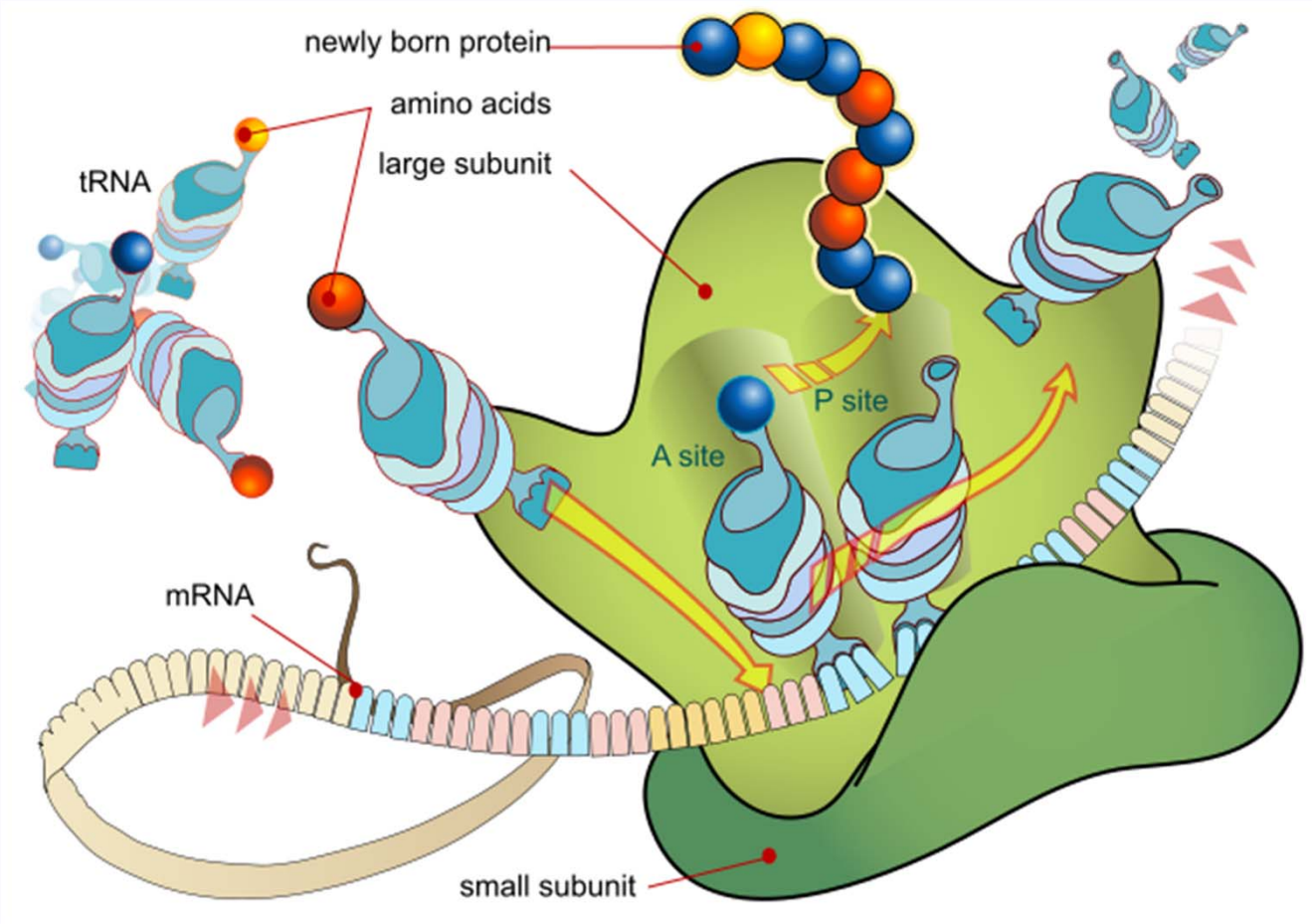
# Chapter 30:
# Protein Synthesis

海生所呂健宏5316

# How is the nucleotide sequence of an mRNA molecule translated into the amino acid sequence of a protein molecule?

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# A Generalized Secondary Structure of tRNA

**Aminoacyl-tRNA synthetase**

tRNA: adaptor molecule bridging the information gap between mRNA and amino acid

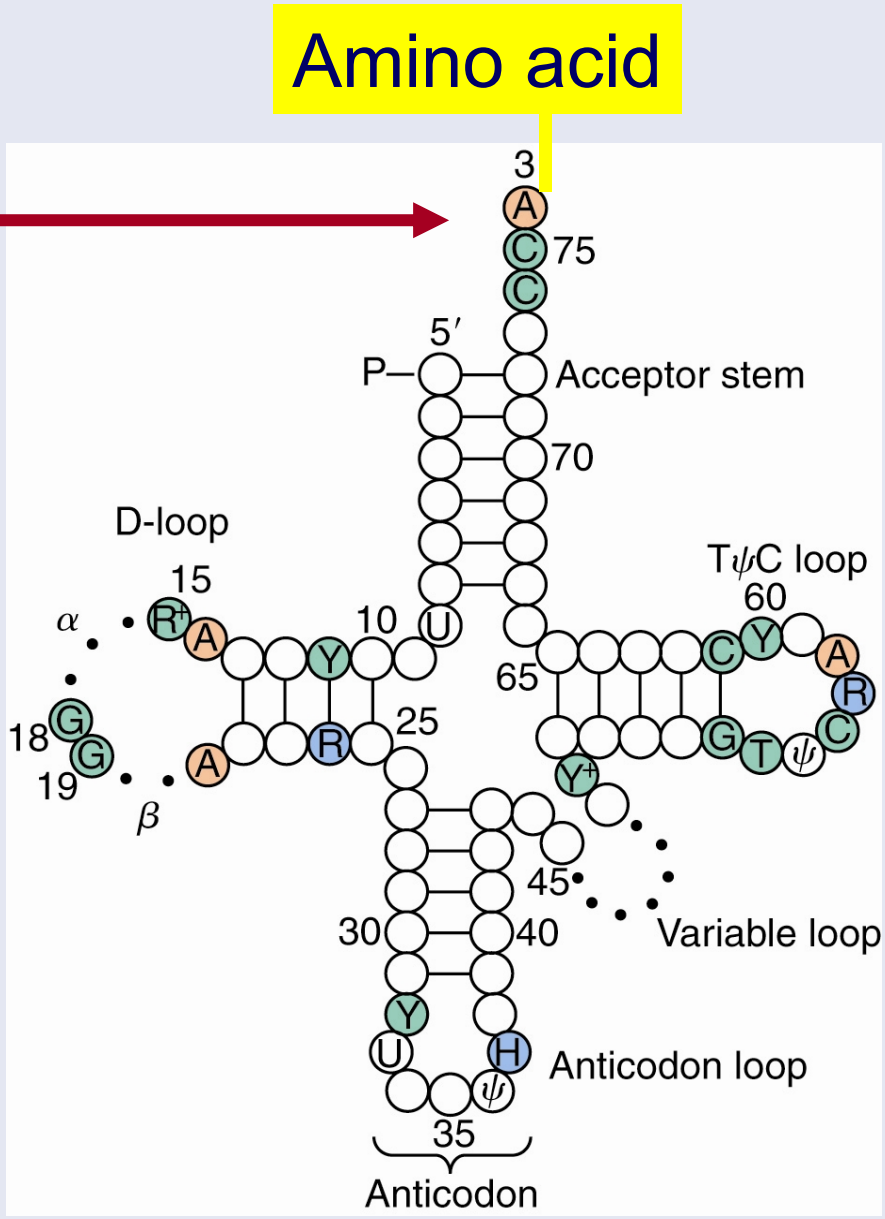



Figure 30.1
Circles: nucleotides in the tRNA sequence.
The numbers: standardized numbering for tRNAs.
Dots: places where the number of nucleotides may vary in different tRNA species.

# Outline

1. What is the genetic code?
2. How is an amino acid matched with its proper tRNA?
3. What are the rules in codon-anticodon pairing?
4. What is the structure of ribosomes, and how are they assembled?
5. What are the mechanics of mRNA translation?
6. How are proteins synthesized in eukaryotic cells?

# 30.1 What Is the Genetic Code?

- Allows the information in a nucleotide sequence to be changed into amino acid of a protein

The genetic code is a **triplet code**

- A group of 3 bases (a **codon**) codes for one amino acid
- The code is not overlapping
- The base sequence is read from a fixed starting point without punctuation
- The code **is degenerate**, meaning that some amino acids can be coded by more than one triplet

# Features of the Code

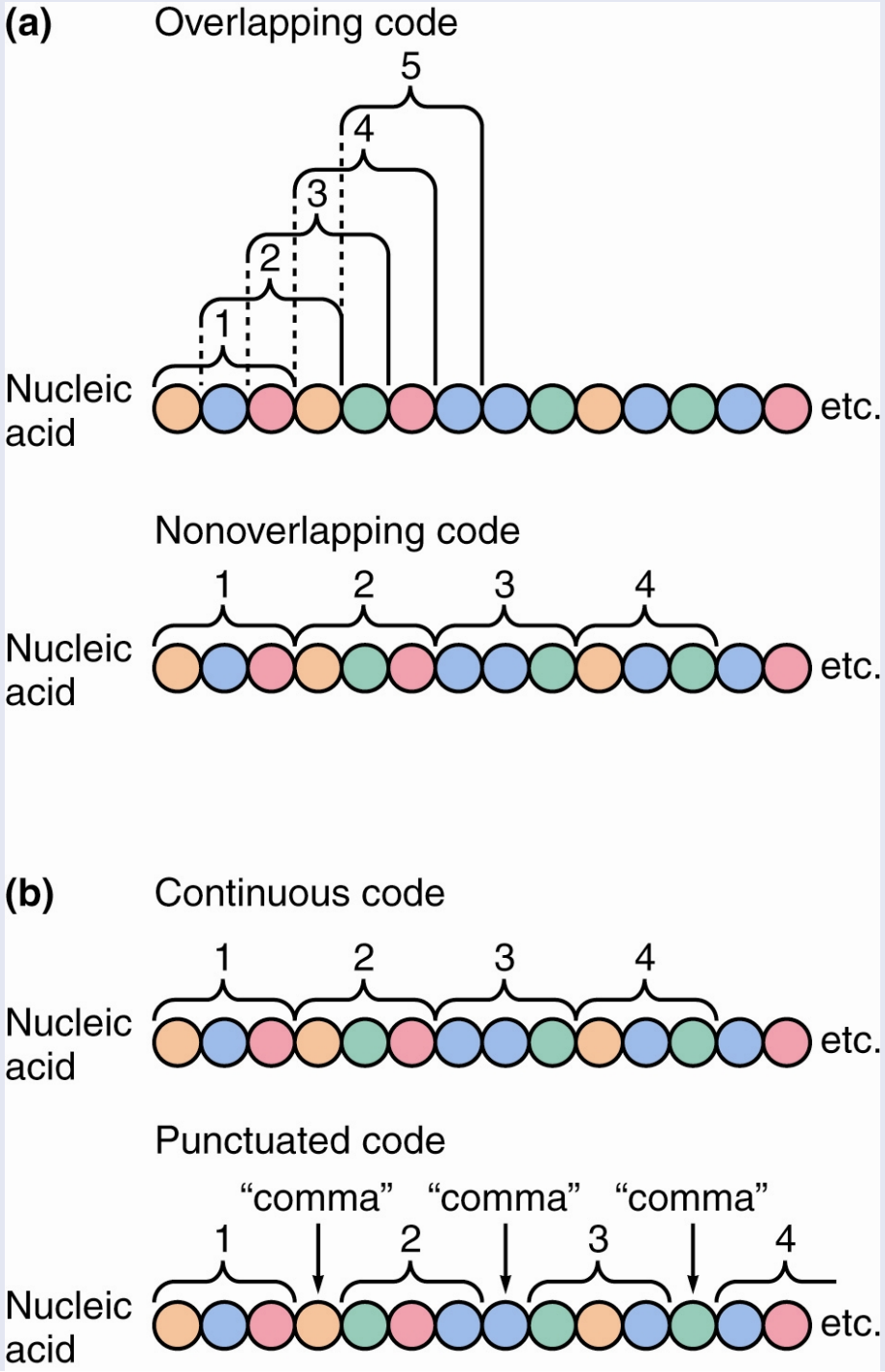



Figure 30.2 (a) An overlapping versus a nonoverlapping code. (b) A continuous versus a punctuated code.

# Codons Specify Amino Acids

- All the codons have meaning: 61 specify particular amino acids. 3 specify no a. a., **nonsense codons**, or **termination codons**
- The genetic code is **unambiguous**: each sense codon encodes only on amino acid.
- The genetic code is **degenerate.** Except Met, Trp, every amino acid is coded by > one codon. Codons coding for the same amino acid are called **synonymous codons**.
- Codons representing the same amino acid or chemically similar amino acids tend to be similar in sequence; **third-base degeneracy**
- The genetic code is "**universal**"

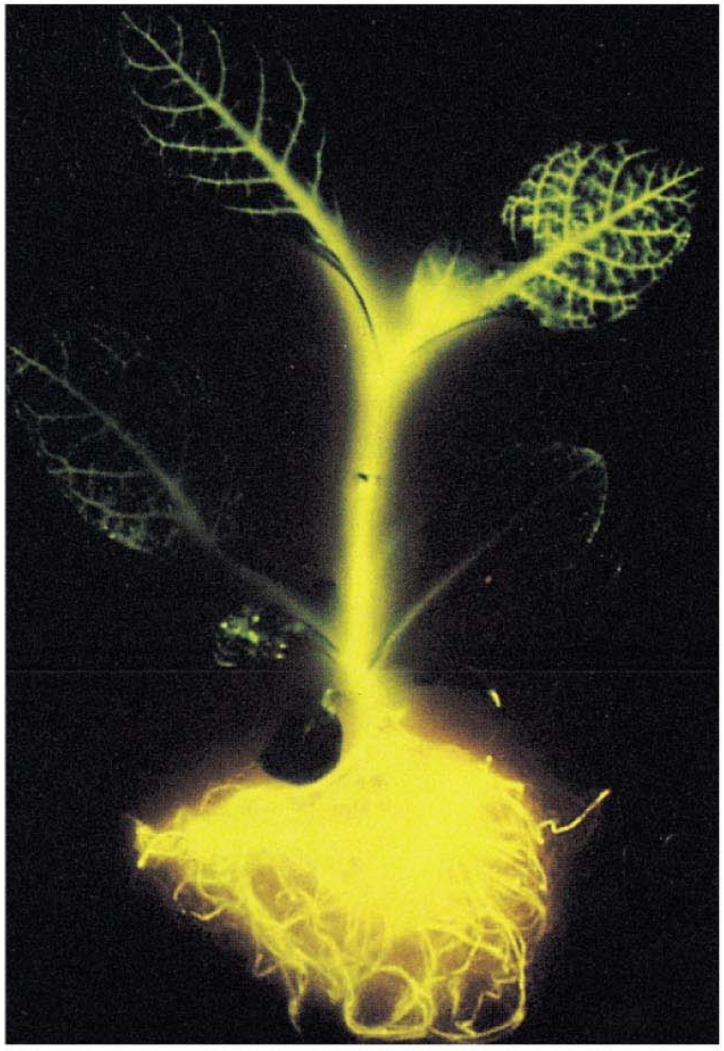
**TABLE 30.1** The Genetic Code

| First Position (5′-end) | Second Position: U | Second Position: C | Second Position: A | Second Position: G | Third Position (3′-end) |
|---|---|---|---|---|---|
| U | UUU Phe | UCU Ser | UAU Tyr | UGU Cys | U |
| | UUC Phe | UCC Ser | UAC Tyr | UGC Cys | C |
| | UUA Leu | UCA Ser | UAA Stop | UGA Stop | A |
| | UUG Leu | UCG Ser | UAG Stop | UGG Trp | G |
| C | CUU Leu | CCU Pro | CAU His | CGU Arg | U |
| | CUC Leu | CCC Pro | CAC His | CGC Arg | C |
| | CUA Leu | CCA Pro | CAA Gln | CGA Arg | A |
| | CUG Leu | CCG Pro | CAG Gln | CGG Arg | G |
| A | AUU Ile | ACU Thr | AAU Asn | AGU Ser | U |
| | AUC Ile | ACC Thr | AAC Asn | AGC Ser | C |
| | AUA Ile | ACA Thr | AAA Lys | AGA Arg | A |
| | AUG Met* | ACG Thr | AAG Lys | AGG Arg | G |
| G | GUU Val | GCU Ala | GAU Asp | GGU Gly | U |
| | GUC Val | GCC Ala | GAC Asp | GGC Gly | C |
| | GUA Val | GCA Ala | GAA Glu | GGA Gly | A |
| | GUG Val | GCG Ala | GAG Glu | GGG Gly | G |

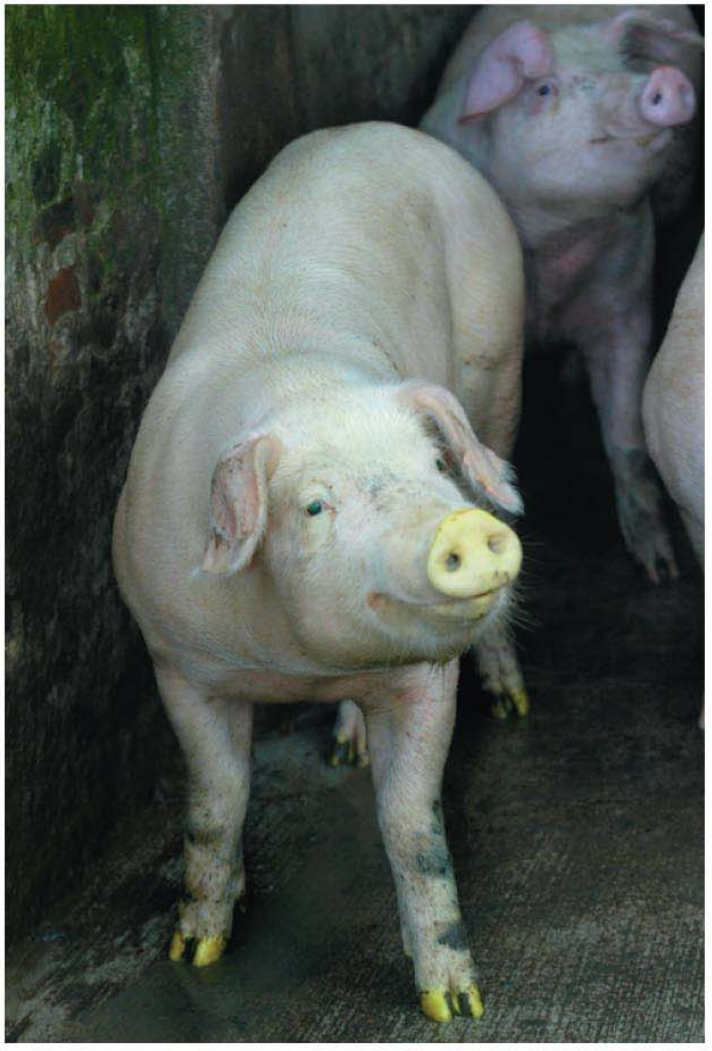
Third-Base Degeneracy Is Color-Coded

| Third-Base Relationship | Third Bases with Same Meaning | Number of Codons |
|---|---|---|
| Third base irrelevant | U, C, A, G | 32 (8 families) |
| Purines | A or G | 12 (6 pairs) |
| Pyrimidines | U or C | 14 (7 pairs) |
| Three out of four | U, C, A | 3 (AUX = Ile) |
| Unique definitions | G only | 2 (AUG = Met) (UGG = Trp) |
| Unique definition | A only | 1 (UGA = Stop) |

# Universal genetic code



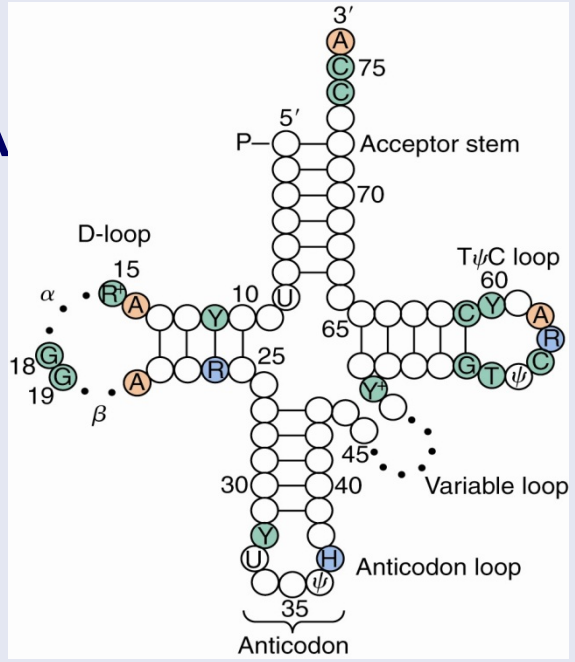
**(a) Tobacco plant expressing a firefly gene**



**(b) Pig expressing a jellyfish gene**

# 30.2 How Is an Amino Acid Matched with Its Proper tRNA?

- Aminoacyl-tRNA: tRNA attached with amino acid at 3'-OH at 3'-CCA end; catalyzed by **aminoacyl-tRNA synthetase**
- Aminoacyl-tRNA synthetases **discriminate between the various tRNAs and amino acids**
- The **features** by which each aminoacyl-tRNA synthetase matches up its amino acid with tRNAs constitutes a **second genetic code**



# Two Distinct Classes of Aminoacyl-tRNA Synthetases

- The **20 different aminoacyl-tRNA synthetases** fall into two classes based on **similar amino acid sequence motifs, oligomeric state,** and **acylation function**
- **Class I** enzymes first add the amino acid to the **2'-OH** of the terminal adenylate residue of tRNA before shifting it to the **3'-OH**
- **Class II** add it directly to the **3'-OH**
- Oligomeric: Class I enzymes are mostly monomeric; class II mostly dimeric or multimeric.

**TABLE 30.2** The Two Classes of Aminoacyl-tRNA Synthetases

| Class I | Class II |
|---|---|
| Arg | Ala |
| Cys | Asn |
| Gln | Asp |
| Glu | Gly |
| Ile | His |
| Leu | Lys |
| Met | Phe |
| Trp | Pro |
| Tyr | Ser |
| Val | Thr |

# The Aminoacyl-tRNA Synthetase Reaction

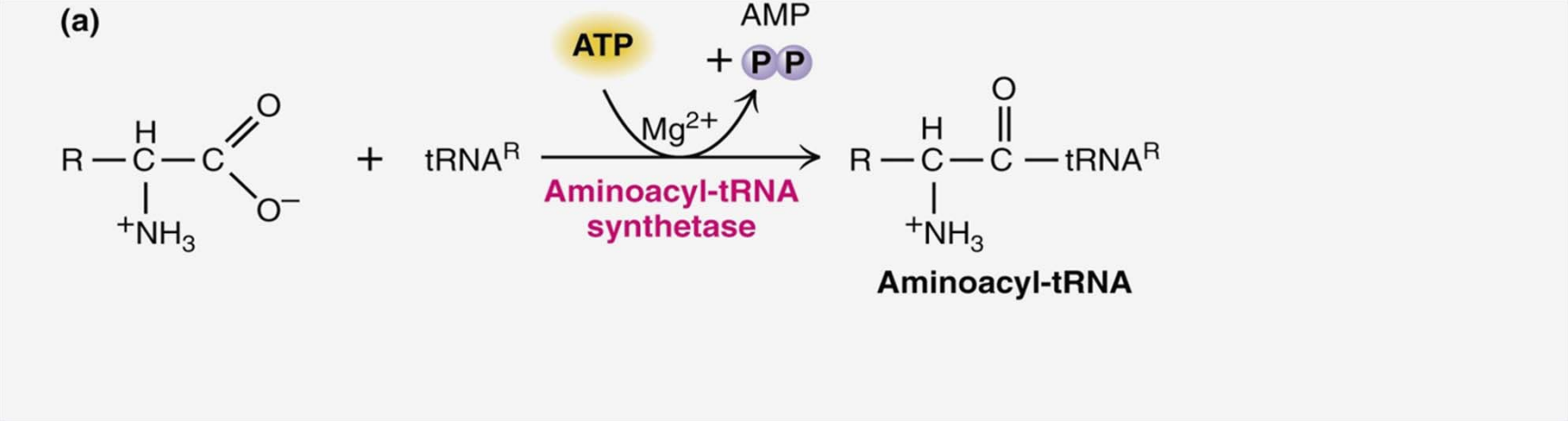



Figure 30.3 (a) the overall reaction.
(b) Aminoacyl-tRNA formation proceeds in two steps: (i) formation of the aminoacyl-adenylate and (ii) transfer of the activated amino acid to either the 2'-OH or 3'-OH at A residue of tRNA.

