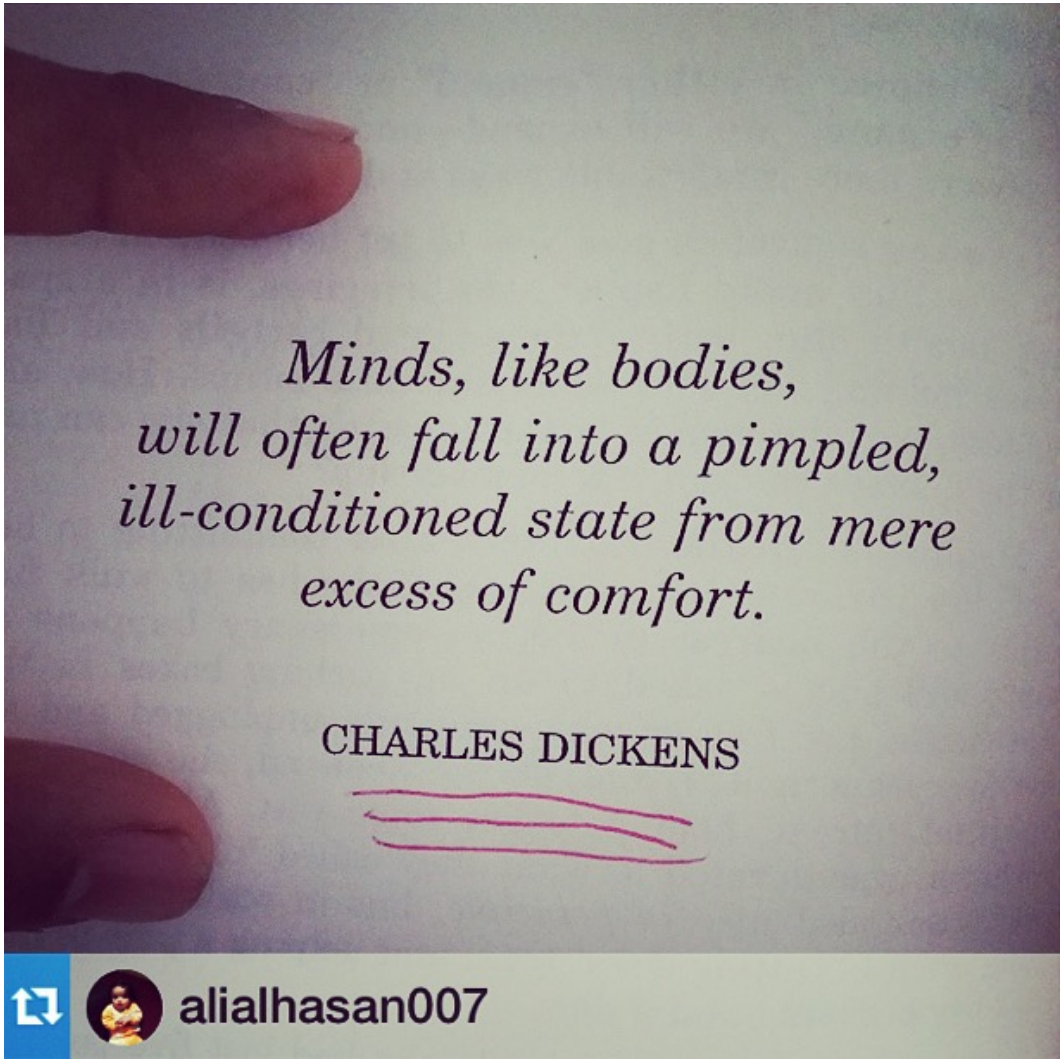
Lecture 18:

Translation in eukaryotes

Course 281

Lessons for life



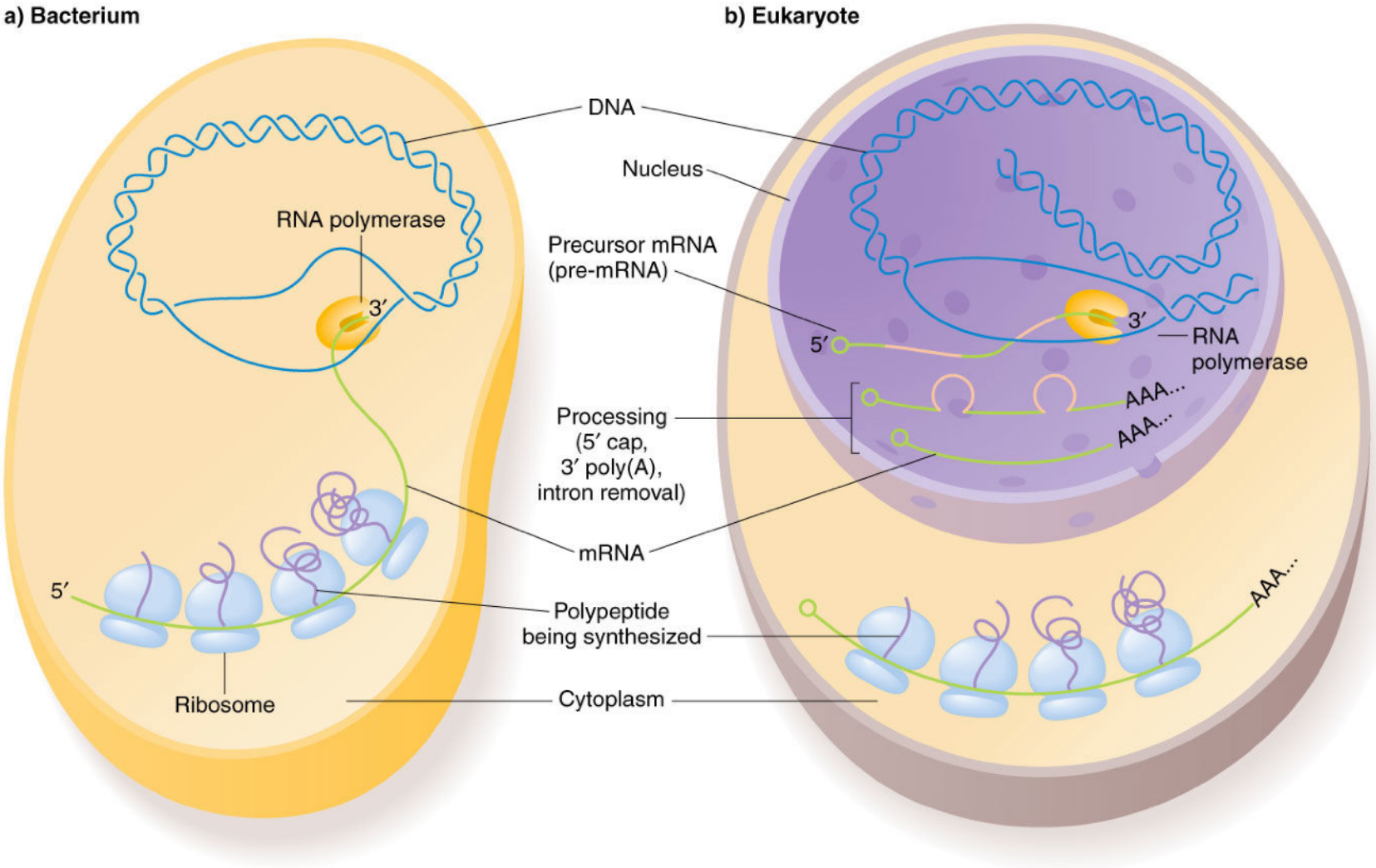
AIMS

- Understand the process of translation in eukaryotes.
- Understand the molecular requirements to translate a eukaryotic mRNA into a protein.
- Understand the sequence of events in eukaryotic translation.
- Understand the differences between prokaryotic and eukaryotic translation.

