

BCH 4054 Fall 2000 Chapter 33 Lecture Notes

Recall that sizes of macromolecular complexes is operationally defined in terms of the sedimentation rate of the particle in a centrifugation experiment. "S" stands for Svedberg units. It is related to size, but not directly proportional because shape factors also affect

sedimentation rate. (See page 157)

The chapter covers degradation of proteins as well. We will not have

time to get into that subject.

Ribosomal Complex with mRNA and tRNA

- The ribosome is the site where mRNA and tRNA are bound in such a way for the anticodon of the tRNA to base pair with the codon of the mRNA
- mRNA and protein thread through a "tunnel" formed between the ribosomes.
- See Fig 33.4 for a scale model of these interactions.

Slide 5

Mechanics of Protein Synthesis

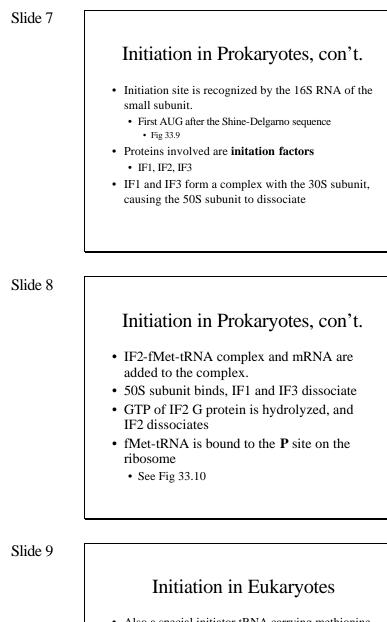
- Three phases:
- initiation, elongation, terminationEach step involves G proteins, and is driven by hydrolysis of GTP
- Peptide bond formation catalyzed by RNA component of 50S subunit
- Sequence of events is similar in prokaryotes and eukaryotes, but details and protein factors differ

Slide 6

Initiation in Prokaryotes

- Initiator tRNA carries formyl methionine.
 - Two tRNA's: tRNA_f^{Met}, tRNA_m^{Met}
 - + $tRNA_{f}^{Met}$ reads the **initial** AUG
 - + $tRNA_{\!m}^{Met}\, reads$ internal AUG's
 - + tRNA $_{\rm f}^{\rm Met}$ is **formylated** by a specific enzyme. (See Fig. 33.8)
- F-Met-tRNA_f^{Met} is bound to **IF2**, a G protein.

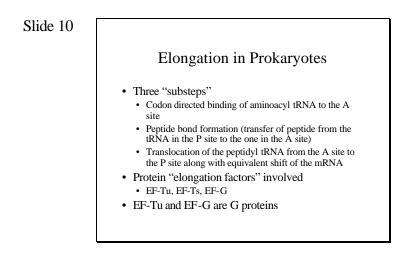
Its interesting that most amino acids have several codons, while Met is coded by a single codon, AUG, yet this codon binds two different tRNA's, depending on its position. While all proteins begin with formyl-methionine in synthesis, many proteins are modified after translation to remove some of the N-terminal sequences. **IF2** stands for initiation factor 2.



- Also a special initiator tRNA carrying methionine, but methionine is not formylated
- Still, different tRNA's for beginning methionine and internal methionine
- Initiation factors recognize the CAP structure. (See Fig 33.22 and 33.24)
- Much more complex set of initiation factors
 (See Table 33.5)
- Phosphorylation of initiation factor involved in some control mechanisms.

Remember that in prokaryotes, operons contain multiple messages, so initiation can occur at several sites along one RNA molecule. Remember also that initiation in prokaryotes can occur before completion of transcription. See Fig 33.21.

Don't worry with details of eukaryotic initiation.



Slide 11

Elongation in Prokaryotes Binding of Aminoacyl-tRNA

• EF-Tu binds aminoacyl-tRNA and GTP

- Most abundant protein in E. coli, about 5% of total
- "Delivers" aminoacyl-tRNA to the vacant A site
- Hydrolysis of GTP releases EF-Tu-GDP from ribosome
- "Proofreading" function—codon-anticodon binding checked both before and after GTP hydrolysis
- EF-Ts displaces GDP from EF-Tu-GDP to form EF-Tu-EF-Ts complex
- GTP displaces EF-Ts to form EF-Tu-GTP to repeat the process

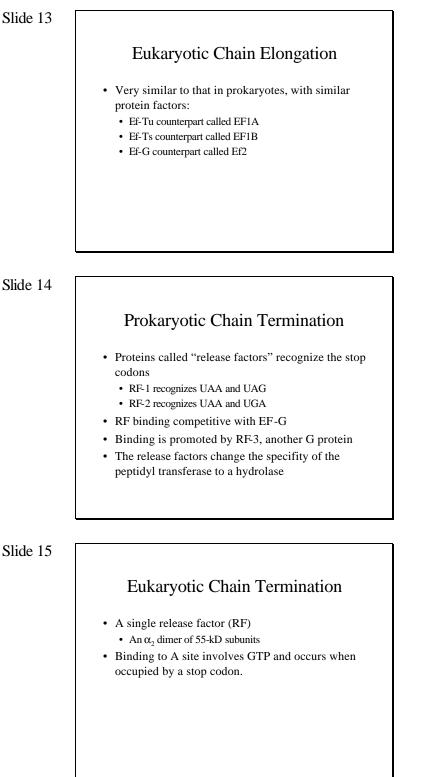
EF-Tu plays the same role with the bulk aminoacyl-tRNA's as IF2 does with the initiator fMet-tRNA. Evidence for the "proofreading" role is that mutants in which the GTPase activity is too rapid show higher mutation rate. The system must strike a balance between too slow (making protein synthesis too slow) or too fast (not allowing time for the second hydrogen bonding check).

Slide 12

Elongation in Prokaryotes Peptide Bond Formation

- "Peptidyl transferase" is actually the 23S RNA
 Fig. 33.13 and 33.14
- 3' ends of tRNA shift sites during peptide bond formation. (Fig 33.15)
- EF-G-GTP complex binds and causes rest of tRNA and the mRNA to shift positions
 - Note similarity in structure to Ef-Tu-tRNA (Fig p 1104)
- GTP hydrolysis helps "drive" completion of translocation, while vacant tRNA dissociates

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RF-3 "delivers" RF-1 or RF-2 to the binding sites in the same way that IF2 and Ef-Tu deliver aminoacyltRNA's to the binding sites.



Other Topics

- Protein synthesis inhibitors
- Protein folding
 - Involvement of "chaperones"
- Proteolytic cleavage in processing
 - Removal of amino terminal
 - Zymogen-protein conversion
 - Removal of leader sequence in membrane or excreted proteins
- Protein Sorting and Translocation
- Protein Degradation

These are all topics which we don't have time to cover, and you won't be responsible for on the exam, but I would encourage you to at least read about.