BME 42-620 Engineering Molecular Cell Biology

Lecture 18:

Gene Expression II: From RNA to Protein

Protein Degradation

Chapter 6

Outline

- Review: from DNA to RNA
- Genetic code
- Ribosome
- Translation cycle
- Chaperones
- Protein degradation

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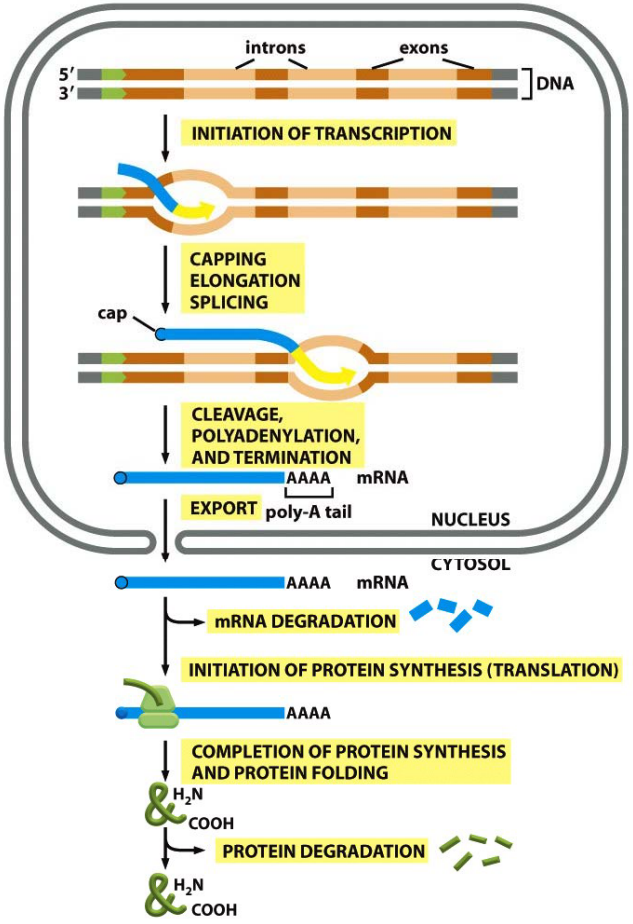
Review: From DNA to RNA

- Steps of transcription in eukaryotic cells:
  - ↓ Initiation
  - ↓ 5' end capping
  - ↓ Elongation
  - ↓ Splicing
  - ↓ Cleavage
  - ↓ 3' end polyadenylation
  - ↓ Termination

  - ↓ Nuclear export of mature mRNA to the cytoplasm

- Proofreading and repair mechanisms for quality control.



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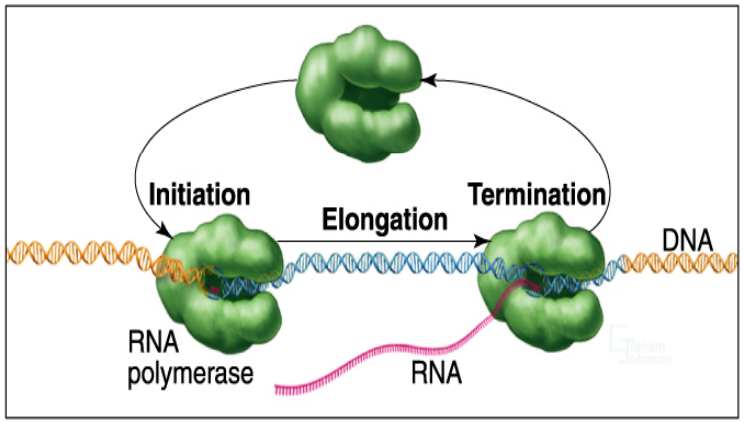
Transcription Elongation

- Elongation phase begins after around 10 bases are synthesized.

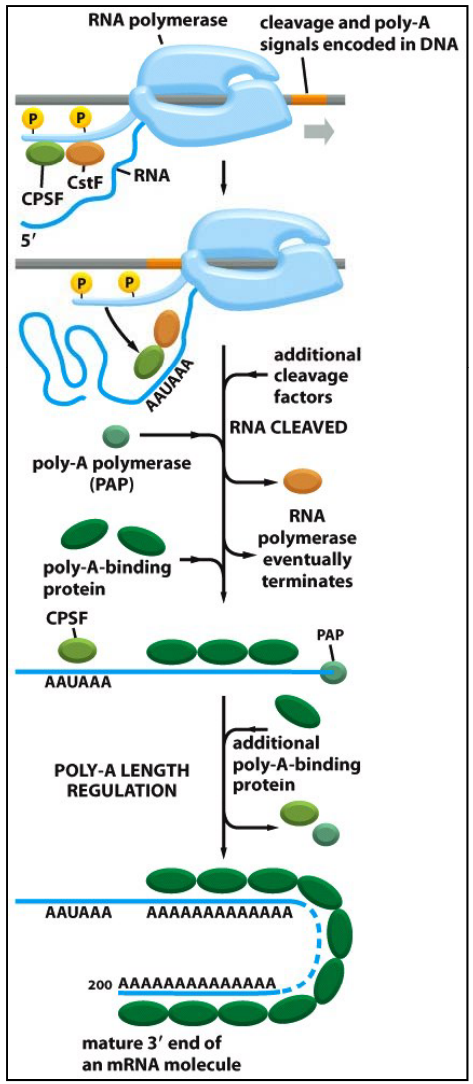
- RNA polymerase conducts multiple processes simultaneously
  - Unwinds DNA in front
  - Reanneals DNA behind
  - Disassociates growing RNA chain from template
  - Performs proofreading



- Elongation factors (proteins) assist movement of RNA polymerases and prevent them from falling off prematurely.

Transcription Termination

- 5' end capping → splicing → 3' end capping.
- RNA polymerase continues to transcribe after cleavage of pre-mRNA.
- The new transcript lacks a 5' cap and is degraded by exonuclease.



CstF: cleavage stimulation factor
CPSF: cleavage and polyadnelyation specificity factor

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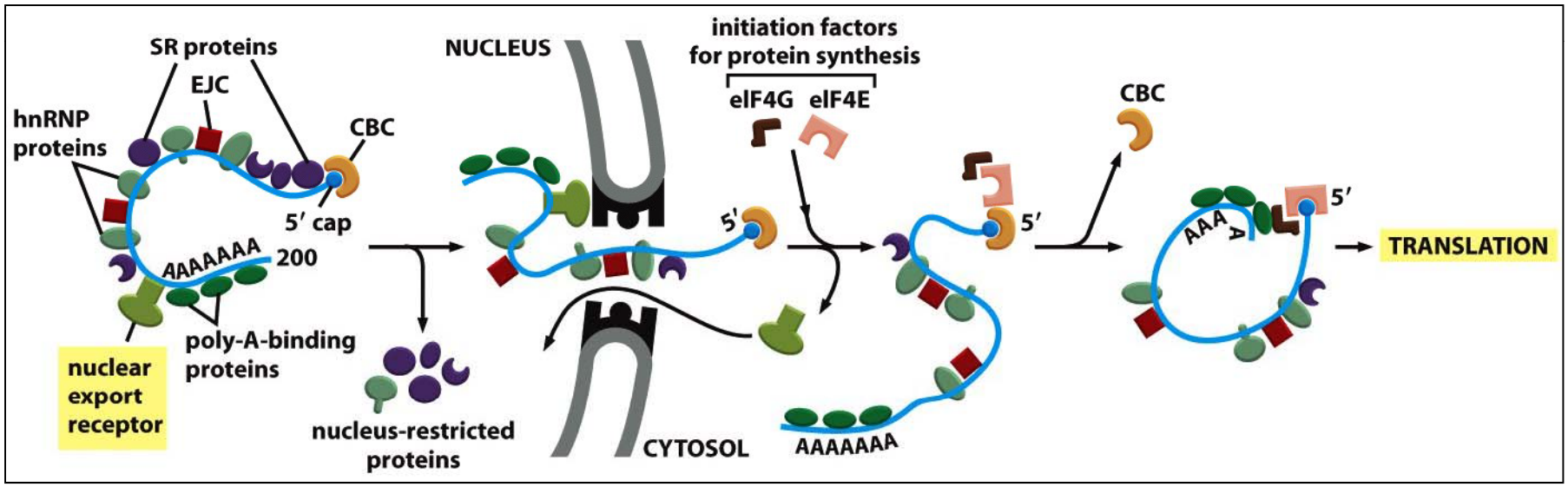
Nuclear Export of RNA

- Export by forming a mRNA-protein complex.
- Nucleus restricted proteins need to unbind.
- Export receptors are re-imported and reused.



