Translation

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What is the genetic code?

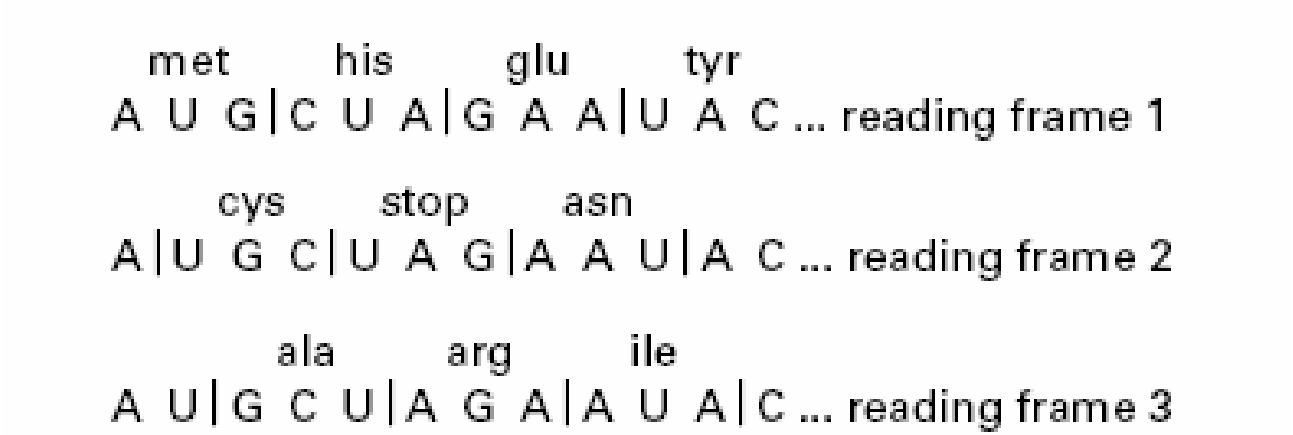
- Triplet code (codon).
- ***Nonoverlapping***: no bases are shared between consecutive (4^3=64)
- ***Commaless*** : ribosome movement with no more than three bases at a time.
- ***Degenerate*** (the opposite is ambiguous) more than one triplet can encode the same amino acid (20 aa + 3 stop signals).
- Third base: wobble base.
- ***Universal:*** same in all organisms.
- Reading frames: different ways of reading message.

First position (5′ end)	Second position U	C	A	G	Third position (3′ end)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Figure 22-3 Principles of Biochemistry, 4/e

Start and Stop Codons and the Reading Frame

- The **start codon AUG.**
- The codons UAA, UAG, and UGA are **stop codons**.
- There are three potential **reading frames in any mRNA.**



Reading frames—the genetic code is read in blocks of three.

Translation

1. Free amino acids are attached to tRNA molecules.
2. Initiation.
3. Elongation: the growing polypeptide chain.
4. Termination.

tRNA molecules carry AA to mRNA on the ribosome

- Each tRNA molecule has an amino acid attachment site at its 3 end and an **anticodon:** three bases that are complementary in sequence to a codon on the mRNA.

- *For example,* the codon for methionine is 5 AUG 3 which will base pair with the anticodon 3 UAC 5.

Table 12.2

Base-Pairing Combinations in the Wobble Scheme

Base at 5′ End of Anticodon	Base at 3′End of Codon
I*	A, C, or U
G	C or U
U	A or G
A	U
C	G

The attachment of an amino acid to its correct tRNA molecule is catalyzed by the enzyme **aminoacyl tRNAsynthase**.

1- The amino acid is joined, via its carboxyl group, to (AMP) and remains bound to the enzyme.

2- **Amino acid activation**: A tRNA that is attached to an amino acid is known as a **charged tRNA.**

There are at least *20 aminoacyl tRNA synthases*, one for each AA and its specific tRNA.

