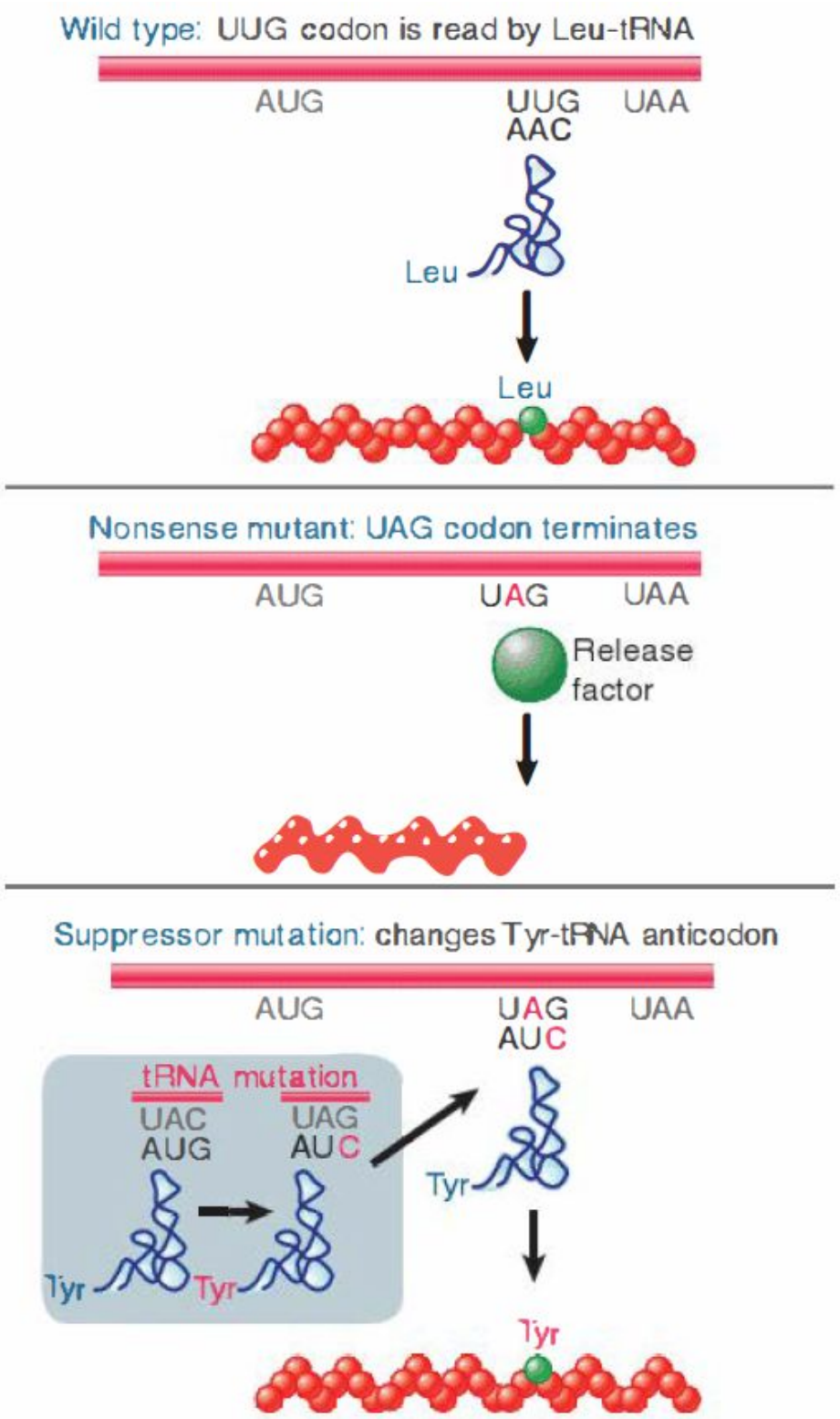
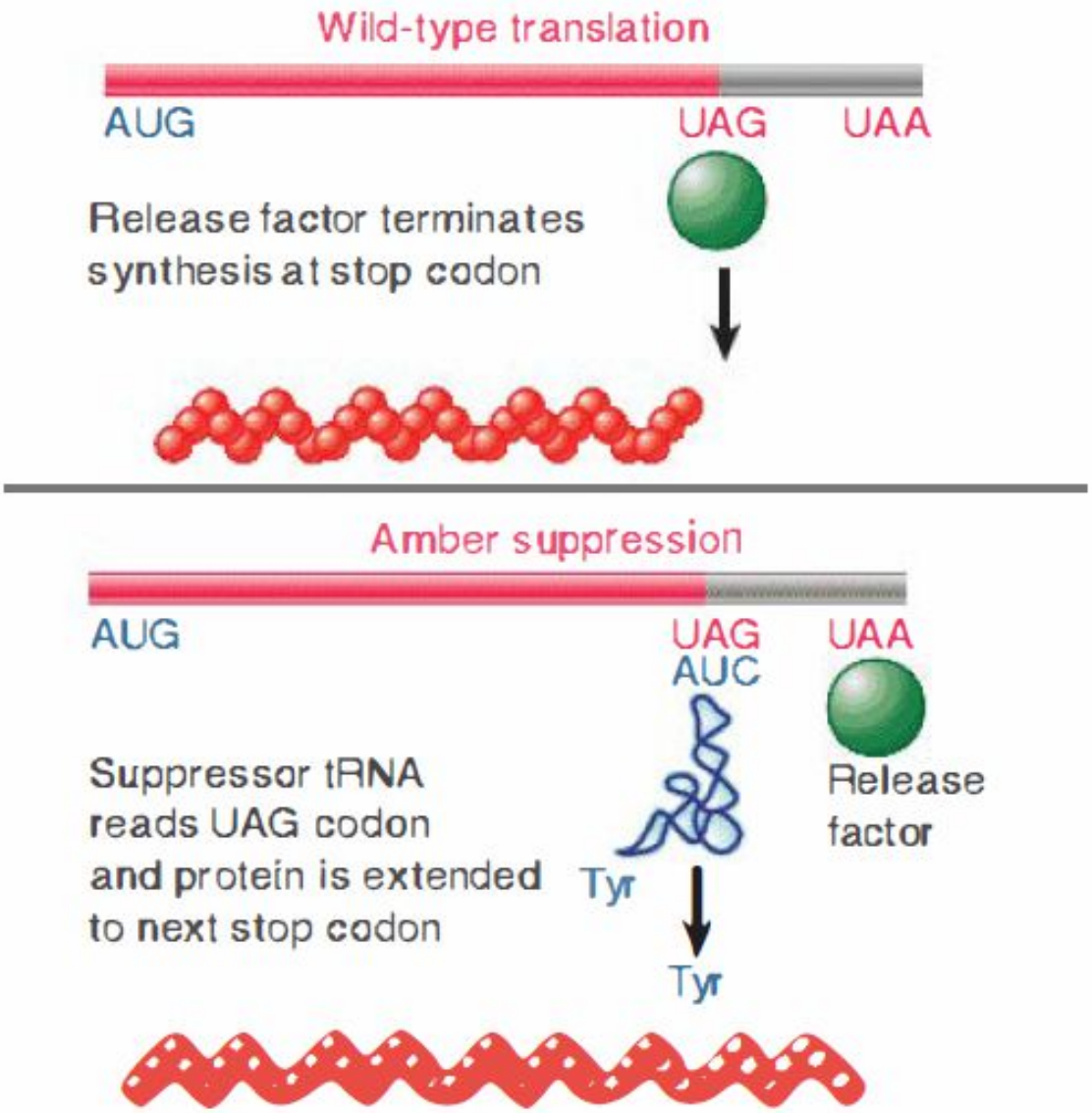
Suppression

In genetic terminology, a mutation that is able to overcome the effects of another mutation is called a suppressor.

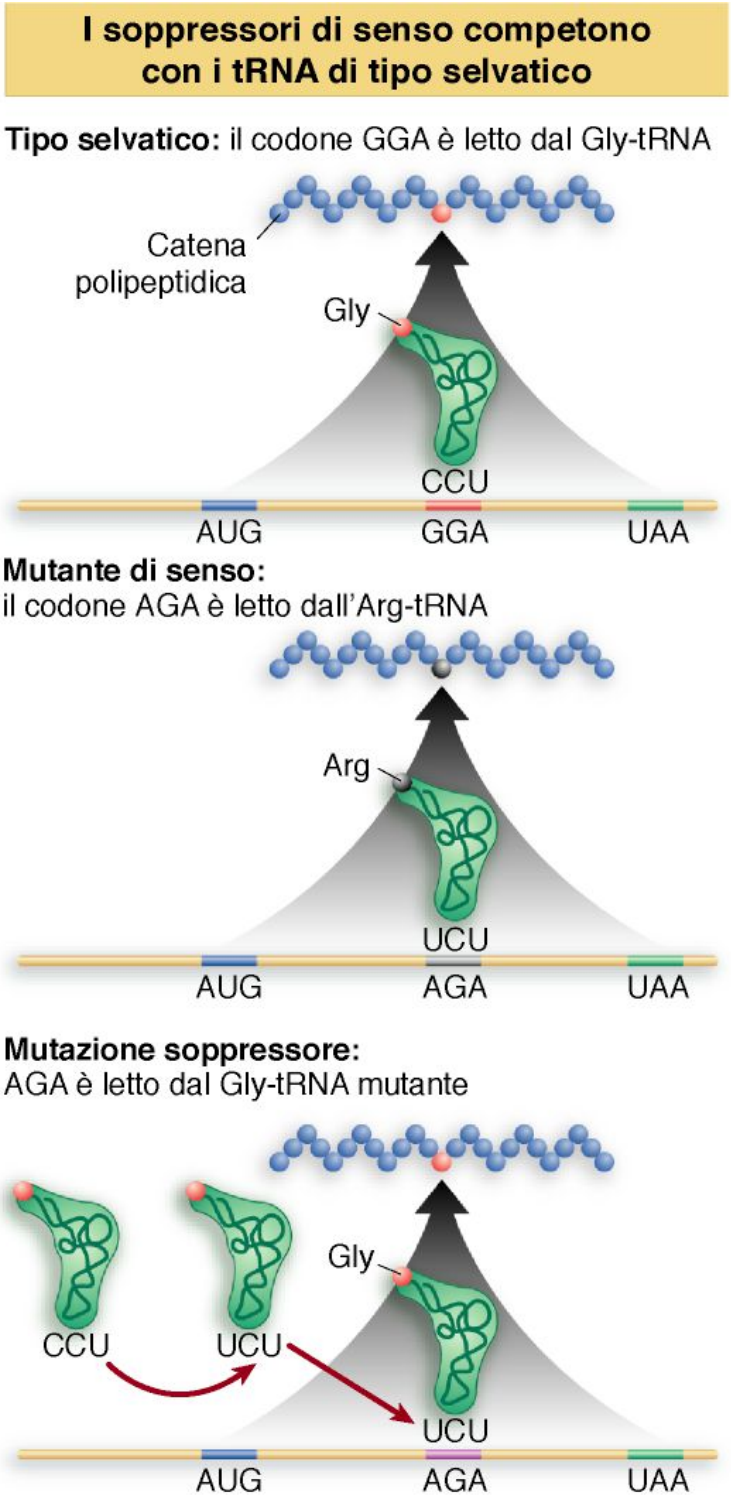
Nonsense mutations can be suppressed by a tRNA with a mutant anticodon, which inserts an amino acid at the mutant codon, producing a full-length polypeptide in which the original Leu residue has been replaced by Tyr.



Nonsense suppressors also read through natural termination codons, synthesizing polypeptides that are longer than the wild type.



Missense suppression occurs when the anticodon of tRNA is mutated so that it responds to the wrong codon. The suppression is only partial because both the wild-type tRNA and the suppressor tRNA can recognize *AGA*.



