Molecular Biology (8)

Translation

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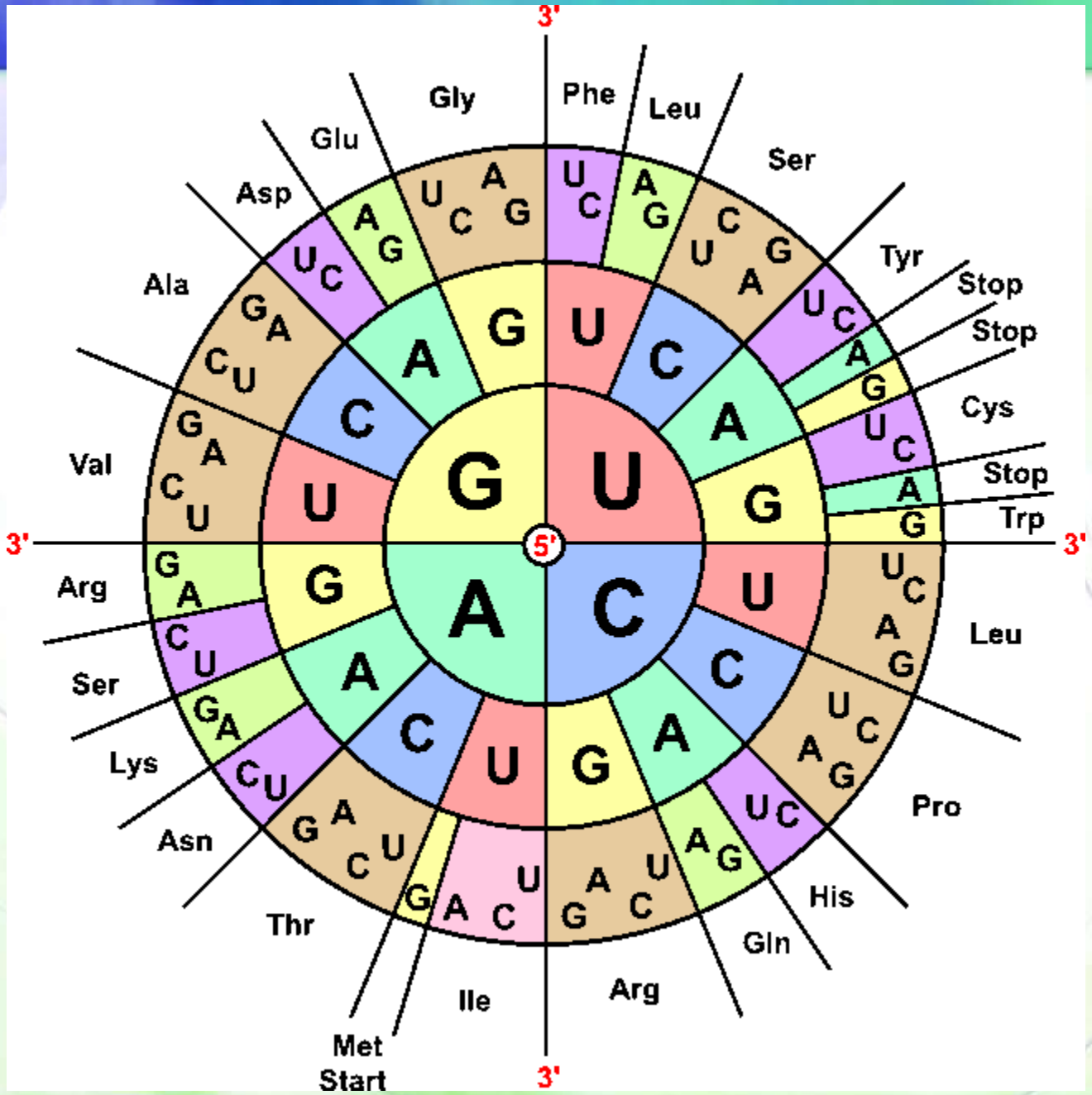
Resources

- 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


3'
5'
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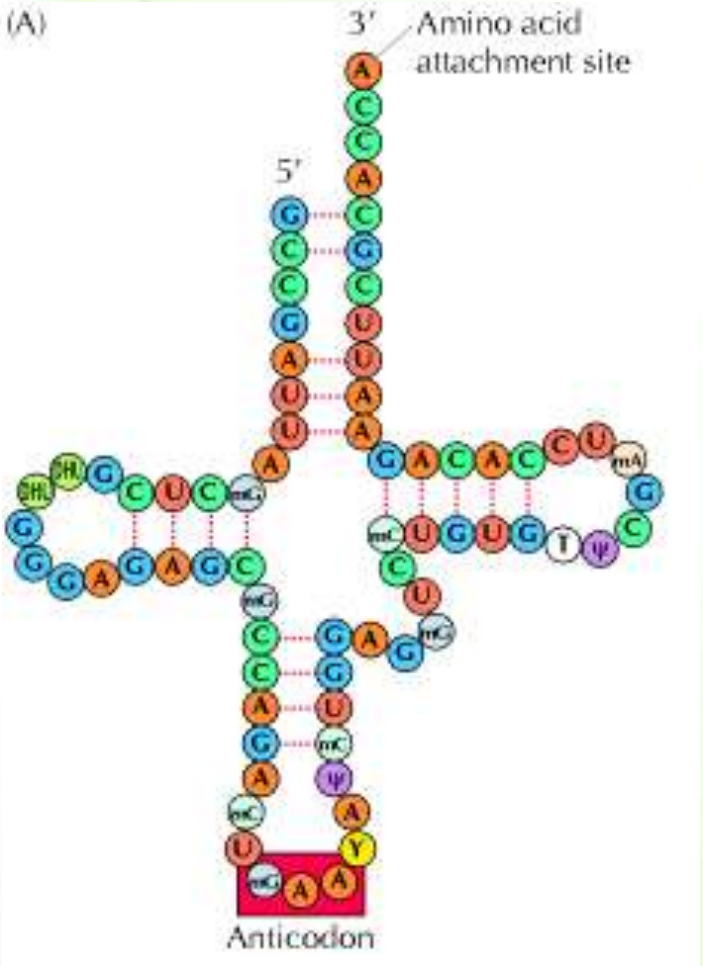
G
U
A
C
Gly
Glu
Asp
Ala
Val
Arg
Ser
Lys
Asn
Thr
Met
Start
Ile
Arg
Gln
His
Pro
Leu
Trp
Stop
Stop
Cys
Tyr
Ser
Leu
Phe

tRNA structure

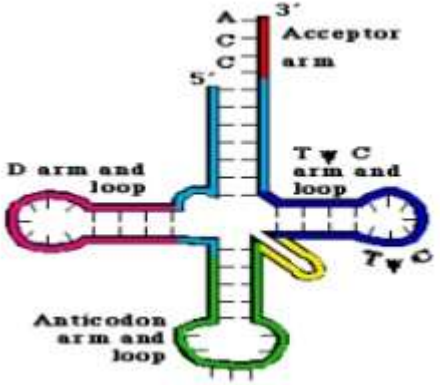
- tRNAs are short single-stranded RNA molecules (80 bases long).
- "Charged" or "activated" tRNA carries one amino acid.
- Twenty Aminoacyl-tRNA synthetases exist for each amino acid.
- An amino acid is covalently attached to the ribose of the terminal adenosine at CCA.
  - The amino acid attached to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



tRNA: coverleaf structure

- In reality, it looks like a cloverleaf (because of the presence of complementary stretches of nucleotides) with internal base pairing.

- Molecules of tRNA are not identical
  - Each carries a specific amino acid on one end (charged tRNA)
  - Each has an **anticodon** on the other end; the anticodon base-pairs with a complementary codon on mRNA



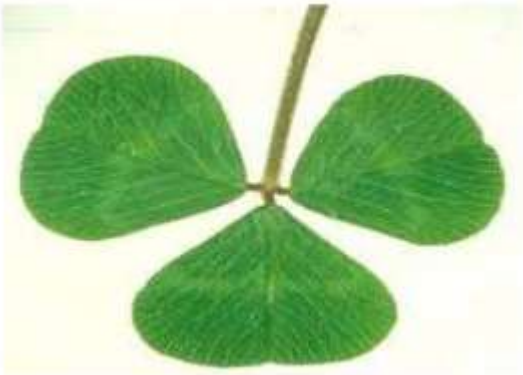
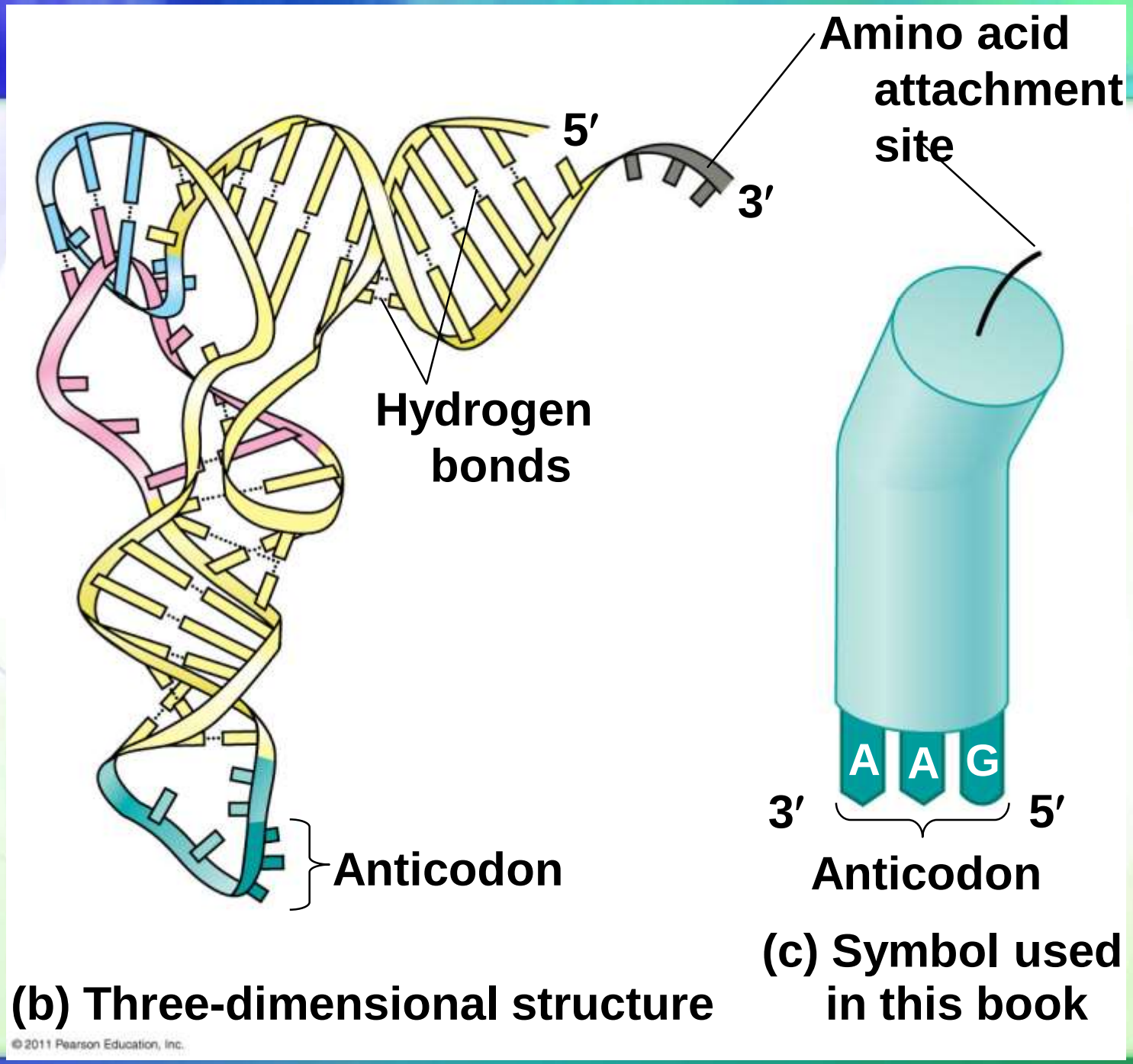


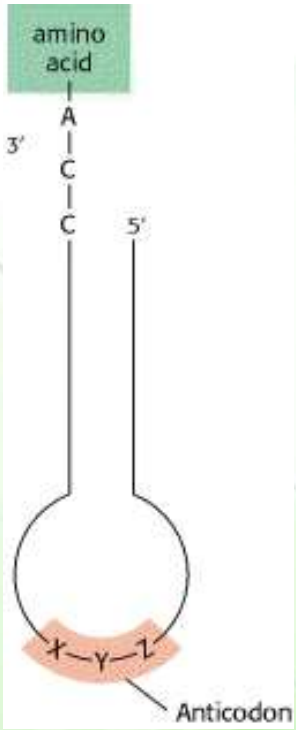
Figure 17.15b

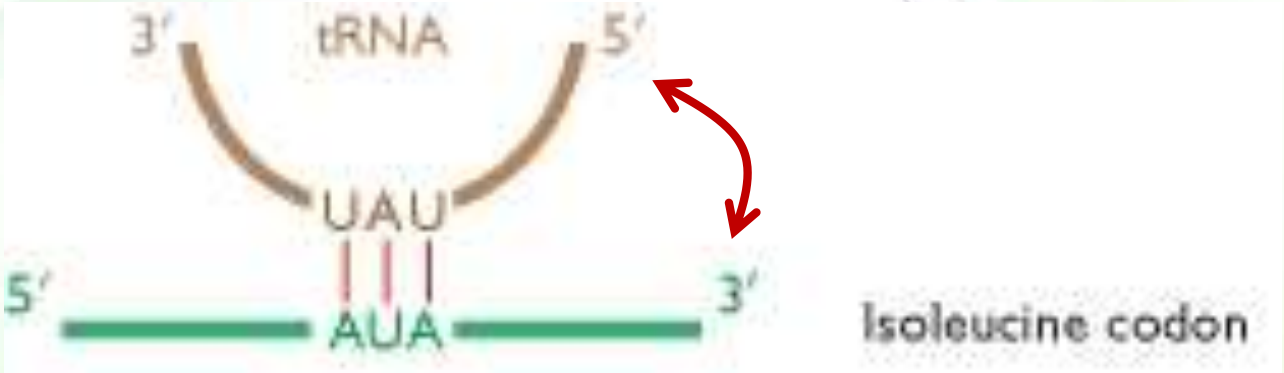


- Because of hydrogen bonds, tRNA actually twists and folds into a three-dimensional molecule
- tRNA is roughly L-shaped
- Linkage of the tRNA and a.a requires ATP
- Active site of each aminoacyl-tRNA synthetase fits only a **specific combination** of aa and tRNA
- There are **20** different synthetases, one for each a.a
- Each synthetase is able to bind all the **different tRNAs that code for its particular aa**
- Synthetase covalently join the aa to its tRNA resulting: **aminoacyl tRNA/ charged tRNA**

Codon vs. anticodon

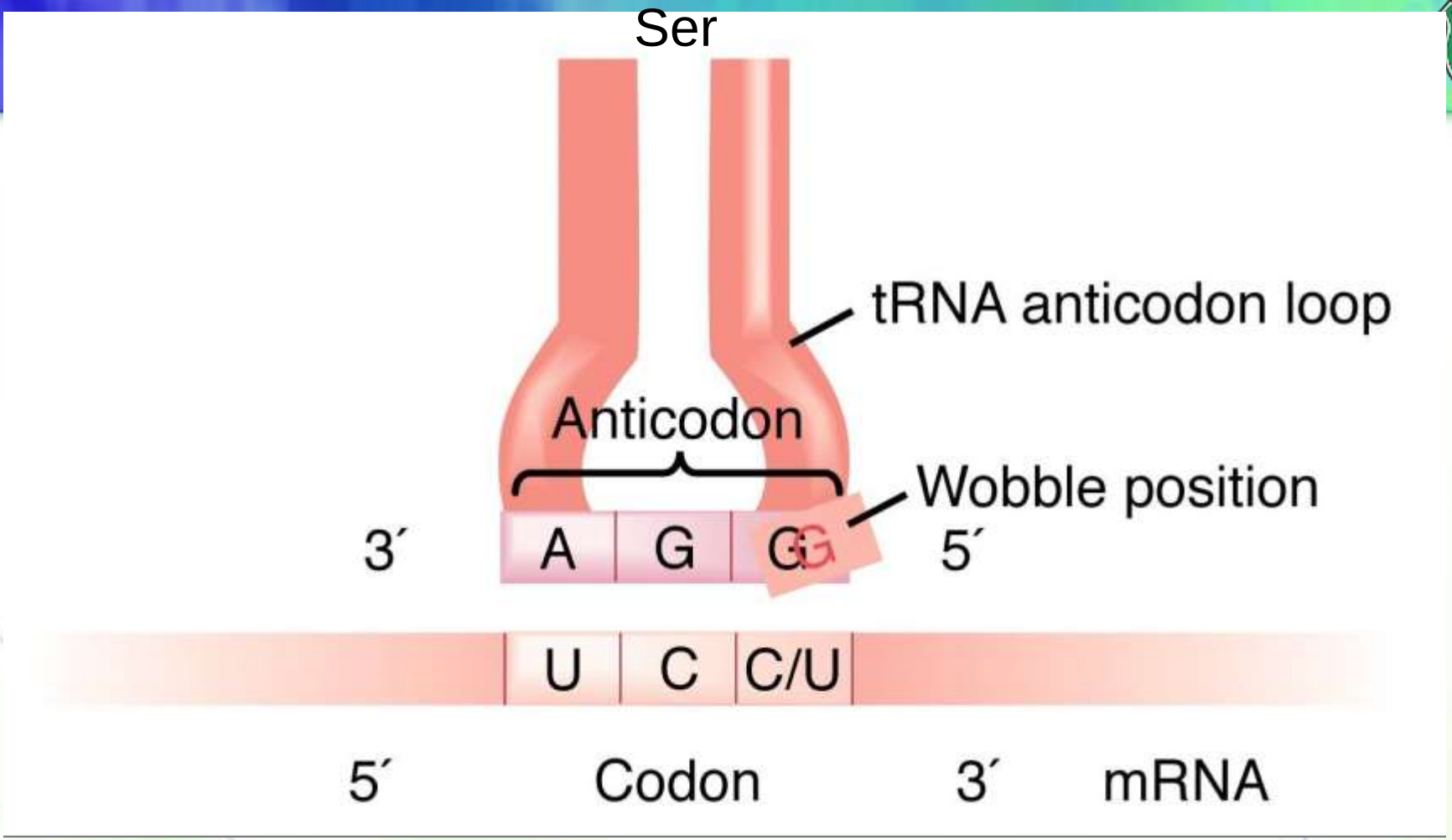
- tRNAs contain a three-nucleotide sequence known as “anticodon” that pairs with the “codon’ or “triplet” mRNA molecules (note the anti-parallel alignment of mRNA-tRNA complex)





Fidelity of translation

- Accurate translation requires two steps
  - First: a correct match between a tRNA and an amino acid, done by the enzyme **aminoacyl-tRNA synthetase**
  - Second: a correct match between the tRNA anticodon and an mRNA codon
- Flexible pairing at the third base of a codon is called **wobble** and allows some tRNAs to bind to more than one codon



This allows **mRNA** to be translated with fewer than the 61 **tRNAs**

There are only 45 tRNAs

e.g. tRNA with anti codon 5'-UCU-3' can base pair with??

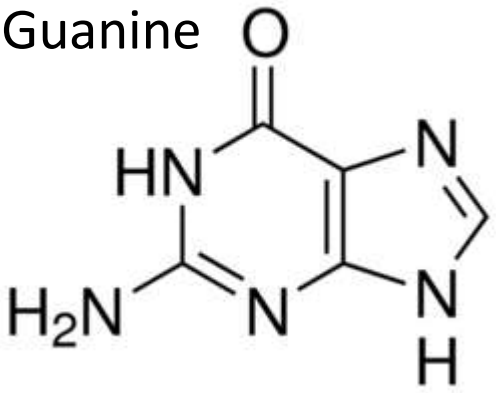
TABLE 1.6 RULES FOR BASE PAIRING CAN BE RELAXED (WOBBLE) AT POSITION 3 OF A CODON

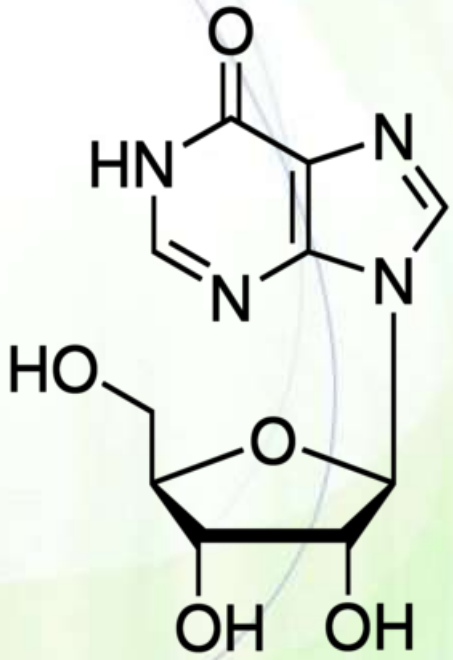
Base at 5′ end of tRNA anticodon	Base recognized at 3′ end of mRNA codon
A	U only
C	G only
G (or I)[a]	C or U
U	A or G

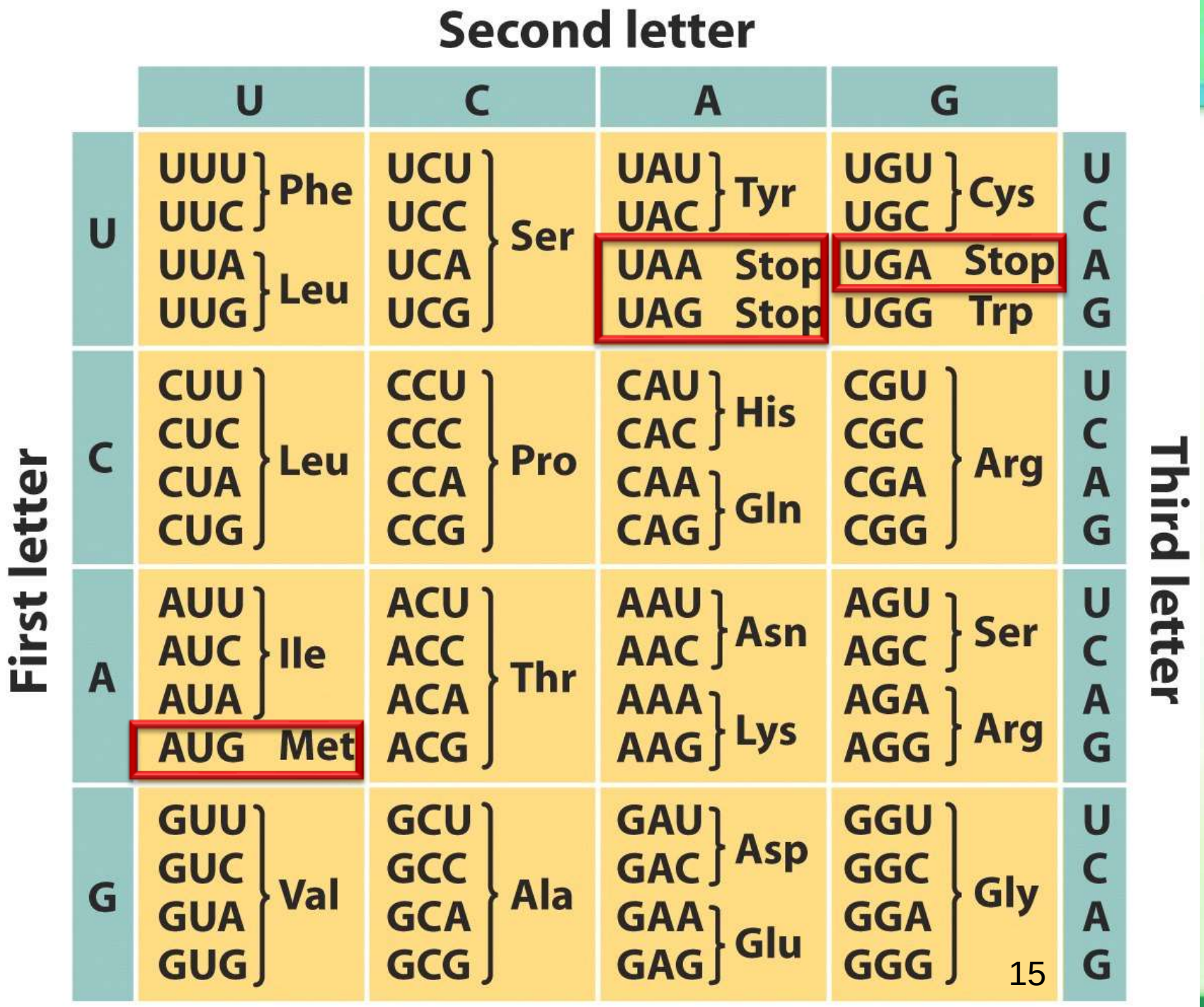
[a]Inosine (I) is a deaminated form of guanosine.

