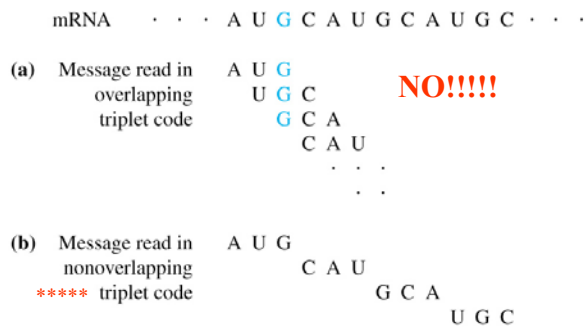




**Fig 22.1 Overlapping vs nonoverlapping reading of the three-letter code**

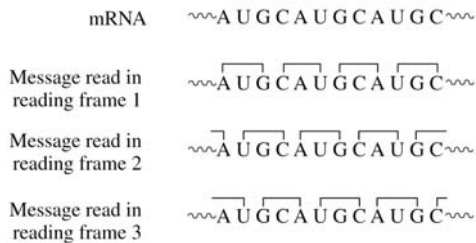


**Fig 22.3 Standard genetic code**

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	*STOP	*STOP	A
	Leu	Ser	*STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	*Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

**Fig 22.2 Three reading frames of mRNA**

- Translation of the correct message requires selection of the correct reading frame



**BCMB 3100 - Chapter 22 Translation**

- translation
- Genetic code
- tRNA
- Amino acyl tRNA
- Ribosomes
- Initiation
- Elongation
- Termination

**Fig. 22.4**

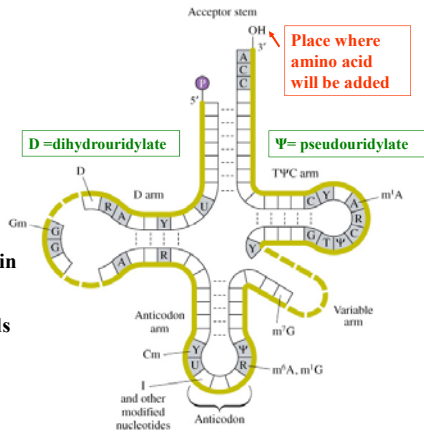
- Cloverleaf structure of tRNA

tRNAs 73-95 nucleotides long.

Anticodon base pairs with codon in mRNA.

3' end always ends in 3'ACC.....5'

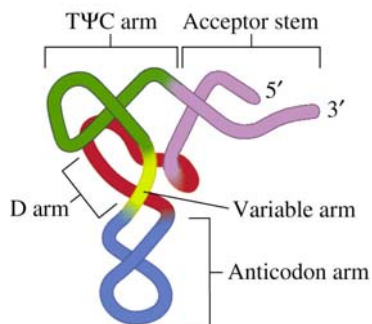
Amino acid is added to A at 3' end



## BCMB 3100 - Chapter 22 Translation

- translation
- Genetic code
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- Amino acyl tRNA
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- Elongation
- Termination

**Fig 22.5 Tertiary structure of tRNA**  
(see also Fig. 22.6)

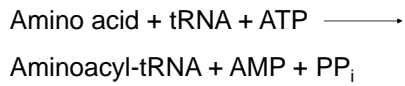


## Aminoacyl-tRNA Synthetases

- synthesize \_\_\_\_\_ (specific amino acid covalently attached to 3' end of specific tRNA (named as: alanyl-tRNA<sup>Ala</sup>)
- At least 20 different aminoacyl-tRNA synthetases (1 per amino acid)
- Each synthetase specific for a particular amino acid, but may recognize isoacceptor tRNAs
- Synonymous codons may be recognized by isoacceptor tRNAs (different tRNAs that attach the same amino acid) (bacteria have 30-60 different tRNAs)

### Aminoacyl-tRNA Synthetase Reaction

- Aminoacyl-tRNAs: high-energy molecules in which the amino acid has been “activated”
- Activation of amino acid by aminoacyl-tRNA synthetase requires ATP



Summary of overall reaction, note **however**, the reaction actually takes place in two steps.

