Organic Chemistry In Biology And Drug Development
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Lecture - 56
Anti - Viral Drugs

Welcome back, we were discussing in the last session the different types of viruses and we have seen that viruses can be classified primarily into DNA virus, RNA virus and retrovirus. In DNA virus, the DNA is the genetic material that is injected into the nucleus and then either it could be integrated or it could remain as separate entities.

It utilizes the host machinery; that means, all the DNA polymerase and the RNA polymerase that is utilised to make the mRNA corresponding to the virus and then the mRNA synthesise the proteins; the virus particle is generated and comes off from the cell.

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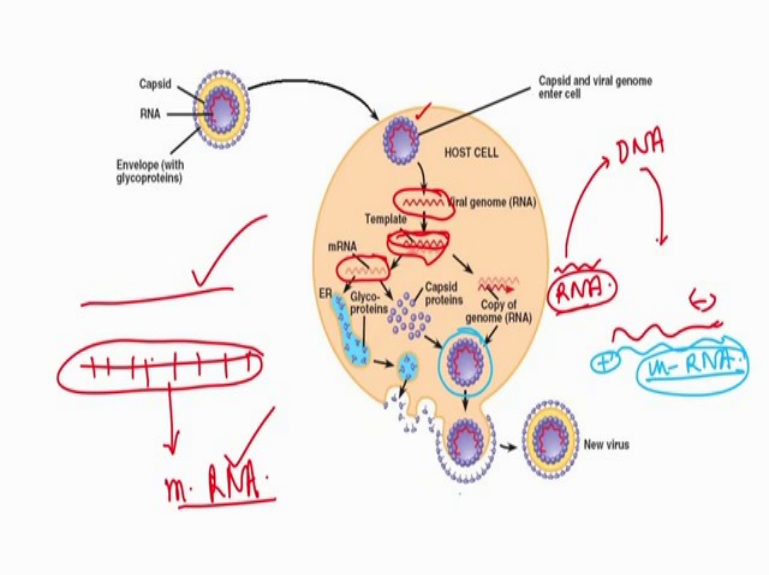

In the case of RNA virus, there are two types. We ended up by saying that RNA virus life cycle depends on whether it is a negative sense RNA virus or a positive sense RNA virus. What is the negative sense RNA virus? That means, the mRNA has the sequence which is complementary to the actual mRNA that is required. You have to understand this. Negative sense RNA virus has the sequence which is basically the sequence of the

negative strand of the corresponding DNA, if you think of the DNA although it does not have the DNA.

The negative strand mRNA means; it is not the correct sequence. Now, when you copy this you have the complementary bases that are taken up. If there is a RNA polymerase, then you get like this as your genetic material and now the genetic material is suppose this. But, if it is negative strand, then when you copy this, you will get the actual mRNA. Positive strand means the actual one, the sequence matches with the RNA that is required to synthesise the proteins.

That means, in RNA virus if it is a negative strand RNA virus, basically you do not have to go to the DNA; you directly copy it to the mRNA which is containing the correct sequence and that mRNA makes whatever proteins are required and then that is packaged and finally, that comes off; that is the negative strand RNA virus. But if you have a positive strand RNA virus, then the job is little bit more. So, first make a negative strand of that; and then negative strand is copied back. So, you get the actual mRNA.

But here it is not required that this virus needs to go to the nucleus. Because the DNA is not involved anywhere in the RNA virus. So, it takes place in the cytosol; that means, outside the nucleus but within the contents of the cell or the contour of the cell. So, that is what RNA virus is and there are 2 types - plus and minus strand. One scientist, a famous biologist David Baltimore first proposed this classification.

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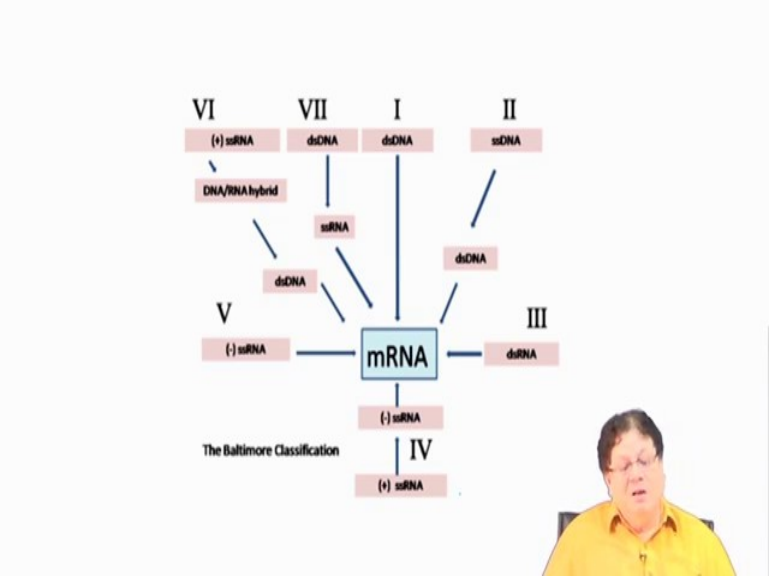

Baltimore's classification, I am not showing it, but you can get it from anywhere in the textbook or in the internet. He actually classified it into 8 types. I told you the DNA could be double strand could be single strand; so there is one classification there. Then the RNA could be single strand, double strand then the RNA could be negative sense, RNA strand positive sense RNA strand. And, then you have retrovirus; we have not discussed the retrovirus.

Thus you can get 8 classifications; that is the famous classification by David Baltimore a Nobel Prize winning biologist. Now let us come to the retrovirus. In retrovirus, again you have this RNA as the genetic material.

