Actin remodelling controls proteasome homeostasis upon stress

Thomas David Williams, Roberta Cacioppo, Alexander Agrotis, Ailsa Black, Houjiang Zhou and Adrien Rousseau

When cells are stressed, bulk translation is often downregulated to reduce energy demands while stress-response proteins are simultaneously upregulated. To promote proteasome assembly and activity and maintain cell viability upon TORC1 inhibition, 19S regulatory-particle assembly chaperones (RPACs) are selectively translated. However, the molecular mechanism for such selective translational upregulation is unclear. Here, using yeast, we discover that remodelling of the actin cytoskeleton is important for RPAC translation following TORC1 inhibition. mRNA of the RPAC ADC17 is associated with actin cables and is enriched at cortical actin patches under stress, dependent upon the early endocytic protein Ede1. ede1Δ cells failed to induce RPACs and proteasome assembly upon TORC1 inhibition. Conversely, artificially tethering ADC17 mRNA to cortical actin patches enhanced its translation upon stress. These findings suggest that actin-dense structures such as cortical actin patches may serve as a translation platform for a subset of stress-induced mRNAs including regulators of proteasome homeostasis.

C

When cells are stressed, bulk translation is often downregulated to reduce energy demands while stress-response proteins are simultaneously upregulated. To promote proteasome assembly and activity and maintain cell viability upon TORC1 inhibition, 19S regulatory-particle assembly chaperones (RPACs) are selectively translated. However, the molecular mechanism for such selective translational upregulation is unclear. Here, using yeast, we discover that remodelling of the actin cytoskeleton is important for RPAC translation following TORC1 inhibition. mRNA of the RPAC ADC17 is associated with actin cables and is enriched at cortical actin patches under stress, dependent upon the early endocytic protein Ede1. ede1Δ cells failed to induce RPACs and proteasome assembly upon TORC1 inhibition. Conversely, artificially tethering ADC17 mRNA to cortical actin patches enhanced its translation upon stress. These findings suggest that actin-dense structures such as cortical actin patches may serve as a translation platform for a subset of stress-induced mRNAs including regulators of proteasome homeostasis.

MRC Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee, UK.

✉ e-mail: arousseau@dundee.ac.uk

Identification of potential RPAC translation regulators. To better understand the mechanisms underlying selective RPAC translation, we established the FGH17 reporter system. The FGH17 reporter encodes two N-terminal Flag epitopes, a green fluorescent protein (GFP) and a C-terminal haemagglutinin tag (HA), under the control of the regulatory elements of the RPAC Adc17 (Fig. 1a). FGH17-containing cells had low basal levels of FGH17, which strongly increased following rapamycin (TORC1 inhibitor) treatment, as for the endogenous RPAC Nas6 (Fig. 1a). We additionally compared the behaviour of endogenous ADC17 mRNA with that of FGH17 to confirm that they share similar translation regulation. Using RiboTag18, we observed that rapamycin increased both the expression and translation of the endogenous ADC17 mRNA, which strongly increased following rapamycin (TORC1 inhibitor) treatment, as for the endogenous RPAC Nas6 (Fig. 1a). We additionally compared the behaviour of endogenous ADC17 mRNA with that of FGH17 to confirm that they share similar translation regulation. Using RiboTag18, we observed that rapamycin increased both the expression and translation of the endogenous ADC17 mRNA, which strongly increased following rapamycin (TORC1 inhibitor) treatment, as for the endogenous RPAC Nas6 (Fig. 1a).
Fig. 1 | Identification of proteins interacting with translating RPAC reporter mRNAs. a, Cartoon depicting the FGH17 reporter, consisting of tandem reporters expressed under control of ADC17 UTRs and western blot analysis of FGH17 expression in untreated cells or cells treated with 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as loading control. Empty vector, EV. b, mRNA levels of endogenous ADC17 and of FGH17 bound to ribosomes after 1.5 h rapamycin treatment compared with untreated cells. Analysis was performed by RiboTag immunoprecipitation (IP) followed by qRT-PCR and normalized to the housekeeping gene ALG9. Ribosome-bound mRNA corresponds to the level of RiboT ag IP mRNA normalized to the level of Input mRNA. Data are presented as mean ± s.d., n = 4, unpaired two-tailed Student’s t-test. c, Western blot analysis of WT and ADC17-70ntΔ untreated yeast cells or cells treated with 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as loading control. d, Cartoon depicting the proteomics experimental design. Step 1, cells were treated with 200 nM rapamycin for 1.5 h or were left untreated; step 2, ribosomes were locked on mRNAs by treating cells with 35 μM CHX; step 3, cells were treated with 1.2 J cm⁻² UV to covalently crosslink proteins to RNA; step 4, translating FGH17 mRNAs were immunoprecipitated; step 5, proteins bound to translating FGH17 mRNAs were recovered by RNase treatment before being subjected to quantitative proteomics (Step 6). e, Volcano plot showing the proteins that were differentially recovered from FGH17 and FGH17-70ntΔ immunoprecipitates. Each dot represents a protein. The red and blue dots are proteins significantly more and less bound to FGH17 mRNA compared with FGH17-70ntΔ mRNA, respectively. n = 5 biologically independent samples per condition; P values were determined by multiple unpaired two-tailed t-test. In a, c, d and f, n = 3 independent biological replicates.
(FGH17-70ntΔ) prevented translation (Fig. 1d). This was not due to alteration of the Kozak sequence, as re-introducing ADC17 Kozak sequence to FGH17-70ntΔ mRNA (FGH17-70ntΔ+Kozak) was not enough to restore FGH17 expression (Extended Data Fig. 1a). The 70-nucleotide region alone was not sufficient for FGH17 reporter expression (Extended Data Fig. 1b). Comparing FGH17 with FGH17-70ntΔ by RiboTag, we observed that the deletion of this 70-nt sequence prevented the recruitment of FGH17-70ntΔ mRNA to ribosomes upon rapamycin treatment (Fig. 1b,c) and decreased its stability by about twofold (Extended Data Fig. 1c). Deleting this 70-nt region at the endogenous ADC17 locus with clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9, we similarly observed abrogation of Adc17 expression (Fig. 1f). These findings indicated that the FGH17 reporter reflects the regulation of the endogenous ADC17 gene.

To discover new RPAC translation regulators, we identified RNA-binding proteins with increased recruitment to translating wild-type (WT) FGH17 mRNAs compared with non-translatable FGH17-70ntΔ mRNAs in vivo. To this end, we treated yeast cells with rapamycin to stimulate FGH17 translation (Fig. 1g, step 1). Polysomes were stabilized by adding cycloheximide (CHX) to the cells (Fig. 1g, step 2), and UV-crosslinking covalently linked the RNA and any bound proteins together (Fig. 1g, step 3). We next used anti-FLAG beads to immunoprecipitate translating FGH17 mRNA complexes where locked ribosomes had already synthesized one or both N-terminal FLAG tags (Fig. 1g, step 4). As FGH17-70ntΔ mRNAs are not translated, these samples should only immunoprecipitate proteins that bind non-specifically to the anti-FLAG beads. On the basis of the prediction that potential regulators of RPAC translation would be UV-crosslinked to translating FGH17 mRNA (Fig. 1g), we used RNases to specifically elute these potential regulators (Fig. 1g, step 5). We identified proteins in the RNase elution by tandem mass tag (TMT)-based quantitative proteomics (Fig. 1g, step 6). Quantitative analysis (Fig. 1h) revealed that two proteins, Ede1 and Cup1, were enriched in the WT compared with FGH17-70ntΔ samples. In contrast, two proteins were significantly depleted in the WT samples (Rps6a and Rps18b), suggesting they could be translational repressors (Fig. 1h). Taken together, these results identify a region of ADC17 mRNA essential for translation and discover potential regulators of selective RPAC translation.

