Introduction to Complex Biological Systems
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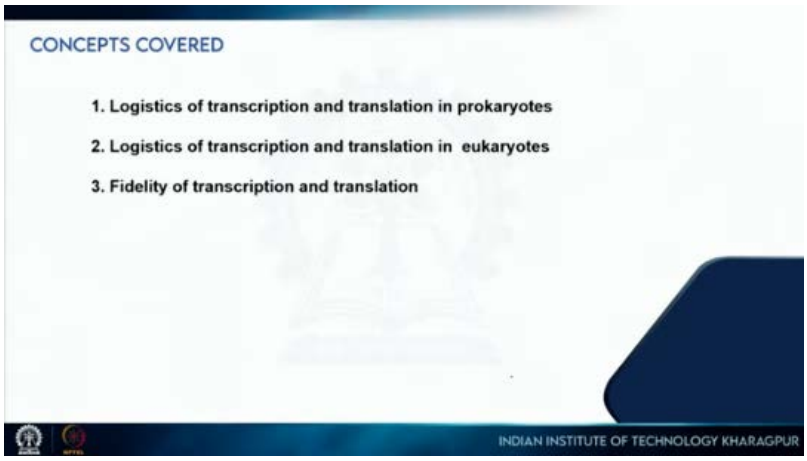
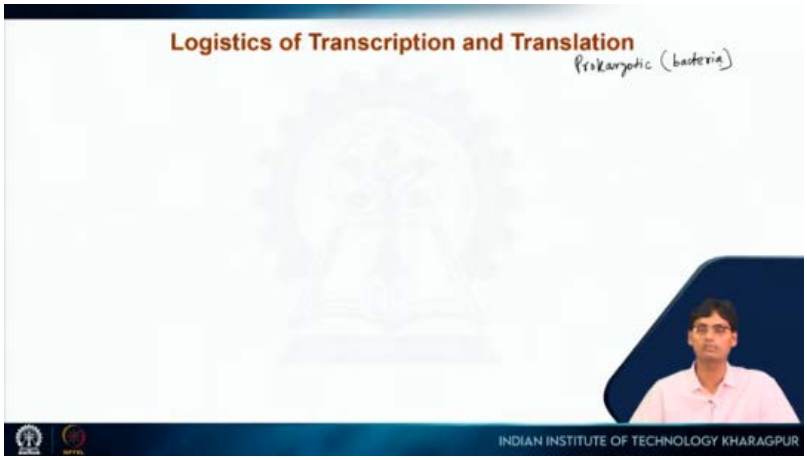
Lecture 9
Time-space correlation and fidelity of transcription and translation

Hello everyone, I am Dibyendu Samanta from IIT Kharagpur, and I am now discussing the fourth lecture of module 2. Today, I am going to discuss time-space correlation and the fidelity of transcription and translation. During the last lecture, I mostly discussed the basic mechanism of protein synthesis. So, during that time, I mentioned that there are much more complex things present there. So, today in this class, I will discuss some of those complexities, some of those fundamental understandings, but still, they are much more complex.



So, particularly, I will concentrate on the logistics of transcription and translation in both prokaryotes as well as eukaryotes, and also the fidelity of transcription and translation. So, those are very important steps in the central dogma. Now, first, if I start with the logistics of transcription as well as translation in the prokaryotic system. So, here I am going to discuss the prokaryotic system, such as in the case of bacteria. Most of the studies are actually conducted in E. coli because it is a beautiful model organism; non-pathogenic E. coli is the model organism in the lab.



So, now, as the name suggests, prokaryotic means they do not have a nucleus. Let us see where those steps are happening. So, if I draw, this is a bacterium. This is E. coli, Escherichia coli, E. coli Adactylia. So, bacteria, particularly E. coli and many other bacteria, have just one DNA molecule, and that DNA is also circular in nature. So, as a result of that, they have circular DNA.

