Biochemical Engineering

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Module No. # 01

Lecture No. # 35

General Model for Receptor-Mediated Endocytosis

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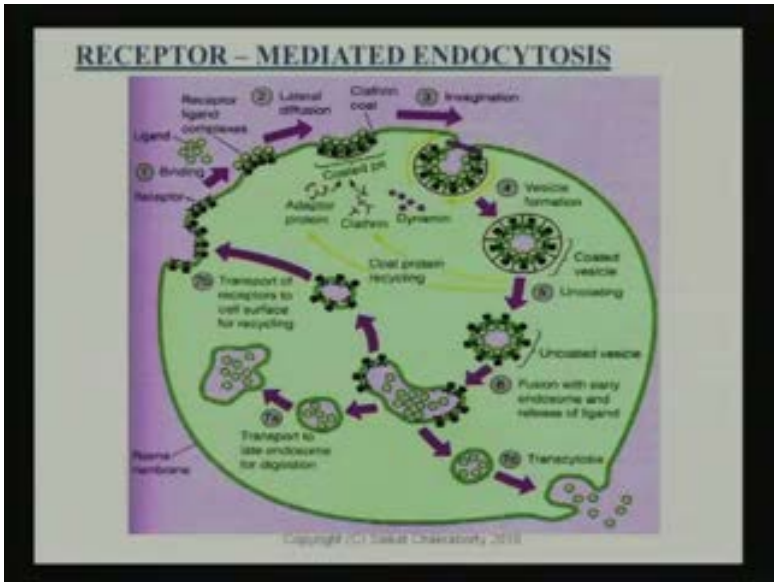

Cytosis in the last couple of lectures, but one of the assumptions that we had continued to make all through was the fact that the receptor number is a constant. And this is assumption that we have made time and again when we are doing looking at bio you know the enzymes, when we are looking at all kinds of places; we have made the assumption that the total number of that of a certain certain species is a constant; be the total amount of enzymes, be it the total number of receptors in the system.

Now, in case of enzymes, it is may be true, but what happens in case of receptors? It is this is not necessarily true. Why this not necessarily true is that, I think I talked about this a little bit before, is that the body needs to regulate the number of receptors. And we

talked about certain diseases and for example, I think we did talk about the pulmonary immune function, and we said that in certain cases, there is a body needs to engage more receptors. In certain cases, the body might need to toned down; lower the number of receptor. So, the cell has a mechanism of the body in general but, cell in particular, has a mechanism of regulating the receptor number which means that, the assumption that the total number of receptors is a constant is not necessarily assumption that holds that all points of time.

So, what we are going to do in today's lecture is that, all the model that we did in the previous lecture, was based on the assumption that the total amount of receptor is a constant if you remember. And I will just remind you one more time that one, but what we are going to do in today's lecture is, how we model the system when the total number of receptor is not a constant or in other words, receptor itself grows and die.

So, there is something you know there is a process in the number of receptors is involved in the process as well because till now, the number of receptors had not been involved in the process. So, this is this is the old picture that we give (( )) and. So, here essentially that the number of receptors that you have in the system; the total number of receptors in the system here was assumed to be constant in every model that we did before, but now we are not going to assume that.

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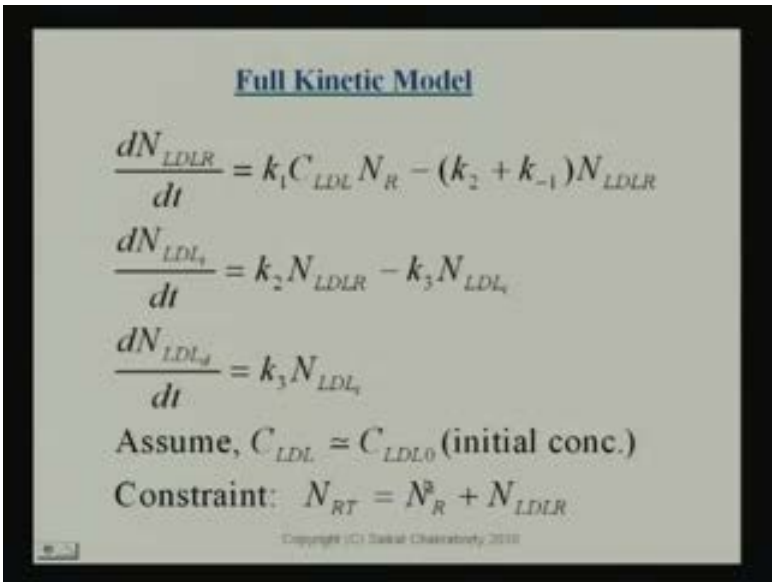

So, let me just go back to the model we did in the last class; this one sorry, full kinetic model. So, this was the constraint that I am talking of N RT equals N R plus N LDLR. So, the total number of receptor present as free and as complex is a constant. How did it help us? We could pull this back into this equation and therefore, it became you know when C LDL is a constant equals the initial concentration, then it became a first order reaction and we could solve it.

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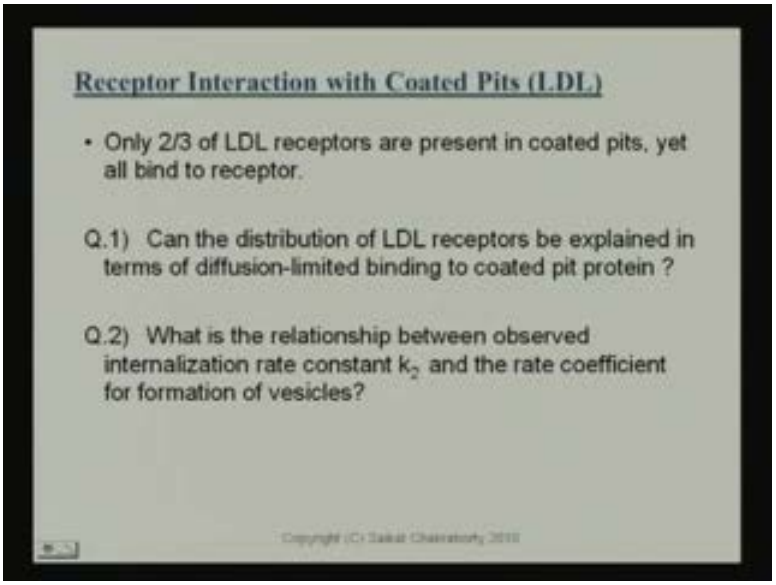

Now, we do not want to make that assumption. Before we go ahead and do that modelling, there are couple of questions that are necessary to answer. So, remember one of the points when we talked about the mutations that results in familial hypercholesterolemia, there were three points right. One was the point; first point was that the dissociation rate constant may be increased in patients with familial hypercholesterolemia, may be you have larger dissociations constant. What does the second point?

The fact is that the internalization rate constant may be lower. So, in patient with familial hypercholesterolemia, the internalization rate constant may be lower. And what was the third point? The third point was maybe the dissociation rate constant and the internalization constants are all right, but what is defective is the localisation in the coated pits.

So, the process of localisation in the coated pit is not good enough. So, that is why we want to ask couple of questions based on that. So, it turns out that only two third of the LDL receptors are present in coated pits, yet all bind to receptor. These, all the ligands that bind to these receptors; that is the question. So, can the distributions, so, the questions; couple of questions we have over here is a) can the distribution of the LDL receptors be explained in terms of diffusion limited binding to coated pit? I discussed this a little bit, but we are going to talk about this in greater detail. And second one is, what is the relationship between the observed internalization rate constant k 2 and the rate of formation of the vesicles?

