Advanced Cell Biology. Lecture 22

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March 11th, 2011

Outline

Questions and answers

Translation

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Translation

Previous final question: the answer

What is bigger, eukaryotic or prokaryotic ribosome?

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What is bigger, eukaryotic or prokaryotic ribosome?

- Eukaryotic: 80S (vs. 70S in prokaryotes)

Hoskins et al.: spliceosome visualization

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RESEARCH ARTICLE

Ordered and Dynamic Assembly of Single Spliceosomes

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ABSTRACT

The spliceosome is the complex macromolecular machine responsible for removing introns from precursors to messenger RNAs (pre-mRNAs). We combined yeast genetic engineering, chemical biology, and multiwavelength fluorescence microscopy to follow assembly of single spliceosomes in real time in whole-cell extracts. We find that individual spliceosomal subcomplexes associate with pre-mRNA sequentially via an ordered pathway to yield functional spliceosomes and that association of every subcomplex is reversible. Further, early subcomplex binding events do not fully commit a pre-mRNA to splicing; rather, commitment increases as assembly proceeds. These findings have important implications for the regulation of alternative splicing. This experimental strategy should prove widely useful for mechanistic analysis of other macromolecular machines in environments approaching the complexity of living cells.

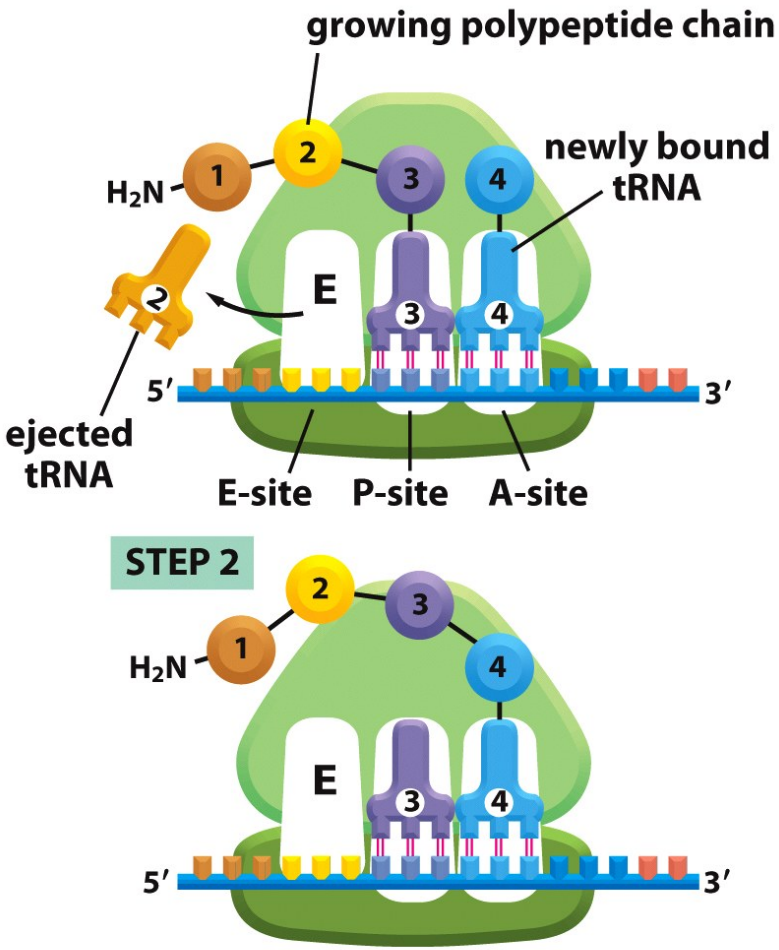
Spliceosomes whithout ATP movie

Spliceosomes with ATP movie

Four steps of translation cycle

1. Binding of tRNA
2. Moving protein chain
3. Translocation of large subunit
4. Translocation of small subunit

Translation



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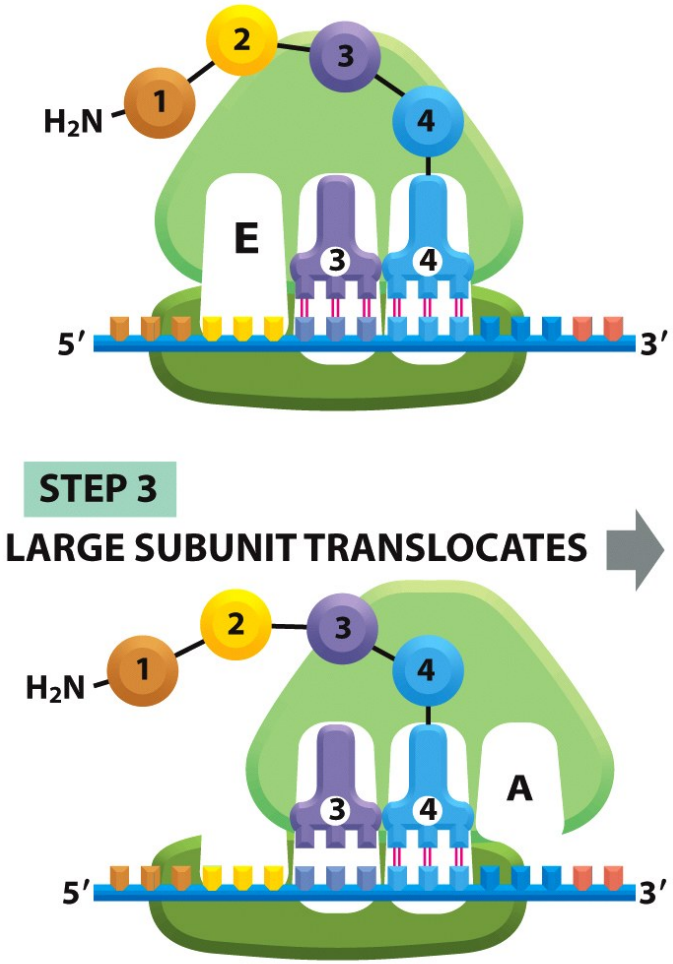

