Translation

Translation: Assembly of polypeptides on a ribosome

- Living cells devote more energy to the synthesis of proteins than to any other aspect of metabolism.
- About a third of the dry mass of a cell consists of molecules that directly participate in protein synthesis
- This reflects the importance of protein synthesis to the existence of the organism.

Translation: An Overview

- Ribosomes translate the genetic message of mRNA into proteins.
- The mRNA is translated 5' → 3' producing a corresponding N-terminal → C-terminal polypeptide.
- Amino acids bound to tRNAs are inserted in the proper sequence due to:
  - Specific binding of each amino acid to its tRNA.
  - Specific base-pairing between the mRNA codon and tRNA anticodon.

Components of Translation

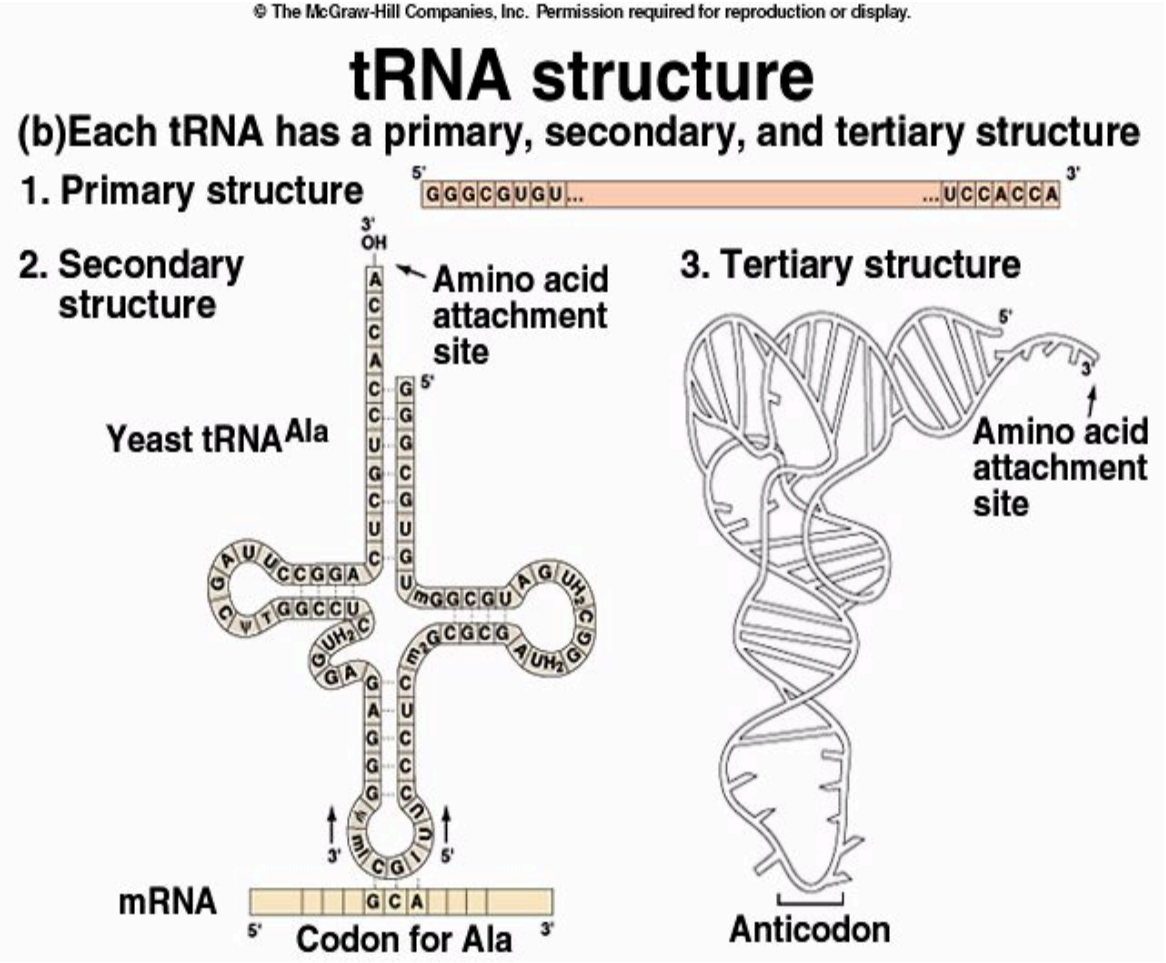
- **mRNA:**
  - Eukaryotes: made in the nucleus, transported to the cytoplasm.
  - Prokaryotes: transcription and translation occur concurrently.
- **tRNA:** Adaptor molecules that mediate the transfer of information from nucleic acids to protein
- **Ribosomes**: manufacturing units of a cell; located in the cytoplasm. Contain ribosomal RNA and proteins.
- **Enzymes:** required for the attachment of amino acids to the correct tRNA molecule, and for peptide bond formation between amino acids.
- **Proteins:** soluble factors necessary for proper initiation, elongation and termination of translation.

tRNA

- small single stranded RNA molecules of 70-95 nucleotides in length,
  - about 4S (Svedberg units) in size.
- In addition to A, G, C and U, tRNAs have modified bases produced by chemical alteration of the 4 primary nucleotides.
- Each tRNA molecule is a clover leaf structure, which looks like an L-shape in three dimensions.
- At the base of the L, three nucleotides form the anti-codon.
- The sequence of the anti-codon dictates the amino acid that binds to it.
  - The anti-codon sequence is complementary to the codon for that amino acid.
  - For example:
    - GCA is a codon for alanine: the anticodon then is CGU, but in the 3' to 5' direction.
- The amino acid is carried at the 3' hydroxyl end of the tRNA molecule.

tRNA

- Tertiary structure
- Amino acids must be attached to be functional
  - Enzymatic reaction
  - Need ATP
  - Aminoacyl tRNA synthase



