

# TRANSLATION

“The Protein Players” - RNA polymerases, transcription factors, initiation factors, enhancers, repressors

**TABLE 6-1 Principal Types of RNAs Produced in Cells**

TYPE OF RNA	FUNCTION
mRNAs	messenger RNAs, code for proteins
rRNAs	ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	small nucleolar RNAs, used to process and chemically modify rRNAs
Other noncoding RNAs	function in diverse cellular processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the ER

**TABLE 6–2 The Three RNA Polymerases in Eucaryotic Cells**

TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes and some snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

Prokaryotes?

[Prokaryotic transcription](#) video

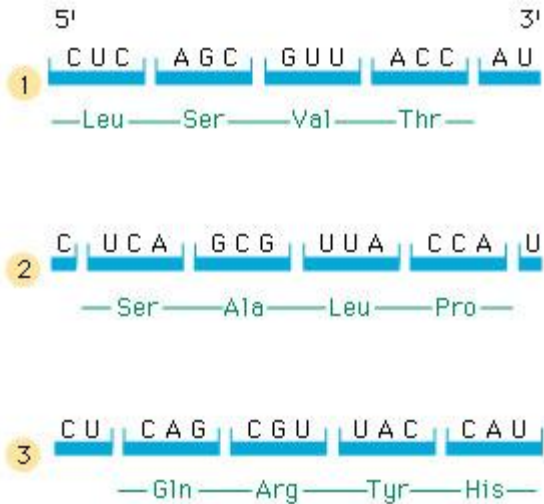
# From RNA to protein: translation

## The genetic code

GCA	AGA																								
	AGG																								
GCC	CGA																								
GCG	CGC																								
GCU	CGU	GAC	AAC	UGC	GAA	CAA	GGA	CAC	AUA	CUA															
	CGG		AAU	UGU	GAG	CAG	GGC	CAU	AUC	CUG	AAA														
	GAU		AAU	UGU	GAG	CAG	GGU	CAU	AUU	CUU	AAG	AUG	UUU	CCA	UCA	ACA									
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop	stop	stop	stop	stop	
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V						

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## Three possible "reading frames"



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THE ABC FOR THE DNA

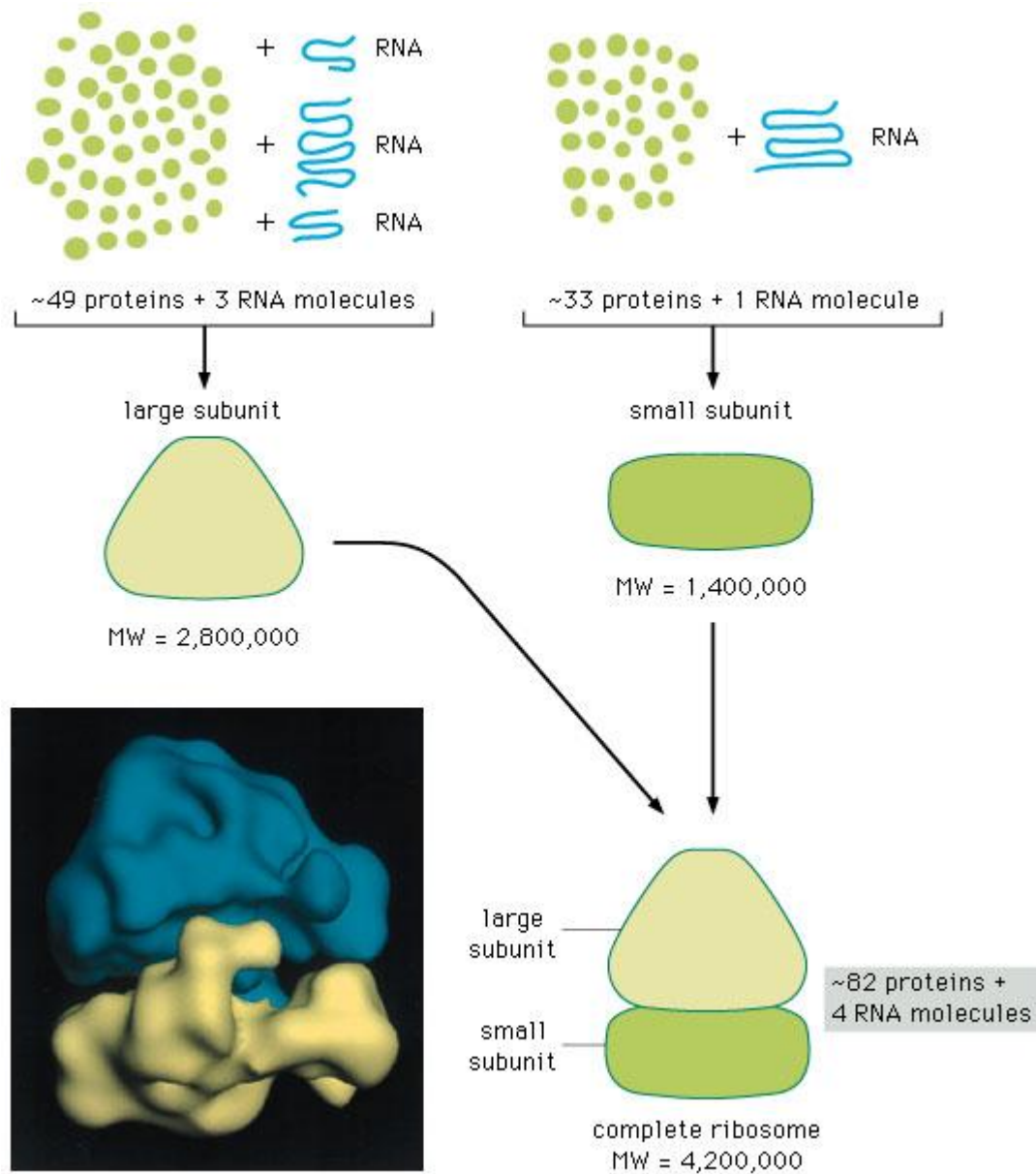
Insertion (X)  
or  
Deletion (B)

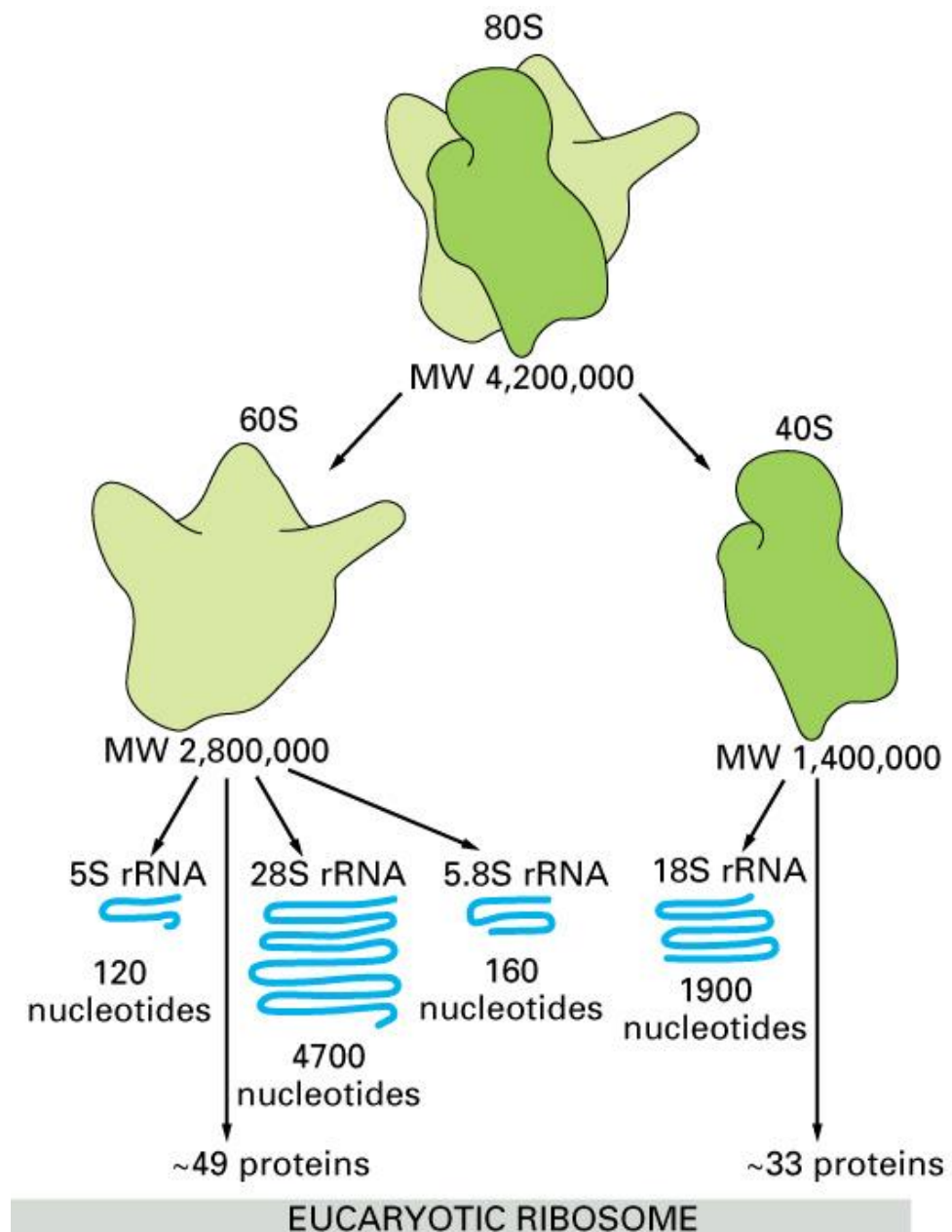
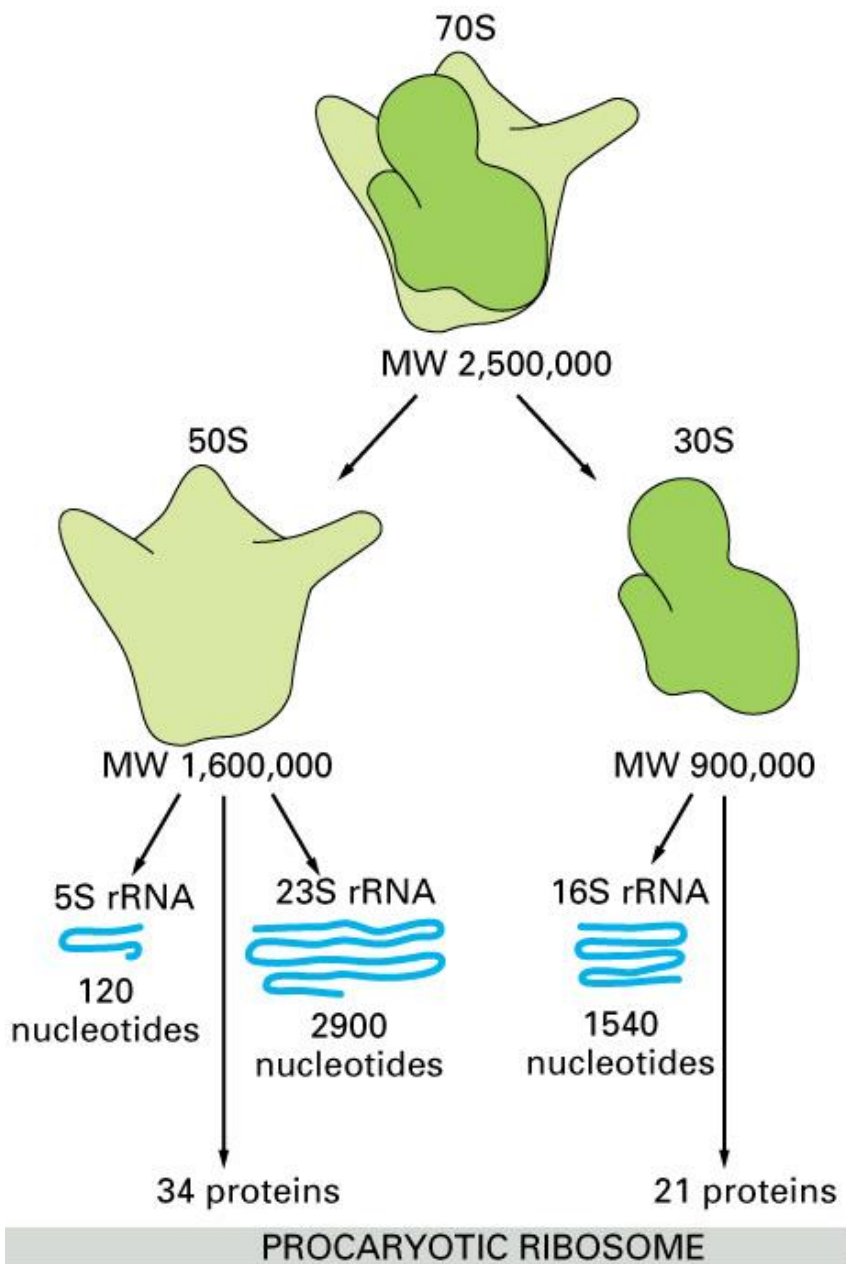


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# Composition of eukaryotic ribosomes

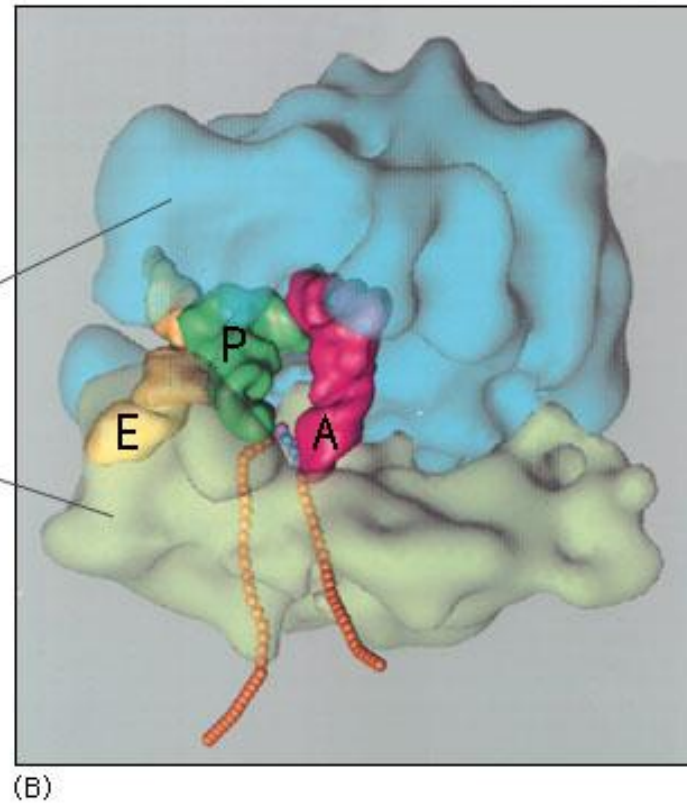
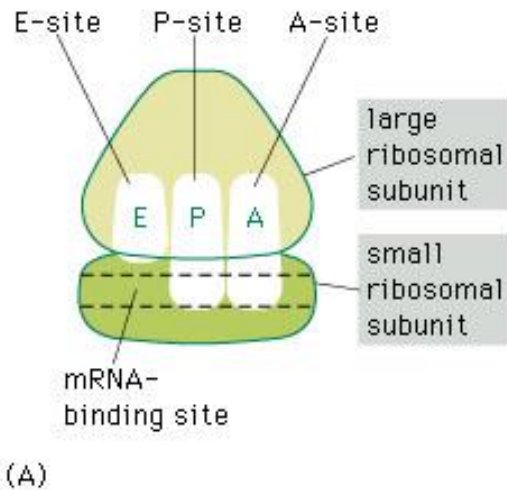




