Introduction to Dynamical Models in Biology

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Lecture 14
Bifurcation in Biological Systems

Hello, welcome to the second module of this third week of our course on interaction to dynamical modules in biology. In the last module we have introduced the basic concept of bifurcation and in this video and this module we will discuss some application of those concepts or bifurcation to understand certain biological phenomena. By concept of bifurcation can be used to understand different phenomena at different levels of biology, for example it can be used to understand population biology, it can also be used to understand molecular process of a phenomena in molecules inside the cell, at cell level also I may act like bifurcation concepts. In this module I will focus more on cellular processes rather than as a whole population behaviour or ecosystem problems of ecosystem and I will focus more on different concept of bifurcation, application of those concepts to understand certain biological phenomena at cellular level.

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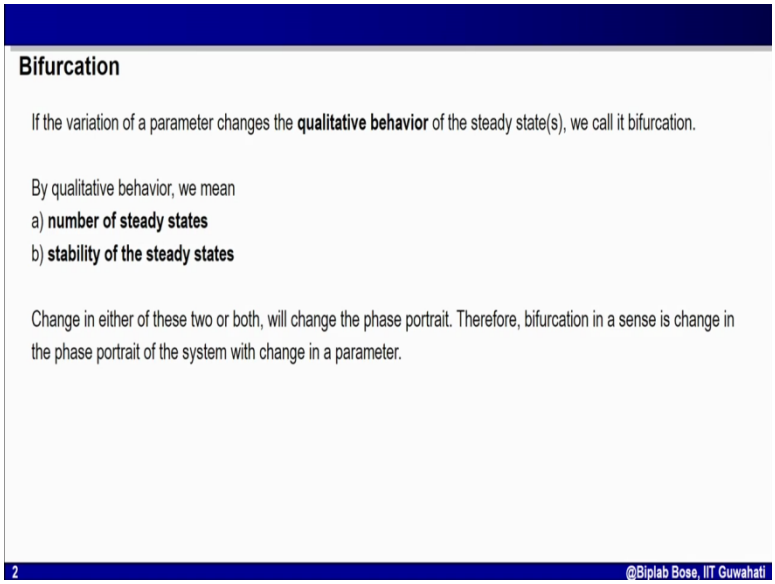

So before we jump into those discussion let us just try to recapitulate what is bifurcation, so if you remember we are trying to understand the dynamics of a system in terms of one or more ODE's and in this ODE's you will have a dependent variables and independent variables, the

independent variable is time and apart form that there will be parameter which remain constant with respect to time and in a system there can be a situation where the qualitative behaviour of the system changes as we change the numerical value of the parameter and if you remember by qualitative behaviour we mean that number of possible steady state in the system and stability of those type of steady states. So there can be situations where if I change the parameter value number of possible steady state in the system changes or the stability of the steady state changes or both of them. In all these 3 cases we will say the parameter is causing bifurcation in the system.

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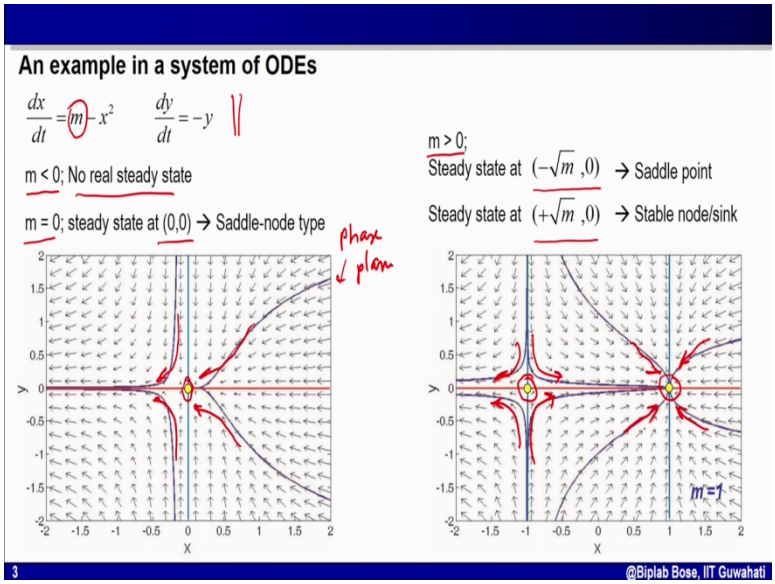

So let us take a old example that we have discussed in the last module, I have a system represented by this set of 2 ODE, $\frac{dx}{dt} = m - x^2$ and $\frac{dy}{dt} = -y$. So as we have discussed earlier if I take m as a parameter and try to check whether it will have any effect on (bi) or it will cause bifurcation in the system or not, I can see that when m < 0 there is no real steady state, so we are not much bothered about that. When $m = 0$ you have 1 steady state that is (0,0) and I have drawn here the phase plan, so phase plan is here and you have seen it earlier. So you have these (0,0) the yellow dot which is a saddle note here on the side trajectory moving away whereas from this side the trajectory collapse.

Now if I change m and make it bigger than 0 there is a positive values then I will have 2 steady state, $(+\sqrt{m},0)$ and $(-\sqrt{m},0)$, in the phase plane you can see them, these 2 are the steady state, possible steady state when $m > 0$ and one of them, this one is a saddle node, it is complete obviously unstable whereas the other one is actually a sync, stable node. So you can see, if you remember what is happening here, as I am changing the parameter m, I have multiple different number of steady state and their stabilities are also changing so I will say this system has bifurcation with respect to the parameter m.

