Lecture 15
3/8/04
Structure references (see these for pretty color pictures of the ribosome)

Module 3, Translation, continued

PLAYERS
1. tRNA (last lecture) see page 1 handout 3a
2. Ribosomes – "same" in all kingdoms

E.coli 70S -> 30S and 50S subunits (small and large subunits)
The #s come from how they sediment when centrifuged
2.5x10^6 Da total (bigger than FAS,PKS)
We now have a 5.5 angstrom resolution atomic structure of the small and large subunits together

25% of dry weight of bacterial cells is ribosome- Bacteria are CHOCK FULL of ribosomes!

Breakdown of the ribosome in E.coli

<table>
<thead>
<tr>
<th>30S</th>
<th>50S</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA (50-60%)</td>
<td>16S (1592 nucleotides)</td>
</tr>
<tr>
<td></td>
<td>23S (2904 nt)</td>
</tr>
<tr>
<td></td>
<td>5S (120 nt)</td>
</tr>
<tr>
<td>Proteins</td>
<td>21 small ribosomal proteins</td>
</tr>
<tr>
<td></td>
<td>Labeled S1-S21</td>
</tr>
<tr>
<td></td>
<td>31-35 proteins</td>
</tr>
<tr>
<td></td>
<td>labeled L1-L31…</td>
</tr>
</tbody>
</table>

Very complex machine!

Eukaryotes 80S total, 40S and 60S subunits, different nomenclature

<table>
<thead>
<tr>
<th>40S</th>
<th>60S</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S (1874 nt)</td>
<td></td>
</tr>
<tr>
<td>28S (4718 nt)</td>
<td></td>
</tr>
<tr>
<td>5.8S (160 nt)</td>
<td></td>
</tr>
<tr>
<td>5.5S (120 nt)</td>
<td></td>
</tr>
</tbody>
</table>

33 small ribosomal proteins 49 large ribosomal proteins

p.3 of handout3a shows conservation of secondary structure of 16S RNA over three kingdoms (yeast, e. coli, archae) – notice how similar they are
We can predict base pairing and secondary structure, however, these flat diagrams can’t tell us about tertiary structure and differences in flexibility, conformational changes in the tertiary and quarternary structures etc

23S RNA has 6 domains
Nomenclature

The 50S is where peptide bonds are formed
CP= central protorudence
Usually drawn with L1 on the left, and (L7,L12)4 on the left (this is a tetramer and is thermally labile- missing in the structure)

3 sites where tRNA can interact
A= acceptor site- where amino acylated tRNA is delivered
P= site for growing polypeptide chain (F-met is delivered directly to the P site)
E= exit site- unmodified tRNA moves here to leave

Exit tunnel – adjacent to the P site is a hole for the growing polypeptide chain (erythromycin functions as an antibiotic by blocking access to this channel)

Drawing of 50S

30 S- decoding site
Shine Dalgarno sequence on mRNA interacts with 16S RNA (of the 30S subunit) to position the start codon in the P site

Drawing of 30S

See 50 S structure- atomic resolution on p. 4 of handout 3a

Notice the groove where tRNAs bind
No protein in the groove! Only RNA
No protein is involved in peptide bond formation!
P 5 of handout 3a
Structures of proteins found in the 50 S ribosome subunit
Notice that many have extended structures (unfolded, not globular)
Act as glue that holds the RNA in tertiary conformation

Lots of RNA in this structure
We learned a lot about RNA-RNA interactions and RNA-protein interactions from these structures

5 angstrom resolution structure of large and small subunits together shows that three tRNAs are visible in the structure

3. Protein factors required to make polypeptides
   A. initiation factors 1-3 (IF_{1,2,3})
   B. elongation factors (EF)
   C. termination-release factors (RF_{1,2,3})

   Only 1 machine, many proteins must interact at the same sites on the ribosome

   Hypothesis: molecular mimicry (or shape selectivity) all of these factors look alike, can all be accommodated in “same site”

CARTOON OVERVIEW – working hypothesis for prokaryotes
p.10 pf handout 3a

1. Initiation
   IF2 (GTP) “delivers” F-met-tRNA into the P site, interacts w/ initiation codon
   Shine-Dalgarno sequence (SD) -located before start codon on mRNA – purine rich, interacts with pyrimidine rich sequence in 16S RNA of the 30S subunit

   Order of binding unknown (F-met tRNA or mRNA binds first?)

   What is the role of IF2(GTP) in delivering F-met to the start site?

   NEXT TIME: elongation