Molecular Biology (9)

Translation

Mamoun Ahram, PhD
Second semester, 2018-2019

Resources

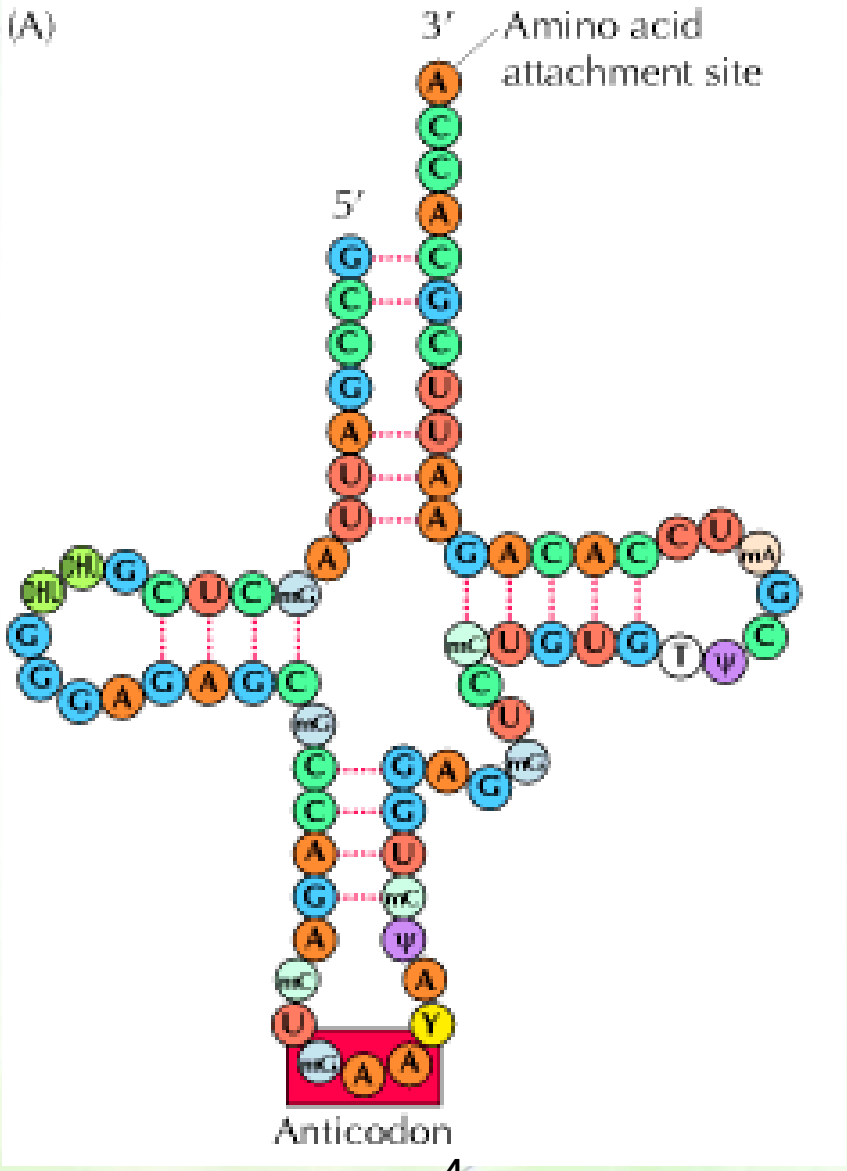
- This lecture
- Cooper, Ch. 8 (297-319)

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

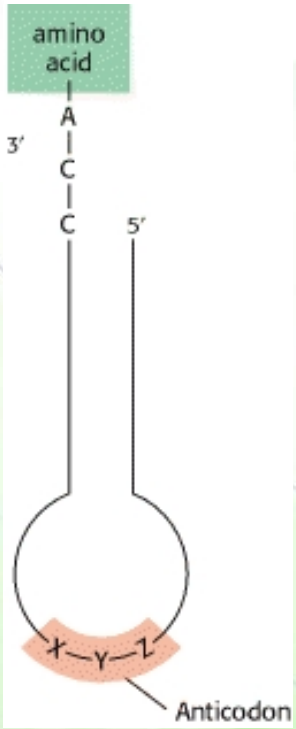
tRNA structure

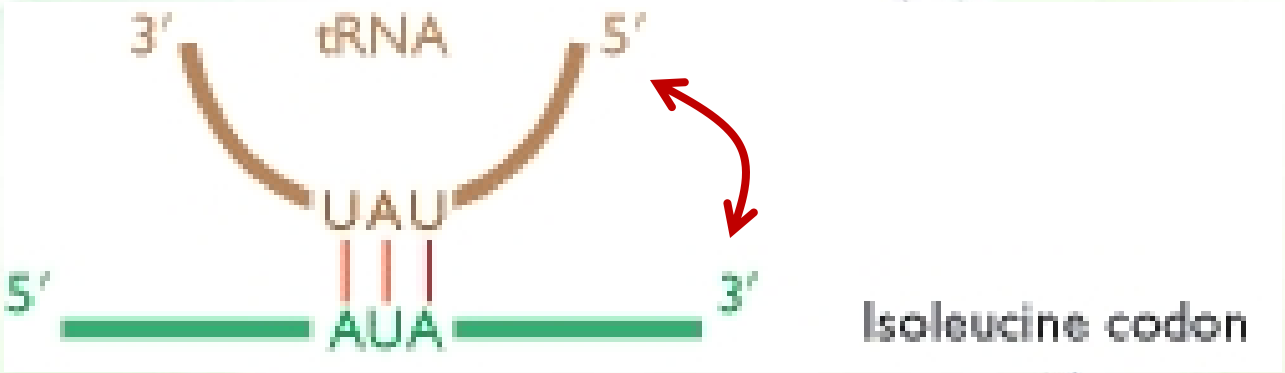
- tRNAs are short RNA molecules (80 bases long).
- "Charged" or "activated" tRNA carries one amino acid.
- Twenty Aminoacyl-tRNA synthetases exist for each amino acid.
- An amino acid is covalently attached to the ribose of the terminal adenosine at CCA.
  - The amino acid attached to tRNA is specified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

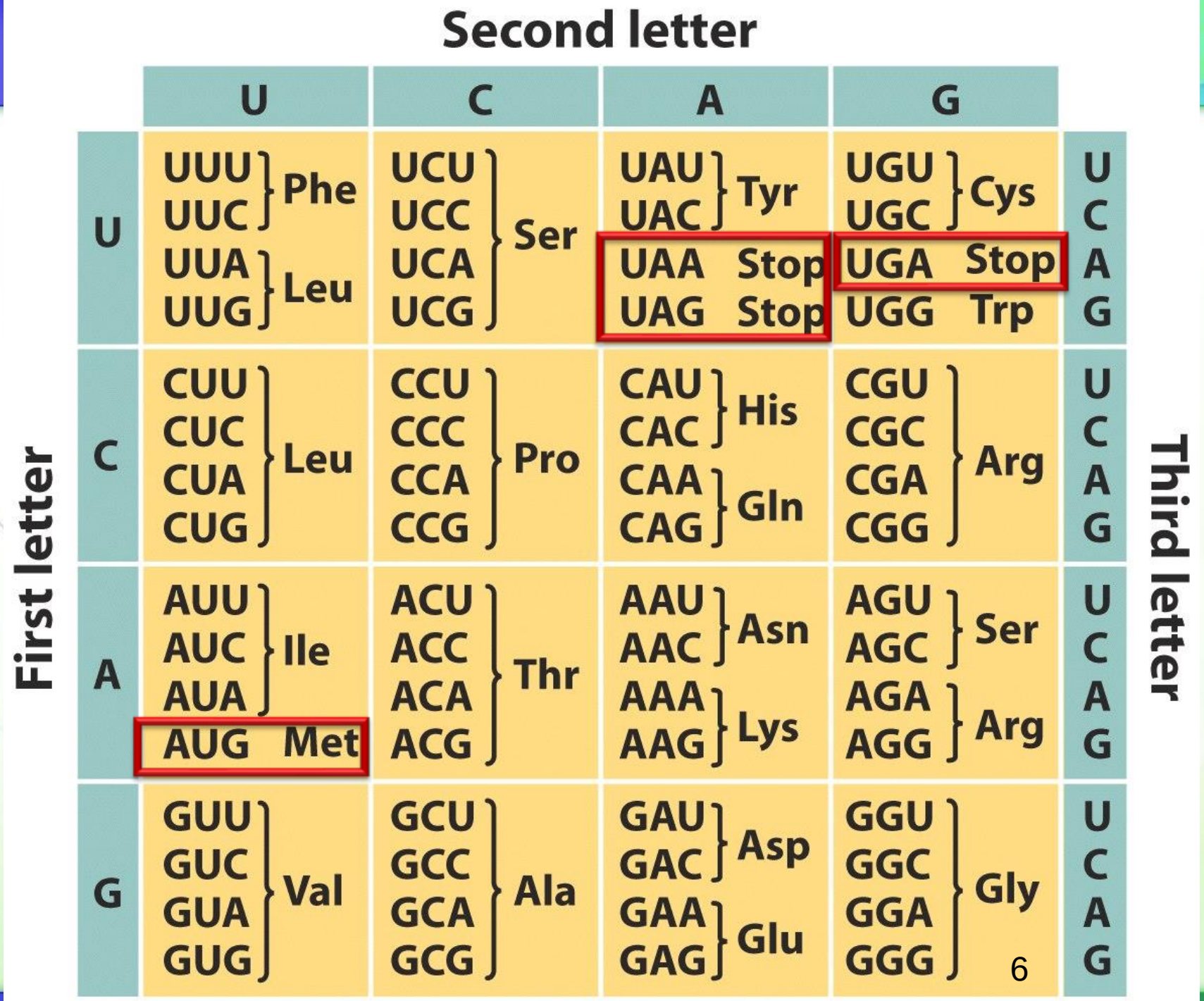


Codon vs. anticodon

- 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)

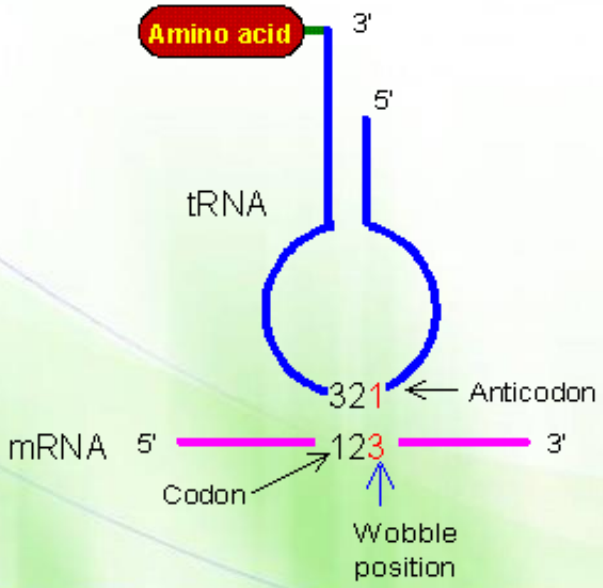





Second letter
U
C
A
G
First letter
Third letter
U
UUU Phe
UUC
UUA Leu
UUG
UCU Ser
UCC
UCA
UCG
UAU Tyr
UAC
UAA Stop
UAG Stop
UGU Cys
UGC
UGA Stop
UGG Trp
C
CUU Leu
CUC
CUA
CUG
CCU Pro
CCC
CCA
CCG
CAU His
CAC
CAA Gln
CAG
CGU Arg
CGC
CGA
CGG
A
AUU Ile
AUC
AUA
AUG Met
ACU Thr
ACC
ACA
ACG
AAU Asn
AAC
AAA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

Features of the genetic

- Not universal
  - Example: AUA in mitochondria (methionine) in cytosol (isoleucine)
- Wobble base pairing (degenerate and nonstandard)
  - 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

