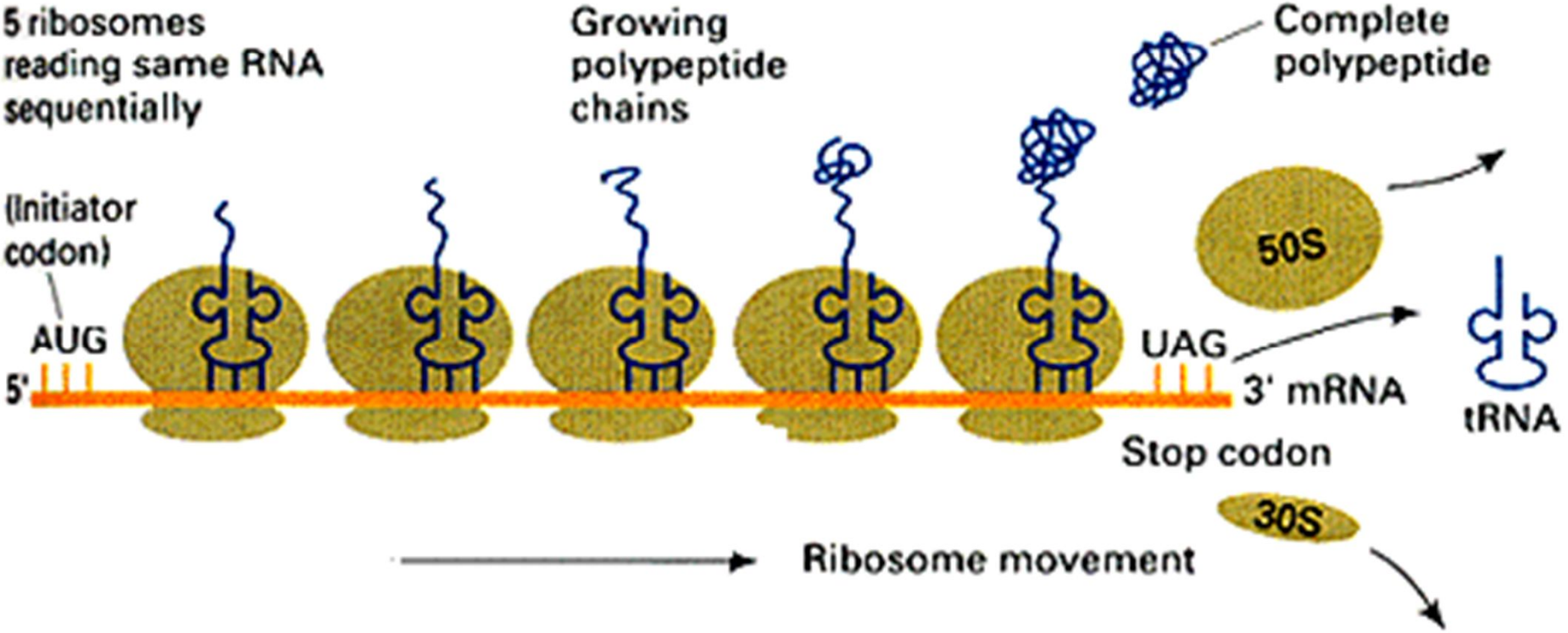
Polyribosomes (polysomes)

mRNAs that are translated at the same time by more than one ribosome


5 ribosomes reading same RNA sequentially
Growing polypeptide chains
Complete polypeptide
(Initiator codon)
AUG
5'
UAG
3' mRNA
50S
tRNA
Stop codon
30S
Ribosome movement

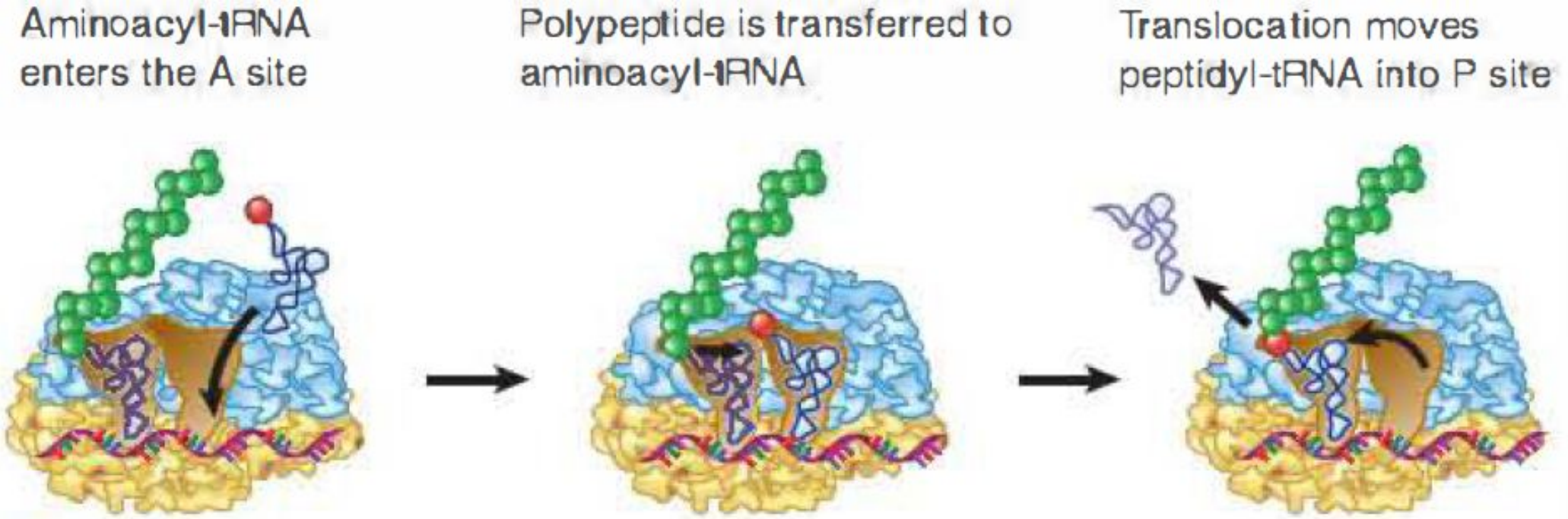
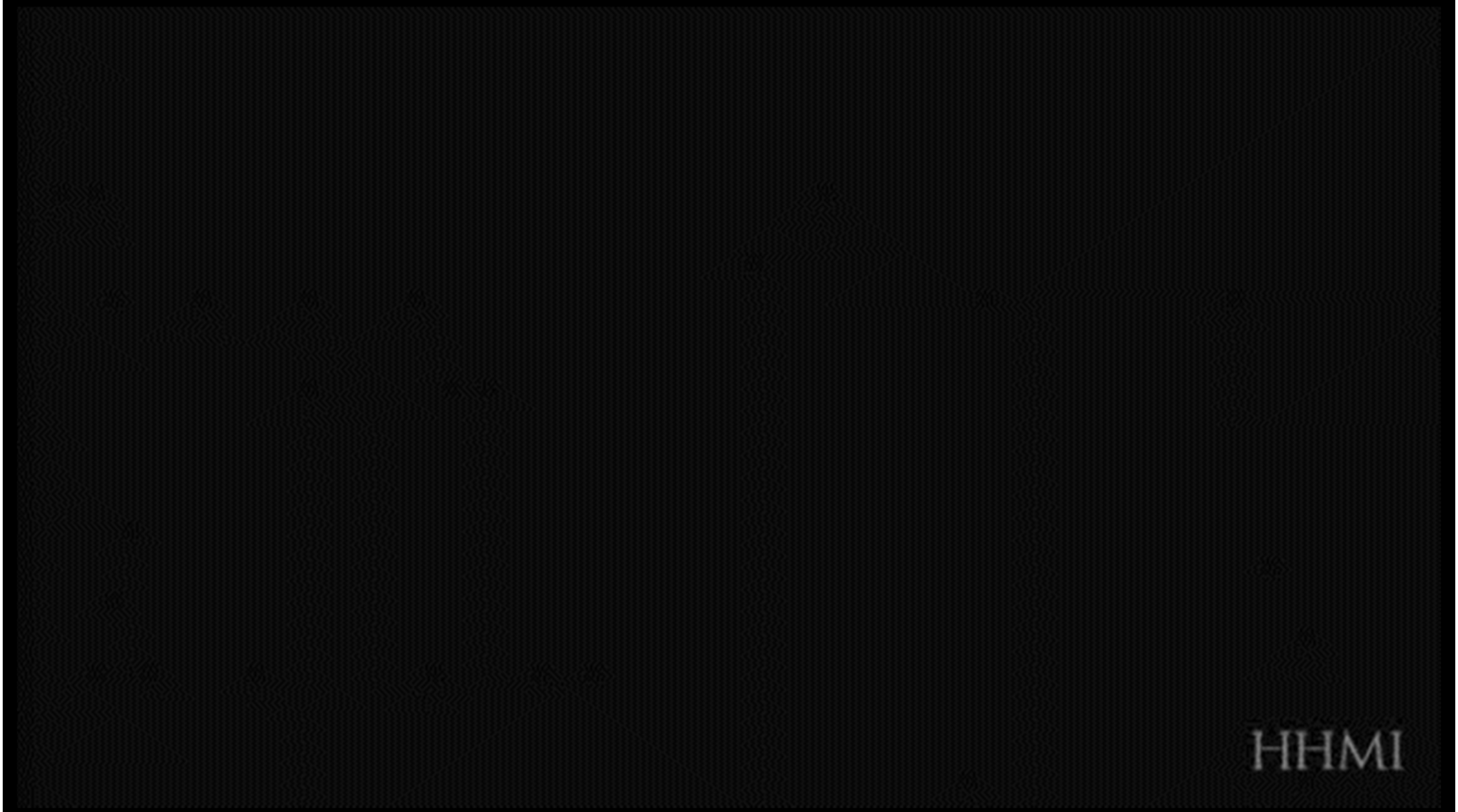


FIGURE 24.5 Aminoacyl-tRNA enters the A site, receives the polypeptide chain from peptidyl-tRNA, and is transferred into the P site for the next cycle of elongation.


HHMI

Translation
(protein synthesis)

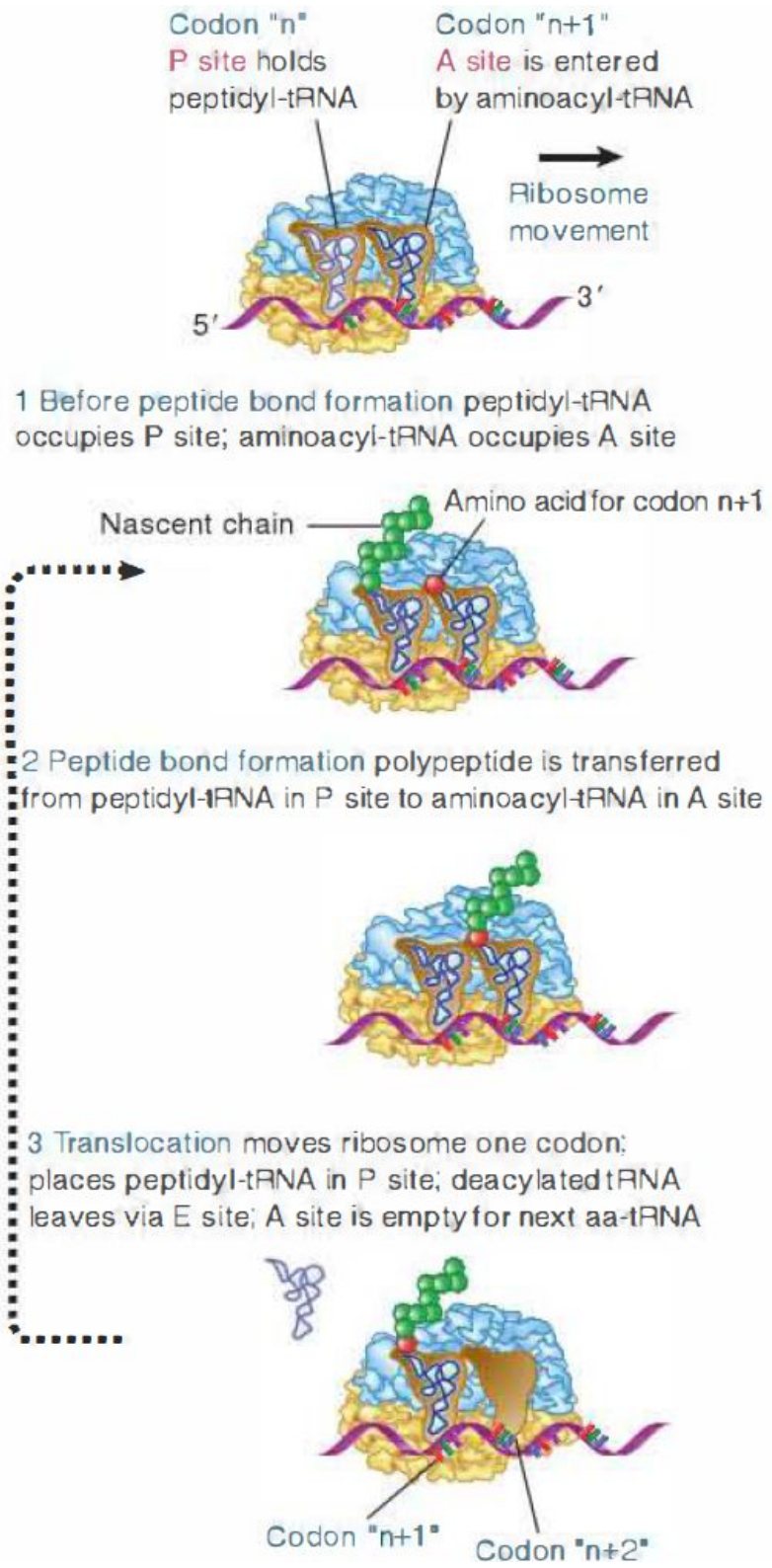


FIGURE 24.3 The ribosome has two sites for binding charged tRNA.