Erkmann & Kutay, Nuclear export of mRNA
Exp. Cell Res. 296:12, 2004

CBC: cap binding complex
hnRNP: heterogeneous ribonucleoprotein
EJC: exon junction complex
SR: serine-arginine rich protein

- Review: from DNA to RNA
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Overview: From RNA to Protein

- Translation: nucleotides → amino acids.
- Caps protect exported mRNA in the cytoplasm.
  - 5' end protects mRNA from degradation in the nucleus and defines the starting point of translation.
  - 3' end protects mRNA from degradation in the cytoplasm.
- Each eukaryotic RNA encodes one protein.
  - Exceptions: RNA can also be the end product, e.g. snRNA, rRNA
- Most proteins are synthesized in the cytoplasm by ribosomes.
- A few proteins are synthesized in mitochondria.

Genetic Code (I)

- mRNA is translated in the unit of 3 nucleotides (a codon).
- Translation is performed according to the genetic code ($4 \times 4 \times 4 \rightarrow 20$).
- AUG serves as the start codon and codes for methionine.
- UAA, UGA, UAG are stop codons.

1st position (5' end)	2nd position U	2nd position C	2nd position A	2nd position G	3rd position (3' end)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

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	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
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Trp: tryptophan

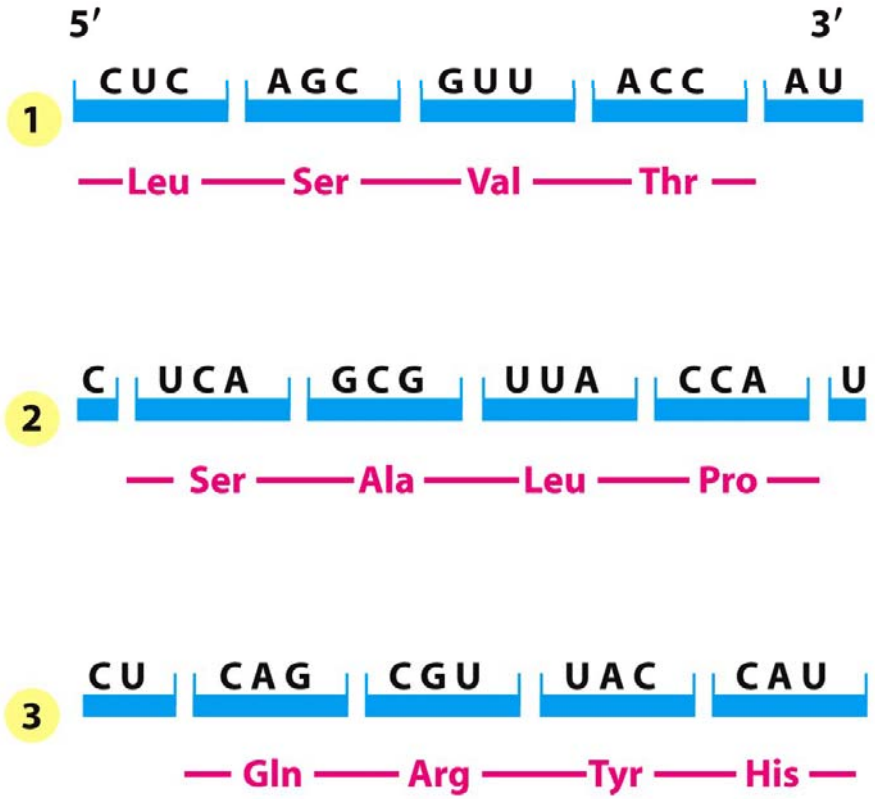
Genetic Code (II)

- mRNA is read from the 5’ end to the 3’ end.

- Leu: leucine
  Ser: serine
  Val: valine
  Thr: threonine

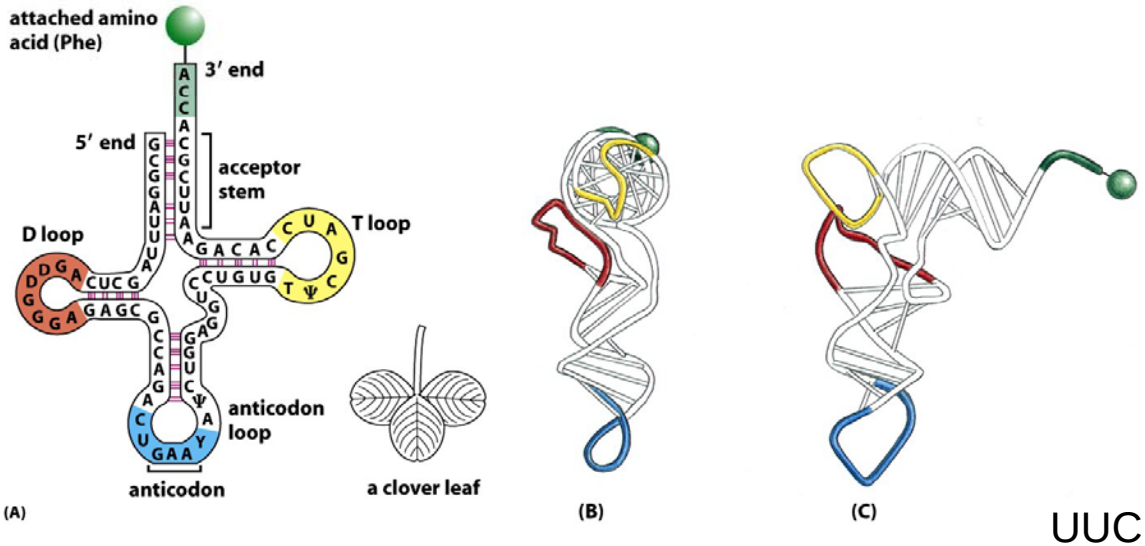
  Ala: alanine
  Pro: proline

  Gln: glutamine
  Arg: arginine
  Tyr: tyrosine
  His: histidine



tRNA Function & Structure

- A tRNA serves as an adaptor between amino acids and codons.
- Each tRNA is ~76 nucleotides in length
- Two important regions:
  - anticodon region
  - amino acid acceptor region

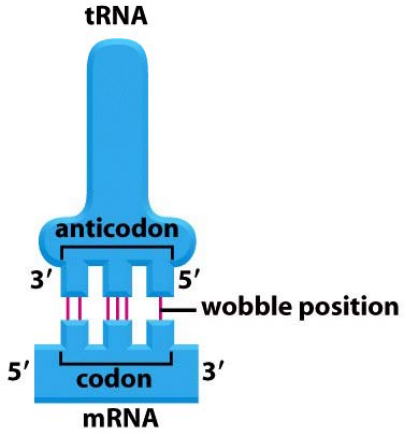


UUC: phenylalanine

5′ GCGGAUUUAGCUCAGDDGGGAGAGCGCCAGACUGAAYAΨCUGGAGGUCCUGUGTΨCGAUCCACAGAAUUCGCACCA 3′
(D)
anticodon

Codon and Anticodon Base-Pairing

- Some amino acids have more than one tRNA
- Some tRNAs can base-pair with more than one codons. That is, a mismatch (wobble) at the third position can be tolerated. (I: inosine)
- Humans have ~500 tRNA genes but only ~48 anticodons.



bacteria

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

eucaryotes

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

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	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
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

tRNA Synthetase

- tRNAs are synthesized by RNA polymerase III in eukaryotic cells.
- tRNA synthetases link amino acids to corresponding tRNA.
- Most cells have 20 tRNA synthetases.

