Genetics
& Cell biology

◉ Sheet ○ Slides

Number

14

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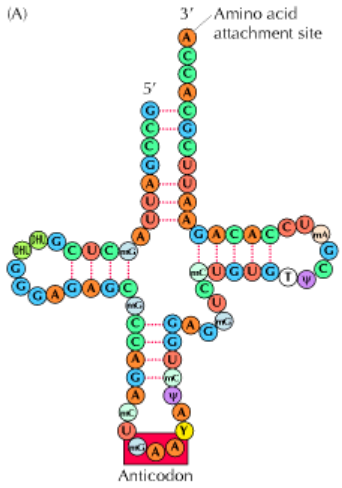
In this lecture we'll be talking about **translation**

- Translation: is the process of protein synthesis from messenger RNA (mRNA) We have three major components that are involved in translation in protein synthesis which are:
    1. tRNAs.
    2. rRNA, which exist in ribosomes (the factories of protein synthesis).
    3. mRNA templates.

1. **tRNAs**
    a. **tRNA strucrure**

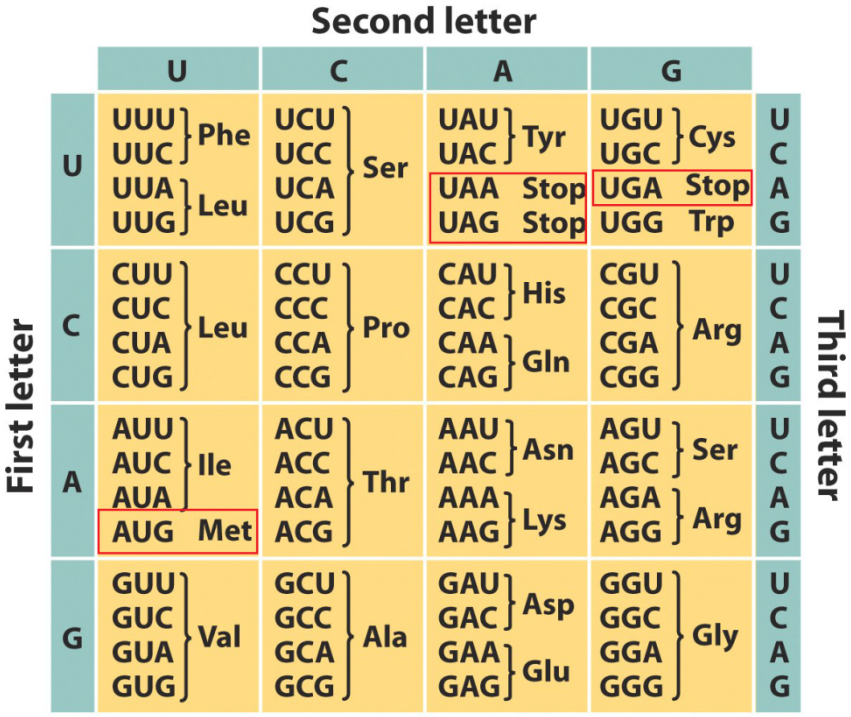


- tRNAs are short RNA molecules (80 bases long).
- It is activated or charged when it is associated with amino acid.
- The amino acid is linked to the 3' end of the tRNA. All the tRNA has the sequence "CCA" at the end and the amino acid is covalently attached to the ribose of the terminal adenosine at "CCA".
- **Aminoacyl-tRNA synthetase** determines which amino acid will be attached to a specific tRNA. What does these enzymes look for? In other words the amino acid attached to the tRNA is specified by:
    - ✓ Anticodon → it is a three nucleotide sequence, that pairs with the “codon’ or “triplet” mRNA molecules.
    - ✓ Identifier sequences → there are specific sequences in each tRNA and this enzyme can identify that this sequence is specific to a particular amino acid.

  Twenty Aminoacyl-tRNA synthetases exist for each amino acid.

    b. **Codon vs anticodon**

What happens during translation is that the tRNA **pairs** with the mRNA. The anticodon (it's located at the bottom of the tRNA) **pairs** with the codon (three nucleotides that specifies the amino acid). Notice that the base pairing between the tRNA (3' → 5') and the mRNA (5'→ 3') are **antiparallel alignment**. The doctor might bring a question with a structure of the tRNA giving us the anticodon and asking for the attached amino acid (he'll give us the table) so you should pay attention that the alignment between the tRNA and mRNA should be antiparallel.



- We have 64 codon specific to the amino acids.
- 3 of them are stop codons (stop codon is a nucleotide triplet within messenger RNA that signals a **termination of translation** into proteins) and there's no tRNA that has an anticodon to these stop codons.
  you have to memorize the sequences of the stop codons (UGA, UAA, UAG).
- AUG → code for Methionine, and usually it is the first codon that is read by the ribosome and the tRNA. You'll notice that in protein, in general, the first amino acid is the methionine but it's not always the case. you have to memorize the sequence.

c. Features of the genetics.

Regarding the genetic codon table:

How to read the codon table? You start with the first letter on the left side of the table for example "A" (the 3rd row) and then the second letter on the top of the table for example "A" (the 3rd row and the 3rd column) and then the third letter on the right of the table for example "G" (the 3rd row and the 3rd column and the last letter) → AAG → Lys

✓ We always have exceptions so it's **not universal**. Eukaryotic cells, Prokaryotic cells almost have the same system but there are exceptions in mitochondria and some bacteria, for example in the mitochondria AUA codes for methionine but in the cytosol codes for isoleucine.

✓ Notice in the table that there are several codons that code for a single amino acid, for example CUU, CUC, CUA, CUG codes for Leucine, **in general** the last nucleotide is flexible, because the base pairing between the tRNA and mRNA is flexible in a way so the interaction between the codon and the anticodon can permit other interactions allowing a phenomenon called **Wobble Base Pairing (degenerate codon):** the interaction between the third nucleotide of the codon with the first nucleotide in the anticodons can permit other interactions or addition of different amino acid, it is called degeneracy of the codon. So if we changed CUU to CUC we won't have dramatic effect.

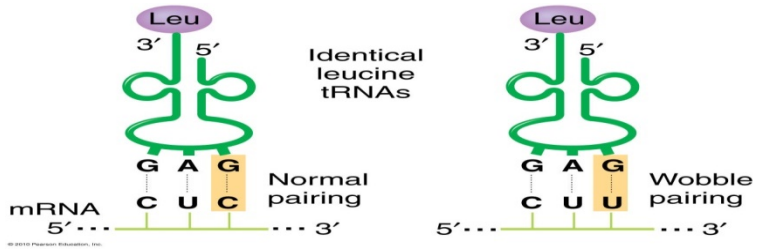
10 minutes

- In the first column in the table if we changed (mutated) the third base what will happen ?

It might be Phe or Leu or Ile or Met or Val. Notice that all of them are aliphatic, non-polar amino acids, so there's some kind of protection mechanism, if a mutation occurred in the 3rd base a great effect in the protein won't occur.