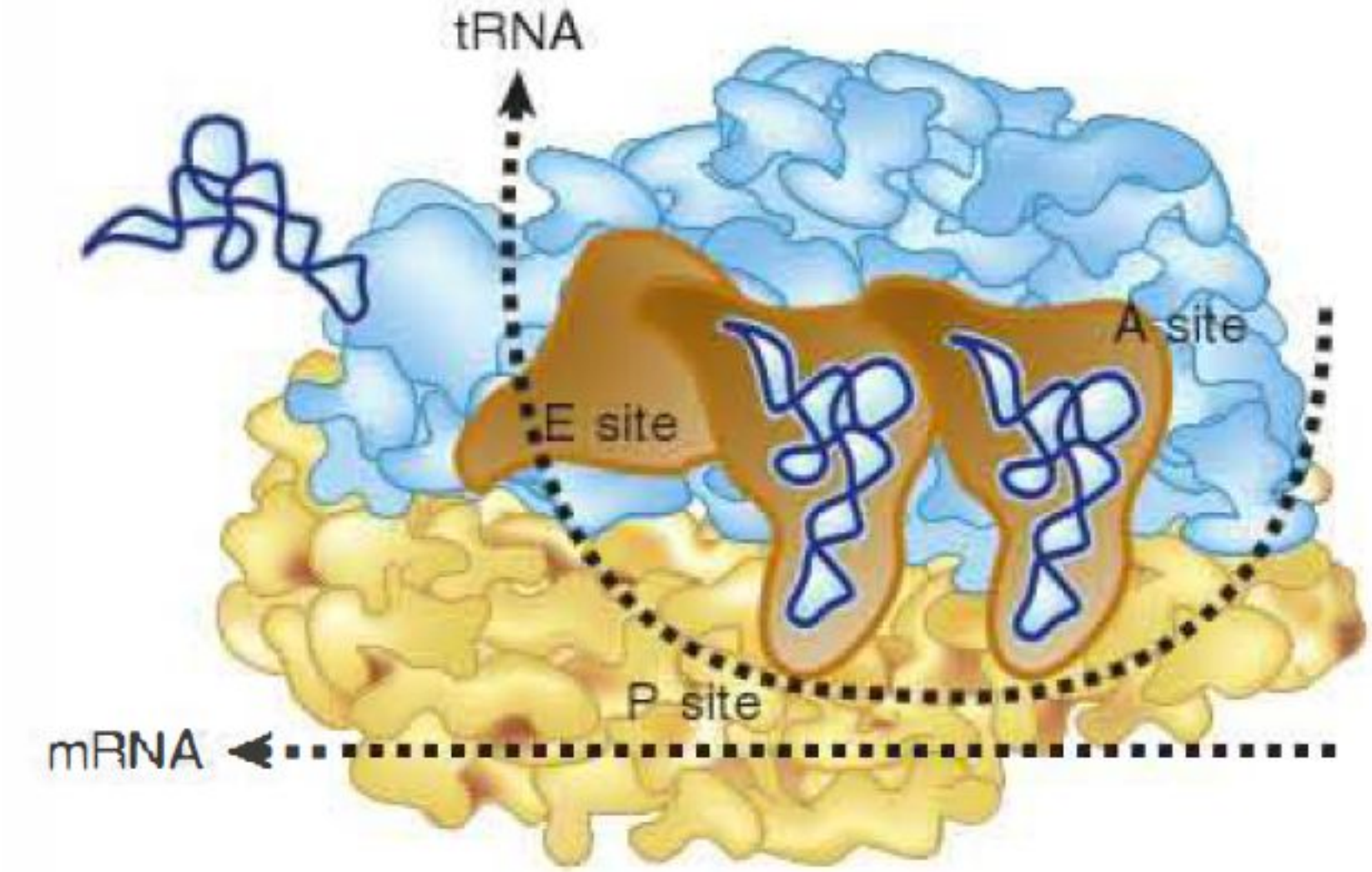


FIGURE 24.6 tRNA and mRNA move through the ribosome in the same direction.

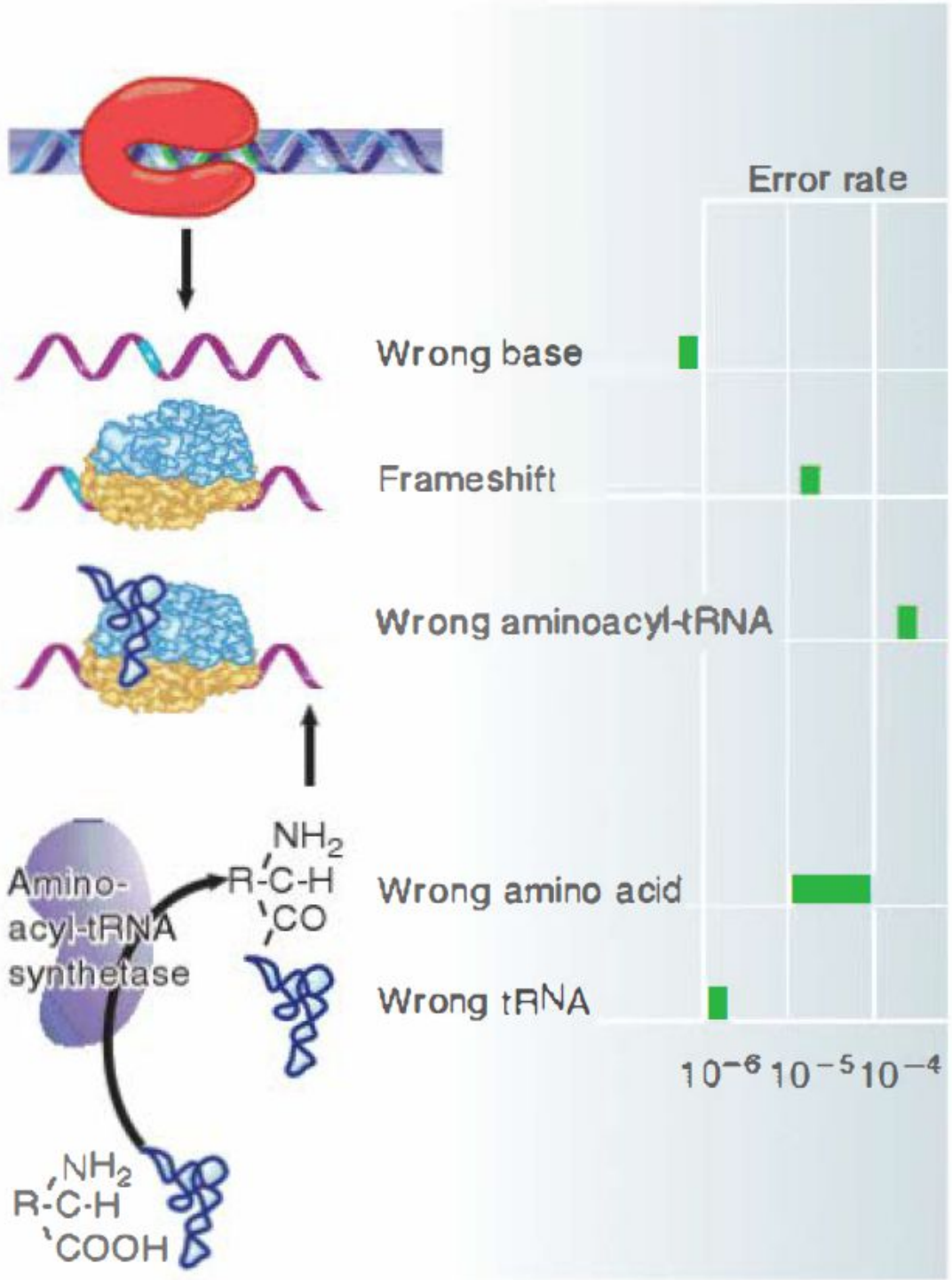
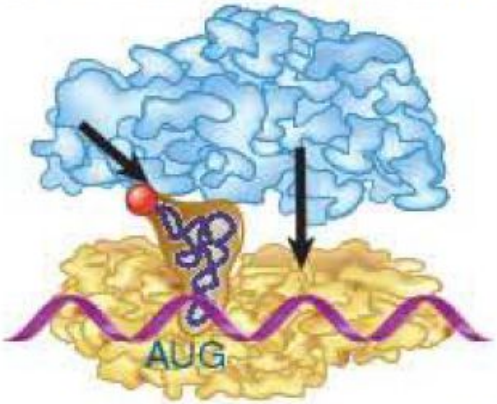


FIGURE 24.8 Errors occur at rates from 10^{-6} to 5×10^{-4} at different stages of translation.

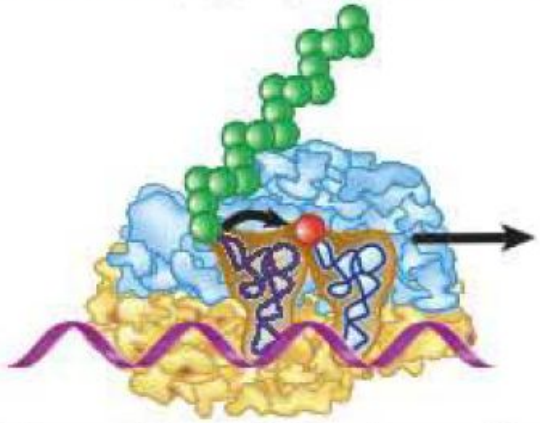
Translation has three stages:

- initiation
- elongation
- termination

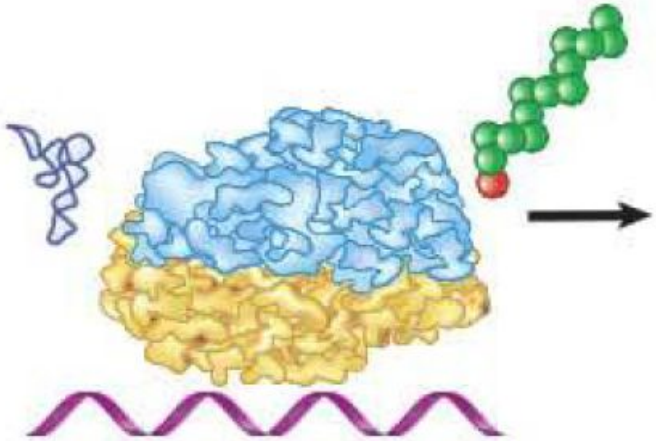
Initiation small subunit on mRNA binding site is joined by large subunit and aminoacyl-tRNA binds



Elongation Ribosome moves along mRNA, extending protein by transfer from peptidyl-tRNA to aminoacyl-tRNA



Termination Polypeptide chain is released from tRNA, and ribosome dissociates from mRNA



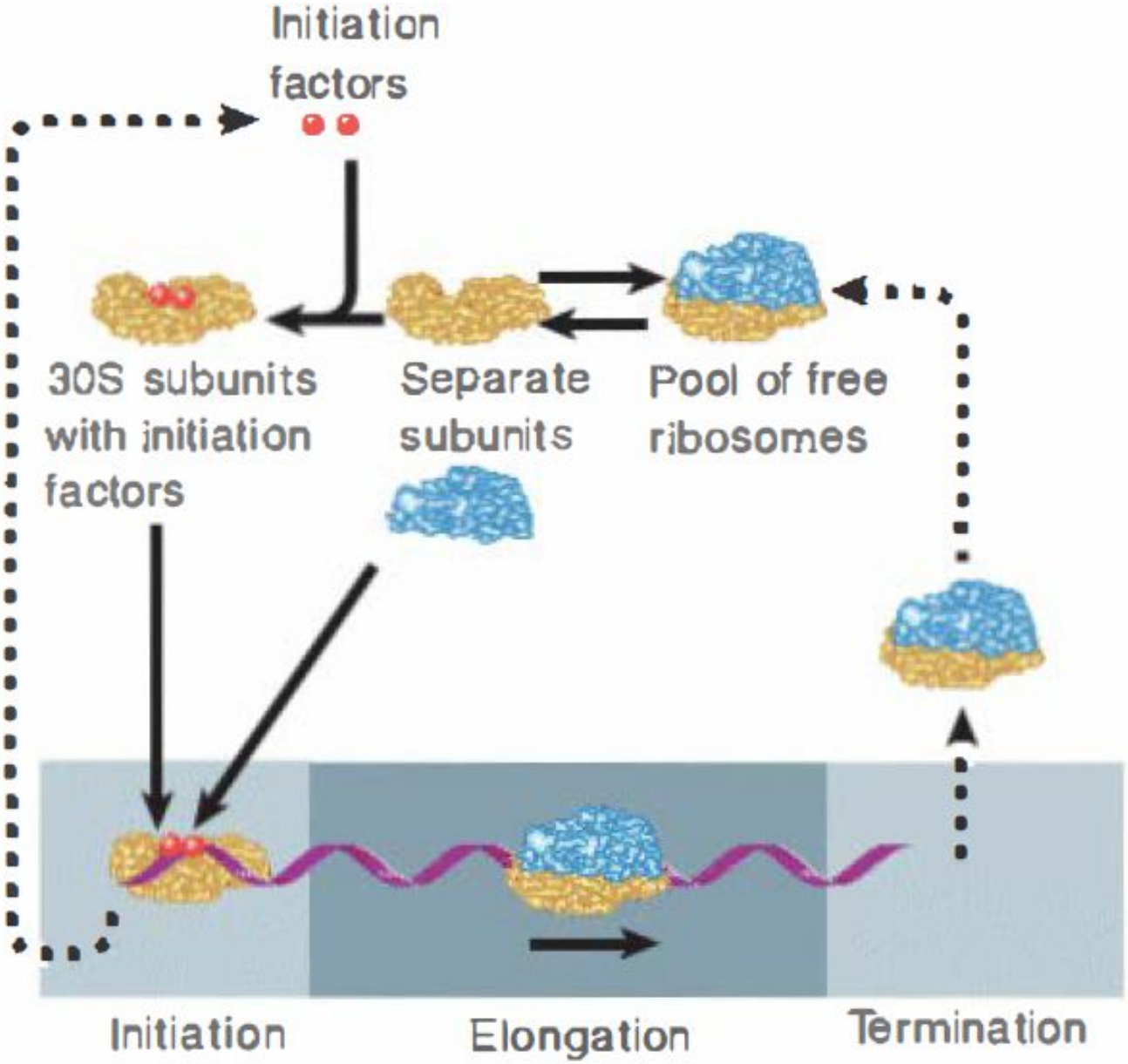


FIGURE 24.9 Initiation requires free ribosome subunits. When ribosomes are released at termination, the 30S subunits bind initiation factors and dissociate to generate free subunits. When subunits reassociate to give a functional ribosome at initiation, they release the factors.

