RNA Biology
Prof. Rajesh Ramachandran
Department of Biological Sciences
Indian Institute of Science Education and Research, Mohali

Lecture - 28
snRNA, rRNA, miRNA, siRNA Processing, Export and Function: Introns and Link to Splicing

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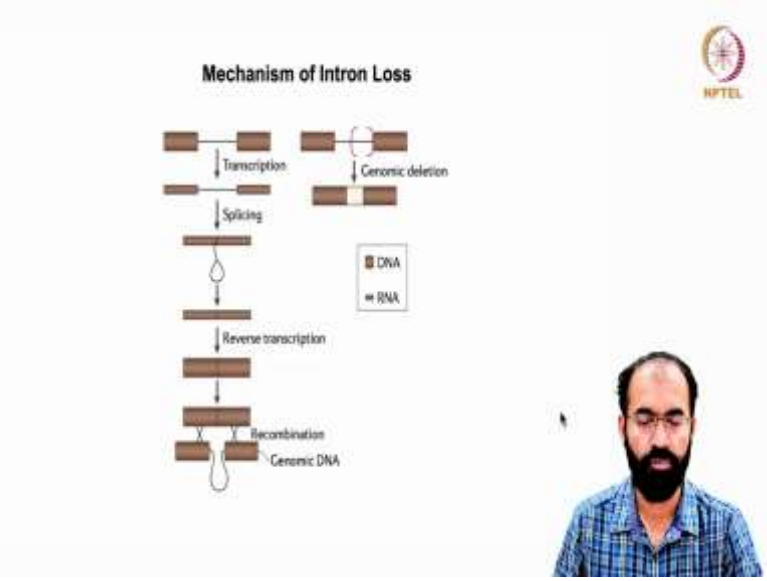

Hello, everyone. Welcome back to another session of RNA biology. So, we were here in the previous class that is mechanism of intron loss and we had proposed two possible situation. One is the reverse transcription mediated loss that is when a cell has encountered a reverse transcriptase and it can recombine back into the genome where the exons are there.

Recombination will happen only at the place of exons because the reverse transcribed gene is from a RNA and the recombination will integrate into the genome and as a result the intron will be lost. Another second category is the loss of part of the intron or full intron through recombination or replication error mediated loss and you can have loss of intron.

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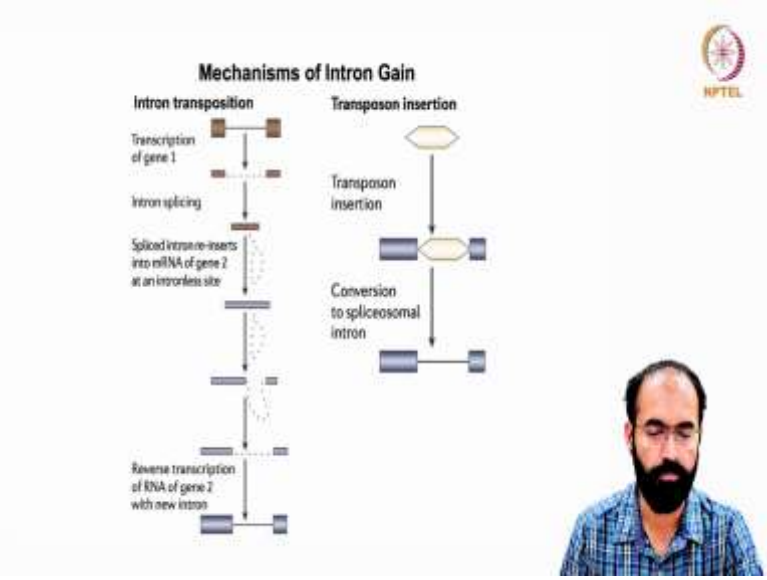

Now, let us see how can we have intron gain, how can you get gain of intron. So, you can see here exon 1, exon 2 and the transcription takes place and introns get removed in the process of splicing and this spliced intron reinserts into mRNA of a gene 2 in an intron less site. So, you have an intron that is removed from a given gene and it is freely moving in the cytoplasm and it can access into the nucleus and it can facilitate itself into recombination into elsewhere into an intron less gene.

Say you can see a RNA which is now recombined with the intron because intron is an RNA sequence which recombines into another RNA and this RNA with intron should be able to and remember this RNA need not be in a pre-spliced form. It is a post-spliced form, a different gene it can recombine into the RNA spontaneously just like an insertion. So, this RNA is now split and the intron is inserted.