Table 1.6 Human Molecular Genetics, 4ed. (© Garland Science)

Guanine

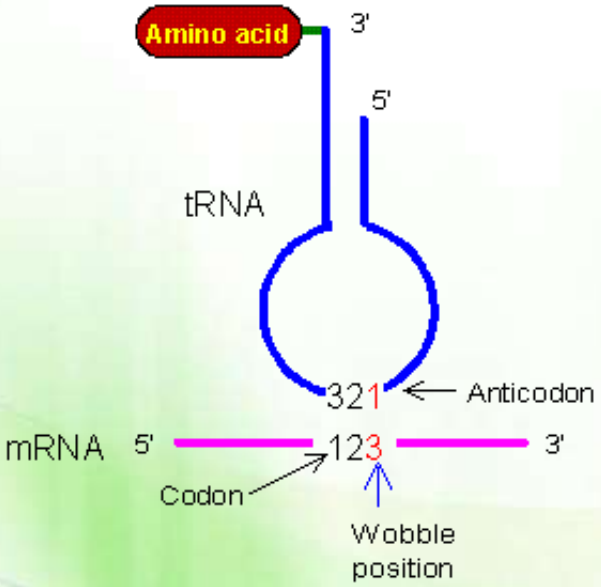





Second letter
U
C
A
G
First letter
Third letter
U
UUU Phe
UUC
UUA Leu
UUG
UCU Ser
UCC
UCA
UCG
UAU Tyr
UAC
UAA Stop
UAG Stop
UGU Cys
UGC
UGA Stop
UGG Trp
C
CUU Leu
CUC
CUA
CUG
CCU Pro
CCC
CCA
CCG
CAU His
CAC
CAA Gln
CAG
CGU Arg
CGC
CGA
CGG
A
AUU Ile
AUC
AUA
AUG Met
ACU Thr
ACC
ACA
ACG
AAU Asn
AAC
AAA 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 codon

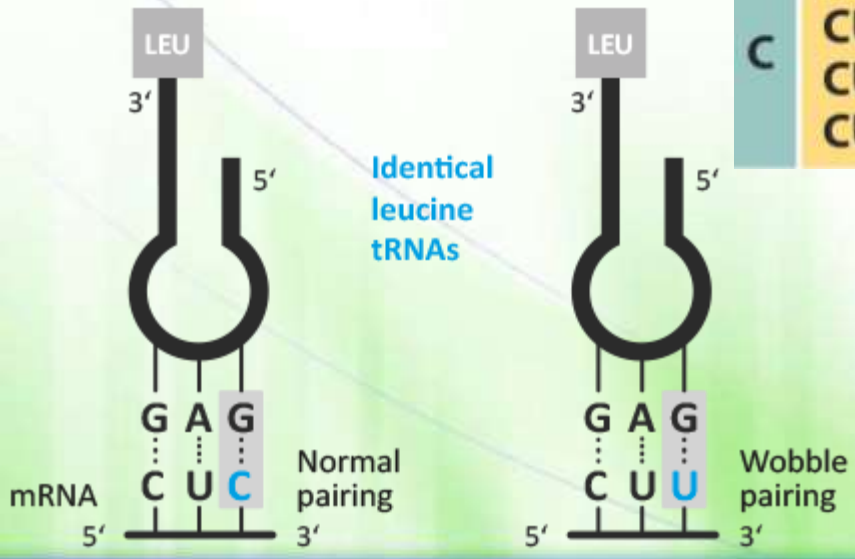
- Not universal
  - Example: AUA in mitochondria (methionine) in cytosol (isoleucine)
- Wobble base pairing (degenerate and nonstandard)
  - The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base, which is called the “wobble”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



Examples of wobble base pairing

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

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



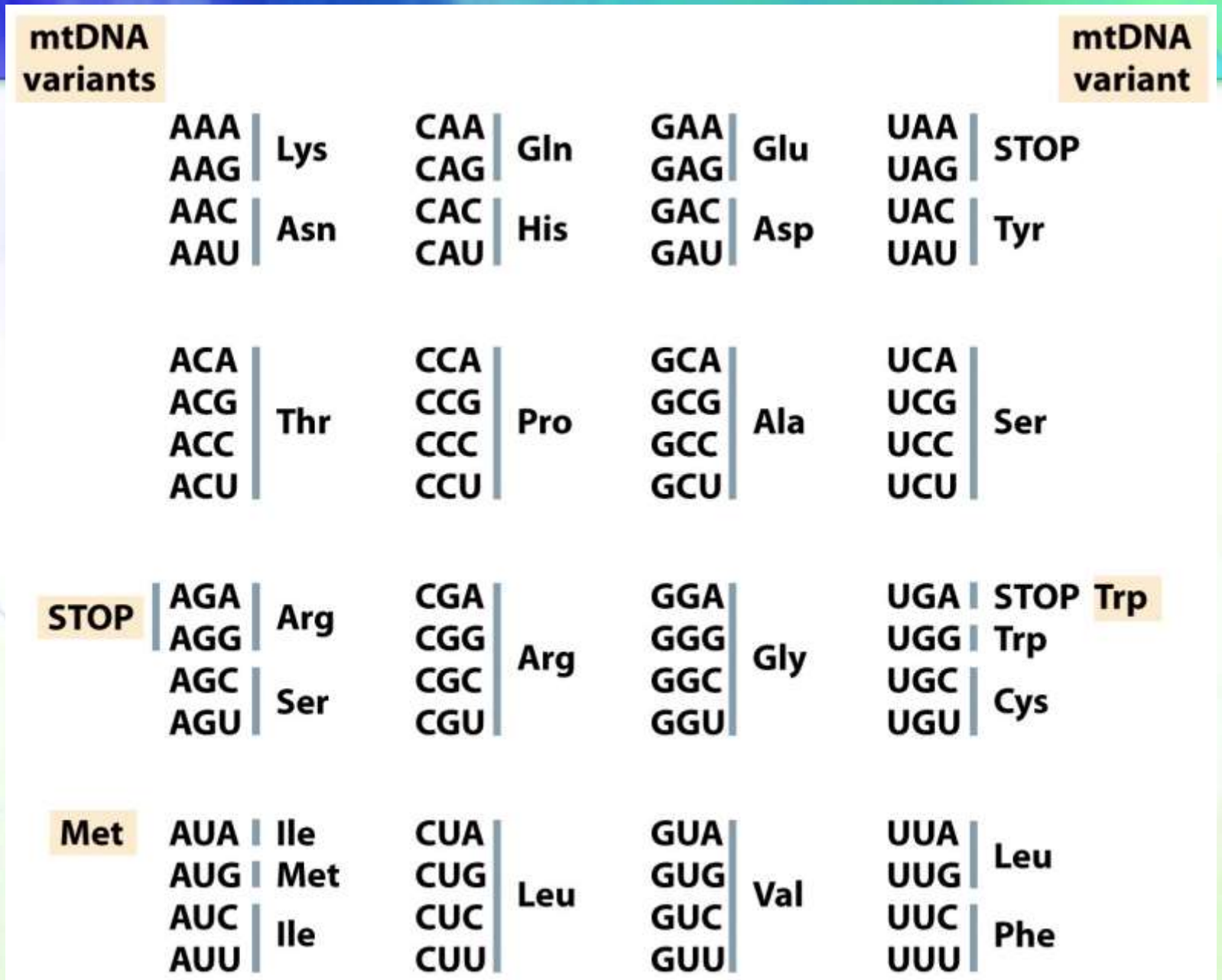


Figure 1.25 Human Molecular Genetics, 4ed. (© Garland Science)

All 64 possible codons of the genetic code and the amino acid specified by each, as read in the 5′→3′ direction from the mRNA sequence.

The interpretations of the 64 codons in the 'universal' genetic code are shown in black immediately to the right of the codons.

Sixty-one codons specify an amino acid.

Three STOP codons (UAA, UAG, and UGA) do not encode any amino acid.

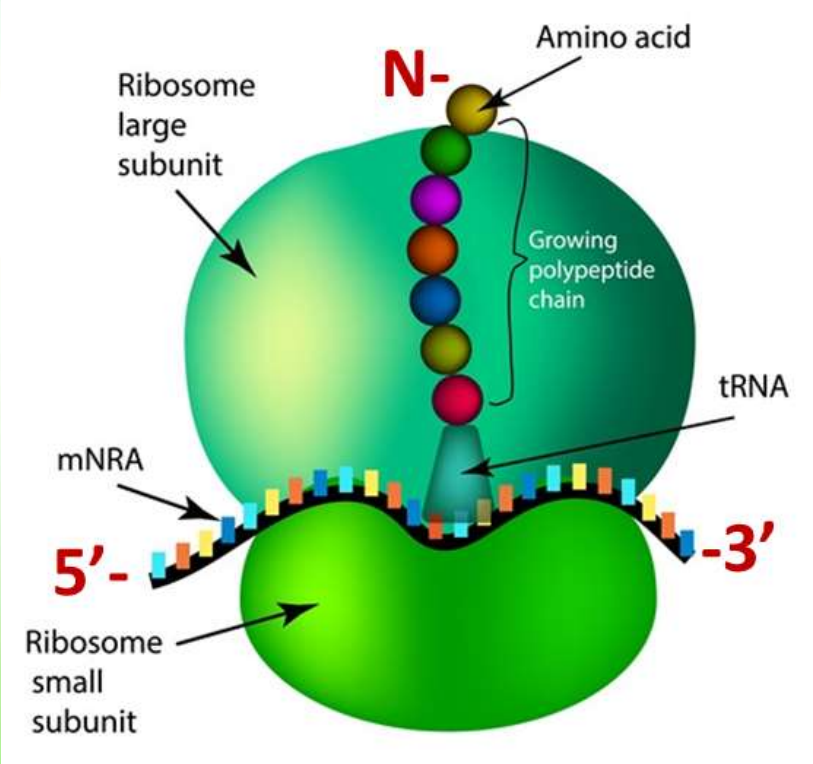
The genetic code for mitochondrial mRNA (mtDNA) conforms to the universal code except for a few variants.

For example, in the mitochondrial genetic code in humans and many other species four codons are used differently:
UGA encodes tryptophan instead of being a STOP codon,
AUA encodes methionine,
and instead of encoding arginine, AGA and AGG are STOP codons.

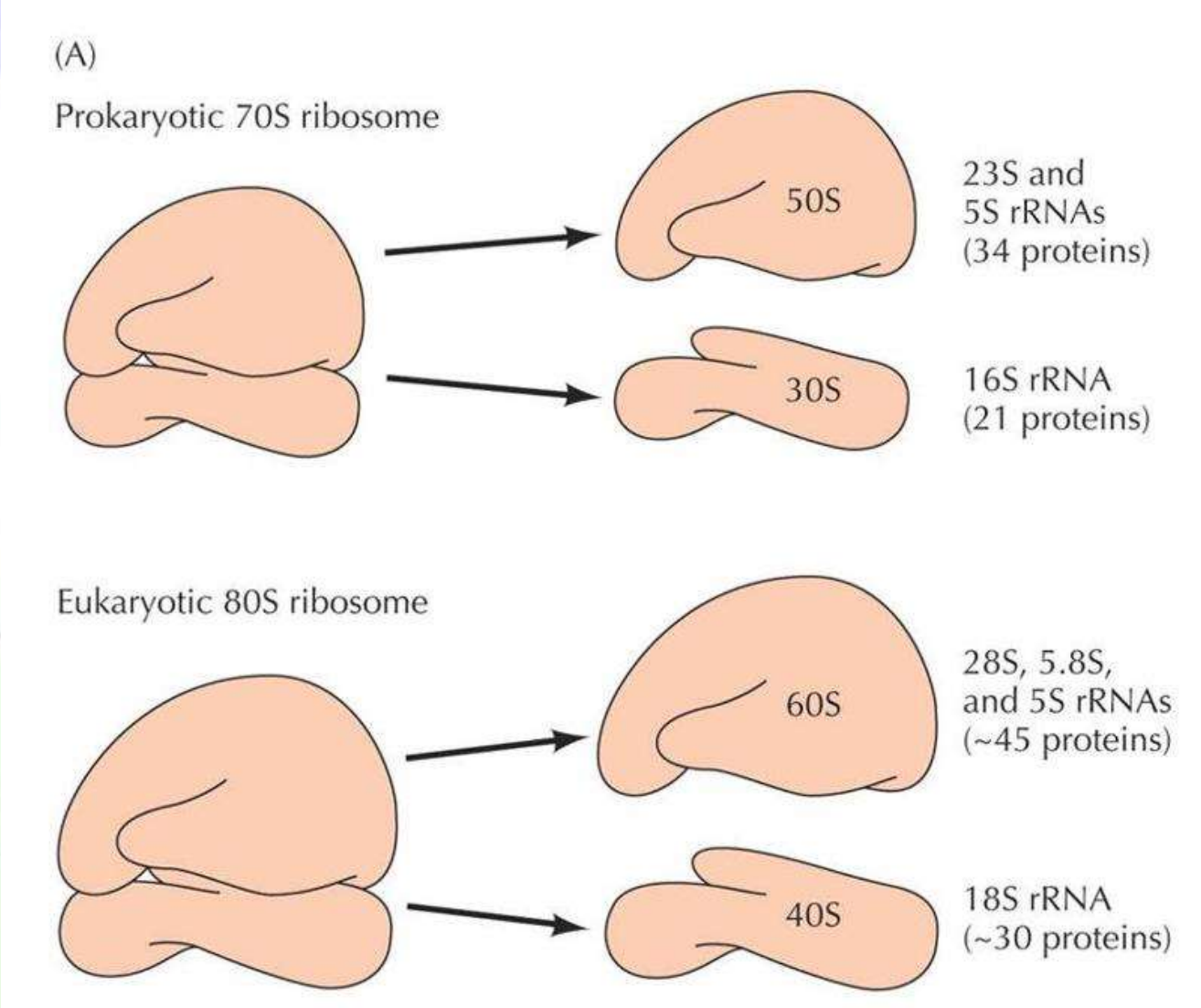
Ribosomes

- 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 major rRNA species are synthesized by cleavage of a shared primary transcript

(A) In human cells, the 18S, 5.8S, and 28S rRNAs are encoded by a single transcription unit that is **13 kb long**. It occurs within tandem repeat units of about **40 kb** that also includes a roughly **27 kb non-transcribed (intergenic) spacer**.

(B) Transcription by RNA polymerase I produces a **13 kb primary transcript** (45S rRNA) that then undergoes a **complex series of post-transcriptional cleavages**

(C–E) Ultimately, individual 18S, 28S, and 5.8S rRNA molecules are released
The 18S rRNA will form part of the small ribosomal subunit
The 5.8S rRNA binds to a complementary segment of the 28S rRNA; the resulting complex will form part of the large ribosomal subunit

The latter also contains 5S rRNA, which is encoded separately by dedicated genes transcribed by RNA polymerase III.

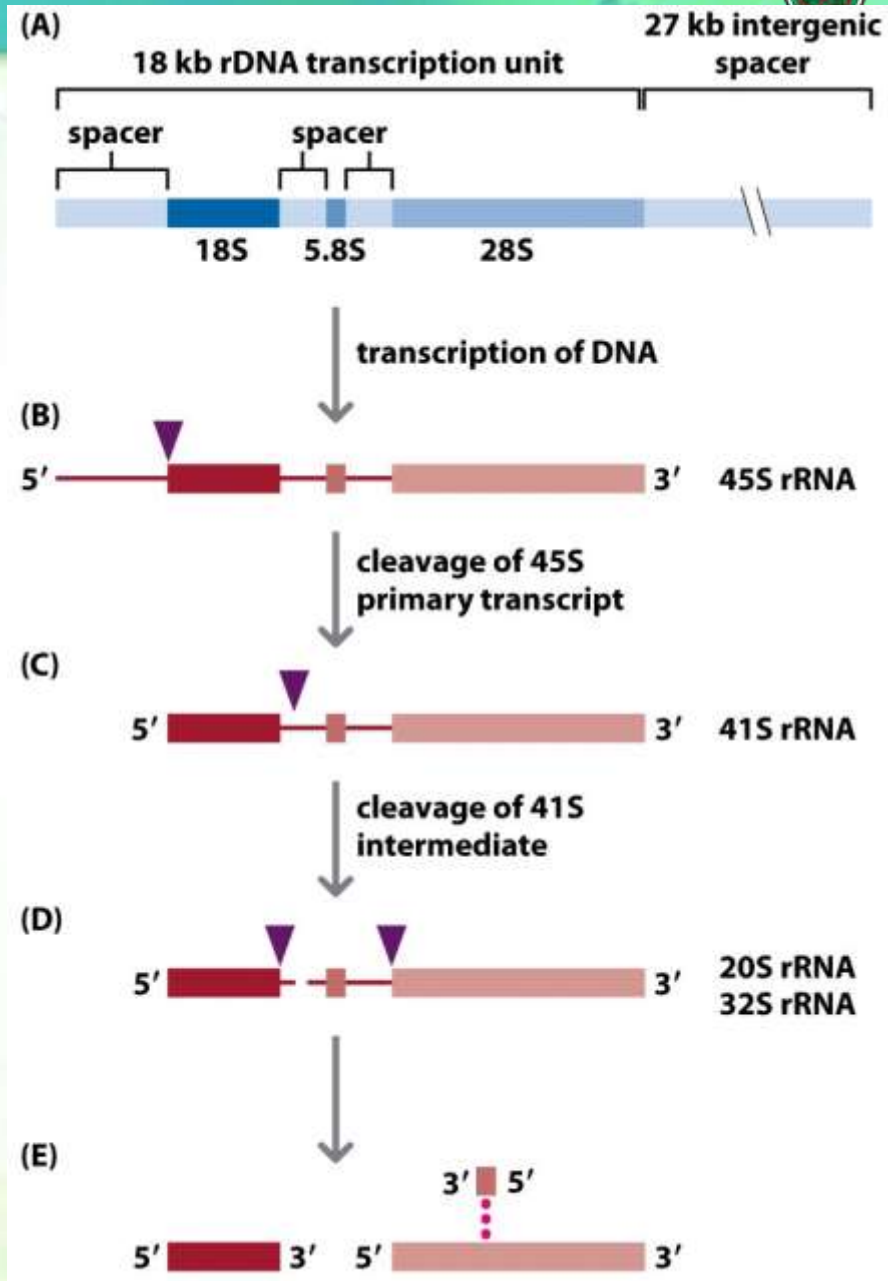
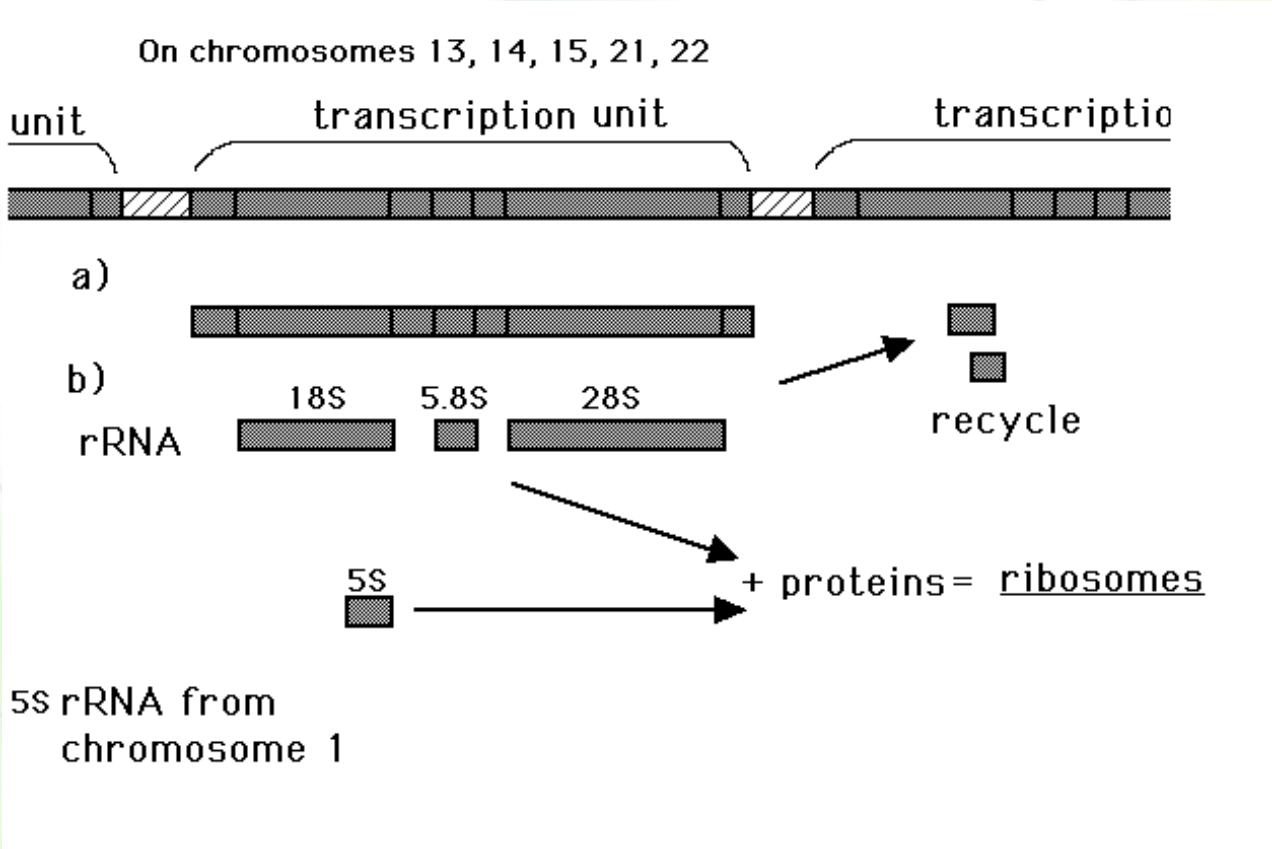


Figure 1.22 Human Molecular Genetics, 4ed. (© Garland Science)

