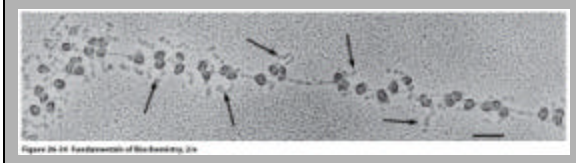
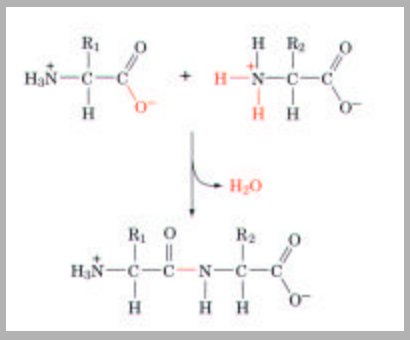
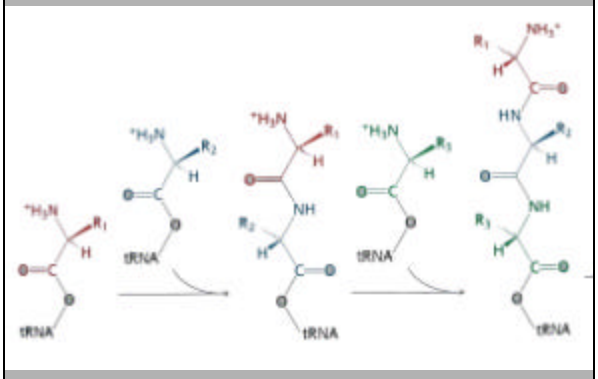
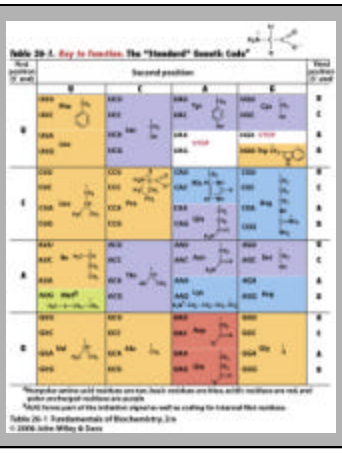
16: Protein Synthesis

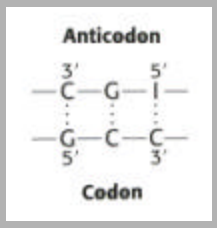


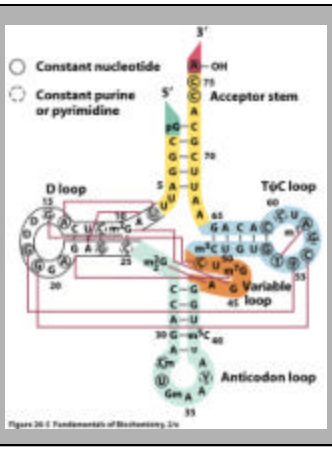
1. mRNA
2. rRNA
3. tRNA

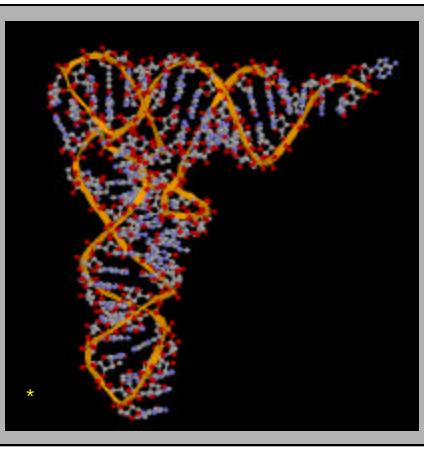


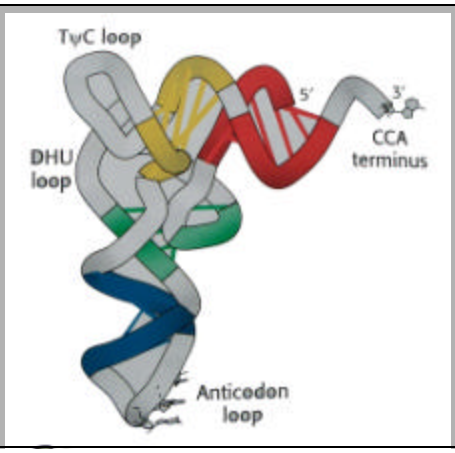





Anticodon
3′
5′
C
G
I
G
C
C
5′
3′
Codon

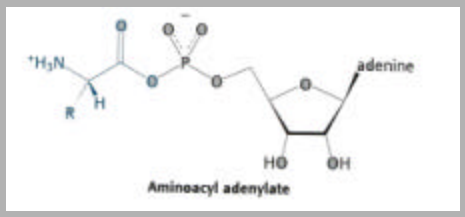

3′
Constant nucleotide
Constant purine or pyrimidine
5′
Acceptor stem
D loop
TψC loop
Variable loop
Anticodon loop

