SUMMARY:

Proteins are synthesized as polymer of amino acids at the peptidyl transference center (PTC) of ribosome and their functional forms require distinct 3D organization (folding), unique for each protein. The rRNA sequence of PTC is one of the most conserved RNA sequences present in the living cell in a well defined tertiary conformation inside the ribosome. This RNA enzyme successfully synthesizes a full length protein by utilizing considerable amount of energy in terms of GTP hydrolysis. When it comes to folding the newly synthesized proteins, chaperones were identified as the class of proteins within the cell. But they assist folding of only a few proteins in an energy dependent manner. Though ribosome bound chaperones are implicated in folding nascent proteins, no universal protein folding activity could be assigned to them. Thus we are handicapped here, for not having any information about the genera mechanism of folding newly synthesized proteins; we cannot solve Levinthal’s paradox which basically indicates that the pathway of folding can not be random in the physiological time scale. It was demonstrated from our lab and later from few others’ that the in vitro transcribed PTC RNA (like the complete 70S or its 50S subunit or the 23S rRNA) can efficiently fold chemically unfolded proteins of diverse characteristics. In vitro, the process does not require any expenditure of energy. It was observed that the unfolded form of a number of unrelated proteins interact specifically with the five sites of this RNA sequence. By the term folding we mean biological activity gain of a protein and it has been demonstrated using proper mutant cells that the PTC mediated folding of nascent protein, in vivo, is independent of ribosome associated chaperones like trigger factor (TF), DnaK etc. In this thesis, we have compared PTC (domain V of 23S rRNA) mediated folding of nascent and chemically unfolded proteins in vivo and in vitro. Our observations indicated that the release of nascent protein from ribosome is a slower even compared to translation and its termination. During this time, the full length nascent proteins stick to and probably recycles the 50S ribosome as the translation factor IF3 (initiation factor 3) recycles the 30S to initiate a fresh round of translation. Interaction of unfolded protein with the PTC RNA is characterized by the release of its different segments sequentially from the PTC. Our study on the time course of specific nucleotide (rRNA)-amino acid (protein)
interaction shows its crucial role for nucleating the early events of folding inside the ribosome. Final native structure formation and the consequent activity gain of the nascent protein takes place post-release of it from the ribosome.

To analyze the PTC-nascent protein interaction we first isolated nascent protein bound population of ribosome from log phase growing bacterial cells. Interestingly we isolated a large population of 50S to be associated with the full length nascent protein along with a relatively small amount of 70S. The first question (chapter 2) we asked was whether the nascent protein interacts with the PTC identically as its chemically unfolded analogue does in vitro? We compared in vitro activity gain of ‘folding’ protein in presence of folding modulators (in vitro transcribed PTC -RNA, 23S rRNA, 50S or 70S ribosome) with that of the activity gain of same nascent protein while synthesizing in vivo. Results of these experiments suggested that: (A) ribosome associated behavior of a nascent protein that is related to its gain of activity, can be predicted from the in vitro experiments using chemically unfolded form of the same native protein; (B) release of a nascent protein through the 50S into the cytosol is a slower process than translation elongation and termination. In the purified in vitro system when chemically unfolded protein was allowed to refold in presence of 70S, it was also found to be associated with the 50S subunit before being released into the medium. We questioned (chapter 3) whether the unfolded protein has any role in splitting ribosomal subunits and how might that be physiologically relevant with the existing model of ribosome recycling. Using filter binding experiment, we observed a temporal interaction pattern of in vitro transcribed PTC RNA with the unfolded protein; activity gain of the protein takes place after it is released in a “folding competent” state from the 50S subunit. Combining Toe printing and MALDI TOF MS/MS analysis, we could suggest a kinetic mechanism (chapter 4) of in vitro protein folding by the PTC linked region of rRNA. Different portions of the protein are released sequentially, basically from the N- to the C-terminus of the protein, from the rRNA which sets the topology of biologically active 3D-structure formation of the protein. How this sequential model of folding is relevant to the direction of protein synthesis in the ribosome is discussed. Therefore, the present work attempts to put together the elongation and termination of translation and ribosome recycling to explain the folding of the newly synthesized protein.