Medicinal Chemistry
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Module 02
Lecture 12
Tutorial-04 Receptors, Binding Interactions, Ion channels

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Welcome to the tutorials session. So in today's session we will look at some aspects of receptor structure and function like we have learnt in the past couple of lectures.

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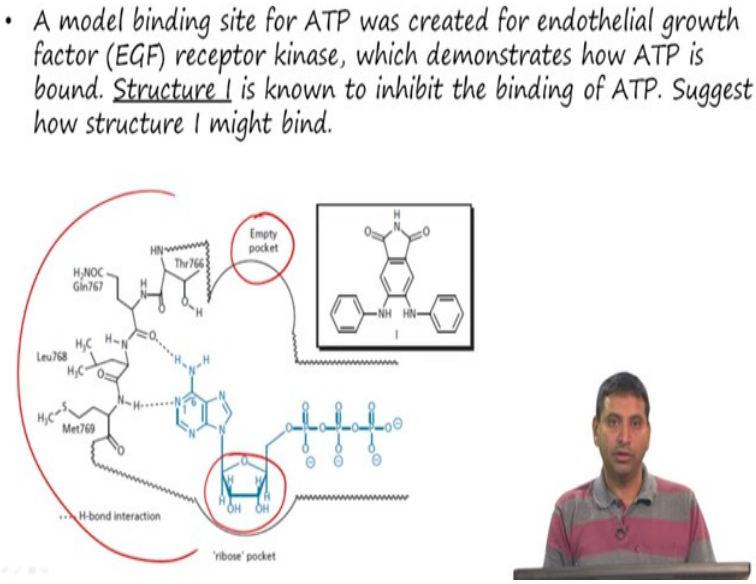

So we will start with some problem solving. So in the first problem we are going to look at how one can design an inhibitor and what are the various aspects of how it binds to the protein. So the question is a model binding site for ATP was created for endothelial growth factor receptor kinase (EGF), which demonstrates how ATP is bound. So this is the model that was that has been proposed and so you have here the ribose binding pocket, you have an empty pocket here and you have a series of amino acids which are shown here and there are some hydrogen bonds that are proposed to be happening.

Structure 1 is known to inhibit the binding of ATP, so the question is suggest how structure 1 might bind. So in order to address this question we shall first look at since we already have the structure of ATP bound to the protein pocket, we will be able to suggest some important interactions.

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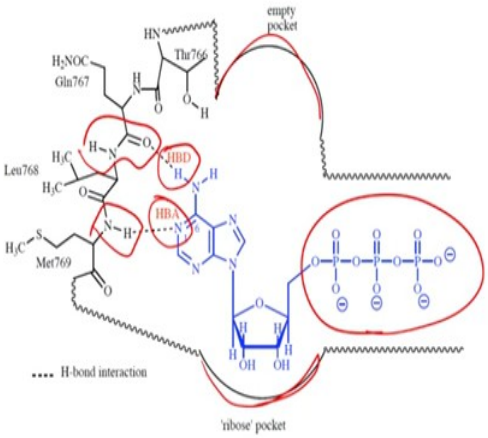

So if you look at the molecule, there is a hydrogen bond donor over here and there is a hydrogen bond acceptor over here in this nitrogen and correspondingly the hydrogen bond donor and hydrogen bond acceptor is shown over here, right. So and of course there is a ribose binding pocket over here and this seems to be an empty pocket and the triphosphate actually goes outside the binding pocket. So this is what we understand from the structure. So we shall assume that these hydrogen bonding interactions are important and proceed with trying to solve the problem.

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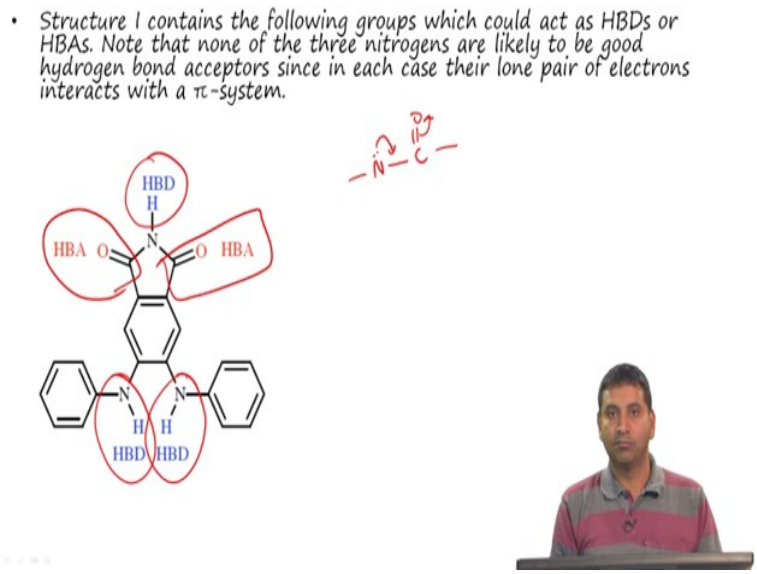

So if you look at structure 1, it contains the following groups that is this can be act as a hydrogen bond donor, this nitrogen can act as a hydrogen bond donor and so can this nitrogen. So you have 3 potential hydrogen bond donors in the molecule. You also have a couple of hydrogen bond acceptors in the form of carbonyls. So note that none of the 3 nitrogens are likely to be good hydrogen bond acceptors since in each case the lone pair of electrons interacts with the pi system.

So we have already seen that when there is a pi bond that is potentially possible then this goes in here and it interacts with this carbonyl and therefore these are not very good hydrogen bond acceptors. So that basically makes only the carbonyls as hydrogen bond acceptors.

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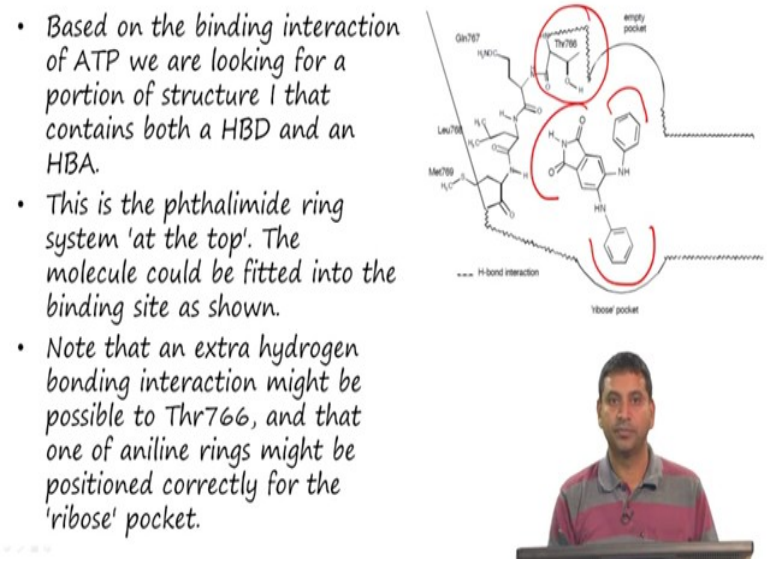

So with this in mind now let's look at the how to solve this problem, so based on the binding interaction of ATP so we are looking for a portion of the structure that contains both a hydrogen bond donor and a hydrogen bond acceptor. So there is only one choice here that is the phthalimide ring which is at the top, so the molecule can be fitted into the binding site as shown here.

