

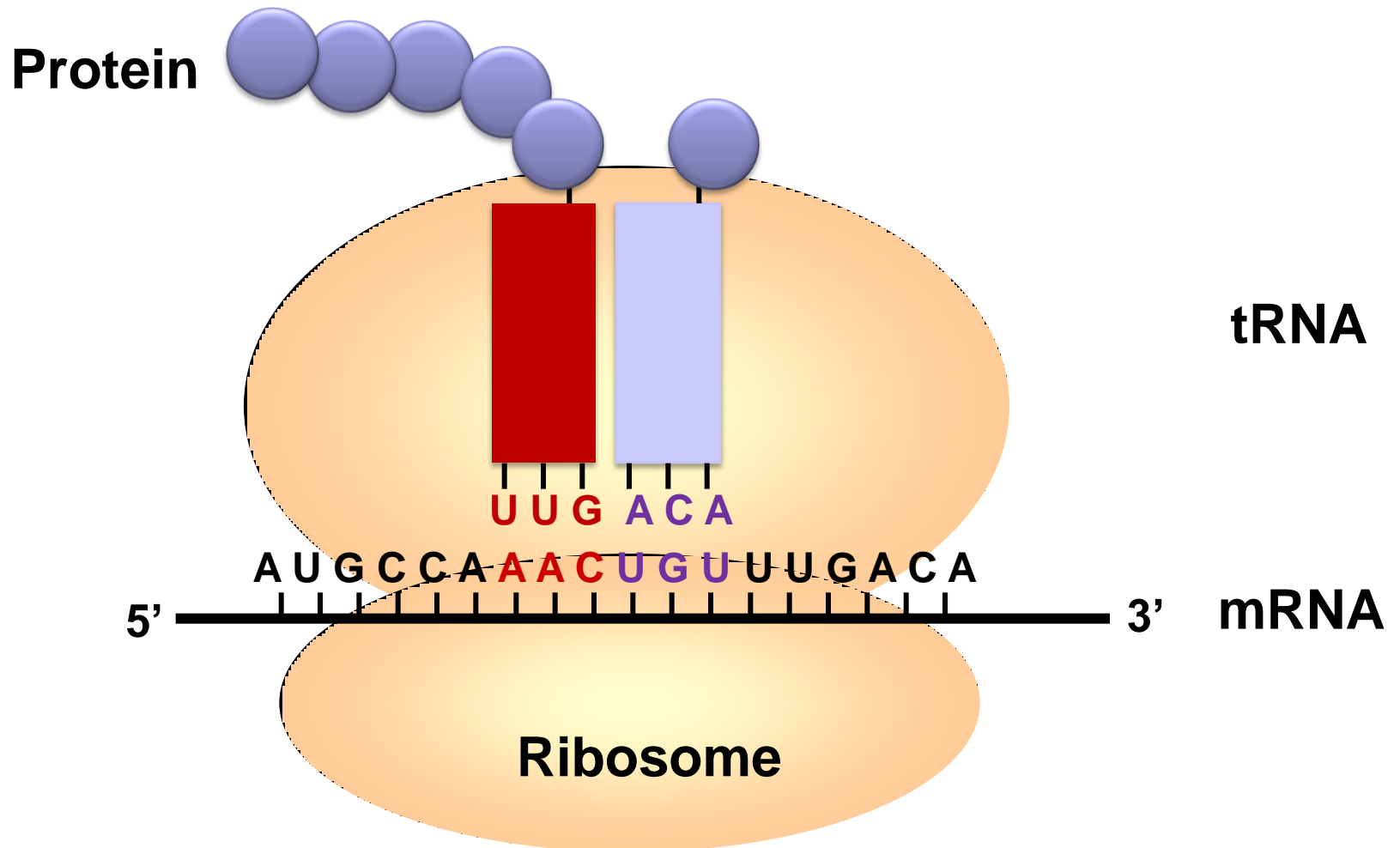
# Chapter 3. DNA, RNA, and Protein Synthesis



# 6. Translation



# Translation: From mRNA to Protein



# Genetic Code: 3 nt to 1 amino acid

5' AUG CGC AUC UAG UCG 3'

- Codon
- Reading Frame

20 amino acids in nature

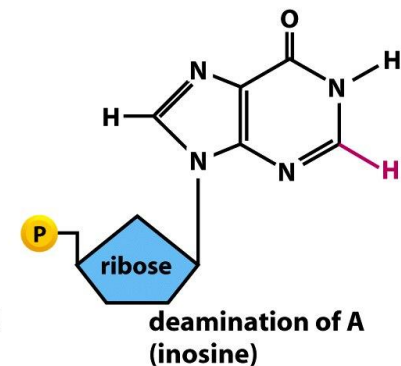
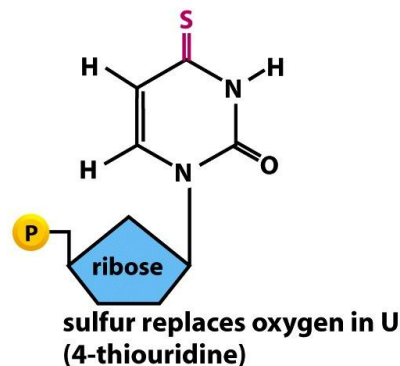
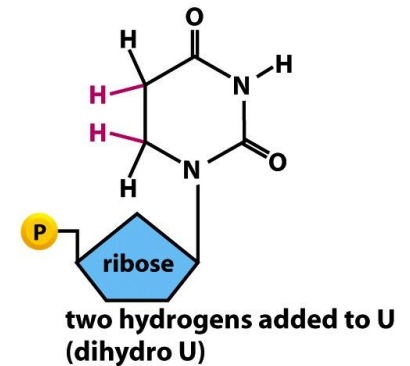
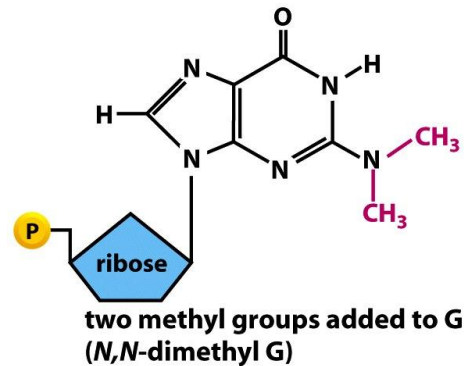
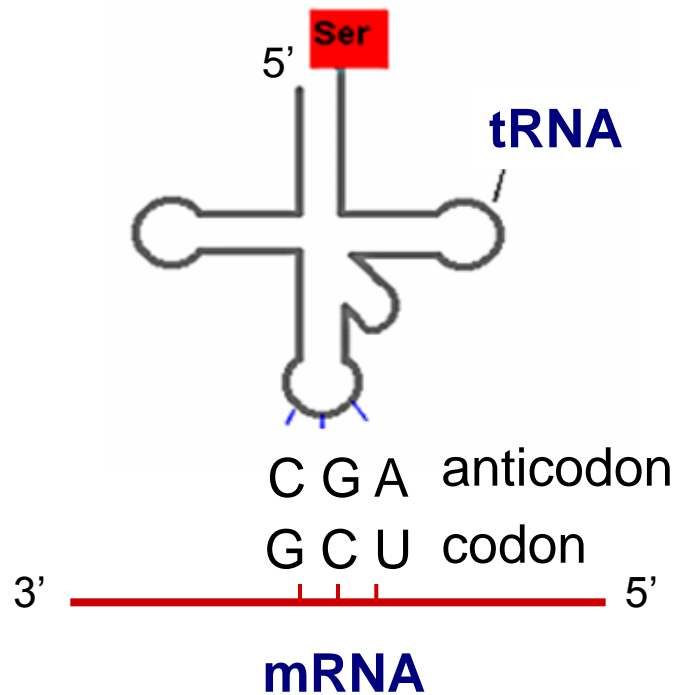
Codon (nt)	Amino acid
1	4
2	16
3	64