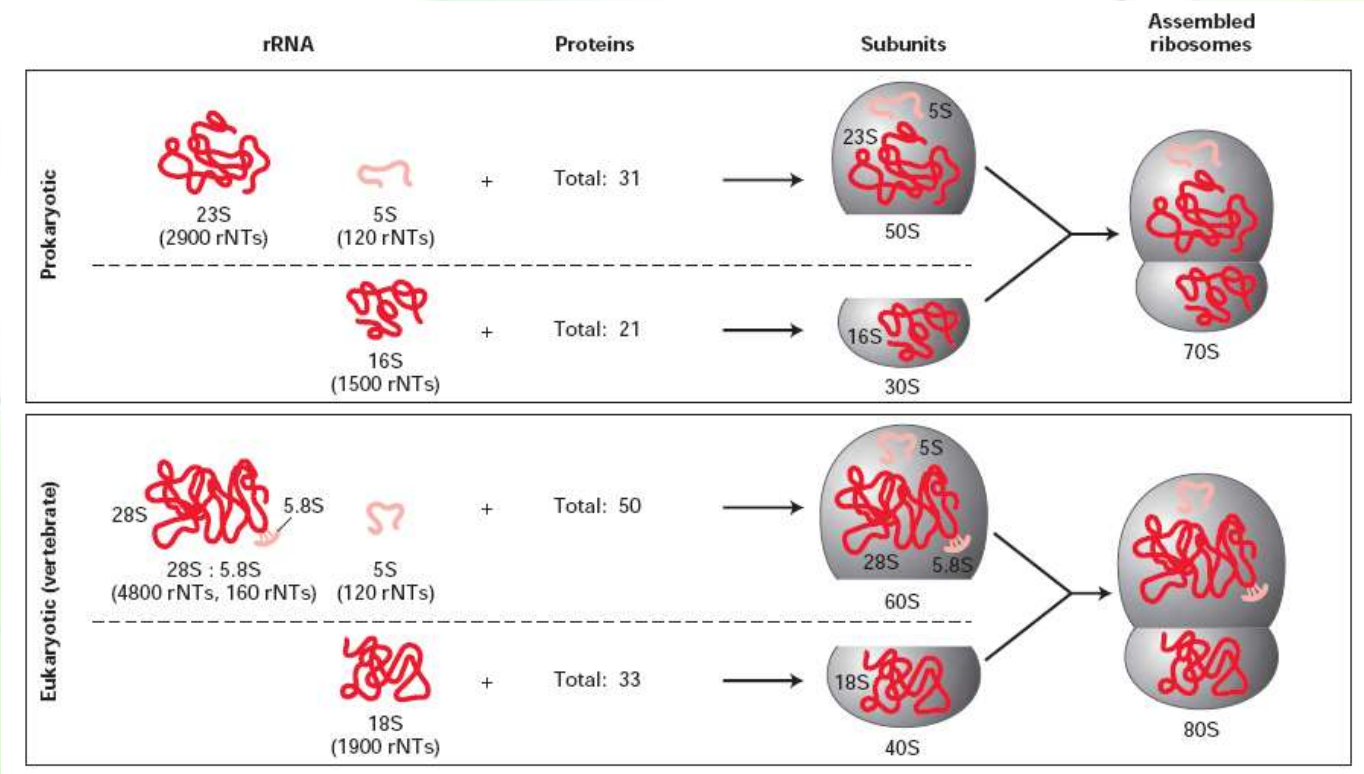
Ribosomal 5s rRNA

- In human cells, a cluster of around 250 genes on several chromosomes synthesize 5S rRNA using Pol III



Ribosomes

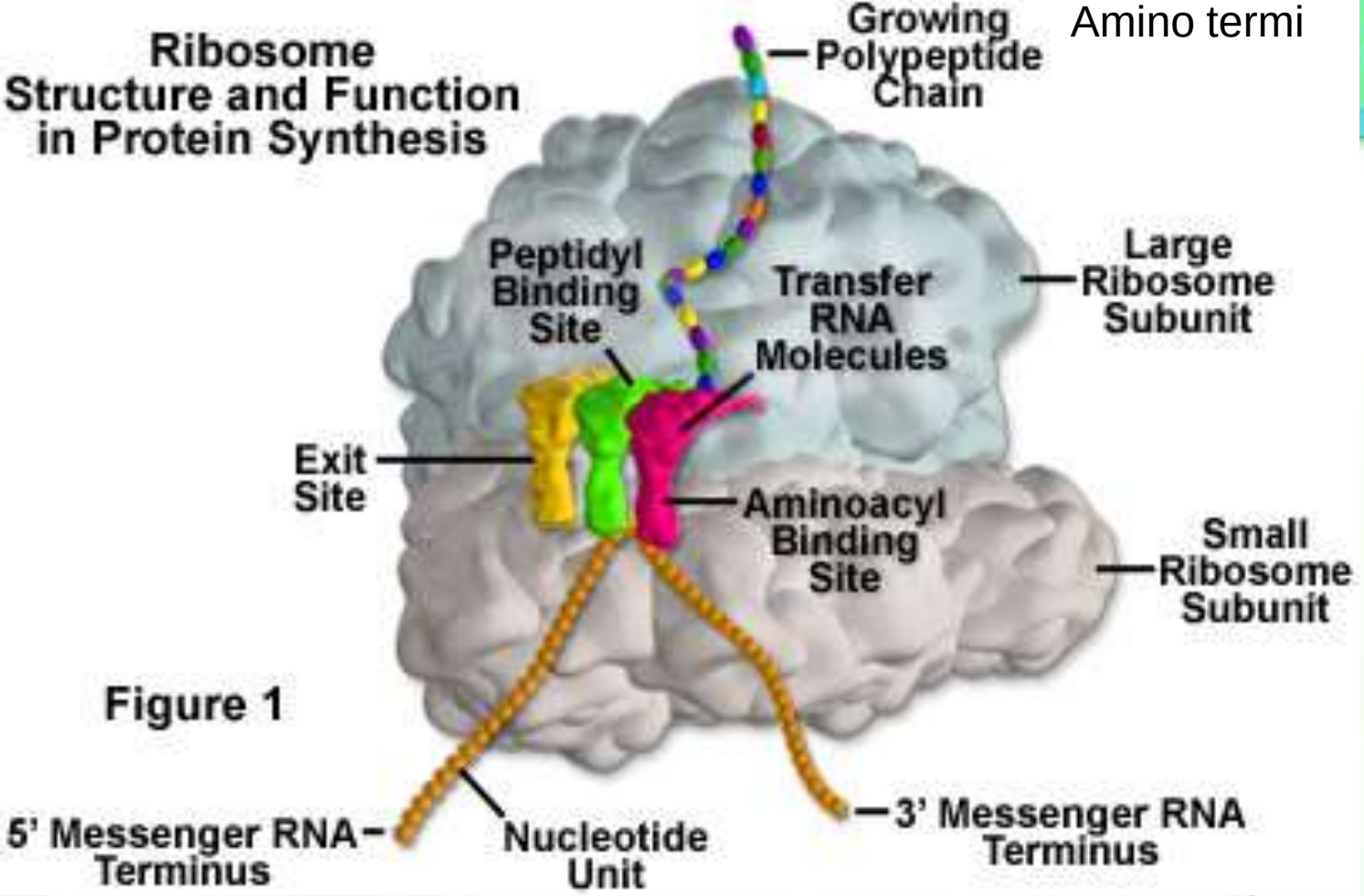
- Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis
- The RNA components are predominantly responsible for the catalytic function of the ribosome, the protein components enhance the function of the rRNA molecules



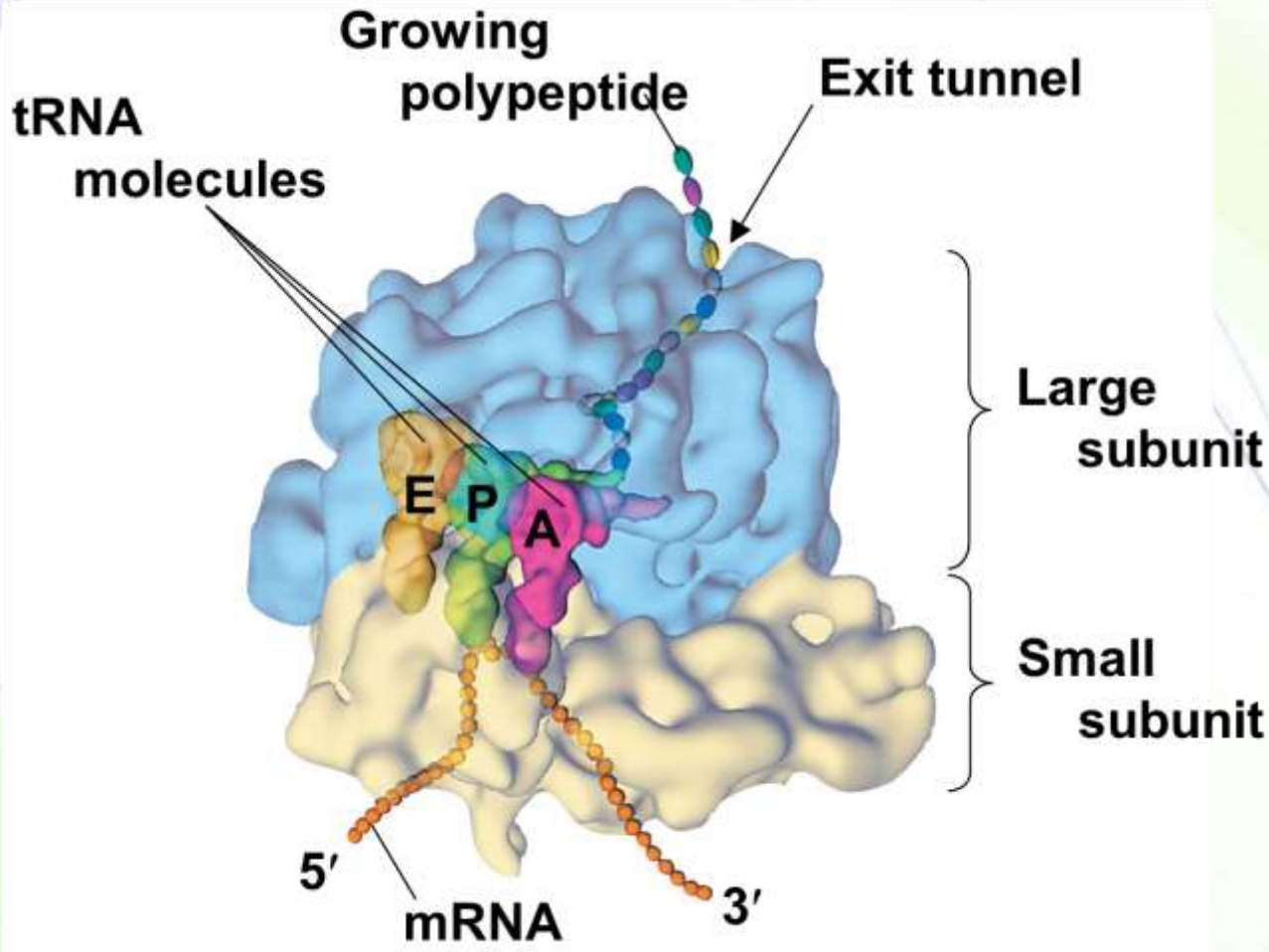
Ribosomes

- rRNA is the most **abundant** type of cellular RNA
- rRNA genes are transcribed, the RNA is processed and assembled with proteins **imported** from the cytoplasm, the resulting ribosomal subunits are then **exported** via nuclear pores to the cytoplasm
- Large and small subunits **join** to form a functional ribomes only when they attach to the mRNA
- Bacterial and eukaryotic ribosomes are somewhat similar but have significant differences: some antibiotic drugs (tetracycline and streptomycin) specifically target bacterial ribosomes and inhibit protein synthesis without harming eukaryotic ribosomes
- They can be regarded as ribozymes

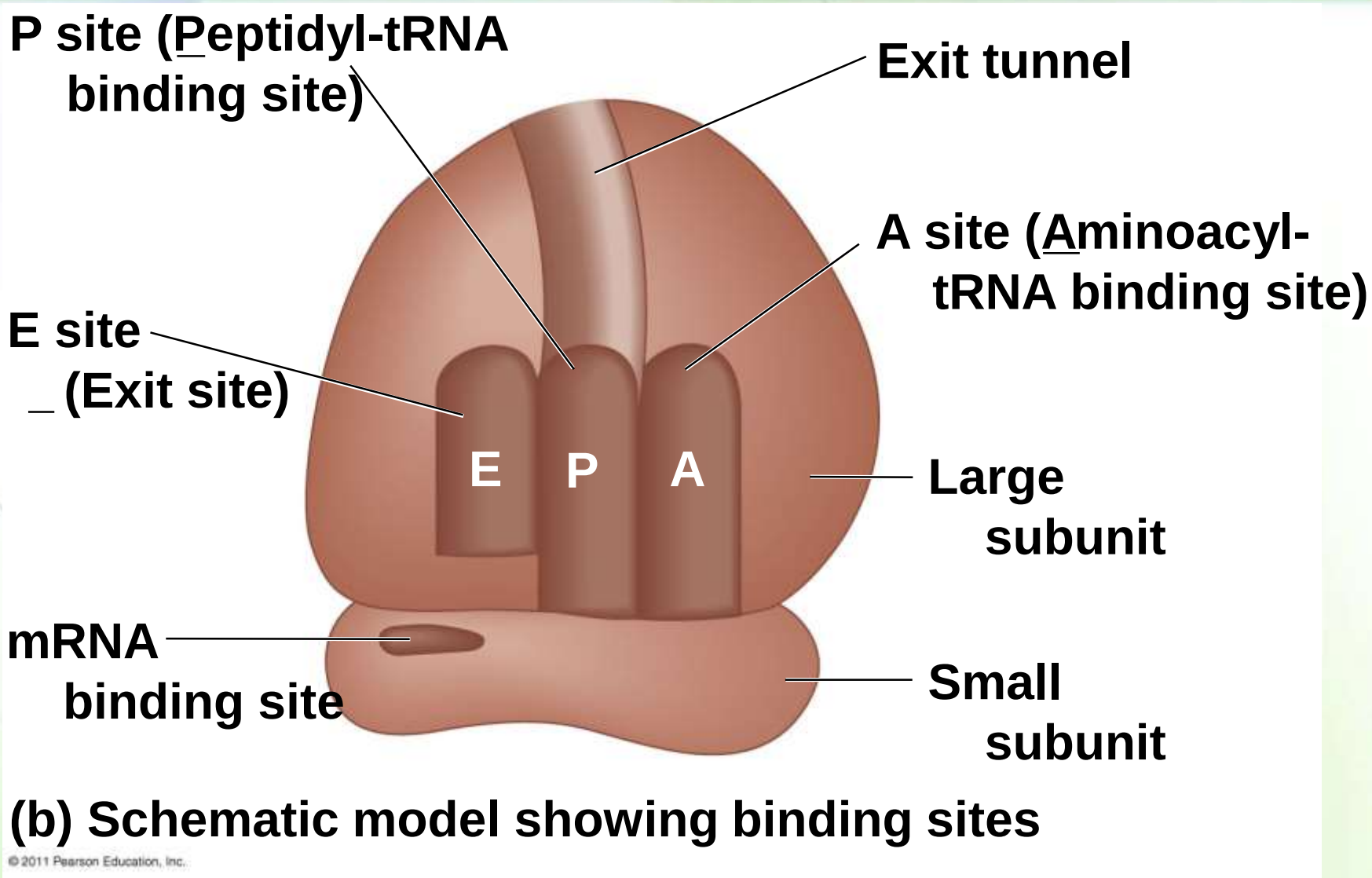
Amino termi



tRNA binding sites on a ribosome:
Aminoacyl-tRNA binding site for a tRNA molecule attached to the next amino acid in the protein (mRNA enters the ribosome at the A site)
Peptidyl-tRNA binding site for the central tRNA molecule containing the growing peptide chain (peptidyl-tRNA, i.e. the tRNA carrying the growing peptide chain)
exit binding site to discharge used tRNA molecules from the ribosome.



(a) Computer model of functioning ribosome


P site (Peptidyl-tRNA binding site)
Exit tunnel
A site (Aminoacyl-tRNA binding site)
E site (Exit site)
E
P
A
Large subunit
mRNA binding site
Small subunit
(b) Schematic model showing binding sites
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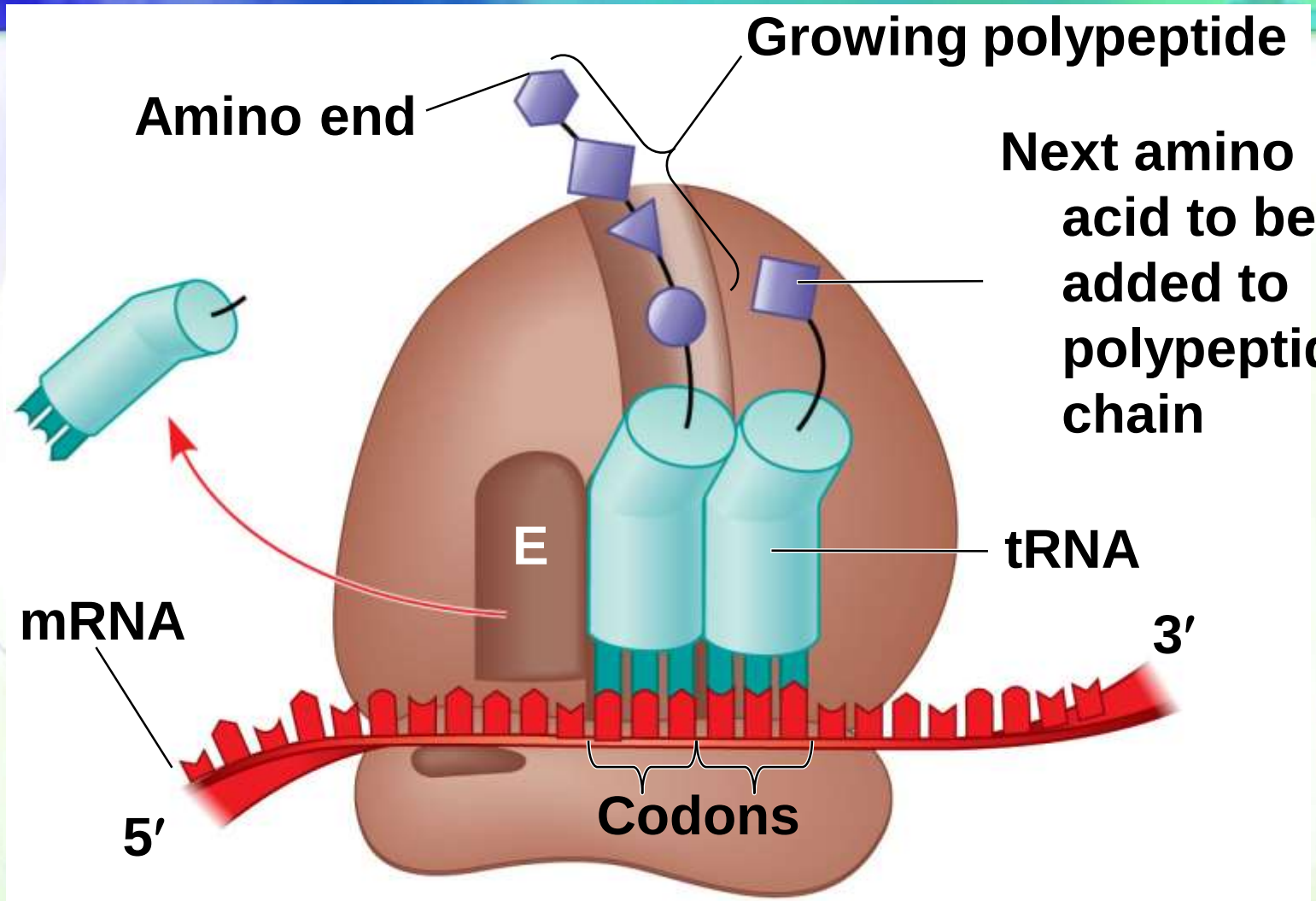
The tRNA-binding sites of ribosomes

- A ribosome has three binding sites for tRNA
  - The **P site** holds the tRNA that carries the growing polypeptide chain
  - The **A site** holds the tRNA that carries the next amino acid to be added to the chain
  - The **E site** is the exit site, where discharged tRNAs leave the ribosome

Figure 17.17c



(c) Schematic model with mRNA and tRNA

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.

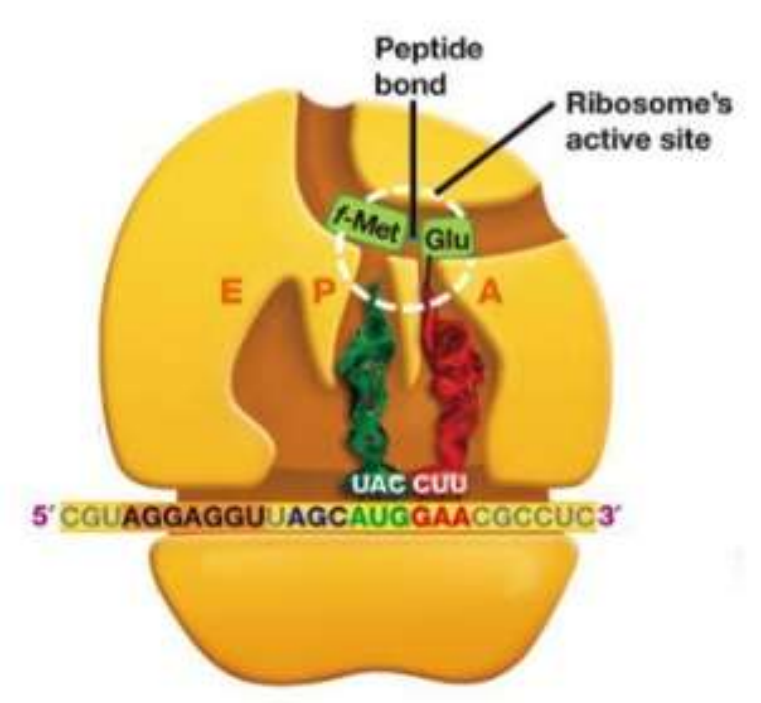


Fig1.24 In translation, the genetic code is deciphered on ribosomes by codon–anticodon recognition