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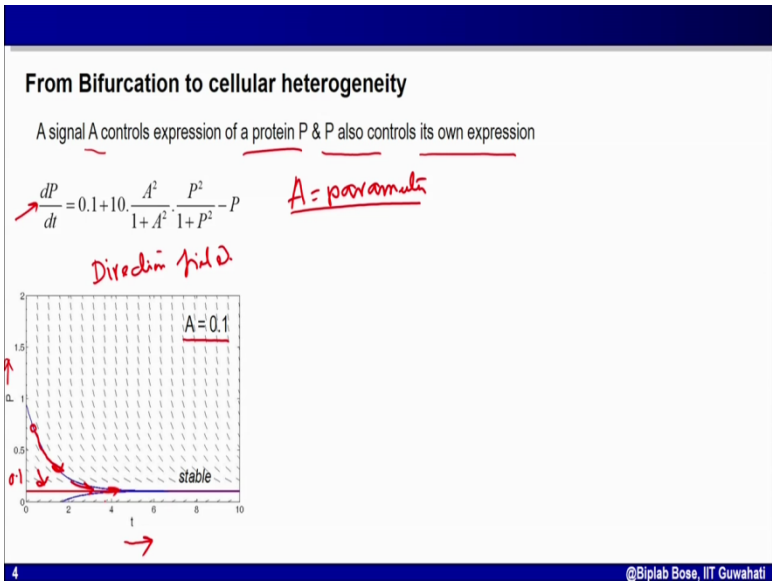

Now let us try to use this concepts on a biological process, imagine there is a protein P which whose expression is controlled by external signal A, it may be like that you have a plasmid, you are going to introduce your plasmid inside a bacteria which has a lac operon and you can induce the expression of that gene under that lac operon by giving IPTG from outside, that can be your experimental system, so that type of system happen in naturally in normal cells also. So you have a signal A which controls expression of a protein P and there is another issue here, P also controls its own expression, so it is just like a positive feedback.

I will not go in details about how to write the ODE for this system, I have written down the ODE and I will not for the time being will not explore different issues why I have written that type of ODE, plus look at the ODE that I written here to model the dynamics of P with respect to time

how P will change its time. $\frac{dP}{dt}$, that is the rate of change of concentration of P, so that is the ODE, I am writing $\frac{dP}{dt}=0.1+10.\frac{A^2}{1+A^2}.\frac{P^2}{1+P^2}-P$. Obviously minus P part is for degradation of the protein you can easily assume.

Now let us look into the behaviour of this ODE, now when I say I want to understand the behaviour of the ODE, most of the time I want to understand the steady state behaviour of the ODE. Now in this case P is a dependent variable, P obviously is an independent variable and A is a parameter. So I want to see the behaviour of steady states of P or the behaviour of P with respect different values of A. So I will use the concept of direction, so I have drawn the direction field here, I hope you remember, what is directional field, in direction field I have T in the horizontal axis and P the concentration of the protein in the vertical axis and I have this whole plane I have divided in grid and at each I have drawn a arrow, here the arrow grid has not been shown there sonly, the line has been and the slope of that each line at each grid point is equal to the derivative of P with respect to time $\frac{dP}{dt}$ at that position.

So in this way you can see these red line shown here is actually a steady state and in this case I have taken A = 0.1, so when I take take A = 0.1, the system has only 1 steady state here the way I have shown the red line and it is horizontal that is why and the looking at the direction field you can easily see that these steady state is actually a stable steady state. The steady state maybe around 0.1 something and it is stable because if I start from suppose here I collapse with time, follow the strategy and collapse the steady state, if I start from lower value then also I move up and which is that steady state, so from both sides I am reaching towards the steady so obviously this is stable steady state but notice the value of P is very low, very close to 0 i.e 0.1.

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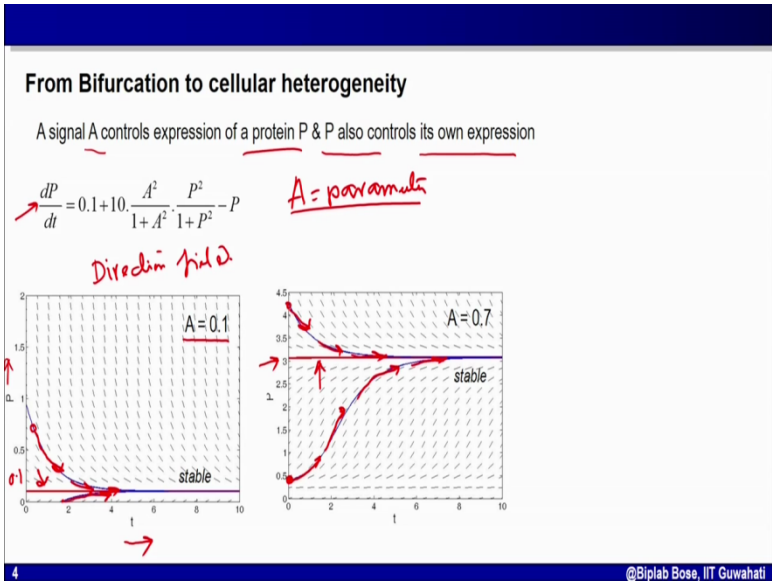

Now what I will do, I will change the value of A to higher value, so let us take A = 0.7 and again I have drawn the direction field with P in the horizontal axis and P in the vertical axis, you can see here again I have only 1 steady state that is shown by this red horizontal line and the value is near 3. So now the steady state has increased its value, it is at higher value of P and in this situation also if you look at the direction field you can easily understand that this steady state near 3 is also at stable steady state because if I start at a lower value suppose at t = 0 I am starting at this position then the trajectory will be like this and ultimately collapsing at the steady state.