# The Aminoacyl-tRNA Synthetase Reaction: two steps

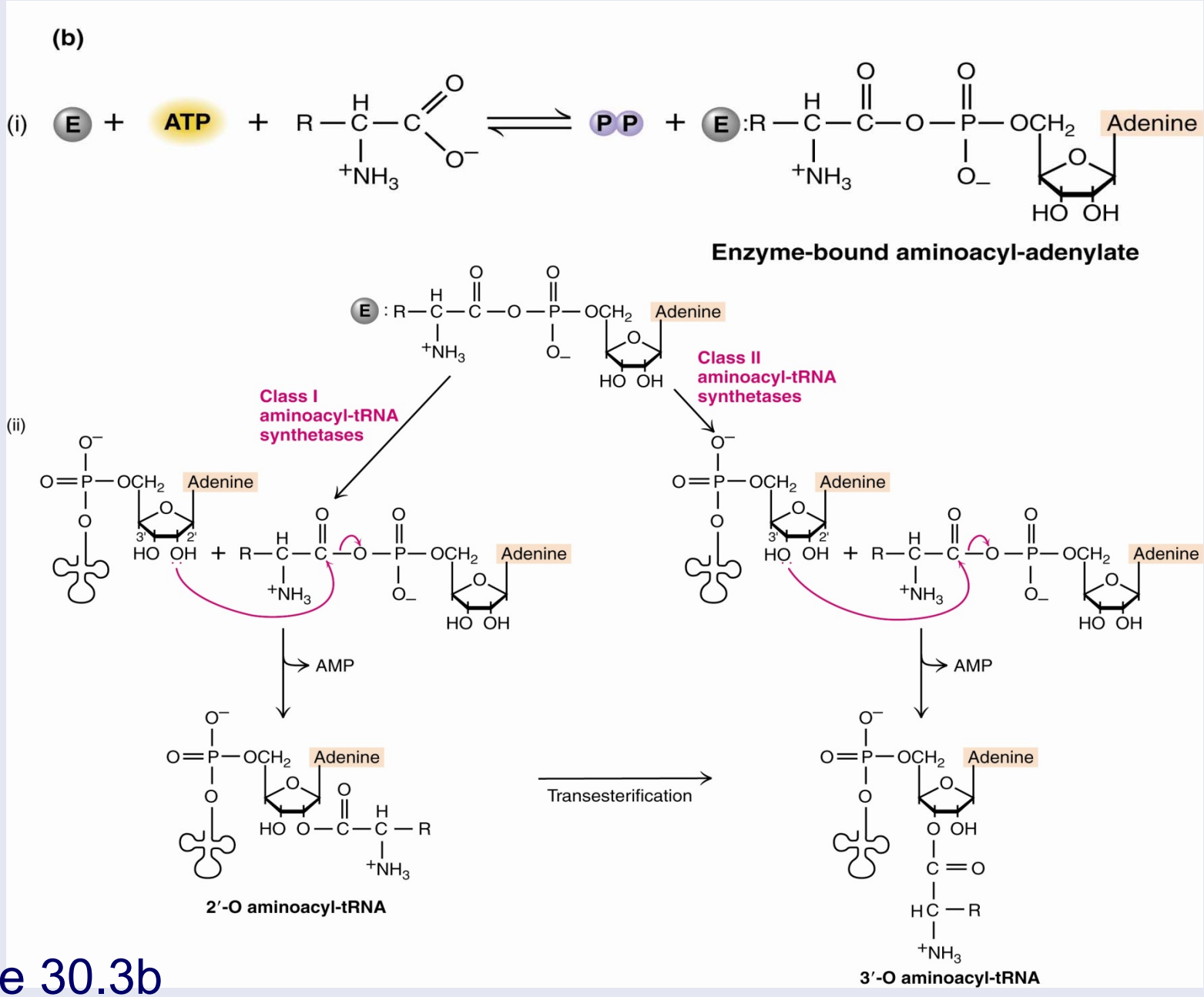



Figure 30.3b

# Mirror-symmetric interactions of class I versus class II aminoacyl-tRNA synthetases

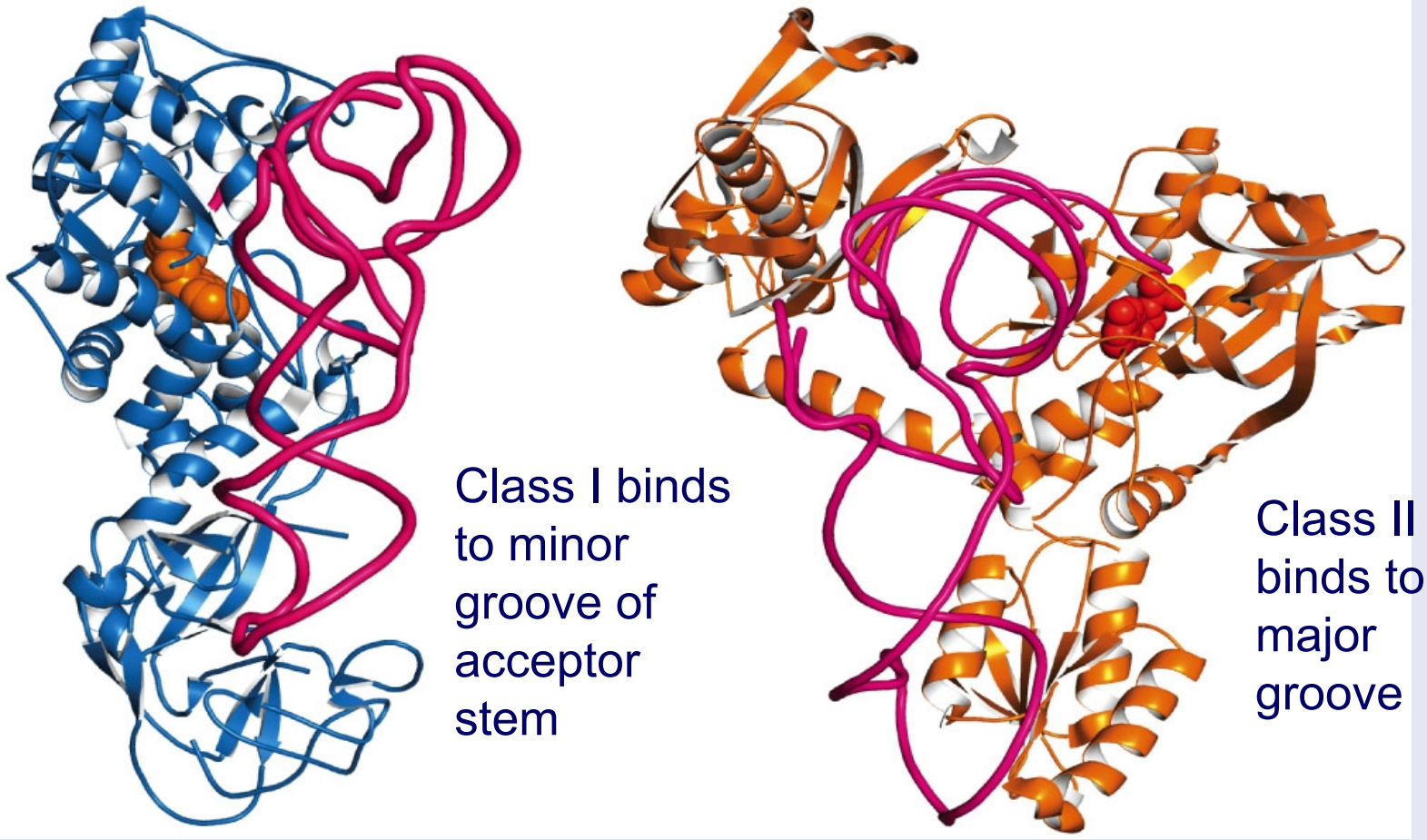



Figure 30.4 the two different enzymes bind to opposite faces of tRNA at 3' terminal CCA and acceptor stem.

# Aminoacyl-tRNA Synthetase Can Discriminate Between the Various tRNAs

- The synthetase need to recognize the cognate amino acids, and tRNA
- The recognized features in tRNA are **not universal**
- **A set of sequence elements (一組序列特徵)**

# The set of sequence elements

- At least one base in the **anticodon**
- One or more of the three base pairs in **acceptor stem**
- The base at canonical position 73, **discriminator base.** This base is fixed in the tRNAs for a particular amino acid.

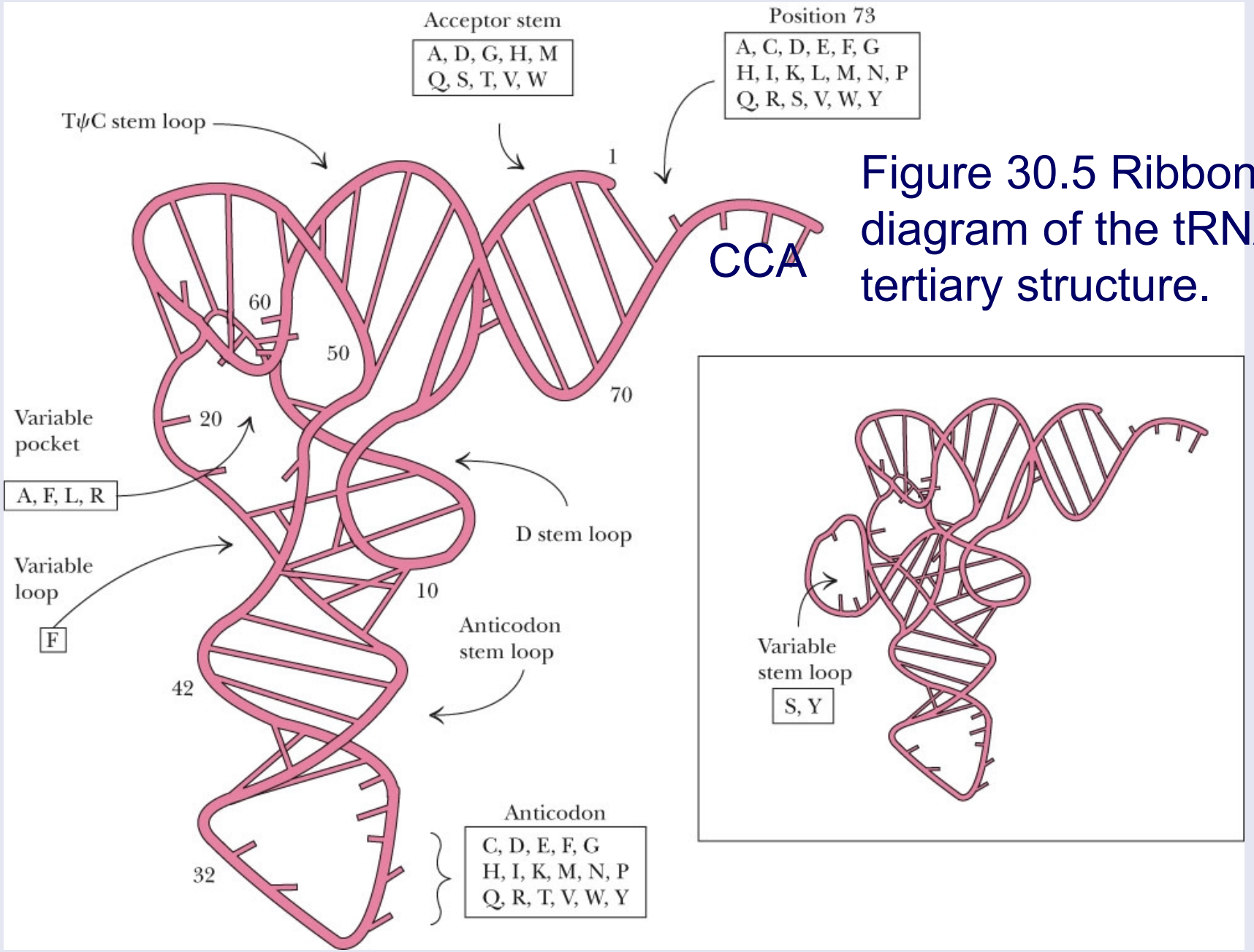
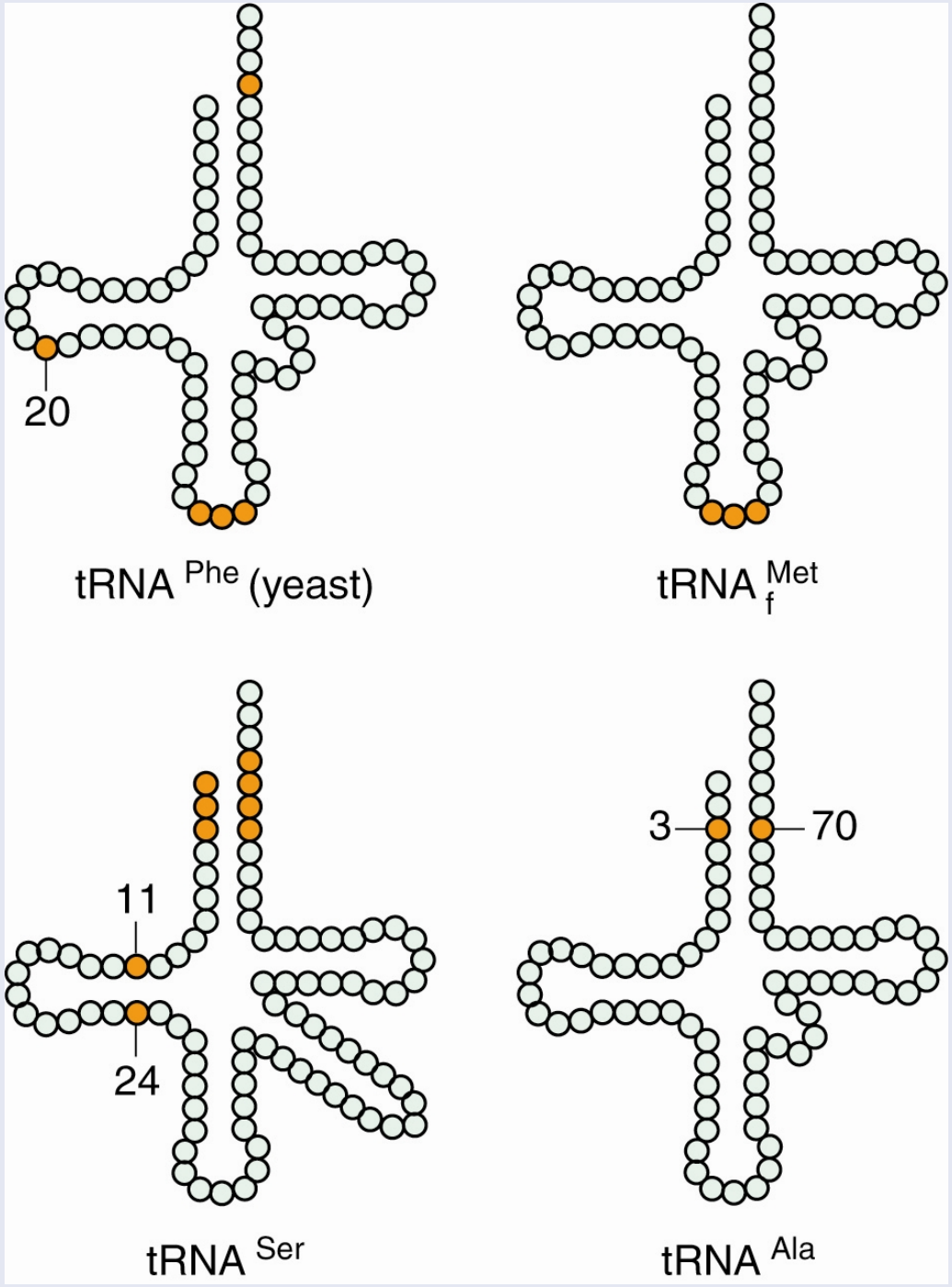



Figure 30.5 Ribbon diagram of the tRNA tertiary structure.

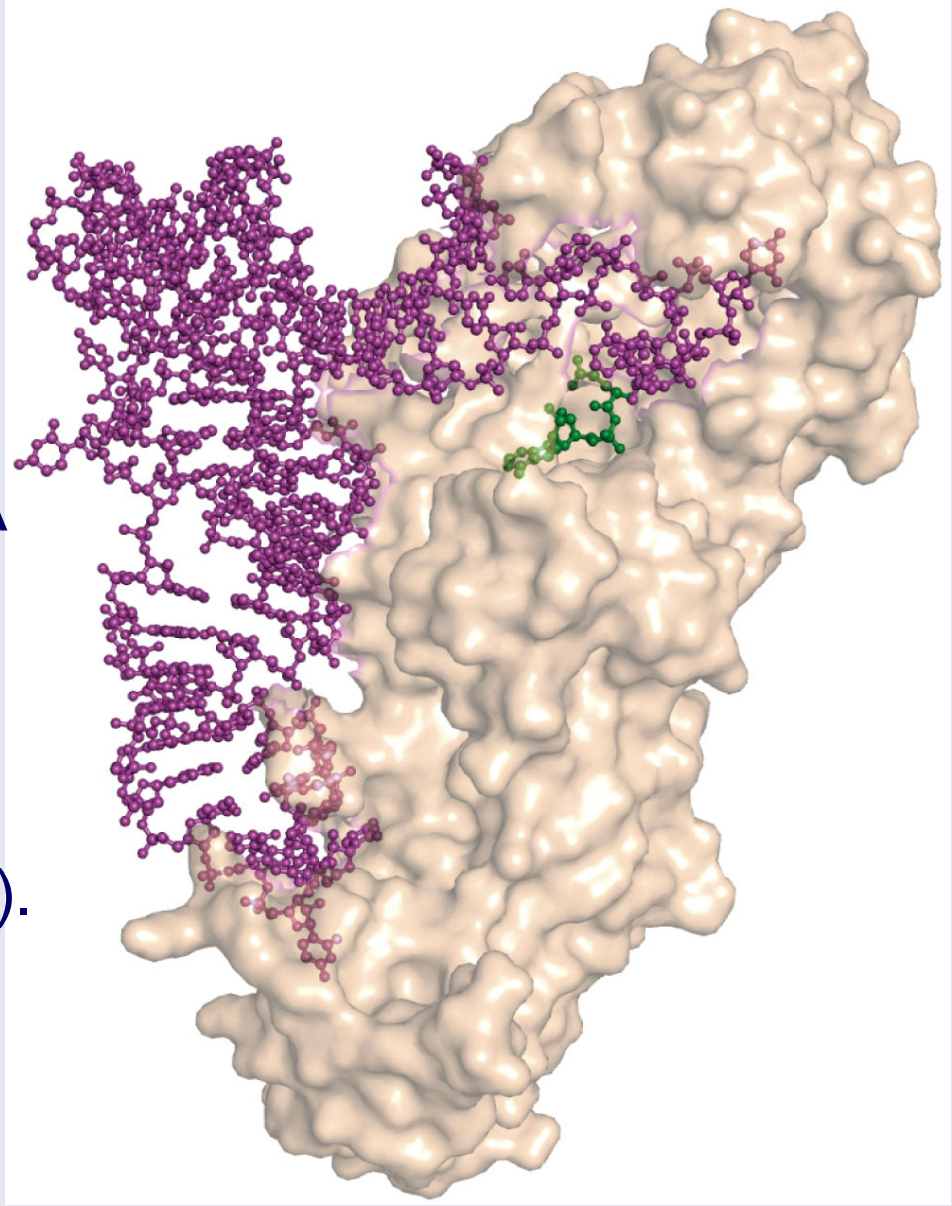
# tRNA Recognition

Figure 30.6 Major identify elements in four tRNA species. Numbered filled circles indicate positions of identity elements within the tRNA that are recognized by its specific aminoacyl-tRNA synthetase.



# Structure of an *E. coli* Glutaminyl-tRNA Synthetase Complexed with tRNA

Figure 30.7. The protein: tRNA contact region extends along one side of the entire length of this extended protein from acceptor stem to anticodon. The acceptor stem of the tRNA and the ATP (green) fit into a cleft at the top of the protein in this view. The enzyme also interacts extensively with the anticodon (lower tip of $tRNA^{Gln}$).



# A Single G:U Base Pair Defines $tRNA^{Ala}S$

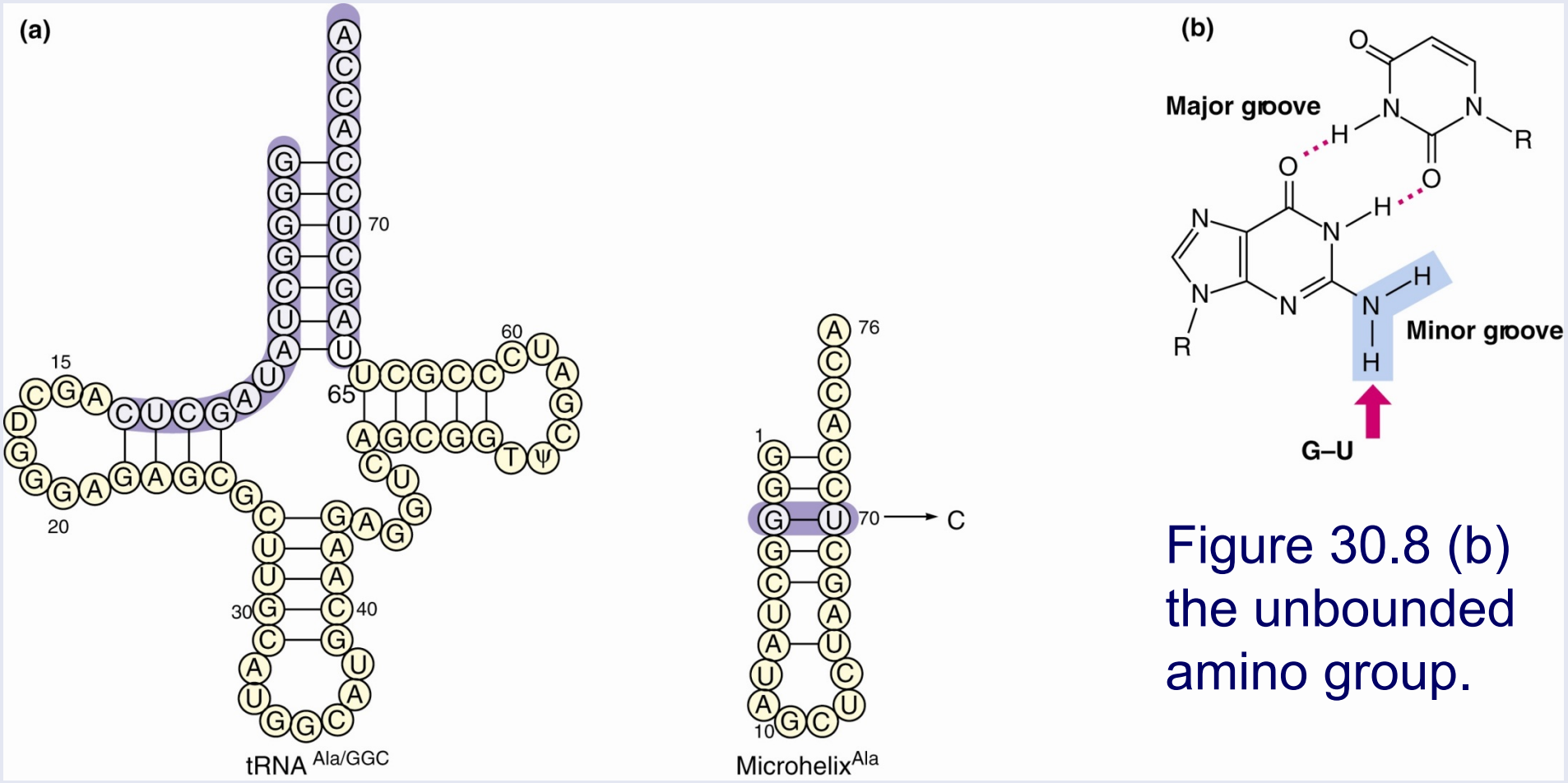



Figure 30.8 (b) the unbounded amino group.

