An evolutionarily conserved pathway controls proteasome homeostasis

Adrien Rousseau1 & Anne Bertolotti1

The proteasome is essential for the selective degradation of most cellular proteins, but how cells maintain adequate amounts of proteasome is unclear. Here we show that there is an evolutionarily conserved signalling pathway controlling proteasome homeostasis. Central to this pathway is TORC1, the inhibition of which induced all known yeast 19S regulatory particle assembly–chaperones (RACs), as well as proteasome subunits. Downstream of TORC1 inhibition, the yeast mitogen–activated protein kinase, Mpk1, acts to increase the supply of RACs and proteasome subunits under challenging conditions in order to maintain proteasomal degradation and cell viability. This adaptive pathway was evolutionarily conserved, with mTOR and ERK5 controlling the levels of the four mammalian RACs and proteasome abundance. Thus, the central growth and stress controllers, TORC1 and Mpk1/ERK5, endow cells with a rapid and vital adaptive response to adjust proteasome abundance in response to the rising needs of cells. Enhancing this pathway may be a useful therapeutic approach for diseases resulting from impaired proteasomal degradation.

Cell survival depends on adaptive signalling pathways to ensure that the supply of vital components matches fluctuating needs. The proteasome is essential for the selective degradation of most cellular proteins and thereby has a key role in most cellular processes1–3. Proteasome abundance is crucial for cell fitness, but how cells maintain adequate amounts of proteasome is unclear. Failure to degrade mutant or misfolded proteins causes diverse diseases, including devastating neurodegenerative diseases, which might potentially be prevented by increasing proteasome degradation4. Although the idea is attractive, increasing proteasome capacity remains a challenge. Thus, a better understanding of the mechanisms regulating proteasome abundance is required.

The proteasome is composed of 33 subunits assembled in two sub-complexes, the 20S core particle (CP), flanked at one or both ends by the 19S regulatory particle (RP) to form the 26S proteasome5. Proteasome assembly requires the assistance of proteasome assembly chaperones6. Four evolutionarily conserved 19S RACs: Nas2, Nas6, Hsm3 and Rpn14 in yeast, and p27 (also known as PSMD9), p28 (also known as PSMD10), S5b (also known as PSMD5) and Rpn14 (also known as PAAF1) in mammals are needed for regulatory particle assembly5–9. In addition, yeast cells have Adc17, a stress-inducible RAC, which is vital for cells to survive conditions, such as accumulation of misfolded proteins, which overwhelm the proteasome10. This suggests that cells have evolved adaptive signalling pathways to adjust proteasome assembly to arising needs, but how this is achieved is unknown.

**TORC1 inhibition increases Adc17 and the proteasome**

To determine how yeast cells maintain proteasome homeostasis, we decided to investigate the pathway regulating Adc17. Adc17 is upregulated by diverse stresses that impose a high burden on the proteasome, indicating that it is a component of an unknown generic stress response. Because Adc17 is induced by tunicamycin, an inducer of the unfolded protein response (UPR)11, we deleted the UPR genes *IRE1* or *HAC1* (ref. 11). This prevented tunicamycin-mediated induction of the UPR marker Kar2, as expected11, but not that of Adc17 (Fig. 1a), indicating that *ADC17* was not a UPR target gene. We tested Adc17 induction by tunicamycin in mutants thought to regulate Adc17 from a genome-wide regulation study12, and found that deletion of *SFP1* abolished Adc17 but not Kar2 induction by tunicamycin (Fig. 1b). Adc17 induction by tunicamycin was higher in a strain carrying a hypomorphic allele of *MRS6*, a negative regulator of Sfp1 (Extended Data Fig. 1a). Sfp1 is a stress- and nutrient-sensitive regulator of cell growth with dual function13–15. Under optimal growth conditions, Sfp1 is located in the nucleus and can activate transcription of ribosomal protein genes, but it re-localizes to the cytosol upon stress13,14. Sfp1 is activated by TORC1, and in turn negatively regulates TORC1 signalling, as a feedback mechanism15. In the absence of Sfp1, TORC1 is hyperactive15. Thus, SFP1 deletion could prevent Adc17 induction directly or by over-activating TORC1. Adc17 induction by tunicamycin (Fig. 1a, b) coincided with Sfp1 re-localization from the nucleus to the cytosol (Extended Data Fig. 1b), suggesting that Sfp1 may regulate Adc17 not directly, but instead indirectly through TORC1. Tunicamycin inhibits TORC1 signalling15, as observed (Fig. 1c) with the phosphorylation of the TORC1 effector Sch9 (ref. 16). In the absence of Sfp1, TORC1 was hyperactive15 (Fig. 1c), and it remained active during tunicamycin-mediated stress, while Adc17 induction was abolished (Fig. 1c), suggesting that Sfp1 regulated Adc17 via TORC1. Confirming this, rapamycin, a selective inhibitor of TORC1 (ref. 17) induced Adc17 (Fig. 1d). Deletion of SFP1 abolished induction of Adc17 by tunicamycin but not by rapamycin (Fig. 1e) because SFP1 deletion affected Adc17 expression by hyperactivating TORC1 (Fig. 1f). To confirm this using a genetic approach, we examined Adc17 regulation in the thermosensitive *kog1-1* mutant. Kog1 (Fig. 1f) is the yeast homologue of Raptor, a subunit of TORC1 (ref. 18). Inactivation of *KOG1* inhibited TORC1, as expected18, and induced Adc17 (Fig. 1g), indicating that selective TORC1 inhibition induces Adc17. We investigated whether rapamycin increased proteasome abundance. Consistent with our previous results for tunicamycin10, proteasome levels increased by more than twofold after 3 h of rapamycin treatment (Fig. 1h, i). Thus, inhibition of the central stress and growth controller, TORC1, increases abundance of Adc17 and of the proteasome in yeast.

