

# TGN/endosomal system perturbation elicits an autophagic response through a nutrient sensing defect

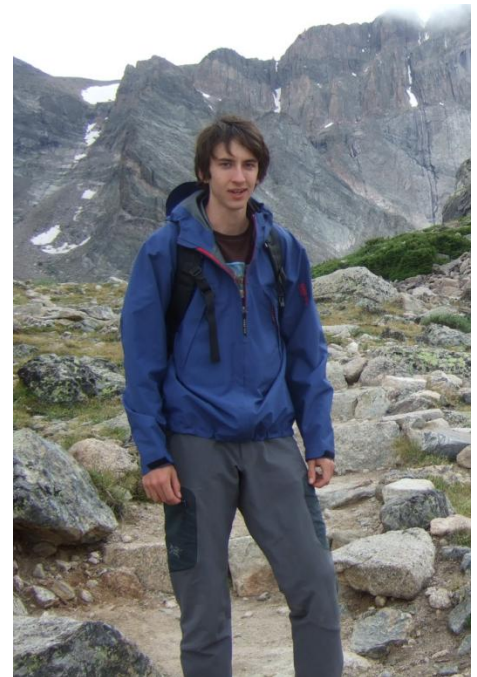
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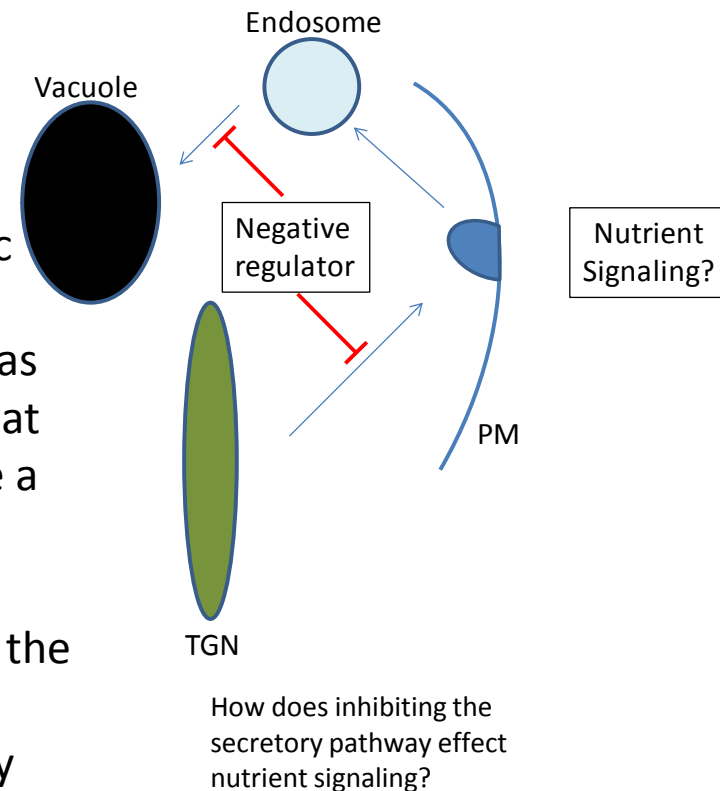
(Chair: Cell and Developmental Biology)



# Introduction

Previous work done in the Bankaitis lab has found a protein which when over expressed perturbs the TGN/endosomal system in yeast. A preliminary observation indicated that upon disruption of the TGN/endosomal system, yeast undergo an autophagic response despite the presence of a sufficient growth medium. The initiation of a starvation response such as autophagy, in standard growth conditions suggests that interfering with the secretory pathway must generate a nutrient sensing defect. The goals of this project are primarily twofold:

- Confirm that morphological changes are due to the induction of autophagy
- Elucidate any connection between the secretory pathway and nutrient sensing



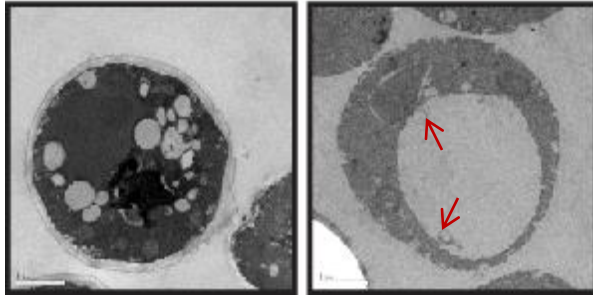
# Results

EM

*P<sub>Dox</sub> KES1*

+Dox

-Dox



Disruption of the secretory pathway leads to morphological changes.

- Dramatically enlarged vacuole
- Internal vesicular structures in vacuole (indicated by arrows)

Both are typical of cells undergoing autophagy.

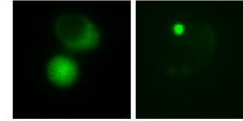
Atg18

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

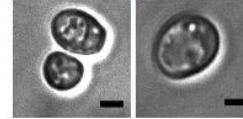
+

-

Atg18 - GFP



DIC



Ammonium starvation in wild type cells results in the redistribution of Atg18-GFP from the cytosol to punctate as autophagy is induced.

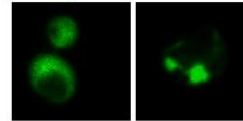
Doxycycline

*P<sub>Dox</sub> KES1*

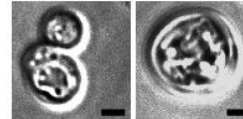
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Atg18 - GFP

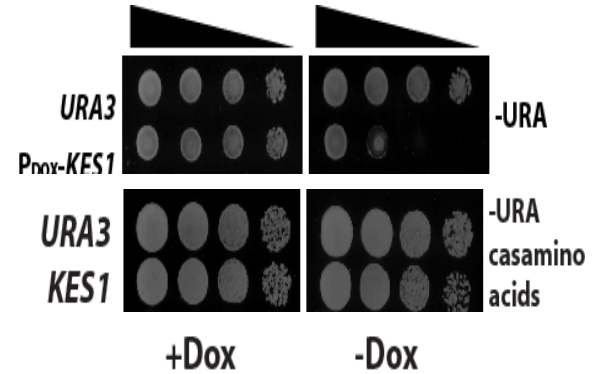


DIC



The redistribution of Atg18-GFP in secretory pathway deficient cells resembles that of starved cells, confirming autophagy.

AA remediation



Upon perturbation of the secretory pathway, a significant growth defect is presented.

Amino acid supplementation suppresses this growth defect, confirming that disruption of the secretory pathway results in a nutrient sensing/signaling defect which is in turn responsible for the starvation response.