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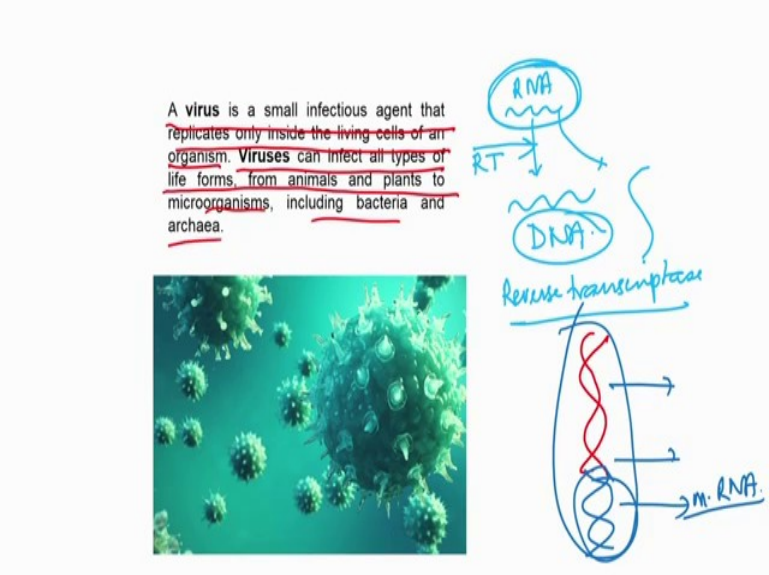

Just like the DNA virus, this RNA is injected inside the nucleus. And, then this RNA is copied into the DNA first; that is a reversal of the transcription process. What is transcription? DNA is transcribed to the RNA, but this is a process where RNA is copied back into the DNA. So, this is the viral DNA.

Now, there must be some enzyme to do that. We do not have this machinery; our system runs from DNA to RNA to protein. So, this enzyme is called reverse transcriptase. So, this DNA is now entering into the nucleus and then it will be integrated into the host DNA.

So, there is a question of integration here. So, if this is the host DNA. So, now you have the viral DNA. Let me just repeat that what is the difference between retrovirus and RNA virus? RNA virus enters and it has its own machinery. It does not need to go to the nucleus, directly the RNA is copied to the mRNA. In retrovirus, the RNA has to be copied into the DNA. The DNA then goes into the nucleus and then integrates with the host DNA and this then is utilised for the information flow.

So, the information flows from this part you will get mRNA of the virus. That is what you need; what the virus needs to replicate is to have the mRNA. And, once the mRNA is formed, then new protein particles will be made and proteins which are required for the virus replication. So, the virus particle will be formed and then it will come out of the

cell and finally infect another cell. So, that is how the infection goes. So, now we know what the different types are. Now, the question is how to treat a patient who is suffering from viral infection?

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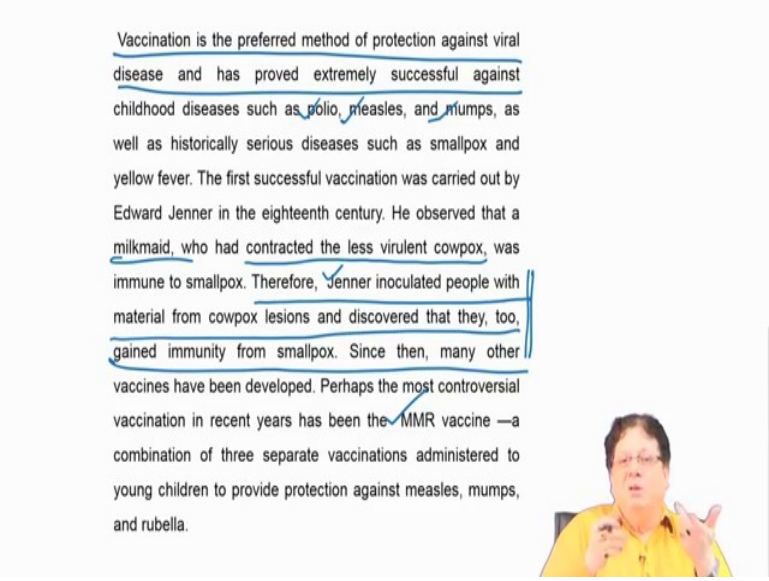

One thing is that vaccination; originally what happened? For viral infection, people realised that many of the viral infections are cured by taking rest for 1 week. If you take any medicine, you will be cured within 7 days and if you do not take medicines, it will take 1 week to get cured; that means, it is same without medicine or with medicine; it does not matter.

What ultimately makes me cured if I am suffering from viral infection usually like cold, some flue type symptoms I have? I am cured through that immune response, the immunity in the body; that ultimately takes care of these virus particles; whatever is in my body and then ultimately destroys them. So, basically it is the immunity that is very important.

Now, the problem is if a person has less immunity, then what will happen? Then you have to provide him with vaccination; see vaccination is something which basically boosts up the immune function; means, if you have a virus and if you take a dead virus of that, inject it, so what will happen? Some antibodies will be generated; antibodies are proteins; and that will generate the memory in the immune system. So, whenever the

next virus particle enters, it immediately knows that this is the type of virus particle and then immediately the body will make these antibodies and destroy the virus particles.

So, vaccination is the general route for protecting against the viral infection. And, there are different vaccines which are very successful; polio vaccine, measles, mumps, MMR. This MMR vaccine is a very standard vaccine which is given to the babies. Not all viral infections have vaccines. The vaccines were discovered way back about I think 300 years ago by Edward Jenner.

He developed the vaccine for the smallpox. At that time nothing was known. In science, only physics and mathematics came into force, chemistry was started by the discovery of Lavoisier and other people of the different gases oxygen, hydrogen, nitrogen. So, nothing was known but just by shear observation he proceeded. What he observed? That the milkmaid (who actually trades with the milk) they are generally contracted the less virulent cowpox. They actually have some type of pox, but that is not fatal.

So, they had some blisters in their hands; that was what Jenner noticed. And, they never had any smallpox. So, those persons had the disease what is called cowpox. They were handling the cow and they get a pox which gives blister, but that is not life threatening. But that protects the person from getting smallpox which is life threatening for the humans. So, he took the contents inside that blister and then injected into the other people and that is the start of the vaccination.

