Introduction to Dynamical Models in Biology
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Lecture 23
Modeling Transcription Circuits - 2

Hello, welcome to module 5 of 4th week of our course. In this module we will built a model for a small genetic circuit as we have done in the earlier module.

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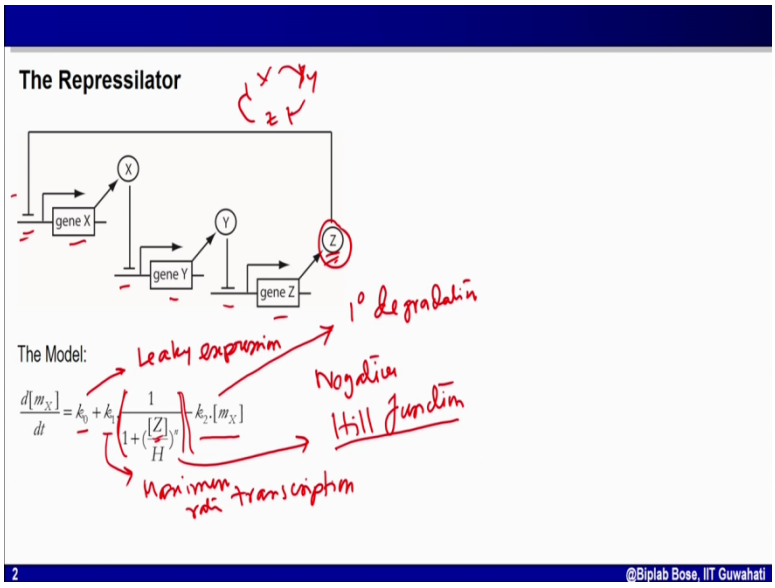

Let us look into the circuit first. In this circuit we have 3 genes x, y, and z. In the earlier one we had two X and y. Now in this module we will have a circuit made up of 3 genes x, y and z. In the earlier module where we have built a repressor of repressor so, X was repressing y and y was repressing x. In this case is repressing y and y is repressing z and z in turn is repressing x. So, I have X repressing y, y repressing z and z repressing x. It is the mutual repression involving 3 different genes. Each of them can be consider transcription factor and each of them control other one x, y and z in the series. This type of circuit has been created artificially in equali and other system and the dynamic has been studied both theoretically as well as by experiment and in biological natural biological system also we many a time find equivalent circuit which are equivalent to these type of 3 gene network.

Now these types of circuit has a particular name it is called repressilator. Why it is called repressilator? It will be cleared as we keep on discussing today about the behavior of this model as we analyze this model using ODE based equations. So, let us start making the model for this system. Here in this model I will consider mRNA and the protein for each of these 3 genes separately. So, I will have mRNA for x, I will have mRNA for y, I will have mRNA for z, 3 mRNA and I will have 3 protein x, y and z that means I will have 6 different ODE's. So, for the first one for the mRNA of X let us consider mRNA of X is MX so that is mRNA of X I will use the same convention MY will be the mRNA of y and MZ will be the mRNA of z.

So, if I write the first ODE for rate of change in concentration of mRNA of X that is DMXDT that will be equal to what I have written here. Look at the equation carefully K0 plus k1 into Hill function minus K2 into MX. So, let us look into each of this thing carefully. What are they? The first one is for leaky expression. If you remember many promoter like even a lac promoter is not a very tight promoter. It is a leaky promoter that means even in absence of control molecule there will be always a sustain expression of the gene maybe at a very low level, so I have consider leaky expression for gene X that is K0. The next term is K1 that is the maximum rate of transcription and in this bracket I have shown a Hill function which is nothing but a negative Hill function.

Why I have considered negative Hill function? Because usually this type of inhibitory system where the transcription factor comes and buying to a promoter this inhibition or even in case of activation the behaviors and non linear as we have discussed earlier and many a times this non linear behavior follow is sigmoidal pattern where initially when the inhibitor is less expression will be very high and when the inhibitor increases there is a drop of rate of transcription in a sigmoidal fashion and if you remember we have discussed in inverse Hill function is a very good simple function to capture this behavior.

So, I have considered Hill function using z here. Z is the inhibitor because z is inhibiting x. So, as you see in the diagram as xz is inhibiting X here so, z divided by h, h is my Hill constant to the power and so, 1 by 1 plus z divided by h to the power N is my negative Hill function. This K2 into MX is nothing but rate of degradation that is first order degradation. So, K2 is the rate constant for degradation and it is multiplied with the concentration of mRNA. So, that's all for

my ODE for mRNA of X, let me clear it up so that we can write the ODE for protein.

