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RESEARCH HIGHLIGHT

A separation that's for the best: coming together at the PAS

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The phagophore assembly site is an organizing center for several proteins that facilitate the initiation and expansion of autophagic membranes. Fujioka and colleagues report that this structure is not a rigid complex but, instead, a liquid-like condensate, the stability of which relies heavily on the interactions of the disordered protein Atg13.

Macroautophagy, hereafter referred to as autophagy, is a highly conserved eukaryotic mechanism for degrading a wide array of intracellular cargo. In this process, everything from individual proteins to entire organelles is sequestered by a transient double-membrane compartment termed the phagophore; the phagophore subsequently matures into an autophagosome, which fuses with the degradative organelle (vacuole in yeast, lysosome in mammals) allowing the intralumenal contents to be broken down into building blocks such as amino acids and lipids that are then released to the cytosol for eventual reuse by the cell. This process inherently necessitates complex and, in many ways, biologically unique membrane dynamics including nucleation of the phagophore through the incorporation of membranes from a variety of sources. In yeast, this nucleation process occurs at a peri-vacuolar site termed the phagophore assembly site (PAS) and involves an intricate cascade of protein recruitment and interaction events. The PAS was first reported ~20 years ago by the Ohsumi and Klionsky laboratories where they observed the localization of several fluorescently tagged autophagy proteins to a peri-vacuolar punctate structure that appeared to give rise to autophagic vesicles.[1,2] Subsequent studies have yielded an extensive list of proteins known to associate with this structure as well as a greater, although still very much incomplete, mechanistic understanding of the role of the PAS in phagophore initiation and expansion. However, the nature of how the constituent proteins are recruited to the PAS and the specific interactions they undergo upon arrival has remained poorly understood. Of the many proteins recruited to the PAS, the first are those of the Atg1 complex (Atg1, Atg13, Atg17, Atg29, and Atg31), which acts as a scaffold and initiates the formation of the PAS.[3,4] Recent studies have revealed molecular details of this complex such as its reliance on the phosphorylation status of Atg13, structural details of the Atg1-Atg13 and Atg13-Atg17 interactions and the ability of Atg13 to link Atg17-Atg31-Atg29 dimers through interaction with two distinct binding sites on Atg17.[5,6]

The article recently published by Fujioka and colleagues in *Nature*[7] and highlighted here harmonizes several previous findings resulting in a conceptual leap in our understanding of how this unique intracellular compartment is organized. Fluorescence recovery after photobleaching (FRAP) experiments with GFP-labeled PAS components show rapid recovery times (1.3 s half-time for GFP-Atg13), consistent with those previously reported for biomolecular condensates. Treatment of cells with a liquid-liquid phase separation inhibitor, 1,6-hexanediol, rapidly disperses fluorescent puncta corresponding to the PAS, and removal of this inhibitor leads to the reformation of the PAS. Additionally, the authors reported several liquid droplet-like behaviors such as puncta fusion and maintenance of sphericity. Next, the droplet-forming propensity of the Atg1 complex was tested in vitro. Upon mixing the purified components, droplets readily form. Furthermore, the authors found that although Atg1 is incorporated into these condensates, its presence is not necessary for droplet formation. Conversely, removal of Atg13 or Atg17, or disruption of either of the two binding sites between these two proteins, eliminates or severely impairs droplet formation in vitro. Regulatory aspects of the Atg1 complex droplet formation were also revealed when the authors investigated the phosphorylation status of the constituent proteins. TORC1-phosphorylated Atg13, which was previously reported to have impaired binding to Atg17, shows severely impaired droplet formation in vitro. Additionally, the authors observed that Atg1 kinase activity, induced by addition of ATP to the purified Atg1 complex components, results in the dispersal of Atg1 complex droplets. Further investigation showed that addition of the autophagy-related PP2C phosphatase, Ptc2, reverses the phosphorylation of Atg1 and Atg13 and restores droplet stability. Finally, Fujioka and colleagues investigated the previously reported Atg13-Vac8 interaction as a facilitator of the consistently observed peri-vacuolar localization of the PAS. They find that deletion of *VAC8* or merely the disruption of the Vac8 interaction with Atg13 greatly impairs the recruitment of the PAS both to the vacuole in vivo and to Vac8-coated giant unilamellar vesicles (GUVs) in vitro.

