Translation

Resources

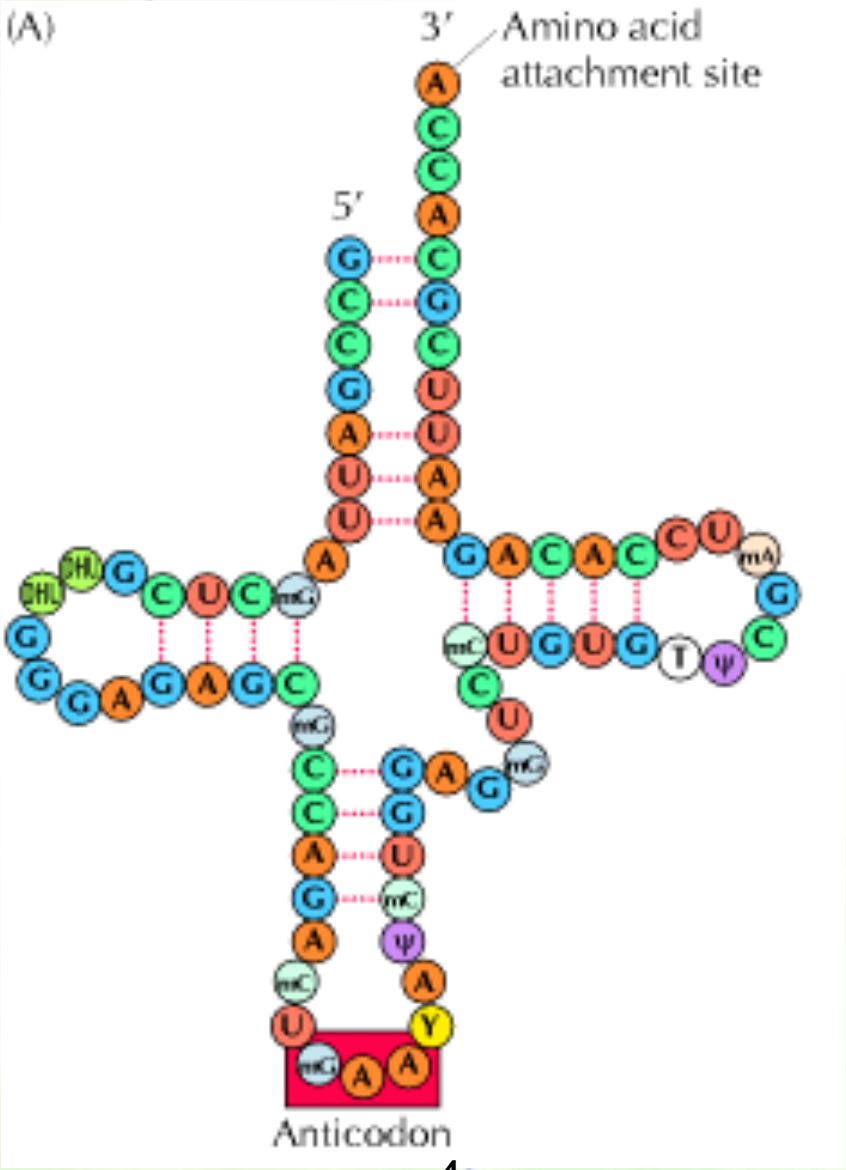
- This lecture
- Cooper, Ch. 8 (297-319)

General information

- Protein synthesis involves interactions between three types of RNA molecules:
  - tRNAs
  - rRNAs, which exist in ribosomes (the factories of protein synthesis)
  - mRNA templates

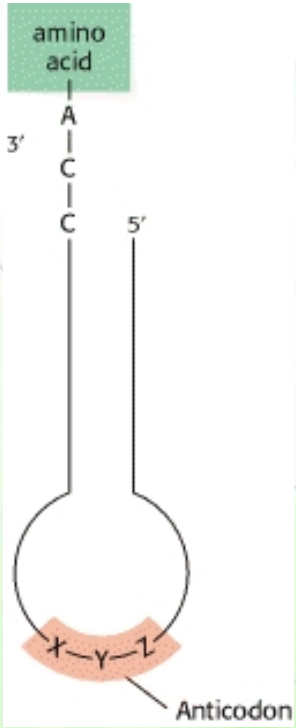
tRNA structure

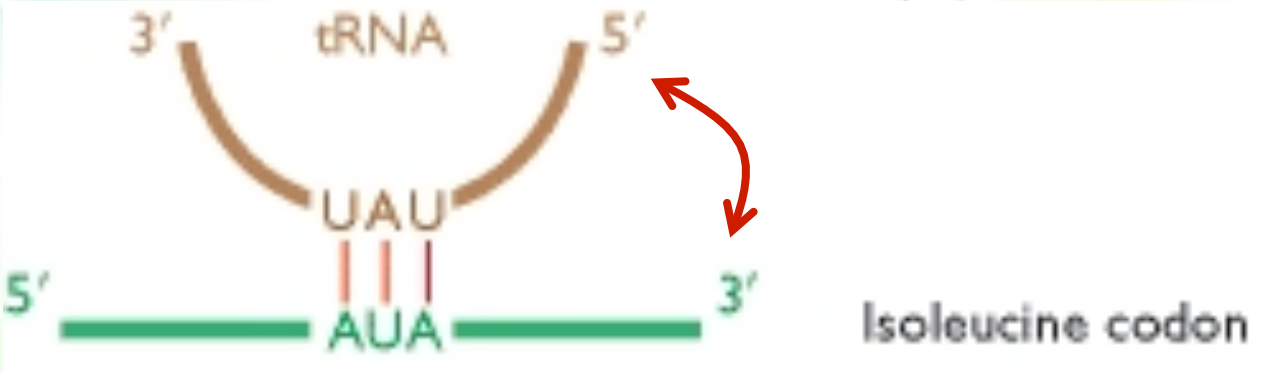
- tRNAs are short RNA molecules (80 bases long).
- "Charged" or "activated" tRNA carries one amino acid.
- Twenty Aminoacyl-tRNA synthetases exist for each amino acid.
- An amino acid is covalently attached to the ribose of the terminal adenosine at CCA.
  - The amino acid attached to tRNA is specified not only by the anticodon, but also identifier sequences.



Codon vs. anticodon

- tRNAs contain a three-nucleotide sequence known as "anticodon" that pairs with the "codon' or "triplet" mRNA molecules (note the anti-parallel alignment of mRNA-tRNA complex)



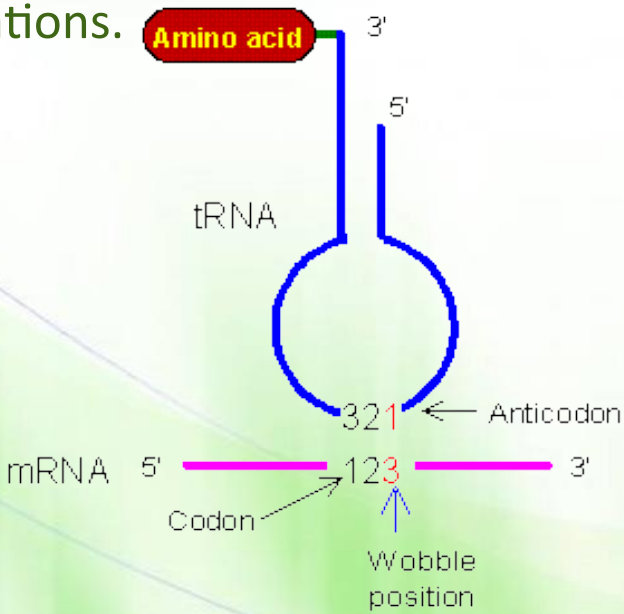


Second letter

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

Features of the genetic

- Not universal
  - Example: AUA in mitochondria (methionine) in cytosol (isoleucine)
- Wobble base pairing (degenerate codon)
  - The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base, which is called the "wobble" base.
  - The degeneracy of the code acts as a buffer against deleterious mutations.



Wobble bases

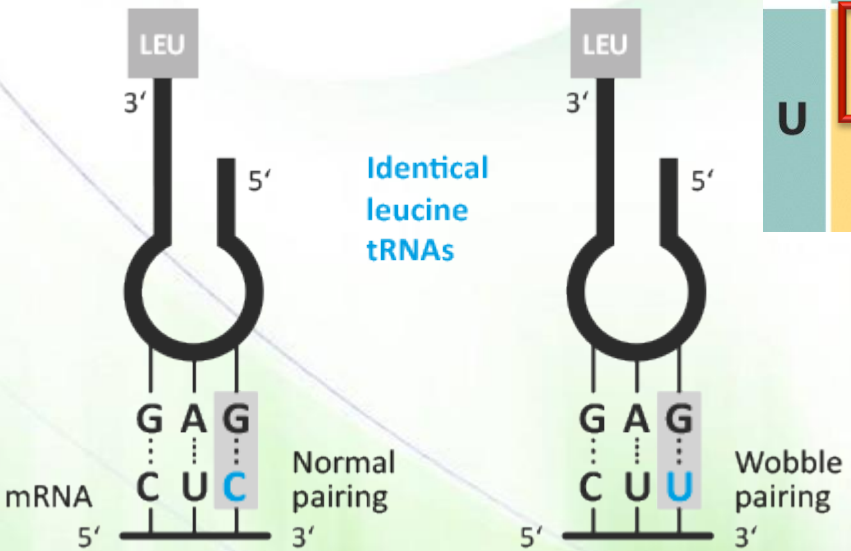
tRNA	C	A	G	U	I
mRNA	G	U	C U	A G	C A U

Wobble bases

mRNA	C	A	G	U
tRNA	G I	U I	C U	A G I

Examples of wobble base pairing

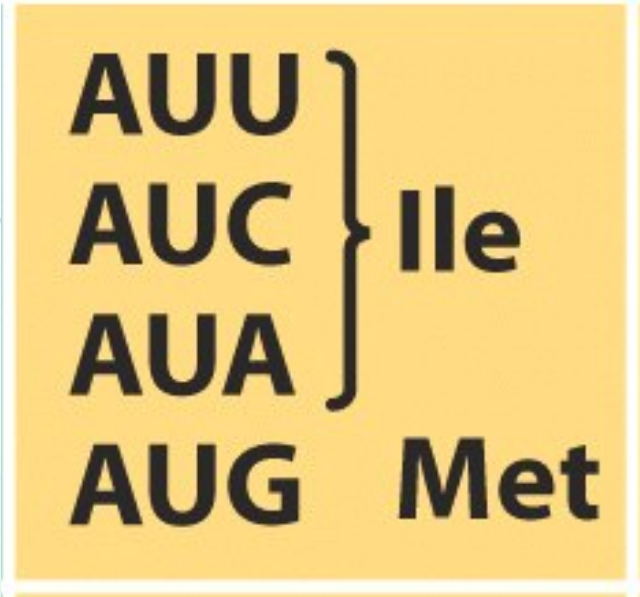
- Relaxed base pairing at this position results from the formation of G-U base pairs.

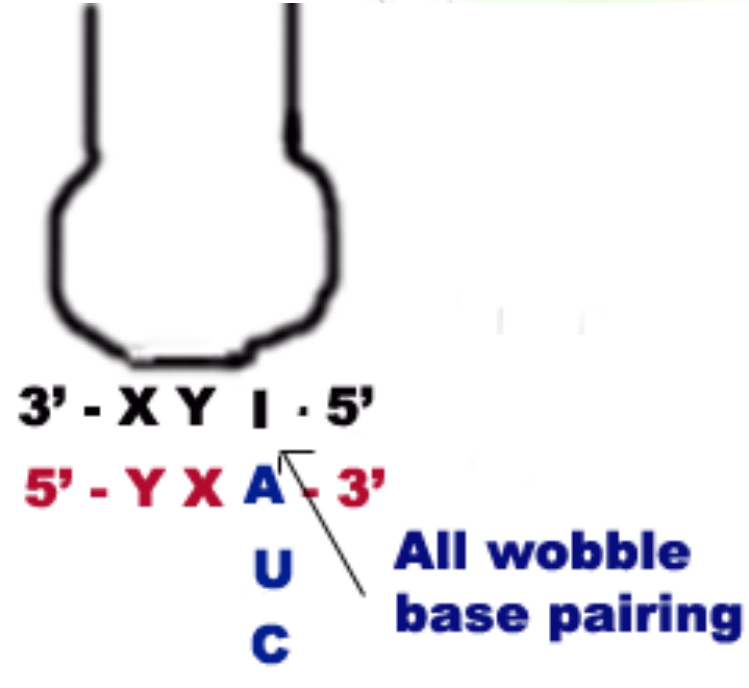


	U	C	A	G	
U	UUU, UUC } Phe UUA, UUG } Leu	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr UAA Stop UAG Stop	UGU, UGC } Cys UGA Stop UGG Trp	U C A G

Inosine

- Also, guanosine is modified in several tRNAs to inosine, which can exist in the anticodons and base-pair with either C, U, or A in the third position.

