Physics of Biological Systems
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Lecture - 27
Protein modification problem

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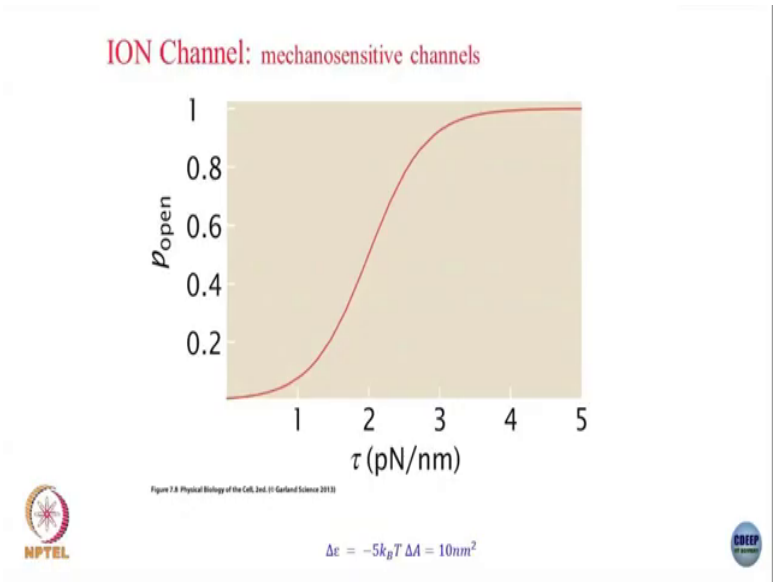

The next along the similar lines a similar idea that I want to look show you is this concept of Protein modification.

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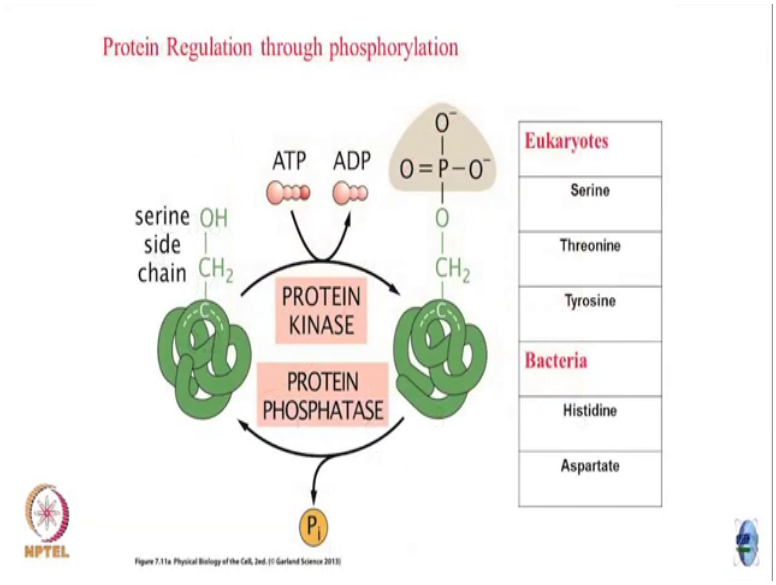

So, let me start off with this very common post translational modification of proteins which is called phosphorylation ok. So, often you will find that here is for example, of native structure of a folded protein, which let us say has some serine side chain. There exist enzymes which are called protein kinases which with the help of ATP will come and put a phosphate group on the top of the serine sidechain. The process like this is called phosphorylation.

You can have the reverse thing as well where another enzyme called the protein phosphatase will come and it will strip this phosphate group from this phosphorylated protein. So, it will eject out the phosphate and it will go back to this unphosphorylated state. So, this protein then can exist in two state; in two state one of two states, either in unphosphorylated state or a phosphorylated state ok.

And you can imagine that depending on whether it is phosphorylated or not, the structure of the protein will be different right. Why because you have introduced some additional negative charges over here. So, that will change the electrostatic interactions between the differently charged amino acids and that you can of course assume will change the structure of the protein.

If you change the structure of the protein you can change the function of the protein. So, something which was less likely to bind earlier can now become more likely to bind simply by virtue of this phosphorylation. So, this is a sort of a class of changes which are called post translational modifications. So, you can have phosphorylation you can have acetylation there is a whole bunch of post translational modifications that you can do to these amino acids, sorry that you can do to these proteins and that will change the function of these proteins.

