Abstract

Translation initiation is the first step of protein synthesis that captures the flow of gene expression pathway in all living organisms. The advantage of regulation of gene expression at the level of translation initiation is that it allows for more rapid changes in the proteome and serves as the rate limiting step under certain conditions such as stress. This process is masterminded by many initiation factors. One of them, a multisubunit eukaryotic initiation factor 3 (eIF3), is a very efficient player in this field taking a part in the most of the initiation steps. The largest subunit of the eIF3 complex is called eIF3a p170 and TIF32 in mammals and yeast, respectively, and at least in yeast, it was shown to represent an essential constituent of the translational machinery. This work is based on all that has been learned about the eIF3a roles in translation initiation in the model organism of yeast *Saccharomyces cerevisiae* in effort to examine the degree of the functional conservation with its human ortholog. This is achieved by the RNAi-mediated knock-down of eIF3a in HeLa and HEK cell lines followed by variety of well established assays to monitor translational status of eIF3a depleted cells. In the first part, I describe optimization of the RNA interference protocol with respect to the choice and concentrations of a transfection reagent as well as the actual siRNAs. In the second part, I focus on investigating the effect of the eIF3a down-regulation on i) cell viability using the MTT assay; ii) the rate of global protein synthesis by ^{35}S-methionin incorporation and iii) formation of polysome profiles.

(In Czech)