Protein synthesis I
Biochemistry 302

February 17, 2006
Key features and components involved in protein biosynthesis

- **High energy cost (essential metabolic activity of cell)**
  - Consumes ~90% of the chemical energy (high energy phosphate groups of ATP and GTP).
  - Net free energy change during peptide bond synthesis in terms of \( \Delta G^\circ \) of hydrolysis:
    \[ (-30.5 \text{ kJ/mol} \times 4) \text{ PDE hydrolysis} - (-21 \text{ kJ/mol}) \text{ peptide bond hydrolysis} \approx -101 \text{ kJ/mol}. \]
  - Components of translational machinery account for ~35% of the dry weight of the cell.

- **Fast and accurate**
  - Polypeptide of 100 amino acids synthesized in ~5 sec.
  - Error rate of ~1 amino acid in 10,000 to 50,000.

- **Highly regulated**
  - Coordination of rRNA, tRNA, and protein synthesis
  - Ribosome activity/assembly
  - Transcript-specific regulation

<table>
<thead>
<tr>
<th>TABLE 27-5</th>
<th>Components Required for the Five Major Stages of Protein Synthesis in E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>Essential components</td>
</tr>
<tr>
<td>1. Activation of amino acids</td>
<td>20 amino acids, 20 aminoacyl-tRNA synthetases, 32 or more tRNAs, ATP, Mg(^{2+})</td>
</tr>
<tr>
<td>2. Initiation</td>
<td>mRNA, N-Formylmethionyl-tRNA(^{\text{met}}), Initiation codon in mRNA (AUG), 30S ribosomal subunit, 50S ribosomal subunit, Initiation factors (IF-1, IF-2, IF-3), GTP, Mg(^{2+})</td>
</tr>
<tr>
<td>3. Elongation</td>
<td>Functional 70S ribosome (initiation complex), Aminoacyl-tRNAs specified by codons, Elongation factors (EF-Tu, EF-Ts, EF-G), GTP, Mg(^{2+})</td>
</tr>
<tr>
<td>4. Termination and release</td>
<td>Termination codon in mRNA, Release factors (RF-1, RF-2, RF-3)</td>
</tr>
<tr>
<td>5. Folding and posttranslational processing</td>
<td>Specific enzymes, cofactors, and other components for removal of initiating residues and signal sequences, additional proteolytic processing, modification of terminal residues, and attachment of phosphate, methyl, carboxyl, carbohydrate, or prosthetic groups</td>
</tr>
</tbody>
</table>
Subunit composition of the prokaryotic ribosome (~2/3 rRNA, 1/3 protein)

*E. coli* ribosome:

~15,000/cell

~25% dry wt

70S → 2.7 MDa

\[ S = \frac{M(1-\nu_p)}{N_f} \]

Why are S values not additive?

L1-L33 and S1-21 proteins vary greatly in size and structure (Mr ~6000 to 75,000) although individual proteins are highly conserved from organism to organism.

Fig. 27.13
Tale of the tape: bacterial (E. coli) vs eukaryotic (mammals) ribosome

<table>
<thead>
<tr>
<th></th>
<th>Prokaryotic 70S</th>
<th>Eukaryotic 80S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large Subunit</strong></td>
<td>50S</td>
<td>60S</td>
</tr>
<tr>
<td><strong>RNA</strong></td>
<td>23S rRNA (3.2 kb)</td>
<td>28S rRNA (4.7 kb)</td>
</tr>
<tr>
<td></td>
<td>5S rRNA (120 nt)</td>
<td>5S rRNA (120 nt)</td>
</tr>
<tr>
<td></td>
<td>5.8S rRNA (160)</td>
<td></td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>36 (L1,L2,L3…)</td>
<td>~49</td>
</tr>
<tr>
<td><strong>Small subunit</strong></td>
<td>30S</td>
<td>40S</td>
</tr>
<tr>
<td><strong>RNA</strong></td>
<td>16S rRNA (1.5 kb)</td>
<td>18S rRNA (1.9 kb)</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>21 (S1,S2,S3…)</td>
<td>~33</td>
</tr>
</tbody>
</table>