So, these are couple of questions I want you to think about. One of them; we are going to answer in the next few minutes which is that what is the distribution you know this diffusion limited binding. So, if you go back, I have to go back to the picture again and again here.

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So, here as you see, this is this is a part that we are going to talk about now. So, why is that, because we have already in a in the previous lecture, we have already discussed the modelling of this part; that is, 1 written here binding. We have already discussed the internalization process given here by 3 for example. And the question that is asked now that I just now asked is related to point number 2 and point number 4. Point number 2 what is the question; that how does this let as you can see over here that these most of these receptors go and localize in the coated pits.

So, the question that was asked you know the question that I asked or mentioned was that, the coated pits is only they are very little. The most of the cell membrane is not coated pits. There are only small regions in the cell membrane which consists of coated pits, but it turns out that most of the receptors go and localize in the coated pits. So, what essentially happens you know what is drawing these receptors towards the coated pit either in unbound form or in bound form depending on the class of the receptor? You follow what I am saying. So, that is question number 1.

And question number 2 is here as you see, three to four that was other question was that, is internalization and vesicle formation the same process or in other words, is the rate constant for internalization same as the rate constant for vesicle formation. You follow

the question that whenever you have internalization, does that mean that at the same rate in vesicle formation is also occurring right, because these are processes in series as I told in the morning class, these are processes in series. So, first you have say for example, binding, followed by diffusion into the coated pits, then you have internalization and finally, vesicle formation. So, what is the surety that internalization is equivalent to vesicle formation? So, that is another question.

So, what does that mean to you, when I say that what is the surety that internalization is not same as vesicle formation and if internalization is not same as vesicle formation, what should you do? What you think you should do? This is very simple answer, but I want you know it is not a complicated question neither is answer complicated, but what do you think you should do? So, this process you know, what you see your screen here,

(( )) one more (( ))

Yeah yeah which the as I said, it is very simple answer. So, here we have step number, we have taken into account step number 1, we have taken into account step number 3, 2; we are going to take into account right now, and if you have the rate of vesicle formation is not the same as the rate internalization, just add on one more step and add rate constant 4; irreversible rate constant 4 out there, and you can have your model.

So, what you have to now distinguish between? You have to distinguish between the complex in the invagination or in the internalize complex as opposed to complex in the vesicle. So, we had here, the complex in the here for example, this is a LDL I which is the complex in the internalized form. Then you add one more rate constant and go to the complex in the vesicle LDL v. That is easy and we can take that into account.

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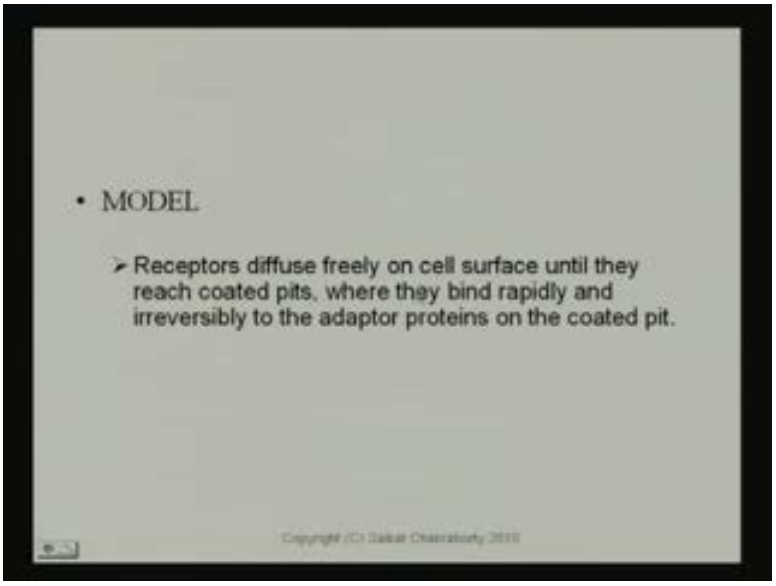

Now, we have to talk about this part, the coated pits and what really happens. So, essentially what happens is what is written on the screen. So, receptors diffuse on cell surface freely till they reach the coated pit, where they bind rapidly and irreversibly to adapter proteins to the coated pit. So, what happens is that these coated pits are there. So, whether it is class 1 or class 2 does not matter, whether the receptor is already bound to the ligand or not bound to the ligand is not important.

So, these receptors will freely float around on the surface of the cell till they reach the coated pit. And coated pit consist of three kinds of proteins you know, we discussed that earlier in the picture we showed. So, this coated pits; these proteins are very quick binders. So, they have this ability that the rate constant for binding is very fast. So, as a result, they as soon as the receptors reach the coated pit, it immediately binds with the coated pit irreversibly and very almost instantaneously to form the receptor on the coated pit.

Now, my question is that so essentially if you try and understand, there are two processes in the system, right. One is the diffusion; lateral diffusion that is taking place, and the second one is the binding; irreversible binding to the coated pits. So, what do you think you know, again it is a very easy question. So, what do you think would be if ask you to model, just you know let me give you this question that I am not telling you how. If I tell you, then its the anticlimax. So, if I just ask you to kind of guess, come up with some

open answers to... this is a system, and this is what is happening in the system, so, how would you model it?

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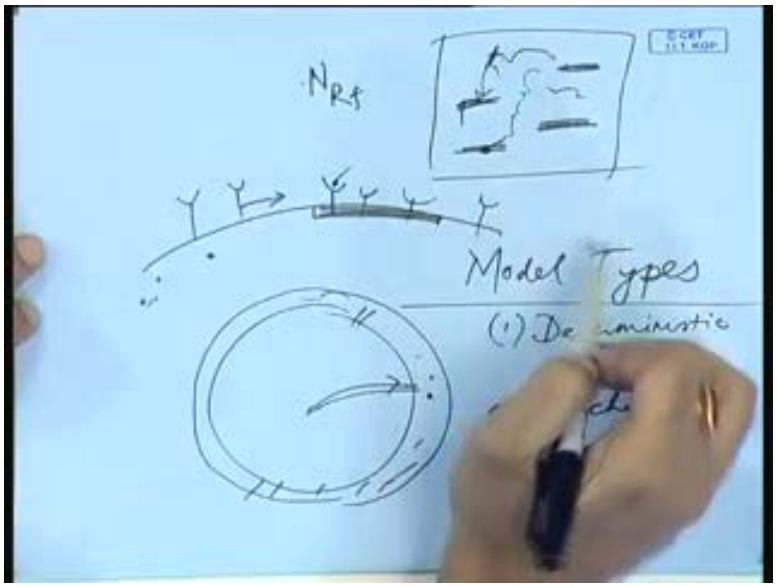

So, this if I am to draw, this is the cell surface, and you have the receptors floating around here like this, and you have this region; certain region which is coated pit. It is like a sticker; as if you know you have put some adhesive on it. So, as soon as the receptor reaches there or with the ligand let us say that reaches there it stuck. It cannot move any further. So, it stuck out here. Otherwise it is freely floating, but as soon as it reaches there, it stuck. So, it is like as if you know you spread some gum or adhesive out there and it comes in. So, now, my you know.

So, this is the system I talked about it and now what do you think, how do you want to model this, if I give you know it is give an open problem. So, ideally you know let us say, do not worry about how difficult the computation or calculation or the solution of the model is going to be. So, if I just give you this problem in the test and say that how you going to model the system, this is the system how you going to model it. So, what are the processes? Tell me what are the fundamental processes?

