Introduction to Cell Biology
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Central Dogma: Translation - Part 3

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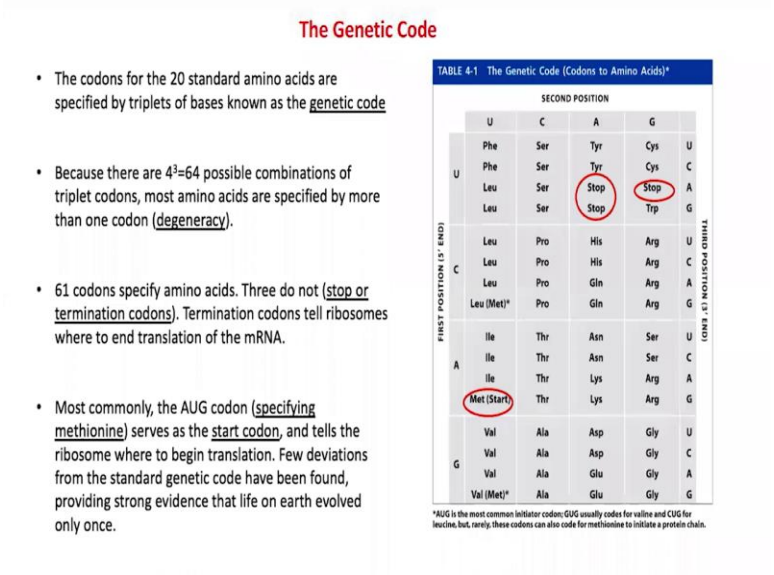

Student: Sir, can you please explain the last line, few deviations from the standard genetic code that line.

Professor: So, there are deviations in terms of what, but these are very minor deviations. For example, the start codon, they can be an alternate start codon, which is basically CUG. It can sometimes code for methionine. So, there are variations, but there are exceptions. So, what I mean is 1 percent of the time if you go to some very exotic animal, maybe archaic animal, which has been caught from hot springs from somewhere deep inside the ocean, it may have some variability as compared to this code which is written over here.

But more often than not this is the reading of the genetic code in all organisms, which means that even if there are small variations here and there, mostly all life follows this code and this code only. So, mitochondria may have slight variation, for example, as compared to what is there in the eukaryotic cytoplasm. But these variations are very small, which is why the line says that there is strong evidence that life on Earth evolved only once using this genetic code. There are

not multiple genetic codes which were evolved and in life forms we do not have multiple species with different genetic codes.

If you take a gene from bacteria, and if you express it in humans, the human ribosome will recognize the bacterial DNA, because the genetic code is the same and DNA is the same. If you take a human gene and you express it in bacteria which is by the way done very routinely all the time, all biologists do that, bacteria will have no problem in recognizing the mRNA sequence simply because the genetic code is universal.

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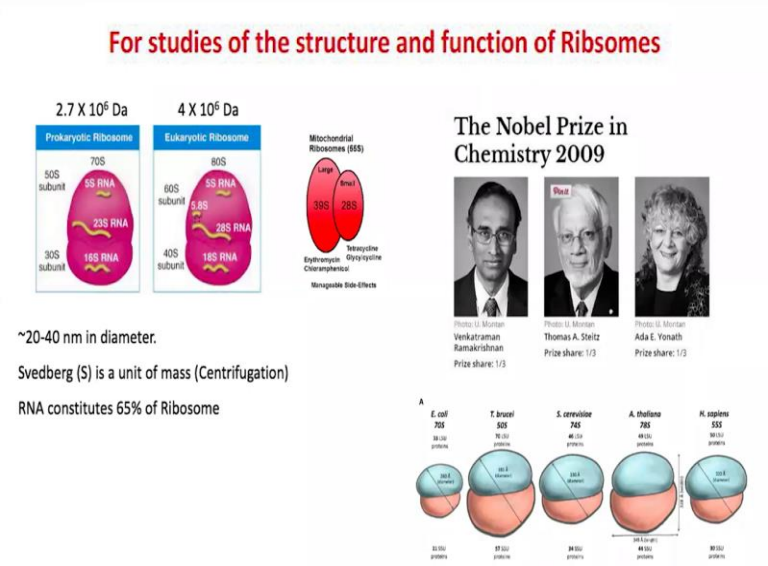

