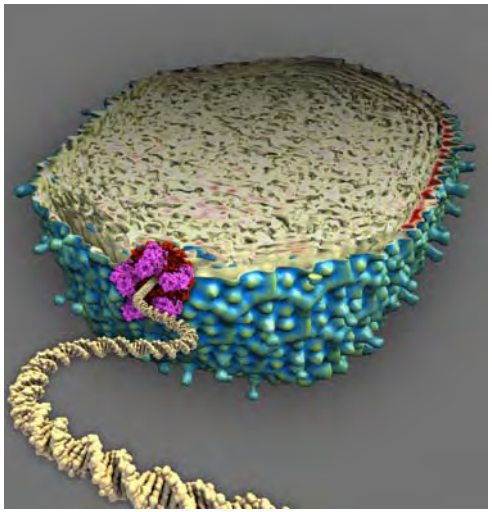
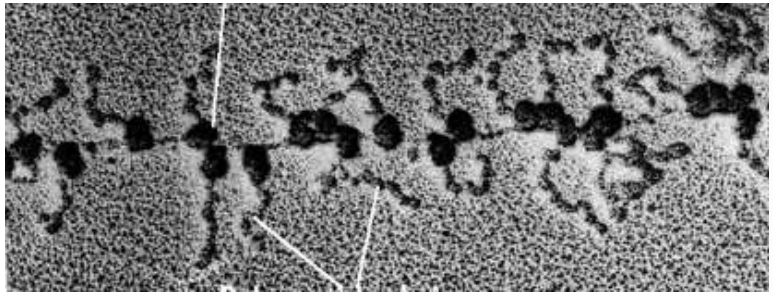
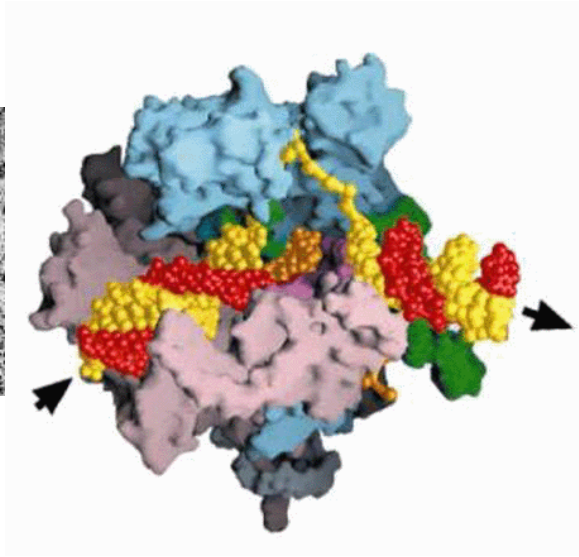
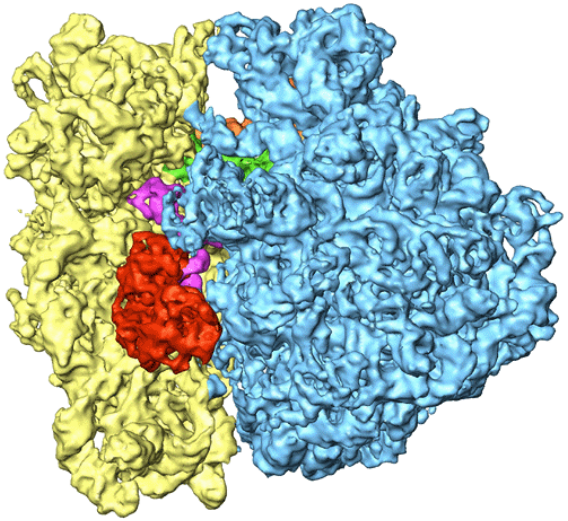
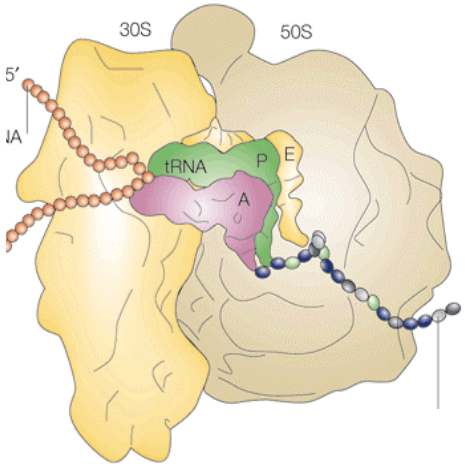
Biomolekulární stroje – výzva pro současnou (a budoucí) chemii a fyziku.

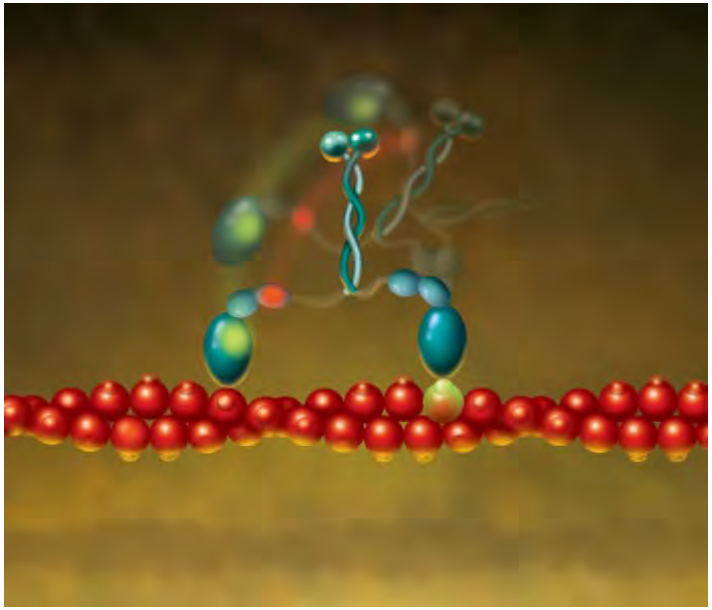




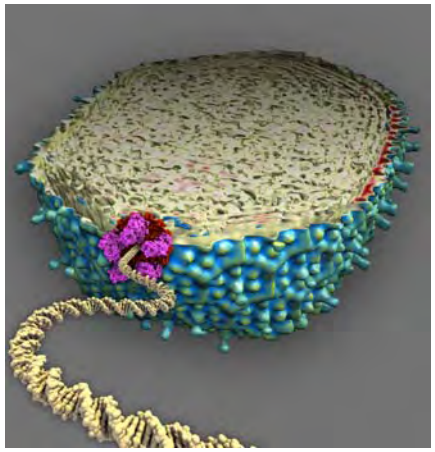


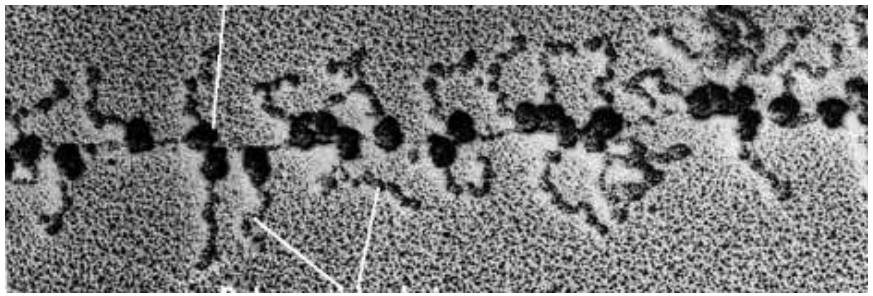


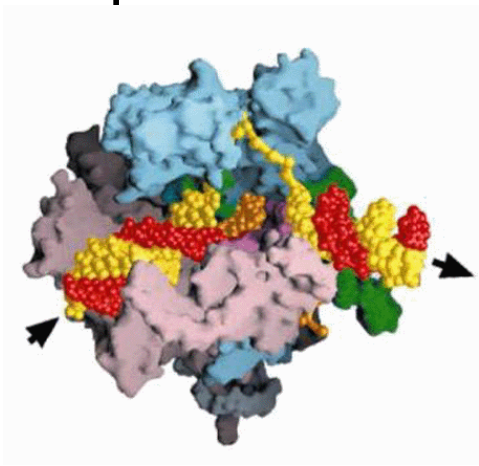


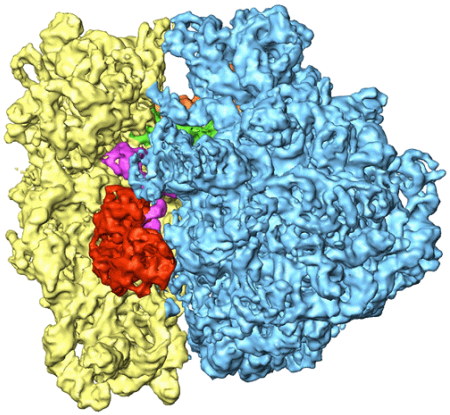


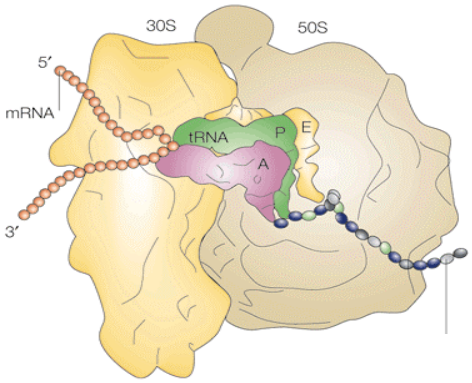
V buňkách fungují stovky různých molekulárních strojů a motorů, které provádějí klíčové funkce jako jsou svalové stahy, pohyb chromozomů během buněčného dělení, „nabíjení“ nervových buněk, replikace DNA, transkripce DNA, syntéza bílkovin…...

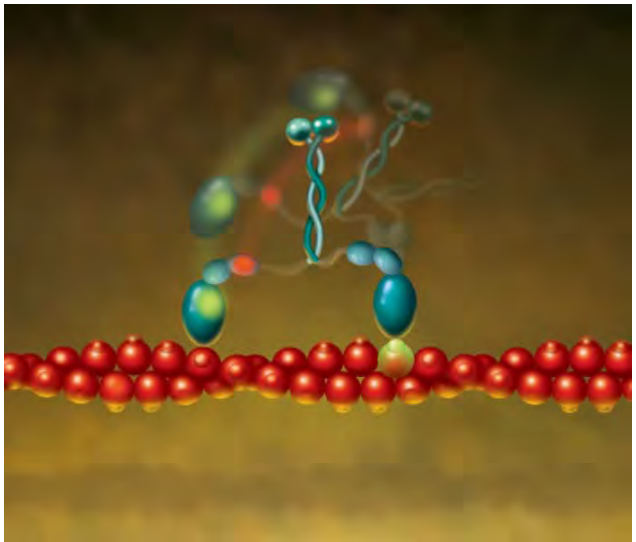




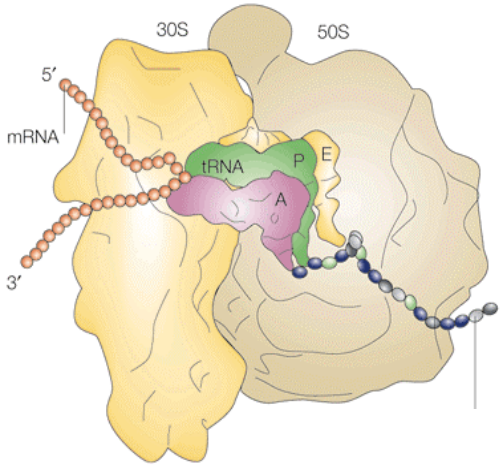








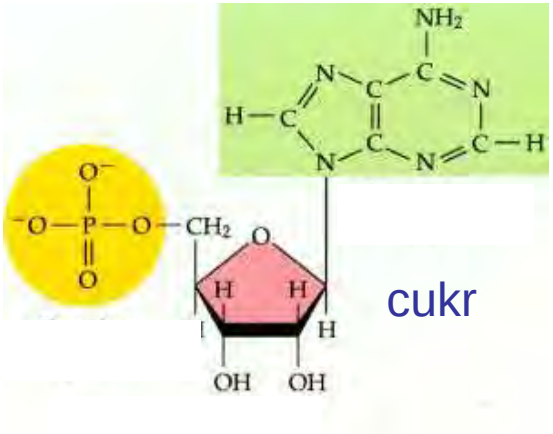
Ribosom – továrna na bílkoviny



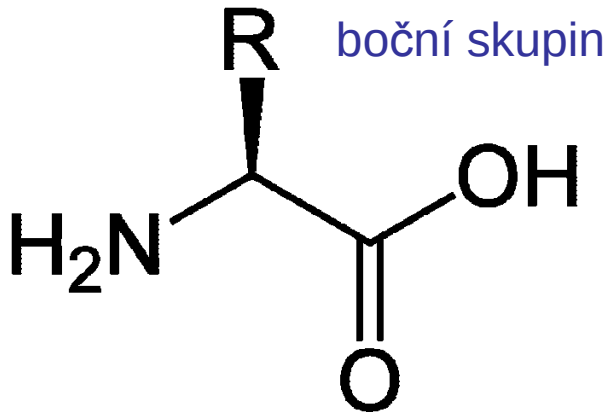
- Co bylo zjištěno po roce 1995
- Co nevíme a hned tak vědět nebudeme.
- Obecné principy biomolekulárních strojů.

Omluva……

- Nukleové kyseliny (DNA a RNA) mají jako základní stavební jednotku nukleotidy.
- DNA má cukr deoxyribózu, RNA ribózu



- Bílkoviny (proteiny) jsou tvořeny aminokyselinami.



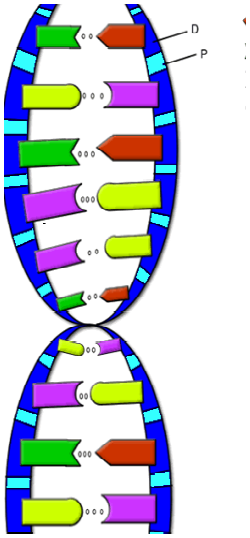
Genetická informace je kódovaná v molekule DNA (v genomu).

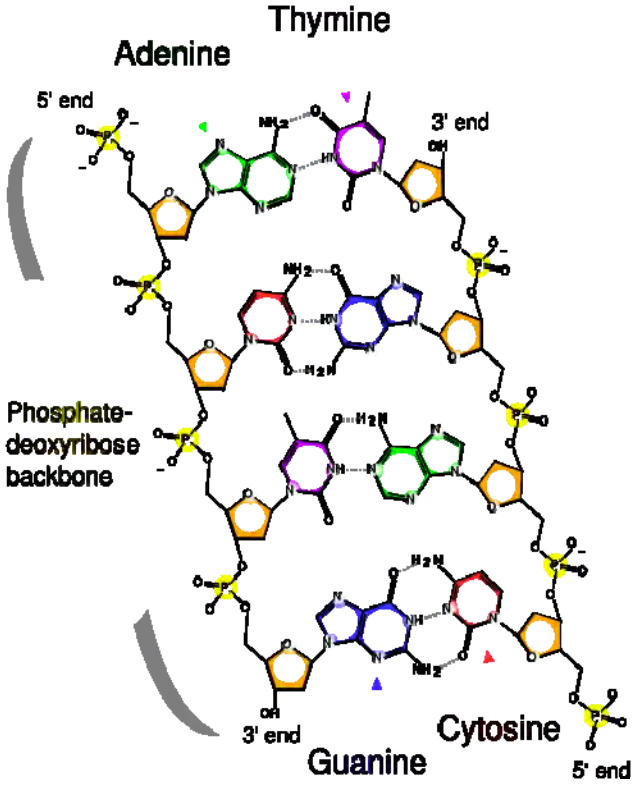
DNA má 4 písmenka, A, C, G a T.

Tři po sobě jdoucí písmenka (kodon, triplet) kódují jednu aminokyselinu nebo interpunkční znaménko.

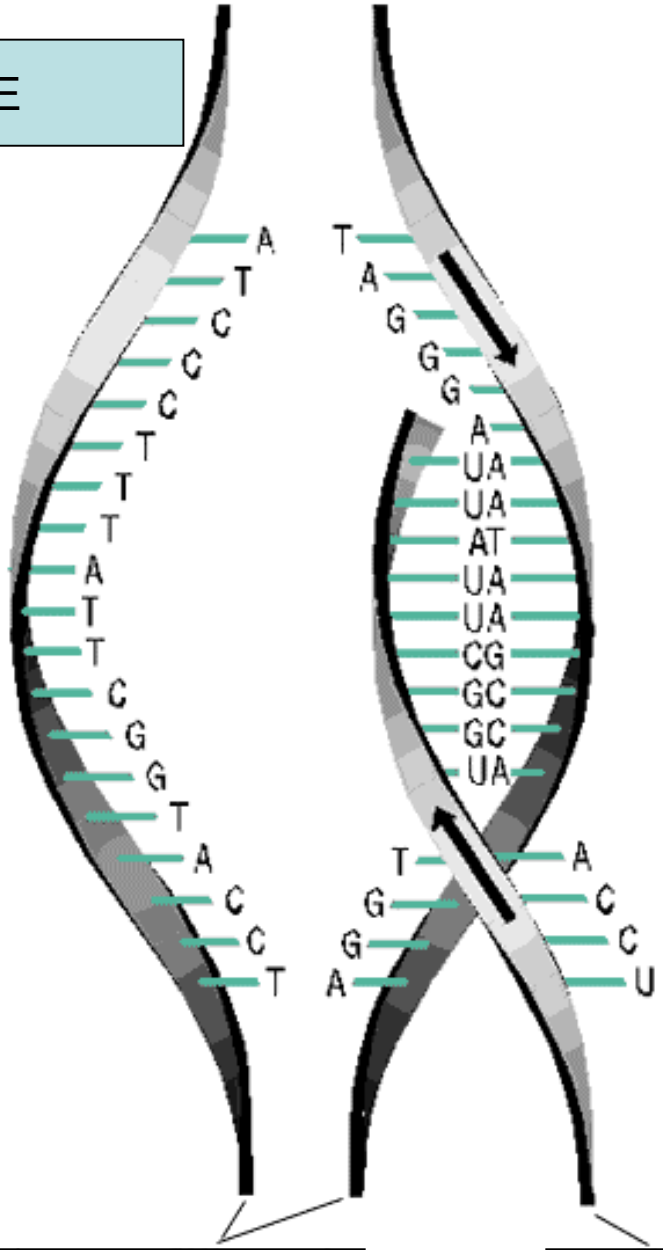
$4^3 = 64$, aminokyselin je 20, genetický kód je degenerovaný.

Genetická informace se přepisuje z DNA do messenger RNA (transkripce).





TRANSKRIPCE



Komplementární DNA řetězce

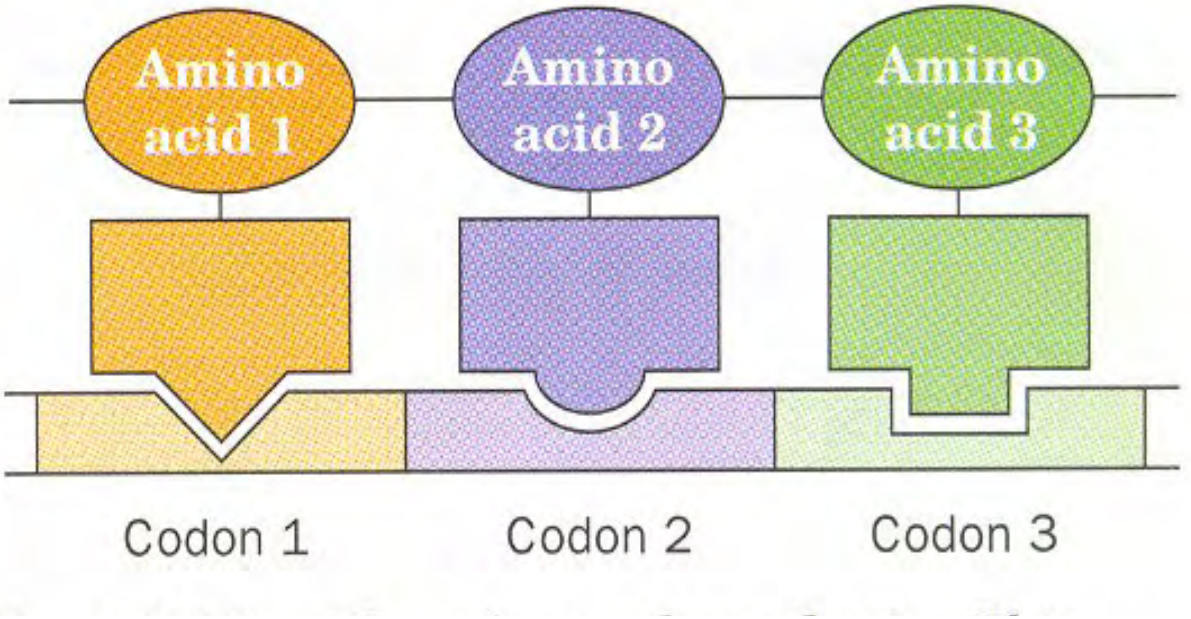
RNA řetězec

Převod mezi světem nukleových kyselin a bílkovin zajišťují molekuly – adaptéry: transferové RNA (tRNA)

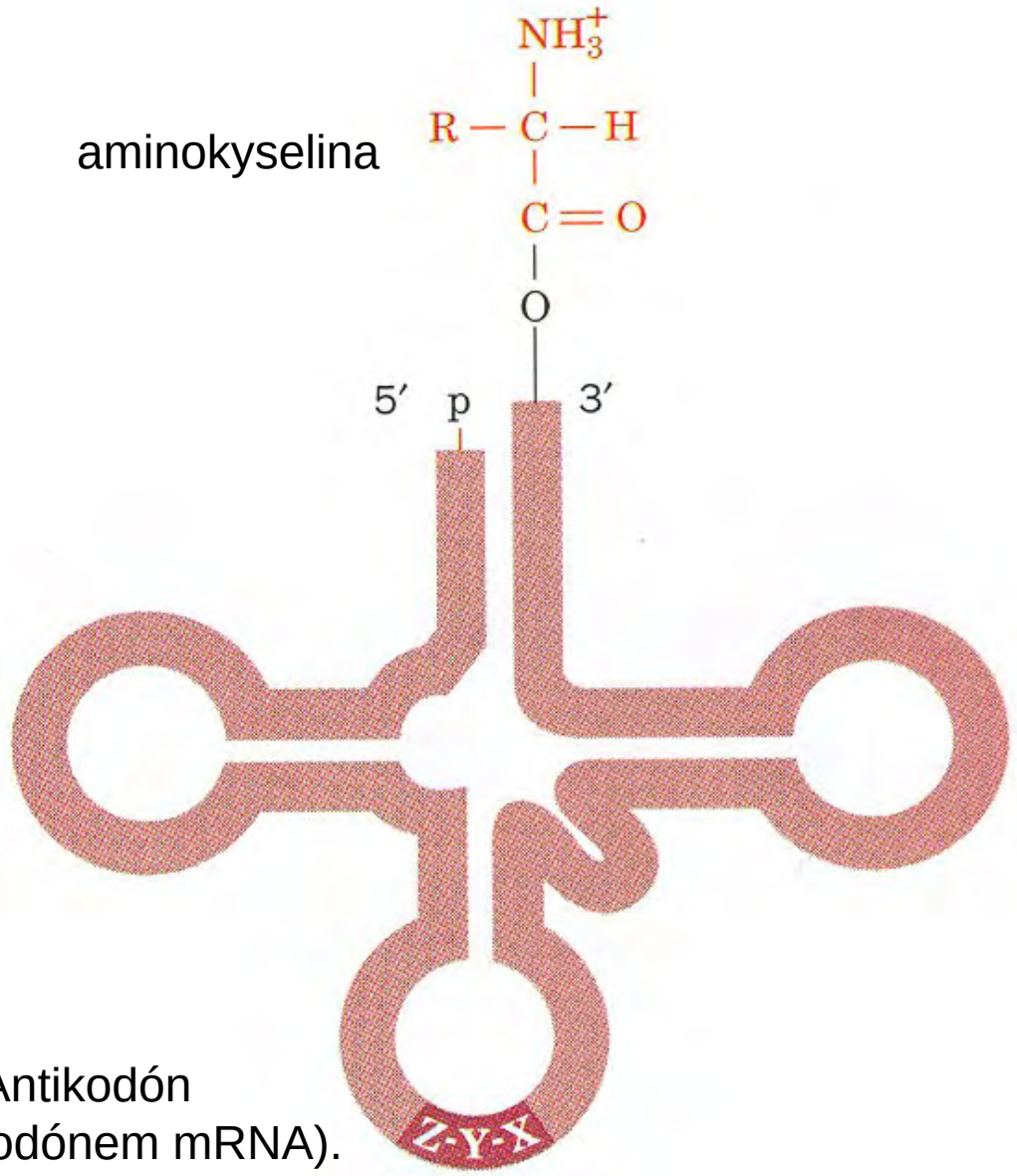
Bílkovina (aminokyseliny)

tRNA

mRNA

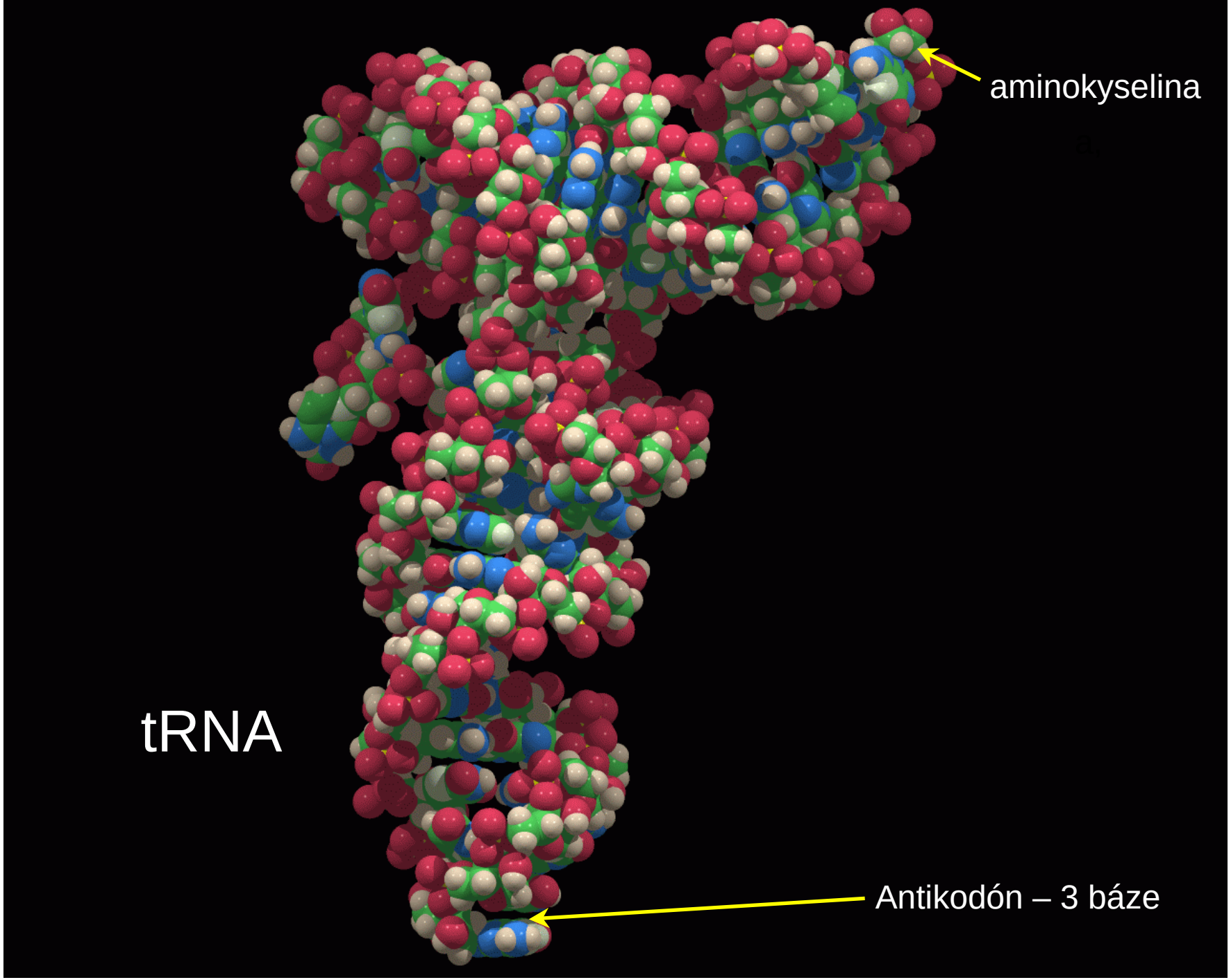


aminokyselina

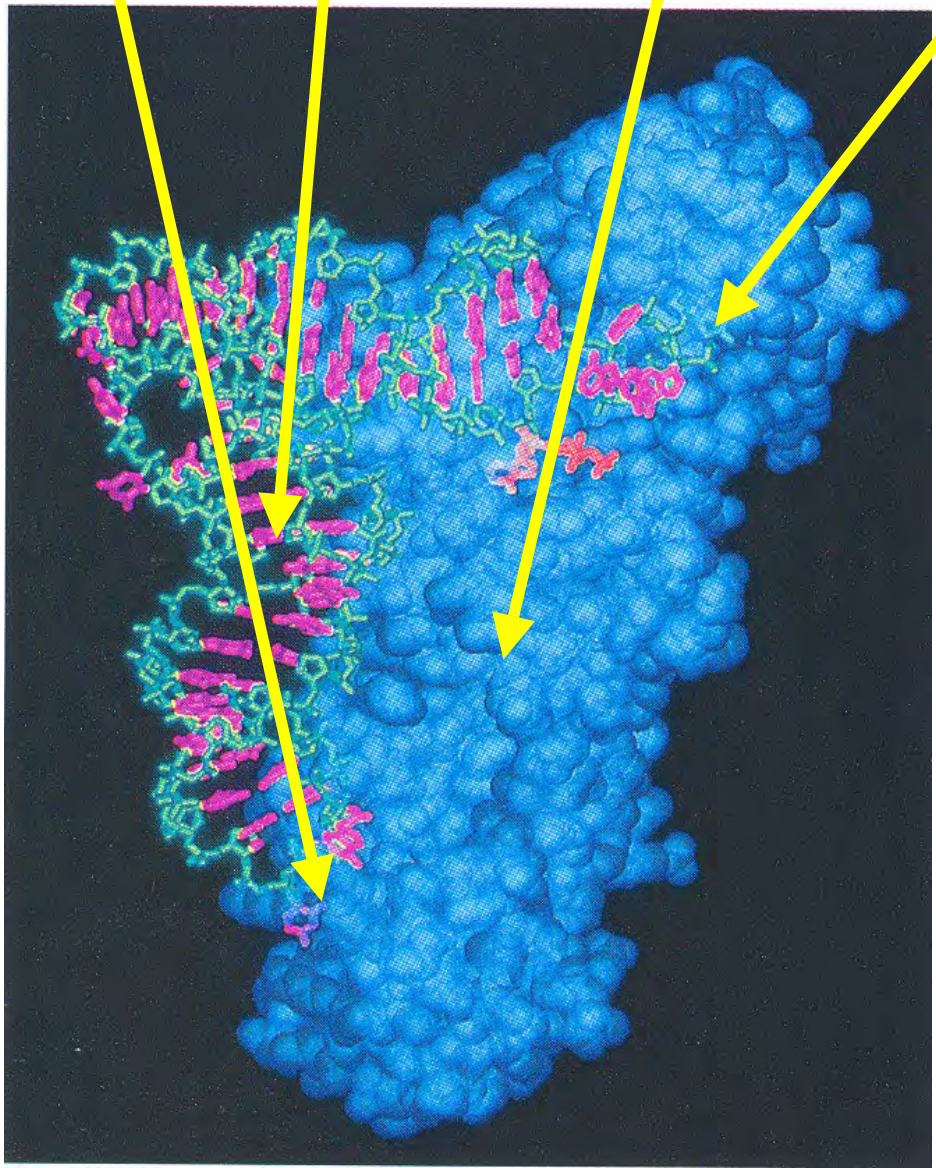


tRNA

Antikodón
(páruje se s kodónem mRNA).


aminokyselina
tRNA
Antikodón – 3 báze

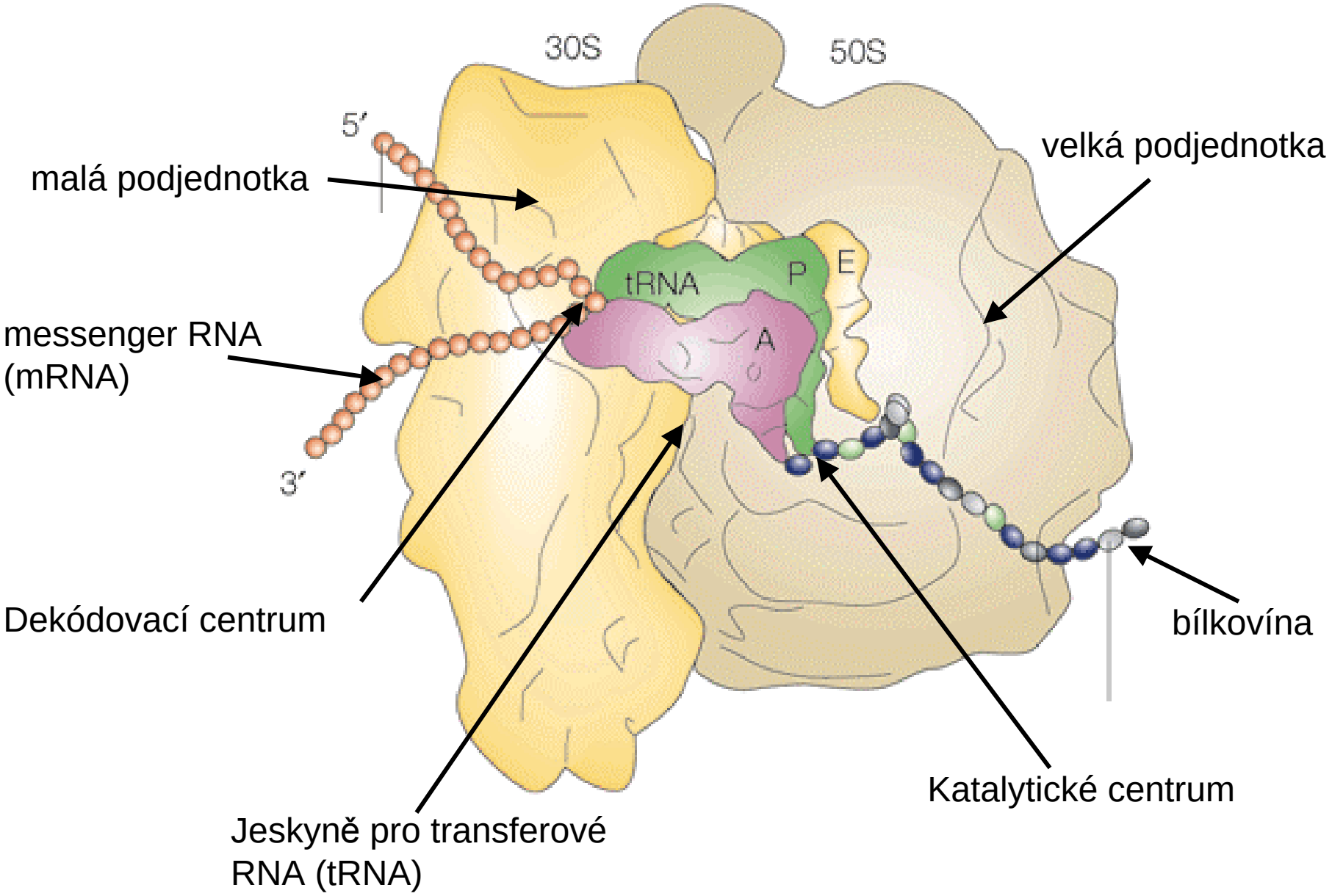
V buňce jsou přítomny vždy jen **tRNA**, kde je připevněná **správná aminokyselina** odpovídající **antikodónu**…
(o což se starají bílkoviny **amino-acyl syntetázy**.)