(( ))

Diffusion and reaction; irreversible reaction it is already said and lateral diffusion. So, it is not it is not like a normal diffusion in the sense that it is a slightly guided diffusion,

slightly guided diffusion. It is not a normal diffusion, but its slightly guided diffusion. Why it is slightly guided, because it is directed diffusion. There is it is a lateral diffusion, but still it some kind of diffusion. So, what do you think, how should you do it? I mean if you if you can if you want come to think of it, think of diffusion in a guided channel like you can have a guided channel, and there can there can be diffusion in that guided channel, right. So, that is the something like that. So, lets. So, you know just talk about the basic processes first, and then we can talk about guided channels and stuffs like that. What is the basic process; diffusion and reaction. So, are they occurring in parallel or in series?

In series.

Series. So, we already did this problem before in this course itself, we did this problem of diffusion followed by reaction.

Now, this is a tricky part you know, this part that we are going to do. Its a slightly tricky part. Now if I am to actually do this whole modelling of diffusion followed by reaction, it is a very complicated process. Why, because you do not know where the cell is, where the receptors are diffusing a, and then you know at what point it is reacting. There is no direct you know there is no clear cut information on to that there is available to us. Do you understand?

How is it different from what we are done before what we have done before is for example, you know I remember we doing this immobilised enzyme, right. So, for example, they are some reaction that is going to occur on the surface, and materials were diffusing out, and the reactions are going to occur here. So, you what was the advantage there? You had clear geographical understanding of what area the diffusion is going to take place; which is this area, followed by where exactly the reaction is going to occur. Here the problem is that you do not have clear understanding of where the diffusion is going to occur, which direction the diffusion is going to occur a, and b where exactly this coated pits are. You are not aware.

I can tell you that coated pits form five percent or ten percent of the entire cell membrane. I think I gave you number already which is like that say ten percent of the entire cell membrane is coated pit. But you do not know where the ten percent is, right. So, you do not have clear cut information available to you as to where diffusion is going

to occur, and where reaction is going to occur. So, what is the process in that case, if you do not have clear cut clear cut information available? What is this kind of modelling that we have done in the enzyme and in the immobilised enzyme? What is this modelling called? What is this? There are two broad kinds of models model types; two very broad types of models, and we are at the end of the course, and we have done this kind of modelling also. What.

Matrix

No, two very broad kind of models. Let me write down.

(( ))

Deterministic model and the second one is probabilistic or stochastic. What is the difference between the deterministic model and the stochastic model?

(( ))

Everything is deterministic. What did Einstein say? He said that God does not play dice which means that there is nothing that is probabilistic in the universe, everything is deterministic, but if you can determine everything down to the last you know, if all information is available, then you can determine everything. For example, you know you have to all know about the butterfly effect right, that if a butterfly flaps its wings in the Amazon, then there could be thunderstorms in Australia, rains in Australia. So, that is the butterfly effect that mean the cause and effect. So, every little thing leads to every little thing.

So, can you predict deterministically this whole process of what is happening in Amazon and what is happening in Australia? Yes you can provided, all kinds of data is available to you. What do you mean by all kinds of data? You have to know what is the ocean currents, what is fluid mechanics of the ocean is, you have to know what is the fluid mechanics of the wind is, you have to know what kind of impedances are there in the system. So, you have to know all kinds of things.

So, once all these information is available to you, then you can write a full deterministic model that can predict from the starting from the t equals 0, the butterfly flaps its wings may be twenty four hours later or couple of days later; that is thunderstorm in Australia

and everything could be described, but when you cannot describe that, then you resort to probabilistic methods.

What I want to impress upon you the fact is that there is nothing probabilistic. Everything is deterministic, but the fact is that we do not have access to such large amount of information and as a result, we deserve to probabilistic or stochastic model right. Is it clear? So, probabilistic or stochastic model is a result of the fact that we deficient in information.

Similarly, here in this example that I was showing, this is the deterministic model, but this model is, we have to go for something like stochastic model. The reason we have to go for something like a stochastic model is because we do not have enough information on the system. We do not have complete information that where my coated pits are, what is the diffusion pathway that the receptor it take. If I had this information, if I was monitoring a particular cell and the whole receptor ligand binding process and the migration of the coated pits to a certain region, then I would be able to come up with the deterministic model.

If I want to do deterministic model, then what I would do is that write a diffusion model; free diffusion followed by these certain area. So, essentially if you look at the cell membrane, if you something like this, then my receptor is, if this is my receptor, the receptor is moving everywhere, and you have these small regions over here which are like glues. You will have this small region. So, whenever the receptor comes over here, it sticks and then you what did you do model, what did you keep account on in that case? You keep account on the... what would be what would be the thing that you model? Is it the concentration, what is it?

(( )).

Number of what?

(( ))

What receptors?

That can be attached.

Free receptors, number of free receptors. So, you are going keep an account on the N R N R f; let say or something like this. So, number of free receptors. So, the number of free receptors is roaming around everywhere, and as soon as they come and there is a coated pit out here, they stick to the coated pit which means, then you immediately. So, that is like a sink term. So, that is like a sink term in your model and you get rid of the... you remove. So, that would be deterministic way of looking at it. But given this you know this kind of modelling would take a lot of time and it is possible, but again it is not impossible, but it is possible, but it will take lot of time.

So, given those constraints, what we come up with is a stochastic kind of model. And the stochastic kind of model is essentially based on Brownian motion, random motion theory you know Brownian or kinetic or whatever. So, and I will show you that in a minute what we do, but what we do, it is a first, probably it is a first time you are encountering something like this is that we model the entire diffusion and reaction process that happens over here as a reaction process, but is that a reaction process? No, is it limited by the diffusion or the reaction? What do you say?

Diffusion.

Diffusion of course, it is limited by the diffusion which means that diffusion is the dominant process over here, but we model it as a reactive process. So, one of the things I do not want to you have an impression is that, when I write rate constant; these are not rate constants of the receptor binding to the coated pit; no, because those we have already discussed, those are very large numbers, large rate constants and those are not the numbers. So, this is the overall diffusion and the reaction model together as a diffusion process.

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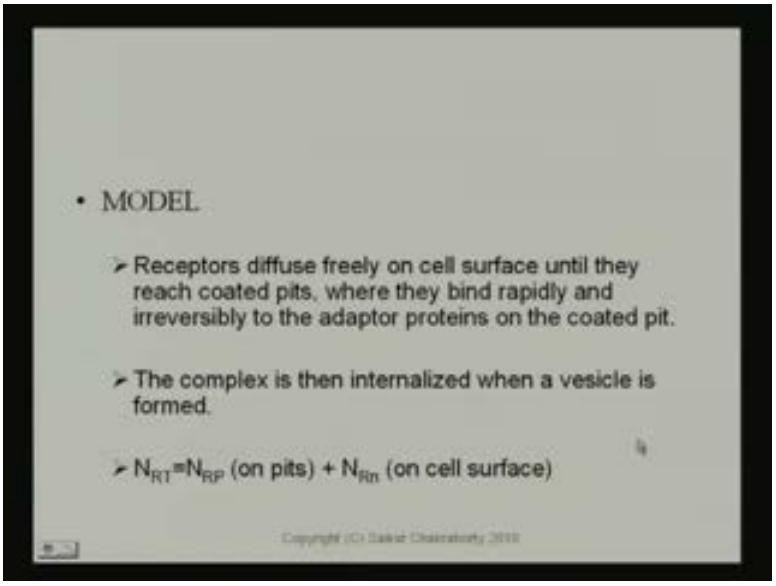

So, let us go back to the screen. So, this happens and the complex is then internalized when a vesicle is formed. Now this is, before we go in to the model, this is this is that definition that you need to have. Now, the total number of receptors that are there; apart from the one say let us forget the ones that have been internalized. This is just a total number of receptors at the start of the process are, we divide them into two different kinds; one which are on the pits and one which are not on the pits; rest of the cell surface. So, N RT is the total number of receptors at time t equals 0, which means that nothing has been internalized yet, equals N RP which is on the coated pits plus N Rn which is on the cell surface, fine.