**The MAPK Mpk1 induces Adc17**

TORC1 integrates multiple signalling pathways17,19. We searched for the pathway downstream of TORC1 controlling Adc17 and proteasome abundance. Adc17 is not a UPR gene (Fig. 1a), but *adc17Δ* cells are sensitive to tunicamycin-mediated stress10. Therefore, we examined...
non-UPR mutants sensitive to tunicamycin. The mitogen-activated protein kinases (MAPKs) Hog1 and Mpk1 were important for tunicamycin-stress survival in yeast (Fig. 2a), as expected, unlike the other MAPKs Fus3, Kss1 and Ssk1 (Fig. 2a); Hog1 being advantageous and Mpk1 essential for stress survival (Fig. 2b). Adc17 induction by tunicamycin was compromised in HOG1 deleted cells and abolished in cells lacking a functional allele of MPK1 (Fig. 2c and Extended Data Fig. 2a, b) revealing a perfect correlation between tunicamycin stress-resistance and Adc17 induction. Genetic interaction studies showed that overexpression of HOG1 failed to restore tunicamycin resistance and Adc17 induction in mpk1Δ cells (Fig. 2c, e), while overexpression of MPK1 increased both tunicamycin resistance and Adc17 induction in hog1Δ cells (Fig. 2f, g). Thus, signalling through Mpk1 is required for Adc17 induction and tunicamycin survival.

We examined if Mpk1 was required for Adc17 induction by rapamycin. MPK1 is negatively regulated by TORC1 and essential for rapamycin survival. Unlike the other MAPK, Mpk1 was essential for both cell viability and Adc17 induction in the presence of rapamycin (Extended Data Fig. 2c–e). HOG1 contributed to Adc17 upregulation by tunicamycin (Fig. 2c) but not by rapamycin (Extended Data Fig. 2d). HOG1 was dispensable for survival in the presence of rapamycin (Extended Data Fig. 2c). Thus, induction of Adc17 and rapamycin-resistance are perfectly correlated (Extended Data Fig. 2d, c). In agreement with a previous study, the levels of Mpk1 increased in response to tunicamycin treatment (Extended Data Fig. 2d), but this increase was markedly attenuated in hog1Δ cells (Extended Data Fig. 2d). Thus, one key function of Hog1 is to regulate Mpk1 levels (Fig. 2h), providing an explanation for why Mpk1 overexpression in hog1Δ cells rescued tunicamycin-resistance and Adc17 induction (Fig. 2f, g). Over time, both Mpk1 phosphorylation and abundance were increased by tunicamycin and rapamycin treatment and this preceded Adc17 induction (Extended Data Fig. 3a, b). Bck1, Mkk1 and Mkk2, three kinases that are upstream of Mpk1 (ref. 24), were also required for Adc17 induction by tunicamycin and rapamycin treatment (Extended Data Fig. 3c, d). Congo red, a cell-wall-damaging agent and known inducer of the Mpk1 MAPK pathway, also induced Adc17, in a Mpk1-dependent manner (Extended Data Fig. 3e). These results indicate that diverse challenges inhibiting TORC1 signal to the Mpk1 MAPK to induce the proteasome assembly chaperone Adc17.

**Mpk1 is a master regulator of the proteasome**

We focused on Mpk1 because it is essential for Adc17 induction (Fig. 3a) and examined whether Mpk1 regulated proteasome abundance. Deleting MPK1 completely abolished the tunicamycin- or rapamycin-induced increase of 26S proteasomes while increasing the abundance of the free core particles (Fig. 3b–d). This defect is symptomatic of regulatory particle assembly defects8–11, and a hallmark of adc17Δ cells in response to stress.10 However, the mpk1Δ cells (Fig. 3b–d) appeared more severely affected than adc17Δ cells, suggesting that other MPK1-regulated factors assist regulatory particle assembly. We found that all the known yeast RACs: Nas2, Nas6, Hsm3 and Rpn14 were induced by treatment with tunicamycin, rapamycin or Congo red in wild-type cells (Fig. 3e and Extended Data Fig. 3f). Genetic inactivation of TORC1 in kog1-1 cells also induced all RACs at the non-permissive temperature (Fig. 3f). Induction of all yeast RACs by tunicamycin and rapamycin was abolished in mpk1Δ, bck1Δ and mkk1/k2Δ cells (Fig. 3g and Extended Data Fig. 3g, h). Overexpression of different combinations of three RACs markedly improved tunicamycin resistance in mpk1Δ cells (Extended Data Fig. 4a). Conversely, the deletion of three RACs severely impaired cell viability in the presence of rapamycin (Extended Data Fig. 4b). Thus, regulating the expression of RACs is a key function of Mpk1. These results reveal that downstream of TORC1 inhibition, signalling through the Mpk1 MAPK pathway coordinates the induction of all RACs to control proteasome abundance and viability upon various stresses.

