Biosinteza proteinov
- nadaljevanje -

Voet 3: poglavje 32

Stryer 5: poglavje 29

Krebs 3: poglavje 24

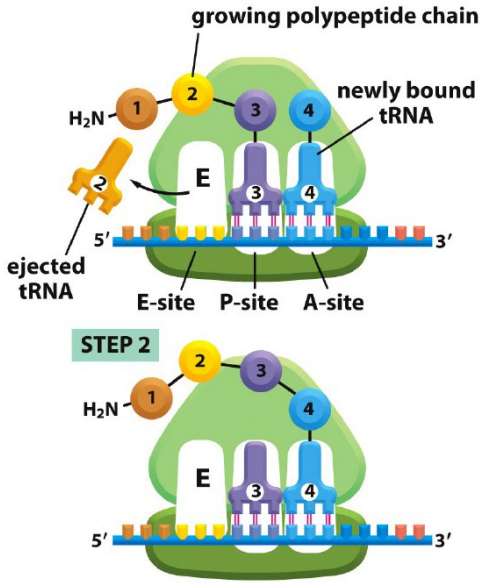


Figure 7-33 (part 2 of 5) *Essential Cell Biology* (© Garland Science 2010)

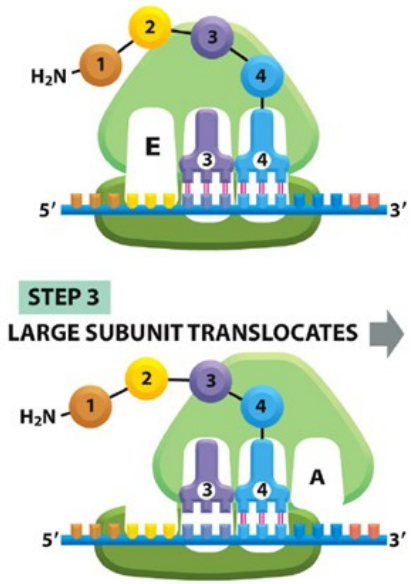


Figure 7-33 (part 3 of 5) *Essential Cell Biology* (© Garland Science 2010)

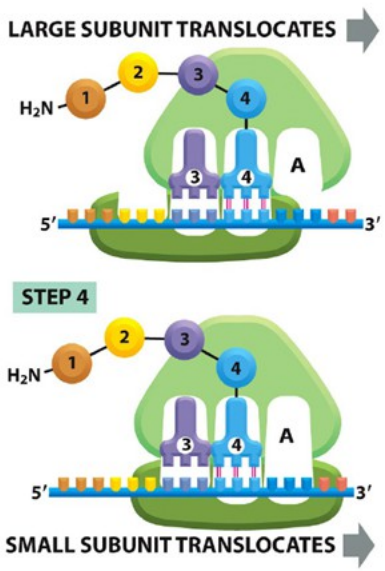


Figure 7-33 (part 4 of 5) *Essential Cell Biology* (© Garland Science 2010)

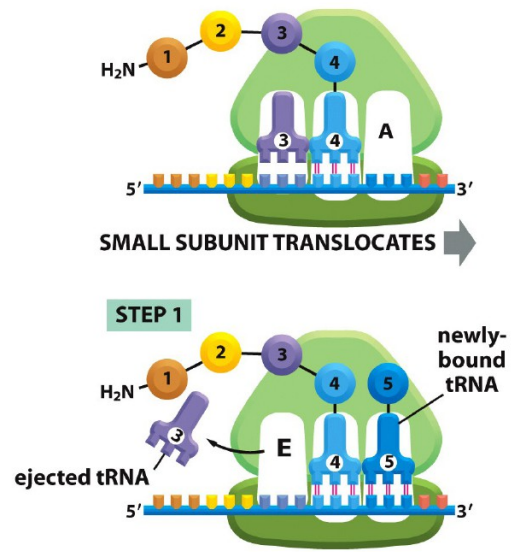
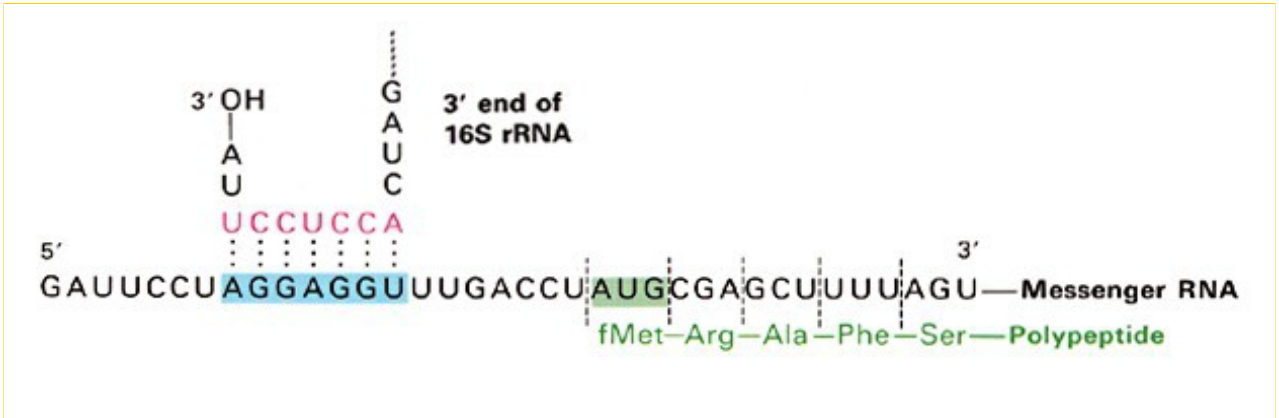


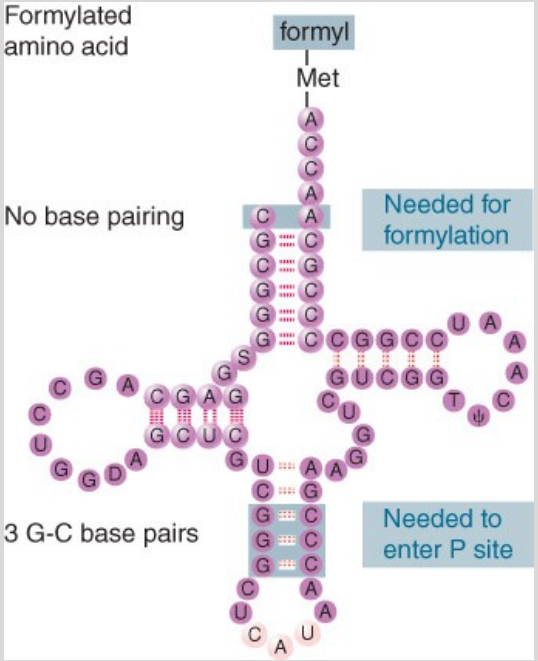
Figure 7-33 (part 5 of 5) *Essential Cell Biology* (© Garland Science 2010)

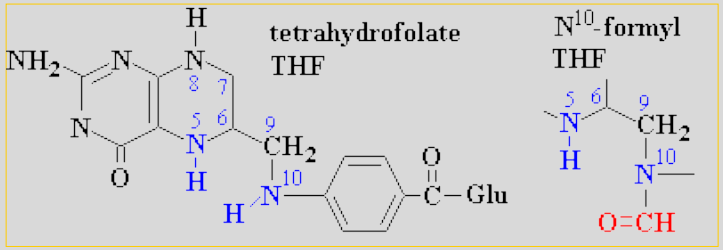
5′ … 3′	
AGCACGAGGGGAAAUCUGAUGGAACGCUAC	*E. coli trpA*
UUUGGAUGGAGUGAAACGAUGGCGAUUGCA	*E. coli araB*
GGUAACCAGGUAACAACCAUGCGAGUGUUG	*E. coli thrA*
CAAUUCAGGGUGGUGAAUGUGAAACCAGUA	*E. coli lacI*
AAUCUUGGAGGCUUUUUUAUGGUUCGUUCU	ϕX174 phage A protein
UAACUAAGGAUGAAAUGCAUGUCUAAGACA	Qβ phage replicase
UCCUAGGAGGUUUGACCUAUGCGAGCUUUU	R17 phage A protein
AUGUACUAAGGAGGUUGUAUGGAACAACGC	λ phage *cro*

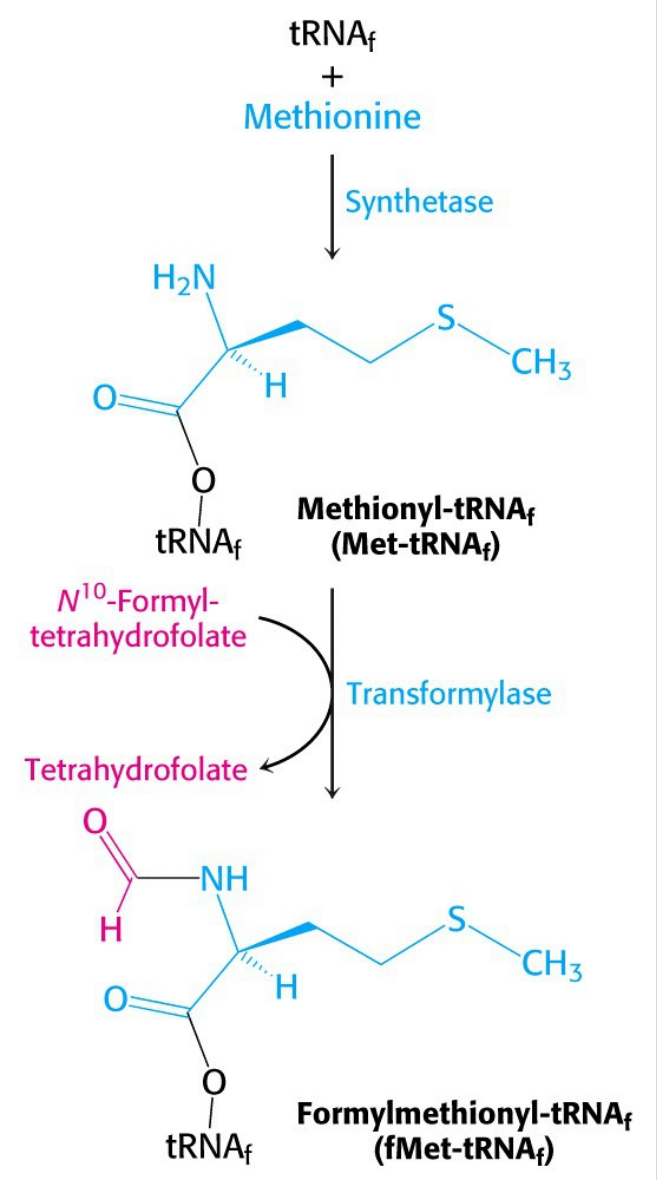
Pairs with 16S rRNA

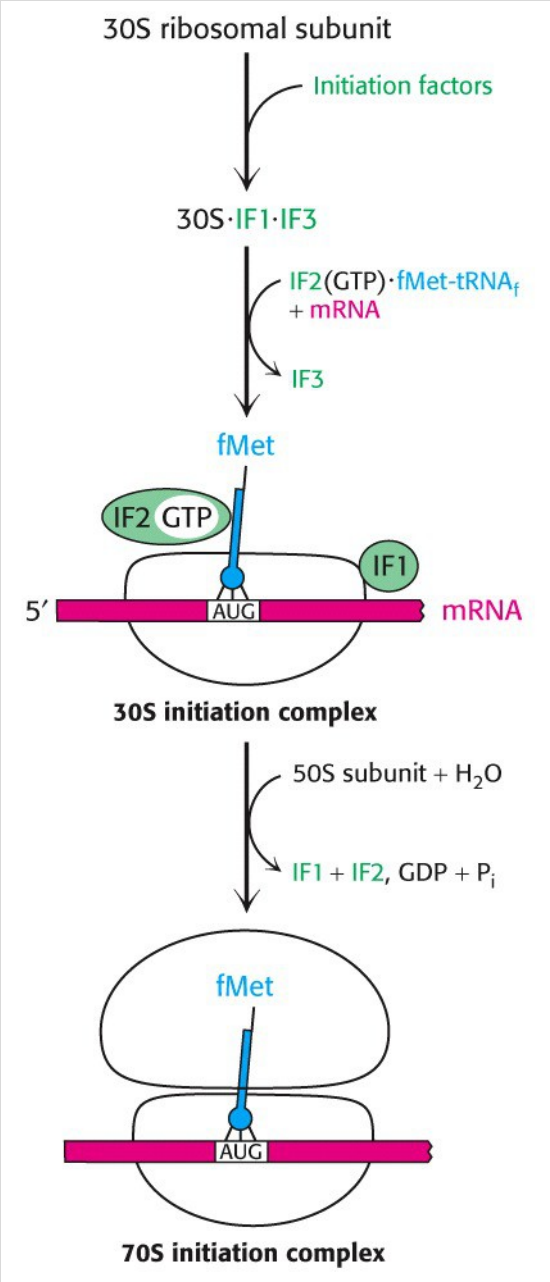
Pairs with initiator tRNA

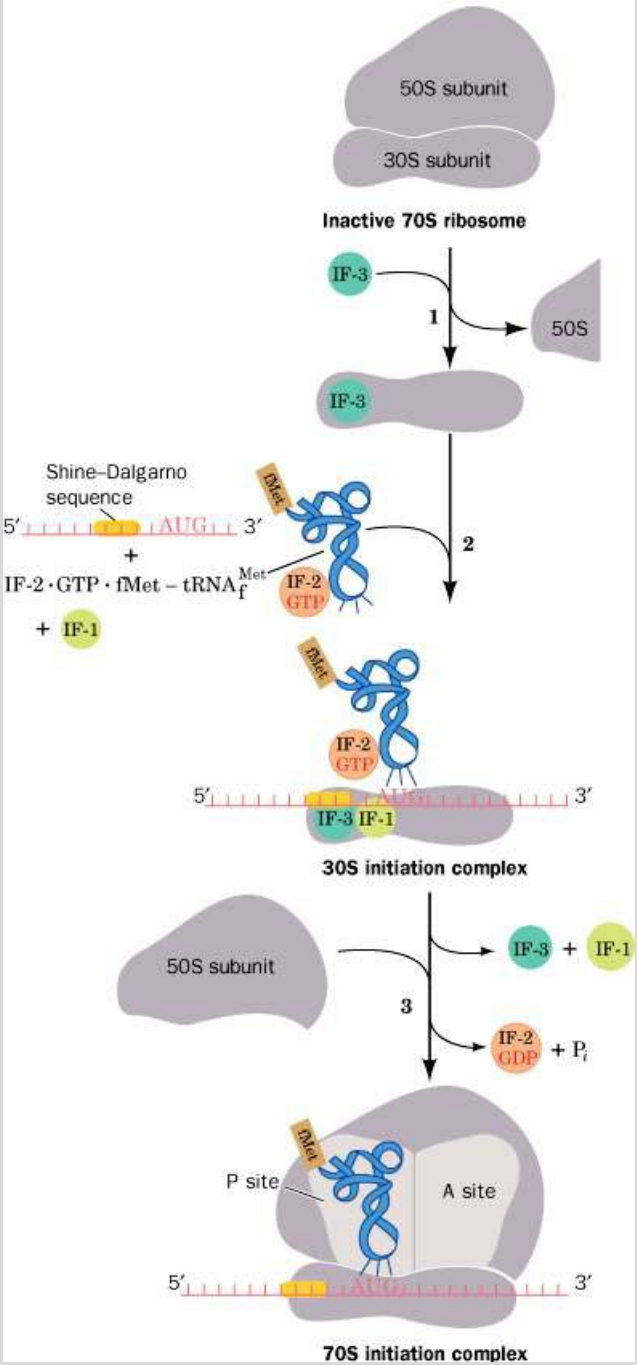



Formylated amino acid
formyl
Met
No base pairing
Needed for formylation
3 G-C base pairs
Needed to enter P site


tetrahydrofolate
THF
N10-formyl
THF
Glu
O=CH


tRNAf
+
Methionine
Synthetase
Methionyl-tRNAf
(Met-tRNAf)
N10-Formyl-tetrahydrofolate
Transformylase
Tetrahydrofolate
Formylmethionyl-tRNAf
(fMet-tRNAf)