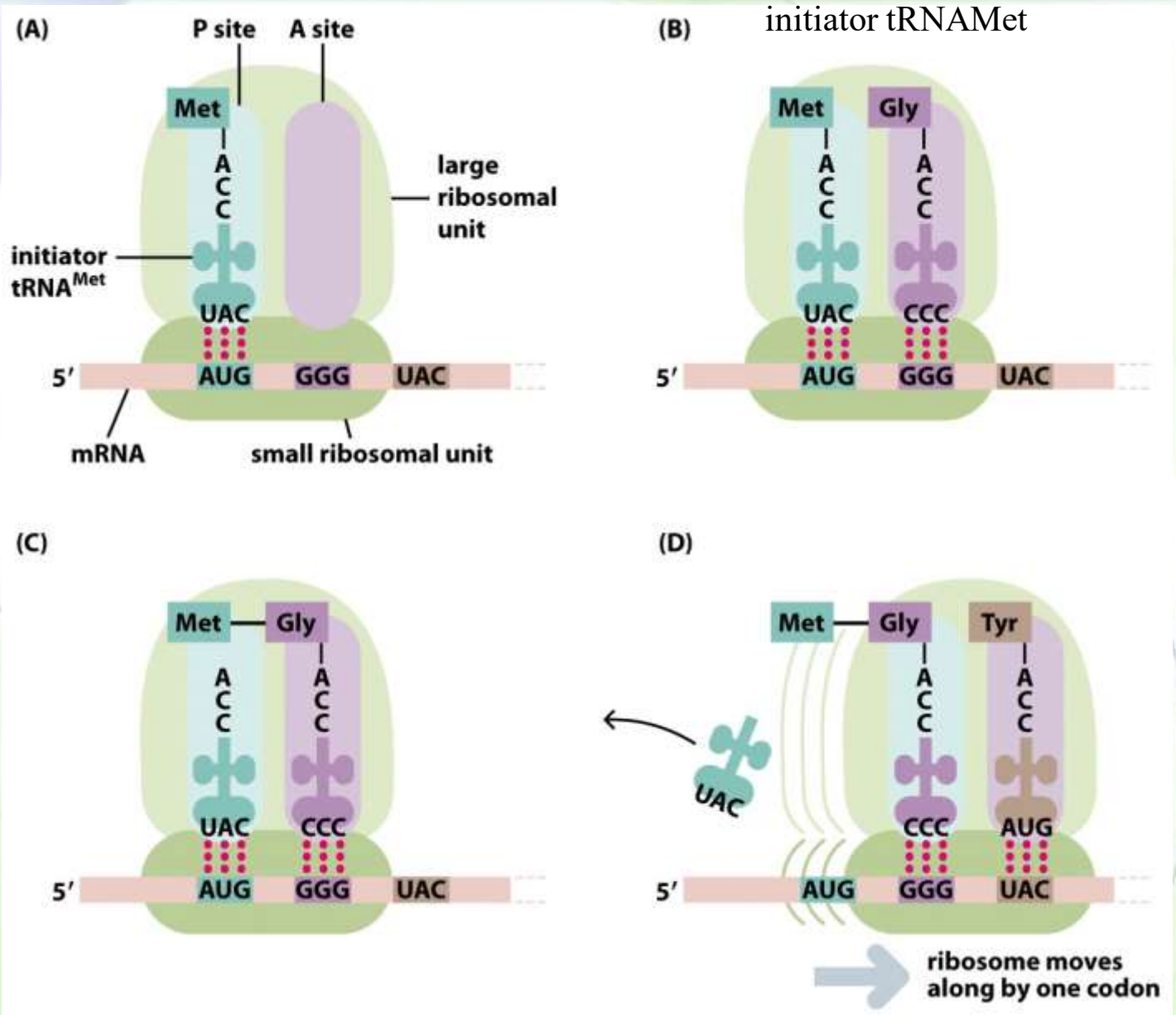
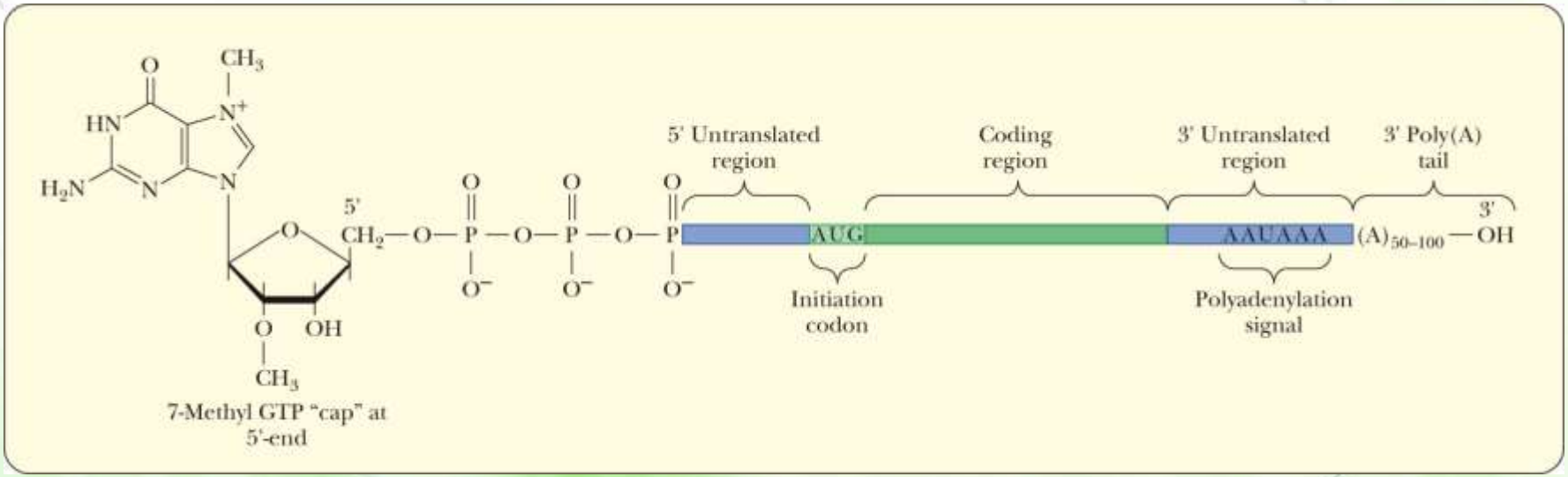


Figure 1.24a-d Human Molecular Genetics, 4ed. (© Garland Science)

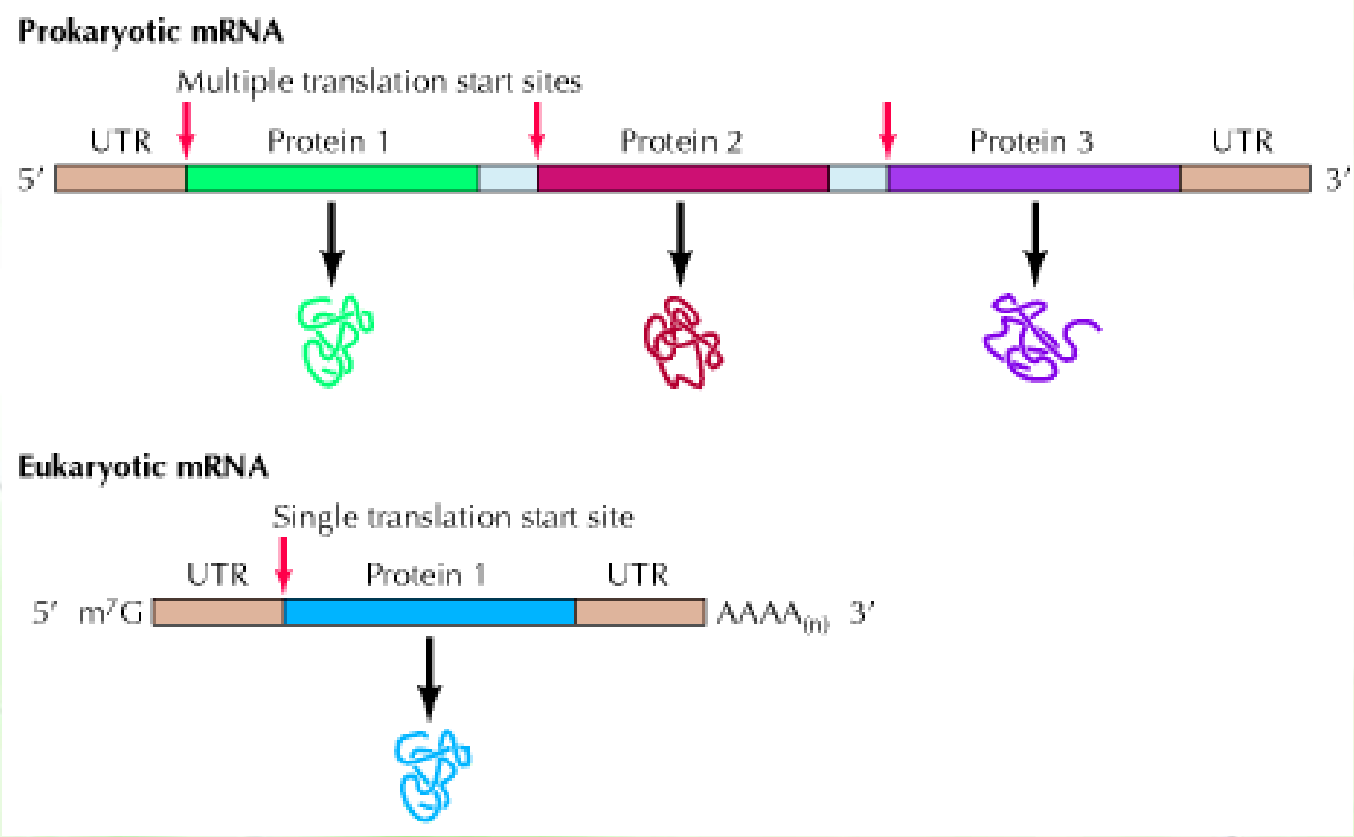
Start of translation

- 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.

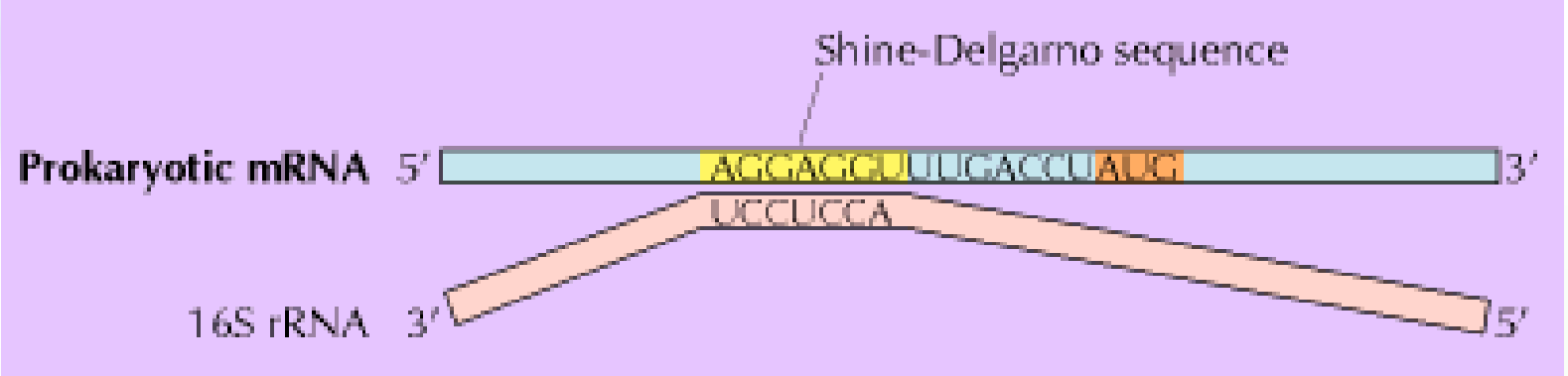


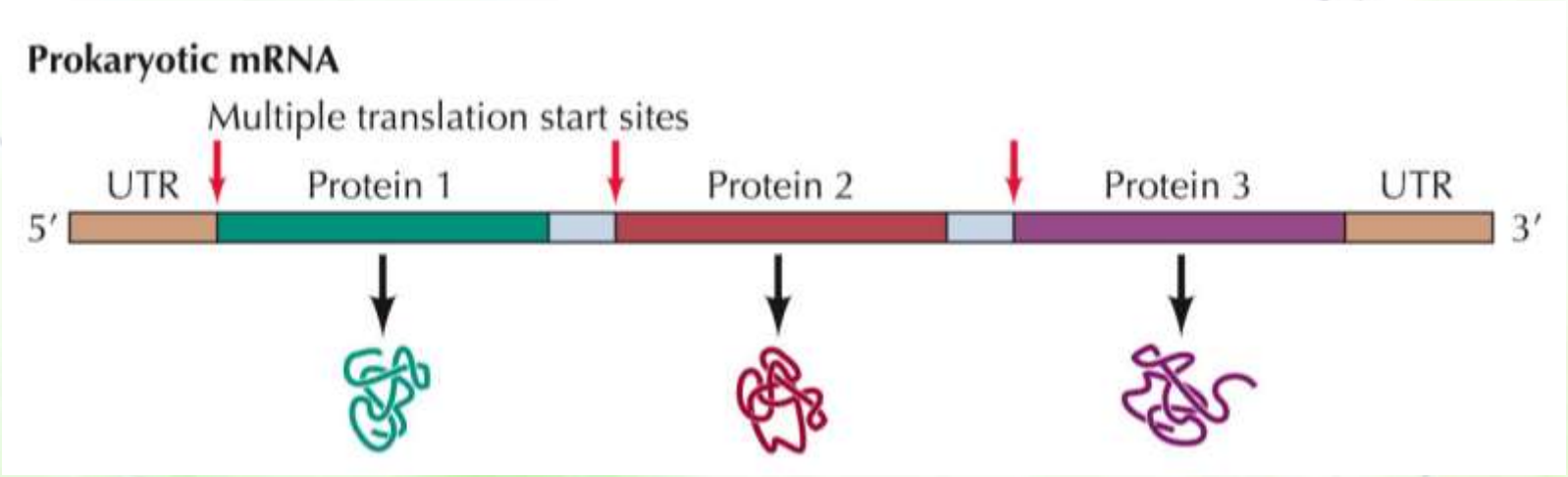
Remember…

- Bacterial mRNA is polycistronic
- Eukaryotic mRNA is monocistronic



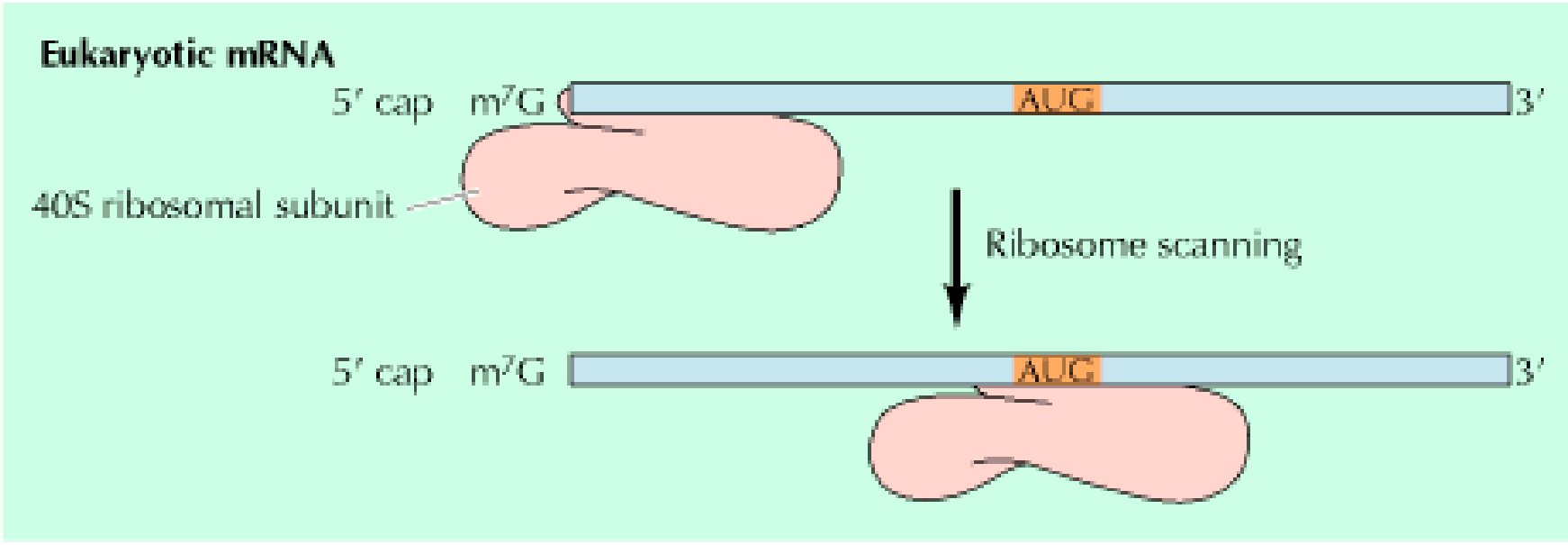
Shine-Dalgarno sequence





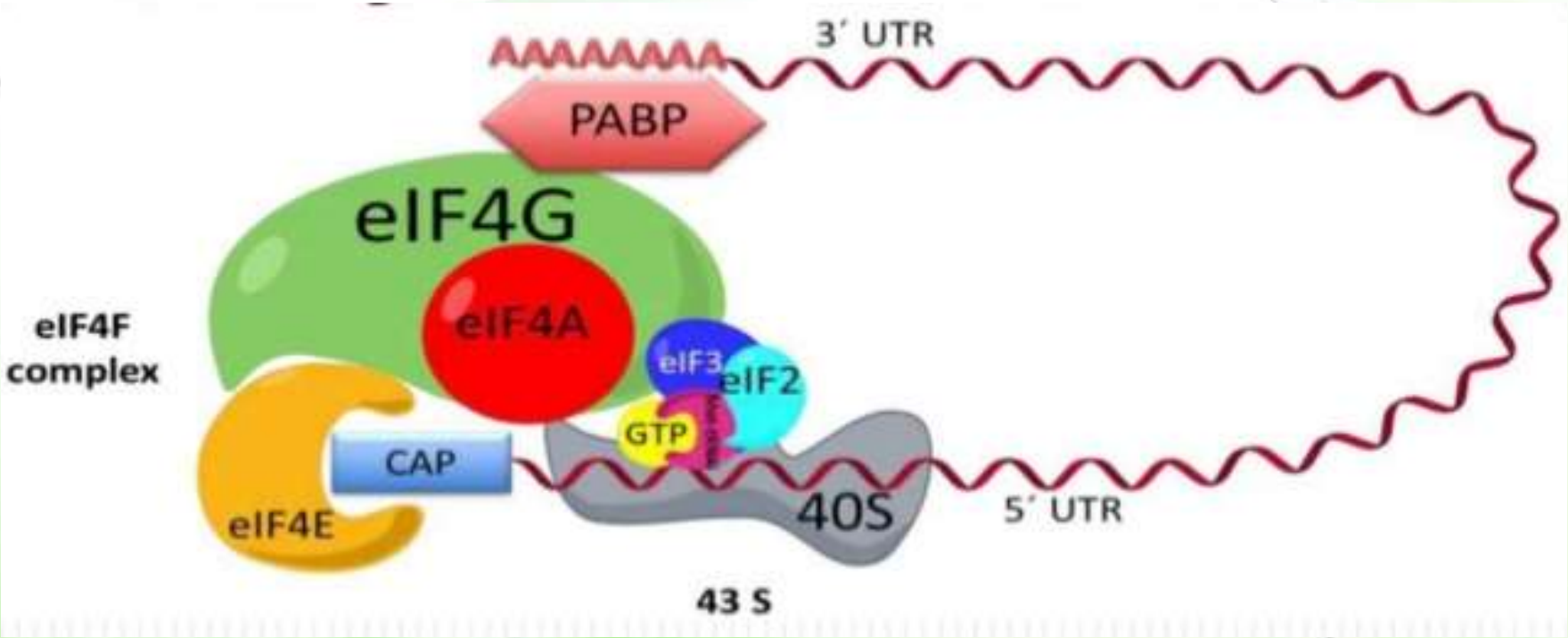
But in eukaryotes…

- 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.

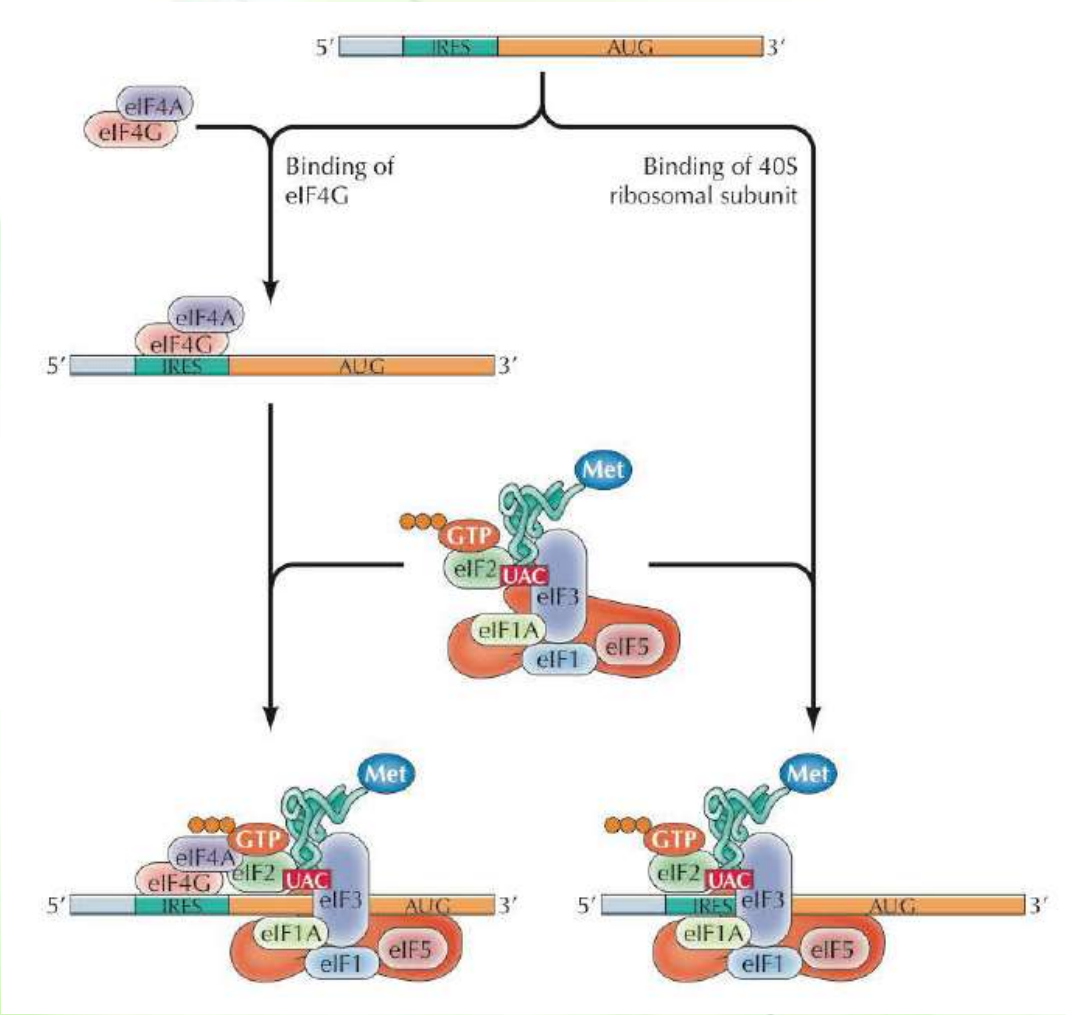
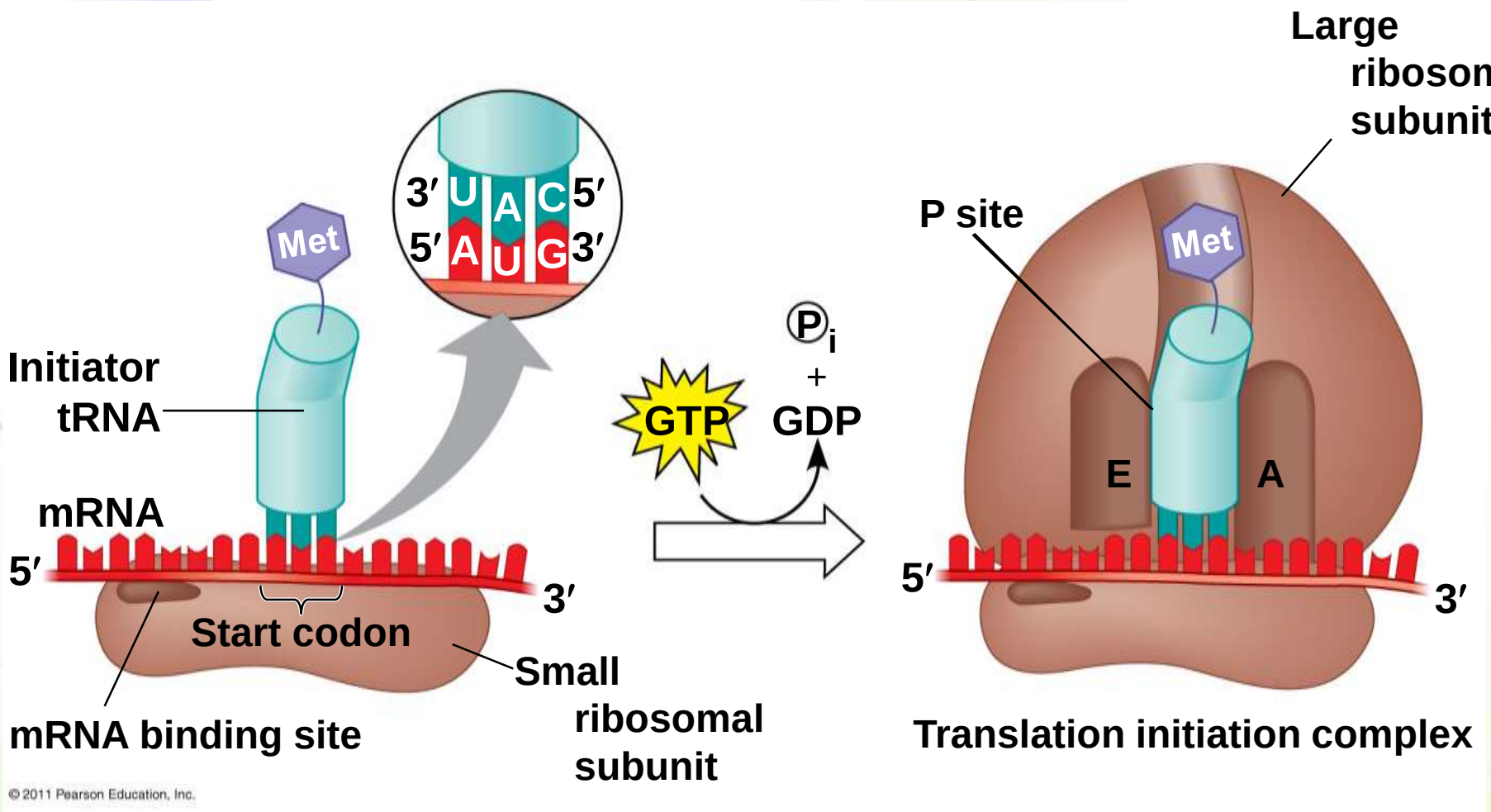


