Abstract

Dysregulation of the ubiquitin-proteasome system (UPS) leads to the accumulation of aggregation-prone proteins, which have been linked to neurodegeneration and cancer. The 26S proteasome is a central component of the UPS. It is assembled by the successive recruitment of the 33 different proteasome subunits, which is assisted by proteasome assembly chaperones: five for the CP and five for the RP. RPACs are translationally induced upon stress to assemble more functional proteasomes. Most recently, it has been shown that the deletion of the 5' untranslated region (UTR) of one RPAC mRNA, Adc17, completely abolished its translation. This study aims to investigate the impact of UTR deletion on RPACs translation and monitor mRNA localisation relative to the actin cytoskeleton. Using the CRISPR/Cas9 system, we revealed that the 5'UTR is responsible for both increased mRNA translation upon stress and mRNA stability in Nas6, while the 3'UTR mainly regulates mRNA stability. Furthermore, confocal microscopy analysis showed that NAS6 mRNA is interacting with the actin cytoskeleton, as previously reported for ADC17. Previous data showed that ADC17 mRNA is recruited to cortical actin patches for translation upon stress in an Ede1-dependent manner. Therefore, the second part of this study seeks to know if Ede1 phase separation is important for this process. CRISPR/Cas9 deletion of the phase-separating region of Ede1 phenocopied Ede1Δ cells, confirming that phase separation is important for Ede1 function in regulating proteasome assembly. Together, these results improve our current understanding of proteasome biology, a key step in developing a new strategy to restore proteasome function in diseases.

Keyword: Proteasome, Proteasome Assembly Chaperone, Stress response, RNA regulation, CRISPR-Cas9