Ede1 is important for RPAC induction upon TORC1 inhibition. Defects in RPAC regulation sensitize yeast to rapamycin. Therefore, to examine the involvement of identified proteins in RPAC regulation, we first tested knockout mutants for rapamycin sensitivity. Mutants of the ribosomal subunits Rps6a and Rps18b, which were less associated with translating FGH17 mRNA, were similarly sensitive to rapamycin as WT cells (Fig. 2a). Cup1 and Ede1 were found to be associated more with translating FGH17 mRNA, and while the cup1Δ mutant showed a similar level of sensitivity to rapamycin as WT cells, ede1Δ cells were highly sensitive (Fig. 2a).

We next tested whether these mutants were defective in rapamycin-induced RPAC expression. Unlike rps6aΔ, rps18bΔ and cup1Δ cells, ede1Δ cells were severely impaired in both Adc17 and Nas6 induction following rapamycin treatment (Fig. 2b), which, together with the rapamycin sensitivity, was rescued by re-introducing Ede1 (Extended Data Fig. 2a,b). TORC1-mediated Rps6 phosphorylation was still inhibited following rapamycin.
treatment in ede1Δ cells, indicating that Ede1 acts downstream of TORC1 inhibition (Extended Data Fig. 2c). A defect in RPAC induction following rapamycin treatment is expected to lead to a defect in increased proteasome assembly and activity\(^1\). Accordingly, ede1Δ cells failed to increase assembly and activity of the 26S proteasome, although there was an increase in 20S CPs (Fig. 2c).
Fig. 4 Ede1, Sla1 and Vrp1 are important for proteasome assembly and activity. a, Screen for rapamycin sensitivity with deletion strains covering all non-essential endocytic genes. Left: schematic representation of YEPD plates indicating the position of deletion strains. WT yeast was used as a control in the indicated positions. Right: yeast growth for 3 days on YEPD plate with or without 20 ng ml⁻¹ rapamycin from the indicated strains. Strains that failed to grow on rapamycin are indicated in coloured boxes in both the schematic and plate image. b, Western blot analysis of RPACs in WT and deletion strains that were untreated or treated with 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as a loading control. Asterisk indicates non-specific band. c, Frequency of ADC17 mRNAs undergoing translation in WT, sla1Δ and vps1Δ cells that were untreated or treated with 200 nM rapamycin (Rapa) for 3 h using the SunTag labelling method. Data are presented as mean ± s.d. n = 4 biologically independent experiments with 511 ADC17 mRNAs for each condition. Statistical analysis was carried out using two-way ANOVA i-test (Tukey multiple comparison test). d, Gradient Native PAGE (3.8–5%) of yeast total RNA from control and rapamycin-treated WT cells. CP, RPCP, RP2CP and Blm10-CP proteasome complexes are indicated. Rpt5 and 20S antibodies recognize the RP and the CP, respectively. In a, b and d, data are representative of three independent biological replicates.

defect is symptomatic of RP assembly defects and a hallmark of RPAC-deleted cells. Ede1 is therefore necessary for enhanced proteasome assembly following rapamycin treatment, by increasing the amount of RPACs available.

Ede1 interacts with ADC17 mRNA to regulate its translation. As Ede1 associates with translating FG11 mutant mRNA and is critical for proteasome homeostasis upon TORC1 inhibition, we predicted that Ede1 will be in contact with ADC17 mRNAs upon rapamycin treatment to play a role in their translation. To explore this possibility, P77 stem loops were introduced into the endogenous ADC17 mRNA, allowing it to be labelled with P77 bacteriophage coat protein (PCP) fused to mKate2 in cells expressing GFPEnvy-tagged Ede1 (Fig. 3a). We tracked single molecules of labelled ADC17 mRNA and observed frequent contact of Ede1 and ADC17 mRNA, demonstrating that this interaction is occurring in vivo (Fig. 3b and Supplementary Videos 1 and 2). Around 29% of ADC17 mRNAs were associated to Ede1 under basal conditions, and this significantly increased to about 40% following rapamycin treatment (Fig. 3c). This is consistent with a recent study that identified Ede1 as a potential RNA-binding protein. To confirm that Ede1 is regulating ADC17 mRNA at the level of translation, we employed the SunTag labelling method. This method uses the multimerization of Gcn4 epitope (SunTag) that, when translated, is recognized by multiple single-chain antibodies coupled to a fluorescent protein (scFv-mCherry), enabling quantitative visualization of the translation of individual mRNA molecules in living cells (Fig. 3d). We identified two populations of ADC17 mRNAs: those
co-localizing with SunTag signal that are translationally active (GFP and mCherry signal) (Fig. 3e, arrowheads 1 and 2) and those devoid of SunTag signal that are translationally inactive (GFP only) (Fig. 3e, arrowheads 3 and 4) (Supplementary Videos 3 and 4). On average, ~23% of ADC17 mRNAs were translationally active in untreated cells, increasing to ~40% in rapamycin-treated cells, attesting that ADC17 mRNA translation is increased upon TORC1 inhibition (Fig. 3f). This increased translation of ADC17 mRNA was lost in ede1Δ cells treated with rapamycin, confirming the importance of Ede1 for Adc17 translation regulation (Fig. 3f). Taken together, these results show that ADC17 mRNAs partially localize to Ede1 sites and demonstrate that Ede1 is critical for ADC17 mRNA translation upon TORC1 inhibition.

Ede1, Sla1 and Vrp1 are important for RPAC translation. Having established that Ede1 regulates RPAC translation, we investigated the underlying mechanism. Ede1 is an early coat protein involved in clathrin-mediated endocytosis (CME)24. To determine whether endocytosis is important for stress-mediated proteasome assembly, we tested if mutants of other non-essential proteins involved in CME mimicked the defects of ede1Δ cells. We initially screened mutants for increased rapamycin sensitivity, revealing six further proteins that were essential for growth on rapamycin (Fig. 3a). We examined whether these proteins were also involved in RPAC induction following rapamycin treatment. Chc1, Cdc1, Pah1 and Vps1 were dispensable for induction of Adc17 and Nas6 after rapamycin treatment; however, sla1Δ and vrp1Δ cells were severely impaired in RPAC induction (Fig. 3b). Like for ede1Δ cells, this was due to a translation defect, as observed using the SunTag labelling method (Fig. 3c). We next confirmed that sla1Δ and vrp1Δ cells had similar defects to ede1Δ in proteasome assembly and activity in response to rapamycin (Fig. 3d).