Re coding

Changes in the universal genetic code have occurred in some species and this is known as re coding process

SelB is the elongation factor in *E. coli* for the sel-cys

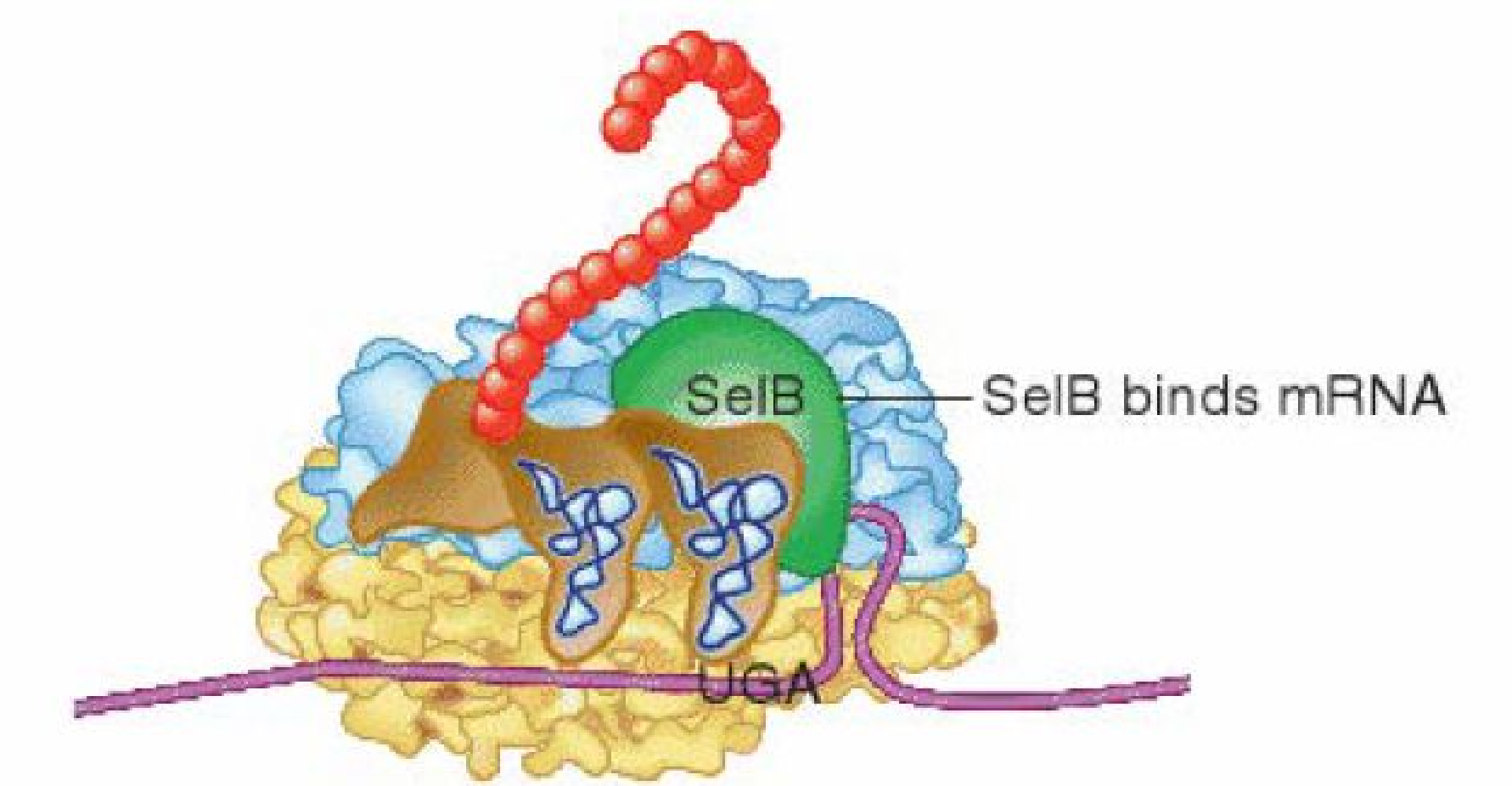


FIGURE 25.12 SelB is an elongation factor that specifically binds $tRNA^{Sec}$ to a UGA codon that is followed by a stem-loop structure in mRNA.

Procaryotic Selenocysteine Insertion

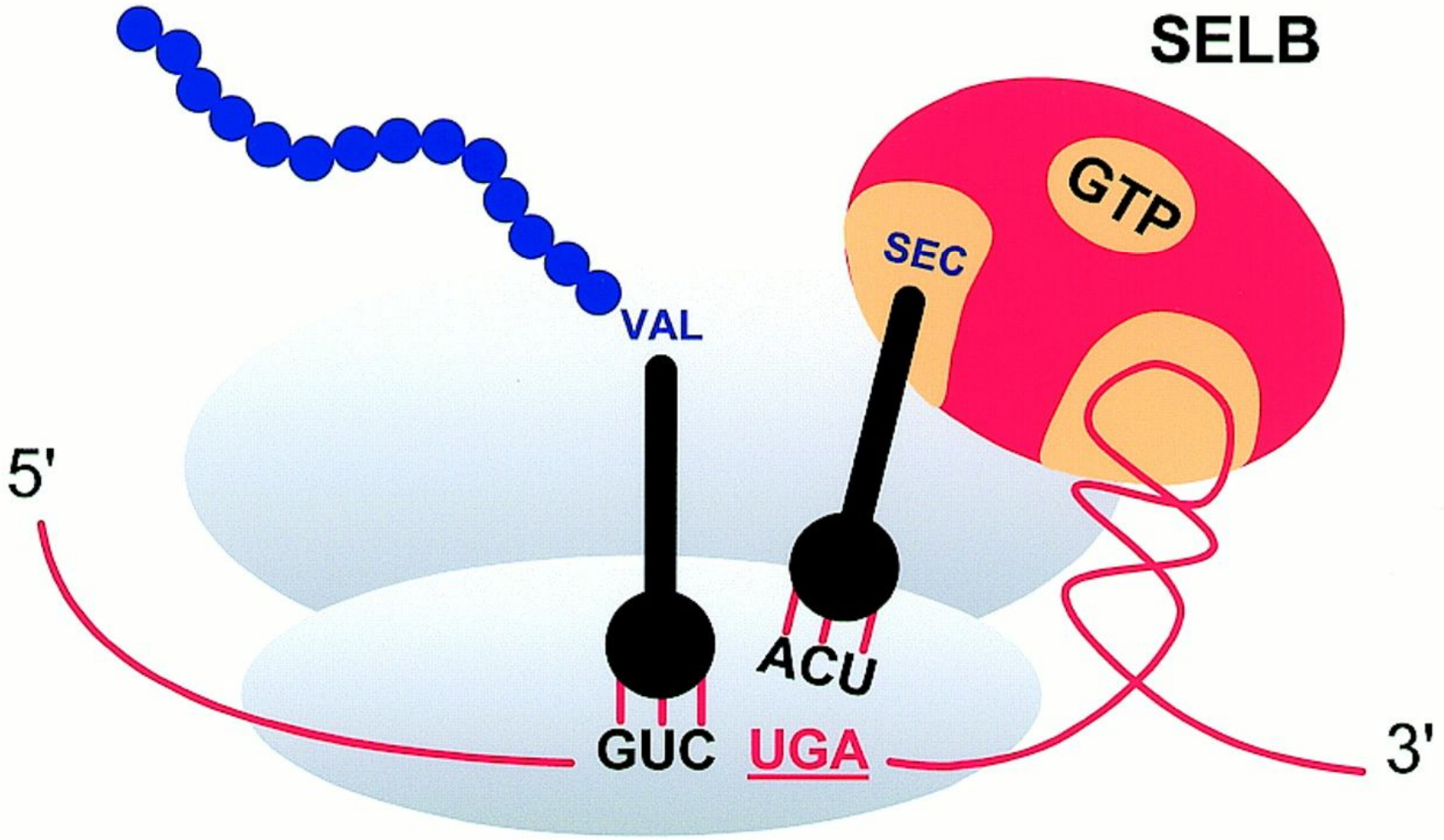


FIGURE 25.26 A +1 frameshift is required for expression of the *tyb* gene of the yeast Ty element. The shift occurs at a 7-base sequence at which two Leu codon(s) are followed by a scarce Arg codon.

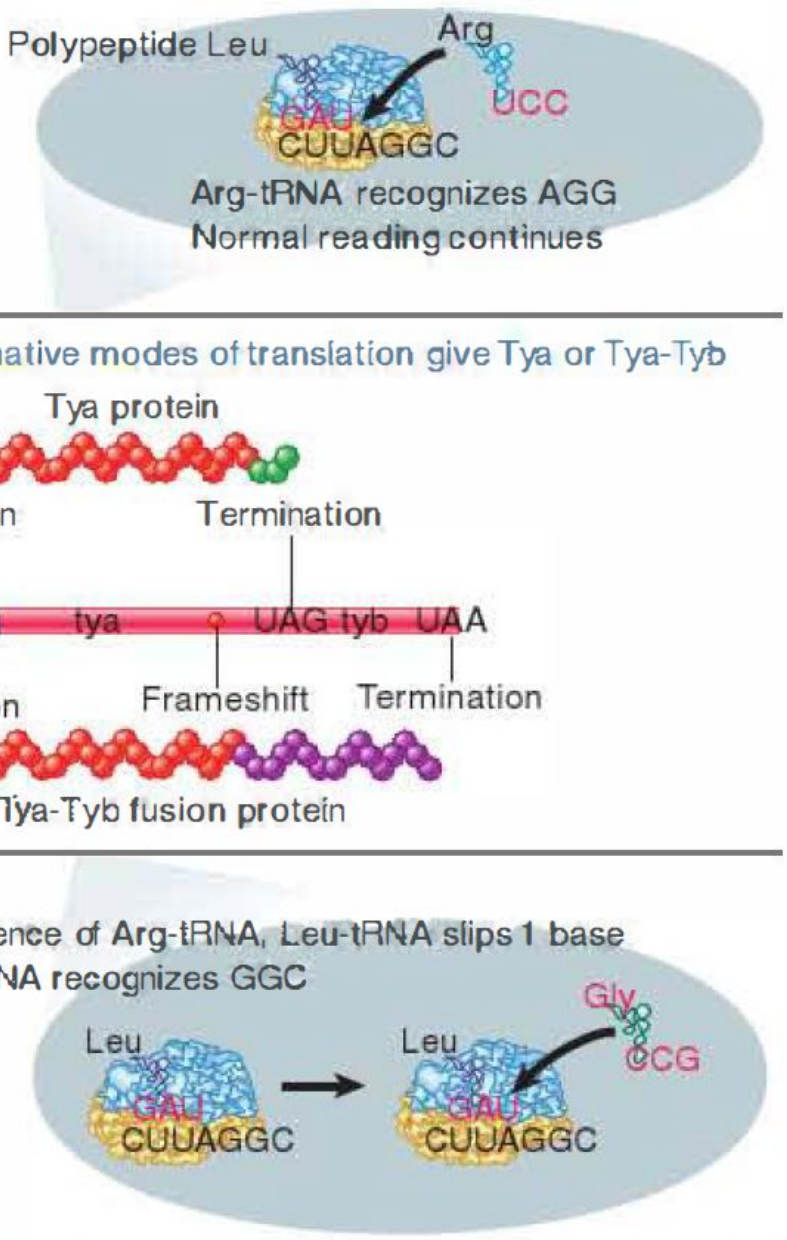


FIGURE 25.25 A tRNA that slips one base in pairing with a codon causes a frameshift that can suppress termination. The efficiency is usually ~5%.

Frameshift efficiency is low (< 5 %)

A very rare event is the <u>ribosome bypass</u> that takes place when a GGA codon is near a stop codon, both placed inside a stem loop structure (only three cases are known).

