Translation

The genetic code

- The evidence that the genetic code is a triplet code—that a set of three nucleotides (a codon) in mRNA code form one amino acid in a polypeptide chain.

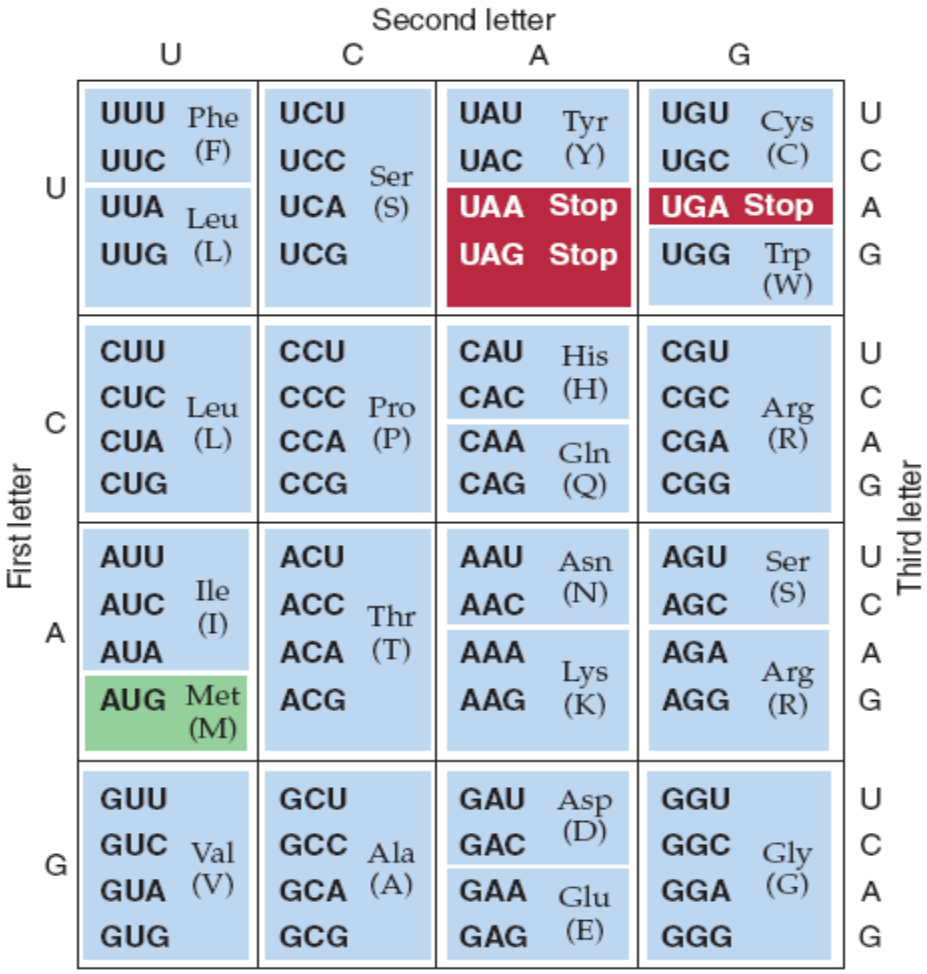
- If it were a two-letter code, then only 16 (4x4) amino acids could be encoded. A three letter code, however, generates 64 (4x4x4) possible codes, more than enough to code for the 20 amino acids found in living cells.

Paradox on Non specific appearance of ribosome

- Ribosome concentration increased in actively translating cells. So the hypothesis was that the rRNA are templates for amino acids.
- Challenges-

i. Ribosomes have two unequal subunits, each containing rRNA, that associate and dissociate depending on salt concentration.
ii. rRNA of small subunits from different species have almost same chain length (~ 1500bp) and that for the large subunit (~ 3000bp).
iii. The base composition of rRNA from all known species are same.

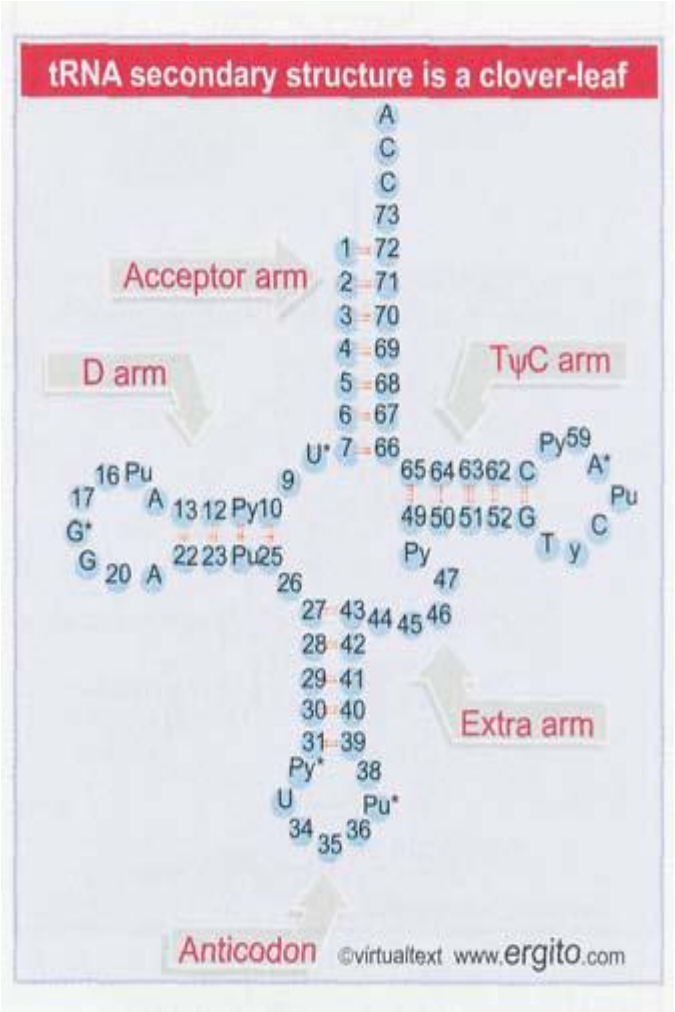
The genetic code



Characteristics of the genetic code

- *The code is a triplet code.*
- *The code is comma free; that is, it is continuous.*
- *The code is nonoverlapping.*
- *The code is almost universal.*
- *The code is "degenerate."*- With two exceptions, more than one codon occurs for each amino acid; the exceptions are **AUG**, which alone codes for methionine, and **UGG**, which alone codes for tryptophan. This multiple coding is called the degeneracy or *redundancy* of the code.
- *The code has start (AUG) and stop signals (***UAG** (amber), **UAA** (ochre), and **UGA** (opal)*).*
- *Wobble occurs in the anticodon.* – According to the wobble hypothesis proposed by Francis Crick, the complete set of 61 sense codons can be read by fewer than 61 distinct tRNAs, because of pairing properties of the bases in the anticodon.

t RNA STRUCTURE



- The tRNA secondary structure can be written in the form of a cloverleaf.
- Complementary base pairing forms stems for single-stranded loops.
- The stem-loop structures are called the arms of tRNA.
- The acceptor arm consists of a base-paired stem that ends in an unpaired sequence whose free 2'- or 3'-OH group can be linked to an amino acid.

• The *TΨC arm* is named for the presence of this triplet sequence. *(Ψ* stands for pseudouridine, a modified base.)

• The anticodon arm always contains the anticodon triplet in the center of the loop.

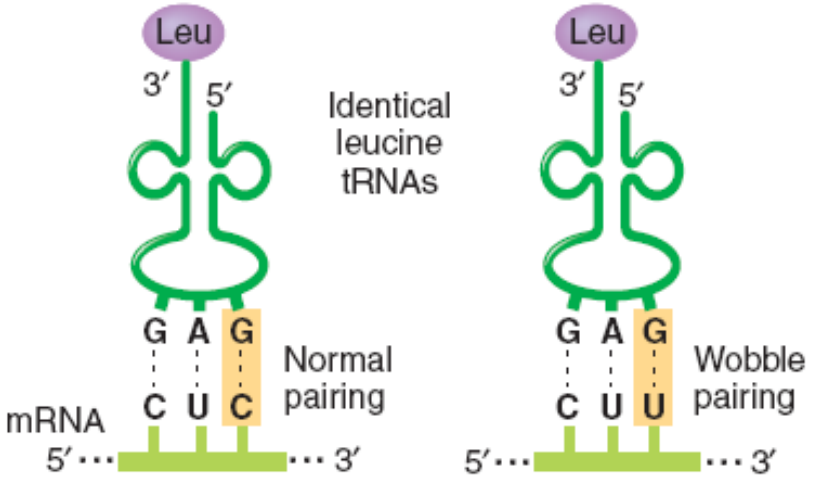
• The D arm is named for its content of the base dihydrouridine (another of the modified bases in tRNA).

• The extra arm lies between the T*Ψ* C and anticodon arms and varies from 3-21 bases.

Wobble hypothesis

Table 6.1 Wobble in the Genetic Code

Nucleotide at 5′ End of Anticodon		Nucleotide at 3′ End of Codon
G	can pair with	U or C
C	can pair with	G
A	can pair with	U
U	can pair with	A or G
I (inosine)	can pair with	A, U, or C



Ambiguities in genetic code

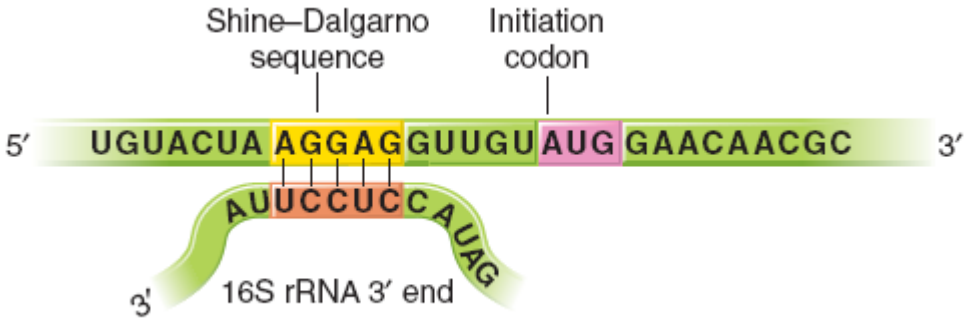
- AUG & GUG codes for formylmethionine at start but codes for methionine or valine at the internal region.
- CUG, UUG and AUU sometimes code for formylmethionine.
- UGA codes for selenocysteine and tryptophan in few bacteria

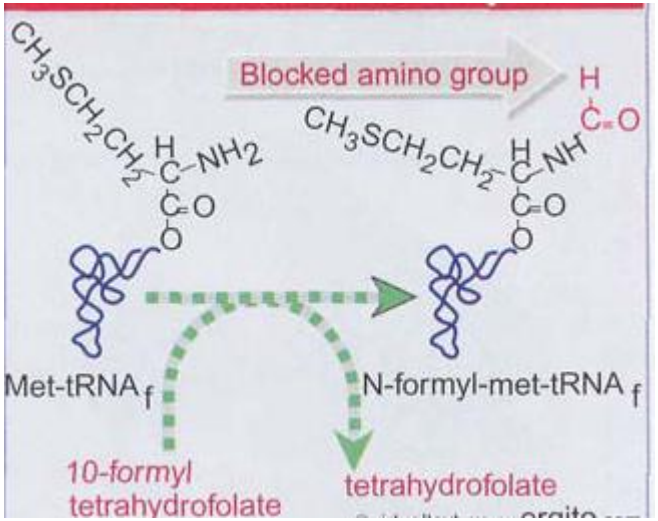
Codon Usage

- Same amino acids may be encoded by different codons in different organisms depending on their GC content.

