Aim: How is mRNA translated?

Ribosome function

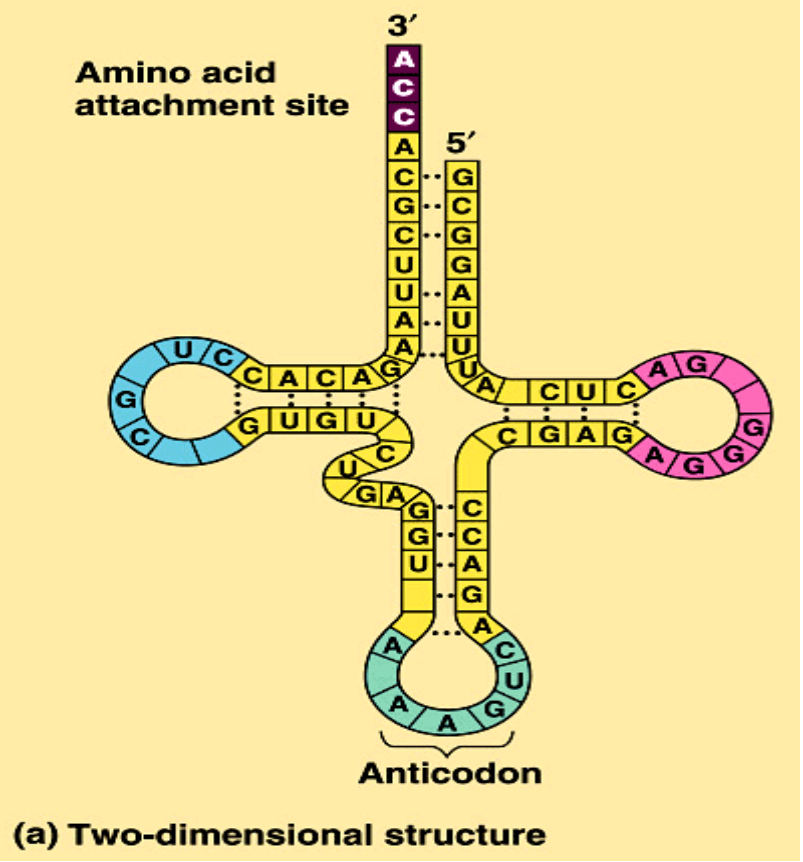
- Free ribosomes synthesize cytoplasmic proteins/enzymes.
- ER-bound ribosomes make proteins for the nucleus, ER, golgi, lysosomes, vacuoles, and cell membranes.

Ribosome structure

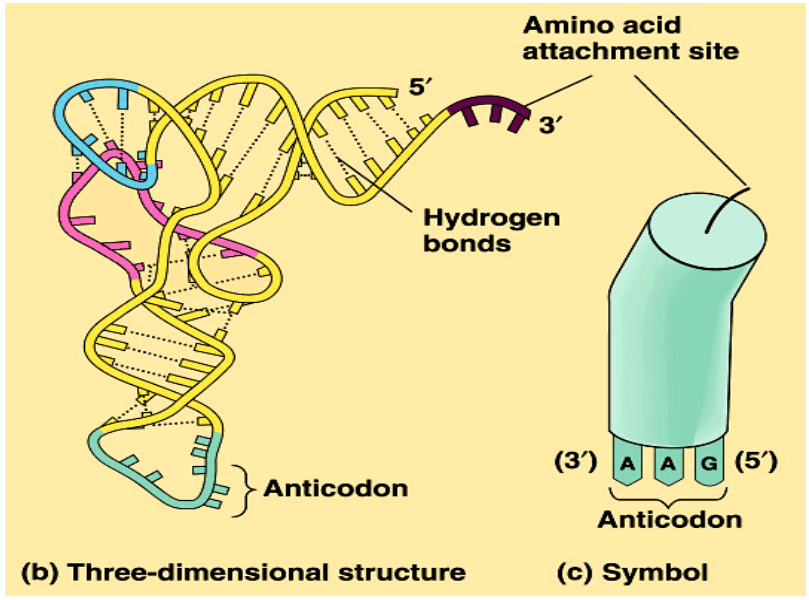
- 1) made up of 2 subunits (large and small) that unite only when mRNA are present.
- 2) subunits are made up of rRNA, structural proteins and enzymes
  - Large = 2 rRNA + 35 proteins
  - Small = 1 rRNA + 20 proteins
- 3) rRNA is made in the nucleoli

