RNA Biology
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Lecture - 26
RNA Splicing, Export and Stability: Different Spliceosomes

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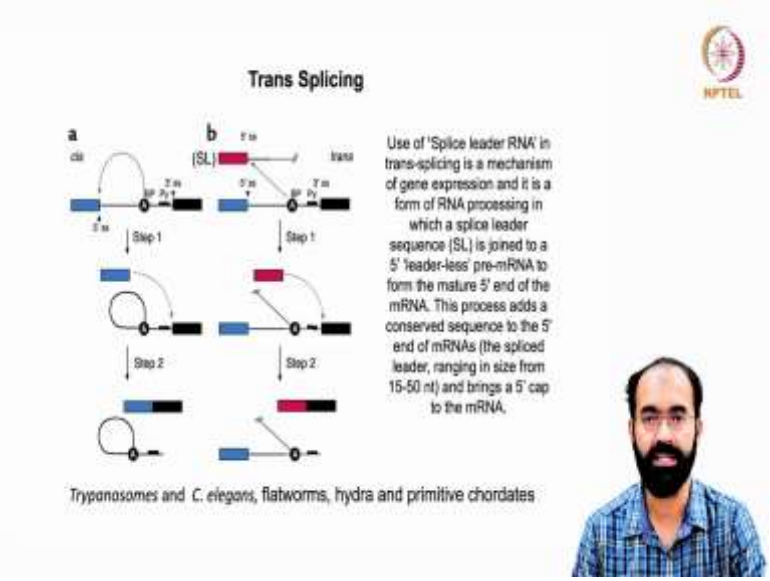

Hello everyone, welcome back to another session of RNA Biology. In the previous class, we were discussing the Trans splicing. And Trans splicing as the name itself indicates it is splicing on to another exon which is not similar to the cis splicing what normally occurs in both group 1, group 2 and also the regular splicing.

So, Trans splicing has got mainly 2 purpose. Purpose number 1 sometimes the RNA lacks the 5 prime regulatory elements such as required for a specific location onto of the RNA in the cytoplasm. And which is by virtue of that gene itself it is lacking those information.

Say for example, you usually wear a shoes, right? So, you do not know to make shoes. So, you rather purchase the shoe from the shop and you wear it and you enjoy the benefit of all those who know to make the shoes. Same way cloth and anything. So, you get the advantage, but you do not to make any of them.

Same way this Trans splicing provides some sequence to a given RNA which is lacking the ability to have those sequence this sequence is a must for the further processing of this RNA. So, we have to understand the piece of RNA that provides this exon and we call it as a splice leader RNA that is contributing to the Trans splicing.

It is a mechanism of gene expression and it is a form of RNA processing. And you can see here this red colored exon which is a splice leader sequence. Now, the branch point from one of this gene one of this RNA that is present on the intron of one of this gene is instead of going to this splice site it goes on to the Trans splice site and subsequently the splice leader sequence is going to do the second transesterification reaction on to the RNA's second exon.

So, in this way it will have the advantage of having this extra exon pink colored exon which is missing from this blue colored exon. So, this is a way of living for many organisms such as trypanosomes, C. elegance, flatworms, hydra and also in several primitive chordates.