Fig 22.9

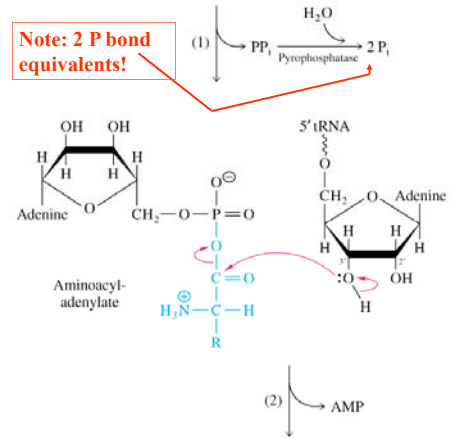


Fig 22.9

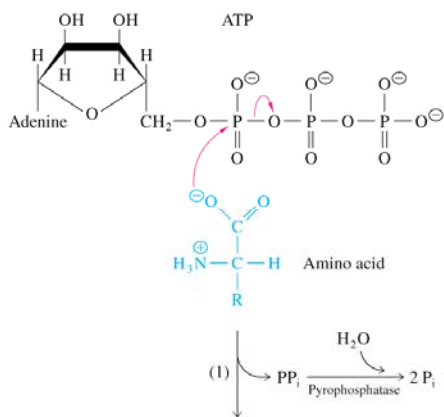
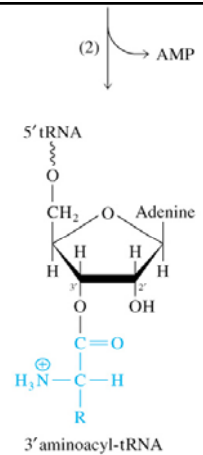


Fig 22.9

Step 1:  $\text{ATP} + \text{amino acid} \rightarrow \text{aminoacyl-adenylate intermediate} + \text{PP}_i$

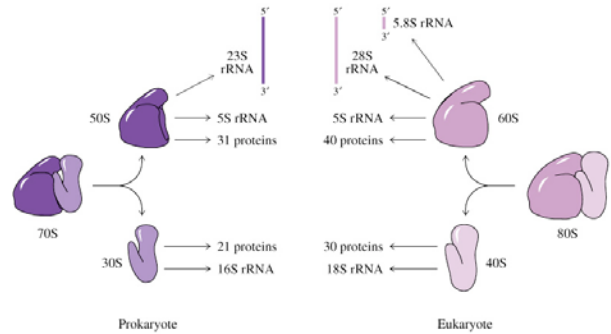
Step 2:  
 $\text{aminoacyl-adenylate} + \text{tRNA} \rightarrow \text{aminoacyl-tRNA} + \text{AMP}$



**BCMB 3100 - Chapter 22**  
**Translation**

- translation
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- tRNA
- Amino acyl tRNA
- Ribosomes
- Initiation
- Elongation
- Termination

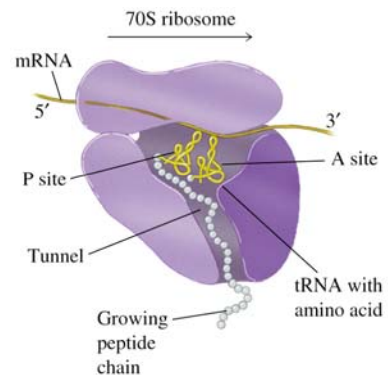
**Fig 22.12 Comparison of prokaryotic and eukaryotic ribosomes**



**Ribosomes**

- \_\_\_\_\_: RNA-protein complex that interacts with accessory protein factors, mRNA and charged tRNA to synthesize proteins
- **Initiation complex**: assembles at first mRNA codon; disassembles at termination step
- **Ribosome moves 5' → 3' along mRNA**
- **Polypeptide** synthesized in **N → C direction**

**Fig 22.15 Sites for tRNA binding in ribosomes**



## BCMB 3100 - Chapter 22 Translation

- translation
- Genetic code
- tRNA
- Amino acyl tRNA
- Ribosomes
- **Initiation**
- Elongation
- Termination