Mahlon Hoagland (Harvard) discovered tRNA

- tRNA contains 73-93 nucleotides
- Clover-leaf shape (2D)
- tRNA binds to specific amino acid molecule
- 45 types of tRNA (redundancy)



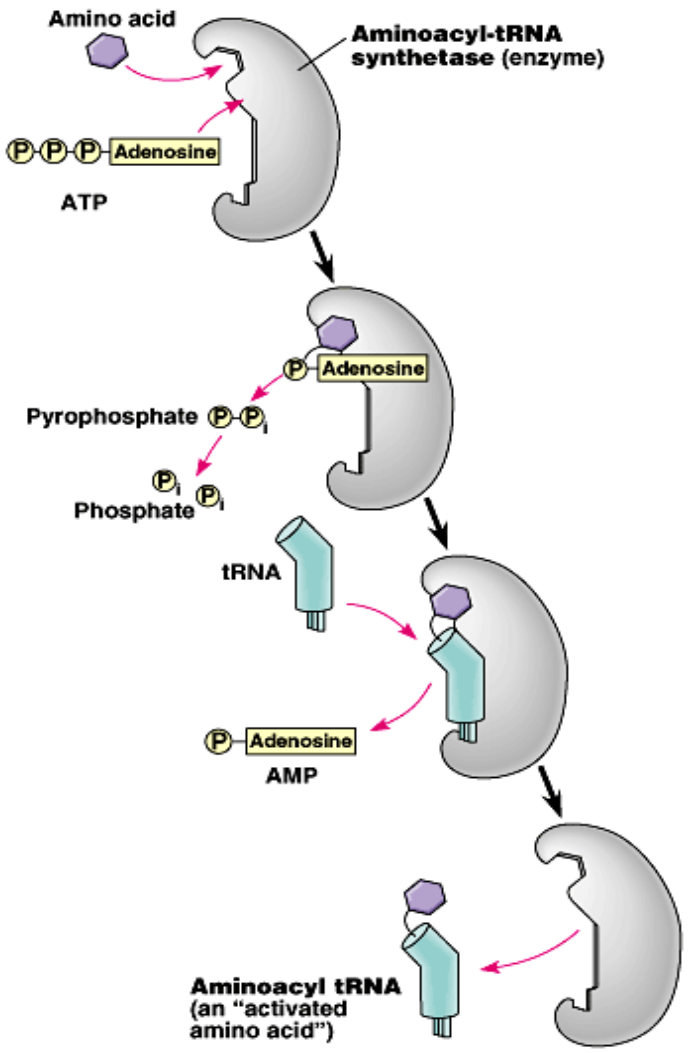
tRNA



- An enzyme, amino-acyl-tRNA-synthetase, catalyzes the union of tRNA-amino acid at the 3’ end.
- The anticodons of some tRNAs recognize more than one codon. (wobble effect)

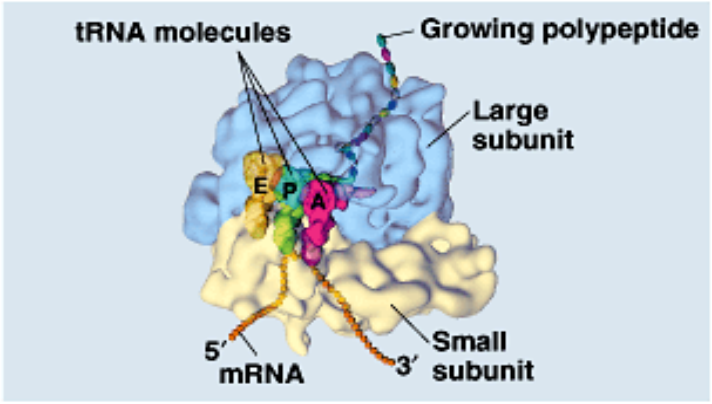
amino-acyl-tRNA-synthetase,

- The 20 different synthetases match the 20 different amino acids.
- Each has active sites for only a specific tRNA and amino acid combination.