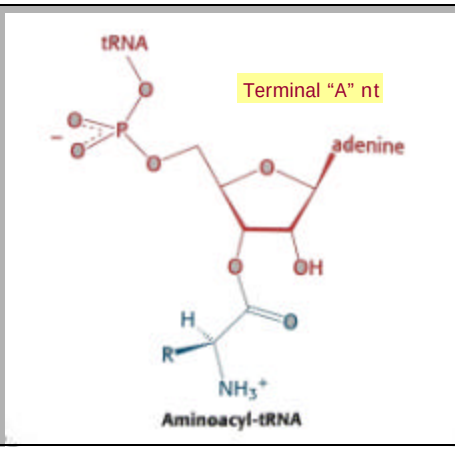


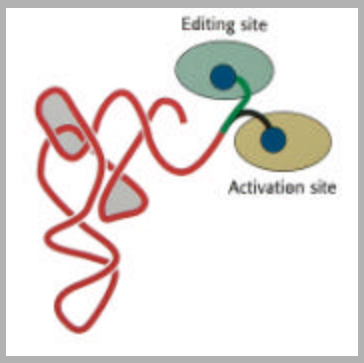


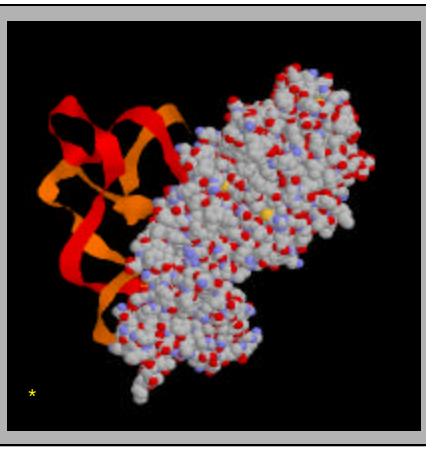
Amino Acid Charging

- AA + ATP → AAA + PPi









Ribosomes; rRNA

Table 26-4 Components of *E. coli* Ribosomes

	Ribosome	Small Subunit	Large Subunit
Sedimentation coefficient	70S	30S	50S
Mass (kD)	2520	930	1590
RNA			
Major		16S, 1542 nucleotides	23S, 2904 nucleotides
Minor			5S, 120 nucleotides
RNA mass (kD)	1664	560	1104
Proportion of mass	66%	60%	70%
Protein		21 polypeptides	31 polypeptides
Protein mass (kD)	857	370	487
Proportion of mass	34%	40%	30%

Table 26-4 Fundamentals of Biochemistry, 2/e
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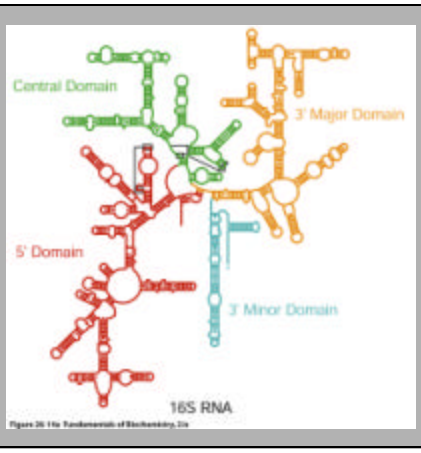
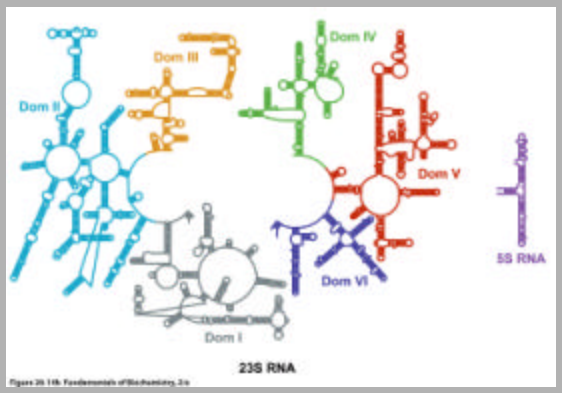
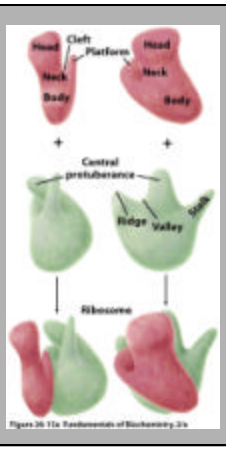




