RNA TRANSLATION

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Information contained in the nucleotide sequence of mRNA instructs the synthesis of a particular polypeptide

Three phases:

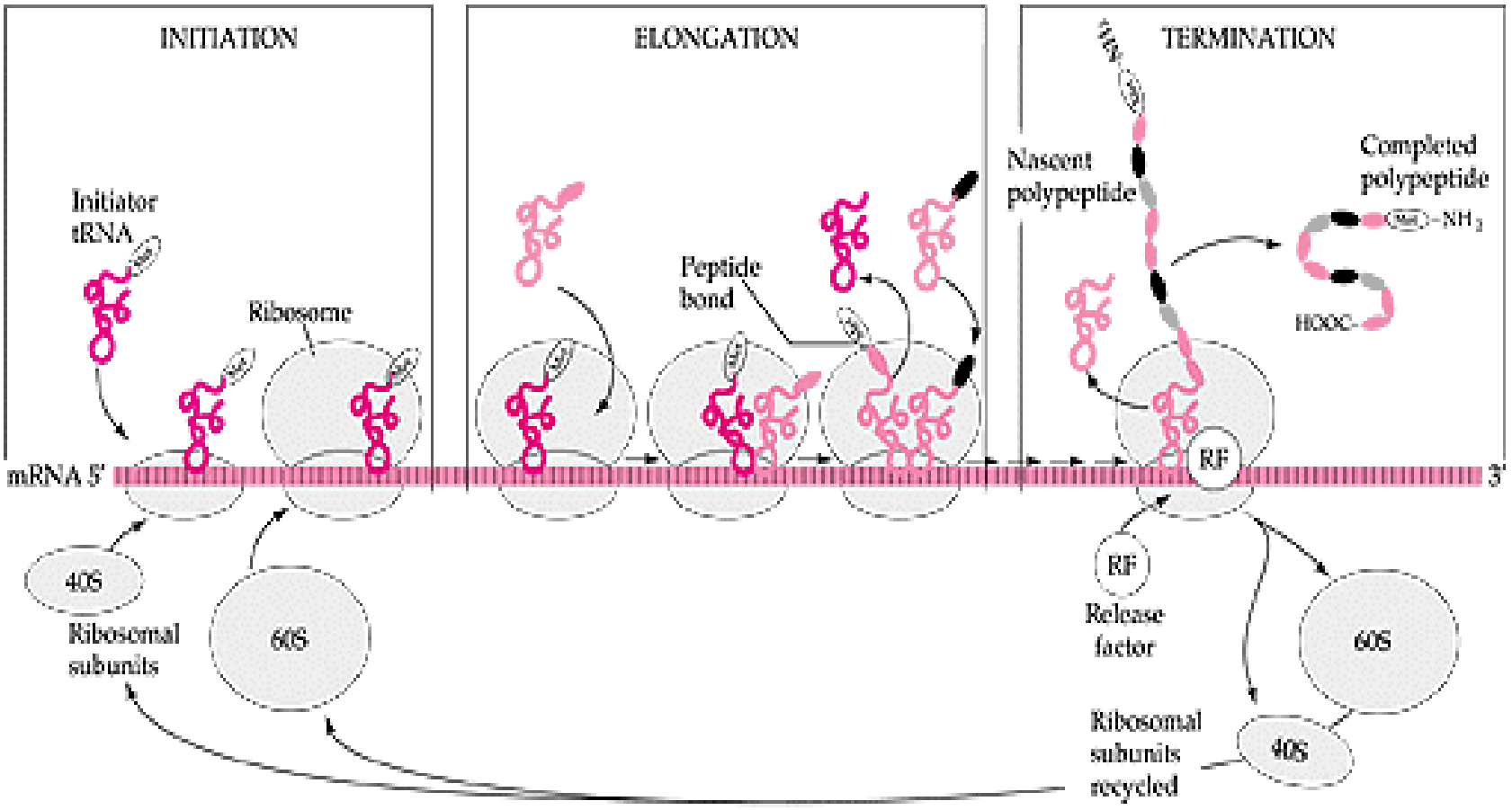
Initiation

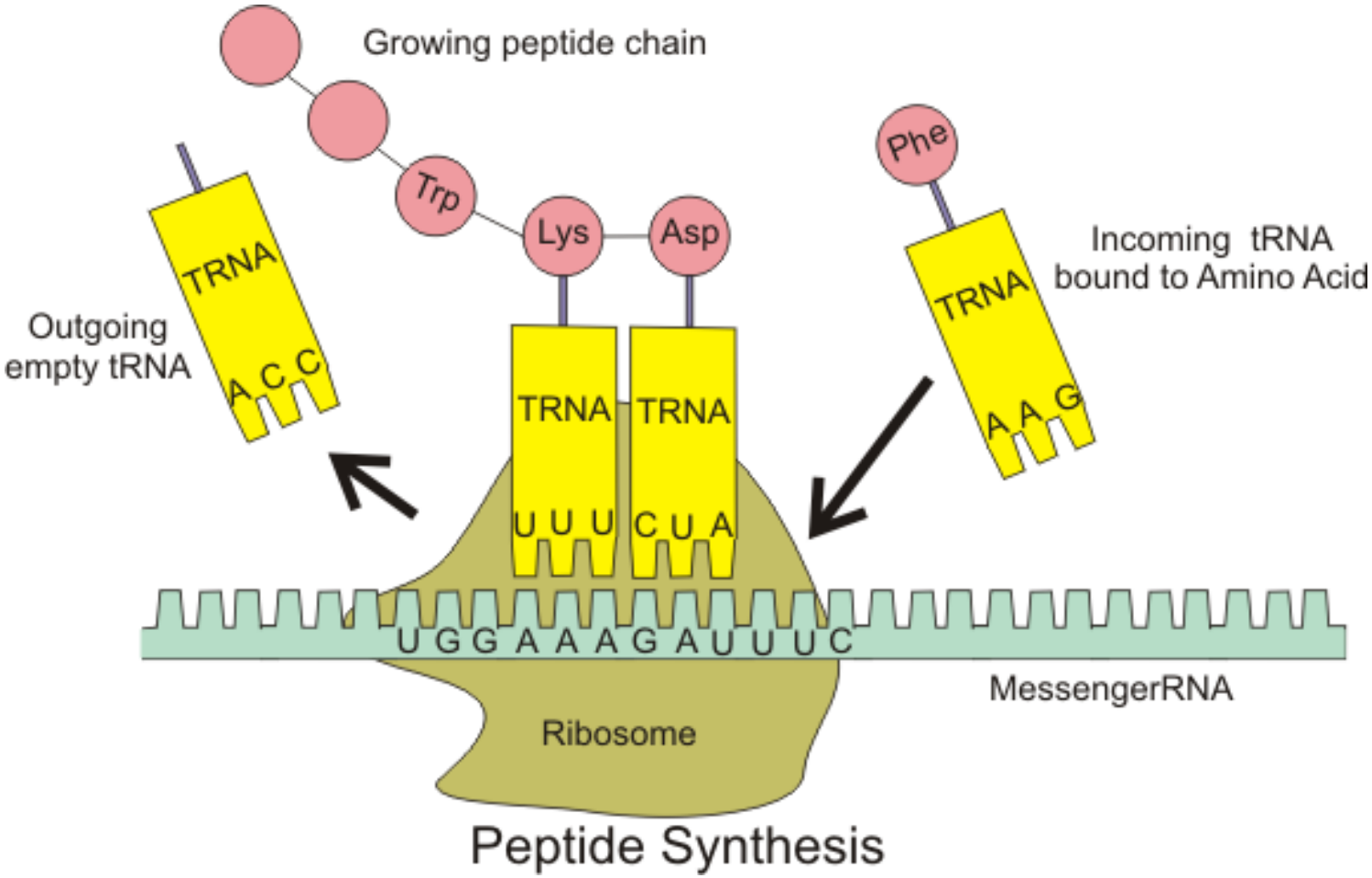
Elongation

Termination

Regulated by proteins: initiation, elongation and termination factors

EVENTS OF EUCARYOTIC TRANSLATION




Growing peptide chain
Trp
Lys
Asp
Phe
TRNA
A C C
Outgoing
empty tRNA
TRNA
TRNA
U U U C U A
TRNA
A A G
Incoming tRNA
bound to Amino Acid
U G G A A A G A U U U C
MessengerRNA
Ribosome
Peptide Synthesis

