

Open and closed HORMAAs regulate autophagy initiation

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The Atg1/ULK complex functions as the most upstream factor among Atg proteins to initiate autophagy. ATG101 is a constitutive component of the Atg1/ULK complex in most eukaryotes except for budding yeast, and plays an essential role in autophagy; however, the structure and functions of ATG101 were largely unknown. Recently, we determined the crystal structure of fission yeast Atg101 in complex with the closed HORMA domain of Atg13, revealing that Atg101 is also a HORMA protein with an open conformation. These 2 HORMA proteins play essential roles in autophagy initiation through recruiting downstream factors to the autophagosome formation site.

In budding yeast, autophagy is mediated by dozens of Atg proteins, among which 5 (Atg1, Atg13, Atg17, Atg29, and Atg31) constitute the Atg1 complex, which functions as the most upstream factor to initiate autophagy. In the case of higher eukaryotes such as mammals, the ULK complex is the equivalent of the Atg1 complex and regulates autophagy initiation. The mammalian ULK complex consists of ULK1/2 (Atg1 ortholog), ATG13, RB1CC1/FIP200 (functional counterpart of Atg17) and ATG101, but lacks Atg29 and Atg31 orthologs. Importantly, most eukaryotes including fission yeast possess ATG101/Atg101 instead of Atg29 and Atg31 and utilize it as an essential factor for autophagy. Nevertheless, Atg101 has received little attention from autophagy researchers simply because it was not conserved in budding yeast, and studies on Atg101 have been delayed in comparison with the other components of the ULK complex.

Using proteins from fission yeast, we recently reported the crystal structure of Atg101 in a complex with the HORMA (Hop1, Rev7 and MAD2) domain of Atg13. Intriguingly, the 2 proteins have a similar structure to each other, and the Atg101 structure is also topologically classified as a HORMA domain. MAD2, a representative of HORMA, possesses 2 topologically different conformations, open MAD2 (O-MAD2) and closed MAD2 (C-MAD2). These 2 conformations of the protein form a functionally important O-MAD2-C-MAD2 asymmetric homodimer that affects the conformational stability of each protein. Intriguingly, Atg101 and Atg13^{HORMA} are topologically similar to O-MAD2 and C-MAD2, respectively, and form the Atg101-Atg13 heterodimer that is quite similar to the O-MAD2-C-MAD2 homodimer. It has been established that C-MAD2 is stabilized by O-MAD2 via the formation of the homodimer. In a quite analogous manner, Atg13 is stabilized by Atg101 via the formation of the heterodimer. In the case of Atg13 from budding yeast, it does not require Atg101 for stabilization. Hurley's group previously reported the crystal structure of Atg13^{HORMA} from a budding yeast, *Lachancea thermotolerans*, which explains the lack of necessity of Atg101 for stabilization in these species; LtAtg13^{HORMA} has a 3-strand β -sheet insertion named the "cap" that has never been observed in canonical HORMA domains and utilizes the cap to stabilize the C-MAD2-like conformation without the help of Atg101. This insertion is not conserved in ATG13^{HORMA} from other eukaryotes that conserve ATG101, explaining the necessity of Atg101/ATG101 for stabilization of Atg13/ATG13 in these species.

Keywords: Atg13, Atg101, autophagy initiation, crystal structure, HORMA, ULK complex

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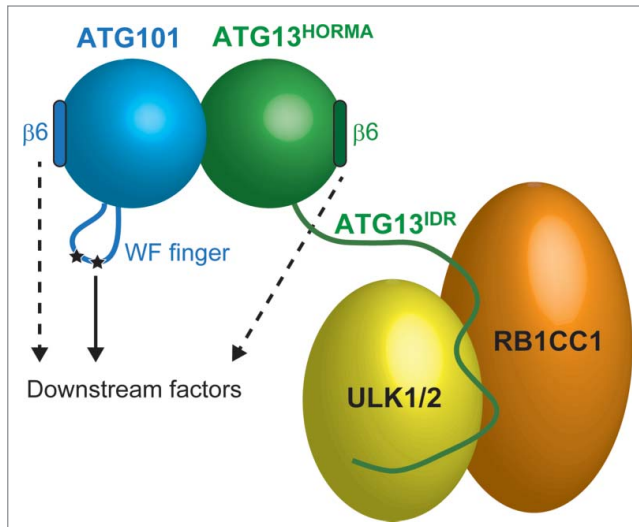


Figure 1. Schematic model of the ULK complex. The WF finger of ATG101 (stars indicate Trp and Phe) recruits downstream factors, in which $\beta 6$ of ATG101 and/or ATG13 might also be involved. IDR, intrinsically disordered region.

Upon conformational transition between O-MAD2 and C-MAD2, the N-terminal segment of MAD2 has to pass under the loop between $\beta 5$ and αC (the $\beta 5$ - αC loop consisting of 13 residues). Therefore, the adequate length of the $\beta 5$ - αC loop is essential for the conformational transition. In the case of Atg101 and Atg13^{HORMA}, the $\beta 5$ - αC loop is too short (5 and 7 residues for Atg101 and Atg13^{HORMA}, respectively) to undergo such a conformational transition. Thus, it is likely that Atg101 and Atg13^{HORMA} are locked in the O-MAD2-like and the C-MAD2-like conformations, respectively. It should be noted that there remains a possibility that these 2 proteins undergo a conformational change into as yet-unknown states that are not observed for MAD2.

MAD2 recognizes its targets using 2 mechanisms: forming an intermolecular β -sheet with the targets using $\beta 6$, and fas-

tening them using a loop region named the safety belt, which require MAD2 to transform from O-MAD2 to C-MAD2. In the case of Atg101 and Atg13^{HORMA}, they are locked to the O-MAD2-like and C-MAD2-like conformations, respectively, and appear not to be able to recognize their targets using the safety belt. Atg101 possesses a long loop between $\beta 4$ and $\beta 5$, which conserves Trp and Phe residues and exposes their aromatic side chains. Mutational studies on human ATG101 revealed that this $\beta 4$ - $\beta 5$ loop, named the WF finger, is dispensable for the ATG101-ATG13 interaction and for ATG13 stabilization, but is required for recruiting downstream autophagy-related factors, such as LC3, WIPI1 and ZFYVE1/DFCP1, to the autophagosome formation site. This observation clearly shows that ATG101 has an important, direct role in autophagy other than stabi-

lizing ATG13. $\beta 6$, which is important for target recognition in MAD2, is located in the proximity of the WF finger and exposed; therefore, it might be possible that $\beta 6$ is also involved in target recognition in collaboration with the WF finger. Mutations that destroy the ATG101-ATG13 interaction also impair the targeting of downstream factors to the autophagosome formation site. ATG13 might also be directly involved in recruiting downstream factors together with ATG101, and in that case, the recognition mode of targets might be distinct from that adopted by MAD2.

The established role of HORMA domains is to mediate protein-protein interactions. Two HORMA domains in the ULK complex, one from ATG101 and the other from ATG13, would mediate various combinations of protein-protein interactions, which would enable the ULK complex to regulate autophagy initiation in higher eukaryotes in a sophisticated manner. Further identification and characterization of the interactions mediated by these 2 HORMAs are critical to unveil the molecular mechanisms of autophagy initiation (Figure 1).

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No potential conflicts of interest were disclosed.

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