If I start at a higher value from the steady state again I come down along this trajectory and collapse at the steady state, so from both the direction I am collapsing, I am moving, with respect when I am moving time, I a moving towards the steady state, so it is stable steady state. So what I have observed in this 2 direction field that for a lower value of A that is the input signal, lower value of input signal I have a stable steady state whose value is lower around point 1 or something whereas when I increase A value to higher like 0.7, I have a another steady state which is stable but its value is higher around 3.

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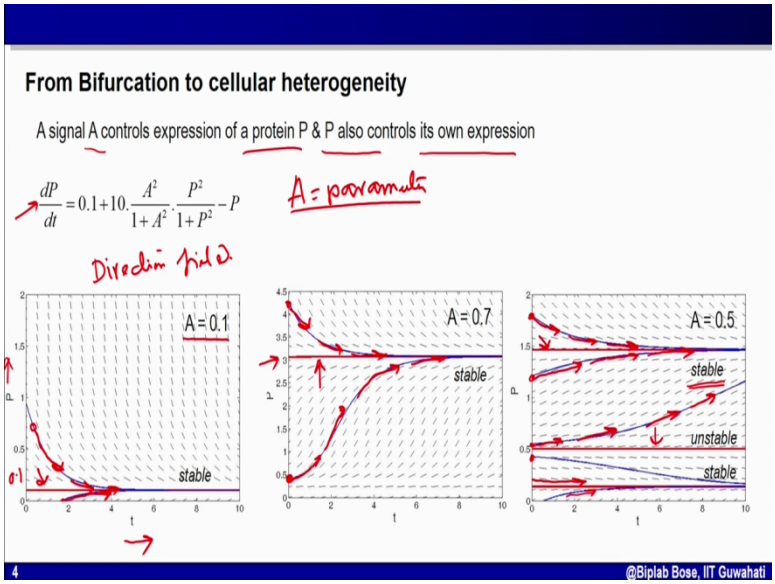

Now take us A value in between for A and what I have drawn here is A = 0.5, now notice the direction field, now we have 3 steady states, here near 1.5 another one is here around 0.5, another one around 0.1, so I have now as I have changed the value of A to 0.5, I have all of a sudden not 1 steady state but 3 steady states. Look at the stability let us follow the direction field to understand the stability, if I start from this higher value suppose 1.7 or 1.8, the direction field arrow says that I will move along this trajectory come down and collapse at this steady state near 1.5. If I start slightly lower value I will follow this trajectory and move up and eventually with time essentially reach the steady state, so obviously this steady state is a stable steady state.

So a steady state near 1.5 is a stable steady state. What about the middle one, near 0.5, if I start near it but not at that steady state, the trajectories are telling me that I am moving away and going towards the higher steady state, if I start slightly lower than the trajectory that I am following is going down, so that means at in this steady state if I slightly deviate from this steady state I divert, so obviously these are instable steady state.

What happens for the lower one let us clean a bit to understand what is happening, the lowest steady state which is around point 1, the trajectories are such that if I start from a higher value actually I will collapse, if I start from a lower value I will again collapse to that steady state, so that means this lower steady state, the third steady state in this system is also stable because from both the side with time I am moving towards the steady state, so what I have observed in this 3

direction field, when I have a lower value of A at 0.1, the signal is 0.1, I have only 1 steady state, when I move to a higher value of 0.5 A equal to 0.5 the external signal equal to 0.5 I have 3 steady state out of which 2 are stable 1 is unstable, the middle one is unstable. Then if you further increase A to 0.7 then again 3 disappears and only 1 steady state comes and that had a higher value and it is a stable steady state.

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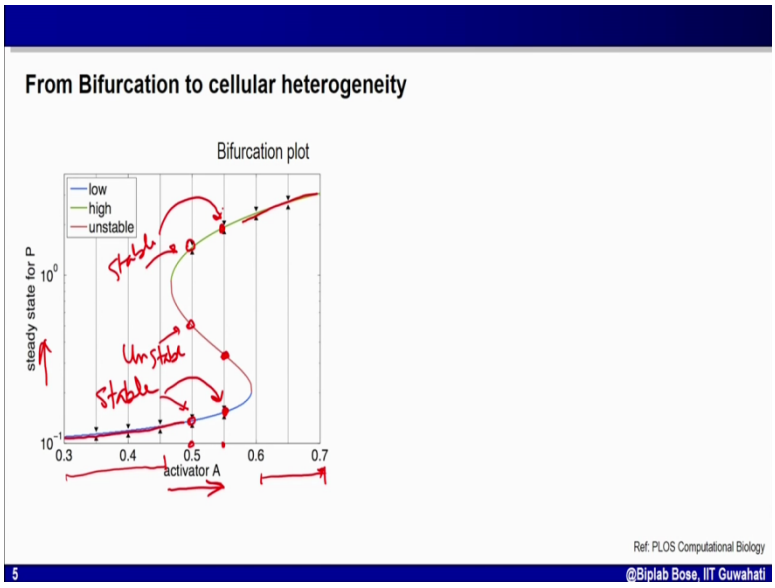