Actin remodelling affects ADC17 mRNA localization. Ede1 is one of the first proteins to be recruited to endocytic sites (Fig. 5a, step 1). Sla1 forms a heterodimeric complex with the actin nucleation promoting factor (NPF) Las17, which is recruited to endocytic patches via Sla1–Ede1 interaction (Fig. 5a, step 2). Vrp1 is recruited to the endocytic site by Las17 (Fig. 5a, step 3), and contributes to the recruitment and the activation of Myo3 and Myo5 that have both NPF and motor activities (Fig. 5a, step 4). NPFs further recruit and activate the actin nucleator complex Arp2/3 to initiate actin nucleation (Fig. 5a, step 5)28–30. As Ede1, Sla1 and Vrp1 localize to and regulate cortical actin patches at the endocytic site, and ADC17 mRNA makes contacts with Ede1, Sla1 and Vrp1 (Fig. 3c and Extended Data Fig. 3a,b), it seemed likely there might be a role for actin in ADC17 mRNA regulation. To test this, we fixed cells expressing endogenous PCP-GFP-labelled ADC17 mRNA and stained them with phalloidin to visualize actin. Yeast has two major actin structures: actin cables, which are polarized linear bundles of parallel actin filaments extending along the long axis of cells, and cortical actin patches, which are dense dendritic networks of branched actin filaments localized at the plasma membrane30. ADC17 mRNA was mainly seen to localize on actin cables (~70%), with the remainder either on cortical actin patches (~26.5%) or not associated with any phalloidin staining (~3.5%) (Fig. 5b,c). We next sought to examine whether ADC17 mRNA is associated with the actin cytoskeleton using live-cell imaging. Tagged Abp1 and Abp140 were used to visualize cortical actin patches and cables, respectively. We observed that ADC17 mRNA is often associated with actin cables in vivo (Fig. 5d and Supplementary Videos 5 and 6), while its interaction with patches is more transient and dynamic, as previously observed for Ede1 (Extended Data Fig. 3c and Supplementary Videos 7 and 8). Overall, these data might suggest movement of ADC17 mRNA along actin cables, although clear determination of direction and mechanism of movement remain to be determined.

The distribution of cortical actin patches and cables is polarized in budding yeast, with cortical actin patches being found almost exclusively in the bud, and cables being aligned longitudinally from the mother cell into the bud26. It has been reported that rapamycin depolarizes the actin cytoskeleton24, and we have shown that ADC17 mRNA is largely localized to actin structures (Fig. 5b–d). It is possible, therefore, that actin depolarization is a key step in RPAC induction. We first monitored the kinetics of actin depolarization after rapamycin treatment. Budding cells containing more than six cortical actin patches in the larger mother cell were considered to have a depolarized actin cytoskeleton, as previously described24–26. Rapamycin rapidly induced actin depolarization, peaking at 1 h and returning to near-normal levels at 4 h (Fig. 5e,f). RPAC induction coincides with actin depolarization, indicating that actin remodelling may relocate RPAC mRNAs to trigger their translation (Fig. 5g).

To test this possibility, we tracked ADC17 mRNA in cells stained for actin. Rapamycin treatment induced a shift of ADC17 mRNA localization from actin cables to patches, from 1 h (1.7-fold increase) onward compared with untreated cells (Fig. 5h). These results show that actin depolarization upon TORC1 inhibition relocates ADC17 mRNAs from actin cables to cortical actin patches, which could be important for its selective translation.

Actin disruption induces proteasome assembly and activity. We next tested whether an alternative means of selectively disrupting actin cables induced RPAC translation. Cells were therefore treated with 25 μM latrunculin B (Lat-B), which at this concentration only disrupts actin cables19, as observed upon rapamycin treatment. Lat-B treatment completely abolished actin cables and thereby relocated ADC17 mRNA to either cortical actin patches or to an unbound
state (Fig. 6a,b). Analysing the pathway regulating RPAC levels, we showed that Lat-B activates Mpk1 kinase (Fig. 6c). RPAC expression is induced straight after Mpk1 activation, as previously reported for rapamycin\textsuperscript{13} (Fig. 6c). This was further confirmed using genetic disruption of actin cables in the temperature-sensitive mutant act1-101 (Extended Data Fig. 4a,b). Moreover, actin nucleation at the surface...
of cortical actin patches was not required for RPAC translation. Latrunculin-A treatment, which disrupts actin patches as well as cables, had similar effects to that of Lat-B (Extended Data Fig. 4c–e). As the RPAC level mirrors the level of proteasome assembly, we monitored the impact of Lat-B on proteasome activity. In-gel peptidase assays showed that, as for rapamycin, Lat-B is a potent inducer of proteasome assembly and activity (Fig. 6d), attesting that actin remodelling regulates proteasome homeostasis.

**Ede1-tethered ADC17 mRNAs are more translated upon stress.** Having found that Lat-B induces RPAC expression, we sought to determine whether Ede1 is required for this process, as for rapamycin. Despite actin becoming depolarized after Lat-B treatment (Fig. 7a), RPACs were not induced in ede1 Δ cells (Fig. 7b). This result shows that Ede1 plays a role downstream of actin depolarization, possibly by stabilizing ADC17 mRNAs at cortical actin patches. While rapamycin treatment induced a shift of ADC17 mRNA localization from actin cables to cortical actin patches in WT cells, this re-localization was lost in ede1 Δ cells (Fig. 7c). This confirms Ede1 helps stabilize ADC17 mRNA at cortical actin patches following TORC1 inhibition.

If stabilization of ADC17 mRNAs at Ede1 sites is important for RPAC translation, artificially targeting ADC17 mRNA to this location may impact its translation levels upon TORC1 inhibition. Therefore, we set out to establish a targeting system in which Ede1 is localized (Fig. 7d). Using a doubly tagged version of Ede1 (Ede1-tdimer2-aGFP), we also demonstrated that the PCP-GFP proteins are co-localizing with Ede1 proteins (Extended Data Fig. 5a,b). In this system, Ede1 sites are decorated with PCP-GFP and, consequently, all PCP-GFP dots do not correspond to one ADC17 mRNA molecule. Because of this, we validated that ADC17 mRNAs are indeed tethered to Ede1 sites using a doubly tagged version of
ADC17 mRNA (Adc17-PP7SL-MS2SL). We observed that ADC17 mRNAs are robustly tethered to Edel-aGFP/PCP-GFP sites, indicating that our mRNA targeting system is efficient (Extended Data Fig. 5c,d). Moreover, the tethering of ADC17 mRNA to Edel sites had no impact on its stability (Extended Data Fig. 5e). We thus used this system to monitor the impact of artificially tethering ADC17
Fig. 7 | Tethering of ADC17 mRNA to actin patches enhances its translation upon stress. a, Representative microscopy images (maximum-intensity Z-projection) of WT and ede1Δ cells treated with 25 μM Lat-B for 1 h and stained for actin (hot red LUT). Scale bars, 3 μm. n = 3 biologically independent experiments. b, Western blot analysis of RPACs in WT and ede1Δ cells that are untreated or treated with 25 μM Lat-B for 3 h. Ponceau S staining was used as loading control. n = 3 biologically independent experiments. c, Frequency of ADC17 mRNA bound to actin cable, cortical actin patch or not associated to actin in WT and ede1Δ cells that are untreated or treated with 200 nM rapamycin (Rapa) for 1 h. Data are presented as mean ± s.d. n = 5 biologically independent experiments (n = 212 ADC17 mRNAs for each condition). d, Schematic representation of the system used to artificially tether ADC17 mRNA to Edel1-aGFP, nanobody against GFP. e, Representative microscopy images of yeast cells containing PCP-GFP-labelled ADC17 mRNA (green) and expressing either WT Edel1 or Edel1 tagged with a nanobody against GFP (Edel1-aGFP). Scale bars, 3 μm. n = 3 biologically independent experiments. f, Western blot analysis of RPACs in cells shown in e that are untreated or treated with 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as loading control. n = 4 biologically independent experiments. g, Quantification of Adc17 protein level from experiments represented in f. Data are presented as mean ± s.d. n = 4 biologically independent experiments. h, Western blot analysis of RPACs in the indicated cells that are untreated or treated with 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as loading control. n = 5 biologically independent experiments. i, Quantification of Adc17 protein level from experiments represented in h. Data are presented as mean ± s.d. n = 5 biologically independent experiments. j, Western blot analysis of RPACs in the indicated cells that are untreated or treated with 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as loading control. n = 5 biologically independent experiments. k, Quantification of Adc17 protein level from experiments represented in j. Data are presented as mean ± s.d. n = 5 biologically independent experiments. In c, g, i and k, two-way ANOVA t-test (Tukey multiple comparison test).

mRNAs to Edel1 sites on their translation level. We observed that Adc1 induction upon rapamycin treatment was increased around twofold when tethered to Edel1-aGFP/PCP-GFP compared with untethered mRNAs, indicating that recruitment of ADC17 mRNA to Edel1 sites is important for its translation regulation (Fig. 7f,g). As NAs6 mRNA does not possess the PP7 stem loops, its induction by rapamycin was unaffected by Edel1-aGFP (Fig. 7f).

