Indian Institute of Science Education and Research, Pune
National Programme on Technology on Technology Enhanced Learning
Medicinal Chemistry
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Tutorial – 02
Basic Concepts of Thermodynamics and Kinetics

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Welcome back in the last class we looked at how receptors are very important for the body in transmitting signals. In today's lecture we look at the various functions and types of receptors.

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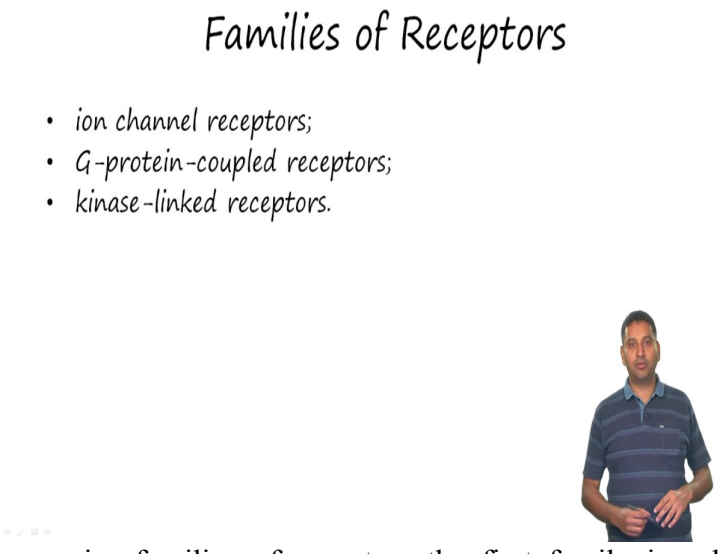

So there are three major families of receptors the first family is called the Ion channel receptors and the second one is called the G protein coupled receptor and the last one is called kinase linked receptors. All three of these are extracellular receptors and we shall also look at certain types of intracellular receptors.

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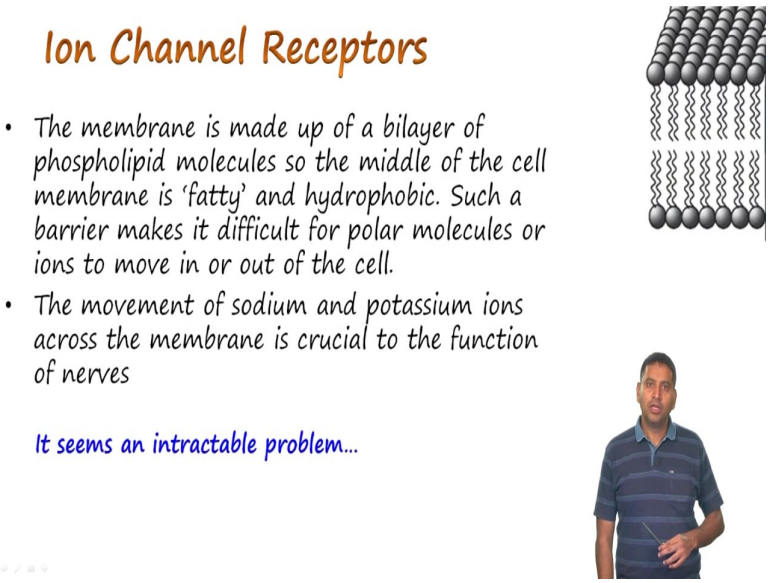

So the first class is Ion channels so as we looked at earlier the membrane is made up of a bilayer okay so it has a phospholipid molecules in the bilayer and so what happens this that this makes the membrane quite hydrophobic. So now the cells has an aqueous environment inside it which has many ions so these ions cannot cross the hydrophobic barrier because of the repulsion between ions and the lipids okay.

So for example the movement of sodium and potassium ions across the membrane is really crucial for the function of nerves so this looks like and intractable problem however the way this problem is solved.

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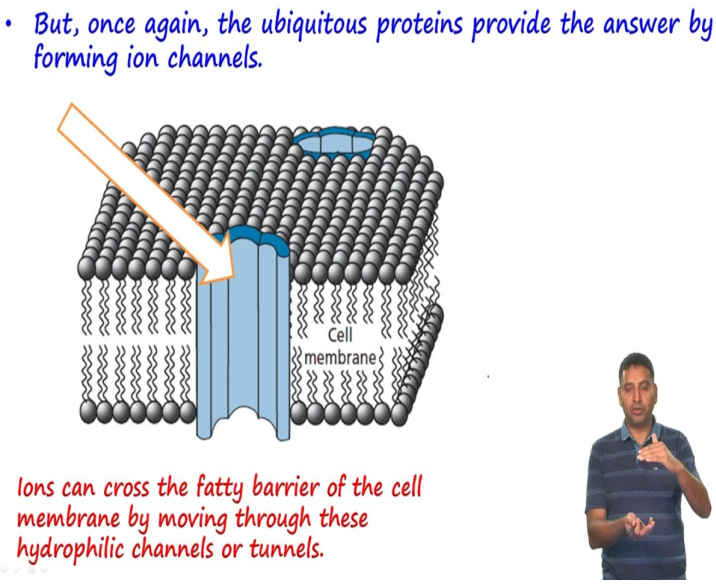

Is that you have proteins which are embedded in the Lipid bilayer and these proteins and as we know proteins have made up of amino acids and amino acids have polar functional groups

and these polar functional groups can interact with ions and enable their transport so the arrow here indicates a typical protein which can transport ions through it.

So these channels through which ions are transported are called as ion channels ok so ions therefore can cross the fatty barrier of the cell by moving across these hydrophobic channels or tunnels.

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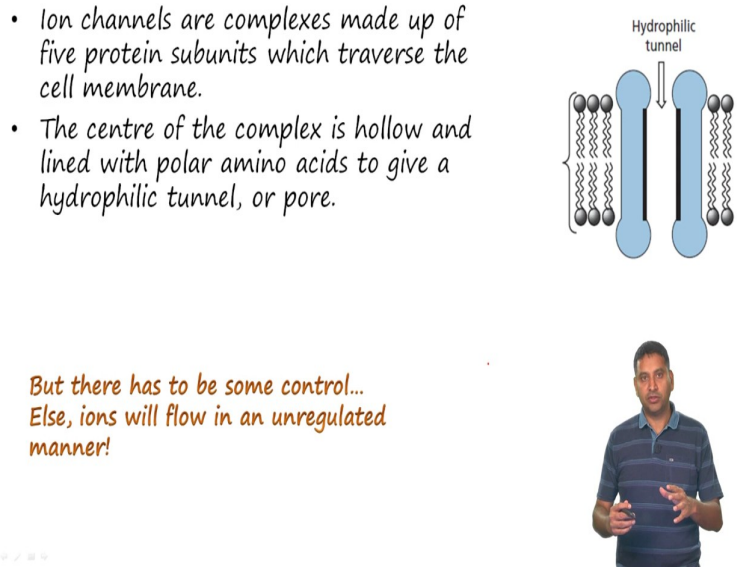

Now ion channels are actually complexes made up of typically five protein subunits and these five protein subunits traverse the membrane. The centre of this complex is hollow and its lined typically with highly polar amino acids so that it provides a nice area through which the ion can go. Now because this is arranged in this particular way the ions can easily travel from outside to inside or from inside to outside.

But if the ions are if the channels are always open there is no control. So in order for that to be controlled there is an extra mechanism that operates.

