Unraveling multiple checkpoints for accurate selection of initiator tRNA on ribosomes for translation initiation

Ribosomes comprise a large (50S) and a small (30S) subunits. To begin (initiate) protein synthesis, the two subunits associate on an mRNA (70S). Organisms possess two classes of tRNAs, the initiators (i-tRNA) and the elongators. The i-tRNA usually binds directly to ribosomal P-site during initiation, whereas the elongators bind the neighboring A-site following proper initiation. Initiation of protein synthesis determines the reading frame in the mRNA coding sequence. Any deficiencies in i-tRNA selection in P-site would cause production of mis-translated proteins/peptides, which could be detrimental to the cell.

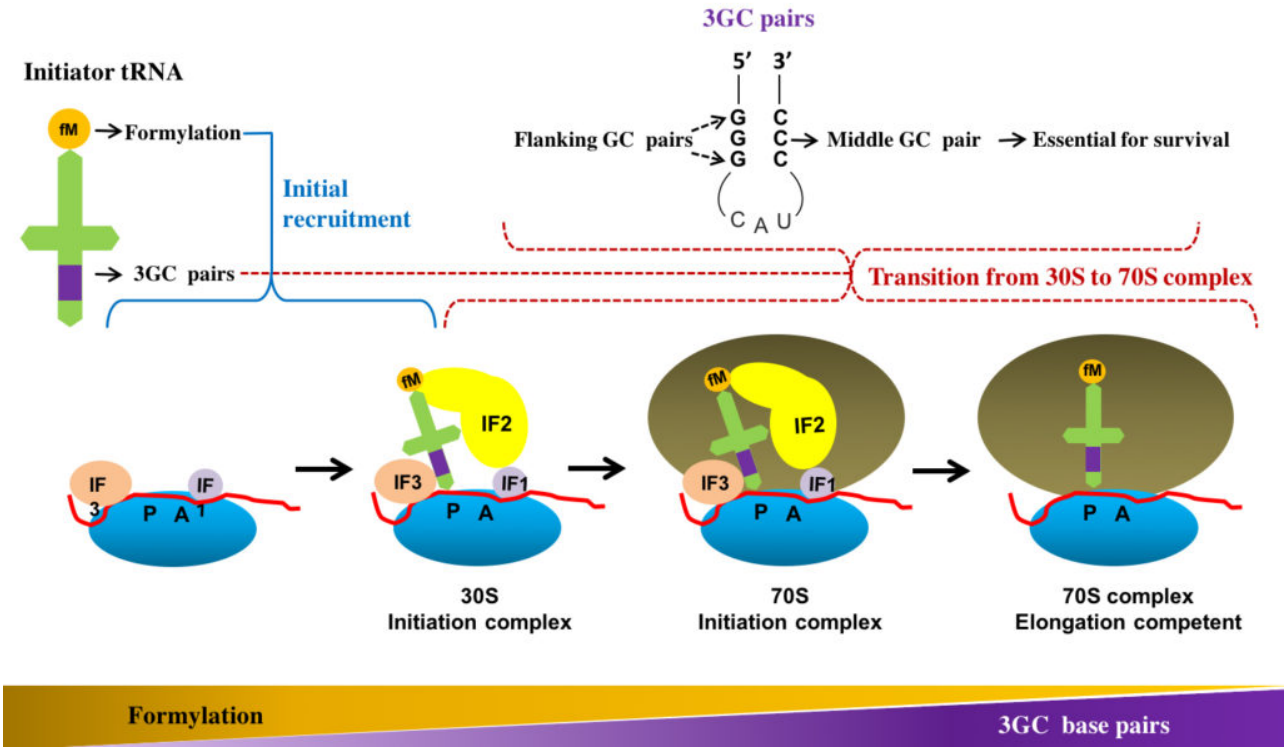

Fig. 1.

Selection of i-tRNA (and exclusion of elongator tRNA) in the P-site is assisted by its special structural features, various ribosomal P-site elements, and the initiation factors (IF1, IF2 and IF3). The bacterial i-tRNAs possesses two unique features: (i) a Watson:Crick mismatch at the top of the acceptor stem (1x72 position), which (together with the second and the third base pairs) guides the formylation of the amino acid attached to it; and (ii) the presence of 3 consecutive GC base pairs (conserved in all the three domains of life) in the anticodon stem. The two features were shown to

be important for i-tRNA function, and their transplantation into elongator tRNA could convert it to an initiator but the mechanistic details remained obscure. Initiation in bacteria involves conformational rearrangements of i-tRNA, GTP hydrolysis, release of initiation factors etc.

Although the deficiency of formylation of the amino acid on i-tRNA results in a severe growth defect, it is not essential in *E. coli*. Interestingly, the growth defects could be rescued by increasing the cellular i-tRNA amounts. On the other hand, the lack of 3GC pairs mainly affected their abundance in 70S complex, and this defect is not rescued by increasing the cellular amount of the i-tRNA mutant lacking the 3GC pairs. Systematic mutagenesis of individual GC base pairs suggested that although for the transition of i-tRNA into 70S complex as well as for the cell survival, the minimal requirement among the three consecutive GC pairs is that of the middle GC pair but the flanking GC pairs enhance the efficiency. Interestingly, in the absence of middle GC pair, the IF3 was still retained in the 70S complex. IF3 acts as anti-association factor during initiation and it leaves the 30S upon formation of 70S complex. Thus, the absence of 3GC pairs impairs the release of IF3 rendering the 70S complex unstable.

How relevant are these observations in nature? A few species of mycoplasmas and rhizobium do possess variation to these sequences in the flanking pairs. Why do these organisms retain such variations if these tRNAs are less efficient in forming 70S complex? Analysis of polysomes from the species of rhizobium showed efficient formation of the 70S complex harboring i-tRNA, suggesting co-evolution of the ribosomes to compensate for the lack of the flanking GC pairs. Our genetic and the biochemical analyses suggest that the unique features of i-tRNA assist it in passing through the multiple checkpoints during initiation, in a sequential manner. Formylation primarily facilitates the initial recruitment of i-tRNA on the 30S ribosome while the 3GC pairs assist in the later steps of formation of 70S complex. Such a two-step scrutiny would ensure accurate and efficient selection of i-tRNA (over elongator tRNA) for initiation of protein synthesis.

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