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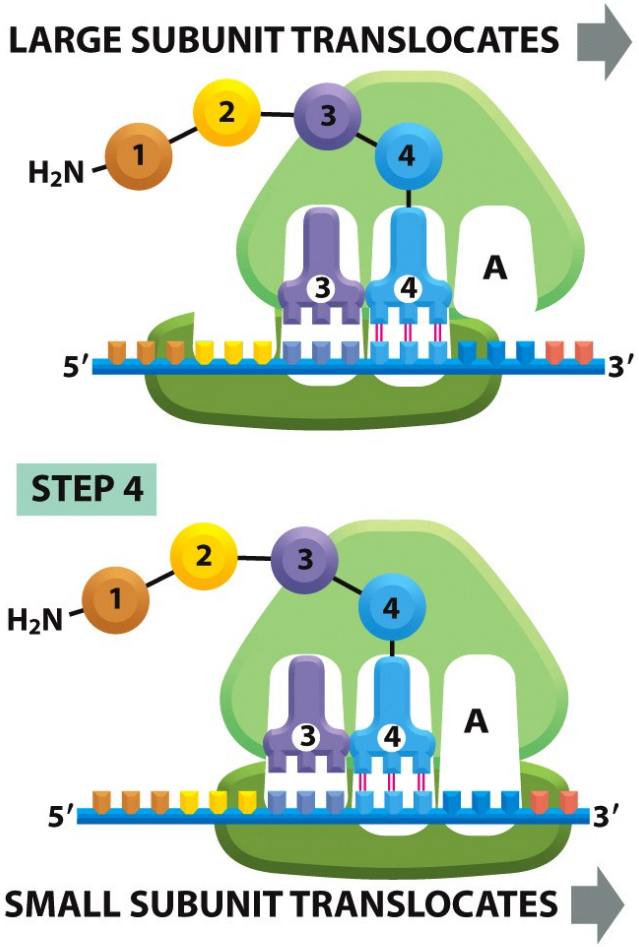

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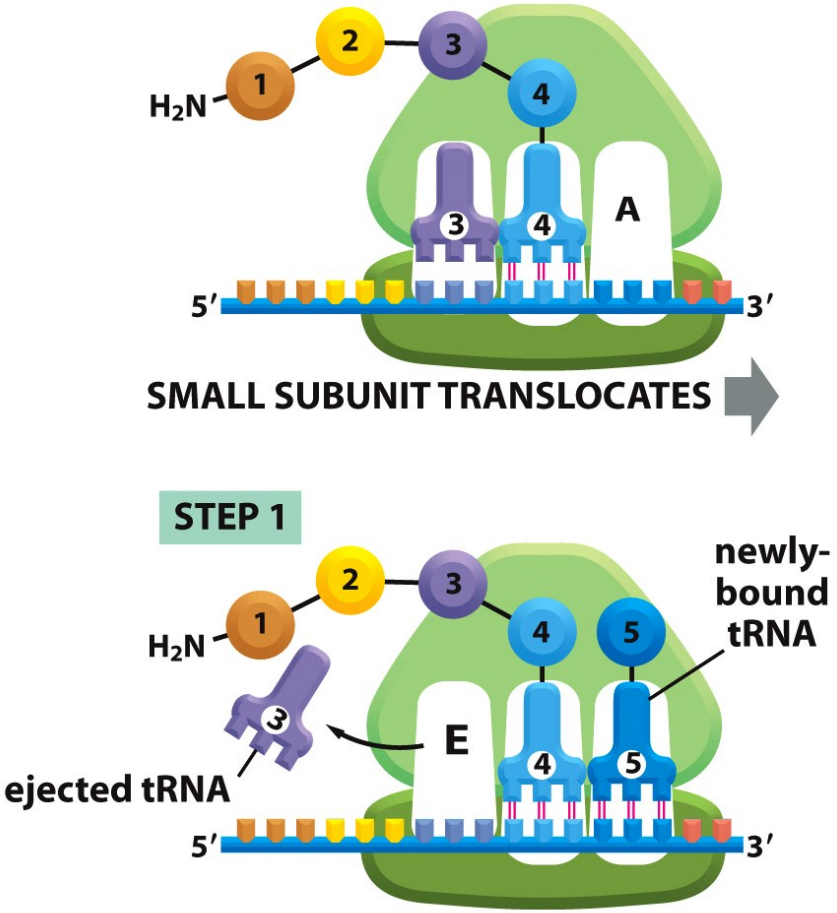