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So what the analogy that we can look at is our gates so when a building needs to be secure or when a campus needs to be secure what we do when we surrounded by a wall but now we cannot pass through this wall and therefore there is gates that are installed so once if the adequate permission is obtained the gate opens and you can enter and the gate closes right so this kind of analogy is what we would used to understand how this kind of ion channels are regulated so once the external chemical messengers transmits the signal this is exactly what happens in an ion channel receptor system.

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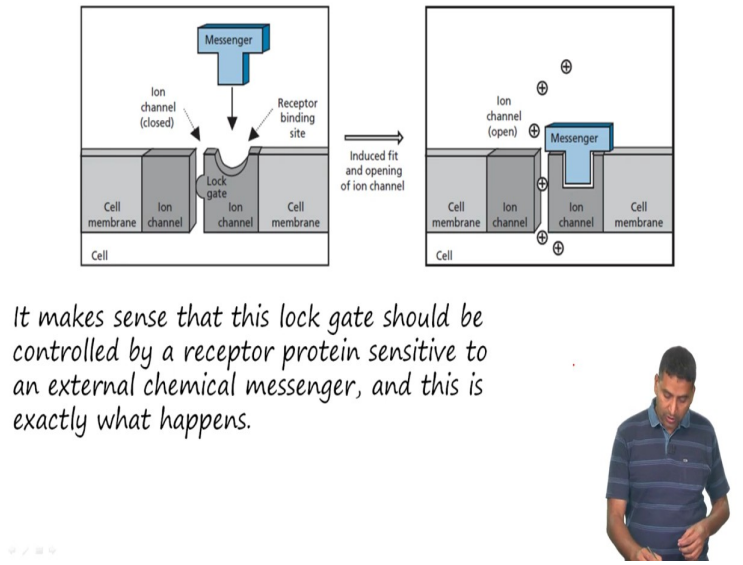

So in this cartoon we shall look at how the ion channel receptors system works. So in here you initially have an ion channel wherein it is locked ok so this is the gate that needs to be opened and here is the cell membrane which is highly lipid rich region and now once a

neurotransmitter or a messenger comes in there is a site for the binding of this messenger right.

And so we already look at how this binding is an induced fit model and once there is an induced fit that occurs this results in a conformational change which permits the ions to go from outside to inside ok so therefore the lock the lock gate can be controlled by the messenger.

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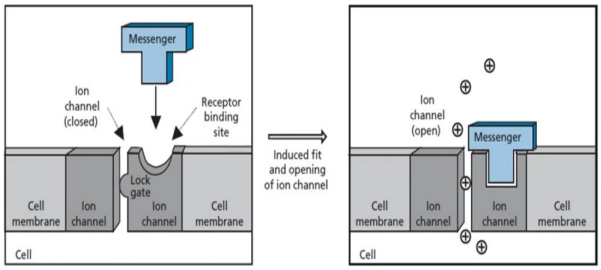

However, when a chemical messenger binds to the external binding site of the receptor protein, it causes an induced fit which causes the protein to change shape. This, in turn, causes the overall protein complex to change shape, opening up the lock gate and allowing ions to pass through the ion channel

However when the chemical messengers binds to the external binding site okay so this in turn causes the overall protein complex to change shape opening of the lock gate okay.

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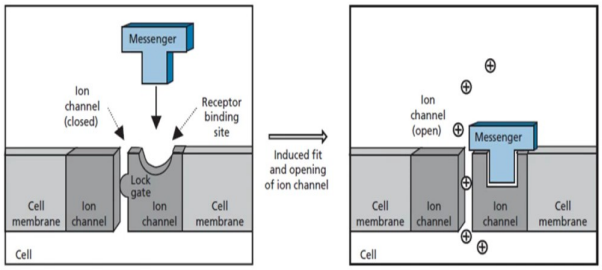

In fact, the receptor protein is an integral part of the ion channel complex and is one or more of the constituent protein subunits. In the resting state, the ion channel is closed (i.e. the lock gate is shut).

So now in fact the receptor protein is an integral part of the ion channel complex so as you can see here the lock gate is over here and the receptor binding site is over here and this

together is a protein complex so any conformational change that occurs in after binding is also reflected in the lock gate.

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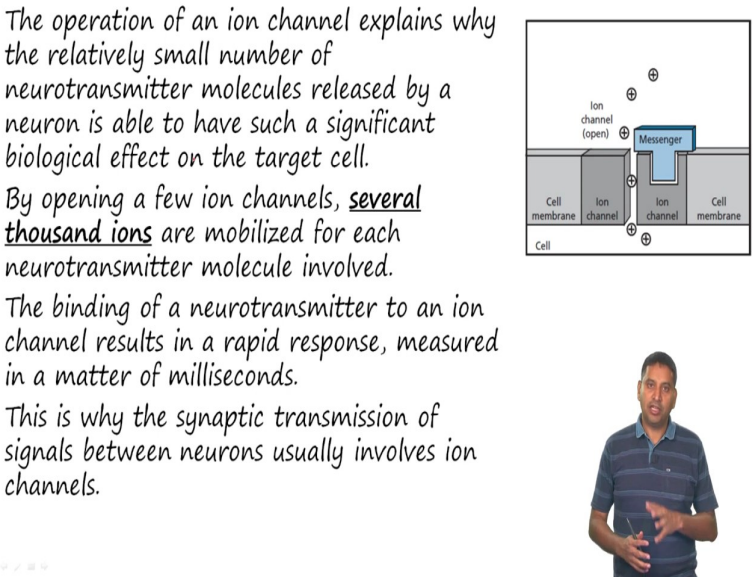

So the operation of this ion channel explains why a small number of neurotransmitter molecules can have a tremendous effect in the target cell. So once you have the lock gate open typically thousands of ions can traverse the barrier therefore the binding of a neurotransmitter to an ion channel results in not just a rapid response but also a amplified response okay the time of response is typically in milliseconds so ion channels are therefore the preferred mode of signal transmission in synaptic nerve systems.

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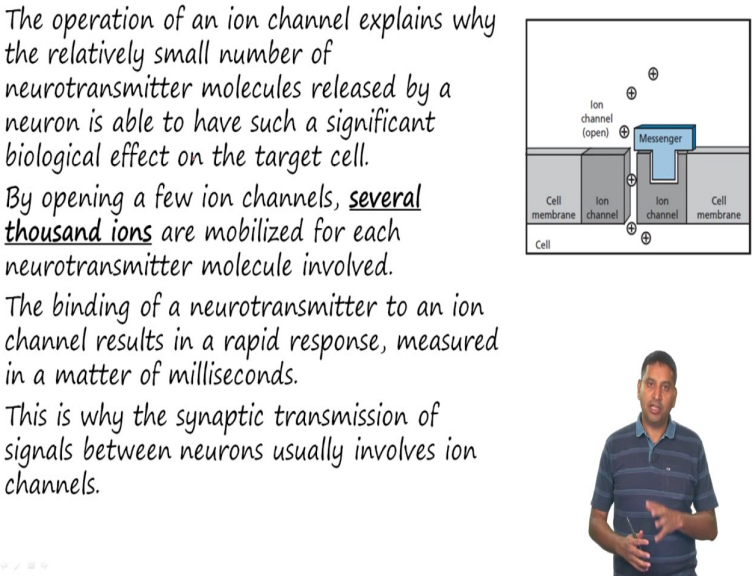

Okay ion channels are specific for certain ions for example they could be channels for sodium ions, potassium ions or calcium ions which are all cationic or they could also be an anionic ion channels for chloride ions okay the ion selectivity will depend on the amino acids

that are lining the ion channel. So for example if you have amino acids which are which have a positively charged lets have an amine residue they would preferentially bind to chloride.

