Actin dynamics regulate proteasome homeostasis

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Actin dynamics regulate protein homeostasis

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Cells must maintain the right amounts of functional protein at all times. Damaged proteins must be degraded. Certain stresses inhibit TORC1, increasing proteasome assembly by translational upregulation of regulatory particle assembly chaperones (RPACs). Using yeast, we identify a protein, Ede1, associated with translating RPAC mRNA following TORC1 inhibition. Following rapamycin/latrunculin-B treatment, cellular actin depolarises and RPAC mRNA localises more to actin patches, dependent on Ede1. Together with TORC1 inhibition, this shift is required for RPAC translation.

1 Proteasome increase maintains protein homeostasis under stress

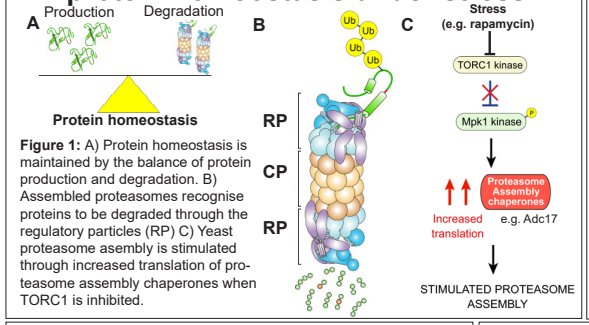


Figure 1: A) Protein homeostasis is maintained by the balance of protein production and degradation. B) Assembled proteasomes recognise proteins to be degraded through the regulatory particles (RP) C) Yeast proteasome asembly is stimulated through increased translation of proteasome assembly chaperones when TORC1 is inhibited.

2 Translating assembly chaperone mRNA interactor identification

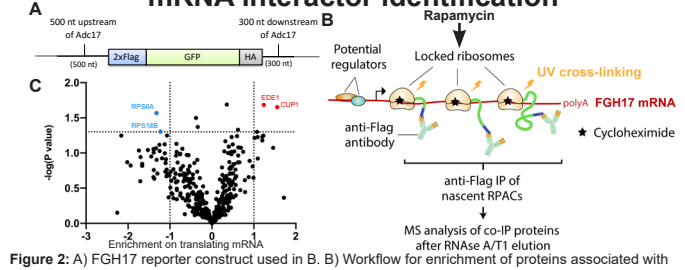


Figure 2: A) FGH17 reporter construct used in B. B) Workflow for enrichment of proteins associated with translating FGH17 mRNA. Nascent chain-ribosome-mRNA-regulatory protein complexes were immunoprecipitated following rapamycin treatment. mRNA-bound proteins were released by RNAse digestion and identified by mass spectrometry. C) Ede1 and Cup1 were identified as potential Adc17 regulators.

3 Ede1 is required for increased assembly chaperone translation

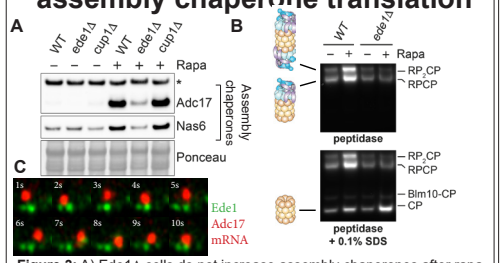


Figure 3: A) Ede1Δ cells do not increase assembly chaperones after rapamycin treatment. B) This results in no proteasome activity increase, viewed using a fluorogenic peptide on a native protein gel. C) Ede1 transiently interacts with Adc17 mRNA.

4 Tethering mRNA to Ede1 increases translation induction

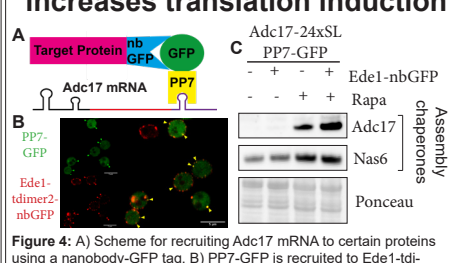


Figure 4: A) Scheme for recruiting Adc17 mRNA to certain proteins using a nanobody-GFP tag. B) PP7-GFP is recruited to Ede1-tdimer2-nbGFP (arrows). C) Adc17 translation is increased following rapamycin treatment when Adc17 mRNA is recruited to Ede1. Translation of Nas6, which is not recruited, is unaffected.