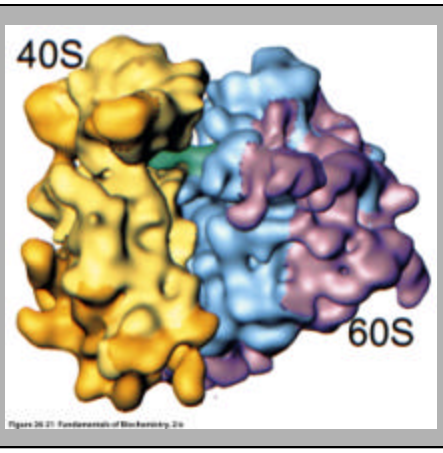


Prokaryotic Ribosomes

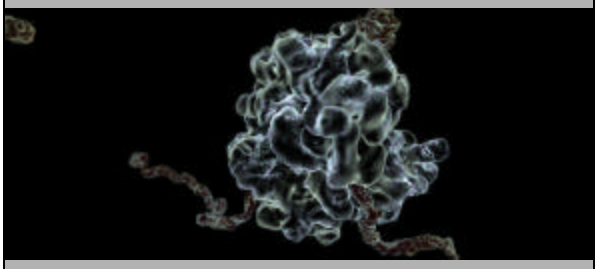
	SS-rRNA	LS-rRNA	Ribosome
Sedi-Coef	30S	50S	70S
RNA	1,542 nt, (16S)	2,904 nt, (23S)	
		120 nt, (5S)	60%
Protein mass %	40%	30%	34%

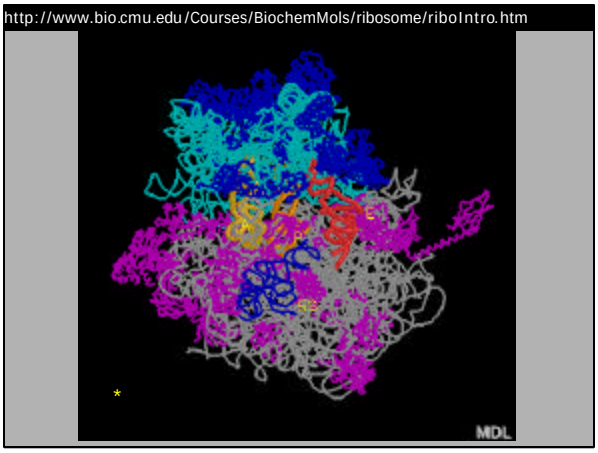
Eukaryotic Ribosomes

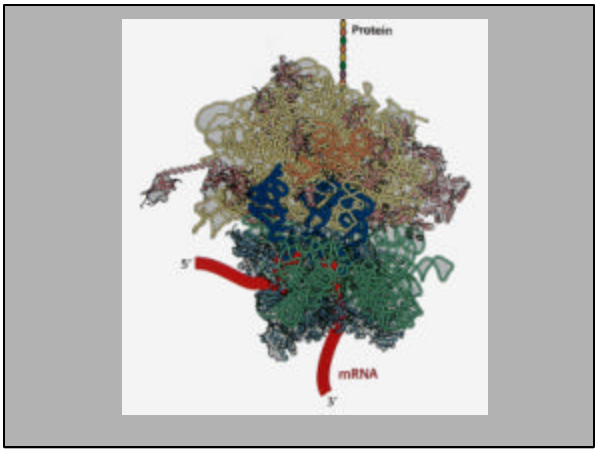
	SS-rRNA	LS-rRNA	Ribosome
Sedi-Coef	40S	60S	80S
RNA	1,874 nt, (18S)	4,718 nt, (28S)	
		160 nt, (5S)	60%
Protein mass %	50%	35%	40%

