**RNA Biology**
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**Lecture - 15**
**RNA Processing and Life Cycle: RNA Maturation and RNPs**

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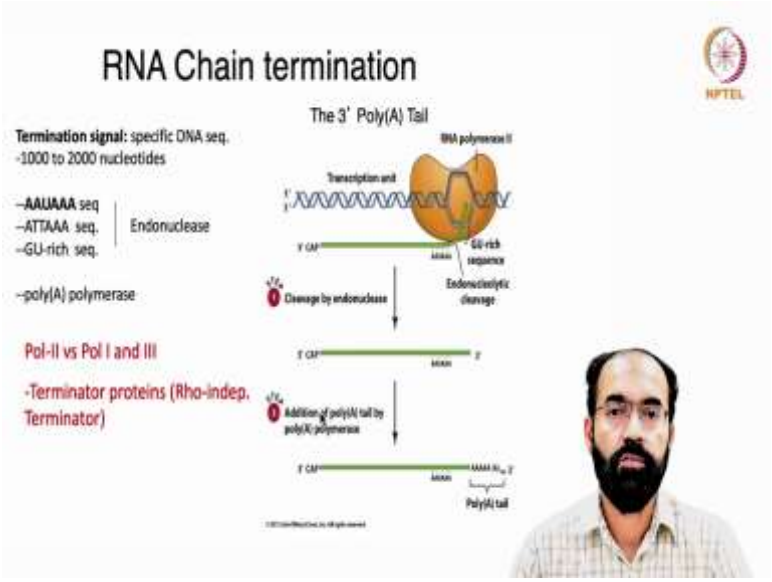


Welcome back to another session of RNA Biology. So, we were looking into the transcription, initiation, elongation and termination in eukaryotes and we were here in the previous class that is the termination of transcription in RNA production in eukaryotes.

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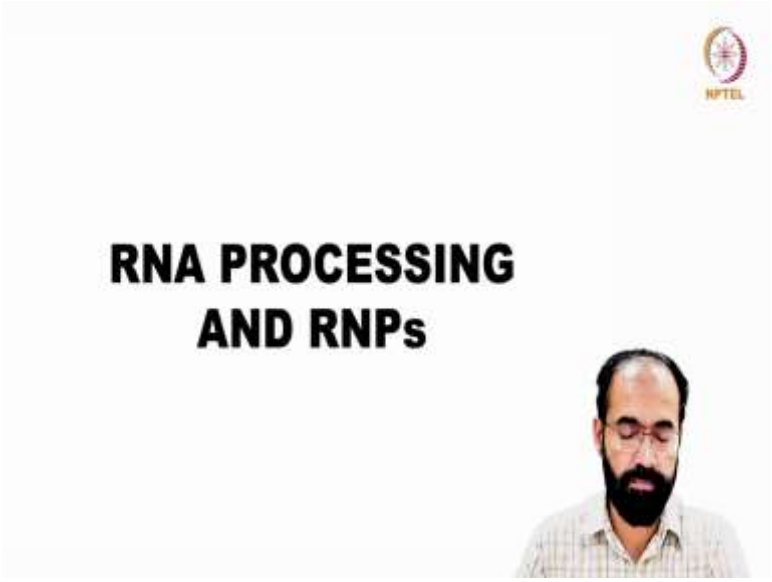


And the next section is RNA processing and RNPs. So, RNA processing is a very intense field and the RNPs are RNA protein complexes. They are very important for the normal functioning of RNA and we will see them in detail what is what.

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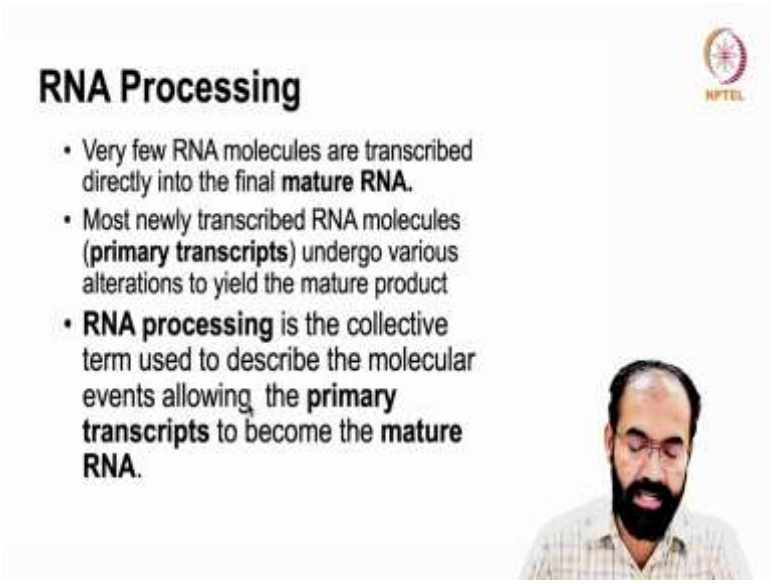


RNA processing is a very deep and still we are learning a lot about the RNA processing. And very few RNA molecules are transcribed directly into final mature RNA means, every RNA some or the other requires some kind of processing before it can function.

And most newly transcribed RNA molecules they are referred to as primary transcript undergo various alterations to yield the mature functional RNA molecule.

And RNA processing is the collective term that we use to describe the molecular events that is following the primary transcript that is the production of the primary transcript to the functional transcript that is the mature transcript.

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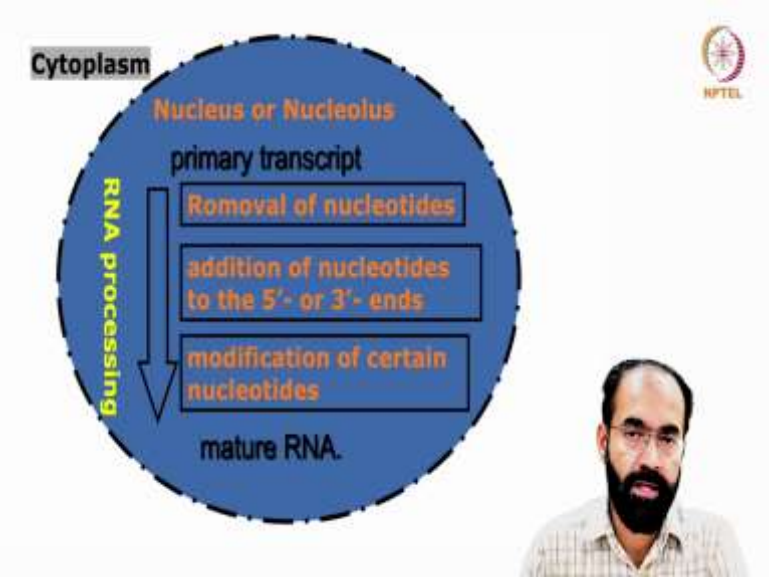


So, if you see this is a cartoon that described how the RNA is processed at various steps. So, what you see this blue circle is the nucleus or nucleolus because both place different types of RNA is being transcribed. And remember, nucleolus is a structure within the nucleus ok its not a separate structure. And outside of this blue circle is the cytoplasm.

