



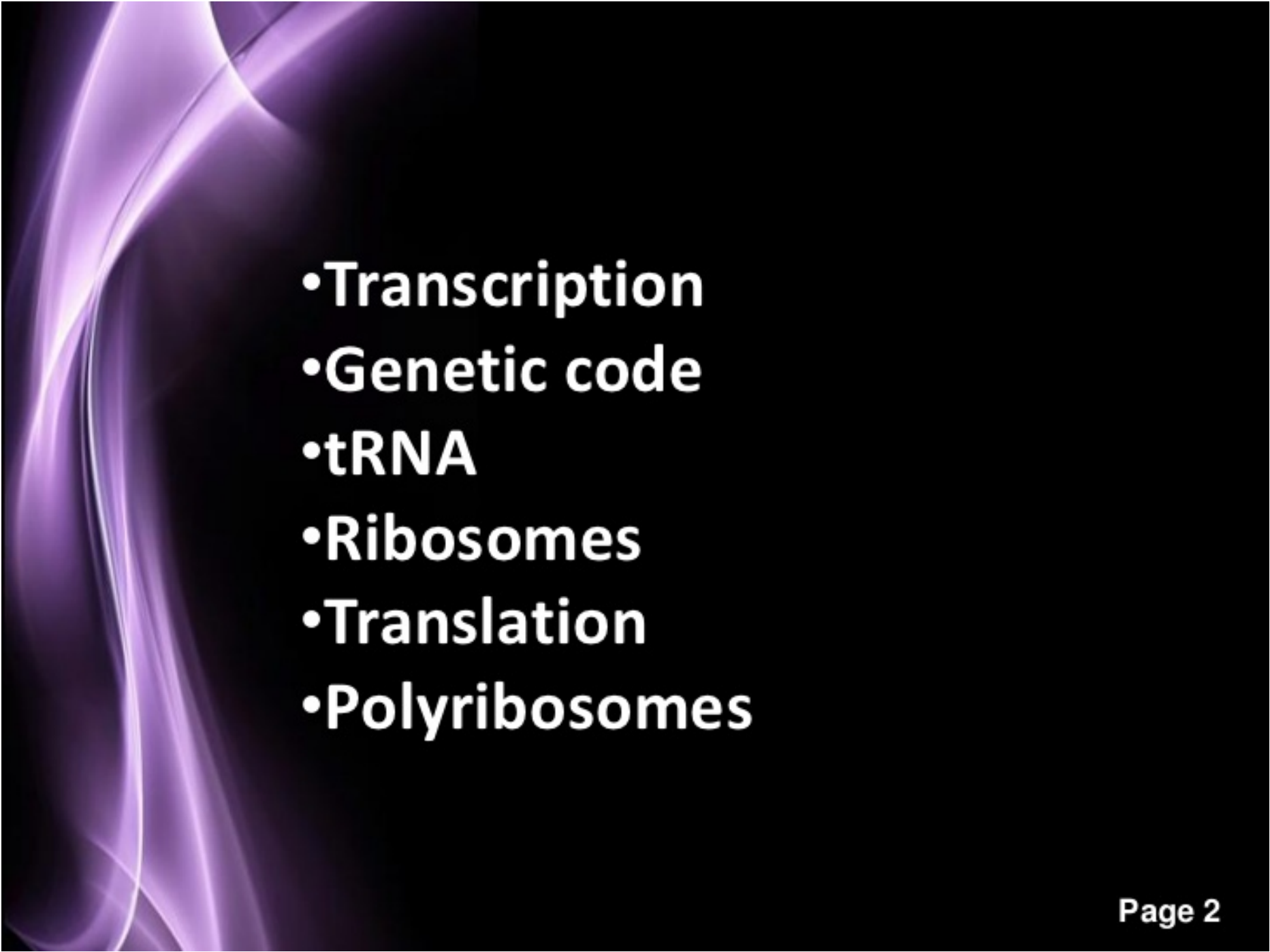
Protein synthesis

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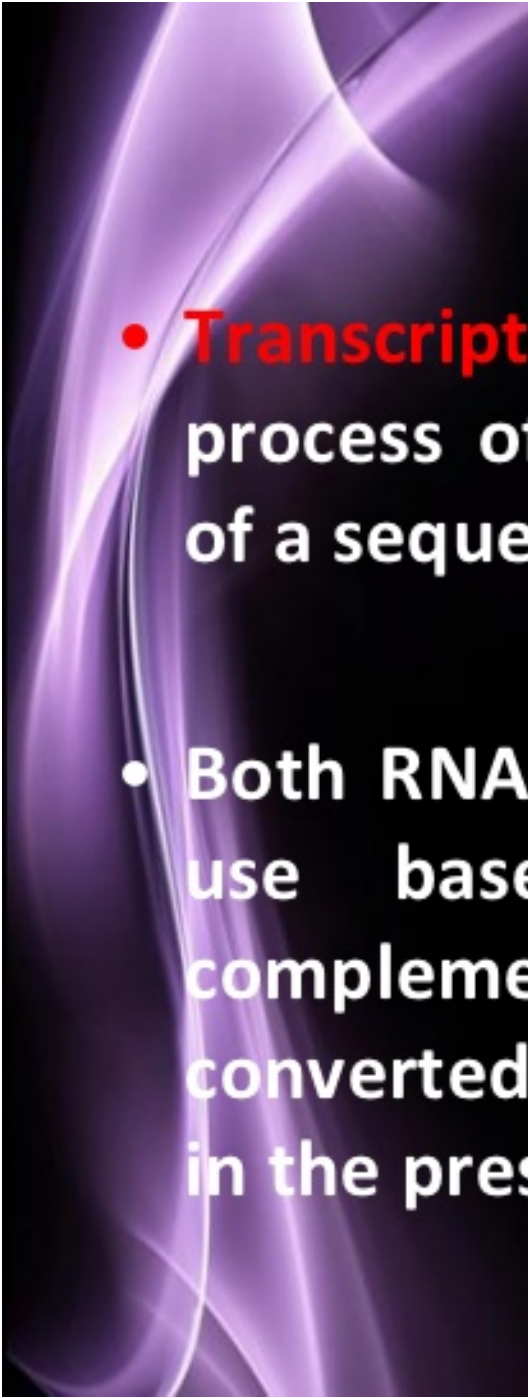
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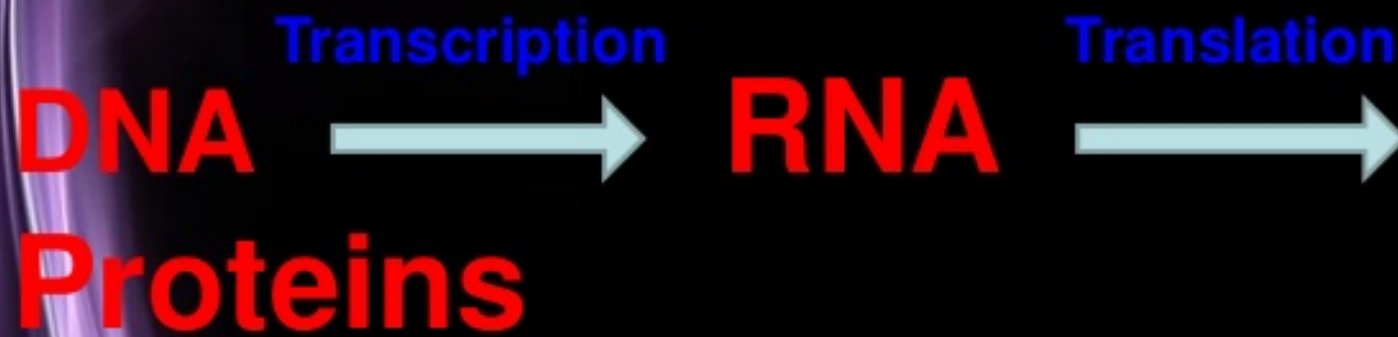
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- **Transcription**
 - **Genetic code**
 - **tRNA**
 - **Ribosomes**
 - **Translation**
 - **Polyribosomes**

The background of the slide is black with a purple abstract design on the left side. The design consists of several overlapping, flowing, wavy lines that create a sense of movement and depth. The lines are in various shades of purple, from light lavender to deep violet. The word "Transcription" is centered in the middle of the slide in a large, white, bold, sans-serif font. The word is underlined with a thick white horizontal line.

Transcription

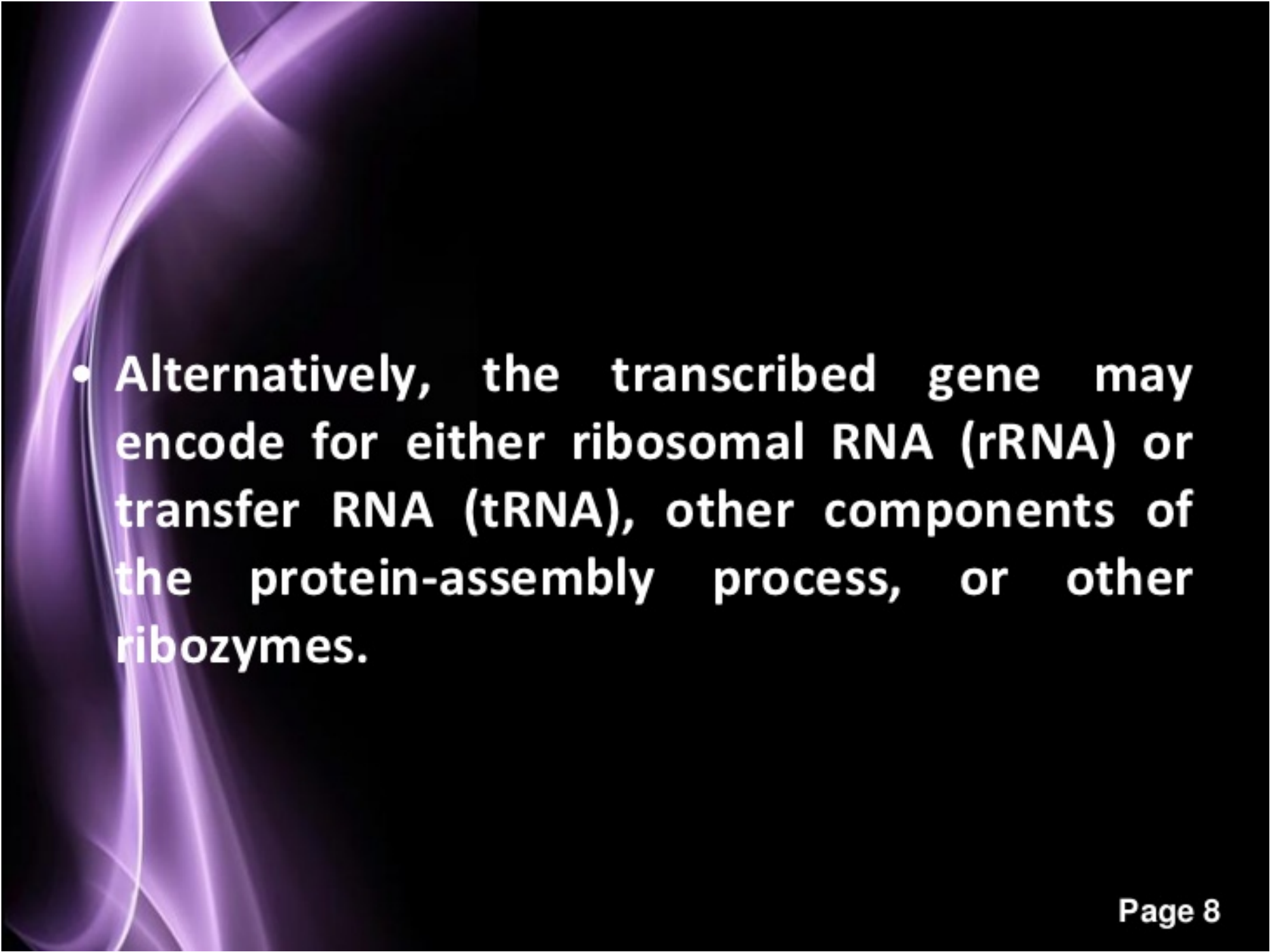
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- **Transcription**, or RNA synthesis, is the process of creating an equivalent RNA copy of a sequence of DNA.
 - Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language that can be converted back and forth from DNA to RNA in the presence of the correct enzymes.

