

Protein's intracellular adventure

Protein biosynthesis
Correct protein folding
Non-functional protein degradation
Intracellular directing of proteins

Talking about

Ribosome – structure and function
Chaperones – definition and role
Proteasome – structure and function
Import of proteins into organelles

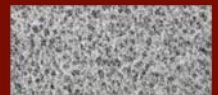
Protein biosynthesis

- Project – mRNA
- Machinery – ribosome
- Raw material – aminoacyl-tRNA

Genetic code (degenerated or redundant)

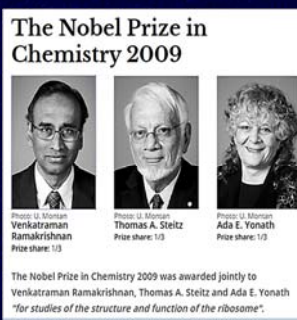
AGA	AGG	CGA	GAC	AAC	UGC	GAA	CAA	GGA	UUA	UUG	CUA	AAA	UUC	CCA	AGU	ACA	GUA	UAA
GCC	COC	GAU	AUU	UGU	GAG	CAG	GGG	GGC	AUA	CUC	AUU	AUG	UUU	CCC	UCC	ACC	GUC	UAG
GCG	CGG	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU	CGU
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y

RIBOSOMES

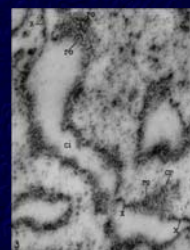


- Electron-dense particles 15-25 nm, free in cytosol or attached to endoplasmic reticulum (membrane bound).
- Macromolecular assemblies of ribosomal RNA and ribosomal proteins.
- Biogenesis in nucleolus as subunits and released in cytosol.
- Organelles responsible for protein biosynthesis.

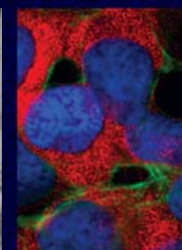
RIBOSOMES



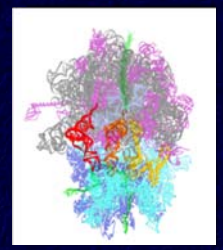
RIBOSOMES



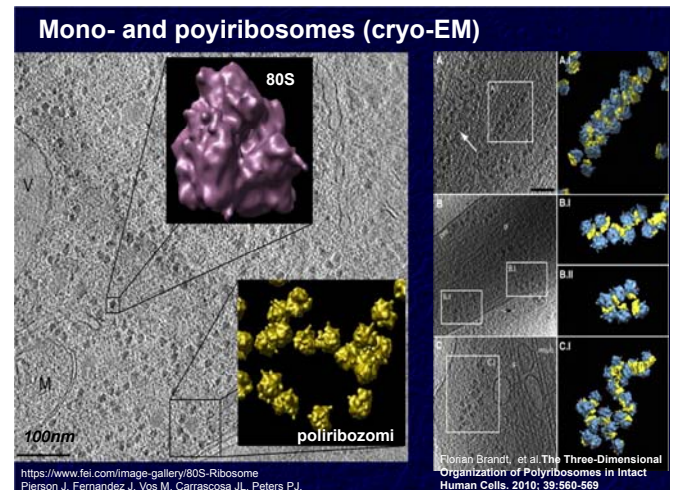
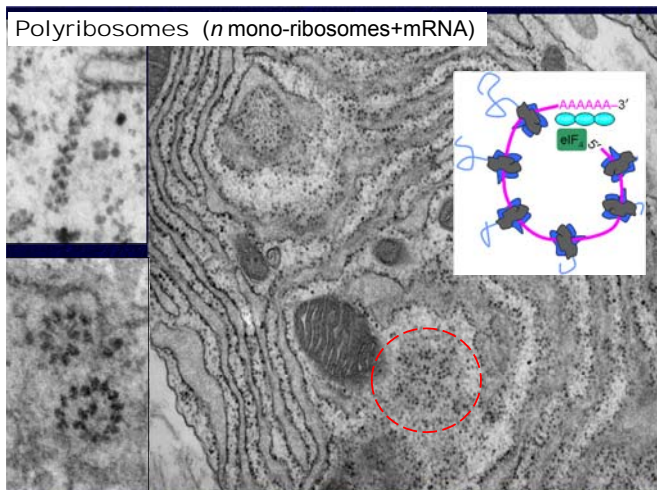
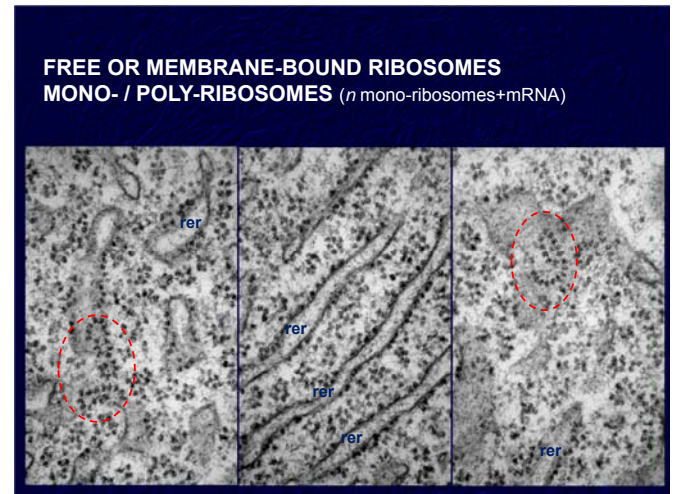
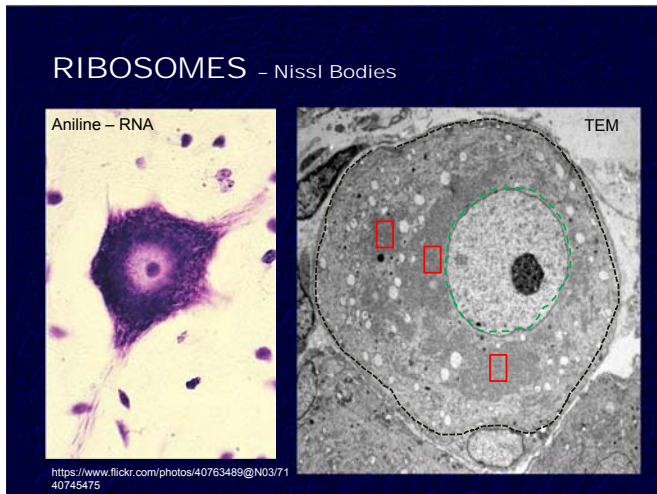
Palade's granules
PALADE GE* 1955