### Initiation Complexes Assemble at Initiation Codons

In prokaryotes 30S ribosome binds to a region of the mRNA (Shine-Dalgarno sequence; purine-rich sequence) upstream of the initiation sequence

- Ribosome-binding sites at 5' end of mRNA for *E. coli* proteins
- S-D sequences (red) occur immediately upstream of initiation codons (blue)

Fig 22.17



### Initiation: Structure of fMet-tRNA<sup>Met</sup>

\*First codon in mRNA is usually AUG

\*recognized by **initiator tRNA**

\***Bacteria:** N-formylmethionyl-tRNA<sup>fMet</sup>

**Eukaryotes:** methionyl-tRNA<sup>iMet</sup>

Fig 22.16

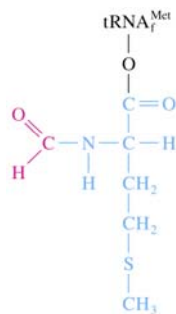
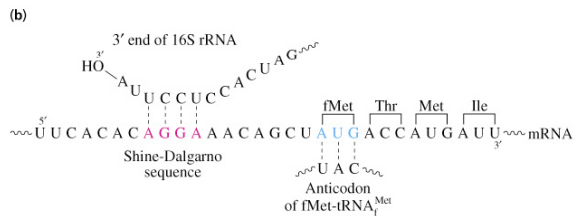
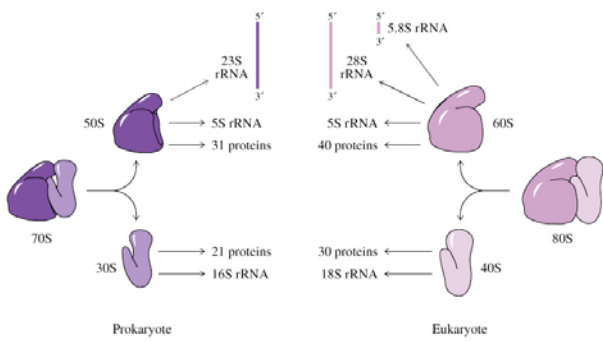


Fig 22.17

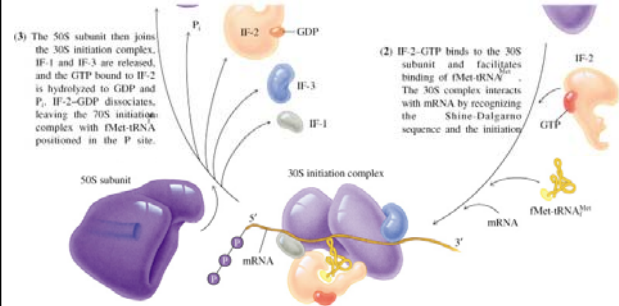
- Complementary base pairing of S-D sequence



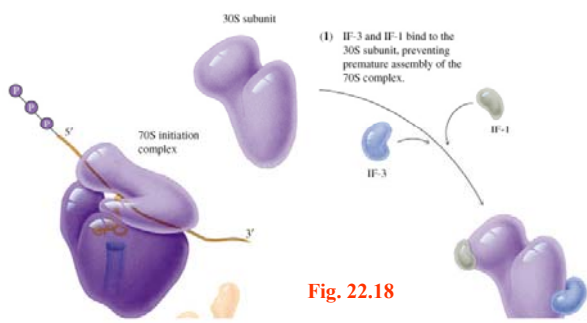
**Fig 22.12 Comparison of prokaryotic and eukaryotic ribosomes**



