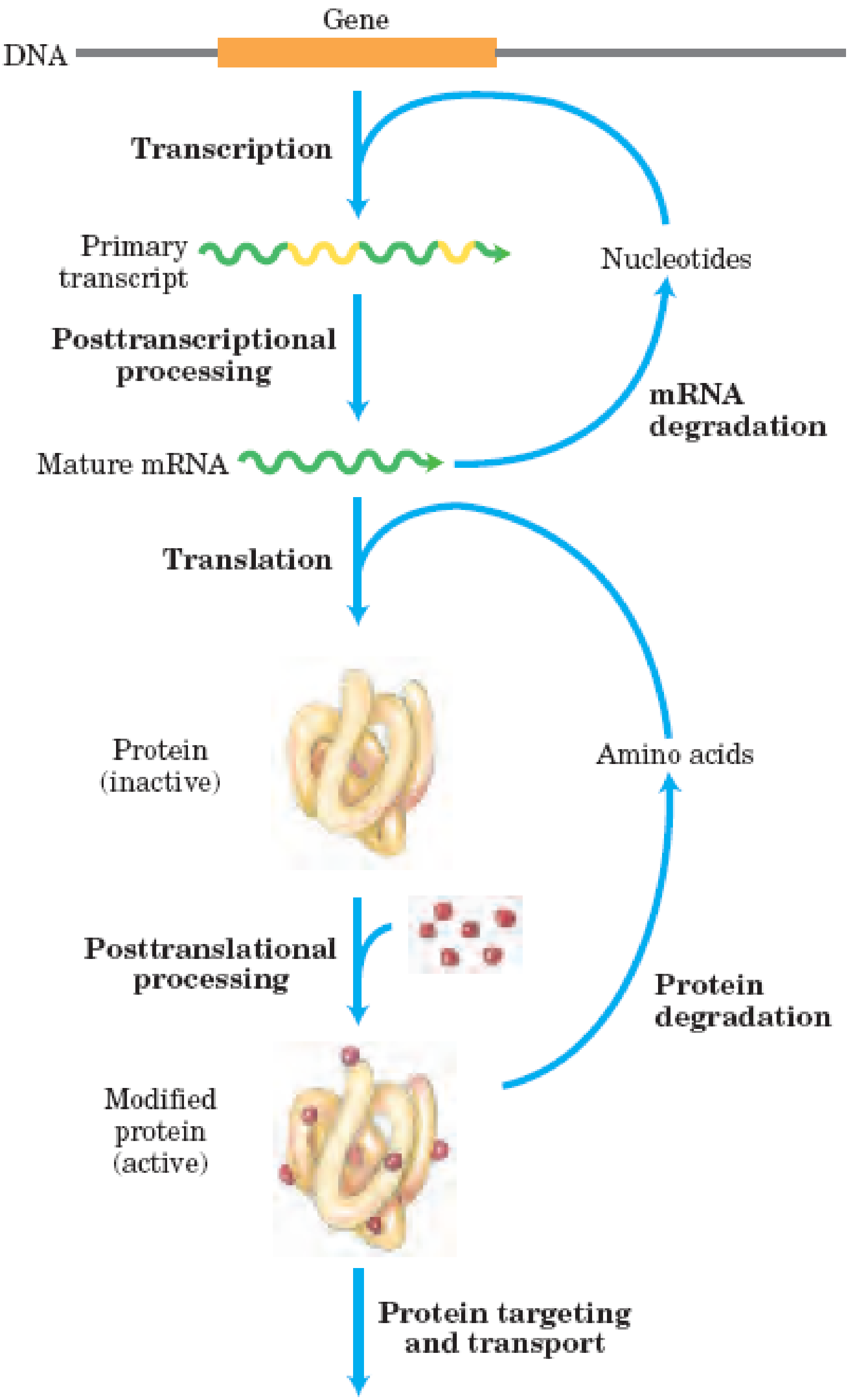
TRANSLATION

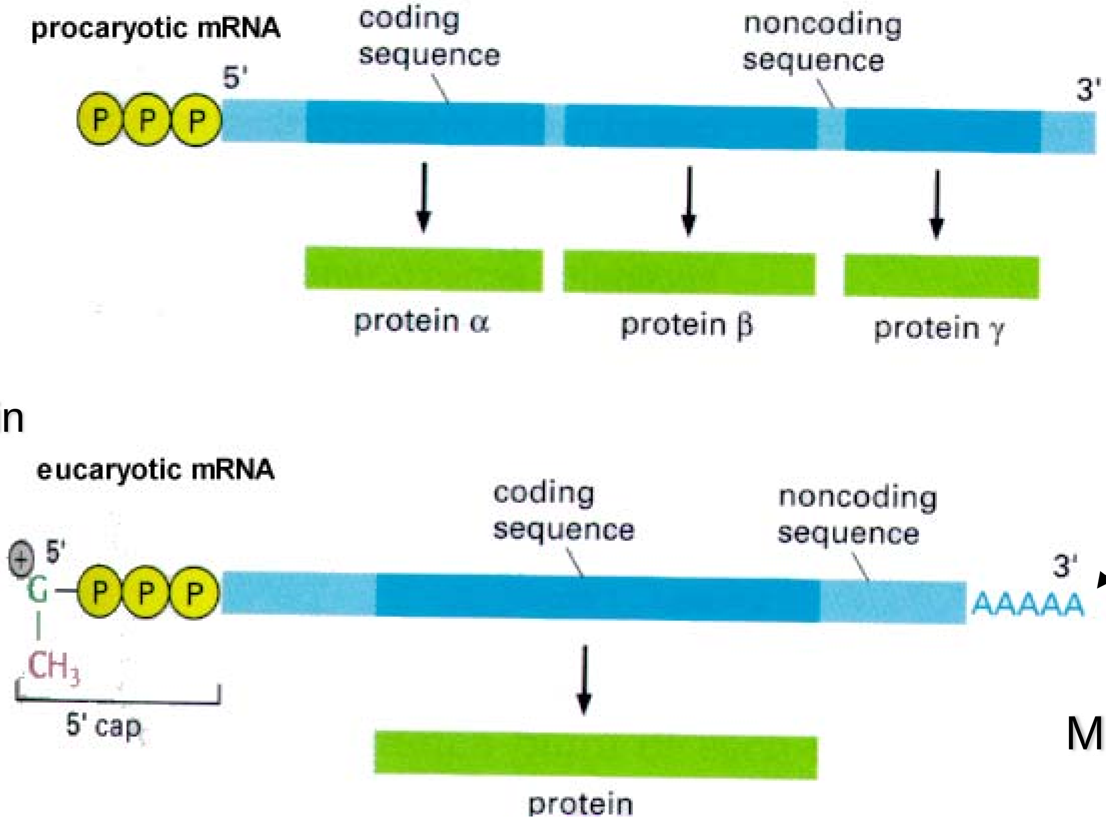
By : Lucia Dhiantika Witasari

Translation

= decoding of the information written in RNA into the amino acid sequence of the protein



A Comparison of Procaryotic and Eucaryotic mRNAs



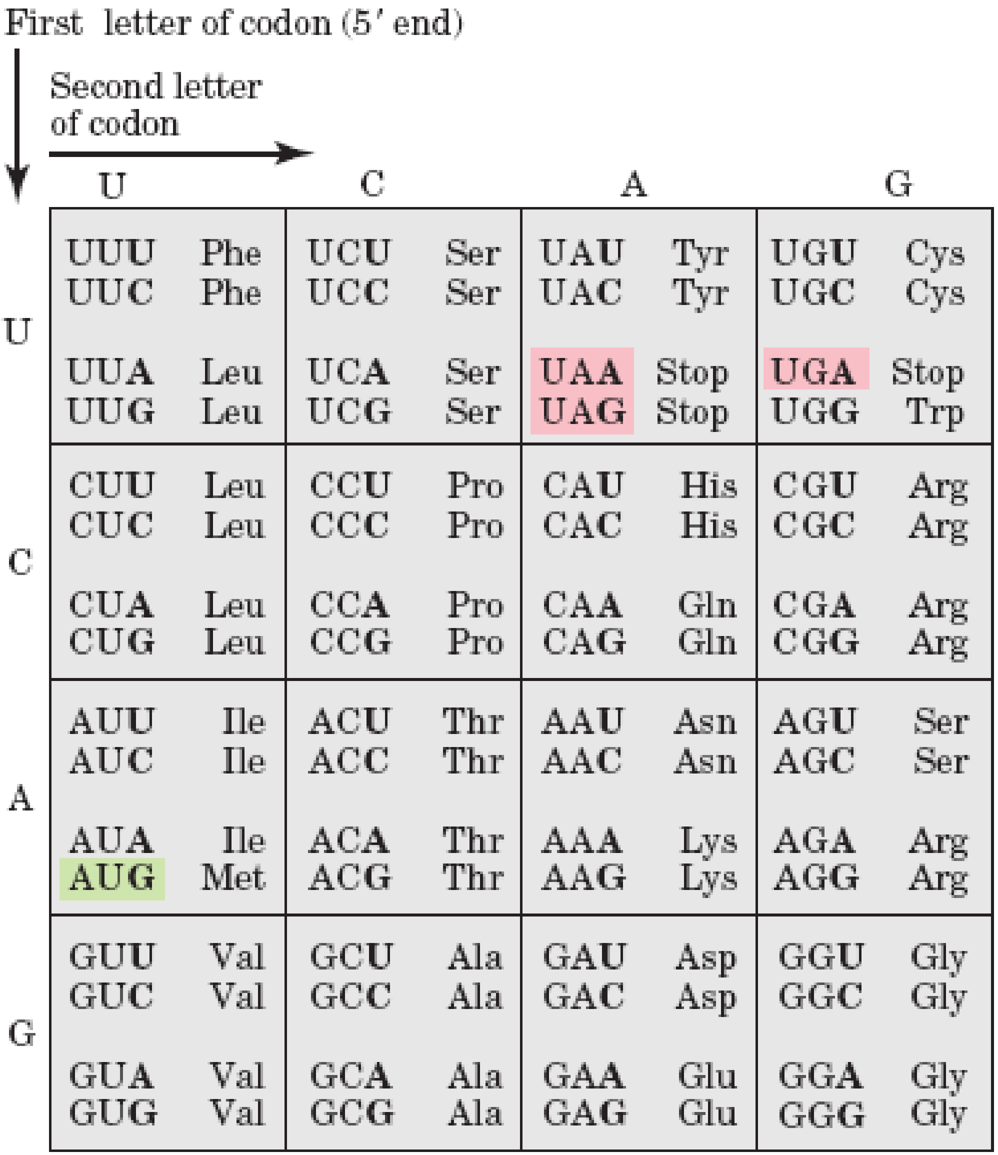
Polygenic

Cap-
7-methylguanosin

Poly-A-tail

Monogenic

The Genetic Code



First letter of codon (5′ end)

Second letter of codon

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

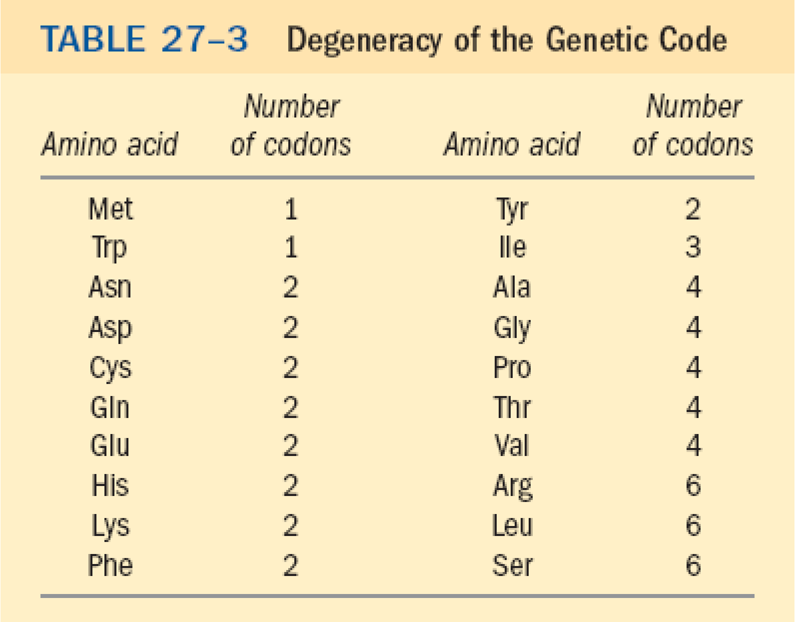
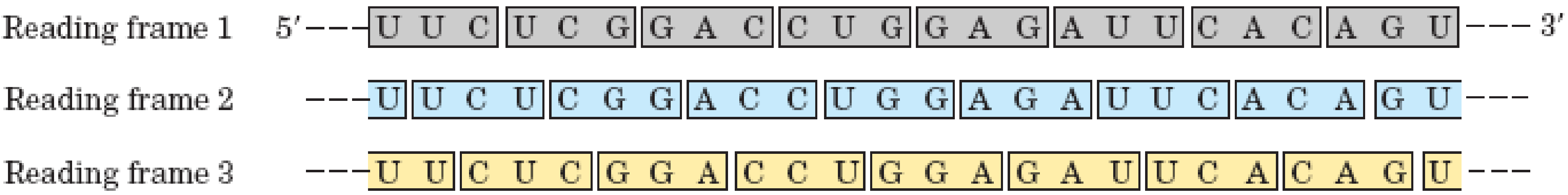


TABLE 27–3 Degeneracy of the Genetic Code

Amino acid	*Number of codons*	*Amino acid*	*Number of codons*
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6

Reading frames in the genetic code



Reading frame 1 5′---UUC UCG GAC CUG GAG AUU CAC AGU---3′

Reading frame 2 ---U UCU CGG ACC UGG AGA UUC ACA GU---

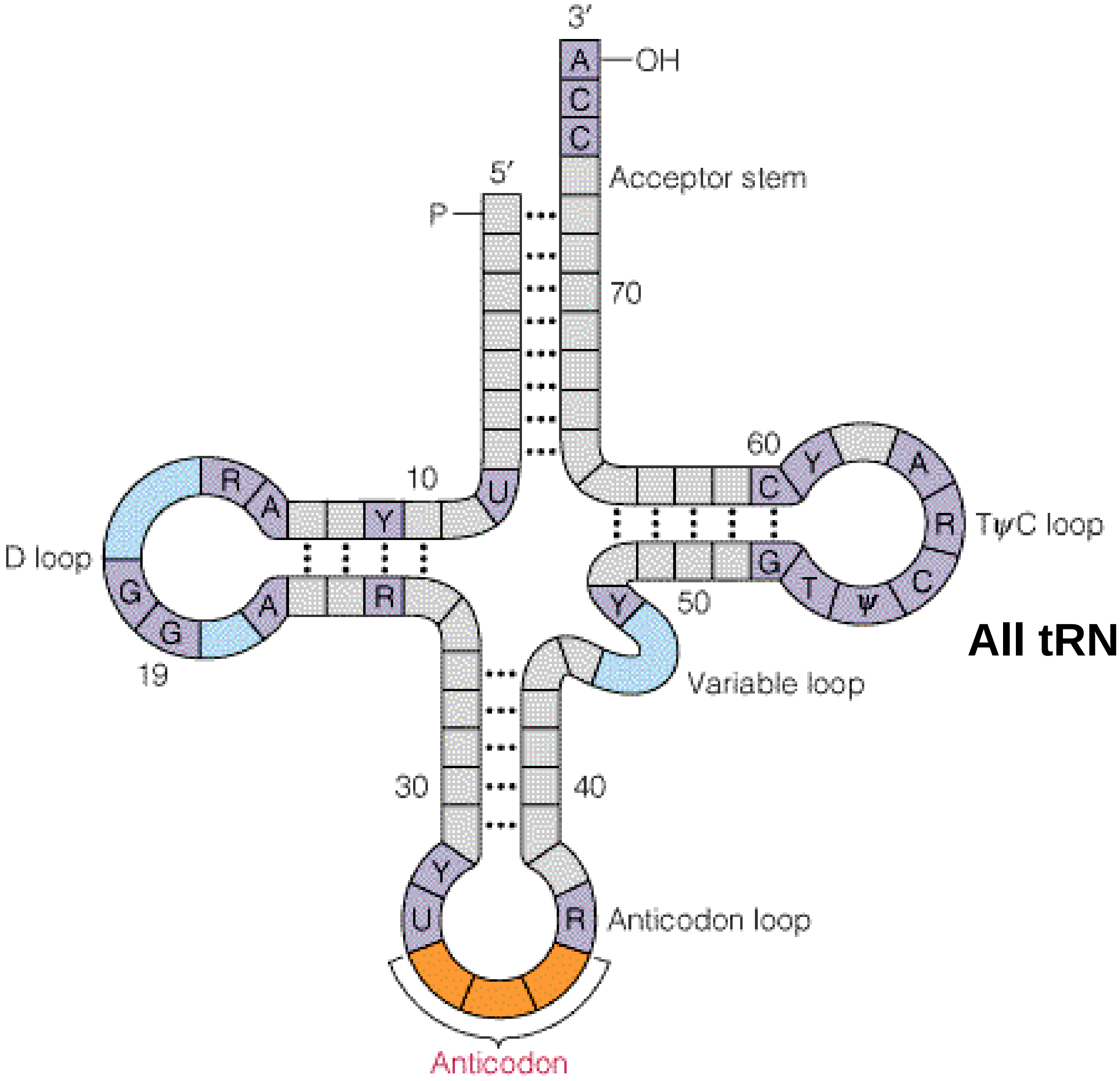
Reading frame 3 ---UU CUC GGA CCU GGA GAU UCA CAG U---

In a triplet, nonoverlapping code, all mRNAs have three potential reading frames

Transfer RNAs (tRNAs)

- Adaptor molecules that match amino acids to codons in mRNA.
- Any cell contains different types of tRNA molecules sufficient to incorporate all 20 amino acids into protein.
- Some tRNAs can recognise more than one codon.
- About 80 nucleotides in length.