Lehninger Principles of Biochemistry, 4th ed., Ch 27
Early EM studies reveal 3D topography of large and small ribosomal subunits

Fig. 27.16

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>30S</td>
<td>~18 nm for 70S</td>
</tr>
<tr>
<td>50S</td>
<td>~25 nm for 80S</td>
</tr>
<tr>
<td>70S</td>
<td></td>
</tr>
</tbody>
</table>

Note how a groove separates the two subunits.
Ribosomal subunits have distinct function roles in protein synthesis

• **Small subunit (recognition & specificity)**
  – Initiates mRNA engagement
  – Decodes the mRNA (along with aa-tRNA of course)
  – Mediates mRNA and tRNA translocation
  – Ensures high fidelity codon-anticodon interaction

• **Large subunit (catalysis & regulation)**
  – Catalyzes peptide bond formation
  – Provides a route for nascent peptide growth (tunnel)
  – Provides binding sites for GTPases and other factors that assist in elongation and termination phases of protein synthesis
Assembly of small ribosomal subunit is an ordered process *in vitro*

Reconstitution of 30S subunit from individual rRNA & protein components 1st reported by P. Traub and M. Nomura in 1968.

Reconstitution of 50S subunit proceeds by a more complex pathway that requires careful temperature control.

Fig. 27.19
Monovalent & divalent cations modulate 70S ribosome assembly \textit{in vitro}

\[ 30S + 50S \xrightarrow{\uparrow [\text{Mg}^{2+}]} 70S \]
\[ 30S + 50S \xleftarrow{\uparrow [\text{Na}^+/\text{K}^+]} 70S \]

Under the ionic conditions present in the cell, ribosomes exist primarily as dissociated subunits.
Putative secondary structure of *E. coli* 16S rRNA

- Many regions of self-complementarity facilitate intrastrand base pairing revealing four major domains of folding (I-IV).
- Predicted double-stranded regions are highly conserved among related 16S rRNA sequences but primary sequences are not.
- Additional folding of rRNA and contribution of ribosomal proteins generate a more realistic 3D structure.

Fig. 27.15
3D-structure of small ribosomal subunit of *Thermus thermophilus* (shape determined by RNA component)

H: head, Be: Beak, N: neck, P: platform, Sh: shoulder, Sp: Spur, Bo: body


Note asymmetric arrangement of proteins and RNA.
T. Steitz, P. Moore and coworkers solve crystal structure of 50S subunit of *Haloarcula marismortui*

Monolithic structure with two lateral protuberances

The surface of the subunit that interacts with the small 30S subunit faces you. Some proteins “snake” through the helices of the rRNA core.

Peptidyl transferase inhibitor

Note that proteins are remote from active site, primary role in stabilizing 3D rRNA structure.

N. Ban et al. *Science* 289:905-920, 2000  2.4 angstrom resolution
Chemical cross-linking studies reveal orientation of tRNA in the ribosome

A site, triangles
P site, circles
E site, squares

Anticodon end contacts 30S subunit near bottom of 70S ribosome cavity.

Acceptor end interacts with 50S subunit near the top of the 70S ribosome cavity.

Fig. 27.21
Modeling of “active” bacterial ribosome based on subunit structures

Viewing interaction surfaces

Complete ribosome

A = aminoacyl site
P = peptidyl site
E = exit site

Removal of tRNA to show cleft where protein synthesis occurs & mRNA winding through channels on the 30S surface.

