Atg11

A Rab-dependent, coiled-coil membrane protein that acts as a tether for autophagy

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Selective macroautophagy uses double-membrane vesicles, termed autophagosomes, to transport cytoplasmic pathogens, organelles and protein complexes to the vacuole for degradation. Autophagosomes are formed de novo by membrane fusion events at the phagophore assembly site (PAS). Therefore, precursor membrane material must be targeted and transported to the PAS. While some autophagy-related (Atg) proteins, such as Atg9 and Atg11, are known to be involved in this process, most of the mechanistic details are not understood. Previous work has also implicated the small Rab-family GTPase Ypt1 in the process, identifying Trs85 as a unique subunit of the TRAPPIII targeting complex and showing that it plays a macroautophagy-specific role; however, the relationship between Ypt1, Atg9 and Atg11 was not clear. Now, a recent report shows that Atg11 is a Trs85-specific effector of the Rab Ypt1, and may act as a classic coiled-coil membrane tether that targets Atg9-containing membranes to the PAS. Here, we review this finding in the context of what is known about Atg11, other Rab-dependent coiled-coil tethers, and other tethering complexes involved in autophagosome formation.

The eukaryotic cell is a busy place, full of membrane-bound carriers zipping from site to site, budding and fusing and morphing in shape. One of the central challenges faced by the secretory system is how to target each carrier to its proper destination. This is particularly challenging if the destination in question is not a large organelle but a single small spot, such as the phagophore assembly site, where autophagosomes (and similar types of sequestering vesicles) are made. One layer of specificity is usually provided by the SNARE proteins that drive membrane fusion, but that is probably not enough for efficient recognition of the target site. So cells need a second layer of specificity, and tethering factors provide that.

Tethering factors act upstream of SNARE proteins to help vesicles find their destination, and in some cases help to organize the SNARES as well. They can interact both with the vesicle and with the target, and use these interactions to bring the two together prior to fusion. Often they are recruited to the vesicle by interactions with Rab-family GTPases.[1,2] Rab-family GTPases act as molecular markers of specific membrane compartments, and, in their GTP-bound (active) form, can recruit various effectors, such as tethers. Some tethers are also targeted by interactions with the vesicle coat; having multiple interactions with different vesicle components presumably helps to ensure accurate targeting.[3,4]

Tethers come in two flavors: multi-subunit tethering complexes and single protein coiled-coil tethers. As their name suggests, the latter are characterized by an abundance of coiled-coil domains that typically give them an extended, rod-like structure and mediate their interactions with various other proteins (**Fig. 1**).[5,6] Now, in a recent paper, Lipatova et al.[7] define a new coiled-coil tether, Atg11, which acts as an effector of the Rab Ypt1 and the TRAPPIII complex during selective macroautophagy (see the punctum by Lipatova and Segev in this issue).
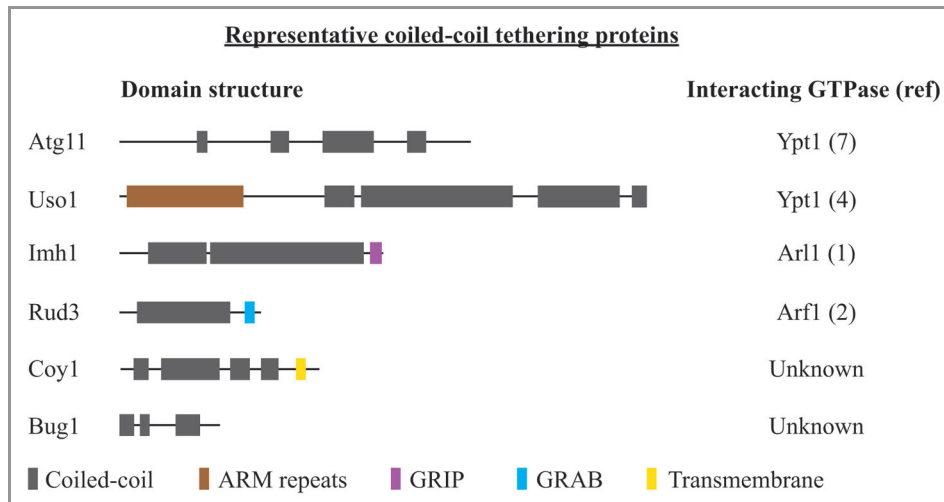
Figure 1. Atg11, like other GTPase-recruited tethering proteins involved in membrane trafficking, contains multiple coiled-coil domains. Location of coiled-coil domains is based on predictions from the Parcoil2 program.[5]

Atg11 in Autophagy

Macroautophagy (hereafter referred to simply as autophagy) is a conserved eukaryotic stress response that involves the construction of double-membrane autophagosomes. The construction of the autophagosome requires the delivery of membrane material to the PAS; that membrane is thought to arrive in the form of tubulovesicular clusters containing the transmembrane protein Atg9.[8] Autophagosomes can either envelop bulk cytoplasm for nutrient recycling, as in the case of starvation-induced autophagy, or specific molecules/organelles such as malfunctioning mitochondria that the cell wants to send to the vacuole for degradation. One specific target of autophagy is the precursor form of the vacuolar peptidase aminopeptidase I (prApe1), which forms oligomers that are constitutively trafficked to the vacuole by selective autophagy even in nutrient-rich conditions.