Phospho-S6 Ribosomal Protein
ThermoFisher



Atomic model of ribosome
T. STEITZ* 2000



Types of ribosomes

55 S – MITOCHONDRIA (~3 MDa)
39S and 28S subunits

70 S – PROKARIOTES (2,7 MDa)
50S and 30S subunits

80 S – EUKARIOTES (4,3 MDa)
60S and 40S subunits

S = Svedberg unit

1 Svedberg (S, Sv) = 10^{-13} seconds (100 fs)
= sedimentation rate

The sedimentation rate for a particle of a given size and shape measures how fast the particle 'settles', the sedimentation.

It is often used to reflect the rate at which a molecule travels to the bottom of a test tube under the centrifugal force of a centrifuge.

Analyze sucrose gradient

heavy half ribosomes

heavy full ribosomes

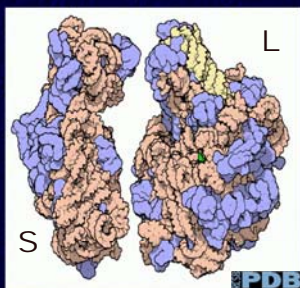
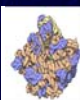
Ribosomes - molecular organization

2 structural and functional distinct subunits

• **SMALL (S)**



• **LARGE (L)**



PDB

Organization of the machinery

Molecular organization

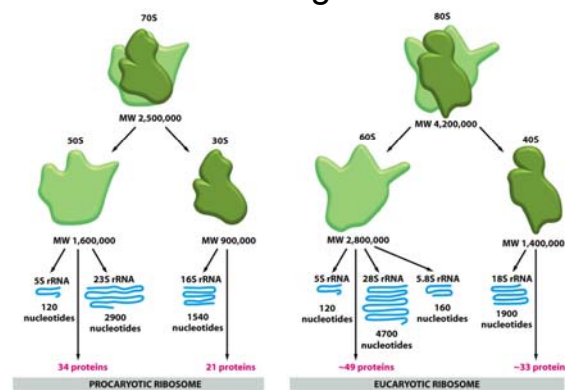


Figure 6-43 Molecular Biology of the Cell 5/e (© Garland Science 2008)

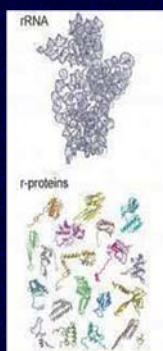
Ribosomes - molecular organization

RIBOZOMAL ARN

- 4 molecules rRNA - eukariotes
- 3 molecules rRNA - prokariotes

RIBOZOMAL PROTEINES

- *uS (17), bS(21), eS(31), RACK1
- uL(30), bL(36), eL(43), P1, P2
- *b- bacterial, e- eukaryotic, u- universal



