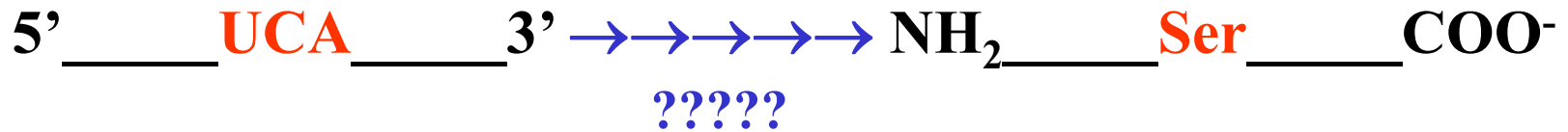
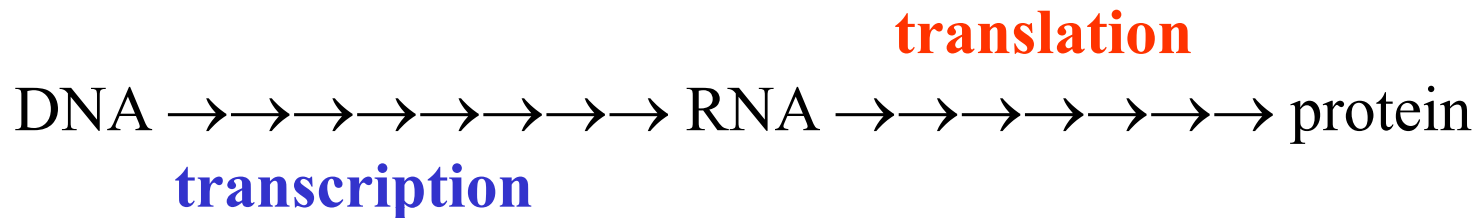


BCMB 3100 - Chapter 22

Translation

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

How is the nucleotide code translated into a protein code?



Adapter Molecule Hypothesis (Crick, 1958)

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Translation

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3 letter code 1st proposed
by George Gamow

2 letter RNA code: $4^2 = 16$

3 letter RNA code: $4^3 = 64$

4 letter RNA code: $4^4 = 254$

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

mRNA . . . A U G C A U G C A U G C . . .

(a) Message read in overlapping triplet code

A U G
 U G C
 G C A
 C A U
 . . .
 . .

NO!!!!!!

(b) Message read in nonoverlapping triplet code

A U G
 C A U
 G C A
 U G C

Fig 22.2 Three reading frames of mRNA

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

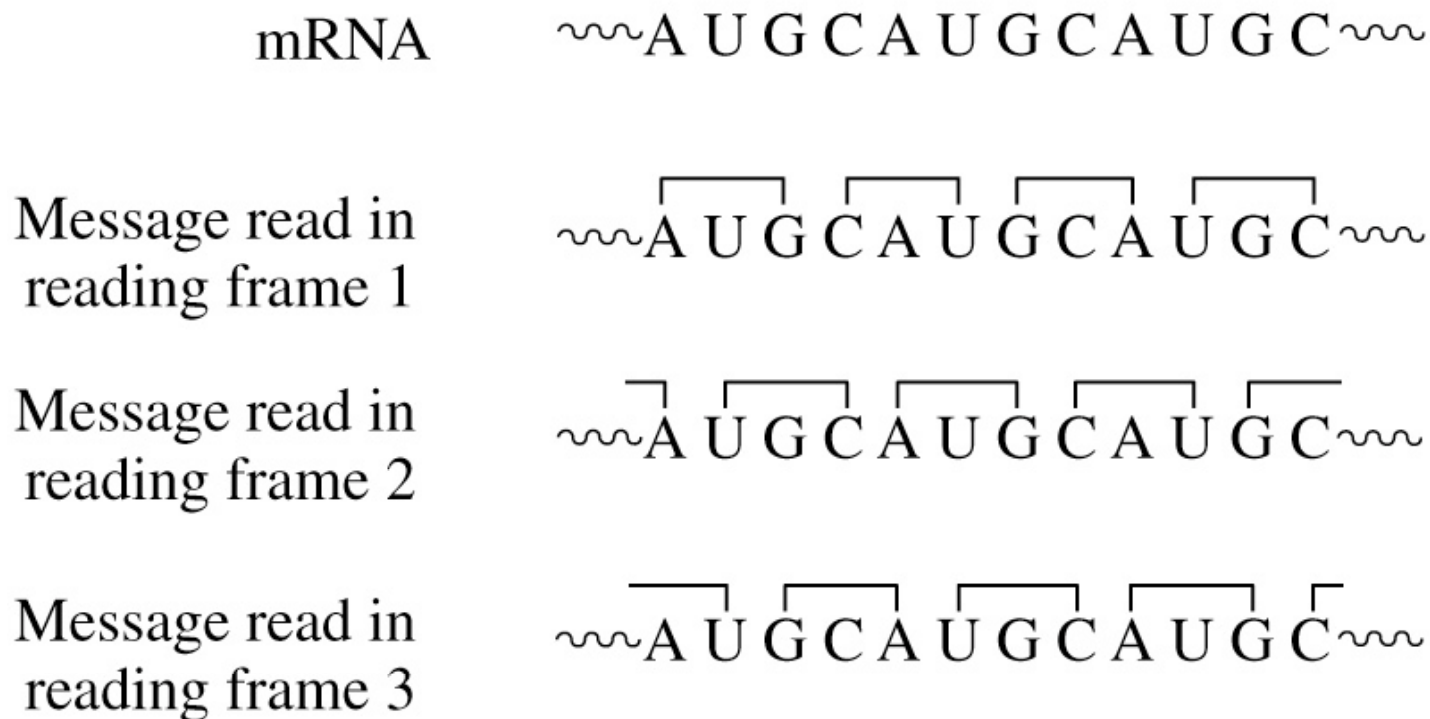


Fig 22.3 Standard genetic code

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

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Translation

- translation
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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