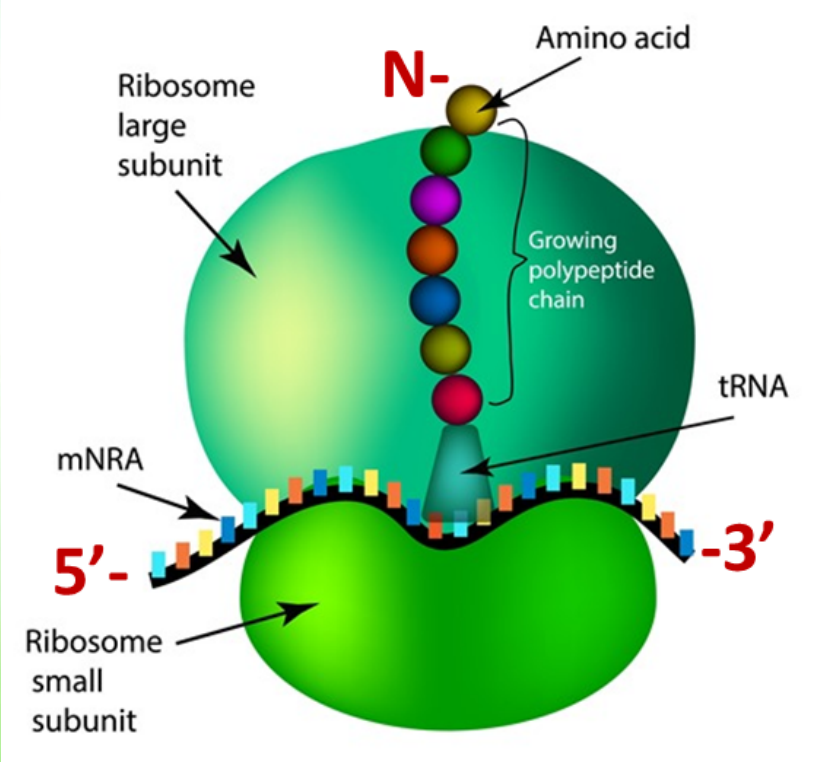




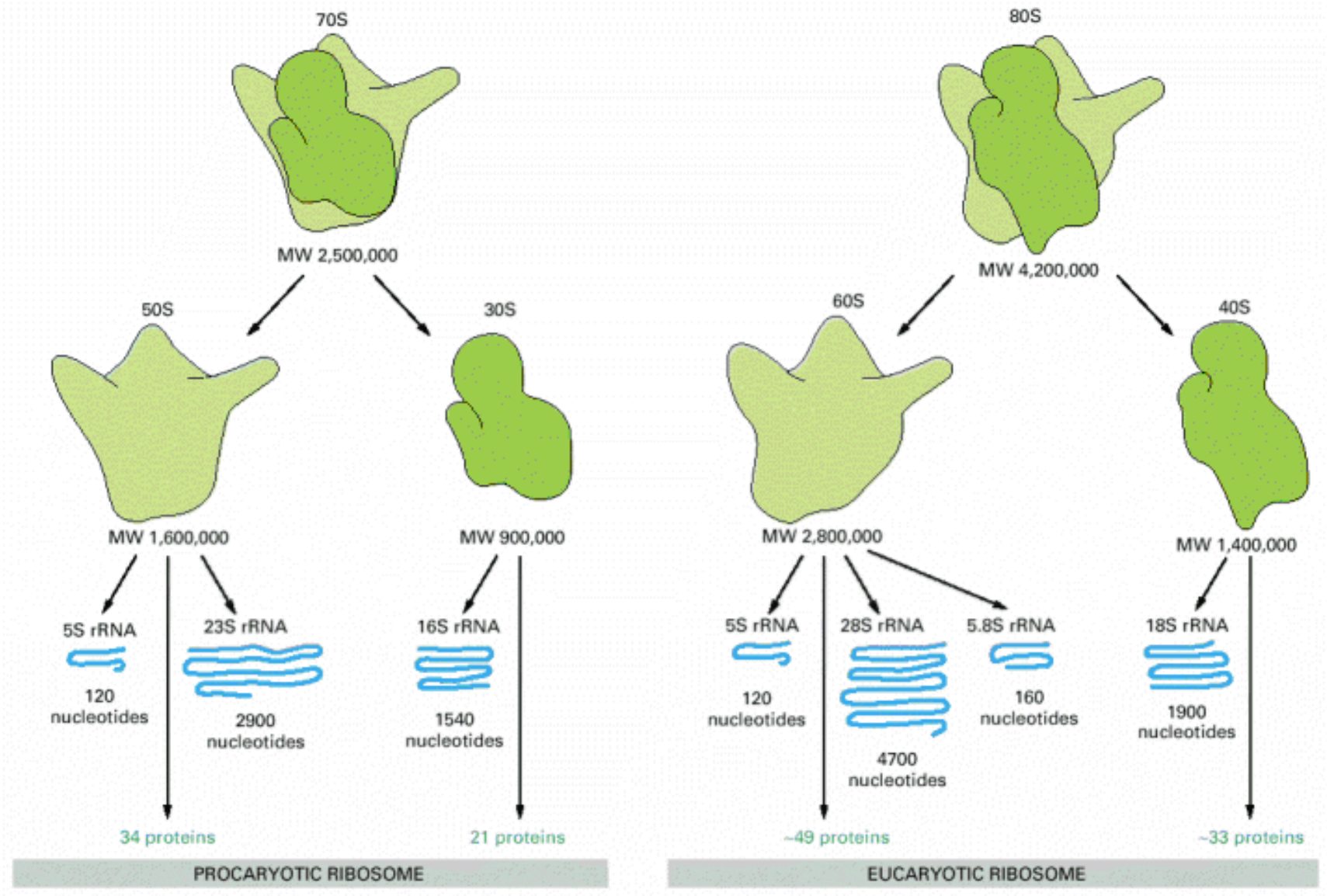
Ribosomes

- Ribosomes are the sites of protein synthesis in both prokaryotic and eukaryotic cells.
- *E. coli* contain about 20,000 ribosomes, which account for approximately 25% of the dry weight of the cell, and rapidly growing mammalian cells contain about 10 million ribosomes.

The peptidyl transferase reaction of a peptide bond is catalyzed by the rRNA of the large ribosomal subunit.

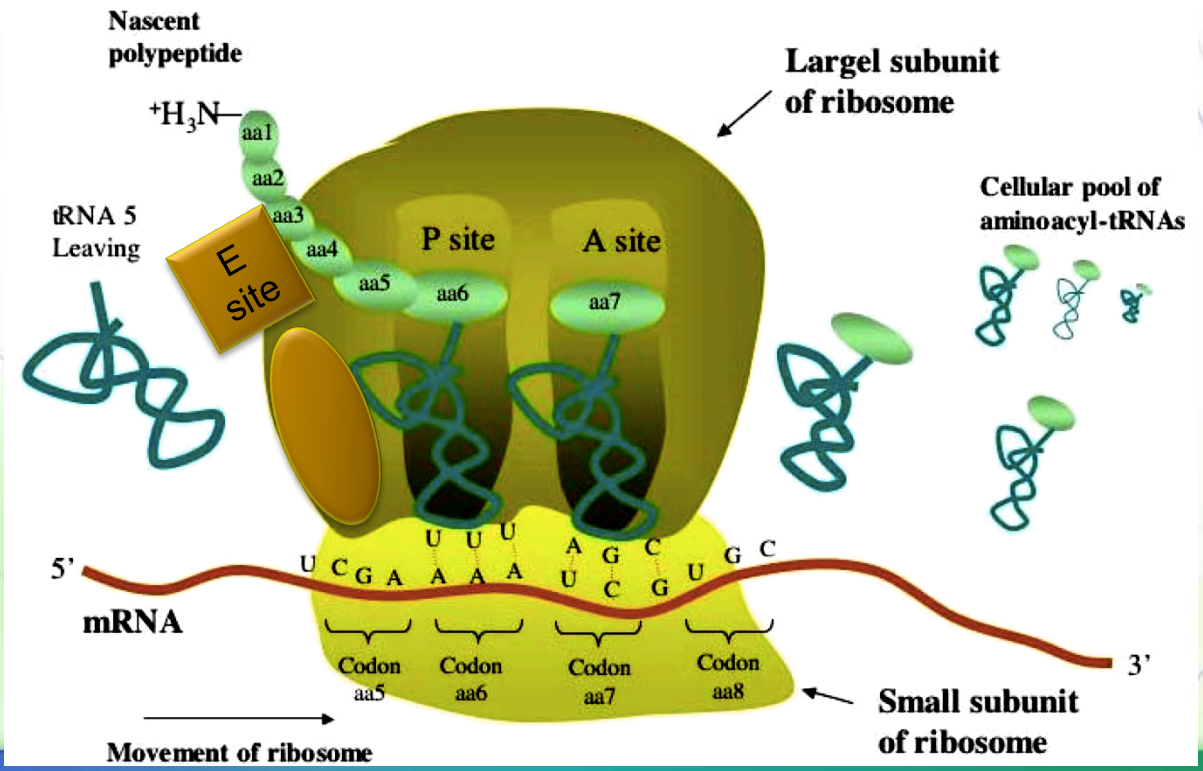


Ribosome structure



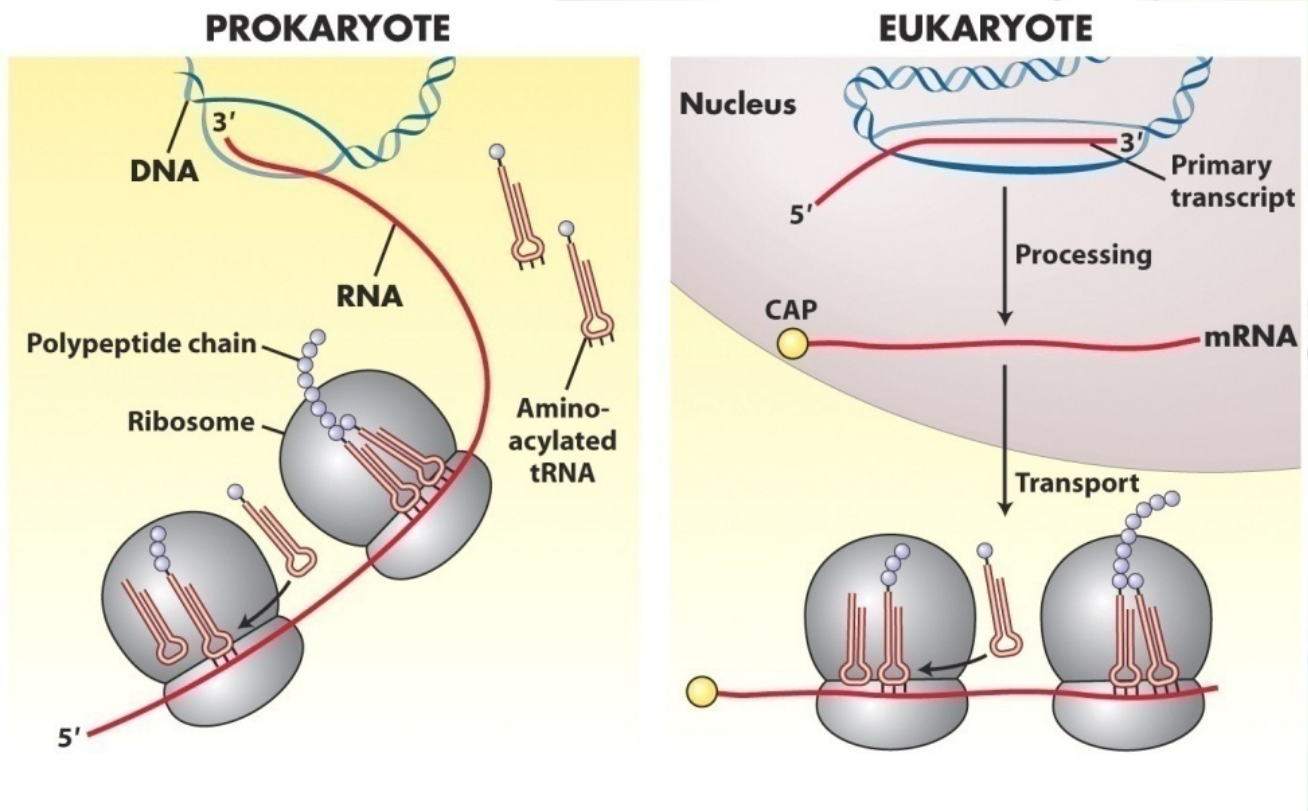
The general mechanism of translation

- Three stages: initiation, elongation, and termination.
- The direction is 5’ → 3’.
- Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus.



