Introduction to Cell Biology
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Central Dogma: Translation - Part 2

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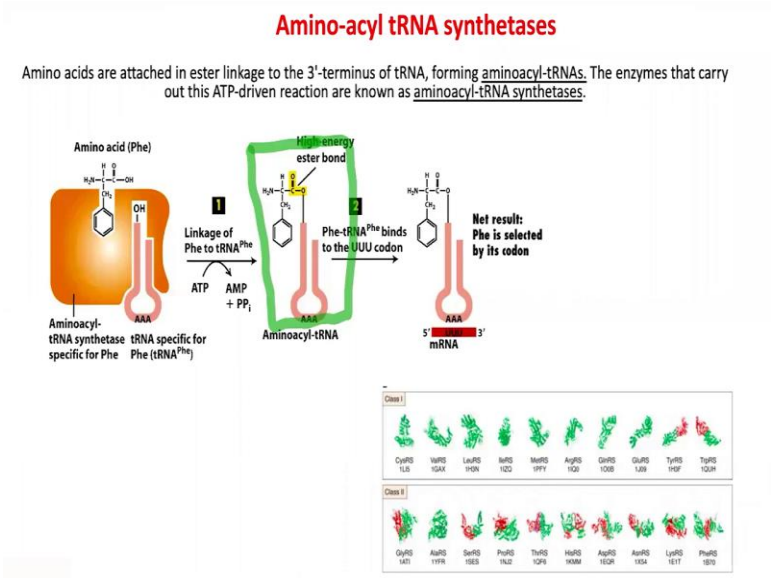

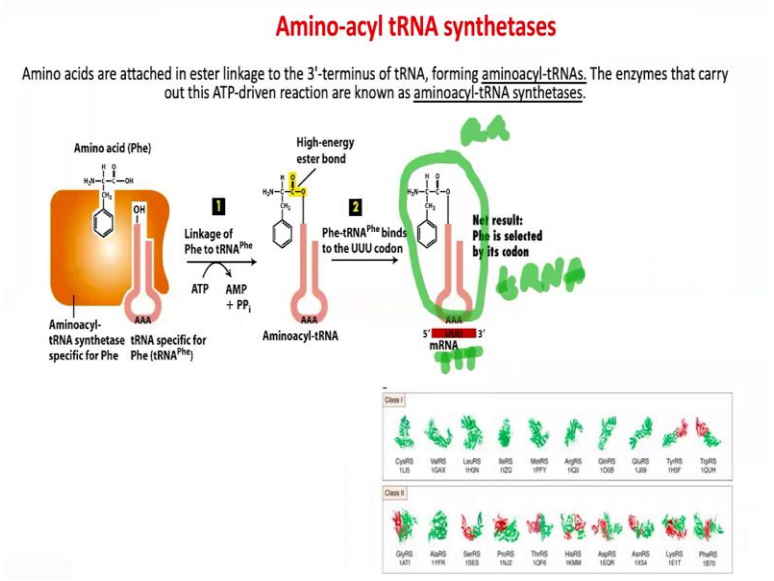

So, we have looked at the genetic code, we have looked at the ribosome. Now, the next question is, what is transfer RNA? Now, there is one transfer RNA for each amino acid and shown in, not in a very big zoom over here, and each transfer RNA has the ability to charge an amino acid and

what charging means is shown over here. Basically, this is a tRNA and a tRNA can be hooked up to an amino acid shown over here through a high energy ester bond.

So, for each amino acid there will be a tRNA which can specifically charge to that amino acid with a relationship with the anti-codon shown over here. And for example, for, this should be tyrosine, and for tyrosine the code is AAA. Now, there is an enzyme called as an aminoacyl tRNA synthetase which is a protein which works to take the amino acid phenylalanine, not tyrosine, and hook it up to partner tRNA.

So, the charging function, the chemical reaction between the amino acid to the tRNA is done by aminoacyl-tRNA synthetase. There is one aminoacyl-tRNA synthetase for each amino acid. There is one tRNA for each amino acid. Aminoacyl tRNA synthetase hooked these two up together which is the charging step.