And increasingly sort of people are realizing that you know earlier we have this paradigm of transcription and translations, you have the genetic code from there you produce the MRNA. From the MRNA you produce the protein and that is it that protein does some stuff does whatever it is supposed to do.

But increasingly people are realizing that the function of the protein is often very closely tied to these post translational modifications. Whether you introduce a phosphate or a acetylase or something else. Depending on whether this protein is phosphorylated or not or the degree to which it is phosphorylated you can have different levels of functionality of the protein.

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So, for example, there are a couple of papers if you look at microtubules you will see that this alpha beta tubulin which constitutes the microtubule, that has a whole variety of post translational modifications. And these actually regulate to a very large extent the functions of the microtubule, this is in nature moll cell biology.

Again similar sort of report but now to do with the proteins that comprise the circadian clock, that maintains the time the inside yourselves and again the post translational modifications of these proteins are essential to regulate the circ adian clock. And this is now widely sort of accepted to be true for a whole variety of different functions and different proteins, that it is not just the native structure that is important. But it is all these post translational modifications that play a very important role.

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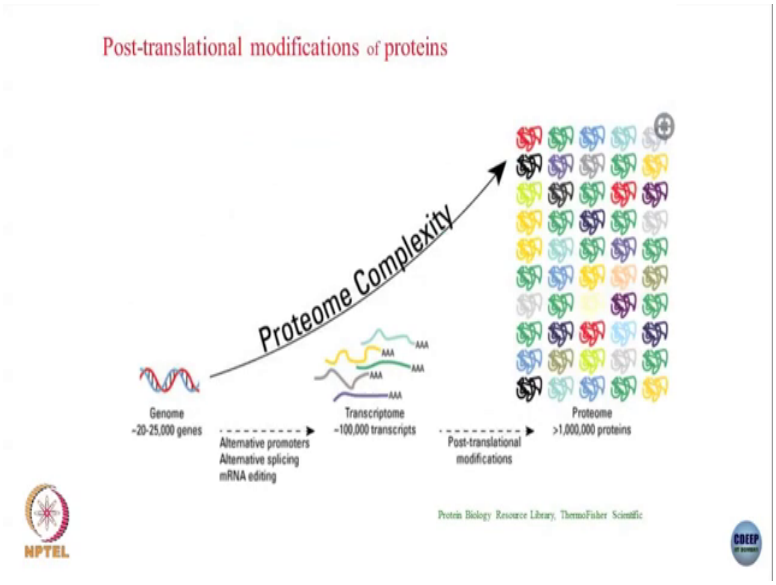

So, you can sort of imagine the increase in complexity that results in. So, you have your genome which anyway has a large number of genes, these genes can code for a large number of proteins and by sort of having alternative promoters binding and so on. The number of transcriptomes this is called is anyway large much larger than the number of genes.

But now if you introduced this post translational modifications, that each protein can exist in many many different states, you have this whole zoo out here which is like some greater than what 10 to the power of 6 possibilities ok. And each of this has some sort of function functional role to play ok.

So, it is an increasingly important area where people are trying to find out, exactly the role of these post translational modifications. So, what I will try to do is that I will try to look at this phosphorylation and we will try to build a very simple model of this phosphorylation.

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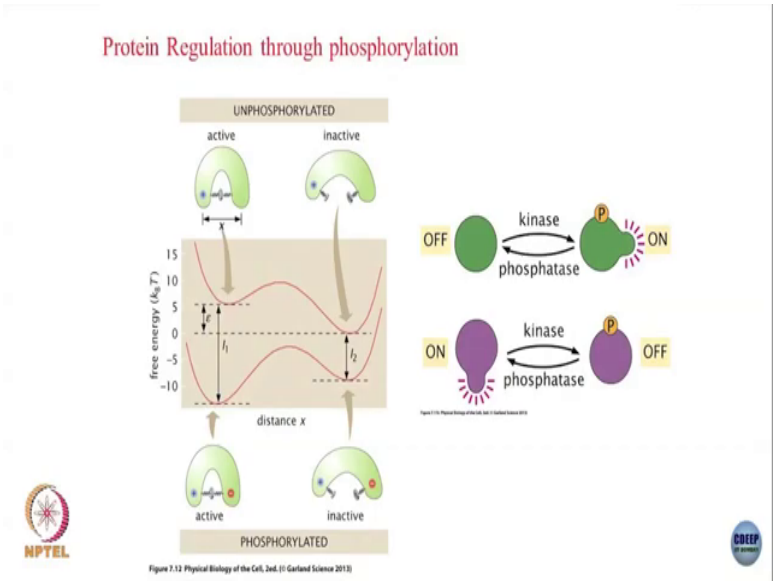

So, here is the basic idea right, that I have this protein which exists in which can exist in two sort of states something like this and something sorry something like this.