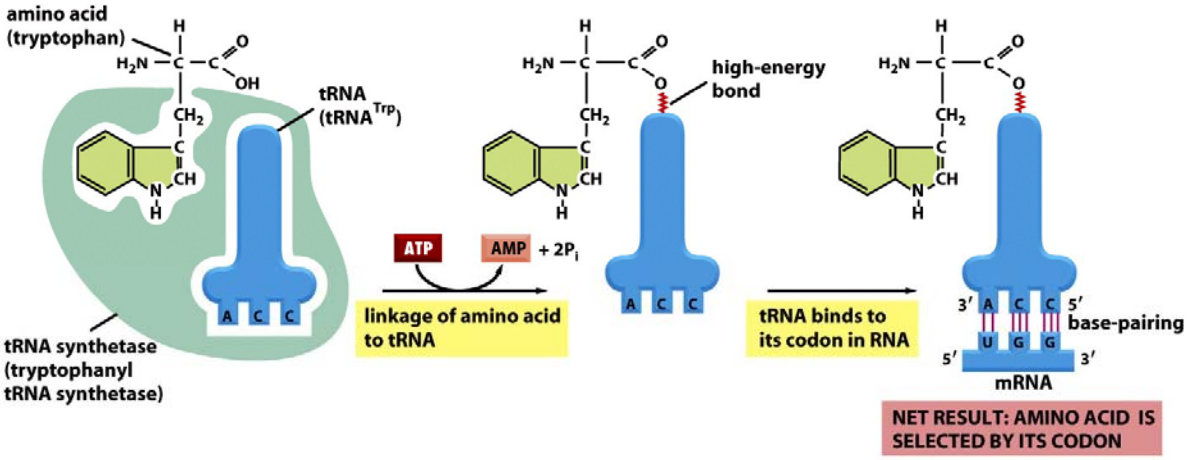


Table 6–2 The Three RNA Polymerases in Eucaryotic Cells

TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

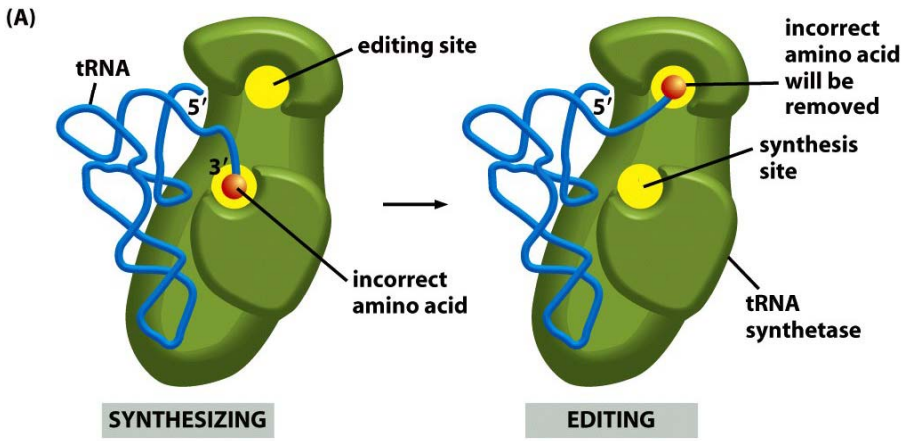
The rRNAs are named according to their "S" values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA.

tRNA Synthetase Uses Editing for Accuracy

- Overall coupling error rate: 1 in 40,000

- First level of amino acid selection
  - the correct amino-acid has the highest affinity for the binding pocket.

- Second level of amino acid control
  - Correct amino-acid is excluded from the editing pocket.
  - Incorrect amino-acid binds the editing pocket but then is hydrolyzed and released.



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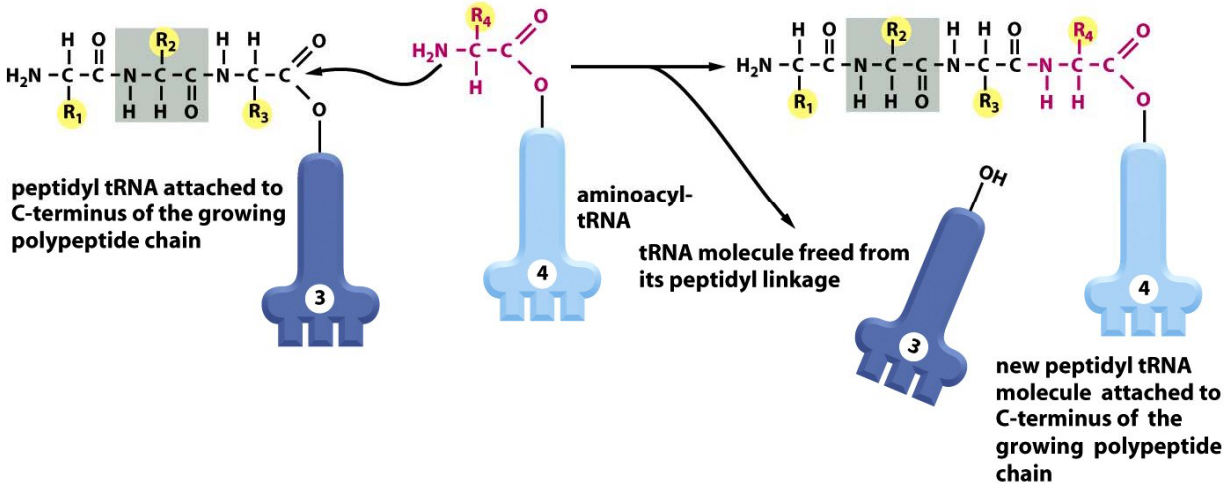
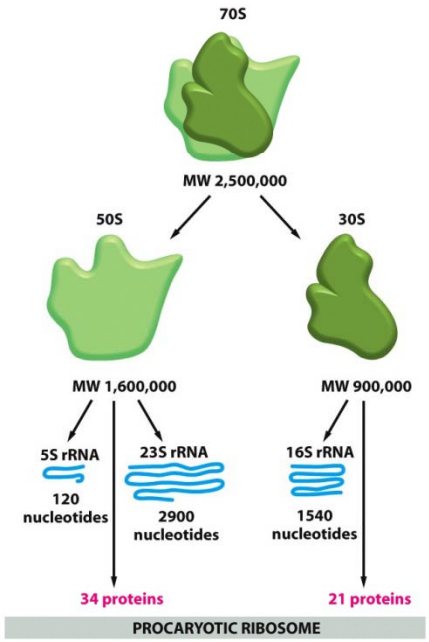
Growth of Polypeptide Chain

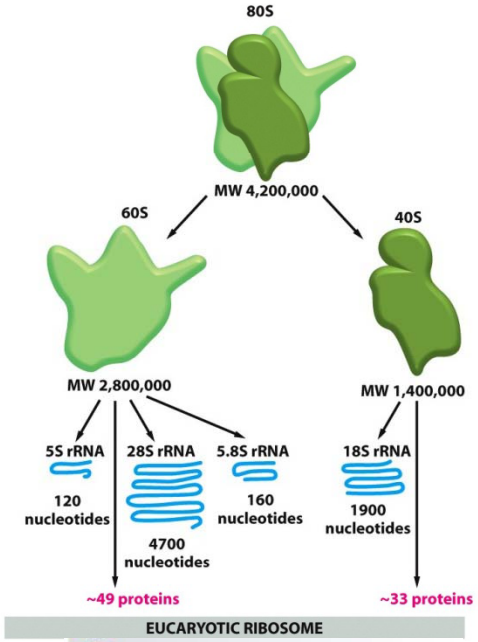
- Polypeptide chains grow by adding amino acids to the C-terminal.
- This process is undertaken in the ribosome.
- Synthesis error rate: 1 in 10,000 amino acids.