Primární genetická informace se přepisuje do mRNA. tRNA (adaptor) přiřadí správnému kodonu mRNA správnou aminokyselinu.

Syntézu bílkovin dle sekvence mRNA zabezpečují obří molekulární továrny zvané ribosomy.

Velikost ~250 A = 25 nm = 1000 x menší než šířka vlasu



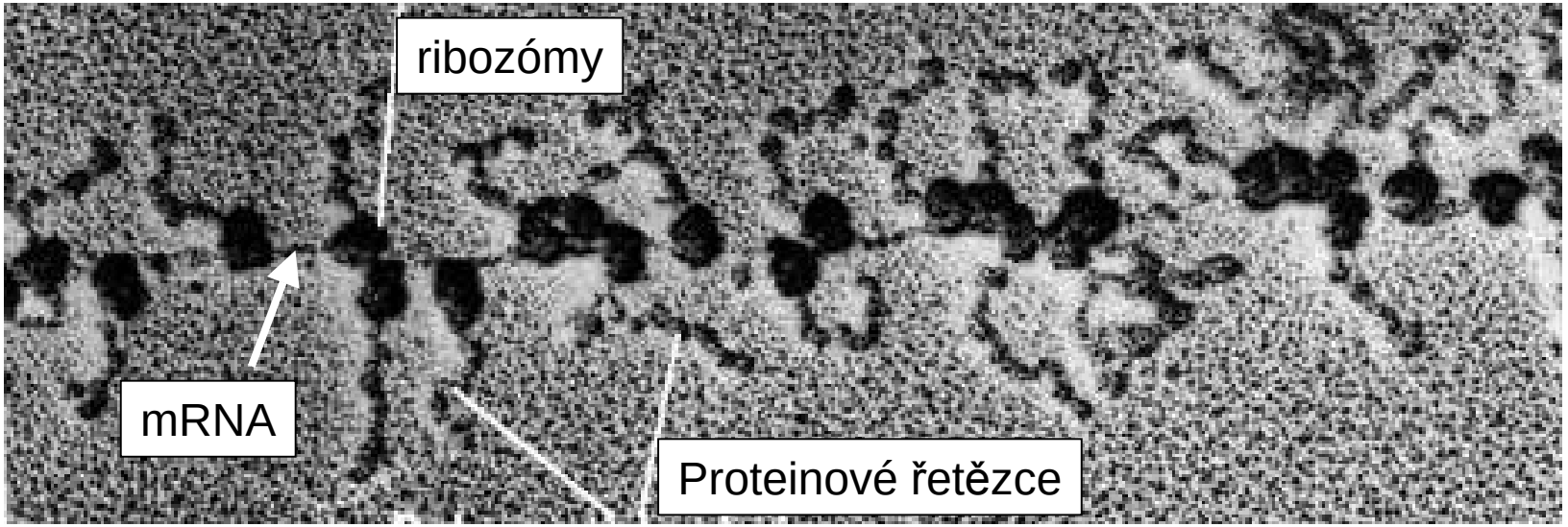
Ribosomy jsou obří částice tvořené RNA a desítkami bílkovinami v poměru 2:1

Přesnost ribosomu je ~10^{-4}, rychlost několik desítek aminokyselin za 1 sekundu

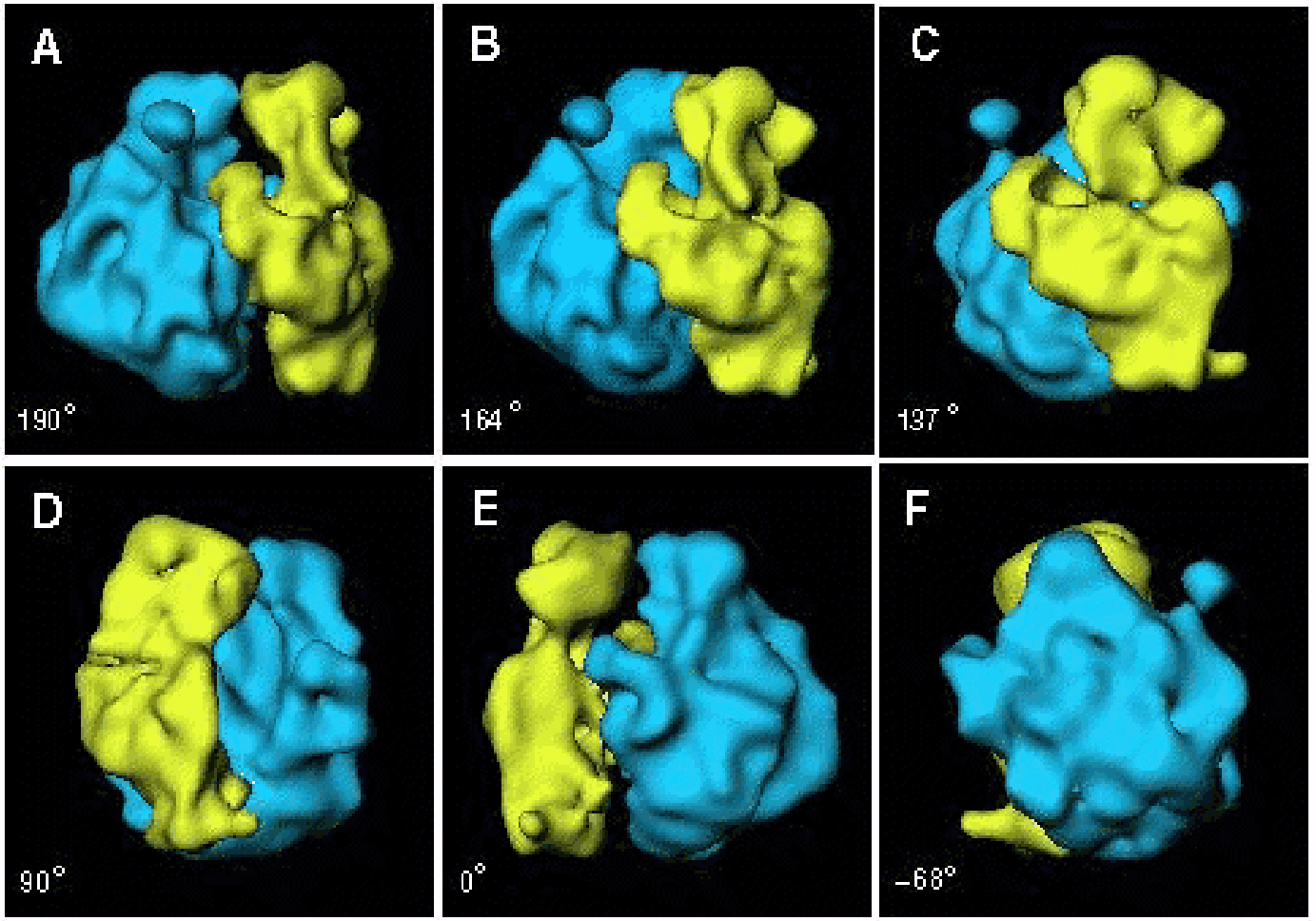
80% hmotnosti RNA v E. coli je ribosomální RNA, 20 000 ribosomů v buňce……

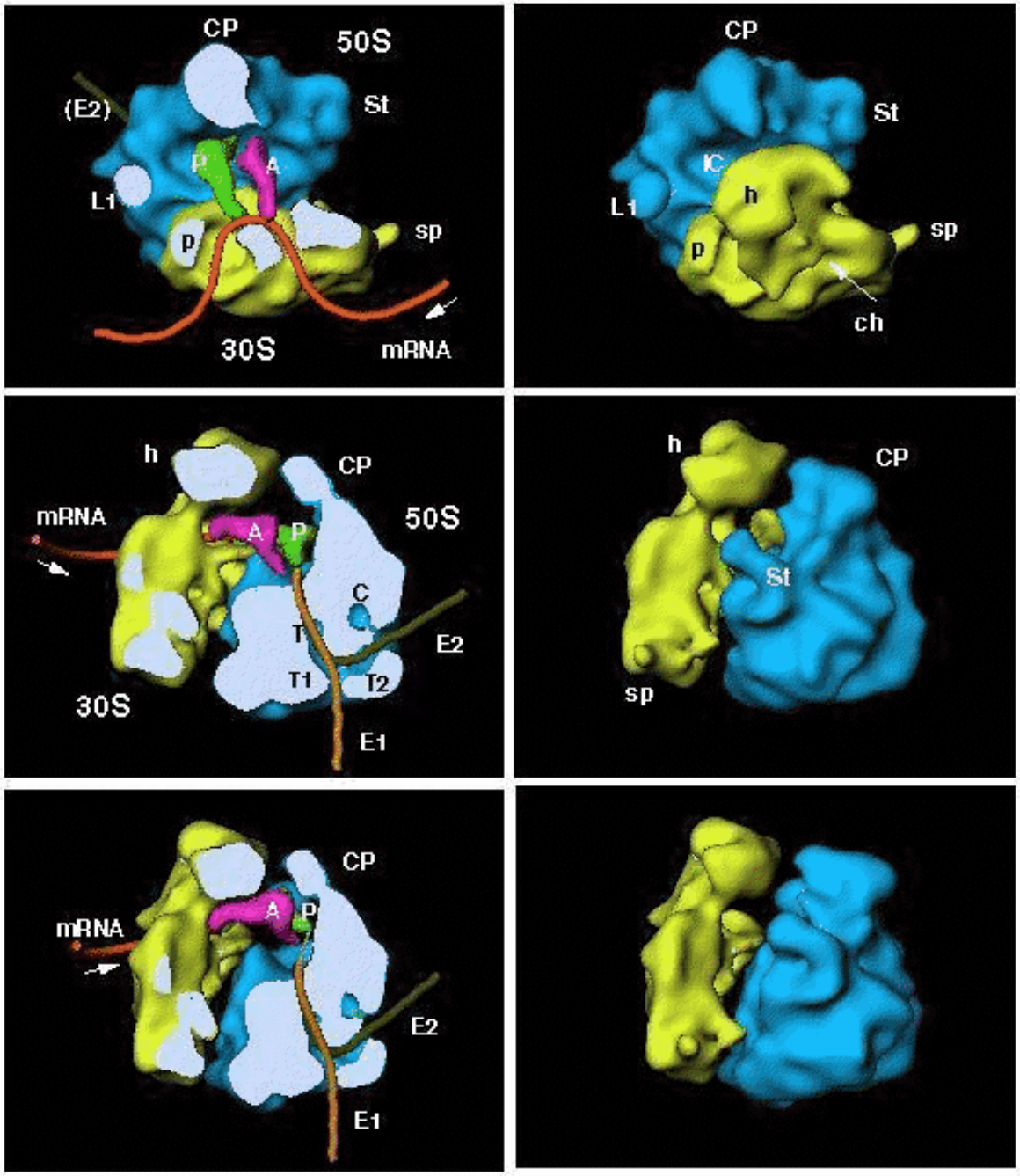
Ribosomy byly kdysi považovány za buněčný odpad…..

Translace (syntéza bílkovin ribosomy) u prokaryotu

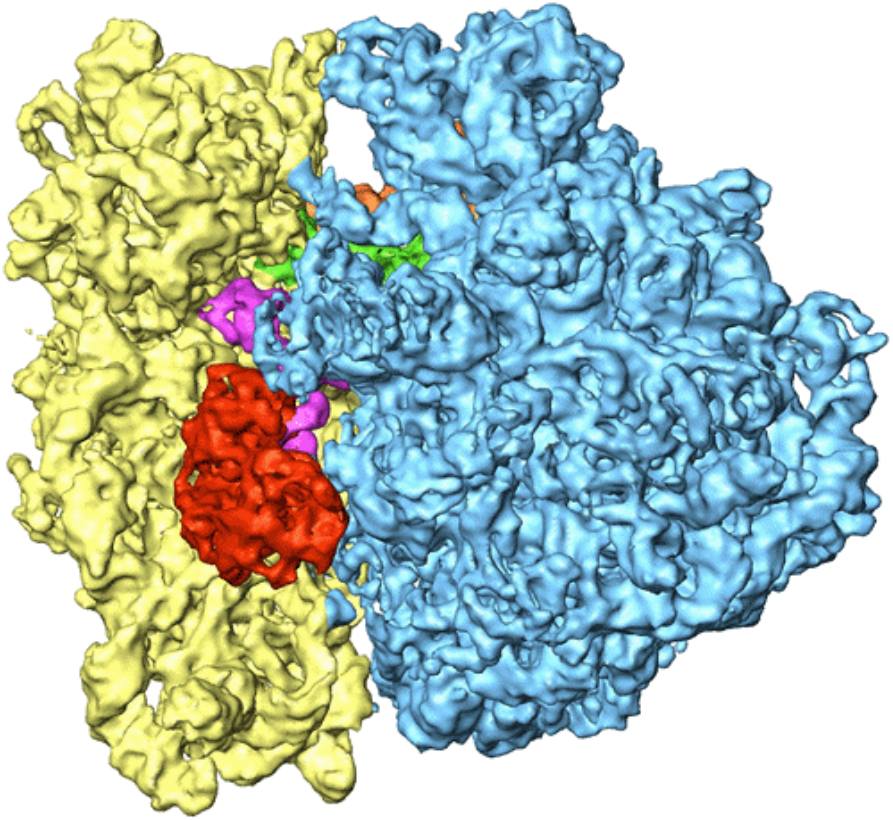


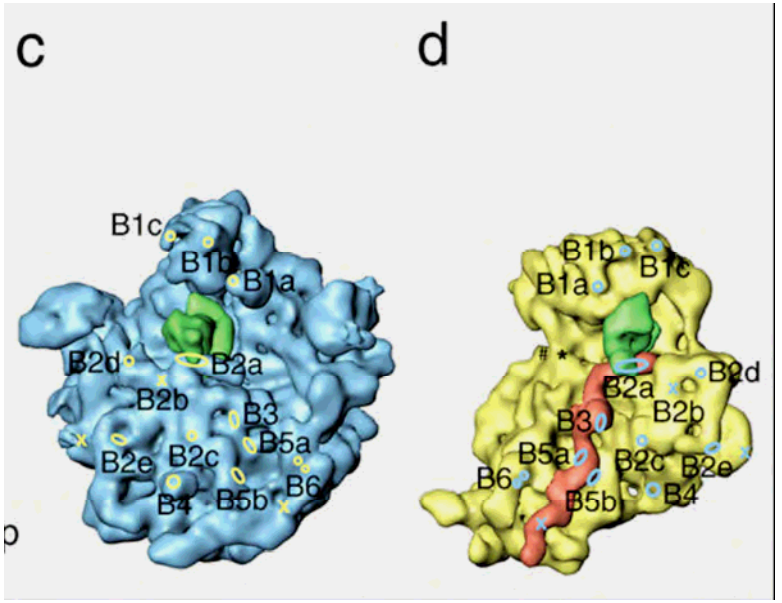
Cryoelektronová mikroskopie – kolem r. 1995



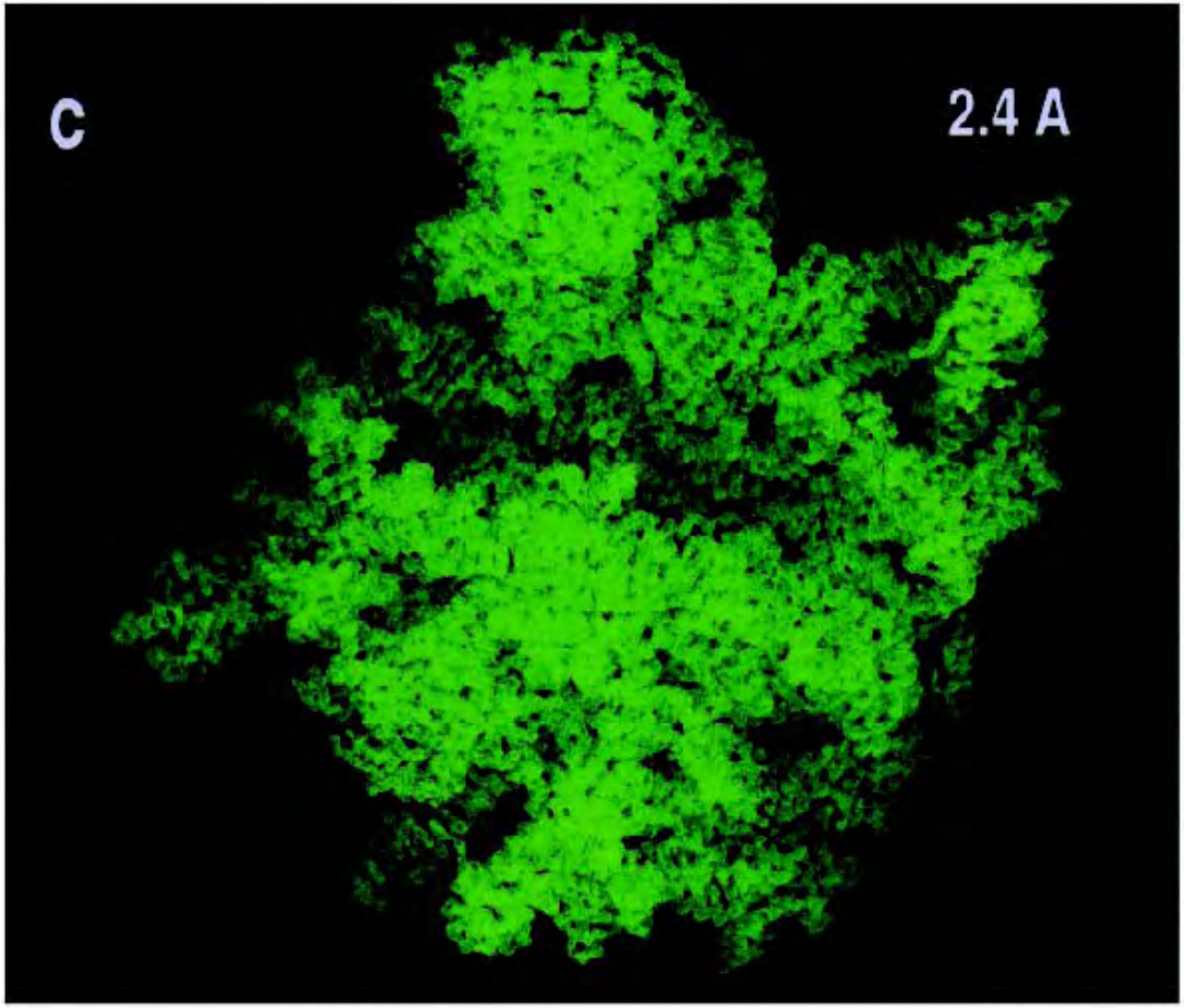

CP
50S
(E2)
St
P
A
L1
p
sp
30S
mRNA
CP
St
IC
h
L1
p
sp
ch
h
CP
mRNA
A
P
50S
C
T
E2
T1
T2
30S
E1
h
CP
St
sp
CP
mRNA
A
P
E2
E1

Cryoelektronová mikroskopie po r. 2000

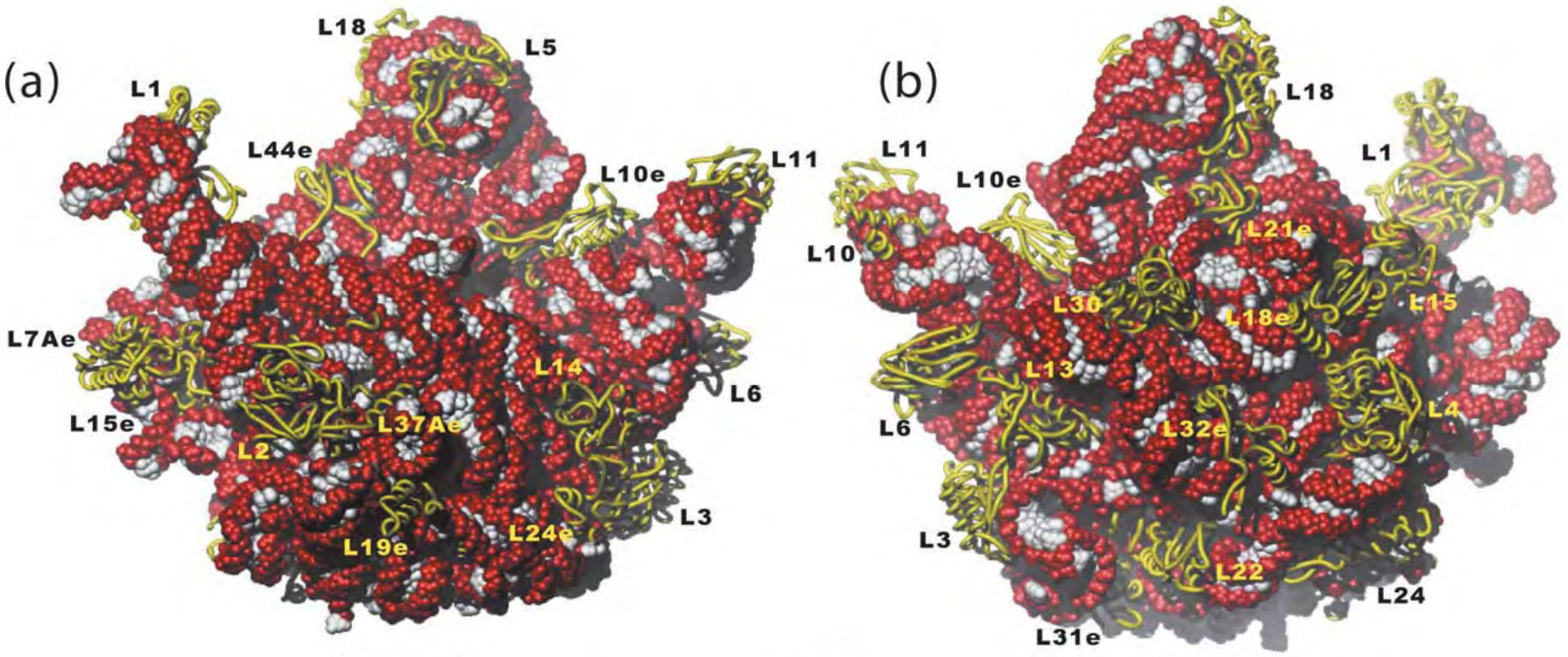




X-ray krystalografie - 2000



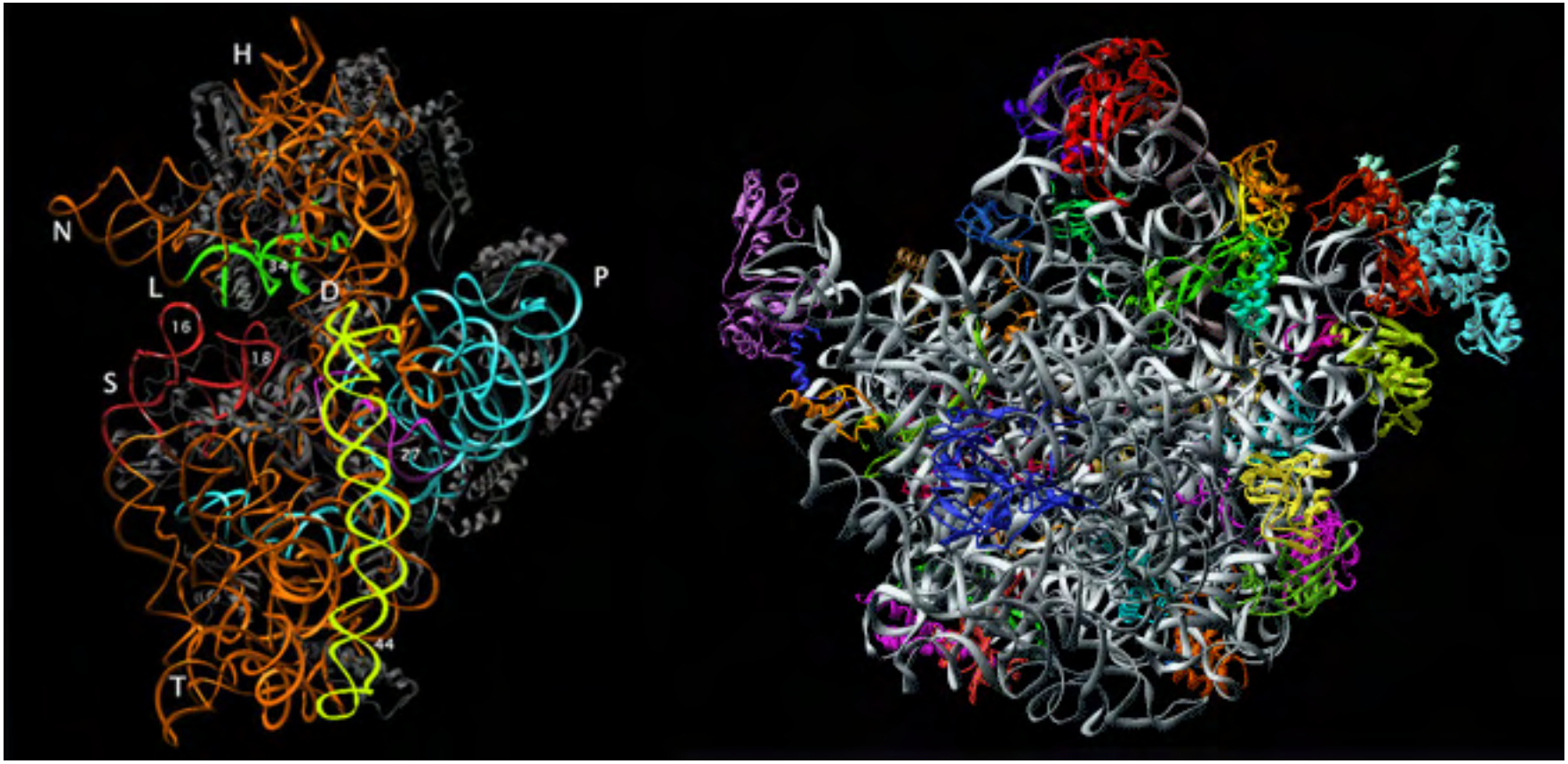
Atomární krystalografická struktura velké podjednotky.
Červená + šedá – RNA – přes 3000 nukleotidů
Žluté – přes 30 bílkovin



pohled zepředu

pohled zezadu

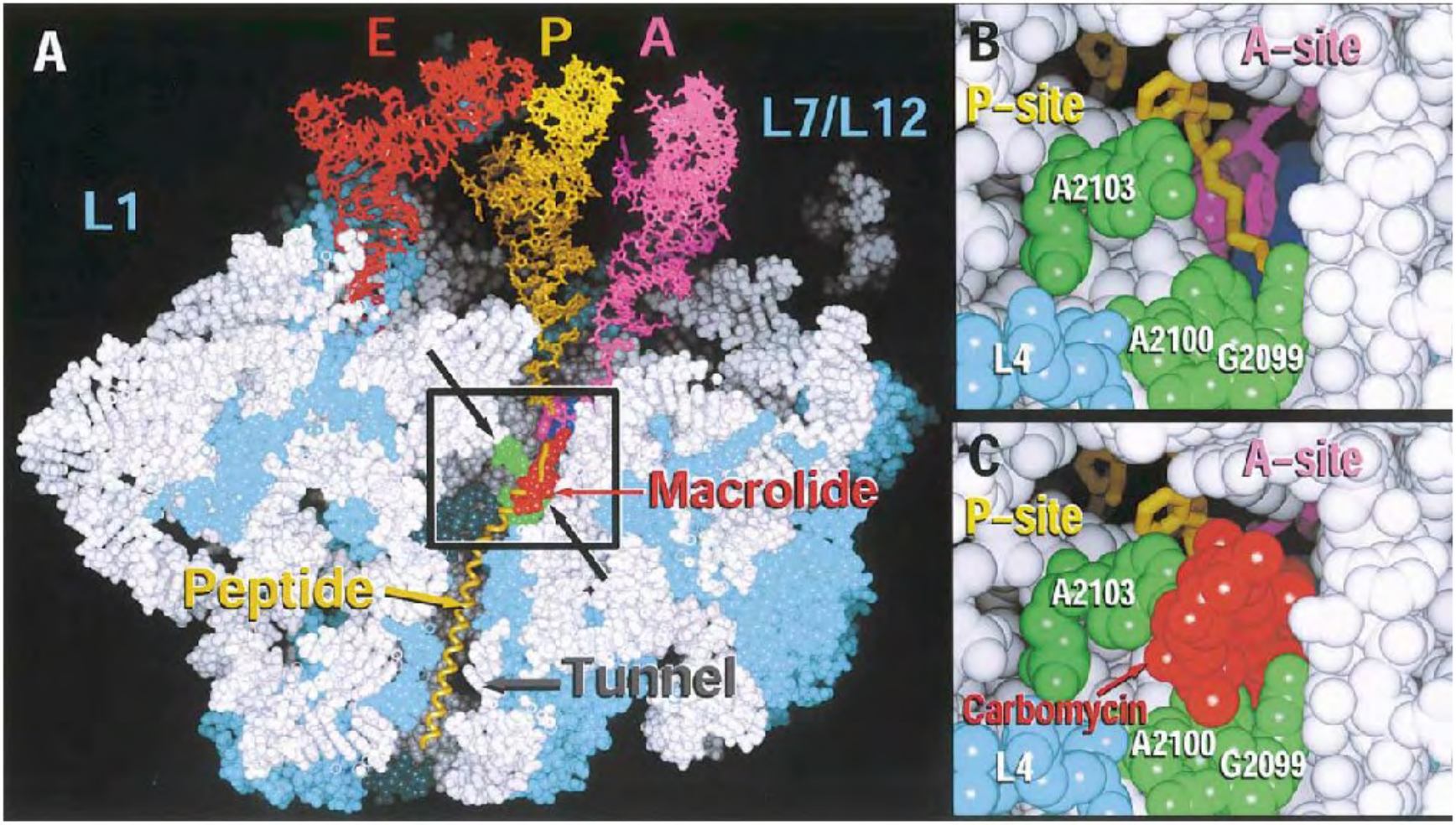
Atomární struktura malé (vlevo) a velké podjednotky.



Co se zjistilo:

- Ribosom je obří katalytická RNA doplněná během evoluce o desítky bílkovin.
- Všechna hlavní funkční centra ribosomu drží pevně v rukou stále RNA.
- Ribosom je vystavěn na modulárním principu (LEGO) a jeho RNA často užívá universální stavební bloky a interakce. Extrémně propracovaná stavebnice.

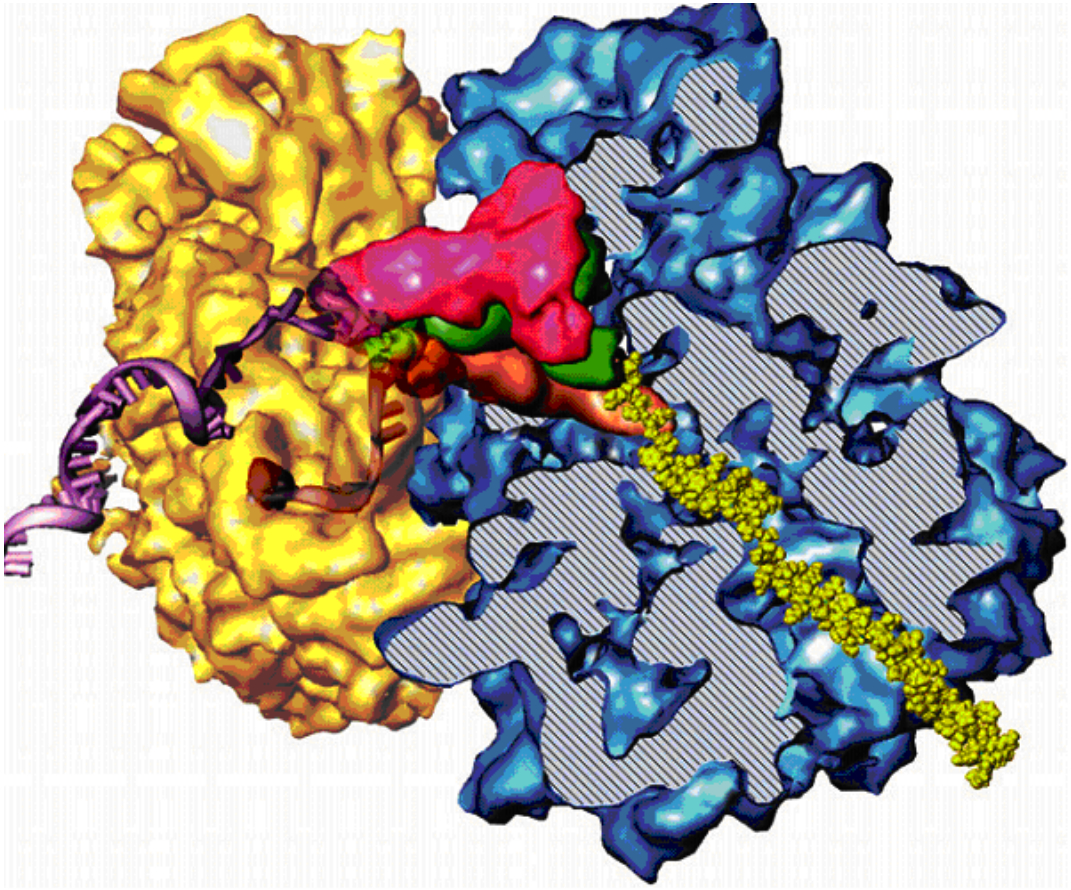
Antibiotika blokují výstupní tunel



Biomolekulární stroje jsou:

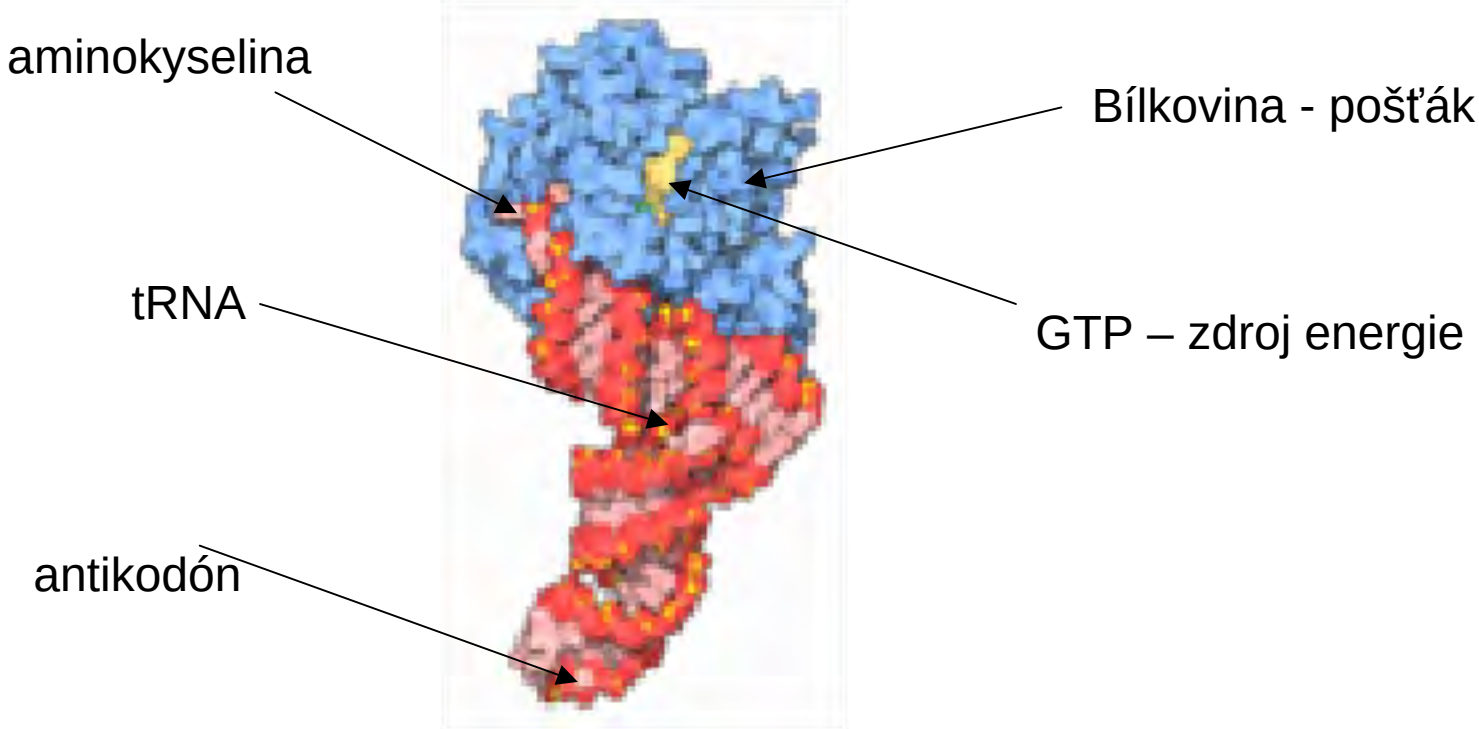
- V přírodě se vyskytující „molekulární zařízení“ = makromolekulární komplexy, které transformují chemickou nebo elektrochemickou energii na usměrněný molekulární pohyb.