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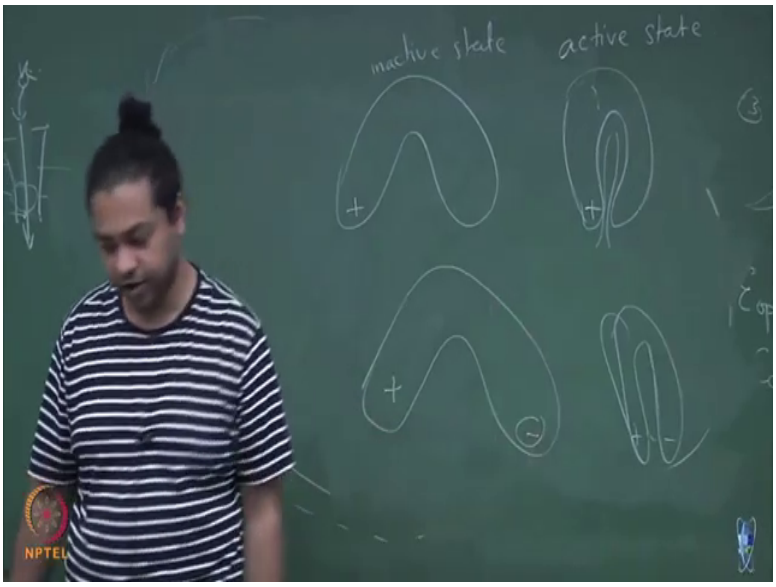

These are two distinct conformational states and let us say that the functionality I am looking at. For example, maybe I want the ligand which is shaped like this to come about just as an example ok. So, it is through a lock and key sort of a mechanism, that functionality is sort of preferred when the protein is in this particular state ok. So, this let us call this as the active state this call this as the active state and let us call this as the inactive state of the protein ok.

Now, you can imagine that let us say some amino acid over here is let us say positively charged ok. Now you can imagine that if I now phosphorylate this protein. So, if I phosphorylate these two configurations there is a positive charge over here and let us say I phosphorylate a residue over here. So, introduce a sort of negative charge over here right.

I think like this will now be much more stable because they are introducing this electrostatic interaction between the negative charge of the phosphate group and this positive charge of

whatever amino acid was there on the other side. So, you can change the energy balance between this active and inactive state by through this phosphorylation of this particular protein right. So, that is sort of what is shown over here. Now let us say that here is my active state here is my inactive state.

So, generally for the unphosphorylated protein the inactive state is energetically favorable right. So, the free energy minimum corresponding to the inactive state is lower than the free energy minimum corresponding to the active state ok. These are both energy minimums because these are both stable structures of the protein, is just that this one is more stable when it is unphosphorylated.

However, if you phosphorylate it so you introduce this negative charge let us say over here, this becomes this active state of the protein becomes much more stable than this inactive state. So, you switch the order of stability of this active versus this inactive state by depending on whether your protein is phosphorylated or unphosphorylated.

So, that is the sort of basic idea and then you know it can be helpful for your function depending. So for example, it could be that in the phosphorylated state it perform the desired function or it could be that in the unphosphorylated state for some other protein you perform the desired function clear. So, that is the basic thing that we will try to sort of amount.

