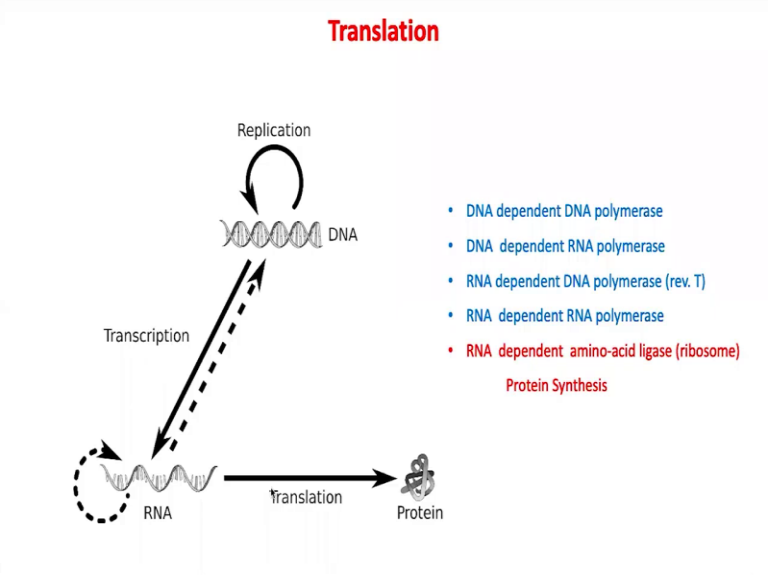
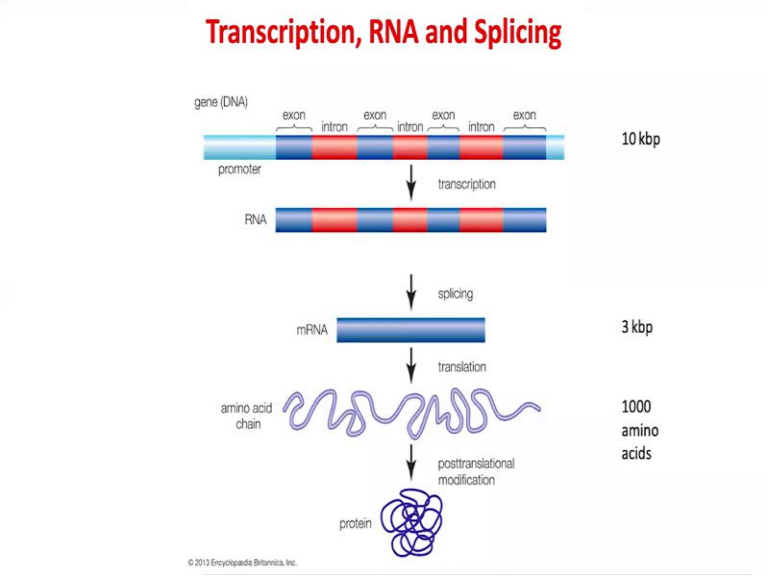
Introduction to Cell Biology
Professor Girish Ratnaparkhi and Nagaraj Balasubramanian
Department of Biology
Indian Institute of Science Education and Research Pune
Central Dogma: Translation - Part 1

(Refer Slide Time: 00:15)





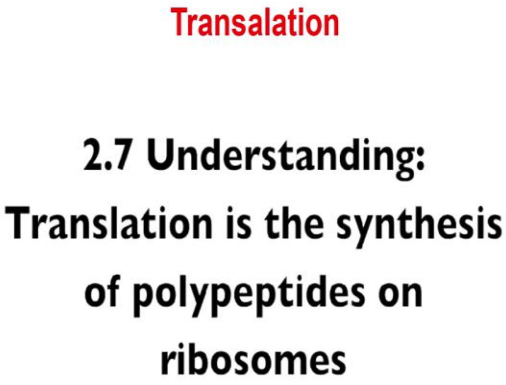
Today's topic is translation and so you are very familiar with this slide. You have seen it multiple times. You know what split genes are now, the definition of introns and exons. You have a broad idea of what a unit of a gene is. You do know that genes can take up a lot of space in the linear double stranded helix which folds into a chromosome. You know that they are very large genes,

they are very small genes, but the gene size has nothing really to do with the size of the mRNA because there are a lot of introns.