tRNA

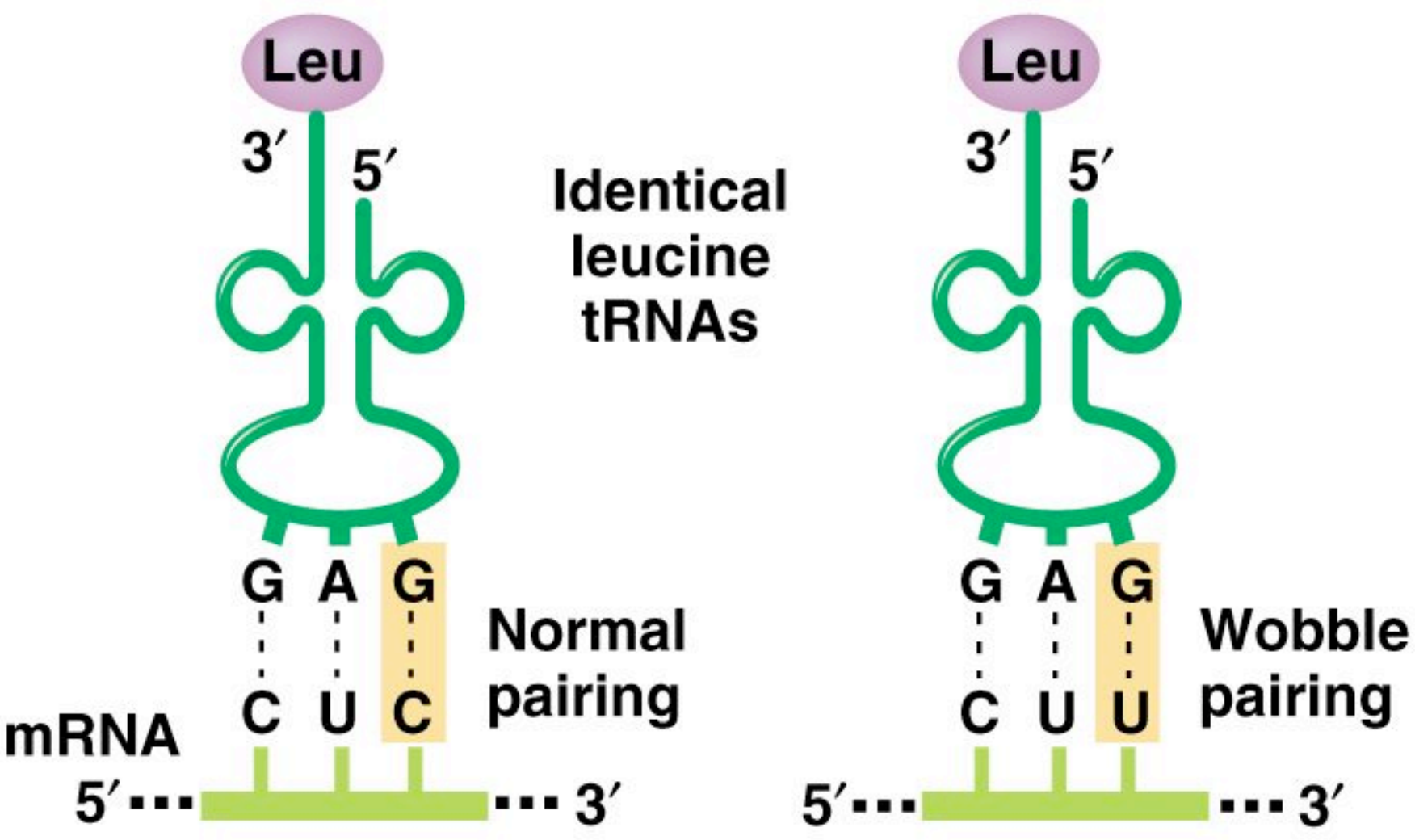
- Recognition of codon is important
- Are there tRNAs for every codon?
  - So are there 61 tRNA's?
    - No actually about 40
    - "Wobble" in third position of anticodon
- One anticodon can recognize several codons…
- What are the wobble rules?

Characteristics of the Genetic Code: The Wobble Hypothesis

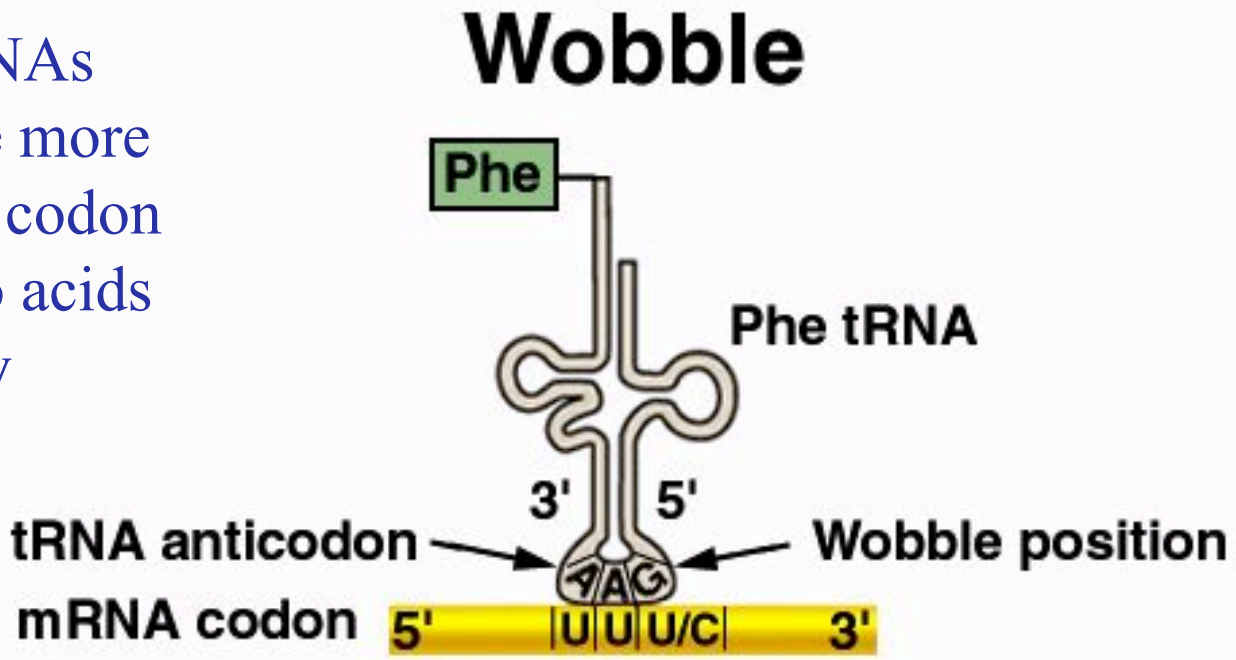
- Wobble occurs in the anticodon.
  - The third base in the codon is able to base-pair less specifically
  - because it is less constrained three-dimensionally.

- allows a tRNA anticodon to recognize up to three different codons (Figure 14.8 and Table 14.1).
  - Does not obey complementary base pairing in certain cases.
  - YIKES!

Fig. 6.9 Example of base-pairing wobble



Some tRNAs recognize more than one codon for amino acids they carry



Wobble rules		
5' end of anticodon	can pair with	3' end of codon
G		U or C
C		G
A		U
U		A or G
I		U,C, or A

Question

- The anti-codon ACG can pair with which codon(s)?
  - 1) UGG
  - 2) UGU
  - 3) UGC
  - 4) 1 and 2
  - 5) 2 and 3

Question

- The anti-codon ACC can pair with which codon(s)?
  - 1) UGG
  - 2) UGU
  - 3) UGC
  - 4) 1 and 2
  - 5) 2 and 3

Enzymes

- **Aminoacyl-tRNA synthetases** catalyze the attachment of a tRNA molecule to its respective amino acid.
  - There is at least one amino acyl tRNA synthetase for each amino acid.
  - The attachment of the amino acid **activates/ charges** the tRNA molecule.
  - The attachment of the aminoacid is at its carboxyl terminal. (NH2-CH2-CO-3'tRNA5')

- **Peptidyl Transferase:**
  - catalyzes the sequential transfer of amino acids to the growing chain.
  - Forms the peptide bonds between amino acids

Ribosomes: Functions

- They are the sites of polypeptide synthesis
- They recognize features that signal the start of translation
- They ensure the accurate interpretation of the genetic code by stabilizing the interaction between tRNA and the mRNA.
- They supply the enzymatic activity that covalently links the amino acids in the polypeptide chain.
- They facilitate the linear reading of the genetic code by sliding along the mRNA molecule.

