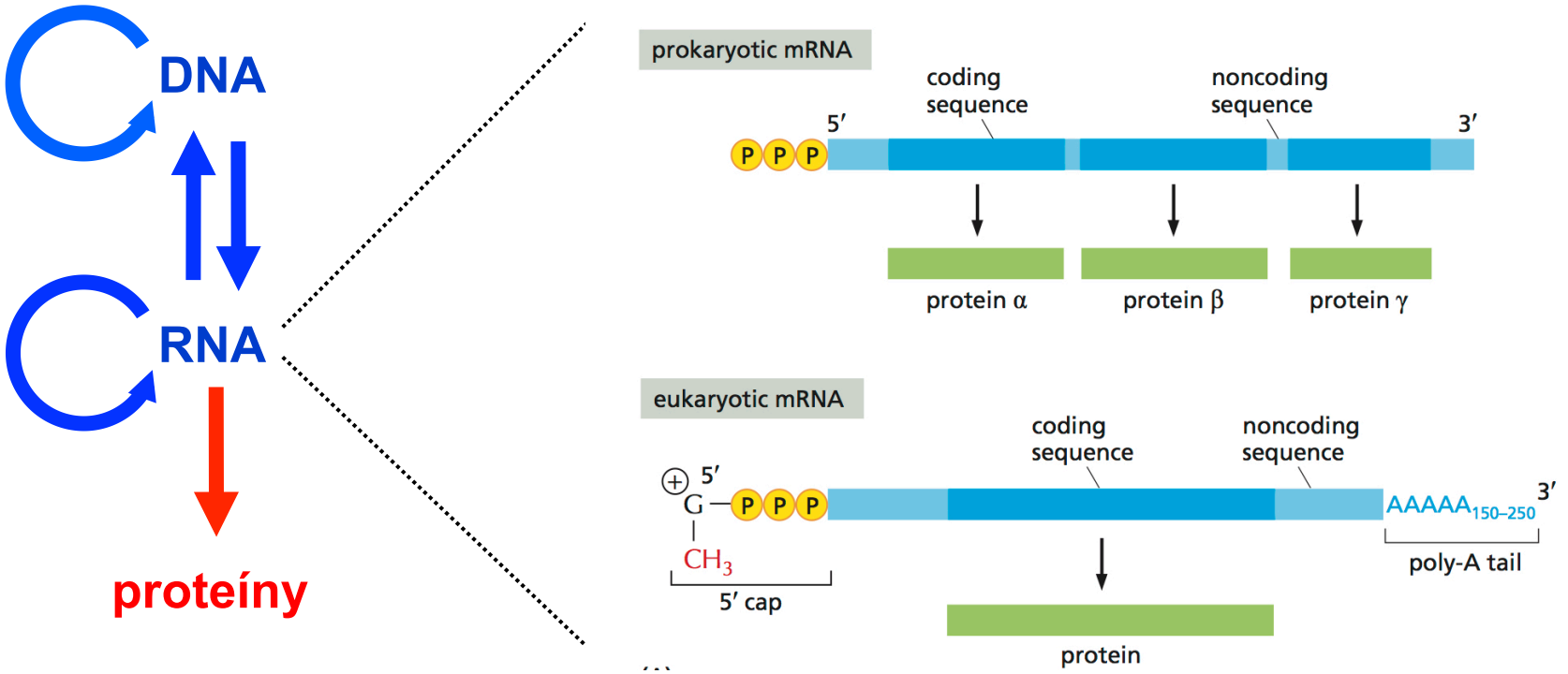
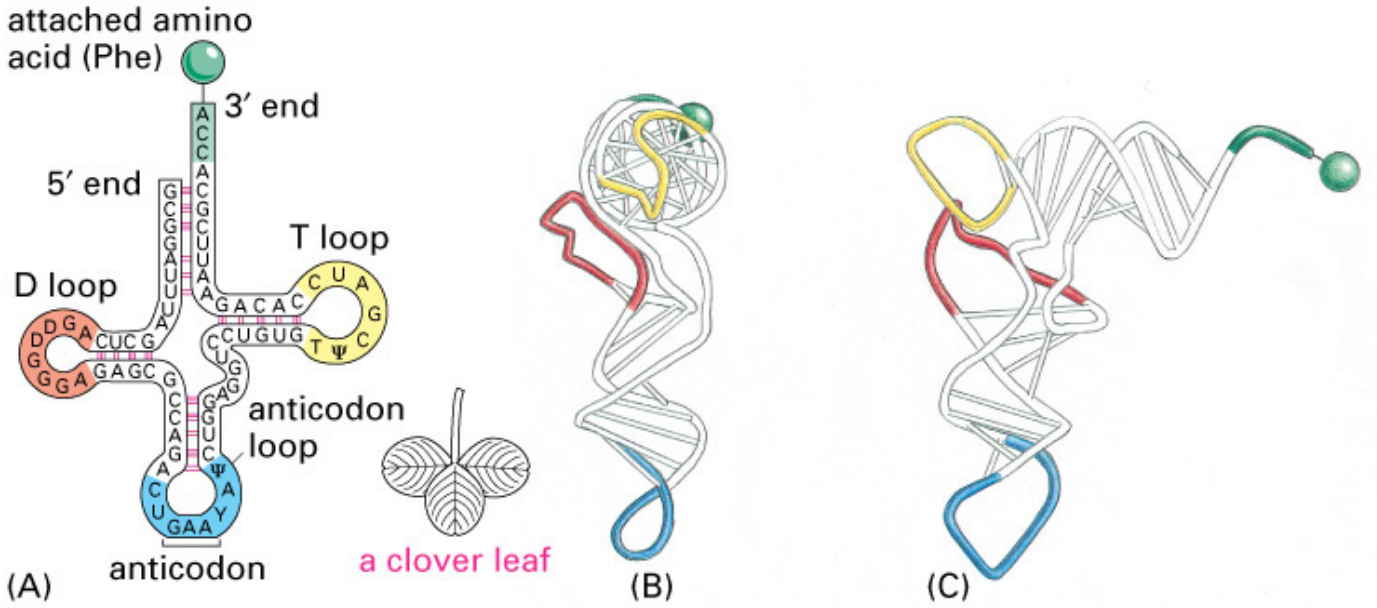
5. Syntéza a distribúcia proteínov v bunkách

- Objav a vlastnosti genetického kódu.
- Štruktúra a vlastnosti tRNA.
- Štruktúra a funkcie ribozómov.
- Ribozomálne RNA a proteínové komponenty ribozómu.
- Základné etapy translácie (iniciácia, elongácia a terminácia).
- Porovnanie prokaryotickej a eukaryotickej proteosyntézy.
- Inhibítory proteosyntézy.
- Vnútrobunková lokalizácia proteosyntézy.
- Distribúcia proteínov v bunke.

Mediátorová (*messenger*) RNA (mRNA)



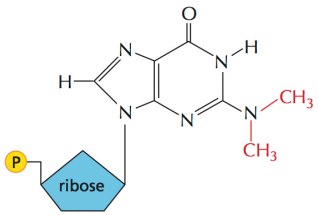
Transferová RNA (tRNA)



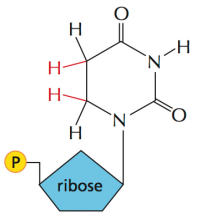
5′ GCGGAUUUAGCUCAGDDGGGAGAGCGCCAGACUGAAYAΨCUGGAGGUCCUGUGTΨCGAUCCACAGAAUUCGCACCA 3′

(D)

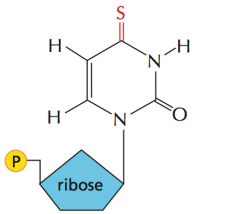
anticodon



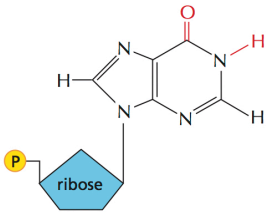
two methyl groups added to G
(*N,N*-dimethyl G)



two hydrogens added to U
(dihydro U)



sulfur replaces oxygen in U
(4-thiouridine)



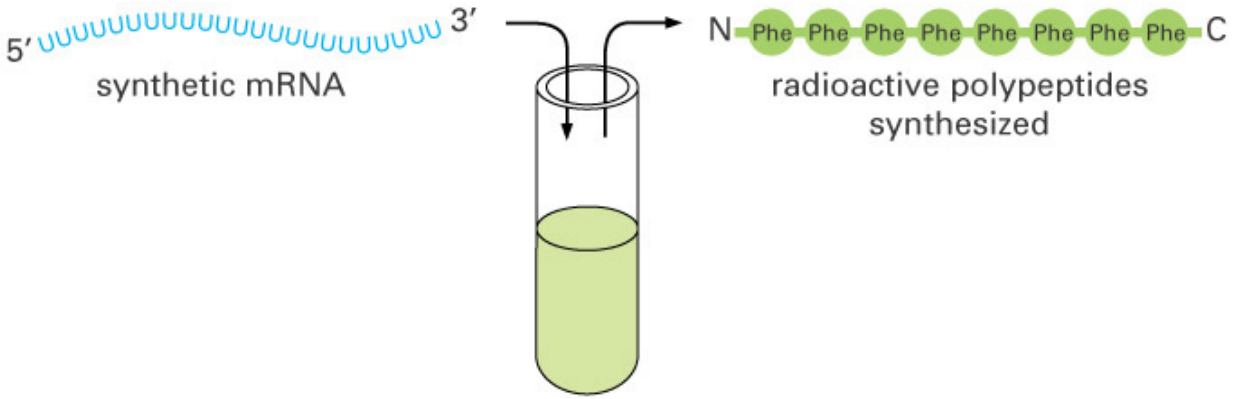
deamination of A
(inosine)

The Nobel Prize in Physiology or Medicine 1968 was awarded jointly to Robert W. Holley, Har Gobind Khorana and Marshall W. Nirenberg ***"for their interpretation of the genetic code and its function in protein synthesis"***

Robert W. Holley
(1922-1993)
USA

Har Gobind Khorana
(1922-2011)
India / USA

Marshall W. Nirenberg
(1927-2010)
USA



MESSAGE	PEPTIDES PRODUCED	CODON ASSIGNMENTS
poly UG	...Cys–Val–Cys–Val...	UGU, GUG — Cys, Val*
poly AG	...Arg–Glu–Arg–Glu...	AGA, GAG — Arg, Glu
poly UUC	...Phe–Phe–Phe... + ...Ser–Ser–Ser... + ...Leu–Leu–Leu...	UUC, UCU, CUU — Phe, Ser, Leu
poly UAUC	...Tyr–Leu–Ser–Ile...	UAU, CUA, UCU, AUC — Tyr, Leu, Ser, Ile

* One codon specifies Cys, the other Val. The same ambiguity exists for the other codon assignments shown here.