Initiation in Bacteria Needs 30S Subunits and Accessory Factors

E. coli Initiation Factors:

- IF1
- IF2
- IF3

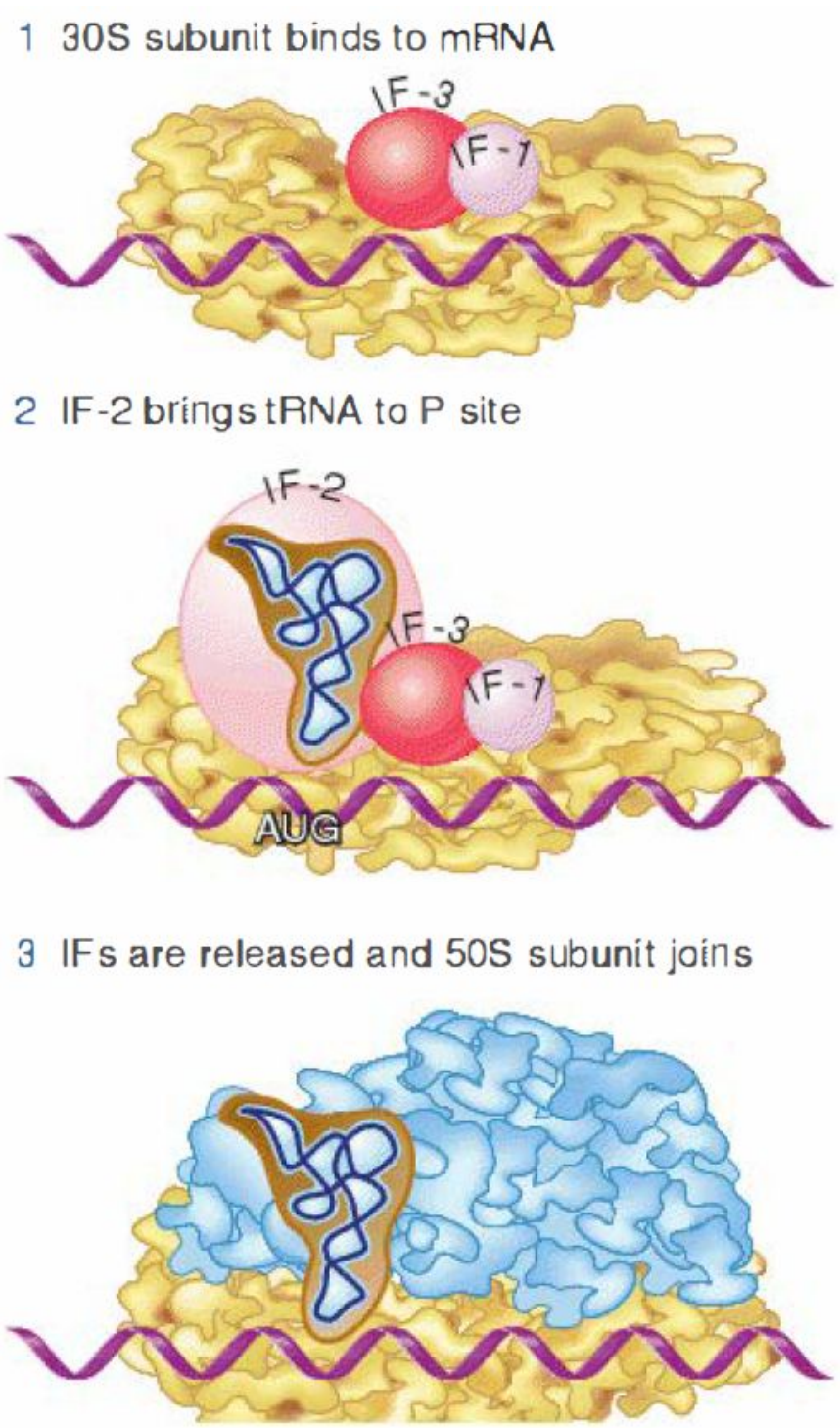


FIGURE 24.10 Initiation factors stabilize free 30S subunits and bind initiator tRNA to the 30S-mRNA complex.

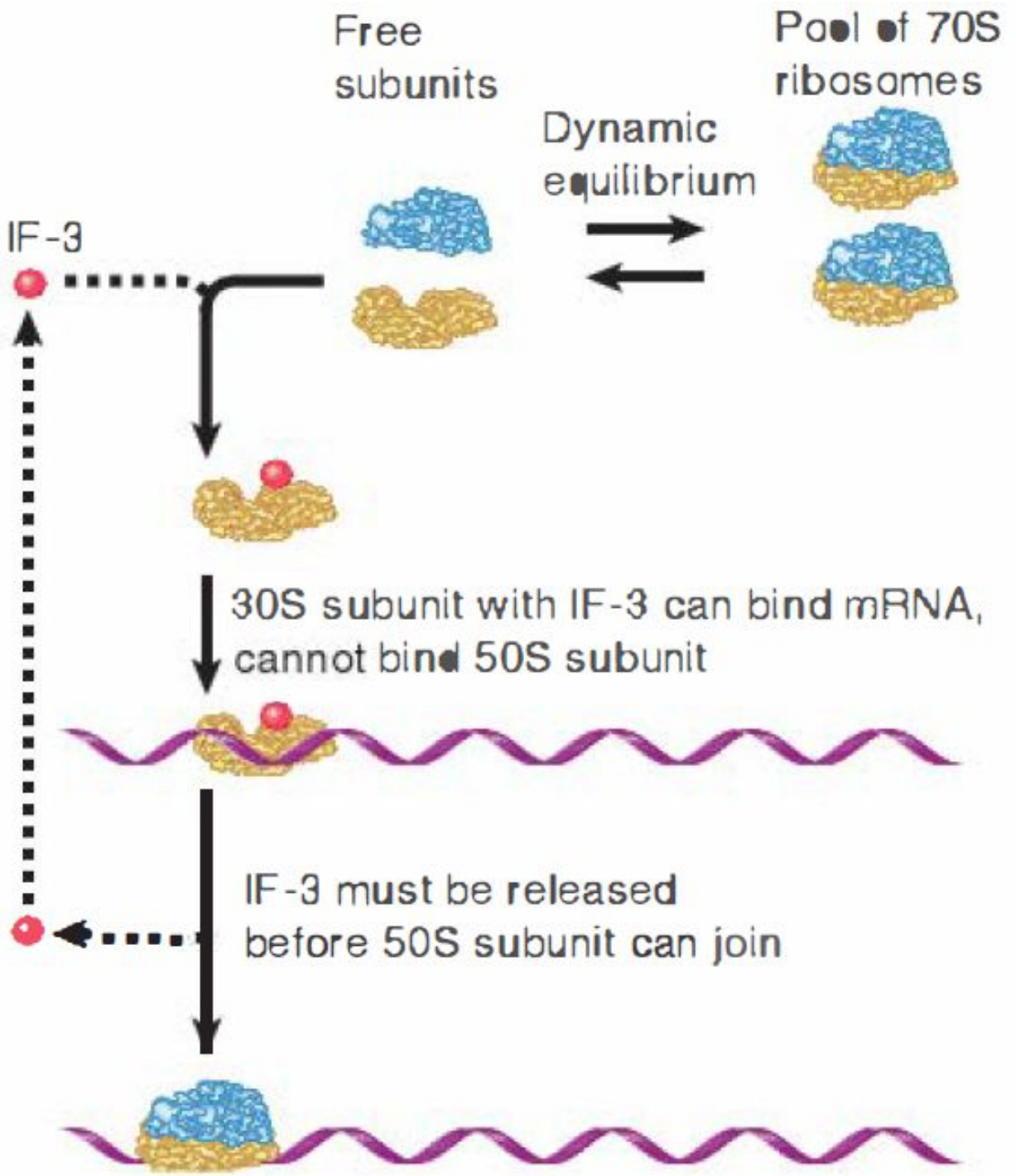


FIGURE 24.11 Initiation requires 30S subunits that carry IF-3.

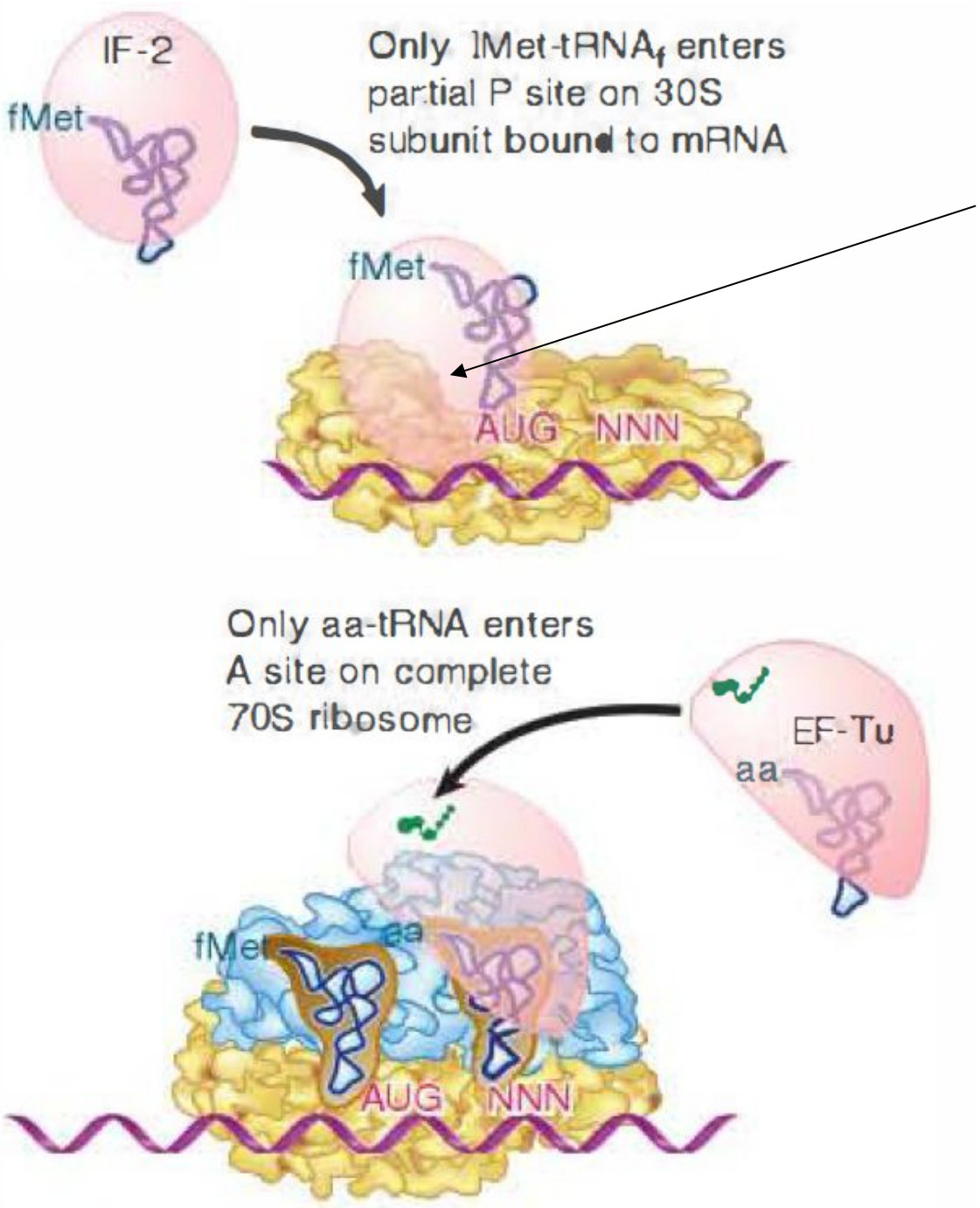


FIGURE 24.16 Only fMet-tRNA$_f$ can be used for initiation by 30S subunits; other aminoacyl-tRNAs (aa-tRNA) must be used for elongation by 70S ribosomes.

fMet-tRNA: special tRNA needed for the initiation of eukaryotic translation, reads AUG or GUG (Met o Val inside the mRNA sequence)

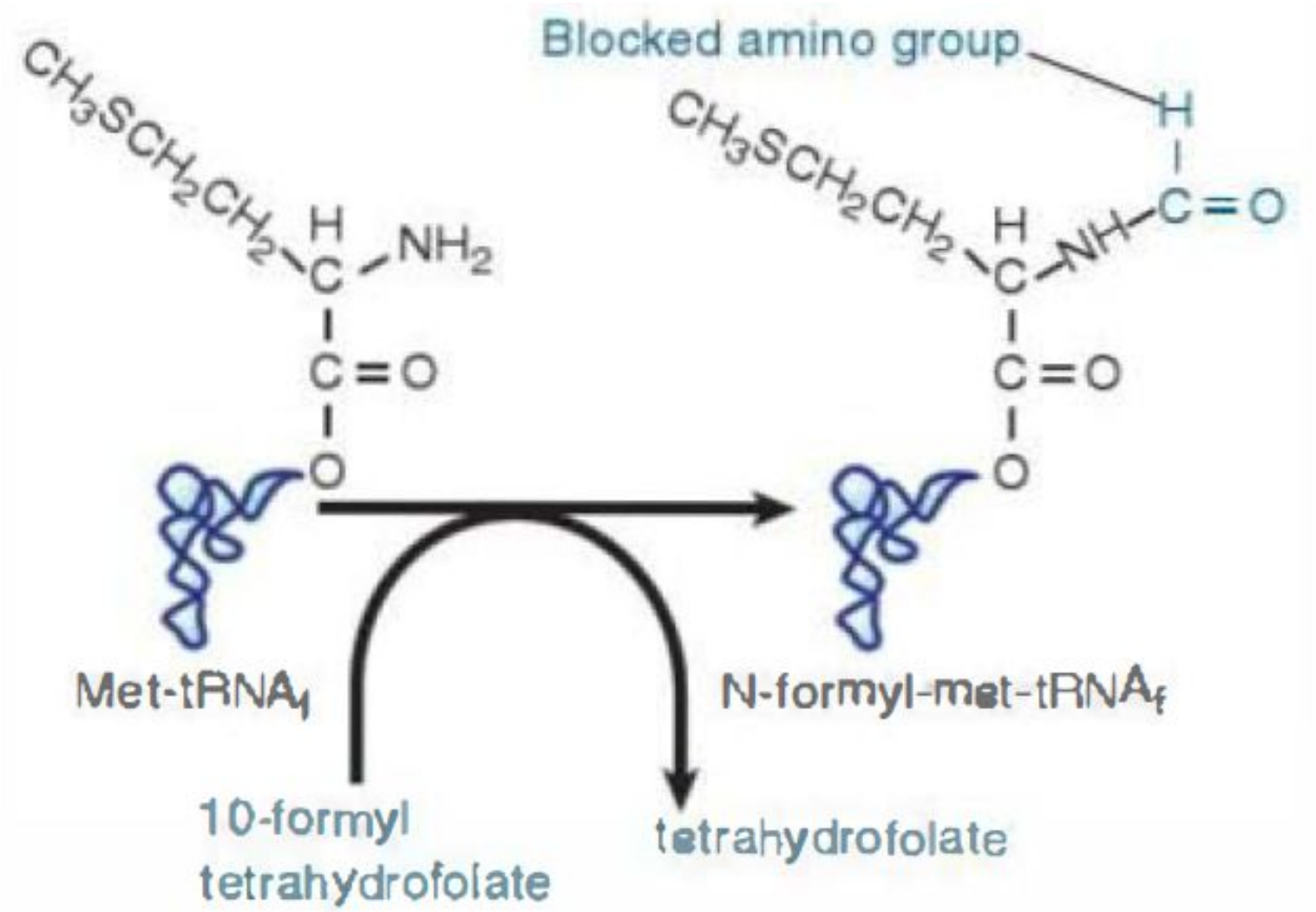


FIGURE 24.14 The initiator N-formyl-methionyl-tRNA ($fMet\text{-}tRNA_f$) is generated by formylation of methionyl-tRNA using formyl-tetrahydrofolate as a cofactor.