Now, this intron has to reverse transcribe. So, one option is intron can access into the nucleus, but many a times it can recombine in the cytoplasm and undergoes reverse transcription and again it should go back into the nucleus and it can integrate. So, the principle is same that a removed intron gets access back into the genome into another gene that is also a gain of intron and another flip side of this is the integration portion need not be intron.

Integration portion can be any other fragment of the DNA, but it may not have the signals required for an intron which can evolve later. So, whenever the integration of a

foreign DNA into a existing gene happens it may not become an intron to start with, but it can eventually acquire the features of an intron because all it need is a polypyrimidine track a branch point and also a GU to start with. So, this is very much possible in thousands of years of evolution

But, if a already spliced out intron is integrating that is having all the features required for an intron. So, that is the plus point. So, here we are discussing about a spliced out intron integrating into another gene because this intron has got all the signatures. So, that is much easy and convenient to explain. And, another possibility of gain of intron is transposon insertion. So, what are transposon? Transposons are jumping genes.

So, transposons usually have a inverted repeats on their ends. An inverted repeats allow the recombination at a faster way. So, transposons also contain a single enzyme encoded inside the transposon that is called transposase. And, transposase when it is expressed it will allow jumping off this fragment at the inverted repeat site and it will be released out and it can integrate elsewhere in the genome. That is why transposons are called jumping genes.

And, this inserted transposon need not have any features of an intron. So, this should gradually gets converted into a spliceosomal intron. Like I told you in the previous example also, that any junk DNA or non-useful or not so helpful DNA fragment is present in the nucleus of a cell, it can integrate randomly anywhere. If it integrates into an exon, first it will disrupt that exon. But over the years, this sequence may acquire may be a part of exon also will be included in the intron. So, anything is possible.

So, it can acquire the features like in the case of transposon you are acquiring the features of a spliceosomal intron. This is very much possible. Remember intron gain or intron loss does not happen overnight. It happens thousands of years of evolution. So, that is why if you see across animals you see unique features in the introns, but common features in the exons.

So, why unique features in intron? Because in that particular species which delineated like mouse and human would have separated millions of years ago. They separated these two branches, but it can still retain exonic part common features. Many enzymes or proteins may not even have more than 2-amino acid difference between human and mouse because this is important to retain the functionality.

But, that is not the case with intron. Intron size will be different, intron sequence will be different. But crucial areas like branch point polypyrimidine track etcetera will be retained more or less the same because any alteration if it is happening to those sequence can be too deleterious for the animals survival itself based on which gene we are talking about. So, we should understand the intron gain and intron loss is very much possible spontaneously means via recombination, but it takes thousands of years.

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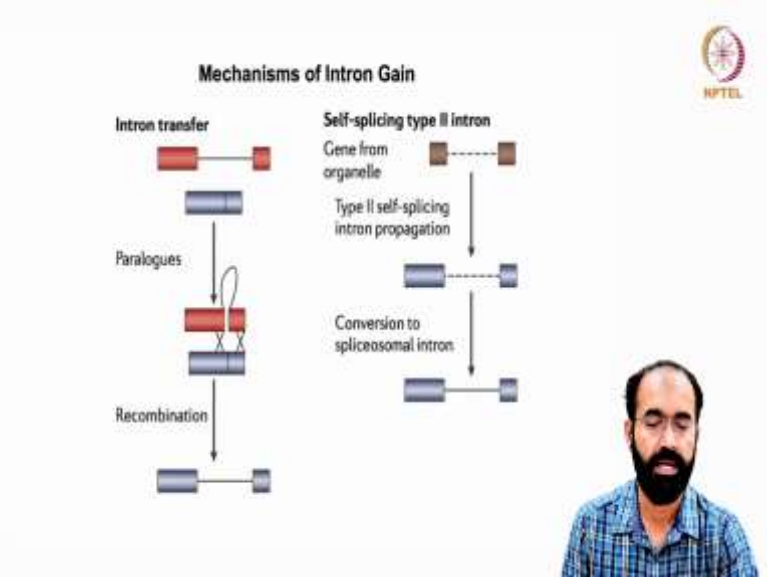

Now, let us see the mechanism of intron gain in a different perspective. So, intron transfer, how does it is possible? So, you have an exon you have another exon and you have an intron in between, it is spliced properly and then there is something called paralogs. So, paralogs means they have common features, but they are now doing identical function. Similarity is there. It is just like if I want to give an example you can distinguish two humans.