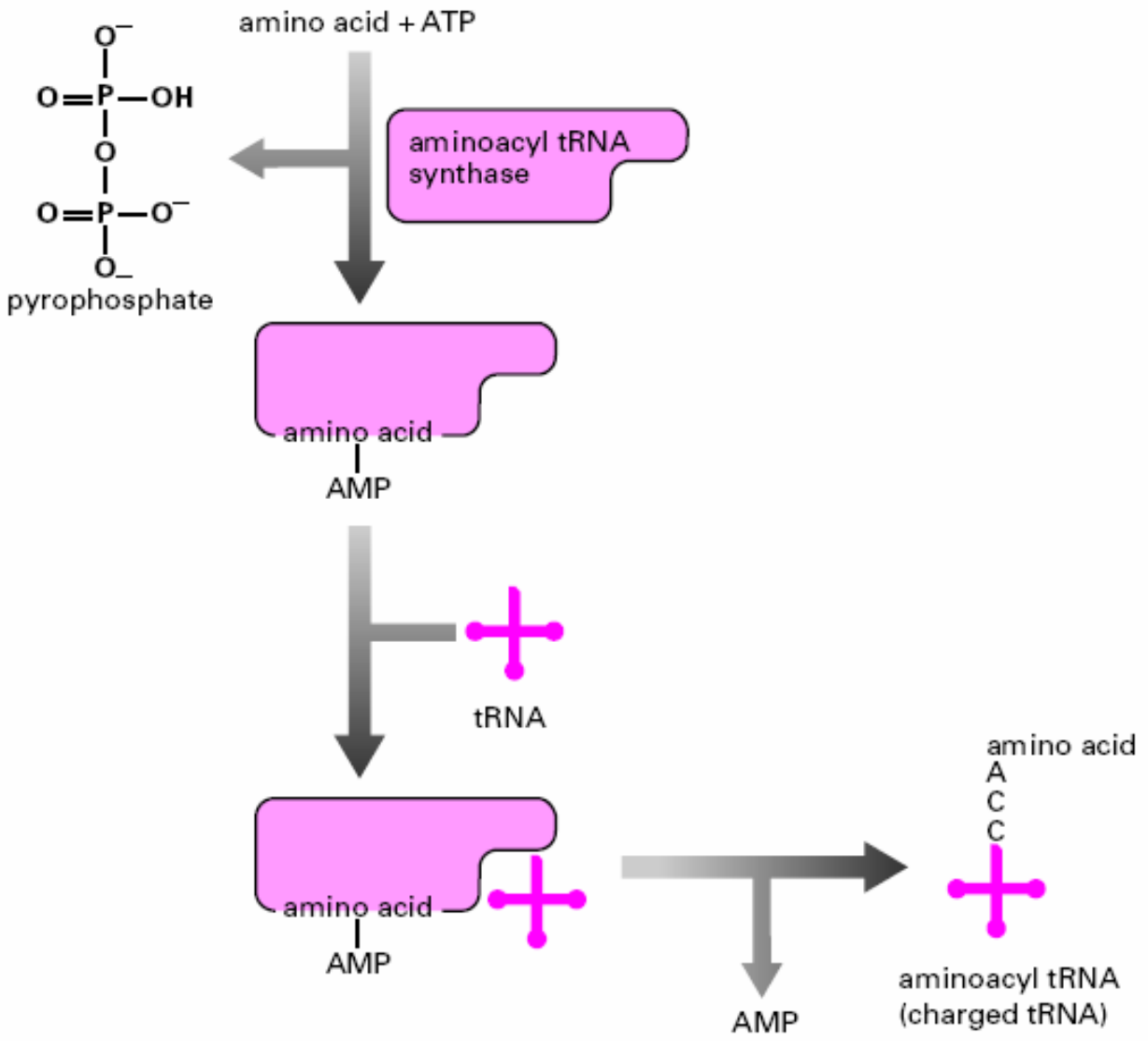


Fig: Attachment of AA to its tRNA.

Protein synthesis occurs on the surface of ribosomes:

- Ribosomes : rRNA+ proteins located in the cytoplasm of eukaryotic and bacterial cells.

- Ribosomes :small subunit binds mRNA.

  large subunit joins mRNA/small subunit complex.

- Large subunit has:

➢ P site “peptidyl” that holds the growing polypeptide chain.

➢ A site “aminoacyl”that binds the next AA to be added to the growing polypeptide chain.