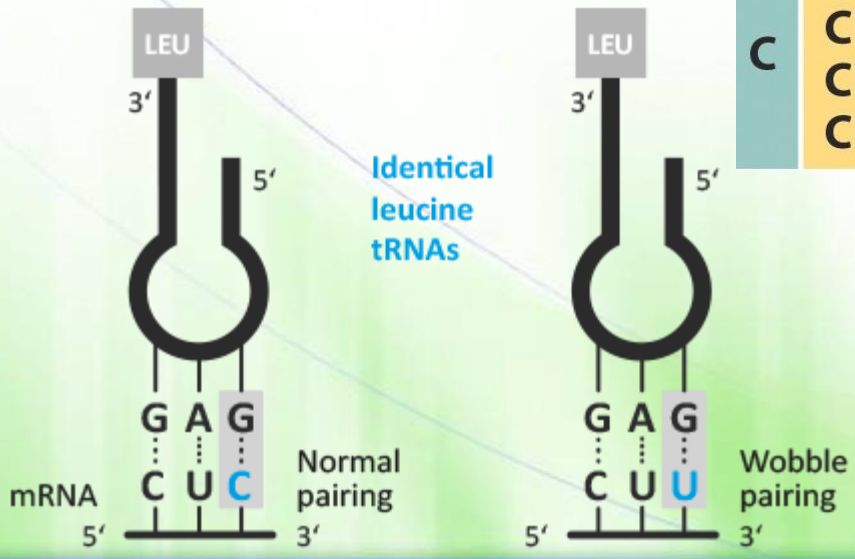
Wobble bases

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

Examples of wobble base pairing

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

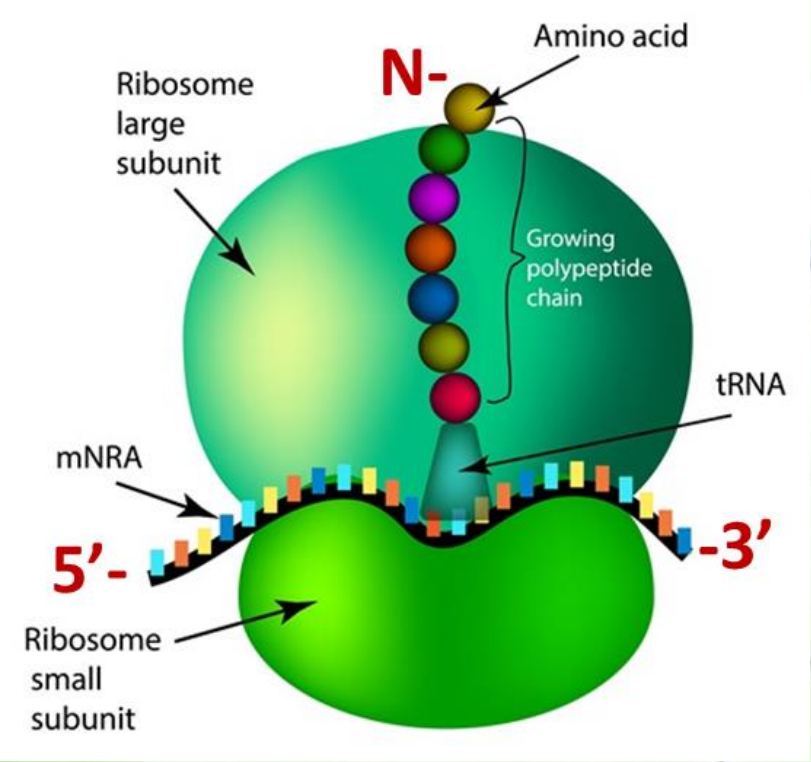
	U	C	A	G	
U	UUU, UUC } Phe	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr	UGU, UGC } Cys	U
	UUA, UUG } Leu		UAA Stop, UAG Stop	UGA Stop, UGG Trp	C, A, G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His; CAA, CAG } Gln	CGU, CGC, CGA, CGG } Arg	U, C, A, G



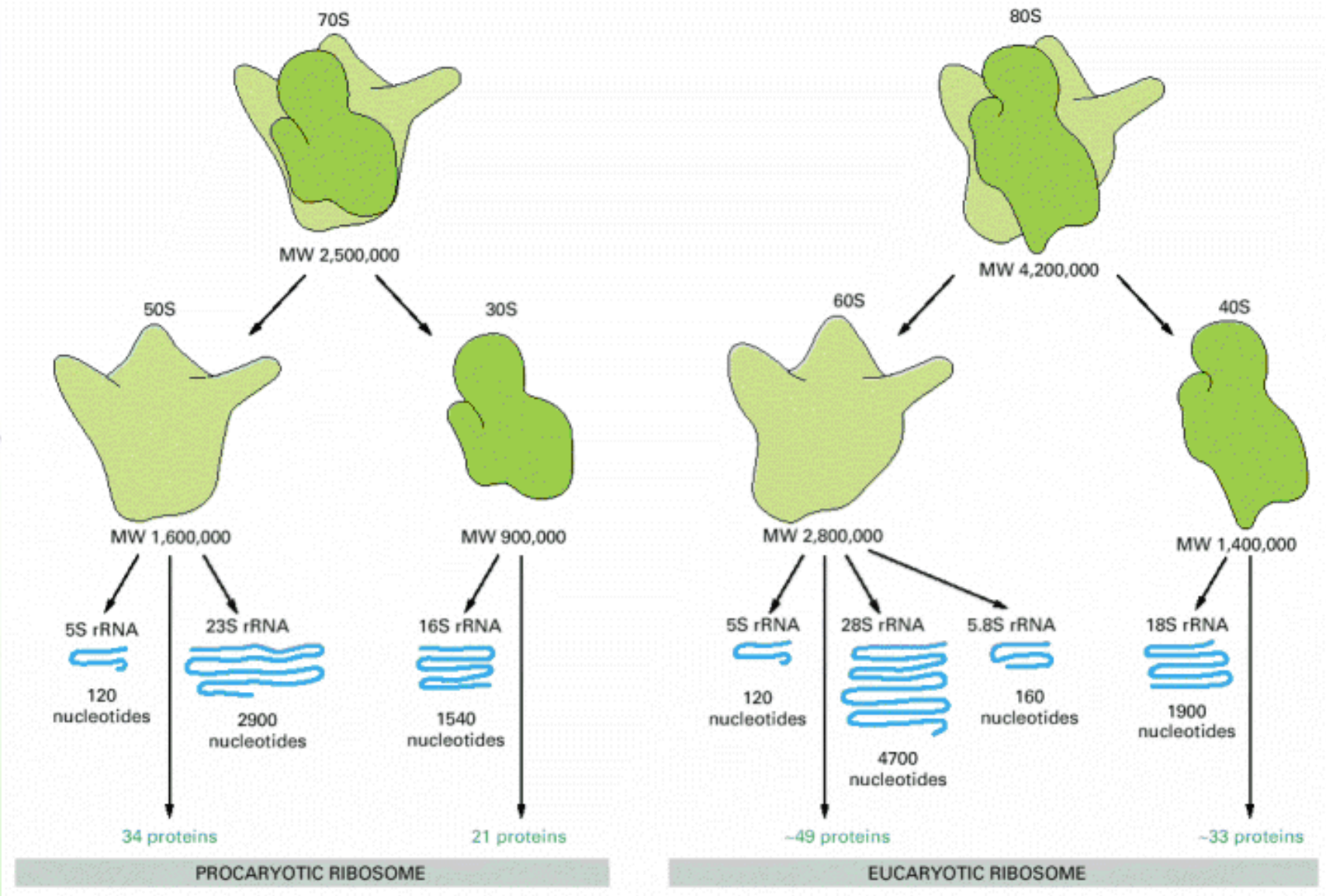
Ribosomes

- 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.