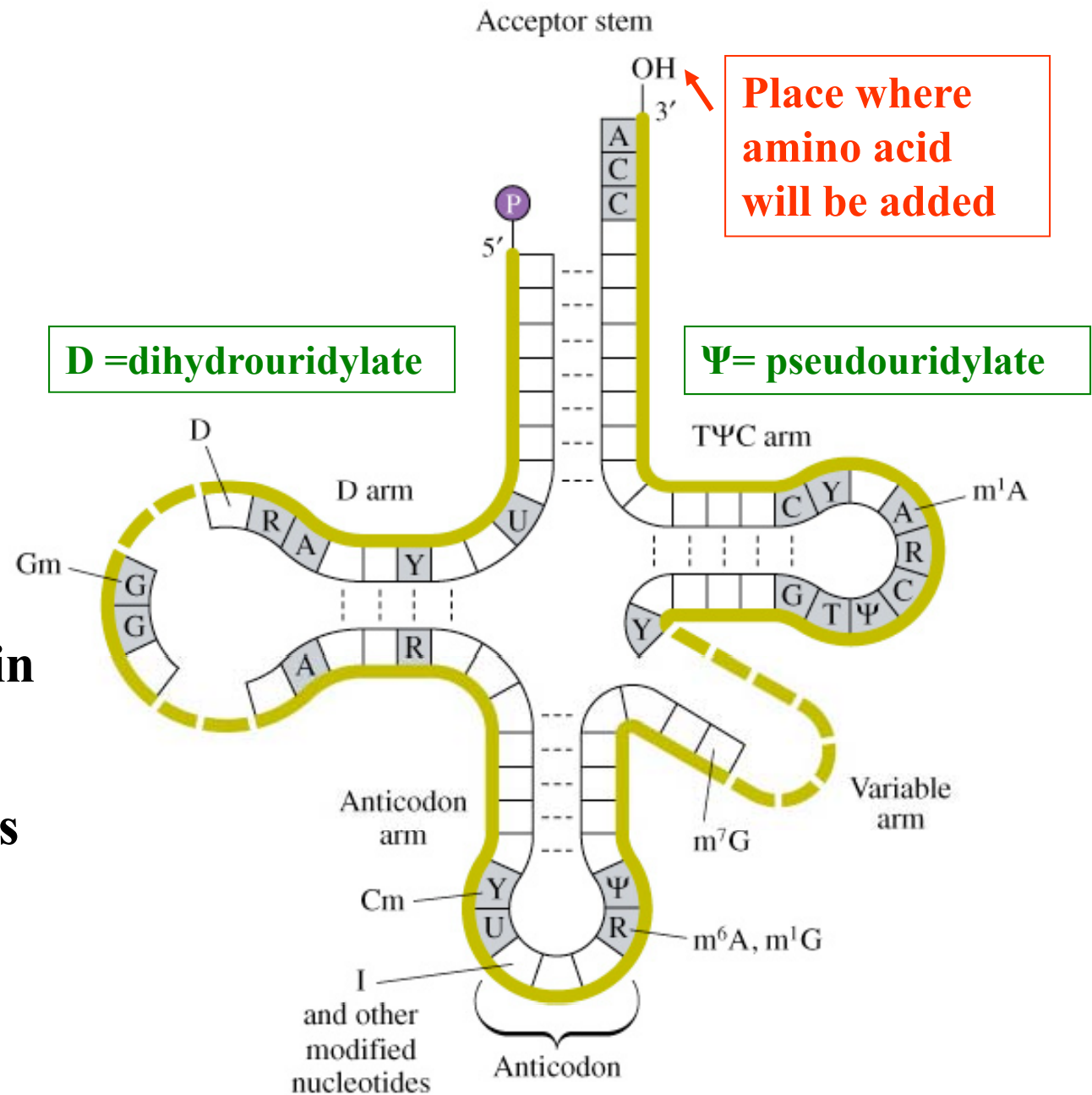
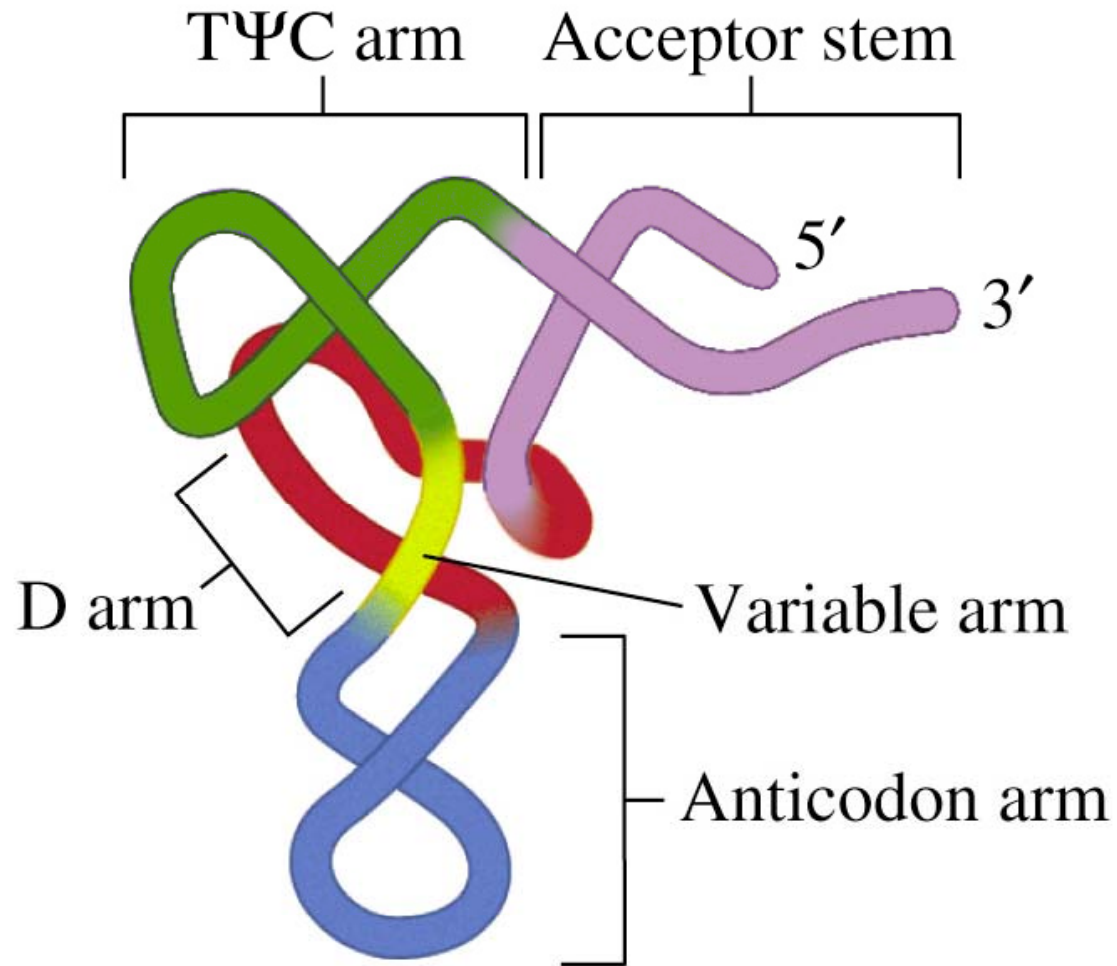


Fig 22.5 Tertiary structure of tRNA
(see also Fig. 22.6)



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Translation

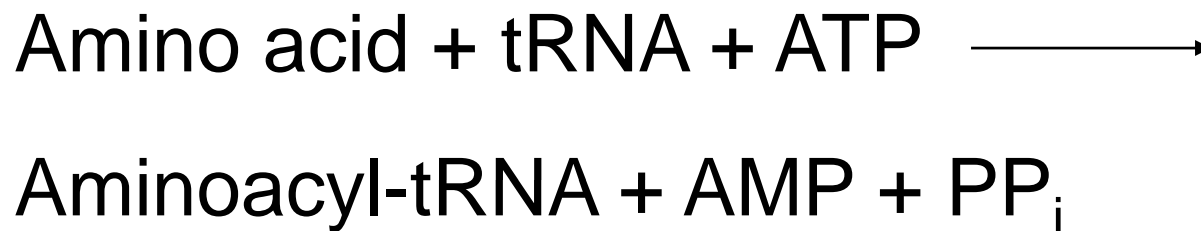
- translation
- Genetic code
- tRNA
- Amino acyl tRNA
- Ribosomes
- Initiation
- Elongation
- Termination

Aminoacyl-tRNA Synthetases

- synthesize _____ (specific amino acid covalently attached to 3' end of specific tRNA (named as: alanyl-tRNA^{Ala})
- 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

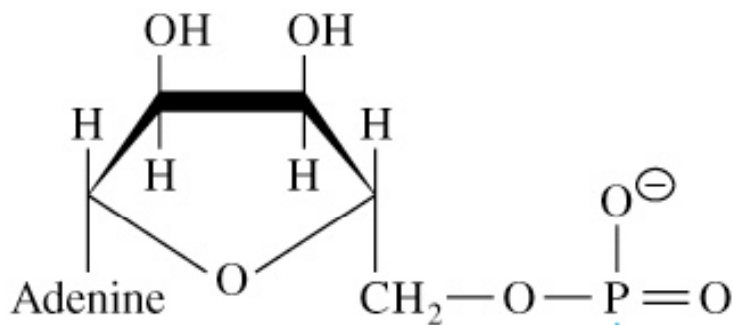
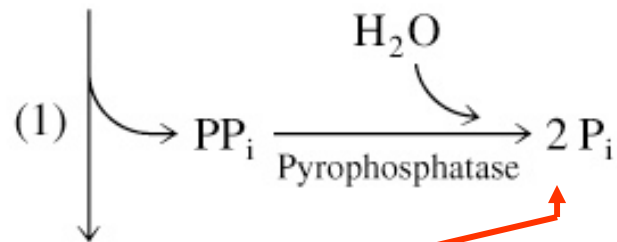
- Aminoacyl-tRNAs: high-energy molecules in which the amino acid has been “activated”
- Activation of amino acid by aminoacyl-tRNA synthetase requires ATP



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

Fig 22.9

Note: 2 P bond equivalents!



Aminoacyl-adenylate

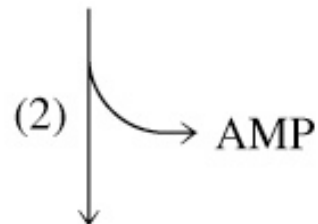
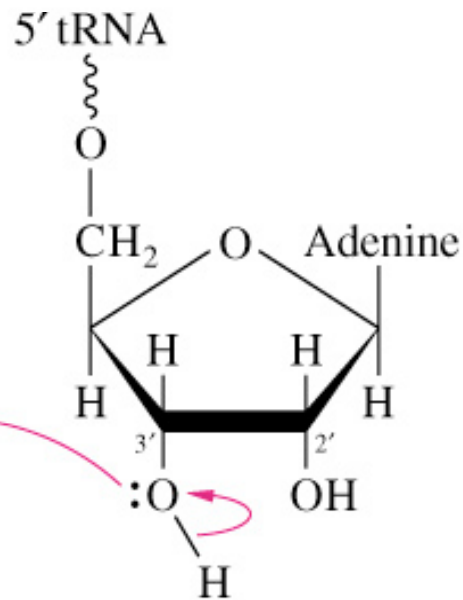
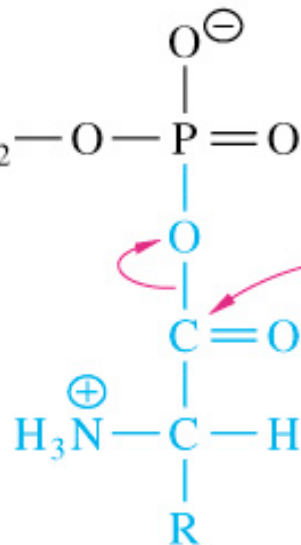
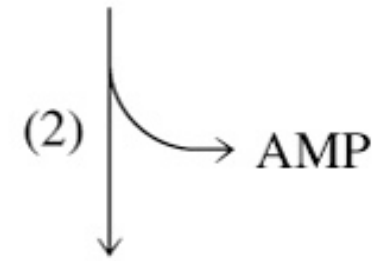
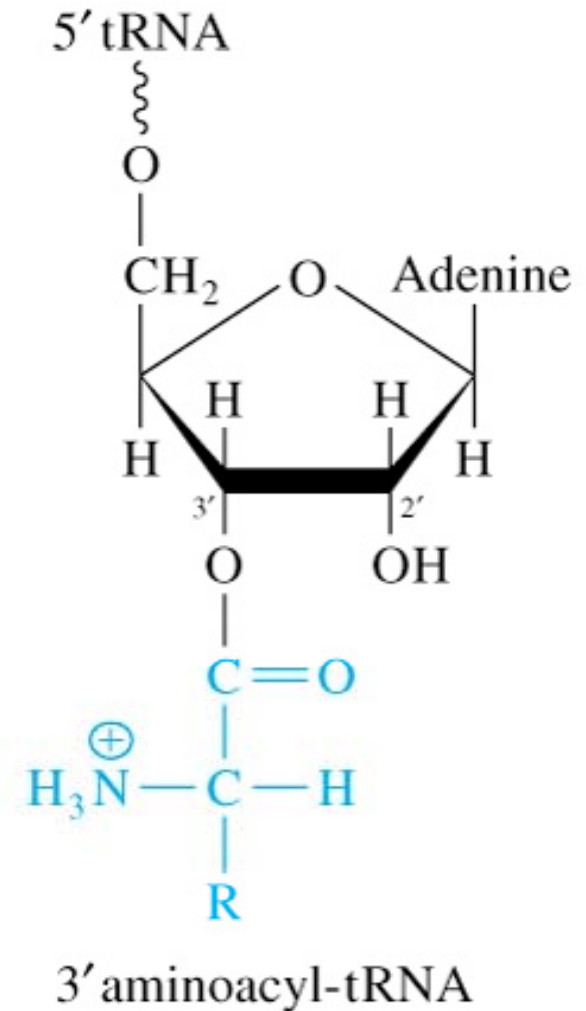