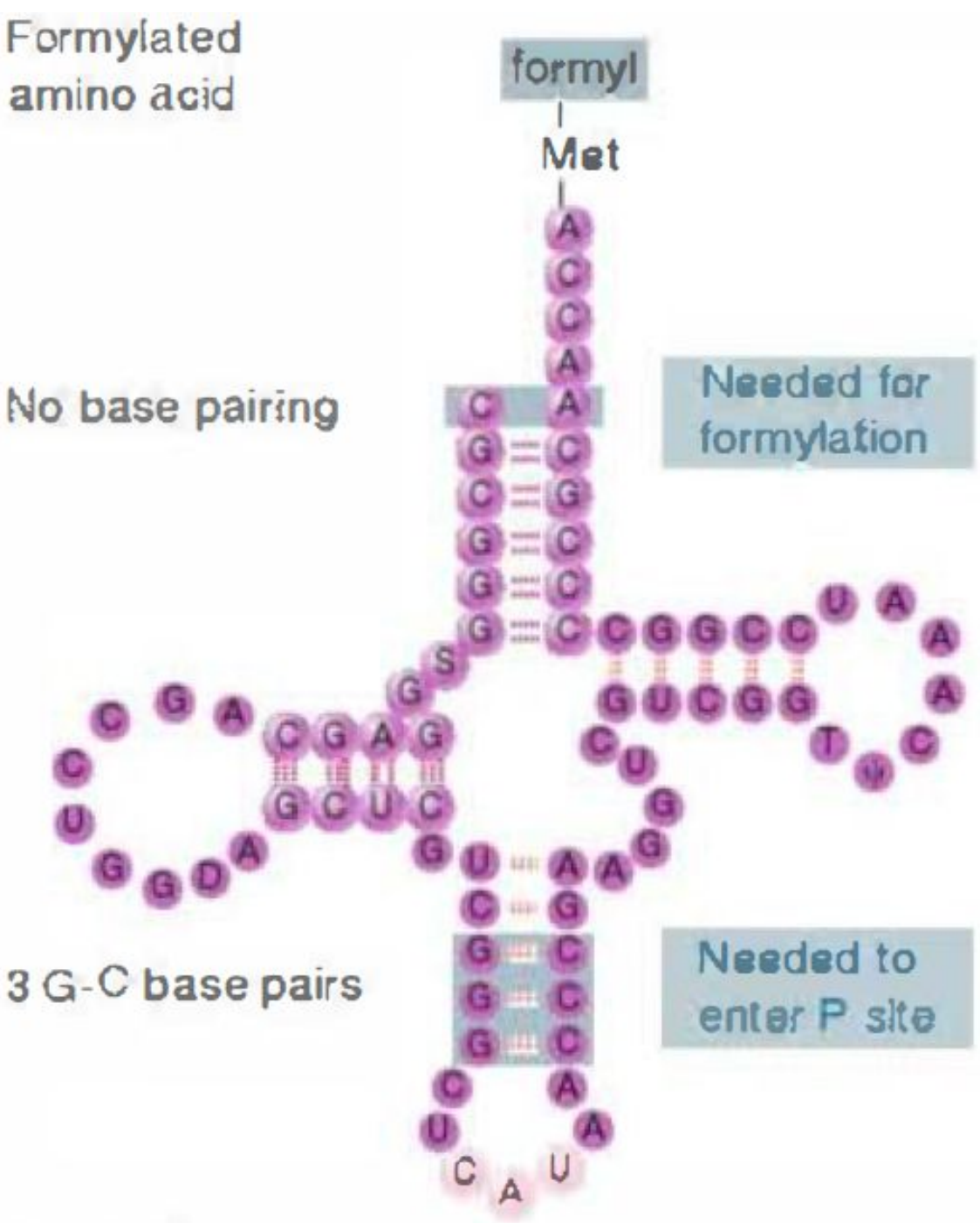


FIGURE 24.15 fMet-tRNA$_f$ has unique features that distinguish it as the initiator tRNA.

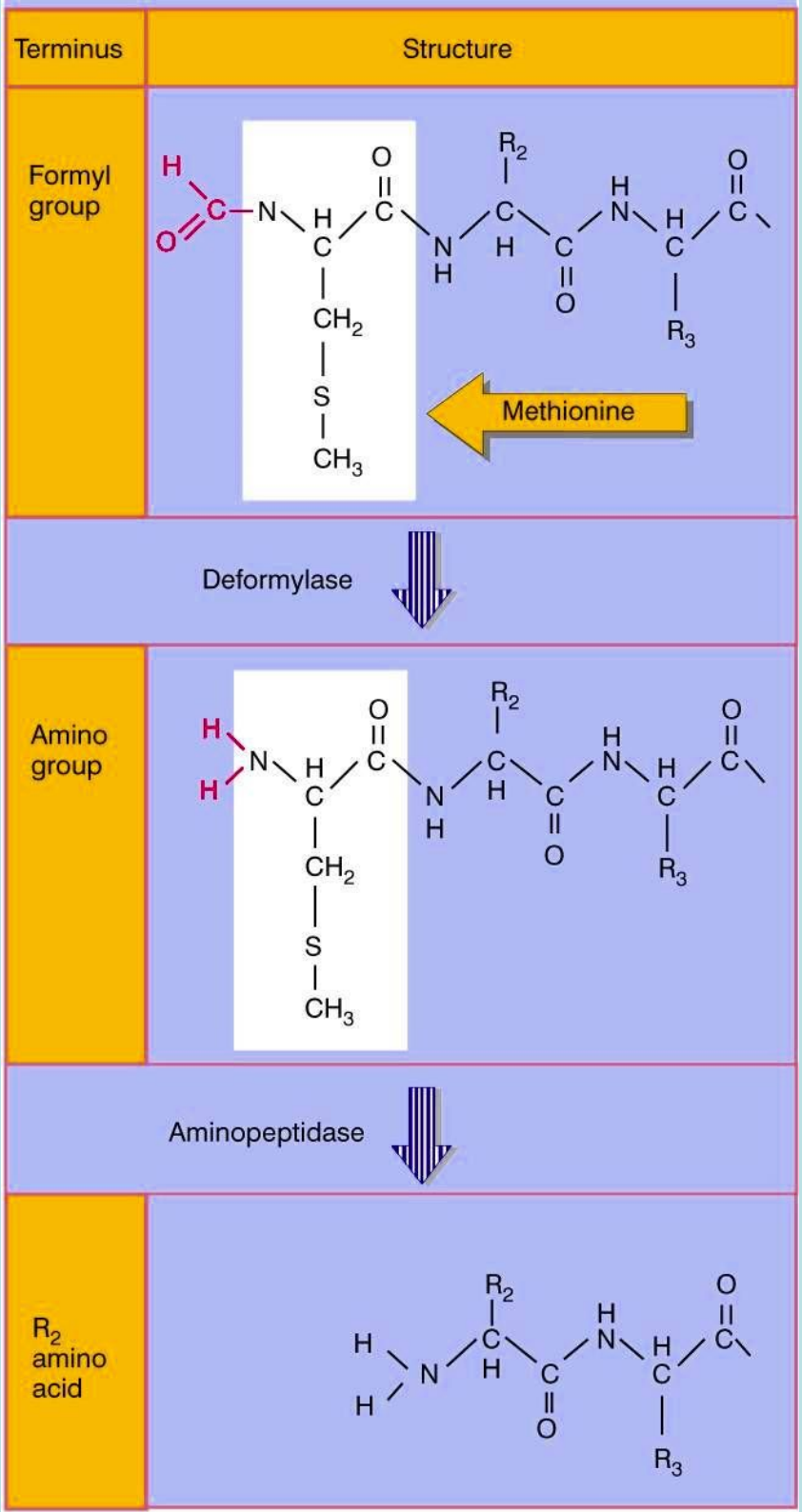


Figure 6.13 Newly synthesized proteins in bacteria start with formyl-methionine, but the formyl group, and sometimes the methionine, is removed during protein synthesis.

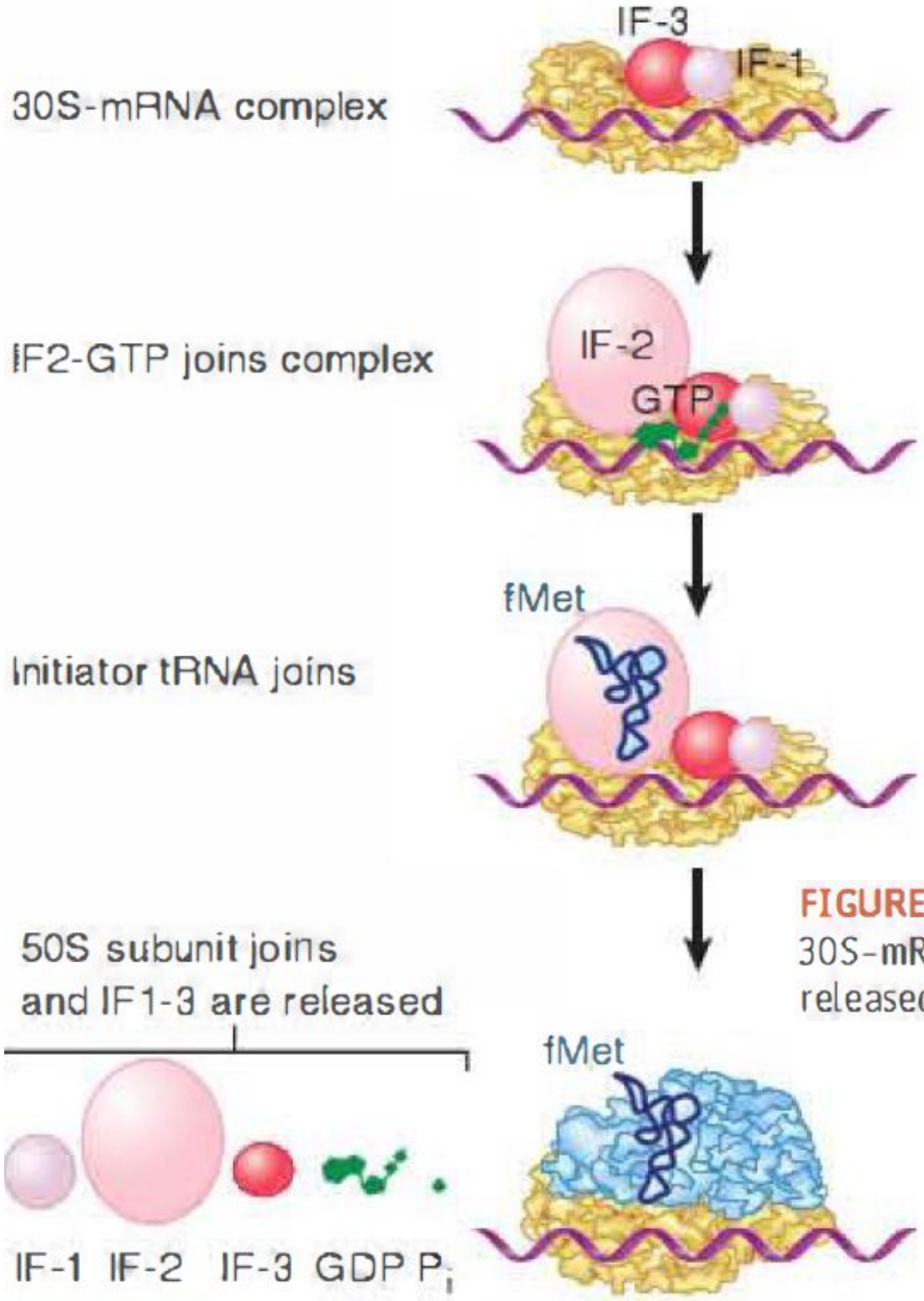


FIGURE 24.17 IF-2 is needed to bind fMet-$tRNA_f$ to the 30S-mRNA complex. After 50S binding, all IF factors are released and GTP is cleaved.