Structure of tRNAs



All tRNAs share a general common structure that includes:

- **an anticodon triplet loop (pairs with mRNA codons)**

- **an acceptor stem (to which the amino acid is attached)**

THE WOBBLE HYPOTHESIS

- **The first two bases of an mRNA codon always form strong Watson-Crick base pairs with the corresponding bases of the tRNA anticodon and confer most of the coding specificity.**
- **The first base of the anticodon (reading in the 5’→3’ direction; this pairs with the third base of the codon) determines the number of codons recognized by the tRNA.**
  - **When the first base of the anticodon is C or A, base pairing is specific and only one codon is recognized by that tRNA.**
  - **When the first base is U or G, binding is lessspecific and two different codons may be read.**
  - **When inosine (I) is the first (wobble) nucleotide of an anticodon, three different codons can be recognized—the maximum number for any tRNA.**
- **When an amino acid is specified by several different codons, the codons that differ in either of the first two bases require different tRNAs**
- **A minimum of 32 tRNAs are required to translate all 61 codons (31 to encode the amino acids and 1 for initiation).**

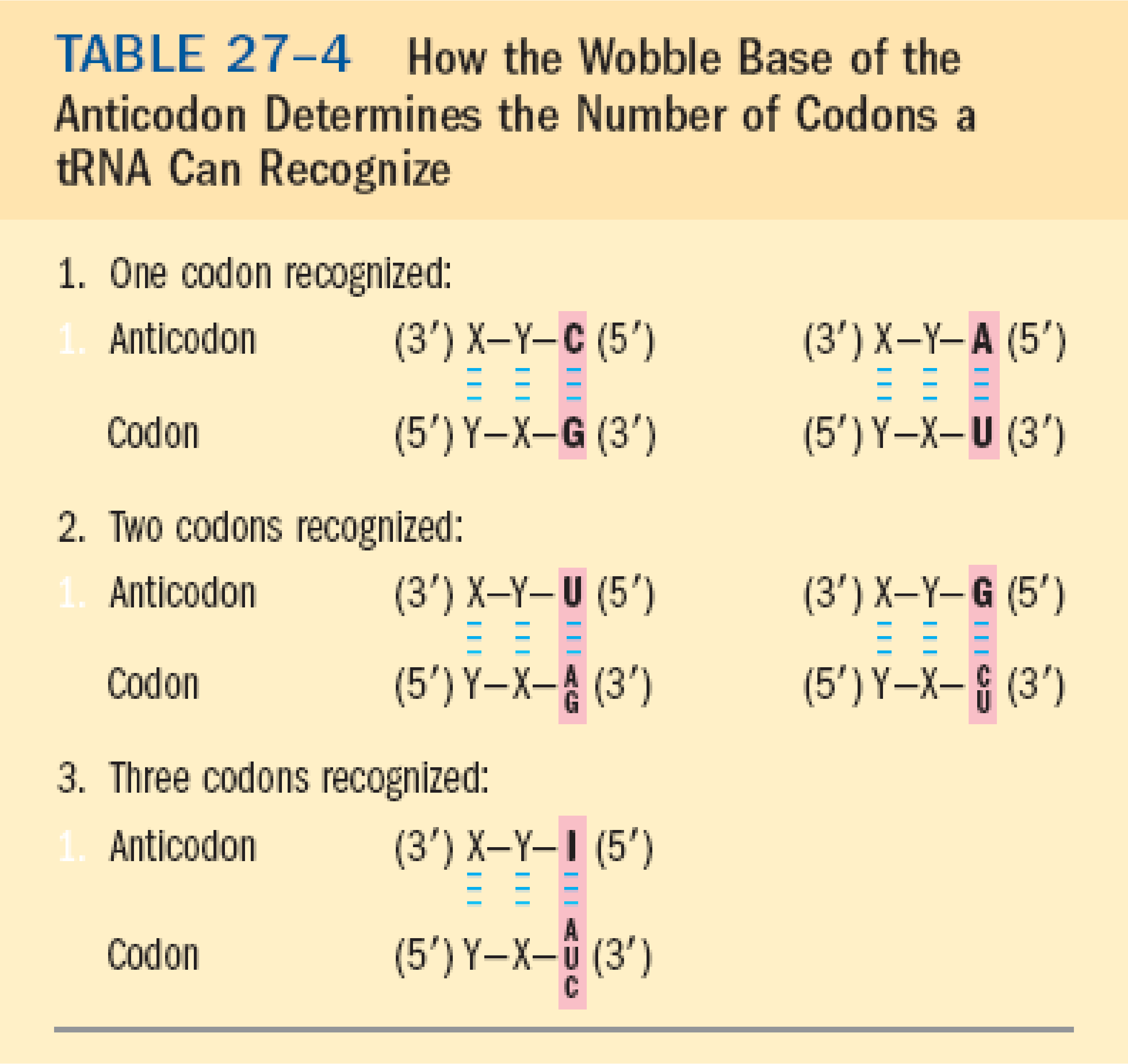


TABLE 27–4 How the Wobble Base of the Anticodon Determines the Number of Codons a tRNA Can Recognize

1. One codon recognized:

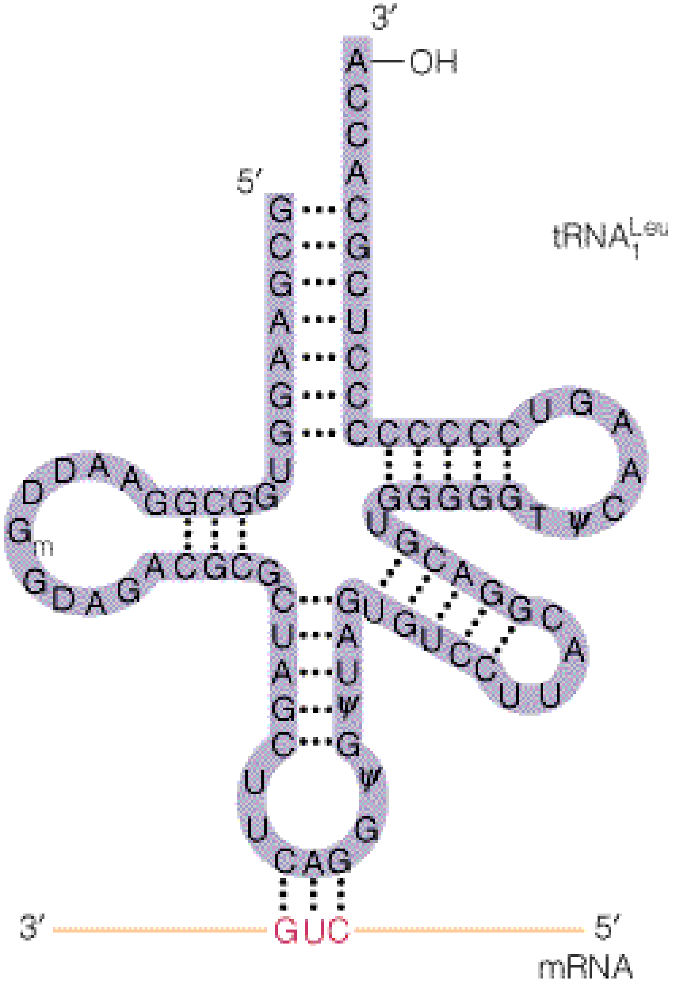
1. Anticodon	(3′) X–Y–**C** (5′)	(3′) X–Y–**A** (5′)
Codon	(5′) Y–X–**G** (3′)	(5′) Y–X–**U** (3′)

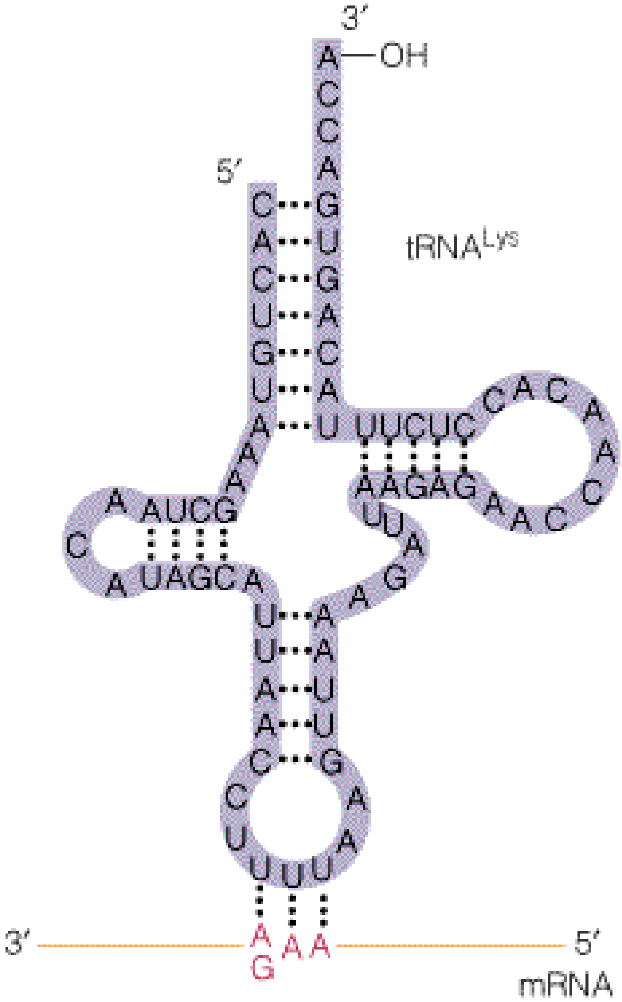
2. Two codons recognized:

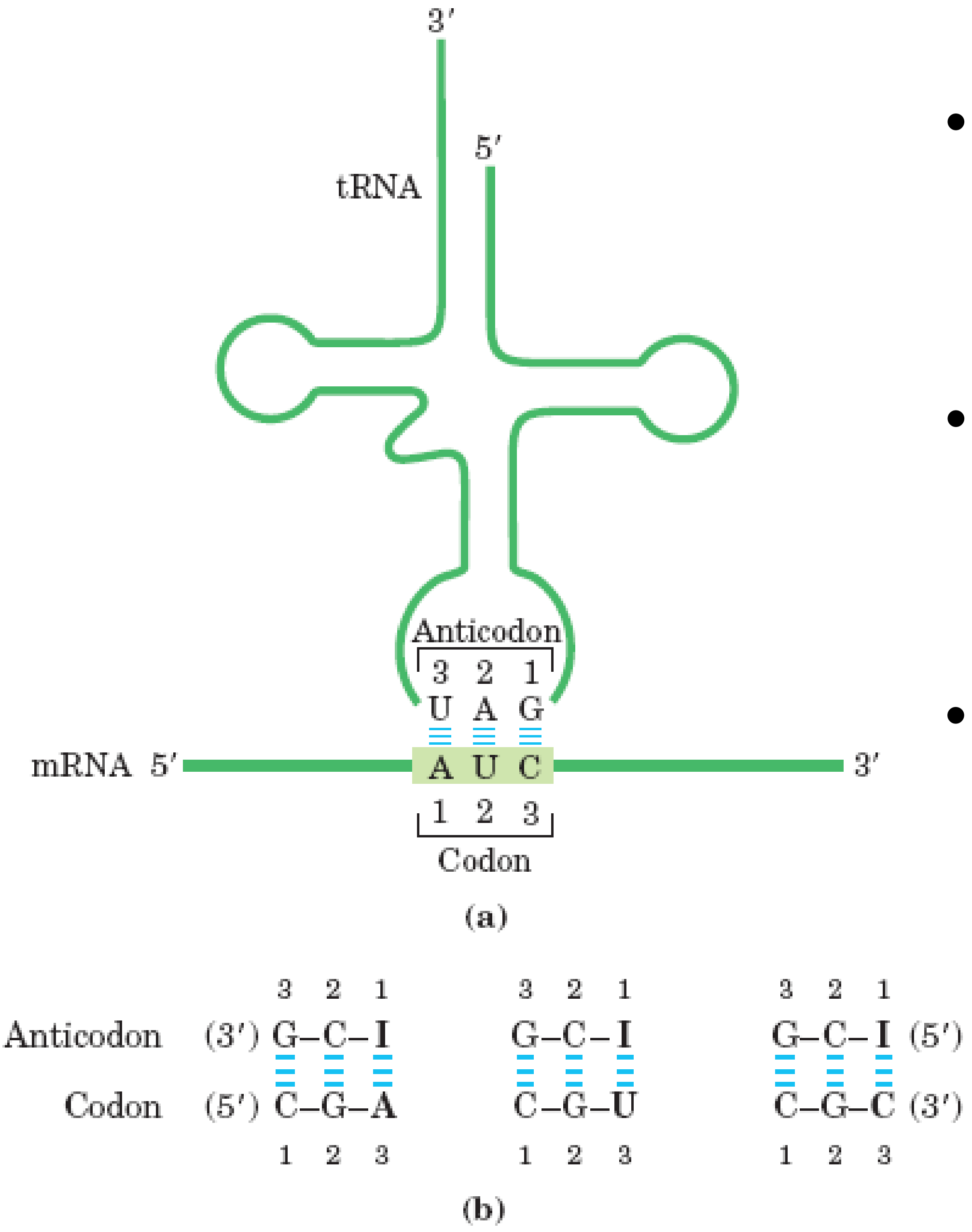
1. Anticodon	(3′) X–Y–**U** (5′)	(3′) X–Y–**G** (5′)
Codon	(5′) Y–X–$\begin{smallmatrix}\mathbf{A}\\\mathbf{G}\end{smallmatrix}$ (3′)	(5′) Y–X–$\begin{smallmatrix}\mathbf{C}\\\mathbf{U}\end{smallmatrix}$ (3′)

3. Three codons recognized:

1. Anticodon	(3′) X–Y–**I** (5′)
Codon	(5′) Y–X–$\begin{smallmatrix}\mathbf{A}\\\mathbf{U}\\\mathbf{C}\end{smallmatrix}$ (3′)

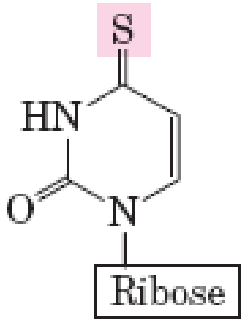

3′
A—OH
5′
tRNA$_1^{Leu}$
CAG
GUC
3′
5′
mRNA


3′
A—OH
5′
tRNA^{Lys}
UUU
AAA
G
3′
5′
mRNA

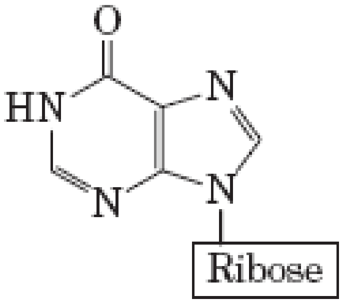


- Transfer RNAs base-pair with mRNA codons at a three-base sequence on the tRNA called the **anticodon**
- the anticodons in some tRNAs include the nucleotide **inosinate (I)**, which contains the uncommon base hypoxanthine
- Inosinate can form hydrogen bonds with three different nucleotides (U, C, and A)