And once these are hooked up together, the tRNA moves to the ribosome and on the mRNA with the help of the ribosome. It basically looks for the codon. And on one side of the tRNA is the anti-codon. And once the codon, anti-codon compatibility is met the amino acid is then presented to the ribosome for ligating to the next amino acid. Is this clear? Are there questions from this slide?

Student: Sir, can you please explain this again? I could not understand.

Professor: So, the goal is that in protein synthesis you need the transfer RNA to take an amino acid to the ribosome, there are 20 transfer RNAs, each transfer RNA is specific for one amino acid. And shown over here is the chemical complex of a tRNA over here and a amino acid over here which is tyrosine and they are connected by a high energy ester bond.

Now, this tRNA has on one side the amino acid on the top and at the bottom it has three nucleotides which are there for recognizing the complementary nucleotides on the mRNA. So, remember, on mRNA you do not have TTT, you have UUU. So, this AAA instead of recognizing TTT recognizes UUU on the mRNA.

Now, since there are 20 transfer RNAs and there are 20 amino acids, there is a pairing. You can only have the same transfer RNA with that anti-codon triplet nucleotide for each amino acid. Now, the conjugation of these two is carried out by an enzyme called the aminoacyl-tRNA

synthetase. And again, rather than having a single aminoacyl-tRNA synthetase, there are 20 aminoacyl-tRNA synthetases which do this function. So, 20 enzymes pair one amino acid to one tRNA. And once this complex is formed, it moves to the ribosome.

So, let us not continue here. I will go ahead, show you the whole process again. And let us see if you still have questions. Let me just go ahead and let us see if you still have questions. I will show you the entire process.

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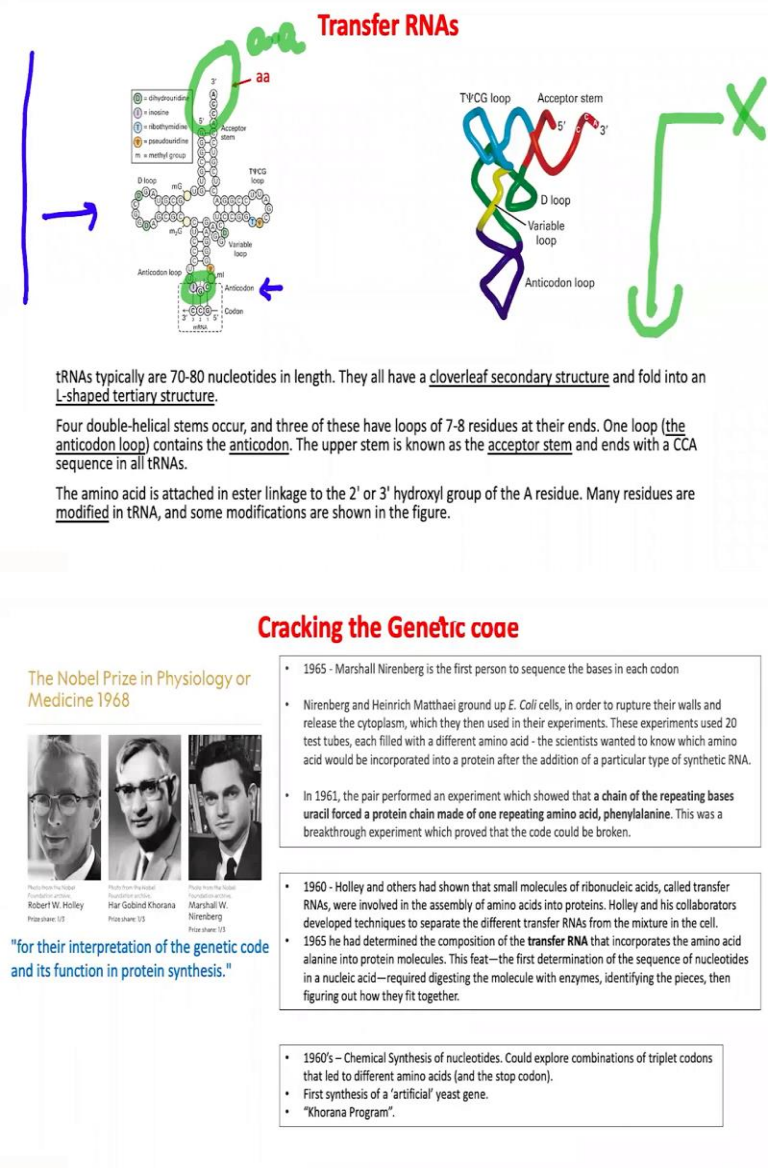
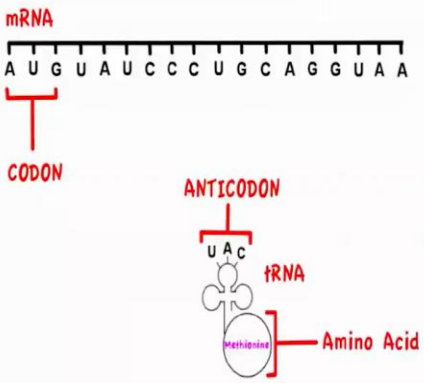


