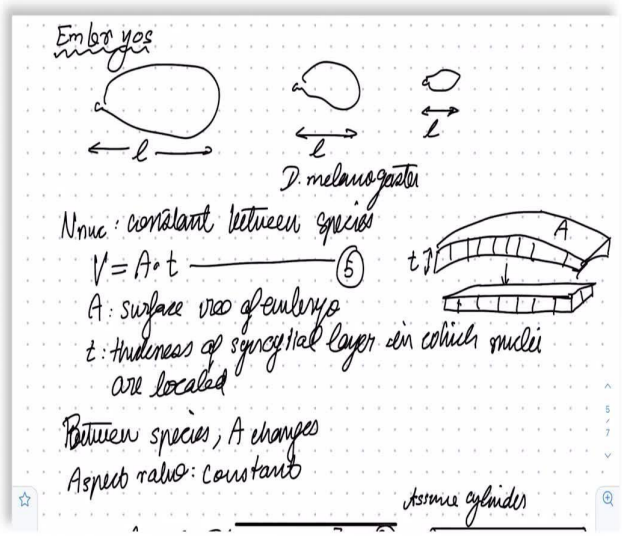


These embryos as we can see are of different lengths. So, somehow we want to incorporate this question what happens when the length, can λ be changed as a function of length? In order to do that, the author Gregor company, Thomas Gregor and company in their PNAS paper, demonstrated that if you now consider that the volume and surface area change with embryos in such a way that volume is A times t.

t is the thickness of a syncytial layer, because the nuclei are actually all over the embryo in this stage, they are in fact, in one single layer, one cell thick layer under the embryonic wall. Between the species area changes, thickness does not and the aspect ratio remains constant. So which aspect ratio are we talking about, we are talking about the diameter to the length.

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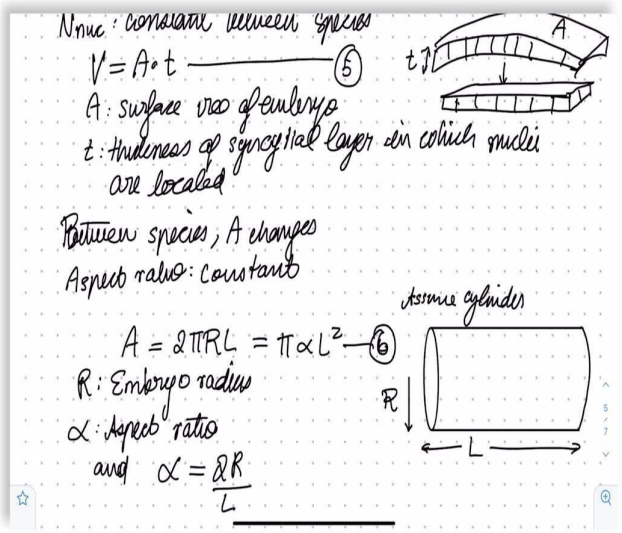


And for this we basically assume that our very nice and unusually shaped drosophila embryo can be simplified into thing being thought of as a cylindrical shaped, cylindrical shape with radius R, length L, L cap and so A becomes $2\pi RL$ which we replace, where we replace R and L by α and end up with the term 2R/L, which is the aspect ratio, the diameter divided by the length.

And this, in fact gives us $\pi\alpha L^2$. Just note that by, in order to get L in the denominator, we have to multiply and divide by L, that is how we got L square.

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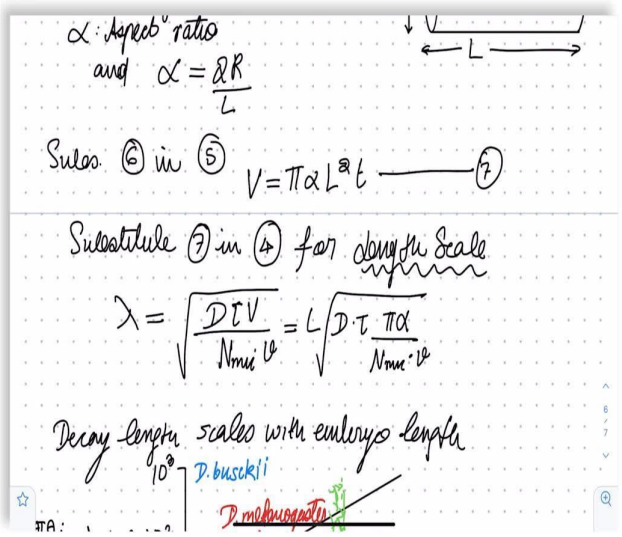