So, if I say this, their DNA is double-stranded. So, better put this double-stranded, something you know, schematic diagram of double-stranded DNA. So, now, I am discussing transcription and translation. So, that is why I kept this portion a little bit in a different way.

So, I am going to show here that the transcription is happening. During the transcription class, I mentioned that transcription happens like this. So, this is the 5' end of mRNA, and this is the 3'. The direction is going from 5' to 3', and here, you can imagine that you have RNA polymerase, the enzyme responsible for transcription. Now, since bacteria do not have a nucleus,

They have just one compartment: the cytoplasm. So, DNA is present there, and transcription is happening. During the last lecture, I mentioned that mRNA is the template for translation. So, if I consider that this RNA, which is being transcribed now, is an mRNA, it can immediately start the translation process because the template is already available here. And the ribosomes are present in the cytoplasm.

So, you can see that ribosomes are present here in this bacterium. So, these are ribosomes. So, as a result, ribosomes can bind here to this already available mRNA, which is present in the cytosol. But this mRNA is still being synthesized; transcription is still going on. So, what I am trying to say is that, in the case of bacteria, in the case of prokaryotes, transcription And translation coupled processes happen together. So, transcription and translation are coupled together because all the necessary ingredients are available in the cytosol of bacteria. So, even before the completion of transcription, the mRNA can still be translated by the ribosome. So, as a result, the ribosomes are going like this.

I mentioned particularly here that mRNA synthesis, or transcription, is a process that means one RNA polymerase is synthesizing the whole gene and the whole mRNA is being synthesized. Now, that mRNA can be translated by multiple ribosomes. So that is kind of amplifying the signal also. Amplifying the signal means from one mRNA, we can have multiple proteins. So as a result of that, if I just draw here, this is one mRNA.

This is just one mRNA, 5' to 3'. So, the ribosome started translating this mRNA here. And after some time, this ribosome will be here. And this protein is being synthesized here. So, this is our ribosome, which started translation a little bit before this ribosome.

So, here the protein is very small, and here the protein is very big now because it is already synthesizing a protein, kind of a little bit longer than this. So, this is also another ribosome. But what I am trying to say is this mRNA, which I drew here, is mRNA, but this mRNA is still being synthesized in E. coli. So, why I am saying this is that there should be some kind of relation between the speed of transcription and translation. So, for example, if the translation is so fast here, the process of translation, that means how ribosomes are reading the genetic code or the code present in mRNA, if that is much faster, then this ribosome will hit here.

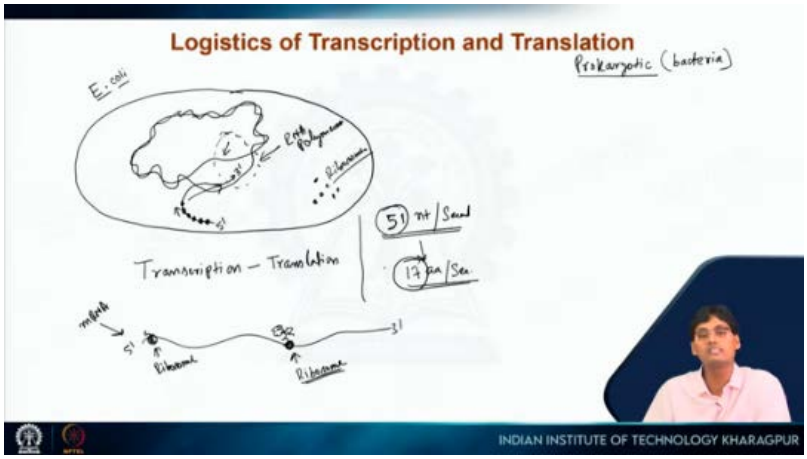
In the transcription complex. So, it will create a problem. So, that is why I am saying that there is some kind of relation in their speed also, so that everything is happening optimally in our living system. So what I mean to say here is that in the case of bacteria in favorable conditions, in optimal conditions, for example, in E. coli, the rate of transcription is, I

would say, 51. This is not so specific; I am just taking this number. So, it will be easy to explain.

