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The molecular mechanism of Atg13 function in autophagy induction: What is hidden behind the data?

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ABSTRACT

Atg13 is an essential subunit of the Atg1 autophagy initiation complex in yeast and its mammalian counterpart, ATG13, is indispensable for autophagy induction by the ULK1 complex. The N terminus of the protein folds into a HORMA domain, an architecture that has been revealed by crystallography.[1-4] In human cells, the ATG13 HORMA domain interacts directly with ATG14, a subunit of the class III phosphatidylinositol 3-kinase complex.[5] In budding yeast, the HORMA domain of Atg13 recruits Atg14, but a direct interaction remains to be proven.[1] The amino acid sequence that follows the HORMA domain does not adopt any 3-dimensional structure on its own; therefore, it is termed an intrinsically disordered region (IDR). Here we discuss the results of 2 recent studies in light of previous reports on Atg13 from yeast. Together, they yield an insight into the molecular mechanism for the function of this intriguing protein, and reveal why Atg13, as well as the mammalian homolog ATG13, cannot have a structurally rigid architecture.

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A recent report by Rao et al.[6] significantly advanced our understanding of the function and structure of the Atg1 complex. This paper is the first study where this complex was reconstituted in vitro from purified components. One interesting discovery in this work is that not only Atg1, but Atg13 as well, is a lipid binding protein that recognizes highly curved membranes. This can be interpreted to indicate that Atg13 binds to Atg9-enriched membrane vesicles, as does a part of the C-terminal EAT domain of Atg1 (the microtubule-interacting and transport [MIT] domain), although how Atg13 interacts with lipids still remains unknown. In contrast to Atg13, Atg17 does not bind lipid membranes.[6,7] In agreement with an earlier study,[8] Atg17 interacts with Atg9. Specifically, the central crescent (α4 helix) of Atg17 binds to the cytoplasmic domain (residues 424–507 in *S. cerevisiae*) and a short N-terminal region (residues 281–316 in *S. cerevisiae*) of Atg9. Since the very same part of the α4 helix of Atg17 also harbors the Atg31 binding site, based on the crystal structure of the Atg17-Atg31-Atg29 trimer,[7] this leads to the hypothesis that Atg9 and Atg31 could compete for the binding site on Atg17. Indeed, the experimental data by Rao et al. show that the C-terminal region of Atg31 (residues 160–196 in *S. cerevisiae*) is a strong competitive inhibitor of the Atg9-Atg17 interaction. This observation means that the C-terminal α1 helix of Atg31 must dissociate from Atg17—and thereby the Atg31-Atg29 subcomplex dislocates from its "obstacle" position in the center of the Atg17 crescent—so that Atg9 can bind to the Atg17 α4 helix.

A steric bloc created by the Atg31-Atg29 dimer in the center of the Atg17 crescent has been proposed earlier from the crystal structure.[7] What is new in the study by Rao et al. is the molecular mechanism for removal of the steric bloc. An earlier hypothesis was that Atg31 pivots in the β7-α1 loop,[7] assuming that the α1 helix of Atg31 stays bound to Atg17. A new model arising from the data of Rao et al. suggests that another domain of the Atg31-Atg29 subcomplex pivots it into a new position, and thereby activates the Atg17-Atg31-Atg29 trimer. One possibility can be that the flexible C terminus of Atg29[9] is a pivoting point; the C terminus of Atg29 was recently shown to interact with Atg17.[10] This new model leads to an important question: what causes the Atg31-Atg29 subcomplex to pivot? An answer to the question comes in the form of the Atg13 protein. Rao et al. showed that the activation of the Atg17-Atg31-Atg29 trimer is Atg13 dependent; in the absence of Atg13, Atg9 binds to Atg17 inefficiently. Moreover, this activation also requires Atg17 dimerization. Both the presence of Atg13 and dimerization of Atg17 being needed for activation of the trimer are difficult to reconcile into a molecular mechanism without considering a study that was published recently by Yamamoto et al.[11] Although the final model in this study does not incorporate some of the findings from the work of Rao et al., it shows that besides the previously reported[12] Atg13 binding region for Atg17 (17BR; residues 424–436), the Atg13 protein harbors an additional binding site for Atg17, named the 17LR, which is regulated by dephosphorylation of Ser379. More importantly, binding of Atg13 to Atg17 via 17LR requires dimerization of Atg17. Putting the results together reveals a possible molecular mechanism of how Atg13 activates the Atg17-Atg31-Atg29 trimer during autophagy induction. Enhanced binding of Atg13