Previous studies show that Atg11 functions as a scaffold protein in the targeting of Atg9 to the PAS,[9] as well as the PAS recruitment of prApe1 via its receptor Atg19. Atg11 interacts directly via its coiled-coil domains with both Atg9 and Atg19 as well as the PAS-localized Atg1, which allows it to bring Atg9-containing membrane and Atg19-prApe1 oligomers to the PAS.[10] What Lipatova et al.[7] now show is that Atg11 also interacts with Ypt1

in a GTP-dependent manner, and as such appears to function as a classical Rab effector membrane tether. They first demonstrate this interaction with a yeast two-hybrid assay, and map the interacting region to coiled-coil domains 2 and 3 (CC2-3) of Atg11. They further verify the interaction using in vitro binding assays with purified GST-Ypt1 and either native Atg11-HA from yeast lysates or purified His-Atg11-CC2-3, as well as with bimolecular fluorescence complementation (BiFC). BiFC also allows them to localize the interaction between Ypt1 and Atg11, and they show that it takes place at the PAS.

This interaction is lost in the *ypt1-1* point mutant, which has been previously shown to be defective in autophagy.[11] Ypt1 plays roles in multiple membrane-trafficking processes, recruiting distinct tethers to both COPII and COPI vesicles as well as playing a previously undefined role in autophagy. Specificity is provided by the TRAPP complex, a multisubunit tethering complex that also acts as a GEF to activate Ypt1. COPII vesicle trafficking requires the TRAPPI complex, whereas COPI requires the slightly larger TRAPPII complex. Autophagy utilizes the TRAPPIII complex, which is the only TRAPP complex that contains the Trs85 subunit.[11] Trs85, and the BiFC Trs85-Ypt1 interaction, colocalize with Atg11 at the PAS.[7] In addition, both are found at a subset of the peripheral Atg9-containing membrane compartments.

Together, these findings suggest a model where Atg9, or some other component of the Atg9 peripheral compartment, first recruits Trs85-Ypt1, which then subsequently recruits Atg11. Atg11 then in turn targets Atg9 and its associated membrane to the PAS via its association with Atg1.

Untangling Atg11's Role

Both the *trs85Δ* and the *ypt1-1* mutant have a stronger defect in autophagosome formation than can be explained by Atg11 alone: Atg11 is necessary only for selective autophagy, during growing conditions, not for starvation-induced bulk autophagy, whereas Trs85 and Ypt1 are necessary for both.[11,12] During starvation, Atg17 is able to compensate for the lack of Atg11 and recruit Atg9 to the PAS. It remains to be seen whether or not Atg17 is likewise a Ypt1 effector, or whether it is recruited independently.

Atg11 interacts with at least six Atg proteins, including Atg17 and itself.[10] A major question about Atg11's tethering activity is whether these multiple interactions are complementary or competitive. The ability to engage multiple components of the Atg9 compartment and the PAS simultaneously might be a way to enhance Atg11's efficiency and selectivity, similar to the way that other tethering factors often bind both active Rabs as well as components of the vesicle coat.[4] It is not

clear, however, that this is what is happening. The interactions of Atg11 with Ypt1, Atg1 and itself are all mediated by the CC2-3 region; similarly, the interactions with Atg9, Atg17 and Atg20 are all mediated by CC2. Can the CC2-3 region engage in multiple interactions simultaneously, or are these mutually exclusive? Only the interaction with Atg19 seems to be completely separate, mapping to CC4.[10]

Particularly interesting is Atg11's self-interaction. Self-interaction could aid in formation of larger complexes, as it does for some other coiled-coiled tethers, such as the dimeric Uso1.[3] Conversely, it could represent some sort of auto-inhibition. Interestingly, the deletion of *ATG19* in a wild-type strain causes GFP-Atg11 to disperse into the cytoplasm,[10] but when all other *ATG* genes are deleted in addition to *ATG19*, GFP-Atg11 forms a bright cytoplasmic punctum,[13] suggesting that Atg11's other interactions limit its ability to self-assemble into an oligomeric complex. The use of in vitro binding studies of Atg11 and its interactors to determine which interactions are competitive and which are not should clear up these questions and help us better understand Atg11's role.

A Tale of Many Tethers

Atg11 is not the only tethering factor that plays a role in autophagy. The conserved oligomeric Golgi (COG) complex, the exocyst complex, the coiled-coil protein Bug1 and Atg8 all potentially have autophagy-specific tethering roles.