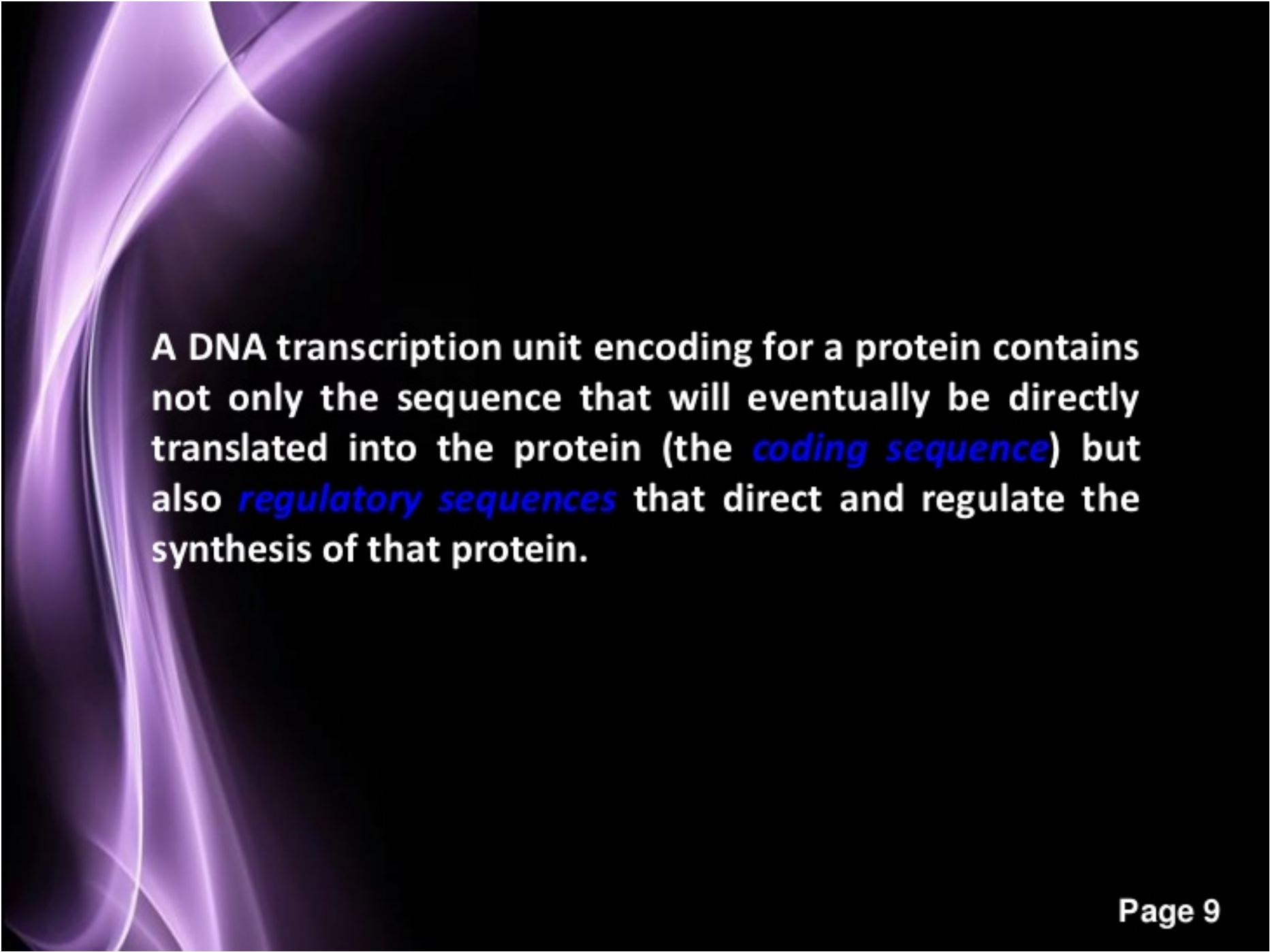


Central dogma

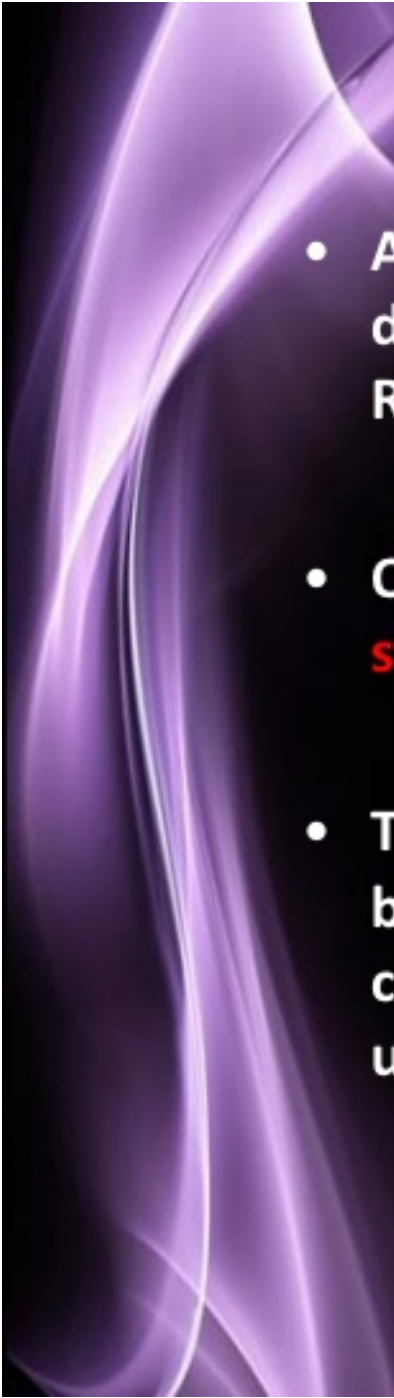
- During **transcription**, a DNA sequence is read by **RNA polymerase**, which produces a complementary, antiparallel RNA strand.
- As opposed to DNA replication, transcription results in an RNA compliment that includes **uracil (U)** in all instances where thymine (T) would have occurred in a DNA compliment.


- **Transcription** is the first step leading to **gene expression**.
- The stretch of DNA transcribed into an RNA molecule is called a ***transcription unit*** and encodes at least one gene.
- If the gene transcribed encodes for a protein, the result of transcription is messenger RNA (mRNA), which will then be used to create that protein via the process of translation.

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- **Alternatively, the transcribed gene may encode for either ribosomal RNA (rRNA) or transfer RNA (tRNA), other components of the protein-assembly process, or other ribozymes.**



A DNA transcription unit encoding for a protein contains not only the sequence that will eventually be directly translated into the protein (the *coding sequence*) but also *regulatory sequences* that direct and regulate the synthesis of that protein.

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- As in DNA replication, DNA is read from 3' → 5' during transcription. Meanwhile, the complementary RNA is created from the 5' → 3' direction.
 - Only one of the two DNA strands, called the **template strand**, is used for transcription.
 - The other DNA strand is called the **coding strand**, because its sequence is the same as the newly created RNA transcript (except for the substitution of uracil for thymine).

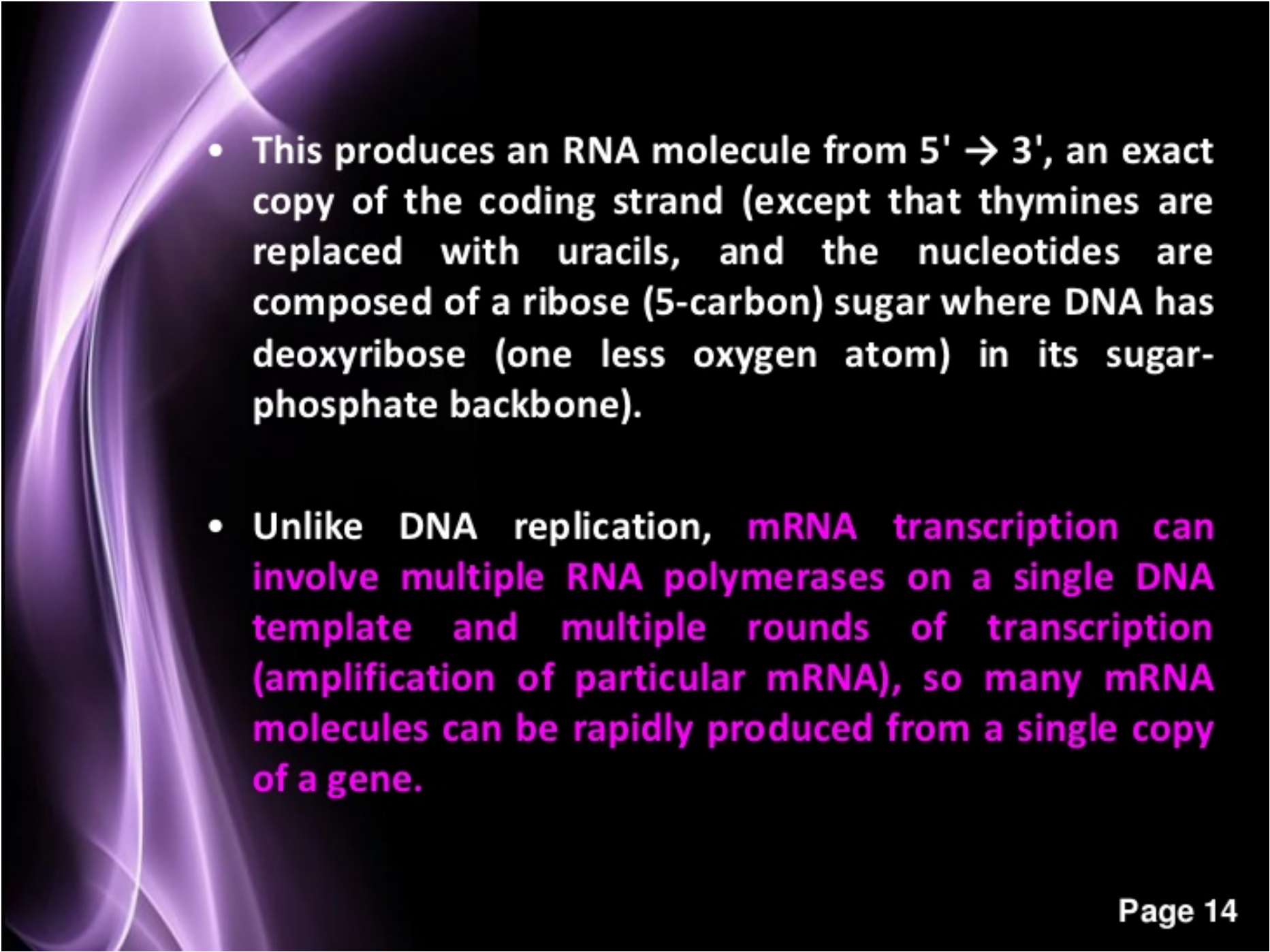
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- Transcription is divided into 3 stages:
 1. *initiation*
 2. *elongation*
 3. *termination.*