Štandardný genetický kód

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu
A	R	D	N	C	E	Q	G	H	I	L

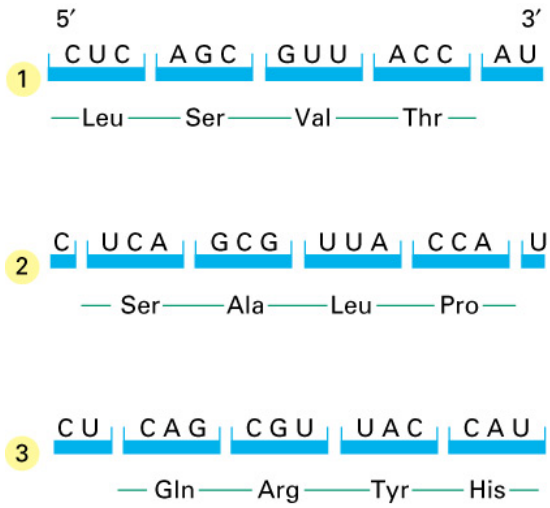
AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
K	M	F	P	S	T	W	Y	V	

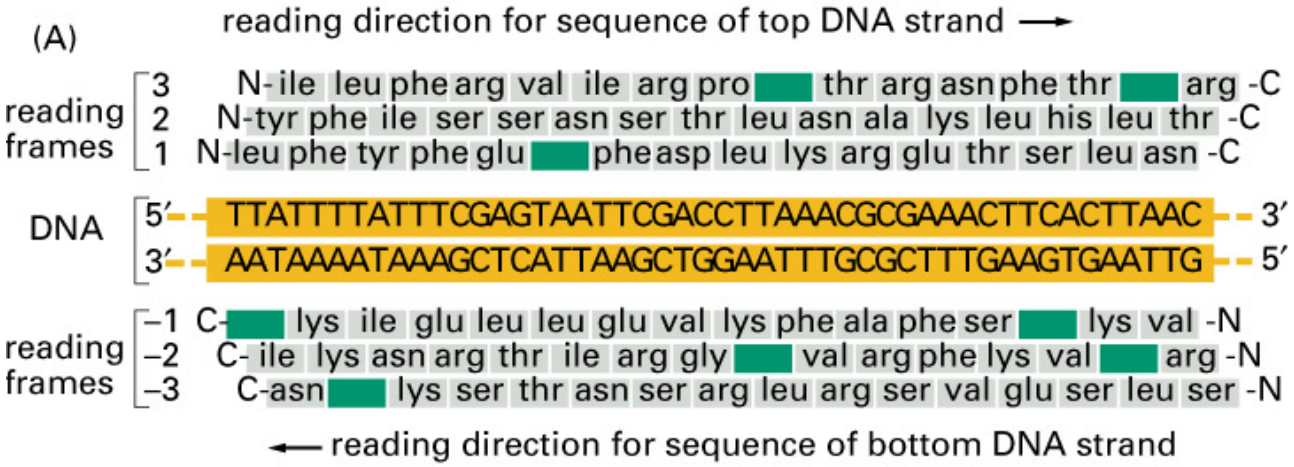
Vlastnosti genetického kódu:

- tripletový (4^3 = 64 kodónov)
- neprekrývavý
- degenerovaný
- „univerzálny“ - sú výnimky
- triplety pre štart (**AUG**, GUG) a stop (**UAA, UAG, UGA**)

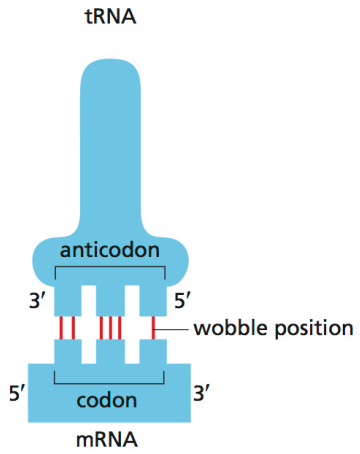
Ako je možné rozšíriť možnosti genetického kódu?
Ako vznikol genetický kód v evolúcii?

Otvorené čítacie rámce (Open Reading Frames, ORF)





„kolísanie“ (*wobble*) tRNA pri párovaní tripletov kodónu a antikodónu



bacteria

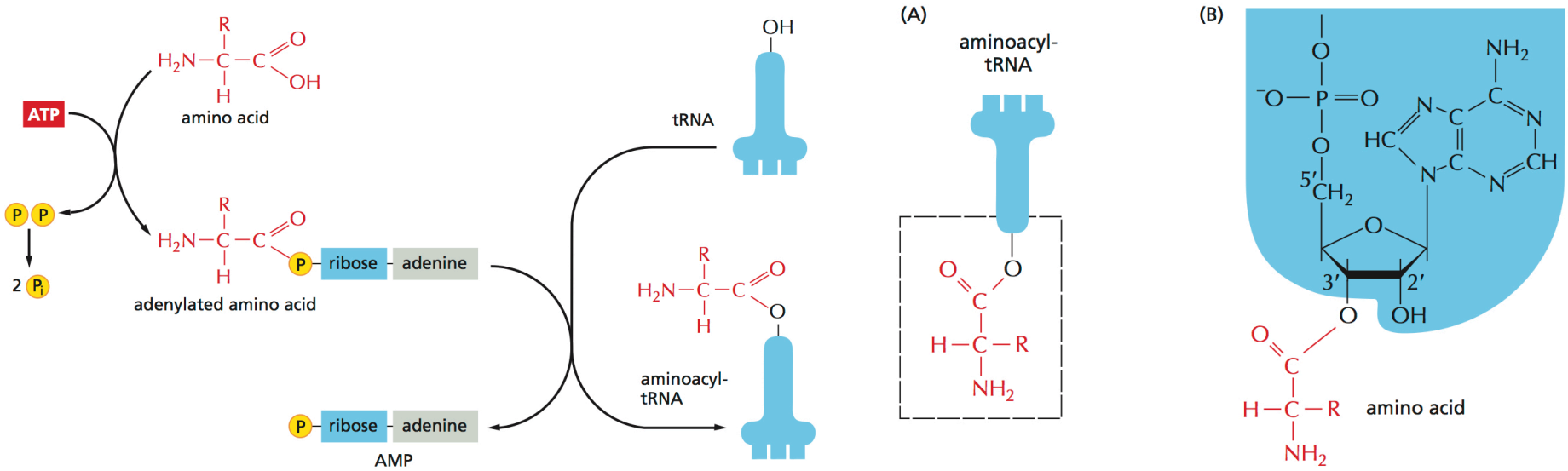
wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U or I
G	C or U

eukaryotes

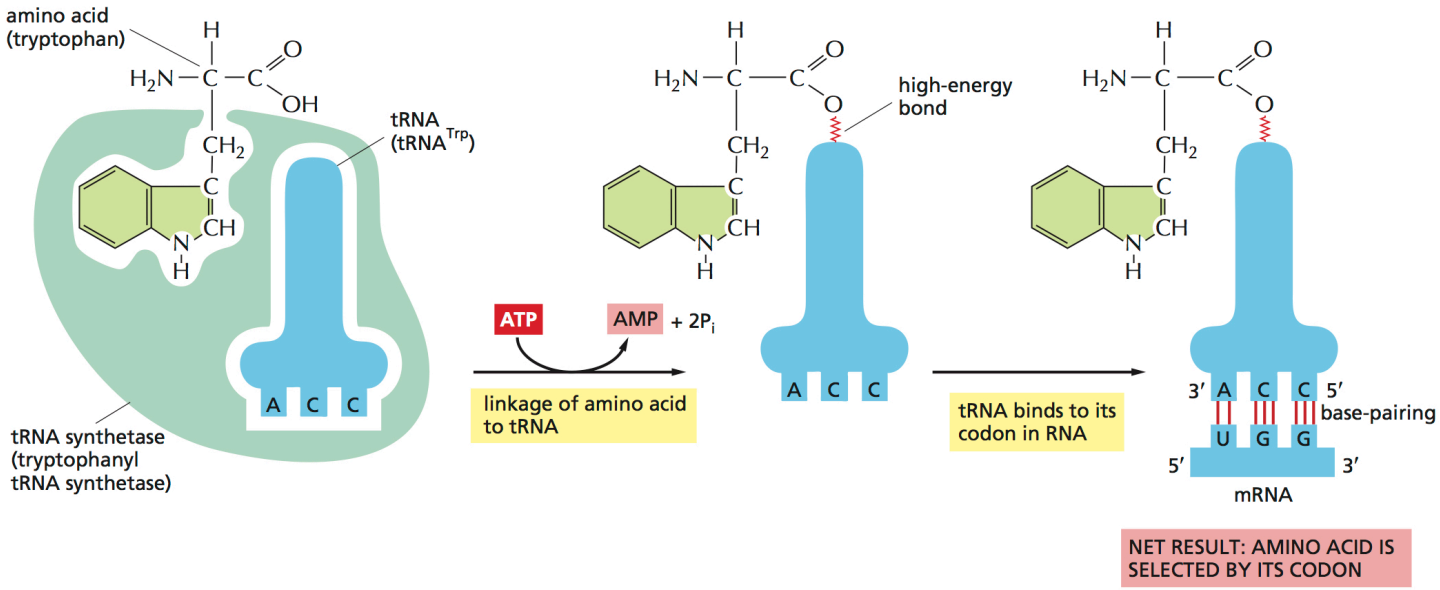
wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U
G	C

61+3 kodónov by vyžadovalo 61 typov tRNA, ale väčšina organizmov má menej typov tRNA, t.j. niektoré z nich musia rozpoznávať viac ako 1 kodón

