RNA Biology
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Lecture - 22
Alternative RNA Processing and Editing Relevance of RNA Editing

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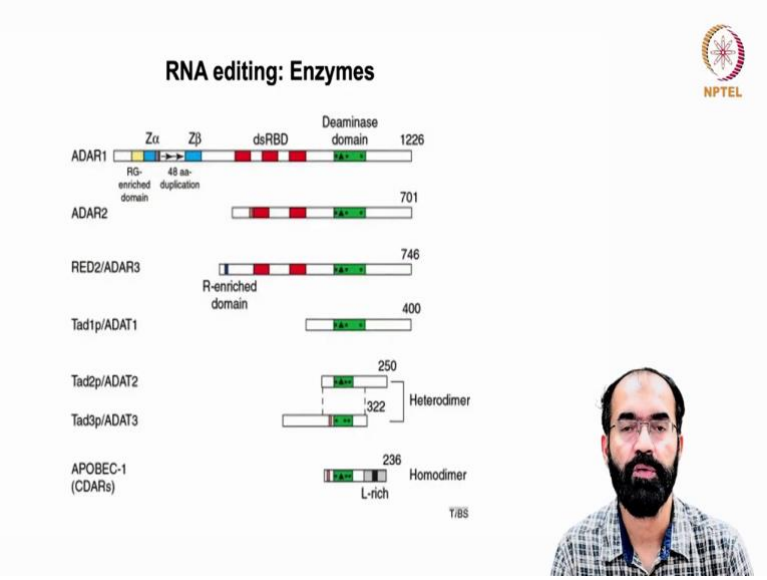

Hello everyone, welcome back to another session of RNA Biology. And we were studying about the RNA Editing Enzymes and different types are there and their names are listed here and they some of them have the RNA binding domain and some of them do not have, but they will take the help of other proteins and they find an RNA target that is called the cooperativity of proteins. But all of them must have the Deaminase domain.

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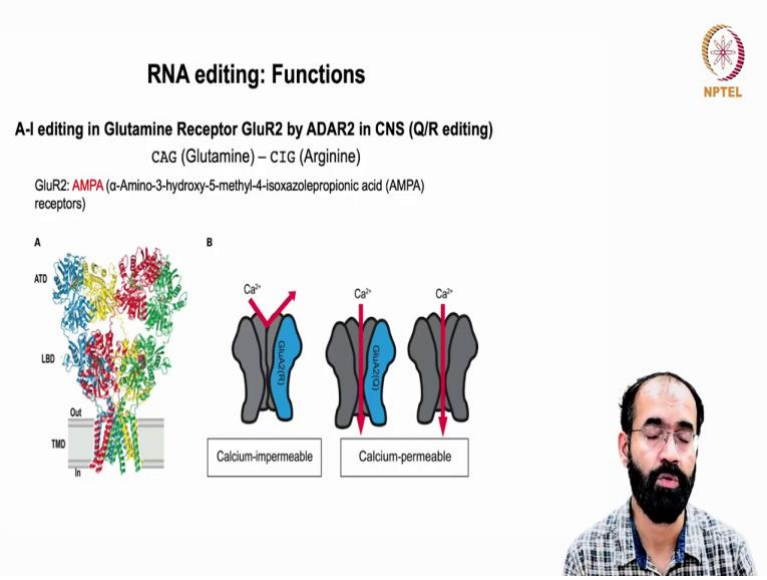

And RNA editing functions we need to know that how important the RNA editing is. So, A to I we know that adenosine gets deaminated becomes ionosine or inosine. So, this A to I editing in glutamine receptor is very very important. So, GluR2 because glutamine is a neurotransmitter and it has to be acted upon by specific receptor that is like we will not go into the neuroscience more in detail.

But time being you understand that neurotransmitters are bound by specific receptors and based on this binding these receptors open up their ion channel and this opening will allow the influx of ions into the neuron that is causing a depolarization and an action potential.

Like I am speaking you are hearing it is because of constant action potential being created in your auditoria. You are able to see because constant action potential is being created in your eyes because of the photons hitting the retina. So, same way in our central nervous system you are able to understand, you are able to think, you are able to perform a task everything because of the communication. And 80 percent of our central nervous system synapse as usually have glutamate as their synapse.