And it's also very interesting that mutation what is mutation. Mutation is basically replacement of one amino acid with another is sometimes sufficient to completely change the selectivity of an ion channel. So therefore the primary structure of these ion channels are significantly important for their function.

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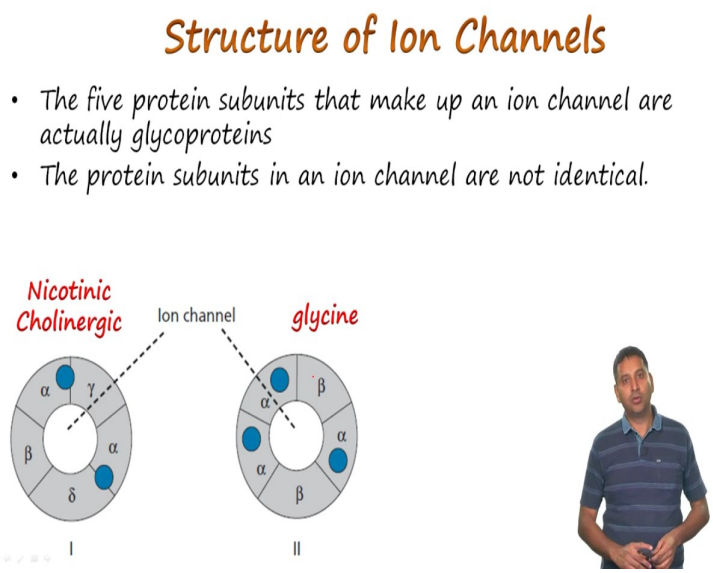

Now let's look into a little bit of detail of these ion channels these ion channels are actually glycoproteins. What we mean by glycoproteins are basically these are proteins which are conjugated to sugars and they have five sub units and not all these 5 sub units are identical so here you have examples of two different kinds of ion channels which both have 5 sub units and one of them response is a nicotinic cholinergic ion channel where as the other one responds to glycine.

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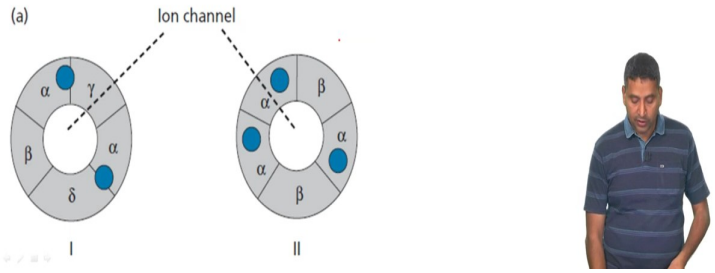
- For example, the ion channel controlled by the nicotinic cholinergic receptor is made up of five subunits of four different types [α (×2) β, γ, δ];
- the ion channel controlled by the glycine receptor is made up of five subunits of two different types [α (×3), β (×2)]



So the area and these are controlled by so for example the nicotinic cholinergic receptor has two alpha subunit, one beta subunit, one Gamma subunit and one Delta subunit where as the glycine receptor ion channel is made up of three alpha subunits and two Beta subunits over here is actually the in the coloured the light blue coloured circle indicates the ligand binding site.

So in this particular case this is where glycine binds.

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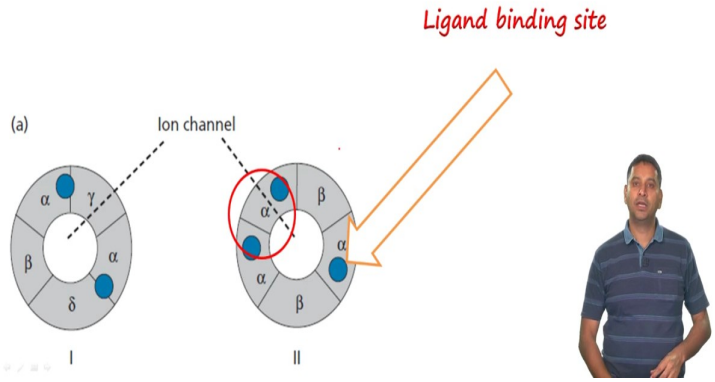
- The receptor protein in the ion channel controlled by glycine is the α-subunit.



Now if this glycine binding the receptor protein in the ion channel that is controlled by glycine is the alpha subunit as shown here.

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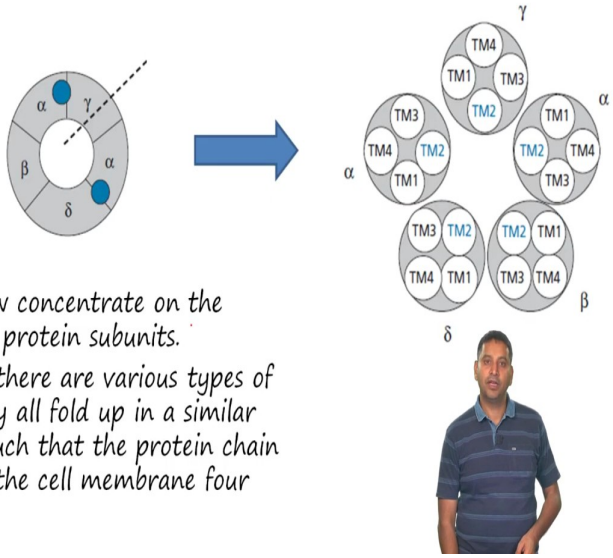

Now let's look at the individual protein subunits in this complex. Although there are various types of these they all fold up in very very similar manner such that the protein chain traverses the cell membrane so when you have a cell membrane these subunits actually go across and come back and so on. We will look at it in little bit more detail later ok.

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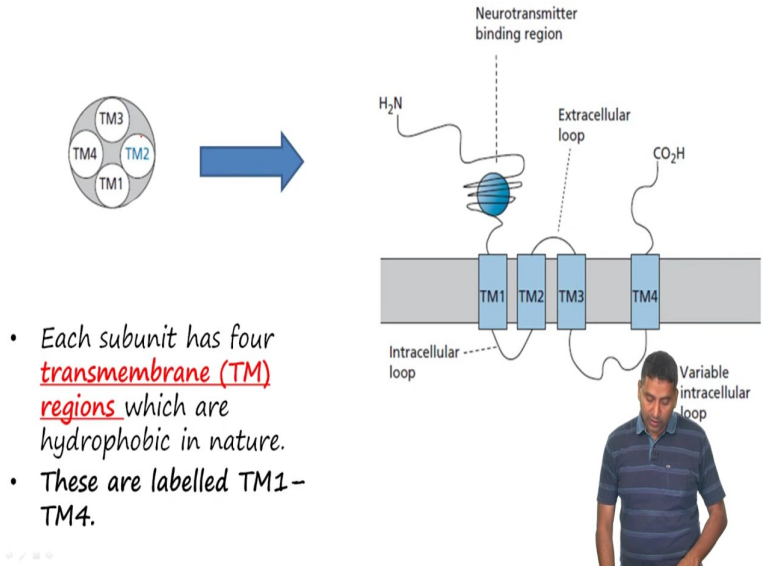

So this domains are called the transmembrane regions. Each subunit has four transmembrane regions which are hydrophobic in nature and one can reason out why this need to be hydrophobic because they need to interact with lipids on the membrane and their labelled as TM1, TM2, TM3 and TM4. They also have a neurotransmitter binding region so here which is located outside and these are the extracellular loops and these are the intracellular loops.