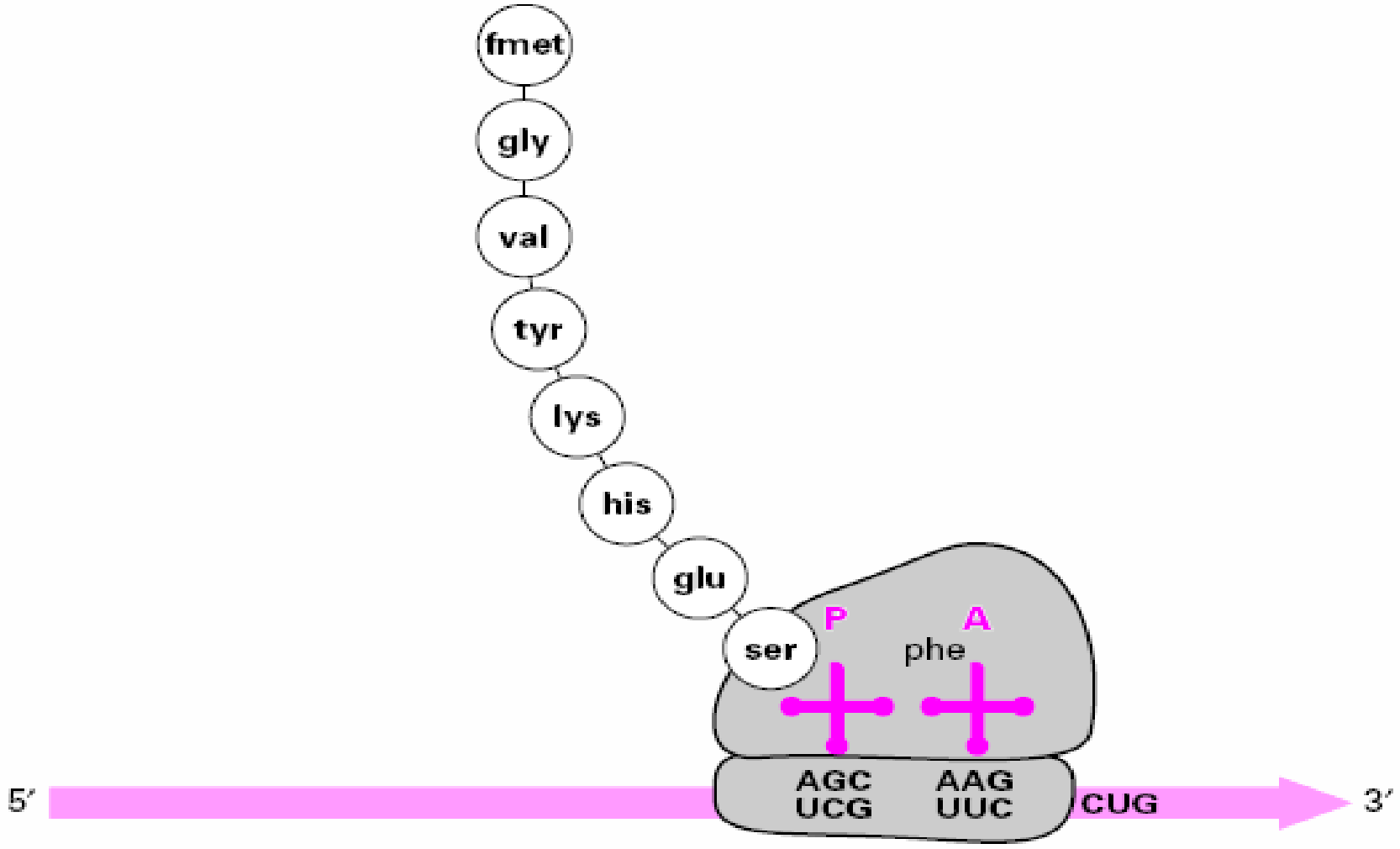


Fig: The P and A sites on the ribosome.