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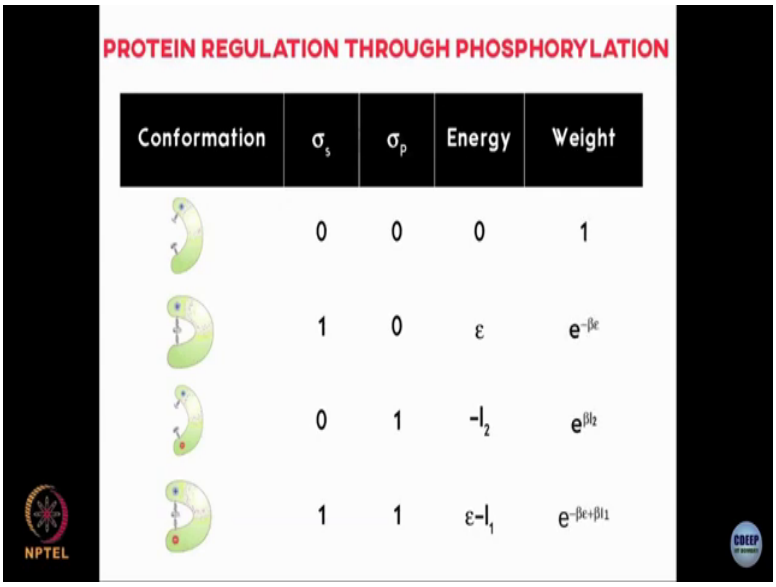

So, again I need to; I need to sort of label my microstates of the system right. So, let me say earlier I was using only one state variable remember for the ion channel.

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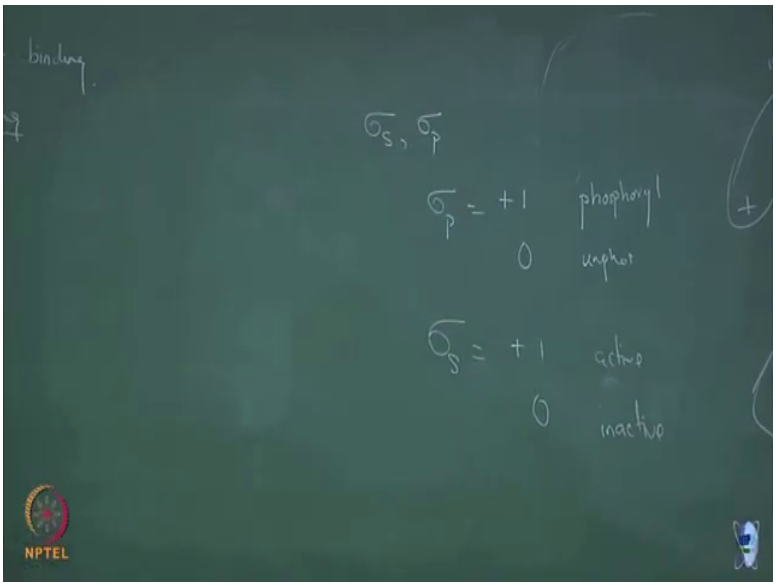

Now, I will use two state variable sigma S and sigma P, one which tells me that whether the protein is phosphorylated or not. So, sigma P is going to be equal to plus 1 if the protein is phosphorylated right, it is I will say that this is 0 if it is unphosphorylated right. Similarly, the sigma S denotes the active versus the inactive state of the protein. So, sigma S is equal to plus 1 it is in the active state it is equal to 0 if it is in the inactive state. Together these two variables specify the microstates of my system right. So, how many possible microstates will I have for a system like this?

Student: 4.

4 right, 2 into 2 2 for each state, so this figure I only cannot see so let me switch here. So, these are my four sort of possible states of the system inactive unphosphorylated active unphosphorylated inactive phosphorylated and active phosphorylated and the corresponding.

So, this is the inactive state active state inactive state active state and for the phosphorylation the top two or unphosphorylated the bottom two are phosphorylated.

And I can write down let us say a corresponding energy for each of this right. So, if I go back to this if I keep in mind this energy level diagram. So, then I can write down an energy for each of this. So, I assume that the inactive phosphorylated state, I say I consider as the 0 of my energy. It does not matter because all ultimately all I will be interested in is the differences of these energies.

So, I can set the 0 to be anywhere. So, I say that this inactive phosphorylated state is my 0, the active unphosphorylated state is plus epsilon because it is energetically unfavorable the inactive phosphorylated state is 0 minus I 2. So, minus I 2 right and the active phosphorylated state is epsilon minus I 1 right.

So, in terms of this epsilon I 1 and I 2 I know what is the energies of each of these states, so that I can write down right. So, this is 0 epsilon minus I 2 epsilon minus I 1 these are the four energies and then each of these will have a weight which is given by the corresponding Boltzmann factor.