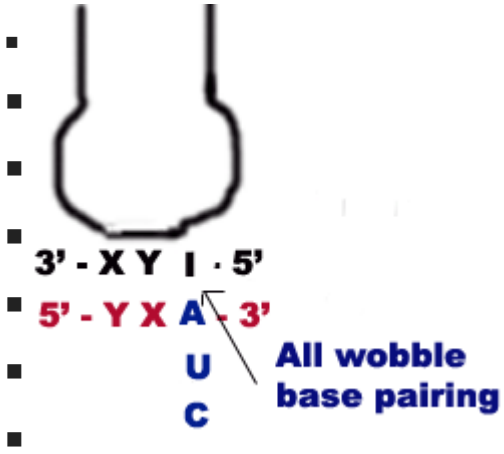
The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base, which is called the "wobble" base.

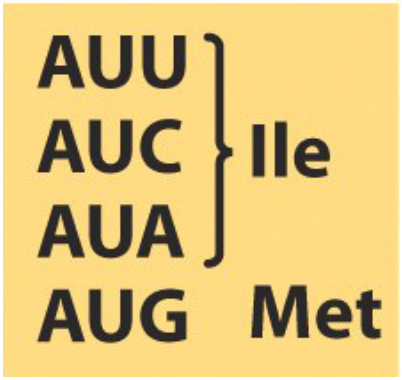
The degeneracy of the code acts as a buffer against deleterious mutations.



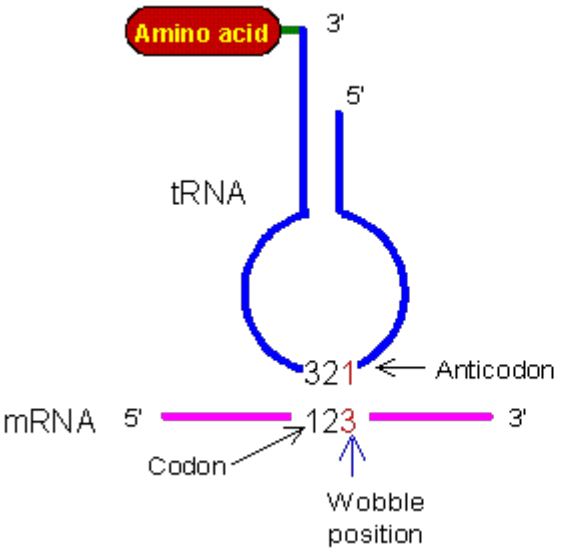
	U	C	A	G	
U	UUU, UUC } Phe	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr	UGU, UGC } Cys	U
	UUA, UUG } Leu		UAA Stop, UAG Stop	UGA Stop, UGG Trp	C, A, G

- Relaxed base pairing at this position results from the formation of G-U base pairs.
- Notice that the U & C always code for the same amino acid so if a mutation happened to the base C and changed it to U and vice versa → it will code for the same amino acid.





- 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.



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

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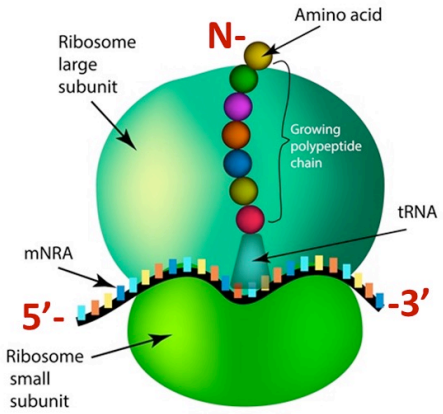
2. Ribosomes

a. Ribosomes



- Ribosomes are collection of rRNAs as well as proteins.
- Are composed of two units: the large ribosomal subunit and the small ribosomal subunit, they're both separated from each other but during initiation of translation they're attached to each other and form single complex.
- Ribosomes are the sites of protein synthesis in both prokaryotic and eukaryotic cells.
- To form a peptide bond between amino acids carried by the tRNA (chemical reaction) it requires an enzyme → **peptidyl transferase** it exist in the large ribosomal subunit, several experiments was made and it was discovered that no protein is responsible for the enzymatic activity so they noticed that the ribosomal RNA acts as an enzyme (the RNA that are capable of acting as an enzyme are called **ribozymes)** and catalyze the peptidyl transferase.
- 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.

b. Ribosomes structure

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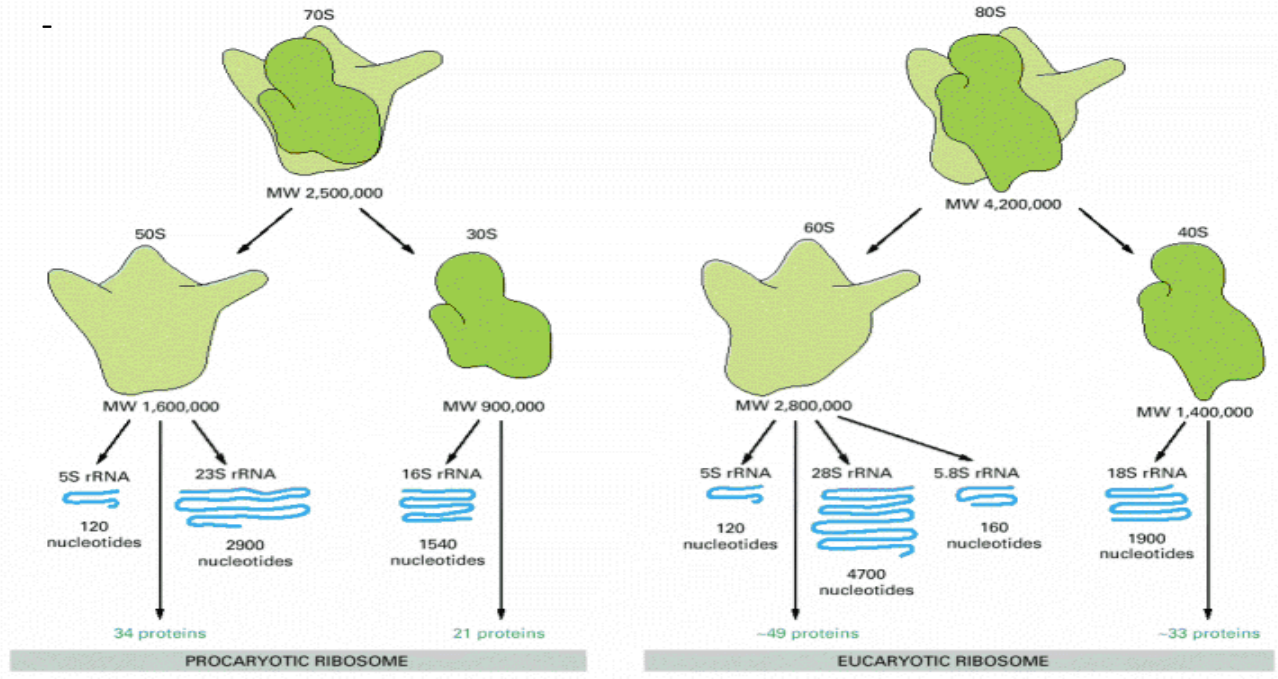
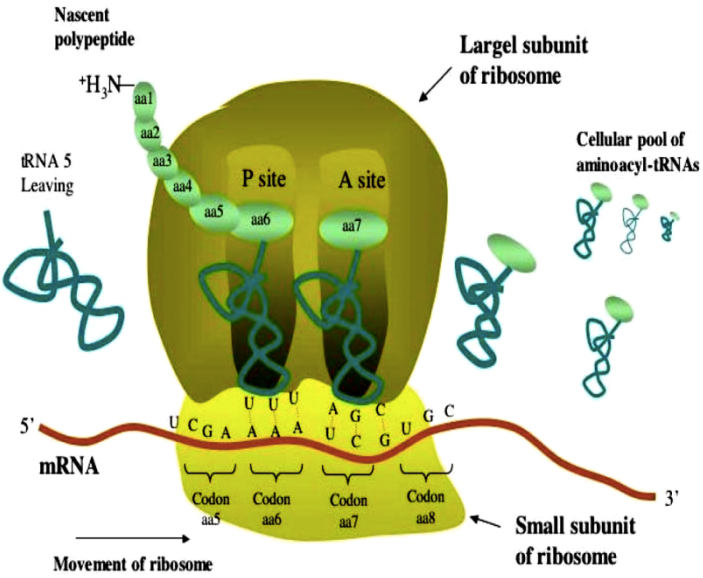