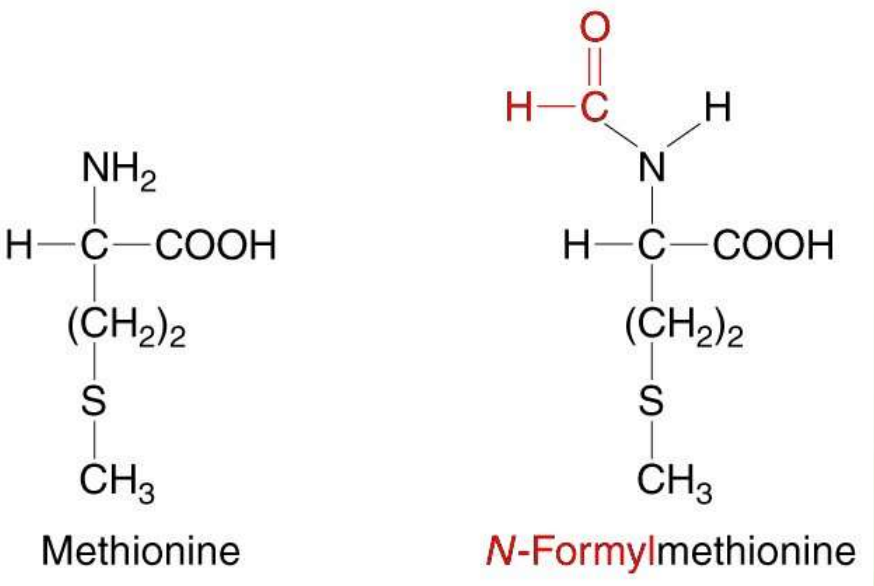
Figure 17.18



The first amino acid

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

- In most bacteria, it is N-formylmethionine.



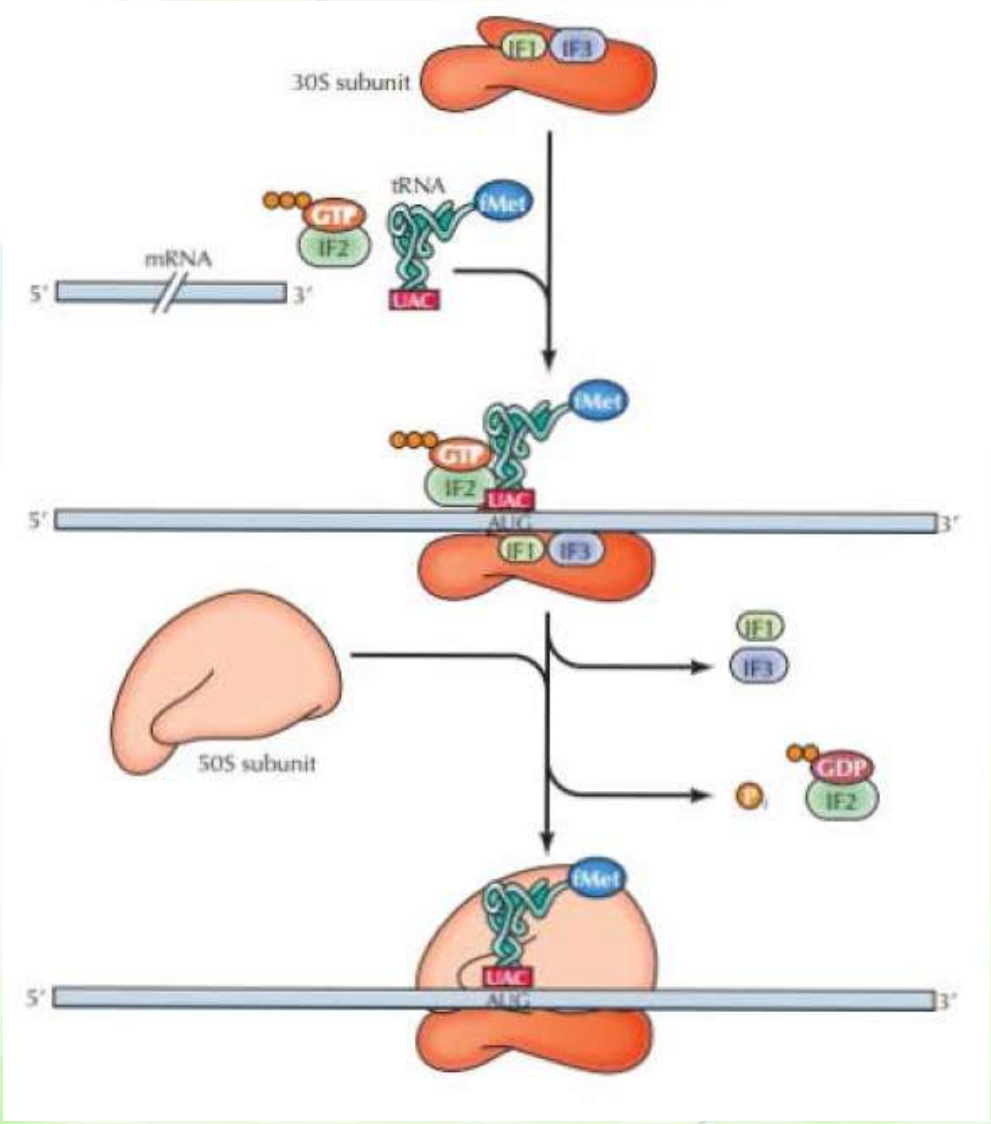
Building a Polypeptide

- The three stages of translation
  - Initiation
  - Elongation
  - Termination
- All three stages require protein “factors” that aid in the translation process

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.

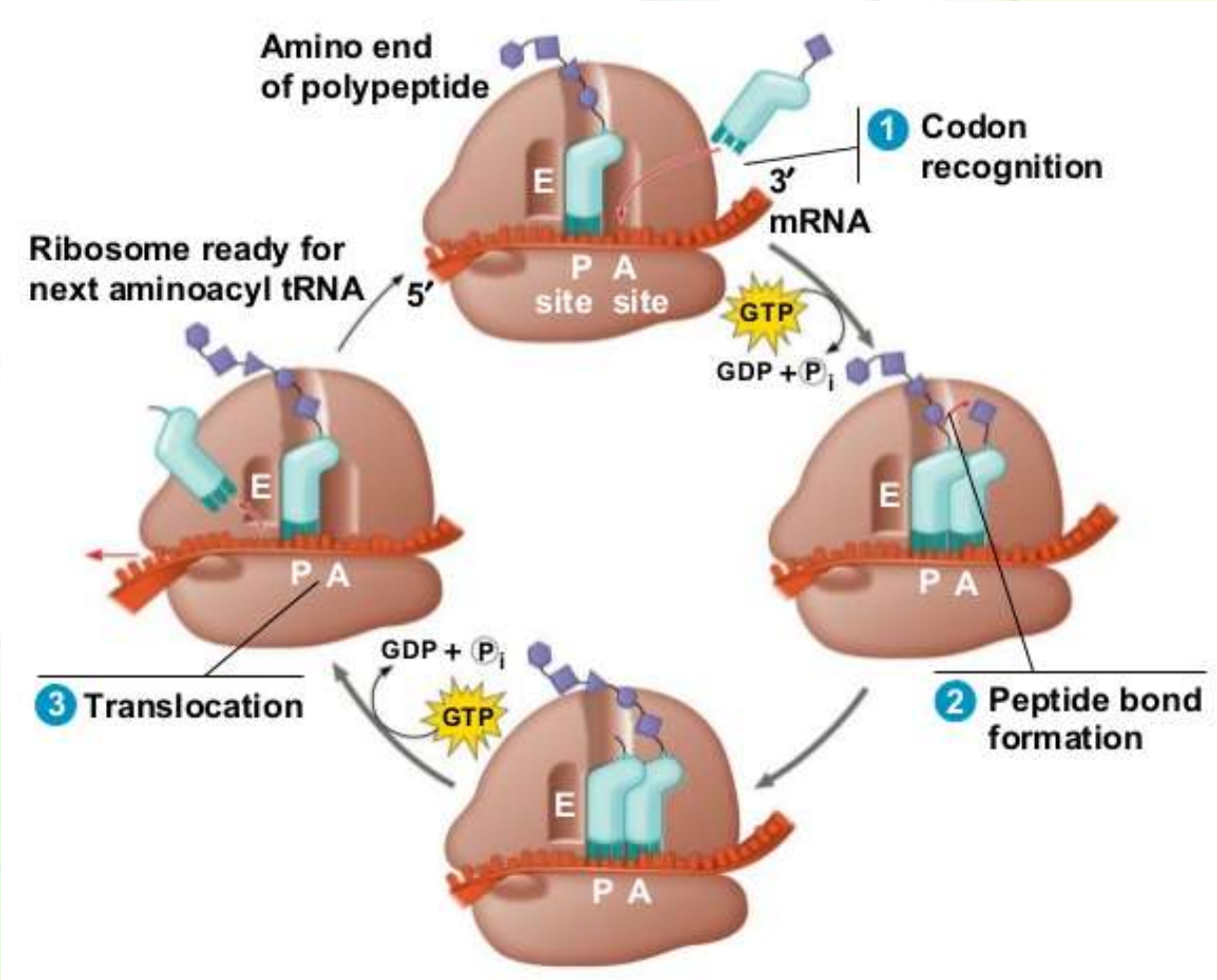


Figure 17.19-1

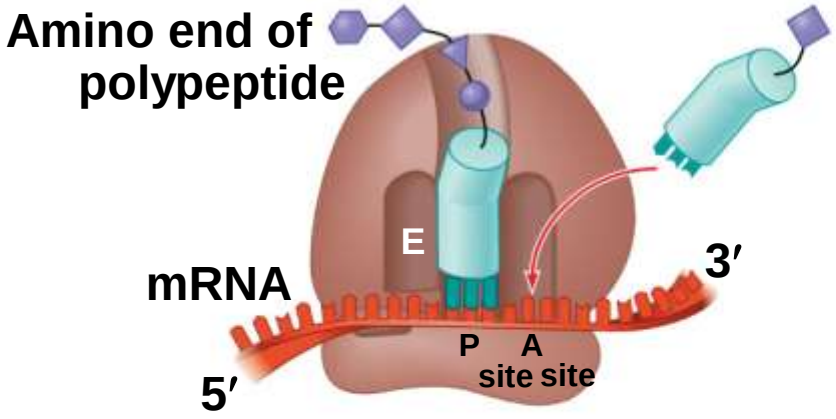


Figure 17.19-2

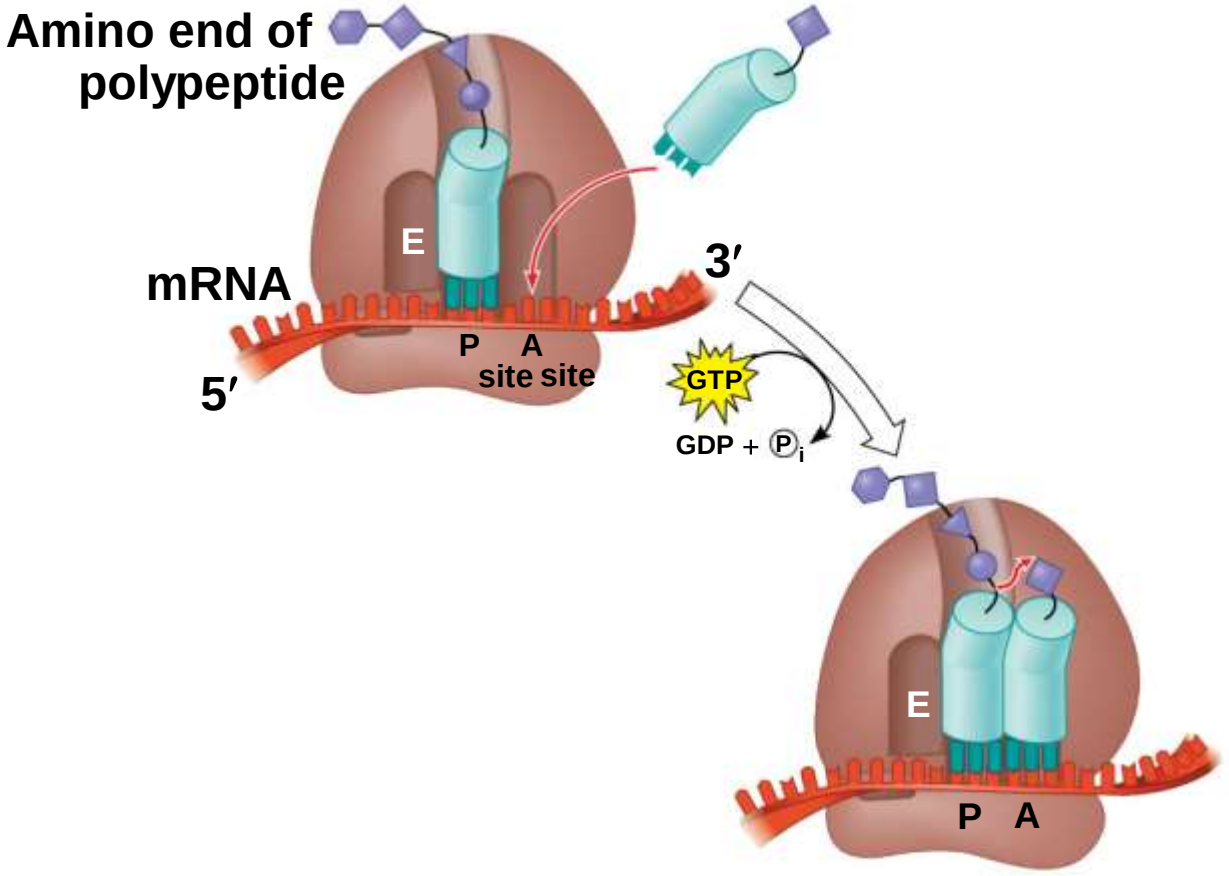


Figure 17.19-3

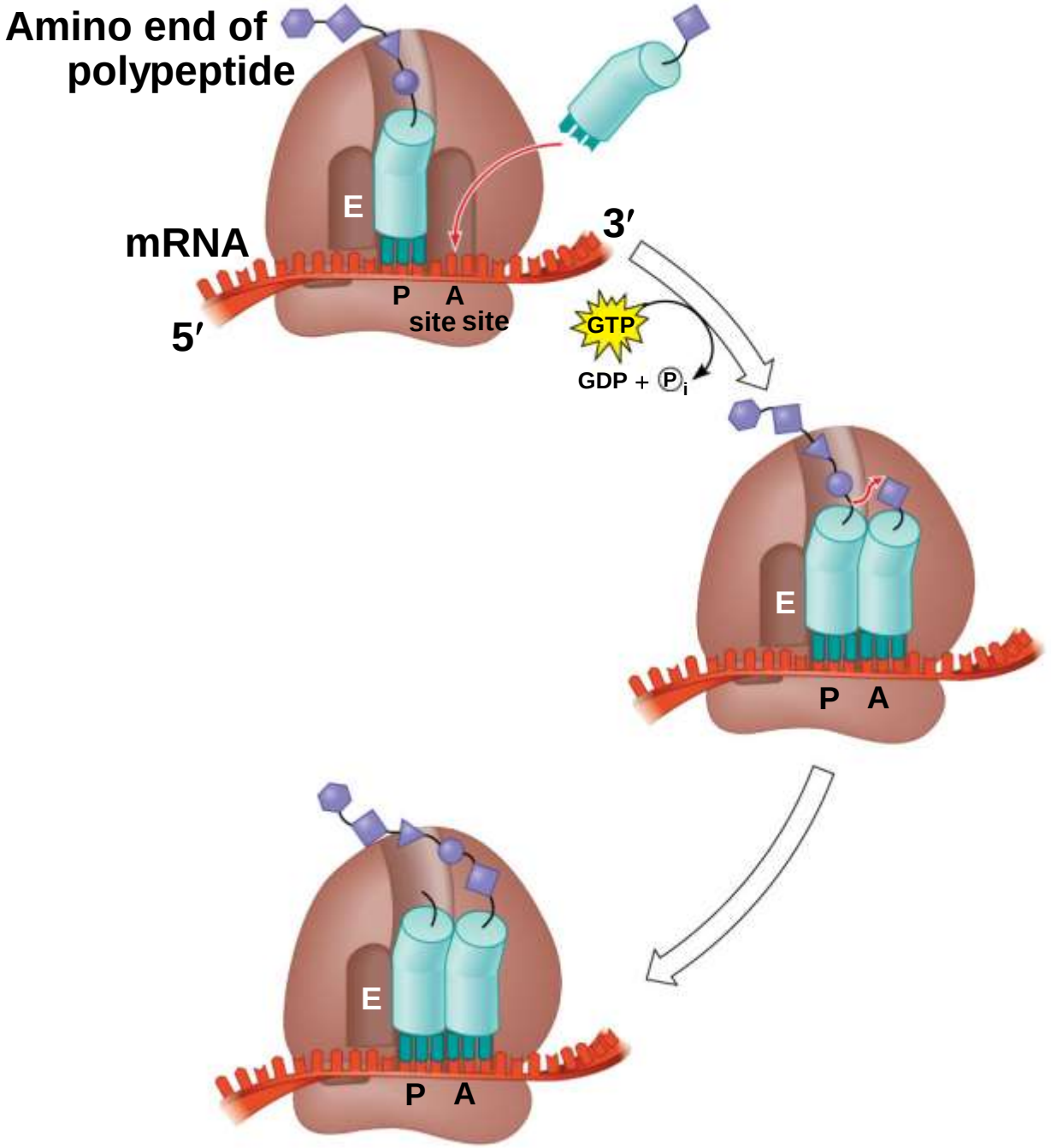
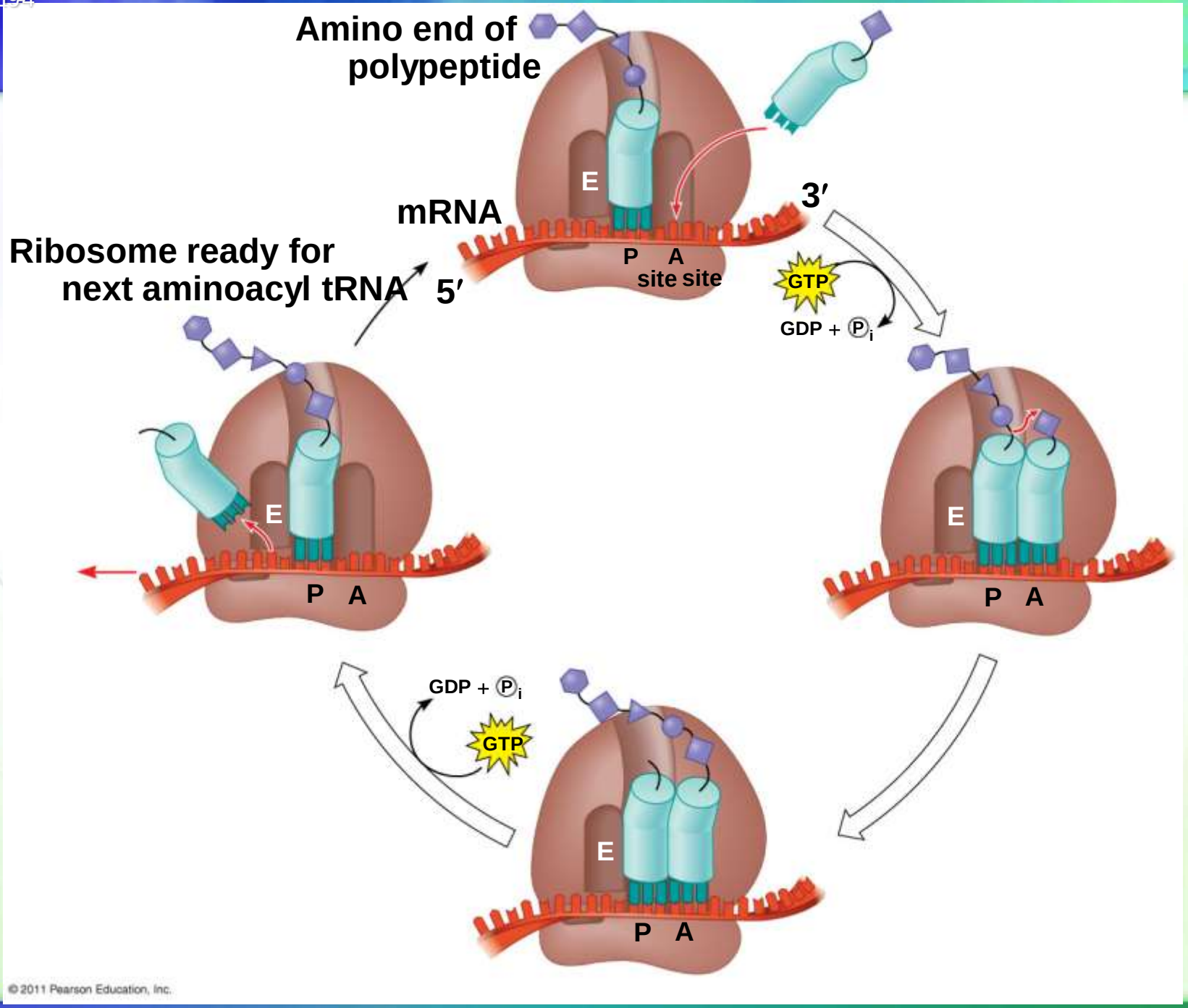


