1. Johnsson, E. S. *Annu. Rev. Biochem.* **74,** 355–382 (2004).
2. Kang, J. S., Saunier, E. F., Akhurst, R. J. & Derynck, R. *Nature Cell Biol.* **10,** 654–664 (2008).
3. Shi, Y. & Massagué, J. *Cell* **113,** 685–700 (2003).
4. Kavsak, O. *et al. Mol. Cell* **6,** 1365–1375 (2000).
5. Ebisawa, T. *et al. J. Biol. Chem.* **276,** 12477–12480 (2001).
6. Lin, X., Chen, Y.-G. & Feng, X.-F. in *The TGF-β Family* 287-332 (Cold Spring Harbor Laboratory Press, NY, 2008).
7. Feng, X.-H. & Derynck, R. *Annu. Rev. Cell Dev. Biol.* **21,** 659–93 (2005).
8. Shimizu, A. *et al. Genes Cells* **3,** 125–134 (1998).
9. Shi, W. *et al. J. Cell Biol.* **164,** 291–300 (2004).
10. Zhang, Y. E. in *The TGF-β Family* 419-438 (Cold Spring Harbor Laboratory Press, NY, 2008).
11. Chen, T., Carter, D., Garrigue-Antar, L. & Reiss, M. *Cancer Res.* **58,** 4805–4810 (1998).
12. Chen, T. *et al. Int. J. Cancer* **93,** 653–661 (2001).
13. Roberts, A. B. & Wakefield, L. M. *Proc. Natl Acad. Sci. USA* **100,** 8621–8623 (2003).
14. Martin, S., Nishimune, A., Mellor, J. R. & Henley, J. M. *Nature* **447,** 321–325 (2007).
15. Yingling, J. M., Blanchard, K. L., & Sawyer, J. S. *Nature Rev. Drug. Discov.* **3,** 1011–1022 (2004).

p53: The Janus of autophagy?

Beth Levine and John Abrams

The autophagy pathway functions in adaptation to nutrient stress and tumour suppression. The p53 tumour suppressor, previously thought to positively regulate autophagy, may also inhibit it. This dual interplay between p53 and autophagy regulation is enigmatic, but may underlie key aspects of metabolism and cancer biology.

p53, the 'guardian of the cellular genome', is the most commonly mutated gene in human cancers[1]. In response to DNA damage, oncogenic activation, hypoxia or other forms of stress, p53 acts through both transcription-dependent and -independent mechanisms to coordinate cellular responses, which either prevent or repair genomic damage or eliminate potentially oncogenic cells. Although the best-studied functions of p53 relate to its control of cell-cycle arrest and cell death, increasing evidence suggests that this protein represents a central node in stress- and nutritional-response networks. These diverse activities of p53 are important not only in tumour suppression but also in metabolism, development, ageing and neurodegeneration[1–3]. Another recently described p53-regulated cellular process is autophagy, a lysosomal pathway of cellular self-digestion, which represents an ancient mechanism used by eukaryotic cells to adapt to different forms of cellular stresses[4]. Previously, p53 activation was shown to induce autophagy[5–10]; however, on page 676 of this issue, Tasdemir *et al.* show that basal levels of p53 inhibit autophagy[11].

Autophagy is induced in response to various stress stimuli, including starvation, trophic factor deprivation, hypoxia, endoplasmic reticulum (ER) stress and oxidative stress[4]. Under these conditions, autophagy is induced through signalling events that commonly, but not invariably, involve activation of the nutrient energy sensor AMP kinase (AMPK), and inhibition of TOR (target of rapamycin). Formation of the autophagosome, a double-membraned vesicle that sequesters the cargo destined for degradation inside the lysosome, is mediated by a set of evolutionarily conserved proteins known as the Atg (autophagy-related) proteins. Through catabolism, autophagy supplies cells with amino acids and energy, allowing them to maintain vital functions and successfully adapt to environmental stress. Autophagy also has an essential role in cellular housekeeping, through routine protein and organelle turnover and the degradation of damaged organelles, toxic aggregate-prone mutant proteins and intracellular pathogens. Thus, autophagy has diverse physiological functions, including stress adaptation, development, lifespan extension, immunity and protection against neurodegeneration.