So, when a primary transcript is formed then it undergoes the maturation either by removal of certain nucleotides no matter whether it is 5 prime or 3 prime. It undergoes some maturation by the removal of certain nucleotides. And addition of nucleotides to the 5 prime or 3 prime ends another level of modification. And then third level of modification is the modification of certain nucleotides at specific locations and this give rise to a mature RNA.

So, all the RNA modifications come under one of these three category without which an RNA cannot function effectively and those unprocessed RNA will be sent for degradation.

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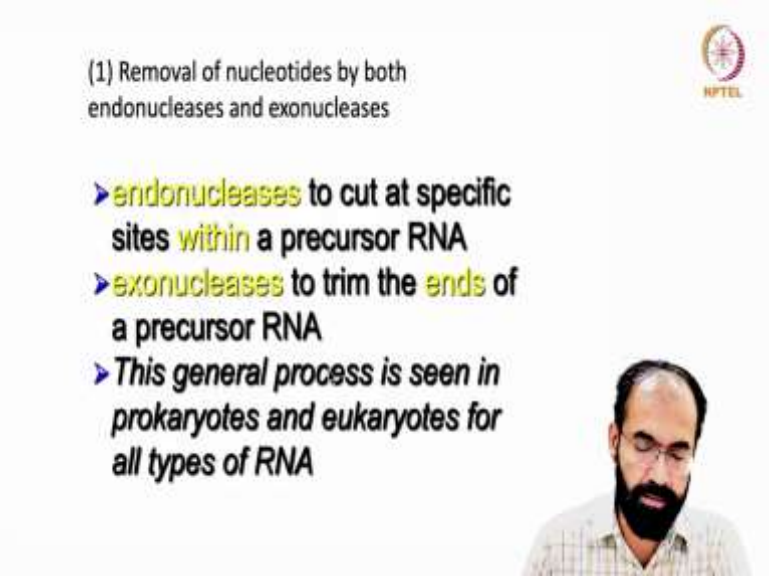


So, let us see the removal of nucleotides, how and when they happen. Removal of nucleotides by both endonuclease and exonuclease they are the enzymes that contribute to the degradation or the cleaving of the RNA. And endo the word itself indicates it is acting inside endonuclease. And exo indicates it is acting on ends either 5 prime end of the RNA or 3 prime end of the RNA.

So, endonuclease are helpful in cutting specific sites within the precursor RNA. That means it can cut like we saw soon after the poly A signal you have the endonuclease action. So, that the growing RNA will be released. And exonuclease are helpful in trimming the ends of the precursor RNA molecule. Some extra basis that it has to be cut short so, that the ideal length is maintained. This general process is seen both in prokaryotes and also in eukaryotes for almost all types of RNA.

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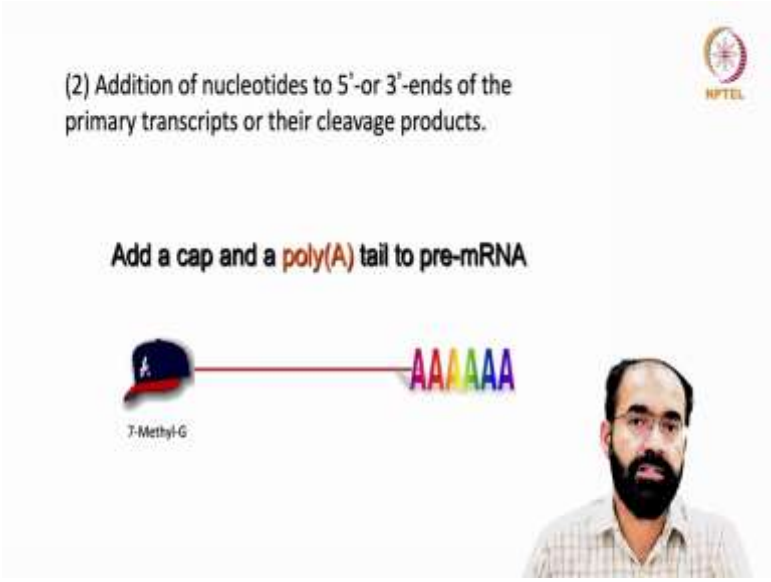


And then comes the addition of nucleotides to the 5 prime or 3 prime ends of the primary transcript or their cleavage products. So, add a cap or a poly A tail both happens onto an RNA you can see there is a poly A tail and also addition of a 7 methyl guanosine cap onto the 5 prime end. This is a feature of addition of nucleotides to either of the ends of the RNA molecule.

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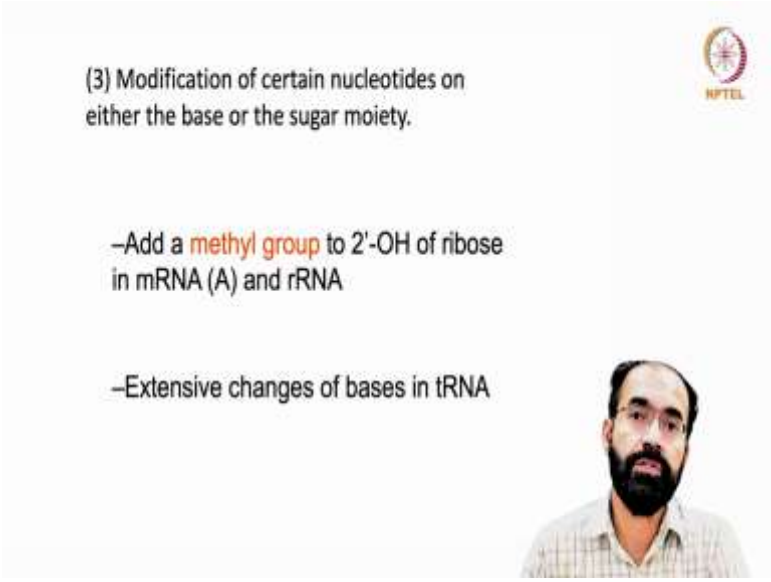


And the third modification is certain nucleotides on either the base or the sugar because nucleotide when you say you have nitrogen base or it also have sugar, (Refer Time:

05:22) sugar, moiety is there. Sometimes it add a methyl group to the 2 prime OH of the ribosome of the given mRNA or ribosomal RNA.

This is a modification which you can see. And also, it can bring in extensive changes in the bases of the tRNA. Sometimes it will introduce a new base which is not any of this A, U, G or C, a total in new base say an inosine base is added or a psi normally you can see the psi arm of the tRNA some unique bases or there will see them more in detail.

But understand that the changes in the base or modification onto specific regions on base or ribose sugar is very important. Many of them provide protection from the degradation. Some of them allow recognition by a enzyme that has to recognize this RNA molecule as a template. Say for example, a tRNA is formed and how a amino acid tRNA synthetase enzyme will know this tRNA is a good tRNA.