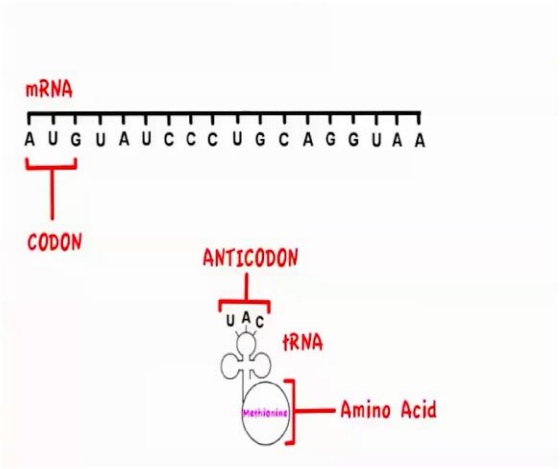
You know what the process of transcription is and you know what the pre-RNA is and you know that there is an event called splicing, Nobel for splicing, Nobel Prize for transcription, Nobel Prize for split genes which Phillip Sharp and others, which I have not talked about, and you finally get a piece of RNA with leader sequences and lagging sequences, which finally is translated into a linear amino acid chain.

The 5 prime to 3 prime basically leads to a N terminal to C terminal polypeptide chain. And this polypeptide chain has an ability to compact itself not stay linear into a folded state which we call as a protein. And over the years, we have solved many, many protein structures of folded protein structures using primarily X-ray crystallography, but also a second technique which became popular in the 90s, which is nuclear magnetic resonance.

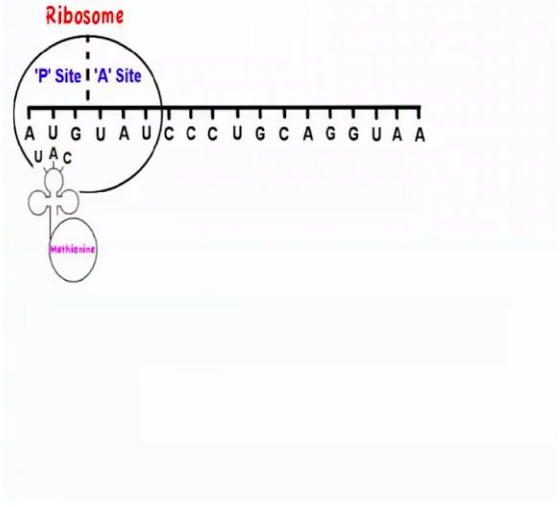
(Refer Slide Time: 01:30)