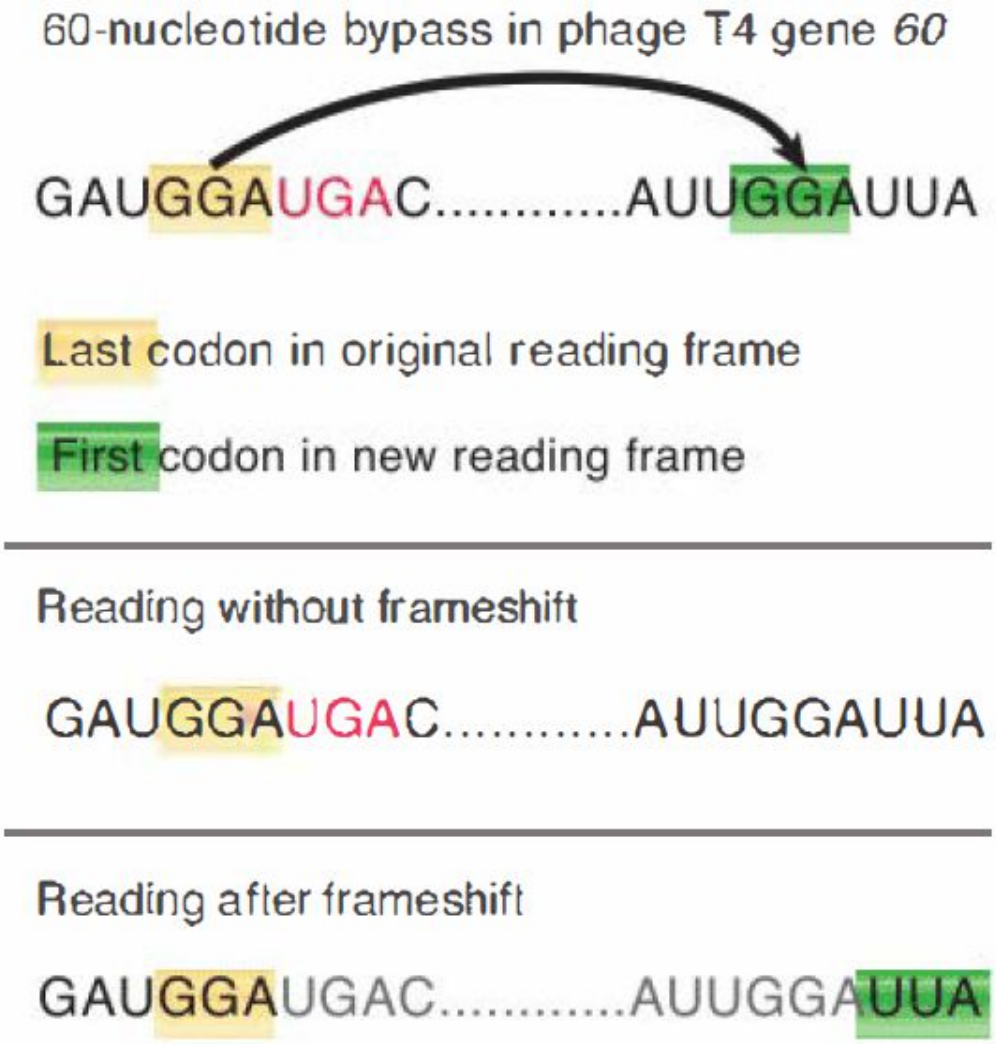


FIGURE 25.27 Bypassing occurs when the ribosome moves along mRNA so that the peptidyl-tRNA in the P site is released from pairing with its codon and then repairs with another codon farther along.

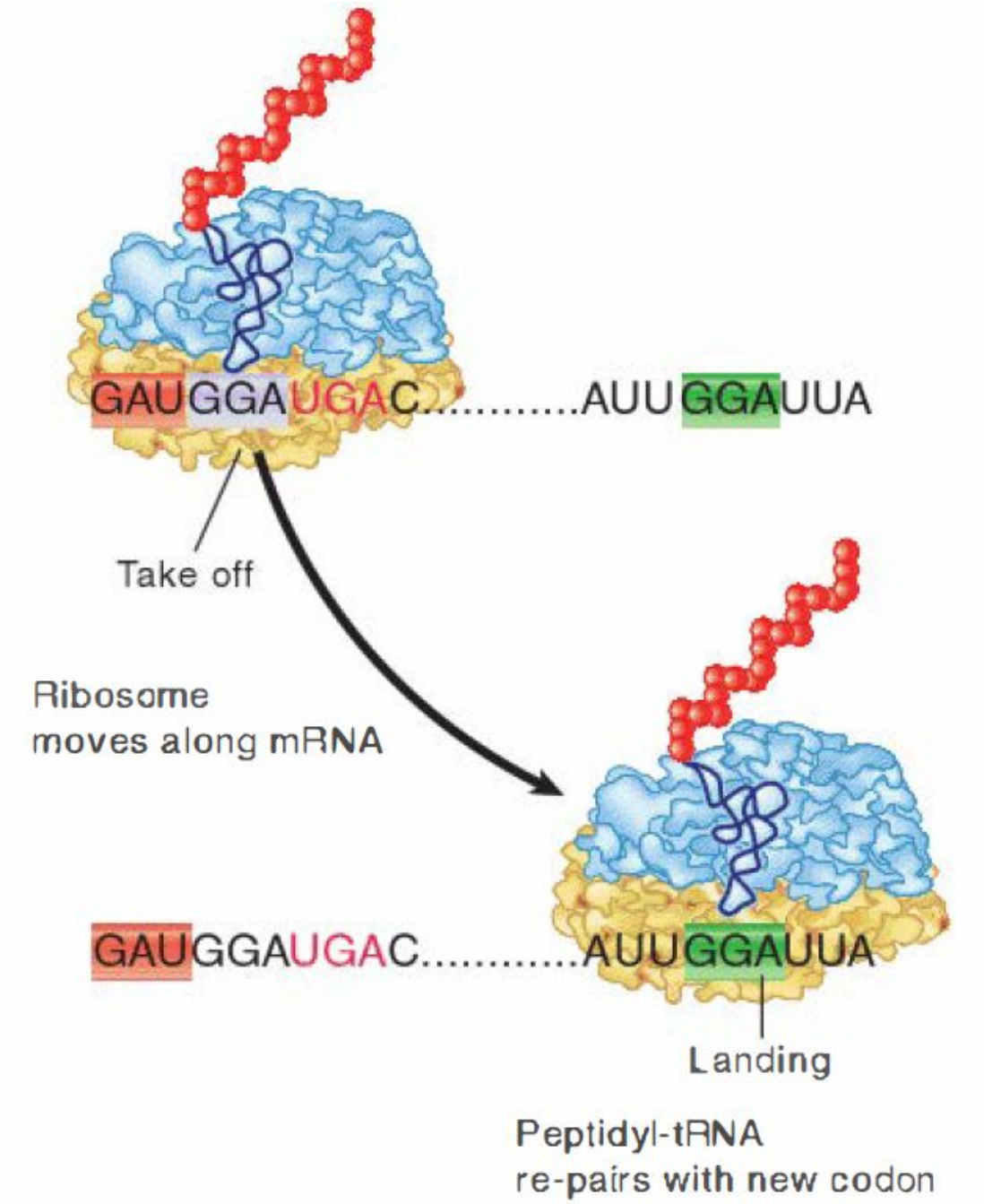


FIGURE 25.28 In bypass mode, a ribosome with its P site occupied can stop translation. It slides along mRNA to a site where peptidyl-tRNA pairs with a new codon in the P site. Then translation is resumed.

The ribosome

Ribosomes are complexes of RNA and proteins. Its function is the expression of the genetic code from nucleic acid into protein, in a process called ***translation***.

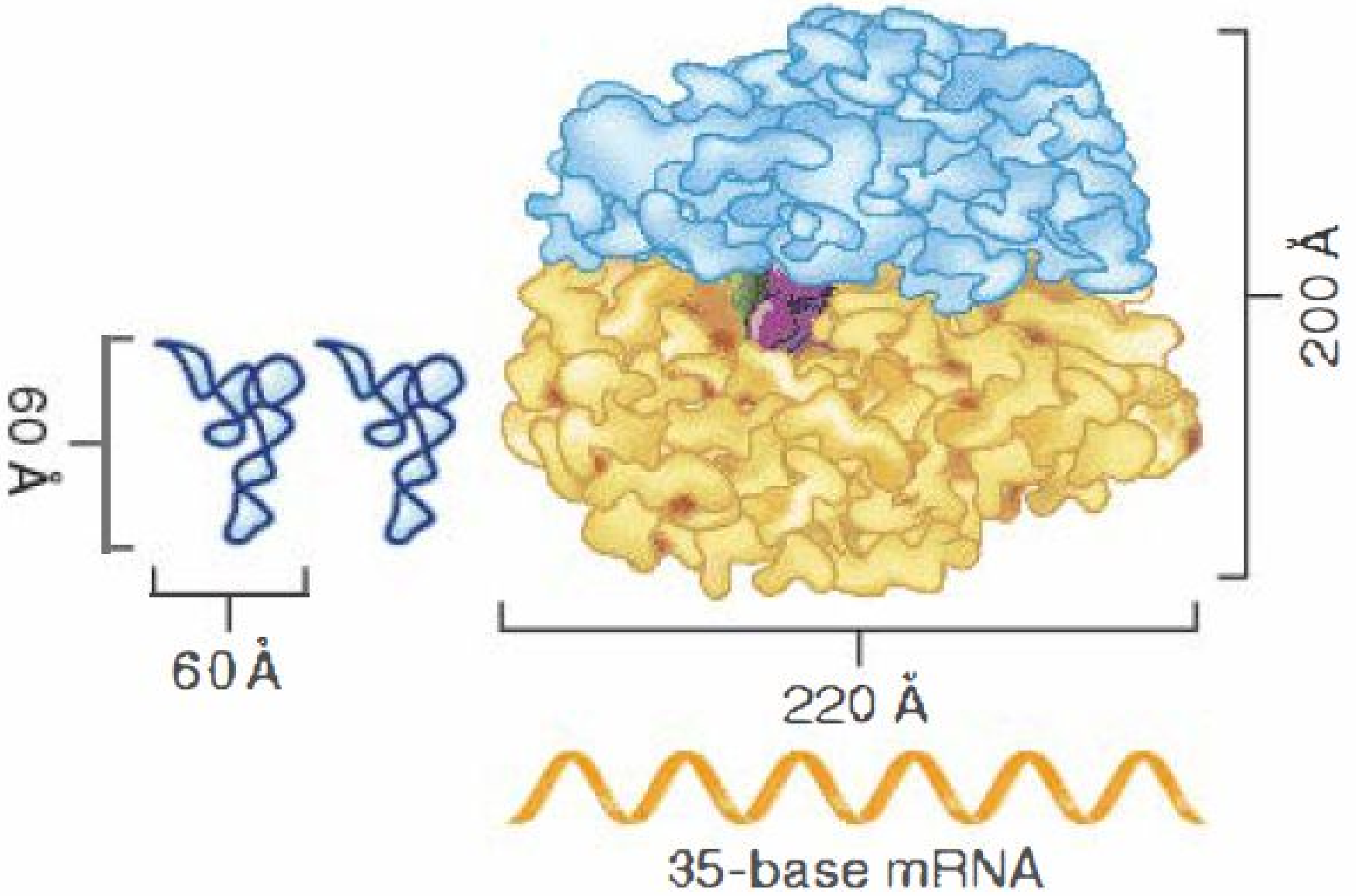
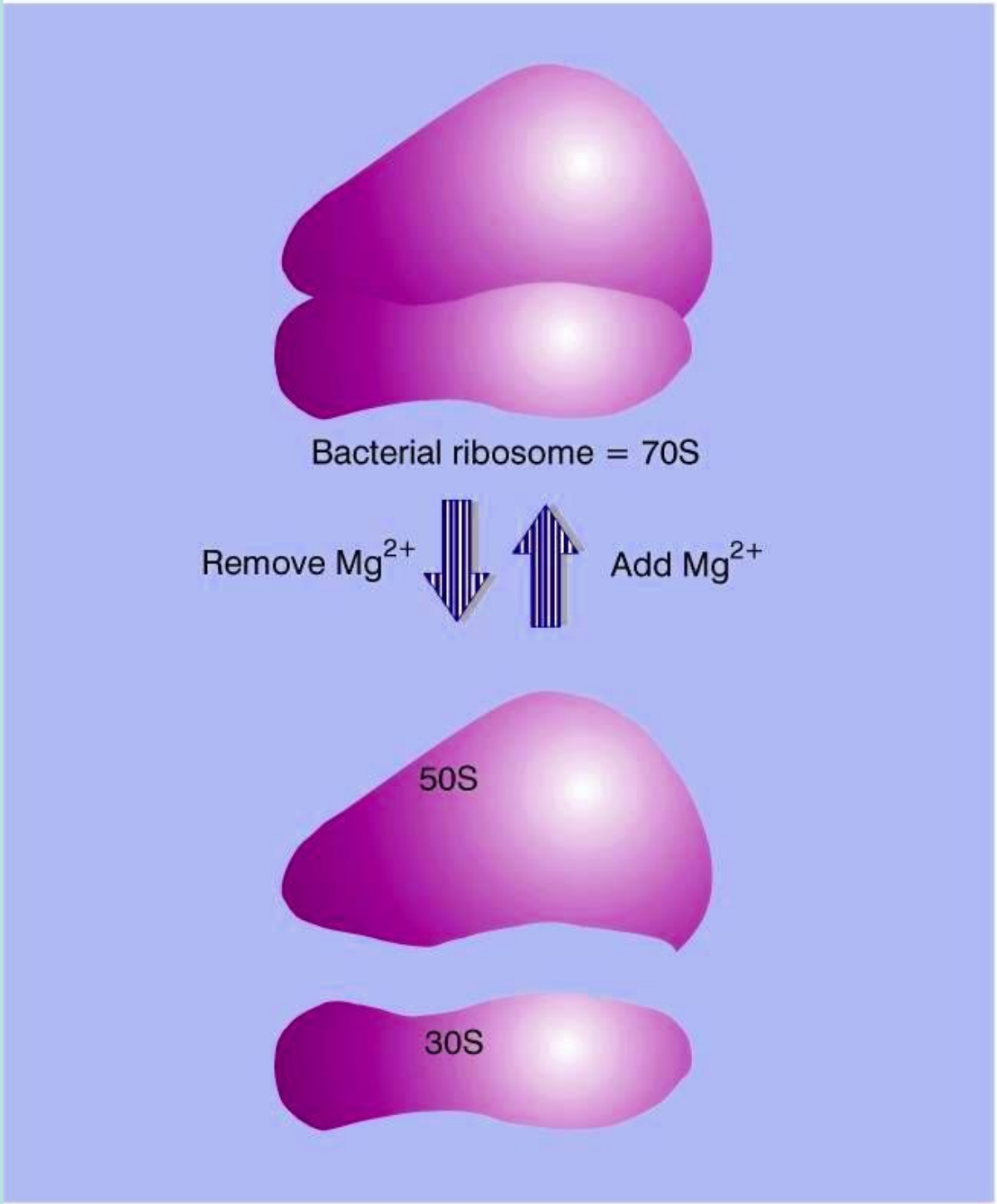