So, if a tRNA has got certain modified bases then the amino acid tRNA synthetase will recognize ok this is a good tRNA. Let me add a amino acid group onto this tRNA. So, that the proteins synthesis can take place. So, identity of a RNA molecule also relies or present onto certain bases modification on the post-production RNAs.

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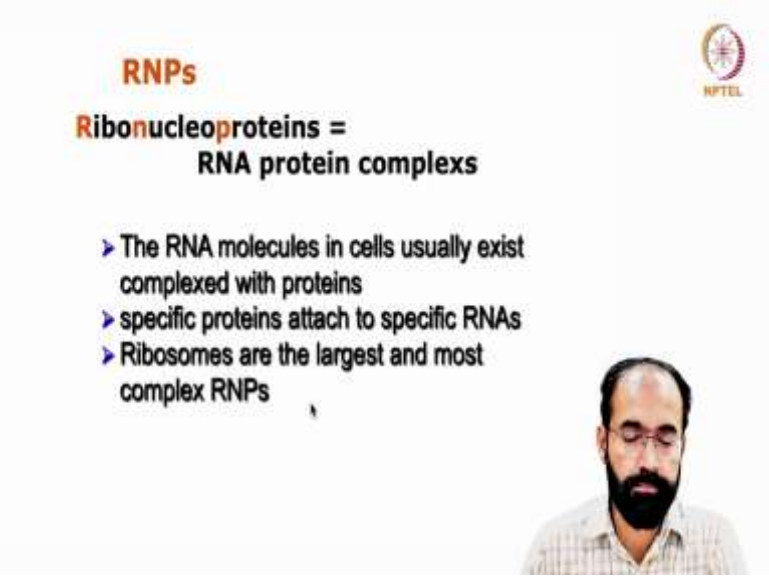


And RNP; RNP the name itself indicates ribonucleo protein that is RNA protein complexes. We know many such example ribosome is a classic example ribosome is nothing but an RNP ribo nucleic acid plus protein. So, RNA molecules in cells usually

exist complexed with proteins and specific proteins they attach to specific RNA not that any protein can bind onto any RNA.

Some of these interactions will stabilize it, some of these interactions will take this RNA to a specific location for its functionality not that every RNA is meant for protein translation like I already told you many non-coding RNAs have roles in regulating gene expression. So, they need to be carried to specific locations.

So, in some cases some RNA need to go to the cytoplasm and then come back into the nucleus for its functionality. So, they are all done with the help of protein interaction. So, RNPs are very important for the functioning of a cell. So, ribosomes are the largest and most complex of the RNPs and they have a functional role that is the protein translation. But there are many RNPs like I told you they interact some of the interactions are transient and some of the interactions are quite deep in the sense they stay for long time for the RNAs lifespan.

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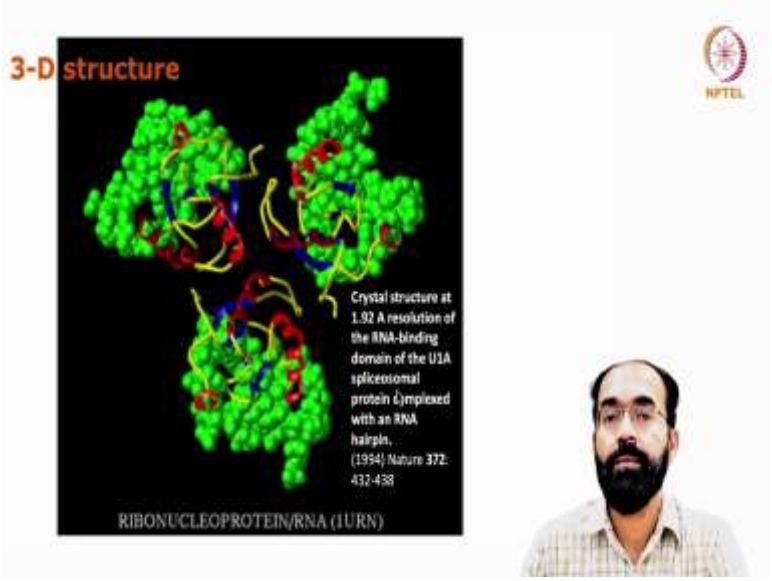


This is a 3D structure 3D structure of the RNP and it is a crystal structure up to 1.92 angstrom resolution of an RNA binding domain of a U1A spliceosomal protein. What is spliceosome? The RNA protein complex that allow the removal of the intron. Different types of spliceosomal complexes are there. This is structure of a U1A spliceosomal protein complex with an RNA hairpin.

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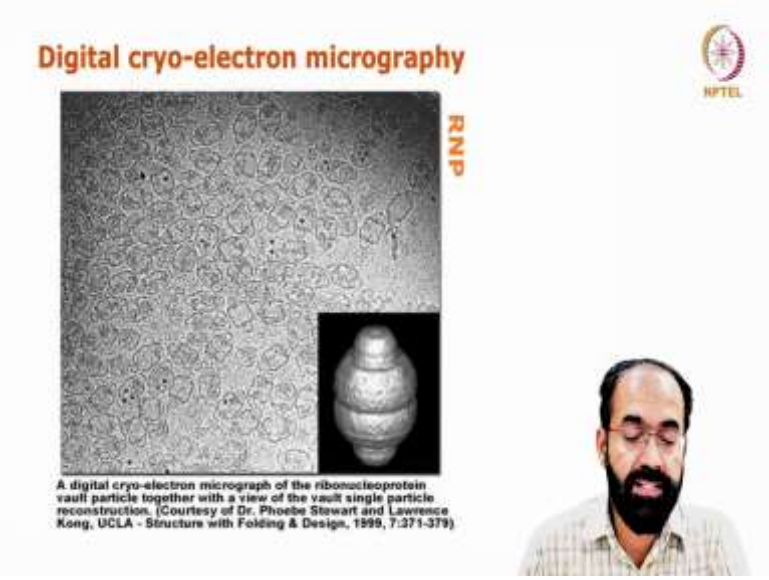


And this is a digital cryo-electron micrography of a another RNP. And what you see in this panel like a barrel shaped structure you can see throughout this section they are RNPs. They are cryo-electron micrograph of a ribonucleoprotein and it is together with a word single particle reconstruction is used. And what you see in the inset and enlarged version of individual ribonucleoprotein.