30S ribosomal subunit
Initiation factors
30S·IF1·IF3
IF2(GTP)·fMet-tRNA$_f$
+ mRNA
IF3
fMet
IF2 GTP
IF1
5′
AUG
mRNA
30S initiation complex
50S subunit + H_2O
IF1 + IF2, GDP + P_i
fMet
AUG
70S initiation complex

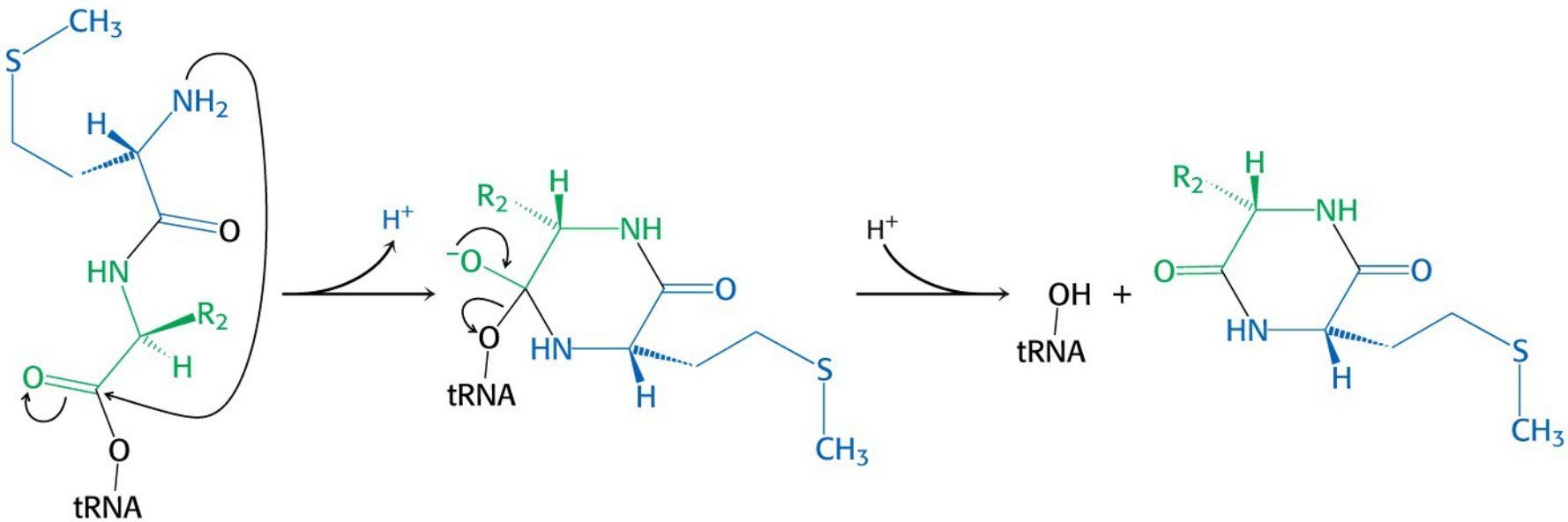

50S subunit
30S subunit
Inactive 70S ribosome
IF-3
1
50S
IF-3
Shine–Dalgarno
sequence
5′
AUG
3′
+
IF-2·GTP·fMet – tRNA$_f^{Met}$
+ IF-1
fMet
IF-2
GTP
2
fMet
IF-2
GTP
5′
AUG
3′
IF-3 IF-1
30S initiation complex
50S subunit
3
IF-3 + IF-1
IF-2
GDP
+ P_i
fMet
P site
A site
5′
AUG
3′
70S initiation complex

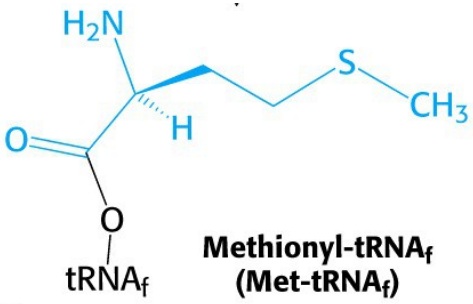
Topni proteinski faktorji pri sintezi proteinov *E. coli*

Factor	Number of Residues[a]	Function
Initiation Factors		
IF-1	71	Assists IF-3 binding
IF-2	890	Binds initiator tRNA and GTP
IF-3	180	Releases mRNA and tRNA from recycled 30S subunit and aids new mRNA binding
Elongation Factors		
EF-Tu	393	Binds aminoacyl–tRNA and GTP
EF-Ts	282	Displaces GDP from EF-Tu
EF-G	703	Promotes translocation through GTP binding and hydrolysis
Release Factors		
RF-1	360	Recognizes UAA and UAG Stop codons
RF-2	365	Recognizes UAA and UGA Stop codons
RF-3	528	Stimulates RF-1/RF-2 release via GTP hydrolysis
RRF	185	Together with EF-G, induces ribosomal dissociation to small and large subunits

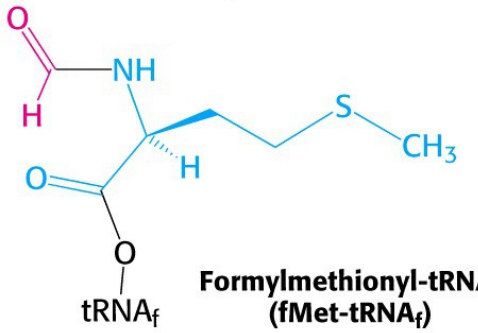
[a]All *E. coli* translational factors are monomeric proteins.

Kaj, če Met1 ne bi bil formiliran…?

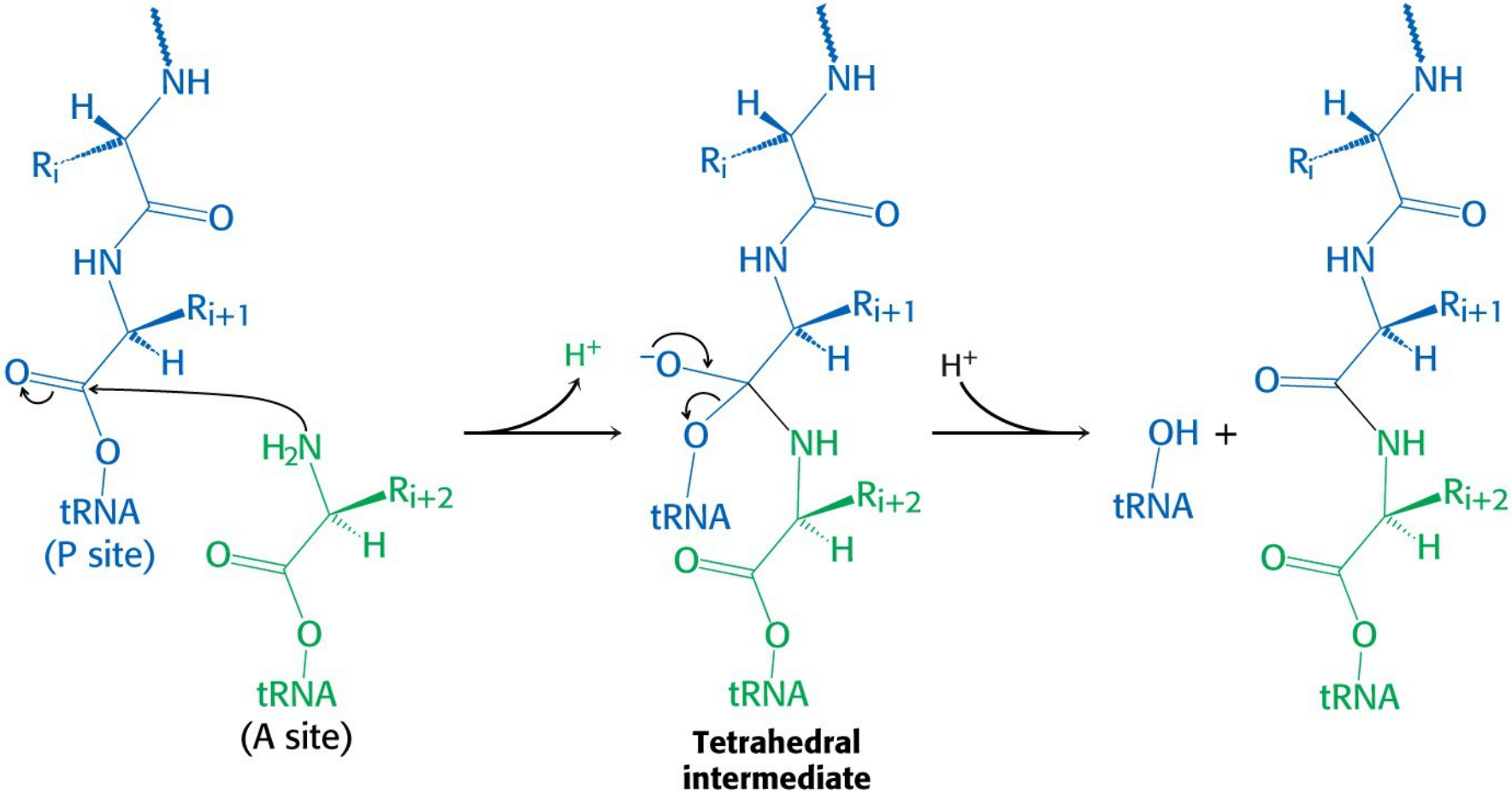


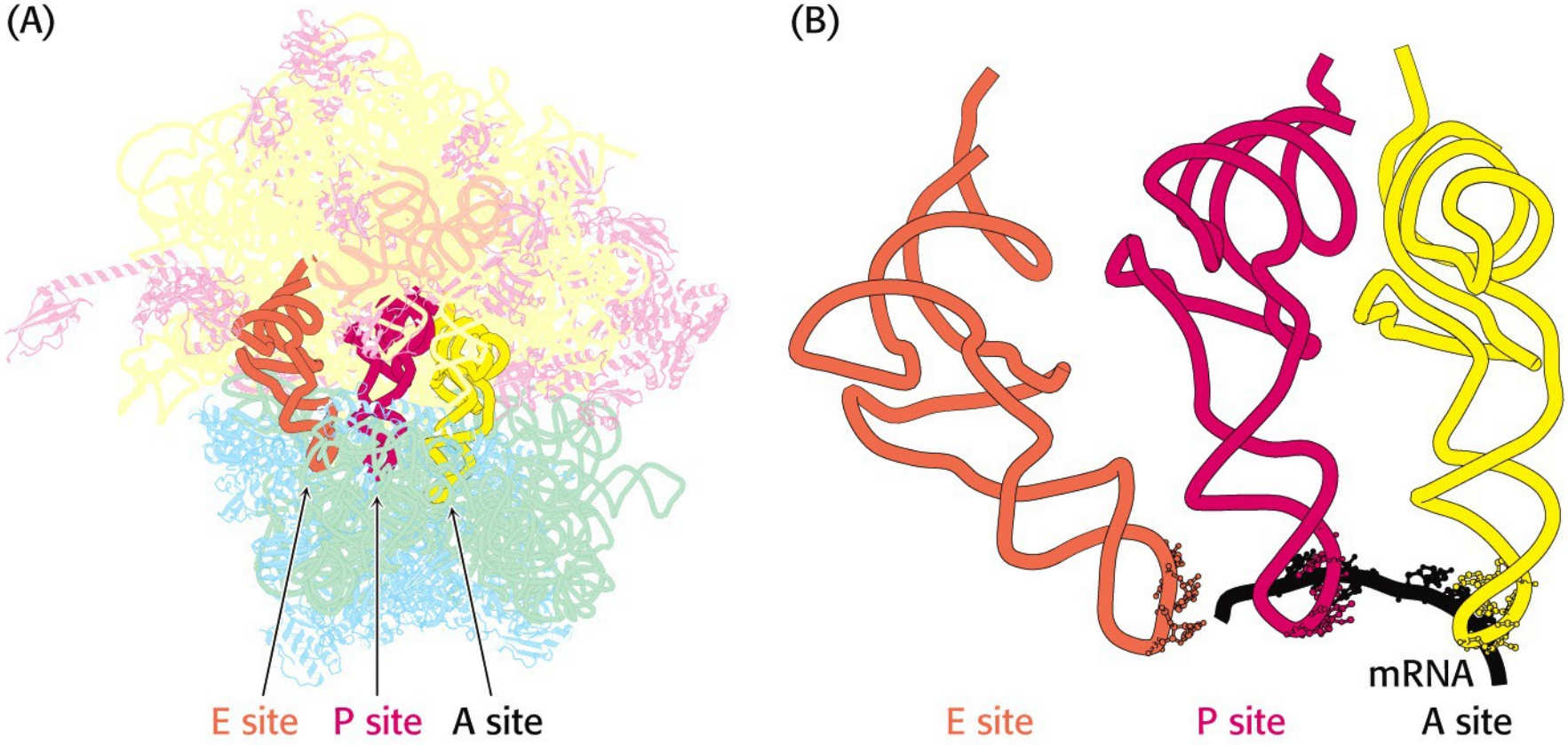


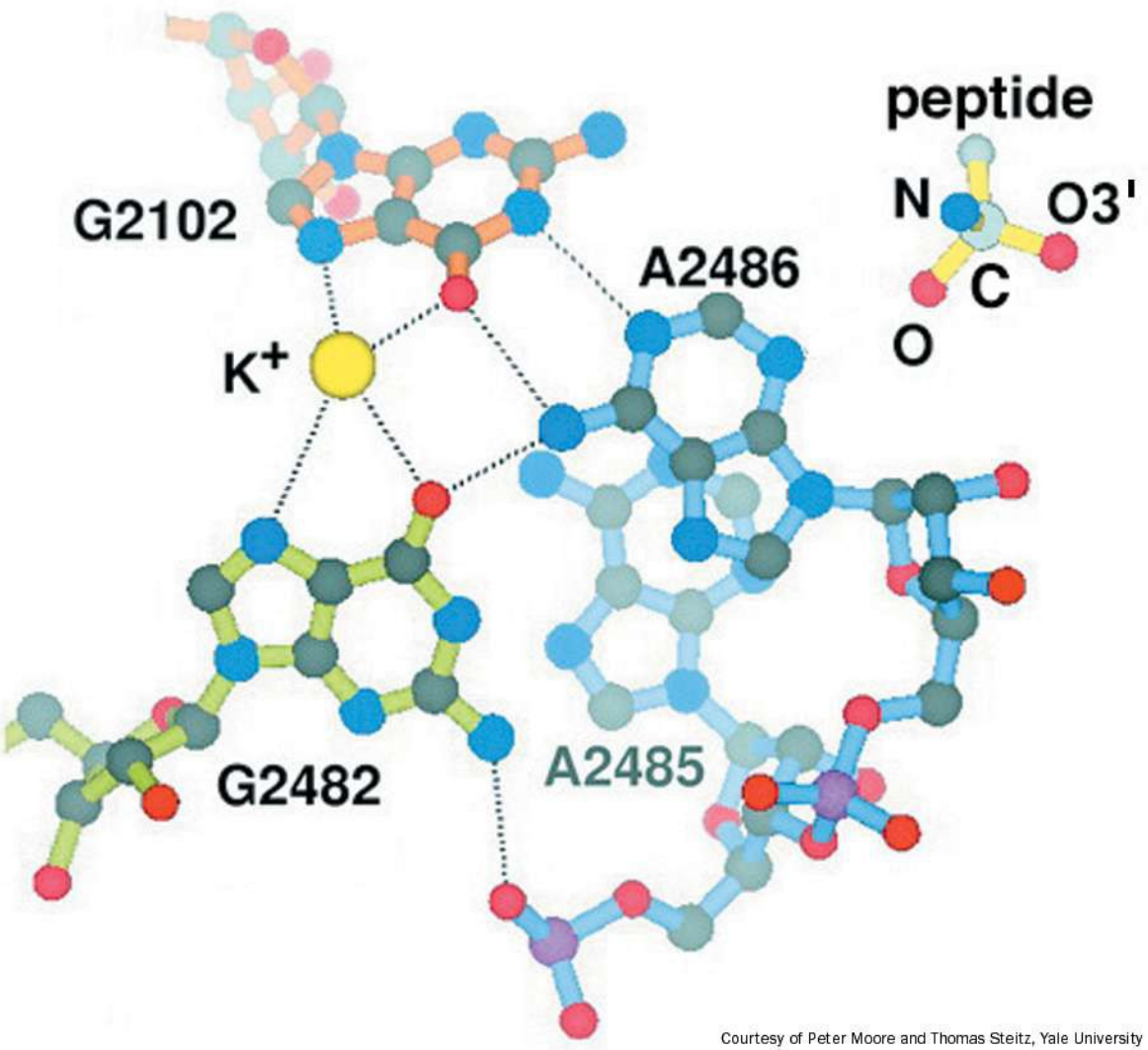
Methionyl-tRNA$_f$
(Met-tRNA$_f$)