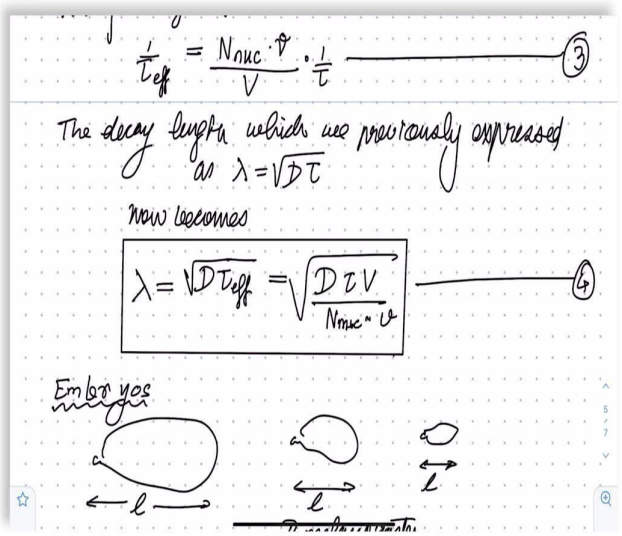


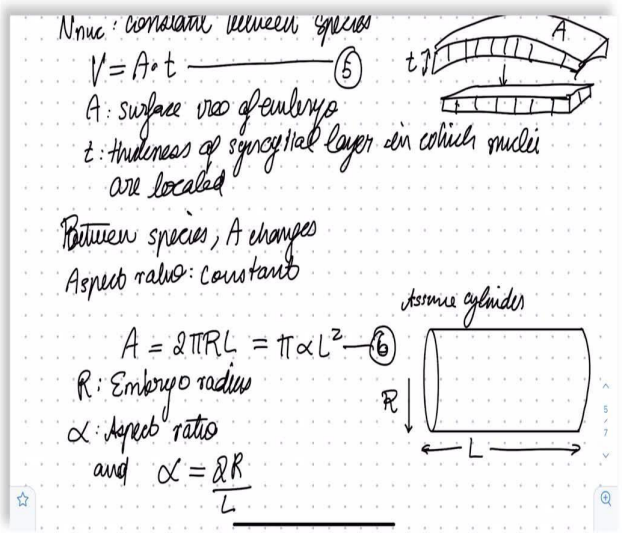


So, substituting this equation in 5, which was the volume term, that is

$$V = A \times t$$

t, the thickness, we get,

$$V = A\pi\alpha L^2 t$$

And now substituting the 7 in 4, which is this equation, the expression of the λ, the scaling length with the number of nuclei and volume of the cell and the nucleus, we end up with something that looks like this.

$$\lambda = \sqrt{\frac{D\tau V}{N_{nuc} v}} = L\sqrt{\frac{D\tau\pi\alpha}{N_{nuc} v}}$$

Lambda is equal to under root $\frac{D\tau V}{N_{nuc} v}$, which opens up if we substitute the terms for V as outside the brackets, outside the square root L, L under root $\frac{D\tau\pi\alpha}{N_{nuc} v}$.

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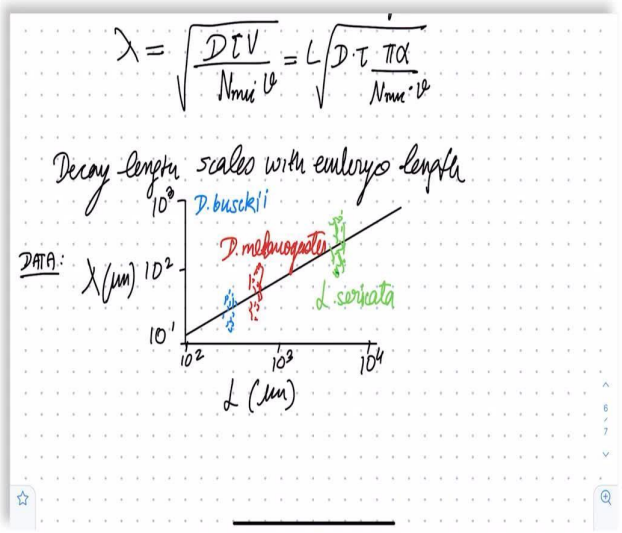


This basically means that the decay length scales with the embryo length. While this is consistent with the graph here, there is of course a tricky part, which is that this graph is a log-log curve. But, at least qualitatively now, we have an expression which allows the scaling of the embryo size and the decay length.

