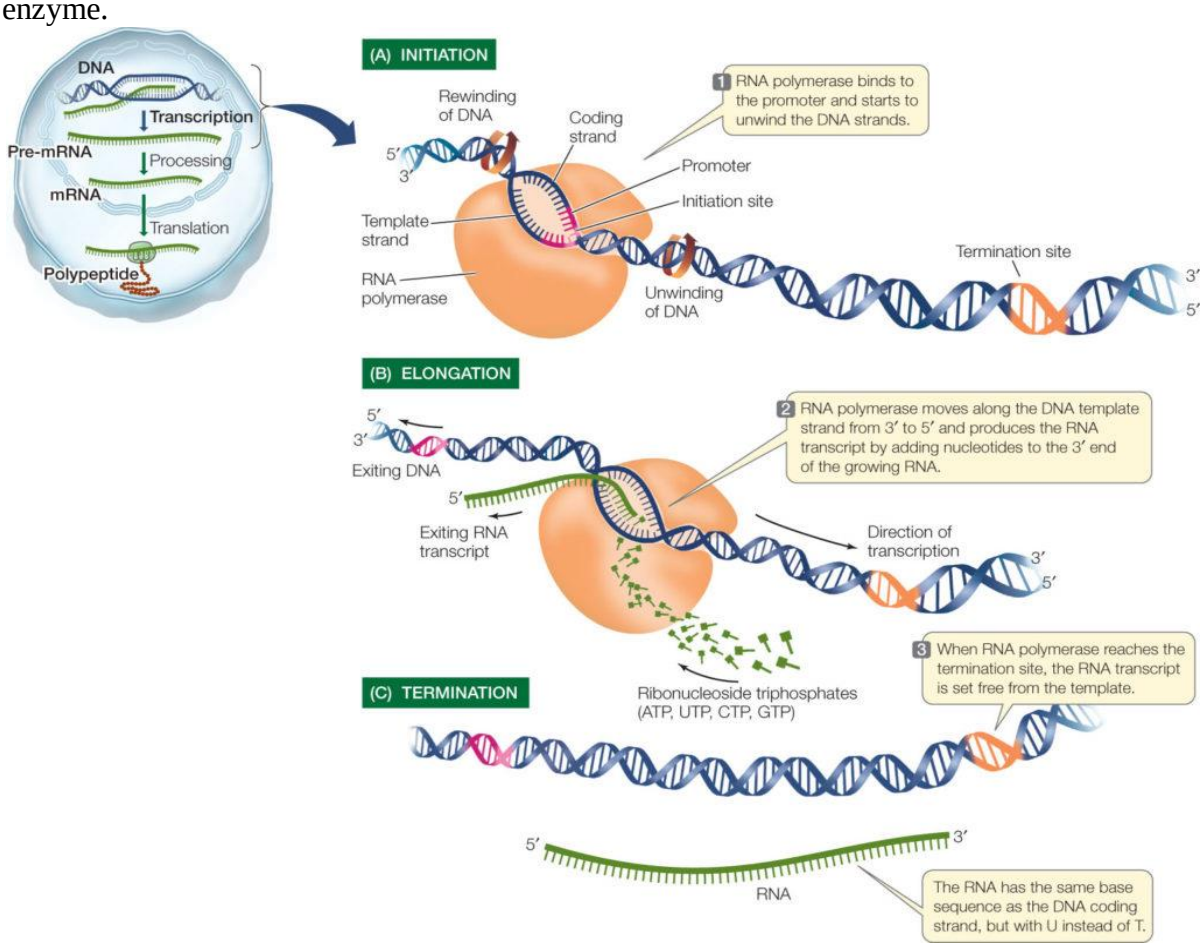
Prokaryotic Transcription- Enzymes, Steps, Significance

- Transcription is the process by which the information in a strand of **DNA** is copied into a new molecule of messenger **RNA** (mRNA).
- In prokaryotic organisms transcription occurs in three phases known as initiation, elongation and termination.

Enzyme(s) Involved

- RNA is synthesized by a single RNA polymerase enzyme which contains multiple polypeptide subunits.
- In *E. coli*, the RNA polymerase has subunits: two α, one β, one β' and one ω and σ subunit (α2ββ'ωσ). This complete enzyme is called as the holoenzyme.
- The σ subunit may dissociate from the other subunits to leave a form known as the core enzyme.



Initiation Phase

During initiation, RNA polymerase recognizes a specific site on the DNA, upstream from the gene that will be transcribed, called a **promoter site** and then unwinds the DNA locally.

Promoters and Initiation

- The holoenzyme binds to a promoter region about 40–60 bp in size and then initiates transcription a short distance downstream (i.e. 3 to the promoter).
- Within the promoter lie two 6 base pair sequences that are particularly important for promoter function.
- They are highly conserved between species.
- Using the convention of calling the first nucleotide of a transcribed sequence as +1, these two promoter elements lie at positions –10 and –35, that is about 10 and 35 bp, respectively, upstream of where transcription will begin.
- The –10 sequence has the consensus Because this element was discovered by Pribnow, it is also known as the Pribnow box. It is an important recognition site that interacts with the σ factor of RNA polymerase.
- The –35 sequence has the consensus **TTGACA** and is important in DNA unwinding during transcriptional initiation.
- RNA polymerase does not need a primer to begin transcription; having bound to the promoter site, the RNA polymerase begins transcription directly.

Elongation Phase

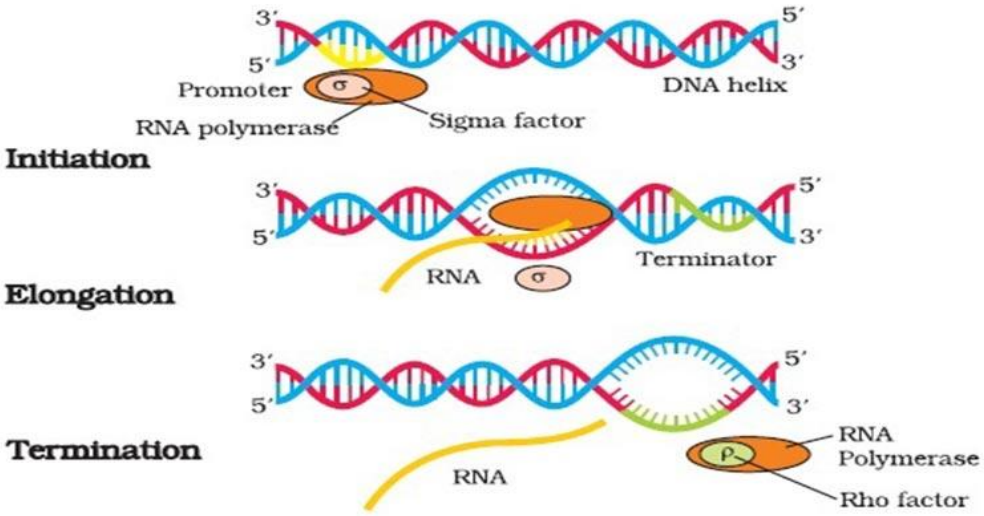
- After transcription initiation, the σ factor is released from the transcriptional complex to leave the core enzyme (α2 ββω) which continues elongation of the RNA transcript.
- The core enzyme contains the catalytic site for polymerization, probably within the β subunit.
- The first nucleotide in the RNA transcript is usually pppG or pppA.
- The RNA polymerase then synthesizes RNA in the 5’ →3’ direction, using the four ribonucleoside 5-triphosphates (ATP, CTP, GTP, UTP) as precursors.

- The 3-OH at the end of the growing RNA chain attacks the α phosphate group of the incoming ribonucleoside 5-triphosphate to form a 3’5′ phosphodiester bond.
- The complex of RNA polymerase, DNA template and new RNA transcript is called a **ternary complex** (i.e. three components) and the region of unwound DNA that is undergoing transcription is called the transcription bubble.
- The RNA transcript forms a transient RNA–DNA hybrid helix with its template strand but then peels away from the DNA as transcription proceeds.
- The DNA is unwound ahead of the transcription bubble and after the transcription complex has passed, the DNA rewinds.
- Thus, during the elongation, the RNA polymerase uses the antisense (-) strand of DNA as template and synthesizes a complementary RNA molecule.
- The RNA produced has the same sequence as the non-template strand, called the sense (+) strand (or coding strand) except that the RNA contains U instead of T.
- At different locations on the bacterial chromosome, sometimes one strand is used as template, sometimes the other, depending on which strand is the coding strand for the gene in question.
- The correct strand to be used as template is identified for the RNA polymerase by the presence of the promoter site.