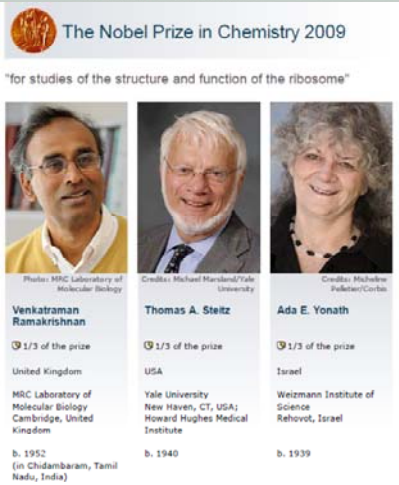


Ribosome Structure

- Main function: base pairing mRNA codon with tRNA anticodons to synthesize the polypeptide chain.
- Overall, more than 50 ribosomal proteins and rRNAs.
- Structure well conserved between bacteria and eukaryotes.
- Small subunit & large subunit first synthesized in the nucleus.







Ribosome (II)

- Small subunit:

  - Mediates the base pairing of mRNA codons with tRNA anticodons.

- Large subunit:

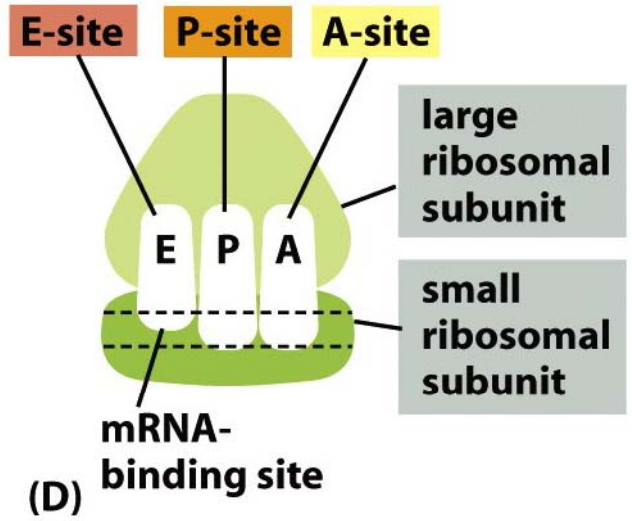
  - Catalyze the synthesis of polypeptide chains.

- Small and large subunit are separate until being joined by an mRNA.

- Approximately 2 amino acids per second in eukaryotic cells; 20 amino acids per second in bacterial cells.

Ribosome (III)

- A ribosome has four binding sites.
  - mRNA binding sites
  - tRNA binding sites: E, P, A



- A tRNA binds tightly at A-site and P-site only if its anticodon base pairing with a codon.

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Translation Cycle

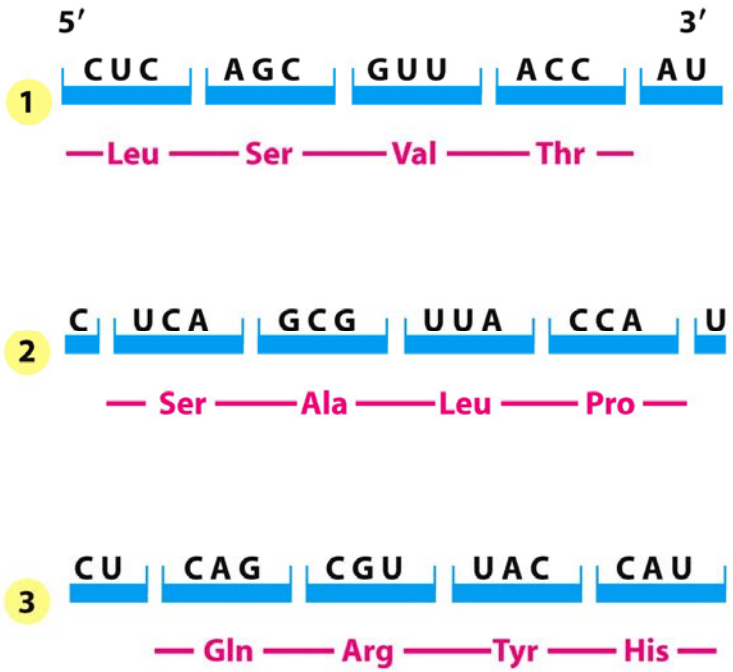
- Initiation
  - ribosome small subunit and initiator tRNA bind the start codon (AUG) of mRNA.

- Elongation
  - tRNA brings the correct amino acid to the ribosome according to the sequence of mRNA codon.
  - Ribosome catalyzes the formation of the polypeptide chain.

- Termination
  - A protein factor (not tRNA) binds the mRNA.
  - C-terminal of the polypeptide chain is hydrolyzed; the chain is released.
  - Ribosome subunits dissociate for recycling.

Initiation of Translation (I)

- The importance of correct initiation
- Initiator tRNA carries methionine (AUG) so that newly synthesized protein always has methionine as the first amino acid.
- The initial methionine is often removed later on.



Ala: alanine
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Gln: glutamine
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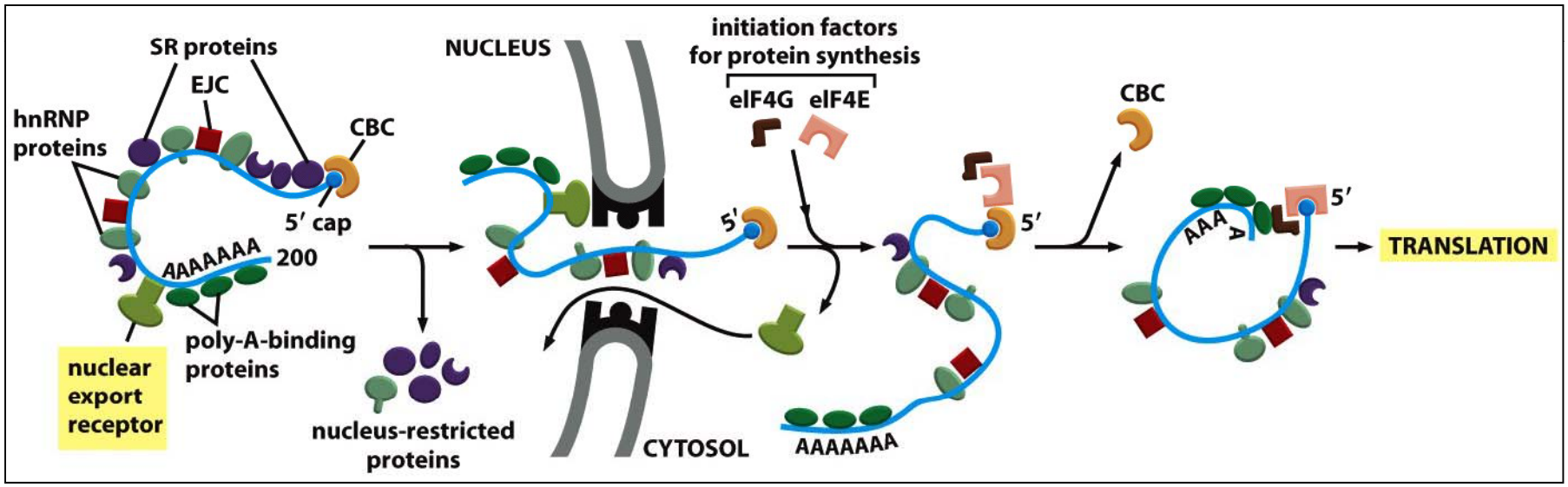
Nuclear Export of RNA

- Export by forming a mRNA-protein complex.
- Nucleus restricted proteins need to unbind.
- Export receptors are re-imported and reused.