So I can compile all this data and draw something which you call bifurcation plot and that is what I have done here, so if you remember in the bifurcation plot usually we put the parameter which is causing bifurcation in the horizontal axis and the steady state value, remember the steady state value of the dependent variable in the vertical axis. So you have the steady state value of P in the vertical axis here and the activator A or the signal A in the horizontal axis and I can show mathematically, I can draw this one and from the previous data also I can understand easily that when activator A, the signal A is at lower value say up to this range, I have only 1 steady state which is stable and has a lower value, this region, this blue region.

Whereas when I have a higher value I have for like this one 0.6 to 0.7, I have this one, the steady state is stable but its value is higher. In between, in between suppose when I take 0.5 or 5.55 I have 3 steady state, 1, 2, 3 for 0.5 we checked, this one is unstable, this one is stable and this one is also stable. Let us take another value here around 0.55 then also I have a stable steady state

here, I have a unstable steady state here, you can check by calculating the different direction field and I have another stable steady state here.

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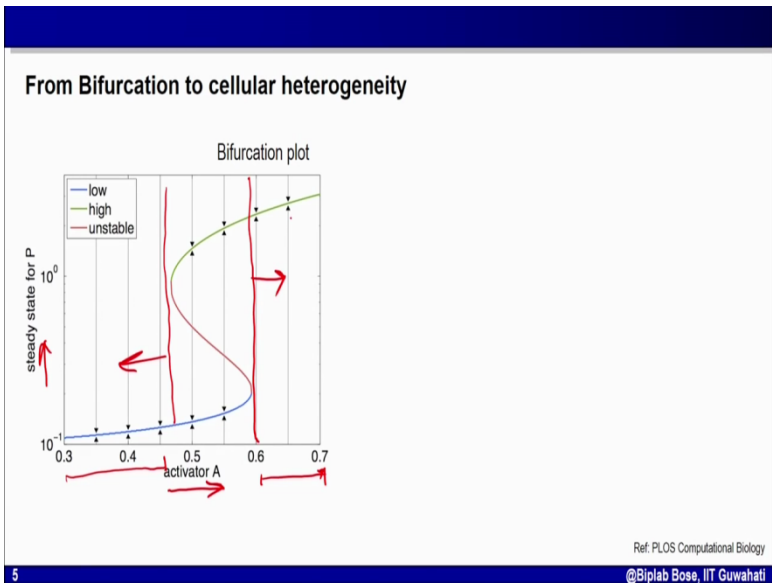

So what we are observing, if I look in the whole map here, if I clean a bit, I can see that depending upon the value of external signal or activator A, I have 3 regimes, 1 regime is here when the system in this regime, I have only 1 steady state, the system has only 1 steady state, P has only 1 steady state and that is the lower value, whereas I have another regime here when A is bigger than this value, I have 1 steady state only for P and that is the higher value, in between this 2 I have a region where depending the for any value of A I will have 3 steady state out of which 2 will be stable 1 will be unstable. So now how does this can affect the behaviour of cells in a population, let us try to understand this.

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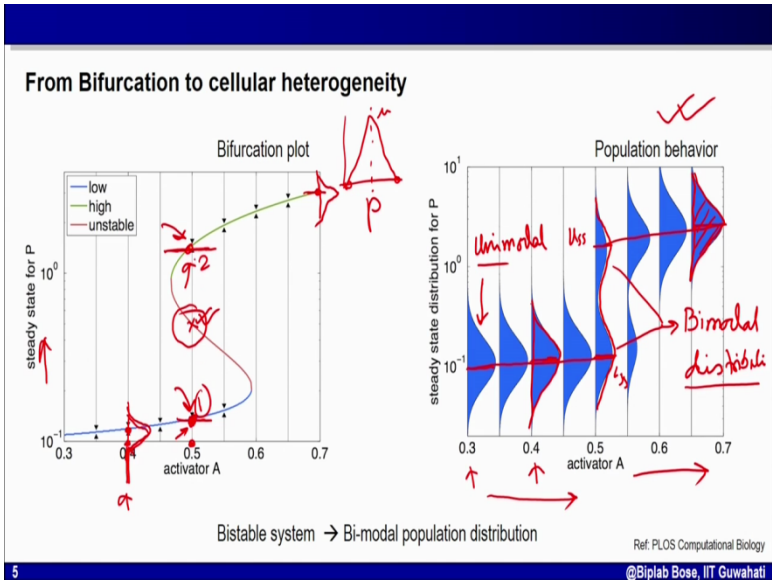

See when I am, suppose I am growing cells in a flask, all these cells are isogenic that means this all cells are genetically identical but that does not mean at any moment of time all cells will have same amount of particular protein, there will be slight variation, so if I can measure the amount of protein P at a particular time point in a in symbol of cell in a population of cells, suppose I have one million cell and if I can measure amount of P in each of this cell then I will not get exactly same value in each cell, rather I will get something like a distribution, suppose this is a protein concentration and I will have a histogram where majority of them has these new value and some of them will have higher value of the protein and some of them will have the lower value of the protein.