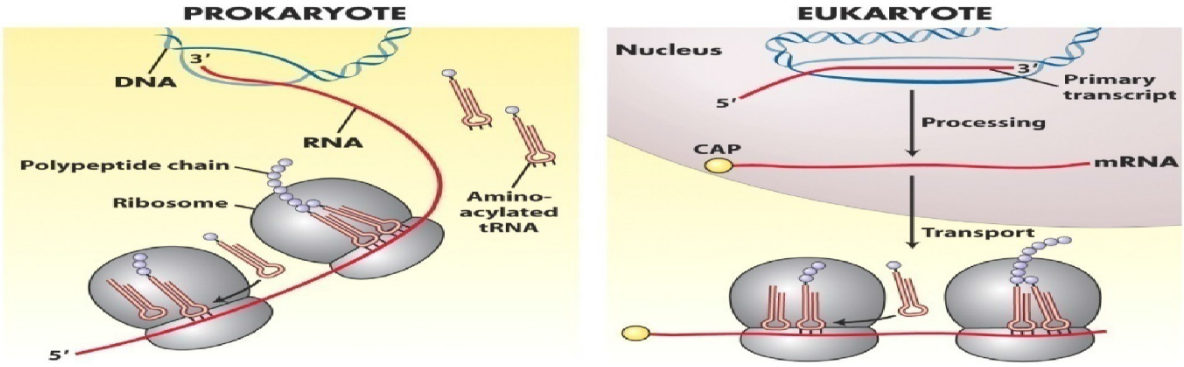
- Prokaryotic and Eukaryotic ribosomes are almost identical→ it tells us that it is an important molecule that should not be changed throughout evolution.
- Large subunit and small subunit are made of a lot proteins as well as ribosomal RNA molecules.

c. General mechanism of translation



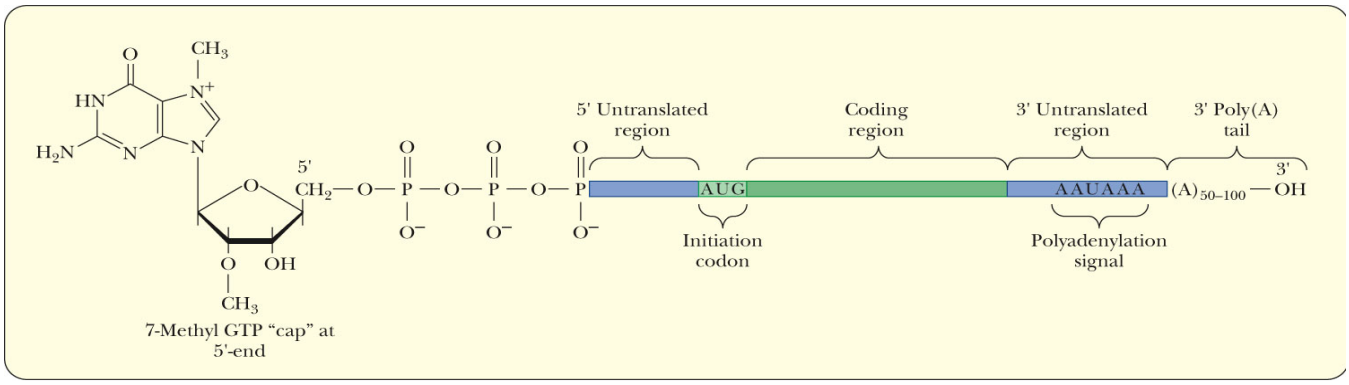
- Is divided into three stages: initiation, elongation, and termination.
- The direction of reading mRNA is 5' → 3'.
- Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus. First amino acid is methionine.

d. Transcription/translation coupling



- This actually works out well for the bacteria because transcription and translation in bacteria are coupled, occurs at the same time since they don't have nuclear membrane to separate the transcription and translation processes. As the bacterial cell is transcribing RNA, the RNA that is synthesized, the ribosomes comes and start synthesizes the poly peptide from the mRNA. Transcription and translation are coupled in space and time.
- In eukaryotic cells mRNA is synthesized in the nucleus and it processed, capped, polyadenylated, spliced → mature mRNA → move to the cytosol → translation.