- The S value**, is a sedimentation rate. (**Eukaryotic ribosomes: 80S, 60S and 40S).
- The formation of a peptide bond between two amino acids takes place on the ribosome.
- An incoming tRNA with its linked amino acid occupies the **aminoacyl site (A site), and the tRNA attached to the growing polypeptide chain occupies the peptidyl site (P site).**

BACTERIAL PROTEIN SYNTHESIS

- Bacterial mRNA molecules usually have a nucleotide sequence

•5` **GGAGGU 3** ` called the **ribosome-binding site (RBS) = (Shine–Dalgarno sequence):** whose center is about 8 to 13 nucleotides upstream of (5 to) the AUG start codon.

•This sequence is complementary to a short stretch of sequence, 3 ` **CCUCC** 5 `, found at the 3 end of the **rRNA** molecule within the **30S** ribosomal subunit.

•There are three possible reading frames, the **reading frame** that is actually used by the cell is defined by the first AUG that the ribosome encounters **downstream of the RBS.**

Chain Initiation

- The first amino acid in a new bacterial polypeptide is always **formyl methionine (fmet).**
- Methionine first attaches to a specific tRNA molecule, tRNA fmet, and is then **modified by the addition of a formyl group** that attaches to its amino group.
- $tRNA^{fmet}$ has the anticodon sequence 5` CAU 3` that binds to its complementary codon.

Initiation complex

- mRNA, 30S, fmet $tRNA^{fmet}$ (30S initiation complex) , GTP, three protein intiation factors called IF-1, IF-2 and IF-3 (facilitates the binding of mRNA to 30S ribosomal subunit and prevent premature binding of the 50S subunit).

- IF-2 binds GTP and aids in the selection of fmet $tRNA^{fmet}$

- IF-1 binds to IF-2 and IF-3 and it facilitates the actions of both, catalyses the separation of the 30S and 50S.

- 70 intiation complex : 30S initiation complex + 50S.