Fig 22.9



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

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



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Translation

- translation
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Fig 22.12 Comparison of prokaryotic and eukaryotic ribosomes

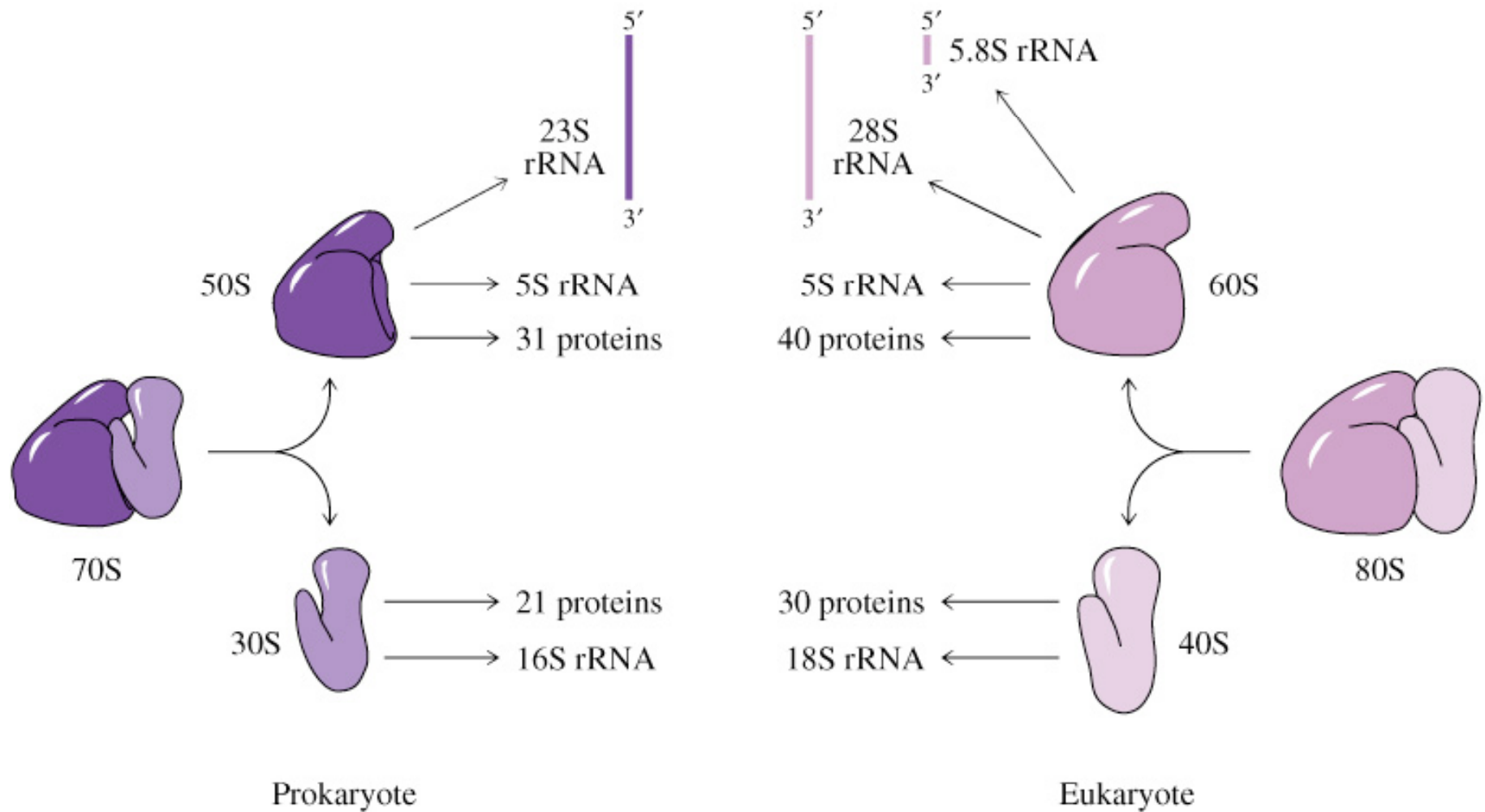
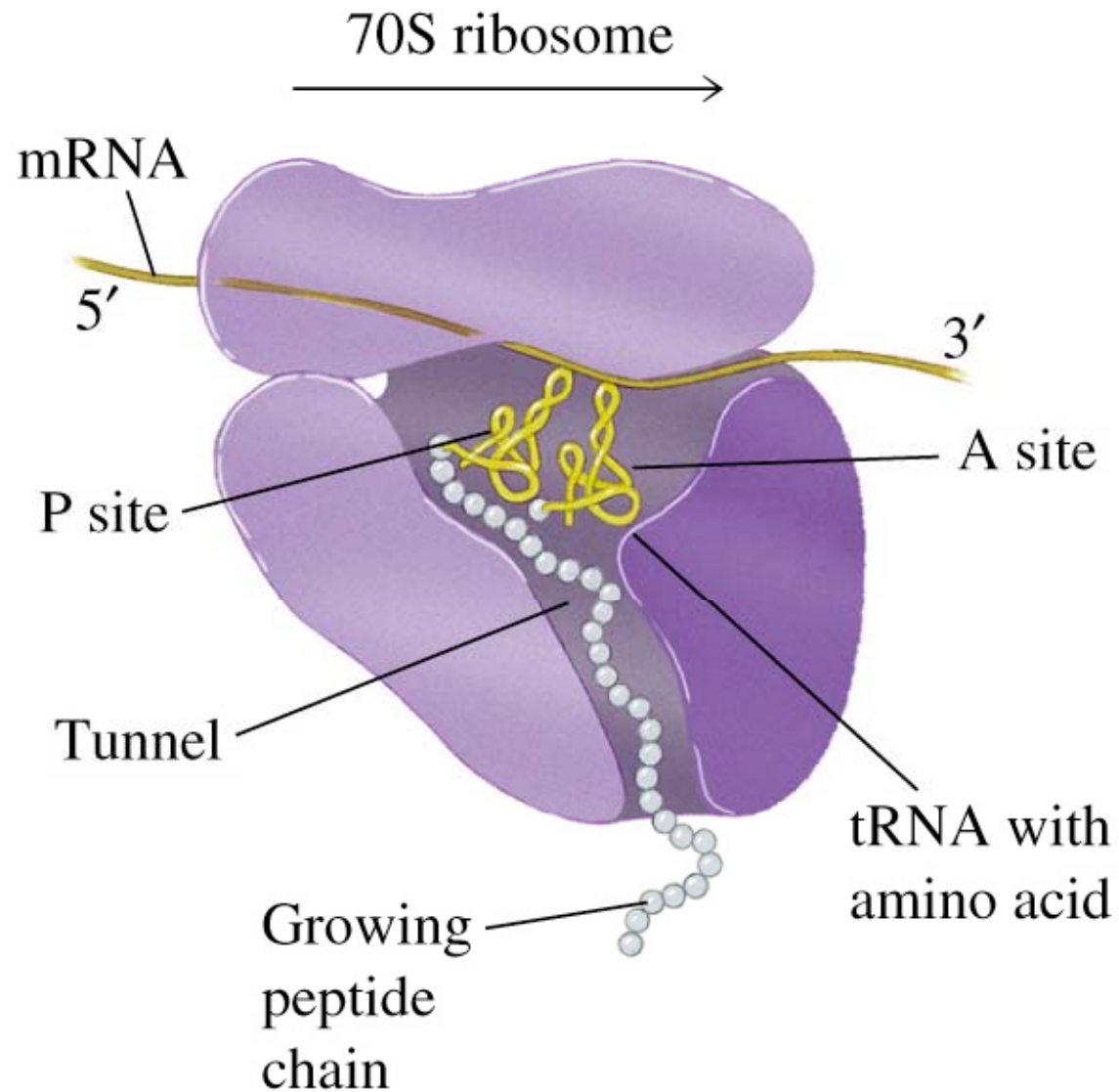