Ribosomes: Components

- two subunits: large and small.
  - Prokaryotes: 50S + 30S = 70S
  - eukaryotes: 60S + 40S = 80S.
- Prokaryotes: overall smaller
  - large subunit contains one rRNAs and ~31 different proteins.
  - small subunit contains two rRNAs and 21 different proteins.
- Eukaryotes: overall bigger
  - large subunit contains three rRNAs and 45 proteins.
  - small subunit consists of one rRNAs and 33 different proteins.

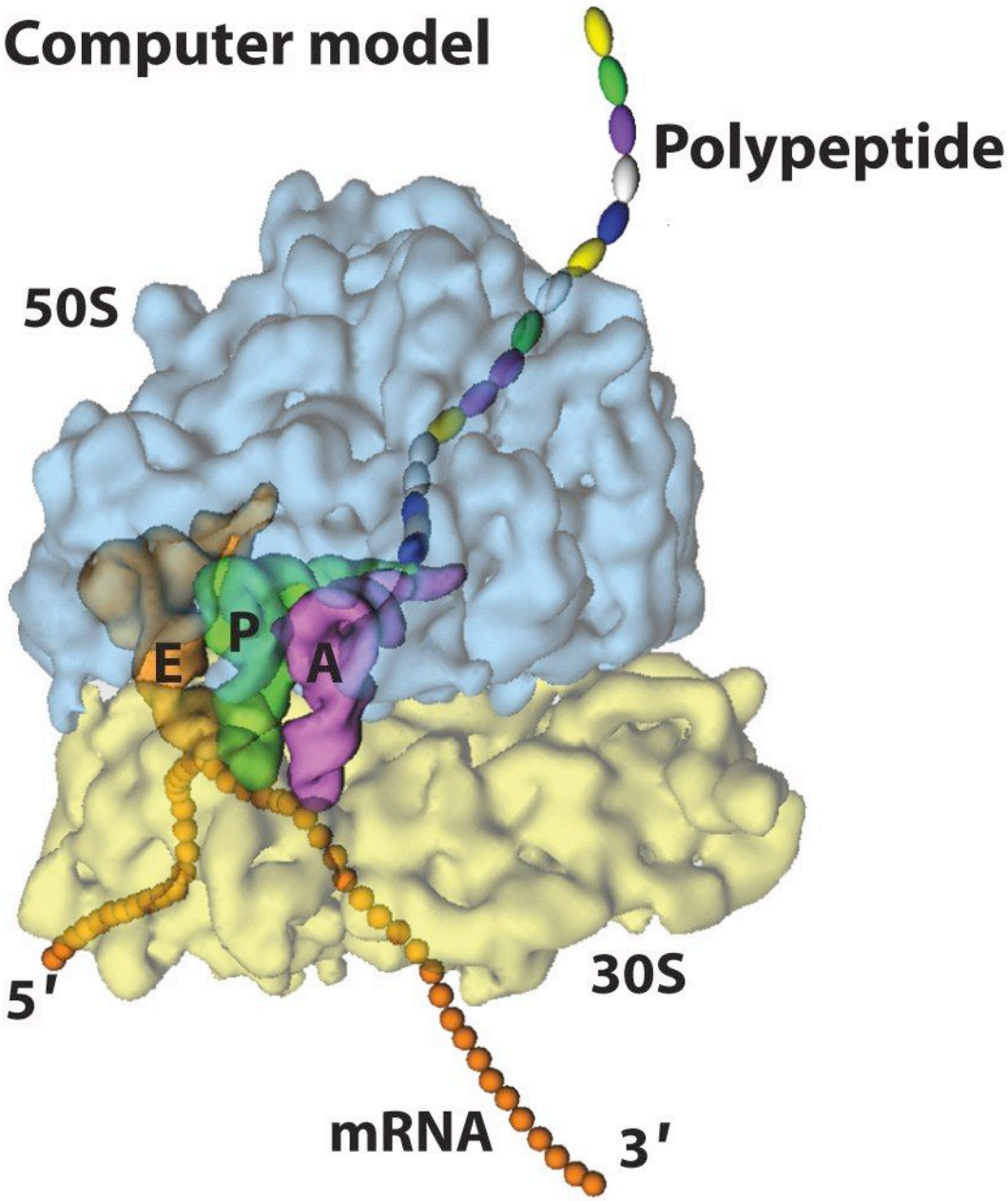
Ribosomes: Synthesis

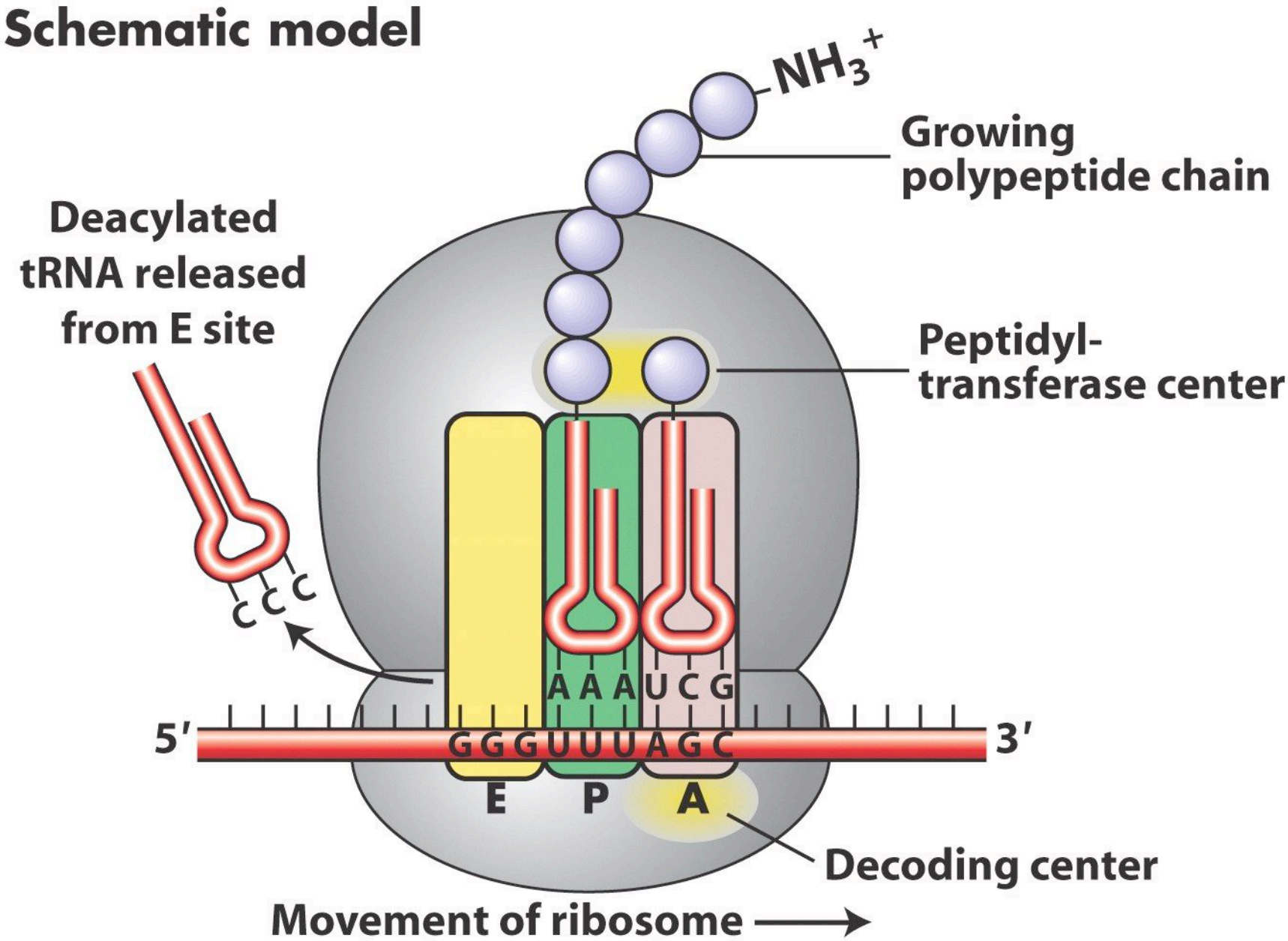
- In eukaryotes, rRNA synthesis and ribosome assembly takes place in the nucleolus.
- Before translation begins, the two ribosomal subunits exist as separate entities in the cytoplasm.
- Soon after the start of translation, they come together.