So always get a distribution of protein expressed in a set of cell the population of cell and that is what you observe when you do flow cytometry experiment, you don't get a single value of reading for a particular protein in a population of cell rather you get a histogram looks which looks like a invert like a bell shaped histogram. So now imagine, let me clean this part a bit, it will be easier for us to understand, so suppose I am giving a signal A to a population of cell and the value of that signal is suppose at 0.4 here, so then the steady state value of P on an average will be this one because there is only 1 possible steady state.

Now as I said in a population of cell, all cell will not have the same amount of protein but rather I will have a distribution so I can configure this steady state value that is shown in the bifurcation

plot is the mean value and the amount of protein in the population will be distributed around this so I can draw a histogram like this, so that is what is drawn here. So this is the mean value of the protein. Now when you increase the value of the signal suppose from external agent I am adding extra signal and make this signal A to 0.7 then the new steady state will come after some time and all cell will move toward this particular steady state, so at this steady state the average value of protein in the cell population will be this value but again I will have a distribution so all cell will not have the same value but rather you have a distribution around this, so that is what we get here, so the mean value is this one and you have distribution around this.

So when I have a low signal I have a population distribution like this when I have a high signal I have a population distribution like this one. In between what will happen, if I give a signal of 0.5, I have 2 possible steady state, so suppose initially cells were here and the signal I am giving from outside was 0.4 and then I have added few drops of a signal from outside and the signal increase to 0.5 now, not if I allow this system to go to a steady state I will have 2 possible steady state, 1 is here, the other one is here, you have a unstable steady state here but as the steady state is unstable, no cell will go there, so based on the initial value of protein present in the cell, some of the cell will move to this steady state, some of the cells will move to this steady state, no one will move to this unstable steady state.

So I will have a population whose average value will be this one lower state, stable steady state and there will be another sub population which will have average value of protein P in them at these upper stable states. So what I will observe, if I measure protein P in each of the cell and then draw a histogram, now I will have histogram like this with 2 peaks, this lower peak is the average value coming from this lower steady state, so this is coming from the lower steady state and this peak, this is coming from the upper steady state and both of them are stable. No cell will reach this intermediate steady state which is unstable.

So what is happening here, if you follow this population behaviour data when a signal is low I have a population of cell where all the cell amount of protein in this cells are distributed around a single mean and as I increase the amount of A or signal A a situation comes when from one peak I get 2 peaks, so I have now 2 sub population, so the whole population is now divided into 2 part, 2 sub population and again if I increase further towards high value of 0.7, 2 population will merge and a new population will arise which is homogeneous population having higher value of

P. So I have initially like this and then this one. So this type of behaviour is called bimodal distribution, initially I have uni-model population distribution, in between when I have 2 peaks this is called bimodal distribution.

This is called bimodal because you can see there are 2 modes, there are 2 peaks, so what is happening here if I brief, if I have a system in which depending upon external signal I have bi-stability because if you look at the bifurcation plot here I have 1 stable steady state here another stable steady state here, so this system is called bi-stable, so it has 2 steady state which are stable, so if I have bi-stability in the system then if I start with a homogeneous population initially and as a I keep on changing the signal I may get for a homogenous population I may get 2 sub population with a bimodal distribution and again if I keep on increasing the signal, again I may get a homogenous population with higher expression. So from unimodal, I get bimodal then again I get unimodal distribution. So these example shows that a simple feedback circuit the ODE which I have written is essentially nothing but a simple feedback circuit for protein expression P that has bi-stability and that give rise to a bimodal population distribution uploaded.

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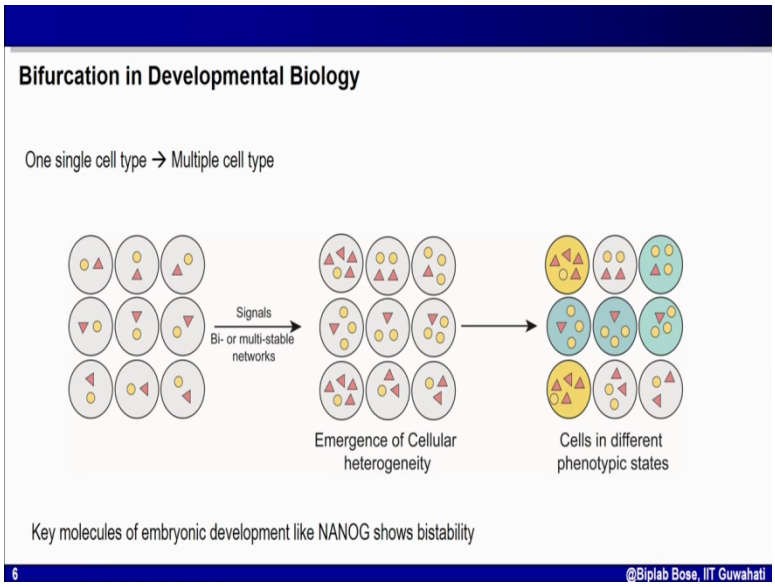

Now let us try to use it further, we know that cells differentiate, you may have a multipotent stem cell from that multi protein stem cell a signal comes and the stem cell differentiate into other type of cell but all cell does not differentiate. If all cell differentiate completely into a new type of cell that will not give this body organisation during our embryonic development. Some of the cell

will differentiate into type A, some of the cell will differentiate into type B something like that, so this type of phenomena can be explained by the bifurcation and bi-stability and bimodality that we discussed just now.