Translation -

2.7 Understanding: Translation is the synthesis of polypeptides on ribosomes

Translation -



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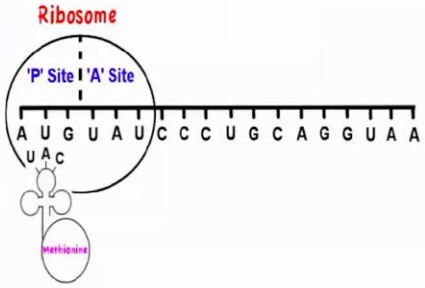
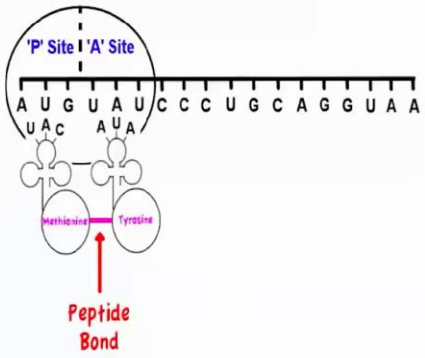
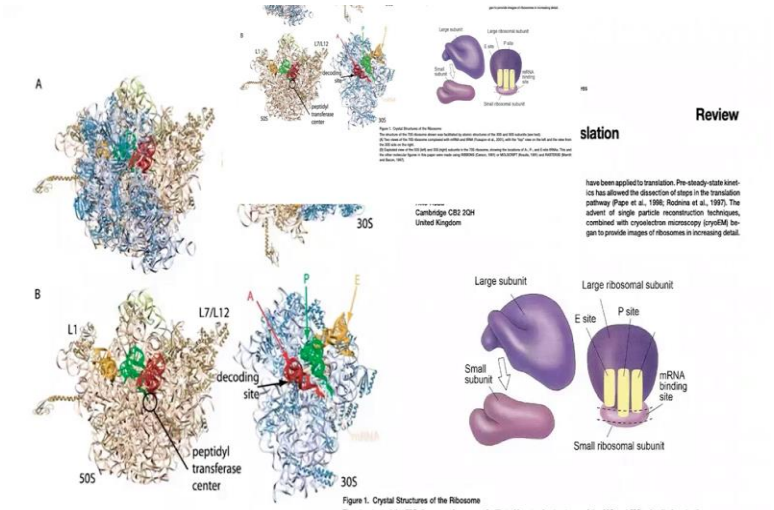


Translation -





Review

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have been applied to translation. Pre-steady-state kinetics has allowed the dissection of steps in the translation pathway (Pape et al., 1998; Rodnina et al., 1997). The advent of single particle reconstruction techniques, combined with cryoelectron microscopy (cryoEM) began to provide images of ribosomes in increasing detail.