Ribosom je stroj. Má funkce: dekódovací, regulační ,katalytickou (chemickou) a **transportní**

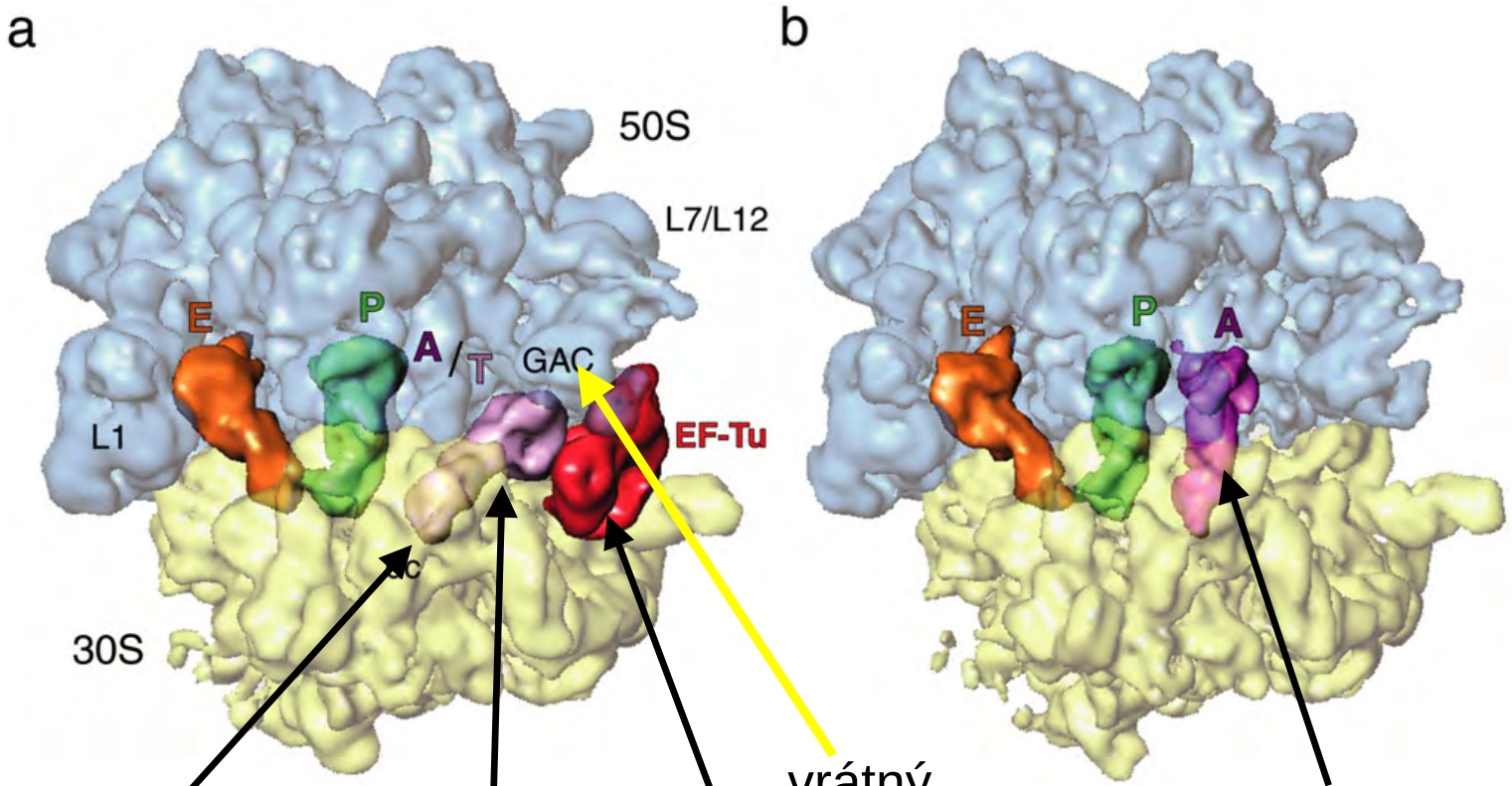


- Pro funkci ribosomu je důležitá ještě řada doprovodných obslužných bílkovin, tzv. faktorů.
- Ukážeme si dva: „pošťák“ a „nakopávač“

tRNA+aminokyselina přichází na ribosom v komlexu s bílkovinou (elongační faktor) + GTP (zdroj energie)



Pošťák přinesl tRNA



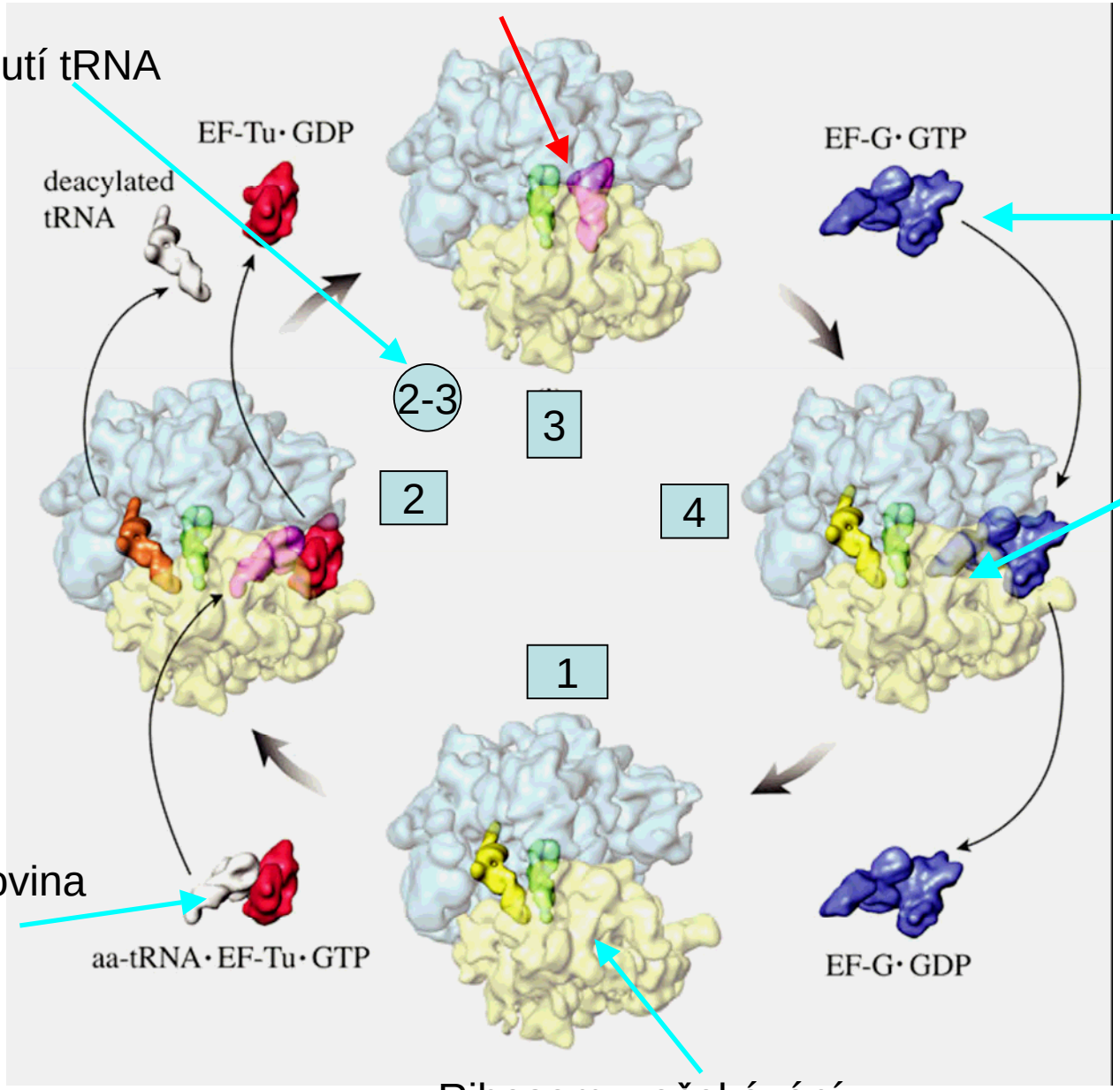
vrátný

Dekódovací centrum

Po uvolnění pošťákem tRNA zapadne jako pružina dovnitř.

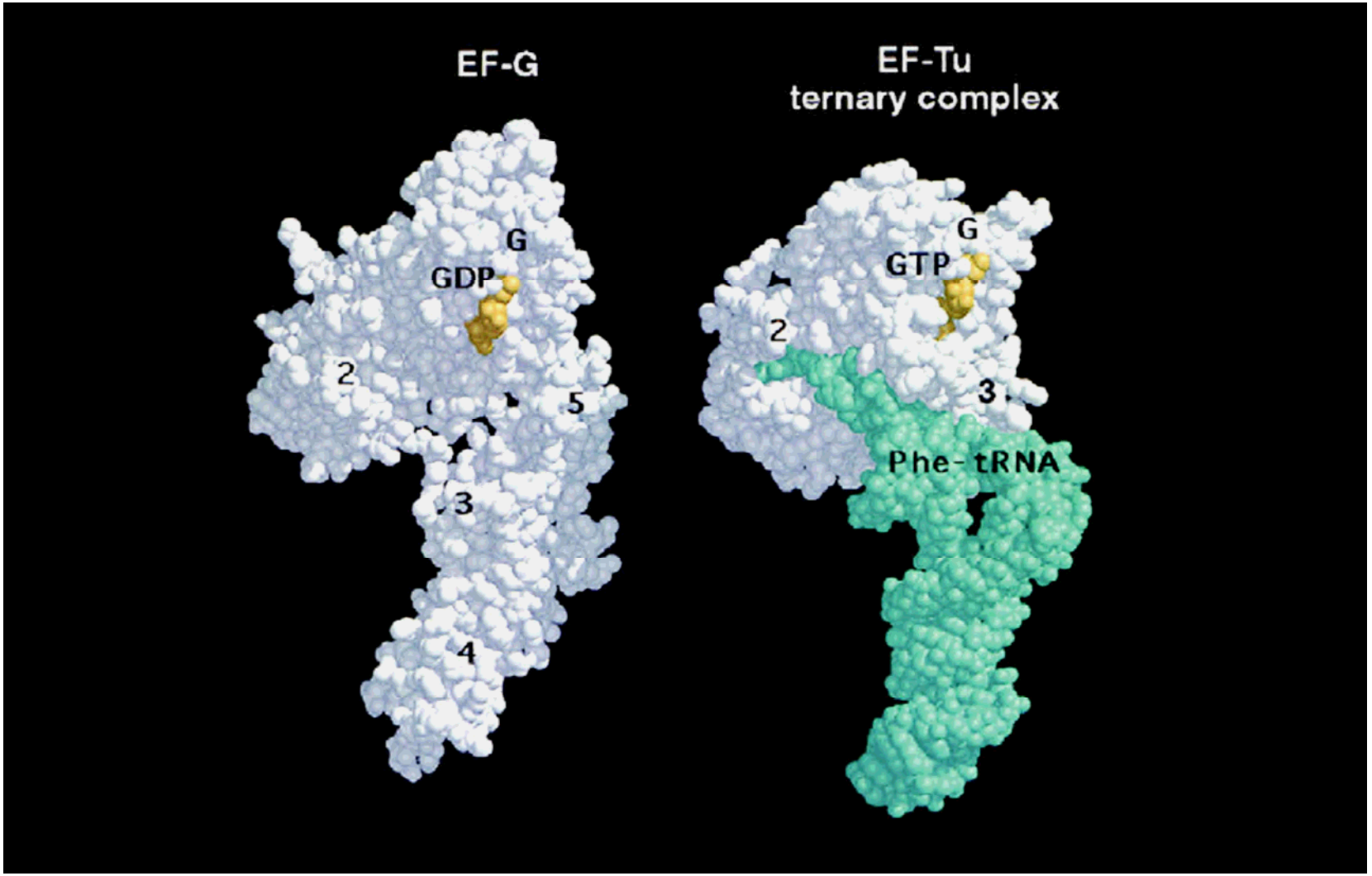
tRNA v komplexu s pošťákem

Proběhne vlastní chemická reakce

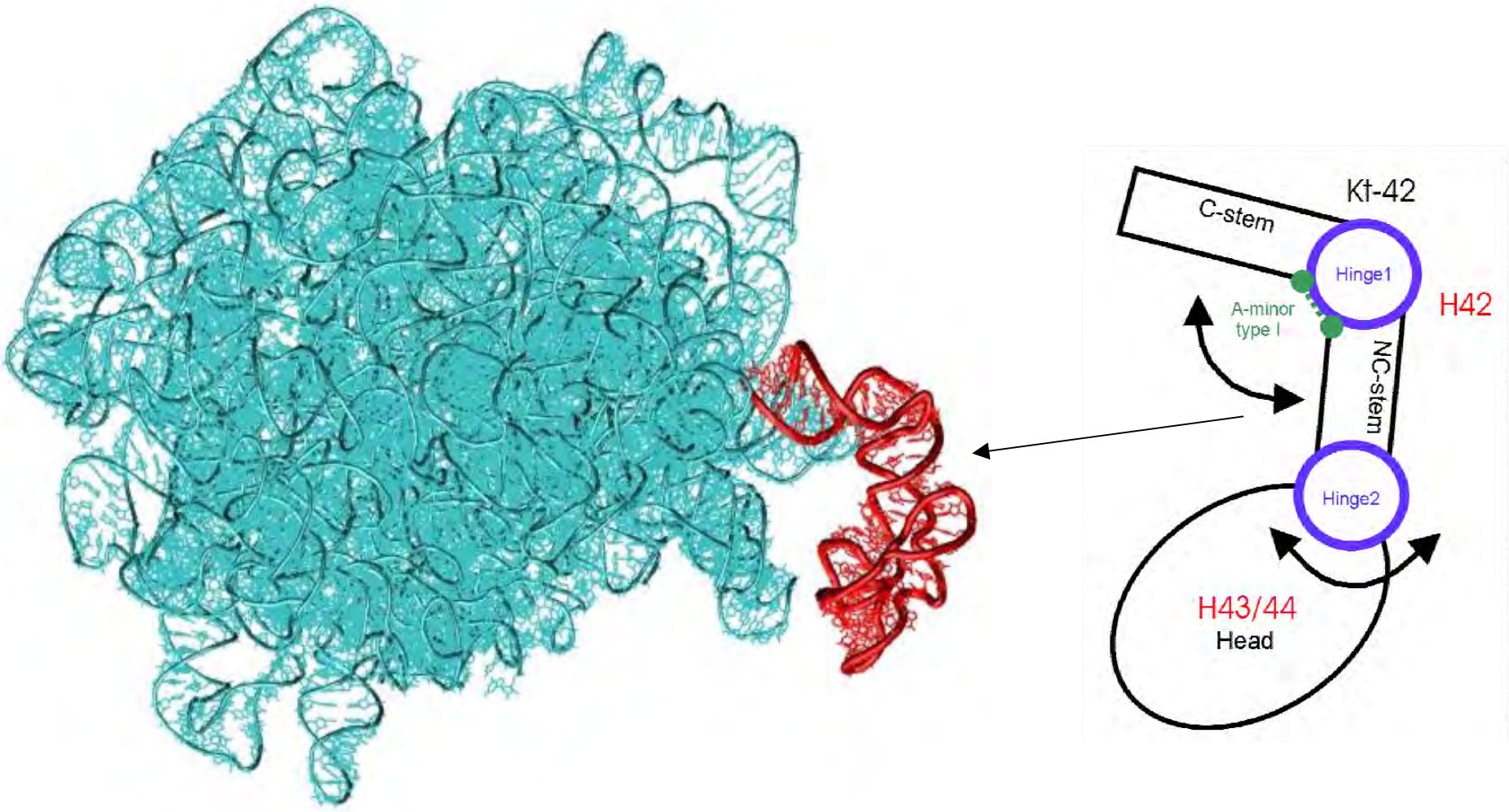


Molekulové napodobování

„nakopávač“ vs. tRNA + pošťák

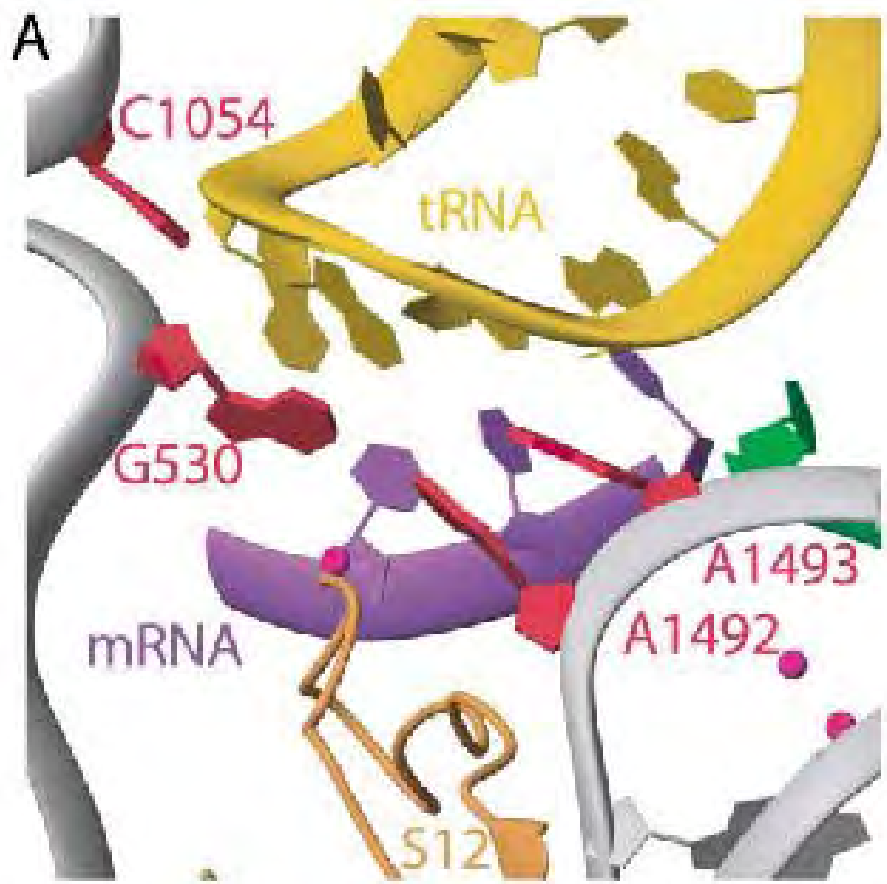


„Vrátný“ je sofistikovaná RNA nanopaže.
2 flexibilní klouby, 2 rigidní segmenty, adjustovatelná hlavice

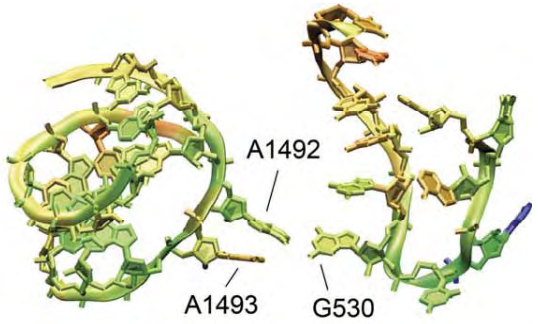


Dekódovací centrum ribosomu

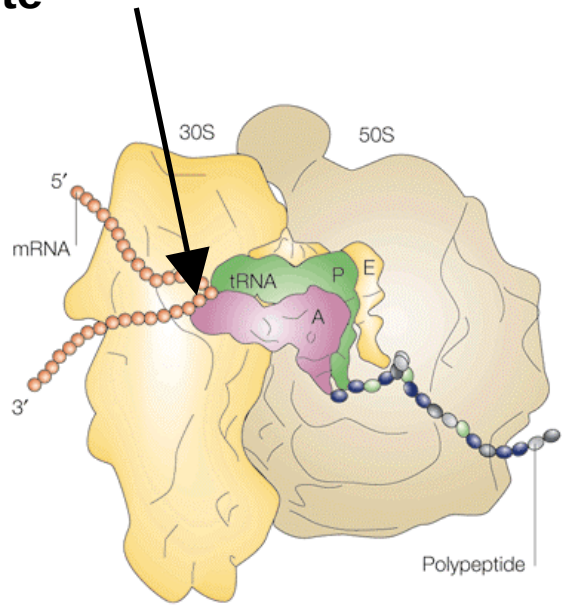
Mezi tRNA a mRNA se vytváří krátká dvoušrobovice mezi kodónem a antikodónem, a ta má tři páry bází.
Ribosom si pomocí několika bází přesně kontroluje tvar prvních dvou z nich.



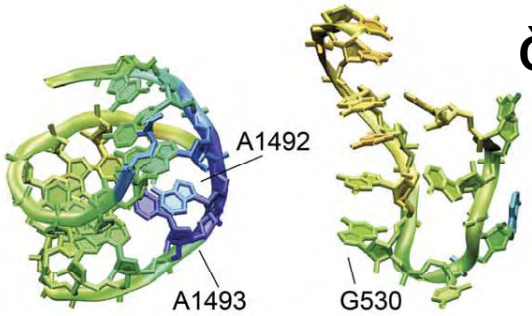
a



Čtecí zařízení zaklapnuté



b

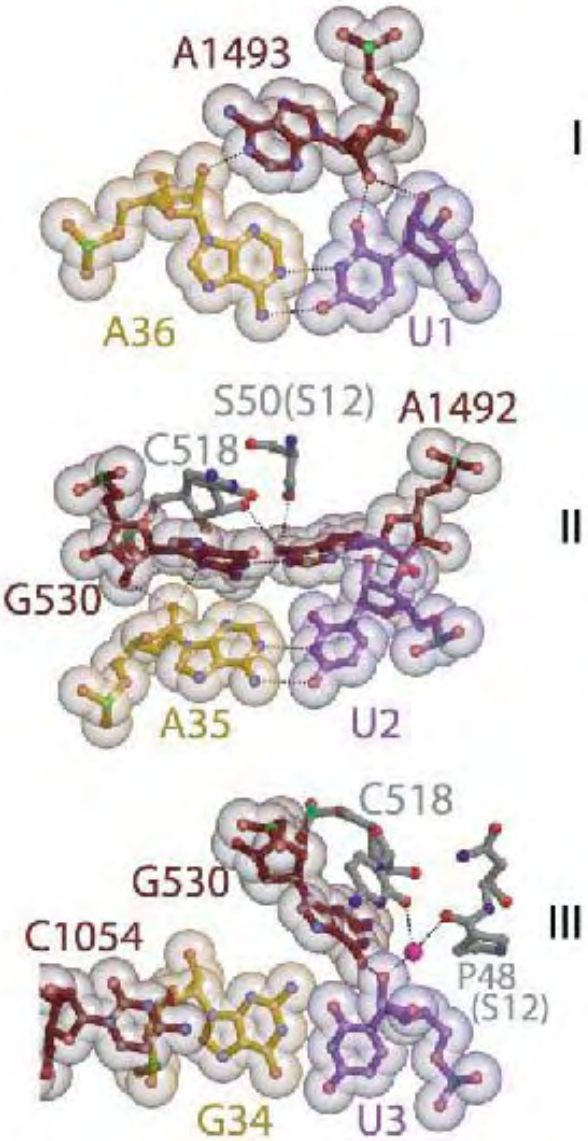


Čtecí zařízení neaktivní

První antikodón – kodón pár

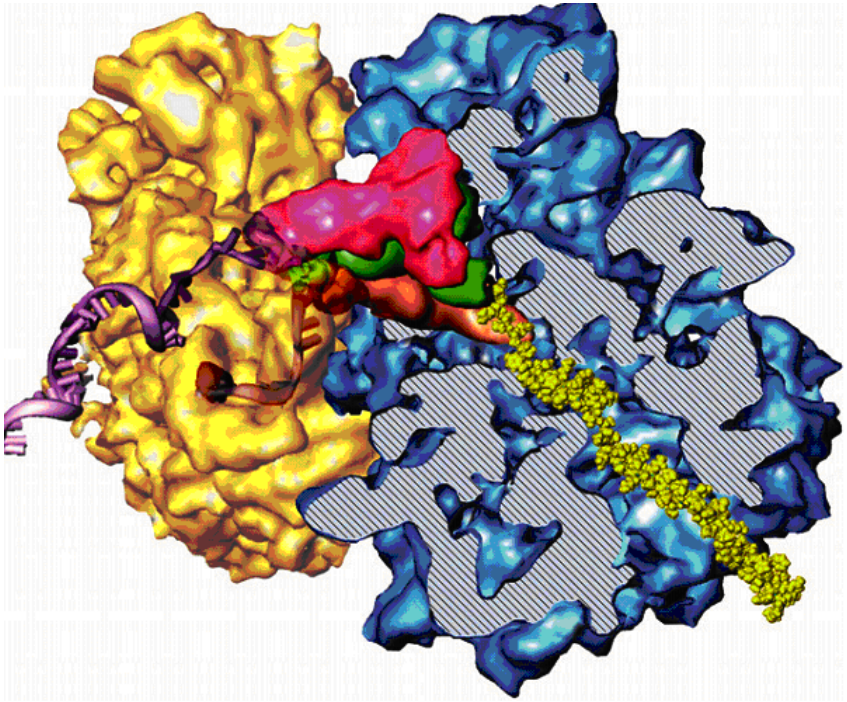
Druhý antikodón – kodón pár

Třetí antikodón – kodón pár



- Redukcionismus ano či ne?

Ribosom je stroj stochastický!!



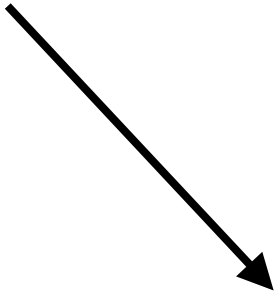
- Biomolekulární stroje působí v režimu vysoké viskozity a jsou „atomární“. Jsou v neustálém tepelném pohybu a pod vlivem tepelného pohybu okolí.
- Nedokáží akumulovat mechanickou energii (žádné páky, kladky, závaží….) a nemohou spoléhat ani na přesné součástky (ozubená kolečka….)
- Energii k pohybu berou z tepelného fluktuačního pohybu a využívají chemické energie (GTP) na usměrnění tepelného pohybu.

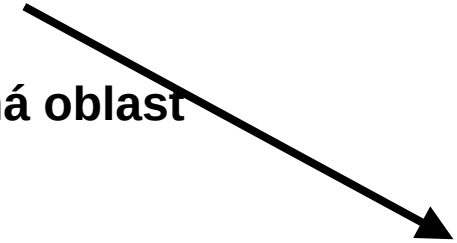
- Díky Cryoelektronové mikroskopii a rentgenostrukturní analýze známe strukturu ribosomu.

  Ve skutečnosti se jedná pouze o pár statických zprůměrovaných „fotografií“.

Potřebovali bychom mít místo toho film filmů.

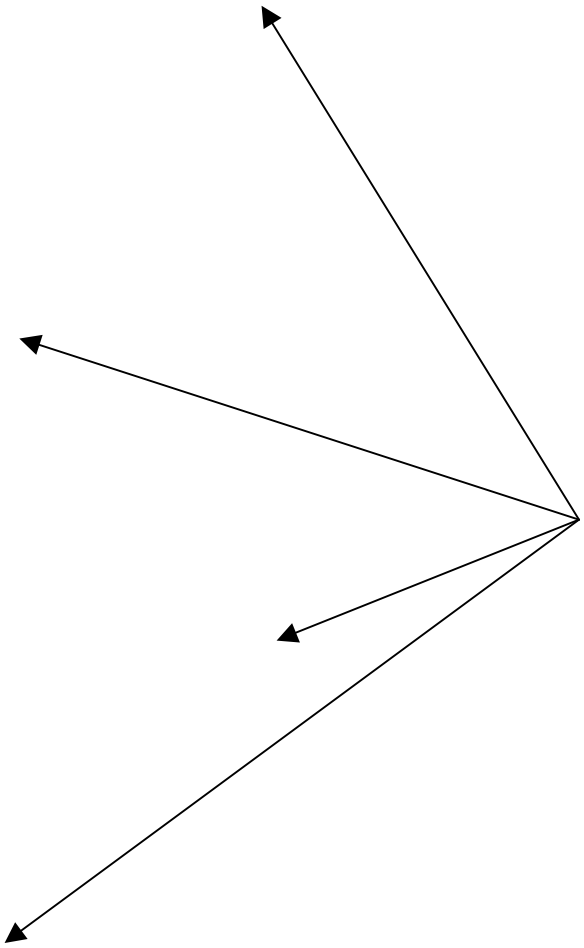
Zcela neviditelná oblast





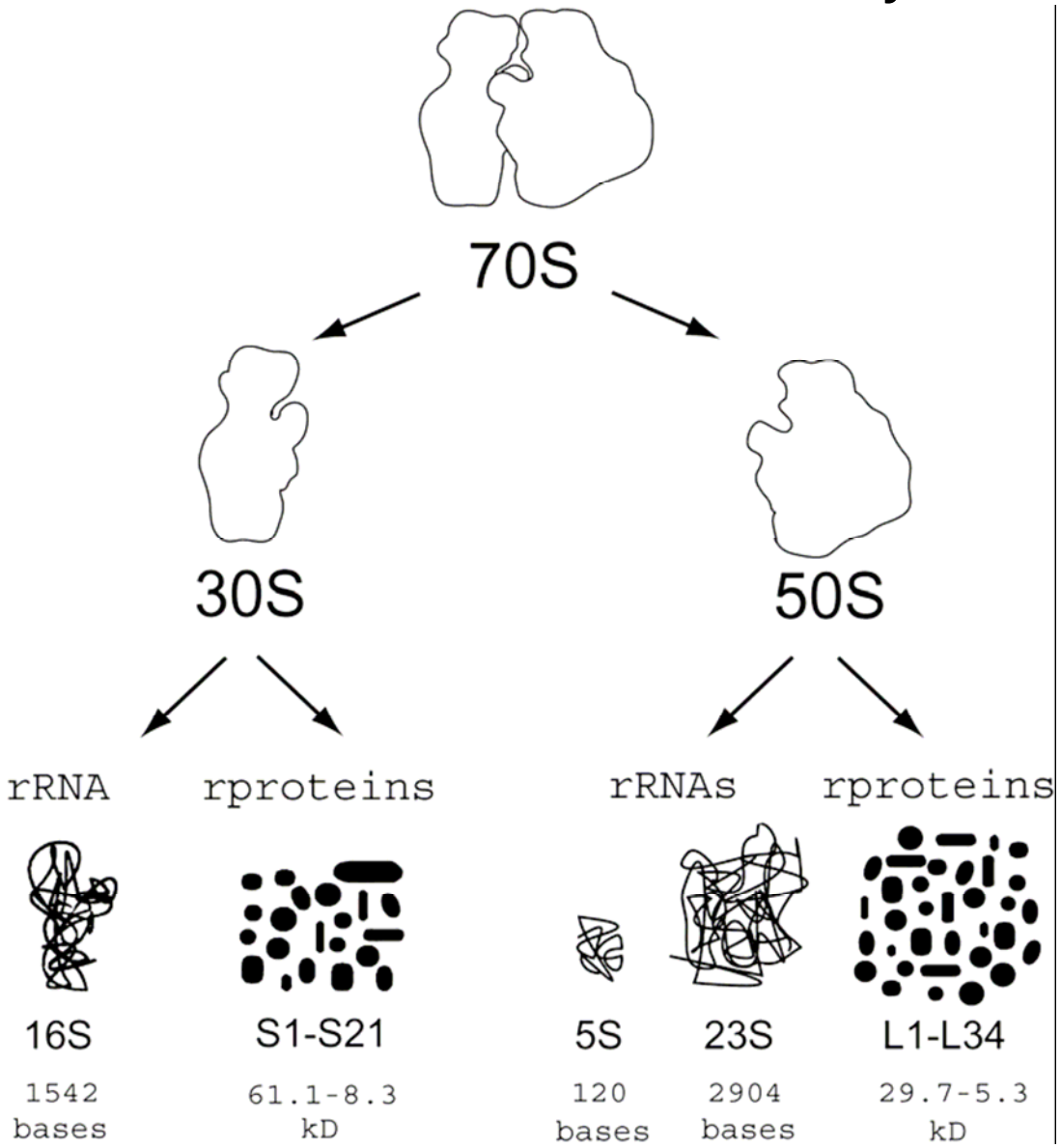
neviditelná oblast

Toto vidíme



L7/12 stalk = „vrátný“

Jak se to vše složí dohromady ???