# RNA-binding sites in the ribosome

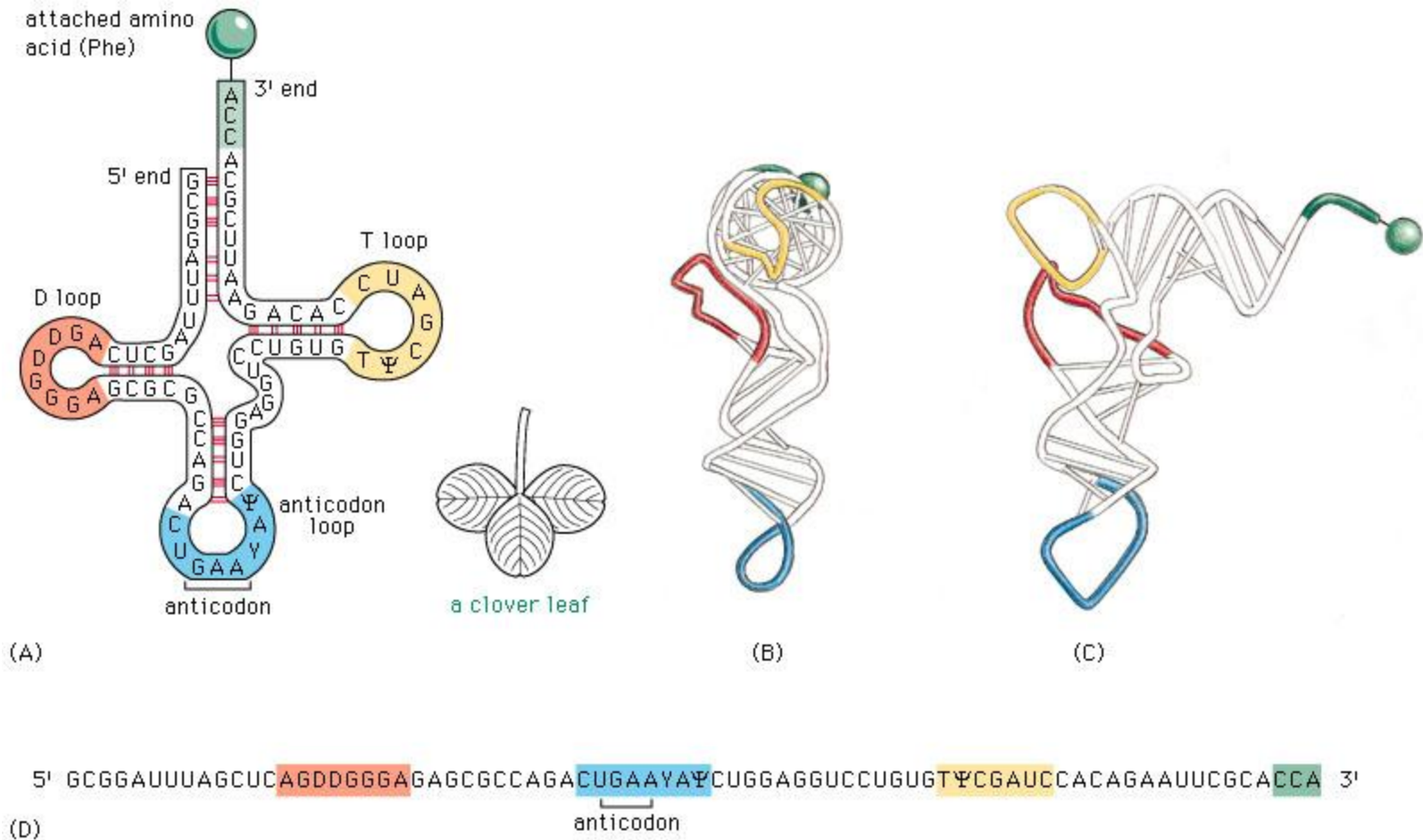
Each ribosome has:

- a binding site for mRNA
- three binding sites for tRNA

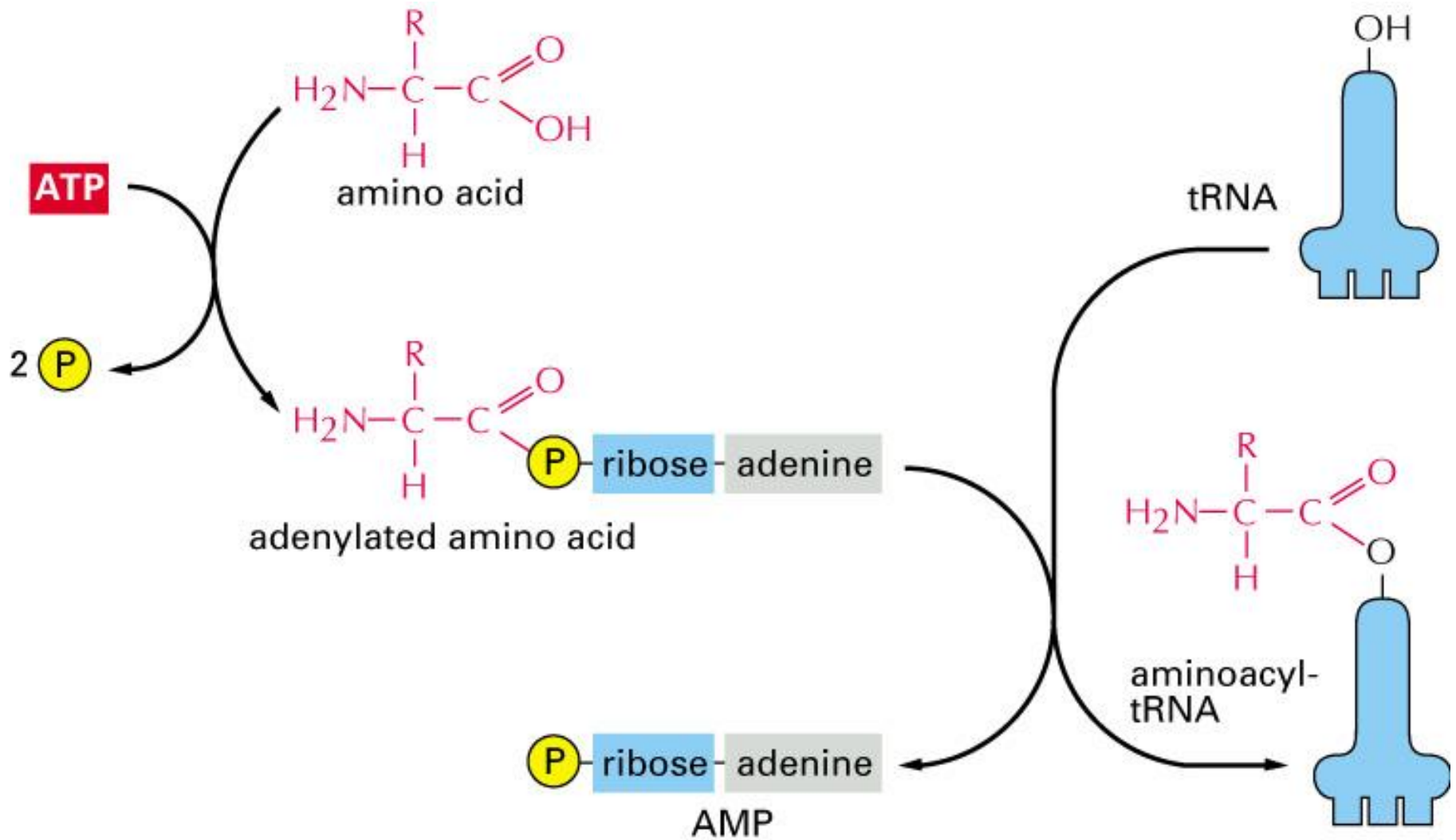


- A-site: aminoacyl-tRNA
- P-site: peptidyl-tRNA
- E-site: exit

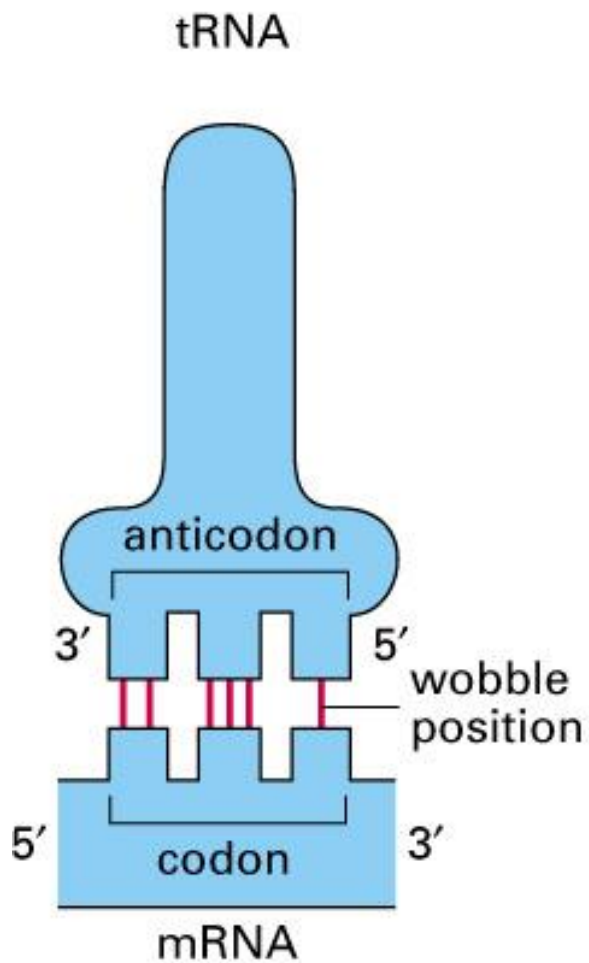
# tRNA molecules: matching amino acids to codons in mRNA







A different aminoacyl-tRNA synthetase enzyme for each amino acid



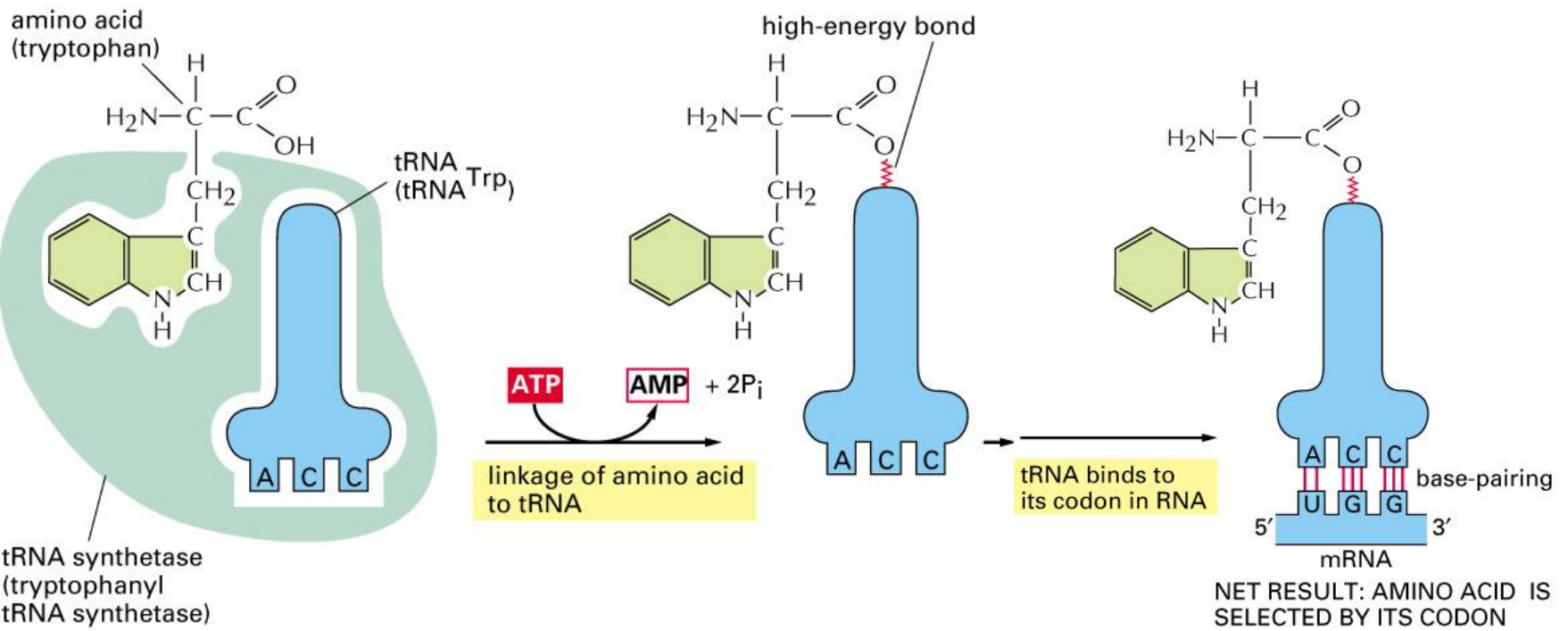
bacteria

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

eucaryotes

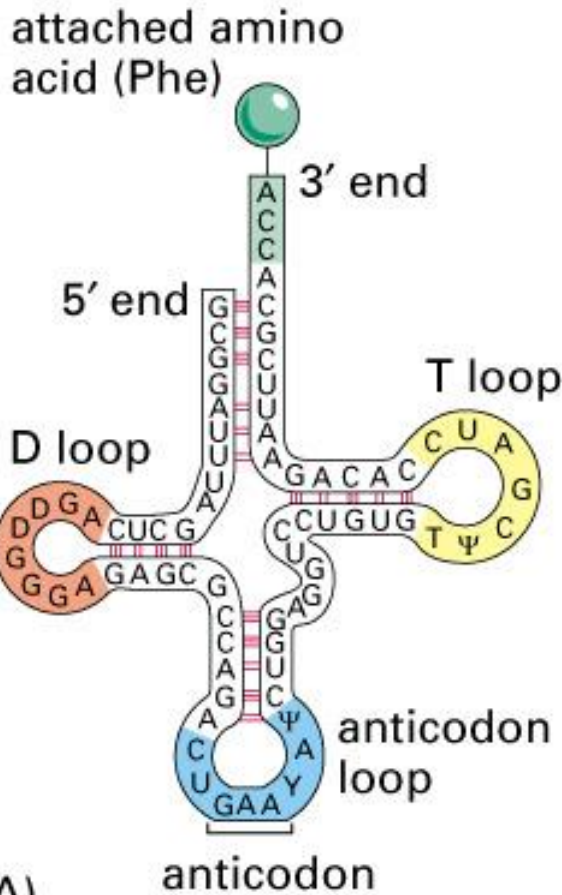
wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

Figure 6-53. Molecular Biology of the Cell, 4th Edition.