DNA Translation

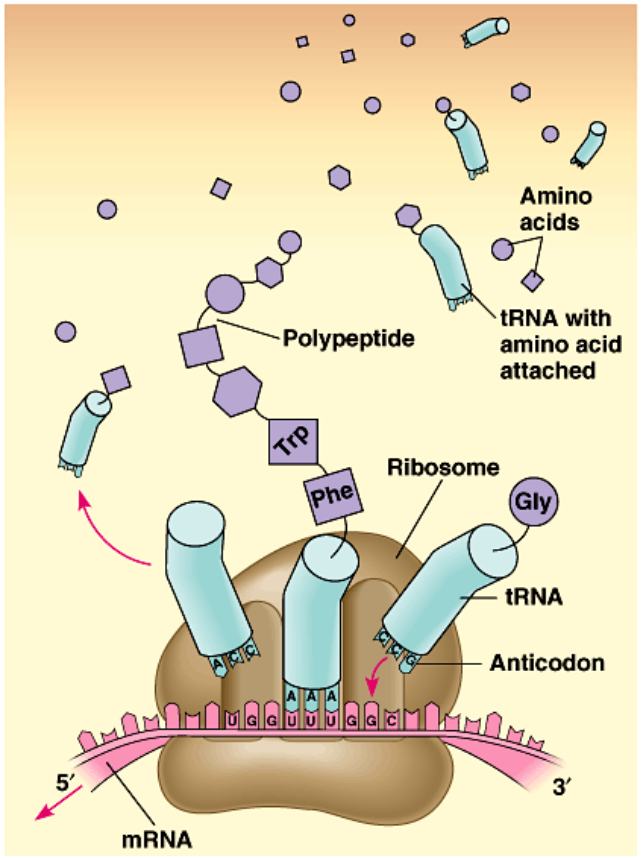
- Polypeptides are made by ribosomes.
- This process involves:
  - Small ribosomal subunit
  - Large ribosomal subunit
  - Ribosomal enzymes
  - mRNA + tRNA
  - ATP & GTP (guanosine triphosphate)
- GTP is another energy molecule like ATP.



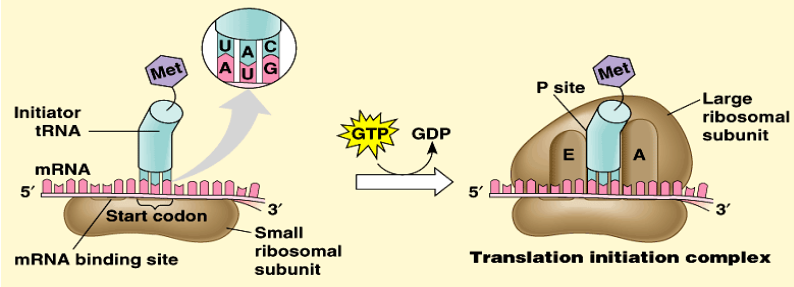
(a) Computer model of functioning ribosome