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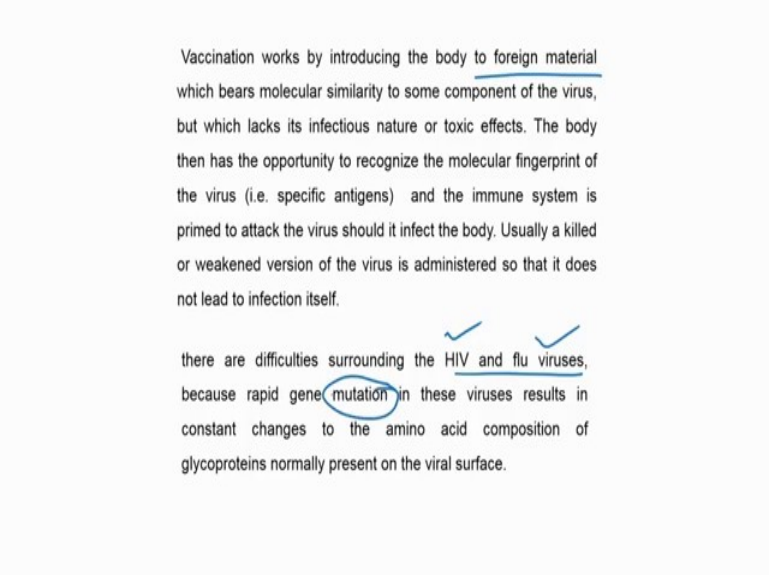

However, as I told you how vaccination works; either you take dead virus or you can take a part of the virus particle, so that antibodies can be generated. But the problem is with some of these viruses like HIV which caused the AIDS epidemic. And, then some of the flu viruses are very dangerous; like birds flu or the Nipah virus or the swine flu; there are different types of viruses are there. So, for them, it is very difficult to have a vaccination, because of two reasons. First reason is very important that the virus changes its character; so there is mutation.

Today the virus looks like this and then you generate a vaccine and the next day you see the virus has changed the surface and has different characteristics. So, that vaccination would not work. So, that is the mutation problem. The reason why I am saying all these is to stress the fact that we need antiviral drugs.

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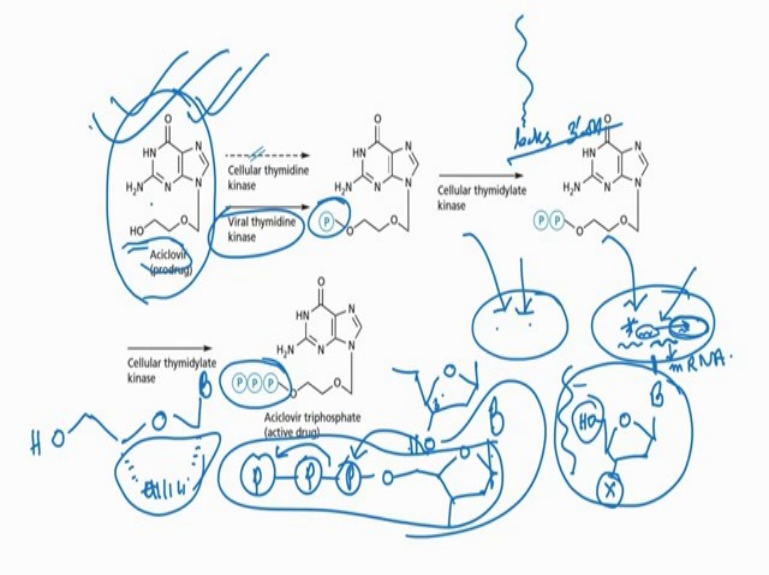

Now, the question is how do we now get the antiviral drugs? I told you it took a long time to come up, because people thought that antiviral drugs will be very difficult, because it is utilising the host machinery. But still there are certain differences; what are the differences? Let's consider a DNA virus. Now, in DNA virus what happens? I have normal cells and I have say virus infected cells; suppose this is virus infected.

In the virus infected cells, the virus will inject its DNA into the nucleus, if it is a DNA virus. What is the example of a DNA virus? Like the blisters that we get in the lips which is caused by a virus call simplex herpes virus. So, that is a DNA virus. Now what happens? Sometimes there may be little bit pain and also cause lot discomfort after having the simplex herpes virus; they also can infect the eye, which is called herpes infection in the eye. So, you need to treat this herpes infection which is a DNA virus infection.

Now what happens here? The DNA is injected into the nucleus and then there are 2 ways as I told you; it could be integrated into the DNA or it could just remain there and then it will utilise the host machinery involving the polymerase, and then make the mRNA. Now, how the polymerase works? The polymerase works by attack of the 3'-OH into the 5'- triphosphate; and then form the phosphodiester bond. So, this phosphate is attacked and you kick out a diphosphate. So, that is the reaction we are talking about.

So, now suppose we talk about the DNA which is not integrated, which is which is released, but not integrated with the DNA. Now, what will happen? This DNA has to be has to be copied. And if you want to do that, I take a compound which is a drug called acyclovir. One of the components of the increase of the polymerization reaction is the triphosphate.

Suppose you take a nucleoside which does not have the 3'-OH; the base is there. And, you are giving it in the form of OH and you have a group here X which is not able to attack the phosphate here to make the phosphodiester bond. If that be the case then what will happen if this is not given as the triphosphate, it is given as the alcohol; that means, only the nucleoside?

But to participate in this polymerization reaction, this has to be transformed into the triphosphate. So, what I have said that suppose I want to stop this process; that means stop DNA going to the mRNA of the virus so what I what I need to do? During this transcription process, I need to add a nucleoside, but not the triphosphate. Again I repeat a nucleoside which lacks the 3'-OH. That means the 3'-OH cannot any further continue that reaction involving the phosphodiester bond formation.

Now, the first drug that was made on this principle is this acyclovir. You see O and then you have a base, I am just not writing, this guanine. So, I just wrote the B. Now, what will happen? This is basically a truncated version of the sugar. But you do not have this part, but it is a truncated version of the sugar.