So, 51 nucleotides per second. It is kind of varying from I would say, from 40 to 70, 80 nucleotides per second in E. coli, depending on the situation. So, if this is the rate of transcription, then what should be the rate of protein synthesis? Scientists observe that the rate of protein synthesis in bacterial systems is approximately 15 to 20 amino acids per second.

So here, for example, 17 amino acids per second. This is not so specific, but for explanation, I am saying 17 amino acids per second. Then why did I put 17 amino acids per second? Because I already mentioned in the last lecture that 3 consecutive nucleotides that is called a codon, encode 1 amino acid. So, as a result of that, if this is the space, this is the speed, and then this ribosome will never hit this transcription complex.

So, everything will be happening smoothly. So, that is why here I am saying that 51 nucleotides per second is the rate of transcription, and the rate of translation in that case is 17 amino acids per second. So, this way, transcription and translation are coupled processes in bacterial systems. So, now we should look into the eukaryotic system in our body, in human cells, what is happening. So, this is now I will explain in the eukaryotic system.



So, it can be a plant, it can be an animal, or it can be a human cell; that does not matter. Eukaryotic cell, eukaryote means they have a true nucleus; they have a nucleus. Let us see what those steps are happening. So, DNA is present where in eukaryotic cells it is present

inside the nucleus, not in the cytosol. So, as a result of that if I draw a very big eukaryotic cell.

So, it will help me to explain the stuff. So, this is a eukaryotic cell. A eukaryotic cell, this one, and it has a nucleus also. The nucleus is a different compartment. This is separated from the cytoplasm. This is cytosol. So, the nucleus is present inside the cell, but it is a different compartment. The nucleus harbors the DNA or deoxyribonucleic acid. So, DNA is present here in the nucleus. It is linear DNA in the eukaryotic system, and in the case of humans, different species have different numbers of DNA molecules present in each cell.

Now, after transcription, we will get RNA. It can be mRNA, ribosomal RNA, transfer RNA, everything. So, I am just mentioning mRNA here. I mentioned during the transcription class that this mRNA needs to be processed. That means the addition of the 7Mg cap at the 5' end, so here, the 7Mg cap and here, at the 3' end, a poly-A tail, and also splicing, the removal of introns. All those steps are happening inside the nucleus, processing is happening, processing of pre-mRNA, this is called pre-mRNA. So, processing of mRNA is happening, and finally, we have mature mRNA. with a 7Mg cap, poly-A tail, and introns are already removed.

So, those mRNAs will come out from the nucleus. So, the nucleus, although I told you it is a different compartment. But if you see, they have some, you know, pores there called nuclear pores. So, they have some pores here. So, things can go inside and outside. So, as a result of that, this mRNA will come out through these pores outside, outside means the cytoplasm.

This is now mRNA coming into the cytoplasm, mRNA export from the nucleus to the cytoplasm. Why does it need to come? It is because the ribosome is actually waiting in the cytoplasm. Ribosomes are present in the cytosol. So, here ribosomes are present.

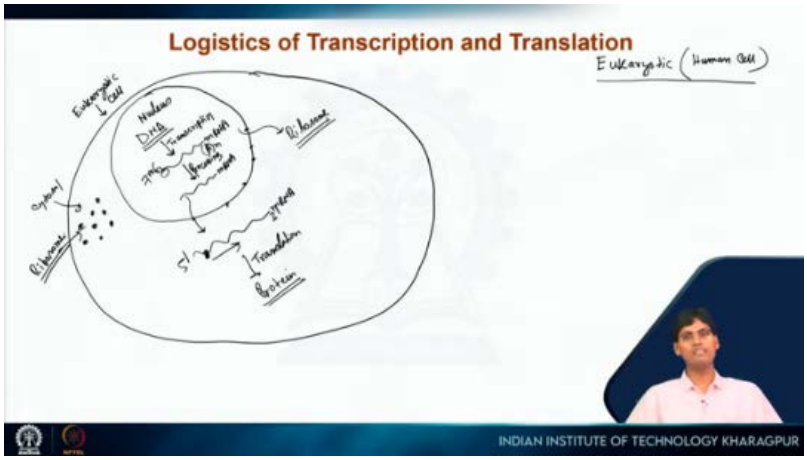
So, these are our ribosomes. So, now these ribosomes, whatever I told that 30S, 50S in case of bacteria, and 40S and 60S. So, those are different, you know, size particles based on their centrifugal force, like they can be separated differently, right? Those are called Svedberg units based on the scientist's name. So, those subunits are available in this cytoplasm freely; they are not together.