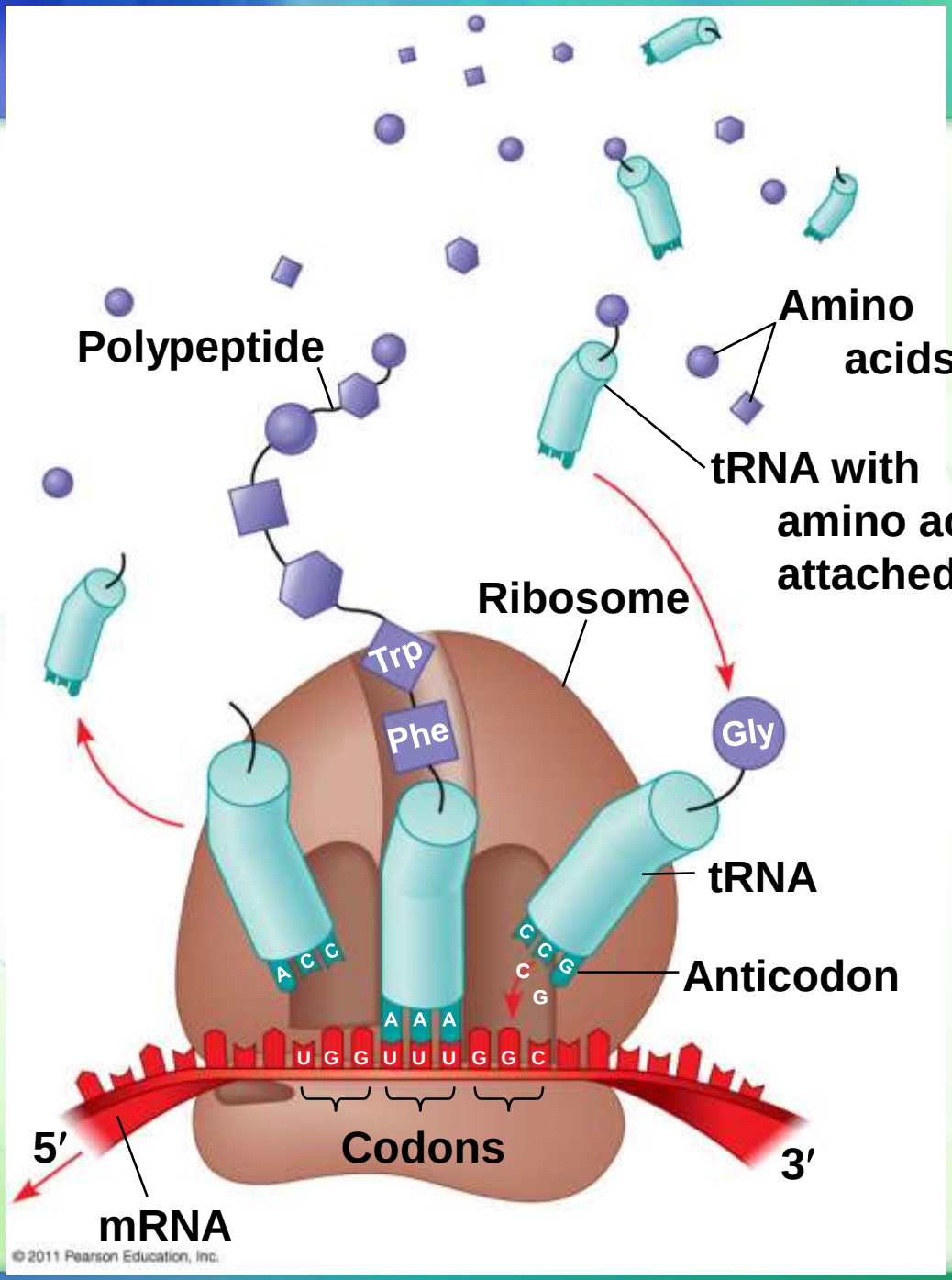
Figure 17.19-4



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.

Figure 17.14


Polypeptide
Amino acids
tRNA with amino acid attached
Ribosome
Trp
Phe
Gly
tRNA
Anticodon
A C C
C C G
C G
A A A
U G G U U U G G C
Codons
5′
3′
mRNA
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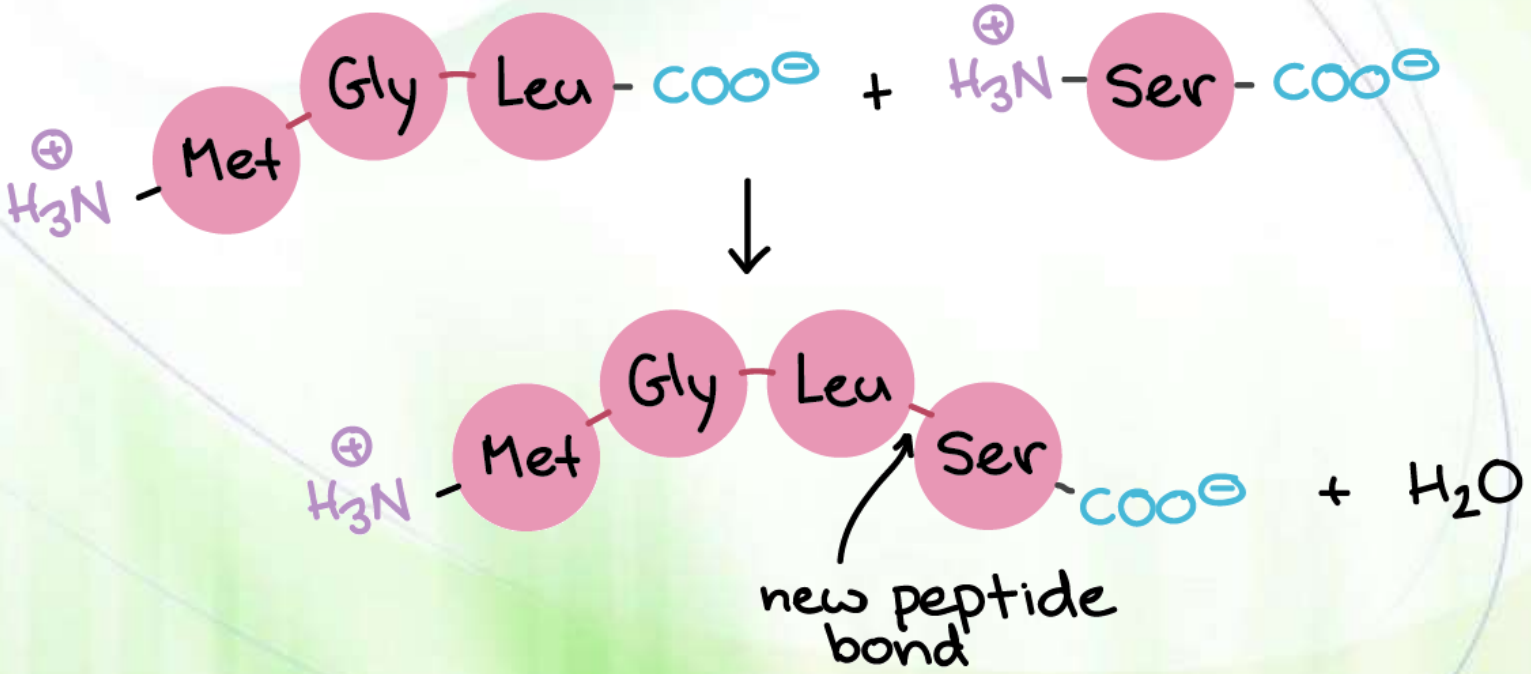
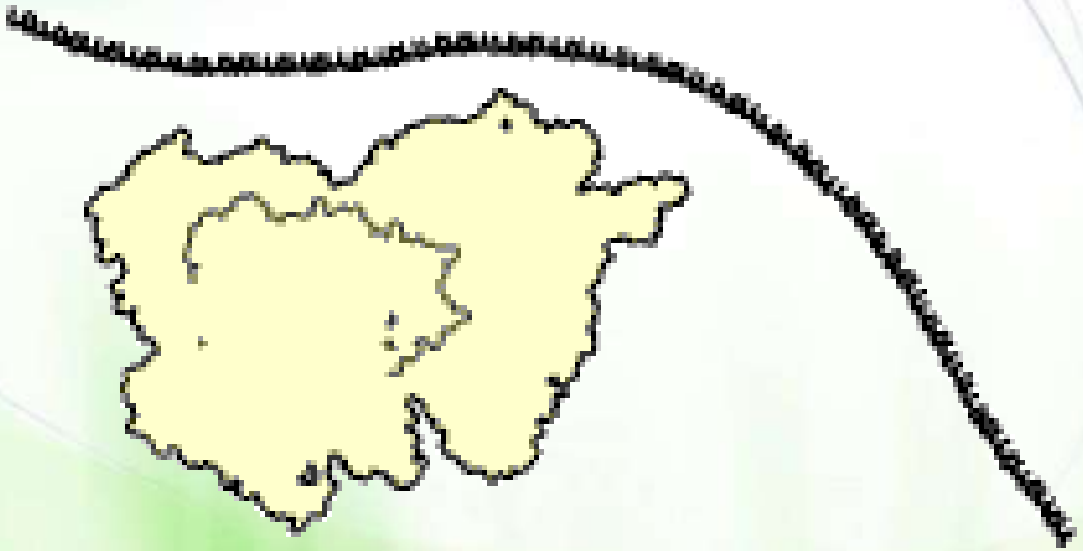
Elongation of the Polypeptide Chain

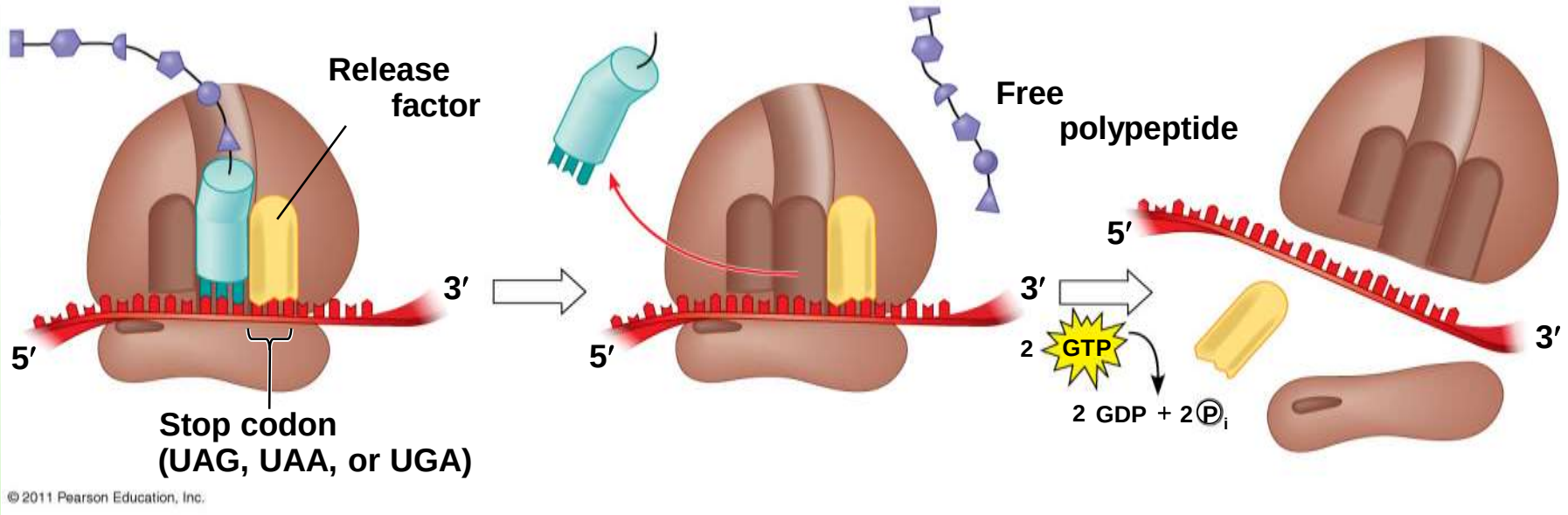
- During the elongation stage, amino acids are added one by one to the preceding amino acid at the C-terminus of the growing chain





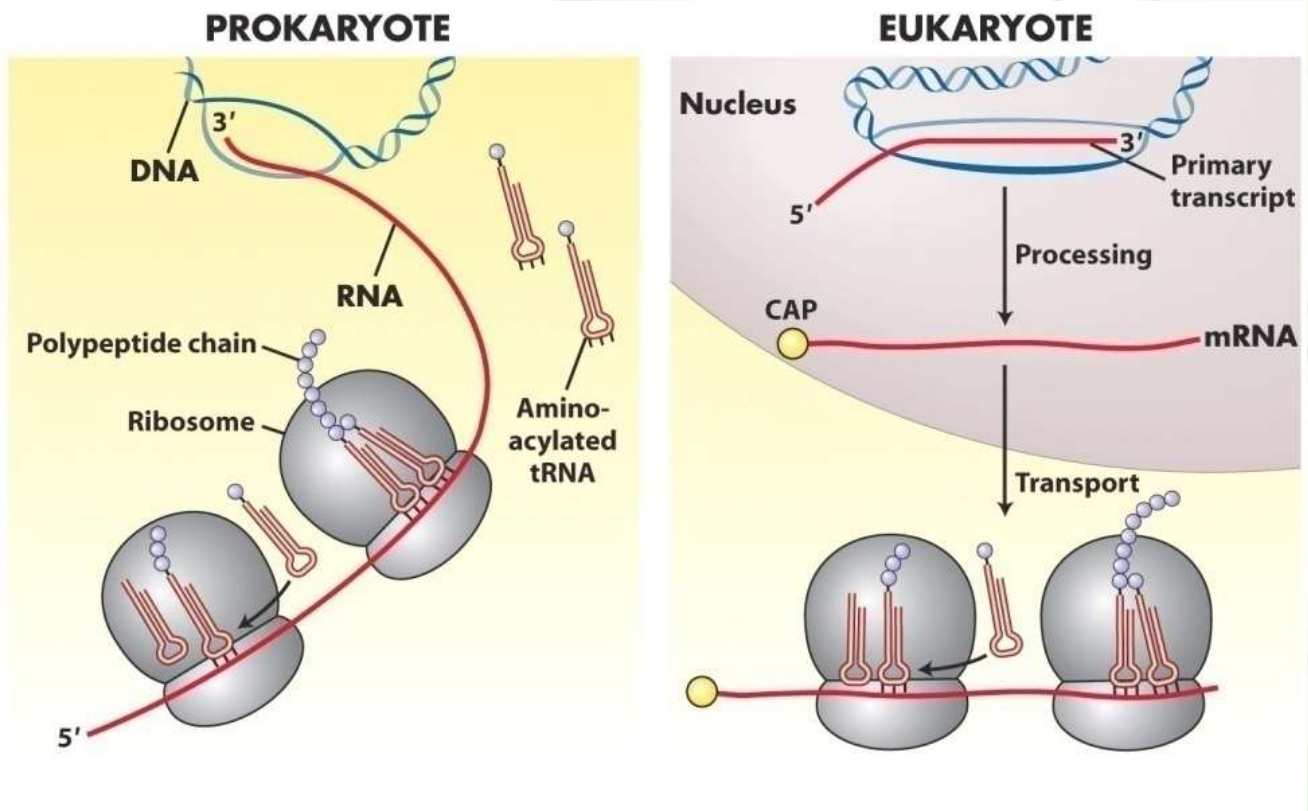
Termination of Translation

- The codons UAA, UAG, and UGA are the stop signals. They are not recognized by any tRNAs, but a release factor protein
- The A site accepts the release factor, which causes the addition of a water molecule instead of an amino acid
- This reaction releases the polypeptide, and the translation assembly then comes apart



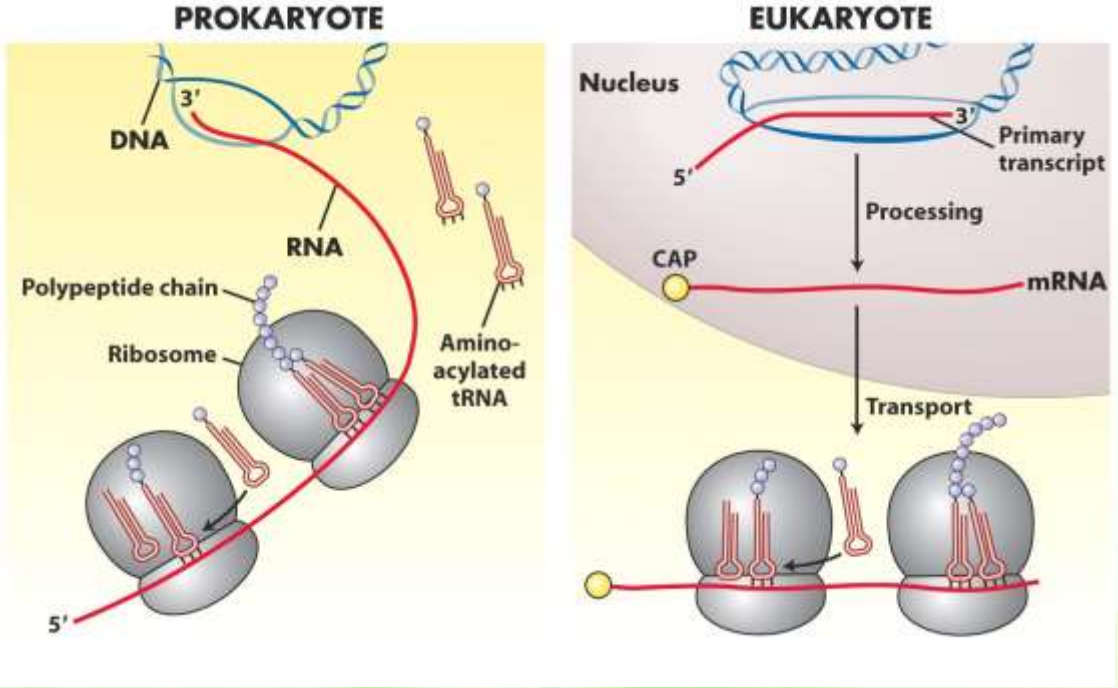
Transcription/translation Coupling

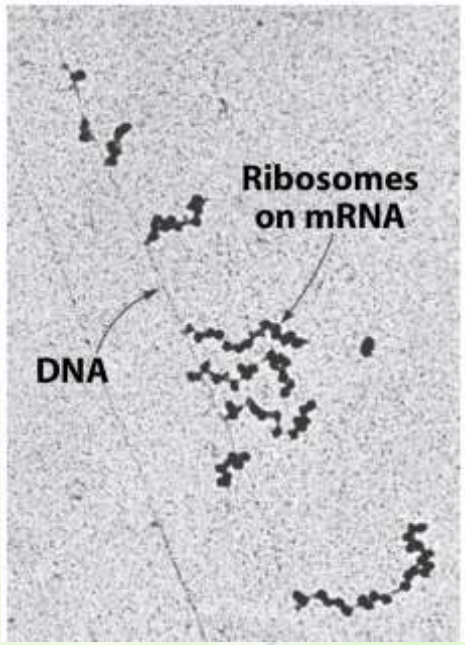
- Translation and transcription are coupled in space and time in prokaryotes.



Polyribosomes (polysomes)

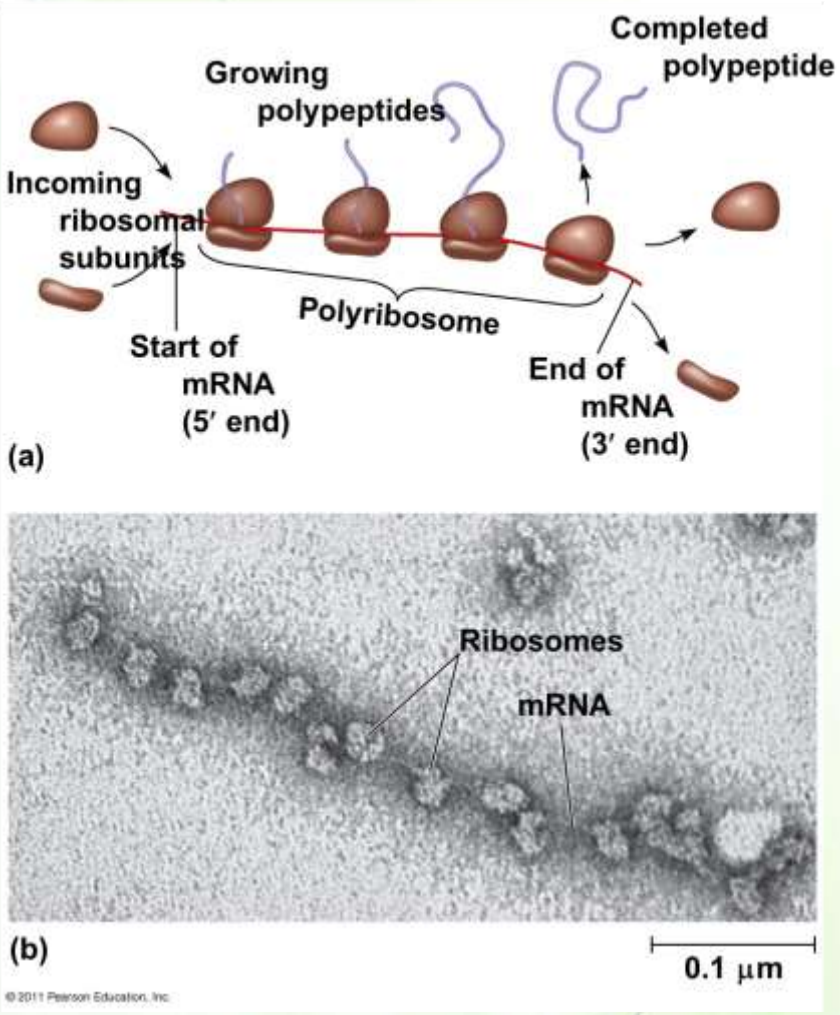
- A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.