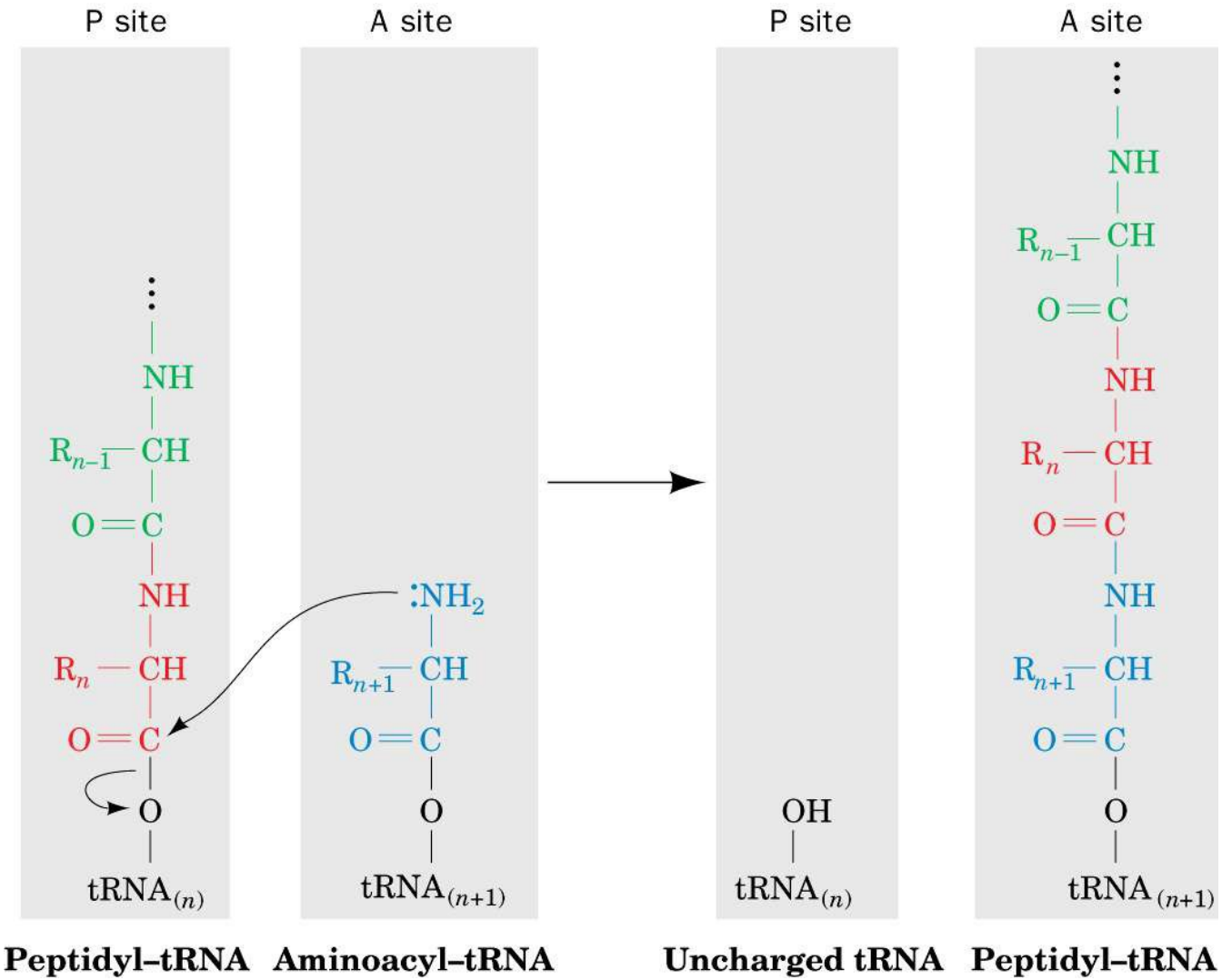
Formylmethionyl-tRNA$_f$
(fMet-tRNA$_f$)


NH
H
Ri
O
HN
Ri+1
H
O
O
tRNA
(P site)
H2N
Ri+2
H
O
O
tRNA
(A site)
H+
−O
O
NH
Ri+2
H
O
O
tRNA
Tetrahedral
intermediate
H+
OH +
tRNA

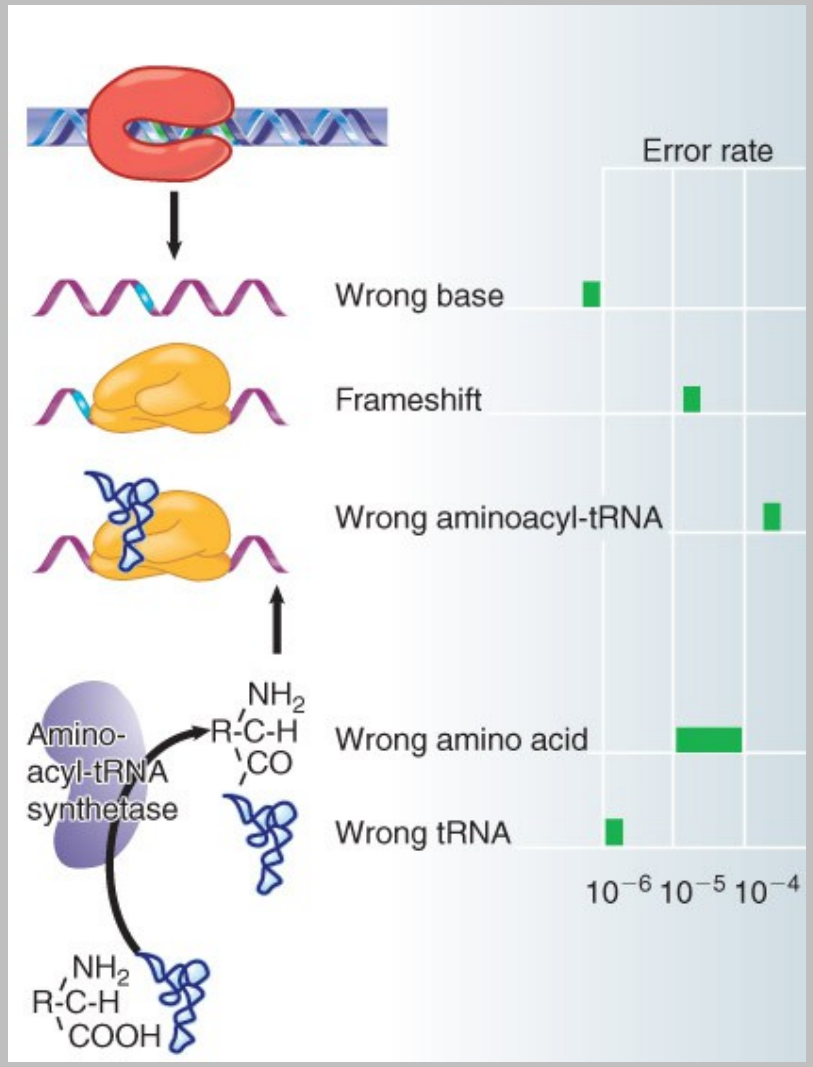

(A)
E site
P site
A site
(B)
mRNA
E site
P site
A site

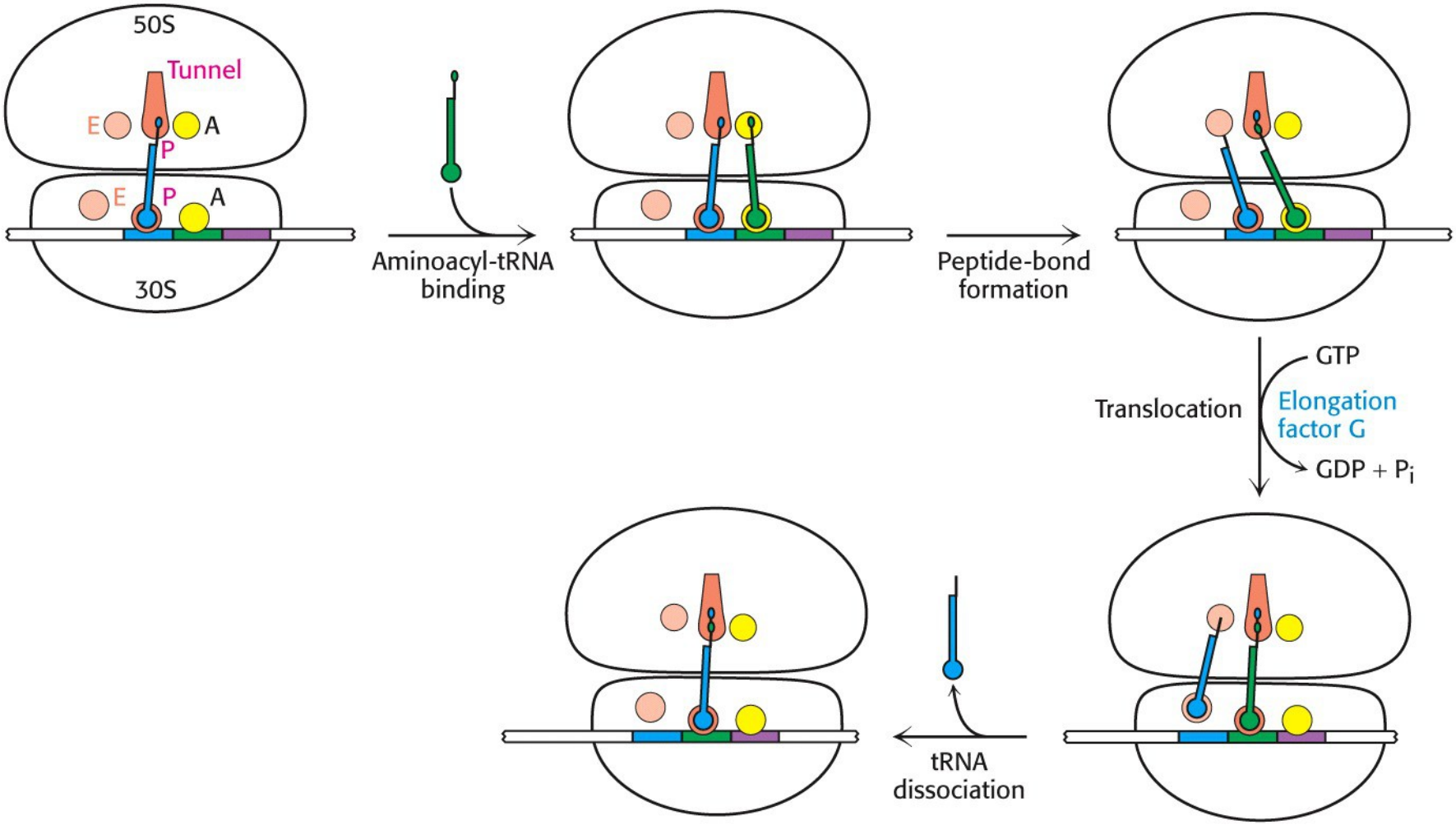


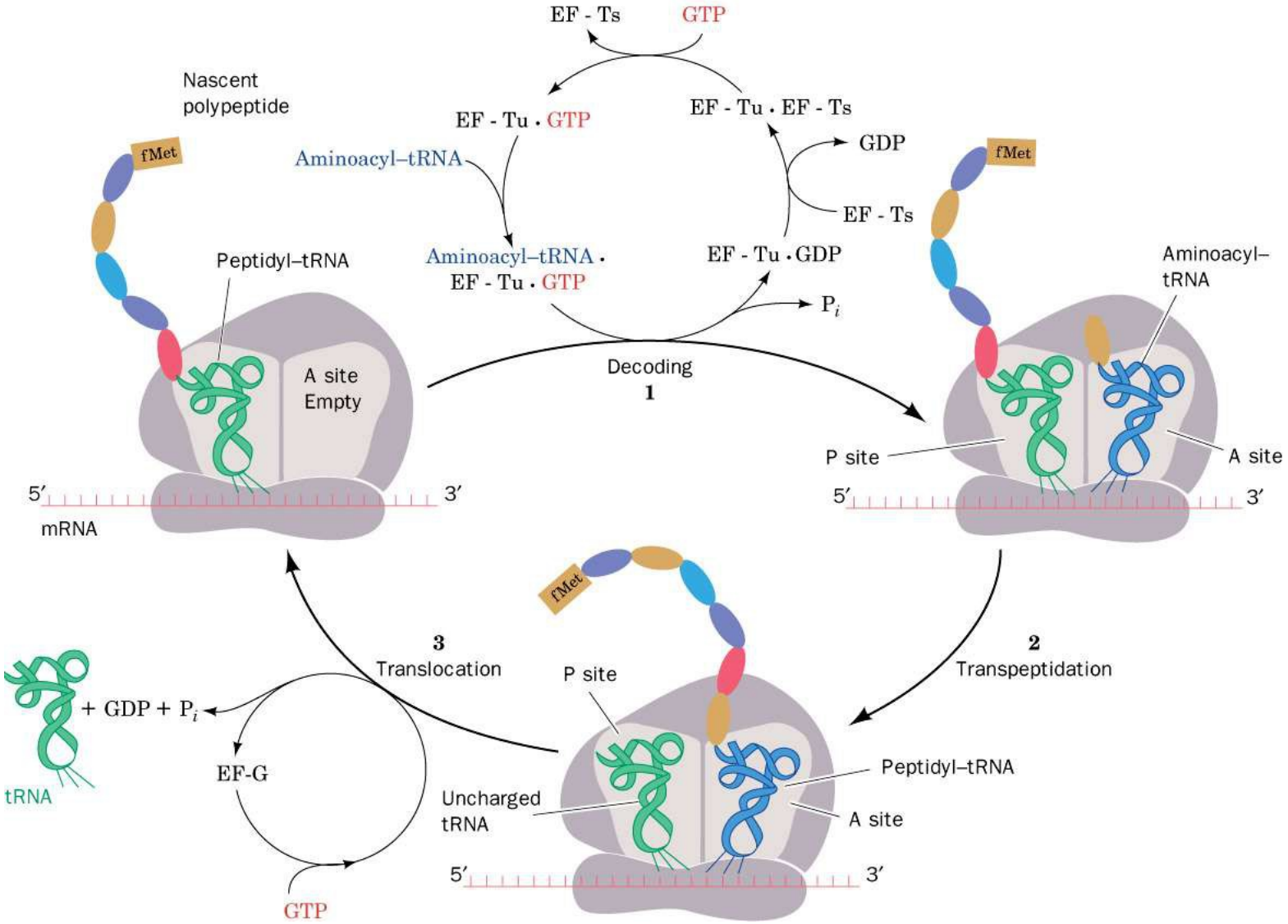
Aktivni center peptidiltransferaze.