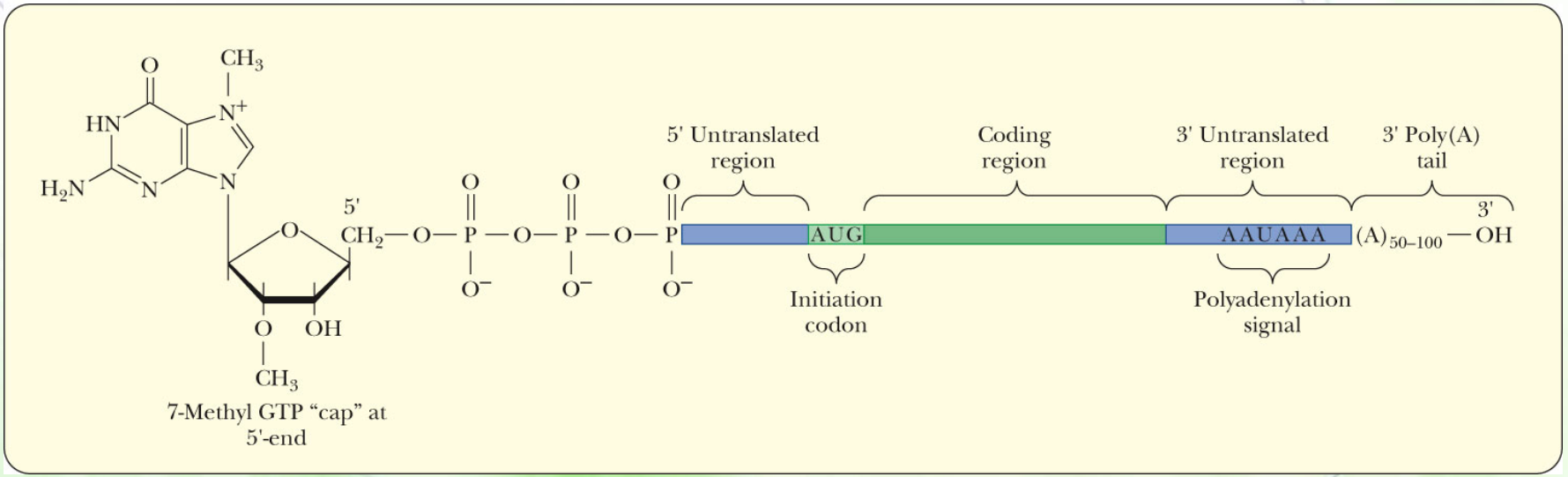
Transcription/translation Coupling

- Translation and transcription are coupled in space and time in prokaryotes.



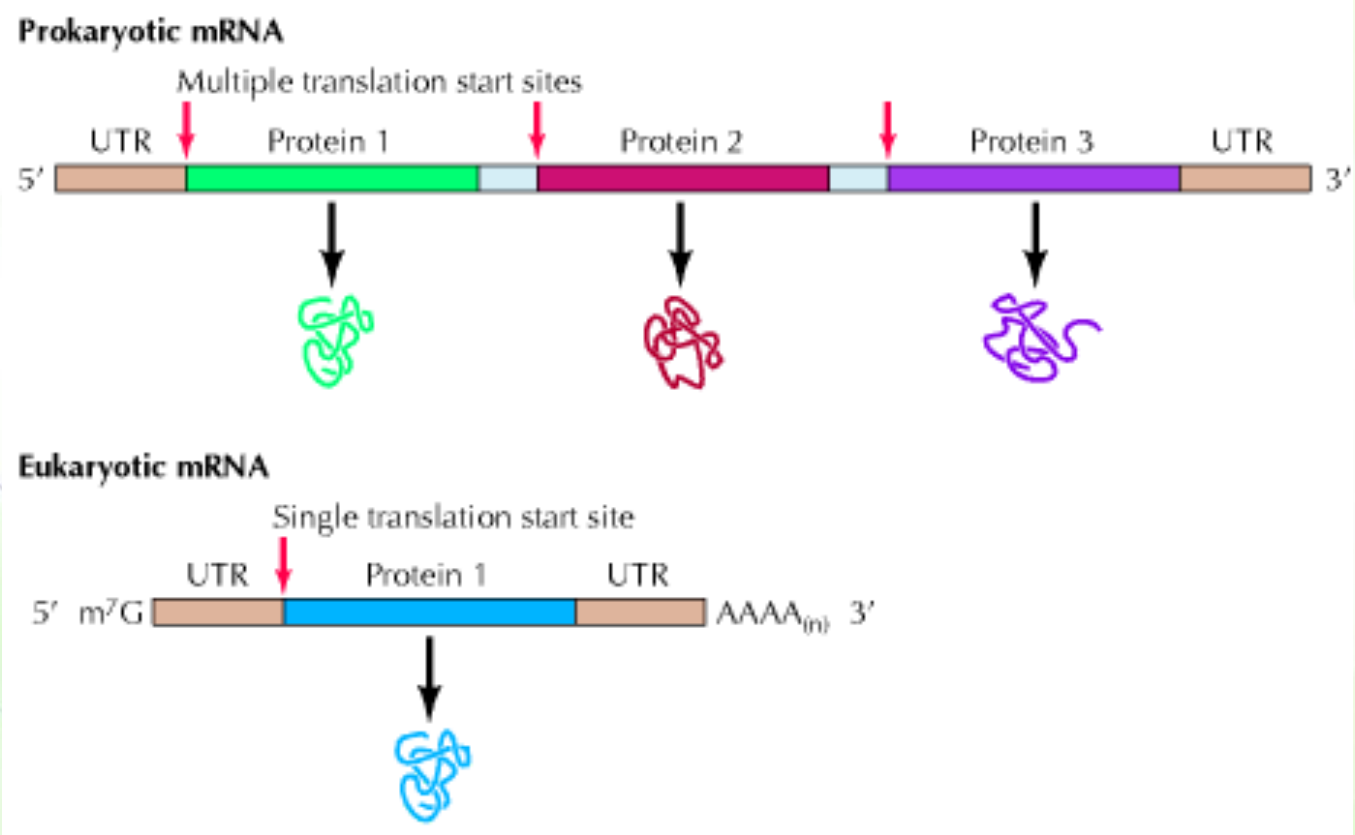
Start of translation

- In both prokaryotes and eukaryotes, translation starts at specific initiation sites, and not from the first codon of the mRNA.
- The 5´ terminal portions upstream of the initiation sites of both prokaryotic and eukaryotic mRNAs contain noncoding sequences, referred to as 5´ untranslated regions (UTRs).
- There is also a 3'-untranslated region.