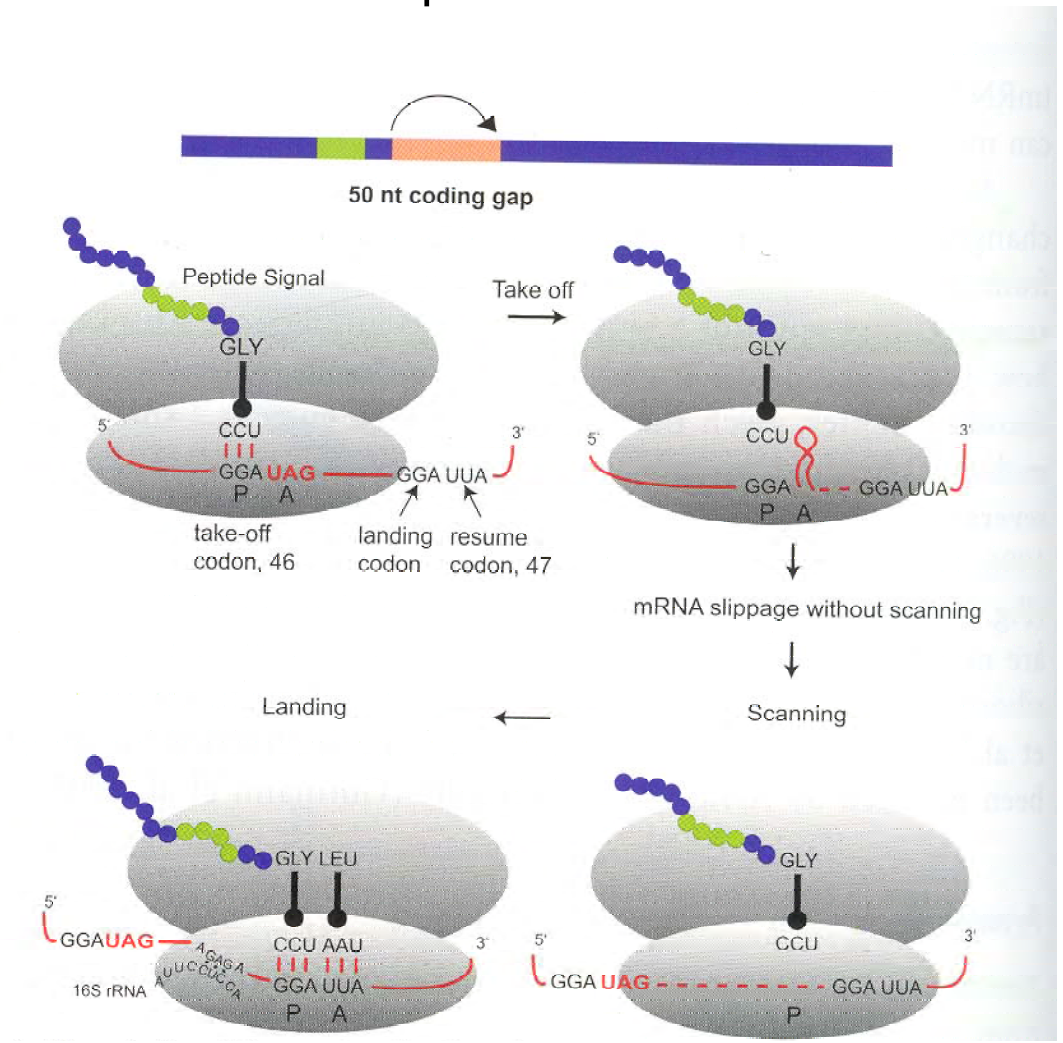


Trochu syrové reality, aneb jak se manipuluje ribosom…….

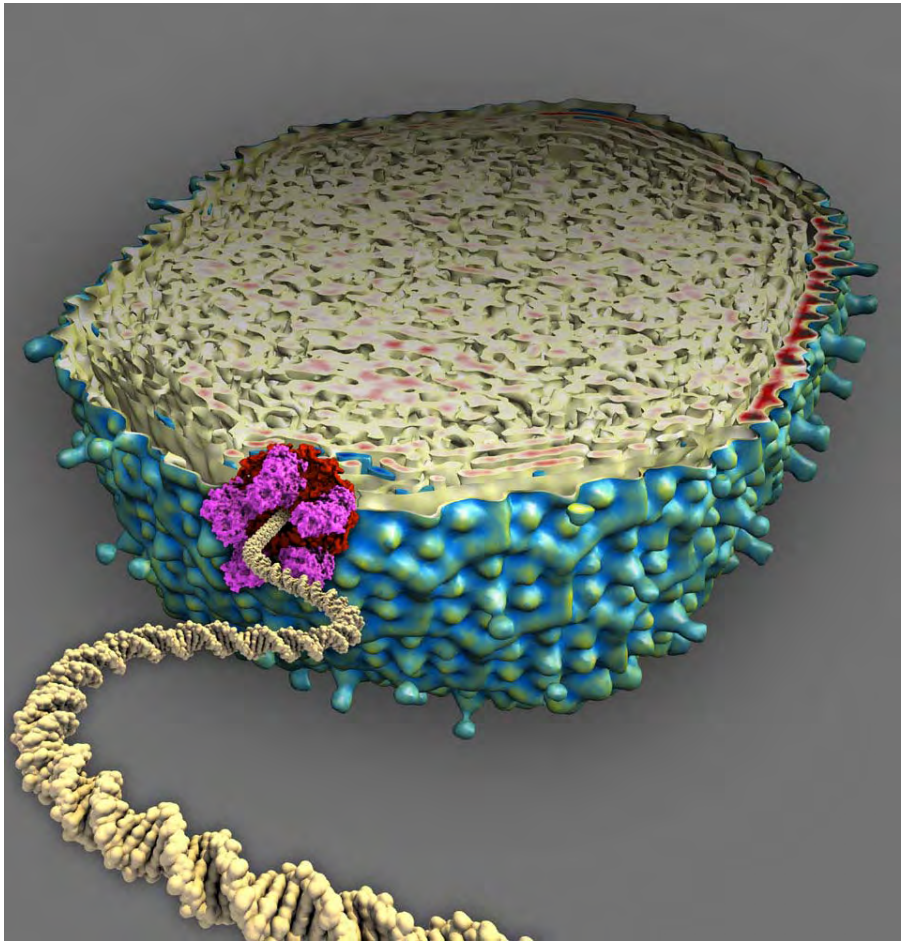
Proklouznutí mRNA na ribosomu. (T4 phage gen 60)

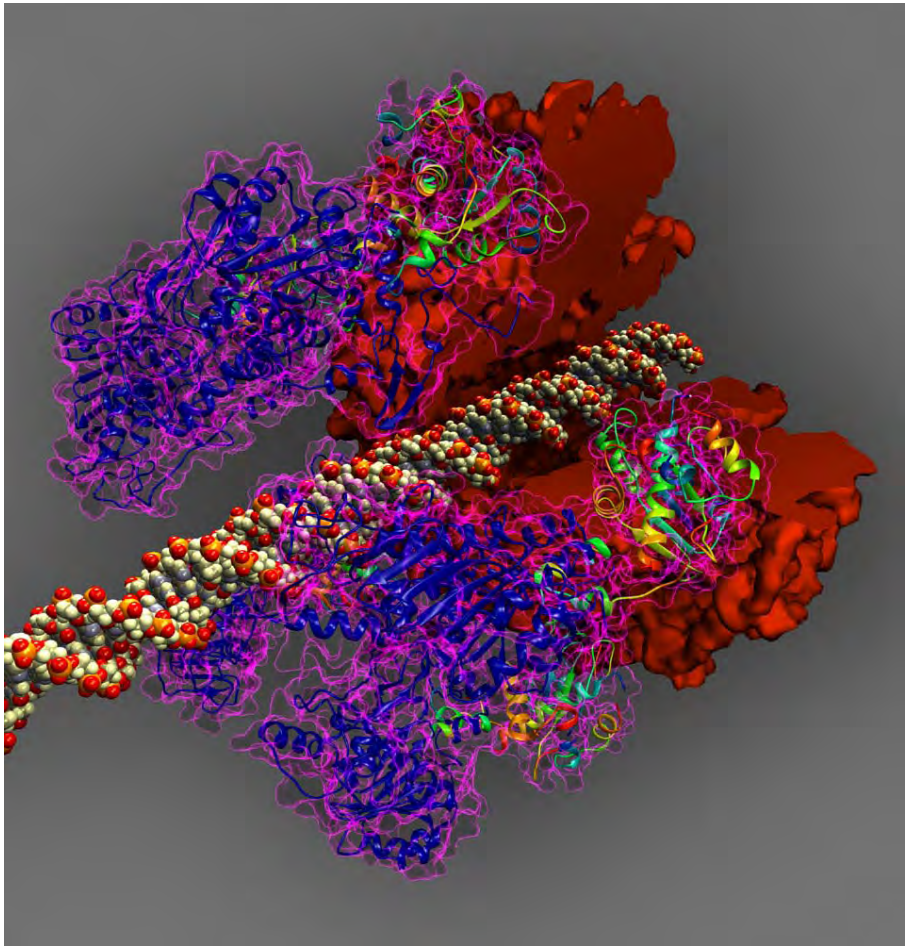
Syntéza bílkoviny se na 50 kroků přeruší a pak pokračuje.

Regulace přes tvar mRNA a dále přes interakci bílkovina – tunel.



Nejúčinnější motor, navinutí DNA dovnitř bakteriofágu.





Myosin nejsou jen svaly.

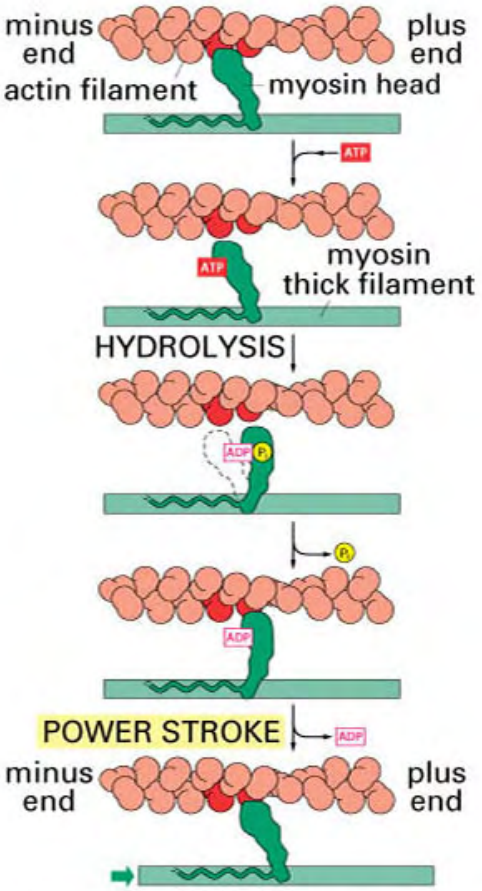
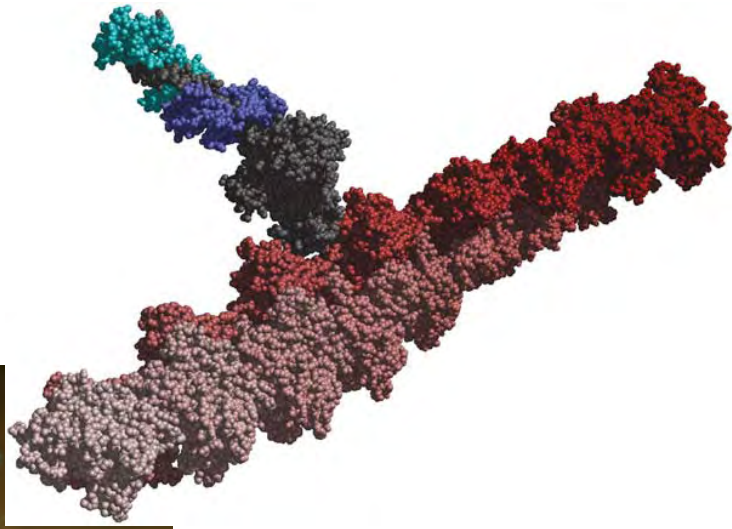
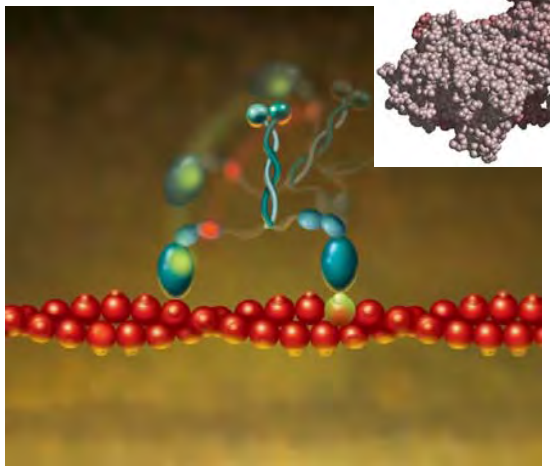
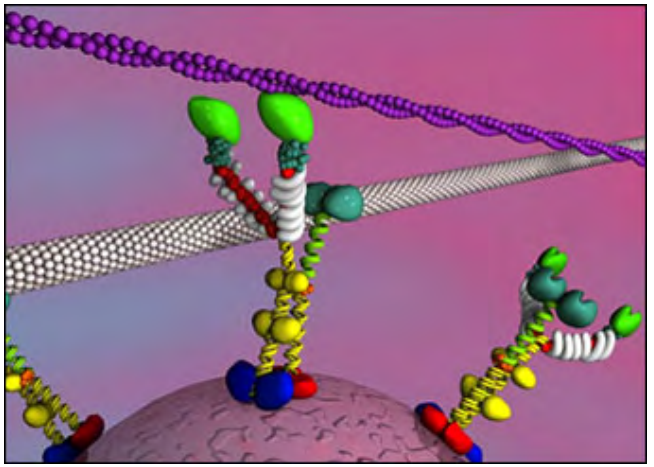


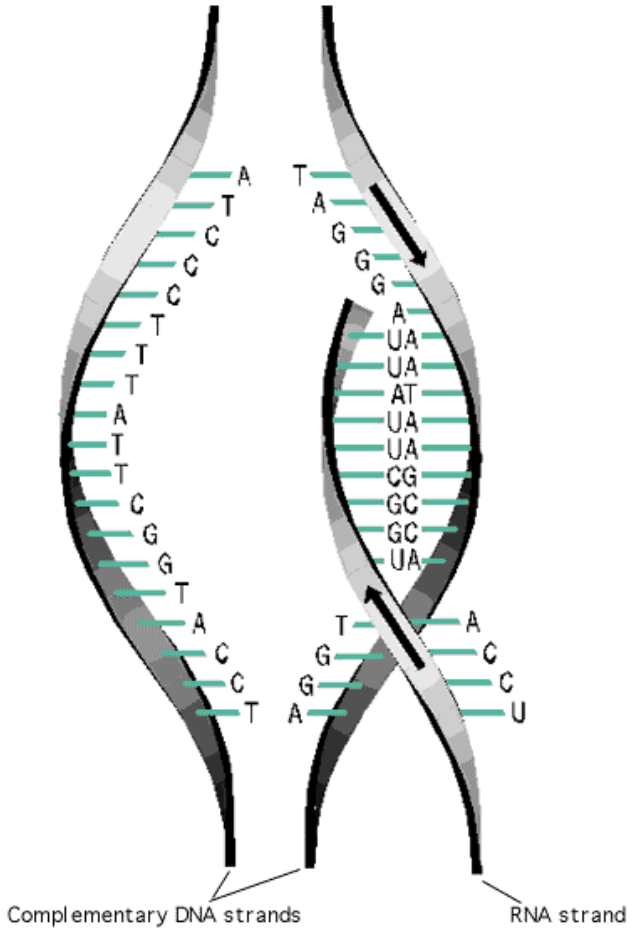
Figure 17-45 Essential Cell Biology, 2/e. (© 2004 Garland Science)

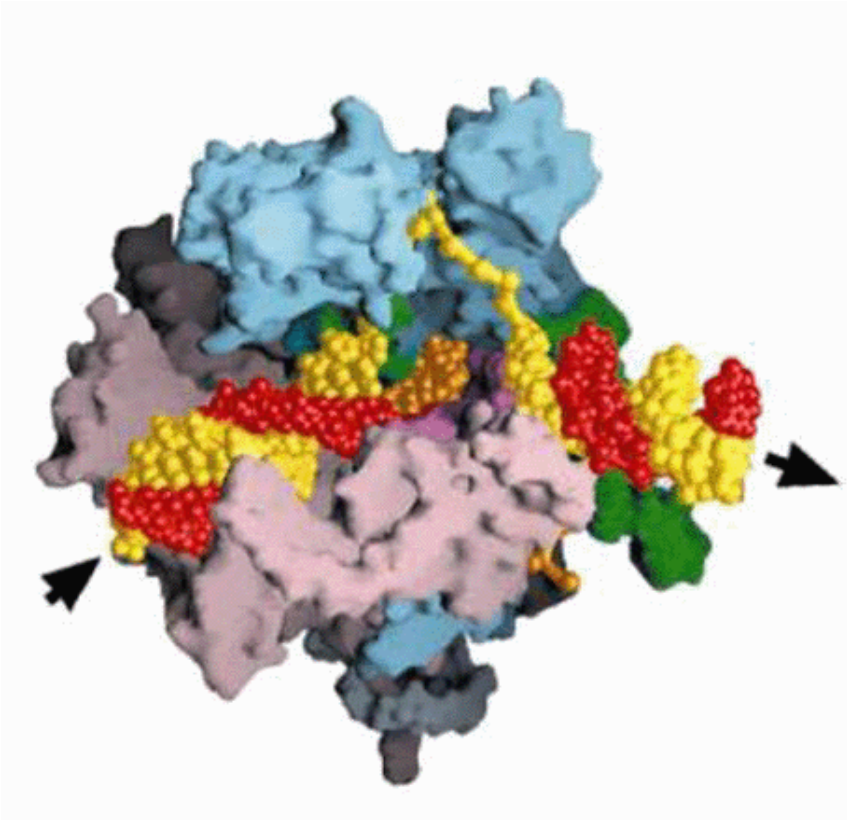


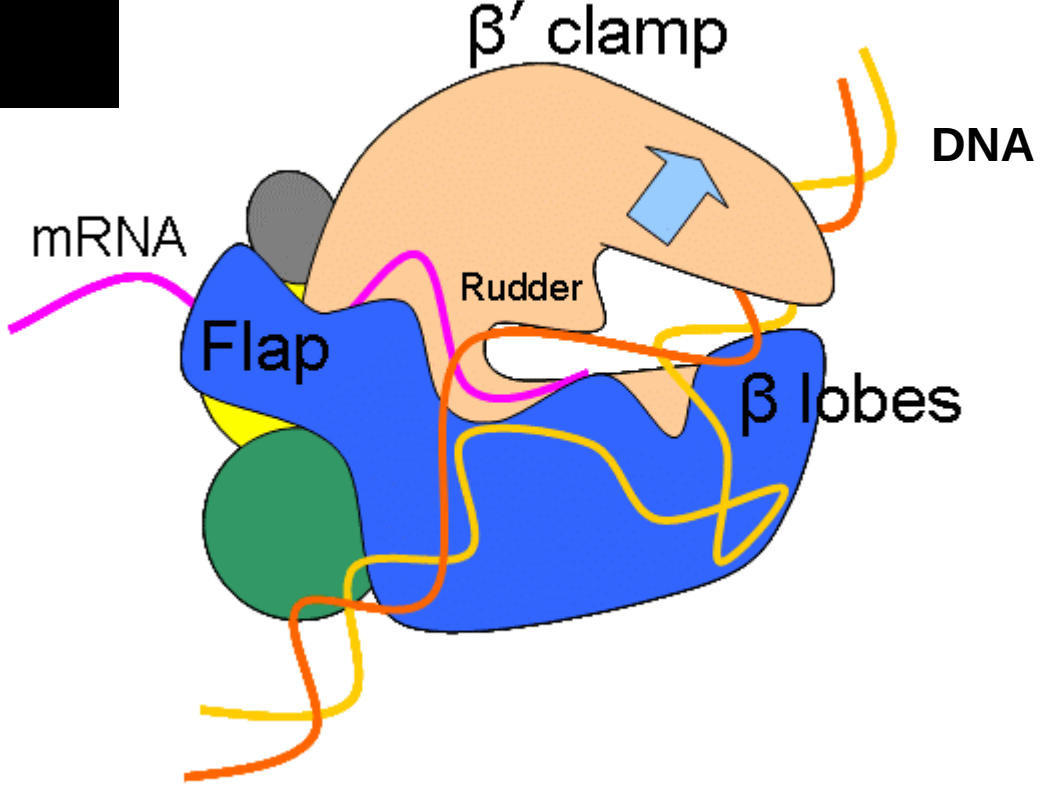




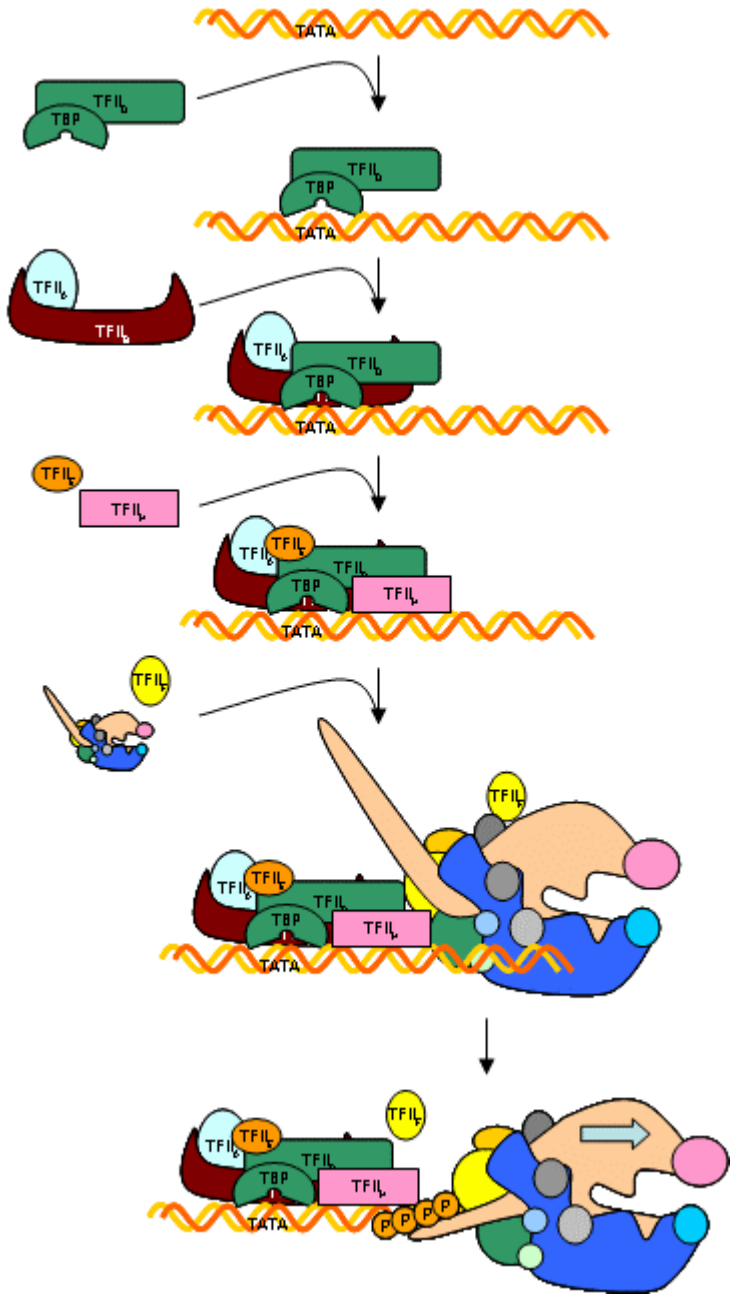
RNA polymeráza



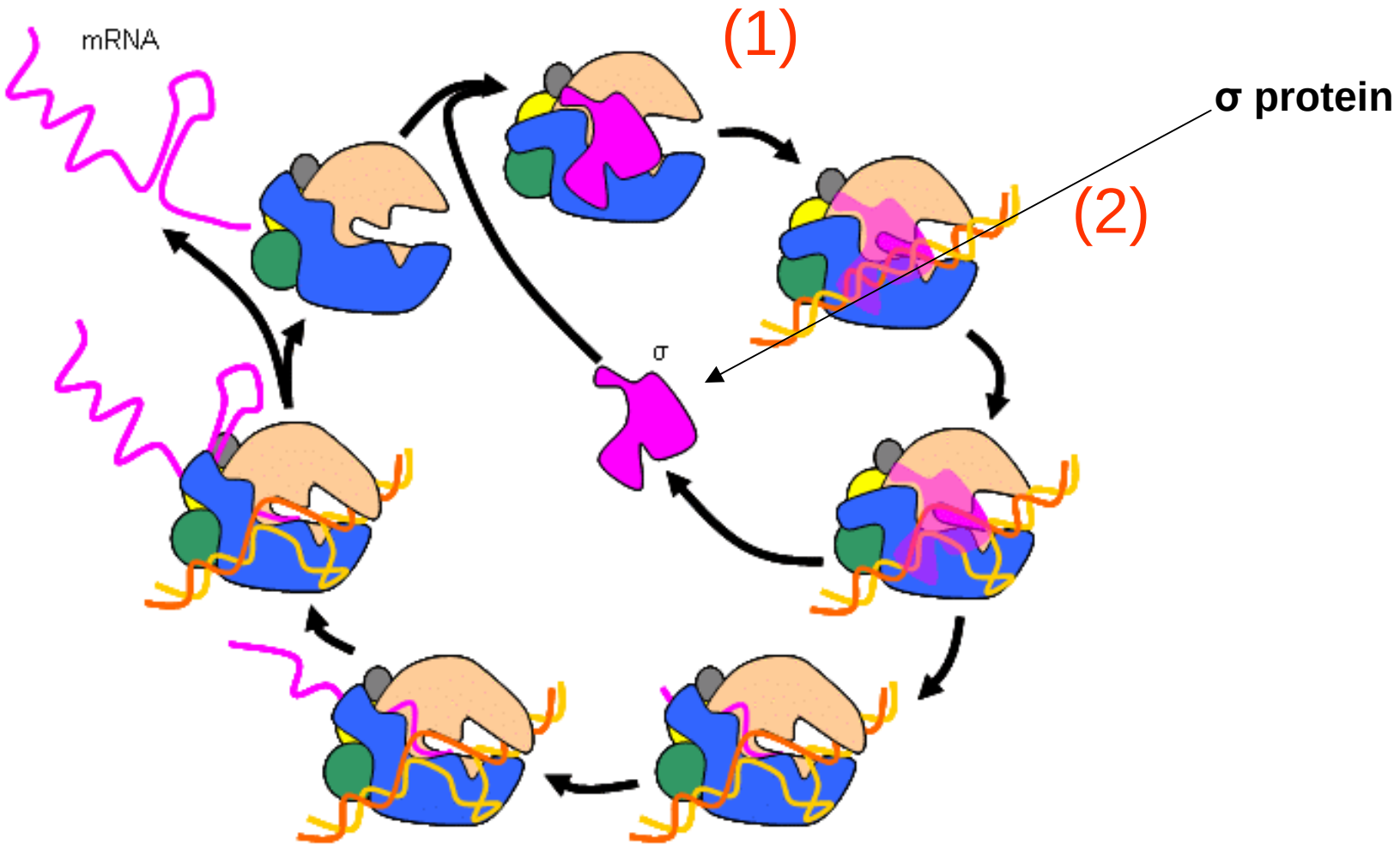



β′ clamp
DNA
mRNA
Rudder
Flap
β lobes

Jak vlastně najde RNA polymeráza počátek genu?



Funkce RNA polymerázy: **jádro** RNA polymerázy a **σ protein** vytvoří holoenzym, ten se naváže na DNA, vytvoří otevřený komplex, σ otevře DNA, pak se σ odpoutá a RNA polymeráza zaklapne kolem DNA. Následně je syntetyzována mRNA, syntéze je většinou ukončena tím, že mRNA vytvoří terminační smyčku.



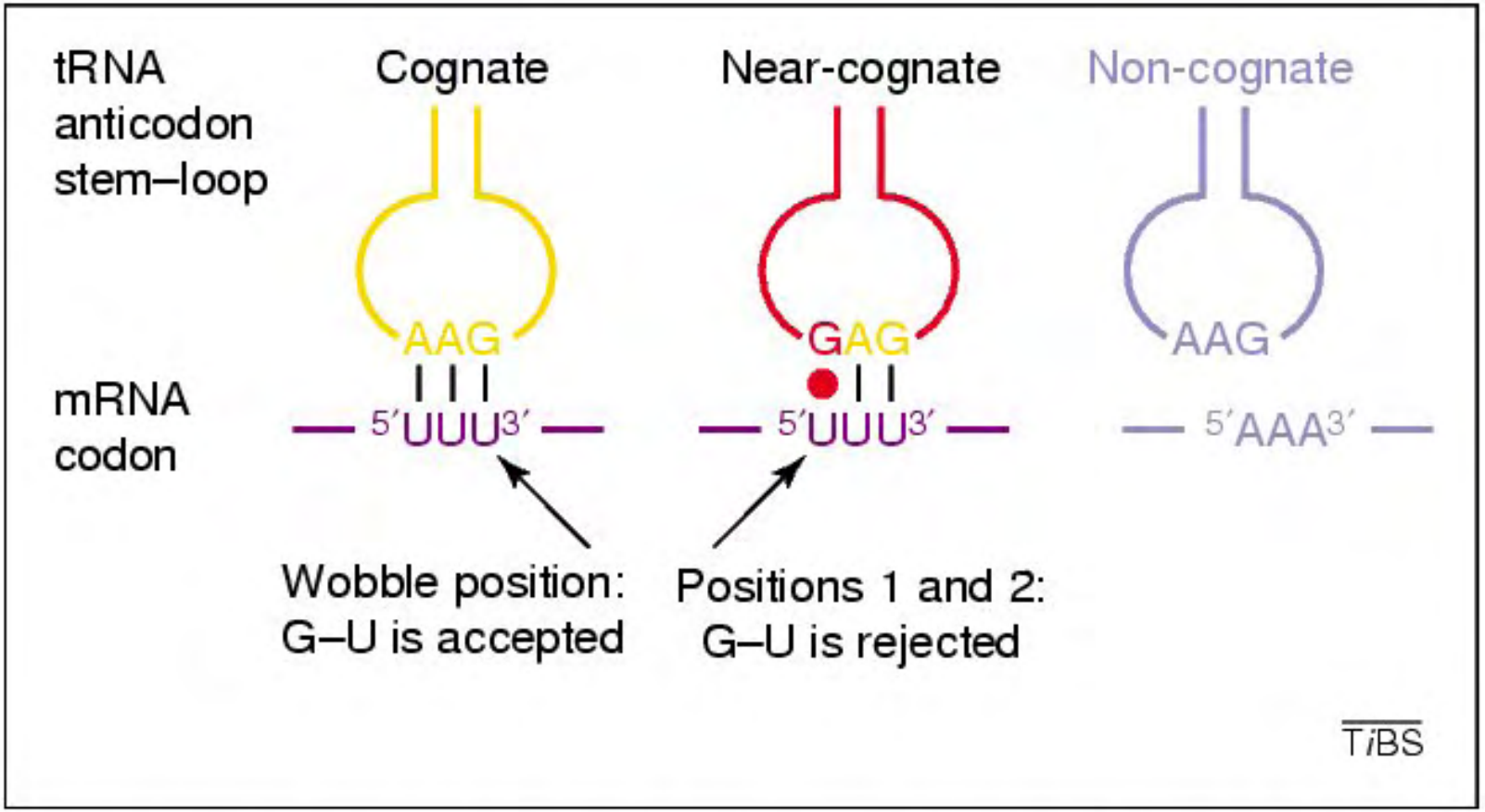


Fig. 1. Cognate, near-cognate and non-cognate codon–anticodon pairing. The anticodon must interact with the first two codon positions according to the Watson–Crick base pairing rules, whereas some deviation, e.g. the G–U pair, is allowed at the third, or 'wobble' position.