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Figure 1. Crystal Structures of the Ribosome

The structure of the 70S ribosome shown was facilitated by atomic structures of the 30S and 50S subunits (see text).
(A) Two views of the 70S ribosome complexed with mRNA and tRNA (Yusupov et al., 2001), with the "top" view on the left and the view from the 30S side on the right.
(B) Exploded view of the 50S (left) and 30S (right) subunits in the 70S ribosome, showing the locations of A-, P-, and E-site tRNAs. This and the other molecular figures in this paper were made using RIBBONS (Carson, 1991) or MOLSCRIPT (Kraulis, 1991) and RASTER3D (Merritt and Bacon, 1997).

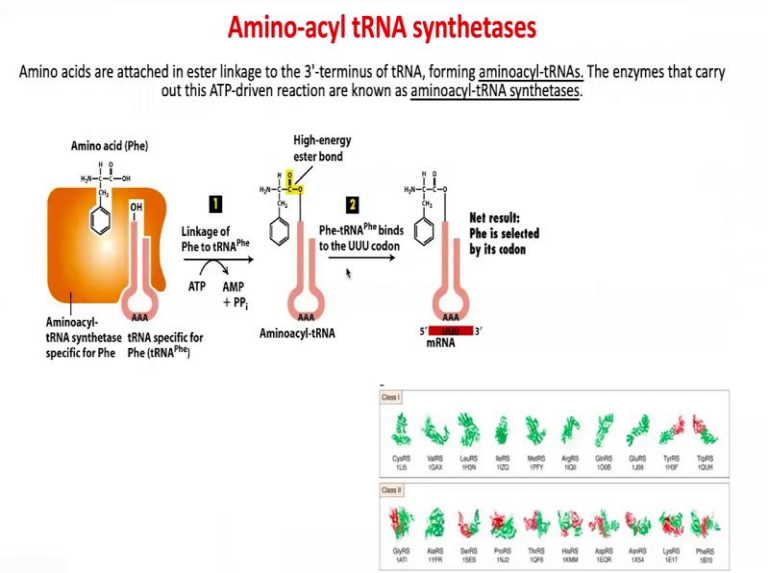
The Genetic Code

- The codons for the 20 standard amino acids are specified by triplets of bases known as the genetic code
- Because there are 4^3=64 possible combinations of triplet codons, most amino acids are specified by more than one codon (degeneracy).
- 61 codons specify amino acids. Three do not (stop or termination codons). Termination codons tell ribosomes where to end translation of the mRNA.
- Most commonly, the AUG codon (specifying methionine) serves as the start codon, and tells the ribosome where to begin translation. Few deviations from the standard genetic code have been found, providing strong evidence that life on earth evolved only once.

TABLE 4-1 The Genetic Code (Codons to Amino Acids)*

FIRST POSITION (5′ END)	SECOND POSITION U	SECOND POSITION C	SECOND POSITION A	SECOND POSITION G	THIRD POSITION (3′ END)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu (Met)*	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met (Start)	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val (Met)*	Ala	Glu	Gly	G

*AUG is the most common initiator codon; GUG usually codes for valine and CUG for leucine, but, rarely, these codons can also code for methionine to initiate a protein chain.



Now, this is what a transfer RNA looks like. A transfer RNA, remember, Holley did a lot of work in terms of defining what a transfer RNA is. And he and his group actually identified all the transfer RNAs in the cell. But the transfer RNA is a piece of RNA, we are calling it tRNA, which is approximately 70 to 80 nucleotides in length, and it folds into a very classic cloverleaf structure. And this was also major discovery.

One end of the transfer RNA has the anti-codon three bases, which will recognize the codon on the mRNA. And this, as you can see, is in the middle of the RNA strand. So this is basically a straight mRNA strand, which folds into a cloverleaf structure. This is where the three bases are which are going to basically be form the anti-codon.