Human number 1, human number 2, human number 3. But, when it comes you want to distinguish identical twins you may struggle, but still they have difference, but you have to keep seeing them for say maybe for few weeks then you will start recognizing them slowly. Same with if I give you two dogs, same skin color – you will struggle to start with to distinguish between them.

So, you keep looking at them for several days although they look the same you will be able. They need not be twins, they are just two dogs. They may not even have any

genetic connection maybe same breed, but they say just because they have the same skin color you will get confused. It is a talking about another species, but we have tremendous ability to distinguish humans, but if they become identical twins your concept falls apart. Same logic applies with paralogs.

They have lot of sequence in common, but their expressivity, tissue specificity etcetera can vary. But because they have lot in common that also allow the recombination. So, you can understand that when introns are present in a gene, it is very much possible to integrate into the paralogs. Say one paralog have sequence similarity not identical to a gene A and gene A has intron because it has got the flanked sequence to its paralog it can always recombine and contribute this intron into the new gene.

And, also self splicing type II intron we have seen it gene from one organelle it can self splicing can happen and this self splicing intron can into a convert into a spliceosomal intron because group II introns have all the features of normal spliceosomal intron because it has got a branch point etcetera it is a question of retaining other features like 5 prime end GU, 3 prime end AG, polypyrimidine tract etcetera and this can now act as a spliceosomal intron. So, this is another way of intron gain.

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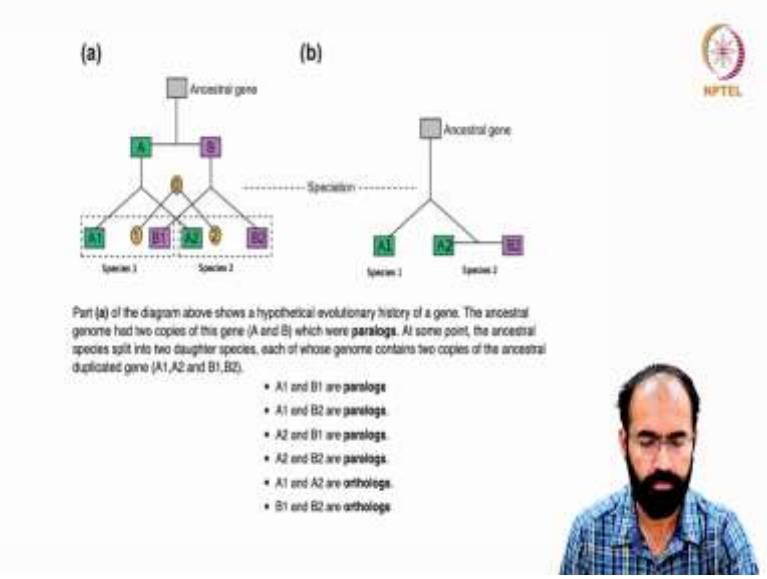

I was talking about paralogs. So, you need to have some clarity or understanding about the orthologs and paralogs. All genes have evolved by gene duplication that the genes during replication it will either make a copy of some part of its exons or it will make a

copy extra copy of the full gene itself. One classical example I do not remember whether I told you in this class or not it is the human growth hormone, placental lactogen and prolactin.

So, these are from one master gene ancient master gene. Growth hormone you know it is expressed in every animal required for the growth. Whereas, placental lactogen is needed only in females expressed in the placenta during the implantation of the embryo in the uterus and prolactin is needed in lactating women for producing the milk. So, technically prolactin and placental lactogen are functionless in males.

In females it is needed, but they have originated. Growth hormone is an essential gene essential protein in all living organisms, but placental lactogen and prolactin are derivatives much later stage in evolution. So, they are originated from one master gene. Let us see how one ancestral gene is giving rise to new genes. So, one ancestral gene giving rise to A and B. Actually ancestral gene was making its own copy, but it ended up making extra copy that is B.

So, that will do the job of that gene and slowly since A is making or doing the function of the ancestral gene already. So, B has the freedom to start doing. It is just like your mother has got a maid in the kitchen. So, mother do not need to break her head in cooking. Just you know overall looking and she can use her talent in doing something else maybe in some other artwork or maybe some other passion which she want to follow.

