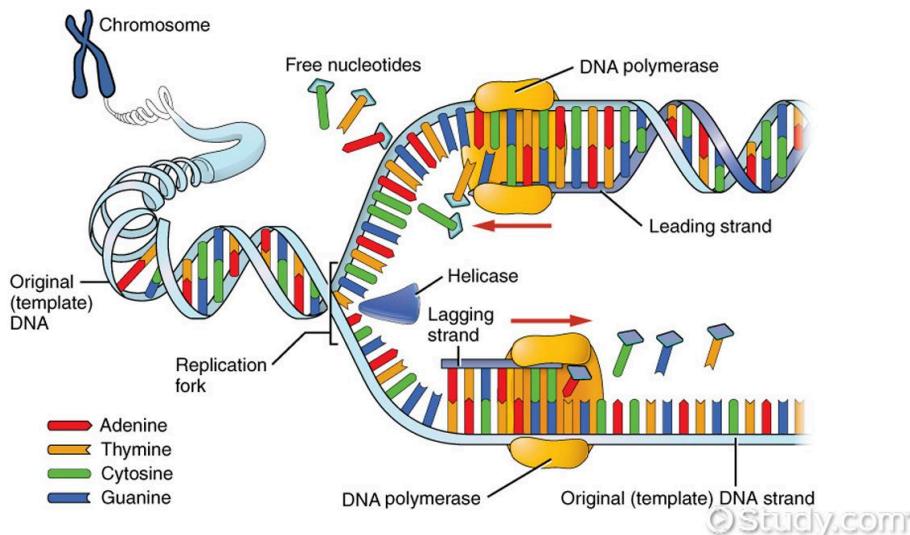
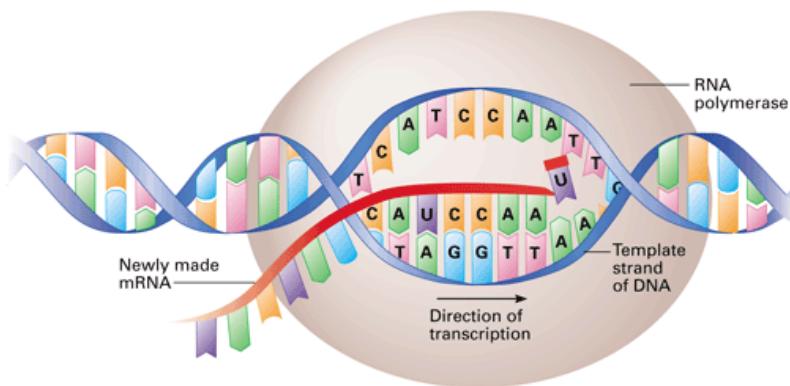


1. **gDNA (Genomic DNA):** Includes both **exons (coding regions)** and **introns (non-coding regions)**. If you're amplifying from gDNA, **primers can be designed to target exonic regions**, but the **amplicon will include both exons and introns**, which can result in very large fragments if the introns are long. You may need multiple primer pairs to amplify large genes.
2. **cDNA (Complementary DNA):** Made from mRNA, so it only includes **exons (no introns)**. Primers should target exonic regions, usually spanning **exon-exon junctions** to avoid amplifying any contaminating gDNA.
3. **Primer Design:**
  - **gDNA:** Primers amplify exons + introns.
  - **cDNA:** Primers amplify only exons.
  - Use **exon-exon junction primers** for cDNA to avoid gDNA contamination.

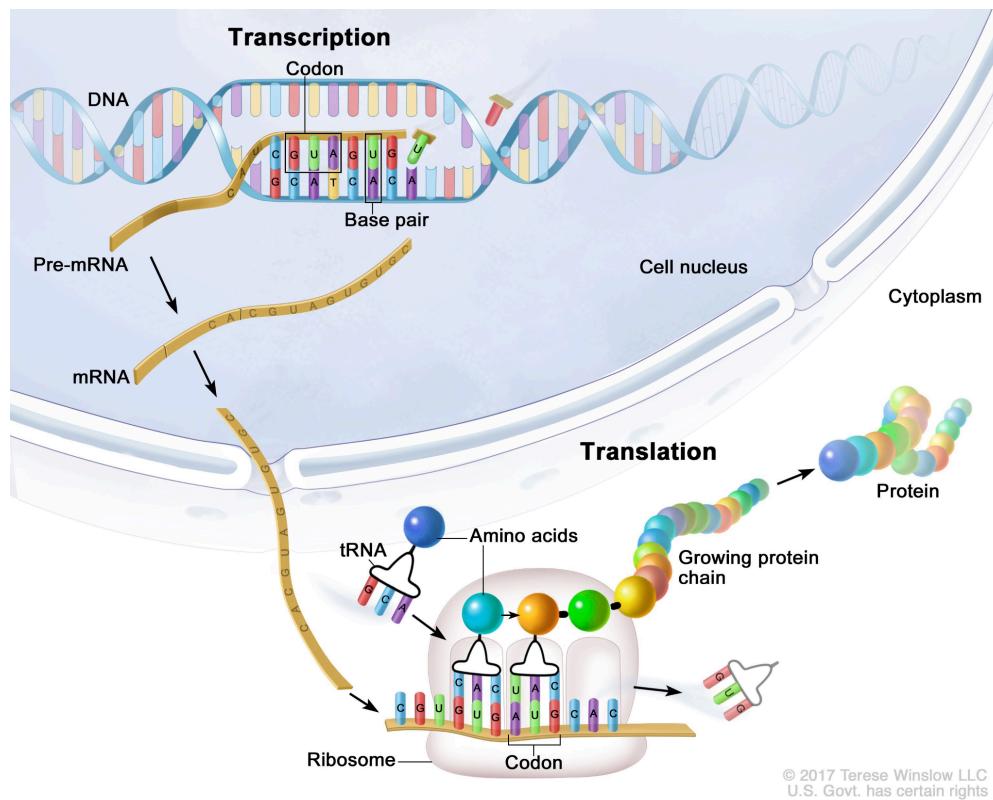
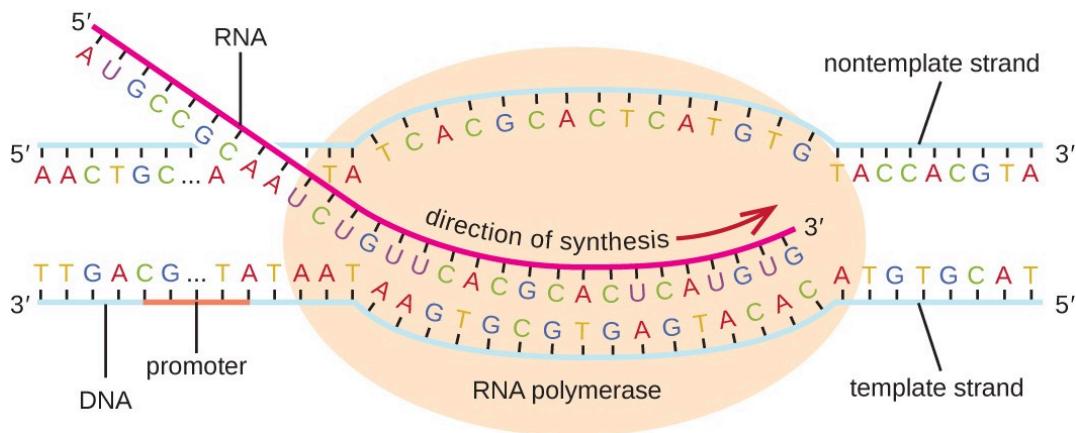


Identify the process in these Images:



**Unlike DNA replication, which copies the entire genome, transcription only copies specific parts of the genome. Unlike DNA polymerase, RNA polymerase doesn't need primase to start; it is DNA-dependent.**

**Transcription:** the process by which a cell makes an **RNA copy of a piece of DNA**. **RNA Polymerase:** RNA polymerases are DNA-dependent because they use a DNA template to synthesize RNA. Just like DNA polymerase uses a DNA strand to create a complementary strand, RNA polymerase uses the DNA template to create a complementary RNA strand. The RNA is synthesized in the  $5' \rightarrow 3'$  direction, with uracil (U) replacing thymine (T) in base pairing.