Figure 30.8 (a) a microhelix analog of $tRNA^{Ala}$ is aminoacylated by alanyl-$tRNA^{Ala}$ synthetase, provided it has the characteristic $tRNA^{Ala}$ G3:U70 acceptor stem base pair.

# 30.3 What Are the Rules in Codon-Anticodon Pairing?

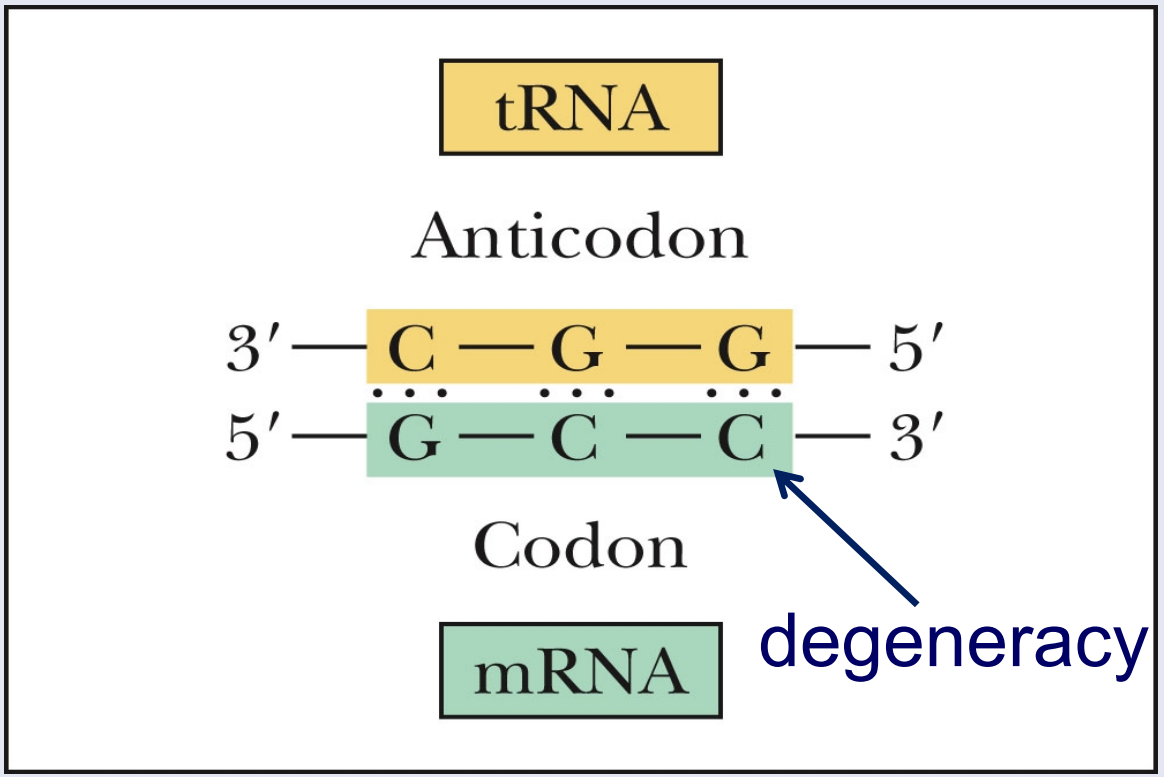



Figure 30.9 Codon-anticodon pairing. Complementary trinucleotide sequence elements align in antiparallel fashion.

# The “wobble” hypothesis for codon: anticodon pairing

- F. Crick hypothesized that the first two bases of the codon and the last two bases of the anticodon form canonical Watson-Crick base pairs
- But pairing between the third base of the codon and the first base of the anticodon follows less stringent rules
- That is, there is **wobble** in base pairing at this position. The third base is called “**wobble position**”

**TABLE 30.3** Base-Pairing Possibilities at the Third Position of the Codon

| Base on the Anticodon | Bases Recognized on the Codon |
|---|---|
| U | A, G |
| C | G |
| A | U |
| G | U, C |
| I | U, C, A |

- The wobble rules predict that four-codon families require at least two different tRNAs.
- **Isoacceptor tRNAs:** all members of the set of tRNAs for a particular a.a. They are served by one aminoacyl-tRNA synthetase.

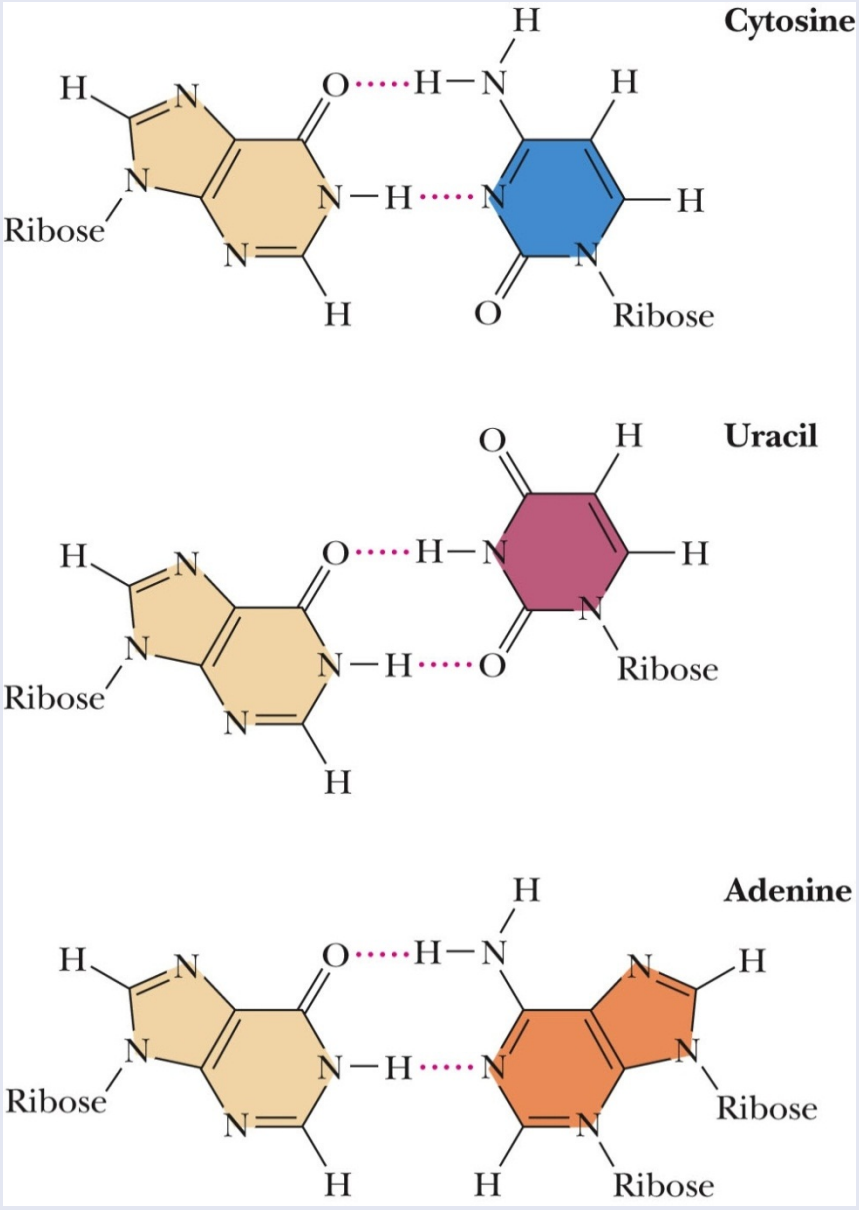



Figure 30.10 Pairing of anticodon inosine (I, left) with C, U, or A as the codon third base. Note that I is the keto tautomeric form.

# Some Codons Are Used More Than Others

- Because more than one codon exists for most amino acids, **variation in codon usage** is possible
- Might be due to the DNA of different organisms varies in relative A:T/G:C content
- Even in organisms of average base composition, codon usage may be biased

**TABLE 30.4** Representative Examples of Codon Usage in *E. coli* and Human Genes

The results are expressed as frequency of occurrence of a codon per 1000 codons tabulated in 1562 *E. coli* genes and 2681 human genes, respectively. (Because *E. coli* and human proteins differ somewhat in amino acid composition, the frequencies for a particular amino acid do not correspond exactly between the two species.)

| Amino Acid | Codon | *E. coli* Gene Frequency/1000 | Human Gene Frequency/1000 |
|---|---|---|---|
| Leu | CUA | 3.2 | 6.1 |
| | CUC | 9.9 | 20.1 |
| | CUG | 54.6 | 42.1 |
| | CUU | 10.2 | 10.8 |
| | UUA | 10.9 | 5.4 |
| | UUG | 11.5 | 11.1 |
| Pro | CCA | 8.2 | 15.4 |
| | CCC | 4.3 | 20.6 |
| | CCG | 23.8 | 6.8 |
| | CCU | 6.6 | 16.1 |
| Ala | GCA | 15.6 | 14.4 |
| | GCC | 34.4 | 29.7 |
| | GCG | 32.9 | 7.2 |
| | GCU | 13.4 | 18.9 |
| Lys | AAA | 36.5 | 21.9 |
| | AAG | 12.0 | 35.2 |
| Glu | GAA | 43.5 | 26.4 |
| | GAG | 19.2 | 41.6 |

- Preferred codons are represented by the most abundant isoacceptor tRNAs

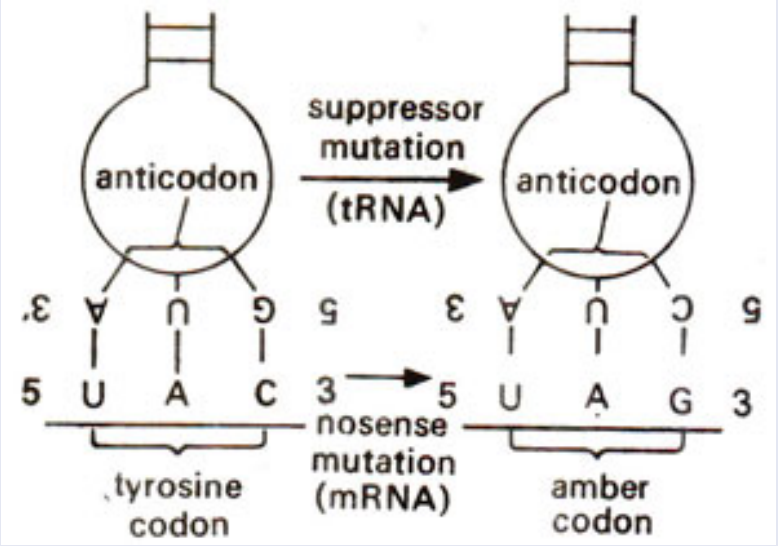
The effect of codon usage:

- mRNAs for proteins that are synthesized in abundance tend to employ preferred codons
- Synthetic human genes with *E. coli* preferred codons

# Nonsense Suppression Occurs When Suppressor tRNAs Read Nonsense Codons

- **Nonsense mutations**: a sense codon is altered to become nonsense codons – UAA, UAG, or UGA
- Geneticists have found that second mutations can suppress the effects of nonsense mutations, a phenomenon termed **nonsense suppression**
- **Suppressors** are mutations in tRNA genes that alter the anticodon so that the mutant tRNA could now read a particular “stop” codon and insert an amino acid

For example:
$tRNA^{Tyr}$ , anticodon GUA
mutate to CUA,
add Tyr at codon
UAG




- Suppressor tRNAs are typically generated from minor tRNA species within a set of isoacceptor tRNAs, causing no serious harm to organism.

# 30.4 What Is the Structure of Ribosomes, and How Are They Assembled?

- **Ribosomes** are compact **ribonucleoprotein** particles found in the cytosol of all cells, and in the matrix of mitochondria and the stroma of chloroplast
- The *E. coli* ribosome is a globular particle with 25 nm diameter, sedimentation coefficient of 70S, 2520 kD in mass, and consists of **two unequal subunits** that dissociate at < 1m*M* $Mg^{2+}$

# *E. coli* Ribosomes Are Composed of 30S and 50S Subunits

- **30S** subunit is 930 kD with 21 different proteins and a **16S rRNA**
- **50S** subunit is 1590 kD with 31 different proteins and two rRNAs: **23S rRNA** and **5S rRNA**
- Ribosomes are roughly 2/3 RNA by mass
- 20,000 ribosomes in an *E. coli* cell, 20% of cell's mass

**TABLE 30.5** Structural Organization of *E. coli* Ribosomes

| | Ribosome | Small Subunit | Large Subunit |
|---|---|---|---|
| Sedimentation coefficient | 70S | 30S | 50S |
| Mass (kD) | 2520 | 930 | 1590 |
| Major RNAs | | 16S = 1542 bases | 23S = 2904 bases |
| Minor RNAs | | | 5S = 120 bases |
| RNA mass (kD) | 1664 | 560 | 1104 |
| RNA proportion | 66% | 60% | 70% |
| Protein number | | 21 polypeptides* | 31 polypeptides† |
| Protein mass (kD) | 857 | 370 | 487 |
| Protein proportion | 34% | 40% | 30% |

* S proteins

+ L proteins

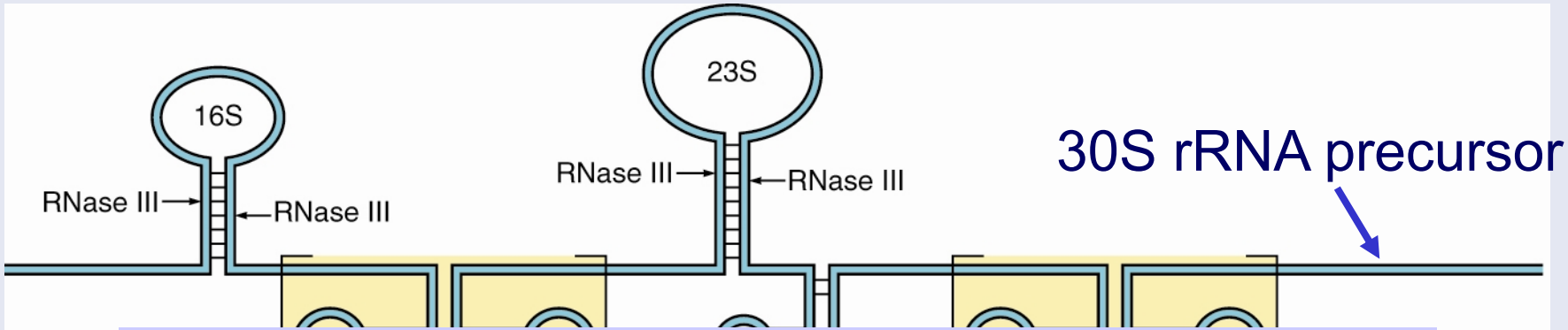
# Prokaryotic Ribosomes Are Made from 50 Different Proteins and Three Different RNAs

## Ribosomal proteins

- One copy of each ribosomal protein per 70S ribosome, with 3 exceptions.
- L7/L12: they are identical, except for extent of acetylation at N-terminus
- S20 = L26: a protein common to large and small subunits

- The largest ribosomal protein is S1 (557 residues, 61.2 kD)
- The smallest ribosomal protein is L34 (46 residues, 5.4 kD)
- The sequences of ribosomal proteins share little similarity
- Rich in **cationic amino acids** Lys and Arg, and few aromatic amino acids
- Properties suitable **for interaction with polyanionic RNAs**

# The rRNAs of *E. coli* Are Encoded by a Set of Seven Operons




Transcription of rRNA accounts for 80% to 90% of total cellular RNA synthesis

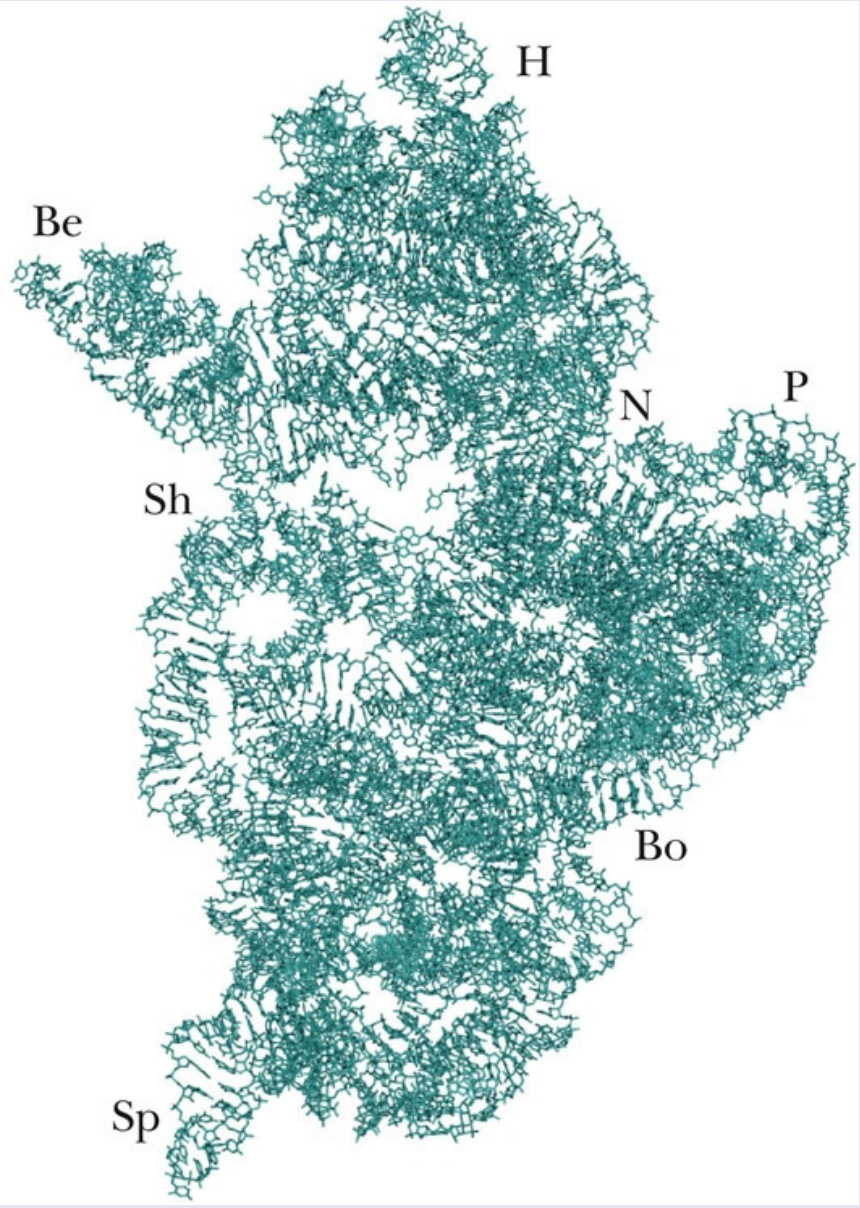
| Operon | Spacer tRNA | Trailer tRNA | Chromosomal location (min) |
|---|---|---|---|
| *rrnA* | Ile, Ala | - - - - - - - - | 86 |
| *rrnB* | Glu | - - - - - - - - | 89 |
| *rrnC* | Glu | Asp, Trp | 84 |
| *rrnD* | Ile, Ala | - - - - - - - - | 71 |
| *rrnE* | Glu | - - - - - - - - | 90 |
| *rrnG* | Glu | Asp | 56 |
| *rrnH* | Ile, Ala | | 5 |

Figure 30.11 The seven ribosomal RNA operons in *E. coli*. Numerals to the right of the brackets indicate the number of species of tRNA encoded by each transcript.

- Ribosomal RNAs form extensive secondary structures and double helix
- Conformation of rRNA molecules determine the general shapes of the ribosomal subunits
- Ribosomal proteins serve a structural role in ribosomes by bracing and stabilizing rRNA conformations

# The Shapes of Ribosomal Subunits Are Determined by the rRNA Conformations

Figure 30.12 Tertiary structure of the 16S rRNA within the *Thermus thermophilus* 30S ribosomal subunit. This view is of the face that interacts with the 50S subunit. H = head; Be = beak; N = neck; P = platform; Sh =shoulder; Sp = spur; Bo = body.



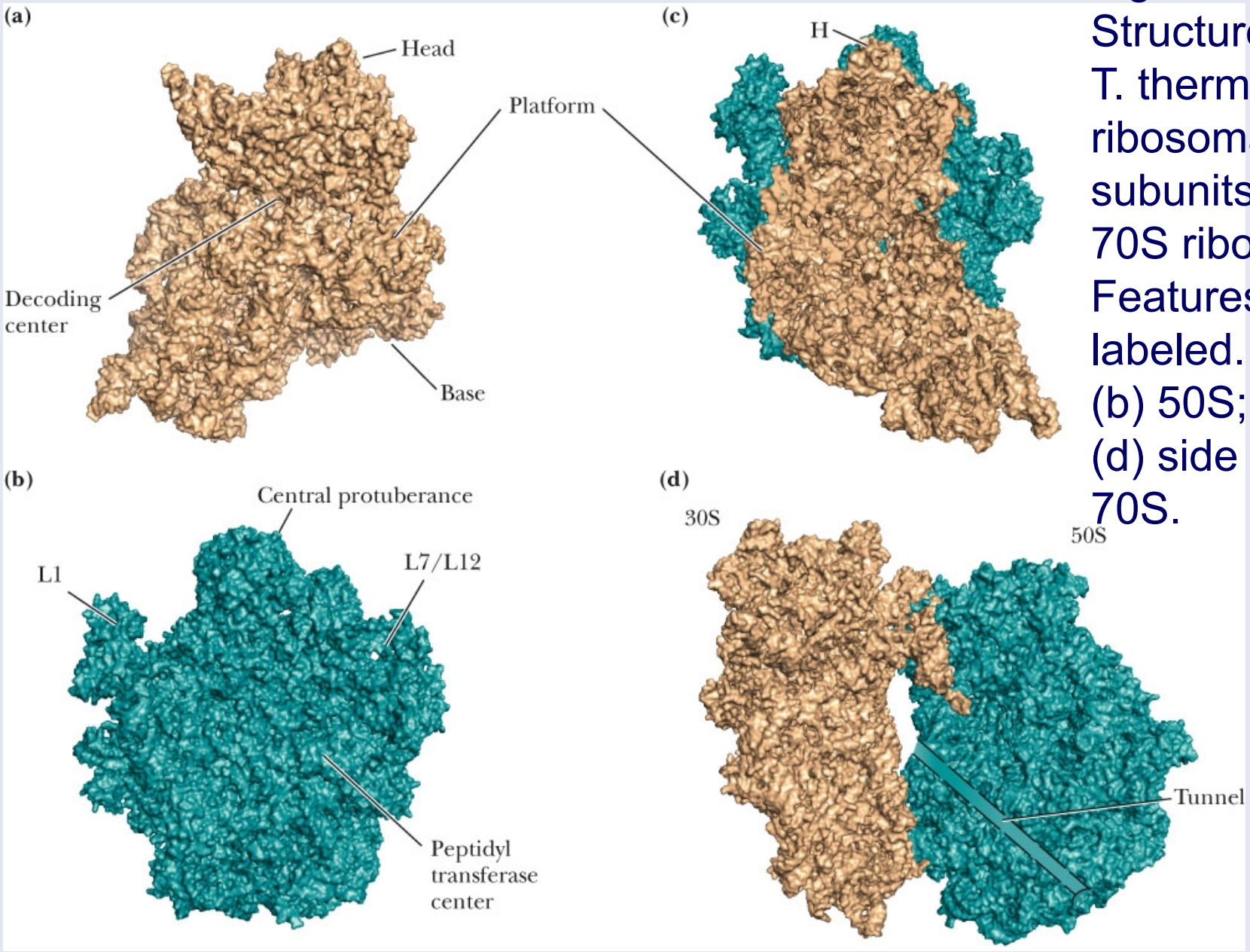



Figure 30.13 Structure of the T. thermophilus ribosomal subunits and 70S ribosome. Features are labeled. (a) 30S; (b) 50S; (c) 70S; (d) side view of 70S.