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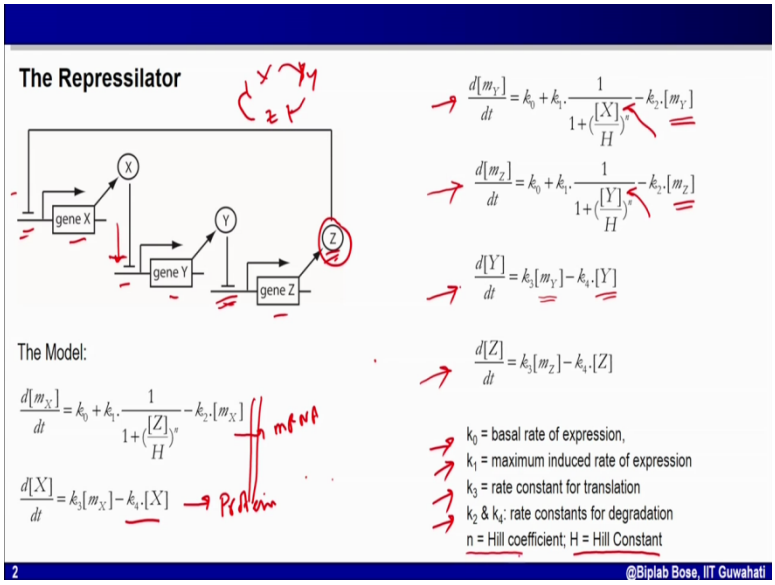

For ODE of protein it will be much easier so, DXDT that is the rate of change in concentration of X depends upon how much mRNA of X is there so, K3 into MX, K3 is the rate of translation. Rate constant of translation into a concentration of MRNA of x minus K4 into X that is the value of degradation of the protein X. So, these two are my ODE for mRNA of X and protein of X. So, this is for mRNA and this is for my protein. We will have similar set of two ODE's for y and z and let us consider that all the parameter K0, K1, Hill constant, Hill coefficient, rate constant for degradation are all equal for these 3 genes that will make our life easier to analyze. You can consider different values further and see the behavior for the time being let us considered that the parameters K0, K1 and other are identical for all the genes.

So, essentially I will have triplicate of this setup equation. So, for the mRNA of y I will have DMYDT that is exactly similar to DMXDT, only difference is you have the X here because X is inhibiting y so in the Hill function you have X and obviously in the degradation term you have MY. Similarly for the mRNA of z I have y in the Hill function because y is inhibiting z and MZ here is for the degradation part. For the protein y DYDt is similar the equation is similar to DXDT only thing is that after K3 it is multiplied with MRNA concentration of y and the degradation rate is K4 into y. Similarly DZDT is for rate of change of concentration of z DZDT

is similar to DXDT which is here it is equal to K3 into MZ the concentration of MRNA for x minus K4 into z the concentration of the protein z. So, that are my 6 ODE almost similar to each other and all the parameters I have kept identical for all the 3 genes to make our life bit easier in this analysis.

So, if I just brief K0 is the basal rate of expression which is coming because of the leaky expression from this promoter. K1 in all cases is the maximum induced rate of expression. There is a maximum rate of transcription when it is induced. K3 is the rate constant for rate translation, K2 and K4 are rate constant for degradation of mRNA and protein, h is the Hill constant in the inverse Hill function or the negative Hill function and N is the Hill coefficient. So, I have this set of 6 ODE's. As I have 6 ODE's large number of them I will not try to do any graphical analysis or something rather I will directly go into JSim and write down the model in JSim for this particular problem and try to do the simulation with different numerical values and try to understand the behavior of the system.

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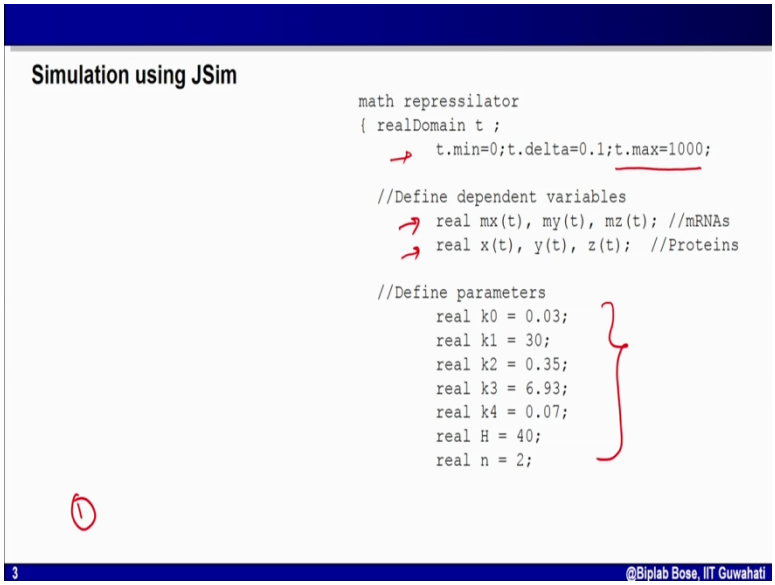