Termination Phase

- Transcription continues until a termination sequence is reached.
- The most common termination signal is a GC-rich region that is a palindrome, followed by an AT-rich sequence.
- The RNA made from the DNA palindrome is self- complementary and so base pairs internally to form a hairpin structure rich in GC base pairs followed by four or more U residues.

- However, not all termination sites have this hairpin structure. Those that lack such a structure require an additional protein, called **rho**, to help recognize the termination site and stop transcription.
- Thus the RNA polymerase encounters a termination signal and ceases transcription, releasing the RNA transcript and dissociating from the DNA.



RNA processing

- In prokaryotes, RNA transcribed from protein-coding genes (messenger RNA, mRNA), requires little or no modification prior to translation.
- Many mRNA molecules begin to be translated even before RNA synthesis has finished.
- However, since ribosomal RNA (rRNA) and transfer RNA (tRNA) are synthesized as precursor molecules, they require post-transcriptional processing.

Significance

- Transcription of DNA is the method for regulating gene expression.
- It occurs in preparation for and is necessary for protein translation.

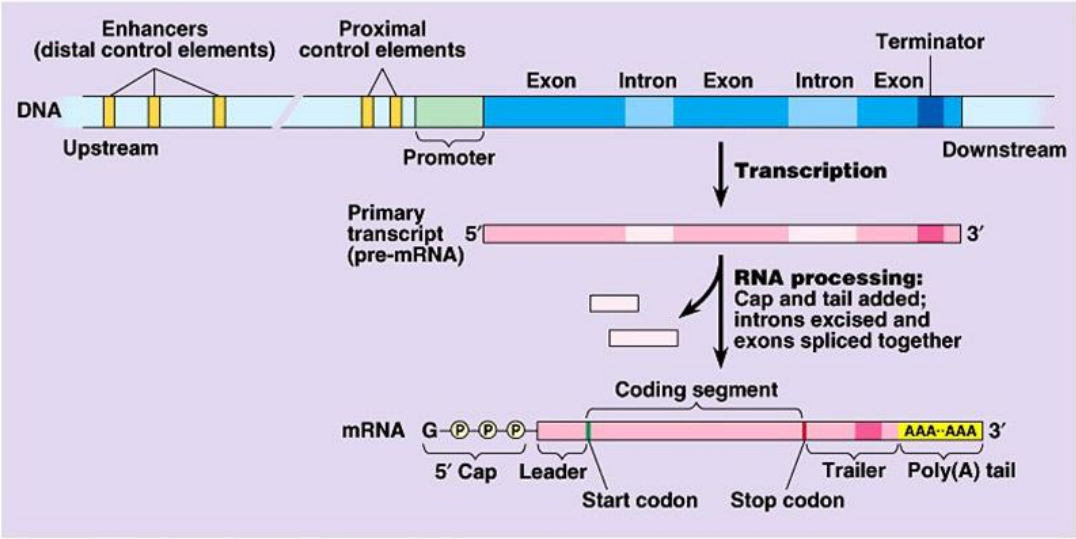
Eukaryotic Transcription

- Transcription is the process by which the information in a strand of DNA is copied into a new molecule of **RNA**.
- It is the first step of gene expression, in which a particular segment of DNA is copied into RNA (especially mRNA) by the enzyme RNA polymerase.
- It results in a complementary, antiparallel RNA strand called a primary transcript.

Transcription in Eukaryotes

Transcription occurs in eukaryotes in a way that is similar to prokaryotes with reference to the basic steps involved. However, some major differences between them include:

- Initiation is more complex.
- Termination does not involve stem-loop structures.
- Transcription is carried out by three enzymes (RNA polymerases I, II and III).
- The regulation of transcription is more extensive than prokaryotes.



Enzyme(s) Involved in Eukaryotic Transcription

Unlike prokaryotes where all RNA is synthesized by a single RNA polymerase, the nucleus of a eukaryotic cell has three RNA polymerases responsible for transcribing different types of RNA.

- **RNA polymerase I (RNA Pol I)** is located in the nucleolus and transcribes the 28S, 18S, and 5.8S rRNA genes.
- **RNA polymerase II (RNA Pol II)** is located in the nucleoplasm and transcribes protein-coding genes, to yield pre-mRNA, and also the genes encoding small nucleolar RNAs (snoRNAs) involved in rRNA processing and small nuclear RNAs (snRNAs) involved in mRNA processing, except for U6 snRNA.
- **RNA polymerase III (RNA Pol III)** is also located in the nucleoplasm. It transcribes the genes for tRNA, 5S rRNA, U6 snRNA, and the 7S RNA associated with the signal recognition particle (SRP) involved in the translocation of proteins across the endoplasmic reticulum membrane.
- Each of the three eukaryotic RNA polymerases contains 12 or more subunits and so these are large complex enzymes.
- The genes encoding some of the subunits of each eukaryotic enzyme show DNA sequence similarities to genes encoding subunits of the core enzyme of*E. coli* RNA polymerase.
- However, four to seven other subunits of each eukaryotic RNA polymerase are unique in that they show no similarity either with bacterial RNA polymerase subunits or with the subunits of other eukaryotic RNA polymerases.

Features of Eukaryotic Transcription

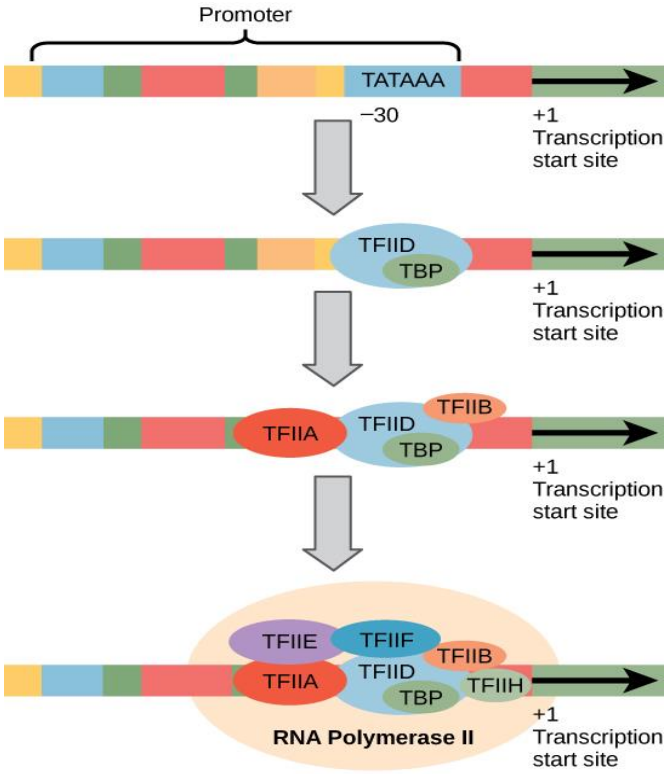
- Transcription in eukaryotes occurs within the nucleus and mRNA moves out of the nucleus into the cytoplasm for translation.
- The initiation of RNA synthesis by RNA polymerase is directed by the presence of a promoter site on the 5’ side of the transcriptional start site.

- The RNA polymerase transcribes one strand, the antisense (-) strand, of the DNA template.
- RNA synthesis does not require a primer.
- RNA synthesis occurs in the 5' → 3' direction with the RNA polymerase catalyzing a nucleophilic attack by the 3-OH of the growing RNA chain on the alpha-phosphorus atom on an incoming ribonucleoside 5-triphosphate.
- mRNA in eukaryotes is processed from the primary RNA transcript, a process called maturation.