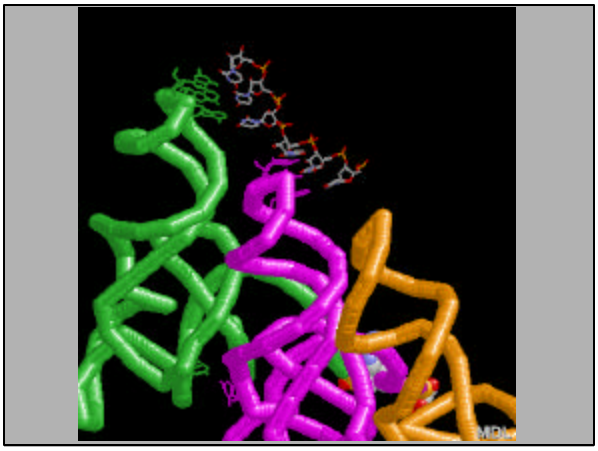


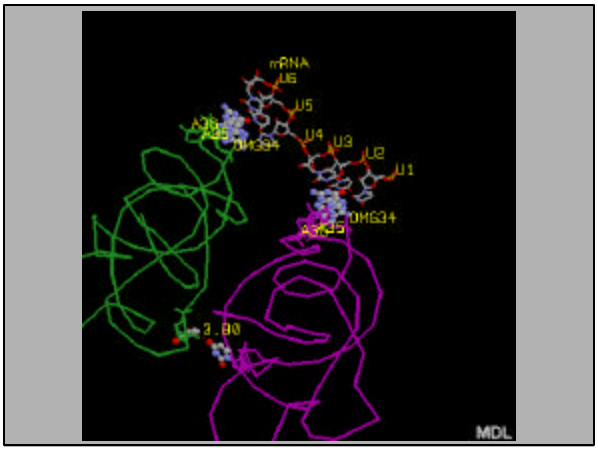
Protein Synthesis: TRANSLATION

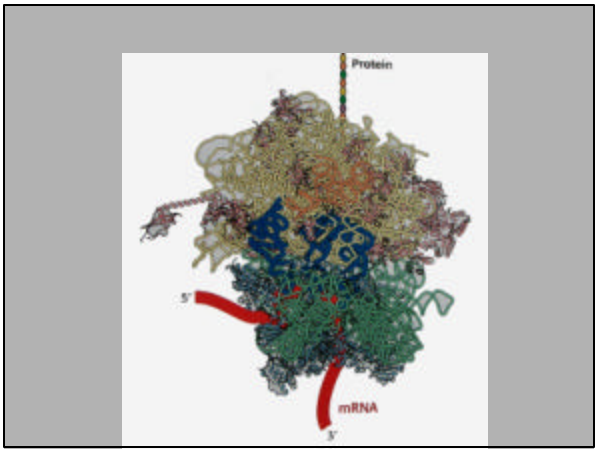


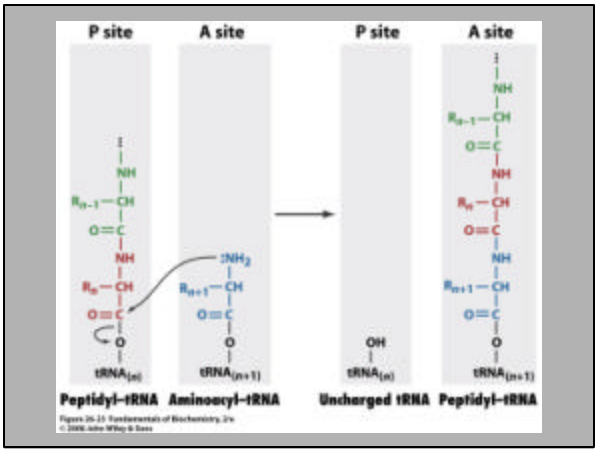

http://www.bio.cmu.edu/Courses/BiochemMols/ribosome/riboIntro.htm
MDL

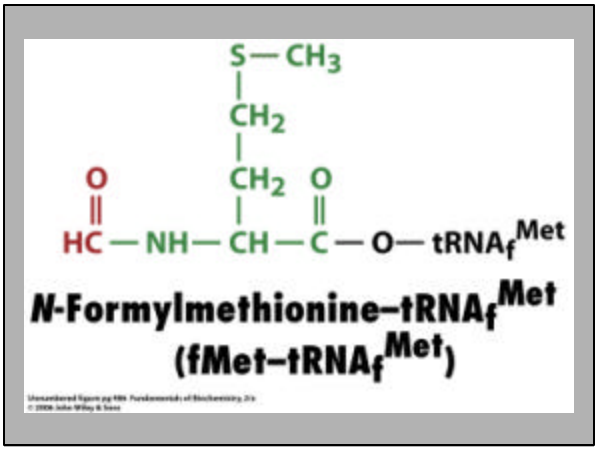

Protein
5'
mRNA
3'




mRNA
U6
U5
U4
U3
U2
U1
MDL







Shine-Delgado Sequence

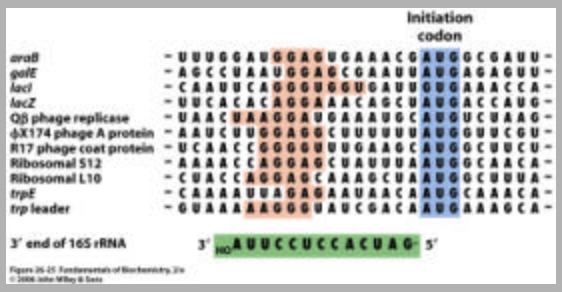


Table 26-6 The Soluble Protein Factors of *E. coli* Protein Synthesis

Factor	Number of Residues*	Function
Initiation Factors		
IF-1	71	Assists IF-3 binding
IF-2	890	Binds initiator tRNA and GTP
IF-3	180	Releases mRNA and tRNA from recycled 30S subunit and aids new mRNA binding
Elongation Factors		