Aminoacylácia tRNA

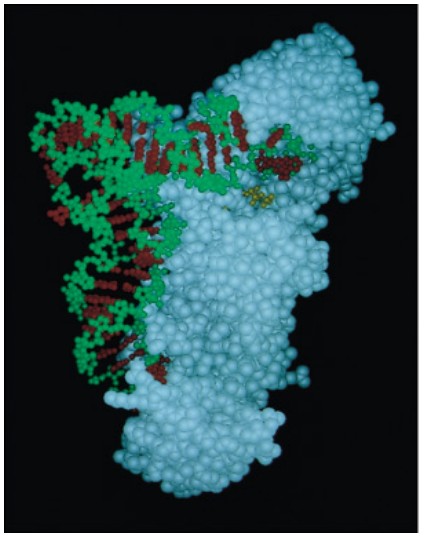


Aminoacyl tRNA syntetázy

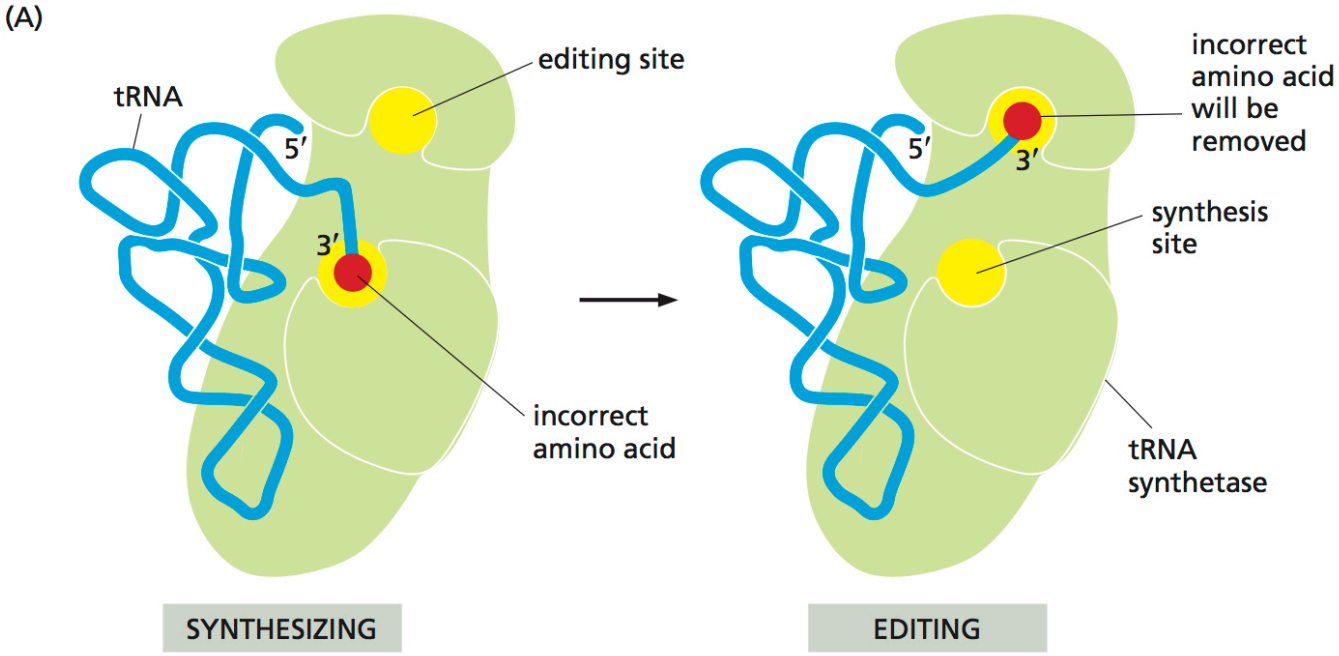


- **20 AK**
- **20 AA-tRNA syntetáz**

Enzým aminoacyl-tRNA syntetáza rozpoznáva antikodónové a aminoacylové rameno tRNA



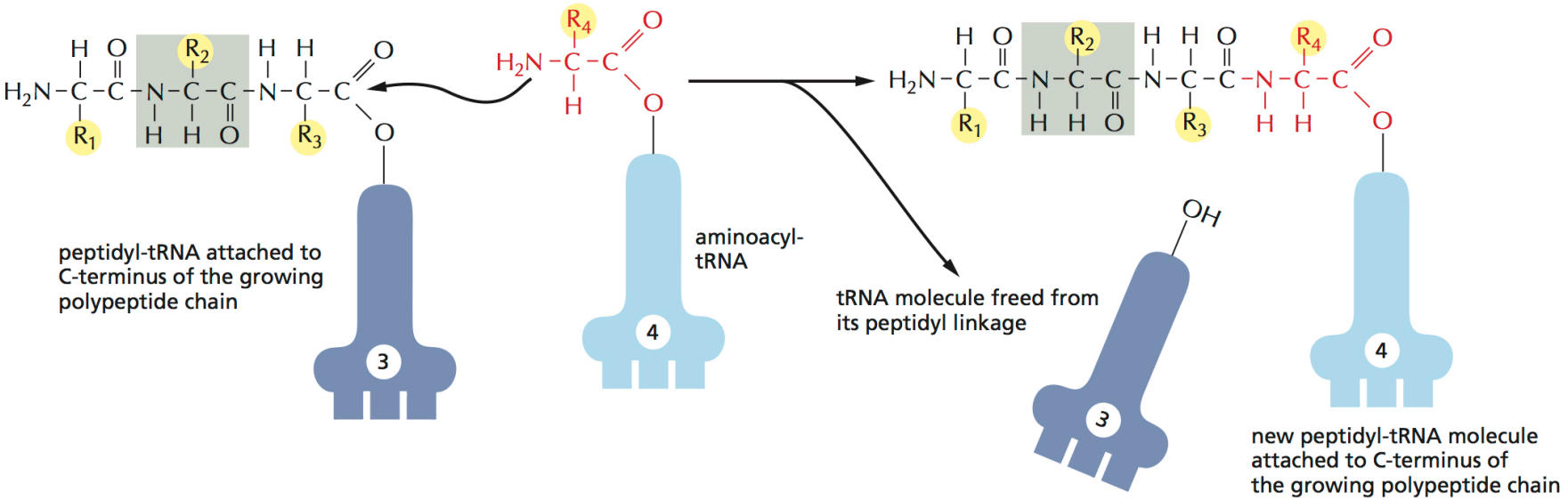
Kontrola AA-tRNA



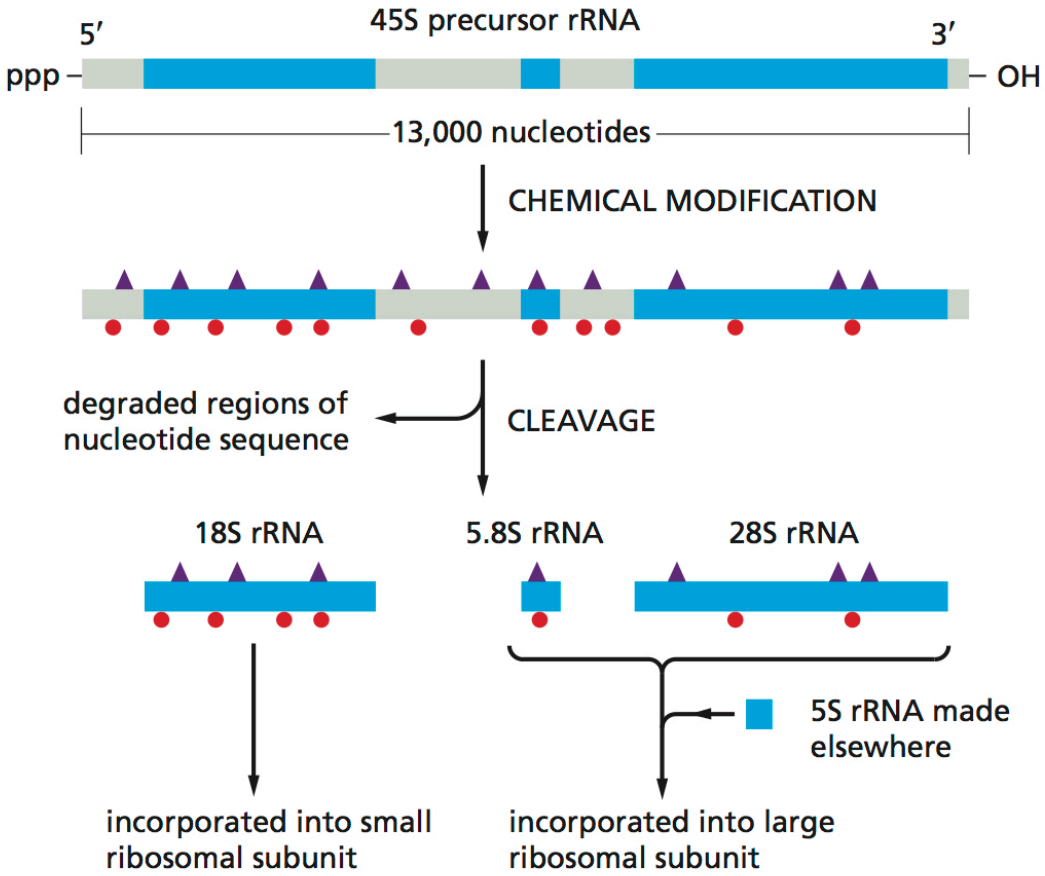
Frekvencia chýb 1: 40.000

Syntéza peptidovej väzby

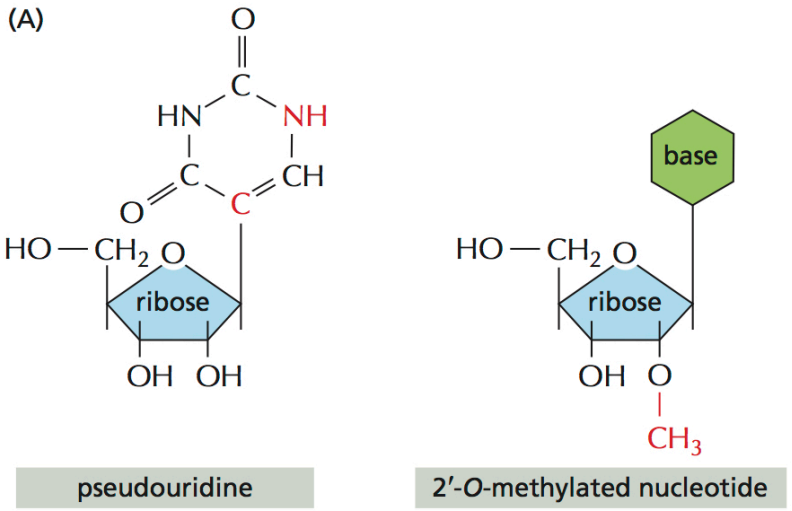
(reťazec rastie v smere NH_2-koniec → COOH-koniec polypeptidu)