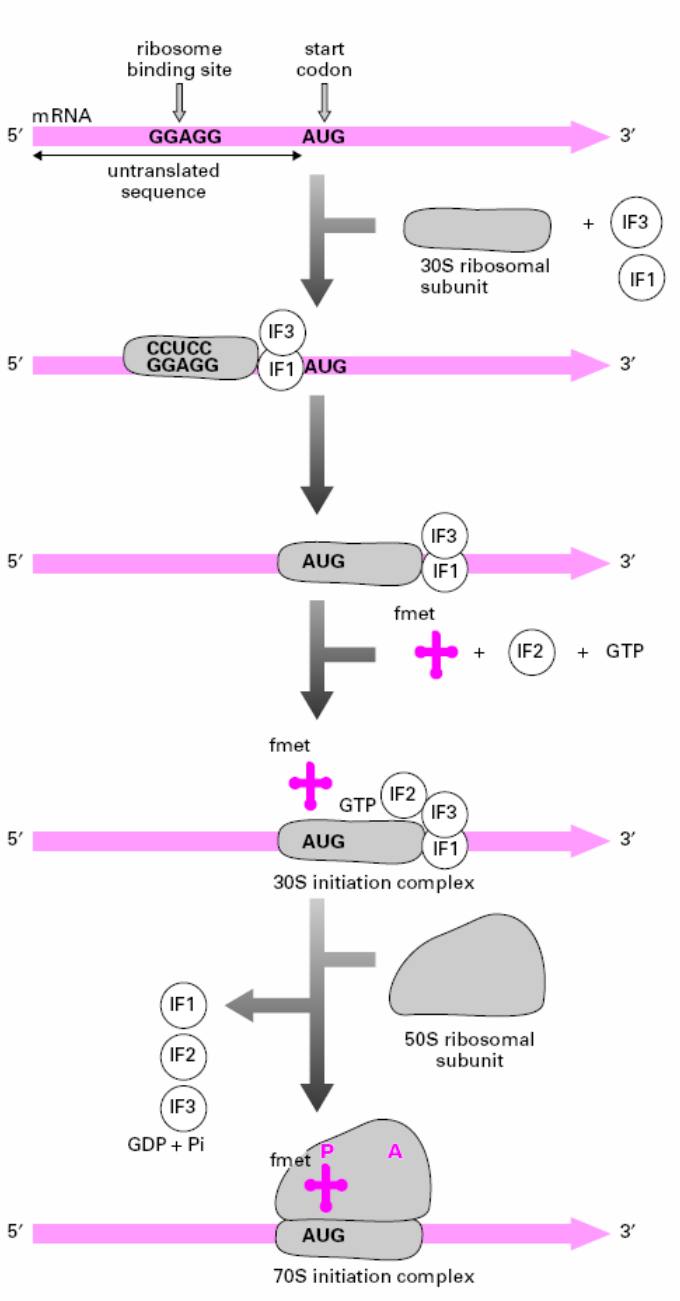


Fig: Formation of the 70S initiation complex.

Elongation of the Protein Chain

1- The identity of the incoming aminoacyl tRNA is determined by the codon on the mRNA. If, for example, the second codon is 5` UUC 3`, then phenylalanyl $tRNA^{phe}$ (whose anticodon is 5` GAA 3`) will occupy the A site.

2- The P site has, of course, already been occupied by $tRNA^{fmet}$ during the formation of the initiation complex.

3-Both the A and P sites are occupied, the enzyme **peptidyl transferase catalyzes the formation of** a peptide bond between the two amino acids (fmet and phe in this example).

4- The ribosome moves along the mRNA to the right so that the tRNA dipeptide now occupies the P site.

Translocation: the movement of the ribosome, three nucleotides at a time, relative to the mRNA, is called**.**

5- Elongation of a polypeptide chain needs the help of three proteins called **elongation factors (**TU, TS and G) and a molecule of guanosine triphosphate (GTP).