EF-Tu	393	Binds aminoacyl-tRNA and GTP
EF-Ts	282	Displaces GDP from EF-Tu
EF-G	703	Promotes translocation through GTP binding and hydrolysis
Release Factors		
RF-1	360	Recognizes UAA and UAG Stop codons
RF-2	365	Recognizes UAA and UGA Stop codons
RF-3	528	Stimulates RF-1/RF-2 release via GTP hydrolysis
RRF	185	Together with EF-G, induces ribosomal dissociation to small and large subunits

*All *E. coli* translational factors are monomeric proteins.

Table 26-6 Fundamentals of Biochemistry, 2/e
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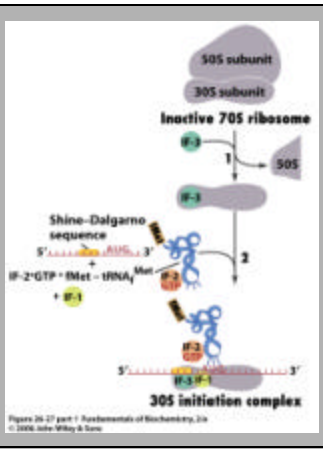

Figure 26-27 part 1 Fundamentals of Biochemistry, 2/e
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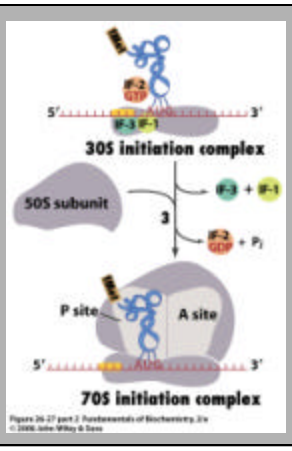