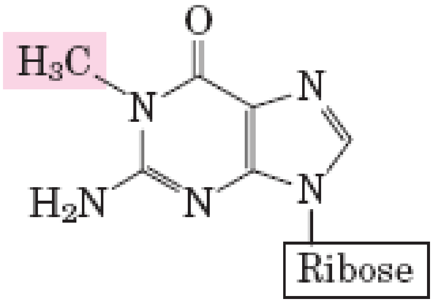
Some modified bases of tRNAs, produced in posttranscriptional reactions



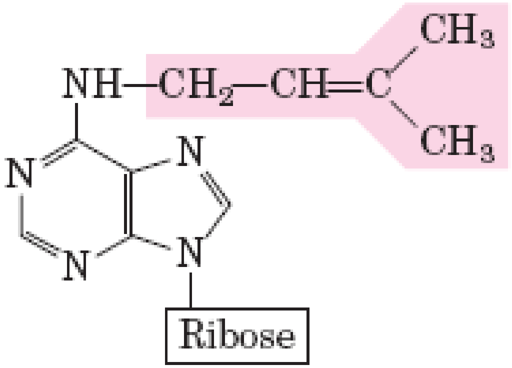
4-Thiouridine (S^{4}U)



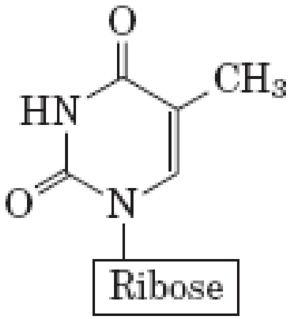
Inosine (I)



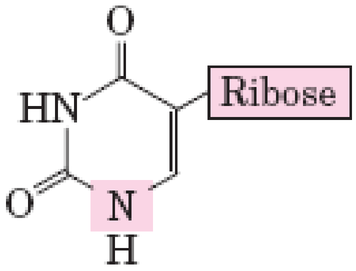
1-Methylguanosine (m^{1}G)



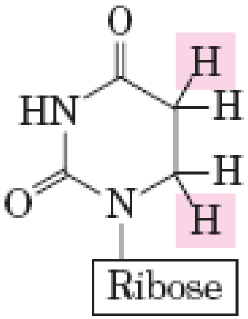
N^6-Isopentenyladenosine (i^{6}A)



Ribothymidine (T)

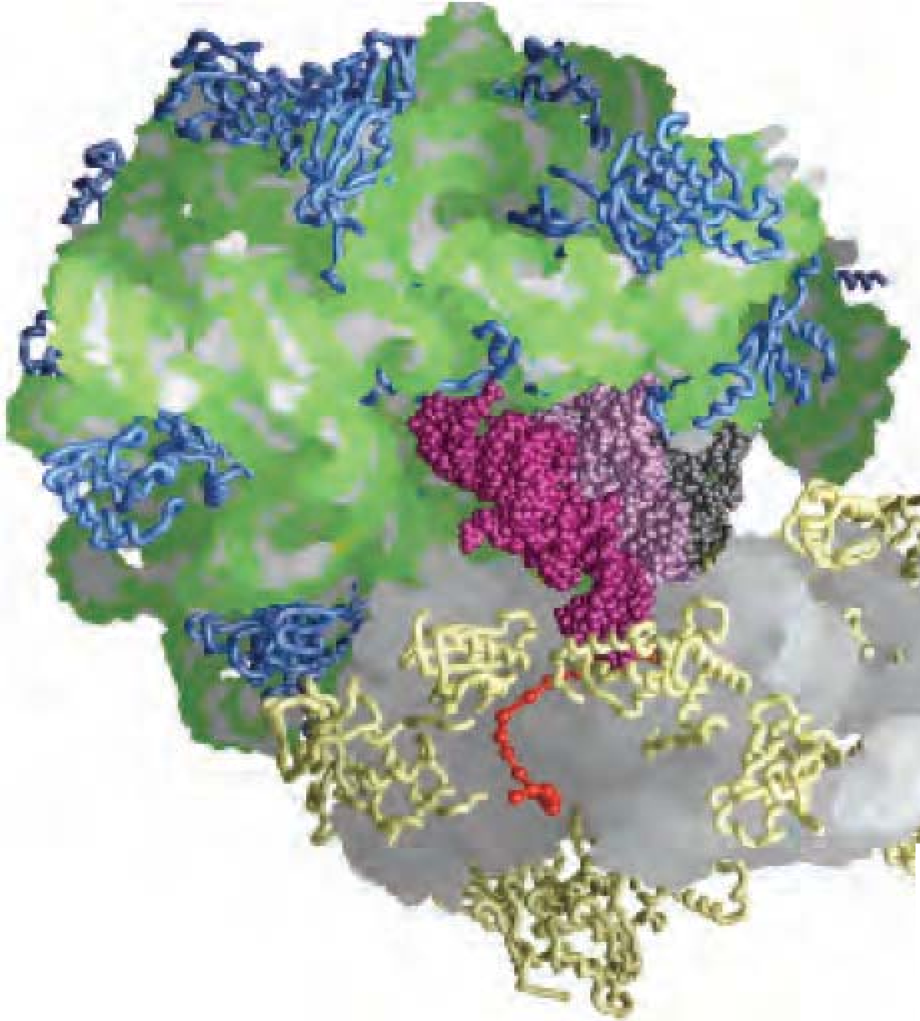


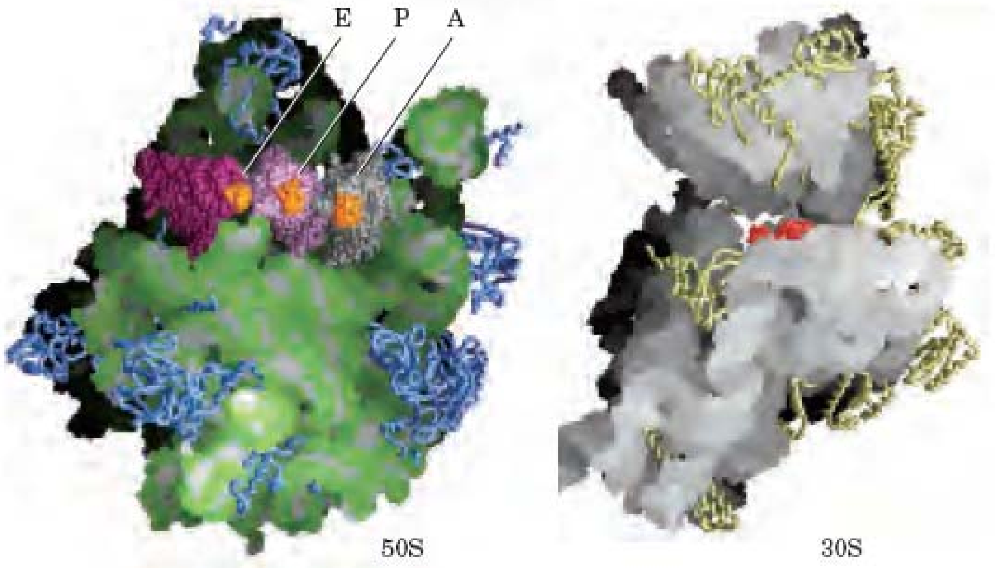
Pseudouridine (ψ)



Dihydrouridine (D)

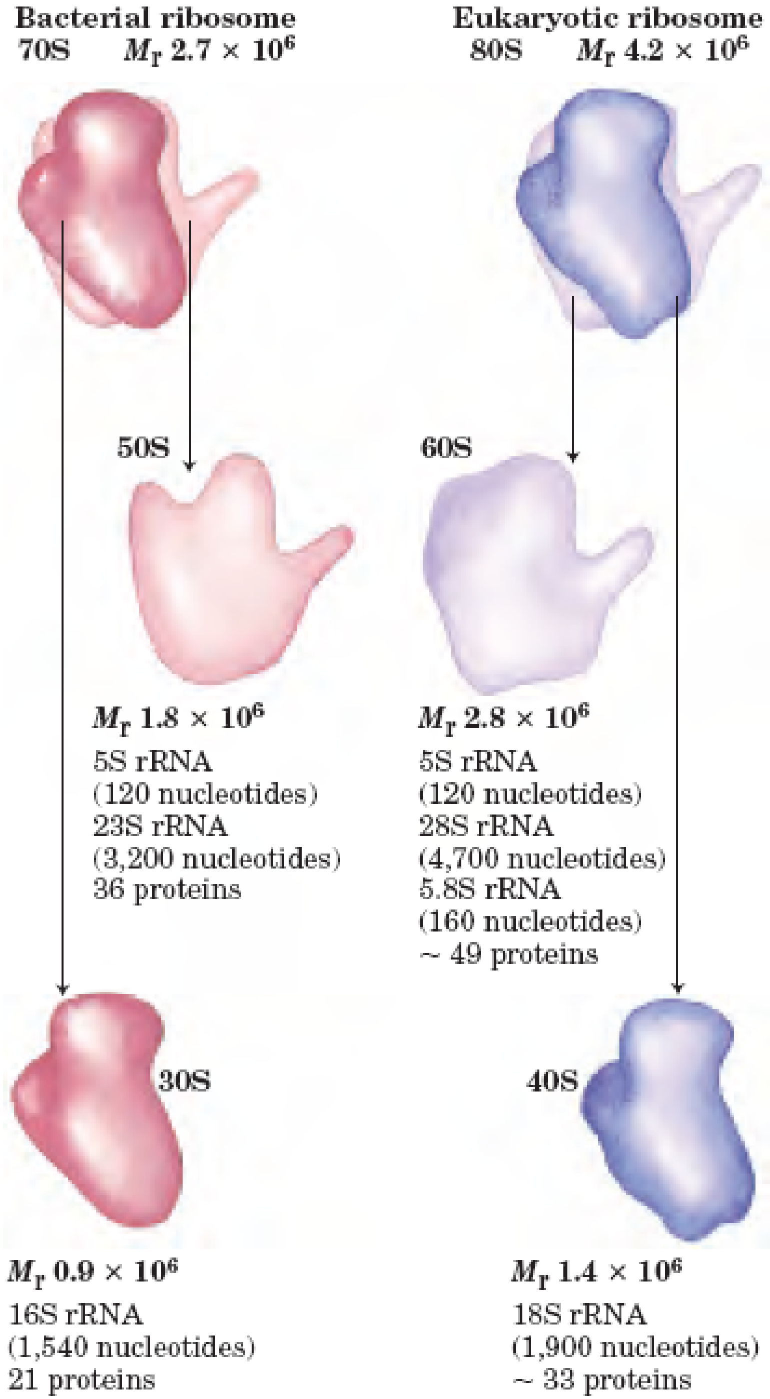
Ribosomes



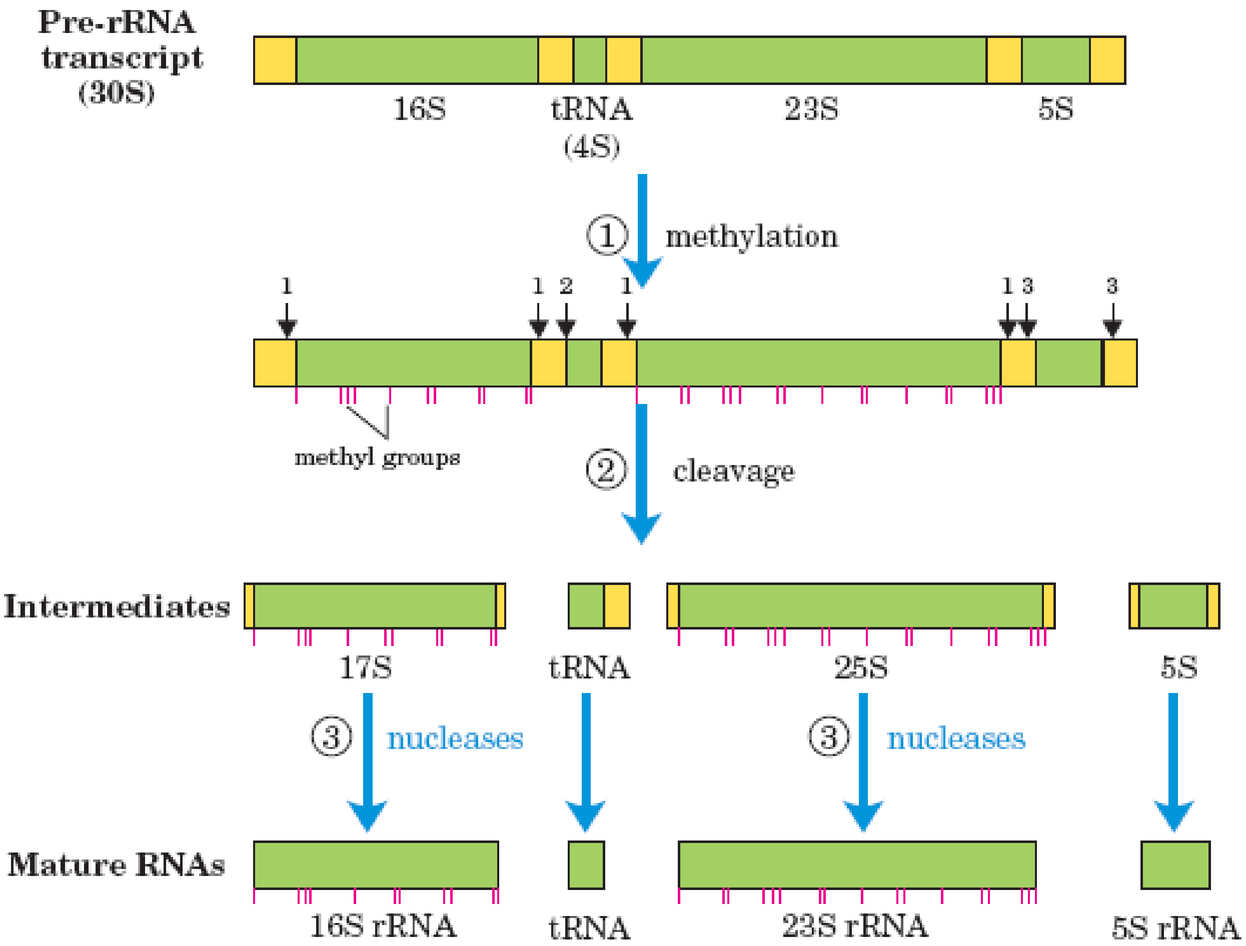


Ribosome Structure

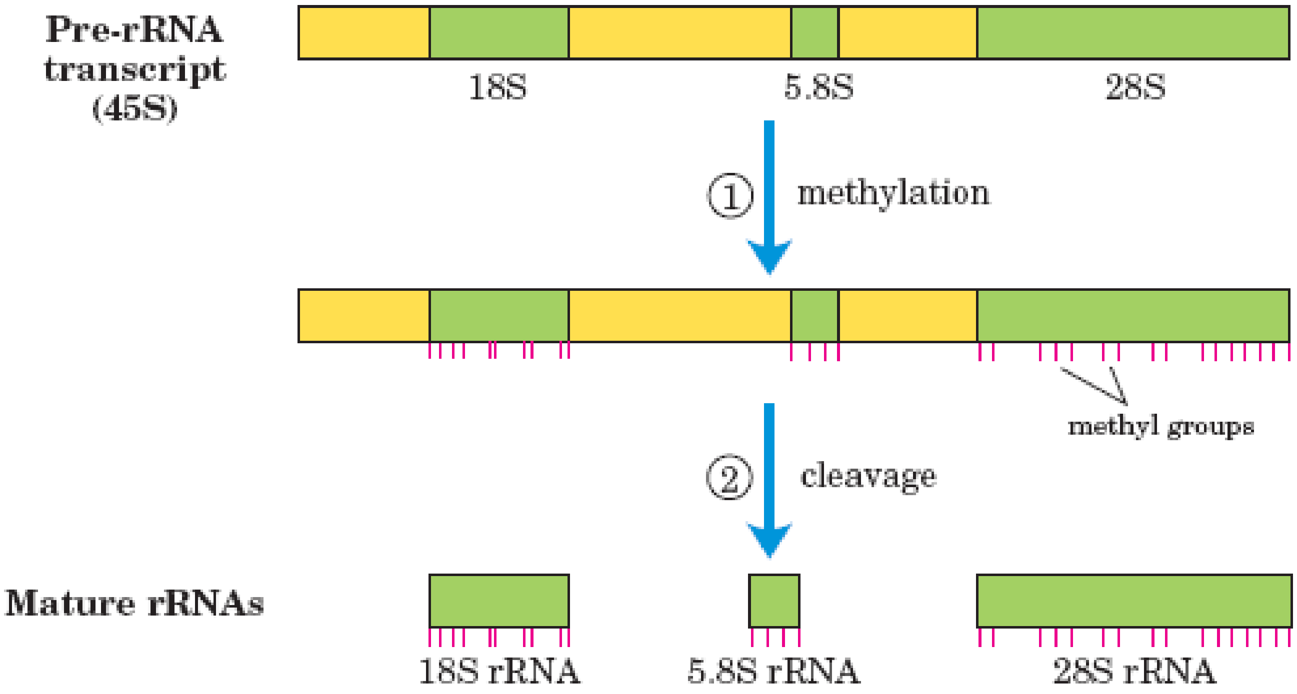
Property	Prokaryote	Eukaryote
Overall Size	70S	80S
Small subunit	30S	40S
No. of proteins	~21	~30
RNA size (No. of bases)	16S (1500)	18S (2300)
Large subunit	50S	60S
No. of proteins	~34	~50
RNA size (No of bases)	23S (2900)	28s (4200
	5S (120	5.8S (160)
		5S (120)