**Transcription and translation** are processes that turn **DNA into proteins**. DNA and RNA are made up of four building blocks (C, A, T/U, and G).

- **Transcription:** In the **cell's nucleus**, a gene in DNA is copied into mRNA.
- **Translation:** The mRNA moves to the **cytoplasm**, where ribosomes read the mRNA. tRNA brings amino acids to the ribosome, matching its sequence to the mRNA. The

ribosome links the amino acids together to form a protein. Once all amino acids are joined, the protein is complete and released.

**RNA**, made up of nucleotides (adenine, guanine, cytosine, and uracil), mostly exists as a **single strand** and can vary in length and structure. RNA viruses use RNA instead of DNA for genetic material and can cause diseases. **Transcription is the process of making RNA from DNA, and translation is the process of making proteins from RNA.**

#### Types of RNA:

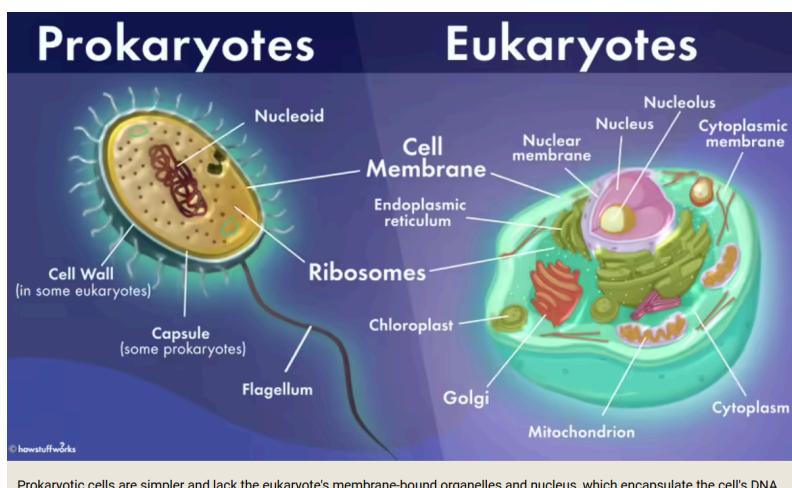
1. **mRNA**: Carries the **genetic code from DNA for protein creation**. In eukaryotes, **mRNA is processed (introns are removed, exons joined, and caps/tails are added)** before being used in protein synthesis.
2. **tRNA**: Brings **amino acids to the ribosome to build proteins**, matching mRNA codons with specific amino acids.
3. **rRNA**: **Forms ribosomes, which assemble proteins** from amino acids.

#### RNA Mutations:

RNA mutations can cause diseases like myotonic dystrophy, spinal muscular atrophy, and several other conditions. RNA viruses have high mutation rates, making them evolve quickly and resist treatments, as seen in diseases like HIV and influenza.

#### RNA Functions:

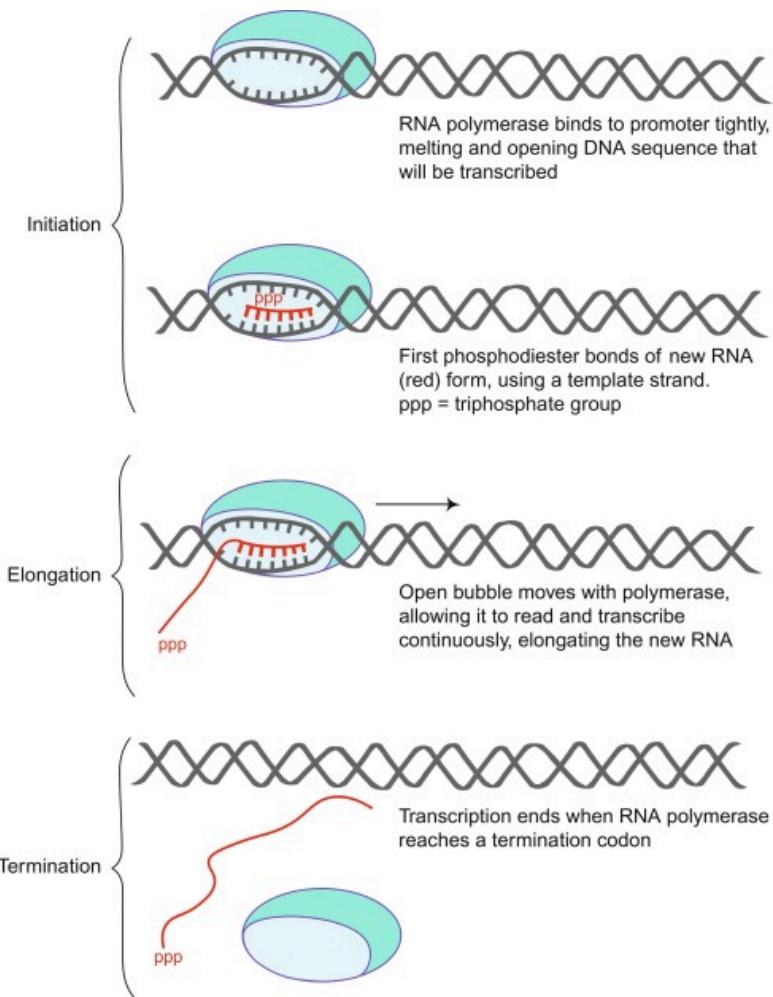
RNA helps in protein synthesis, gene regulation, and RNA interference. Small nuclear RNAs (**snRNA**) help splice introns, **microRNAs (miRNA)** regulate gene expression, and small interfering RNAs (**siRNA**) silence genes.



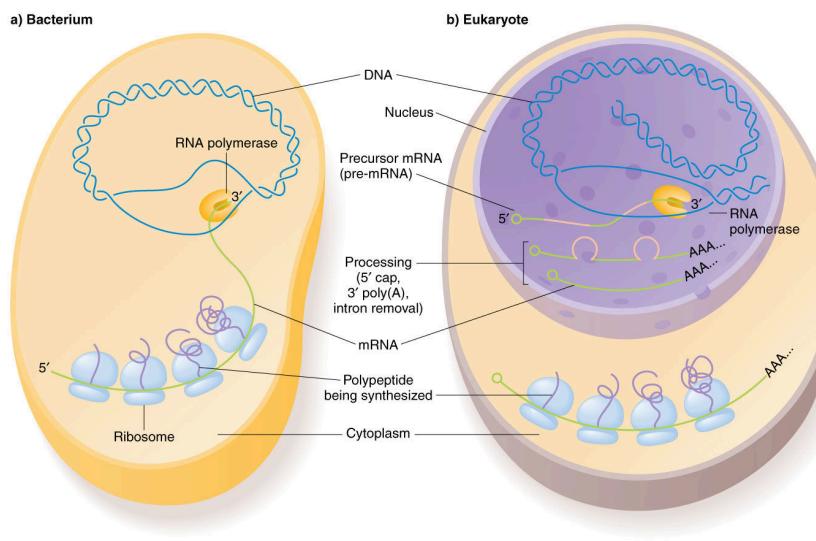
In **prokaryotes** (organisms without a nuclear membrane), **DNA replication, transcription, and RNA translation all happen in the same cell** and can occur at the same time.