Fig 22.15 Sites for tRNA binding in ribosomes



BCMB 3100 - Chapter 22

Translation

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

Initiation: Structure of fMet-tRNA_f^{Met}

*First codon in mRNA is usually AUG

*recognized by **initiator tRNA**

*Bacteria: **N-formylmethionyl-tRNA_fMet**

Eukaryotes: **methionyl-tRNA_iMet**

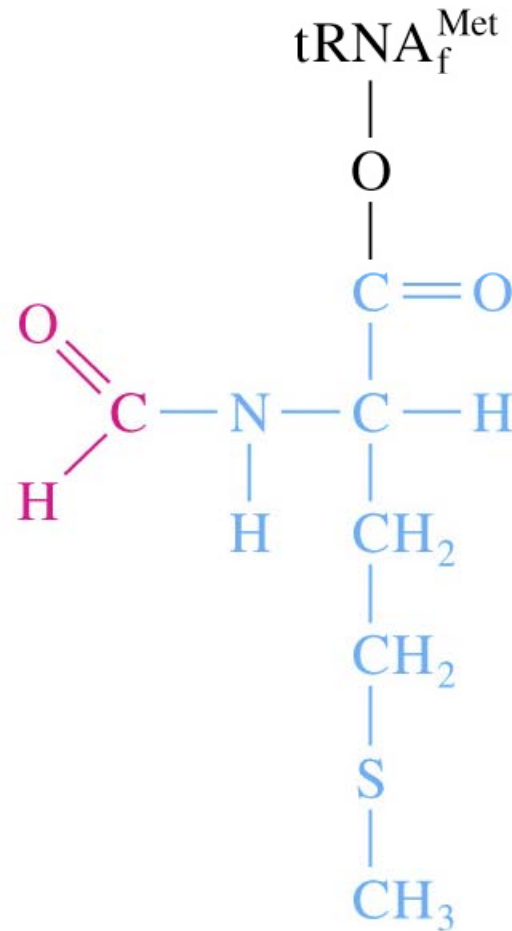


Fig 22.16

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

(a)



Fig 22.17

- Complementary base pairing of S-D sequence

(b)

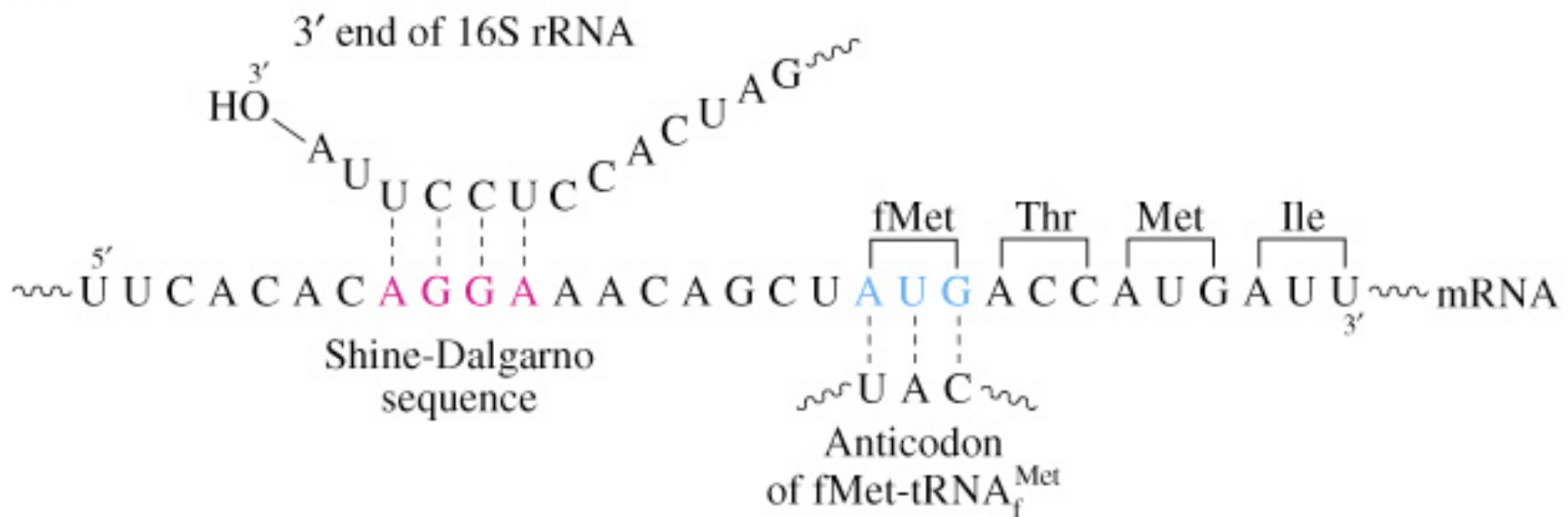
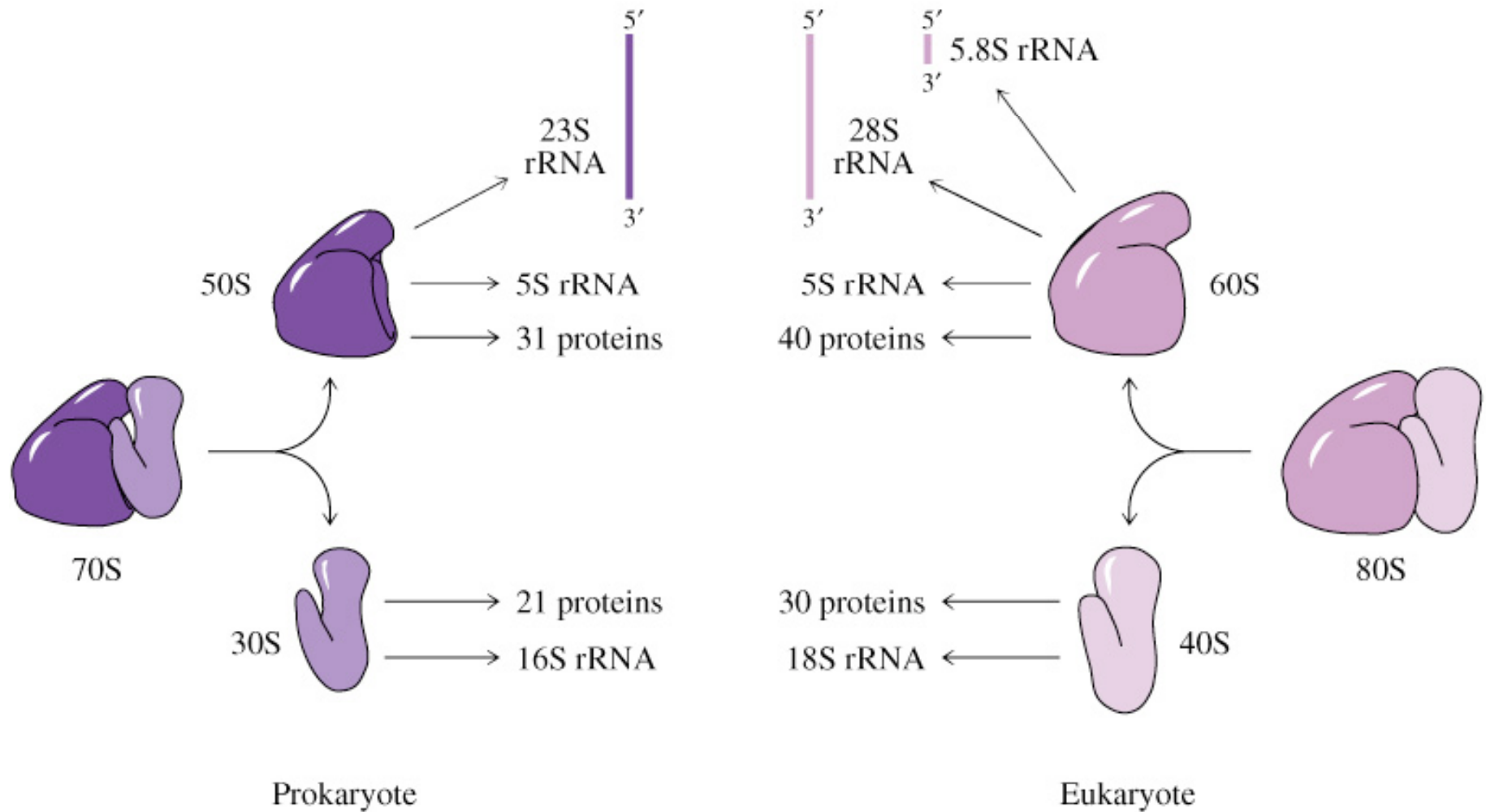


Fig 22.12 Comparison of prokaryotic and eukaryotic ribosomes



Initiation: formation of the prokaryotic 70S ribosome

Initiation factors are required to form a complex
(IF-1, IF-2, IF 3 in prokaryotes)

