Structural Basis of Protein Synthesis by the Ribosome

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In all organisms mRNA directed protein synthesis is catalyzed by a ribonucleoprotein particle called the ribosome. Bacterial ribosomes, or 70S ribosomes according to their sedimentation coefficient, are two-subunit structures that have molecular weights of approximately 2.5 million Daltons and contain 50-60 different protein molecules and three different RNAs. The 70S ribosome is formed from two unequally sized subunits : the large, 50S, and the small, 30S, subunit. A huge leap in our understanding of ribosome structure occurred with the publication of the complete atomic structure of the large and small ribosomal subunits in 2000. In recent years we have witnessed an explosion of results in structural studies of protein synthesis, which are based on the existing structures and structure-based design of biochemical experiments. Some of the recent exciting results, which gave further insight in the molecular mechanism of protein synthesis include structure determinations of several ribosomal complexes with tRNA analogues, translation factors and antibiotics.

The structure of the large ribosomal subunit with the active site highlighted.

The work of my group mostly focused on structural studies of the large ribosomal subunit. The 50S subunit of the ribosome catalyzes the peptidedyl-transferase reaction of protein synthesis and binds initiation, elongation and release factors critical in this process. The 50S subunit consists of two RNA molecules and approximately 31 to 35 proteins (L1 – L35). The atomic structure of the large ribosomal subunit in complex with substrates and inhibitors demonstrated that the active site of the ribosome is exclusively composed of RNA. This result shows that ribosomal RNA is responsible for the catalysis of peptide bond formation and that, therefore, the ribosome is a ribozyme. This finding strongly supports the pre-protein RNA world hypothesis.

Co-translational protein folding

The synthesis of peptide bonds, however, is only the first step in the complex process of obtaining a functional, folded and thus active protein. How exactly the folding of a polypeptide chain to its unique native structure is achieved, is one of the fundamental questions in molecular biology. To date, little is known about the extension of the nascent polypeptide through the ribosome and the role that the ribosome or various factors might play in folding of the growing polypeptide. Newly synthesized protein chains leave the ribosome through a molecular tunnel. Although some degree of secondary structure may be formed within the ribosomal exit tunnel during the synthesis of a new polypeptide, complete folding of the protein into native confirmation occurs either post-translationaly in the cytosol or co-translationally immediately outside the tunnel exit while the protein is still attached to the active site of the ribosome. In both cases, chaperones play a major role to facilitate correct folding.

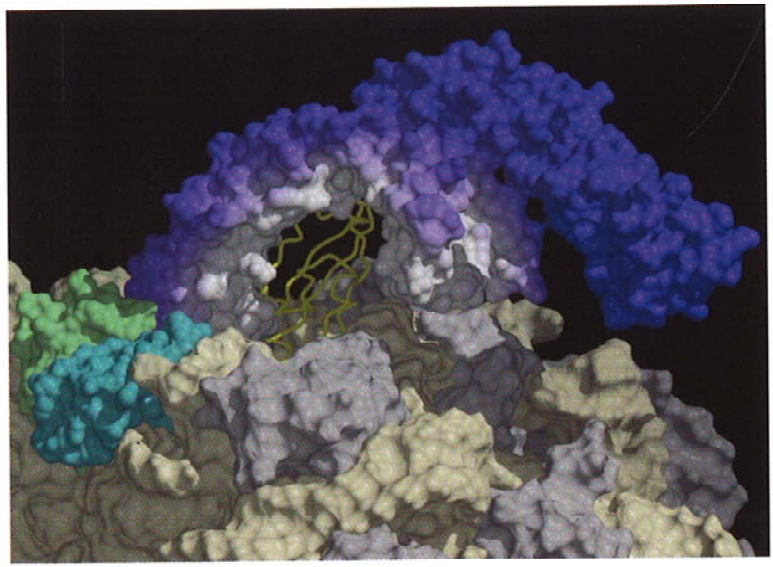
Trigger factor is a ribosome-associated chaperone

In case of co-translational folding, the nascent protein chain is welcomed by chaperones that bind to the ribosome just outside the tunnel exit and interact with the emerging polypeptide chain. One example of such a ribosome-associated chaperone in bacteria is 'trigger factor'. Recently, in collaboration with the group of Elke Deuerling and Bernd

Bukau in Heidelberg, we have solved the structure of the trigger factor and of its ribosome-binding domain in complex with the large ribosomal subunit. These results suggest an unexpected mechanism of action for ribosome-associated chaperones.

A dragon shaped molecule forms a cradle

The structure of the isolated Trigger factor resembles a dragon. Structure analysis of the trigger factor in complex with the large ribosomal subunit revealed that trigger factor hunches over the tunnel exit of the ribosome. The trigger factor attaches to the ribosome with its "tail" and forms a cradle with a hydrophobic inner surface in the "belly" region between the 'arms' and the 'tail'. Combining these structural results with biochemical evidence suggests that the nascent protein chain interacts with the hydrophobic cradle, which protects it from degradation and aggregation. Therefore, the space inside the cradle provides a protected environment for protein folding. It appears that the protected environment of the "cradle" would be able to accommodate an average size protein domain.



Trigger factor hunches over the tunnel exit of the ribosome.

The mechanism of trigger factor action

The presence of a protective space of sufficient size to accommodate a folding protein domain at the tunnel exit is surprising, since it was previously assumed that trigger factor can only bind small polypeptide stretches. This suggests a new mechanism of action for this ribosome-associated chaperone ; Trigger factor has weak affinity to the ribosome, and it dissociates relatively easy from vacant ribosomes. However, the trigger factor-ribosome complex will be stabilized through hydrophobic interactions with an unfolded polypeptide chain emerging from the tunnel. Trigger factor might therefore promote co-translational folding by providing a shielded environment, in which the folding is initially postponed by hydrophobic contacts between the trigger factor and the polypeptide chain. When sufficient sequence information becomes available, folding will proceed in a self-promoted fashion. As the trigger factor-ribosome complex would no longer be stabilized by hydrophobic contacts to the nascent chain, trigger factor would dissociate from the ribosome and release the folded protein domain. Once a next portion of unfolded protein emerges from the tunnel, trigger factor may re-bind for a new folding cycle.

This text has been based on :

1) Ban, N., Nissen, P., Hansen, J., Moore, P. B., and Steitz, T. A., 2000, The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 289 : 905-920.
2) Nissen P, Hansen J, Ban N, Moore PB, Steitz TA (2000) The structural basis of ribosome activity in peptide bond synthesis. Science, **289(5481)** : 920-30.
3) Ferbitz, L., Maier, T., Patzelt, H., Bukau, B., Deuerling, E., and Ban, N., 2004, Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. Nature 431 : 590-596.