So you have the hydrogen bond donor acceptor system over here and here might be the ribose binding pocket and since there is an empty binding pocket one can propose that the aniline would interact there. So that if you fit it in the following manner it appears that it could be an optimal fitting. So that an extra hydrogen interaction is possible with this Threonine 766 as shown here and that might increase the fitting or it might increase the stability of the interactions.

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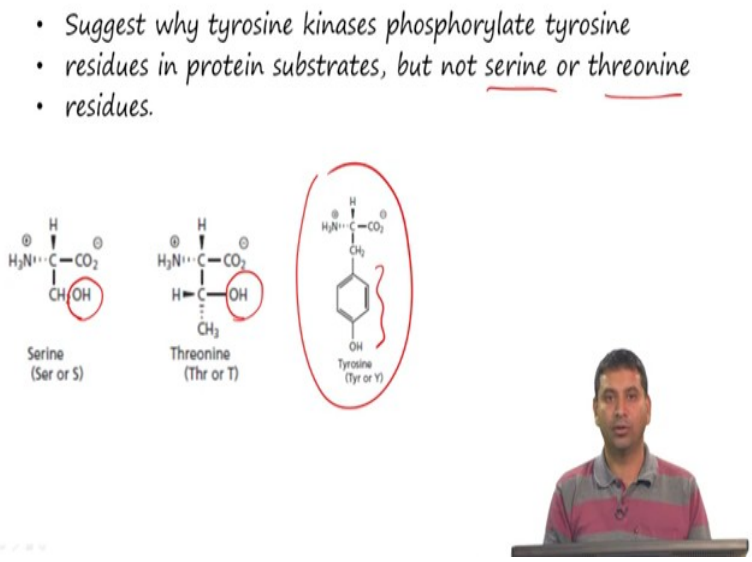

So the next question is suggest why tyrosine kinases phosphorylate tyrosine residues in protein substrates, but not serine or threonine? okay. So the structure of tyrosine is shown here which we are already quite familiar with, it has a phenol ring in this molecule and the question is that if this phosphorylation occurs specifically with tyrosine and if you see here that serine and threonine both have hydroxyl group which is very similar to tyrosine and they both can a principle be phosphorylated but what happens in this particular example is that tyrosine kinases are very selective. So the question is what is the origin of selectivity?

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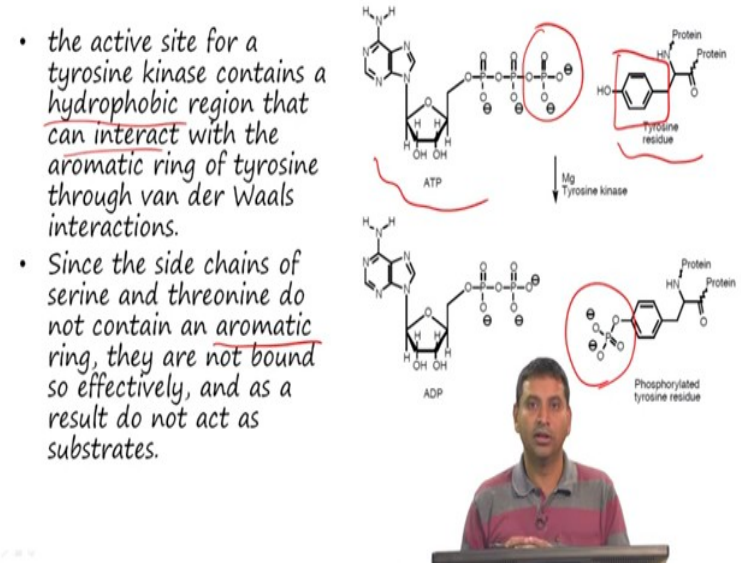

So in order to understand let's first look at how this tyrosine kinase functions? So you have a tyrosine residue in a particular protein which is the target of the tyrosine kinase and here is ATP so the protein will have to or the enzyme will have to bind to this ATP and transfer a phosphate over here as shown here and then it is going to react with the tyrosine and produce a phosphorylated tyrosine.

So as we know phosphorylation is very important reaction that occurs inside the cell and it is a major signalling event because phosphorylation of a protein changes the way in which the residue is going to function. So for example previously the residue was a neutral phenol and may be it will be little bit ionized to O minus depending on what other functional groups are there but once it undergoes phosphorylation, it introduces two negative charges on the protein and that is going to either attract or repel the corresponding groups around it and that is going to result in a major shift in the conformation.

So this is how the signalling in the cell occurs and now the active site for a tyrosine kinase contains a hydrophobic region is what we would infer because that hydrophobic region must interact with the aromatic ring of tyrosine. So if that aromatic ring if the hydrophobic region is not very or if this hydrophobic interaction is not very strong then it is unlikely that it would be selective.

So since the side chains of serine and threonine do not contain an aromatic ring they are not bound so effectively and so because they are not bound so effectively it is possible that phosphorylation does not occur to a significant level. So what we can understand from this

selectivity data is that there must be a hydrophobic interaction region in the active site of the tyrosine kinase and that region must be important in binding.

So if it is not present or if it is not important then perhaps the selectivity may not be as large, of course keep in mind it is a more complicated issue then this but one could at the minimal at a very simple level can propose such a model.