Figure 26-27 part 2 Fundamentals of Biochemistry, 2/e
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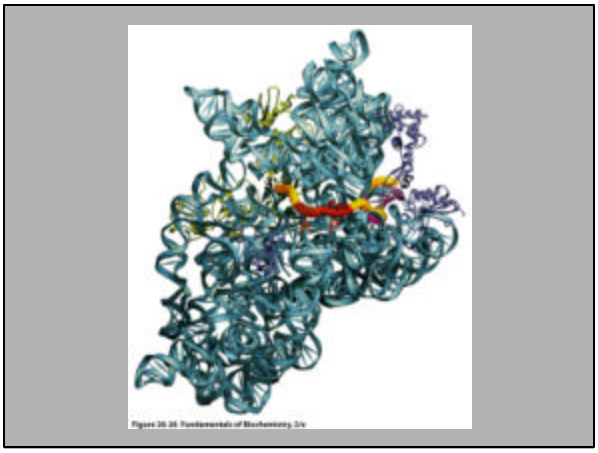
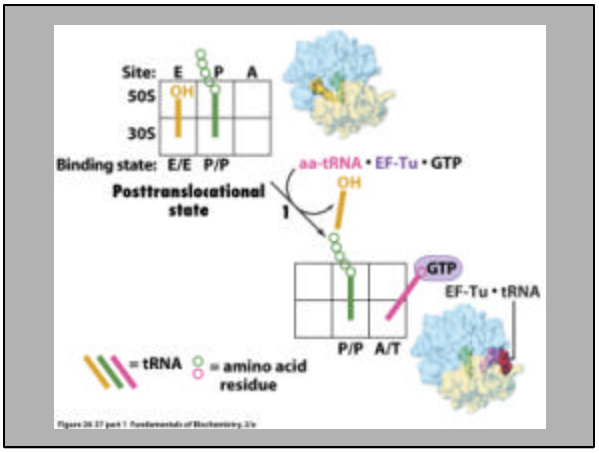
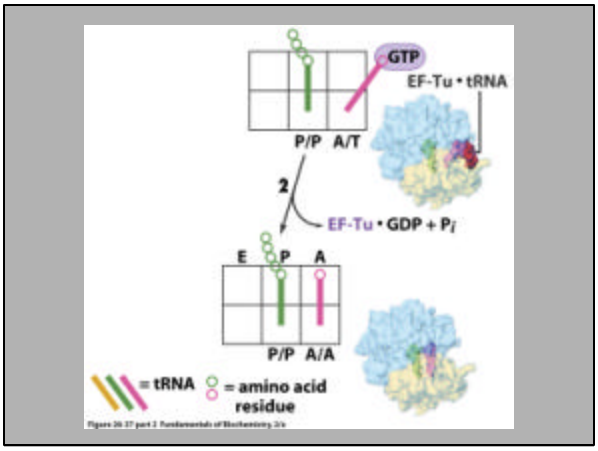
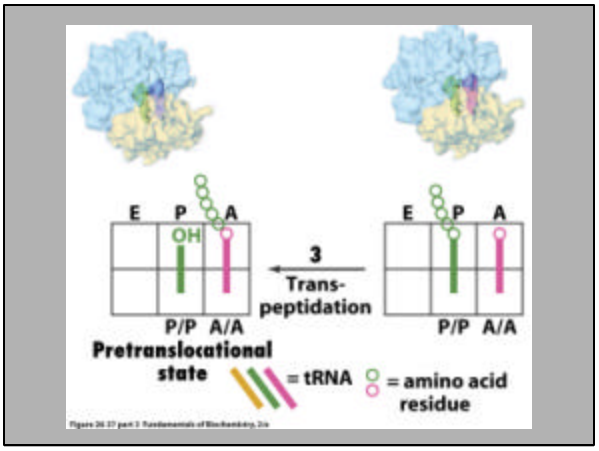
Initiation Factors

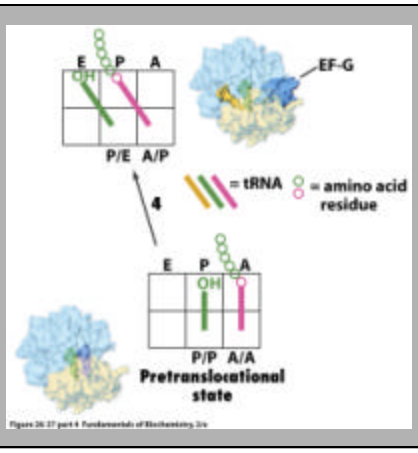
- IF-1:
  - assists with IF-3 binding
- IF-2:
  - Binds initiator tRNA and GTP
- IF-3:
  - Releases 30S subunit from inactive ribosome and promotes mRNA binding