# Genetic Code: 64 Codons

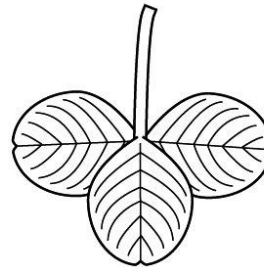
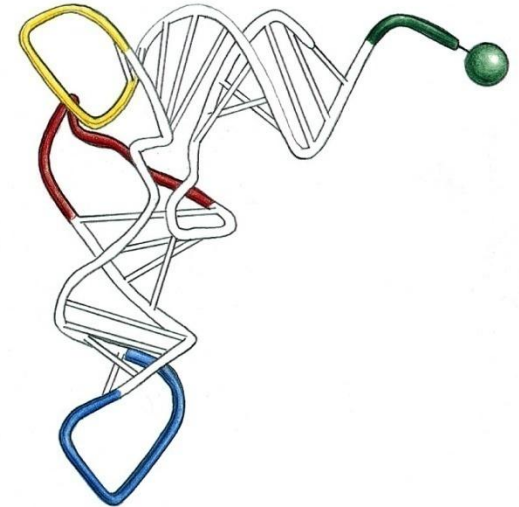
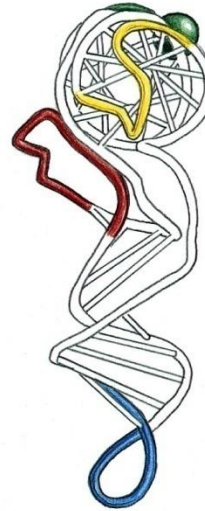
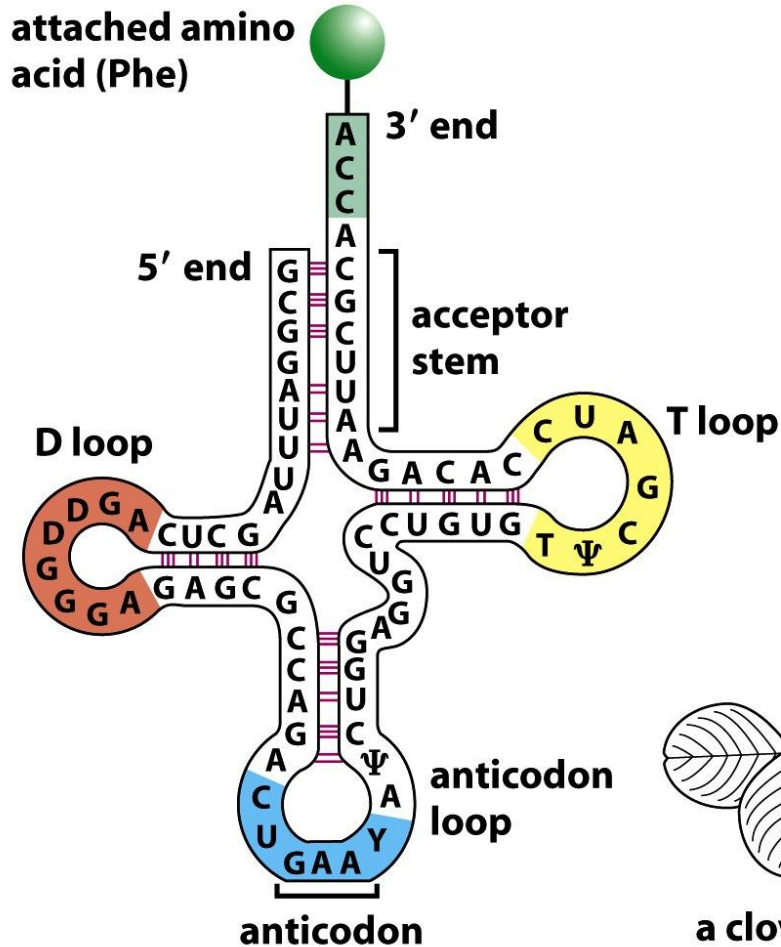
		Second base							
		U	C	A	G				
First base	U	UUU } Phenyl- UUC } alanine UUA } Leucine UUG }	UCU } UCC } Serine UCA } UCG }	UAU } Tyrosine UAC } UAA } Stop codon UAG } Stop codon	UGU } Cysteine UGC } UGA } Stop codon UGG } Tryptophan	U			
	C	CUU } Leucine CUC } CUA } CUG }	CCU } Proline CCC } CCA } CCG }	CAU } Histidine CAC } CAA } Glutamine CAG }	CGU } Arginine CGC } CGA } CGG }	C			
	A	AUU } Isoleucine AUC } AUA } AUG } Methionine start codon	ACU } Threonine ACC } ACA } ACG }	AAU } Asparagine AAC } AAA } Lysine AAG }	AGU } Serine AGC } AGA } Arginine AGG }	A			
	G	GUU } Valine GUC } GUA } GUG }	GCU } Alanine GCC } GCA } GCG }	GAU } Aspartic GAC } acid GAA } Glutamic GAG } acid	GGU } Glycine GGC } GGA } GGG }	G			
					U	C	A	G	Third base

# Structure of tRNA: transfer RNA

- 73 to 93 nt
- Unusual modified bases



# Structure of tRNA



a clover leaf

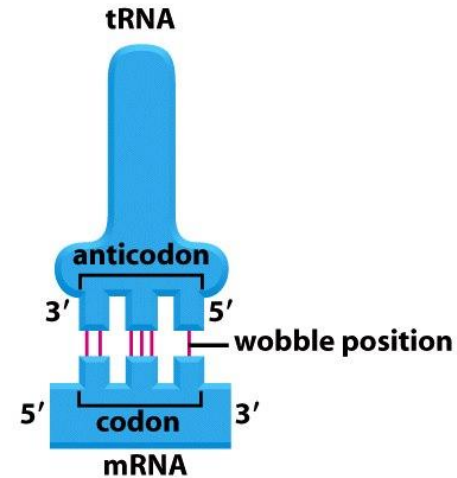
Y: pyrimidine nucleotide

D: dihydrouridylate

Ψ: pseudouridylate

# Wobble Hypothesis

Nonstandard base-pairing between the first base of anticodon and the third base of codon



## bacteria

wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U or I
G	C or U

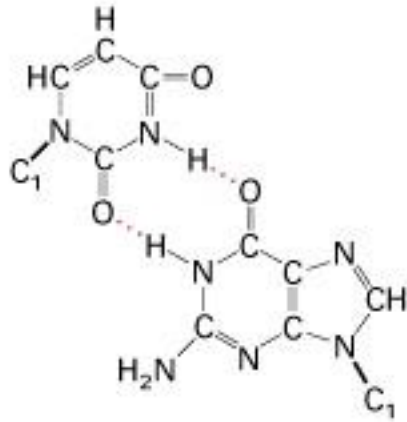
## eucaryotes

wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U
G	C



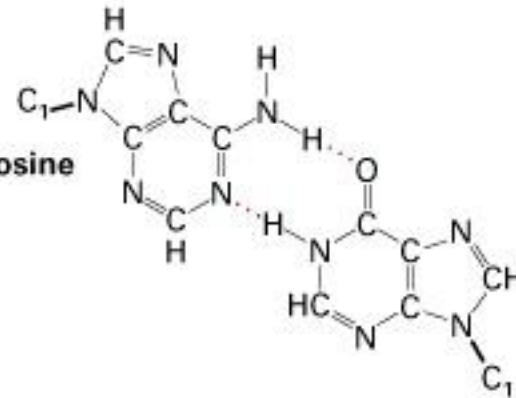
# Wobble Base-pairing

Uridine



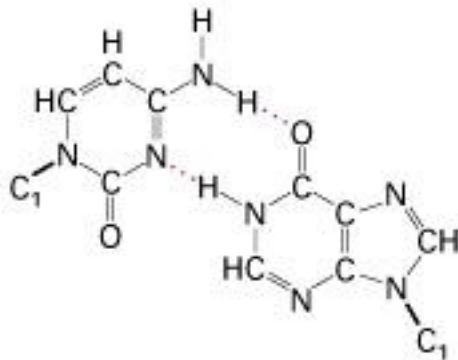
Guanine

Adenosine



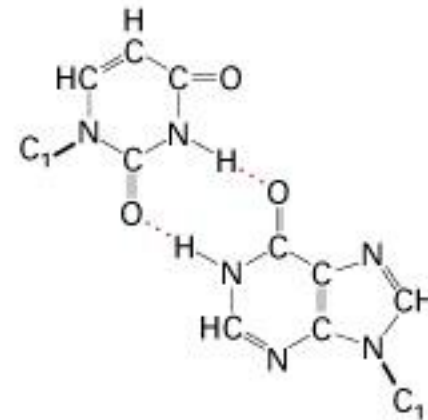
Inosine

Cytidine



Inosine

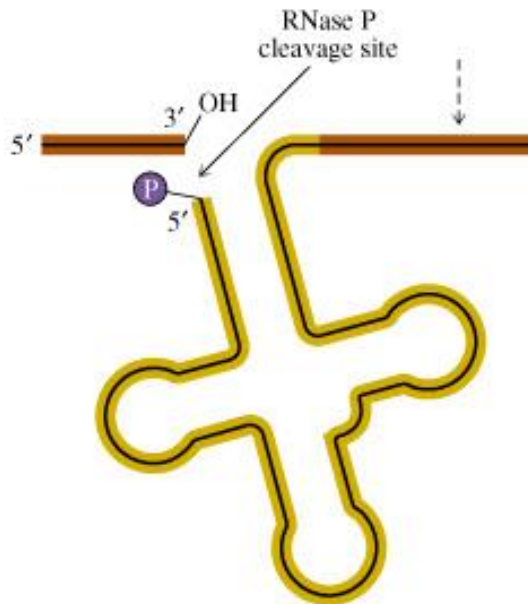
Uridine



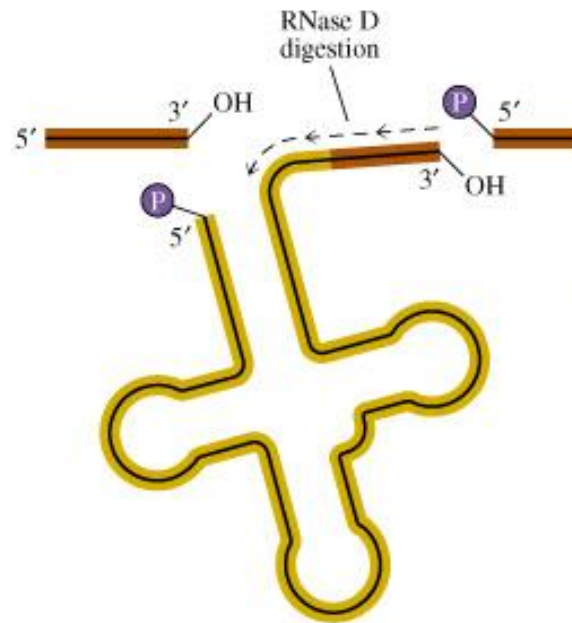
Inosine

# Prokaryotic tRNA processing

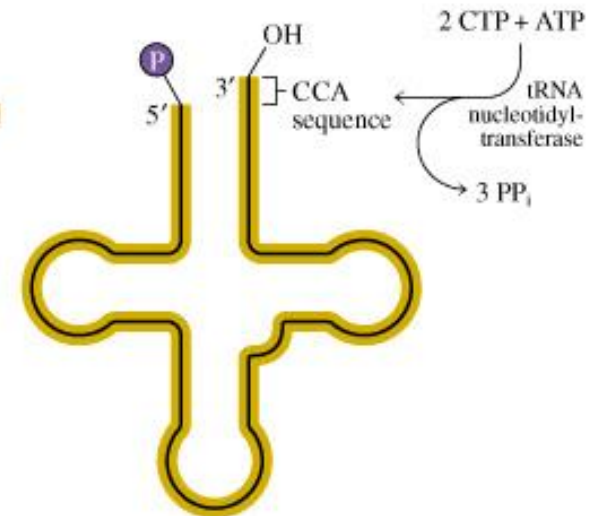
(a) RNase P and other endonucleases cleave the primary transcript.