As Edel1 localizes to cortical actin patches, its main function in regulating RPAC translation could be to stabilize RPAC mRNAs at these sites. Therefore, we artificially tethered ADC17 mRNA to the cortical patch marker Abp1 and monitored RPAC levels. We observed that Adc1 induction upon rapamycin treatment was increased by 1.84-fold when tethered to Abp1 (Abp1-mK-aGFP) compared with untethered mRNAs (Abp1), which is similar to that of Edel1 targeting (Fig. 7h,i). When ADC17 mRNA was targeted to cortical actin patches in ede1Δ cells, Adc1 induction upon rapamycin was restored to WT levels, indicating that an important function of Edel1 is to recruit ADC17 mRNA to cortical actin patches (Fig. 7j,k). Taken together, these results show that Edel1-mediated recruitment of ADC17 mRNAs at cortical actin patches following rapamycin treatment is important for stimulating Adc1 translation.

Discussion

Local mRNA translation has been described as important for various processes, including development, cell migration, stress resistance and neuron function. Yeast mRNAs have been reported to localize to the bud tip, the endoplasmic reticulum (ER), mitochondria and cortical actin patches, where they have either been shown, or are predicted, to be locally translated. The most well characterized of these is the ASH1 mRNA, which is transported along the actin cytoskeleton in a translationally repressed state to the bud tip, where it is anchored, and translated activated. It is possible that a similar mechanism is responsible for RPAC induction. In this scenario, RPAC mRNA is transported along actin cables in a translationally repressed state. When actin cables are disrupted by stress, ADC17 mRNA re-localizes to Edel1 sites and translation inhibition is relieved. Likewise, in human cells, mRNAs have been reported to localize to the ER, mitochondria, distal parts of neurons and actin-dense structures such as focal adhesions, which are somewhat akin to cortical actin patches. The localization of certain mRNAs has been shown to be dependent on F-actin, while other mRNAs are transported along microtubules. We have shown that ADC17 RPAC mRNA may be transported on actin cables and interacts with cortical actin patches. Upon rapamycin and Lat-B treatment, which respectively weaken and remove actin cables, the mRNA is stabilized at cortical actin patches. This re-localization of RPAC mRNA allows increased RPAC translation and, ultimately, proteasome assembly in the presence of Edel1, Sla1 and Vrp1.

As cortical actin patches have a higher density of F-actin and are less sensitive to stress than actin cables, they may serve directly as a stress platform or indirectly by recruiting mRNA to a translationally active cellular compartment, helping to translate stress-induced proteins such as RPACs. In agreement with this possibility, it has recently been reported that Edel1 localizes to both cytosolic and ER-targeted proteins. These observations have been reported in diverse organisms and using different methodologies, suggesting that the ER is a favourable environment for translation. Furthermore, ER-localized mRNA translation is less inhibited by stress than their cytosolic counterparts, suggesting the ER represents a protective environment for translation upon stress, allowing the cell to synthesize a specific set of proteins under these conditions. This would support a model in which the role of Edel1 in RPAC translation upon stress may be to recruit their mRNAs to cortical actin patches, so they are near ER-associated ribosomes for translation. As the ER has been reported to be an important site for 20S proteasome assembly, it is possible to imagine that proteasome components are co-translationally assembled at the surface of the ER membrane.

Multiple stresses impact upon the actin cytoskeleton, leading it to be proposed as a biosensor for detecting stress. In this work, we showed that rapamycin and Lat-B treatment perturb the actin cytoskeleton and re-localize a stress-responsive mRNA to cortical actin patches. This could potentially allow local translation of the mRNA throughout the mother cell, helping it to cope with the stress before resuming cell growth. While it is becoming clear that local and selective translation is crucially important in regulating cellular functions, less is known about how it is regulated under stress. This work illustrates that actin remodelling controls mRNA localization and translation under stress, helping to adapt the proteome to environmental and physiological challenges. As perturbation of the actin cytoskeleton has been associated with various human diseases such as cancer and autoimmunity, it will be important to better understand the impact of actin cytoskeleton remodelling in controlling selective translation under pathophysiologival conditions.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplemental information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of
data and code availability are available at https://doi.org/10.1038/s41556-022-00938-4.

Received: 29 July 2021; Accepted: 10 May 2022; Published online: 23 June 2022

References

1. Hipp, M. S., Kasturi, P. & Hartl, F. U. The proteostasis network and its decline in aging. Nat. Rev. Mol. Cell Biol. 20, 421–435 (2019).

2. Labbadia, J. & Morimoto, R. I. The biology of proteinostasis in aging and disease. Annu. Rev. Biochem. 84, 435–464 (2015).

3. Pillai, E., Schneider, K. & Bertolotti, A. Coping with protein quality control failure. Annu. Rev. Cell Dev. Biol. 33, 439–465 (2017).

4. Rousseau, A. & Bertolotti, A. Regulation of proteasome assembly and activity in health and disease. Nat. Rev. Mol. Cell Biol. 19, 697–712 (2018).

5. Dikic, I. Proteasomal and autophagic degradation systems. Annu. Rev. Biochem. 86, 193–224 (2017).

6. Yu, H. & Matouschek, A. Recognition of client proteins by the proteasome. Annu. Rev. Biophys. 46, 149–173 (2017).

7. Budenholzer, L., Cheng, C. L., Li, Y. & Hochstrasser, M. Proteasome structure and assembly. J. Mol. Biol. 429, 3500–3524 (2017).

8. Ben-Sahra, I. & Manning, B. D. mTORC1 signaling and the metabolic control of cell growth. Curr. Opin. Cell Biol. 45, 72–82 (2017).

9. González, A. & Hall, M. N. Nutrient sensing and TOR signaling in yeast and mammals. EMBO J. 36, 3976–3997 (2017).

10. Saxton, R. A. & Sabatini, D. M. mTOR signaling in growth, metabolism, and disease. Cell 168, 960–976 (2017).

11. Noda, T. Regulation of autophagy through TORC1 and mTORC1. Biomolecules 7, 52 (2017).

12. Zhao, J., Zhai, B., Gygi, S. P. & Goldberg, A. L. mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. Proc. Natl Acad. Sci. USA 112, 15790–15797 (2015).

13. Rousseau, A. & Bertolotti, A. An evolutionarily conserved pathway controls proteasome homeostasis. Nature 536, 184–189 (2016).

14. Suraweera, C., Münch, C., Hanssum, A. & Bertolotti, A. Failure of amino acid homeostasis causes cell death following proteasome inhibition. Mol. Cell 48, 242–253 (2015).

15. Vabulas, R. M. & Hartl, F. U. Protein synthesis upon acute nutrient restriction relies on proteasome function. Science 310, 1960–1963 (2005).