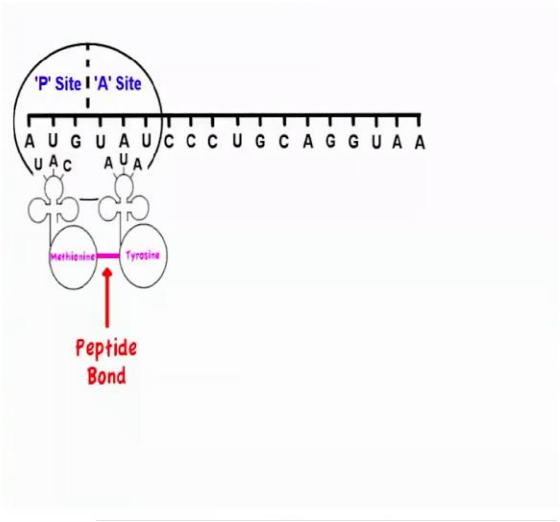
Translation



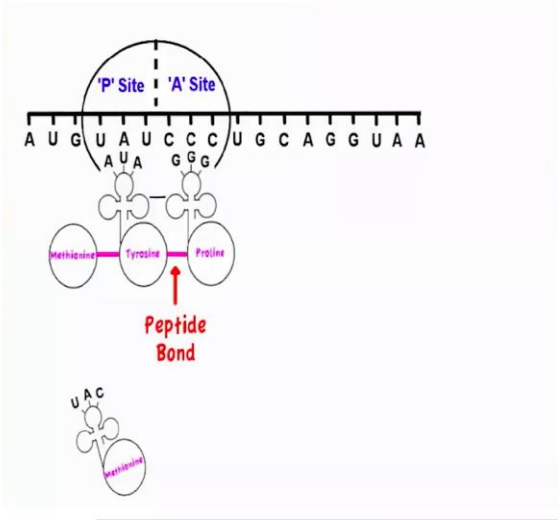
Translation



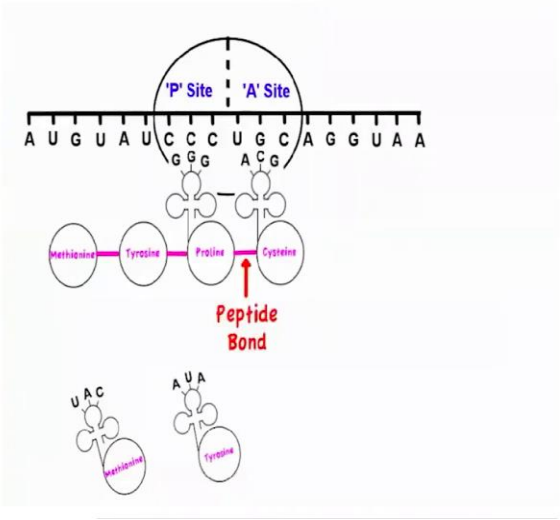
Translation



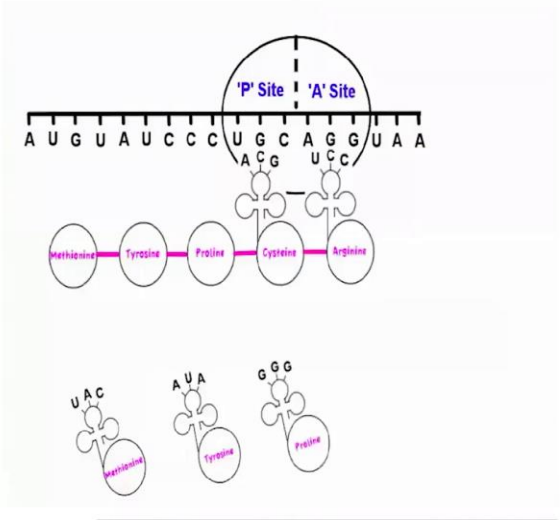
Translation



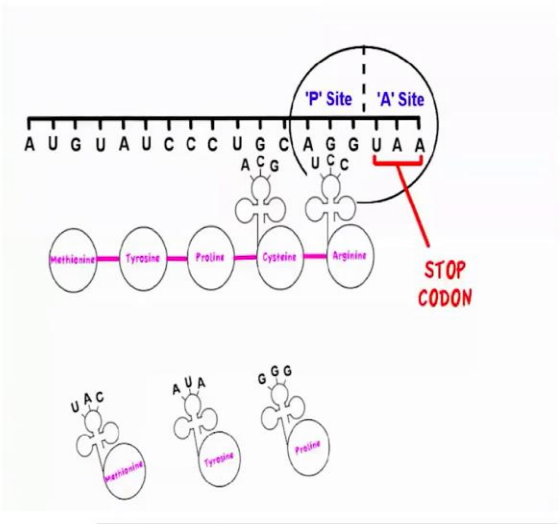
Translation



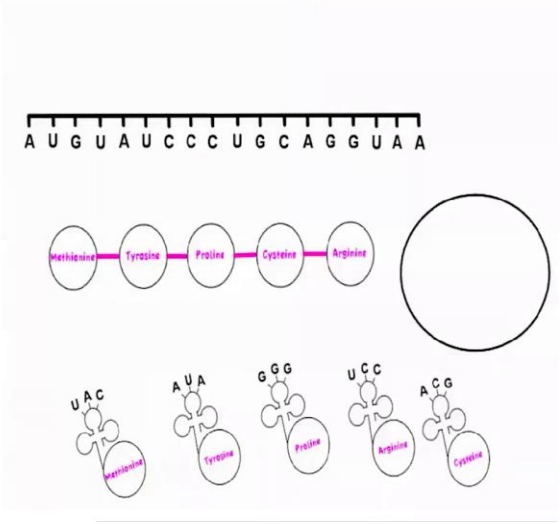
Translation

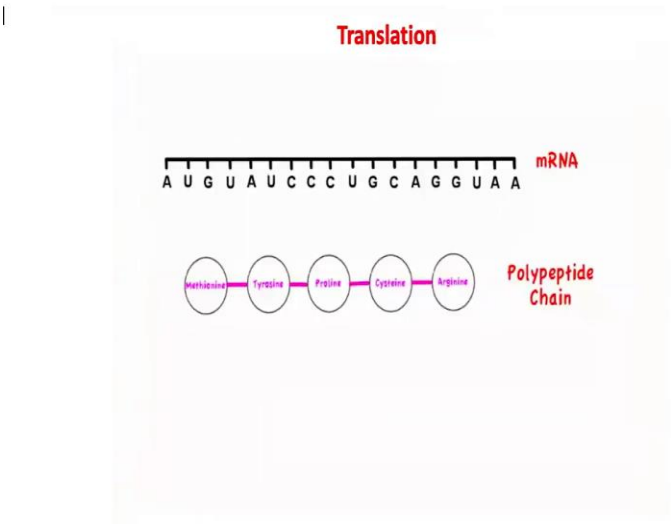


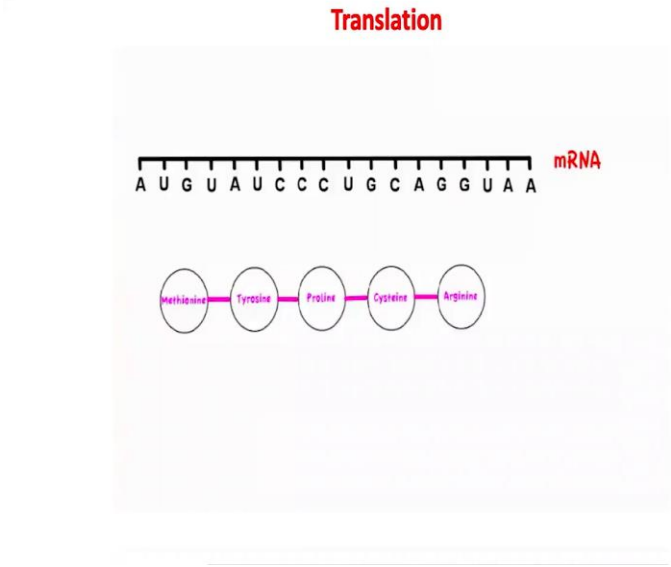
Translation



Translation







So, let us start with a simple view of translation, which gives you a overall idea. And then I will fill in all the details in the next few slides. Translation is the second stage of protein synthesis where a piece of mRNA is used to create the polypeptide chain. Firstly, let us cover the key terms. Remember that mRNA or messenger RNA is made up of codons. tRNA or transfer RNA has an anti-codon on it. And the anti-codon defines the amino acid carried by the tRNA.

So this is a fairly important point which I have not really introduced for the non-biologist that the sequence on the mRNA is read as a triplet that is three nucleotides at a time define a code and these three nucleotides over here define one code and the next three and the next three and the next three. This is something which is very familiar to people who have done biology.

So what this movie is going to focus on is, of course, it will talk about the ribosome, but I would like you to pay attention to how following molecules, the mRNA, or the transfer RNA, which is carrying an amino acid, it is a carrier of amino acid, how the codon and the anti-codon recognize each other and how, as you will see, this leads to protein synthesis using the mRNA as a template.

Translation takes place in the cytoplasm, and the first thing that happens is that the ribosome attaches to the mRNA at the start codon, which is AUG. The ribosome is made up of ribosomal RNA, rRNA and ribosomal protein and has two sites on it the P site and the A site.