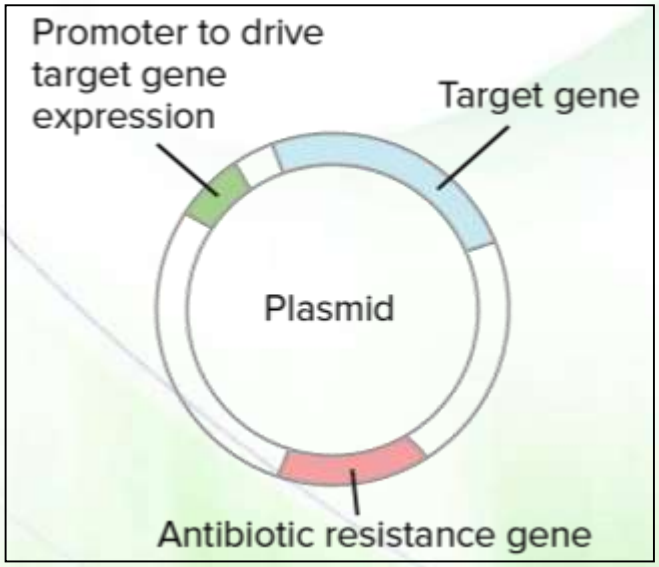


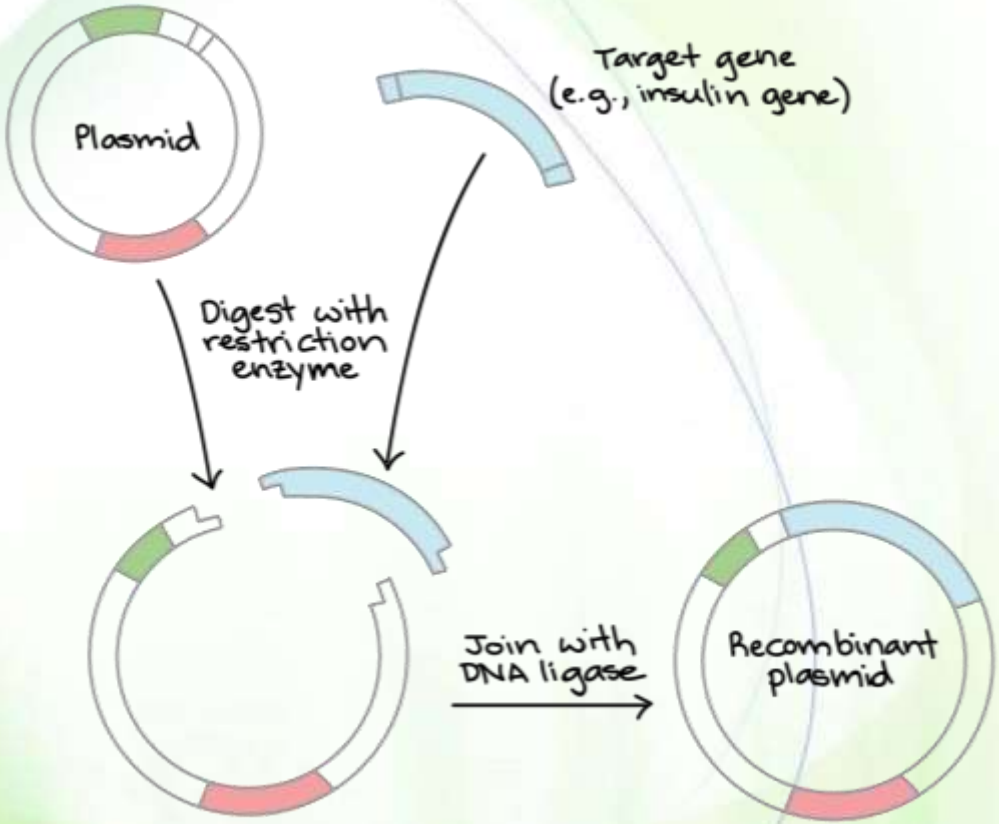
Polysomes (in eukaryotes)

- A number of ribosomes can translate a single mRNA simultaneously, forming a **polyribosome** (or **polysome**).
- Polyribosomes enable a cell to make many copies of a polypeptide very quickly.



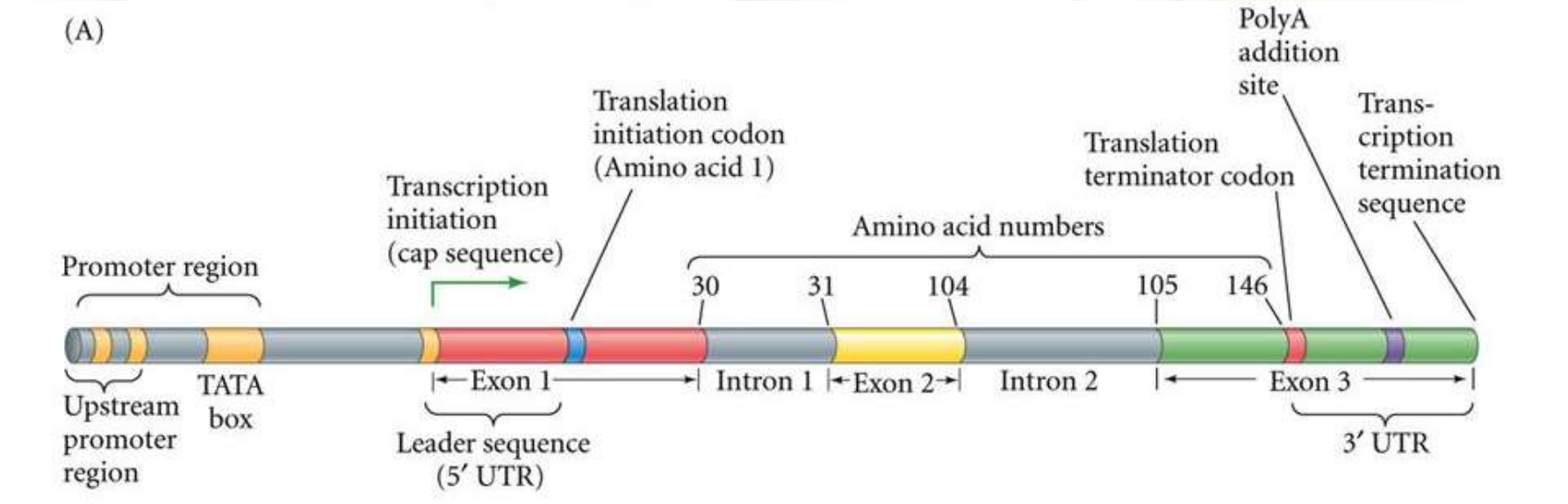
A benefit of cloning (production of hormones)





From DNA to protein: an example

Anatomy of a eukaryotic gene



Synthesis of β-globin

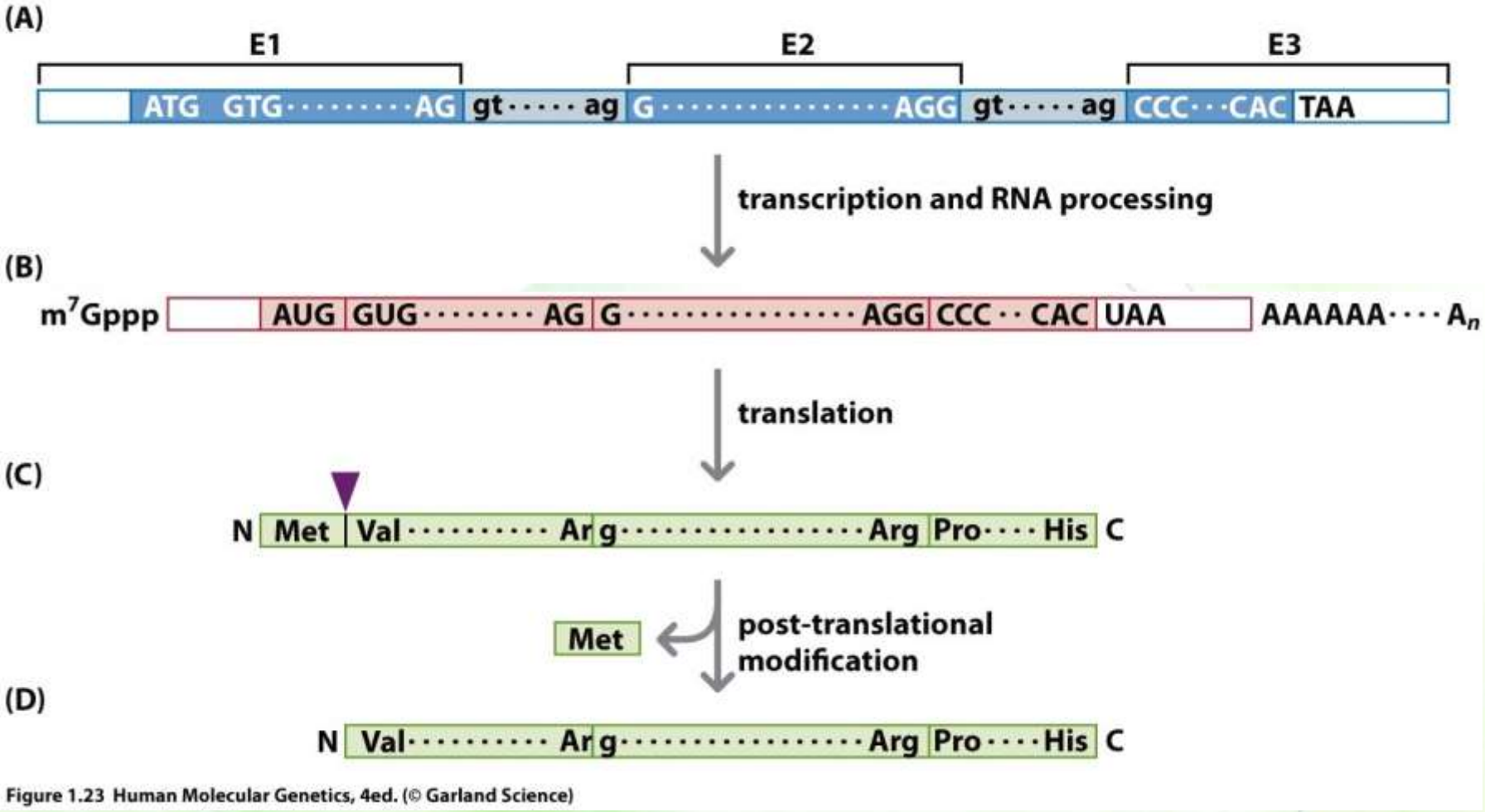
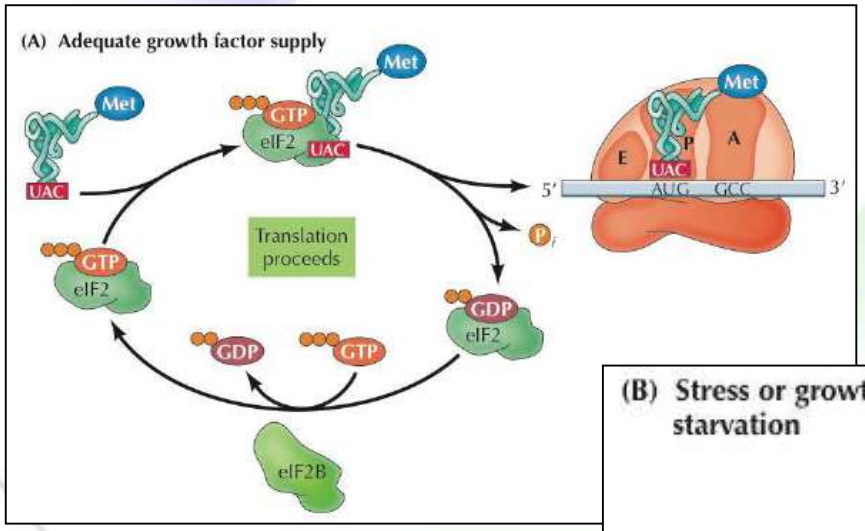
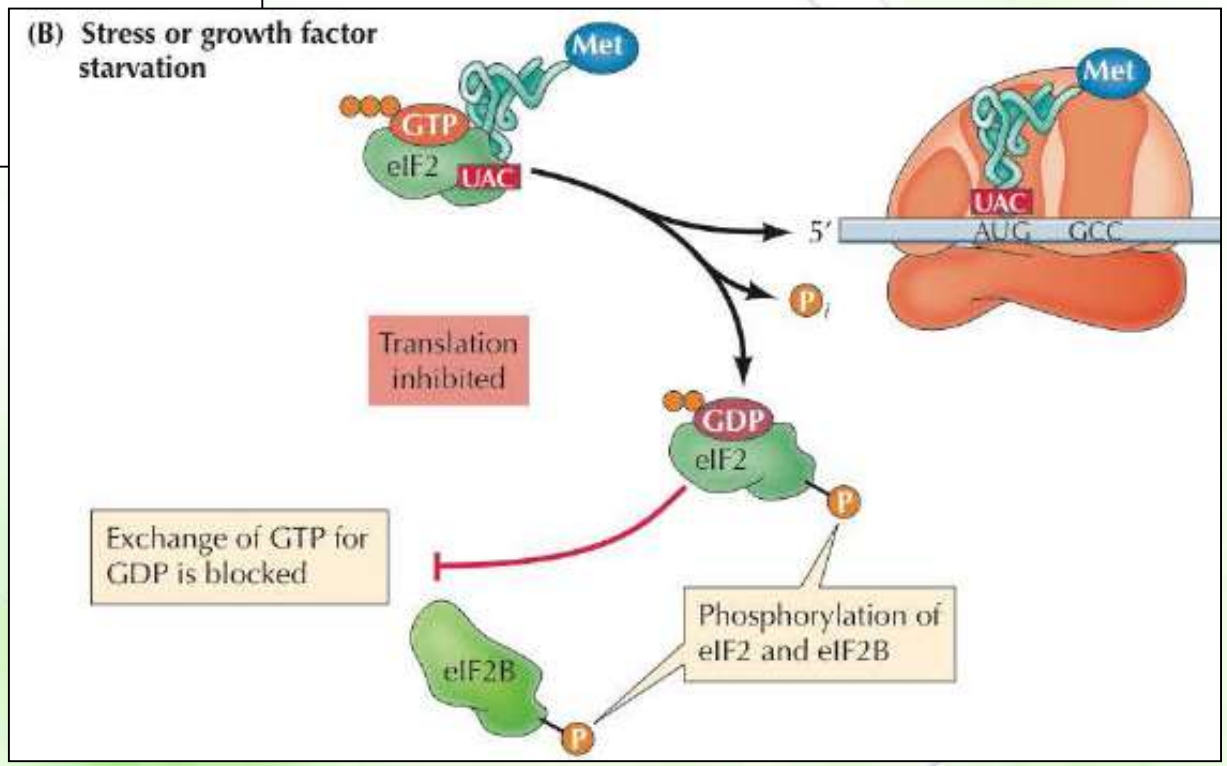


Figure 1.23 Human Molecular Genetics, 4ed. (© Garland Science)

Regulation of translation…globally



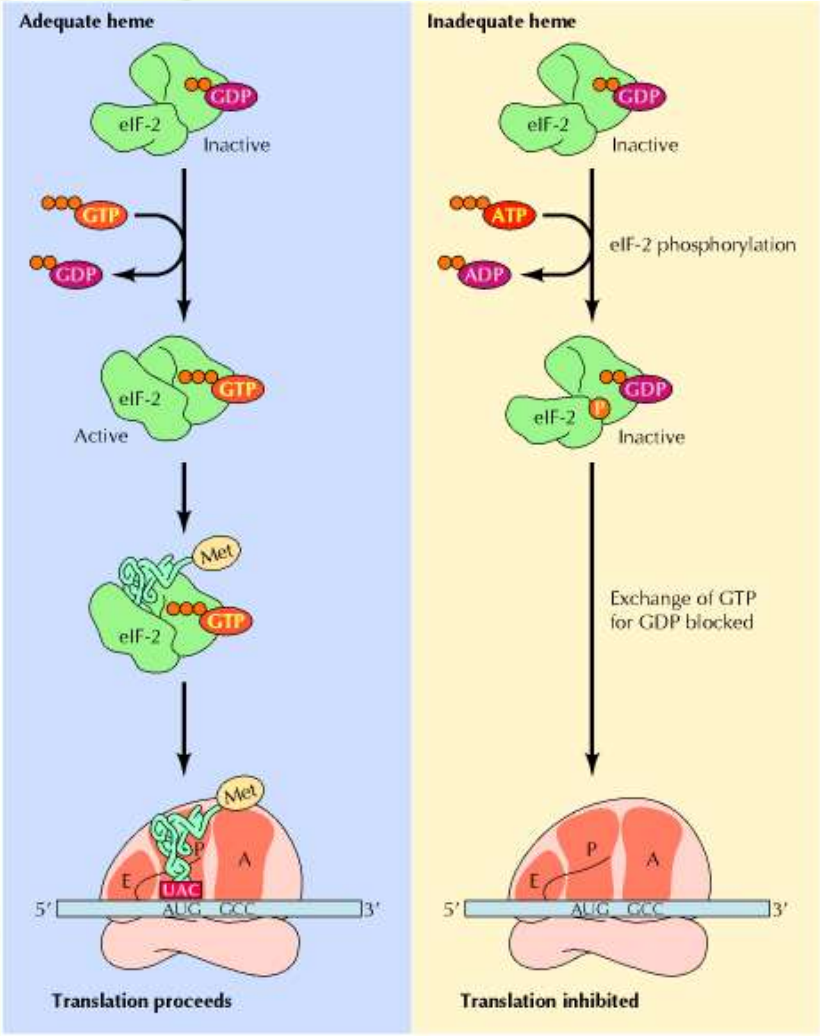


Heme and protein synthesis

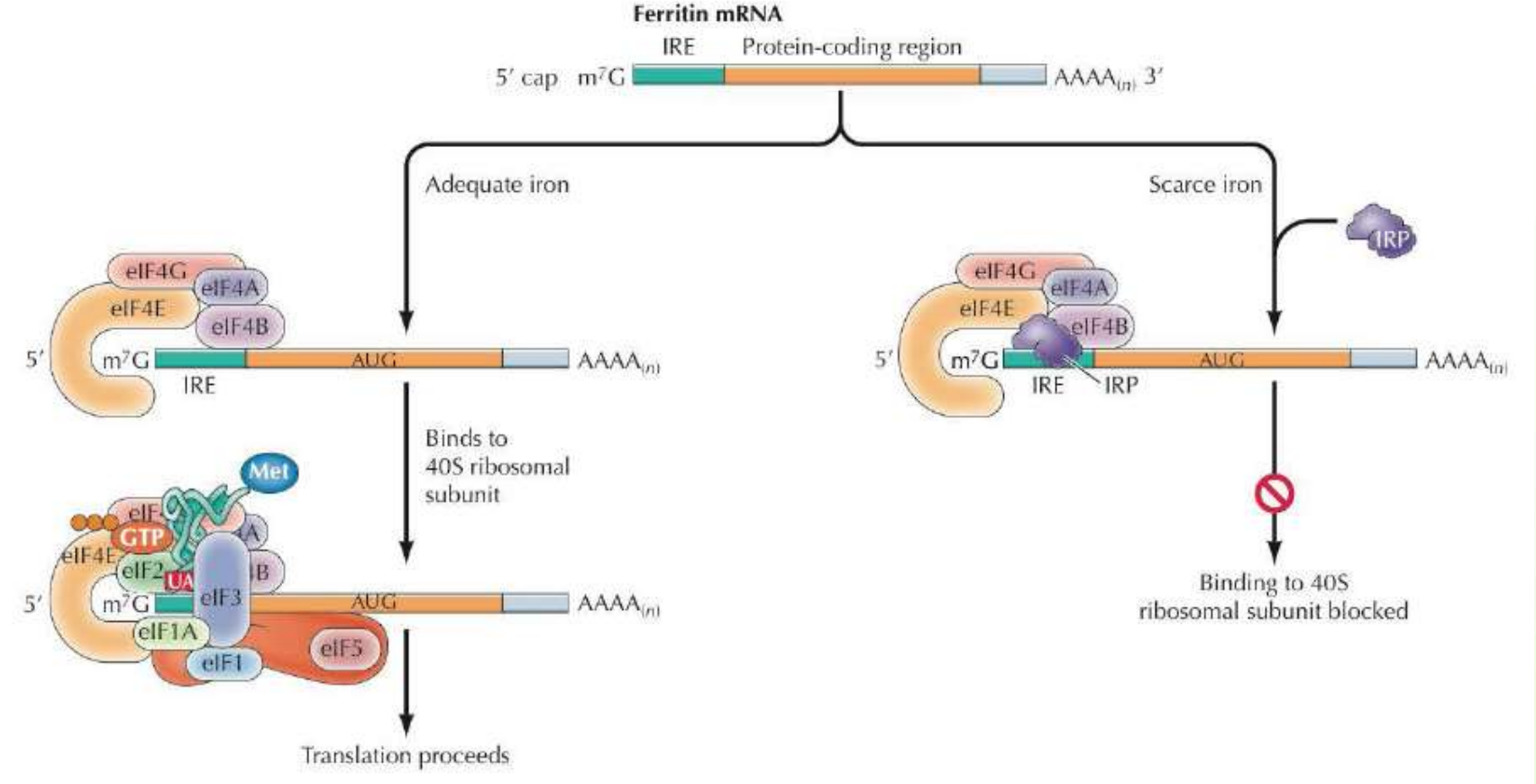
- In reticulocytes (immature erythrocytes), heme stimulates protein synthesis.
- The mRNA is translated only if adequate heme is available to form functional hemoglobin molecules.
- This is done via regulating the activity of eIF-2, which is responsible for escorting initiator methionyl tRNA to the ribosome.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

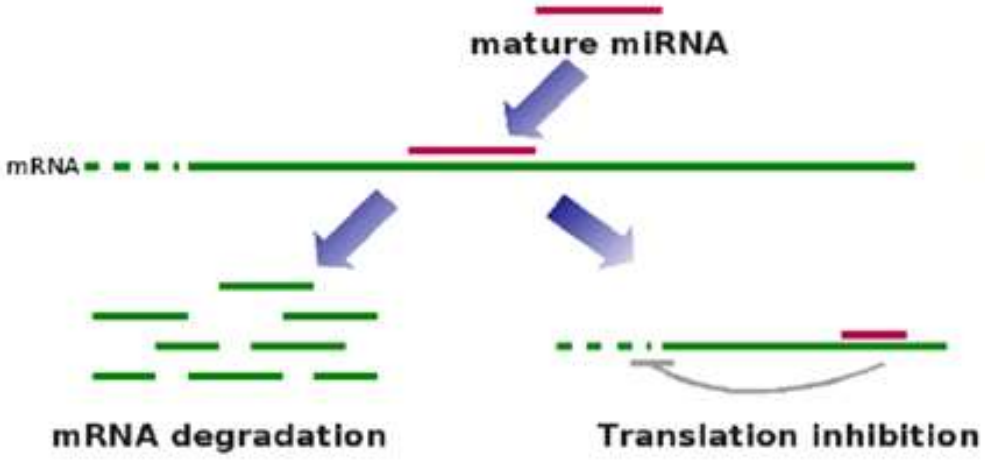


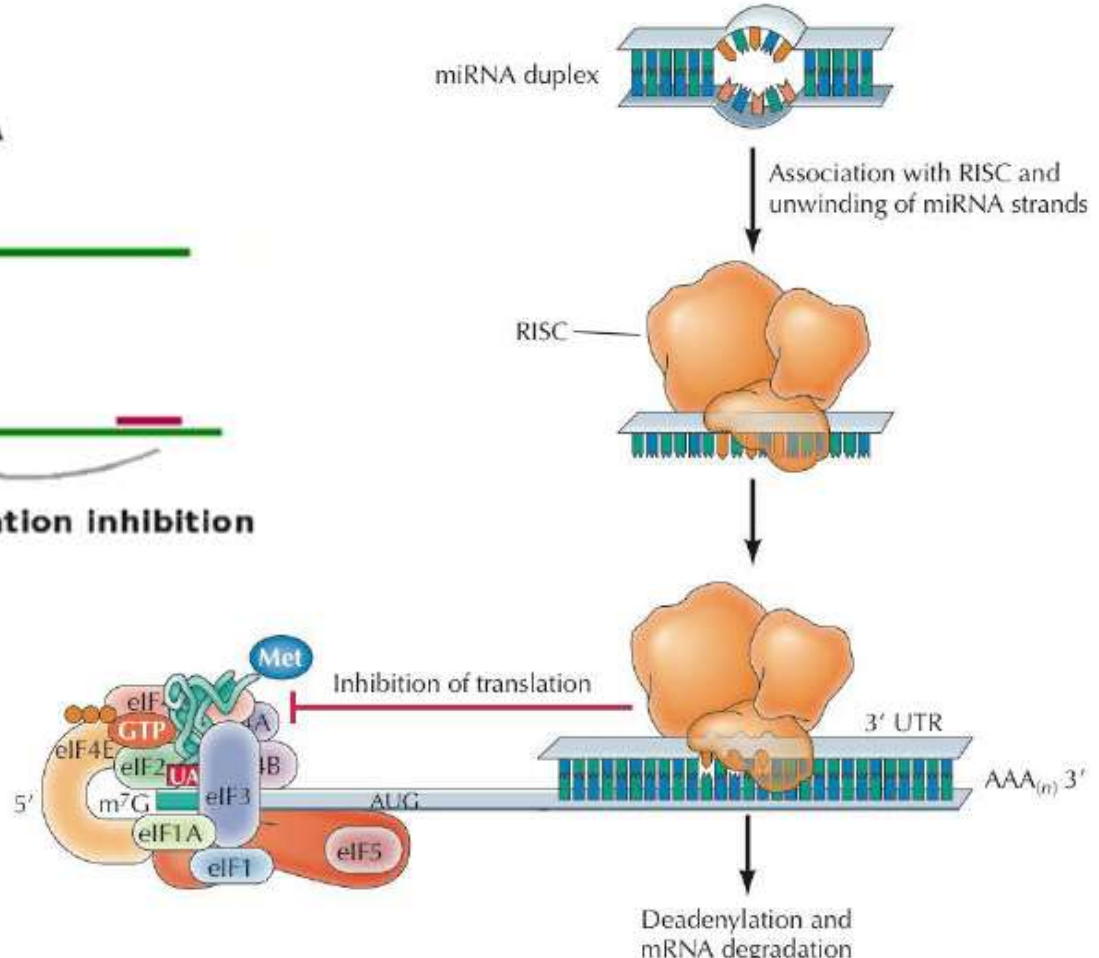
Also, remember…ferritin



Regulation by microRNA (miRNA)