*Ban N et al.
A new system for naming ribosomal proteins. *Curr Opin Struct Biol.* 2014; 24:165.

eRibosomal RNA

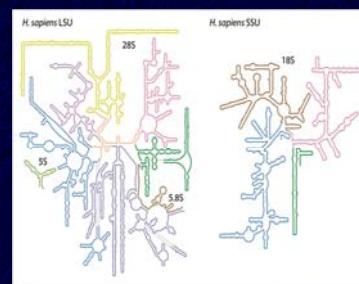
LARGE SUBUNIT

5S, 5.8S, 28S

(peptidyl-transferase –
ribozyme activity –
accomplished by rRNA)

SMALL SUBUNIT

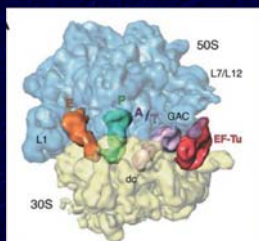
18S (links mRNA)



RiboVision
<http://apollo.chemistry.gatech.edu/RiboVision/index.html>

Ribosomes – ribonucleoproteins

Ribonucleoproteins – complexes of RNA and proteins



https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2009/advanced-chemistryprize2009.pdf

The Eukaryotic Ribosome

Structure of the 60S and 40S subunits
in complex with initiation factors 1 and 2

Klinge S, Voigts-Hoffmann F, Leibundgut M, Ban N.
Atomic structures of the eukaryotic ribosome.
Trends Biochem Sci. 2012 ;37(5):189-98

RIBOSOME – FUNCTIONAL SITES

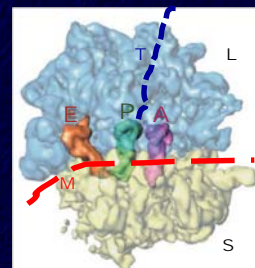
A site: available/occupied by aminoacyl-tRNA. The aminoacyl-tRNA in the A-site functions as the acceptor for the growing polypeptide during peptide bond formation.

P site: occupied by peptidyl-tRNA – a tRNA carrying the growing polypeptide chain.

E site: it harbors released tRNA, on transit out from the ribosome.

M groove – mRNA site spanning the ribosome

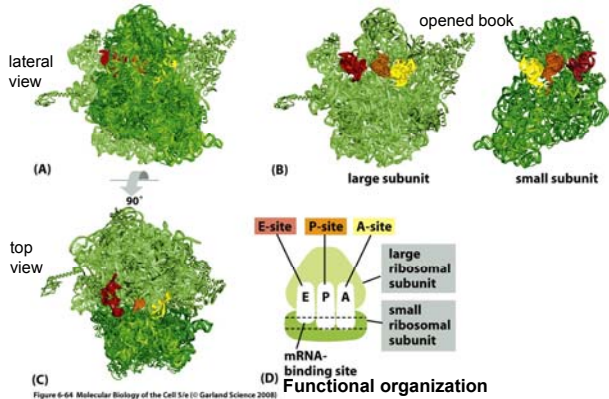
T pathway – polypeptide exit tunnel



https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2009/advanced-chemistryprize2009.pdf

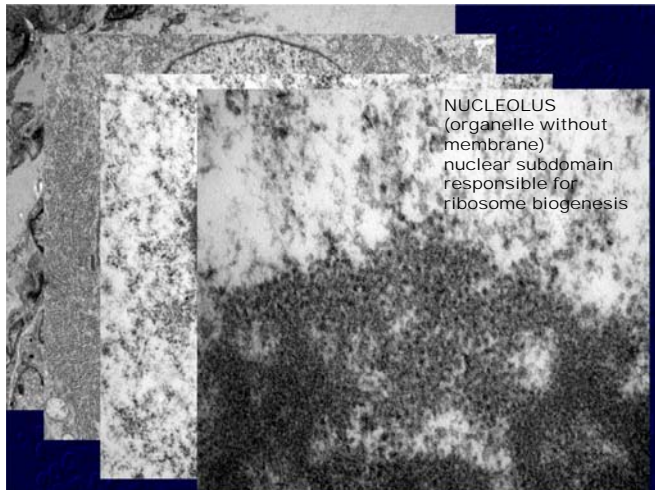
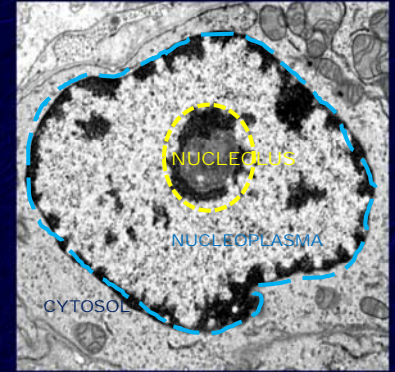
Organization of the machinery

