Abstract

Protein synthesis or mRNA translation is a complex and highly conserved process. Translation consists of initiation, elongation, termination, and ribosome recycling stages. Since most regulation occurs during initiation, its mechanism is being studied intensively to elucidate the molecular basis of every potential control point. The initiation factor eIF3, which in yeast consists of five essential core subunits (eIF3a/TIF32, b/PRT1, c/NIP1, g/TIF35, and i/TIF34) and one transiently associated, non-essential subunit (j/HCR1), is undisputedly one of the key promoters of initiation. In addition, it has also been implicated in playing a critical role during ribosomal recycling, reinitiation, signal transduction, NMD etc.

We have focused on determining the molecular mechanism of the roles of eIF3 and its associated eIFs not only in translation initiation but also in termination and in reinitiation. This included the biochemical and genetic mapping of yeast eIF3 binding site on the small ribosomal subunit, among others.

We showed that the interaction between the residues 200–400 of a/TIF32-NTD and flexible C-terminal tail RPS0A significantly stimulates attachment of eIF3 and its associated eIFs to small ribosomal subunits *in vivo*, thus a/TIF32-NTD together with the recently published PCI (proteasome component) domain in c/NIP1-CTD form important intermolecular bridges between eIF3 and the 40S. Moreover, we demonstrated that the partial deletion of the RPS0A-binding domain of a/TIF32 also severely blocks the induction of *GCN4* translation that occurs via reinitiation. Genetic analysis reveals a functional interaction between 5′ *cis*-acting sequences of the *GCN4* mRNA and a/TIF32-NTD. This interaction facilitates stabilizing post-termination 40S subunits on upstream ORF1 of the *GCN4* mRNA and resuming of scanning downstream.

Furthermore, another part of my Ph.D. thesis reveals functional characterization of two essential eIF3 subunits, g/TIF35 and i/TIF34, previously suggested to be dispensable for formation of the 48S preinitiation complexes (PICs) *in vitro*, hallmark function of eIF3. We showed that both subunits are involved in promoting the rate and processivity of scanning in living cells. Moreover, we demonstrated that g/TIF35 specifically interacts with ribosomal proteins RPS3 and RPS20 located near the ribosomal mRNA entry channel and its RRM domain plays role in reinitiation by stabilizing uORF1 post-termination 40S ribosomes on *GCN4*, although by different molecular mechanism than a/TIF32-NTD. Besides, we reported

the 2.2A˚ resolution crystal structure of i/TIF34 subunit in complex with the minimal CTD of b/PRT1 (654–700), the boundaries of which were defined by solution NMR spectroscopy

In my last part of this thesis we identified and defined a role for eIF3 in the stop codon selection process in vivo and uncovered its active roles in translation termination, defining a communication bridge between initiation and termination/recycling phases of protein synthesis.