We will not go further into the detail. But in this case what happened? This CAG which is bringing in an amino acid glutamine is now changed into CIG in into Arginine. And this is an important interesting change that is occurring into the GluR2, GluR2 receptor. So, GluR2 normally the neurotransmitter receptors are made of multiple sub unit GluR1,

GluR2, GluR3, GluR4 normally 5 units come together and can act like a like you take 5 pens and put a rubber band.

So, if you twist them, you can see it will in the centre portion where the rubber band is there it will seal. But if you keep all the 5 straight then you can see in the centre. There is a opening or hole. So, like that simplistic way I can explain that is how the modifications happen in the protein structure to open and close the ion channel.

So, what happens is GluR2 sub unit other sub units of this glutamine receptor is unaffected, ok. So, this has to be read as not glutamine receptor it is glutamate receptor. And the amino acid that changes that is causing the that is causing this change because of this RNA editing is glutamine to arginine.

So, CAG is changed into CIG. So, GluR2 sub unit of the AMPA receptor and they have Amino-3-hydroxy-5-methyl. So, this is the long name of the AMPA. So, normally glutamate receptors are of 3 types. One is NMDA receptor another is AMPA receptor another is kainate receptor. So, AMPA receptor is the one which is governing the RNA editing and which cause the survival of the organism. So, the AMPA's name is alpha-amino-3 hydroxy-5-methyl-4-isoxazolepropionic acid.

This indicates that some neurotransmitter receptors can bind onto certain chemicals. Do not think AMPA is a neurotransmitter, do not think Glu NMDA is a neurotransmitter. These are all molecules that can actively bind. But the neurotransmitter endogenously produced a neurotransmitter that binds is the glutamate which is derived from the glutamic acid amino acid. Many many amino acids are used to create certain derivatives that can act as neurotransmitters.

And those glutamate receptor that have a high affinity for AMPA chemical AMPA we call them as AMPA receptor. And we are talking about the GluR2 subunit of AMPA receptor. So, this is how it look like. So, in the left-hand side A panel A you have the structure that resembles a typical space filling model that is basically the crystallographic structure. This is derived using X-ray crystallography.

And at B what you are seeing is calcium-impermeable or calcium-permeable. So, what we should understand what is the mutation? This blue colour one is the subunit that is M that is the Glu glutamate subunit that is GluR2 of the AMPA receptor. And there are

other subunits which are unaffected. So, what happens when you are having arginine that is edited that glutamine should have been there that single-electric code is Q.

And if glutamine is there that is the normal situation that the gene encoding GluR2 is giving rise to GluR2 subunit protein. And normally it is supposed to contain glutamine that is written as Q. And this is what the protein you should get it. And this subunit if it comes into the AMPA receptor it will allow calcium ion. Of course, it allows sodium also. It allows calcium ion. Calcium ion is bigger than sodium ion. So, like in your house you can happily go.

Mouse also can go happily. But if an elephant has to come into your house your room should be big and the door should be at least the size of an elephant or the width of an elephant then only it can go. So, sodium can easily go. But calcium if it has to go it has to have a bigger opening.

So, the normal case it allows the entry of calcium. Whereas if this glutamine from here is changed into arginine because of RNA editing where it is editing CAG is becoming CIG. And glutamine is no more coming, but instead arginine is coming in the M GluR2 subunit of this AMPA receptor.

You do not allow entry of this calcium. It will allow sodium, ok. Remember do not think that this channel is closed. Channel is not closed. Channel is open. Provided the glutamate neurotransmitter is binding. In both case whether it is changed or not changed glutamate should be there in order to otherwise by default the channel remains closed. No entry for any ions.

But when glutamate is bound it will open or the opening becomes such a way that you are able to allow only sodium. Calcium is not welcome. Like in houses if you have toddlers in the house small kids are there in the house. You may have seen in the door they will make a half way stoppage. Like around maybe some one and a half feet height a door they will make it. So, that the adults can just put their leg across and go. The toddler cannot jump. Toddler will be stuck in the room.

So, door is closed, but not closed. So, instead of closing the door you make a half way door. Half way door you make like in offices also you can see. Like big officials are

there. They will have some half door. That provides some privacy to the officer who is inside.

So, people cannot just dash in or cannot peep in, but the door is not fully closed. So, something similar logic applies. When the arginine is present because of the mutation or because of the editing of the CAG to CIG A is changing into I inosine and now you are having arginine then it allows sodium, but calcium is not welcome. And if it is such a editing did not happen calcium also welcome. So, calcium permeability is a decisive factor for whether or not this mutation or this alteration is welcome or not.