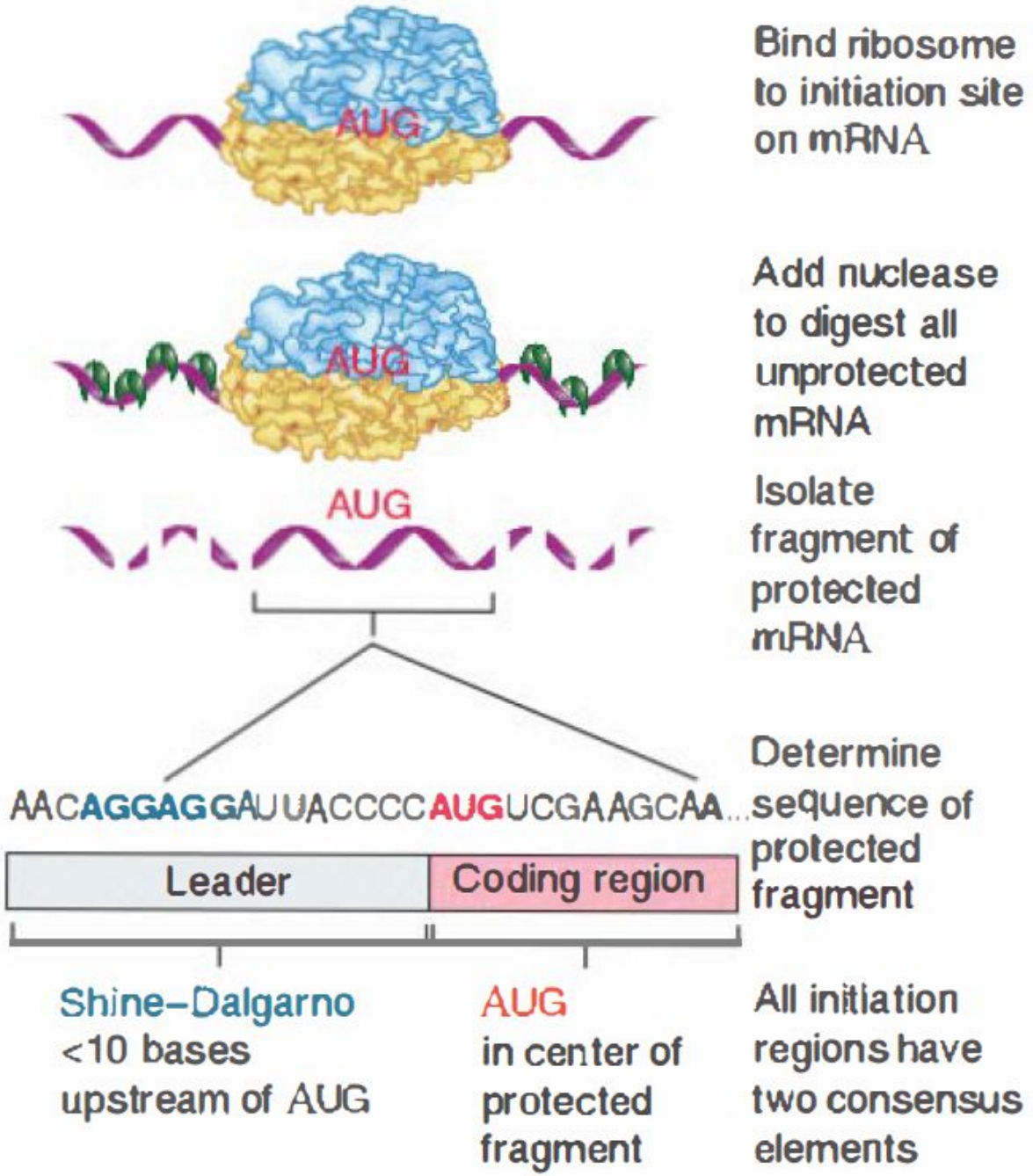
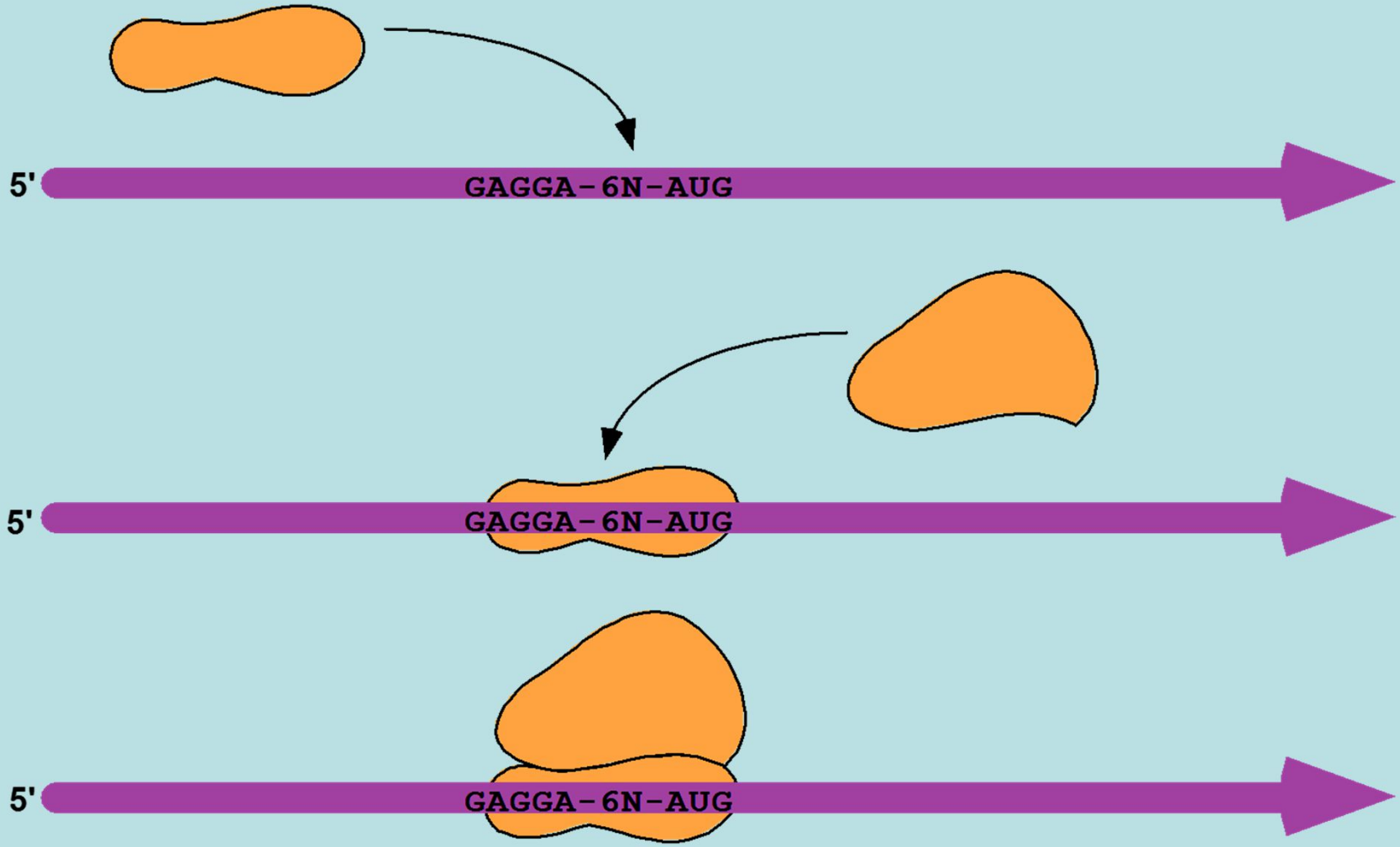
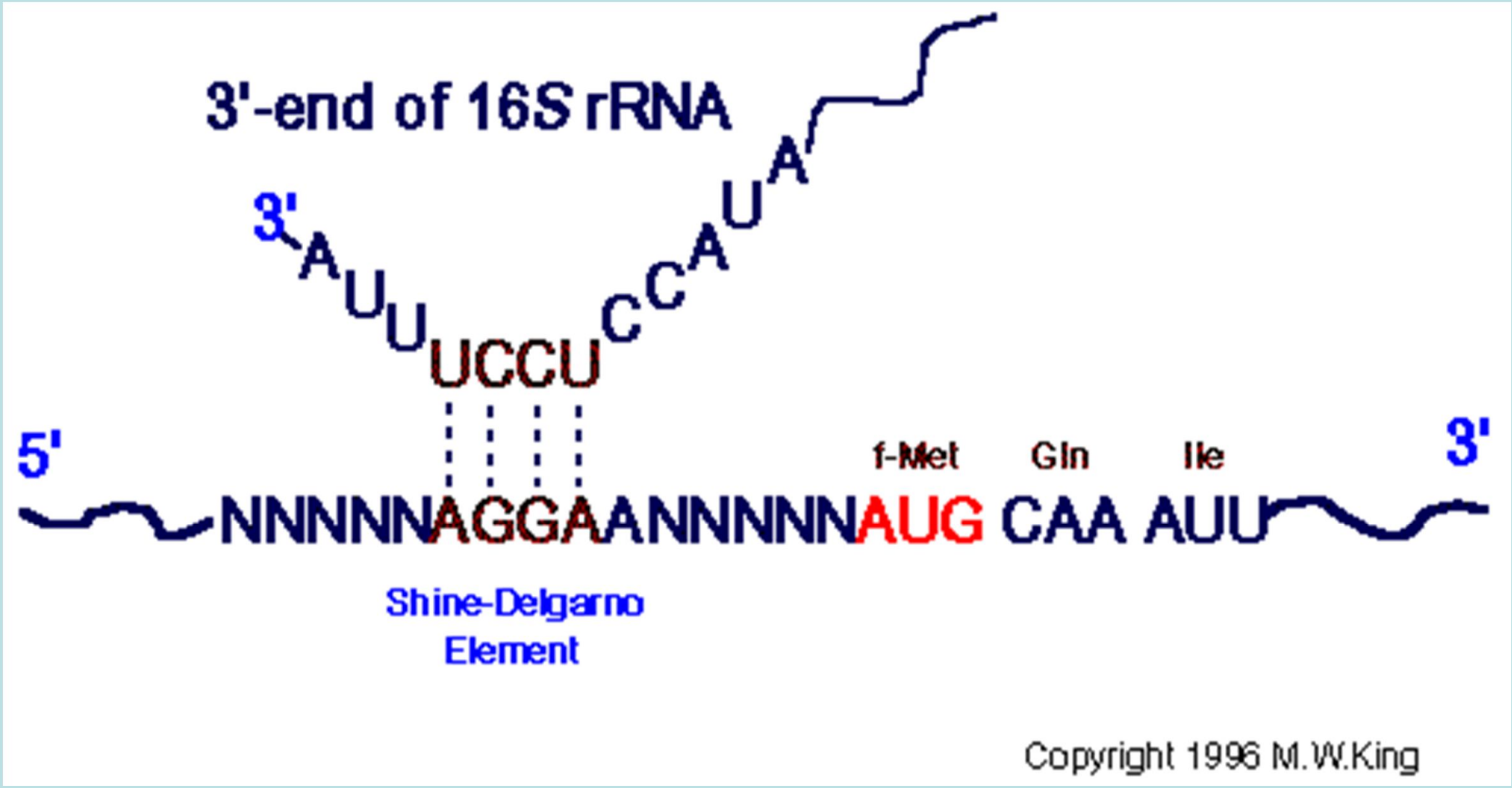


FIGURE 24.12 Ribosome-binding sites on mRNA can be recovered from initiation complexes. They include the upstream Shine–Dalgarno sequence and the initiation codon.

Shine-Dalgarno sequence




3'-end of 16S rRNA
3'
AUUUCCUCCAUA
5'
NNNNNAGGAANNNNNAUG CAA AUU
3'
f-Met
Gln
Ile
Shine-Delgarno
Element
Copyright 1996 M.W.King

Polycistronic mRNA translation in prokaryotes

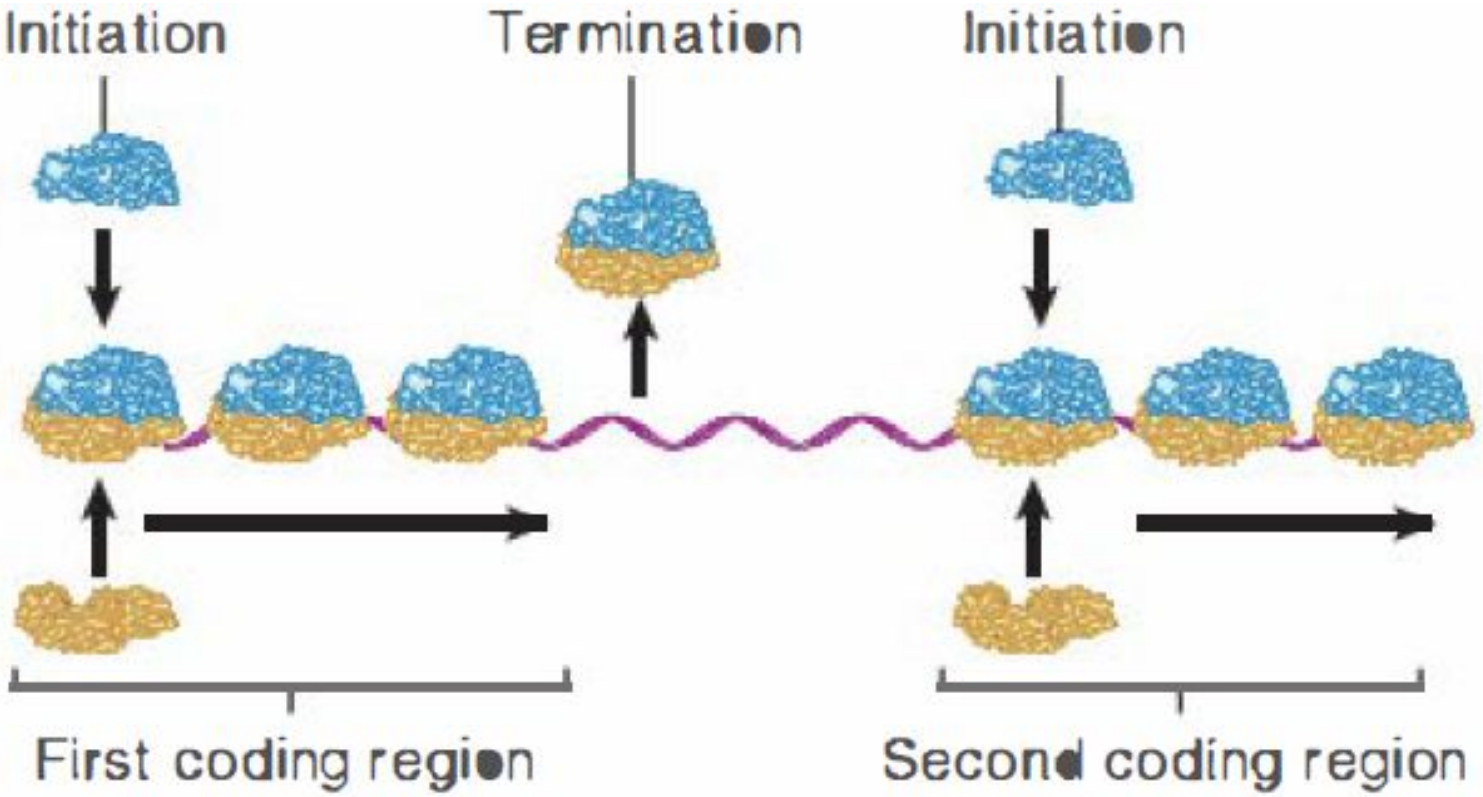
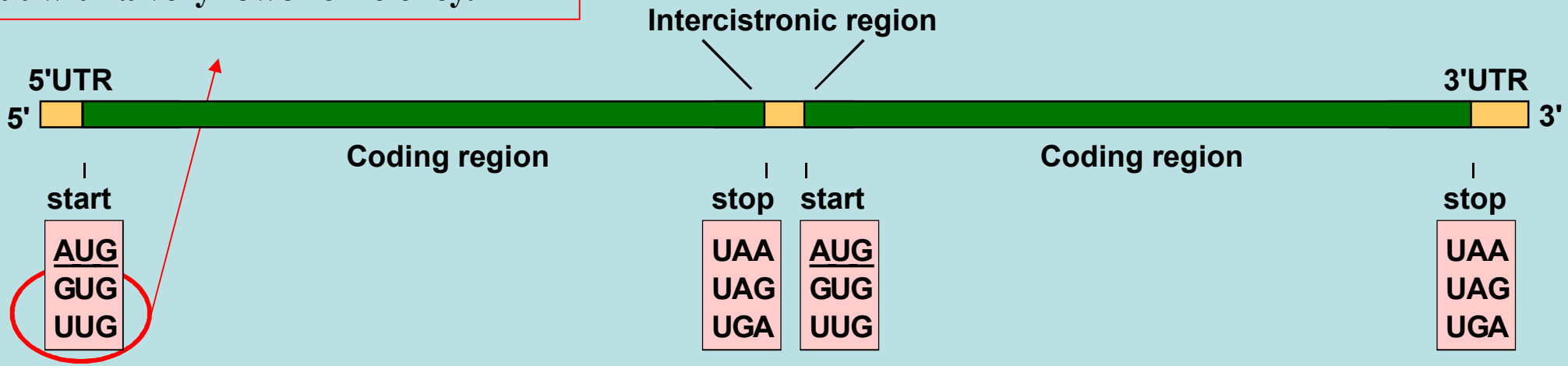