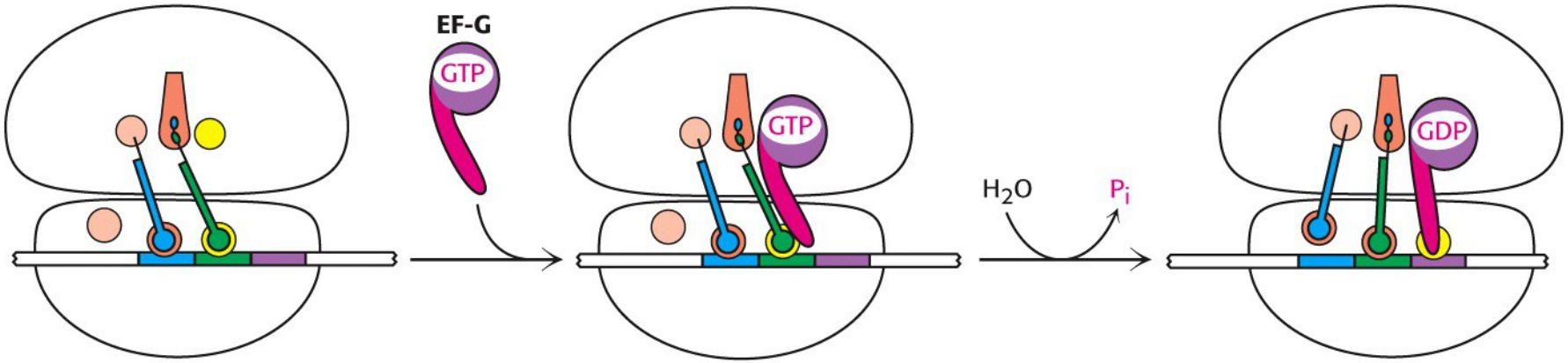
Nastanek peptidne vezi s posredovanjem ribosomske peptidiltrasferaze.

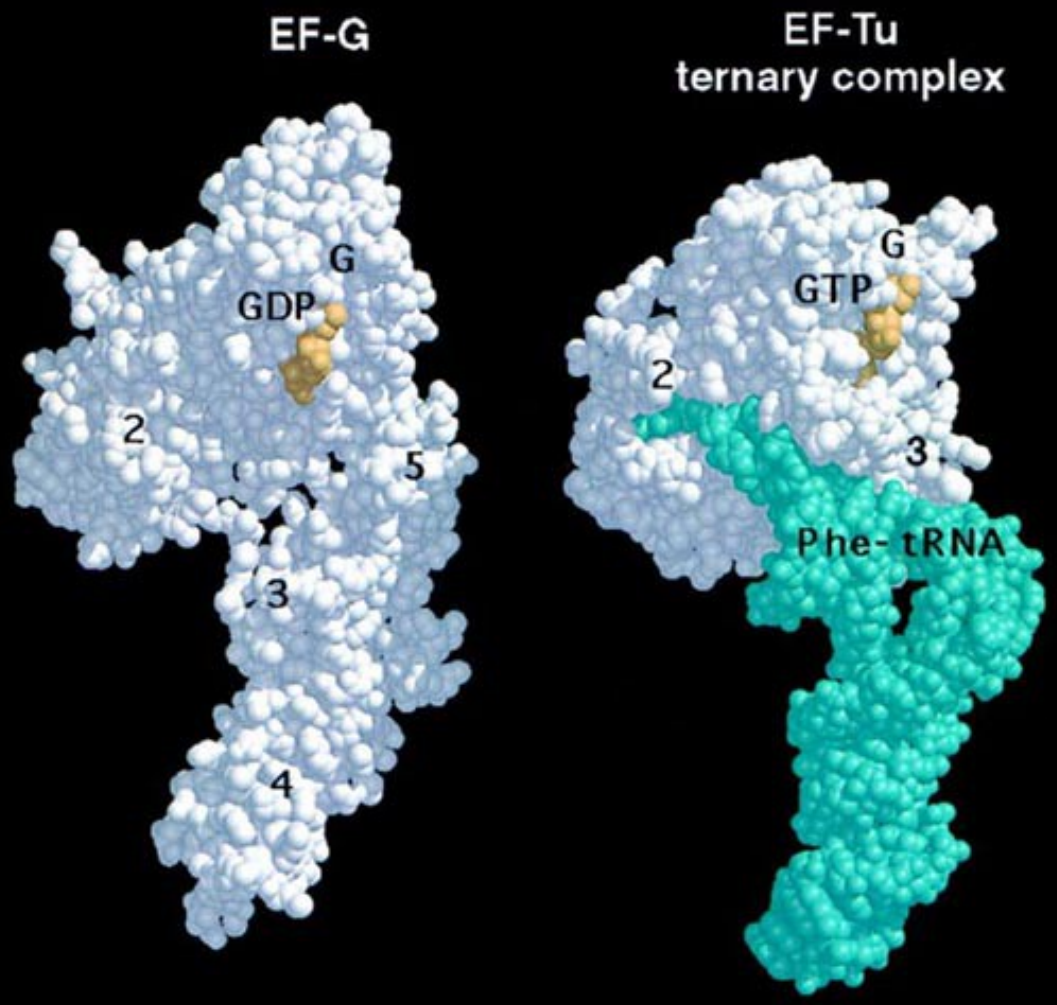

Error rate
Wrong base
Frameshift
Wrong aminoacyl-tRNA
Wrong amino acid
Wrong tRNA
10^{-6} 10^{-5} 10^{-4}
Amino-
acyl-tRNA
synthetase
NH_2
R-C-H
CO
NH_2
R-C-H
COOH

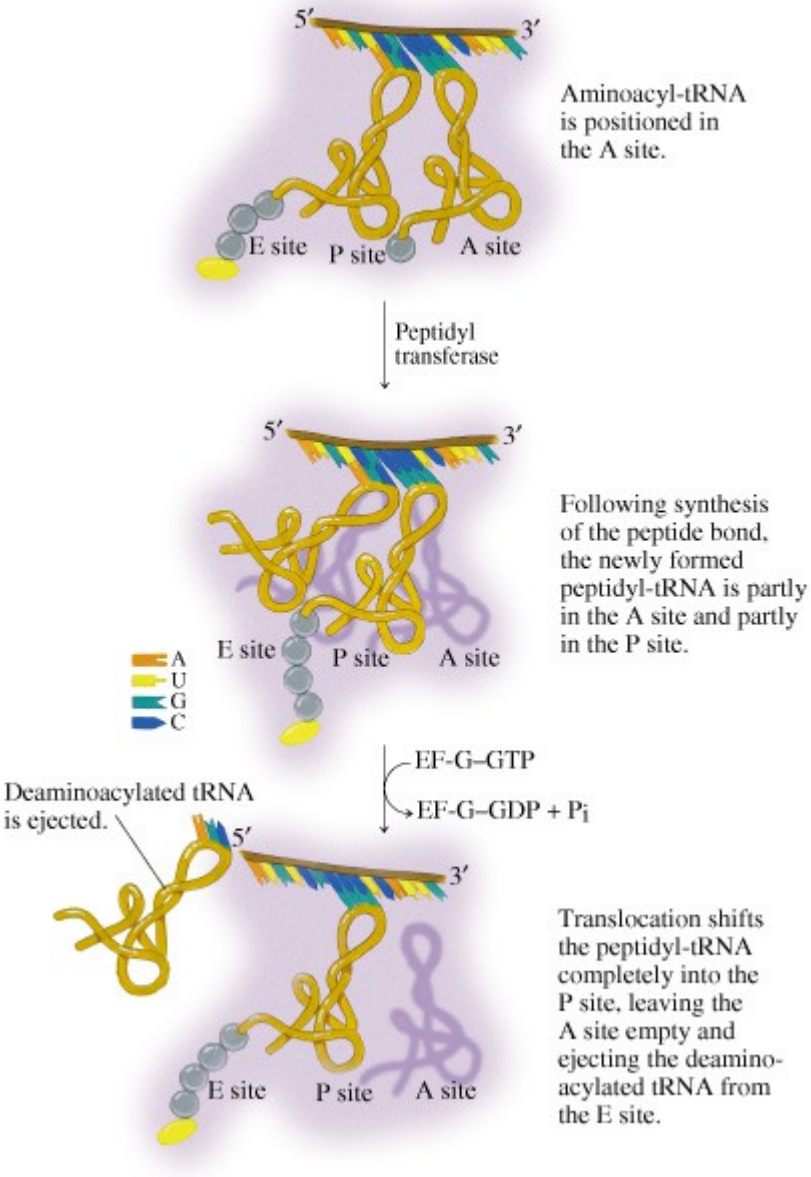

50S
Tunnel
E
A
P
E
P
A
30S
Aminoacyl-tRNA
binding
Peptide-bond
formation
GTP
Translocation
Elongation
factor G
GDP + Pi
tRNA
dissociation



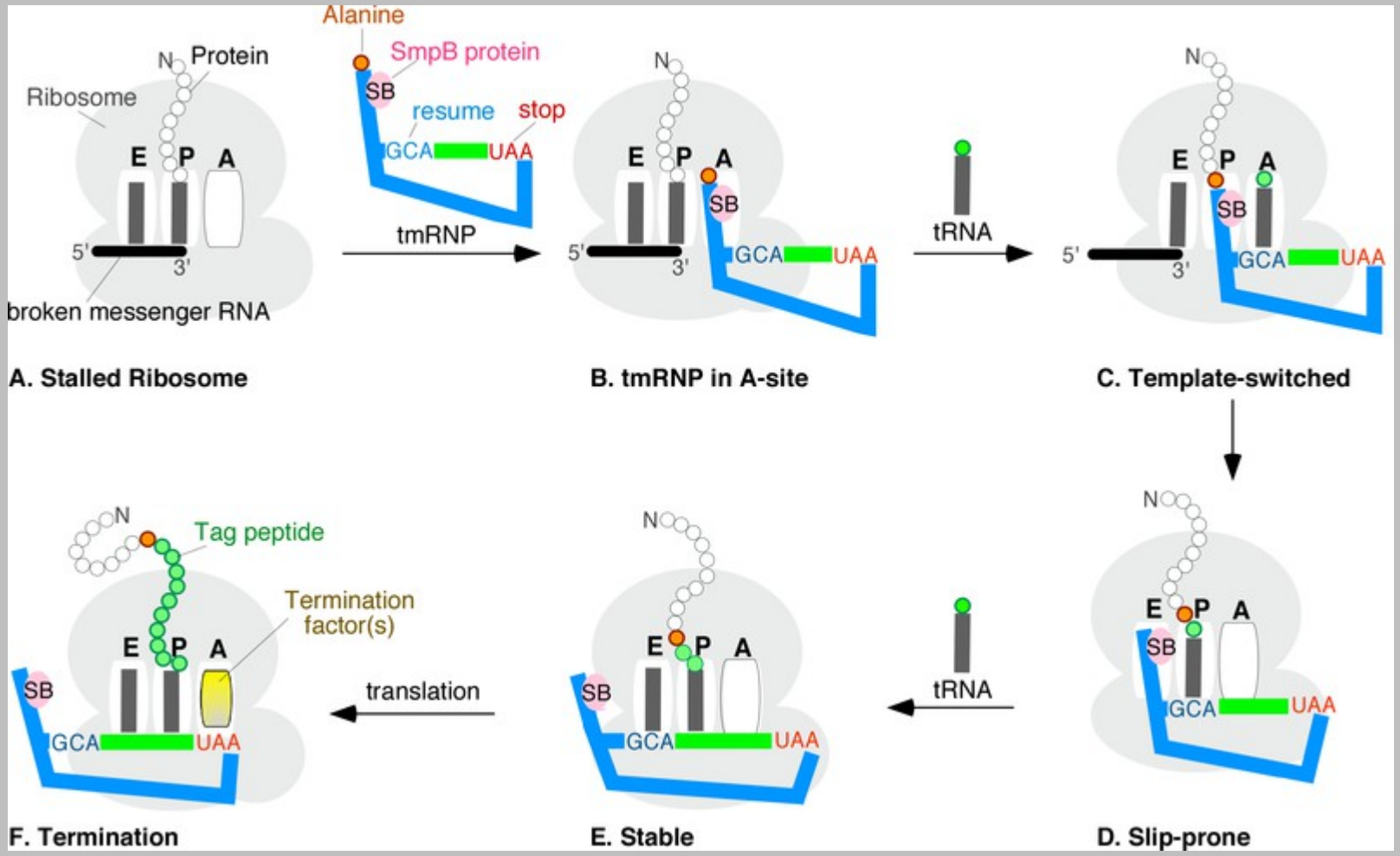
Shematski prikaz elongacije polipeptidne verige pri prokariontih.