And here at the 3 prime or the 5 prime end is where the amino acid is going to be attached. And the rest of the structure is important for stability and for recognition. And I will not go into what it recognizes at this point. And this is a sort of a schematic which is an L shaped, an inverse L shape of the transfer RNA with the amino acid being over here and the anti-codon loop being over here.

So, let us go over this whole process again. I will show you the movie again. Translation is the second stage of protein synthesis where a piece of mRNA is used to create the polypeptide chain. Firstly, let us go over 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.

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. 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 associated with this 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 in 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 site is no longer really required, because its amino acid is attached to the amino acid on the second tRNA by the covalent peptide bond.

The ribosomes then moves along the mRNA by one codon. This place is the second tRNA in the P site and the first tRNA is then freed 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.

So, hopefully with this movie which is a simplistic explanation of the core process of ligation of different amino acids, the definition of what the ribosome is of having two subunits with the mRNA moving in between the two subunits in one direction, the fact that there are defined sites in this large structure where events are going on and the fact that amino acids are brought in by the tRNA anti-codon end of the tRNA recognizes the codon on the mRNA that amino acid is then ligated to the next amino acid and this whole process happens in sequence.

The fact that you understand what the relationship between the codes and amino acids is which is the genetic code, the fact that you understand what tRNAs are which is shown over here, and there are 20 tRNAs each charge two amino acid and this charging step, this connective, chemical connectivity is done by an enzyme called aminoacyl-tRNA synthetase, again one for every amino acid, and you have a broad idea of what the tRNA structure is which is a cloverleaf structure. And remember there is a tRNA with the anti-codon one each for each amino acid. So there are 20 of them and they all function together with the ribosome to make protein.

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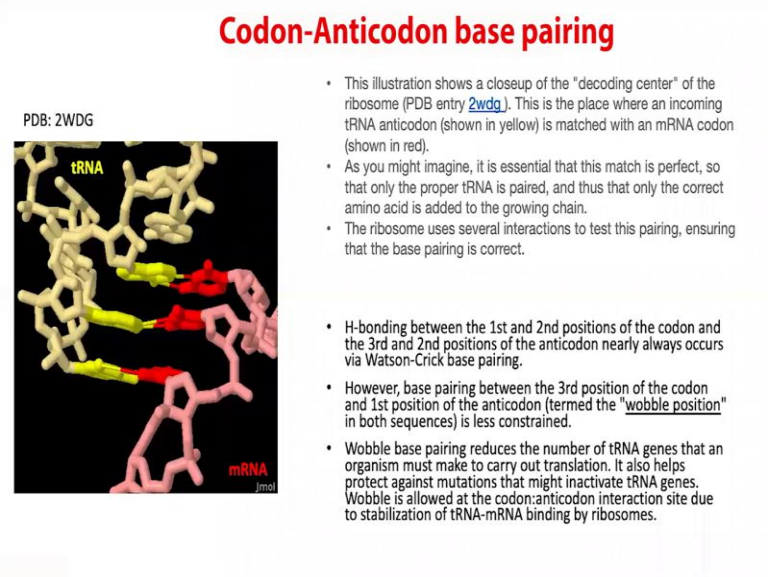

Let me now sort of end this part by showing you a higher resolution structure from the protein databank. There is the mRNA on the right, there is the tRNA on the left, and the tRNA will have these three anti-codons and the mRNA will have the codons, and you can see Watson-Crick hydrogen bonding between the codon and the anti-codon. This zoomed in structure is very important, because if this recognition works, then the tRNA is carrying an amino acid, which is related to the codon on the mRNA. And this amino acid will then be ligated to the next amino acid based on the code on the mRNA.

One additional point I would like to leave you with is that the third base, as we know, has a wobble. It is flexible. And you will ask how is it that all three are Watson-Crick base pairs, all three are nucleotides, how is it that the third has a bit of a wobble, and the first two do not. The first two, you cannot have any flexibility. The reason is very, pretty much to do with the ribosomal structure.

Remember this whole recognition is surrounded by the ribosome. And the ribosome sort of tweaks the third base for the codon anti-codon recognition site, allowing more flexibility. And this is part of the biological process. And we do not really understand why this is true only for the third codon.