**Fig 22.18 (cont)**



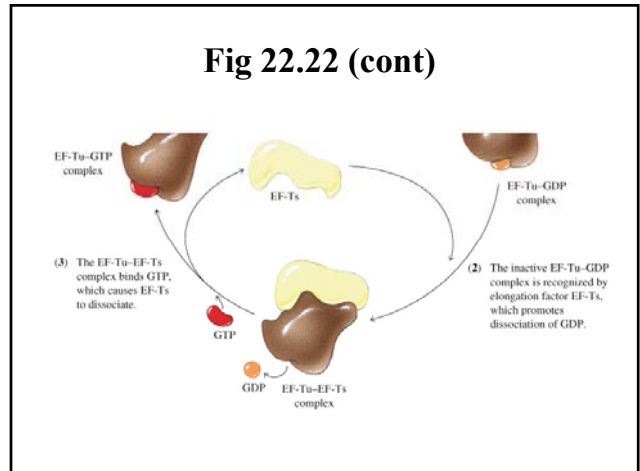
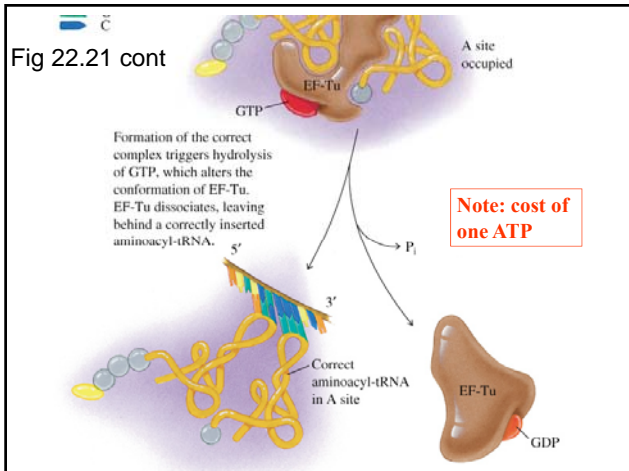
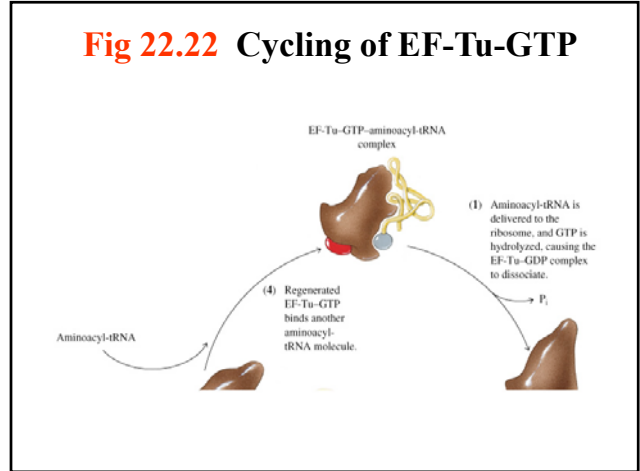
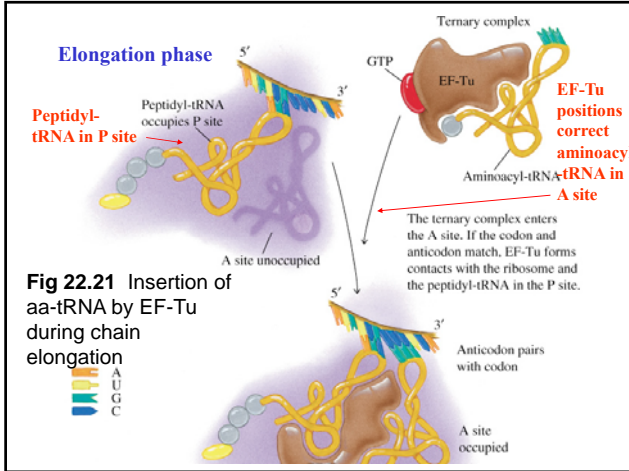
**Initiation: formation of the prokaryotic 70S ribosome**  
**Initiation factors are required to form a complex**  
**(IF-1, IF-2, IF 3 in prokaryotes)**