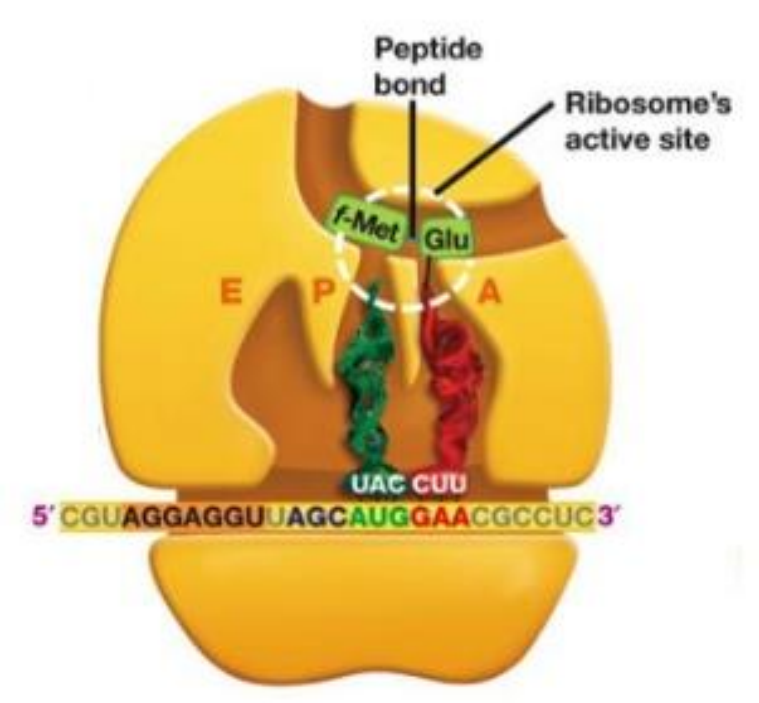


Ribosome structure



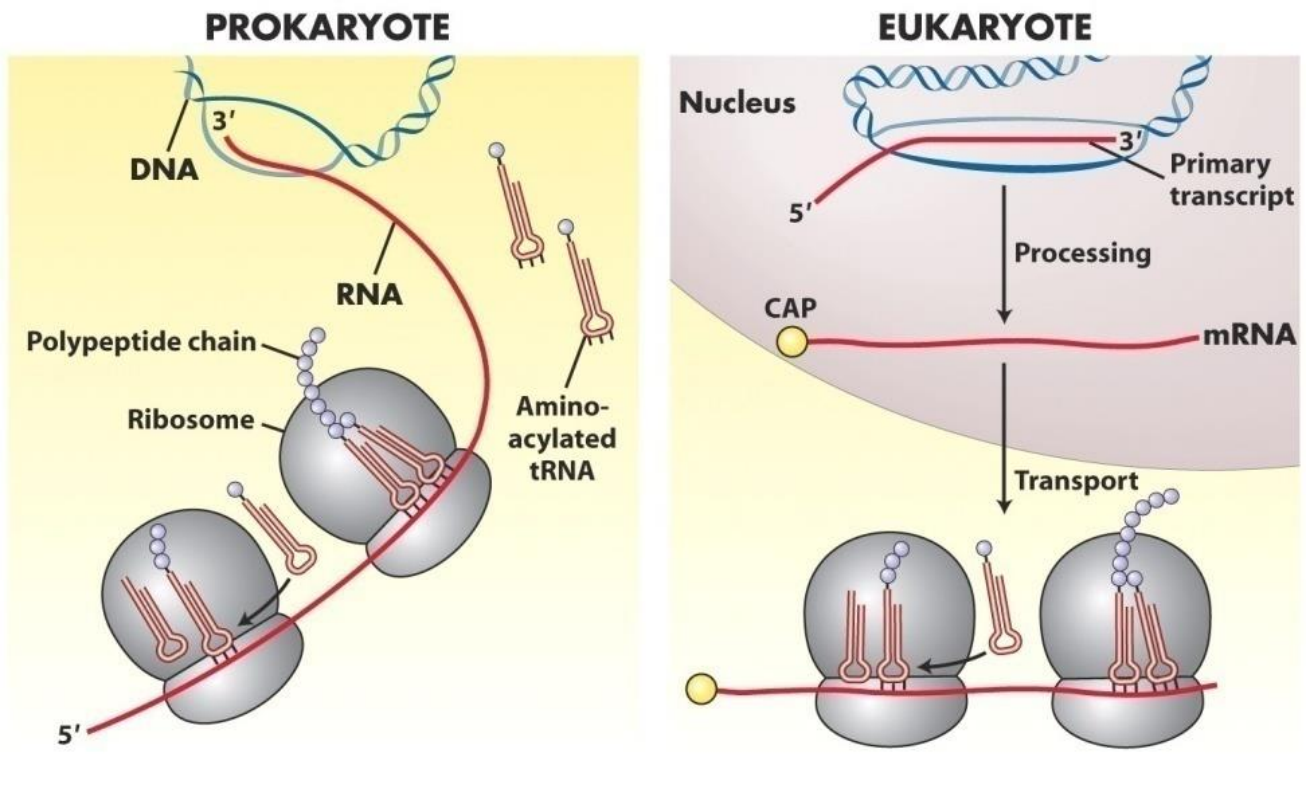
The general mechanism of translation

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



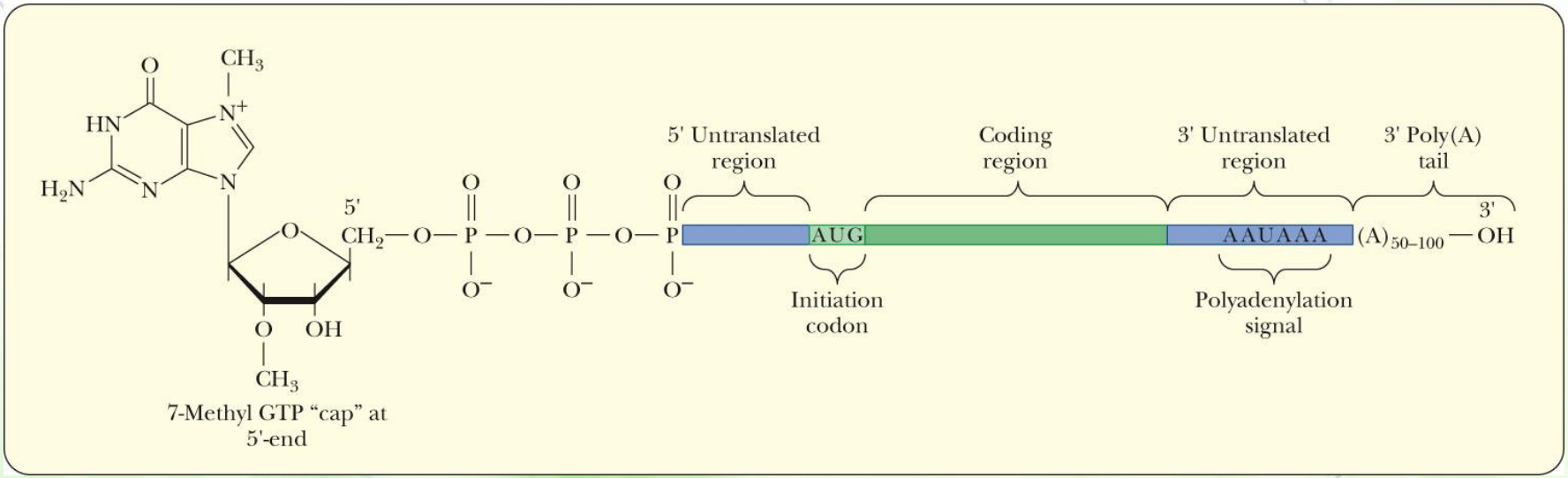
Transcription/translation Coupling

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



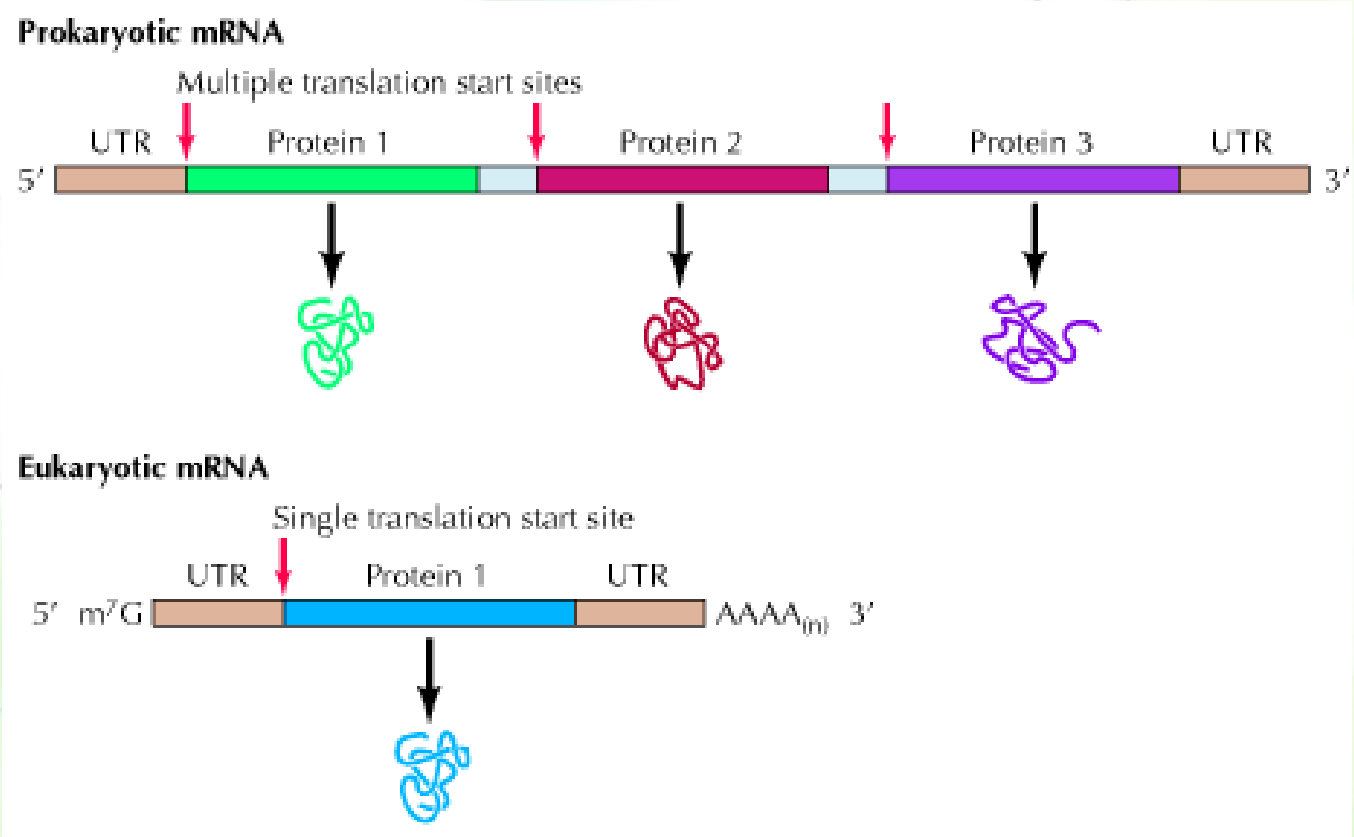
Start of translation

- 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.