Tunicamycin and rapamycin increased 26S abundance in wild-type cells and induced free core particles in mpk1Δ cells (Fig. 3b), suggesting that core particle assembly might also be regulated. We analysed the levels of the core particle assembly chaperones proteasome biogenesis-associated (Pba1–4) (refs 25, 26) after tunicamycin treatment, the most potent inducer of core particles in mpk1Δ cells (Fig. 3b, d). In wild-type cells, tunicamycin treatment increased the level of Pba1 and Pba2 but not the level of Pba3 and Pba4 (Extended Data Fig. 5a–d). Thus, the increase in core particles was accompanied by an increase of the assembly chaperones Pba1 and Pba2. This increase was unaltered upon MPK1 deletion (Extended Data Fig. 5a–d). This demonstrates that Pba1 and Pba2 are upregulated by the stress caused by tunicamycin treatment and their regulation is independent of Mpk1. The mechanism of Mpk1-independent regulation of Pba1 and Pba2 will be an important topic for future study.

We examined the regulation of proteasome subunits. Both tunicamycin and rapamycin treatment increased the levels of proteasome subunits, and this increase required Rpn4, the transcription factor controlling expression of proteasome subunits (Extended Data Fig. 6a, b). Rpn4 increased upon tunicamycin or rapamycin treatment (Extended Data Fig. 6c). In contrast, Adc17 is upregulated independently of Rpn4 upon diverse stresses (ref. 10), and all yeast RACs show the same pattern of regulation (Extended Data Fig. 6b). Upregulation of proteasome subunits depends on Rpn4, and upregulation of all known RACs is independent of Rpn4. Deletion of MPK1 completely abrogated the tunicamycin- and rapamycin-induced upregulation of proteasome subunits, indicating that Mpk1 is a master regulator of proteasome homeostasis (Fig. 4a and Extended Data Fig. 6d).
We identified a weak genetic interaction between RPN4 and MPK1, and found that both were required for survival in response to tunicamycin treatment (Extended Data Fig. 6e, f). Tunicamycin and rapamycin increased Rpn4 levels to wild-type levels in Δmpk1Δ cells (Extended Data Fig. 6g), suggesting that Mpk1 is acting downstream of the transcription factor Rpn4, possibly post-transcriptionally. At the protein level, MPK1 deletion completely abrogated the induction of proteasome subunits and RACs by rapamycin treatment (Fig. 4a). At the mRNA level, rapamycin only modestly, yet reproducibly, increased abundance of RACs and proteasome subunits mRNA (Fig. 4b), and this increase was similar in wild-type and mpk1Δ cells (Fig. 4b). Rpn4 induction was similar in both strains (Extended Data Fig. 6g). Blocking the synthesis of new proteins with cycloheximide for 4 h did not change the abundance of proteasome subunits and RACs, indicating that they

**Figure 3** Mpk1 coordinates the expression of all yeast RACs to control proteasome abundance. a, Immunoblots of lysates from yeast cells cultured ± tunicamycin or rapamycin for 4 h. b, Native PAGE (4.2%) of yeast cells cultured ± tunicamycin or rapamycin, monitored by Suc-LLVY-AMC and by immunoblots. Rpt5’ (Rpt5 intermediates). c, d, Quantifications from experiments as in b. Data are mean ± s.d. of four biological replicates. **P ≤ 0.01; ***P ≤ 0.001; NS, not significant (two-way ANOVA). e, Immunoblots from lysates of yeast cells cultured ± tunicamycin or rapamycin for 4 h. f, Immunoblots from lysates of yeast cells cultured at 30°C or 37°C for 4 h. g, Immunoblots from lysates of yeast cells cultured ± tunicamycin or rapamycin for 4 h.
were stable over this time period (Extended Data Fig. 6h, lanes 1 and 4). Likewise, the stability of proteasome subunits and RACs appeared similar in mpk1Δ cells and wild-type cells (Extended Data Fig. 6i). However, cycloheximide completely blocked induction of proteasome subunits and RACs by tunicamycin and rapamycin in wild-type cells (Extended Data Fig. 6h). Together these results reveal that the MAPK Mpk1 coordinates the translation of proteasome subunits and RACs to provide the increased proteasome abundance required to sustain cell viability.

**Mpk1 adapts proteasome degradation to rising needs**

We analysed the consequences of the MPK1-dependent increase of proteasome abundance on protein degradation. Polyubiquitinated conjugates represent a hallmark of impaired proteasomal degradation and were slightly elevated in mpk1Δ cells compared to wild type (Fig. 5a, b). This defect was exacerbated upon tunicamycin or rapamycin treatment (Fig. 5a, b), suggesting impaired proteasomal degradation, and providing an explanation for why mpk1Δ cells failed to survive tunicamycin (Fig. 2a) or rapamycin treatment (Extended Data Fig. 2c).