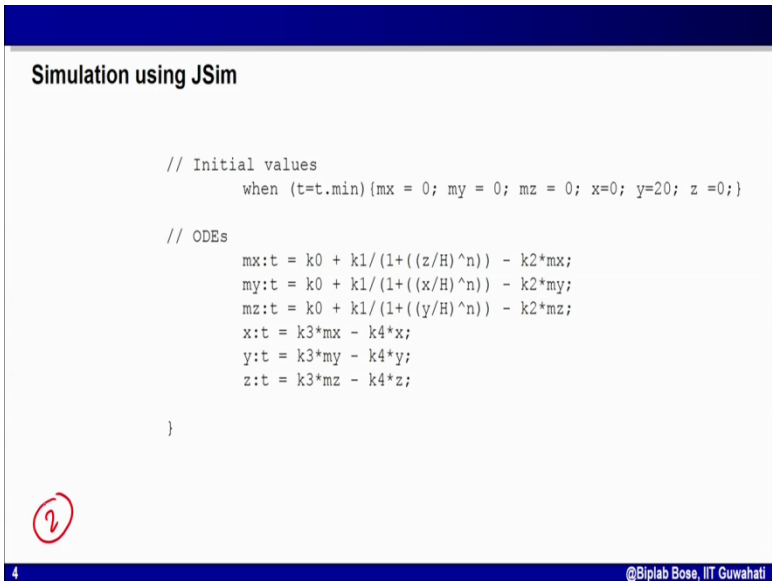

Let us do that. The code for this system it bit longer and I could not accommodate in one slide that's why I have broken them in two part. This is the part one so it start with obviously defining time here and note one important thing t.max have considered as 1000 that's bit higher and in this type this particular model sometimes we may have to take t.max at the maximum duration of simulation into 10,000 to understand the behavior of the system. I have 6 variables; 3 for mRNA that is Mx, My and Mz. These are dependent variables and 3 for protein x, y and z and I have a set of parameter values here and will play around with this parameters so that we can understand dynamics of this system at different parameter regime.

Once you have define this is the second part the continuation of the code so, once you have defined the parameter we have to define the initial valuation and before that let me tell you we will show that this system is very sensitive to initial condition how, where you are starting at. So, we may have to vary at x, what is the value of x, value of y, value of z at t equal to 0 here what I have done I have consider all mRNA at 0, X the protein at 0, z the protein I have kept it 0, y I have kept it 20 and these are my six ODE based on this the model will be simulated. That's all for the JSim code you can try to type that in JSim and try to simulate as I discussed this one.

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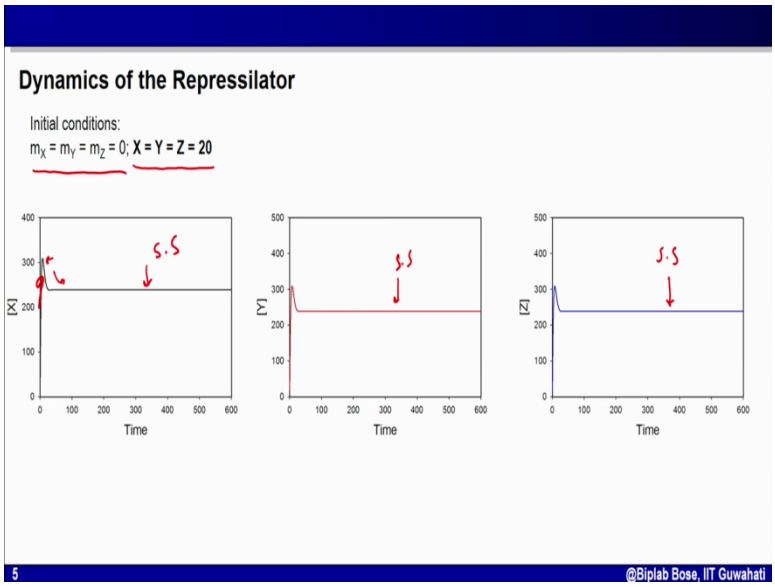