But the most interesting thing is that entire AMPA receptor in the central nervous system entire AMPA receptor undergoes this change. Means we should understand the gene of gene of this individual have default is glutamine which allows calcium, but you do not want it. So, in entire case wherever this sub unit is expressed you have to have this editing and you convert it into arginine to make sure that calcium is not welcome, sodium is welcome to cause this depolarization of the neuron.

It has got lot of other implication because calcium is divalent, sodium is monovalent, if calcium also comes in depolarization becomes much faster. So, just like you think about it. If you are able to eat one chapati right, you will make one chapati into maybe some four pieces or five pieces or six pieces and you eat.

What if you make one enter chapati hold it and put it into your mouth then you may hold it, but you simply will not be able to chew it and you will not be able to gulp it, if you do that there is a good chance that you will get chocking. Same logic applies if the depolarization happens way too high because sodium is also coming and calcium also is coming. That can create lot of problems to the depolarization.

Depolarization happens much more than what is required. So, the conductance there will be a shortage of ions and depolarization become hay-wired and there can have lot of problems to the action potential creation in the neurons. So, you want sodium, but you do not want potassium.

So, the way of controlling sorry, not potassium calcium sodium is welcome and you do not want calcium along with sodium. So, the you are kind of controlling the size of the

whole of this channel. That is what you should understand that the editing is very important for the functioning of a neurotransmitter receptor.

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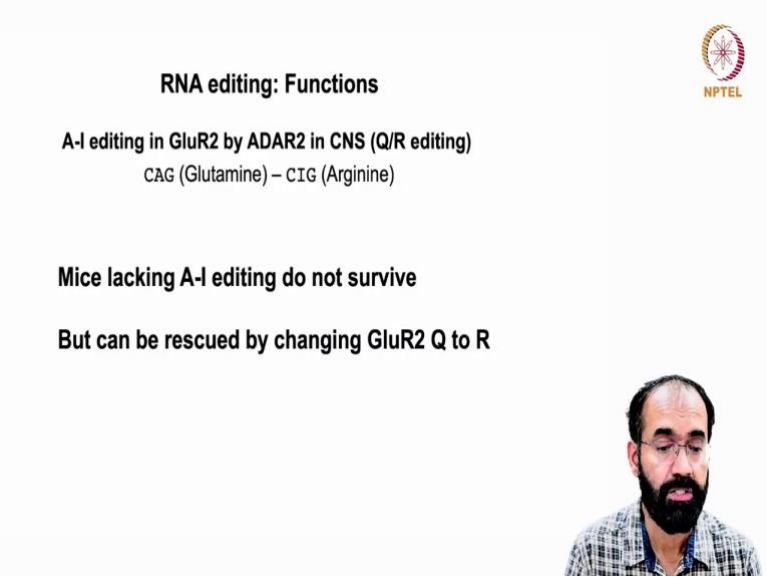

Now, let us see how important it is. So, A to I editing in GluR2 by ADAR2 that is the enzyme. Adenosine deaminase acting on RNA and two basically is the number of genes which you are at many times if you study genomics you will say gene 1, gene 2, gene 3, gene 4 like that many plenty many isoforms etcetera will be there and you simply give them the numbering. ADAR2 is one of the ADAR family enzymes that acts in central nervous systems.

The CNS stands for central nervous system and it is important Q R editing; that means, converting Q to arginine. So, this editing is very important. So, CAG is bringing in glutamine and CIG is bringing in arginine. And the Mice lacking A to I editing do not survive. How important it is?

That is quite throat provoking; that means, you simply have to get rid of this ADAR2 enzyme or you create a mutation such that the glutamine remains as glutamine it is not available for editing. Then if A to I editing is prevented, what happened? The mice will not survive. You can imagine how crucial this modification is. But it can be rescued by changing the GluR2 Q to R.

So, if you are changing the gene GluR2 Q to R by artificially creating a change for a mutation. You are not depending on the because the organism is mutant because it is no more producing ADAR2. ADAR2 is not being produced it will not recognize this as a substrate. But you may wonder how you can think that ADAR2 is doing only this function maybe it is doing 10 another function. Of course, it might be doing ten another function, but none are as important as this Q to R editing.