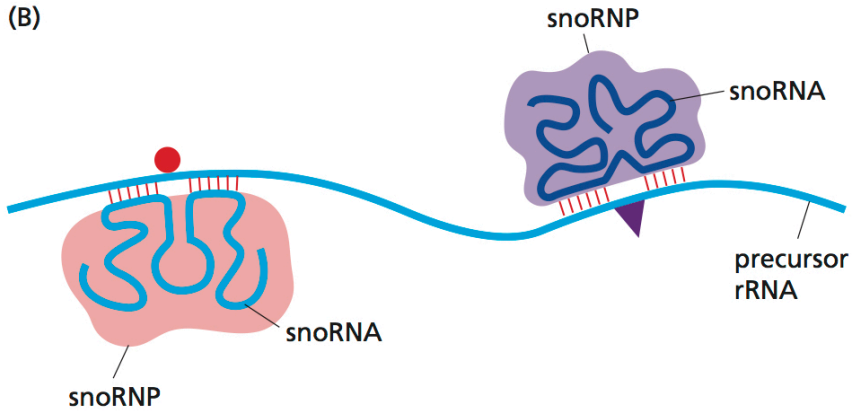


Ribozomálne RNA (rRNA)

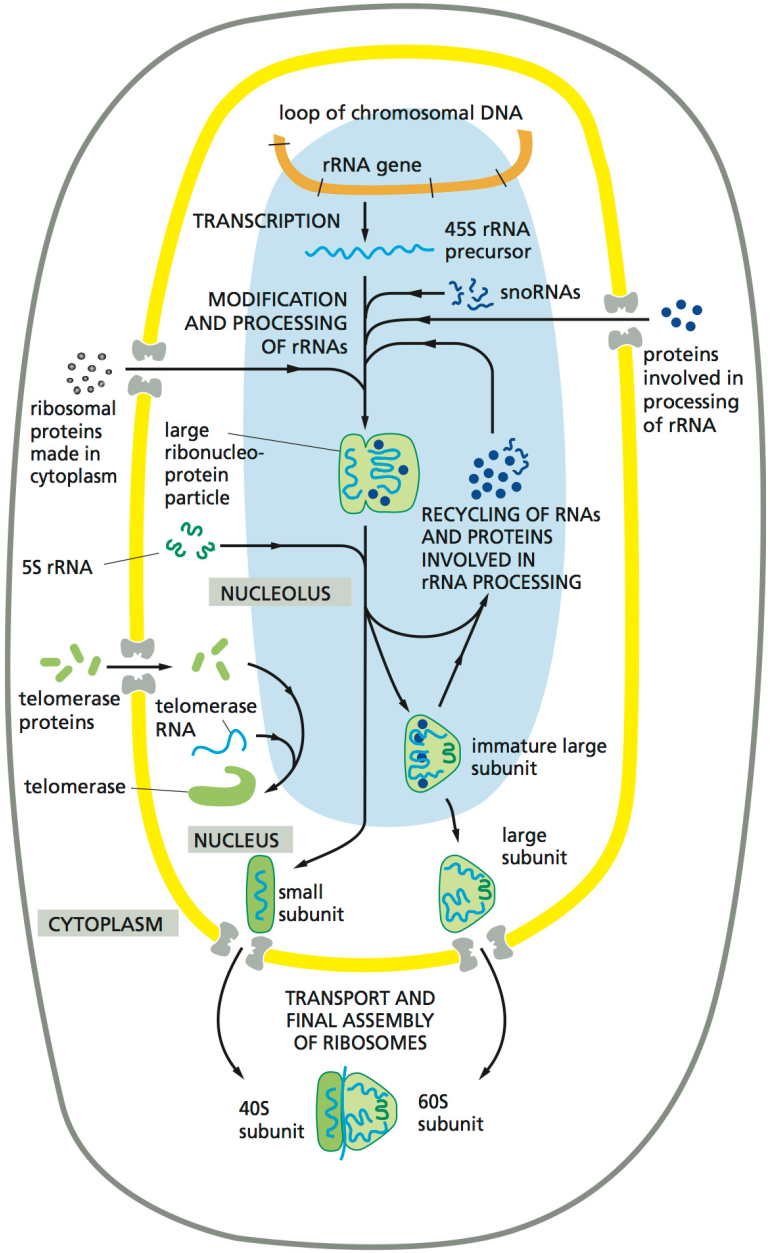


rRNA (aj tRNA) sú posttranskripčne modifikované

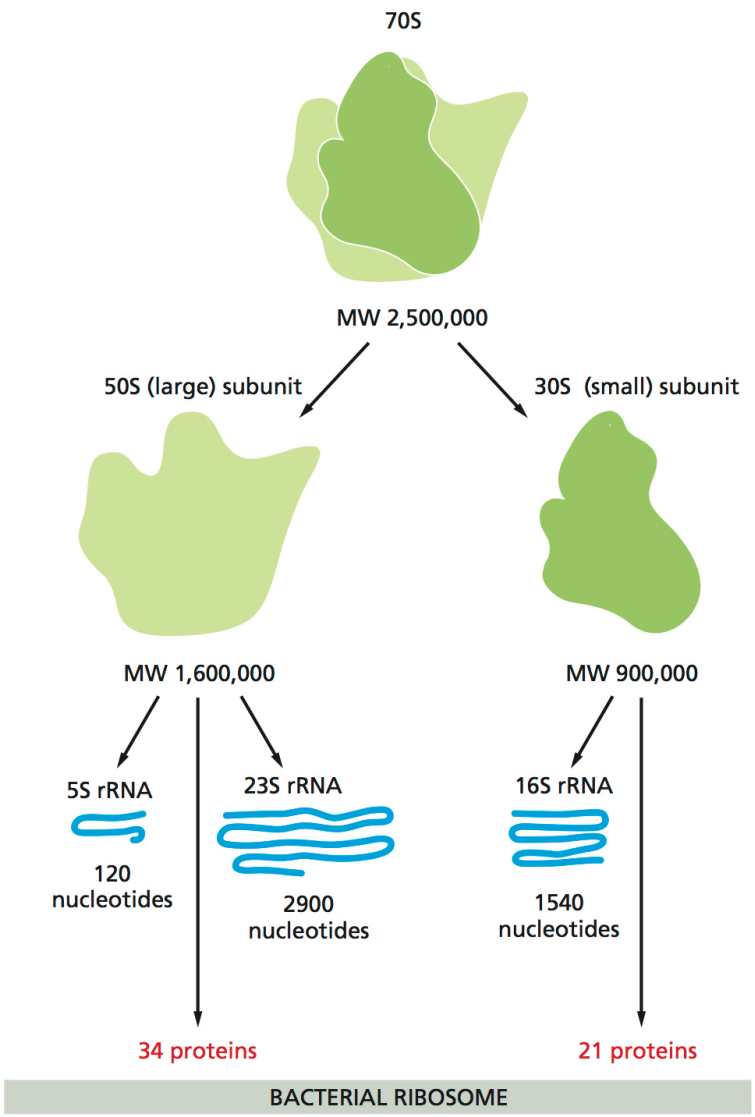


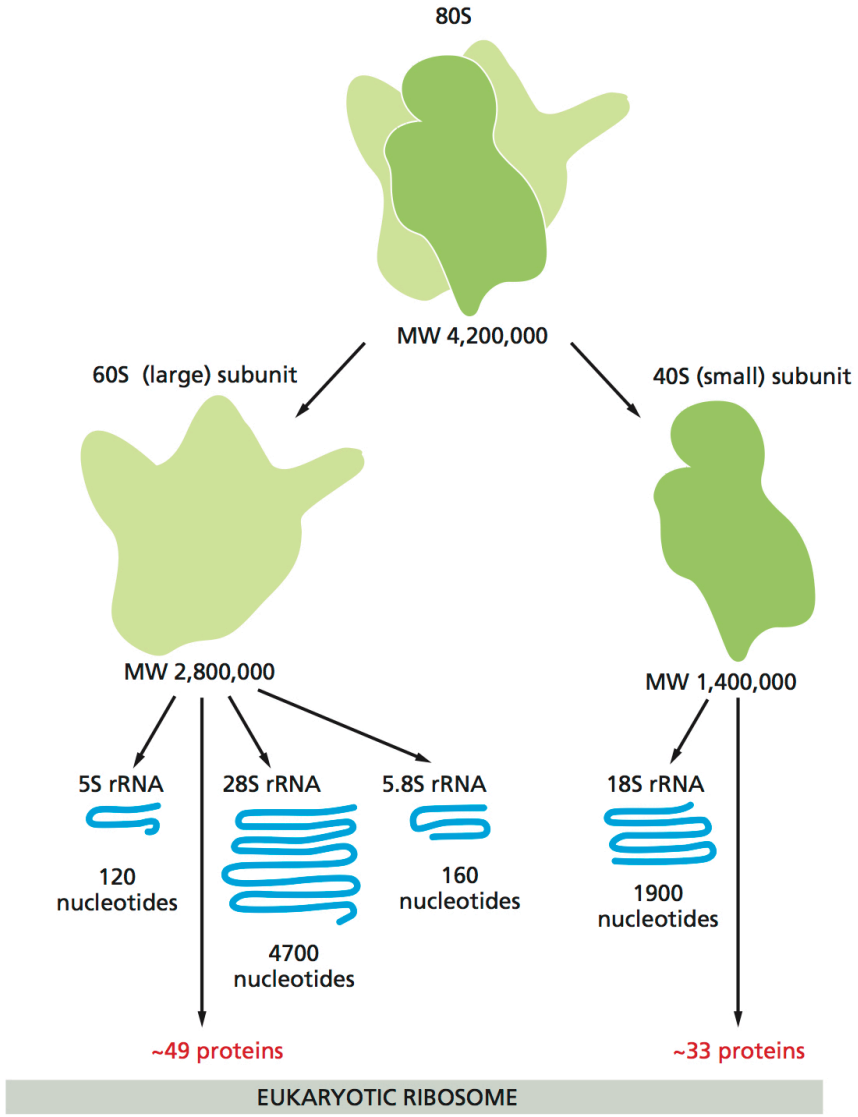


Biogenéza ribozómov v eukaryotickej bunke

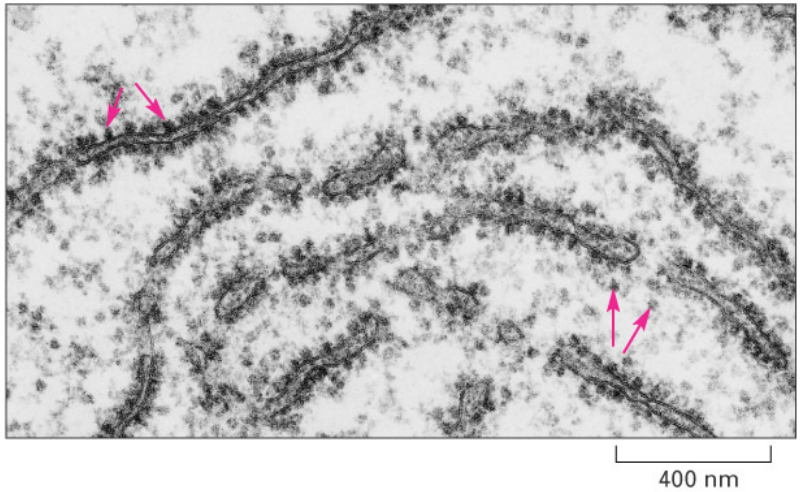


Bakteriálny vs. eukaryotický ribozóm

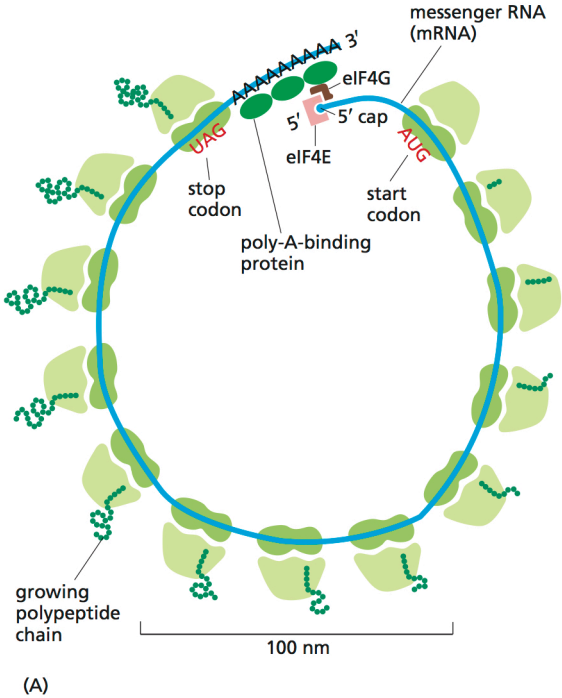


