ABSTRACT

Translation represents a highly regulated, interconnected process of protein synthesis in the cell. It could be divided into 4 phases: initiation, elongation, termination, and ribosomal recycling.

Our laboratory is involved in in-depth studies of a complex eukaryotic initiation factor 3 protein (eIF3). We are interested not only in revealing its molecular roles in the translational cycle in general but also in specific mechanisms that allow translational regulation according to specific cellular needs.

In the budding yeast, the eIF3 is composed of five essential subunits (a/Tif32, b/Prt1, c/Nip1, g/Tif35 and i/Tif34). In mammals, the protein is even more complex, comprising of 12 subunits (a-i, k-m).

eIF3 is a key player not only in translation initiation but also in ribosomal recycling and, surprisingly, in translation termination and stop codon readthrough as well. The latter process harbors important clinical potential, as approximately 1/3 of genetically inherited diseases is caused by the presence of a premature termination codon in the protein-coding region. Therefore, understanding the molecular mechanism underlying this phenomenon provides important tools for the targeted and less toxic drug development approaches needed for patient therapy.

In this Ph.D. Thesis, I uncovered the role of yeast small subunit ribosomal protein Rps3 in the control of stop codon recognition efficiency. I identified Rps3 residues involved in maintaining the termination fidelity and proved that eIF3 and Rps3 co-operate during the mechanism of the programmed stop codon readthrough. Precisely, I identified that the fine-tunning of the termination fidelity occurs by a minimum of three different contacts that the C-terminal domain of a/Tif32 subunit of eIF3 establishes with the Rps3 protein.

Additionally, I participated in two other eIF3-oriented projects in mammals. One dealt with the gene-specific mechanism of translational control called the reinitiation (REI) and its conservation between yeast and higher eukaryotes. We found out that the eIF3´s role in REI is indeed very well conserved, however, in mammals the eIF3h subunit is specifically involved instead of the a/Tif32 subunit, which has been demonstrated in yeast.

The second project focused on the fundamental role of mammalian eIF3 subunits in the 43S and 48S pre-initiation complex assembly. We proved the key role of the eIF3d subunit in

the recruitment of the 40S subunit to eIF3 and defined the roles of eIF3c, eIF3k and eIF3l subunits in the recruitment of the mRNA to the 43S pre-initiation complex.

As a whole, this Ph.D. Thesis extends the knowledge concerning the involvement of ribosomal proteins in translational control and reveals molecular details about translation stages influenced by eIF3 not only in yeast but also in mammalian cells.