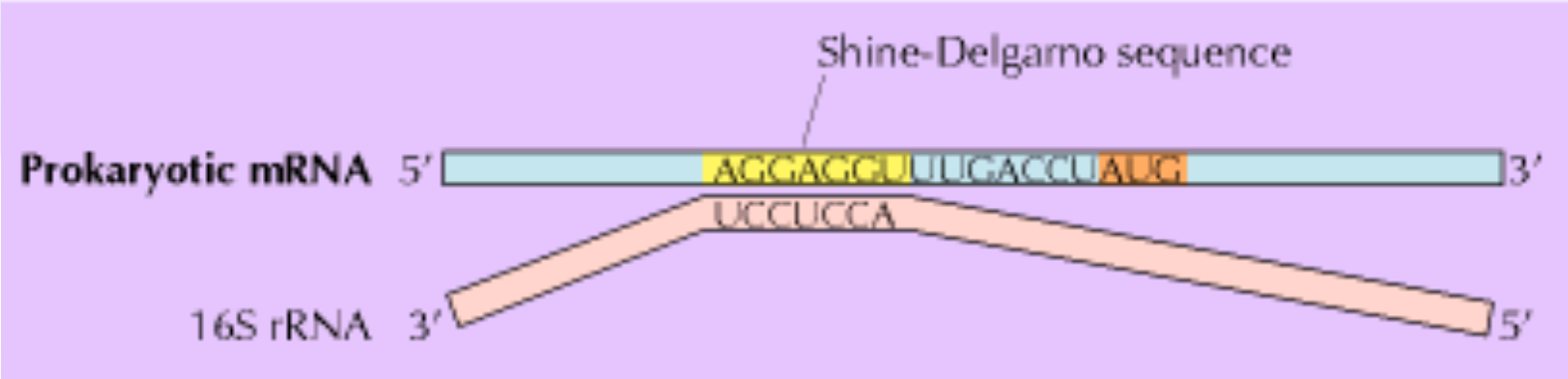


Remember…

- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic

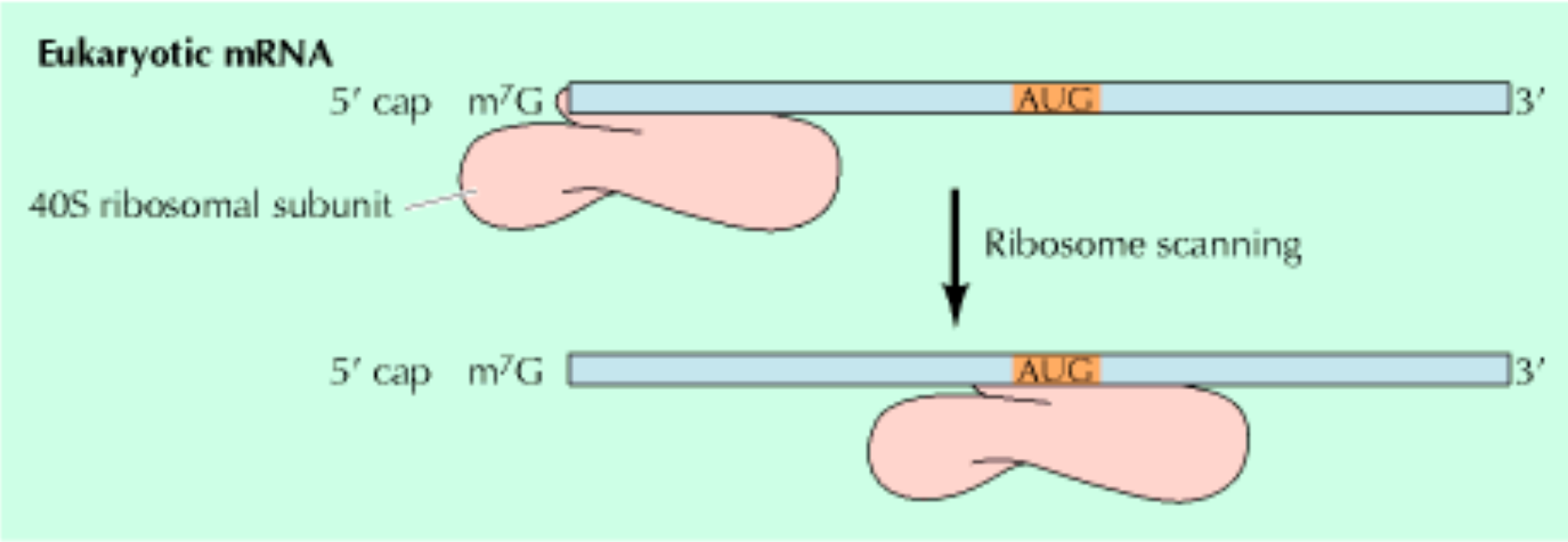


Shine-Dalgarno sequence



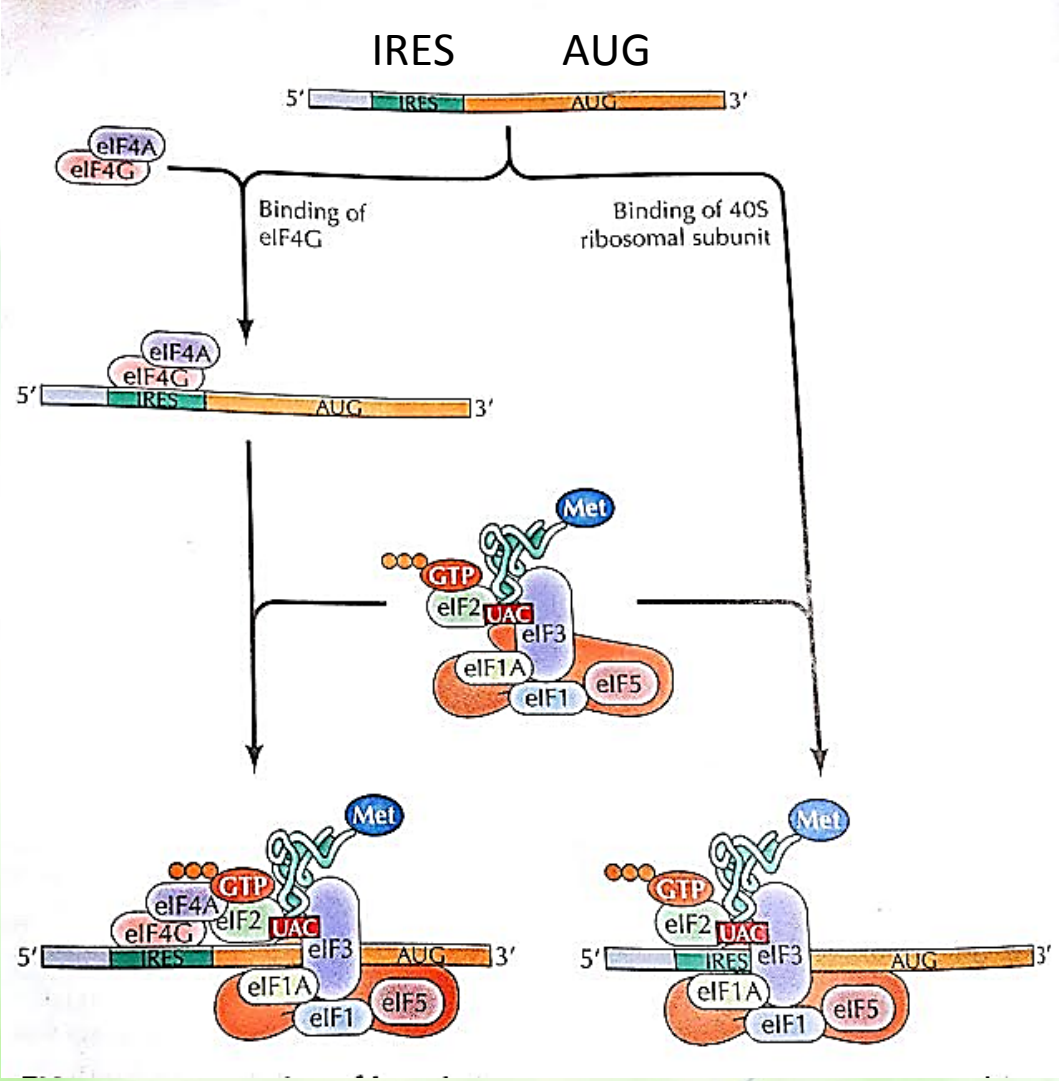
But in eukaryotes…

- Eukaryotic ribosomes recognize mRNAs by binding to the 7-methylguanosine cap at their 5´ terminus



internal ribosome entry site (IRES)

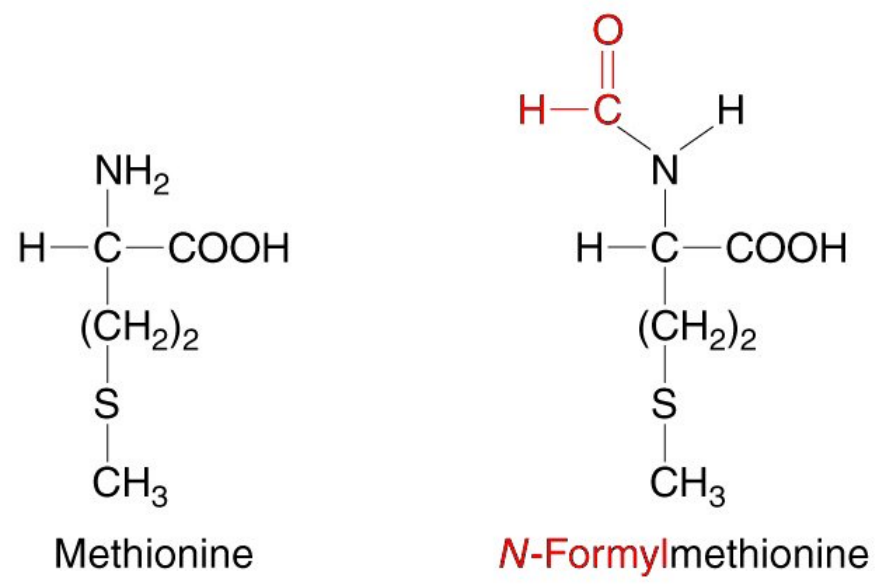
- Alternatively, internal ribosome entry site (IRES) is recognized by the 40S ribosome or eIF4G protein followed by recruitment of the 40S ribosome.



The first amino acid

- Translation always initiates with the amino acid methionine, usually encoded by AUG.

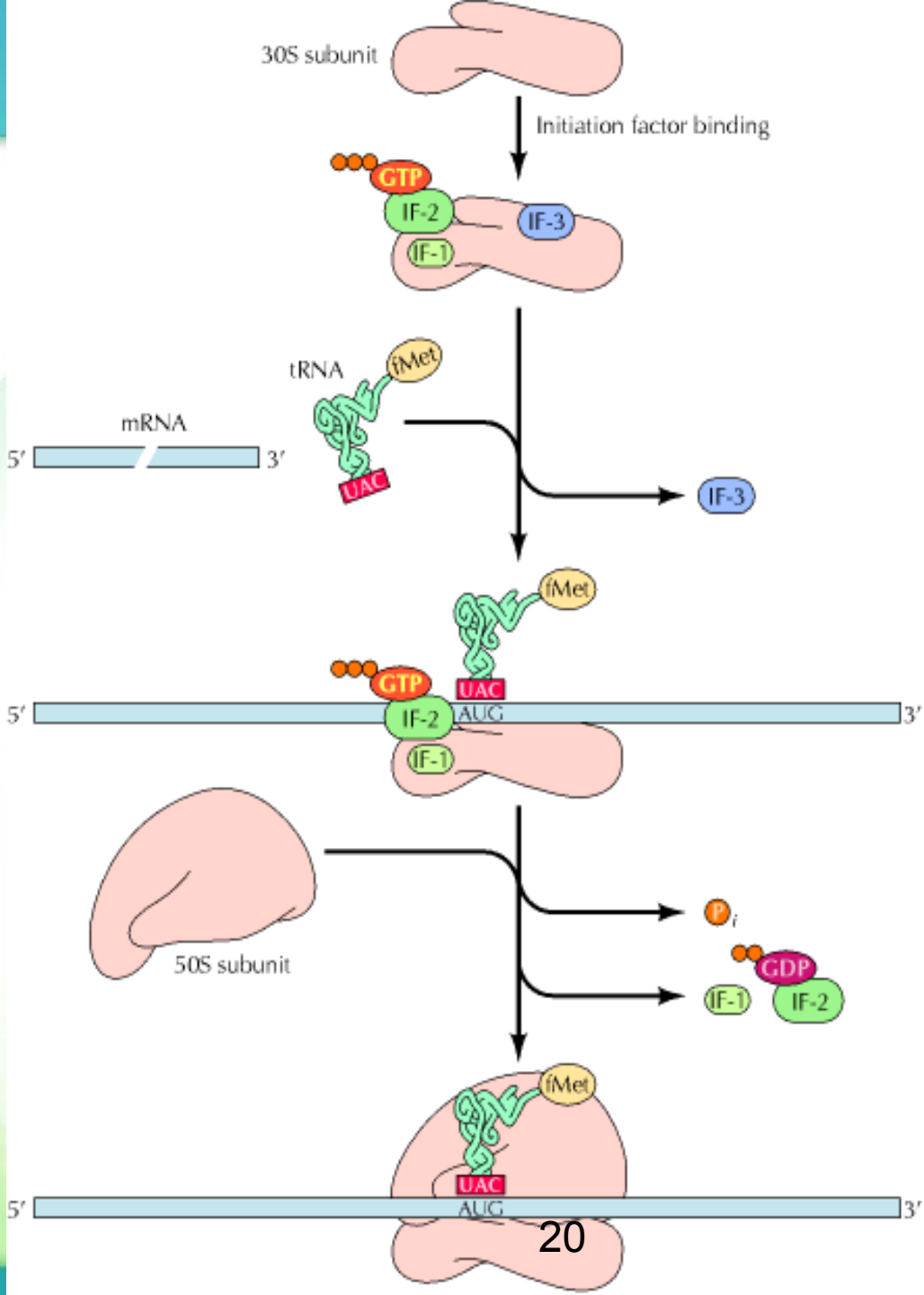
- In most bacteria, it is N-formylmethionine.



Translation initiation

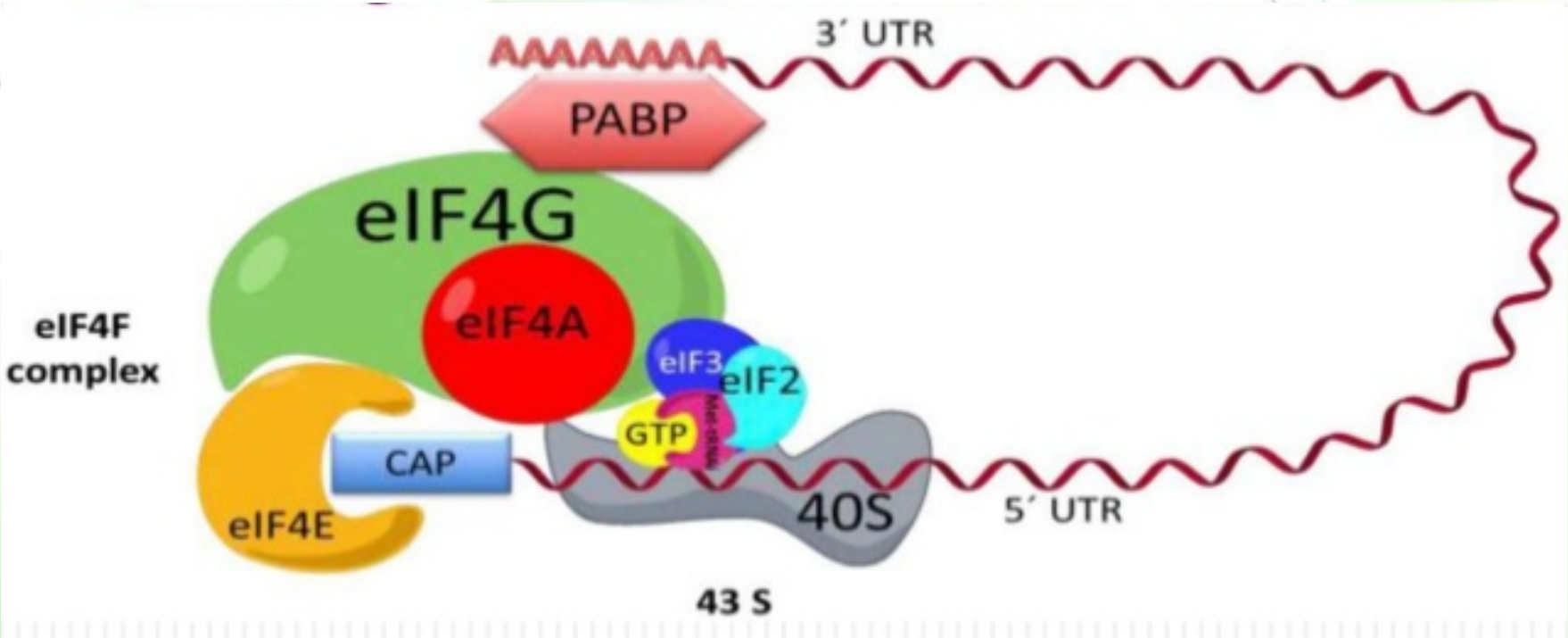
Prokaryotes

- The 30S ribosomal subunit binds to mRNA and fmet-tRNA in the presence of GTP and the three initiation factors, IF-1, IF-2, and IF-3, forming the 30S initiation complex.
- The 50S ribosomal subunit is added, forming the 70S initiation complex.