Let us imagine initially I have a homogenous population expressing this yellow protein and this red color protein in triangle shape is a homogenous population. Now these proteins are controlled by a network or circuit which shows suppose bi-stability or multi-stability, so if it is showing bi-stability then as we give signal increase the signal a situation will come where from a homogenous sub population two sub population will only appear, one having higher expression of protein another having lower expression of the protein, so in this case you can see for this cell for example, the red proteins are higher whereas for this cell the yellow protein are higher but red protein are lower.

So what is happening, from a homogenous population where most of the cell has almost close number of red and yellow protein as the signal is given and as the system has bi-stability for both the red and yellow protein I have two population, sub population now, 1 having higher red protein and another having higher yellow protein. So imagine this yellow protein and the yellow protein controls differentiation of the cell, so suppose may be the red protein pushes the cell towards numeral cell type whereas the red one the yellow one pushes it to different cell type, type B. So what will happen, this cells which is expressing more of red now which is a sub population expression more red now will become one type of cell whereas the other cells of sub population which is expressing more yellow will become another type of cell.

So from a homogenous population I get two sub population and their protein expression behaviour are different and ultimately they are moving to different path of differentiation. This type of observation we have made not just theoretically, people have studied this type of behaviour for different molecules like nanog which are key transcription factor involved in differentiation of in mammalian system but even in human, so in those cases we have observed that the network which controls the expression of this protein are bi-stable and at a particular signal with a proper signal these bi-stability can give rise to two sub population of cell having a bimodal distribution.

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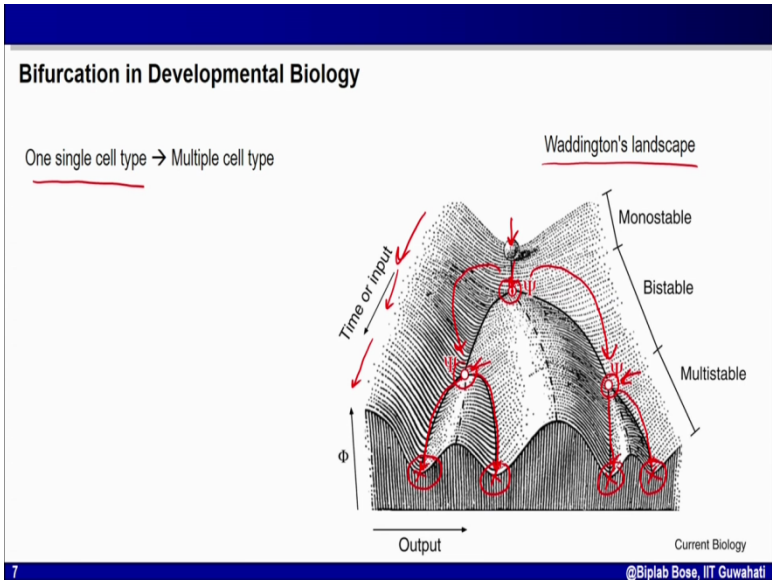

Now these idea can be extended further to understand the embryonic development, we know that we all start form a lump of mass, lump of cell where all cells are identical then this cell differentiate in different path and we get this body organisation, so it is a long standing problem that how this differentiation happened and bifurcation happened that different type of cell originate form one single cell type, what is the dynamical reason behind it? Long back waiting term proposed something called waiting term epigenetic lands, he used the metaphor of valleys and river, suppose you can imagine the whole space has valleys and hills, so initially the embryo maybe somewhere here then as the time progress, different input signal comes, the cells move in this trajectory just like they move on phase plane and they have only one stable steady state here, then as time pass further and input changes.

Now the system may have two stable steady state one here and one here, so some of the cell in the population will move towards this steady state and the other will move to the other steady state, obviously depending upon their initial condition in the phase plane. Suppose there are further down with time and depending upon the input signal the system now can have four steady states, stable steady state here, here, here and here, all this four are stable steady state, so the cells which are here in these particular steady state now either will move towards this one or will move to this one based on their initial conditions and cells present in the other steady state will either move to this steady state and this steady state.

Each of this steady state suppose represent one particular type of cells, so using the concept of bifurcation we can easily understand that from a one single cell type as the time progress and the input signal changes depending upon the input signal or the parameter value the system goes to multi-stable or bi-stable situation and as you go through bi-stable or multi-stable situations cells will bifurcate and then will move into one of this stable steady state and they will differentiate into different cell types.