Other functions may be compromised because ADAR2 is lacking, but the moment you complement the animal that is called rescue experiment complement this GluR2 with arginine containing version of the gene then the organism is able to survive. So, this is what you should understand that some RNA editing are so crucial and the life and death hang by a balance of RNA editing and to a highly developed mammal such as Mice.

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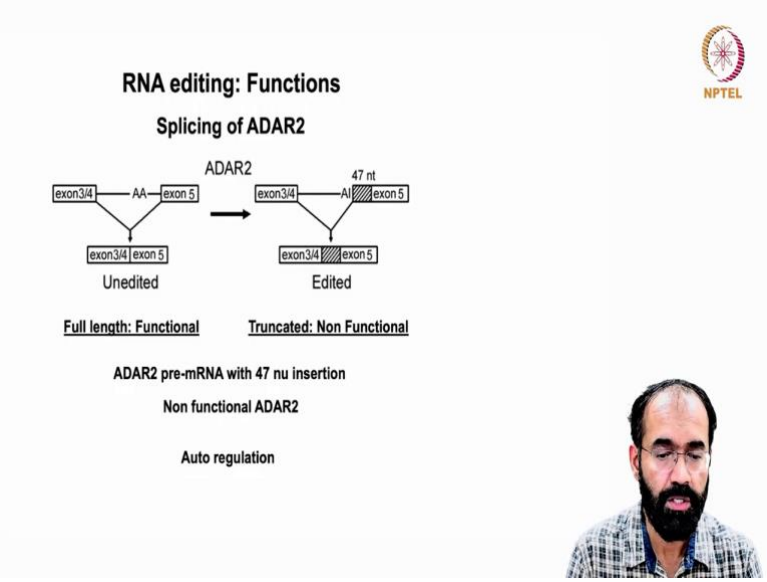

So, RNA editing. When you are looking further into this AMPA receptor and ADAR2 is the enzyme and you have got this region is between exon 3 4 and exon 5. And in the unedited condition you have exon 3 4 and 5 and you end up getting full length which is functional. And when you have a truncated one truncated version say you are introducing a extra 47 nucleotide in between edited which can become non-functional. So, this is you are doing a change onto the ADAR2 enzyme itself.

So, how enzyme itself is regulating itself. So, ADAR2 pre-mRNA with the 47 nucleotide insertion is seen in this case and it is non-functional and this can create cause its own

auto regulation. So, exon 3 4 of ADAR2 enzyme and it has got a exon 5 downstream and normal splicing happens and you end up getting a full length functional version. But if ADAR2 is present it can create a A to I change and this is happening in the branch point.

So, branch point of the intron you know there is an A. And now this A is not an A the adenosine is no more an adenosine that adenosine is now edited into ionosine inosine. And because of this it will skip the portion from the branch point until the end of the intron. Because of this it has got extra 47 nucleotides comes in and this is another way of editing.

That is you are incorporating extra bases from the intron. That is adding 47 nucleotide and this is another way of editing or which caused a complete stretch of amino acid in between exon 3 4 and exon 5. And this truncated this truncation or this modification creates a non-functional ADAR2.

So, this also can be seen as a auto regulation; that means, ADAR2 adequate level is there it will do the job what it is supposed to do that is AMPA receptor editing etcetera. If ADAR2 more is there, how will you control it? Someone has to control the splicing of the ADAR2 itself. How you control it? The ADAR2 will start acting on itself. It is just like cutting the tree the branch of the tree where you are sitting.

So, if you are sitting on a branch and you are cutting wisely side branch, top branch etcetera you will be able to gather fire woods. What if you are cutting the branch where you are sitting actually? There is a good chance you will fall down and it may cause real injury to you.

Same thing this ADAR2 is doing as a way of auto regulation one of the way of regulating it. Normal ADAR2 is needed for normal functioning, normal editing one we already saw AMPA receptor modification. But if there is excess more is there, how will you reduce it? So, to reduce that you will always go back to its own RNA. And this is one way of auto regulation.

