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Should I bend or should I grow: the mechanisms of droplet-mediated autophagosome formation

Sebastian W. Schultz[a,b,*], Jaime Agudo-Canalejo[c,d,e,*], Haruka Chino[f], Simona M. Migliano[a,b], Chieko Saito[f], Ikuko Koyama-Honda[f], Harald Stenmark[a,b], Andreas Brech[a,b], Noboru Mizushima [f], Roland L. Knorr[f,g], and Alexander I. May[h,i]

[a]Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [b]Centre for Cancer Cell Reprogramming, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; [c]Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford, UK; [d]Department of Chemistry, The Pennsylvania State University, University Park, PA, USA; [e]Department of Living Matter Physics, Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany; [f]Graduate School and Faculty of Medicine, The University of Tokyo, Tokyo, Japan; [g]Department of Molecular Physiology, Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany; [h]Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan; [i]Tokyo Tech World Research Hub Initiative (WRHI), Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan

ABSTRACT

Phase-separated droplets with liquid-like properties can be degraded by macroautophagy/autophagy, but the mechanism underlying this degradation is poorly understood. We have recently derived a physical model to investigate the interaction between autophagic membranes and such droplets, uncovering that intrinsic wetting interactions underlie droplet-membrane contacts. We found that the competition between droplet surface tension and the increasing tendency of growing membrane sheets to bend determines whether a droplet is completely engulfed or isolated in a piecemeal fashion, a process we term fluidophagy. Intriguingly, we found that another critical parameter of droplet-membrane interactions, the spontaneous curvature of the membrane, determines whether the droplet is degraded by autophagy or – counterintuitively – serves as a platform from which autophagic membranes expand into the cytosol. We also discovered that the interaction of membrane-associated LC3 with the LC3-interacting region (LIR) found in the autophagic cargo receptor protein SQSTM1/p62 and many other autophagy-related proteins influences the preferred bending directionality of forming autophagosomes in living cells. Our study provides a physical account of how droplet-membrane wetting underpins the structure and fate of forming autophagosomes.

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With the core autophagy machinery now broadly characterized, the focus of autophagy research has recently turned to how this machinery recognizes and interacts with its cargo and, subsequently, how autophagic membranes are recruited to sequester cargo. Cargo receptor proteins that interact with lipidated Atg8-family proteins, such as SQSTM1/p62, are thought to play an important role by bringing autophagic membranes into contact with cargo. However, the availability of receptor proteins alone does not ensure efficient cargo sequestration. For example, SQSTM1 phase separates under certain conditions and the resulting cytosolic SQSTM1-containing droplets can be degraded by autophagy. Accumulations of SQSTM1-positive protein structures in contrast are a hallmark of aging and are implicated in the progression of neurodegenerative diseases, liver diseases, and myopathies. As a well-characterized protein known to interact with the autophagy machinery, SQSTM1 is therefore an excellent candidate for the study of conditions allowing interactions between phagophore membranes and cargoes destined for degradation.

The process of phase separation has recently garnered great interest as a key mechanism of intracellular organization. An additional and important conceptual implication of phase separation is that the liquid-like nature of "droplet" compartments (also known as biomolecular condensates or membrane-less organelles) is associated with unique physical properties, including surface tension and viscoelasticity, that are important for their function in cells. Surface tension is responsible for the spherical shape of droplets as it forces droplets to minimize surface area; the ability of a droplet to resist perturbations in sphericity depends on the magnitude of its surface tension. Meanwhile, viscoelasticity describes the dynamics of liquid-like droplet deformations upon stress. Over long timescales, viscoelastic materials exhibit characteristics of liquids, including the ability to flow and assume new shapes. In contrast, short-term stress causes these materials to exhibit solid-like elasticity, with an immediate return to the original shape upon stress removal. Both surface tension and viscoelasticity are influenced by a range of factors, including

CONTACT Roland L. Knorr knorr@m.u-tokyo.ac.jp Graduate School and Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan; Alexander I. May may.a.ab@m.titech.ac.jp Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology, 4259-S2-12 Nagatsuta-cho, Midori-ku, Yokohama, 226-8503, Japan
*These authors contributed equally to this work.

droplet age and composition. However, how the relative fluidity of SQSTM1-containing and other droplets contributes to their fate remains unexplored.

A hallmark of autophagy is the formation of flattened membrane sheets known as phagophores. Initially, a prohibitively large energy barrier ensures stability of the flattened shape against bending. Subsequent sheet expansion increases the area of the peripheral rim of the sheet, which is energetically expensive due to its strong curvature. For isolated flattened sheets, growth beyond a critical size causes morphological instability of the sheet; in this case, sheet bending and remodeling into an autophagosome greatly reduces the area of the rim, strongly favoring autophagosome formation. Importantly, this simple picture neglects the influence of phagophore-droplet wetting interactions, which can affect the energy balance determining sheet closure into an autophagosome.

