that tumour-induced necroptosis enhanced tumour-cell migration across the endothelial barrier. The authors made similar findings in *in vivo* experiments using genetic inactivation of the RIPK3 kinase enzyme, a necroptosis regulator, in endothelial cells. Inactivation of RIPK3 prevented endothelial-cell death, and reduced the ability of cancer cells to cross the endothelial barrier and enter the lung. Metastastic tumour-colony formation was reduced upon genetic or pharmacological inhibition of endothelial-cell necroptosis, indicating that tumour-induced endothelial-cell killing exerted control over metastasis.

How does endothelial-cell death enhance tumour-cell migration across the endothelial barrier? Strilic and colleagues propose various mechanisms. Tumour cells could migrate through gaps left in the endothelial barrier by dead endothelial cells. Another possibility is that damage-associated molecular pattern molecules (DAMPs), such as ATP released from necroptotic endothelial cells, could act on neighbouring endothelial cells to open the endothelial barrier by enabling tumour-cell migration between neighbouring endothelial cells that are usually bound together to form an impermeable barrier, and/or these signals could act directly on tumour cells to enhance their migration across the barrier[8].

How do tumour cells induce endothelial necroptosis? The authors used a combination of molecular, pharmacological and genetic approaches to show that amyloid precursor protein (APP) on the surface of tumour cells induces necroptotic cell death by interacting with death receptor 6 (DR6) on endothelial cells (Fig. 1b). Consistent with this, pharmacological inhibition of DR6 signalling — achieved by injecting mice with a 'decoy' version of the DR6 receptor — inhibited metastasis.

Strilic *et al.* provide compelling evidence to support the existence of intricate signalling interactions between migrating tumour cells in the bloodstream and the blood-vessel endothelium that promote tumour-cell metastatic migration into tissue and subsequent tissue colonization. These findings raise a series of intriguing issues. Only a small fraction of endothelial cells cultured *in vitro* with tumour cells are induced to undergo necroptotic death. Discovering the molecular determinant that governs which endothelial cells die is a key challenge. The authors reveal that only approximately 10% of endothelial cells express DR6 and are thus susceptible to APP-mediated cell death.

It will be important to understand the mechanisms that regulate which fraction of endothelial cells express DR6, and whether cancer cells can regulate the susceptibility of endothelial cells to necroptosis by modulating DR6 expression on the cells. Microscopy analysis of human tumours could be used to reveal whether an increased fraction of DR6-expressing endothelial cells is associated with the propensity for lung metastatic progression. Perhaps molecular signals from the endothelium to tumour cells regulate expression or cleavage of APP on tumour cells — thus having an effect on endothelial-cell necroptosis. Such endothelial-cell-derived signals have roles in epithelial-cell fate and function[9].

In addition to the mechanisms proposed by the authors, another mechanism by which endothelial-cell necroptosis might enhance tumour migration into tissue could be mediated by ATP. Release of ATP from dying endothelial cells might promote the survival of tumour cells during their migration through the endothelial barrier into the tissue[10] — a process that can cause traumatic tumour-cell deformation and death. Live-cell microscopy imaging of tumour- and endothelial-cell dynamics during this interaction[11] may be an ideal means of determining which of the intriguing potential cellular mechanisms proposed by the authors might underlie tumour-cell migration across the endothelial barrier. ■

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1. Strilic, B. *et al. Nature* **536,** 215–218 (2016).
2. Folkman, J. *Cancer Res.* **46,** 467–473 (1986).
3. Dvorak, H. F. *Cancer J.* **21,** 237–243 (2015).
4. Ferrara, N. *Arterioscler. Thromb. Vasc. Biol.* **29,** 789–791 (2009).
5. Minn, A. J. *et al. Nature* **436,** 518–524 (2005).
6. Gupta, G. P. *et al. Nature* **446,** 765–770 (2007).
7. Pasparakis, M. & Vandenabeele, P. *Nature* **517,** 311–320 (2015).
8. Schumacher, D., Strilic, B., Sivaraj, K. K., Wettschureck, N. & Offermanns, S. *Cancer Cell* **24,** 130–137 (2013).
9. Ding, B. S. *et al. Nature* **505,** 97–102 (2014).
10. Furlow, P. W. *et al. Nature Cell Biol.* **17,** 943–952 (2015).
11. Headley, M. B. *et al. Nature* **531,** 513–517 (2016).

