

Abstract

In eukaryotic translation, eukaryotic initiation factors (eIFs) are at least as important as the ribosome itself. Some of these factors play different roles throughout the entire process to ensure proper assembly of the preinitiation complex on mRNA, accurate selection of the initiation codon, errorless production of the encoded polypeptide and its proper termination. Perhaps, the most important factor integrating signals from others and coordinating their functions on the ribosome is eIF3. In *Saccharomyces cerevisiae*, eIF3 is formed by five subunits. All these subunits contain structural motifs responsible for contact with ribosomal proteins and RNAs. In addition to these highly structured parts, the rest of eIF3 is unstructured and very flexible. Therefore, despite the recent progress thanks to the use of a cryo-electron microscopy, a precise structure and position of eIF3 on the 40S ribosomal subunit are still not known. Also, the presence of eIF3 on 80S during early elongation and its role in reinitiation and readthrough are not fully understood.

In order to crack mysteries of yeast eIF3, we used x-ray crystallography, chemical cross-linking coupled to mass spectrometry, and various biochemical and genetic assays.

We demonstrated that eIF3 is very compactly packed when free in solution. This finding is in sharp contrast with the situation when eIF3 interacts with the 40S and embrace it almost completely from both the mRNA entry and exit channels. Considering that eIF3 association with its major interacting partners, namely eIF1 and eIF5, do not seem to dramatically change the globular shape of ribosome-free eIF3, we conclude that it is most probably the initial contact of eIF3 with the 40S that triggers its dramatic structural rearrangement. Importantly, using the same approach we determined the so far unknown binding site of eIF5 on 40S.

With the help of the newly developed pull-down assay, we also demonstrated that eIF3 stays bound on ribosomes elongating and terminating on short upstream open reading frames and promotes reinitiation in both yeast and mammals. On top of that, we designed and verified an *in vivo* assay for the comprehensive study of translational readthrough.

This thesis thus markedly extends the knowledge of yeast eIF3, its geometry, structural rearrangements provoked by its different binding partners, and its roles in reinitiation and readthrough.