Now, what happens? Interestingly this is also taken up as a kind of nucleoside triphosphate for lengthening the chain. But then what happens if this is taken up by the polymerase? Then the next reaction occurs; basically if this is taken up as a base then what will happen? The sugar that is here that lacks the 3'-OH. So, if it lacks the 3'-OH then the chain cannot grow any further. So, chain growth will be stopped.

The question is how this is taken as a substrate for the polymerization when it is not present in the triphosphate form? You have given it only in the free alcohol form. So, in order to have this taken up by the polymerase enzymes and recognise it, it has to be transformed into a triphosphate. Now, here is the interesting part. You have 2 cells; one cell is virus infected, another is the normal cell, no virus.

Now, this acyclovir molecule enters both the cells. Now, it has to be converted into a triphosphate, but this takes place in stages; what are the stages? First it will undergo monophosphorylation. And, then it will undergo diphosphorylation finally, triphosphate then that will be taken up as the substrate, ok.

Interestingly, the first phosphorylation is done by a viral kinase. Our body does not have any kinase. Kinases basically make the phosphate; they does the phosphorylation. We do not have any corresponding kinase to do the mono phosphorylation of this. So, once this is monophosphorylated, then the host kinase takes up the further phosphorylation. So, it makes the diphosphate then the triphosphate.

So, what happens, when this molecule enters the 2 cells; one is infected, another is uninfected. In the infected cell, there is viral kinase present. So, that will be converted into the monophosphate. And, if it is converted to monophosphate then only the host cell machinery converts into the triphosphate and if it is converted to triphosphate, it is taken up as substrate by the RNA polymerase; if it is taken up as a substrate, then it is incorporated into the chain, the chain stops form further elongation. So, the mRNA will be truncated mRNA. So, it will not be a proper mRNA.

Now, there are two mechanisms by which this acyclovir can work. One is that it makes the triphosphate *via* this process involving the viral kinase followed by the host kinase, but either it acts as a competitive inhibitor; it goes and binds to the RNA polymerase stays there or it can react to form the to phosphodiester, but after that, the RNA cannot grow any further. So, there are two ways it can it can stop the RNA polymerase; one is acting as an inhibitor, goes binds to the active site and the other is it is actually incorporated in the growing chain, but once it is incorporated the chain growth stops.

So, basically virus cannot make the mRNA. So, that is the first landmark discovery, that an antivirus drug was discovered. So, that means, if you have a very good biological knowledge about the virus and its life cycle, you can make compounds which are antiviral. Basically you have to identify the processes which are different from the host.

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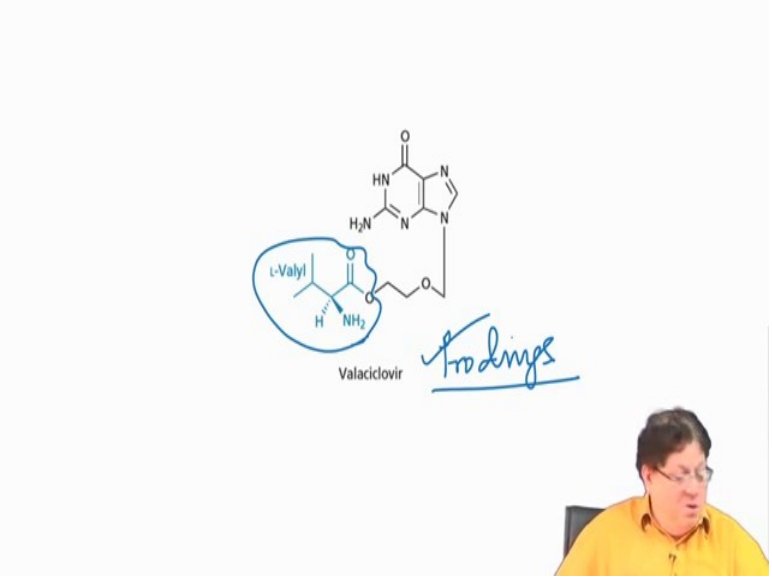

Now, problem with acyclovir is that it has got some bioavailability issue. It is not absorbed properly from the gastrointestinal tract. So, see it has to be absorbed and then it goes into the bloodstream and then attack the cells which are virus infected. In order to have that absorption better, people have put an ester which is an ester of valine.

Now, valine has recognition; valine is a natural amino acid. So, there are receptors to hold the valine part; so in the GI tract; the valine is recognised so that is taken up and the absorption is better. And, than that goes into the bloodstream. So, these are prodrugs. See all are prodrugs. If I say what is acyclovir?

That is a prodrug, because it has to be converted into the triphosphate before it actually stops the RNA polymerase; so it is a prodrug, all are prodrug. This is this is basically another step before the prodrug, because you have to cleave the valine ester bond and then you have to make a triphosphate. So, that concludes our discussion on the DNA virus. Similarly, now people have developed compounds which are anti-HIV drugs. What is HIV? HIV is a retrovirus.

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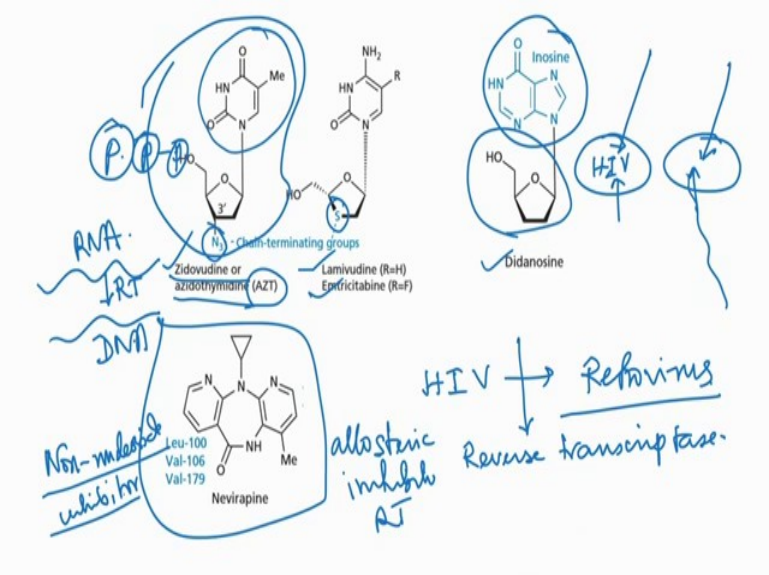

Again I remind you, in retrovirus you have to integrate the DNA which is obtained by reverse transcription process from RNA to DNA and these DNA gets integrated into the host. So, that is what a retrovirus is; then as it is integrated to the host DNA, so the host DNA will copy to the mRNA. Here one new enzyme that is there which is called reverse transcriptase; what is reverse transcriptase?