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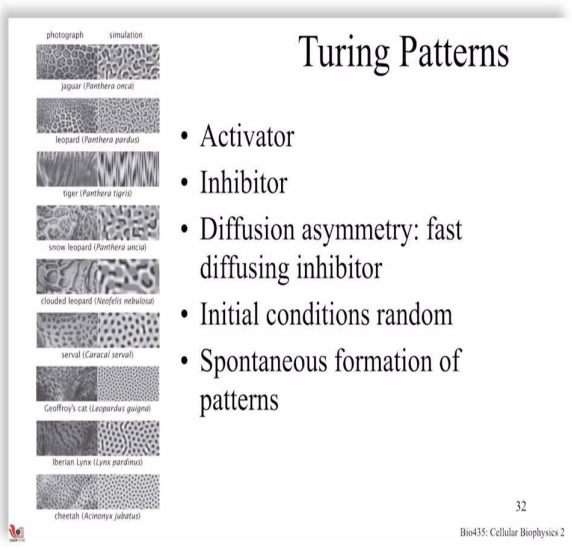


So, with that, I come to the last part, which is the idea that what we have spoken about so far involves reaction diffusion patterns, which are predetermined. Remember, I said at the beginning that the mother lays down the gradient of Bicoid, the Bicoid gradient leads to downstream elements of Hunchback, Krupple and Giant, Giant lead, and all these together,

integrate into the expression of even-skipped, even-skipped, in fact, forms 7 stripes and then this cascade goes on and on.

It is almost like a deterministic cartesian universe. There is a second, at least second possibility for reaction diffusion patterns and how they can influence embryo cell and tissue development, and that is called Turing patterns. These Turing patterns I think those of you have taken the IISER Biology second year course called Systems Biology, we have come across it. I believe they spent quite some time solving the equations of reaction and diffusion.

In essence, Alan Turing was a mathematician who got bored of making computers and hacking German radio communication, and decided to turn to the most exciting topic in the 1930s and 40s, namely, biological pattern formation. So in that, he came up with this fantastic model. And being a mathematician, and applied mathematician at that, he came up with a model that was elegant and simple.

It had only two variables activator and inhibitor, it is out of equilibrium, it involved production of both activator inhibitor and diffusion of both of them, and solved the longer one dimensional boundary with boundary conditions that were periodic. He found that if the initial conditions were random, that these structures can form spontaneous patterns.

In fact, some people have shown in the right hand column on the, over here, you can see simulations that were used to predict the differences between jaguars, leopards, what in Marathi called Bibtia, tiger stripes, snow leopard, coat patterns, clouded leopard, serval, Geoffrey's cat, Iberian Lynx, and cheetah patterns.

So this is amazing. If a mathematical model can produce these kinds of diverse code patterns, there must be something to it. But the strange part is that coat formation, while it can be simplified phenomenologically into a reaction diffusion pattern, we know that there is a lot more going on. So is there any way we can at least try to find a mechanism where actually we find an activator and inhibitor. Turns out after decades of search, one system showed that.

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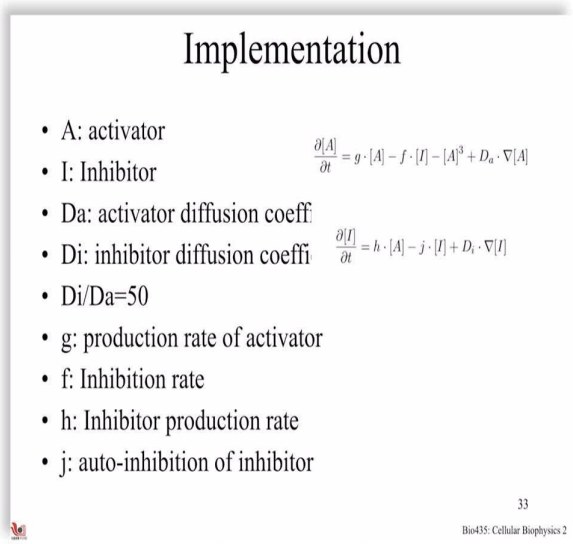


And we will come to that in a bit, but if we are to look at the basic mathematical implementation, or a numerical implementation to that, then we need activator variable 1, inhibitor variable 2, and then parameters activator diffusion coefficient, inhibitor diffusion coefficient, a ratio of the inhibitor to activator diffusion coefficient of about 50.