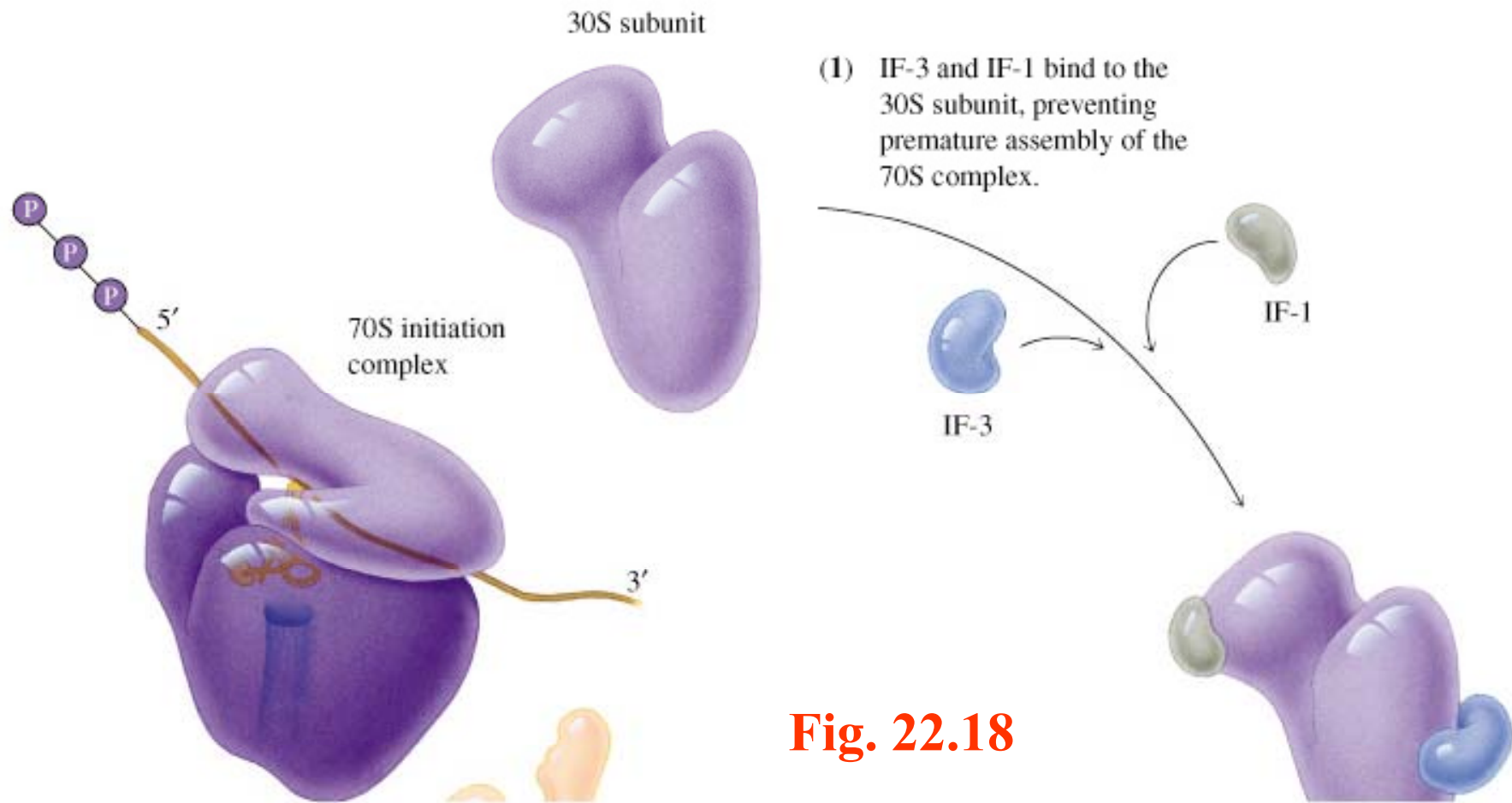
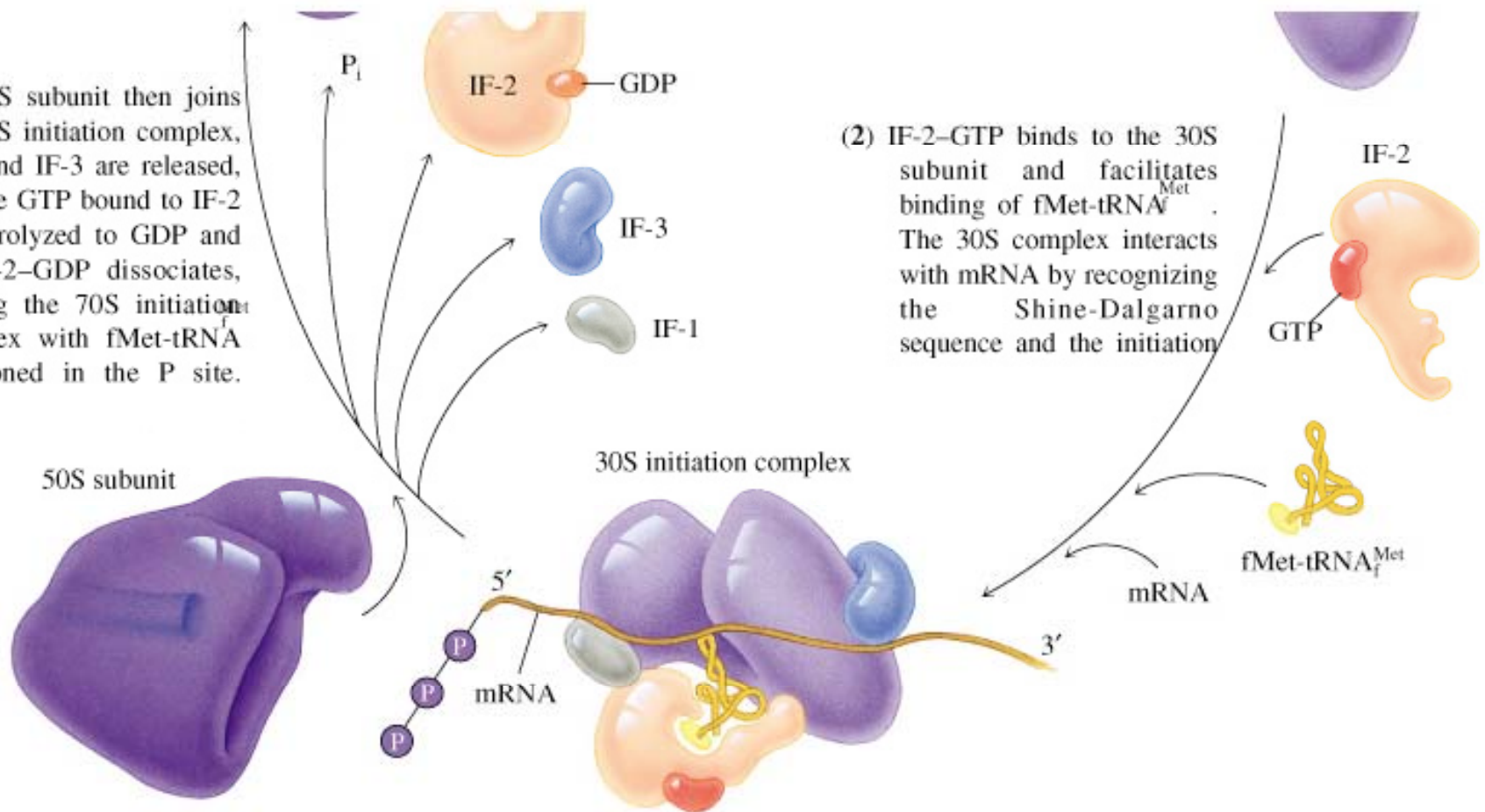


Fig 22.18 (cont)

(3) The 50S subunit then joins the 30S initiation complex, IF-1 and IF-3 are released, and the GTP bound to IF-2 is hydrolyzed to GDP and P_i . IF-2-GDP dissociates, leaving the 70S initiation complex with fMet-tRNA^{Met} positioned in the P site.

(2) IF-2-GTP binds to the 30S subunit and facilitates binding of fMet-tRNA^{Met}. The 30S complex interacts with mRNA by recognizing the Shine-Dalgarno sequence and the initiation



BCMB 3100 - Chapter 22

Translation

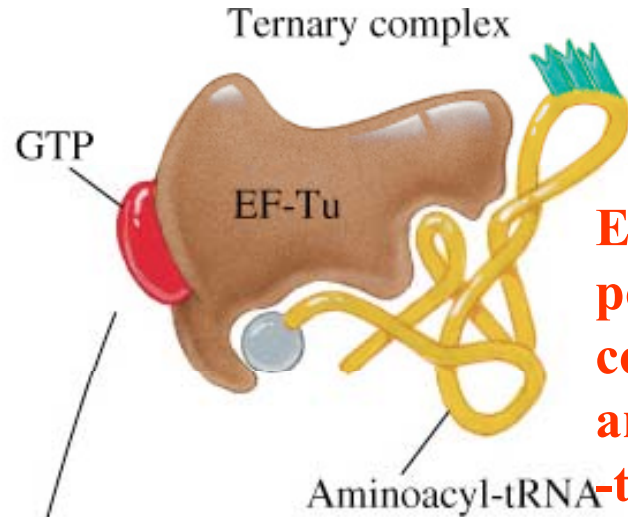
- **translation**
- **Genetic code**
- **tRNA**
- **Amino acyl tRNA**
- **Ribosomes**
- **Initiation**
- **Elongation**
- **Termination**

Elongation phase

Peptidyl-tRNA in P site

Peptidyl-tRNA occupies P site

A site unoccupied



EF-Tu positions correct aminoacyl-tRNA in A site

The ternary complex enters the A site. If the codon and anticodon match, EF-Tu forms contacts with the ribosome and the peptidyl-tRNA in the P site.