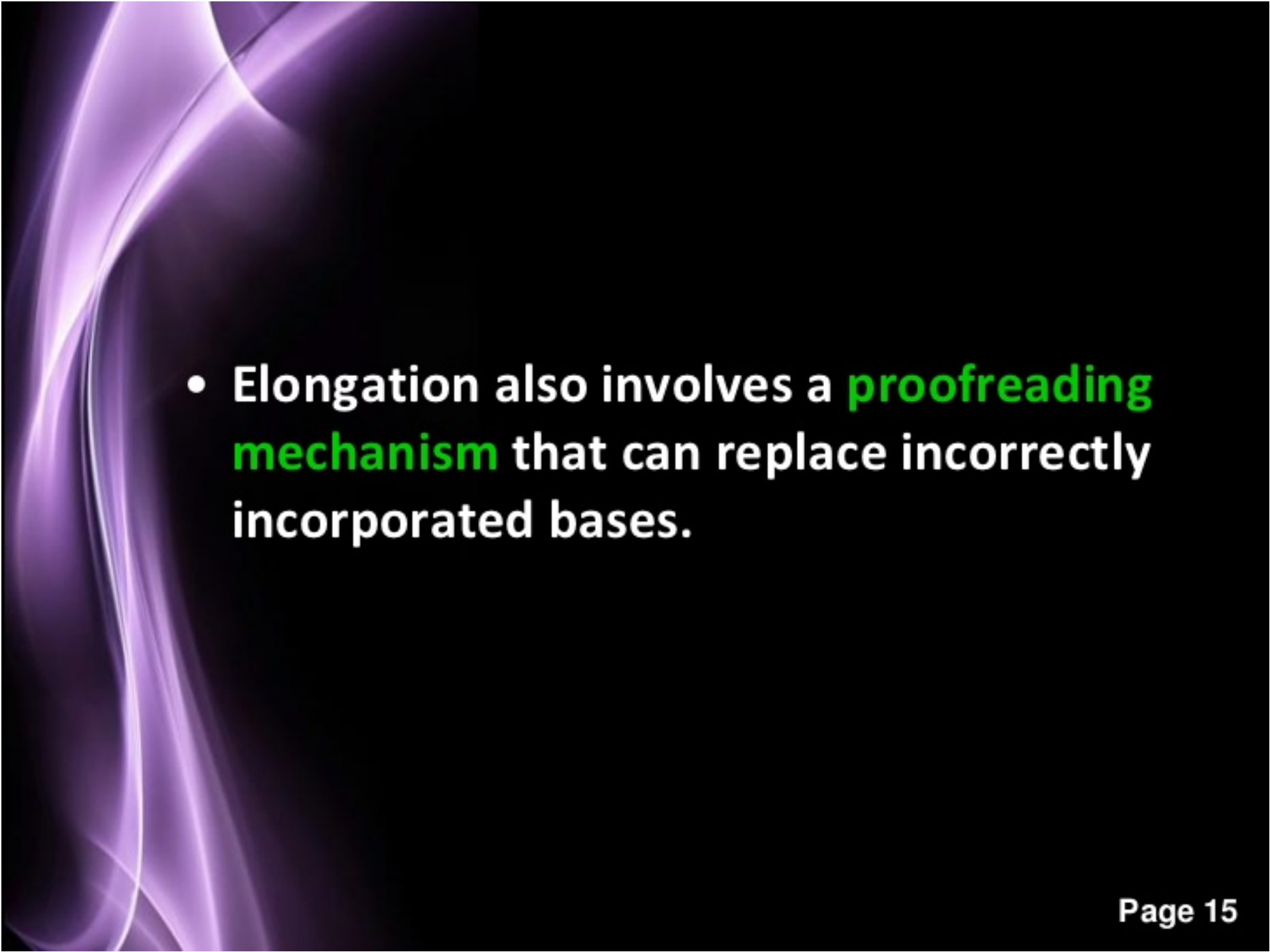
Initiation

- In bacteria, transcription begins with the binding of RNA polymerase to the promoter in DNA.
- **RNA polymerase** is a core enzyme consisting of five subunits: **2 α subunits, 1 β subunit, 1 β' subunit, and 1 ω subunit.**
- At the start of initiation, the core enzyme is associated with a **sigma factor** that aids in finding the appropriate **-35** and **-10 base pairs** downstream of promoter sequences.

Elongation

- One strand of DNA, the *template strand* (or noncoding strand), is used as a template for RNA synthesis.
- As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy.
- Although RNA polymerase traverses the template strand from $3' \rightarrow 5'$, the coding (non-template) strand and newly-formed RNA can also be used as reference points, so transcription can be described as occurring $5' \rightarrow 3'$.

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- This produces an RNA molecule from 5' → 3', an exact copy of the coding strand (except that thymines are replaced with uracils, and the nucleotides are composed of a ribose (5-carbon) sugar where DNA has deoxyribose (one less oxygen atom) in its sugar-phosphate backbone).
 - Unlike DNA replication, mRNA transcription can involve multiple RNA polymerases on a single DNA template and multiple rounds of transcription (amplification of particular mRNA), so many mRNA molecules can be rapidly produced from a single copy of a gene.

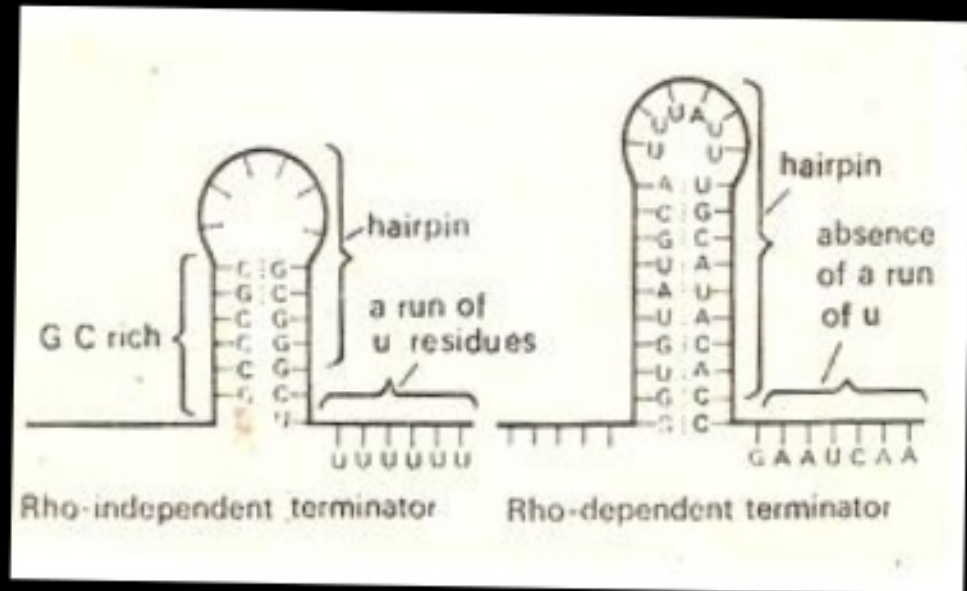
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- Elongation also involves a **proofreading mechanism** that can replace incorrectly incorporated bases.

Termination

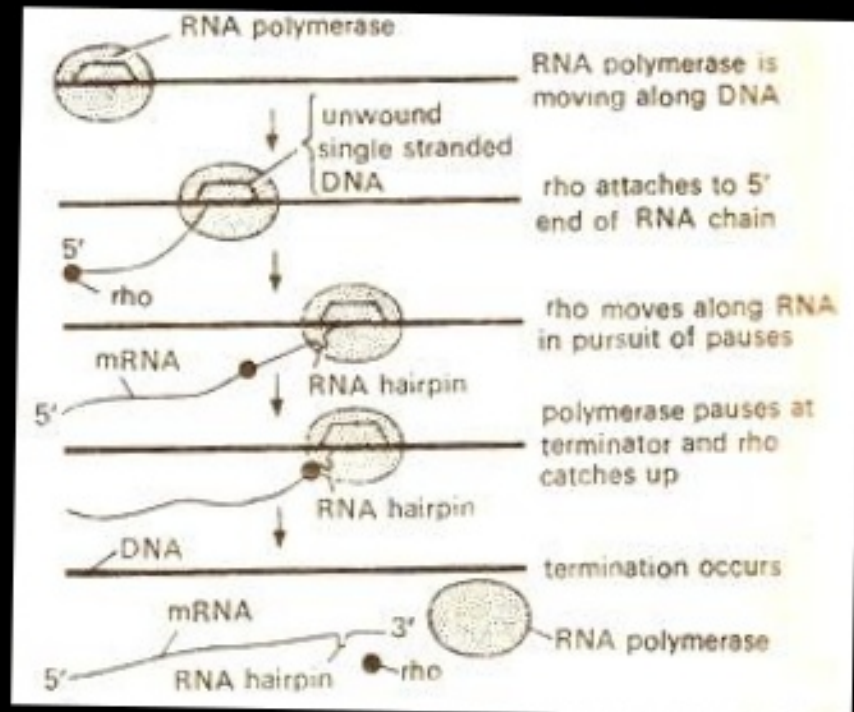
- Bacteria use two different strategies for transcription termination: **Rho-independent** and **Rho-dependent**

In **Rho-independent**
transcription

termination, RNA
transcription stops
when the newly
synthesized RNA
molecule forms a G-C
rich hairpin loop
followed by a run of
U's, which makes it
detach from the DNA
template.



In the **"Rho-dependent"** type of termination, a protein factor called "Rho" destabilizes the interaction between the template and the mRNA, thus releasing the newly synthesized mRNA from the elongation complex.