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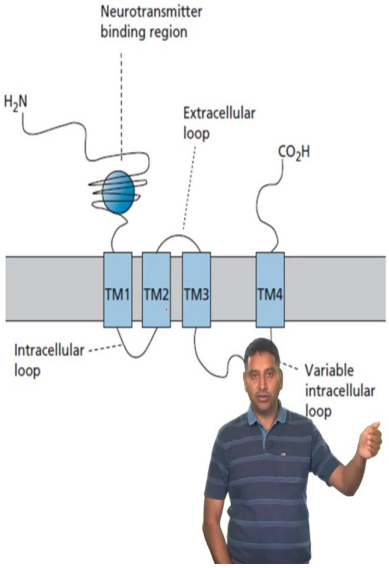

- There is also a lengthy N - terminal extracellular chain which (in the case of the α-subunit) contains the ligand-binding site

And there is also a lengthy N-terminal extracellular chain which contains the ligand binding site which I already described just now.

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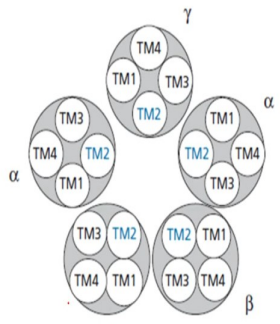
- The subunits are arranged such that the second transmembrane region of each subunit faces the central pore of the ion channel



Alright now each of the subunits are arrange such that the second transmembrane region of each unit faces the central pore of the ion channel. So if you look at it this TM2 is actually the one that faces the ions.

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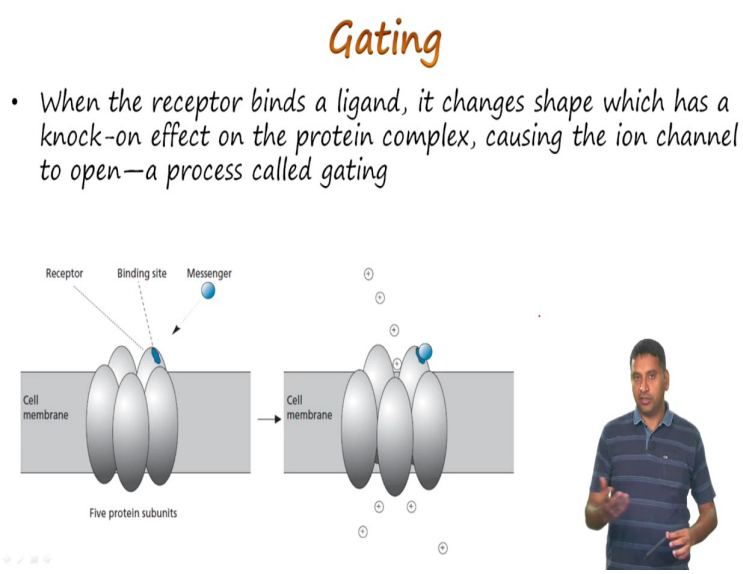

Now let's understand how the gating mechanism works so when the receptor binds the ligand so over here this is the messenger and this is the receptor binding region what happens is that there is a conformational change okay first there is an induced fit and then there is a conformational change and this conformational change results in ions flowing into the cell this process is called as gating.

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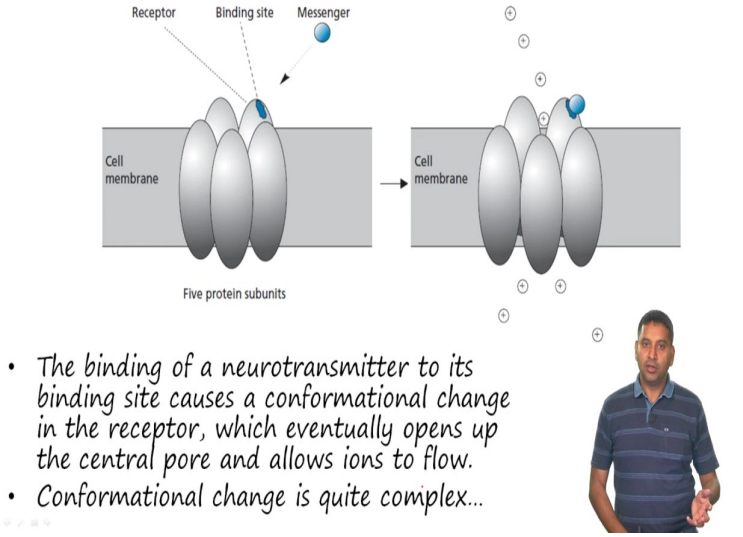

And the binding of the neurotransmitter eventually opens up this central pore allowing the ions to flow. This conformational change is quite complex and we will not be discussing this in this lecture but what happens is that.

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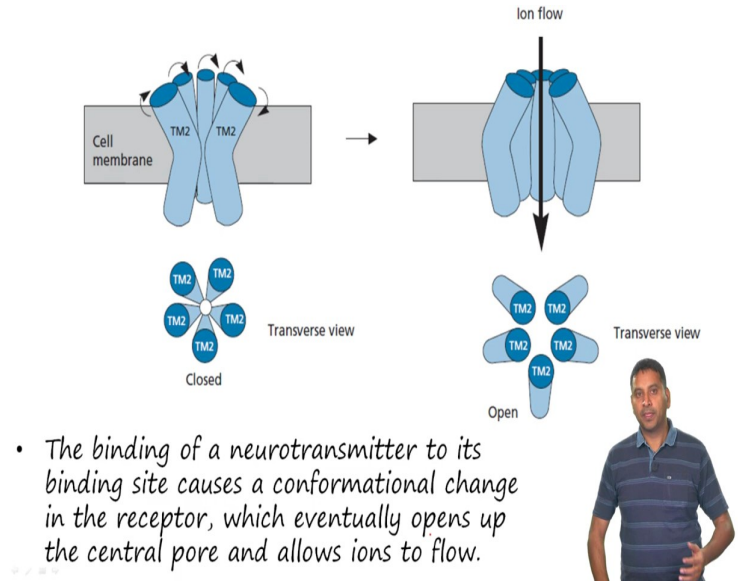

There is a sort of change that occurs which helps in opening it up so you can imagine that this is the area where the ion will not be able to go through when these open up like this then what happens is that they assume an open conformation which allows the ions to flow. So eventually the central pore is through which through which the ions flow has to be created by conformational changes.

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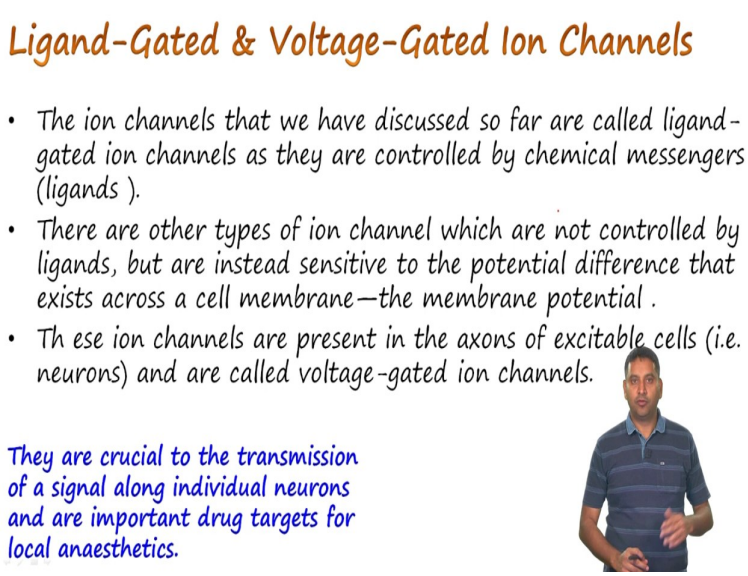

Now there are two types of gated ion channels. The first one which we have already described just now is the ligand gated ion channel so where the opening and closing of the gate is controlled by ligands. The other type of ion channels are not controlled by ligands but instead are sensitive to differences in potential that that exist across the cell membrane which is also known as the membrane potential. These ion channels are actually present in the axons of excitable cells.