Let us start look in the results I have consider initial condition Mx, My, Mz all mRNA at t equal to 0 at 0 but all protein x, y, z are equal amount non zero values are there. I have taken 20 you can take some other value and you can check the behavior so, if I have this situation and then if I allow the system to proceed with time then what I see I have 3 plots here for x, y and z. So, X will increase slightly and then it reaches the maximum with slight peak and then it falls and reaches the steady state. The same behavior for y it increases initially slightly and then falls back to the steady state and then the same thing for z also. It increases initially slightly then falls to a steady state.

What is happening here? As the time progress there is leaky expression there is initial amount of x, y and z so initially there is some amount of expression and that accumulate more and more x, y and z and then that negative Hill function take control and it try to pull down the rate of expression, rate of transcription down and rate of transcription goes down and after some time rate of production of mRNA, rate of translation, rate of degradation of mRNA and protein matches so, I get a steady state so, initially there was rise but as after sometime all of them x, y and z try to repress each other they all pull them down and reaches the steady state.

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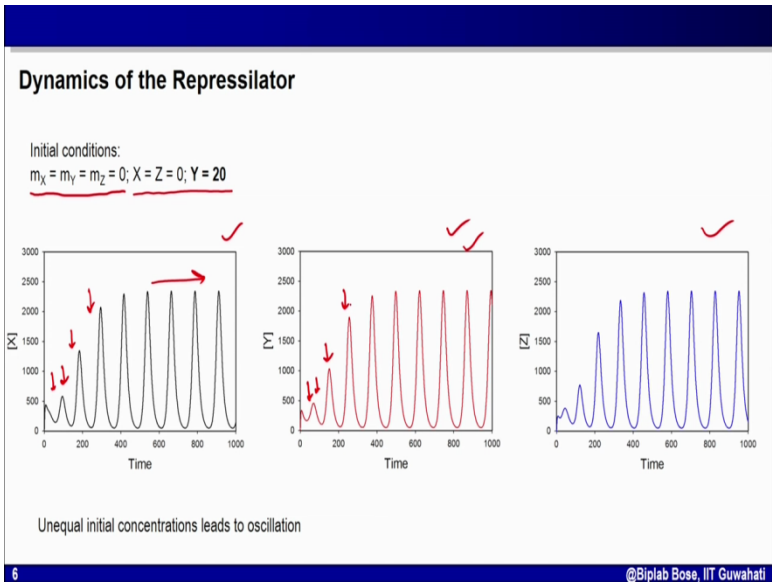

Now let us simulate the same system with a bit differed initial condition. Pay attention to this. T equal to 0, all MRNA I have considered m equal to 0, but I have imbalanced in x, y and z at protein level so, what I consider X and z I have kept at 0 they don't exist in the cell. Y I have kept at 20. In the previous simulation y have x, y and X equal to 20, in this case only y equal to 20, X and z are 0. Now if you simulate the thing you can see a interesting behavior. Here x, y and z they do not reach a steady state rather they show oscillation and the oscillation also has some interesting feature. Initially the amplitude of oscillation if you look at 0 to 200 the amplitude of oscillation is smaller. Then the amplitude increases it increases further and then it stabilizes so, X is oscillating with time, initially its less oscillation amplitude is low then amplitude increases then it further increases and stabilize at that higher amplitude. 4

The similar behavior is observed for y initially lower amplitude, amplitude increase with time, it increase further and then it reaches steady value of amplitude. Same thing is for z. So what has happened here? In the earlier case x, y, z were present in equal amount so, all the repression was equally balanced so, there were equal repression of y by X that has equivalent repression on z by y and z has equivalent repression on X so, all are matched together and we got a steady state but in this case we have imbalanced from very beginning so rather than going into a steady state we get unstable oscillatory behavior and that oscillation has interesting thing, initially the amplitude

is lower with time the amplitude grows and then it stabilizes.