Lehninger Principles of Biochemistry, 4th ed., Ch 27
Primary steps/stages involved in synthesizing a functional protein

- **Stage 1: Activation of amino acids**
  - Joining amino acids to their cognate tRNA
- **Stage 2: Initiation of protein synthesis**
  - Assembling the ribosome on the mRNA
- **Stage 3: Elongation of polypeptide chain**
  - Creating peptide bonds between amino acids
- **Stage 4: Termination of translation**
  - Completing the polypeptide chain and releasing ribosomes
- **Stage 5: Folding and Processing**
  - Covalent modification of certain amino acids
A representative prokaryotic mRNA: the lac operon (a polycistronic message)

How many open reading frames?

Signals for ribosome binding and translation initiation, some better (↑affinity) than others
Initiation requires alignment of 30S subunit: Shine-Dalgarno sequences (~8-13 nt to 5′ side of start codon)

SD sequences: Purine-rich sequences that function as attachment sites for 3′ end of 16S rRNA (30S subunit).
Initiator Met-tRNA\textsuperscript{Met} is special

- All organisms have 2 tRNAs for Met, one special tRNA\textsuperscript{Met} for initiation at start 5' AUG codon, the other to decode internal AUG codons in ORF.

- Bacteria: Formyl group is added after charging of tRNA\textsuperscript{fMet} with Met.

- Transformylase catalyzes the transfer of a formyl group from N\textsuperscript{10-}formylTHF to Met-tRNA\textsuperscript{fMet}.

- All bacterial proteins (and proteins synthesized by mitochondrial or chloroplast ribosomes) begin with N-formylmethionine.

- Addition of N-formyl group prevents fMet-tRNA\textsuperscript{fMet} from entering A site. Met-tRNA\textsuperscript{Met} or any other charged tRNA are not accepted in 30S initiation site.

- Formyl group is removed during peptide chain elongation by deformylase.
Essential prokaryotic protein factors involved in translation

<table>
<thead>
<tr>
<th>Factor</th>
<th>Approximate Number per Ribosome in Cell</th>
<th>Binds GTP?</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initiation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* IF1</td>
<td>1/7</td>
<td>No</td>
<td>Promotes dissociation of 70S ribosome</td>
</tr>
<tr>
<td>IF2</td>
<td>1/7</td>
<td>Yes</td>
<td>Helps attach initiator tRNA</td>
</tr>
<tr>
<td>IF3</td>
<td>1/7</td>
<td>No</td>
<td>Similar to IF1</td>
</tr>
<tr>
<td><strong>Elongation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF-Tu</td>
<td>~10</td>
<td>Yes</td>
<td>Carries tRNA into A site</td>
</tr>
<tr>
<td>EF-Ts</td>
<td>1</td>
<td>Yes</td>
<td>Participates in recharging EF-Tu with GTP</td>
</tr>
<tr>
<td>EF-G</td>
<td>1</td>
<td>Yes</td>
<td>Facilitates translocation</td>
</tr>
<tr>
<td><strong>Termination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF1</td>
<td>1/20</td>
<td>No</td>
<td>Release factor (UAA, UAG)</td>
</tr>
<tr>
<td>RF2</td>
<td>1/20</td>
<td>No</td>
<td>Release factor (UAA, UGA)</td>
</tr>
<tr>
<td>RF3</td>
<td>?</td>
<td>Yes</td>
<td>A GTPase that promotes release</td>
</tr>
</tbody>
</table>

*EF-Ts not a GTPase per se*

**key role: blocks A site**
Model for formation of initiation complex in bacteria

• IF-1 and IF-3 interact with 30S subunit to block A site and to prevent pre-mature ribosome assembly. mRNA binding and AUG guidance to correct initiation position (P site) follows.

• Pre-initiation complex is joined by GTP bound IF-2 and fMet-tRNA^{fMet}. This charged tRNA is the only one that binds first to the P site. IF-2 is a G protein (GTPase).

• 50S subunit then joins the 30S pre-initiation complex. This occurs with simultaneous hydrolysis of GTP and release of IF-1, IF-2, and IF3.