Such as neurons and these are called voltage gated ion channels and voltage gated ion channels are really crucial to the transmission of signal along individual neurons and therefore they are very important targets for pain killers and local anaesthetics.

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- Receptors controlling ion channels are an integral part of the ion channel. Binding of a messenger induces a change in shape, which results in the rapid opening of the ion channel.

So, to summarise receptors controlling ion channels are an integral part of the ion channel and binding of the messenger induces a change in shape which results in opening of the ion channel.

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

And receptors controlling the ion channels are called ligand gated ion channels and they contain five protein subunits with the receptor binding site being present in one or more of these subunits.

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- Receptors controlling ion channels are called ligand-gated ion channel receptors. They consist of five protein subunits with the receptor binding site being present on one or more of the subunits.
- Binding of a neurotransmitter to an ion channel receptor causes a conformational change in the protein subunits such that the second transmembrane domain of each subunit rotates to open the channel.

Now binding of a neurotransmitter to an ion channel causes a conformational change in the protein such that the second transmembrane domain of each subunit rotates and open the channel. This allows the ions to go down.

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G-Protein Coupled Receptors

- The G-protein-coupled receptors are some of the most important drug targets in medicinal chemistry.
- Indeed, some 30% of all drugs on the market act by binding to these receptors.
- In general, they are activated by hormones and slow-acting neurotransmitters.
- They include the muscarinic receptor, adrenergic receptors, and opioid receptors.

The response from activated G-protein-coupled receptors is measured in seconds.

The next class of receptors are called as the G protein coupled receptors to give you some ideas as to how important these are. About 30% of all drugs on the market act by binding to these receptors. In general these are pretty slow and their activated by hormones and these include the muscarinic receptor, the adrenegic receptor and the opioid receptor and these are the the times timescales of this of these receptors are typically measured in seconds.

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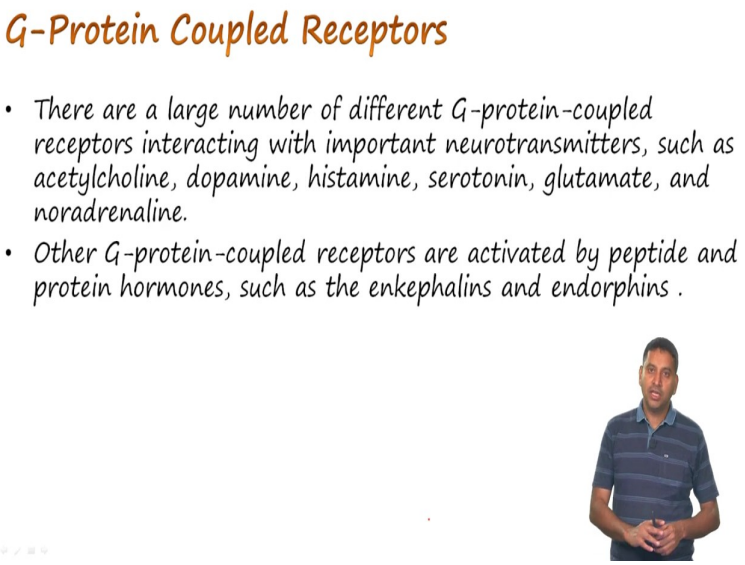

There are a large number of different G protein coupled receptors which interact with important neurotransmitters such as acetylcholine, dopamine, histamine, serotonin, glutamate or and noradrenaline. Other G protein coupled receptors are activated by peptides or peptides hormones which we looked at earlier such as enkephalins and endorphins.

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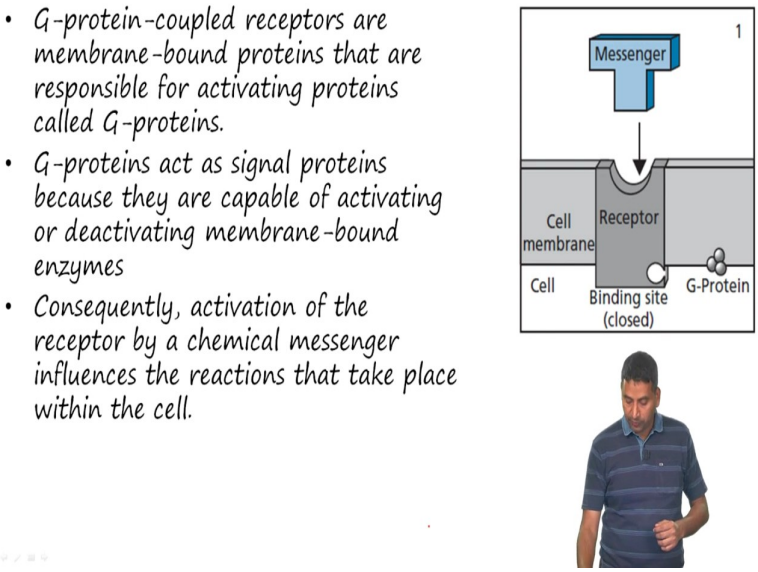

G protein coupled receptors are membrane bound proteins that are responsible for activating proteins known as the G proteins. G proteins act as signal proteins and they are capable of activating or deactivating membrane-bound enzymes. Consequently the activation of the receptor by chemical messengers influences the reaction that take place within the cell so let's look at this process now.

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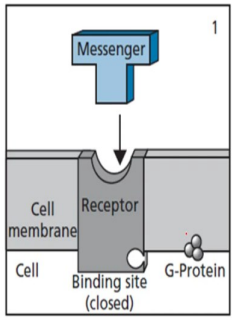

- The receptor protein is embedded within the membrane, with the binding site for the chemical messenger exposed on the outer surface.
- On the inner surface, there is another binding site which is normally closed...

Now what happens initially is that the receptor is embedded within the membrane and it has a binding site okay now on the inner surface there is another binding site which is normally closed right.

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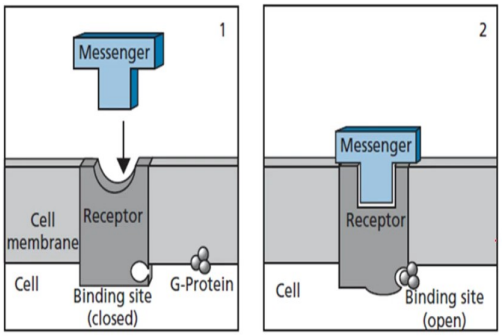

- When the chemical messenger binds to its binding site, the receptor protein changes shape, opening up the binding site on the inner surface.

Once the messenger binds to the receptor what happens is that there is an induced fit which helps the binding of the messenger once this binding happens the inner binding site which was previously closed is now open.