Fig 22.21 Insertion of aa-tRNA by EF-Tu during chain elongation



Anticodon pairs with codon

A site occupied



Fig 22.21 cont

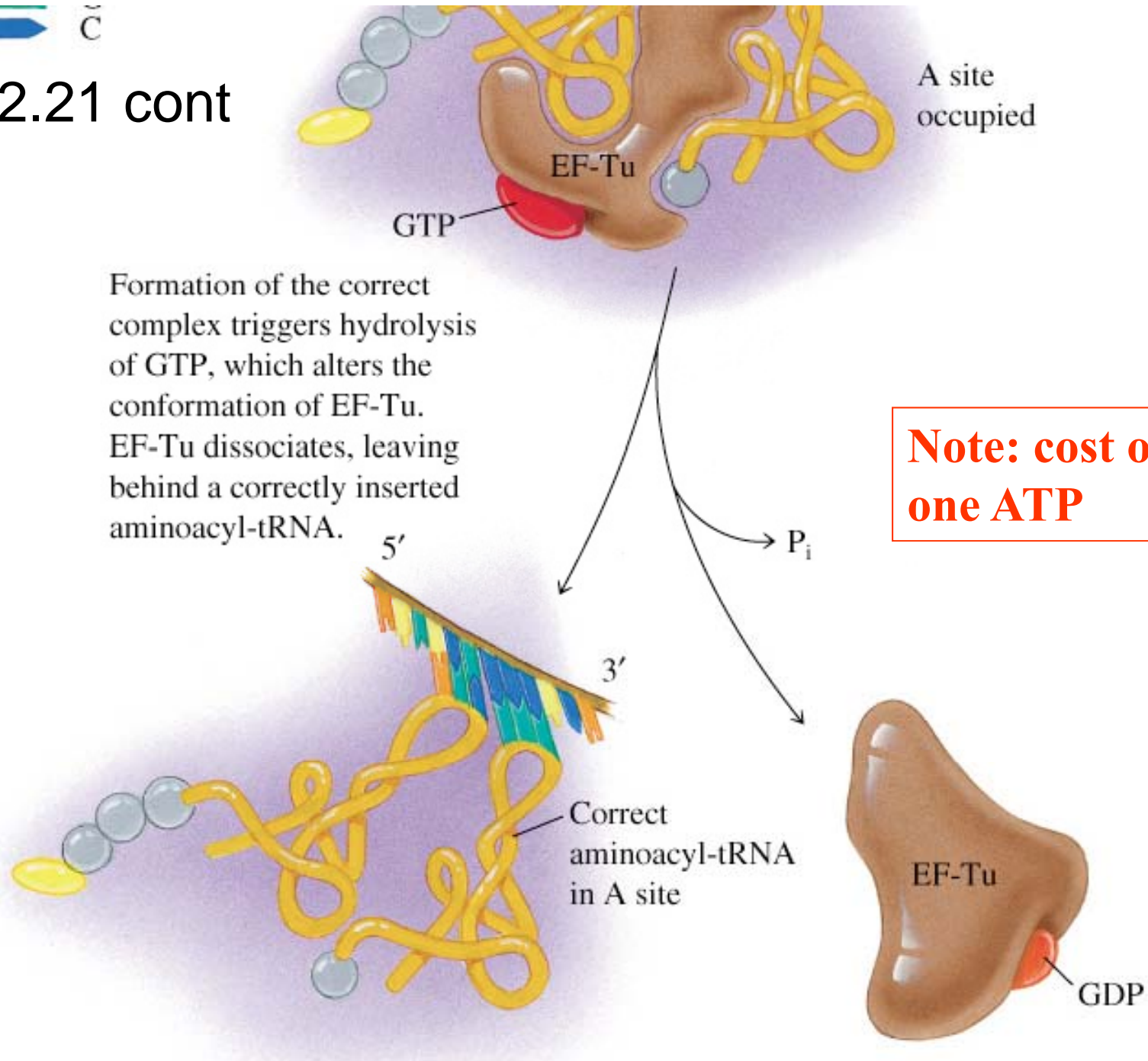


Fig 22.22 Cycling of EF-Tu-GTP

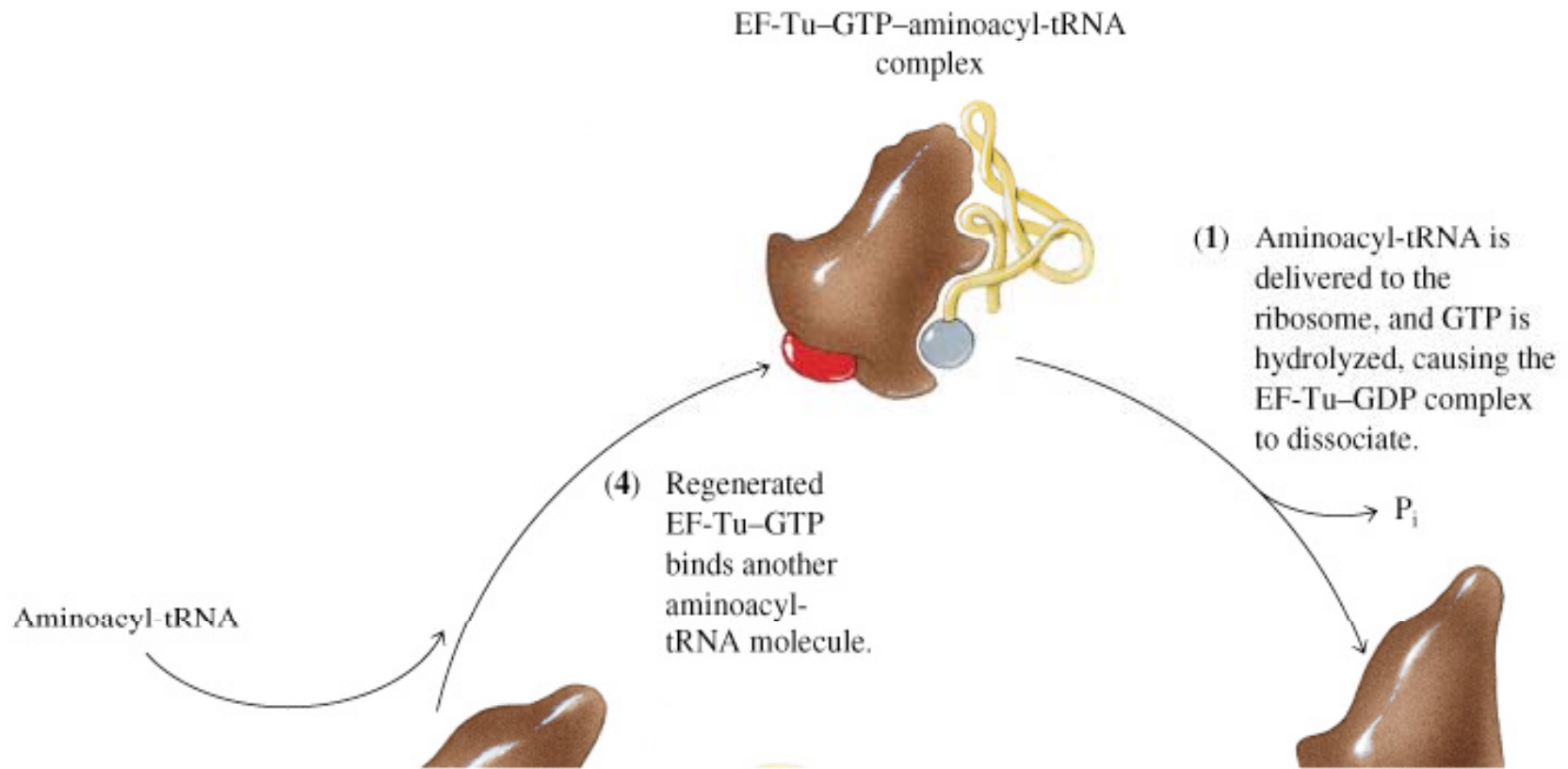


Fig 22.22 (cont)