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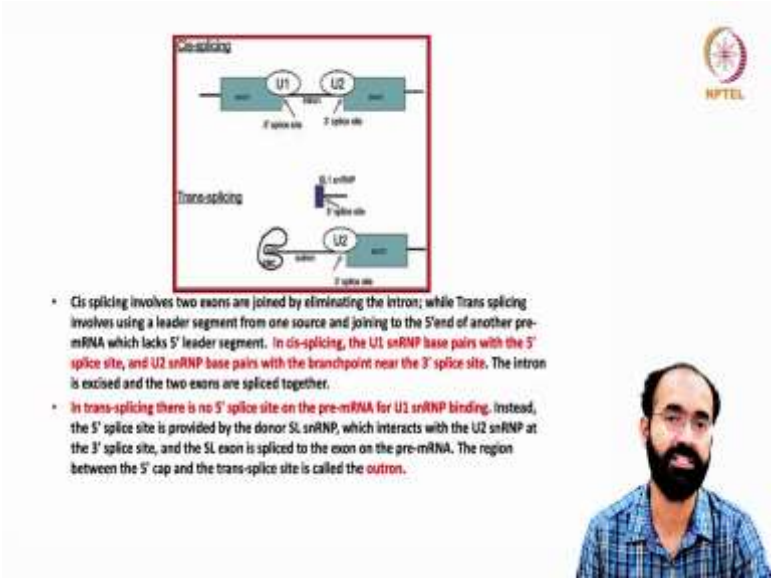

Now, let us quickly see more in detail about the Cis splicing versus Trans splicing. Cis splicing involves two exons that are joined by eliminating one intron while Trans splicing involves using a leader segment from one source; that means, some another RNA joining the 5 prime end of another mRNA which lacks the 5 prime leader segment.

What we understand from this is several RNAs need this. Just like you need a shoes several RNA need this 5 prime leader sequence, but it is incapable of having in its own sequence or incapable of having in its own genome. It is produced elsewhere and it is provided by this Trans splicing.

In Cis splicing the U1 snRNP base pairs with the 5 prime splice site whereas, U2 snRNP pairs with the branch point where the a is there at the 3 prime splice site. The intron is excised and the 2 exons are spliced together which we know very well we have seen this with group 1, group 2 introns and also with the regular splicing. In Trans splicing there is no 5 prime splice site on the pre mRNA for the U1 snRNP to bind.

In many cases the 5 prime splice site is missing. Instead, the 5 prime splice site is provided by a donor SL snRNP which contains the SL RNP or leader sequence. And also, it is recognizable by the U1 snRNP that is why we call it as snRNP which interacts now with the U2 snRNP which is located on another RNAs branch point which is present in the intron at the 3 prime splice site. As you can see here U2 is situated here and U1 is now pairing here.

And normally the branch point is at the 3 prime splice site and the SL exon that is the splice leader sequence bearing exon is now spliced to the exon onto the pre mRNA by during the second transesterification reaction. The region between the 5 prime cap and the Trans splice site is now called outron. Because it is not intron it is standing outside the exon. Normally intron lies between two exons. So, since this is standing outside one exon, we call it as an outron.

So, be clear with this terminology. Normally intron also is removed out, outron also is removed out. Here the outron is removed as a small piece or a fragment which is expelled from the existing RNA. And that is why we refer to as an outron. And it contains both the exonic part if any or the unwanted intronic part.

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What are the functions of Trans splicing? First of all, it has a function in the regulation of gene expression. What way it is regulating the gene expression? If a gene is lacking this leader sequence it may not be able to function in many cases.

So, it makes sure that the RNA is capable of performing the task what it is supposed to be doing. If it is an mRNA, it should be able to participate in translation. If it is a non coding RNA, it should be able to target its actual destination. So, the 5 prime cap is provided by the splice leader sequence.

Because if the splice leader sequence is completely lacking many times when the RNA is processed it may not be able to get the cap. If the cap is not there 7 methyl guanosine cap is not there in the 5 prime end it is a basically a death sentence for the RNA.

So, you do not want that to happen. So, the splice leader bearing extra RNA or a fragment that is added becomes handy and a life saviour for this RNA that lacks this information. And it also helps in the separation of cistrons. Say for example, what is cistron?

Cistron is the coding sequence present in one RNA like we kind of discussed in earlier class that the prokaryotic genes are polycistronic whereas, eukaryotic genes are monocistronic. And polycistron basically means one large RNA can contain multiple genes located with a gap in between.