Process of Eukaryotic Transcription

The basic mechanism of RNA synthesis by these eukaryotic RNA polymerases can be divided into the following phases:

Initiation Phase



- During initiation, RNA polymerase recognizes a specific site on the DNA, upstream from the gene that will be transcribed, called a **promoter site** and then unwinds the DNA locally.
- Most promoter sites for RNA polymerase II include a highly conserved sequence located about 25–35 bp upstream (i.e. to the 5 side) of the start site which has the consensus TATA(A/T)A(A/T) and is called the TATA box.
- Since the start site is denoted as position +1, the TATA box position is said to be located at about position -25.
- The TATA box sequence resembles the -10 sequence in prokaryotes (TATAAT) except that it is located further upstream.
- Both elements have essentially the same function, namely recognition by the RNA polymerase in order to position the enzyme at the correct location to initiate transcription.
- The sequence around the TATA box is also important in that it influences the efficiency of initiation. Transcription is also regulated by upstream control elements that lie 5′ to the TATA box.
- Some eukaryotic protein-coding genes lack a TATA box and have an initiator element instead, centered around the transcriptional initiation site.
- In order to initiate transcription, RNA polymerase II requires the assistance of several other proteins or protein complexes, called general (or basal) transcription factors, which must assemble into a complex on the promoter in order for RNA polymerase to bind and start transcription.
- These all have the generic name of TFII (for Transcription Factor for RNA polymerase II).
- The first event in initiation is the binding of the transcription factor IID (TFIID) protein complex to the TATA box via one its subunits called TBP (TATA box binding protein).
- As soon as the TFIID complex has bound, TFIIA binds and stabilizes the TFIID-TATA box interaction. Next, TFIIB binds to TFIID.

- However, TFIIB can also bind to RNA polymerase II and so acts as a bridging protein. Thus,
- RNA polymerase II, which has already complexed with TFIIF, now binds.
- This is followed by the binding of TFIIE and H. This final protein complex contains at least 40 polypeptides and is called the **transcription initiation complex.**
- Those protein-coding genes that have an initiator element instead of a TATA box appear to need another protein(s) that binds to the initiator element.
- The other transcription factors then bind to form the transcription initiation complex in a similar manner to that described above for genes possessing a TATA box promoter.

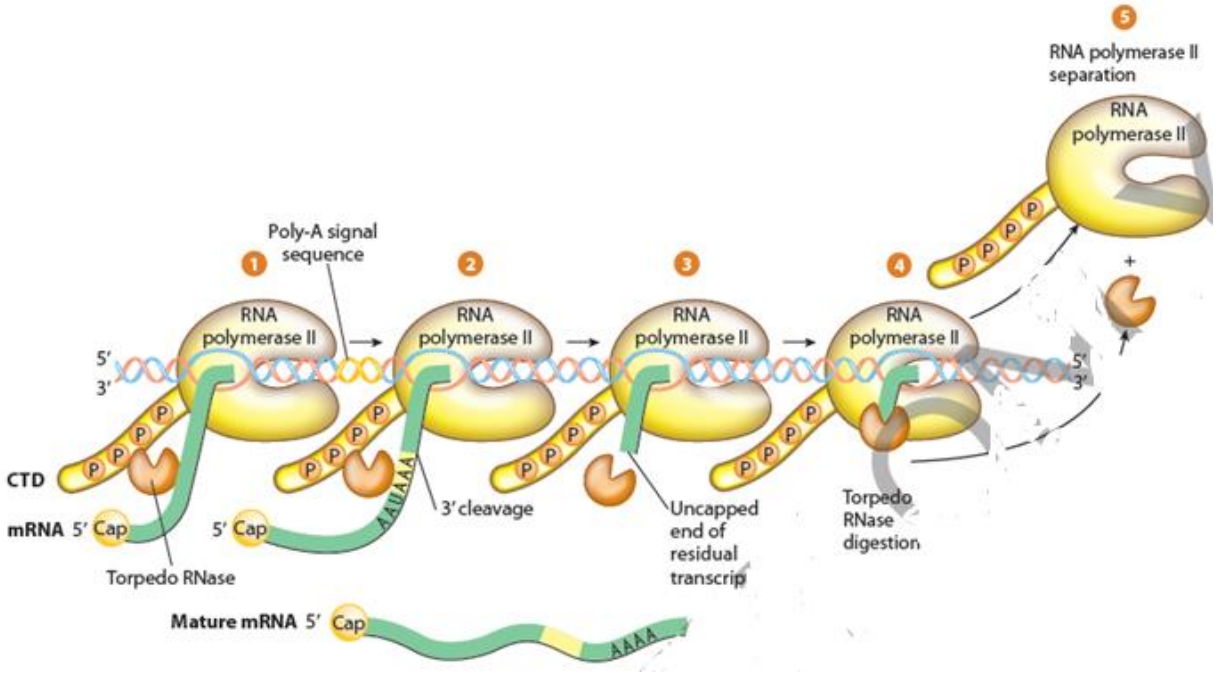
Elongation Phase

TFIIH has two functions:

1. It is a helicase, which means that it can use ATP to unwind the DNA helix, allowing transcription to begin.
2. In addition, it phosphorylates RNA polymerase II which causes this enzyme to change its conformation and dissociate from other proteins in the initiation complex.

- The key phosphorylation occurs on a long C-terminal tail called the C-terminal domain (CTD) of the RNA polymerase II molecule.
- Interestingly, only RNA polymerase II that has a non-phosphorylated CTD can initiate transcription but only an RNA polymerase II with a phosphorylated CTD can elongate RNA.
- RNA polymerase II now starts moving along the DNA template, synthesizing RNA, that is, the process enters the elongation phase.
- RNA synthesis occurs in the 5' → 3' direction with the RNA polymerase catalyzing a nucleophilic attack by the 3-OH of the growing RNA chain on the alpha-phosphorus atom on an incoming ribonucleoside 5-triphosphate.
- The RNA molecule made from a protein-coding gene by RNA polymerase II is called a primary transcript.

Termination Phase



- Elongation of the RNA chain continues until termination occurs.
- Unlike RNA polymerase in prokaryotes, RNA polymerase II does not terminate transcription at a specific site but rather transcription can stop at varying distances downstream of the gene.
- RNA genes transcribed by RNA Polymerse II lack any specific signals or sequences that direct RNA Polymerase II to terminate at specific locations.
- RNA Polymerase II can continue to transcribe RNA anywhere from a few bp to thousands of bp past the actual end of the gene.
- The transcript is cleaved at an internal site before RNA Polymerase II finishes transcribing. This releases the upstream portion of the transcript, which will serve as the initial RNA prior to further processing (the pre-mRNA in the case of protein-encoding genes.)
- This cleavage site is considered the “end” of the gene. The remainder of the transcript is digested by a 5′-exonuclease (called Xrn2 in humans) while it is still being transcribed by the RNA Polymerase II.

- When the 5′-exonulease “catches up” to RNA Polymerase II by digesting away all the overhanging RNA, it helps disengage the polymerase from its DNA template strand, finally terminating that round of transcription.

RNA processing

The primary eukaryotic mRNA transcript is much longer and localised into the nucleus, when it is also called heterogenous nuclear RNA (hnRNA) or pre- mRNA.

It undergoes various processing steps to change into a mature RNA:

Cleavage

- Larger RNA precursors are cleaved to form smaller RNAs.
- Primary transcript is cleaved by ribonuclease-P (an RNA enzyme) to form 5-7 tRNA precursors.

Capping and Tailing

- Initially at the 5′ end a cap (consisting of 7-methyl guanosine or 7 mG) and a tail of poly A at the 3′ end are added.
- The cap is a chemically modified molecule of guanosine triphosphate (GTP).

Splicing

- The eukaryotic primary mRNAs are made up of two types of segments; non-coding introns and the coding exons.
- The introns are removed by a process called RNA splicing where ATP is used to cut the RNA, releasing the introns and joining two adjacent exons to produce mature mRNA.

Nucleotide Modifications

- They are most common in tRNA-methylation (e.g., methyl cytosine, methyl guanosine), deamination (e.g., inosine from adenine), dihydrouracil, pseudouracil, etc.