16. Torres, J., Di Como, C. J., Herrero, E. & de la Torre-Ruiz, M. A. Regulation of the cell integrity pathway by rapamycin-sensitive TOR function in budding yeast. J. Biol. Chem. 277, 43495–43504 (2002).

17. Waite, K. A., Burris, A., Vontz, G., Lang, A. & Roelofs, J. Proteaphagy is specifically regulated and requires factors dispensable for general autophagy. J. Biol. Chem. 298, 101494 (2022).

18. Sanz, E. et al. Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. Proc. Natl Acad. Sci. USA 106, 13939–13944 (2009).

19. Pan, T. Adaptive translation as a mechanism of stress response and adaptation. Ann. Rev. Genet. 47, 121–137 (2013).

20. Salazar, M. J. et al. Widespread mRNA association with cytoskeletal motor proteins and identification and dynamics of myosin-associated mRNAs in S. cerevisiae. PLoS ONE 7, e31912 (2012).

21. Medina-Munoz, H. C., Lapointe, C. P., Porter, D. F. & Wickens, M. Records of RNA locations in living yeast revealed through covalent marks. Proc. Natl Acad. Sci. USA 117, 23539–23547 (2020).

22. Mulet, J. M., Martin, D. E., Loewith, R. & Hall, M. N. Mutual antagonism of target of rapamycin and calcium/calcineurin signaling. J. Biol. Chem. 281, 33000–33007 (2006).

23. Jezouk, J. E., Howell, A. S., Thesfeld, C. L. & Lew, D. J. Opposing roles for actin in Cdc42 polarization. Mol. Biol. Cell 16, 1296–1304 (2005).

24. Su, K.-H. & Dai, C. mTORC1 senses stresses: coupling stress to proteostasis. BioEssays News Rev. Mol. Cell. Dev. Biol. 39, 1600268 (2017).

25. Medioni, C., Mowry, K. & Besse, F. Principles and roles of mRNA localization in animal development. Dev. Camb. Engl. 139, 3263–3276 (2012).

26. Herbert, S. P. & Costa, G. Sending messages in moving cells: mRNA localization and the regulation of cell migration. Essays Biochem. 63, 595–606 (2019).

27. Gock, C., Heunmüller, M. & Schuman, E. M. mRNA transport & local translation in neurons. Curr. Opin. Neurobiol. 45, 169–177 (2017).

28. Kraut-Cohen, J. et al. Translation- and SRP-independent mRNA targeting to the endoplasmic reticulum in the yeast Saccharomyces cerevisiae. Mol. Biol. Cell 24, 3069–3084 (2013).

29. Katz, Z. B. et al. β-Actin mRNA compartmentalization enhances actin messenger RNA. Mol. Biol. Cell 26, 221–231 (2015).

30. Lesnik, C., Golani-Armon, A. & Arava, Y. Localized translation near the mitochondrial outer membrane: an update. RNA Biol. 12, 801–809 (2015).

31. Rangaraju, V. & Schuman, E. M. Local translation in neuronal compartments: how local is local? EMBO Rep. 18, 693–711 (2017).

32. Vedula, P. et al. Different translation dynamics of β- and γ-actin regulate cell migration. Life 10, e68712 (2021).

33. Sundell, C. L. & Singer, R. H. Requirement of microfilaments in sorting of actin messenger RNA. Science 253, 1275–1277 (1991).

34. Katz, Z. B. et al. β-Actin mRNA compartmentalization enhances focal adhesion stability and directs cell migration. Genes Dev. 26, 1855–1890 (2012).

35. Scholz, D. et al. Microtubule-dependent distribution of mRNA in adult cardiocytes. Am. J. Physiol. Heart. Circ. Physiol. 294, H1135–H1144 (2008).

36. Willing, F. et al. A selective autophagy pathway for phase-separated endocytic protein deposits. Mol. Cell 80, 764–778 e7 (2020).

37. Priebe, B., Heinik, S., Steffen, I., Kloetzel, P.-M. & Krüger, E. The proteasome maturation protein POMP facilitates major steps of 20S proteasome formation at the endoplasmic reticulum. EMBO Rep. 8, 1170–1175 (2007).

38. Smethurst, D. G. J., Dawes, I. W. & Gourlay, C. W. Actin—a biosensor that adapts translation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/. © The Author(s) 2022.
Methods

Yeast strains, plasmid and growth conditions. Yeast strains and plasmids used in this study are listed in Supplementary Table 1. All plasmids during this study were made with In-Fusion HD Cloning Plus (Takara, 638999). All yeast strains are iso-1 and anti-Act17 from Bertolotti laboratory were used in all experiments except for Figs. 1f and 7d and Extended Data Fig. 4b, where sheep anti-Act17 antibody was used instead owing to stock availability. Anti-mouse IgG, HRP-linked antibody (Cell Signaling Technology; 1:10,000; #7076) and anti-rabbit IgG, HRP-linked antibody (Cell Signaling Technology; 1:10,000; #7074).

Protoplasts assay. Yeast was grown in YEPD medium overnight at 30 °C, then diluted to OD<sub>600nm</sub> 0.2 in 30 ml YEPD, grown at 32 °C to OD<sub>600nm</sub> 0.5–0.7 and then diluted back to OD<sub>600nm</sub> 0.2. Treatments were then performed (30 ml with 200 μM Lat-B, 20 ml with 25 μM Lat-B and 20 ml untreated control) and cells returned to 32 °C for 3 h. Then, 15 OD<sub>600nm</sub> of cells were spun down (3,200 × g, 4 min), resuspended in 800 μl ice-cold water, transferred to a 2 ml tube and spun down again (6,200 g, 30 s, 4 °C). The pellet was resuspended in 300 μl native lysis buffer (50 mM Tris pH 8, 5 mM MgCl<sub>2</sub>, 0.5 mM EDTA, 5% glycerol, 1 mM DTT and 5 mM ATP) and lysed with 250 μl acid-washed beads (Sigma, G-8772) (Bio-Rad, MP; 3 × 30 s on, 4 °C). Beads and cell debris were removed by centrifugation (17,000 g, 2 min, 4 °C), the supernatant was transferred to a fresh tube and centrifuged again (17,000 g, 10 min, 4 °C). Protein concentration was determined on a NanoDrop (A<sub>260</sub>, Thermo) and standardized between samples. Then, 75 μg protein in 1x native sample buffer (50 mM Tris–HCl pH 6.8, 10% glycerol and 0.01% bromophenol blue) was loaded onto a 15 cm 3.8–5% acrylamide gradient native gels (prepared in duplicate as for the western blot gels (above), using 10 ml solutions of 90 mM Tris, 90 mM boric acid, 1 mM MgCl<sub>2</sub>, 0.12% APS and 0.12% TEMED with acrylamide added to either 3.8% or 5%). Gels were run for 2.5 h, at 110 V and 4 °C, in ice-cold native running buffer (9.9 T). After transfer to a nitrocellulose membrane (Bio-Rad), the membrane was incubated in 15 ml antibody buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 5 mM MgCl<sub>2</sub> and 10% glycerol) containing 100 μl 10 μM suc-LVLY-AMC fluorogenic substrate (Cambridge Biosciences, 4011369), 30 °C in the dark for 15–20 min and imaged using a ChemiDoc Touch imaging system (Bio-Rad). To image CP assembly, SDS was added to the antibody buffer at a final concentration of 0.05% and the gel left to incubate for 15 min before imaging again. Gels were transferred onto 0.2 μm nitrocellulose membrane (Bio-Rad; 1620112) using a TransBlot Turbo (Bio-Rad) for western blot analysis.