Autophagy can also function as a tumour suppressor or cell-survival pathway[4,12]. Deletion of autophagy genes, such as *UVRAG* and *beclin 1*, are common in human cancer. Many of these genes, including *beclin 1*, *atg4C* and *atg5*, function as tumour suppressors in knockout or tumour xenograft mouse models. Loss of autophagy genes leads to increased DNA damage, chromosomal instability and deregulated control of cell growth, indicating a potential overlap in tumour suppressor-related autophagy effects and p53 actions. Paradoxically, elevated autophagy, often associated with the tumour microenvironment and/or treatment with cytotoxic agents, can also increase tumour cell survival and in this sense, is pro-oncogenic.

The role of autophagy in tumour suppression is consistent with previous studies indicating that p53 positively regulates autophagy (Fig. 1a). For example, genotoxic stress caused by DNA-damaging agents induces p53-dependent autophagy[5,6]. Similarly, oncogenic activation, simulated by forced expression of ARF or p53, induces autophagy in human cancer cells[7]. The mechanisms of p53-dependent induction of autophagy are still incompletely understood, but are thought to involve both transcription-independent functions (for example, AMPK activation), as well as transcription-dependent functions (for example, upregulation of mTOR inhibitors, PTEN and TSC1, or the p53-regulated autophagy and cell death gene, DRAM)[5,8]. In some cases, p53-induced autophagy may lead to cell death and this can be blocked by *DRAM* siRNA[8]. However, in *c-myc*-driven lymphomas, p53-mediated autophagy increases cell survival, as blockade of autophagosomal maturation enhances p53-mediated tumour regression and tumour-cell death[9,10]. These seemingly disparate effects of p53-mediated autophagy on life and death decisions of the cell may be cell-type or stimulus-specific, and/or reflect the activation of a different constellation of p53 signals.

The mysteries underlying p53 regulation of autophagy extend beyond the question of whether p53-mediated autophagy is pro-death or pro-survival. Tasdemir *et al.* directly challenge the notion that p53 is a positive regulator

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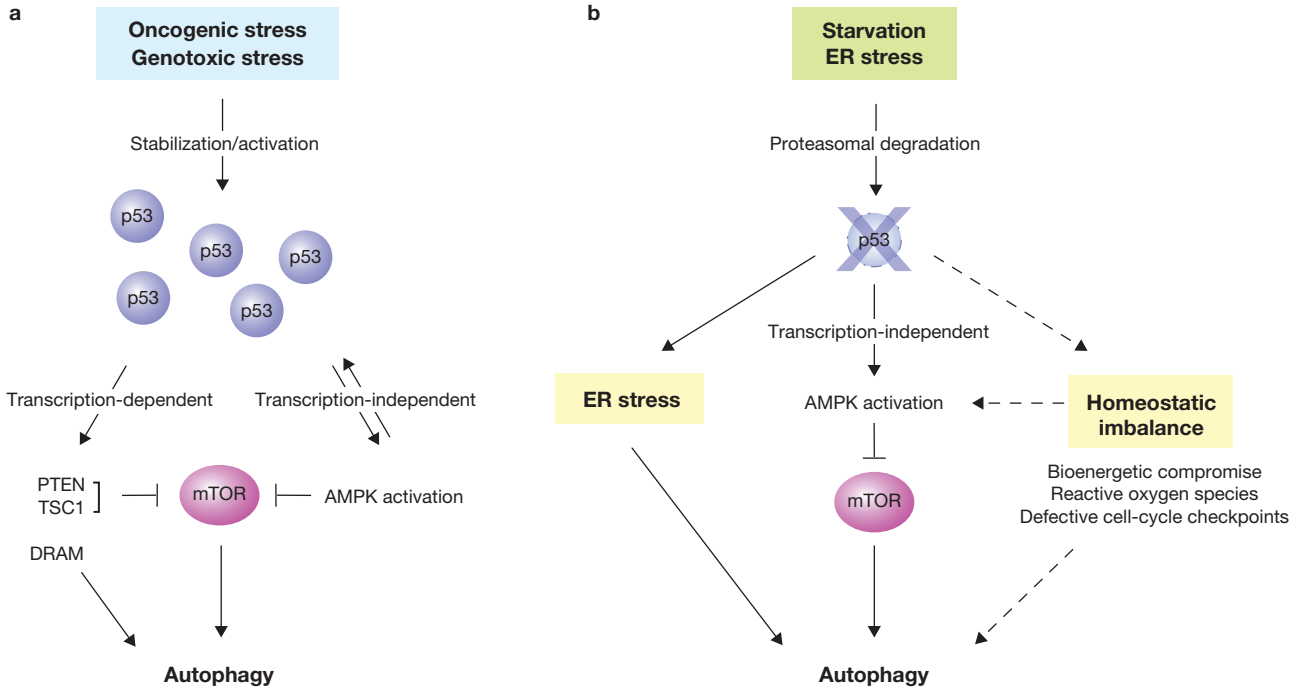