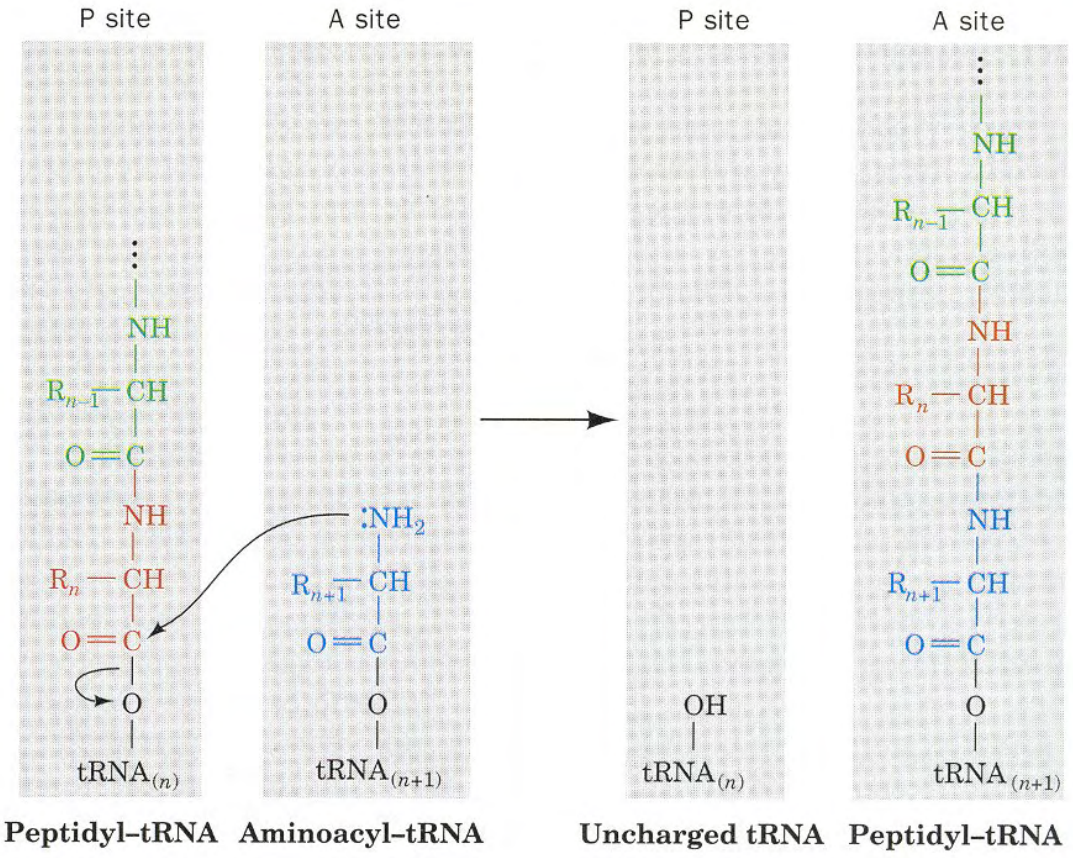
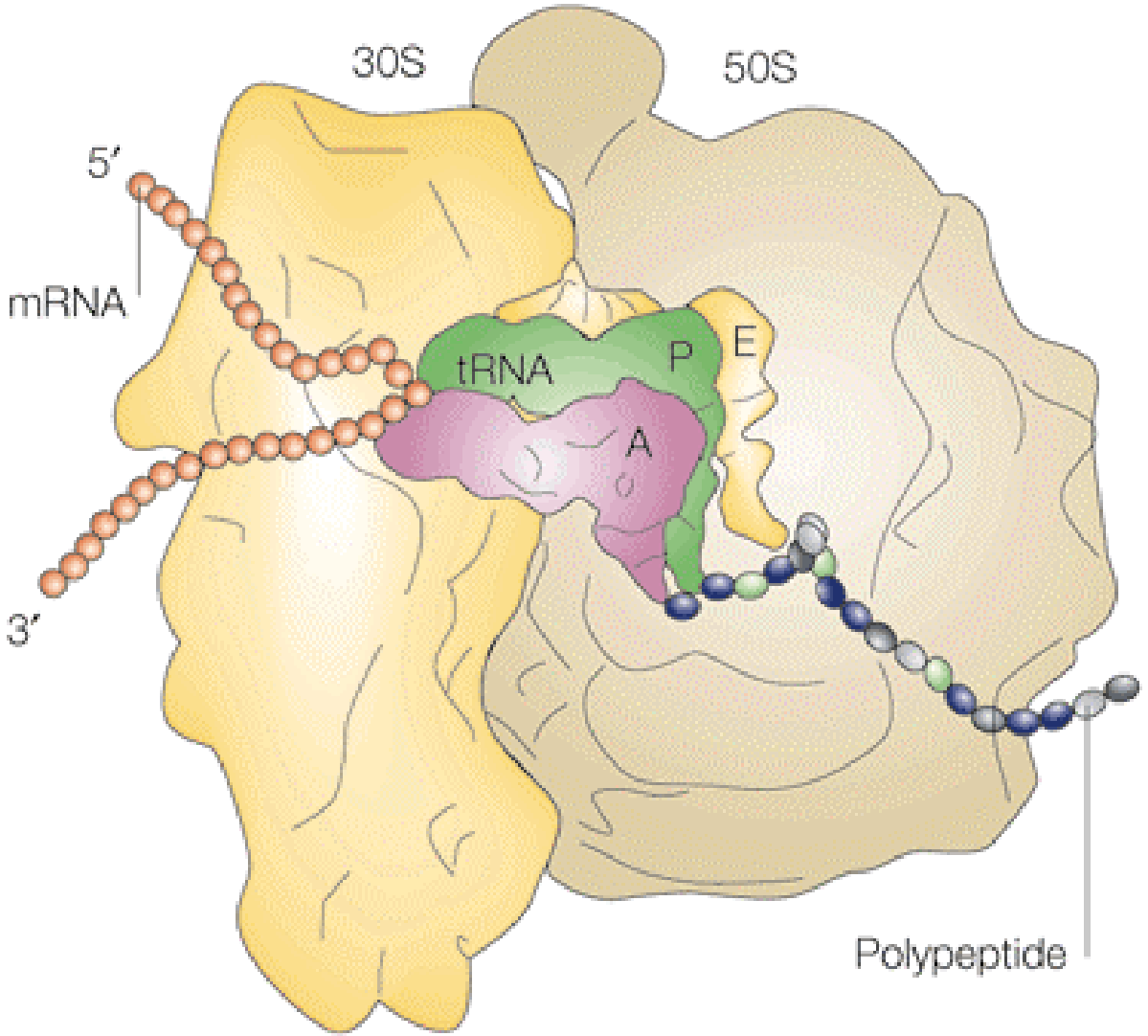
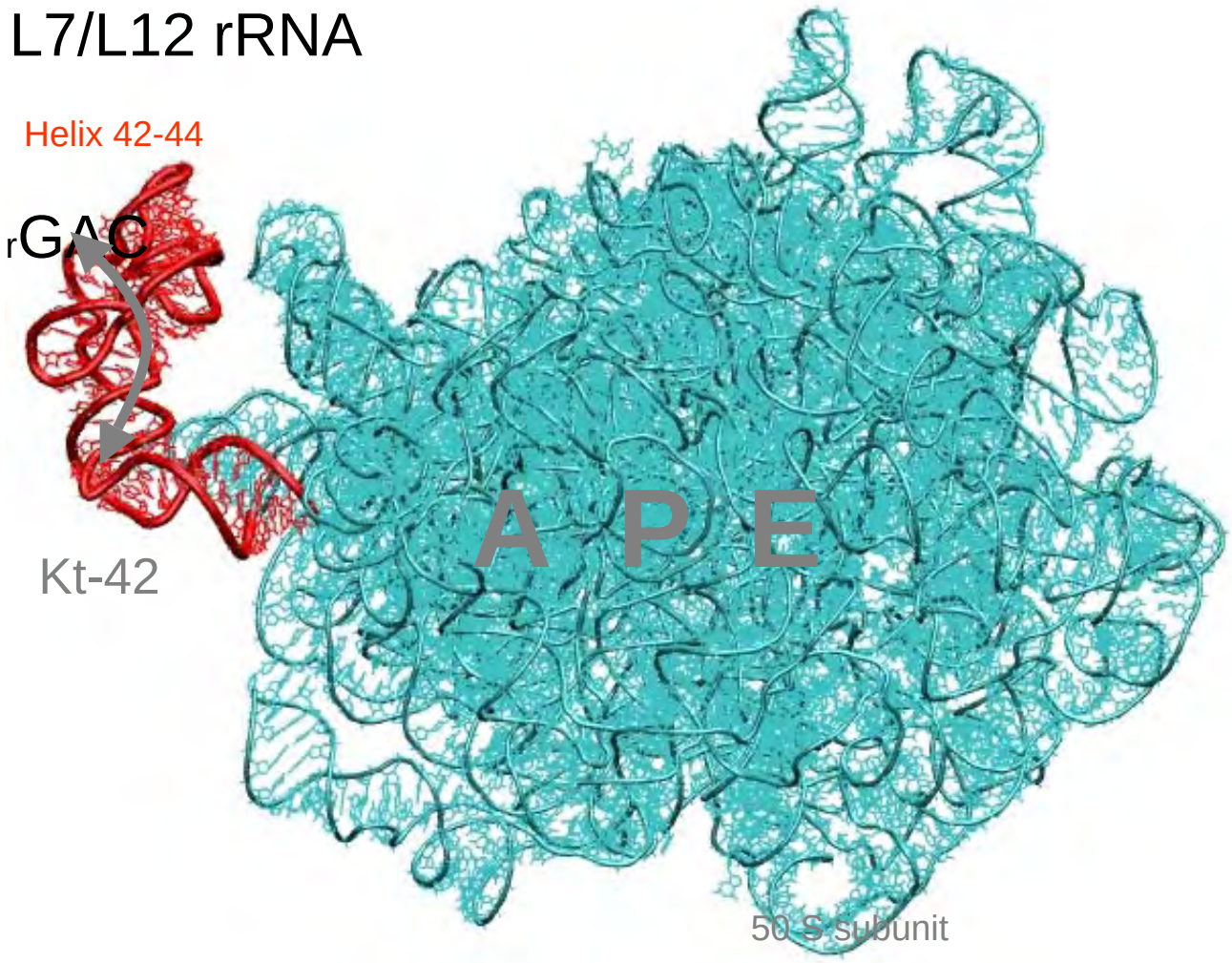
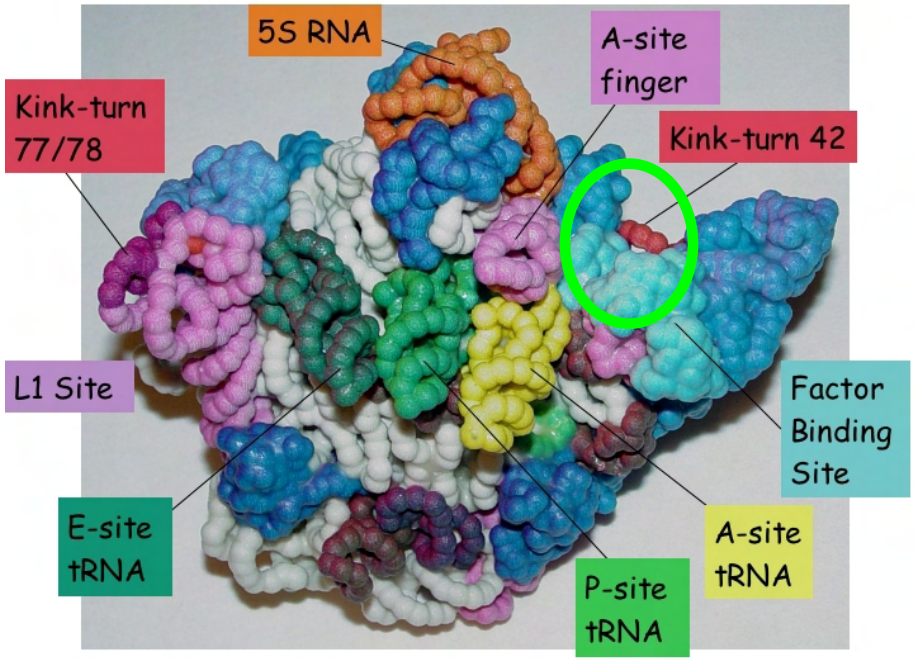


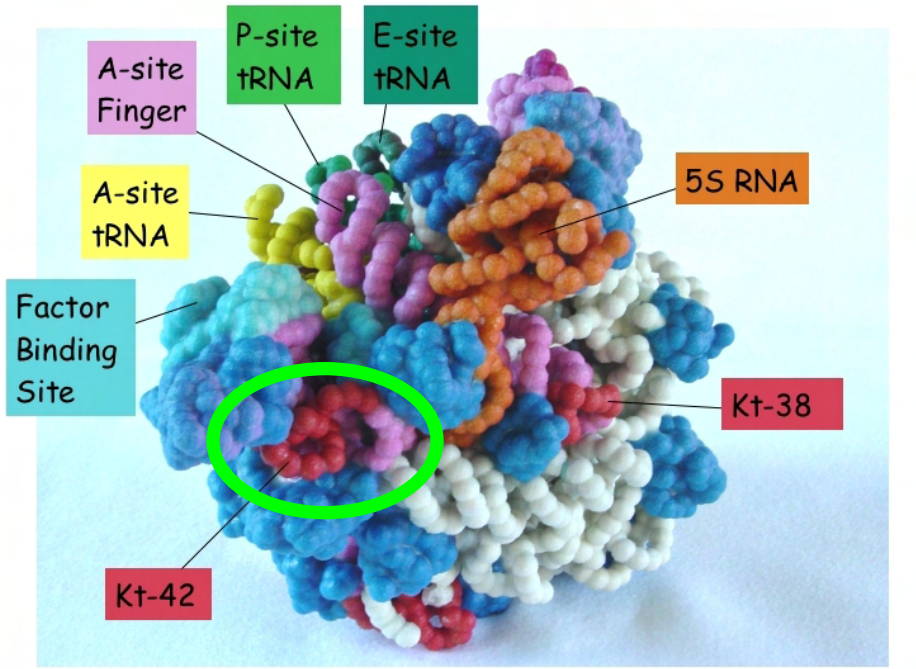
***Figure* 26-23. The ribosomal peptidyl transferase reaction forming a peptide bond.** The amino group of the aminoacyl–tRNA in the A site nucleophilically displaces the tRNA of the peptidyl–tRNA ester in the P site, thereby forming a new peptide bond and transferring the nascent polypeptide to the A-site tRNA.


30S
50S
5′
mRNA
tRNA
P
E
A
3′
Polypeptide

L7/L12 rRNA




5S RNA
A-site finger
Kink-turn 77/78
Kink-turn 42
L1 Site
Factor Binding Site
E-site tRNA
A-site tRNA
P-site tRNA


P-site tRNA
E-site tRNA
A-site Finger
A-site tRNA
5S RNA
Factor Binding Site
Kt-38
Kt-42

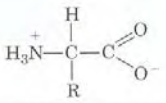
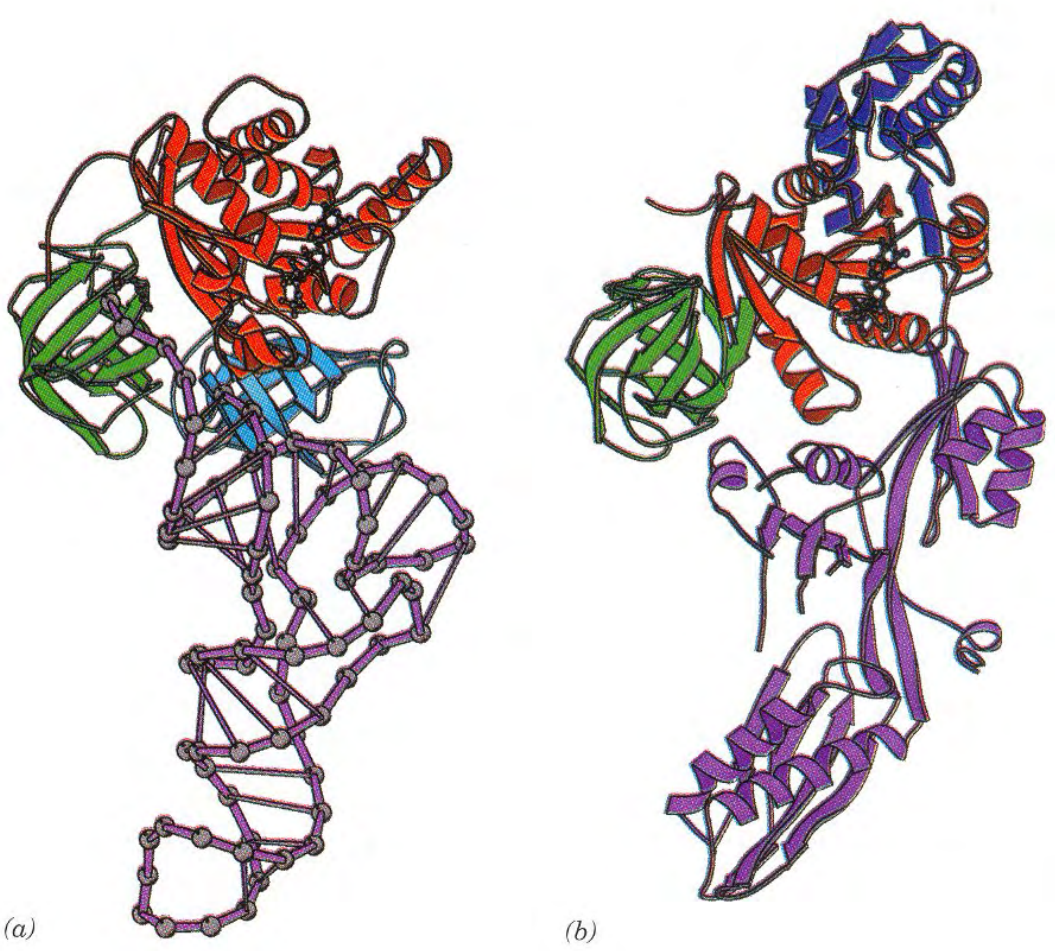


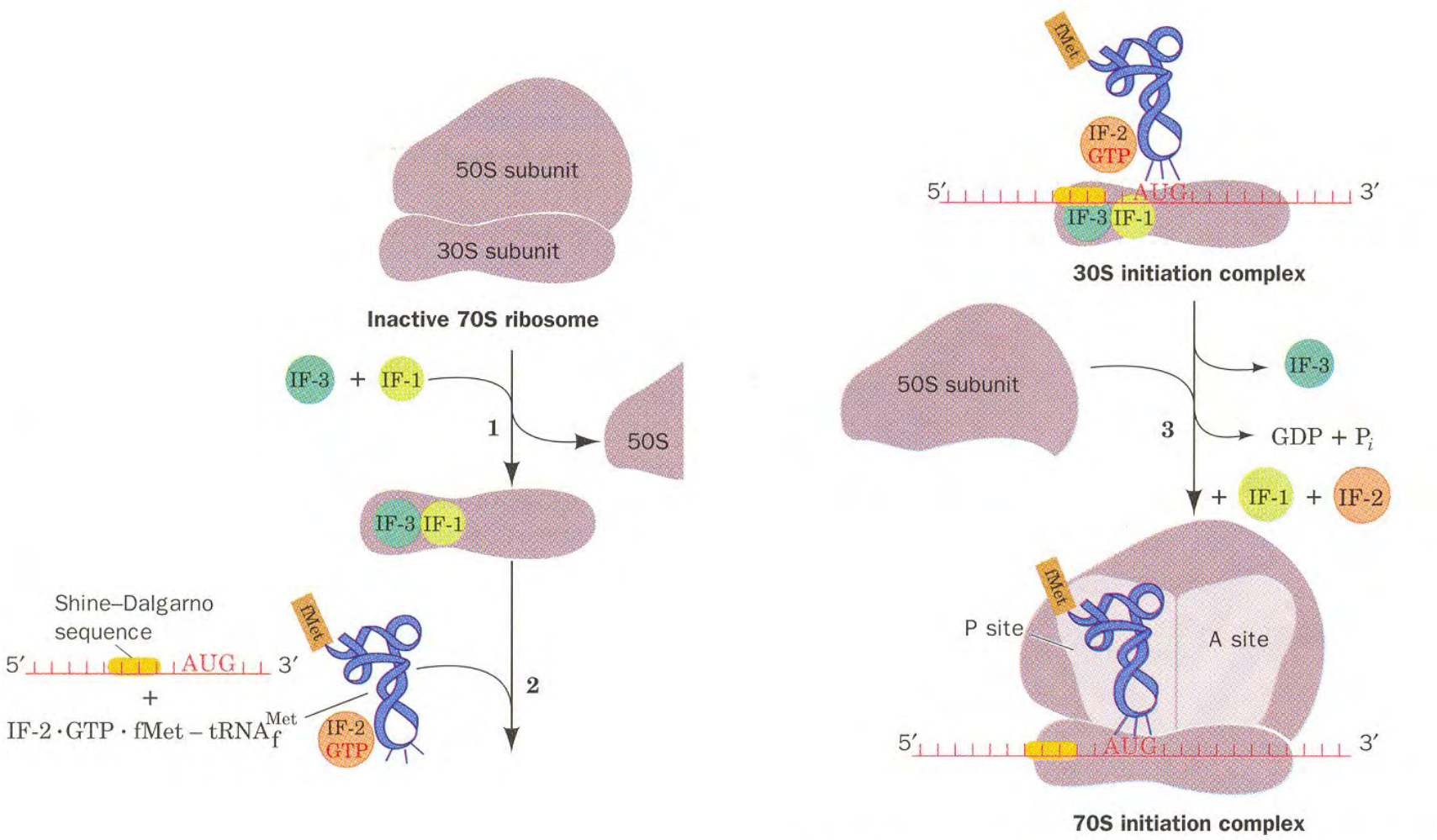
Table 26-1. **Key to Function. The "Standard" Genetic Code**[a]

First position (5′ end)	Second position U	Second position C	Second position A	Second position G	Third position (3′ end)
U	UUU Phe	UCU	UAU Tyr	UGU Cys	U
	UUC Phe	UCC	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met[b]	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

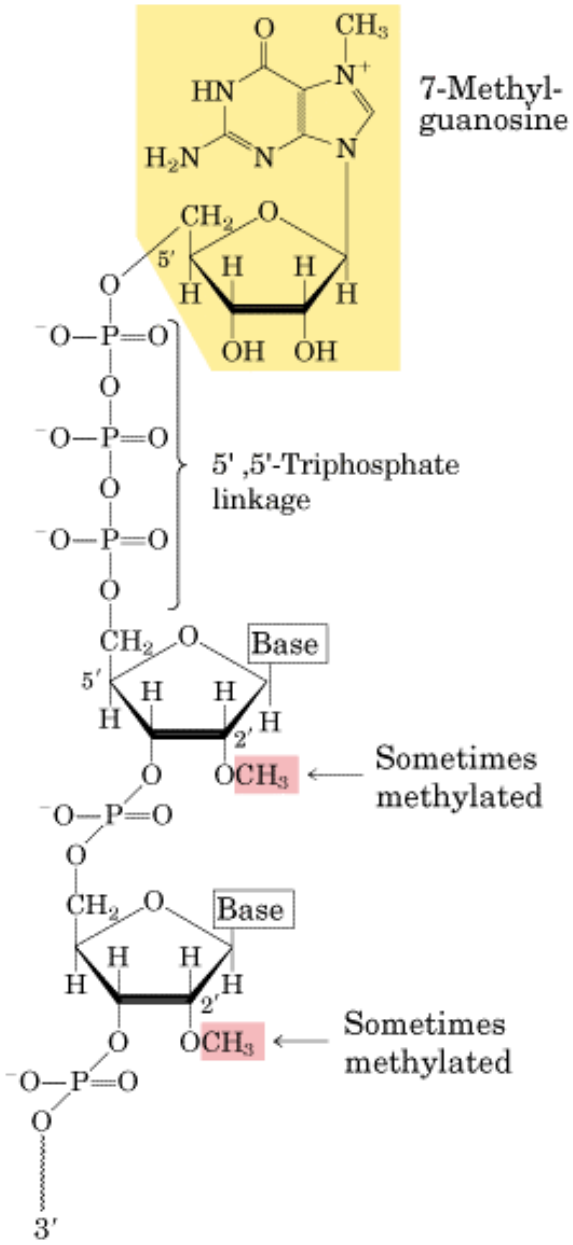
[a] Nonpolar amino acid residues are tan, basic residues are blue, acidic residues are red, and polar uncharged residues are purple.
[b] AUG forms part of the initiation signal as well as coding for internal Met residues.

Figure 26-30. **A comparison of the ternary structures of EF-Tu · tRNA and EF-G.** (*a*) In EF-Tu, domain 1 is red, domain 2 is green, and domain 3 is blue. The tRNA backbone of yeast $tRNA^{Phe}$ is shown in purple. (*b*) In EF-G, domain 1 is red, domain 2 is green, an extra domain is dark blue, and domains 3, 4, and 5 are purple. A 25-residue segment of EF-G's domain 1 is not visible in its X-ray structure and domain 3 is poorly defined, thereby accounting for the several polypeptide segments seen in this model. Courtesy of Jens Nyborg, Aarhus University, Århus, Denmark.] **See the Interactive Exercises.**

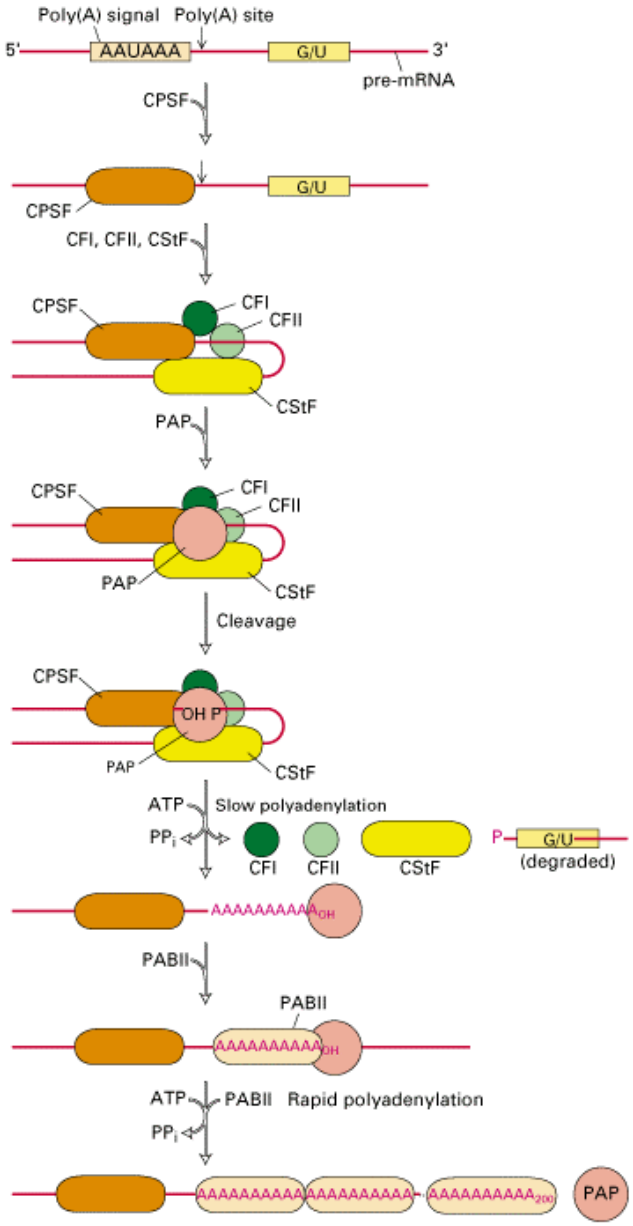


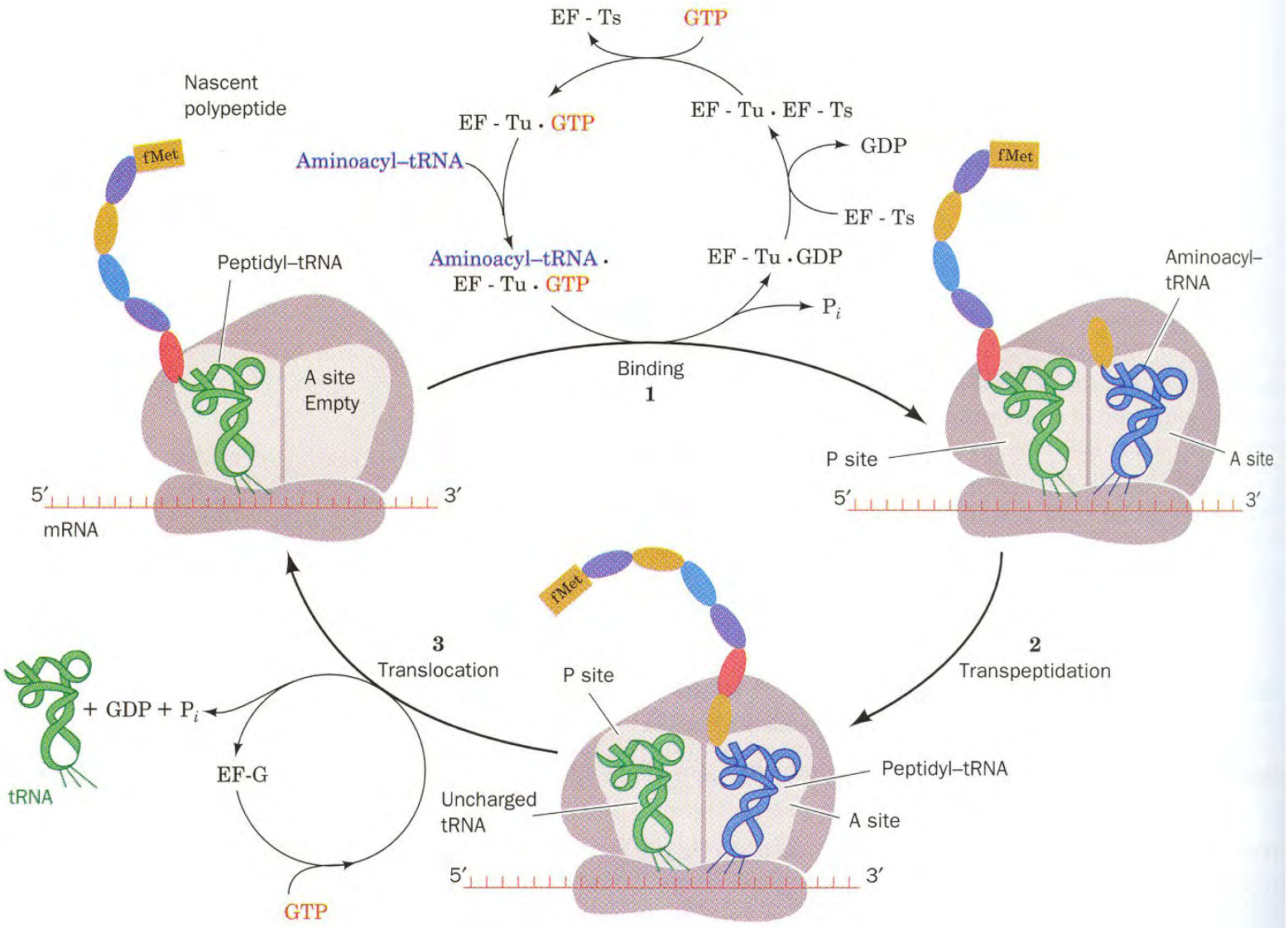


The translation initiation pathway in *E. coli* ribosomes.


7-Methyl-
guanosine
5' ,5'-Triphosphate
linkage
Base
Sometimes
methylated
Base
Sometimes
methylated
3'

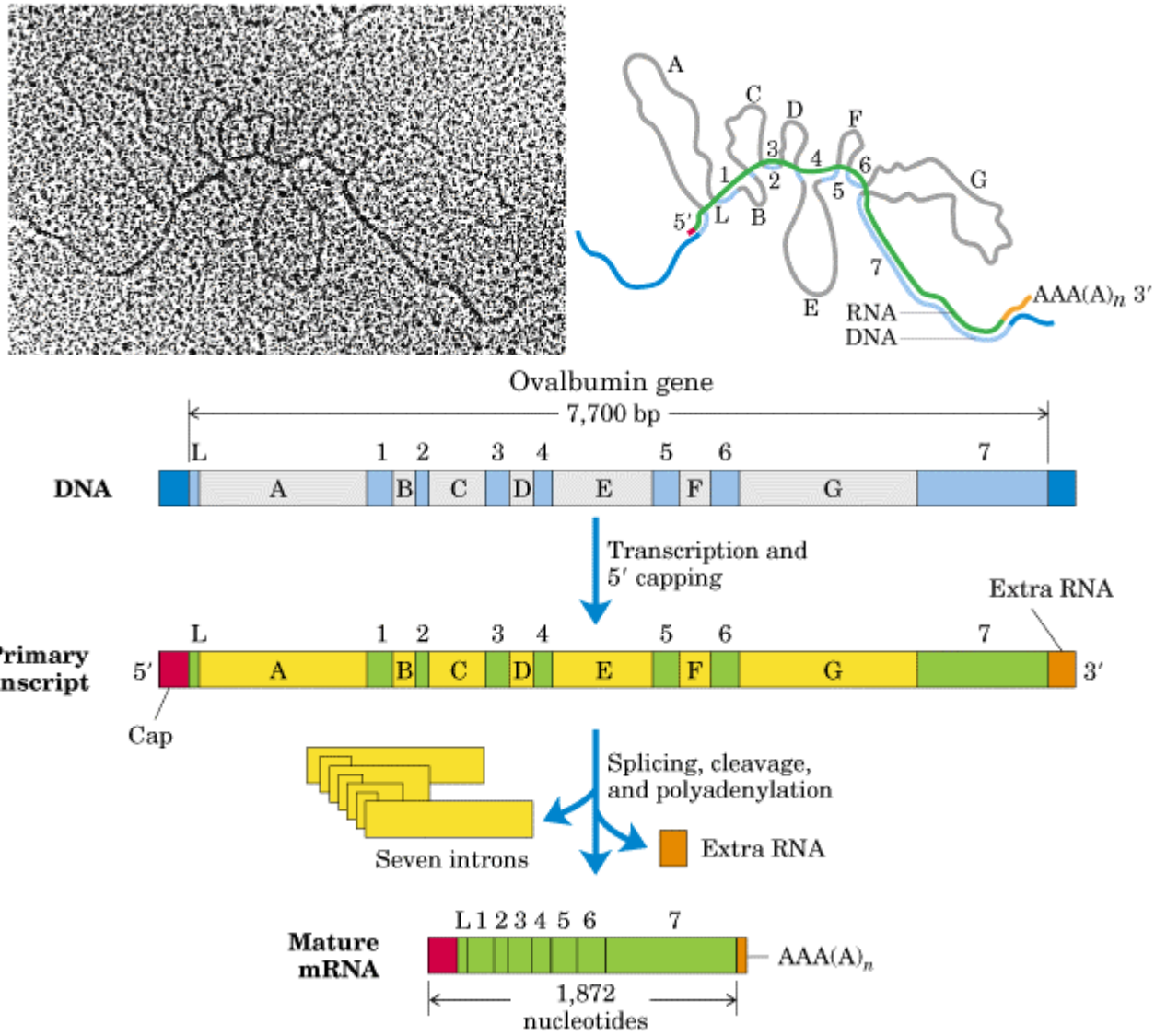
(a)

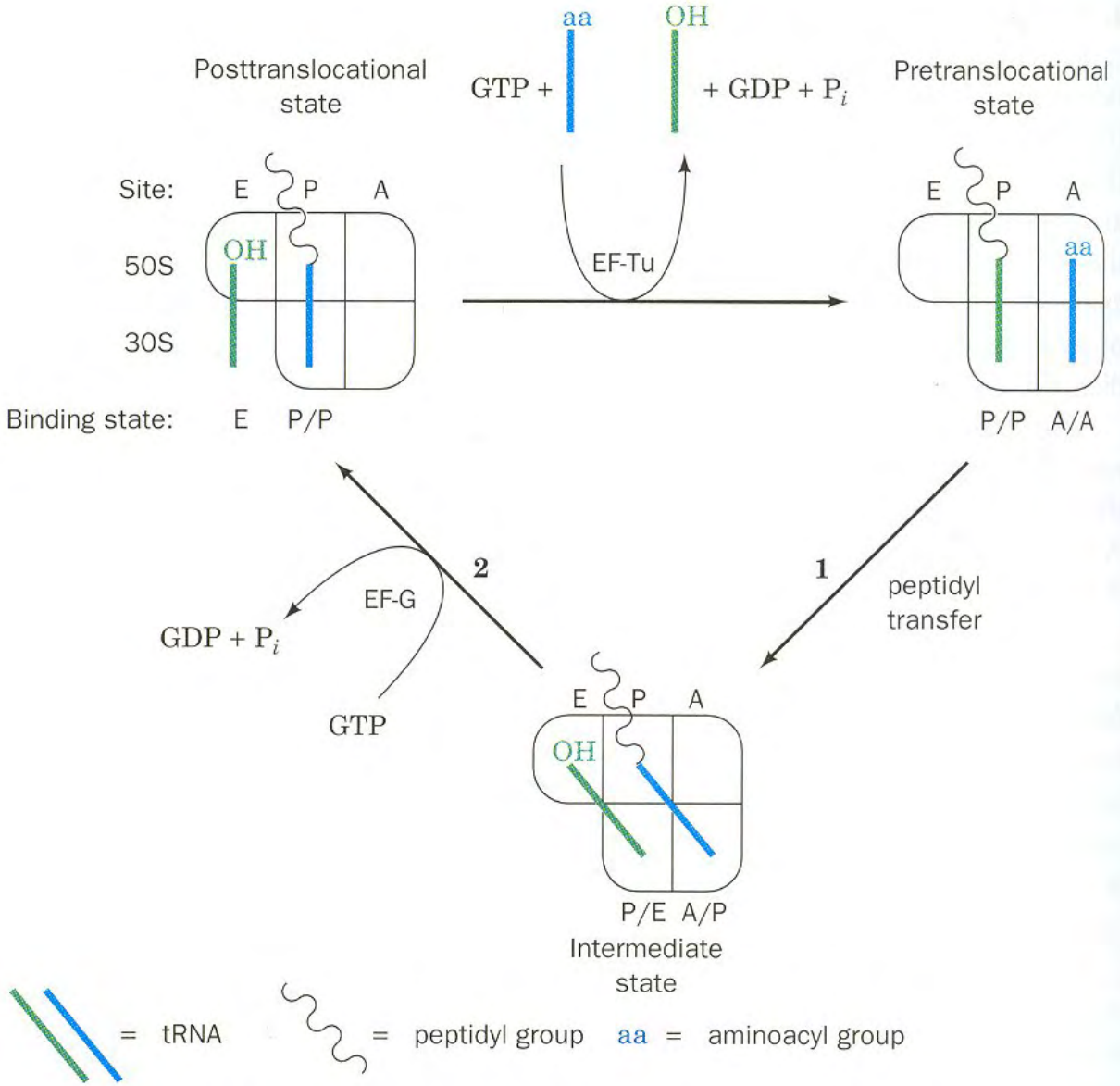

Poly(A) signal
Poly(A) site
5'
AAUAAA
G/U
3'
pre-mRNA
CPSF
CPSF
G/U
CFI, CFII, CStF
CPSF
CFI
CFII
CStF
PAP
CPSF
CFI
CFII
PAP
CStF
Cleavage
CPSF
OH P
PAP
CStF
ATP
PPi
Slow polyadenylation
CFI
CFII
CStF
P
G/U
(degraded)
AAAAAAAAAA
OH
PABII
PABII
AAAAAAAAAA
OH
ATP
PABII
Rapid polyadenylation
PPi
AAAAAAAAAA
AAAAAAAAAA
AAAAAAAAAA
200
PAP



The elongation cycle in *E. coli* ribosomes.

There are 4 classes of Introns (splicing mechanisms): group I, group II, snRNP requiring, tRNA





The ribosomal binding states in the elongation cycle.