Summary and review

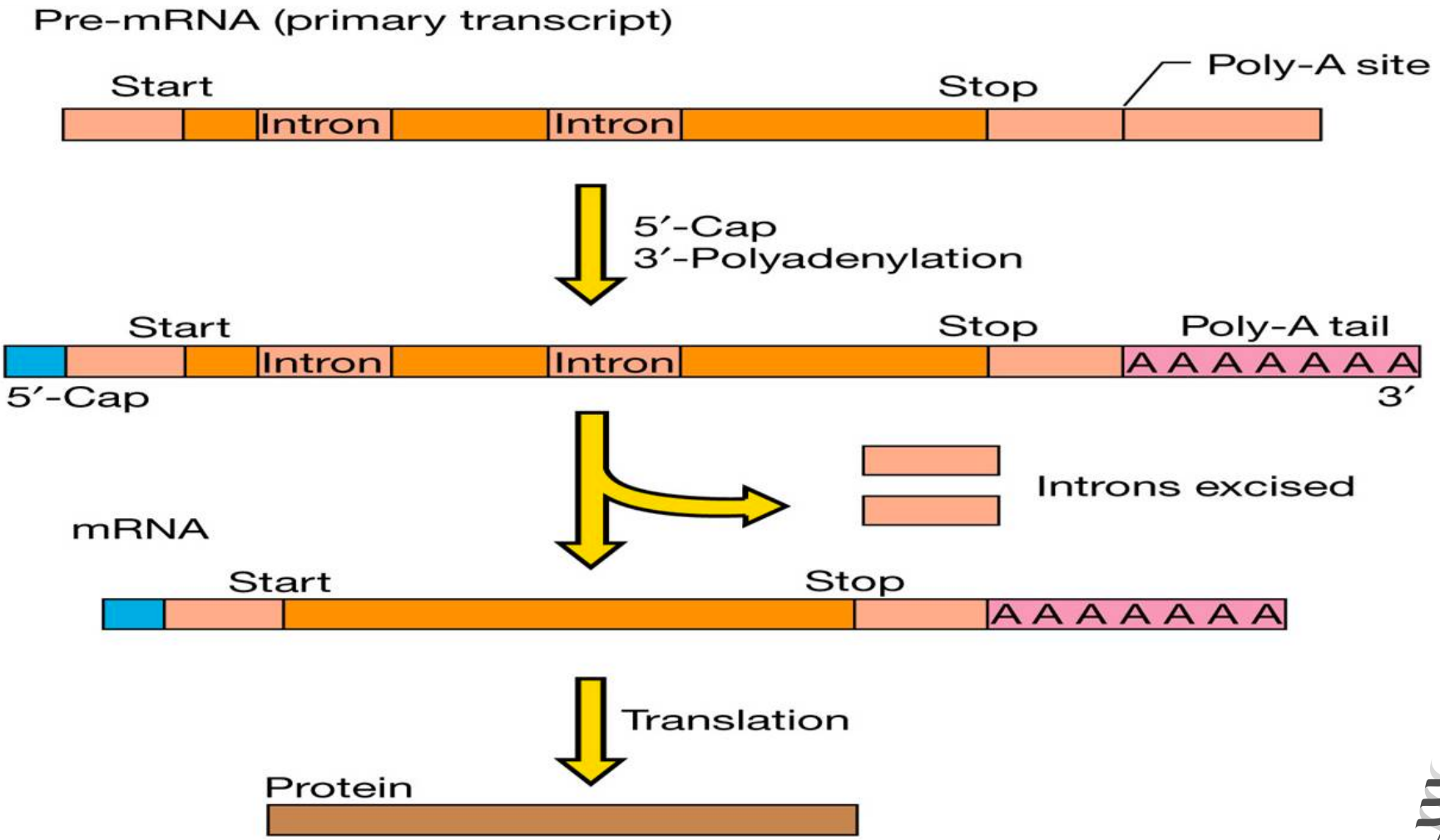
Prokaryotes	Eukaryotes
No nucleus	Nucleus
DNA in cytoplasm	DNA in nucleus
Transcription in cytoplasm	Transcription in nucleus
Translation in cytoplasm	Translation in cytoplasm
Polcistronic transcripts (one transcript many genes)	**Monocistronic** transcript (one transcript one gene)
Coupled transcription and translation	Transcription and translation **NOT** coupled
mRNA not processed	mRNA processed
One RNA polymerase	Many RNA polymerases

Summary and review



Review

Eukaryotic mRNA



Eukaryotic translation

- Eukaryotic translation is similar to that of prokaryotes but a bit more complex.
- Differences in the translation process are due to differences in transcript structure and location of translation.

Eukaryotic translation

What are the stages of protein synthesis in eukaryotes?

(1) Initiation
(2) Elongation
(3) Termination

Eukaryotic translation initiation

What is needed for translation initiation?

1. Mature mRNA
2. Ribosome
3. Eukaryotic initiation factors (eIFs)
4. GTP
5. Initiator tRNA (Met-tRNA)

Eukaryotic translation initiation

Differences between eukaryotic and prokaryotic translation initiation?

1. No Shine-Dalgarno sequence in eukaryotes

2. No modified initiator tRNA (fMet-tRNA)

How does the ribosome know where to start?

Eukaryotic translation initiation

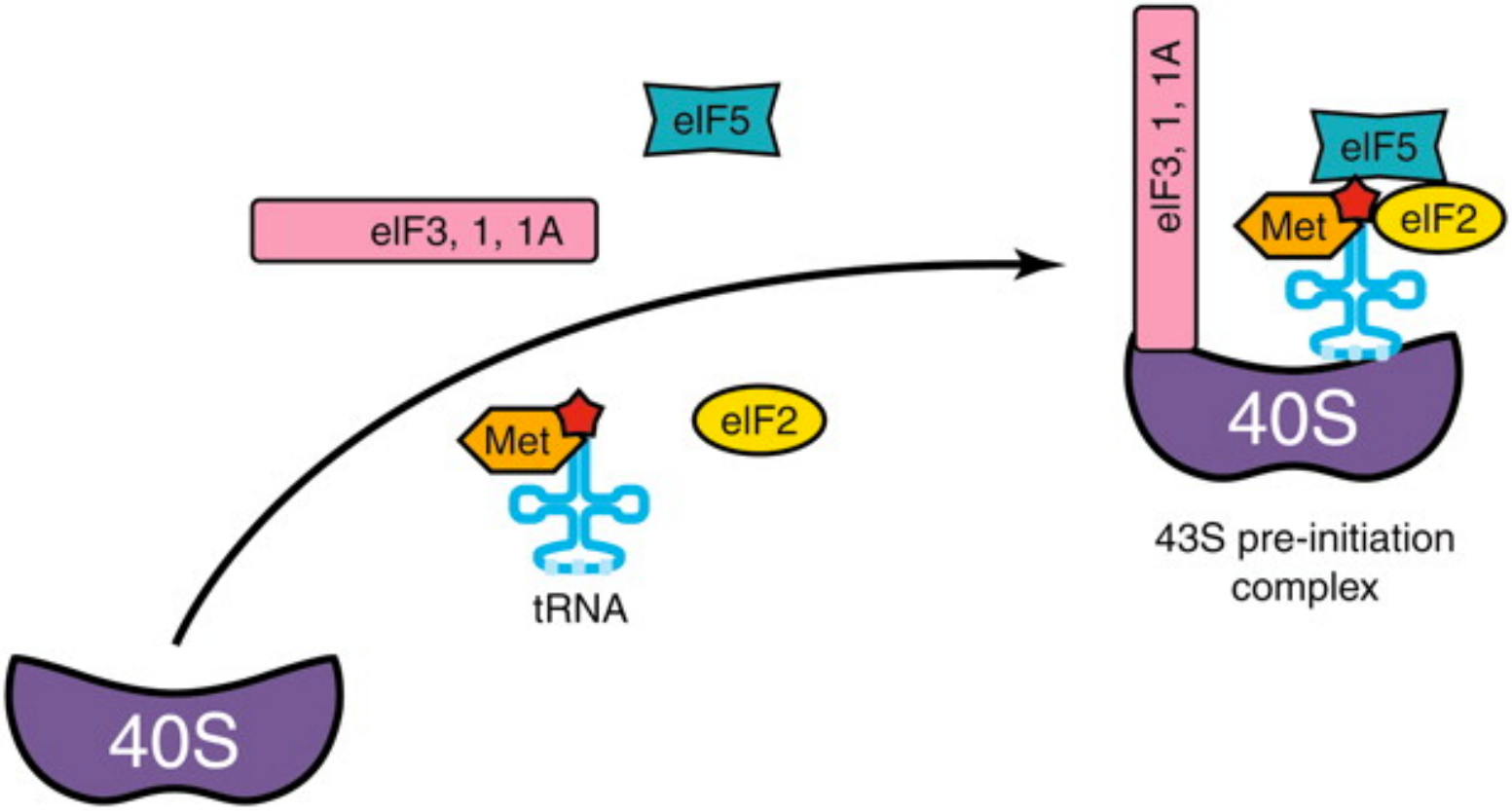
The process:

1.Initiator tRNA (Met-tRNA) gets loaded onto the small ribosomal subunit (40S).