Figure 1 Models for positive (**a**) and negative (**b**) regulation of autophagy by p53. (**a**) Onocogenic and genotoxic stress result in p53 stabilization and activation, which is believed to stimulate autophagy through both transcription-independent mechanisms (for example, AMPK activation, mTOR inhibition) and transcription-dependent mechanisms (for example, PTEN, TSC1 and DRAM transcriptional upregulation). (**b**) Genetic or chemical inhibition of p53 (not depicted here), or proteasomal depletion of p53 during starvation and ER stress, activates autophagy through transcription-independent mechanisms involving AMPK activation and mTOR inhibition. P53 loss also leads to ER stress-induced autophagy. Dotted lines represent a speculative pathway by which p53 depletion may also result in autophagy. p53 loss leads to homeostatic imbalance (for example, bioenergetic compromise, reactive oxygen species, defective cell-cycle checkpoints), which leads to autophagy; therefore, p53 depletion may induce autophagy indirectly through homeostatic imbalance.

of autophagy[11] (Fig. 1b). The authors show that chemical inhibition of p53 with pfithrin-α, knockdown of *p53* with siRNA or genetic deletion of *p53* increases autophagy in both normal and transformed cells. Autophagy induced by p53 loss is canonical in that it is associated with AMPK activation and TOR repression, and is inhibited by knockdown of *AMPK* or autophagy genes, including *atg5*, *beclin 1*, or *atg10*. Pharmacological inhibition, silencing or knockout of p53 also resulted in enhanced basal levels of autophagy in mice and in nematodes, suggesting that p53 negatively regulates autophagy *in vivo* and in a phylogenetically conserved fashion. This negative regulation seems to involve transcription-independent effects of p53, at least *in vitro*, as cytoplasmic or ER-targeted (but not nuclear-targeted) p53 inhibits autophagy in p53-deficient cells.

Thus, similar to the Roman God Janus, who had two heads facing opposite directions, p53 regulates autophagy in a two-faced fashion — p53 turns autophagy on and p53 turns autophagy off. Are these two functions of p53 reconcilable? To date, the contexts of p53-mediated induction and inhibition of autophagy seem to be distinct. Stimulation of autophagy by p53 occurs when cells are subjected to oncogenic activation or genotoxic stress and p53 is activated[5–7]. In contrast, p53 loss induces autophagy in the absence of stress signals, suggesting that basal levels of p53 activity (rather than activated p53) inhibit autophagy[11]. There are other precedents for the dichotomous functions of basal versus activated p53; for example, basal levels of p53 promote cell survival under normal growth conditions, whereas high levels of p53 promote cell death in response to acute stress[1]. One way to reconcile the p53/autophagy 'Janus conundrum' would be to postulate that basal levels of p53 coordinate regulatory outputs that are distinct from those propagated in stimulus-activated contexts. Consistent with this notion, expression profiling in *Drosophila melanogaster* indicates that the scope of p53-dependent expression in development far exceeds stimulus-induced p53-dependent signatures[13]. Tasdemir *et al.* compared the transcriptomes of wild-type and p53-deficient cells, and observed comparable expression of autophagy-related transcripts[11]. An analysis of differences that do exist in the transcriptomes may provide some clues regarding autophagy inhibition by basal p53 in mammalian cells.