The strongest evidence for an autophagy-specific tethering role exists for the COG complex, which is a multisubunit tether that functions in Golgi transport, and, like Atg11, is a Ypt1 effector. Mutant analysis has demonstrated a role for COG subunits 1 through 4 in both specific and starvation-induced bulk autophagy.[14] These subunits localize to the PAS (albeit at a low frequency), and interact with certain Atg proteins, including Atg17, Atg20 and Atg24. Like Atg11, the COG complex is necessary for normal trafficking of Atg9 to the PAS.[14] Neither the COG mutants nor an *atg11Δ* strain are completely defective for autophagy, suggesting

that these two tethers may be partially redundant in Atg9 trafficking. However, to date there has been no direct exploration of the relationship between these two tethers, and so this remains a subject for future study.

The exocyst complex is a multisubunit tether that is recruited to exocytic vesicles by the Rab-family GTPase Sec4 and promotes exocytosis. Both Sec4[15] and several subunits of the exocyst[16] also play a role in autophagy. The nature of this role has not been determined, but in mammalian cells the exocyst complex was recently shown to interact with a number of ATG proteins and proposed to act as a scaffold for the induction of autophagy during starvation.[17]

Bug1 is a coiled-coil tether whose activating GTPase is unknown. Bug1 forms an obligate complex with Grh1, which is recruited to COPII vesicles by an interaction with the Sec23-Sec24 complex, and to the *cis*-Golgi by an acetylated N-terminal amphipathic helix.[18] Its normal role is in ER-to-Golgi trafficking, and it has not been implicated directly in autophagy. However, during starvation conditions, Grh1 relocalizes in a Bug1-dependent manner to a novel organelle adjacent to ER exit sites that is involved in an unconventional secretory pathway. This pathway involves much of the autophagy machinery, and Atg9 (but not prApe1) partially localizes to the Grh1 compartment during starvation,[19] suggesting that Bug1 might play a starvation-specific role in Atg9 targeting. Deletion of *GRH1* does not block nonspecific autophagy, as measured by GFP-Atg8 processing,[20] but it is possible that a more quantitative assay would show a partial defect. At the moment, however, a role for Bug1 in conventional autophagy remains completely speculative.

Finally, there is evidence that Atg8 might act as an unconventional membrane tether. Atg8 is a small ubiquitin-like protein that is covalently conjugated to the lipid phosphatidylethanolamine (PE) at the PAS. Atg8 conjugation is necessary for autophagosome formation, and Atg8 is thought to play a role in the expansion of the autophagosome since cellular levels of Atg8 help control autophagosome size.[21] In vitro work has shown that Atg8

self-interacts, and can tether artificial membranes and cause liposome hemifusion[22] or fusion.[23] A recent report, however, disputes the idea that Atg8 can cause membrane fusion under physiological conditions,[16] and this point remains controversial. However, all reports agree that Atg8 can self-interact and tether liposomes, suggesting that Atg8 in vivo might likewise play a membrane-tethering role, perhaps upstream of SNARE-mediated membrane fusion. Curiously, successful autophagosome formation also requires the ability of Atg8 to be deconjugated from PE, suggesting that Atg8 acts both as a positive and a negative factor, or that cycles of conjugation and deconjugation are somehow important.[24] Likely, much more work will be required to understand the as-of-yet rather elusive role of Atg8 in autophagosome formation, but a potential role as a membrane tether should be considered.

SNARE Connections

Many tethers, including Uso1, the COG complex and the exocyst complex, interact with SNARE proteins. This interaction presumably allows them to organize the SNARE proteins and help with SNARE complex formation and, thus, membrane fusion.[3] The mechanisms of membrane fusion during autophagosome formation have long been mysterious, but recently the yeast Q-SNARES Sso1, Sso2, Sec9 and Tlg2 and the R SNARES Sec22 and Ykt6 have been shown to play a role.[16] Ultrastructural analysis of the yeast *sso1Δ/sso2ts* mutant shows clusters of Atg9-positive carriers resembling small secretory vesicles. This suggests that Sso1 acts in the very early stages of autophagosome formation, during the formation of the Atg9-containing tubulovesicular clusters. In addition, it may also act later in the expansion of the phagophore, as suggested by the fact that overexpressed Sso1 can partially localize to the PAS. Given the frequent links between membrane tethers and SNARE complexes, it seems reasonable to look for interactions between Atg11 or the COG complex and these SNARES. Mapping these interactions and their cellular location should give further insight into the roles these tethers play.

Conclusion: Membrane Trafficking in Autophagy

The mechanisms of membrane trafficking during autophagy have long been mysterious, and they remain a subject of much debate. Many aspects are still unclear, and doubtless many surprises remain. Nevertheless, in the last few years a burst of research activity has given some clues as to how this process might work. There appears to be a combination of two approaches: the redirection and repurposing of known membrane trafficking machinery, such as SNARES and the COG complex, as well as the use of novel machinery such as Atg11 and Atg8. The recent identification of Atg11 as a potential Ypt1 effector coiled-coil membrane tether is another piece in the puzzle, and prepares the way for additional work.

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