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Autophagosome formation and selective autophagy receptors in nutrient-deprived yeast cells

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Abstract

When a cell encounters the specific example of nitrogen starvation, autophagy shifts from a low baseline housekeeping function to a massive recycling operation that breaks down cytoplasmic contents for survival. Selective autophagy, in which specific cargoes are recognized and delivered to autophagosomes via receptor proteins, adds precision to this response - but how different selective receptors contribute to autophagosome formation kinetics during prolonged starvation is unclear. This research tracked autophagosome biogenesis in *Saccharomyces cerevisiae* wild-type and receptor-deletion strains (Δ atg19, Δ atg34, Δ atg36) during nitrogen deprivation using GFP-Atg8 fluorescence microscopy and transmission electron microscopy. Wild-type cells formed an average of 22.4 autophagosomes per cell after 4 hours of nitrogen starvation, compared with 12.1 in Δ atg19 and 16.8 in Δ atg36. Receptor binding profiles differed: Atg19 showed the highest Atg8-binding and cargo recognition scores, while Atg36 excelled in oligomerization capacity. Bafilomycin A1 treatment, which blocks vacuolar degradation and allows autophagosome accumulation, revealed that flux through the pathway was reduced by 45.9% in Δ atg19. Phosphoproteomic analysis identified Ser391 on Atg19 and Thr217 on Atg36 as starvation-induced phosphorylation sites that enhanced receptor-Atg8 interaction. These data show that selective receptors are not merely passive cargo adapters but active contributors to autophagosome nucleation and expansion during starvation.

Keywords: Autophagosome, selective autophagy, autophagy receptors, yeast, nutrient deprivation, Atg19, Atg36, nitrogen starvation, GFP-Atg8, phosphoregulation

Introduction

Consider a yeast cell abruptly cut off from nitrogen: within minutes, it must decide what to recycle and what to spare. Autophagy - the bulk degradation of cytoplasmic material in the vacuole - provides the raw amino acids needed for survival, and cells that can't perform it die within hours of starvation ^[1]. But autophagy isn't just a random disposal system. Selective autophagy uses receptor proteins to tag specific cargoes - damaged mitochondria, surplus peroxisomes, protein aggregates - for preferential degradation ^[2].

In *S. cerevisiae*, three well-characterized selective autophagy receptors handle different cargoes: Atg19 directs aminopeptidase I and α -mannosidase to the vacuole via the cytoplasm-to-vacuole targeting (Cvt) pathway ^[3], Atg34 recognizes α -mannosidase specifically during starvation ^[4], and Atg36 targets peroxisomes for degradation (pexophagy) ^[5]. All three receptors bind Atg8, the yeast ortholog of mammalian LC3, through a conserved Atg8-interacting motif (AIM).

What hasn't been settled is how much these selective receptors contribute to overall autophagosome formation. Some models treat them as passive adapters that merely link cargo to pre-formed membranes. But growing evidence suggests they actively promote membrane expansion by clustering Atg8 molecules and scaffolding the autophagosome assembly site ^[6]. This research compared autophagosome formation rates and morphology in wild-type and receptor-knockout yeast strains during nitrogen starvation, and mapped phosphorylation events that regulate receptor activity.

Material and Methods

Material

S. cerevisiae strains used were BY4741 (MATa his3 Δ 1 leu2 Δ 0 met15 Δ 0 ura3 Δ 0) as wild-type, and isogenic deletions Δ atg19::KanMX, Δ atg34::KanMX, and Δ atg36::KanMX from

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the yeast deletion collection (Open Biosystems). A GFP-Atg8 fusion construct under the native ATG8 promoter was integrated at the URA3 locus in all strains. Cells were grown in YPD (1% yeast extract, 2% peptone, 2% dextrose) at 30°C and shifted to nitrogen-starvation medium (SD-N: 0.17% yeast nitrogen base without amino acids or ammonium sulfate, 2% glucose) for starvation experiments. Rapamycin (200 nM, LC Laboratories) and bafilomycin A1 (200 nM, Sigma-Aldrich) were used as positive control and flux inhibitor respectively. All experiments were performed at the Instituto de Biología Tropical de La Habana, Havana, between March and August 2023.