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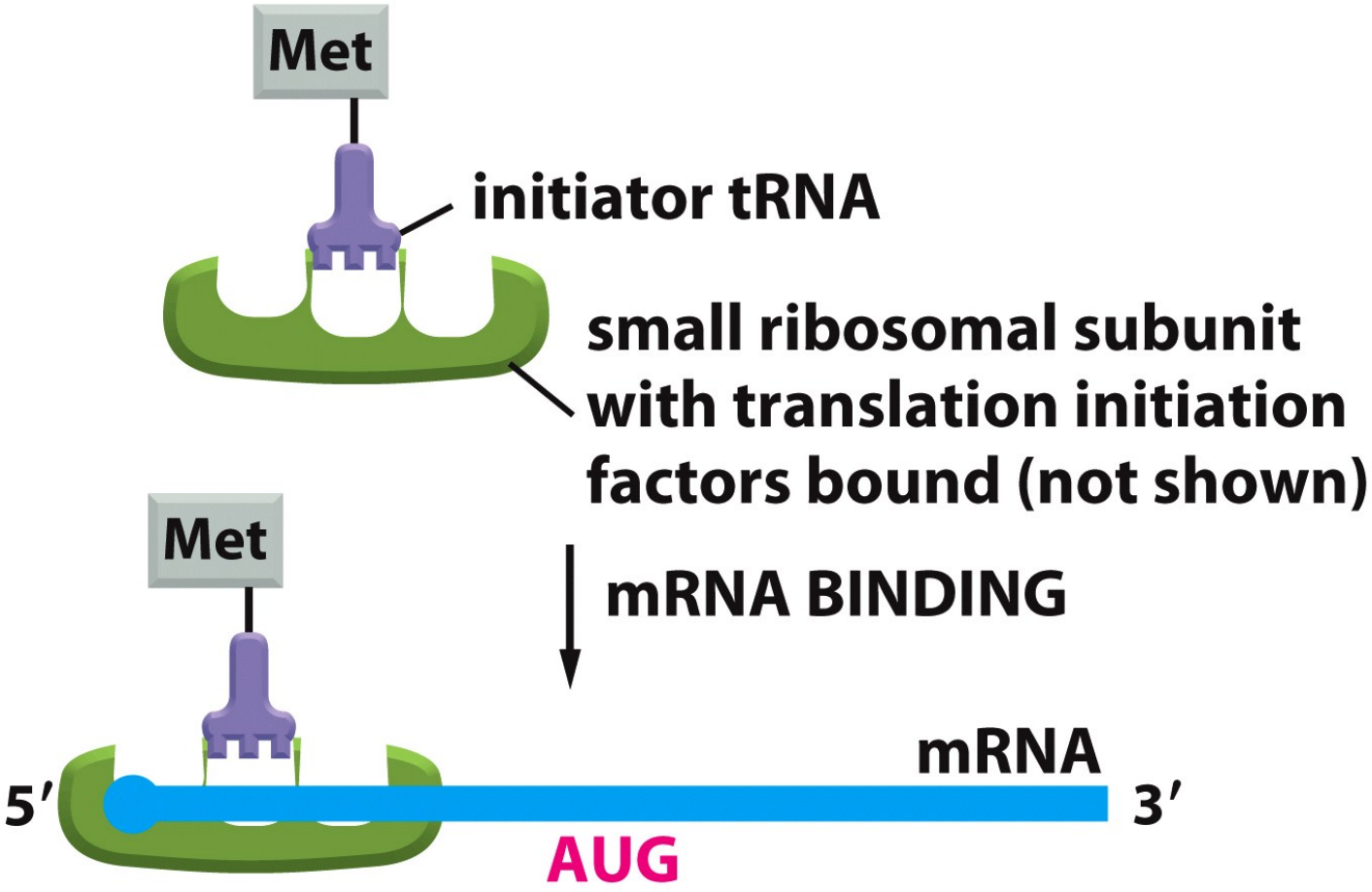
Translation movie I

Translation movie II

Initiation of translation

- Initiator tRNA carries methionine and establishes complex with small subunit (at P site) and translation initiation factors
- This complex founds AUG start codon, releases factors and attaches large subunit

Initiation of translation



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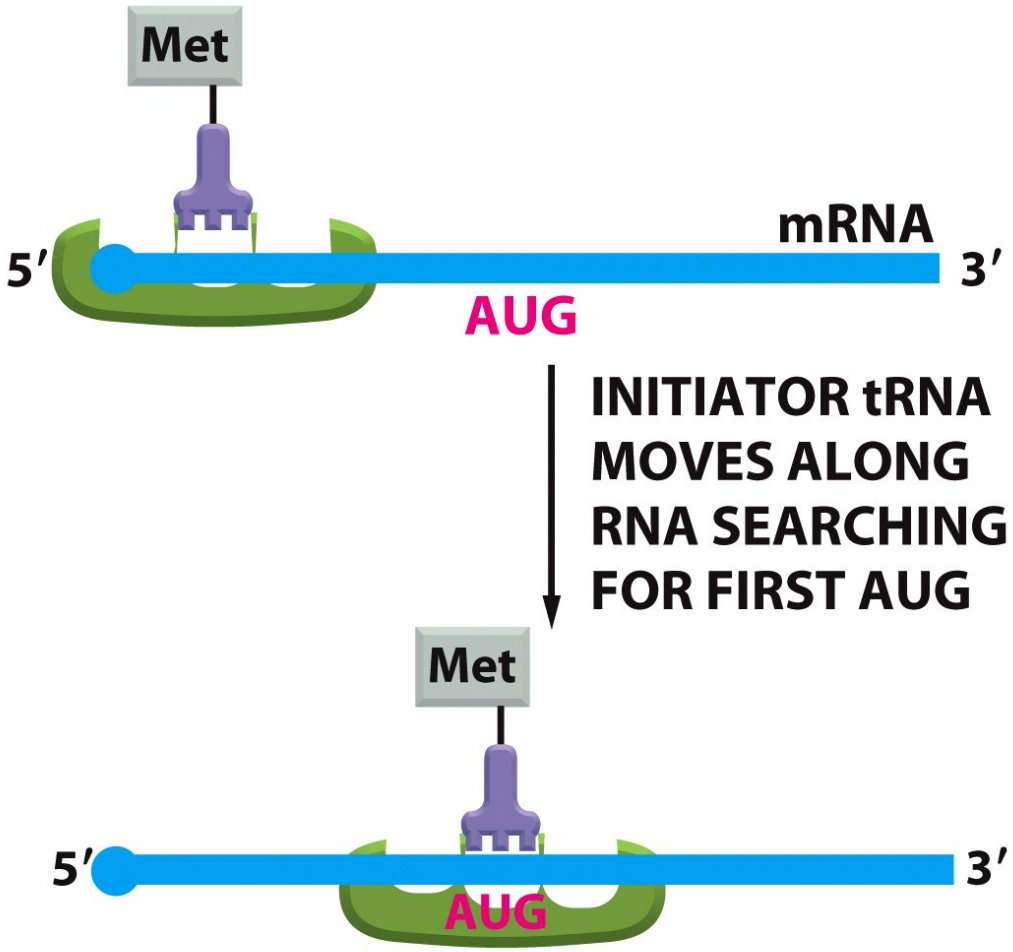
Initiation of translation