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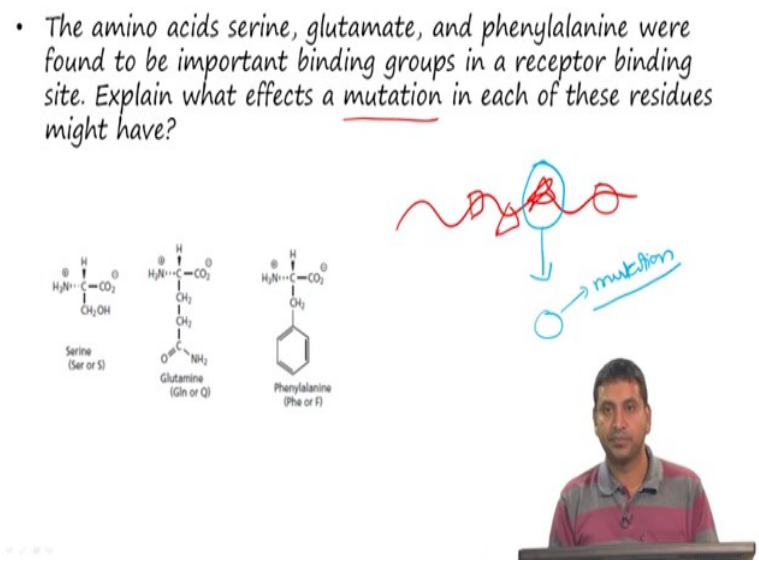

The next question is the amino acids serine, glutamate, and phenylalanine were found to be important binding groups in a receptor binding site. Explain what effects a mutation in each of these residues might have? First let us try to understand what a mutation is? So let's say you have a primary structure of a protein and there are many residues that are happening that are going on here which I am representing by various shapes.

Now if I change one of these residues let's say I have a something that looks like a star over here into let us say a circle then that is called a mutation. So mutation studies are very important and they can be done in a very engineered manner that means that I can do site directed mutagenesis or specific residue in a protein can be modified with great precession in today's technology, so carrying out mutations is not a problem.

So therefore the question here is if we mutate out serine, glutamate and phenylalanine which are important binding groups what would you expect to see?

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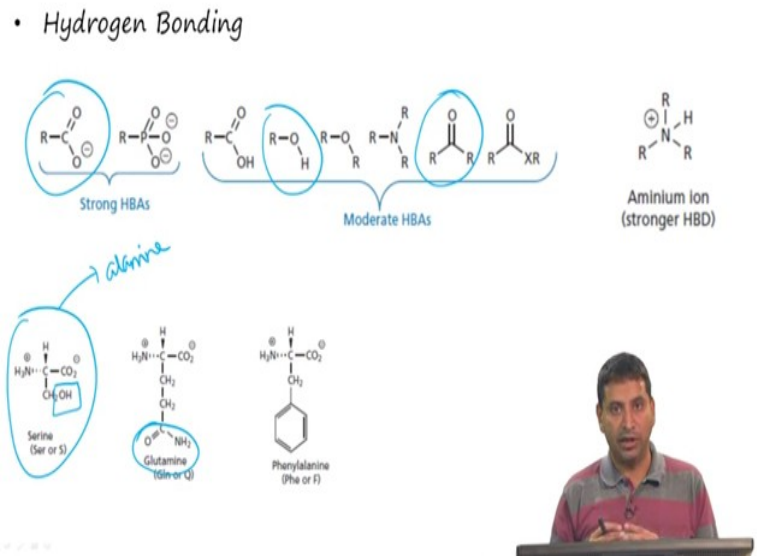

So let's look at for example what a various hydrogen bonding capabilities of these groups are? So we have already looked at that carboxylate ions are excellent hydrogen bonding acceptors, whereas carbonyls are moderate hydrogen bond acceptors and alcohols are also (moderate bond) hydrogen bond acceptors. So in this system here we have been shown we have been told that the serine residue is very important and as you can see the serine residue has a hydroxyl group which is a moderate hydrogen bond acceptor.

So now if you mutate this out and make remove the hydroxyl group altogether and convert this to let us say alanine which has basically a methyl group then if the hydrogen bond interaction is important for the binding then you would assume that particular binding is going to become weak or absent and may be the receptor or the substrate will not act as the same way as we would expect it.

In the next case you have a glutamine, so glutamine has an amide and the amide as you know is a moderate hydrogen bond acceptor in compare to a carboxylic acid and so in the case of an amide, you would have an if you replace this amide let's say with a carboxylic acid then you are increasing the binding interaction and if that binding interaction is important then you would find that the compound is going to bind stronger.

On the other hand if you completely remove it and you make it into a aliphatic side chain or if you converted into let's say serine then you are going to reduce the hydrogen bond acceptance or eliminate it all together and that is going to result in perhaps poorer binding.

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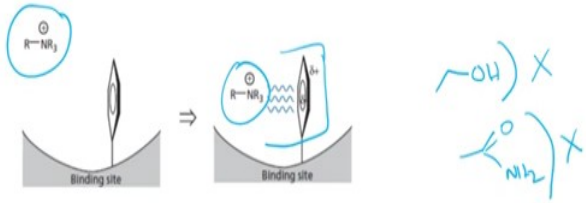

Induced dipole interaction between an alkylammonium ion and an aromatic ring.

In the last case you have phenylalanine, now phenylalanine can interact with many of the receptor binding ligands have ammonium ions on it and so this can actually induce a dipole interaction between an alkylammonium ion and an aromatic ring. So here is the induced dipole system which can be quite effective in binding ligands. So if you mutate out this phenylalanine let's say if you are going to remove this phenylalanine altogether then this kind of an interaction may not occur and therefore you might find that the residue or the ligand is not binding to the same extent.

So together if you take serine you have a hydrogen bonding capability and if you eliminate this systematically let's say one by one and if this is important and it has been shown that it is important then you will find that the ligand is not binding. Similarly, in the next case if you have a glutamine which has an NH_2 again if you remove this and if the binding is going to decrease then you can infer that it is important.

And in the last case you have phenylalanine which can involve itself in induced dipole interactions or even in vander waals interaction and therefore the receptor ligand must contain these functional groups or functional groups which are positioned in such a manner that they interact with these residues in a favourable manner. So based on this one can start constructing the various structures of potential receptor binding ligands which will interact with these residues.