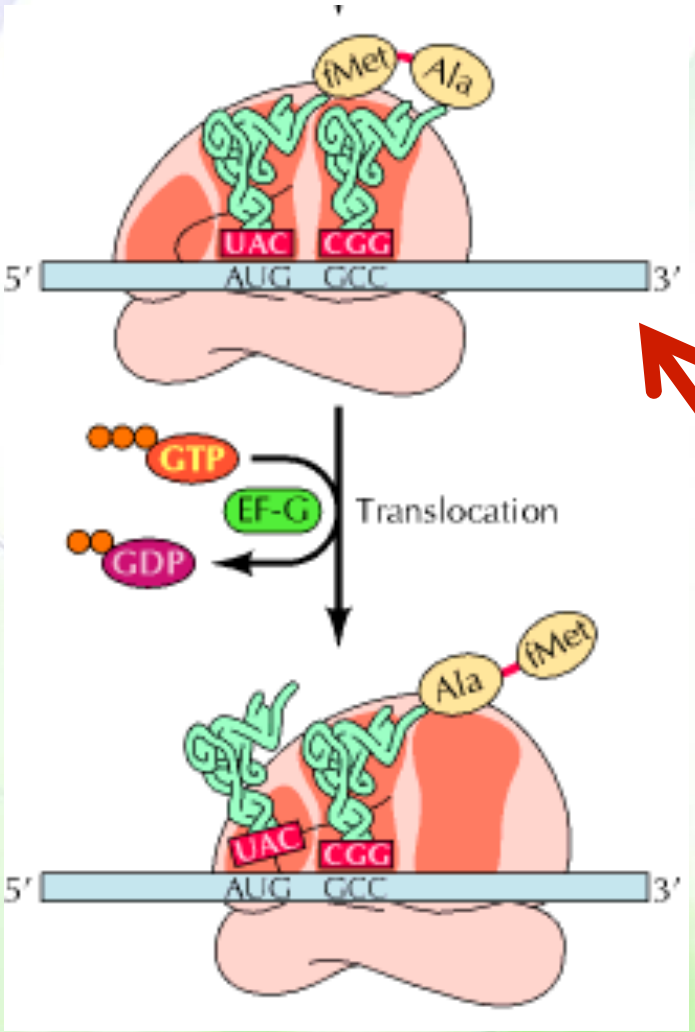
Translation iitiation in eukaryotes

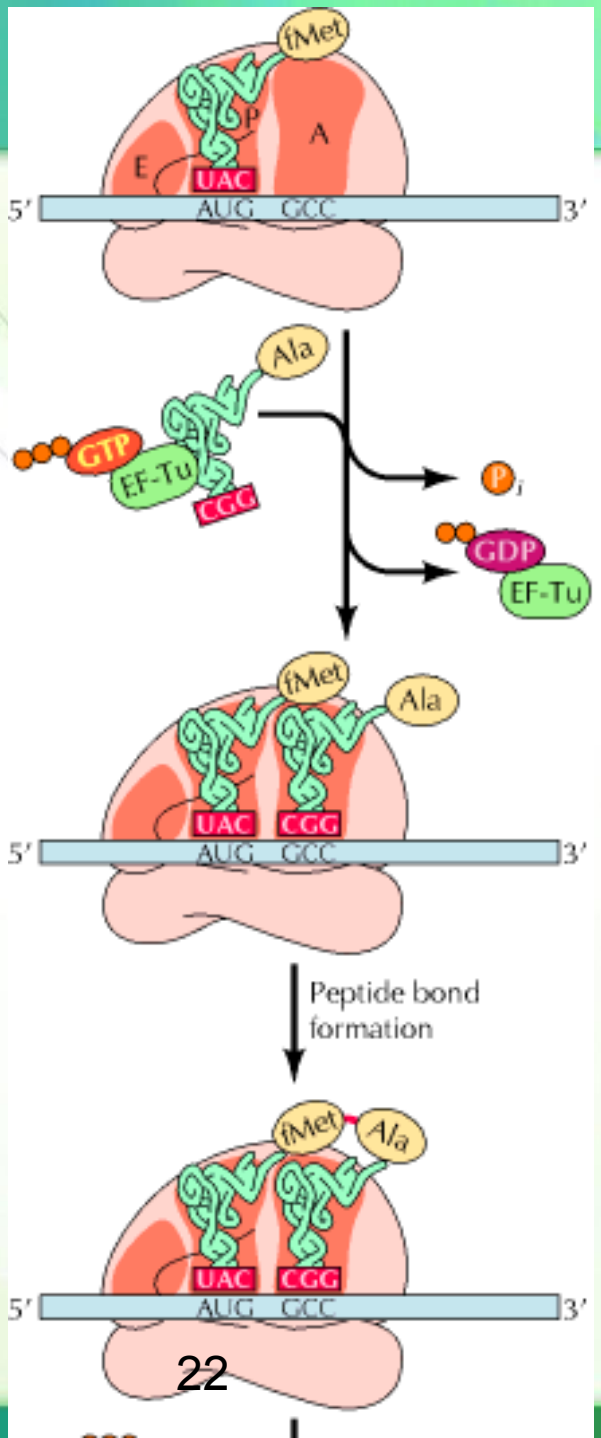
- The initiation factor, eIF4G, is member of a complex that includes poly-A binding protein (PABP), which binds to the poly-A tail via and eIF4E, which binds to the CAP.



Translation elongation I

Three steps: aminoacyl-tRNA binding, peptide bond formation, and translocation.



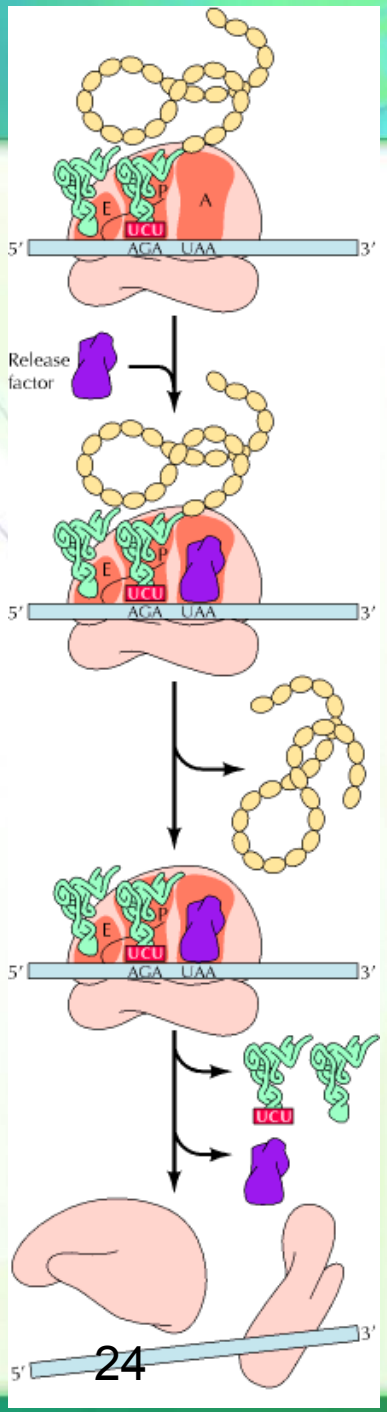


Details of elongation

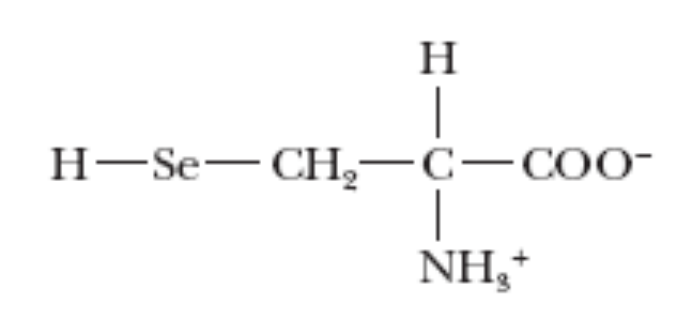
- Step 1: An aminoacyl-tRNA is bound to the A site on the ribosome. Elongation factor EF-Tu (Tu) and GTP are required. The P site on the ribosome is already occupied.
- Step 2: Elongation factor EF-Tu is released from the ribosome and regenerated
- Step 3: The peptide bond is formed, leaving an uncharged tRNA at the P site.
- Step 4: the uncharged tRNA is released. The peptidyl-tRNA is translocated to the P site, leaving an empty A site. The uncharged tRNA is translocated to the E site and subsequently released.

Translation termination

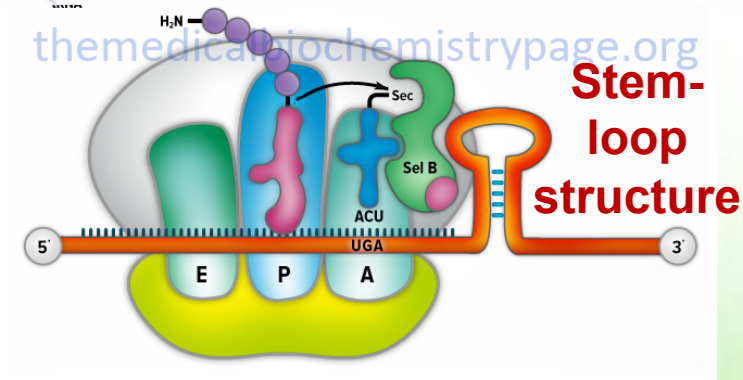
- A stop signal is required for the termination of protein synthesis. The codons UAA, UAG, and UGA are the stop signals. These codons are not recognized by any tRNAs, but they are recognized by proteins called release factors.
- The release factor blocks the binding of a new aminoacyltRNA and facilitates the hydrolysis of the bond between the carboxyl end of the peptide and the tRNA.
- Then, the whole complex dissociates.