In **eukaryotes** (organisms with a nuclear membrane), **DNA replication and transcription happen in the nucleus, while protein production occurs in the cytoplasm**. RNA has to move out of the nucleus before it can be translated. This separates transcription and translation.

#### STEPS in Transcription



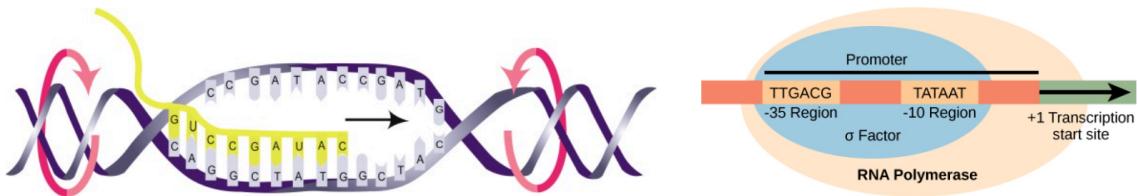
### Transcription in Prokaryotes vs Eukaryotes



### Transcription in Prokaryotes:

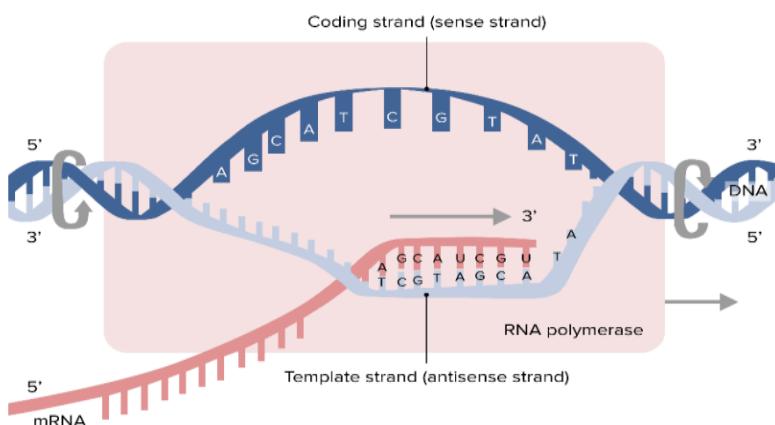
#### 1. Initiation: Transcription Bubble

- **Topoisomerase** relaxes both positive (before replication bubble) and negative supercoiling (after) of DNA. **RNA polymerase follows the template strand** around the duplex, so that it can avoid any supercoiling of DNA
- **RNA Polymerase** binds to the **promoter region** on the DNA (in E. coli, it recognizes -35 and -10 regions).
- The RNA polymerase unwinds the DNA to form an "open complex."
- It starts making a short RNA. **If it makes more than 10 nucleotides, it leaves the initiation phase and continues.**



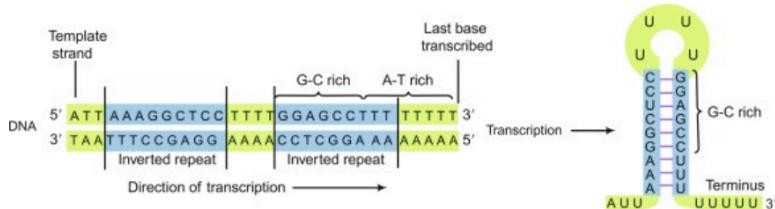
## 2. Elongation:

- After the **RNA is long enough, the σ-factor is released**, and **RNA polymerase moves along the DNA** to make the RNA in the 5' to 3' direction.
- **The RNA exits the polymerase.**

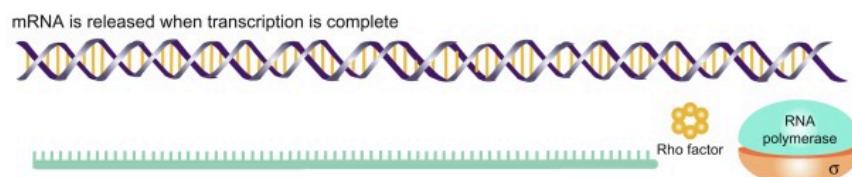
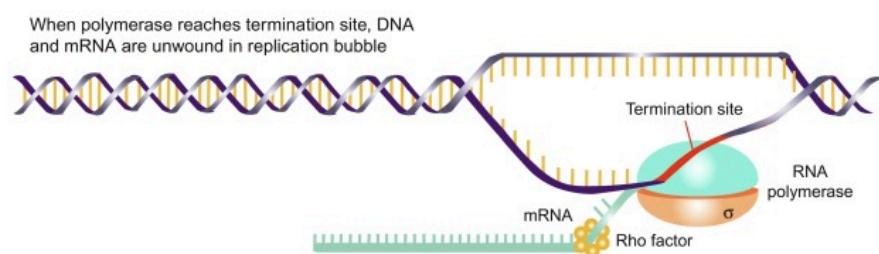
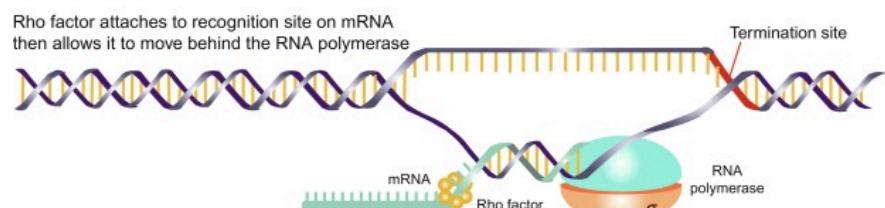
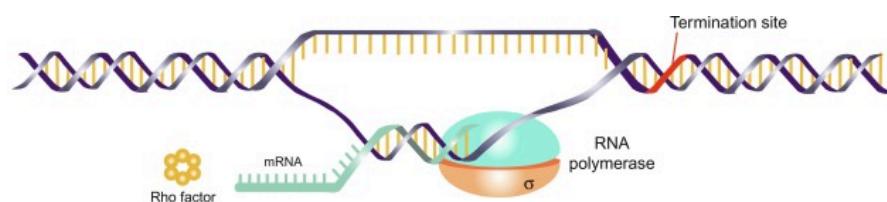
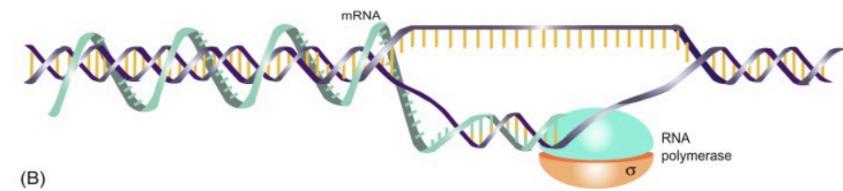
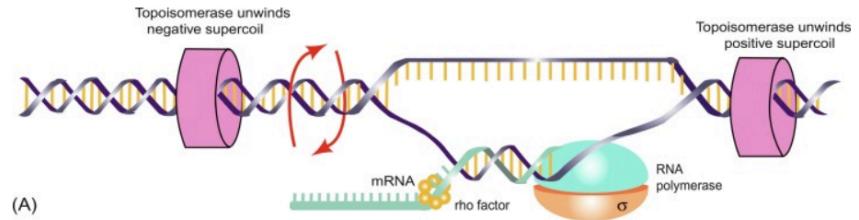


## 3. Termination:

- **Rho-Independent:** The RNA forms a hairpin structure due to a GC-rich sequence, causing it to detach from the DNA and stop transcription.