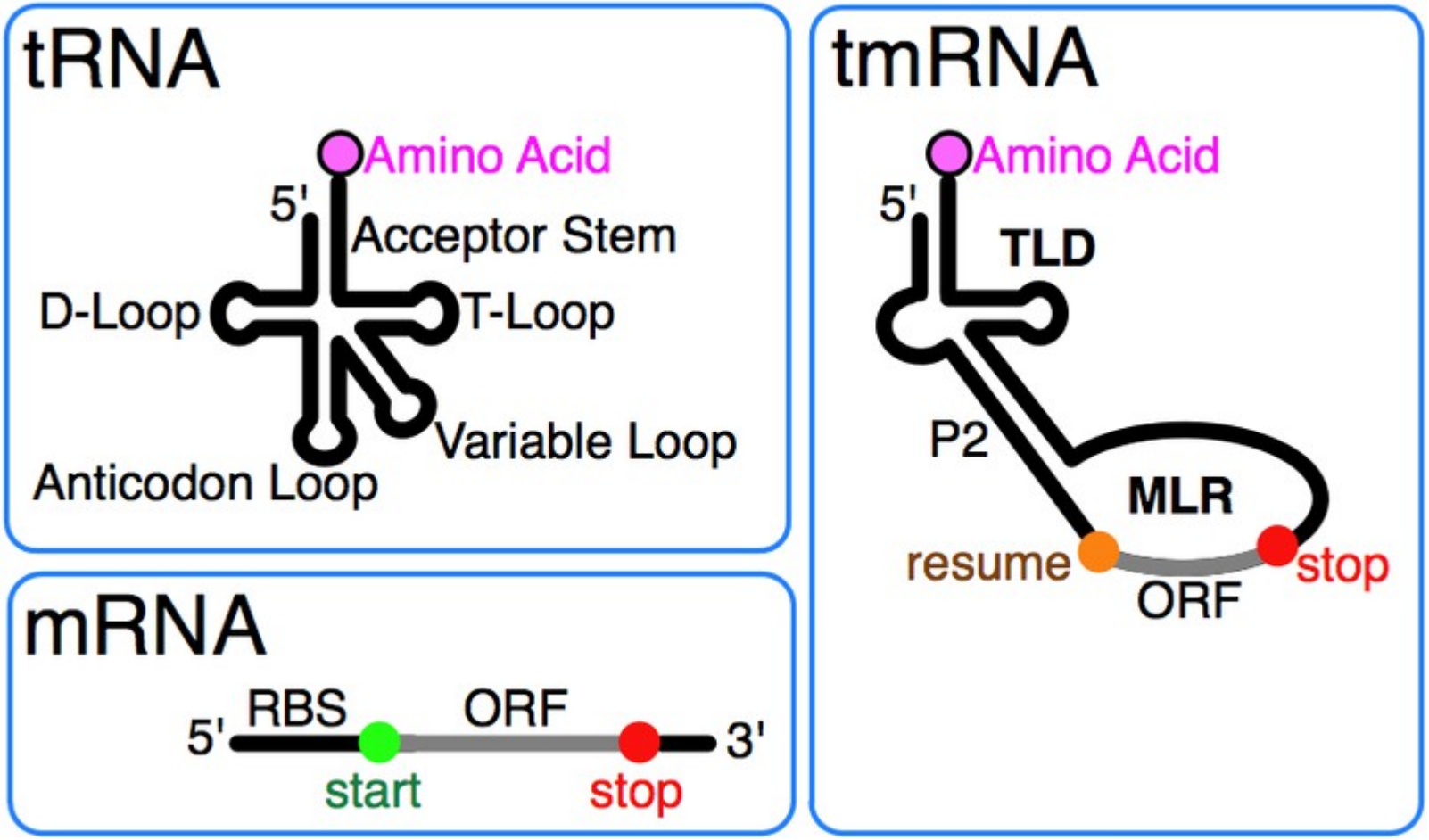

EF-G
GTP
GTP
H_2O
P_i
GDP


EF-G
G
GDP
2
5
3
4
EF-Tu
ternary complex
G
GTP
2
3
Phe- tRNA


5′
3′
Aminoacyl-tRNA is positioned in the A site.
E site
P site
A site
Peptidyl transferase
5′
3′
Following synthesis of the peptide bond, the newly formed peptidyl-tRNA is partly in the A site and partly in the P site.
E site
P site
A site
A
U
G
C
EF-G–GTP
EF-G–GDP + Pi
Deaminoacylated tRNA is ejected.
5′
3′
Translocation shifts the peptidyl-tRNA completely into the P site, leaving the A site empty and ejecting the deamino-acylated tRNA from the E site.
E site
P site
A site

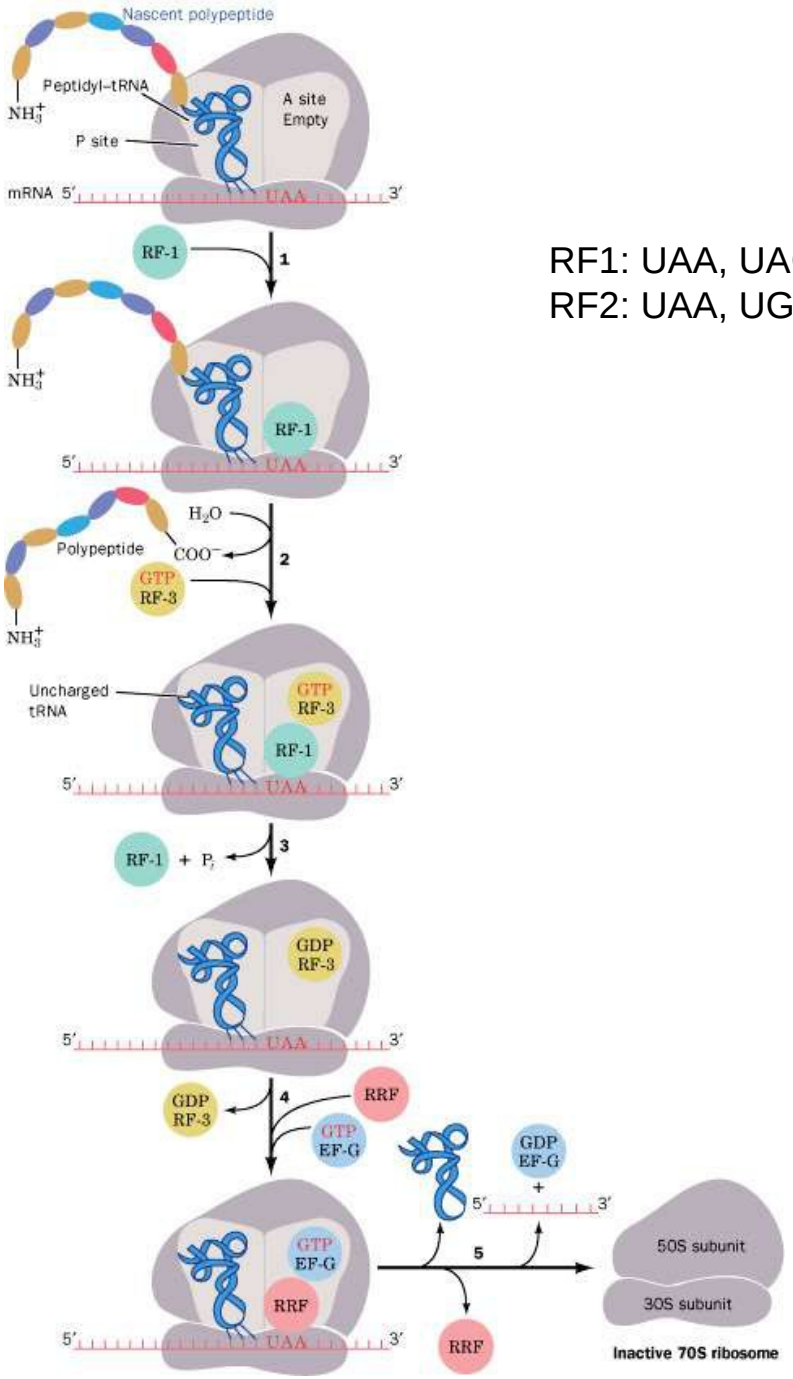
Dogajanje na ribosomu pri poškodovani mRNA:
Trans-translacija