# Transfer RNA

- anticodon- 3' to 5' sequence that matches the complementary 5' to 3' sequence (codon) on the mRNA
- Acceptor arm - Amino acid code on 3' end
- T and D loops – provide structure for interface with aminoacyl-tRNA synthetase



(A)

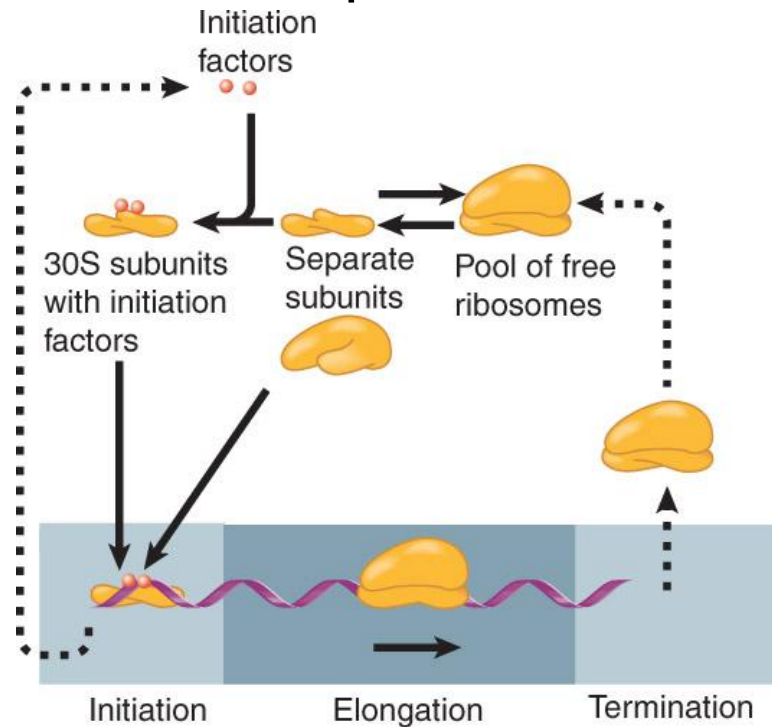
?

GCA	AGA						GGA		
GCC	AGG						GGC		AUA
GCG	CGA						GGG	CAC	AUC
GCU	CGC	GAC	AAC	UGC	GAA	CAA	GGU	CAU	AUU
	CGU	GAU	AAU	UGU	GAG	CAG			
<b>Ala</b>	<b>Arg</b>	<b>Asp</b>	<b>Asn</b>	<b>Cys</b>	<b>Glu</b>	<b>Gln</b>	<b>Gly</b>	<b>His</b>	<b>Ile</b>
<b>A</b>	<b>R</b>	<b>D</b>	<b>N</b>	<b>C</b>	<b>E</b>	<b>Q</b>	<b>G</b>	<b>H</b>	<b>I</b>

UUA						AGC				
UUG						AGU				
CUA				CCA		UCA	ACA			GUA
CUC				CCC		UCC	ACC			GUC
CUG	AAA		UUC	CCG		UCG	ACG		UAC	GUG
CUU	AAG	AUG	UUU	CCU		UCU	ACU	UGG	UAU	GUU
<b>Leu</b>	<b>Lys</b>	<b>Met</b>	<b>Phe</b>	<b>Pro</b>	<b>Ser</b>	<b>Thr</b>	<b>Trp</b>	<b>Tyr</b>	<b>Val</b>	<b>stop</b>
<b>L</b>	<b>K</b>	<b>M</b>	<b>F</b>	<b>P</b>	<b>S</b>	<b>T</b>	<b>W</b>	<b>Y</b>	<b>V</b>	

- **ribosome-binding site** — A sequence on bacterial mRNA that includes an initiation codon that is bound by a 30S subunit in the initiation phase of polypeptide translation.

FIGURE: Ribosome subunits  
recycle

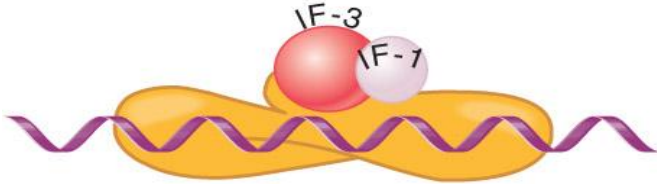


# Initiation in Bacteria Needs 30S Subunits and Accessory Factors

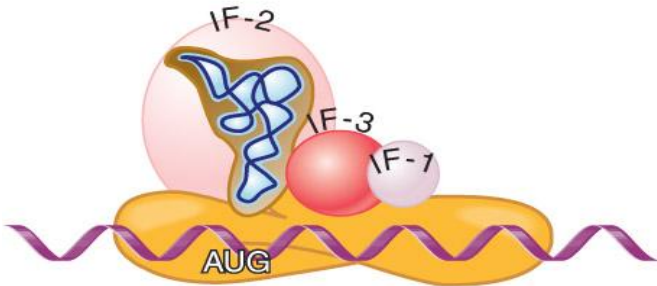
- **Shine–Dalgarno sequence** – The polypurine sequence AGGAGG centered about 10 bp before the AUG initiation codon on bacterial mRNA.
  - It is complementary to the sequence at the 3' end of 16S rRNA.

# Initiation in Bacteria Needs 30S Subunits and Accessory Factors

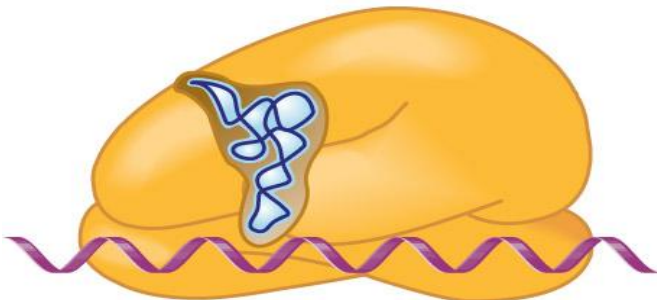
1 30S subunit binds to mRNA



2 IF-2 brings tRNA to P site

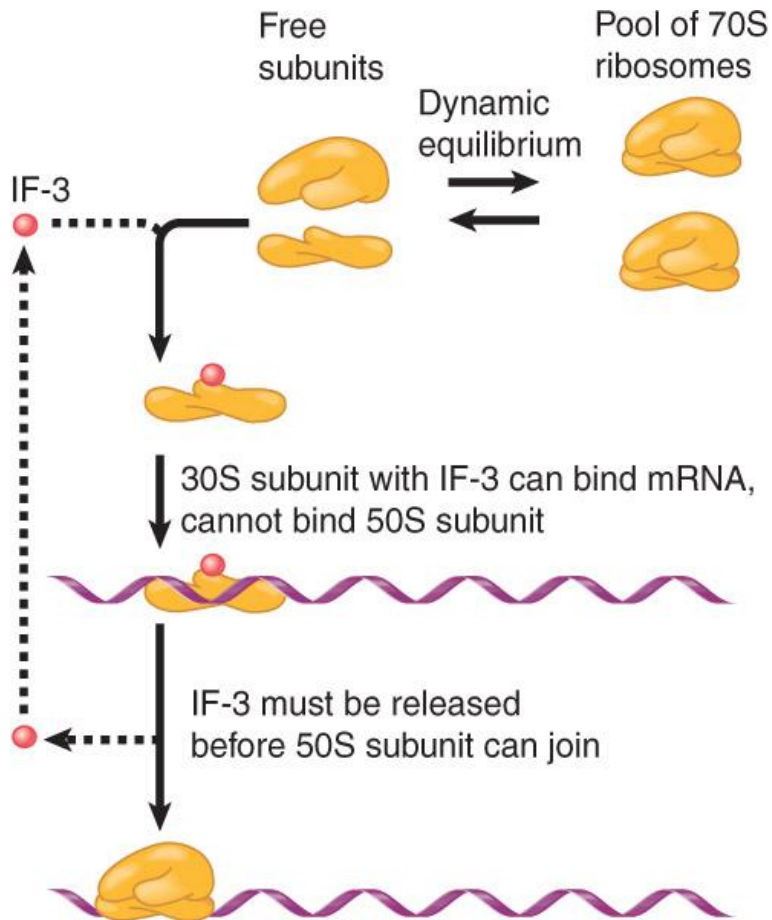


3 IFs are released and 50S subunit joins



- Initiation of translation requires separate 30S and 50S ribosome subunits.
- **Initiation factors (IF-1, IF-2, and IF-3)**, which bind to 30S subunits, are also required.

# Initiation in Bacteria Needs 30S Subunits and Accessory Factors



- A 30S subunit carrying initiation factors binds to an initiation site on mRNA to form an initiation complex.
- IF-3 must be released to allow 50S subunits to join the 30S-mRNA complex.

**FIGURE :** IF3 controls the ribosome-subunit equilibrium



## 24.5 Initiation Involves Base Pairing between mRNA and rRNA

- An initiation site on bacterial mRNA consists of the AUG initiation codon preceded with a gap of ~10 bases by the Shine–Dalgarno polypurine hexamer.
- The rRNA of the 30S bacterial ribosomal subunit has a complementary sequence that base pairs with the Shine–Dalgarno sequence during initiation.

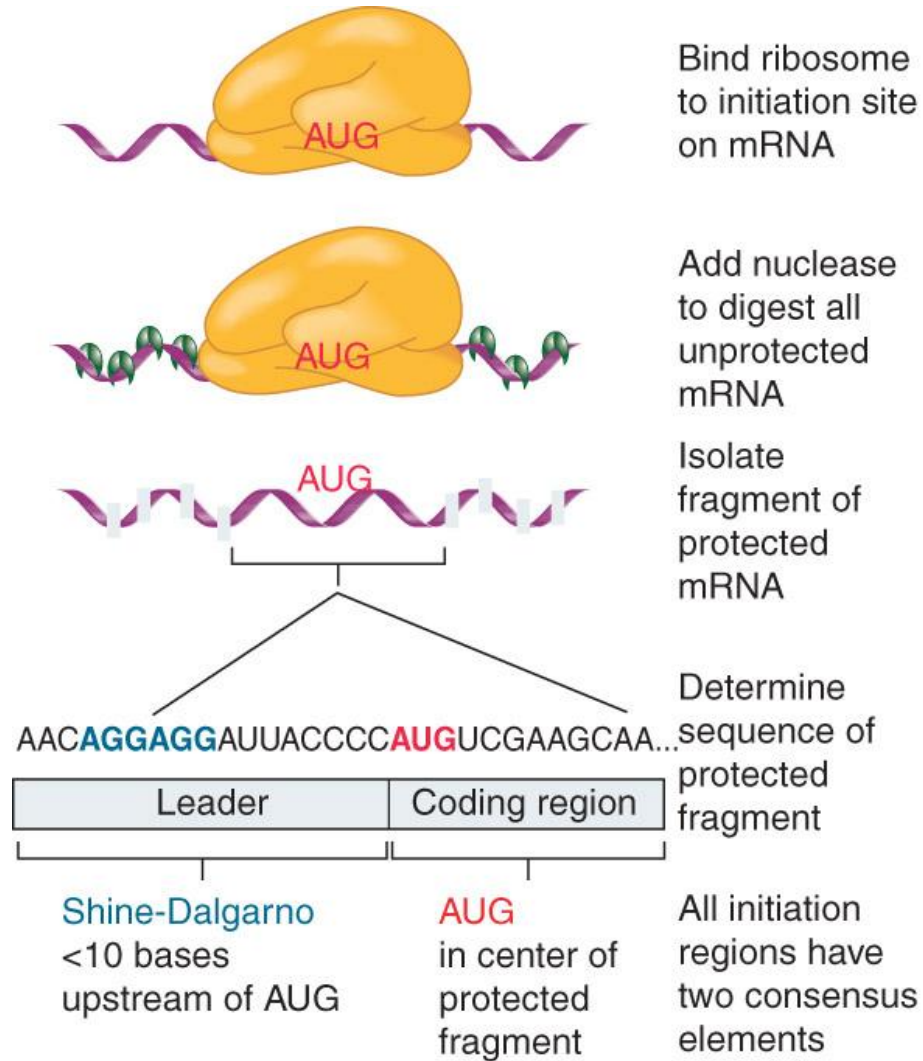


FIGURE 12: The AUG is preceded by a Shine-Dalgarno sequence.

## 24.6 A Special Initiator tRNA Starts the Polypeptide Chain

- Translation starts with a methionine amino acid usually coded by AUG.
- Different methionine tRNAs are involved in initiation and elongation.

## 24.6 A Special Initiator tRNA Starts the Polypeptide Chain

- **N-formyl-methionyl-tRNA (tRNA<sub>f</sub><sup>Met</sup>)** – The aminoacyl-tRNA that initiates bacterial polypeptide translation.
  - The amino group of the methionine is formylated.