So, these are just arbitrary nomenclature. In this particular movie, the lady who is speaking will define the P site as a packing site, that is not official nomenclature, and the A site is the so called attachment.

A tRNA carrying the complementary anti-codon to the start codon, which is always AUG comes and attaches in the P site. The first amino acid is always methionine. A piece of tRNA having an anti-codon complementary to the mRNA second codon then floats in from the cytoplasm carrying the next specific amino acid. In this case, that is tyrosine. And this tRNA then attaches at the A site. A peptide bond is then formed between the first two amino acids.

At this point, the tRNA that is in the P site or you might want to think of that like the parking sign is no longer really required because its amino acid is attached to the amino acid on the second tRNA by the covalent peptide bond. Ribosome then moves along the mRNA by one codon.

This place is the second tRNA in the P site and the first tRNA is then free to break away, while it does so a new tRNA with an anti-codon matching the mRNA's codon and carrying a specific amino acid can then come and join in the A site that is been freed up consider this an attachment site. A peptide bond then joins this new amino acid to the existing chain. And so this process continues.

The ribosome always moves down by one codon. And once it is done so it means that the previous site is able to break away free from its amino acid and allow a new tRNA with a new amino acid to come and join in A site that is freed up. The new amino acid can then join the

existing polypeptide chain with a peptide bond and the tRNA that was freed up goes back to the cytoplasm and collects another specific amino acid ready to be used again.

The amino acids used are specific and defined by the codons on the mRNA and complementary anti-codons on the tRNA. The process is somewhat repetitive and continues until the ribosome encounters a stop codon on the mRNA. The stop codon does not code for an amino acid, but merely indicates where the process of translation terminated.