Ribosomes: Role in translation

- The small subunit is the one that initially binds to the mRNA.
- The larger subunit provides the enzyme activity:
  - Peptidyl transferase,
  - catalyzes formation of peptide bonds joining amino acids
- The assembled structure of the ribosome creates three pockets for the binding of two molecules of tRNA.

- The far left pocket is the Exit site or E site
  - It binds the deacylated tRNA (no amino acid attached)
- The one in the middle is referred to as the peptidyl or the P site:
  - it binds to the tRNA holding the growing chain of polypeptide.
- The site on the right is termed the amino acyl, or the A site,
  - it binds to the incoming tRNA molecule.


Computer model
Polypeptide
50S
P
E
A
5′
30S
mRNA
3′


Schematic model
NH_3^+
Growing polypeptide chain
Deacylated tRNA released from E site
Peptidyl-transferase center
C C C
A A A U C G
5′
G G G U U U A G C
3′
E
P
A
Decoding center
Movement of ribosome

Question

- One difference between prokaryote and eukaryote ribosomes is:
  - 1. Their function
  - 2. Prokaryotes do not have ribosomes because they do not have organelles
  - 3. Their size
  - 4. How they work

Mechanism of Translation

- Three steps of translation:

  - Initiation: sets the stage for polypeptide synthesis.

  - Elongation: causes the sequential addition of amino acids to the polypeptide chain in a colinear fashion as determined by the sequence of mRNA.

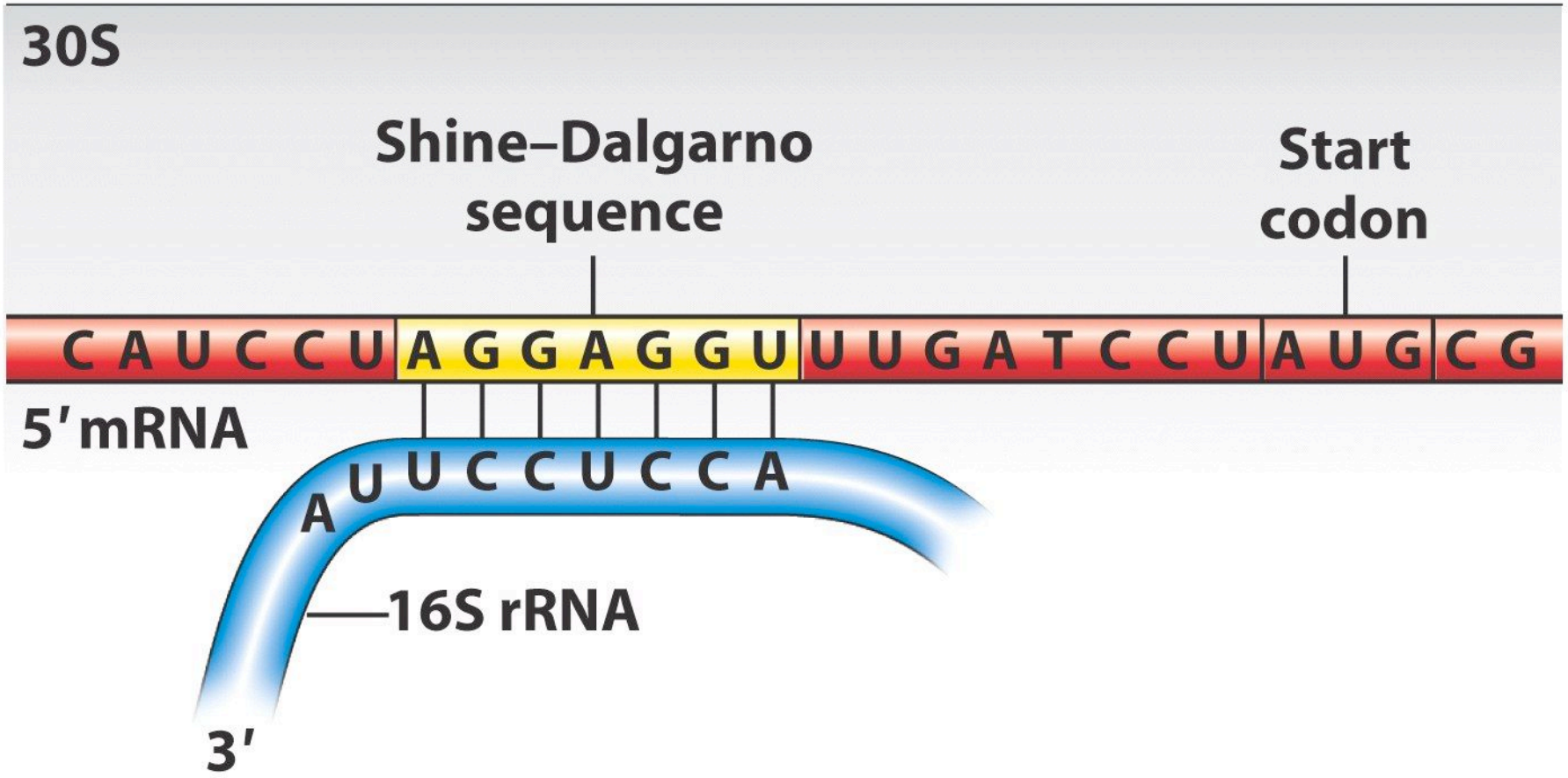
  - Termination: Brings the polypeptide synthesis to a halt.