Reverse transcriptase is making of the DNA from the RNA. RNA is the genetic material, it goes to the DNA and that is what is done by the RT (reverse transcriptase. What does making of DNA mean? It involves a polymerase, but it is an RNA dependent DNA polymerase. Because the template is RNA and you are making a DNA; you have to be careful about naming all these. If I say RNA dependent DNA polymerase; that means, I am making a DNA utilising an RNA template so that it is a reverse transcriptase and what is a transcriptase? It is a DNA as a template, but you are making an RNA so that means, it is a RNA polymerase.

People found that the same strategy worked for HIV. The first drug that was made was called zidovudine or it is popularly known as AZT (azidothymidine). What it has? It has got a thymine base. It lacks the 3'-OH, but it has got an azide and it has got the OH.

But the basic principle is same that if it is taken up as a substrate, chain elongation will not take place. Now the question is how does the triphosphorylation work? Like in the

earlier acyclovir; it is the viral infected cells where the monophosphorylation is done and then the diphosphate and the triphosphate is formed by the host kinase. In case of AZT, contrary to the earlier case; here if you have 2 cells suppose, HIV infected and HIV non-infected. This AZT enters, here the entire phosphorylation is done by the host kinase.

Why it will be very specific? Because since the triphosphorylation is done by the host kinase, so this AZT will be converted into the triphosphate in the normal cell as well as in the HIV infected cell, but fortunately this triphosphate of AZT has got much higher affinity for the reverse transcriptase then towards the other polymerase enzymes that are present in the host. I hope this is clear. There is a difference in mechanism as here the phosphorylation is done by the host kinase.

So, phosphorylation will be done in both the cells; infected, non-infected. But the triphosphate that is made up from the AZT is recognised by reverse transcriptase, much more than the transcriptase or other polymerase enzymes that are present in the in the host.

So, basically what will happen? After the triphosphate formation, it is the HIV infected cells that will be affected. The AZT will work against the HIV infected cells where it is having the phosphorylation that will ultimately come out of the cell. There is a huge difference between the affinities for the reverse transcriptase via-a-vis the polymerases that are present in the host. So, that is the mechanism of AZT.

On the same line you have got different drugs. First drug was AZT, then somebody put a sulphur here; then somebody removed the sulphur nothing is there only di-deoxy. And, also you can have different bases. So, there are many permutations, combination that you can get; and in fact, there are lot of reverse transcriptase inhibitors now available for treating the HIV. You know AIDS is a deadly disease; it destroys the immune system and ultimately the patient dies of very opportunistic infections; some infections that we have are called opportunistic.

Opportunistic means they are all waiting to invade the body, but because of the immunity, they cannot do that. So, when the immunity goes down, these opportunistic micro-organisms invade. And, since immunity is not there, ultimately nothing works so the person dies of that. So, basically these are like hyenas, they are opportunistic animal;

hyenas never kill any other animal, it is the lioness who kills the animals and then the hyenas come and try to take over whatever remains are, there remove that; kick out this lioness, because they are in large numbers and then the lioness goes leaving the the dead animal and the hyenas eat those dead animals. So, they are opportunistic animals; you have similar opportunistic infections also. So, this is one way to combat them - reverse transcriptase inhibitors.

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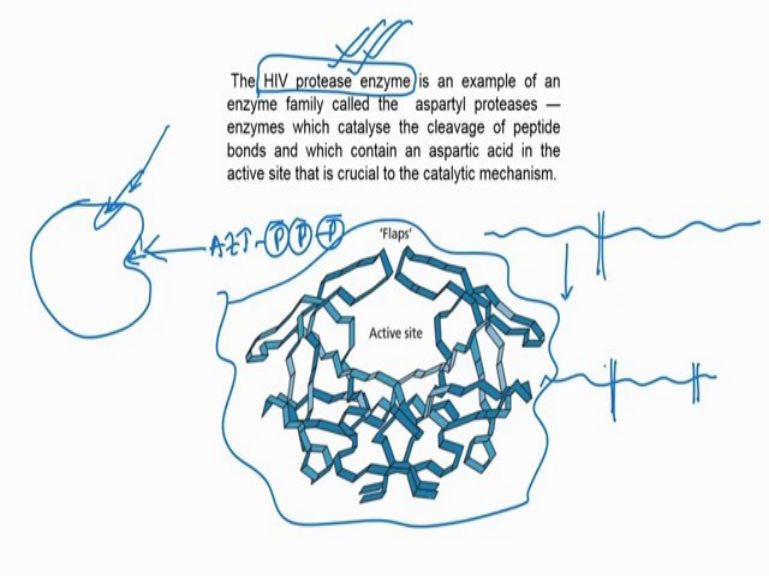

Reverse transcriptase is an enzyme, it must be having some active site, where this di deoxy derivative or the azidothymine triphosphate is binding; and then the polymerase works, so basically the AZT triphosphate has to bind to the active site then that will be incorporated into the growing chain.

Now, there is a process which is called allosteric inhibition. If you remember, I told you that allosteric inhibition is basically where the inhibitor binds to a different site, but when it binds to this site, it closes the normal site; that is allosteric inhibition. So, basically it is non-competitive, because they are not going into the same active site, the normal active site is there, and there is an allosteric site. So, there is a molecule which goes here, it does not bind to the normal active site, but as it binds to the allosteric binding site, as a result, the normal binding pocket is closed.