Only during the translation process, they get united, like 30S and 50S, they get united and they carry out translation in bacteria. Similarly, 40S and 60S, they get united to make the ribosome and that translates protein inside eukaryotic cells. Now, this ribosome will start translation from the 5' to 3' direction. I am not showing here the cap and the poly-A tail, but that should be present here. So, this way translation should be happening in the cytosol.

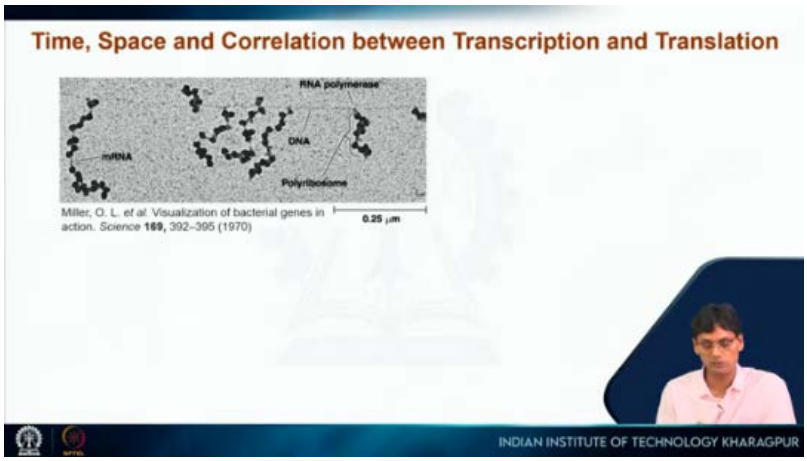
Here I would like to mention one thing that translation is happening inside, or I would say, in the cytoplasm, right? So, that is why after translation, you will get a protein molecule in the cytoplasm. Now, the replication, transcription, everything is happening inside the nucleus, and that requires enzymes DNA polymerase, RNA polymerase. So, that means we have a very complex system so that the required enzymes, which are actually required, Inside the nucleus after translation, will be transported to the nucleus again.

For example, RNA polymerase is synthesized in the cytoplasm, then it will be transported into the nucleus so that it can transcribe RNA molecules, right? So, that is why I mentioned that it is a very complex scenario. Similarly, another level of complexity is like even the ribosomal RNA, which constitutes the ribosome along with many different proteins. Ribosomal RNA will also be synthesized inside the nucleus because it is the result of transcription.

So, because of that, it is very interesting to know that ribosomes are assembled and generated inside the nucleus itself. So, for example, the ribosome particles, in this case, the 40S and 60S particles of the ribosome in the eukaryotic system, they get assembled inside the nucleus and then those ribosome particles are exported to the cytoplasm, where they perform translation. So, if you see all those things, they are actually really complex, and everything is happening in a coordinated fashion so that life moves very smoothly. But all those things are because of many, many years of evolution, and it makes a very robust system. So, now I would like to show you something else here, the time, space, and correlation between transcription and translation. I already mentioned a little bit of this when I was talking about the presence and absence of a nucleus in prokaryotes and eukaryotes.