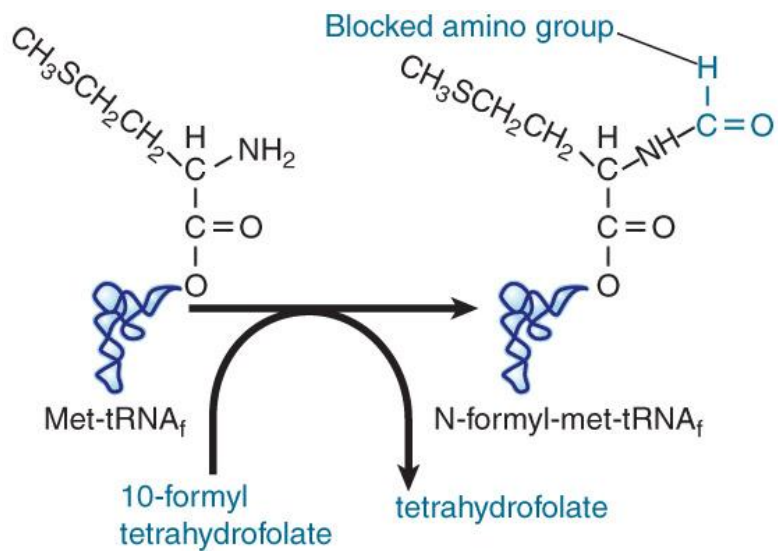


FIGURE 14: Initiator Met-tRNA is formylated.

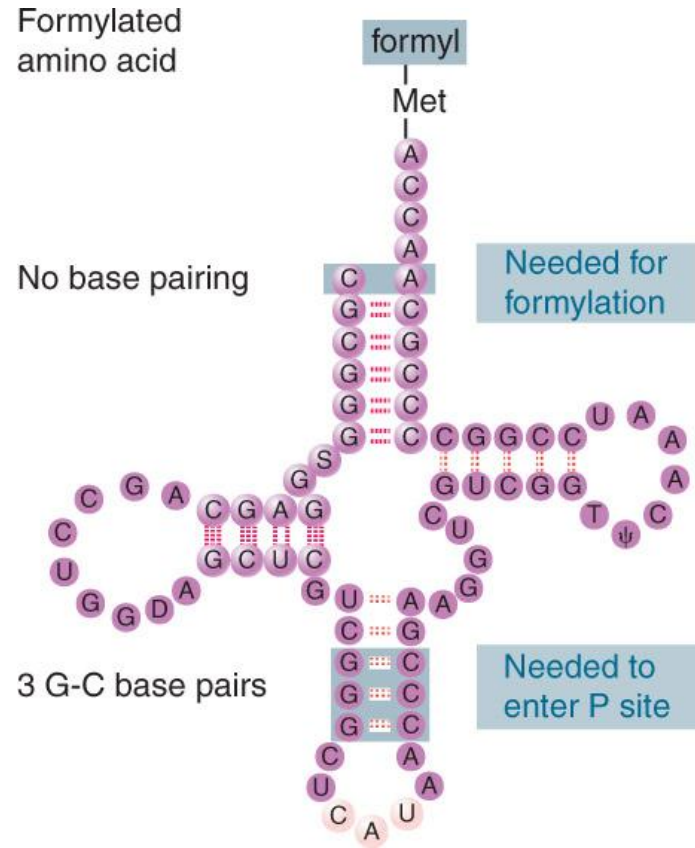
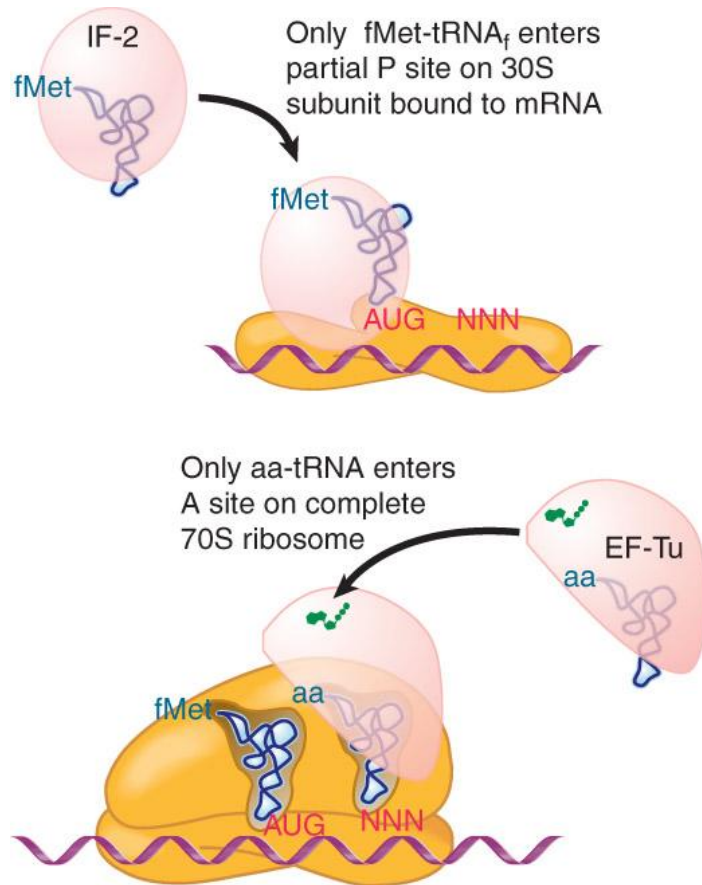


FIGURE 15: Initiator tRNA has distinct features.

## 24.6 A Special Initiator tRNA Starts the Polypeptide Chain

- **tRNA<sub>m</sub><sup>Met</sup>** – The bacterial tRNA that inserts methionine at internal AUG codons.
- The initiator tRNA has unique structural features that distinguish it from all other tRNAs.
- The NH<sub>2</sub> group of the methionine bound to bacterial initiator tRNA is formylated.

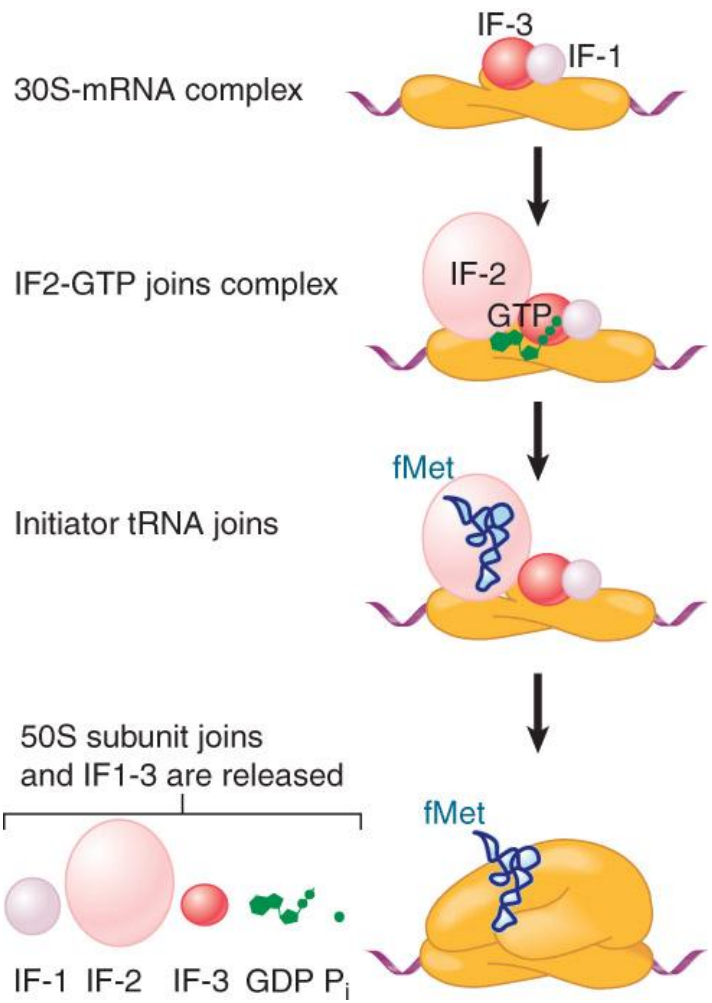
## 24.7 Use of fMet-tRNA<sub>f</sub> Is Controlled by IF-2 and the Ribosome



- **context** – The fact that neighboring sequences may change the efficiency with which a codon is recognized by its aminoacyl-tRNA or is used to terminate polypeptide translation.

FIGURE 16: 30S subunits initiate; ribosomes elongate

# 24.7 Use of fMet-tRNA<sub>f</sub> Is Controlled by IF-2 and the Ribosome



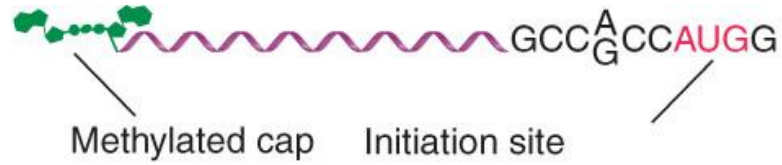
- IF-2 binds the initiator fMet-tRNA<sub>f</sub> and allows it to enter the partial P site on the 30S subunit.

FIGURE 17: Initiation is controlled by three factors



## 24.8 Small Subunits Scan for Initiation Sites on Eukaryotic mRNA

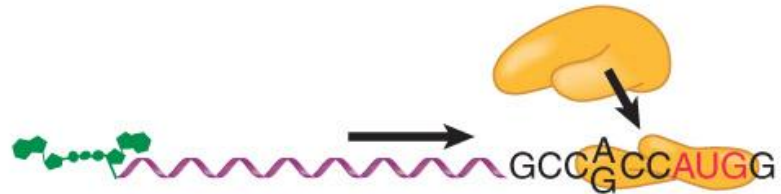
- Eukaryotic 40S ribosomal subunits bind to the 5' end of mRNA and scan the mRNA until they reach an initiation site.
- A eukaryotic initiation site consists of a ten-nucleotide sequence that includes an AUG codon.
- 60S ribosomal subunits join the complex at the initiation site.



1 Small subunit binds to methylated cap



2 Small subunit migrates to initiation site



3 If leader is long, subunits may form queue



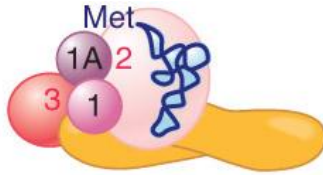
FIGURE 18: mRNA has two features recognized by ribosomes

## 24.8 Small Subunits Scan for Initiation Sites on Eukaryotic mRNA

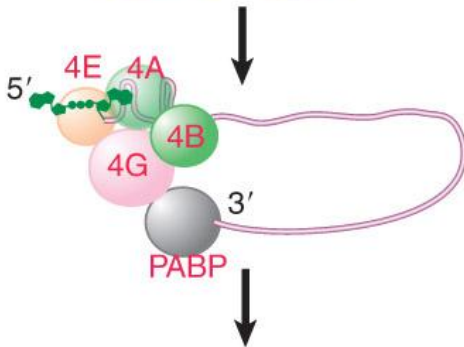
- **IRES (internal ribosome entry site)** – A eukaryotic messenger RNA sequence that allows a ribosome to initiate polypeptide translation without migrating from the 5' end.

# 24.9 Eukaryotes Use a Complex of Many Initiation Factors

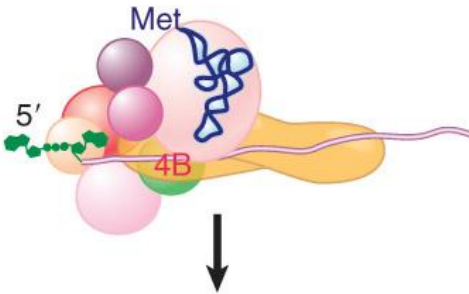
43S preinitiation complex eIF2, eIF3, Met-tRNA<sub>i</sub>, eIF1, eIF1A



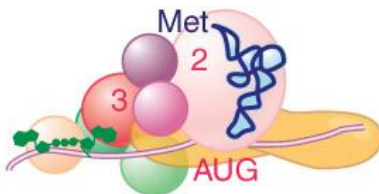
Cap-binding complex + mRNA eIF4A, B, E, G



43S complex binds to 5' end of mRNA



48S complex forms at initiation codon eIF2, EIF3 eIF1, 1A eIF4A, B, F



- Initiation factors are required for all stages of initiation, including binding the initiator tRNA, 40S subunit attachment to mRNA, movement along the mRNA, and joining of the 60S subunit.
- Eukaryotic initiator tRNA is a Met-tRNA that is different from the Met-tRNA used in elongation, but the methionine is not formylated.

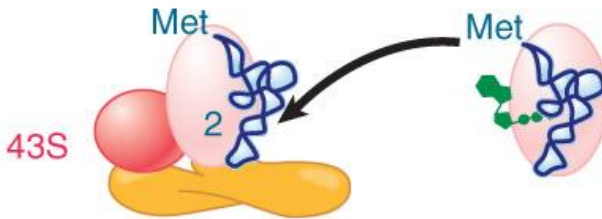
FIGURE 19: Eukaryotic initiation uses several complexes

# 24.9 Eukaryotes Use a Complex of Many Initiation Factors

eIF3 maintains free 40S subunits

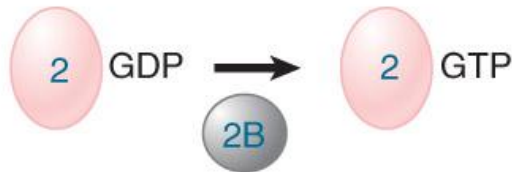


eIF2 binds Met-tRNA to 40S



eIF2 is a GTPase

eIF2B is the exchange factor



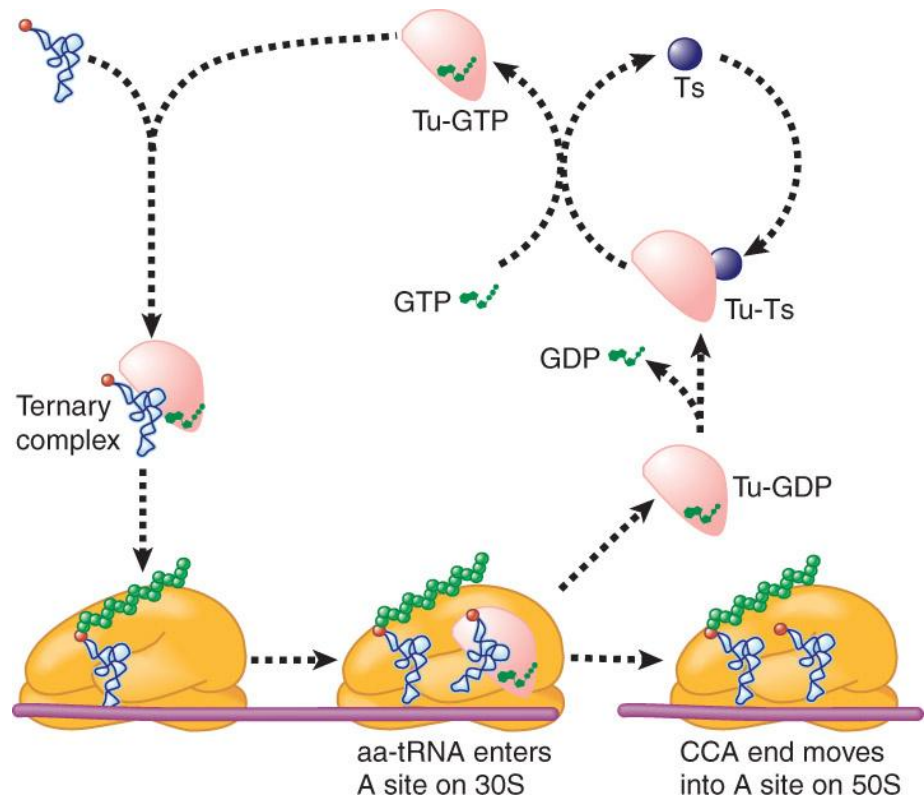
- eIF2 binds the initiator Met-tRNA<sub>i</sub> and GTP, forming a ternary complex that binds to the 40S subunit before it associates with mRNA.
- A cap-binding complex binds to the 5' end of mRNA prior to association of the mRNA with the 40S subunit.