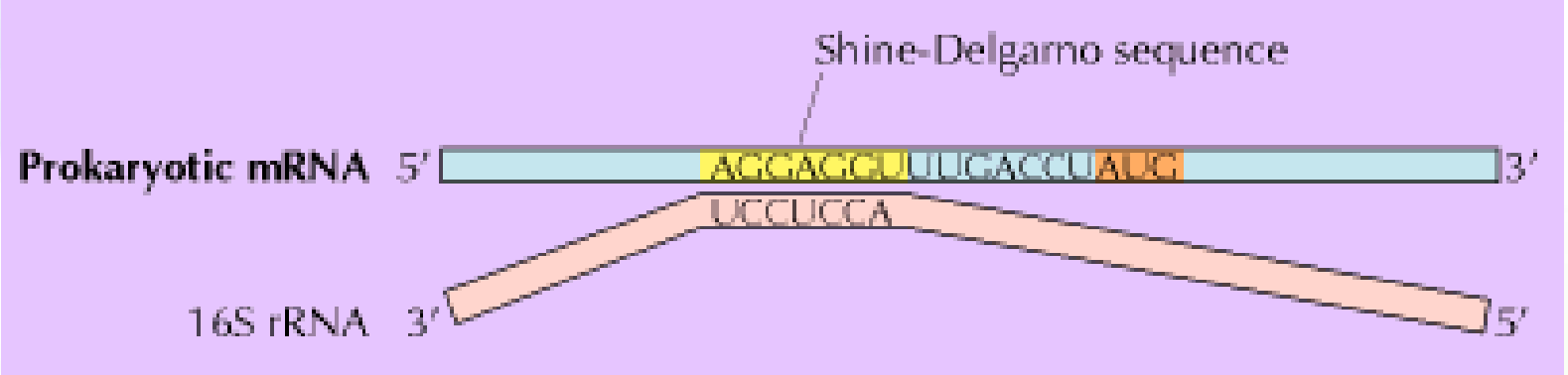


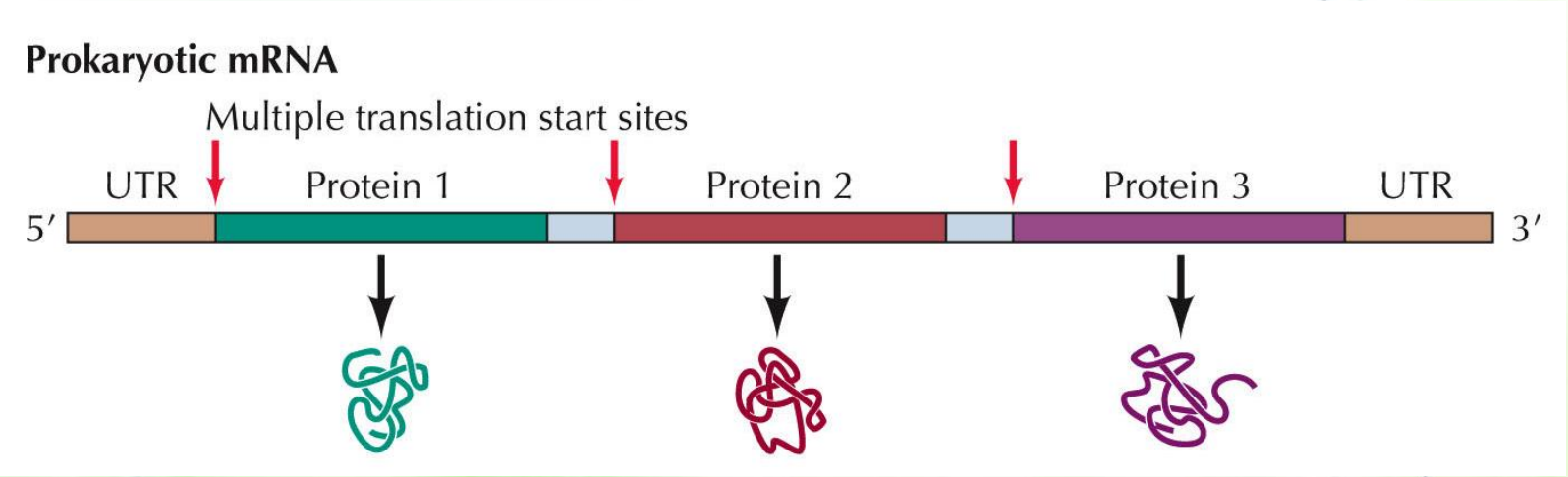
Remember…

- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic



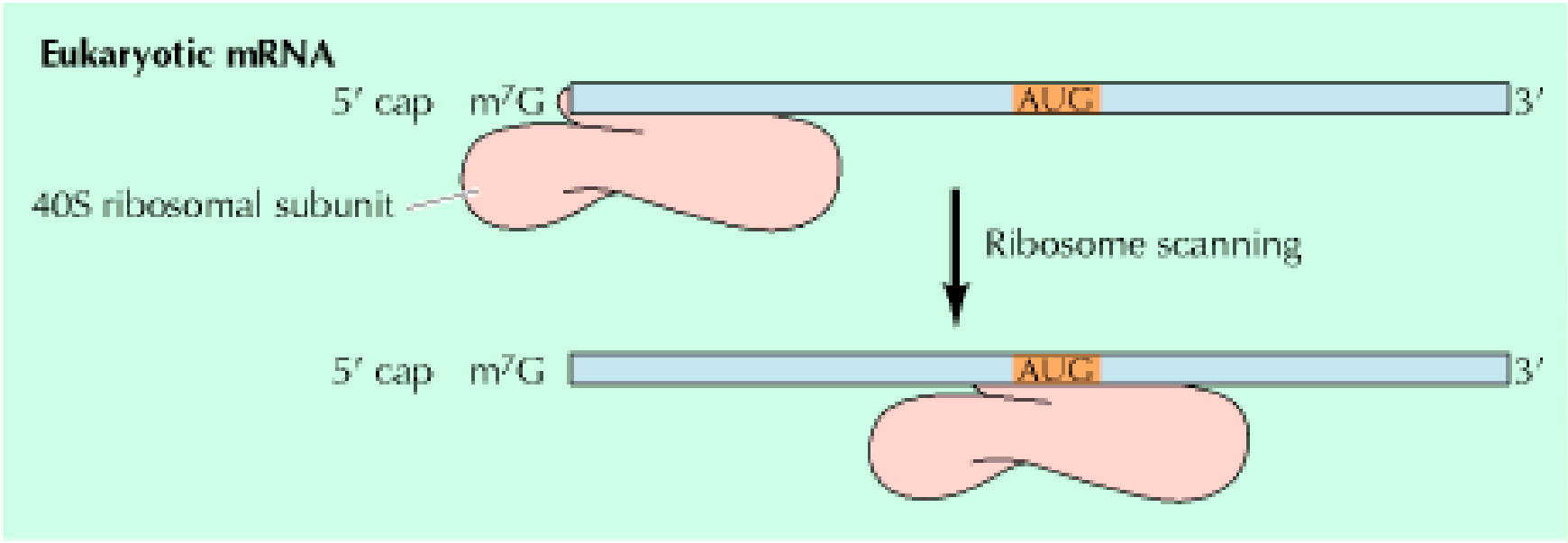
Shine-Dalgarno sequence





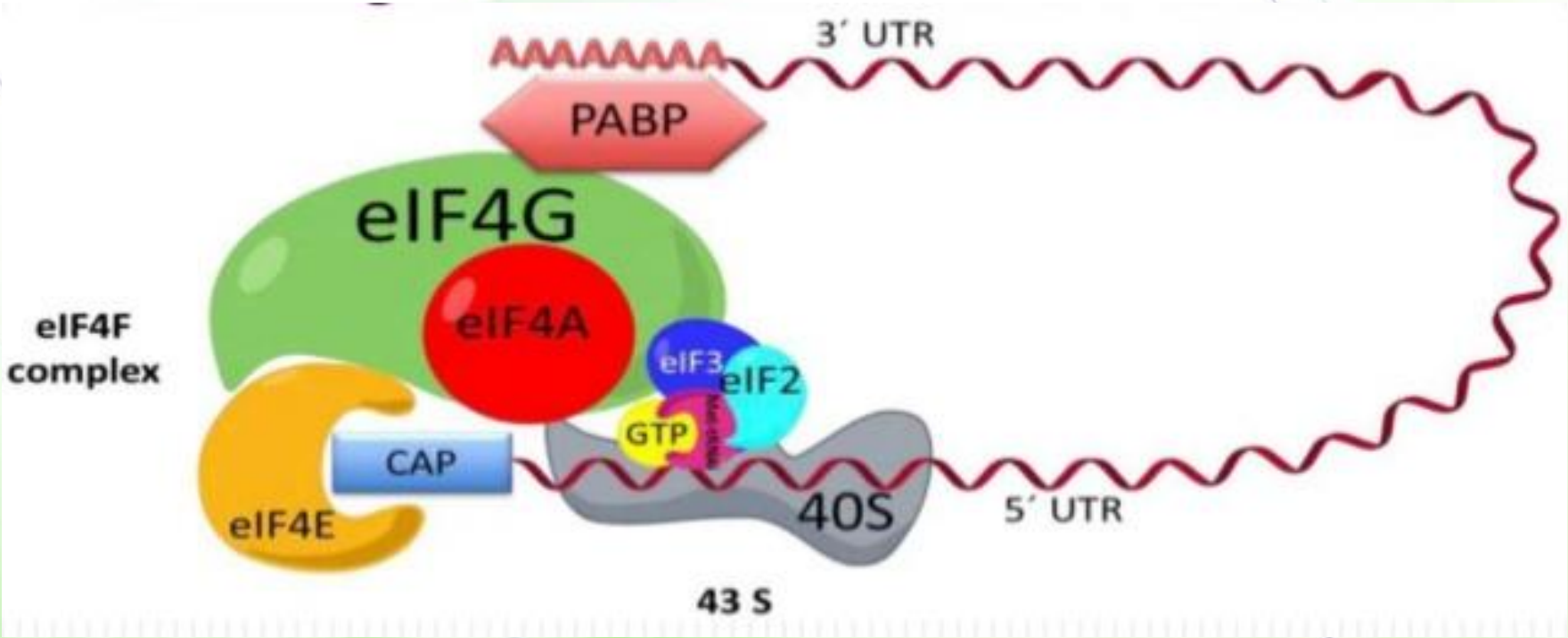
But in eukaryotes...

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



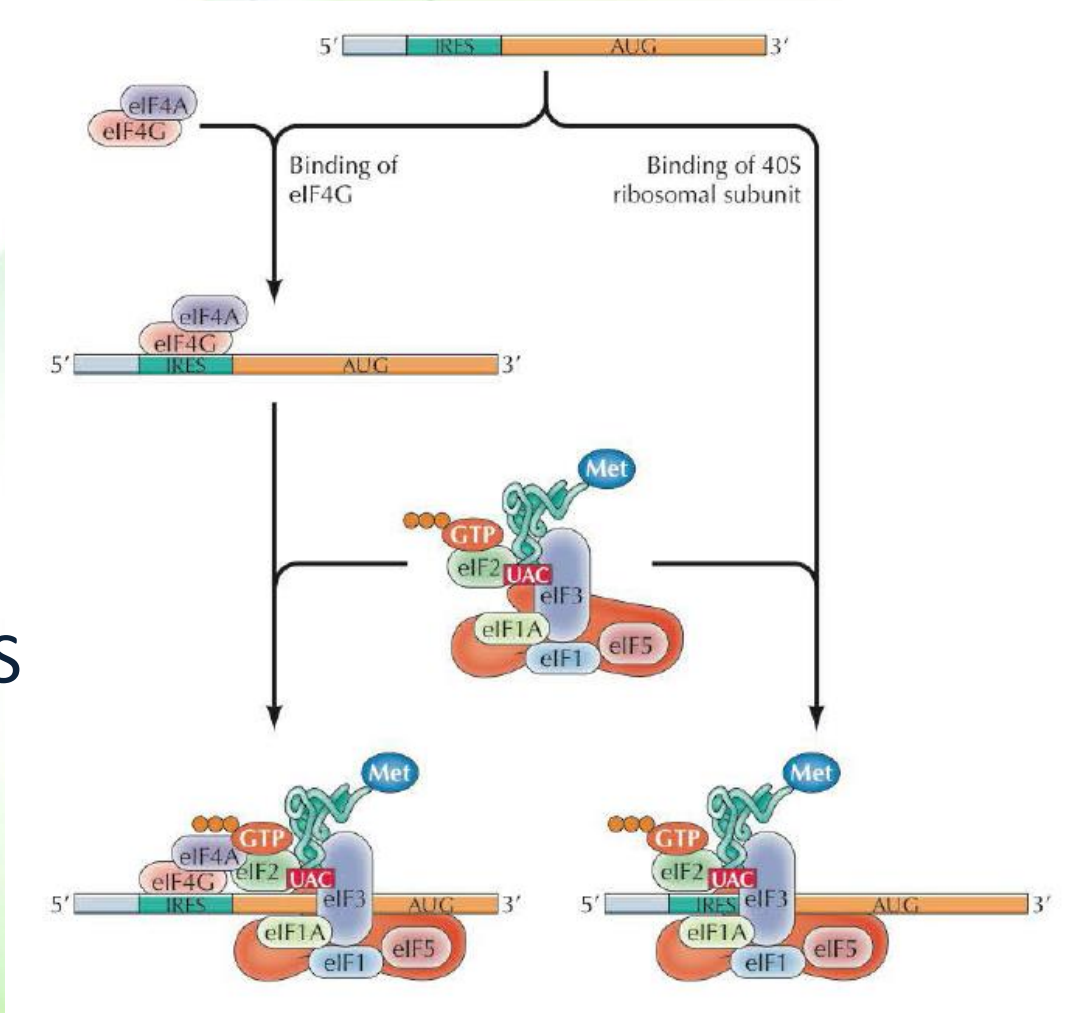
Translation initiation in eukaryotes

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



Internal ribosome entry site (IRES)

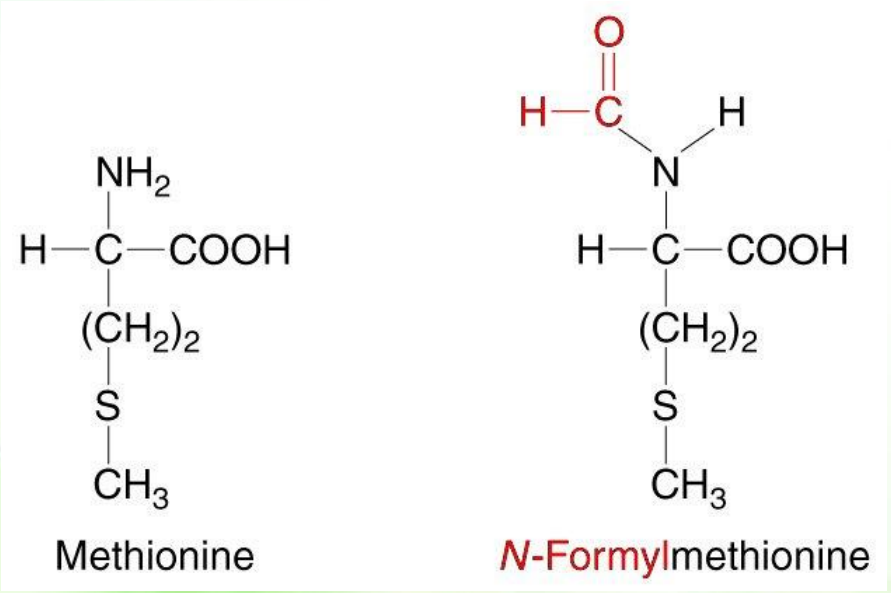
- Alternatively, internal ribosome entry site (IRES) exist in some other mRNAs and is recognized by the 40S ribosome or eIF4G protein followed by recruitment of the 40S ribosome.



The first amino acid

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

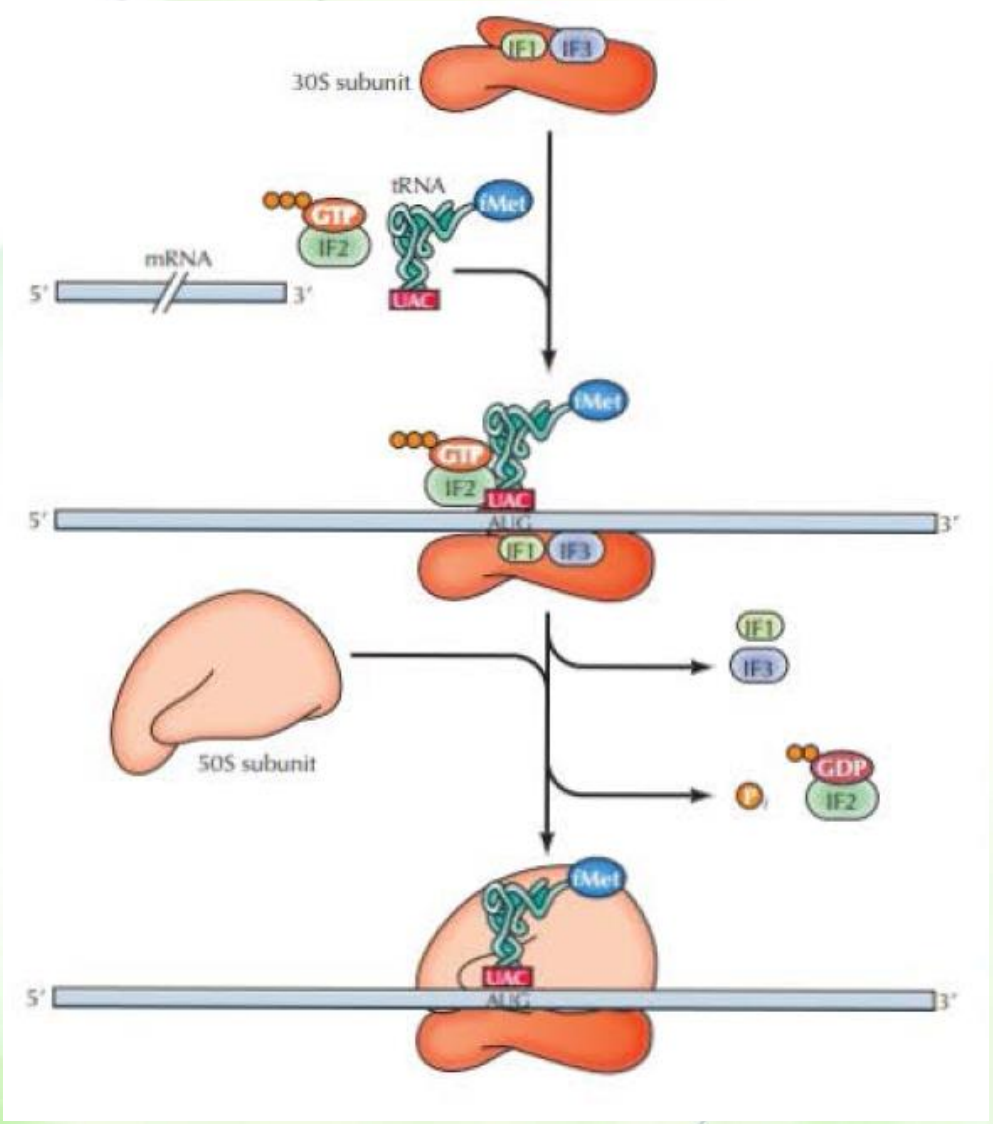
- In most bacteria, it is N-formylmethionine.