5 Actin patch regulators in RPAC translation

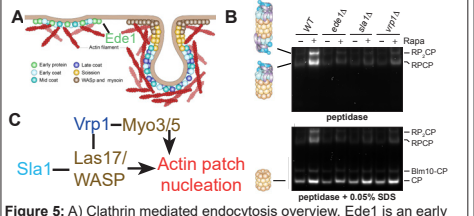


Figure 5: A) Clathrin mediated endocytosis overview. Ede1 is an early endocytic protein, so we screened endocytic mutants for rapamycin induced RPAC translation and proteasome assembly defects. B) 2 mutants, Sla1Δ and Vrp1Δ, were identified. C) Sla1 and Vrp1 recruit actin nucleators for actin patch nucleation.

6 Actin patches interact with RPAC mRNA

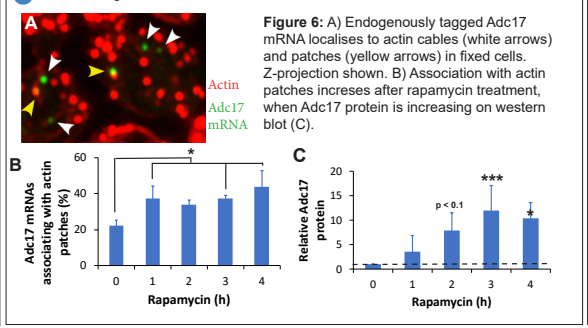


Figure 6: A) Endogenously tagged Adc17 mRNA localises to actin cables (white arrows) and patches (yellow arrows) in fixed cells. Z-projection shown. B) Association with actin patches increses after rapamycin treatment, when Adc17 protein is increasing on western blot (C).

7 Actin depolarisation increases RPAC mRNA-actin patch interaction

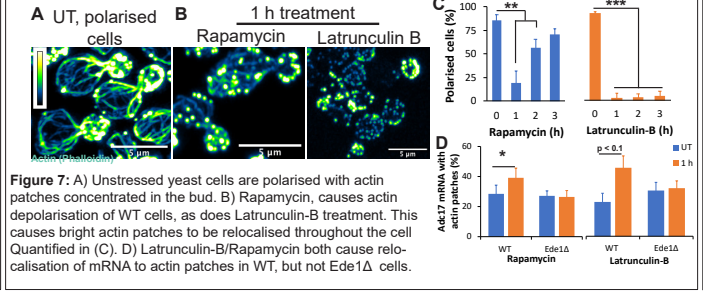


Figure 7: A) Unstressed yeast cells are polarised with actin patches concentrated in the bud. B) Rapamycin, causes actin depolarisation of WT cells, as does Latrunculin-B treatment. This causes bright actin patches to be relocalised throughout the cell Quantified in (C). D) Latrunculin-B/Rapamycin both cause relocalisation of mRNA to actin patches in WT, but not Ede1Δ cells.

8 Depolarisation causes Ede1-dependent proteasome assembly

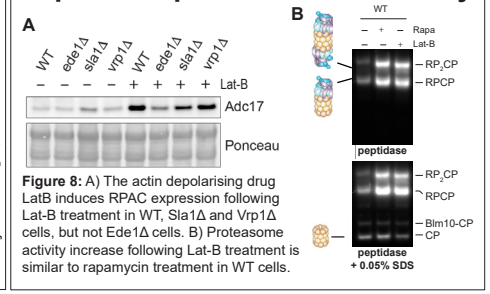
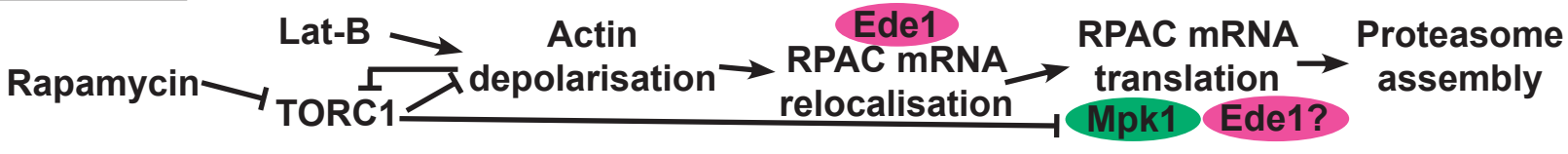


Figure 8: A) The actin depolarising drug LatB induces RPAC expression following Lat-B treatment in WT, Sla1Δ and Vrp1Δ cells, but not Ede1Δ cells. B) Proteasome activity increase following Lat-B treatment is similar to rapamycin treatment in WT cells.



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