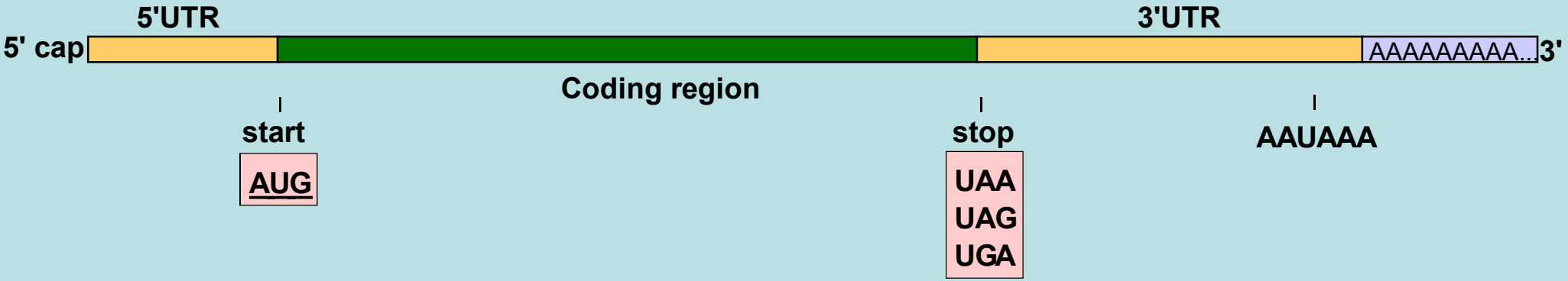
FIGURE 24.13 Initiation occurs independently at each cistron in a polycistronic mRNA. When the intercistronic region is longer than the span of the ribosome, dissociation at the termination site is followed by independent reinitiation at the next cistron.

Small Subunits Scan for Initiation Sites on Eukaryotic mRNA

Very rarely recognized as start codons for fMET-tRNA (even if they are read as codons for different a.a. when they are inside a coding region) but with a very lower efficiency.

Prokaryotes





Eukaryotes

Initiation Factors		Activity
prokaryotes	eukaryotes	
IF3	eIF-1	Fidelity of AUG codon recognition
IF2	eIF-1A	Facilitate Met-tRNAiMet binding to small subunit
	eIF-2	Ternary complex formation
	eIF-2B (GEF)	GTP/GDP exchange during eIF-2 recycling
	eIF-3 (12 subunits)	Ribosome antiassociation, binding to 40S
	eIF-4F (4E, 4A, 4G)	mRNA binding to 40S, RNA helicase activity
	eIF-4A	ATPase-dependent RNA helicase
	eIF-4E	5' cap recognition
	eIF-4G	Scaffold for of eIF-4E and -4A
	eIF-4B	Stimulates helicase, binds with eIF-4F
	eIF-4H	Similar to eIF4B
	eIF-5	Release of eIF-2 and eIF-3, GTPase
IF1	eIF5B	Subunit joining
	eIF-6	Ribosome subunit antiassociation

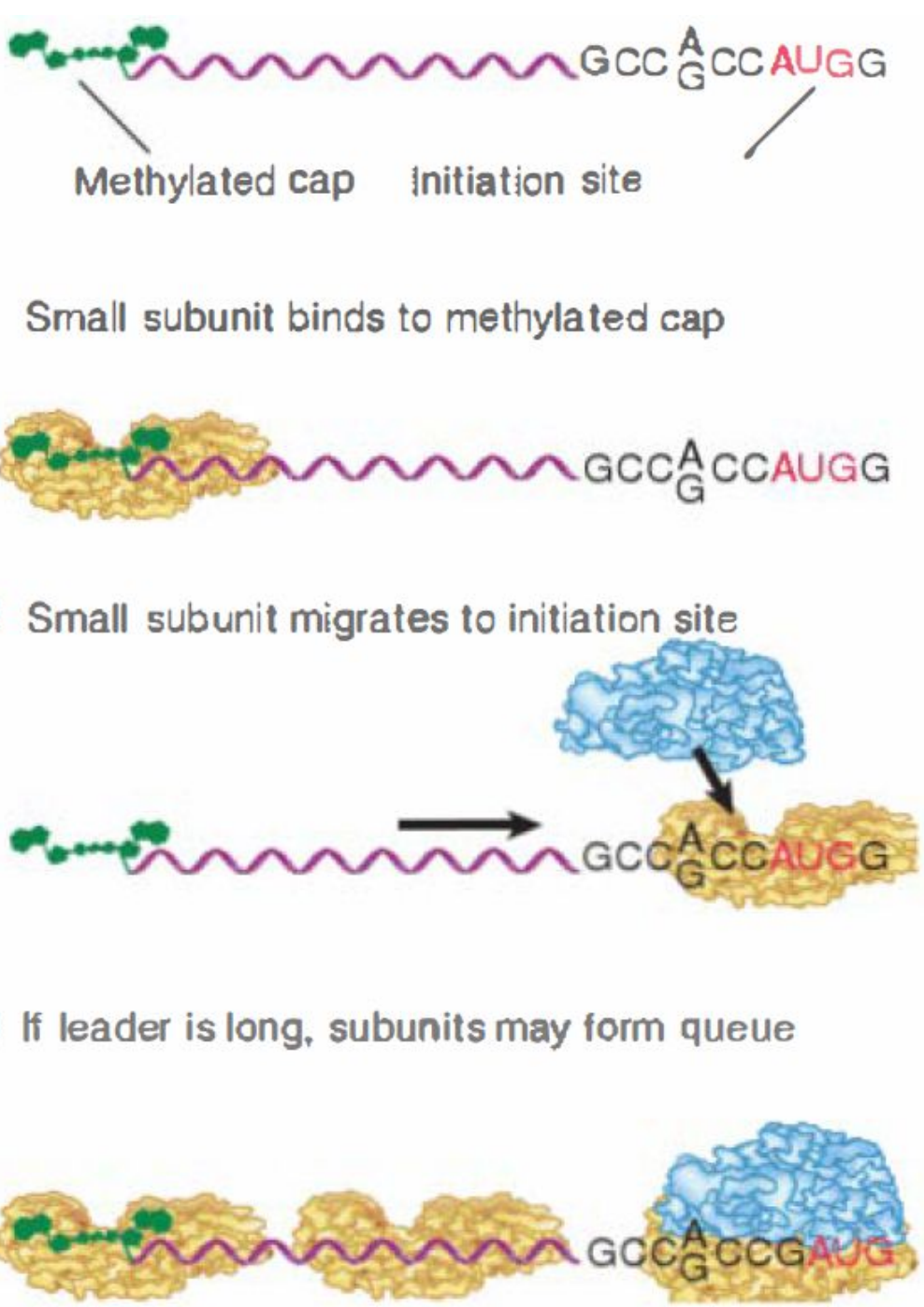
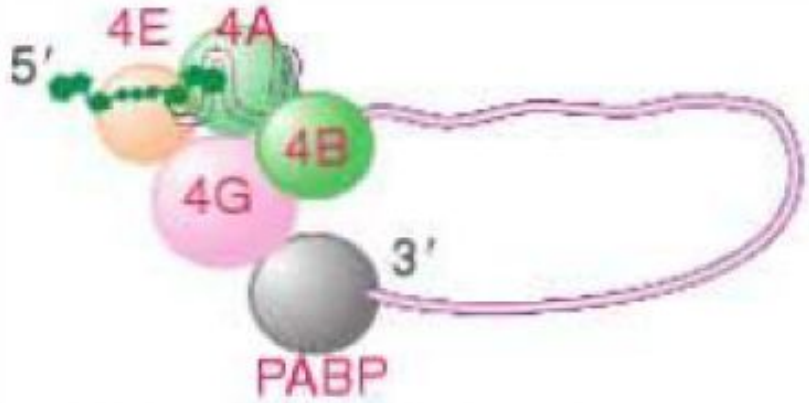


FIGURE 24.18 Eukaryotic ribosomes migrate from the 5′ end of mRNA to the ribosome binding site, which includes an AUG initiation codon.

Kozak consensus sequence

eIF4F is a heterotrimer consisting of:

eIF4G is a scaffold protein
eiF4E binds the 5' methyl cap
eIF4A is a helicase that unwinds the 5' structure



eiF4G binds two further factors
eIF4B stimulates eIF4A helicase
PABP binds 3' poly(A)

FIGURE 24.22 The heterotrimer eIF4F binds the 5' end of mRNA as well as other factors.

In Eukaryotes

- eIF-2 forms a ternary complex with Met-tRNA$_f$
- The ternary complex binds to the 40S **free** subunit than binding to the 5′ mRNA end
- GTP is idolized when eIF2 is released as eIF2-GDP
- eIF-2B regenerate the active form

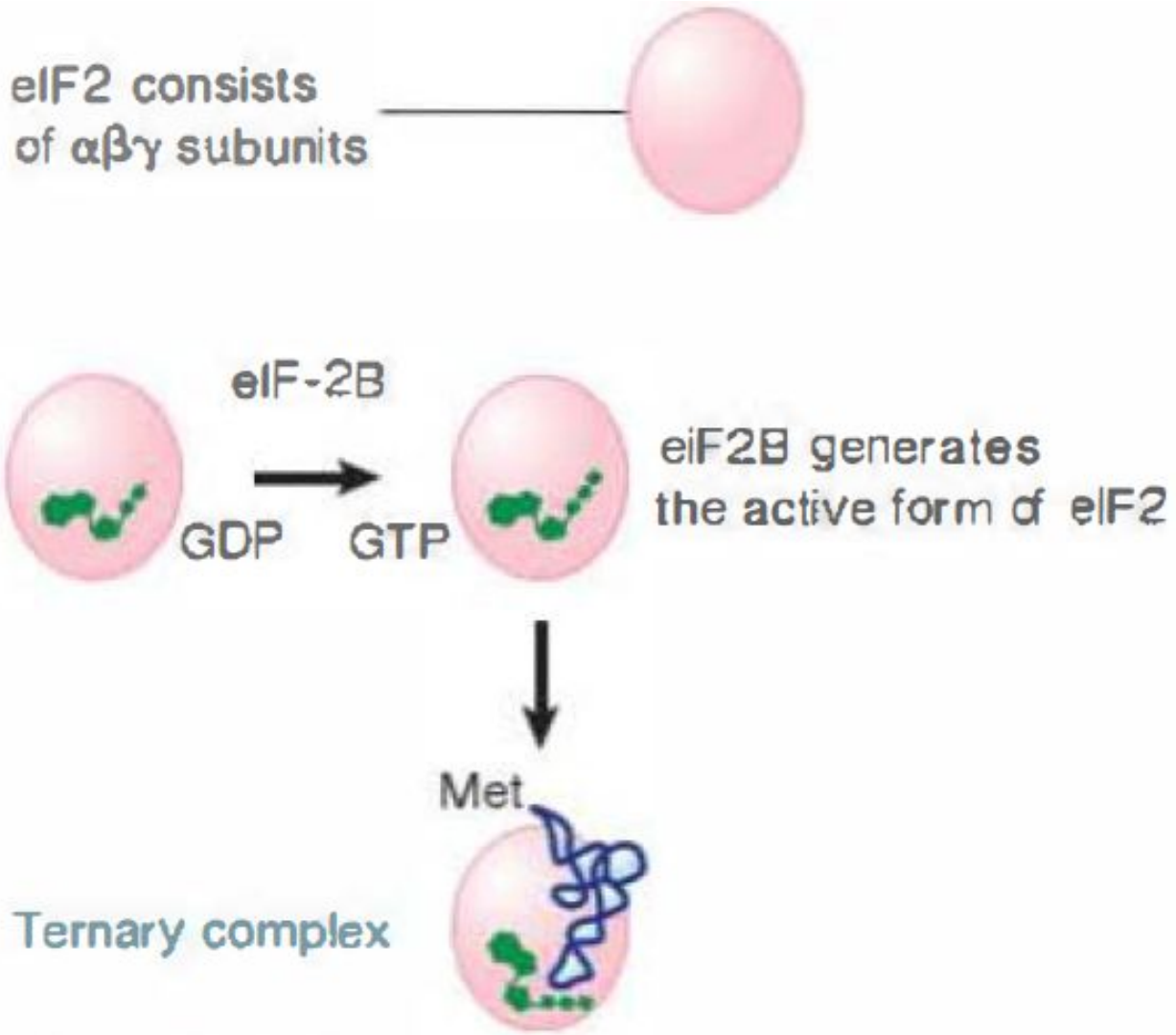


FIGURE 24.20 In eukaryotic initiation, eIF-2 forms a ternary complex with Met-tRNA$_i$ and GTP. The ternary complex binds to free 40S subunits, which attach to the 5′ end of mRNA. Later in the reaction, GTP is hydrolyzed and eIF2 is released in the form of eIF2-GDP. eIF2B regenerates the active form.