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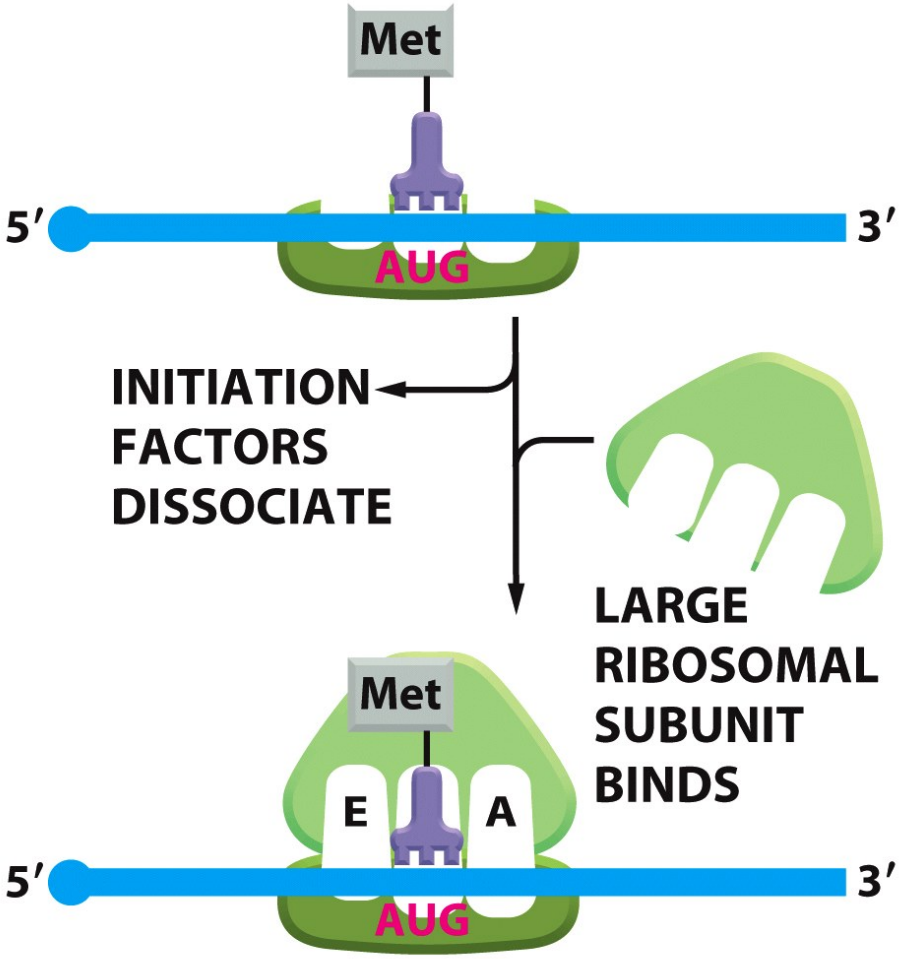
Initiation of translation



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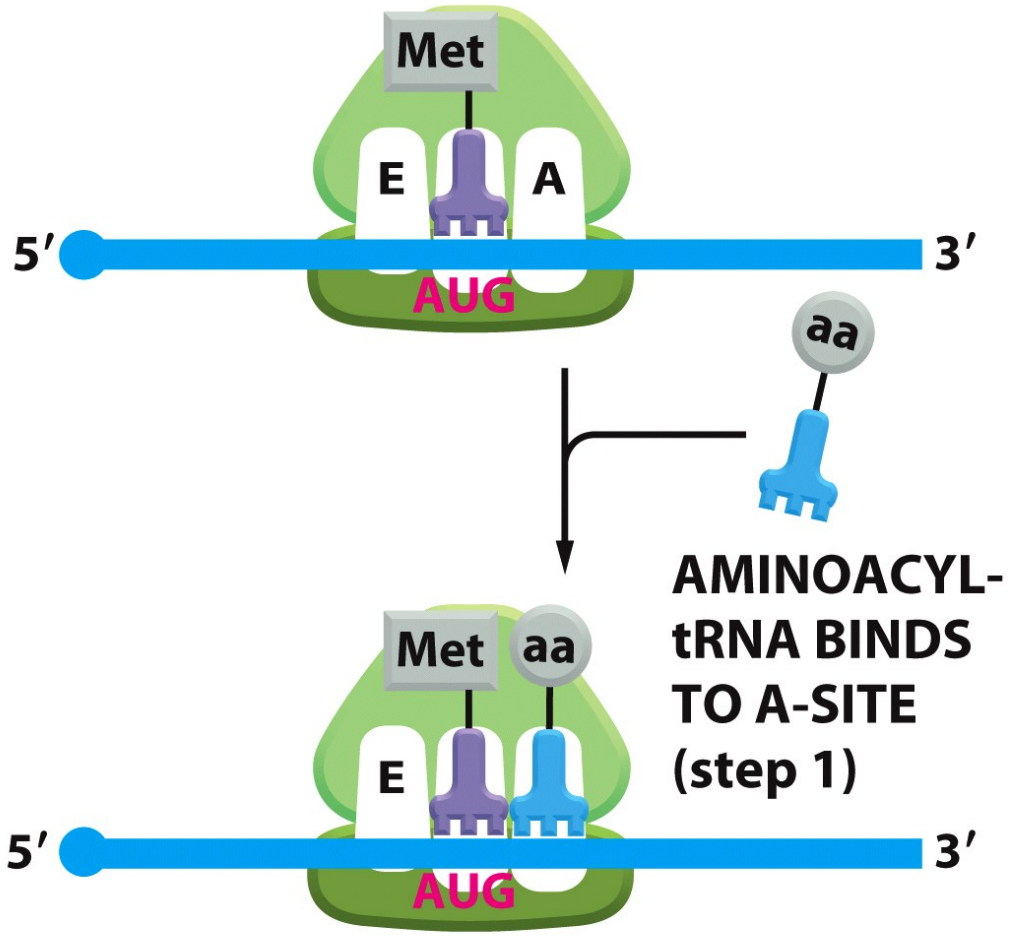
Initiation of translation