Initiation

- The initiation codon is an AUG
  - is towards the 5' end of the mRNA molecule that is being translated.
  - NOT the first 3 nucleotides!
  - It determines the reading frame.

- In prokaryotes, there is a conserved region about 7 nucleotides upstream from the initiating AUG:
  - this region contains a 6-nucleotide sequence
  - **Shine-Dalgarno box:** AGGAGG.

- The Shine-Dalgarno sequence is **complementary to a region at the 3' end of the 16 rRNA of the small subunit**;
  - base pairing between these complementary sequences stabilizes the binding of the small ribosomal subunit to the mRNA for proper assembly.


30S
Shine–Dalgarno sequence
Start codon
C A U C C U A G G A G G U U U G A T C C U A U G C G
5′ mRNA
A U U C C U C C A
16S rRNA
3′

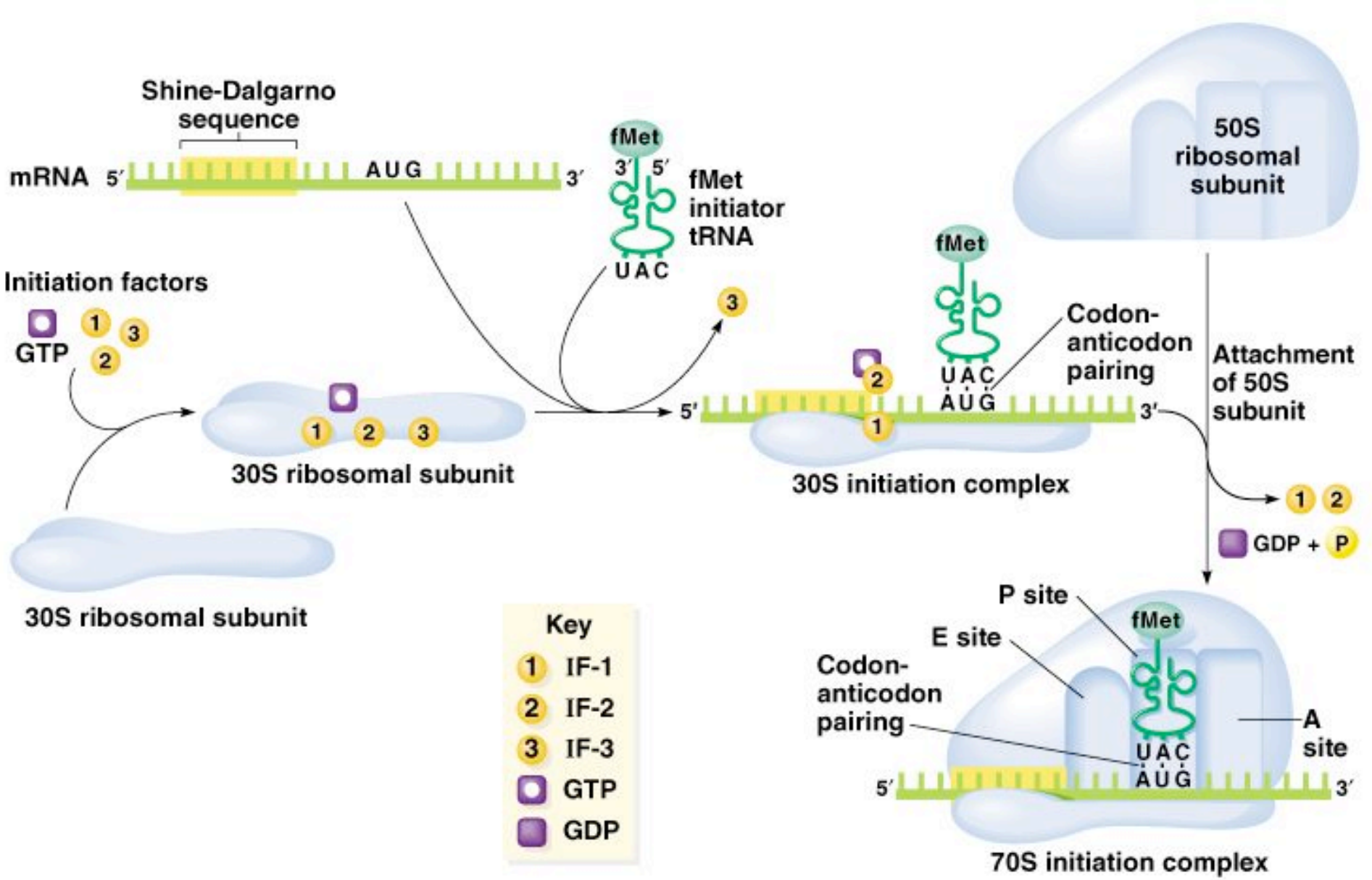
Initiation: *continued*

- In prokaryotes, the first AUG is recognized by a special tRNA ($tRNA_f^{Met}$) carrying a modified methionine: formyl methionine.
  - The large subunit of the ribosome now attaches to the small subunit, to complete the initiation process.

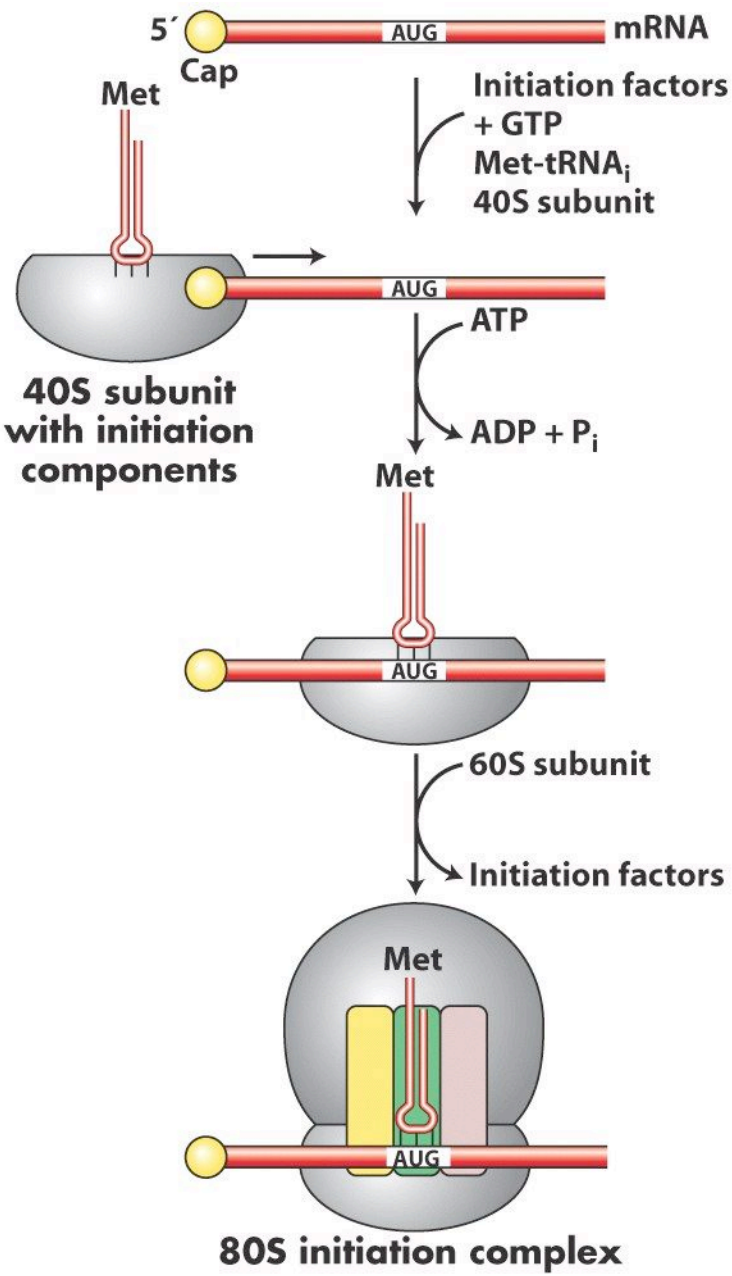
- In eukaryotes, the small ribosomal unit binds first to the methylated cap (7-methyl guanosine) at the 5' end of the mRNA.
  - It then migrates to the initiation site, usually the first AUG it encounters as it scans the mRNA in the 5' to 3' direction.