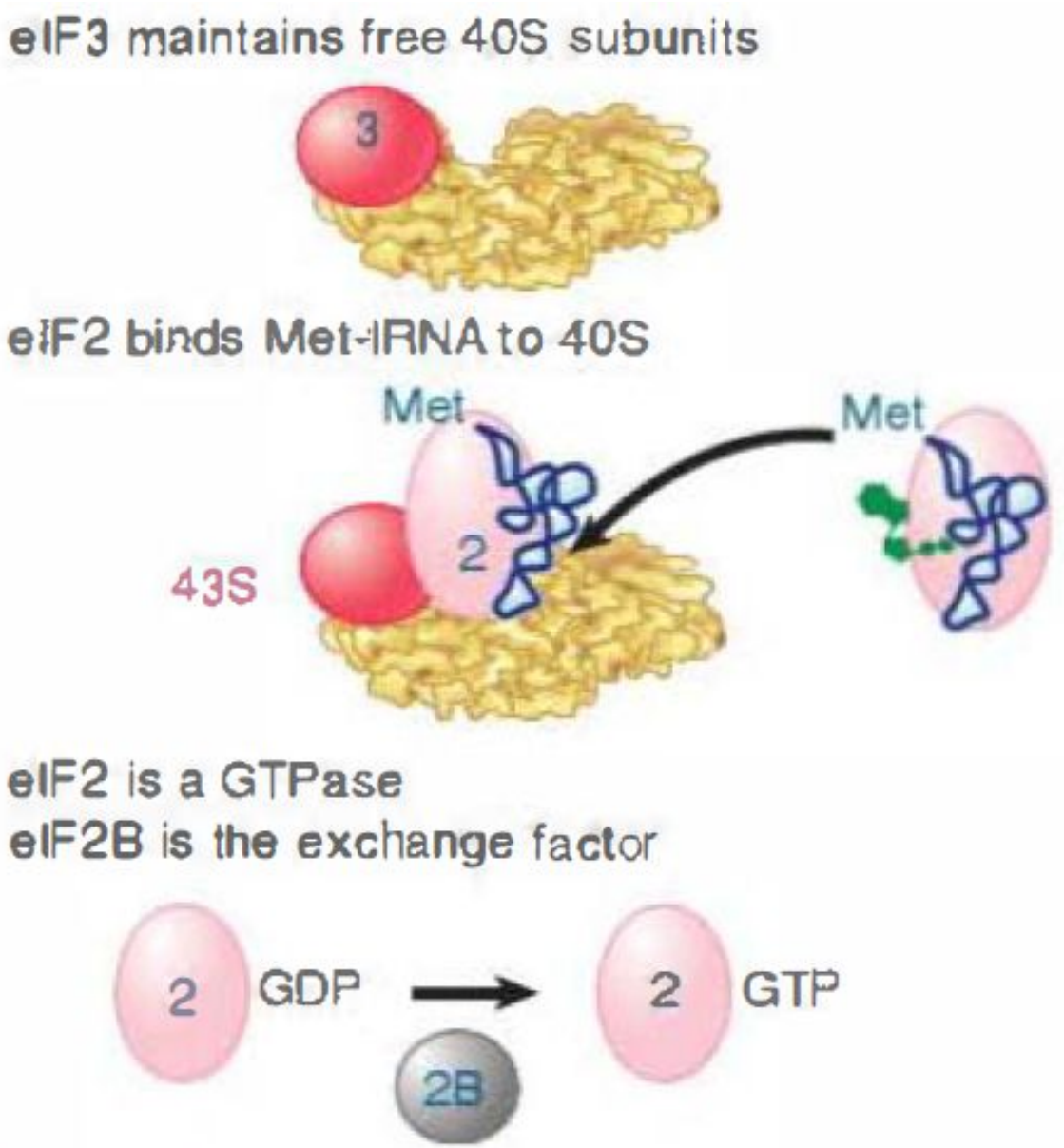
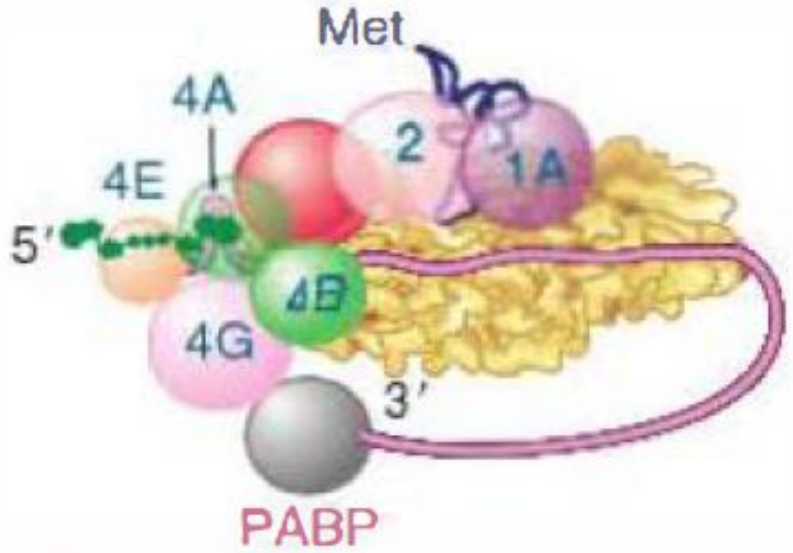


FIGURE 24.21 Initiation factors bind the initiator Met-tRNA to the 40S subunit to form a 43S complex. Later in the reaction, GTP is hydrolyzed and eIF2 is released in the form of eIF2-GDP. eIF2B regenerates the active form.



Possible interactions:
eIF4G binds to eIF3
mRNA binds eIF4G, eIF3, and 40S subunit

FIGURE 24.23 Interactions involving initiation factors are important when mRNA binds to the 43S complex.

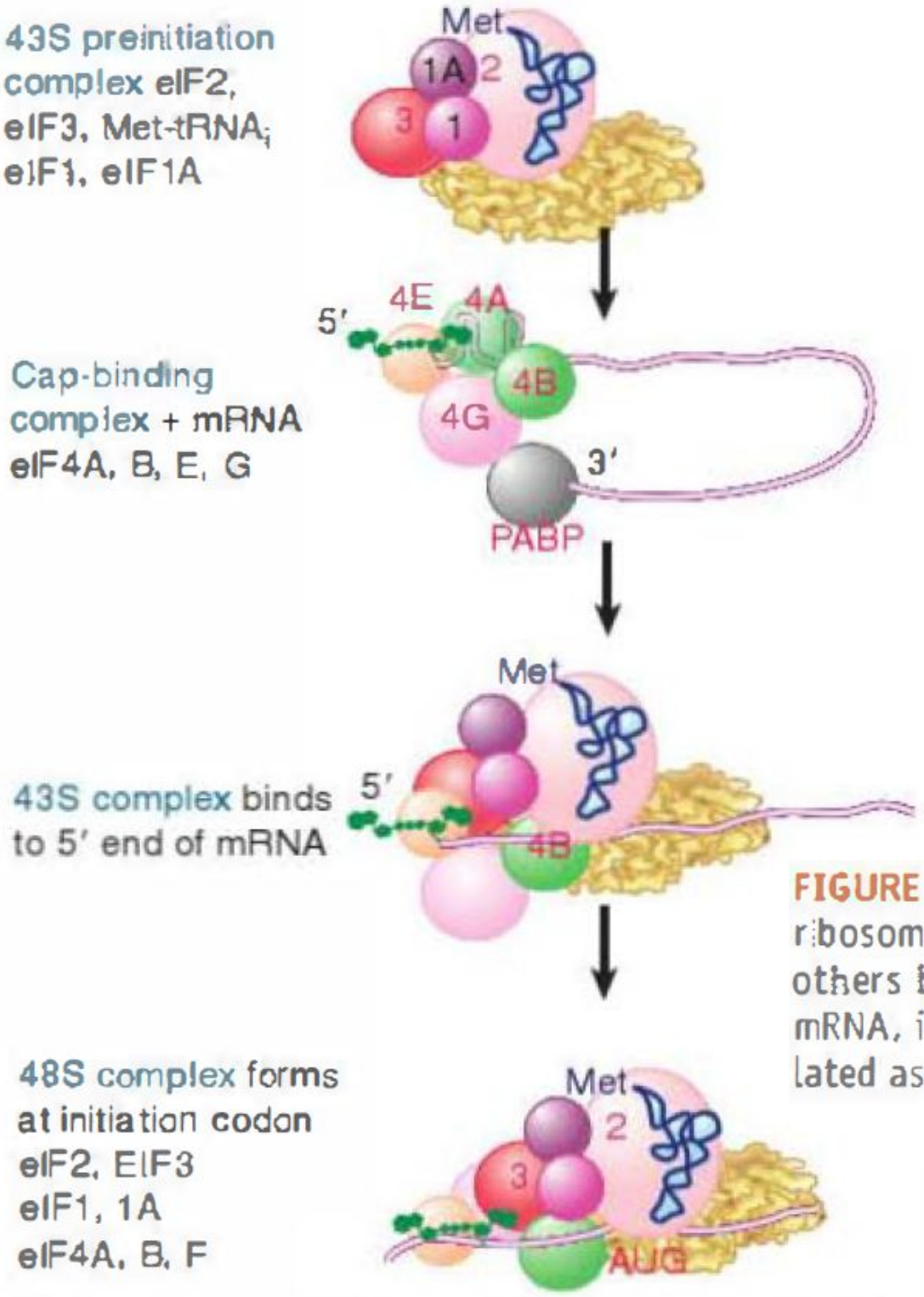


FIGURE 24.19 Some initiation factors bind to the 40S ribosome subunit to form the 43S preinitiation complex; others bind to mRNA. When the 43S complex binds to mRNA, it scans for the initiation codon and can be isolated as the 48S complex.