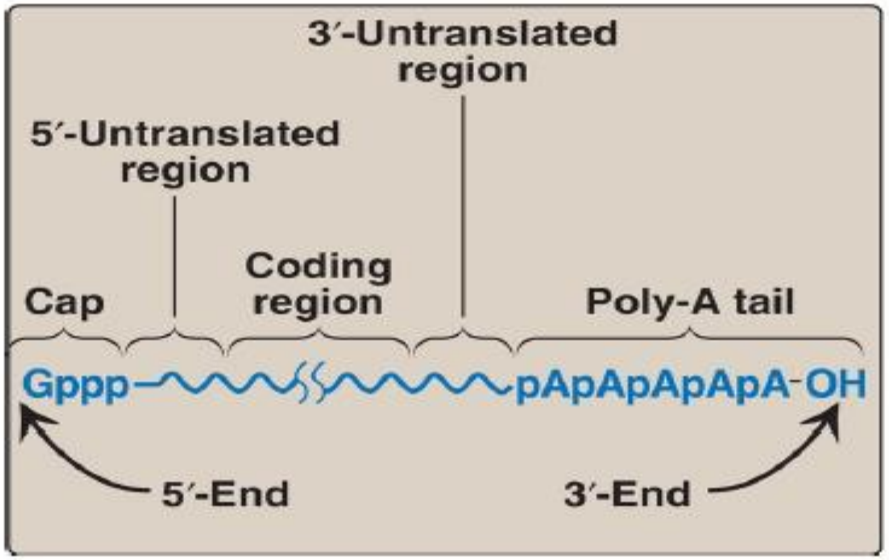


Fig: Structure of eukaryotic messenger RNA. G = guanine; A = adenine

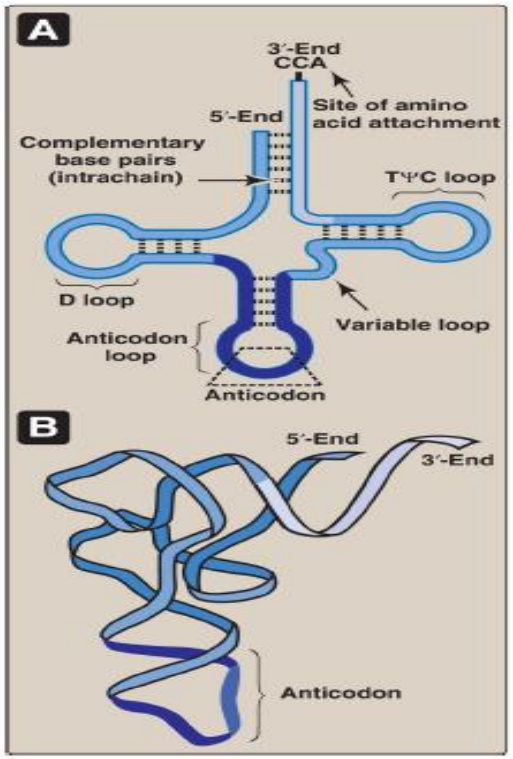


Fig A. Characteristic transfer RNA (tRNA) secondary structure (cloverleaf). B. Folded (tertiary) tRNA structure found in cells. D =dihydrouracil; Ψ = pseudouracil; T = thymine; C = cytosine; A = adenine.

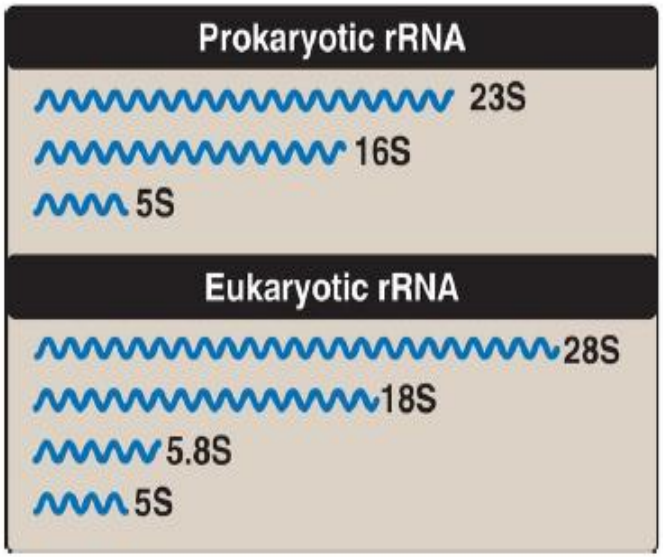


Fig: Prokaryotic and eukaryotic ribosomal RNA (rRNA). S = Svedberg unit.