Fluorescence microscopy. Yeast was grown on YEPD plates overnight at 30 °C, resuspended to OD<sub>600nm</sub> 0.2 in YEPD medium and grown at 30 °C to OD<sub>600nm</sub> 0.5–0.7. Cultures were split into 4 ml samples, rapamycin (200 mM final) or Lat-B (250 μM final) was added and samples were returned to 30 °C for the indicated time. Formaldehyde (Sigma Aldrich; F8775) was then added to 3.7%, and the sample returned to 30 °C for 30 min. Samples were spun down (3,200 g, 4 min), washed twice with 7 ml PBS, transferred to a 1.5 ml tube, spun down (7,800 g, 2 min), resuspended in 100 μl PBS/0.1% Triton X-100 containing 1:10,000 dilution of rhodamine phallidin (Abcam; ab255138) or phallidin-iFluor 647 (Abcam; ab176759) and incubated at 4 °C on a Stuart SB3 vertical rotator in the dark. After 1 h, samples were spun down as before, washed in 1 ml PBS and resuspended in 10 μl ProLong Glass antifade (Thermo Fisher, P36980). Then, 3–4 μl was mounted on a SuperFrost microscope slide (VWR; 631-0847), covered with a glass cover slip (VWR; 631-0119) and cured in the dark overnight before imaging on a Zeiss 880 Airyscan microscope (Airyscan mode, Alpha Plan-AP 100X/1.40 DIC VIS objective (Zeiss)) and Zen 2.3 SP1 FP software was used to acquire images. For the quantification of mRNAs per cell, actin cables (linear structures, twofold brighter than cell background) and no mRNAs (circular punctae, >30% brighter than the local cell background) were detected and assayed for co-localization using the ComDet v0.5.1 plugin with the standard settings. All detected particles were manually checked. For quantification of co-localization of red (translating mRNA) and green (all mRNA) puncta in SunTag experiments, the ComDet v0.5.1 plugin was used as above. ADC17 mRNA interaction with actin in fixed cells was analysed by performing a maximum-intensity Z-projection, then the number of PP7-GFP-labelled ADC17 mRNAs (circular punctae, >0.3 μm diameter, >50% brighter than the local cell background) were detected in cortex with cortical actin patches (circular punctae, >0.5 μm diameter, twofold brighter than actin cables), actin cables (linear structures, twofold brighter than cell background) and no mRNA interaction (ADC17 punctae) was counted manually. Quantified maximum-intensity projections were again performed and budding cells with more than six cortical actin patches in the larger mother cell were counted as depolarized, while those with six or fewer were counted as polarized. To quantify
mRNAs per cell, a standard deviation Z-projection was performed and ComDet v0.5.1 was used to detect mRNAs (MCP-mCherry), while cells were counted manually.

**RiboTag RNA isolation.** Rpl10-GFP yeast expressing either FGH17 or FGH17-70ntΔ was grown in YEPD medium overnight at 30°C, then diluted to OD600nm 0.5 in 50 ml YEPD medium and grown at 30°C to OD600nm ~1 and diluted back to OD600nm 0.5 before being treated with 200 nM rapamycin for 1.5 h at 30°C, or remaining untreated. After treatment, polysomes were stabilized by washing cells in 20 ml cold HCl-containing 50 mM ammonium bicarbonate (90 min with shaking before quenching with 1 μl of 5% hydroxylamine, after which 1 μl of labelled sample from each channel was analysed by liquid chromatography with tandem mass spectrometry (LC–MS/MS) to ensure complete labelling before reporting. Data were normalized by the 1-chamber reference channel and transcription levels were calculated for each reporter ion from each peptide spectral match.Reporter ion abundance (S/N) values were used to represent the reporter ion abundance for the normalization. Protein ratios were calculated from medians of summed reporter ion abundances (S/N) values. The significance of the identified differences was determined by the two-tailed unpaired t-test. Each experiment was repeated independently a minimum of five times. No data were excluded from the analyses, and the experiments were not randomized. The investigators were not blinded to allocation during conduct or reporting. Further details are available in the Methods and the data underlying the results presented in this paper have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE101317.
**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All the data generated or analysed during the current study are included in this published article and its supplementary files (Supplementary Information and source data). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD027655. All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

**References**

52. Janke, C. et al. A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast* **21**, 947–962 (2004).

**Acknowledgements**

We thank A. Bertolotti for the gift of Ade17 antibody. We thank the MRC PPU reagents and services, including Cloning team and DNA sequencing services, MRC-PPU Mass Spectrometry and the Dundee Imaging facility. We thank K. Labib and D. Alessi for reading and discussing the manuscript. This research was supported by the Medical Research Council (grant number MC_UU_00018/8 to A.R.).

**Author contributions**

A.R. and T.W. designed experiments and wrote the manuscript. A.R., T.W., R.C., A.A., A.B. and H.Z. performed experiments and analysed data. All authors edited the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41556-022-00938-4.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41556-022-00938-4.

Correspondence and requests for materials should be addressed to Adrien Rousseau.

Peer review information Nature Cell Biology thanks Ivan Topisirovic, and the other, anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | Characterisation of the region important for FGH17 regulation. a, Schematic showing the introduction of the ADC17 5’UTR Kozak sequence into the FGH17-70ntΔ vector. Western blot analysis showing the impact on FGH17 levels in cells treated ± 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as a loading control. b, FGH17 vectors with the full 5’UTR, the 5’UTR lacking the 70 nucleotides upstream of the start codon (FGH17-70ntΔ) or containing only the 70 nucleotides upstream of the start codon (FGH17-70nt only). Western blot analysis showing the impact on FGH17 levels in cells treated ± 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as a loading control. c, Relative abundance of FGH17 and FGH17-70ntΔ mRNA (mRNA of interest normalised to ALG9 housekeeping mRNA) in yeast cells. Data are presented as mean ± s.d., n = 4 biologically independent experiments. Statistical analysis was carried out using unpaired two-tailed Student’s t-test. a, b, Data are representative of three independent biological replicates.
Extended Data Fig. 2 | Ede1 regulates RPAC levels downstream of TORC1 inhibition. a, Cells spotted in a fivefold dilution and grown for 3 days on plates ± 20 ng/ml rapamycin. b, Western blot analysis of RPACs in WT and ede1Δ cells treated ± 200 nM rapamycin (Rapa) for 4 h. Ponceau S staining was used as a loading control. c, Western blot analysis of RPACs and P-Rps6 in WT and ede1Δ cells treated ± 200 nM rapamycin (Rapa) for the indicated time. Ponceau S staining was used as a loading control. a-c, Data are representative of three independent biological replicates.
Extended Data Fig. 3 | ADC17 mRNA interacts with cortical actin patch proteins. a, Frequency of ADC17 mRNAs colocalizing with Sla1-mKate2 in cells grown for 3 h ± 200 nM rapamycin (Rapa). Untreated (UT). Data are presented as mean ± s.d., n = 4 biologically independent experiments. (n = 529 ADC17 mRNAs per condition). Statistical analysis was carried out using unpaired two-tailed Student’s t-test. b, Frequency of ADC17 mRNAs colocalizing with Vrp1-mKate2 in cells grown for 3 h ± 200 nM rapamycin (Rapa). Untreated (UT). Data are presented as mean ± s.d., n = 4 biologically independent experiments. (n = 408 ADC17 mRNAs per condition). Statistical analysis was carried out using unpaired two-tailed Student’s t-test. c, Representative single frames from time-lapse imaging showing contacts between Abp1-mKate2 (red) and PCP-GFP-labelled ADC17 mRNA (cyan). Scale bars, 1 μm. n = 3 biologically independent experiments.
Extended Data Fig. 4 | Genetic and chemical disruptions of actin induces RPAC levels. Representative microscopy images (maximum intensity Z-projection) of WT (ACT1) and act1-101 cells either grown at the permissive temperature (25 °C) or shifted to the non-permissive temperature (37 °C) for 4 h and stained with Rhodamine phalloidin to visualise actin (hot red LUT). Scale bars, 3 μm. n = 3 biologically independent experiments. b, Western blot analysis of RPACs in WT and act1-101 cells either grown at the permissive temperature (25 °C) or shifted to the non-permissive temperature (37 °C) for 4 h. Ponceau S staining was used as a loading control. n = 3 biologically independent experiments. c, Representative microscopy images (maximum intensity Z-projection) of WT cells treated or not with either 25 μM Latrunculin-B or 12.5 μM Latrunculin-A for 3 h and stained for actin (hot red LUT). Scale bars, 3 mm. n = 3 biologically independent experiments. d, Frequency of ADC17 mRNAs colocalising with Ede1-timer2 in cells treated with either 25 μM Latrunculin-B (Lat-B) or 12.5 μM Latrunculin-A (Lat-A) for 3 h. Data are presented as mean ± s.d., n = 4 biologically independent experiments (>500 ADC17 mRNAs per condition). Statistical analysis was carried out using unpaired two-tailed Student’s t-test. ns not significant. e, Western blot analysis of RPACs and Mpk1 kinase in WT cells treated or not with either 25 μM Latrunculin-B (Lat-B) or 12.5 μM Latrunculin-A (Lat-A) for 3 h. Ponceau S staining was used as a loading control. n = 3 biologically independent experiments.
Extended Data Fig. 5 | Validation of ADC17 mRNA tethering to Ede1. 