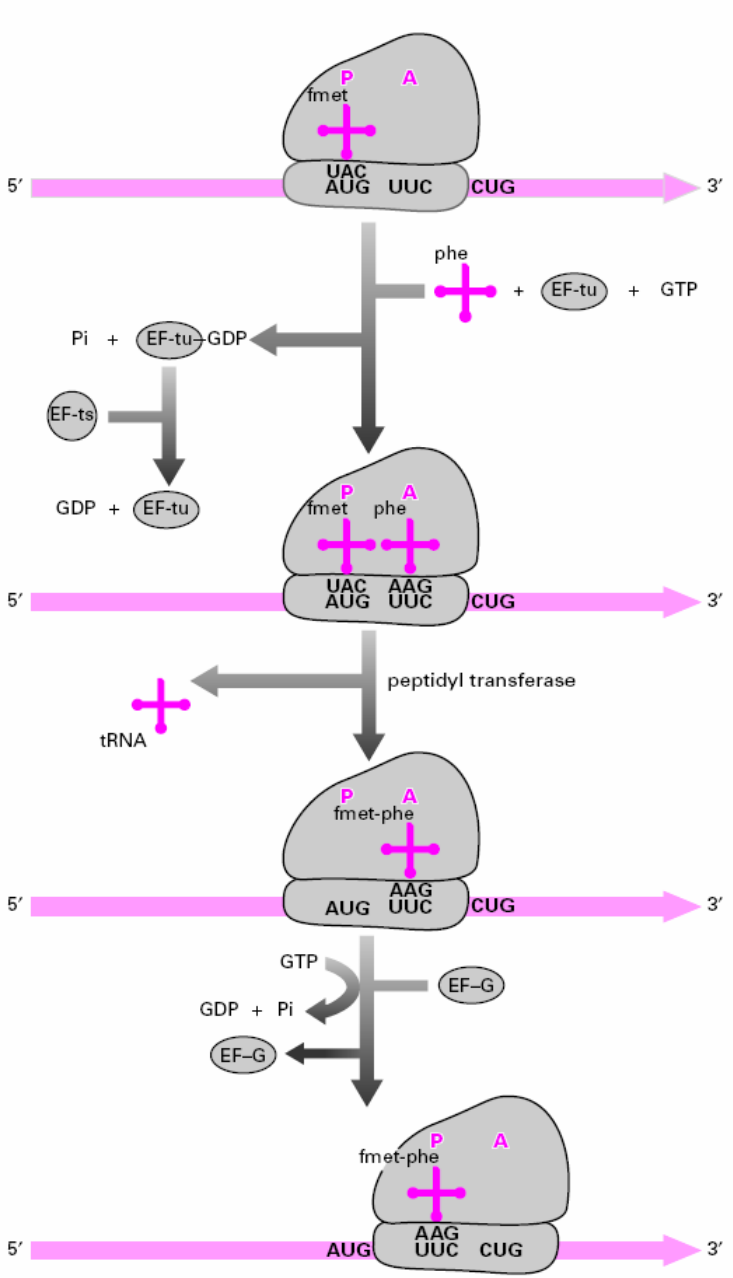


Fig: Elongation of the protein chain. Pi represents the inorganic phosphate ion HPO2− 4 .

- Proteins are synthesized beginning at their amino or **N terminus**.

- The last amino acid in the chain has a free carboxyl group and is known as the carboxyl or C terminus.

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Termination of Protein Synthesis

- There are three stop codons, UAG, UAA, and UGA, that have no corresponding tRNA molecule.
- Instead of interacting with tRNAs, the A site occupied by one of these codons is filled by proteins known as **chain release factors.**
- **In the presence** of these factors the newly synthesized polypeptide chain is freed from the ribosome, and the mRNA, tRNA, and the 30S and 50S ribosomal subunits dissociate.

Chain Termination

UAA, UAG and UGA stop signals (not recognised by any tRNA, but they are recognised by release factors.

1. RF-1 binds to UAA and UAG.
2. RF-2 binds to UAA and UGA.
3. RF-3 facilitates the activity of the other two release factors.

GTP.