2.Eukaryotic initiation factors (eIFs) gets loaded as well on the 40S ribosomal subunit.

3.A pre-initiation complex is formed containing (40S – Met-tRNA – eIFs).

Eukaryotic translation initiation



Eukaryotic translation initiation

Note:

Why only Met-tRNA binds to the 40S ribosomal subunit?

What about other charged tRNAs?

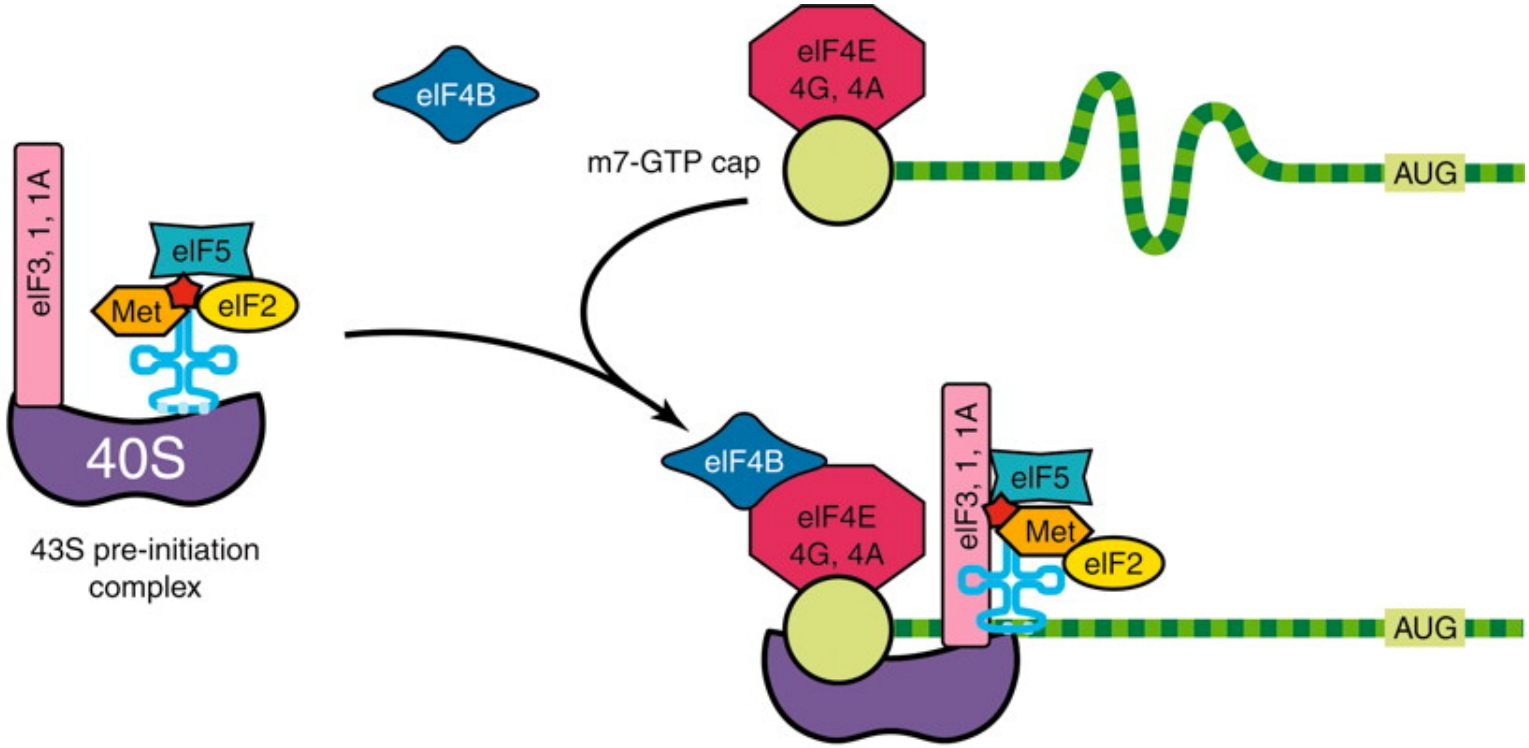
Only Met-tRNA can bind tightly to the small ribosomal subunit without the assembly of the entire ribosome.

Eukaryotic translation initiation

The process:

4.The complex (40S – Met-tRNA – eIFs) binds to the 5’ end of the mRNA (5’ Cap).

5.More eIFs are bound to the 5’ Cap.



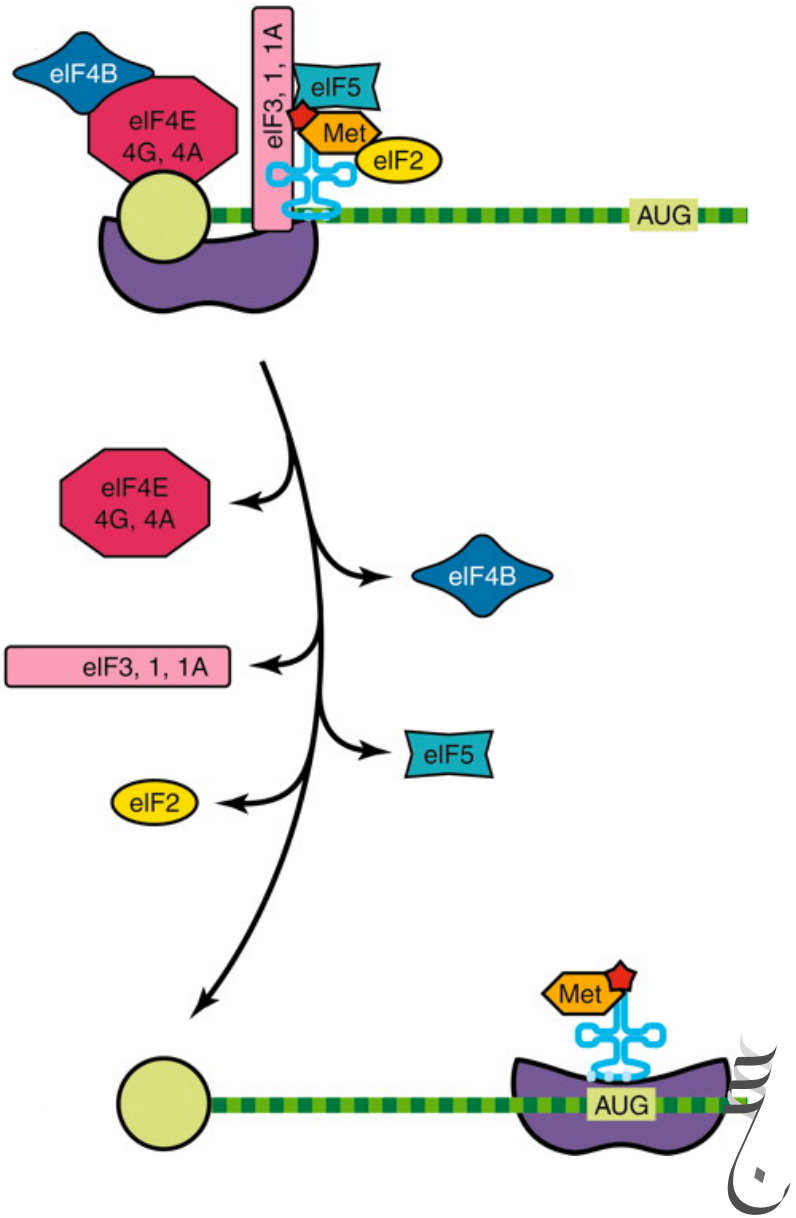
Eukaryotic translation initiation

The process:

6. The complex (40S – Met-tRNA – eIFs) moves along the transcript (5' – 3') in search for the first AUG.

7. The process is called "scanning" of mRNA.

8. When first AUG is found, eIFs dissociate.

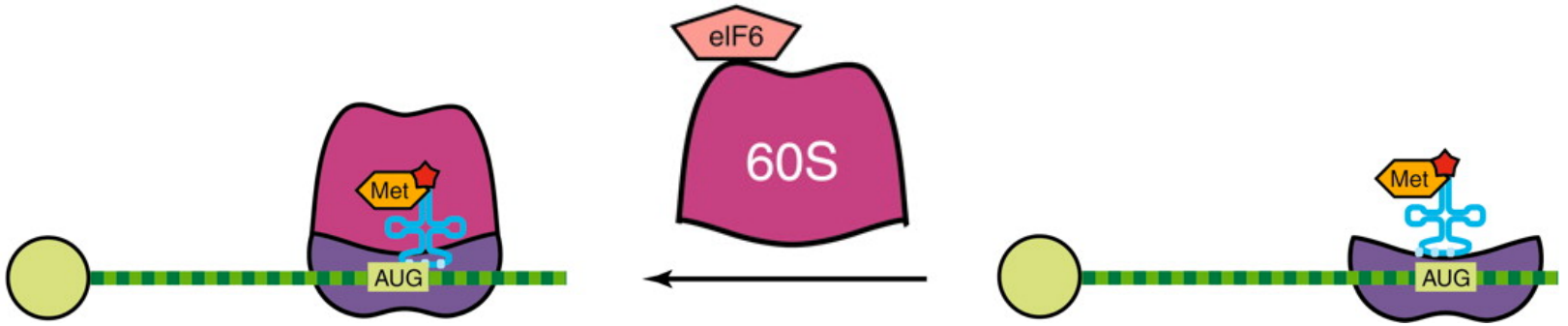


Eukaryotic translation initiation

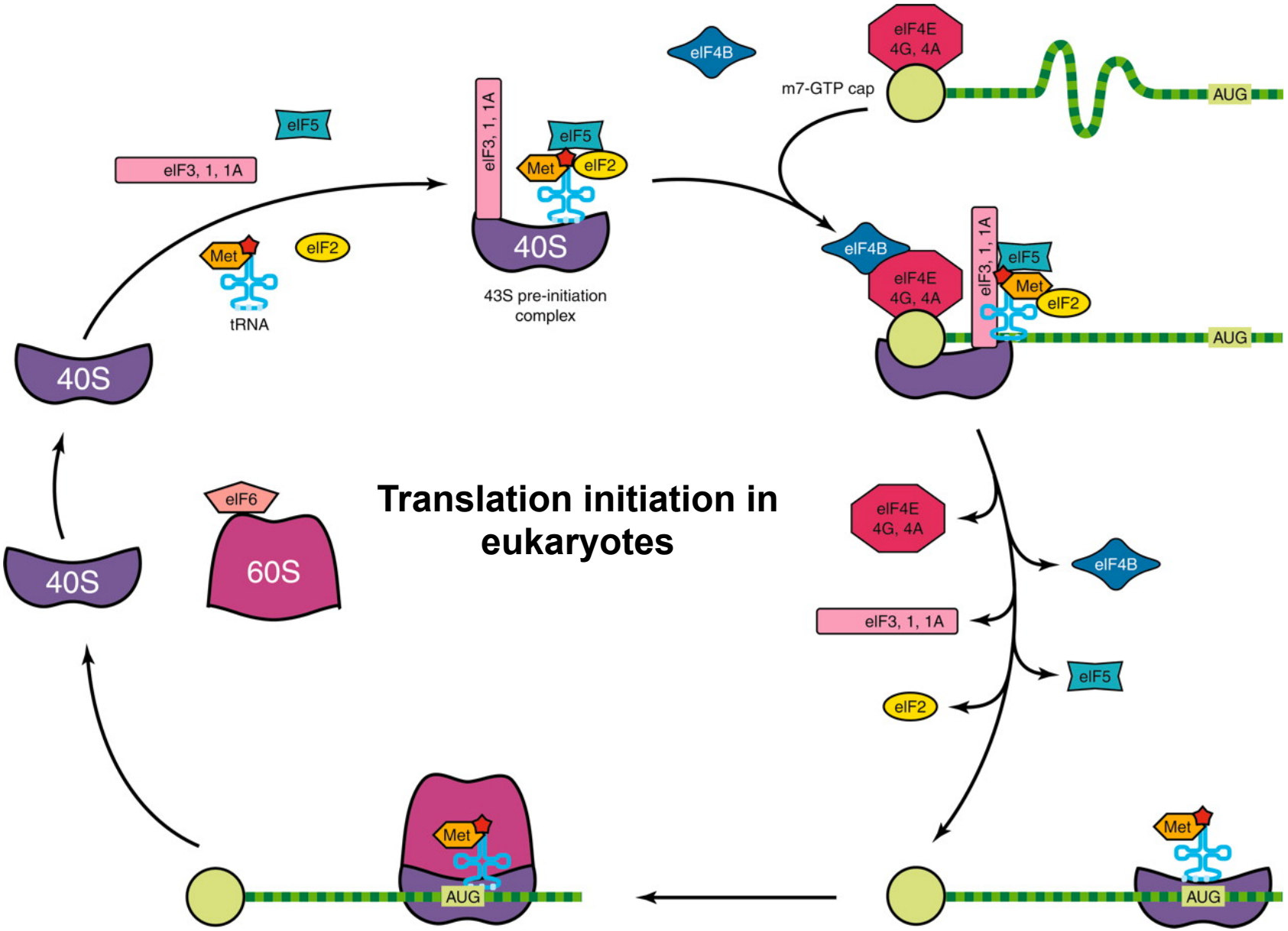
The process:

9.The large ribosomal subunit (60S) assembles to the complex at the start codon.

10. The initiator tRNA is positioned in the P site of the ribosome and the A site is available to receive to next charged tRNA.

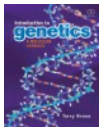


Summary of initiation



Initiation in eukaryotes

- Only a small number of eukaryotic mRNAs have internal ribosome binding sites
- With most mRNAs the small subunit of the ribosome makes its initial attachment at the 5’ end of the molecule
- Then **scans** along the sequence until it locates the initiation codon
- The first step involves assembly of the **pre-initiation complex**, the principal components of which are:
  - The small subunit of the ribosome
  - The initiator tRNA