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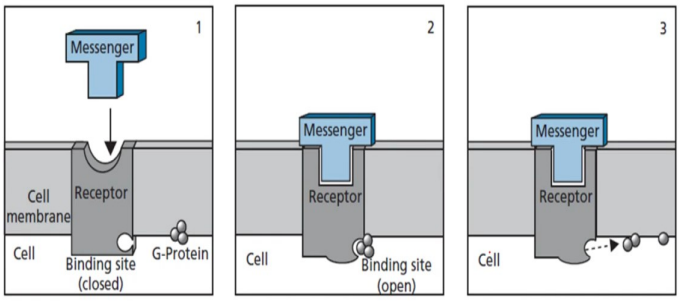

- This new binding site is recognized by the G-protein, which then binds.
- The G-protein is attached to the inner surface of the cell membrane and is made up of three protein subunits, but once it binds to the receptor the complex is destabilized and fragments to a monomer and a dimer

After it opens it becomes a site for recognition by G proteins and once the the G protein which is attached to the inner surface of the cell membrane and is made up of three protein subunits but once it binds to the receptor the complex is destabilized and fragments in to a monomer and dimer.

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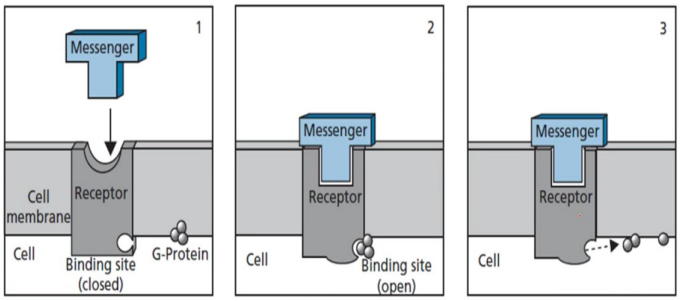

- These then interact with membrane-bound enzymes to continue the signal transduction process

Ok then these interact with membrane bound enzymes and continue the signal transduction process.

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- There are several different G-proteins, which are recognized by different types of receptor.
- Some of the activated subunits from these G-proteins have an inhibitory effect on a membrane-bound enzyme, while others have a stimulatory effect.
- Nevertheless, the mechanism by which the G-protein is activated by fragmentation is the same.

There is a substantial amplification of the signal in this process, as one activated receptor activates several G-proteins.

There are several different G proteins which are recognised by different types of receptors some of the activated subunit from this G-proteins have an inhibitory effect on membrane bound enzyme while others have a stimulatory effect. However the mechanism by which G protein is activated by fragmentation remains same irrespective of whether it has it is a stimulatory or an inhibitory effect.

So how does a signal get amplified. The amplification of the signal in this process as one activated receptor activates several G proteins.

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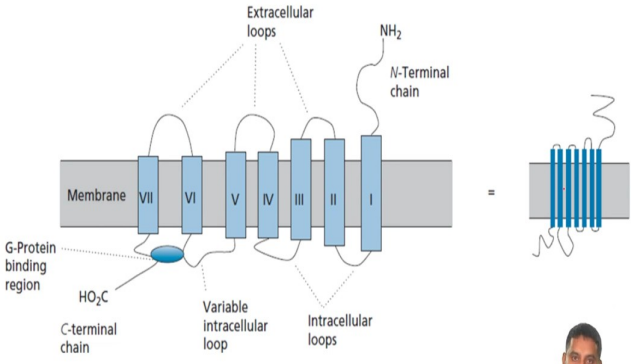

- The G-protein-coupled receptors fold up within the cell membrane such that the protein chain winds back and forth through the cell membrane seven times G-proteins.

Let's now look at the structure of this G proteins. The G proteins receptors fold up within the cell membrane such that the protein chain winds back and forth through the cell membrane 7 times as shown here ok so again similar to the ion channel receptors there are extracellular domains and intracellular domains.

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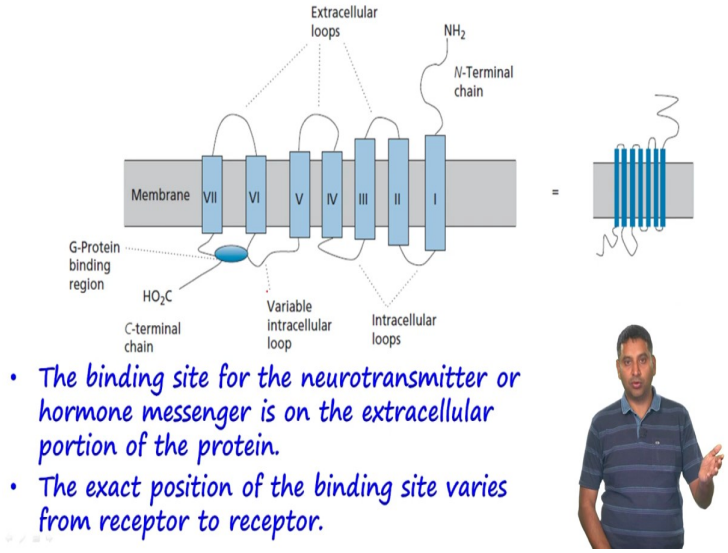

There is a binding site for the neurotransmitter or the hormone which is on the external extracellular portion of the protein and the exact position of the binding site varies from receptor to receptor. There is also as you can see a G protein binding region which is shown here. We shall look into detail about both ion channel receptors as well as G protein receptor later in the course.

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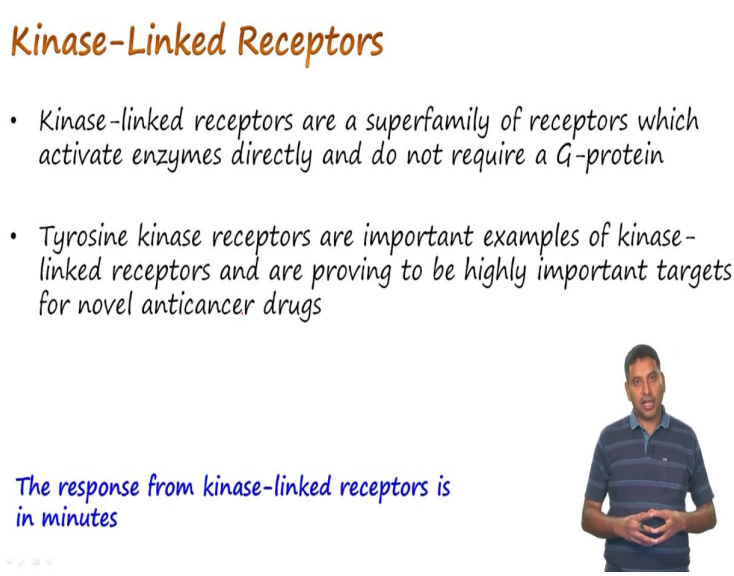

The third type of receptors are kinase linked receptors. Kinase linked receptors are super family of receptors which activate enzymes again but they do not require a G protein. Tyrosine kinase are important examples of kinase linked receptors. Tyrosine as you know has a phenolic residue and their very important targets in developing novel anticancer agent. The response from kinase linked receptors is typically in minutes.

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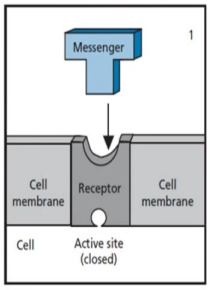
- In these structures, the protein concerned plays the dual role of receptor and enzyme.
- Th e receptor protein is embedded within the cell membrane, with part of its structure exposed on the outer surface of the cell and part exposed on the inner surface.
- Th e outer surface contains the binding site for the chemical messenger and the inner surface has an active site that is closed in the resting state.