Post-transcription processing is required to convert primary transcript into functional RNAs.

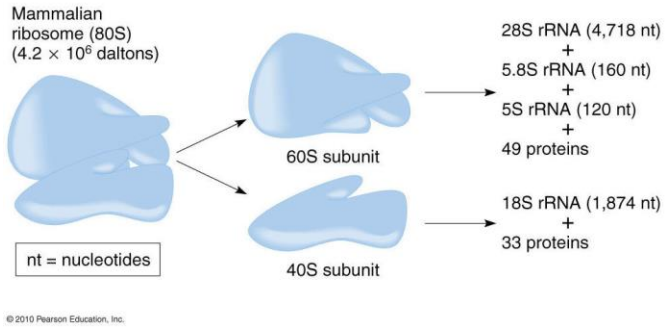
Significance

- Transcription of DNA is the method for regulating gene expression.
- It occurs in preparation for and is necessary for protein translation.

Translation (Protein Synthesis) in Eukaryotes

- Translation involves translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis.
- It is the process in which ribosomes in the cytoplasm or ER synthesize proteins after the process of transcription of DNA to RNA.

The Ribosomes



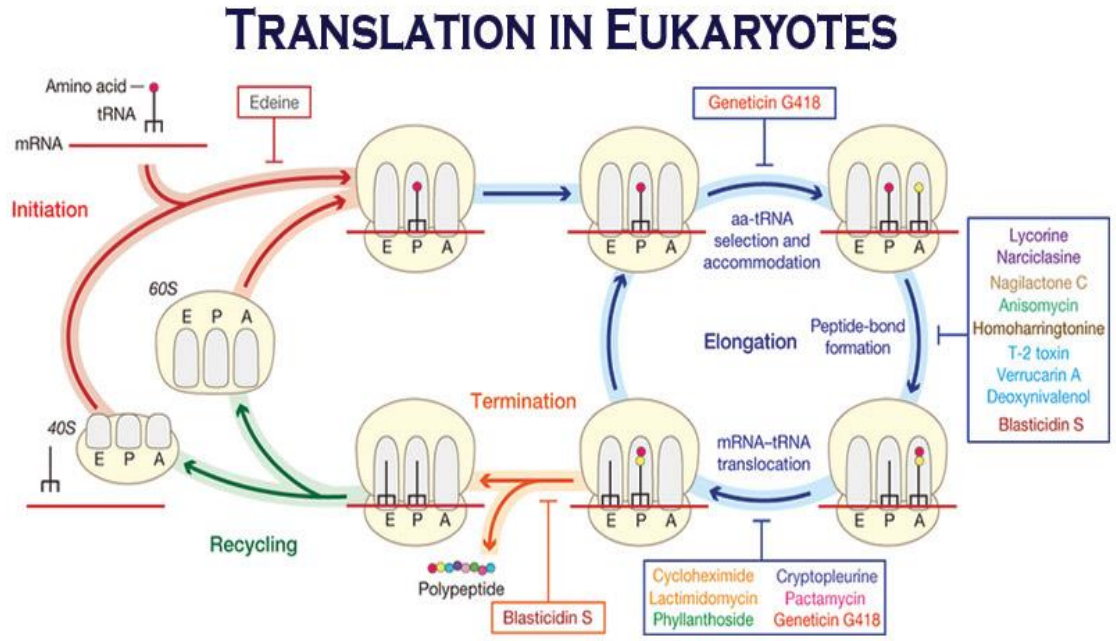
- Ribosomes exist normally as separate subunits that are composed of protein and rRNA.
- Eukaryotic ribosomes are larger (80S) and more complex than prokaryotic ribosomes (70S).
- The subunits come together to form a ribosome when they bind to an mRNA, near its 5' end.
- On binding to an mRNA, the ribosome reads the nucleotide sequence from the 5' to 3' direction, synthesizing the corresponding protein from amino acids in an N-terminal (amino-terminal) to C-terminal (carboxyl terminal) direction.
- Ribosomes are located in the cytosol, either freely floating or associated with the endoplasmic reticulum.
- They serve to synthesize proteins.

Ribosomal Sites for Protein Translation

Each prokaryotic ribosome, shown schematically, has three binding sites for tRNAs.

1. **The aminoacyl-tRNA binding site** (or A site) is where, during elongation, the incoming aminoacyl-tRNA binds.
2. **The peptidyl-tRNA binding site** (or P site) is where the tRNA linked to the growing polypeptide chain is bound.
3. **The exit site** (or E site) is a binding site for tRNA following its role in translation and prior to its release from the ribosome.

All three sites (A, P and E) are formed by the rRNA molecules in the ribosome.



THE PROCESS OF TRANSLATION

The overall mechanism of protein synthesis in eukaryotes is basically the same as in **prokaryotes**.

However, there are some significant differences:

- Whereas a prokaryotic ribosome has a sedimentation coefficient of 70S and subunits of 30S and 50S, a eukaryotic ribosome has a sedimentation coefficient of 80S with subunits of 40S and 60S.

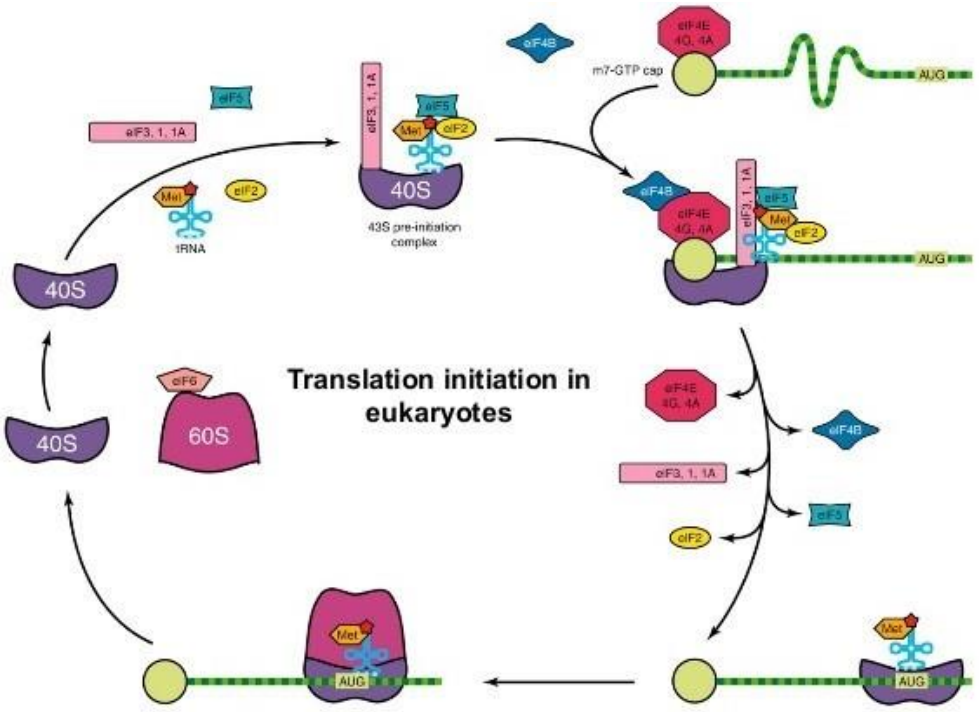
- The composition of eukaryotic ribosomal subunits is also more complex than prokaryotic subunits but the function of each subunit is essentially the same as in prokaryotes.
- In eukaryotes, each mRNA is monocistronic that is, discounting any subsequent post translational cleavage reactions that may occur; the mRNA encodes a single protein. In prokaryotes, many mRNAs are polycistronic that is they encode several proteins. Each coding sequence in a prokaryotic mRNA has its own initiation and termination codons.
- Initiation of protein synthesis in eukaryotes requires at least nine distinct eukaryotic initiation factors (eIFs) compared with the three initiation factors (IFs) in prokaryotes.
- In eukaryotes, the initiating amino acid is methionine, not N-formylmethionine as in prokaryotes.
- As in prokaryotes, a special initiator tRNA is required for initiation and is distinct from the tRNA that recognizes and binds to codons for methionine at internal positions in the mRNA. When charged with methionine ready to begin initiation, this is known as Met-$tRNA_i^{met}$
- The main difference between initiation of translation in prokaryotes and eukaryotes is that in bacteria, a Shine–Dalgarno sequence lies 5' to the AUG initiation codon and is the binding site for the 30S ribosomal subunit.
- In contrast, most eukaryotic mRNAs do not contain Shine–Dalgarno sequences. Instead, a 40S ribosomal subunit attaches at the 5' end of the mRNA and moves downstream (i.e. in a 5' to 3' direction) until it finds the AUG initiation codon. This process is called scanning.
- Prokaryotic translation requires no helicase, presumably because protein synthesis in bacteria can start even as the mRNA is still being synthesized whereas in eukaryotes, transcription in the nucleus and translation in the cytoplasm are separate events which allows time for mRNA secondary structure to form.