- **Rho-Dependent:** The **Rho protein** moves up the RNA, reaches the RNA polymerase, and **causes it to release the RNA, stopping transcription.**



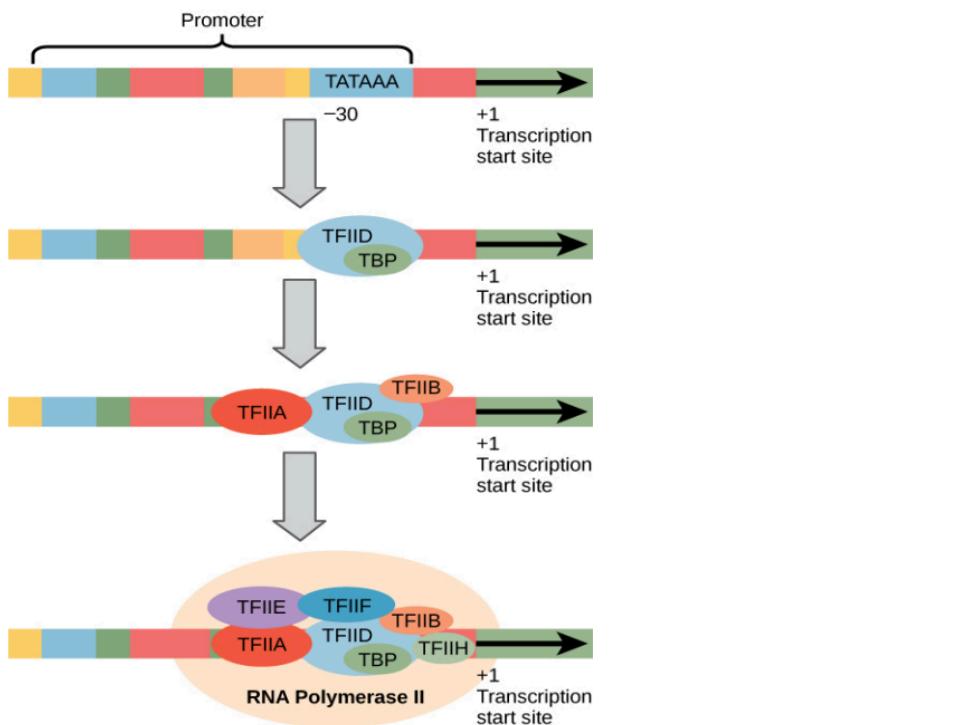
### Transcription in Eukaryotes:

### 1. Steps of Transcription: Initiation:

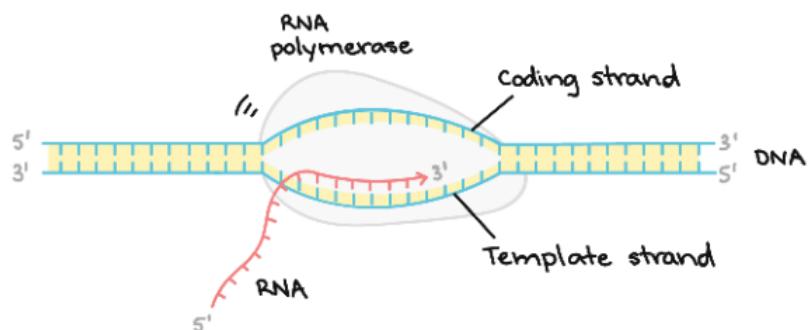
The TATA-binding protein (TBP) binds to the **TATA element** in the **promoter region** of the gene.

Transcription factors (TFs) bind sequentially to the promoter and form the pre-initiation complex: **TFIID** binds first, followed by other factors:

- **TFIIB** stabilizes TFIID binding,
- **TFIIE** recruits **TFIIH**,
- **TFIIF** helps recruit **RNA polymerase II** to the promoter.

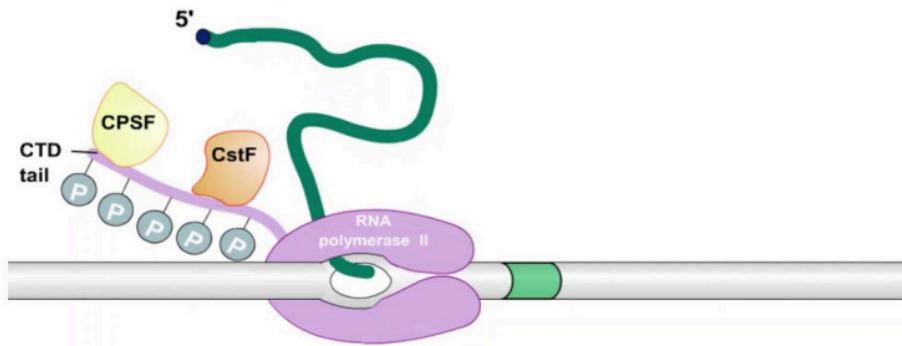


- **Elongation:** After initiation, RNA polymerase is released from transcription factors. The pre-mRNA is synthesized in the 5' to 3' direction.



- **Termination:**

- As RNA polymerase II reaches the end of the gene, two protein complexes (**CPSF** and **CSTF**) attached to the C-terminal domain (CTD) recognize the poly-A signal in the RNA.
- CPSF and CSTF help recruit other proteins to cut the RNA and end transcription.



Enzyme	Transcription Product	Minimum Required Initiation Factors
RNA Pol I (14 subunits)	rRNA 45S	SL1*/TIF1B/core factor TIF1A/RRN3 UBF/UAF
RNA Pol II (12 subunits)	mRNA snRNA miRNA	TFIIA TFIIB TFIID* TFIIE TFIIF TFIIC
RNA Pol III (17 subunits)	tRNA rRNA 5S small RNAs	TFIIC TFIIC*

### **RNA Transcription and Processing:**

- **RNA Synthesis:**

Only one of the two DNA strands (the template strand) is used by RNA polymerase, so the RNA produced is single-stranded. **Transcription ends when RNA polymerase reaches a specific sequence at the end of the gene**, and the RNA is then called precursor mRNA (**pre-mRNA**).

- **Error Rate:**

**RNA polymerases make more errors than DNA polymerases**, incorporating an incorrect base about **once every 100,000 nucleotides transcribed**.

- **Post-Transcriptional Modifications:**

After transcription, pre-mRNA undergoes several changes to become mature mRNA:

1. **5' Cap:** A methylated guanine (7-methylguanosine) is added to the 5'-end to protect it and **help ribosomes attach for translation**.