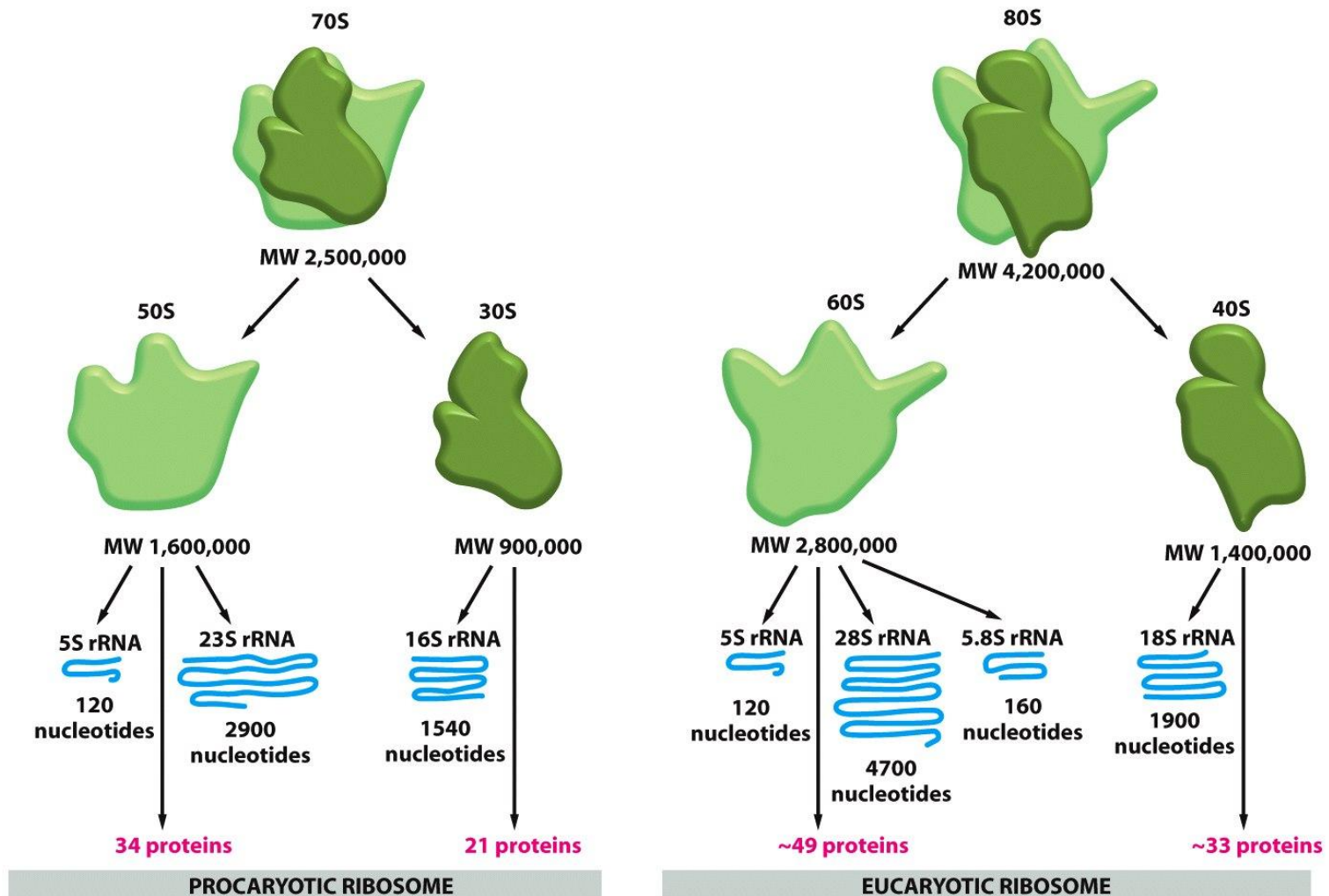
(b) RNase D trims the 5' end.



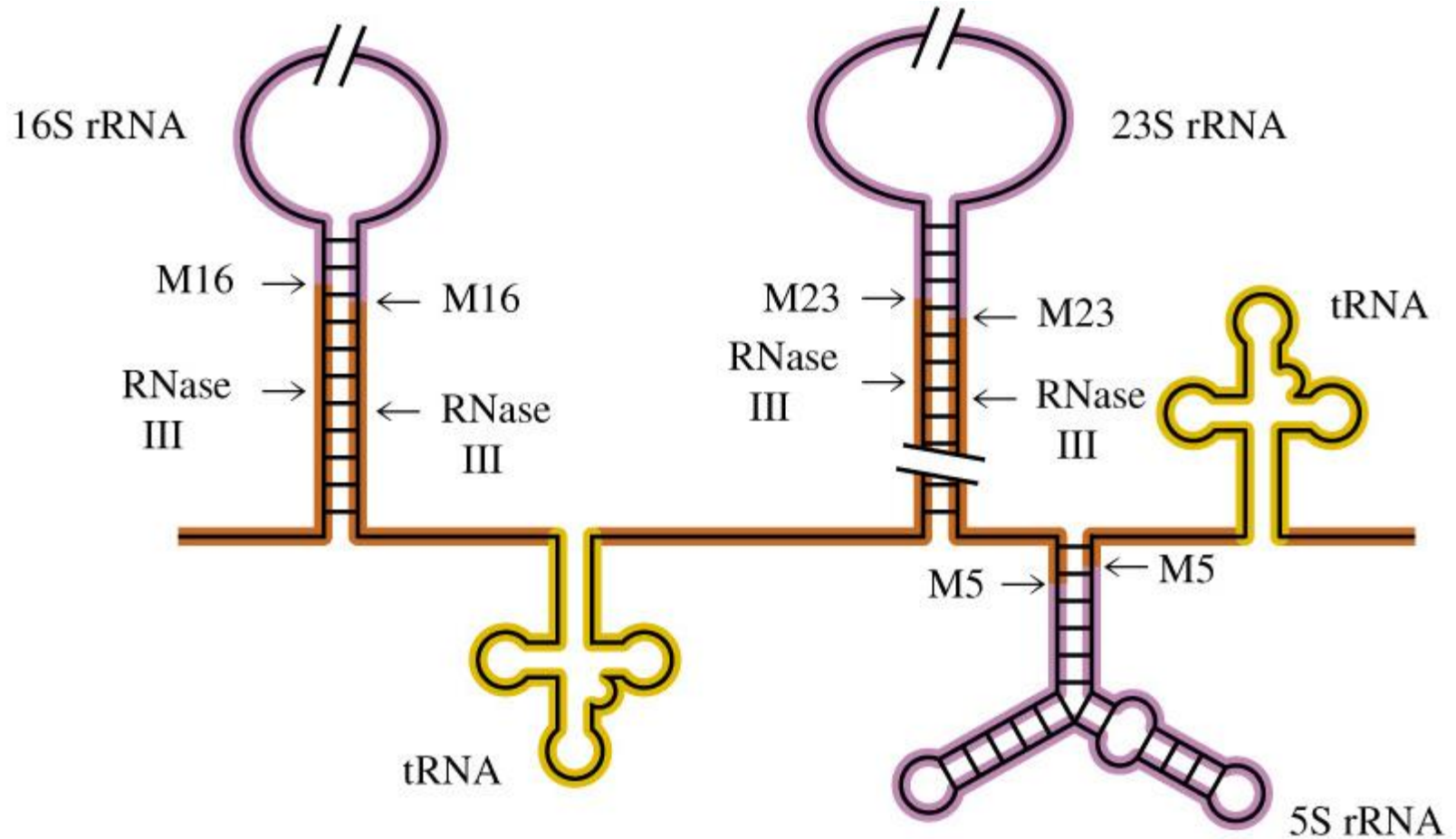
(c) tRNA nucleotidyl transferase adds CCA to the 3' end.



