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Quality control autophagy

A joint effort of ubiquitin, protein deacetylase and actin cytoskeleton

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Autophagy has been predominantly studied as a nonselective self-digestion process that recycles macromolecules and produces energy in response to starvation. However, autophagy independent of nutrient status has long been known to exist. Recent evidence suggests that this form of autophagy enforces intracellular quality control by selectively disposing of aberrant protein aggregates and damaged organelles—common denominators in various forms of neurodegenerative diseases. By definition, this form of autophagy, termed *quality-control (QC) autophagy*, must be different from nutrient-regulated autophagy in substrate selectivity, regulation and function. We have recently identified the ubiquitin-binding deacetylase, HDAC6, as a key component that establishes QC. HDAC6 is not required for autophagy activation per se; rather, it is recruited to ubiquitinated autophagic substrates where it stimulates autophagosome-lysosome fusion by promoting F-actin remodeling in a cortactin-dependent manner. Remarkably, HDAC6 and cortactin are dispensable for starvation-induced autophagy. These findings reveal that autophagosomes associated with QC are molecularly and biochemically distinct from those associated with starvation autophagy, thereby providing a new molecular framework to understand the emerging complexity of autophagy and therapeutic potential of this unique machinery.

Key words: quality control autophagy, actin, ubiquitin, HDAC6, autophagosome-lysosome fusion

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For decades, autophagy has been known simply as degradative machinery that, by nonselective self-digestion of cytosolic contents and organelles, supplies essential macromolecules to cells subject to starvation. However, it has become apparent that autophagy is not solely dedicated to nutrient management. One main function of this nutrient-independent, or often-called "basal," autophagy is to enforce intracellular quality control by eliminating toxic protein aggregates or damaged organelles, two common denominators in age-related disorders such as neurodegenerative disease. We therefore refer to this form of autophagy as *quality-control* (QC) autophagy to highlight this function. By definition, QC autophagy and starvation-induced autophagy would be distinct in their substrate selectivity, regulation and function. For example, QC autophagy would only process damaged proteins or mitochondria but not their functional counterparts. Elucidating the molecular and biochemical basis that establishes features unique to these autophagic modes would be fundamental for understanding the complexity as well as disease implications of autophagy.

In our recent study, we identified the ubiquitin-binding deacetylase HDAC6 and cortactin-dependent actin cytoskeleton as two central components that define and distinguish QC autophagy from starvation-induced autophagy. Most interestingly, we find that HDAC6-dependent actin-remodeling machinery promotes QC autophagy not by influencing formation or targeting of autophagosomes to substrates, but rather by stimulating their eventual fusion to lysosomes. These findings show that the fusion of autophagosomes to lysosomes, a crucial step for productive autophagy, is actively regulated.