- In eukaryotes, the methionine need not be modified.

Fig. 14.12 Initiation of protein synthesis in prokaryotes



Translation Initiation in Eukaryotes



Elongation

- At the start of elongation, the mRNA is bound to the complete two subunit ribosome,
  - with the initiating tRNA in the P site,
  - and the A site free for binding to the next tRNA.

- The ribosome moves along the mRNA in a 5' to 3' direction, in a step-wise process, recognizing each subsequent codon.

- The peptidyl transferase enzyme then catalyzes the formation of a peptide bond between
  - the free N terminal of the amino acid at the A site,
  - and the Carboxyl end of the amino acid at the P site, which is actually connected to the tRNA.

- This disconnects the tRNA fMet from the amino acid, and the tRNA at the A site now carries two amino acids,
  - with a free N terminal and the Carboxyl terminal of the second aa connected to its tRNA.

Translation Elongation

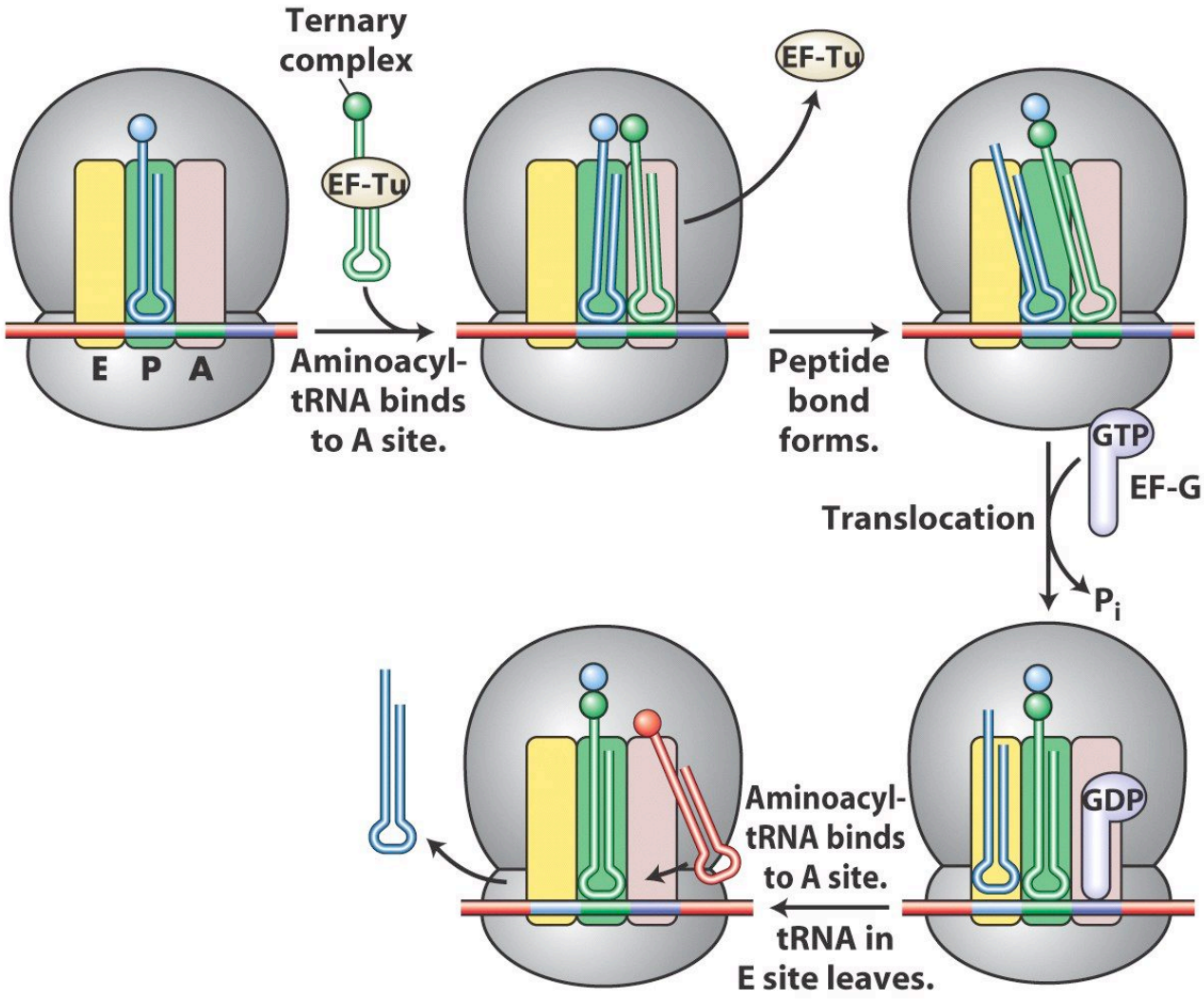
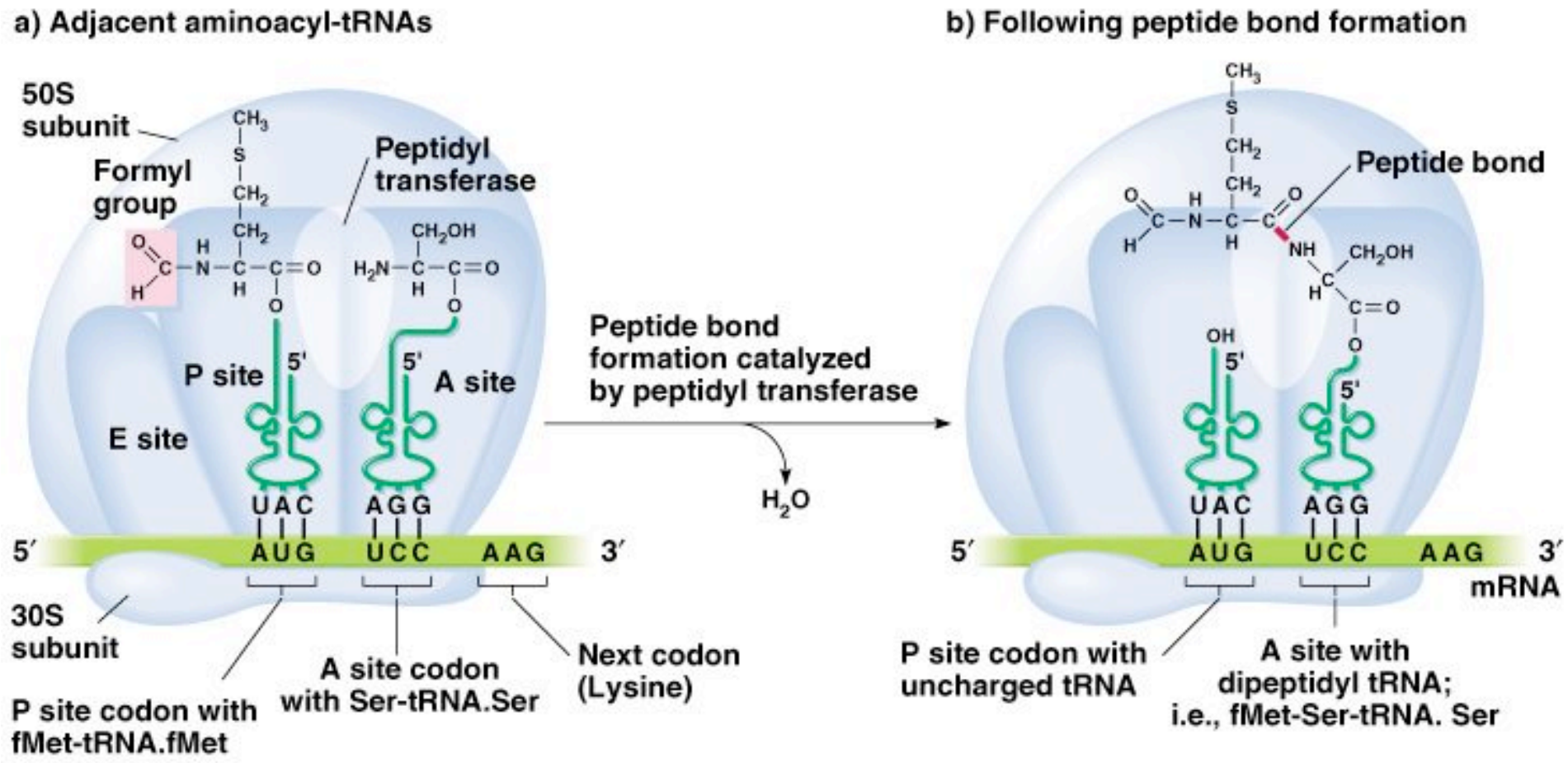