FIGURE 21: 43S complex = 40S subunit + factors +tRNA

# 24.10 Elongation Factor Tu Loads Aminoacyl-tRNA into the A Site

- **EF-Tu** ( an **elongation factor**) is a monomeric G protein whose active form (bound to GTP) binds to aminoacyl-tRNA.
- The EF-Tu-GTP-aminoacyl-tRNA complex binds to the ribosome A site.

FIGURE 25: Ef-Tu recycles between GTP-bound and GDP-bound forms.



## 24.10 Elongation Factor Tu Loads Aminoacyl-tRNA into the A Site

- **GMP-PCP** – An analog of GTP that cannot be hydrolyzed.
  - It is used to test which stage in a reaction requires hydrolysis of GTP.
- **kirromycin** – An antibiotic that inhibits protein synthesis by acting on EF-Tu.

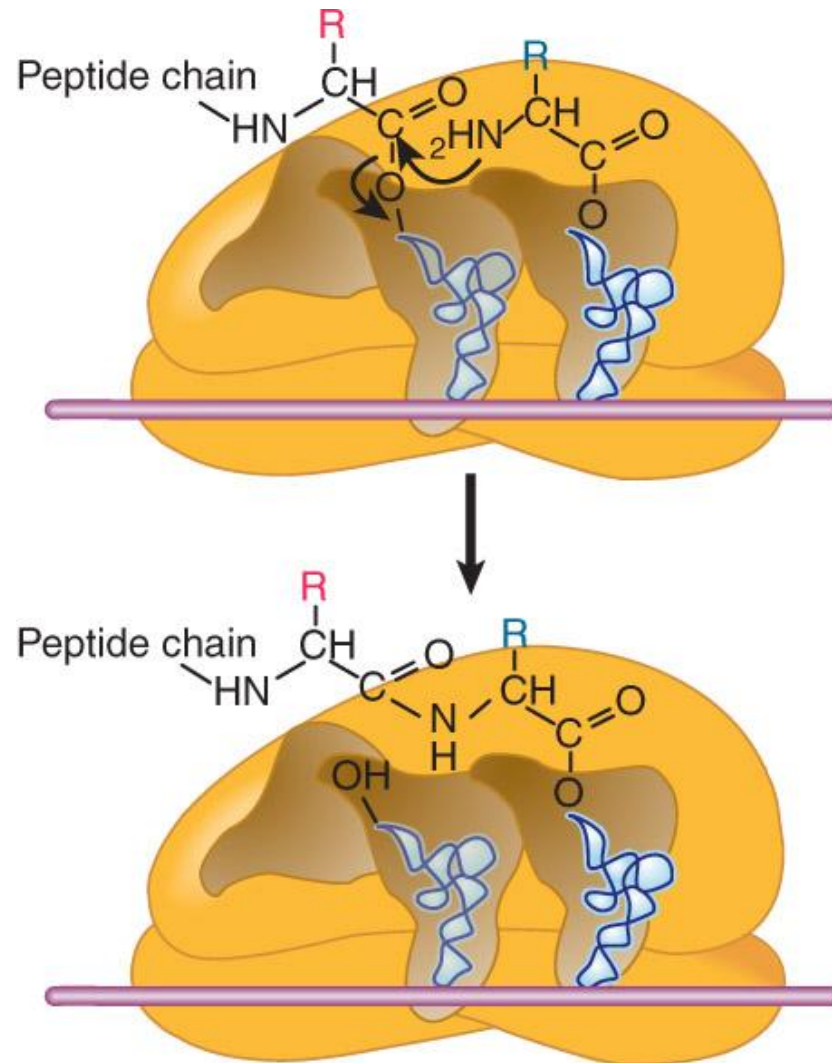
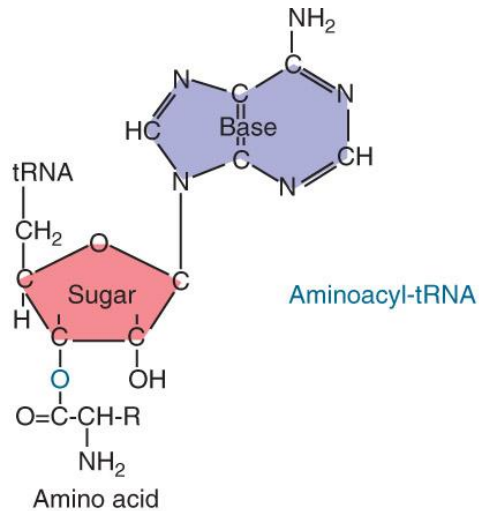


FIGURE 26: Nascent polypeptide is transferred to aminoacyl tRNA.



# 24.11 The Polypeptide Chain Is Transferred to Aminoacyl-tRNA



- **puromycin** – An antibiotic that terminates protein synthesis by mimicking a tRNA and becoming linked to the nascent protein chain.

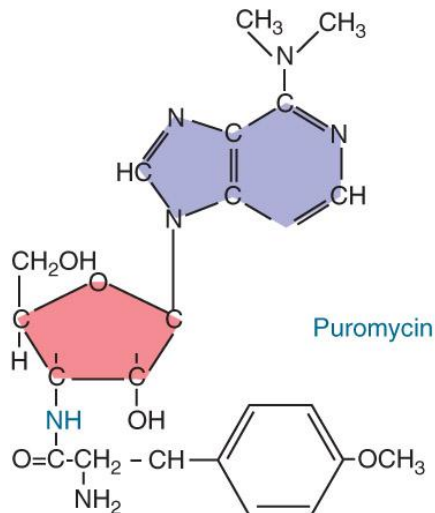
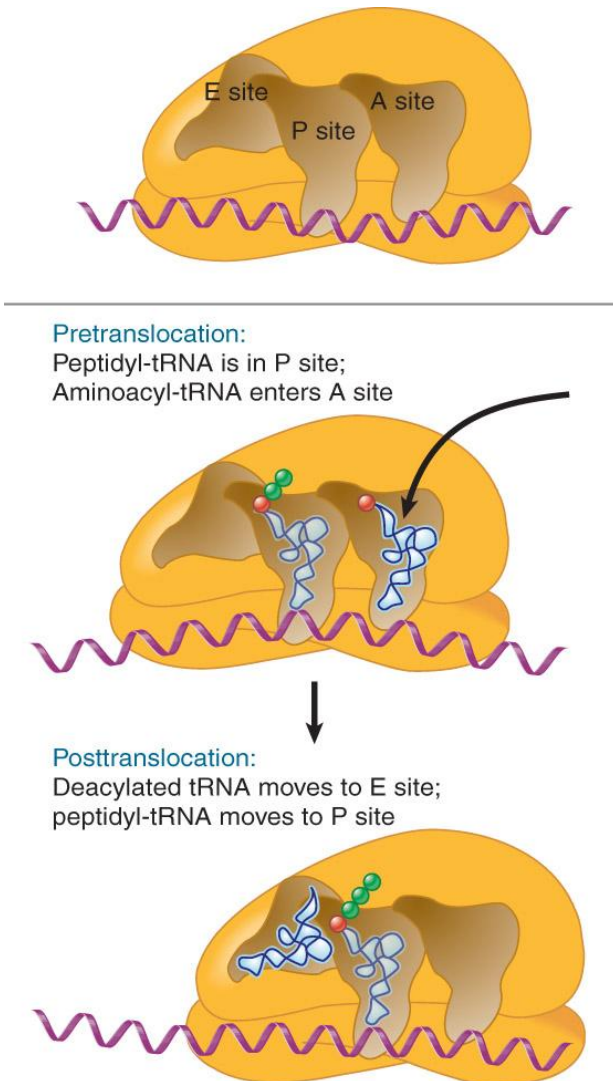


FIGURE 27: Puromycin resembles aminoacyl-tRNA.

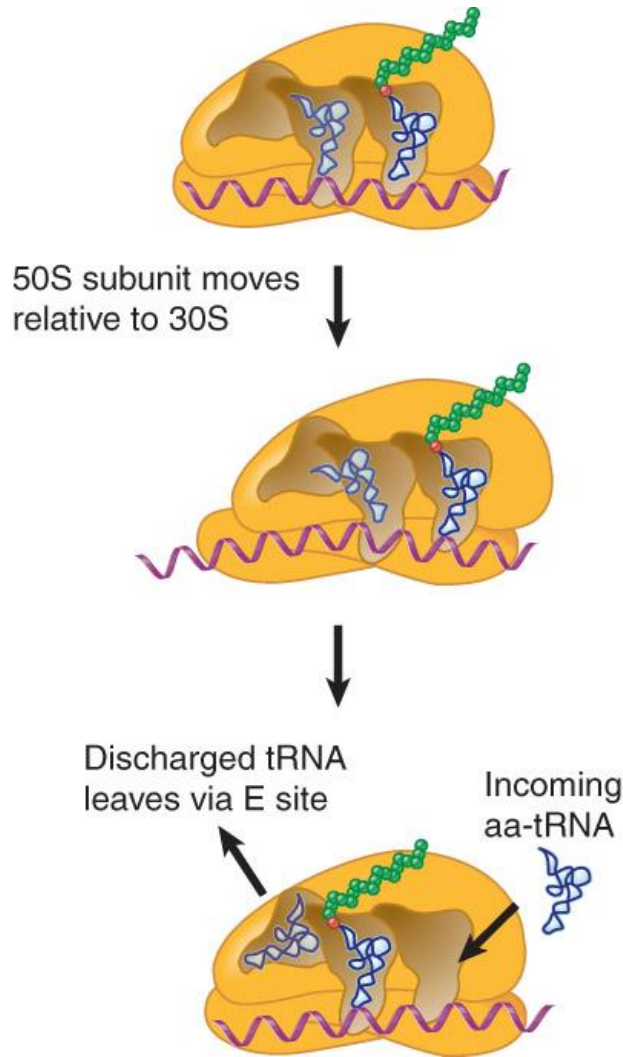
# 24.12 Translocation Moves the Ribosome



- Ribosomal translocation moves the mRNA through the ribosome by three bases.
- Translocation moves deacylated tRNA into the E site and peptidyl-tRNA into the P site, and empties the A site.

FIGURE 28: tRNA moves through 3 ribosome sites.

# 24.12 Translocation Moves the Ribosome



- The hybrid state model proposes that translocation occurs in two stages, in which the 50S moves relative to the 30S, and then the 30S moves along mRNA to restore the original conformation.

FIGURE 29: Translocation occurs in two stages.

## 24.13 Elongation Factors Bind Alternately to the Ribosome

- Translocation requires EF-G, whose structure resembles the aminoacyl-tRNA-EF-Tu-GTP complex.
- Binding of EF-Tu and EF-G to the ribosome is mutually exclusive.
- Translocation requires GTP hydrolysis, which triggers a change in EF-G, which in turn triggers a change in ribosome structure.

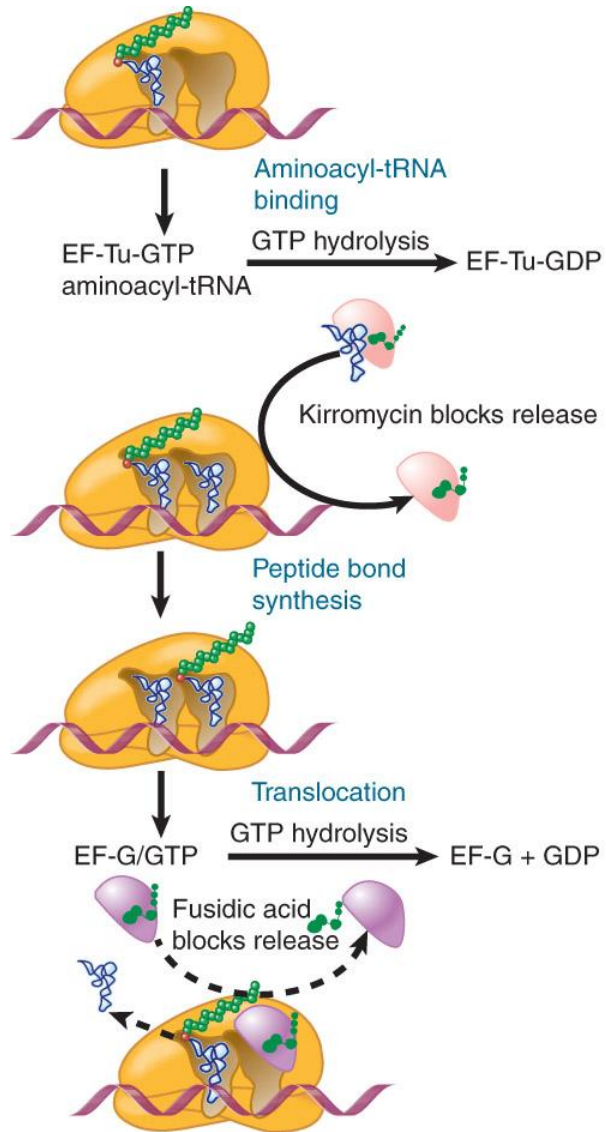


FIGURE 30: EFs have alternating interactions.