Bacterial ribosome
70S M_r 2.7 × 10^6
50S
M_r 1.8 × 10^6
5S rRNA
(120 nucleotides)
23S rRNA
(3,200 nucleotides)
36 proteins
30S
M_r 0.9 × 10^6
16S rRNA
(1,540 nucleotides)
21 proteins
Eukaryotic ribosome
80S M_r 4.2 × 10^6
60S
M_r 2.8 × 10^6
5S rRNA
(120 nucleotides)
28S rRNA
(4,700 nucleotides)
5.8S rRNA
(160 nucleotides)
~ 49 proteins
40S
M_r 1.4 × 10^6
18S rRNA
(1,900 nucleotides)
~ 33 proteins

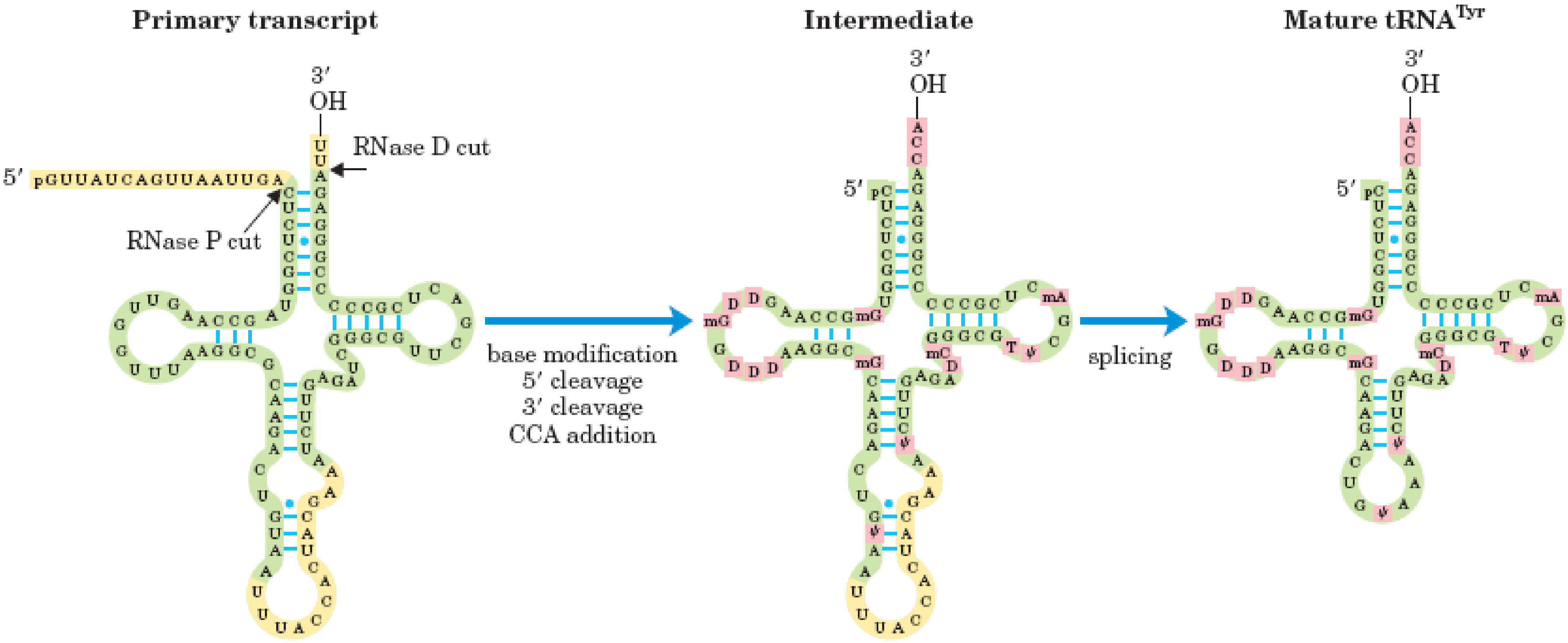
Processing of pre-rRNA transcripts in bacteria



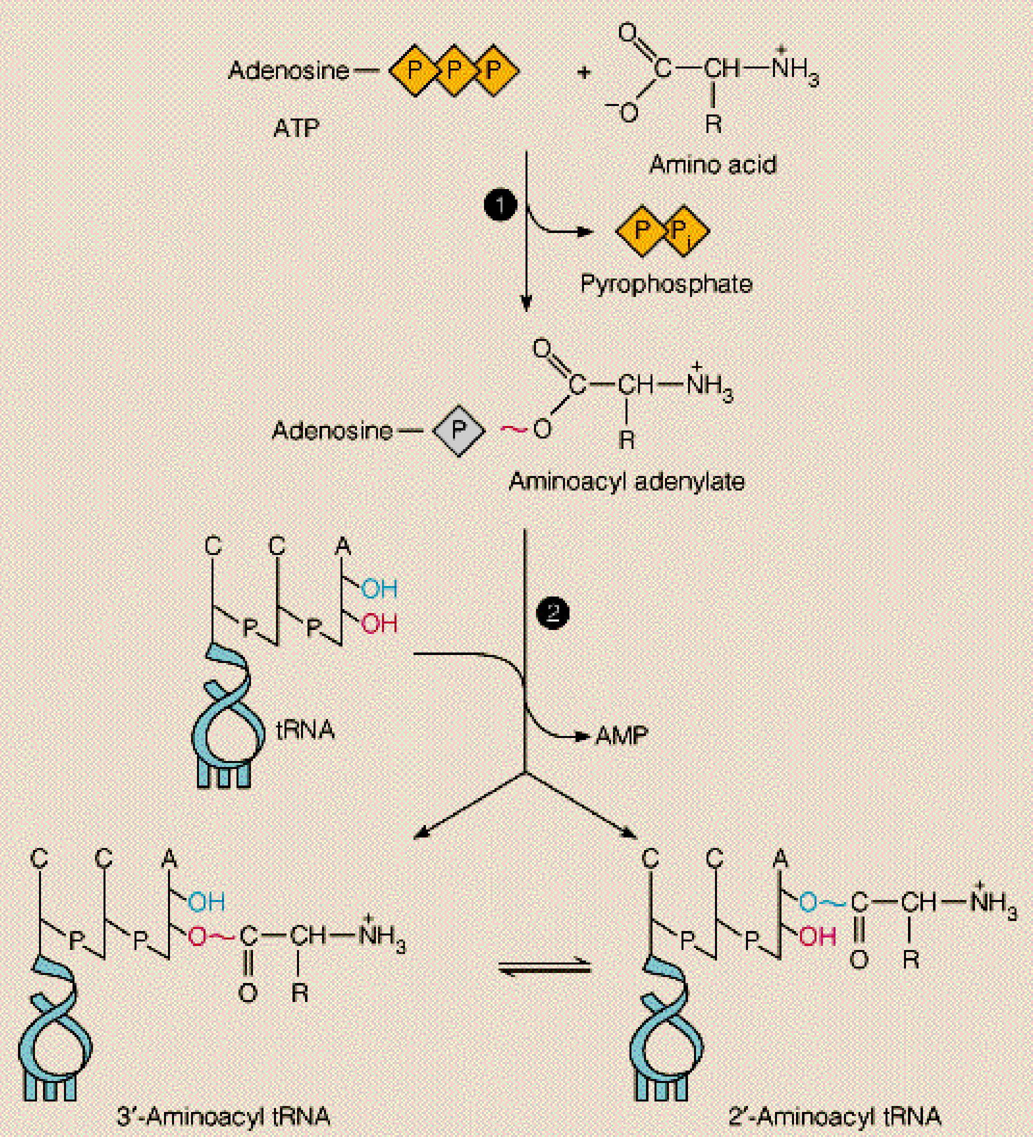
Processing of pre-rRNA transcripts in vertebrates



Processing of tRNAs in bacteria and eukaryotes



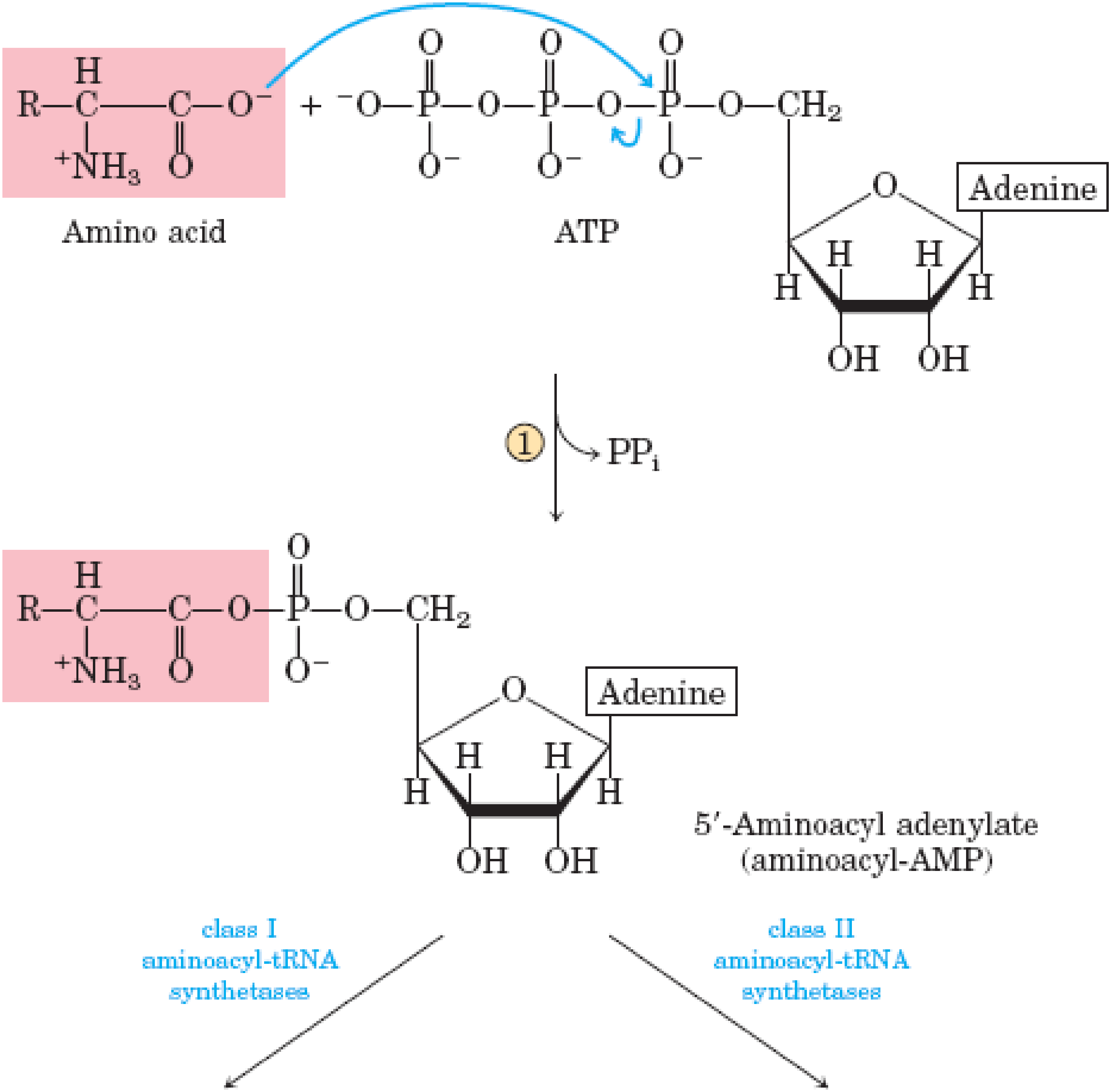
Coupling of amino acids to tRNAs

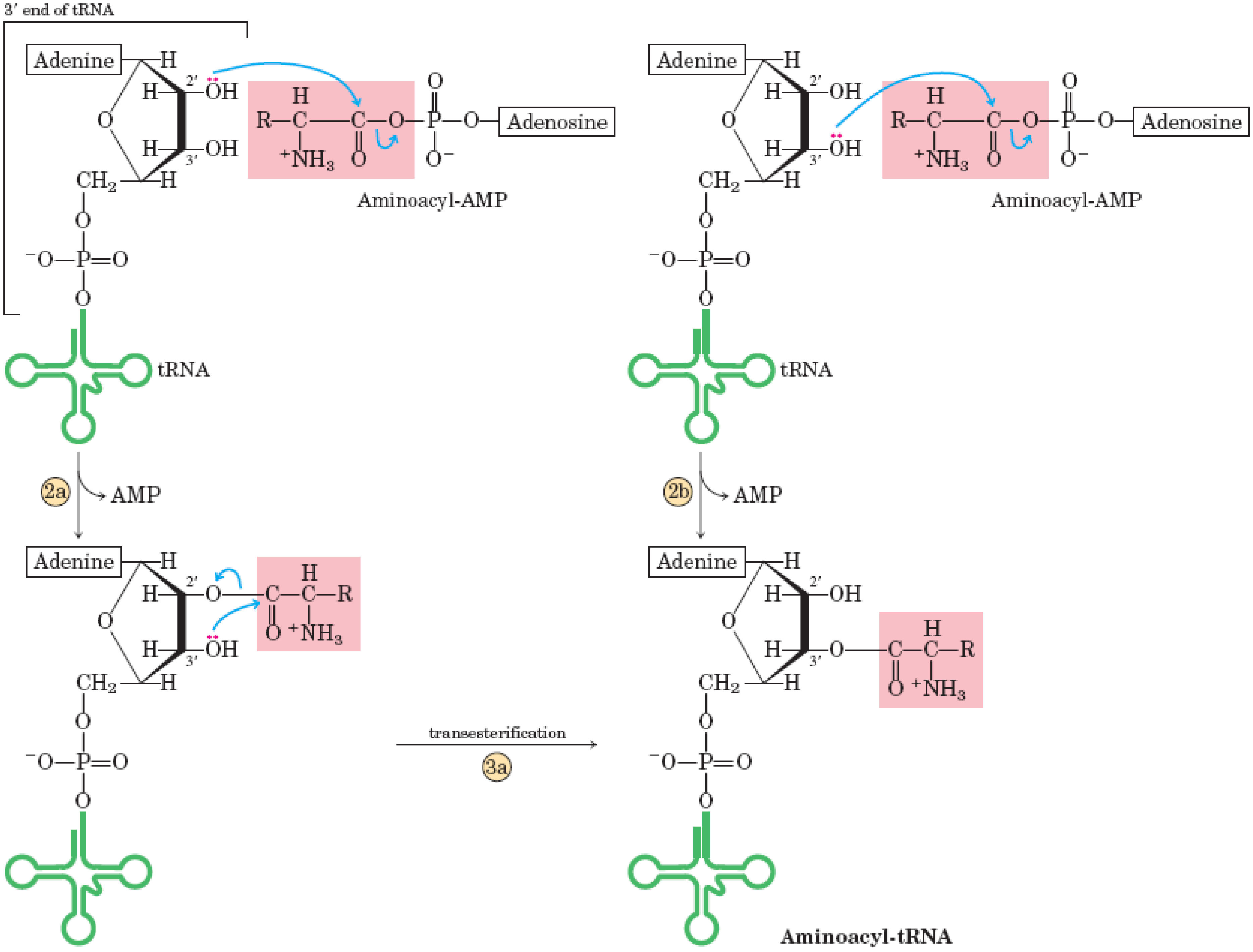


1. **The amino acid is accepted by the aminoacyl-tRNA synthetase enzyme and is adenylated**