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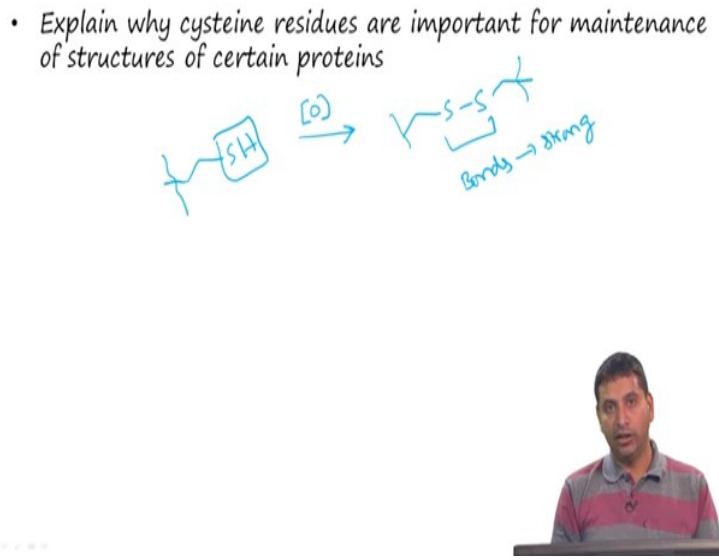

So the next question is explain why cysteine residues are important for maintenance of structures of certain proteins. So in order to address this question let's first look at what is the structure of cysteine. Cysteine has a thiol group in it which is shown here and this thiol actually is very important because it is one of the residues which can undergo a covalent modification.

So you have a potential covalent bond that can be found and as we already know covalent bonds are extremely strong and are difficult to break, so these kinds of disulphide bonds as they are called are quite strong. So if a protein structure depends on this kind of disulphide forming capability then it would contribute to the maintenance of the structure.

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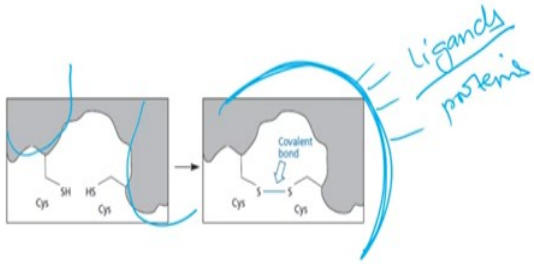

So if you had a covalent bond between let's say this part of the molecule and this part of the molecule then it is possible that the two residues because it forms a covalent bond can be brought together and this will provide a very unique shape to this molecule which can then interact with various ligands or other proteins. So therefore this shape that is (imp) that is brought by this covalent bond might be very important in its function. So certain proteins rely on the formation of disulphide bonds to maintain their structure.

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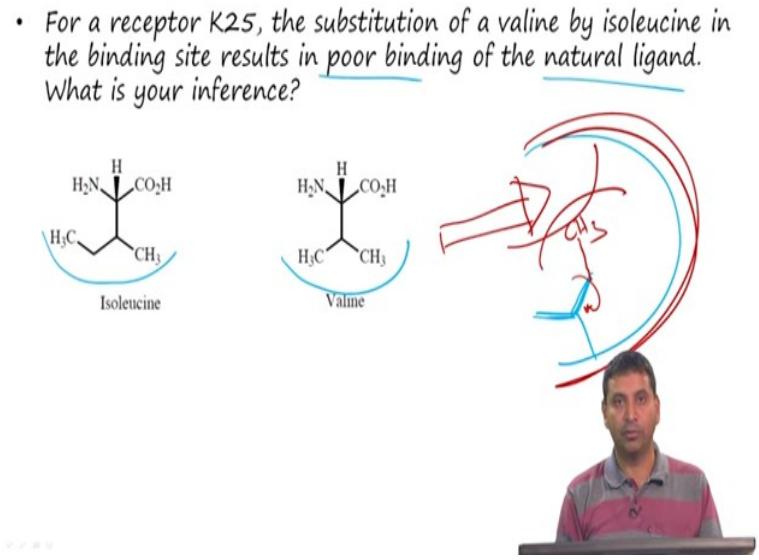

Next question for a receptor K25, the substitution of a valine by a isoleucine in the binding site results in poor binding of the natural ligand. What is your inference? Again this question is a the technique that we would use here is mutation, so you will substitute a valine which is shown here by isoleucine, okay. So let's imagine that the binding pocket of the receptor is shaped like this and may be the valine is over here sitting over here.

So from this experiment it seems like if you change this valine, an increase a methyl group over here and make it into an isoleucine then it results in poor binding. So it is possible that in the binding site of the receptor by introducing this methyl group although this carbon is going to rotate you are increasing the steric hindrance over here and so the ligand which is going to approach the receptor may be hindered. So by carrying out a simple experiment where you replace one of the units of valine by isoleucine it is possible to make some inference about the binding site.

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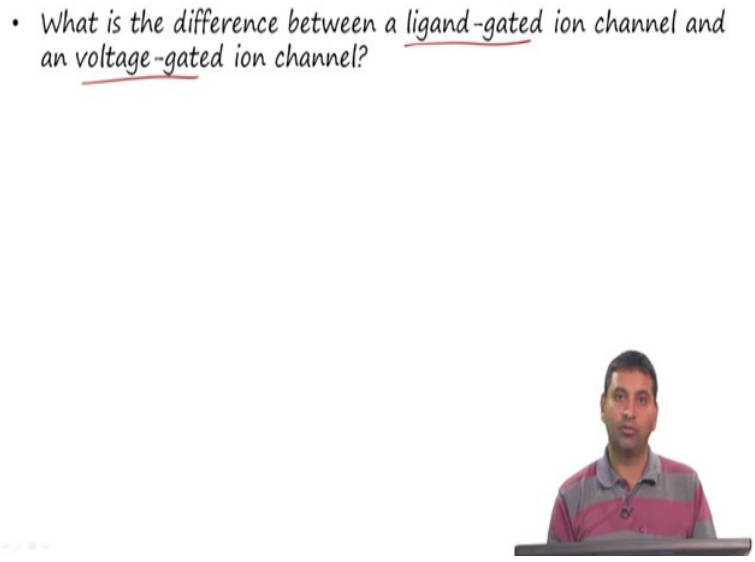

Next question is what is the difference between a ligand-gated ion channel and a voltage-gated ion channel? So we have already looked at in detail what ion channels are and this is just a recap of what we did in class.