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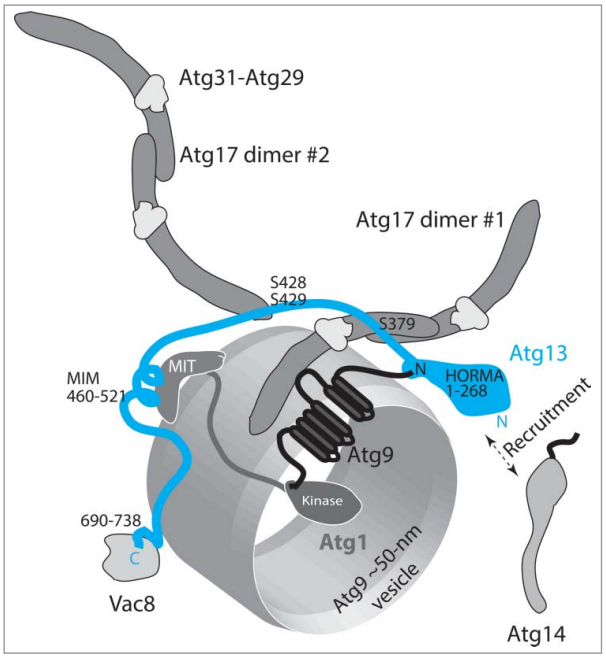

Figure 1. Model for the function of Atg13 in yeast during autophagy induction. Atg13 is depicted interacting with currently known binding partners. The HORMA domain of Atg13 binds the N terminus of Atg9,[2] and recruits Atg14, directly or indirectly.[1] The IDR of Atg13 contains at least 2 Atg17 binding motifs that are regulated by dephosphorylation upon starvation. Specifically, dephosphorylation of phosphoserines S379 in the 17LR motif (359–389) and S428 and S429 in the 17BR motif (424–436) enhances the interaction between Atg13 and Atg17.[11,12] The 17BR and 17LR motifs of Atg13 bind to 2 distinct Atg17 dimers. Binding of the Atg13[17LR] to the Atg17 dimer interface facilitates a pivoting movement of the Atg31-Atg29 subcomplex away from the center of the Atg17 crescent, which activates the Atg17-Atg31-Atg29 trimer and leads to the Atg9-Atg17 interaction.[6] Downstream from 17BR, the Atg13 IDR carries the inducible *alpha*-helical MIT-interacting motif/MIM (460–521) that binds the MIT region of Atg1. The affinity of this interaction is also enhanced by dephosphorylation of phosphoserines in the MIT-interacting motif region.[12] The C terminus of Atg13 is occupied by Vac8.[19] The position of the membrane binding site(s) on Atg13 is unknown.

to the Atg17 dimer interface via the dephosphorylated 17LR region initiates the movement of the Atg31-Atg29 subcomplex from its inhibitory position, and thereby allows for binding of Atg9 to Atg17. This explains why Rao et al. found that the monomeric Atg17-Atg31-Atg29 trimer and the monomeric Atg17-Atg31-Atg29-Atg13-Atg1 pentamer cannot bind and tether Atg9-protein liposomes. These monomeric complexes simply do not have the binding site for the Atg13 17LR domain.

Combining the results of Rao et al.[6] and Yamamoto et al.[11] in reverse chronological order along with the previous research yields a model of Atg13 function within the Atg1 complex for tethering an Atg9 vesicle (Fig. 1). This model shows that Atg13 interacts with at least 6 structurally diverse partners, and thereby functions as a type of "glue" bringing all the components optimally together. This type of function was observed in hub proteins that, in contrast to non-hub proteins, make numerous binding connections.[13] Analysis of hubs revealed that intrinsic disorder is an important feature for their function.[14,15] Thus, Atg13 appears to operate as a disordered assembly component or scaffold[16] that provides a backbone regulating spatiotemporal assembly of the components in the autophagy induction complex. This model also explains why Atg13 needs its architectural plasticity—this is the only way to prevent steric hindrance when accommodating structurally diverse binding partners. This conclusion translates also to mammalian ATG13, which is so far known to interact with RB1CC1, ULK1, LC3, ATG101, ATG14, and lipid membranes.[5,17] The ATG13 HORMA is unstable in the absence of ATG101,[3] suggesting a structural plasticity even at the N terminus of the protein. Given the recently reported nonautophagic function of ATG13 in cardiac development,[18] there are likely other binding partners that ATG13 must accommodate and that are waiting to be discovered.

Abbreviations

IDR intrinsically disordered region
MIT microtubule-interacting and transport

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No potential conflicts of interest were disclosed

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