We examined the degradation of diverse proteasome reporter substrates. The metabolizable Ura3 reporter was rapidly degraded in wild-type cells cultured at 37 °C, but not in cells harbouring a thermosensitive mutation in the proteasome subunit Rpt4 (Extended Data Fig. 7a, b). Similarly, the degradation of the reporter substrate was strikingly compromised in mpk1Δ cells (Extended Data Fig. 7c, d). The degradation of the two well-characterized proteasome reporter substrates, CPY*–HA and Δss–CPY*–GFP, which are localized in the endoplasmic reticulum and in the cytosol, respectively, was also compromised in mpk1Δ cells (Fig. 5c–f). The protein degradation defect of mpk1Δ cells was more pronounced in cells challenged with tunicamycin and rapamycin treatment (Extended Data Fig. 7e–l). Together with the previous findings, this demonstrates that Mpk1 maintains adequate levels of proteasome required to sustain protein degradation and cell viability under challenging conditions.

**Evolutionary conservation of proteasome regulation**

Four RACs are evolutionarily conserved with p27, p28, S5b and Rpn14 being human orthologues of the yeast Nas2, Nas6, Hsm3 and Rpn14, respectively. We investigated whether the TORC1 and Mpk1 regulation of RACs was evolutionarily conserved. Inhibition of mTOR by Torin-1 rapidly increased the levels of all mammalian RACs (Fig. 6a, b), similar to what was found in the experiments in yeast (Fig. 3e, f). mTOR inhibition resulting from nutrient starvation also increased the RACs (Extended Data Fig. 8a, b). As in yeast, the concerted increase of the RACs was accompanied by an upregulation of proteasome subunits (Fig. 6a, b), and resulted in an increase in the levels of 26S proteasome (Fig. 6c, d and Extended Data Fig. 8c, d). This response was acute, with a rapid return to basal levels (Fig. 6a–d). As previously reported, singly capped proteasome RPCP (CP, core particle; RP, regulatory particle) was more abundant than doubly capped proteasome RP2CP in mammalian cells (Fig. 6c).

Conversely, medium replenishment to increase nutrient supply and activate mTORC1 had the opposite effect resulting in S6K1 phosphorylation (Extended Data Fig. 9a) and decreasing abundance of both RACs (Extended Data Fig. 9a, b) and proteasome (Extended Data Fig. 9c, d). Rapamycin, a selective mTORC1 inhibitor, also acutely and transiently induced the RACs as well as proteasome subunits (Extended Data Fig. 10), confirming that, as in yeast, mTORC1 controls proteasome homeostasis. We wondered whether ERK5 (also known as MAPK7) (ref. 32), the mammalian orthologue of Mpk1, also regulates proteasome abundance. ERK5 overexpression in yeast rescued tunicamycin resistance in mpk1Δ cells (Fig. 6e). Knocking down ERK5 with short interfering RNA (siRNA) in human cells resulted in a decrease of the four mammalian RACs p27, p28, S5b and Rpn14 (Fig. 6f, g), as well as the 26S proteasome (Fig. 6h, i). Thus, mammalian ERK5, like yeast Mpk1, controls RACs and thereby acts as a switch to control proteasome abundance.

**Discussion**

Here we report a general and evolutionarily conserved homeostatic response that increases proteasome abundance as needed, through the coordinated upregulation of regulatory particle assembly chaperones and proteasome subunits. The master regulators of growth and stress, TORC1 and Mpk1/ERK5 are central to this response. Consistent with the general principle of homeostatic responses, we observed that proteasome increase is an acute and rapidly reversible response. Trying to identify the other components of this proteasome homeostatic response was beyond the scope of this study.
were spotted in a sixfold dilution and grown on plates transformed with a plasmid encoding the human ERK5 or an empty vector revealed with Suc–LLVY–AMC and by immunoblots.

**Figure 6 | Evolutionary conservation of the pathway controlling RACs and proteasome abundance.** a, b, Immunoblots (a) and quantifications (b) of the indicated proteins in lysates of HeLa cells treated with 250 nM Torin-1 for the indicated time. c, d, Native PAGE (4.2%) (c) and quantifications (d) of HeLa cell lysates following treatment as in a and revealed with Suc–LLVY–AMC and by immunoblots. e, mkp1Δ cells transformed with a plasmid encoding the human ERK5 or an empty vector were spotted in a sixfold dilution and grown on plates ± tunicamycin for 3 days. f, g, Immunoblots (f) and quantifications (g) of the indicated proteins in lysates of HeLa cells 3 days after transfection with a non-target siRNA (siCTL) or a siRNA targeting ERK5 (siERK5). h, i, Native PAGE (4.2%) (h) and quantifications (i) of HeLa cell extracts 3 days after transfection with siCTL or siERK5 monitored by Suc–LLVY–AMC or by immunoblots. b, d, g, i, Data are mean ± s.d.; n = 3 biological replicates. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS, not significant (b, d, g, i, one-way ANOVA; g, two-way ANOVA; i, two-tailed Student’s t-test).