So, gene a, gene b, gene c can be present in one huge mRNA. This happens in prokaryotes. Or in other words this polycistronic gene can be compared to the multiple exons present in a eukaryotic gene. Prokaryotes do not have exons and introns. We know that they have a single coding region, but prokaryotic RNA can have multiple genes located in one RNA. And that we call it as polycistronic RNA.

In many cases in advanced or metazoans they may not have this polycistronic feature. So, if at all it existed then you should have a way of separating. Because without separating this way they will not be able to participate in translation. Because eukaryotes once the ribosomes start protein translation it will complete until the stop codon is reached. The moment stop codon is reached the ribosomes disassemble from the RNA. There is no way of proceeding further.

So, the second coding region or a second cistron if it is there it will fail to get translated usually, viruses come with a some elegant strategy that is called internal ribosome entry site where the ribosomes can re-enter into the RNA because of their unique sequence feature.

So, that is what is called IRES Internal Ribosome Entry Site which can fool the eukaryotic system. Because viruses also produce this polycistronic RNA, but a typical eukaryote do not produce. If it produced the Trans splicing becomes handy. Because say gene 1 and gene 2 present, 2 genes are present in one RNA.

So, gene 1 can be spliced onto an spliced leader bearing RNA and it can get rescued. And then gene 2 also can get Trans spliced onto a SL bearing RNA and it can save both the cistron separately. So, that is with another advantage of having Trans splicing.

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And now let us look into the RNA splicing little bit more in detail with spliceosome in focus.

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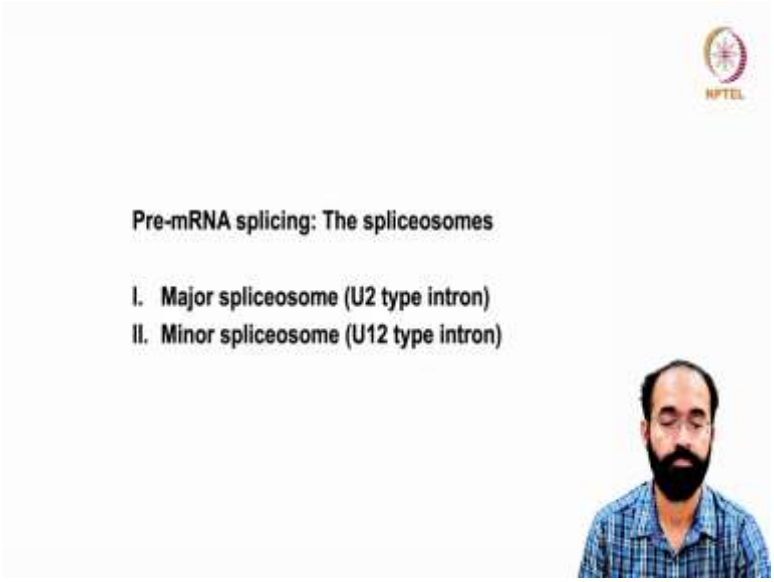

And pre-mRNA splicing is broadly classified into two. One is via major spliceosome and another via minor spliceosome. So, the major and minor is given nothing to do with their size, but based on their frequency.

So, major spliceosome is the handling of bulk of the splicing whereas, the minor one handles a few of like in many universities you may have heard you can major in some

subject and you can also take minor in subject. And their major and minor means how intensive you are studying that subject? But here major and minor you are naming based on the prevalence how frequently. It is just like in your house you have human and also you have got a cat.

So, who is major in bulk? And who is occupying more space? Who is occupying more eating more food? Naturally human is the one who is eating occupying more space and cat require less food less space etcetera. So, human can be called major, cat can be called as minor.

Same way the prevalence of major spliceosome is more obvious whereas, the minor spliceosome is a tiny fraction of it. But the main difference is U2, U1 and U2 are used in the major ah spliceosome whereas, U11 and U12 are used in the minor spliceosome.