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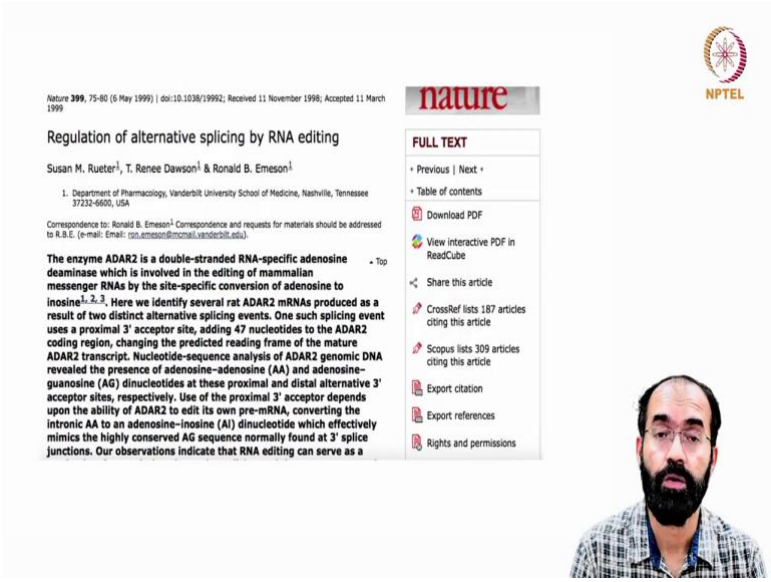

Now, you can also read this article that is published in Nature few years ago almost 20 21 years ago that is Regulation of alternative splicing by RNA editing. So, it is a very interesting article those who are interested can read and get more insight and more deep knowledge about the subject.

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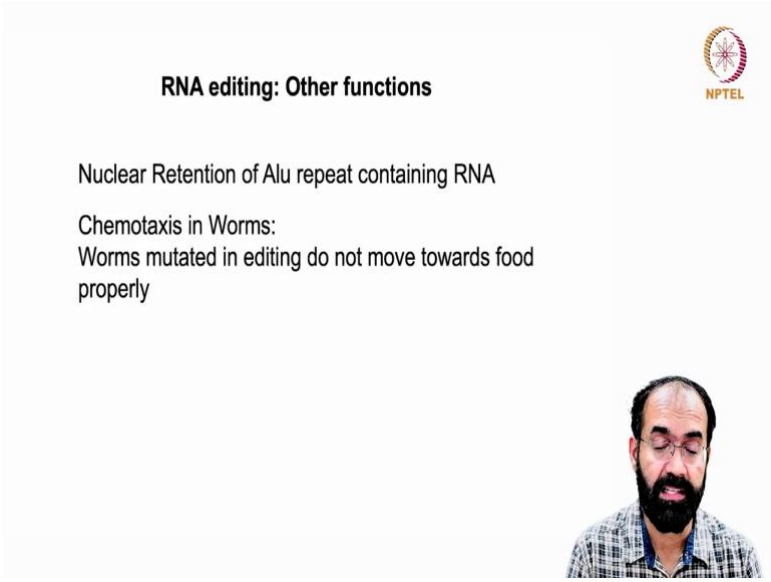

Let us see what are the other functions of RNA editing. So, nuclear retention of Alu repeat containing RNA. So, Alu repeat is basically Alu is a restriction enzyme. So, which can cut randomly at sequence of course, sequence specific manner, but plenty of Alu

binding or Alu recognizing restriction Alu enzyme recognizing nucleotide sequences can be there plenty in the genome restriction.

And the nucleus I guess all of you know they are enzymes that can cut in in inside the DNA. So, some repeats are there long repeats they do not have a single Alu cutting site. So, if you take the genomic DNA of an organism cut it with Alu restriction enzyme then you will end up getting bands, you will get long smear and among the smear, you will get strong bands like glowing addendum bromide-stained bands you can see which means they do not have cut sites inside and they are repeated.

Sometimes Alu repeat containing RNA are very important for them retaining at specific location etcetera. We will see them a little bit in the RNA export or RNA transportation shuttling between the nucleus and the cytoplasm we will see about that. But time being you understand that the nuclear retention of certain Alu repeat containing RNA have got rolled in the RNA editing it is one of the functions of RNA editing.

Chemotaxis in worms like worms means C. elegans the model organ some Caenorhabditis elegans they are they normally leave on eating the bacteria. So, they have attraction and repulsion for certain chemicals something bacterial derived chemical it will go. So, that it will get its food that is the bacteria. And if something is harmful do not want to go their toxic or maybe it can cause its life then it will have repulsion.