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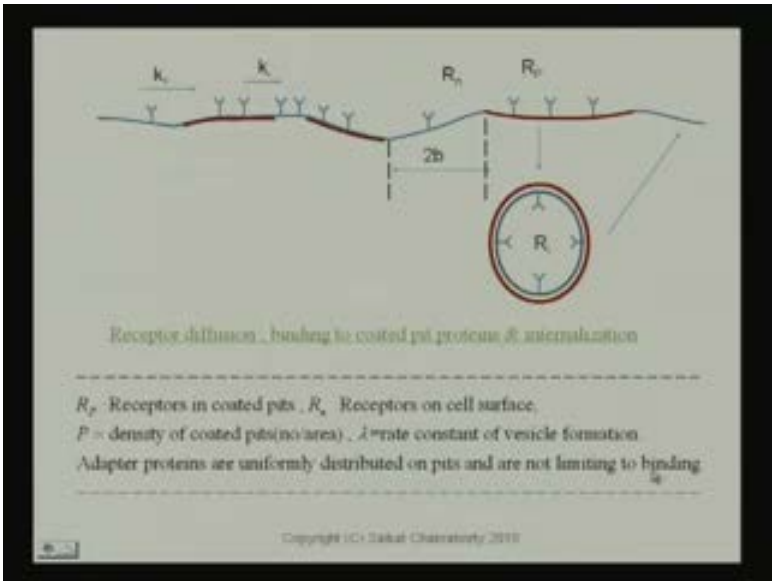

So, now this how the process looks. So, here. So, these are the free receptors N R n I think know N n I yeah N R n, this is the N R n, and this would be N R P now, fine. So, these receptors are moving in this direction and moving away, and this is the coated pit and 2 b is the distance between two coated pits. And we will also have the radius of the coated pit, once the coated pit is formed, we will have the radius of the coated pit.

I will come to that, but look at these two rate constants out here in on the top here; k plus and k minus. So, what are these rate constants, k plus is the affinity of the receptor towards the coated pit. So, it is a random diffusion event out there plus the reactive uptake or reactive binding those two, and k minus is the lack of affinity or the tendency of the receptor to move away from the coated pit because though it is unlikely that receptor that is there is going to move away from the coated pit, but still we include it.

Can you tell me why? It is very unlikely because there is a rate constant is very high in the forward direction, but still we include this k minus. Why is that? And it is something to do with very basics sorry yeah. So, why do we have k plus fine k plus there is no argument or no doubt about k plus, why do we have k minus.

So, though the backward rate constant is low, but still that could be a backward rate constant; that is not the point. The point is that see diffusion is occurring, and diffusion is a random process. Diffusion can occur in any direction. So, you have to keep the possibility that it can diffuse out of the coated pit. Is that clear? See that is the one of the

basic things that, diffusion is a random process and you can take in every direction. There is nothing that stop stopping you that it has to be in this direction or that direction, and these are lateral diffusions that can occur.

So, if there is a concentration or a chemical potential or a number gradient for the receptor out here, you know in this direction, so, the there is a possibility that the receptor might move away from the coated pit. Though that possibility is could be very low, which means that k minus could be much lower than k plus, but still you have to retain that possibility. And then this coated pit, as it is, will form a circle and we will have to take account the radius of the circle.

So, the definition that the symbol that we are going to use over here is R P is a receptor in the coated pit, Rn is the receptor on the cell surface, and P is the density of the coated pit or number of coated pit per unit area, and lambda is the rate constant of vesicle formation. So, what we are trying to do over here is that we are again trying to separate out the internalization from the vesicle formation.

So, why do I need the density of the coated pit? So, coated pit, density of the coated pit is in terms of number per unit area, and why what are we doing with what balance what kind of balance are we doing over here? We doing the number balance, remember. So, keeping a track of the number of receptors, we divided them up into those which are on the cell surface and those which are on coated pit, and why do we need the density of the coated pit, you tell me?

(( )) number attached (( ))

But still why do we need that?

(( )) number of (( ))

Yeah, or in other words, you know that is not exactly true, but conceptually its correct in other words, more is the amount of you know more is the density of the coated pit, then the more is the possibility of the receptor binding to it, right. So, if P is the density of the coated pit, and if that density had is the density, more is the possibility of it binding to it, and that is conceptually correct. The way it will come in our model is likely different and you will look at it what is going on, and lambda is the rate of vesicle formation.

Adapter proteins are uniformly distributed in the coated pit and not limiting to binding. This we already discussed that proteins are there, and the reaction is suppose to be very fast, and this is not what is going to limit. And why are we saying adapter proteins are uniformly distributed? What is the significance of this word? So, that we do not discriminate based on where we are on the coated pit. You know as long as we are on the coated pit, if the adapter protein; the three adapter proteins that are there and uniformly distributed on the coated pit, it does not matter at what geographical location on the coated pit we are. Is that clear?

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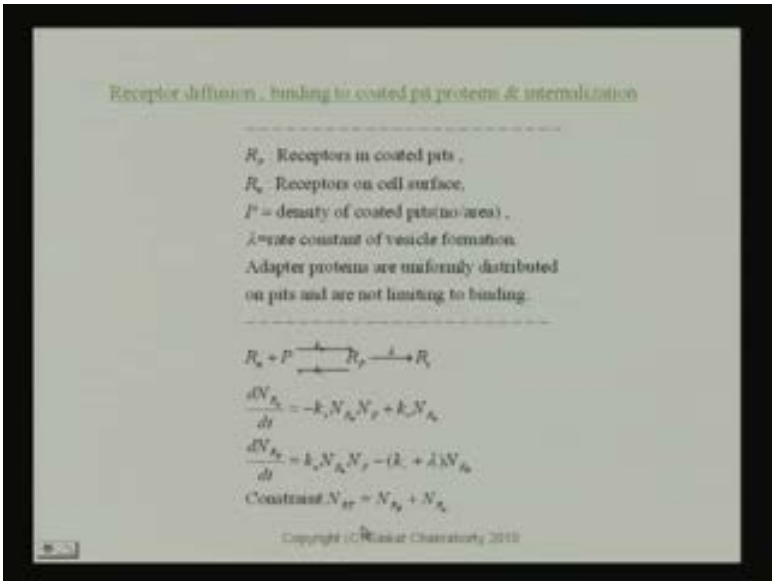

Now, let us go and look at the model (( )) in the arrows over here, but that is not a problem I think. So, as I said, the symbols are: R P is the receptors in the coated pit, R N is the number of receptors on the cell surface; receptor on the cell surface, P is the density of the coated pit or number per unit area and lambda is the internalization vesicle formation right rate constant for vesicle formation. So, you see over here, the way we represent it and what he says you know is correct, that greater is larger is the number of proteins on the cell surface on the coated pit, more is the possibility. And the way we include it is, making it a second order reaction instead of a first order.