DNA Translation - Initiation

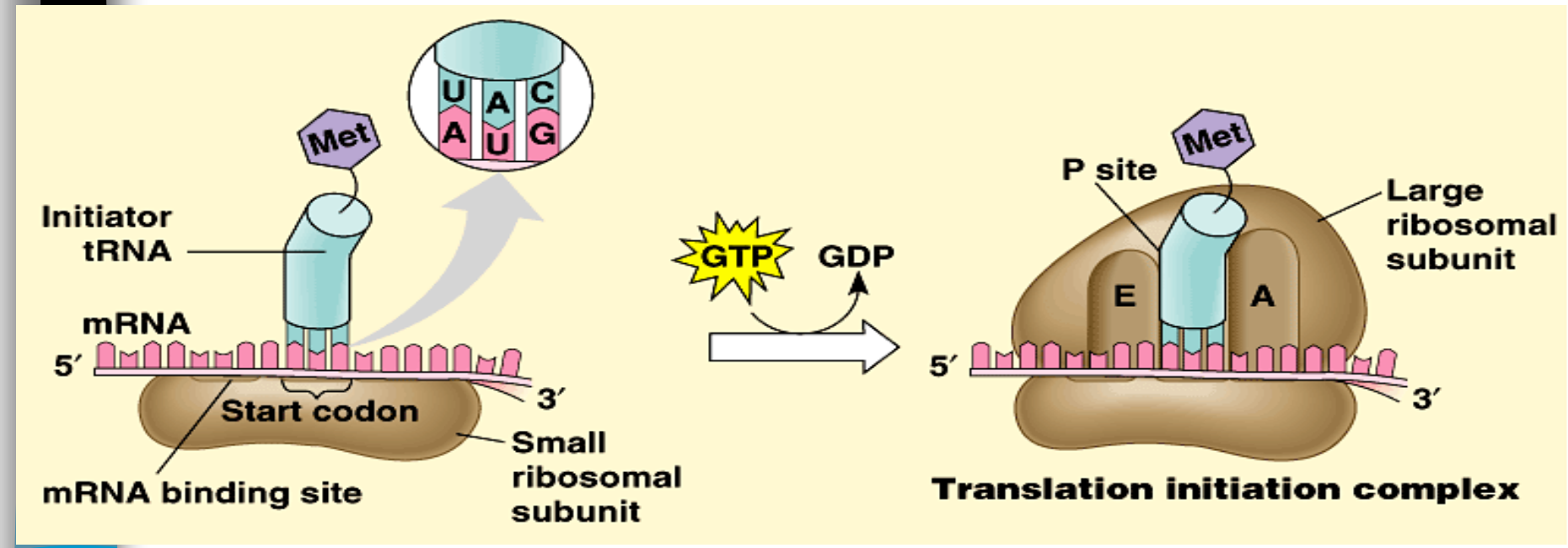
- Translation can be divided into three phases:
  - Initiation
  - Elongation
  - Termination
- Protein factors assist all three phases.



DNA Translation – Initiation (2)

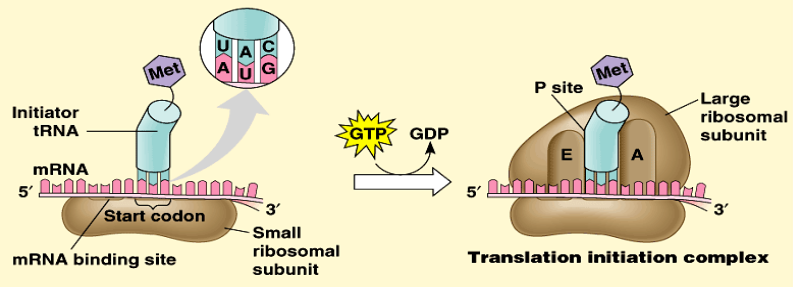


- 1) small ribosomal unit binds to mRNA and a special initiator tRNA.
  - In prokaryotes – at leader of mRNA
  - In eukaryotes – at 5’ cap
- Initiator tRNA attaches to the start codon AUG. It carries methionine amino acid.