So, the chemotaxis in worms is significantly regulated by RNA editing. Worms mutated in editing do not move towards food properly; that means, it becomes a dumb worm it is basically useless; it can also cause life or death situation. Because if a worm is not able to find its food in a natural condition, I do not think this worm will be able to make a good living or it will be even be able to survive.

So, it is very important that it is almost like a lion unable to recognize buffalo or deer. So, who will give food to the lion. So, this is very important that RNA editing function if it is defective the animals suffers.

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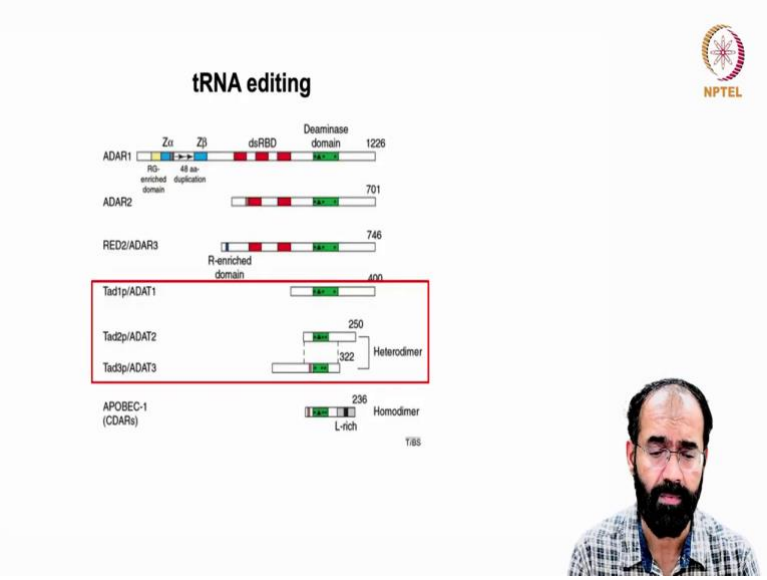

Let us see another example, ADAR1 basically is one of the enzyme like you are able to see which is causing the tRNA editing. We are now going to understand the tRNA editing and some we have seen ADAR1, ADAR2 etcetera that is involved in the mRNA editing.

Whereas some examples we are seeing here they are meant for tRNA editing. And the tRNA editing also is important. We know tRNA have got anticodon sequence. The codons are there in the mRNA and the anticodons are present in the tRNA and anticodon and codon pair together. So, that the amino acids can come into the correct position in the ribosome. So, that the peptide synthesis or the proteins synthesis can take place effectively. So, some examples are put inside this box they are the tRNA editing enzyme. So, how are they doing let us see more in detail.

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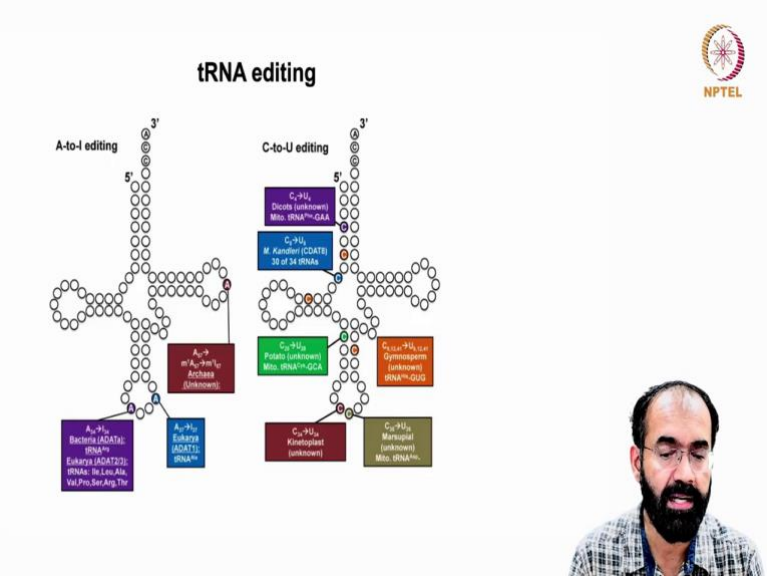

So, tRNA editing is quite interesting. We have already seen that specific base of the tRNA will be edited both for stability and also for recognition by amino acid, tRNA, synthetize etcetera. So, that they can be marked or they can be tagged with the amino acids which can participate with this tRNA or charged tRNA or amino acetylated tRNA can participate in the proteins synthesis.

