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Bidirectional roles of Dhh1 in regulating autophagy

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ABSTRACT

Macroautophagy/autophagy activity is carefully modulated to allow cells to adapt to changing environmental conditions and maintain energy homeostasis. This control notably occurs in part through the regulation of autophagy-related (*ATG*) gene expression. Others and we have jointly shown that under nutrient-rich conditions Dhh1 mediates the degradation of certain *ATG* mRNAs, most significantly that of *ATG8*, through a Dcp2-dependent decapping pathway to maintain gene expression and autophagy activity at a basal level. More recently, we illustrated that under nitrogen-starvation conditions Dhh1 switches its role to become a positive regulator of autophagy, and promotes the translation of *ATG1* and *ATG13* mRNAs to meet the increased demand for autophagy activity. This regulation helps selected *ATG* mRNAs to escape the general repression in translation that occurs when nutrients are limited and TOR is inhibited. Our studies also suggest that Dhh1's nutrient-dependent bidirectional regulation of autophagy is conserved in more complex eukaryotes.

Abbreviations: ATG: autophagy related; EIF4EBP: EIF4E binding protein; UTR: untranslated region.

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Dhh1 belongs to the DExD/H-box-containing RCK family of RNA helicases. This protein plays roles in various aspects of mRNA regulation, including Dcp2-dependent decay, storage in stress granules, and translation. In 2015, collaborators and our lab jointly reported the first study demonstrating Dhh1's role in the regulation of autophagy; we identified Dhh1 as a negative regulator of this process. This study demonstrated that Dhh1 and its orthologs, including Vad1 in the pathogenic fungus *Cryptococcus neoformans* and DDX6 in mammals, directly binds specific *ATG* mRNAs, including *ATG8*, mediating their decapping and subsequent degradation through the action of Dcp2 under nutrient-rich conditions; a subsequent study from our lab showed that the Xrn1 exonuclease plays a role in degrading the decapped transcripts. Based on results using phospho-deficient mutants of Dcp2, we also suggested that the binding of Dhh1/Vad1/DDX6 to *ATG* transcripts is regulated by the TOR-dependent phosphorylation of Dcp2.

Although our work suggested that the inhibitory effects of Dhh1-Dcp2 on *ATG* mRNAs is released after nitrogen starvation when kinase activity of TOR is inhibited, we observed that the *dhh1Δ* cells display a significantly lower viability than wild-type cells after 1 or more days of nitrogen starvation. We initially hypothesized that the loss of viability was due to prolonged excessive autophagy in the mutant cells; however, this hypothesis was not experimentally tested. Recently, using a newly developed assay, we were able to show that contrary to our initial hypothesis, autophagy activity is diminished in the *dhh1Δ* cells, implying that Dhh1 positively regulates autophagy during long-term nitrogen starvation [1].

To address this paradox, we explored the function of Dhh1 in the regulation of autophagy after nitrogen starvation. It was previously known that Dhh1 facilitates the translation of a specific group of mRNAs, which contain structured regions shortly after their start codons. Among 18 *ATG* mRNAs within the core molecular machinery of autophagy, *ATG1* and *ATG13* transcripts display such predicted structured regions. Atg1 and Atg13 protein levels, but not their mRNA levels, significantly decrease in the *dhh1Δ* cells after nitrogen starvation, leading us to hypothesize that Dhh1 positively regulates autophagy through promoting the translation of *ATG1* and *ATG13* mRNAs. Using RNA immunoprecipitation assays, we showed that Dhh1 associates with both the 5′ untranslated region (UTR) and the 3′ UTR of *ATG1* and *ATG13* transcripts. Molecular genetic analysis suggested that the 5′ UTR, the 3′ UTR and the structured regions of the mRNAs, as well as the RNA binding and helicase activity of Dhh1 are required for Dhh1-dependent translational regulation. These data suggest a model in which Dhh1 promotes autophagy during nitrogen starvation by binding to *ATG1* and *ATG13* mRNAs, and possibly remodeling their structured regions to facilitate translation. We also showed that in HEK293A cells during amino acids starvation DDX6

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positively regulates the translation of *ATG16L1* mRNA, which also contains predicted structured regions, suggesting that translational regulation of autophagy-related mRNAs by Dhh1 is conserved in more complex eukaryotes.

Dhh1 associates with the cap-dependent translation initiation machinery containing EIF4E, and the EIF4E binding protein (EIF4EBP) Eap1 has been previously reported to interact with Dhh1 under nutrient-rich conditions. To test whether Eap1 is involved in the Dhh1-dependent translational regulation of *ATG* mRNAs, we first showed that Eap1 also interacts with Dhh1 under nitrogen-starvation conditions in an RNA-dependent manner. In addition, the deletion of *EAP1* leads to decreased Atg1 and Atg13 protein levels, but not the corresponding mRNA levels, as well as to impaired autophagy activity during nitrogen starvation, similar to the results seen with the *dhh1Δ* strain.

EIF4EBPs are known as general translation repressors, but our findings suggest that Eap1 promotes the translation of Atg1 and Atg13, which is rather unexpected. To further investigate Eap1's function as a translation activator, we compared the protein sequence of Eap1 with that of other EIF4EBPs, and found that Eap1 contains a unique long C-terminal region, which is required for its role in promoting Atg1 translation. However, our analysis indicates that the C terminus of Eap1 is not necessary for its interaction with Dhh1. Further studies on this region may reveal more on the intriguing question of how Eap1 positively regulates translation of certain mRNAs under stress conditions.

To summarize, two studies from our lab showed that Dhh1 is a bidirectional regulator of autophagy. In nutrient-rich conditions, Dhh1 coordinates with the mRNA decapping machinery to degrade *ATG* mRNAs to keep autophagy at a basal level. Upon nitrogen starvation, Dhh1 switches its role to facilitate autophagy through promoting Atg1 and Atg13 translation. What triggers the switch of Dhh1's functions in autophagy requires further investigation. It is likely that post-translational modifications on Dhh1 and/or Eap1 are relevant. Supporting this hypothesis, the unique C terminus of Eap1 is highly phosphorylated by TOR and possibly other unknown kinases under nutrient-rich conditions. It is also of interest to note that Snf1 phosphorylates Dhh1 during glucose starvation, although we have not explored whether Dhh1-Eap1 regulates autophagy and *ATG* gene expression under this type of stress. Nonetheless, we think that changes in extracellular nutrient availability may regulate post-translational modifications on Dhh1-Eap1, thus modifying its interactome and switching its functions on different mRNAs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Reference

[1] Liu X, Yao Z, Jin M, et al. Dhh1 promotes autophagy-related protein translation during nitrogen starvation. PLoS Biol. 2019;17:e3000219.