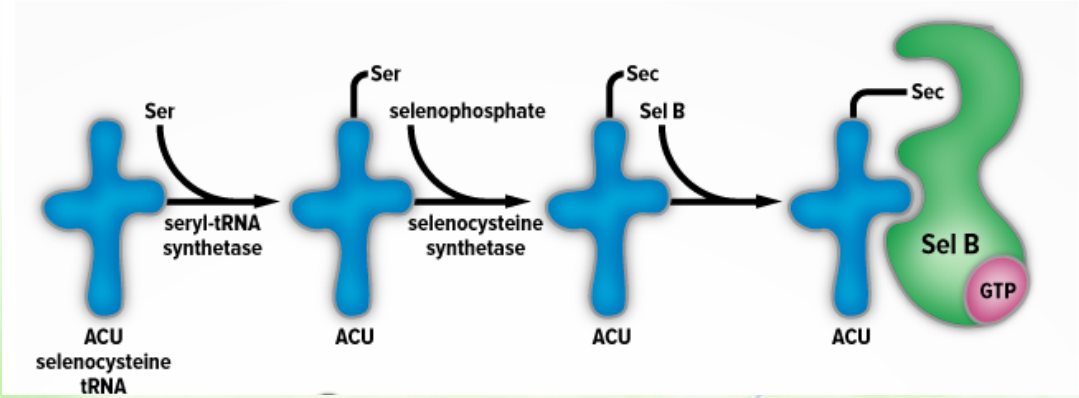


Selenocysteine



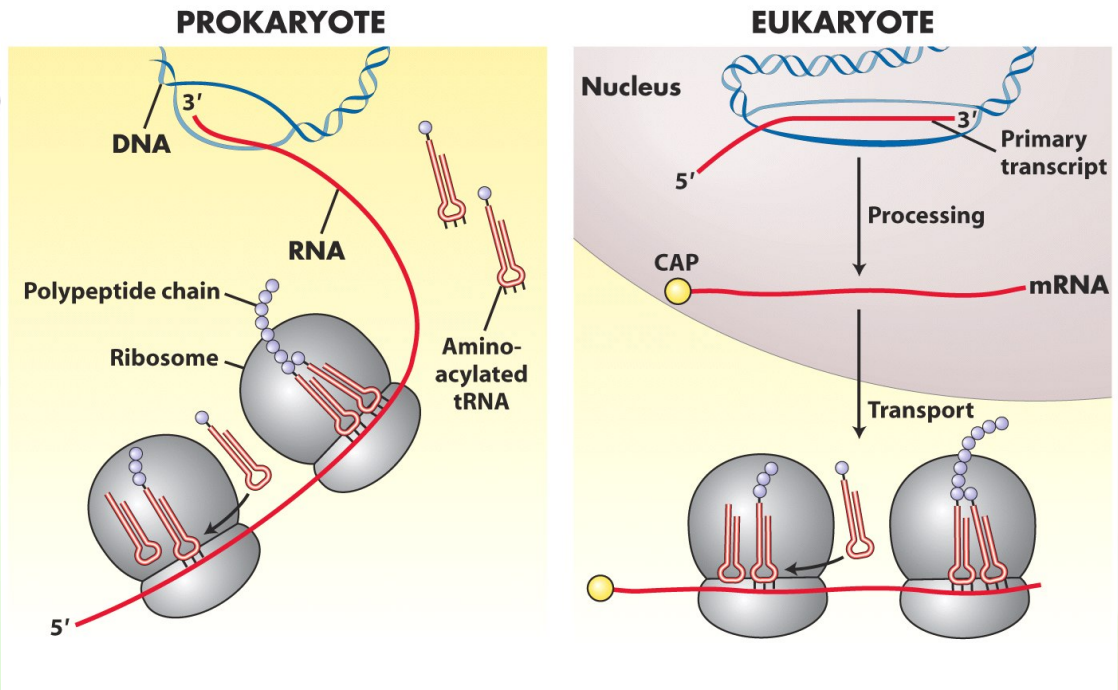
- The oxygen of a serine, which is bound to a special tRNA molecule called $tRNA^{sec}$, is replaced by selenium.
- This tRNA molecule has an anticodon that matches the UGA stop codon. In special cases, the UGA is not read as a stop; rather, the selenocysteinetRNAis loaded into the A site and translation continues.

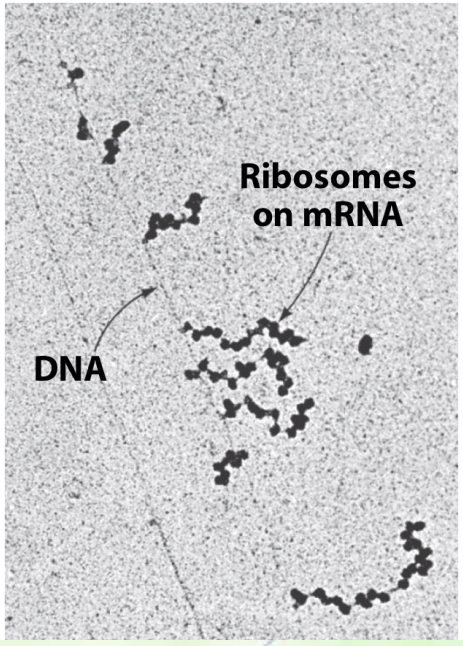




Polyribosomes (polysomes)

- A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.



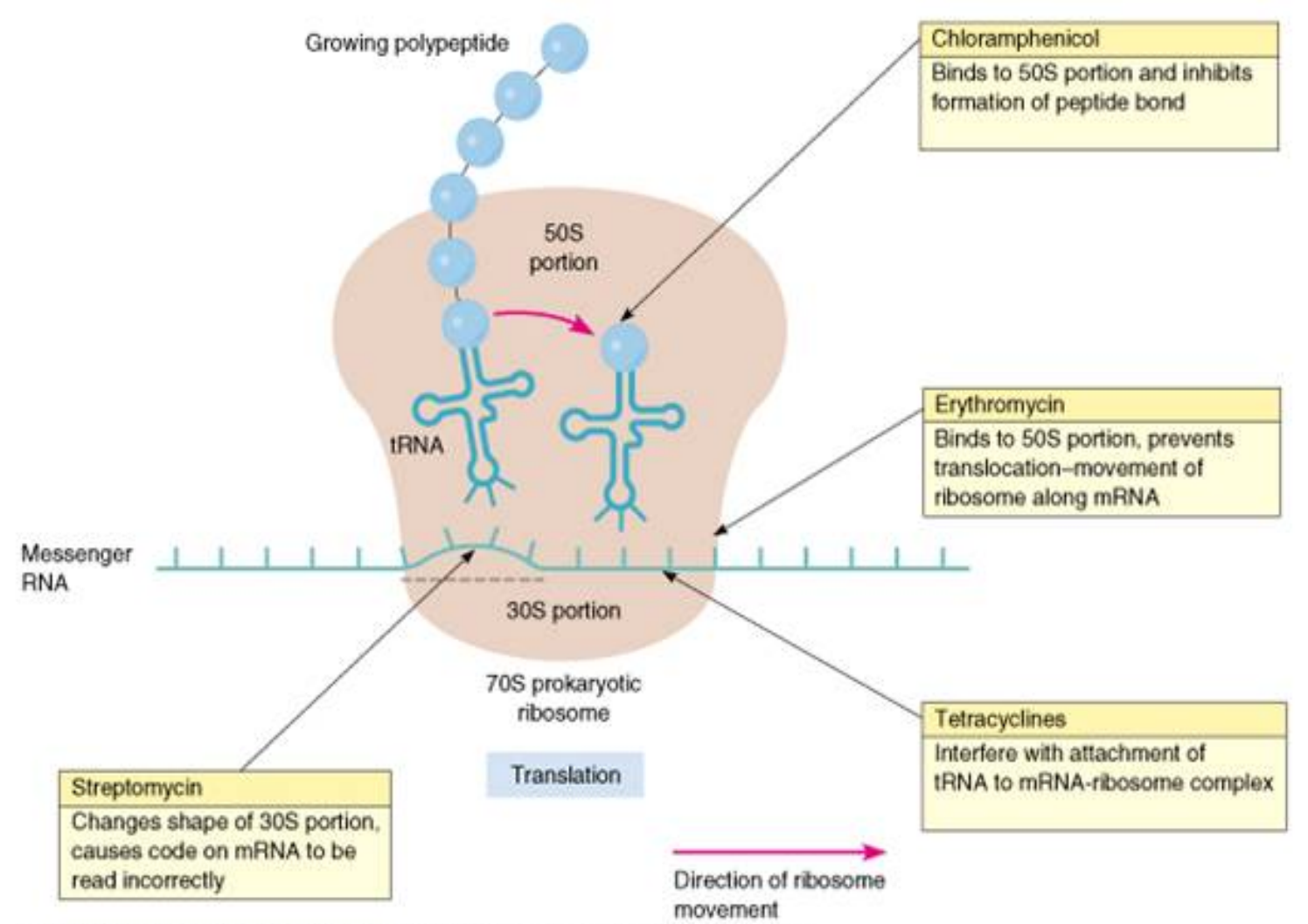


Inhibitors of translation

INHIBITOR	SPECIFIC EFFECT
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	Induces binding of wrong t-RNA-AA complexes resulting in false proteins
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes
Erythromycin	blocks the translocation reaction on ribosomes

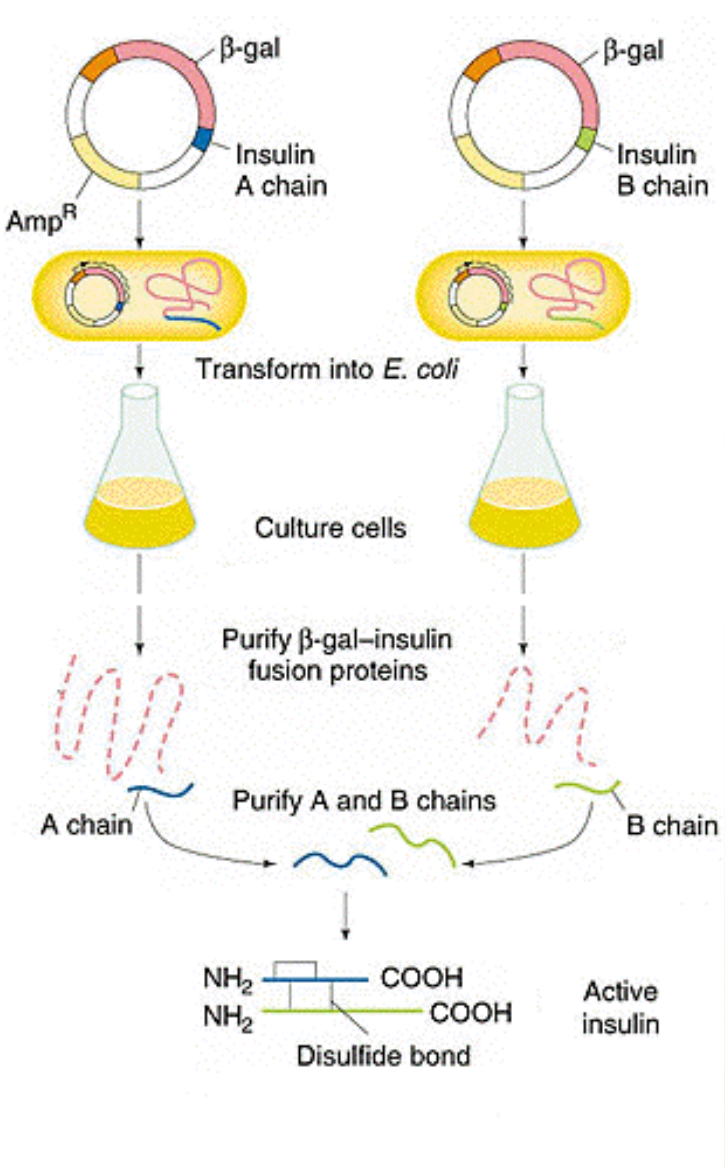
In eukaryotes, diphtheria toxin is a protein that interferes with protein synthesis by decreasing the activity of the eukaryotic elongation factor eEF2.

Inhibitors of translation



A benefit of cloning

- Production of eukaryotic proteins ir bacteria (example: Insulin)
- Challenges: insulin is a dimer linked by disulfide bonds and produced from genes containing introns.
- Solution: synthetic DNA is made for each polypeptide and inserted into bacteria separately. The polypeptides are purified from each bacterial batch and mixed to form the mature insulin protein.