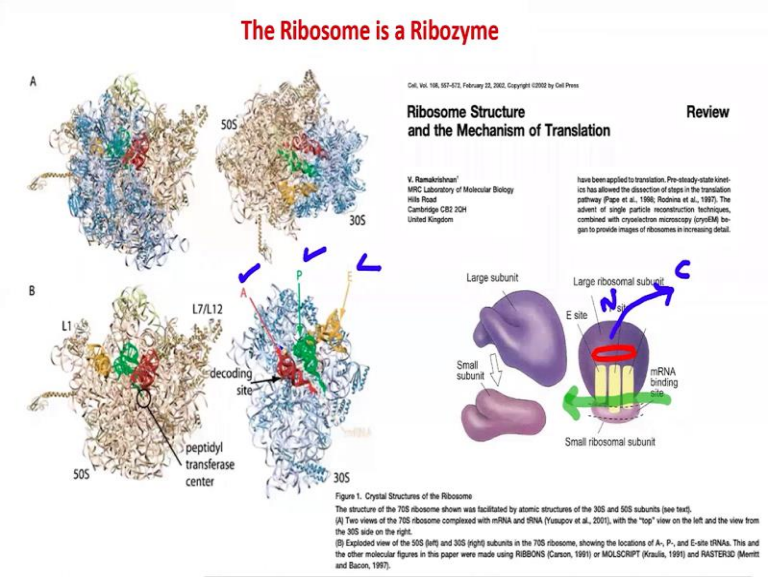
Once the process has been terminated the polypeptide chain, tRNA, ribosome, and mRNA detached and the polypeptide chain becomes free floating in the cytoplasm. Thus, in translation, overall, we have used the mRNA to form a polypeptide chain.

So, hopefully, what this movie does, and one of the reasons I show many of these movies is they are the simplest representation of translation without too many added complications. And this movie gets to the heart of the matter, which is basically the fact that the mRNA is used to trans, to basically information in the mRNA is translated to information in the protein.

There is a three letter codon. The codon is recognized by entity called as a transfer RNA. The transfer RNA at one end has what is called as an anti-codon. The codon and the anti-codon recognize themselves, very critical in biology, this recognition. The tRNA is carrying an amino acid. And the complementary codon, anticodon recognition allows it to specify, allows the mRNA to specify which amino acid is going to come first, which next.

The sequence of 20 amino acids one at a time, is defined by reading the mRNA by the ribosome, which is the translational machinery. So what I am going to do in the next three or four slides is now focus on each of the components. And near the end show you a slightly more complicated molecular a picture of how translation takes place. Now, before I go ahead, are there any questions at this point about this movie, per se? Any terminology questions, for example.

(Refer Slide Time: 07:00)



Let us go over the different elements. The first is of course this large macromolecular complex called as the ribosome. And I have already told you a couple of times that it was a big surprise when researchers realized the ribosome is not actually a protein enzyme. It is an RNA enzyme. So, the ribosome is a mixture of proteins and RNA, we call this RNA, which is part of the structure of the ribosome, and of course, which is the enzymatic activity, active entity as ribosomal RNA or rRNA.

Now, rRNA is very distinct from the tRNA, which you saw in the last movie, and I will talk to you about tRNA also. To give you an idea, now these are of course the actual structures of, which is the atomic level structure solved by x-ray crystallography of the 70S ribosome. Now, I will define what the 70S ribosome is.

But basically what you are seeing over here is different angles of looking at the same structure of a bacterium, E. coli ribosome. It has two units and this is a simplified version. There is a large subunit and there is a small subunit. And in order to execute protein synthesis these two subunits have to sit on, well, the large subunit has to sit on the small subunit.

Now, you will also realize that when these two subunits come together, many years of molecular biology with a lot of experiments done blind mostly have made scientists realize that they can define three sites, the A site, which is somehow not clear over here, the P site, which you have heard of, and there is also an E site and the simplest definition of an E site is an exit site. So, you

