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# AMPK and autophagy get connected

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**The energy sensor AMP-activated protein kinase (AMPK), which is activated by ATP depletion or glucose starvation, was previously thought to activate autophagy entirely through its ability to inactivate mTOR complex-1. Two recent papers now provide evidence that it also mediates this by direct phosphorylation of the protein kinase that initiate autophagy, ULK1. Surprisingly, however, the two groups identified different sites on ULK1.**

Autophagy ('self-eating') is a process in eukaryotic cells in which regions of cytoplasm, insoluble protein aggregates, damaged mitochondria, or even invading pathogens are engulfed by a double membrane, forming vesicles that then fuse with lysosomes to degrade the contents. It is also induced by amino-acid starvation or by energy stress, most likely to recycle amino acids for synthesis of essential proteins, or to generate catabolic substrates for ATP production. Although first observed by electron microscopists over 50 years ago (De Duve and Wattiaux, 1966), the field took off in the early 1990s when numerous yeast genes were identified (most of which have orthologues in humans) in which mutations interfere with autophagy. The first gene identified was *ATG1* (originally *APG1* (Tsukada and Ohsumi, 1993)), which is fitting because it encodes the catalytic subunit of a protein kinase complex that triggers autophagy. Two recent papers from the groups of Shaw (Egan *et al*, 2011) and Guan (Kim *et al*, 2011) suggest that energy stress triggers autophagy in mammalian cells by activating AMPK, which then directly phosphorylates ULK1, one of the two mammalian orthologues of Atg1 that forms a complex with ATG13 and FIP200.

AMPK is activated by increases in ADP and AMP, signals that the energy state of the cell is compromised. It is only active when phosphorylated at T172 on the catalytic (α) subunit by upstream kinases such as LKB1, and binding of ADP and AMP to the γ subunit cause conformational changes that inhibit T172 dephosphorylation and cause further allosteric activation (Hardie, 2007). The key amino-acid sensor in eukaryotic cells is target-of-rapamycin complex-1 (TORC1), which is activated by amino acids and inhibited by the antibiotic rapamycin (Wullschleger *et al*, 2006). Experiments with rapamycin showed that TORC1 suppresses autophagy, and since AMPK is known to inhibit TORC1 by phosphorylating both TSC2 (Inoki *et al*, 2003) and Raptor (Gwinn *et al*, 2008), it had been widely assumed that this was how energy stress activated autophagy. However, the two new papers suggest an additional, more direct mechanism (Figure 1), although there are some surprising discrepancies.

The two groups approached from different directions. Shaw's group were searching for new AMPK targets, and used a two part screen, first looking for proteins that interacted with 14-3-3 proteins (a common consequence of phosphorylation) when AMPK was activated, and then examining whether these had conserved sequences fitting the established recognition motif for AMPK (Scott *et al*, 2002; Gwinn *et al*, 2008). By contrast, Guan's groups started with