Translation initiation

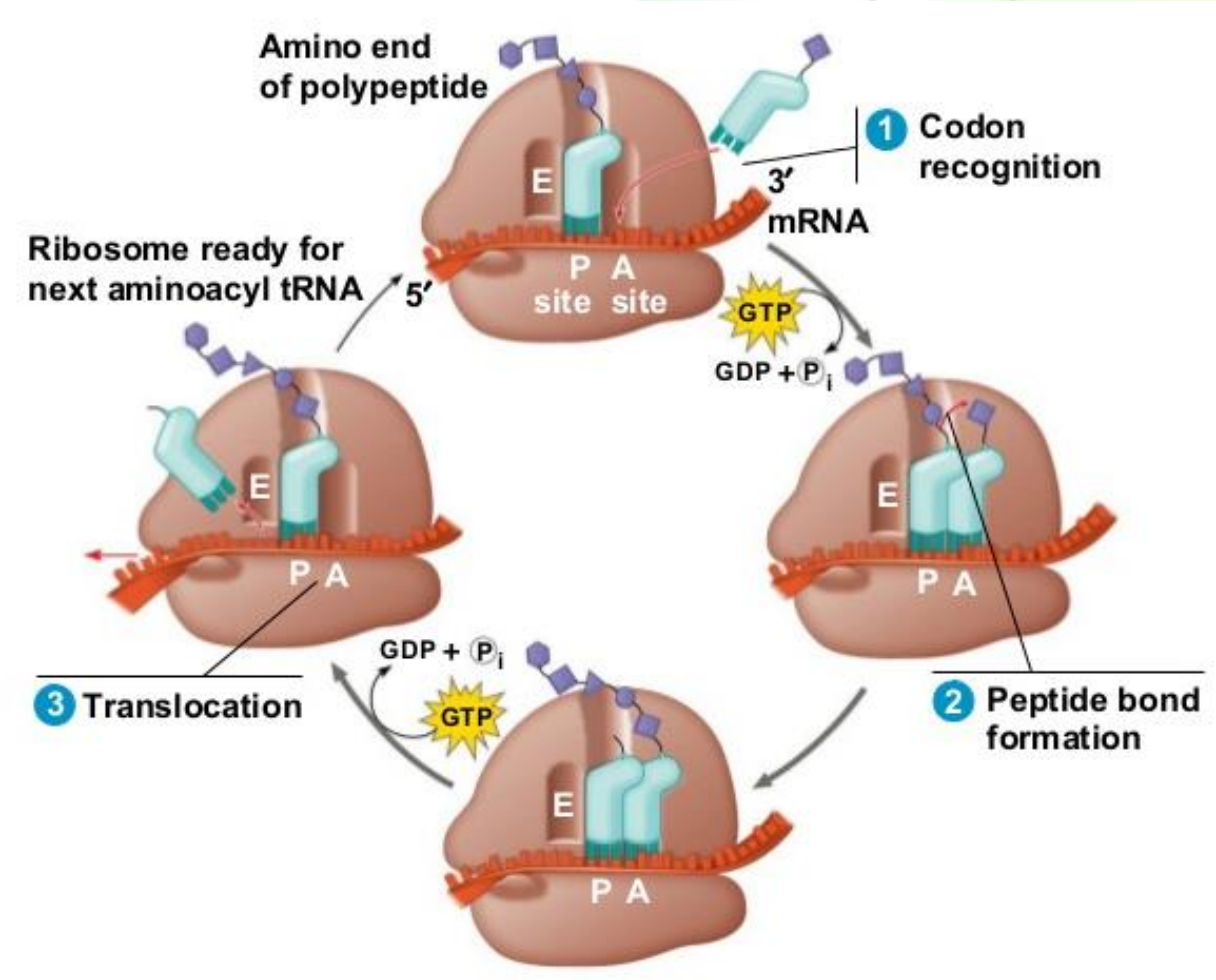
Prokaryotes

- 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 elongation I

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

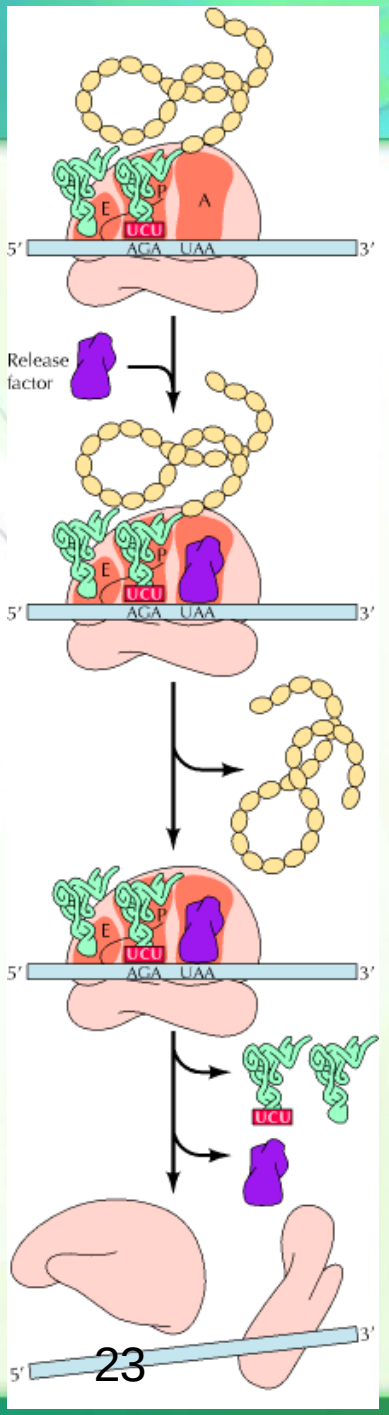


Details of elongation

- 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.

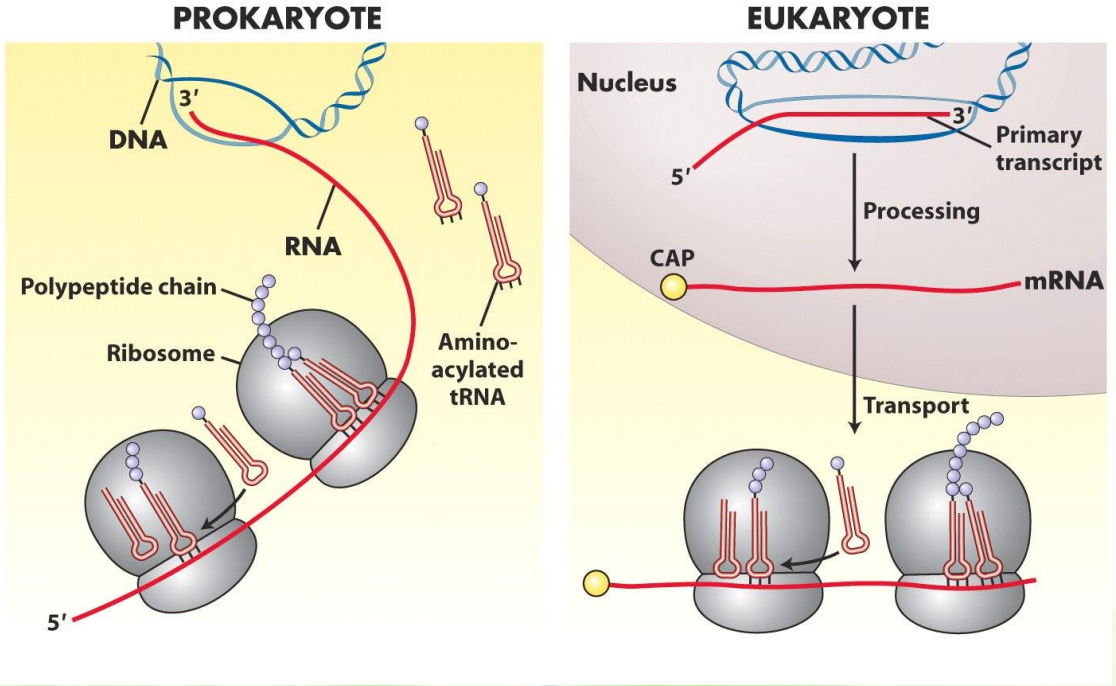
Translation termination

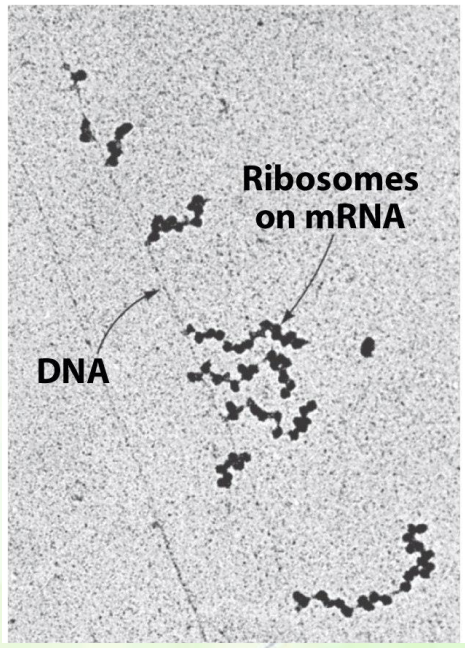
- The codons UAA, UAG, and UGA are the stop signals. They are not recognized by any tRNAs, but a release factor protein
- The release factor protein facilitates the hydrolysis of the peptide from the tRNA.
- Then, the whole complex dissociates.



Polyribosomes (polysomes)

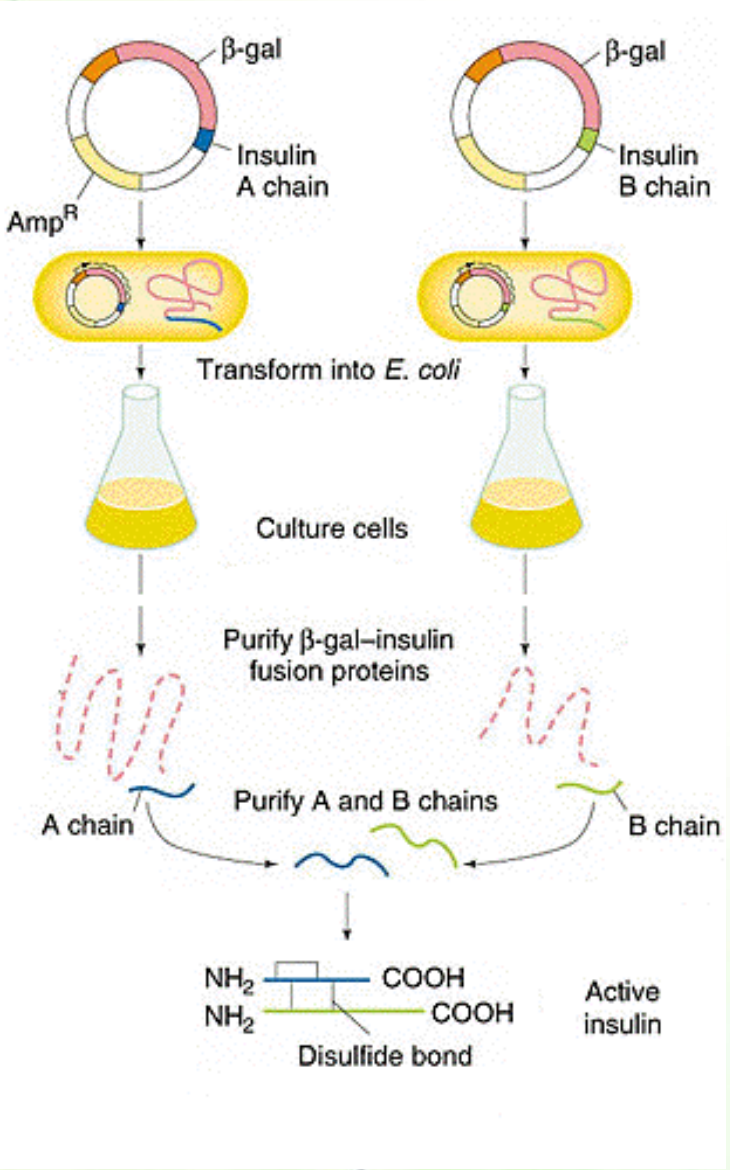
- A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.