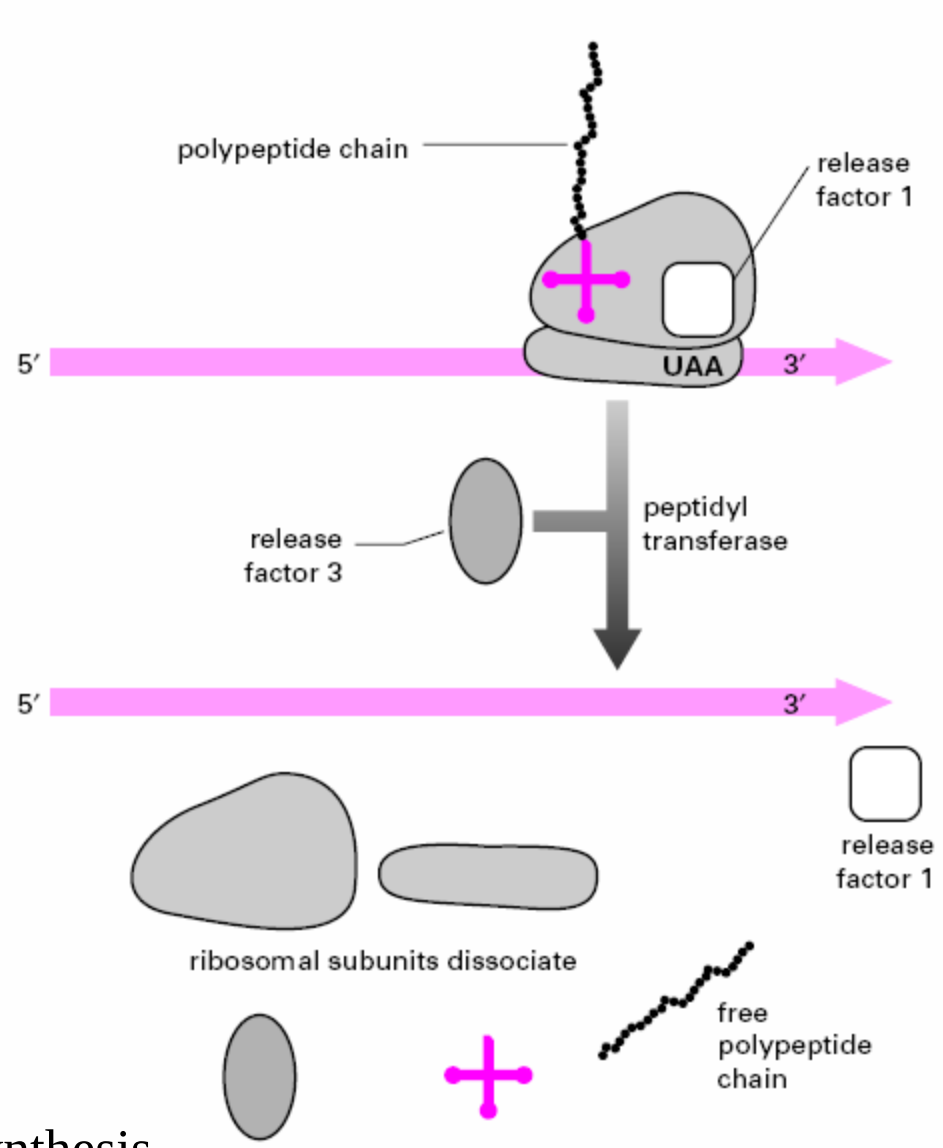


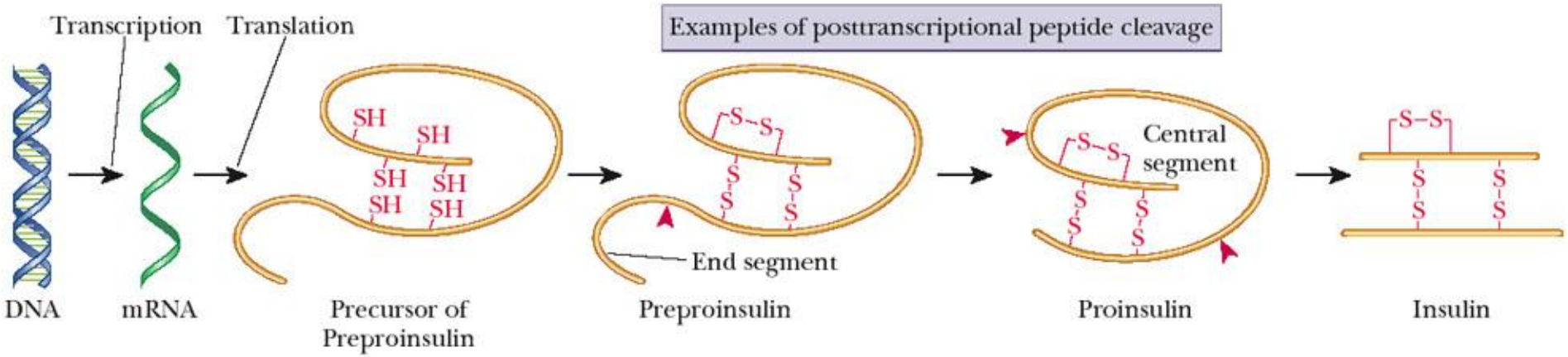
Fig: Termination of protein synthesis.

EUKARYOTIC PROTEIN SYNTHESIS

- The initiation of protein synthesis is more complex in eukaryotes.
- Eukaryotic mRNAs have at their 5 end a **7-methyl guanosine** cap.
- Their proteins always start with **methionine**. The methionine is often removed from the protein after synthesis.
- The $tRNA^{met}$ binds to the **40S** subunit and then the large **60S** ribosomal subunit attaches to form the **80S** initiation complex.
- Compartmentalized transcriptin/translation.

Posttranslational Modification of Proteins

- N-formylmethionine is cleaved off (in prokaryotes).
- Breaking bonds: (cleavage of preproinsulin to proinsulin and then to insulin).
- Various cofactors (such as heme groups) are added, and disulfide bonds are formed.
- Some amino acids are covalently modified (proline to hydroxyproline).
- Other covalent modifications: addition of carbohydrates or lipids. Protein can be methylated, phosphorylated and ubiquitinylated.



Antibiotics and Protein Synthesis

- Chloramphenicol, which blocks the peptidyl transferase reaction.
- Tetracycline inhibits the binding of an aminoacyl tRNA to the A site of the ribosome.
- Streptomycin inhibits the formation of the 70S initiation complex because it prevents tRNAfmet from binding to the P site of the ribosome.