So in these structures the protein concerned place a dual role of both receptor as well as an enzyme. The receptor here is embedded in the cell membrane and one portion of this is exposed to the inner surface and the other one is exposed to the outer surface. The outer surface contains the binding site for the chemical messenger while the inner surface has an active site in the resting state.

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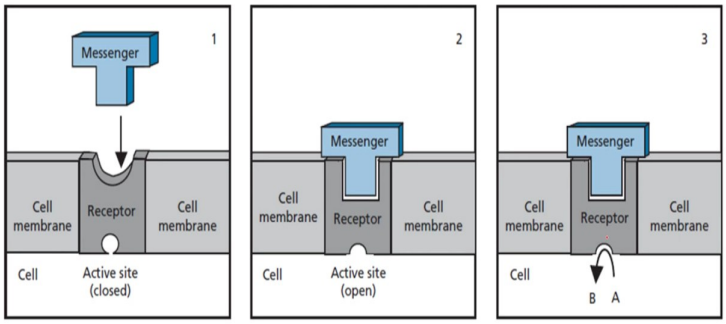

- When a chemical messenger binds to the receptor it causes the protein to change shape.
- This results in the active site being opened up, allowing the protein to act as an enzyme within the cell.

Again very similar to the other receptor ligand interaction, once the messenger binds to the receptor it causes the protein to undergo a change in shape and once this change in shape happens the active site which is previously closed is now opened up and this is an enzyme active site and therefore it can catalyse reaction A goes to B.

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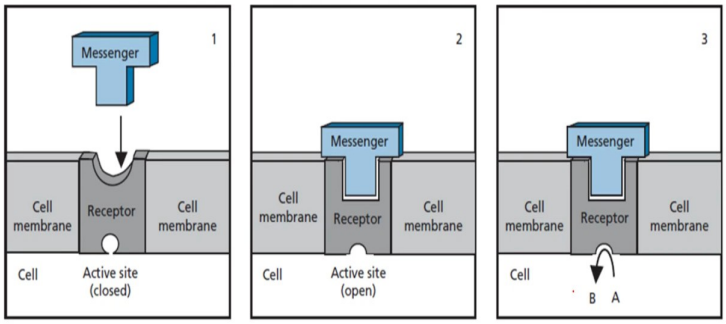

- The reaction that is catalysed is a phosphorylation reaction where tyrosine residues on a protein substrate are phosphorylated.

And reaction here that is catalysed is a phosphorylation reaction where a tyrosine residue is on a protein substrate is phosphorylated.

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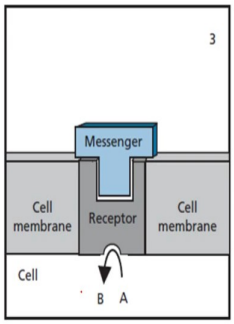
- An enzyme that catalyses phosphorylation reactions is known as a kinase enzyme and so the protein is referred to as a tyrosine kinase receptor.
- ATP is required as a cofactor to provide the necessary phosphate group.
- The active site remains open for as long as the messenger molecule is bound to the receptor, and so several phosphorylation reactions can occur, resulting in an amplification of the signal.



We look at earlier the various classes of enzymes that I have that carry out various functions and enzyme that catalyzes the phosphorylation reaction is known as a kinase. Ok so therefore this protein is referred to as a tyrosine kinase receptor. Here ATP is required as a cofactor to provide the necessary phosphate group and the active site remains long remains open as long as the messenger molecule is bound to the receptor. So, therefore several phosphorylation reactions can occur resulting in amplification of the signal.

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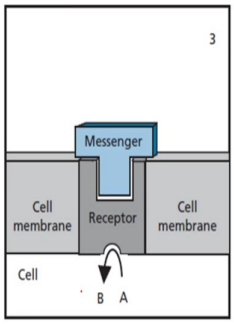
- The kinase-linked receptors are activated by a large number of polypeptide hormones, growth factors, and cytokines.
- Loss of function of these receptors can lead to developmental defects or hormone resistance.
- Overexpression can result in malignant growth disorders.



This kinase linked receptors are activated by large number of polypeptide hormones, growth factors and cytokines. Loss of function of these receptors can lead to several developmental defects or hormone related problems and also over expression can result in malignant growth disorders so therefore these constitute important target for drug discovery.

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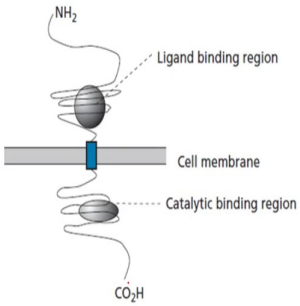
- The basic structure of a tyrosine kinase receptor consists of a single extracellular region (the N -terminal chain)
- that includes the binding site for the chemical messenger, a single hydrophobic region that traverses the membrane as an α-helix of seven turns (just sufficient to traverse the membrane), and a C - terminal chain on the inside of the cell membrane



Let's look at the structure of these tyrosine kinase receptors. These contain a single extracellular region which is the N-terminal region which also contains the ligand binding region and the binding site of this chemical messenger is a single hydrophobic region that traverses the membrane as an alpha helix of several turns and the C-terminal chain is inside the cell membrane as shown here and this has the catalytic binding region.

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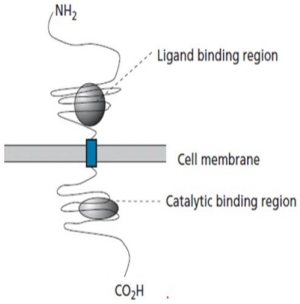
- The important point to grasp at this stage is that an external chemical messenger has managed to convey its message to the interior of the cell without itself being altered or having to enter the cell.



The important point here is that an external chemical messengers has managed to convey its signal or message to the interior of the cell without the messenger getting into the cell or without it being altered.

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Intracellular Receptors

- Not all receptors are located in the cell membrane. Some receptors are within the cell and are defined as intracellular receptors.
- There are about 50 members of this group and they are particularly important in directly regulating gene transcription.
- As a result, they are often called nuclear hormone receptors or nuclear transcription factors.

The response from kinase-linked receptors is in hours to days...

There are other classes of receptors known as which I mentioned earlier which are known as intracellular receptors and these are receptors are about of 50 member family in this or 50 members of this group and their very important indirectly regulating gene transcription so to recap gene transcription is nothing but the process where in something goes and binds to a DNA inside the cell and initiates the transcription process.

So these are are sometimes called as nuclear hormone receptors or nuclear transcription factors. The response from these intracellular receptors is in hours to days.

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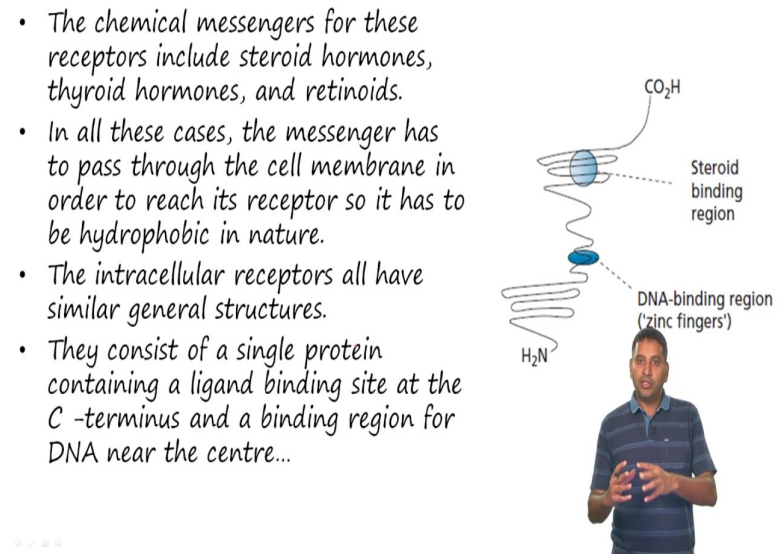

Okay so when we look at the structure of these chemical messengers for these receptors include steroid hormones, thyroid hormones and retinoids. In all these cases you the compound or the messenger has to cross the cell membrane, get inside and then find the domain where it can bind to this receptors.