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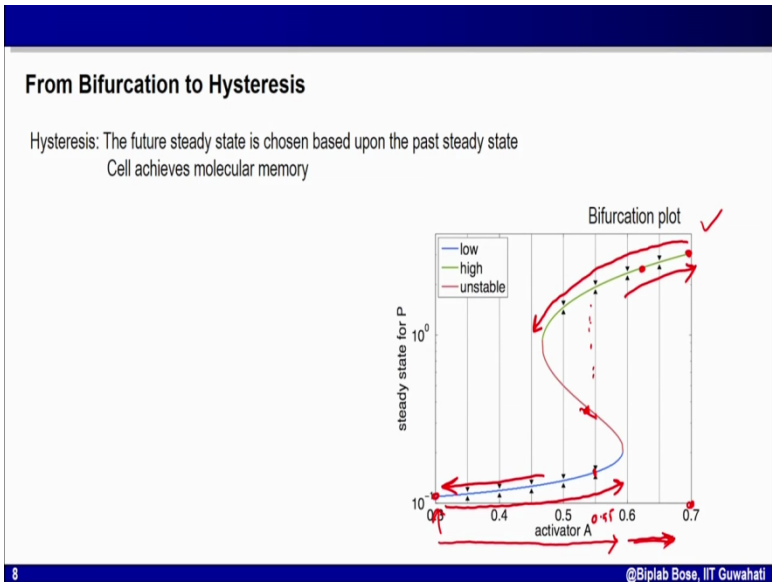

Let us look into another interesting thing that can happen in cell biology due to bifurcation. I have drawn a same bifurcation plot here that we discussed some time back, suppose initially I am treating the cells with a signal 0.3 then if I allow the system to reach steady state, the steady state value of P will reach here then I add some more amount of the signal A or activator A and suppose the total concentration becomes this one 0.55, so now if I allow the system to reach steady state by waiting for some time, then cells will have three possibility, the system will have three possibilities three steady state.

Obviously the middle one is unstable, the system will not move there but the system has option to either stay in this lower value or to jump into the higher one. Using common sense and if you draw looking into the direction field or in case of a more than one ODE, you can easily understand that as initially the system is in the lower steady state, this lower blue line, it will try to stay at this lower steady state, it will not jump into the higher one, this will happen till I reach

this point near 0.6. So in this whole range up to this value of A up near 0.6, the system will always remain on this blue line although for some value of A it has a chance to go to higher value but if you will not go there because of the phase plane or direction field it is far away, so the system is already in the lower steady state, so it will stay in the lower steady state.

If I increase the value of A further there is no other option but the system will jump to the upper steady state in the green line, so if I keep on increasing the value of A towards 0.7 the system will jump along these green line and may reach to this highest value. So the trajectory is initially it will move like this and then it will move like this. Now reverse the process, suppose already the cells are at this higher steady state and the signal is also at 0.7, I reduce the signal, I change the media or I do something so that the amount of A in the culture media decreases so that the cells will see less amount of the activator and allow the system again to reach steady state. So where it will go, obviously it follow this green line and drop somewhere here then if I reduce it further it will go somewhere here.

Now if I reduce A further, then suppose I reduce A to this value at 0.55 so at 0.55 again the system has two option, either it can stay on this green line which is a stable steady state or it can also jump to this lower one which is also a steady state on the blue line. So in this 0.55 again the system has two steady state which are stable, one is the higher one, one is the lower one, the question is will it divide or it will jump to lower one, in fact if you look into the direction field or phase plane plot for this type of problem you can easily understand that as the system is already on a stable steady state it will not jump into the lower blue line rather it will remain in this green line.

So what will happen, when I am decreasing this values of the activator A from higher value, it will follow the green line up to this and then it will have no option, so it will follow this path, the lower blue line, so if I draw it with clarity when I increase the value of A up to this value, near 0.6 from 0 from lower value to 0.6, the system will stay in the blue or the lower steady state and then it will follow this path. So my path is like this and then jump and increase, when I start form a higher value of the steady state and decrease the signal or activator A.

It will follow this path and then it will jump to this lower one and if I decrease A further it will go follow the blue path. So you can easily see here, the path followed by the system when I am increasing A from lower value to higher value it is different from the path followed by the system

when it is coming back from a higher value of the signal A to a lower value of signal A. This typical behaviour of two different path is called hysteresis, hysteresis you may have seen in case of polymers in other materials and discussion, there also the paths of change in physical properties of those material are different depending upon how you are applying the force. So here also as I increase the input signal, the system follow one steady state path and when it decrease signal from a higher value it follows another path.

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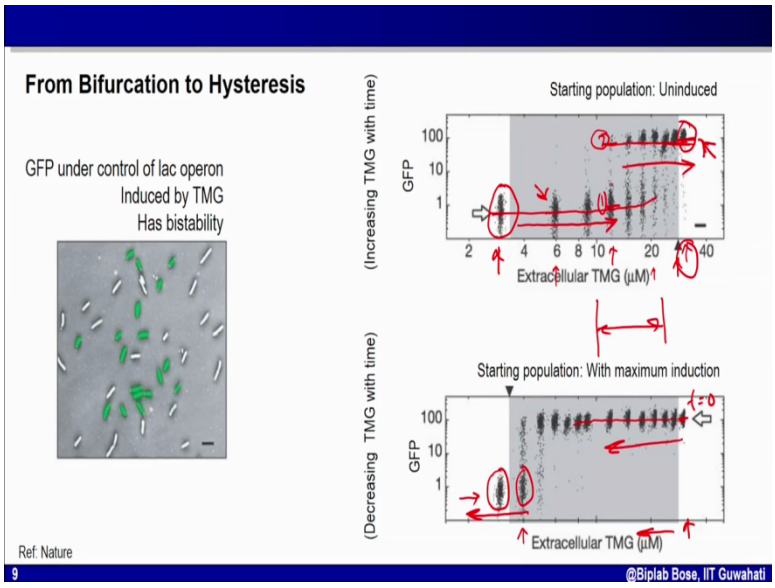

Now let us look into a concrete example of this experimental system and I have taken this from a general paper in nature, so what they have, they have taken bacterial cell and have introduced a plasmid system there which will express GFP and the GFP is under the control of a network of positive feedbacks, I will not go in detail of that, in a sense the GFP is controlled primarily by a lac operon, so from outside I can give IPTG or TMG to induce the whole system and GFP will be expressed and the figure here shown GFP expressing bacteria and if you remember all cell will not express same amount of GFP based on the same induction value because I will always have some amount of difference in the expression of protein from cell to cell. So what they did, they did a very simple experiment, when the consent they used initially, they took cells which are uninduced, so those uninduced cell has very low expression of GFP and you get very low value of GFP intensity.