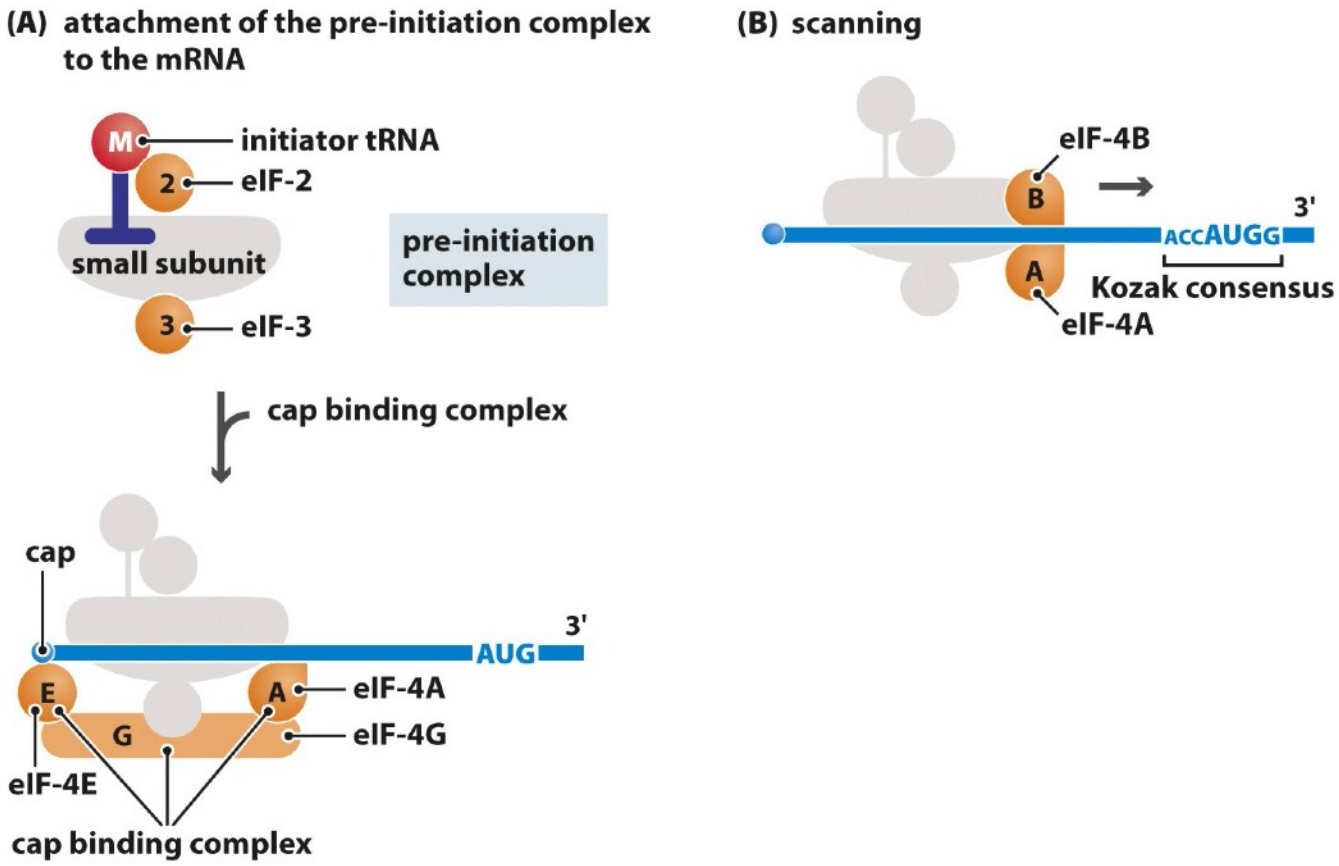
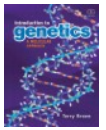
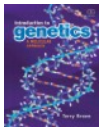


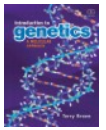
Figure. Initiation of translation in eukaryotes



- As in bacteria, the initiator tRNA is different from the normal $tRNA^{Met}$ that recognizes internal 5'-AUG-3' codons
- initiator tRNA is aminoacylated with normal methionine
- After assembly, the pre-initiation complex attaches to the cap structure at the extreme 5' end of the mRNA
- The **initiation complex** then scans along the molecule to find the initiation codon

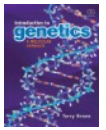


- The leader regions of eukaryotic mRNAs can be several tens, or even hundreds, of nucleotides in length
- Often contain several 5’-AUG-3’ triplets that the complex must ignore before it reaches the true initiation codon
- The initiation codon 5’-AUG-3’ triplets, is contained in a short consensus sequence, 5’-ACCAUGG-3’, referred to as the **Kozak consensus**



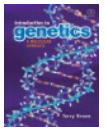
There are two further aspects of eukaryotic translation initiation

- The involvement of the poly(A) tail of the mRNA
- The role of the plethora of initiation factors possessed by eukaryotes



Poly(A) tail of the mRNA

- it promotes the binding of the pre-initiation complex to the cap structure
- It is involved at this stage because the length of the tail influences the extent of translation initiation that occurs with a particular mRNA
- Removal of the tail is one of the steps that lead to inactivation of an mRNA whose translation product is no longer needed



Initiation factors

- At least 13 initiation factors involved
- Five of these are components of the pre-initiation complex:
  - eIF-1,
  - eIF-1A
  - eIF-2
  - eIF-2B
  - eIF-3—

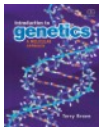
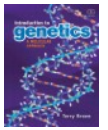


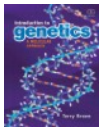
TABLE 8.3 FUNCTIONS OF INITIATION FACTORS IN EUKARYOTES

Initiation factor	Function
eIF-1	Component of the pre-initiation complex
eIF-1A	Component of the pre-initiation complex
eIF-2	Binds to the initiator tRNA within the pre-initiation complex; phosphorylation of eIF-2 results in a global repression of translation
eIF-2B	Component of the pre-initiation complex
eIF-3	Component of the pre-initiation complex; makes direct contact with eIF-4G and so forms the link with the cap binding complex
eIF-4A	Component of the cap binding complex; a helicase that aids scanning by breaking intramolecular base pairs in the mRNA
eIF-4B	Aids scanning, possibly by acting as a helicase that breaks intramolecular base pairs in the mRNA
eIF-4E	Component of the cap binding complex, possibly the component that makes direct contact with the cap structure at the 5′ end of the mRNA
eIF-4F	The cap binding complex, comprising eIF-4A, eIF-4E, and eIF-4G, which makes the primary contact with the cap structure at the 5′ end of the mRNA
eIF-4G	Component of the cap binding complex; forms a bridge between the cap binding complex and eIF-3 in the pre-initiation complex; in at least some organisms, eIF-4G also forms an association with the poly(A) tail
eIF-4H	In mammals, aids scanning in a manner similar to eIF-4B
eIF-5	Aids release of the other initiation factors at the completion of initiation
eIF-6	Associated with the large subunit of the ribosome; prevents large subunits from attaching to small subunits in the cytoplasm

Table 8.3 Introduction to Genetics (© Garland Science 2012)

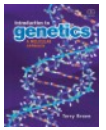


- Three of these factors form a structure called the **cap binding complex** :
  - eIF-4A
  - eIF-4E
  - eIF-4G—



The cap binding complex

- Makes the initial contact with the 5’ end of the mRNA
- Mediates subsequent attachment of the pre-initiation complex
- Two factors, eIF-4A and eIF-4B, are involved in the scanning process
- eIF-4A and possibly eIF-4B having a helicase activity
    - Which enables them to break intramolecular base pairs that sometimes form in the mRNA leader region
- eIF-5 aids the release of all the other initiation factors at the end of the initiation phase
- eIF-6 plays the same role as IF-3 in bacteria, preventing the large subunit of the ribosome from joining the initiation complex until it is needed