So, here is one important and real diagram that I have taken from this paper. So, this is very interesting to understand the complexity that I just mentioned, but this is the experiment they are showing. So, in this figure, if we concentrate you will see a very light, thread-like structure in this figure. I am just drawing over it



So that it will be easy for everyone to see. So, for example, here you have a very thin line going like this and like this. So, this thin line is nothing but what is this? This is DNA. This is the DNA. I will explain everything mentioned here. Right, this is DNA. Now, in this DNA, you can see some small, not very prominent features here, here, here, and here.

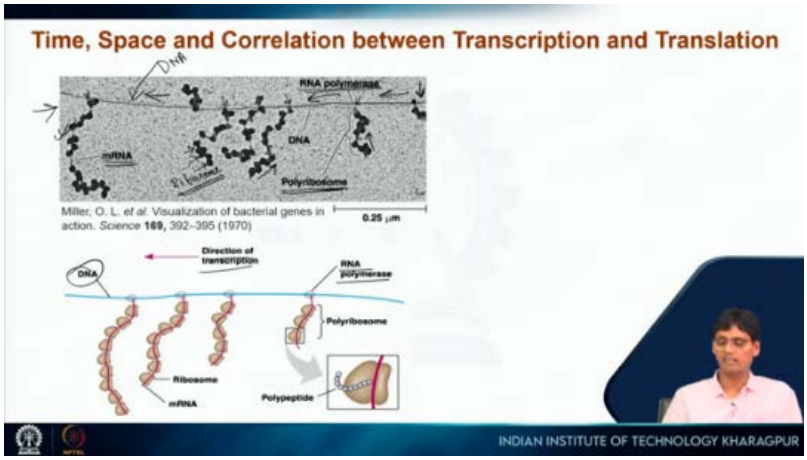
So, those are RNA polymerases. This figure is an electron micrograph, a very high-resolution image of a translation event in a bacterial system, in a prokaryotic system, right? So, this is RNA polymerase. So, RNA polymerase is actually transcribing RNA. It is transcribing RNA; it is making the RNA. DNA is the template in this case. Now, then, what are those particles? So, this particle, these are nothing but ribosomes. They are

ribosomes. Ribosomes, and these ribosomes together—like many ribosomes—are together. They are attached to a single mRNA, as I mentioned during the explanation of coupled transcription and translation in a bacterial system.

So, these many ribosomes attached to the same mRNA are called polyribosomes. So, multiple ribosomes are together; they are translating the same mRNA. This is a polyribosome. So, now the question is, you can see it is the same DNA, a single DNA molecule, right? And we have, like, we can show here multiple RNA polymerases. So, this RNA polymerase, for example, here after some time it will transcribe, transcribe, transcribe, and it will go in this direction, and it will reach here. Then the length of mRNA will be more, and more ribosomes will be attached here.

So, this is, you know, this right side here, from this position, from this side, transcription started. Why? Because you can see the length of mRNA is small here; that is why there are fewer ribosomes here. When this is going in this direction, then the length of mRNA will increase, as you can see here. The length of mRNA is more here, and more ribosomes are attached to this mRNA. So, as a result of that, from this figure, we can say that transcription is happening in this direction. Then, what is the direction of translation? So, the translation direction should be here. Ribosomes are going like this; ribosomes are reading the mRNA in this direction.

So, because of this experiment, because of this data only, we were able to understand this coupled transcription and translation, which I already explained. But this is the actual experiment from where we came to know all of these things. Now, the same thing, this is a reconstituted image for all of us. So, we can, like, if our eye is not very specialized to see this kind of electron micrograph, this is much easier here. So, as you can see, this is our DNA, and this is RNA polymerase, and the direction of transcription, whatever I already explained, And then you can see that, like, here the length of this mRNA is not that big yet, but here the mRNA is much bigger, and so many ribosomes are attached together, and coupled transcription and translation are happening in this way.



But now if I discuss a similar thing in eukaryotes. In eukaryotes, this coupled transcription and translation cannot happen because, as you know, transcription occurs inside the nucleus and translation occurs outside the nucleus. So, this is not a coupled process. So, as a result of that, if I draw this, it is an mRNA.