A benefit of cloning

- Production of eukaryotic proteins in 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.

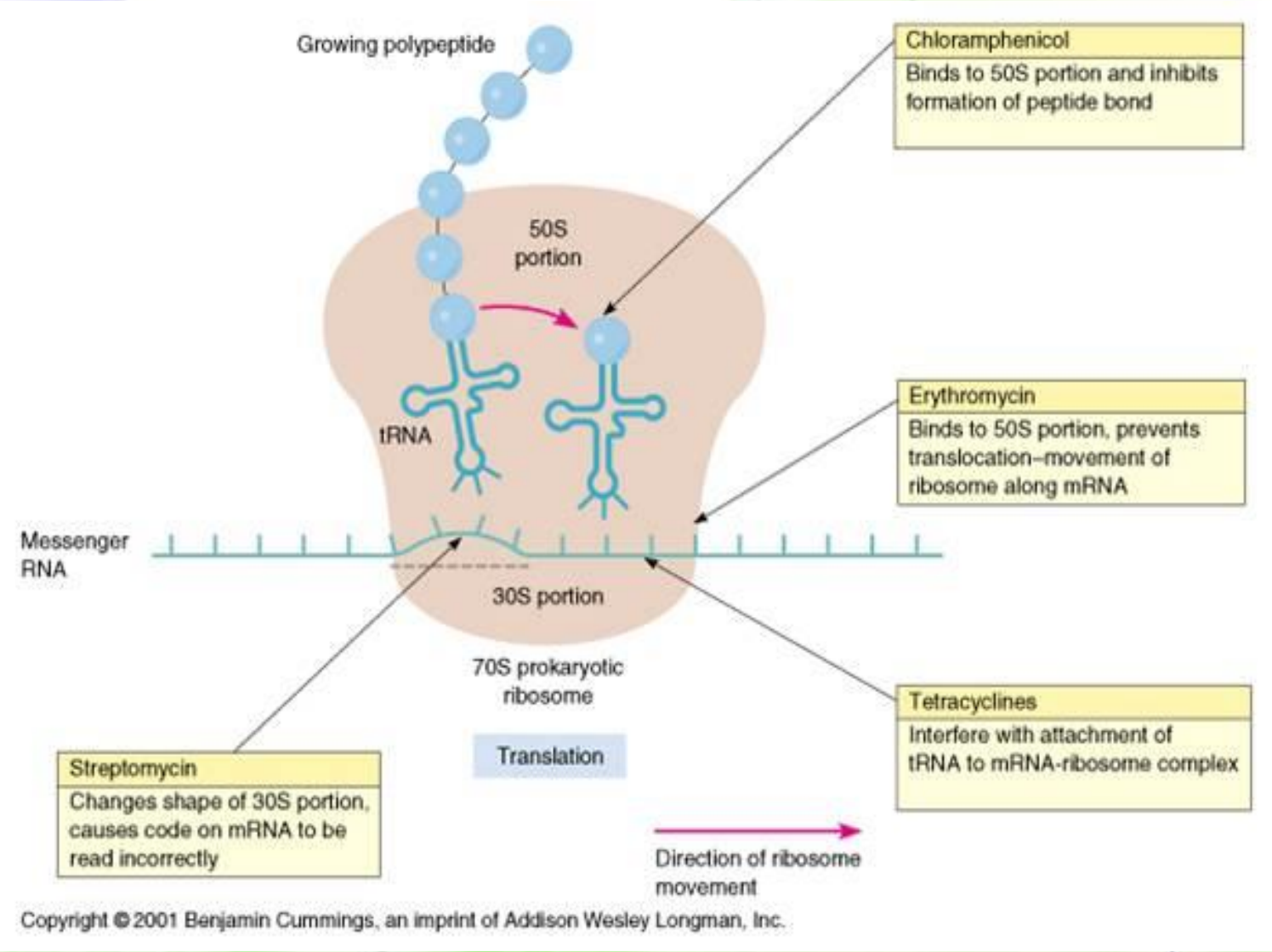


Inhibitors of translation

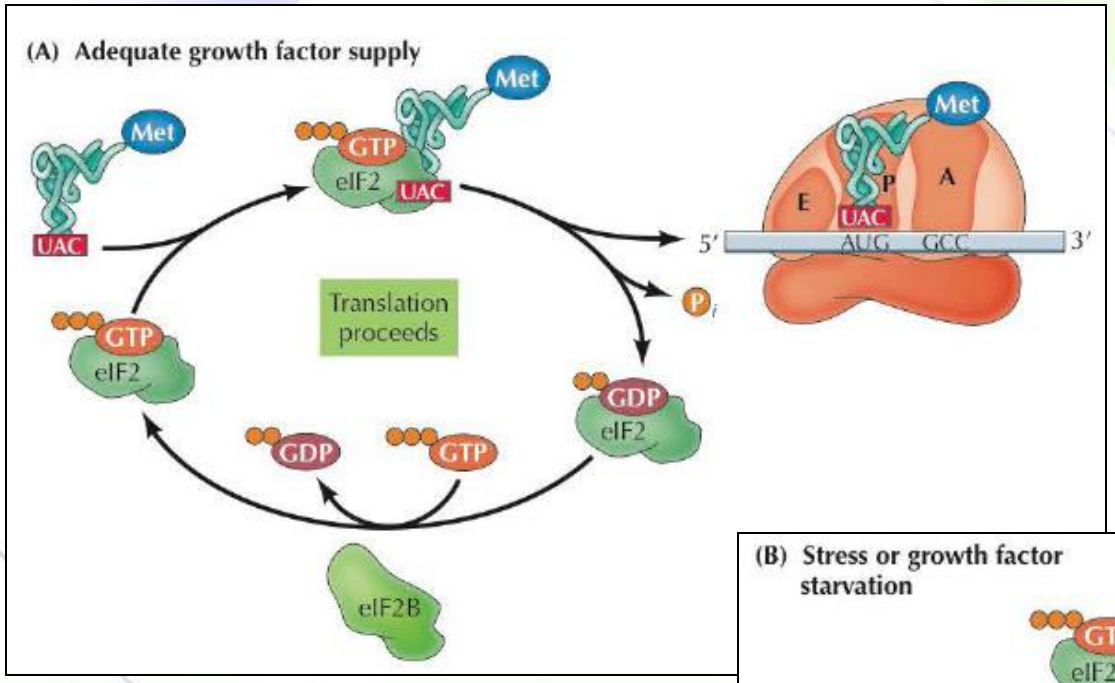
INHIBITOR	SPECIFIC EFFECT
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	Induces binding of wrong t-RNA-AA complexes resulting in false proteins
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes
Erythromycin	blocks the translocation reaction on ribosomes

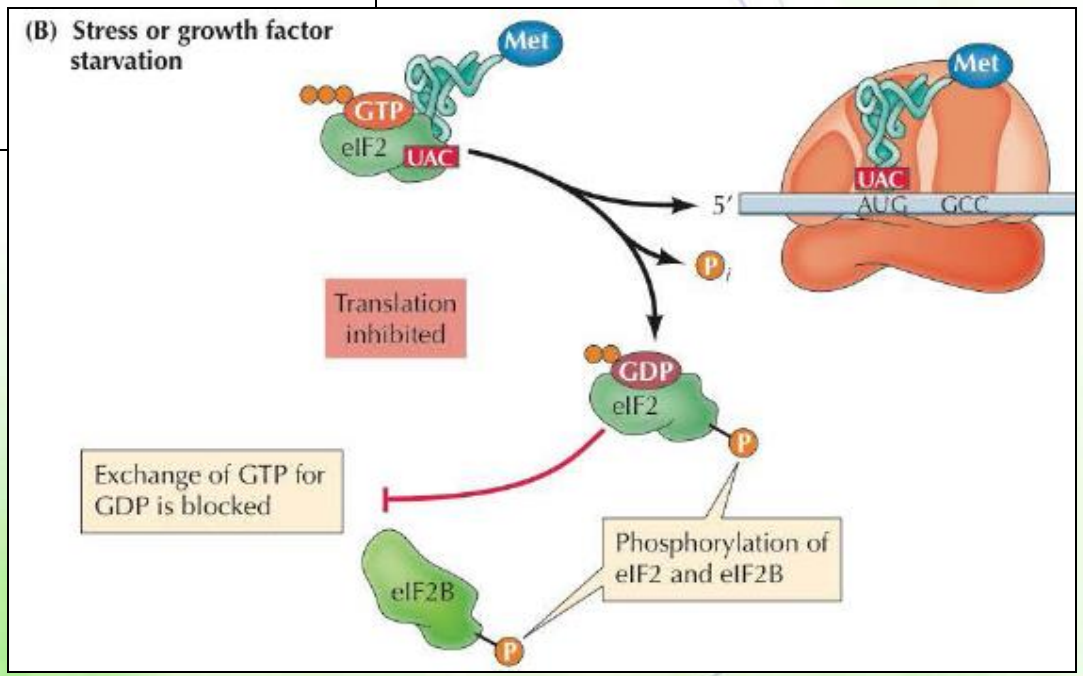
In eukaryotes, diphtheria toxin is a protein that interferes with protein synthesis by decreasing the activity of the eukaryotic elongation factor eEF2.