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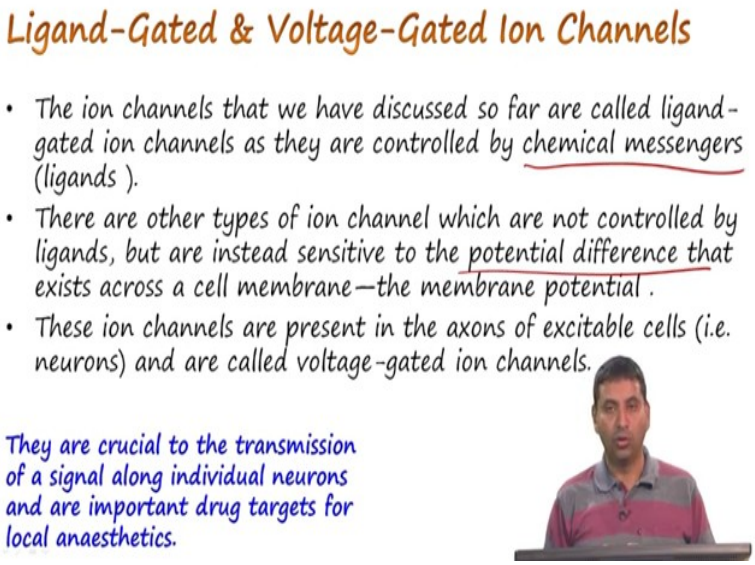

So there are two types of ion channels as we have discussed and they are called ligand-gated that means that they are controlled by chemical messengers or neurotransmitters which are called as ligands and there is another class of ion channels which are not controlled by ligands but instead they are controlled by potential difference that occurs across cell membranes which is also called as the membrane potential.

So these ion channels are present in axons of excitable cells such as neurons. So where there is going to be a substantial difference in the membrane potential during neurotransmission and these are very crucial for transmission of a signal along individual neurons and as we have looked at earlier they are important targets for local anaesthetics.

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So in the example that the picture that we looked at earlier we had looked at this 'lock gate' as shown here that can be opened or closed by an external chemical messenger or by potential difference, so this gate can open if you have a difference in potential between the inside and outside. So this is the difference between a ligand-gated ion channel and a voltage-gated ion channel.

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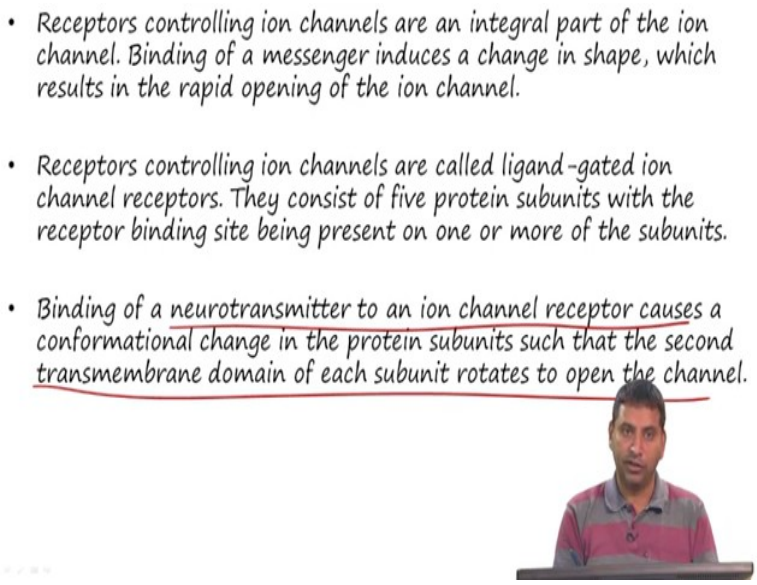

So receptors controlling ion channels are an integral part of the ion channel. So binding of a messenger induces the change in the shape, which results in rapid opening of the ion channel. Receptors controlling ion channels are called ligand-gated ion channels and they consist of five proteins subunits with the receptor binding site being present on one or more of the subunits.

So binding of the neurotransmitter to an ion channel causes a conformational change in the protein subunits such that the second transmembrane domain of each subunit rotates to open the channel. So therefore this is something that we have looked at previously it is just a recap of what we looked at in the previous lecture.

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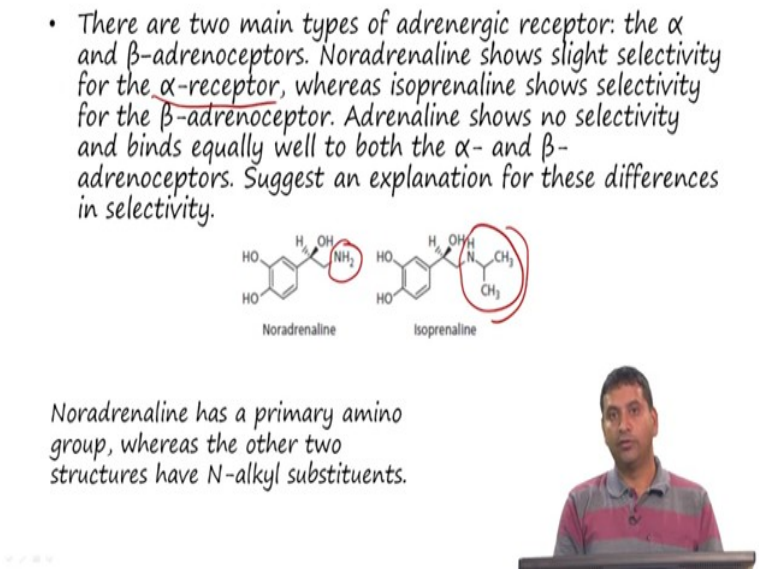

The next question there are two main types of adrenergic receptors the alpha and the beta. Noradrenaline shows slight selectivity for the alpha receptor, whereas isoprenaline shows selectivity for the beta adrenoceptor. Adrenaline shows no selectivity and binds equally well to both alpha and beta receptors. So suggest an explanation for these differences in selectivity. So here is a structure of noradrenaline and here is the structure of isoprenaline.

So as you can see clearly this has an NH_2 group, whereas this has a isoprenaline as a functional group here. So that is one major difference or that is the only difference between these two molecules.

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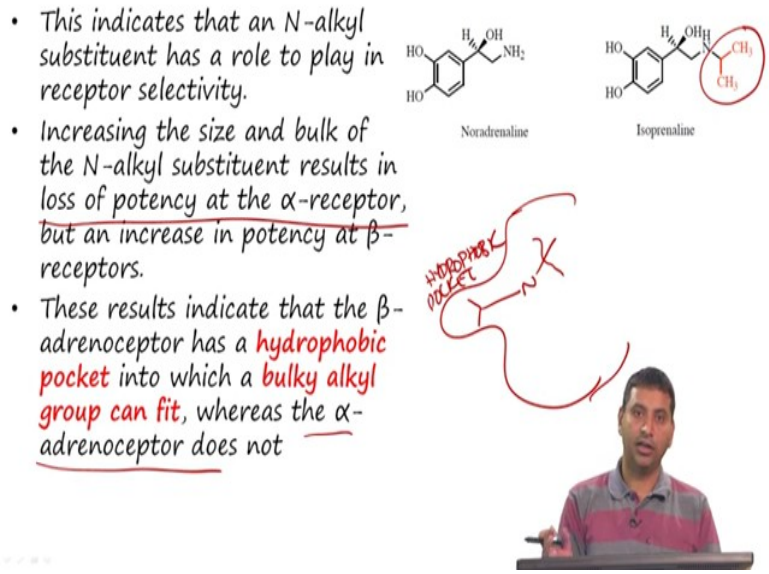

So this indicates that the N-alkyl substituent as shown here is important, okay. So increasing the size and the bulk of the N-alkyl substituent results in the loss of potency at the alpha receptor, but an increase in potency at the beta receptor, so what this suggest is that the beta receptor has a hydrophobic pocket into which the bulky alkyl group can fit, whereas the alpha receptor does not. So this is something that we can infer as differences between the alpha and beta.