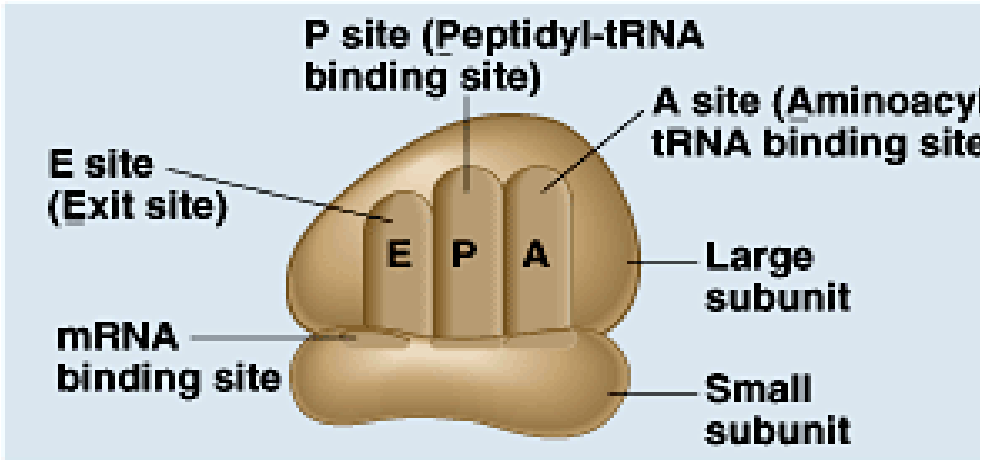

U A C
A U G
Met
Initiator tRNA
mRNA
5′
3′
Start codon
mRNA binding site
Small ribosomal subunit
GTP
GDP
Met
P site
Large ribosomal subunit
E
A
5′
3′
Translation initiation complex

DNA Translation – Initiation (3)

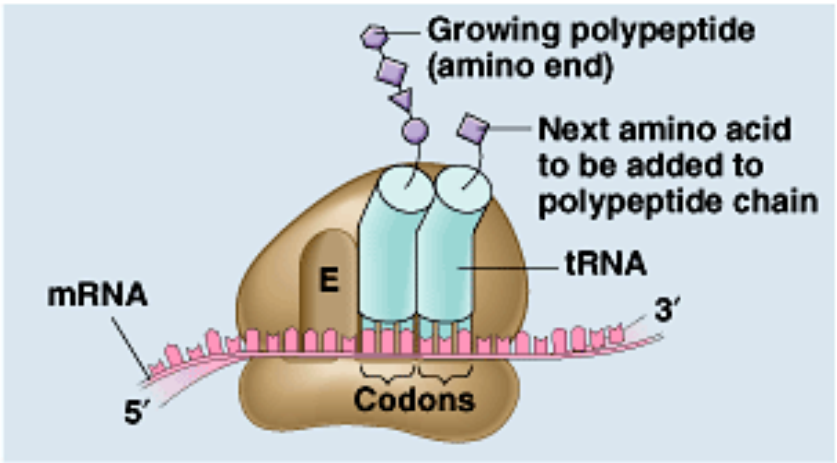
- 2) large ribosomal unit binds to small ribosomal unit creating a translation initiation complex (TIC)
- Proteins called initiation factors bring the components of TIC together. Energy for the process comes from GTP



- Each ribosome has a binding site for mRNA and three binding sites for tRNA molecules.
  - The **P site** holds the tRNA carrying the growing polypeptide chain.
  - The **A site** carries the tRNA with the next amino acid.
  - Discharged tRNAs leave the ribosome at the **E site**.