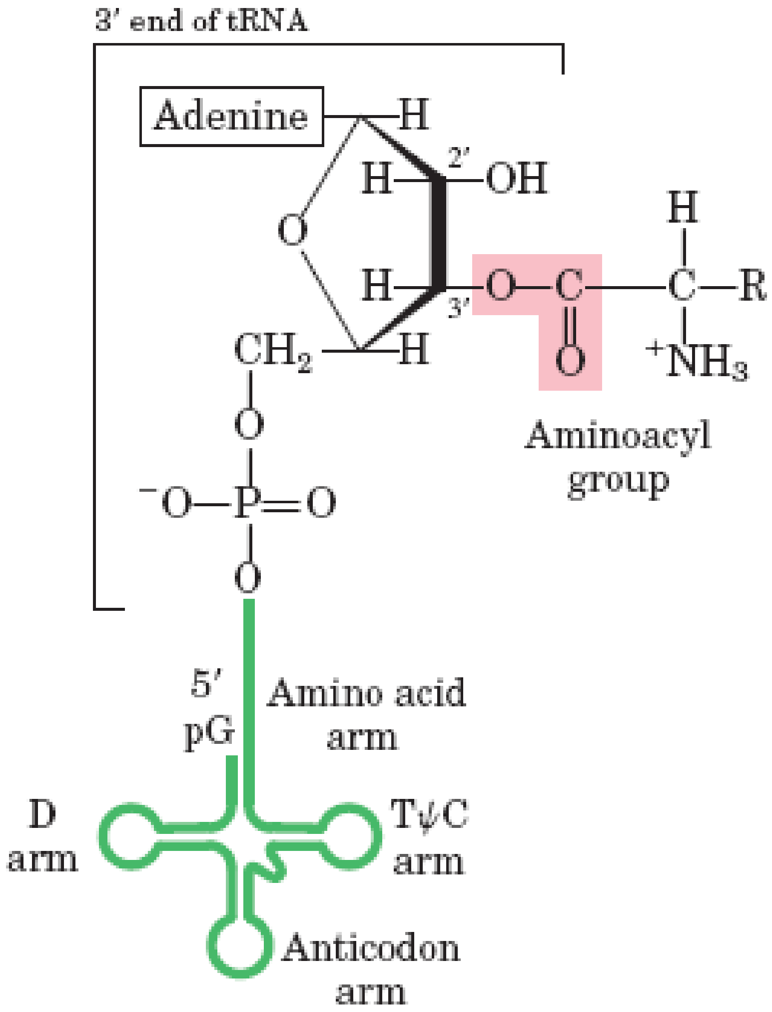
2. **The proper tRNA is accepted by the enzyme and the amino acid residue is transferred to the 2’ or 3’ OH of the 3’-terminal residue of the RNA**

All reactions occur on the synthetase enzyme.

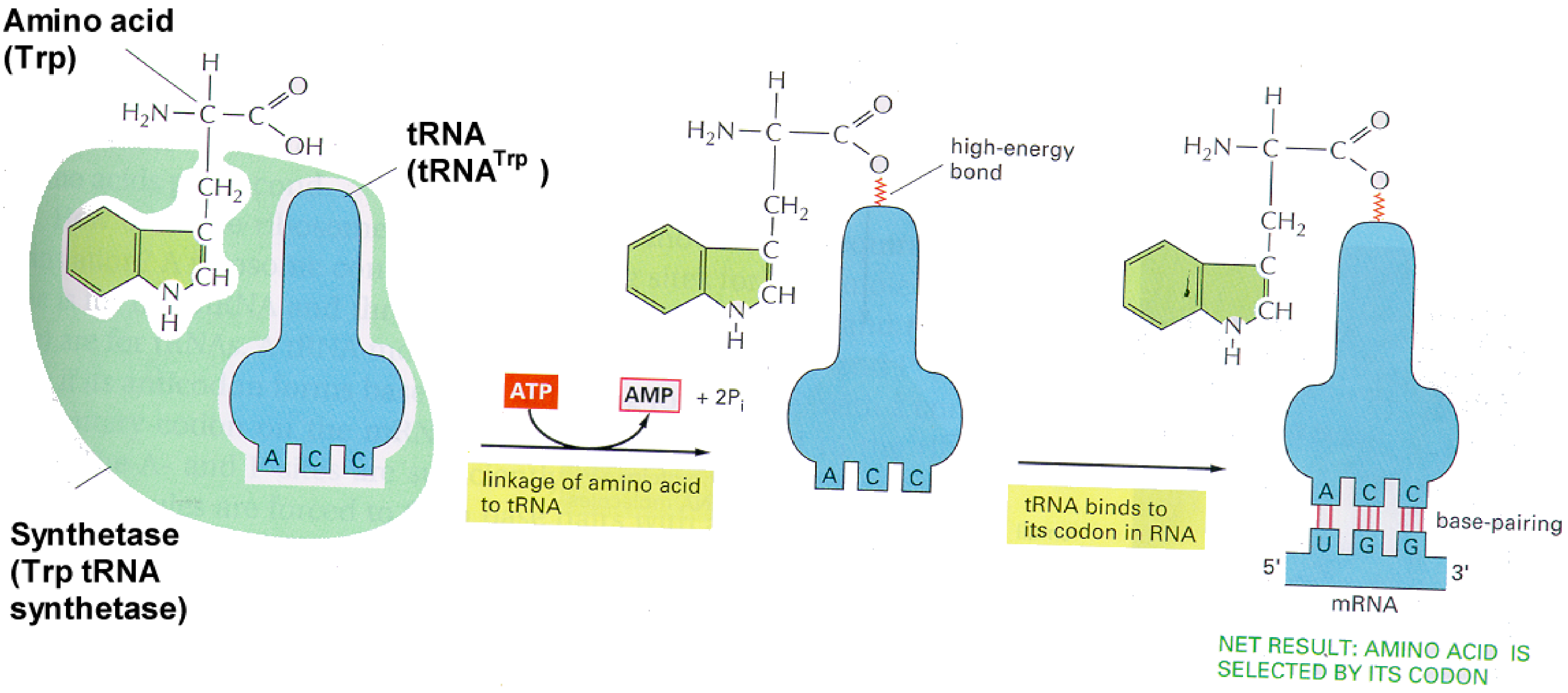

Amino acid
ATP
Adenine
①
PPᵢ
Adenine
5′-Aminoacyl adenylate
(aminoacyl-AMP)
class I
aminoacyl-tRNA
synthetases
class II
aminoacyl-tRNA
synthetases


3′ end of tRNA
Adenine
2′
3′
OH
CH2
tRNA
Aminoacyl-AMP
Adenosine
R
+NH3
2a
2b
AMP
transesterification
3a
Aminoacyl-tRNA

General structure of aminoacyl-tRNAs



The Two Steps of Decoding

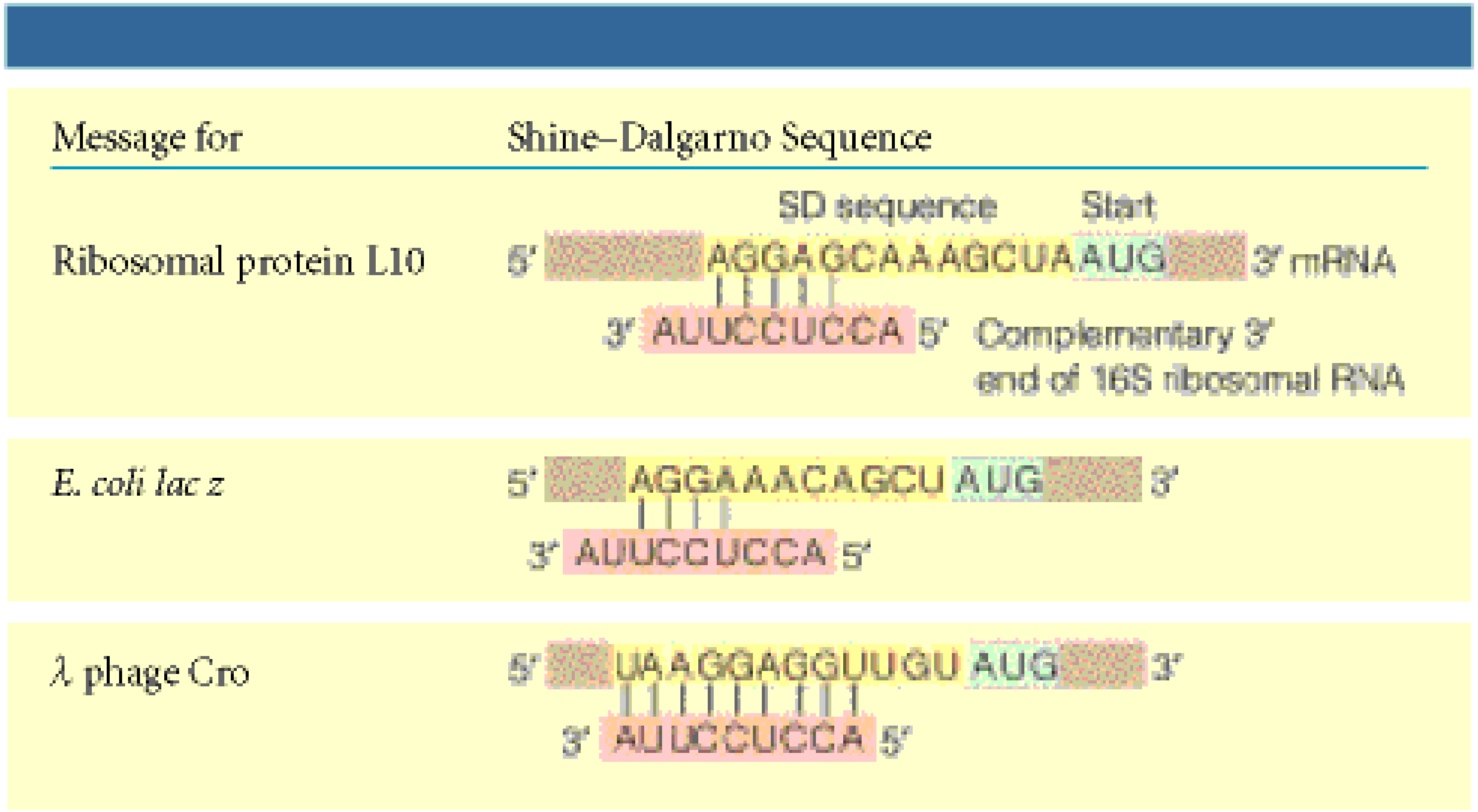


Shine-Dalgarno Sequences

(named for Australian researchers John Shine and Lynn Dalgarno)

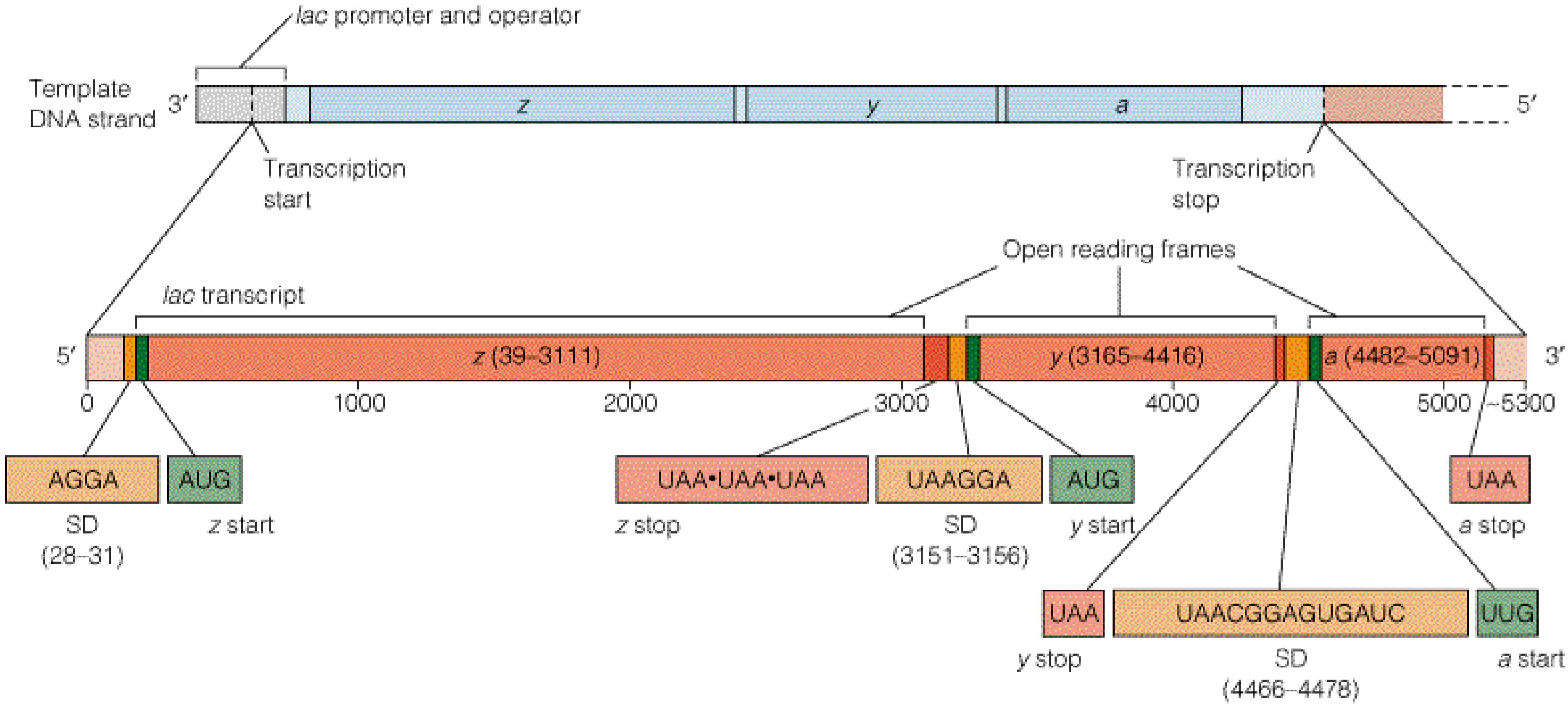
Help align ribosomes on mRNA to properly start translation

Can base-pair with a sequence contained in the ribosomal RNA



Message for	Shine–Dalgarno Sequence
Ribosomal protein L10	SD sequence Start 5′ AGGAGCAAAGCUA AUG 3′ mRNA 3′ AUUCCUCCA 5′ Complementary 3′ end of 16S ribosomal RNA
E. coli lac z	5′ AGGAAACAGCU AUG 3′ 3′ AUUCCUCCA 5′
λ phage Cro	5′ UAAGGAGGUUGU AUG 3′ 3′ AUUCCUCCA 5′