Now as we have looked at previously the differences across various cell types is very important in new ligand design or drug design because if all the receptors are identical in nature then it is very difficult for us to achieve selectivity but if there are small differences in the protein which are let's say between the heart and the lung for example then one can specifically look at the lung and not (bind) develop a drug which targets the heart and vice versa.

So here is an example (which) of a compound which has a slight structural modification but results in improved selectivity. So from this example what we can infer is that there might be a hydrophobic pocket where this isopropyl group comes in, binds and this hydrophobic group seems to be important for the binding, whereas in the case of the alpha adrenoreceptor this hydrophobic group does not seem to be present which results in no selectivity.

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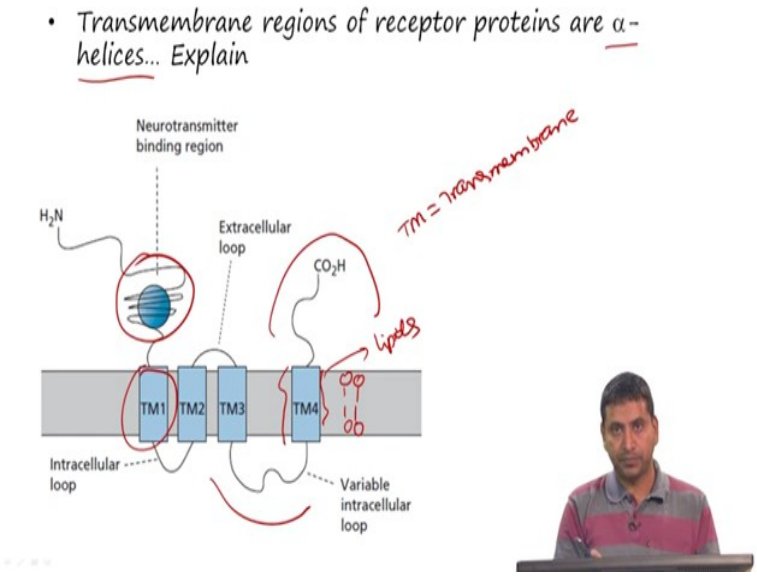

Next question is transmembrane region of receptor proteins are alpha helices, explain. So here is the cartoon that depicts transmembrane or a receptor protein. So these regions which are known as TM1 to TM4 are transmembrane regions and these are alpha helices so the question is why is it that they are alpha helices how would we understand that formation of an alpha helix in this context.

So just to recap so here is the neurotransmitter binding region and so once the neurotransmitter binds to this region there is a conformational change that occurs which results in the signal transmissions. We have already looked at all of these in details. So the important question here is about the transmembrane region, so the transmembrane region is very unique, so why it is unique if because here is the aqueous region, here is the aqueous region and we already know that there are lipid bilayer in this area, so therefore this area must be compatible with lipids or they should have some hydrophobic interactions that are occurring.

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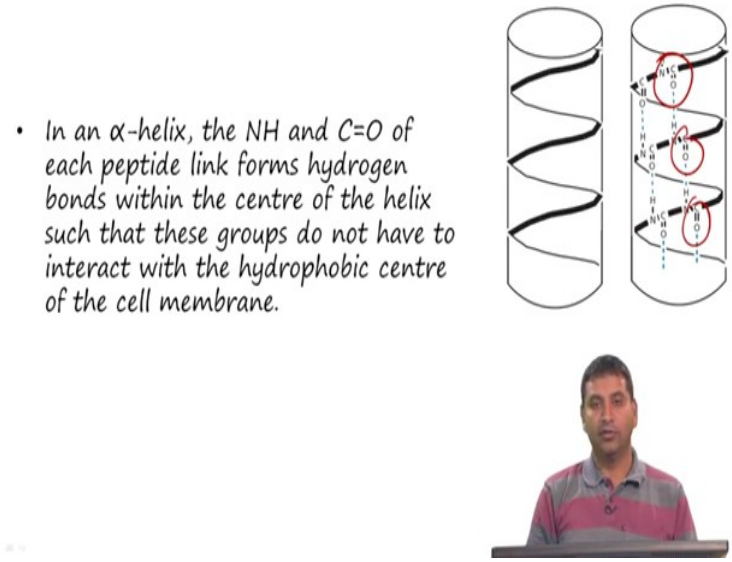

So with this in mind let us look at the structure of an alpha helix. An alpha helix basically has peptide region where it forms hydrogen bonds within the centre of the helix such that these groups do not have to interact with the hydrophobic centre of the cell membrane. So if you see here they have hydrogen bonding regions over here which results in a very regular arrangement.

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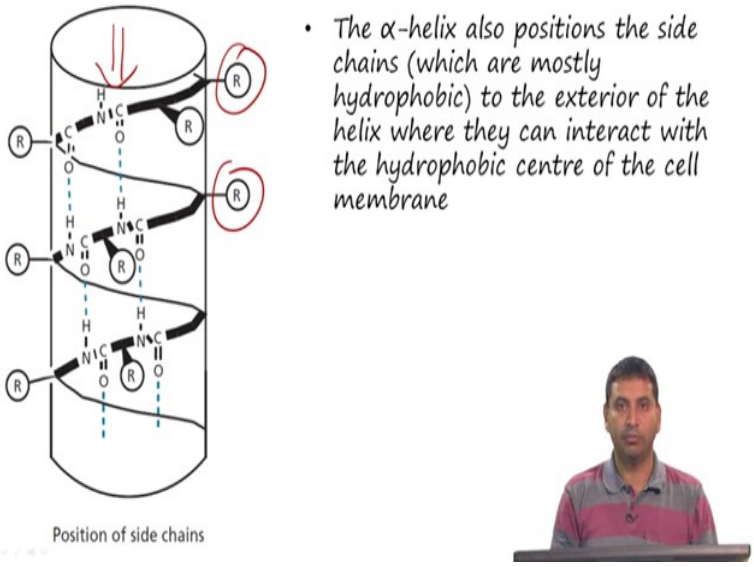

But the interesting thing about the alpha helix is that the side chains which is represented by R is actually on the outside, so if the side chains are positioned outside and the regions the hydrogen bonding regions are on the inside then the side chains which can be hydrophobic in nature can actually interact with the cell membrane, this is why we have alpha helices in the

transmembrane region because they have a hydrophilic interior and the hydrophobic exterior which can bind to the transmembrane region.