Important features of translation initiation

- Strat codon AUG
- First amino acid formyl methionine.
- The **AUG** initiation codon alone is not sufficient to indicate where the 30S subunit should bind to the mRNA;
- A sequence upstream (to the 5’ side in the leader of the mRNA) of the **AUG** called the ribosome-binding site (RBS) is also needed.
- The mRNA RBS region is now commonly known as the Shine–Dalgarno sequence.



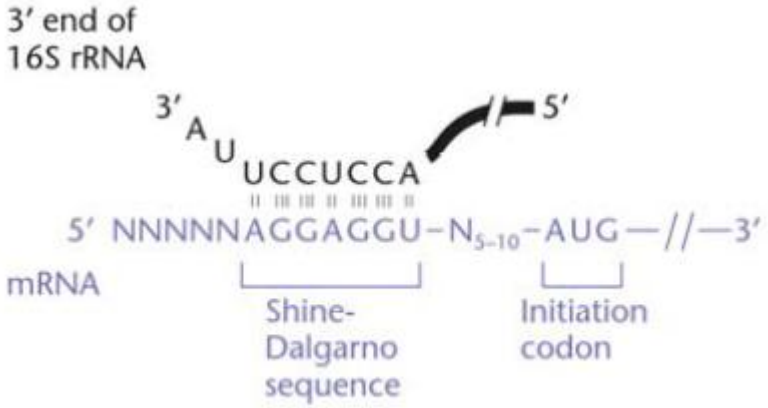


Initiation codons

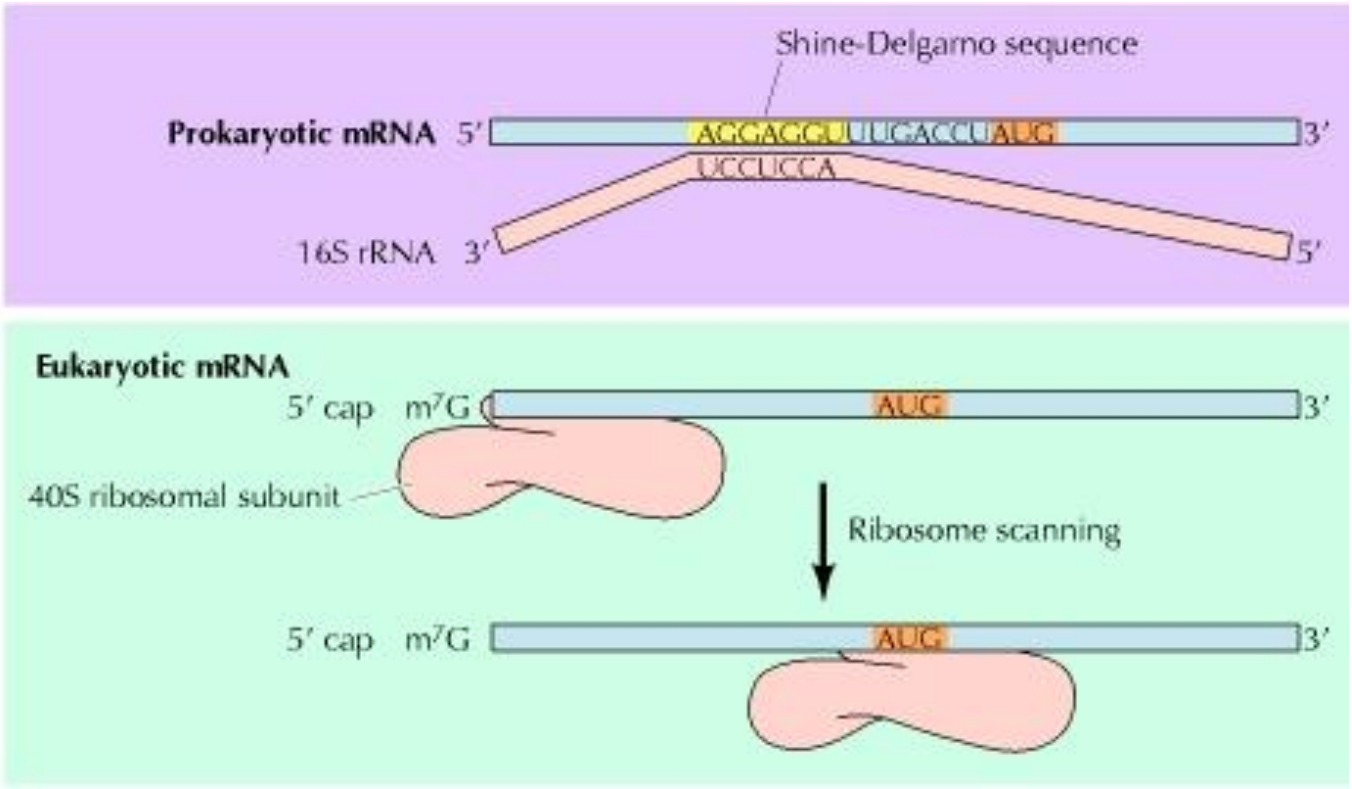
- AUG / GUG / UUG / AUA / AUU.
- All codes for methionine at start.
- After translation this methionine sometimes cut off.
- Wobble in reverse.
- This codons are recognized in the P site rather than A site.

Shine – Dalgarno sequence

- 5 to 10 nucleotide upstream of initiation codon.
- Complementary to 16SrRNA.
- In few bacterial gene it resides at downstream of the initiation codon.
- Not all bacterial gene have SD sequence.

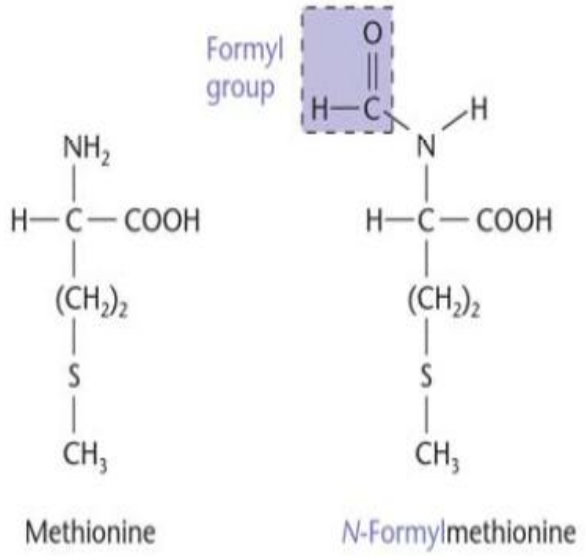


5’ cap is rcognized by ribosome in eukaryotes

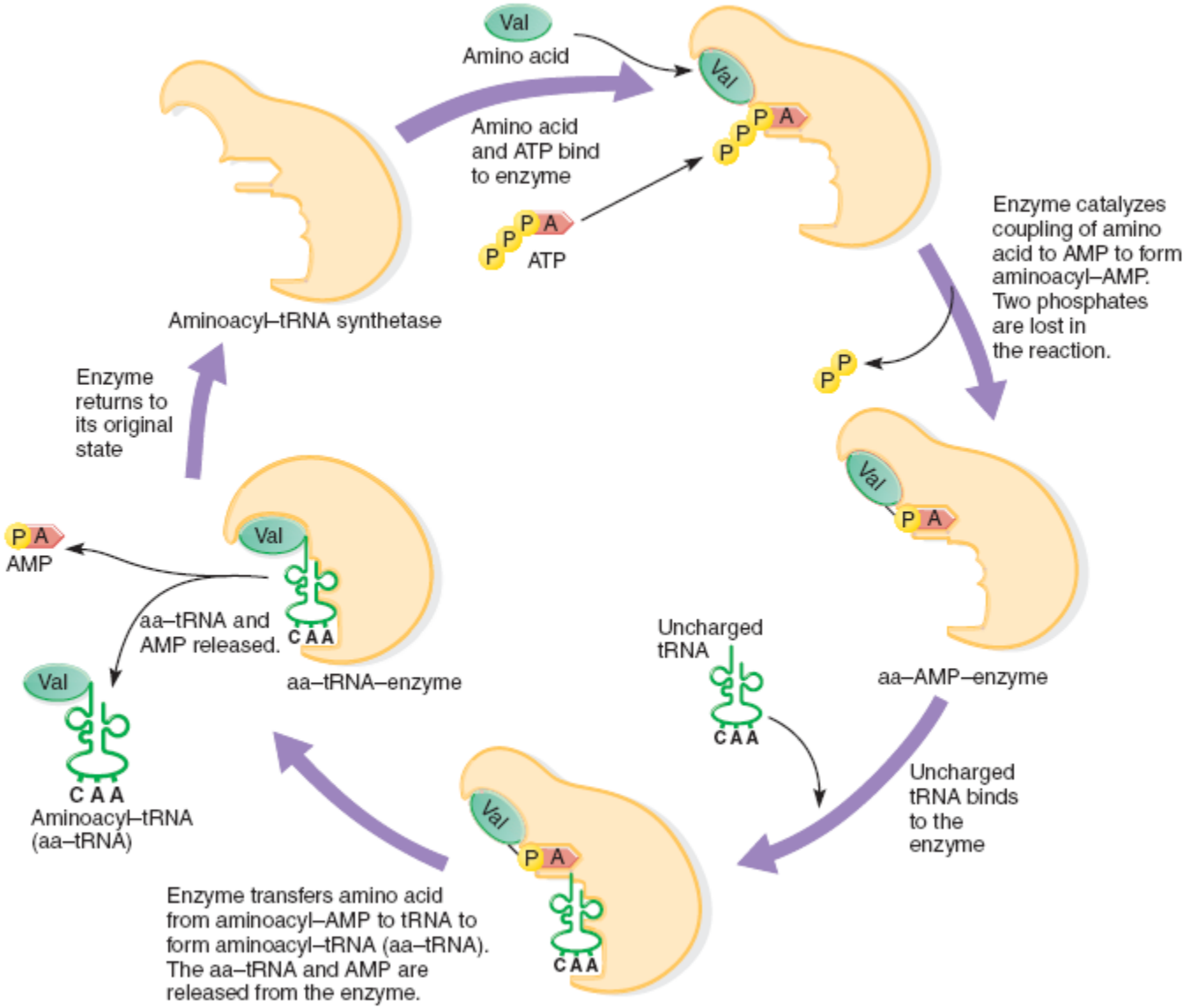


Initiator tRNA

- fMet tRNA, peptidyl tRNA rather than aminoacyl tRNA.
- That's why it can bind to P site rather than A site.
- Does not have its own aminoacyltransferase, shared the same with tRNAmet.
- Transformylase add formyl group to methionine.