e. Start of translation

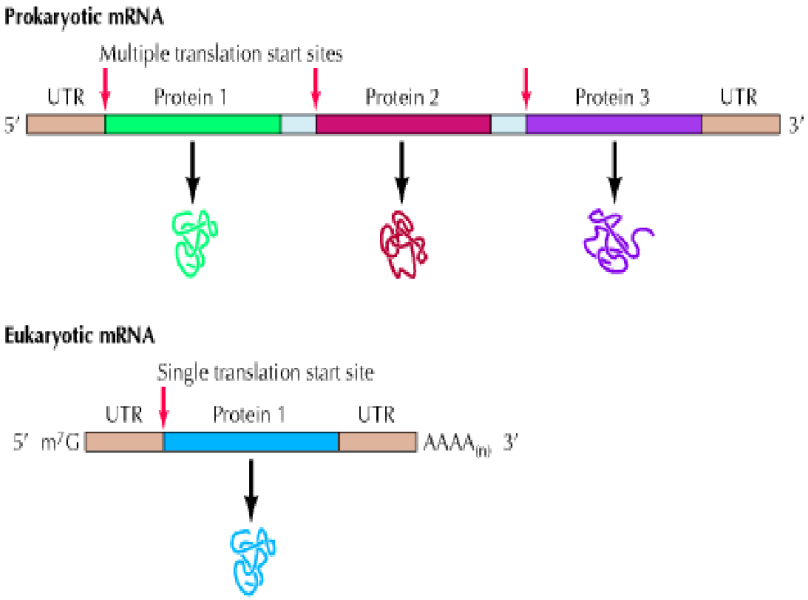


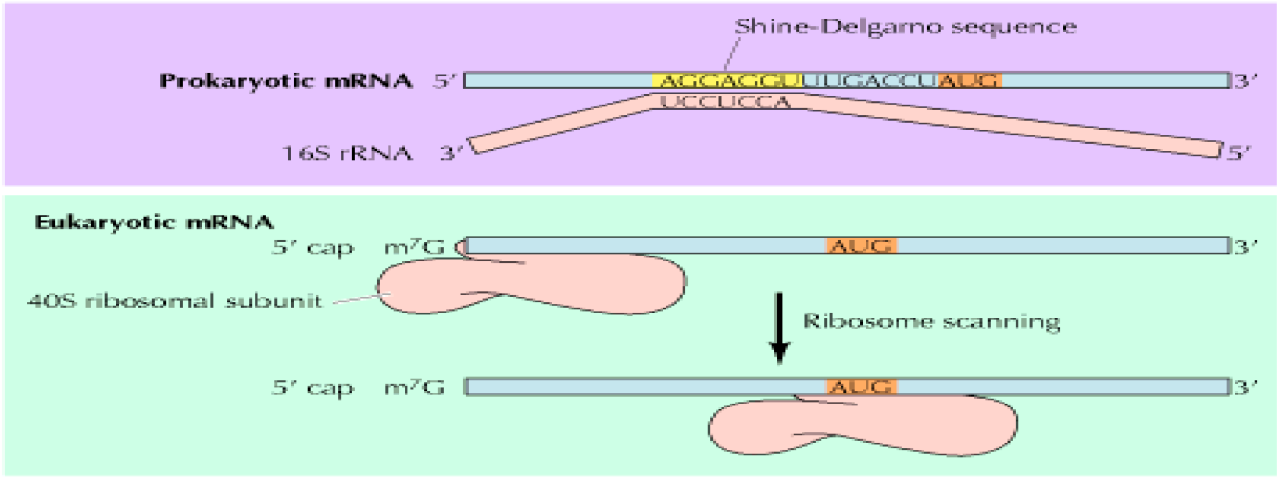
- In both prokaryotes and eukaryotes, translation starts at specific initiation sites, and not from the first codon of the mRNA. The first codon to be read → **AUG** which codes for methionine. So the ribosomes scan the mRNA until it reads the AUG and then start synthesis, so there's region that is not translated and not used for protein synthesis.
- 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)** which is transcribed but not translated.
- Ribosomes continue reading the codons until they reach a stop codon and it's not usually the last codon in mRNA in the 3' end so we will stop translation and we'll have a region called **3'-untranslated region** that is transcribed but not translated.

20 minutes

- Those regions (untranslated regions) might contain regulatory sequence which are Iron binding protein and it is attached to the 5' untranslated region and 3' untranslated region.

- **Remember** Bacterial mRNA is **polycistronic** (one mRNA from different part I can generate different polypeptide) and Eukaryotic mRNA is **monocistronic** (one mature mRNA can generate one polypeptode).



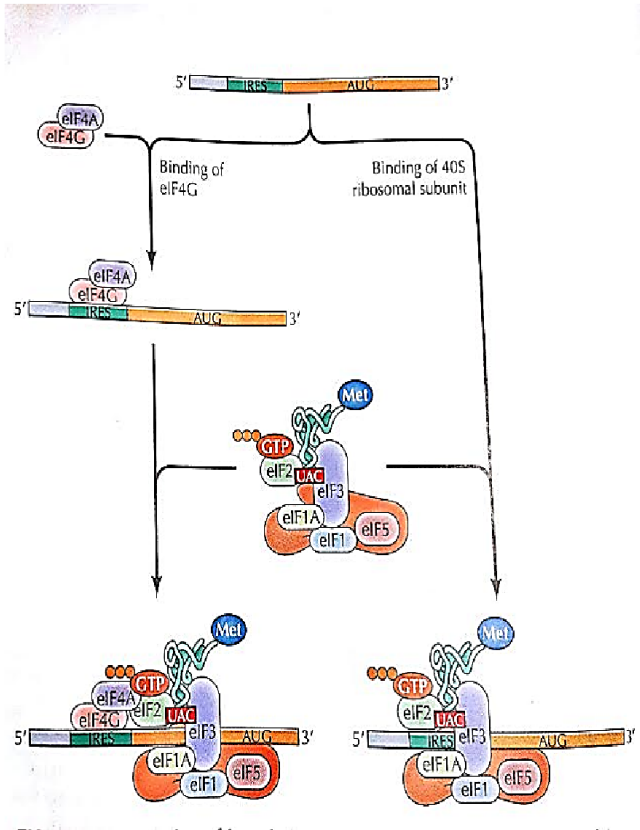


In prokaryotic:

Shine-Delgarnio sequence: this sequence precedes the AUG sequence, the ribosomes attaches to the 5'end and scan the mRNA and when it recognize the Shine-Delgarnio sequence ,the sequence is not for memorization, 16s rRNA which exist in the small ribosomal subunit recognize it and it hybridize it (binding of the small ribosomal subunit and Shine-Delgarnio sequence on the mRNA) and then the large ribosomal subunit attaches and search for the AUG sequence to start translation. If there's AUG but there's no Shine-Delgarnio sequence → no initiation of translation. That's why the same mRNA is used to synthesize different polypeptides.

In eukaryotic:

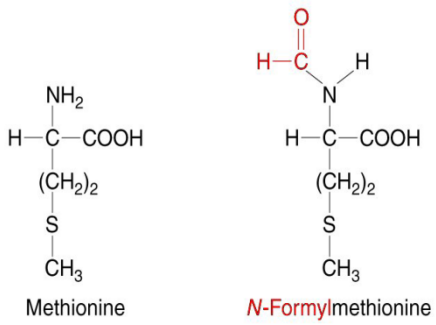
First mechanism: There're two processing in eukaryotic and one of the processing is the addition of cap (inverted guanosine), small ribosomal subunit recognizes mRNAs by binding to the **7-methylguanosine (inverted guanosine) cap at their 5´ terminus** and then start searching for the first AUG sequence following the cap.
Second mechanism: there's a specific sequence before the AUG called **internal ribosome entry site (IRES)** is recognized either by the 40S ribosome alone or initiation factor number 4 (eIF4G) protein followed by recruitment of the 40S ribosome. For memorization.



