

**7.88 Lecture Notes - 15**  
**7.24/7.88J/5.48J**  
**The Protein Folding and Human Disease**

**Ribosomes and In Vivo Folding**

**A. Ribosomes**

Ribosomes: Source of all Proteins!

In prokaryotes, all ribosomes within cytoplasm;  
However, multiple delivery compartments:

- PP chains >> cytoplasm
- PP Chains >> inner membrane
- PP chains >> organelles e.g. flagellum
- PP chains >> through membrane to periplasm
- PP chains >> through membrane and periplasm to outer membrane
- PP chains >> secreted outside cell.

Export pathways use protein channels - poorly defined, very active area of research;

Polypeptide chains have a leader sequence whose recognition brings them to channel; this is recognized by a leader peptidase, which cleaves off leader sequence, coupled to transit.

In Eukaryotes:

- Cytoplasm
- Exported through Endoplasmic reticulum (Signal Recognition Particle) > Golgi > export
- Imported into Mitochondria or chloroplasts
- Translocated to Nucleus
- Inserted into Membranes:
- Many others, e.g. lysosomes

Rate of Prokaryotic Cytoplasmic Protein Synthesis:

Forschhammer and Lindahl (1971) J. Mol. Biol. 55, 563-568.

- About 20,000 ribosomes/cell in rapidly growing E. coli
- Rate of synthesis = 21aa/second/ribosome in coli growing in broth at 37°C.

Slower growth rates:

Carbon Source	Rate
Succinate	16/sec
Glucose	21/sec
Acetate	12/sec
Casamino acids	20/sec

Ribosomes proceed 5' to 3' down mRNA.

In eukaryotes, generally 3-5 fold slower – {3-6 amino acids/second (soft number):

Morphology and Composition of 70s Prokaryotic Ribosomes:

E. coli bacteria:

- Small 30s and large 50s subunits;
- $2.6 \times 10^6$  Daltons total;
- RNA: Protein 2:1 by weight;
- RNA as  $Mg^{++}$  salt;
- Only  $1gmH_2O/gm$  ribosomes - compact, dense;
- 1 each of 53 protein chains

**30S subunit:**

<u>RNA</u>	<u>Protein</u>	<u>Mol.</u>	<u>wt.</u>
16s RNA - 1542 bases:	21 diff. Chains	$1.0 \times 10^6$	120A x 230 A

**50S subunit:**

23s RNA - 2904 bases	34 diff. Chains	$1.7 \times 10^6$	60A x 220 x 220A
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5s RNA -120 bases.

Largest protein: S1, 557aa  
 Smallest: L34, 46 aa

Only one protein in common to both subunits (S20 = L26).  
 Little sequence similarity.

### **Composition Eukaryotic 80S Ribosome:**

Two subunits small 40s and large 60s;

More protein than prokaryotes:

RNA: Protein ratio, closer to 1:1;

Eighty + species of protein chains!

### **Subunits:**

#### **40S:**

18s RNA -1874 bases; 33 proteins

#### **60S:**

28s – 4718 bases; 49 proteins

5.8Ss - 160; and 5S (\*120) RNAs

Chloroplast and Mitochondrial ribosomes closer to prokaryotic.

A note on ribosomal proteins:

- Not globular soluble; not fibrous:
- Fold together with RNA! True two component system, one is not chaperone of the other
- Masayasu Nomura in 1970s.

### **Anatomy of 50S:**

Central protuberance or head: (location of 5SRNA)

L7/L12 stalk

L1 Ridge

Peptidyl transferase site; central protuberance of large subunit

### **Functional Anatomy of 30S:**

Base or body; Distinctive head: lateral platform, defined by groove.

30S Platform opposes L1 ridge in 70S; head to head.

Head and stalk of 70S not covered by 50S

Initiation factor binding + Cleft of small subunit

Messenger binding site = platform of small subunit

Translocation Factors, Peptidyltransferase, 5S sites = inside face of head of 50S, base of L7/L12 stalk

GTP dependent translocation steps = L7/L12 stalk:

### **Where does Peptide Bond Formation Occur?**

tRNA; anticodon loop on 30S subunit encountering mRNA; but amino acyl site down in L7/L12 stalk

What is environment of newly synthesized chain?

Aqueous or protected from solvent?

## **B. Stereochemistry of synthesis**

V. I. Lim and A. S. Spirin (1986) J. MB 188, 565-577:

Assume  $S_N2$  nucleophilic substitution reaction;

- Peptidyl tRNA is in donor site;
- amino acyl tRNA is in acceptor site.
- After covalent bond formed nascent chain is passed to amino acyl tRNA;
- Amino group of amino acyl tRNA is active nucleophile which attacks carbonyl of ester of donor tRNA;
- generates acyl of generating tetrahedral intermediate;
- Has to be sterically acceptable for all 400 pairwise reactions for 20 amino acids.