In other words, the inhibitor moves faster than the, is more mobile than the activator or you can say activator is slow moving or somehow hindered in its mobility. And g, f, h and j are parameters of production of activator, inhibition rate of inhibitor, inhibitor production rate and auto inhibition of inhibitor.

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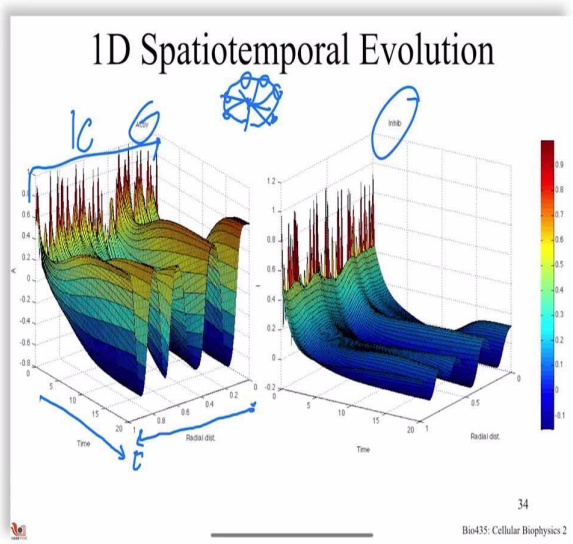

So under such circumstances, the 1D spatiotemporal evolution, so what you are looking at is a 3D plot. This is our time axis, and this is a spatial domain. This is a little flipped, but it does not really matter. We are going in a radial direction, this equation is solved in such a way that the idea is that the grid cells are located on radius.

And in such a situation, please note that the initial conditions are random. And in the case of activator, as well as the case of inhibitor. And in such random initializations, and evolution in time, for the right choice of parameters, we indeed see spontaneous emergence of steady state peaks. But what of it?

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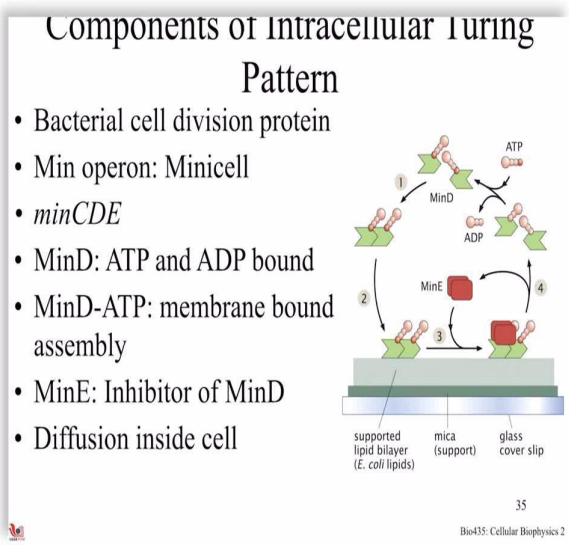


So turns out that decades of experimental biology and genetics, it was found that in *E. coli*, bacterial cell division protein, which belongs to the Min operon, Min, because mutating any of those three proteins results in mini cells, tiny E. coli cells, tiny here and they should be in the wild type then this operon, minCDE produces a MinD, which can be ATP and ADP bound and when it is ATP bound, it is also immobilized on the membrane, or slower in its mobility.

And the same time MinE is produced by a same operon, which is an inhibitor of MinD and removes the MinD if it is found to be bound on a lipid bilayer, resulting now, in a kinetic and a oscillatory behavior, where MinD attaches to ATP, binds to the membrane, and then the moment it does that, if it has any MinE around it will result in its dislodgement and its release of ATP in exchange for ADP, which of course then starts the cycle again.

So together with this activator and inhibitor relation, there is also diffusion that couples all these, because all these proteins are diffusible both in the membrane as well as in the cytoplasm. And, and this might set the stage for something that we had like to probably examine.

