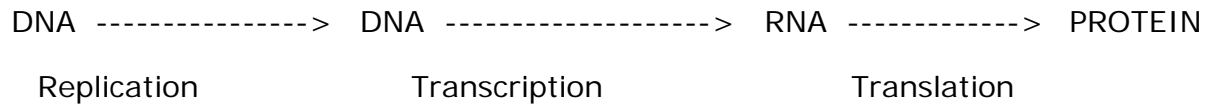


TRANSCRIPTION – TRANSLATION - GENETIC CODE AND OUTLINE OF PROTEIN SYNTHESIS

Central Dogma of Protein Synthesis

Proteins constitute the major part by dry weight of an actively growing cell. They are widely distributed in living matter. All enzymes are proteins. Proteins are built up from about 20 amino acids which constitute the basic building blocks. In proteins the amino acids are linked up by peptide bonds to form long chains called polypeptides.

The sequence of amino acids has a bearing on the properties of a protein, and is characteristic for a particular protein. The basic mechanism of protein synthesis is that DNA makes RNA, which in turn makes protein. The central dogma of protein synthesis is expressed as follows:



Noble Prize Winners in Protein Synthesis

Nobel prize winners. In the last 15 years several Nobel prizes in physiology and medicine have been awarded for work done on nucleic acids and protein synthesis. In 1975 Alexander Todd of Great Britain was awarded the prize for his studies on nucleotides and nucleotidic coenzymes.

The 1958 prize went to Beadle and Tatum for their work showing that one gene is responsible for one enzyme. Lederberg also shared the prize for his work on genetic recombination. The 1959 Nobel prize was shared by Ochoa and Kornberg for successful synthesis of RNA and DNA, respectively.

Watson of U. S. A and Crick and Wilkins of Great Britain received the 1962 prize for elucidating the structure of DNA. In 1965 the prize was awarded to Jacob, Monod and Lwoff of France for their discovery of regulator genes.

The 1968 prize went to the Americans Nirenberg and Khorana for their work on the genetic code, and to Holley (also of U. S. A.) for his finding out the nucleotide sequence of tRNA. In 1969 Delbruck, Hershey and Luria received the prize for their

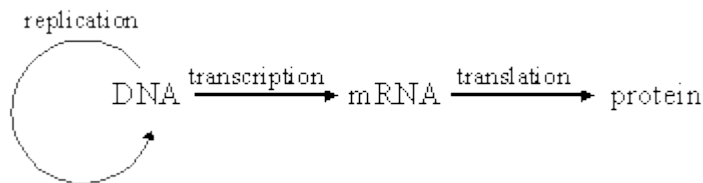
work on the reproductive pattern of viruses, In 1975 Temin was awarded the Nobel prize for his work on RNA directed DNA synthesis.

PROTEIN SYNTHESIS

Proteins are widely used in cells to serve diverse functions. Some proteins provide the structural support for cells while others act as enzymes to catalyze certain reactions. We have already seen the roles that different enzymes play in building the cell's structure and in catalyzing metabolic reactions, but where do proteins come from?

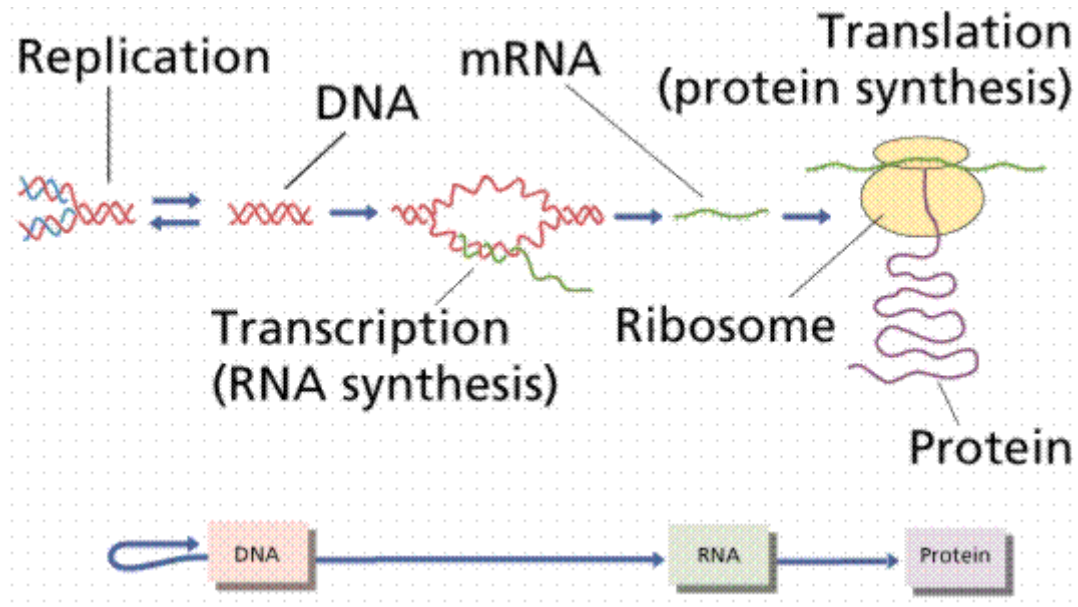
Since the beginning of evolution, cells have developed the ability to synthesize proteins. They can produce new proteins either for reproduction or to simply replace a degraded one. To manufacture proteins, cells follow a very systematic procedure that first transcribes DNA into mRNA and then translates the mRNA into chains of amino acids. The amino acid chain then folds into specific proteins.

Protein synthesis requires two steps: ***transcription*** and ***translation***.



Ribonucleic acid (RNA) was discovered after DNA. DNA, with exceptions in chloroplasts and mitochondria, is restricted to the nucleus (in eukaryotes, the nucleoid region in prokaryotes). RNA occurs in the nucleus as well as in the cytoplasm (also remember that it occurs as part of the ribosomes that line the rough endoplasmic reticulum).