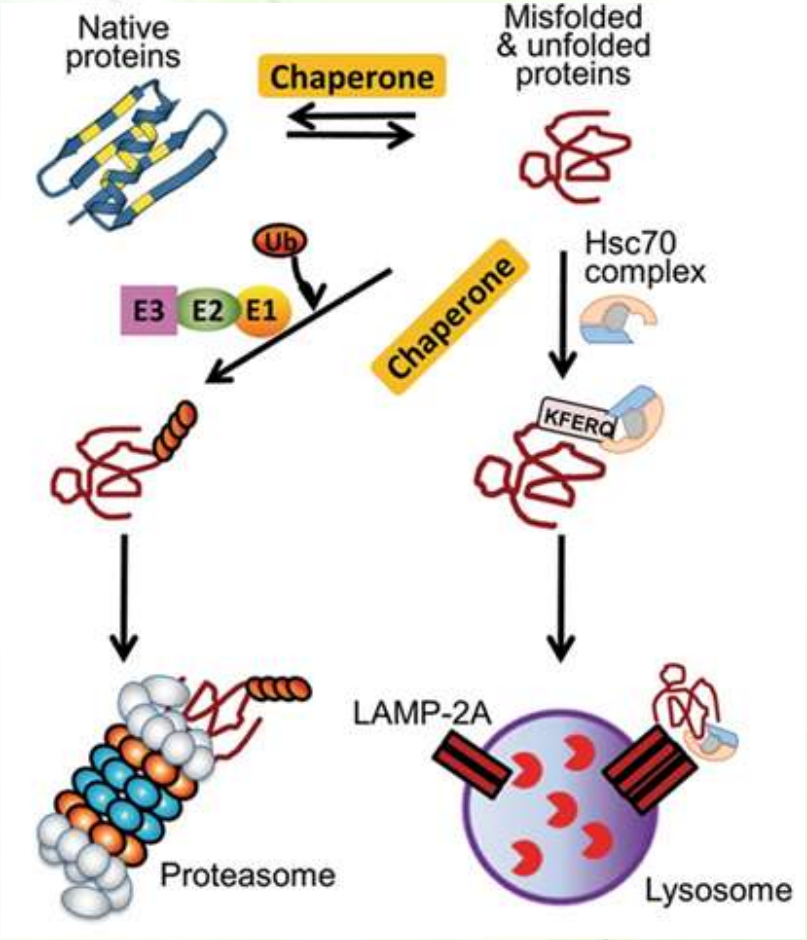
- MicroRNA is synthesized by RNA Pol II into single-stranded, primary miRNA (pri-miRNA) transcript.
- It gets processed and one strand is loaded onto RISC complex where miRNA is targeted to mRNA.





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.

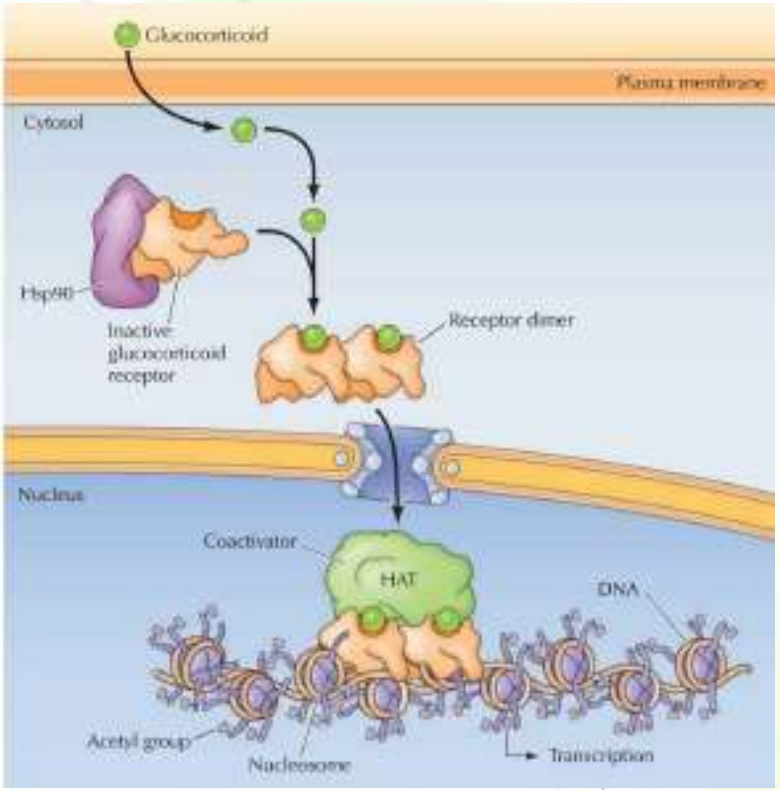


Connecting outside to inside: from cell siganling to protein synthesis

Different modes of regulating protein synthesis

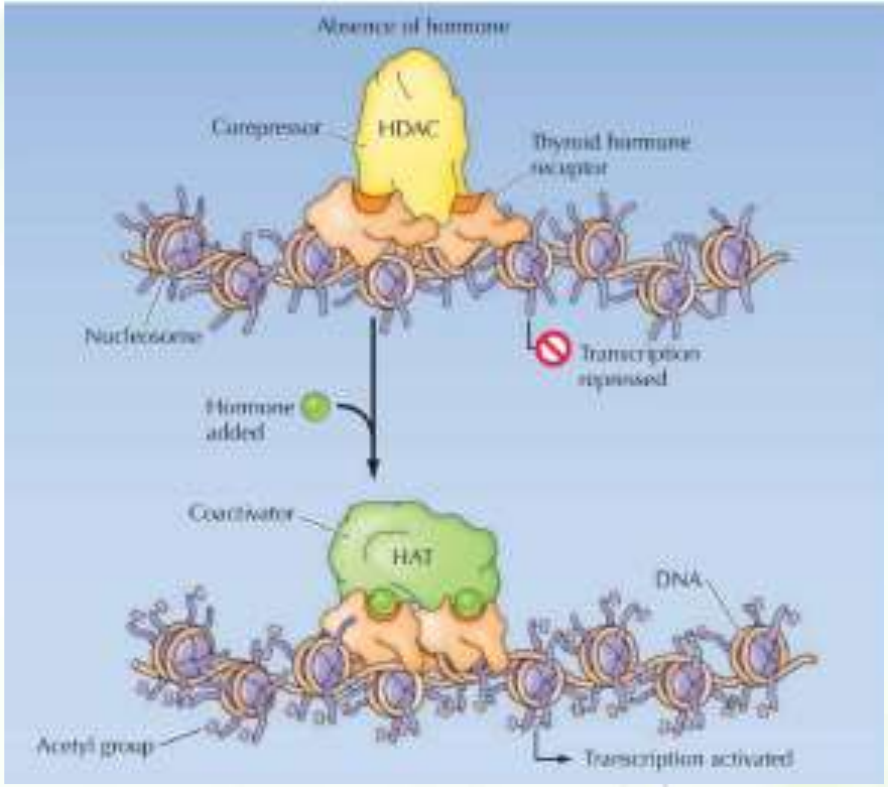
Glucocorticoids

- Glucocorticoids diffuse across the plasma membrane and bind to the glucocorticoid receptor.
- Glucocorticoid binding allows the formation of receptor dimers.
- The activated receptors translocate to the nucleus, bind DNA, and associate with coactivators (example: histone acetyltransferase (HAT)) to stimulate transcription of their target genes.



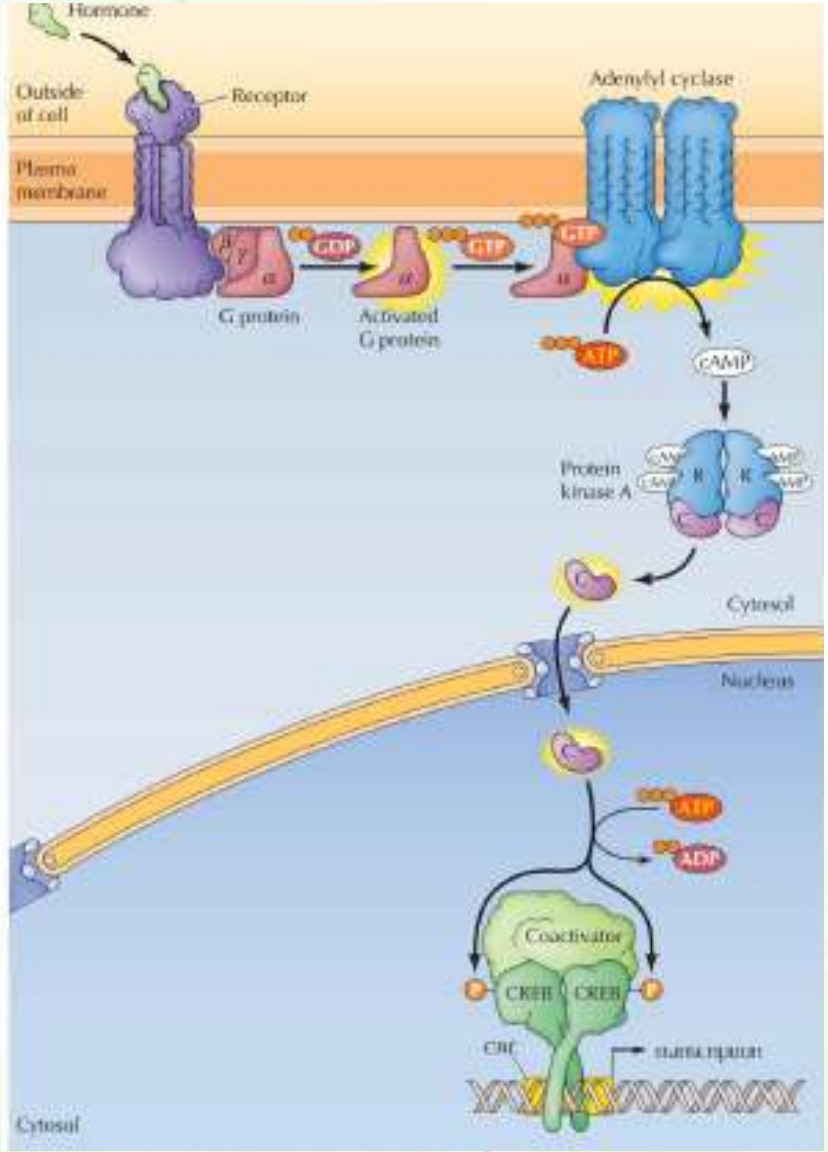
Thyroid hormone

- Thyroid hormone receptor binds DNA in either the presence or absence of hormone.
- In the absence of hormone, the receptor associates with corepressors with histone deacetylase (HDAC) activity.
- In the presence of hormone, the receptor associates with coactivators with histone acetyltransferase (HAT) activity.



cAMP-inducible gene expression

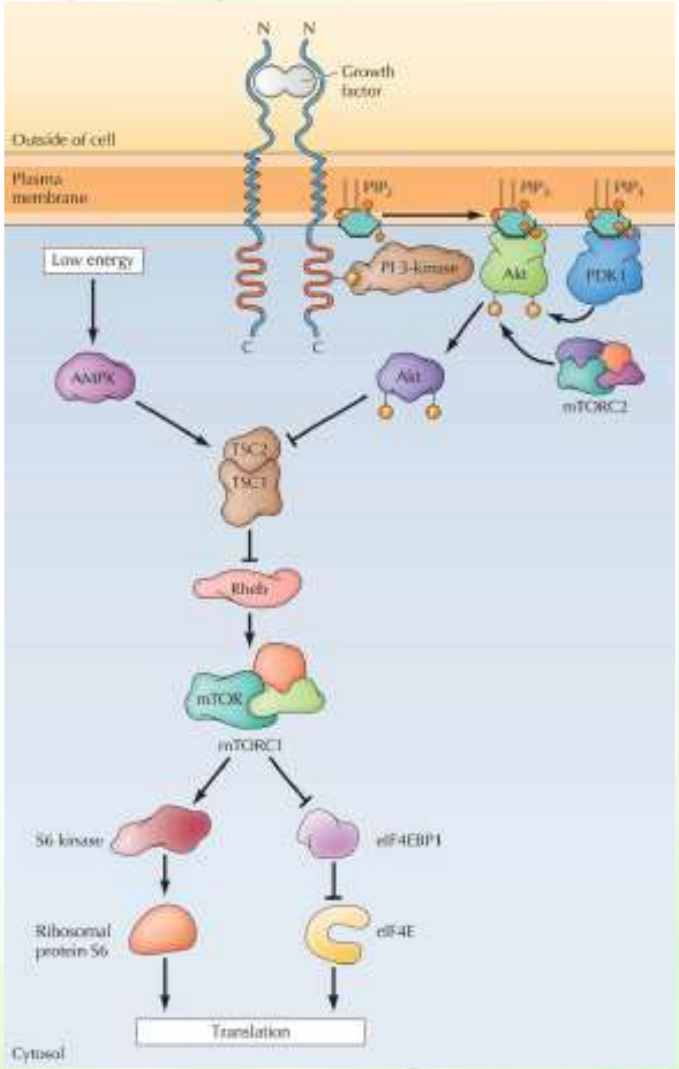
- Receptor stimulation leads to activation of adenylyl cyclase, synthesis of cAMP, and activation of protein kinase A.
- The catalytic subunit of protein kinase A is freed, translocates into the nucleus, and phosphorylates the transcription factor CREB (CRE-binding protein), leading to the recruitment of coactivators and expression of cAMP-inducible genes.



The mTOR pathway

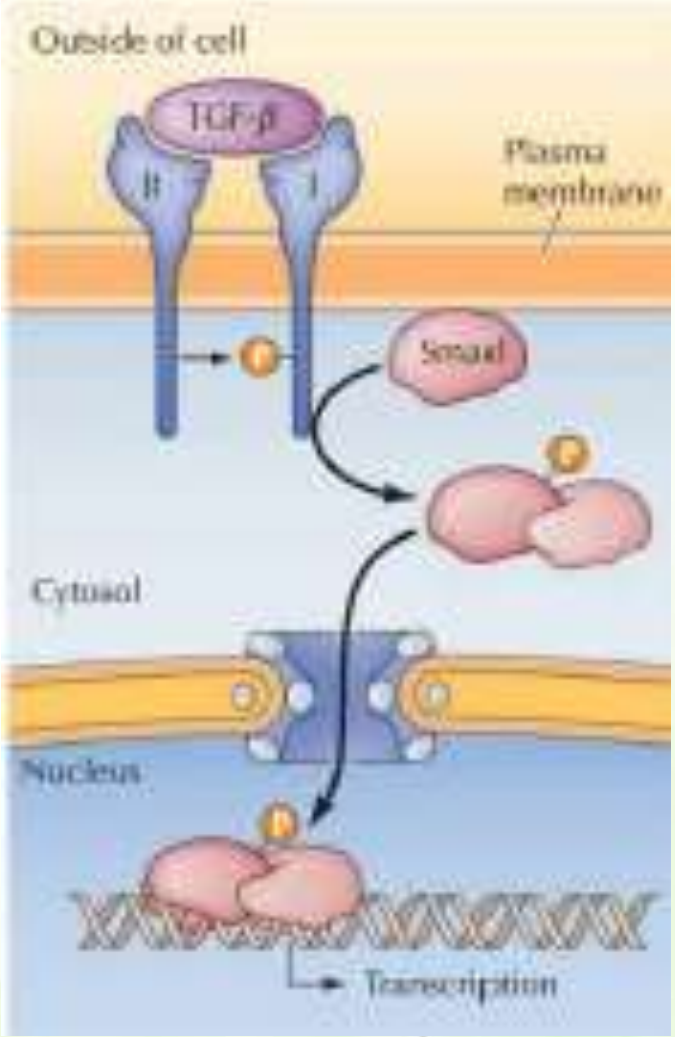
Coupling growth to energy stores

- mTORC1 stimulates translation by phosphorylating 56 kinase (which phosphorylates ribosomal protein 56) and by phosphorylating eiF4E binding protein-1 (4E-BP1), relieving inhibition of translation initiation factor eiF4E.
- At high energy, Akt is activated leading to activation of mTORC1 and, hence, translation.
- At low energy stores (AMP>ATP), AMP kinase (AMPK) inhibits translation by inhibiting mTORC1.



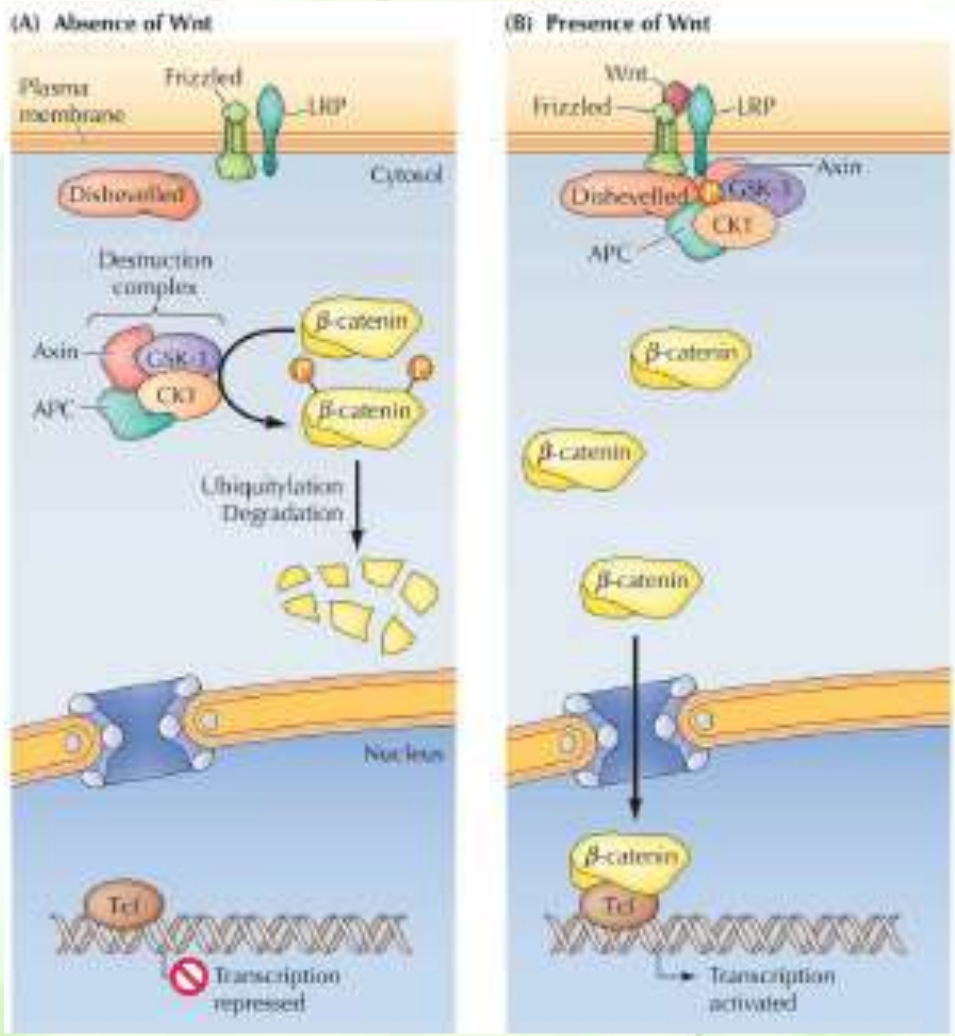
Direct activation of transcription factors (TGF-β receptors → Smad)

- When bound to their ligand, transforming growth factor receptors (TGF-β receptors) phosphorylate a Smad protein.
- Phosphorylated Smads form complexes and translocate into the nucleus to activate transcription of target genes.



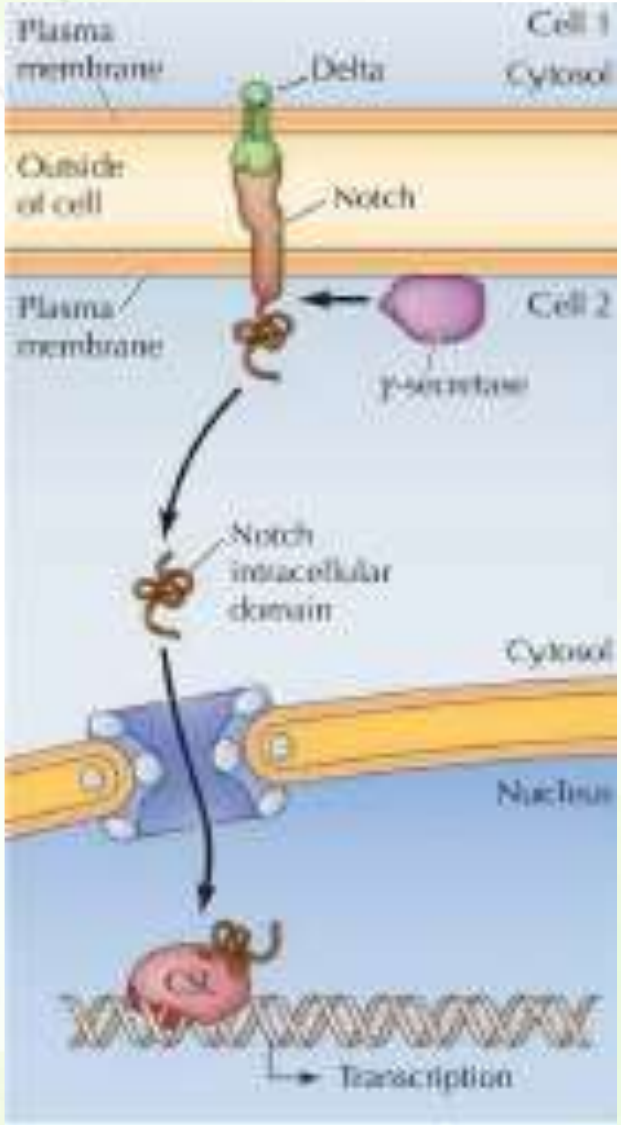
Trapping of transcription factors (The Wnt pathway)

- (A) In the absence of the ligand Wnt, β-catenin is ubiquitylated and degraded.
- (B) When Wnt is present, the destruction complex is inactivated and β–catenin is stabilized, translocates into the nucleus and forms a complex with other transcription factors activating transcription.



The TF is within the membrane receptor (The Notch pathway)

- Notch is a receptor.
- The binding of its ligand leads to proteolytic cleavage of Notch by y-secretase.
- This releases the Notch intracellular domain, which translocates to the nucleus and interacts with a transcription factor to induce gene expression.



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)