Protein Folding on the Ribosome

LD Cabrita, CM Dobson & J Christodoulou, Curr Opin Struct Biol 2010

CBB 752
February 2010
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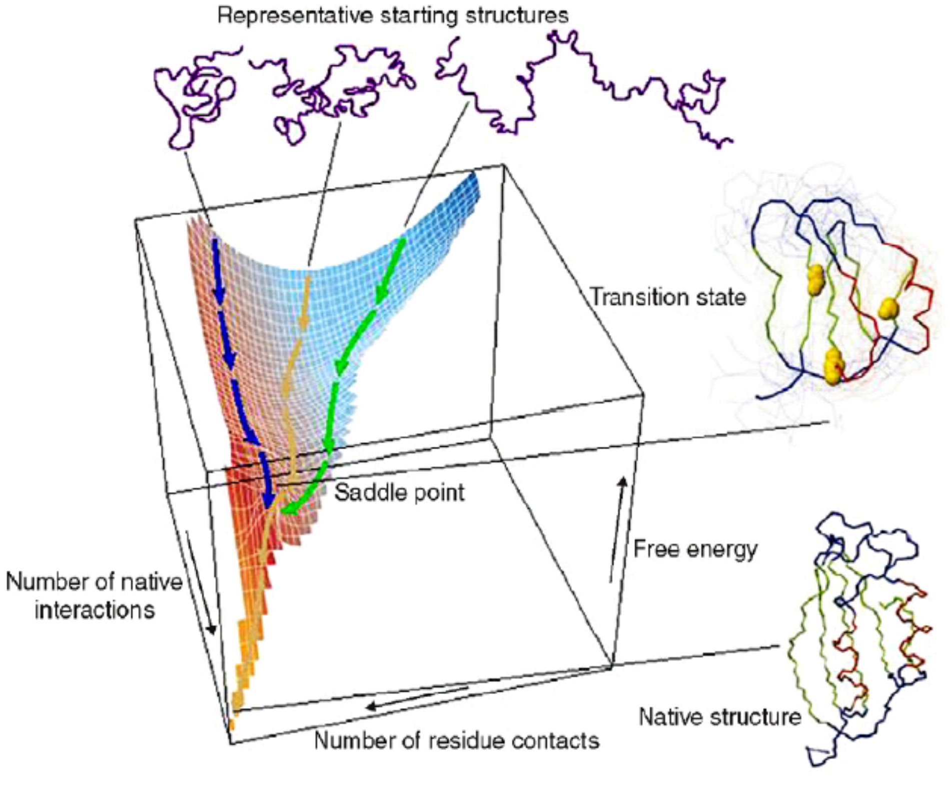
Outline

- Levinthal‘s paradox and cotranslational folding
- Experimental evidence
    - Ribosome structure
    - Biochemical properties
    - Associated proteins
- Summary