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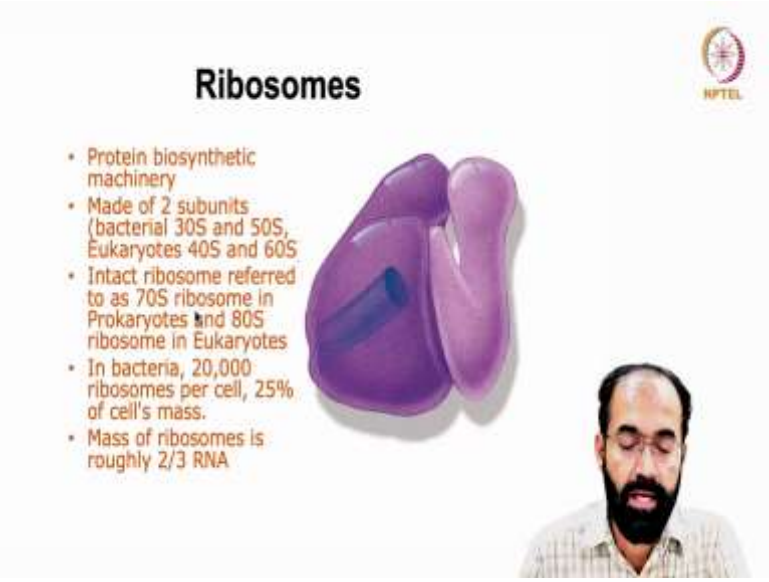


And if you come to ribosomes and they are the protein biosynthetic machinery. By now we know a lot about ribosomes because we have seen it is also a ribozyme, we have seen

it and it is made of 2 subunits of ribosomal or ribosomal subunits consists of RNA plus protein and they are broadly 2 subunits are there. And in bacteria they the 2 subunits are one is 30S and another is 50S.

S stands for Svedberg unit after the scientist who discovered it say if you take the ribosomes and spin it and in a centrifuge based on their size they will settle down at specific band region. So, that notation is given a Svedberg value. So, that is what 30S 50S like that. So, normally the bacterial ribosome is of 70S the intact ribosome is 70S 7 0, but if you split it into 2 subunits give rise to 30S and 50S.

So, that is why 30 and 50 should have an 80, but it would not why? Because this is not total mathematical addition it is the sedimentation where it is sedimenting. So, the 50S and 30S together will not give 80S rather it is giving 70S that is the place where it is sedimenting.

So, intact ribosome in bacteria is referred to as 70S ribosome in prokaryotes and whereas, in eukaryotes it is 80S it is little larger than bacteria in eukaryotes including humans. And in bacteria around 20000 ribosomes are present per cell and which is constituting around 25 percent of the cells mass that is 1 by 4th of a bacterial cell mass is ribosome that shows how important the ribosomes are and it also supports the RNA world origin. And the mass of the ribosome is roughly around 2 by 3rd of the total RNA.

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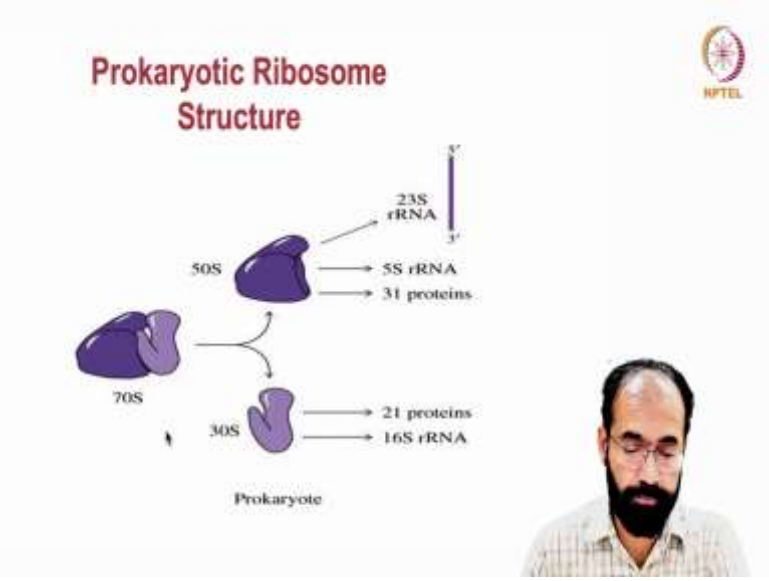

If you split further the prokaryotes ribosome structure, this is the 70S ribosome, it is split into 50S and 30S and 50S can be further split into 23S, 5S and other 31 different types of protein. And the 30S ribosome have got 21 proteins and predominantly 16S ribosomal RNA. So, this is the classification or the subdivision of the prokaryotic ribosome structure. Same way if you come to eukaryotic ribosome structure and it is much larger and more complex than the prokaryotic ribosome.

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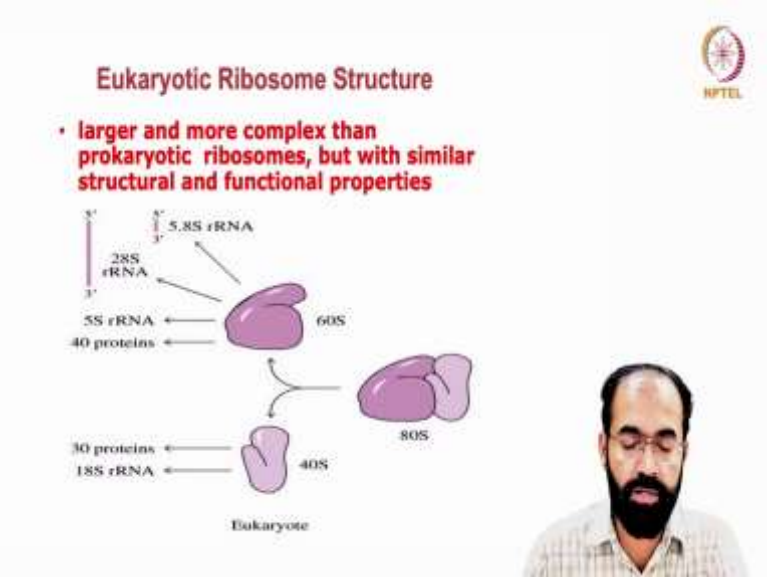


But it has similar structural feature that too have got 2 sub unit. In eukaryote it is called 80S sub unit, 80S ribosomal RNA it has got 2 sub unit, 60S sub unit and 40S sub unit. It is adding up to 100, but actually you would not get 100 you get only 80S because of the sedimentation effect. And the 60S ribosomal sub unit can be further split into 5.8S, 28S, 5S ribosomal RNA and 40 different proteins.

Whereas in 40S you have 18S ribosomal RNA and 30 different types of proteins. Which itself gives you a clue how complex the ribosome structure is and hence it is an important regulatory molecule in every cell present both in prokaryote and eukaryote.

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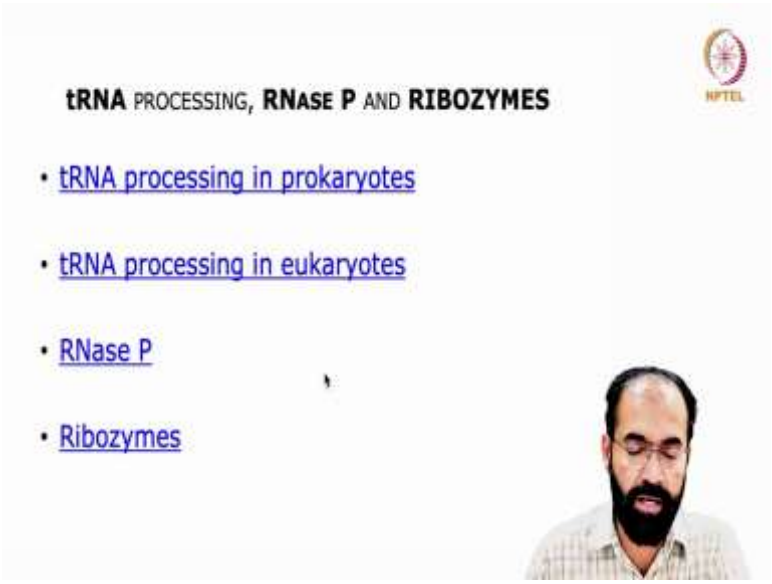


So, now we will see more in detail about how tRNAs are undergoing processing, how RNase P comes into picture. We have already known about RNase P, we studied about RNase P it is one of the ribozyme that helps in the maturation of the tRNA 5 prime end and also we know about ribozymes.