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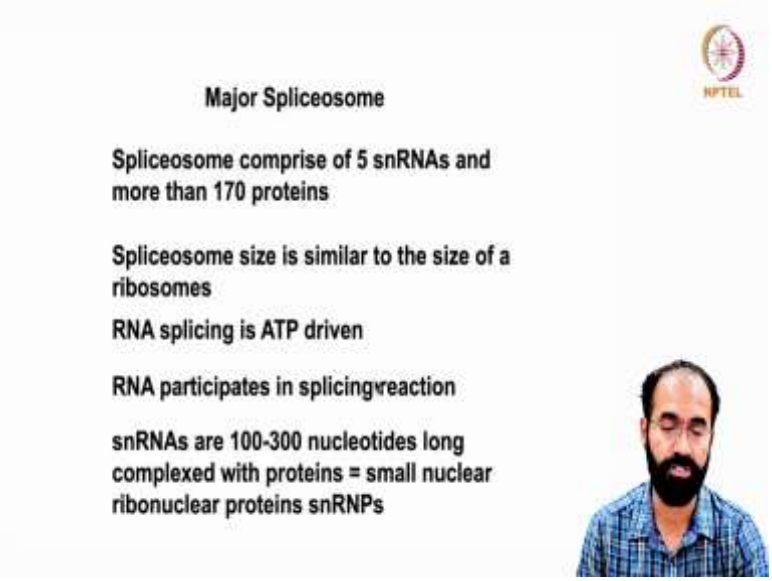

So, major spliceosome consists of 5 snRNA and more than 170 proteins are involved. And the spliceosome size is similar to the size of the ribosome itself. And the RNA splicing is ATP driven and the RNA participates in the splicing reaction, proteins basically stabilize it, RNA participates in the actual splicing. And snRNAs are around 100 to 300 nucleotides long and it complexed with proteins and we call it as small nuclear ribonuclear protein or snRNP and the splicing signals.

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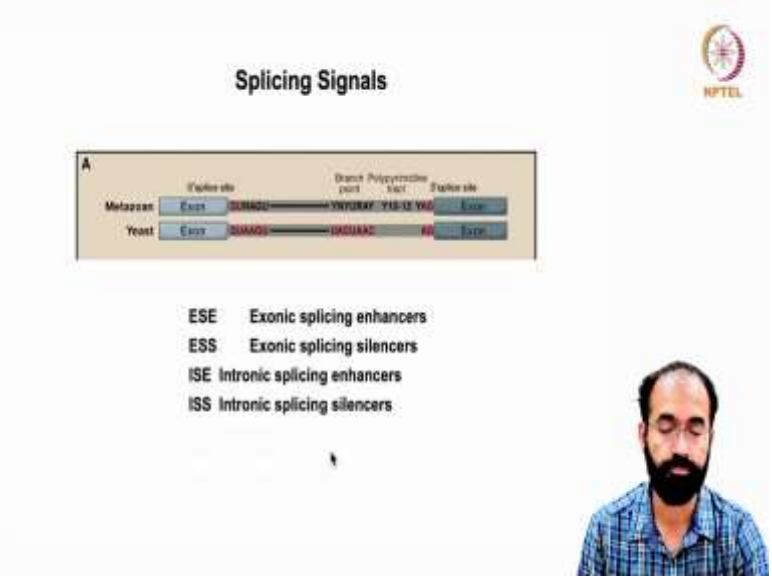

When you are looking further the splicing signal can be classified into 4 different categories. And first of them is ESE Exonic Splicing Enhancers and ESS Exonic Splicing Silencers and ISE Intronic Splicing Enhancers and ISS Intronic Splicing Silencers. So, from this what do you understand from this 4 categories? The name itself indicates exonic splicing enhancers.

So, these are sequences that facilitate the splicing event. And there are exonic splicing silencers. This just like in a car you have got an accelerator as well as brake. You cannot imagine a car that has only accelerator or only brake. So, these enhancers and silencers act antagonistically. So, they will enhance and they also decrease reduce the rate of production of spliced product. And same way intron specific.