Structure of Procaryotic mRNAs



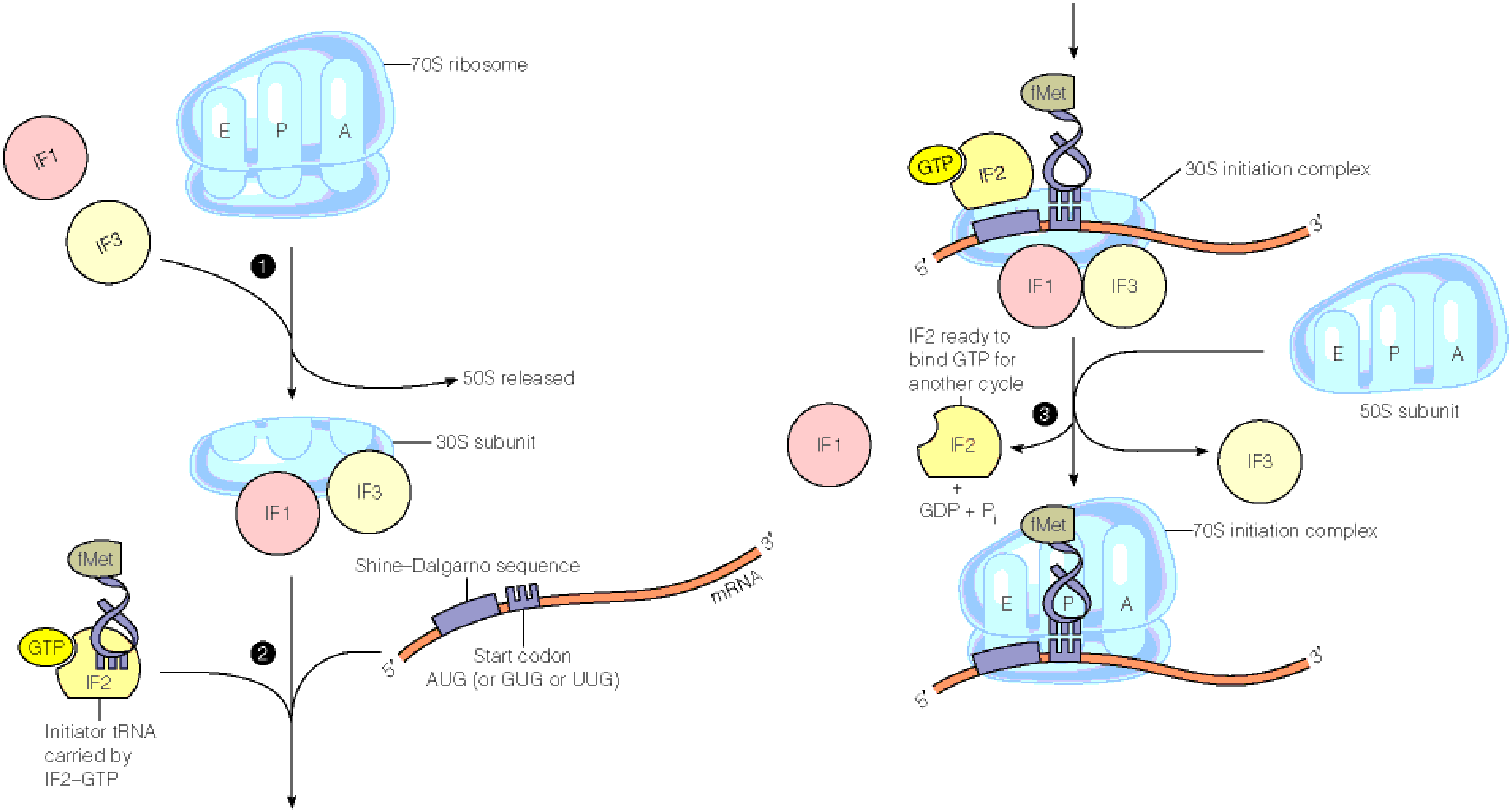
mRNA has also regions that do not encode for a protein

Shine-Dalgarno sequence (SD) = Ribosome Binding Site (RBS)

The first AUG after SD-sequence is interpreted as the start site of translation

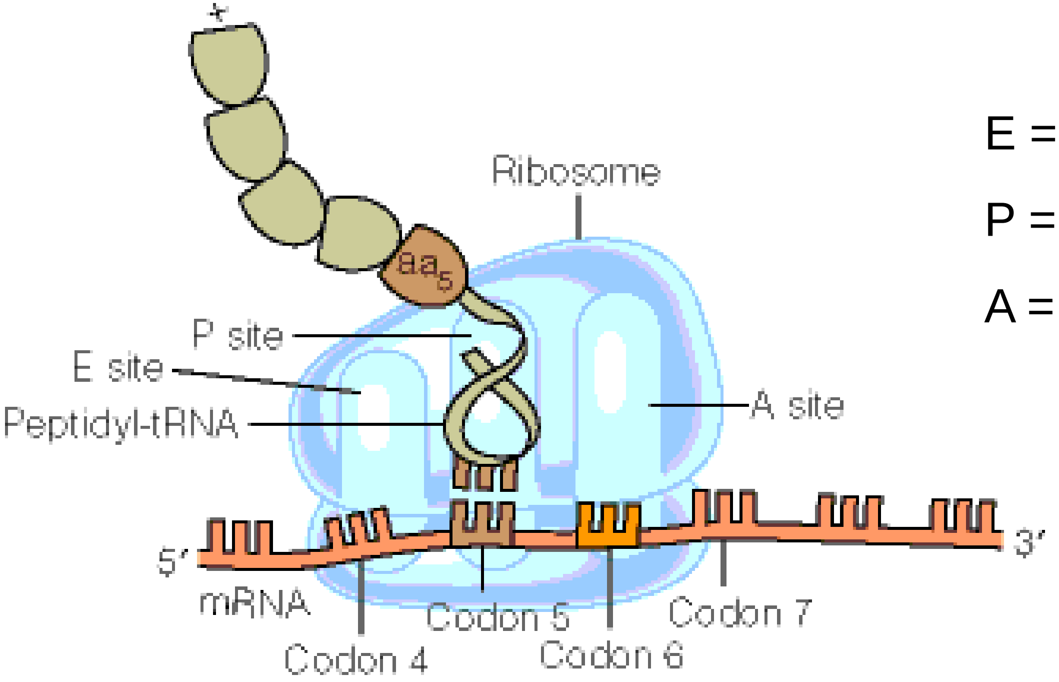
The Mechanism of Translation

Initiation in Procaryotes



The Mechanism of Translation

Elongation in Procaryotes (1)



E = exit site

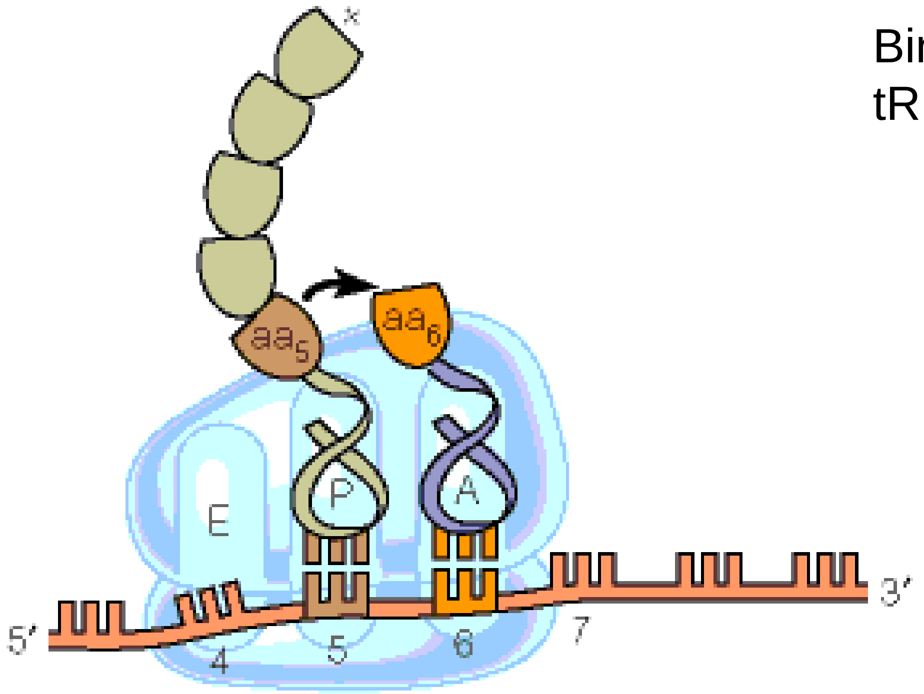
P = peptidyl binding site

A = aminoacyl binding site

The E site is the site from which the “uncharged” tRNAs leave during elongation.

The Mechanism of Translation

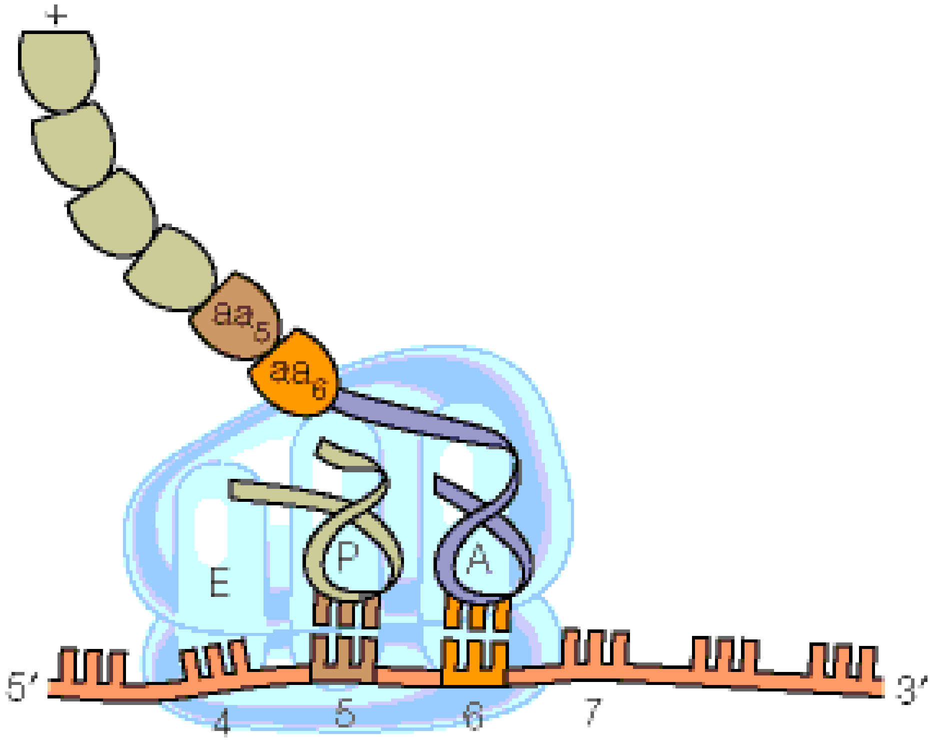
Elongation in Procaryotes (2)



Binding of a spesific amino acid tRNA to A site

The Mechanism of Translation

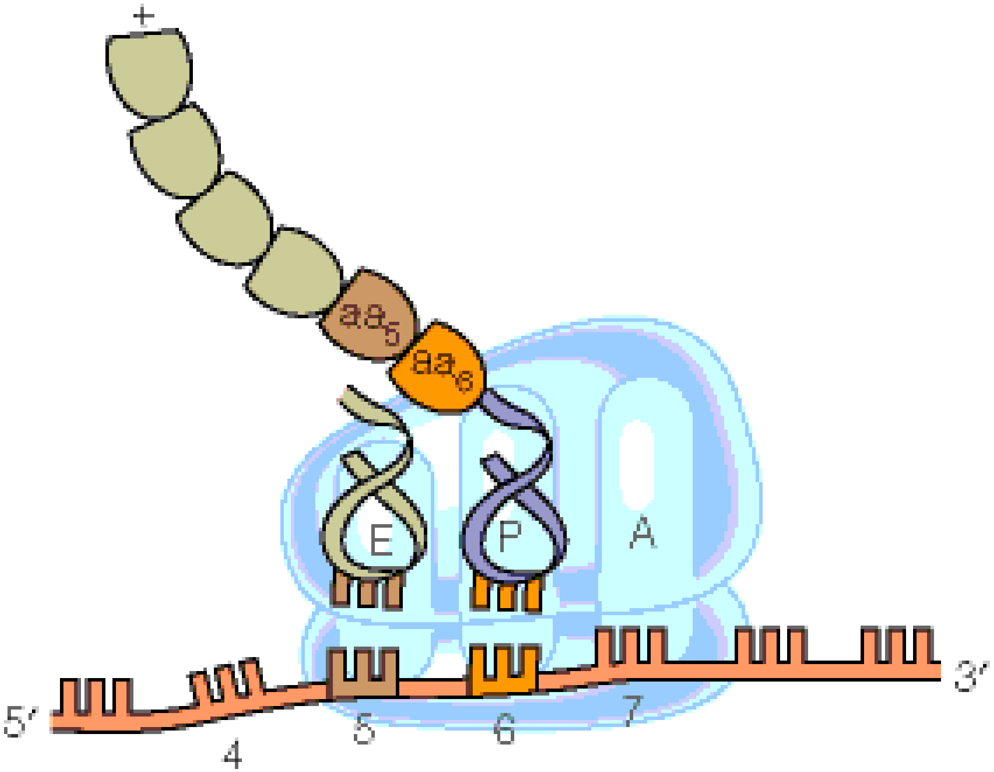
Elongation in Procaryotes (3)



Peptide bond formation: chain transfer from peptidyl tRNA to aminoacyl tRNA

The Mechanism of Translation

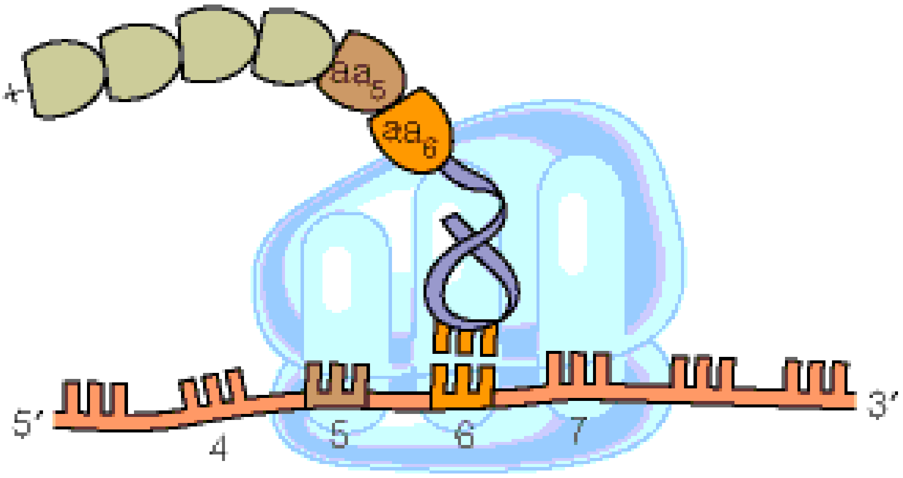
Elongation in Procaryotes (4)



Translocation of peptidyl tRNA from A site to P site. Ribosome moves one codon to the right, and the now uncharged tRNA moves from P site to E site.

The Mechanism of Translation

Elongation in Procaryotes (5)

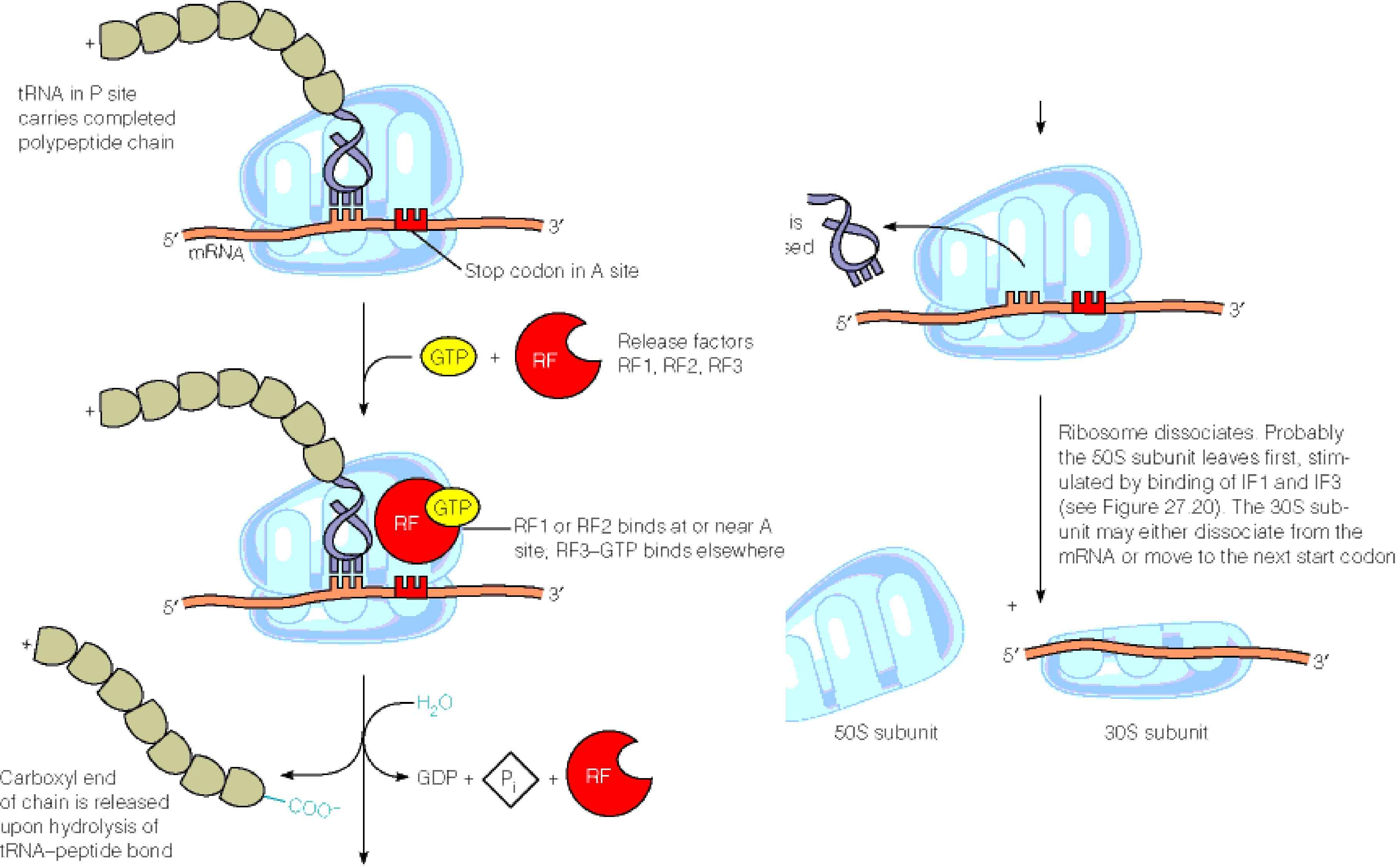


Ribosome is ready to start another cycle.