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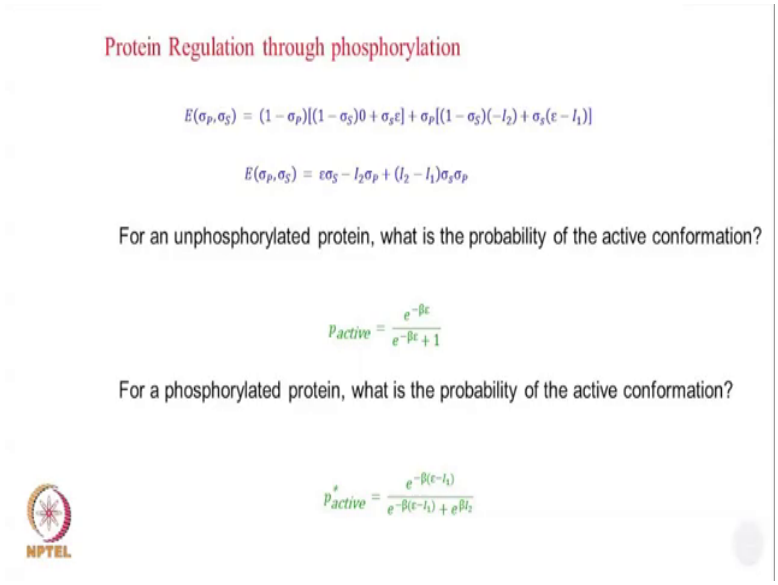

So, you can write down ok. So, this is exactly what I said, but written down in a formulaic form. So, when the protein is phosphorylated then these terms will come into play when the protein is unphosphorylated these terms will come into play and so on right. And these are the four energy 0 epsilon minus I 2 epsilon minus I 1.

So, again you can calculate that so I can ask two things right. So, I can ask that what is the probability that the protein is going to be in the active state. I can ask what is the probability that the protein is going to be in the active state.

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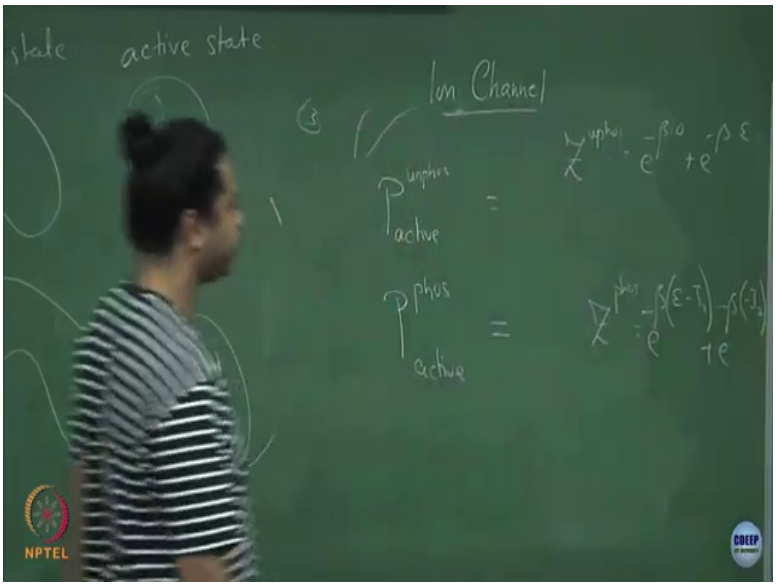

If I know that it exists in it is unphosphorylated form right. Correspondingly I can ask that what is the probability that it will be in the active state. If it now exists in the phosphorylated form ok, so what will this be? When it is unphosphorylated remember these are my four energies over here right. So, when it is unphosphorylated these terms come into the picture and it is phosphorylated those terms come into the picture.

So, if it is unphosphorylated what is the probability that it will be in the active state. So, it will just be delta epsilon in this case will be 0 and epsilon is just epsilon and the numerator will be 1 plus e to the power of beta epsilon right. So, here is the probability of it being in the active state, if your protein is unphosphorylated right.

Correspondingly if the protein is phosphorylated then these two energies will come into play right and then again you will get a probability of it being in the active state, which depends on

this epsilon minus e 1 and then in the numerator this partition function e to the power of minus beta epsilon minus c 1 e to the power of beta I 2 right this is clear.

