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Identifying an essential role of nuclear LC3 for autophagy

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MAP1LC3/LC3 (microtubule-associated protein 1 light chain 3), a mammalian ortholog of yeast Atg8, is a key protein contributing to major steps of autophagy. It has been recognized for a long time that LC3 is abundant in the nucleus despite the fact that it functions primarily in the cytoplasm where the autophagosomes and autolysosomes arise. An important question regarding nuclear LC3 is whether and how it participates in autophagy. In this punctum, we discuss our recent findings about the essential role for nuclear LC3 in starvation-induced autophagy. During nutrient-rich conditions, LC3 is distributed in an acetylated form in both the nucleus and cytoplasm. Nutrient deprivation promotes the redistribution of LC3 from the nucleus to the cytoplasm. This relocation depends on a deacetylation of the protein by the activated nuclear deacetylase SIRT1 and the association of the protein with its nuclear interaction partner TP53INP2/DOR. More importantly, the deacetylation is also required for LC3 to bind with ATG7 for its subsequent lipidation. Therefore, the results implicate the nuclear pool of LC3 as the primary source of membrane-conjugated LC3, and a regulation of deacetylation and nucleocytoplasmic translocation of LC3 in priming starved cells for autophagy induction.

Keywords: autophagy, deacetylation, LC3, nucleus, SIRT1

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Autophagy is a major intracellular degradation and recycling system of the eukaryotic cell by which cellular components, including superfluous macromolecules and damaged organelles, are enwrapped within double-membraned autophagosomes and delivered to lysosomes for degradation. Autophagy functions not only in maintaining cellular homeostasis in nutrient-rich conditions, but also in promoting cell survival during starvation or other stress situations. A key regulator of autophagy is LC3, which initiates autophagosome biogenesis after becoming membrane-conjugated through interactions with ATG7 and other autophagic effectors. Although multiple pathways have been confirmed to trigger autophagy during starvation, including the transcriptional upregulation of ATG proteins and lysosomal biogenesis, as well as post-translational modification of ATG proteins via phosphorylation or acetylation, what occurs mechanistically to enable LC3 to interact with autophagy effectors to prime autophagosome formation is unclear. Another puzzling issue is the abundant distribution of LC3 in the nucleus in addition to the cytoplasm where it exerts its functions in autophagy. We set out in our study to investigate the necessity of LC3 in the nucleus and the regulatory mechanism for the relocation of nuclear LC3 to the cytoplasm and its coordination with LC3 membrane conjugation.

First, we confirmed in different types of cells the nuclear distribution of endogenous and exogenous LC3, because of the previous postulation that nuclear LC3 may serve as a reserve for the cytoplasmic pool during autophagy or is alternatively involved in nonautophagy events. We then examined the temporal and spatial localization of LC3 in starved cells, and confirmed a redistribution of nuclear LC3 to the cytoplasm by nutrient depletion. By selectively photobleaching and photoactivating the subcellular pool of LC3, we demonstrated that the nuclear pool of LC3 is the primary source of membrane-conjugated LC3 on autophagosomes. This

conclusion was verified by results showing that fixing LC3 in the cytoplasm through fusing with an exogenous nuclear export signal sequence (NES) dramatically decreased the association of LC3 with pre-autophagic membranes, and the defect in autophagosome formation by LC3 knockdown could only be rescued by expression of wild-type LC3, but neither the NES-LC3 nor the NLS-LC3, the latter a nucleus-fixed LC3 containing a nuclear localization signal (NLS).

Utilizing comprehensive approaches combining site-directed mutagenesis, protein-protein interaction, and protein acetylation analysis, we determined that the starvation-induced LC3 translocation is controlled by deacetylase SIRT1-dependent LC3 deacetylation in the nucleus. Deacetylation of core autophagic machineries including LC3, ATG5, and ATG7 by SIRT1 has been previously shown during cell starvation, but so far the relevance of these deacetylation events to their functions in autophagy induction has not been determined. We demonstrated that activated SIRT1 deacetylates LC3 at K49 and K51, identified by mass spectroscopy and mutagenesis, allowing LC3 specifically to interact with nuclear TP53INP2 and be exported together with TP53INP2 from the nucleus.

We speculate that deacetylation of LC3 is not only required for the export of nuclear LC3 but is also a prerequisite for the membrane association of LC3, as the LC3 lysine-to-glutamine mutant, which is fixed in the acetylated state fails to form puncta despite its distribution in both the nucleus and cytoplasm. We then checked the interaction of LC3 and ATG7 by affinity isolation experiments and in vitro binding assay using purified recombinant ATG7 and LC3. As expected, deacetylation of LC3 at Lys49 and Lys51 is essential for LC3 to bind with ATG7.

Thus, by identifying an autophagic signal-controlled nucleocytoplasmic redistribution mechanism, we uncovered the role of nuclear localized LC3 and its deacetylation in starvation-induced autophagy. However, an important unanswered question is how SIRT1 is activated so rapidly in response to acute nutrient starvation. Because the starvation condition applied in the experiments depletes both glucose and amino acids from the culture medium, how the activation of AMPK and/or inactivation of MTOR influence SIRT1 activity need to be elucidated in the future. Another interesting question is whether ATG5 and/or ATG7 is activated by SIRT1 in a similar mode during starvation, as both of these proteins have been found in the nucleus.