In prokaryotic **IF#** → stands for initiation factor
In eukaryotic **eIF#** → e stands for eukaryotic ,IF→ initiation factor, # → number

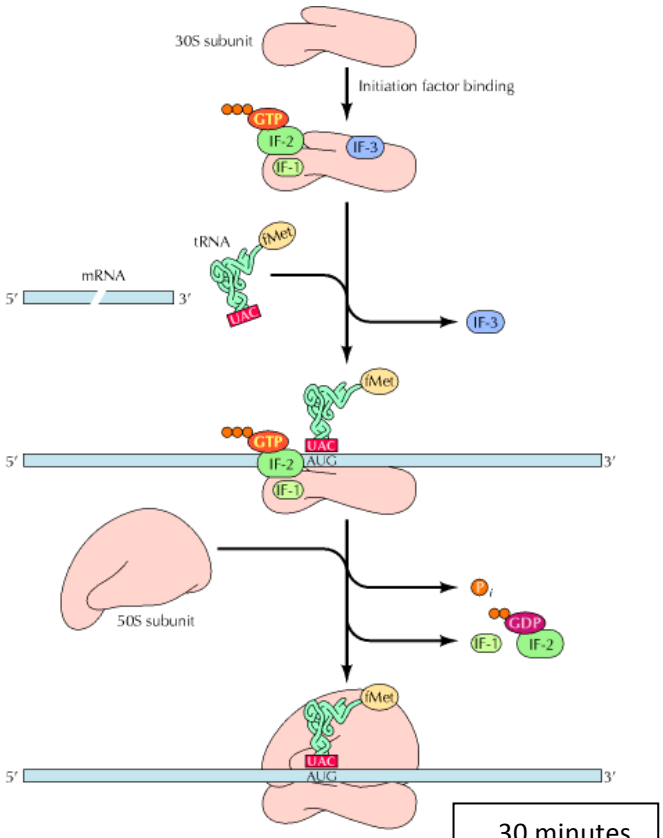
f. The first amino acid

Translation always initiates with AUG which codes for the amino acid **methionine**. In most bacteria, AUG codes for modified methionine → **N-Formylmethionine**.



g. Initiation of translation in prokaryotic cells.

- The 30S small ribosomal subunit binds to mRNA and fmet-tRNA (formyl-methionin-tRNA) in the presence of GTP (provide energy) 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.
- When the body needs energy in general for metabolism → ATP, protein function → GTP, lipid regulation → CTP, sugar activation or metabolism → UTP.



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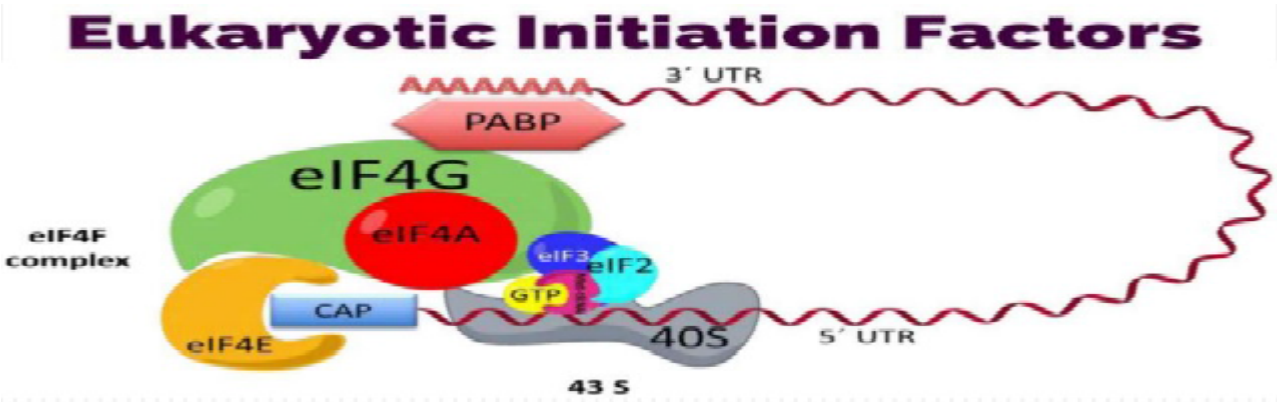
h. Initiation of translation in eukaryotic cells



- We have a lot of initiation factors in eukaryotic cells but we will concentrate on the initiation factor **eIF4G,** it binds to poly-A binding protein (PABP), which

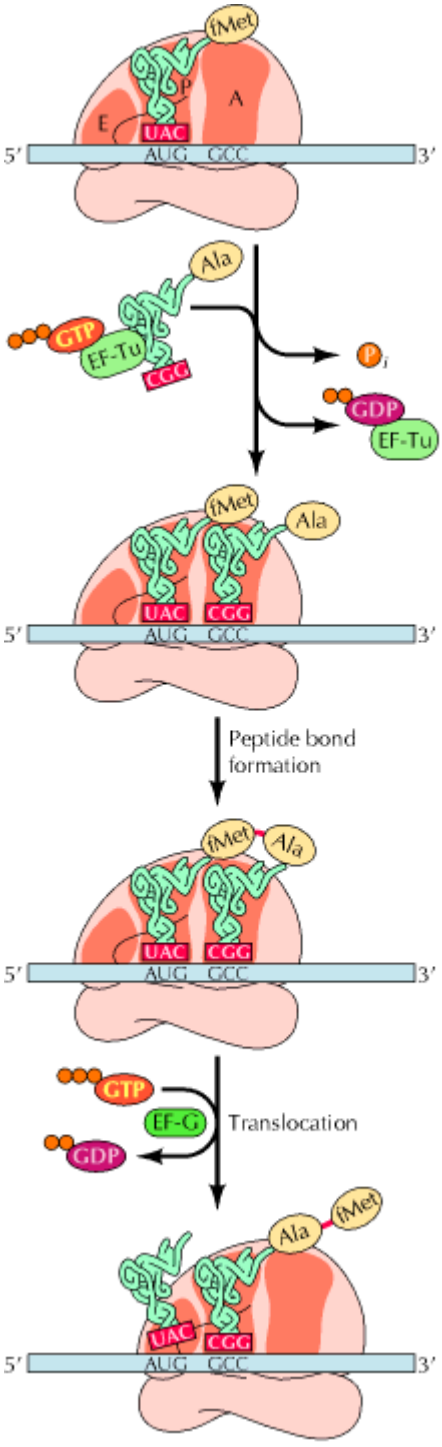
binds to the poly-A tail, **eIF4G** again bind with **eIF4E**, which recognizes the cap so it links between the poly-A-tail and the cap.

- The poly A tail stabilizes the mRNA and helps in the process of translation, it is important to recognize that a specific mRNA needs to be translated.

i. Translation elongation in prokaryotics

Large ribosomal subunit has 3 sites where the tRNA molecules bind to and they're called chambers.