In our study [1], we explored this mechanism of droplet autophagy. Our theoretical model reduces the interaction of an expanding sheet pinned to the surface of droplets to just two key competing forces: droplet surface tension and sheet instability. Our model predicts two outcomes: if the surface tension of the droplet is below a critical value, the increasing sheet instability of the growing membrane will induce sheet bending and droplet disruption, resulting in piecemeal droplet sequestration (Figure 1, upper panel). Alternatively, droplet surface tensions above a critical value work to stabilize sheets: the membrane is unable to remodel and continues to grow along the droplet surface, until the entire droplet is engulfed (Figure 1, center panel) or membrane supply is exhausted, resulting in incomplete autophagosome formation.

We set out to observe the predicted membrane-droplet interplay experimentally. To ensure that our model applies in vivo, we confirmed that SQSTM1-containing droplets are liquid-like condensates on the timescale of autophagosome formation and observed that LC3-positive membranes interact with these droplets. Critically, we observed the piecemeal sequestration of droplets predicted by our model. Two key conclusions arise from these results: (i) the predicted droplet-membrane interplay occurs in cells, and (ii) the surface tension of intracellular SQSTM1-containing droplets is below the critical value that allows for their piecemeal sequestration.

As our model describes the droplet-sheet mechanical interplay using coarse-grained physical parameters, similar droplet-membrane behavior should be observed in a protein-free model system. We therefore reconstituted fluid interphases with wetting membrane sheets in vitro using phase separating synthetic polymers. By manipulating droplet surface tension, we were able to control sheet remodeling into autophagosome-like structures. This finding suggests that, in general, the formation of autophagosome-like structures does not depend on proteins. A further implication is that changes in a droplet's physical properties might be linked to its degradation in vivo.

The SQSTM1 LC3-interacting region (LIR) brings SQSTM1 into physical contact with the machinery of autophagy and is critical for SQSTM1 turnover. We next asked if the SQSTM1 LIR has any effect on droplet autophagy via the SQSTM1-LC3 interaction. Importantly, LIR disruption does not prevent wetting of SQSTM1 droplets on phagophore membranes: neither deletion of the LIR-domain nor blockage of LC3 lipidation and membrane incorporation in *ATG3* knockout cells prevent wetting. However, phagophore membranes often fail to engulf SQSTM1-containing droplets under these conditions, instead isolating portions of the cytosol, a phenomenon also observed in vivo by the Komatsu group in a very recent paper. This surprising result may have mechanistic implications for the finding last year that the nucleation site of expanding phagophore membranes, the PAS, is in fact a phase-separated droplet.

While counterintuitive, the isolation of cytosol is predicted by our theoretical model (Figure 1, lower panel). During droplet autophagy, a membrane sheet interacts with the droplet on one side and the cytosol on the other. This likely

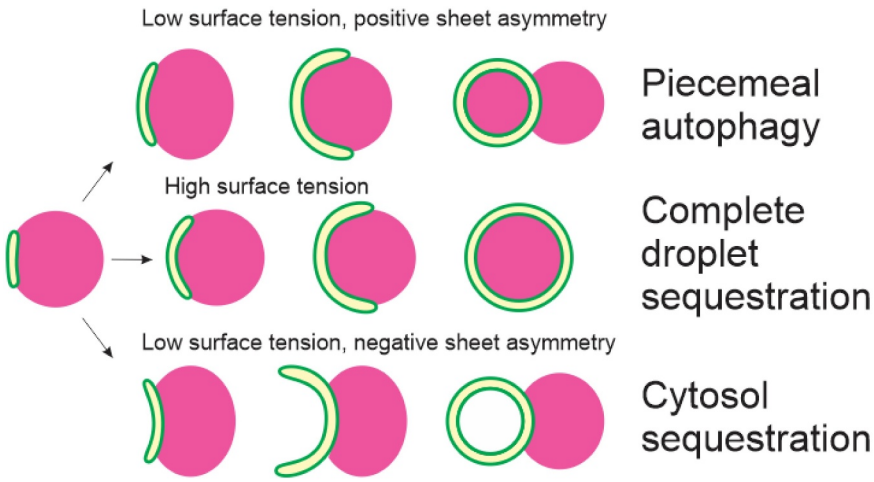

Figure 1. Wetting interactions between droplets and autophagosomal membranes determine the fate of forming autophagosomes. Following wetting of a liquid-like droplet by autophagosomal membrane, droplet and membrane properties specify the subsequent mode of degradation, with piecemeal droplet autophagy, complete droplet autophagy and even cytosolic autophagy possible.

results in an asymmetry in membrane spontaneous curvatures – the inherent degree and direction of bending of the two membranes that make up the phagophore – that arises from divergent cytosol and droplet compositions. If the spontaneous curvature differential favors curvature toward the droplet, the sheet will sequester the droplet, and vice versa. Our modeling therefore suggests that the LIR-mediated LC3-SQSTM1 interaction serves to alter net membrane spontaneous curvature, thereby exerting an influence over bending direction and ultimately cargo specification. This finding highlights that droplets can act as an origin of cytosol-degrading autophagic membranes, but are also subject to specific degradation by two distinct modes of sequestration. We anticipate that further research will reveal the relative importance of cargo-machinery interactions in both droplet and bulk autophagy pathways, further highlighting the important role of physical forces in determining the fate of forming autophagosomes in cells.

Disclosure statement

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ORCID

Noboru Mizushima http://orcid.org/0000-0002-6258-6444

Reference

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