AUTOPHAGY
2017, VOL. 13, NO. 3, 629–630
http://dx.doi.org/10.1080/15548627.2016.1276678

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AUTOPHAGIC PUNCTUM

Passing membranes to autophagy: Unconventional membrane tethering by Atg17

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ABSTRACT

Macroautophagy delivers cytoplasmic material to lysosomal/vacuolar compartments for degradation. Conserved multisubunit complexes, composed of autophagy-related (Atg) proteins, initiate the formation of membrane precursors, termed phagophores. Under physiological conditions these cup-shaped structures can capture cytoplasmic material highly selectively. Starvation or cytotoxic stresses, however, initiate the formation of much larger phagophores to enclose cytoplasm nonselectively. The biogenesis of nonselective autophagosomes is initiated by the hierarchical assembly of the Atg1 kinase complex and the recruitment of Atg9 vesicles at the phagophore assembly site (PAS). In this punctum we summarize our recent findings regarding tethering of Atg9 vesicles by the Atg1 kinase complex. We discuss membrane tethering by and activation of its central subunit Atg17 in the context of other canonical membrane tethering factors. Our results show that Atg17 suffices to bind and tether Atg9 vesicles. The Atg31-Atg29 subcomplex inhibits Atg17 activity, and activation of Atg17 depends on the formation of the Atg1 kinase complex that involves recruiting Atg1-Atg13. Our studies lead to a model of unconventional membrane tethering in autophagy.

ARTICLE HISTORY
Received 8 December 2016
Revised 13 December 2016
Accepted 19 December 2016

KEYWORDS
Atg1 kinase complex; Atg9 vesicles; autophagy; initiation; tether

Maintaining cellular homeostasis is one of the most important tasks of cells to adapt to cytotoxic stresses. Nonselective macroautophagy (autophagy in the following) is initiated by such stresses and recycles bulk cytoplasm by enclosing it within a double-membrane vesicle, termed an autophagosome. These de novo-formed organelles deliver their content to the vacuole for degradation. The molecular mechanism of autophagy is best understood in yeast but appears to be highly conserved in humans. Early steps of autophagy depend on the Atg1 kinase complex that assembles at the PAS, where it initiates the formation of cup-shaped phagophores. Membranes are donated by small Atg9-containing vesicles and their recruitment to the PAS depends on Atg17, a central scaffolding subunit of the Atg1 kinase complex. Atg1 kinase complex assembly is closely intertwined with the nutrient status of the cell and regulated by the target of rapamycin complex (TORC) 1. Upon starvation, inactivation of TORC1 leads to partial dephosphorylation of Atg13 thereby promoting Atg1 kinase complex formation.

Atg17 is the earliest component of the complex to arrive at the PAS upon starvation. Atg17 forms S-shaped dimers, which constitutively associate with the 2 regulatory subunits Atg31 and Atg29 to form a ternary complex (Atg17TC). Donor vesicles that contain the membrane protein Atg9 are recruited to the PAS by Atg17 independently of Atg13 or Atg1. However, Atg1 and Atg13 are essential to form phagophores suggesting that fusion of these vesicles depends on these 2 subunits. Interestingly, each monomer of Atg17 adapts a crescent shape with a radius of approximately 10 nm, fitting well to the 30- to 60-nm diameters of Atg9 vesicles. This observation led to the hypothesis that dimeric Atg17 tethers 2 Atg9 vesicles to initiate autophagy. Although the assembly, disassembly and phosphoregulation of the Atg1 kinase complex has been characterized extensively in vitro, its interaction with Atg9 vesicles remained poorly understood.

We reconstituted the full-length Atg1 kinase complex in vitro from its purified subunits and studied its interaction with Atg9-proteoliposomes. We found that the core domain of Atg9, lacking parts of its unstructured N- and C-termini, interacts with Atg17 in vivo and in vitro. This interaction is regulated by Atg31-Atg29, which occupies the Atg9 binding region involving helix α4 in Atg17. Atg31-Atg29 thus prevents Atg17 from binding Atg9 during vegetative growth. Upon starvation-induced assembly of the Atg1 kinase complex Atg17TC becomes active. As a result, the Atg1 kinase complex binds Atg9 as strongly as non-inhibited Atg17 does. Moreover, Atg17 and the fully assembled Atg1 kinase complex are able to tether Atg9 vesicles in vitro, whereas Atg17TC is not. Furthermore, a phosphomimetic variant of Atg13 that blocks Atg1 kinase complex formation in vitro cannot restore tethering.