So, one can one set can be exon specific another can be intron specific. But starting from yeast to metazoan you have same signal that is starting with GU ending with AG you need to have a branch point and you also need to have poly pyrimidine track in the case of metazoan.

And the requirement remains the same, but there are specific sequences on exons as well as intron that can accelerate and decelerate the splicing event. Of course, they will be recognized by specific proteins and we will see them how they become handy in the degradation of RNA.

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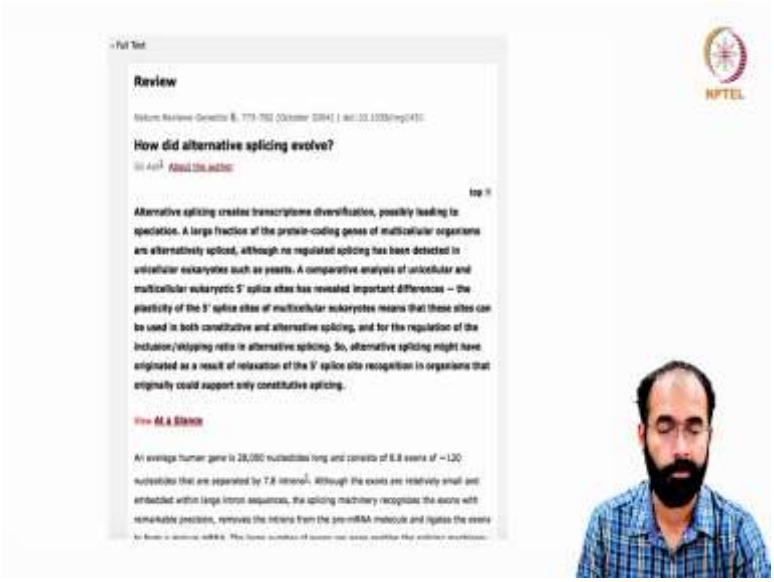

And how did the alternative splicing evolve? So, those who are interested can read this review that is published in Nature Reviews Genetics few years ago and alternative splicing is a very interesting topic. And you will get to know more about it how different organisms make use of alternative splicing not only for introducing complexity but also for various other cell biological needs.

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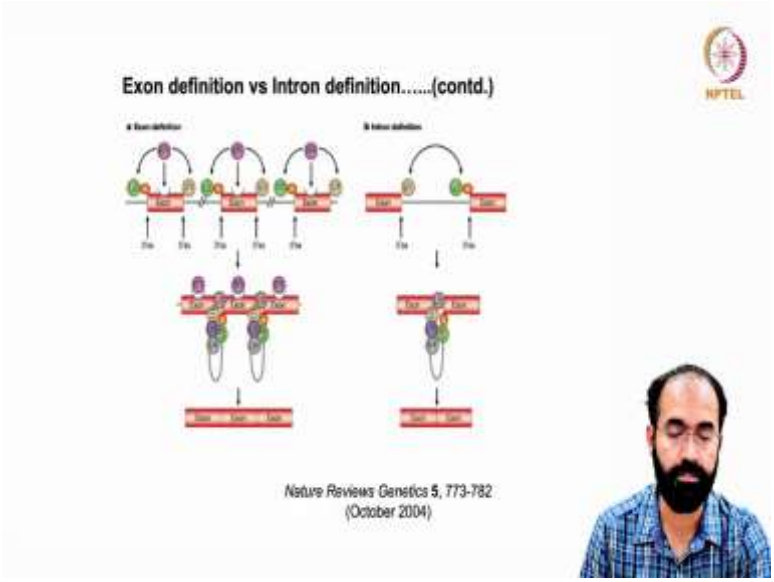

Now, let us think how can we define how can we define exon or how can we define intron. So, if you see here, you can see that a typical exon, typical exon have got a 3

prime boundary and also a 5 prime boundary. And it also have got splicing recognition signal proteins located bound on to the exonic sequence. And individual exons have these molecule and we should understand in every exon 5 prime to that exon you always have got this U2 branch point.