The background of the slide features a dark, almost black, field with flowing, ethereal waves of light purple and white. These waves originate from the left side and curve towards the right, creating a sense of movement and depth. The overall aesthetic is clean and modern, typical of a professional presentation.

Genetic code

Properties of genetic code

- **The code is universal.** All prokaryotic and eukaryotic organisms use the same codon to specify each amino acid.
- **The code is triplet.** Three nucleotides make one codon. 61 of them code for amino acids and 3 viz., UAA, UAG and UGA are nonsense codons or chain termination codons.
- **The code is degenerate.** For a particular amino acid more than one word can be used

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- **The code is non overlapping.** A base in mRNA is not used for two different codons
 - **The code is commaless.** There is no special signal or commas between codons.
 - **The code is non ambiguous.** A particular codon will always code for the same amino acid, wherever it is found.

The Genetic Code

	U	C	A	G				
U	<p>UUU Phenylalanine</p> <p>UUC Phenylalanine</p> <p>UUG Leucine</p> <p>UUA Leucine</p>	<p>UCU Serine</p> <p>UCC Serine</p> <p>UCA Serine</p> <p>UCG Serine</p>	<p>UAU Tyrosine</p> <p>UAC Tyrosine</p> <p>UAA Stop</p> <p>UAG Stop</p>	<p>UGU Cysteine</p> <p>UGC Cysteine</p> <p>UGA Stop</p> <p>UGG Tryptophan</p>	U	C	A	G
C	<p>CUU Leucine</p> <p>CUC Leucine</p> <p>CUA Leucine</p> <p>CUG Leucine</p>	<p>CCU Proline</p> <p>CCC Proline</p> <p>CCA Proline</p> <p>CCG Proline</p>	<p>CAU Histidine</p> <p>CAC Histidine</p> <p>CAA Glutamine</p> <p>CAG Glutamine</p>	<p>CGU Arginine</p> <p>CGC Arginine</p> <p>CGA Arginine</p> <p>CGG Arginine</p>	U	C	A	G
A	<p>AUU Isoleucine</p> <p>AUC Isoleucine</p> <p>AUA Isoleucine</p> <p>AUG Methionine</p>	<p>ACU Threonine</p> <p>ACC Threonine</p> <p>ACA Threonine</p> <p>ACG Threonine</p>	<p>AAU Asparagine</p> <p>AAC Asparagine</p> <p>AAA Lysine</p> <p>AAG Lysine</p>	<p>AGU Serine</p> <p>AGC Serine</p> <p>AGA Arginine</p> <p>AGG Arginine</p>	U	C	A	G
G	<p>GUU Valine</p> <p>GUC Valine</p> <p>GUA Valine</p> <p>GUG Valine</p>	<p>GCU Alanine</p> <p>GCC Alanine</p> <p>GCA Alanine</p> <p>GCG Alanine</p>	<p>GAU Aspartic acid</p> <p>GAC Aspartic acid</p> <p>GAA Glutamic acid</p> <p>GAG Glutamic acid</p>	<p>GGU Glycine</p> <p>GGC Glycine</p> <p>GGA Glycine</p> <p>GGG Glycine</p>	U	C	A	G

tRNA

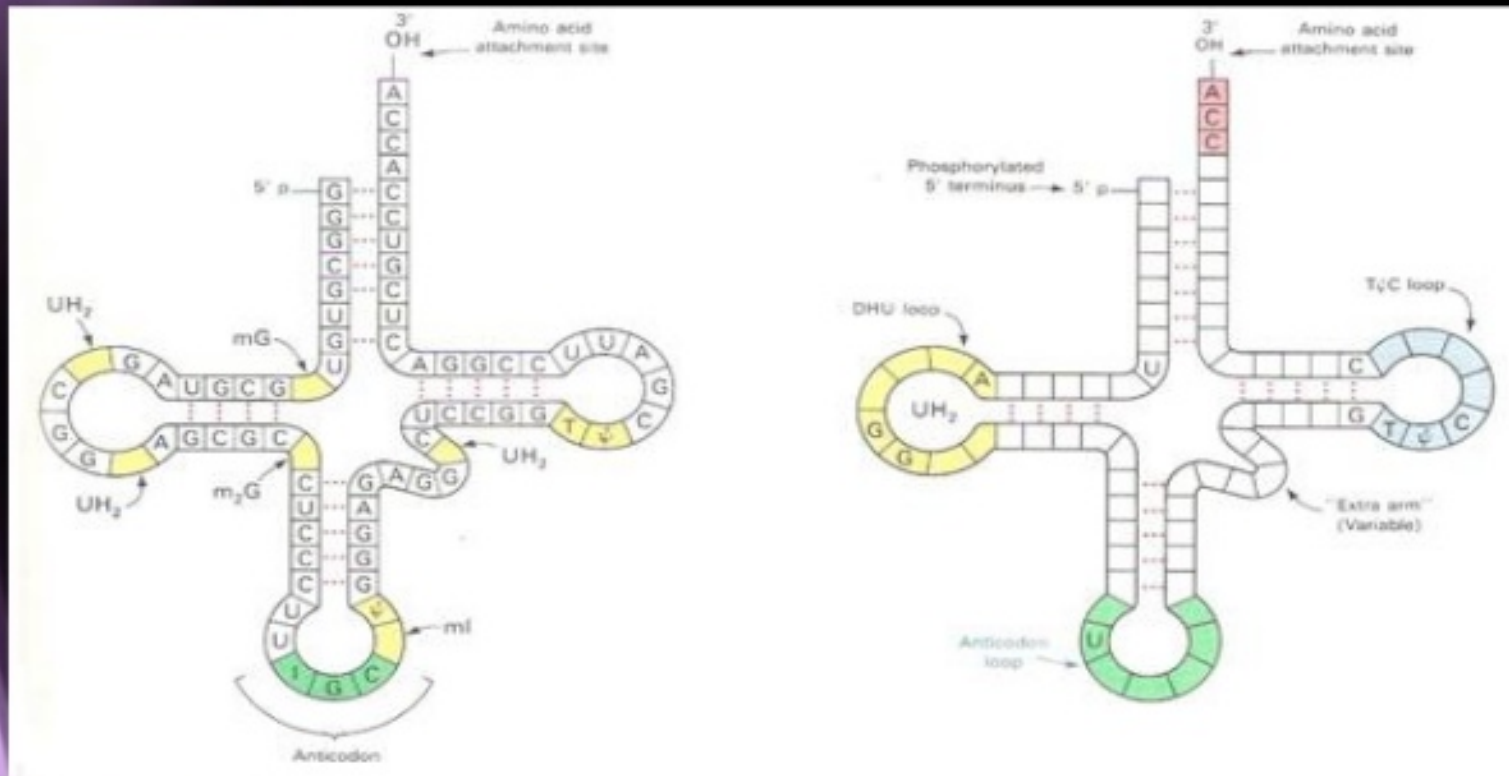


Fig. Base sequence of yeast alanyl tRNA

Fig. Common features of tRNA molecule

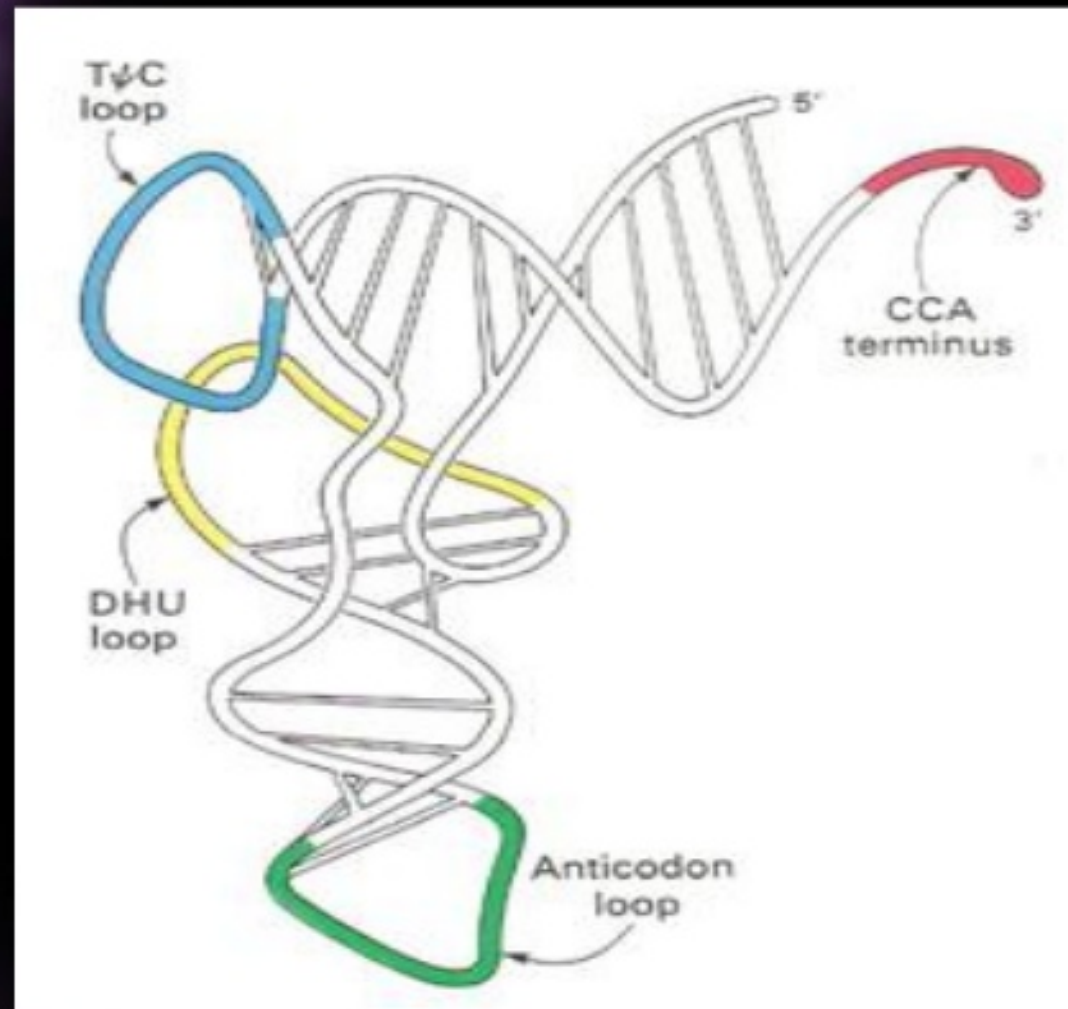


Fig. 3D structure of tRNA

Ribosomes

- Bacterial ribosomes consists of **two subunits** of unequal size, the larger having a sedimentation coefficient of **50S** and the smaller of **30S**.
- The two ribosomal subunits have irregular shapes which fit together in such a way that a cleft is formed through which mRNA passes as the ribosome moves along it during the translation process and from which the newly formed polypeptide chain emerges.