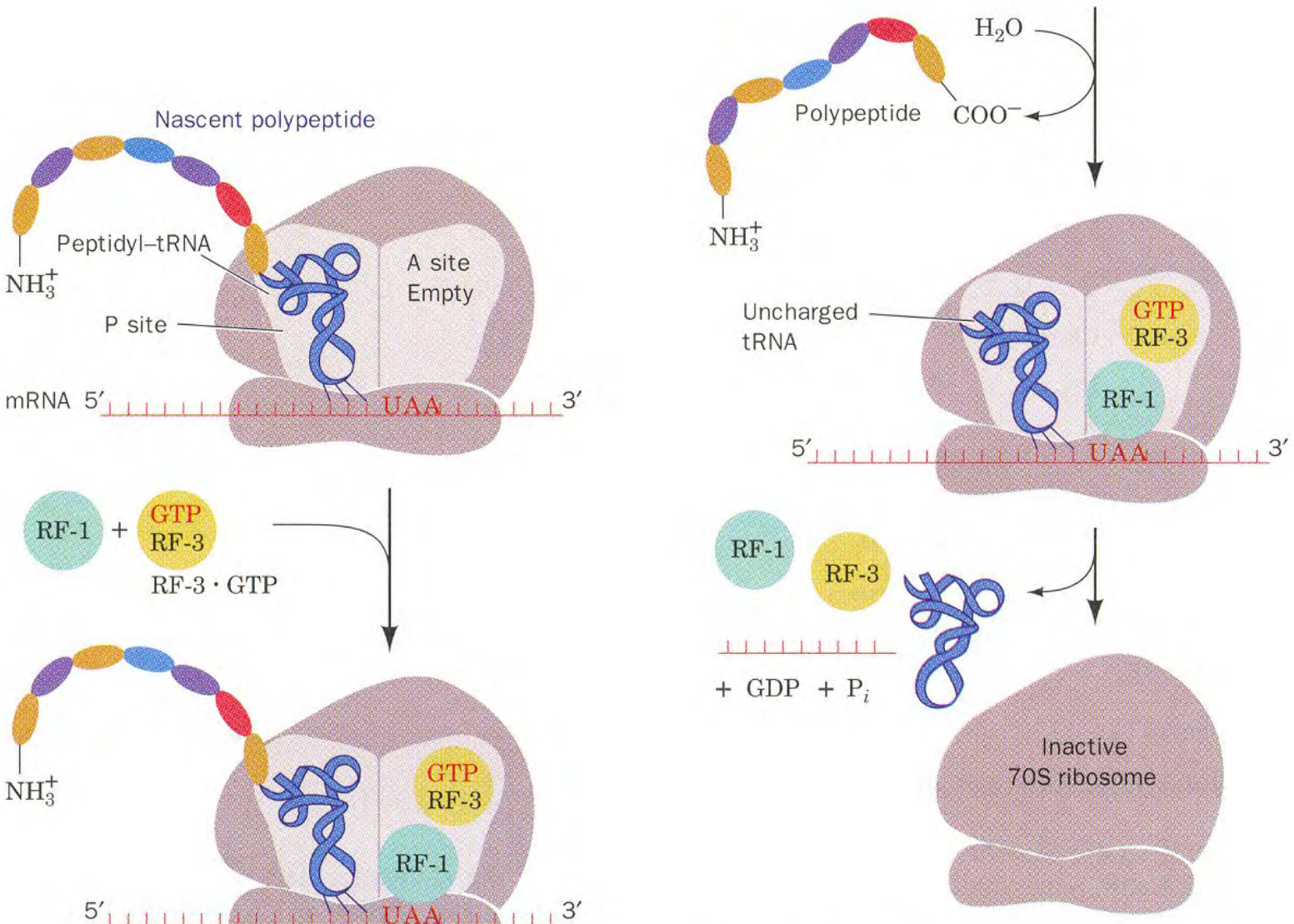


Figure 26-32. **The translation termination pathway in *E. coli* ribosomes.**

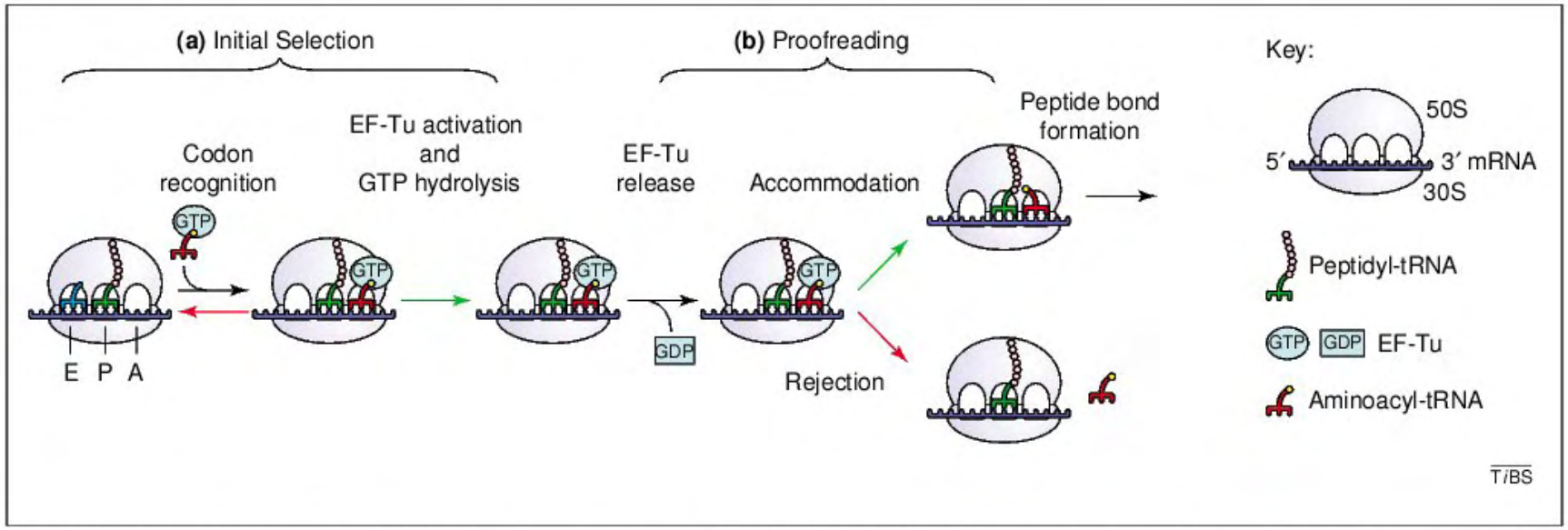


Fig. 2. The aminoacyl-tRNA (aa-tRNA) selection pathway. Ternary complexes of aa-tRNA, EF-Tu and GTP bind reversibly to the ribosome. The anticodon can access mRNA codon in the 30S A site, and if the codon is recognized as correct, the EF-Tu GTPase is activated. After GTP hydrolysis, the GDP form of EF-Tu dissociates, whereupon the aminoacyl end of cognate aa-tRNA moves into the peptidyl transferase site on the 50S (accommodation). Red arrows indicate steps at which non- or near-cognate aa-tRNA are rejected, either during initial selection before GTP hydrolysis or in a proofreading step thereafter. With near-cognate ternary complex, steps represented by green arrows are slower than in the cognate case, but are accelerated by paromomycin or streptomycin [7,8,11].

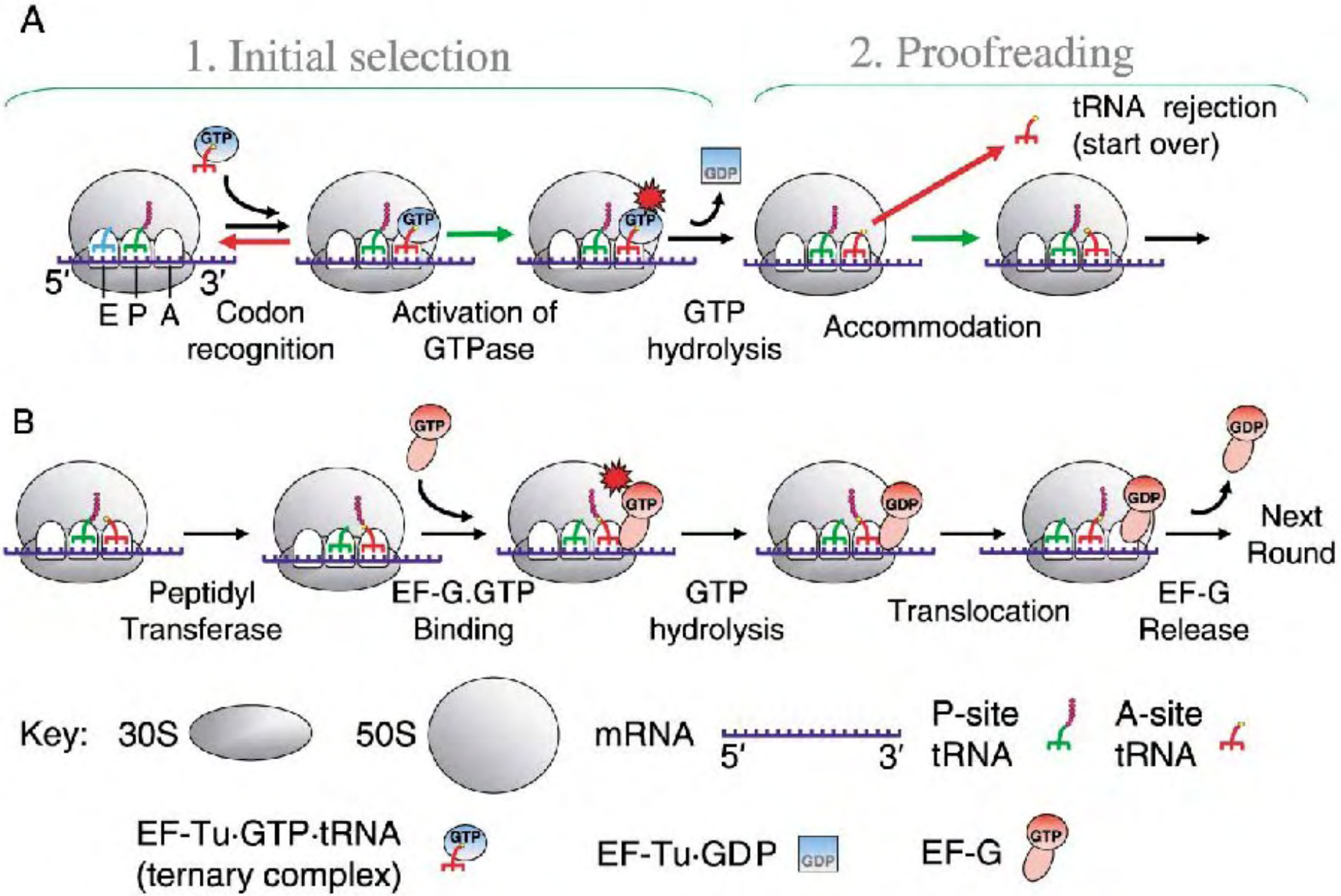


Figure 3. Overview of Elongation Pathway

For simplicity, not all of the steps discerned in kinetic experiments are shown.

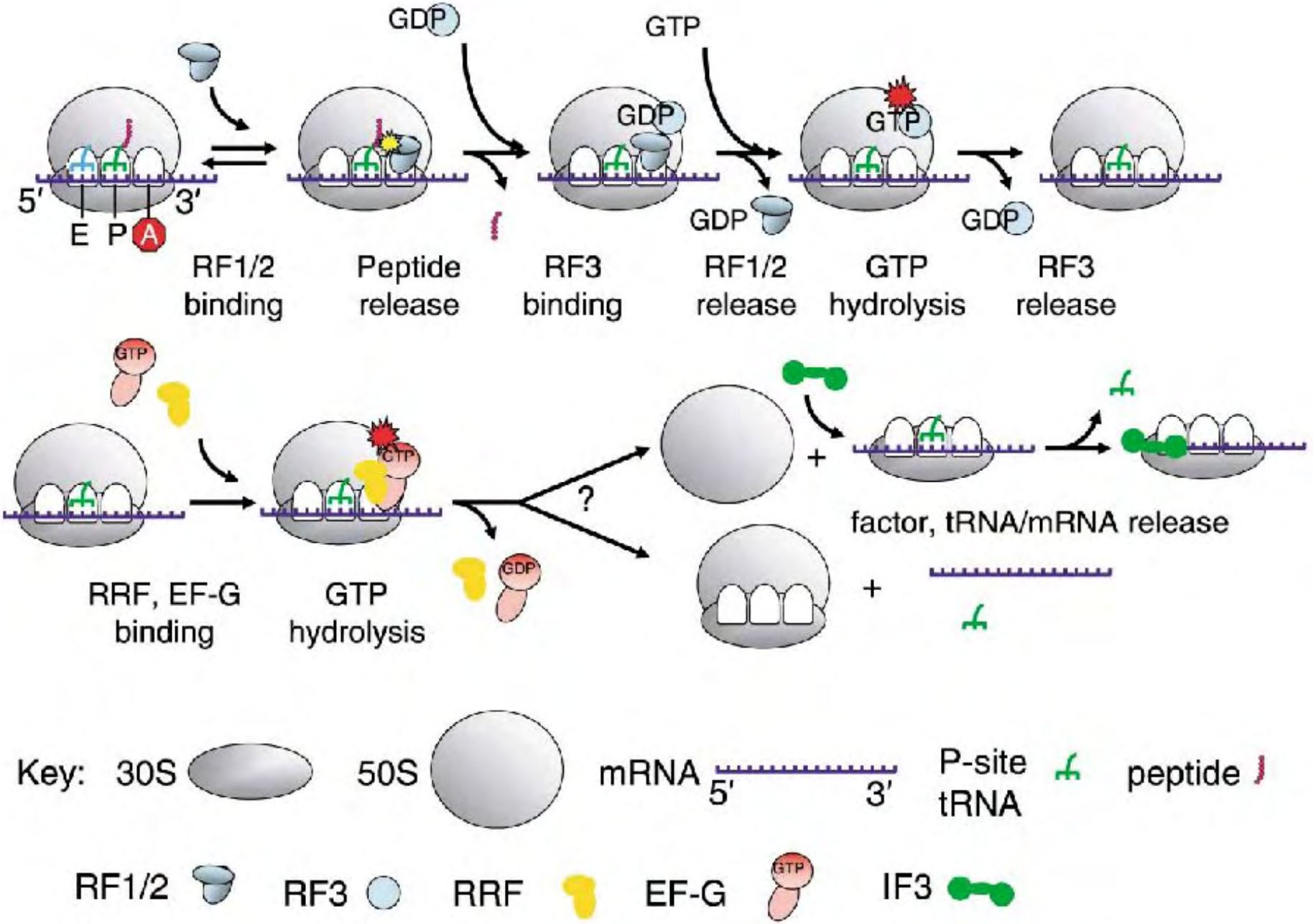
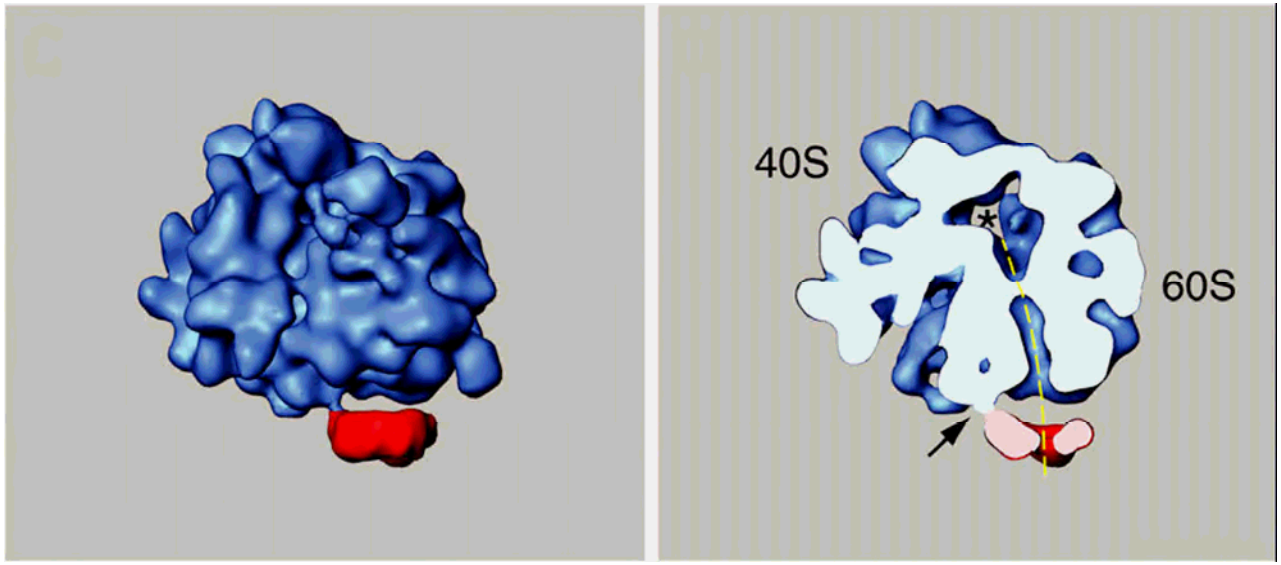


Figure 8. Overview of Termination in Translation


40S
60S

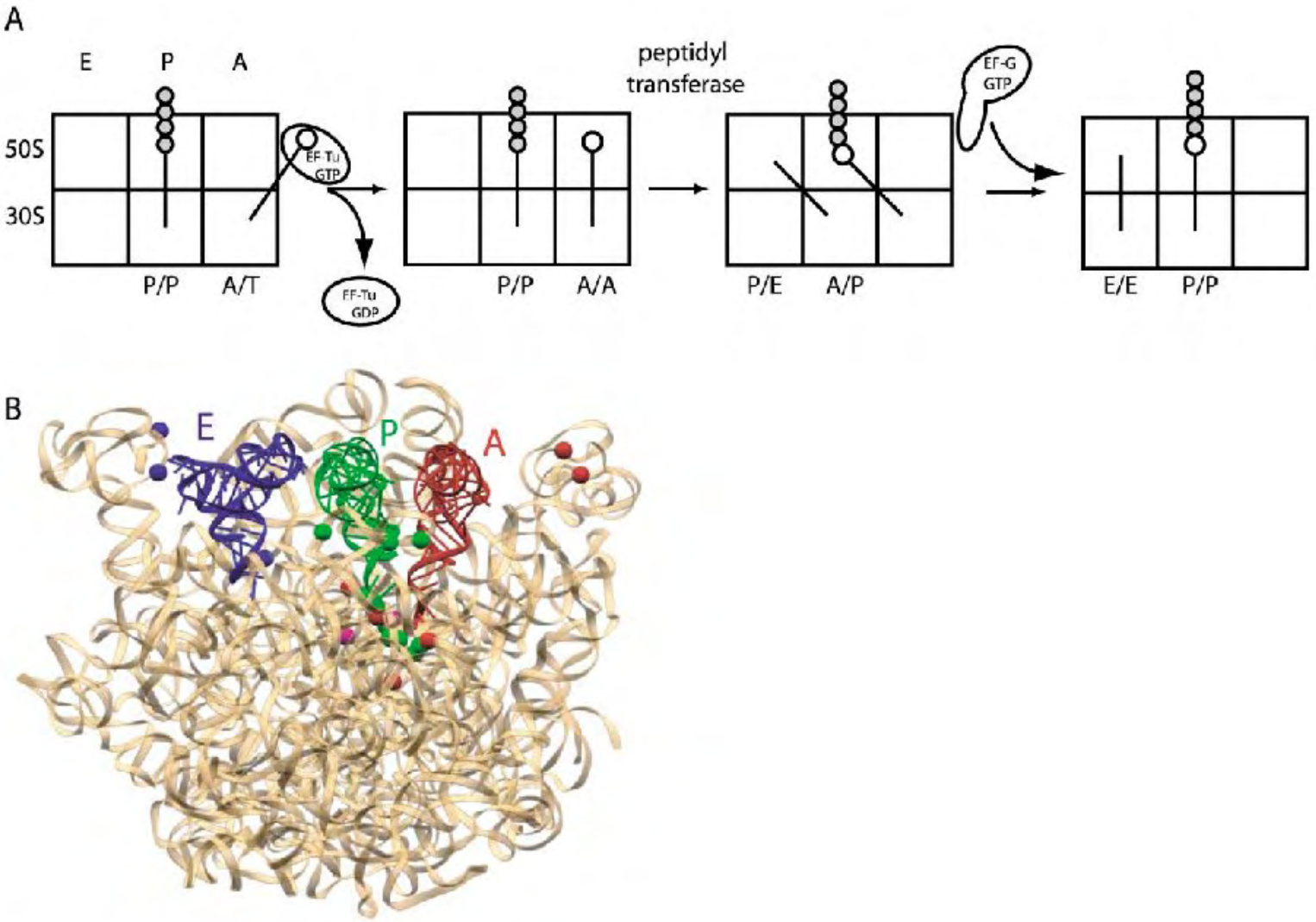
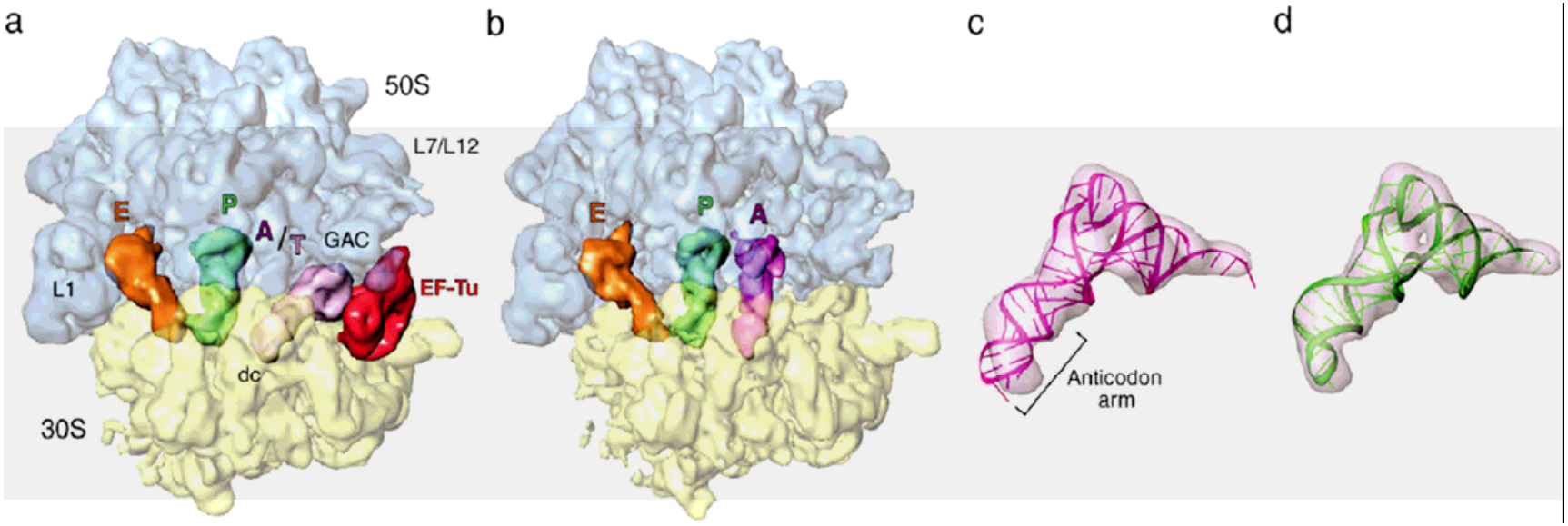
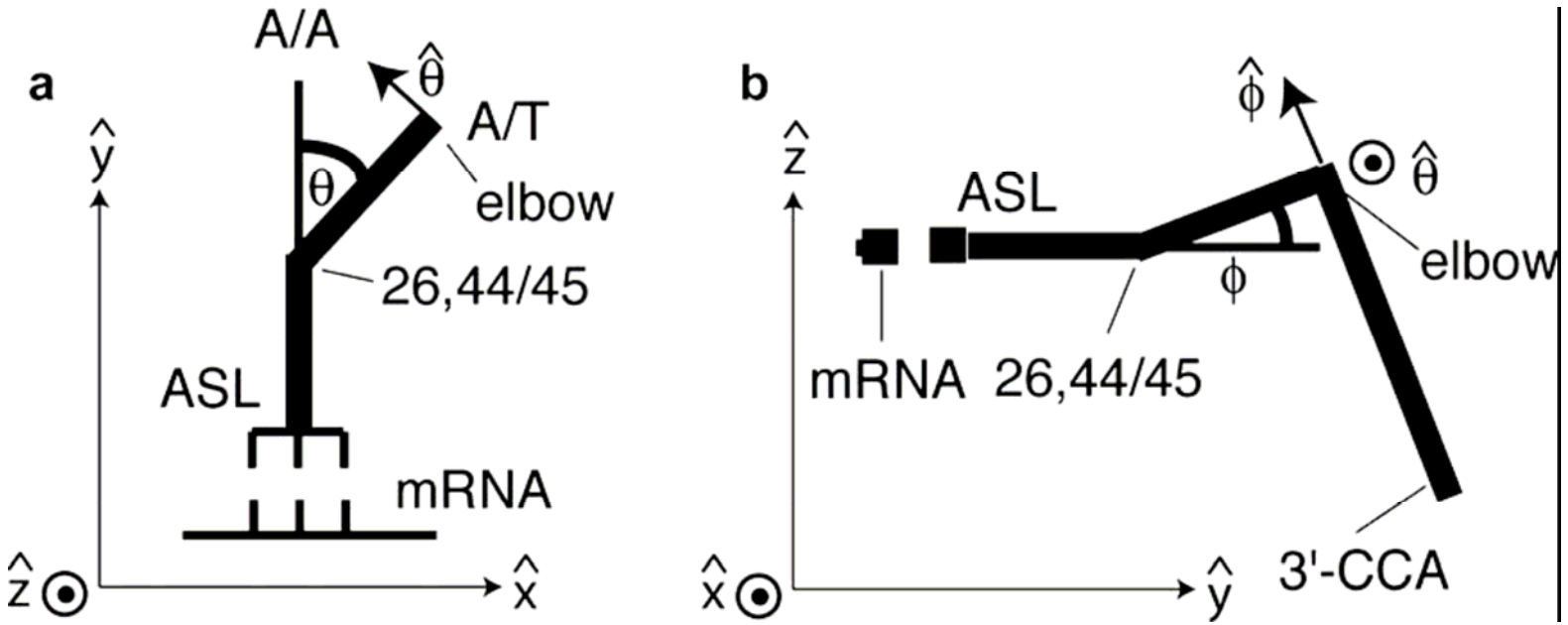


Figure 6. The Hybrid States Model for Translocation

(A) Cartoon of the hybrid states model of translocation as proposed by Moazed and Noller (1989), but now including an E site in the 30S subunit.

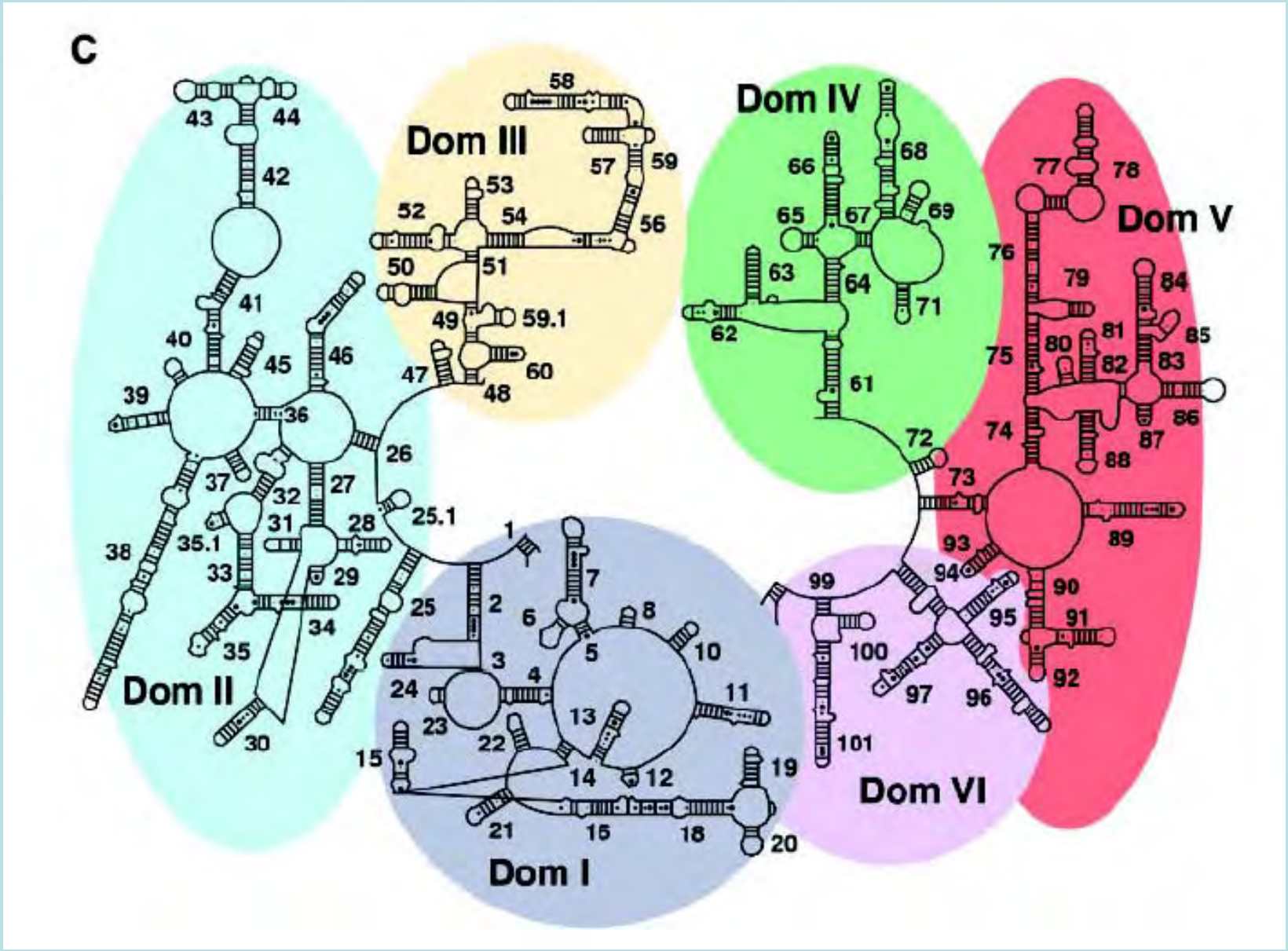
(B) The E-, P- and A-site tRNAs in the 50S subunit of the 70S structure (Yusupov et al., 2001), shown in blue, green, and red respectively. The base footprints from each tRNA (Moazed and Noller, 1989) are mapped onto the 70S structure and shown as spheres of the corresponding color. The two magenta spheres are bases affected by both A- and P-site tRNAs.

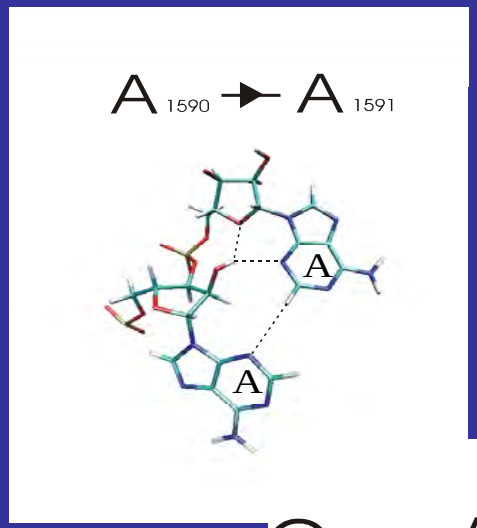

a
b
c
d
50S
L7/L12
E
P
A/T
GAC
L1
EF-Tu
dc
30S
E
P
A
Anticodon
arm

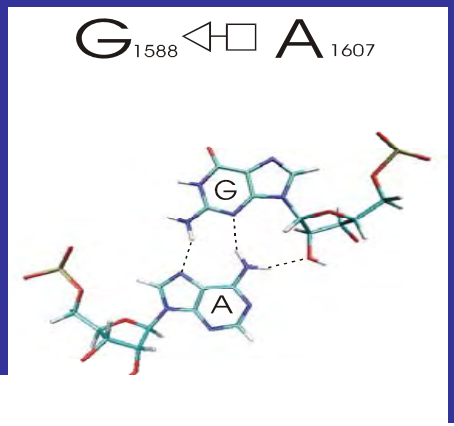

a
A/A
$\hat{\theta}$
A/T
elbow
θ
26,44/45
ASL
mRNA
$\hat{y}$
$\hat{z}$
$\hat{x}$
b
$\hat{\phi}$
$\hat{\theta}$
ASL
elbow
ϕ
mRNA
26,44/45
3'-CCA
$\hat{z}$
$\hat{x}$
$\hat{y}$

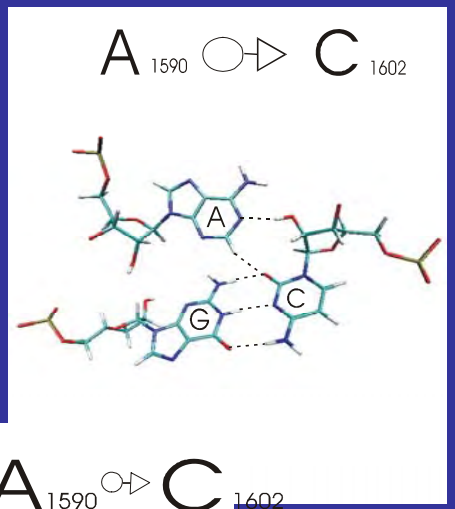
- Párování v RNA

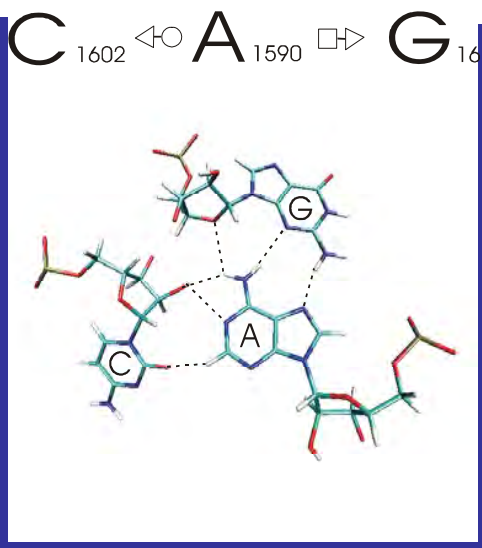
23S rRNA Domains

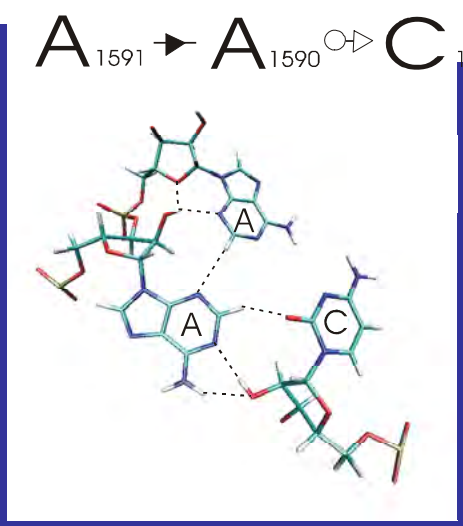






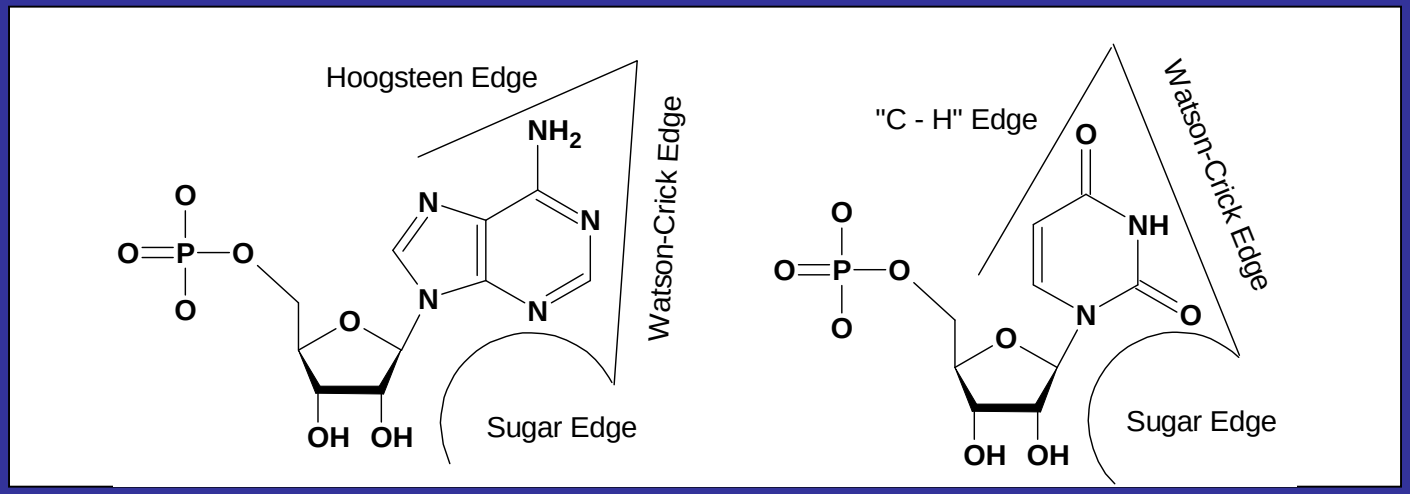


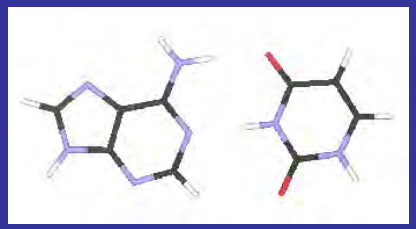




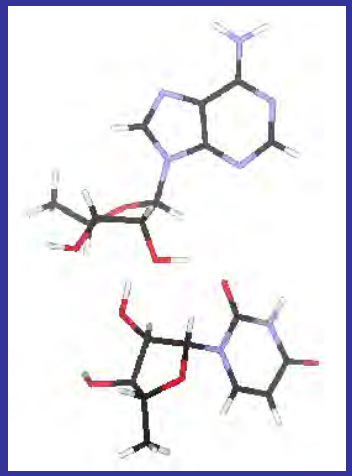
Non Watson-Crick basepairs – examples

Nomenklatura RNA párů bází, 150 – 200 principiálně možných kombinací.