The cycles will continue until a termination codon is reached.

The Mechanism of Translation

Termination in Procaryotes

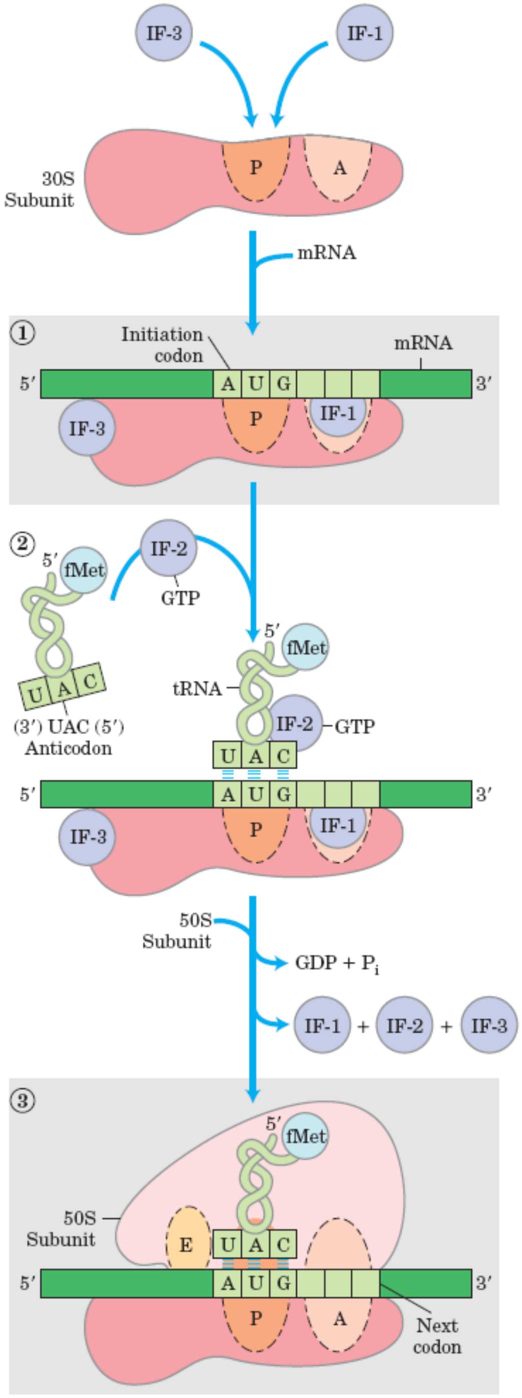


RF1 or RF2 binds at or near A site; RF3–GTP binds elsewhere

Carboxyl end of chain is released upon hydrolysis of tRNA–peptide bond

Ribosome dissociates. Probably the 50S subunit leaves first, stimulated by binding of IF1 and IF3 (see Figure 27.20). The 30S subunit may either dissociate from the mRNA or move to the next start codon

Stage 1 : The initiation of polypeptide synthesis



Step 1

- The 30S ribosomal subunit binds two initiation factors, IF-1 and IF-3. Factor IF-3 prevents the 30S and 50S subunits from combining prematurely. Factor IF-1 binds at the A site and prevents tRNA binding at this site during initiation
- The mRNA then binds to the 30S subunit.
- The initiating (5)AUG where fMet-tRNAfMet is to be bound is guided to its correct position by the **Shine- Dalgarno sequence.** The initiating (5)AUG is positioned at the P site, the only site to which fMettRNAfMet can bind.

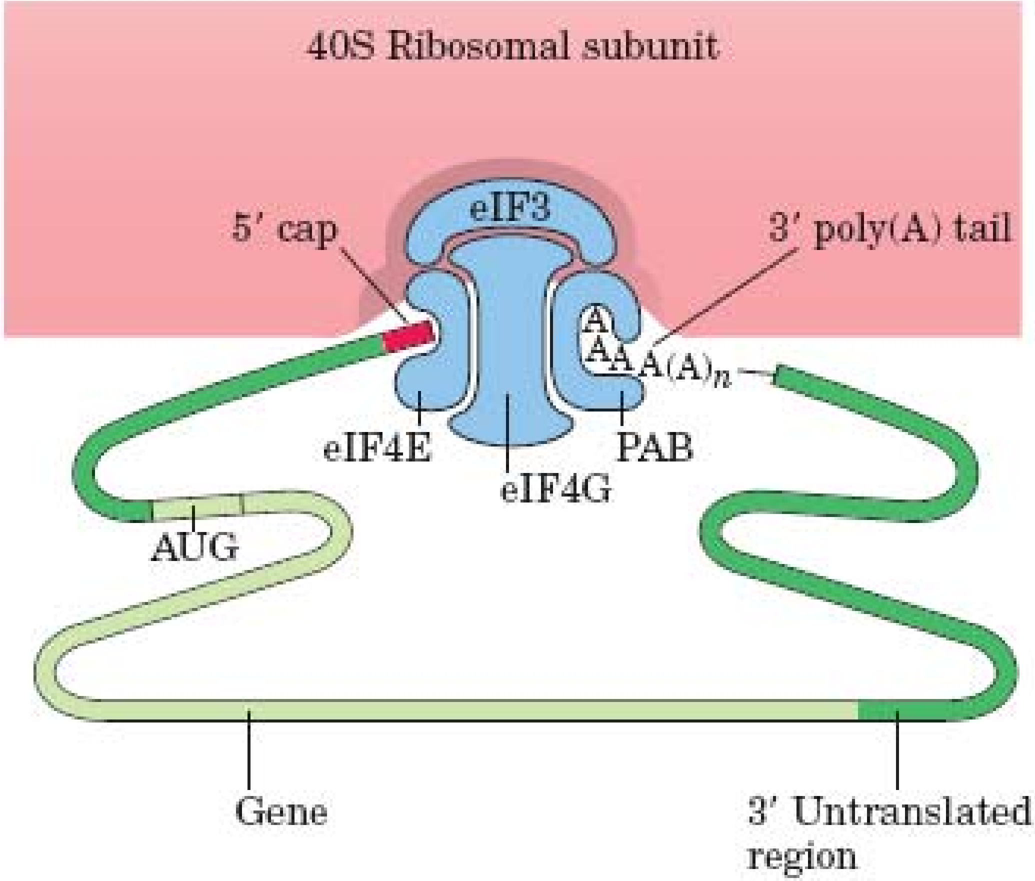
Step 2

- the complex consisting of the 30S ribosomal subunit, IF-3, and mRNA is joined by both GTP-bound IF-2 and the initiating fMet-tRNAfMet.
- The anticodon of this tRNA now pairs correctly with the mRNA's initiation codon.

Step 3

- this large complex combines with the 50S ribosomal subunit; simultaneously, the GTP bound to IF-2 is hydrolyzed to GDP and Pi, which are released from the complex.
- All three initiation factors depart from the ribosome at this point.

Initiation in Eukaryotic Cells



The initiating (5)AUG is detected within the mRNA not by its proximity to a Shine-Dalgarno-like sequence but by a scanning process: a scan of the mRNA from the 5 end until the first AUG is encountered, signaling the beginning of the reading frame.

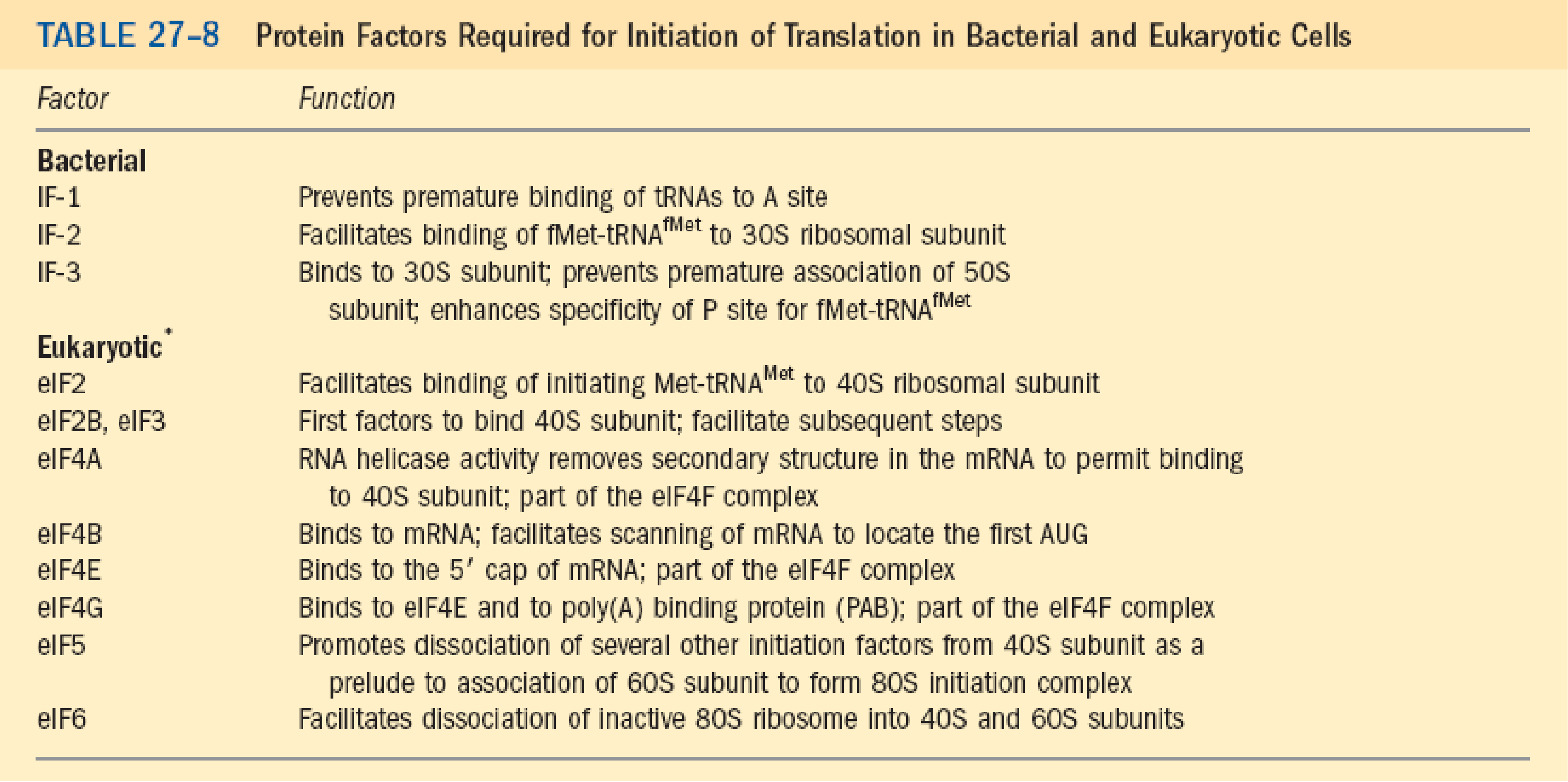
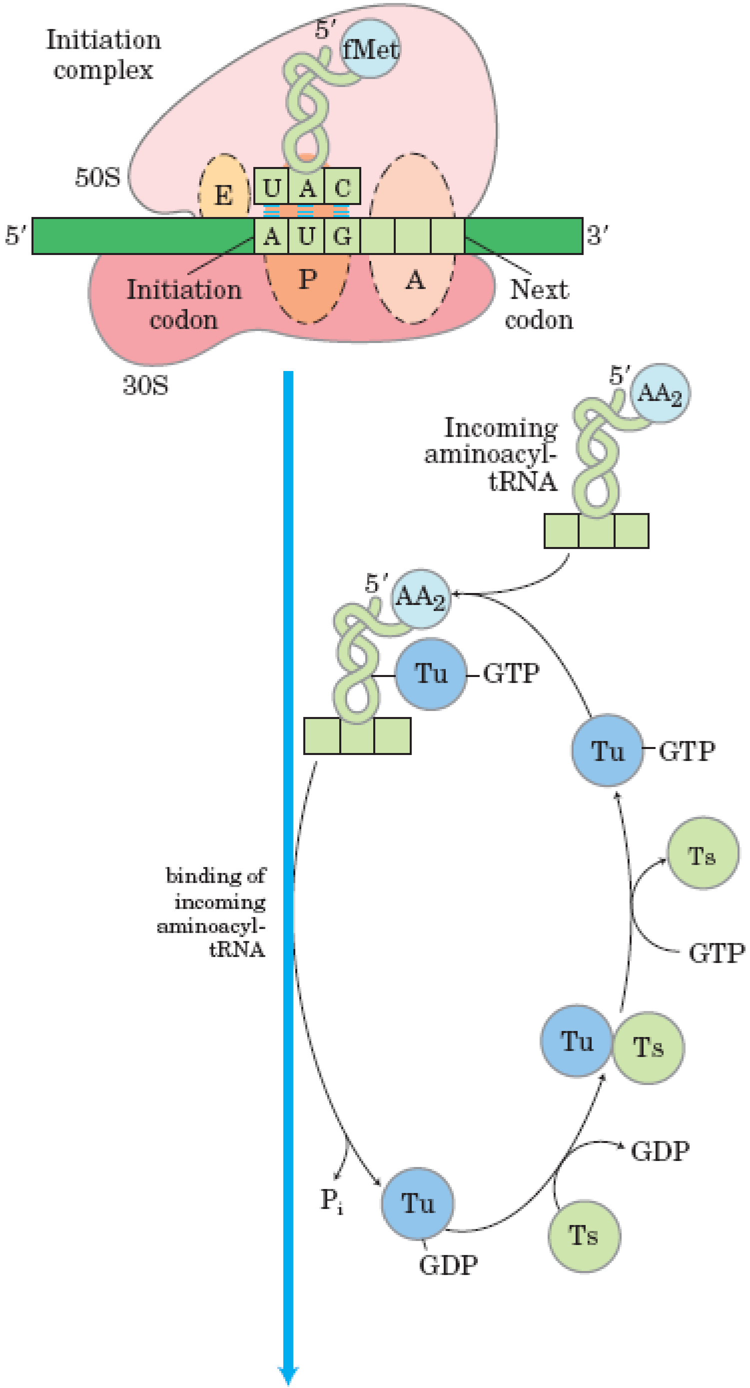