Our data revealed that the initiation of nonselective autophagy involves not only recruitment of Atg9 vesicles but also their tethering by Atg17. Conventional tethers accelerate rate-limited steps in membrane trafficking and ensure delivery of cargo to their correct destinations. These principles hold true

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Punctum to: Rao Y, Perna M, Hofmann B, Beier V, Wollert T. The Atg1–kinase complex tethers Atg9-vesicles to initiate autophagy. Nat Commun 2016; 7:10338; http://dx.doi.org/10.1038/ncomms10338

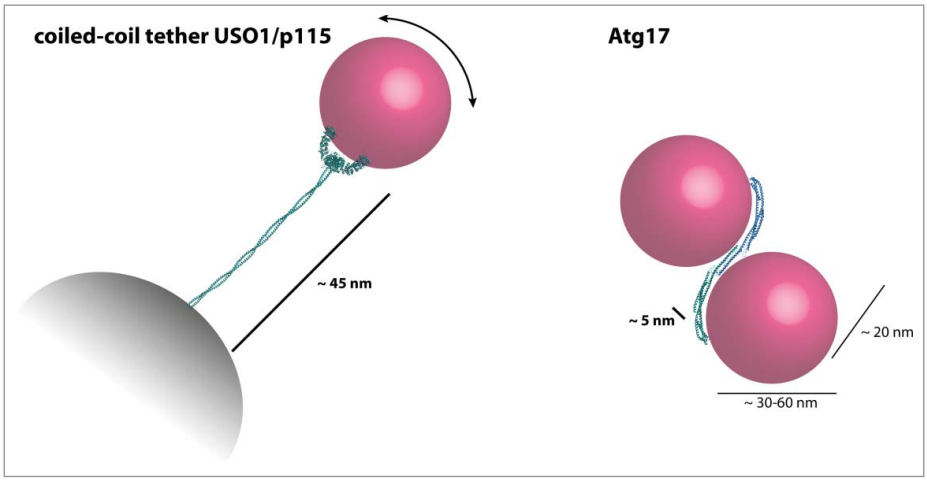

Figure 1. Comparison of the conventional coiled-coil tether USO1/p115 with the unconventional autophagic tether Atg17. The flexible coiled-coil dimer of USO1/p115 (left) tethers COPI-vesicles to the Golgi and spans a distance of ~45 nm. In contrast, the S-shaped Atg17 dimer (right) captures 2 Atg9 vesicles bringing them in close proximity. Structures were prepared using PyMol and pdb-files 2W3C (head domain of USO1/p115), 2ZTA (coiled coil of USO/p115) and 4HPQ (Atg17).

for the biogenesis of the autophagosomal membranes as well. However, Atg17 has no obvious structural similarities to canonical membrane tethering factors that regulate other cellular trafficking pathways. Although Atg17 is comprised of elongated coiled-coils, its unusual S-shaped structure is much more compact and stiff compared to long coiled-coil tethers. Golgins, for example, form flexible rods spanning distances of 100 to 600 nm with binding sites for interaction partners located at their termini. Atg17, by contrast, adopts a slightly crescent shape with a radius of approximately 10 nm as noted above, with the Atg9-binding site located at the center of the crescent. Similar to other coiled-coil tethers, Atg17 exists as a homodimer. However, the 2 monomers are arranged such that the Atg9- binding regions are facing opposite directions, bringing 2 Atg9 vesicles into close proximity. In contrast to coiled-coil tethers, the compact and rigid structure of Atg17 potentially restricts movement of Atg9 vesicles, which would not be achieved by long and flexible tethers (Fig. 1).

Multiple Atg1 kinase complexes are present at the PAS and their assembly into higher order structures is coordinated by subunits of the complex including Atg1. Thus, the Atg1 kinase complex also possesses properties of a second class of membrane tethering complexes termed multisubunit tethering complexes (MTC). Members of the MTC-family include, for example, HOPS, Dsl1, and COG complexes. Compared to coiled-coil tethers, MTCs are more compact spanning 20–40 nm, which compares well to the 30-nm long Atg17 dimers. However, in contrast to the Atg1 kinase complex, which only requires Atg9 to tether membranes, subunits of MTCs interact with different proteins including small GTPases and SNAREs on opposing membranes.

In conclusion, the Atg1 kinase complex combines properties of MTCs and coiled-coil tethers. The identification of Atg17 as a tether for autophagy initiation does not, however, exclude a possible contribution of other membrane tethers in autophagy. Atg9, for example, also interacts with Ypt1 and the TRAPPIII tethering complex, and ER-derived COPII vesicles contribute to phagophore formation as well. To what extent membrane tethering complexes cooperate to coordinate cellular membrane traffic remains to be investigated.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.