So, to recognize a good tRNA versus unprocessed or not ready tRNA this tagging becomes very important. Just like whether you are eligible to fly abroad or travel abroad you will get your passport will have visa of that country. So, that means, you are eligible you are ready having a flight ticket does not allow you to go to any country.

Of course, some countries will allow you, but many countries do not allow you. Same logic applies certain modification in the base is necessary for this tRNA to be recognized as a bonafide substrate for some enzymes that is where tRNA editing becomes important. So, many changes happens you know it can change from C to U or A to I.

So, some A to I editing here like you can see here we will not go into the detail because just we will touch upon the location. You can see here A to I changes is seen in archaea and here in this arm which is basically the anticodon arm the A to I change and here there is another A to I change and this can bring lot of changes.

And that will have a species specificity sometimes even changing the codon itself. And then here comes the C U editing. Both A to I and C to U happens via the deaminases. So, here C to U change possible and here C to U change possible, here it is possible. In many places C to U changes possible.

And remember this is the these three bases are the one two and three these three bases are the anticodon part and this changes brings in lot of and where it is changed is not important. What we should understand which kind of change happens, sometimes it becomes very handy to compensate for lack of certain tRNA.

Because you want a 10 tRNA, but you have only 5 tRNA. So, remaining 5 you can create through this editing. Because with less you can do more again coming back to the Swiss Army knife example with one tool you can do many things let us see more in detail about that.

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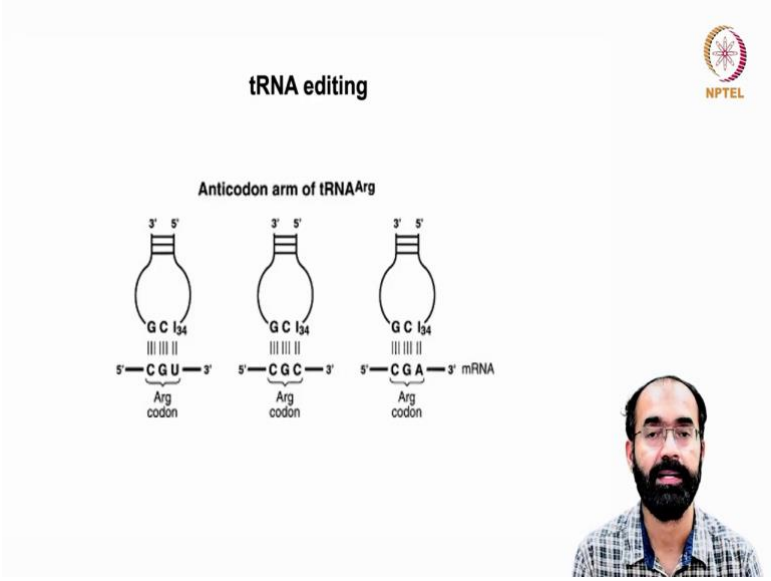

tRNA editing and the anticodon arm of tRNA can bring changes and this tRNA is meant to carry arginine. And here what happens? G C and I is there normally and if G C I this is the loop of the tRNA and this is the mRNA. So, this is the arginine codon in the mRNA. C G U and it is supposed to have G C and A and this a is now edited to I this is one change.

And now let us see another arginine codon C G C and you have G C and I another one. And technically should have been G C and G ok. And, but we do not have that we do not have that tRNA with this codon and then comes C G A another codon of arginine it should have paired with G C and U.

And you do not have that all you have is G C I. But we already mentioned that inosine same have equal affinity for all the bases. So, C G U can easily pair. So, G C I, G C I, G C I pairing tRNA can cater one two three different codon of different sequence. Of course, they all code for arginine C G U, C G C, C G A totally different codons they should have had a unique dedicated tRNA.

But just to buy this conversion of actually this was G C A, G C As A is converted into I by editing and this made it ok, for catering three two more total three codons can be utilized. So, this is how the evolution have taken place in various organism with the less you are able to do more. And this is a big achievement when you are talking about the RNA editing and some of them can be very deleterious and very detrimental etcetera.

So, we will end the class of this topic today and we will continue with more in detail in the subsequent class and you also keep tracking of what you have learned so far. Because RNA editing and mRNA, tRNA and various editing enzyme etcetera also try to read this extra topics what I am giving in the literature form that is also very important not just reading the reference book.

Thank you.