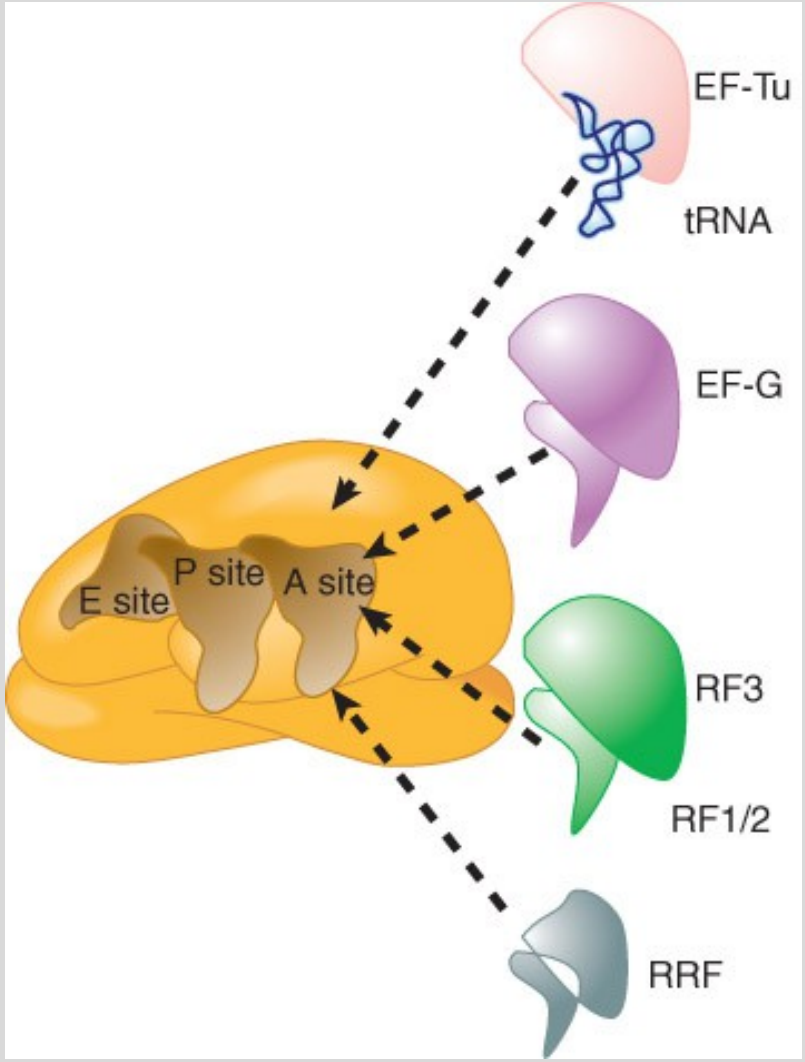
tmRNA = 10 Sa RNA = SsrA

Terminacija pri *E. coli*

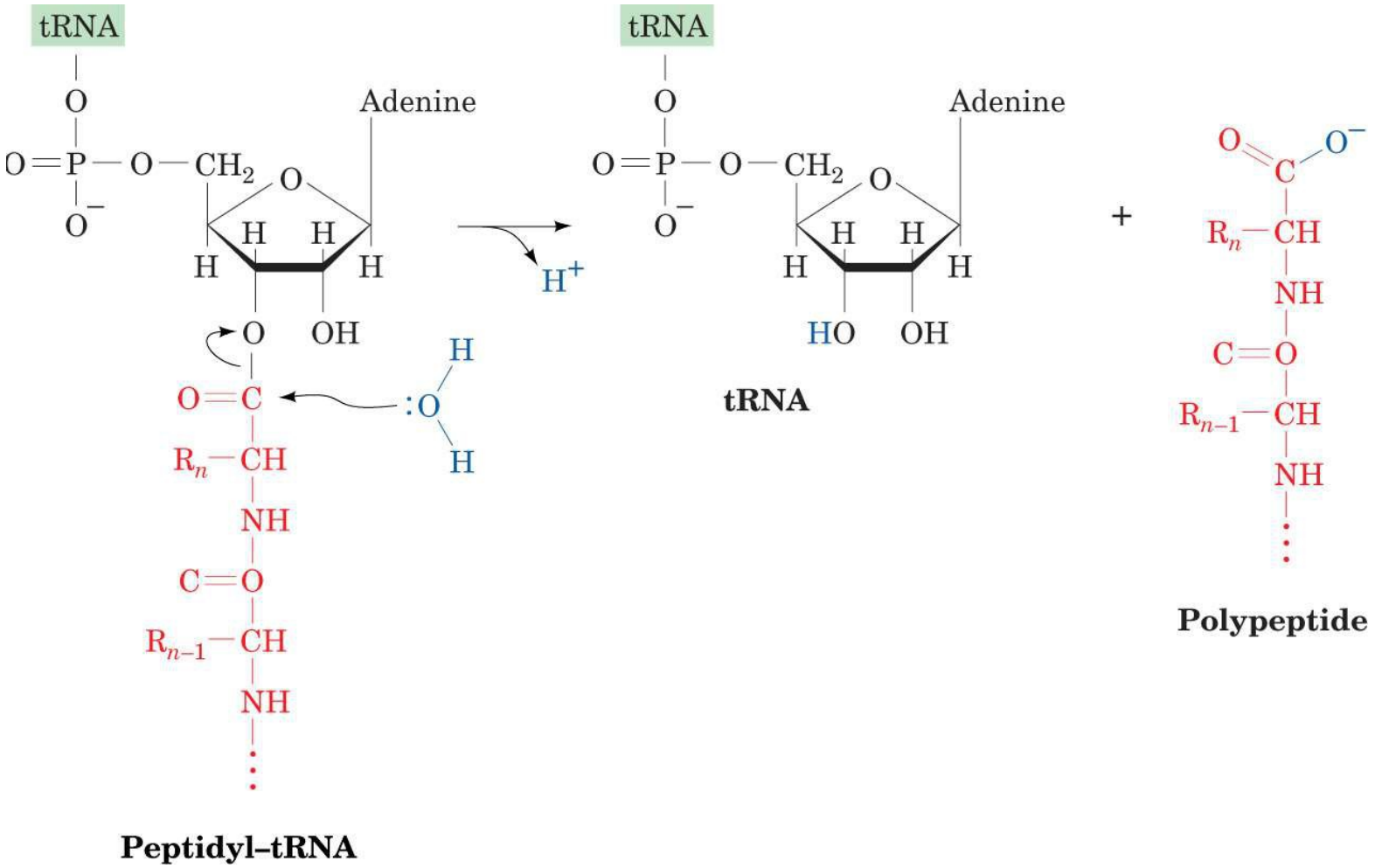


RF1: UAA, UAG
RF2: UAA, UGA

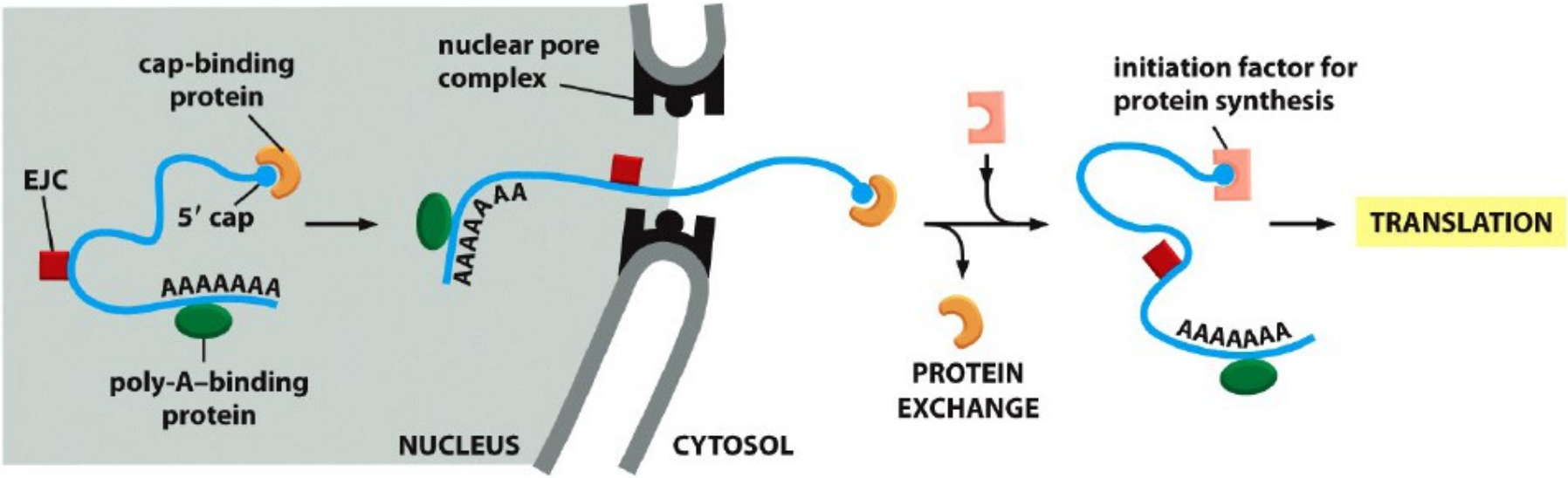
RRF: ribosome recycling factor

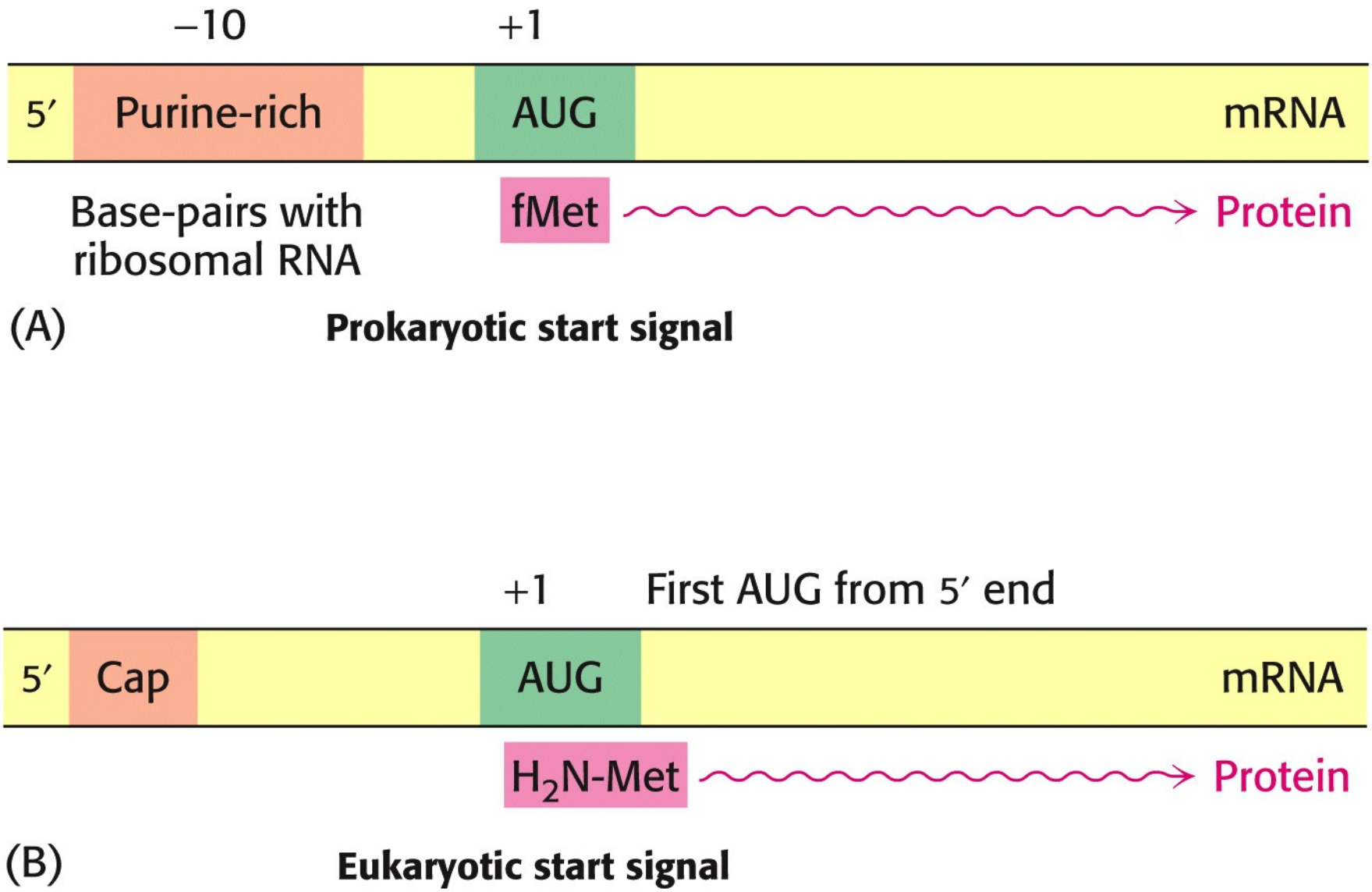


Molekulska mimikrija omogoča vezavo različnih transkripcijskih faktorjev na isto mesto v ribosomu



Hidrolitična sprostitev tRNA s končnega aminokislinskega ostanka.




−10
+1
5′
Purine-rich
AUG
mRNA
Base-pairs with ribosomal RNA
fMet
Protein
(A)
Prokaryotic start signal
+1
First AUG from 5′ end
5′
Cap
AUG
mRNA
H2N-Met
Protein
(B)
Eukaryotic start signal

Consensus RBS Sequences

Prokaryotic (Shine-Dalgarno sequence)

+1

5' – AGGAGGACAGCUAUG – 3'

RBS spacer initiator

Eukaryotic (Kozak sequence)

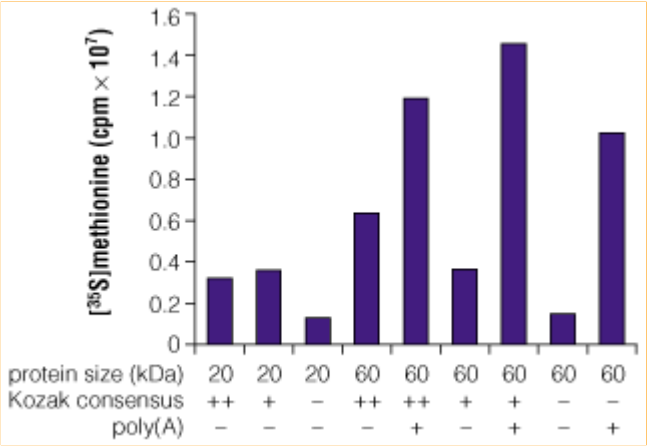
+1

5' – A/GCCACCAUGG – 3'

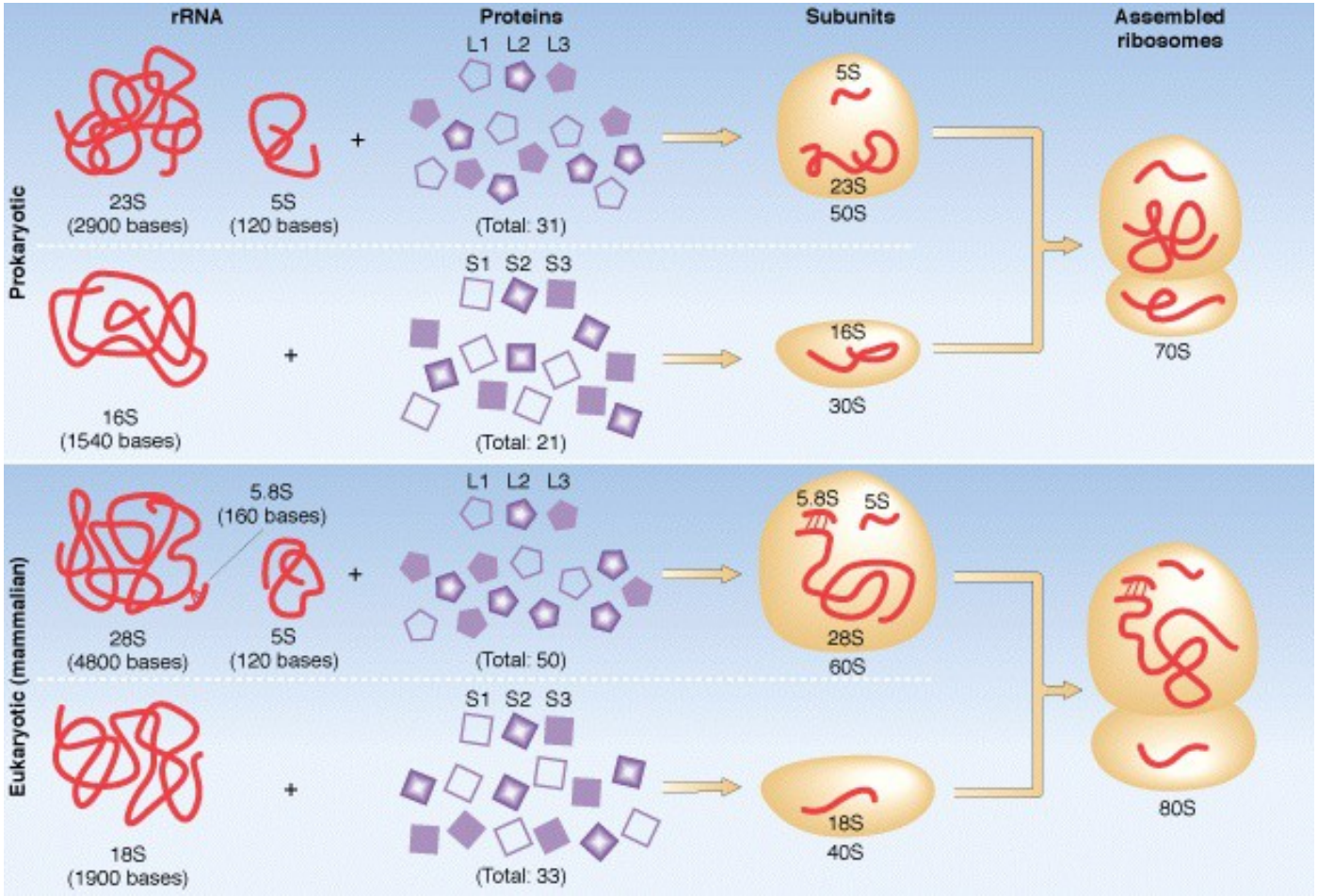
RBS

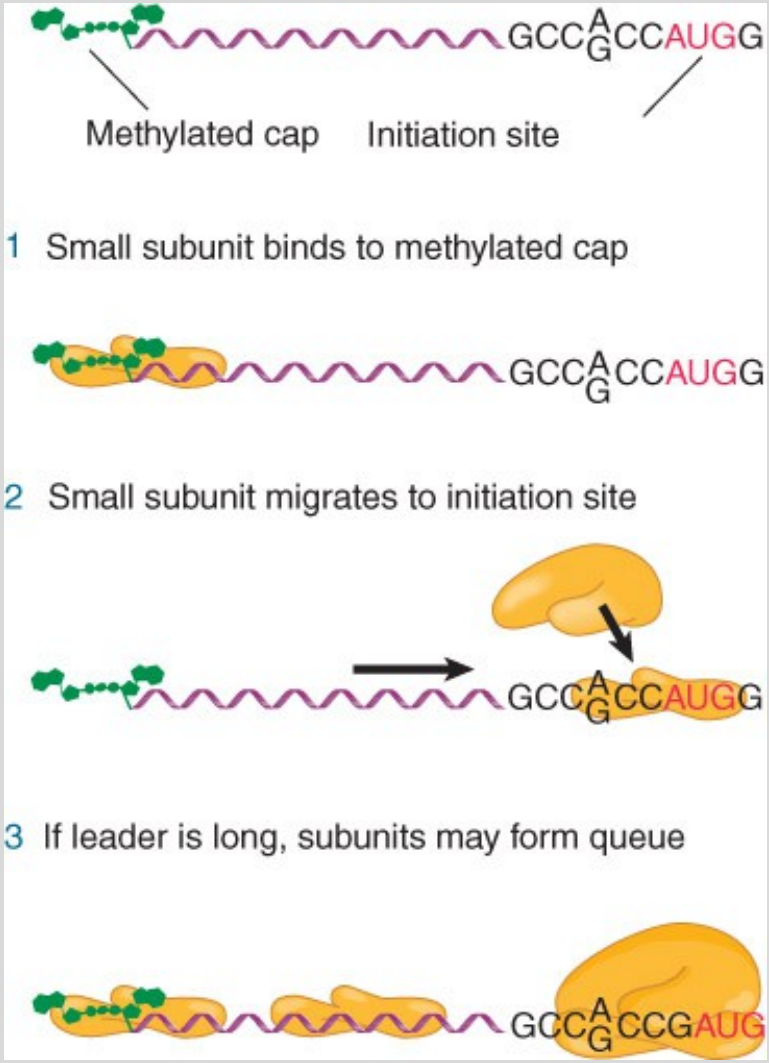
initiator

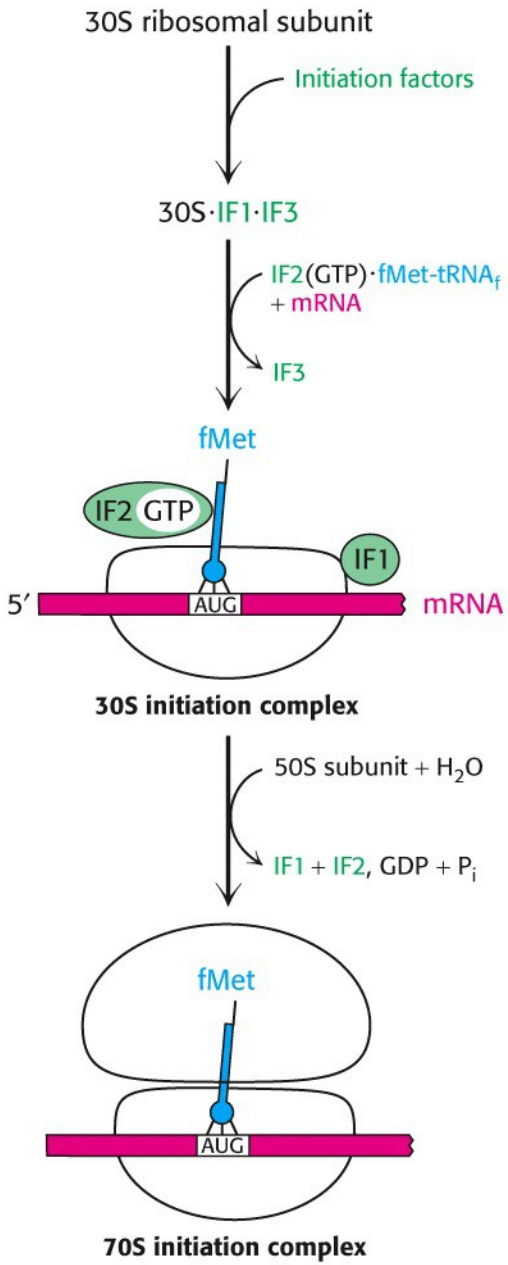
http://www.ambion.com/techlib/append/rbs_requirements.html