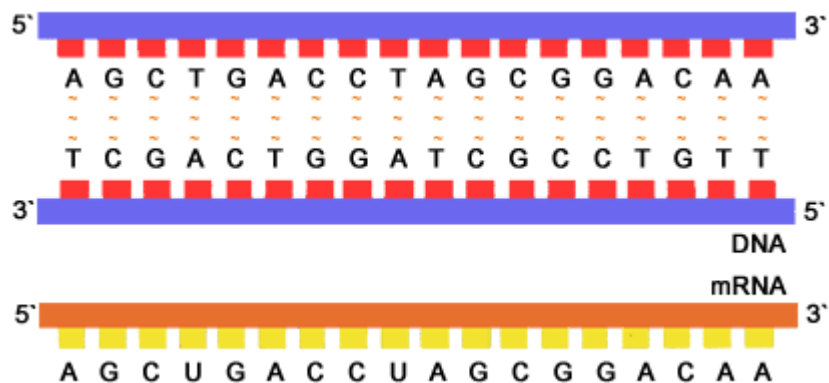
Crick's central dogma. Information flow (with the exception of reverse transcription) is from DNA to RNA via the process of transcription, and thence to protein via translation. Transcription is the making of an RNA molecule off a DNA template. Translation is the construction of an amino acid sequence (polypeptide) from an RNA molecule. Although originally called dogma, this idea has been tested repeatedly with almost no exceptions to the rule being found.



Step 1: DNA Transcription

Protein synthesis begins in the cell's nucleus when the gene encoding a protein is copied into RNA. Genes, in the form of DNA, are embedded in in the cell's chromosomes. The process of transferring the gene's DNA into RNA is called transcription. Transcription helps to magnify the amount of DNA by creating many copies of RNA that can act as the template for protein synthesis. The RNA copy of the gene is called the mRNA.

DNA and RNA are both constructed by a chain of nucleotides. However, RNA differs from DNA by the substitution of uracil (U) for thymine (T). Also, because only one strand of mRNA is needed when synthesizing proteins, mRNA naturally exist in single-stranded forms.



After transcription, the mRNA is transported out of the cell's nucleus through nuclear pores to go to the site of translation, the rough endoplasmic reticulum.

Transcription is a process of making an RNA strand from a DNA template, and the RNA molecule that is made is called transcript. In the synthesis of proteins, there are actually three types of RNA that participate and play different roles:

a. Messenger RNA(mRNA) which carries the genetic information from DNA and is used as a template for protein synthesis.

b. Ribosomal RNA(rRNA) which is a major constituent of the cellular particles called ribosomes on which protein synthesis actually takes place.

c. A set of transfer RNA(tRNA) molecules, each of which incorporates a particular amino acid subunit into the growing protein when it recognizes a specific group of three adjacent bases in the mRNA.

DNA maintains genetic information in the nucleus. RNA takes that information into the cytoplasm, where the cell uses it to construct specific proteins, RNA synthesis is transcription; protein synthesis is translation.

RNA differs from DNA in that it is single stranded, contains Uracil instead of Thymine and ribose instead of deoxyribose, and has different functions. The central dogma depicts RNA as a messenger between gene and protein, but does not adequately describe RNA's other function.

Transcription is highly controlled and complex. In Prokaryotes, genes are expressed as required, and in multicellular organisms, specialized cell types express subsets of gene. Transcription factors recognize sequences near a gene and bind sequentially, creating a binding transcription. Transcription proceeds as RNAP inserts complementary RNA bases opposite the coding strand of DNA. Antisense RNA blocks gene expression.

Messenger RNA transmits information in a gene to cellular structures that build proteins. Each three mRNA bases in a row forms a codon that specifies a particular amino acid. Ribosomal RNA and proteins form ribosomes, which physically support the other participants in protein synthesis and help catalyze formation of bonds between amino acids.

In eukaryotes, RNA is often altered before it is active. Messenger RNA gains a cap of modified nucleotides and a poly A tail. Introns are transcribed and cut out,

and exons are reattached by ribozymes. RNA editing introduced bases changes that alter the protein product in different cell types.

The genetic code is triplet, non-overlapping, continuous, universal, and degenerate. As translation begins, mRNA, tRNA with bound amino acids, ribosomes, energy molecules and protein factors assemble. The mRNA leader sequence binds to rRNA in the small subunit of a ribosome, and the first codon attracts a tRNA bearing methionine. Next, as the chain elongates, the large ribosomal subunit attaches and the appropriate anticodon parts of tRNA molecules form peptide bonds, a polypeptide grows. At a stop codon, protein synthesis ceases. Protein folding begins as translation proceeds, with enzymes and chaperone proteins assisting the amino acid chain in assuming its final functional form. Translation is efficient and economical, as RNA, ribosomes, enzymes, and key proteins are recycled.

RNA transcription requires the following components

The enzyme RNA polymerase

- A DNA template
- All four types of ribonucleoside triphosphates (ATP, GTP and UTP)
- Divalent metal ions Mg^{++} or Mn^{++} as a co-factor
- No primer is needed for RNA synthesis
- RNA transcription is a process that involves the following steps.

Binding of RNA Polymerase to DNA Double Helix

The histone coat protecting the DNA double helix of the gene to be transcribed is removed, on a signal from the cytoplasm, exposing the polynucleotide sequences in this region of DNA. The RNA polymerase enzyme binds to a specific site, called promoter, in the DNA double helix. This site is located on the 5' side of the gene to be transcribed. It signals the beginning of RNA synthesis. The promoter also determines the DNA strand that is to be transcribed.