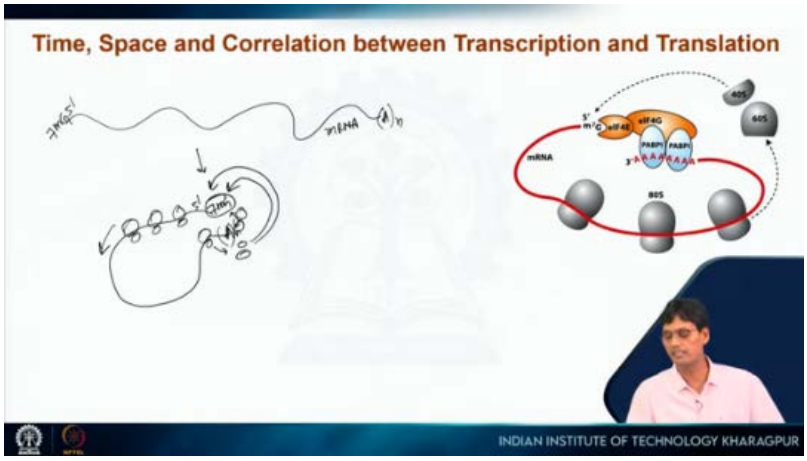
So, this is our 7 mg cap, and here we have a poly-A tail, and this is mRNA of eukaryotes. So, when it is in the cytoplasm, then it will be translated, but to increase the efficiency, we have a different kind of strategy. So, in the case of bacteria, transcription and translation are coupled processes, even before the synthesis of the full mRNA, translation starts. So, the translation rate and transcription rate are kind of matching in such a way. So, that it is a very efficient process, but in this case,

if you see this mRNA, many times they can make some kind of loop-like structure like this. So, here you have a 7 Mg cap at the 5' end, this is our 5' end, and here we have a poly-A tail at the 3' end. How they form this loop-like structure is because some proteins are there. So, specific proteins are there; somehow they interact with each other here, some protein molecules here, like poly-A binding protein, like this is the poly-A tail. So, poly-A binding protein and some eukaryotic initiation factor.

So, they will bind together here, and that way they will make this kind of circle, but what is the advantage of this? The advantage is that when ribosomes are translating this mRNA, they are going in this direction, right? And what will happen when they are done with the translation process? When they are here, then they will be separated. I already told that ribosomes during translation unite in this manner, but otherwise, those two subunits remain

separated. So, here, when the translation is done, that means when they encounter the stop codon, then the translation is done. So, as a result of that, during that time, those ribosomes will split into free ribosome particles, but now the 5' end is very close by.

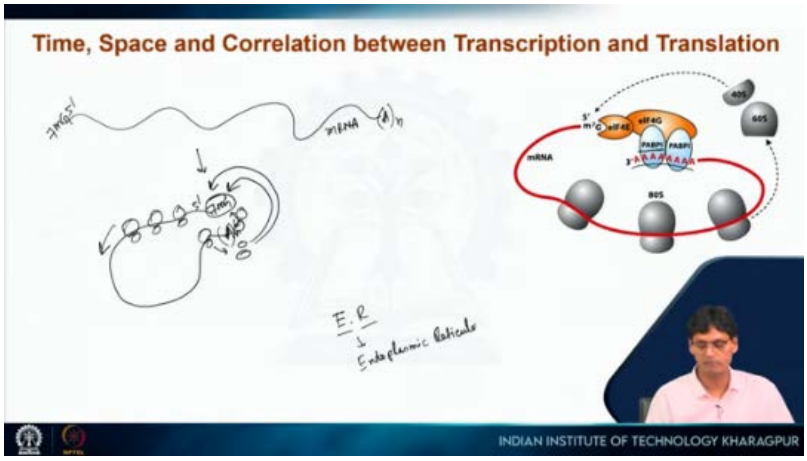
So, again they will start so that way the efficiency and effectiveness of translation is much more. So, the ribosome concentration, the particles of this 40S and 60S subunit, will be more in this region because they are getting dissociated at the 3' end and again they will quickly bind to the 7mg cap and that way it will start the translation. So, that is why it is a very, very effective process. So, here you can see that whatever I explained is the same thing here. This is the poly-A tail and this is the poly-A binding protein which is binding to eukaryotic initiation factors like 4G and 4E. Those are kind of names you do not need to remember, but that way it makes some kind of closed loop and that will increase the efficiency of translation.