- A chamber(stands for aminoacyl).
- P chamber(in the middle and stands for peptidyl transferase)
- E chamber (the smallest and stand for exit)
-



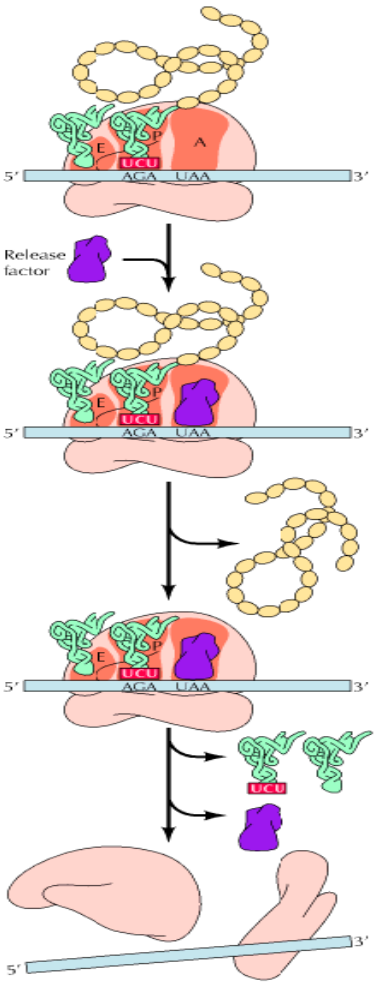
So the first tRNA (lets call it tRNA1) carries the f-met coded by AUG (chamber P), the next tRNA (tRNA2) carries the amino acid (aminoacyl-tRNA) where you have an anticodon that is complementary to the codon (chamber A), those two are next to each other in chamber P and A. Again Elongation factor EF-Tu (Tu) and GTP are required, these elongation factors help in recognizing elongation and they provide energy for the chemical reaction, Elongation factor EF-Tu is released from the ribosome and regenerated, what happens is the f-met jumps on to the other amino acid and form a peptide bond so what happens now is tRNA2 carries two amino acid, and tRNA1 is uncharged. Then translocation happens and the ribosomes moves 3 bases (one codon) until the AUG arrives to the E chamber and the tRNA1 (uncharged) is now in the E chamber to be released, and the tRNA2 which was in A chamber will move to P chamber . A→P→E

.

And then another tRNA3 that has anticodon complementary to the codon enters the A site→ peptidyl transferase reaction in the tRNA2 in the P site and the two amino acid will jump from tRNA2 to tRNA3 located in the A site → translocation etc… continue translation until it reaches a stop codon.

NOTE that there's nothing called tRNA1, 2, 3… I added the numbers to make it easier

There's no tRNA that has anticodon complementary to the stop codon (UAA, UGA, UAG) so the stop codons are not recognized by the tRNA but a protein known as **a release factor** recognizes the stop factor so it binds to A site and blocks the binding of a new aminoacyl tRNA and facilitates the hydrolysis of the bond between the carboxyl end of the peptide and the tRNA and dissociate the mRNA, large rRNA and small rRNA.



35 minutes

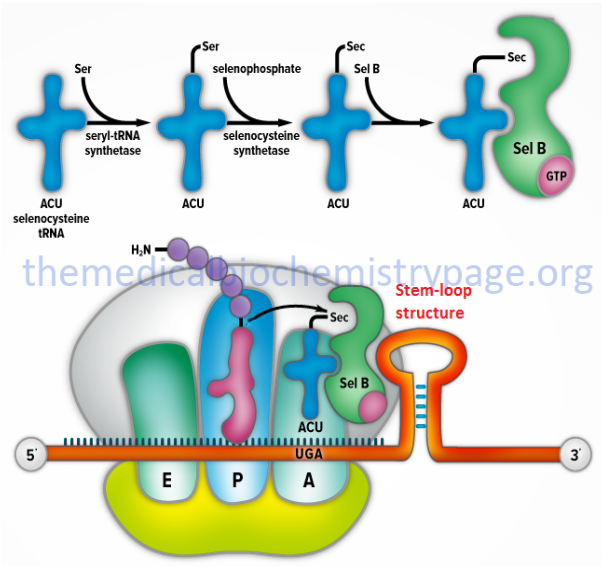
j. Selenocystine

- Is known as the 21st amino acid, it enters in some protein structures in eukaryotic cells.

Selenocysteine

There's specific tRNA that carries serine and this serine is transferred into selenocystine. (The oxygen of a serine, which is bound to a special tRNA molecule called tRNAsec, is replaced by selenium).

- When it reaches a stop codon that is followed by stem loop structure, a specific protein known as **sel B ;** it recognizes the stop codon and the stem loop structure and won't allow stop translation and it will add selenocystine and continue translation. (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 selenocysteine tRNA is loaded into the A site and translation continues).



k. 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.

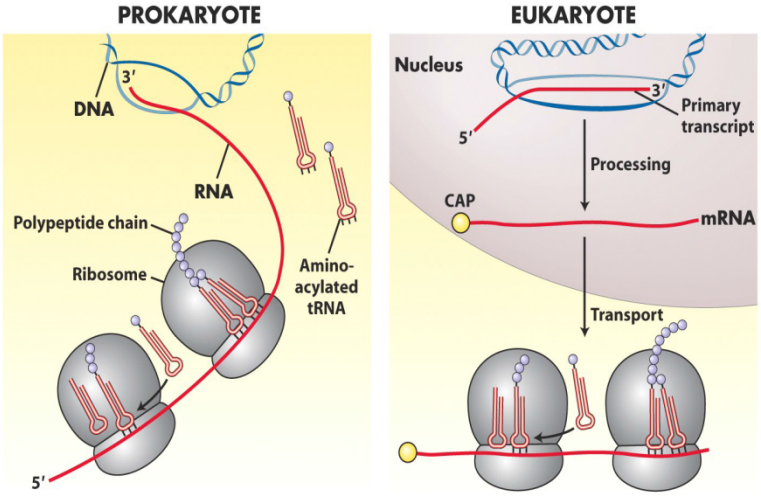


Figure 30-15
Biochemistry, Sixth Edition
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Multiple ribosomes sitting on the same mRNA as the mRNA is being synthesized (polypeptides aren't clear in the picture)

l. Inhibitors of translation

Common antibiotics that target the translation process in bacteria but not eukaryotic.