Fig. 6.13 The formation of a peptide bond between the first two amino acids of a polypeptide chain is catalyzed on the ribosome by peptidyl transferase



Chain Elongation:Translocation

- During translocation the peptidyl-tRNA remains attached to its codon, but is transferred from the ribosomal A site to the P site by an unknown mechanism.

- The vacant A site now contains a new codon, and an aminoacyl-tRNA with the correct anticodon can enter and bind.

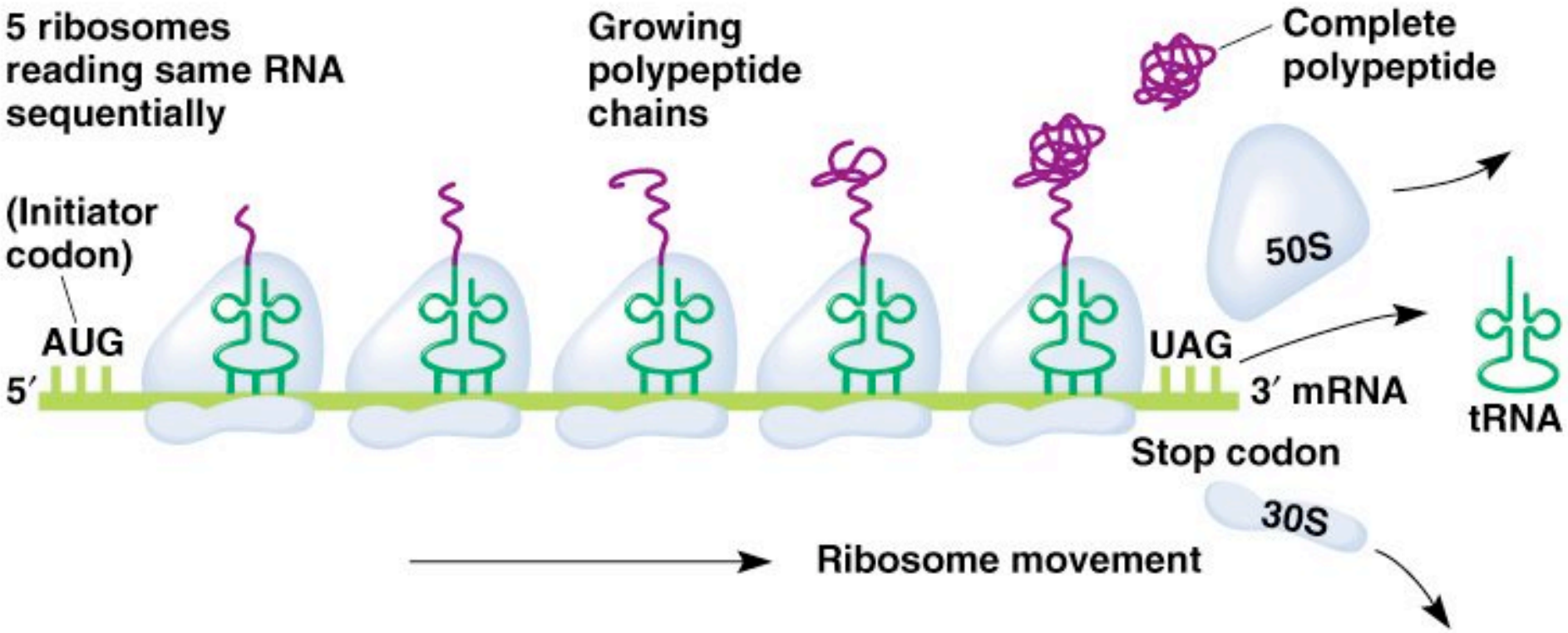
- The process repeats until a stop codon is reached.

Chain Elongation: Translocation

- Elongation and translocation are similar in eukaryotes, except for differences in number and type of elongation factors and the exact sequence of events.

- In both prokaryotes and eukaryotes, simultaneous translation occurs.
  - New ribosomes may initiate as soon as the previous ribosome has moved away from the initiation site, creating a polyribosome (polysome).
  - An average mRNA might have 8–10 ribosomes attached at a given moment (Figure 14.15).