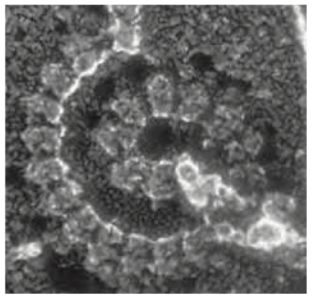


Ribozómy: voľné alebo viazané na (drsné) endoplazmatické retikulum

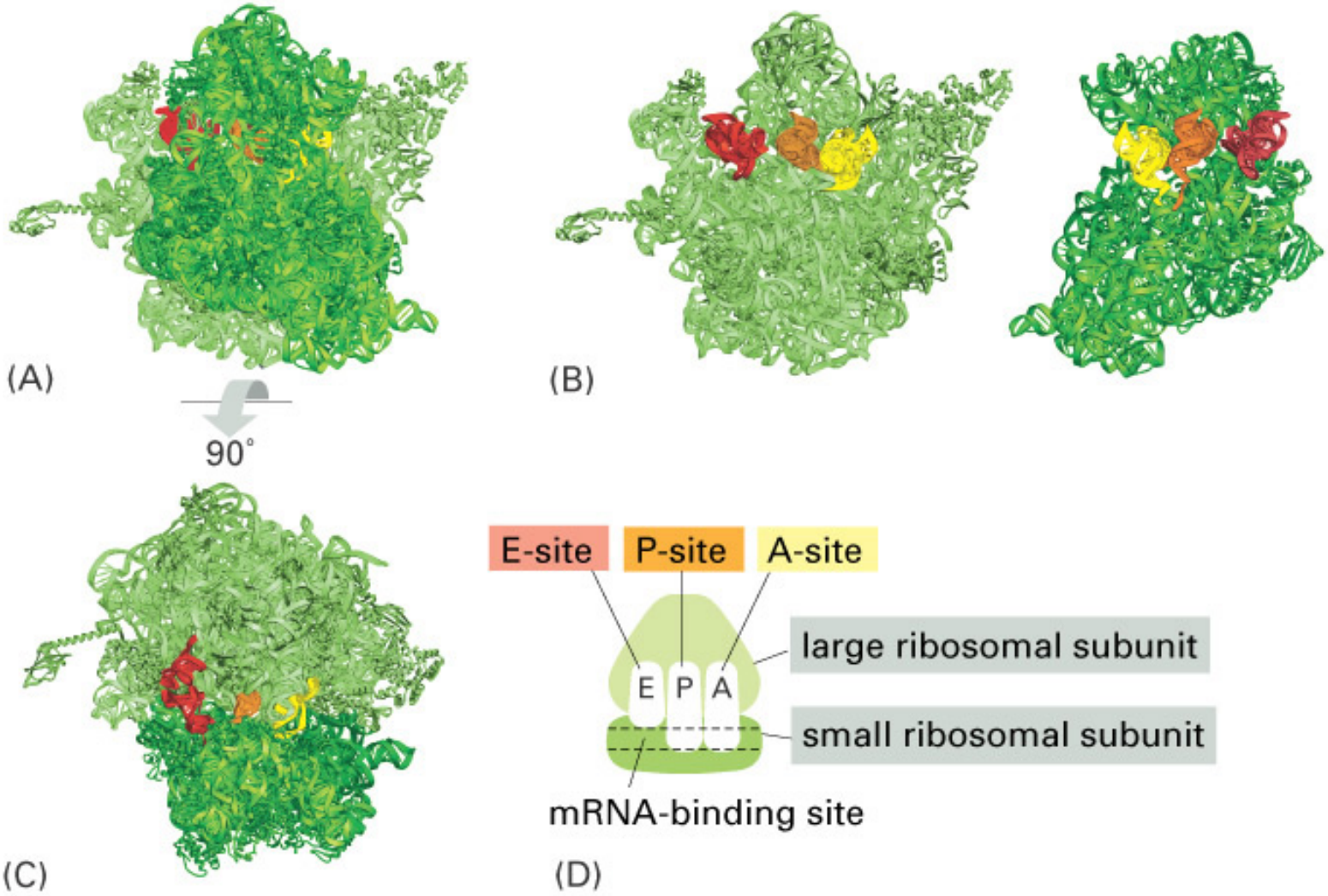


polyribozómy/polyzómy





Ribozóm je ribozým



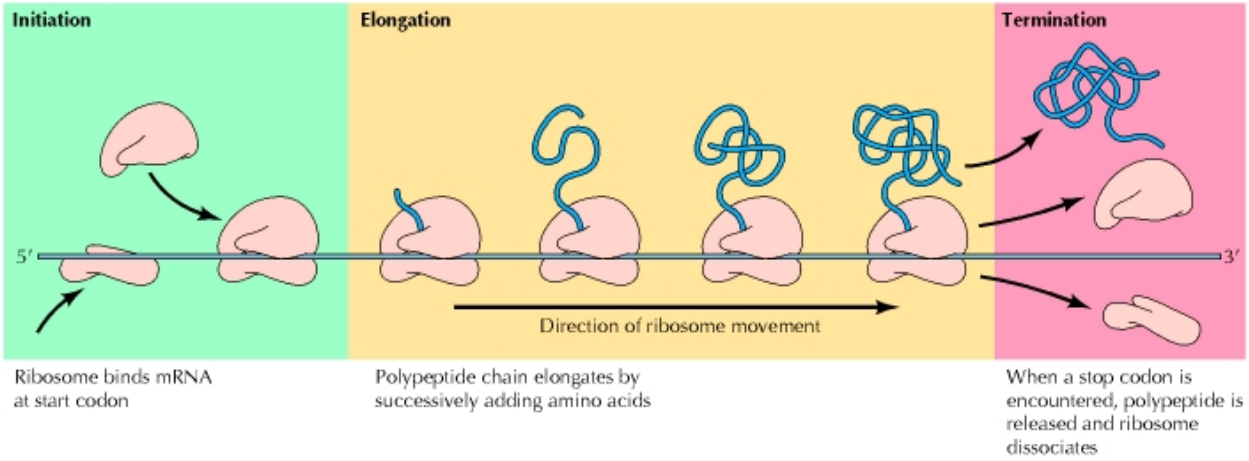
The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath ***"for studies of the structure and function of the ribosome***"