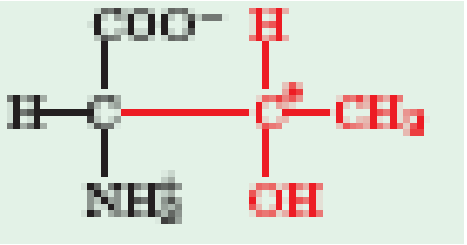


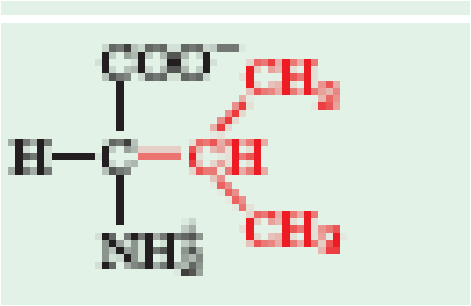
Charging of aminoacyl tRNA

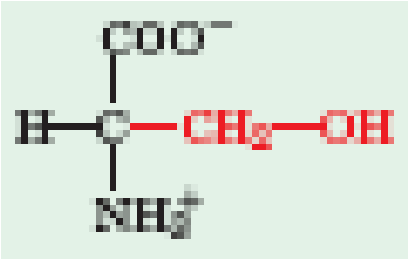


Aminoacyl-tRNA synthetase have highly discriminating active site

- Side chain OH of threonine binds with Zn ion and aspertate at active site.
- Valine CH3 does not promote this reaction.







Proof reading by synthetase

- Rapid hydrolysis
- Editing site increase the fidelity.
- Editing site is 20Å apart from active site.
- Required for smaller amino acids
- CCA with smaller amino acid swings to editing site.

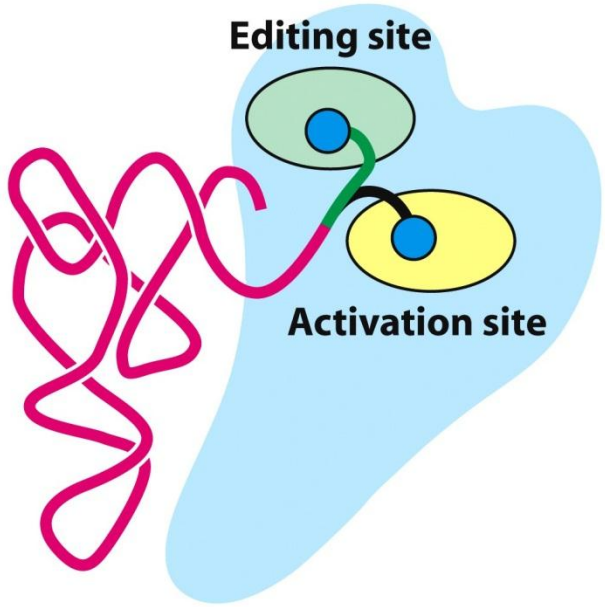


Figure 30.10
Biochemistry, Seventh Edition

It is codon-anticodon interaction between mRNA and tRNA

- Randome copolymer of U and G 5:1.
- Addition of both cys and ala depending on Aminoacyl tRNA.

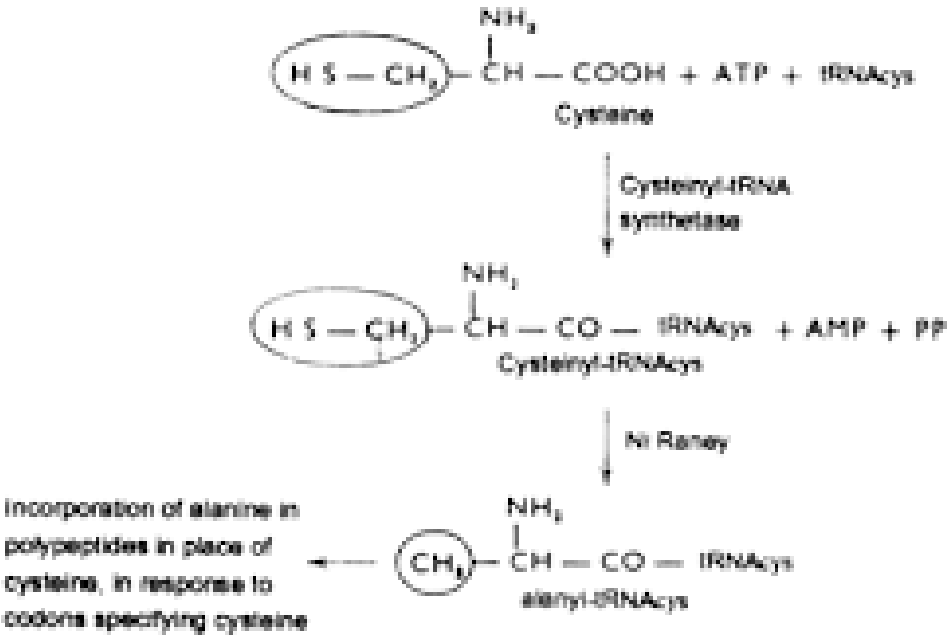


FIG. 6-45. — Diagram of the experiments of Chapeville and co-workers.

Two classes of Aminoacyl-tRNA synthetase

Class I	Class II
Activation domain Rossmann fold	Activation domain β strand
CCA arm take hairpin conformation while interacting with enzyme	CCA arm take helical conformation while interacting with enzyme
Acylate 2’ OH of tRNA	Acylate 3’ OH of tRNA
Monomeric	Dimeric

table 27–8

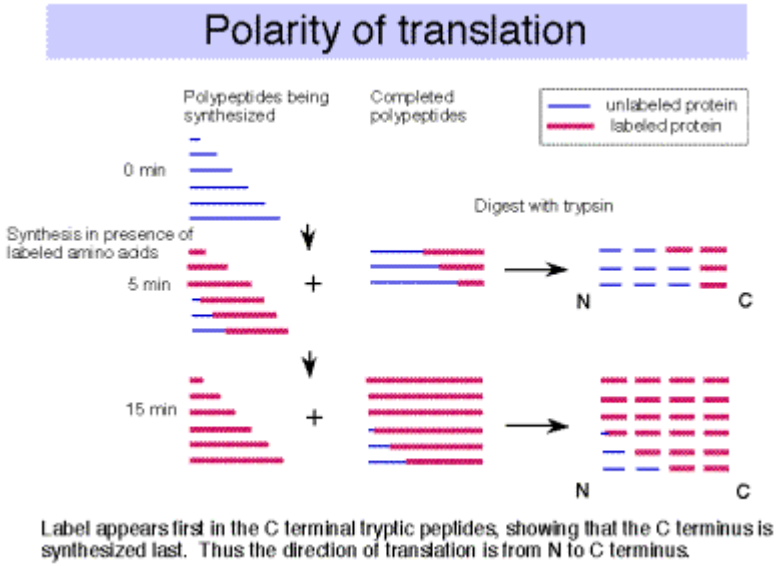
Two Classes of Aminoacyl-tRNA Synthetases*

Class I	Class II
Arg	Ala
Cys	Asn
Gln	Asp
Glu	Gly
Ile	His
Leu	Lys
Met	Phe
Trp	Pro
Tyr	Ser
Val	Thr

*Here, Arg represents arginyl-tRNA synthetase, and so forth. The classification applies to all organisms for which tRNA synthetases have been analyzed and is based on protein structural distinctions and on the mechanistic distinction outlined in Figure 27–16.

Protein synthesis occurs in N – C terminal direction

- Active reticulocyte treated with [3H] leucine.
- Time interval study of Hb α and β chain.
- Initial radioactivity at C terminus.
- Radioactivity appears at N terminus with time.



Label appears first in the C terminal tryptic peptides, showing that the C terminus is synthesized last. Thus the direction of translation is from N to C terminus.