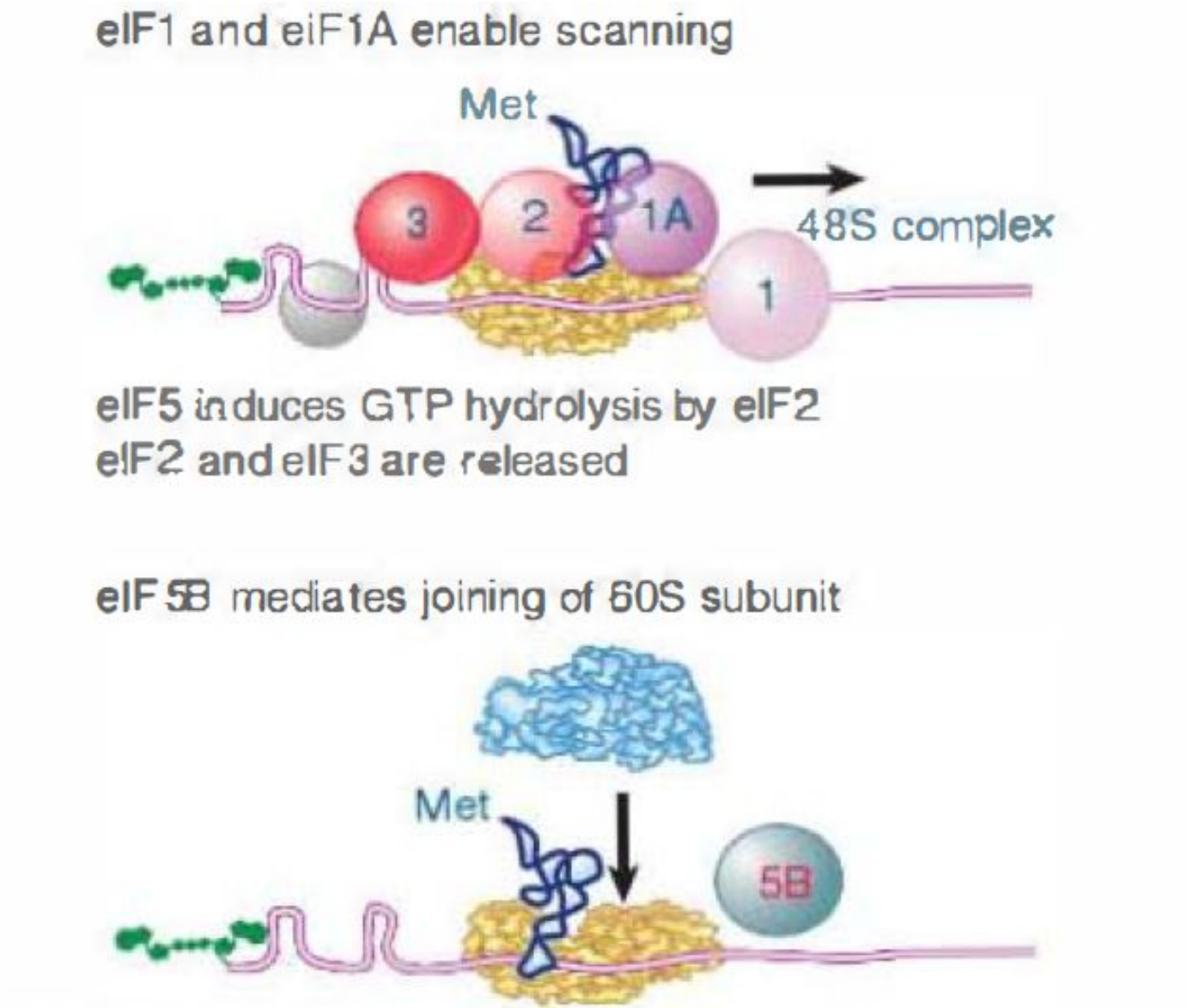
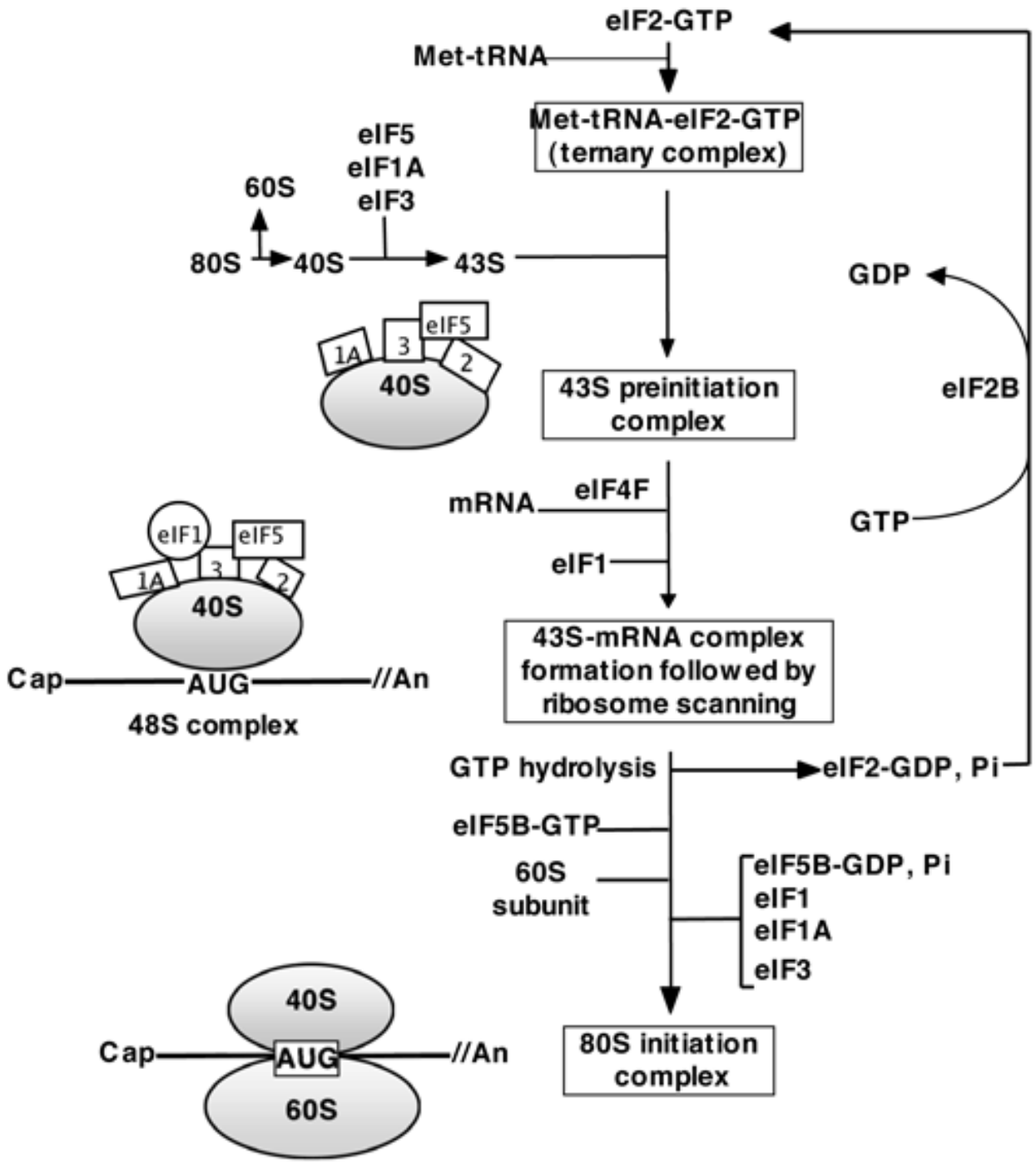
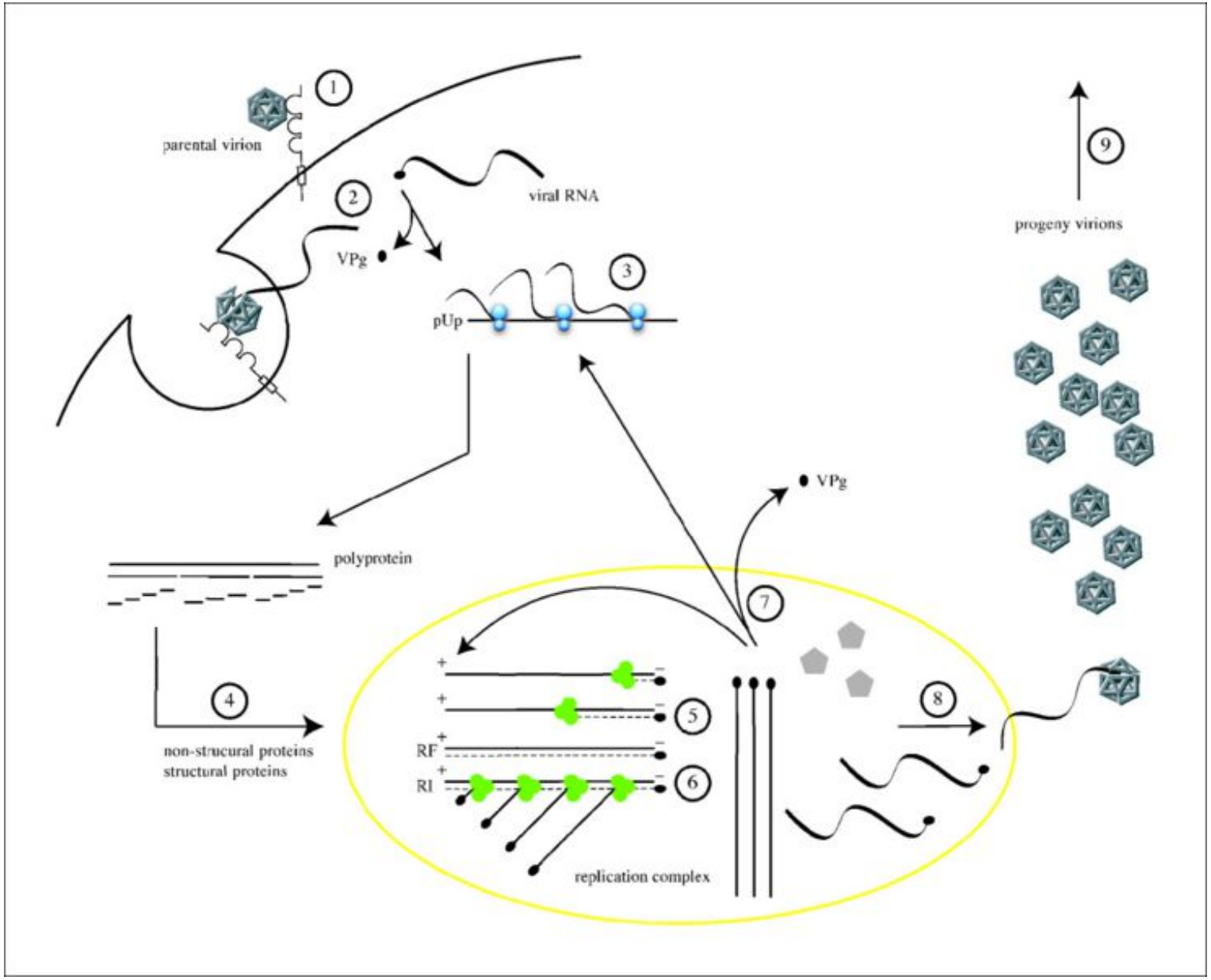


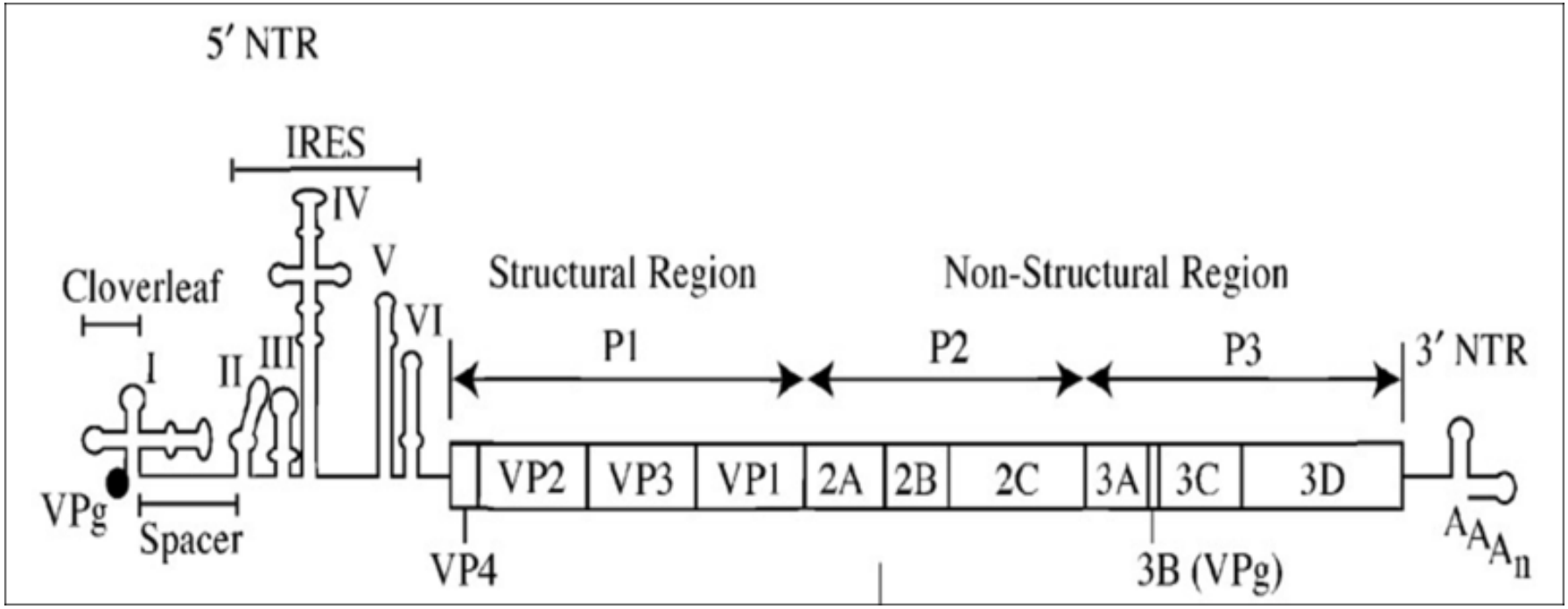
FIGURE 24.24 eIF1 and eIF1A help the 43S initiation complex to scan the mRNA until it reaches an AUG codon. eIF2 hydrolyzes its GTP to enable its release together with IF3. eIF5B mediates 60S–40S joining.

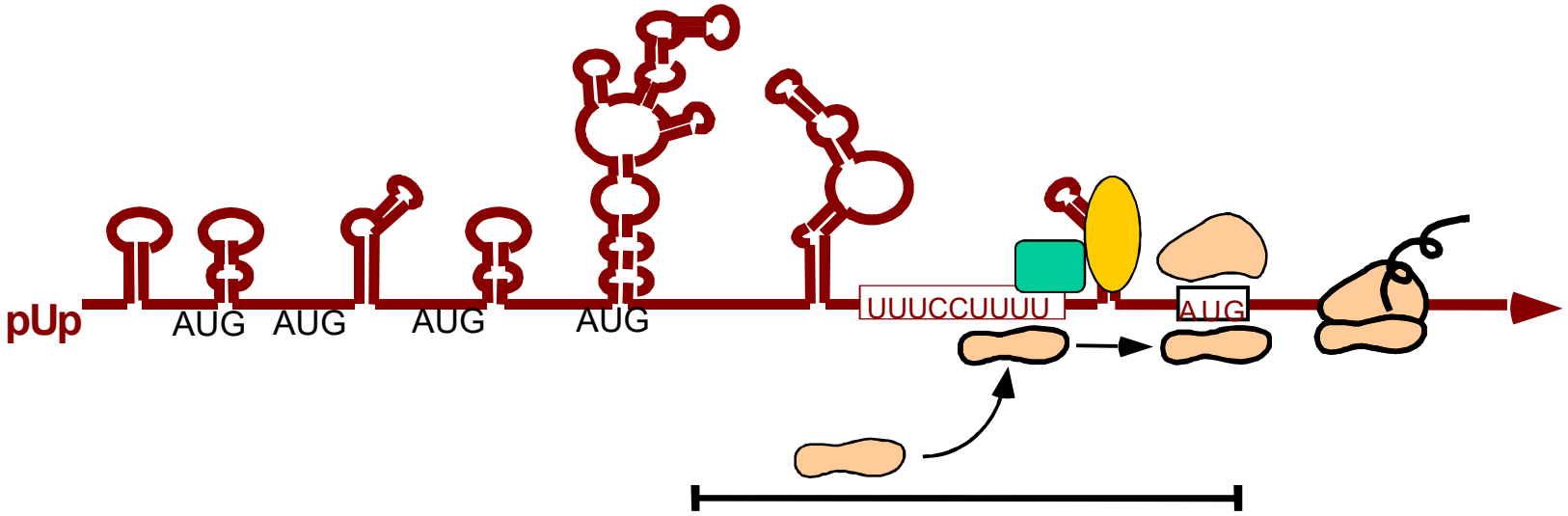
Summarizing the initiation of translation in eukaryotes



Initiation of mRNA translation in poliovirus







IRES= Internal ribosome entry site

The mechanism of viral IRES function is to date better characterized than the mechanism of cellular IRES function, for which no clear mechanism have been proposed yet. Hepatitis C Virus-related IRESs directly bind 40S ribosomal subunit in such a way that their initiator codons are located in ribosomal P-site without mRNA scanning. These IRESs do not require Eukaryotic initiation factors eIF1, 1A, 4A, 4B, and 4E. Picornavirus IRES do not attract 40S directly, but rather through high-affinity eIF4G-binding site. In addition, many viral IRES (as well as cellular IRES) require additional proteins to mediate their function, known as IRES *trans*-acting factors (ITAFs).