So, see we have to understand this that this process, why am I talking about this entire process that we are talking about is the conceptual process. In reality, this is not the reaction. It is not the first order reaction or second order reaction just like that. So, we are

conceptualising thing which is diffusion with respect to reaction. So, when diffusion occurs, if the concentration or the density of the coated pit in a certain region is more, then the probability of receptors migrating to that particular region is also going to be higher right. So, that is what is incorporated through this second order model. Is that clear? There is it is not a reaction taking place as it is, but we are conceptualizing this diffusion followed by reaction process by a reaction process, and these rate constants k plus k minus that we have over are not real rate constants. They are called diffusion limited rate constants and I will come to that.

So, the reason they called diffusion limit because actual reaction is not limiting. As its written over here, the proteins bind very fast and the whole system is limited by diffusion. So, we write it as a second order reaction. So, R N being the free receptors binding with P; the proteins on the on the coated pits, k plus is a forward rate constant I mean; so called rate constant, k minus is a so called backward rate constant, and R P to R i; that you can you know conceptualise, can you see that screen. So, R P to R i is you can conceptualise it as a reaction, and lambda is a rate constant for that.

So, d dt of N R N is just minus k plus N R n times N P plus k minus N R n and dt of N R P then this will both these terms will come in. So, the forward reaction; formation of N R P and N R P loss due to backward reaction and the internalization process.

So, N RT is still that total number of receptors is still N R P plus N R n. Why is that because we still have not considered receptor regulation? We will do that in the let us get done with this model, and then the next part of the model that we do now we will consider receptor regulation.

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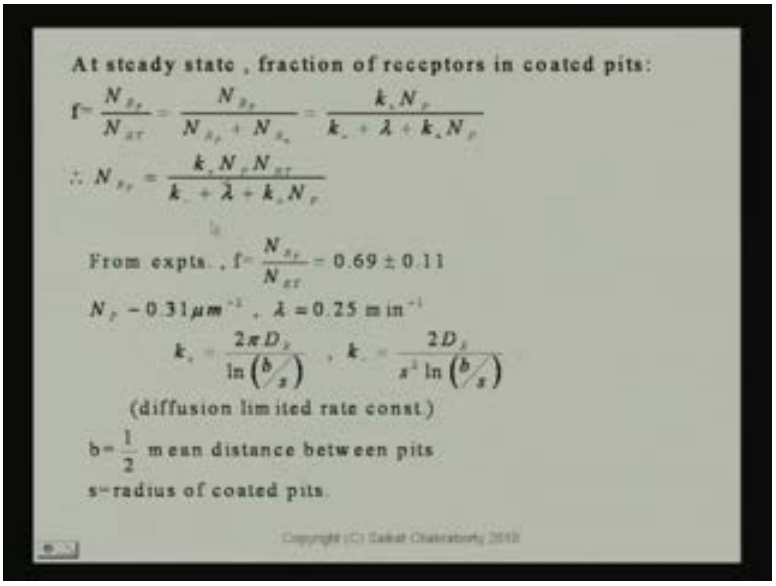

So, at steady state, N R P of the fraction of receptors in the coated pits is given by N R P over N RT. So, which is because of the constraint, this N R P over N R P plus N R N and that is given by from here, at steady state. If you equate these two equations to the left hand side to 0, you can come up with the relationship between N R P and N RT. That is what we have done over here, and the whole thing can be written in this. So, N R P could be written as k plus N P over N RT and N P is related to the protein density on the cells surface.

So, it turns out that from experiments. So, this is this is the fraction that is important and that we can measure, which is the fraction of the total amount of receptors that are on the coated pits. So, that number comes out to be around 0.7, experimentally that is measured to be around 0. P is the concentration of protein concentration. So, though you know no unit of concentration is there. So, it is a number, it is not concentration its number per unit area. So, point three one per micrometer square micron square, and lambda is the rate constant. So, it is 0.25 and inch inverse.

And this is what I think I would like to tract your attention to; this k plus and k minus you see over here. Is this the first time you are encountering something like this, diffusion limited rate constant or have you seen this before? No. So, this is based on probabilistic theory. This is obtained from probabilistic. Does it ring the bell; have you seen something like this ever before?

Yes.

Where?

Heat transfer

Heat transfer where exactly?

(( )) cylinder (( ))

No, not in concentric resistance, but somewhere else. I have talked to you that, in one of the earlier lectures of heat transfer, I taught you whereas, this is not deterministic, this is not obtained from the... that was the deterministic model we did. This is not obtained from the deterministic model; heat transfer in gases, do you remember? Not k plus and k minus, but something similar to this. How do you obtain the thermal conductivity in gases? You remember that, we did that in the class.

So, that had to do, the thermal conductivity in gases had to do with kinetic theory and these parameters that are coming in between here, came in there. So, here b for example, so, the k plus the forward one is given by 2 pi D r over l N b over s and the backward is given by 2D r s square over l N b over s. So, if s is the radius of coated pit is large as compared to b, then the backward rate constant is; obviously, must as you can see; obviously, much smaller than the forward rate constant. Now what is this? b is half the mean distance between pits, and this the analogy that you have is in kinetic theory is the mean free path of the between the molecules.

So, let us go back to the picture and you can see. So, 2 b is the total distance; mean distance between mean distance of course, because this is probabilistic models. So, 2 b is the mean distance between two coated pits and b is half the distance and s is the radius of the coated pit. So, once the coated pit is it is not the radius of the curvature, but once the coated pit becomes the vesicle, so, it is a circular thing and you can get a radius or an effective radius at least. So, s is the radius of the coated pit. And as you can see over here, so, what is D r, what is D r?

(( ))

Diameter of the cell. No.

Diffusivity.

Diffusivity of the receptor, yeah. So, see look at these two rate constants; k plus and k minus over here. There is no reaction parameter out there at all. So, that is what I was trying to say that these are not reaction rate constant any which way, and the reason that reaction parameters do not appear over here is that because reaction is very fast. It is irreversible and very fast. It has nothing to do with the process. And all we are trying to do is complicated diffusion process; we are trying to model using reaction.

So, you can go and look up a little bit more if you are interested in Brownian motion theory or (( )) you know kinetic theory. So, how to do this? So, and that is interesting area and if you are interested, you can look at this. So, we just we are just going to use these two these two rate constants, and once we use this you know they are here k plus k minus and lambda, you can get the fraction of the receptor.

Then you can incorporate that into your model and it is your job is an assignment actually to do that to incorporate that into a model and show what happens, but I gave you this is the model, but what I am asking you is that how do you integrate this into the earlier model, right. We have the earlier model over here.