Ribosome – 3D Structure



RIBOSOME BIOGENESIS

production of ribosomal subunits in the nucleolus



ARN

Biosynthesis in the nucleus by **RNA polymerases** according with DNA template (TRANSCRIPTION)

4 major ribonucleotides - the structural units of RNAs

Nucleotide	Symbols	Nucleoside
Adenylate (adenosine 5'-monophosphate)	A, AMP	Adenosine
Guanylate (guanosine 5'-monophosphate)	G, GMP	Guanosine
Uridylate (uridine 5'-monophosphate)	U, UMP	Uridine
Cytidylate (cytidine 5'-monophosphate)	C, CMP	Cytidine

RNA polymerase

enzymes that produce (assisted by ~200 factors) the primary transcript of RNA.

DNA→RNA

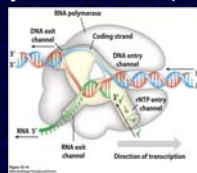
RNApol I – 45S pre-RNAr

(precursor for 28S, 5.8S și 18S)

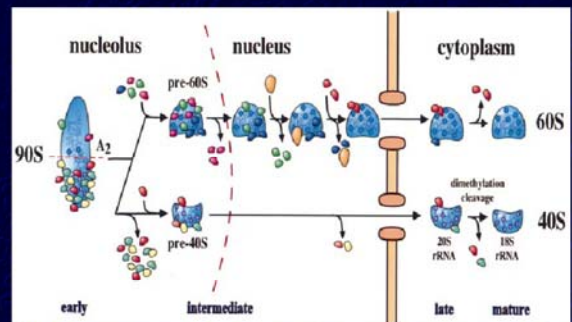
RNApol II – mRNA and microRNA

RNApol III – rRNA 5S and tRNA

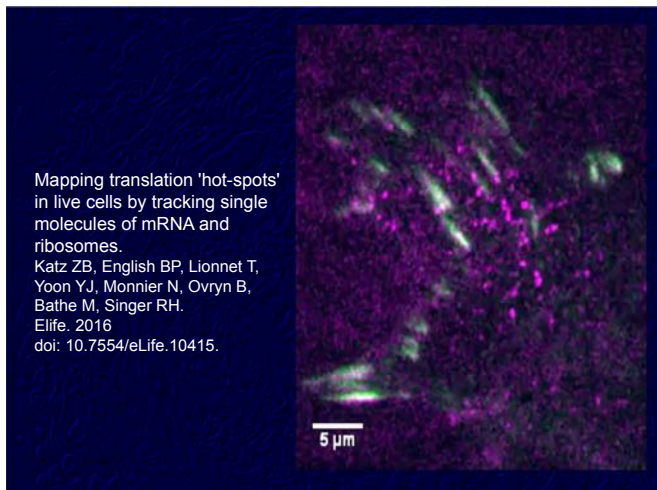
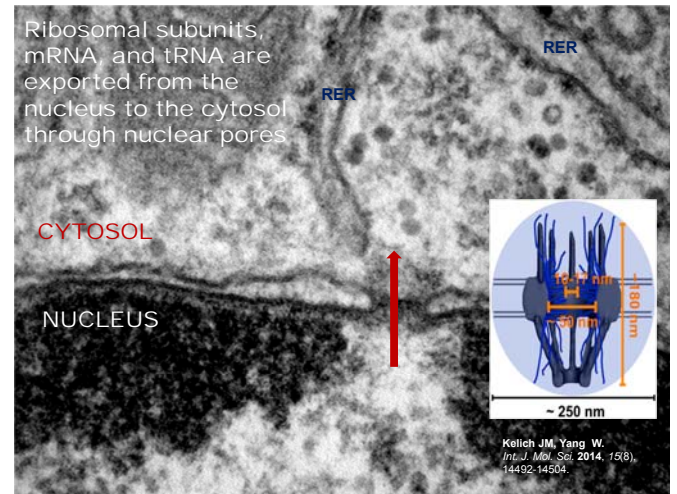
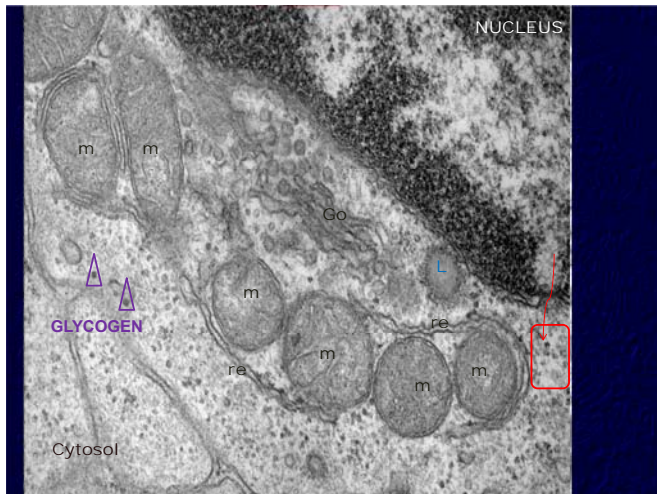
N.B. RNApol II and RNApol III act outside of the nucleolus



RIBOSOME'S BIOGENESIS



The path from nucleolar 90S to cytoplasmic 40S pre-ribosomes. EMBO J. 2003 Mar 17; 22(6): 1370-1380.