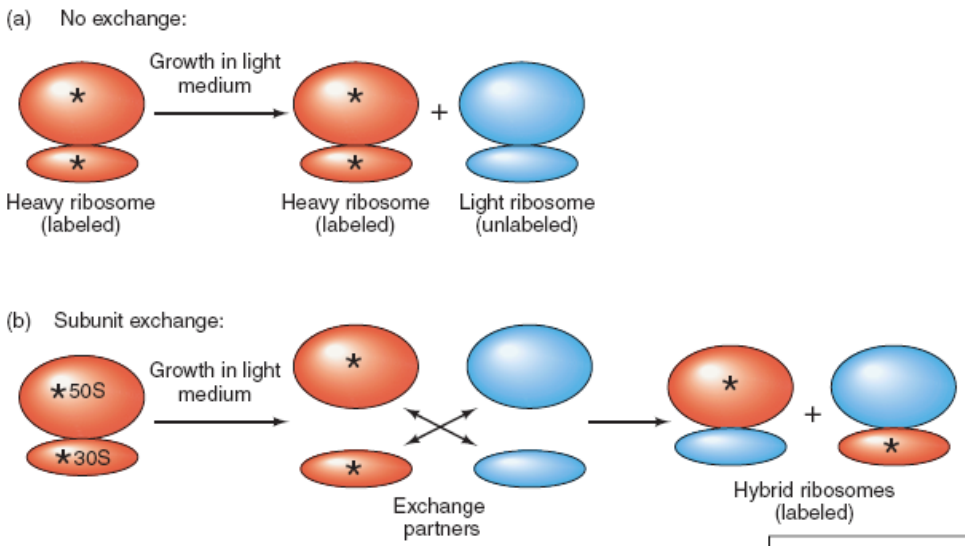
Translation occurs 5’-3’ direction of mRNA

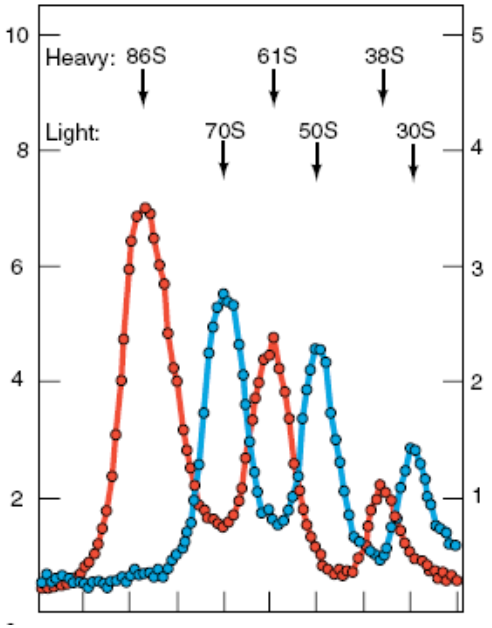
- Synthetic oligonucleotide

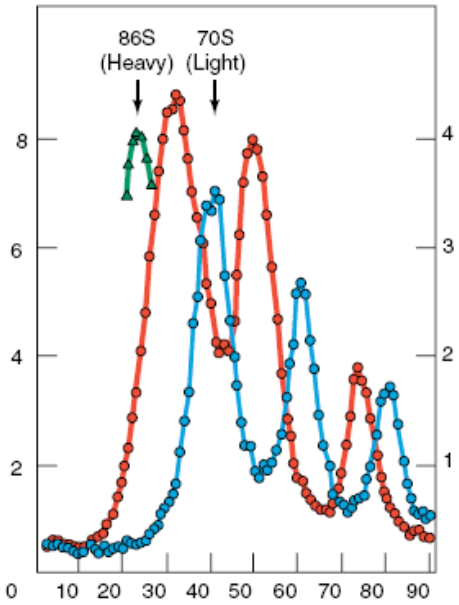
  5’A-A-(A)n-A-A-C 3’

- Cell free protein synthetic machinary.
- Polylysine - Asparagine

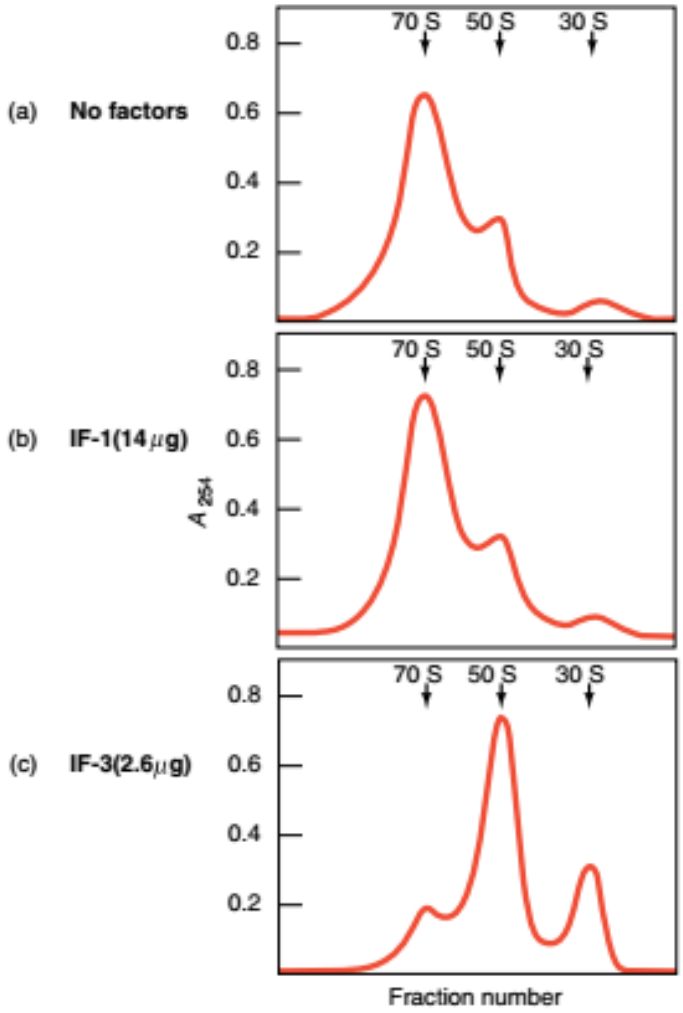
Dissociation of ribosome



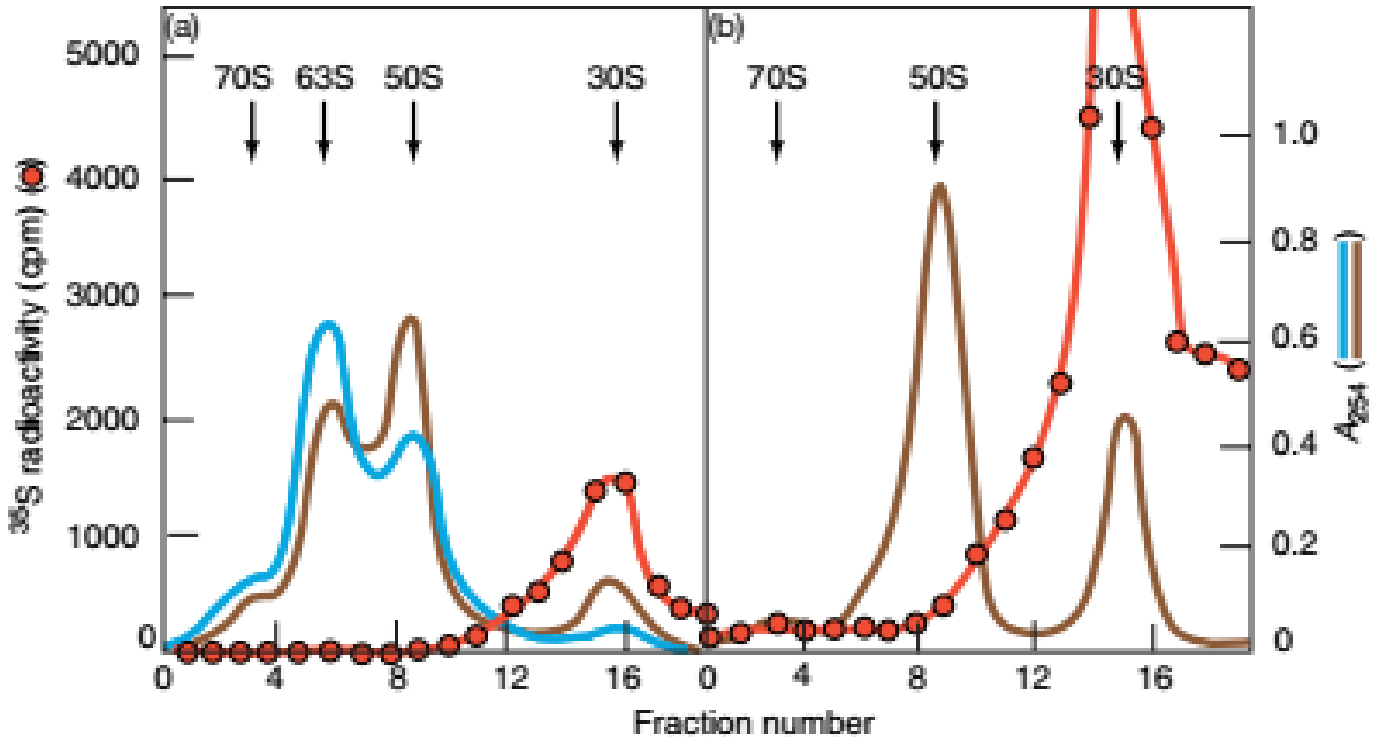




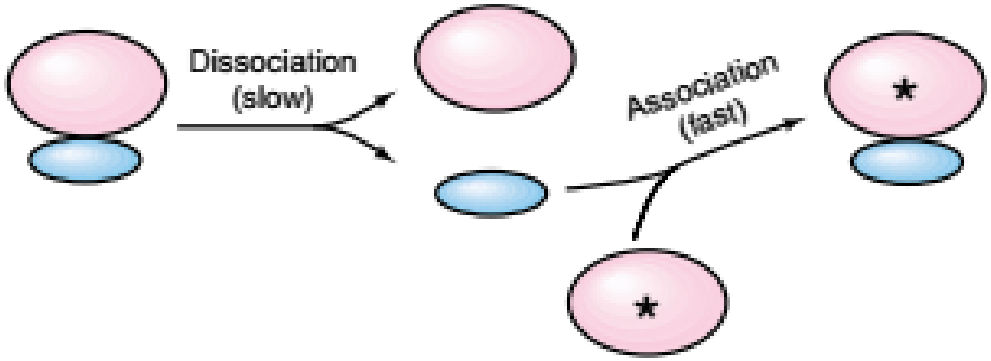
IF3 actively participate in ribosome dissociation