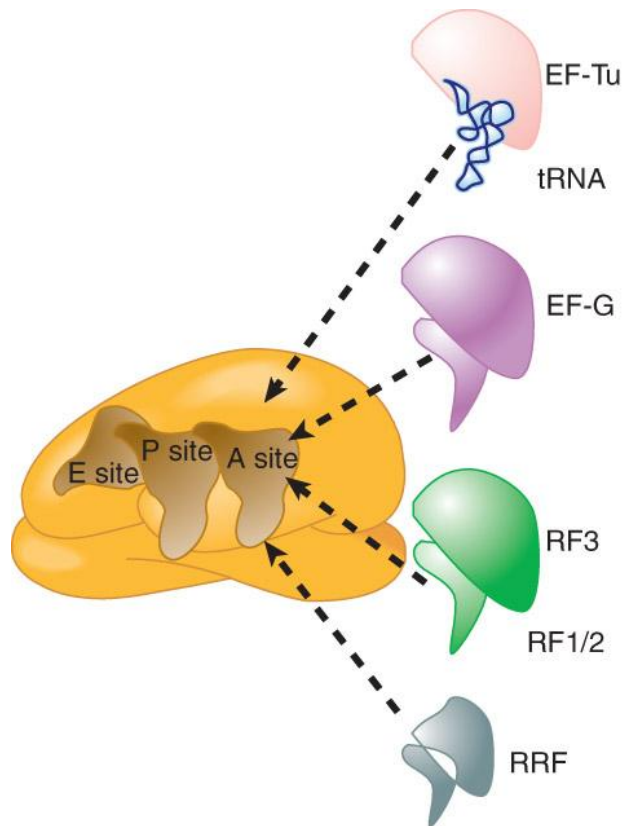
## 24.14 Three Codons Terminate Translation

- The **stop codons** UAA (**ochre**), UAG (**amber**), and UGA (sometimes called **opal**) terminate translation.
- In bacteria, they are used most often with relative frequencies UAA>UGA>UAG.

## 24.14 Three Codons Terminate Translation

- **premature termination** – The termination of protein or of RNA synthesis before the chain has been completed.
  - In translation it can be caused by mutations that create stop codons within the coding region.
  - In RNA synthesis it is caused by various events that act on RNA polymerase.

# 24.15 Termination Codons Are Recognized by Protein Factors



- Termination codons are recognized by protein **release factors**, not by aminoacyl-tRNAs.
- **RF1** – The bacterial release factor that recognizes UAA and UAG as signals to terminate polypeptide translation.
- **RF2** – The bacterial release factor that recognizes UAA and UGA as signals to terminate polypeptide translation.

FIGURE 32: Several factors have similar shapes.



# 24.15 Termination Codons Are Recognized by Protein Factors

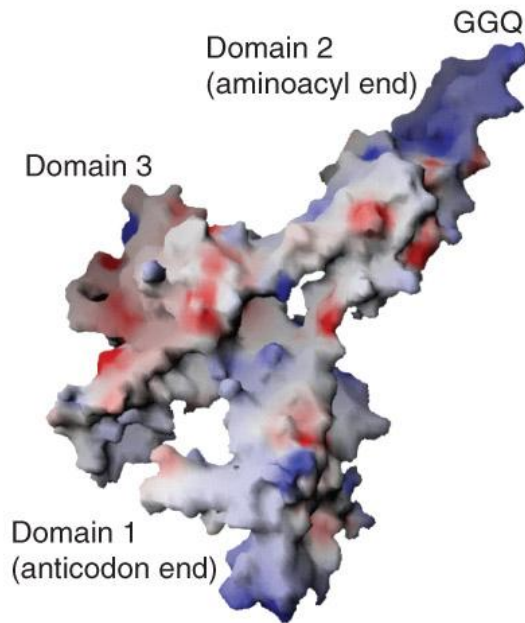


FIGURE 33: eRF1 mimics tRNA.

- **RF3** – A polypeptide translation termination factor related to the elongation factor EF-G.
  - It functions to release the factors RF1 or RF2 from the ribosome when they act to terminate polypeptide translation.
- The structures of the class 1 release factors resemble aminoacyl-tRNA-EF-Tu and EF-G.

## 24.15 Termination Codons Are Recognized by Protein Factors

- The class 1 release factors respond to specific termination codons and hydrolyze the polypeptide-tRNA linkage.
- The class 1 release factors are assisted by class 2 release factors that depend on GTP.
- The mechanism is similar in bacteria (which have two types of class 1 release factors) and eukaryotes (which have only one class 1 release factor).

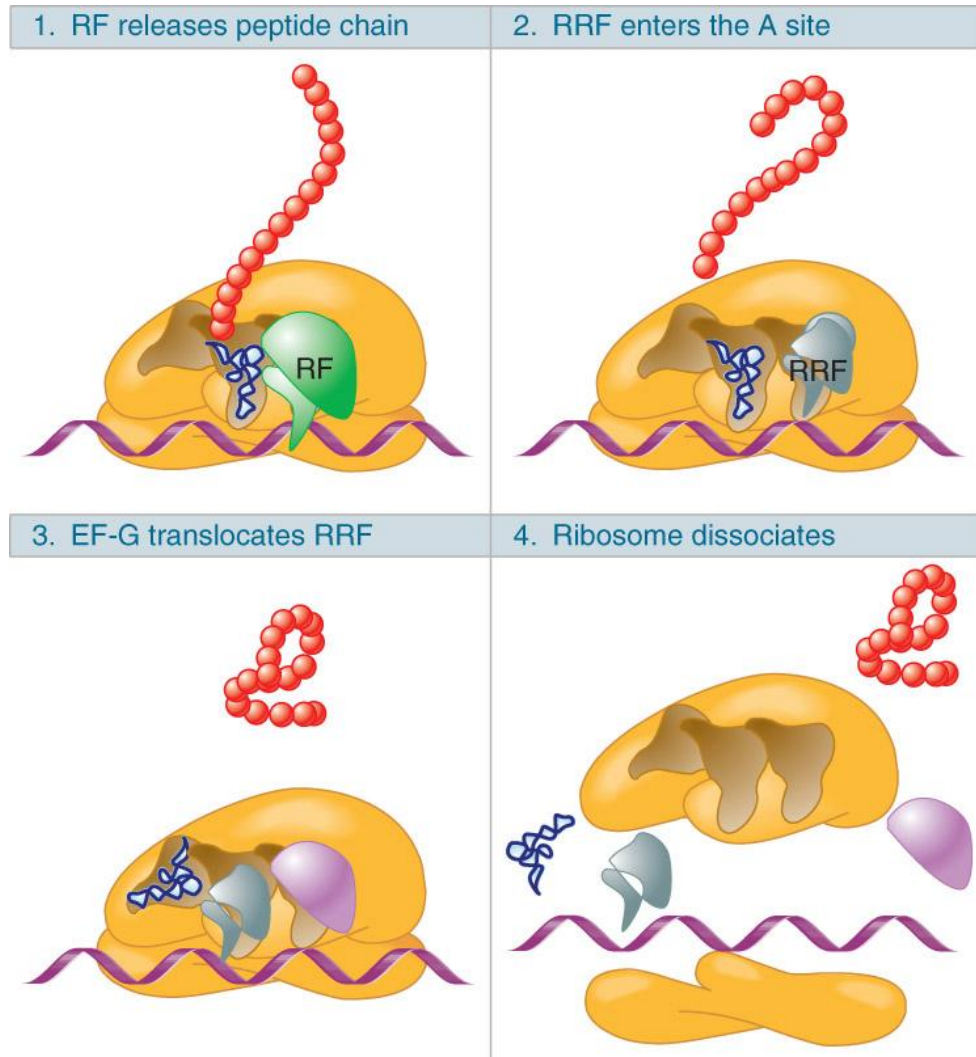


FIGURE 35: Termination requires several protein factors.

# Termination Codons Are Recognized by Protein Factors

Initiation Factors			
Prokaryotic	Eukaryotic	General Function	Notes
IF-1	eIF1A	Blocks A site	eIF1A assists eIF2 in promoting Met-tRNA <sub>i</sub> <sup>Met</sup> to binding to 40S; also promotes subunit dissociation eIF2 is a GTPase eIF3 stimulates formation of the ternary complex, its binding to 40S, and binding and scanning of mRNA eIF5B is involved in initiator tRNA entry and is a GTPase eIF4 complex functions in cap binding
IF-2*†	eIF2, eIF3, eIF5B*	Entry of initiator tRNA	
IF-3	eIF1, eIF4 complex, eIF3	Small subunit binding to mRNA	
Elongation Factors			
Prokaryotic	Eukaryotic	General Function	
EF-Tu <sup>†‡</sup> , EF-G <sup>†</sup> EF-Ts EF-G <sup>§</sup>	eEF1 $\alpha$ <sup>‡</sup> eEF1 $\beta$ , eEF1 $\gamma$ eEF2 <sup>§</sup>	GTP-binding GDP-exchanging Ribosome translocation	
Release Factors			
Prokaryotic	Eukaryotic	General Function	
RF1 RF2 RF3 <sup>†</sup>	eRF1 eRF1 eRF3	UAA/UAG recognition UAA/UGA recognition Stimulation of other RF(s)	

\* IF-2 and eIF5B have sequence homology.

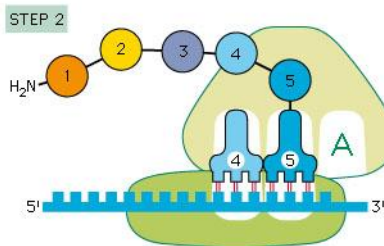
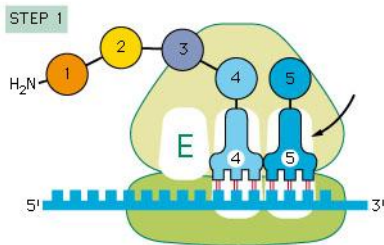
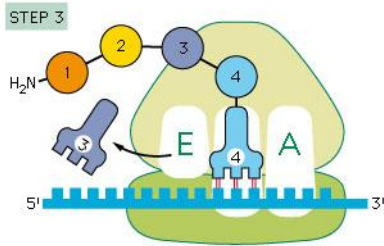
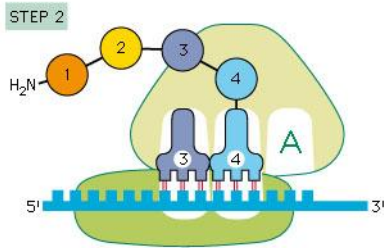
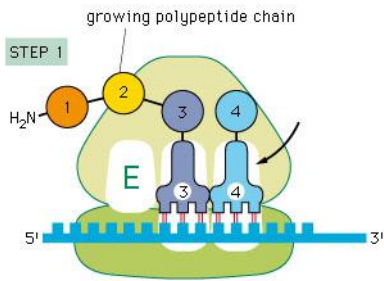
† IF-2, EF-Tu, EF-G, and RF3 have sequence homology.

‡ EF-Tu and eEF1 $\alpha$  have sequence homology.

§ EF-G and eEF2 have sequence homology.

Functional homologies of prokaryotic and eukaryotic translation factors.

# mRNA translation mechanism



Step1: An aminoacyl-tRNA molecule binds to the A-site on the ribosome

Step2: A new peptide bond is formed

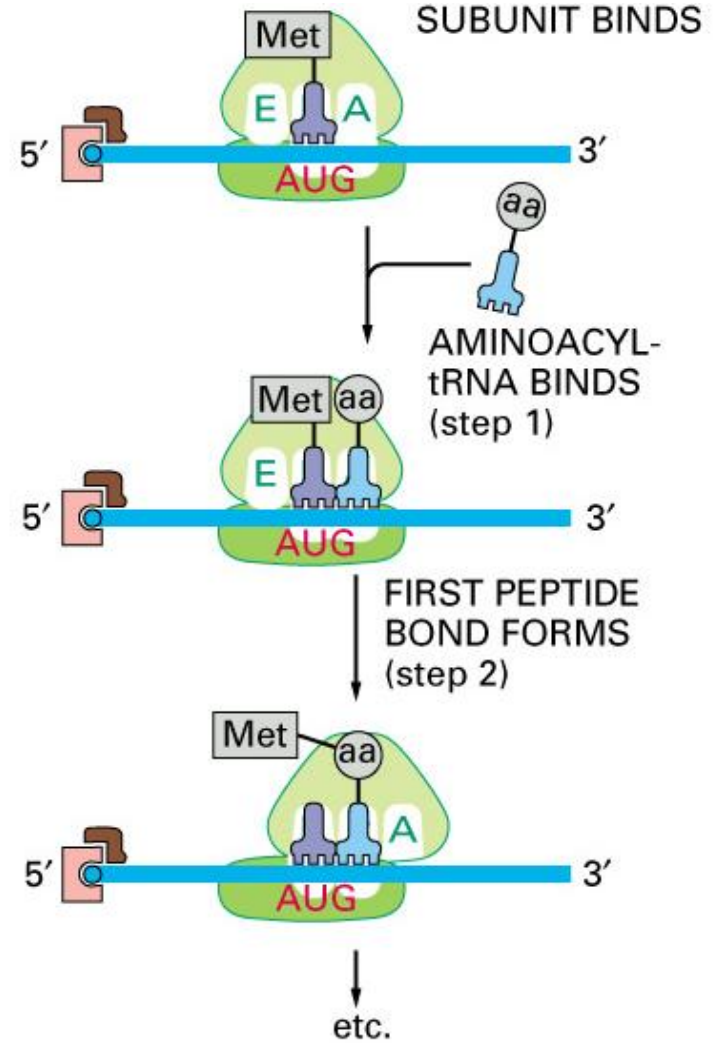
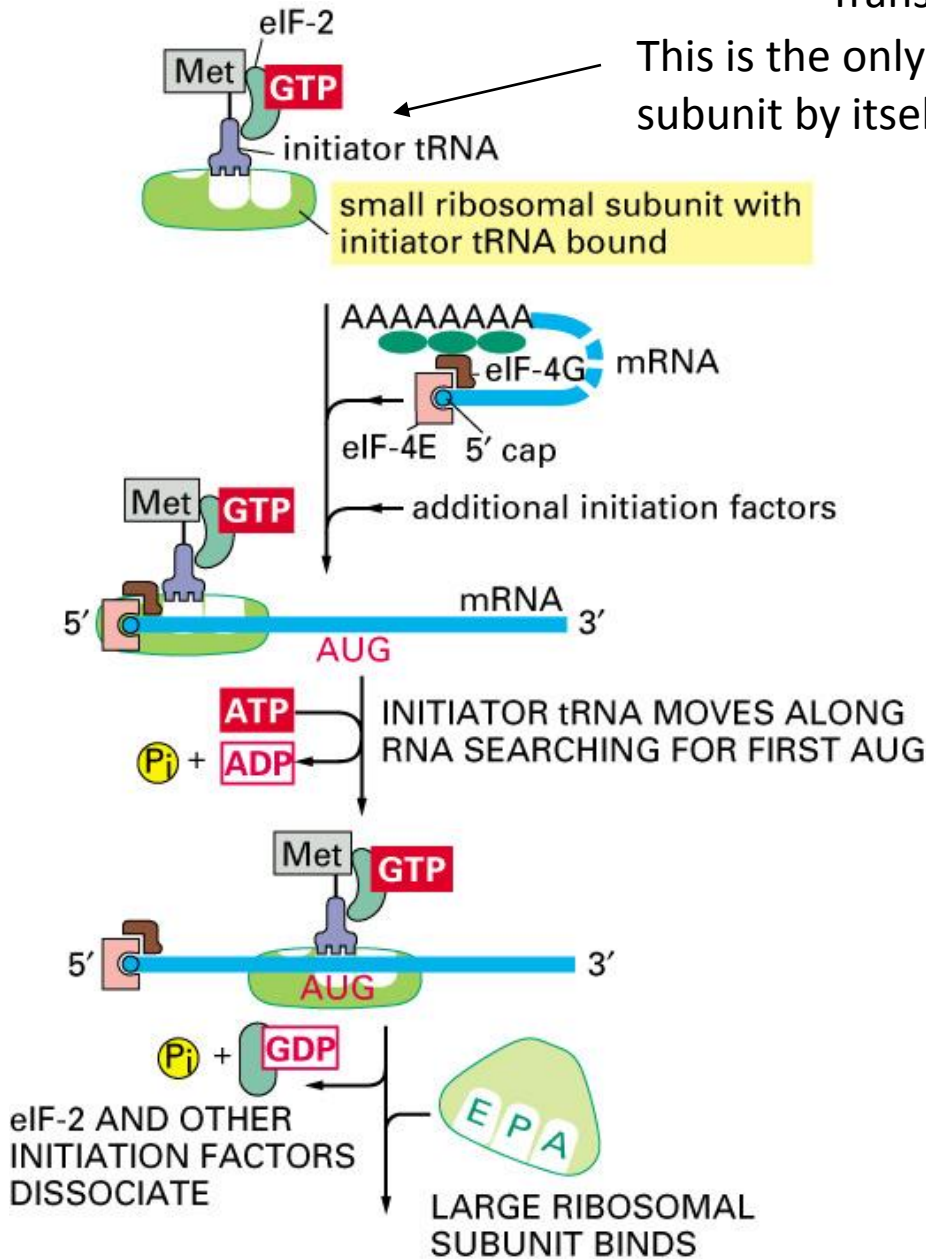
Step3: The small subunit moves a distance of three nucleotides along the mRNA chain ejecting the spent tRNA molecule