RNA – coding/non-coding

Involved in transcription, translation, regulation

- MESSENGER – **mRNA** <5% (variable length)
- TRANSFER – **tRNA** – 10-15% (80nt)
- RIBOSOMAL – **rRNA** – 80-90% (e: 18S / 5S, 5.8S, 28S)
- SIGNAL RECOGNITION PARTICLE - **SRP** (4.5S)

RNA #:

- snoRNA, snRNA,
- microARN (21-22nt),
- siRNA (20-25nt),
- piRNA (29-30nt), ...

https://en.wikipedia.org/wiki/List_of_RNAs

<http://learn.genetics.utah.edu/content/basics/centraldogma/>

Messenger RNA - mRNA

a large family of RNA molecules that convey genetic information from **DNA to the ribosome**, where they **specify the amino acid sequence of the protein** products of gene expression.

mRNA genetic information is in the sequence of nucleotides, which are arranged into **CODONS** (three bases).

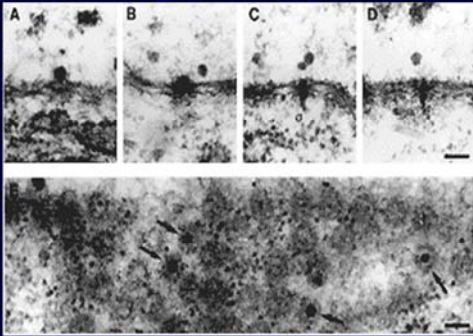
<http://laneogenetics.pbworks.com/w/page/58023767/Mutation>

Messenger RNA - mRNA

- 5' cap - 7-methylguanosine
- 5' UTR – untranslated region (translation control)
- **START** codon - AUG [*Kozak consensus sequence* (ACCAUGG)]
- **CODING SEQUENCE**
- **STOP** codons - UGA, UAG, UAA
- 3' UTR (zip code for subcellular localization of mRNA)
- 3' poly(A) tail (stability)

http://mol-biol4masters.masters.gkrjaj.org/html/Ribose_Nucleic_Acid8-Stability_of_mRNAs_and_its_Regulation.htm

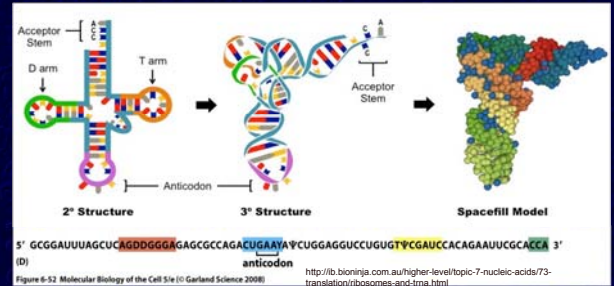
Hans Mehlín, Bertil Daneholt, Ulf Sköglund. Cell 69: 605-613 1992.
"Translocation of a Specific PREMESSENGER RIBONUCLEOPROTEIN PARTICLE through the Nuclear Pore Studied with Electron Microscope Tomography".



<https://www.nobelprize.org/educational/medicine/dna/index.html>

Eukaryotic transfer RNA - tRNA

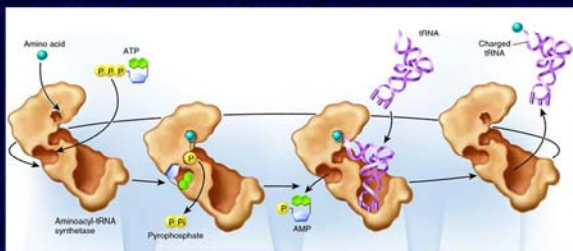
- The **acceptor stem** (3'-CCA) carries the amino acid
- The **anticodon** associates with the mRNA codon (via complementary base pairing)
- The **T arm** associates with the ribosome (via the E, P and A binding sites)
- The **D arm** associates with the tRNA activating enzyme (responsible for adding the amino acid to the acceptor stem – elongation factor)



Aminoacyl-tRNA-Synthetase

Reaction:

1. amino acid + ATP → aminoacyl-AMP + PP_i
2. aminoacyl-AMP + tRNA → **aminoacyl-tRNA** + AMP