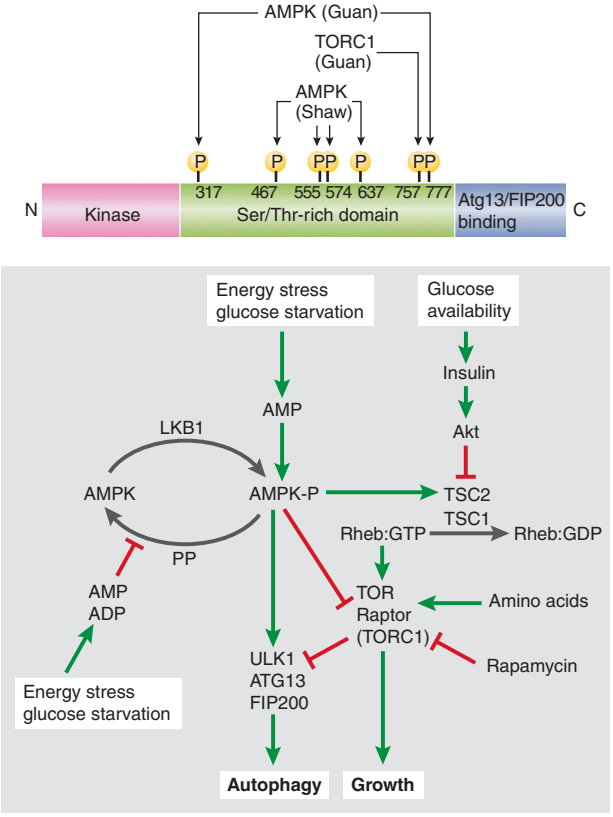


**Figure 1** Domain structure of ULK1 with locations of phosphorylation sites identified by the Shaw and Guan groups (top); and summary of signalling pathways impacting on autophagy or cell growth (bottom). Numbering of ULK1 residues is based on the human sequence; most of the putative phosphorylation sites are conserved across mammals but, because of difficulties in making alignments of the poorly conserved S/T-rich domain, it is not easy to assess which are conserved in lower eukaryotes, including yeast.

observations of a shift in ULK1 electrophoretic mobility in cells starved of glucose. Both provided evidence that AMPK would phosphorylate ULK1 in cell-free assays and intact cells. Shaw identified four sites (S467, S555, T574, and S637) based on the use of mass spectrometry and phosphospecific antibodies. Surprisingly, Guan found that mutations of two of the sites identified by Shaw (S555 and T574) had no effect on phosphorylation by AMPK, and proposed instead, by making systematic truncations and point mutations, two completely different sites (S317 and S777). All six sites lie within the central Ser/Thr-rich region, which is less well conserved than the kinase domain, or the C-terminal domain that binds ATG13 and FIP200 (Figure 1). To address the role of the sites, Shaw introduced wild-type ULK1 or a quadruple mutant (S467A, S555A, T574A, S637A), into human cells in which endogenous ULK1 had been knocked down or ULK1$^{-/-}$ mouse embryo fibroblasts (MEFs), while Guan's group introduced a double mutant (S317A, S777A) into ULK1$^{-/-}$ MEFs. Despite the fact that there was no overlap in the mutations made, both reported that markers of autophagy were reduced in the mutant cells, either under basal conditions or during nutrient starvation.

Guan also studied interactions between AMPK, TORC1, and ULK1. As recently reported (Behrends *et al*, 2010), ULK1 forms a stable complex with AMPK, but Guan found that this was disrupted by activating TORC1 via overexpression of Rheb, an effect blocked by rapamycin. By making more truncations and point mutations, they pinpointed S757 as a site phosphorylated by TORC1 on ULK1, and they propose that this disrupts the AMPK–ULK1 interaction.

Taken together, these results suggest that AMPK activation activates autophagy via a dual 'fail-safe' mechanism involving not only inactivation of TORC1 but also direct phosphorylation of ULK1 (Figure 1). However, the fact that the two groups identified multiple, yet different, AMPK sites on ULK1 is rather perplexing, and suggests that the story is not as simple as either group suggests. This discrepancy also illustrates the limitations of the methods used to identify phosphorylation sites. The mass spectrometry methods used by Shaw (and the phosphospecific antibodies used by both groups) confirm that a site is phosphorylated but do not give much information about stoichiometry. Conversely, the mutational approach used by Guan is open to artefacts, since mutations can affect phosphorylation indirectly through structural alterations. The fact that neither of the sites detected by Guan are good fits to the established AMPK recognition motif (Scott *et al*, 2002; Gwinn *et al*, 2008) casts doubt on whether they are directly phosphorylated by AMPK. One possibility is that Guan's sites are phosphorylated by different kinases, as a secondary response to phosphorylation by AMPK at other sites, possibly including some of those identified by Shaw. Clearly, further work is required to resolve these issues.

## Conflict of interest

The authors declare that they have no conflict of interest.

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