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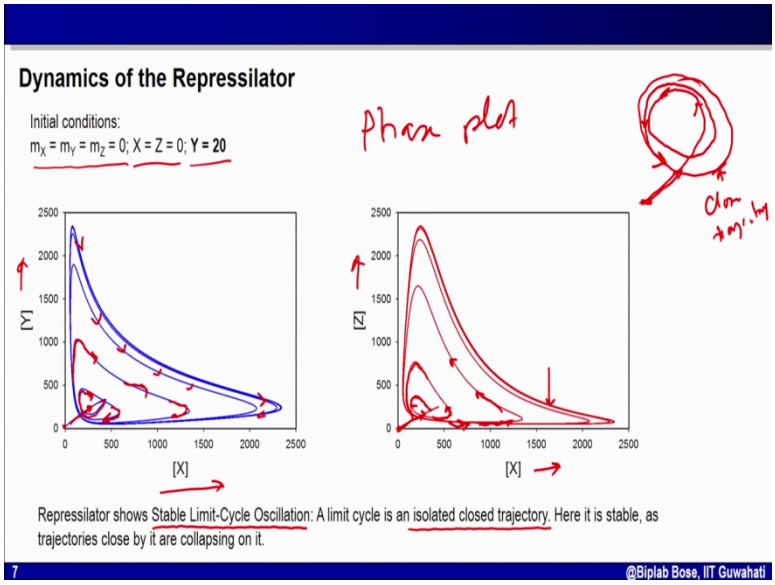

Let us look further into this amplitude behavior of this oscillation. To show this behavior what I have done I have made the phase plot. So, if you remember in phase plot I will have in one axis one dependent variable in another axis the other dependent variable. So, the same thing, same data where t equal to 0, mRNA equal to 0, XZ are 0 but y are 20. Here X is in the horizontal axis, y is in the vertical axis, you have started somewhere here y equal to 20, x equal to 0 with time we have moved along these, plane with time to trajectory node like this, then like this, then like this one the amplitude is increasing and slowly we reach this trajectory where the amplitude is much higher and eventually we reach this final trajectory where the system keeps on moving in the same loop.

The same thing for the X versus Z. this X in horizontal, Z in the vertical. You start at that 00 position you follow this trajectory then you come along this. This trajectory takes you to the larger trajectory so, the amplitude of oscillation increases so as you follow this trajectory, this trajectory mix with the another new trajectory you follow this bigger trajectory now and increases the amplitude and eventually you reaches this outer trajectory where it stabilizes. And this outer trajectory both the cases for XZ and XY has the highest biggest one so the amplitude is the bigger one and another thing which you may carefully notice here is that the outer trajectory

is closed view.

So, what is happening I have outer trajectory which is a close loop. I have started from somewhere here I entered into one trajectory that trajectory has is not close lop rather that was open loop and that has taken to me ultimately to this outer trajectory which is a closed loop. This type of behavior is called stable limit cycle oscillation. What happens in case of stable limit cycle oscillation, you have oscillating system obviously as it is oscillating is called limit cycle oscillation. The phase plane of that has multiple trajectories one of the trajectory is actually isolated closed trajectory so, once you reach that trajectory you will move around this trajectory and not be able to come out. All other trajectories around it are such that they collapse at that closed trajectory. So, here this one the outer one I have shown as a closed trajectory.

And I have started from here and I have moved along this I have trajectories here which gives oscillation but they are not closed they eventually collapse at this closed trajectory. So, as the trajectories slightly away from this closed trajectories are collapsing in this closed trajectory it is a stable trajectory. These are the stable steady state but is a stable trajectory which will give oscillation so, this type of system is called stable limit cycle because it is stable because things are collapsing at this closed trajectory and is limit cycle because initially you have cycle oscillation but those are not in the closed trajectory but on the open trajectory so, this open trajectory eventually takes the system to a close trajectory which is the limit of the whole thing so, is a closed limit cycle. So, there is limit cycle oscillation.

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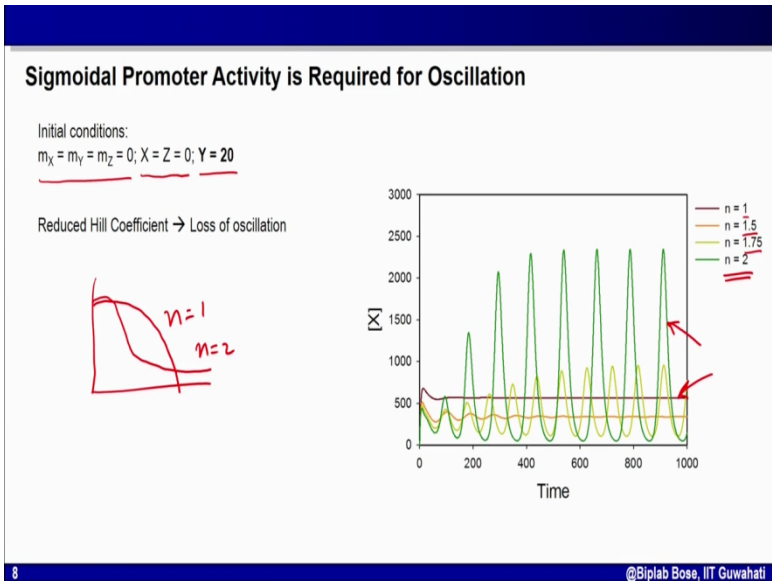