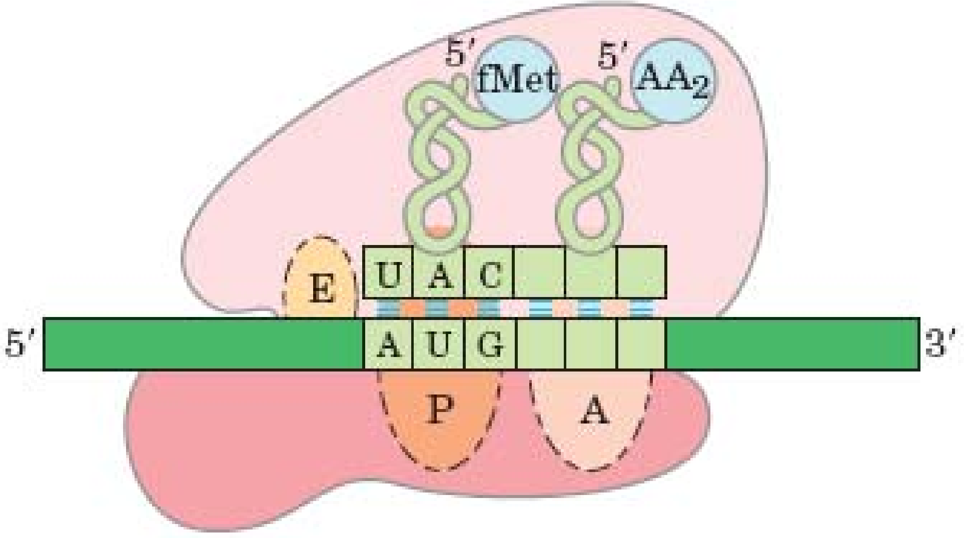
TABLE 27–8 Protein Factors Required for Initiation of Translation in Bacterial and Eukaryotic Cells

Factor	*Function*
Bacterial	
IF-1	Prevents premature binding of tRNAs to A site
IF-2	Facilitates binding of fMet-tRNA$^{\text{fMet}}$ to 30S ribosomal subunit
IF-3	Binds to 30S subunit; prevents premature association of 50S subunit; enhances specificity of P site for fMet-tRNA$^{\text{fMet}}$
Eukaryotic[*]	
eIF2	Facilitates binding of initiating Met-tRNA$^{\text{Met}}$ to 40S ribosomal subunit
eIF2B, eIF3	First factors to bind 40S subunit; facilitate subsequent steps
eIF4A	RNA helicase activity removes secondary structure in the mRNA to permit binding to 40S subunit; part of the eIF4F complex
eIF4B	Binds to mRNA; facilitates scanning of mRNA to locate the first AUG
eIF4E	Binds to the 5′ cap of mRNA; part of the eIF4F complex
eIF4G	Binds to eIF4E and to poly(A) binding protein (PAB); part of the eIF4F complex
eIF5	Promotes dissociation of several other initiation factors from 40S subunit as a prelude to association of 60S subunit to form 80S initiation complex
eIF6	Facilitates dissociation of inactive 80S ribosome into 40S and 60S subunits

Elongation Step 1: Binding of an Incoming Aminoacyl-tRNA

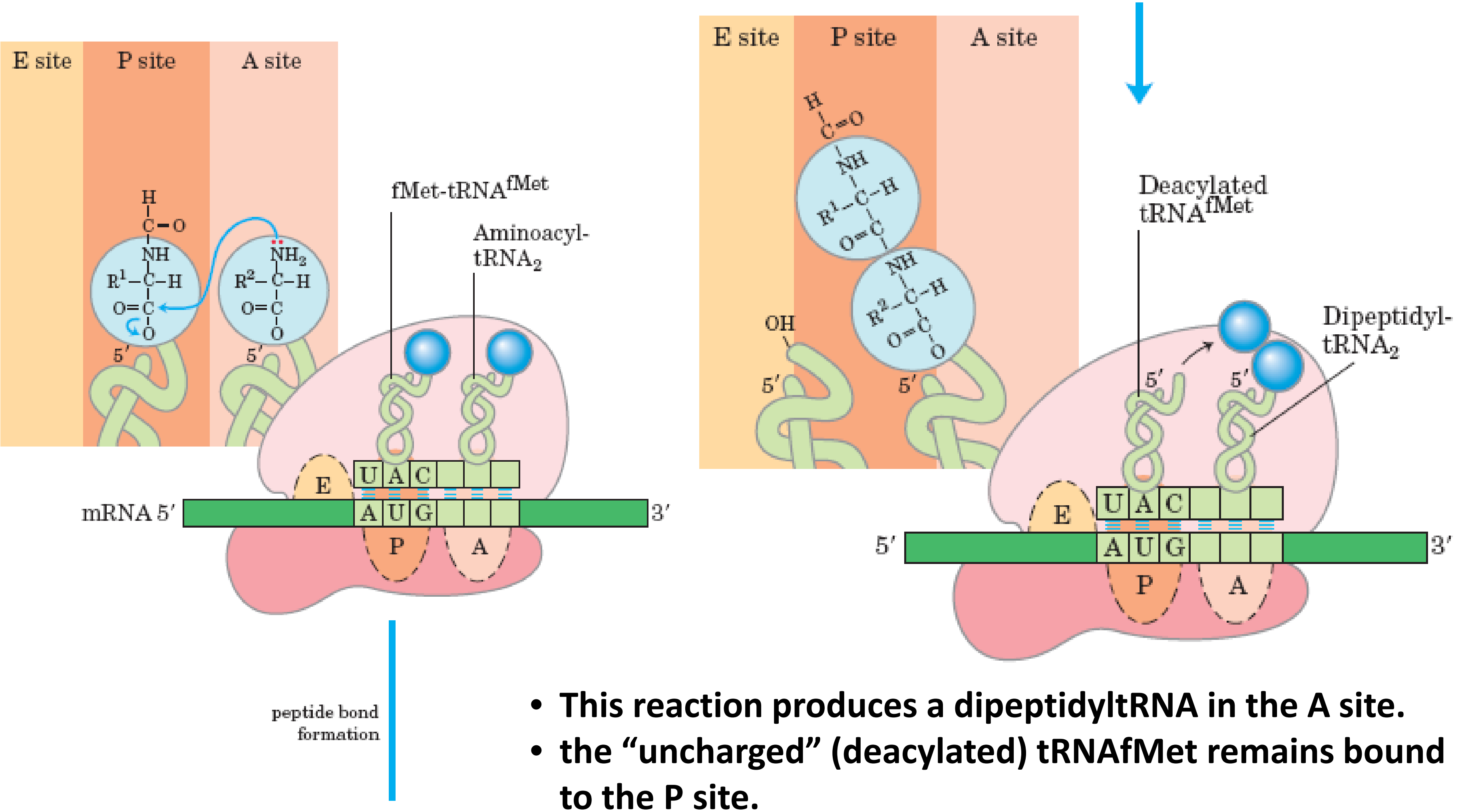
elongation factors in bacteria :
EF-Tu, EF-Ts, and EF-G





Elongation Step 2: Peptide Bond Formation

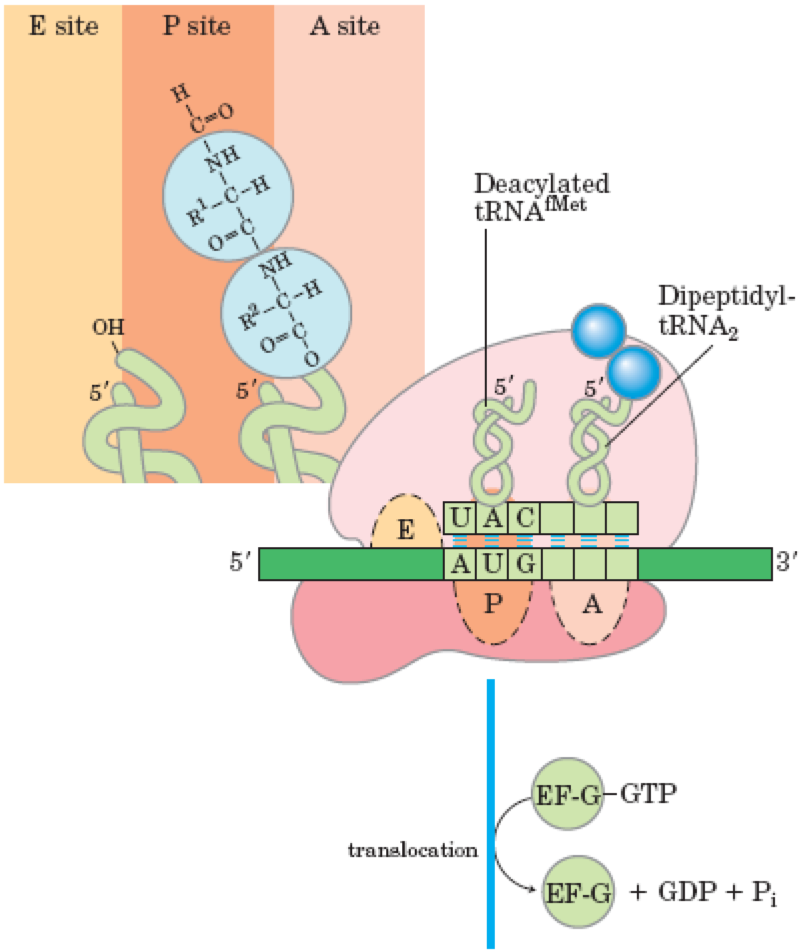
The peptidyl transferase catalyzing this reaction is the 23S rRNA ribozyme



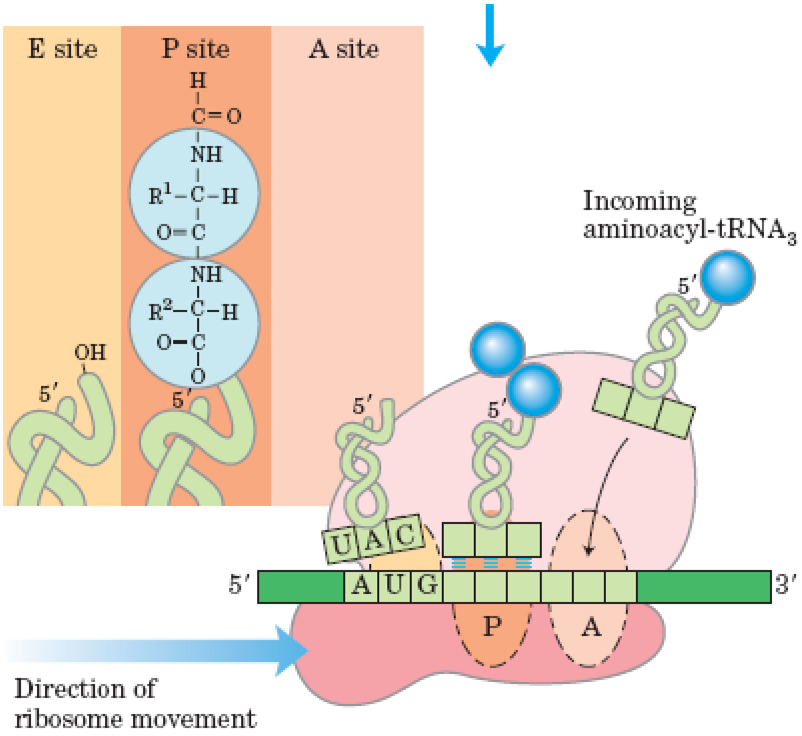
- **This reaction produces a dipeptidyltRNA in the A site.**
- **the “uncharged” (deacylated) tRNAfMet remains bound to the P site.**
- **The tRNAs then shift to a hybrid binding state**

Elongation Step 3: Translocation

the ribosome moves one codon toward the 3 end of the mRNA



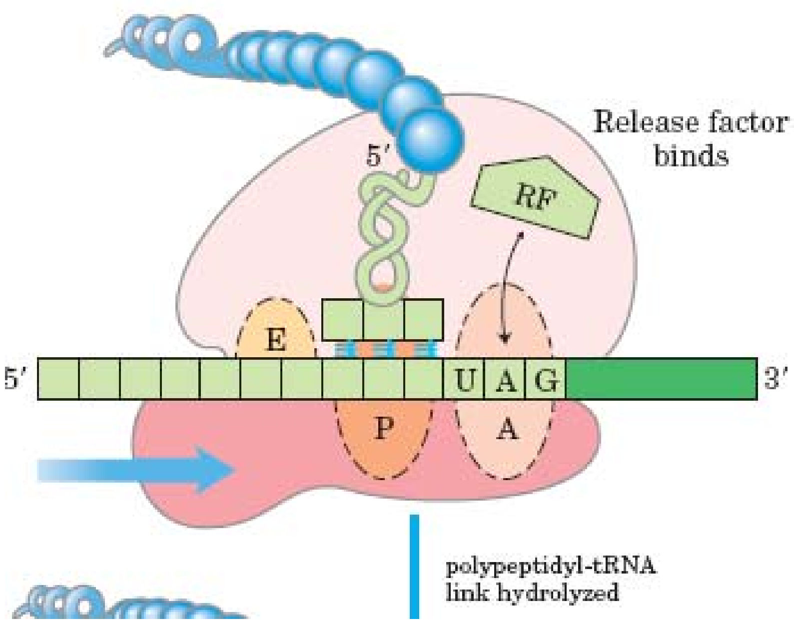
EF-G (translocase)

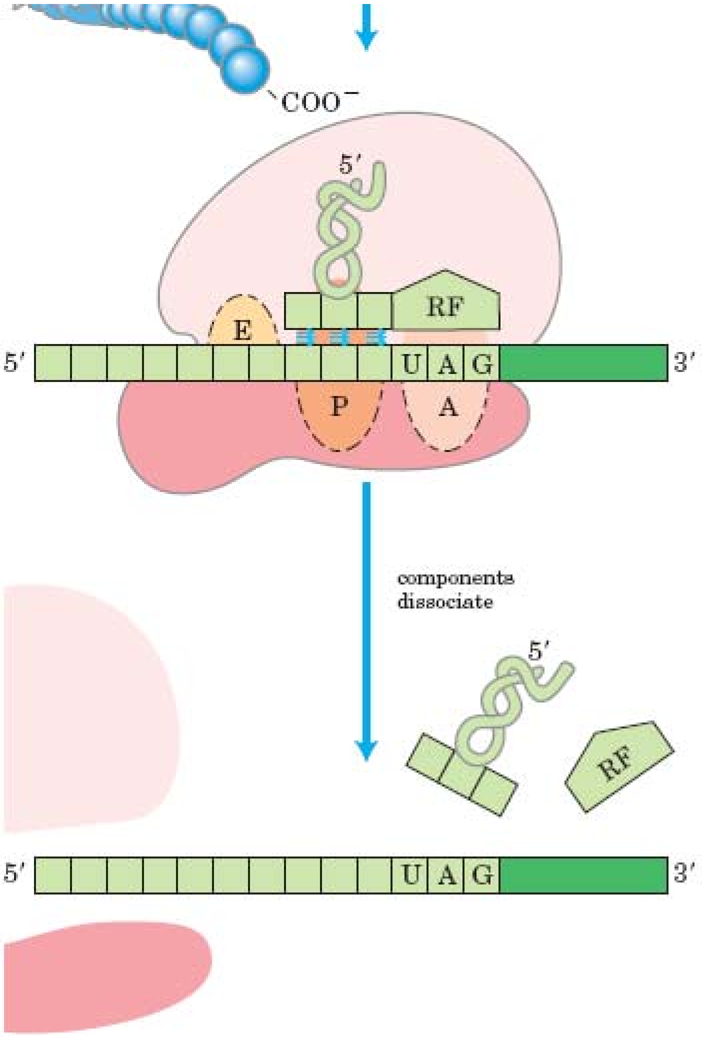


The elongation cycle in eukaryotes

- The elongation cycle in eukaryotes is quite similar to that in prokaryotes.
- Three eukaryotic elongation factors (eEF1, eEF1, and eEF2) have functions analogous to those of the bacterial elongation factors (EF-Tu, EF-Ts, and EF-G, respectively).
- Eukaryotic ribosomes do not have an E site; uncharged tRNAs are expelled directly from the P site.

Stage 4: Termination of Polypeptide Synthesis Requires a Special Signal





(1) hydrolysis of the terminal peptidyltRNA bond
(2) release of the free polypeptide and the last tRNA, now uncharged, from the P site;
(3) dissociation of the 70S ribosome into its 30S and 50S subunits, ready to start a new cycle of polypeptide synthesis

Terminator factors

- **In bacteria → three termination factors, or release factors (RF-1, RF-2, and RF-3)**
  - RF-1 recognizes the termination codons UAG and UAA
  - RF-2 recognizes UGA and UAA
  - RF-3 → release the ribosomal subunit
- **In eukaryotes, a single release factor, eRF, recognizes all three termination codons.**