And the tRNA processing in prokaryotes, tRNA processing in eukaryotes and the importance of RNase P and a few of the ribozymes we will see subsequently.

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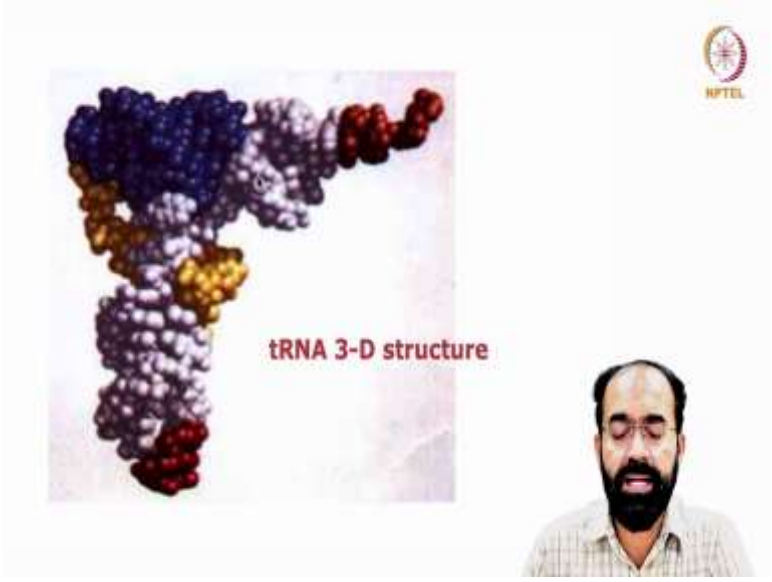

So, tRNA 3-D structure is somewhat like its called clover leaf structure, but in a simplistic form it look like a inverted L shape.

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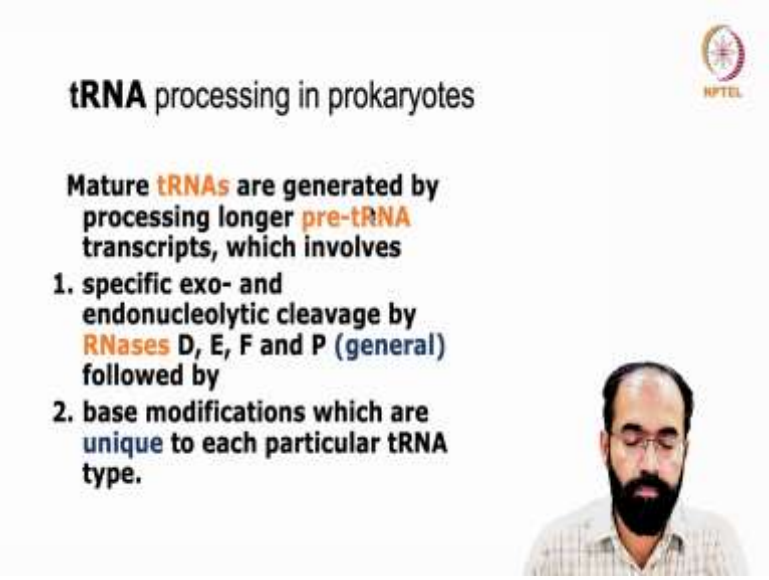


And tRNA processing in prokaryotes is quite interesting because the mature tRNA are generated by processing of longer pre-tRNA transcripts which has to undergo further maturation. This maturation involves specific exo and endonucleolytic cleavage by RNases of the category RNase D, RNase E, RNase F and RNase P and these are all the general RNase which is further followed by base modifications which are quite unique in each particular tRNA. tRNAs are 20 different types, we know 20 different amino acids are there.

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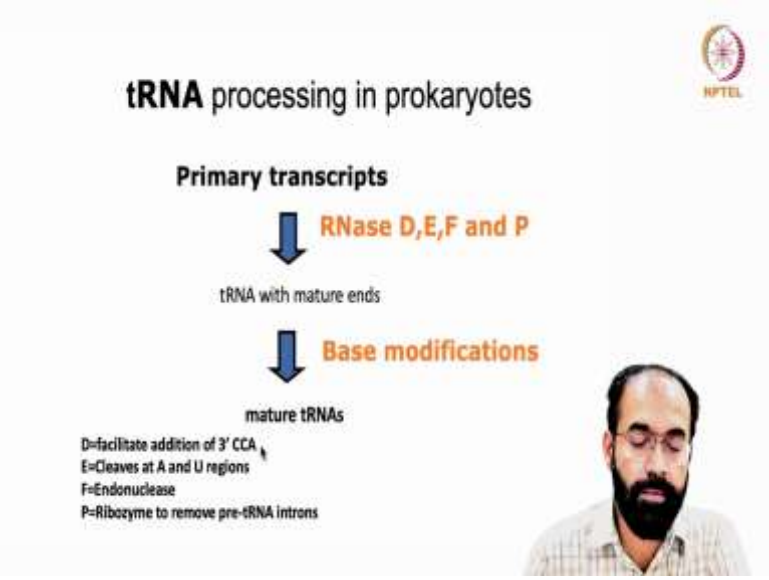


So, they are of different types are there. And tRNA processing in prokaryotes let us see the primary transcript when it is produced it is acted upon by RNase D, E, F and P and this causes a proper maturation of the transcription ends. And this will further undergo base modifications and you end up getting a mature tRNA and RNase D will do facilitate the addition of 3-prime CCA end to the tRNA.

RNAse-E cleaves at A and U regions in the TRNA. RNase-F is a typical endonuclease RNA and P is a ribozyme to remove the pre-tRNA introns. So, these 4 RNase are important for the maturation of tRNA in prokaryotes and the.