So then they keep on increasing the amount of TMG that is a inducer in this flask, so initially they have added this amount of TMG that may be 3 μM and then they measure the GFP intensity in a population of cell, so here the data is shown as dots, each dot represent one cell, you can see on an average the value of intensity is somewhere here. Now after sometime they increase the amount of TMG in the system to 6 μM and allow the system again to reach its steady state then I get this data. Still the average value on an average, the value of steady state value of the GFP is at a lower steady state value.

They kept on doing it after 10 μM you can see I am they are getting two population, population 1 here second population here, that means the system is reaching the zone where I have bi-stability, one stable steady state is here, another stable steady state is here. As they keep on increasing amount of TMG you can see two population distinctly arise and then at these arrow after that when they increase TMG further, we have only one population here which has a higher steady state.

So what is happening, they took uninduced cell and they started adding TMG to reach a particular value of TMG concentration and allow this cells to reach a steady state and measure the intensity of florescence of GFP in each of the cell and plotted the data and you can see initially the system remain at this lower steady state and then bi-stability arise and majority cells are in the lower one then slowly they move to the higher one. Now what will happen if I take this cells which are already induced with high amount of TMG, so already I have increased TMG in the system, so already I have a population which is inductive high amount of TMG around 30 μM.

Now if I start reducing the amount of TMG in the system that is the input signal I am reducing the system what type of profile I will see, that is the experiment started with, so here you have the starting point t = 0 where cells are induced with high amount of TMG around 30 μM. So they have high amount of expression of GFP as they fall, decrease the amount of TMG, you can see the cells are still remaining at the higher value, they are not dropping in any lower value of steady state value of GFP although they have reached the region of this region where bi-stability is possible.

So as they started with a higher value of GFP expression, higher steady state of GFP they are staying there till these value of around 4 μM of TMG where the lower steady state GFP cells

appears and here at the end I have only one population having the lower expression of GFP. So you can easily see when I am increasing TMG my path is this one, when I am decreasing TMG my path is this one. So this two path are distinctly different that means this system has hysteresis, the cells which were induced slowly from lower level to higher level and eventually have seen large amount of TMG they remember the molecular information.

And when you try to reduce the amount of TMG, they don't fall back to lower steady state value but they remain at the higher steady state value of GAP for prolonged period and then eventually when they go out of the bi-stable region, they drop down to lower steady state value, so here I have a very simple example by which bi-stability give rise to hysteresis and hysteresis is nothing in this case but a form of molecular memory.

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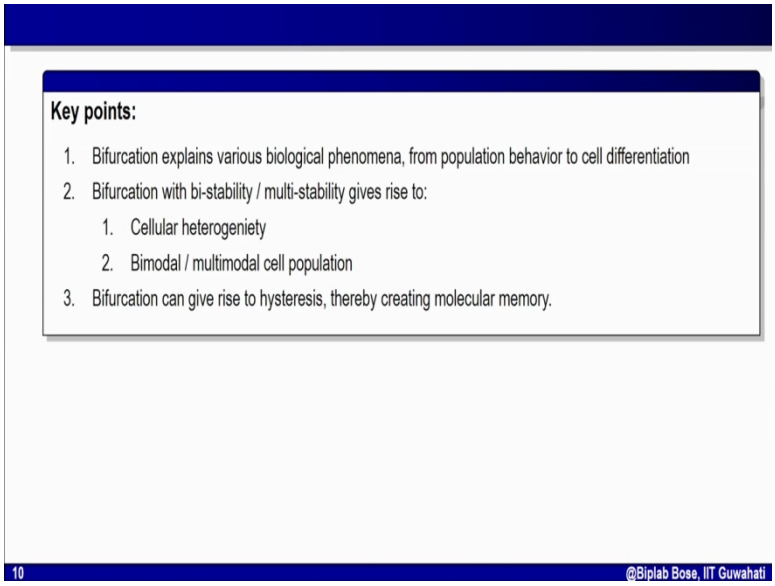

Let us look in jot down what we have discussed in this module, first we have discussed that bifurcation can happen in many system in biology, I have focus mostly in case of cellular system, for example when a particular protein is controlled by positive feedback or I have hysteresis in the system. Now bifurcation with bi-stability and multi-stability can happen, in case of bi-stability you have two stable steady state, in case of multi-stability you have multiple stable steady state.

Now this is a bi-stable or multiple stable system can give rise to cellular heterogeneity, we have discussed that and this type of cellular heterogeneity eventually may give rise to a bimodal or

multimodal cell population depending upon the number of possible steady state available to the cells and finally we have discussed that bifurcation can give rise to hysteresis and hysteresis in a way give rise to molecular memory in cells, that is all for this module, see you again.