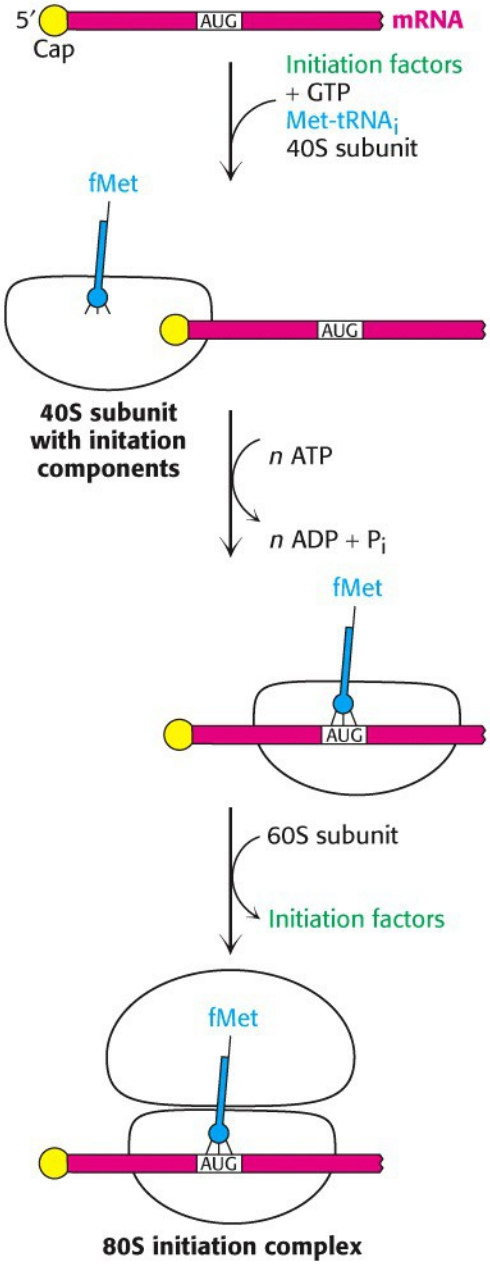


http://www.promega.com/enotes/features/0012/images/fe0009_fig4.gif

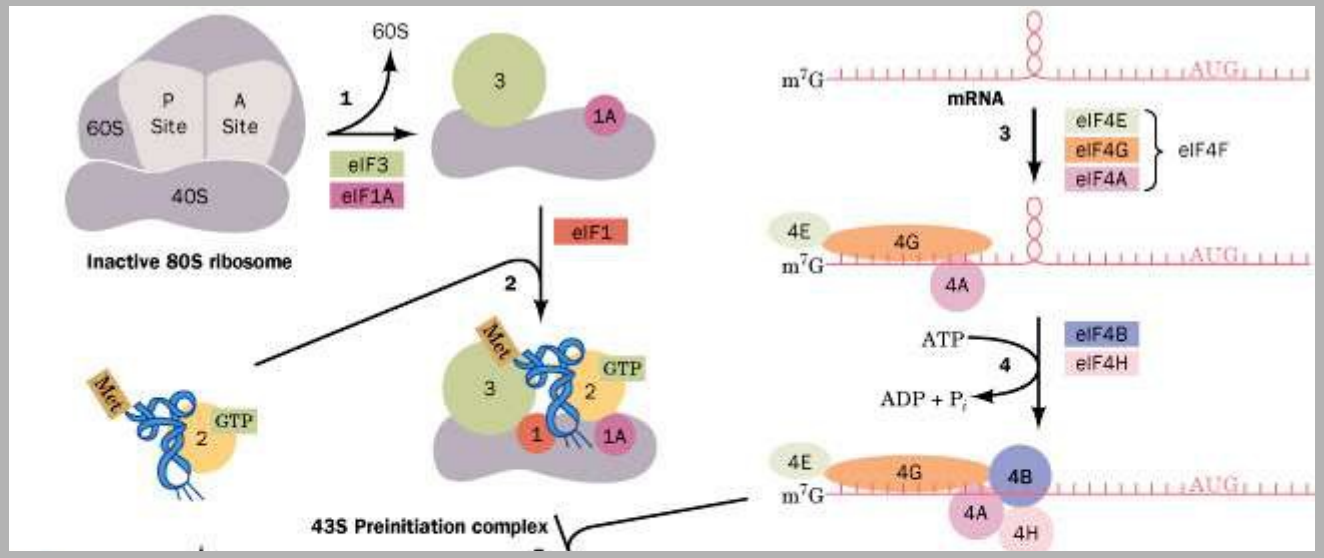

rRNA
Proteins
Subunits
Assembled ribosomes
Prokaryotic
23S
(2900 bases)
5S
(120 bases)
L1 L2 L3
(Total: 31)
5S
23S
50S
16S
(1540 bases)
S1 S2 S3
(Total: 21)
16S
30S
70S
Eukaryotic (mammalian)
5.8S
(160 bases)
28S
(4800 bases)
5S
(120 bases)
L1 L2 L3
(Total: 50)
5.8S 5S
28S
60S
18S
(1900 bases)
S1 S2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


GCCACCAUGG
Methylated cap
Initiation site
1 Small subunit binds to methylated cap
GCCACCAUGG
2 Small subunit migrates to initiation site
GCCACCAUGG
3 If leader is long, subunits may form queue
GCCACCGAUG

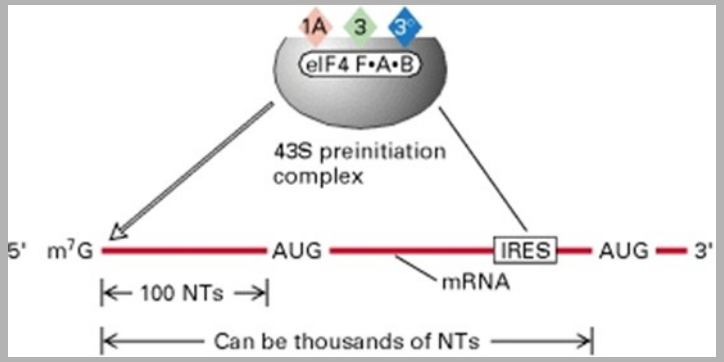

30S ribosomal subunit
Initiation factors
30S·IF1·IF3
IF2(GTP)·fMet-tRNA$_f$
+ mRNA
IF3
fMet
IF2 GTP
IF1
5′
AUG
mRNA
30S initiation complex
50S subunit + H_2O
IF1 + IF2, GDP + P_i
fMet
AUG
70S initiation complex

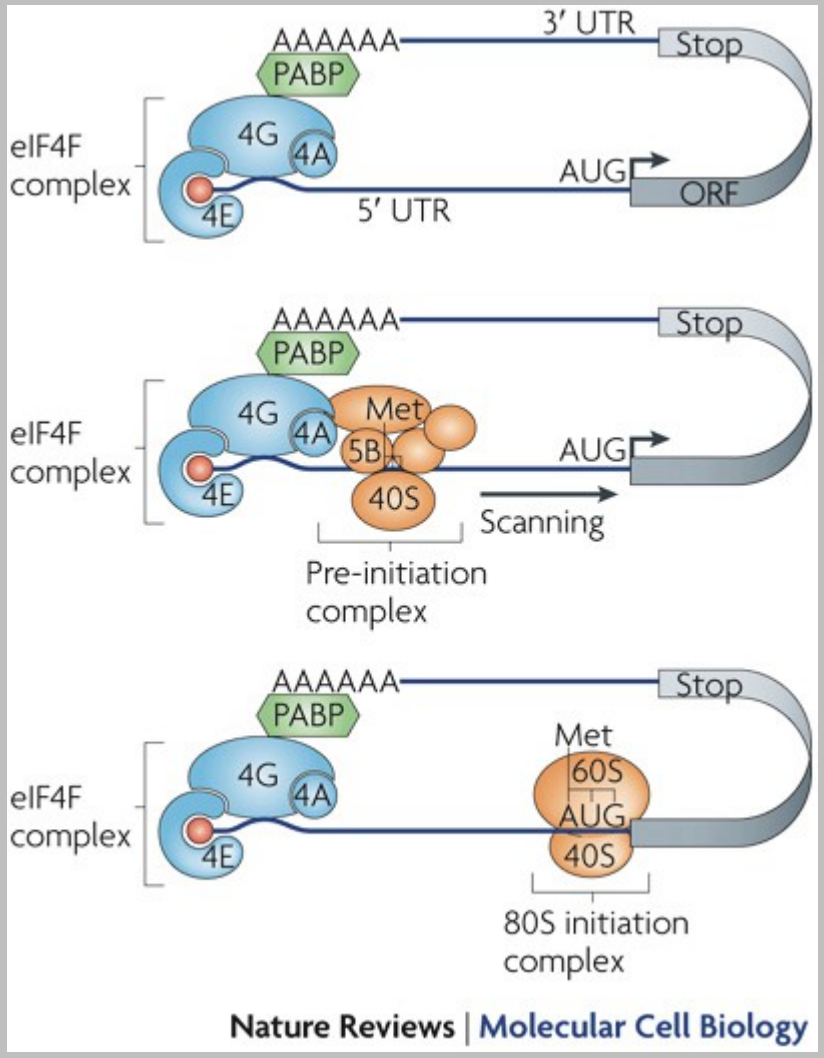

5′
Cap
AUG
mRNA
Initiation factors
+ GTP
Met-tRNA$_i$
40S subunit
fMet
AUG
40S subunit
with initation
components
n ATP
n ADP + P_i
fMet
AUG
60S subunit
Initiation factors
fMet
AUG
80S initiation complex

Sestavljanje prediniciacijskega kompleksa 43 S



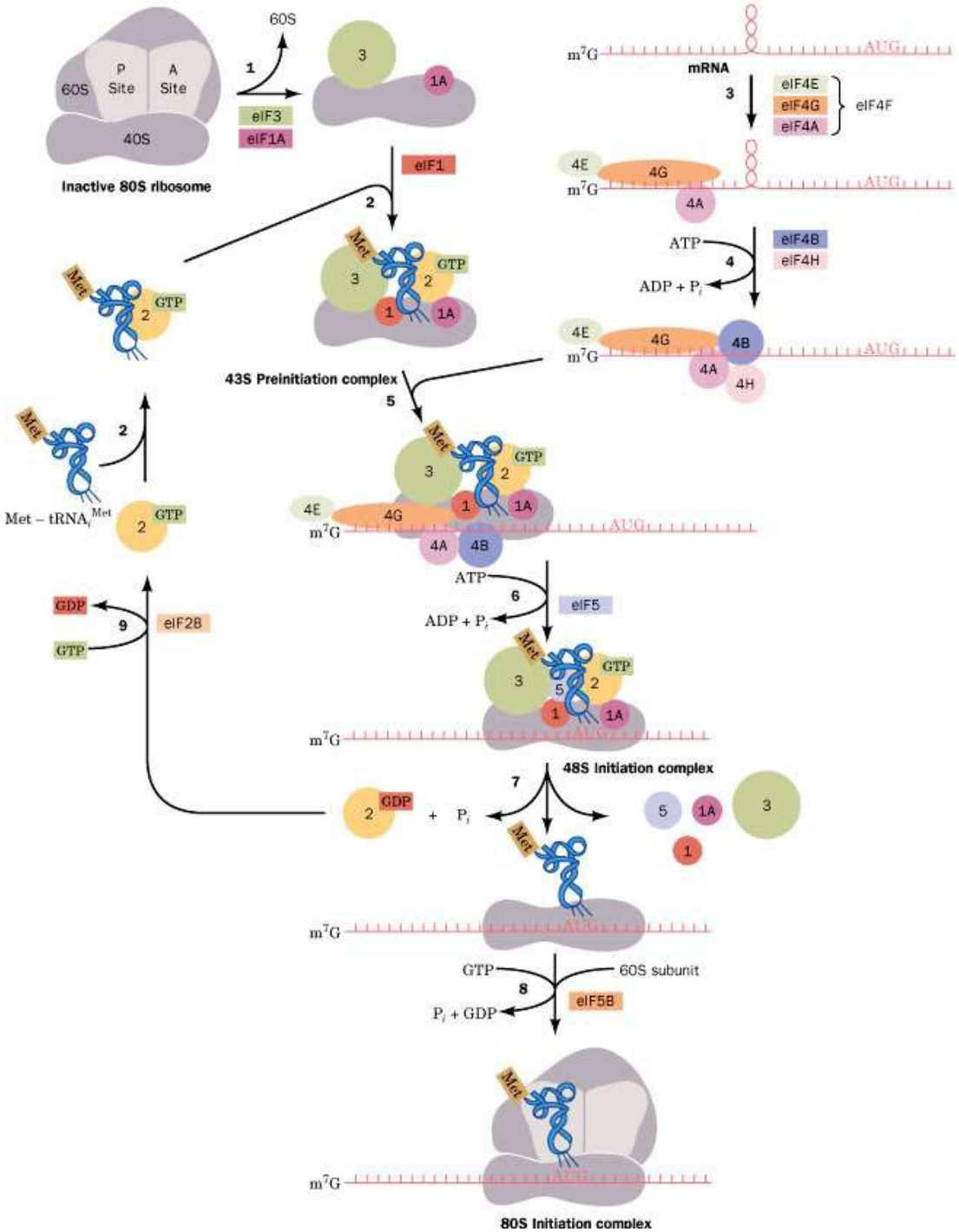
IRES = notranje vstopno mesto za ribosom
(internal ribosomal entry site)

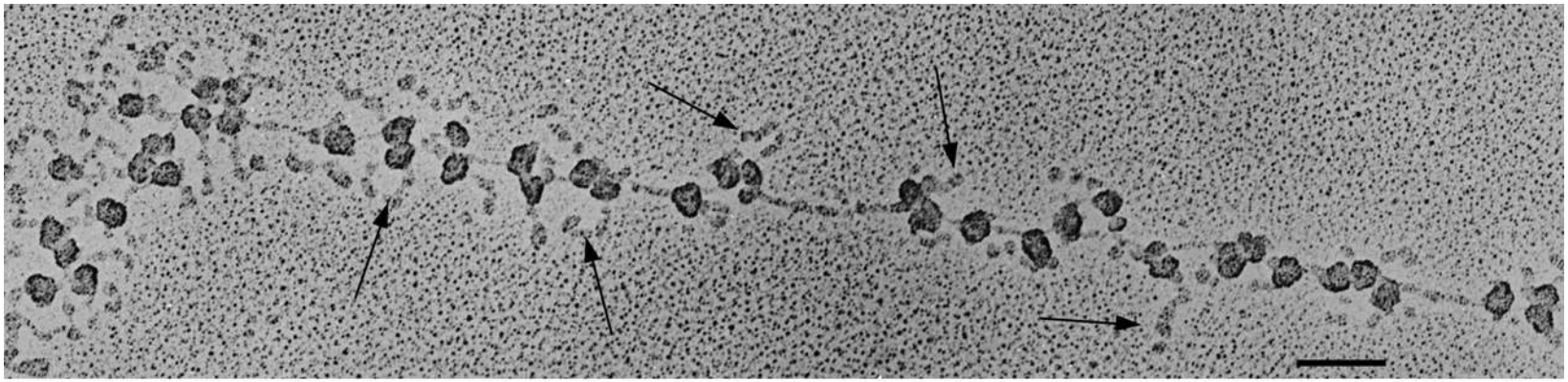




9, 971-980 (December 2008)

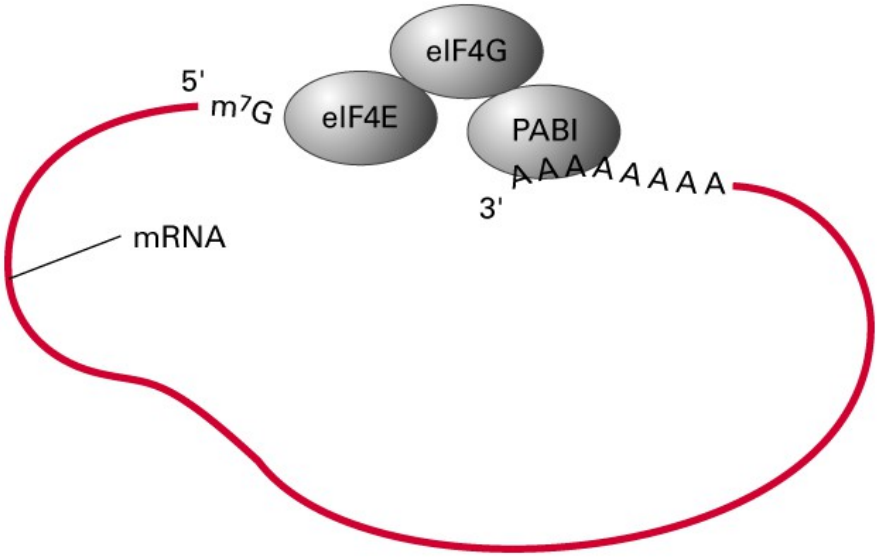
4E – vezava kape, 4A – RNA-helikaza, 4G – ogrodje, PABP – protein, ki se veže na poli-A

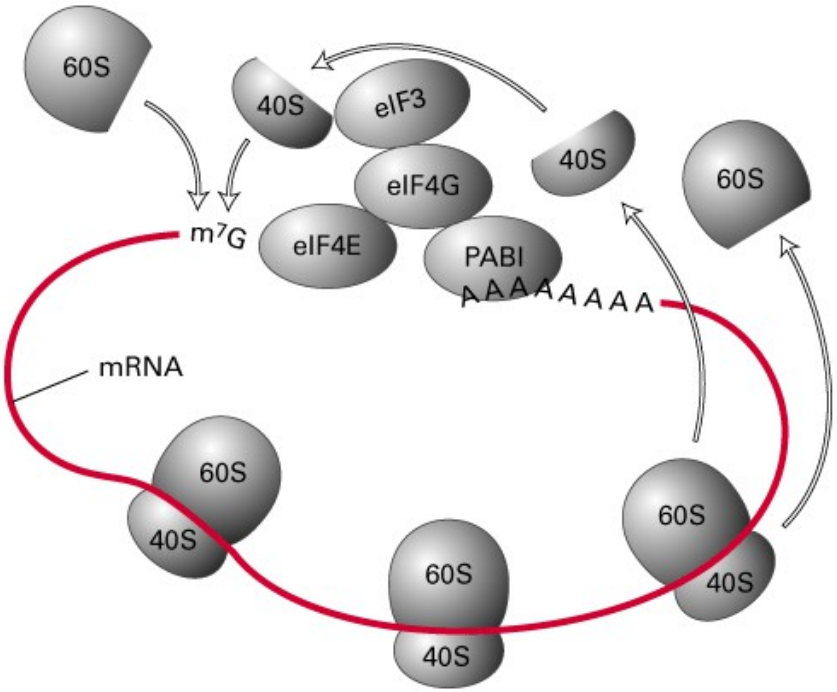

60S
P Site
A Site
40S
Inactive 80S ribosome
1
60S
3
1A
eIF3
eIF1A
eIF1
2
Met
GTP
3
2
1
1A
43S Preinitiation complex
m7G
AUG
mRNA
3
eIF4E
eIF4G
eIF4A
eIF4F
4E
4G
4A
ATP
ADP + Pi
4
eIF4B
eIF4H
4B
4H
5
Met – tRNAiMet
6
eIF5
48S Initiation complex
7
GDP
+ Pi
5
9
eIF2B
GTP
60S subunit
8
eIF5B
Pi + GDP
80S Initiation complex



Courtesy of Oscar L. Miller, Jr. and Steven L. McKnight, University of Virginia

Polisomi v svilnih žleznih celicah sviloprejke (elektronska mikroskopija).