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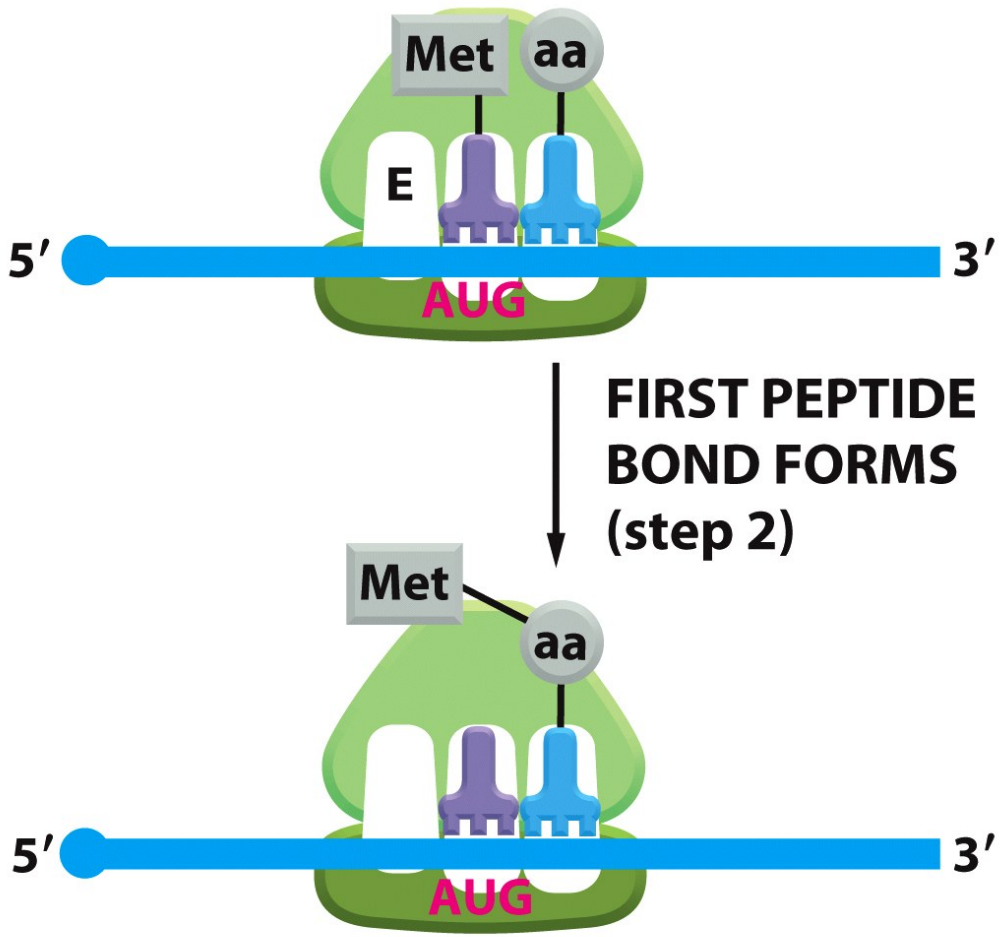
Initiation of translation



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Termination of translation

- Stop codon(s) binds release factor(s)
- Release factor moves to P site deattaching protein chain from last peptydil-tRNA
- Translation complex is dissolving
- In prokaryotes, polycistronic mRNAs bear multiple starting and stopping points