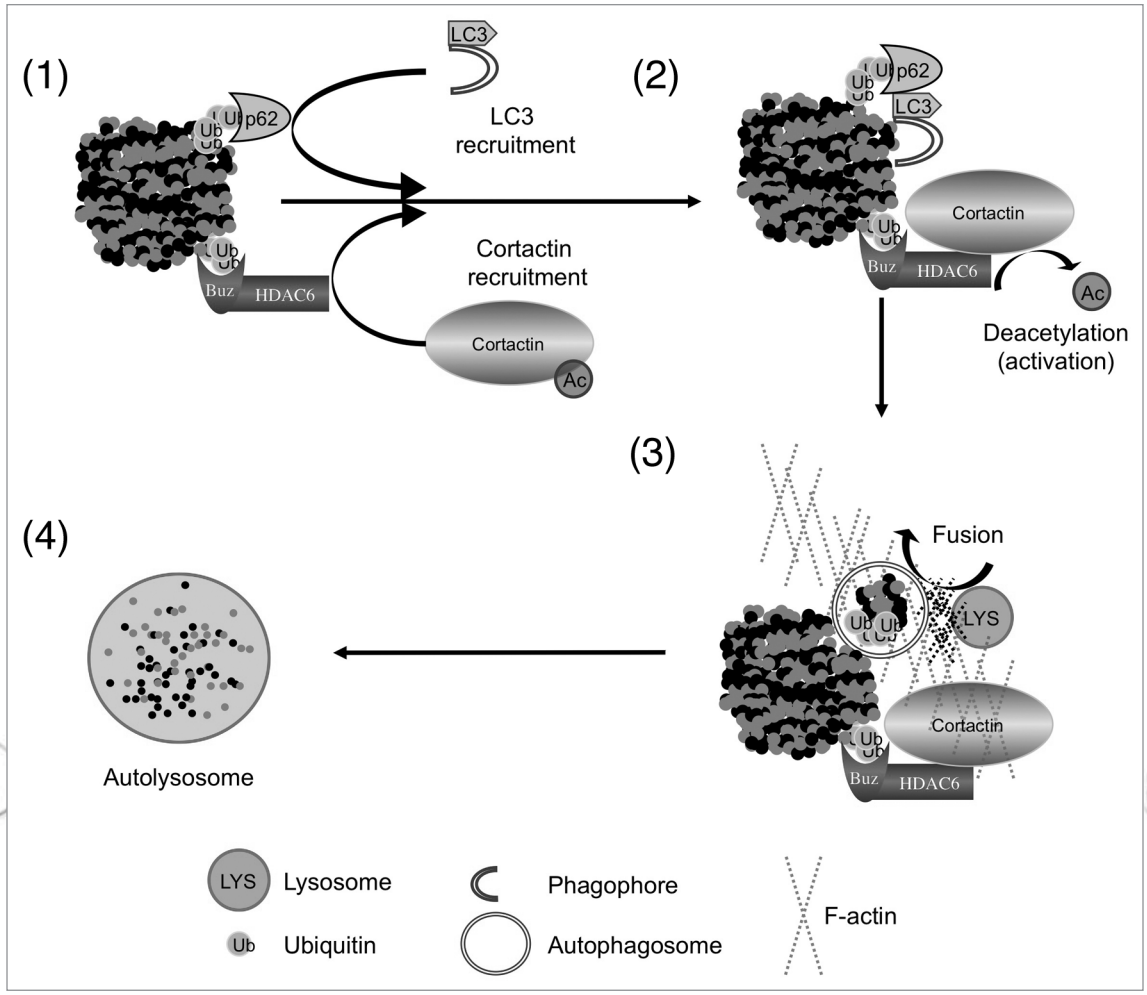

Figure 1. Schematic model for HDAC6-dependent clearance of ubiquitinated protein aggregates by QC autophagy. Our findings indicate that HDAC6 could promote protein aggregate clearance at multiple levels. (1) The protein aggregates are first independently recognized and bound by HDAC6 and p62 through the presence of specific polyubiquitin chains. The bound HDAC6 recruits cortactin to protein aggregates (2) and deacetylates cortactin leading to formation of an actin network. (2) The aggregate-bound p62 would independently recruit LC3 and the formation of autophagosomes that sequester oligomeric or small protein aggregates. (3) The actin network further promotes the fusion of autophagosomes and lysosomes, (4) leading to the eventual degradation of the protein aggregates.

In stark contrast, HDAC6 and actin remodeling are not required for autophagosome-lysosome fusion involved in starvation-induced autophagy. By assessing the fusion capacity of purified autophagosomes and lysosomes in vitro, we find that HDAC6 and actin are required for efficient fusion when autophagosomes are purified from fed animals or cells cultured in full medium. Remarkably, when autophagosomes are purified from starved animals or cells, fusion proceeds normally without HDAC6 or actin. These findings suggest an interesting possibility that autophagosomes induced to dispose of toxic cellular wastes and those used to recycle nutrients are intrinsically distinct! It is notable that actin is preferentially co-purified with autophagosomes under normal nutrient conditions, suggesting that actin may be an integral component of autophagosomes associated with QC autophagy. While this proposition remains to be tested, these findings nonetheless uncover an important link between actin cytoskeleton and QC autophagy. In the future, a detailed comparative survey of the composition of QC- vs. starvation-induced autophagosomes would be crucial to understand their distinct fusion capacity and activity.

The differential requirement of HDAC6 and the actin cytoskeleton could reflect the very distinct nature of the two autophagic modes. Since the main objective of nutrient-regulated autophagy is to rapidly replenish macromolecules for survival under starvation conditions, autophagosomes formed during starvation are logically not equipped with selectivity. This arrangement would allow autophagosomes to nondiscriminatively sequester cytosolic contents and fuse with lysosomes for efficient recycling of macromolecules. In contrast, QC autophagy must possess built-in "selectivity" for aberrant protein aggregates and damaged organelles. The specific involvement of HDAC6, which has intrinsic ubiquitin binding activity, suggests that the substrate selectivity involves ubiquitin modification. Indeed, the ubiquitin-binding BUZ domain is required for HDAC6 to bind ubiquitinated protein aggregates and support autophagosome-lysosome fusion. In fact,

ubiquitin-positive protein aggregates can be found in autophagosomes purified from cells cultured in rich medium. However, HDAC6 is not required for the recruitment of autophagosomes to protein aggregates. This activity is likely mediated by another ubiquitin-binding protein, p62, which binds LC3 with high affinity and promotes protein aggregate clearance. Accordingly, a simple model for QC autophagy would be that p62 and HDAC6 independently recognize specific ubiquitin moieties on protein aggregates or other QC autophagic substrates, where they recruit and assemble the components essential for autophagy (**Fig. 1**). Concentrating autophagic components to the substrates and stimulating the fusion of autophagosomes with lysosomes would enable QC autophagy to achieve specific and efficient clearance of protein aggregates or damaged organelles.

Autophagy has emerged as critical neuroprotective machinery. Failure to form autophagosomes by genetic ablation of ATG5 or ATG7 leads to profound neurodegeneration in mice. As discussed previously, however, unlike ATG5 or ATG7 mutations, HDAC6 deficiency does not affect autophagosome formation. Nevertheless, HDAC6 knockout mice and knockdown Drosophila accumulate ubiquitin-positive aggregates and develop spontaneous neurodegeneration. Thus, a defect in autophagosome-lysosome fusion could contribute to the development of neurodegenerative disease. In HDAC6-deficient cells, EM analysis reveals a marked accumulation of autophagosomes, which in many cases, are morphologically abnormal. Interestingly, similar abnormal autophagic structures also accumulate in dystrophic axons in Alzheimer disease patients and in a mouse model of frontotemporal dementia, the second most common form of presenile dementia. Thus, defects in autophagosome-lysosome fusion might be a common contributing factor to various forms of neurodegenerative disease. If this hypothesis is correct, simply activating the formation of autophagosomes, a step targeted by most of the commonly used autophagy-activating drugs, including the mTOR inhibitor rapamycin, may not be the most effective therapeutic approach for neurodegenerative disease. Indeed, evidence indicates that the neuroprotective effect of rapamycin is lost in HDAC6-deficient animals. As an alternative strategy, agents that stimulate autophagosome-lysosome fusion might enhance the degradative capacity of autophagy to remove toxic protein aggregates or damaged organelles. We suspect that HDAC6-dependent fusion machinery could be a potential therapeutic target for neurodegenerative as well as other diseases with related cellular defects.

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