Now let us look into the affect of different parameters in this system on this limit cycle oscillation. How we will do it? Let us first check the effect of the Hill coefficient. Remember I have kept Hill coefficient same for all the X and y and z genes. So, I have taken same initial condition all mRNA at 0. X and z at 0, y is at 20 so, that makes the system to give rise limit cycle oscillation. Now I will keep on changing and from 2 initial n equal to 2 then reduce it to 1.75 then 1.5 and 1. That means what I am doing I have this type of sigmoidal behavior for n equal to 2 and I am moving towards this one where n equal to 1. So, as I move from sigmoidal to hyperbolic what happens to the behavior of the system. I have only plotted X versus t.

You can plot y versus t, z versus t and you will find the similar behavior. When the Hill coefficient is 2, this green line you have limit system oscillation as we have discussed earlier. When I make n equal to 1, I get this stable steady state. I have lose the oscillatory behavior and you can see in this plot as we are moving from 2 towards 1 from sigmoidal non linear behavior to a hyperbolic behavior and towards linear one we are losing this limit cycle oscillation and we are moving towards a steady state. So, that's how the promoter non linearity on close existing path limit cycle oscillation in the system.

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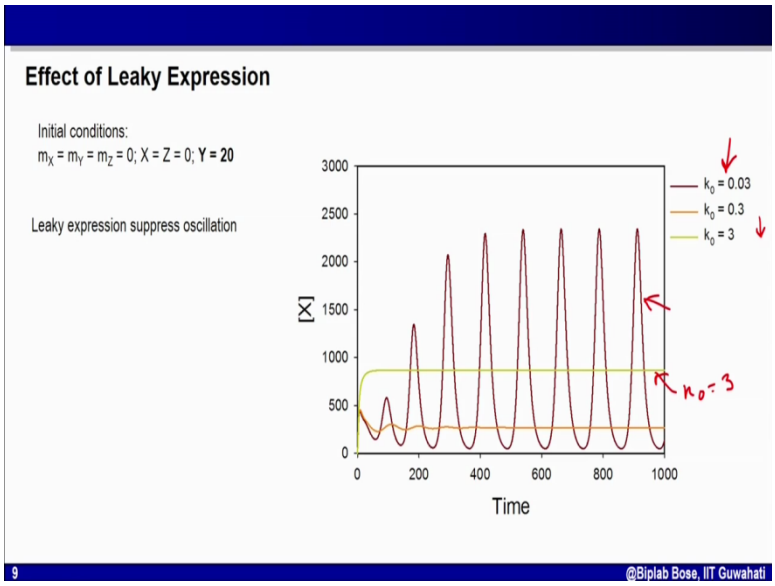

If you remember in this system model we have consider leaky expression K0 so, in general when we are talking about limit cycle oscilaltion we are K0 at a very low value 0.3. Now I want to change the value of K0 to simulate situation leaky expression is very strong that means the promoter is quite leaky so, I am changing the value of K0 from 0.3 towards K0 equal to 3. The same system with imbalance protein t equal to 0, when K0 is 0.3 I get this limit cycle oscillation and as I move towards K0 equal to 3 I get a steady state behavior I lose the oscillation.

That means to get the oscillatory behavior the promoter control should be very tight I should not have much leaky expression because leaky expression nullifies the effect of the repression. But the repression activity is there in the Hill function which is separate from the promoter activity... leaky expression. So, if the leaky expression increase with respect to the induced expression then that effect of that repression may get nullify. So, I do not get any oscillation so, in this system I have to reduce leaky expression to get the limit cycle oscillation.