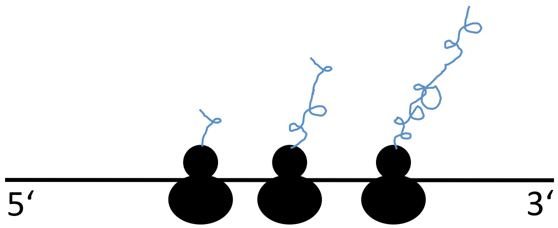
Levinthal‘s Paradox

Problem: too many possible configurations, local mimima



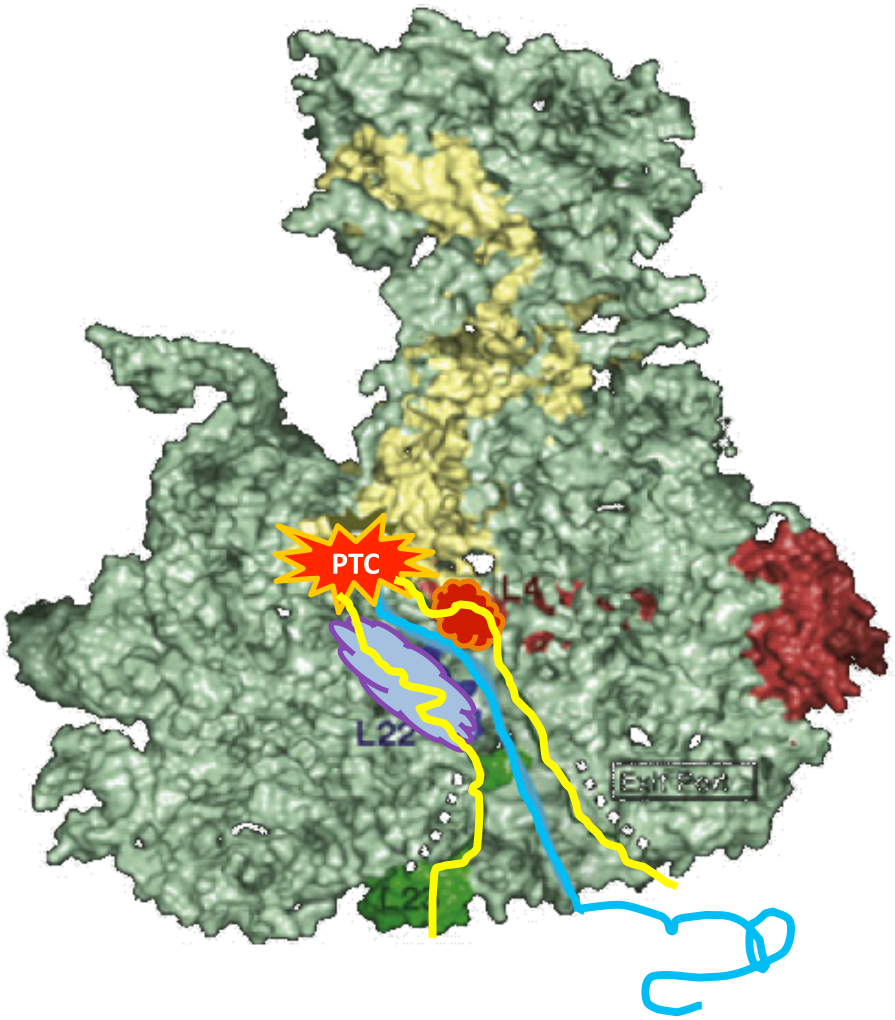
Current Opinion in Structural Biology

Solution: cotranslational folding



- Restrict number of configurations at each point
- Provide native-like initial conditions

Ribosome Structure



Peptide exit tunnel:

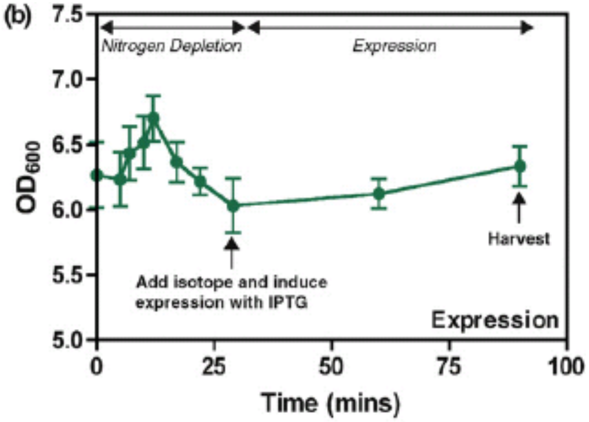
- 80 Å long, 10-20 Å wide
- hyrdrophilic
- 2 ribosomal proteins (L4, L22)
- 1 rRNA
- rigid structure
- many proteins associated with the exit port

Nascent polypeptide:

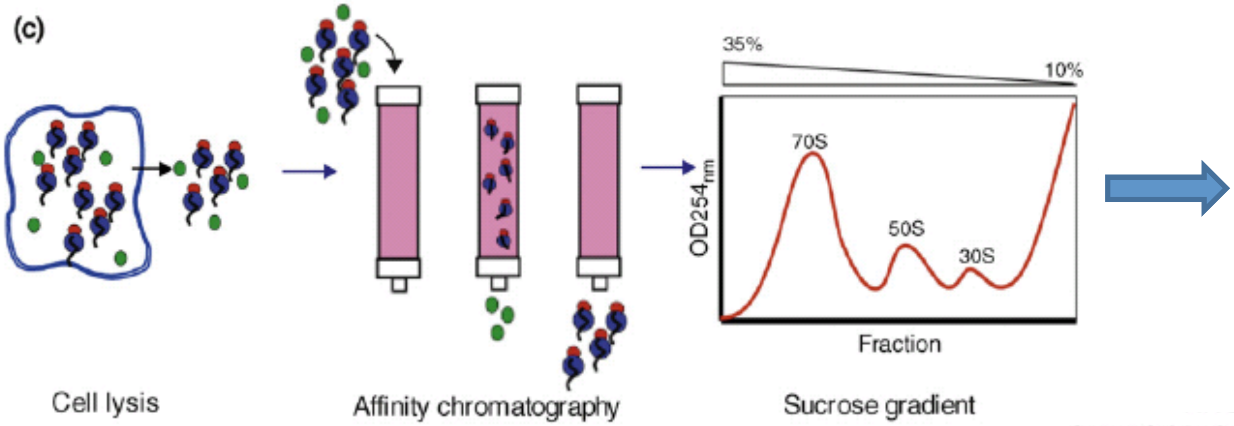
- slightly condensed in tunnel
- confined to few basic structures within tunnel
- diverse folding occurs outside tunnel