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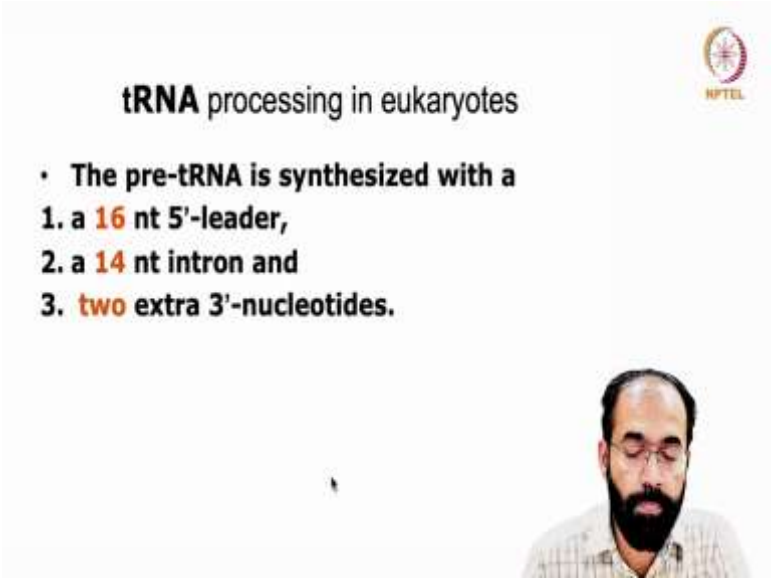


tRNA processing in eukaryotes are also somewhat similar. The pre-tRNA synthesis takes place with the pre- tRNA pre-tRNA when it is produced it has to have or it usually will have a 16 nucleotide 5-prime leader sequence and a 14 nucleotide intron sequence and a two extra 3-prime nucleotide. These 3 features have to undergo maturation.

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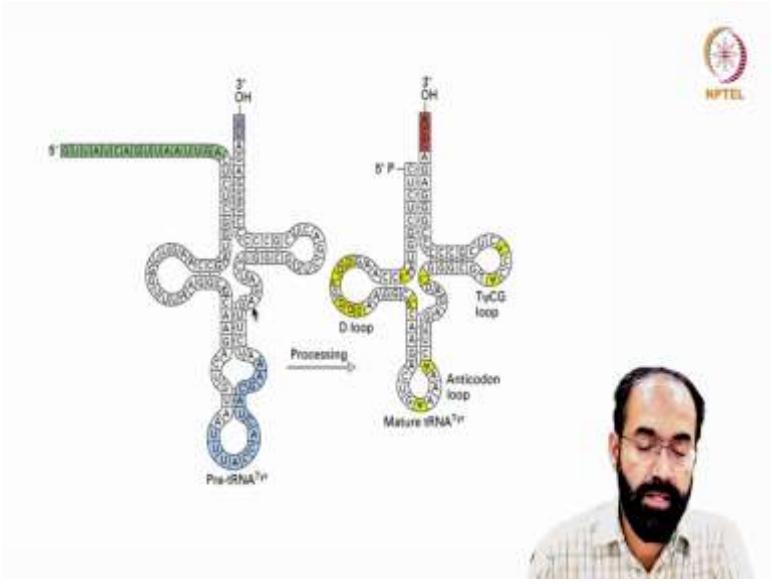


And you can see in this picture can see this is pre-tRNA and it has an extra sequence which is seen in green and this white portion is normal and this blue have to be removed and this extra two use has to be removed. And as the maturation even these all extra

portions are removed and it retain the structure the 3 arms it has and this CCA is added extra by the action of the modifying enzymes.

And then you can see there is a T-psi CG loop and then you also have a in this anti-codon loop you have got modification different bases sometimes the bases are methylated. So, each of these places you are seeing a alteration a change occurs onto the base. And like I told you this allows the recognition by amino acid tRNA synthetase.

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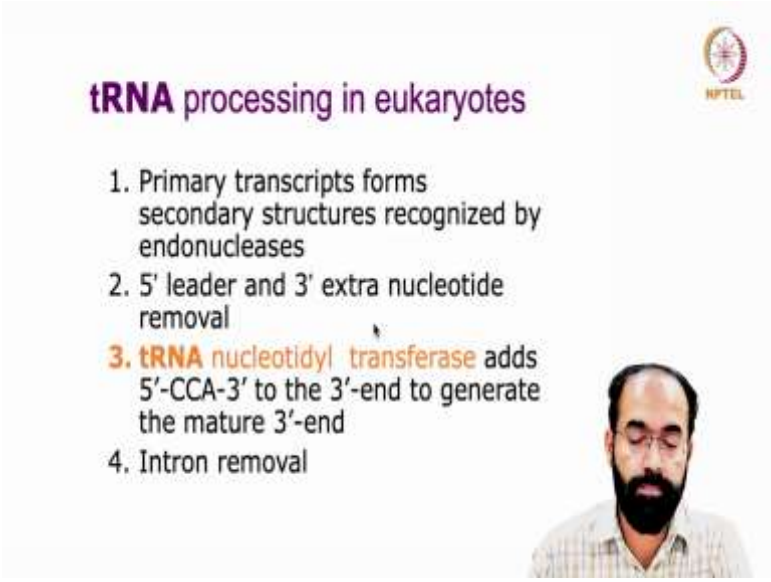


And tRNA processing in eukaryotes happens in a step-first manner. The primary transcripts forms the secondary structures that are recognized by the endonucleases. One thing important we should know about RNA is that RNA is recognized by a enzyme not looking at its sequence. RNA has to bind or bend or form stem and loop.

So, that it will have a typical secondary or tertiary structure which allows them to recognize the protein enzyme will recognize RNA not based on its sequence, but based on its folding and structure. So, that is why the primary transcripts has to form specific secondary structure. So, that will be recognized by protein enzyme.

So, the 5 prime leader and the 3 prime extra nucleotides has to be removed as we saw in the previous picture. And then the tRNA nucleotidyl transferase that adds CCA because the CCA have got a role in the peptide bond formation inside the ribosome which we

saw in the previous classes. The 5 prime CCA 3 prime to the 3 prime end to generate a typical 3 prime end which is common to all tRNAs.

No matter what is the sequence of the tRNA because one tRNA may bring adenine, another tRNA may bring methionine, another tRNA may bring tyrosine, another tRNA may bring tryptophan. Each of them may have a different sequence; however, they all will have a common CCA end which is made by post-transcriptional modification. Then existing introns has to be removed that is a process called intron removal.

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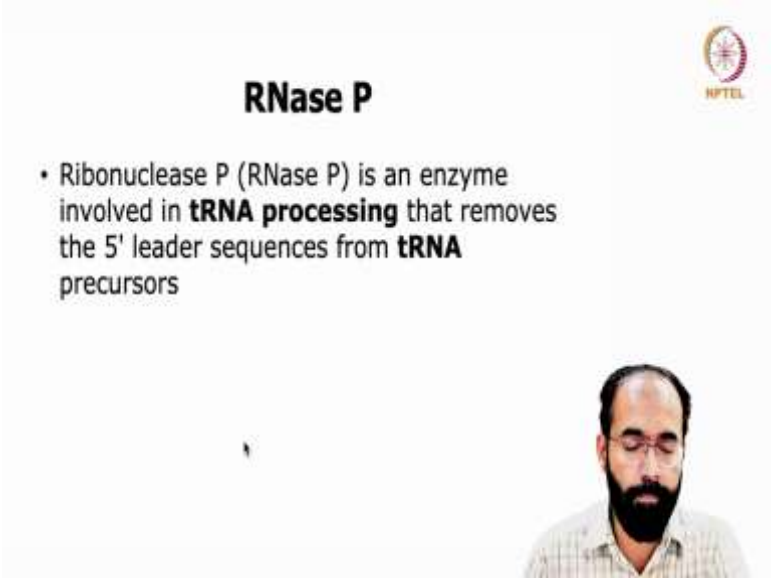


And RNase P we have seen it in few examples earlier when we studied about ribosome. RNase P is an enzyme involved in the tRNA processing that removes the 5 prime leader sequence of the tRNA precursors.