FIGURE 24.1 Size comparisons show that the ribosome is large enough to bind tRNAs and mRNA.

Figure 5.7 A ribosome consists of two subunits.



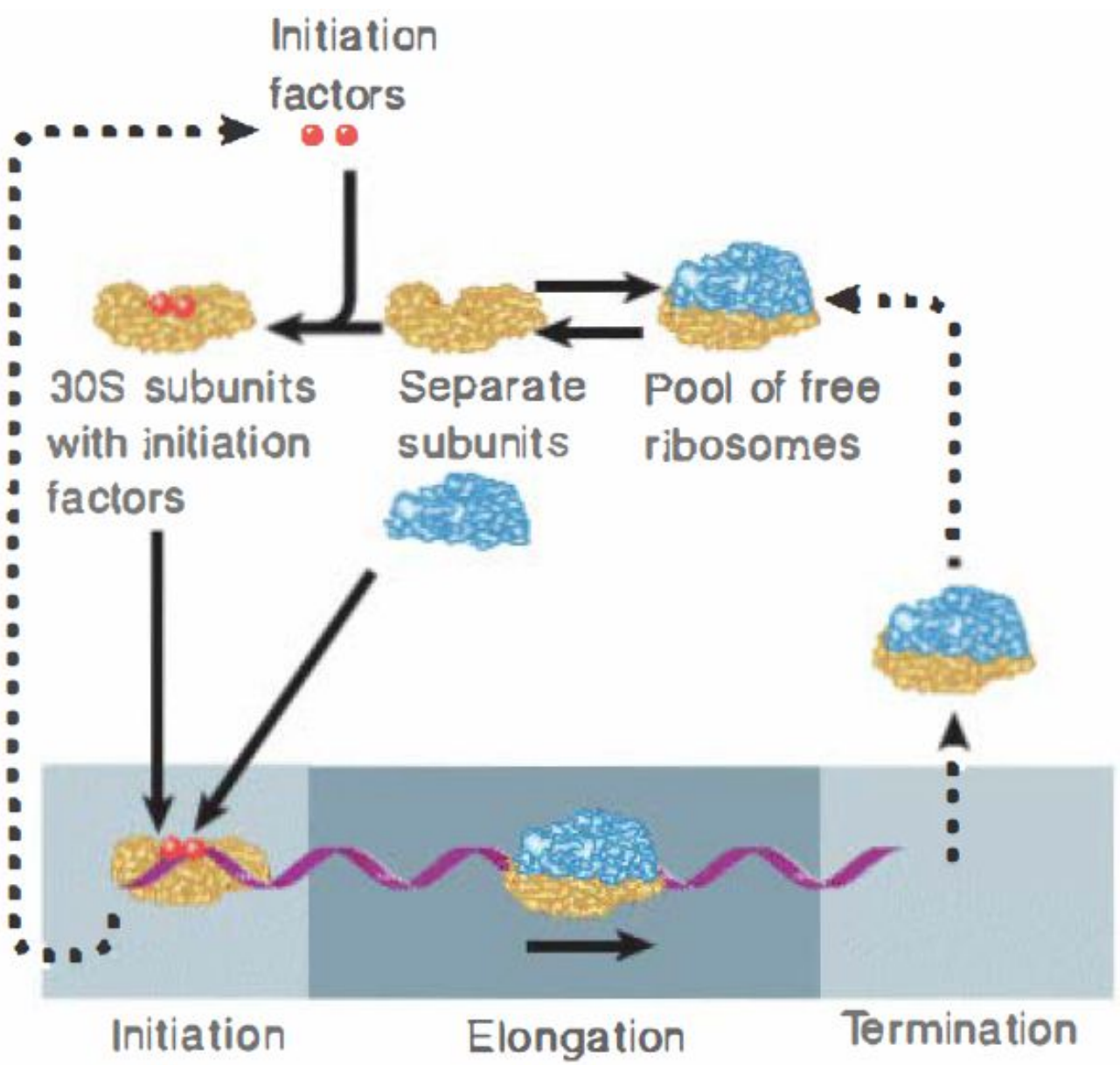


FIGURE 24.9 Initiation requires free ribosome subunits. When ribosomes are released at termination, the 30S subunits bind initiation factors and dissociate to generate free subunits. When subunits reassociate to give a functional ribosome at initiation, they release the factors.

PROKARYOTES

70S

23S rRNA (2904 nt)
5S rRNA (120 nt)

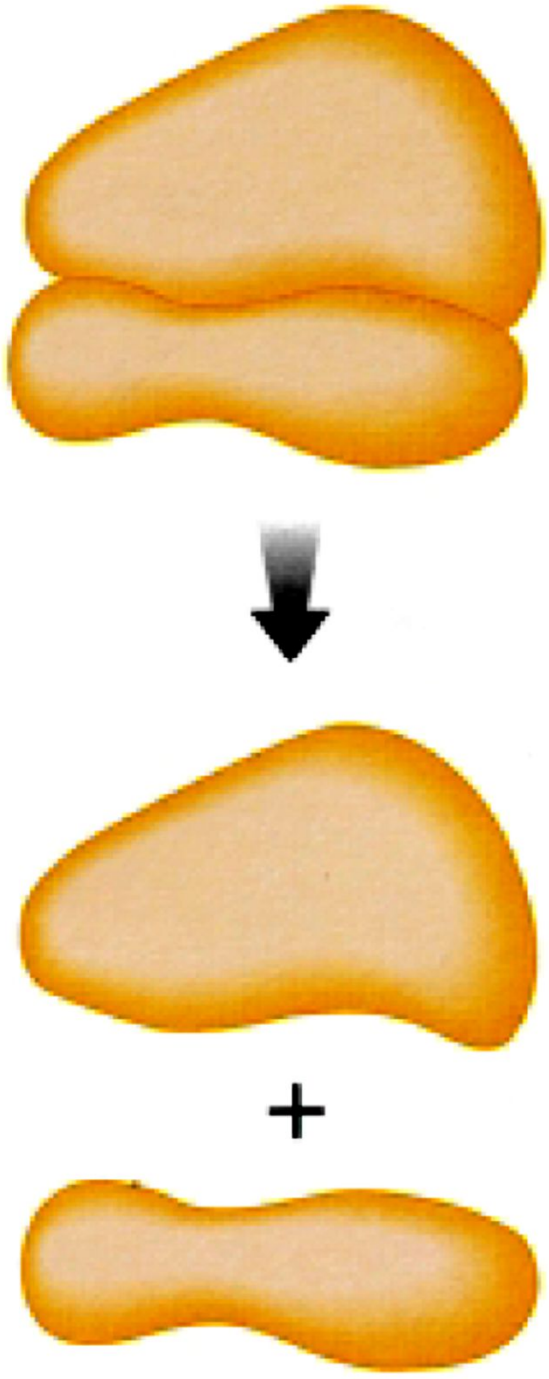
31 proteins

} 50S

16S rRNA (1541 nt)

21 proteins

} 30S



EUKARYOTES

80S

Transcribed by pol III in the nucleoplasm

60S { 28S rRNA (4718 nt)
5,8S rRNA (160 nt)
5S rRNA (120 nt)

50 proteins

Transcribed by pol I in tht nucleolus as a unic pre-rRNA

40S { 18S rRNA (1874 nt)

33 proteins

Origin of the ribosome	Big rRNA	Small rRNA
Trypanosome mitochondria	1152 bp	597 bp
Mammal mitochondria	1559 bp	954 bp
Yeast mitochondria	3273 bp	1686 bp
Tobacco Chloroplast	2904 bp	1485 bp
Bacteria	2904 bp	1542 bp
Yeast cytosol	3392 bp	1799 bp
Amphibian cytosol	4110 bp	1825 bp
Mammal cytosol	4718 bp	1874 bp