Biochemical Experiments

- Study of <u>RNC</u> *in vivo* expression systems



1. Create recombinant lac p/o controlled snapshot strain
2. Grow dense culture enriched in ribosomes
3. Add radioisotopes and IPTG to induce expression



analysis

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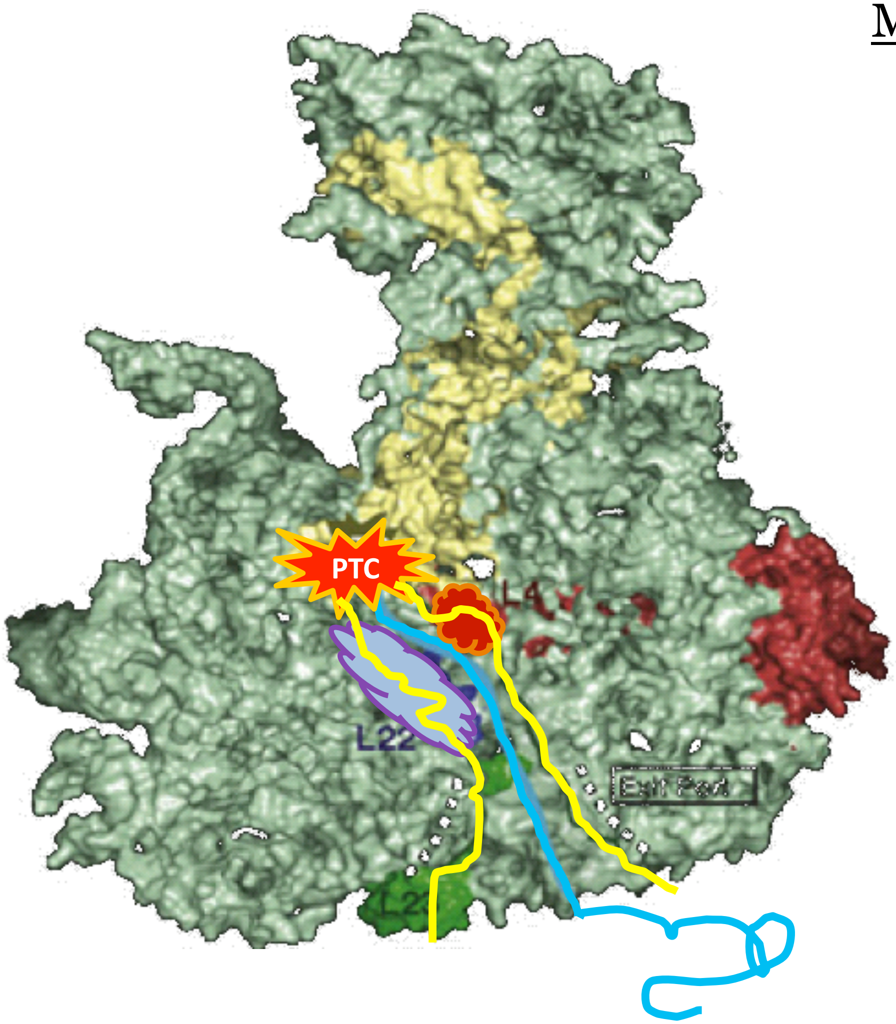
Biochem: Methods and Results

- RNC part outside the tunnel has almost full flexibility
  - QCM(D), NMR
- Compact, native-like structures in near vincinity of the ribosome
  - cryo-EM, NMR, limited proteolysis
- Domain-by-domain folding
  - limited proteolysis
- Post-translational modifications in RNC
  - SDS-PAGE, QCM(D), MS
- Folding dynamics determine structure
  - mutagenesis + any of the above

Associated Proteins



Many proteins interact with the nascent chain

- TF binds and sticks to hydrophobic segments
- Post-translational modifications observed in RNC
- SRP directly threads nascent chain to its destination

➢ Potentially a reason for the observed differences between *in vivo* and *in vitro* expression systems

Summary

- Cotranslational folding is an efficient reduction of sampling space
- Experimental evidence for model of hierarchical folding
- Folding dynamics (rare codons) support search for native structure
- Associated proteins support folding