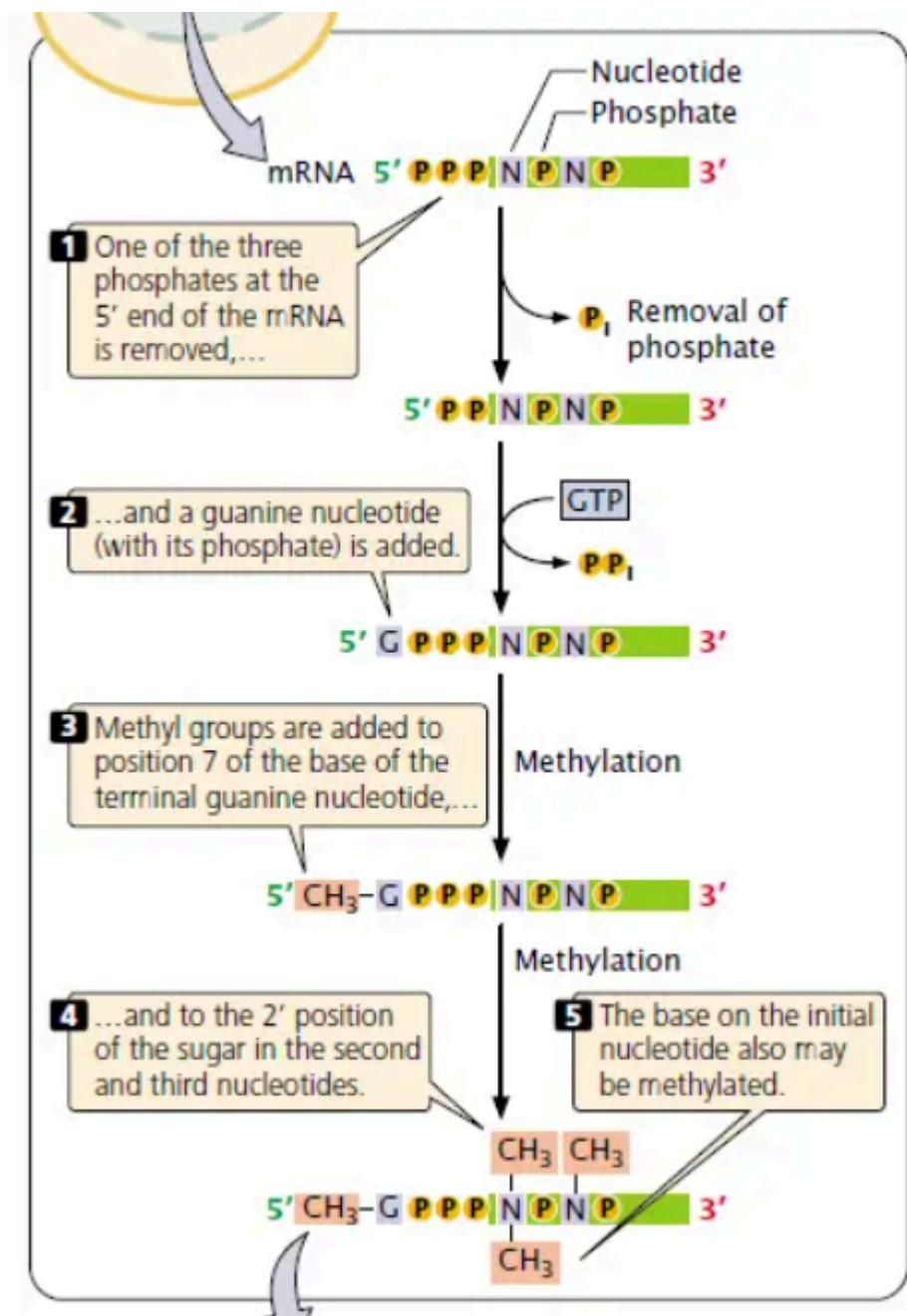


Fig: Addition of 5' cap

2. **3' Poly-A Tail:** A chain of 50–250 adenine nucleotides is added to the 3'-end to protect the mRNA.

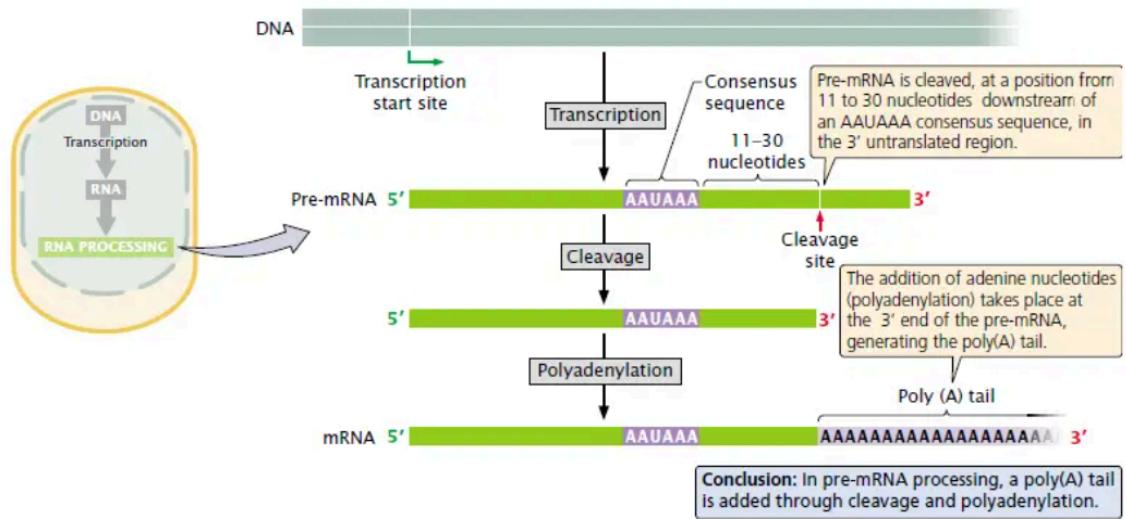
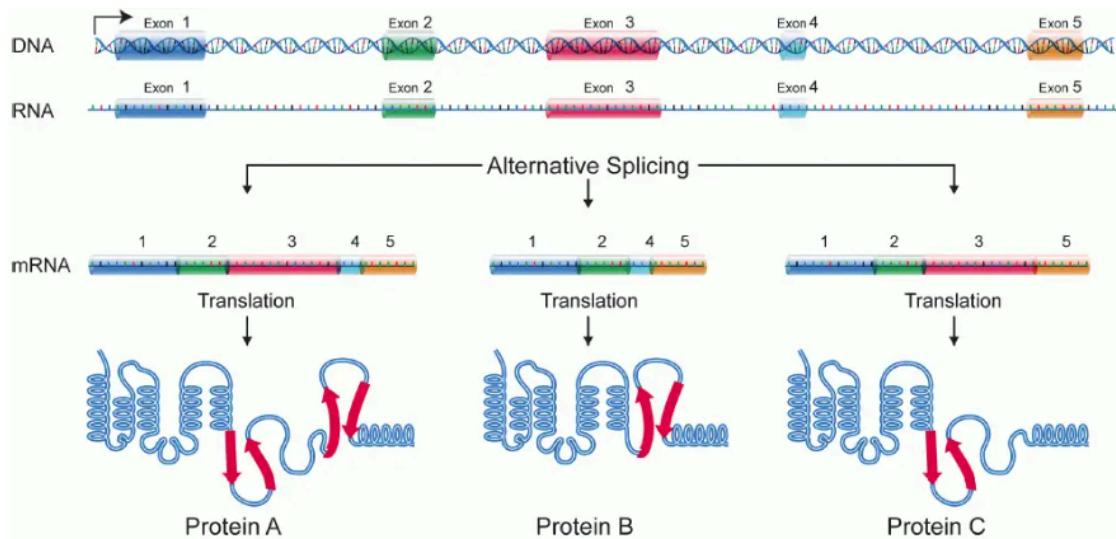


Fig: Poly A tail

3. **RNA Splicing:** Introns (non-coding regions) are removed, and exons (coding regions) are joined together to form the final mRNA sequence.