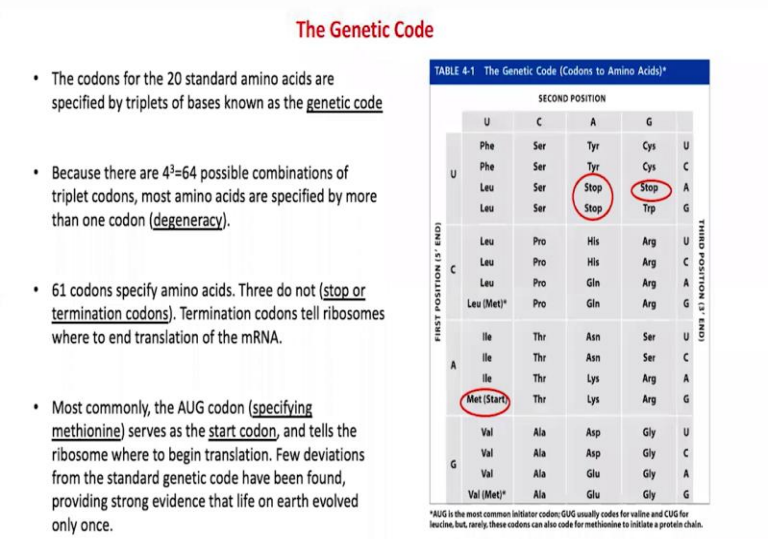

Student: Sir, can you ones go to previous slide where mitochondrial ribosome was directed? Sir, here, below the subunits, why there is written erythromycin, chloramphenlcol.

Professor: So, I would not talk about this. I would encourage you to read up on this. Biology is vast. Every word, every sentence can take you to an interesting direction of new information. Basically, antibiotics which we use, some of the antibiotics we use target the ribosome. And that is how we, when we eat an antibiotic, tetracycline, for example, we kill bacteria inside our body.

But what this slide is also saying is there are sometimes there is a weak effect of tetracycline on mitochondrial ribosomes. So, in that sense, since we all have mitochondria in ourselves, it can have side effects. But wherever I took the slide from, it seems that these side effects are manageable. So, there are variations in the ribosomes which allow drugs to target prokaryotic ribosomes, but not affect eukaryotic ribosomes. And because of these differences, drugs can be used against bacteria in our body without harming the cells in our body.

Student: Sir, like why is the protein sequence, the amino acid sequence always start with methionine, like if the codons codes for something else, even like for leucine and valine like if they are the start coding then the code for methionine in the beginning.

Professor: So, this seems to be the way biology is.

Student: Is there any advantage to that or any specific why that has been use structural advantage?

Professor: So, I have, at least in textbooks, there is nothing. The same question would have been asked by researchers. The moment you ask a question like this, it becomes what is called as an open problem. Why is, why are things like this, and some researchers somewhere would have studied it. And since we know about the genetic code for almost 30, 40 years now, it would have been studied for at least 30, 40 years. But as far as I can figure out, we have to go and look in literature. There is really no clear answer to why methionine is always used as a start codon.

I will be happy to take questions now.

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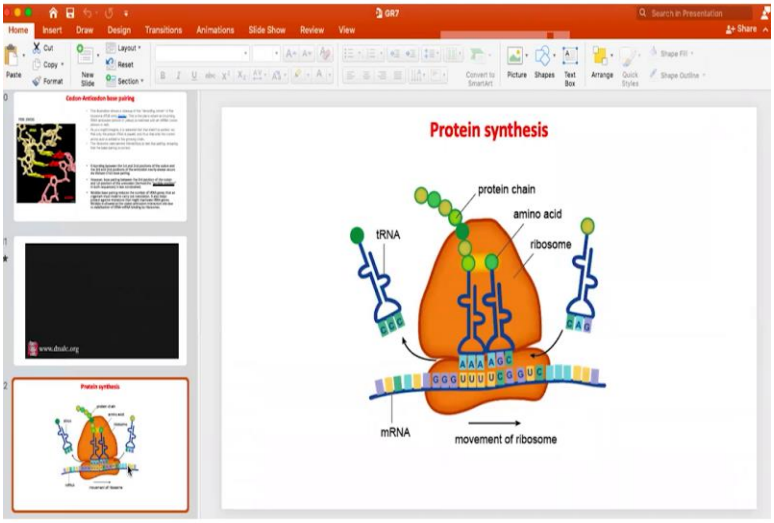

Student: Sir, I have a question. These tRNA are just floating in the liquid, right?

