Chapter 1

Introduction

1.1 Background

The ubiquitin-proteasome system (UPS) regulates protein breakdown and proteostasis (Rousseau & Bertolotti, 2016). Failure of UPS to clear aberrant proteins and their subsequent aggregation is a hallmark of neurodegenerative disorders and cancers (Disorders et al., 2013). The UPS tags proteins with ubiquitin conjugates, providing a recognition signal for protein destruction. The core unit of this system is the proteasome. In eukaryotes, the 26S proteasome consists of the 19S regulatory particle (RP) and the 20S core particle (CP) (Tomko & Hochstrasser, 2013). A 20S proteasome CP is capped on one or both ends by a 19S RP to form the 26S proteasome. The RP is essential for the function of the 26S proteasome, and, as such, the deletion of any of its subunits is lethal in mammals. RP association with one or both ends of the CP is therefore an essential process in cells. Under challenging conditions, the 26S proteasome is regulated to adjust protein degradation to cellular demands. The activity of the proteasome can be increased by either stimulating its catalytic activity or increasing its assembly. Increased proteasome assembly is partly mediated by the coordinated induction of proteasome assembly chaperones. These assembly chaperones consist of five CP assembly chaperones (Pba1-4 and Ump1) and five RP assembly chaperones (RPACs: ADC17, NAS2, HSM3, NAS6, and RPN14). Given that RP is required for proteasomal degradation, altering their assembly chaperones impairs RP integrity and hence proteasome function. Previous research found that inhibition of the nutrient-sensitive TORC1 (target of rapamycin complex 1) kinase complex induces proteasome assembly and activity (Rousseau & Bertolotti, 2016). One key mechanism promoting proteasome assembly upon stress is the concerted increase of RPACs, such as Adc17 and Nas6, which mainly occurs at the level of translation. The authors confirmed that TORC1 is an evolutionary conserved major regulator of proteasome homeostasis; however, the mechanism behind increased RPAC translation remains to be further elucidated.

One study performed deletions in either the 3' or 5' untranslated regions (UTRs) of ADC17 to understand the process that underpins the selective translation upon stress. The findings discovered that deletions of the 5' UTRs completely ablated its expression, indicating that this region contains regulatory elements essential for Adc17 translation (Williams et al., 2022). Interestingly, this study uncovered that the increased translation upon stress is instigated by the RPACs mRNA localisation to the cortical actin patches, while it typically travels via actin cables during normal cellular conditions. Interestingly, this study uncovered that the increased translation upon stress is instigated by the ADC17 mRNA localisation to the cortical actin patches, while it typically travels via actin cables during normal cellular conditions. Furthermore, it was revealed that ADC17 mRNA interacts with Ede1, an endocytic protein, which facilitates its attachment to cortical actin patches, enabling enhanced translation of Adc17. Confirming this, the same previous study performed a deletion of the Ede1 gene, which resulted in a decrease in the translation of ADC17 mRNA. However, to this end, the mechanism underlying this process is still unclear. Therefore, by gaining a deeper understanding of the mechanisms that govern RPAC mRNA translation, including the roles of UTRs and actin remodelling, we can gain insights into proteasome assembly as an adaptive process. This discovery holds important implications for developing therapies that modulate proteasome function in order to restore protein homeostasis in diseases.

1.2 Objective

An important unresolved question is whether ADC17 translation regulation is conserved in other RPACs. The aim of this study is therefore divided into two parts. The first part is to uncover the role of the UTRs of NAS6 and ADC17 (3’UTR) in regulating their mRNA localisation and translation. To achieve this, several experiments were conducted: (1) the impact of the deletion of the UTRs of NAS6 and ADC17 mRNA on their translation was investigated by immunoblot, and (2) Nas6 mRNA localisation relative to the actin cytoskeleton was monitored by confocal microscopy. Finally, having established the role of Ede1 in increasing RPACs mRNA translation upon stress, the second part of the study seeks

to investigate the impact of deleting Ede1's phase-separating domain on both the localisation and translation of ADC17 mRNA. While the contribution of the dysfunction of the proteasome to disease is well appreciated, the underlying mechanisms are often unknown. Overall, these experiments will improve our current understanding of how proteasome assembly and activity are regulated in cells, which could ultimately contribute to the development of new therapeutic strategies for diseases defective in protein homeostasis such as neurodegeneration and cancer.

1.3 Hypothesis

Two main hypotheses have been tested in this study: (1) NAS6 mRNA follows the same mode of regulation that ADC17 mRNA: (1a) The mRNA is interacting with the actin cytoskeleton; (1b) The UTRs are important for mRNA stability and translation; and (2) Phase separation of Ede1 is important in regulating ADC17 mRNA recruitment to cortical actin patches for translation upon stress.