# Ribosomes Self-Assemble Spontaneously in Vitro

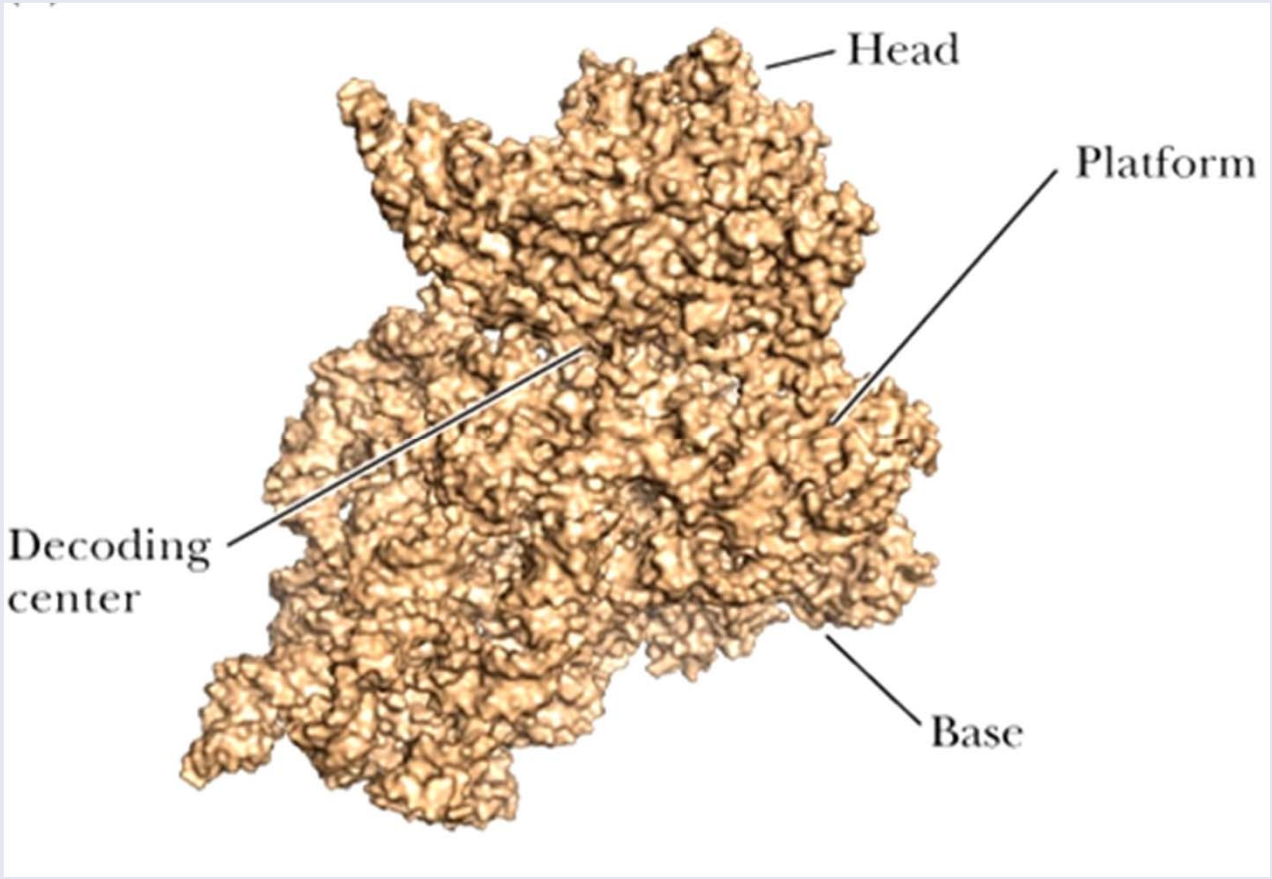
- If individual proteins and rRNAs are mixed under appropriate conditions, functional ribosomes will assemble
- rRNA acts as a scaffold on which the ribosomal proteins convene in a specific order

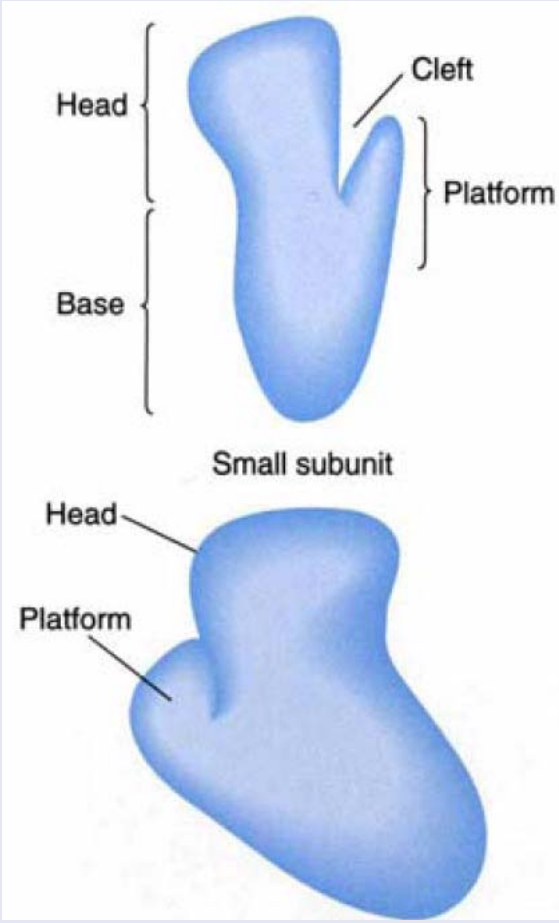
# Ribosomes Have a Characteristic Anatomy

- The 30S subunit: head, body (or base), platform
- A cleft forms between head, body, and platform; mRNA passes across the cleft

- Platform (projecting from the base):
  - the central domain of 30S
  - binds mRNA and anticodon stem-loop of aminoacyl-tRNA
  - mediating codon-anticodon recognition: **decoding center,** composed of only 16S rRNA

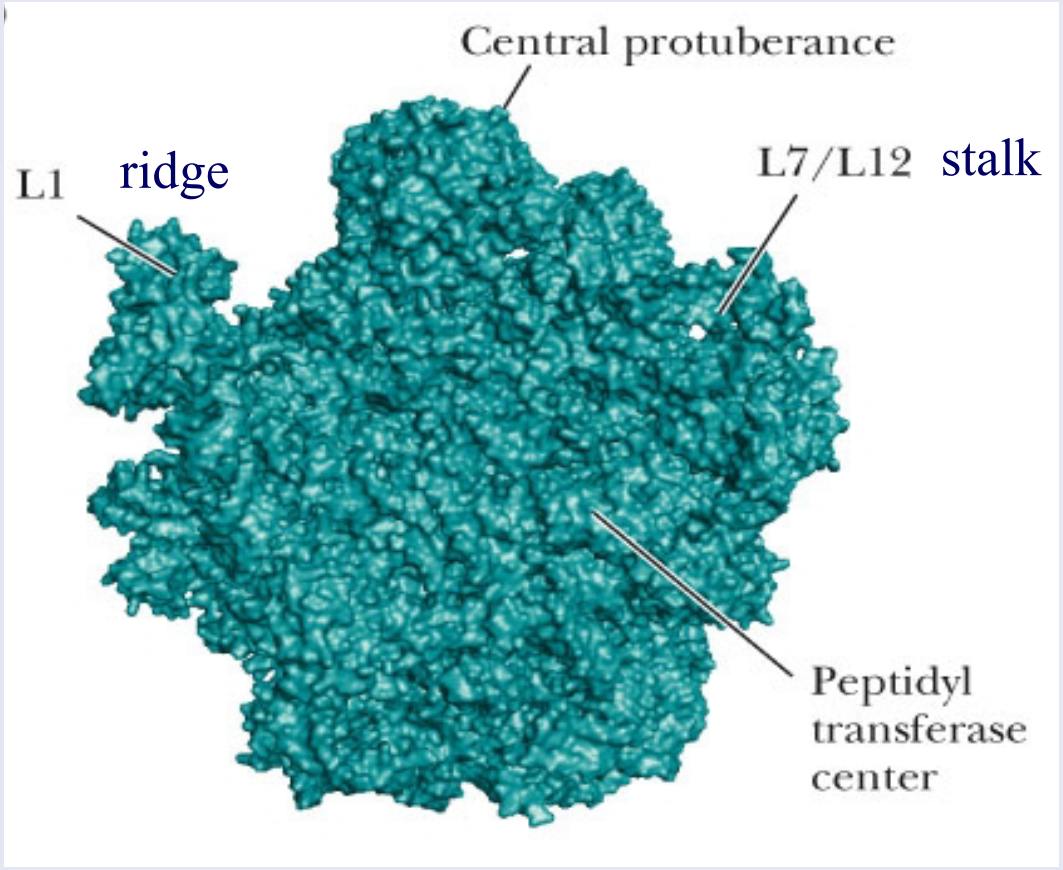
# Inner face

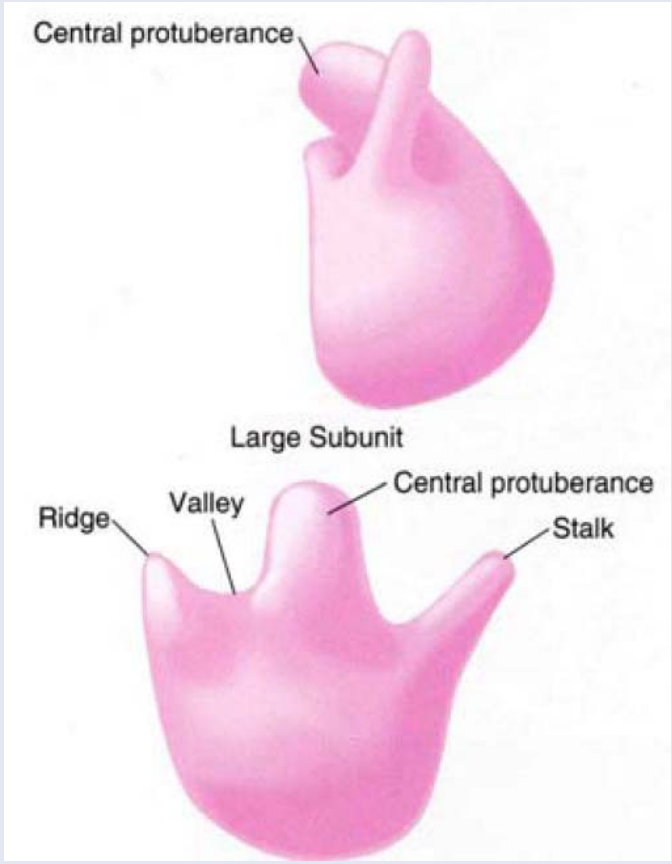

Head
Platform
Decoding center
Base


Head
Cleft
Platform
Base
Small subunit
Head
Platform

- The 50S subunit: a mitt-like globular structure with three distinct projections –central protuberance, stalk, and L7/L12 ridge
- 50S subunit binds the aminoacyl-acceptor ends of tRNA,catalyzing peptide bond formation
- The catalytic center, the **peptidyl transferase**, is located at the bottom of 50S
- From the center, a tunnel runs through the large subunit and the growing peptide chain is threaded through the tunnel during protein synthesis

# Inner face


Central protuberance
L1
ridge
L7/L12
stalk
Peptidyl
transferase
center


Central protuberance
Large Subunit
Ridge
Valley
Central protuberance
Stalk

# The Cytosolic Ribosomes of Eukaryotes Are Larger than Prokaryotic Ribosomes

- Mitochondrial and chloroplast ribosomes are quite similar to prokaryotic ribosomes, reflecting their prokaryotic origin
- Cytoplasmic ribosomes are larger and more complex, but many of the structural and functional properties are similar to prokaryotic ribosomes

**TABLE 30.6** Structural Organization of Mammalian (Rat Liver) Cytosolic Ribosomes

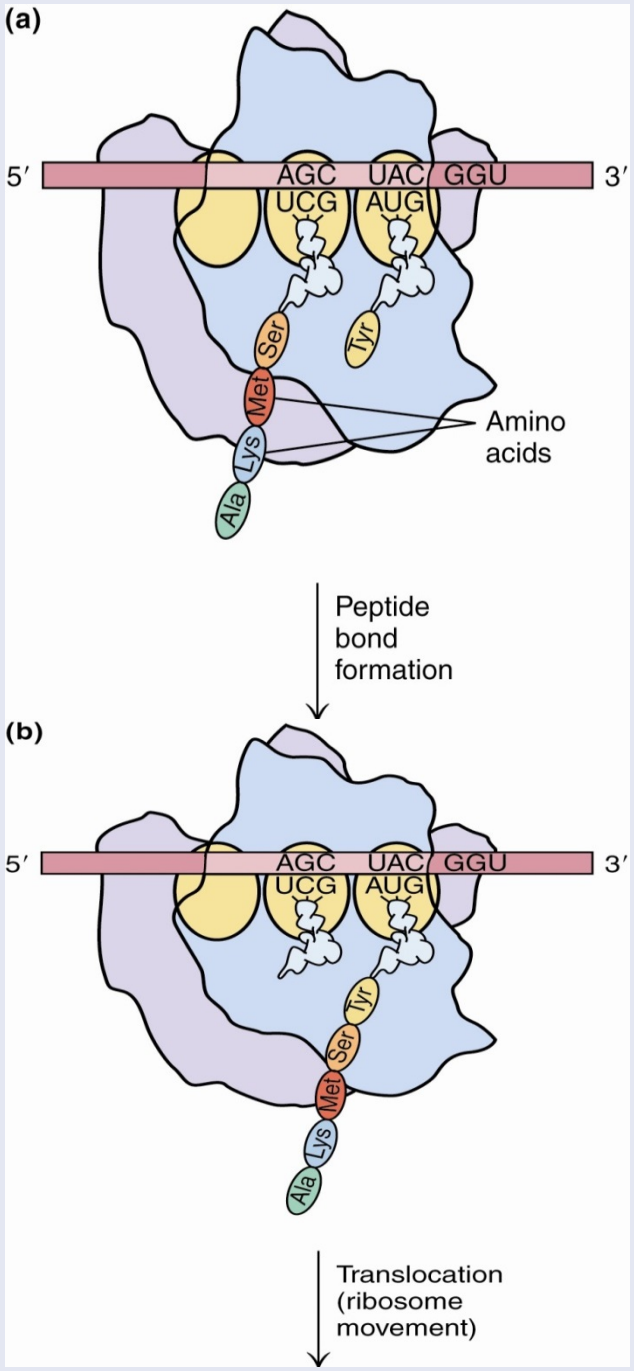
| | Ribosome | Small Subunit | Large Subunit |
|---|---|---|---|
| Sedimentation coefficient | 80S | 40S | 60S |
| Mass (kD) | 4220 | 1400 | 2820 |
| Major RNAs | | 18S = 1874 bases | 28S = 4718 bases |
| Minor RNAs | | | 5.8S = 160 bases |
| | | | 5S = 120 bases |
| RNA mass (kD) | 2520 | 700 | 1820 |
| RNA proportion | 60% | 50% | 65% |
| Protein number | | 33 polypeptides | 49 polypeptides |
| Protein mass (kD) | 1700 | 700 | 1000 |
| Protein proportion | 40% | 50% | 35% |

- The rRNA genes of eukaryotes are present in the form of several hundred tandem clusters:  in humans, 300–400 repeats, five clusters (chromosomes).
- Nucleolus: a distinct region where these clusters are located and transcription of rRNA occurs
- 80% to 90% of eukaryotic transcription is rRNA synthesis

# 30.5 What Are the Mechanics of mRNA Translation?

- Protein synthesis involves three phases: initiation, elongation, and termination
- **GTP** hydrolysis provides the energy driving the process
- **G-protein** family members use released energy to fuel conformational changes

- **Initiation:** Binding of mRNA to small subunit, followed by an initiator aminoacyl-tRNA, then by large subunit
- **Elongation**: Synthesis of all peptide bonds – ribosome moves along mRNA, translating the message into amino acid, with repetitive cycle of adding aminoacyl-tRNAs
- **Termination:** when **stop codon** is reached; the polypeptide chain is released and ribosome dissociate from mRNA




# Elongation steps:

## Ribosome may bind three tRNAs:

A: acceptor site
P: peptidyl site
E: exit site

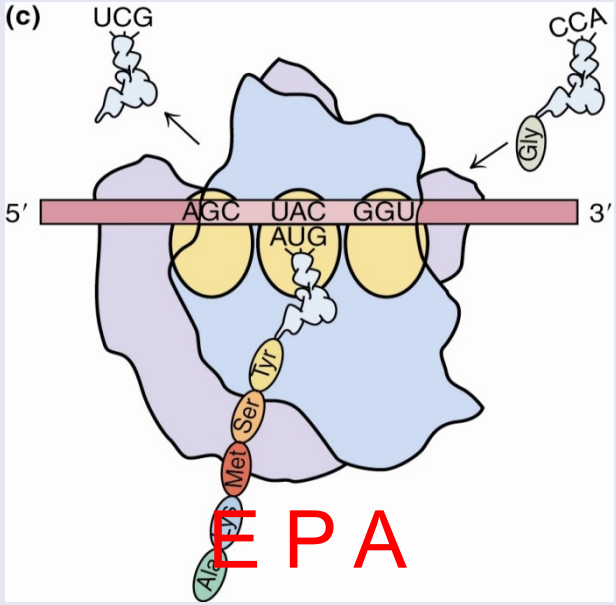



Figure 30.14 the basic steps in protein synthesis.

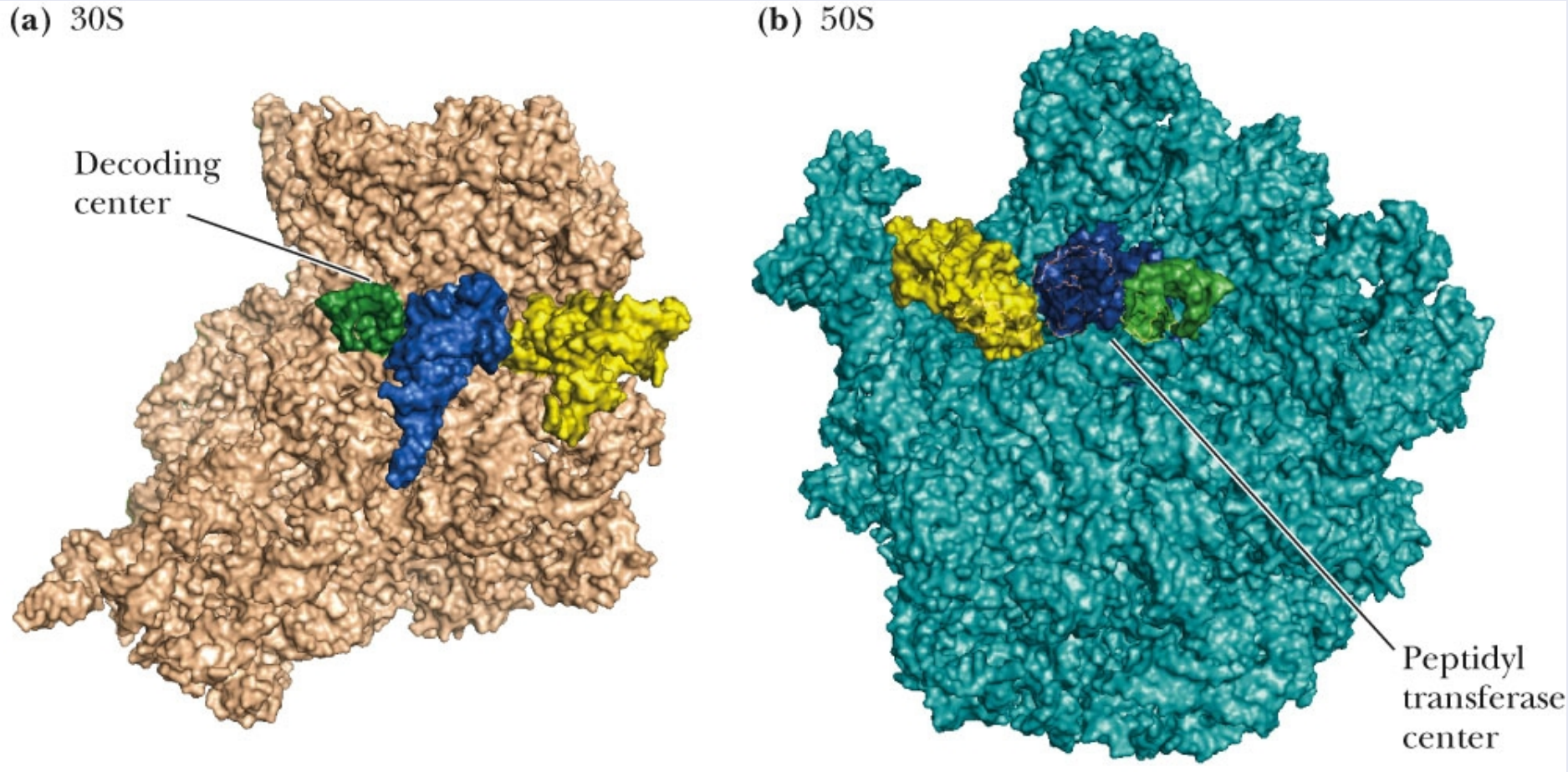



Figure 30.15 The three tRNA binding sites on ribosomes. The view shows the ribosomal surfaces that form the interface between the (a) 30S and (b) 50S subunits in a 70S ribosome. The A (green), P (blue), and E (yellow) sites have bound tRNA.