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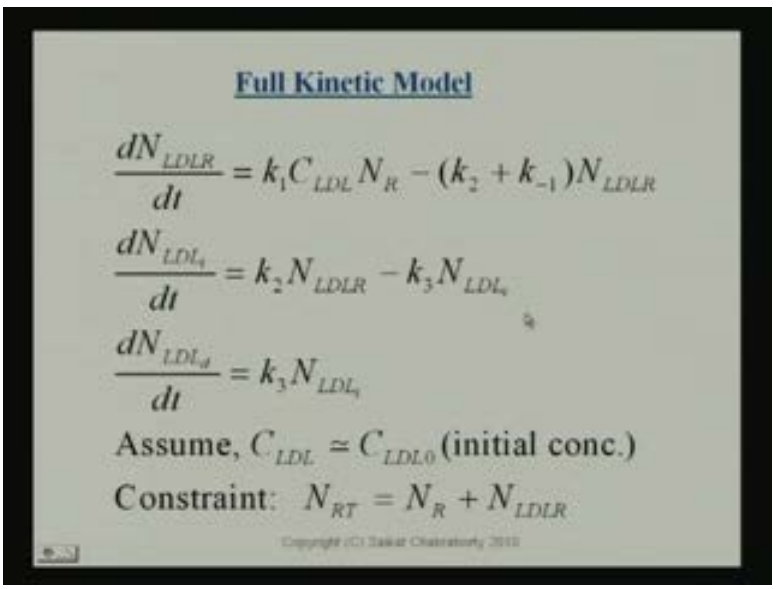

Let me go back here, this was a full kinetic model. So, if it is a, assume lets its say a class 2 reactor receptor. So, how do you this model for the receptor binding to the coated pit? How do you integrate to this model?

The second equation (( ))

By fraction of f into?

Fraction of f into N LDLR

So, N LDRL is essentially is our N RT that we talked of, right. It is essentially, it is not N sorry this is not N LRT. You have to split this up first, you have to split this up into two parts. So, N n LRT would be N R plus N R N plus N R P plus N LDLR, but I do not think you just replace it by fraction times that probably not just a little bit more because...

Internalization (( )) dependent on the (( )) that part which is associated with the coated pit (( ))

Correct, but there is a step of the vesicle formation also. You are skipping that.

Vesicle formation is after internalization.

Yeah after internalization, but we have to differentiate between these. So, what I said is that this is no longer, N LDL is no longer and you know then you have to form the N LDL I, then you have to have the N n LDL I will have the vesicle formation reaction into it. So, just have a look at that you know think about how to integrate. So, there are two steps; one is that coated pit attachment to the coated pit and then the vesicle formation. So, how do you integrate that with this model? So, that is the task that I give you and you can do that on your own.

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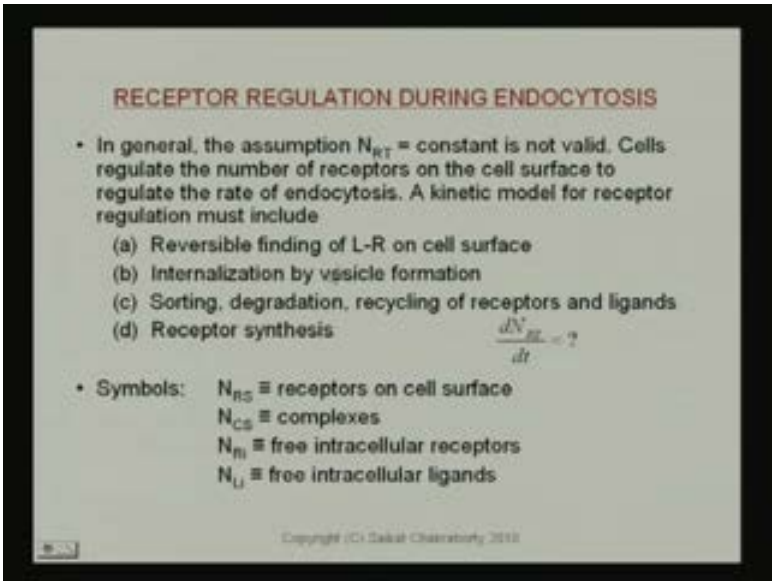

Let us move on to the next the last model that we do in this chapter which is the regulation; receptor regulation and try to finish we do not have a lot of time, but let us try to finish it in today's class. So, regulation receptor regulation during endocytosis. So, as I said that you know receptors are not necessarily receptor number is not necessarily constant. Till now everything that we have assumed, we have assumed that receptor number is not necessarily a constant, but receptor number is regulated. So, here what we saying in general, the assumption that N RT is constant is not valid. Cells regulate when we talked about this; cells regulate the number of receptors on the cell surface to regulate the rate of endocytosis.

So, at points of time when there is a immune attack on the system or anything you know you probably have a lot of protein and the body for some reason wants to internalize this protein, it is going to up the increase the number of receptors that is available for uptake, and as a result increase accelerate the process.

So, kinetic model for receptor regulation should include reversible binding receptor (( )) reversible binding of L-R on the cell surface, internalization by vesicle formation you know all these. Sorting, degradation; these are things that we are not talked about; sorting, degradation, recycle of receptors and ligands and finally receptors synthesis. So, whenever you talking of receptor, so, what have we looked at?

Till now, in this model, what we have looked at was even if you add this was up to the internalization step and you know the coated pit and vesicle formation, but we had not looked at that what happen after internalization. The decrease in the p H; we have talked about. But the fact that once p H is decreased, then it splits up into two parts; one has a ligand, one has a receptor and the receptors are send through microtubules back to the cell surface. So, when you are going to take a total count on the number of receptors, you obviously, have to include all of these all of these effects right, which is the recycling of the reactor back to the cell surface and the final thing is synthesis of reactor. Sorry, one more thing; degradation; degradation and synthesis.

So, receptors are being destroyed. Receptors are being formed. So, three things that are happening and that we have not looked at and we need to look at now. One is recycling back of the reactor to the cell surface a, b is degradation or destruction of the receptors and c is synthesis of these reactor. So, how would we incorporate these into the models? It is not hard and let us do that, quick.

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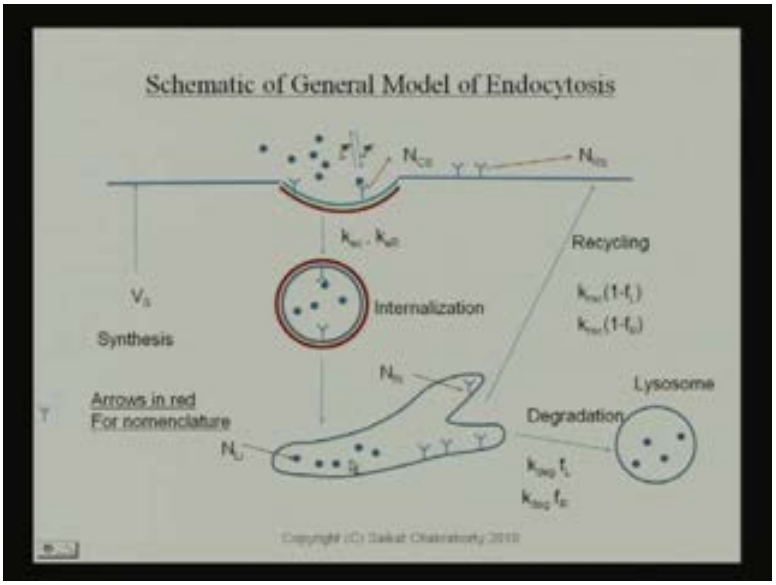

So, this is the picture that we need to look at. This is the picture contains all the effects. So, this is complete model and what is incomplete, what is missing? Can you look have a study second look at the picture and let me know what is missing in the picture. If I ask you to do complete model, this is I wrote this is a schematic of general model. General

model is fine, but if I want to want you to make a complete model, is this is the complete or something missing?

(( ))

Right, the step that we discussed earlier about the binding to the coated pit is missing out here, and if ask you to do a complete model, you have to add that step in and do the kinetics. It is not easy actually you know for not writing the model, but solving this model is not easy actually. So, let go step by step.

