Protein synthesis I
Biochemistry 302

Bob Kelm
February 18, 2005
Key features of protein biosynthesis

• High energy cost
  – Essential metabolic activity of the cell
  – Consumes ~90% of the chemical energy (ATP, GTP). Energy cost paid for forming peptide bond between specified amino acids in terms of $\Delta G^\circ$ of hydrolysis:
    $$(-30.5 \text{ kJ/mol} \times 4)_{\text{PDE bond}} - (-21 \text{ kJ/mol}_{\text{peptide bond}}) = -101 \text{ kJ/mol}.$$  
  – 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 and protein synthesis
  – Ribosome activity/assembly
Subunit composition of the prokaryotic ribosome (~2/3 rRNA, 1/3 protein)

*E. coli* ribosome:

- ~15,000/cell
- ~25% dry wt
- 70S \(\rightarrow\) 2.7 MDa
- S = M(1-\(\nu_p\))/Nf

L1-L33 (33 different proteins but 36 total due to modified forms and extra copies)
- 3200 nt
- S1-S21
- 120 nt
- 1542 nt

Why are S values not additive?

Fig. 27.13

L1-L33 and S1-21 proteins vary greatly in size and structure (~6000 to 75,000) although individual proteins are highly conserved from organism to organism.
### Tale of the tape: bacterial (\textit{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>
Early EM studies reveal 3D topography of large and small ribosomal subunits

**Fig. 27.16**

- Diameter: 
  - ~18 nm for 70S
  - ~25 nm for 80S

- Note how a groove separates the two subunits.

- Side view
- Front view

<table>
<thead>
<tr>
<th>30S</th>
<th>50S</th>
<th>70S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small subunit</td>
<td>Large subunit</td>
<td>Ribosome</td>
</tr>
</tbody>
</table>
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 in vitro

\[ 30S + 50S \xrightarrow{\text{↑}[\text{Mg}^2+]} 70S \]

\[ 30S + 50S \xleftarrow{\text{↑}[\text{Na}^+/\text{K}^+]} 70S \]

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)

Note asymmetric arrangement of proteins and RNA.

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

3D-structure of small ribosomal subunit of *Thermus thermophilus* (decoding center made entirely of RNA)

F. Schluenzen et al. *Cell* 102:615-623, 2000 from Max-Planck Institute, 3.3 angstrom resolution, 1 angstrom = 10^{-10} m
T. Steitz, P. Moore and coworkers solve crystal structure of 50S subunit of *Haloarcula marismortui*

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

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.

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

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

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

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

Viewing interaction surfaces

Complete ribosome

Note how mRNA winds through channels on the 30S surface.

Removal of tRNA to show cleft where protein synthesis occurs.

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

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)

Fig. 27.5

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).
**Table 27.4**

<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>

**Key note:**
- EF-Ts not a GTPase per se
- *IF1 is a 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\textsuperscript{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.
A word about the prokaryotic initiator tRNA, fMet-tRNA$^{f\text{Met}}$

- Formyl group is added after charging of tRNA$^{f\text{Met}}$ with Met.
- Transformylase catalyzes the transfer of a formyl group from $N^{10}$-formylTHF to Met-tRNA$^{f\text{Met}}$.
- All bacterial proteins are synthesized with the same N-terminal residue, $N$-formyl Met.
- Addition of $N$-formyl group prevents fMet-tRNA$^{f\text{Met}}$ from entering A site. Met-tRNA$^{\text{Met}}$ or any other charged tRNA are not accepted in 30S initiation site.
- Formyl group is removed during peptide chain elongation by deformylase.
Initiation of translation in eukaryotic cells

1: Multiple initiation factors with distinct biochemical roles (linking, tethering, recruiting, and scanning)

2: 5’ and 3’ ends of mRNA tied together and tethered to 40S subunit via eIF/PAB complex. Longer poly (A) tract → more efficient translation

3: Identification of start AUG achieved by “scanning” mechanism involving eIF4B and eIF4F(complex of 4E, 4A, and 4G). Initiator tRNA is Met-tRNA\textsuperscript{Met}.

---

**TABLE 27-8** Protein Factors Required for Initiation of Translation in Bacterial and Eukaryotic Cells

<table>
<thead>
<tr>
<th>Factor</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial</strong>&lt;br/&gt;IF-1</td>
<td>Prevents premature binding of tRNAs to A site</td>
</tr>
<tr>
<td>IF-2</td>
<td>Facilitates binding of fMet-tRNA\textsuperscript{Met} to 30S ribosomal subunit</td>
</tr>
<tr>
<td>IF-3</td>
<td>Binds to 30S subunit; prevents premature association of 50S subunit; enhances specificity of P site for fMet-tRNA\textsuperscript{Met}</td>
</tr>
<tr>
<td><strong>Eukaryotic</strong>&lt;br/&gt;eIF2</td>
<td>Facilitates binding of initiating Met-tRNA\textsuperscript{Met} to 40S ribosomal subunit</td>
</tr>
<tr>
<td>eIF2B, eIF3</td>
<td>First factors to bind 40S subunit; facilitate subsequent steps</td>
</tr>
<tr>
<td>eIF4A</td>
<td>RNA helicase activity removes secondary structure in the mRNA to permit binding to 40S subunit; part of the eIF4F complex</td>
</tr>
<tr>
<td>eIF4B</td>
<td>Binds to mRNA: facilitates scanning of mRNA to locate the first AUG</td>
</tr>
<tr>
<td>eIF4E</td>
<td>Binds to the 5’ cap of mRNA; part of the eIF4F complex</td>
</tr>
<tr>
<td>eIF4G</td>
<td>Binds to eIF4E and to poly(A) binding protein (PAB); part of the eIF4F complex</td>
</tr>
<tr>
<td>eIF5</td>
<td>Promotes dissociation of several other initiation factors from 40S subunit as a prelude to association of 60S subunit to form 80S initiation complex</td>
</tr>
<tr>
<td>eIF6</td>
<td>Facilitates dissociation of inactive 80S ribosome into 40S and 60S subunits</td>
</tr>
</tbody>
</table>

*The prefix “e” identifies these as eukaryotic factors.*