The findings of Tsademir *et al.*, however, suggest a more complex model, in which switching autophagy 'on' or 'off' by p53 is not simply dictated by a dichotomy of basal levels versus stress-induced levels, but also by the specific nature of the stress signal. The authors found that not only does inhibition of basal p53 induce autophagy, but also that proteasomal-mediated p53 depletion seems to be a prerequisite for autophagy induction in response to physiologically important stress stimuli, such as starvation or ER stress. Thus, it is possible that the nature of directionality of autophagy regulation by p53 is in part determined by the nature of the stress stimulus, with divergent actions of oncogenic and genotoxic stress versus starvation and ER stress. In this regard, perhaps p53 operates within a broader regulatory network to define autophagic control. Another plausible angle, not yet tested, could involve recently recognized p53 isoforms[14] whose functions are not yet known but may conceivably influence autophagy in context-specific ways.

Although it is perhaps straightforward to imagine how different stressors may activate different downstream signalling pathways, resulting in opposite effects of p53 on autophagy, it is more difficult to imagine how p53 activation or depletion results in similar modulation of identical downstream autophagy effectors, such as AMPK activation and mTOR inhibition. This effect could be indirect and the homeostatic imbalance imposed by p53 loss, such as bioenergetic compromise, oxidative stress or defective cell-cycle checkpoints, could constitute stress stimuli that activate autophagy in a classical manner. Consistent with the hypothesis that p53 loss may indirectly turn on autophagy by activating autophagy-inducing stress pathways, Tasdemir *et al.* show that p53 inhibition results in ER stress, and that blocking ER stress by *IRE-1*α knockdown decreases autophagy induced by p53 loss. It will be important to determine whether ER stress and/or metabolic consequences of p53 loss are mediators of autophagy induction *in vivo* and specifically in the context of tumour development. Perhaps cancer cells meet their energy demands in the setting of impaired mitochondrial function conferred by p53 loss, not only by aerobic glycolysis (the Warburg effect), but also by increased autophagy. Indeed, Tasdemir *et al.* show that maintenance of cytosolic ATP levels (and cellular viability) that occurs selectively in $p53^{-/-}$ but not $p53^{+/+}$ glucose-deprived colon cancer cells is autophagy-dependent.

The bidirectional control of autophagy by p53 raises important questions for future research. At the molecular level, how can we mechanistically resolve the Janus paradox that p53 governs autophagy in seemingly opposing ways? On a more global level, how do we ultimately integrate such knowledge within the broader context of autophagy control, metabolism and cancer biology? To what extent does autophagy induction contribute to p53-dependent tumour suppression, and conversely, to what extent does autophagy induction contribute to tumour cell survival in p53-deficient cells? How does bidirectional control of autophagy by p53 interface with its diverse effects on apoptosis, cell-cycle checkpoints and metabolism? Looking beyond metabolism and cancer, the negative regulation of autophagy by basal p53 may also contribute to p53 effects on premature ageing and neurodegeneration. With these biomedical implications of the p53/autophagy axis, solving the Janus conundrum now becomes a central imperative.