**Fig. 22.18**

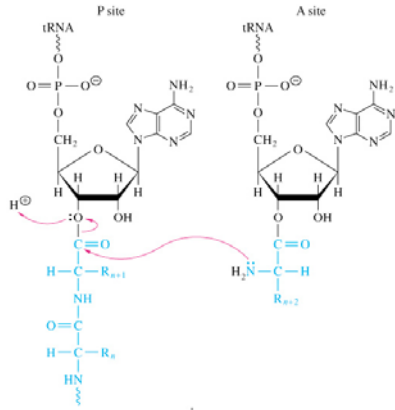
**BCMB 3100 - Chapter 22**  
**Translation**

- translation
- Genetic code
- tRNA
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- Elongation
- Termination



Formation of a peptide bond catalyzed by **Peptidyl transferase** (activity in large ribosomal subunit)

**Catalytic activity from 23S rRNA** (an RNA-catalyzed reaction!)

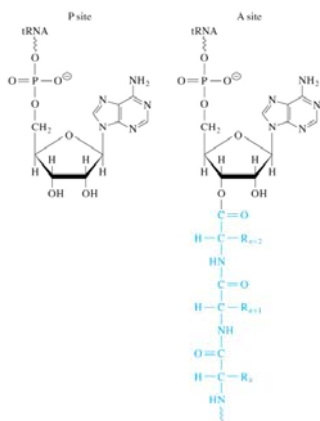


**RNA has the catalytic activity of the ribosome large subunit**

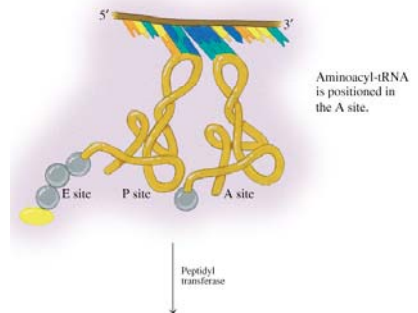
Atomic resolution crystal structures of the large subunit published since the middle of August 2000 prove that **the peptidyl transferase center of the ribosome, which is the site of peptide-bond formation, is composed entirely of RNA; the ribosome is a ribozyme.** They also demonstrate that alignment of the CCA ends of ribosome-bound peptidyl tRNA and aminoacyl tRNA in the peptidyl transferase center contributes significantly to its catalytic power.

Moore P.B. and Steitz T.A. (2003) PNAS

**Fig 22.23 (cont)**



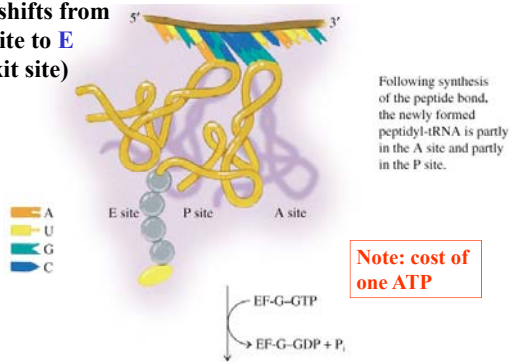
**Translocation step: new peptidyl-tRNA moved from A site to P site; mRNA shifts by one codon**



**Fig 22.24**

**Deaminoacylated tRNA shifts from the P site to E site (exit site)**

**Fig 22.24**

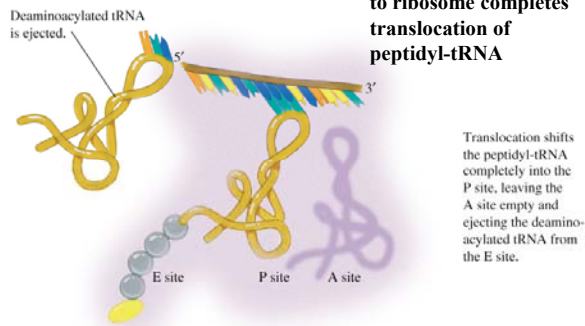


**BCMB 3100 - Chapter 22  
Translation**

- translation
- Genetic code
- tRNA
- Amino acyl tRNA
- Ribosomes
- Initiation
- Elongation
- Termination