Venkatraman Ramakrishnan
(1952-)
India / United Kingdom

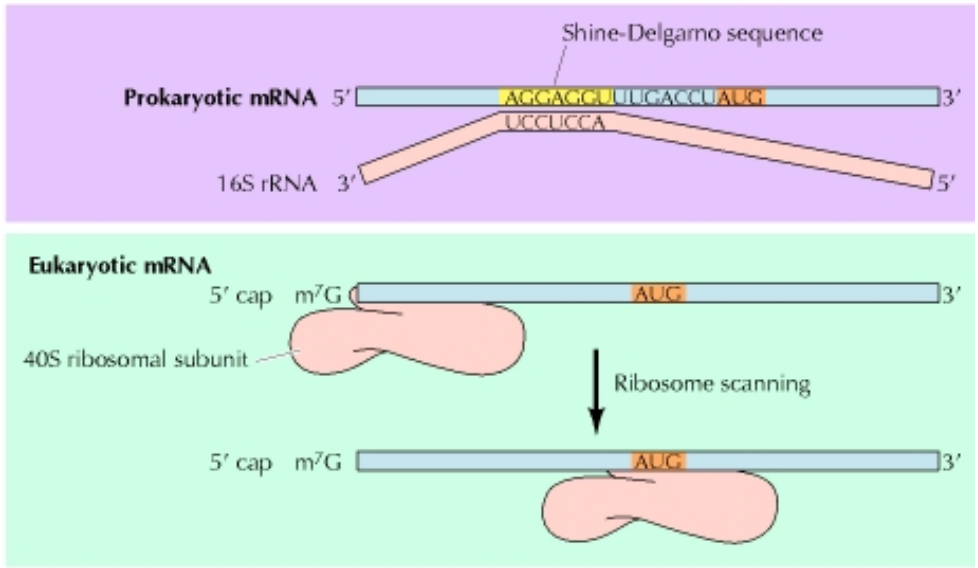
Thomas A. Steitz
(1940-)
USA

Ada E. Yonath
(1939-)
Israel

Etapy translácie



Rozpoznanie 5’ konca mRNA

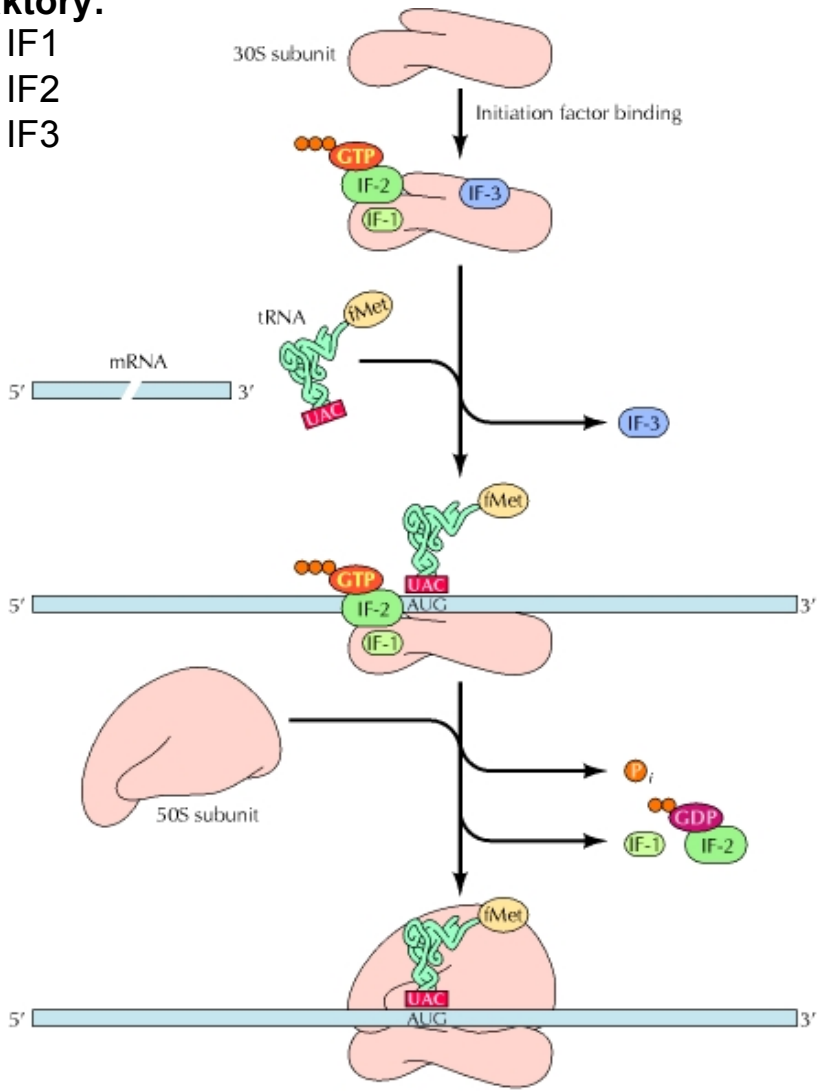


Iniciácia translácie

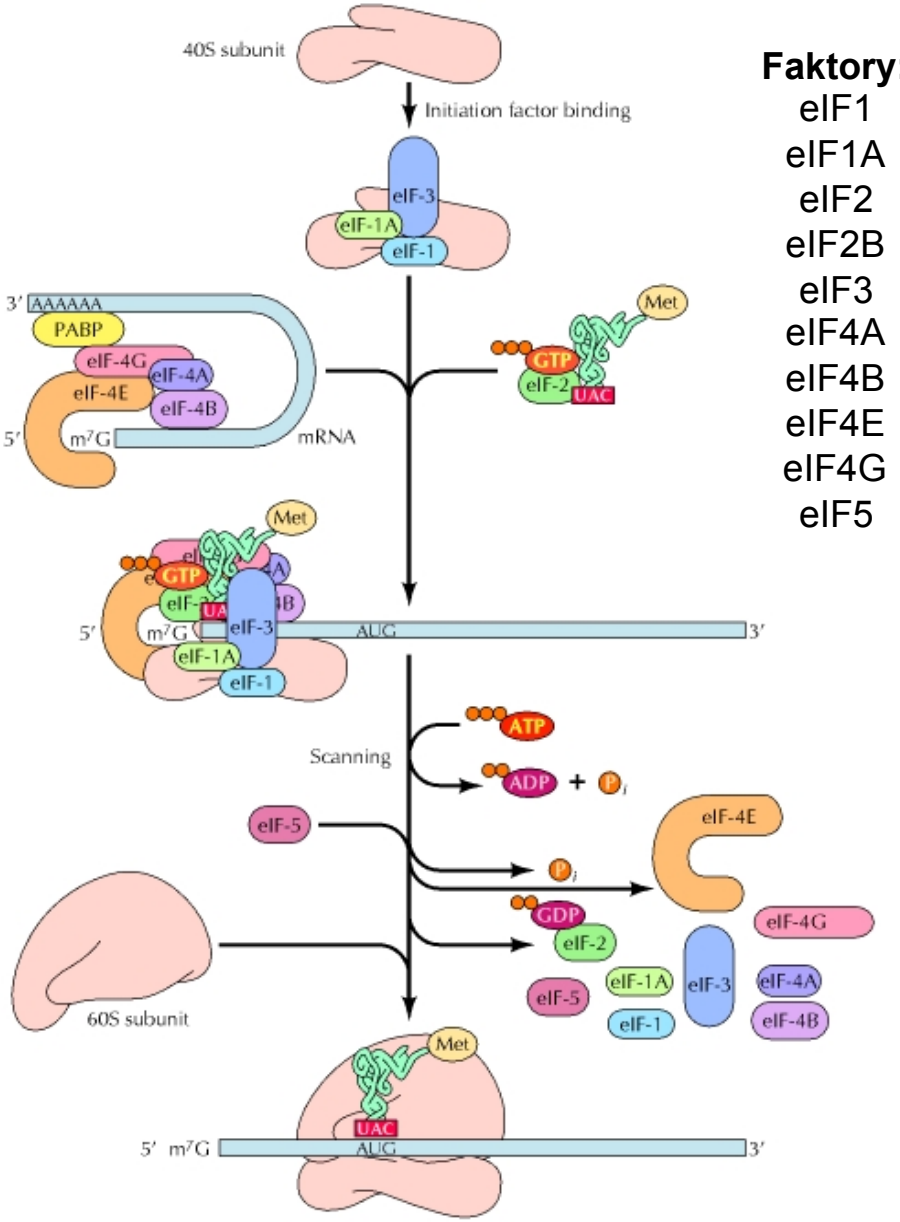
Baktérie

Faktory:

IF1
IF2
IF3



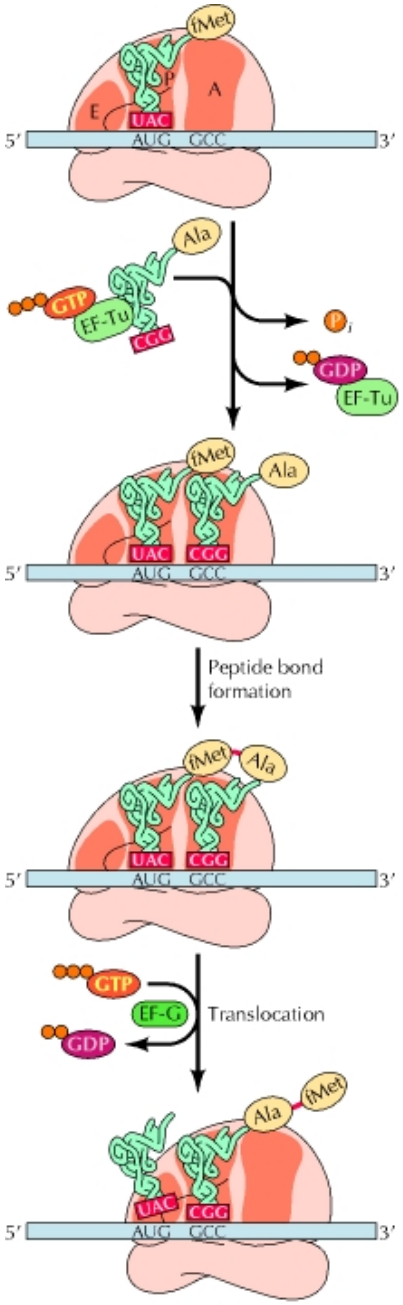
Eukaryoty

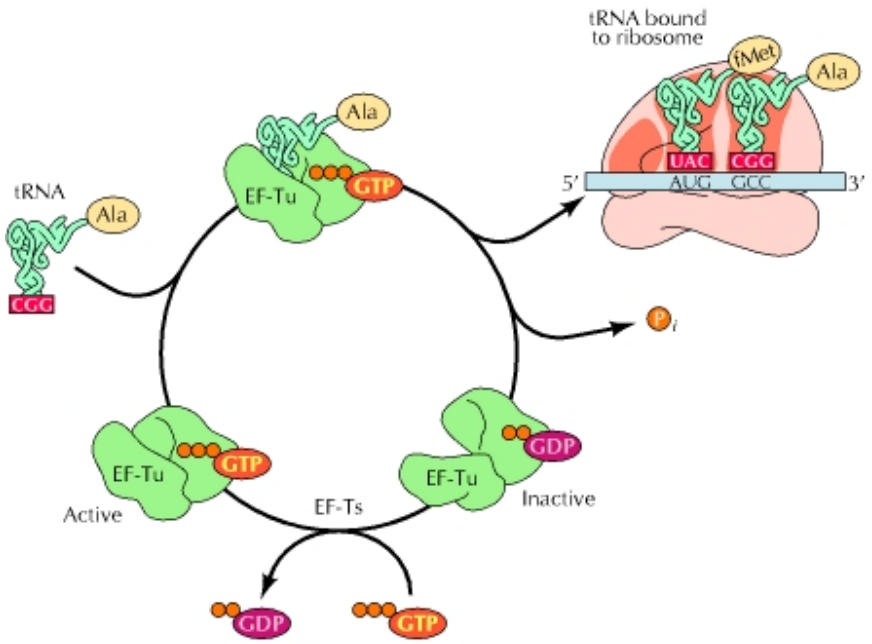


Faktory:

eIF1
eIF1A
eIF2
eIF2B
eIF3
eIF4A
eIF4B
eIF4E
eIF4G
eIF5

Elongácia translácie

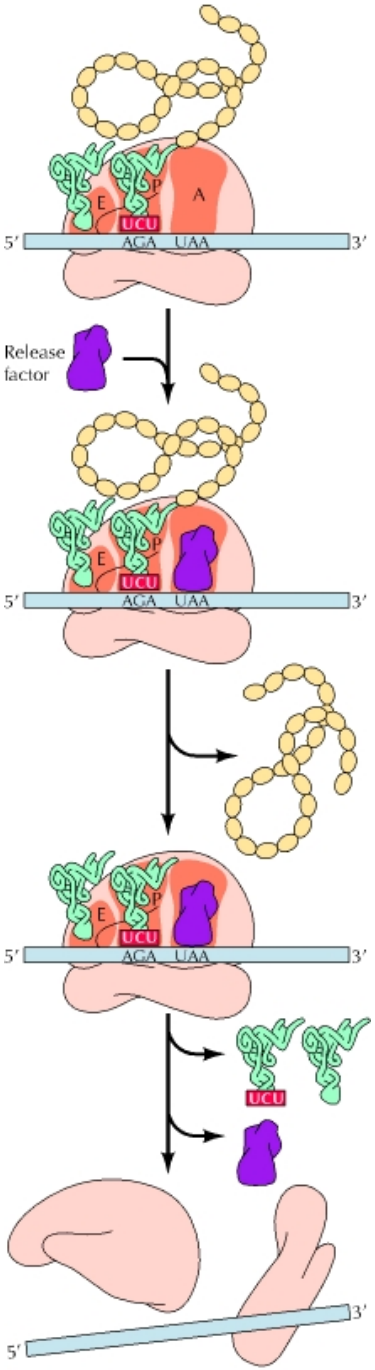




Faktory
Baktérie:
EF-Tu
EF-Ts
EF-G

Eukaryoty:
eEF1α
eEF1βγ
eEF-2

Terminácia translácie

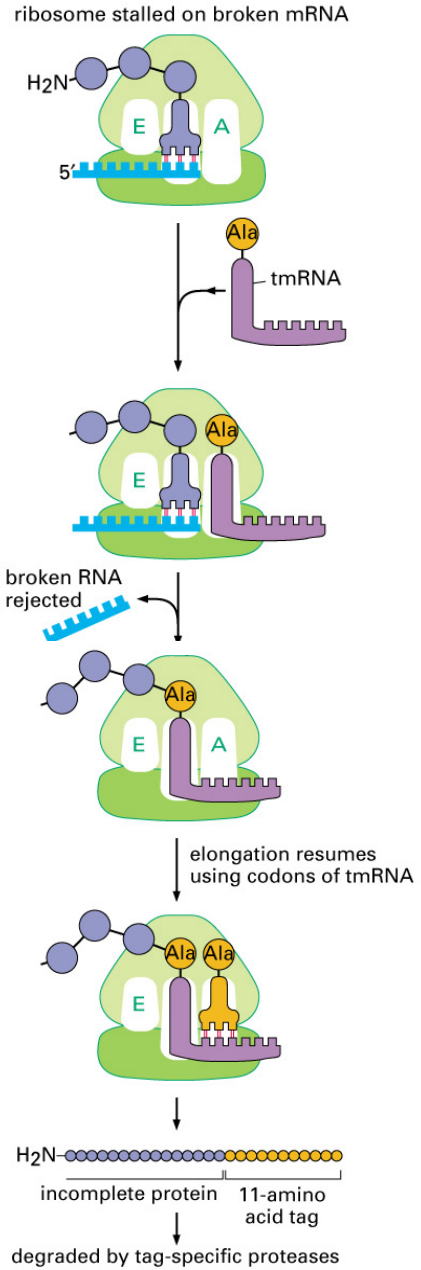


Faktory
Baktérie:
RF-1
RF-2
RF-3

Eukaryoty:
eRF1
eRF-3

Záchrana (rescue) blokovaného ribozómu na poškodenej mRNA (v baktériách)

úloha tmRNA



Inhibítory translácie

Účinok len na baktérie:

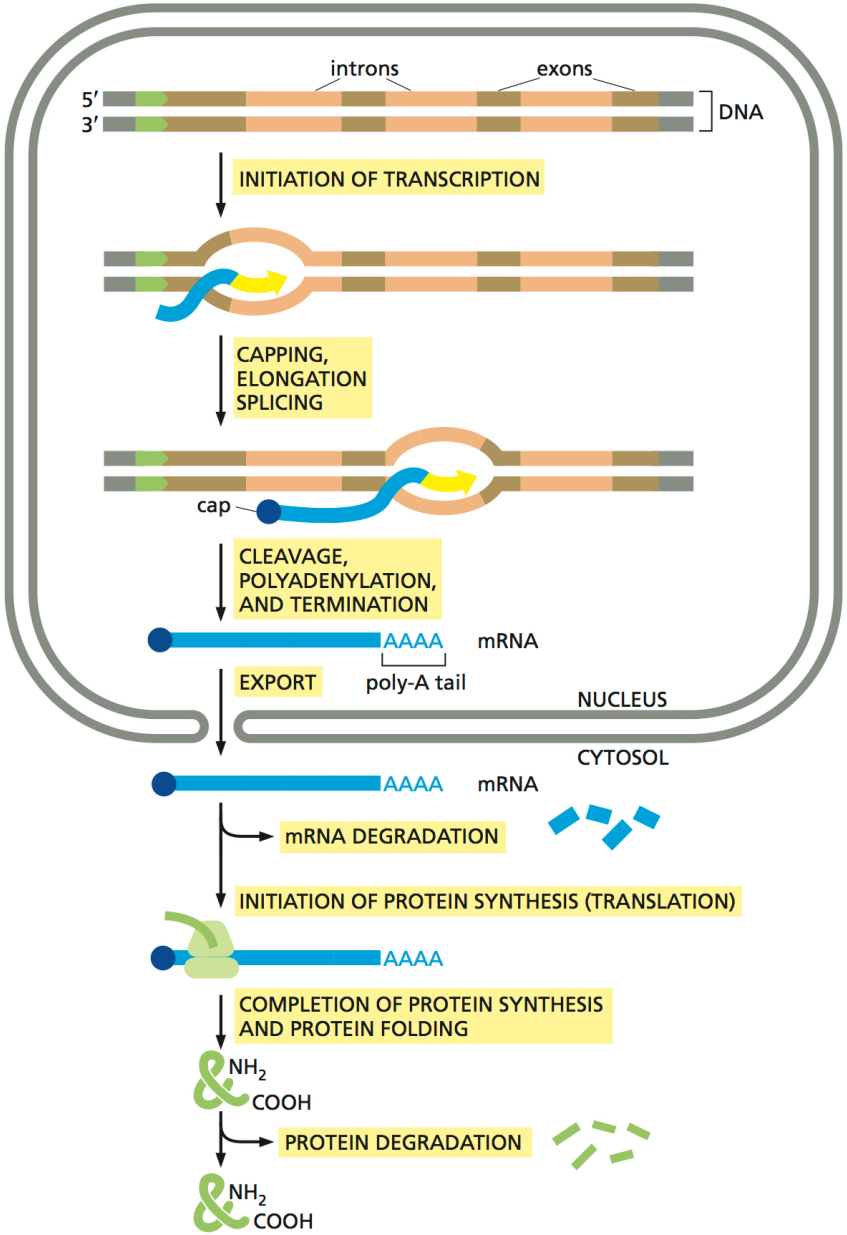
Tetracyklín	Blokuje väzbu aminoacyl-tRNA na A-miesto ribozómu
Streptomycín	Bráni tranzícii iniciačného komplexu na elongáciu, spôsobuje chybné čítanie kódu
Chloramfenikol	Blokuje peptidyltransferázovú reakciu na ribozóme
Erytromycín	Blokuje translokačnú reakciu na ribozóme

Účinok na baktérie a eukaryoty:

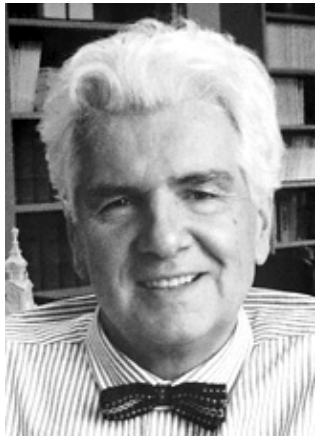
Puromycín	Spôsobuje predčasnú termináciu syntézy polypeptidu, viaže sa na C-koniec reťazca

Účinok len na eukaryoty:

Anizomycín	Blokuje peptidyltransferázovú reakciu na ribozóme
Cykloheximid	Blokuje translokačnú reakciu na ribozóme

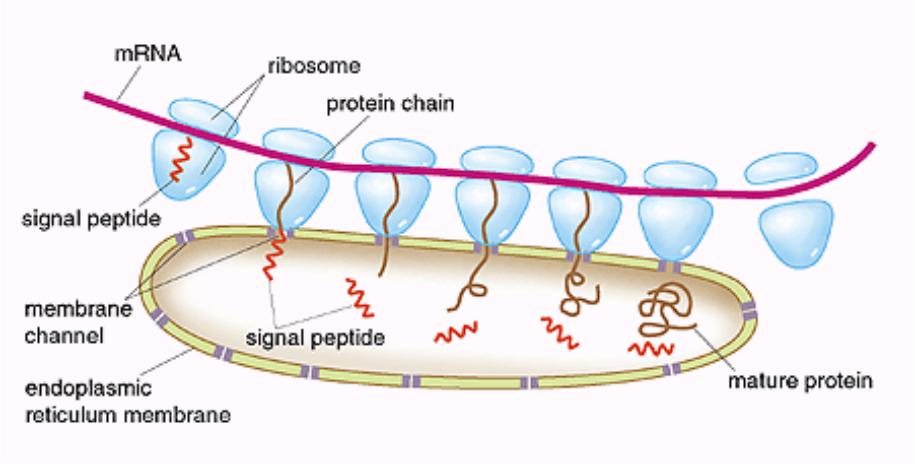

introns
exons
5′
3′
DNA
INITIATION OF TRANSCRIPTION
CAPPING,
ELONGATION
SPLICING
cap
CLEAVAGE,
POLYADENYLATION,
AND TERMINATION
AAAA
mRNA
poly-A tail
EXPORT
NUCLEUS
CYTOSOL
AAAA
mRNA
mRNA DEGRADATION
INITIATION OF PROTEIN SYNTHESIS (TRANSLATION)
AAAA
COMPLETION OF PROTEIN SYNTHESIS
AND PROTEIN FOLDING
NH2
COOH
PROTEIN DEGRADATION
NH2
COOH

The Nobel Prize in Physiology or Medicine 1999 was awarded to Günter Blobel ***"for the discovery that proteins have intrinsic signals that govern their transport and localization in the cell"***.