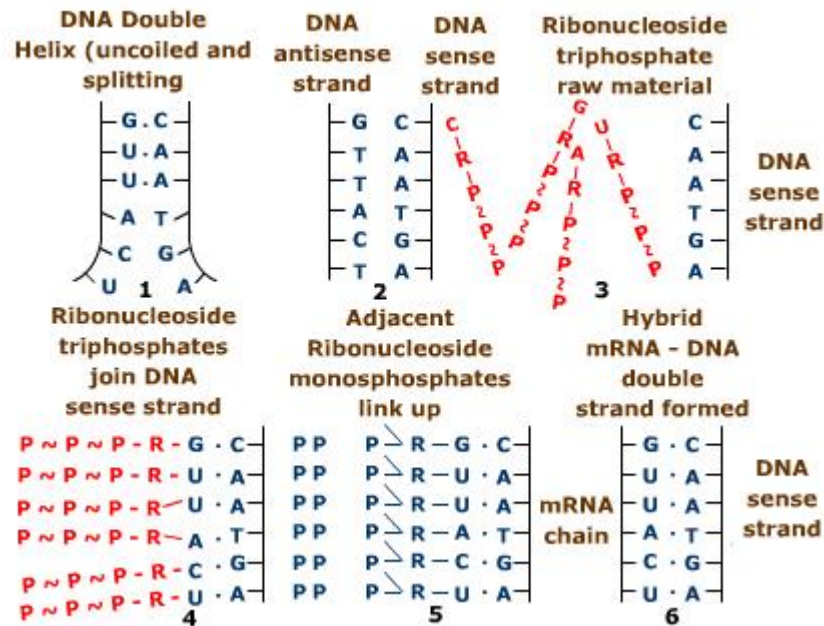
Exposure of RNA Bases

The histone coat protecting the DNA double helix of the gene to be transcribed is removed, on a signal from the cytoplasm, exposing the polynucleotide sequences in this region of DNA. The RNA polymerase enzyme binds to a specific site, called promoter, in the DNA double helix. This site is located on the 5' side of the gene to be transcribed. It signals the beginning of RNA synthesis.

The promoter also determines the DNA strand that is to be transcription is not known.

Base pairing

The ribonucleoside triphosphates, namely, adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP), floating free in the nucleus, serve as the raw material for RNA synthesis. They are formed by activation (phosphorylation) of ribonucleoside monophosphates, viz., adenosine monophosphate (AMP), guanosine monophosphate (GMP), cytidine monophosphate (CMP) and uridine monophosphate (UMP) as a result of their combining with ATP. The enzyme phosphorylase catalyses this activation process. The ribonucleotide triphosphates are joined to the bases of the DNA template chain one by one by hydrogen bonding according to the base pairing rule i.e., A U, U A, C G, G C. This base pairing is brought about by the RNA polymerase.



Synthesis of mRNA from DNA

The nucleotides are added one by one. A=Adenine, T=Thymine, C=Cytosine, G=Guanine, U=Uracil, R=Ribose sugar, P=Phosphate

Conversion to Ribonucleoside Monophosphates

The various ribonucleoside triphosphates on linking to the DNA template chain break off their high-energy bonds. This changes them to ribonucleoside monophosphates which represent the normal components of RNA, and sets free pyrophosphate groups (P~P). Pyrophosphate contains a high-energy bond (~). It undergoes hydrolysis by the enzyme pyrophosphatase, releases energy and sets free inorganic phosphate Pi. The first ribonucleotide phosphate retains all the three phosphates and is, thus, chemically distinct from the other nucleotides added after it



Formation of RNA Chain

Each ribonucleoside monophosphate attached to the DNA template chain then combines with the ribonucleotide arrived earlier, making the RNA chain become longer. The process is catalysed by the enzyme RNA polymerase and requires a divalent ion Mg⁺⁺ or Mn⁺⁺. The RNA chain, thus formed, contains nitrogenous bases that are complementary to those of the template **DNA** chain.

Separation of RNA Chain

As transcription proceeds, the hybrid DNA-RNA molecule dissociates, partly releasing the RNA molecule under synthesis. When polymerase reaches a terminator signal on the DNA, it leaves the DNA. The fully formed RNA chain is now totally released by this process, one gene forms several molecules of RNA, which get released from the DNA template one after the other.

In some cases, such as in E. coli, a specific chain terminating protein, called rho factor (P), stops the synthesis of RNA chain. In most cases, the enzyme RNA polymerase on its own can stop transcription.

Return of DNA Segment to Original Form

As the RNA chain grows, the transcribed region of the DNA molecule gets hydrogen bonded to the opposite strand and the two become spirally coiled to assume the original double helical form. When the last ribonucleotide is added, the RNA polymerase and RNA chain are completely released from the DNA, and now the DNA completes its winding into a double helix. The protective protein coat is added again to the DNA duplex.

The sequence of nitrogen bases from the promoter to the terminator sites form a transcription unit. It may include one or more genes. An entire transcription unit gets transcribed into a single RNA chain.

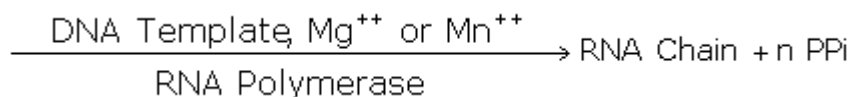
Processing of RNAs

The forms of RNAs originally transcribed from DNA are called primary transcripts. These undergo extensive changes, termed processing or post-transcriptional modification of RNAs, before they can become functional in both prokaryotes and eukaryotes.

In RNA processing,

- Larger RNA precursors are cut into smaller RNAs by a ribonuclease-P cleaving enzyme
- Unwanted nucleotides are removed by enzymes called nucleases (splicing)
- Useful regions are rejoined by ligase enzyme
- Certain nucleotides are added at the terminal ends enzymatically (terminal addition)
- The RNA molecule may fold on itself to assume proper shape (folding) and
- Some nucleotides may be modified (nucleotide modification)

The entire process of RNA transcription may be summed up in the equation
(ATP + GTP +CTP +UTP) n