**a.** Schematic representation of PCP-GFP recruitment to Ede1 fused with tdimer2 and aGFP (nanobody against GFP). 

**b.** Representative microscopy images of yeast cells containing PCP-GFP (green) and Ede1-tdimer2 (magenta) tagged with a nanobody against GFP (Ede1-tdimer2-aGFP). Scale bars, 3 μm. 

**c.** Schematic representation of doubly tagged ADC17 mRNA (magenta) recruitment to Ede1-aGFP/PCP-GFP complex. 

**d.** Representative microscopy images of yeast cells expressing PCP-GFP (green), MCP-mCherry (magenta), Ede1 tagged with a nanobody against GFP (Ede1-aGFP) and ADC17 mRNA containing PP7-stem-loops (PP7SL) and MS2-stem-loops (MS2SL) which are recognised by PCP-GFP and MCP-mCherry, respectively. Scale bars, 3 μm. 

**e.** Quantification of the number of ADC17 mRNAs per cell in adc17Δ Ede1 WT and Ede1-aGFP cells expressing ADC17 mRNA containing MS2 and PCP stem loops, PP7-GFP and MCP-mCherry grown for 2 h ± 200 nM rapamycin (Rapa). Untreated (UT). mRNAs were quantified using MCP-mCherry as a marker. Data are presented as mean ± s.d., n = 4 biologically independent experiments. (n = 261 ADC17 mRNAs per condition). Statistical analysis was carried out using two-way ANOVA t-test (Tukey multiple comparison test). ns, not significant.
Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

Number of all variances tested

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- A description of any covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  *Give P values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

Software and code

| Policy information about | availability of computer code |
|-------------------------|-------------------------------|
| Data collection         | ZEN 2.3 SP1 FP3 version 14.0.21.201, Proteome Discoverer software v.2.2, Chemidoc Touch imaging system, Zeiss LSM 880 Airyscan microscope, CFX384 Real-Time PCR Detection system, and Orbitrap Fusion Lumos mass spectrometer. |
| Data analysis           | Images were acquired with Zeiss LSM 880 AiryScan confocal microscope. Images taken by Zeiss confocal microscope were analysed using ZEN 2.3 SP1 FP3 black software (version 14.0.21.201). For quantifications of confocal images and Western Blot, Fiji imagej 2.1.0/1.53c software was used. For quantification of colocalization of red (translating mRNA) and green (all mRNA) puncta in SunTag experiments, the ComDet v0.5.1 plugin was used. Proteomic data acquired by the Orbitrap Fusion Lumos mass spectrometer were analysed with Proteome Discoverer software v.2.2 with Mascot search engine. Gene expression data collected by the CFX384 Real-Time PCR Detection system was analysed with Bio-Rad CFX Maestro 2.0 (Version 5.0.021.0616). All statistics were performed using Graph Pad Prism 9 software (Version 9.1.2) (Graph Pad Software Inc., La Jolla, CA). |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.
Data

Policy information about availability of data.
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy.

All the data generated or analysed during the current study are included in this published article and its supplementary files. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD027655.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical test or power analysis were performed to predetermine sample size. We defined sample sizes based on routine practice in the similar studying fields. Experiments were repeated three or more time, as indicated.

Data exclusions
No data were excluded from the manuscript.

Replication
All experiments were replicated at least three time with similar findings. Sample sizes are provided in each figure legend.

Randomization
All images were acquired randomly.

Blinding
No blinding was use for this study. Experiments and data analyses were performed by the same investigator.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study | Antibodies | Eukaryotic cell lines | Palaeontology and archaeology | Animals and other organisms | Human research participants | Clinical data | Dual use research of concern |
|-----|----------------------|------------|----------------------|-----------------------------|-----------------------------|-----------------------------|---------------|-----------------------------|

Methods

| n/a | Involved in the study | ChIP-seq | Flow cytometry | MRI-based neuroimaging |
|-----|----------------------|---------|---------------|------------------------|

Antibodies

Antibodies used
Anti-Adc17 (Bertolotti) laboratory; Rabbit, 1:1000; anti-Adc17 (Abber); Sheep, 1:250; DU66321; Fig. 7(h), anti-Nas6 (Abcam; Rabbit, 1:2000; ab91447), anti-Flag (Sigma Aldrich; Mouse, 1:2000; F3165), anti-Rps5 (Enzo life sciences; Rabbit, 1:5000; PW8245), anti-25S (Enzo life sciences; Rabbit, 1:2000; PW9353), anti-Mpk1 (Santa Cruz; Mouse, 1:5000; sc-374434), anti-p-Mpk1 (Cell Signalling Technology; Phospho-p44/42, Rabbit; 1:1000; 9101) and anti-p-Rods (Cell Signalling Technology; Rabbit, 1:1000; 2211), Anti-mouse IgG, HRP-linked Antibody (Cell Signalling Technology, 1:10000; 7076) and Anti-rabbit IgG, HRP-linked Antibody (Cell Signalling Technology, 1:10000; 7074).

Validation
Validation statement and product literature references are available here:
Rabbit and sheep anti-Adc17 antibodies, rabbit anti-Nas6 antibody, mouse anti-Mpk1 and rabbit anti-p-Mpk1 antibodies have been validated using the respective knock out yeast strains. Each antibody gave a specific signal at the expected size in WT cells while the
signal was absent in a strain where the target has been knocked-out. Re-expressing the target on a vector restored the signal, confirming the specificity of detection.