GENETIC CODE

	U	C	A	G
U	UUU = phe UUC = phe UUA = leu UUG = leu	UCU = ser UCC = ser UCA = ser UCG = ser	UAU = tyr UAC = tyr UAA = stop UAG = stop	UGU = cys UGC = cys UGA = stop UGG = trp
C	CUU = leu CUC = leu CUA = leu CUG = leu	CCU = pro CCC = pro CCA = pro CCG = pro	CAU = his CAC = his CAA = gln CAG = gln	CGU = arg CGC = arg CGA = arg CGG = arg
A	AUU = ile AUC = ile AUA = ile AUG = met	ACU = thr ACC = thr ACA = thr ACG = thr	AAU = asn AAC = asn AAA = lys AAG = lys	AGU = ser AGC = ser AGA = arg AGG = arg
G	GUU = val GUC = val GUA = val GUG = val	GCU = ala GCC = ala GCA = ala GCG = ala	GAU = asp GAC = asp GAA = glu GAG = glu	GGU = gly GGC = gly GGA = gly GGG = gly

Characteristic of the Genetic Code:

- Specific
- Universal
- Redundant (degeneracy)
- Non overlapping

Single nucleotide base change:

- Silent mutation
- Missense mutation
- Nonsense mutation

INITIATION

The initiation steps bring together the 40S and 60S ribosomal subunits, mRNA, and the initiator tRNA which is complexed to the amino acid methionine (met)

Protein synthesis is initiated when an mRNA, ribosome, and the first tRNA molecule carrying its methionine amino acid) come together.

- Note that the ribosome is inactive when it exists as two subunits (a large one and a small one) before it contacts an mRNA. The small unit of the ribosome will initiate the process of translation when it encounters an mRNA in the cytoplasm

The first AUG codon on the 5'end of the mRNA acts a "start" signal for the translation machinery.

Codes for methionine amino acid

This codon and thus amino acid will always be the first in any and all mRNA molecules.

Initiation is complete when the methionine tRNA occupies one of the two binding sites on the ribosome

Since this first site is the site where the growing peptide will reside, it's known as the P site.

This is where the growing Protein will be. There is another site just to the 3'direction of the P site; it is known as the A site. This is where the incoming tRNA will Attach itself.

Even though every protein begins with the methionine amino acid, not all proteins will ultimately have methionine at one end. If the "start" methionine is not needed, it is removed before the new protein goes to work (either inside the cell or out side the cell, depending on the type of protein synthesized)

ELONGATION

The incoming tRNA will bind to the A site (next to where the tRNA with the methionine attached is on the P site)

All available tRNAs will approach the site and try to attach, but the only tRNA which will successfully attach is the one whose anticodon is complementary to the codon of the A site on the mRNA

Let us say for example that the second tRNA that lands next to the methionine –tRNA is leucine. The two tRNAs (holding methionine on the P site and leucine on the A site) are now next to each other.

In order for a protein chain to form, the amino acids must be attached, linked together.

Amino acids continue to be linked until the protein is finished. Once the bond has formed between the two amino acids, the tRNA on the P site leaves and passes its amino acid on to the tRNA on the A site.

The tRNA with the two amino acids on it is now sitting on the P site (because it is holding the growing protein). The ribosome slides down three bases (1 codon on the mRNA) exposing a new A site by the action of a translocase

The next appropriate tRNA molecule "lands" bringing its amino acid right next to the tRNA holding the two amino acids. At this point, the process repeats itself: a peptide bond forms between the two amino acid molecules already joined together and the newly brought in amino acid: the tRNA on the P site leaves and the chain of amino acids is passed to the tRNA on the A site by the action of translocase (now this site is called the P site because this tRNA now has the growing protein chains). The ribosome slides down another codon and the procedure repeats itself until the termination event occurs.

