A New Nutritional Effector of Autophagy in the Yeast *Saccharomyces cerevisiae*

Ishani Kapoor, Ranga Rajan, Henrik G. Dohlman
Department of Pharmacology, University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Abstract

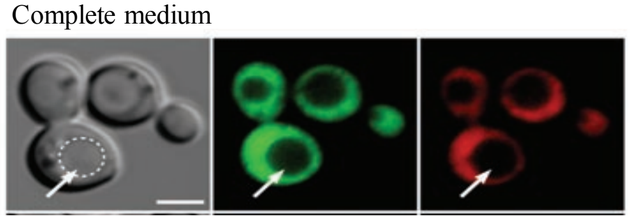
Autophagy is a conserved process that recycles cellular contents to promote survival during nutrient depletion. The genes and regulatory mechanisms of this pathway were first discovered in the budding yeast *Saccharomyces cerevisiae* and subsequently confirmed in higher eukaryotes. Although nitrogen starvation is the canonical inducer of autophagy, recent studies have revealed the important roles of other nutrients. In this work, we used a high-throughput assay to identify potassium starvation as a unique and potent inducer of autophagy. Peak response to potassium is one-third of that induced by nitrogen. We validated our findings using the GFP-Atg8 reporter. A targeted screen of ion channels revealed a new role of the Hal4 kinase in nitrogen and potassium dependent autophagy. Taken together, our studies highlight a new autophagy pathway governed by potassium ions.

Approach: Rosella assay

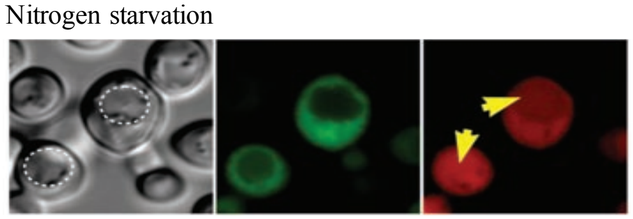
The autophagy biosensor Rosella is comprised of super ecliptic pHluorin (green, pH sensitive) fused with DsRed.T3 (red, pH stable). This reporter is introduced in wild-type BY4741 yeast via a plasmid-based vector. In nutrient rich media, Rosella is present in the cytoplasm. Upon induction of autophagy, Rosella is transported to the vacuole where the lower pH causes quenching of green fluorescence, whereas the red signal remains unaffected. Autophagy is quantified as the ratio of red and green fluorescence.

Yeast growth medium is comprised of a nitrogen source, sugar, amino acids and nucleotides, and a complex mixture of salts, vitamins, and trace elements (yeast nitrogen base, YNB). Since the roles of nitrogen and sugar in autophagy are well understood, we focused our analysis on individual components of YNB. We employed a high-throughput 96-well microplate format to simultaneously monitor autophagy in medium lacking YNB (SCD-YNB) and after adding back each of the individual components. Cells were maintained in logarithmic growth (optical density at 600 nm < 1) in complete medium (SCD) for ~ 24 hours and transferred to the 'starvation' medium (SCD-YNB+component). Fluorescence was measured at 30 minute intervals for 8 hours.

Complete medium



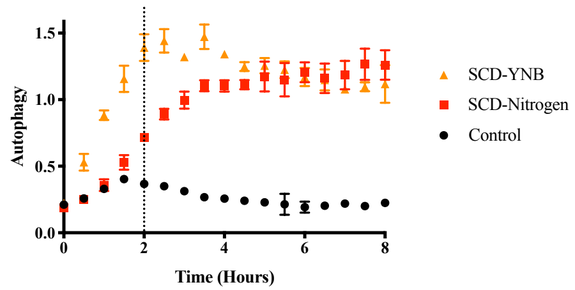
Nitrogen starvation



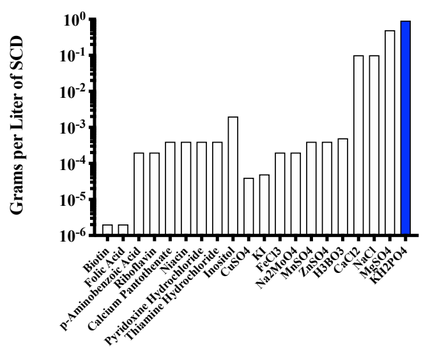
Rosado et. al. *Autophagy* **2008**

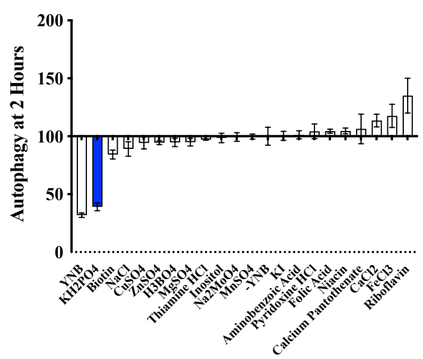
Results: Potassium starvation induces autophagy

YNB starvation induces a substantial early autophagy response that is 2-fold higher than that observed with nitrogen starvation.