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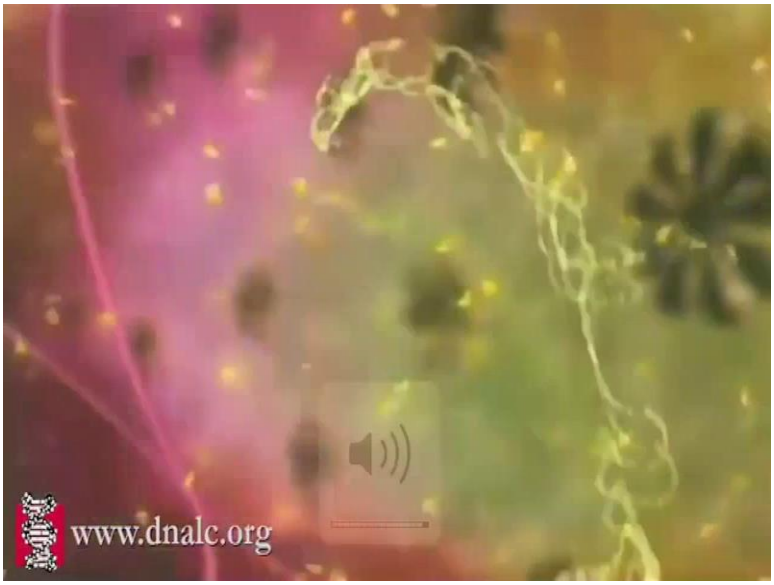


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So, I will now show you a final movie and then I will end and take questions. The job of this mRNA is to carry the genes message from the DNA out of the nucleus to arrive a ribosome for production of the particular protein that this gene codes for. There can be several million ribosomes in a typical eukaryotic cell. These complex catalytic machines use the mRNA copy of the genetic.

So, again, very obvious, you can see that the either the mRNA is moving or the ribosome is moving, probably the mRNA, because the ribosome is really a huge machine. And sometimes it is sitting on the endoplasmic reticulum. You can see like a ticker tape, the mRNA, which is very huge is moving through the ribosome. And there is obviously a large tunnel in the ribosome through which it is moving. And at the bottom is the small subunit, at the top is the large subunit.

And you can see coming out in red the amino acids which have been ligated together based on the unique sequence which this particular mRNA carries. And the unique sequence which this mRNA carries is because of the unique sequence on the DNA from which this mRNA was copied.

These complex catalytic machines use the mRNA copy of the genetic information to assemble amino acid building blocks into the three dimensional proteins that are essential for life. Let us see how it works. The ribosome is composed of one large and one small subunit that assembled around the messenger RNA, which then passes through the ribosome like a computer tape.

The amino acid building blocks that is the small glowing red molecules, are carried into the ribosome attached to specific transfer RNAs. That is the larger green molecules also referred to as tRNA. The small subunit of the ribosome positions the mRNA so that it can be read in groups of three letters known as a codon.

Each codon on the mRNA matches a corresponding anti-codon on the base of a transfer RNA molecule. The larger subunit of the ribosome removes each amino acid and joins it onto the growing protein chain. As the mRNA is ratcheted through the ribosome, the mRNA sequence is translated into an amino acid sequence. There are three locations inside the ribosome designated the A site, the P site and the E site.

The addition of each amino acid is a three step cycle. First, the tRNA enters the ribosome at the A site and is tested for a codon anti-codon match with the mRNA. Next, provided there is a correct match, the tRNA is shifted to the P site, and the amino acid it carries is added to the end of the amino acid chain. The mRNA is also ratcheted on three nucleotides or one codon.

Thirdly, the spent tRNA is moved to the E site and then ejected from the ribosome to be recycled. As the protein synthesis proceeds, the finished chain emerges from the ribosome. It folds up into a precise shape determined by the exact order of amino acids. Thus, the central dogma explains how the four letter DNA code is quite literally turned into flesh and blood.