TERMINATION

The elongation procedure continues until the proper protein is completed. A "stop" codon (UAA, UGA or UAG) signals the end of the process. There is no tRNA that is complementary to the Stop Codon, so the process of building the protein stops.

An enzyme called the releasing factor then frees the newly made polypeptide chain, also known as the protein from the last tRNA. The mRNA molecule is released from the ribosome as the small and large subunits fall apart. The mRNA can then be re-translated or it may be degraded, depending on how much of that particular protein is needed. All mRNA messages are eventually degraded when the protein is no longer needed to be made.

ANTIBIOTIC INHIBITORS OF PROTEIN SYNTHESIS

Many antibiotic inhibitors of protein synthesis are known

Mechanisms of action of several of them have been elucidated.

Streptomycin, tetracycline, chloramphenicol and erythromycin-highly potent anti-bacterial agents

-Block different steps in procaryotic protein synthesis

- Streptomycin-inhibits initiation and causes misreading of mRNA

- Tetracycline- binds to the 30S subunit

- Chloramphenicol- inhibits the peptidyl transferase of the activity of the 50S ribosomal subunit
- Erythromycin- binds to the 50S subunit and inhibits translocation

POST-TRANSLATIONAL EVENTS

Proteins contain signals that determine their ultimate destination. The synthesis of all proteins (except those encoded by mitochondrial and chloroplast DNA) begins on free ribosomes in the cytosol

Covalent modifications:

Trimming:

- Precursor molecules not functionally active
- Zymogens

Phosphorylation:

- Specific amino acid residues- increase or decrease functional activity

Glycosylation:

- Part of the cellular membrane or secreted from the cells

Hydroxylation:

- Proline and lysine residues of the α-chains of collagen

Vitamin Biotin bound to carboxylase enzymes

In eucaryotes protein, synthesis continues in the cytosol unless the nascent chain contains a signal sequence that directs the ribosome to the Endoplasmic reticulum (ER). Amino-terminal signal sequences of the procaryotes as well as eucaryotes consist of a hydrophobic segment of 10 to 15 residues preceded by a basic region.

Signal recognition particle (SRP), a ribonucleoprotein assembly, recognises signal sequences and brings ribosomes bearing them to the ER. The nascent chain is then translocated across the ER membrane. Translocation continues until a stop –transfer sequence is encountered

Multiple signal sequences and stop-transfer sequences give rise to proteins that weave back and forth across the bilayer. Most signal sequences are cleaved after translocation.

Glycosylation also begins in the ER. Glycoproteins acquire the core of their N-linked sugars from a donor in the ER. Transport vesicles carry proteins from the ER to the golgi complex for further modification of the carbohydrate units and for sorting. Resident ER proteins are recognised by their carboxyl-terminal sequence

The golgi consists of a stack of membranous sacs that are differentiated into cis, medial and trans compartments. The cis compartment is closest to the ER and receives vesicles from it.

A different set of vesicles transfers proteins from the cis to the medial and then to the trans compartment of the golgi. Carbohydrate units of the glycoproteins are modified in each of these compartments

Enzymes destined to be delivered to lysosomes contain a conformational motif that leads to the addition of a mannose 6- phosphate unit. This phosphorylated sugar is recognised by a membrane-bound receptor that brings glycoproteins to pre-lysosomes, which then fuse with lysosomes. Sorting takes place primarily in the trans golgi.

Most mitochondrial proteins are encoded by nuclear DNA and are synthesized by free ribosomes in the cytosol. They contain signals that direct their entry into mitochondria and specify whether they are to remain in the outer membrane or be translocated to the inner membrane, the intermembrane space or the matrix

Cytosolic proteins containing a carboxyl-terminal sequence are directed to peroxisomes. Nuclear localisation signals enable proteins to enter the cell nucleus through large pores in the nuclear envelope. Soluble cytosolic proteins can be given a membrane anchor.

Specific proteins are imported into eucaryotic cells by receptor–mediated endocytosis. Many viruses and toxins enter cells by receptor-mediated endocytosis. The acidity of endosomes (PH 5 to 6) induces conformational changes in these proteins and permits their release into cytosol.

Proteins are also targeted for destruction. Ubiquitin, a protein present in all eucaryotes and highly conserved in evolution, becomes covalently linked to proteins destined for degradation