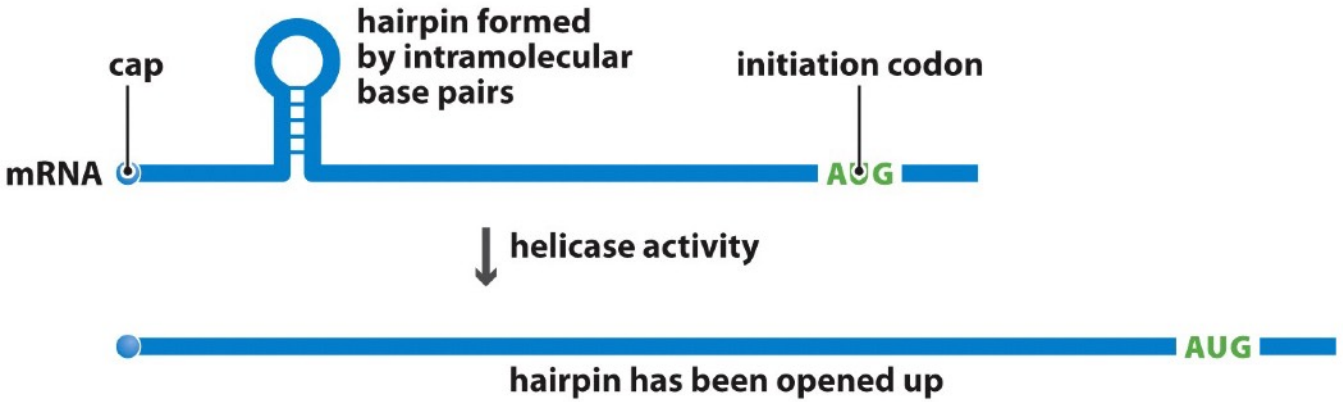
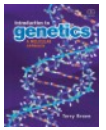
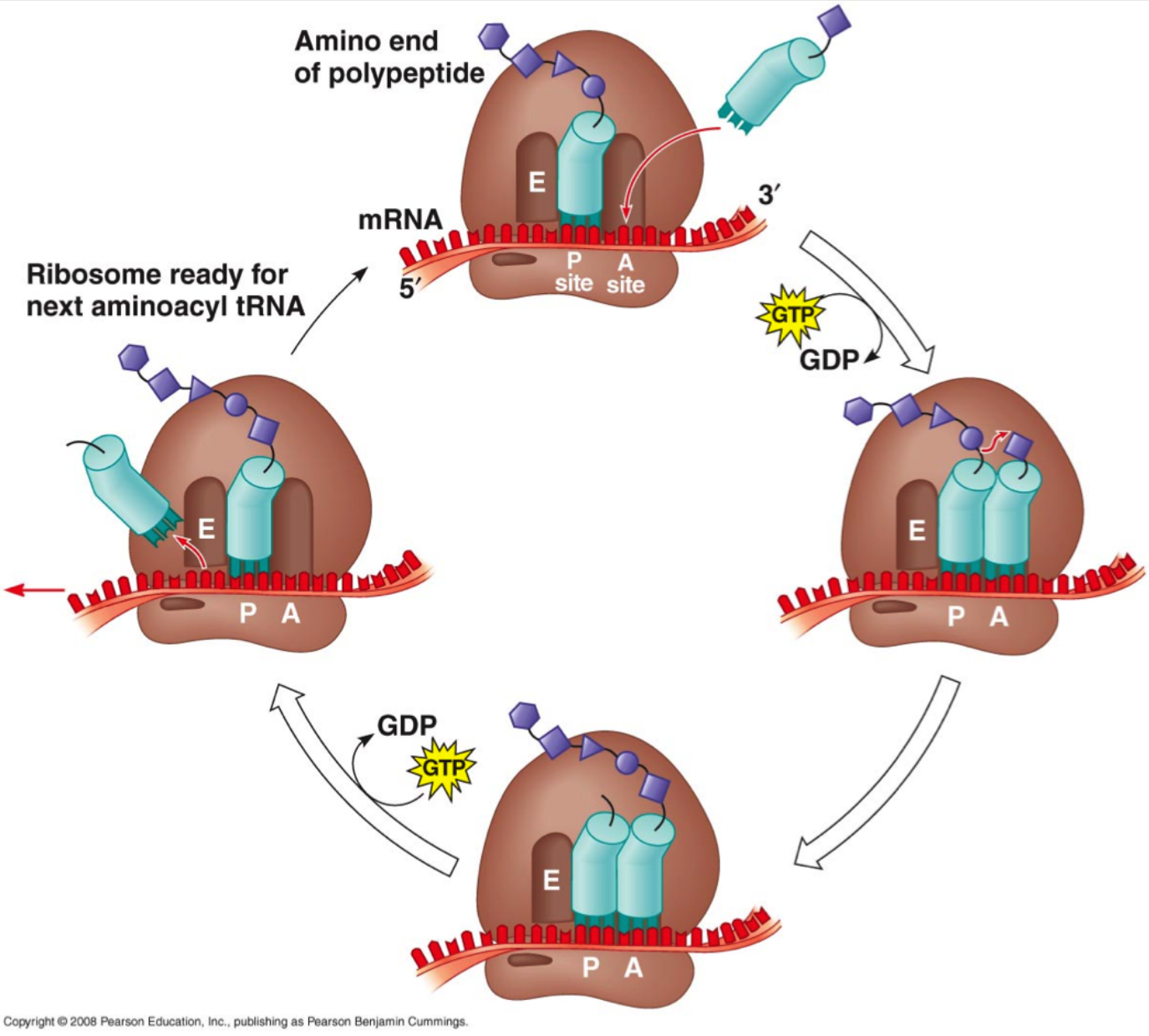


Figure. The helicase activities of eIF-4A and possibly eIF-4B enable hairpin structures resulting from intramolecular base pairing to be broken in the leader region of the mRNA.

