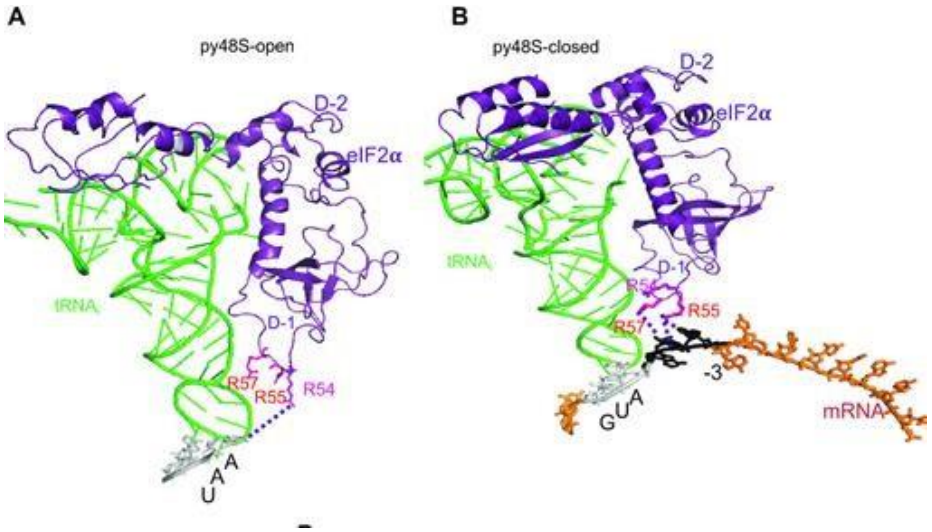
eIF2α interactions with mRNA control accurate start codon selection by the translation preinitiation complex



Scientists at DBT-Regional Centre for Biotechnology (RCB), Faridabad, found that during translation initiation, AUG recognition triggers rearrangement of the 48S preinitiation complex (PIC) from an open conformation to a closed state with more tightly-bound Met-tRNAi. Cryo-EM structures have revealed interactions unique to the closed complex between arginines R55/R57 of eIF2α with mRNA, including the −3 nucleotide of the 'Kozak' context. It was found that R55/R57 substitutions reduced recognition of a UUG start codon at HIS4 in Sui− cells (Ssu− phenotype); and *in vitro*, R55G-R57E accelerated dissociation of the eIF2·GTP·Met-tRNAi ternary complex (TC) from reconstituted PICs with a UUG start codon, indicating destabilization of the closed complex. R55/R57 substitutions also decreased usage of poor-context AUGs in SUI1 and GCN4 mRNAs *in vivo*.

In contrast, eIF2α-R53 interacts with the rRNA backbone only in the open complex, and the R53E substitution enhanced initiation at a UUG codon (Sui− phenotype) and poor-context AUGs, while reducing the rate of TC loading (Gcd− phenotype) in vivo. Consistently, R53E slowed TC binding to the PIC while decreasing TC dissociation at UUG codons in vitro, indicating destabilization of the open complex. Thus, distinct interactions of eIF2α with rRNA or mRNA stabilize first the open, and then closed conformation of the PIC to influence the accuracy of initiation *in vivo*.