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Comprehensive Decoding of Phosphorylation Events Regulated by Atg1/ULK1 Complex and TORC1

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Autophagy is a conserved eukaryotic cellular degradation processes, which targets cytoplasmic contents, macromolecules, and dysfunctional organelles for degradation. Dysregulation of autophagy has been linked to various human diseases, such as Vici syndrome, Parkinson's disease, cancer, and Crohn's disease. This work focussed mainly on macroautophagy, in which cytoplasmic components are sequestered within a double-membrane vesicle called autophagosome for lysosomal degradation. This process is induced under different stresses such as nutrient deprivation, lack of growth factors, and infection. Although substrates of both TORC1 and Atg1/ULK1 complexes have been identified supporting their roles in different stages of autophagy, there is still a need to generate comprehensive signal transduction cascades regulated by the two kinase complexes and being critical for autophagosome formation and fusion. In this thesis, we combined classic MS-based *in vivo* SILAC phosphoproteomics with a newly developed *in vitro* kinase assay to generate comprehensive datasets of protein phosphorylation events in autophagy pathway. Specifically, we identified new bona fide substrates of TORC1 and Atg1 in yeast and those of ULK1 in mammalian cells. Our results revealed, that an exquisitely multilayered regulatory network appears to coordinate TORC1 and Atg1 activities to robustly tune autophagy in response to nutritional cues. Furthermore, we also showed, that ULK1 is not only a PP2A target but also directly phosphorylates the regulatory PP2A subunit striatin, activating PP2A and serving as positive feedback to promote autophagy-dependent protein turnover. Thus, ULK1 and phosphatase activities are tightly coordinated to robustly regulate protein degradation by autophagy.

Jury:
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