But, if she do not have any maid help then she only have to do that. So, this is the advantage of a having an extra duplication or mother has the ability to make a copy of herself. So, one copy will be doing the cooking work, the other copy will be doing the what you call you know other her art or extra kitchen related work she will be doing.

So, same way if you have a gene that is duplicated, then one of them will do identical work of the previous gene and the duplicated gene have the freedom to explore novel things. So, that is what you should know A and B and what happens when it is further splitting in evolution and it is splitting into A1 and A2 and remember by then its speciation has happened, it is split into two different species.

So, now A1 is retaining the function of the A1 in this species and B also same way, it is retaining the function of that particular gene B in the next species. However, what you should understand? This A when it is further separating in a small fraction A1 and A2 A1 is in one species and A2 in another species. So, B also similarly, remember A and B originated from one ancestral gene B is also now separated into B1 and B2 both are in two different species.

Now, what happened? A1 and B1 can be called as paralogs. They were not originated from 1G, but they originated from one great grandfather, not from the grandfather and A1 and B2 also can be called as paralogs. So, A1 and B1 are paralogs, A1 and B2 are paralogs; A2 and B1 also paralogs and A2 and B2 are also paralogs whereas, A1 and A2 are orthologs and B1 and B2 are orthologs. Reason being, they will be doing the same function.

So, when you say ortholog like human growth hormone and mouse growth hormone they are orthologs because they are doing the same function whereas, human growth hormone and mouse prolacting we have to call them as paralogs. Technically, here I am using the example because they are from one common ancestral gene, but happened several millions of years ago. So, that, but still they have lot in common. So, this idea should be present.

So, this diagram hopefully will make the point very clear to you and this example here you can see ancestral gene you have A1, say it is split into A and B and now you have A1 in species 1. Whereas, in species 2 you have A2 and also a B2 because this duplicated gene in this case it is the ideal example what you are saying in the left hand panel, but in right hand panel it is not in the ideal situation because when you have an extra function just like your mother have a maid does not mean that your neighbor also should have a maid, right.

But, if it is there most likely will be there, but it need not that does not mean that whatever your mother and maid doing together function is not happening in that house. So, maybe happening in a compromised manner maybe that mother may not be having this extra you know cultural activity or any other non-kitchen related activity. This logic you should keep it that this other species may or may not have this extra duplicator relic from the ancestral gene.

If it is there it is very good because it will help in the jumping of this introns between these two because that the presence of paralogs are very important for the cross communication without altering or without disrupting the functioning of a given existing gene because while this happening one of them has to be the savior of the cell.

That means it should be taking care of the functioning the actual functioning of that particular gene because of this recombination etcetera, it should not start losing the function. And, one more thing you should understand that such a recombination if it happens in the germ line, if it happened in my finger tip it is useless, if it happened in my nose tip it is useless because it will not go to the next generation.

The recombination should ideally happen in the germ cell and the organism does not suffer. See, if this recombination happen organism do not need a prolactin or growth hormone produced from the gonads because other tissue is producing, but this recombination if it is go to the next generation, next population then it is a question of time that is why you can always see that parents are fertile that is why you are born.

But, you need not be fertile because what went wrong because you got the gene because some recombination even happened in the gonad formation and some of the genes got affected you lost some part of the gene. So, that is why parents were fertile and the offspring or the son or daughter is not able to propagate to the next generation. This I am giving an easier example for you to understand.

You will see in many times in nature like one couple or do not have kids. So, I am not saying that anyone who do not have kids are something to do with the genetic deletion I am not saying that. It can be due to n number of reasons, but it is also possible some part of the chromosome especially Y chromosome etcetera are deleted in the in that organism. But, the father has perfectly fine, but when it became to son – son lost some part of the chromosome which makes him sterile. So, this is very much possible.

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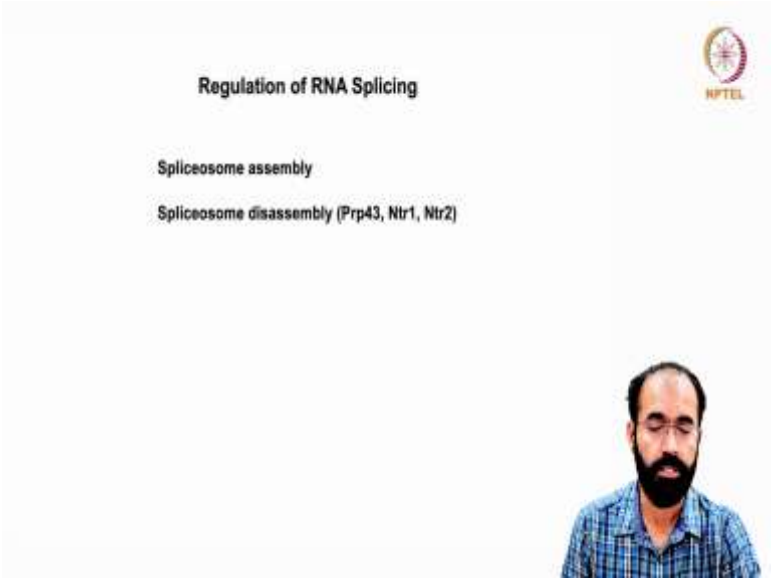

