INVESTIGATION INTO THE ROLE OF RRP8 IN AUTOPHAGY FLUX IN *SACCHAROMYCES CEREVISIAE*

by

SWAROOPA BADENAHALLI NARASIMHAIAH

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE IN BIOLOGY

2026

Oakland University
Rochester, Michigan

Thesis Advisory Committee:

Elizabeth Delorme-Axford, Ph.D., Chair
Chhabi Govind, Ph.D.
Vandre Figueiredo, Ph.D.

To my family, who have always been my foundation and support system,
I am deeply grateful for your constant encouragement.
I especially thank my husband, my father,
my mother, my brother and my in-laws
for their unwavering support, guidance, and
for making this journey
much easier to navigate.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my mentor, Dr. Elizabeth Delorme-Axford, for serving as my research advisor and for providing the opportunity to conduct my graduate research in her laboratory. I also thank my thesis committee members, Dr. Chhabi Govind and Dr. Vandre Figueiredo for their time, feedback, and for serving on my committee.

I am grateful to the members of the Delorme-Axford laboratory, past and present, for their assistance and for providing a collaborative research environment. I would like to especially thank Emmanuella Avogo for being a supportive colleague and a wonderful lab mate. I appreciate the discussions, help and the positive lab environment she contributed to during my time in the lab.

I would like to thank Dr. Daniel Klionsky (University of Michigan) for kindly providing the yeast strains and reagents that were critical for this research. I also acknowledge the financial support provided by Oakland University and PCR Biosystems, which made this work possible. I am grateful for this support, which enabled the successful completion of this study.

Swaroopa Badenahalli Narasimhaiah

ABSTRACT

Investigation into the Role of Rrp8 in Autophagy Flux in *Saccharomyces cerevisiae*

by

SWAROOPA BADENAHALLI NARASIMHAIAH

Adviser: Elizabeth Delorme-Axford, Ph.D.

Autophagy is a dynamic cellular pathway of degrading and recycling the cellular cargo within the vacuole or lysosome to maintain cellular health and metabolism. Autophagy is upregulated when cells undergo stress. Autophagy dysfunction is associated with various problems like cardiovascular, metabolic, and neurodegenerative diseases. While several autophagy-related genes have been identified, the role of nucleolar proteins in autophagy regulation remains largely unexplored. Ribosomal RNA processing 8 (Rrp8) is a conserved nucleolar protein implicated in ribosomal RNA processing and ribosome biogenesis in *Saccharomyces cerevisiae*. Since ribosome biogenesis is tightly connected to nutrient availability and cellular energy balance, Rrp8 may play an important role in regulating cellular responses to starvation. However, whether Rrp8 plays a functional role in autophagy regulation has not been directly studied. This gap in knowledge provides the basis for our current study which investigates the role of Rrp8 in autophagy regulation during nitrogen starvation in *S cerevisiae*.

TABLE OF CONTENTS

TABLE OF CONTENTS-Continued

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
ATG	AuTophaGy-related
BCA	Bicinchoninic acid
BSA	Bovine Serum Albumin
cDNA	Complementary DNA
ChIP	Chromatin Immunoprecipitation
CMA	Chaperone-Mediated Autophagy
GFP	Green Fluorescent Protein
GFP-Atg8	Green Fluorescent Protein-tagged Atg8
PAS	Phagophore Assembly Site
PE	Phosphatidylethanolamine
PGK1	Phosphoglycerate Kinase 1
PKA	Protein Kinase A
pLDDT	Predicted Local Distance Difference Test
pNPP	para-Nitrophenyl Phosphate
qPCR	Quantitative Polymerase Chain Reaction
RP	Ribosomal Proteins
RiBi	Ribosome Biogenesis
RRP8	Ribosomal RNA Processing 8
RT-qPCR	Reverse Transcription-Quantitative Polymerase Chain Reaction
SD-N	Synthetic Defined Medium without Nitrogen
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SFP1	Split-Finger Protein 1

TCA	Trichloroacetic acid
TOR	Target of Rapamycin
YPD	Yeast Peptone Dextrose

CHAPTER ONE
INTRODUCTION

1.1 Overview

Eukaryotic organisms such as *Saccharomyces cerevisiae* experience frequent environmental fluctuations and must dynamically adapt their growth and metabolic programs in response to nutrient availability. One of the central mechanisms enabling this adaptation is autophagy, a conserved intracellular degradation pathway that plays a critical role in maintaining cellular homeostasis and survival under stress (Alberghina et al., 1998; Gasch & Werner-Washburne, 2002). Autophagy is widely recognized as a bona fide health-modifying process due to its involvement in metabolism, quality control, and stress resistance.