Professor: Yes.

Student: So, how does that particular tRNA comes and any tRNA can come, right? So, why does only the correct order tRNA?

Professor: So, these are a lot of interesting questions. When we are taught biology at least in my time in 10th, 11th, standard or even before that, it is very difficult for us to visualize what is going on about molecules in the cell, which is why A, I emphasize on size scales or molecules, B, I am also showing you movies which are animations made in the last 10 years, which gives you a more clearer picture of what is going on inside the cell.

So, the question you are asking which is a very obvious question, one you are asking is what caused these transfer RNAs to the ribosome? Is there a process where there are directed like a traffic police directing traffic is somebody grabbing them and bringing them to the ribosome, is there a compartment where the concentration is so high, like tRNAs are always hitting the ribosome, because there are so many hundred, thousands of them in a very small space. There are answers to these questions, but they are not very clear.

A more interesting question which comes out of your question is how does the exact tRNA with the correct codon anti-codon come there automatically or are 20 amino, 20 tRNAs tried one at a

time till the correct codon anti-codon is found? The answer to that is probably multiple tRNAs are, because multiple tRNAs are tried till the match is completely made.

So, we are looking at a set of molecules in water, mostly water, it is an aqueous buffer, which are all wobbling around, there is a lot of chaos going on at the molecular level. We have these large complexes. We have mRNA coming out of the nuclear pore. It is not clear whether mRNA comes out like a snake as it is shown in the animation because or is it carried by specific proteins. There is a lot of space inside the cell as far as small molecules are concerned. So, how is all of this directed? How do molecules meet each other? So, these are all open questions. We have answers to some of them, but not to all of them.

Questions?

Student: Sir, does this process depend on the concentration of tRNAs present in the.

Professor: tRNA or, yes, it does. So, you need to have the correct concentration of tRNA and you also need to have enough tRNA. So, there is a tRNA charging step which you know about. There will be also a tRNA synthesis step. And tRNAs are synthesized by tRNA genes which are present on the genome. So, transcription of tRNA also has to take place continuously.

So, in order for you and I to be alive, all these processes we have been studying have to happen continuously. Not only continuously, they have to happen at great speed. And all the time you and I have to be, have to eat our breakfast, lunch and dinner. So, that nutrition goes into our stomach from there it is carried to every cell. Biochemistry is taking place in each cell. All these building blocks are being made and used for making DNA, RNA, protein, which are the major macromolecules, and of course, lipids and carbohydrates which will come to.

Student: Sir, if the process, rate of the process increases with the concentration of tRNAs does it not mean that like 20 of them are competing to get attached?

Professor: So, remember each 20 is a unique individual. And for each of those 20 there are thousands of molecules. So, for example, in the slide in front of you CAG anti-codon tRNA carrying an amino acid is a unique tRNA. But there will be thousands of these in the cell and thousands of CCCs, thousands of AGGs, in fact hundreds and thousands. They are all floating

around in the cell and they are continuously being charged and the tRNAs are continuously being synthesized.

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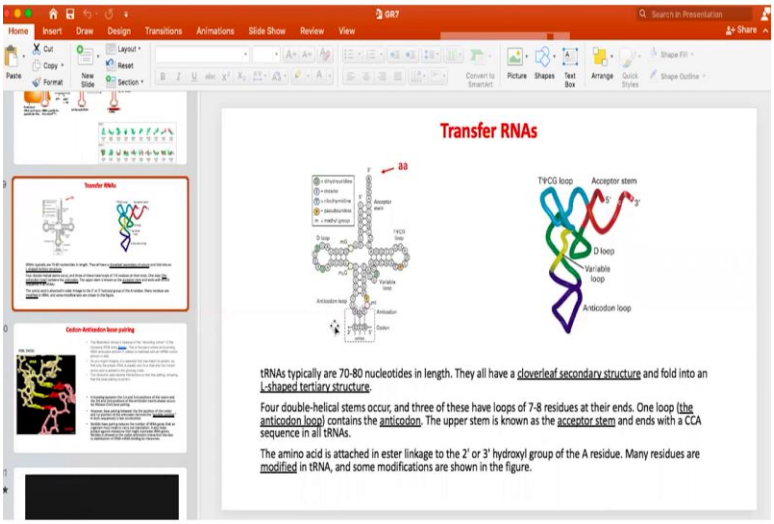

Student: The extra sequences around the cloverleaf of the tRNA, what all significance that hold or is it completely variable?

