Abstract

The biological role of eIF4E2, translation initiation factor, remains unexplored in mitosis. To better understand its role in mitotic translation regulation, I established, with validations, RPE-1 stable cell lines with inducible expression of eIF4E2 tagged with GFP and 3XFLAG respectively. I also created similar cell lines with inducible expression of eIF4E with identical tags, for comparison. I further performed a pilot LC-MS/MS experiment with GFP- and 3XFLAG-eIF4E proteins immunoprecipitated from G2, M, and G1 cell cycle phase of these RPE-1 cells. However, mass spectrometry and Western blot analyses identified only eIF4G translation initiation factor as specific eIF4E-interacting protein, indicating a likely stringent immunoprecipitation condition.

Furthermore, to study the gene-specific translation regulation in mitosis, I took advantage of the ribosome profiling study by Tanenbaum et al. (2015) and selected 10 transcripts with differential translation efficiency (TE) upon mitotic entry. In the two biological replicates of polysome profile of G2, M and G1 conducted, decreased TE of PPP1CC and ARHGAP5 in mitosis was confirmed, whereas a similar trend in TE of MCM2, MCM5 and CDT1 mRNAs was observed in one replicate. The reliability of the qRT-PCR based approach for mRNA TE calculation can be better appreciated with more than two biological replicates and more ribosome profiling datasets.

Key words: translation; translation control; cell cycle; mitosis; polysome profiling; ribosome profiling; human stable cell lines