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Multiple weak interactions through intrinsically disordered regions mediate the recruitment of Atg9 vesicles by Atg11 to the PAS

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Macroautophagy/autophagy is a complex and highly regulated cellular recycling process during which double-membrane vesicles, the autophagosomes, deliver cytoplasmic material for degradation to lysosomes (or the vacuole in yeast). The early step of autophagosome formation, namely the nucleation of the precursors to autophagosomes termed phagophores, involves over a dozen proteins belonging to the conserved autophagy machinery. The molecular mechanisms underlying autophagosome formation and especially the spatiotemporal regulation of the autophagic machinery at the phagophore assembly site (PAS) are still poorly understood.

In selective autophagy during which specific cargo material is targeted for degradation, the cargo itself is thought to trigger the assembly of the upstream acting autophagy machinery. Recognition of the cargo by so-called cargo receptors results in the recruitment of the scaffold protein Atg11, that in turn recruits Atg9 vesicles. These vesicles then serve as a platform for the recruitment of further autophagy proteins as well as membrane seeds for phagophore expansion. Atg9 vesicles contain on average 30 copies of the Atg9 molecule, which consists of a transmembrane core and two disordered cytosolic termini. While the transmembrane domains of Atg9 are important for its scramblase activity supporting phagophore expansion, the disordered termini mediate its interaction with autophagy machinery.

It was previously shown that the recruitment of Atg9 vesicles by Atg11 is mediated by the N terminus of Atg9 (Atg9-NTD). In a recent study we set out to identify the mechanistic and structural basis for the interaction between Atg11 and the Atg9-NTD. The Atg9-NTD is largely devoid of any

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secondary of tertiary structure and qualifies as an intrinsically disordered protein (IDP) [1]. Molecular interactions involving IDPs are notoriously difficult to characterize using high-resolution methods such as X-ray crystallography or cryo-EM. We therefore used state-of-the-art multidimensional solution nuclear magnetic resonance (NMR) to map the regions in the Atg9-NTD, which interact with Atg11. This approach allowed us to identify two short PLF motifs in the Atg9-NTD that are crucial for this interaction. Mutating either of these PLF motifs to PAA lead to a significant decrease of the Atg9-NTD-Atg11 interaction as determined in a microscopy-based protein-protein interaction assay (MBPPI). In particular, mutation of both motifs completely abolished the interaction, suggesting that both PLF motifs contribute to the binding of the Atg9-NTD to Atg11. In line with the in vitro studies, co-immunoprecipitation and fluorescence microscopy-based colocalization assays confirmed that the two PLF motifs are important for the interaction between Atg9 and Atg11 and that they are required to recruit Atg9 to the PAS *in vivo*. Additionally, precursor aminopeptidase 1 (prApe1) processing and Pho8Δ60 assays to measure autophagic flux revealed that mutation of the PLF motifs to PAA reduces the transport of prApe1 to the vacuole in the selective autophagy-like cytoplasm-to-vacuole targeting (Cvt) pathway but had little effect on non-selective bulk autophagy.

A quantitative analysis using ITC showed that the K_D of the interaction between Atg11 and the Atg9-NTD is of the order of 1 μM. With such relatively low affinity isolated Atg9 and Atg11 will only transiently interact in cells. However, both proteins are clustered on surfaces. While Atg11 binds the cargo receptor Atg19 on the clustered prApe1 cargo thereby generating a high local Atg11 concentration, Atg9 is concentrated in several trimeric complexes on Atg9 vesicles. These clustering effects are likely to turn the weak interactions between the individual proteins into strong avidity driven interactions. The result is a tight complex that allows a robust recruitment of the Atg9 vesicle to the cargo (Figure 1). Avidity effects like this play important roles for autophagy at several different stages. For example, the cargo-bound receptor Atg19 recognizes locally concentrated Atg8 at the phagophore membrane. This mechanism enables a selective recognition of the cargo by the growing phagophore and a tight wrapping of the membrane around the targeted material. Another example is the recruitment of early autophagy factors by Vac8. Vac8 is locally confined at the vacuole. This local concentration enables anchoring of the cargo-Atg11 complex and the recruitment of the class III phosphatidylinositol 3-kinase complex 1 to the vacuole. In line with this, we suggest that the clustering of the scaffold protein Atg11 on the cargo-receptor complex triggers the stable recruitment of Atg9 vesicles and thereby becomes a crucial regulator of selective autophagy.