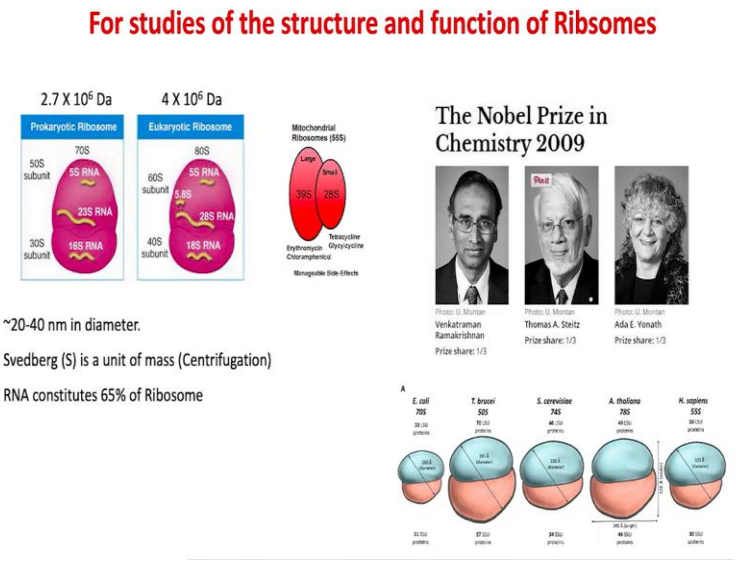
saw in the movie before how the P site and the A site are kind of important. You will realize that the mRNA goes through this large ribosomal complex at the point in which the small and the large subunits meet. So the ribosome is basically going to crossover over here.

You will also realize, and this will become very obvious in movies, that the actual ligation will happen somewhere over here, where I have drawn in red, and now I will just change it to blue and the protein will basically come out over here N terminus to C terminus from the top of the large subunit. So, in a single slide, I am giving you a broad picture of what the large complex looks like, its shape. I am showing you actual x-ray crystallographic structures, showing you the A site over here, the P site over here, and the E site. I know you would not be able to interpret them.

Even researchers who have actually not doing crystallographic cannot look at these structures and easily interpret them, which is why on the right hand side you have a simplistic representation. I have also put for extra reading, this is not mandatory for all of you, review by Venki Ramakrishnan written in 2010 and a few years later, five to six years later, Venki Ramakrishnan got a Nobel Prize for solving the structure of the ribosome.

So, these are the elements. For those of you not used to terminology, again you have to pick up all this terminology of E site, A site, P site, large subunit, small subunit, nothing more complicated than that. So, this now introduces you to one major player in the translation in making proteins.

(Refer Slide Time: 10:26)



Now, a little bit about the structure and function of ribosomes and as always I keep on telling you that you should have a historical timeline in your mind. There was a lot of work in the 50s, 60s, 70s, 80s, 90s, 40-50 years of work. The ribosome is a very, very challenging problem simply because it was a very large complex made up of somewhere between 50 to 100 different independent units and these units will be either RNA or protein.

The size of the ribosome ranges from around 20 to 40 nanometers in diameter. It is really very, very large. Much of the initial work was done on prokaryotic ribosomes and we use the term 70S usually to define the prokaryotic or more specifically the E. coli ribosome. And the generic eukaryotic ribosome is called as the 80S particle. Now, S is basically a unit of mass just like Daltons is and it is based on how heavy a large molecule, because we are dealing with a very large molecule over here, is in during centrifugation. And I will not really go into a definition of all of lt.

The size of the eukaryotic ribosome is 4 into 10 raise to 6 Daltons, you know what Dalton is a unit of mass, and the prokaryotic ribosome is approximately 2.7 into 10 raise to 6 Dalton, really very large complexes inside the cell. Now, in this schematic, you see that there are three major RNAs and, in the prokaryotic ribosome and four major RNAs in the eukaryotic ribosome.

Now, do not be taken by this very simplistic picture of one for prokaryotes, one for eukaryotes. On the right hand side, as you can see, there is a range of ribosomes which from E. coli all the

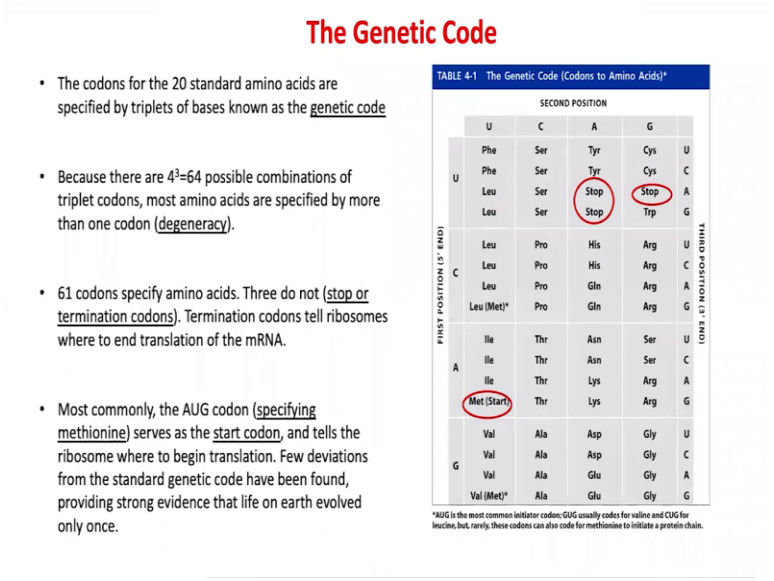
way to Homo sapiens and their sizes, shapes vary. So there is a lot of variation in ribosomes. This is what we have discovered in the last 20 years.

