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AUTOPHAGIC PUNCTUM

Mapping the proximity interactome of ATG9A reveals unexpected dynamics of ULK1 complex proteins

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ABSTRACT

ATG9A is essential for macroautophagy/autophagy and considered to be one of the earliest ATG (autophagy related) proteins recruited to sites of autophagosome biogenesis. Recent data suggest ATG9A vesicles may even form the lipid seed of the autophagosome. However, ATG9A regulation is still poorly understood, which is likely at least partly due to challenges inherent to studying an intracellular transmembrane protein with no apparent enzymatic activity. To help overcome these challenges, we used BioID and quantitative LC-MS/MS to map the proximity interactome of ATG9A, which included entire protein complexes involved in protein trafficking, and proteins implicated in autophagy but previously lacking any physical link to core autophagy machinery. We also unexpectedly found an ATG9A interaction with an ULK1-independent ATG13-ATG101 dimer that promotes autophagy in fed cells.

Current paradigms of ATG9A function and regulation

ATG9A travels in lipid vesicles through a Golgi-endosomal trafficking path and, in response to autophagic signals (e.g., starvation), is routed to sites of autophagosome biogenesis known as phagophore assembly sites (PAS). At these sites, ATG9A is proposed to act as a lipid scramblase, in combination with ATG2A or ATG2B, to move lipids into the growing phagophore. The lipid portion of the ATG9A vesicle may serve as the seed of the autophagosome. Despite the central role of ATG9A in various forms of autophagy, we still lack a clear understanding of how ATG9A is regulated in response to different autophagy triggers.

During starvation-induced autophagy – the most studied form of autophagy – the loss of MTORC1 activity coupled with activation of AMPK results in activation of the ULK1/ULK2 kinase complex, consisting of the kinase ULK1 (or ULK2) and its partners RB1CC1/FIP200, ATG13, and ATG101. In turn, the active ULK1/ULK2 complex orchestrates the next steps in autophagy via direct phosphorylation of autophagy proteins, including ATG9A. These starvation-induced ULK1/ULK2 signals are thought to help reroute ATG9A vesicles to the PAS. In contrast to starvation-induced autophagy, little is known about the regulation of ATG9A in fed cells – referred to here as basal autophagy. For example, basal autophagy is dependent on ATG9A, evidenced by the buildup of SQSTM1/p62-positive, ubiquitin-rich condensates in ATG9A-deficient cells; yet how is ATG9A regulated in the absence of persistent, starvation-induced activation of AMPK and ULK1/ULK2? To begin to address these questions, we became interested in elucidating ATG9A protein-protein interactions in fed conditions.

BioID mapping of the ATG9A proximity interactome

To overcome the challenges of traditional co-IP approaches with a transmembrane protein like ATG9A, we used BioID. In short, we fused the biotin ligase BirA* to the C terminus of ATG9A and identified biotinylated interactors by quantitative LC-MS/MS. We refined our list of ATG9A proximity interactors, crosschecked against BirA* controls, and ended with 283 significant proteins [1]. Most of the proteins match what we expected based on our understanding of ATG9A biology. The largest gene ontology (GO) category of interactors, vesicle and membrane trafficking, included entire (or nearly entire) protein complexes, such as the EARP, GARP, TRAPP, RETROMER, SNARE, and AP1 to AP4 complexes. Another category of interactors includes proteins involved in the synthesis of phosphatidylinositol phosphates (phosphoinositides), such as PIK3C2A/PI3K-C2α UVRAG, and PIKFYVE, suggesting that ATG9A may help organize phosphatidylinositol-3-phosphate synthesis at the phagophore membrane. Interestingly, one of the most heavily biotinylated proteins in our dataset is the BEACH domain protein LRBA, mutations in which cause immunodeficiency in humans. B cells from LRBA-deficient patients show defects in autophagy, which, together with our data, suggest that LRBA may cooperate with ATG9A in autophagy.