Inhibitor	Specific effect
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome (f-met tRNA is there but aminoacyl tRNA will be blocked)
Streptomycin	Induces binding of wrong t-RNA-AA complexes resulting in false proteins (protein is not functional and the bacterial cell dies)
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (there's aminoacyl-tRNA in the A site and waiting for the f-met(amino acid) to jump from the P site to the A site but it's not happening since it blocked the peptidyl transferase)
Erythromycin	blocks the translocation reaction on ribosomes (inhibition to the elongation factor that helps in ribosomal movement along the mRNA)

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

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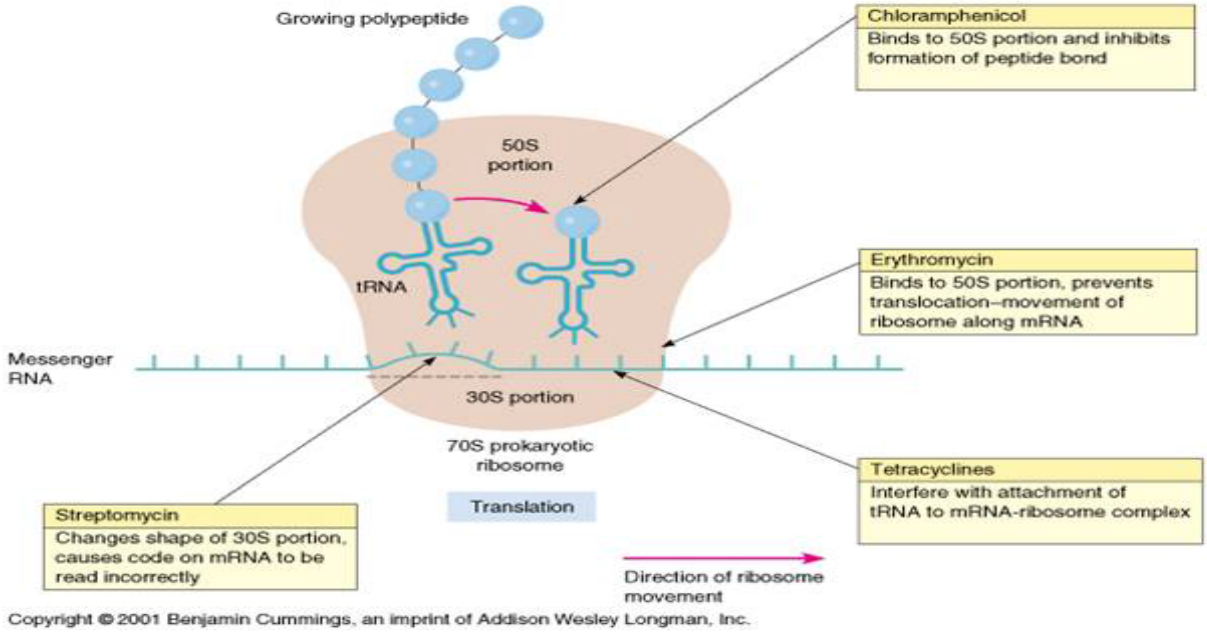
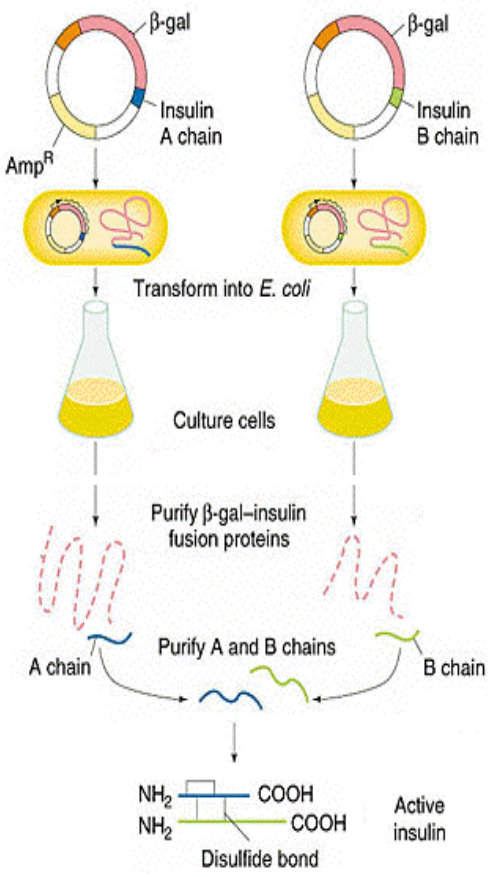


j. Benefits of cloning

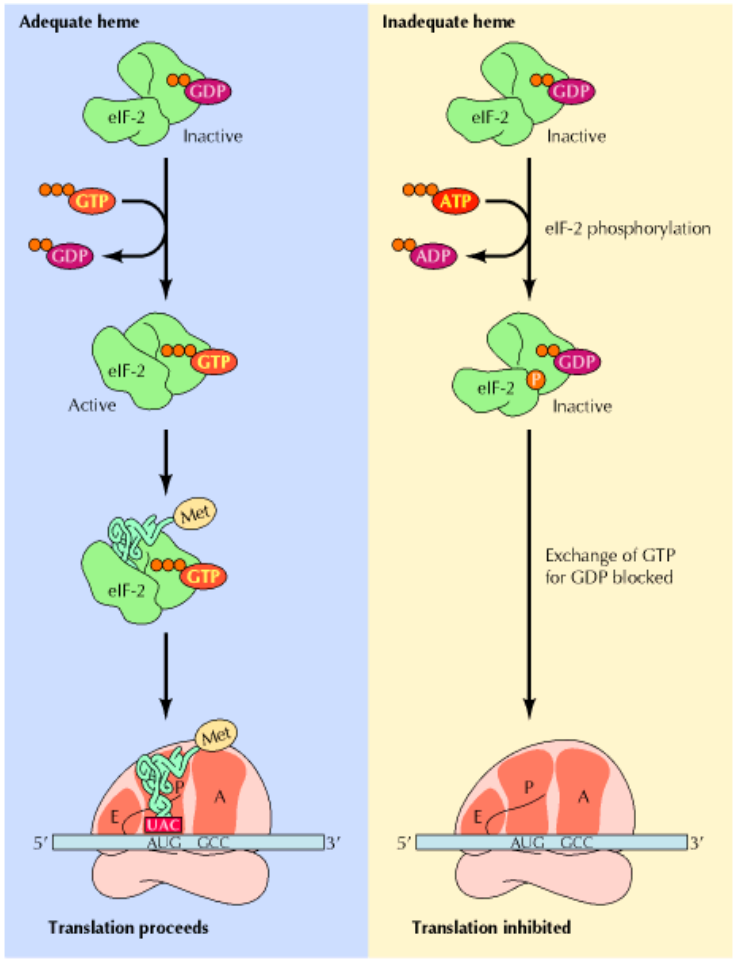


- We can use plasmid to synthesize eukaryotic proteins in bacteria
- like when we synthesize artificial insulin in the bacteria
- Insulin is a dimer linked by disulfide bonds and produced from genes containing introns.
- synthetic DNA is made for each polypeptide and inserted into bacteria separately.
- we add the synthetic insulin gene without introns, we add the alpha chain and beta chain under the control of promoter in the plasmid → the promoter is always active and it will give us the alpha and beta subunit →purification to the alpha and beta subunit → combine them → forming active insulin
- Growth factors is another example