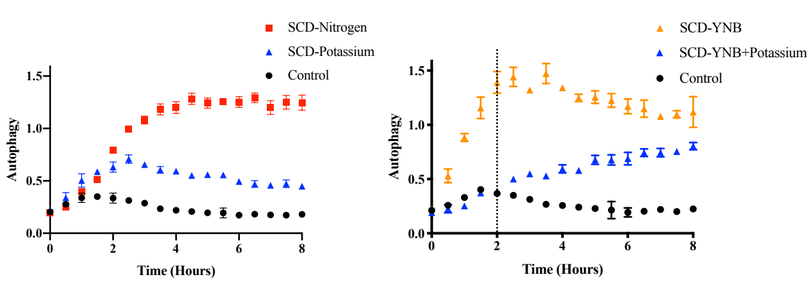


To identify the source(s) of the autophagy response in SCD-YNB, we added back each individual component to SCD-YNB and monitored Rosella fluorescence. As shown in the waterfall plot, addition of potassium ions (alone) shifts the response to basal levels.



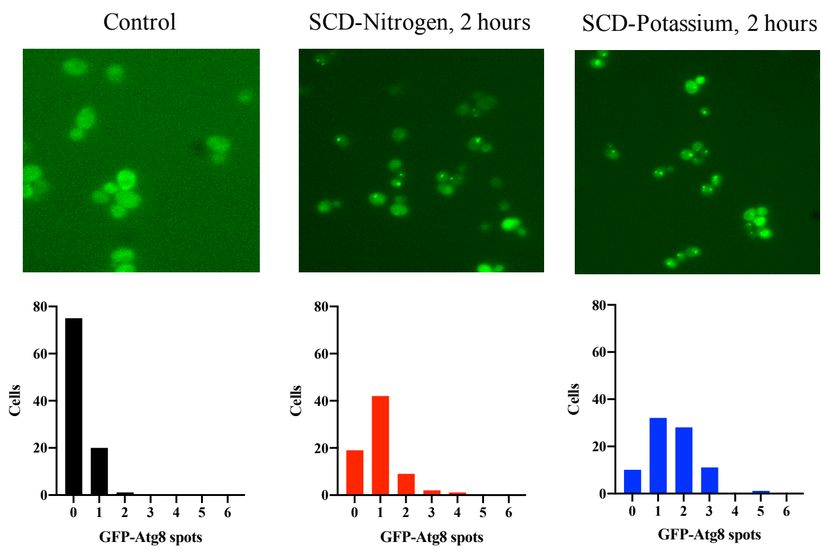


Using a complementary approach, we found that potassium starvation induces a significant autophagy response, which is one-third of that observed with nitrogen limitation.



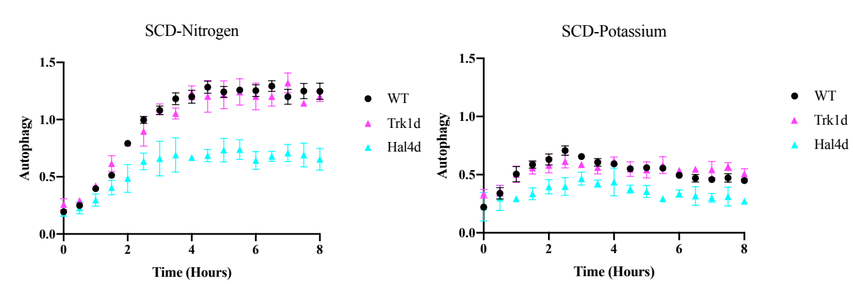
Results: Potassium starvation promotes clustering of Atg8

Atg8 is an autophagy-related protein essential for the formation of autophagy-related vesicles (autophagosomes) that engulf and transport cytoplasmic materials to the vacuole for degradation. GFP-labeled Atg8 has been used by other researchers to study autophagy using microscopy and immunoblotting. GFP-Atg8 is dispersed in the cytoplasm under normal conditions, and assembles into bright spots upon induction of autophagy. In agreement with our Rosella data, we observed multiple GFP-Atg8 spots in potassium-starved cells. These data confirm that the potassium response is mediated by the canonical autophagy machinery.



Results: Hal4 kinase promotes nitrogen and potassium dependent autophagy

Trk1 and Trk2 are the major ion channels that allow potassium ions to enter the cell. Hal4/5 kinases are required for Trk1/2 function.



Conclusions

Our systematic studies highlight the importance of potassium ions in the regulation of autophagy. In humans, ion homeostasis is important for many fundamental physiological processes such as muscle contraction, heart function and memory. Similarly, autophagy is implicated in cell differentiation, embryonic development and aging. Therefore, a thorough understanding of the regulatory mechanisms by which ions affect autophagy is crucial to develop effective therapeutic interventions.