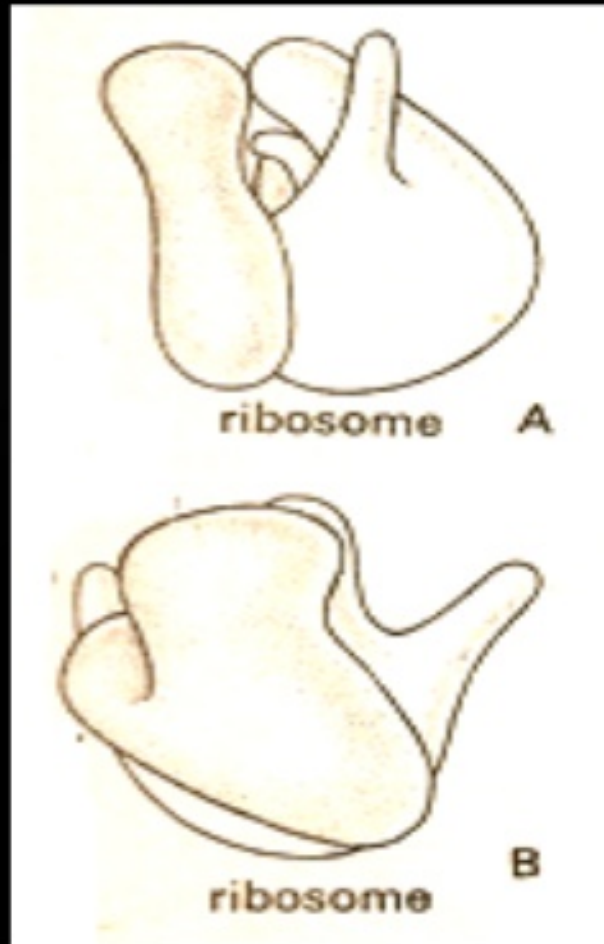





Fig. Ribosomes

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Translation

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- **Translation** is the first stage of protein biosynthesis (part of the overall process of gene expression).
 - Translation is the production of proteins by decoding mRNA produced in transcription.
 - It occurs in the cytoplasm where the ribosomes are located.
 - Ribosomes are made of a small and large subunit which surrounds the mRNA.

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- In **translation**, messenger RNA (mRNA) is decoded to produce a specific polypeptide according to the rules specified by the genetic code.
 - This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acid that form a protein.
 - Many types of transcribed RNA, such as transfer RNA, ribosomal RNA, and small nuclear RNA are not necessarily translated into an amino acid sequence.



- **Translation** proceeds in four phases:

- activation,
- initiation,
- elongation and
- termination

(all describing the growth of the amino acid chain, or polypeptide that is the product of translation).

Amino acids are brought to ribosomes and assembled into proteins.

Activation

- In **activation**, the correct amino acid is covalently bonded to the correct transfer RNA (tRNA).
- While this is not technically a step in translation, it is required for translation to proceed.
- The amino acid is joined by its carboxyl group to the 3' OH of the tRNA by an **ester bond**.
- When the tRNA has an amino acid linked to it, it is termed "**charged**".

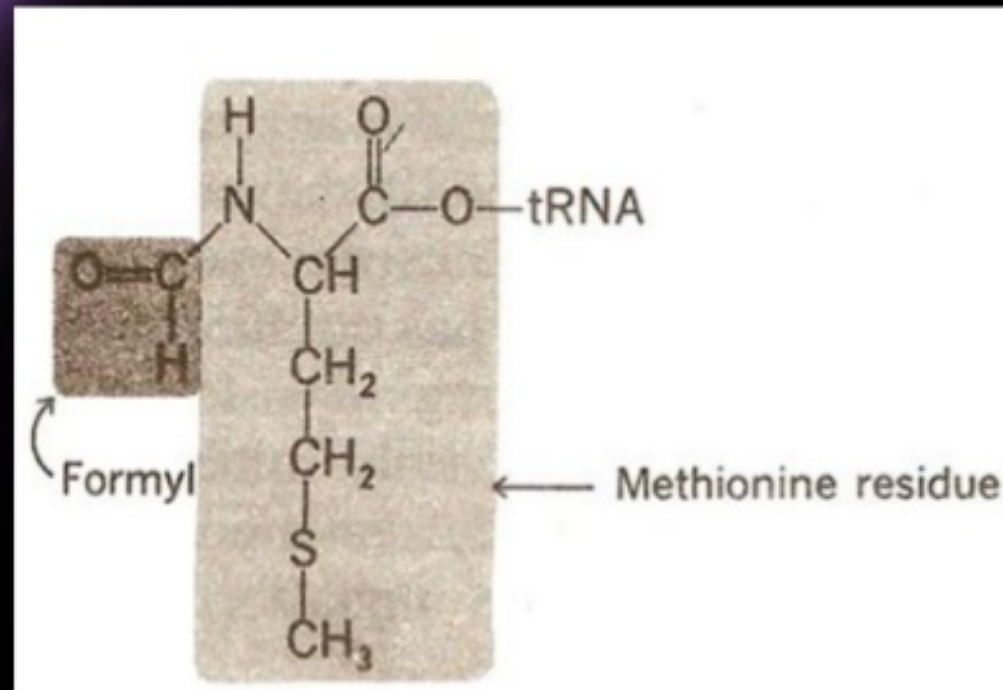
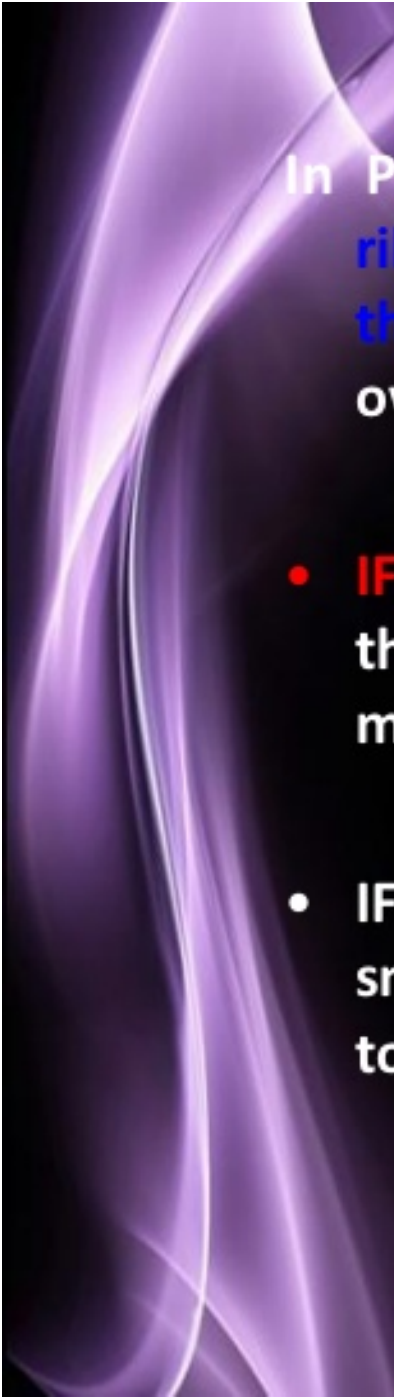
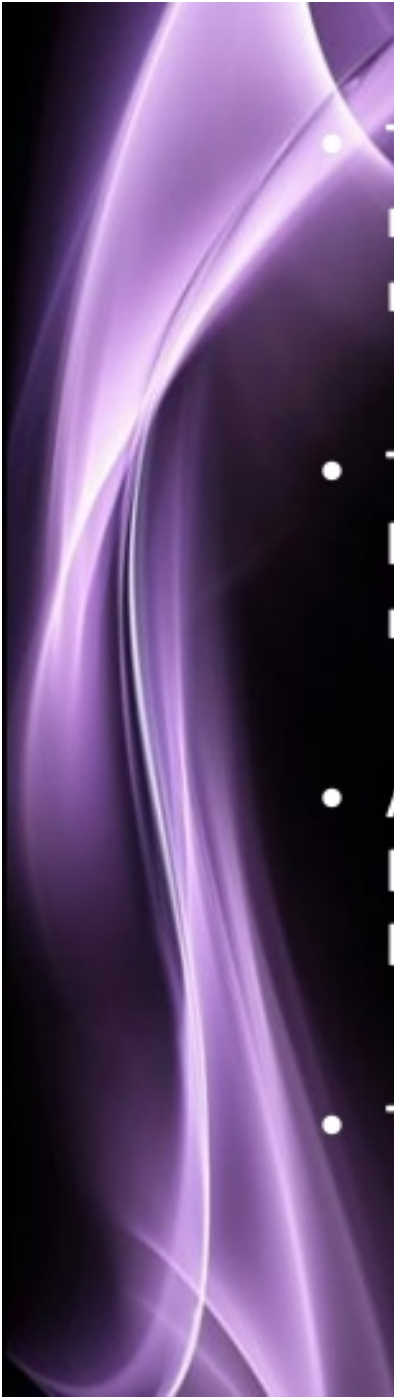


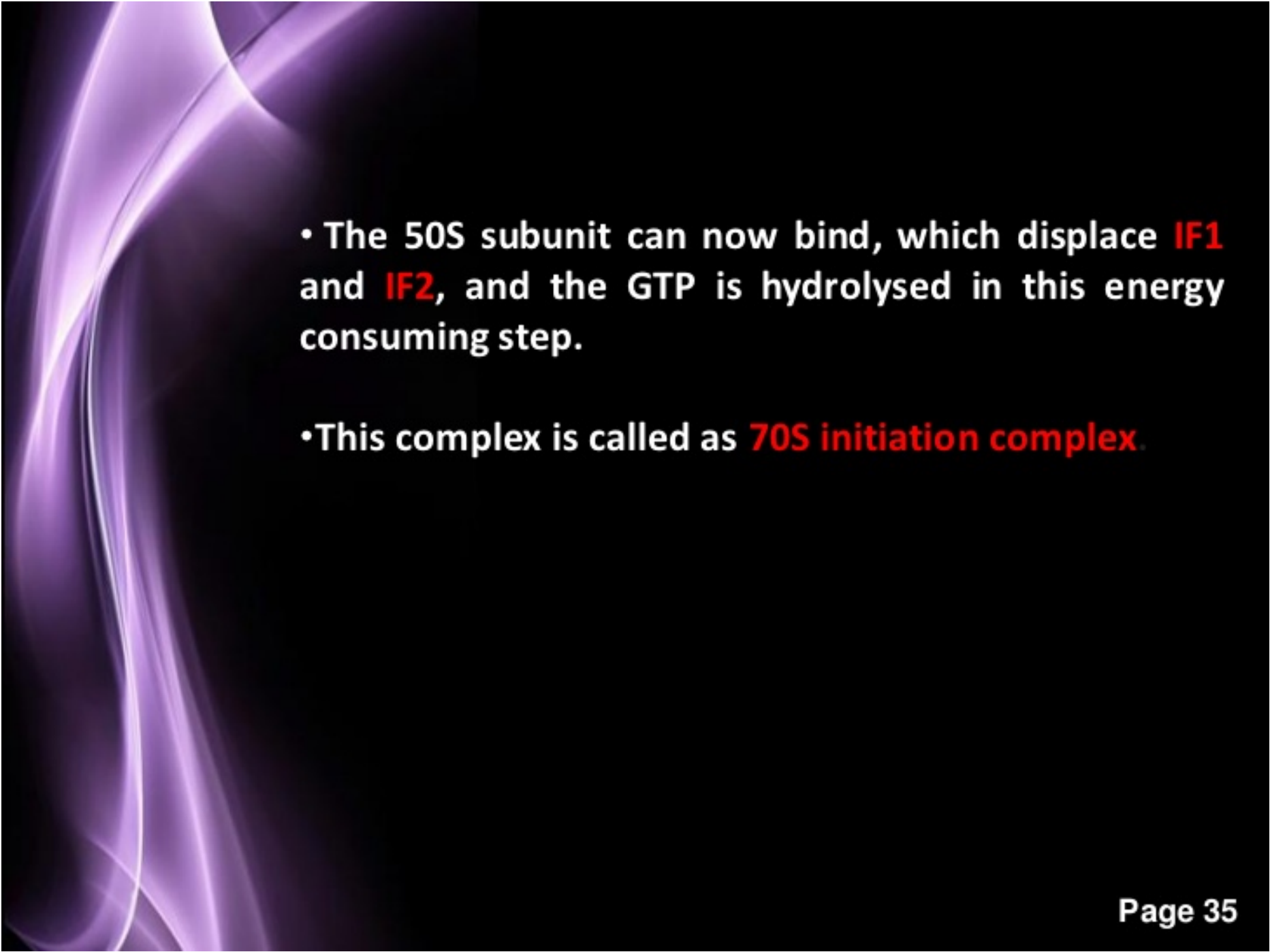
Fig. Charged tRNA (N-formyl methionyl tRNA_f^{met})



