CHAPTER - V

GENERAL DISCUSSION
In the ribosome mediated protein folding process, the domain V region of the large RNA plays the central role. Two parts of this domain V region perform two independent role which are complementary for the protein folding process. We can schematically present this folding process as follows.

Unfolded protein
  ↓
Formation of secondary structures and some tertiary structure within 10-15 seconds
  ↓
Folding intermediates
  ↓
Central loop region of domain V
  ↓
Domain V intermediate complex
  ↓
Tertiary organization of the folding intermediate
  ↓
Domain V competent folding intermediates
  ↓
Release of competent folding intermediates by stem loop region of domain V
  ↓
Competent folding intermediate
  ↓
Chaperones?
  ↓
Spontaneous attainment of further tertiary/quaternary structure.
  ↓
Folded protein

It was found that the last step of this schematic representation is the rate-limiting step and takes several minutes to occur. This timing agreed with the slow folding of β-galactosidase present in the cell when further synthesis of the enzyme was blocked by
the 50S-subunit specific antibiotics (Chattopadhyay et al., 1999). The basic characterization of this ribosome mediated folding process is the stoichiometric requirement of folding modulators with respect to denatured proteins. This also indicates that a single polypeptide chain is the substrate for a single ribosome, which means that for oligomeric proteins individual monomers become folded independently. We have observed this phenomenon in the ribosome mediated folding of dimeric cytoplasmic malate dehydrogenase (Suparna Chandra Sanyal, personal communication). In case of folding of denatured malate dehydrogenase, 23S rRNA catalyze the folding of individual monomers and release them in a folding competent state so that they can immediately form the dimeric active enzyme.

The ribosome mediated refolding process is independent of ATP hydrolysis, whereas chaperones generally need ATP to refold unfolded proteins (Hartl, 1996) with some exceptions (Ellis, 1991). It may be possible that a conformational switch is present in 23SrRNA which works during binding and release of unfolded polypeptide through alternating base paired arrangements just like the switch in 16SrRNA during decoding of messenger RNA (Lodmell and Dahlberg, 1997). Perhaps the interaction of RNA2 with RNA1-denatured protein complex mimics the situation and helps to release the folding intermediates from RNA1.

Since ribosome or its part act as a general protein folding modulator, the domain V RNA could have very specific reaction with all the nascent polypeptides within the cell. This prediction is also supported by the conserved secondary structure of central loop region (RNA1) all over the living world. Besides, many nucleotides in the central loop region is conserved throughout the evolution from mitochondria, the chloroplast, the
smallest prokaryotic cells to higher eukaryotes (Douthwaite, 1992). This specific reaction requires the specific structure of the domain V RNA which is evident from the complete inhibition of domain V mediated refolding of denatured proteins in presence of 0.75 mM EDTA. The non-competitive inhibition of binding of denatured carbonic anhydrase with RNA1 in presence of chloramphenicol and lincomycin also reinforce this idea.

This general protein folding activity also indicates that probably protein molecules evolved through the natural selection attributed by achieving their functional conformation through the interaction set by the central loop of domain V RNA.

The central loop of domain V RNA thus presents a 'molecular mould' where a specific three-dimensional motif of a protein-folding intermediate would fit properly to give rise to a productive folding intermediate (DasGupta, 1999).

This point should be discussed in a little more detail. We found the same sites (nucleotides) of interaction in RNA1 With a number of proteins. This implies that these RNA1 bound protein-folding intermediates have identical topographic arrangements of similar (identical?) amino acids which bind to specific nucleotides in road block experiments. If this turns out to be true for all proteins, we have a RNA-protein folding intermediate interaction frozen in evolution. Most informations on molecular evolution in life comes from one kind of molecule eg., 16S rRNA, similar classes of proteins etc. But here we have a RNA-protein folding intermediate complex carrying an imprint of natural selection. Changes in amino acid sequences in protein can be made as long as this interaction is not violated, that is what happened in evolution, and we have to abide by this rule if we want to design proteins by alternating , adding and deleting amino acids. When we struggle to find out whether protein folding is co-translational or post
translational, we must keep in mind that the intermediate of folding on ribosome is a RNA-folding intermediate complex. This folding intermediate occurs unassisted (takes less than 15 seconds) in our in vitro experiment. But in vivo, the growing polypeptide chain is always in contact with the ribosome. Then the most important question that remains to be answered is what role, if any, is played by ribosome in the cell in the formation of this folding intermediate. To stretch the argument further, we would like to know whether domain V RNA screens the amino acids to be added to a growing polypeptide chain. In other words did the primary structure of protein evolve following some dictates of the domain V region of large ribosomal RNA, and that this was subsequently relayed to DNA via RNA in arranging codons in the genes? Is the domain V RNA the architect of the protein world?

The overlap of the peptidyl transferase activity and protein folding activity of 23S ribosomal RNA also suggests an evolutionary relationship of this RNA with ribosomal proteins, e.g., those of 50S particles. Perhaps these proteins were synthesized and folded by 23S rRNA leading to the morphogenesis of 50S particle. Recent crystal structure of the 50S particle clearly showed that the ribosomal proteins are present to form a scaffold around the 23S ribosomal RNA (Cate et al., 1999; Ben et al., 2000) and more interestingly, the vicinity of domain V region is almost free of ribosomal proteins providing a strong support that this ribozyme only binds intermediates and not completely folded native proteins.