RNA TYPES

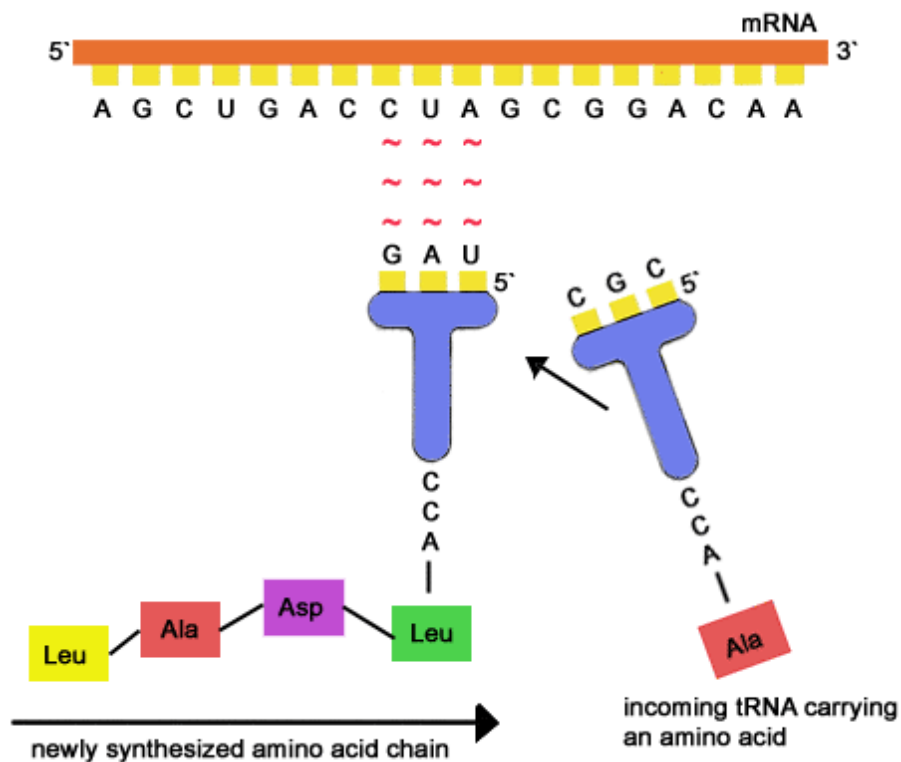
The three different types of RNA, namely, messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA) are transcribed from different regions of the DNA molecule. Three different RNA polymerases: I, II and III catalyses the transcription of rRNA, mRNA and tRNA respectively in eukaryotes. In prokaryotes, a single RNA polymerase composed of different subunits does this work. Transcription of RNA also occurs in the 5-3 direction like the replication of DNA.

Step 2: RNA Translation

After the mRNA has been transported to the rough endoplasmic reticulum, it is fed into the ribosomal translation machineries. Ribosomes begins to read the mRNA sequence from the 5` end to the 3` end. To convert the mRNA into protein, tRNA is used to read the mRNA sequence, 3 nucleotides at a time.

Amino acids are represented by codons, which are 3-nucleotide RNA sequences. The mRNA sequence is matched three nucleotides at a time to a complementary set of three nucleotides in the anticodon region of the corresponding tRNA molecule. Opposite the anticodon region of each tRNA, an amino acid is attached and as the mRNA is read off, the amino acids on each tRNA are joined together through peptide bonds.

Translation is the mechanism by which the triplet base sequence of an mRNA guides the linking of a specific sequence of amino acids to form a polypeptide (protein) on ribosomes. All the proteins a cell needs are synthesized by the cell within itself.



Machinery for Protein Synthesis

Protein synthesis requires amino acids, DNA, RNAs, ribosomes and enzymes, enzyme activators and ATP molecules

Amino Acids

Proteins are the polymers of amino acids. Therefore, amino acids form the raw material for protein synthesis. The proteins found in living organisms need about 20 amino acids as building blocks or monomers. These are available in the cytoplasmic matrix as an amino acid pool.

DNA as Specificity Control

In order to maintain its own special characteristics a cell must manufacture proteins exactly similar to those already present in it. Thus, protein synthesis requires specificity control to provide instructions about the exact sequence in which the given numbers and kinds of amino acids should be linked to form the desired polypeptides. The specificity control is exercised by DNA through mRNA. Sequences of 3 consecutive nitrogenous bases in the DNA double helix form the biochemical or genetic code. Each base triplet codes for a specific amino acid. Since the DNA is more or less stable, the proteins formed in a cell are exactly like the preexisting proteins

RNAs

RNA molecule is a long, unbranched, single-stranded polymer of ribonucleotides. Each nucleotide unit is composed of three smaller molecules, a phosphate group, a 5-carbon ribose sugar, and a nitrogen-containing base. The bases in RNA are adenine, guanine, uracil and cytosine. The various components are linked up as in DNA. There are three types of RNA in every cell: messenger RNA or mRNA, ribosomal RNA or rRNA and transfer RNA or tRNA. The three types of RNAs are transcribed from different regions of DNA template. RNA chain is complementary to the DNA strand, which produces it. All the three kinds of RNAs play a role in protein synthesis.

Differences Between RNA Types

Features	Ribosomal RNA (rRNA)	Messenger RNA (mRNA)	Transfer RNA (tRNA)
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1. Percentage of cell's total RNA	About 80	About 5	About 15
2. Length of molecule	Variable	Longest	Shortest
3. Shape of molecule	Greatly coiled	Linear	Clover leaf- like, folded into L- shape
4. Types	Six	Numerous	About 60
5. Role	Form greater part of ribosome's	Carry information from DNA	Carry amino acids to mRNA codons
6. Life	Long, used again and again in translation	Very short, 2 minutes to 4 hours, degraded after translation	Long, used again and again in translation

mRNA

The DNA, that controls protein synthesis, is located in the chromosomes within the nucleus, whereas the ribosomes, on which the protein synthesis actually occurs, are placed in the cytoplasm. Therefore, some sort of agency must exist to carry instructions from the DNA to the ribosomes. This agency does exist in the form of mRNA. The mRNA molecule carries the message (information) from DNA about the sequence of particular amino acids to be form a polypeptide, hence its name. It is also called informational RNA or template RNA. The mRNA forms about 5% of the total RNA of a cell. Its molecule is linear and the longest of all the three RNA types. Its length is related to the size of the polypeptide to be synthesized with its information. There is a specific mRNA for each polypeptide. Because of the variation is size in mRNA population in a cell; the mRNA is often called heterogeneous nuclear RNA, or hnRNA

It has at its 5 end a cap of methylated guanine followed successively by an initiation codon (AUG or GUG), a long coding region, a termination codon (UAA or UAG or UGA) and a poly-A tail of many adenine-containing nucleotides at 3 end. A small non-coding region may be present after the head and before tail

In eukaryotes, mRNA carries information for one polypeptide only. It is monocistronic (monogenic) because it is transcribed from a single cistron (gene) and has a single terminator codon

Bacterial mRNA often carries information for more than one polypeptide chain. Such an mRNA is said to be polycistronic (polygenic) because it is transcribed from many continuous (adjacent) genes. A polycistronic mRNA has an initiator codon and a terminator codon for each polypeptide to be formed by it.