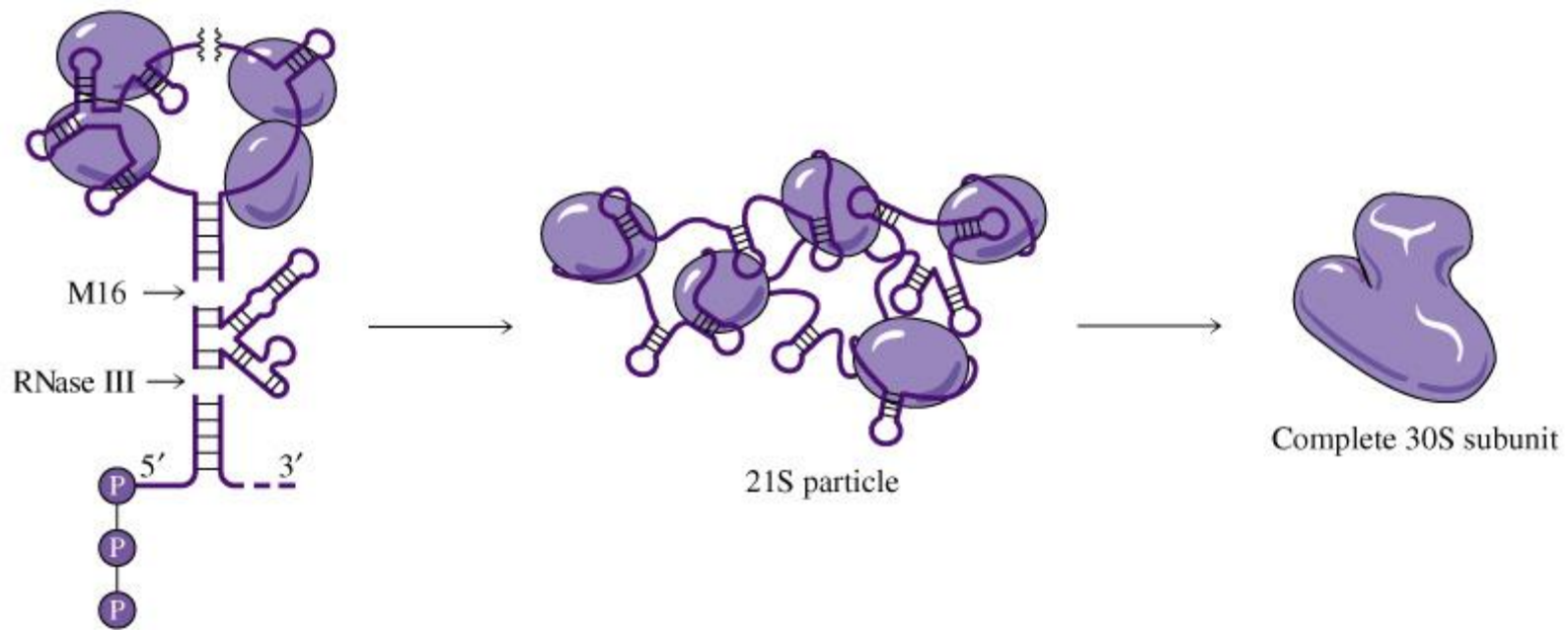
# Ribosome: The site of protein synthesis



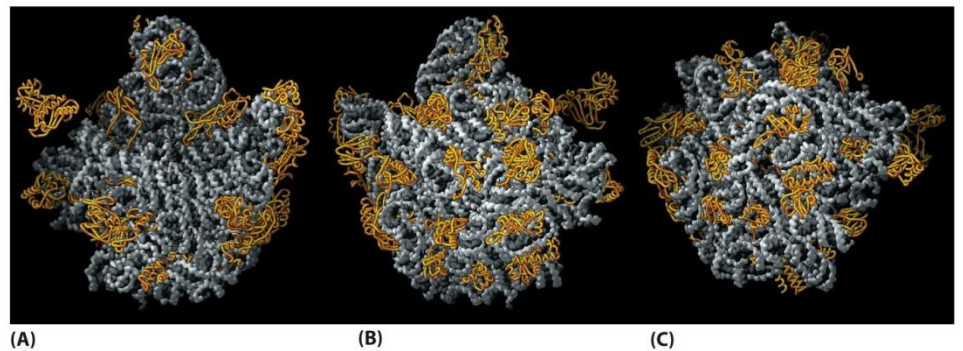
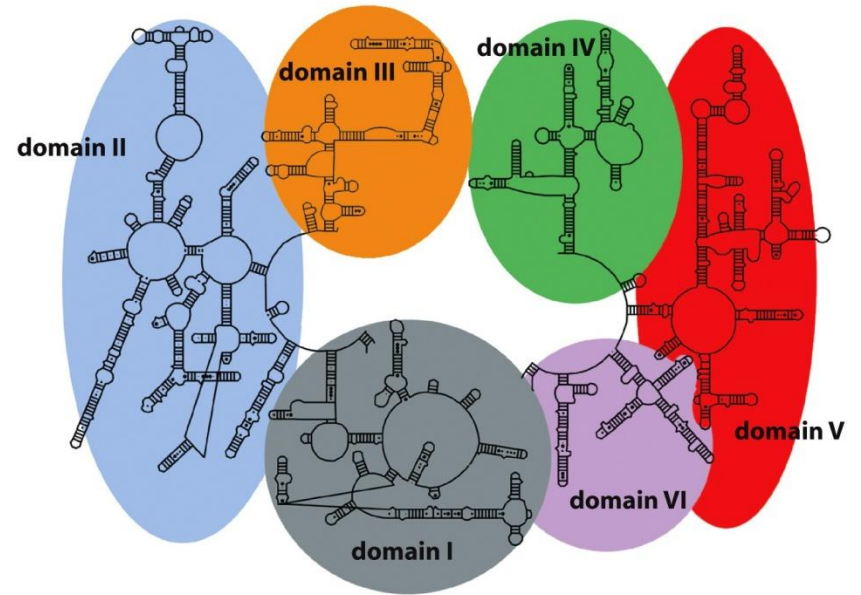
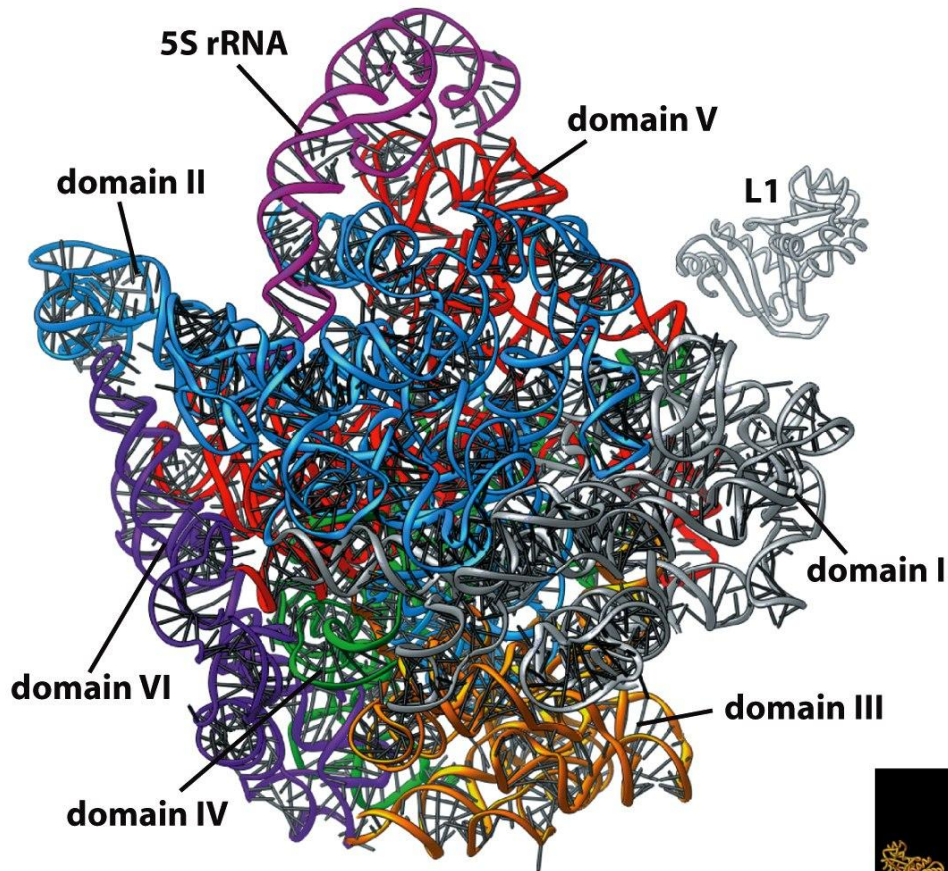
# Processing of *E.coli* rRNA