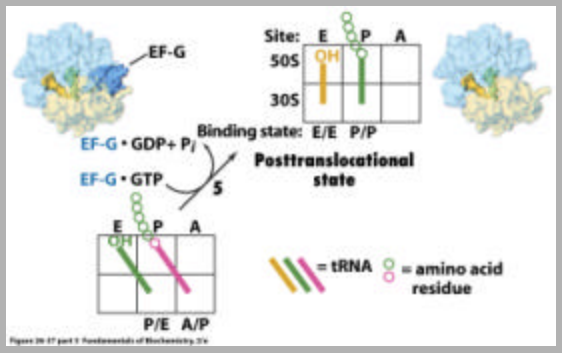


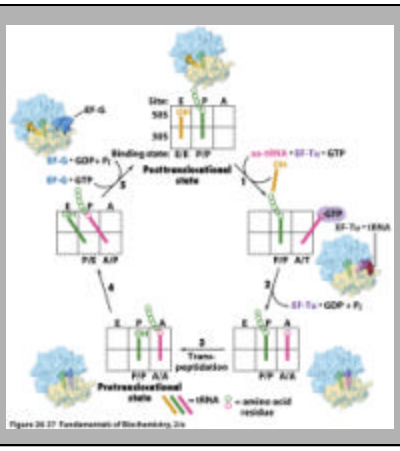

Site: E P A
50S
30S
OH
Binding state: E/E P/P
aa-tRNA • EF-Tu • GTP
Posttranslocational state
1
OH
GTP
EF-Tu • tRNA
P/P A/T
= tRNA
= amino acid residue


GTP
EF-Tu • tRNA
P/P A/T
2
EF-Tu • GDP + Pi
E P A
P/P A/A
= tRNA
= amino acid residue


E P A
OH
P/P A/A
3
Trans-peptidation
E P A
P/P A/A
Pretranslocational state
= tRNA
= amino acid residue

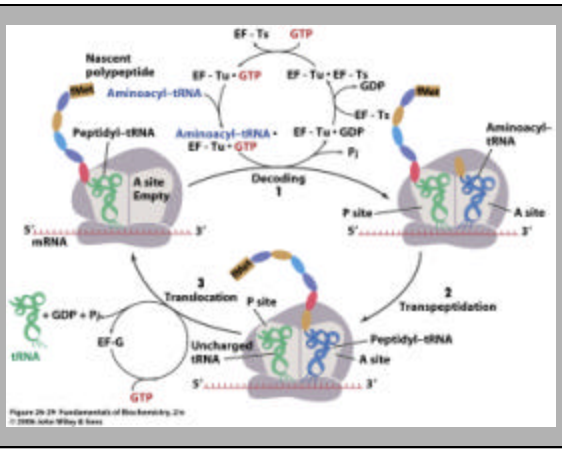




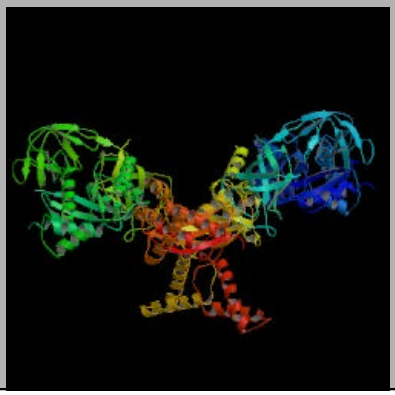


Elongation Factors

- EF-Tu:
  - Binds aminoacyl-tRNA and GTP
- EF-Ts:
  - Displaces GDP from EF-Tu (interaction)
- EF-G:
  - Promotes translocation by binding GTP to the ribosome for subsequent hydrolysis