But now, while I was discussing that, I told that when it encounters the stop codon, then the translation will stop. During the translation lecture, I forgot to mention that, but how will it stop? Very simply, we can imagine that whatever those three stop codons I mentioned, like UAA, UAG, and UGA. Maybe the matching tRNA is not there. The matching tRNA means the anticodon loop with the matching sequence is not present in our system. So, as a result of that, no tRNA is available. They cannot bring any specific amino acid, and during that time, a specialized protein called a release factor comes into play and it terminates the protein synthesis. That is all. Now, here are some more interesting complexities. I am not going into all the details of that, but just to highlight here, during

translation, another interesting question is that which one is moving? Is the mRNA moving or is the ribosome actually reading every three nucleotides, that means codons, one after one? Which is true? Maybe both are right because as you know, ribosomes are present in the eukaryotic system. They can be present freely in the cytosol or the cytoplasm in the eukaryotic system.

Similarly, inside the eukaryotic cell, there are so many organelles. One of those is named ER. ER stands for endoplasmic reticulum, right? Endoplasmic reticulum. This is a kind of sac-like structure present in the eukaryotic system. So, on the surface of the endoplasmic reticulum, many ribosomes are attached together and specially they synthesize proteins which will be exported from the cell, which will come out from the cell. There are many proteins that should be secreted outside the cell, so they are being synthesized by ribosomes which are bound to the endoplasmic reticulum. Then, which one is moving, mRNA or the ribosome? So, those are kind of complex and challenging questions, but anyway.



So, now, this is the last slide of this presentation. So, here I am trying to explain the time-space correlation between transcription and translation from an evolutionary perspective, not just inside one cell, like whether it is a eukaryote or a prokaryote, whatever I mention. From an evolutionary perspective, I want to say. Since I already mentioned that translation is a very fundamental process to make all proteins in our body, but it is being catalyzed by RNA. And at the same time, RNA can act as genetic material also.

So, scientists presumed that initially when life formed at the very, very beginning, maybe we had an RNA-based system where RNA could go into RNA. So, it can act as our genetic material as well as catalyze reactions, and over time, the evolution of RNAs could direct protein synthesis, right? So, as you can see, finally, RNA is making protein. So, this is something that will not happen in just a year or two. This is evolution over thousands and thousands of years, leading to this kind of complex system.

And present-day cells, what we see today, are the evolution of new enzymes that synthesize DNA and make RNA copies from it. Replication, transcription, translation—everything is happening properly. But now, if you see, as I already mentioned, the main catalysis is done by RNA, not by any protein enzyme. So, this is a very interesting aspect. And I want to mention here additionally that, as I mentioned, bacteria do not have a nucleus, and eukaryotic cells have a nucleus, and transcription and translation happen there.

Inside eukaryotic cells, some organelles, for example, mitochondria, are present. Inside mitochondria, they have their own small DNA, a small genome, which is very surprising, right? Other organelles, like chloroplasts in plants and mitochondria in plants and animals, have their own DNA molecules that get transcribed to make important RNA, which also makes some proteins. And this is the idea that maybe at the very beginning, during evolution, some eukaryotic cells or bigger cells actually consumed those bacteria, and they are still maintained inside the eukaryotic cell. And they still have their protein-synthesizing machinery, their transcription machinery, but they also rely on cytoplasmic proteins that can go from the cytoplasm to mitochondria and chloroplasts. Still, they maintain this

situation where inside mitochondria and chloroplasts, transcription and translation are happening, and they are making very important products.