Erkmann & Kutay, Nuclear export of mRNA
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CBC: cap binding complex
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EJC: exon junction complex
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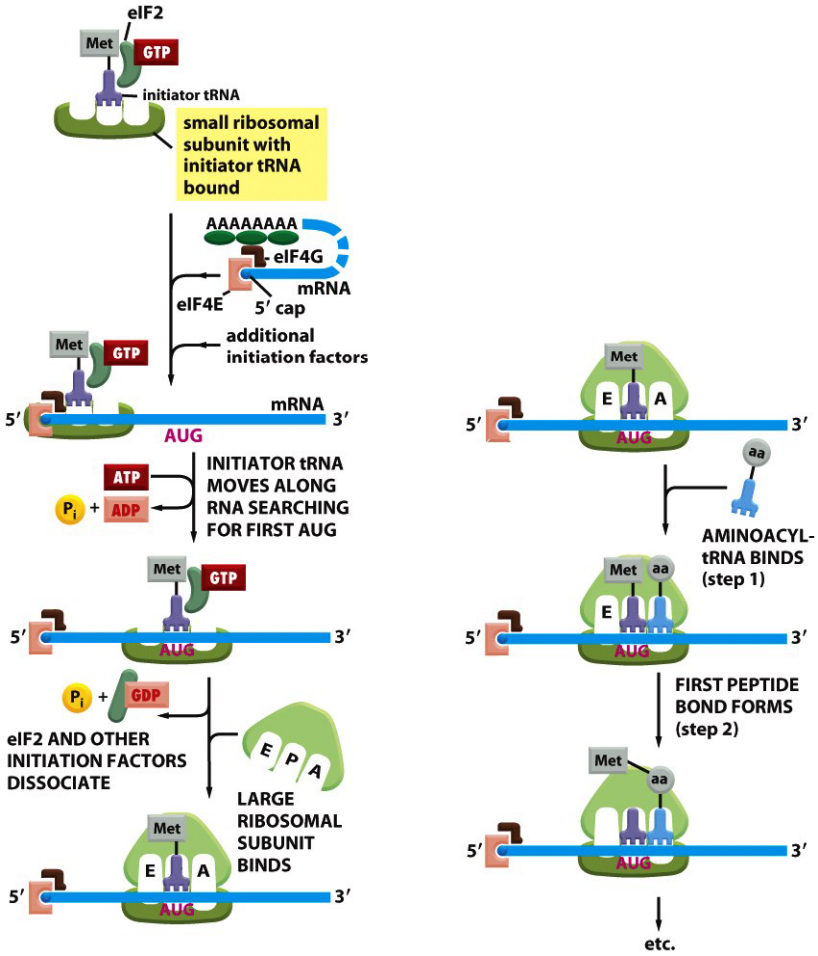
Initiation of Translation (II)

- Eukaryotic initiation factors (eIFs)
- First, initiator tRNA is loaded into the ribosome small subunit with eIFs. The complex binds the 5' end.
- Second, search for the first AUG.
- Third, translation starts at the first AUG. eIFs come off so that the large subunit can bind.
- Fourth, ribosome catalyzes the formation of the first peptide bond.



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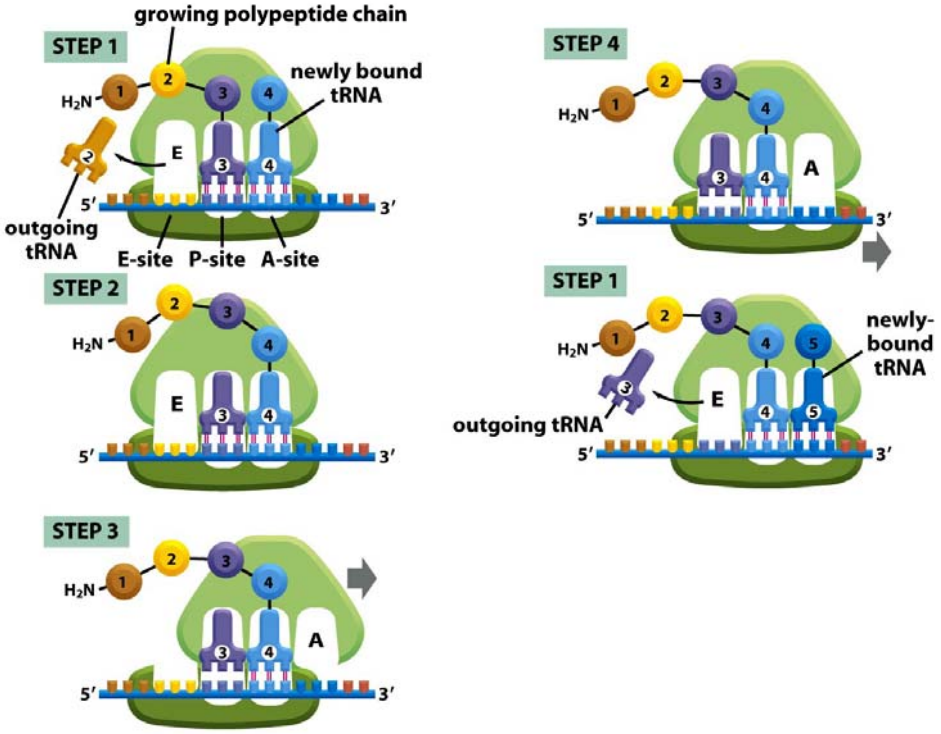
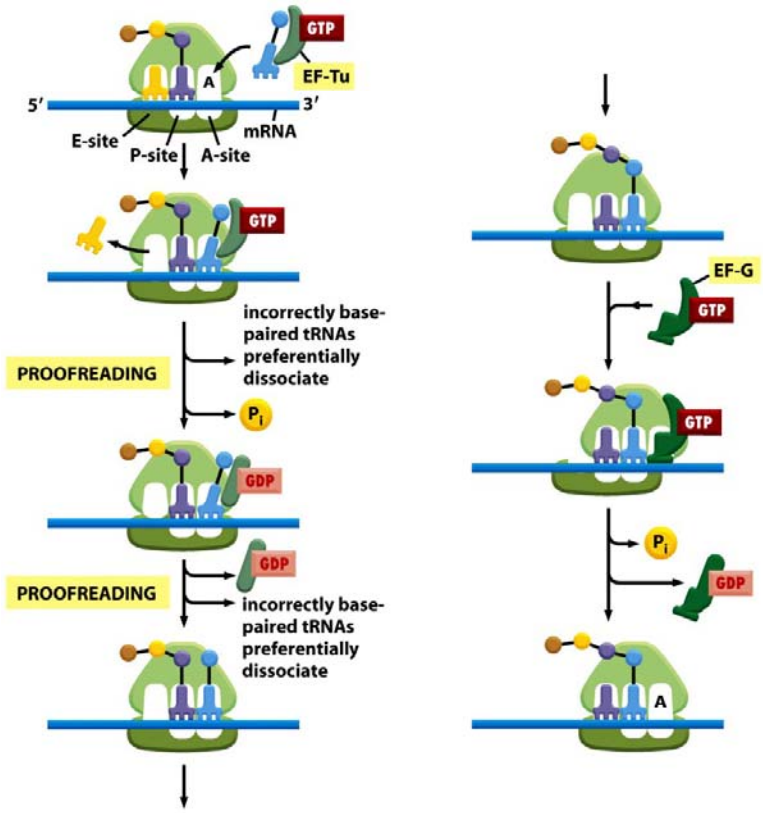
Translation Cycle: Elongation (I)

- Step 1: tRNA binds a vacant A-site. Used tRNA dissociates from the E-site.
- Step 2: a new polypeptide bond is formed.
- Step 3: the large subunit translocates by one codon.
- Step 4: the small subunit translocates by one codon and reset the ribosome with an empty A-site.