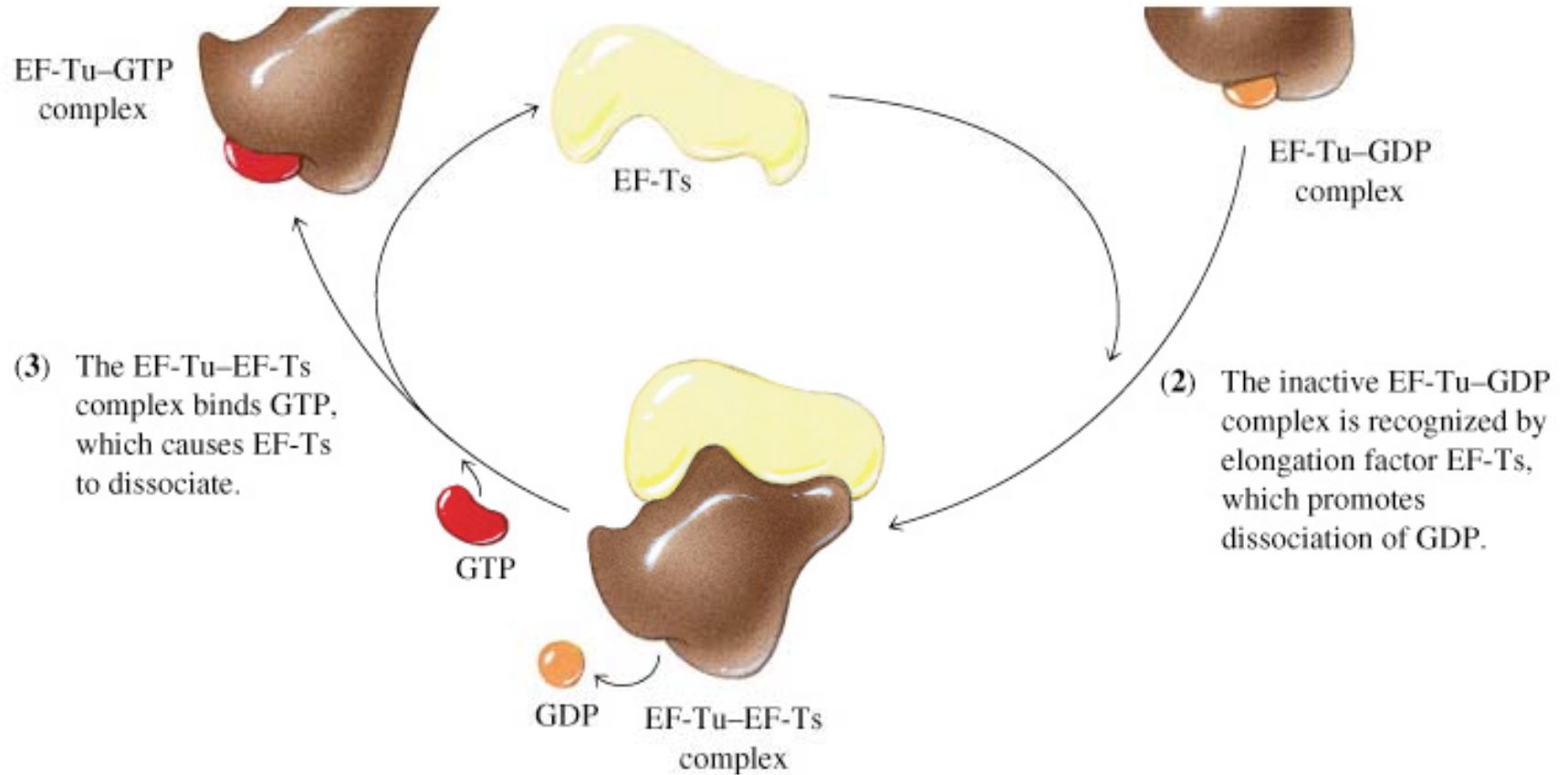
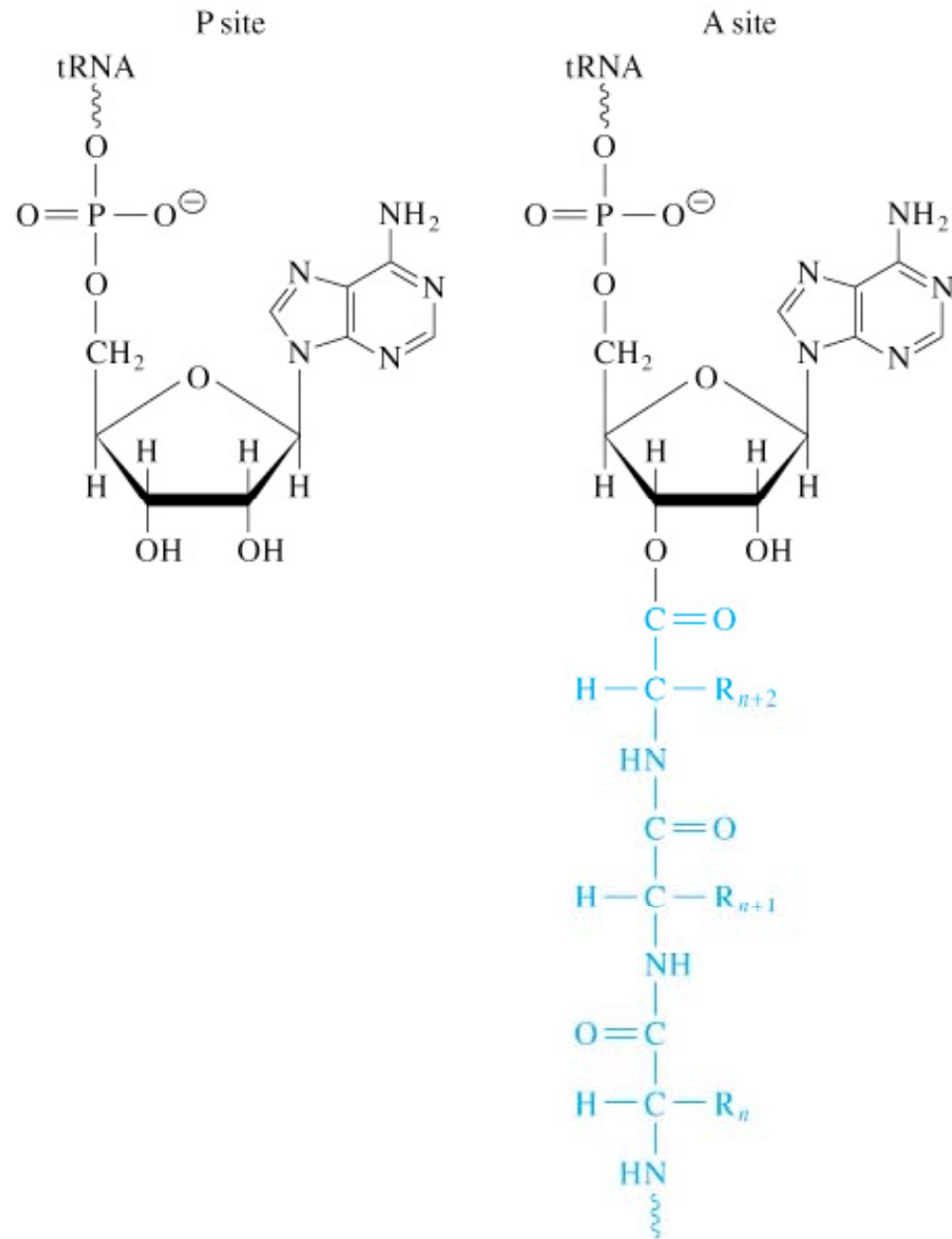


Fig 22.23
(cont)