Professor: This one?

Student: Yes.

Professor: So, you are using the term extra because your logic is that if there is a shark and I am not pulling your leg, I am just being trying to make sure everybody understands your question. What you are saying is if there is a shark, only its teeth are important. So, these are not really extra. These make sure that the teeth of the shark are sharp and per pointed in the right direction. Do you get the point I am trying to make?

Student: Yeah.

Professor: So, they are very important in the folded state of this structure. And of course there is variability over here. And by the way, studying each one of this variability is a PhD problem, which is approximately five to six years. So, I would argue and my numbers will be off by an order of magnitude that about 3,000 to 5,000 students have worked only on the tRNA structure

and the meaning of the individual nucleotides and how much variability is there between, let us say, prokaryotes, invertebrates, vertebrates, in this cloverleaf structure.

So it is conserved, but conservation does not mean identity. There is variation and what is the importance of this variation in terms of function. And whether evolution has made things more efficient as the system becomes more and more complex? So, it is a very valid question and the identity of these amino acids, the conservation is also very, very important or non-conservation is also very important.

Student: Okay, thank you sir.

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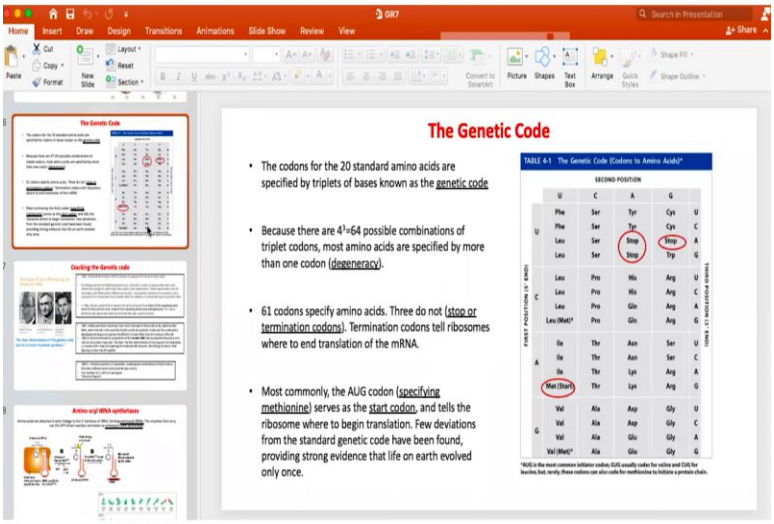

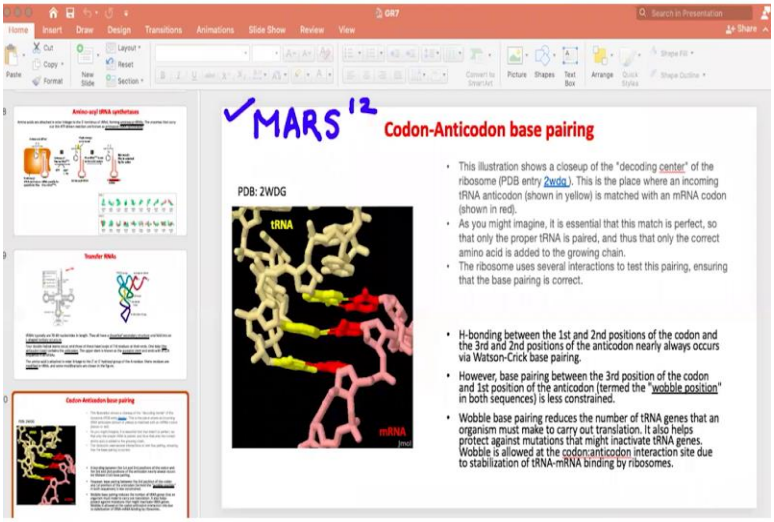

Student: Sir, can you please again talk about the wobble position, why third codon is called wobble?

Professor: So, this is what is happening. This is the reality in the genetic code. In the translation process for 20 amino acids, we could have done with 20 codons plus 1 stop and 1 start. So, you probably needed 22 codons minimal. But it turns out for whatever the reason evolution has chosen a three letter code. And if you use a three letter code, you now have 64 instead of 22 possibilities.