Also to remind you that mitochondria and chloroplasts are inside eukaryotes. And the origin of these mitochondria and chloroplasts is actually a prokaryotic cell. So the mitochondria have their own independent ribosome which is very distinct from the prokaryotic ribosome and the eukaryotic ribosome. And these ribosomes are functional. And in the mitochondria or in the chloroplast protein synthesis takes place just like it takes place in the eukaryotic cytoplasm.

Now, the Nobel Prize for events related to structure and function of ribosome was given to these three scientists Venki Ramakrishnan, Tom Steitz and Ada Yonath. All three of them contributed in different ways, but much of their contribution, for example, Ada, Thomas and Venki were all crystallographers. They were also biochemists. They did a lot of biochemical studies.

And as I said, there is a lot of wet biochemistry over here. Purification of these 50 to 70 units, each unit had to be purified and ribosomal complex formed that had to be crystallized. And even the solution of the interpretation of the x-ray pattern was not trivial. This was one of the most challenging problems in the last 30 or 40 years.

(Refer Slide Time: 13:06)



Now, let me move on to another aspect of what we are talking about that there is a genetic code, universal genetic code, which exists in all plants and animals and prokaryotes, which basically

defines the relationship between the three letter codon on the mRNA and the way it is interpreted and converted into an amino acid.

And many, many tables on the Internet, this is just one of them, and what this does is it basically says that if you have a three letter code, they are 64 possible combination of triplet codons. But there are not 64 amino acids. In nature, there are only 20 main native amino acids. There are non native amino acids which we will not talk about in this particular class.

And the way the scientists who worked out this code and I will define who these are in the next slide worked it out was that you have a three letter codon, but it is the first two letters which are very important, because this is the first position, this is the second position and this is the third position of a three letter codon.

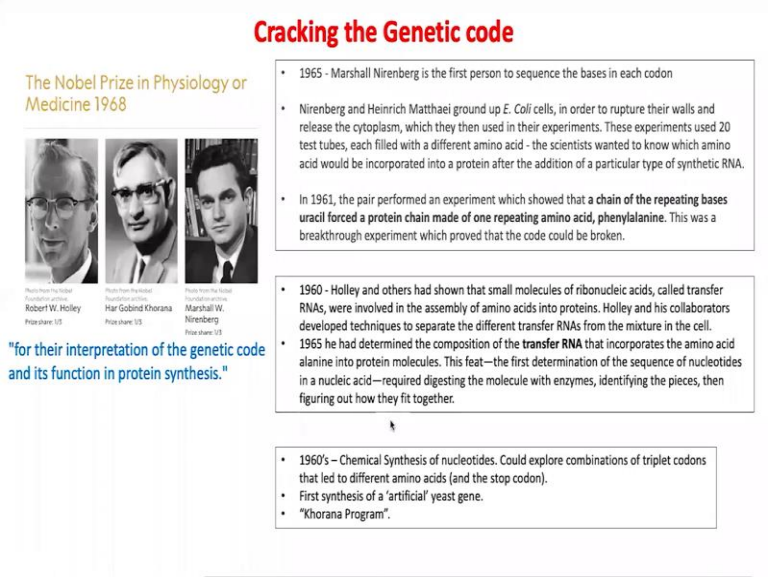
So, if we take any one, let us say, we take UU, so UU and U is phenylalanine. So, if you have a UUU on an mRNA that basically means that during the translation process, the transfer RNA which has an anti-codon equivalent to UUU will carry phenylalanine or leucine in this case. So, you have UUU is phenylalanine, UUC is phenylalanine, UUA is leucine and UUG is again leucine.