And because the U2 snRNA pairs with the branch point and it also can have a association or a link with the proteins bound to the exon. Because that is why the branch point cannot be way too far upstream from the second exon or downstream exon. So, you can see individual exons can have specific proteins bound on to them which can interact with its 5 prime and U2 and the 3 prime and U1. And 5 prime of the exon have got the 3 prime splice site whereas, 3 prime of the exon you have the 5 prime splice site.

So, that you should keep in mind and each of these exons will have a U2 snRNA which is bound to the branch point and interacting to the exon bound protein which is specific type of protein that can recognize the exon. And this complex is important that it plays or it contributes a major role in the actual splicing event to get rid of this huge chunk of intron, this is one exon, another exon, another exon.

So, these proteins can influence or interact on either side or to U2 as well as U1. U1 anyway binds on to the exon intron boundary. And this is the next phase in which these proteins exon bound proteins are still located and this splicing is occurring and end of the show you have all 3 exons are fused together.

Now, let us see how intron you are defining, how introns definition can be brought into this context. So, you can see exons and exons are present which are separated by the intron. And you have U1 and U2. U2 pairs in the branch point U1 pairs in the exon intron boundary and U1 pairs in the 5 prime splice site whereas, U2 is close to the 3 prime splice site.

And now this complex is formed such a way that the U1 and U2 have got an interrelationship. Because the boundary of an intron is defined by the gap between the U1 and U2. U2 is not on exactly on to the 3 prime splice site, but it is very close to the vicinity of the exon intron boundary.

So, here U1 binds on to the exon intron boundary whereas, U2 interact with the downstream exon via a protein linkage. So, the boundary of an intron is in between these

2. Ideally, you can say gap between U1 and U2. So, this is the intermediate complex and viscose of which the intron is expelled out and the exons get spliced.

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So, let us see the definition in a verbal manner. So, exon to define they contains the SR protein which is in the previous picture we saw bound onto the exon which is given in purple color. And they bind to the exonic splicing enhancers and which is referred to as ESE and which is given in blue color in this picture.

And they could recruit the U1 snRNA to the downstream 5 prime splice site and the splicing factor U2AF and which is given in orange color as you see here. And what happens? And this is located to the downstream of the polypyrimidine tract.

So, the branch point followed by the polypyrimidine tract followed by the downstream exon and this is happening or this is occurring at the 3 prime splice site. So, U2AF then recruits the U2 actual U2 snRNA to the branch site. Therefore, when the SR proteins bind to the ESE that is exon specific enhancer, they promote the formation of a cross exon recognition complex by placing the basal splicing machinery in the splice site that flank the same exon as you saw in this picture.

So, this SR protein have got a unique interact interrelationship with U2 and U1. And if you want to bring this context into intron definition what happened? The binding of U1 to the upstream of the splice site which is given as ss in the picture and the U2AF and U2

to the downstream of the polypyrimidine tract that is the gap between the branch point and the polypyrimidine tract and further downstream you have got the second exon. And the branch site respectively of the same intron.

So, the boundaries of introns are defined by this U2AF, U2 towards the 3 prime and U1 to the 5 prime of the intron. So, therefore, intron definition selects a pair of splice sites located on both the ends of the same intron and the SR proteins can also mediate this process as you saw in this picture.

So, exon boundaries are defined by U2 AF and U2 and the U1 in the downstream. Whereas in the case of intron U1 in the upstream and U2AF and U2 in the downstream. Those who are interested can read this nature reviews genetics which appeared few years ago.