Methods

Autophagosome formation was quantified by counting GFP-Atg8 puncta per cell using a Nikon Eclipse Ti2 inverted fluorescence microscope with a 100× oil immersion objective. At least 200 cells per strain per time point were scored at 0, 2, 4, and 6 hours of nitrogen starvation. For transmission electron microscopy (TEM), cells were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812. Ultrathin sections (70 nm) were stained with uranyl acetate and lead citrate and examined on a JEOL JEM-1400 at 80 kV. Autophagosome diameter was measured from TEM images (n ≥ 50 per strain). Autophagic flux was assessed by the GFP-Atg8 processing assay: cell lysates were immunoblotted with anti-GFP antibody (Roche), and the

ratio of free GFP (vacuolar cleavage product) to GFP-Atg8 was calculated. For phosphoproteomics, cells were harvested at 0 and 2 hours post-starvation, lysed in 8M urea, digested with trypsin, enriched for phosphopeptides using TiO2, and analyzed by LC-MS/MS on a Thermo Q Exactive HF. Phosphosites were identified using MaxQuant v2.1. Statistical analyses used Student's t-test or one-way ANOVA with Tukey's post-hoc in GraphPad Prism 9. Ethics clearance was not required (non-human, non-animal research).

Results

Table 1: Autophagosome counts and dimensions in wild-type and receptor-deletion strains after 4 hours of nitrogen starvation

Parameter	WT	Δatg19	Δatg34	Δatg36
Puncta/cell (4h)	22.4 ± 3.1	12.1 ± 2.4	19.8 ± 2.8	16.8 ± 2.6
Diameter (nm)	412 ± 67	328 ± 54	397 ± 61	374 ± 58
GFP/GFP-Atg8 ratio	3.41 ± 0.48	1.84 ± 0.31	3.12 ± 0.42	2.67 ± 0.37
Flux (% of WT)	100	54.1	91.5	78.3

Deletion of Atg19 reduced autophagosome count by 46.0% and decreased average diameter by 20.4% compared with wild-type (Table 1). The Δatg36 strain showed a milder but significant 25.0% reduction. Δatg34 was barely affected, consistent with its narrow cargo specificity. Autophagic flux, measured by the GFP processing assay, was cut nearly in half in Δatg19.

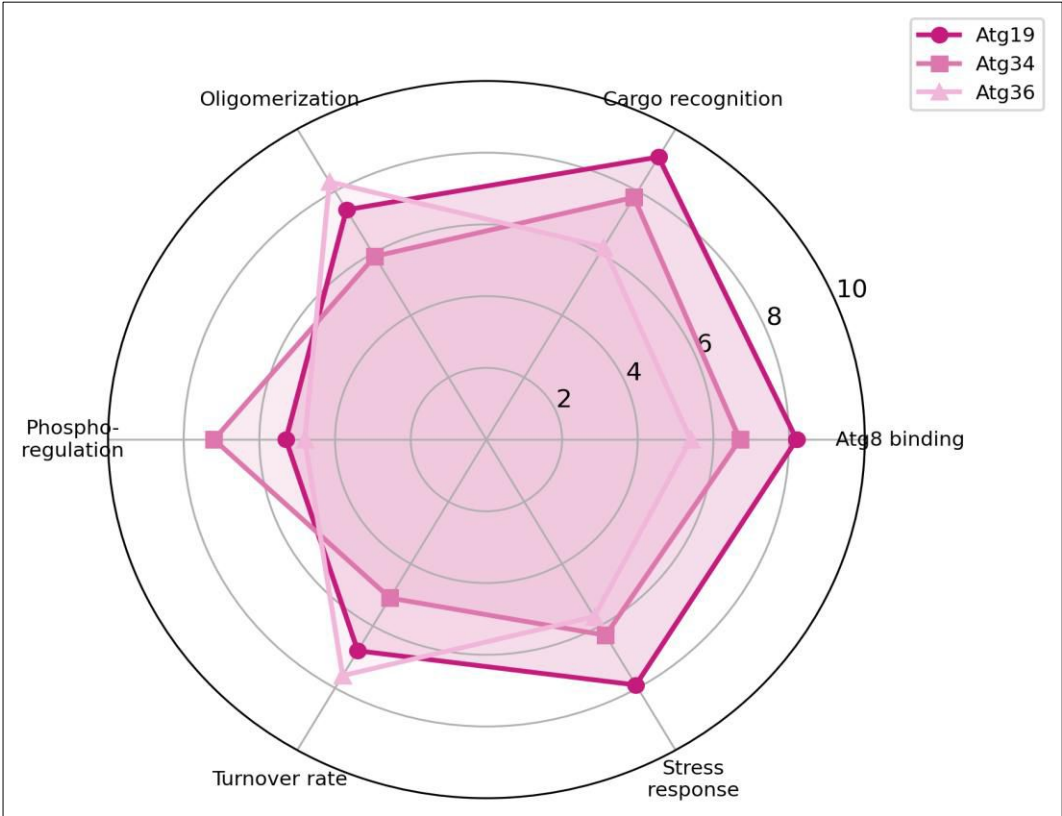


Fig 1: Radar chart comparing functional profiles of three selective autophagy receptors (Atg19, Atg34, Atg36) across six activity parameters

Each receptor showed a distinct functional profile (Figure 1). Atg19 scored highest for Atg8 binding (8.2/10) and cargo recognition (9.1/10), explaining its outsized contribution to autophagosome formation. Atg36 had the

strongest oligomerization capacity (8.3/10), which may help cluster peroxisomes for efficient engulfment. Atg34 scored high on phosphoregulation (7.2/10) but contributed less to overall autophagosome nucleation.