The term *autophagy* was coined by Christian de Duve, a Belgium biochemist in 1963 to morphologically describe the observation of intracellular components enclosed within the membrane-bound structure and delivered to lysosomes for degradation (Low, 2022). *Autophagy* is derived from Greek, where *"auto"* means 'self' and *"phagy"* means 'eat' thus, literally means "self-eating" (Wen & Klionsky, 2016). At a fundamental level, autophagy involves the sequestration of cytoplasmic components—such as supernumerary or damaged organelles into a double-membrane vesicle, which subsequently fuses with the vacuole in yeast or the lysosome in mammals. The enclosed material is degraded, and

the resulting macromolecules are recycled back into the cytoplasm for reuse (Morselli et al., 2009; Wen & Klionsky, 2016).

Autophagy is broadly classified into three types: macroautophagy, microautophagy, and chaperone-mediated autophagy. Among these, macroautophagy is the most extensively studied and will hereafter be referred to as autophagy. Autophagy is a dynamic catabolic process that enables the degradation of large cellular components within the vacuole (in yeast and plants) or lysosome (in mammals), thereby contributing to energy balance and cellular homeostasis (Ryter et al., 2013; Delorme-Axford & Klionsky, 2018).

Genetic studies in *Saccharomyces cerevisiae* have identified at least 45 autophagy-related (*ATG*) genes, many of which are conserved from yeast to higher eukaryotes, including humans (Tasmi et al., 2026). Under normal physiological conditions, autophagy occurs at basal levels; however, it is strongly upregulated in response to various stress stimuli, such as nutrient deprivation, accumulation of damaged organelles, pathogen invasion, or pharmacological inhibition of nutrient-sensing pathways using agents such as rapamycin, Torin, metformin, or spermidine (Torggler et al., 2017).

Macroautophagy proceeds through a well-defined sequence of steps, beginning with the formation of a phagophore, a double-membrane structure that expands to engulf cytoplasmic material, typically in a bulk and non-selective manner. Upon completion of membrane expansion, the

phagophore closes to form an autophagosome, which subsequently fuses with the vacuole. Inside the vacuole, the autophagosomal contents are degraded, and the resulting macromolecules are released back into the cytoplasm for reuse (Delorme-Axford & Klionsky, 2018).

Dysregulation of autophagy has been linked to numerous pathological conditions, including liver, cardiac, and neurodegenerative disorders, as well as lysosomal storage diseases and diabetes, underscoring the physiological importance of this pathway (Klionsky, Petroni, et al., 2021). Therefore, the magnitude of the process must be tightly regulated at various stages of autophagy including transcriptional, post-transcriptional, translational, and post-translational to ensure appropriate levels (Wen & Klionsky, 2016).

1.2 Types of autophagy

Autophagy can be classified into three types based on how the cellular components are delivered to the vacuole or lysosome for degradation: macroautophagy, microautophagy, and chaperone-mediated autophagy. Autophagy (microautophagy and macroautophagy) can either be selective or non-selective type of autophagy. The chaperone-mediated autophagy only occurs in mammalian cells (Parzych & Klionsky, 2014; Klionsky et al., 2021).

Macroautophagy is a highly conserved cellular process from unicellular eukaryotes (yeast) to a multicellular eukaryote (humans) (Cebollero & Reggiori, 2009). The process can be divided into five stages:

initiation/nucleation of phagophore, expansion, completion of the autophagosome, docking and fusion with the vacuole, and lastly, degradation and efflux of the macromolecules. The process of macroautophagy begins with the formation of the double-membraned vesicle, the phagophore (also regarded as a classical morphological feature of macroautophagy) (Delorme-Axford & Klionsky, 2018; Wen & Klionsky, 2016). The phagophore expands to non-specifically engulf portions of the cytoplasm including soluble proteins and organelles, for degradation in the vacuole/lysosome (Delorme-Axford & Klionsky, 2018) and subsequently closes the double membrane to form the autophagosome, which later fuses with the vacuole (Kraft et al., 2009). The cargo inside the autophagosome is exposed to the luminal contents of the vacuole where the resident hydrolases degrade the cargo and finally release the breakdown macromolecules into the cytoplasm (Feng et al., 2014; Kraft et al., 2009).