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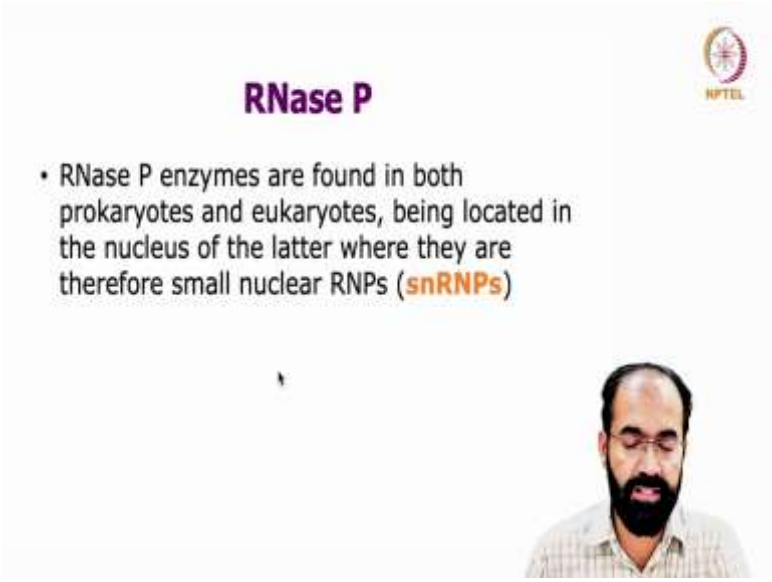


And RNase P enzymes are found both in prokaryotes and eukaryotes and it is being located in the nucleus the latter where they are therefore, small RNPs. So, RNase P can be called as RNP because they remain attached with the proteins. RNA component can catalyze the pre-tRNA in vitro in the absence of protein.

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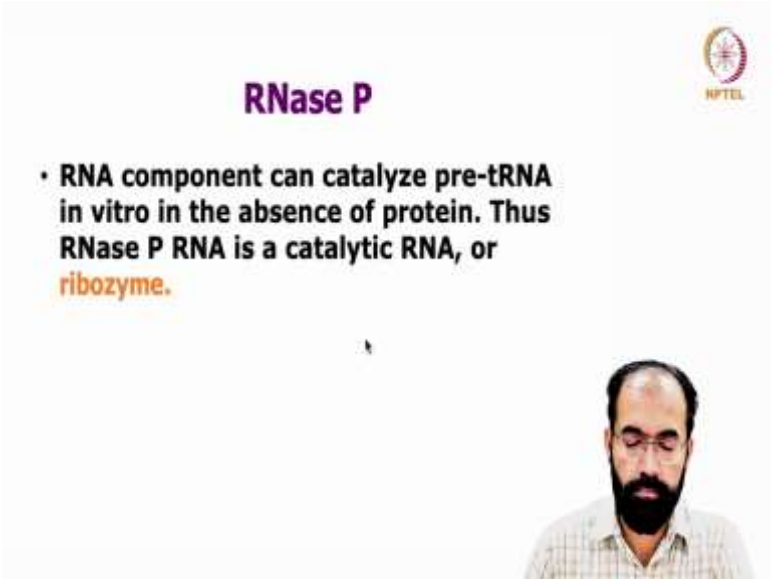


So, that is why we can call RNase P as a typical ribozyme. Why? Because protein provides structural stability and the RNase P is basically a ribozyme because the RNA component of RNase P or the RNP is the one which is participating in the removal

function or the removal of the leader sequence. So, that is why we call RNase P as a typical ribozyme in the maturation of tRNA ends.

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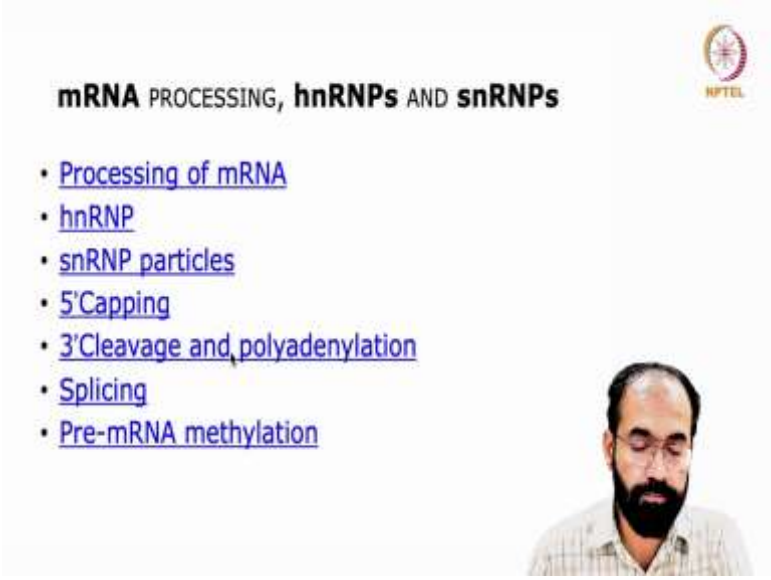


And if you look further mRNA processing hnRNP and snRNPs are also need to be studied in detail and they are classified in this following sections. The processing of mRNA need to be understood properly and what is the importance of hnRNP, heteronuclear RNP, ribonucleoprotein or snRNP, small nuclear RNP and importance of 5 prime capping, 3-prime cleavage and polyadenylation and RNA splicing and also pre-mRNA methylation.

All these things are the processes that undergoes modification onto the various RNA molecules at various levels. And there are different types of RNA modifications are there which we will see them one by one and because all of them if you address together it can leads to confusion also.

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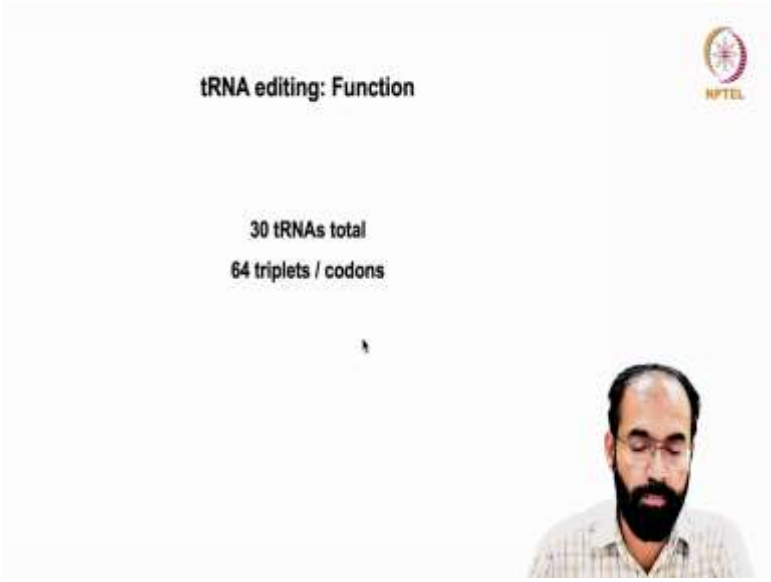


So, let us quickly see what is tRNA editing. tRNA editing, we kind of discussed in the earlier class that is a existing base now undergoes an alteration with a purpose. Total 30 different tRNAs are there which has to cater 64 triplet codons of which 61 brings in amino acid 3 of them do not bring in amino acids and we have around 30 different tRNAs genes are there.