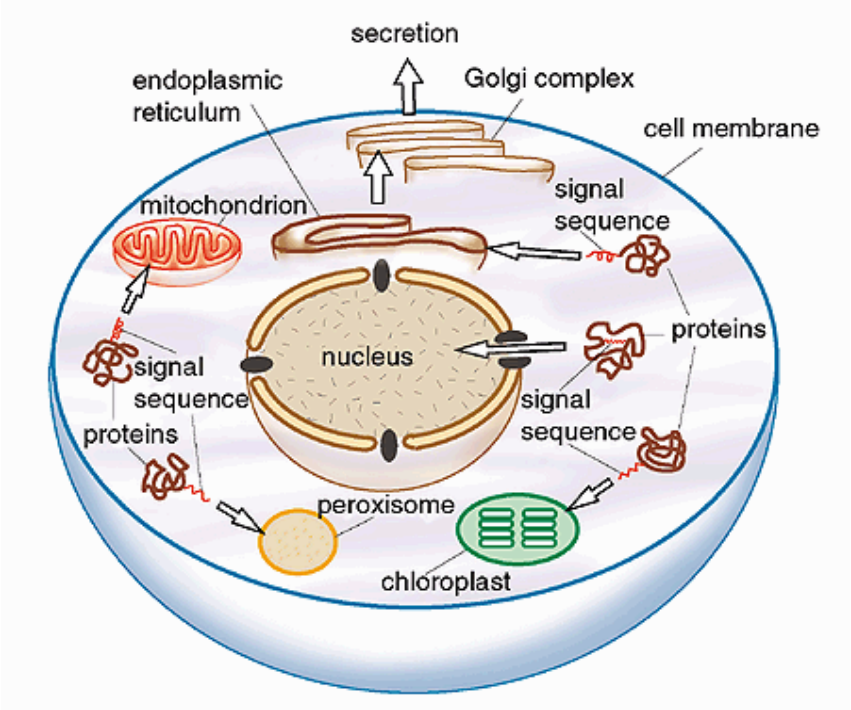


Günter Blobell
(1936-)
Germany / USA

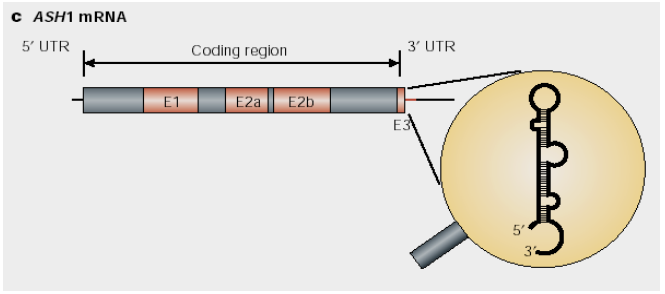
Signálna hypotéza



Polypeptidy obsahujú signálne sekvencie (topogénne signály), ktoré ich smerujú na miesto určenia



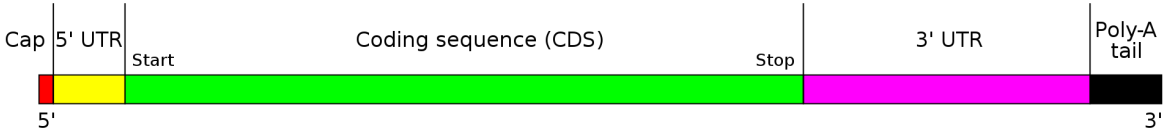
Vnútrobunkový transport mRNA

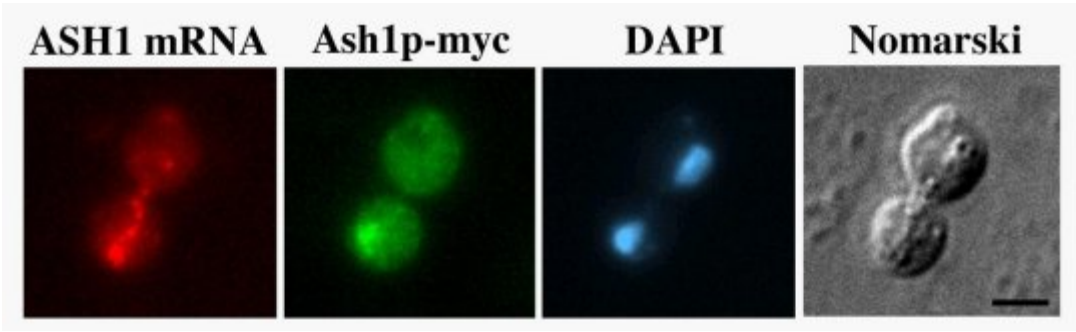


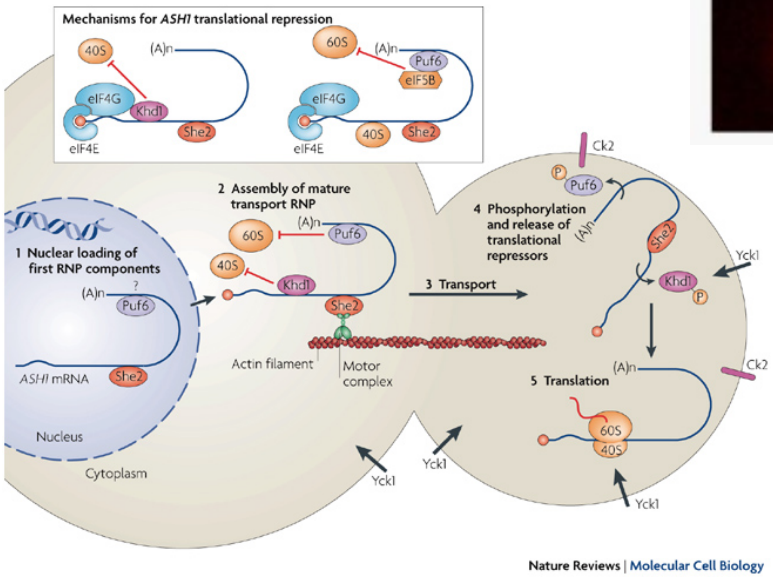
Molecular Cell, Vol. 2, 437–445, October, 1998, Copyright ©1998 by Cell Press

Localization of *ASH1* mRNA Particles in Living Yeast

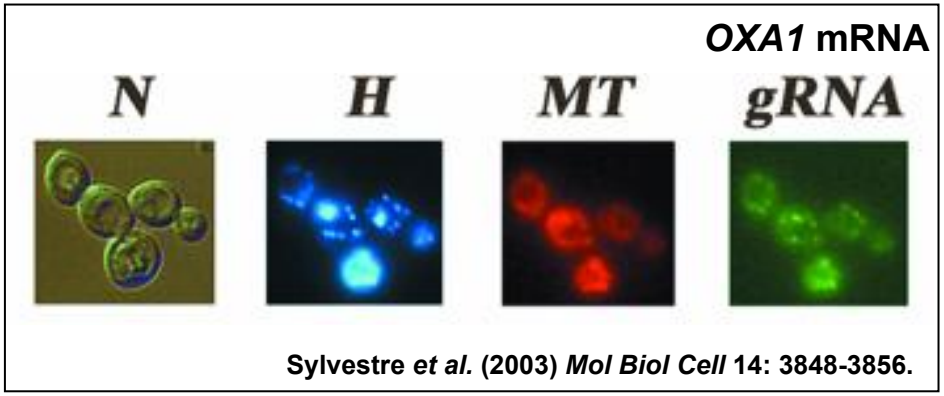
Edouard Bertrand,† Pascal Chartrand, Matthias Schaefer,‡ Shailesh M. Shenoy, Robert H. Singer,* and Roy M. Long§
Department of Anatomy and Structural Biology and Cell Biology
Albert Einstein College of Medicine
Bronx, New York 10461





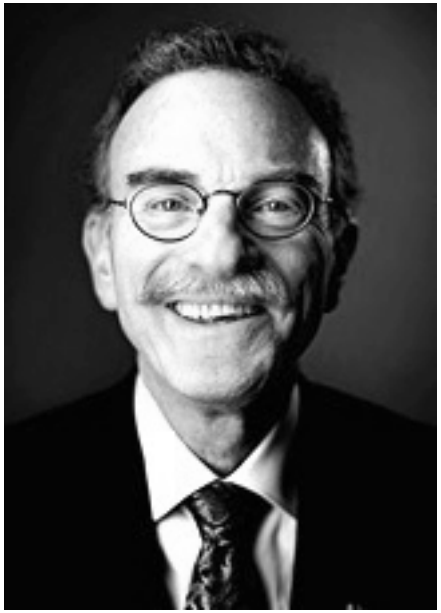


- signál je v oblasti 3’ UTR mRNA
- je rozpoznaný proteínmi She1-5
- mRNA je transportovaná na miesto určenia



The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof "**for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells**".

James E. Rothman
(1950 -)
USA



Randy W. Schekman
(1948 -)
USA

Thomas C. Südhof
(1955 -)
Germany

Nabudúce:

6. Princípy kontroly expresie génov.

- Úrovne kontroly expresie génov.
- Operónový model. Pokusy Jacoba a Monoda.
- Negatívna a pozitívna kontrola expresie.
- Katabolická represia.
- Atenuácia.
- Regulácia životného cyklu fága lambda.
- Porovnanie kontroly génovej expresie v prokaryotických a eukaryotických bunkách.
- Kontrola na úrovni transkripcie a posttranskripčné úpravy RNA.
- Kontrola na úrovni translácie a posttranslačné úpravy proteínov.