Termination of translation

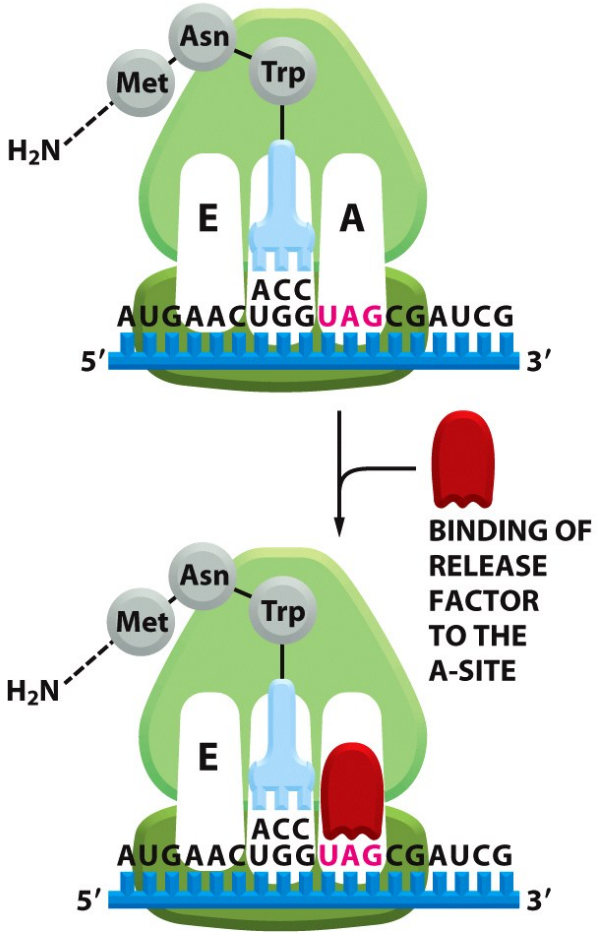
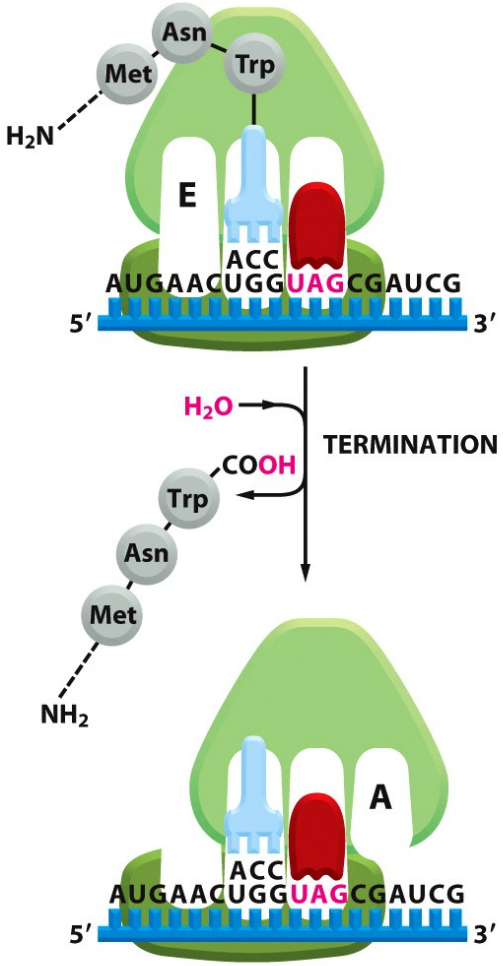


Figure 7-37 part 1 of 3 Essential Cell Biology 3/e (© Garland Science 2010)

Termination of translation



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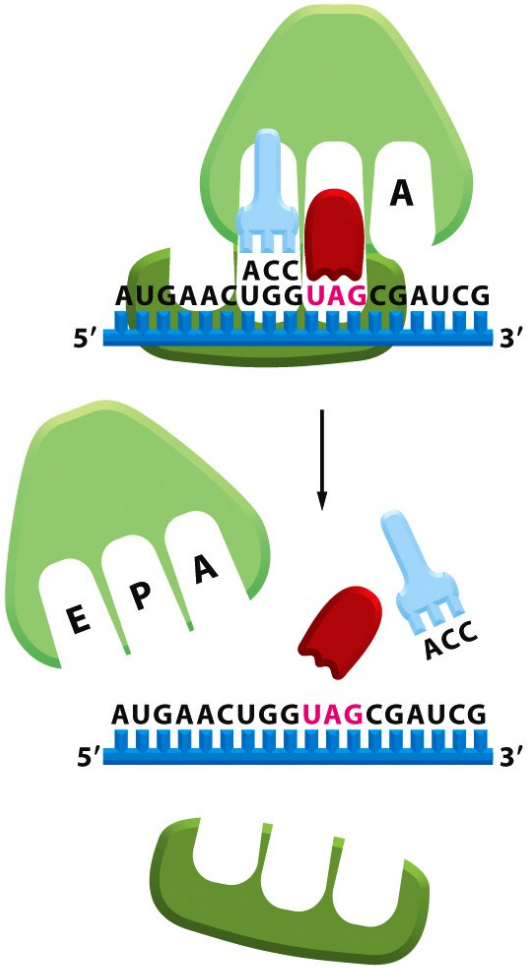
Termination of translation



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Polyribosomes

- Ribosomal complexes which increase the rate of protein synthesis
- The other name is polysomes

Polyribosomes

- Ribosomal complexes which increase the rate of protein synthesis
- The other name is polysomes
- Why ribosomes are located somewhat loosely on mRNA?