In Prokaryotes initiation requires the **large and small ribosome subunits**, the **mRNA**, the **initiator tRNA** and **three initiation factors (IF1, IF2, IF3)** and **GTP**. The overall sequence of the event is as follows

- **IF3** bind to the free 30S subunit, this helps to prevent the large subunit binding to it without an mRNA molecule and forming an inactive ribosome
- **IF2** complexed with **GTP** and **IF1** then binds to the small subunit. It will assist the charged initiator tRNA to bind.

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- The 30S subunit attached to an mRNA molecule making use of the **ribosome binding site (RBS)** on the mRNA
 - The initiator tRNA can then bind to the complex by base pairing of its anticodon with the AUG codon on mRNA.
 - At this point, IF3 can be released, as its role in keeping the subunits apart and helping the mRNA to bind are complete.
 - This complex is called **30S initiation complex**

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- The 50S subunit can now bind, which displace **IF1** and **IF2**, and the GTP is hydrolysed in this energy consuming step.
 - This complex is called as **70S initiation complex**.

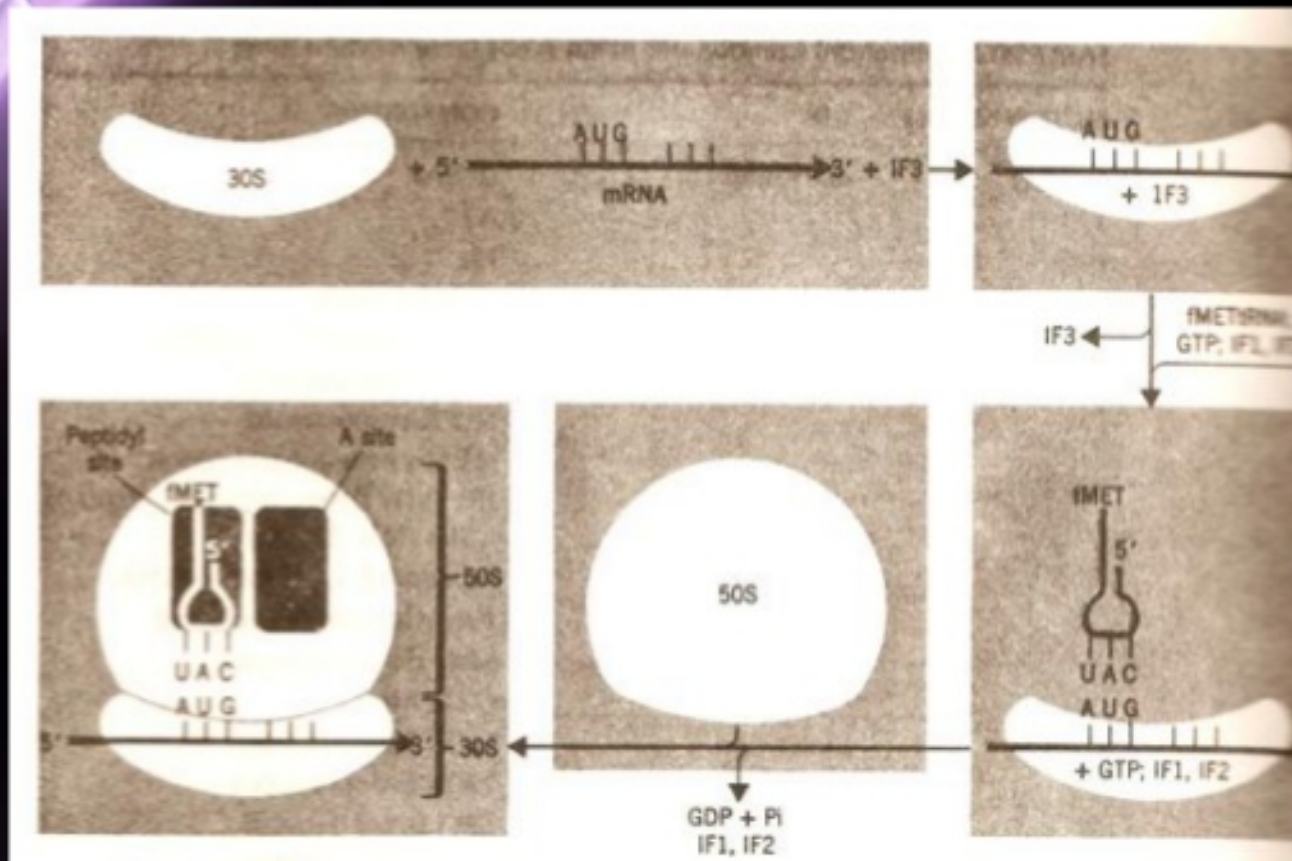

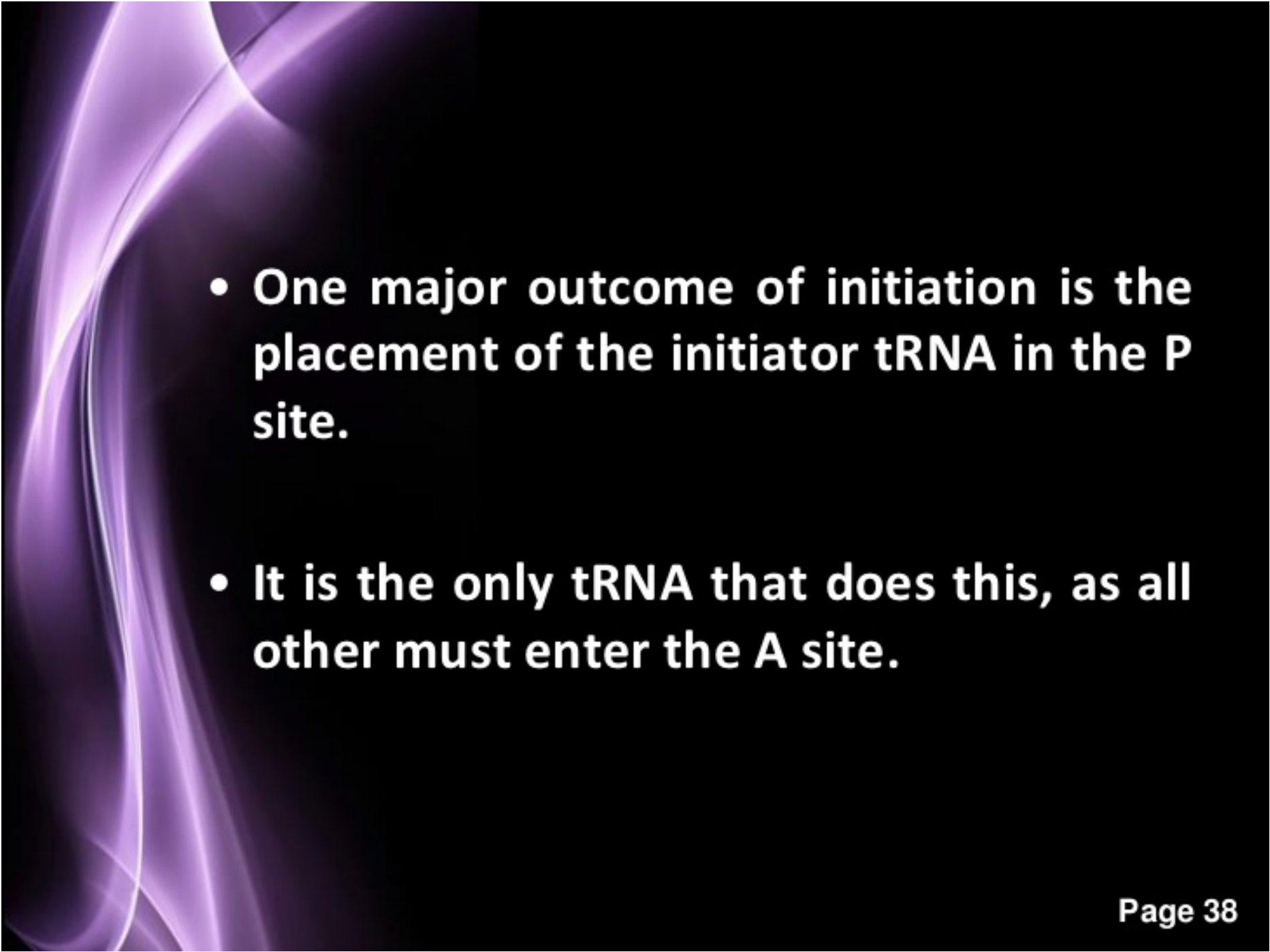


Fig. Formation of the 70S initiation complex

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- The assembled ribosome has two tRNA binding sites.
 - These are called the **A** and **P sites**, for **amino acyl** and **peptidyl** sites.
 - The **A site** is where incoming amino acyl tRNA molecules bind, and the **P site** is where the growing polypeptide chain is usually found.
 - These sites are in the cleft of small subunit and contain adjacent codons that are being translated.

