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Rph1 mediates the nutrient-limitation signaling pathway leading to transcriptional activation of autophagy

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To maintain proper cellular homeostasis, the magnitude of autophagy activity has to be finely tuned in response to environmental changes. Many aspects of autophagy regulation have been extensively studied: pathways integrating signals through the master regulators TORC1 and PKA lead to multiple post-translational modifications affecting the functions, protein-protein interactions, and localization of Atg proteins. The expression of several *ATG* genes increases sharply upon autophagy induction conditions, and defects in *ATG* gene expression are associated with various diseases, pointing to the importance of transcriptional regulation of autophagy. Yet, how changes in *ATG* gene expression affect the rate of autophagy is not well characterized, and transcriptional regulators of the autophagy pathway remain largely unknown. To identify such regulators, we analyzed the expression of several *ATG* genes in a library of DNA-binding protein mutants. This led to the identification of Rph1 as a master transcriptional regulator of autophagy.

Cells lacking *RPH1* show a higher expression of several *ATG* genes and their corresponding proteins specifically in physiological conditions. This upregulation is associated with an increase in autophagy flux soon after nitrogen starvation. Conversely, overexpressing *RPH1* blocks the induction of expression normally observed for these genes after starvation, and consequently results in a large defect in autophagy activity. Together these data demonstrate that Rph1 functions as a negative regulator of autophagy by repressing the expression of *ATG* genes in rich conditions. The expression of *ATG8* and *ATG9* is highly increased upon autophagy induction, and the products of these genes control the size and number of autophagosomes, respectively.

Here, we show that although *RPH1* regulates several *ATG* genes including *ATG8* and *ATG9*, it particularly affects the expression of *ATG7*. Lowering the level of Atg7 leads to reduced autophagy activity suggesting that a concomitant increase of Atg7 and Atg8 is required for proper autophagy induction upon nitrogen starvation. Consistent with this idea is the finding that the single overexpression of *ATG7* is not sufficient to promote autophagy, suggesting that the latter requires a concerted upregulation of multiple *ATG* genes. Our results further indicate that the overexpression of *RPH1* causes a drastic reduction in the number of autophagosomes, but does not affect their size. These results are surprising given that a reduction in the level of Atg8 was previously associated with a reduction in autophagosome size. However, we hypothesize here that a block in the induction of multiple *ATG* genes, including *ATG9*, might slow the overall autophagosome biogenesis process thus allowing the formation of full-sized vesicles.

How does Rph1 control *ATG* gene expression? Rph1 is a DNA-binding protein, which contains a Jumonji C histone demethylase catalytic domain. Cells deprived of trimethylation at H3K36 or cells expressing a catalytically inactive mutant of Rph1 have normal autophagic capacity demonstrating that Rph1 functions mostly independently of its histone demethylase activity in regulating autophagy. Conversely, an Rph1 mutant lacking its DNA-binding domain, which is not able to bind the *ATG7* promoter, is not able to rescue the *rph1Δ* phenotype.

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These results suggest that Rph1 controls the expression of *ATG* genes by directly binding to their promoters, thus restricting the access of potential activators, or of the general transcriptional machinery.

How is Rph1 function released to promote autophagy under starvation conditions? We noticed that the *RPH1* deletion strain shows higher expression of *ATG* genes only in rich conditions suggesting that the repressive effect of Rph1 is released after nitrogen starvation. Furthermore, the fact that overexpressing the protein blocked the induction of *ATG* gene expression upon nutrient limitation pointed to the strict requirement of such a release for the proper induction of autophagy. We found that Rph1 is highly phosphorylated upon nitrogen starvation and we identified some of the residues involved in the post-translational modification; these data indicate that a reduction in Rph1 phosphorylation leads to a partial block in autophagy flux. In addition, we identified Rim15 as the kinase mediating Rph1 phosphorylation under these conditions, thereby inhibiting its function and allowing for autophagy induction. Rim15 controls autophagy, notably by integrating signals from TORC1 and PKA. TORC1 prevents Rim15 nuclear localization through its phosphorylation by Sch9, whereas PKA directly phosphorylates Rim15 thereby inhibiting its kinase activity. If the post-translational regulation of autophagy involving the master regulator TORC1 is well defined, its transcriptional effect on autophagy is less understood. Moreover, the mechanisms by which PKA and Sch9 inhibit autophagy are still mostly elusive; Rim15 positively regulates autophagy upon PKA-Sch9 inactivation, yet little was known about the downstream effectors in this signaling pathway. Here, we characterize Rph1 as one of these effectors and uncover a Rim15-dependent Rph1-mediated transcriptional control of autophagy similar to the Rim15-dependent phosphorylation of Ume6 after nitrogen starvation, which leads to a release of its repression of the expression of *ATG8* and an induction of autophagy.

Finally, our work shows that the function of Rph1 is conserved in higher eukaryotes. A reduction in the expression of one mammalian homolog of *RPH1, KDM4A*, leads to an increase of autophagy flux whereas, conversely, its overexpression blocks autophagy activity. The level of KDM4A decreases after autophagy induction while the phosphorylation of the protein is increased, suggesting that, like Rph1, the inhibition of KDM4A activity acts as a switch to promote autophagy in stress conditions.

Rph1 was previously identified as a negative regulator of several DNA-damage inducible genes and is therefore thought to be part of the signaling pathway leading to the cell response upon genotoxic stress. Rph1 is phosphorylated in these conditions as well as upon osmotic stress, suggesting that it might mediate broader stress signal responses. DNA damage induces autophagy in both yeast and mammalian cells; it would therefore be of interest to determine whether the role of Rph1/KDM4A in autophagy induction is conserved in these conditions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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