Polysome

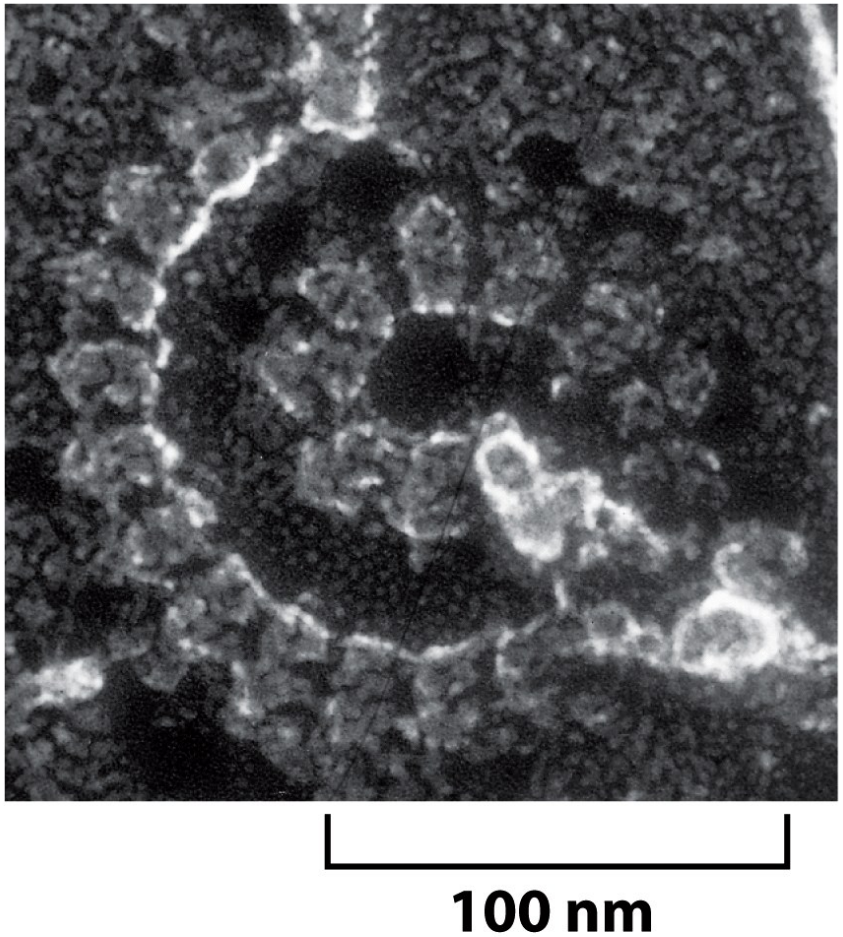

Figure 7-38b Essential Cell Biology 3/e (© Garland Science 2010)

Antibiotics

- Most of antibiotics break parts of translation machinery (and others—cell wall synthesis)
- This is the result of competition between bacteria and fungi, and simplification of bacterial transcription and translation
- Antibiotics often have influence on chloroplasts and/or mitochondria

Antibiotics

TABLE 7–3 ANTIBIOTICS THAT INHIBIT PROTEIN OR RNA SYNTHESIS

ANTIBIOTIC	SPECIFIC EFFECT
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome (step 1 in Figure 7–33)
Streptomycin	prevents the transition from initiation complex to chain-elongating ribosome (see Figure 7–35); also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 7–33)
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 7–33)
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase

Table 7-3 Essential Cell Biology 3/e (© Garland Science 2010)

Protein maturation

- Chaperones spent ATP to help proteins fold correctly
- Phosphorylation and cofactor binding modify proteins further
- Quaternary integration produce protein complexes where separate proteins are subunits

Protein degradation

- Proteases destroy misfolded and broken proteins
- Ubiquitin marks proteins for the destruction
- Proteasome recognized ubiquitin and takes proteins inside for complete degradation

Proteasome

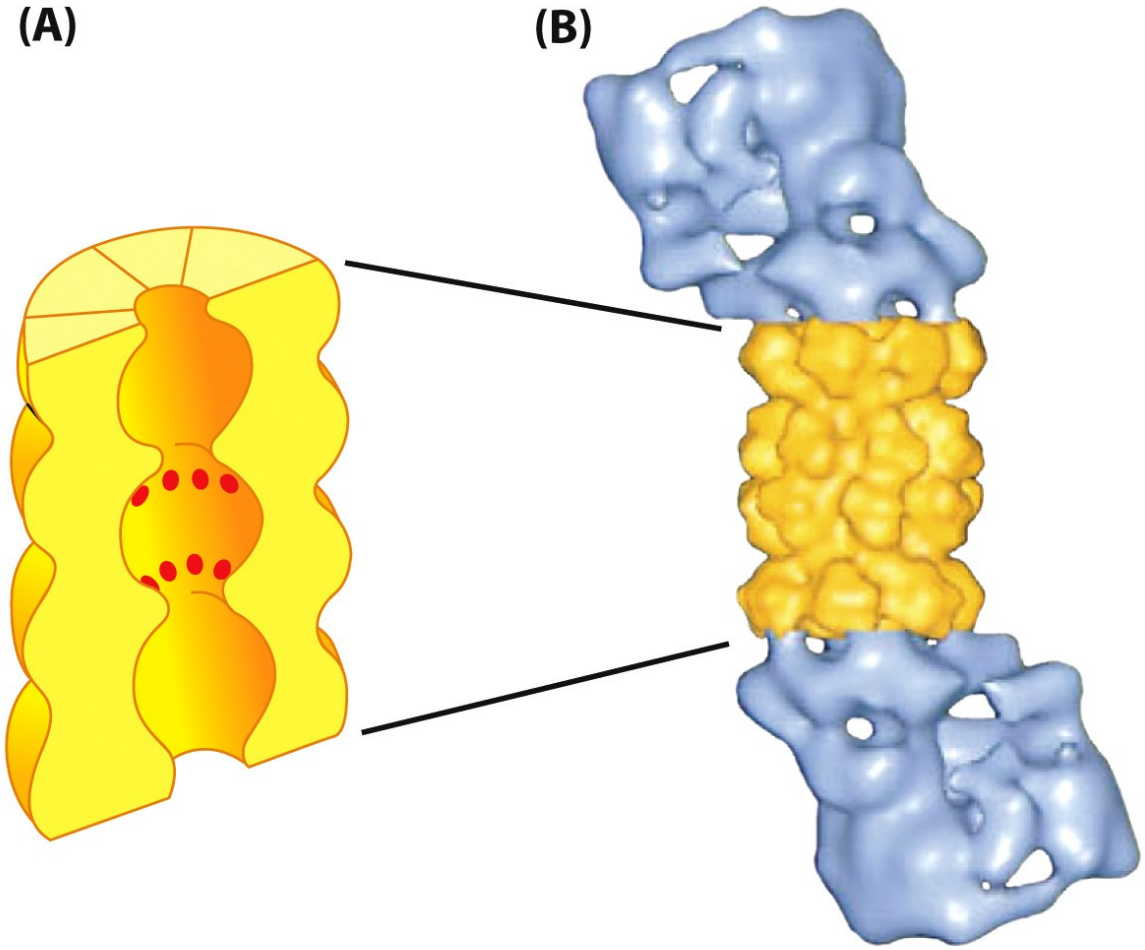

Figure 7-39 Essential Cell Biology 3/e (© Garland Science 2010)