And we have around 20 different amino acids are there which will bring in this tRNA and amino acids together and which has to cater 61 codons of the total 64 codons.

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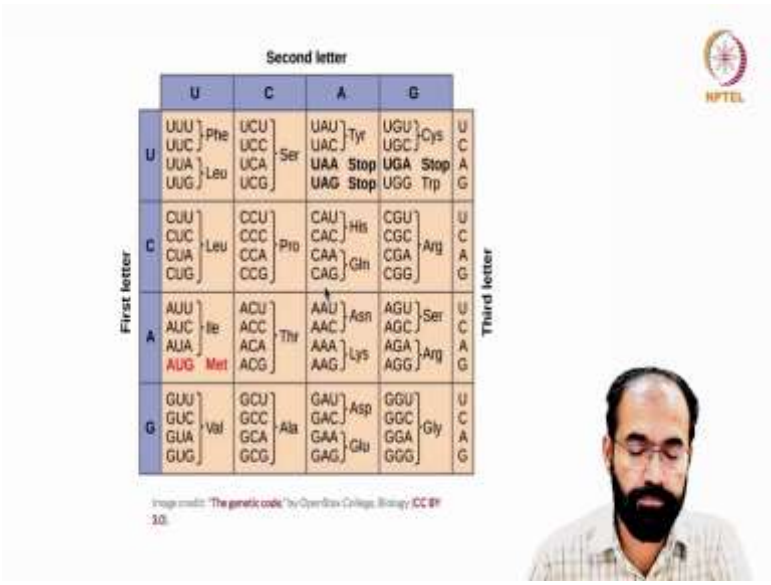

And this is the codon table as you can see here this is the first letter of the codon, this is the second letter of the codon, this is the third letter of the codon its very easy to write. See first letter is written U that indicates all these codons have the first letter as U and the second letter U means second letter also is U here U U.

And the third letter is here U. So, U U U first second and the third and then first is U and second is C. So, in this section you have got U C and then you have got UCU, UCC, UCA, UCG here. You have UUU, UUC, UUA, UUG like that you can keep continuing and each of them have got amino acid written here UUU UUC brings in phenylalanine UUA, UUG brings in leucine like that you can keep looking some amino acids are having around 6 different codons.

That means wherever UUA comes, UUG comes, CUU comes, CUC comes, CUA comes, CUG comes in the mRNA you will have leucine and another such example is arginine; arginine also have got total 6 codons, but otherwise many of them have got 4 or 2 and 2 amino acids have got only one.

One is methionine that is AUG which is the start codon and another example is tryptophan somewhere here it should be tryptophan also have got only one codon remaining all of them have got at least 2 codons are there see here UGG tryptophan have got only one codon.

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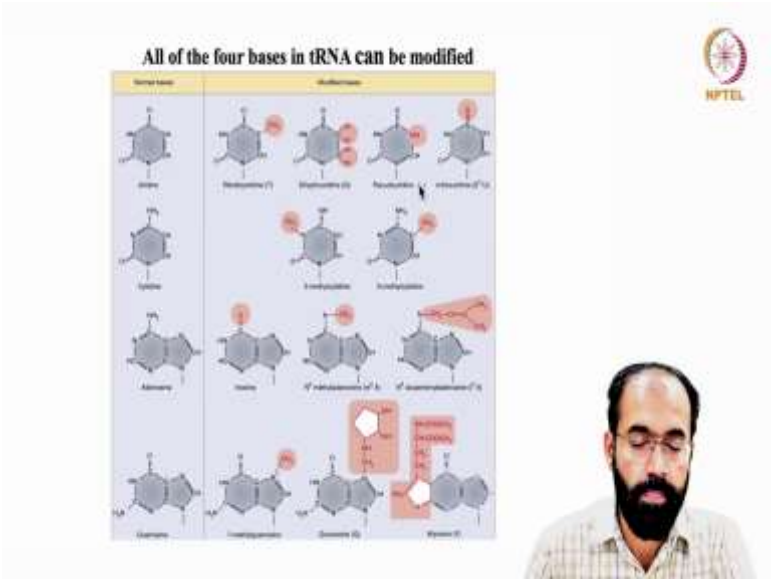

And all of the 4 bases in the tRNA can undergo modification.

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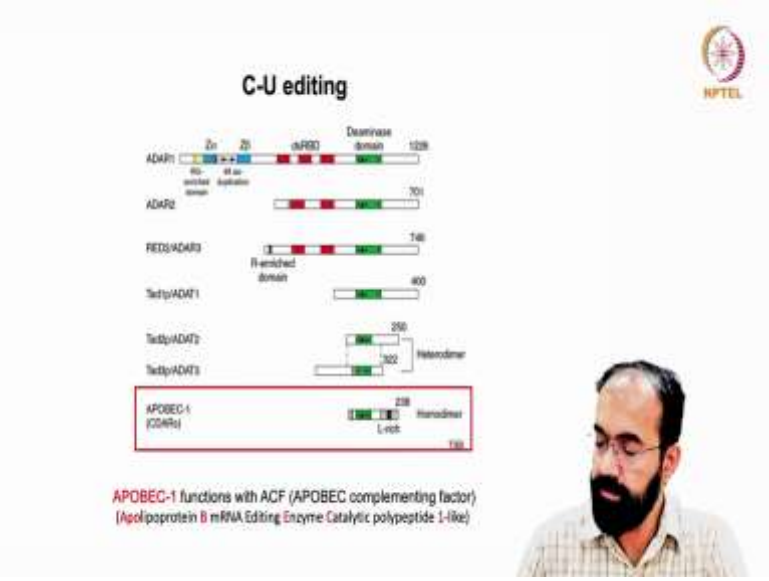


Like tRNA have got; tRNA have got specific bases that is uridine, cytosine, adenosine and guanosine and that can undergo modifications that is different base modifications can come into individual bases and that can be quite handy for the recognition of these bases by various enzymes and we will study about that more in detail in the next class.

Because this need quite detailed description and we may not be able to complete that in today's class; however, we should revise quite strong and we should be on the same page as the classes are going. So, look each of these topics one by one on a day-to-day basis. So, that you will not find it quite difficult and also try to remember the terminology or the technical terms.

Because whenever you read a research article or a text book these words will become handy for you to understand the article to understand the subject quite easily. So, I will end this topic and we will continue with the RNA modification in the next class.

Thank you.