# Peptide Chain Initiation in Prokaryotes Requires a G-Protein Family Member

The components required for peptide chain initiation:

1) mRNA
2) 30S and 50S ribosomal subunits
3) a set of proteins known as **initiation factors**
4) **GTP**
5) **f-Met-tRNA$_i^{fMet}$**

# Initiator tRNA

- $tRNA_i^{fMet}$ reads AUG (or GUG, UUG) codon that signals the start site of a polypeptide
- The initiator tRNA is charged with a formylated methionine to become f-Met-$tRNA_i^{fMet}$
- **N-formyl-Met-$tRNA_i^{fMet}$** is only used for initiation, and regular Met-$tRNA^{Met}$ is used for incorporation of Met internally
- The N-formyl methionine is removed in about half of the *E.coli* proteins post-translationally

# Initiator tRNA

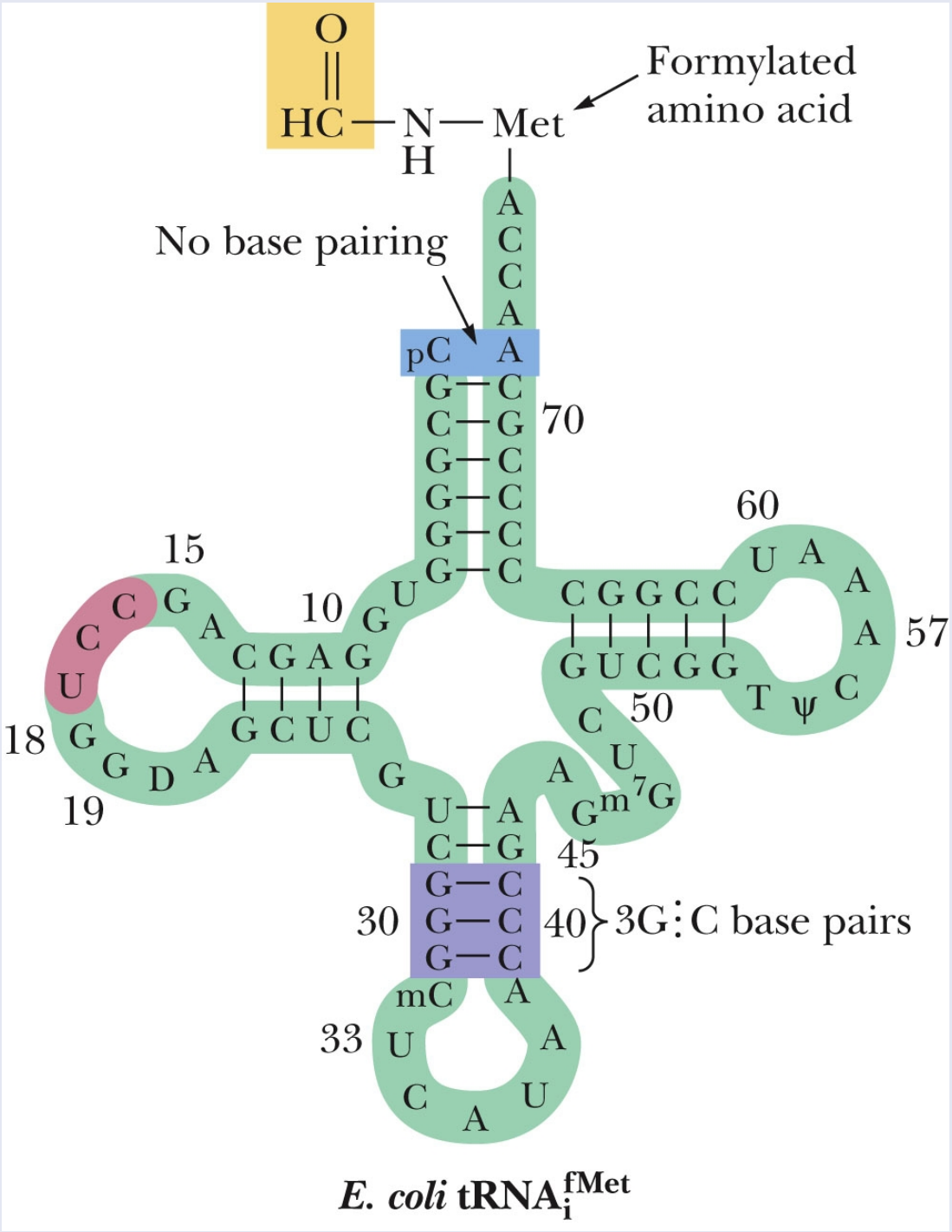



Figure 30.16 The secondary structure of *E. coli* N-formyl-methionyl-$tRNA_i^{fMet}$. The features distinguishing it from noninitiator tRNAs are highlighted.

# The Transformylation of Methionyl-tRNA$_i^{fMet}$

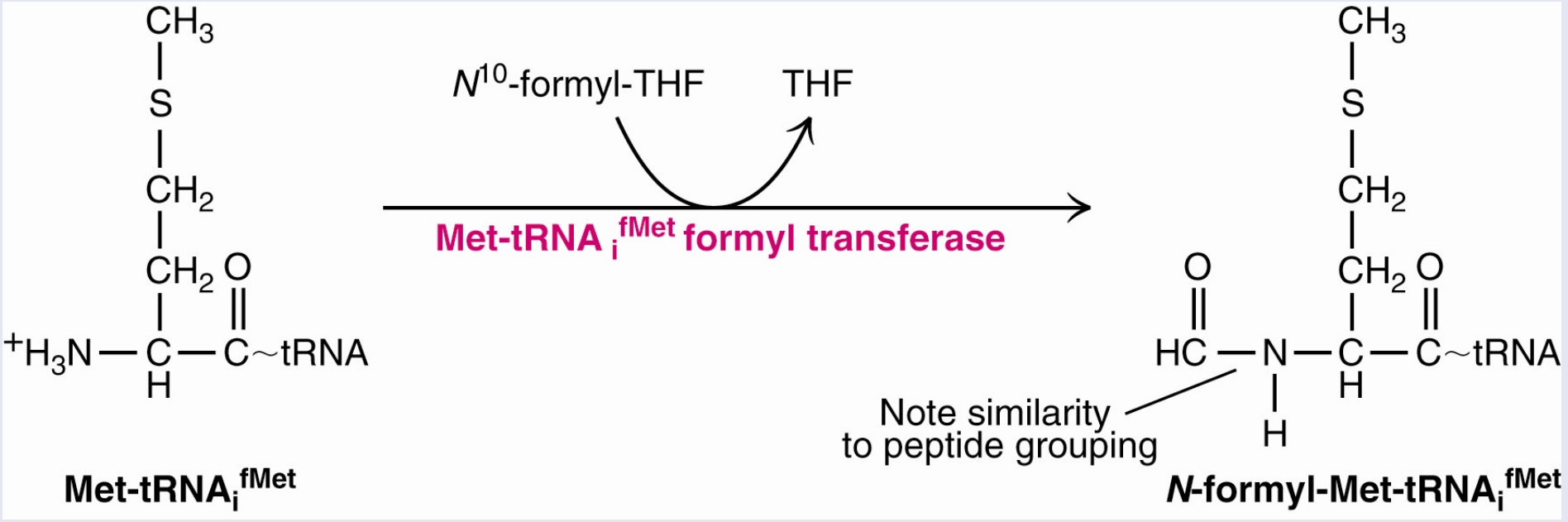



Figure 30.17 Methionyl-tRNA$_i^{fMet}$ formyl transferase catalyzes the transformylation of methionyl-tRNA$_i^{fMet}$. **The tRNA for reading Met codons within a protein (tRNA$^{Met}$) is not a substrate for this transformylase.**

# mRNA AUG Recognition and Alignment

- Recognition of translation initiation codon on mRNAs involves the 16S rRNA of the 30S subunit
- A pyrimidine-rich sequence on 3'-end of 16S RNA is aligned with a purine-rich part of 5'-end of mRNA
- The purine-rich segment - the ribosome-binding site - is known as the **Shine-Dalgarno sequence**
- These sequences lie about 10 nt upstream from their respective AUG initiation codon

# Various Shine-Dalgarno Sequences Recognized by *E. coli* Ribosomes


Figure 30.18 These sequences lie about 10 nucleotides upstream from their respective AUG initiation codon and are complementary to the UCCU core sequence element of *E. coli* 16S rRNA.

# Properties of *E. coli* Initiation Factors

**TABLE 30.7** Properties of *E. coli* Initiation Factors

| Factor | Mass (kD) | Molecules/ Ribosome | Function |
|---|---|---|---|
| IF-1 | 9 | 0.15 | Binds to 30S A site and prevents tRNA binding |
| IF-2 | 97 | | G-protein that binds fMet-$tRNA_i^{fMet}$; interacts with IF-1 |
| IF-3 | 23 | 0.25 | Binds to 30S E site; prevents 50S binding; mRNA binding |

- Initiation factor (IF) proteins combine with GTP, *N*-formyl-Met-$tRNA_i^{fMet}$, mRNA and 30S ribosome form the **30S initiation complex**
- The 50S subunit then binds to form a **70S initiation complex**
- The **initiation factors** are soluble proteins required for assembly of these initiation complexes

# Events of Initiation

- 30S subunit:(IF-3:IF-1) complex binds mRNA, and a complex of IF-2, GTP and f-Met-tRNA$_i^{fMet}$ ; 30 S initiation complex
- IF-3 losses
- 50S subunit binding triggers GTP hydrolysis
- GDP, IF-2, IF-1 release
- Formation of active 70S initiation complex

# The Sequence of Events in Peptide Chain Initiation

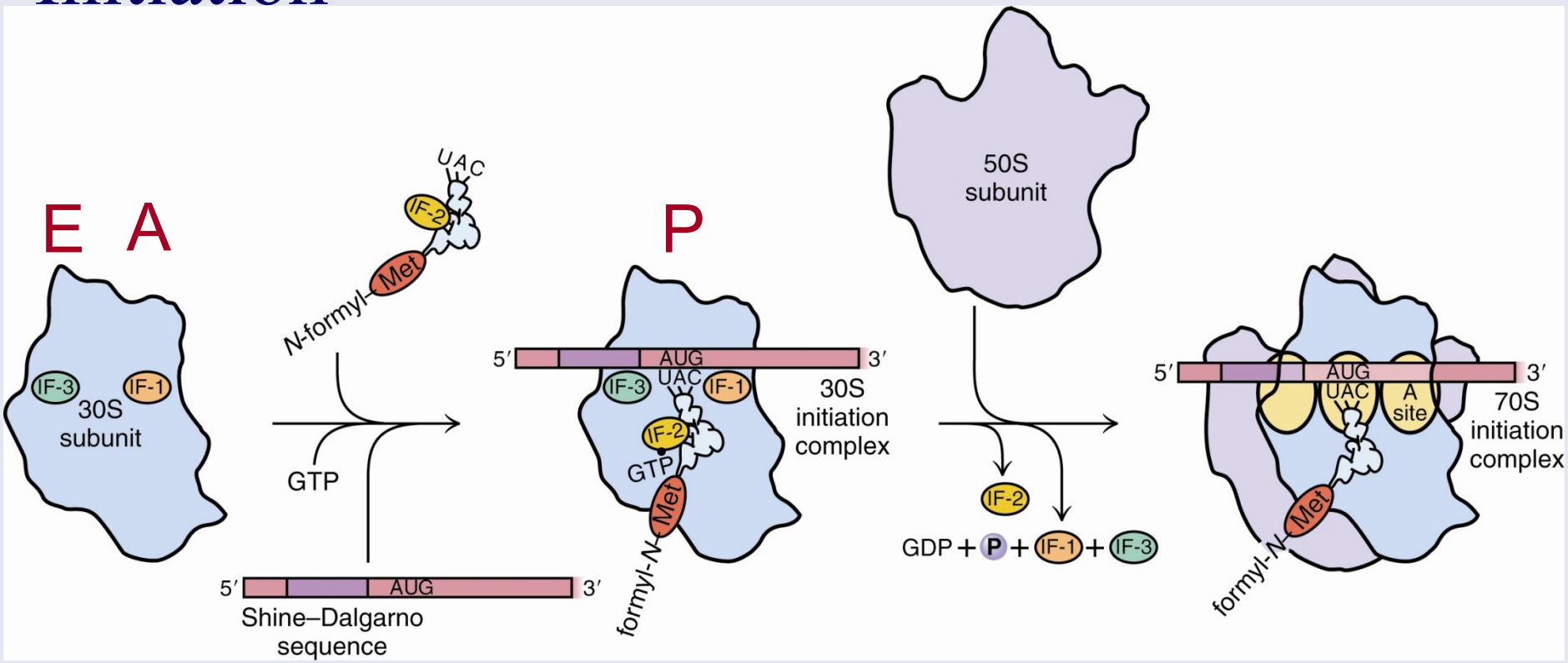



Figure 30.19 Initiation begins when a 30S subunit:(IF-3:IF-1) complex binds mRNA and a complex of IF-2, GTP, and f-Met-$tRNA_i^{fMet}$.

# Peptide Chain Elongation Requires Two G-Protein Family Members

The requirements for peptide chain elongation are:

1) An mRNA:70S ribosome:peptidyl-tRNA complex (peptidyl-tRNA in the P site)
2) Aminoacyl-tRNAs
3) A set of proteins known as **elongation factors**
4) GTP

# Chain elongation can be divided into 3 steps:

1) **Binding of the incoming aminoacyl-tRNA** at the A site. Proper aminoacyl-tRNA being confirmed by decoding center
2) **Peptide bond formation**: transfer of the peptidyl chain from the tRNA to the $-NH_2$ group of the new amino acid
3) **Translocation:** Ribosome translocates one codon further along the mRNA, shifting the peptidyl-tRNA to the P site to empty the A site

# Elongation Factors

**TABLE 30.8** Properties of *E. coli* Elongation Factors

| Factor | Mass (kD) | Molecules/ Cell | Function |
|---|---|---|---|
| EF-Tu | 43 | 70,000 | G protein that binds aminoacyl-tRNA and delivers it to the A site |
| EF-Ts | 74 | 10,000 | Guanine-nucleotide exchange factor (GEF) that replaces GDP on EF-Tu with GTP |
| EF-G | 77 | 20,000 | G protein that promotes translocation of mRNA |

- These proteins are present in large quantities, reflecting its importance to cell viability.

# Aminoacyl-tRNA Binding

- **EF-Tu** binds aminoacyl-tRNA and GTP forming ternary complex
- The complex binds to stalk region of 50S
- Correct paring between codon-anticodon stimulate EF-Tu to hydrolyze GTP
- EF-Tu:GDP complex release
- **EF-Ts** recycles EF-Tu by exchanging GTP for GDP by forming EF-Tu/EF-Ts transient complex
- GAC: GTPase-activating center, in 50 S ribosome, at A2662 in 23S rRNA

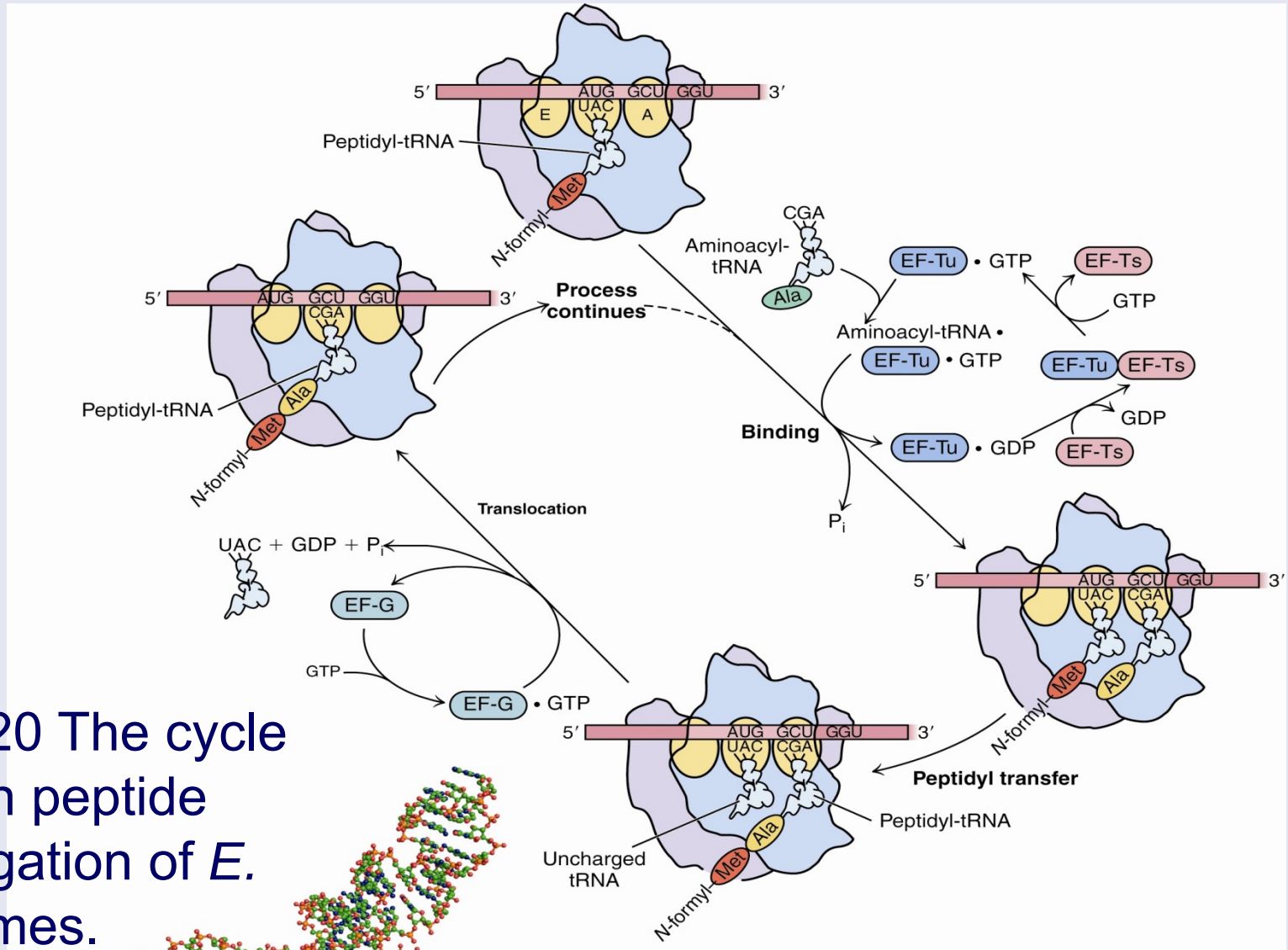
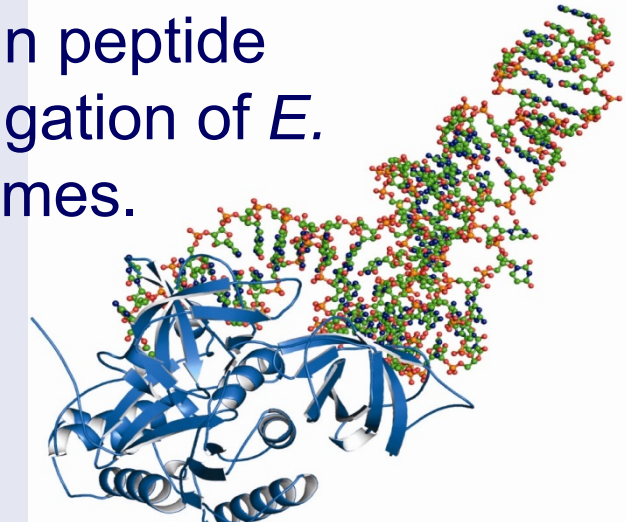



Figure 30.20 The cycle of events in peptide chain elongation of *E. coli* ribosomes.



The structure at lower left is an EF-Tu:aminoacyl-tRNA complex.

# The **Decoding Center**: A 16S rRNA function

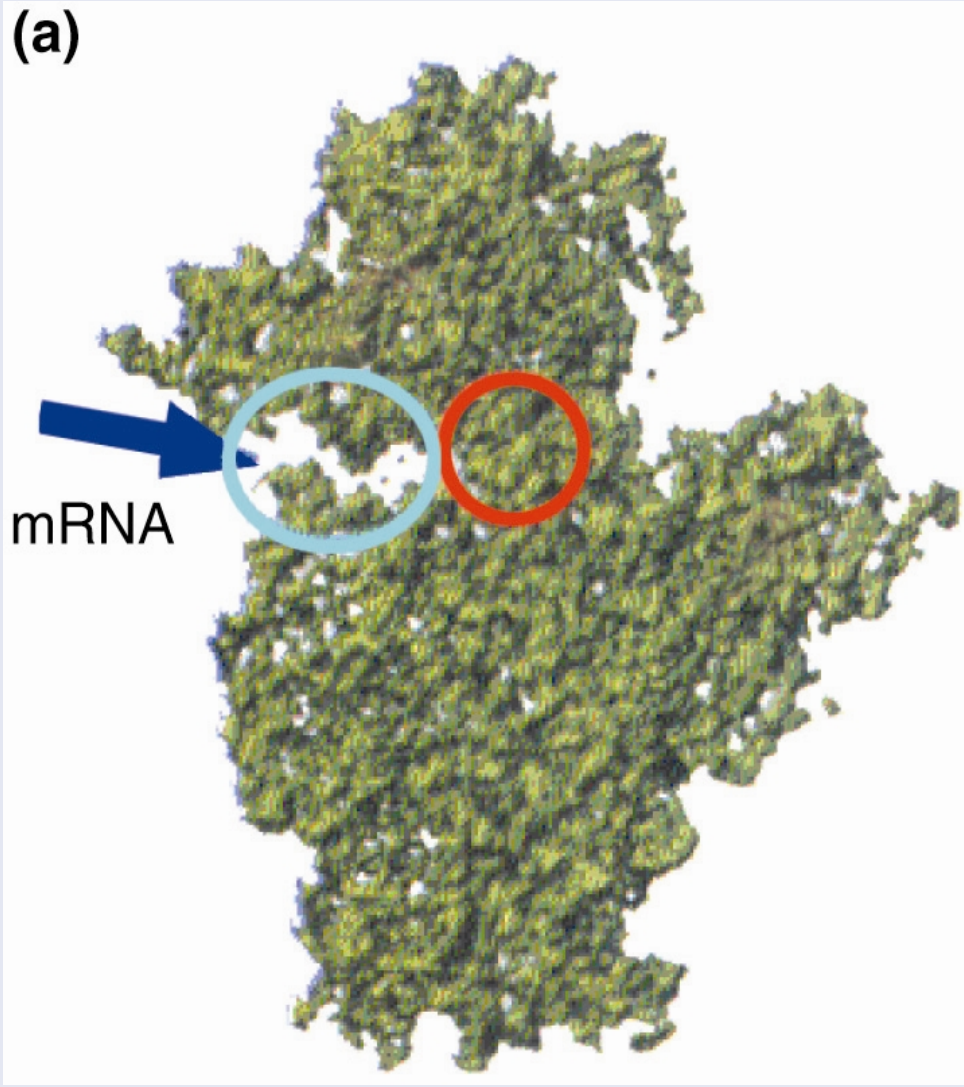
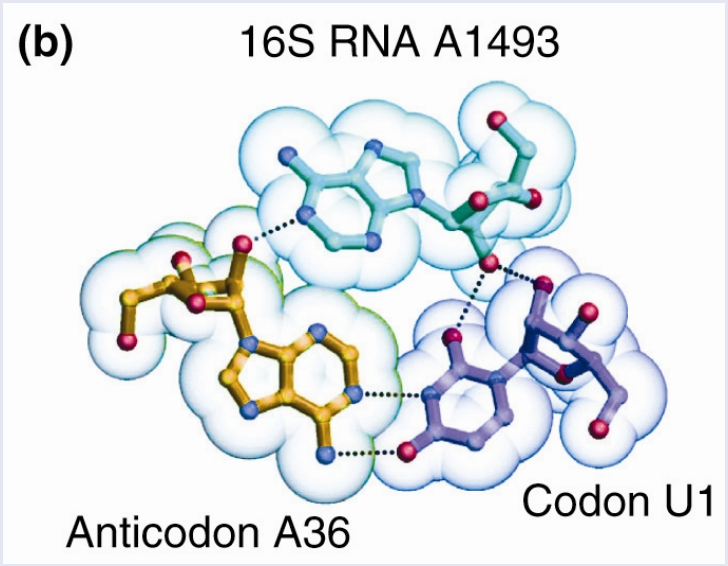



Figure 30.21 (a) The 30S subunit, as viewed from the 50S subunit. Red circle indicates the location of decoding center.