Life cycle of protein

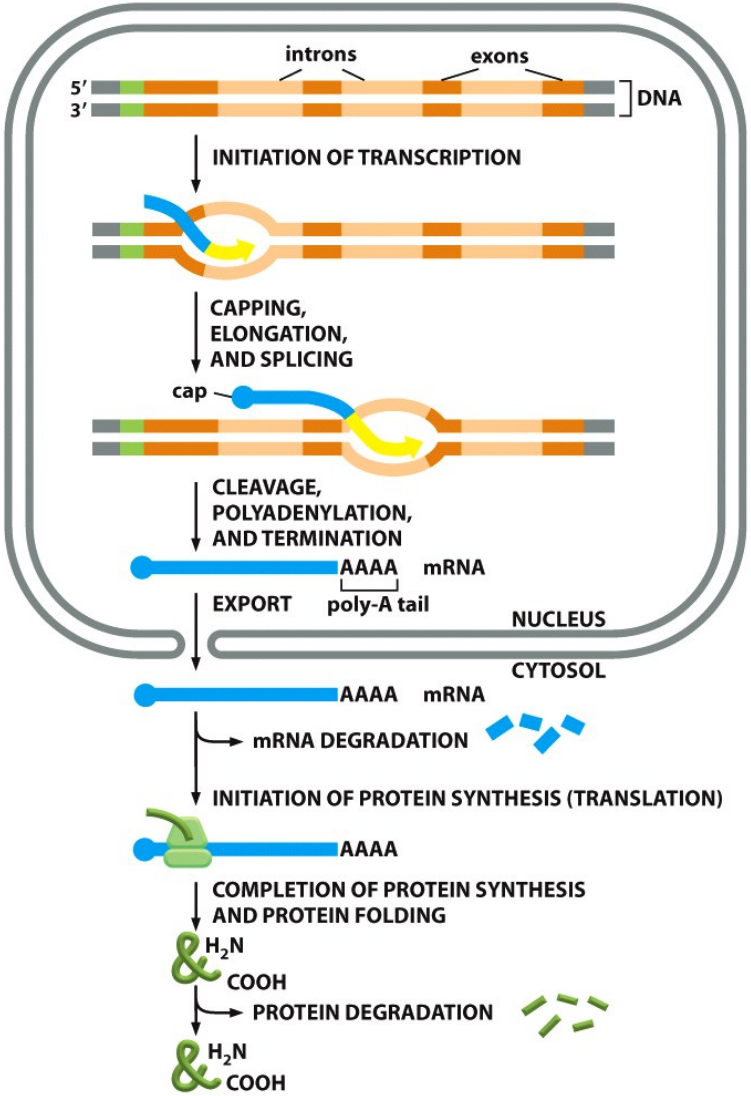

Figure 7-40 Essential Cell Biology 3/e (© Garland Science 2010)

RNA world

- The first living things should be autocatalytic systems
- RNAs bear both information an catalytic activity

RNA world evolution

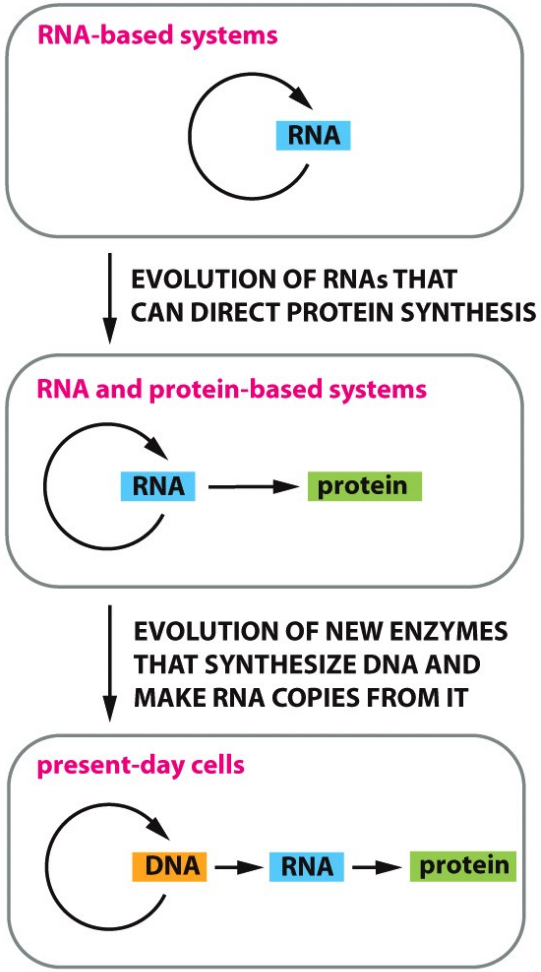

Figure 7-46 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (1 point)

Final question (1 point)

What is chaperone?

Summary

- Translation machinery is more similar between pro- and eukaryotes than transcription
- Ancient RNAs were probably capable of auto-catalysis and therefore may be basic to first living systems

For Further Reading

A. Shipunov.
Advanced Cell Biology [Electronic resource].
2011—onwards.
Mode of access: http://ashipunov.info/shipunov/school/biol_250.

B. Alberts et al.
Essential Cell Biology. 3rd edition.
Garland Science, 2009.
Chapter 7.