tRNA

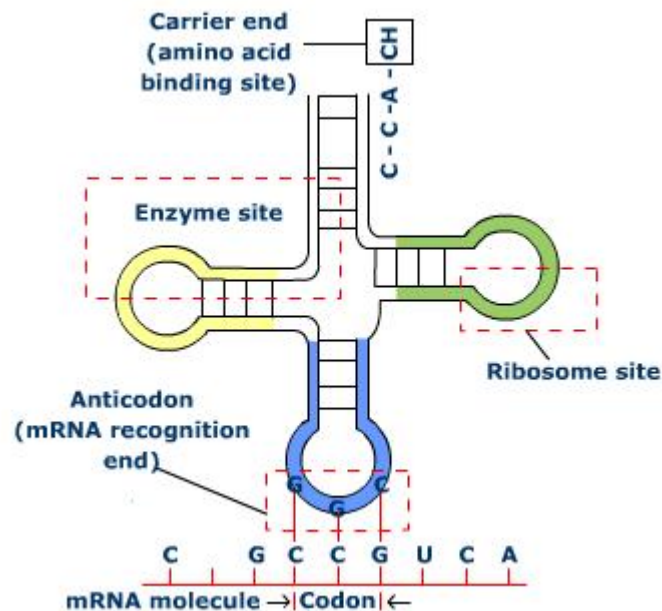
The tRNA carries a specific amino acid from the amino acid pool to the mRNA on the ribosomes to form a polypeptide, hence its name. The tRNAs form about 15% of the total RNA of a cell. Its molecule is the smallest and has the form of a cloverleaf. It has four regions.

Carrier End: This is the 3' end of the molecule. Here a specific amino acid becomes attached. The tRNA molecule has a base triplet CCA with OH group at the tip. The COOH of amino acid joins the OH group.

Recognition End: It is the opposite end of the molecule. It has 3 unpaired ribonucleotides. The bases of these ribonucleotides are complementary bases of the triplet found on mRNA chain called a codon. This triplet base sequence in tRNA is called as an anticodon. The anticodon binds with the codon at the time of translation.

Enzyme Site: It is on one lateral side of the molecule. It is meant for a specific charging enzyme which catalyses the binding of a specific amino acid to tRNA molecule.

Ribosome Site: It is on the other lateral side of the molecule. It is meant for attachment to a ribosome.



rRNA

The rRNA molecule is greatly coiled. In combination with proteins, it forms the small and large subunits of the ribosomes, hence its name. It forms about 80% of the total RNA of a cell. A eukaryotic ribosome is 80S; with a large 60S subunit consists of 28S, 5.8S and 5S rRNAs and over 45 different basic proteins, the smaller 40S subunit comprises 18S RNA and about 33 different basic proteins. A prokaryotic ribosome is 70S; its large 50S subunit consists of 23S and 5S rRNAs and about 34 different basic proteins; its small 30S subunit comprises 16S rRNA and about 21 different basic proteins. The 3' end of 18S rRNA (16S rRNA in prokaryotes) has a binding site for the mRNA cap. The 5S rRNA has a binding site for tRNA. The rRNA also seems to play some general role in protein synthesis. It is involved in assembling the amino acid molecules brought by tRNA, into a polypeptide chain

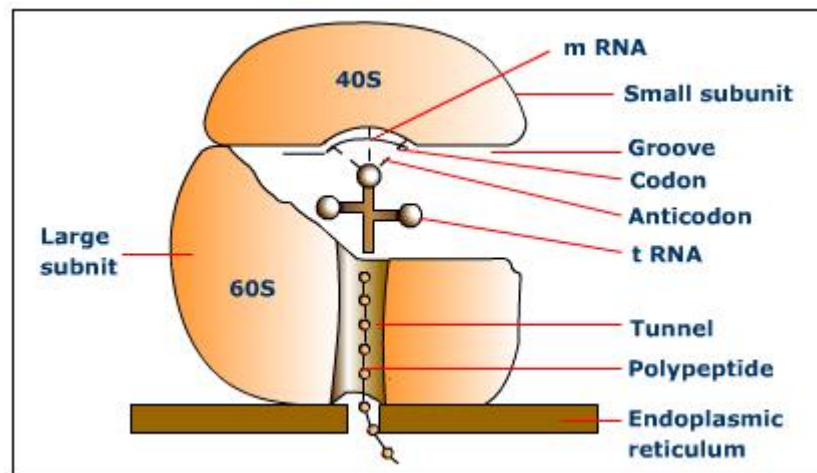
There are two more types of RNA, recognised in the cell namely

Small nuclear RNA (snRNA) that helps in processing of rRNA and mRNA and

Small cytoplasmic RNA (scRNA) which helps in binding the ribosome to ER

Ribosomes

Ribosomes are tiny ribonucleoprotein particles without a covering membrane. They serve as the site for protein synthesis. Hence, they are called protein factories of the cell. Each ribosome consists of larger and smaller subunits. The subunits of ribosome occur separately when ribosomes are not involved in protein synthesis. The two subunits join when protein synthesis starts, and undergo dissociation (separate) when protein synthesis stops. Many ribosomes line up on the mRNA chain during protein synthesis. Such a group of active ribosomes is called a polyribosome, or a polysome. In a polysome, the adjacent ribosomes are about 340 Å apart. The number of ribosomes in a polysome is related to the length of the mRNA molecule, which reflects the length of the polypeptides to be synthesized. It is now known that polypeptide synthesis occurs at the polysomes and not at the single free ribosomes, in both prokaryotes and eukaryotes.



A ribosome has two binding sites for tRNA molecules. One is called A site (acceptor or aminoacyl) and the other is termed P site (peptidyl). These sites span across the larger and smaller subunits of the ribosome. The A site receives the tRNA amino acid complex. The tRNA leaves from P site, after releasing its amino acid. However, the first tRNA amino acid complex directly enters the P site of the ribosome.

A eukaryotic ribosome has a groove at the junction of the two subunits. From this groove, a tunnel extends through the large subunit and opens into a canal of

the endoplasmic reticulum. The polypeptides are synthesized in the groove between the two ribosomal subunits and pass through the tunnel of the large subunit into the endoplasmic reticulum. While in the groove, the developing polypeptide is protected from the cellular enzymes.

The smaller subunit forms a cap over the larger subunit. The larger subunit attaches to the endoplasmic reticulum by two glycoproteins named ribophorin I and II.