Mouse anti-Flag antibody is a gold standard extensively used in science (https://www.sigmaaldrich.com/Gil/en/product/sigma/F3165?gclid=EAIaiaWQChl_Hjs9Hic71ygIEWEAYASAAEgiopvO_BwE)

Rabbit anti-Rpt5 (https://www.enzolifesciences.com/8ML-PW8245/proteasome-19s-rpt5-s6a-subunit-polycyclonal-antibody/)

Rabbit anti-20S (https://www.enzolifesciences.com/8ML-PW9355/proteasome-20s-core-subunits-polycyclonal-antibody/)

Rabbit anti-Nas6 antibody (https://www.abcam.com/nas6-antibody-ab91447.html)

Mouse anti-Mpk1 antibody (https://www.scbt.com/fr/p/mpk1-antibody-d-1)

Mouse anti-p-Mpk1 antibody [https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-antibody/s101] is extensively used in publications to monitor Mpk1 activation, including [Torres J et al., 2002; Liu L et al., 2018 and Sellers-Moya et al., 2021]

Rabbit anti-p-Rps6 antibody has been validated in yeast in these publications (Yerikaya et al., 2015 and Gonzalez A et al., 2015)

Anti-mouse IgG, HRP-linked Antibody (https://www.cellsignal.com/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076)

Anti-rabbit IgG, HRP-linked Antibody (https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

BY4741 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0] Horizon Discovery
BY4741 + FGH17 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416:FGH17::URA3]] This study
BY4741 + FGH17-5’UTRA [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416:FGH17-5’UTRA::URA3]] This study
BY4741 + FGH17-3’UTRA [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416:FGH17-3’UTRA::URA3]] This study
BY4741 + FGH17-40ntA [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416:FGH17-40ntA::URA3]] This study
BY4741 + FGH17-30ntA [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416:FGH17-30ntA::URA3]] This study
BY4741 + FGH17-23ntA [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416:FGH17-23ntA::URA3]] This study
rps5AΔ [MATa his3Δ1 met15Δ0 ura3Δ0 rps6Δ::LEU2] This study
rps18AΔ [MATa his3Δ1 met15Δ0 ura3Δ0 rps18Δ::LEU2] This study
ede1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ede1::kanMX] Horizon Discovery
cup1-128 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cup1-128::kanMX] Horizon Discovery
Adc1-124kPP7S1 + Pcp-mKate2 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC1-124kPP7S1::LoxP [pFA6:yc1p-PCP-mKate2::HIS3]] This study
Adc1-124kPP7S1 + Ede1-3xHA-GFPenv + Pcp-mKate2 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC1-24kPP7S1::LoxP EDE1-3xHA-GFPenv:KanMX [pFA6:yc1p-PCP-mKate2::HIS3]] This study
Adc1-12SunTag [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416:adc1p-Cdc17-12SunTag [24k]-PP7S1 [24k]:::URA3 + pFA6:yc1p-PCP-EGFP [24k]:::yc1p-scF-VGCF4::mCherry::HIS3]] This study
Adc1-12SunTag ede1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ede1::kanMX [p416:adc1p-Cdc17-12SunTag [24k]-PP7S1 [24k]:::URA3 + pFA6:yc1p-PCP-EGFP [24k]:::yc1p-scF-VGCF4::mCherry::HIS3]] This study
syplΔ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sypl::kanMX] Horizon Discovery
dcl1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dcl1::kanMX] Horizon Discovery
chc1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 chc1::kanMX] Horizon Discovery
pap1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pap1::kanMX] Horizon Discovery
yap180Δ1 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 yap180::kanMX] Horizon Discovery
yap180Δ2 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 yap180::kanMX] Horizon Discovery
alg1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 alg1::kanMX] Horizon Discovery
alg13Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 alg1::kanMX] Horizon Discovery
aps2Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 aps2::kanMX] Horizon Discovery
app4Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 app4::kanMX] Horizon Discovery
ent1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ent1::kanMX] Horizon Discovery
ent2Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ent2::kanMX] Horizon Discovery
end3Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 end3::kanMX] Horizon Discovery
sia1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sia1::kanMX] Horizon Discovery
lsb3Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lsb3::kanMX] Horizon Discovery
lsb4Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lsb4::kanMX] Horizon Discovery
lsb5Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lsb5::kanMX] Horizon Discovery
ubx3Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ubx3::kanMX] Horizon Discovery
gts1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gts1::kanMX] Horizon Discovery
lbd1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lbd1::kanMX] Horizon Discovery
bbc1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bbc1::kanMX] Horizon Discovery
aim1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 aim1::kanMX] Horizon Discovery
ub70Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ub70::kanMX] Horizon Discovery
bz1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bz1::kanMX] Horizon Discovery
vpr1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vpr1::kanMX] Horizon Discovery
myo3Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 myo3Δ::kanMX] Horizon Discovery
myo5Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 myo5Δ::kanMX] Horizon Discovery
rsv161Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rsv161Δ::kanMX] Horizon Discovery
rsv167Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rsv167Δ::kanMX] Horizon Discovery
vps1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vps1Δ::kanMX] Horizon Discovery
Adcd124ΔPP7SL + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Abp1-3Kha-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Abp1-3Kha-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL ede1Δ + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Ede1-4GFP + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
eded1Δ + p416 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 eded1Δ::kanMX [p416]] This study
eded1Δ + p416-Eded1 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 eded1Δ::kanMX [p416-ede1p-Ede1]] This study
Adcd124ΔPP7SL Ede1-tdimer2-4GFP + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Ede1-4GFP::Leu2 [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Ede1-4GFP + p416-Adcd124ΔPP7SL-Stop-24xM52SL + PEP3-GFP [2X] + MCP-mCherry [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Ede1-4GFP::Leu2 [p416-Adcd124ΔPP7SL-Stop-24xM52SL + pFA6-cyc1p-PCP-EFP(2X):cyc1p-MCP-mCherry::HIS3]] This study
Adcd124ΔPP7SL Abp1-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Abp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Abp1-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Abp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Abp1-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Abp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Abp1-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Abp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Ede1-tdimer2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Ede1-tdimer2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Sla1-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Sla1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Vrp1-mKate2 + PEP3-GFP [2X] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Vrp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd124ΔPP7SL Abp1-mKate2-4GFP + PEP3-GFP [2X] ede1Δ [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp ede1Δ::Leu2 Abp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
Adcd17-70Δ [CRISPR CAS9] [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 5'UTR-70nΔ-ADC17-70Δ] This study
WT + FGH17-70Δ + Kozak [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Abp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
WT + FGH17-70Δ only [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ADC124ΔPP7SL-loxp Abp1-mKate2::kanMX [pFA6-cyc1p-PCP-EFP(2X):HIS3]] This study
B9/471 + FGH17-70nΔ + Kozak [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416;FGH17-70nΔ + Kozak::URA3]] This study
B9/471 + FGH17-70Δ only [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 [p416;FGH17-70Δ only::URA3]] This study
acr1-151 [MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 act1-101::kanMX] Euroscarf
Rpl10-GFP + FGH17 [MATa leu2Δ0 met15Δ0 ura3Δ0 Rpl10-GFP::His3MX6 [p416;FGH17-URA3]] This study
Rpl10-GFP + FGH17-70nΔ [MATa leu2Δ0 met15Δ0 ura3Δ0 Rpl10-GFP::His3MX6 [p416;FGH17-70nΔ::URA3]] This study
Authentication
Authentication of cell lines that were not generated in this study, was done by PCR and on the basis of the expected phenotype.

Mycoplasma contamination
Testing for Mycoplasma contamination was not needed, as only yeast has been used in this study

Commonly misidentified lines
(See ILAC register)
No commonly misidentified cell lines were used