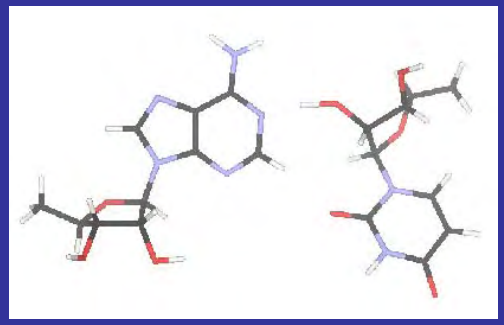




WC/WC AU

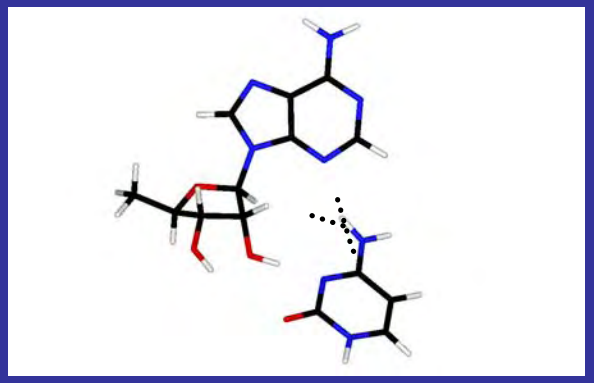


cis SE/SE rA/rU

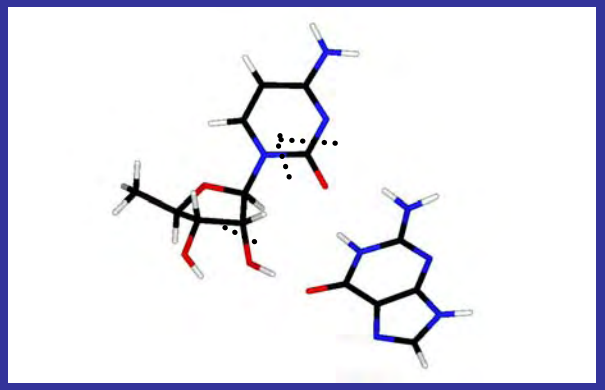


trans SE/SE rA/rU

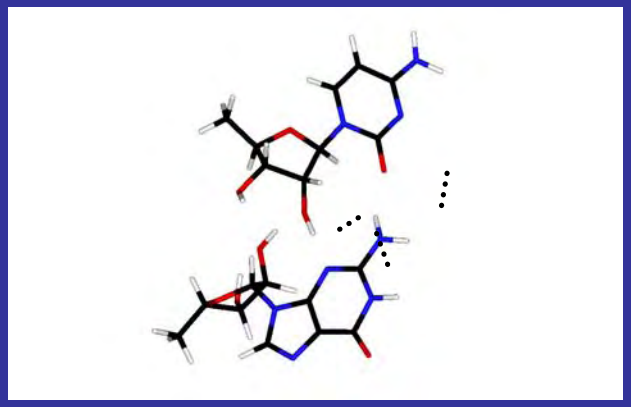
Kvantová chemie umožňuje i) přiřadit strukturám energii, ii) předpovědět nové páry bází, iii) spočíst NMR parametry, iv)……..



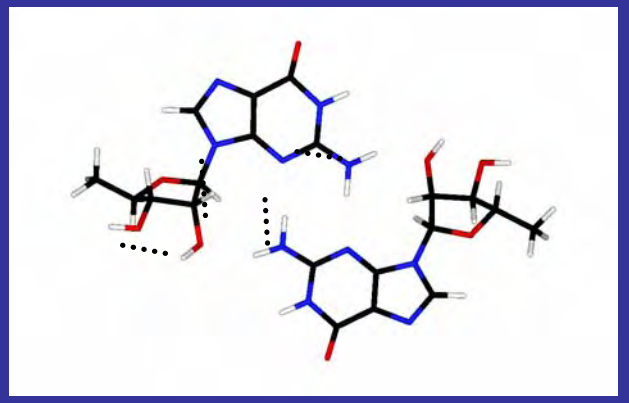
cis WC/SE C/rA
E_{int} = -19.5 kcal/mol



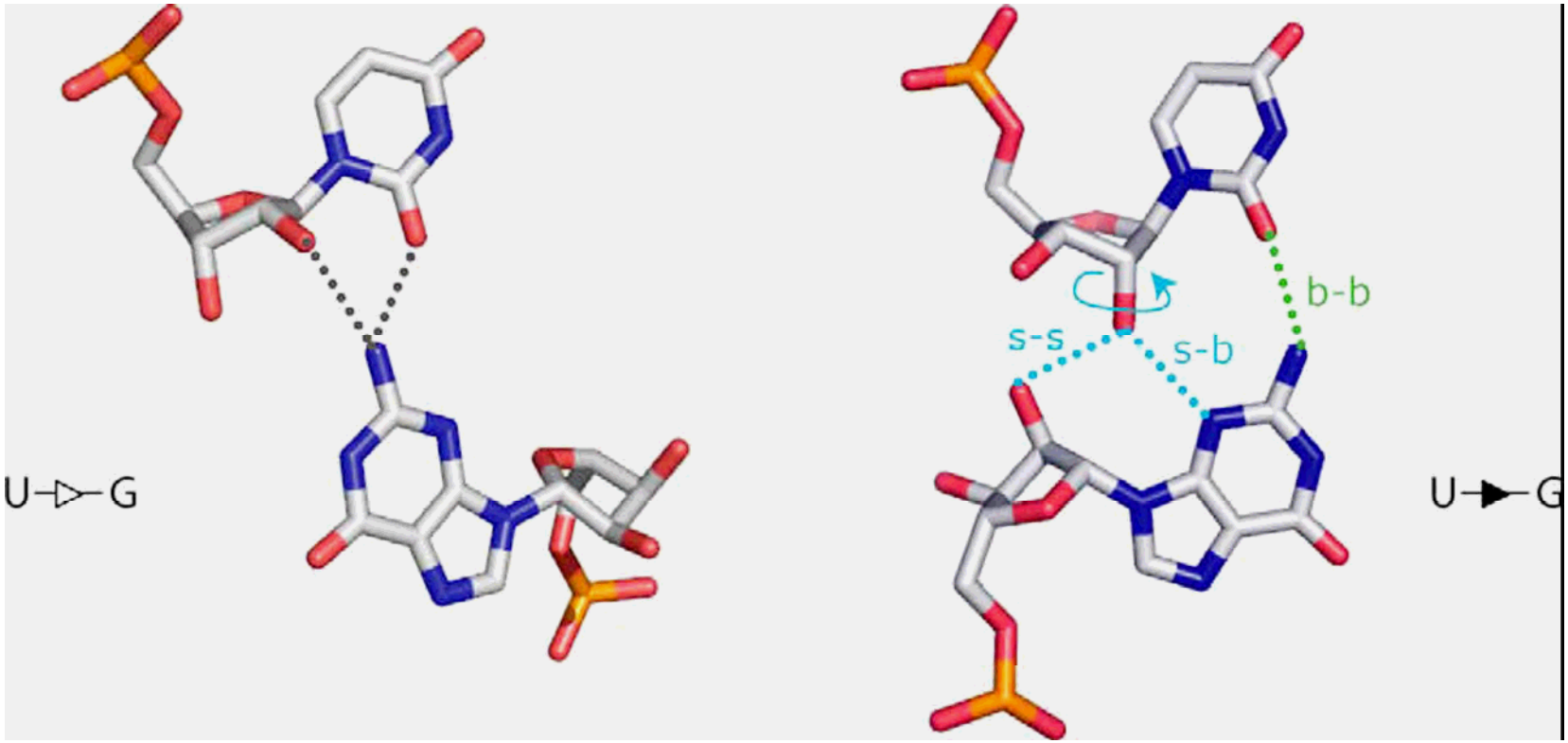
trans WC/SE G/rC
E_{int} = -28.4 kcal/mol

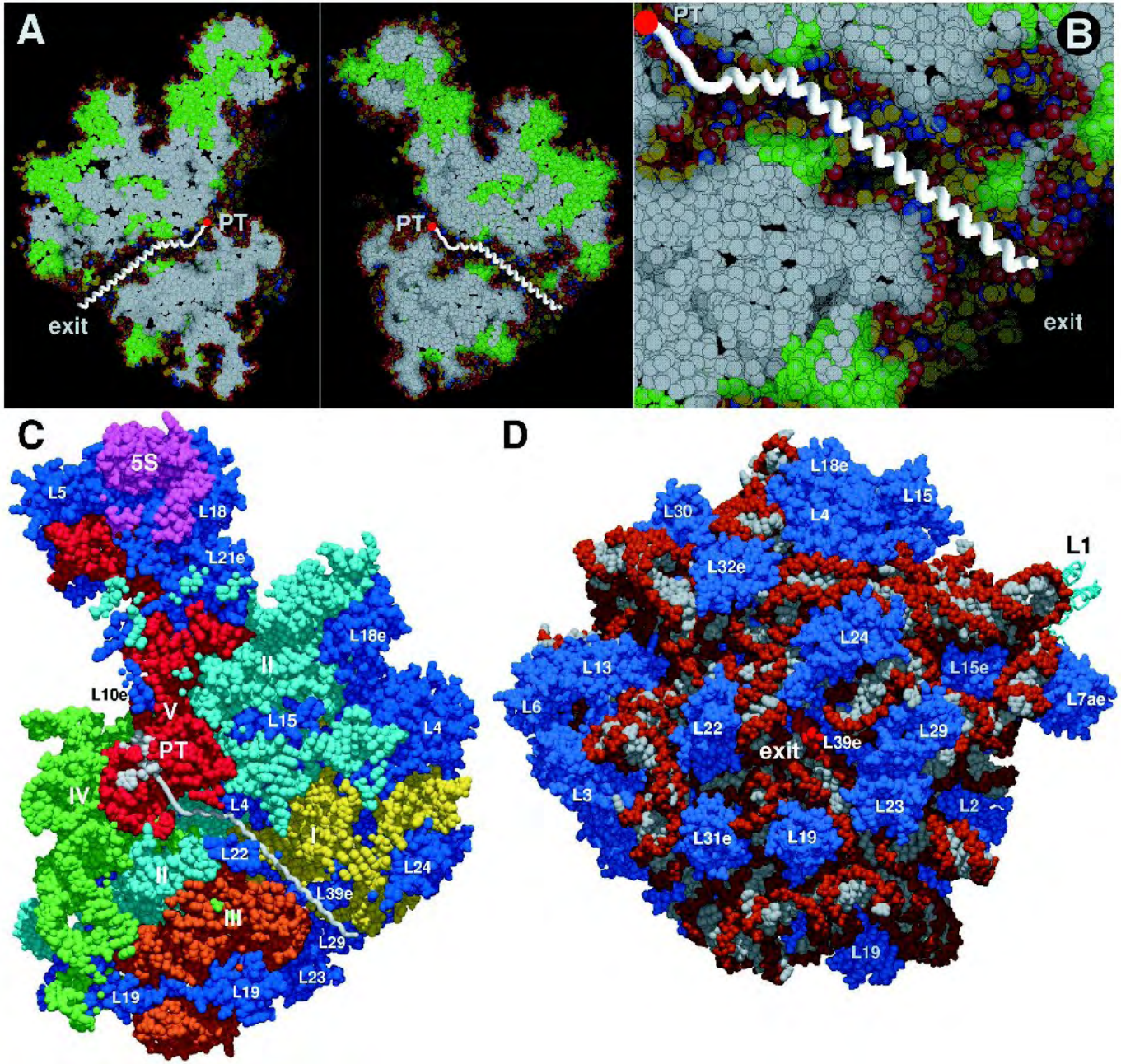


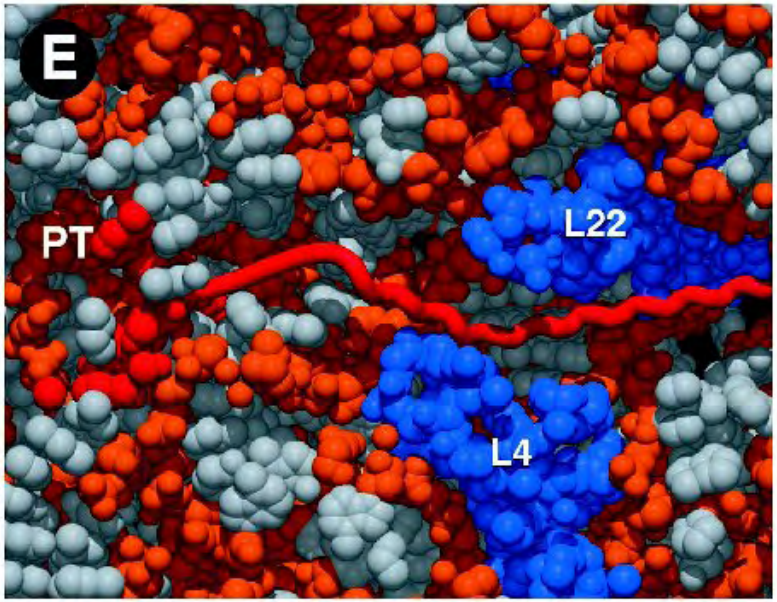
cis SE/SE rC/rG
E_{int} = -26.9 kcal/mol

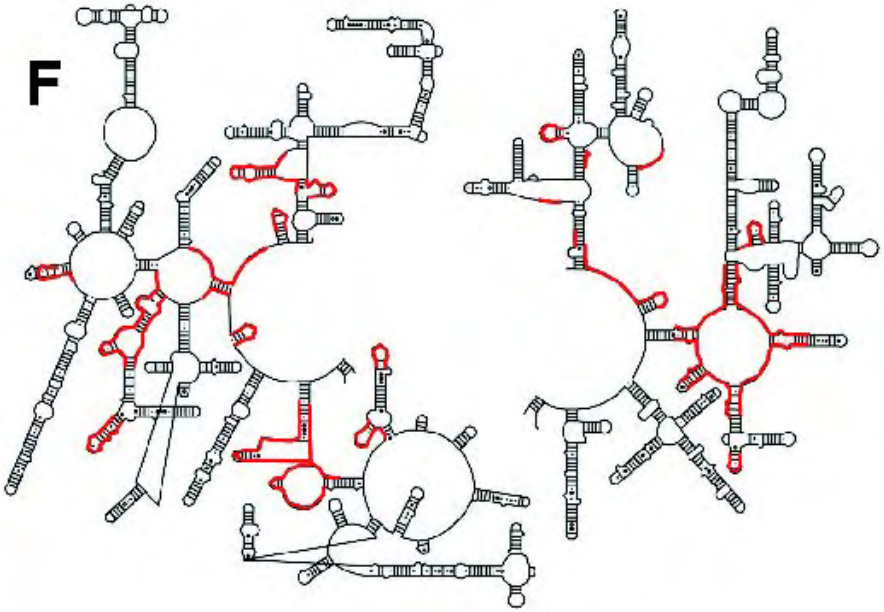


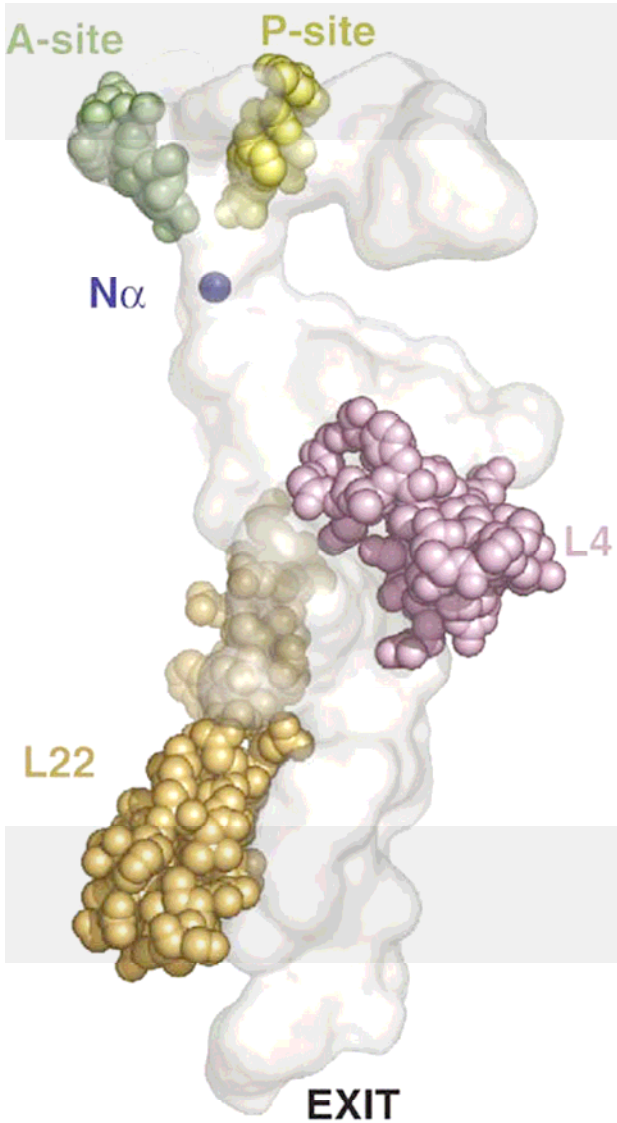
trans SE/SE rG/rG
E_{int} = -21.2 kcal/mol


U
G
b-b
s-s
s-b
U
G


A
PT
exit
PT
B
PT
exit
C
5S
L5
L18
L21e
L18e
II
L10e
V
L15
L4
PT
IV
L4
I
L22
II
L24
L39e
III
L29
L23
L19
L19
D
L18e
L15
L30
L4
L1
L32e
L24
L13
L15e
L6
L7ae
L22
exit
L39e
L29
L3
L23
L2
L31e
L19
L19


E
PT
L22
L4


F



20 Å

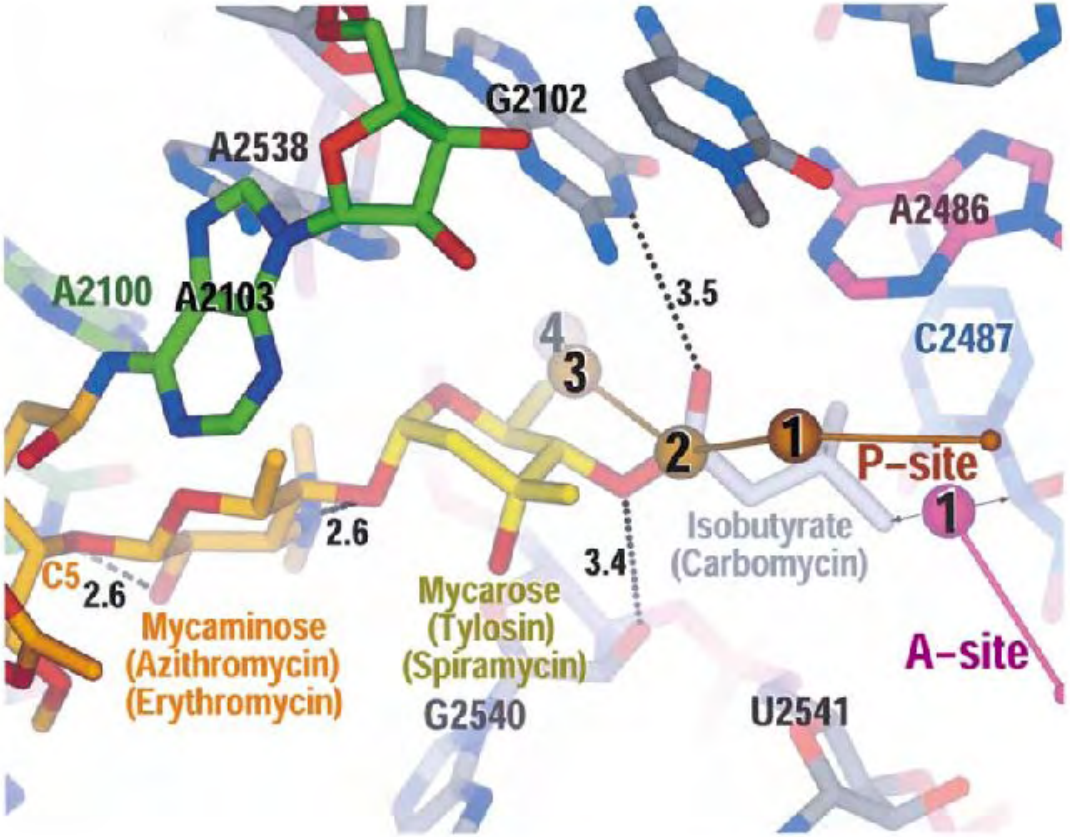
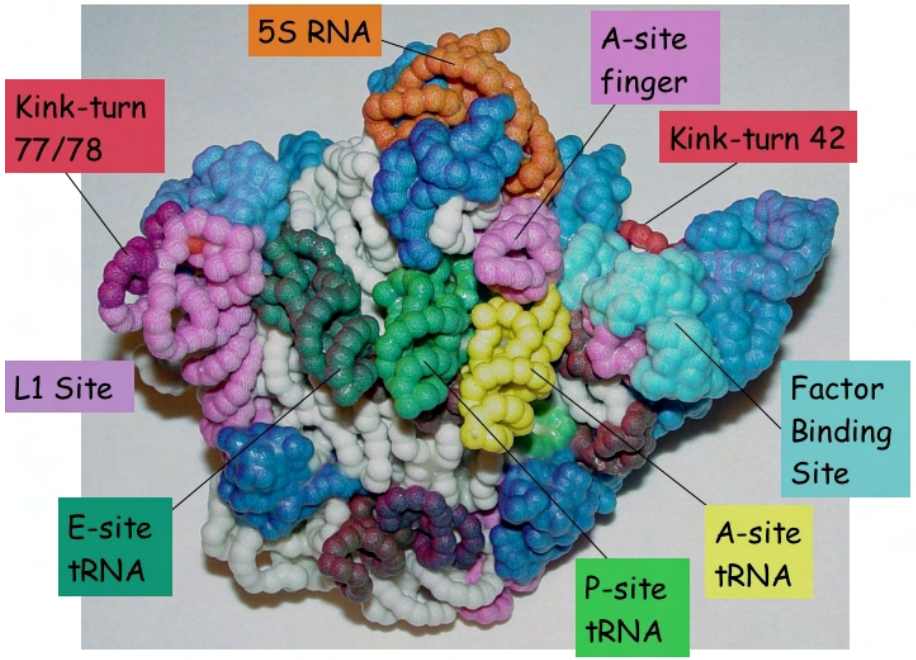
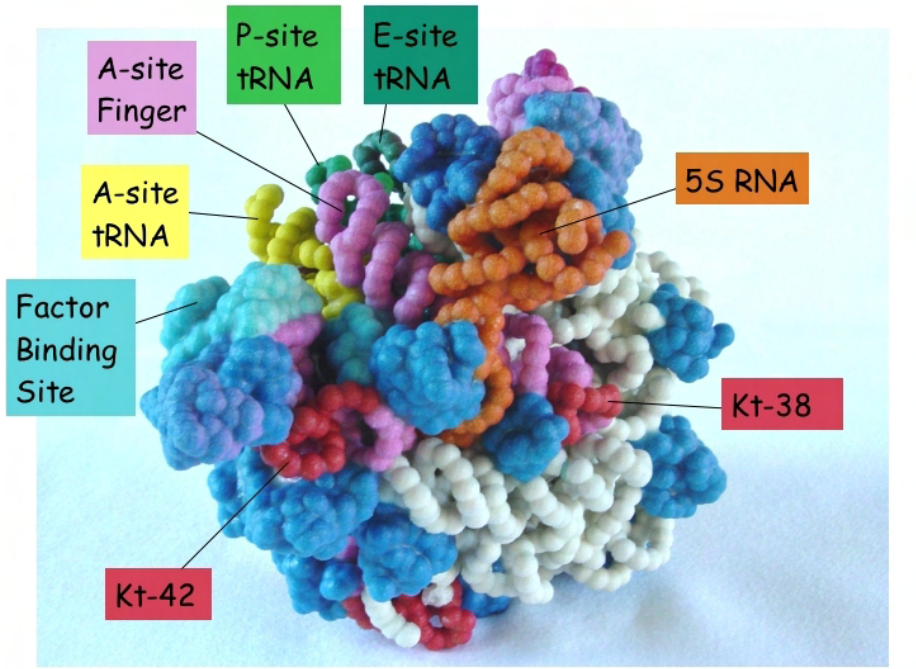


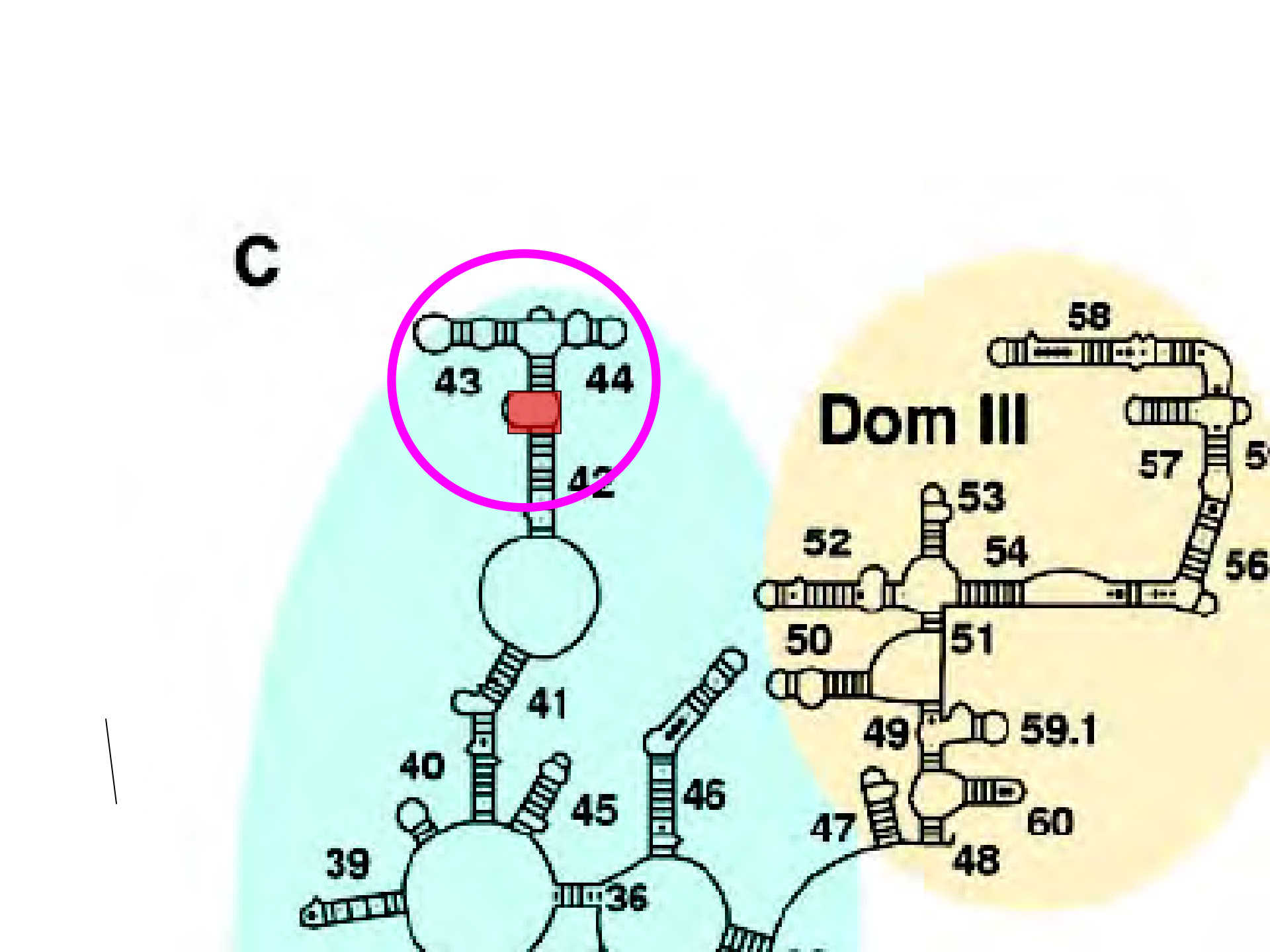
Figure 7. The Correlation between Macrolide Structures and Their Effect on Protein Synthesis

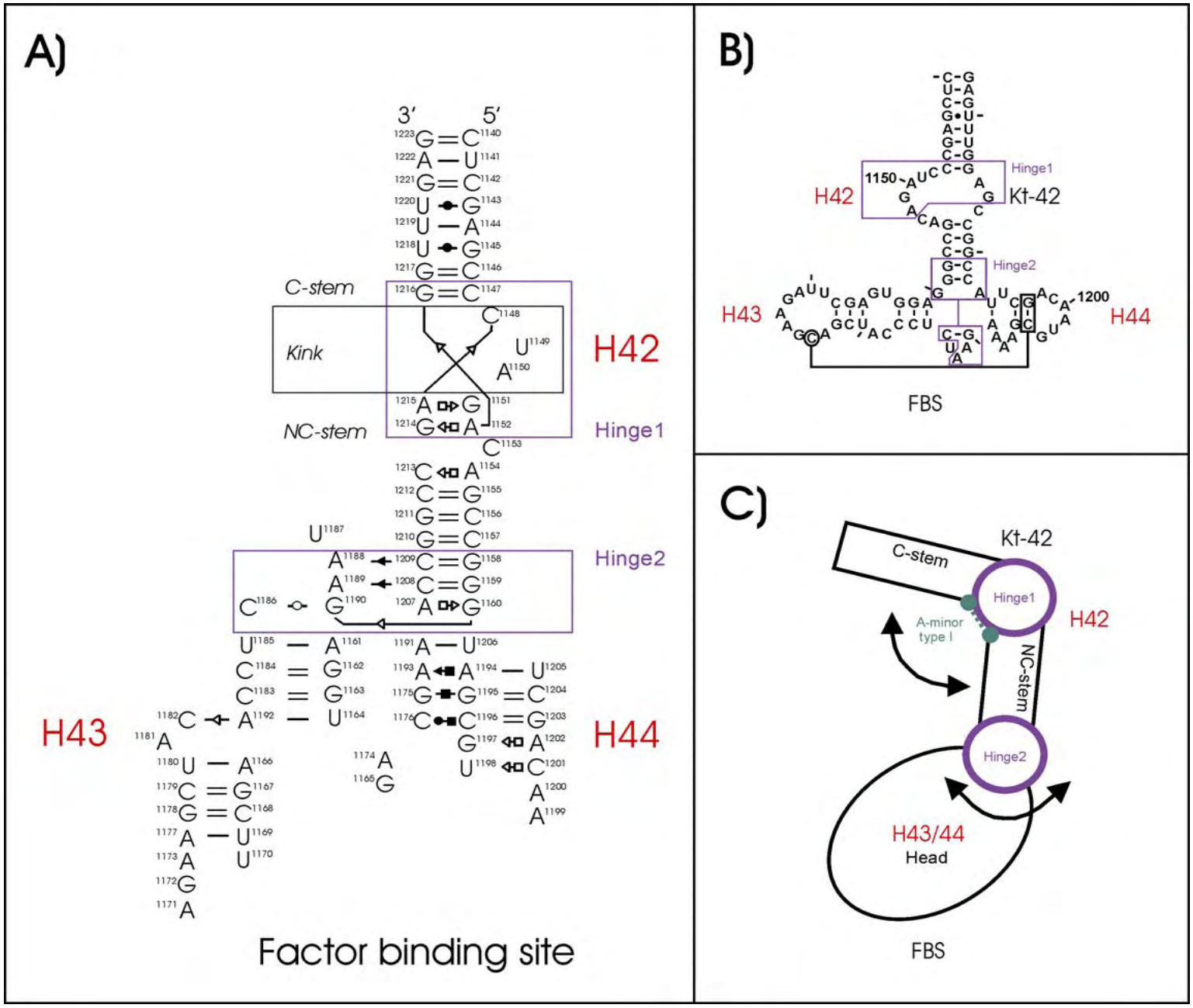
Carbomycin A in stick representation with the three moieties attached to the C5 of carbomycin lactone ring, mycaminose (orange), mycarose (yellow), and isobutyrate (gray) in order to highlight the inverse relationship between the length of the C5 branch of a macrolide and the length of an oligopeptide (brown α-carbon spheres) that can be synthesized in the presence of the macrolide. The isobutyrate group of carbomycin reaches the peptidyl transferase center and occupies the A-site amino-acid binding pocket, which would prevent binding of an A-site substrate (purple α-carbon sphere number 1) and prevents formation of even a dipeptide on a P-site substrate (brown α-carbon spheres number 1 and 2). Tylosin extends to the mycarose sugar (yellow) and allows only dipeptide formation. Azithromycin (and erythromycin) which have only a monosaccharide extension from C5 of the lactone ring (orange) allow formation of up to tetra-peptides.