The function of the ribosome is to hold the mRNA, tRNA and the associated enzymes controlling the process in position, until a peptide bond is formed between the adjacent amino acids

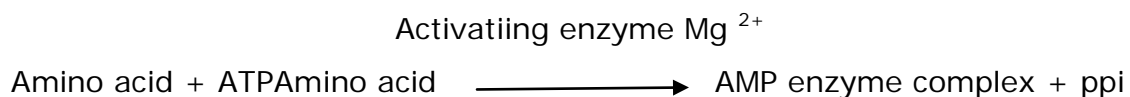
Mechanism of Protein Synthesis

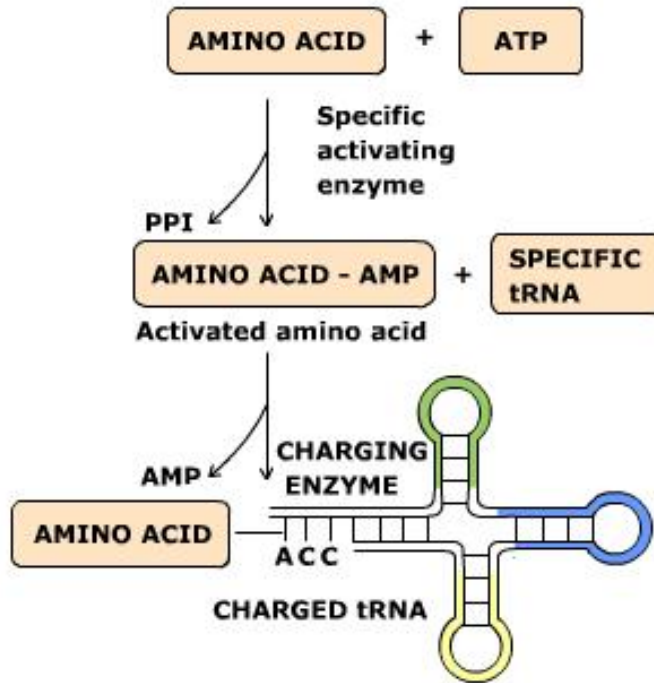
The events in protein synthesis are better known in bacteria than in eukaryotes. Although these are thought to be similar in the two groups there are some differences. The following description refers mainly to protein synthesis in bacteria on the 70S ribosome.

Protein synthesis is a highly complex and an elaborate process and involves the following steps:

Activation of Amino Acids

It is the step in which each of the participating amino acid reacts with ATP to form amino acid AMP complex and pyrophosphate. The reaction is catalyzed by a specific amino acid activating enzyme called aminoacyl-tRNA synthetase in the presence of Mg^{2+} . There is a separate aminoacyl tRNA synthetase enzyme for each kind of amino acid. Much of the energy released by the separation of phosphate groups from ATP is trapped in the amino acid AMP complex. The complex remains temporarily associated with the enzyme. The amino acid AMP enzyme complex is called an activated amino acid. The pyrophosphate is hydrolyzed to two inorganic phosphates (2pi)



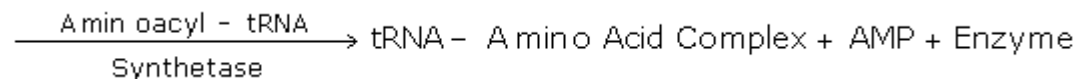


Activation of Amino Acids

Charging of tRNA

It is the step in which the amino acid AMP-enzyme complex joins with the amino acid binding site of its specific tRNA, where its COOH group bonds with the OH group of the terminal base triplet CCA. The reaction is catalyzed by the same enzyme, aminoacyl tRNA synthetase. The resulting tRNA-amino acid complex is called a charged tRNA. AMP and enzyme are released. The released enzyme can activate and attach another amino acid molecule to another tRNA molecule. The energy released by change of ATP to AMP is retained in the amino acid-tRNA complex. This energy is later used to drive the formation of peptide bond when amino acids link together and form a polypeptide

Amino acid AMP Enzyme complex + tRNA



The tRNA amino acid complex moves to the ribosomes, the site of protein synthesis.

Activation of Ribosome

It is the step in which the smaller and the larger subunits of ribosome are joined together. This is brought about by mRNA chain. The latter joins the smaller ribosomal subunit with the help of the first codon by a base pairing with an appropriate sequence on rRNA. The combination of the two is called initiation complex. The larger subunit later joins the small subunit, forming active ribosome. Activation of ribosome by mRNA requires proper concentration of Mg^{++}

Assembly of Amino Acids (Polypeptide Formation)

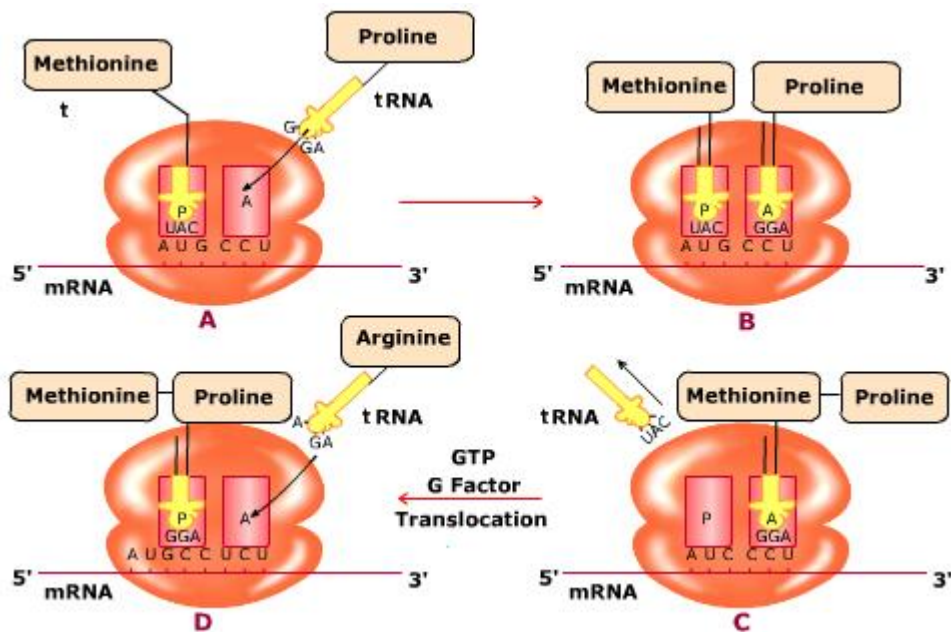
It is the step in which the amino acids are assembled into a polypeptide chain. It involves 3 events: initiation, elongation and termination of polypeptide chain

Initiation of Polypeptide Chain

The mRNA chain has at its 5 end an "initiator" or "start" codon (AUG or GUG) that signals the beginning of polypeptide formation. This codon lies close to the P site of the ribosome. The amino acid formylmethionine (methionine in eukaryotes) initiates the process. It is carried by tRNA having an anticodon UAC which bonds with the initiator codon AUG of mRNA. Initiation factors (IF1, IF2 and IF3) and GTP promote the initiation process.