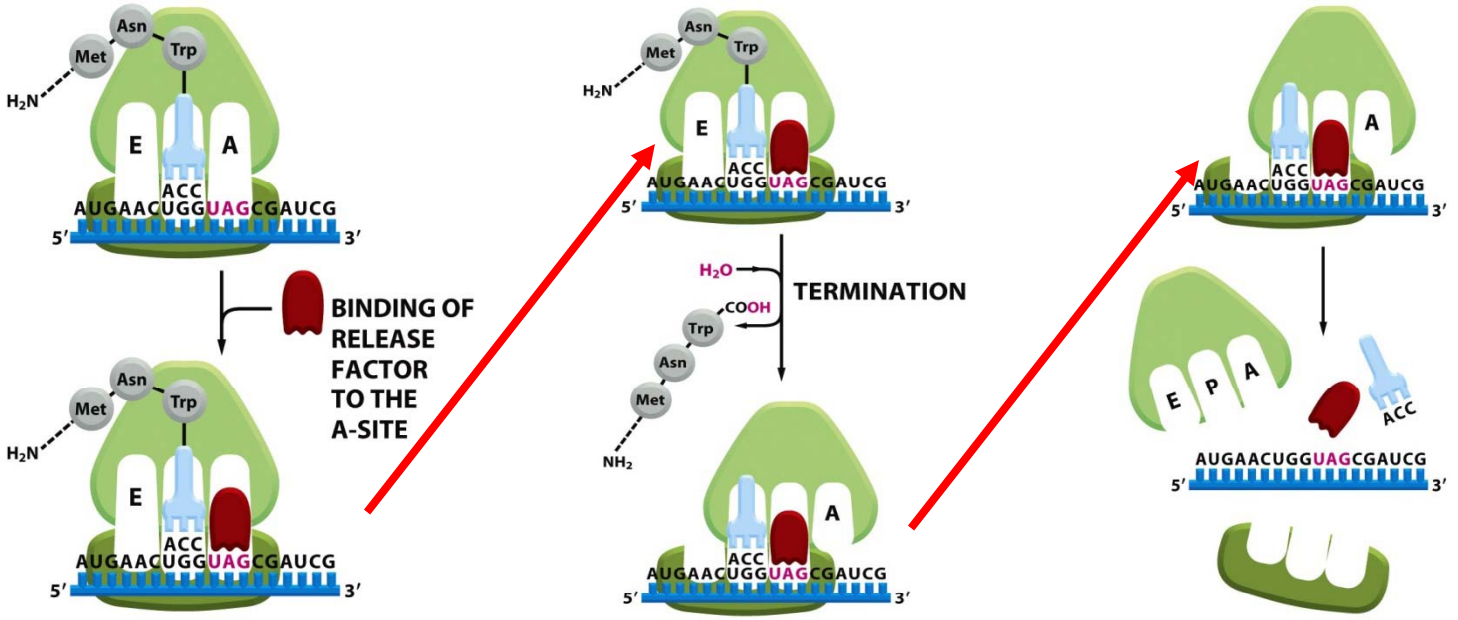
Translation Cycle: Elongation (II)

- Elongation factors provide important assistance to the elongation process.
- EF-Tu and EF-G in bacteria.
- EF1 and EF2 in eukaryotes.
- Functions
  - Proofreading
  - Accelerating translation by helping translation move forward



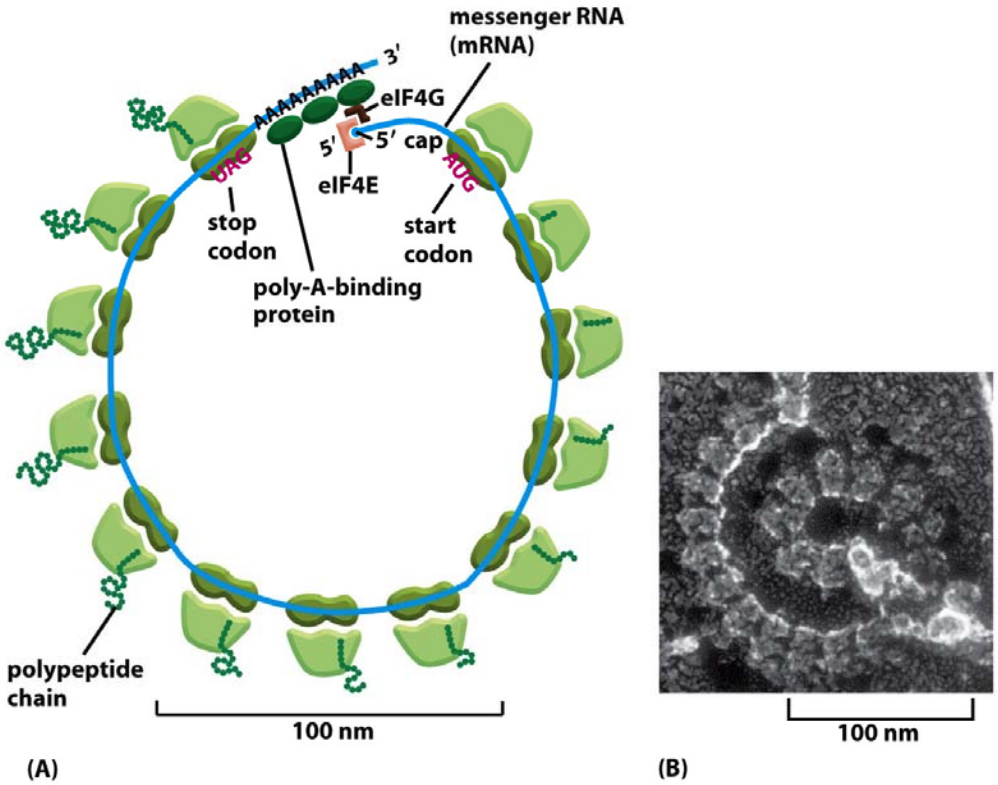
Termination of Translation

- When a stop codon is encountered, release factors bind to A-site and induces ribosome to cleave the polypeptide chain.



Translation by Multiple Ribosomes

- Multiple ribosomes can space as close as 80 nucleotides apart.
- Used by both bacteria and eukaryotes.



Ribosome & Antibiotics

- To design antibiotics to preferentially block bacterial protein synthesis.

Table 6–4 Inhibitors of Protein or RNA Synthesis

INHIBITOR	SPECIFIC EFFECT
Acting only on bacteria	
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from translation initiation to chain elongation and also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
Erythromycin	binds in the exit channel of the ribosome and thereby inhibits elongation of the peptide chain
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
Acting on bacteria and eucaryotes	
Puromycin	causes the premature release of nascent polypeptide chains by its addition to the growing chain end
Actinomycin D	binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
Acting on eucaryotes but not bacteria	
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 6–66)
Anisomycin	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
α-Amanitin	blocks mRNA synthesis by binding preferentially to RNA polymerase II

The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.