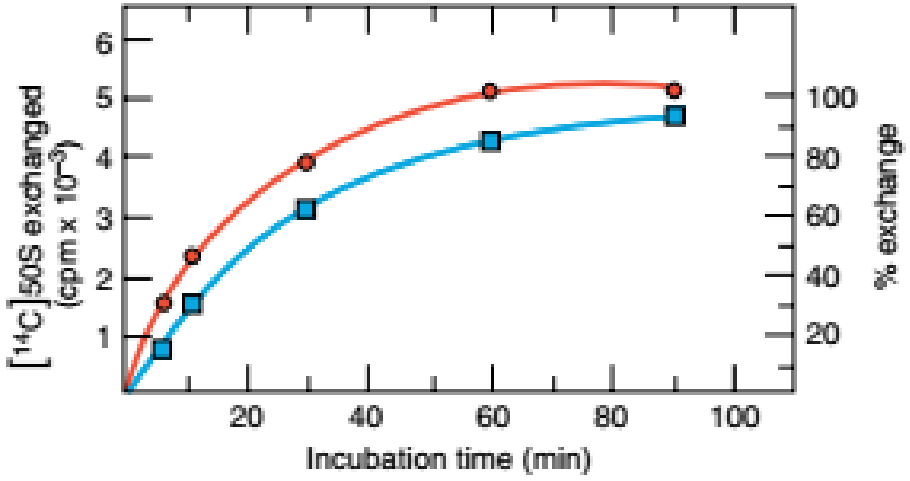


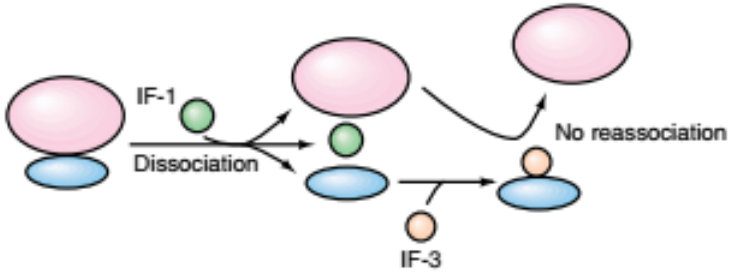
IF3 binds to 30S subunit



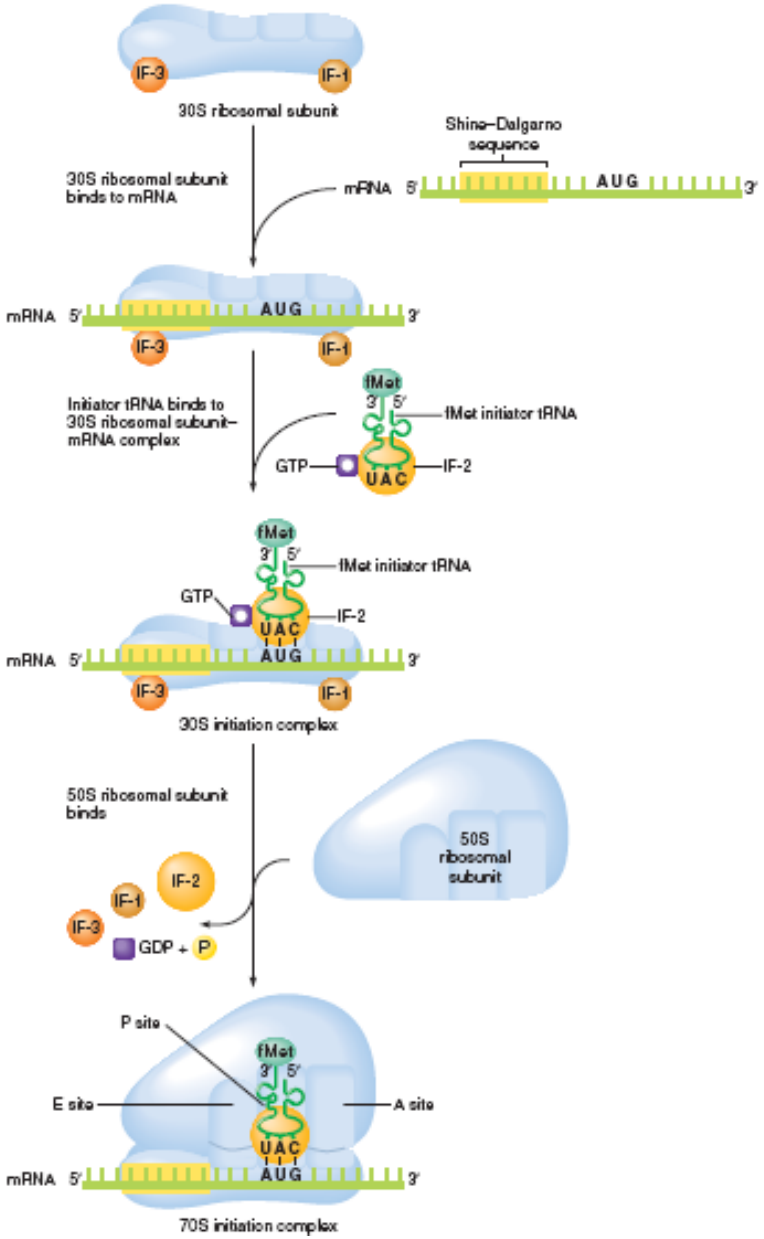
Role of IF1 is observed through association



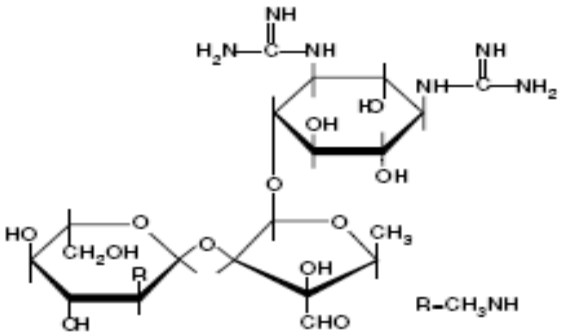




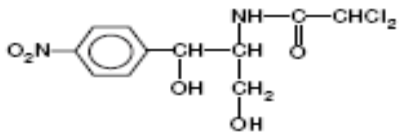
Initiation of prokaryotic translation



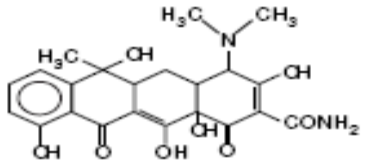
Antibiotics that inhibit protein synthesis



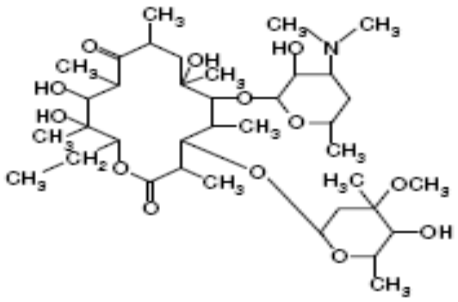
Streptomycin



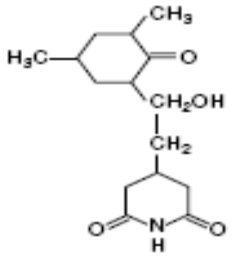
Chloramphenicol



Tetracycline

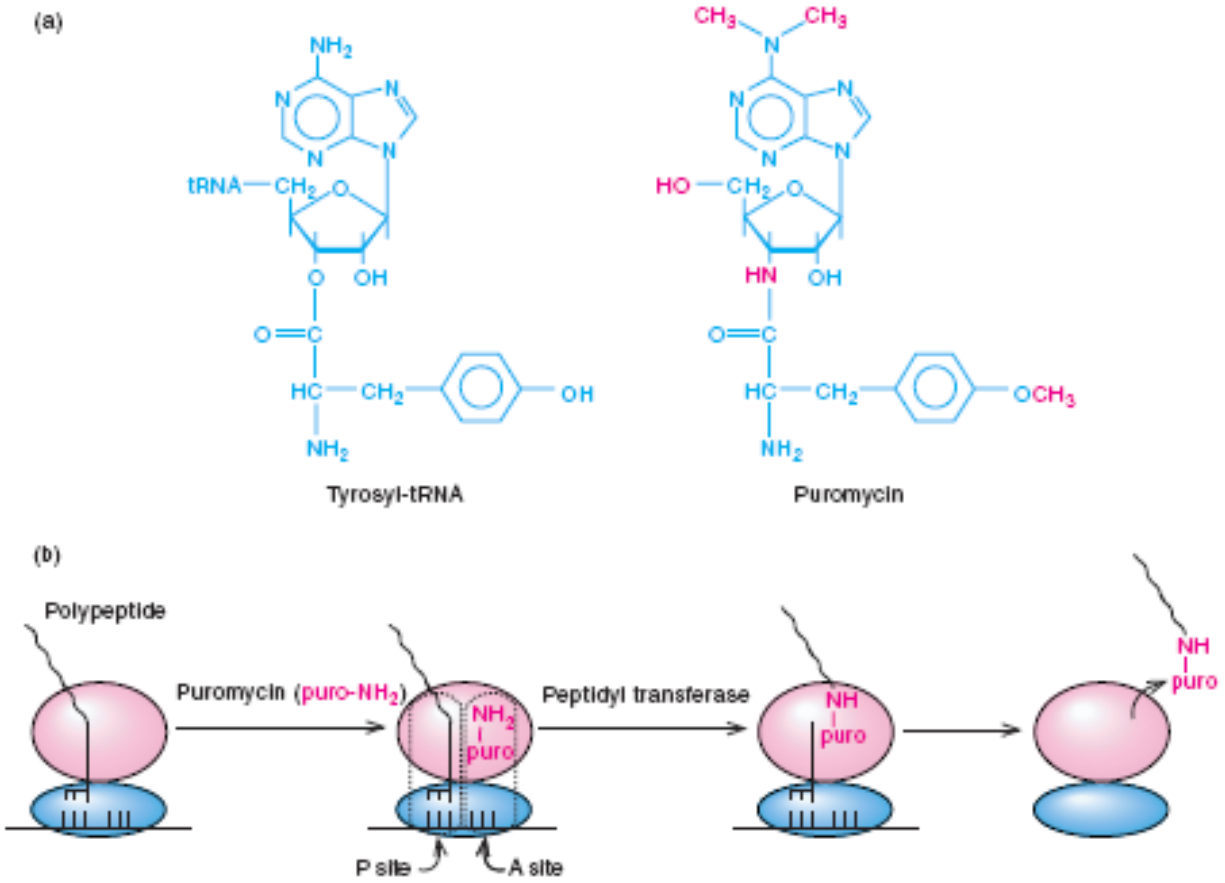


Erythromycin



Cycloheximide

Initial discovery of ribosome A & P site

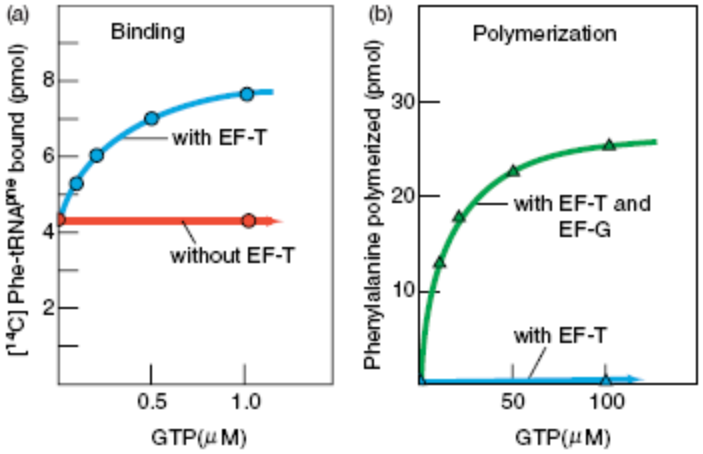


Evidence against E site of ribosome

- Experimental strategy was to bind radioactive deacylated tRNAPhe (tRNAPhe lackingphenylalanine), or Phe-tRNAPhe, or acetyl-Phe-tRNAPhe to *E. coli* ribosomes and to measure the number of molecules bound per 70S ribosome.