# The Decoding Center



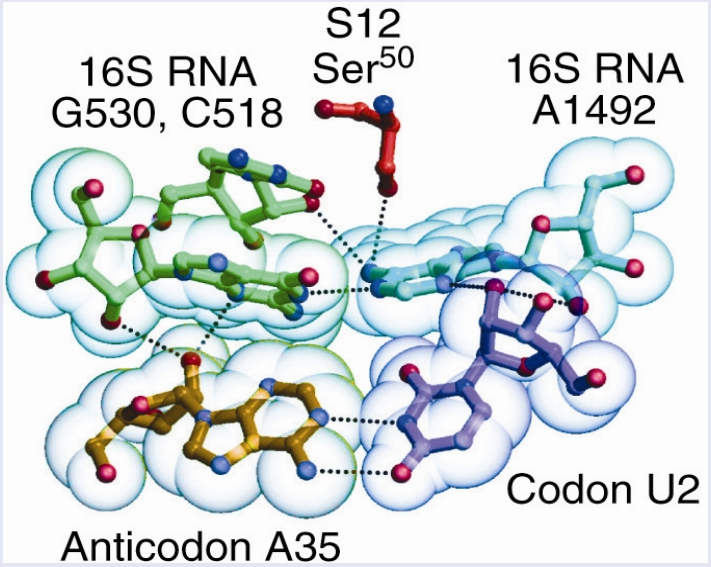
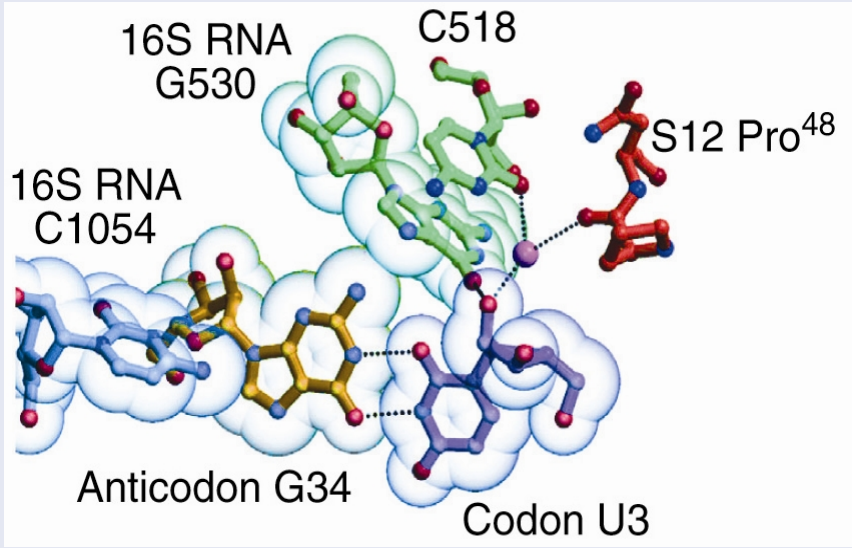




Figure 30.21 (b) 30S ribosomal subunit interactions with the codon-anticodon duplex during cognate tRNA recognition.



# **Peptidyl Transfer** – the Central Reaction of Protein Synthesis

- Peptidyl transfer, or **transpeptidation**, is the peptide bond-forming step
- No energy input is needed; the ester bond linking the peptidyl moiety to tRNA is intrinsically reactive
- The **peptidyl transferase** is associated with the **23S rRNA** in 50S ribosomal subunit
- The **peptidyl transferase center (PTC)** of 23S rRNA

# Catalytic Power of the Ribosome Depends on Tight Binding of Its Substrates

- At PTC, base-pairing of rRNA of PTC with peptidyl-tRNA and with aminoacyl-tRNA occurs leading to tight binding
- The 3'-acceptor ends of peptidyl-tRNA and aminoacyl-tRNA meet
- The reactive groups, aminoacyl and peptidyl, are juxtaposed and properly oriented for reaction to occur

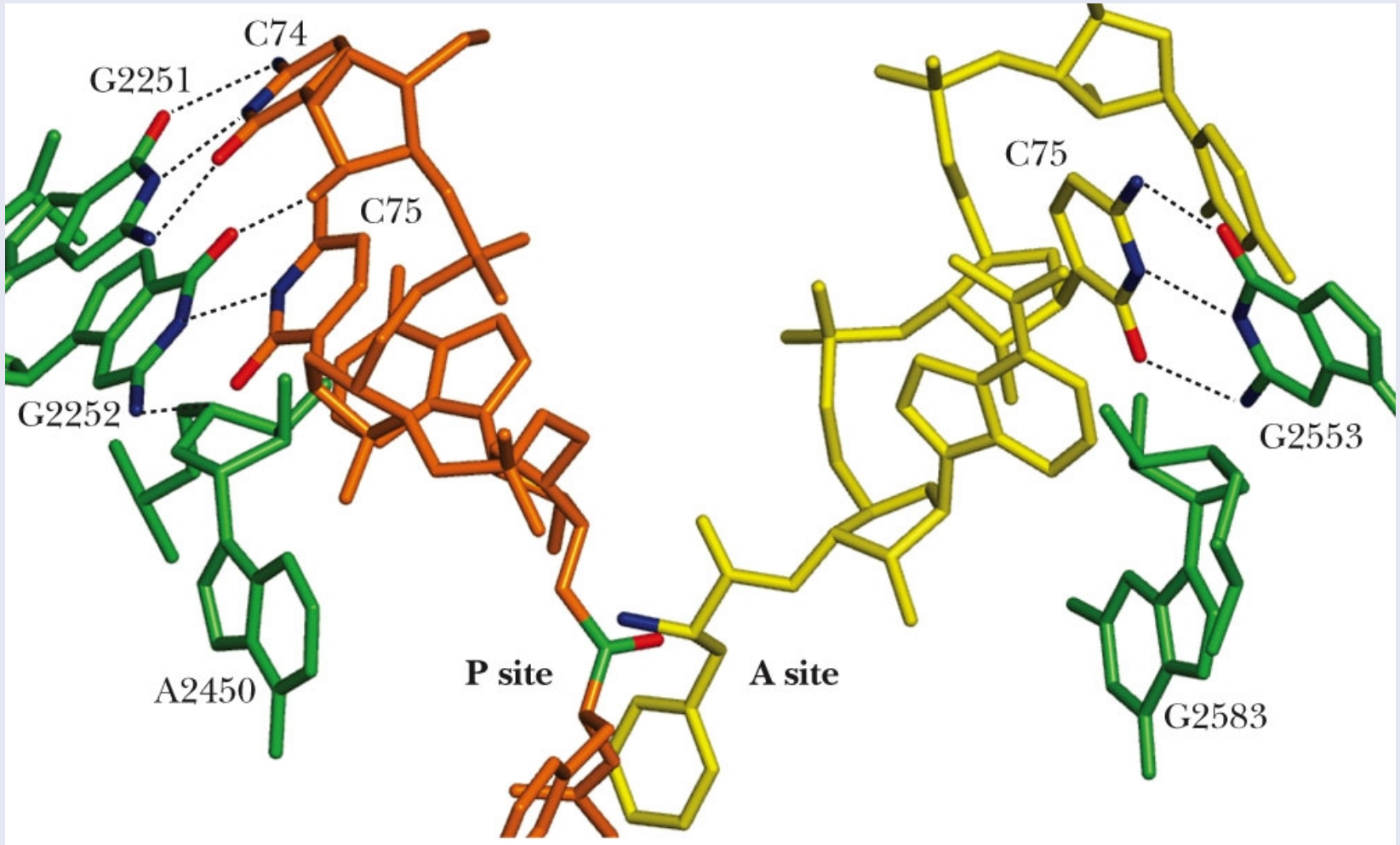



Figure 30.22 The peptidyl transferase active site.

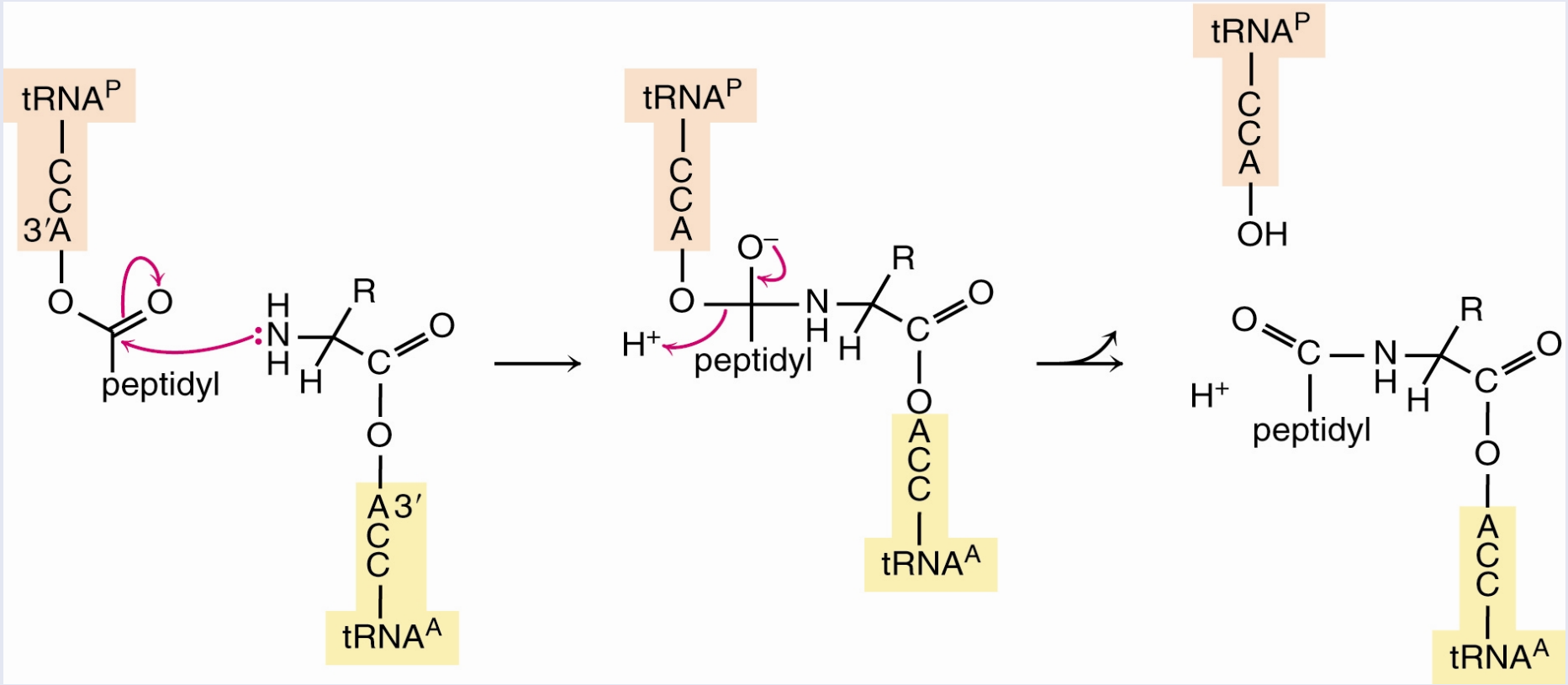



Figure 30.23 The protein synthesis reaction proceeds via deprotonation of the α-amino group of the aminoacyl-tRNA, followed by nucleophilic attack of the α-amino on the peptidyl-tRNA carbonyl carbon to form the polar tetrahedral intermediate. A proton is transferred to 3'-O of $tRNA^P$ to release the $tRNA^P$.

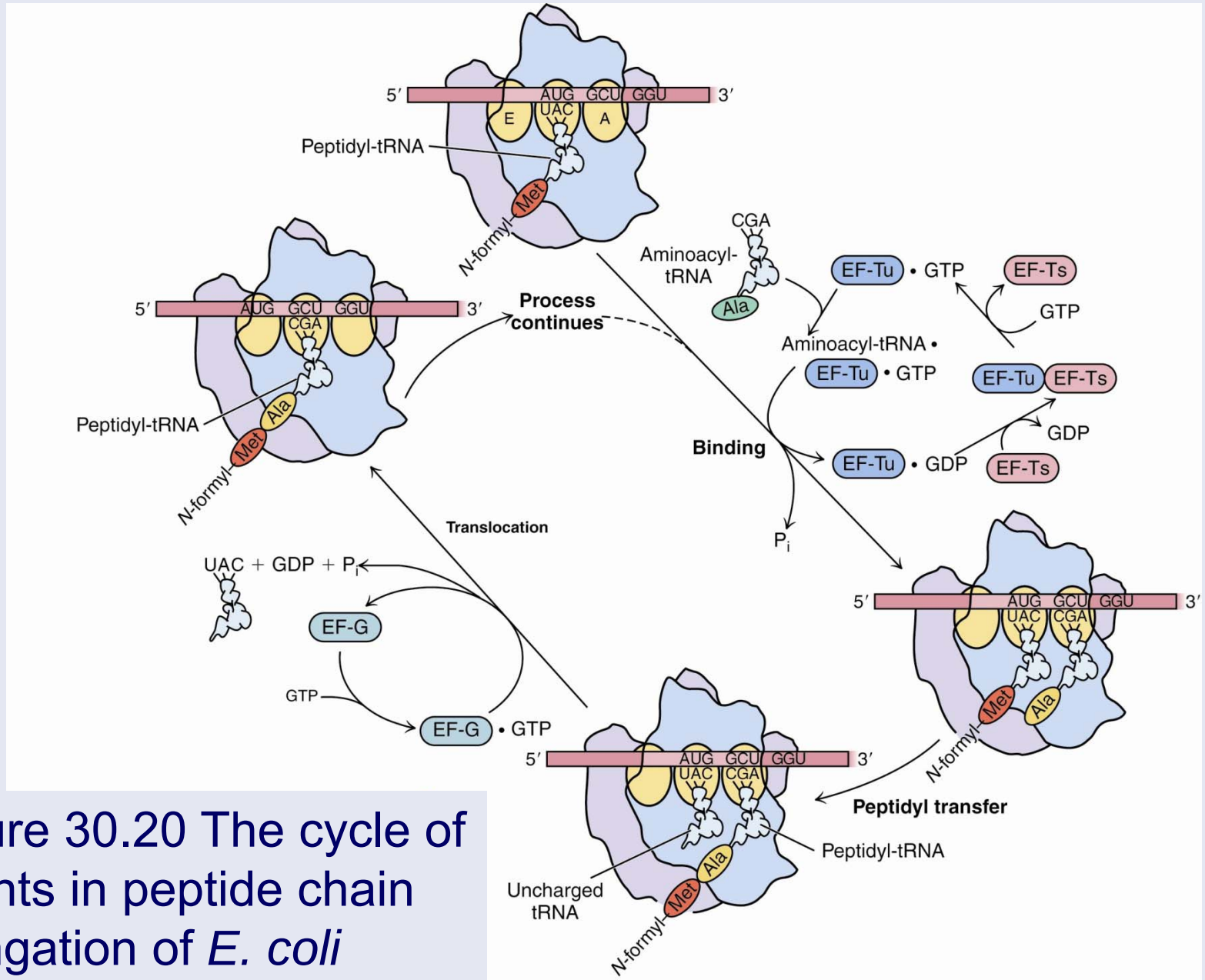



Figure 30.20 The cycle of events in peptide chain elongation of *E. coli* ribosomes.

# Translocation

- The deacylated tRNA removes from P site to E site
- The peptidyl-tRNA moves from A site to P site
- The ribosome moves one codon down the mRNA to position next codon in the A site
- The movement is catalyzed by the translocation protein **elongation factor G** (EF-G)

# Elongation factor G (EF-G)

- EF-G:GTP complex binds to ribosome
- GTP hydrolysis powers the translocation of ribosome and is essential for subsequent dissociation of EF-G
- EF-G release is a prerequisite for starting new elongation step

✓ EF-G and EF-Tu bind to the same site: factor-binding center or the GAC (GTPase-activating center)

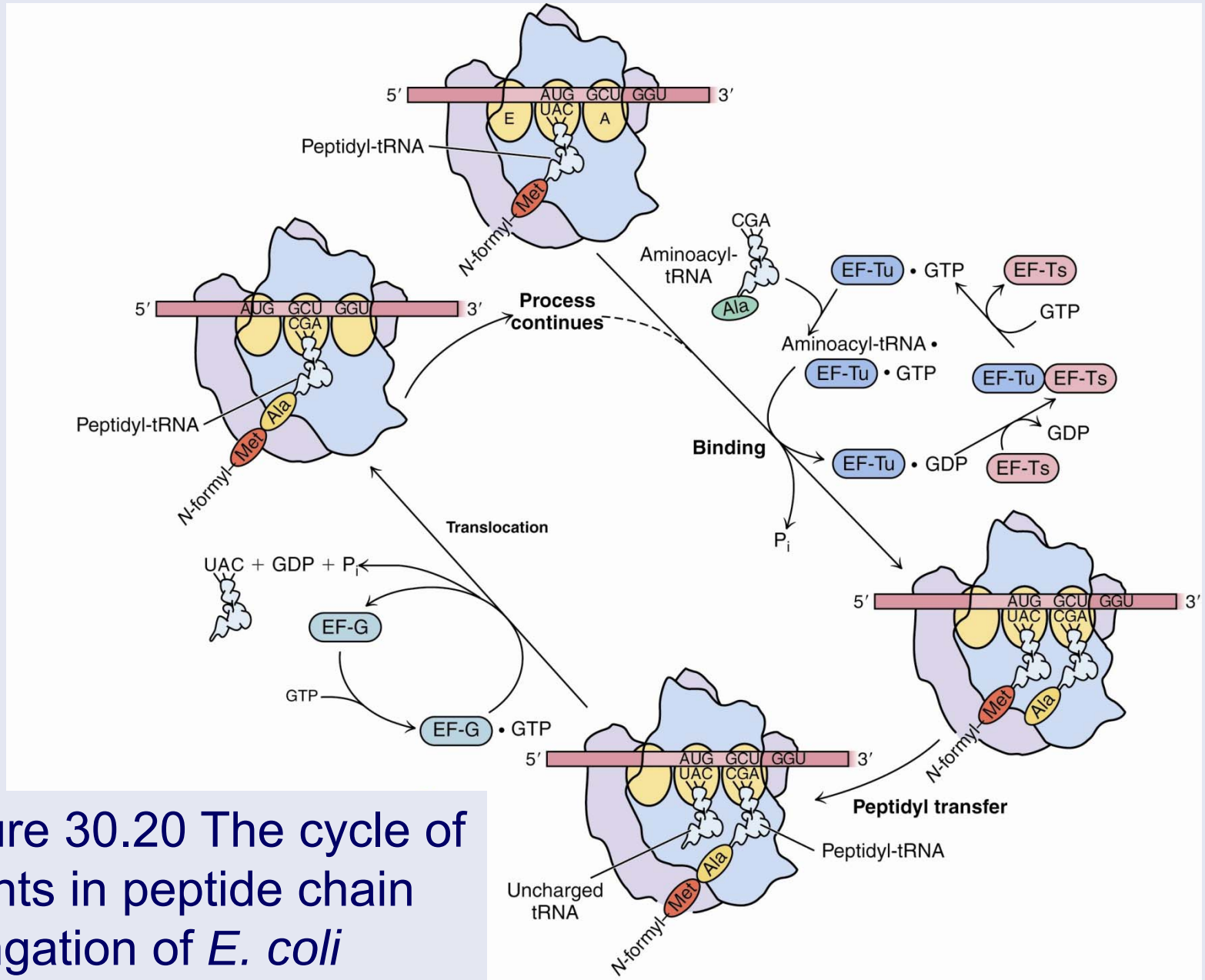



Figure 30.20 The cycle of events in peptide chain elongation of *E. coli* ribosomes.

# GTP Hydrolysis Fuels the Conformational Changes That Drive Ribosomal Functions

- GTP-binding induces conformational changes for protein synthesis; hydrolysis drives conformation back to initial state.
- Two GTPs are hydrolyzed for each amino acid incorporated into peptide.
- Total of four high-energy phosphate bonds are expended per amino acid residue added - two GTP here and two in amino acid activation via aminoacyl-tRNA synthesis

# Peptide Chain Termination Requires a G-Protein Family Member

- Elongation continues until the 70S ribosome encounters a “stop” codon
- **Release factors** recognize the stop codon at the A site
- Presence of release factors at A site transforms the peptidyl transferase into a **hydrolase**, which cleaves the peptidyl chain from the tRNA
- Dissemble ribosome:mRNA:P-site tRNA by **ribosome recycling factor (RRF)** with the help of EF-G

# Release factors (RF)

- RF1: recognize UAA and UAG, member of guanine nucleotide exchange factor (GEF); recruit RF3
- RF2: recognize UAA and UGA, GEF; recruit RF3
- RF3: G-protein family member, complexed with GTP, mimic of tRNA, binds to factor-binding center on ribosome

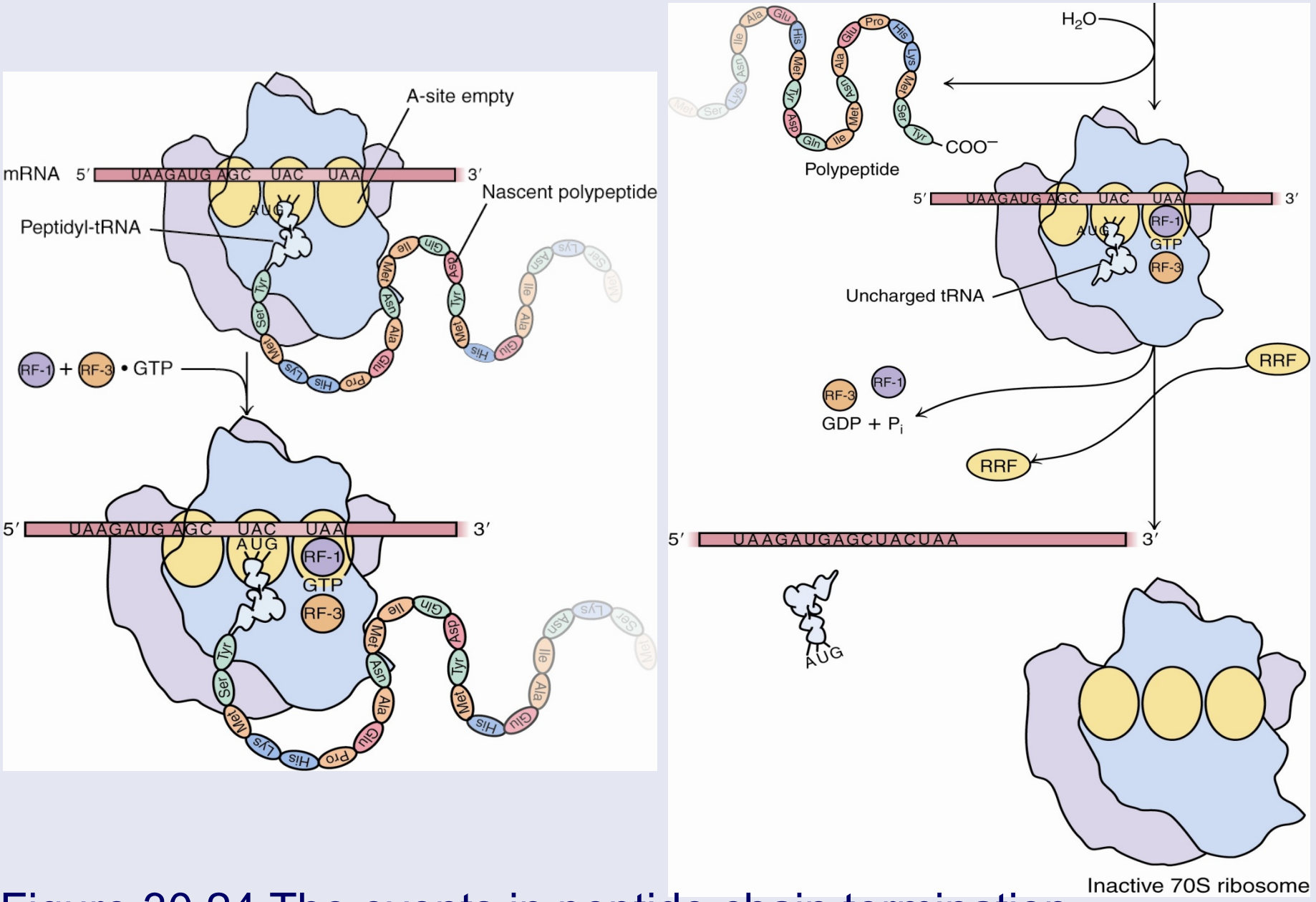



Figure 30.24 The events in peptide chain termination.