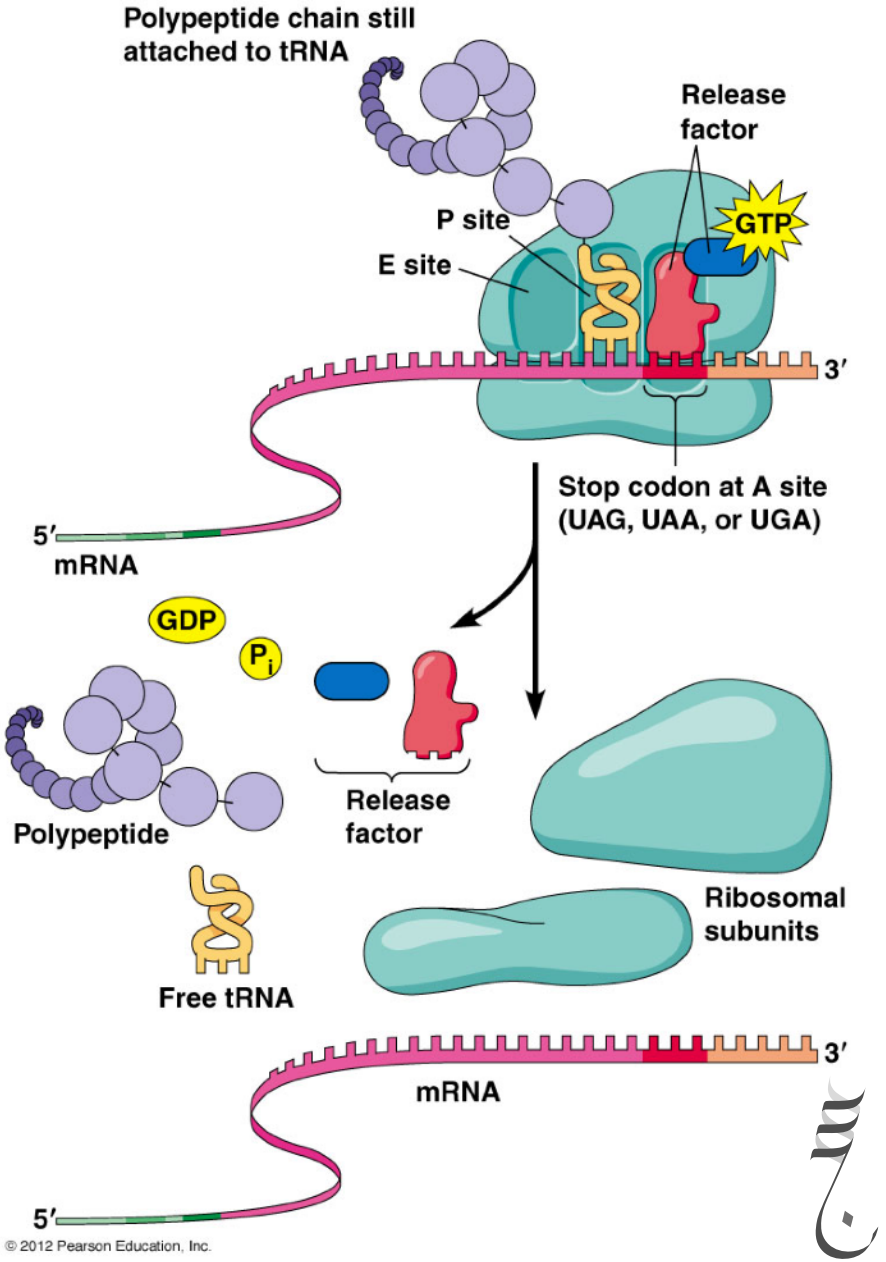


Eukaryotic translation elongation

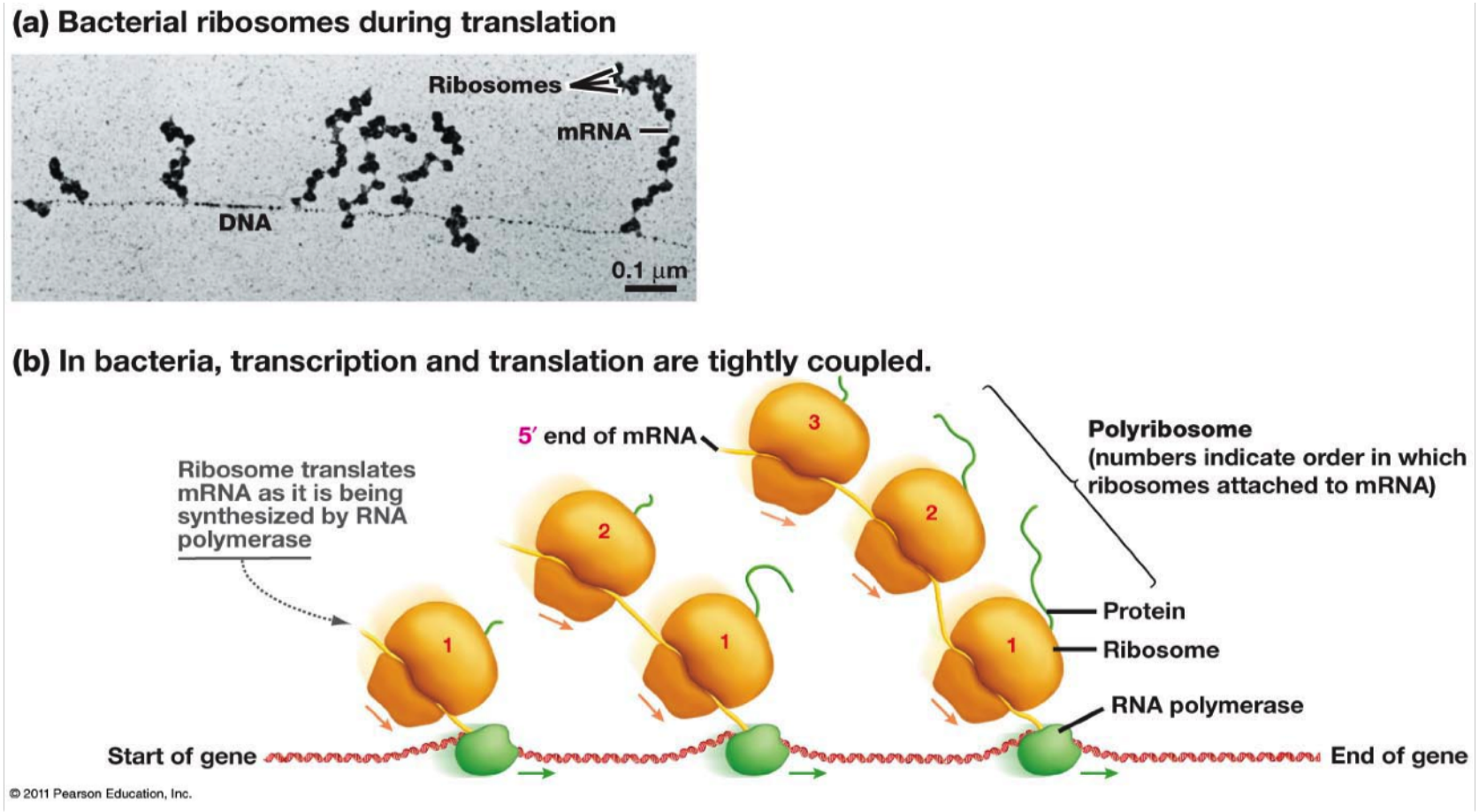


Eukaryotic translation termination

- Termination in eukaryotes is similar to that in prokaryote.
- Eukaryotic Release factor (eRF1) recognizes all stop codons.
- Other release factors help in the termination and disassembling of the ribosome.

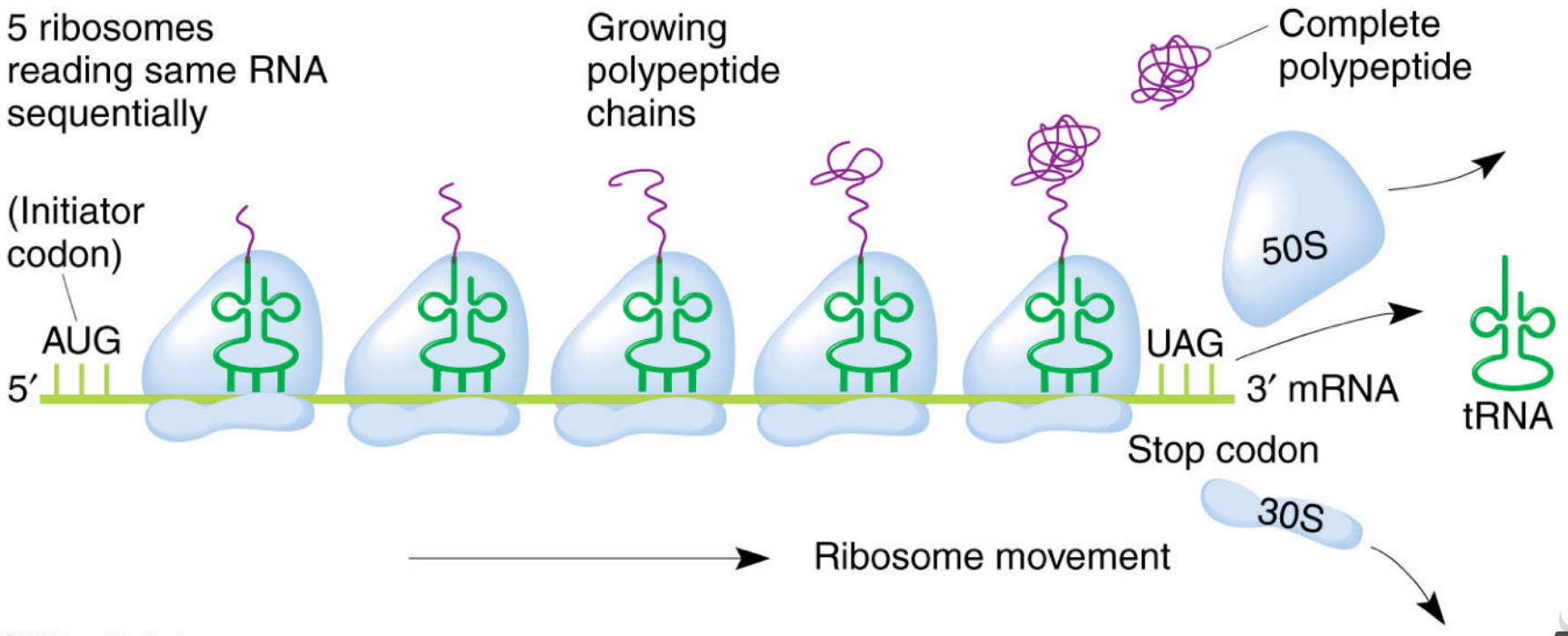


Coupled transcription translation in bacteria



Polysome

Polysome or polyribosome: a number of ribosomes translating the same transcript.