So, this is the case where the receptor ligand binding is occurring on the coated pit. You add the binding of the receptor; free receptor to the coated pit or the ligand complex through the coated pit depending on the class of the reactor, add that part over here, then this is the internalization that is taking place. Now as you see over here that we have these two rate constants here; k e c and k e R. Can you intuit what these two rate constants are? Why two? Why do we have two rate constants; k e c and k e R? What is k e c, what is the name suggest?

(( ))

No it is a complex. c for the complex, that is what...

(( ))

Yeah, one for the complex, one for the receptor, but why that?

There would be some free receptors.

There would be some free receptors. If it is you know if the receptors are already bound to the coated pit, then there could be some free receptors which are there, which are not binding to the complex, but they are on the coated pit. So, we have to incorporate them when you go to the internalization process.

So, the rate of... when the internalization of the vesicle formation is there, the rate at which the, this is free this is for free and this is complex. So, the total amount of receptors that are there is not necessarily equal to everything that is there in the complex form, some are free. So, then this is this is formed. Now then this vesicle that is formed and you know then the late endosome and all that stuff and the early endosome and then

what happens between here and here? The what process, acidification happens, and these hydrogen pumps open up and pump in hydrogen as a result the system is acidified, and when the system is acidified, what happens, then the here what I am pointing to?

(( ))

The k minus 1 will increase. The dissociation rate constant will increase. As a result, the complexes that are there now are going to dissociate, and then they are going to go to two different parts. So, this receptors will as usual is sticking to the cell surface and the ligands will be floating around.

Now this ligand that you have, ligand and receptor both are partially... So, this is general model and it takes into account all kinds of possibilities. So, there are two possibilities; one is that the receptors could be recycled back and there is also possibility that some of the receptors are degraded. So, you have to account for that possibility. So, what we have over here is that k f l is the fraction of these receptors.

So, if the total number of receptors say N or something whatever that is there in N R I, then f l is the f R, sorry f R is the fraction of receptors that are being degraded, that are being lost in the process because there is always going to be some receptors that are going to be lost, and also you remember couple of lectures back, we talked about the lifetime of receptors. How many rounds did we say the receptors can do? 150 rounds, 150 rounds of receptors can do at the end of which it is going to be degraded.

So, at any point of time there is going to be some receptors which we have finished the lifetime you know and they are going to retire. So, f l is the f R is the fraction of receptors which are degraded, and if f R is a fraction of receptors that is degraded, 1 minus f R is the fraction of receptors that are going to be recycled. Now these are then preceded by the rate constants because fraction is fine, fraction is just concentration, but the rate at which this degradation or the rate at which recycling is occurring is also important. You have to take that into account.

Similarly, for ligands also, not all the ligands all the ligands preferably they would like all the ligands to go into the endosome and be used, but not all the ligands are degraded or entrapped in the form of lysosomes and used up. Some of them can be recycled back. There is a possibility that some of them can be recycled back.

So, this is the overall process. The last thing that we need to add is the synthesis; V s is the synthesis of the reactor that is that is happening. So, at any point of time, you can assume that V s this is amount of receptors that is synthesising. So, I wish I could keep this, do you want to give it a short or shall I let see how much time I think.

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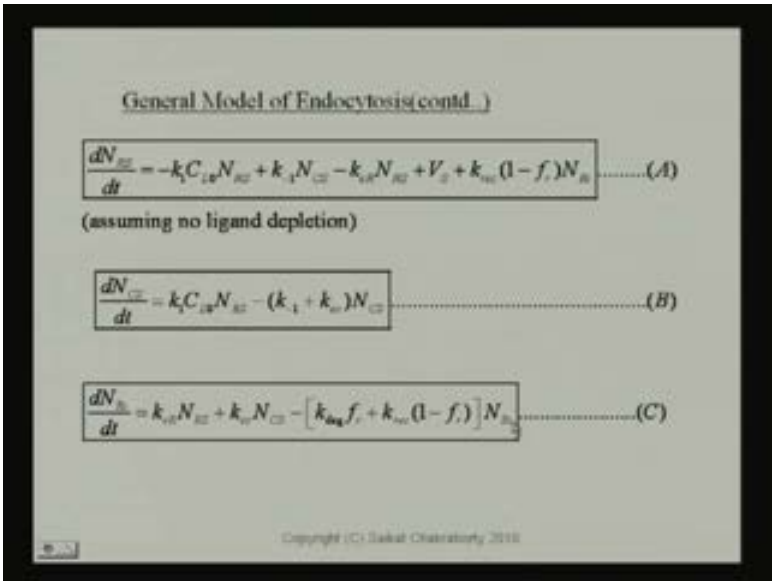

So, shall I just give the model because lets go back and back and forth from the model to picture. So, N RS is the receptor you know. So, let us N RS is the receptor on the cell surface. So, that is the nomenclature. So, the receptor on the cell surface, this is what is minus k 1 C L naught N RS; that is the formation of the complex right, and this backward reaction k minus 1 N c s, let us go back again. So, k minus 1 N c s is the backward reaction and forward reaction is C L naught times N RS times k 1 fine. So, these two terms, then k e R N RS; that is that is that is what is taken up here fine; the receptor the number of receptors that would go that are going into the vesicles, fine.

So, receptors are formed and combining with combining with this to form complexes, but some of the receptors free receptors are as it is going into the vesicles. So, that is this k e R times N RS. V s is the synthesis of the receptor on the on the cell surface and the last term k R e c f r 1 minus f r times N R i is the recycle that occurs. Let us go back to the picture again. So, this is the recycle that is occurring.

So, N RS if I am keeping a track on the number of receptors on the cell surface, so, what is happening is these two processes; backward and forward reaction, internalization or

encapsulation in the vesicle and recycle back and of course, the synthesis. So, this is the total number on the cell surface. Complex is easier by the assumption here is that C L naught you know that no ligands depletion that assumption is there. Therefore, C L naught is used here.

So, complex formation is more straightforward. So, k 1 C L naught N RS minus this. So, there are two processes; one is formation; that is, forward reaction, one backward reaction and the other one is internalization; just three. Then what else do you think what are the... just go back to the picture what are the things that we have to take do a balance on you have to do a balance on N R i; that is the internal receptors in the internalized form. So, that is what we will do now.

N R i, N R i is it does not have anything to do with N R i would not have anything to do with what is actually happening on the cell surface. It had would have to start from the invagination or the encapsulation process. So, N n R N R i is given as k e R times N RS times k e C times N c s minus these two. Why this added, why is is k e R times N RS, fine agreed. I think nobody would have a problem about this. Why k e C times N c s, let us go back to the picture then you can see. So, what is N R i? N R i is a receptor all kinds of receptors that is inside the endosome.

(( ))

Right yeah. So, what happens here that combined receptors that are there in with the ligand, once the pH is decreased with the system is acidified, they also gives this receptors within the internalized form. So, that is resultant both from the both from the free receptors and the combined, and these two are things that you know. One is the these two are the losses that are happening in the internalized receptors; one is because of degradation of the receptors which is given as k degradation times f r times N R i, and the other thing that is happening is the recycle which is k R e C times 1 minus f r times N R i.