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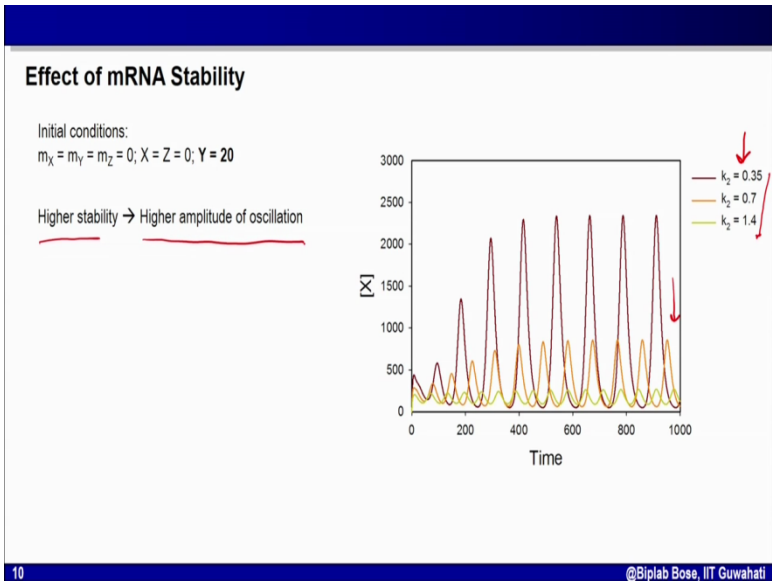

Now, this particular system of oscillation has two other key parameter one is the stability of mRNA, rate of degradation of mRNA and the rate of degradation of protein. Rate of degradation of mRNA depends upon the rate constant for degradation of mRNA and the rate of degradation of protein also depends upon the rate constant of degradation of that protein. So, let us look into the effect of mRNA stability and protein stability on the oscillatory behavior of this 3 genes system. What I will do I have taken the same system where I will get oscillation. In this oscillation... when we get oscillation our K2 is at 0.35. Now I want to increase K2 so, that's what I am doing I am increasing K2 towards 0.7 then 1.4. If I increase k2, that means mRNA will become less right because K2 is the rate constant for degradation of the mRNA so, if I increase K2 more and more mRNA will get degraded.

So, here as I increasing the rate constant for degradation of mRNA I can see the amplitude of oscillation is decreasing. That means if I have higher stability of mRNA then I have higher amplitude of oscillation and that quite easy to understand because I have considered mRNA, formation and translation separately so the translation efficiency... effective rate of translation how many proteins will produce per minute depends upon how many mRNA's are there. So, if the mRNA is very unstable then the ribosomes will not get much time to produce protein. So, if you increase K2 the rate constant for degradation of mRNA, mRNA becomes very unstable so, effective rate of production of protein decreases so, my amplitude of oscillation of x, y and z also decreases because I cannot have higher amount of x, y and z.

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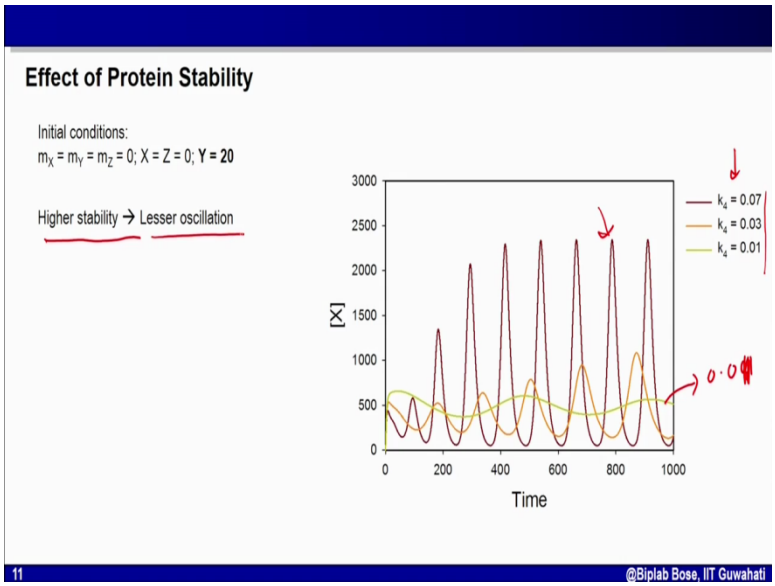

Now let us look into what will happen if I make the protein stable or change the stability of protein in this case I am varying K4, the rate constant for degradation of protein from 0.07 to 0.01 that means what I am doing. I am increasing the stability of protein 0.07 to 0.01 when K4 is 0.01 it will be much more stable. So, if you increase the value of K4, and do the same simulation you will see when K4 is 0.07 I get this limit cycle oscillation. But when K4 is reduced to 0.01 that is 0.01 that means you have decreased the rate constant for degradation of the protein that means now the protein will be more stable. I lose this oscillation. Why is it happening?