Protein synthesis (or translation) takes place in three stages:

1. Initiation

2. Elongation and
3. Termination

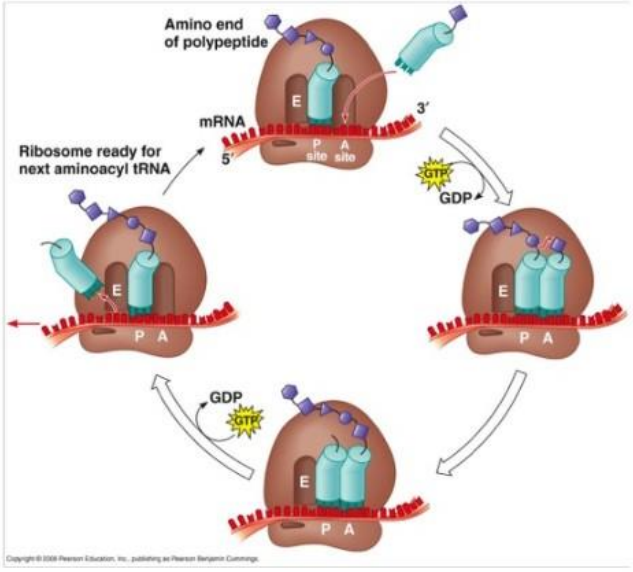
Initiation of Protein Synthesis



- The first step is the formation of a pre-initiation complex consisting of the 40S small ribosomal subunit, Met-$tRNA_i^{met}$, eIF-2 and GTP.
- The pre-initiation complex binds to the 5' end of the eukaryotic mRNA, a step that requires eIF-4F (also called cap binding complex) and eIF-3.
- The eIF-4F complex consists of eIF-4A, eIF-4E, and eIF-4G; eIF-4E binds to the 5' cap on the mRNA whilst eIF-4G interacts with the poly (A) binding protein on the poly (A) tail.
- The eIF-4A is an ATP-dependent RNA helicase that unwinds any secondary structures in the mRNA, preparing it for translation.
- The complex then moves along the mRNA in a 5' to 3' direction until it locates the AUG initiation codon (i.e. scanning).

- The 5’ untranslated regions of eukaryotic mRNAs vary in length but can be several hundred nucleotides long and may contain secondary structures such as hairpin loops. These secondary structures are probably removed by initiation factors of the scanning complex.
- The initiation codon is usually recognizable because it is often (but not always) contained in a short sequence called the **Kozak consensus** (5’-ACCAUGG-3’).
- Once the complex is positioned over the initiation codon, the 60S large ribosomal subunit binds to form an 80S initiation complex, a step that requires the hydrolysis of GTP and leads to the release of several initiation factors.

Elongation of Protein Synthesis



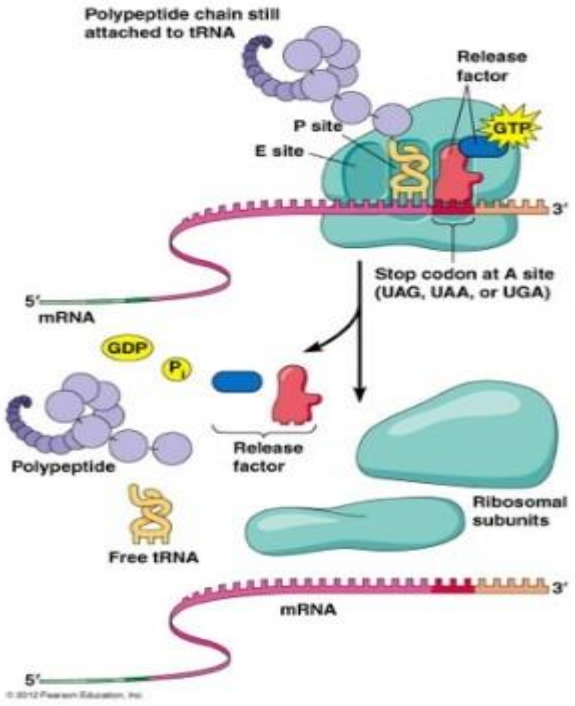
- Elongation depends on eukaryotic elongation factors.
- Three elongation factors, eEF-1A, eEF-IB and eEF-2, are involved which have similar functions to their prokaryotic counterparts EF-Tu, EF-Ts and EF-G.
- At the end of the initiation step, the mRNA is positioned so that the next codon can be translated during the elongation stage of protein synthesis.

- The initiator tRNA occupies the P site in the ribosome, and the A site is ready to receive an aminoacyl-tRNA.
- During chain elongation, each additional amino acid is added to the nascent polypeptide chain in a three-step microcycle.
- The steps in this microcycle are:

1. Positioning the correct aminoacyl-tRNA in the A site of the ribosome,
2. Forming the peptide bond and
3. Shifting the mRNA by one codon relative to the ribosome.

- Although most codons encode the same amino acids in both prokaryotes and eukaryotes, the mRNAs synthesized within the organelles of some eukaryotes use a variant of the genetic code.
- During elongation in bacteria, the deacylated tRNA in the P site moves to the E site prior to leaving the ribosome. In contrast, although the situation is still not completely clear, in eukaryotes the deacylated tRNA appears to be ejected directly from the ribosome.

Termination of Protein Synthesis



- Termination of elongation depends on eukaryotic release factors.
- In eukaryotes, eukaryotic release factor eRF-1 recognizes all three termination codons (UAA, UAG and UGA) and, with the help of protein eRF-3, terminates translation.
- Upon termination, the ribosome is disassembled and the completed polypeptide is released.

Prokaryotic Translation (Protein Synthesis)

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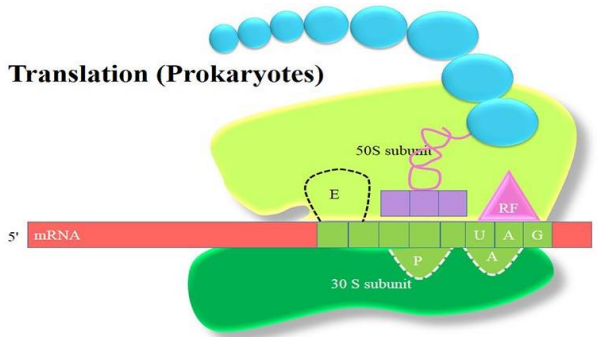