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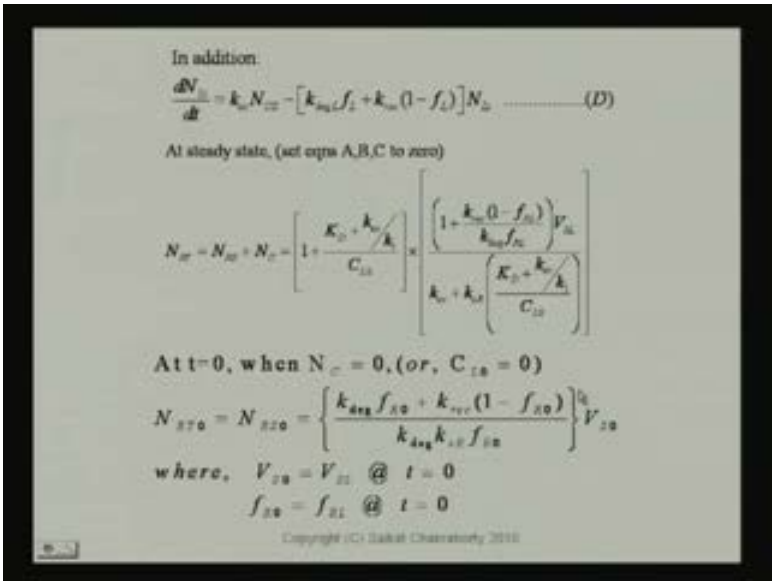

Now, in addition, the last balance that we writing over here is N l I, which is the ligand in the internalized form. Why do we have to the (( )) the ligand in the internal form because unlike before where we had assumed that all ligand that are internalized are being used, here that is not the case because some of it is being degraded and some of it is being recycled back. So, the first part is all right like we had before k e c times N c s, but these are terms we did not have before because we are we did not take into account the recycling and the degradation process.

So, minus k degradation times f l. This is the degradation and minus R e c k R e c times 1 minus f l and both multiplied by N l N l i. So, this is what we have. Now if we want to study the dynamics of the process, we simply go and simulate this process. As you can see over here, the solution of this is not as easy as the solutions before and we have already made this assumption that the ligand depletion does not occur, but still it is the solution of this is not as simple; the reason being that you know first N R I is its coupled you know the entire system is coupled. So, we have N R s over here, N RS over here N c s, N c s over here, N R i over here, N R I over here all cross terms out there a, and b is also because we have quantity is like V s which can...

(( ))

Vary with?

with time.

With time, right.

So, V s itself the synthesis is that that is how regulation processes is you know effected because essentially what you do, what is the cell do, it regulate the system by synthesising more at some point of time or less at some point of time. You can regulate what could be the other ways to regulate that you have the sort of equations in front of you. What would you think would be could be other ways for regulating? ((No audio from 47:54 to 48:01)) What are the other parameters? If the cell wants to regulate at a certain point of time, it just decides that it wants to increase the number, how you regulate. You have for example, immune attack on the system what would the cell do it would like to have like to increase what which variable, you have four variables out here, which one would you like to increase?

V s

No variable. V s is not a variable, you have four variables out here N R s, N c s, N R i and N l I which variable would the cell like to increase.

(( ))

N R s, right. It would like to maximise the total number of free receptors on the surface so that they can take up the ligand. So, if it wants to do that then you see the screen. So, what would be my parameters what. So, N RS did it. So, essentially what would you like to do, it would increase d dt of N RS, right on the cell surface. It would mean like to increase the number suddenly. If it wants to do that, so, this is that is your clue. So, equation a, what parameters can you tune you know; it is like a control system. So, what parameters would the body or the cell tune so that you can increase the number of receptors? Fraction is not possible, but yes you should tune these rate constants.

So, k r e c and the k e r; not the k e r sorry, k r e c essentially. So, k r e c is something that you can tune. You may be able to tune the fraction, but you know that is hard to tune because this rate constants are functions of time. The cell can increase the rate constants; cell can decrease the rate constants. Rate constant again you know what you what you mean by rate constant is that, really a rate constant; it is probably not a rate constant, it is

just the speed at which this is this whole thing rate constant is a inverse you know this would have the (( )) this is first order rate constant. So, this will have just inverse of time.

So, rate constant is just an inverse of the speed at which this whole recycling process is occurring. So, that it can change. It can accelerate the process as a result of which, the rate constant will decrease or increase, and rate constant will increase; it accelerates the process. If it wants to decelerate the process, then the rate constant will decrease. So, that is something is easier to easier to control rather than the rather than the fraction.

So, you can do that analysis, but at steady state, this is what we do just a simple steady state analysis. You set all the equations to 0, and you obtain N RT which is now the total number of receptor which is N RS plus N c; that is in the complex form because the complex form takes into account all the things. So, the free receptors on the surface plus everything that is in the complex form and this is what it comes out to be, but yes of course, the thing that is important here to notice to note is that there is there some is there some meaning in what we are doing this steady state analysis.

The is there some you now is there some significance to the steady state analysis, that the steady state steady state function thing that we gave, function that we gave, is there some significance to it? Are you getting?

(( ))

Yeah I mean. So, what only thing that is yeah that is correct to some extent. See there is not much sense in doing this steady state analysis right here, what we did up to that steady state analysis makes the maximum sense. Why is that? Because when the cell is regulating the system that is hardly a steady state you know how can we talk about a steady state when the system is regulating? The only thing that it the steady state analysis is make sense is that you can measure N RT right. You can level the ligands and the receptors and you can you can measure N RT.

So, what is the only thing that is important that you can get out here; not all parameters this is one particular parameter that you can measure easily which is V s l; the synthesis rate of steady state. You want to measure what you synthesis rate is steady stated, and that at unsteady state, you can similarly measure the synthesis rate and then you can compare whether the synthesis rate is increased or decreased. Otherwise a steady state

analysis does not have you know does not cut my choice because it when you talking of regulation, it is essentially intrinsically unsteady state process.

So, then we you know we are talking now we are talking about the unsteady state process and if we are to solve this unsteady state process, this is these are my initial conditions. you need initial conditions to solve. So, t equals 0, we assume that the number of complexes is 0, N RT naught which is the initial condition for the reactor; that you can get from this steady state. Is it clear?

So, what is happening is that steady state is when the system is not requiring itself to regulate. There is no immune attack nothing is happening in the system and the system is questioned and then you have the steady state. For example, sudden suddenly a immune attack occurs and the system wants to regulate, increase your V s or whatever increase your k r e c or whatever, it wants to do. So, then for that your initial condition is going to be your steady state. Is that clear? So, this is in no way similar to what we did this perturbation we did this in the chemical the chemo stat problem. We took the steady state and we perturb the steady state.

So, essentially you can think of this as physiological perturbation to the steady state. That is what happens right, when you when you get sick. So, you have a steady state, when you are normal when you are doing all right, you body functions at a at a particular level you know some sort of homeostasis there, then you when you get sick, it there is a perturbation from the steady state. So, that is exactly what happens. So, whenever you get sick or there is an immune attack on the system, this is this is your steady state in general; normal condition this is the perturbation, but to evaluate that initial condition from where the system would start? You would need this steady state values V s naught is the synthesis at the steady state values, and then you can evaluate the rest.

So, I think that is all I wanted to talk about in this chapter I hope that you know this follow something there is yeah. So, do you have any question in a minute or so, we can we have, we have almost run out of time, but you know this is the entire process; that is, why put the picture out there and one of the things we learned from the processes that you know it depends on the level of complexity. You want to go through, you can come up with the very simple model and then you can increase the level of complexity one step at a time. So, is there any question on this? So, otherwise we will conclude this

chapter which is on the receptor-mediated endocytosis. So, if there no questions, then we will conclude.