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CELL BIOLOGY

The TORC1 pathway to protein destruction

A study of the proteasome — a protein-degradation complex — reveals an evolutionarily conserved pathway that acts through the protein kinase TORC1 to adjust proteasome levels in response to cellular needs. SEE ARTICLE P.184

LYNNE CHANTRANUPONG & DAVID M. SABATINI

To maintain amino-acid and protein levels, cells must couple nutrient availability to protein synthesis and turnover. Central to this process is the enzyme called target of rapamycin complex 1 (TORC1) kinase, a master growth controller that integrates diverse environmental inputs to coordinate many metabolic processes[1]. Rousseau and Bertolotti[2] reveal on page 184 that inhibition of TORC1 increases levels of the proteasome — a large protein complex involved in cellular protein degradation — to promote cell survival under stressful conditions. Consistent with previous reports[3–5], the new work identifies TORC1 as a central regulator of proteasome homeostasis. However, the relationship between TORC1 and the control of proteasome function seems to be complex, because TORC1 can regulate the proteasome through multiple mechanisms that depend on the particular cellular context[3–5].

The proteasome functions in one of the main protein-degradation pathways in cells, the ubiquitin–proteasome system[6]. In this pathway, a multi-enzymatic cascade covalently links the small polypeptide ubiquitin to proteins. This modification is recognized by the proteasome, which degrades ubiquitinated proteins to produce peptide mixtures that can then replenish the intracellular pool of amino acids[6].

The proteasome comprises a multisubunit core particle, which carries out protein degradation, and up to two additional regulatory particle components that facilitate substrate recognition, removal of ubiquitin, and protein unfolding and translocation into the proteasome[6]. Inhibition of the proteasome results in a lethal shortage of amino acids[7]; therefore cells must maintain adequate proteasome levels to survive. However, the mechanisms that govern the assembly and regulation of this complex molecular machine, particularly under stressful conditions, are not fully understood.

The discovery[8] in yeast of Adc17, a stress-induced regulatory particle assembly chaperone protein (RAC), offers an insight into the mechanism of proteasome regulation.

Rousseau and Bertolotti used Adc17 as a starting point to investigate the proteasome. They treated yeast with the antibiotic tunicamycin to induce the unfolded-protein response, a cellular stress response to the presence of misfolded or unfolded proteins. They found that yeast upregulates Adc17 levels in the presence of tunicamycin, and that loss of the protein Sfp1, a negative regulator of TORC1, abrogates this increase of Adc17. The authors established that the increase in Adc17 requires inhibition of TORC1. Pharmacological suppression of TORC1 by the compound rapamycin or genetic inhibition of TORC1 by inactivation of *KOG1*, which encodes an essential TORC1 subunit, are sufficient to increase the expression not only of Adc17, but also of all other known RACs and of proteasome subunits.

To understand how TORC1 might mediate an increase in proteasome abundance, the authors focused on Mpk1, a yeast enzyme known as a mitogen-activated protein kinase (MAPK) that functions downstream of TORC1, and which is essential for the survival of cells in which tunicamycin has induced a stressful increase of unfolded proteins. Rousseau and Bertolotti found that Mpk1 is required for the TORC1-mediated increase in RACs and proteasome subunits (Fig. 1a). Neither the abundance of their messenger RNAs nor the protein stability of these RACs and proteasome subunits was altered in response to rapamycin-mediated inhibition of TORC1, which indicates that the increased levels of these proteins probably occur though regulation of mRNA translation.

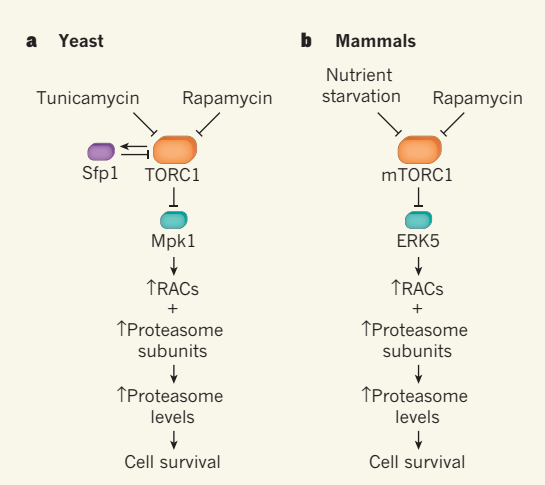

Figure 1 | Evolutionarily conserved regulation of proteasome abundance. **a**, Rousseau and Bertolotti[2] report that activation of the yeast mitogen-activated protein kinase enzyme (MAPK) known as Mpk1 mediates an increase in the levels of regulatory particle assembly chaperone proteins (RACs) and subunits required for the formation of the proteasome complexes that mediate protein degradation. Mpk1 is activated by inhibition of TORC1 protein kinase activity. TORC1 can be inhibited by tunicamycin or rapamycin treatment or by the action of the protein Sfp1. **b**, The authors also show that this process of proteasomal regulation is conserved in mammals — ERK5, the mammalian protein kinase most similar to Mpk1, is required to upregulate mammalian proteasome levels through an increase in RACs and proteasome subunits upon mTORC1 inhibition by compounds such as rapamycin or by nutrient starvation. In both yeast and mammals, an increase in proteasome abundance is necessary for cell survival under stress.