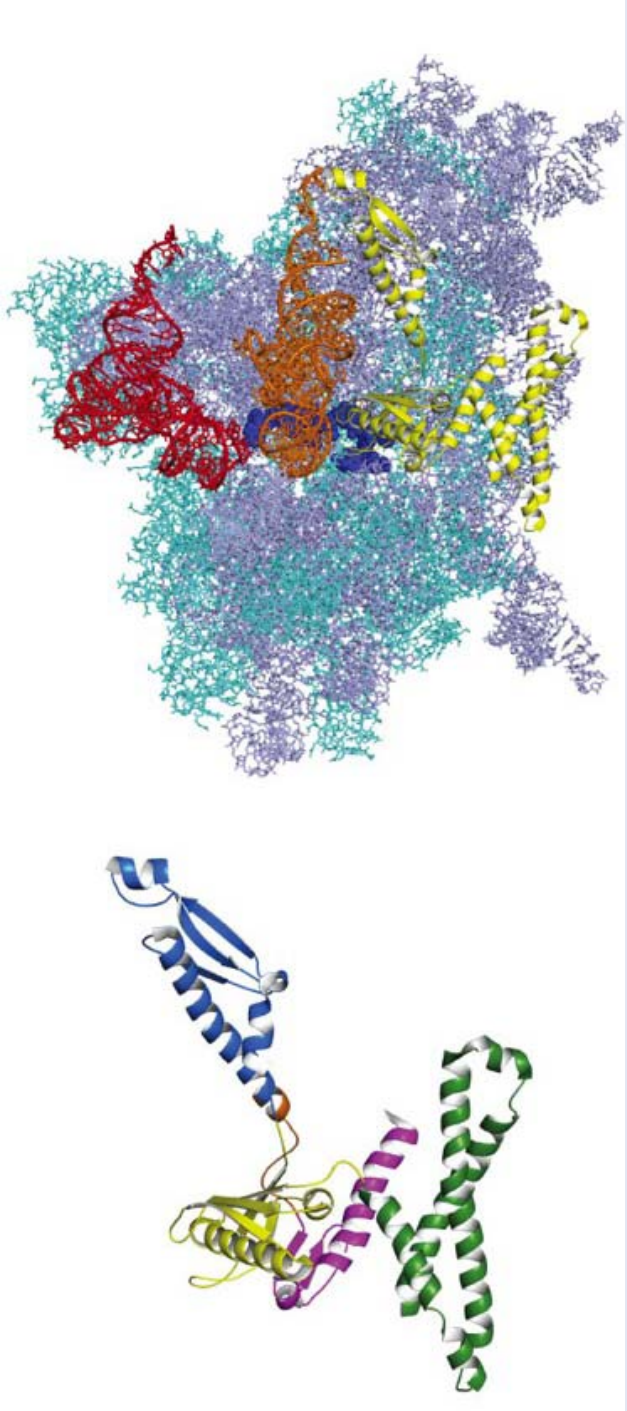


Figure 30.24 Ribosome:RF-2 termination complex. RF-2 is shown in yellow, the P-site tRNA in orange, the E-site tRNA in red, mRNA in dark blue, the 30S proteins in cyan, and the 30S ribosomal subunit 16S rRNA in light purple. Lower: The ribosome-bound conformation of RF-2.

# The four G-proteins in protein synthesis

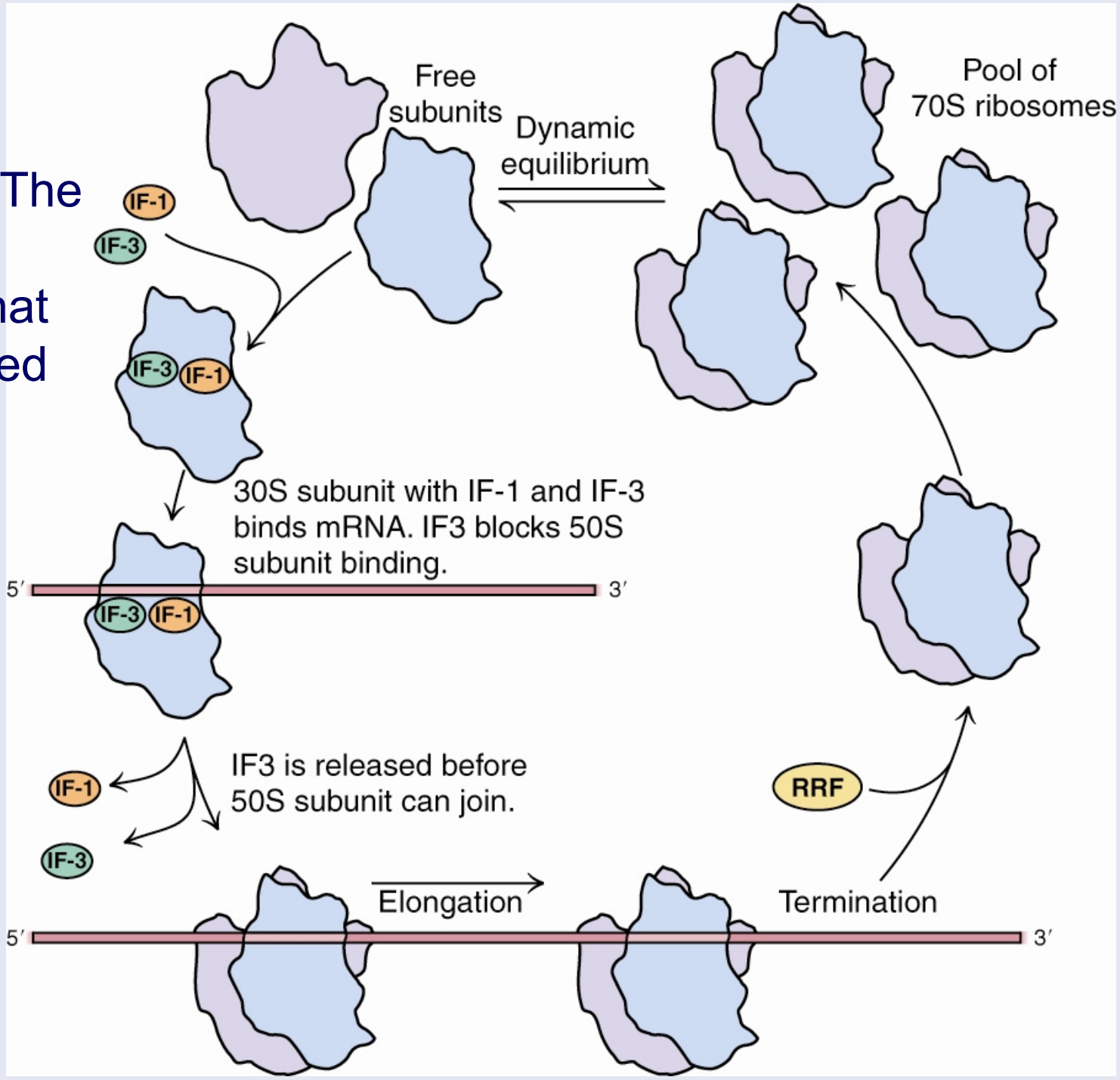
IF-2
EF-Tu
EF-G
RF-3

- involved in different stages of proteins synthesis
- Same binding site on 50 S subunit
- GTPase activity is activated after binding

# The Ribosome Subunits Cycle Between 70S Complexes and a Pool of Free Subunits

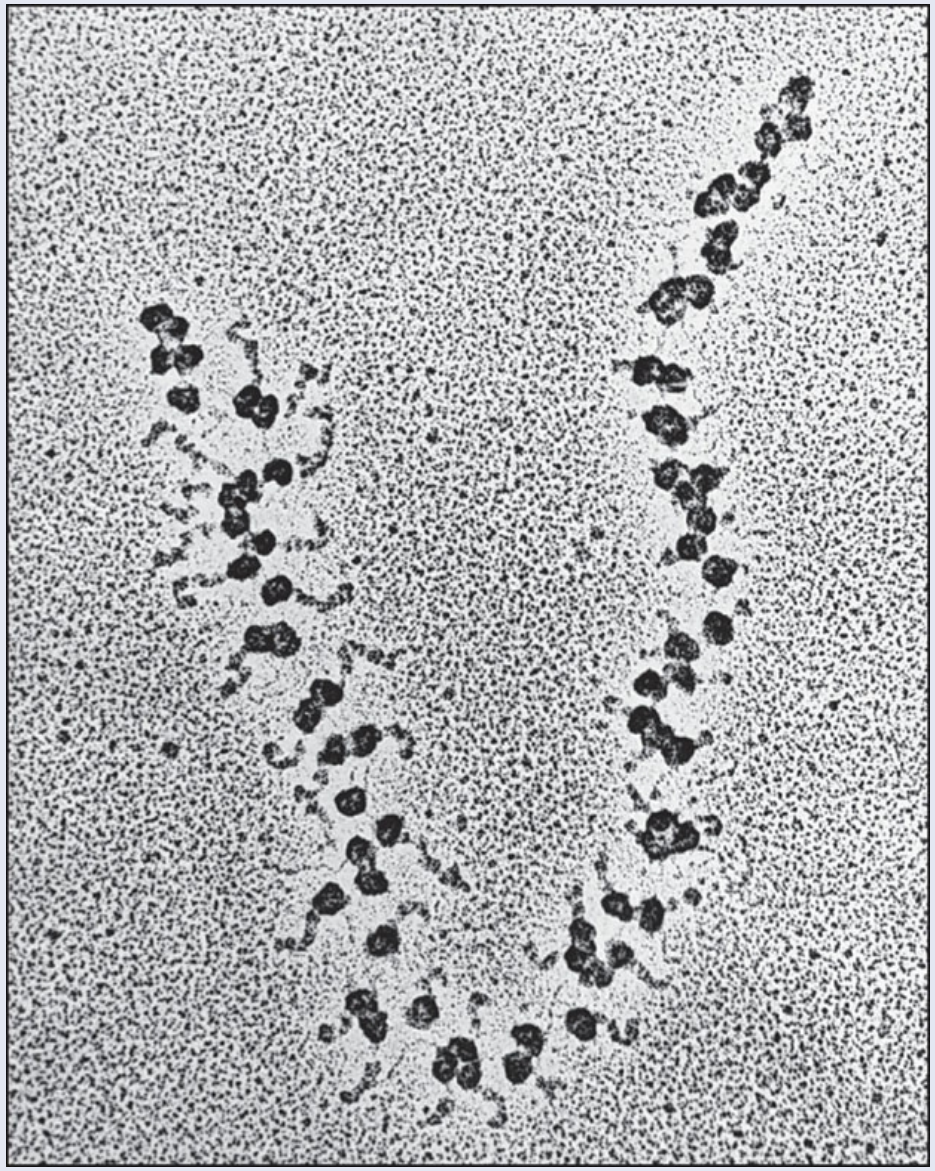
- After protein synthesis ,the 70S ribosome dissociates from mRNA, and separates into free 30S and 50S subunits
- Intact 70S ribosomes are inactive in protein synthesis, only 30S subunits can interact with initiation factors....

Figure 30.25 The ribosome life cycle. Note that IF-3 is released prior to 50S addition.



# Multiple Ribosomes Can Translate the Same mRNA at a Given Time

Figure 30.26 EM of polyribosomes or **polysomes**– multiple ribosomes translating the same mRNA.



# 30.6 How Are Proteins Synthesized in Eukaryotic Cells?

- Eukaryotic mRNAs are post-transcriptionally modified:
  - The 5'-terminal $^{7}$**methyl-GTP cap**
  - The 3'-terminal **poly(A) tail**
- 5'-methyl-GTP cap: mRNA binding by eukaryotic ribosomes; enhances stability by preventing degradation by 5'-exonucleases
- Poly(A) tail: enhance stability and translational efficiency of eukaryotic mRNAs.

# The Characteristic Structure of Eukaryotic mRNAs

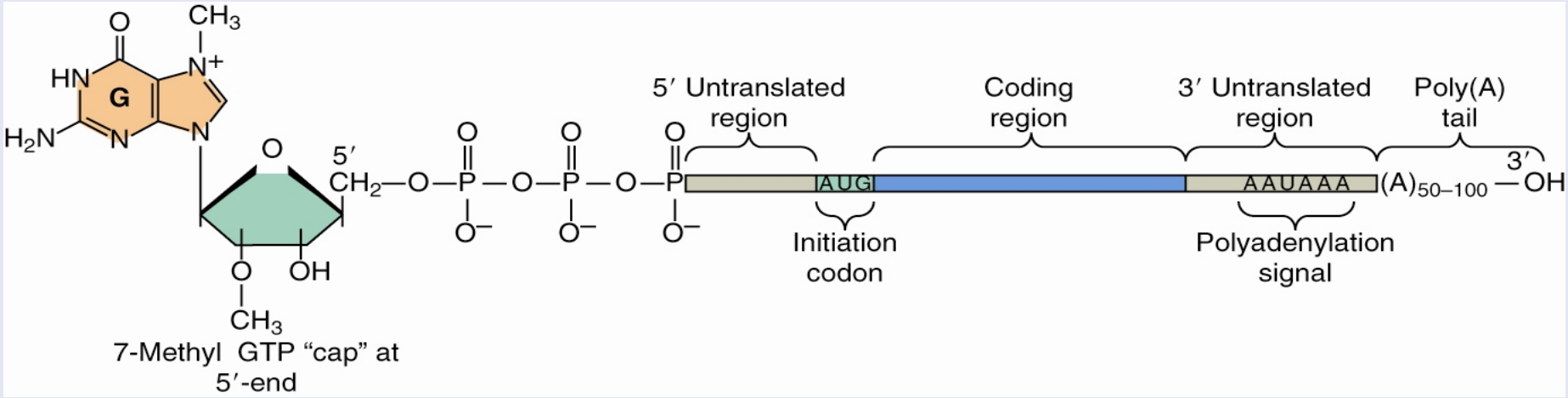



Figure 30.27 Untranslated regions ranging between 40 and 150 bases in length occur at both the 5'- and 3'-ends of the mature mRNA. An initiation codon at the 5'-end, invariably AUG, signals the translation start site.

# Peptide Chain Initiation in Eukaryotes

- Eukaryotic protein synthesis is much more complex than prokaryotic protein synthesis
- Initiation of protein synthesis in eukaryotes involves more than a dozen **eukaryotic initiation factors (eIFs)**
- **The overall process is similar**
- The initiator tRNA that carries only Met and functions only in initiation - it is called $tRNA_i^{Met}$ but **it is not formylated**

# Properties of Eukaryotic Translation Initiation Factors

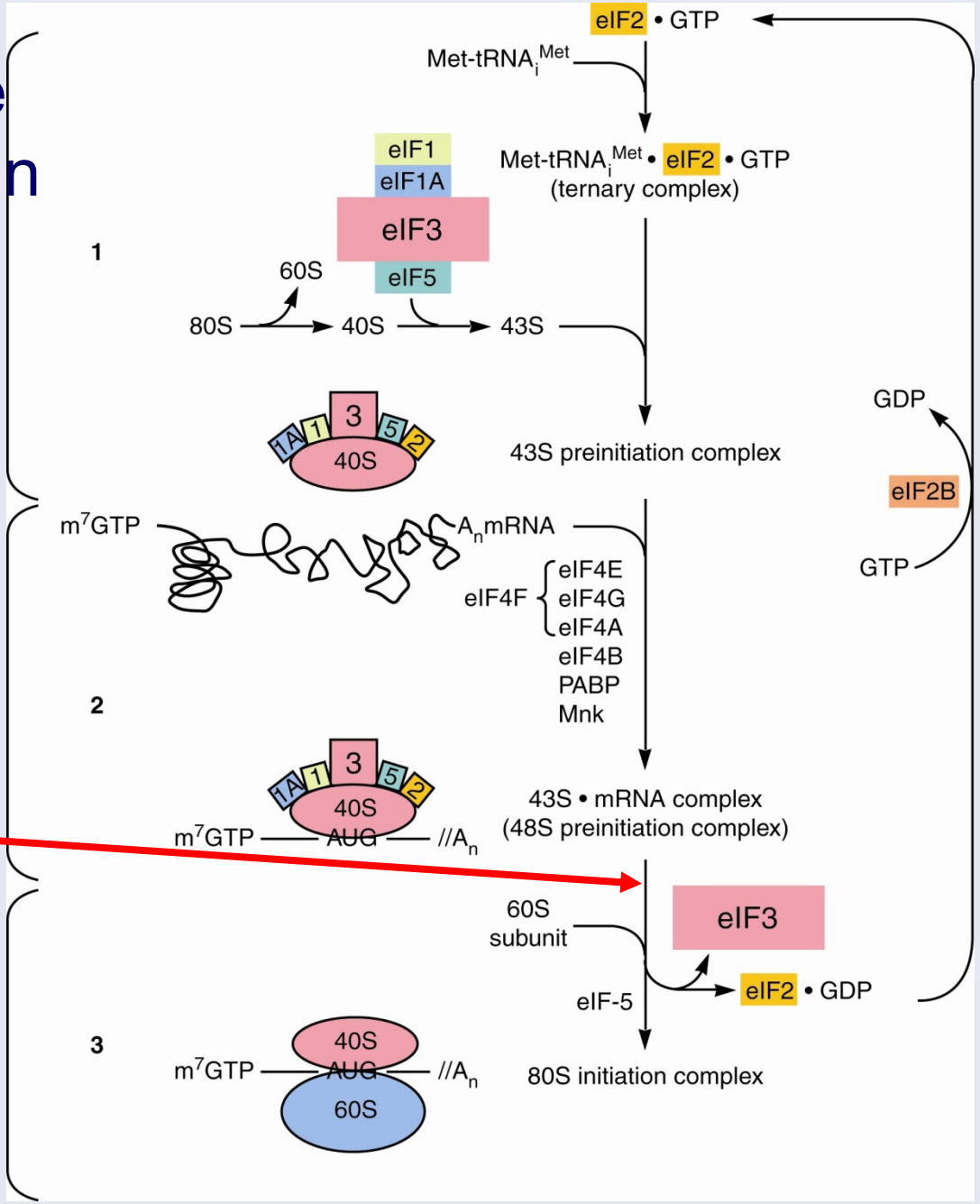
**TABLE 30.9** Properties of Eukaryotic Translation Initiation Factors

| Factor | Subunit | Size (kD) | Function |
|---|---|---|---|
| eIF1 | | 15 | Enhances initiation complex formation |
| eIF1A | | 17 | Stabilizes Met-tRNA$_i$ binding to 40S ribosomes |
| eIF2 | | 125 | GTP-dependent Met-tRNA$_i$ binding to 40S ribosomes |
| | $\alpha$ | 36 | Regulated by phosphorylation |
| | $\beta$ | 50 | Binds Met-tRNA$_i$ |
| | $\gamma$ | 55 | Binds GTP, Met-tRNA$_i$ |
| eIF2B | | 270 | Promotes guanine nucleotide exchange on eIF2 |
| eIF2C | | 94 | Stabilizes ternary complex in presence of RNA |
| eIF3 | | 800 | Promotes Met-tRNA$_i$ and mRNA binding |
| eIF4F | | 243 | Binds to mRNA caps and poly(A) tails; consists of eIF4A, eIF4E, and eIF4G; RNA helicase activity unwinds mRNA 2° structure |
| eIF4A | | 46 | Binds RNA; ATP-dependent RNA helicase; promotes mRNA binding to 40S ribosomes |
| eIF4E | | 24 | Binds to 5′-terminal $^7$methyl-GTP cap on mRNA |
| eIF4G | | 173 | A scaffolding protein that associates the mRNA with ribosome-bound eIF3, binds to PABP |
| eIF4B | | 80 | Binds mRNA; promotes RNA helicase activity and mRNA binding to 40S ribosomes |
| eIF4H | | 25 | Acts with eIF4B to stimulate eIF4A helicase activity |
| eIF5 | | 49 | Promotes GTPase of eIF2, ejection of eIF2 and eIF3 |
| eIF5B | | 175 | Ribosome-dependent GTPase activity; mediates 40S and 60S joining |
| eIF6 | | | Dissociates 80S; binds to 60S |

# Figure 30.28
# The three stages in the initiation of translation in eukaryotic cells.

PABP: (poly)A-binding protein

40S scans mRNA to search AUG. GTP hydrolysis to release eIFs. 60S binds



# Peptide Chain Initiation in Eukaryotes

- Eukaryotic translation begins with formation of **ternary complex** of eIF-2, GTP and Met-tRNA$_i^{Met}$
- This binds to eIF-1/1A/3/5:40S subunit complex to form the **43S preinitiation complex**
- **eIF3 serves as scaffold for binding mRNA and other proteins**

# Peptide Chain Initiation in Eukaryotes

- **mRNA then adds** with several other factors (eIF4, PABP etc), forming the **48S preinitiation complex**
- **40S subunit scans to find the first AUG (start) codon**
- GTP hydrolysis to release eIFs
- 60S adds to form **80S initiation complex**; translation begin