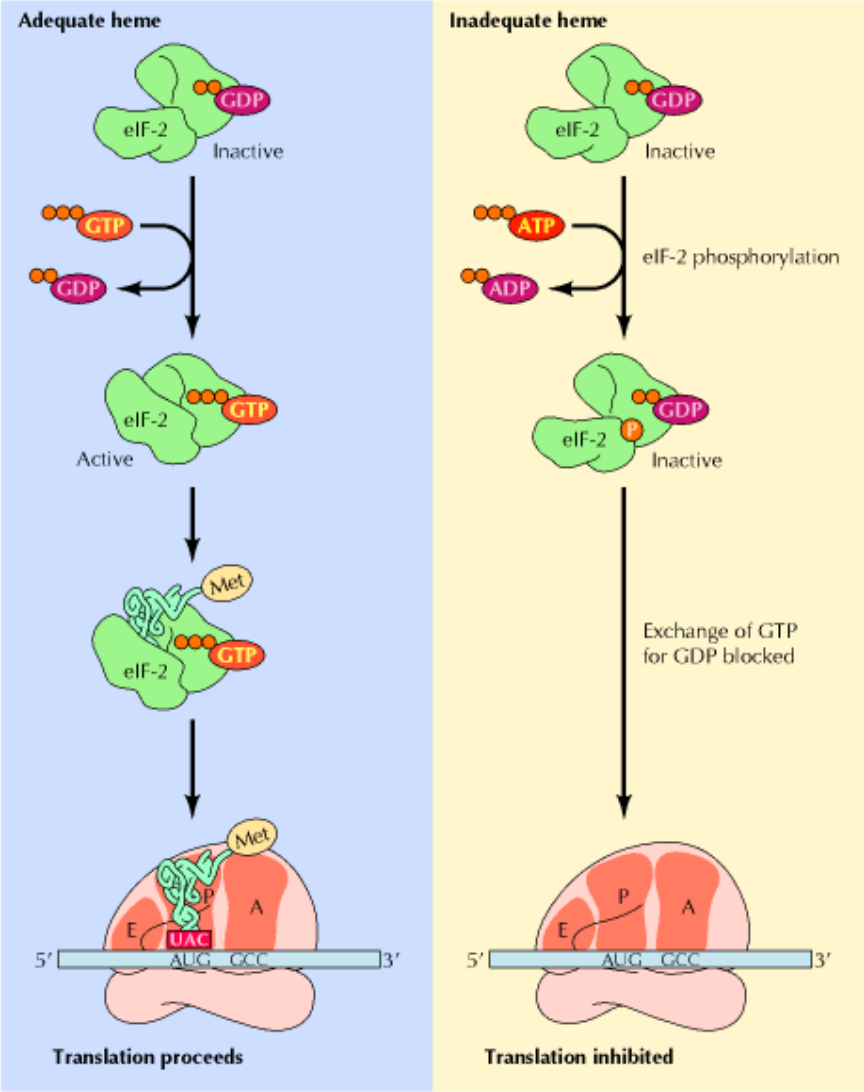


Heme and protein synthesis

- In reticulocytes (immature erythrocytes), heme stimulates protein synthesis.
- The mRNA is translated only if adequate heme is available to form functional hemoglobin molecules.
- This is done via regulating the activity of eIF-2, which is responsible for escorting initiator methionyl tRNA to the ribosome.
- eIF-2 must be bound to GTP to be active. When it is released from the ribosome, GTP is hydrolyzed to GDP, which must be exchanged with GTP for eIF-2 to be active again.

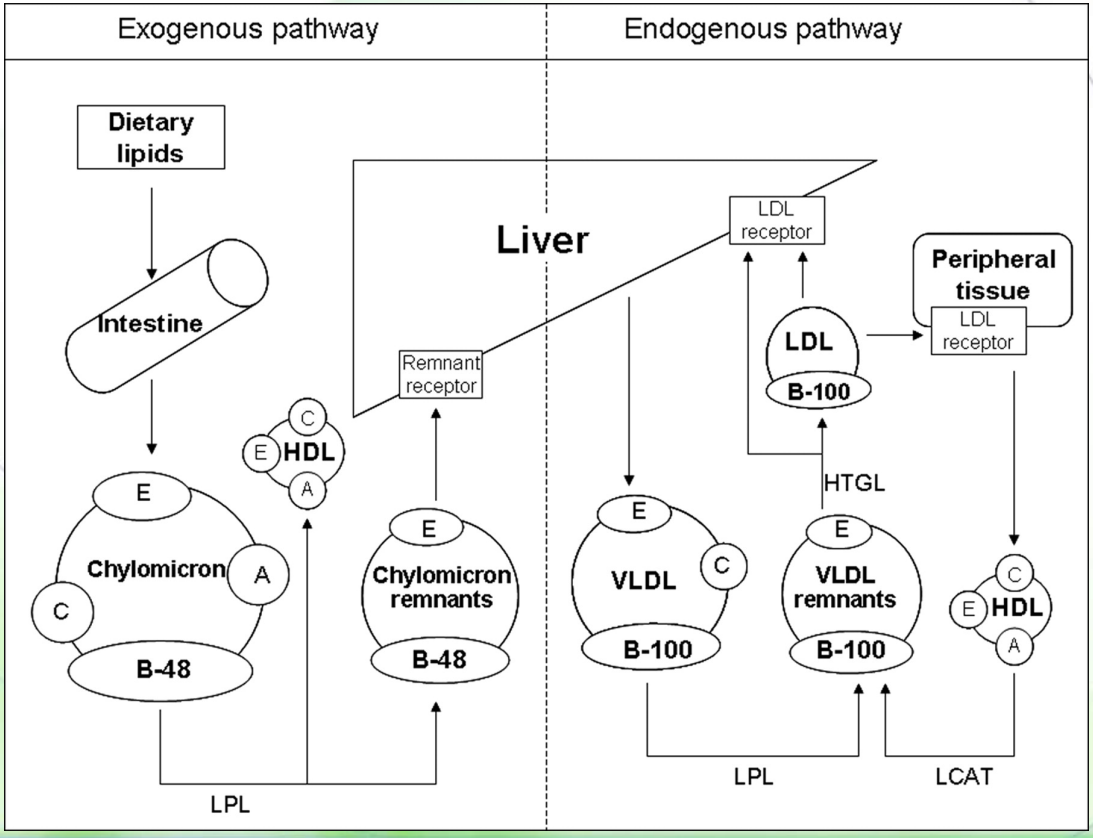
Regulation

- If adequate heme is available, GDP-GTP exchange occurs and translation is able to proceed.
- If heme supplies are inadequate, a protein kinase that phosphorylates eIF-2 is activated. Phosphorylation of eIF-2 blocks the exchange of GTP for GDP, so eIF-2/GTP cannot be regenerated and translation is inhibited.

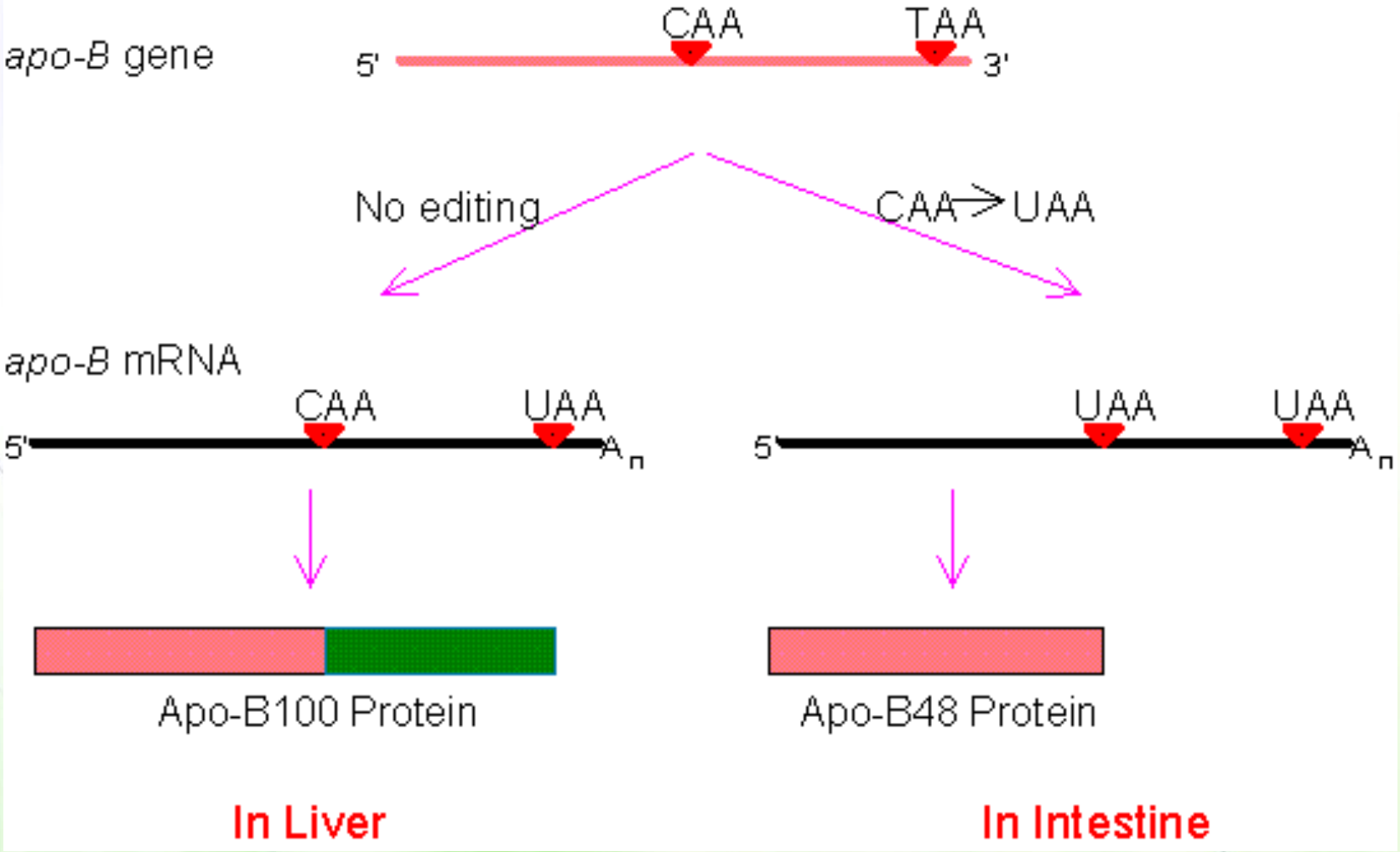


ApoB-100 vs. apoB-48

- These proteins make up specific lipoprotiens that are responsible for lipid transport.
  - ApoB-100 is a liver proteins that is part of low-density lipoproteins
  - ApoB-48 is an intestinal proteins that is part of chylomicrons
- Both proteins are synthesized from the same gene.