Fig. 6.14 Diagram of a polysome, a number of ribosomes each translating the same mRNA sequentially

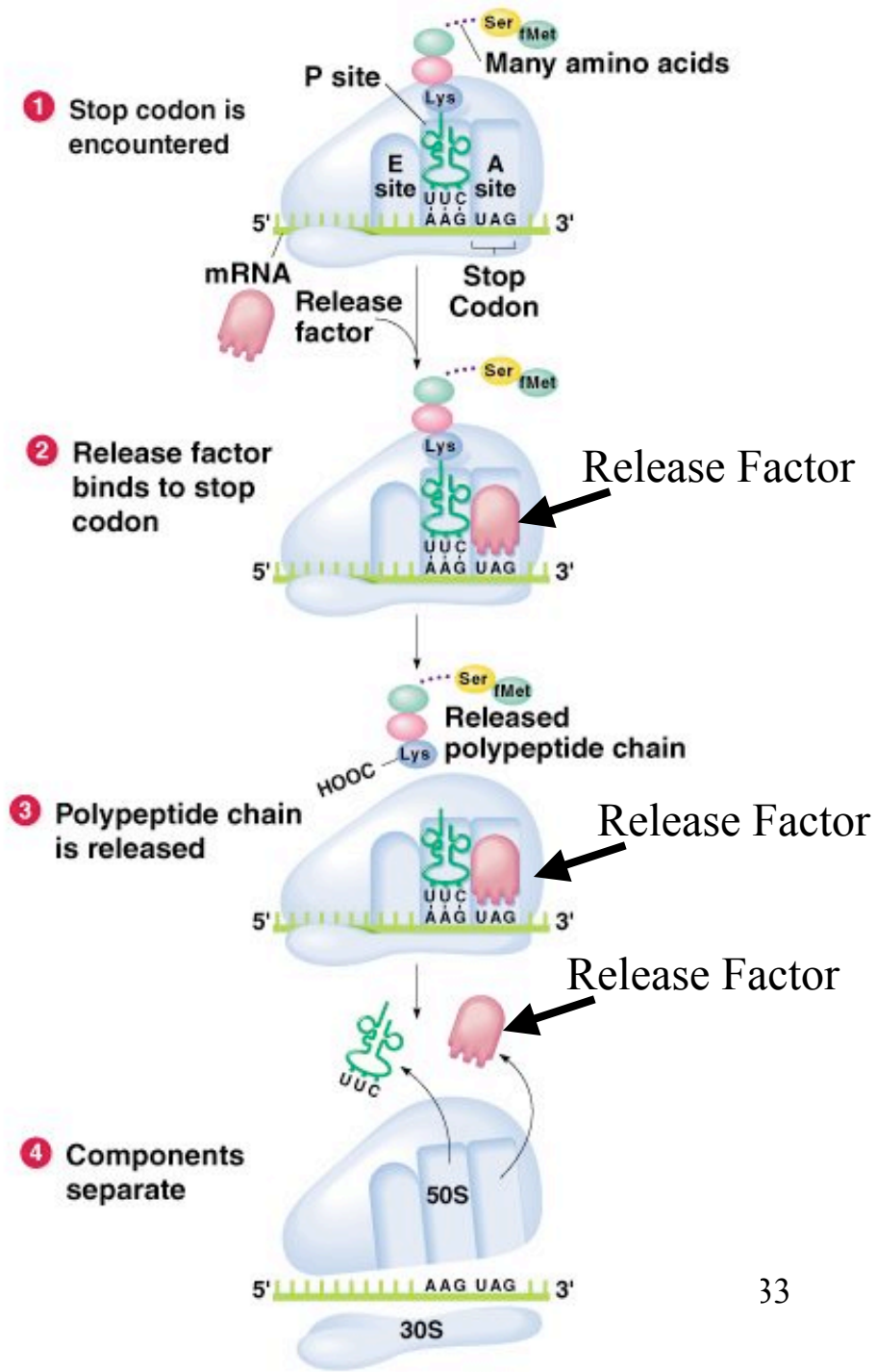


Termination

- When the ribosome encounters a stop codon,
  - there is no tRNA available to bind to the A site of the ribosome,
  - instead a release factor binds to it.

- The details are not very clear, but once the release factor binds, the ribosome unit falls apart,
  - releasing the large and small subunits,
  - the tRNA carrying the polypeptide is also released, freeing up the polypeptide product.

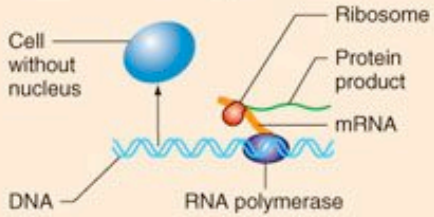
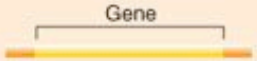
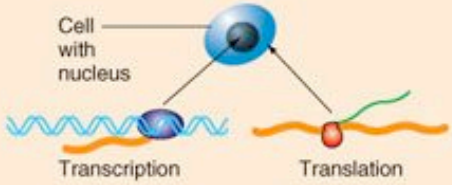
Fig. 14.15 Termination of translation



Question

- All of the following are necessary components for translation except:
  - 1) Rho protein
  - 2) Peptidyl transferase
  - 3) rRNA
  - 4) tRNA

TABLE 8.1 **Differences Between Prokaryotes and Eukaryotes in the Details of Gene Expression**

	Prokaryotes	Eukaryotes
Overview	1. No nucleus. Transcription and translation thus take place in the same cellular compartments, and translation is often coupled to transcription.  2. Genes are not divided into exons and introns. 	1. Nucleus separated from the cytoplasm by a nuclear membrane. Transcription takes place in the nucleus, while translation occurs in the cytoplasm. Direct coupling of transcription and translation is thus not possible.  2. The DNA of a gene consists of exons separated by introns; the exons are defined by posttranscriptional splicing, which deletes the introns. 
Transcription	1. One RNA polymerase consisting of five subunits. 2. Primary transcripts are the actual mRNAs; they have a triphosphate start at the 5′ end and no tail at the 3′ end. 	1. Several kinds of RNA polymerase, each containing 10 or more subunits; different polymerases transcribe different genes. 2. Primary transcripts undergo processing to produce mature mRNAs that have a methylated cap at the 5′ end and a poly-A tail at the 3′ end. 
Translation	1. Unique initiator tRNA carries formylmethionine. 2. mRNAs have multiple ribosome binding sites and can thus direct the synthesis of several different polypeptides.  3. Small ribosomal subunit immediately binds to the mRNA's ribosome binding site. 	1. Initiator tRNA carries methionine. 2. mRNAs have only one start site and can thus direct the synthesis of only one kind of polypeptide.  3. Small ribosomal subunit binds first to the methylated cap at the 5′ end of the mature mRNA and then scans the mRNA to find the ribosome binding site. 

Homework Problems

- Chapter 14
- # 23, 26, 27,
- DON'T forget to take the online QUIZ!
- DON'T forget to submit the iActivity
  - "Cause of CF"