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Autophagy up and down by outsmarting the incredible ULK

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ABSTRACT

Macroautophagy/autophagy initiation is finely regulated by post-translational modifications of key proteins, to comply with the fast kinetics of the cellular response to several stress stimuli. Phosphorylation and ubiquitination play a central role in controlling autophagy by influencing the activity, recruitment and turnover of autophagic components. Recently, we found that, upon autophagy progression, ULK1 kinase is specifically ubiquitinated by the E3 ligase NEDD4L and then degraded via the proteasome. However, during prolonged autophagy, while the ULK1 protein undergoes this inhibition, *ULK1* mRNA is actively transcribed, translated and then inhibited again by MTOR-dependent inhibitory phosphorylation. This regulation is essential to promptly restore the ULK1 protein to its original levels to keep autophagy under a safe and physiological threshold.

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Physiological levels of autophagy promote cellular survival in response to a variety of stress conditions, including starvation, hypoxia, mitochondrial damage and pathogen infection. Autophagy is rapidly upregulated when cells need to generate intracellular nutrients and energy, as well as when they are preparing to undergo structural remodelling, as occurs during development and differentiation. Post-translational modifications are essential for modulating autophagy to adapt rapidly to different types of environmental stress, regarding both the amplitude of autophagy activity and its duration. These modifications help regulate autophagy at multiple levels; autophagy core components can be directly modified, either being activated or inhibited. The master regulator of autophagy is MTOR (mechanistic target of rapamycin), which drives the major inhibitory signal that shuts autophagy off in the presence of growth factors and nutrients. In conditions of nutrient abundance, the ULK1-ATG13-RB1CC1/FIP200 complex is inhibited through a direct MTOR-mediated phosphorylation on both ULK1 and ATG13. During autophagy induction, MTOR dissociates from the ULK1 complex, leading to its dephosphorylation and, consequently, activation. However, how transcriptional, post-transcriptional, and post-translational mechanisms function to regulate autophagy under inducing conditions (i.e., to prevent excessive autophagy) remains to be fully elucidated. In our paper, we have uncovered new pieces of this tangled regulation.

We found that, after the first few hours of starvation, ULK1 protein levels are downregulated by the E3 ligase NEDD4L; in more detail, NEDD4L ubiquitinates ULK1 by both K27- and K29-linked ubiquitin chains and thus mediates its proteasomal degradation (Fig. 1). Acting on an upstream player of autophagy such as ULK1, autophagy could be easily turned off. Meanwhile, *ULK1* mRNA is actively transcribed and, after that, translated upon MTOR-dependent reactivation of translation. According to this model, MTORC1 activity can drive alternating periods of mutually exclusive autophagy and protein synthesis. It is notable that restored levels of ULK1 are quite similar to original levels in wild-type cells, and are also maintained in an inhibited state by a direct rephosphorylation by MTOR. Negative and positive regulation of ULK1 can be viewed as a regulatory mechanism that protects a cell against excessive autophagy, which could compromise cell viability. It is intriguing to consider the possibility that, in combination with mutual inhibition of MTORC1 and ULK1, this regulation may generate pulses of autophagy separated by periods of translation that allow the use of amino acids generated through autophagy and recovery from self-eating. Of note, through preventing ULK1 protein rescue by inhibiting transcription by means of actinomycin D, we blocked autophagy re-induction after nutrient replenishment. On the contrary, by maintaining high levels of ULK1, by NEDD4L downregulation, autophagy re-induction results in higher activity than in control conditions, supporting the importance of a ULK1 balance in autophagy modulation.

Moreover, our findings also underline the important role played by autophagy as an amino acid producer. Indeed, autophagy-derived amino acids generated during starvation are not only used for general protein synthesis but also to produce specifically key autophagy molecules, such as ULK1, to have them ready again to be used.

Furthermore, thinking about autophagy as an oscillatory process, we cannot but take into account the role of

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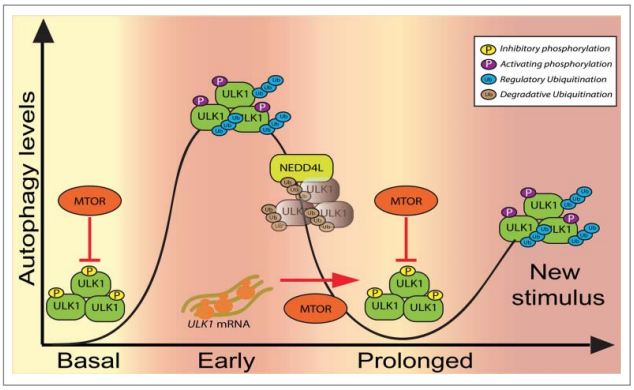

Figure 1. Autophagy oscillates through ULK1-MTOR crosstalk. In basal conditions, the ULK1 complex is maintained in an inhibited state by MTOR. During the early phase of autophagy induction, ULK1 is activated by both phosphorylation and regulatory ubiquitination; in enduring starvation, NEDD4L promotes ULK1 degradation by the proteasome, while *ULK1* mRNA is actively transcribed. During prolonged starvation, MTOR is reactivated and *ULK1* mRNA is translated and then inhibited again by MTOR, preventing excessive autophagy. When a new stimulus occurs, the system is thus ready for a new round of autophagy. This regulation permits an oscillatory autophagy pattern.

autophagy in circadian rhythm. Many advantages for multicellular organisms to maintain cellular homeostasis and tissue metabolism come, indeed, from the strict coupling of autophagy-mediated molecular degradation to the biological clock. However, the significance of autophagy cycles in physiology and pathology is far from being clear.

Finally, these findings shed new light on the strong interplay existing between protein ubiquitination and autophagy regulation. ULK1 itself is modulated by both regulatory ubiquitination (by TRAF6) and by at least 3 other E3 ubiquitin ligases controlling its stability, in different circumstances and conditions. For example, the RING E3 ligase MUL1 mediates ULK1 degradation after its translocation to mitochondria in selenite-treated cells. Moreover, recent research has shown that ULK1 is targeted by the E3 ligase CUL3/CULLIN-3 for proteasomal degradation at the end of the autophagy response. In addition, the "incredible" kinase ULK1 is not the only autophagy upstream regulator affected by ubiquitination. Together with ULK1, the AMBRA1, BECN1/Beclin 1 and PIK3C3/Vps34 proteins are the "Fantastic Four" of autophagy regulation that undergo a crucial control by ubiquitin. Obviously, the deep consequences of such a crosstalk between proteasome-mediate proteostasis and autophagy represent a new frontier in biomedicine.