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COMMENTARY

RUBCNL/Pacer and RUBCN/Rubicon in regulation of autolysosome formation and lipid metabolism

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ABSTRACT

Recently, we identified a vertebrate-specific macroautophagy/autophagy regulator, RUBCNL/Pacer, which promotes autolysosome formation by engaging the class III phosphatidylinositol 3-kinase (PtdIns3K) and HOPS complexes. Hepatocyte-specific *rubcnl* knockout in mice results in impaired autophagy flux, glycogen and lipid accumulation, and liver fibrosis. We further showed that under nutrient-rich conditions RUBCNL is inactivated by MTORC1-mediated phosphorylation. When nutrients are insufficient, RUBCNL is dephosphorylated, which facilitates its acetylation by the activated GSK3-KAT 5/TIP60 pathway. RUBCNL acetylation significantly enhances HOPS complex recruitment, which eventually results in more efficient autophagosome maturation and lipid metabolism both in vitro and in vivo. Therefore, our work not only demonstrates that RUBCNL is essential for hepatic autophagy and liver homeostasis, but also reveals a signal integration mechanism involved in late stages of autophagy and lipid metabolism. Interestingly, these in vitro and in vivo functional data on RUBCNL are partially the opposite of the results from RUBCN/Rubicon studies that were either obtained by us or others. This implies a dual molecular switch model that is controlled by RUBCNL and RUBCN in modulation of autophagosome maturation and lipid metabolism.

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The PtdIns3K is an essential regulator of autophagy. Two distinct mammalian PtdIns3K subcomplexes, namely the ATG14- and UVRAG-subcomplexes, were characterized independently by different groups including us. RUBCN was initially identified as an inhibitor of the UVRAG-PtdIns3K subcomplex. We and others have shown that RUBCN inhibits endocytic trafficking and autophagy by repressing HOPS recruitment, RAB7 GTPase activation and PtdIns3K kinase activity. However, it remains unclear how RUBCN, an endosome-resident protein, inhibits autophagy. Moreover, RUBCN depletion significantly induces autophagy without additional stresses, implying that other factors may directly contribute to RUBCN-regulated autophagy.

The missing piece of this puzzle appears to be RUBCNL (RUN and cysteine rich domain containing beclin 1 interacting protein like), a novel accessory protein of PtdIns3K complexes that was recently identified in our laboratory [1]. RUBCNL primarily localizes to ER and autophagic structures and positively regulates autophagosome maturation. Mechanistically, RUBCNL antagonizes RUBCN to stimulate PIK3C3/Vps34 kinase activity and to recruit PtdIns3K and HOPS complexes to the autophagosome for their site-specific activation by anchoring to the autophagosomal SNARE STX17. From these observations, we propose a molecular switch model in autophagy regulation, which consists of 2 molecules with opposite biological activities. RUBCN behaves as a 'brake' by sequestering UVRAG-PtdIns3K during autophagy progression. In contrast, RUBCNL may be able to release this 'brake' after receiving upstream signaling to facilitate autophagosome maturation. Therefore, the next obvious question is how the dual molecular switch perceives upstream stimuli, in particular, the metabolic signals to regulate autophagosome maturation.

Systematic studies have been performed to elucidate how the dynamic fluctuation of environmental nutrients and energy regulates autophagy initiation. In contrast, how metabolic signals affect the late stage of autophagy is less understood. We found that under nutrient-rich conditions MTORC1 phosphorylates RUBCNL at serine 157 to disrupt the association of RUBCNL with STX17 and the HOPS complex, and thus abolishes RUBCNL-mediated autophagosome maturation. In contrast, dephosphorylation of RUBCNL under nutrient-deprived conditions promotes GSK3-KAT5/TIP60-mediated RUBCNL acetylation, which facilitates HOPS complex recruitment and is required for autophagosome maturation. These results are in line with the observations that MTOR inhibition dissociates RUBCN from UVRAG-PtdIns3K to facilitate autophagosome maturation. Therefore, the molecular switch of RUBCNL and RUBCN is indeed regulated by upstream metabolic signals.

To investigate RUBCNL's physiological function in vivo, we conducted mouse studies and we observed that hepatocyte-specific RUBCNL ablation in mice results in impaired autophagy flux, glycogen and lipid accumulation and liver

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fibrosis. Importantly, RUBCNL overexpression in mouse livers is able to alleviate nonalcoholic fatty liver disease in mice. In contrast, liver-specific knockout of *Rubcn* leads to autophagy overactivation and displays significant improvement of both liver steatosis and injury. Therefore, this molecular switch plays important physiological roles in vivo.

RUBCN plays a positive role in LC3-associated phagocytosis, one type of non-canonical autophagy, by recruiting PtdIns3K to phagosomes. In addition, RUBCN appears to be crucial in innate immunity. Therefore, it would be interesting to investigate whether RUBCNL has a function in these pathways in a future study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Reference

[1] Cheng X, Ma X, Zhu Q, et al. Pacer is a mediator of mTORC1 and GSK3-TIP60 signaling in regulation of autophagosome maturation and lipid metabolism. Mol Cell. 2019 Jan 19;73:788–802.e7.