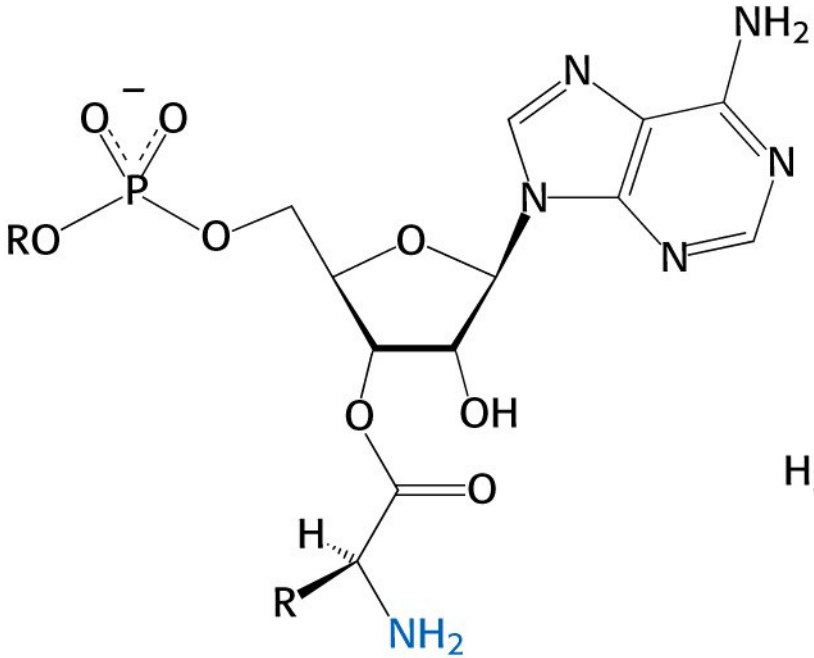


Protein PABI (vezavni protein za poli-A-rep I) skupaj z nekaterimi podenotami eIF4 poveže kapo in poli-A-rep in s tem cirkularizira mRNA. Ribosomi tako lažje vstopajo v naslednji cikel sinteze proteina.

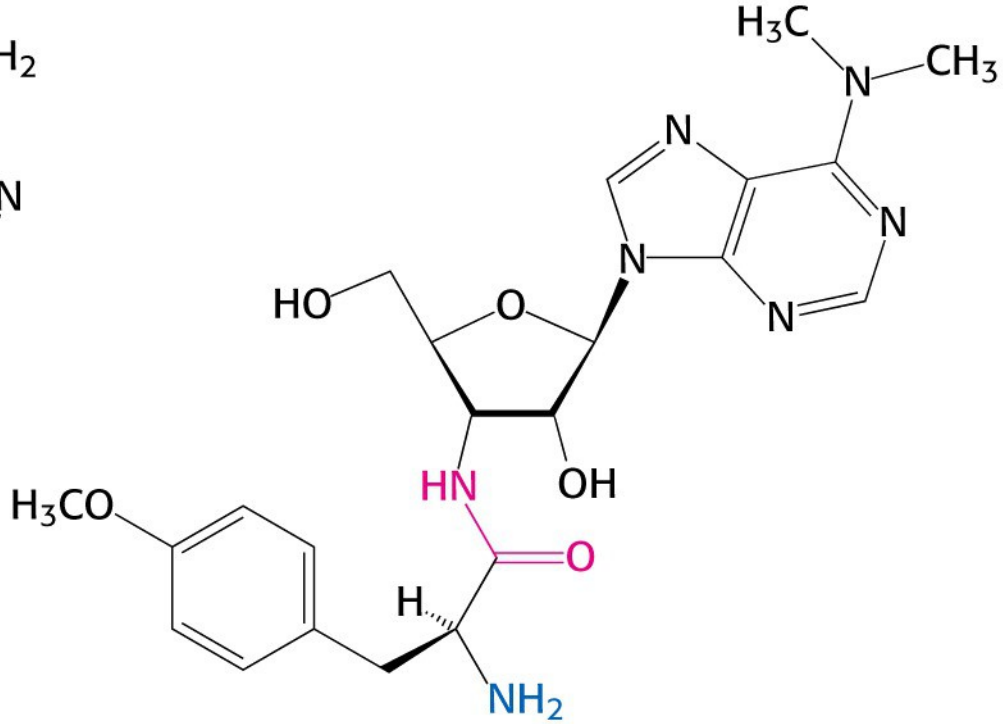
Lodish *et al*.: Molecular Cell Biology, 4. izdaja, slika 4-42

TABLE 29.4 Antibiotic inhibitors of protein synthesis

Antibiotic	Action
Streptomycin and other aminoglycosides	Inhibit initiation and cause misreading of mRNA (prokaryotes)
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl-tRNAs (prokaryotes)
Chloramphenicol	Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (prokaryotes)
Cycloheximide	Inhibits the peptidyl transferase activity of the 60S ribosomal subunit (eukaryotes)
Erythromycin	Binds to the 50S subunit and inhibits translocation (prokaryotes)
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA (prokaryotes and eukaryotes)

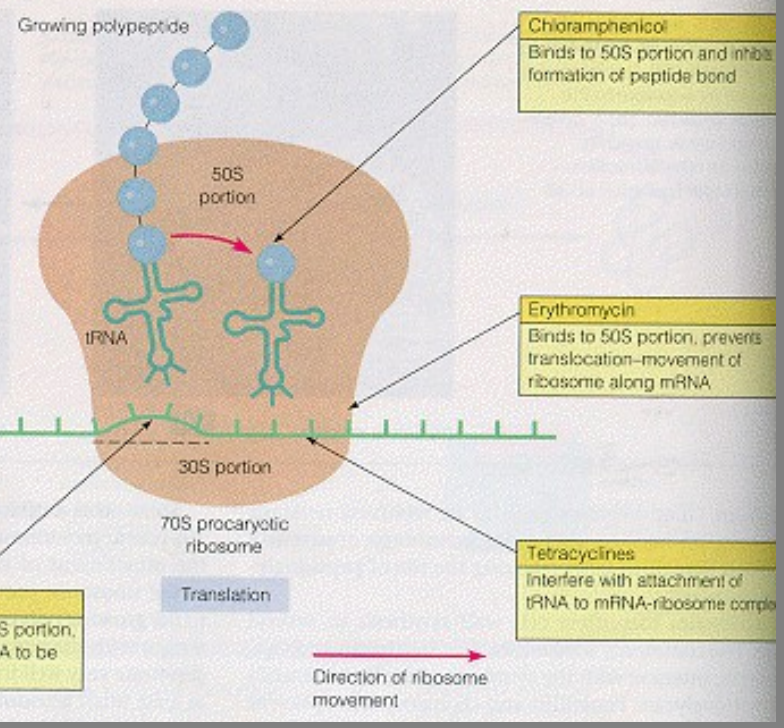


Aminoacyl-tRNA



Puromycin

FIGURE 20.4 Inhibition of protein synthesis by antibiotics. The black arrows indicate the specific points at which chloramphenicol, erythromycin, the tetracyclines, and streptomycin exert their activities.

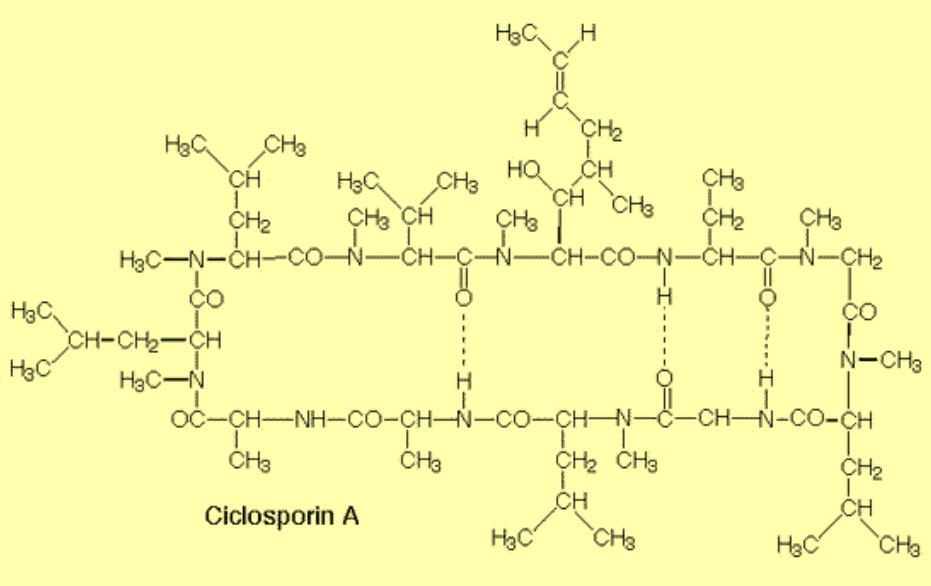


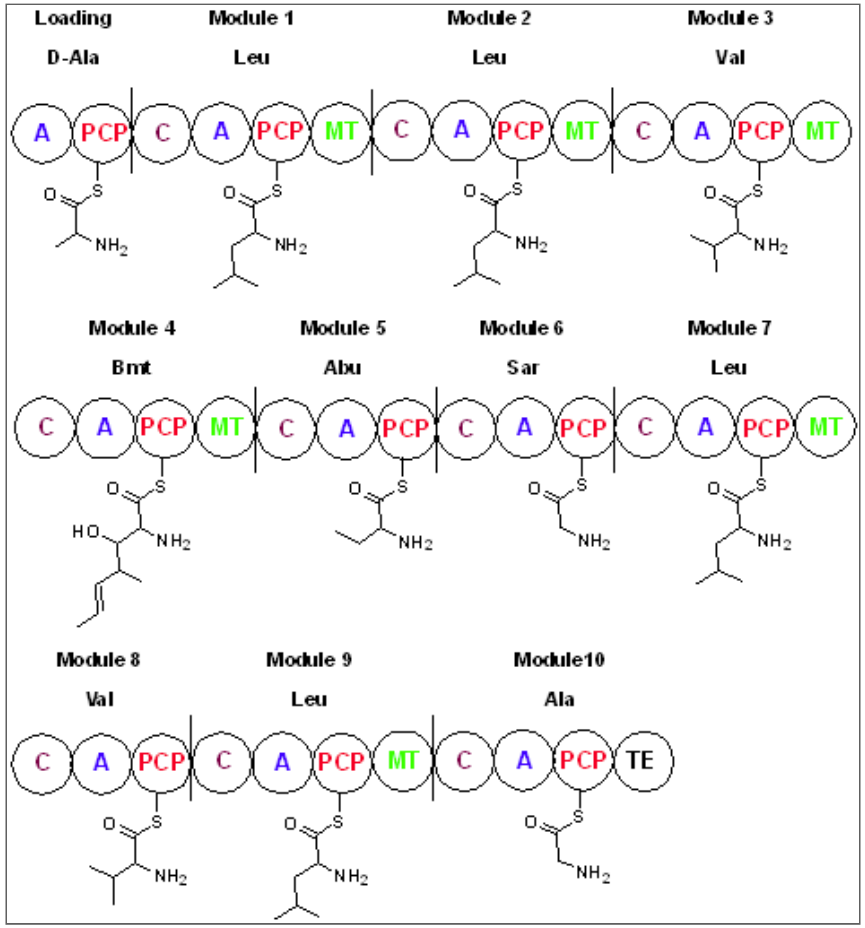
Neribosomska sinteza peptidov

- predvsem pri bakterijah in glivah
- predvsem za peptidne sekundarne metabolite
- peptidi so pogosto ciklični
- sintezo katalizirajo peptid-sintaze, ki delujejo neodvisno od RNA (NRPS)
- encimi so specifični za vsak posamezen peptid
- možna je vgradnja modificiranih aminokislin in D-aminokislin
- geni za NPRS enega peptida so pogosto organizirani kot en operon oz. grozd
- znanih je ~500 monomerov, ki jih NRPS lahko vgrajujejo
- razen aminokislin NRPS lahko vključujejo tudi mašč. kisline in alfa-hidroksi kisline

Ciklosporin: zgradba in biosinteza

Ciklosporin-sintetaza: 4 domene, 1,6 MDa
- organizirana po modulih
- vsak modul sestavlja več domen, povezanih z linkerji
- sinteza se zaključi s ciklizacijo in sprostitvijo produkta





Bmt = butenil-metil-treonin, Abu = L-alfa-aminomaslena kislina, Sar = sarkozin

Mehanizem delovanja:

1. aktivacija monomera (domena A)
2. rast verige (domena PCP [peptidyl carrier protein])
3. kondenzacija aminokislin (domena C)
4. sprostitev produkta (domena TE [tioesteraza])

Struktura modulov:

- sestavljeni iz več katalitičnih domen
- 3 domene so esencialne: A, PCP in C
- domena TE je samo v terminacijskem modulu
- znane so le strukture posameznih modulov

Energijske zahteve za ribosomsko sintezo proteina, ki ga sestavlja N aminokislinskih ostankov:

2N	ATP za vezavo AK na tRNA (ATP -> AMP + PPi -> AMP + 2Pi)
1	GTP za iniciacijo translacije (IF2)
N-1	GTP za usmerjanje aa-tRNA v ribosom za N-1 pept. vezi (EF-Tu)
N-1	GTP za N-1 translokacijskih korakov (EF-G)
1	GTP za terminacijo (RF-3)
=========	
4N	

Za vgradnjo vsake aminokisline je potrebna cepitev 4 visokoenergijskih fosfatnih vezi. Vsaka taka cepitev sprosti ~40 kJ/mol → skupaj 160 kJ/mol za eno peptidno vez.

Če reakcija ne bi bila katalizirana, bi bil vložek energije samo ~20 kJ/mol.

Zakaj je sinteza peptidov na ribosomih tako energetsko potratna?
Zato, da lahko nastane natančno definirano zaporedje aminokislin.

Hitrost sinteze: 15 AK/s, usklajena s hitrostjo sinteze mRNA (45 nt/s).