Inhibitors of translation



Regulation of translation…globally



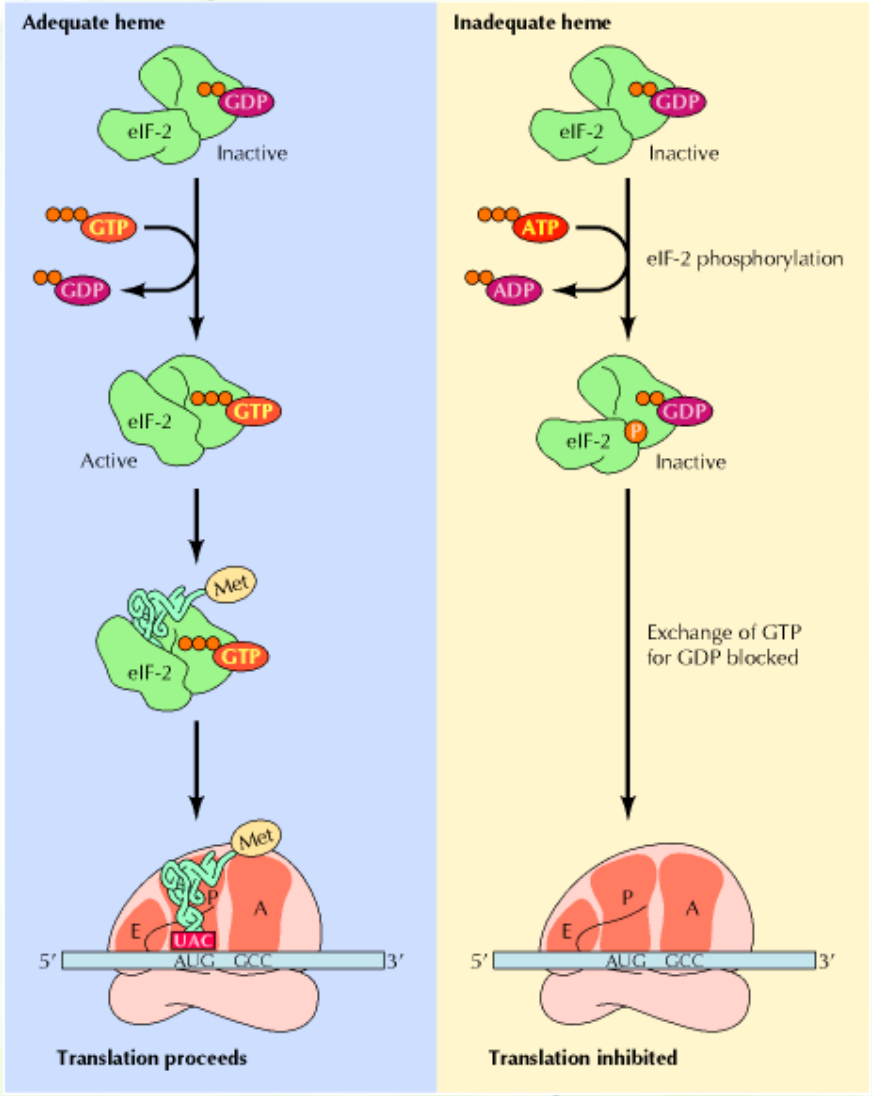


Heme and protein synthesis

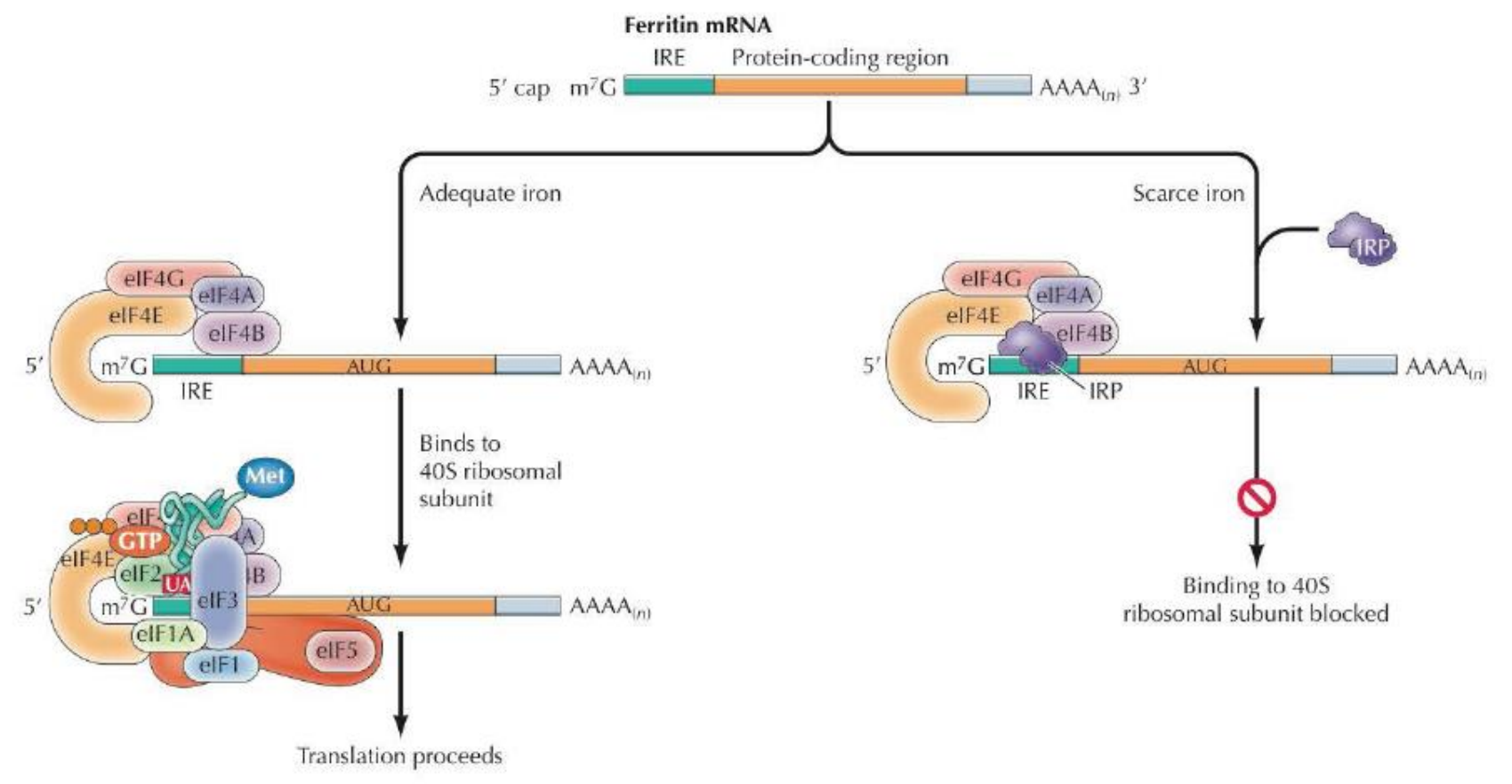
- In reticulocytes (immature erythrocytes), heme stimulates protein synthesis.
- The mRNA is translated only if adequate heme is available to form functional hemoglobin molecules.
- This is done via regulating the activity of eIF-2, which is responsible for escorting initiator methionyl tRNA to the ribosome.
- 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.

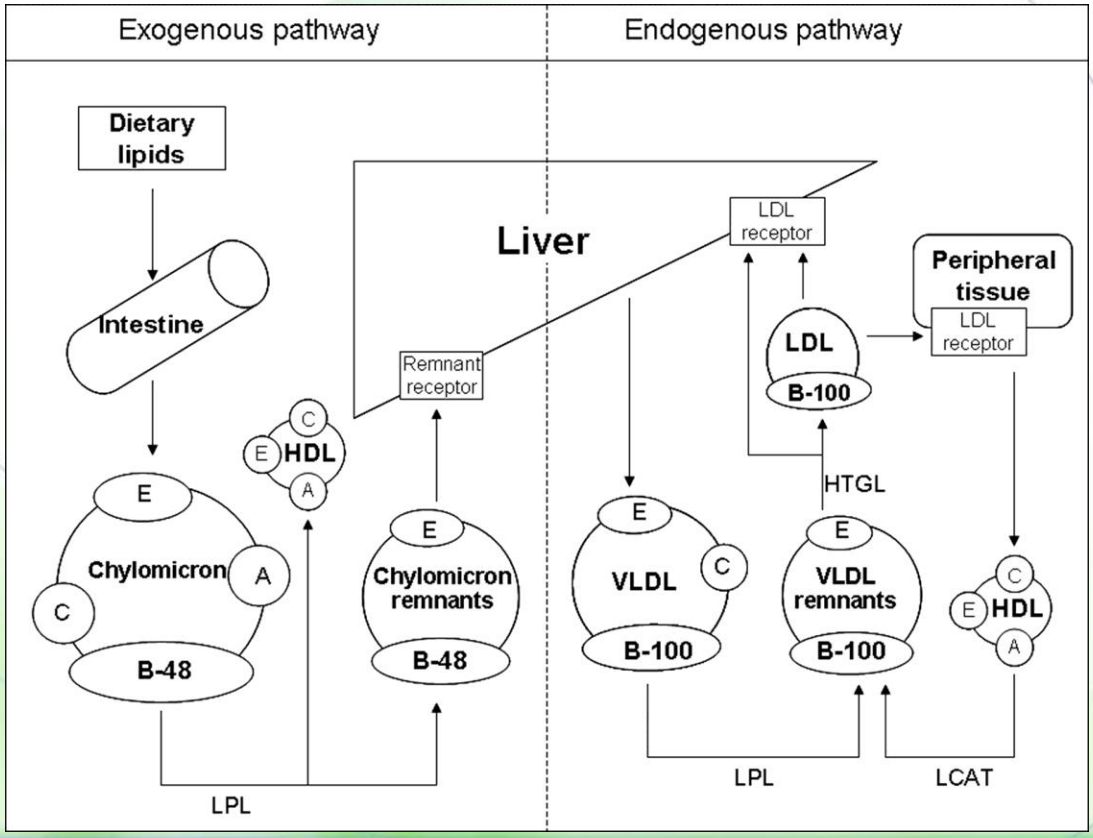


Also, remember…ferritin

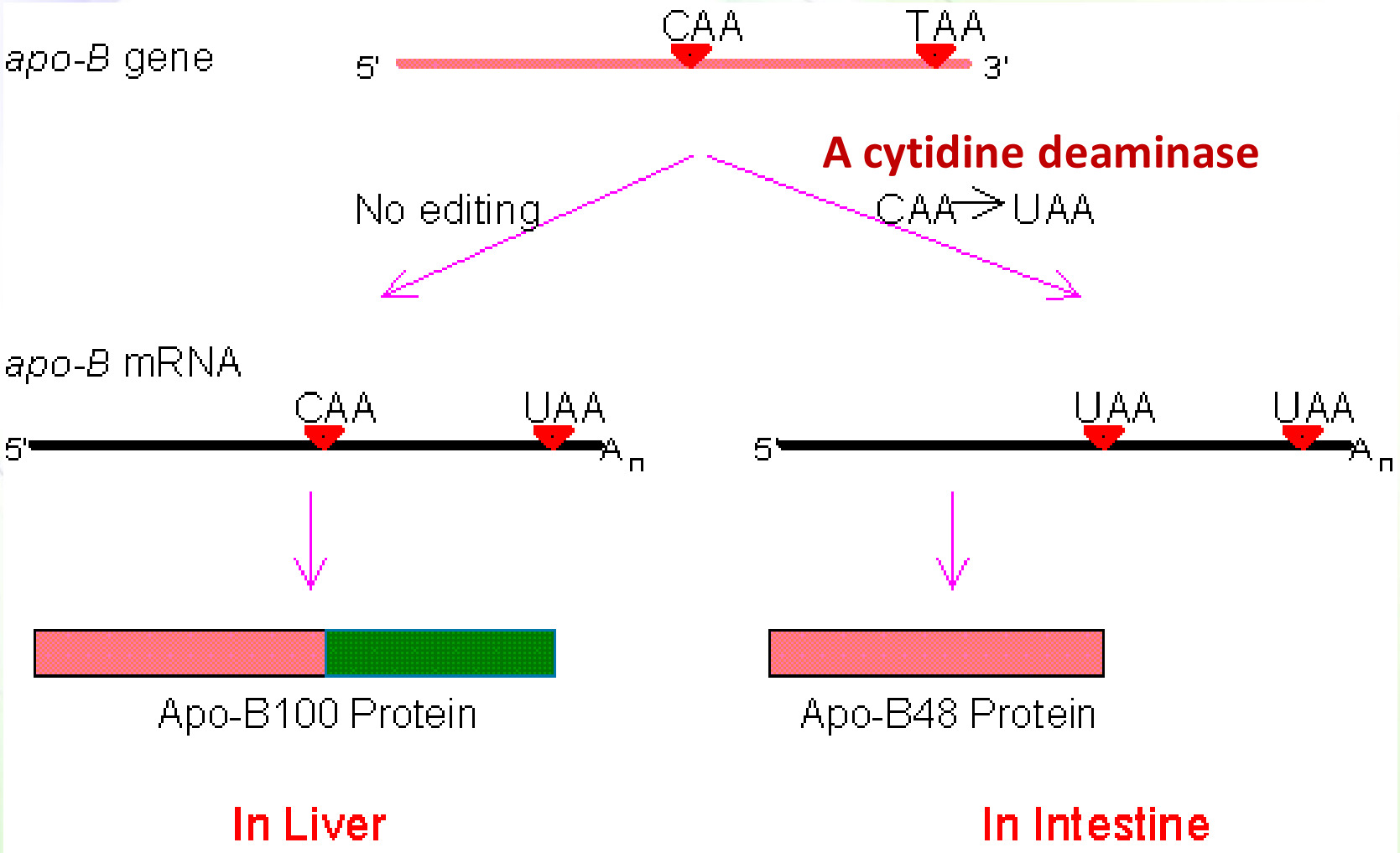


ApoB-100 vs. apoB-48

- 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.