k. Heme and protein synthesis

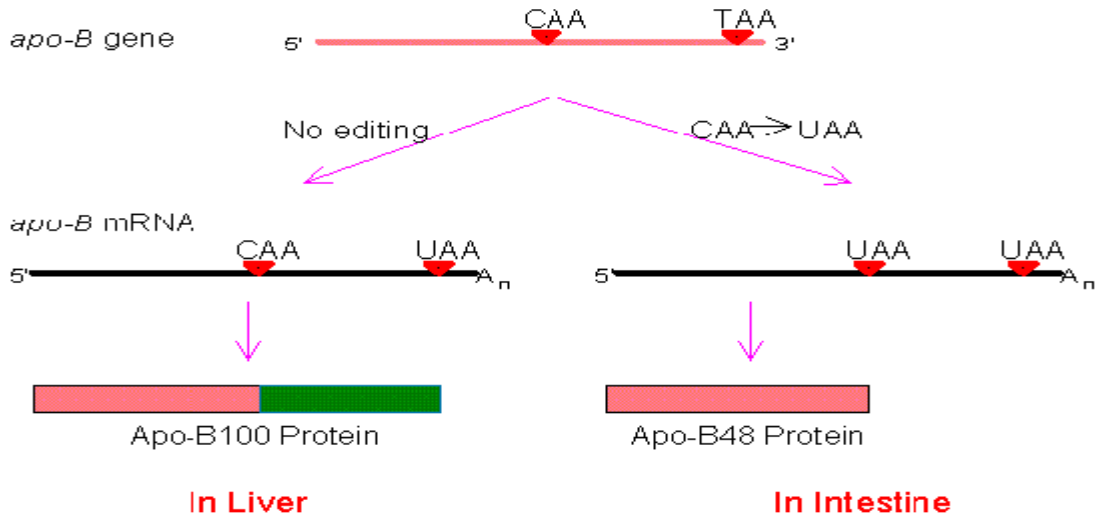
- In reticulocytes (immature erythrocytes)→ protein synthesis →they synthesize hemoglobin (heme + globin protein).
- Regulation of translation :

- The mRNA is translated only if **adequate heme** is available to form functional hemoglobin molecules.
- 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.
- If adequate heme is available, GDP-GTP exchange occurs and translation is able to proceed.
- If there's **inadequate supply of heme** in the cell, a certain protein kinase will phosphorylate the initiation factor inactivating it, the phosphorylated initiation factor won't be able to detach from the GDP and will not be able to exchange GDP for GTP so the translation is inhibited until there an adequate heme is supplied to the cell which will lead to lowering the kinase activity.



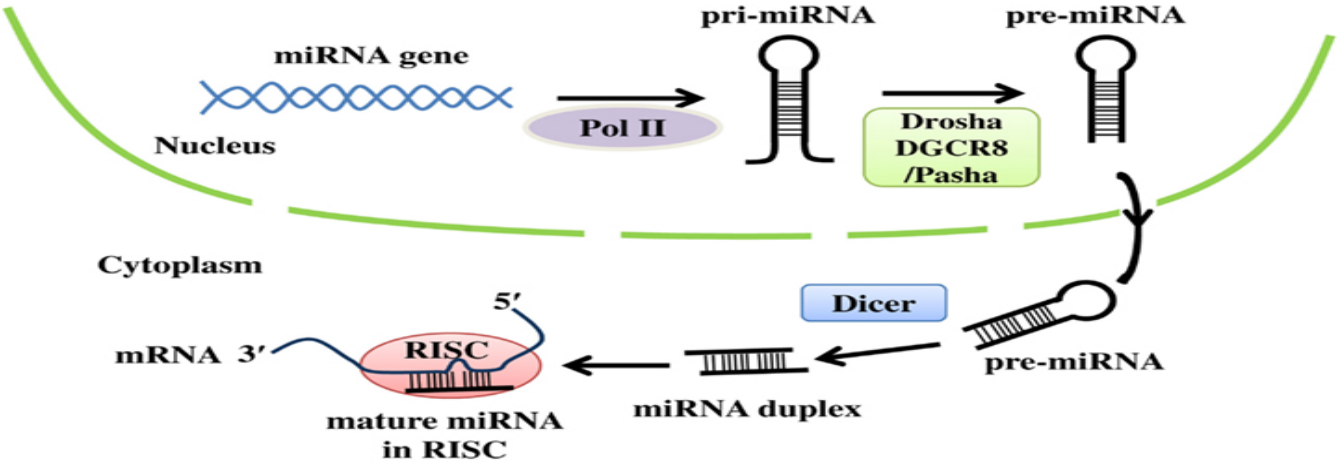
l. Apo-B100 vs Apo-B48

- These proteins make up specific lipoprotiens that are responsible for lipid transport.
- Apo-B100 is a liver proteins that is part of low-density lipoproteins and it is 100 kilo Daltons.
- Apo-B48 is an intestinal proteins that is part of chylomicrons and it is 48 kilo Daltons and shorter.
- Both proteins are synthesized from the same gene.



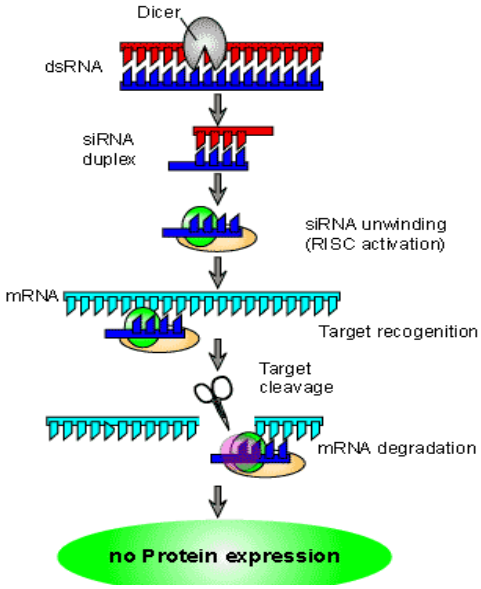
In liver:
Apo-B gene has CAA → mRNA has CAA → large protein →Apo-B100 protein
In intestine:
Apo-B gene has CAA→ **gene editing** → C is deaminated and transform to U → CAA wil be UAA which is a stop codon → shorter protein → Apo-B48

m. microRNA (miRNA)



- MicroRNA is synthesized by RNA Pol II into single-stranded, primary miRNA (pri-miRNA) transcript. (hair pin like structure)
- 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.
- Dorsha → processing, Dicer→ double stranded miRNA
- One strand is loaded onto **RISC complex** where miRNA is targeted to mRNA resulting in either translation repression or mRNA degradation.

- **siRNA** → short interfering RNA, the difference is they are synthesized as double stranded RNA molecules, they could be natural or artificial, same process as miRNA happens to it. where it is processed by Dicer and escorted by RISC to perfectly bind to complementary mRNA and induce mRNA degradation.



n. 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.

o. What make us human?

- We have the same genes as mice, drosophila, yeast etc..
- Different level of regulation is what makes us… us.
  - ✓ Transcription
  - ✓ RNA processing
  - ✓ RNA transport
  - ✓ mRNA stability
  - ✓ Translation
  - ✓ Post-translational modification
  - ✓ Protein activity
  - ✓ Protein degradation

Being human is given, but keeping our humanity is a choice.

خلقنا أناس, ولكن الحفاظ على إنسانيتنا هو خيار.