Explore all angles; conclude only one conformation of newly synthesized peptide doesn't violate: alpha helix!

- Newly synthesized sequence is alpha helical independently of sequence.
- Ribosome channel is designed to transmit alpha-helical conformation.

Where does newly synthesized polypeptide chain emerge from ribosome?

Location of exit site:

- 30-40 amino acids protected from protease degradation by ribosome itself (Smith, Tai, and Davis, 1978, PNAS, 75, 5922-5925.)>>Channel?
- If there is a channel, where does nascent chain exit?

Answered with recent solution of ribosome structure by EM and X-ray diffraction:

Prepare antibody to beta-galactosidase;

>>Isolate ribosomes synthesizing beta gal; >>treat fractions with antibody;  
>>centrifuge and examine dimers in EM

Use E. coli synthesizing high level of beta galactosidase: Tetramer of 465,000 total.

Lyse cells, fractionate ribosomes and polysomes by sucrose gradient centrifugation:

Absorption at 260 on left axis

Fraction number on X axis, 1-15.

Monosome peak followed by polysome peak peaking at about 10 ribosomes.

Activity assay peaks at fraction 7-8

Now incubate with RNase to break down messenger, generate all monosomes:

Incubate with anti-beta gal, recentrifuge:

Most ribosomes not shifted, but beta galactosidase activity now shifts to disome sedimentation.

This activity is released by treatment of puromycin which releases nascent chains:L

**Now for morphological experiment:**

Incubate polysomes with antibody; Resediment and look for disome peak:

Prepare for electron microscopy:

Find lots of pairs; butts facing each other. So back site is exist:

They estimate 150 A from transpeptidation site. If 30-40 protected, can't be alpha helical,  $40 \times 1.5 = 60A^\circ$ .

What about epitope and antibody??

Exit site= membrane binding site.

Technical problem; require epitope; perhaps epitope does not correspond to exit sites.

Most recent work:

A.S. Spirin Ryabova et al, FEBS Letters (1988) 226, 255-260.

They conclude exit site is close to peptidyl transferase site, may wind on ribosome surface.

Perhaps exit site is protein folding site.!!??

Nenad Ban, Poul Nissen, Jeffrey Hansen, Malcolm Capel, Peter B. Moore and Thomas A. Steitz (1999) "Placement of protein and RNA structures into a 5A resolution map of the 50S ribosomal subunit" Nature 400, 841-847.

"At the bottom of the active-site cleft is a tunnel that runs straight through the subunit. In one of our heavy-atom derivatized crystals, the path of this tunnel is

marked by the positions of four bond eleven-tungsten (W11)-cluster molecules, which have a van der Waals diameter of nearly 20Å and lie on an almost straight line (Fig.2). This tunnel is thought to be the path used by the nascent chain polypeptide chain to traverse from the peptidyltransferase centre to the point where it leaves the ribosome, an idea supported by the location at the tunnel entrance of the amino acyl end of formyl-methionine f(Met)-tRNA bound at the P site and the alignment of the tunnel exit on the back of the large subunit with the central pore of the Sec61 channel in yeast.”

Conformation of nascent chain:

Not enough known to be worth reporting;

- Epitopes can fold in nascent chain
- Many proteins, N-terminus is dispensable, so not playing key nucleating role.

To what compartment is protein targeted?

- Cytoplasm
- Periplasm
- Export

So successful transit requires the chain not reach native conformation:

SecB >>tetramer; binds newly synthesized chain for many proteins; Does not bind correctly folded native state.

Chaperonin>>retard folding (Linda Randall)

SecB protein rec

### **C. Folding Pathways evolved through biological evolution**

Biologically evolved process: No reason to expect or believe that there is a unitary mechanism! Reptiles evolved in hot climates; but living North American reptiles and amphibians – frogs, salamanders, turtles - adapted to ice ages; may well retain features of folding intermediates only needed at higher temperatures, but still retained in current physiology: “relic features”

NS >> [I]<sub>1</sub> >>[I]<sub>2</sub> >> N = f(salts, concentration, temperature, pH, cofactors)

May have one mechanism, pathway dominant at one temperature, second under other conditions;

Example: Collagen;

In mammals, clearly folding and chain association f (extent of hydroxylation).

But! Collagens from cold-blooded creatures have very low levels of hydroxyproline; yet still form collagen, still Tm f {proline rings}

Triple helix driven at low temp by glycines and prolines

Driven at high temperature by extent of hydroxylation of prolines!

One pathway in bacterial cells, another in red blood cells!

Very general result that folding dependent on environmental context:

When faced with problems in recovering folded protein, understanding process is critical:

- In what cells are proteins synthesized?
- What cell compartments does the chain pass through?
- In which compartment does it achieve its native state?
- What is form of chain that is released from ribosomes:
- cf collagen/cytochrome c/insulin

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