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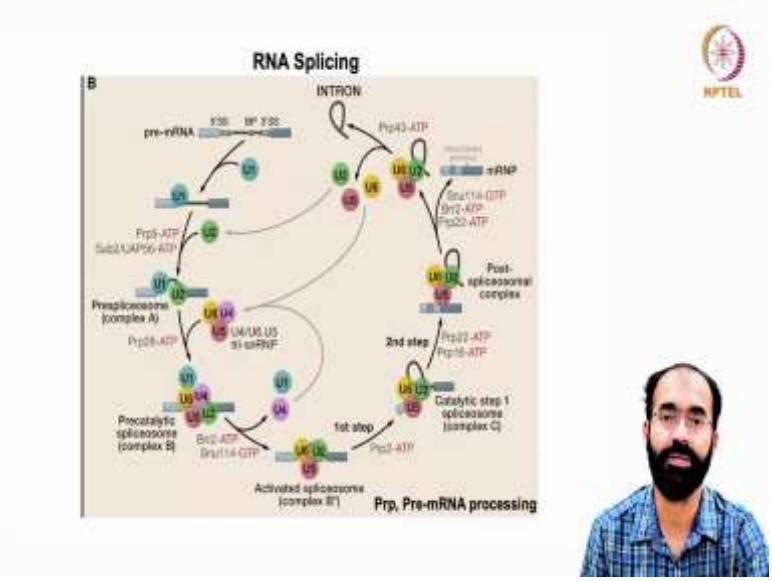

And let us revisit the RNA splicing. What happens? In a pre mRNA you have intron and exon that is present and intron and exons boundaries are specified and you have the incorporation of U1 in the first exon intron boundary and then incorporation of U2. And this stage there is utilization of ATP in this when the a U2 is joint.

Because that is facilitating the folding of this intron so that U1 and U2 can come together. Because their pairing also is important without which the first transesterification reaction cannot happen between the branch point and the first exon

intron boundary. And we call it as pre spliceosome complex A and then again you have got this Prp28 another ATP utilizing enzyme.

And in this complex you have U6, U4 and U5 proteins joint and you end up getting a bigger complex complex B and this is pre catalytic spliceosomal complex which contain U1, U2, U6, U4 and U5 together. Soon after this complex is formed that is complex B is formed you have two more proteins that is Brr2 and Snu114 and one utilize the ATP another utilize the GTP. Both are energy yielding reactions.

And as a result, you lose U4 and U1 from this complex which can continue the cycle and it can participate further and further. Whereas this U1 and U4 is lost you have U2, U6 and U5 and this is called activated spliceosome that is complex B star. So, it is an intermediate complex. And this proceeds into the first step of the transesterification reaction and in this it it has the Prp2 another ATP utilizing enzyme. And then you have the catalytic step of the spliceosome we call it as complex C.

And once the complex C is formed; that means, the first transesterification reaction happened between the branch point and the 5 prime splice site then the second step. Because it is free the exon is free the 3 prime OH of the first exon is free and it participate in second reaction provided you have this Prp22 ATP and the Prp16 ATP joins this complex and you end up getting a U6, U2 and U5 and which is called post spliceosomal complex.

Because first step is done, second step is done, the RNA spliced and you got a post spliceosomal complex. At this stage there is snu114 GTP, Brr2 ATP, Prp22 ATP these molecules are involved and they release the mRNP that is quite a lot of the proteins are associated still with the spliced exon bearing RNA. That is why we call it as instead of mRNA we call it as mRNP. And again, there is utilization of ATP to disassemble this complex U6, U2 and U5 disassemble.

So, that the intron lariat is released and once the intron lariat is released this U2, U5 and U6 are ready to participate in one more round of reaction. So, this is the actual splicing event that takes place in a sequential manner. You can calculate how many ATP's are utilized in one splicing or a one circle of splicing reaction 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ATP's are used and 2 GTP's are used.

So, total 12 are used in per splicing reaction between between 2 exons. You need roughly 12 energy yielding molecule 10 ATP and 2 GTP are utilized. So, we will continue in detail about the snRNP's and the spliceosome more in detail in the next class.

Thank you.