AMPK Activates Autophagy by Phosphorylating ULK1

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Phosphorylation of ULK1 (hATG1) by AMP-Activated Protein Kinase Connects Energy Sensing to Mitophagy


In eukaryotes, macroautophagy (hereafter, autophagy) functions as a highly conserved catabolic mechanism in which cytoplasm, including damaged organelles and protein aggregates, is sequestered into autophagosomes, double-membrane vesicles, for ultimate degradation and recycling in lysosomes. Autophagy has attracted increasing attention in recent years because of its significance in various aspects of cell physiology, including survival during nutrient or energy limitation, and in the clearance of excess or dysfunctional proteins and organelles. In addition, autophagy is associated with a range of human pathophysiological conditions, including cancer, neurodegeneration, and cardiomyopathies. Autophagy is tightly regulated because either too much or too little can be detrimental to cell physiology. The ULK1 (mammalian homolog of yeast Atg1) kinase complex plays a central role in autophagy induction; the mTORC1 kinase complex, one of the primary negative regulators of autophagy, functions, in part, by phosphorylating and inhibiting ULK1. In contrast, AMPK is a positive regulator that stimulates autophagy in response to energy depletion. Two recent studies suggest that AMPK directly regulates autophagy through phosphorylating and activating ULK1.1,2 mTORC1-dependent phosphorylation appears to work antagonistically to that of AMPK and prevents the physical association of AMPK with ULK1.

AMPK is an evolutionarily conserved cellular energy manager controlling nutrient sensing and energy homeostasis. Previous studies suggest a role for AMPK in autophagy induction,3,4 dependent on ULK1.5 However, it has been thought that AMPK triggers autophagy through an indirect mechanism, inhibiting mTORC1 activity by phosphorylating TSC2 and Raptor.6,7 Research by the Shaw and Guan groups indicate a more direct mechanism whereby AMPK regulates autophagy through phosphorylation of ULK1. This finding significantly improves our understanding of the mechanism that connects cellular energy homeostasis and autophagy. The two groups initiated their studies based on different strategies. Shaw’s group utilized a two-part screen to identify the substrates of AMPK by first focusing on proteins containing AMPK phosphorylation sites. Experiments on mTORC1 kinase complex with ULK1-mATG13-FIP200.11,12 ATG101 is a mATG13 binding protein that forms a stable complex with AMPK and mATG13, reducing ULK1 kinase activity. B, Following glucose starvation, mTORC1 dissociates from the complex allowing AMPK to bind and phosphorylate ULK1. The autophosphorylation activity of ULK1 increases, allowing ULK1 to phosphorylate mAtg13 and FIP200 (the potential candidates based on their ability to bind a recombinant 14-3-3 protein in an AMPK-dependent manner. In contrast, Guan’s group observed an electrophoretic phosphorylation-dependent mobility shift of ULK1 in glucose-starved cells and sought to directly map the relevant sites, as well as examine the role of AMPK. Both groups confirmed the AMPK phosphorylation of ULK1 in vivo and in vitro. Interestingly, different phosphorylated sites (S467, S555, S574, and S637 from Shaw’s group; S317 and S777 from Guan’s group) in ULK1 were identified. Although both groups demonstrated the necessity of these target sites for efficient autophagy induction, neither used phosphomimetic analyses to show their sufficiency. Thus, multiple sites are likely phosphorylated, and the two studies are not necessarily contradictory.

Egan et al observed defects in selective mitochondrial degradation by autophagy (mitophagy) in AMPK- or ULK1-deficient hepatocytes, whereas Kim et al showed an autophagy defect in ULK1-deficient MEFs upon glucose starvation. Both labs provided evidence that the nonphosphorylatable ULK1 (4SA or 2SA from the Shaw and Guan groups, respectively) is unable to rescue the mitophagy defect in ULK1-deficient hepatocytes, whereas Kim et al showed an autophagy defect in ULK1-deficient MEFs upon glucose starvation. Both labs provided evidence that the nonphosphorylatable ULK1 (4SA or 2SA from the Shaw and Guan groups, respectively) is unable to rescue the mitophagy defect in ULK1-deficient hepatocytes, whereas Kim et al showed an autophagy defect in ULK1-deficient MEFs upon glucose starvation. Both labs provided evidence that the nonphosphorylatable ULK1 (4SA or 2SA from the Shaw and Guan groups, respectively) is unable to rescue the mitophagy defect in ULK1-deficient hepatocytes, whereas Kim et al showed an autophagy defect in ULK1-deficient MEFs upon glucose starvation. Both labs provided evidence that the nonphosphorylatable ULK1 (4SA or 2SA from the Shaw and Guan groups, respectively) is unable to rescue the mitophagy defect in ULK1-deficient hepatocytes, whereas Kim et al showed an autophagy defect in ULK1-deficient MEFs upon glucose starvation. Both labs provided evidence that the nonphosphorylatable ULK1 (4SA or 2SA from the Shaw and Guan groups, respectively) is unable to rescue the mitophagy defect in ULK1-deficient hepatocytes, whereas Kim et al showed an autophagy defect in ULK1-deficient MEFs upon glucose starvation. Both labs provided evidence that the nonphosphorylatable ULK1 (4SA or 2SA from the Shaw and Guan groups, respectively) is unable to rescue the mitophagy defect in ULK1-deficient hepatocytes, whereas Kim et al showed an autophagy defect in ULK1-deficient MEFs upon glucose starvation.

Both studies reveal a direct connection between energy sensing and autophagy, although there are many further questions that remain unanswered. For example, under autophagy-inducing conditions, mTORC1 is inactivated and dissociates from the ULK1-mAtg13-FIP200 complex, which allows ULK1 to phosphorylate mAtg13 and FIP200 (the
homolog of yeast Atg17). However, autophagy cannot be activated continually or it will result in cell death. Thus, if the cells are still maintained in conditions of glucose starvation, how is autophagy downregulated? Does mTORC1 become reactivated to block AMPK binding to ULK1? Does another factor inactivate AMPK? The mechanism by which ULK1 phosphorylation is controlled to avoid excessive autophagy is as important to understand as that which regulates autophagy induction.

One significant role of autophagy is to maintain cardiomyocyte structure and function in basal conditions and to protect the heart during myocardial ischemia. In particular, ischemia-induced autophagy requires activation of AMPK and inhibition of mTORC1, which is a classical starvation-induced autophagy model. Confirming the phosphorylation of ULK1 by AMPK in myocardial cells and determining how to take advantage of this event to enhance autophagy and protect cardiomyocytes during ischemia might be beneficial with regard to future therapeutic applications. Furthermore, the autophagy that occurs during myocardial reperfusion after ischemia, which is Beclin 1-dependent and AMPK-independent, might play a negative role for cell survival. Understanding the mechanism by which AMPK-activated autophagy is downregulated might allow clinicians to reduce the autophagic flux before reperfusion, which might alleviate the deleterious aspects of autophagy and protect the heart.

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References
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