So, you can have allosteric inhibition of reverse transcriptase. And, one example is shown here. Nevirapine is a drug; which is an allosteric inhibitor of reverse transcriptase. Look at the structure; it is also called a non-nucleoside inhibitor.

Because, earlier AZT and all these things were nucleoside, base and sugar. But now you have a non-nucleoside inhibitor that is the Nevirapine. Since the virus changes the characteristics, it mutates very rapidly so you have to target the virus by different techniques, if you give the same type of molecule like; only AZT or other nucleoside, it may not work in the long run.

So better you have a cocktail of drugs; that one targets the active site of this reverse transcriptase, another targets the allosteric site. And, if there is another mechanism by which this reverse transcriptase can be inhibited then better do that also, add that into the cocktail. Now yes, there is there are again scope of making new antiviral agents for HIV, because remember HIV is a retrovirus.

So, retrovirus what it does? It has got a reverse transcriptase which is not present in the host. And, I told you that in the retrovirus, that RNA has to be copied into the DNA and the DNA has to be integrated into the host DNA. Who does the integration? That is also another process. Because your host DNA is here so somewhere you have to insert your viral DNA.

So, there must be some protein which is doing that; chopping at some point, putting in the virus DNA here and then sealing it. So, that is what is done by an enzyme called integrase. So, now we have a scope; you can inhibit the integrase also; that is possible however, not much success is yet achieved by inhibiting the integrase, but it is a good target. But there is another target and that is how the virus enters the host cell; how does the virus enter the host cell? I told you that there is a recognition point; there has to be recognition between the cell surface and the virus particle.

The virus envelope that made up of glycoproteins. Who makes that glycoprotein for the virus? There must be some enzyme that is present in the virus and that was a protease enzyme which is very crucial for the virus to replicate and to enter into the cell. Because this HIV protease makes the glycoprotein; so initially the viral mRNA that comes out of

the nucleus is very big. It has to make all the proteins, so first it makes a big protein and then the protein needs to be chopped off into the actual functional components

This entire big protein has to be chopped off; that means, you have to need a protease to do that; so the virus HIV protease is very crucial for the virus to amplify; to replicate, because if you do not allow this chopping to be done, then the virus particle will not have the proper glycoprotein which allows it to anchor on to the surface of a cell. Thus the protease enzyme is very vital for this replication process of the virus to make the proper glycoprotein. So people started studying what is the structure of this HIV protease? And after the advent of this X-ray crystallography and the Cryo-EM, HIV protease structure was solved and it was found that it is a dimer.

It is basically has two similar units forming a dimer. And, the active site is basically in the cavity that is there when the dimer is formed. So, basically you can dissect it into this; so one subunit is here, another subunit is here and you see there is an empty site here which is the active site and this is the flap region through which the molecule enters.

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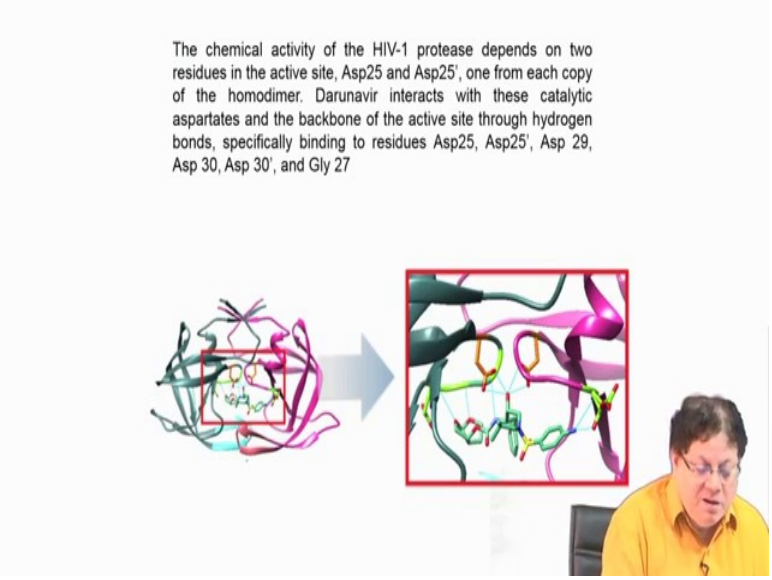

I think I might have a better picture of HIV protease; this is a crystal structure you see this is one part and this is the other part. So, it is a basically a C_2-symmetric dimeric enzyme which does cleavage of the peptide bond. Now, question is proteases we know

are 4 different types: one is serine protease, cysteine protease, aspartyl protease, metalloprotease.

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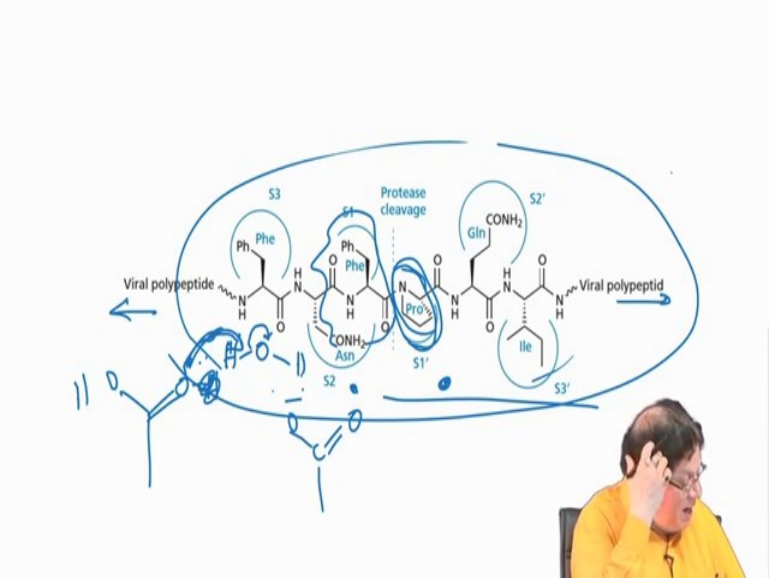

So, first question is what type of proteases is this? They found that this is an aspartyl protease; that means, the aspartate is present like this. So, the aspartate helps the water to remove the hydrogen from here and then thereby increasing the nucleophilicity of water.