- **Alternative Splicing:**

Different proteins can be made from the same gene by removing different introns, creating multiple RNA sequences from one transcript. This process is known as **alternative splicing** and occurs in some viruses, like HIV, as well



In eukaryotes, the initial RNA (hnRNA, **heterogenous nuclear RNA**) is processed in the nucleus to become mature mRNA before it can exit the nucleus for translation. **Unmodified RNA** made from the DNA template is called **pre-mRNA or hnRNA**. This RNA contains **non-coding sequences called introns**, which interrupt the coding parts. These **introns are removed during RNA processing**, which can remove 30% to 90% of the pre-mRNA. The remaining **coding sequences, called exons**, are joined together by **splicing enzymes** to form **mature mRNA**. This splicing process, along with other modifications like capping and adding a **polyA tail**, happens in the nucleus. The **5' cap and 3' end** are usually kept in the final mRNA. **Introns vary in size and number across different genes and are typically longer than exons.**

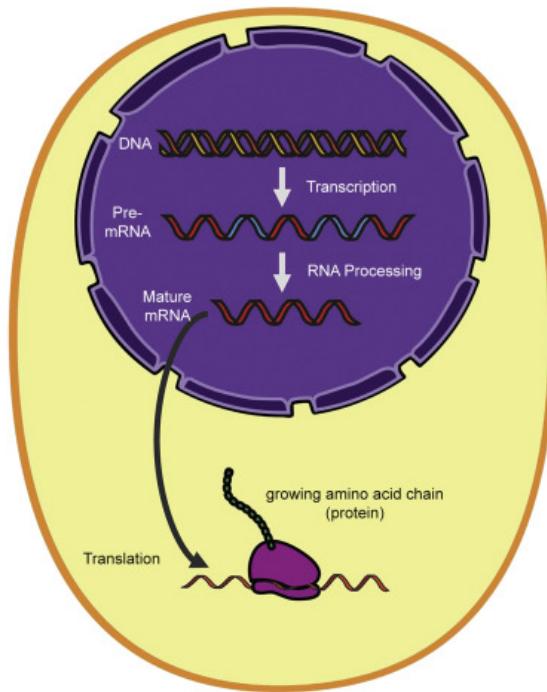
## Role of Introns

Introns help **control how often genes are turned on to make proteins and RNA**. They can affect the amount of RNA produced by influencing transcription, export, and stability, and can also improve how efficiently mRNA is translated into protein.

Introns allow a **single gene to produce multiple mRNA versions**, enabling genes to carry out various complex functions in the cell.

Introns help **organize the genome and regulate transcription**. Introns may control the speed of splicing, especially during **stress or starvation**, helping cells manage growth and survival. Introns may have contributed to the development of complex structures in higher organisms and play a role in **species-specific traits and complexity**.

Central Dogma is not this Simple!



The "central dogma" of molecular biology, first proposed by Francis Crick in 1957, explains how genetic information flows from **DNA to RNA to proteins**.

In **1960**, scientists discovered the first **RNA polymerase** activity in bacteria and rat liver cells. A year later, **mRNA** was identified as the link between DNA and protein, followed by the discovery of **tRNA** (adaptor molecule) and **rRNA** (ribosomal RNA).

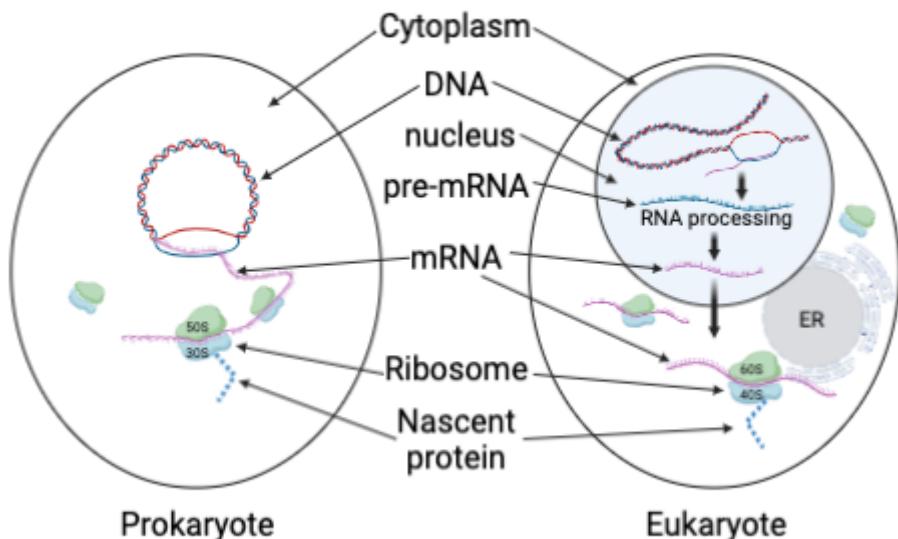
However, how RNA was made remained unclear until **Robert Roeder's** groundbreaking work in **1969**, where he identified three distinct **mammalian RNA polymerases**. His research paved the way for understanding how different types of RNA are produced.

+++++

## Translation:

- **Translation** is the process of protein synthesis inside a living cell. tRNA and ribosomes work together to decode mRNA into a protein.
- It converts the **nucleotide sequence of mRNA into an amino acid sequence**.
- Translation occurs in the **cytoplasm** where ribosomes are located.
- It is a **universal process** found in all living organisms.
- **mRNA** is decoded to produce a specific polypeptide (protein chain).
- **Ribosomes** use the mRNA sequence as a template to build proteins.
- Some RNAs like **tRNA, rRNA, and snRNA** are not translated into proteins.
- **Steps of Translation:**

- **Activation** (prepares amino acids)
- **Initiation** (ribosome binds to mRNA): A ribosome binds to mRNA and the start codon (AUG) is read to start the protein.
- **Elongation** (amino acids are linked): The ribosome moves along the mRNA, adding amino acids.
- **Termination** (protein synthesis stops): The ribosome reaches a stop codon (UAG, UAA, or UGA) and the protein is released.
- **Activation** is sometimes excluded as a formal step in translation.



Feature	Prokaryotic Translation	Eukaryotic Translation
<b>Process</b>	Synchronous (with transcription)	Discontinuous (separate from transcription)
<b>mRNA Location</b>	Cytoplasm	Nucleus
<b>Cap Initiation</b>	Cap-independent	Cap-dependent & Cap-independent
<b>Ribosomes Used</b>	70S (30S + 50S)	80S (40S + 60S)
<b>mRNA Stability</b>	Unstable	Stable
<b>mRNA Lifespan</b>	Seconds to minutes	Hours to days
<b>Occurrence</b>	No specific phase	G1 & G2 phases of the cell cycle
<b>Process Speed</b>	Fast	Slow
<b>Release Factors</b>	RF1, RF2	eRF
<b>Initiation Factors</b>	3	9

mRNA translation turns nucleotide sequences into amino acids to form proteins. Each amino acid is coded by a set of three nucleotides called a **codon**. Since there are 64 codon combinations but only 20 amino acids, multiple codons can code for the same amino acid. This is known as **degeneracy**.

	second letter				
	U	C	A	G	
first letter	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA }	UAU } Tyr UAC } <b>UAA stop</b> <b>UAG stop</b>	UGU } Cys UGC } <b>UGA stop</b> UGG Trp	U C A G
C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } CCA } Pro CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
A	AUU } AUC } Ile AUA } <b>AUG Met</b>	ACU } ACC } ACA } Thr ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
G	GUU } GUC } Val GUA } GUG }	GCU } GCC } GCA } Ala GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G

This figure shows the genetic code for translating each nucleotide triplet in mRNA into an amino acid or a termination signal in a nascent protein.

Proteins are made up of 20 amino acids. Each amino acid has an  $\alpha$ -carboxyl group, a primary  $\alpha$ -amino group, and a side chain called the R group

Protein synthesis requires **mRNA (seen in detail), ribosomes, tRNA, and enzymes**.