Considering this in light of our results, it may be the adaptive response to the stress resulting from the lack of Tsc2 combined with serum starvation that increases proteasomal degradation in Tsc2−/− cells, rather than Tsc2 deletion per se.

In line with our findings is the well-established notion that mTOR activation enhances anabolic processes and represses catabolic processes. mTORC1 is known to repress autophagy. We show here that TORC1 restricts proteasome abundance and this is rapidly alleviated upon TORC1 inhibition. Therefore, the same controller TORC1...
restricts the abundance of the two cellular proteolytic systems, the proteasome and autophagy. Our findings integrate the regulation of proteasome assembly and abundance with growth and cellular metabolism, and suggest that the increased proteasome capacity resulting from TORC1 inhibition may also contribute to the benefit of the widely used TORC1 inhibitors.

The current prevailing view is that protein degradation is largely regulated at the level of ubiquitination. Here we demonstrate that modulating proteasome abundance is an important component of regulation of proteasomal degradation. Adapting proteasome abundance is vital to cope with overwhelming cellular needs, implying that proteasome abundance can be rate limiting under critical conditions. The evolutionary conservation of the TORC1 and Mpk1/ERK5 pathway controlling proteasome abundance further highlights the importance of this regulation.

The pathway identified here can be used as a unique switch to increase proteasome assembly and abundance on demand. Because many human diseases are associated with accumulation of misfolded proteins, increasing proteasome abundance by manipulating the switches identified here could be used as a generic strategy to reduce the burden of misfolded proteins that accumulate in such age-related diseases.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

**Received 21 January; accepted 20 June 2016.**

**Published online 27 July 2016.**

1. Goldberg, A. L. Functions of the proteasome: from protein degradation and immune surveillance to cancer therapy. *Biochem. Soc. Trans.* **35**, 12–17 (2007).
2. Finley, D. Recognition and processing of ubiquitin–protein conjugates by the proteasome. *Annu. Rev. Biochem.* **78**, 477–513 (2009).
3. Tanaka, K., Mizushima, T. & Saeki, Y. The proteasome: molecular machinery and pathophysiological roles. *Biol. Chem.* **393**, 217–234 (2012).
4. Tomko, R. J. J. & Hochstrasser, M. Molecular architecture and assembly of the eukaryotic proteasome. *Annu. Rev. Biochem.* **82**, 415–445 (2013).
5. Le Tallec, B., Barrault, M. B., Guérois, R., Carré, T. & Peyroche, A. HspM3/S5b participates in the assembly pathway of the 19S regulatory particle of the proteasome. *Mol. Cell* **33**, 389–399 (2009).
6. Saeki, Y., Toh–E, A., Kudo, T., Kawamura, H. & Tanaka, K. Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle. *Mol. Cell* **33**, 900–913 (2009).
7. Funakoshi, M., Tomko, R. J. Jr, Kobayashi, H. & Hochstrasser, M. Multiple assembly chaperones govern biogenesis of the proteasome regulatory particle base cell. *Cell* **137**, 887–899 (2009).
8. Rolfs, J. et al. Chaperone-mediated pathway of proteasome regulatory particle assembly. *Nature* **459**, 861–865 (2009).
9. Kaneko, T. et al. Assembly pathway of the mammalian proteasome base subcomplex is mediated by multiple specific chaperones. *Cell* **137**, 914–925 (2009).
10. Hanssum, A. et al. An inducible chaperone adapts proteasome assembly to stress. *Mol. Cell* **55**, 566–577 (2014).
11. Wiseman, R. L., Haynes, C. M. & Ron, D. SnapShot: The unfolded protein response. *Cell* **140**, 590–590.e2 (2010).
12. Venets, B. J. et al. A comprehensive genomic binding map of gene and chromatin regulatory proteins in Saccharomyces. *Mol. Cell* **41**, 480–492 (2011).
13. Marion, R. M. et al. Sfp1 is an stress- and nutrient-sensitive regulator of ribosomal protein gene expression. *Proc. Natl Acad. Sci. USA* **101**, 14315–14322 (2004).
14. Jørgensen, P. et al. A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. *Genes Dev.* **18**, 2491–2505 (2004).
15. Lempinen, H. et al. Sfp1 interaction with TORC1 and Mrs6 reveals feedback regulation on TOR signaling. *Mol. Cell* **33**, 704–716 (2009).
16. Takahara, T. & Maeda, T. Transient sequestertration of TORC1 into stress granules during heat stress. *Mol. Cell* **47**, 242–252 (2012).
17. Soular, A. & Hall, M. N. Snapshot: mTOR signaling. *Cell* **129**, 434.e1–434.e2 (2007).
18. Loeith, R. & Hall, M. N. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* **189**, 1177–1201 (2011).
19. Zoncu, R., Efeyan, A. & Sabatini, D. M. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nature Rev. Mol. Cell Biol.* **12**, 21–35 (2011).
20. Bonilla, M. & Cunningham, K. W. Mitogen-activated protein kinase stimulation of Ca2+ signaling is required for survival of endoplasmic reticulum stress in yeast. *Mol. Biol. Cell* **14**, 4296–4305 (2003).
21. Kreuse, S. A. & Gray, J. V. The protein kinase C pathway is required for viability in quiescence in Saccharomyces cerevisiae. *Curr. Biol.* **12**, 588–593 (2002).
22. Torres, J., Di Como, C. J., Herrera, 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).
23. Babour, A., Bicknell, A. A., Tourtellotte, J. & Niwa, M. A surveillance pathway monitors the function of the endoplasmic reticulum to control its inheritance. *Cell* **142**, 256–269 (2010).
24. Levin, D. E. Regulation of cell wall biogenesis in Saccharomyces cerevisiae: the cell wall integrity signaling pathway. *Genetics* **189**, 1145–1175 (2011).
25. Hirano, Y. et al. A heterodimeric complex that promotes the assembly of mammalian 20S proteasomes. *Nature* **437**, 1381–1385 (2005).
26. Le Tallec, B. et al. 20S proteasome assembly is orchestrated by two distinct pairs of chaperones in yeast and in mammals. *Mol. Cell* **27**, 660–674 (2007).
27. Xie, Y. & Varshavsky, A. RPNA is a ligand, substrate, and transcriptional regulator of the 26S proteasome: a negative feedback circuit. *Proc. Natl Acad. Sci. USA* **98**, 3056–3061 (2001).
28. Surawere, A., Münch, C., Hanssum, A. & Bertolotti, A. Failure of amino acid homeostasis causes cell death following proteasome inhibition. *Mol. Cell* **48**, 242–253 (2012).
29. Hiller, M. M., Finger, A., Schweiger, M. & Wolf, D. H. ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. *Science* **273**, 1725–1728 (1996).
30. Medicherla, B., Kostova, Z., Schaefer, A. & Wolf, D. H. A genomic screen identifies Dsk2p and Res2p as essential components of ER-associated degradation. *EMBO Rep.* **5**, 692–697 (2004).
31. Asano, S. et al. A molecular census of 26S proteasomes in intact neurons. *Science* **347**, 439–442 (2015).
32. Truman, A. W. et al. Inhibited in the yeast Saccharomyces cerevisiae, human ERK5 is a client of the Hsp90 chaperone that complements loss of the Slt2p (Mpk1p) cell integrity stress-activated protein kinase. *Eukaryot. Cell* **5**, 1914–1924 (2006).
33. Zhang, Y. et al. Coordinated regulation of protein synthesis and degradation by mTORC1. *Nature* **513**, 440–443 (2014).
34. 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).
35. Albert, V. & Hall, M. N. mTOR signaling in cellular and organismal energetics. *Curr. Opin. Cell Biol.* **33**, 55–66 (2015).