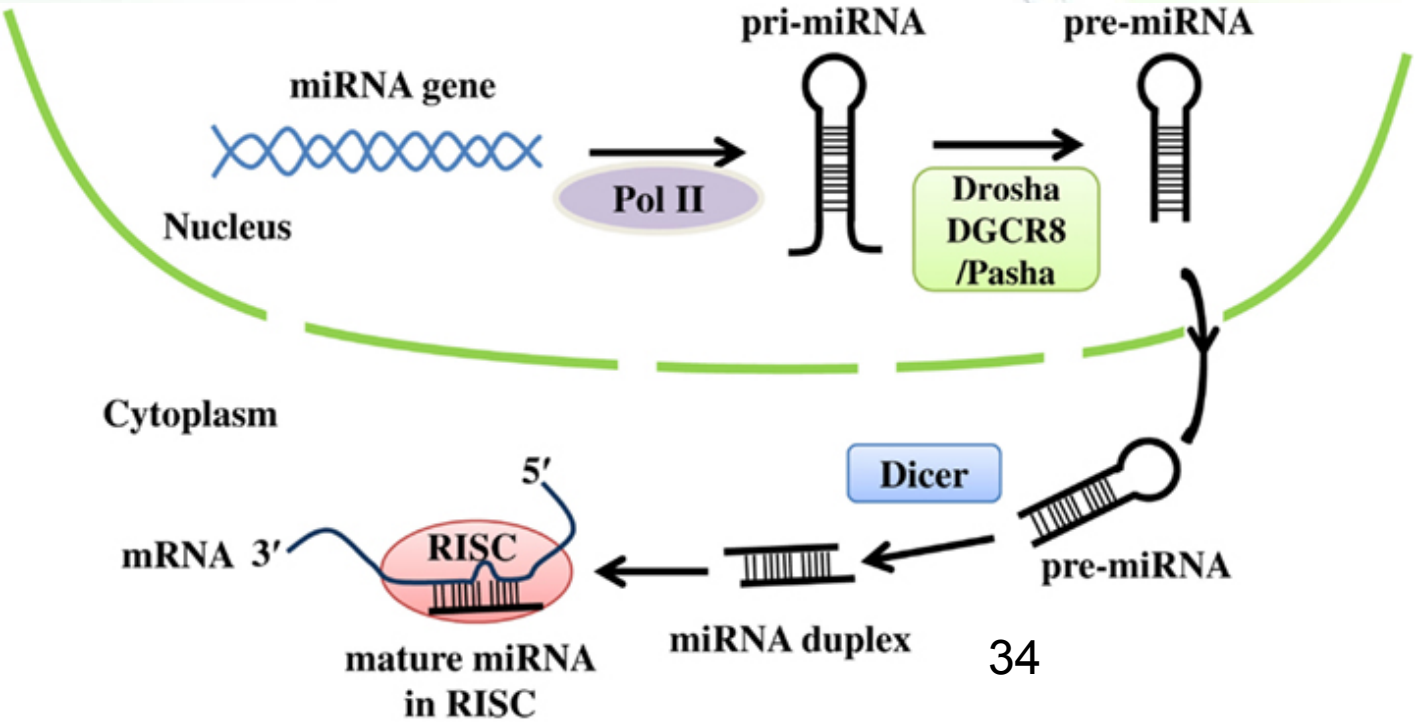


Gene editing



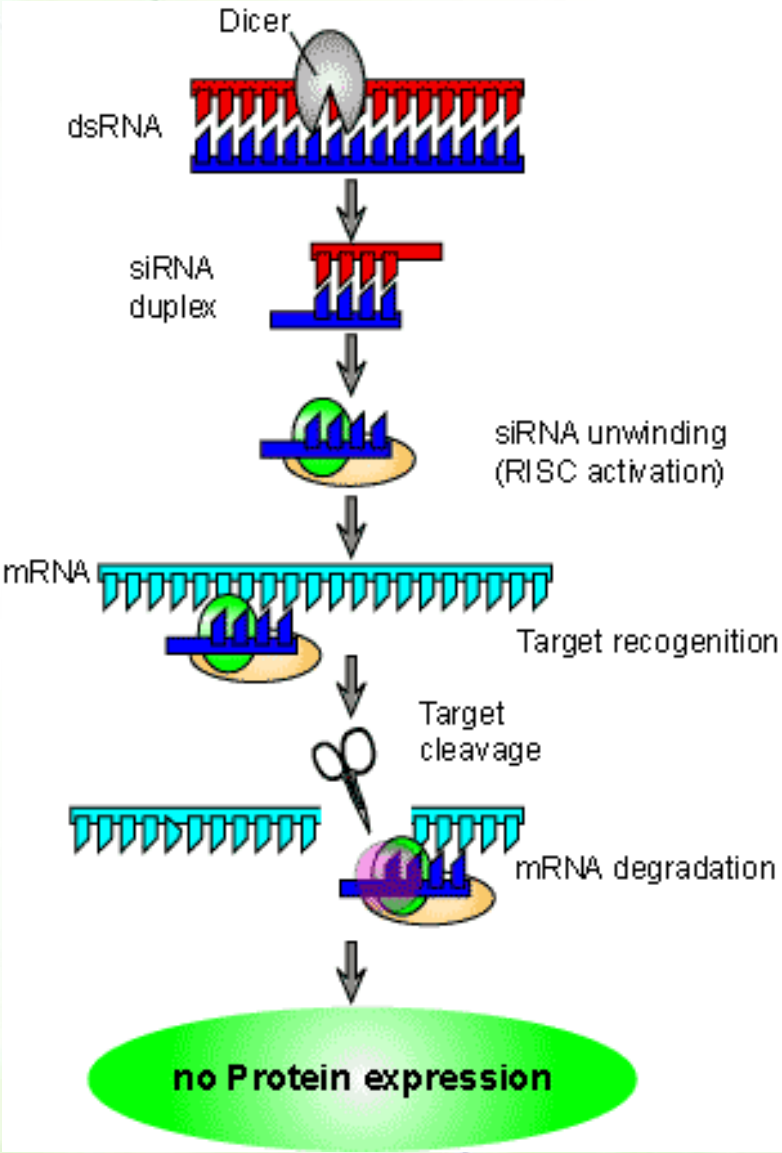
Regulation by microRNA (miRNA)

- MicroRNA is synthesized by RNA Pol II into single-stranded, primary miRNA (pri-miRNA) transcript.
- Pri-miRNA is processed in the nucleus by Drosha and exported to the cytoplasm, modified by an endonuclease complex containing Dicer to generate a mature miRNA duplex.
- One strand is loaded onto RISC complex where miRNA is targeted to mRNA resulting in either translation repression of mRNA degradation.

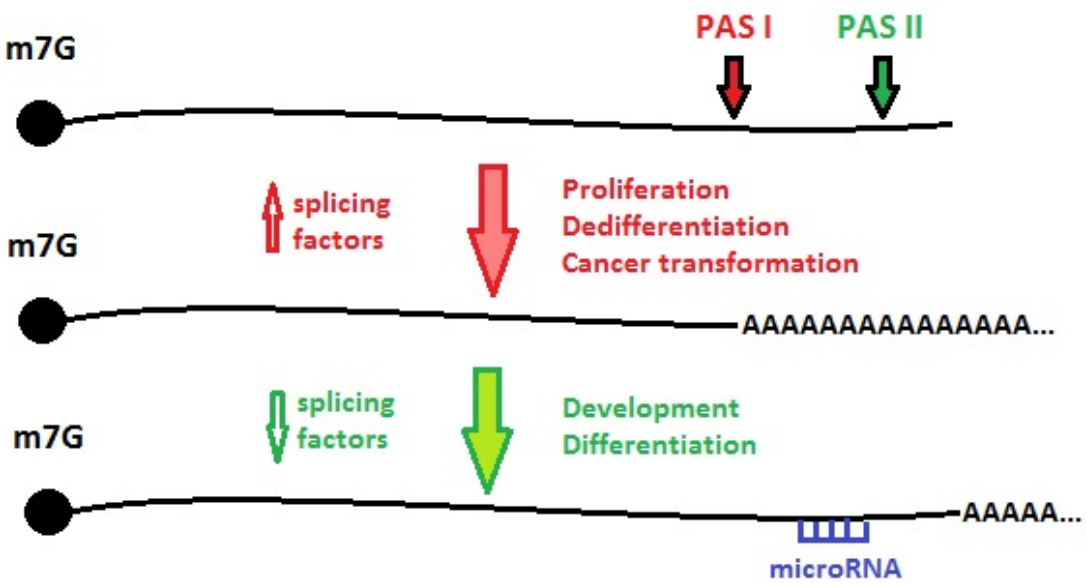


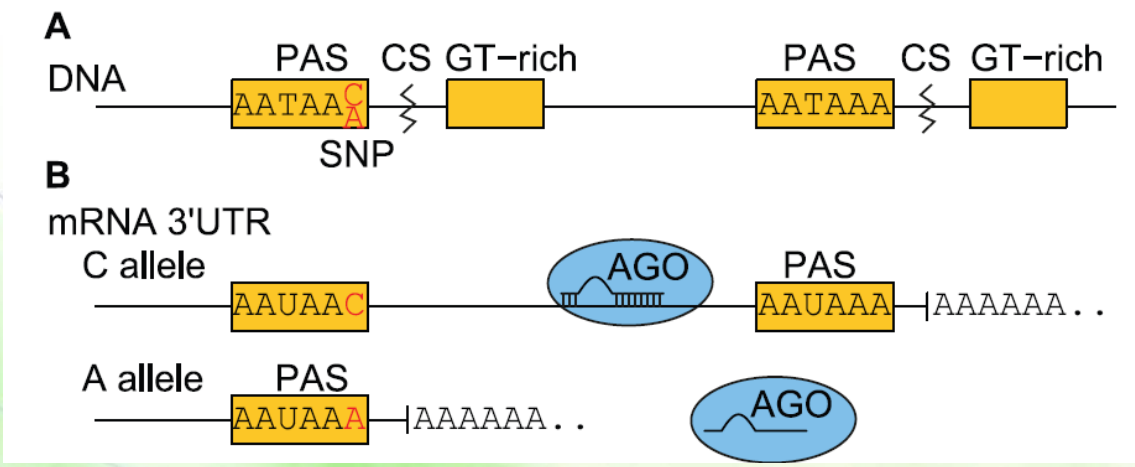
RNA interference (RNAi)

- Alternatively, short-interfering RNA is injected into cells as double-stranded RNA (or produced naturally by cells) where it is processed by Dicer and escorted by RISC to perfectly bind to complementary mRNA and induce mRNA degradation.



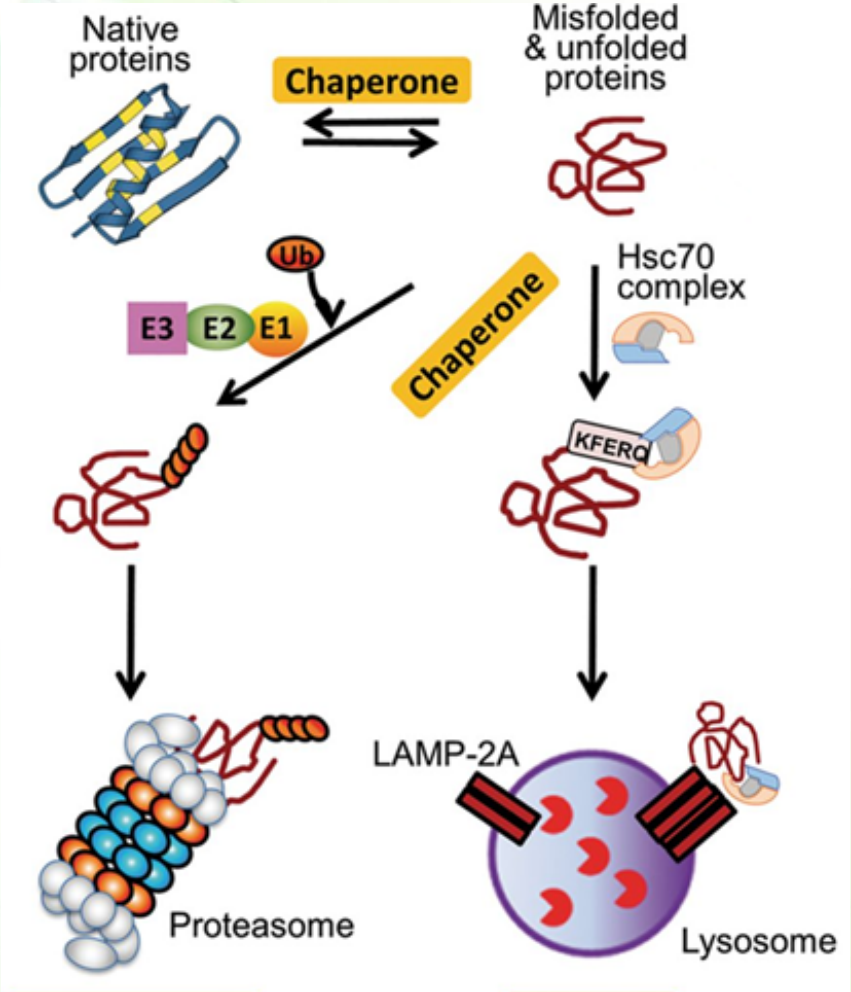
Alternative polyadenylation





Fate of (mis)- and (un)-folded proteins

- Proteins are degraded either in degradative subcellular organelles like lysosomes or by the macromolecular **proteasomes**.
- Proteins are targeted for destruction in a proteasome by **ubiquitinylation** which involves labeling by small polypeptides known as ubiquitin.



Levels of regulation

- Transcription
- RNA processing
- RNA transport
- mRNA stability
- Translation
- Post-translational modification
- Protein activity
- Protein degradation