	tRNA	Binding sites	
mRNA	Species	No.	Location
Poly(U)	Acetyl-Phe-tRNA^{Phe}	1	P or A
Poly(U)	Phe-tRNA^{Phe}	2	P and A
Poly(U)	tRNA^{Phe}	3	P, E, and A
None	tRNA^{Phe}	1	P
None	Phe-tRNA^{Phe}	0	—
None	Acetyl-Phe-tRNA^{Phe}	1	P

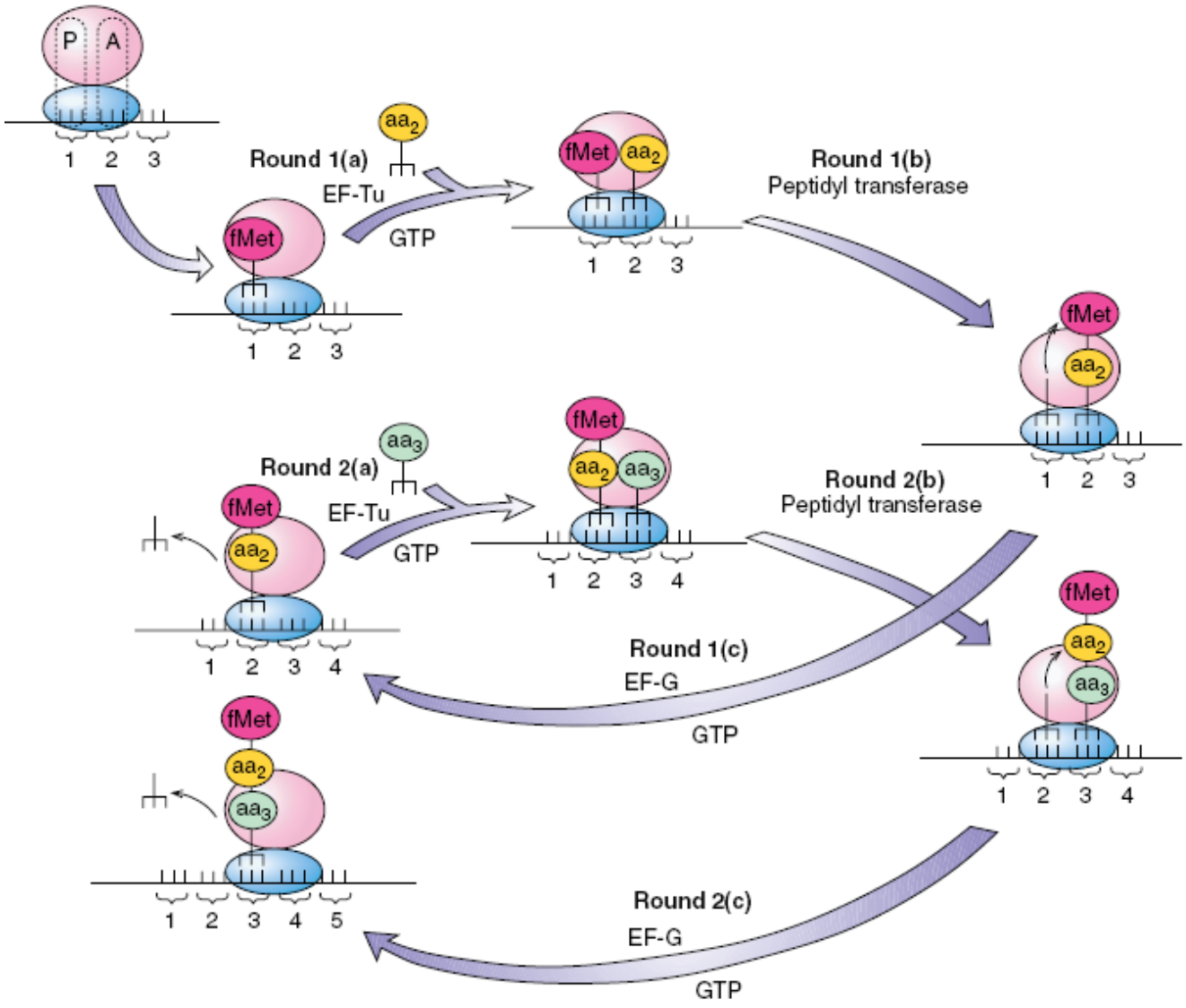
Elongation factors play important role



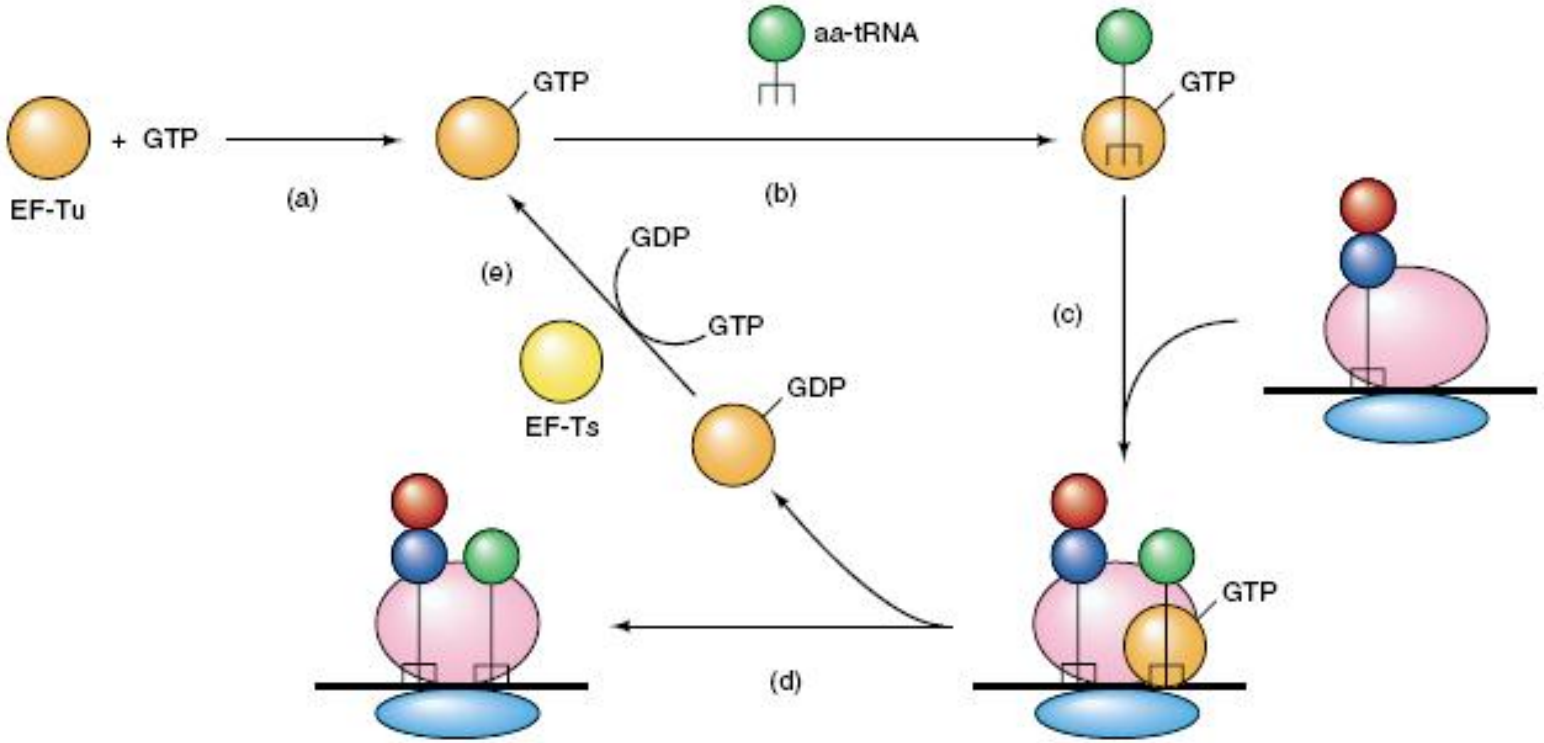
Additions	[^{3}H]Phe-tRNA bound (pmol)
None	2.8
EF-Ts + GTP	2.8
EF-Tu + GTP	5.2
EF-Ts + EF-Tu + GTP	11.6

- The ester bond joining the amino acid to its cognate tRNA is easily broken, and sequestering the aminoacyl-tRNA within the EF-Tu protein protects this labile compound from hydrolysis.

