ABSTRACT

Translation initiation in eukaryotes is a complex process promoted by numerous proteins or protein complexes called eukaryotic initiation factors (eIFs). The eukaryotic initiation factor 3 (eIF3) is a multisubunit complex that has been implicated in several steps of the translation initiation pathway. In yeast *Saccharomyces cerevisiae*, eIF3 is composed of five essential subunits (a/TIF32, b/PRT1, c/NIP1, g/TIF35, i/TIF34) and one nonessential subunit (j/HCR1). It is our long-term goal to understand how eIF3 promotes different stages of translation initiation and which subunits or their domains play a critical role in this process as well as to map the binding sites of eIF3 on 40S ribosomal subunit and to create a structural model of eIF3 complex.

Here I present two structural studies showing interactions between the RNA recognition motif of eIF3b and a short peptide of eIF3j subunits of human eIF3 solved by NMR spectroscopy, and a crystal structure of the C-terminal fragment of yeast b/PRT1 in complex with the full length i/TIF34 subunit at 2.2 Å resolution. In the former study, me and my colleagues showed that the critical determinants mediating this eIF3b–eIF3j interaction are evolutionary conserved, since their mutations in yeast proteins reduced cellular growth rate, eliminated j/HCR1 association with b/PRT1 *in vitro* and *in vivo*, affected 40S-binding of the entire eIF3 complex, and finally produced a leaky scanning defect (skipping the AUG start codon) indicating impairment of the AUG selection process. In the latter study, I revealed that the b/PRT1-i/TIF34 interaction is mediated by two major contacts. Site-directed mutagenesis of these contacts eliminated association of i/TIF34 and g/TIF35 with the rest of eIF3 *in vivo*, affected 40S-binding of the remaining trimeric subcomplex of eIF3 and its binding partner eIF5, and, as a result, led to accumulation of aberrant preinitiation complexes containing only eIF1 and eIF2 causing a severe leaky scanning defect.

In other studies, we identified novel contacts between the a/TIF32 C-terminal domain (a/TIF32-CTD) and yeast small ribosomal proteins RPS2 and RPS3, and between j/HCR1 and RPS2 and RPS23, which place this mutually interacting eIF3 subunits in the vicinity of the mRNA entry channel, where they can contribute to regulation of the start codon recognition, and at least in case of the a/TIF32-CTD, also in mRNA recruitment, as we demonstrated. We also identified RPS3 and RPS20 as the
binding partners of g/TIF35, which places the g/TIF35-i/TIF34 subcomplex on the solvent side of the 40S subunit right above the mRNA entry channel, where it can contribute to the proper assembly of the 48S pre-initiation complex and influence subsequent scanning for AUG, as we showed. We also mapped the binding sites of two factors critically involved in AUG recognition within the c/NIP1 N-terminal domain, namely eIF1 and eIF5, and characterized how these contacts promote the ternary complex recruitment and modulate start codon selection.

Finally, in our functional genetic studies, we showed that a/TIF32 N-terminal domain and g/TIF35 play important roles in the process of gene-specific regulation called reinitiation, although by a different molecular mechanism.