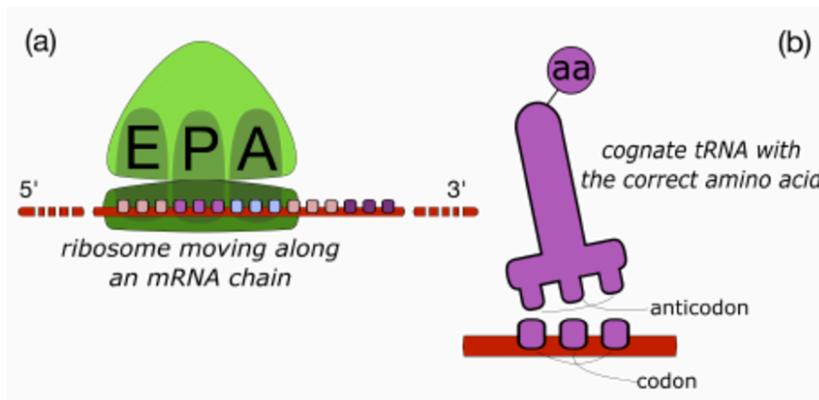
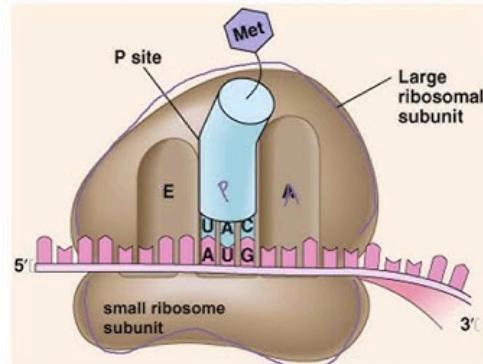
**Ribosomes** : macromolecules made of **rRNAs and proteins**.

- **Prokaryotic ribosomes**: 70S (30S small + 50S large subunit).
- **Eukaryotic ribosomes**:
  - **Cytoplasm & Rough ER**: 80S (40S small + 60S large subunit).
  - **Mitochondria & Chloroplasts**: 70S (similar to prokaryotes).
- The **small subunit** binds mRNA, while the **large subunit** binds tRNA.

# What is the Structure of a ribosome?

Key Concepts:

- A ribosome has an mRNA binding site and three tRNA binding sites.
- The tRNA binding sites are A, P, E:
  - A: aminoacyl-tRNA
  - P: peptidyl-tRNA
  - E: exit



**Transfer RNAs (tRNAs)** tRNAs are small RNA molecules that **help translate mRNA into proteins**. Each **tRNA carries a specific amino acid and binds to a matching codon on the mRNA**. Bacteria have 60-90 types of tRNAs.

- tRNAs fold into a 3D shape, with:
  - **Amino acid binding site (CCA sequence)** at the 3' end
  - **Anticodon** that pairs with an mRNA codon

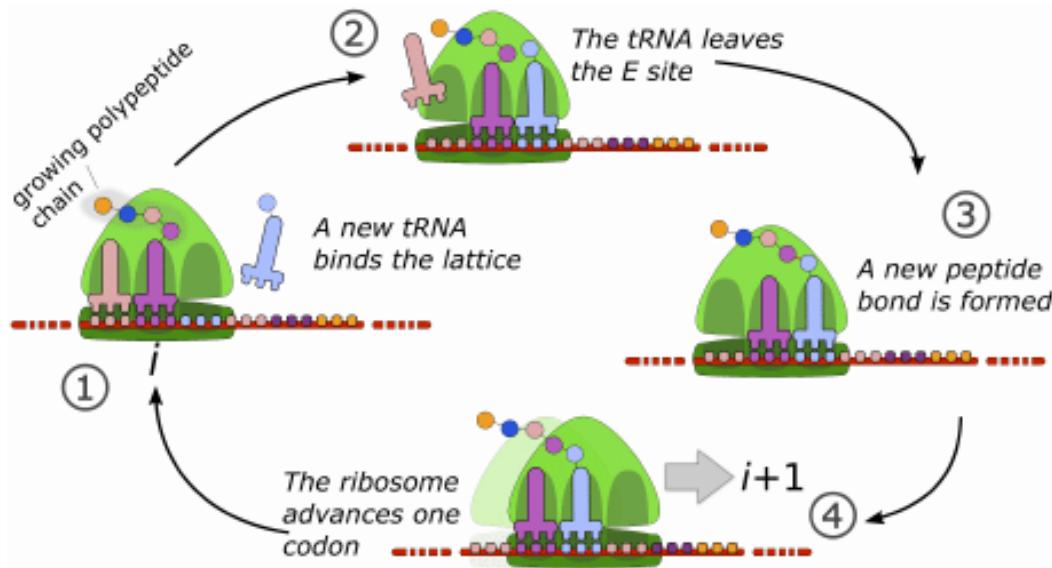
## tRNA Charging

tRNAs are loaded with their correct amino acid by enzymes called **aminoacyl tRNA synthetases**. Each amino acid has a specific synthetase. The process involves:

1. Activating the amino acid with **AMP**
2. Attaching it to the tRNA, forming a **charged tRNA**
3. Releasing AMP

Charged tRNAs then deliver amino acids to the ribosome for protein synthesis.

[https://www.youtube.com/watch?v=WNZf4ip\\_R9s](https://www.youtube.com/watch?v=WNZf4ip_R9s)



## Protein Synthesis: Translation Mechanism

Translation is similar in both bacteria and eukaryotes. Here's how it works in *E. coli* (a bacteria), with key differences in eukaryotes.

### 1. Initiation (Starting Protein Synthesis)

#### 1. Formation of the Initiation Complex

- In *E. coli*, translation starts when:
  - The **small ribosome (30S)** binds to mRNA
  - Three **initiation factors** help assembly
  - **GTP** provides energy
  - A special tRNA (**fMet-tRNA<sup>fMet</sup>**) carries **formyl-methionine (fMet)** and binds to the **AUG start codon**
  - The **Shine-Dalgarno sequence (AGGAGG)** on mRNA helps position the ribosome
  - The **large ribosome (50S)** joins, forming a complete ribosome

#### 2. Eukaryotic Differences:

Uses a different **initiator tRNA (Met-tRNA<sup>i</sup>) carrying methionine**

- No Shine-Dalgarno sequence; instead, the ribosome **recognizes the 5' cap** of mRNA and moves along until it finds **AUG**
- Uses **40S and 60S ribosome subunits** instead of 30S and 50S

Most eukaryotic mRNAs contain a short recognition sequence that greatly facilitate the initial binding of mRNA to the small subunit of the ribosome. The consensus sequence for initiation of translation in vertebrates (also called Kozak sequence) is:

**ACCATGG**

More general it is:

**(GCC)RCCATGG**

where R is a purine (A or G).

To improve expression levels, it may be advantageous to design the cloned insert according to Kozak's rules.

#### **References:**

Kozak, M. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell.* 1986, 44(2):283-92.

Kozak, M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 1987, 15(20):8125-48

## **Shine-Dalgarno Sequence**

In prokaryotes, the signal for initiation of protein synthesis consists primarily, but not exclusively, of an AUG codon and a rRNA-complementary sequence, the Shine Dalgarno sequence:

**AGGAGG**

This sequence usually locates 4-7 nucleotides 5' of the initiator AUG of many mRNAs. The sequence is complementary to gaucaCCUCCUuaOH at the 3' end of 16S rRNA.

**2. Elongation (Building the Protein Chain)** The process is similar in both bacteria and eukaryotes. In *E. coli*:

1. The **large ribosome (50S)** joins, creating three key sites:

- **A site:** Receives incoming tRNA with an amino acid.
- **P site:** Holds the growing protein chain.

- **E site:** Releases empty tRNA after it drops off its amino acid.
2. **Step-by-step process:**

- tRNA with an amino acid enters the **A site**.
- The ribosome moves forward (3 bases at a time).
- The growing protein chain is transferred from the **P site tRNA** to the **A site tRNA**.
- The empty tRNA moves to the **E site** and exits.
- This cycle repeats, adding one amino acid at a time.