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

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

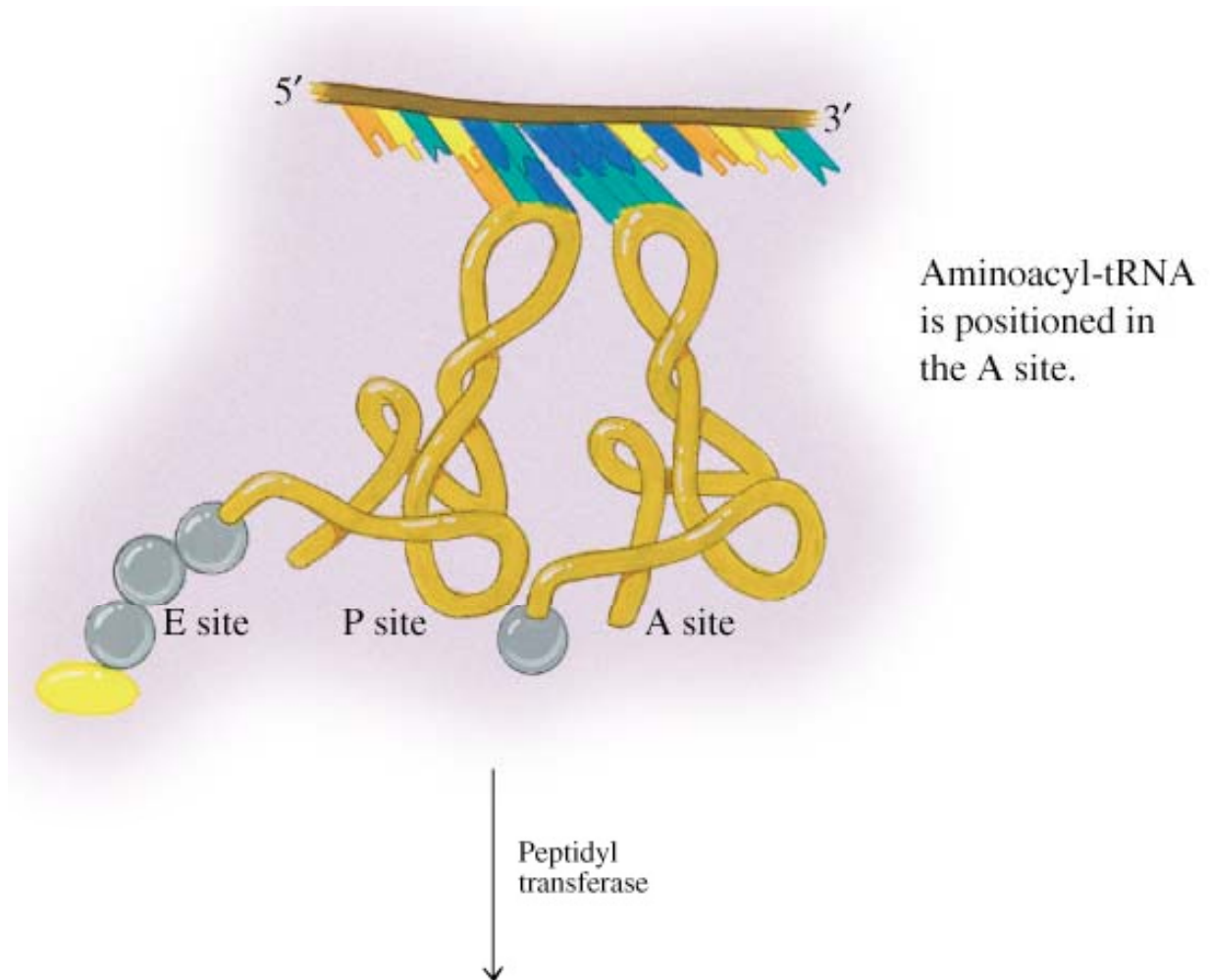


Fig 22.24

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

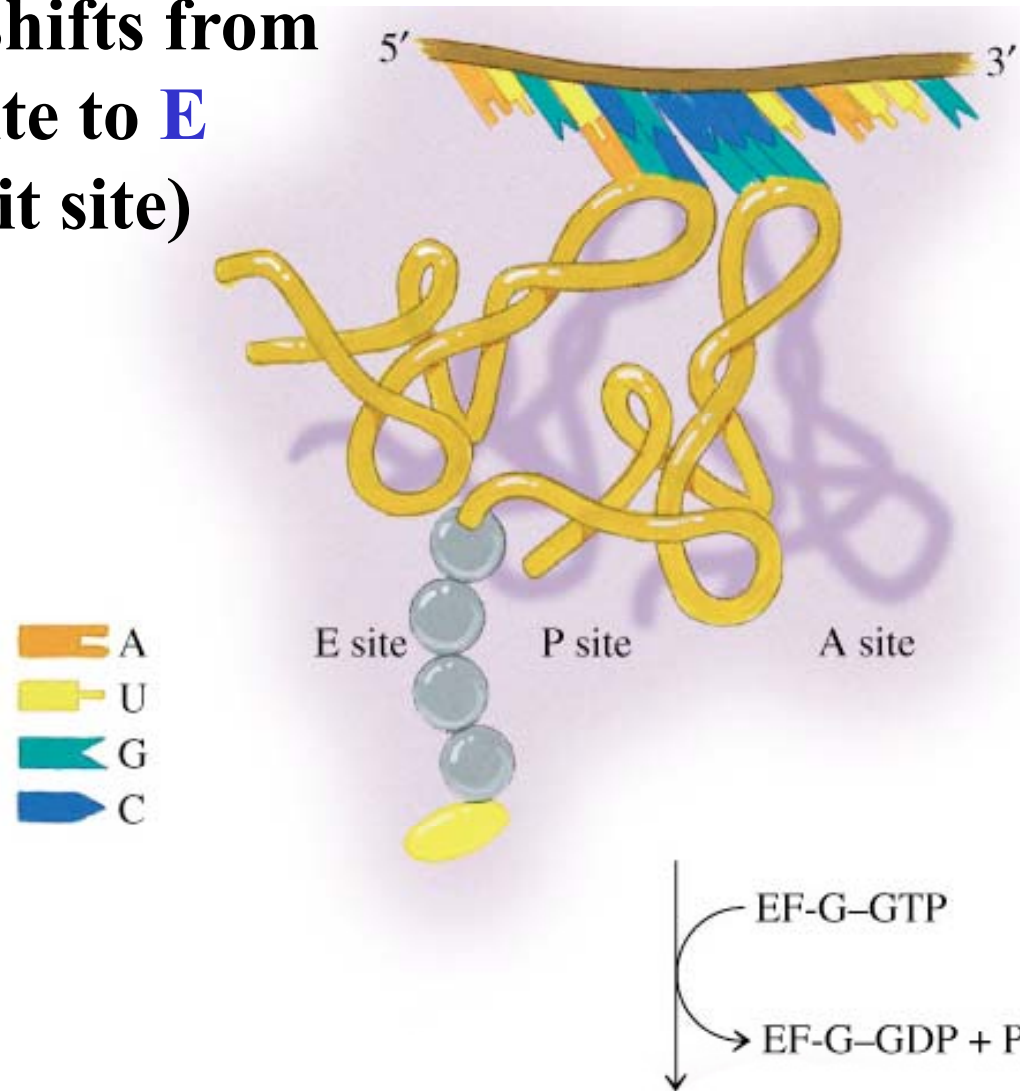


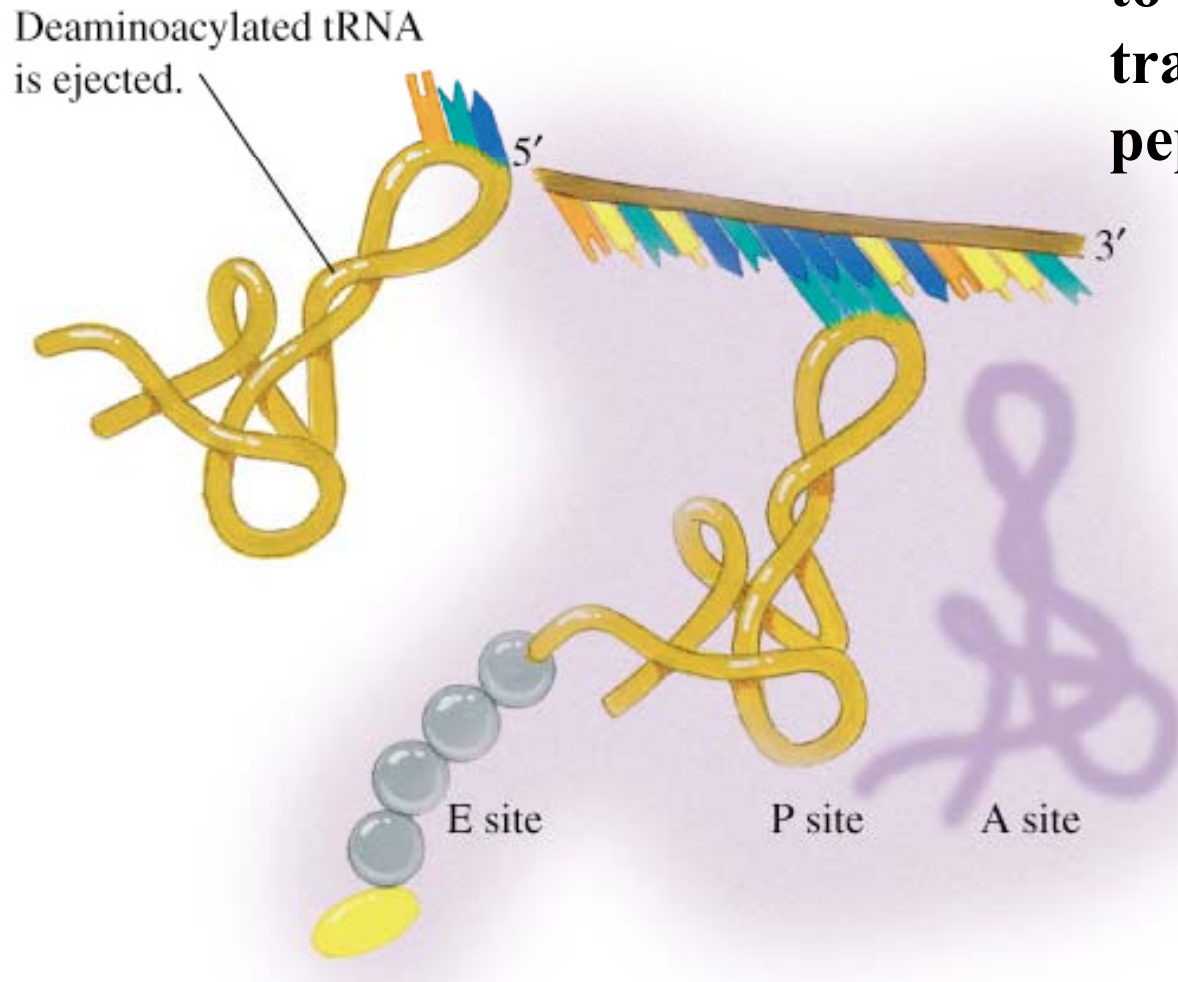
Fig 22.24

Following synthesis of the peptide bond, the newly formed peptidyl-tRNA is partly in the A site and partly in the P site.

Note: cost of one ATP

Fig 22.24

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



Translocation shifts the peptidyl-tRNA completely into the P site, leaving the A site empty and ejecting the deaminoacylated tRNA from the E site.

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Translation

- translation
- Genetic code
- tRNA
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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

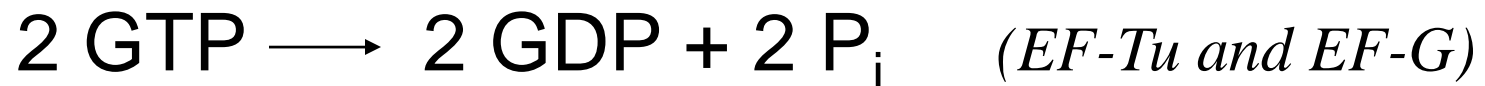
Protein Synthesis is Energetically Expensive

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

Amino acid activation: Two ~P bonds



Chain elongation: Two ~P bonds



**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

Enjoy metabolism!!!

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^{Ala})
- 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

Initiator tRNA

- First codon translated is usually AUG
- Each cell contains at least two methionyl-tRNA^{Met} molecules which recognize AUG
- The **initiator tRNA** recognizes initiation codons
- Second tRNA^{Met} recognizes only internal AUG
- Bacteria: **N-formylmethionyl-tRNA_f^{Met}**
- Eukaryotes: **methionyl-tRNA_i^{Met}**

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

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_f^{Met} (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

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

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)

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