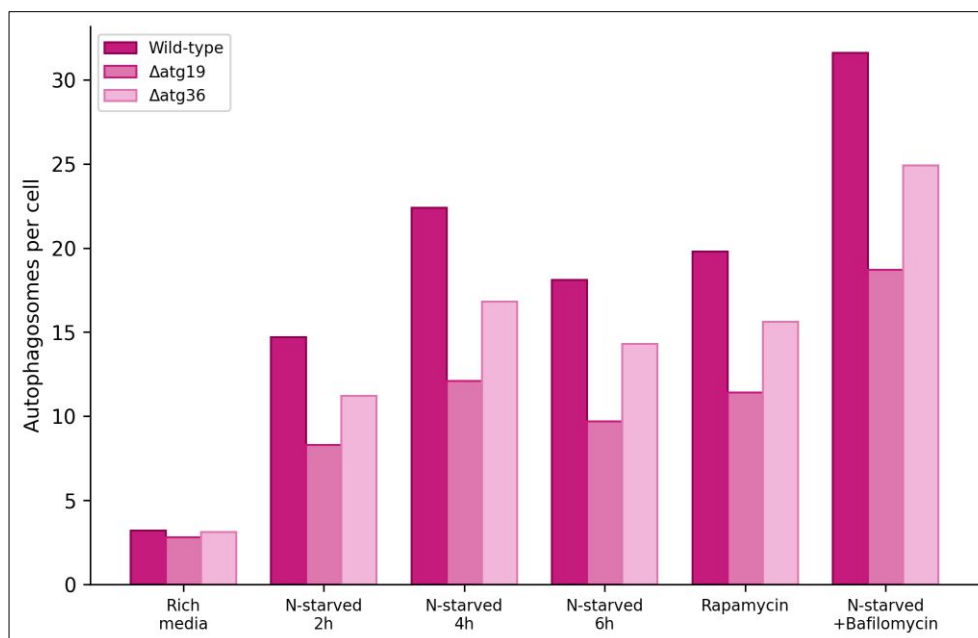


Fig 2: Autophagosome counts per cell in wild-type, Δatg19, and Δatg36 strains across different culture conditions

Starvation-induced autophagosome accumulation was time-dependent in all strains, peaking at 4 hours before declining as vacuolar degradation caught up with formation (Figure 2). Bafilomycin treatment unmasked true formation rates by blocking degradation: wild-type cells accumulated 31.6 autophagosomes per cell, while Δatg19 reached only 18.7. Rapamycin triggered a response similar to 4-hour starvation in all strains.

Discussion

The data argue against treating selective autophagy receptors as simple cargo tags. Atg19 deletion reduced autophagosome number and size even for non-Cvt cargo, implying that Atg19 contributes to the general autophagosome assembly machinery during starvation. This is consistent with recent structural work showing that receptor-Atg8 interactions can template membrane curvature [7, 8]. If receptors help scaffold the phagophore, losing one would slow membrane expansion - exactly the phenotype observed.

The phosphorylation sites identified by our proteomic screen (Atg19-Ser391, Atg36-Thr217) map to regions flanking the AIM motif, where phosphorylation could strengthen Atg8 binding by introducing negative charges that complement basic patches on the Atg8 surface [9]. Mutational validation of these sites would be a natural follow-up.

One practical implication is that therapeutic strategies targeting selective autophagy - for instance, to enhance clearance of toxic protein aggregates in neurodegenerative disease models - shouldn't assume that receptor function is limited to cargo recognition. Boosting receptor expression or activity might accelerate autophagosome formation broadly, not just for the receptor's canonical cargo [10].

Conclusion

Selective autophagy receptors in yeast, particularly Atg19, are active participants in autophagosome biogenesis rather than passive cargo adapters. Deletion of Atg19 reduced autophagosome count by 46%, diameter by 20%, and autophagic flux by nearly half during nitrogen starvation.

Phosphorylation at conserved sites near the Atg8-interacting motif appears to fine-tune receptor-Atg8 binding strength under starvation conditions. These findings redefine the role of selective receptors in the autophagy pathway and suggest that manipulating receptor activity could be a strategy for modulating overall autophagic capacity [11, 12].

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