3. **Speed:**

- *E. coli* adds **one amino acid every 0.05 seconds**, making a **200-amino-acid protein in just 10 seconds!**
- Energy for this process comes from **GTP**.

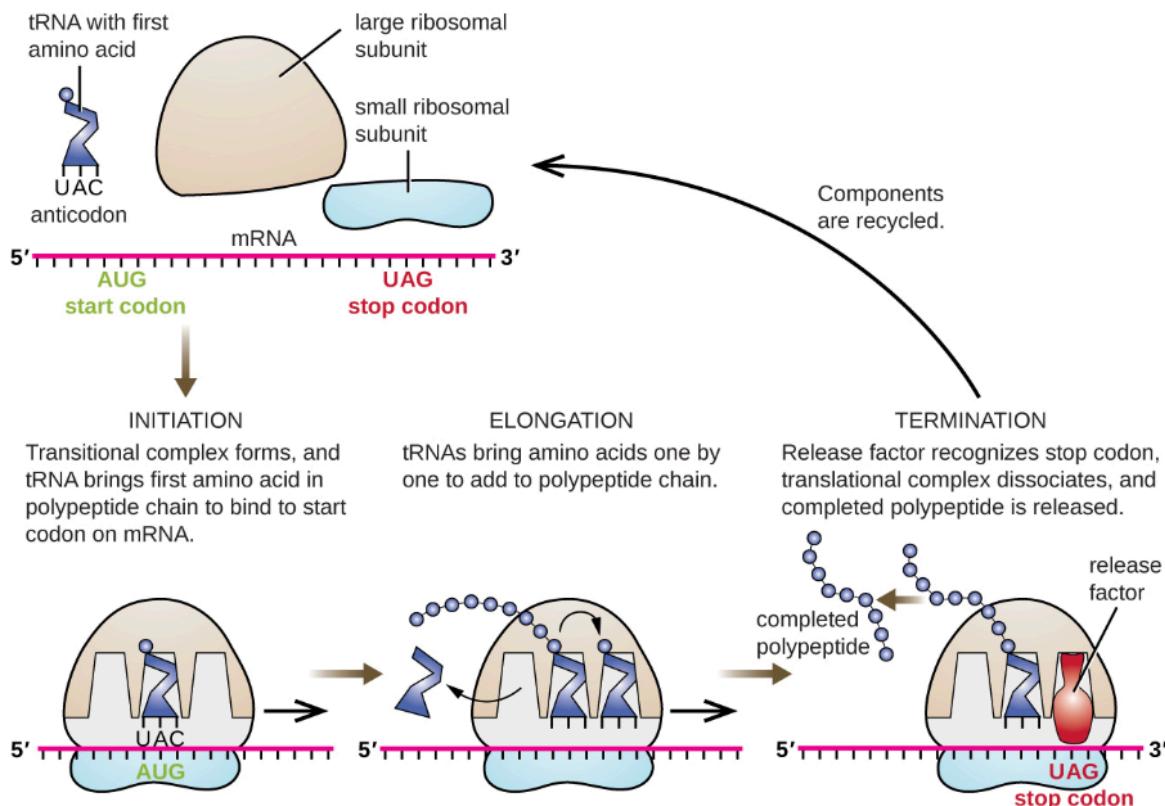


Figure 9.4.4: Translation in bacteria begins with the formation of the initiation complex, which includes the small ribosomal subunit, the mRNA, the initiator tRNA carrying N-formyl-methionine, and initiation factors. Then the 50S subunit binds, forming an intact ribosome.

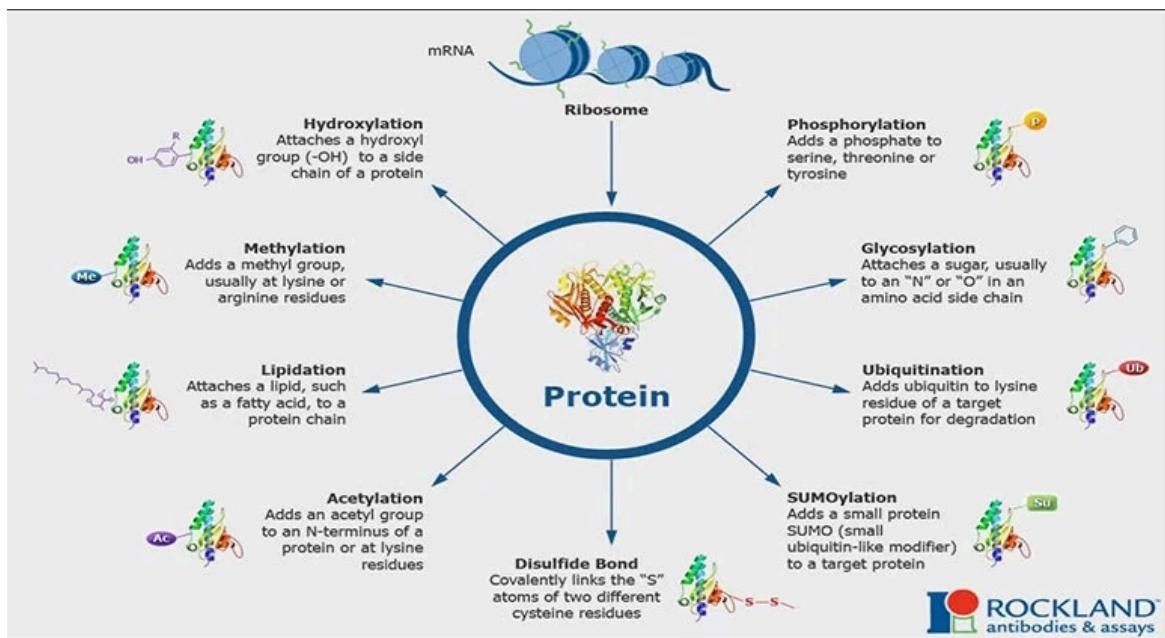
### 3. Termination (Stopping Protein Synthesis)

1. The ribosome stops when it reaches a "stop" codon (UAA, UAG, or UGA).
2. Since no tRNA matches the stop codon, **release factors** step in.
3. The new protein is released, and the ribosome **breaks apart**, ready to start again.

Comparison of Translation in Bacteria Versus Eukaryotes		
Property	Bacteria	Eukaryotes
Ribosomes	70S <ul style="list-style-type: none"> <li>• 30S (small subunit) with 16S rRNA subunit</li> <li>• 50S (large subunit) with 5S and 23S rRNA subunits</li> </ul>	80S <ul style="list-style-type: none"> <li>• 40S (small subunit) with 18S rRNA subunit</li> <li>• 60S (large subunit) with 5S, 5.8S, and 28S rRNA subunits</li> </ul>
Amino acid carried by initiator tRNA	fMet	Met
Shine-Dalgarno sequence in mRNA	Present	Absent
Simultaneous transcription and translation	Yes	No

### Post-Translational Processing

After proteins are made, they undergo modifications that shape their function, structure, and location. Some key modifications include:



These modifications expand protein diversity and ensure they function properly in the cell.

**Significance of Post-Transcriptional Modifications (PTMs)** :crucial for cell function, communication, and adaptation to the environment. Understanding PTMs helps in medicine and biotechnology.

- **Helping proteins fold correctly** (e.g., calnexin assists glycosylated proteins).
- **Sorting proteins to the right location** (e.g., phosphorylated mannose directs proteins to lysosomes).
- **Regulating protein activity** (e.g., phosphorylation can turn proteins on or off).
- **Responding to oxidative stress** (redox modifications adjust protein function).
- **Increasing protein variety** by introducing new chemical changes.