Step4: The next aminoacyl-tRNA molecule binds to the A-site on the ribosome

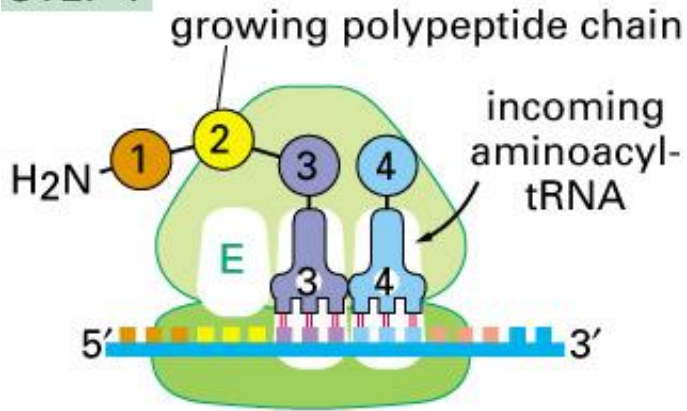
Step5: ...

# Translation Initiation

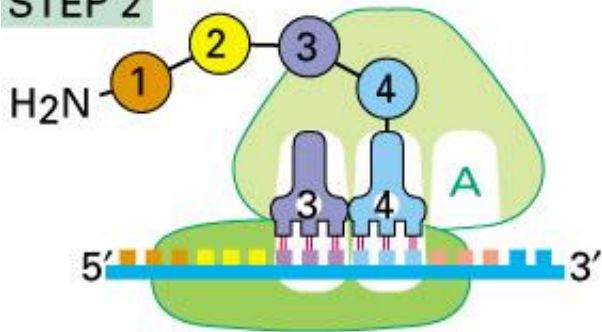
This is the only tRNA that can bind to the small ribosomal subunit by itself



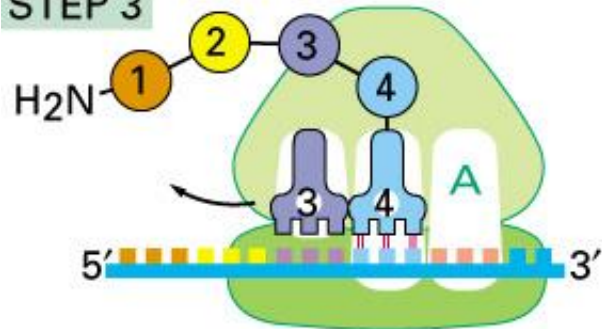
### STEP 1



### STEP 2



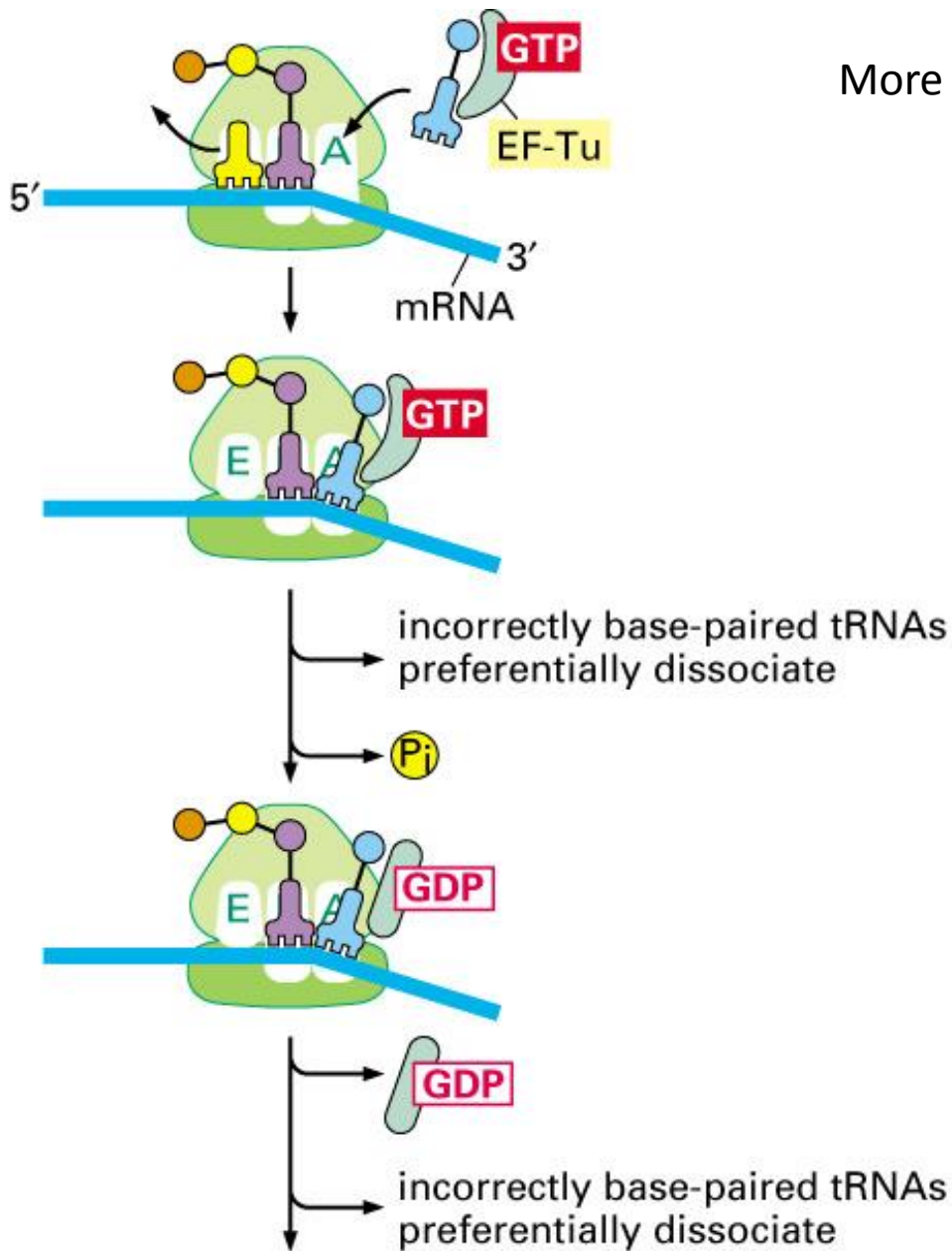
### STEP 3



Protein made in 5' to 3' direction, with N-terminal end made first

### General Mechanism

- A site is where new codon is translated
- P site is where the growing peptide chain is kept and new aa are attached
- E site is where “naked” t RNA exit the ribosome



## More Detailed View

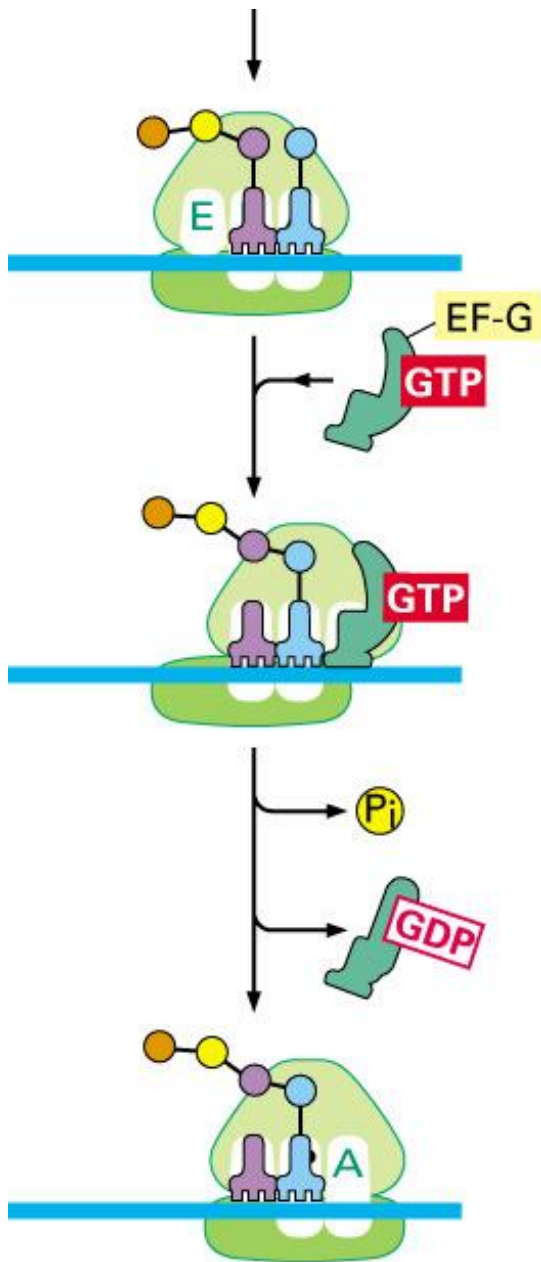
New tRNA carrying amino acids are accompanied by elongation factor called EF-Tu

The tRNA-ETu occupies a hybrid binding site (not quite in A)

Correct codon-anticodon pairing triggers ETu to split GTP and fall off, and tRNA moves into the A position

The delay caused by the association/dissociation of ETu helps increase accuracy of translation





Elongation factor G (EF-G) then binds near the A site, forcing the tRNAs containing the new amino acid and the growing chain into the next (P and E) sites on the ribosome

EF-G splits GTP, changes conformation and falls off, thus increasing the speed of translation.

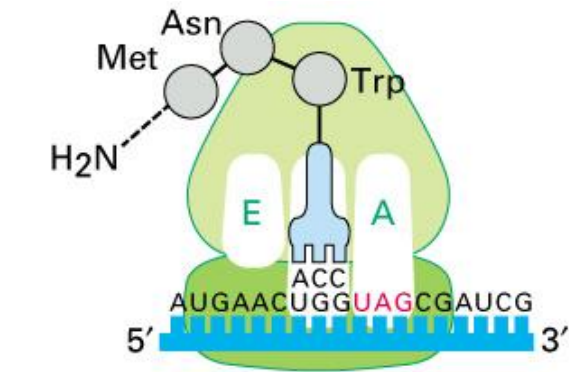
GTP exchange factors continually recharge the GTP on both of the elongation factors.