1. Vousden, K. H. & Lane, D. P. *Nature Rev. Mol. Cell Biol.* **8,** 275–283 (2007).
2. Brensaad, K. & Vousden, K. H. *Trends Cell Biol.* **17,** 286–291 (2007).
3. Green, D. R. & Chipuck, J. E. *Cell* **126,** 30–32 (2006).
4. Levine, B. & Kroemer, G. *Cell* **132,** 27–42 (2008).
5. Feng, Z., Zhang, H., Levine, A. J. & Jin, S. *Proc. Natl Acad. Sci. USA* **102,** 8204–8209 (2005).
6. Zeng, X., Yan, T., Schupp, J. E., Seo, Y. & Kinsella, T. J. *Clin. Cancer Res.* **13,** 1315–1321 (2007).
7. Abida, W. M. & Gu, W. *Cancer Res.* **68**, 352–357 (2008).
8. Crighton, D. *et al. Cell* **126,** 121–134 (2006).
9. Amaravadi, R. K. *et al. J. Clin. Invest.* **117,** 326–336 (2007).
10. Maclean, K. H., Dorsey, F. C., Cleveland, J. L. & Kastan, M. B. *J. Clin. Invest.* **118,** 79–88 (2008).
11. Tasdemir, E. *et al. Nature Cell Biol.* **10**, 676–687 (2008).
12. Maiuri, M. C., Zalckvar, E., Kimchi, A. & Kroemer, G. *Nature Rev. Mol. Cell Biol.* **8,** 741–752 (2007).
13. Akdemir, F., Christich, A., Sogame, N., Chapo, J. & Abrams, J. M. *Oncogene* **26,** 5184–5193 (2007).
14. Bourdon, J. C. *et al. Genes Dev.* **19,** 2122–2137 (2005).

Skeletal muscle dressed in SOCs

Dong Min Shin and Shmuel Muallem

Store-operated Ca^{2+} channels (SOCs) are activated in response to Ca^{2+} release from the endoplasmic reticulum (ER). The stromal interaction molecule 1 (STIM1) is the ER sensor that transmits the stored Ca^{2+} content to the pore-forming SOCs Orai and TRPC channels. Recent studies reveal high levels of Orai1 and STIM1 in skeletal muscle, and a prominent role of SOCs in muscle development and function.

Skeletal muscle, Ca^{2+} and contraction are associated with L-type Ca^{2+} channels (LTCCs), ryanodine receptors (Ryrs), sarcoplasmic reticulum and ER Ca^{2+} ATPase (SERCA) pumps and excitation-contraction (E–C) coupling, but not with SOCs. However, this notion will have to be modified in view of recent studies showing that skeletal muscle is a prime site of expression and function of SOCs. On page 688 of this issue, Stiber *et al.*[1] report that the Ca^{2+} sensor STIM1 is expressed at a particularly high level in skeletal muscle, and that it is crucial for muscle development and function. Skeletal muscle also expresses a very high level of Orai1, the pore-forming subunit of SOCs[2].

SOCs have mostly been associated with non-excitable cells. Release of Ca^{2+} from the ER activates SOCs, which mediate receptor-stimulated influx of extracellular Ca^{2+} (ref. 3). The molecular identity of SOCs and how they are activated by the release of stored Ca^{2+} has been recently elucidated with the discovery of STIM1 and the Orai family of Ca^{2+} channels[4,5]. STIM1 is characterized by an EF hand Ca^{2+}-binding domain and a sterile-alpha-motif (SAM) domain that reside in the ER lumen, a single transmembrane domain and cytoplasmic Ezrin-radixin-moesin (ERM), Ser/Pro-rich and Lys-rich domains. The *Orai* gene family codes for the channels that mediate the Ca^{2+}-release-activated Ca^{2+} (CRAC) current I_{crac} (refs 6, 7). SOCs also include TRPC channels, which mediate a large fraction of the agonist-activated Ca^{2+} influx in many cell types[3]. In response to Ca^{2+} release from the ER, STIM1 clusters into punctae at ER/plasma membrane microdomains[8], where it interacts with and activates Orai[6,7] and

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