Because if you remember the... if I increase X the extend of repression on y will increase then also extend of repression on z will increase and the repression of z on X will also increase. This repression if there low then also I don't get oscillation. If the repression is very high then also I lose the oscillation. So, I have to make a balance and that balance is controlled obviously by how much protein is there in the cells. If you make the system such that the proteins are very stable then this oscillation will get reduced so, higher stability of protein giving rise to lesser oscillation.

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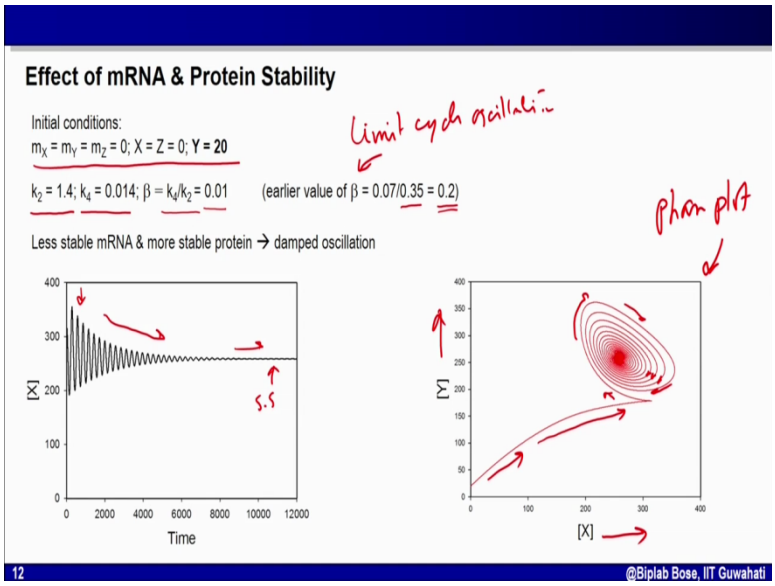

Now what if I mix both of them together I want to change the mRNA stability and the protein stability together in the system and I want to compare with what I have got earlier in the limit cycle oscillation. So, I am keeping the initial condition same where I got limit cycle oscillation mx, my, mz equal to 0, the mRNA's are 0, xz is kept at 0, y is kept equal to 20 and if I keep all the parameters same as I have shown in the model initially then I should get a limit cycle oscillation but what I did in this simulation I increase K2 to 1.4 from 0.07 initially K2 was 0.07 and I decrease K4 from 0.35 to 0.04 so, I am increasing the K2 that means I am making mRNA less stable. I am decreasing K4 that means I am making protein more stable. So, if I look into the ratio of K4 and K2 I have beta equal to K4 by K2 and that is equal to 0.01.

When I have the limit cycle oscillation initially my beta was this K4 was 0.07 and K2 was 0.35 and the ratio was 0.2. So, now beta has changed from 0.2 to 0.01 essentially I have made the protein more stable, mRNA less stable. So, what type of behavior do I get if you look into the behavior if you simulate with this parameter in JSim and then plot X versus time, y versus time z versus time I have plotted only X versus time here you can see initially you have oscillation but then this oscillation dk and then I reach at stable steady state. So, I have damped oscillation initially I have oscillation then it get damped to a steady state.

If I draw the phase plane of that so I have phase plane plot here with X in the horizontal axis, y in the vertical axis so this is my trajectory initially things increases then a oscillation start the trajectory of this oscillation are collapsing because they are becoming smaller in amplitude and

they are slowly collapsing and they eventually collapse accentor of this trajectory which is a stable steady state. So, what I have learnt from this particular simulation is that if I increase the stability of protein reduce the stability of the MRNA rather than get a limit cycle oscillation I get a damped oscillation.

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Let us jot down all the points so, what I have discussed here we have discussed 3 mutually repressing genes that is forming a repressionate. So, it is matter of represses and it has oscillating behavior that's why this type of circuit is called repressivate. Depending on the initial condition and the parameter values as we have shown discussed earlier this system can show stable limit cycle oscillation. One key point in this system if you have to have stable limit cycle oscillation then there should be some imbalance in the initial concentration of molecules at t equal to 0 otherwise you never get oscillation in the system.

We have observed by simulation that if you have higher leaky expression then you do not get oscillation and also we have observed the oscillation require non linear sigmoidal behavior in the promoter activity otherwise you lose the oscillation and finally one key issue we have discussed through simulation is that the ratio of protein to MRNA stability is the key parameter. If you decrease the ratio that means you are increasing the stability of protein, decreasing the stability of MRNA rather getting from stable limit cycle you get damped oscillation. So, based on this

relative stability of protein with respective MRNA either you will get damped oscillation or you will get a stable limit cycle oscillation. That's all for this module thank you for watching.