BCH 4054 Fall 2000 Chapter 33 Lecture Notes

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Chapter 33

Protein Synthesis

The chapter covers degradation of proteins as well. We will not have time to get into that subject.

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Prokaryotic Ribosomes

- 70S particle with 30S and 50S subunits
  - 30S contains 16S RNA (1542 bases) and 21 peptides
  - 50S contains 23S RNA (2904 bases), 5S RNA (120 bases) and 31 peptides.
- RNA is cut from a precursor operon
  - (Fig 33.1)
- Shapes have been discerned from both EM and X-ray studies.
  - Fig 33.3

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)

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Eukaryotic Ribosomes

- 80S particle with 40S and 60S subunits
  - 40S contains 18S RNA (1874 bases) and 33 polypeptides
  - 60S contains 28S RNA (4718 bases), 5.8S RNA (160 bases), 5S RNA (120 bases) and 49 polypeptides
- Mitochondria have ribosomes similar to prokaryotic ribosomes

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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.

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Mechanics of Protein Synthesis

- Three phases:
  initiation, elongation, termination
- Each 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

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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
  - $tRNA_f^{Met}$ 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.

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Initiation in Prokaryotes, con't.

- Initiation site is recognized by the 16S RNA of the small subunit.
  - First AUG after the Shine-Delgarno sequence
    - Fig 33.9
- Proteins involved are **initation factors**
  - IF1, IF2, IF3
- IF1 and IF3 form a complex with the 30S subunit, causing the 50S subunit to dissociate

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.

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Initiation in Prokaryotes, con't.

- IF2-fMet-tRNA complex and mRNA are added to the complex.
- 50S subunit binds, IF1 and IF3 dissociate
- GTP of IF2 G protein is hydrolyzed, and IF2 dissociates
- fMet-tRNA is bound to the **P** site on the ribosome
  - See Fig 33.10

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Initiation in Eukaryotes

- 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.

Don't worry with details of eukaryotic initiation.

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Elongation in Prokaryotes

- Three "substeps"
  - Codon directed binding of aminoacyl tRNA to the A site
  - Peptide bond formation (transfer of peptide from the tRNA in the P site to the one in the A site)
  - Translocation of the peptidyl tRNA from the A site to the P site along with equivalent shift of the mRNA
- Protein "elongation factors" involved
  - EF-Tu, EF-Ts, EF-G
- EF-Tu and EF-G are G proteins

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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).

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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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Eukaryotic Chain Elongation

- Very similar to that in prokaryotes, with similar protein factors:
  - Ef-Tu counterpart called EF1A
  - Ef-Ts counterpart called EF1B
  - Ef-G counterpart called Ef2

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Prokaryotic Chain Termination

- Proteins called "release factors" recognize the stop codons
  - RF-1 recognizes UAA and UAG
  - RF-2 recognizes UAA and UGA
- RF binding competitive with EF-G
- Binding is promoted by RF-3, another G protein
- The release factors change the specifity of the peptidyl transferase to a hydrolase

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

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Eukaryotic Chain Termination

- A single release factor (RF)
  - An α_2 dimer of 55-kD subunits
- Binding to A site involves GTP and occurs when occupied by a stop codon.

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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.