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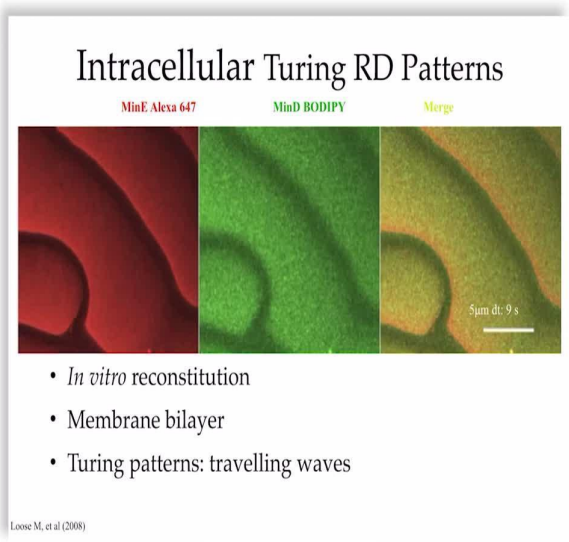


And it turns out when taking all of these ideas on board, a group in Munich that of Perta Schwille, she and her coworkers assembled a lipid bilayer, where MinE was labeled with Alexa 647 dye, minD with green dye called BODIPY and they were merged, what they saw were these amazing pretty time series of the evolution of traveling waves of MinD and MinE. And now, one can, of course, admire these patterns, but one would really like to know what is driving them.

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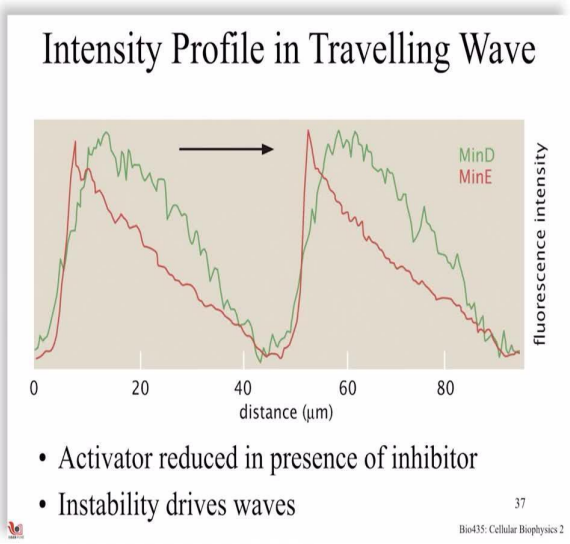


And when we look at the density profile at any given time point along a certain axis, then you see that the MinD and MinE are offset. They are not always colocalized although by eye it might have actually appeared like that. The activitor reduces in the presence of inhibitor, and instability drives waves, this is seen in terms of where the profiles peak.

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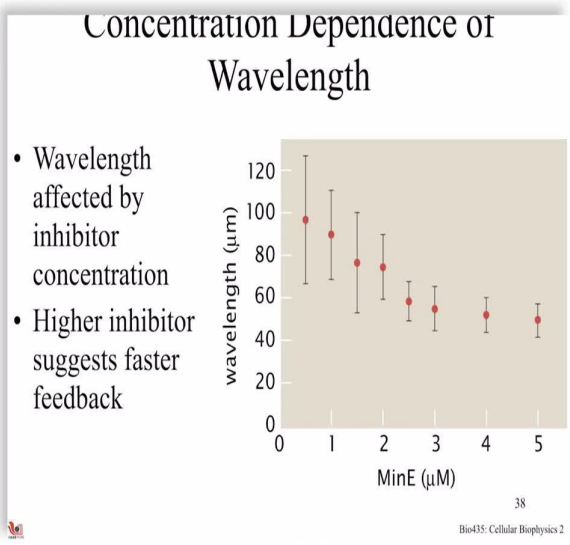

