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The maturation pathway of flaviviruses studied by crystallography and electron microscopy

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Many viruses go through a maturation step in the final stages of assembly before being transmitted to another host. The maturation process of flaviviruses is directed by the proteolytic cleavage of the precursor membrane protein (prM), turning inert virus into infectious particles. We have determined the crystal structure of a recombinant protein in which the dengue virus prM is linked to the envelope glycoprotein E. The structure represents the prM-E heterodimer and fits well into the cryo-electron microscopy density of immature virus at neutral pH. The pr peptide beta-barrel structure covers the fusion loop in E, preventing fusion with host cell membranes. The structure provides a basis for identifying the stages of its pH-directed, conformational metamorphosis during maturation, ending with release of pr when budding from the host.

Keywords: virus structure, flaviviruses, maturation

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Structural insights into molecular chaperone activity

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Anfinsen established that the polypeptide sequence dictates the folded conformation of a native protein. Protein folding in the crowded cellular milieu is often frustrated by off-reaction aggregations, however, and especially so under destabilizing stresses such as thermal shock. Moreover, the native state may require an intimate co-folding with other components for assembly into multimeric complexes. Molecular chaperones, many of which are heat shock proteins (Hsps), provide a machinery to assist protein folding in the cell. We present studies on two kinds of molecular chaperones. Trigger factor (TF) is a molecular chaperone that associates with bacterial ribosomes, where it is thought to assist in the folding of nascent polypeptides. We also find that ribosome-free TF stably associates with a large repertoire of full-length proteins, including ribosomal protein S7. The crystal structure of a TF:S7 complex from T. maritima reveals the molecular basis of promiscuous substrate recognition by TF, indicates how TF could accelerate protein folding, and suggests a role for TF in the biogenesis of ribosomes and other protein complexes. Hsp70 chaperones use ATP-driven cycles of binding and release of unfolded polypeptides to assist in diverse processes of protein folding and translocation. We deduced hypotheses about the mechanism of Hsp70 chaperone activity from the crystal structure of an ATP complex of yeast Sse1, an Hsp110 chaperone from the Hsp70 superfamily. Mutational tests in Hsp70 chaperones yeast Ssa1 and E. coli DnaK define an Hsp70 chaperone cycle in which radically different Hsp70 conformations are engaged during the chaperone cycle. Allosteric coupling between the ATP and polypeptide binding sites promotes disaggregation and protein folding.

Keywords: conformational change, protein folding, MAD phasing

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Structures of the ribosome on different functional states

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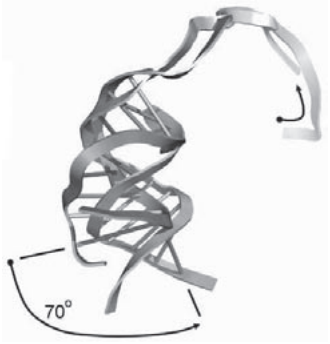
Recent crystal structures of bacterial 70S ribosome containing functional ligands provided information about the general organization of the ribosome and its functional centres. Ribosomes co-crystallized with messenger RNA (mRNA) containing strong Shine-Dalgarno (SD) sequence and transfer RNAs (tRNA) have shown diffraction to 2.8Å resolution. This ribosome complex with initiator tRNA in peptidyl-tRNA binding site represents functional state of translation initiation. We have compared x-ray structures of ribosome complexes modelling translation initiation, post-initiation and elongation states. In the initiation and post-initiation complexes, the presence of the SD duplex causes strong anchoring of the 5 ´ -end of mRNA on the platform of the 30S subunit, where numerous interactions between mRNA and the ribosome take place. Conversely, the 5 ´ -end of the elongator mRNA lacking SD interactions is flexible during elongation. The post-initiation ribosome complex reveals that after initiation of translation, while SD interaction is still present, mRNA moves in the 3´-5´ direction with simultaneous clockwise rotation and lengthening of the SD duplex (Figure).



Keywords: ribosome structure, functional complexes, mRNA and tRNA

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Structural basis of transcription: Structures of the bacterial RNA polymerase elongation complexes

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Understanding the mechanisms of transcription elongation and its regulation requires detailed structural information. The structure of the bacterial EC (2.5Å) revealed the post-translocated intermediate