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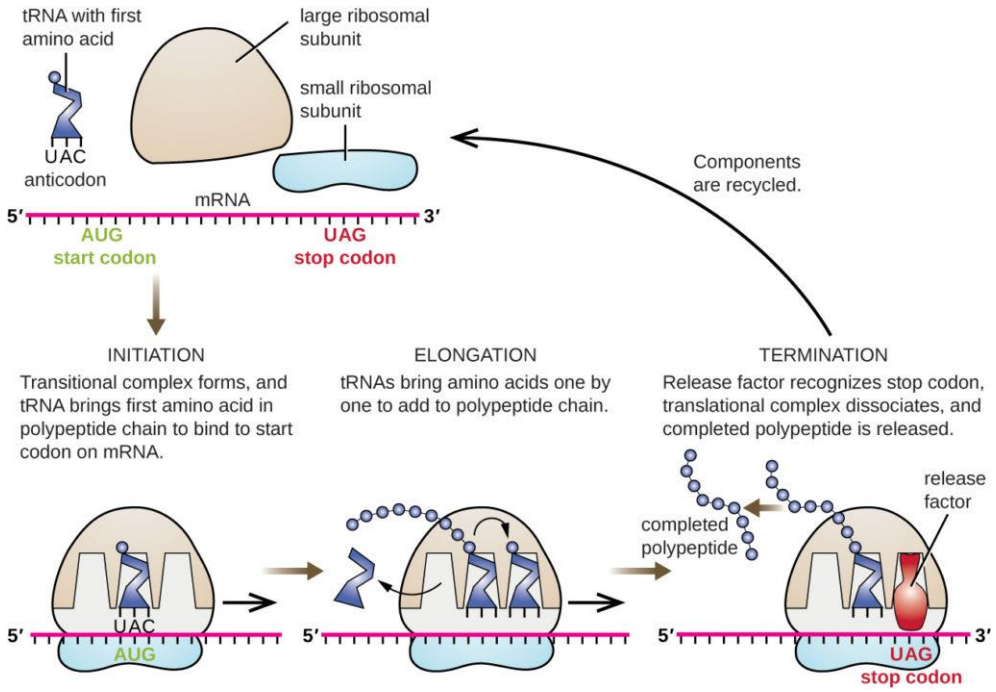
THE PROCESS OF TRANSLATION



Protein synthesis (or translation) takes place in three stages:

1. Initiation
2. Elongation and
3. Termination.

- During initiation, the mRNA–ribosome complex is formed and the first codon (always AUG) binds the first aminoacyltRNA (called initiator tRNA).
- During the elongation phase, the other codons are read sequentially and the polypeptide grows by addition of amino acids to its C-terminal end.
- This process continues until a termination codon (Stop codon), which does not have a corresponding aminoacyl-tRNA with which to base pair, is reached.
- At this point, protein synthesis ceases (termination phase) and the finished polypeptide is released from the ribosome.

Synthesis of aminoacyl-tRNA

- Synthesis of aminoacyl-tRNAs is crucially important for two reasons:

1. Each amino acid must be covalently linked to a tRNA molecule in order to take part in protein synthesis, which depends upon the 'adaptor' function of tRNA to ensure that the correct amino acids are incorporated.
2. The covalent bond that is formed between the amino acid and the tRNA is a high energy bond that enables the amino acid to react with the end of the growing polypeptide chain to form a new peptide bond.

For this reason, the synthesis of aminoacyl-tRNA is also referred to as **amino acid activation.**

- Each tRNA molecule has a cloverleaf secondary structure with the anticodon accessible at the end of the anticodon stem loop.
- During synthesis of the aminoacyl-tRNA, the amino acid is covalently bound to the A residue of the CCA sequence at the 3' end.
- Each tRNA molecule carries only a single amino acid.
- The attachment of an amino acid to a tRNA is catalyzed by an enzyme called**aminoacyl-tRNA synthetase.**
- A separate aminoacyl-tRNA synthetase exists for every amino acid, making 20 synthetases in total.

The synthesis reaction occurs in two steps.

1. The first step is the reaction of an amino acid and ATP to form an aminoacyl-adenylate (also known as aminoacyl-AMP).
2. In the second step, without leaving the enzyme, the aminoacyl group of aminoacyl-AMP is transferred to the 3' end of the tRNA molecule to form aminoacyl-tRNA

The overall reaction is:

Amino acid + ATP + tRNA → aminoacyl-tRNA + AMP + PP_i

Initiation of Protein Synthesis

- The first codon translated in all mRNAs is the start codon or initiation codon, AUG which codes for methionine.
- Two different tRNAs are used for the two types of AUG codon; $tRNA_f^{Met}$ is used for the initiation codon and is called the initiator tRNA whereas $tRNA_m^{Met}$ is used for internal AUG codons.
- In prokaryotes the first amino acid of a new protein is N-formylmethionine (abbreviated fMet). Hence the aminoacyl-tRNA used in initiation is fMet-$tRNA_f^{Met}$.
- A short sequence rich in purines (5'-AGGAGGU-3'), called the **Shine–Dalgarno sequence**, lies 5' to the AUG initiation codon and is complementary to part of the 16S rRNA in the small ribosomal subunit.
- Therefore this is the binding site for the 30S ribosomal subunit which then migrates in a 3' direction along the mRNA until it encounters the AUG initiation codon.
- Initiation of protein synthesis requires proteins called initiation factors (IFs).
- In prokaryotes, three initiation factors (IF-1, IF-2 and IF-3) are essential.
- Because of the complexity of the process, the exact order of binding of IF-1, IF-2, IF-3, fMet-tRNAf is controversial.

Steps Involved

1. Initiation begins with the binding of IF-1 and IF-3 to the small (30S) ribosomal subunit.
- Their role is to stop the 30S subunit binding to the 50S subunit in the absence of mRNA and fMet-$tRNA_f^{Met}$ which would result in a nonfunctional ribosome.

2. The small subunit then binds to the mRNA via the Shine–Dalgarno sequence and moves 3’ along the mRNA until it locates the AUG initiation codon.
3. The initiator tRNA charged with N-formylmethionine and in a complex with IF-2 and GTP (fMet-tRNAfMet/IF-2/GTP) now binds.
4. IF-3 is released.
5. The complex of mRNA, fMet-tRNAf Met, IF-1, IF-2 and the 30S ribosomal subunit is called the 30S initiation complex.
6. The large (50S) ribosomal subunit now binds, with the release of IF-1 and IF-2 and hydrolysis of GTP, to form a 70S initiation complex.

Elongation of Protein Synthesis

- At the start of the first round of elongation, the initiation codon (AUG) is positioned in the P site with fMet-tRNA$_f^{Met}$ bound to it via codon–anticodon base pairing.
- The next codon in the mRNA is positioned in the A site.
- Elongation of the polypeptide chain occurs in three steps called the elongation cycle, namely aminoacyl-tRNA binding, peptide bond formation and translocation:

Aminoacyl-tRNA binding

- The corresponding aminoacyl-tRNA for the second codon binds to the A site via codon–anticodon interaction.
- Binding of the aminoacyl-tRNA requires elongation factor EF-Tu and GTP which bind as an aminoacyl-tRNA/EF-Tu/GTP complex.
- Following binding, the GTP is hydrolyzed and the EF-Tu is released, now bound to GDP.
- Before the EF-Tu molecule can catalyze the binding of another charged tRNA to the ribosome, it must be regenerated by a process involving another elongation factor, EF-Ts.

This regeneration is called the EF-Tu–EF-Ts exchange cycle.

- First, EF-Ts binds to EF-Tu and displaces the GDP. Then GTP binds to the EF-Tu and displaces EF-Ts. The EF-Tu-GTP is now ready to take part in another round of elongation.

Peptide bond formation

- The second step, peptide bond formation, is catalyzed by peptidyl transferase.
- In this reaction the carboxyl end of the amino acid bound to the tRNA in the P site is uncoupled from the tRNA and becomes joined by a peptide bond to the amino group of the amino acid linked to the tRNA in the A site.

Translocation

- In the third step, a complex of elongation factor EF-G (also called translocase) and GTP (i.e. EF-G/GTP) binds to the ribosome.
- Three concerted movements now occur, collectively called translocation:

1. the deacylated tRNA moves from the P site to the E site
2. the dipeptidyl-tRNA in the A site moves to the P site, and
3. the ribosome moves along the mRNA (5' to 3') by three nucleotides to place the next codon in the A site.

- During the translocation events, GTP is hydrolyzed to GDP and inorganic phosphate, and EF-G is released ready to bind more GTP for another round of elongation.
- After translocation, the A site is empty and ready to receive the next aminoacyltRNA.
- The A site and the E site cannot be occupied simultaneously. Thus the deacylated tRNA is released from the E site before the next aminoacyl-tRNA binds to the A site to start a new round of elongation.
- Elongation continues, adding one amino acid to the C-terminal end of the growing polypeptide for each codon that is read, with the peptidyl-tRNA moving back and forth from the P site to the A site as it grows.