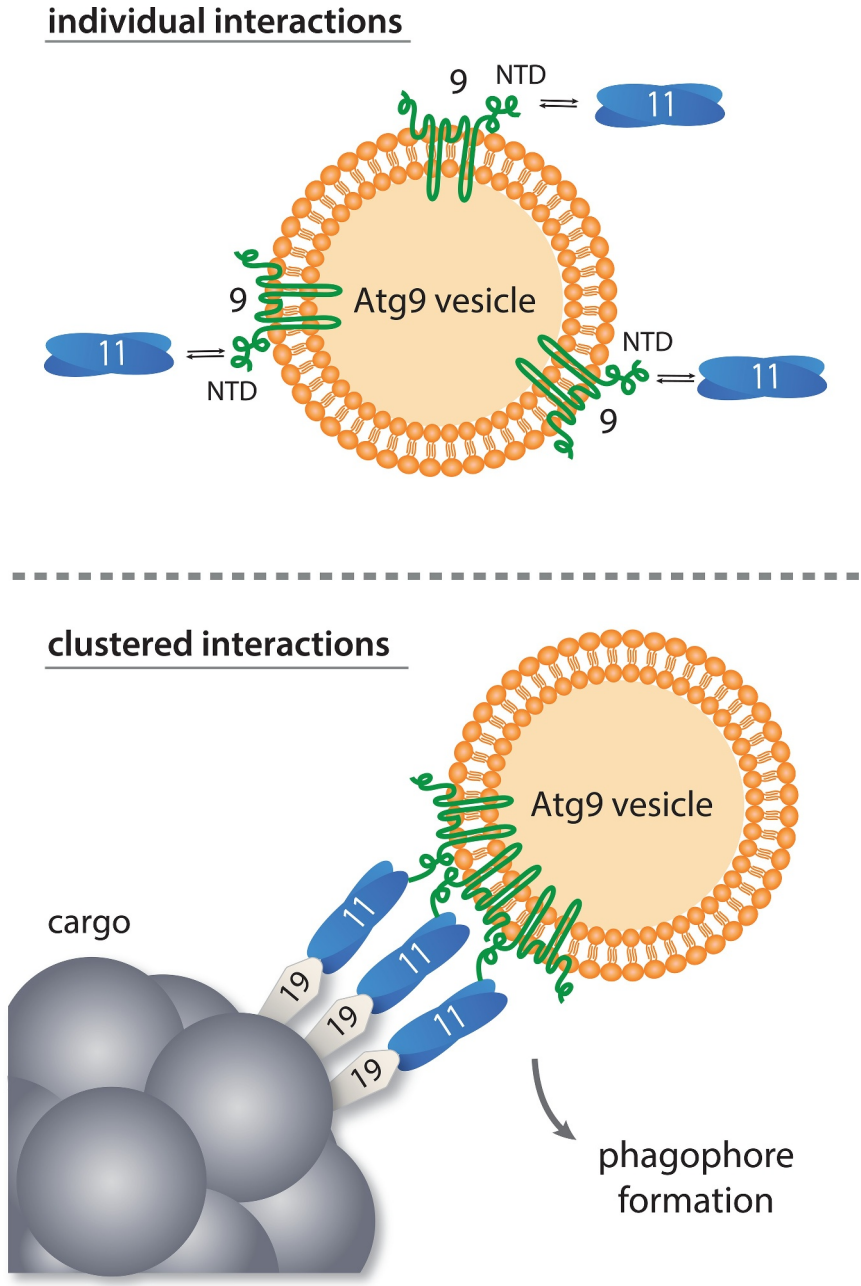

Figure 1. Model for the stable recruitment of Atg9 vesicles to the prApe1 cargo by Atg11. The recruitment is facilitated by the clustering of Atg11 on the cargo and of Atg9 on the vesicle.

IDPs such as the Atg9-NTD are known to engage in multiple interactions. In addition to Atg11, it was shown to interact with Atg17 and Atg13. The ability of IDPs to sample a large conformational space allows them to form complexes with different ligands and to act as interacting hubs orchestrating cellular processes. It is therefore not surprising that many autophagy factors contain disordered regions. Their ability to form redundant weak interactions with multiple partners and their amenability to regulation by post-translational modifications such as phosphorylation are key to the assembly of the phagophore. For example, many subunits of the yeast Atg1 and the mammalian UKL1 complexes contain IDRs that are crucial for their activity.

Understanding the properties of the IDPs and IDRs found in the autophagy machinery is fundamental to understanding the fine and subtle mechanisms that dictate the assembly of the phagophore and the spatiotemporal regulation of this process.

Disclosure statement

Sascha Martens is member of the scientific advisory board of Casma Therapeutics.

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Reference

[1] Coudevylle N, Banaś B, Baumann V, *et al*. Mechanism of Atg9 recruitment by Atg11 in the cytoplasm-to-vacuole targeting pathway. J Biol Chem 2022; 298(2), 101573.