So, that attacks the carbonyl of the peptide. So, it is an aspartyl protease; again I just say aspartyl protease is basically, one aspartic acid and one aspartate. So, first the aspartate takes up the hydrogen from the water. So, making OH minus; virtual OH minus; that goes to attack the carbonyl, kicks out the nitrogen and then the OH minus and then it has to put back the hydrogen to the to the aspartic acid that is there.

Anyway I think that has been covered earlier, the aspartyl protease. So, two aspartic acid are there; one is here, another is there. And, then the peptide bond, that is hydrolysed; it is very interesting; that the peptide bond is on this side; that means, on the C-terminal and this is the N-terminal.

So, now this is a proline. And, this is usually an aromatic ring phenylalanine; what we know that like all the enzymes they cannot cleave the peptide bond if there is a proline. But here this is an enzyme which likes to have a proline and cleaves the bond between phenylalanine and proline, that gives a very good handle because; that means, if you can

inhibit this protease, human enzymes because they do not cleave the peptide bond involving proline, so they will not be affected much because they will be entirely different character.

Even if we have aspartyl protease, but that is not able to hydrolyse the peptide bond involving a proline. So, that is why you can expect selectivity. If you can inhibit this HIV protease; so HIV protease one means, it can hydrolyse many other sites; but one of the primary site is this that hydrolysis of peptide bond between a phenylalanine and a proline of a glycoprotein.

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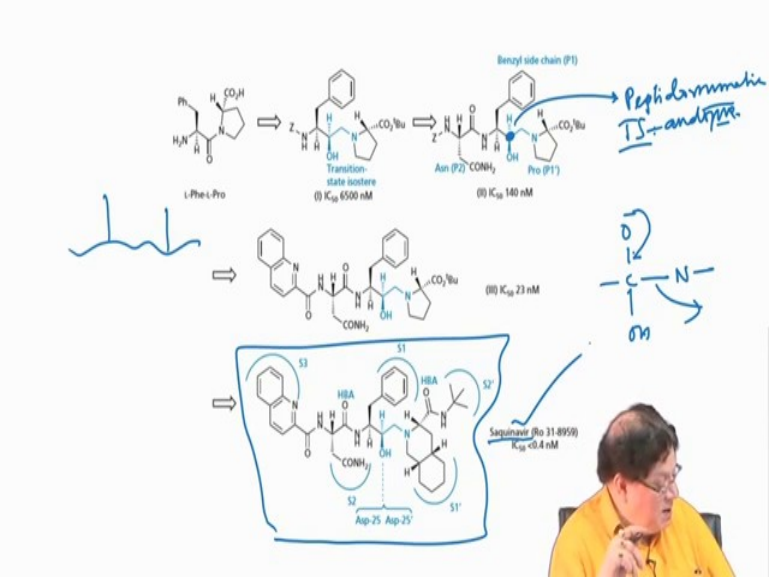

Based on the structure of this peptide, they have modelled different compounds and made HIV protease inhibitors. They started with a proline. Remember the enzyme is HIV protease which recognizes a proline and then hydrolyses the amide bond. So, basically your compound should start with a proline and a phenylalanine.

Now, let see whether L-phenylalanine-L-proline is an inhibitor. Or you increase the size; put different groups. So, slowly the size of the peptide got increased. And finally, what happened? You know that when the hydrolysis takes place, it goes *via* a tetrahedral intermediate. This was the case, if water comes then this goes out; it is a tetrahedral carbon.

So, you want to have a tetrahedral carbon at the site of the carbonyl, but it cannot be a carbonyl because if it has got a leaving group then the attack will take place. So, what you want is what is called transition state analogue. So, you have a sp^3 carbon with OH and then the carbon. So, this is called peptidomimetics. It is not a peptide bond, but you are mimicking a peptide, but this peptidomimetic is basically based on transition state analogue.

So, they have put proline and then they change the proline; finally, they found that a fully hydrogenated isoquinoline is better than proline. So, we are not going into the details; it must be a trial and error, but they started with L-proline and L-phenylalanine and finally, the drug that is now approved is called Saquinavir.

Saquinavir is a compound for which the design is based on that peptide which it hydrolyzes; there is a proline and there is a phenylalanine. So, it starts from there and slowly elaborated that; only thing you have to remember that it has to use a transition state analogue; you cannot use a peptide bond. So, that crucial peptide bond you have to replace by what is called peptidomimetic approach.

So, this is the first drug against the HIV protease inhibitor. Then a success story from a Bengali scientist followed.

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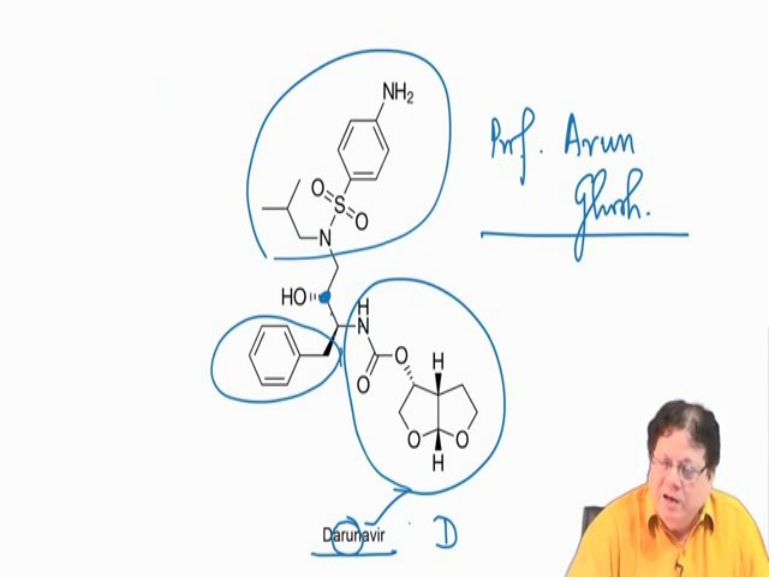

Many people do not know this. Professor Arun Ghosh, he was the student of Narendrapur Ramakrishna Mission College in Calcutta. Studied chemistry, went to IIT Kanpur then went to went to Harvard did his PhD and then went to first Illinois and then now he is in Purdue University.