rRNA 16S from the 30S subunit

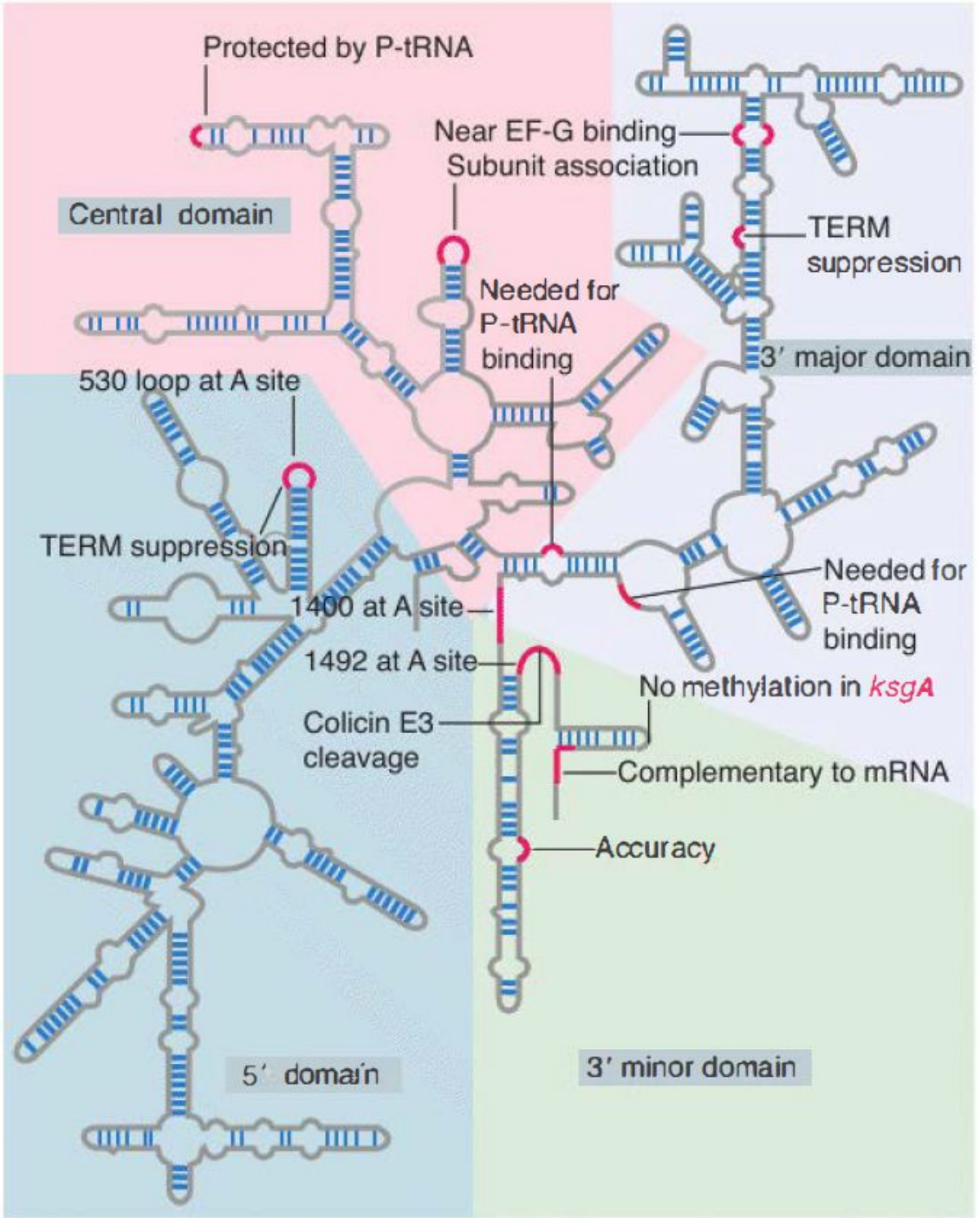


FIGURE 24.46 Some sites in 16S rRNA are protected from chemical probes when 50S subunits join 30S subunits or when aminoacyl-tRNA binds to the A site. Others are the sites of mutations that affect translation. TERM suppression sites may affect termination at some or several termination codons. The large colored blocks indicate the four domains of the rRNA.

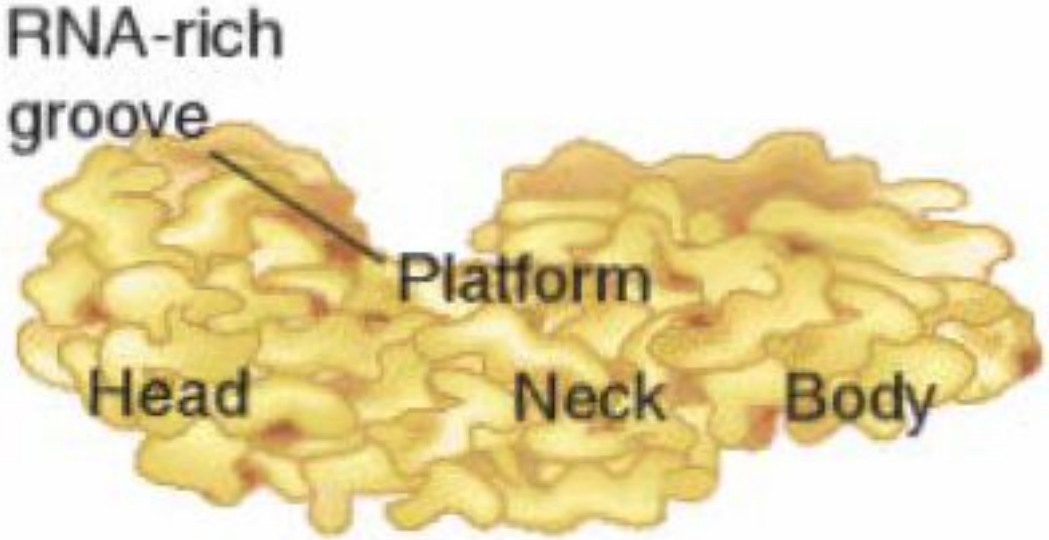


FIGURE 24.37 The 30S subunit has a head separated by a neck from the body, with a protruding platform.

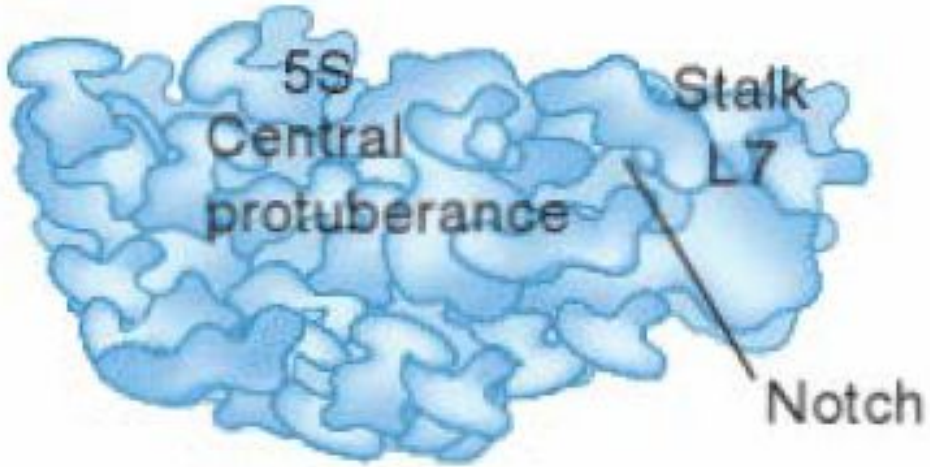


FIGURE 24.38 The 50S subunit has a central protuberance where 5S rRNA is located, separated by a notch from a stalk made of copies of the protein L7.

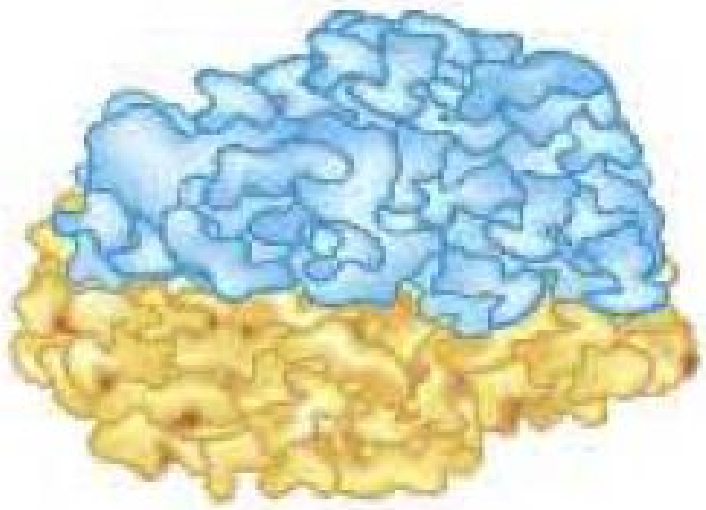
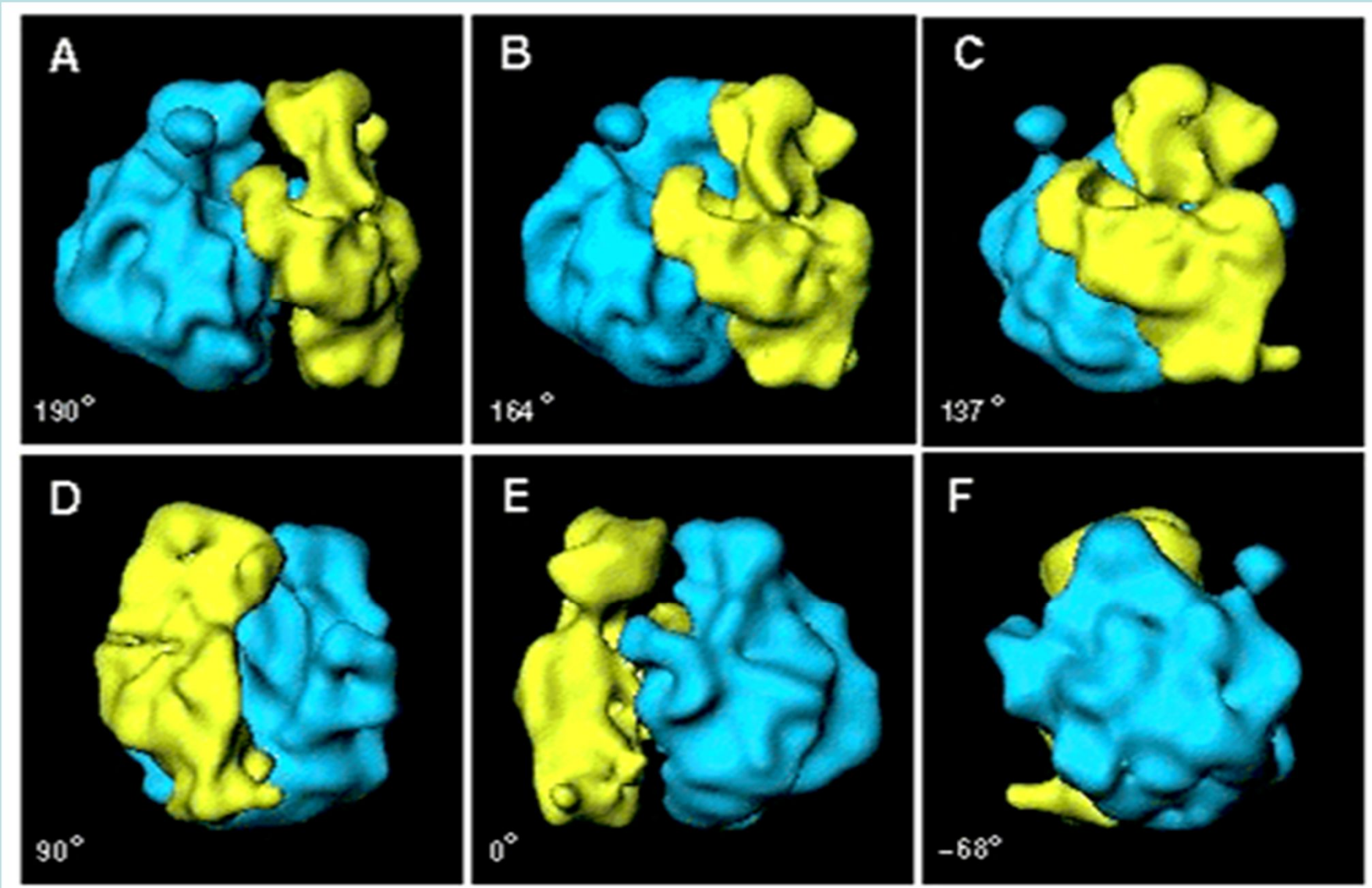


FIGURE 24.39 The platform of the 30S subunit fits into the notch of the 50S subunit to form the 70S ribosome.