**Supplementary Information** is available in the online version of the paper.

**Acknowledgements** We thank Y. Lee and M. Hochstrasser for the kind gift of Nas2, Nas3, Hsm3 and Rpn14 antibodies; D. H. Wolf for CPY–HA and Δss-CPY–GFP constructs; T. Maeda for the P-Sch9 antibody; and members of the Bertolotti laboratory for discussion. A.B. is an honorary fellow of the University of Cambridge Clinical Neurosciences Department. This work was supported by the Medical Research Council (UK) MC_U105185860. A.R. is supported by an EMBO long-term fellowship.

**Author Contributions** A.R. designed, performed and analysed all experiments, prepared the figures and helped with the manuscript. A.B. designed and supervised the study and wrote the manuscript.

**Author Information** Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.B. (aberto@mrc-lmb.cam.ac.uk).

**Reviewer Information** Nature thanks S. Murata and D. Sabatini and the other anonymous reviewer(s) for their contribution to the peer review of this work.
Quantitative RT–PCR. Total yeast RNA was extracted as previously described, 15 μg of purified RNA was treated with the Turbo DNase kit (Ambion) and 1 μg of DNA-free RNA was synthesized into cDNA using the iScript cDNA synthesis kit (Bio-Rad laboratories). cDNA was diluted 1:10 before the quantitative RT–PCR was performed.

Mammalian cell culture. HeLa cells were from IGBMC (Strasbourg, France) with authentication and they were not used beyond passage 20 from original derivation. HeLa cells were routinely tested for mycoplasma contaminations. HeLa cells were cultured in minimum essential media (MEM) (11095-080; Life Technologies) supplemented with 1-glutamine-penicillin-streptomycin solution (G6784; Sigma-Aldrich) and containing 10% fetal bovine serum (FBS). The medium was changed every 24 h. Medium replenishment experiment was carried out using DMEM (11960-044; Life Technologies) (high glucose, no glutamine) supplemented with 1-glutamine-penicillin-streptomycin solution (G6784; Sigma-Aldrich) and containing 10% FBS.