Termination of Protein Synthesis

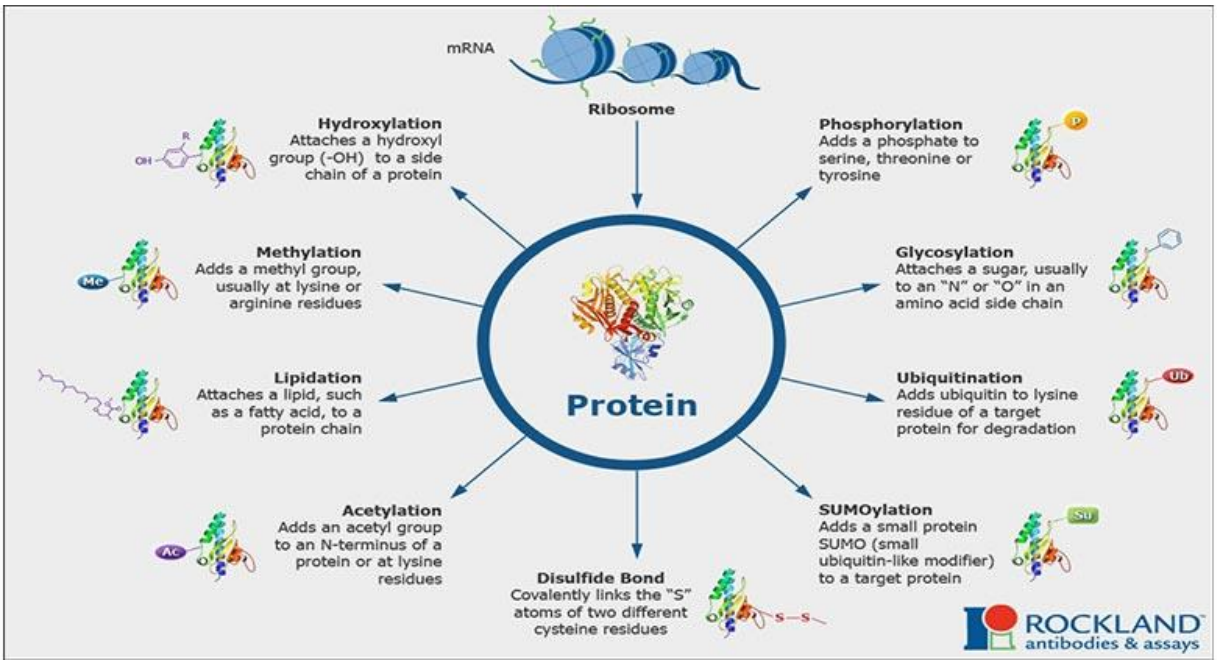
- Eventually, one of three termination codons (also called Stop codons) becomes positioned in the A site. These are UAG, UAA and UGA.
- Unlike other codons, prokaryotic cells do not contain aminoacyl-tRNAs complementary to

- Stop codons. Instead, one of two release factors (RF-1 and RF-2) binds instead.
- RF-1 recognizes UAA and UAG whereas RF-2 recognizes UAA and UGA. A third release factor, RF-3, is also needed to assist RF-1 or RF-2 interaction with the ribosome. Thus either RF-1 + RF-3 or RF-2 + RF-3 bind depending on the exact termination codon in the A site.
- RF-1 (or RF-2) binds at or near the A site whereas RF-3/GTP binds elsewhere on the ribosome.
- The release factors cause the peptidyl transferase activity to transfer the polypeptide to a water molecule instead of to aminoacyl-tRNA, effectively cleaving the bond between the polypeptide and tRNA in the P site.

The free polypeptide now leaves the ribosome, followed by the mRNA and free tRNA, and the ribosome dissociates into 30S and 50S subunits ready to start translation again.

Post Translational Modification

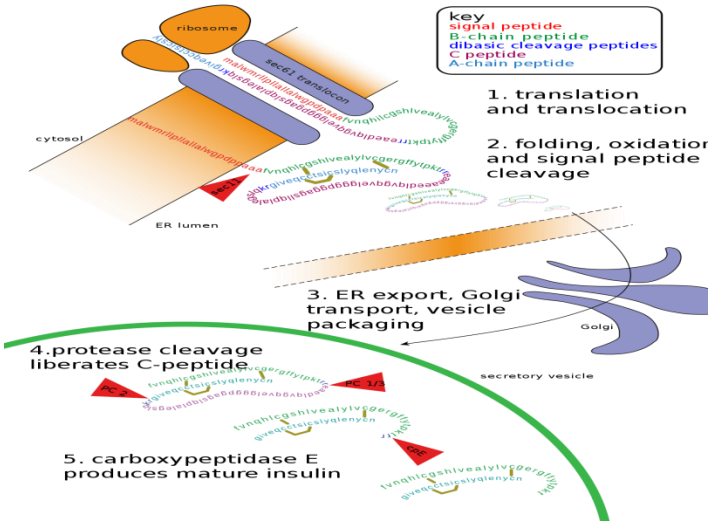
- Post translational modifications refer to any alteration in the **amino acid**sequence of the protein after its synthesis.
- It may involve the modification of the amino acid side chain, terminal amino or carboxyl group by means of covalent or enzymatic means following protein biosynthesis.
- Generally, these modifications influence the structure, stability, activity, cellular localization or substrate specificity of the protein.
- Post translational modification provides complexity to proteome for diverse function with limited number of genes.



Location

Post-translational modifications (PTMs) mainly occur in the endoplasmic reticulum of the cell but sometimes continue in the golgi bodies as well.

Post-translational processing



After synthesis is completed, proteins can be modified by various methods such as phosphorylation, glycosylation, ADP ribosylation, hydroxylation, and addition of other groups.

1. Proteolysis

As the newly synthesized protein is released in the lumen of the ER, signal peptidases cleave peptide sequence. Apart from signal peptide, some polypeptide sequence of the protein is also cleaved resulting in the final sequence.

Example:

Insulin is synthesized in the cells in its inactive form which cannot perform its function. Post translational modifications ensure proper function which involves the removal of the part of protein to convert it into a three dimensional and fully active form.

2. Phosphorylation

Phosphoryalation is the addition of one or more phosphate groups to the protein. Post Translational Phosphorylation is one of the most common protein modifications that occur in animal cells. Majority of phosphorylation occurs as a mechanism to regulate the biological activity of a protein. In animal cells Serine, tyrosine and thereonine are the amino acids that subjected to the phosphorylation.

3.Glycosylation

Glycosylation is the addition of carbohydrate molecules to the polypeptide chain and modifying it into glycoproteins. Many of the proteins that are destined to become a part of plasma membrane or to be secreted from the cell, have carbohydrate chains attached to the amide nitrogen of asparagine(N linked) or the hydroxyl groups of serine, threonine(O linked). N glycosylation occurs in ER and O glycosylation occurs in the golgi complex.

4. Sulfation

Sulfate modidication takes place by the addition of sulphate molecules and these modifications of proteins occurs at tyrosine residues. Tyrosine sulfation accomplished via the activity of tyrosylproteinsulfotransferases (TPST) which are membrane associated enzymes of trans-Golgi network. There are two known TPSTs. TPST-1 TPST-2 The universal phosphate donor is 3'-phosphoadenosyl- 5'-phosphosulphate (PSPA).

5. Methylation

The transfer of one-carbon methyl groups to nitrogen or oxygen to amino acid side chains increases the hydrophobicity of the protein and can neutralize a negative amino acid charge when bound to carboxylic acids. Methylation is mediated by methyltransferases and S-adenosyl methionine (SAM) is the primary methyl group donor.

6. Hydroxylation

The biological process of addition of a hydroxy group to a protein amino acid is called Hydroxylation. Protein hydroxylation is one type of PTM that involves the conversion of –CH group into –COH group and these hydroxylated amino acids are involved in the regulation of some important factors called transcription factors. Among the 20 amino acids, the two amino acids regulated by this method are proline and lysine.