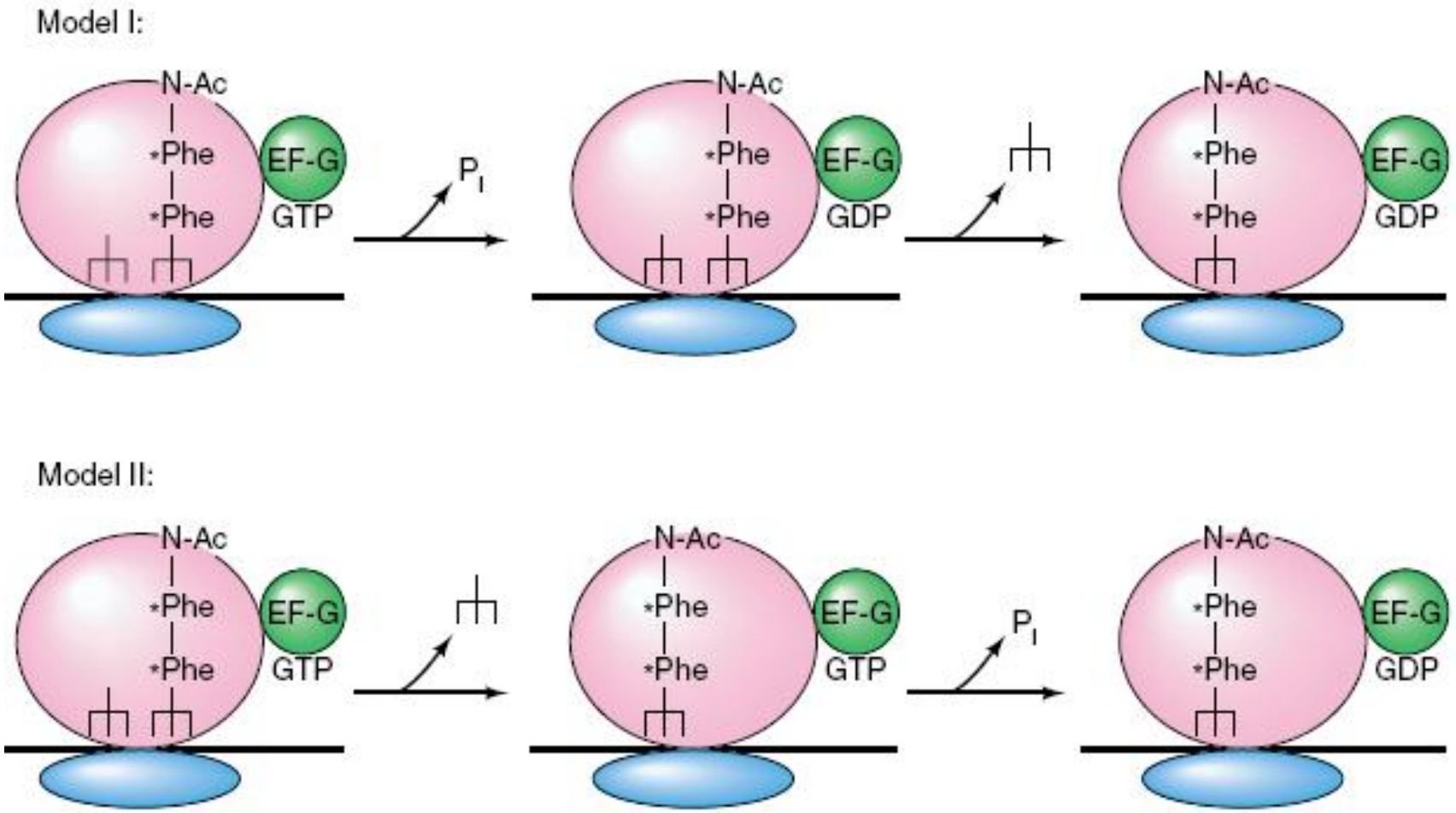
Elongation


P
A
1 2 3
Round 1(a)
aa2
EF-Tu
GTP
fMet
fMet aa2
Round 1(b)
Peptidyl transferase
Round 2(a)
aa3
Round 2(b)
Round 1(c)
EF-G
GTP
Round 2(c)
1 2 3 4
1 2 3 4 5

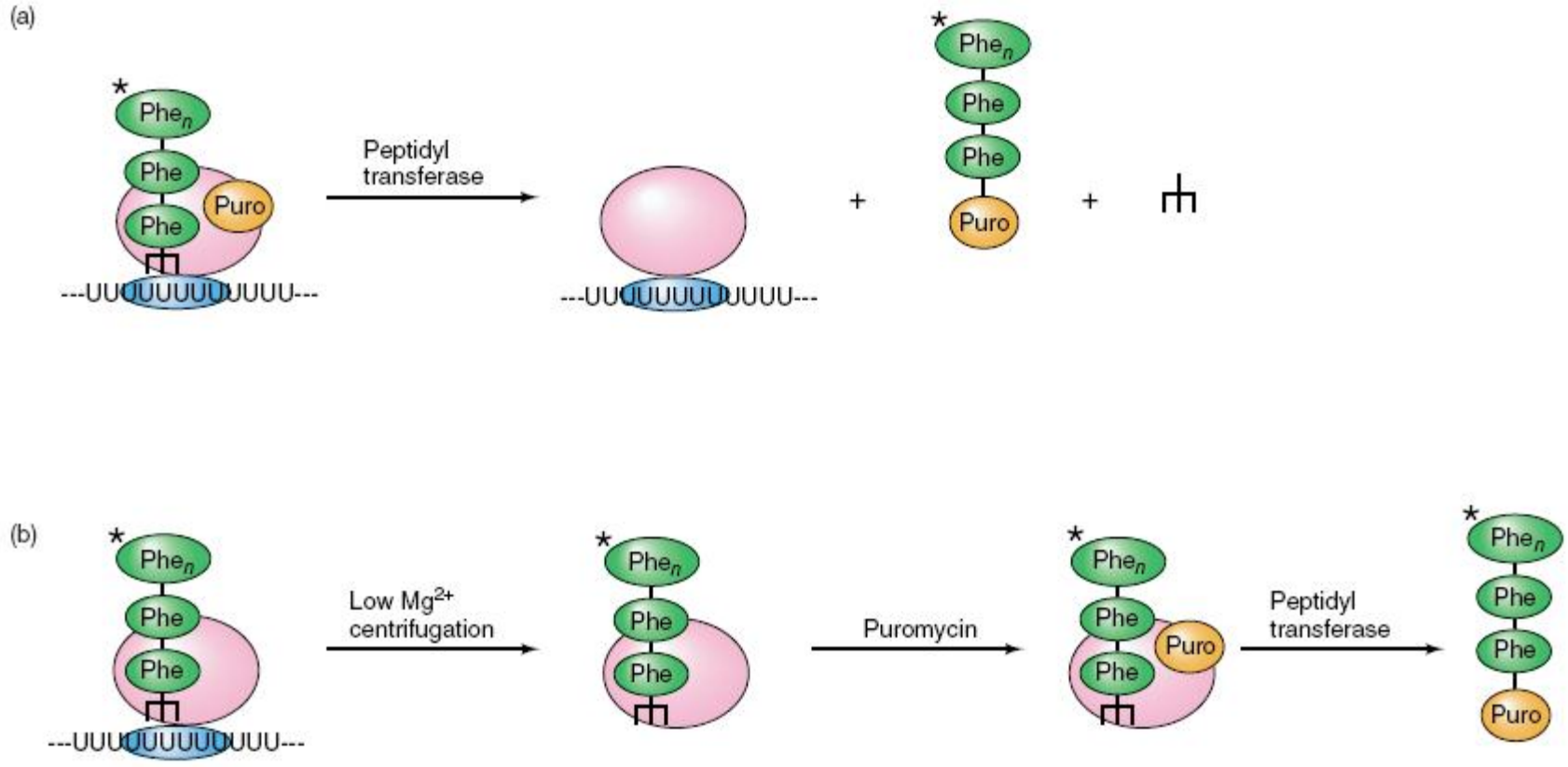
EF-Tu EF-Ts cycling



Models for EFG activity



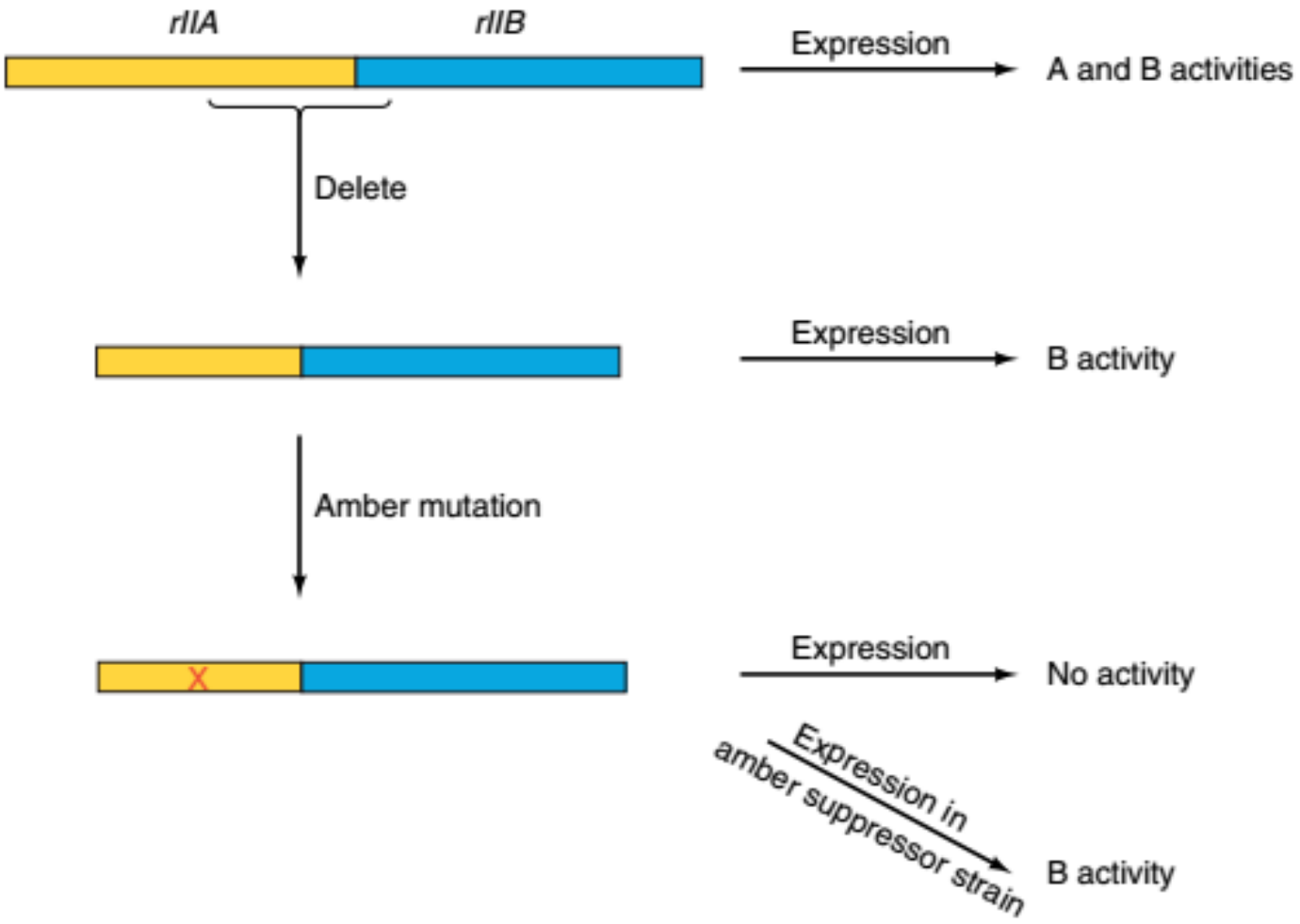
But 50S subunit solely responsible for peptidyl transferase reaction