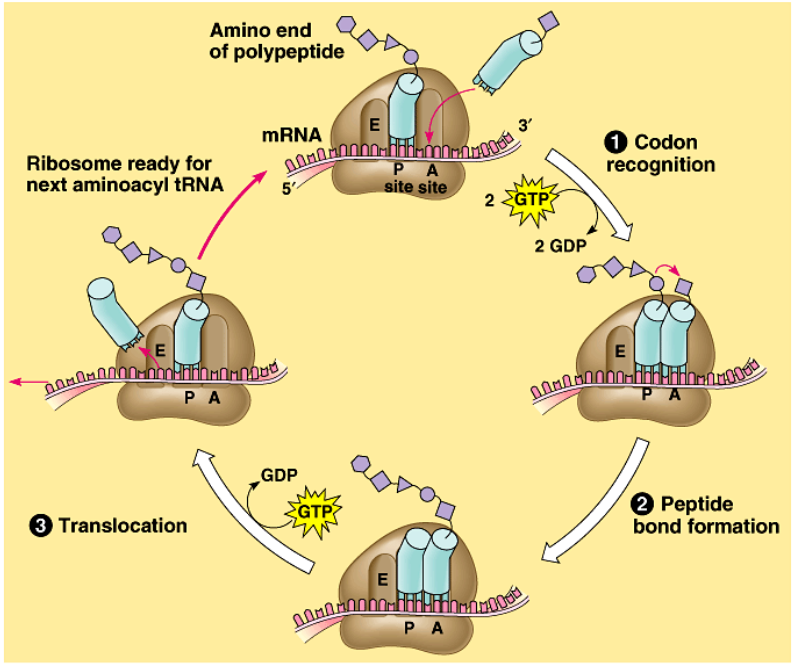


(b) Schematic model showing binding sites



(c) Schematic model with mRNA and tRNA

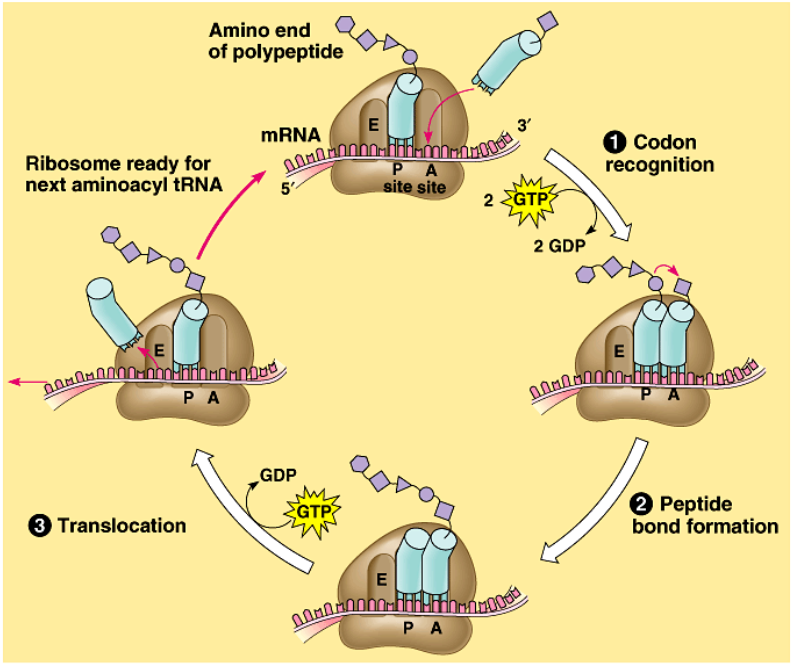
DNA Translation – Elongation (1)



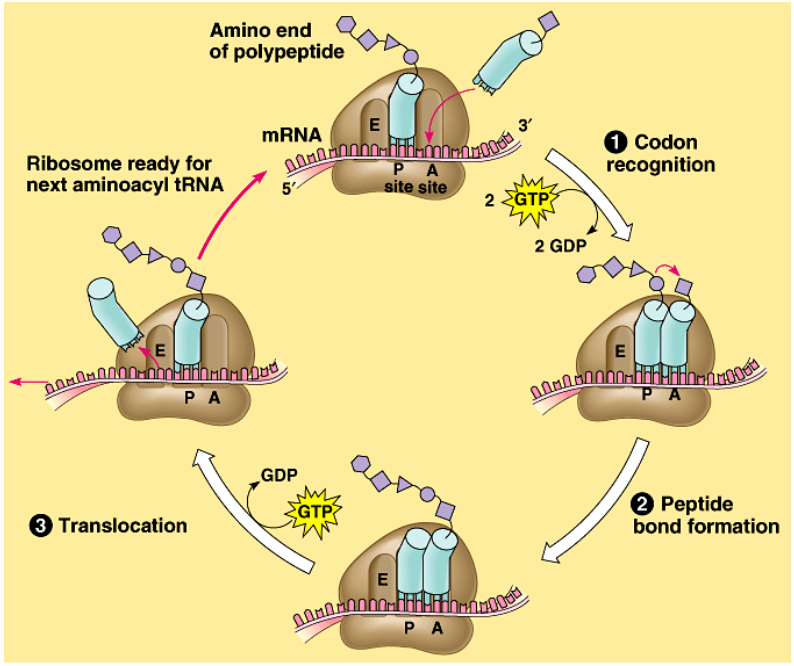
- 1) Codon recognition – at A site, the anitcodon of a tRNA binds to the mRNA codon by hydrogen bonds.
- Two molecules of GTP are needed.
- Elongation factors assist.

