Abstract

Translation initiation is a multistep process resulting in the formation of the elongation-competent 80S ribosome at the AUG start codon of the mRNA to be translated into a polypeptide chain. This process is orchestrated by numerous proteins called eukaryotic initiation factors (eIFs), out of which the most multitasking one is the eukaryotic initiation factor 3 (eIF3). The main focus of our laboratory aims at the complex characterization of the multisubunit protein eIF3 and the mechanisms of its contribution to various steps of translation initiation. Besides this, we also study one of the gene-specific translational control mechanisms called reinitiation which was, at least in yeast, also shown to be promoted by eIF3.

Here I show that the N-terminal domain (NTD) of the largest subunit of yeast eIF3, a/Tif32, plays an important role not only in anchoring the eIF3 complex to the 40S small ribosomal subunit but it also critically contributes to mRNA recruitment to the 43S preinitiation complexes *in vivo*. The mRNA stabilization role of the a/Tif32-NTD at the mRNA exit channel of the 40S subunit was further confirmed in our following study by biophysical experiments. There, using *in vivo* approaches, we also demonstrated that mRNAs with longer 5´UTRs are more dependent on the stabilization role of the a/Tif32-NTD than those containing short 5´UTRs.

In other studies, where I turned my attention to the mechanism of reinitiation, we revealed novel *cis*-determinants contributing to the efficiency of reinitiation on the yeast model *GCN4* mRNA and, importantly, brought the first insights into this gene-specific regulatory mechanism in human cells on the model human *ATF4* mRNA. In detail, we discovered that similarly to the yeast *GCN4* mRNA, the reinitiation-permissive upstream ORF1 (uORF1) of *ATF4* is also surrounded by sequences that contribute to high reinitiation efficiency that this uORF allows. Moreover, we computationally predicted that the sequence immediately preceding uORF1 of *ATF4* probably folds into a specific secondary structure that seems to be conserved among mammals. Computationally designed mutations disrupting this structure obliterated the reinitiation potential of uORF1 suggesting that formation of this secondary structure critically contributes to the yet-to-be-described molecular mechanism underlying reinitiation in humans. Finally, we also demonstrated that in analogy with the reinitiation mechanism in yeasts, reinitiation in humans is also promoted by eIF3; only the contributing subunit is not eIF3a/Tif32, but eIF3h.

Thus, this PhD thesis contributed not only to our understanding of basic principles of general translation initiation in eukaryotes but also shed new light onto the molecular mechanism of reinitiation in human cells, revealing that many mechanistic aspects of this process are conserved both in higher and lower eukaryotes.