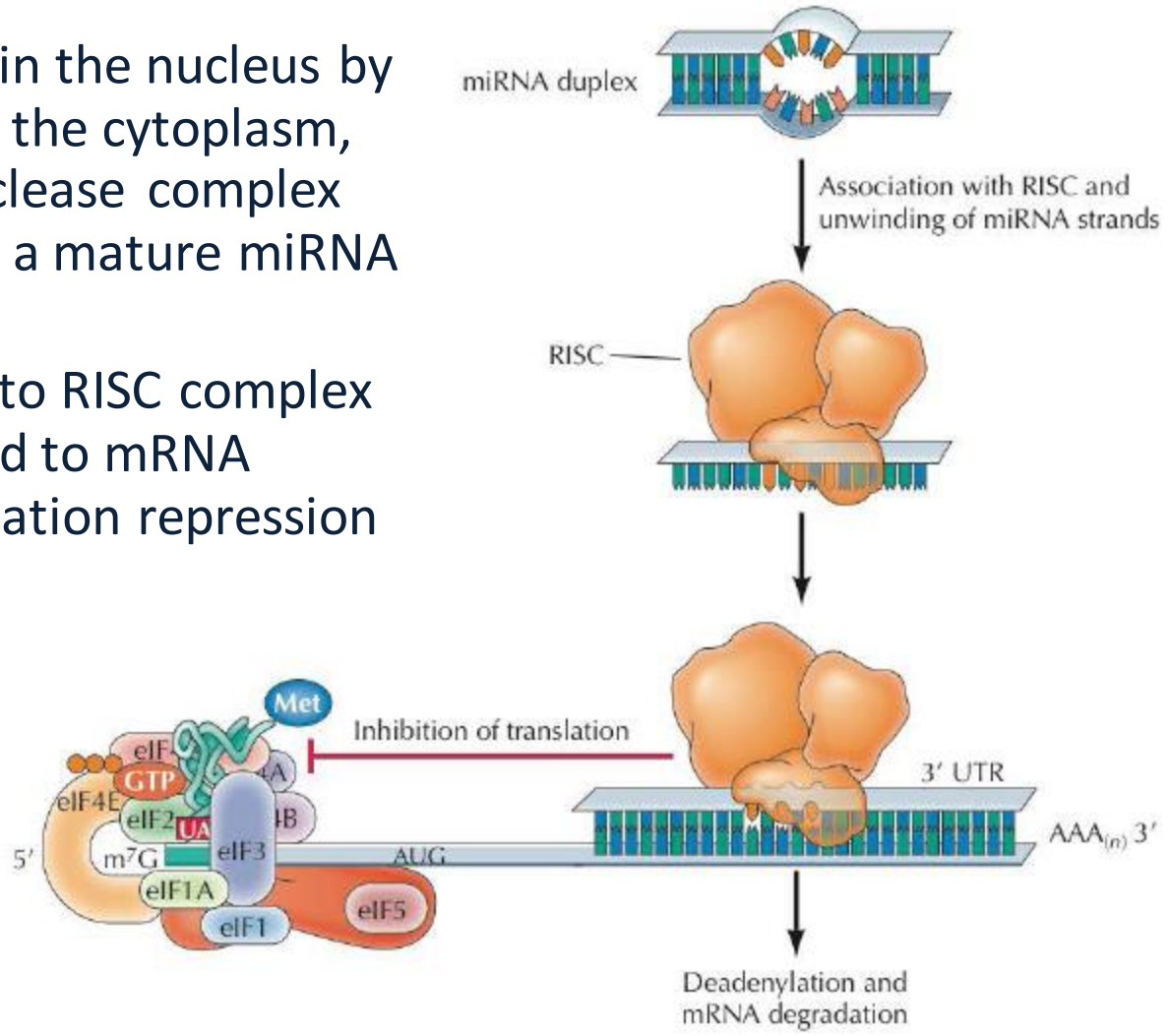


Gene editing

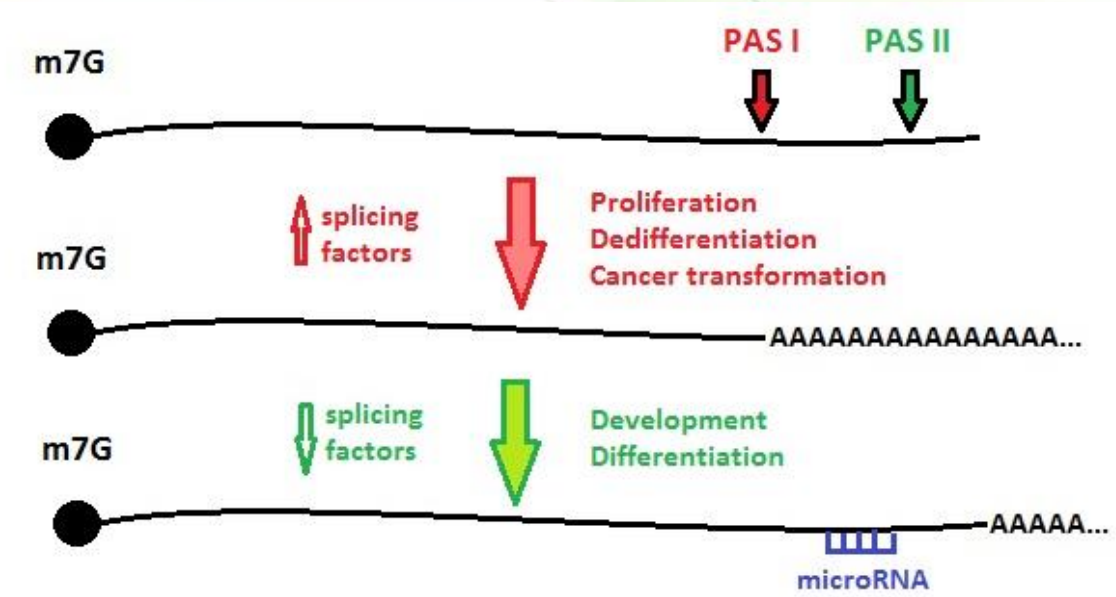


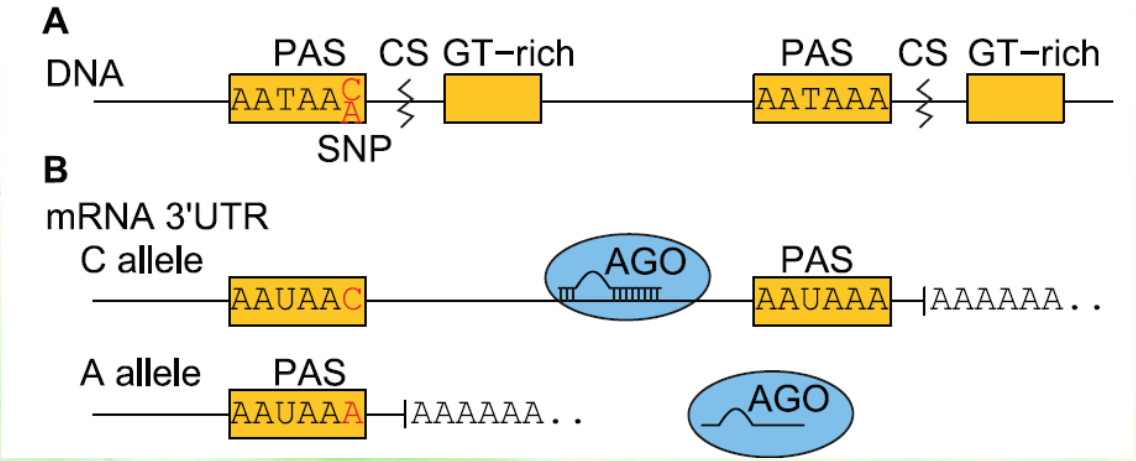
Regulation by microRNA (miRNA)

- 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 called 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.



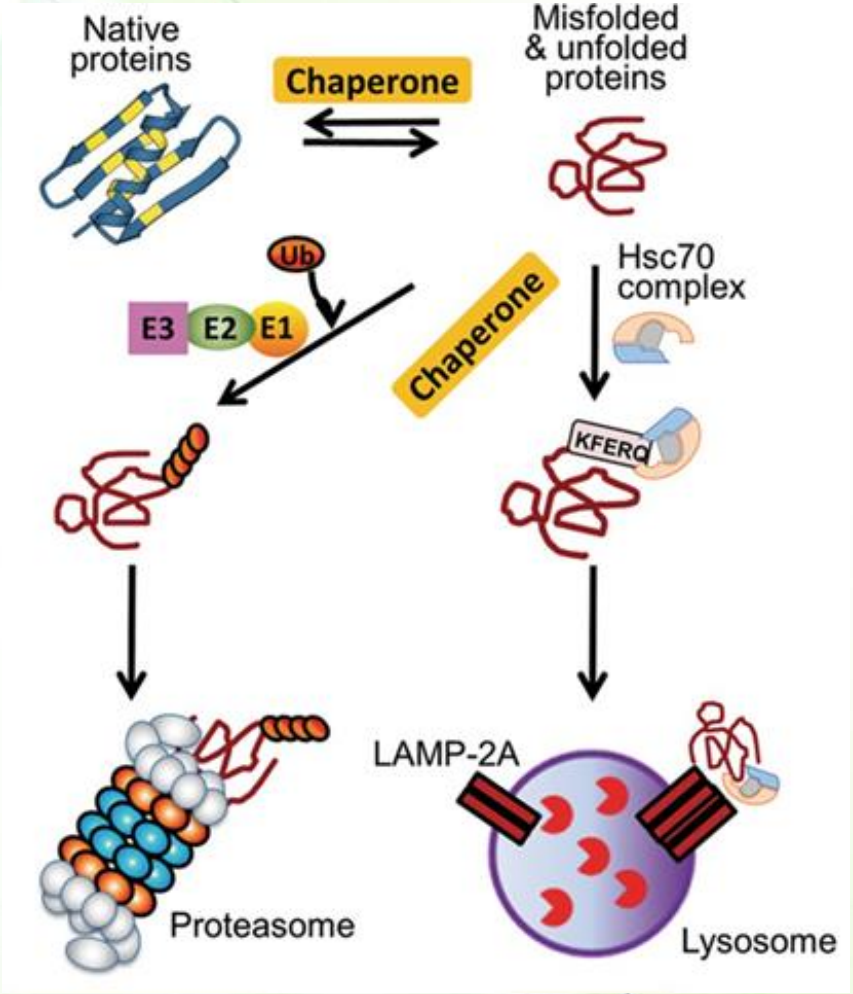
Alternative polyadenylation



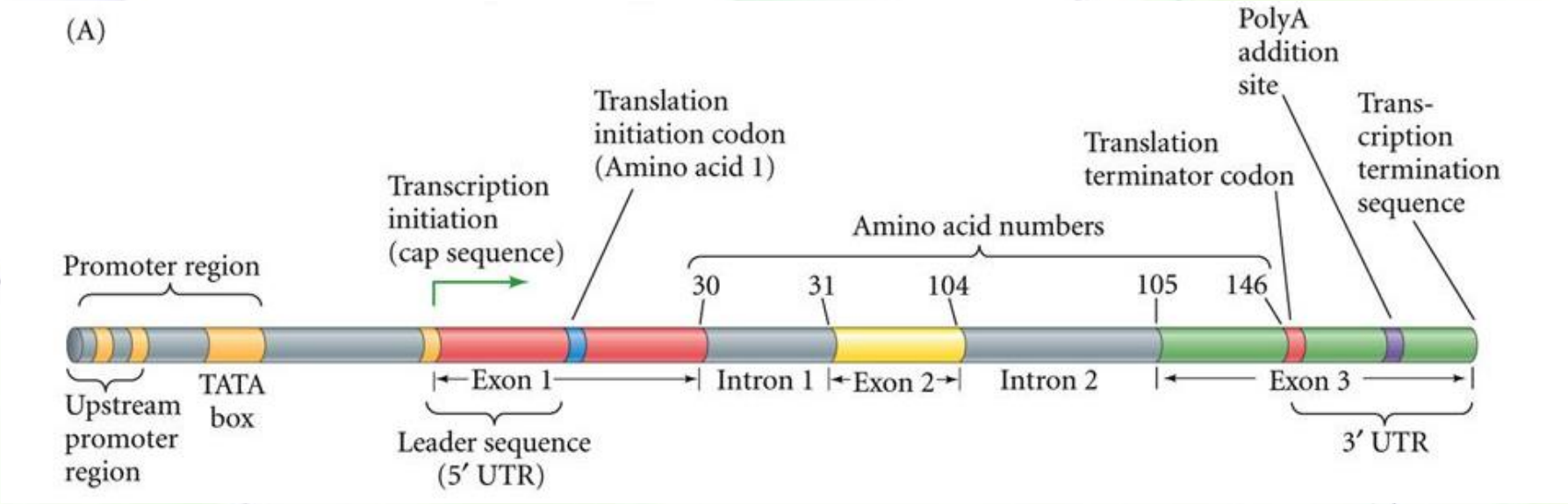


Fate of (mis)- and (un)-folded proteins

- 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.



Anatomy of a eukaryotic gene



Levels of regulation

- Transcription (cis- and trans-acting elements)
- RNA processing (splicing)
- RNA transport
- mRNA stability (degradation; miRNA)
- Translation (ferritin)
- Post-translational modification (phosphorylation, etc.)
- Protein activity (inhibitors)
- Protein degradation (e.g. ubiquitination)