Abstract

The presence of the three consecutive GC base pairs in the anticodon stems of the initiator tRNAs (3GC base pairs) are a highly conserved and a vital feature of the initiator tRNAs in all the domains of life. How this feature is involved in the process of translation initiation is not very clear. The work described in this thesis entitled as "Initiation of protein synthesis: Role of the three consecutive GC base pairs in the anticodon stem of initiator tRNAs" provides new insights into the role of the 3GC base pairs in translation. Chapter 1 provides the relevant information regarding protein synthesis, initiator tRNA selection and the ribosome biogenesis. The experimental procedures as well as the materials used to carry out the study are mentioned in the Chapter 2. The remaining 3 chapters (Chapters 3 to 5) describe the results of the research findings on the role of 3GC base pairs in translation initiation. The brief summary of the research work described in thesis is summarized as follows.

1: Crosstalk between the initiator tRNA and the mRNA.

Initiator tRNAs are special in their direct binding to the ribosomal P-site due to the hallmark occurrence of the three consecutive GC base pairs (3GC base pairs) in their anticodon stems. How the 3GC base pairs function in this role, has remained unsolved. The research described in Chapter 3 shows that mutations in either the mRNA or rRNA leading to extended interaction between the Shine-Dalgarno (SD) and anti-SD sequences compensate for the vital need of the 3GC base pairs in tRNAfMet for its function in *Escherichia coli*. *In vivo*, the 3GC mutant tRNAfMet occurred less abundantly in 70S ribosomes but normally on 30S subunits. However, the extended SD: anti-SD interaction increased its occurrence in 70S ribosomes. We propose that the 3GC base pairs play a critical role in tRNAfMet retention in ribosome during the conformational changes that mark the transition of 30S pre-initiation complex into elongation competent 70S complex. Furthermore, treating cells with kasugamycin, decreasing ribosome recycling factor (RRF) activity or increasing initiation factor 2 (IF2) levels enhanced initiation with the 3GC mutant transmits, suggesting that the 70S mode of initiation is less dependent on the 3GC base pairs in tRNAfMet.

2: Essentiality of the 3GC base pairs in the initiator tRNA.

Translation initiation involves binding of mRNA to the small ribosomal subunit along with the initiation factors and the initiator tRNA followed by recruitment of 50S subunit to form 70S initiation complex. In eubacteria selection of initiator tRNA in the P- site of the ribosome mainly depends upon the formylation of the amino acid attached to it and the presence of 3GC base pairs in the anticodon stem of the tRNA. Unlike the property of formylation, which is conserved only in eubacteria, the presence of the 3GC base pairs in the initiator tRNA is highly conserved in all the three domains of life. The presence of natural variants lacking 1st or 3rd GC pair in mycoplasmal and rhizobial species implicates the essentiality of middle GC pair. This poses a

question of why 3GC base pairs are retained in all three domains of life. In this study (Chapter 4), we show that the middle GC pair, particularly mid G (G30) residue is indeed essential for the initiator tRNA to sustain *E. coli*. However, the flanking GC pairs also contribute to the efficiency of the initiator tRNA function. Further, most of the mutants in the 3GC base pairs were abundant in 30S ribosomes but showed defect in the 70S ribosomal occupancy to varied extent suggesting their role in transition from 30S initiation complex (IC) to the 70S complex. Our in vivo genetic analysis shows that formylation of the amino acid attached to the initiator tRNA is mainly needed for the initial binding of the tRNA to the 30S
ribosome while later steps are mainly dependent on the 3GC base pairs to form functional 70S complex. One such step could well be the eviction of IF3 from the 70S IC to convert it into an elongation competent 70S complex. Thus, the two highly conserved features of the eubacterial initiator tRNAs function at two distinct stages of initiation and help it to proceed through the various checkpoints during the step of initiation. Further, we provide evidence to suggest that the rhizobial and mycoplasmal species could do away with the full complement of 3 GC base pairs as they might have co-evolved ribosomes to utilize variant of initiator tRNA.

3: Role of the 3GC base pairs in the ribosome biogenesis.

Ribosomes are the largest molecules of the cell which play a central role in the synthesis of the polypeptides and are also a hub of various regulatory mechanisms. The biogenesis of the ribosome is a complex and multistep process which requires proper folding of rRNAs as well as incorporation of more than 50 proteins. Any hampering in these processes can generate defective ribosomes which can be detrimental to the cell as they could lead to inefficient and/or mistranslation of mRNAs. As ribosomes are complex molecules, how the cell ensures their proper synthesis and their functional fidelity has remained unclear. In the genetic study described in Chapter 5, we find that the 3GC base pairs of the initiator tRNA play an important role in final maturation of ribosomes. Using a number of diverse genetic analyses in *E. coli*, we show that the formation of initiation complex by initiator tRNA contributes to ensure a proper biogenesis of the ribosomes. Ribosomal complexes defective in the formation of initiation complex were found to retain immature 5‟ and 3‟ ends of the 16S rRNAs in the complex.