And lead to activation they all have typical typical is very similar general structures. They all have a steroid or ligand binding region and they have a DNA binding region will look at we will look into this more details shortly and here is the N-terminus. Now these are a single protein containing all these various components.

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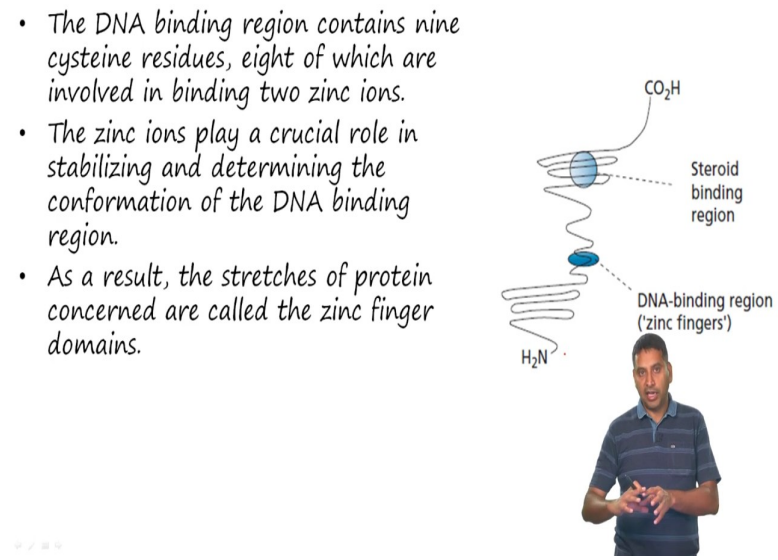

The DNA binding region contains nine cysteine residues, eight of which are involved in binding to zinc ions okay zinc ions play a very crucial role in stabilizing and determining the conformation of the DNA binding region so one this this protein has to bind to specific

stretches of DNA. As a result the stretches of protein are sometimes referred to as zinc finger domains.

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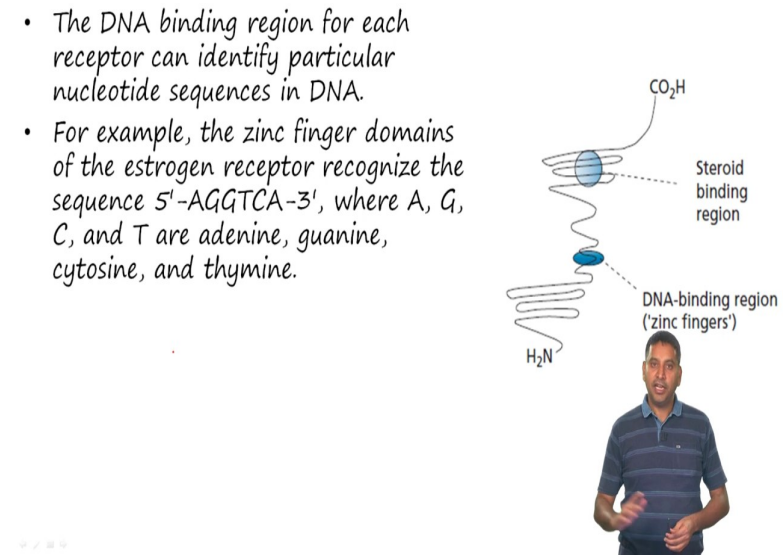

The DNA binding reason for each receptor can identify a specific nucleotides sequence in DNA. For example the zinc finger domains for the estrogen receptor recognise the sequence shown here where A, G, C and T are adenine, guanine, cytosine, thymine, respectively.

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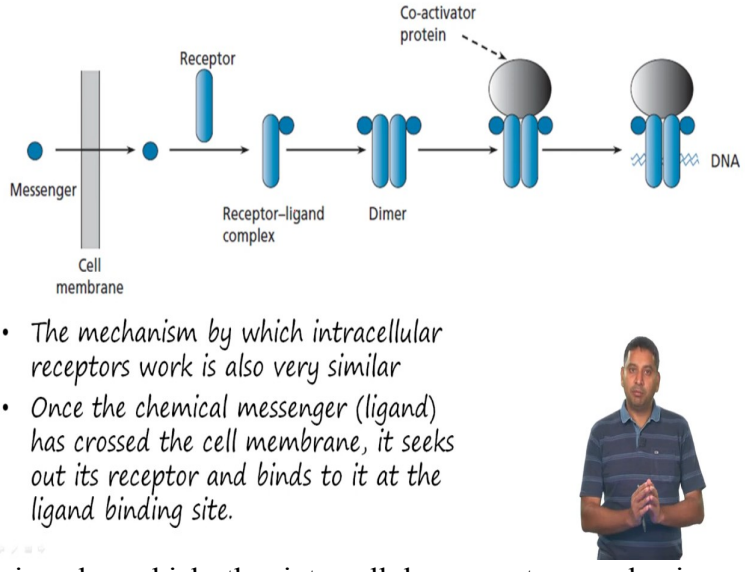

Now the mechanism by which the intracellular receptor works is very similar to the extracellular receptors. The major difference being that the messenger has to cross the cell membrane so once it crosses the cell membrane it can bind to the receptor and once the receptor ligand complex is formed this can dimerize.

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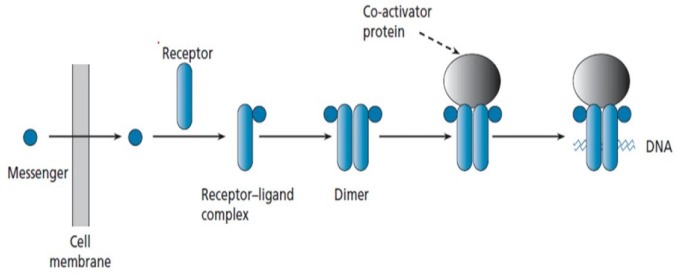

- An induced fit takes place which causes the receptor to change shape.
- This, in turn, leads to a dimerization of the ligand–receptor complex.
- The dimer then binds to a protein called a co-activator and, finally, the whole complex binds to a particular region of the cell's DNA.

Okay after it dimerizes of course and induced fit take place first which causes the receptor to change shape and this in turn leads to dimerization of the ligand receptor complex. The dimer then binds to a protein known as the coactivator protein and this together can go and bind to particular region of the cells DNA.

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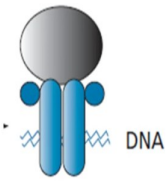
- The dimer then binds to a protein called a co-activator and, finally, the whole complex binds to a particular region of the cell's DNA.
- Depending on the complex involved, binding of the complex to DNA either triggers or inhibits the start of transcription, and affects the eventual synthesis of a protein.



The depending on the complex involved the binding of the complex to DNA either triggers or inhibit the start of transcription and this will affect the let's say the synthesis of a protein and that protein may have important function in inside the cell.