# Assembly of 30S ribosomal subunit in *E.coli*



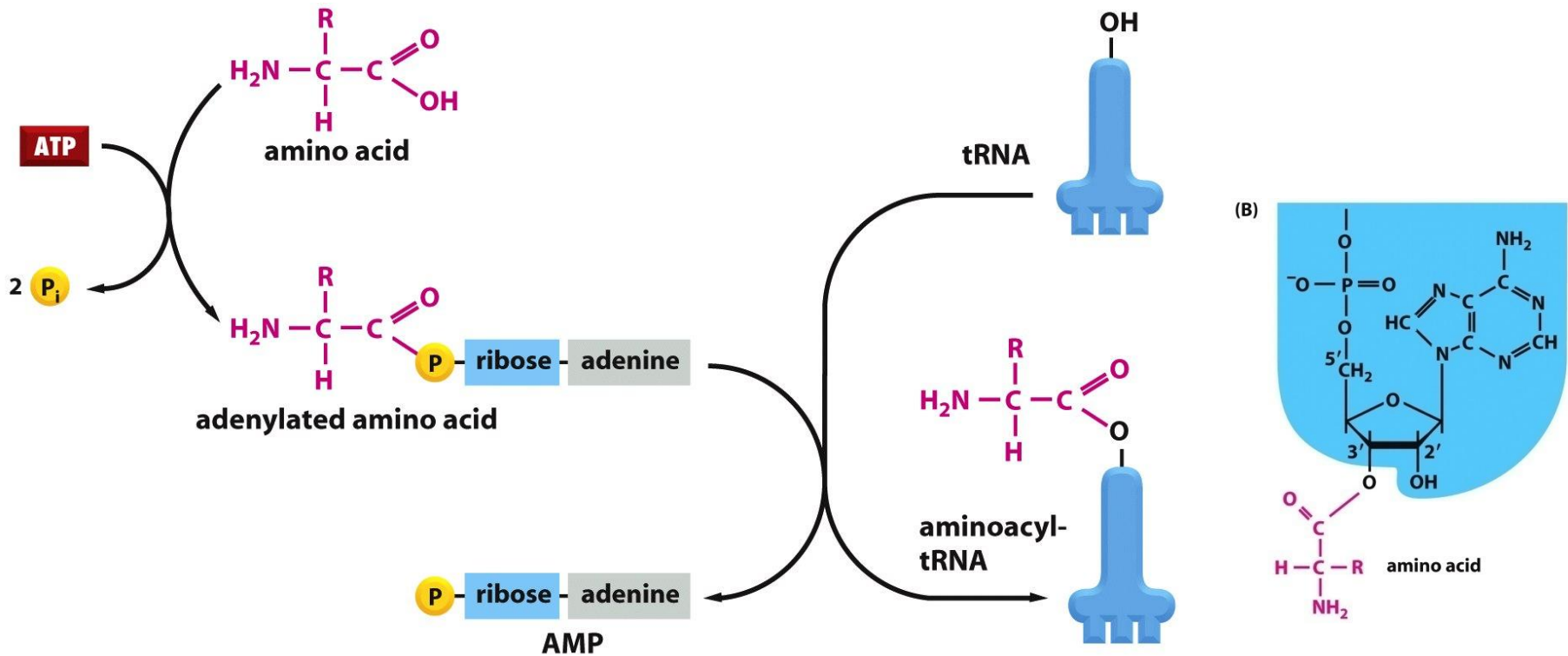
# Structure of 50S Subunit



# Four Steps in Translation

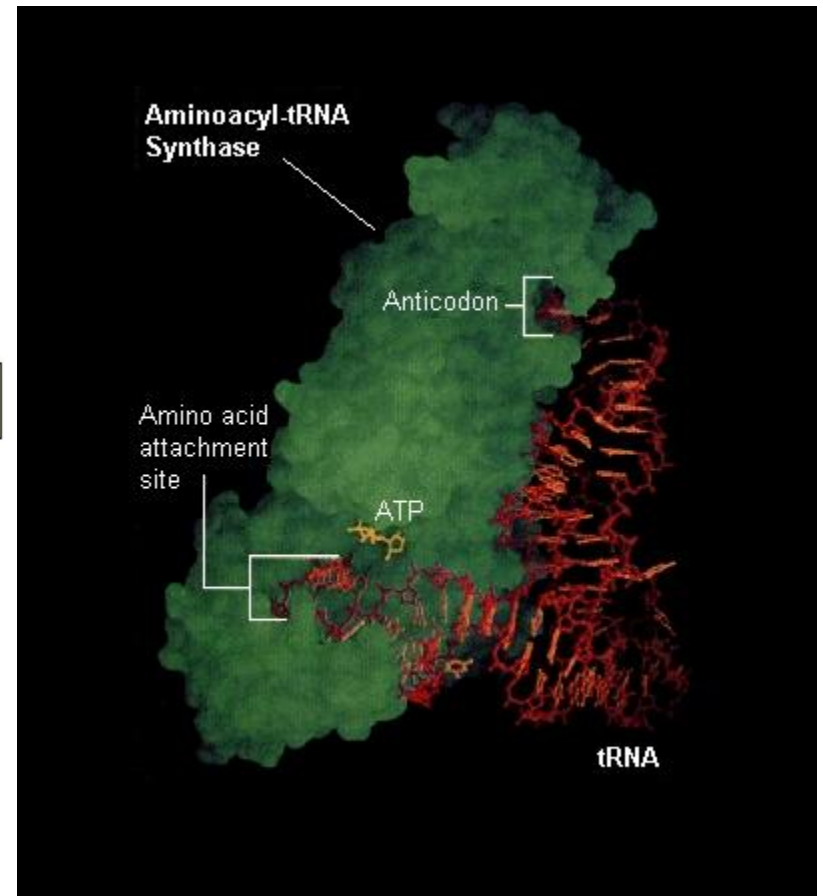
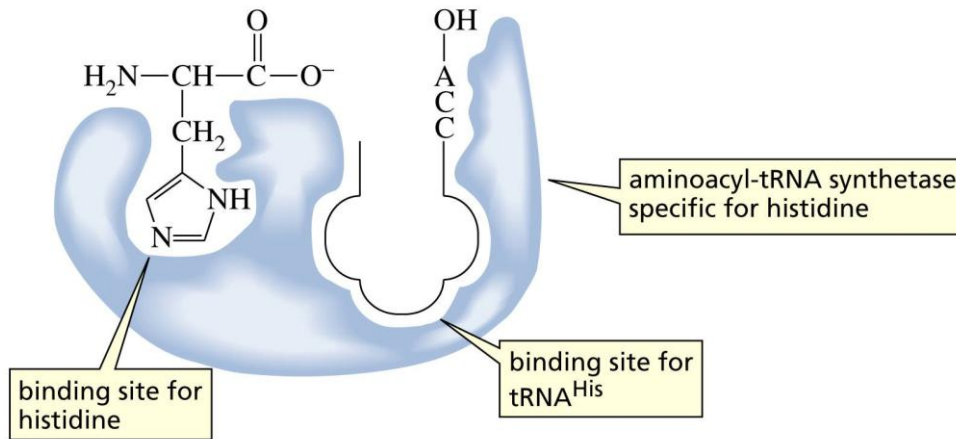
- 1. Activation
  - Adding an amino acid to tRNA (aminoacyl-tRNA)
  
- 2. Initiation
  - Assembling players : ribosome, mRNA, aa-tRNA
  
- 3. Elongation
  - Adding new aa's
  
- 4. Termination
  - Stopping the process

# Activation of Amino Acid





# Structure of Aminoacyl tRNA Synthase



# Translational Initiation

## ■ Prokaryote

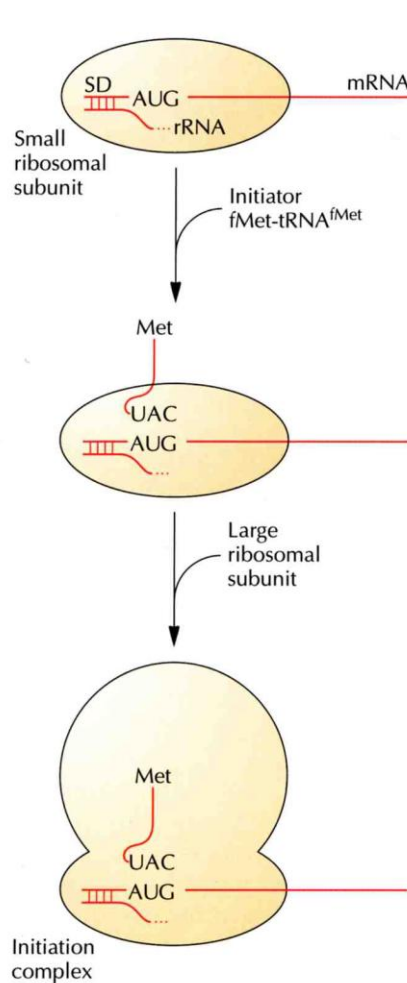
- Binding of small ribosomal subunit to mRNA by Shine-Dalgarno (SD) sequence
- Binding of initiator fMet-tRNA<sup>fMet</sup>
  - N-formylmethionine
  - Release of formyl group (deformylase) after protein synthesis

## ■ Eukaryote

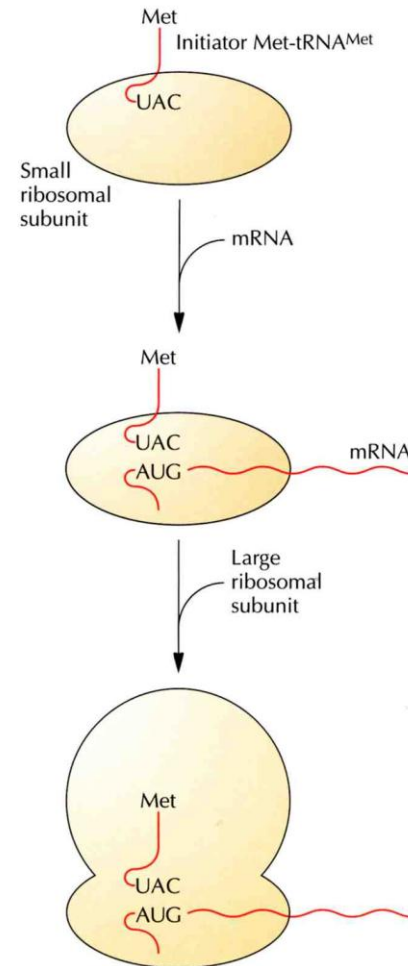
- Assembly of small ribosomal subunit and initiator Met-tRNA<sup>Met</sup>
- Scanning along the mRNA until the complex encounter AUG start codon