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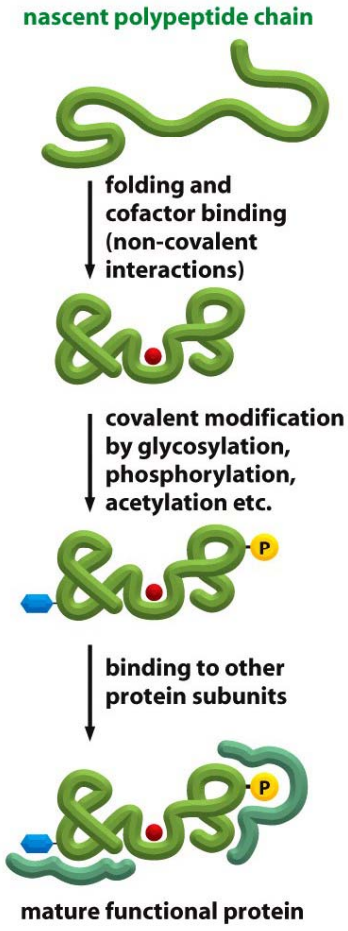
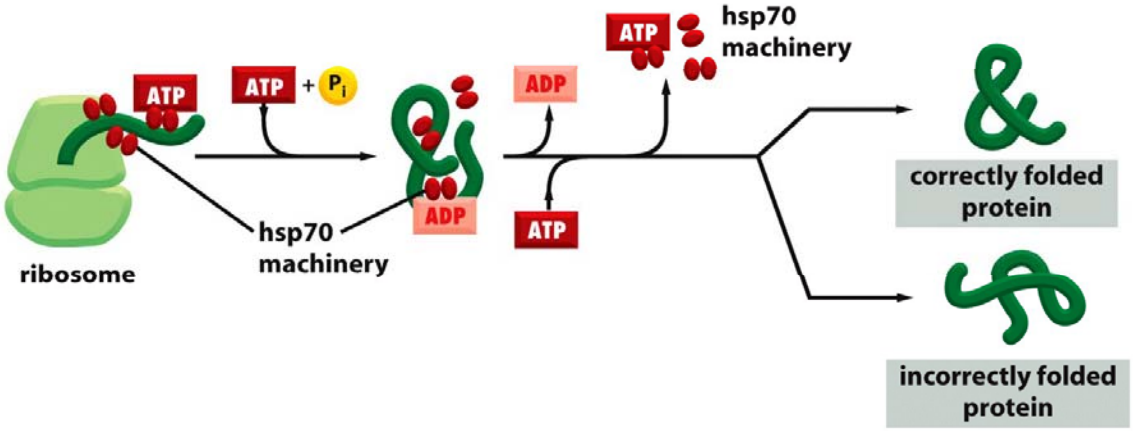
Chaperons-Assisted Protein Folding (I)

- To help prevent aggregation of hydrophobic regions
- To assist folding
- Many chaperones are heat-shock proteins.
- There are multiple families of chaperones
  - Hsp60
  - Hsp70
  - Hsp90



Chaperons-Assisted Protein Folding (II)

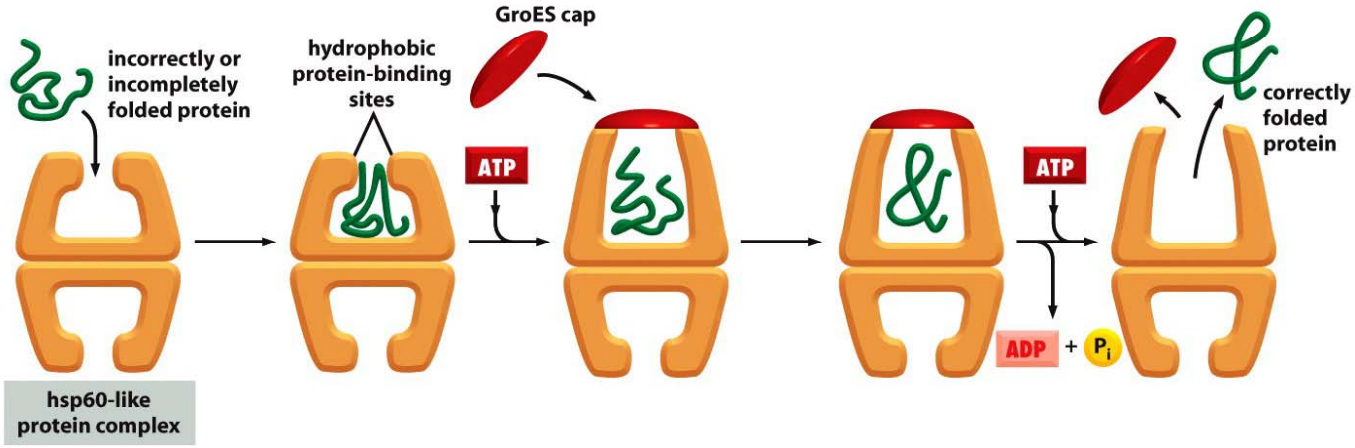
- Hsp70 chaperons
  - Most prevalent. Act early in the synthesis of protein.
  - Binds and releases peptides with hydrophobic residues.



- Hsp90 Chaperons
  - Stabilizes steroid-hormone receptors.

Chaperons-Assisted Protein Folding (III)

- Chaperonins (Hsp60 in mitochondria; GroEL in bacteria) serves as a protein misfolding correction site.