ATG9A interacts with an ATG13-ATG101 dimer independently of ULK1

Given our interest in ATG9A regulation in fed cells, we were intrigued by the presence of the entire ULK1 complex in our BioID dataset. Through a series of BioID and co-IP
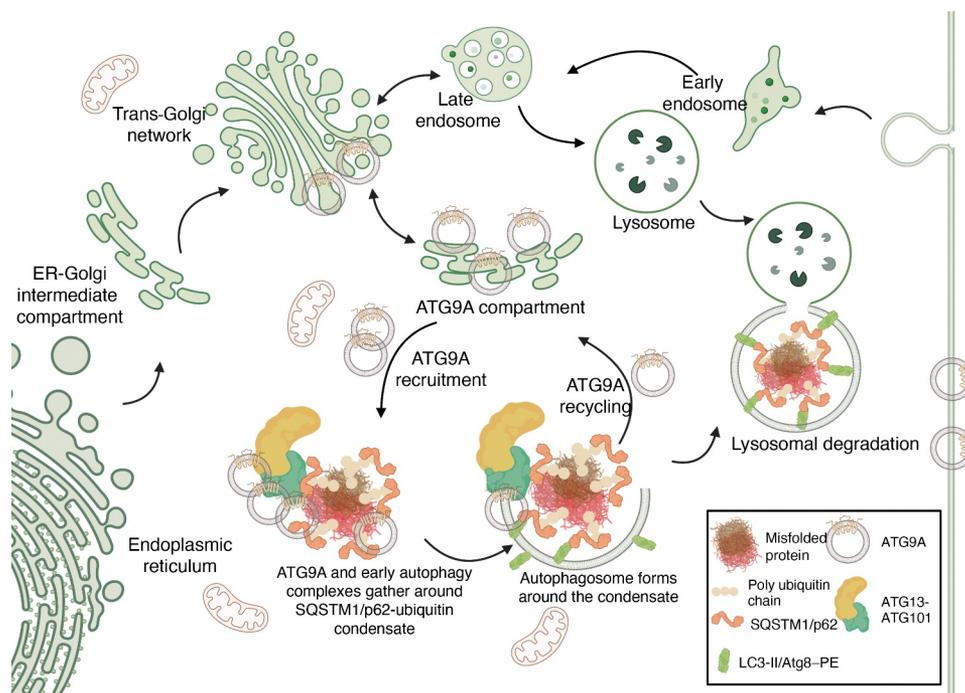
Figure 1. ATG9A interacts with a ULK1-independent ATG13-ATG101 complex in basal autophagy. ATG9A traffics through the Golgi-endosomal system and resides in peripheral ATG9A-containing compartments. The accumulation of ubiquitin-SQSTM1 condensates signals recruitment of ATG9A from peripheral compartments. At the condensate, ATG9A interacts with an ATG13-ATG101 subcomplex to begin assembly of the early autophagosomal machinery (created with BioRender.com).

studies to probe the nature of the ATG9A-ULK1 complex interaction, we were surprised to find that ATG9A interacts with ATG13 in the absence of ULK1. Furthermore, deletion of *ATG13* abrogates the interaction between ATG9A and ATG101, but has no effect on the interaction of ATG9A with ULK1, suggesting that ATG9A interacts with different iterations or subcomplexes of ULK1 complex proteins.

We suspected that ATG13 may help recruit ATG9A to SQSTM1-positive, ubiquitin-rich condensates to promote basal autophagy (or aggrephagy). Instead, we found that deletion of *ATG13* causes an accumulation of ATG9A at the condensates. Furthermore, we found that essentially any means of autophagy inhibition that increases the size of ubiquitin-rich condensates causes a corresponding accumulation of ATG9A at the condensate where ATG9A and ATG13 colocalize. First, this suggests the intriguing idea that a signal intrinsic to the condensate is recruiting and/or helping to tether ATG9A at those sites. Second, taken together with our other data, it suggests that ATG9A and an ATG13-ATG101 dimer independently assemble at the condensate to initiate basal autophagy.

Importantly, our data do not rule out the possibility that ULK1 also assembles with the ATG13-ATG101 dimer and ATG9A at the condensates. In fact, we observed ULK1 colocalizing with an ATG13-ATG101 dimer at condensate-like puncta. Interestingly, ULK1 and the ATG13-ATG101 dimer colocalize at these sites even when ATG13 lacks the ability to bind ULK1. Thus, we suspect that the physical interaction between ATG13 and ULK1 may not be necessary for the intact ULK1 complex to assemble at the condensate. Instead, components of the ULK1/

ULK2 complex may independently coalesce at condensates, perhaps scaffolded by RB1CC1, as previous data suggest. In turn, this may allow for a high local concentration of ULK1/ULK2 activity even under fed conditions. Mechanistic details of how this would occur await future study. Nevertheless, these data begin to paint a more dynamic picture of the mammalian ULK1 complex than previously appreciated.

In conclusion, our study provides a catalog of ATG9A proximity interactors that we hope will be a resource to the autophagy community. In addition, our data suggest that SQSTM1-positive condensates recruit ATG9A. In turn, ATG9A assembles with early autophagy machinery, such as the ATG13-ATG101 dimer, to promote phagophore growth and engulfment of the condensate (Figure 1).

Disclosure statement

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Reference

[1] Kannangara AR, Poole DM, McEwan CM, et al. BioID reveals an ATG9A interaction with ATG13-ATG101 in the degradation of p62/SQSTM1-ubiquitin clusters. EMBO Rep. 2021;22(10):e51136. Epub 2021/08/10. PubMed PMID: 34369648.