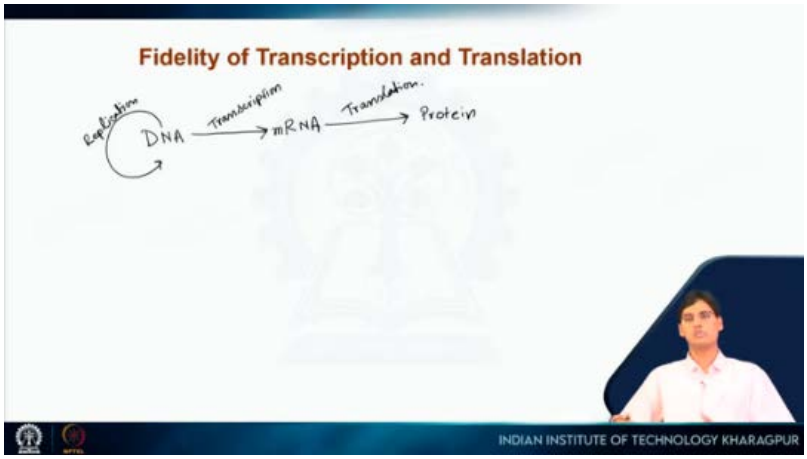
All these things are very complex, but if you are interested, you can refer to some textbooks, and you will get all these ideas. So, this is called the endosymbiont theory. Whatever I just mentioned, bacteria went inside eukaryotic cells and are still present, doing very important work for us in present-day cells. Now, I am going to discuss the fidelity of transcription and translation. So, if we see all these three steps replication, transcription, and translation you will see that during replication, we have a rigorous propagating machinery, which means DNA to DNA, the steps we call replication. So, during replication, the probability activity is very stringent because DNA is a kind of archival copy.

So, whatever information is present there, we have to maintain that information in a proper way. Like any mistake will only lead to a wrong mRNA followed by a wrong protein. So, as a result of that, although during replication some minor mistakes may happen, we have very good machinery to correct those mistakes. So, there are a lot of different types of machinery available inside the cell which will rectify the mistakes. But in the next step, when we are getting RNA, so particularly I am mentioning messenger RNA through the process of transcription. So, during this stage, also, although some level of proofreading activity is there, some kind of correction activity is there, I would say it is not comparable to replication. Here, some minor mistakes may happen, but that is not a big problem because, anyway, from one gene we are getting multiple mRNA molecules.

So, now if one of them contains some kind of mistake, then we have very good machinery. So, that mRNA can be destroyed soon also. On the other hand, if we say from mRNA we are getting finally protein, right. So, this is translation. If because of some mutation, some wrong nucleotide is present in mRNA, that means, something, some problem in some codon.

So, that will code some different amino acid in protein. And because of that amino acid, if the protein structure is not proper, if it is not attaining its native conformation, the protein will also be destroyed. So, as a result of that, what I am trying to say is that DNA is our

archival copy. So, where we have to keep our information safe, and then mRNA is just like a sticky note. For example, in every lecture, I am kind of recommending one textbook, for example, Lehninger's Principles of Biochemistry.



Now, if something is wrong in the textbook itself, then all students who are actually following this textbook will learn something wrong. So, as a result of that, the textbook Lehninger's Principles of Biochemistry should be very much in the correct form, right? So, similarly, the information in DNA should be very much in its correct form. And now, when you are actually writing something from this textbook on a sticky note or on a piece of paper, When you are writing, maybe by mistake, you wrote something wrong.

Nothing will happen. You will just, you know, destroy that piece of paper and again you will write. Right. So that way, I would like to say that transcription and translation, although we have some kind of machinery to take care of it, it is not that essential that you have to make it exactly, you know, just like DNA, how it is getting probed. So, that is all about the fidelity of transcription and translation, which I just discussed, and that is all.

These are the textbooks you can follow and thank you very much.

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