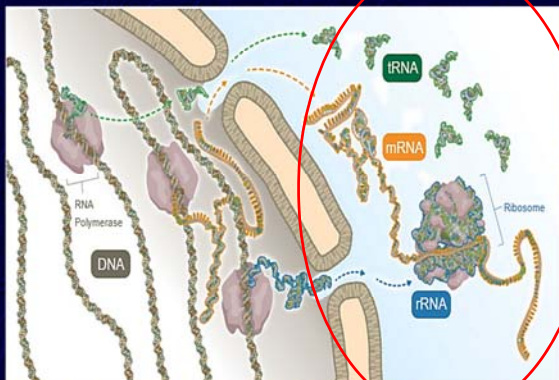
<http://memim.com/aminoacyl-trna-synthetase.html>

Biosynthetic process development

Stages of protein biosynthesis

- Initiation: initiation factors
- Elongation: elongation factors
- End of translation: releasing factors

REQUIRED COMPONENTS



<http://learn.genetics.utah.edu/content/basics/centraldogma/>

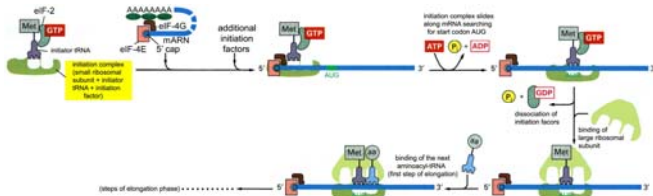
ENERGY SOURCES

Both ATP and GTP are required for the supply of energy in protein biosynthesis.

The reactions involve the breakdown of ATP or GTP (to AMP and GMP) with the liberation of pyrophosphate.

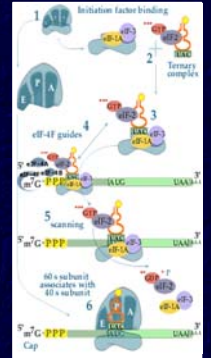
Each one of these reactions consumes two high energy phosphates (equivalent to 2 ATP).

Protein biosynthesis initiation



INITIATION – ribosome assembly

- 1) Three initiation factors (IF) and GTP bind to the ribosomal small subunit (SSU).
- 2) The initiator aminoacyl-tRNA (**Metionin-tRNA-UAC**) is attached on SSU
- 3) mRNA is attached on mRNA-binding site of SSU
- 4) The large ribosomal subunit joins the complex after initiation complex gliding on mRNA reaches the START codon.



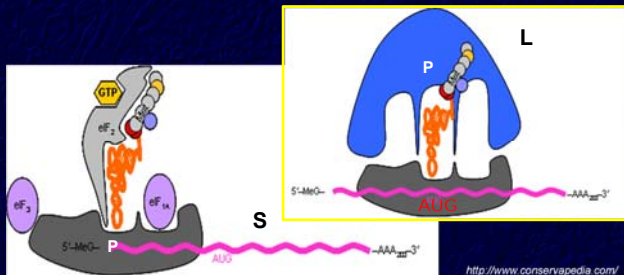
https://www.nobelprize.org/educational/medicine/dna/a/translation/pics/initiation_new.gif

INITIATION – ribosome assembly

Small subunit of ribosome

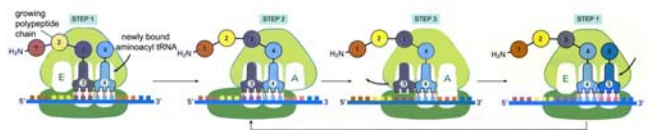
Metionin-tRNA-UAC (in P site)

Initiation factors (GTP-eIF1+eIF3)



http://www.conservapedia.com/Gene_expression

Elongation's steps

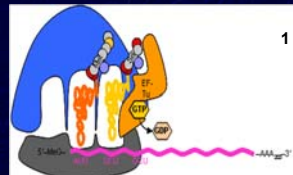


Repeating stage, n cycles

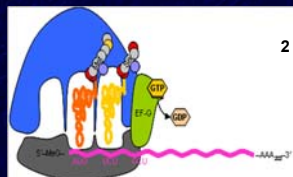
ELONGATION – aa1-aa2-aa3- ... aax

- 1) Elongation begins with the binding of the second aminoacyl-tRNA at the ribosomal aminoacyl (A) site. The tRNA is escorted to the A site by the elongation factor EF-Tu/EF1-GTP.
- 2) A peptide bond is formed between the carboxyl group of the terminal amino acid (Met in the first cycle) at the P site and the amino group of the newly arrived amino acid at the A site (peptidyl-transferase activity of the 28S rRNA, ribozyme, molecule in the large ribosomal subunit).
- 3) After EF-G/2-GTP binds to the ribosome and GTP is hydrolyzed, the tRNA carrying the elongated polypeptide translocates from the A site to the P site.
- 4) The discharged tRNA moves from the P site to the E (exit) site and leaves the ribosome.