EF-Tu and EF-Ts

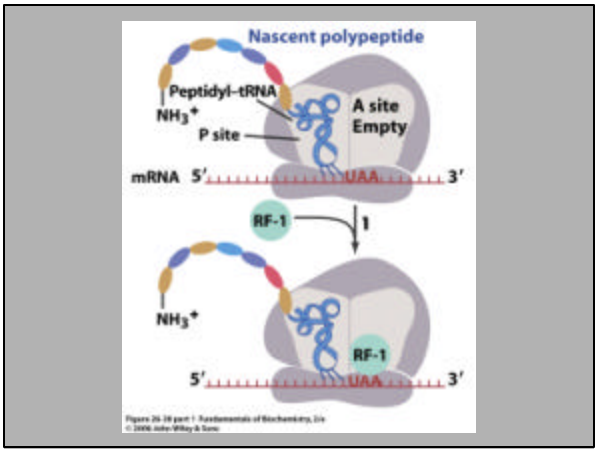


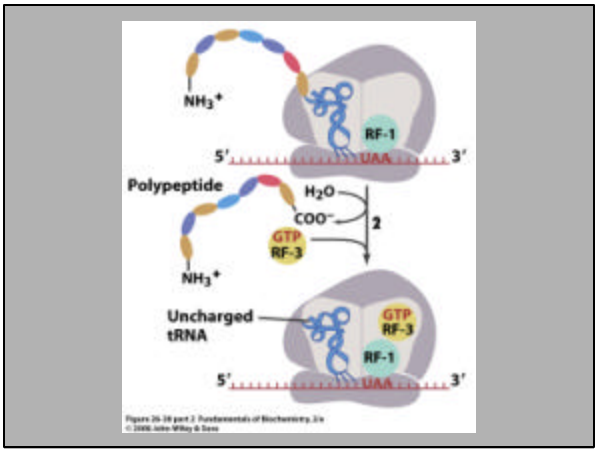
$$[\text{Ribosome}\,(n) \cdot \text{aa–tRNA} \cdot \text{EF-Tu} \cdot \text{GDP}]^{\circ}$$

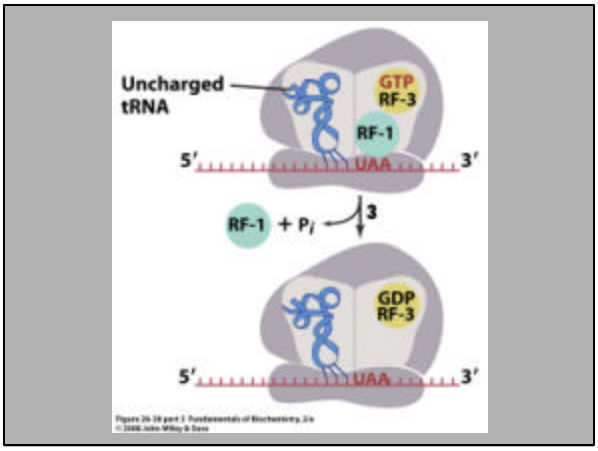
- EF-Tu establishes a proofreading mechanism
- In E.coli:
  - 100000 copies per cell
  - 5% total cellular protein
  - approximtely 1 EF-Tu per tRNA molecule

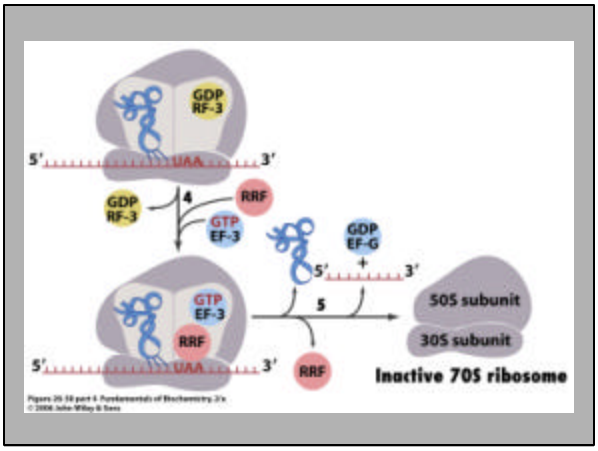
Release Factors

- RF-1:
  - Recognizes UAA and UAG stop codons
- RF-2:
  - Recognizes UAA and UGA stop codons
- RF-3:
  - Binds GTP and stimulates RF-1 & 2 binding


Nascent polypeptide
Peptidyl-tRNA
NH_3^+
A site
Empty
P site
mRNA 5′
UAA
3′
RF-1
1
NH_3^+
RF-1
5′
UAA
3′


NH_3^+
RF-1
5′
UAA
3′
Polypeptide
H_2O
COO^-
2
GTP
RF-3
NH_3^+
Uncharged
tRNA
GTP
RF-3
RF-1
5′
UAA
3′


Uncharged
tRNA
GTP
RF-3
RF-1
5′
UAA
3′
RF-1 + P_i
3
GDP
RF-3
5′
UAA
3′


GDP
RF-3
5′
UAA
3′
GDP
RF-3
4
RRF
GTP
EF-3
GDP
EF-G
+
5′
3′
GTP
EF-3
5
50S subunit
30S subunit
RRF
5′
UAA
3′
RRF
Inactive 70S ribosome

Cost of Protein Synthesis

- Protein synthesis is an ongoing cellular process to replace denatured and degraded cellular components.
- An average mammalian cell consumes 30-40% of it's basal metabolic rate on synthesizing proteins.