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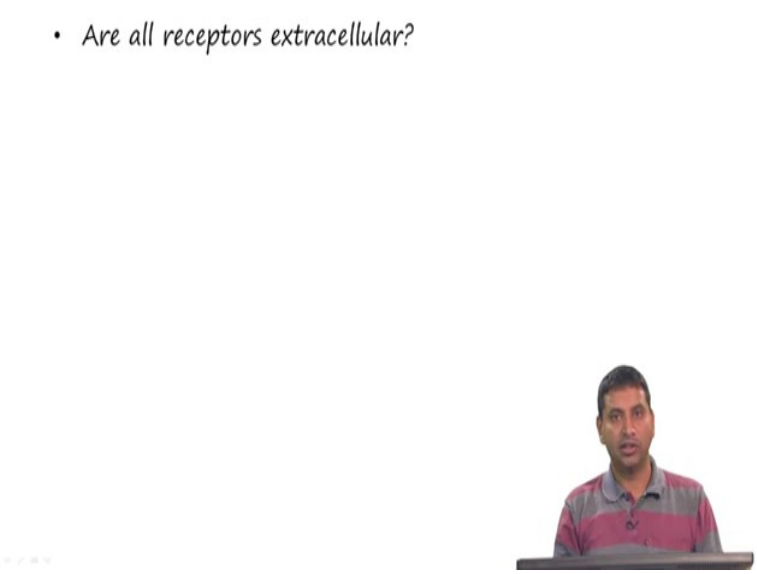

The next question is, are all receptors extracellular?

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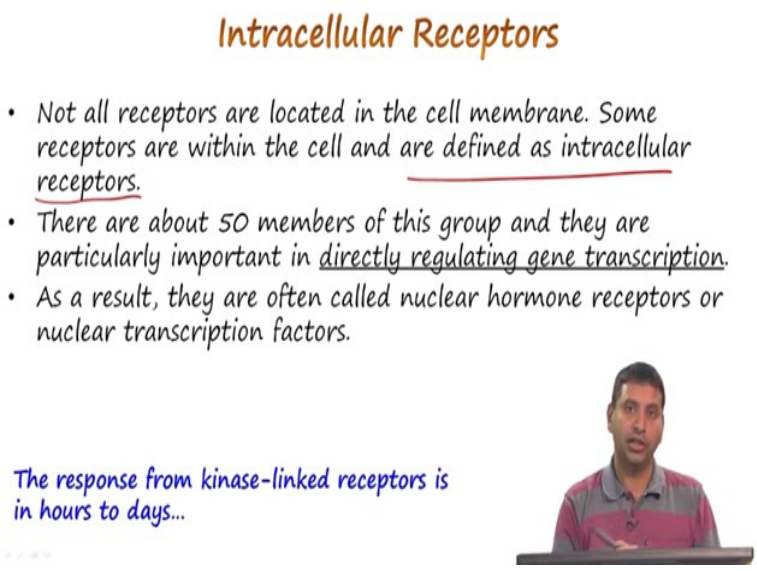

And to answer this question let us go back to our class description where we have looked at intracellular receptors. So again not all receptors are located in the cell membrane, some receptors are within the cell and are defined as intracellular receptors. So one should understand that receptor mediated cell signalling can occur inside the cell as well. So for this to occur the ligand should actually cross the cell membrane get into the cell and interact with

the intracellular receptor and there are about 50 members of this group and they are particularly important in regulating gene transcriptions. So gene transcription is the process by which transcription factor that is going to go and bind to DNA to trigger transcription. So the response from kinase-linked receptor is in hours or days, so it is a very slow process in compared to the other receptors that we have looked at.

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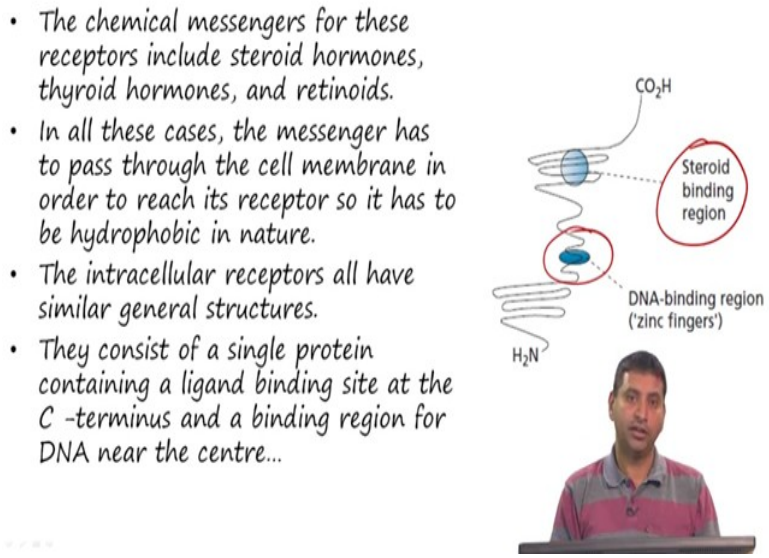

So the way it happens is that there are regions to which there is the ligand comes and binds, so for example here is the steroid binding region and for example thyroid hormones or steroid hormones are quite hydrophobic in nature and so that they can get across cell membrane go and bind to this region and trigger the signalling but they also have a DNA binding region and so these can go and bind to DNA for example they are cysteine based zinc fingers in this and then once they go and bind to this DNA they can initiate transcription.