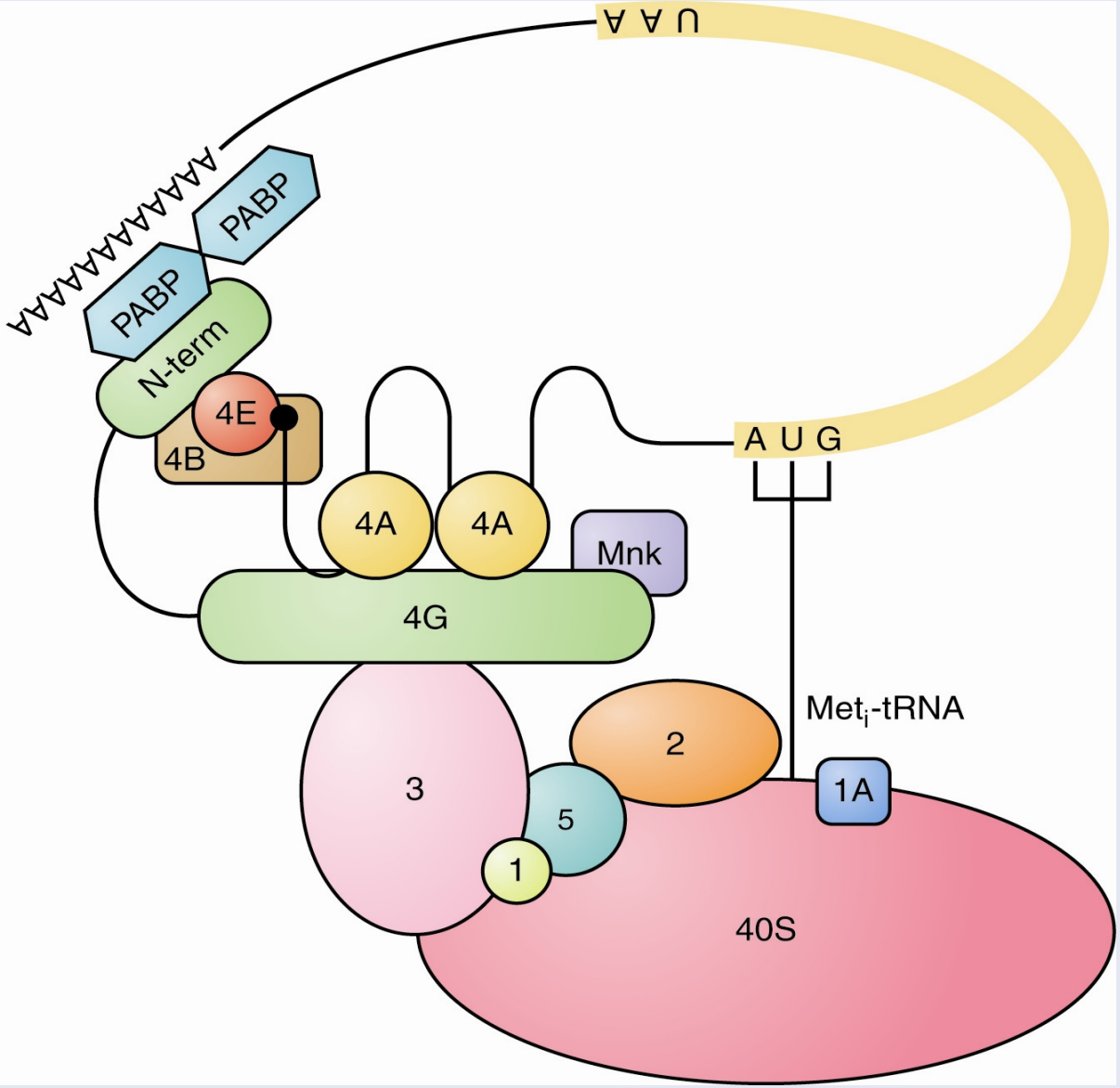
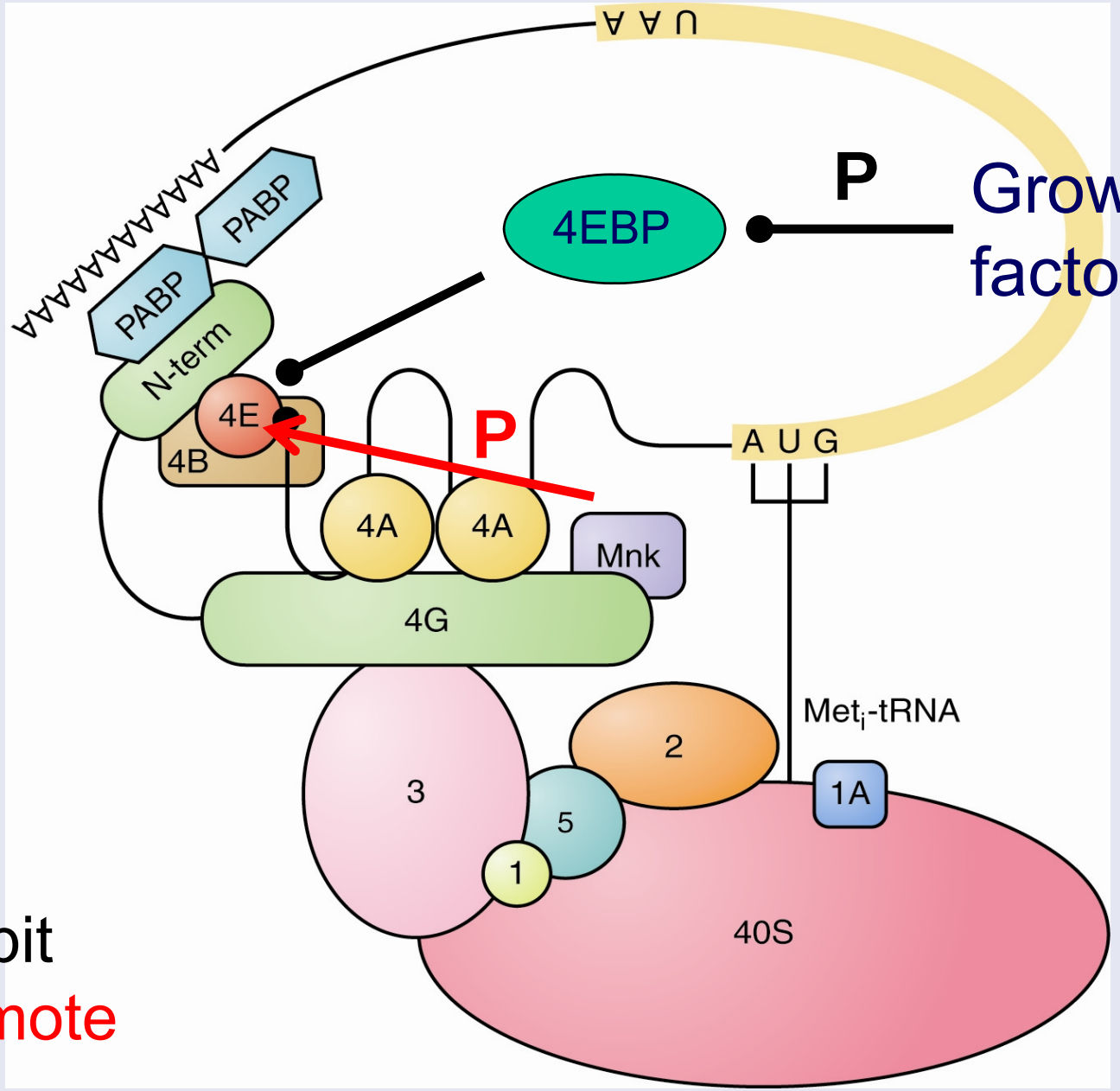



Figure 30.29 The 48S preinitiation complex. mRNA is shown as a black line, and the 5'-cap is a black circle. The coding region is shown as a yellow bar.

- Only the correctly processed mRNAs are translated.
- Protect mRNA from exonucleolytic degradation.

# 4E is regulated by Mnk and 4EBP




Inhibit

promote

# Control of Eukaryotic Peptide Chain Initiation to control Gene Expression

- Phosphorylation/dephosphorylation of translational components is a dominant mechanism for control of protein synthesis
- Four initiation factors (eIF2$\alpha$/2$\beta$/4E/4G), two elongation factors (eEF1/2), and ribosomal protein S6 are regulated by phosphorylation
- Phosphorylation of S6 (by serum growth factors) facilitates initiation of protein synthesis.
- Phosphorylation of eIF-2$\alpha$ causes it to bind eIF-2B and ,rendering eIF-2 unavailable for protein synthesis

# α-Subunit Phosphorylation Controls the Function of eIF2

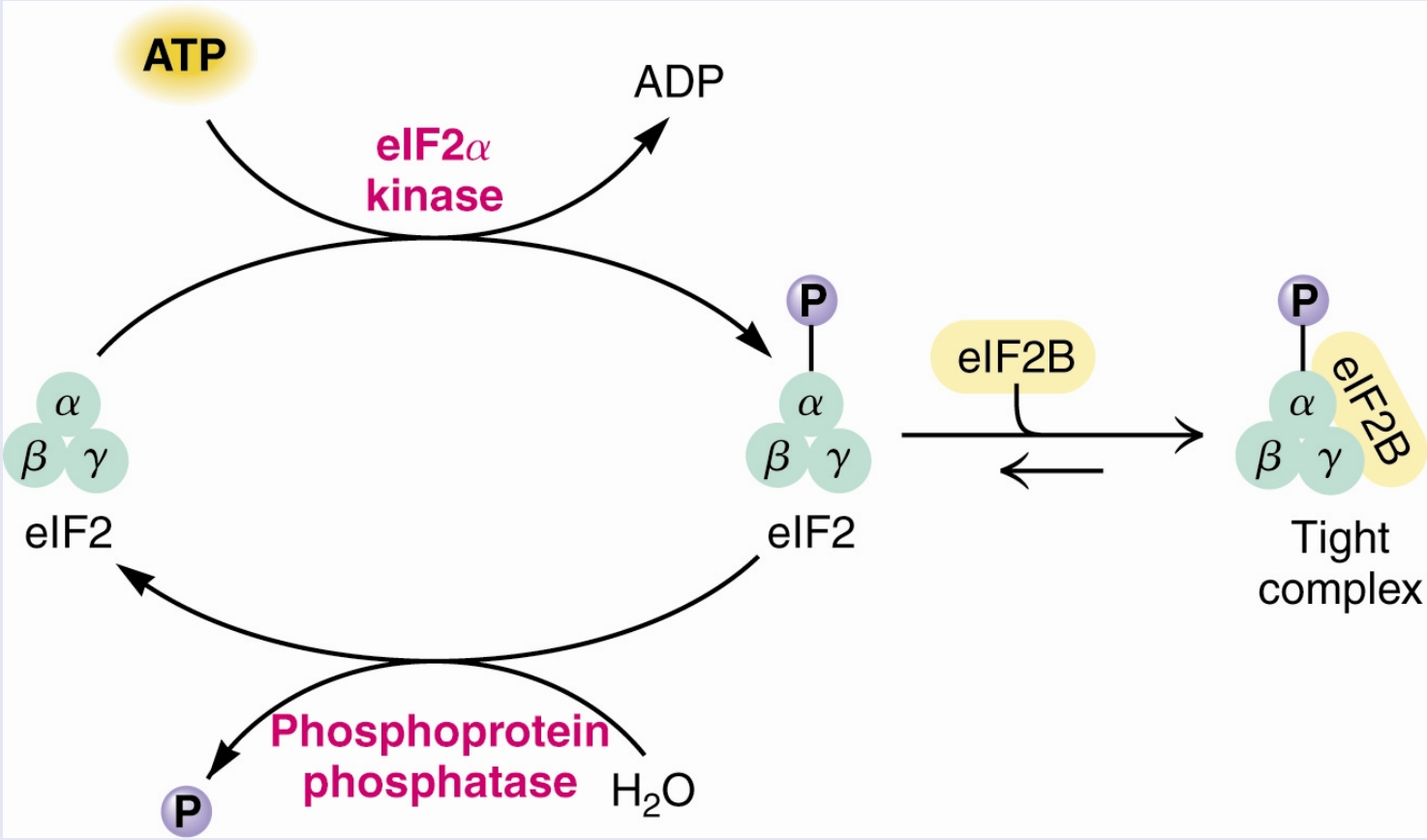



Figure 30.30 Control of eIF2 functions through reversible phosphorylation of a Ser residue on its α-subunit.

# Peptide Chain Elongation in Eukaryotes

- Very similar to prokaryotes
- Aminoacyl-tRNA in A site; peptidyl-tRNA in P site; peptidyl transfer; translocation of the ribosome
- Two elongation factors: eEF1 and eEF2
- eEF1A equal to EF-Tu; eEF1B equal to EF-Ts
- eEF2 equal to EF-G

# Peptide Chain Termination in Eukaryotes

- Only one RF
- Eukaryotic RF:GTP binds to A site with stop codon
- Hydrolysis of peptidyl-tRNA ester bond, hydrolysis of GTP, …

# Inhibitors of Protein Synthesis

*Two important purposes*

- To unravel the biochemical **mechanism** of protein synthesis
- Those that affect prokaryotic but not eukaryotic protein synthesis are **effective antibiotics**

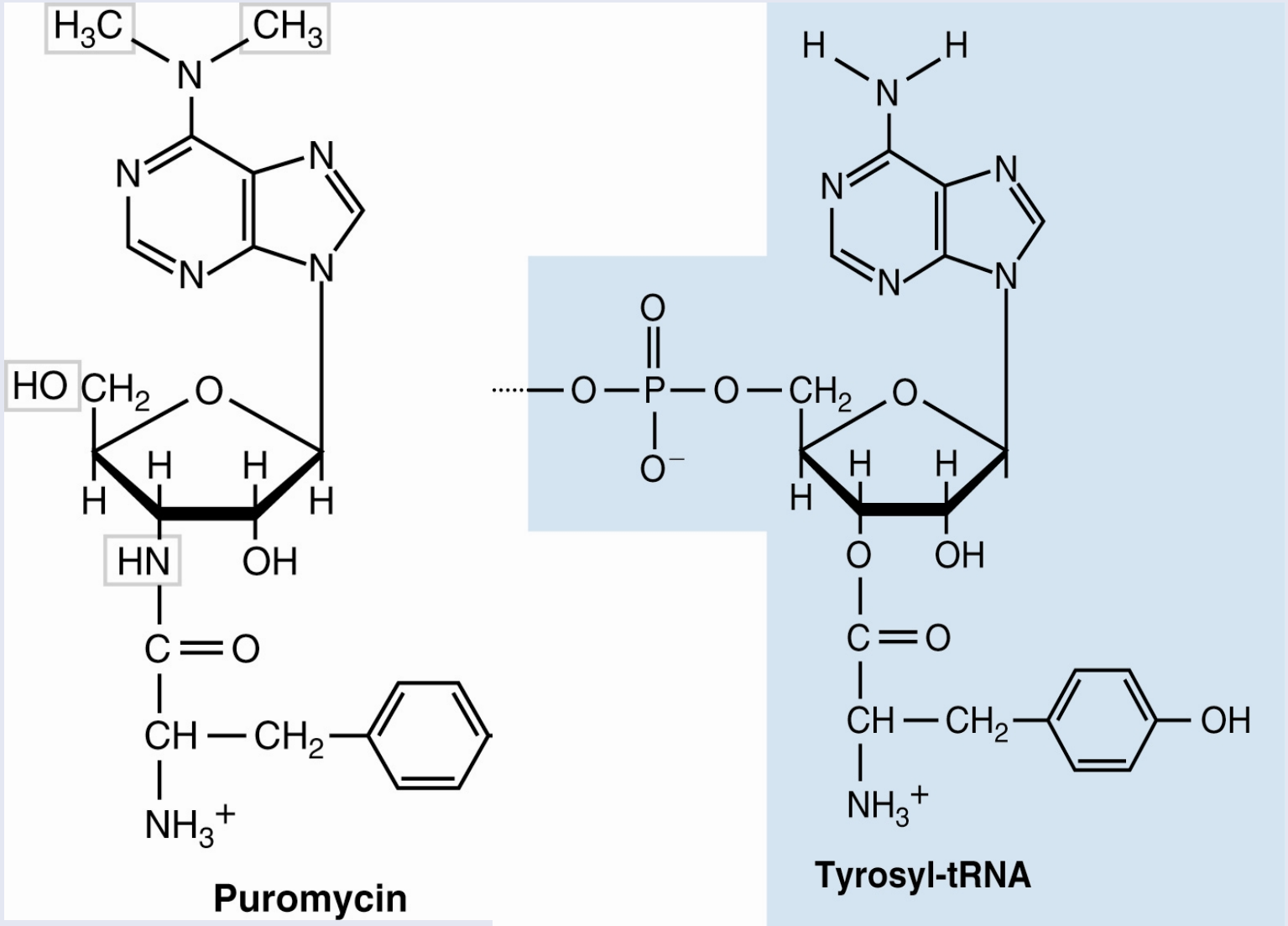



Figure 30.31 Puromycin is an analog of the 3′-end of Tyr-tRNA, shown here.

**TABLE 30.10** Some Protein Synthesis Inhibitors

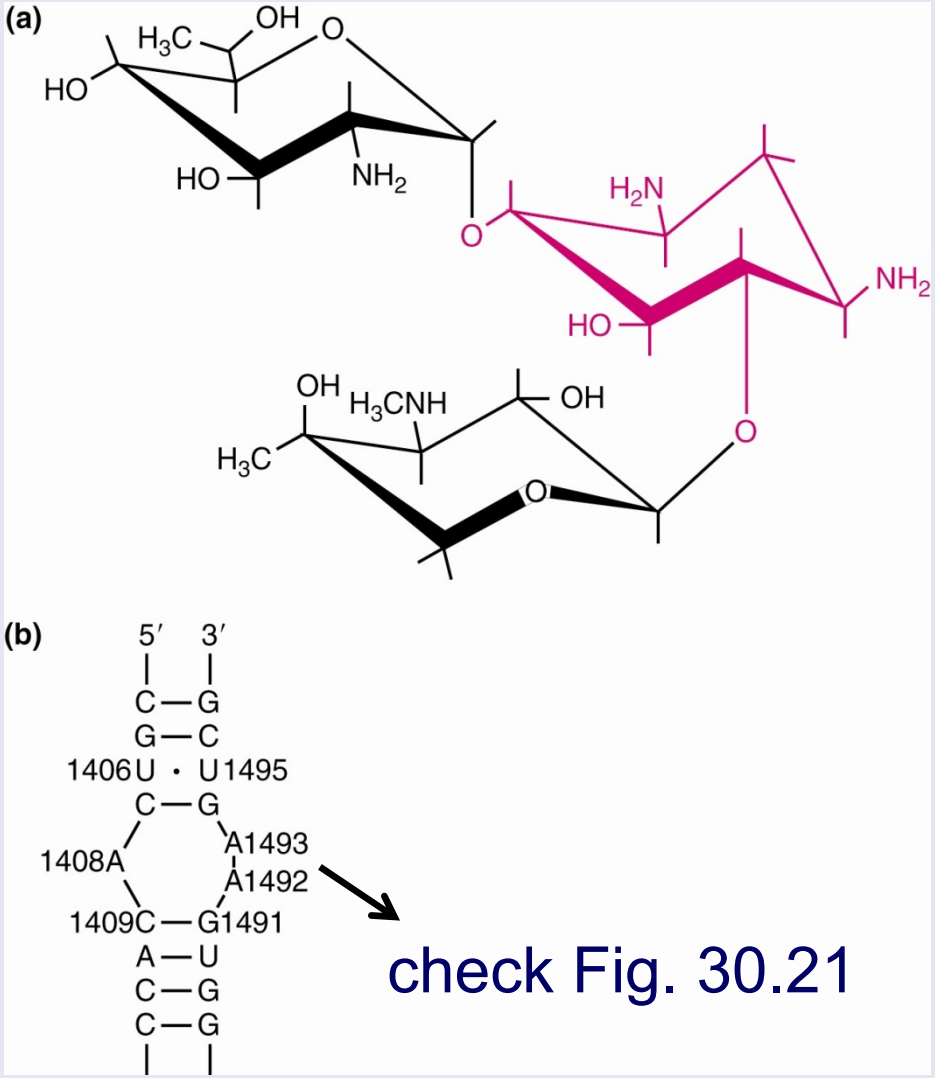
| Inhibitor | Cells Inhibited | Mode of Action |
|---|---|---|
| *Initiation* | | |
| Aurintricarboxylic acid | Prokaryotic | Prevents IF binding to 30S subunit |
| Kasugamycin | Prokaryotic | Inhibits fMet-tRNA$_i^{fMet}$ binding |
| Streptomycin | Prokaryotic | Prevents formation of initiation complexes |
| *Elongation: Aminoacyl-tRNA Binding* | | |
| Tetracycline | Prokaryotic | Inhibits aminoacyl-tRNA binding at A site |
| Streptomycin | Prokaryotic | Codon misreading, insertion of improper amino acid |
| Kirromycin | | |
| *Elongation:* | | |
| Sparsomyci | | |
| Chloramphe | | |
| Clindamyci | | ctivity |
| Erythromyc | | |
| *Elongation:* | | |
| Fusidic acid | | |
| Thiostrepto | | |
| Diphtheria toxin | Eukaryotic | Inactivates eEF-2 through ADP-ribosylation |
| Cycloheximide | Eukaryotic | Inhibits translocation of peptidyl-tRNA |
| *Premature Termination* | | |
| Puromycin | Both | Aminoacyl-tRNA analog, binds at A site and acts as peptidyl acceptor, aborting peptide elongation |
| *Ribosome Inactivation* | | |
| Ricin | Eukaryotic | Catalytic inactivation of 28S rRNA via *N*-glycosidase action on A$^{4256}$ |

These chemicals inhibit protein synthesis by binding to key rRNA components:

- 16S rRNA of decoding center
- PTC of the 23S rRNA

# Decoding site is a target of aminoglycoside antibiotics

- Compounds with a 2-deoxy-streptamine (2-DOS) core structure
- These antibiotics bind to $A^{1408}$ that limit the flexibility of $A^{1492}$ and $A^{1493}$
- This flexibility loss results in errors in translation fidelity, where the **codon is misread, the wrong amino acid is inserted**, the protein being made is nonfunctional.
- For example, Streptomycin,geneticin




check Fig. 30.21

Figure 30.32 (a) Structure of geneticin, a representative aminoglycoside antibiotic. Note the characteristic 2-deoxystreptamine (2-DOS) core structure, in red. (b) the base sequence of the small RNA loop within the 16S rRNA decoding center.

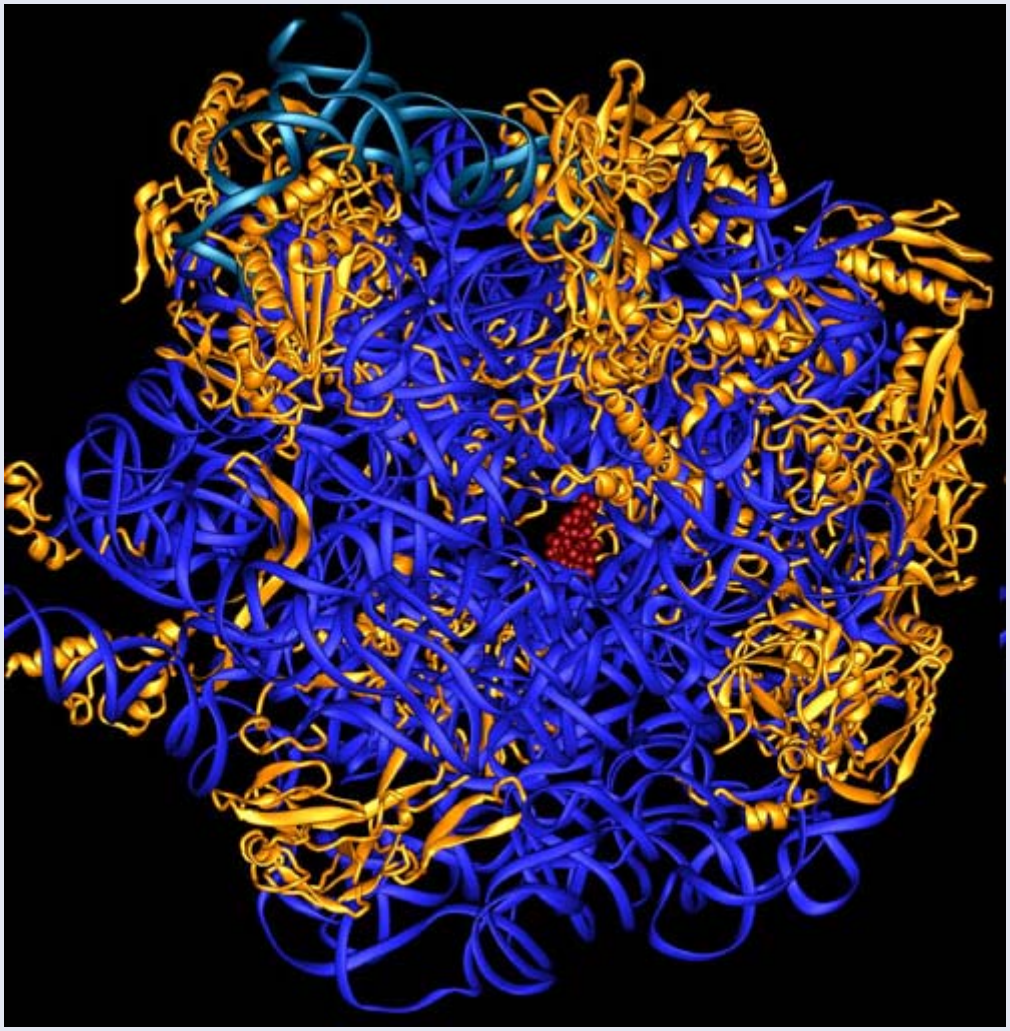
# Many Antibiotics target the PTC and the Peptide Exit Tunnel

- The **macrolide antibiotics** are one of the clinically most important classes, such as **erythromycin**, which bind within the peptide exit tunnel and plug the tunnel
- The antibiotics binds to 23S RNA

  ✓ the movement of growing peptide chain is stopped

  ✓ protein synthesis is aborted

  ✓ The peptidyl-tRNA dissociates from ribosome



Erythromycin binds to A $^{2058}$ of 23S rRNA

Top view of the 50S ribosomal subunit from *D. radiodurans* showing erythromycin (red) bound to the entrance of the tunnel. Blue, 23S rRNA and 5S rRNA. Gold, ribosomal proteins. http://www.molgen.mpg.de

# Many Antibiotics target the PTC and the Peptide Exit Tunnel

- Macrolide antibiotics targeting PTC occupy space within this center
- Therefore, the amino acid or peptide chain linked at 3’-end of tRNA cannot be positioned properly for peptide bond formation
- This effect is more common for aminoacyl-tRNAs in the A site
- For example, chloramphenicol, erythromycin