ELONGATION – aa1-aa2

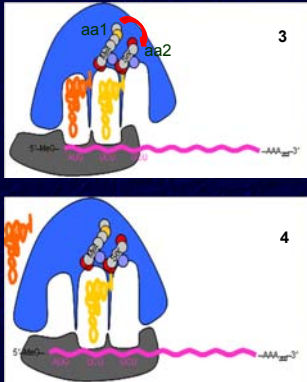


ELONGATION FACTORS - GTP



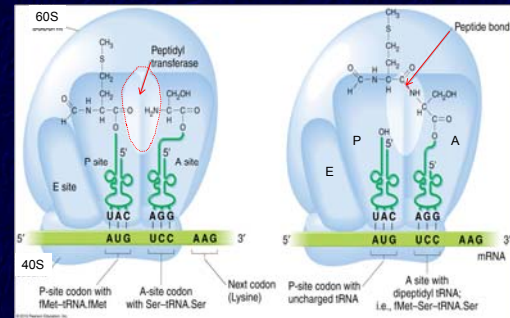
http://www.conservapedia.com/Gene_expression

ELONGATION – aa1-aa2

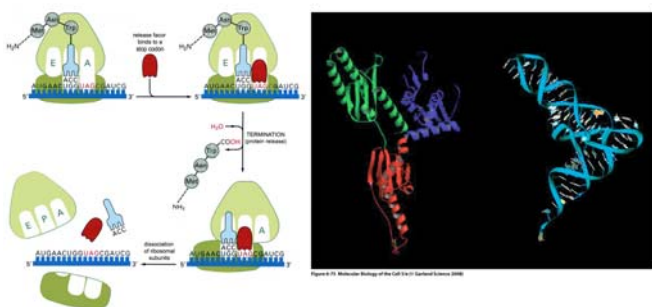


PETIDYL-TRANSFERASE
(RIBOZYME – RNA molecule with catalytic activity)

Ribozyme – peptidyl-transferase ARNr 28S



End of translation



Fooling the ribosome; releasing factor accomplishes a mimetic function

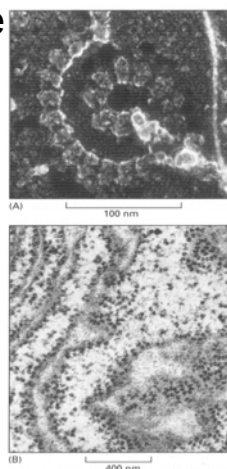
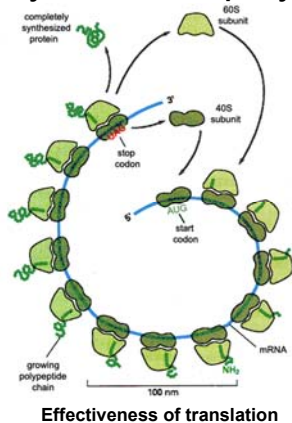
RIBOSOME – protein biosynthesis

V prokaryotes ~ 20 aa/sec
V eukaryotes ~ 2 aa/sec
P ~ 350 aa [1 protein/2-3min]



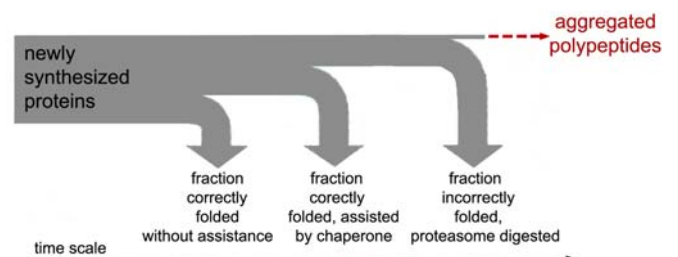
By Bensaccount at en.wikipedia, CC BY 3.0, <https://commons.wikimedia.org/w/index.php?curid=8287100>

Polyribosome/polysome

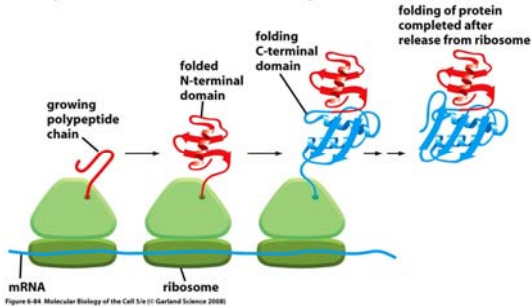


Protein folding and destiny

- Spontaneous folding
- Assisted folding – chaperones
 - Hsp60
 - Hsp70
- Folding failure and/or nonfunctional – proteasome