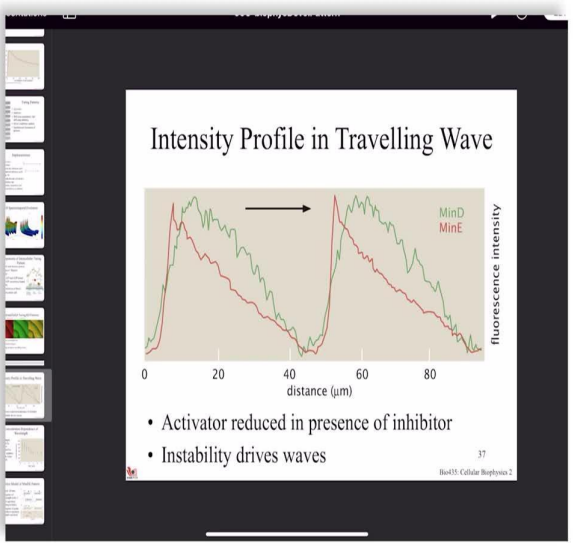


So when you do all that, then it turns out that there appears to be a concentration dependence of the wavelength. The wavelength is nothing but the distance between the peaks. So over here, the distance between the peaks, let us say, of MinD is between 10 and 60, so it is about 50 micrometers.

And 50 micrometers corresponds to one of the lower concentrations of MinE, I am sorry, the highest concentration of MinE. And, and this would suggest that in fact, as MinE becomes more prevalent, the higher inhibitor produces a faster negative feedback into the system removing any MinD that is present, making it more rapidly oscillating in space and time.

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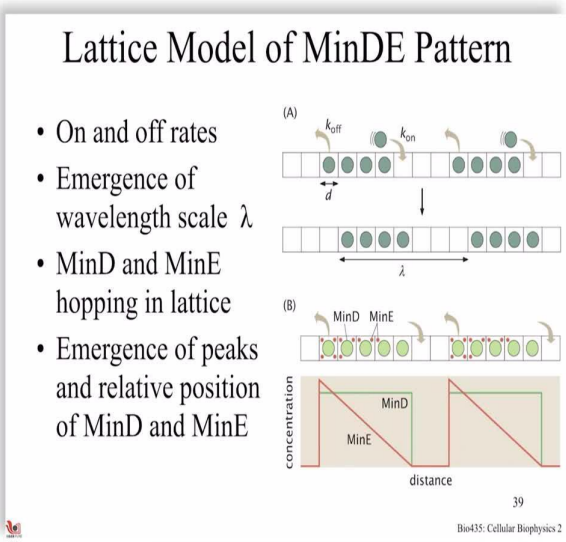

One can of course, write down to a lattice model for these things with an on and off rates and lattice size of d. And the fact that the MinD, and MinE put in the offset in the concentrations comes straight out of that.

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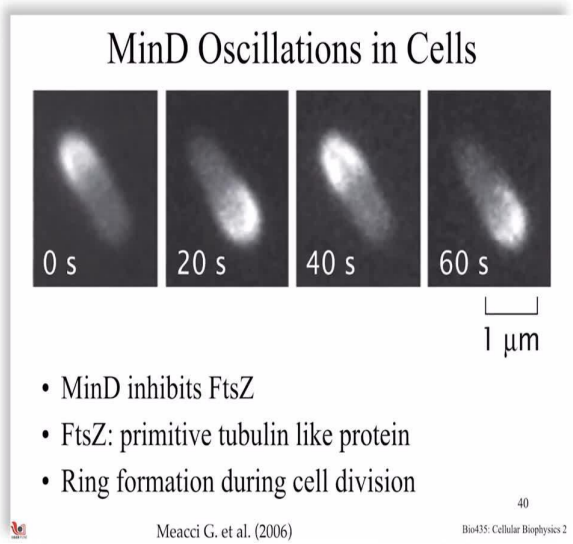


In fact, looking at intact cells with MinD, one can also observe oscillations. MinD itself inhibits FtsZ, which is a primitive tubulin like protein, form a ring, FtsZ, Z ring or something, you call it, during cell division.

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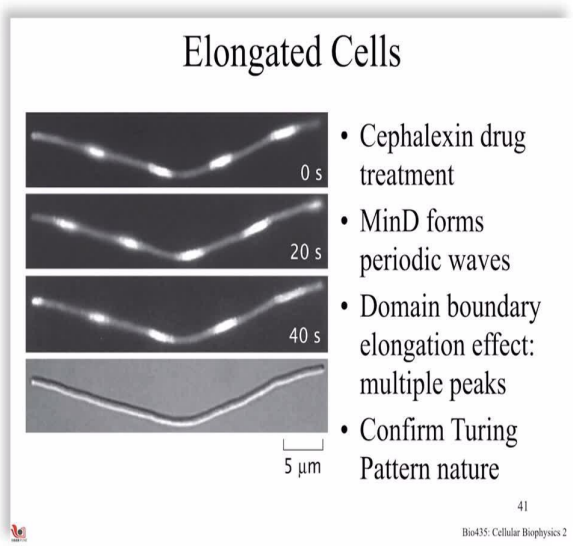


And in fact, when you elongate the E coli cells by another treatment, in this particular case, Cephalexin, then you can find that the MinD protein concentration is almost like steady state

wave all through the embryo. And this is quite exciting. Because if you notice my rough calculations at the very beginning, in our longer domain boundary, calculations demonstrate that they will result in the steady state multi-peak localization pattern.