7. Others

a) SUMOylation

SUMO (small ubiquitin related modifier) proteins are 100 amino acid residue proteins which bind to the target protein in the same way as ubiquitin. They also confer the transcription regulatory activity of the protein and help in the transport of the target protein from cytosol to the nucleus.

b) Disulfide bond formation

Stabilizes protein structure and involved in redox processes.

c) Lipidylation, Acetylation, Prenylation etc.

Significance

Proteins are synthesized by ribosomes translating mRNA into polypeptide chains, which may then undergo modifications to form the mature protein product.

Post-translational modifications of proteins, which are not gene- template based, can regulate the protein functions, by causing changes in protein activity, their cellular locations and dynamic interactions with other proteins.

PTMs have significant biological functions which include:

- Aids in proper protein folding – few lectin molecules called calnexin binds to glycosylated proteins and assist in its folding.
- Confers stability to the protein- glycosylation can modify the stability of the protein by increasing protein half life.
- It protects the protein against cleavage by proteolytic enzyme by blocking the cleavage sites.
- Protein sorting or translocation- If phosphorylated mannose residues are present in the protein it always goes to lysosome.
- It regulates protein activity and function- phosphorylation of protein is a reversible PTM which activates the protein.
- Acetylation regulates many diverse functions, including DNA recognition, protein-protein interaction and protein stability.
- Redox-dependent PTM of proteins is emerging as a key signaling system conserved through evolution, influences many aspects of cellular homeostasis.
- PTMs are important components in cell signaling, as for example when prohormones are converted to hormones.
- It significantly increases the diversity and complexity in the proteome.

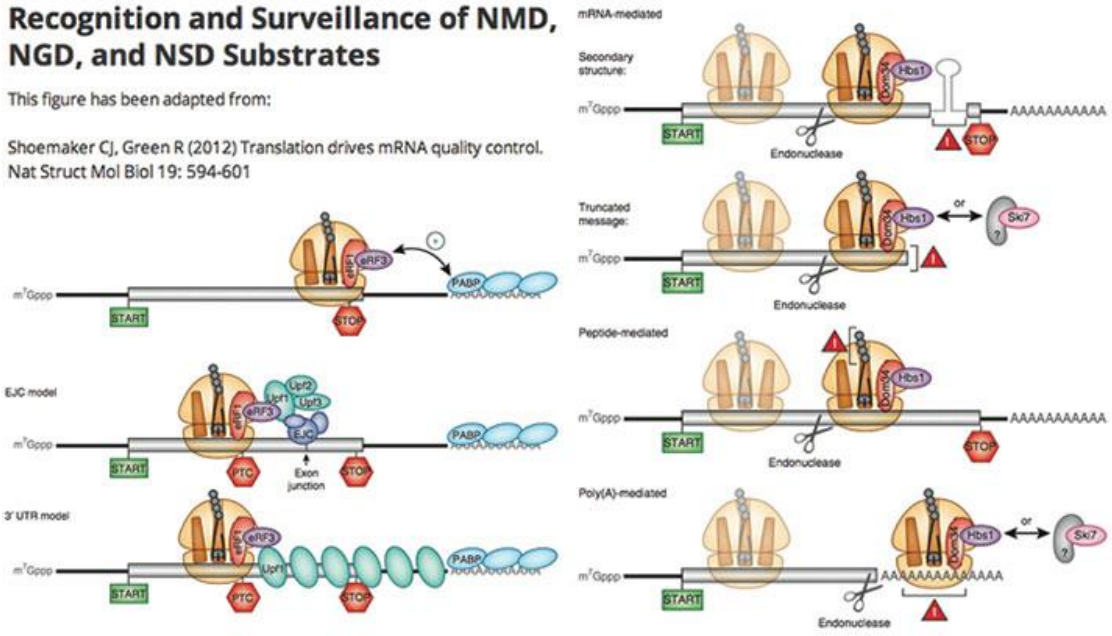
Regulation of Translation In Eukaryotes

- Translational regulation refers to the control of the levels of protein synthesized from its mRNA.
- In eukaryotes, regulation of protein synthesis can occur by modification of DNA or at the level of transcription within the nucleus, processing of mRNA in the nucleus, or translation in the cytoplasm.

A. Regulation through Changes in Genes

1. **Genes can be lost** (or partially lost) from cells so that functional proteins can no longer be produced (e.g., during differentiation of red blood cells).

2. **Genes can be amplified**. For example, the drug methotrexate causes hundreds of copies of the gene for the enzyme dihydrofolate reductase to be produced, which results in resistance to the drug.
3. **Segments of DNA can move** from one location to another on the genome, associating with each other in various ways so that different proteins are produced. **Example:** A number of different potential sequences (or arrangements) occur for various portions of an antibody-producing gene.
4. **Modification of the bases in DNA** affects the transcriptional activity of a gene.
    - Cytosine can be methylated at its 5 position, which often occurs in CpG islands within promoter regions.
    - The greater the extent of methylation, the less readily a gene is transcribed.



B. Regulation of the level of trancription

1. **Histones,** which are small, basic proteins associated with the DNA of eukaryotes, act as non-specific repressors.
    - Histone acetyltransferases, or acetylases (HAT or HAC) will acetylate lysine side chains on histones, which reduces the charge attraction between histones and DNA.

- Histone deacetylases (HDAC) will remove the acetate groups from histones, thereby allowing histones to reassociate with the DNA.
- Heterochromatin is the tight association of histones and DNA, and represents the transcriptionally inactive areas of the genome.
- Euchromatin refers to the transcriptionally active areas of the genome in which histone association with the DNA has been reduced.

2. The expression of specific genes is stimulated by **positive mechanisms**.
3. **Inducers** (e.g., steroid hormones) enter cells, bind to protein receptors, interact with chromatin in the nucleus, and activate specific genes.
4. Some genes have more than one **promoter**. Thus, the promoter that is used can differ under varying physiologic conditions or in different cell types.

C. Regulation by Chromatin Remodeling

Nucleosome displacement such that transcription can occur

1. An ATP-driven chromatin remodeling complex will bind to the regions of DNA that contain acetylated histones. Bromodomains on proteins within the complex recognize the acetylated histones. Once bound, using ATP as an energy source, the complex will move and displace histones to free up an area of DNA for transcription.
2. Histone acetylase activity is often associated with transcription factors that bind to the region of DNA that needs to be transcribed, facilitating the removal of histones from the DNA and binding of the transcription apparatus.

D. Regulation during processing and transport of mRNA

Regulatory mechanisms that occur during capping, polyadenylation, and splicing can alter the amino acid sequence or the quantity of the protein produced from the mRNA.

Editing of mRNA also occurs, and the rate of degradation of mRNA is also regulated.

1. **Alternative splice sites** can be used to produce different mRNAs.
2. **Alternative polyadenylation** sites can be used to generate different mRNAs.

3. **mRNAs can be degraded** by nucleases after their synthesis in the nucleus and before their translation in the cytoplasm.
4. **RNA editing** involves the alteration ("editing") of bases in mRNA after transcription.
5. **Small and interfering RNA** (SiRNA)
    - Gene silencing can occur through the use of small RNA products (miRNA), which can either block the translation of a target mRNA or induce degradation of the target mRNA.
    - miRNA molecules are the products of many genes scattered throughout the chromosome, some even located in the introns of the genes they regulate. miRNAs are synthesized in the nucleus and processed to form an active molecule that will bind to the target RNA and ablate its expression.

E. Regulation at the translational level

It occurs during the initiation or elongation reactions.

1. **Heme** stimulates the synthesis of globin by preventing the phosphorylation and consequent inactivation of eIF-2, a factor involved in the initiation of protein synthesis.
2. **Interferon** stimulates the phosphorylation of eIF-2, causing inhibition of initiation.
3. **Iron-response elements** (IREs) in mRNA for ferritin (an iron storage protein) and the transferrin receptor regulate translation of the respective mRNAs. These elements either destabilize the mRNA (transferrin receptor) or allow translation of the mRNA (ferritin) when iron levels are high.

Significance

Translational control in eukaryotic cells is critical for gene regulation during nutrient deprivation and stress, development and differentiation, nervous system function, aging, and disease.