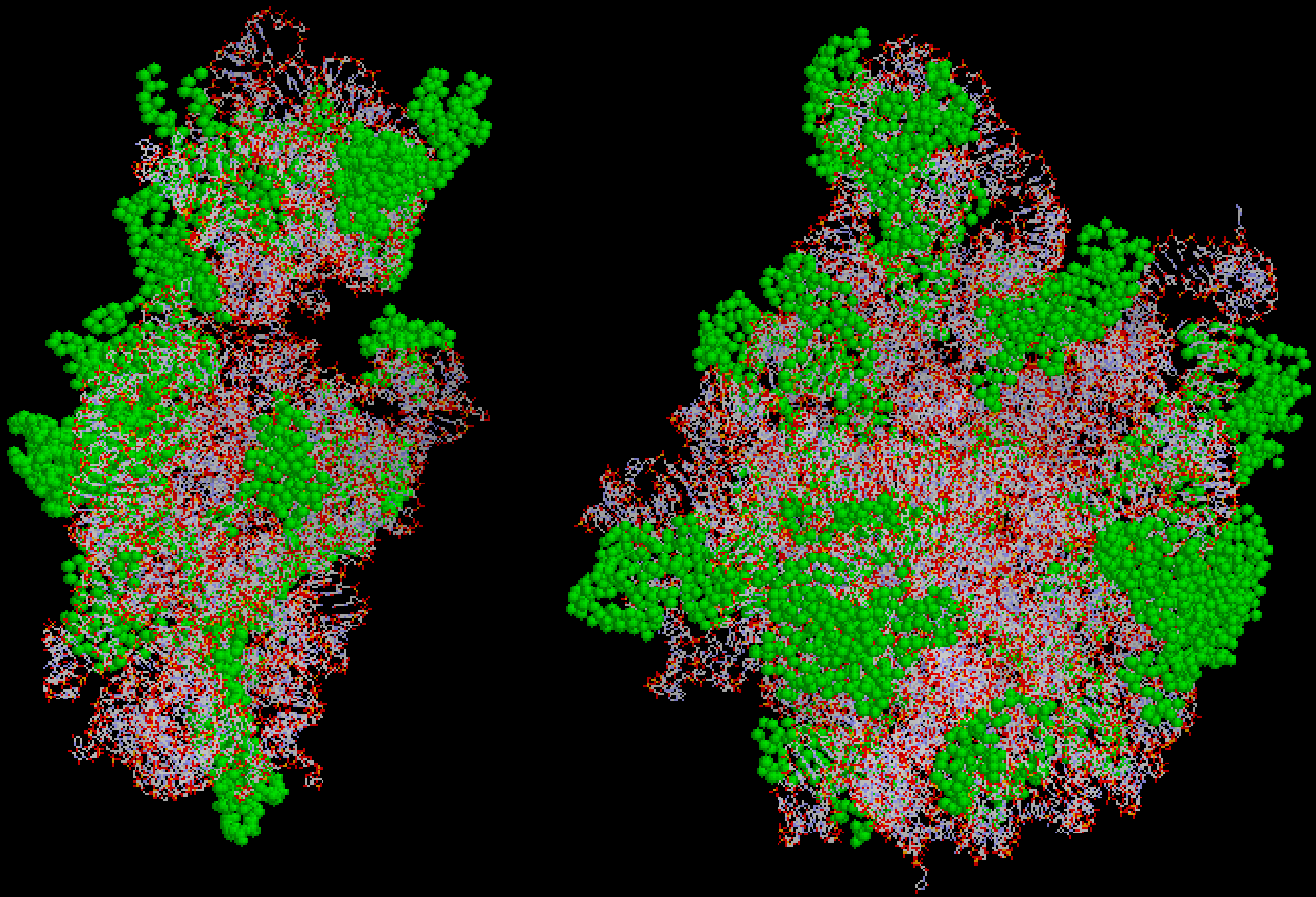
The ribosome of Escherichia coli at 25 A resolution
as obtained by cryo-electron microscopy of single ribosomes
and reconstruction using 4300 projections
(Frank et al., Nature 376 (1995) 441-444).
Yellow: 30S subunit, blue: 50S subunit.



Ribosome structure solving

Crystals of the 50S subunit from Deinococcus radiodurans

30S and 50S subunits from the prokaryotic ribosome



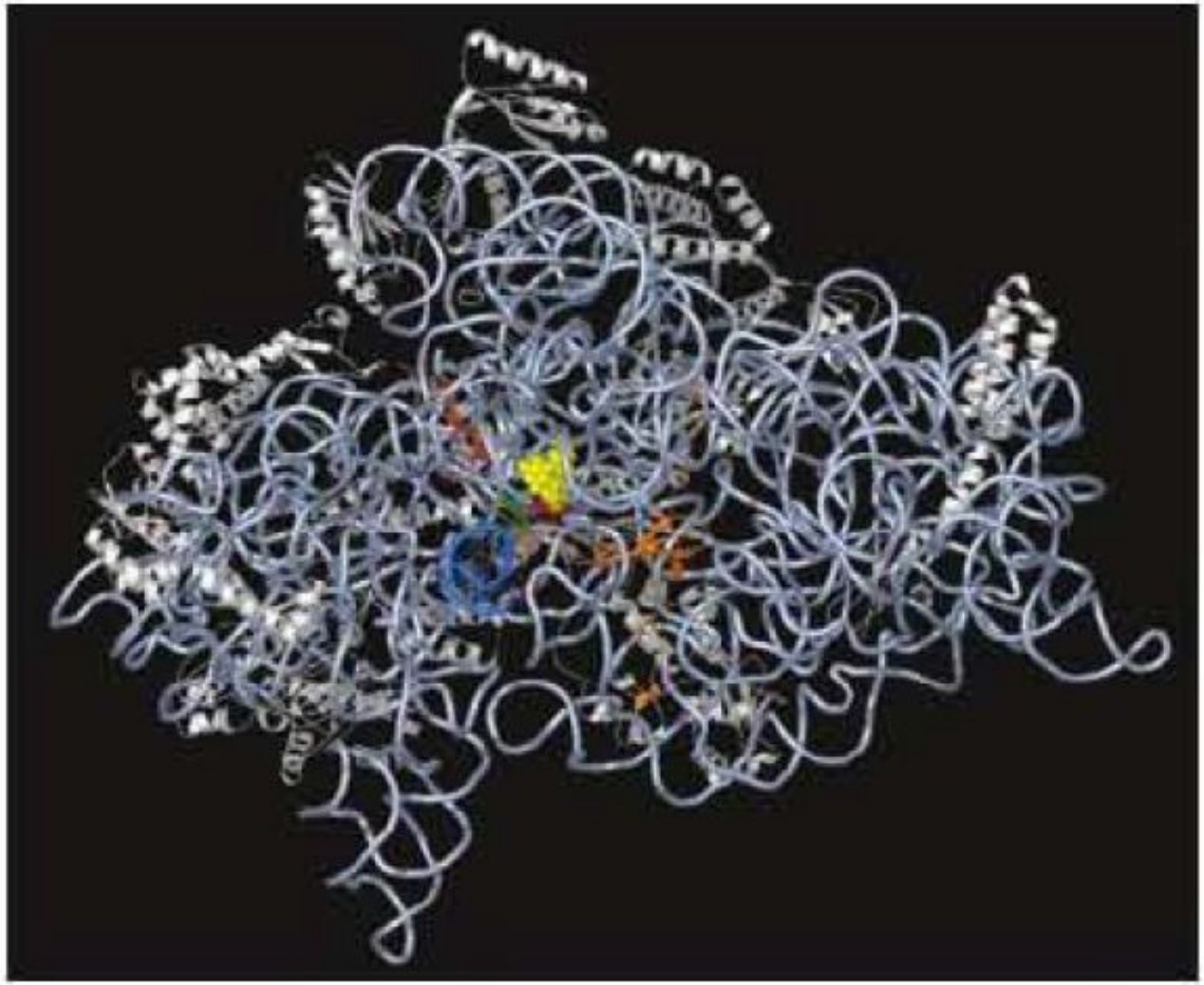


FIGURE 24.40 The 30S ribosomal subunit is a ribonucleoprotein particle. Ribosomal proteins are white and rRNA is light blue. Courtesy of Dr. Kalju Kahn.

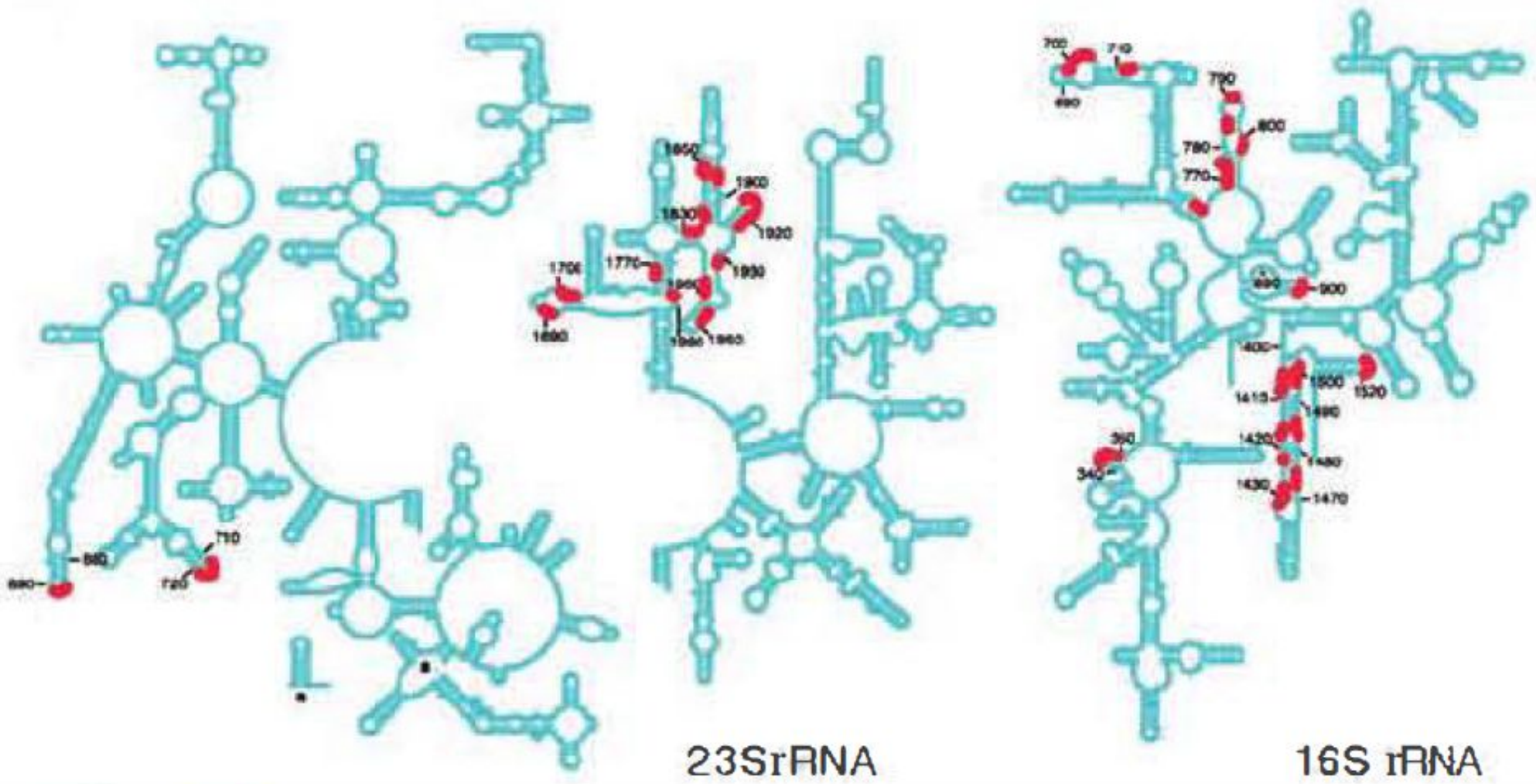
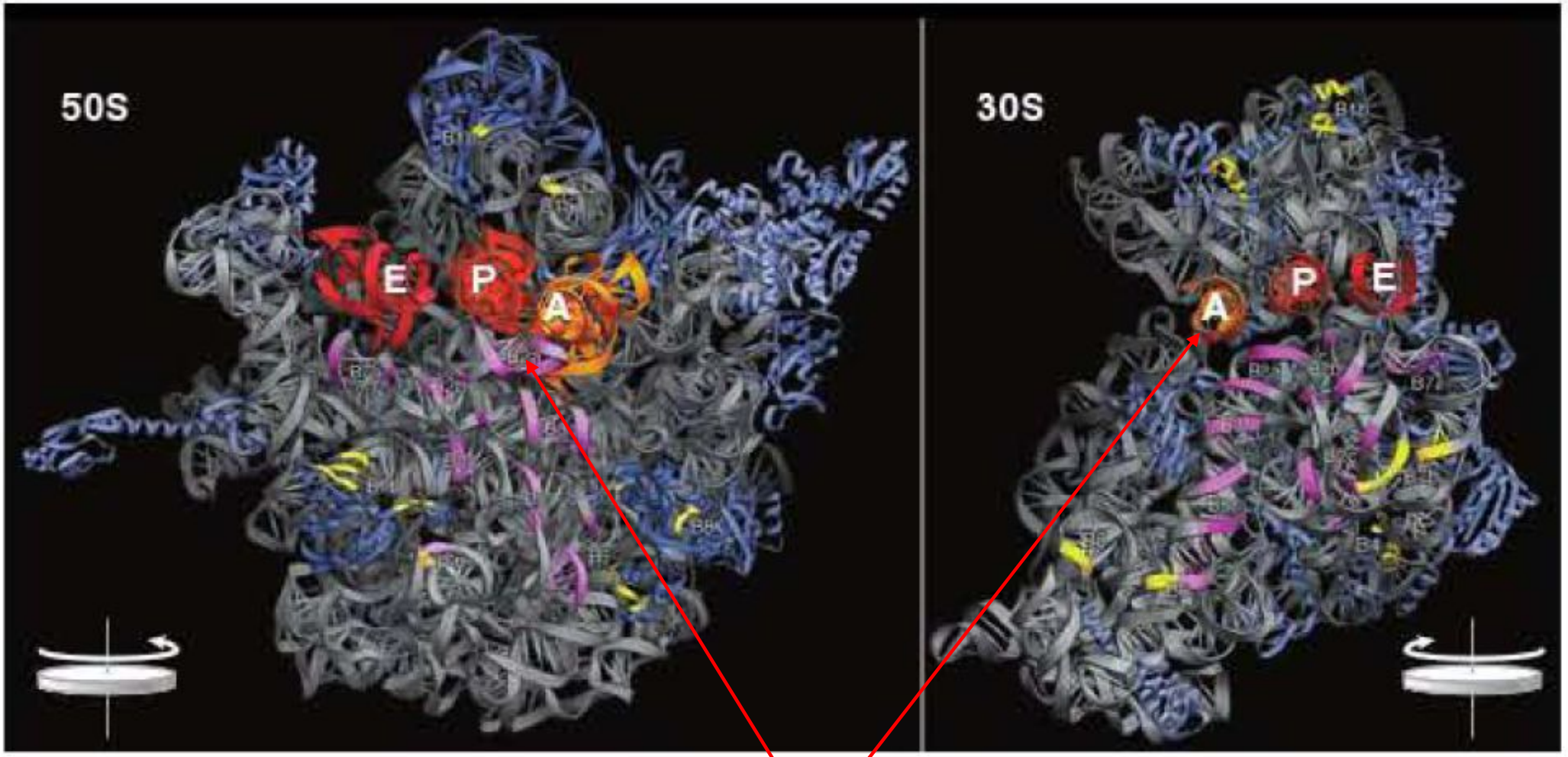