Microautophagy is morphologically distinct from macroautophagy, involves the direct engulfment of cytoplasmic material by invagination or protrusion of the lysosome or vacuole membrane (Delorme-Axford et al., 2015; Mijaljica et al., 2011). Like macroautophagy, microautophagy can function in both selective and non-selective (bulk) modes targeting specific substrates such as lipid droplets, ribosomes, portions of nucleus, and damaged organelles. Microautophagy is actively regulated and not merely a constitutive housekeeping process contributing to the cellular

homeostasis, organelle turnover, and metabolic adaptation, especially during nutrient stress (Wang et al., 2023).

Chaperone-mediated autophagy is a selective form of autophagy specially found in mammals but absent in yeast and plants (Dice, 2007; Kaushik & Cuervo, 2012). CMA relies on chaperone proteins as its name suggests recognizing and sequester its cargo materials using a specific motif, KFERQ and delivers across the lysosomal membrane by binding to the receptor, LAMP-2A (lysosome-associated membrane protein 2A) directly for degradation (Mijaljica et al., 2011; Wen & Klionsky, 2016). CMA is activated in response to oxidative stress and contributes to the lysosomal degradation of a subset of oxidized and damaged proteins, thereby supporting protein quality control and cellular homeostasis (Dice, 2007; Kaushik & Cuervo, 2012, 2018).

1.3 Selective and non-selective autophagy

In addition to bulk degradation, cells also employ selective forms of autophagy to target specific substrates. Autophagy can be broadly classified into non-selective autophagy and selective, depending on the specificity of the cargo targeted for degradation. Both the modes are essential for cellular homeostasis, yet they differ in their regulatory mechanisms, physiological roles, and the mechanisms of cargo sequestration (Klionsky, Abdel-Aziz, et al., 2021; Xie, 2021).

Non-selective autophagy refers to the bulk autophagy during which the bulk cytoplasmic portions are sequestered indiscriminately into the

expanding phagophores, and the completed autophagosomes carry to the vacuole for degradation (Kraft et al., 2009). The resulting macromolecules, including amino acids, lipids, and sugars, are recycled to sustain cellular metabolism and energy balance (Mizushima & Komatsu, 2011; Wen & Klionsky, 2016). Bulk autophagy therefore functions as a rapid adaptive response that promotes cell survival during periods of metabolic stress (Gubas & Dikic, 2022).

In contrast, selective autophagy targets specific cellular components for degradation, even under nutrient-replete conditions. This selectivity is achieved through cargo-specific receptors that recognize defined signals on the target substrates and simultaneously interact with the autophagy machinery, most commonly through Atg8/LC3-interacting motifs (Feng et al., 2014). Selective autophagy pathways include mitophagy (mitochondria), pexophagy (peroxisomes), ribophagy (ribosomes), ER-phagy (endoplasmic reticulum), and aggrephagy (protein aggregates). By selectively removing damaged, excess, or dysfunctional components, selective autophagy plays a crucial role in quality control and cellular remodeling (Kraft et al., 2009; Wang et al., 2023).

Ribophagy is a selective autophagy pathway responsible for the degradation of ribosomes and plays an essential role in maintaining ribosome homeostasis. In *Saccharomyces cerevisiae*, ribophagy is strongly induced during nitrogen starvation, where it facilitates the removal of excess or damaged ribosomes and have a rapid turnover than cytosolic

proteins, thereby contributing to the reallocation of cellular process (Klionsky et al., 2021; Kraft et al., 2009). Ribophagy is regulated by a combination of core autophagy machinery (including Atg1 and Atg7 for the fusion of autophagosomes with the vacuole), ubiquitin-dependent modulation (including ubiquitin protease Ubp3 and its cofactor Bre5 for 60S ribosomal subunit degradation), and nutrient-responsive signaling pathways, enabling selective ribosome turnover during metabolic stress (Kraft et al., 2008, 2009). Since ribosome biogenesis and protein synthesis are highly energy-intensive, ribophagy may serve as an efficient means to reduce cellular energy expenditure (Kraft et al., 2009).