An enhanced proteasomal capacity enables cells to adapt to the rising demand for protein degradation that accompanies stress. The absence of proteasome induction, as tested by Rousseau and Bertolotti using cells in which the gene for Mpk1 had been deleted, severely impairs the clearance of ubiquitinated proteins and of well-characterized reporter substrates used to monitor proteasomal activity.

The authors found that in mammalian cells, ERK5, the mammalian equivalent of Mpk1, also facilitates a rapid rise in RAC and proteasome levels when mTORC1 (the mammalian equivalent of TORC1) is inhibited (Fig. 1b). Thus, the TORC1 and Mpk1 pathway is an evolutionarily conserved regulator of proteasomal homeostasis.

Rousseau and Bertolotti's work contributes an additional perspective to the current debate about the exact relationship between TORC1/mTORC1 and the regulation of proteasome function. Consistent with the model proposed by Rousseau and Bertolotti, a study by Zhao *et al.*[4] found that acute pharmacological inhibition of mTORC1 in the HEK293 mammalian cell line upregulates protein degradation by the proteasome. However, a report by Zhang *et al.*[3] reveals nuances in the regulation of the proteasome by mTORC1, and finds that in the absence of the protein TSC2, a major inhibitor of the mTORC1 pathway, the transcription factor NRF1 mediates an increase in levels of the proteasome and of intracellular amino acids.

The differences between these three studies[2–4] probably arise from variations in the extent to which mTORC1 is perturbed. Under acute mTORC1 inhibition, as studied by Rousseau and Bertolotti[2] and Zhao *et al.*[4], upregulation of the proteasome would increase amino-acid pools and permit the translation of proteins necessary for survival. mTORC1 inhibition induces autophagy, another major intracellular protein-degradation pathway that removes proteins in bulk from the cytoplasm and delivers them to an organelle called the lysosome for breakdown[1]. In combination, the rapid and coordinated activation of both the autophagic and proteasomal arms of protein degradation would be beneficial to cells as a mechanism to increase amino-acid levels under stress or nutrient deprivation.

However, under states of prolonged mTORC1 hyperactivation — for example, when TSC2 is lost, as investigated by Zhang *et al.*[3] — cells may also need to increase proteasomal capacity to counteract unrestrained consumption of resources driven by sustained mTORC1 activity. It will be informative to compare the regulation of the proteasome in genetic models in which mTORC1 is constitutively active but not hyperactivated — for example, in mice that have a constitutively active Rag GTPase enzyme[9].

How TORC1 inhibition increases proteasome-dependent degradation is another question requiring further investigation. Rousseau and Bertolotti found that this upregulated proteolysis depends on elevated proteasome levels, whereas the study by Zhao *et al.*[4] found that enhanced ubiquitination drives protein breakdown without a change in proteasome content or activity. It will also be of interest to determine whether specific proteins are preferentially targeted for proteasomal degradation when TORC1 is inhibited. Consistent with this possibility, Zhao *et al.*[4] found evidence for the selective proteasomal breakdown of growth-related proteins. Finally, given the integral link between ubiquitination and the proteasome, it is probable that both systems are concomitantly regulated under stress. The identification of enzymes called ubiquitin ligases and deubiquitinases, which are necessary to target substrates specifically to the proteasome, may provide a way to address this question.

From all these studies[2–4], it is clear that the TORC1/mTORC1 pathway is a central regulator of proteasome homeostasis. It will be necessary to resolve the differences in current models of how this pathway affects the proteasome, especially given that modulation of the proteasome might be a therapeutic approach for diseases such as cancer and neurodegeneration. ■

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1. Laplante, M. & Sabatini, D. M. *Cell* **149,** 274–293, (2012).
2. Rousseau, A. & Bertolotti, A. *Nature* **536,** 184–189 (2016).
3. Zhang, Y. *et al. Nature* **513,** 440–443 (2014).
4. Zhao, J., Zhai, B., Gygi, S. P. & Goldberg, A. L. *Proc. Natl Acad. Sci. USA* **112,** 15790–15797 (2015).
5. Zhao, J., Garcia, G. A. & Goldberg, A. L. *Nature* **529,** E1–E2 (2016).
6. Finley, D. *Annu. Rev. Biochem.* **78,** 477–513 (2009).
7. Suraweera, A., Münch, C., Hanssum, A. & Bertolotti, A. *Mol. Cell* **48,** 242–253 (2012).
8. Hanssum, A. *et al. Mol. Cell* **55,** 566–577 (2014).
9. Efeyan, A. *et al. Nature* **493,** 679–683 (2012).

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