And what is true is that the first two positions are more important than the third position. And the third position has variability for most amino acids as I said proline for example has, you can have any one of the four nucleotides as the third position. And what I told you is that the level of recognition of the codon anti-codon loop the Watson-Crick base pairing obviously is not wobbling. It is a very well defined base pairing which is there in many, many structures.

But the ribosome itself seems to be willing to accept a lack of compatibility in the third codon as compared to the first two codons. And the reason it is so is that the part of the ribosome which is surrounding this recognition site which I am not showing you allow two codons which are not completely compatible, two match each other. Is that something which I am conveying clearly to you?

Student: Yes, sir. It is clear, thank you.

Student: What happens to the mRNA after translation?

Professor: It is usually degraded or it can be used again. Now, whether it is used again or degraded or not depends on the half life of the mRNA, which means how long can an mRNA survive inside the hostile, I am using the term deliberately, inside the hostile environment of the cytoplasm. And the stability of mRNA depends on its 5 prime and 3 prime leader sequences which are not coding. But mRNAs do not have a very long life per say. We are talking about a few minutes to a few hours max. So, they are recycled.

Student: Sir, where do these tRNAs get aminoacylated?

Professor: Where do they get aminoacylated?

Student: Yes, sir, in which part of the cell do they get amino.

Professor: In the active site of the aminoacyl-tRNA synthetases and these are all cytoplasmic enzymes.

Student: They are floating around?

Professor: They are all floating, well, we think they are floating around. Over the years just like the ribosome is a massive complex, it is like a big industrial park where everything happens. There may be industrial parks, compartments, macromolecular complexes where tRNA synthetases are together.

In fact, there is a known and very well conserved complex called as the MARS complex which is actually bigger than the ribosome. It is called the multi-acyl-tRNA synthetase complex. And it contains, if I remember right, 12 of the 20 tRNA synthetases. And we have no clue why 12 tRNA synthetases are hanging around together as compared to the other 8, but this complex does exists.

Student: And another thing, so there exists 61 different kinds of tRNAs right for every codon?

Professor: 20, no the third codon is wobbling. So, even the recognition by the tRNA allows for wobble. They are not 64, they are 20.

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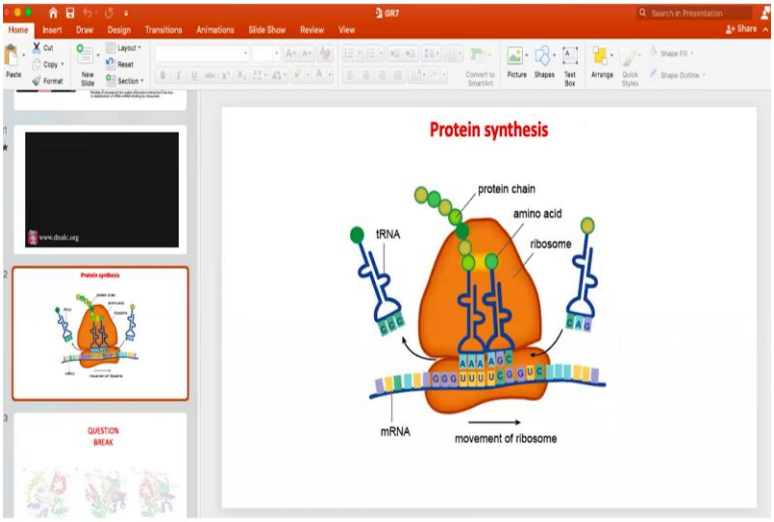

Professor: I want to end with a final question. There is a mistake in this slide. Can anybody point it out?

Student: Sir, the used tRNA on the left side, there is still an amino acid attached so that is wrong.

Professor: Very nice, yeah, so that is what I wanted you to point out. It is kind of obvious whoever made the schematic missed out on that, so which also tells me that you are grasping this whole process very clearly. Thank you everybody. See you in tomorrow's class.

Student: Sir, anti-codon would not be removed from the tRNA, why is it wrong? So, how that CCC thing is wrong here?

Professor: No, no, no, so what the student said is that there should not be an amino acid over here. So, it should leave its amino acid as it exists.

Student: Excuse me sir, is there any relation between degeneracy or the code and the wobble position?

Professor: Yes, yes, there is.