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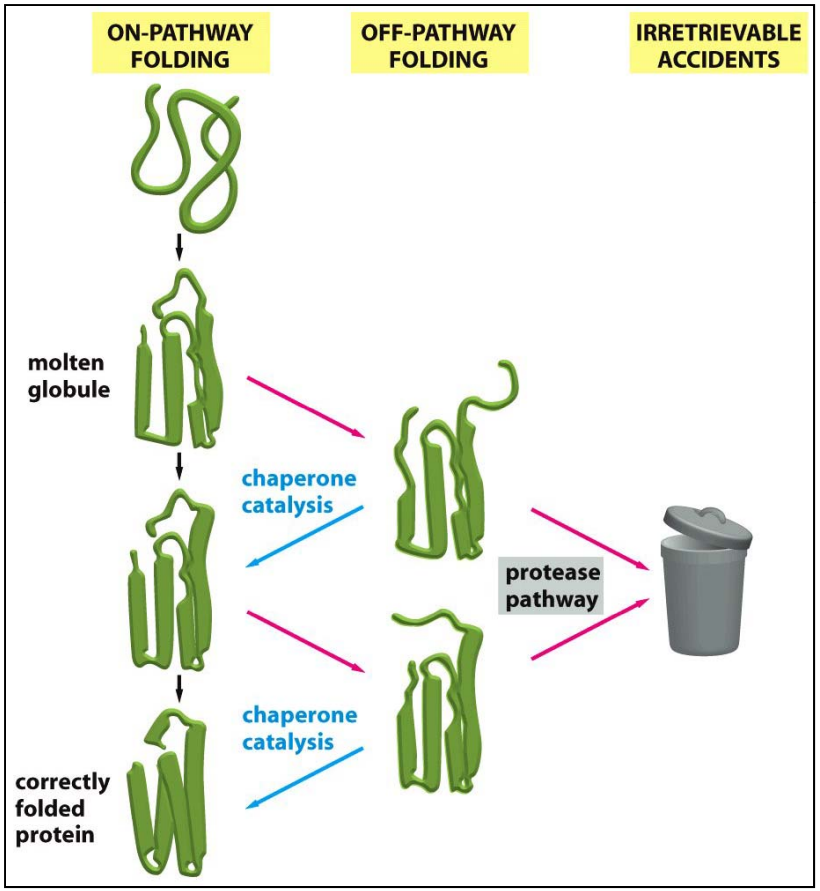
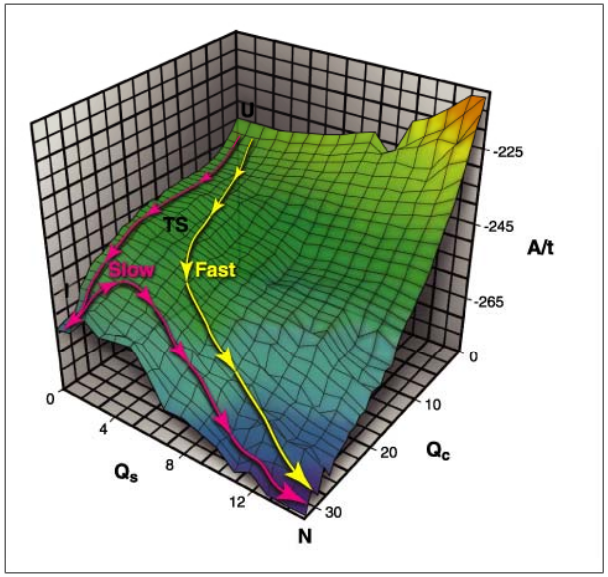
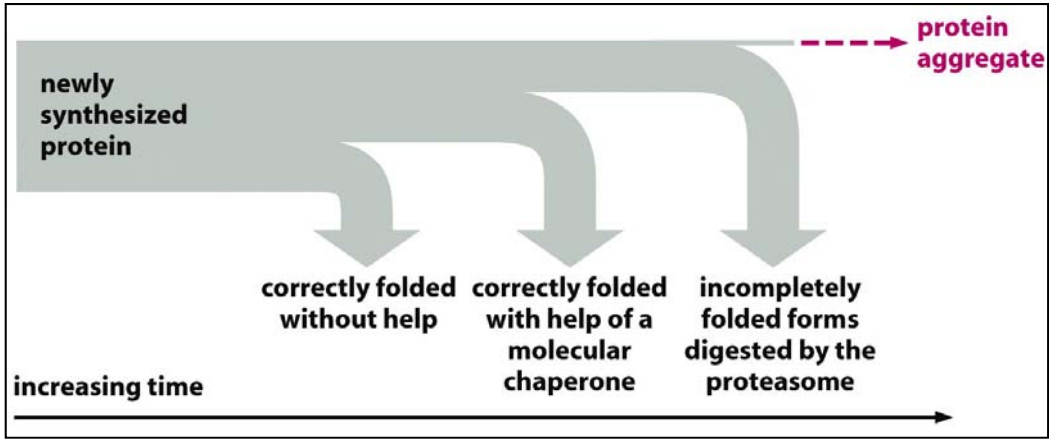
Different Outcomes of Newly Synthesize Proteins

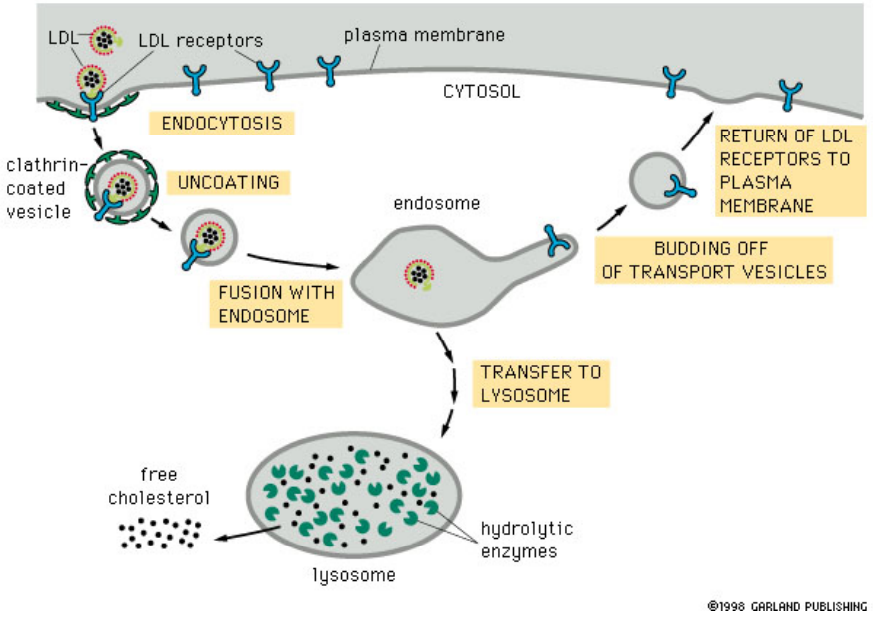




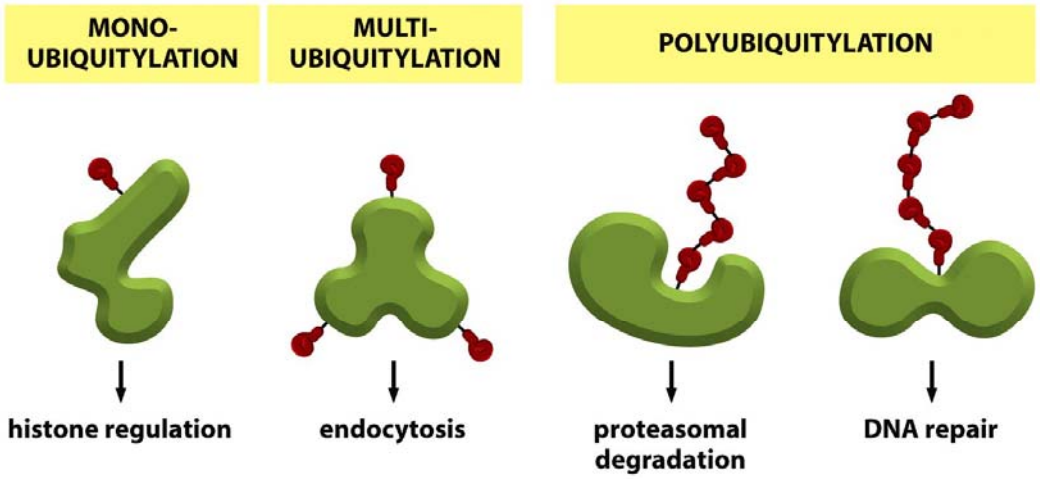


Protein Degradation Pathways

- Approximately 1/3 of newly synthesized proteins are degraded.
- Lysosome degrades proteins and lipids taken in by endocytosis.
- Proteasome is present in both nucleus and cytoplasm.
- Proteasome degrades both cytoplasmic and nuclear proteins after they are marked through conjugation with ubiquitin.



Different Ubiquitylation Related Pathways



Molecular Cell
Review

Histone Ubiquitination: Triggering Gene Activity

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[1]Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA
*Correspondence: jlw@stowers-institute.org
DOI 10.1016/j.molcel.2008.02.014

Recently, many of the enzymes responsible for the addition and removal of ubiquitin from the histones H2A and H2B have been identified and characterized. From these studies, it has become clear that H2A and H2B ubiquitination play critical roles in regulating many processes within the nucleus, including transcription initiation and elongation, silencing, and DNA repair. In this review, we present the enzymes involved in H2A and H2B ubiquitination and discuss new evidence that links histone ubiquitination to other chromatin modifications, which has provided a model for the role of H2B ubiquitination, in particular, in transcription initiation and elongation.

Weake & Workman, Molecular Cell, 29:653:663, 2008

Questions ?