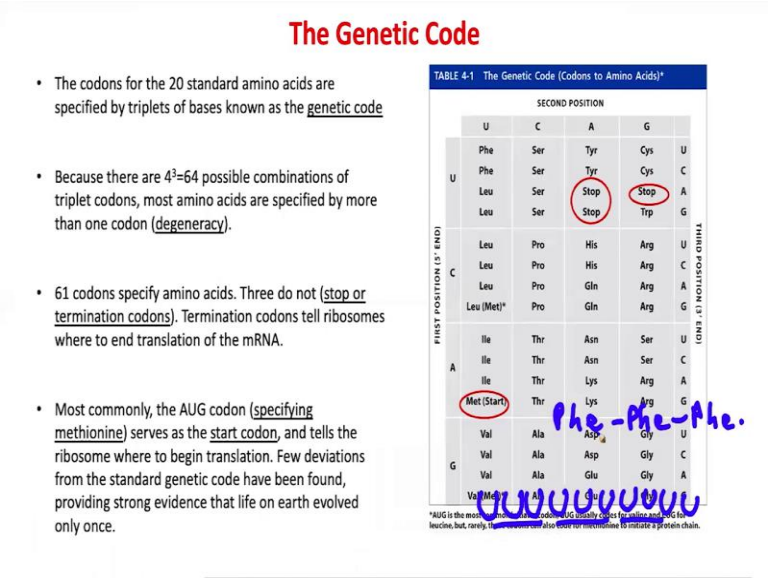
So, if it is UUC it will, there will be an anti-codon on the tRNA. It will recognize the mRNA codon and the phenylalanine amino acid will be placed first in the P site and then in the A site and it will be ligated to the next amino acid in sequence. So, this table basically defines the relationship between the codons and the amino acids and this was established by Nirenberg and Khorana and others which I will basically talk to you in the next slide.

Methionine which is AUG functions as both as a start codon and as addition of methionine which is AUG. And UAA and UAG function as stop codons as thus UGA. One very clear thing which comes out of the solution of the genetic code done by the researchers who are there in the next few slides is that the third position has a so called is more flexible than the first two positions.

So, for example, phenylalanine has two options UUU and UUC. Proline, for example, has four options CCU, CCC, CCA, CCG. So, the third codon has what we call as a wobble or flexibility, which is part of biology. We do not completely understand why this is so. And this also leads to degeneracy in the genetic code. Are there any questions on this slide?

(Refer Slide Time: 15:46)





So, now the genetic code basically relates a triplet codon to an amino acid. And it is very critical for the whole process of translation. And the understanding the cracking of the genetic code, remember, we are talking about 1968 when the prize was given, much of the work was done between 1961 and 1965. Though of course, all work has previous work on which these researchers based on their research on and this was barely a decade after deciphering the structure of DNA.

And remember, in the 1950s also people did not really believe that DNA was the genetic material. And the cracking of the genetic code in the 60s and the Nobel Prize awarded in the late

60s basically brought home completely clearly that at least in prokaryotes, where much of the work was done, that there was a very clear genetic code.

So, here are the three people Robert Holley, Har Gobind Khorana and Marshall Nirenberg who were given credit for cracking the genetic code. Holley basically was the person who discovered, sequenced and found the role for transfer RNA in this process.

Har Gobind Khorana was a chemist who did the first synthesis of nucleotides. And this was a very important step because if you synthesize nucleotides and you put it in an extract which contains the ribosome, then defining on, depending on what kind of nucleotides you synthesize, for example, I will give you an example up there, as I told you, UUU was phenylalanine.

If you make a nucleotide which is UUU, UUU, just a series of Us, and then you put it inside extract with ribosome and you get only a, and you look at what polypeptide is synthesized, and you find out that the only polypeptide, only amino acid which you see is phe-phe-phe, you realize that this triplet code probably relates to phenylalanine.

And because you can now synthesize oligonucleotides done for the first time by Har Gobind Khorana, you can now put any sequence you synthesize of different combinations and look to see which amino acids are being made by the ribosome. Thus, you can then have a relationship between the nucleotide sequence and the amino acid sequence.

So, Nierenberg was the first person to sequence the bases and define each codon and help of course, by work done independently by Har Gobind Khorana and Robert Holley brought in the concept of transfer RNA. So, he discovered that part of the protein synthesis process. For undergrad students you should be aware of a program called as a Khorana Program run by the Government of India, University of Wisconsin Madison. They support undergrad students to move, to basically go to the university in the U.S. for internships. So you can look up that. You can try and apply for the Khorana Program in the future.