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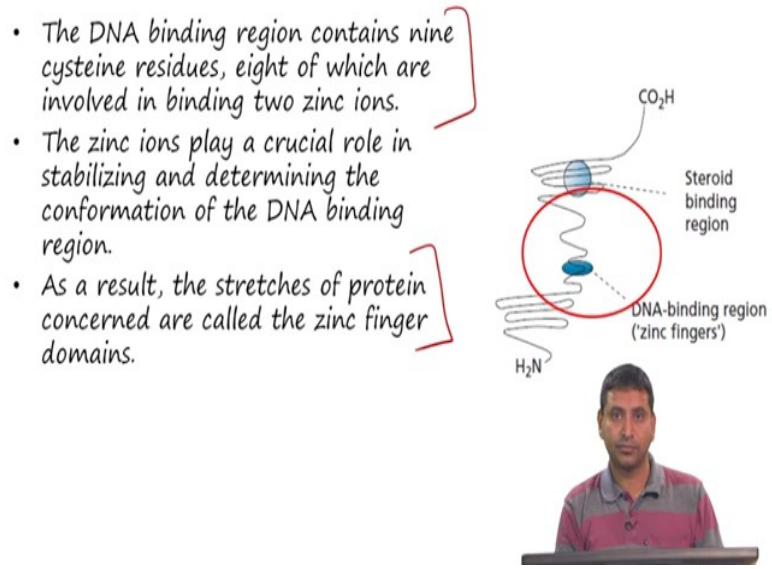

So the DNA binding region contains nine cysteine residues, eight of which are involved in binding to zinc ions. Zinc ions actually play a very crucial role in stabilizing and determining the conformation of the DNA binding region. So as a result the stretches of protein concerned are called the zinc finger domains.

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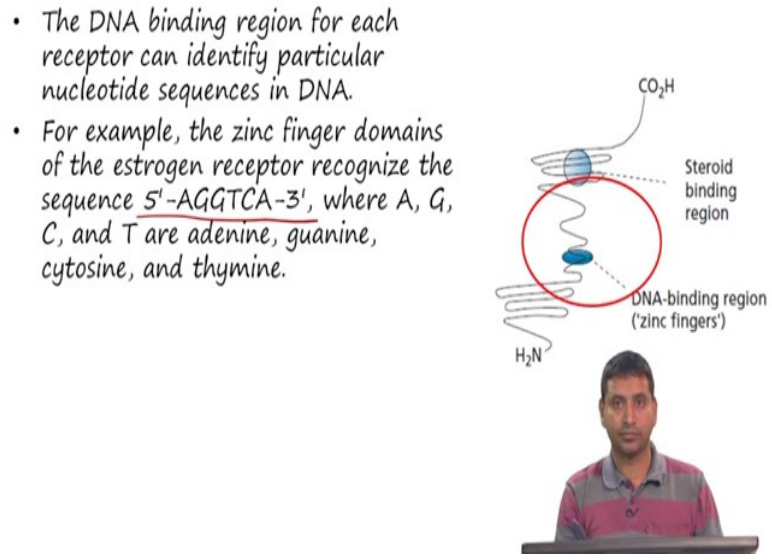

So the DNA binding region for each receptor can identify a pair of nucleotides in the sequence. So for example the zinc finger domains of the estrogen receptor can recognize this sequence as shown here so whenever this the steroid comes and binds it exposes the zinc fingers and the zinc finger is going to go and bind to the DNA at this particular sequence.

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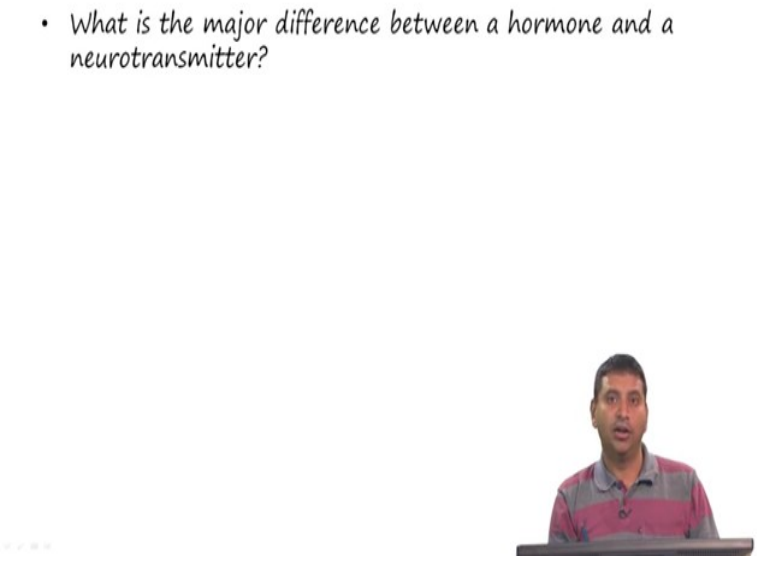

The next question is what is the major difference between a hormone and a neurotransmitter? We have already looked at again different forms of receptors and now we are talking about the ligands.

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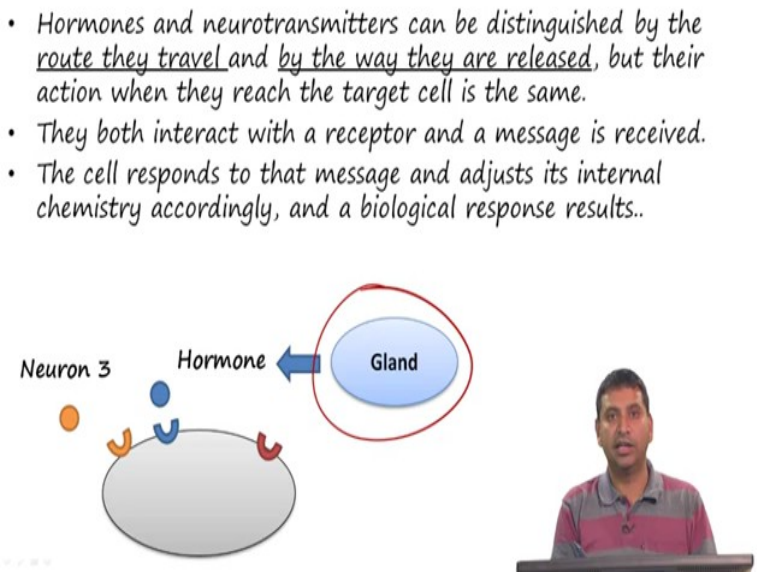

So this is again a recap from the class. So hormones and neurotransmitters can be distinguished by the route that they travel and by the way they are released. So hormones are secreted by glands, whereas neurotransmitters are going to be secreted by neurons and so they both interact with the receptor and message is received inside the cell and they whatever happens in terms of the receptor binding and further signal transmission remains more or less

the same but the major difference is that they have a longer route to travel in the case of hormones and neurotransmitters (are) the route that they travel is much shorter.