Stop Codons = UAA, UAG, UGA

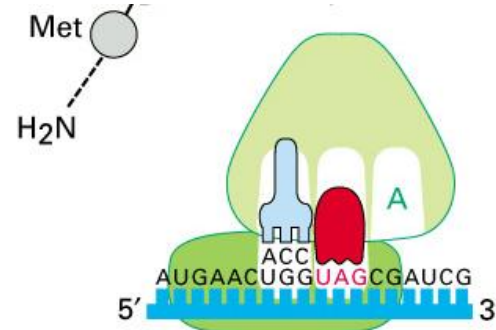
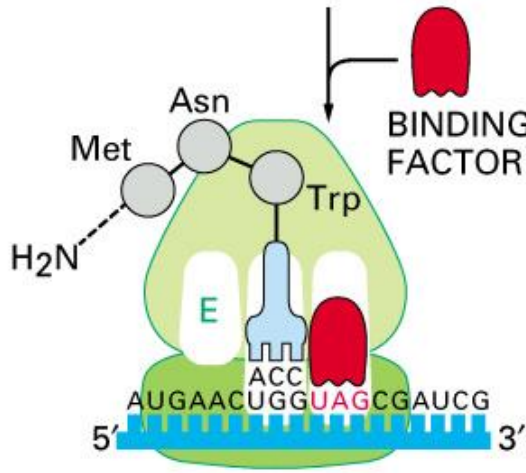
No tRNA binds to this set of codons

One of these codons at the A site attracts a release factor

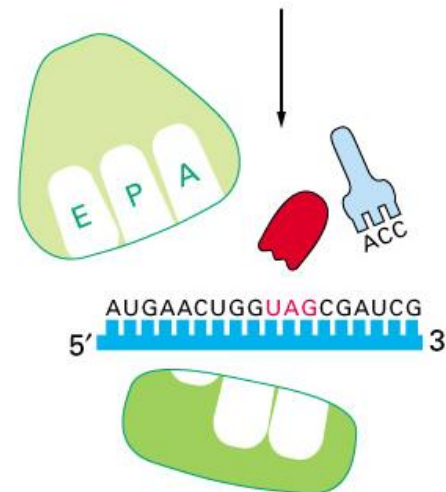
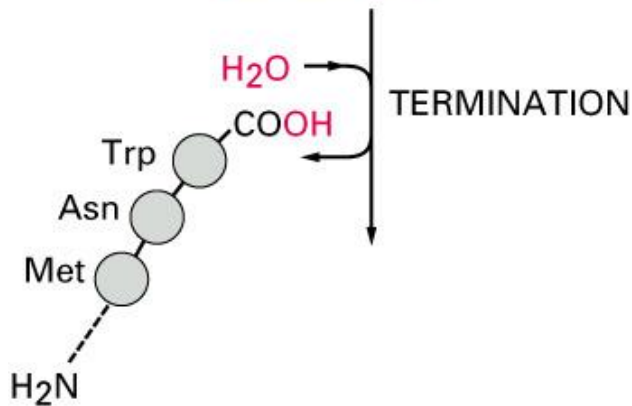
Ribosome adds a water to the last peptide, creating the carboxyl end



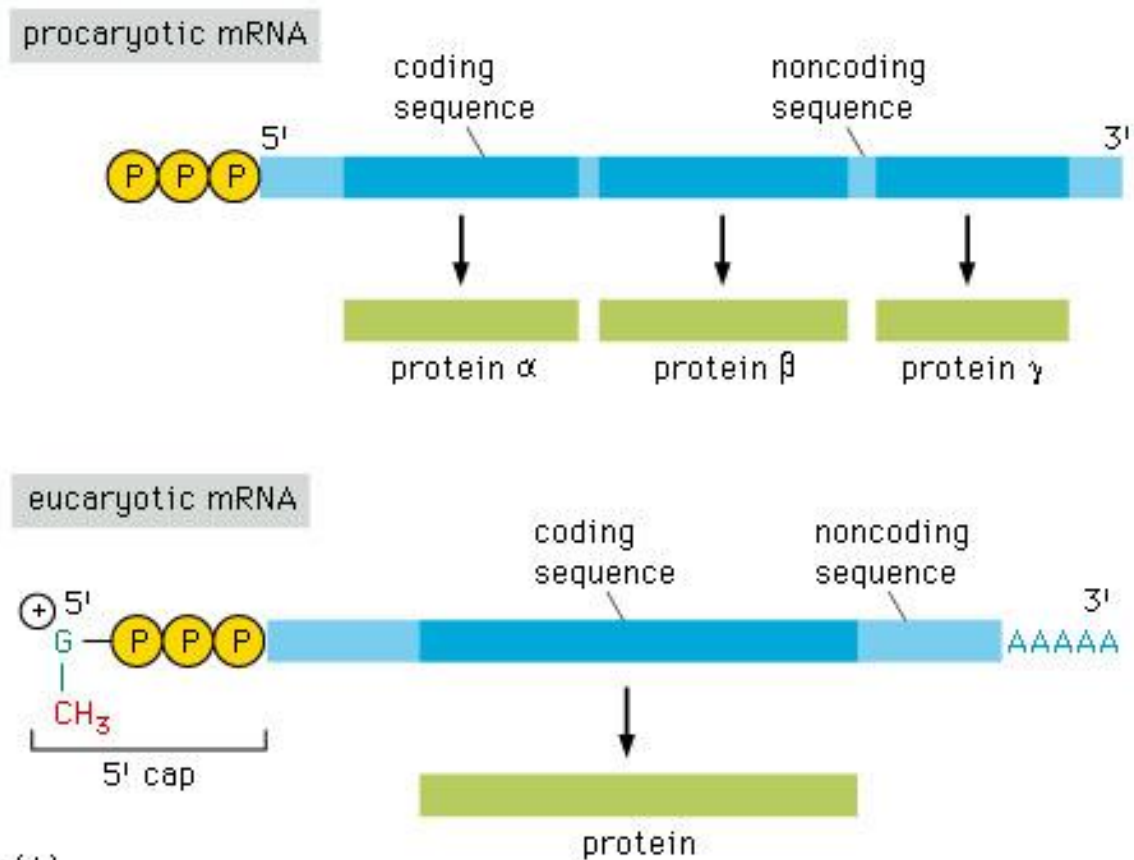
BINDING OF RELEASE FACTOR TO THE A-SITE



TERMINATION

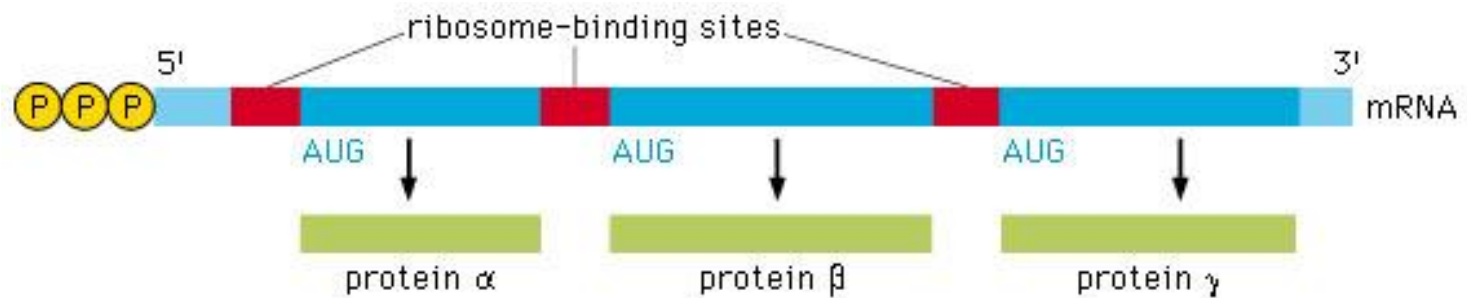


# Prokaryotic vs eukaryotic mRNA molecules



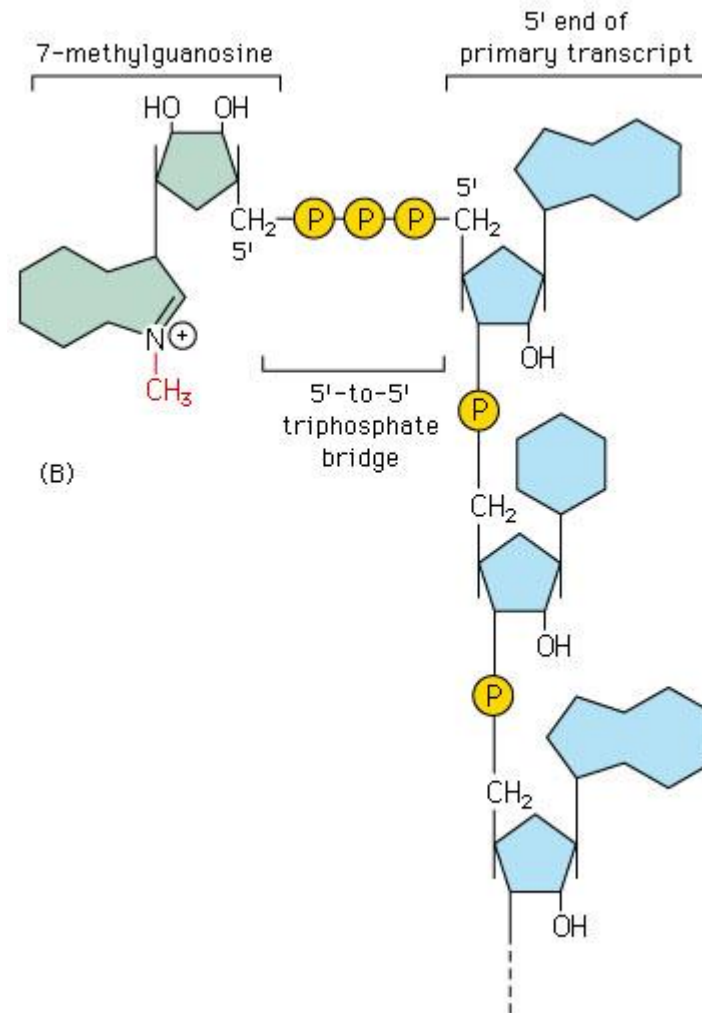
(A)

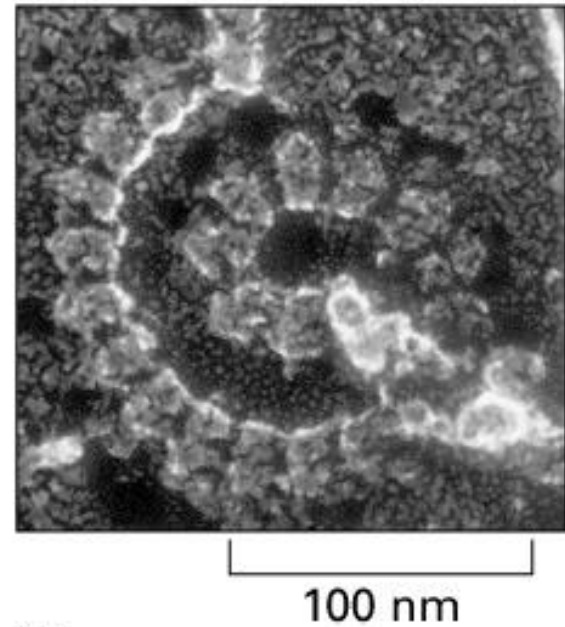
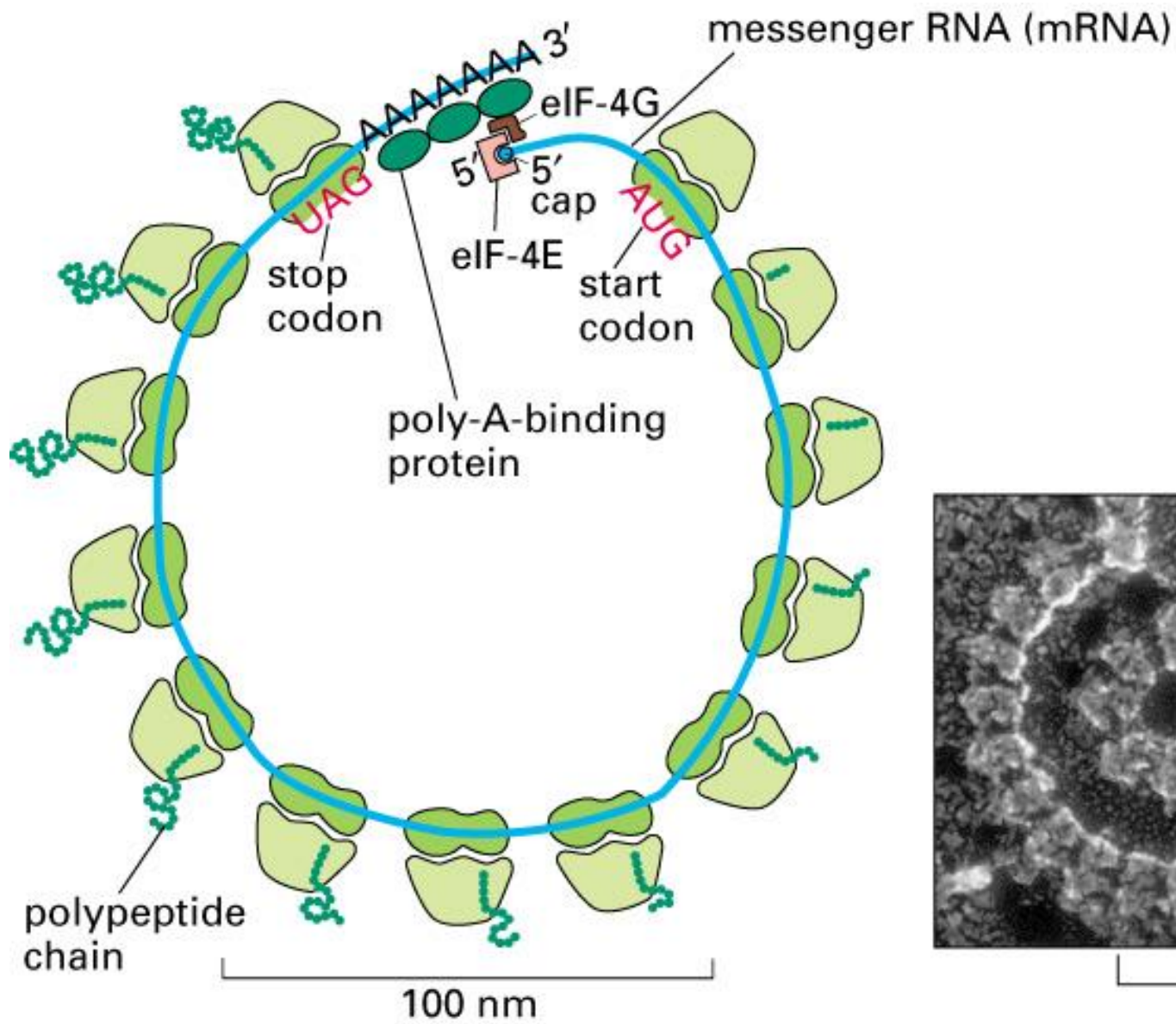
# Structure of a typical prokaryotic mRNA molecule



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# 5' end capping of eukaryotic mRNA molecules





(A) (B)

Figure 6-75. Molecular Biology of the Cell, 4th Edition.

**TABLE 6-3** Inhibitors of Protein or RNA Synthesis

INHIBITOR	SPECIFIC EFFECT
<i>Acting only on bacteria</i>	
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from initiation complex to chain-elongating ribosome and also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6-65)
Erythromycin	blocks the translocation reaction on ribosomes (step 3 in Figure 6-65)
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
<i>Acting on bacteria and eucaryotes</i>	
Puromycin	causes the premature release of nascent polypeptide chains by its addition to growing chain end
Actinomycin D	binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
<i>Acting on eucaryotes but not bacteria</i>	
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 6-65)
Anisomycin	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6-65)
$\alpha$ -Amanitin	blocks mRNA synthesis by binding preferentially to RNA polymerase II
The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.	

**Thank you**