So, the regulation of RNA splicing and we know that the spliceosome assembly we have seen it with SM proteins, SMN complex etcetera. And, spliceosomal disassembly also is equally important which is mainly governed by Prp43, Ntr1, Ntr2 proteins.

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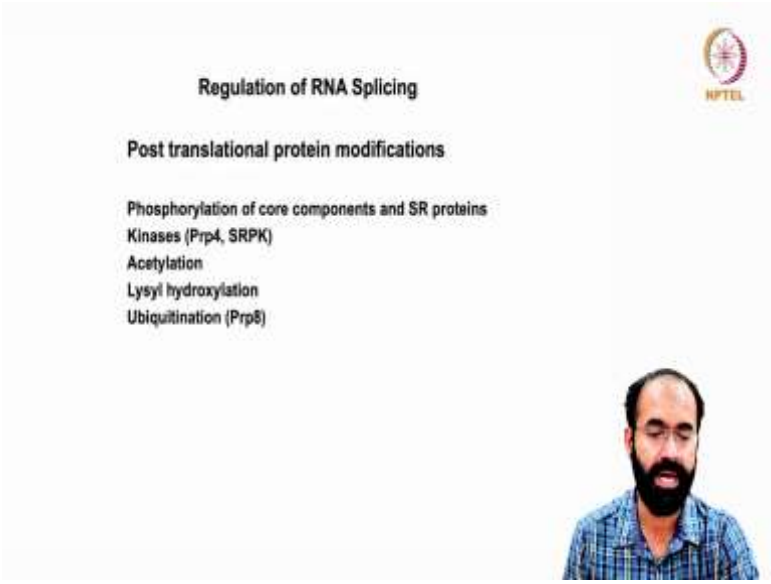

And, the regulation of RNA splicing is a lot of proteins and their post translational modifications are important for whether they should be part of we have seen that one example of SR protein which binds on to the exons we saw in the previous class. The phosphorylation of core components every protein if it is phosphorylated or any protein

translational post translational modification on to the protein can influence its ability to interact with other protein ability to function in a given way everything can get affected.

Because proteins are retaining a particular shape or a particular function credit goes to their current state. If a protein gets phosphorylated, there is a good chance that it will start doing a new function. Sometimes if a protein gets phosphorylated it will be marked for degradation. Say a protein if it is functional if it is phosphorylated it can become non-functional. So, understand if post translational modification alters the status of the protein in the current form.

And, any protein translational modification acetylation is possible, methylation is possible, ubiquitination is possible lot of such changes are there. So, phosphorylation of core components of the SR proteins done by kinases such as Prp4 and SRPK and acetylation is possible, lysyl hydroxylation is possible, ubiquitination is possible by Prp8 many more modifications are possible which will influence the functioning of these proteins.

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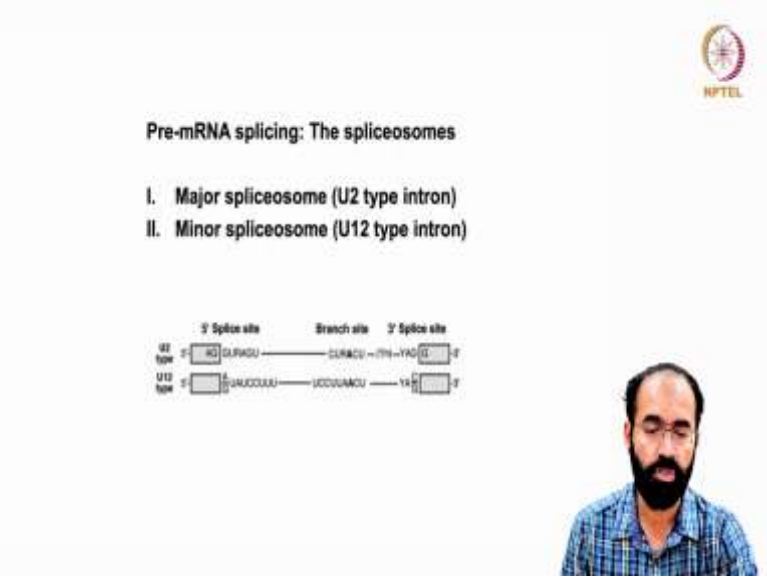