Mammalian cell treatments. For mTOR inhibition by Torin-1, cells were plated in 6-well plates at a density of 400,000 cells per well. The medium was changed 24 h after plating and a final concentration of 250 nM Torin-1, 200 nM rapamycin or DMSO was directly added to the medium 48 h after plating (confluence: 85–95%) for the indicated time. For starvation experiments, cells were plated in 6-well plates at a density of 400,000 cells per well. The medium was changed 24 h after plating. 48 h after plating, HeLa cells were washed twice with PBS before being cultured in Earle’s Balanced Salt Solution (EBSS) for the indicated time points. For medium replenishment experiments, cells were plated in 6-well plates at a density of 400,000 cells per well. The medium was changed 24 h after plating. 48 h after plating, HeLa cells were washed twice with PBS before being cultured in Earle’s Balanced Salt Solution (EBSS) for the indicated time points. For medium replenishment experiments, cells were plated in 6-well plates at a density of 400,000 cells per well. The medium was changed 24 h after plating. 48 h after plating, HeLa cells were washed twice with PBS before being cultured in Earle’s Balanced Salt Solution (EBSS) for the indicated time points.
20 μl of 5× native loading buffer (0.25 M Tris-HCl (pH 6.8), 50% glycerol, 0.05% bromophenol blue). 25 μg of each extract were subjected to 4.2% native PAGE. In-gel peptidase assay was performed as described previously before the samples were transferred to nitrocellulose membranes. Membranes were incubated with antibodies to Alpha7 (PW8110; Biomol, 1:1,000) and Rpt6 (SUG-18; Euromedex, 1:5,000). Proteins were visualized by ECL Prime (GE Healthcare).

**RNA interference.** ON-TARGET plus SMARTpool siRNA for ERK5 or non-targeting control (Dharmacon) were used in knockdown experiments. HeLa cells (200,000 cells per well) were plated in 6-well plates. 24 h after plating, media were replenished and siRNAs were delivered into cells using RNAiMAX (catalogue number 13778075 from Invitrogen) according to the manufacturer’s instructions. The medium was changed every 24 h post-transfection for a total of 3 days. Cells were then harvested and analysed by immunoblot.

**Statistical analysis.** Representative results of at least three independent experiments (biological replicates) are shown in all panels. GraphPad Prism software was used for all statistical analyses. Data are presented as mean and standard deviations. For immunoblot quantifications, level of each protein was normalized to PGK1 in yeast and β-actin in mammalian cells and expressed as fold change. Data were analysed using unpaired Student’s t-test or repeated measures analysis of variance (one-way ANOVA or two-way ANOVA where indicated). The level of significance was set at *P* ≤ 0.05; **P** ≤ 0.01; ***P** ≤ 0.001; NS, not significant.

36. Gietz, R. D. & Woods, R. A. Yeast transformation by the LiAc/SS carrier DNA/PEG method. *Methods Mol. Biol.* **313**, 107–120 (2006).
37. Zhang, T. et al. An improved method for whole protein extraction from yeast *Saccharomyces cerevisiae*. *Yeast* **28**, 795–798 (2011).
38. von der Haar, T. Optimized protein extraction for quantitative proteomics of yeasts. *PLoS One* **2**, e1078 (2007).
39. Urban, J. et al. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol. Cell* **26**, 663–674 (2007).
40. Elsasser, S., Schmidt, M. & Finley, D. Characterization of the proteasome using native gel electrophoresis. *Methods Enzymol.* **398**, 353–363 (2005).
41. Knutson, B. A. & Hahn, S. Domains of Tra1 important for activator recruitment and transcription coactivator functions of SAGA and NuA4 complexes. *Mol. Cell. Biol.* **31**, 818–831 (2011).
Extended Data Figure 1 | Adc17 induction is increased in mrs6-DAmp cells and occurs when Sfp1 is cytosolic. a, Immunoblots of the indicated proteins in lysates of wild-type and Mrs6-hypomorphic (mrs6-DAmp) yeast strains ± tunicamycin for 4 h. b, Representative images of yeast cells carrying a GFP-tagged SFP1 at the endogenous locus, ± tunicamycin for 4 h. Scale bar, 5μm. Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 2 | Mpk1 is essential for tunicamycin and rapamycin survival and Adc17 induction. a, *mpk1Δ* cells transformed with wild-type *MPK1* or a kinase-dead allele (*MPK1-K52R*) or empty vector were spotted in a sixfold dilution and grown on plates containing or lacking tunicamycin. b, Immunoblots of lysates of yeast strains shown in a, cultured for 4 h ± tunicamycin. c, Cells of the indicated genotype were spotted in a sixfold dilution and grown for 3 days at 30 °C on plates containing or lacking rapamycin. d, Immunoblots of lysates from wild-type and MAPK genetic deletion mutant yeast cells cultured for 4 h ± tunicamycin or rapamycin. e, Same as in a, using *mpk1Δ* cells transformed with empty vector or a vector encoding *MPK1* or *HOG1*. Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 3 | Mpk1 MAPK pathway is essential for stress-mediated RACs induction. a, b, Immunoblots of the indicated proteins in lysates of wild-type yeast cells ± tunicamycin (a) or rapamycin (b) for the indicated time. c, g, Immunoblots of the indicated proteins in lysates of wild-type and bck1Δ cells cultured ± tunicamycin or rapamycin for 4 h. d, h, Immunoblots of the indicated proteins in lysates of wild-type and mkk1Δ cells cultured ± tunicamycin or rapamycin for 4 h. e, f, Immunoblots of the indicated proteins in lysates of wild-type or mpk1Δ cells ± 50 μg ml⁻¹ Congo red (CR) for 4 h. Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 4 | Induction of RACs under challenging conditions is an important function of Mpk1. a, Wild-type cells or mpk1Δ cells transformed with one or combinations of two or three RACs were spotted in a sixfold dilution and grown on plates containing or lacking tunicamycin, where indicated. b, Multiple-deletion yeast strains of different RACs were spotted in a sixfold dilution and grown for 3 days at 33°C on plates containing or lacking rapamycin. Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 5 | Pba1 and Pba2 are induced by tunicamycin in a Mpk1-independent manner. a–d, Immunoblots of the indicated proteins in lysates of wild-type yeast cells carrying a TAP-tagged Pba1 (a), Pba2 (b), Pba3 (c) and Pba4 (d) at the endogenous locus ± tunicamycin for 3 h. Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 6 | Mpk1 post-transcriptionally regulates proteasome subunits and RACs. a, b, Immunoblots of the indicated proteins in lysates of wild-type (a) and rpn4Δ (b) cells ± tunicamycin or rapamycin for 4 h. c, Immunoblots of the indicated proteins in lysates of wild-type yeast cells carrying a TAP-tagged RPN4 at the endogenous locus ± tunicamycin or rapamycin for 4 h. d, Immunoblots of the indicated proteins in lysates of wild-type and mpk1Δ cells ± tunicamycin or rapamycin for 4 h. e, rpn4Δ cells transformed with RPN4, MPK1, a kinase-dead allele of MPK1 (MPK1-K52R) or empty vector were spotted in a sixfold dilution and grown on plates containing or lacking tunicamycin. f, mpk1Δ cells transformed with MPK1, RPN4 or empty vector were spotted in a sixfold dilution and grown on plates containing or lacking tunicamycin where indicated. g, Immunoblots of the indicated proteins in lysates of wild-type and mpk1Δ cells carrying a TAP-tagged RPN4 at the endogenous locus ± tunicamycin or rapamycin for 4 h. h, i, Immunoblots of the indicated proteins in lysates of wild-type (h, i) and mpk1Δ (i) cells treated with different combinations of drugs: 5 μg ml⁻¹ tunicamycin, 0.2 μg ml⁻¹ rapamycin and 35 μg ml⁻¹ cycloheximide, where indicated for 4 h. Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 7 | Mpk1 maintains the adequate levels of proteasome required to sustain protein degradation.  