# Translational Initiation

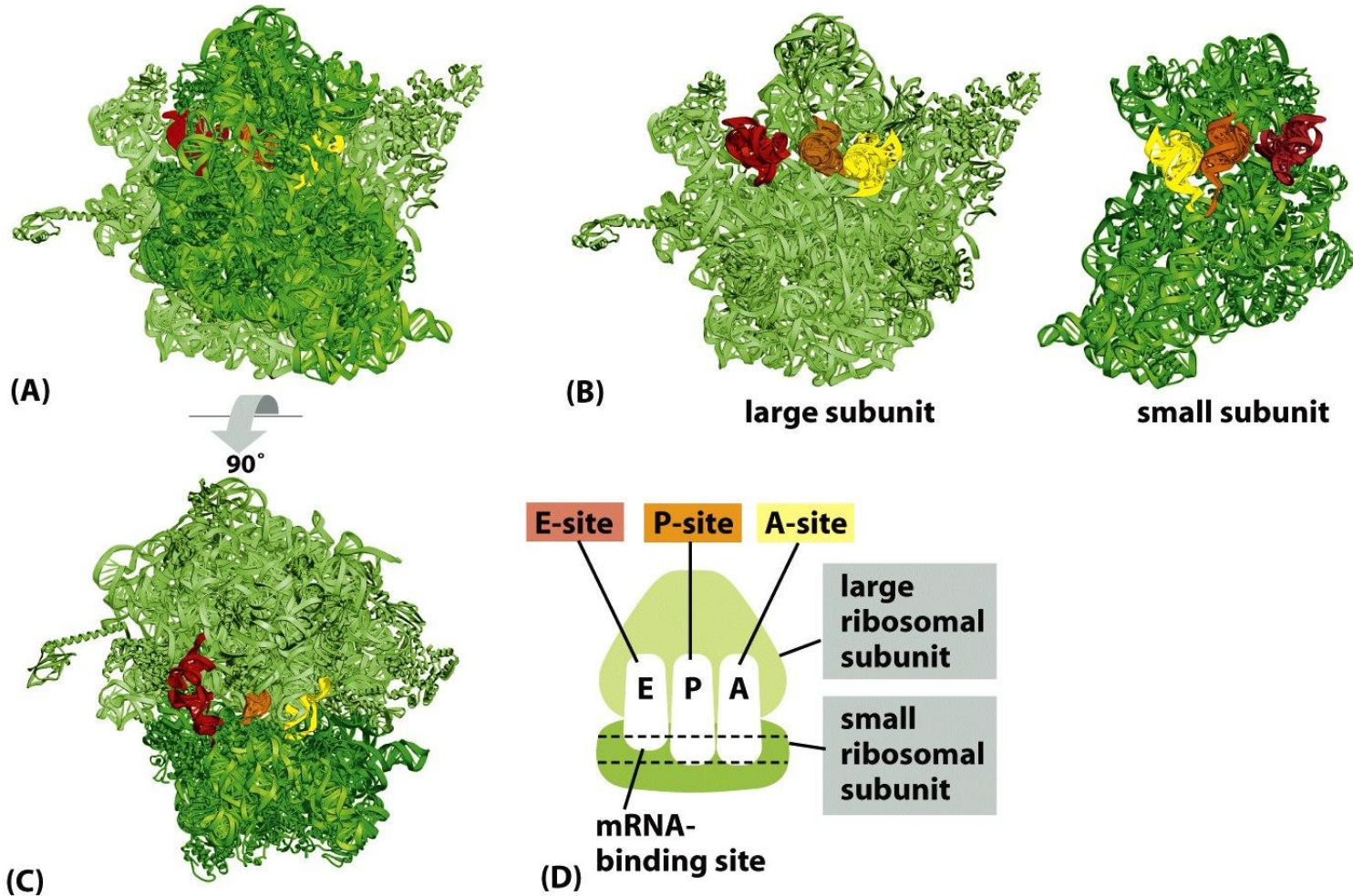
## Prokaryote



## Eukaryote

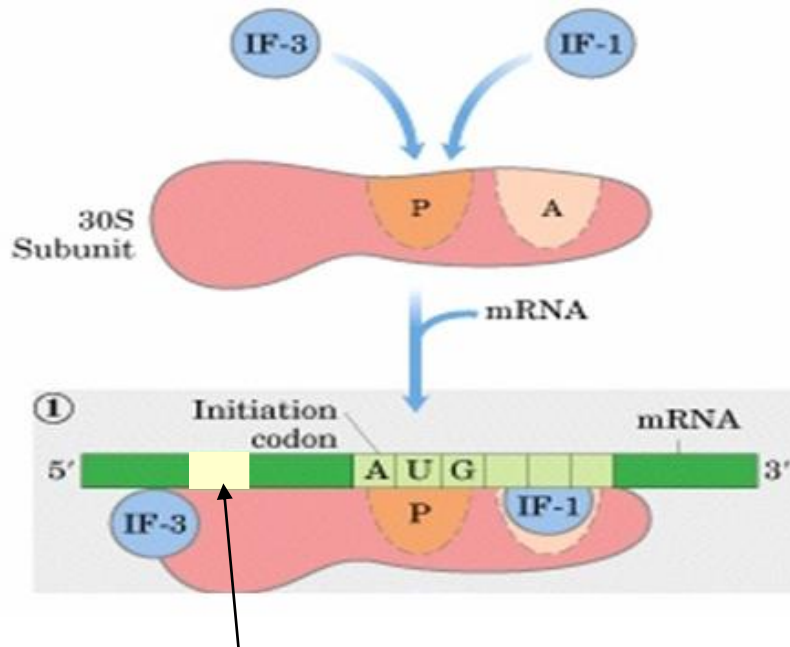


# RNA Binding Sites in the Ribosome



# Initiation in Prokaryote (1)

## Assembly of 30S Ribosomal Subunits with mRNA



- SD sequence

- **IF-3** prevents binding of the 50S subunit

- **Shine-Dalgarno sequence** in the mRNA guides AUG to correct position

- **tRNA Binding Sites**

**A site:** Aminoacyl site

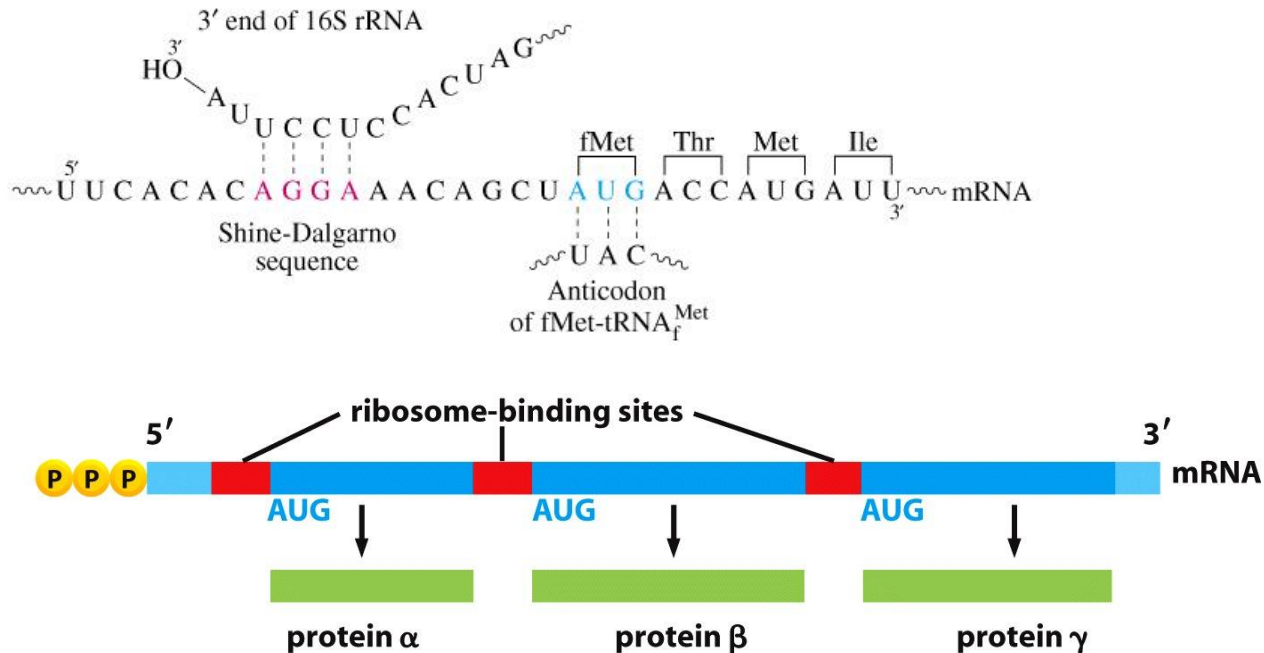
**P site:** Peptidyl site

# Ribosome Binding site: Shine-Dalgarno Sequence

(a)

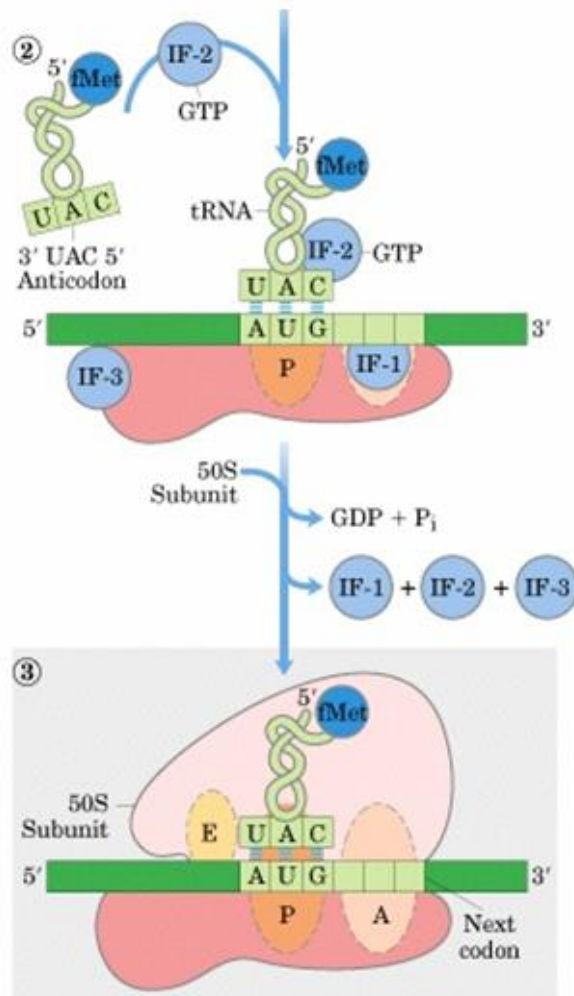
Lipoprotein	~AUCUA <b>GAGG</b> GUAUUAAUA <b>AUG</b> AAAGCUACU~
RecA	~GGCAUGAC <b>GAGG</b> AGUAAAA <b>AUG</b> GCUAUCG~
GalE	~AGCCUAAU <b>GAGG</b> CGAAUU <b>AUG</b> AGAGUUCUG~
GalT	~CCCGAU <b>UAAGGA</b> ACGACCA <b>AUG</b> ACGCAAUUU~
LacI	~CAAUUCAG <b>GUGG</b> UGGUGAAU <b>UGUG</b> AAACCAGUA~
LacZ	~UUCACAC <b>AGGA</b> AACAGCU <b>AUG</b> ACCAUGAUU~
Ribosomal L10	~CAUCA <b>AGGAG</b> CAAAGCUAA <b>AUG</b> GCUUUAUU~
Ribosomal L7/L12	~UAUUC <b>AGGA</b> ACAAUUUAA <b>AUG</b> UCUAUCACU~