So, coming back to the pre-mRNA splicing the major spliceosome you have seen which use the U2 type intron and minus spliceosome use the U12 and otherwise they are basically the same U2 and U12 are the same. Except that in the U2 type which is the major spliceosome you have got the sequence GURAGU in the 5 prime splice site and

you have the branch point you have got CURACU in the branch site and you have a polypyrimidine track and you have got YAG and then the second exon.

In the U12 type you have slightly different sequence. You know every intron start with the GU. Here it can be AU also; GU can be AU no other basis allowed and rest of the sequence as you can see here it can be AUA or GUA, then you have UCCUUU that is the 5 prime branch 5 prime splice site and then at the branch point you have UCCUUAACU slightly different from the branch site of the U2 or the major spliceosome.

And, towards the end you do not have after the post branch point you do not have a polypyrimidine tract rather you have a YAC or YAG. So, GUAG rule is basically relaxed. GU need not be GU, it can be AU and AG need not be AG it can be AC also; one of the major change you can say is this alteration in the minor spliceosome. You can think sometimes if a gene lost it, lost this ability to retain the GUAG, so, minor spliceosome becomes handy for such splicing.

So, this type of evolution also tolerated in, but if it becomes very prevalent then the splicing defect will be seen. If it is a minor change a small portion or small fraction of the RNA minor spliceosome can become handy. So, that is why minor spliceosomes are useful in many such cases.

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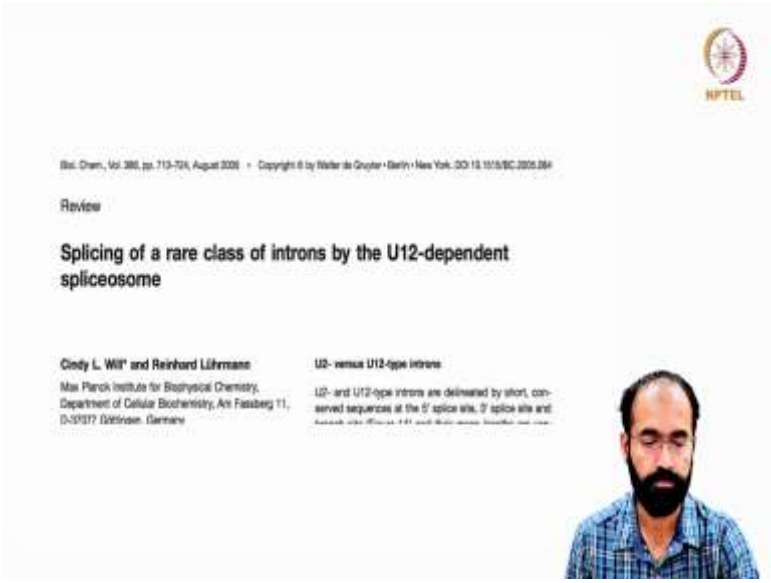

So, you can read this review splicing of rare class of introns by U12 dependent spliceosome, it is a quite interesting article.

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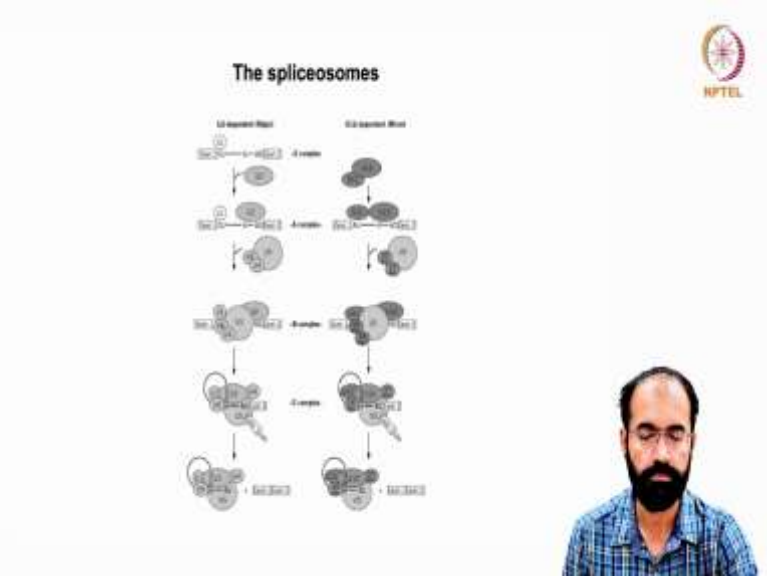