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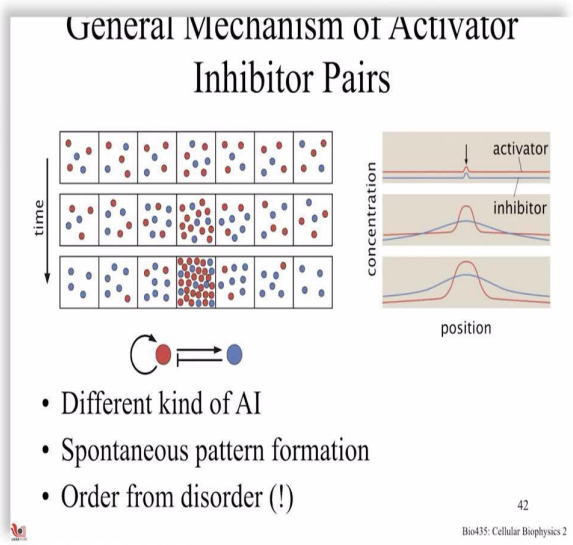


Indeed, one can argue that activator, inhibitor systems are very general. And they can also be employed in oscillatory systems, in cell cycle timings. And we talked a little bit about this very, very briefly in peripheral but if you go back and look, at systems biology textbooks, will find a heck of a lot more about this.

So this kind of activator inhibitor system which I like to think of as a different kind of AI approach, results in spontaneous pattern formation, which in fact, almost resembles this paradoxical order from disorder. But suffice to say, it is auto equilibrium system, which is why it is white.

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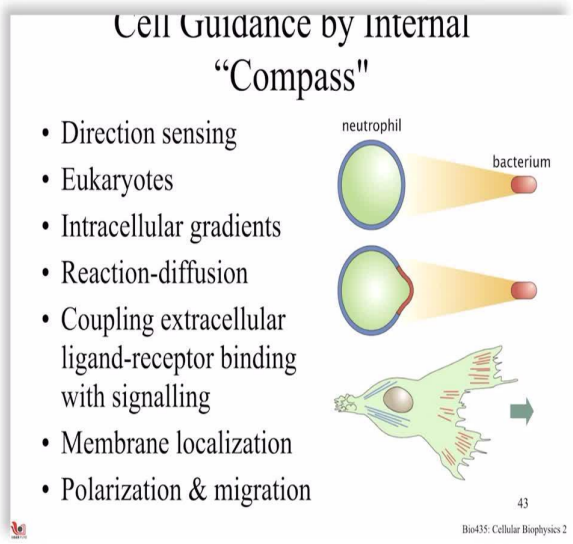


And these things are not just found in bacteria. So neutrophil guidance towards the bacterium to swim and eat it up the kinds that we saw at the very beginning of this course, when we talked about the motivation. In terms of the role of cell guidance and immune cell monitoring of bacteria in the system, or epithelial migration of a gradient during morphogenesis.

All of these results from the coupling of reaction diffusion systems inside the cell with the extracellular ligand receptor binding and signaling, producing membrane localization, and act in assembly, and so on. And so this is something that Ankita Jha spoke in the guest lecture two weeks ago.

So I would say that all these concepts that we have talked about so far, reaction, diffusion, rate constants, cell mechanics, cytoskeleton, lipids, microtubules, all of these integrate to kind of give us this cellular behavior, which we are looking at.