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- **One major outcome of initiation is the placement of the initiator tRNA in the P site.**
 - **It is the only tRNA that does this, as all other must enter the A site.**

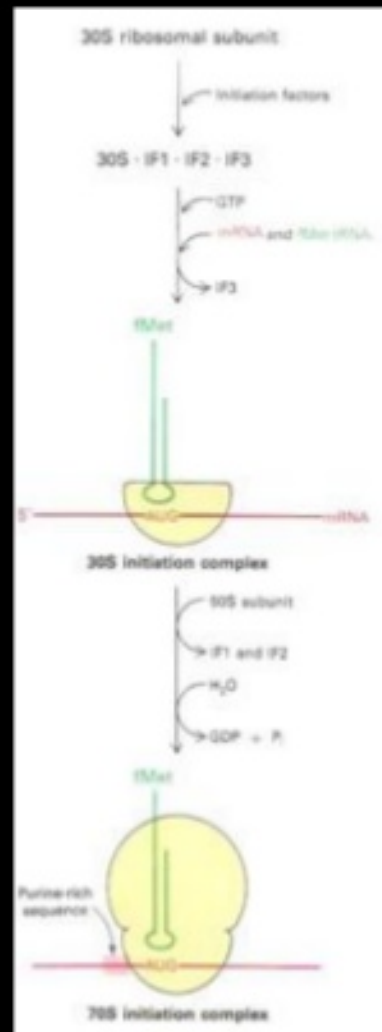
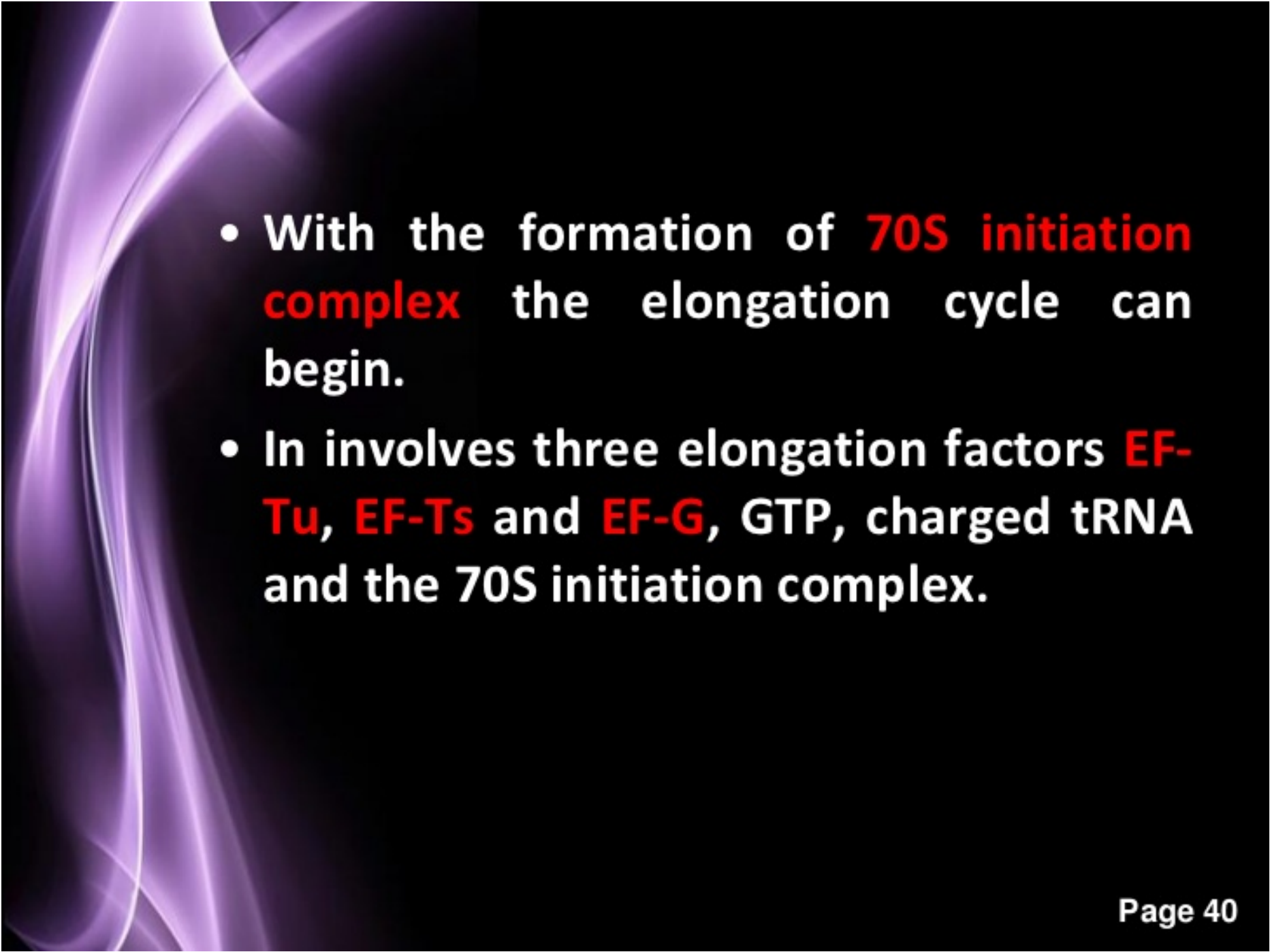


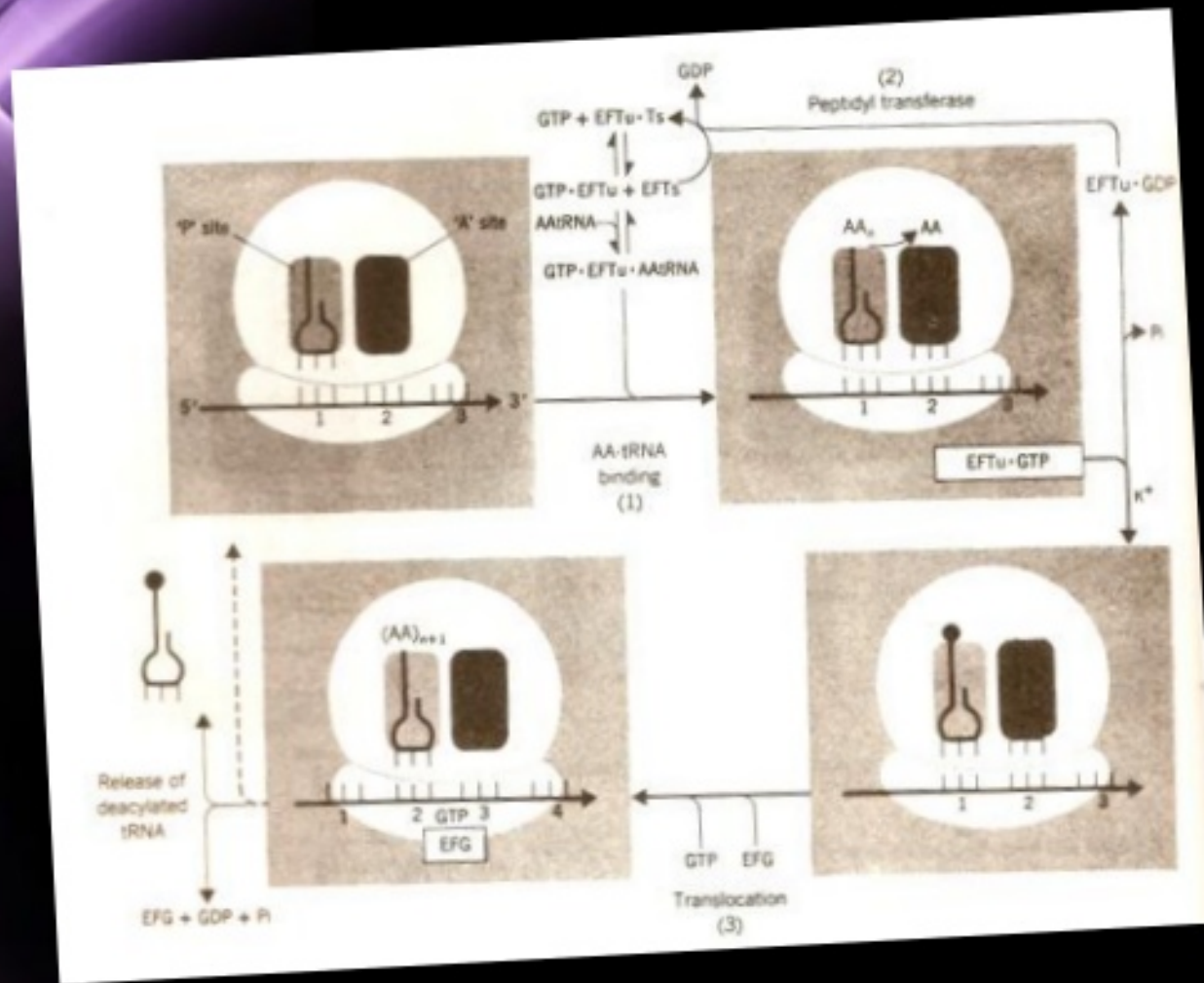
Fig. Initiation phase of protein synthesis

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- With the formation of **70S initiation complex** the elongation cycle can begin.
 - In involves three elongation factors **EF-Tu**, **EF-Ts** and **EF-G**, GTP, charged tRNA and the 70S initiation complex.

Elongation is divided into three steps:

1. Amino acyl tRNA delivery.

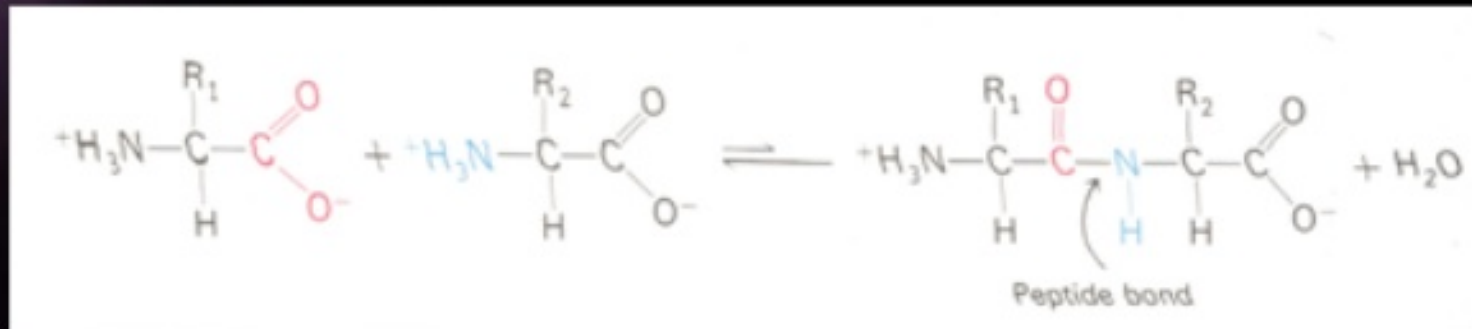
- **EF-Tu** is required to deliver the amino acyl tRNA to the A site and energy is consumed in this step by the hydrolysis of GTP.
- The released **EF-Tu. GDP** complex is regenerated with the help of **EF-Ts**.
- In the **EF-Tu EF-Ts** exchange cycle **EF-Ts** displaces the GDP and subsequently is displaced itself by GTP.
- The resultant **EF-Tu.GTP** complex is now able to bind another amino acyl tRNA and deliver it to the ribosome.
- All amino acyl tRNAs can form this complex with **EF-Tu** except the initiator tRNA.