Polysome

- **Polysome or polyribosome:** a number of ribosomes translating the same transcript.
- Many rounds of translation takes place on the same transcript.

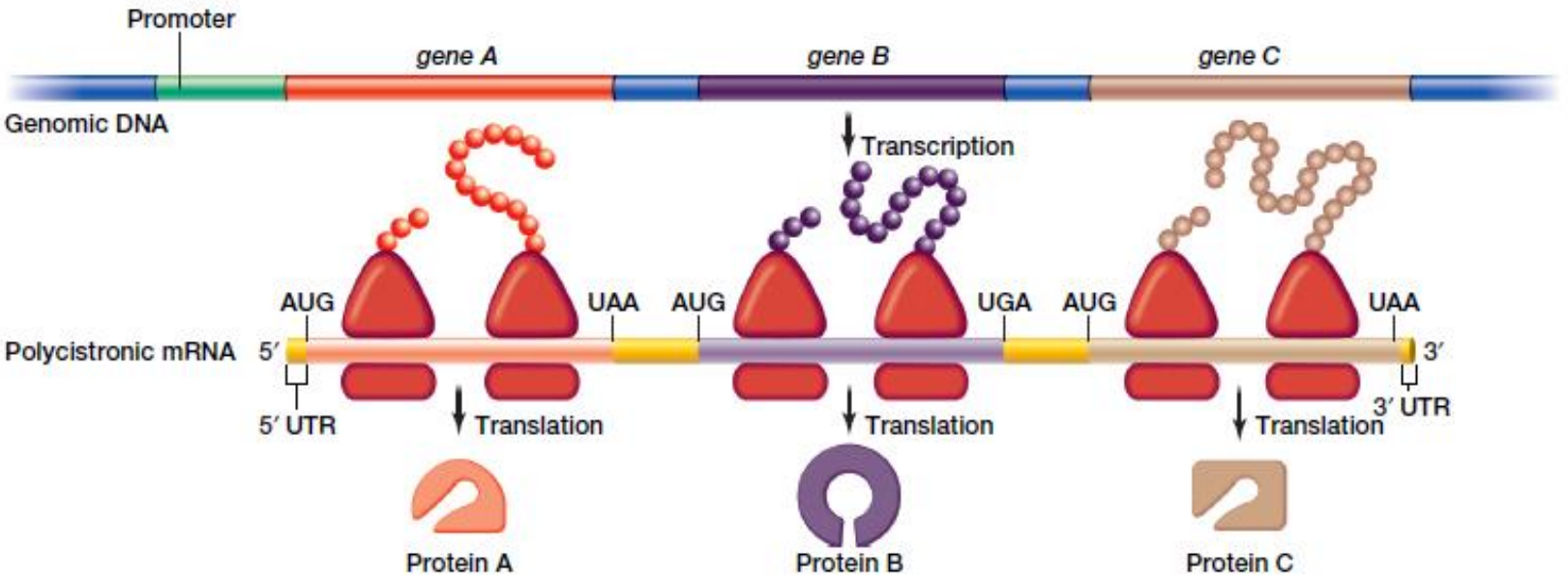
Why is this process beneficial for the cell?

- Saves time (How long)
- Quantity (How much)

Polycistronic transcript

Translating polycistronic prokaryotic transcript:

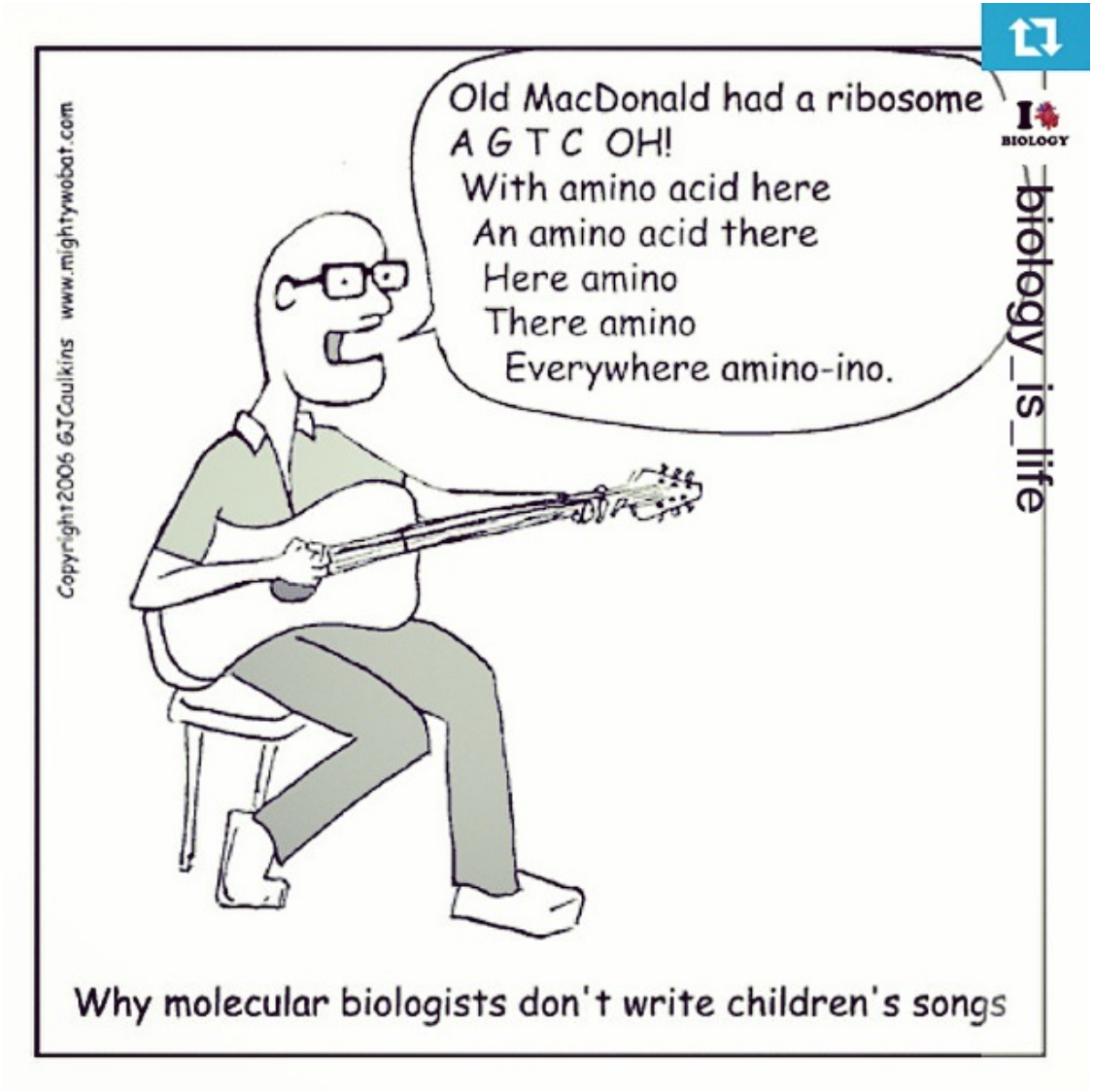
- Each gene gets translated independently because each has its own Shine-Dalgarno sequence.



Expectations

- You know how translation process occurs in eukaryotes.
- You know how ribosome finds the correct location to start translation in eukaryotes.
- You know the molecules needed in every step of translation.
- You the sequence of events in eukaryotic translation.
- You know the differences between prokaryotic and eukaryotic translation.

For a smile


Old MacDonald had a ribosome
A G T C OH!
With amino acid here
An amino acid there
Here amino
There amino
Everywhere amino-ino.
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biology_is_life
Why molecular biologists don't write children's songs