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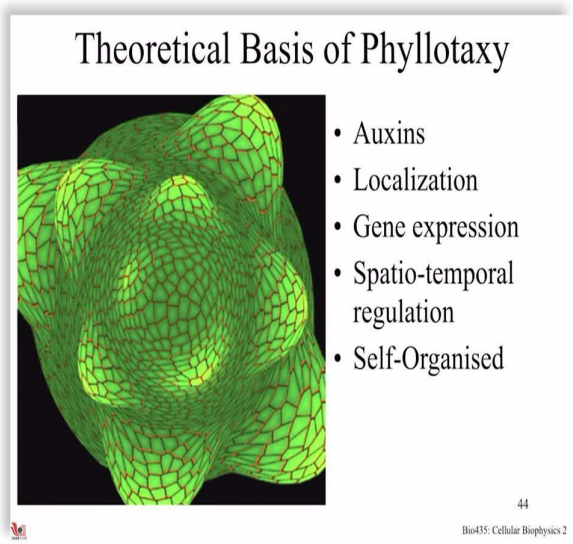


And finally, I am going to just make a little announcement that all these things are now finally allowing us, all these concepts are finally allowing us to understand the theoretical basis of phyllotaxy. Now, those of you who remember your high school plant biology, also remember that plants seem to form localization patterns of their leaves, which follow Fibonacci's series, the addition of two terms followed by the third term and then so on and so forth.

Now, this is mathematically quite nice and it is a beautiful little number puzzle. But the problem is that we know much more than 2020, 2021, about auxins and their role as plant hormones in producing plant like, shoot growth and so on and so forth. So the real question becomes how do auxin localizations get affected by these kinds of reaction diffusion patterns, or developmentory patterns or combination of those in order to regulate the temporal dynamics of development of plants.

And this, in some senses, is the topic that I would love to discuss, at some point if we ever come back for a developmental biophysics course, but for the moment, I am going to leave you to make your own readings. This is more or less now, the end.

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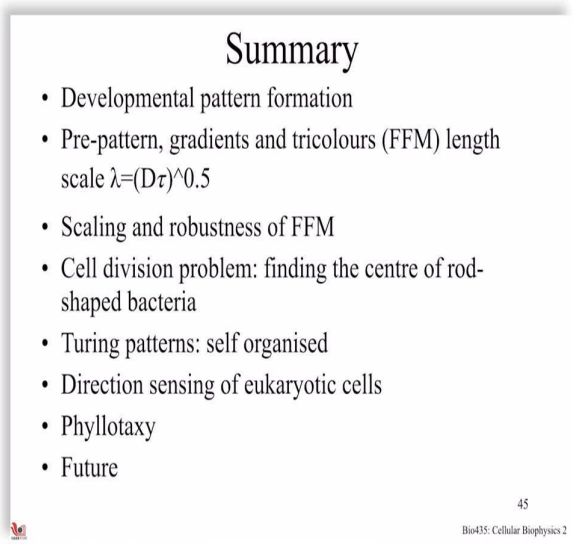


So to summarize, we talked about developmental pattern formation. We said pre-pattern gradients and tricolour, tricolours that is the French Flag Model predicts the scale length, scale of diffusion and degradation model with a $\lambda = \sqrt{D\tau}$ . But the scaling and robustness suggests that there is more going on and that you, if you then further include the role of nuclear localization of these proteins, you can probably predict the length scaling across species.

And so it is a modified French flag model and in fact certainly, going beyond and above what Lewis Wolpert thought of initially. The cell division problem that we spoke about, discussed the finding of center of rod shaped bacteria cells, using a spontaneous reaction diffusion system, or self organized one, where traveling waves in a one dimensional systems seem to find result in a concentration minimum of minD which inhibits FtsZ, which is the primitive cell division protein, which therefore then results in cell division at the center of the cells.

It is interesting to note that MinD oscillations are not necessary for cell division in bacillus, but they are in *E coli.* Nobody knows the answer. I think these are general problems. Scaling or mapping of these standard models to other organisms, ironically, is still a problem in bacterial cell biology, and biophysics. And anyone interested in reading more about it, you are welcome to do so.

So Turing patterns themselves are self organized. They are also involved along with these bacterial cell division systems in sensing of eukaryotic cells of their environment. I have not

talked to you about phyllotaxy. Suffice to say there are new models that have successfully managed to explain how the regularity of plant shoot growth happens.

And for the future, I am going to say that the frontier for biophysics at a cellular scale is probably in two directions. In terms of understanding whole cell behaviors, cell physiology, and of course, going to even higher scales of tissues and tissue morphogenesis in terms of the developmental context.

So I would like to thank you for listening. Thank you.