The large ribosomal subunit now joins the small subunit to complete the ribosome. At this stage, GTP is hydrolysed to GDP. The ribosome has formylmethionine bearing tRNA at the P site. Later, the formylmethionine is changed to normal methionine by the enzyme deformylase in prokaryotes. If not required, methionine is later separated from the polypeptide chain by a proteolytic enzyme aminopeptidase.

Elongation of Polypeptide Chain



The above figure shows,

- A charged tRNA arriving at the A site, reading its codon on the mRNA
- Amino acid of tRNA at P site is ready to be transferred to the amino acid of tRNA at A site
- Amino acids are joined by peptide bond and tRNA is discharged from P site
- Peptide chain-carrying tRNA is translocated to P site, making A site free to receive another charged tRNA

Three elongation factors (EF Tu, EF Ts and EF G) assist in the elongation of the polypeptide chain. A charged tRNA molecule along with its amino acid, proline, for example, enters the ribosome at the A site. Its anticodon GGA locates and binds with the complementary codon CCU of mRNA chain by hydrogen bonds. The amino acid methionine is transferred from its tRNA onto the newly arrived proline tRNA complex where the two amino acids join by a peptide bond. The process is catalyzed by the enzyme peptidyl transferase located on the ribosome. In this process, the linkage between the first amino acid and its tRNA is broken, and the -COOH group now forms a peptide bond with the free -NH₂ group of the second amino acid. Thus, the second tRNA carries a dipeptide, formylmethionineproline.

The energy required for the formation of a peptide bond comes from the free energy released by separation of amino acid (formylmethionine or methionine) from its tRNA.

The first tRNA, now uncharged, separates from mRNA chain at the P site of the ribosome and returns to the mixed pool of tRNAs in the cytoplasm. Here, it is now available to transport another molecule of its specific amino acid.

Now the ribosome moves one codon along the mRNA in the 3' direction. With this, tRNA_{dipeptide} complex at the A site is pulled to the P site. This process is called translocation. It requires GTP and a translocase protein called EF-G factor. The GTP is hydrolysed to GDP and inorganic phosphate to release energy for the process

At this stage, a third tRNA molecule with its own specific amino acid, arginine, for example arrives at the A site of the ribosome and binds with the help of anticodon AGA to the complementary codon UCU of the mRNA chain. The dipeptide formylmethionineproline is shifted from the preceding tRNA on the third tRNA where it joins the amino acid arginine again with the help of peptidyl transferase enzyme. The dipeptide, thus, becomes a tripeptide, formyl-methionine-proline-arginine. The second tRNA being now uncharged, leaves the mRNA chain, vacating the P site. The tRNA_{tripeptide} complex is translocated from A site to P site. The entire process involving arrival of tRNA-amino acid complex, peptide bond formation and translocation is repeated. As the ribosome moves over the mRNA, all the codons of mRNA arrive at the A site one after another, and the peptide chain grows. Thus, the amino acids are linked up into a polypeptide in a sequence communicated by the DNA through the mRNA. A polypeptide chain which is in the process of synthesis is often called a nascent polypeptide

The growing polypeptide chain always remains attached to its original ribosome, and is not transferred from one ribosome to another. Only one polypeptide chain can be synthesized at a time on a given ribosome.

Termination and Release of Polypeptide Chain:

At the terminal end of mRNA chain there is a stop, or terminator codon (UAA, UAG or UGA). It is not joined by the anticodon of any tRNA amino acid complex. Hence, there can be no further addition of amino acids to the polypeptide chain.

The linkage between the last tRNA and the polypeptide chain is broken by three release factors. (RF 1, RF 2 and RF 3) and GTP. The release is catalyzed by the peptidyl transferase enzyme, the same enzyme that forms the peptide bonds. The ribosome jumps off the mRNA chain at the stop codon and dissociates into its two subunits. The completed polypeptide (amino acid chain) becomes free in the cytoplasm.

The ribosomes and the tRNAs on release from the mRNA can function again in the same manner and result in the formation of another polypeptide of the same protein.