And, this spliceosome if you look you know U2 dependent and U12 dependent their mechanism is more or less the same. In U2 dependent you know U1 and U2 join and which is further by U6, U4, U5 complex and then we have seen it. I will not go into the detail too much because we have seen it n number of times.

And, in U12 dependent the U11 and U12 come together and they come and occupy at the 5 prime and the branch point respectively, 5 prime splice site and the branch point respectively. And, then you have this complex formation made of U6, U5 and U4 as happened in the case of U2 complex that is the major spliceosome complex.

They come and join and rest of them happen more or less in a similar fashion and you end up getting exon 1 and exon 2 fused together. So, this is how the major and minor spliceosome differ or how they interact.

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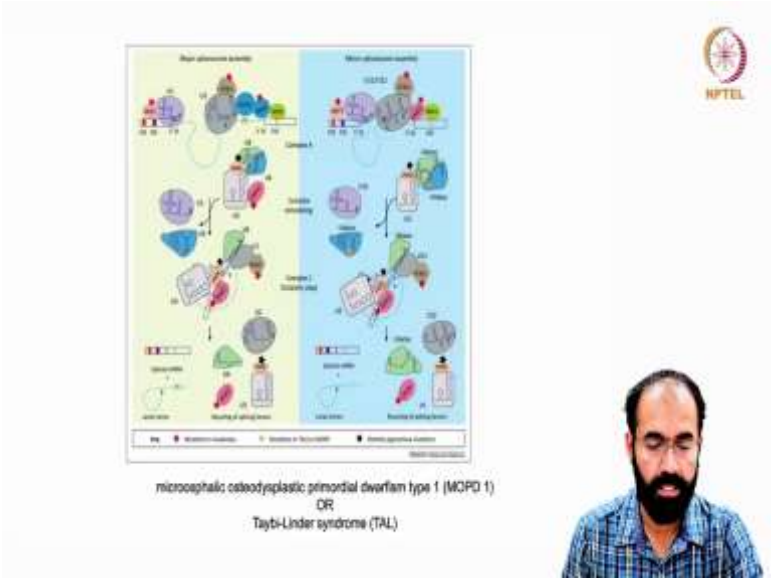

So, many disorders can come. One such example are microcephalic osteo dysplastic primordial dwarfism type 1 MOPD1 MOPD1 or Taybi – Linder syndrome TAL mentioned as TAL. They can come because of the defect in the minor spliceosomal assembly. So, major spliceosomal assembly is been shown here and this is the minor spliceosomal assembly.

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And, let us see some examples of defects that is occurring pathologies resulting from the splicing defects. This we have seen it, we are revisiting once again. Mutations can affect

the splicing factors and that can lead to diseases such as spinal muscular trophy, retinitis pigmentosa, myotonic dystrophy. This is caused because of mutations in the splicing factors or the proteins involved in the splicing and the mutations affecting the splicing pattern of pre-mRNA; that means, it is affecting a given splicing event.

Proteins are intact, but the splicing got affected despite the proteins being intact. So, that can affect in a candidate manner. If the splicing protein is affected it will affect every splicing, but if one splicing is affected then that particular gene is affected. Examples of these diseases include beta thalassemia, Duchenne muscular dystrophy, cystic fibrosis, Frasier syndrome, frontotemporal dementia and Parkinsonism.

So, these are some example and many a time some of them are affected a specific gender like Duchenne muscular dystrophy, cystic fibrosis these are all males are. These are all X-linked genes that affects mainly the male because males have got only one X chromosome.

So, these are all the main reasons, but understand many of these changes, many of these problems are occurring because of not any defect with the gene as such, defect is in the splicing event. So, the many of these diseases are like threatening. So, we will see more in detail about this splicing and also importance of RNA helicases etcetera in the next class.

Thank you.