The partition function for this unphospho in the unphosphorylated state is simply e to the power of minus beta into 0 plus e to the power of minus beta into epsilon. And here for the phosphorylated state it is e to the power of minus beta epsilon minus I 1 plus e to the power of minus beta into minus I 2 right and then you get this corresponding probabilities.

So therefore, you could ask that how much has this probability changed because I have phosphorylated the protein. The probability of finding this protein in the active state how much has it changed, because I have phosphorylated this protein.

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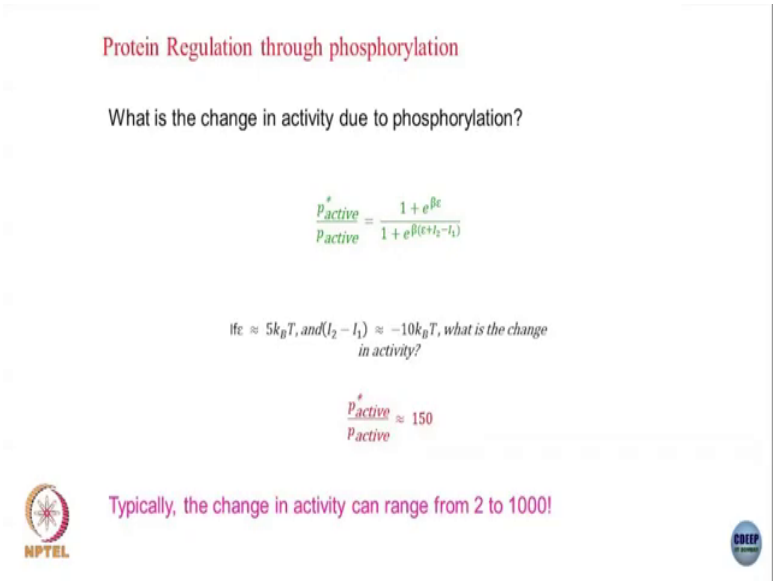

And so that is just the ratios of these two right. So, that comes out to be 1 plus beta epsilon plus 1 plus beta epsilon plus I 2 minus I 1. You can now put in some sort of reasonable numbers, for example, if I put if I say that this epsilon is somewhere around 5. So, all of these will roughly be of the scale of thermal energy K B T.

So, let us say epsilon is 5 K B T and this I 2 what is that I 2 minus I 1 is 10 K B T something which a reasonable numbers to sort of put ok. And then you can calculate how much has this change how much has this activity change due to phosphorylation. So, if I put in these numbers what I get is that this activity has increased around 150 times ok.

And typically, if you look at proteins by phosphorylation you can change the activity anywhere you can double the activity to some you can take it to somewhere around 1000 times as much. So, you can cause huge changes in the probability of this active state by doing this post translational modification of phosphorylation and same for any other post translational modification.

Student: (Refer Time: 14:45).

Room temperature always in biology unless specified otherwise K B T is 300. So, you can try to put in these numbers and hopefully that should come out T is 300 KB not K B T all right.

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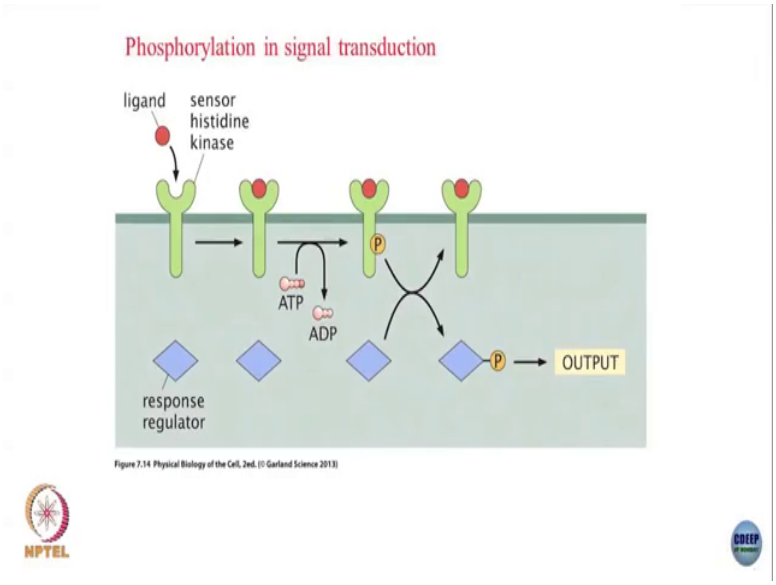

So, for example, here is an example of phosphorylation in signal transduction. So, we saw in various for example, in the ecoli diffusion, we saw that this bacteria was sensing chemical gradients which was outside right. So, it was moving towards the chemo attractant or it was moving away from a chemo repellant and one of the ways it does that is through this signal transduction. So, basically let us say here is your bacterium, it senses some sort of a chemical gradient outside of it.