Spontaneous folding (non-assisted)



Intramolecular chaperones

Peculiar sequences in the polypeptide chain acting in protein folding by itself:
- type I – sequences at N-terminal end; type II – sequences at C-terminal end;
- no other significance in protein function (these sequences are finally removed)

Assisted folding – chaperones

Heat shock proteins family (discovered by Italian researcher Ferruccio Ritossa, in 1962)

Classification according the molecular weight

- small HSP (sHSP)
- HSP40
- HSP60
- HSP70
- HSP90
- HSP100

Various mechanisms of action

Assisted folding – chaperones

(hsp70 mechanism for protein folding)

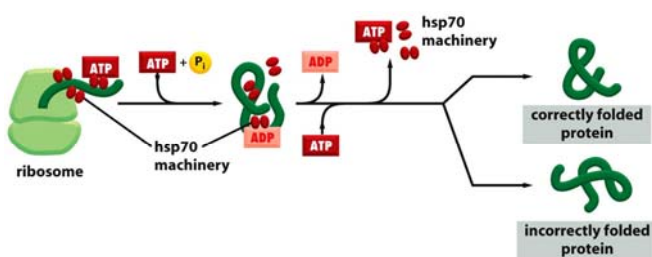


Figure 6-86 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Assisted folding – chaperones

(hsp60 mechanism for protein folding)

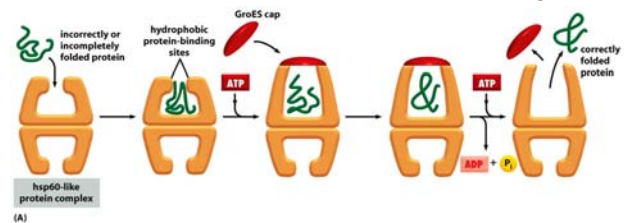
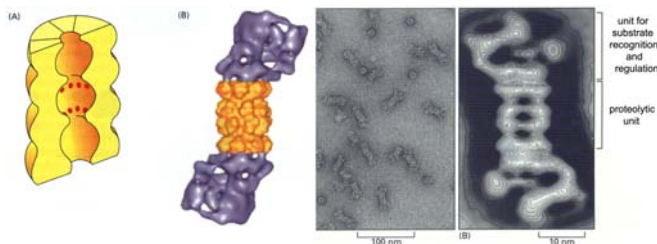


Figure 6-87 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Incorrect folded protein digestion

Proteasome and polyubiquitination



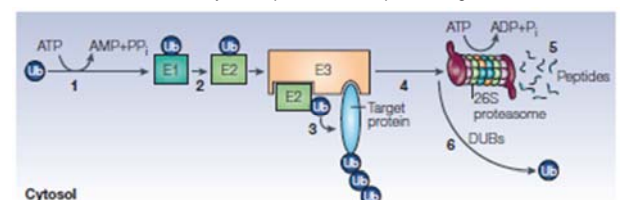
Protein (poly)ubiquitination

E1, Ub-activation enzyme

E2, Ub-conjugation enzyme

E3, Ub ligase

Avram Hershko, Aaron Ciechanover and Irwin Rose, 2004 Nobel Prize in Chemistry
"for the discovery of ubiquitin-mediated protein degradation"



Form: Welchman RL, Gordon C, Mayer RJ. *Nat Rev Mol Cell Biol.* 6(8), 599-609 (2005).

Intracellular distribution of newly biosynthesized proteins

- Import into nucleus
- Import into peroxisome
- Import into mitochondrion
- Import into endoplasmic reticulum
 - cell membrane
 - organelles (ER, Golgi complex, lysosomes)
 - endosomal system
 - extracellular space
- Signal peptide
 - sequential signal (signal sequence)
 - conformational signal (signal patch)

Signal peptides

Table 12-3 Some Typical Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Ala-Lys-Ala-Gly-Asp-
Import into mitochondria	*H ₂ N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	*H ₂ N-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-COO-
Import into ER	*H ₂ N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Val-Gly-Ser-Leu-Phe-Trip-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-COO-

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in red and negatively charged amino acids are shown in green. Similarly, important hydrophobic amino acids are shown in white and hydroxylated amino acids are shown in blue. *H₂N indicates the N-terminus of a protein; COO⁻ indicates the C-terminus.

Table 12-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Summary

- Protein biosynthesis initiates into the cytosol
- Needs cooperation between ribosome, mRNA and tRNA
- Newly biosynthesized proteins need correct folding
- Folding involves spontaneous or chaperone assisted events
- Proteins that are failing correct folding are degraded by proteasome
- Correctly folded proteins are directed toward appropriate cellular locations by specific mechanisms, due to different signal peptides