(b)



# Initiation in Prokaryote (2)

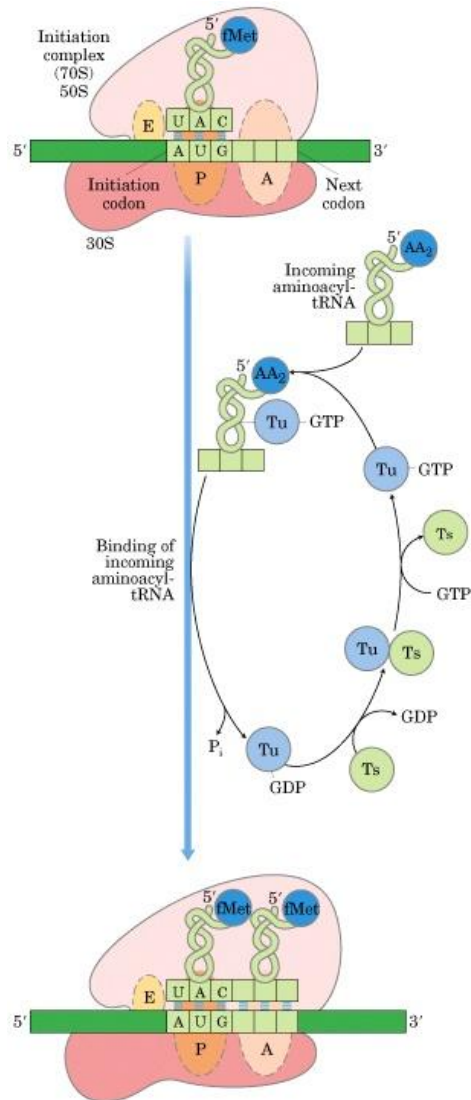
## Assembly of fMet-tRNA<sup>fMet</sup> and 50S Ribosomal Subunit



- Binding of GTP-bound **IF-2** and the initiating **fMet-tRNA<sup>fMet</sup>**
- Assembly of the **50S ribosomal subunit** with simultaneous GTP hydrolysis and release of all initiation factors

# Elongation (1)

## Binding of Incoming aminoacyl tRNA

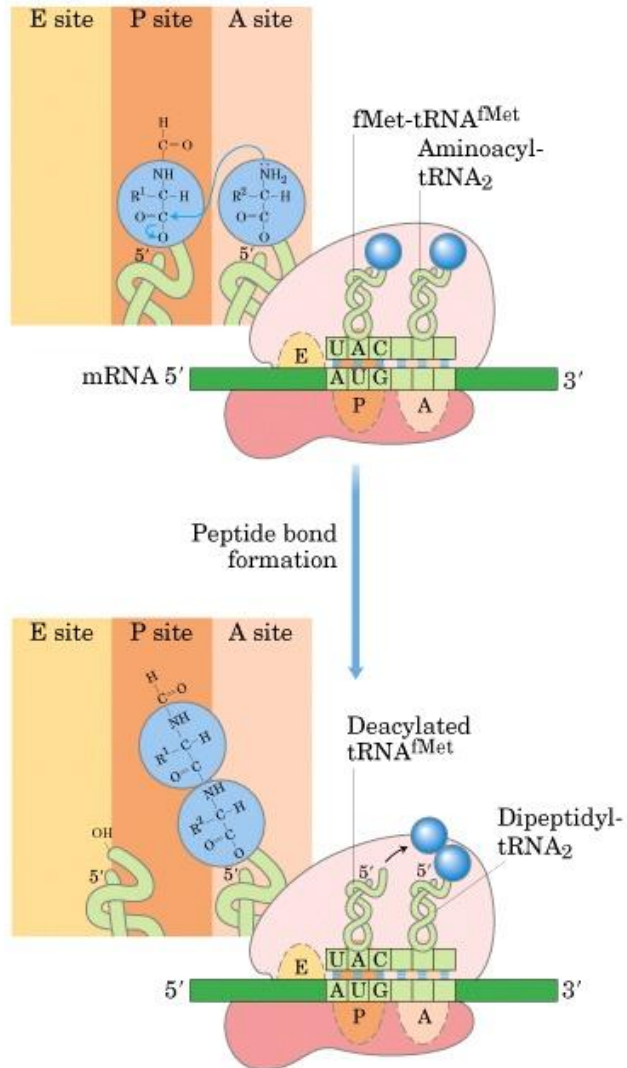


- Binding of aa-tRNA to GTP-bound **EF-Tu** (elongation factor)
- Binding of aa-tRNA to **A site**
- Recycling EF-Tu by **EF-Ts**