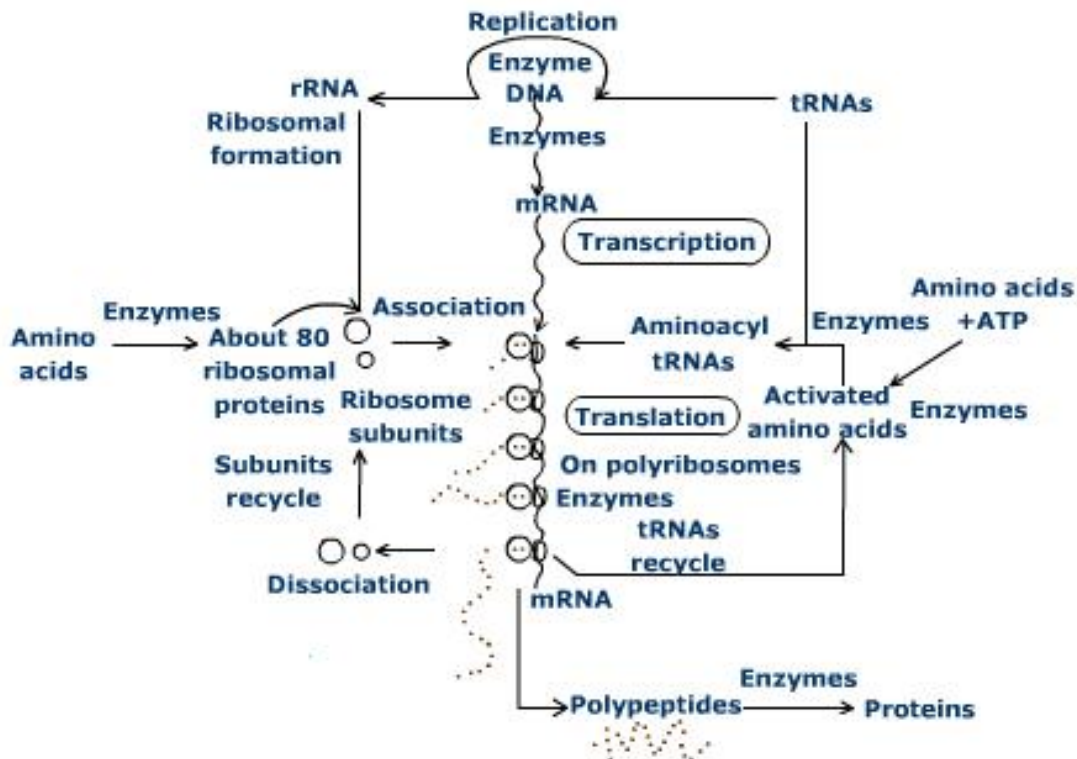
Modification of Released Polypeptide

The just released polypeptide is a straight, linear exhibiting a primary molecule, structure. It may lose some amino acids from the end with the help of a peptidase enzyme, and then coil and fold on itself to acquire secondary and tertiary structure. It may even combine with other polypeptides, to have quaternary structure.

The proteins synthesized on free polysomes are released into the cytoplasm and function as structural and enzymatic proteins. The proteins formed on the polysomes attached to ER pass into the ER channels and are exported as cell secretions by exocytosis after packaging in the Golgi apparatus.

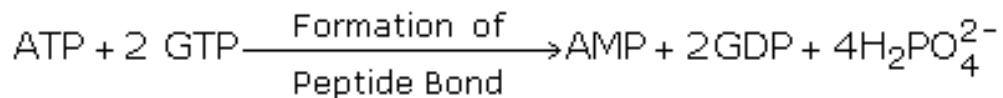
Polysome Formation

When the ribosome has moved sufficiently down the mRNA chain towards 3' end, another ribosome takes up position at the initiator codon of mRNA, and starts synthesis of a second molecule of the same polypeptide chain. At any given time, the mRNA chain will, therefore, carry many ribosomes over which are similar polypeptide chains of varying length, shortest near the initiator codon and longest near the terminator codon. A row of ribosomes joined to the mRNA molecule, is called a polyribosome, or a polysome. Synthesis of many molecules of the same polypeptide simultaneously from one mRNA molecule by a polysome is called translational amplification.



Energy Used for Protein Synthesis

One GTP is hydrolysed to GDP as each successive amino acid-tRNA complex attaches to the A site of the ribosome. A second GTP is broken down to GDP as the ribosome moves to each new codon in the mRNA. One ATP is hydrolysed to AMP during amino acid activation. Thus, the formation of each peptide bond uses 3 high-energy molecules, one ATP and two GTP.



An interesting aspect of protein synthesis is that the DNA and ribosomes are located at different sites in the cell. Location of instruction centre (DNA) and manufacturing centre (ribosomes) at different sites in a cell is advantageous. If both were in the nucleus, the manufacturing centre would be far away from the energy sources and raw materials; and if both were in the cytoplasm, the information centre would be exposed to respiratory breakdown. The nuclear

envelope preserves stability of the DNA by protecting it from respiratory destruction. The message in the DNA in the form of genes (codes) are, permanent, authentic master documents from which working copies are prepared in the form of mRNAs, as and when required by the cell.

The complex process by which the information in RNA is decoded into a polypeptide is one of the exciting discoveries the genetic code

With only four biochemical letters (A, G, C, U) a one letter code could not unambiguously encode 20 amino acids. A two letter code could encode only 16 amino acids. So, a triplet code based on 3 biochemical letters or nucleotide bases could make $4 \times 4 \times 4 = 64$ codons. This will be required to code for 20 or so different amino acids.

The discovery of the genetic code became possible through the contribution of many scientists like Francis Crick, Severo Ochoa, Maxell Ninenberg, Hargobind Khorana and J. H. Malther in the 1960s. Ninenberg and Khorana shared the Nobel Prize in 1968.

Characteristics of Genetic Code

The genetic code is a triplet code: Three adjacent bases, termed as codon, specify one amino acid

Non-overlapping: Adjacent codons do not overlap

No punctuation: The genetic code is comma less

The genetic code is universal i.e., a given codon specifies the same amino acid in all protein synthesising organisms

The genetic code is degenerate: it lacks specificity and one amino acid often has more than one code triplet

Each codon codes for only one amino acid, none for more than one

Three of the 64 codons, names UAA, UAG and UGA do not specify any amino acid but signal the end of the message. They are called nonsense or terminator codons

The codons AUG and GUG are called the initiation or start codons as they begin the synthesis of polypeptide.

		MIDDLE LETTER									
		U	C	A	G						
FIRST LETTER (5' END)	U	UUU	UCU UCC UCA UCG	UAU UAC UAA* UAG*	UGU UGC UGA* UGG	phenyl - alanine	Serine	Tyrosine	Cysteine	Trypto - phan	U C A G
		UUC									
		UUA									
		UUG									
	C	CUU	CCU CCC CCA CCG	CAU CAC CAA CAG	CGU CGC CGA CGG	Leucine	Proline	Histidine	Arginine	U C A G	
		CUC									
		CUA									
		CUG									
	A	AUU	ACU ACC ACA ACG	AAU AAC AAA AAG	AGU AGC AGA AGG	Isoleu - cine	Threonine	Asparagine	Serine	U C A G	
		AUC									
		AUA									
		AUG [†]									
	G	GUU	GCU GCC GCA GCG	GAU GAC GAA GAG	GGU GGU GGA GGG	Valine	Alanine	Aspartic acid	Glycine	U C A G	
		GUC									
		GUA									
		GUG [†]									

THIRD LETTER (3' END) ↓

In the above figure, the sequence of nucleotides in the triplet codons of RNA is indicated; each triplet specifies a particular amino acid.