FIGURE 24.41 Contact points between the rRNAs are located in two domains of 16S rRNA and one domain of 23S rRNA.Reproduced from M. M. Yusupov, et al., 292 (2001): 883-896 [http://www.sciencemag.org]. Reprinted with permission from AAAS. Photo courtesy of Harry Noller, University of California, Santa Cruz.

The prokaryotic ribosome has 3 sites for tRNA: A, P, E



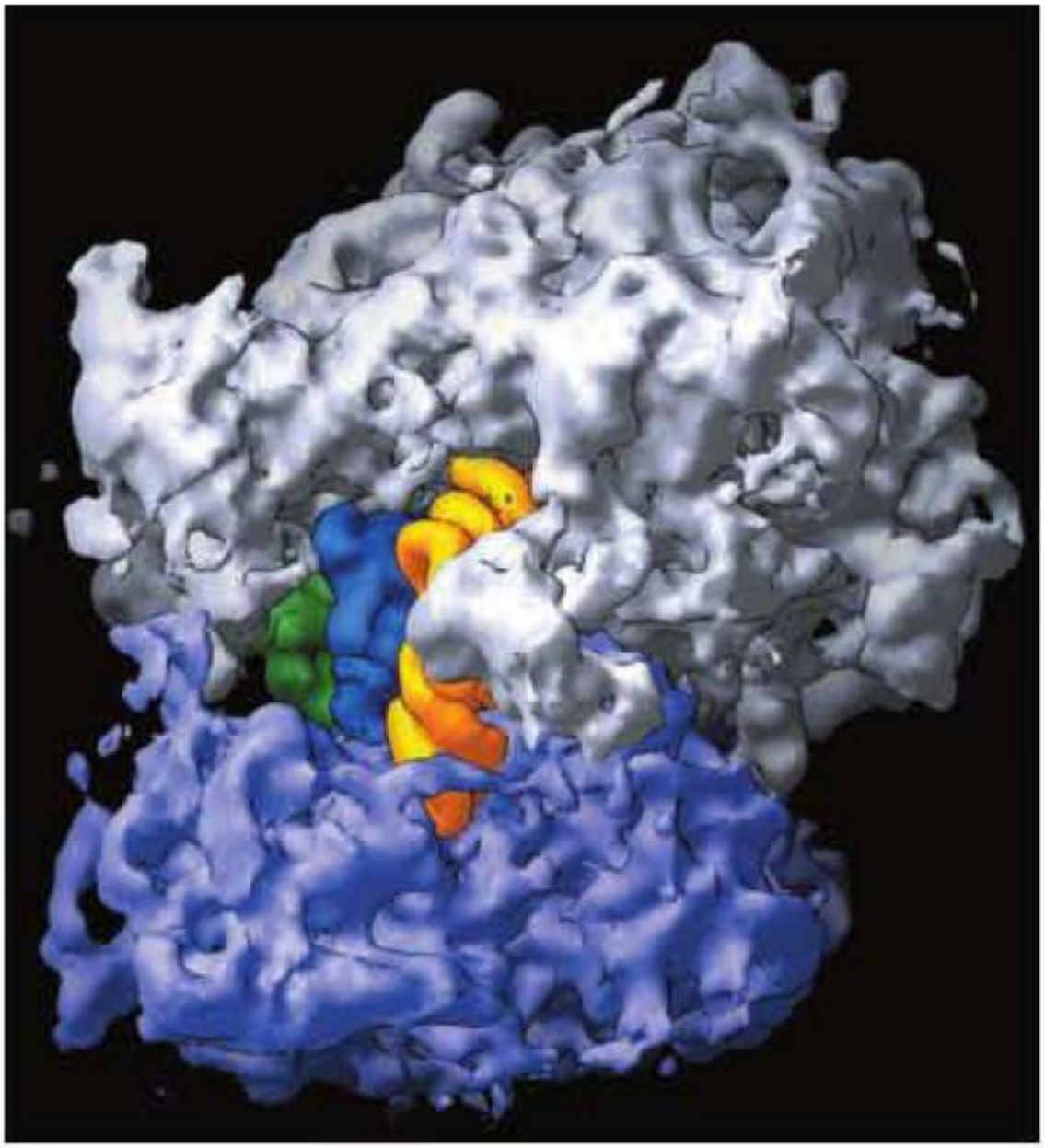


FIGURE 24.43 The 70S ribosome consists of the 50S subunit (white) and the 30S subunit (purple), with three tRNAs located superficially: yellow in the A site, blue in the P site, and green in the E site. Photo courtesy of Harry Noller, University of California, Santa Cruz.

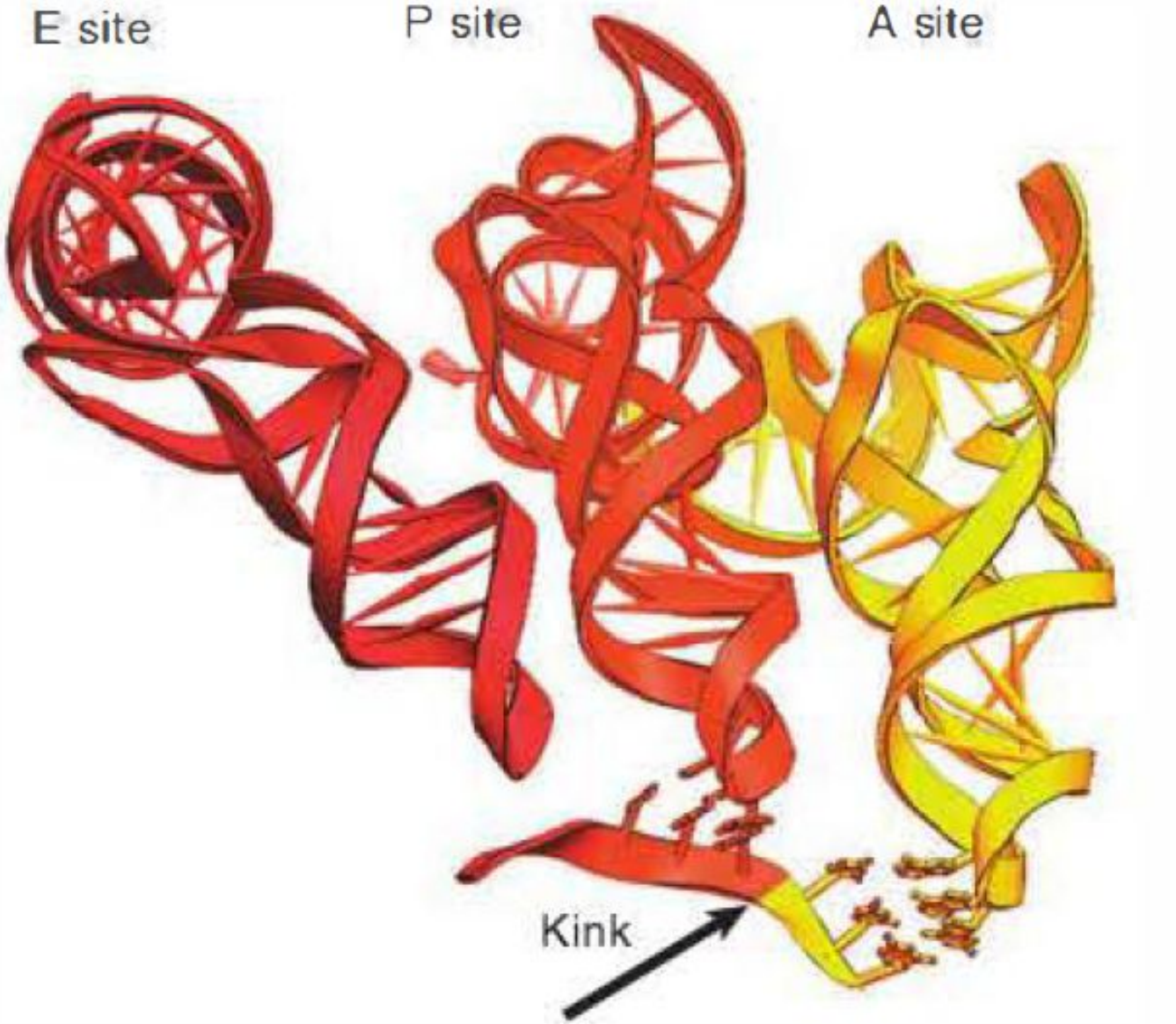


FIGURE 24.44 Three tRNAs have different orientations on the ribosome. mRNA turns between the P and A sites to allow aminoacyl-tRNAs to bind adjacent codons. Photo courtesy of Harry Noller, University of California, Santa Cruz.