DNA Translation – Elongation (2)

- 2) Peptide bond formation:
- Large ribosomal unit acts as a ribozyme and catalyzes a peptide bond formation between the 2 amino acids.
- Bond holding the amino acid on the P site tRNA breaks.



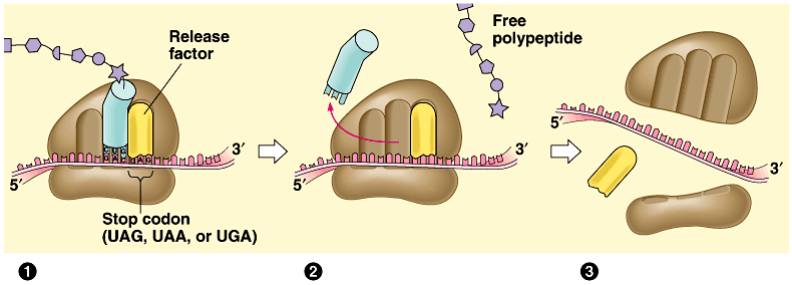
DNA Translation – Elongation (3)

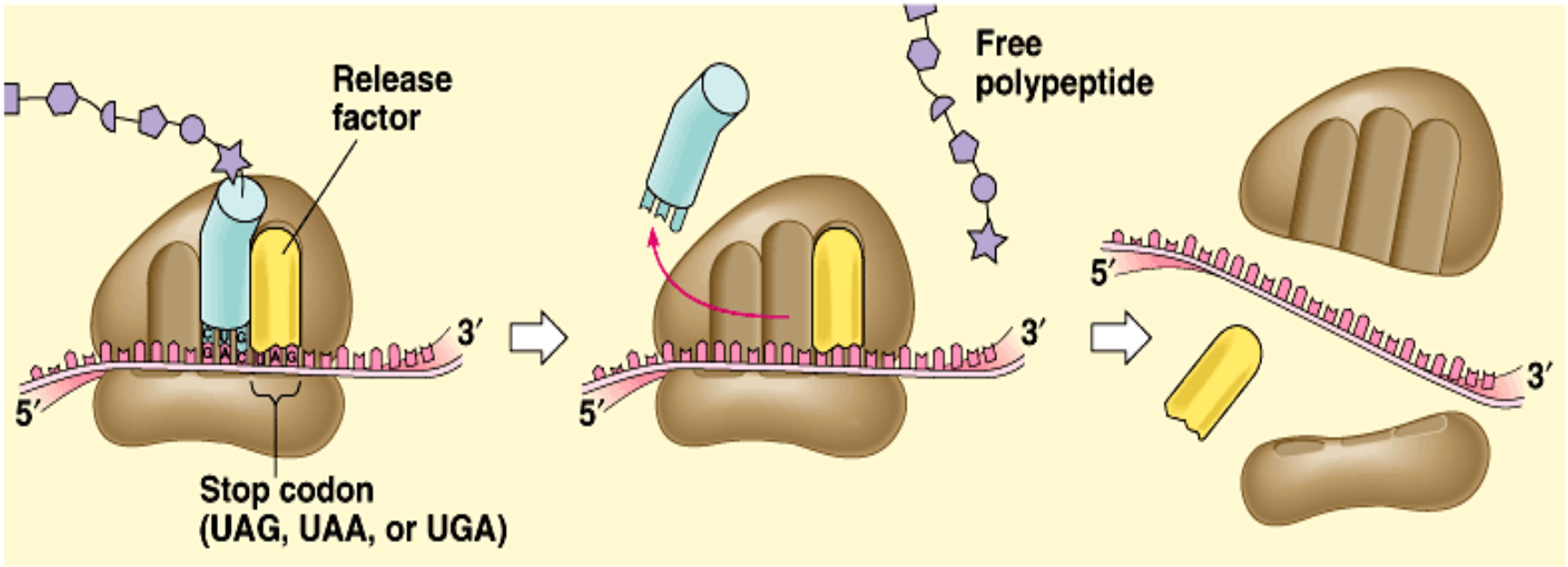


- 3) Translocation:
- Ribosome moves tRNA in A site to P site.
- tRNA from P site moves to E site and then out.
- A GTP molecule provides energy for translocation
- Remember – ribosome moves towards the 3’ end. (5’ to 3’)

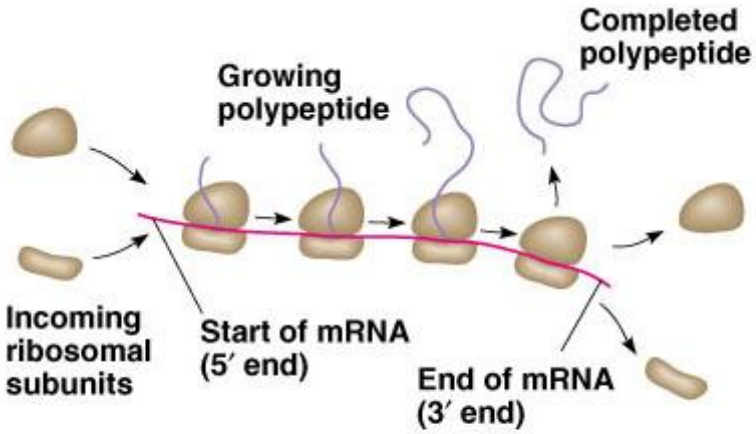
DNA Translation - Termination

- Termination occurs when the ribosome moves the termination codon (stop codon) into the A site.
- A protein called the *release factor* cause water and not tRNA to bind.
- Hydrolysis occurs and everything disconnects.

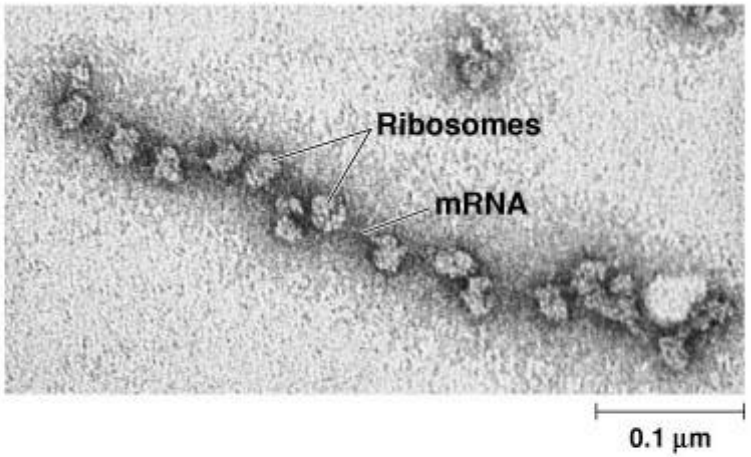



Release factor
3′
5′
Stop codon (UAG, UAA, or UGA)
Free polypeptide
3′
5′
5′
3′
1
2
3

- Typically a single mRNA is used to make many copies of a polypeptide simultaneously.
- Multiple ribosomes, **polyribosomes**, may trail along the same mRNA.
- A ribosome requires less than a minute to translate an average-sized mRNA into a polypeptide.



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

Post-Translational Modifications

- During and after synthesis, a polypeptide coils and folds to its three-dimensional shape spontaneously.
- In addition, proteins may require *post-translational modifications* before doing their particular job.
  - This may require additions like sugars, lipids, or phosphate groups to amino acids.
  - Enzymes may remove some amino acids or cleave whole polypeptide chains.
  - Two or more polypeptides may join to form a protein.