The results of this study support the claims made by the authors and have important implications in the field of autophagy. Previous studies have reported the phase separation of autophagic cargo prior to sequestration into phagophores;[8] however, this is the first report of the autophagic machinery itself undergoing liquid-liquid phase separation—an important distinction. The intrinsic reliance of this phenomenon on the Atg13-Atg17 multivalent interaction, as well as the dispensability of Atg1, add further details to the model of PAS phase separation. Nutrient starvation promotes both Atg1 kinase activity and the formation of the PAS (and subsequently autophagosomes). Consequently, the authors' observation that Atg1 kinase activity results in the dispersal of Atg1 complex droplets seems initially counterintuitive. This was remedied, however, by the interesting demonstration that the Ptc2 phosphatase combats this effect in cells allowing for both highly active Atg1 and PAS formation. Adding further importance to Atg13 in the context of the PAS, the interaction of Atg13 with the peripheral membrane protein Vac8 plays an in important role in the proper localization of the PAS (Fig. 1). The revelation that the PAS is a phase-separated droplet as opposed to

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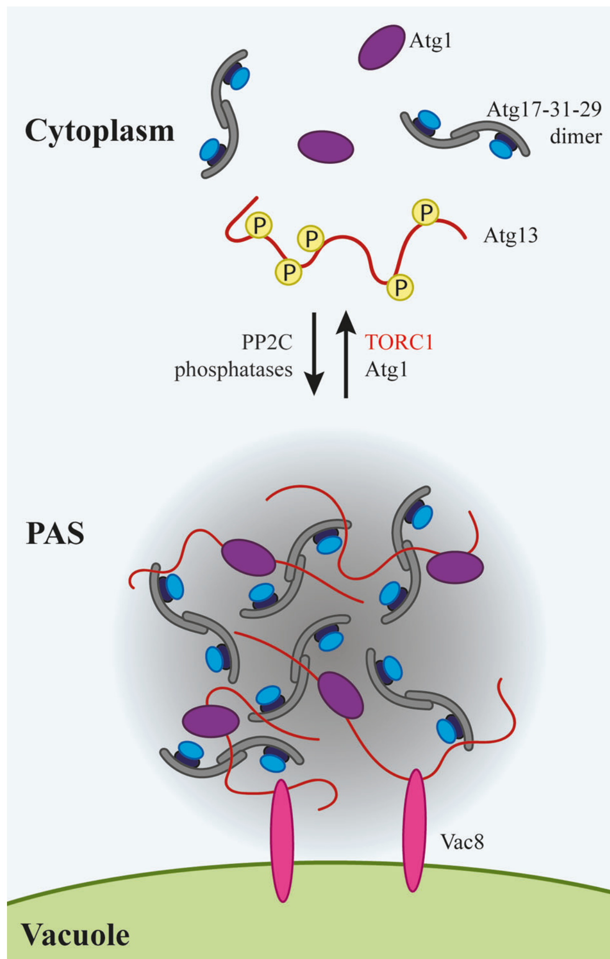

Fig. 1 Diagram of the phase separation of the PAS components and the regulation of this phenomenon by cellular cues. Under high nutrient availability, TORC1 is active and phosphorylates several autophagy proteins including Atg13 at residues S428 and S429, modifications that impair the Atg13-Atg17 interaction, precluding PAS formation in growing conditions. When cells are starved for nutrients, TORC1 is inactivated and the inhibitory phosphoryl groups placed on Atg13 by this complex are removed by PP2C phosphatases. This dephosphorylation allows formation of the PAS droplet resulting in the clustering of Atg1 molecules, which contributes to activating autophosphorylation of this kinase. Activated Atg1 then re-phosphorylates S428 and S429 on Atg13; however, this is reversed by PP2C phosphatases such that the PAS condensate remains stable. The peri-vacuolar localization of the PAS is facilitated at least partially by the interactions between Atg13 and Vac8.

a rigid complex explains some previous observations such as the fragility and dynamic nature of the PAS, but it raises several new questions and avenues for future research. For example, do other PAS-associated proteins also phase separate? Is induction of Atg1 complex phase separation sufficient to induce autophagy? Does the mammalian counterpart of the Atg1 complex, the ULK1 complex, also form a biomolecular condensate? Subsequent studies will no doubt pursue answers to questions such as these and contribute to a more wholistic understanding of autophagy initiation and progression.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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