**Fig 22.24**

**Binding of EF-G-GTP to ribosome completes translocation of peptidyl-tRNA**



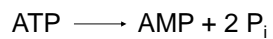
**Termination of Translation**

- One of three termination codons binds to A site: **UGA, UAG, UAA**
- No tRNA molecules recognize these codons; protein synthesis stalls
- One of release factors (**RF-1, RF-2, RF-3 in *E.coli***) binds and causes hydrolysis of the peptidyl-tRNA to release the polypeptide chain

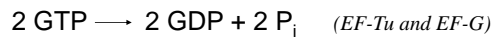
**Protein Synthesis is Energetically Expensive**

**Four phosphoanhydride bonds cleaved for each amino acid added to polypeptide chain**

Amino acid activation: Two ~P bonds



Chain elongation: Two ~P bonds



**Enjoy metabolism!!!**

**Comparison of rates of translation, transcription and DNA replication in *E. coli*.**

**Rate of protein synthesis: 18 aa/sec**

**Rate of transcription: 30-85 nucleotides/sec**

**Rate of DNA synthesis: 1000 nucleotides/sec**

**Additional Class Notes**

### Aminoacyl-tRNA Synthetases

- **Aminoacyl-tRNA** - amino acids are covalently attached to the 3' end of each tRNA molecule (named as: alanyl-tRNA<sup>Ala</sup>)
- Aminoacyl-tRNA synthetases catalyze reactions
- Most species have at least 20 different aminoacyl-tRNA synthetases (1 per amino acid)
- Each synthetase specific for a particular amino acid, but may recognize isoacceptor tRNAs

### Initiation Complexes Assemble Only at Initiation Codons

- Ribosome must recognize protein synthesis start
- In **prokaryotes**, the 30S ribosome binds to a region of the mRNA (**Shine-Dalgarno sequence**; **purine-rich sequence**) upstream of the initiation sequence
- S-D sequence also binds to a complementary base sequence at the 3' end of the 16S rRNA
- Double-stranded RNA structure binds mRNA to the ribosome

### Initiator tRNA

- First codon translated is usually AUG
- Each cell contains at least two methionyl-tRNA<sup>Met</sup> molecules which recognize AUG
- The **initiator tRNA** recognizes initiation codons
- Second tRNA<sup>Met</sup> recognizes only internal AUG
- **Bacteria**: **N-formylmethionyl-tRNA<sub>f</sub><sup>Met</sup>**
- **Eukaryotes**: **methionyl-tRNA<sub>i</sub><sup>Met</sup>**

### Initiation Factors Help Form Initiation Complex

- **Initiation factors** are required to form a complex
- **Prokaryote factors**: IF-1, IF-2, IF-3
- **Eukaryote factors**: eIFs (8 or more factors)

### Elongation Factors Dock an Aminoacyl-tRNA in the A Site

- Bacterial elongation factor EF-Tu helps the correct aa-tRNA insert into site A
- An EF-Tu-GTP complex binds to all aa-tRNA molecules except fMet-tRNA<sup>fMet</sup> (initiator)
- A ternary complex of **EF-Tu-GTP-aa-tRNA** binds in the ribosomal A site
- If the anticodon of the aa-tRNA correctly base pairs with the mRNA codon, complex is stabilized

### Peptidyl Transferase Catalyzes Peptide Bond Formation

- **Peptidyl transferase** activity is contained within the large ribosomal subunit
- Substrate binding site in 23S rRNA and 50S ribosomal proteins
- Catalytic activity from 23S rRNA (an RNA-catalyzed reaction)

### Chain Elongation is a Three-Step Microcycle

- The initiator tRNA is in the P site
- Site A is ready to receive an aminoacyl-tRNA
- Elongation is a three-step cycle:
  - (1) Positioning the correct aa-tRNA in site A
  - (2) Formation of a peptide bond
  - (3) Shifting mRNA by one codon

### Translocation Moves the Ribosome by One Codon

- **Translocation step**: the new peptidyl-tRNA is moved from the A site to the P site, while the mRNA shifts by one codon
- The deaminoacylated tRNA has shifted from the P site to the **E site** (exit site)
- Binding of EF-G-GTP to the ribosome completes translocation of peptidyl-tRNA