So, he started trying to develop HIV protease inhibitors. And, again you see, this was the carbon which is involved in the peptidomimetics; that means, the transition state analogue and he had the phenylalanine already there, but instead of the proline, you have a sulphonamide now. Actually this requires lot of *in-silico* screening those different molecules how do they score when you dock with the HIV protease.

So, ultimately he came up with the molecule which is called Darunavir. So, this is having S configuration. I know that this part comes from his name Arun. So, possibly there is some D-configuration in the molecule that is Darunavir. So, he called it Darunavir and it is now approved as a drug.

And, so that is the contribution of an Indian scientist in developing an anti-HIV agent. This is the crystal structure how Darunavir is interacting with the HIV protease. It is binding with aspartate 25 and 25'. Here prime means one from the subunit and normal numbering is the other subunit.

These are the key amino acids in the enzyme which does the hydrolysis. It actually coordinates to the water molecule and then in addition to that, it has got other binding partner; that is why it works in nano-molar level. So, we have now discussed about how to develop antiviral drugs.

With antiviral drugs, you can target certain aspects of the virus life cycle; like if it is retrovirus, you may reverse transcriptase; if it is a DNA virus, you can target the phosphorylation basically and then you can inhibit the polymerase there; you can target the HIV protease in case of HIV. And, this is important because the glycoproteins are synthesized by the virus.

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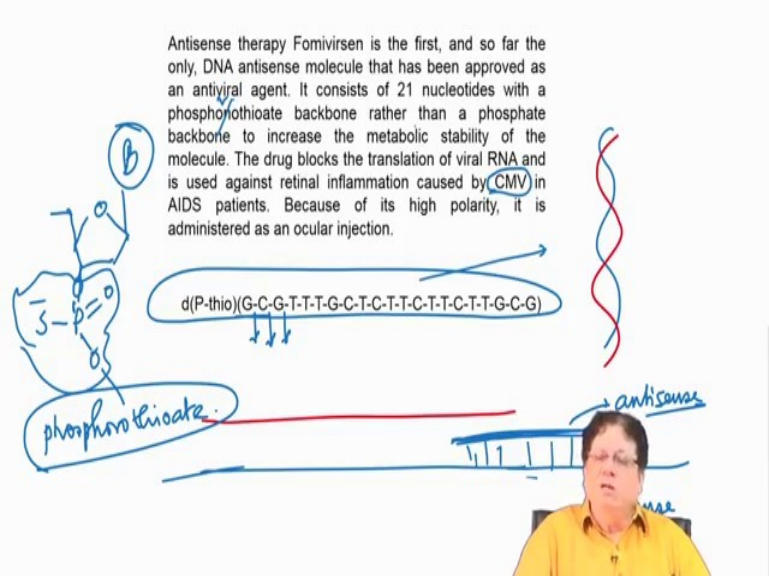

Just the last one I think another strategy which was successful only in one case up till now is what is called antisense therapy. What is antisense? Antisense means, the negative strand; which is copied to the strength strand; so when the virus replicates, it will make the sense strand of the mRNA.

Now, if you know the sequence of the mRNA, what you can do? You can make the complementary strand of that RNA and give it to the system. So, what will happen? Suppose this is the same strand of the mRNA. From outside you are giving the complementary strand of the RNA. So, what will happen? The RNA will now form a double stranded RNA. I write it in linear fashion. Suppose this is the sense strand, and I make antisense part suppose this. So, this will go and block the mRNA for my double strands here like a primer.

So, then what will happen? This translation will be stopped. So, that has to be antisense then, because you have to stop the sense strand of mRNA from working. However, there was a problem in this strategy, the problem was, there are enzymes which are called nuclease enzymes; like ribonuclease. So, when you give some RNA from outside, some RNA piece, that will be immediately chopped up by the by the RNA the ribonuclease; the nuclease enzymes.

Before sense RNA binds to the target, this whole RNA will be hydrolysed. So, that will be ineffective. So, that was the major problem. So, what people did? The sequence has to be the complementary. So, the normal bases have to be taken, but instead of phosphates, what they did, took the thiophosphate.

Now, this is what is called also called phosphorothioate linkage. So, basically what you have done? You have removed the oxygen, put a sulphur; you can say sulpho phosphate, that is not a problem, but in the literature it says phosphorothioate; some books have written phosphonothioate; I think that is wrong. The nuclease cannot hydrolyse this phosphorothioate linkage.

So, these are called antisense with stable phosphorothioate linkage; that linkage has to be there because in order to bypass the action of the ribonuclease which is going to hydrolyse all these things. So, all these linkages are basically phosphorothioate, and this is the drug. So, they made this antisense drug for cytomegalovirus; there is a virus called cytomegalovirus.

Cytomegalovirus causes infection of the eye; very serious conjunctivitis in the eye. So, the strategy is to make an antisense oligonucleotide with a stable phosphorothioate linkage. When the virus wants to replicate, it makes the mRNA; that mRNA is blocked by forming the double strand. So, that is the other strategy.

So, basically vaccine is a strategy, then you have this reverse transcriptase inhibitor, you have the DNA polymerase inhibitor, you have the protease inhibitor and you have the antisense strategy. Besides there are other strategies; I think for our purpose knowing more or less the general strategies and the different drugs that are present or that are used today to treat viral infection, is enough. So, we have come a long way from 80s in developing many of these antiviral agents.

Thank you.