**a, c,** Yeast cells of the indicated genotype expressing GFP-tagged Ura3-3 proteins were treated with cycloheximide and incubated at 37 °C for the indicated time. 

**b, d,** Quantifications from three independent experiments (biological replicates) such as the one shown in a and c.  

**e, g,** Cells of the indicated genotype expressing CPY*-HA (e) or Δss-CPY*-GFP (g) proteins were treated with tunicamycin for 4 h.  

**f, h,** Quantifications from three independent experiments (biological replicates) such as the one shown in e and g.  

**i, k,** Cells of the indicated genotype expressing CPY*-HA (i) or Δss-CPY*-GFP (k) proteins were treated with rapamycin for 4 h.  

**j, l,** Quantifications from three independent experiments (biological replicates) such as the one shown in i and k.  

Data are mean ± s.d. n = 3 biological replicates. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS, not significant (two-way ANOVA).
Extended Data Figure 8 | Starvation inhibits TORC1 signalling, induces mammalian RACs and increases proteasome abundance.

a, b, Immunoblots (a) and quantification (b) of the indicated proteins in lysates of HeLa cells after EBSS (Earle’s balanced salt solution) treatment for the indicated time. c, HeLa cell extracts following EBSS treatment for the indicated time were resolved on native PAGE (4.2%) and monitored using the fluorogenic substrate Suc-LLVY-AMC or by immunoblots. d, Quantification of the 26S proteasome activity (RPCP and RP2CP) of experiments such as the one shown in c, b, d. Data are mean ± s.d. n = 3 biological replicates. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS, not significant (one-way ANOVA). Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 9 | TORC1 activation by nutrient replenishment decreases the abundance of RACs as well as 26S proteasome.

a, b, Immunoblots (a) and quantification (b) of the indicated proteins in lysates of HeLa cells after replenishment with rich complete medium for the indicated time. c, Native PAGE (4.2%) of cell extracts from HeLa cells following media replenishment as in a, monitored by the fluorogenic substrate Suc-LLVY–AMC or by immunoblots. d, Quantification of the 26S proteasome activity (RPCP and RP,CP) of experiments such as the one shown in c, d. Data are mean ± s.d. n = 3 biological replicates. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS, not significant (one-way ANOVA). Representative results of at least three independent experiments (biological replicates) are shown.
Extended Data Figure 10 | mTORC1 inhibition by Torin-1 and rapamycin acutely induced the RACs. a, b, Immunoblots (a) and quantification (b) of the indicated proteins in lysates of HeLa cells treated with 250 nM Torin-1 or 200 nM rapamycin for the indicated time. Data are mean ± s.d. n = 3 biological replicates. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; NS, not significant (two-way ANOVA). Representative results of at least three independent experiments (biological replicates) are shown.