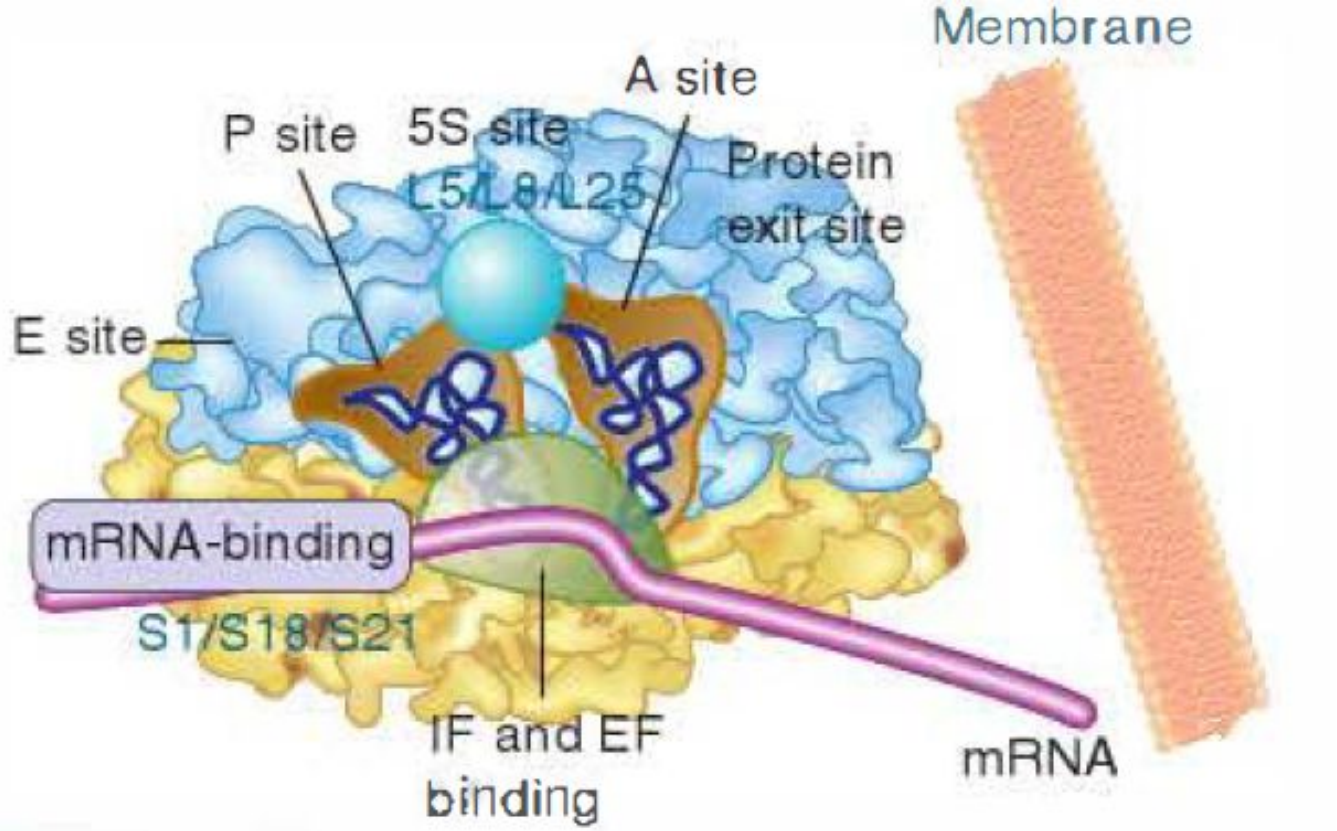


FIGURE 24.45 The ribosome has several active centers. It may be associated with a membrane. mRNA takes a turn as it passes through the A and P sites, which are angled with regard to each other. The E site lies beyond the P site. The peptidyl transferase site (not shown) stretches across the tops of the A and P sites. Part of the site bound by EF-Tu/G lies at the base of the A and P sites.

Ribosome assembly

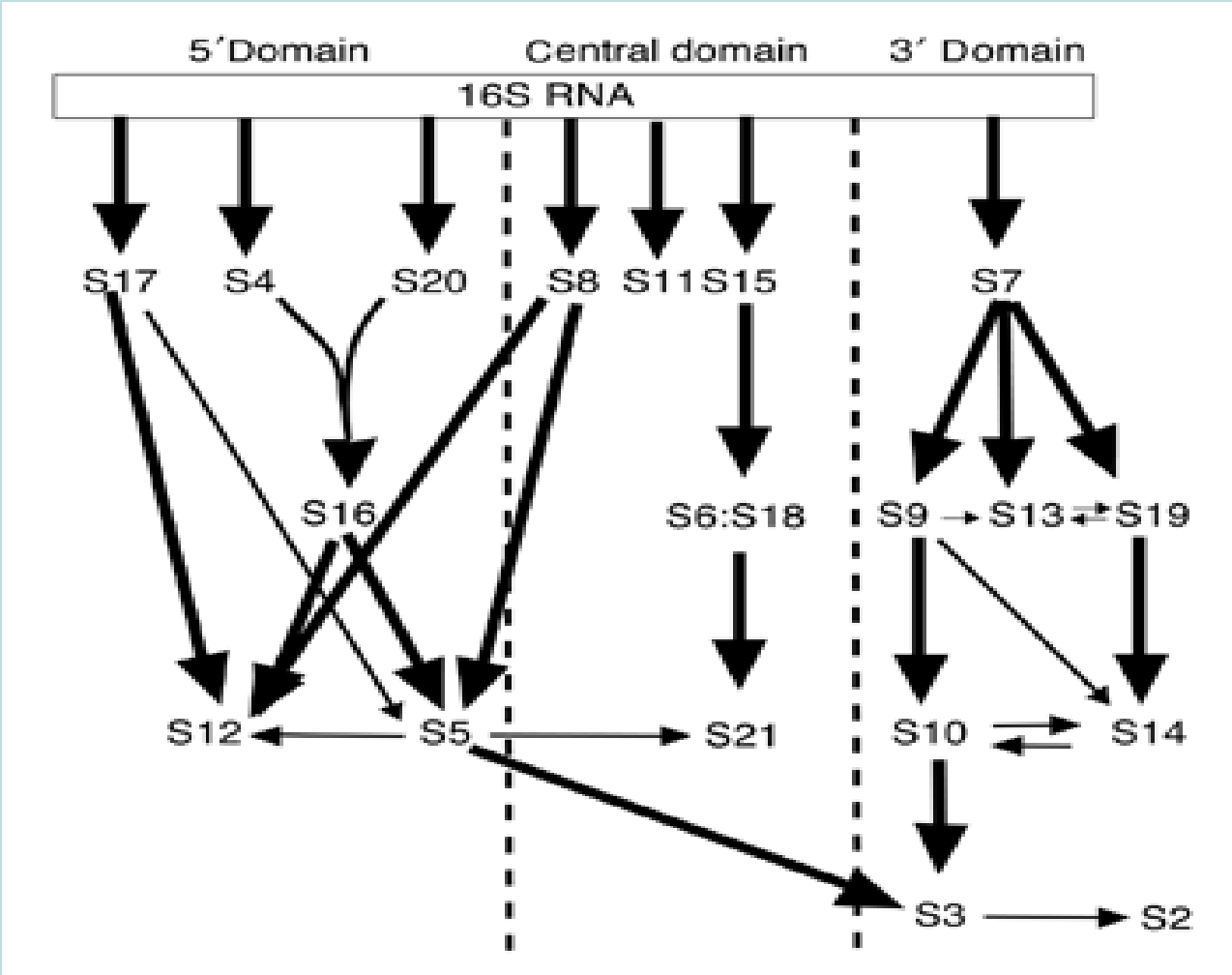
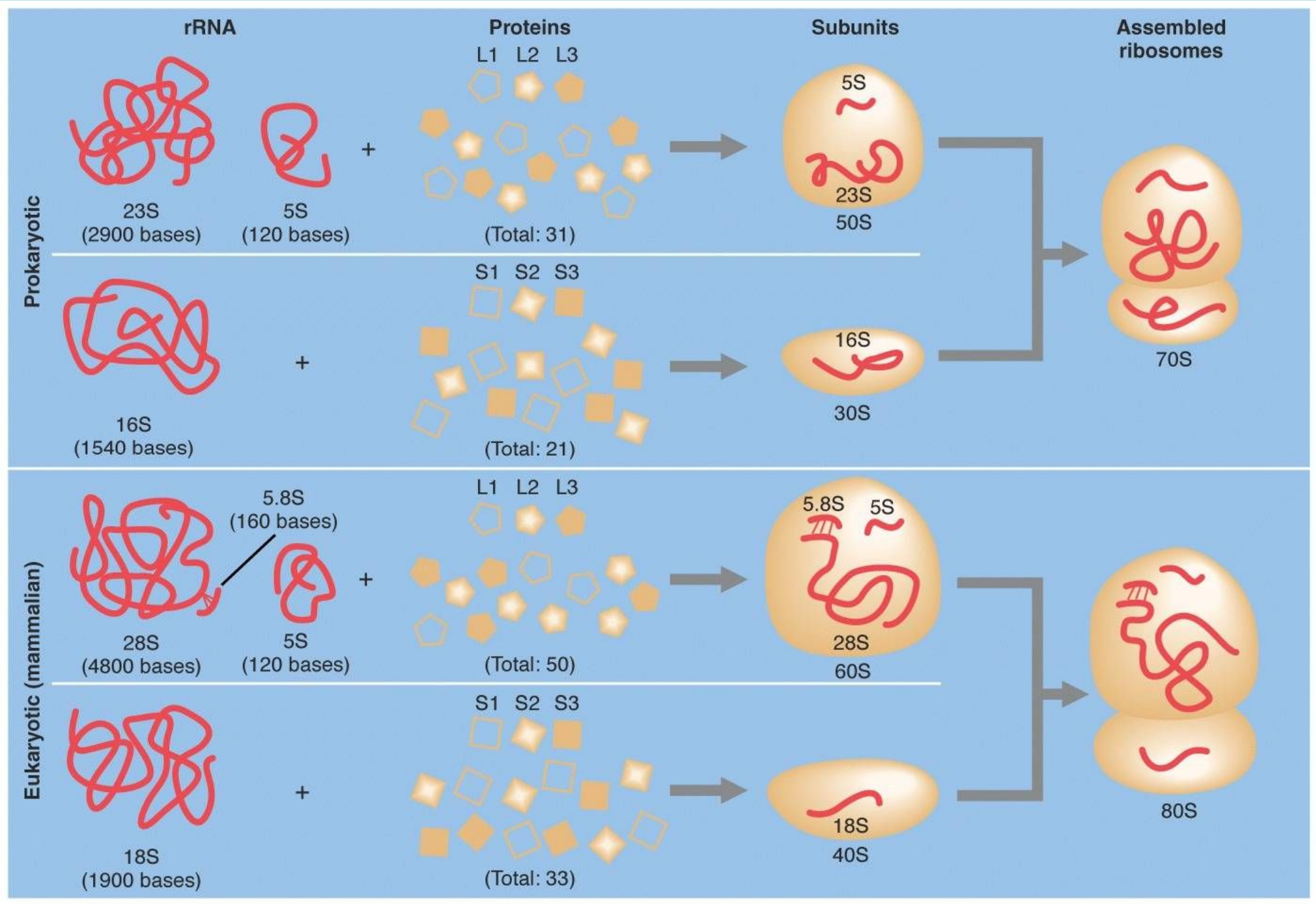


Fig. 1. The revised assembly map of the 30S subunit. The 16S ribosomal RNA is shown at the top, oriented from 5´ to 3´ direction. Each of the arrows indicates an observed dependency of binding for each ribosomal protein. The primary binding proteins depend solely on interactions with 16S rRNA (top row); the secondary and tertiary binding proteins depend on prior binding of other proteins.


rRNA
Proteins
Subunits
Assembled ribosomes
Prokaryotic
23S
(2900 bases)
5S
(120 bases)
+
L1 L2 L3
(Total: 31)
5S
23S
50S
16S
(1540 bases)
+
S1 S2 S3
(Total: 21)
16S
30S
70S
Eukaryotic (mammalian)
28S
(4800 bases)
5.8S
(160 bases)
5S
(120 bases)
+
L1 L2 L3
(Total: 50)
5.8S
5S
28S
60S
18S
(1900 bases)
+
S1 S2 S3
(Total: 33)
18S
40S
80S

E. Coli macromolecular elements

Element	% dry mass	molecules / cell	different types	copies
Cell wall	10	1	1	1
Membrane	10	2	2	1
DNA	1.5	1	1	1
mRNA	1	1,500	600	2-3
tRNA	3	200,000	60	>3000
rRNA	16	38,000	2	20,000
Ribosomal proteins	9	10^6	52	20,000
Soluble proteins	46	2×10^6	1800	>1000
Small molecules	3	8×10^6	800	

	ribosomes / cell
E.coli	20,000
Animal cells:	
Somatic cell	10^6
Xenopus oocyte	10^{12}