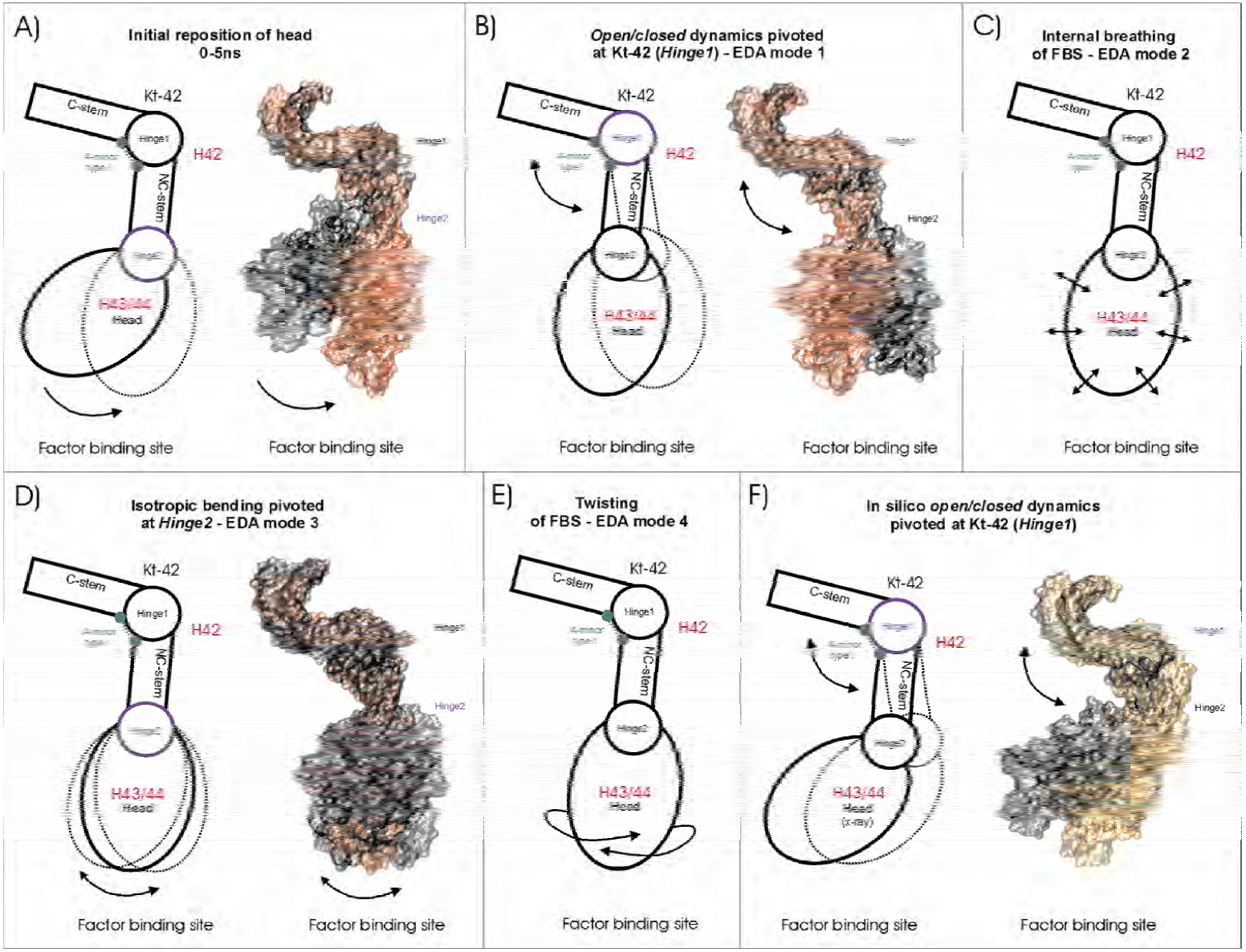
RNA motivy

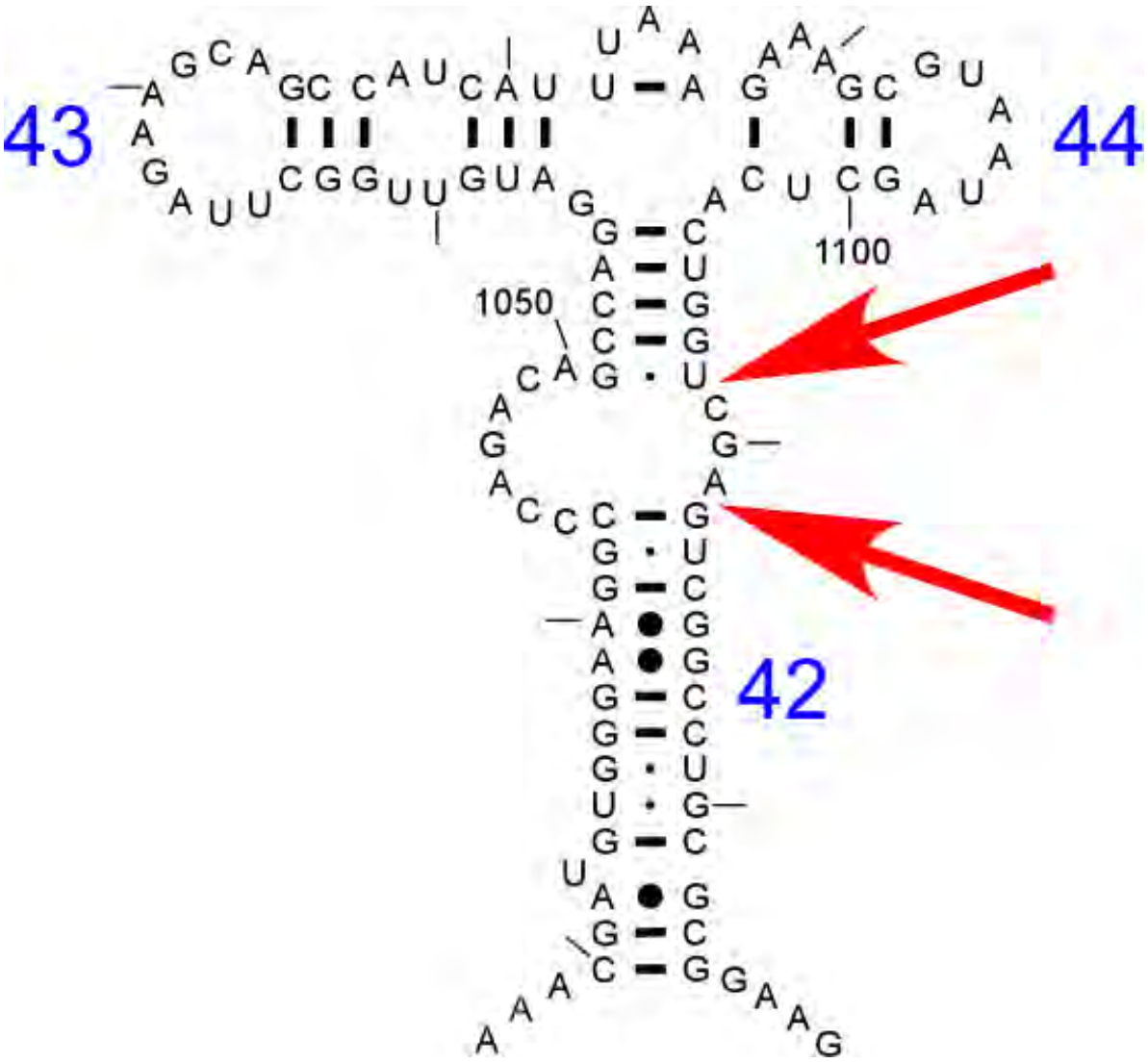

5S RNA
A-site finger
Kink-turn 77/78
Kink-turn 42
L1 Site
Factor Binding Site
E-site tRNA
A-site tRNA
P-site tRNA


P-site tRNA
E-site tRNA
A-site Finger
A-site tRNA
5S RNA
Factor Binding Site
Kt-38
Kt-42

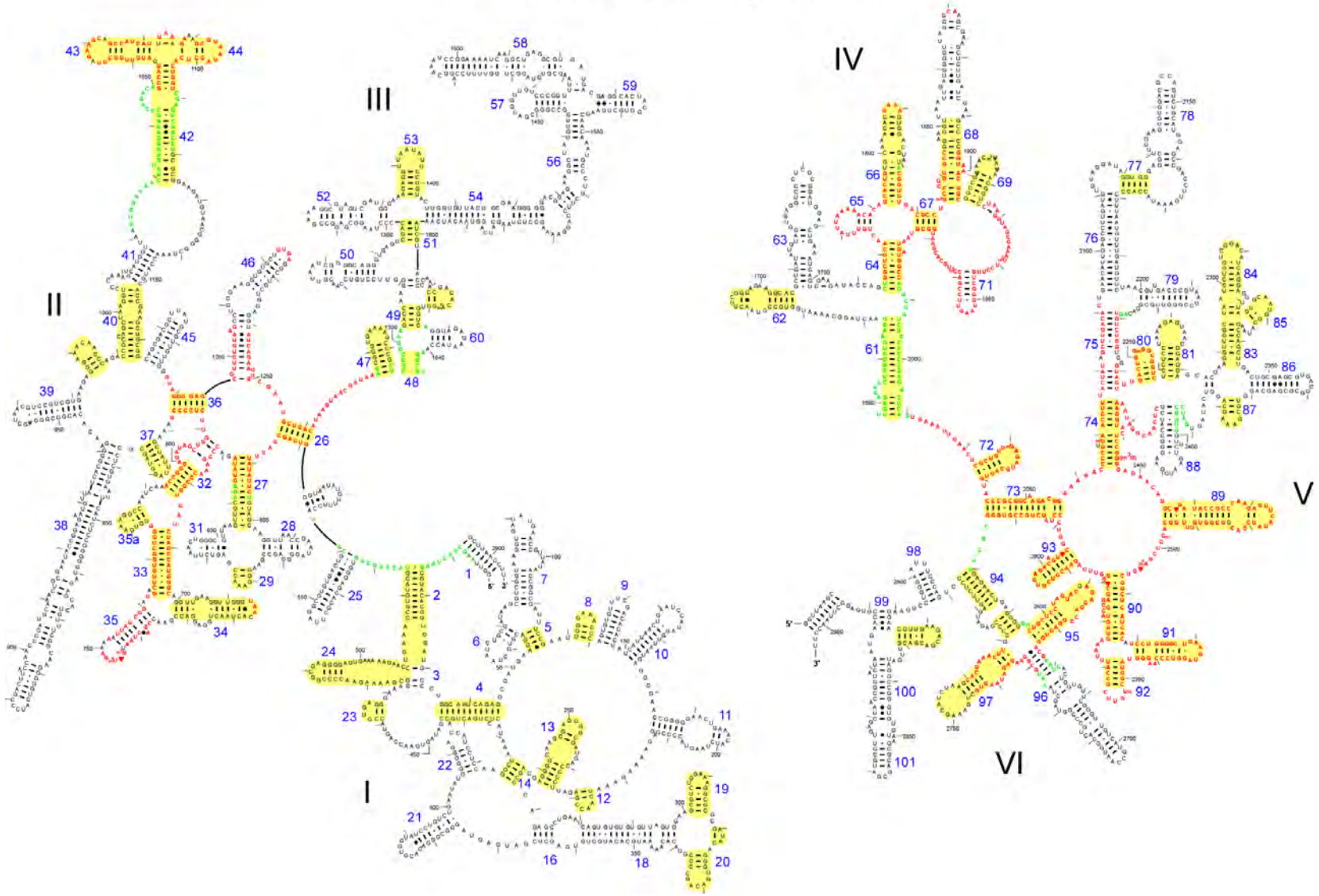

C
Dom III
58
57
53
52
54
56
50
51
49
59.1
60
47
48
43
44
42
41
40
45
46
39
36

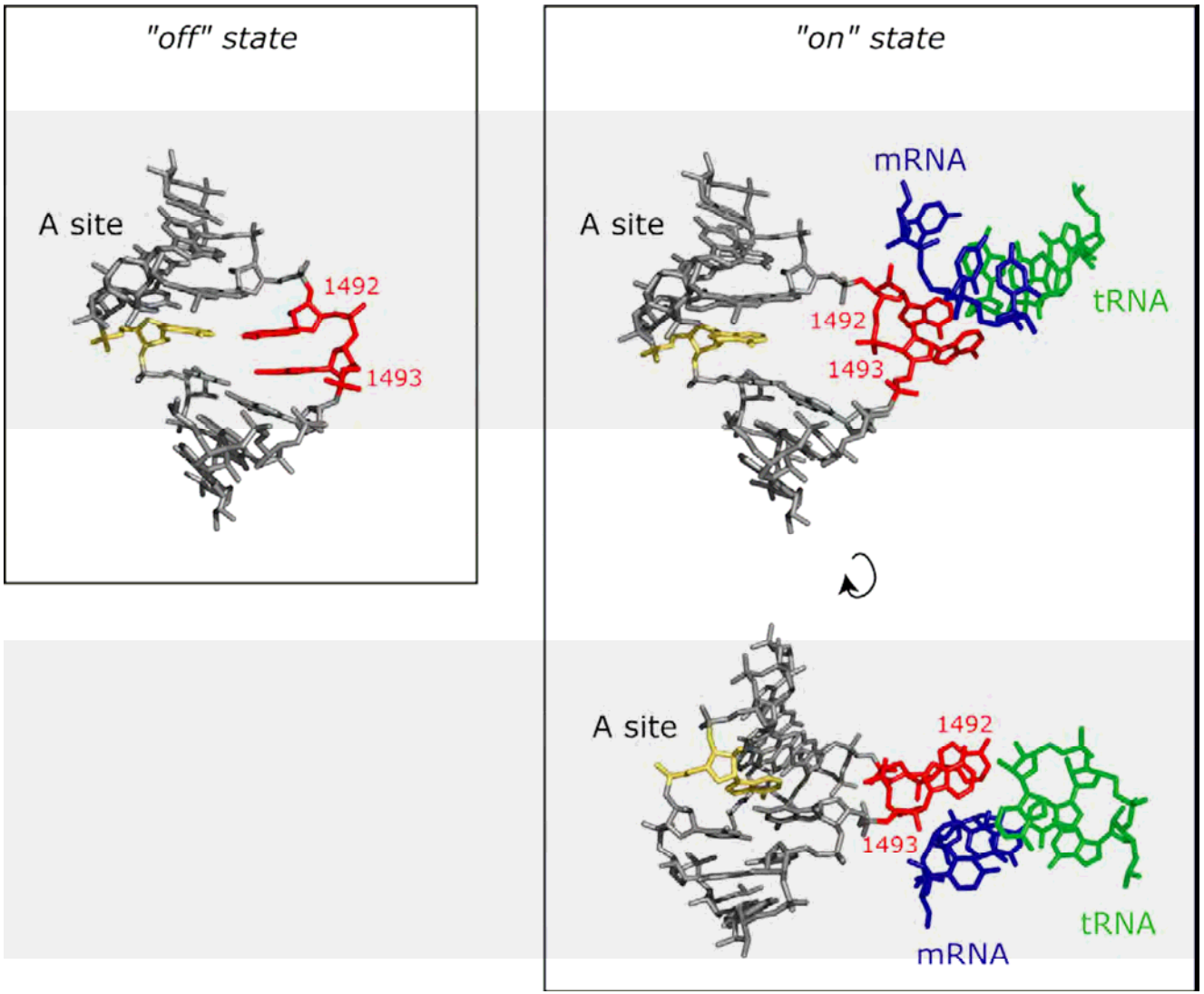

A)
3′
5′
C-stem
Kink
NC-stem
H42
Hinge1
Hinge2
H43
H44
Factor binding site
B)
Hinge1
H42
Kt-42
Hinge2
H43
H44
1200
FBS
C)
Kt-42
C-stem
Hinge1
H42
A-minor
type I
NC-stem
Hinge2
H43/44
Head
FBS


A)
Initial reposition of head
0-5ns
Kt-42
C-stem
Hinge1
H42
NC-stem
H43/44
Head
Hinge2
Factor binding site
Factor binding site
B)
Open/closed dynamics pivoted
at Kt-42 (Hinge1) - EDA mode 1
Kt-42
C-stem
H42
NC-stem
H43/44
Head
Hinge2
Factor binding site
Factor binding site
C)
Internal breathing
of FBS - EDA mode 2
Kt-42
C-stem
H42
NC-stem
H43/44
Head
Factor binding site
D)
Isotropic bending pivoted
at Hinge2 - EDA mode 3
Kt-42
C-stem
Hinge1
H42
NC-stem
H43/44
Head
Hinge2
Factor binding site
Factor binding site
E)
Twisting
of FBS - EDA mode 4
Kt-42
C-stem
Hinge1
H42
NC-stem
H43/44
Head
Factor binding site
F)
In silico open/closed dynamics
pivoted at Kt-42 (Hinge1)
Kt-42
C-stem
H42
NC-stem
H43/44
Head
(x-ray)
Hinge2
Factor binding site
Factor binding site

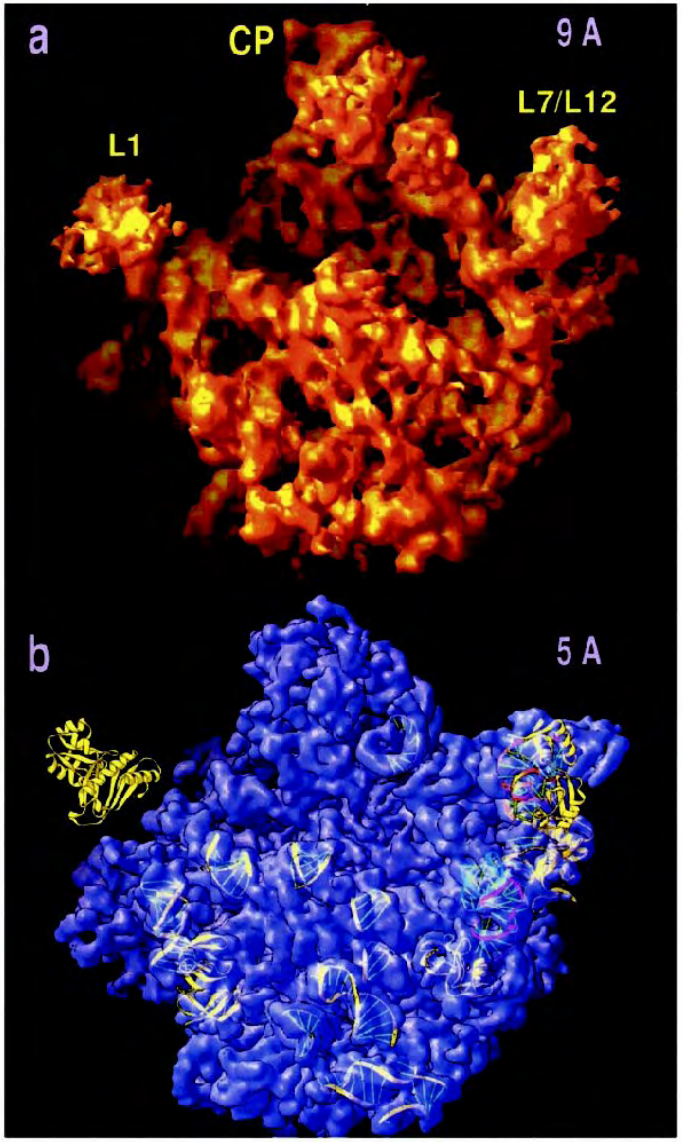

43
44
42
1100
1050

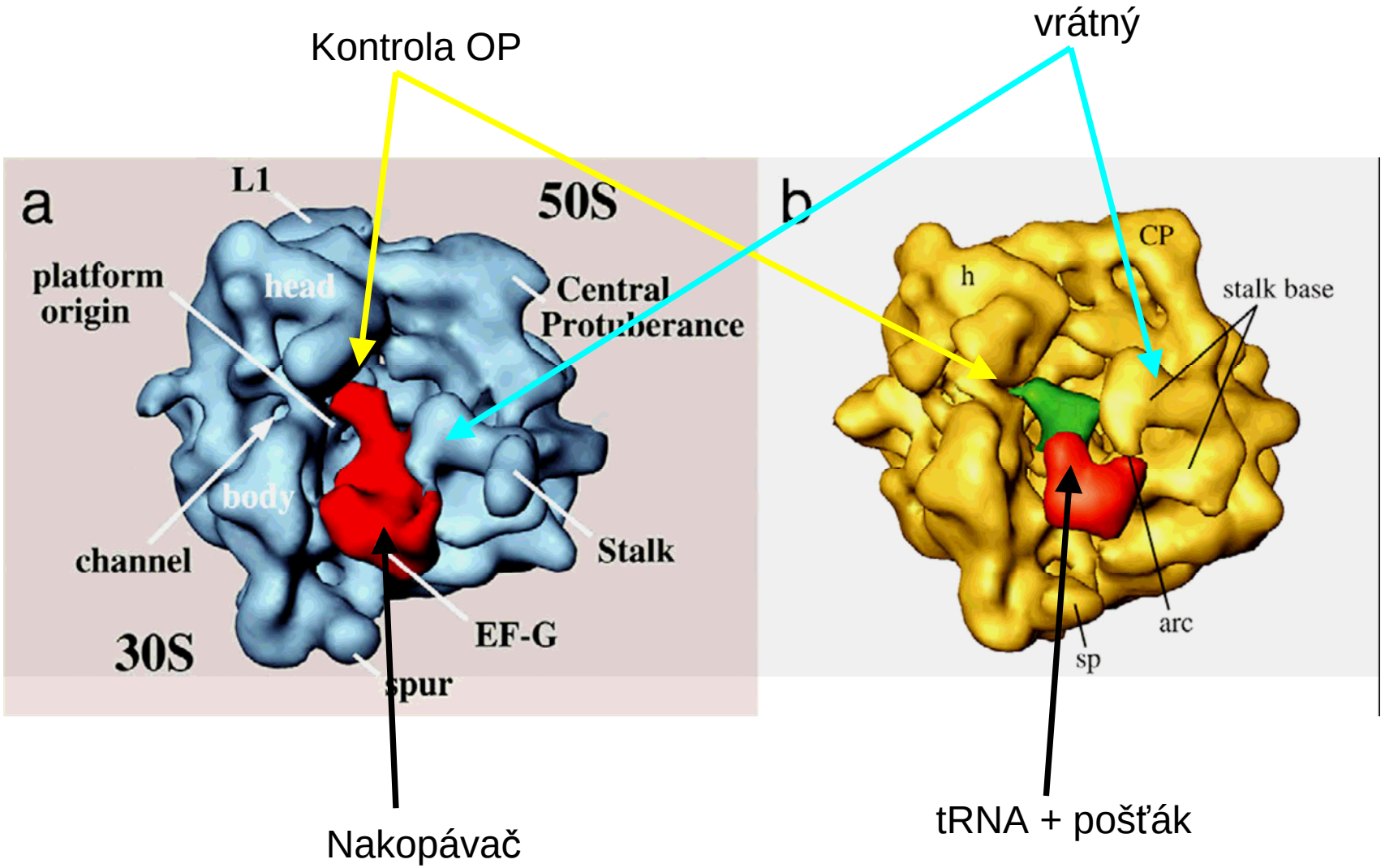
Secondary Structure of Escherichia coli large ribosomal subunit

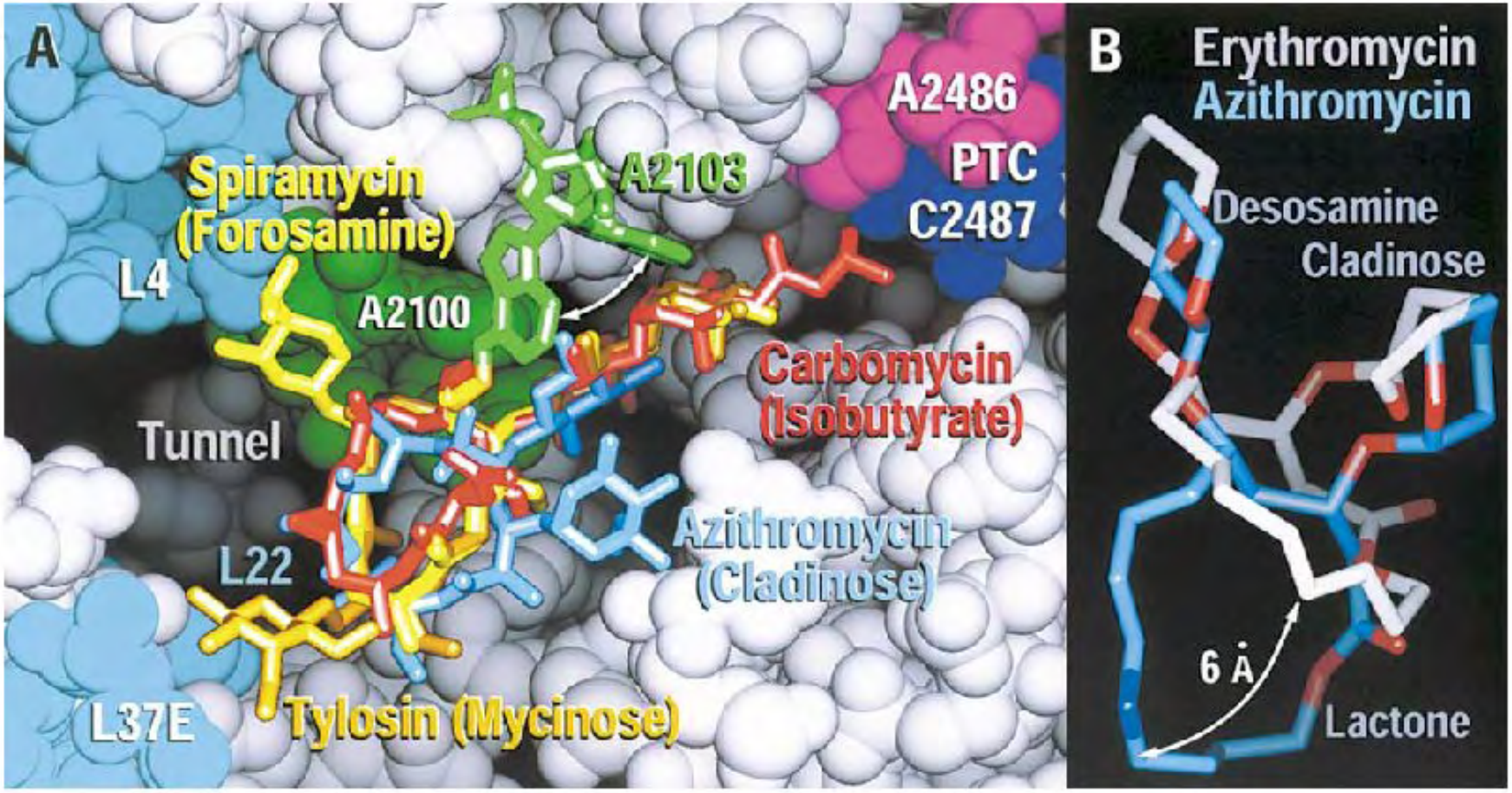


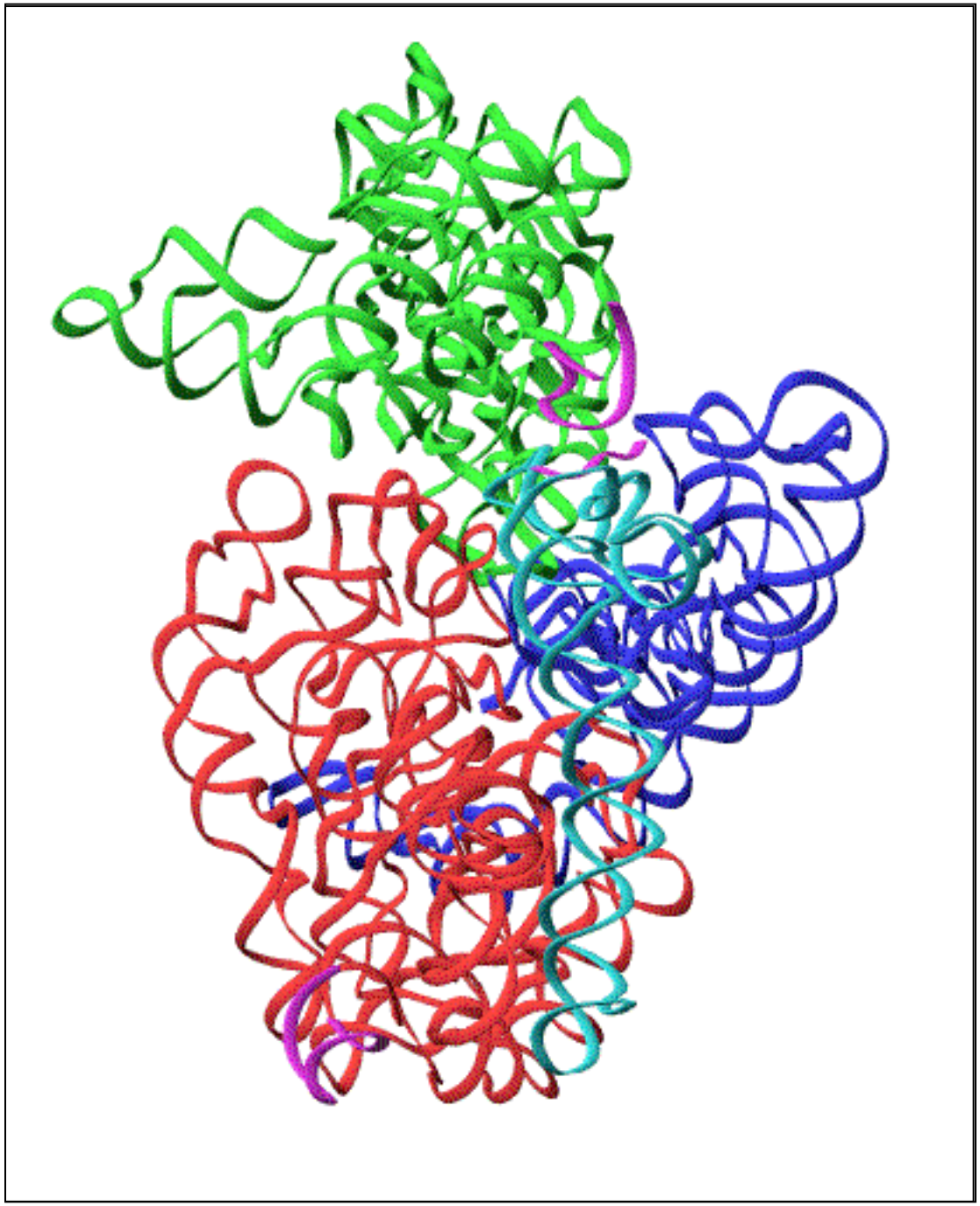

"off" state
A site
1492
1493
"on" state
mRNA
A site
1492
1493
tRNA
A site
1492
1493
tRNA
mRNA

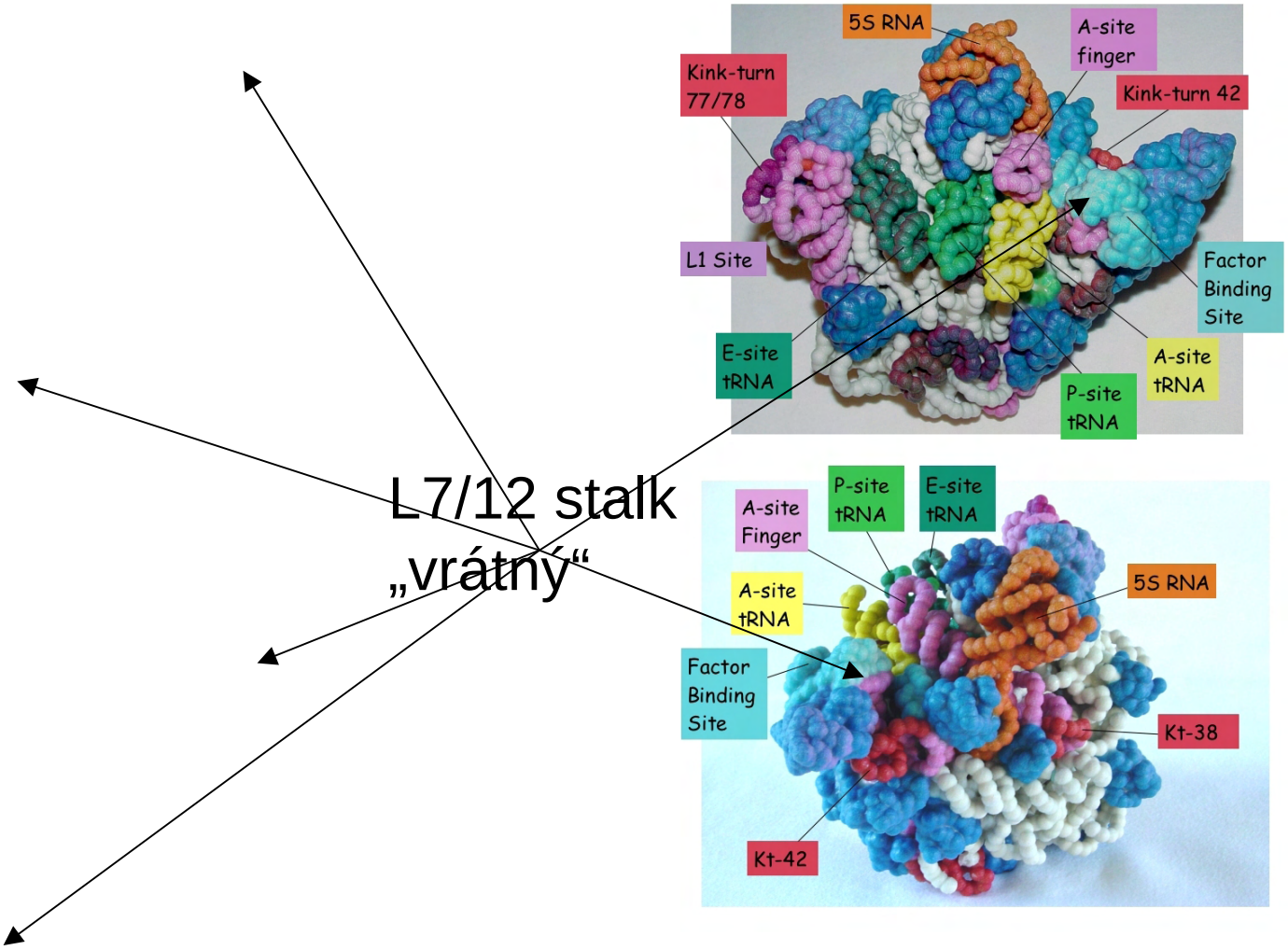
Rentgenostrukturní analýza (krystalografie) v 90tých letech.
Před dosažením atomárního rozlišení.




Kontrola OP
vrátný
a
L1
50S
platform origin
head
Central Protuberance
body
channel
Stalk
EF-G
30S
spur
b
CP
h
stalk base
arc
sp
Nakopávač
tRNA + pošťák


A
A2486
PTC
C2487
A2103
Spiramycin
(Forosamine)
L4
A2100
Carbomycin
(Isobutyrate)
Tunnel
Azithromycin
(Cladinose)
L22
L37E
Tylosin (Mycinose)
B
Erythromycin
Azithromycin
Desosamine
Cladinose
6 Å
Lactone




5S RNA
A-site finger
Kink-turn 77/78
Kink-turn 42
L1 Site
Factor Binding Site
E-site tRNA
A-site tRNA
P-site tRNA
L7/12 stalk
„vrátný“
A-site Finger
P-site tRNA
E-site tRNA
A-site tRNA
5S RNA
Factor Binding Site
Kt-38
Kt-42