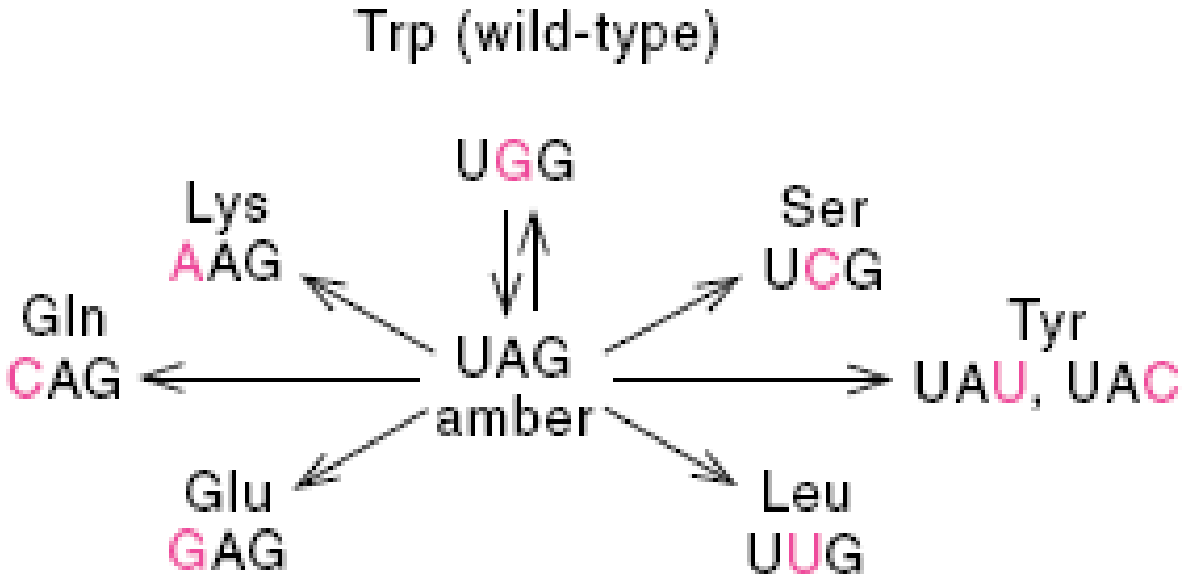
Stop codons-Amber, Ochre, Opal

- Amber mutations caused premature termination, overcome by suppressor.
- Ochre mutations were originally distinguished by the fact that they were not suppressed by amber suppressors.
- Opal mutations are suppressed by **opal suppressors.**

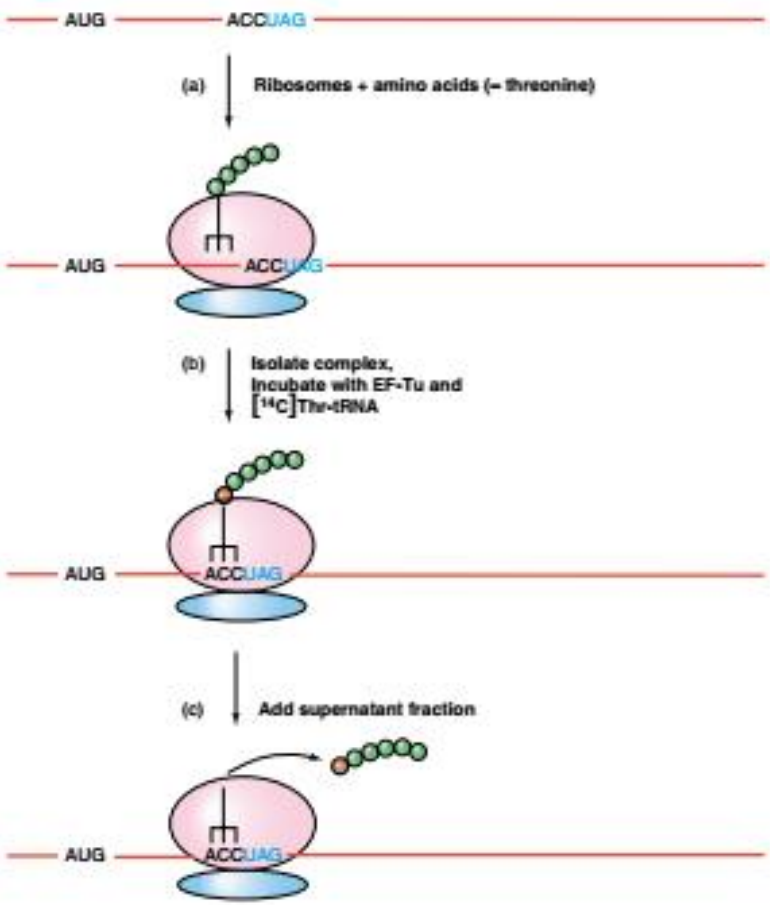
Experiment that shows amber mutation stops translation



UAG is the amber codon

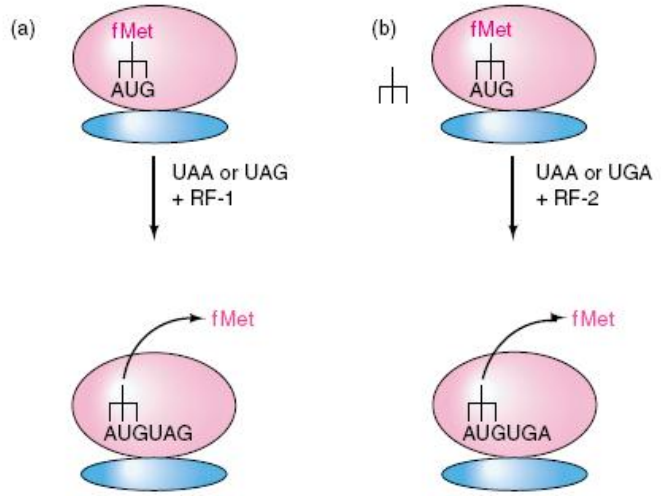


First concept of release factor



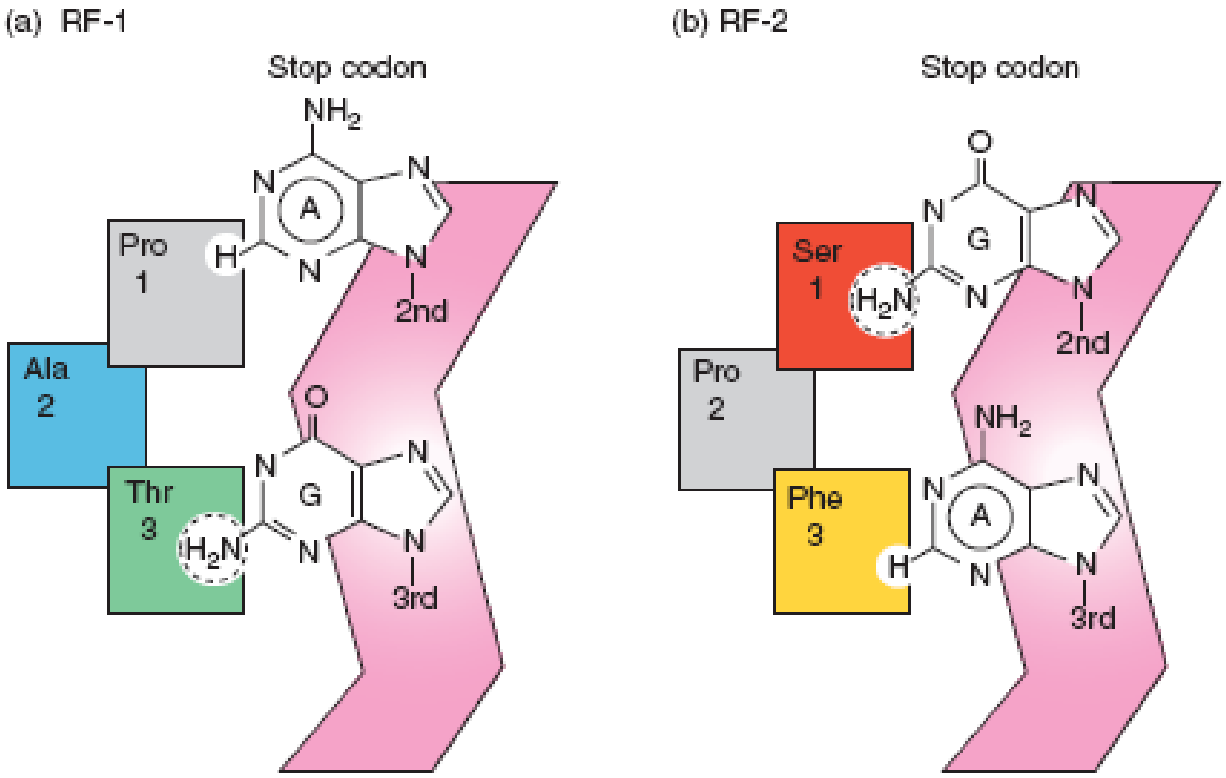
Release factors

- One factor (**RF-1**) cooperated with the stop codons UAA and UAG.
- Another factor (**RF-2**) cooperated with UAA and UGA.
- A third release factor, (**RF-3**), a ribosome-dependent GTPase, binds GTP and helps the other two release factors bind to the ribosome.



Additions		pmol [^{3}H]fMet Released in Presence of:	
Release factor	Stop codon	0.012 M Mg^{2+}	0.030 M Mg^{2+}
RF-1	None	0.12	0.15
RF-1	UAA	0.47	0.86
RF-1	UAG	0.53	1.20
RF-1	UGA	0.08	0.10
RF-2	None	0.02	0.14
RF-2	UAA	0.22	0.77
RF-2	UAG	0.02	0.14
RF-2	UGA	0.33	1.08

Binding of RF with stop codon



Termination