# Elongation (2)

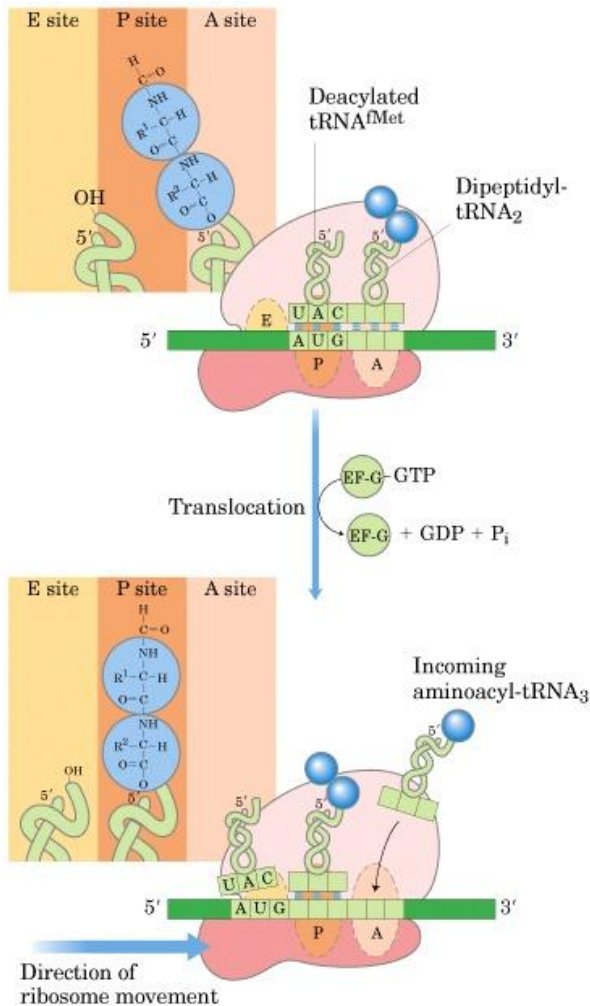
## Peptide Bond Formation



- Transfer of carboxyl group of aa in P site to aa in A site

- **Peptidyl transferase** activity in 50S subunit

# Elongation (3) Translocation

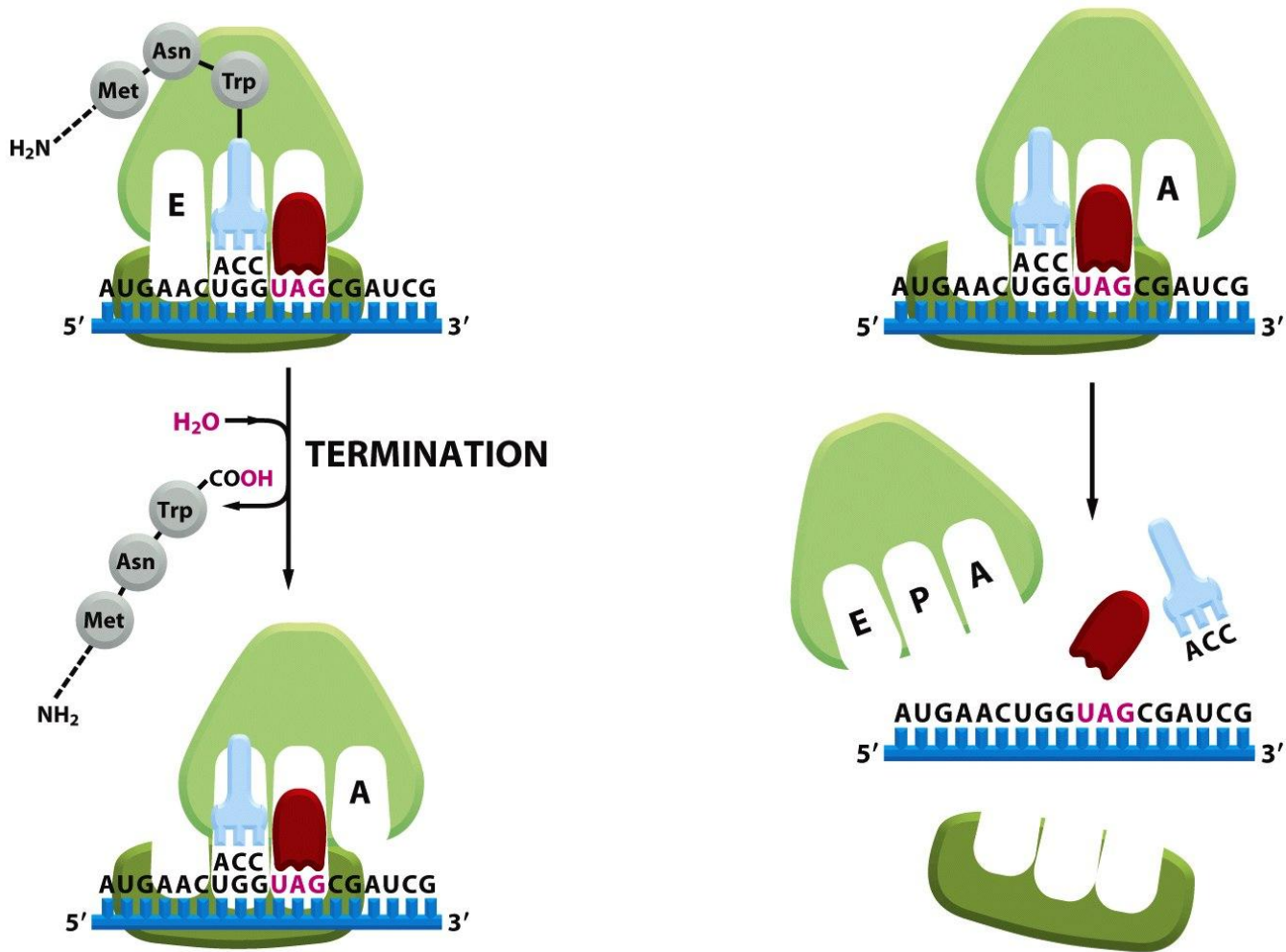


- Translocation of peptidyl-tRNA to P site
- GTP-bound **EF-G**: translocase
- Translocation of mRNA and 30S subunit
- Ejection of empty tRNA

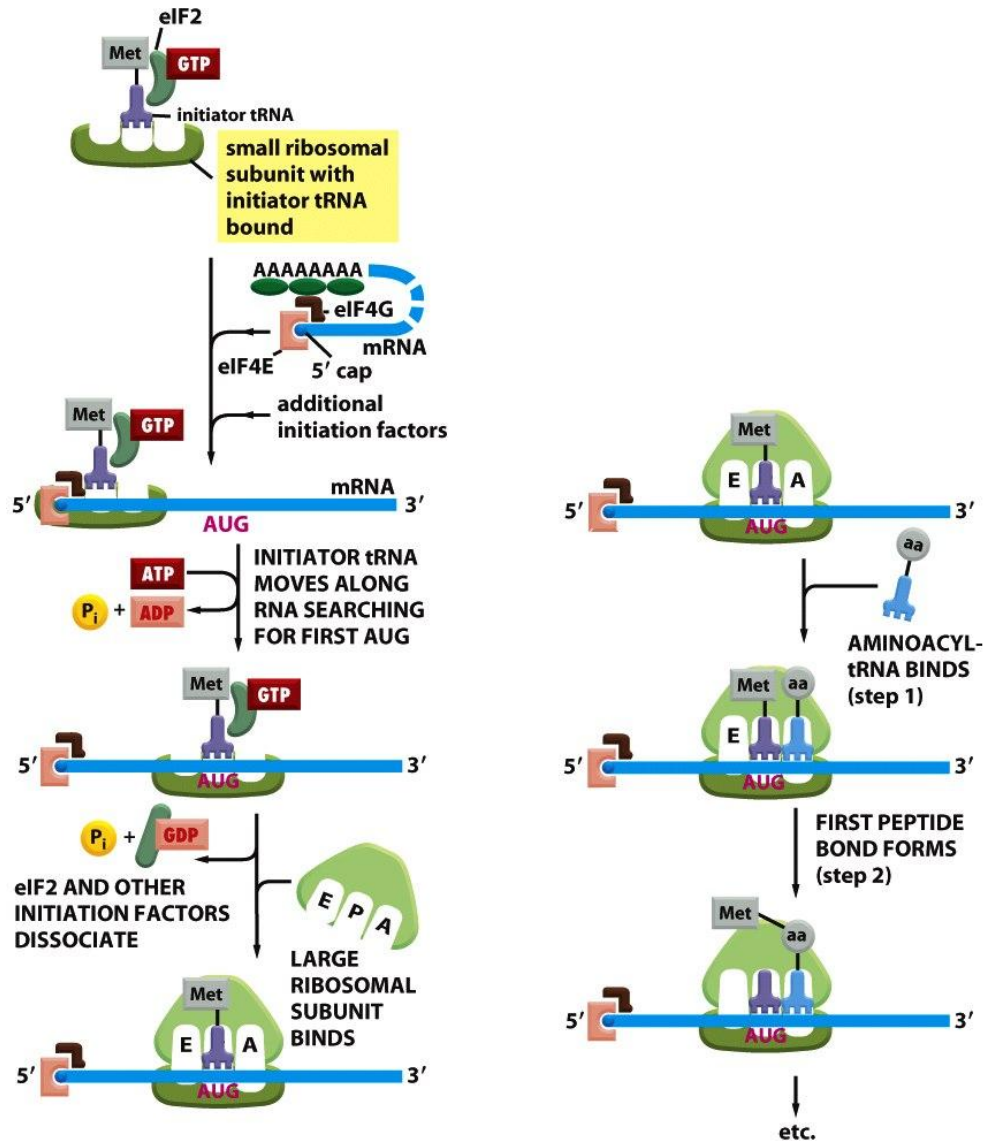
# Termination and Release

- Terminating Codons
  - UAA, UAG, UGA
- Termination or Release Factors
  - Termination codon in A site
  - Release factors (RF1, RF2, & RF3) :
    - Hydrolysis of the terminal peptidyl-tRNA bond
    - Release of the free polypeptide and the last tRNA
    - Dissociation of the 70S ribosome into the 50S and 30S subunits

# Termination and Release



# Translation Initiation in Eukaryotes



# Translation

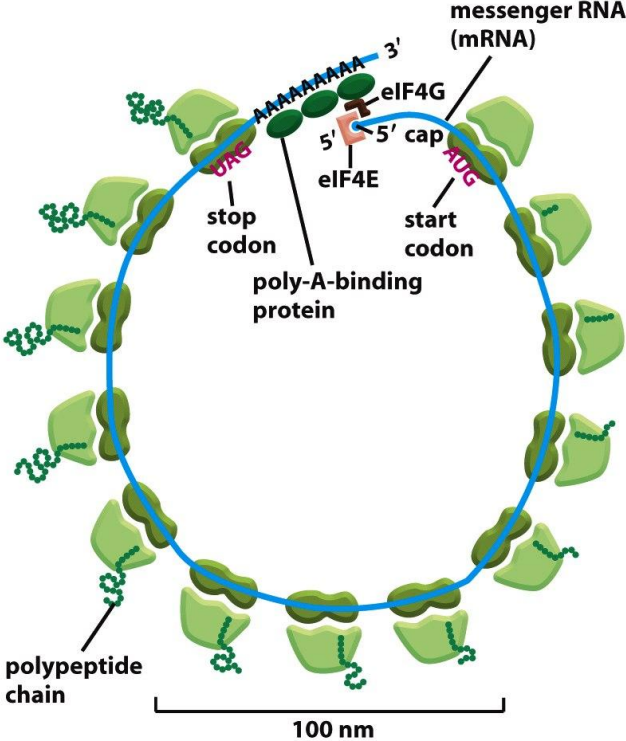
## ■ Energy cost

- **ATP** hydrolysis for the aminoacyl-tRNA formation
- **GTP** hydrolysis during the elongation and translocation step

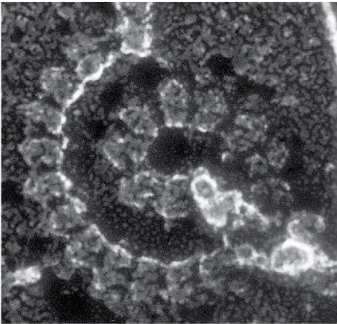
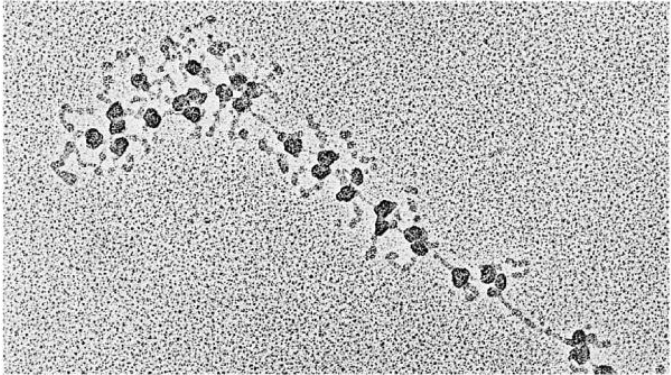
## ■ Translation rate

- 15 amino acids/sec
- Rapidly growing *E. coli*
  - 20,000 ribosomes/cell → 30,000 polypeptides/min
  - Average molecular weight of bacterial protein
    - 1 kb structural gene → 333 amino acids → 35 kDa (105 D/amino acid)

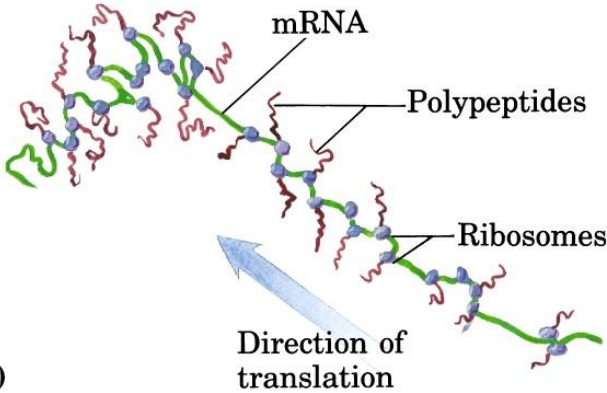
# Polysome: 10 to 100 ribosomes on a single mRNA



(A)



(B)



(b)

# Inhibitors of Bacterial Translation