So, it wants to produce more or less of certain protein in response to that it is responsible environmental stimuli right. How does it do it generally does it in two steps there is a receptor over here a sensor on the cell membrane right. For example, here is a sensor histidine kinase depending on this environmental conditions a ligand can come and bind once it binds it can phosphorylate this sensor this kinase ok.

Once it is phosphorylated this kinase which is trapped in the membrane it has to get that information to the inside right, here is your sensor which is sitting in the cell membrane it is just transmit that information to the inside. So, that the cell can produce more or less of whatever protein that it wants to ok. To get it to the inside what it does is that it talks with a different protein which is called a response regulator, at it can phosphorylate this particular protein the response regulator.

That response regulator can then go and sit on the DNA and it can change the expression levels of a particular gene, saying how much more or how much less of a particular protein is going to be produced in response to that environmental stimuli right. So, this chain of sort of processes you sense the environment in response to that you phosphorylate or unphosphorylated a protein and that information gets conveyed through and that is through some sort of transcription factor binding for example and that changes the expression levels of your gene ok. And bacteria especially in ecoli this system is actually very well study.

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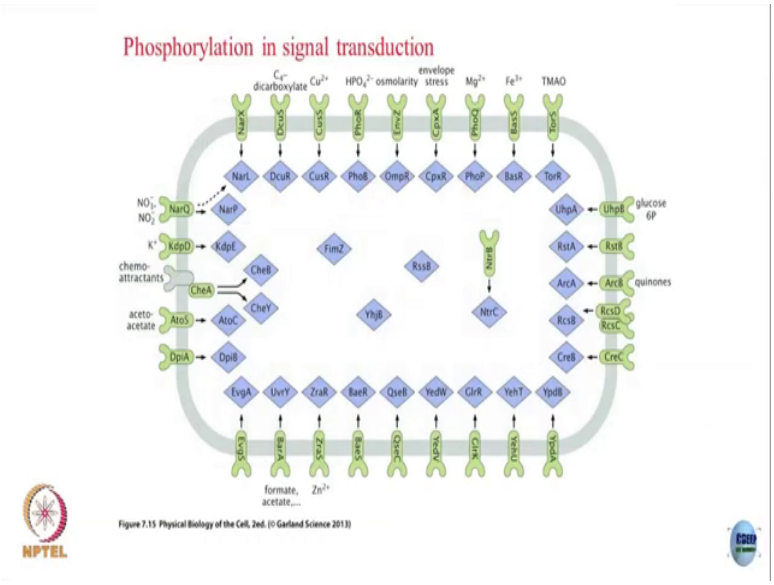

For example you have this whole set of sensors versus this response regulators, that respond to different different chemicals. So, these yellow things over here are the sensors and corresponding to each of these sensors you have a response regulator and then through this for this phosphorylation chain this response regulators go and modify the expression levels of particular protein.

For example, this the chemo attractants are here is the sensor for the chemo attractants and here are the response regulators for the chemo attractants. And similarly for different sort of environmental signals because the bacteria has to respond to many many different signals this fairly well mapped out for ecoli.

And these are also actually remarkably specific, for example you can say that well this sensor was phosphorylated and I have all these different response regulators that are floating around

in the cell R1 R2 R3 and so on, all of these different blue boxes. People have shown that actually this there is a very tight pair relationship between these two.

So, this sensor if this is a sensor corresponding to this R2 it will not phosphorylate this R1 receptor it will not phosphorylate this R3 receptor, it will only phosphorylate this corresponding R2 receptor. At least people have shown that vitro experiments all right. So, again so that was about phosphorylation and you can do the similar sort of machinery for different post translational modifications, at how much is the activity of the protein change to the function of these modifications.