- **Fig. Elongation of polypeptide chain**

2. Peptide bond formation.

- After aminoacyl-tRNA delivery, the **A-** and **P- sites** are both occupied and the two amino acids that are to be joined are in close proximity.
- The **peptidyl transferase** activity of the 50S subunit can now form a peptide bond between these two amino acids without the input of any more energy, since energy in the form of ATP was used to charge the tRNA.



- **Fig. Peptide bond formation**

3. Translocation.

- A complex of **EF-G (translocase)** and GTP binds to the ribosome and, in an energy consuming step, the discharged tRNA is ejected from the **P-site**, the **peptidyl-tRNA** is moved from the A-site to the P-site and the mRNA moves by one codon relative to one codon to the ribosome.
- **GDP** and **EF-G** are released, the latter being reusable. A new codon is now present in the vacant A-site.

The cycle is repeated until one of the termination codons (**UAA, UAG** and **UGA**) appear in the A-site.

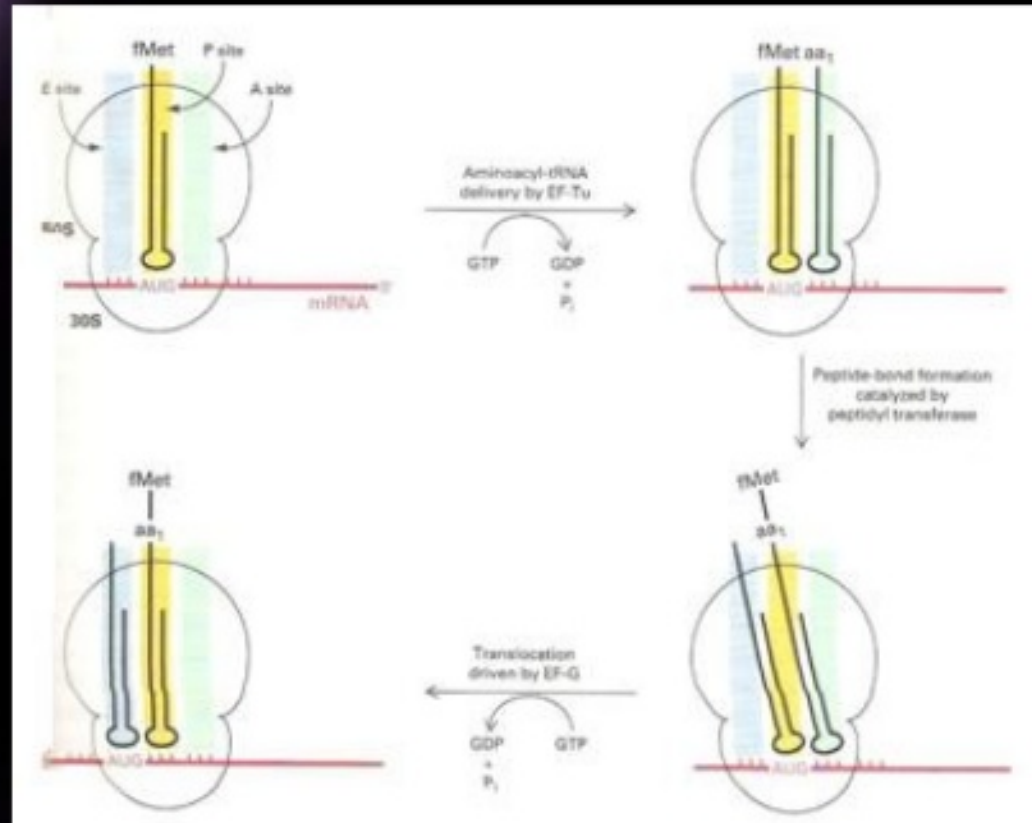
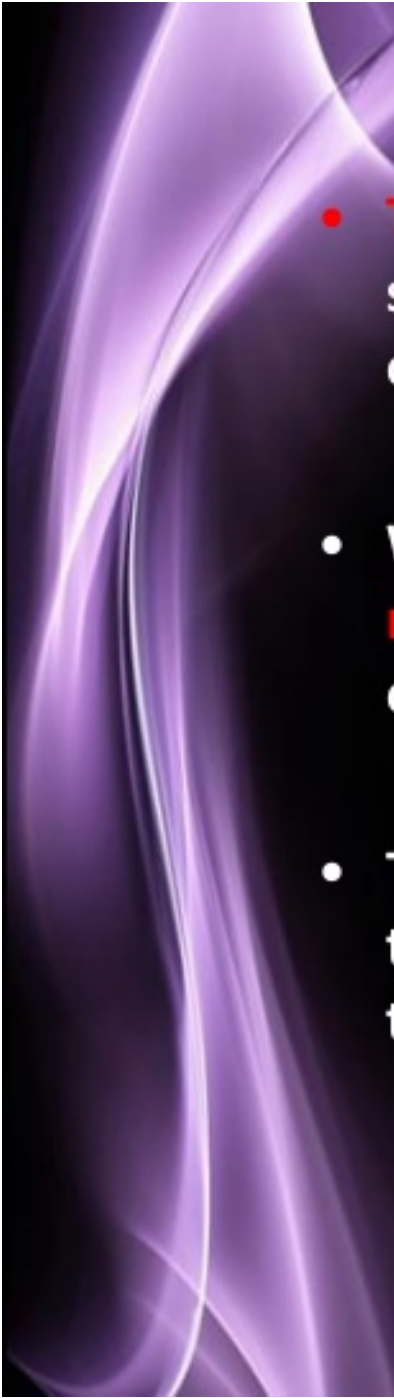



Fig. Translocation

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- **Termination** of the polypeptide happens when the A site of the ribosome faces a stop codon (UAA, UAG, or UGA).
 - When this happens, no tRNA can recognize it, but a **releasing factor** can recognize nonsense codons and causes the release of the polypeptide chain.
 - The 5' end of the mRNA gives rise to the protein's N-terminus, and the direction of translation can therefore be stated as N->C.

- Termination of polypeptide synthesis is signalled by one of the three **termination codons** in the mRNA (**UAA, UAG** and **UGA**) immediately following the last amino acid codon.
- In **prokaryotes**, once a termination codon occupies the ribosomal A-site, three termination or **release factors**, viz., the protein **RF1, RF2** and **RF3** contribute to-
 - The hydrolysis of the terminal peptidyl-tRNA bond.
 - Release of the free polypeptide and the last uncharged tRNA from the P-site
 - The dissociation of the 70S ribosome into its 30S and 50S subunits

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- **RF1** recognizes the termination codon **UAG** and **UAA** and **RF2** recognize **UGA** and **UAA**.
 - Either **RF1** or **RF2** binds at the termination codon and induces peptidyl transferase to transfer the growing peptide chain to a water molecule rather than to another amino acid.
 - Function of **RF3** is not known.

Polyribosomes

- A single strand of mRNA is translated simultaneously by many ribosomes, spaced closely together.
- Such clusters of ribosomes are called **polysomes** or **polyribosomes**.
- The simultaneous translation of a single mRNA by many ribosomes allow **highly efficient use of the mRNA**

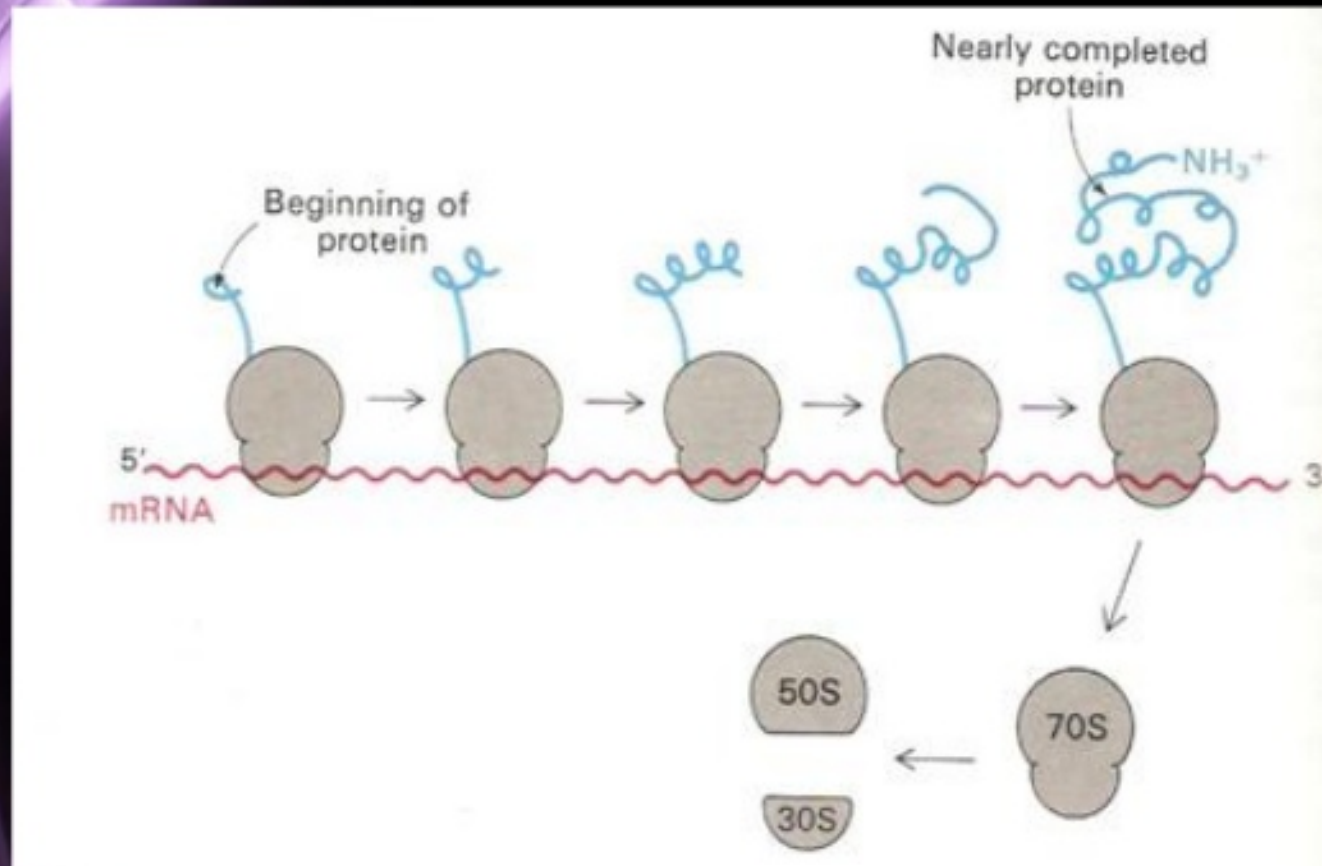


Fig. Polyribosomes



THANK YOU ALL

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