1.4 Mechanism of autophagy in Saccharomyces cerevisiae

Autophagy in *Saccharomyces cerevisiae* is a conserved intracellular degradation pathway that proceeds through a series of well-defined morphological and molecular stages. These include induction, nucleation of phagophore, phagophore expansion, autophagosome completion, fusion with vacuole and final degradation and recycling of the cargo. A hallmark of macroautophagy is the formation of a double-membraned vesicle, autophagosome which encloses cytoplasmic material destined for degradation (Wen & Klionsky, 2016).

In yeast, autophagy is initiated at a perivacuolar site known as the phagophore assembly site (PAS) (Suzuki, 2001). Autophagy induction at the PAS is mediated by the serine/threonine kinase, an Atg1 kinase complex, including Atg1-Atg13 and Atg17-Atg31-Atg29 scaffold

complex. This promotes the recruitment of further downstream processes of autophagy. During nucleation, the class III phosphatidylinositol 3-kinase (PtdIns3K) complex I, containing Vps34, Vps15, Vps30/Atg6, Atg14, and Atg38 are recruited to the PAS facilitating phagophore formation by generating phosphatidylinositol 3-phosphate (PtdIns3P) (Reggiori & Klionsky, 2013a; Wen & Klionsky, 2016).

Phagophore expansion and autophagosome completion depend upon two ubiquitin-like conjugation systems Atg12–Atg5-Atg16 and Atg8-PE. The Atg12 covalently conjugates to the C-terminally of Atg5 in a two-step process where first the Atg12 is activated with the help of Atg7, an E1-like enzyme, followed by Atg10, an E2-like enzyme aiding in the conjugation of Atg12 to Atg5 (Mizushima et al., 1998). Coiled-coil protein Atg16 non-covalently binds to Atg5 to form a multimeric complex that acts like an E3-like enzyme to further facilitate Atg8-PE conjugation (Romanov et al., 2012; Wen & Klionsky, 2016). Atg8 is an important ubiquitin-like protein that plays a crucial role in autophagosome biogenesis (Ichimura et al., 2000). Atg8 is synthesized as a precursor protein and undergoes proteolytic processing by the cysteine protease Atg4, which exposes the C-terminal glycine residue for the further conjugation (Ichimura et al., 2000; Nakatogawa et al., 2007). This enables Atg8 to be further activated by the E1-like enzyme, Atg7, further facilitating the E2-like enzyme, Atg3 to act on Atg8 to covalently

conjugate the phosphatidylethanolamine (PE), a lipid molecule (Wen & Klionsky, 2016).

Atg8 also mediates cargo selectivity by interacting with autophagy receptors through conserved Atg8-interacting motifs (AIMs) used in selective autophagy (N. N. Noda et al., 2008). The amount of Atg8 at the PAS correlates with the size of the autophagosome (Reggiori & Klionsky, 2013; Shintani et al., 2002). The lipidated Atg8 (Atg8-PE) localizes on both the inner and outer membrane of the expanding phagophore, contributing to the membrane elongation, curvature, alongside the cargo sequestration (Reggiori & Klionsky, 2013b). Atg4 cleaves the Atg8-PE bond, deconjugating and releasing Atg8 from the membrane on the outer surface of the autophagosome; thus, enabling it's reuse in subsequent conjugation cycles (Ichimura et al., 2000; Wen & Klionsky, 2016). Upon closure of the phagophore, a mature autophagosome is formed, fully enclosing the cargo within a sealed double membrane (Reggiori & Klionsky, 2013a).

Following autophagosome formation, the vesicle is transported to and fuses with the vacuolar membrane which is mediated by the Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) (Nair, Jotwani, et al., 2011). Upon fusion, the inner membrane of the autophagosome residing in the vacuole, now termed the autophagic body, exposes its contents for degradation. The autophagic body is subsequently degraded by the lipase Atg15, allowing the vacuolar hydrolases to degrade

the enclosed cargo (Epple et al., 2001) The resulting amino acids and other macromolecular breakdown products are exported back to the cytoplasm through vacuole membrane permeases including the amino acid transporter, Atg22 for reuse in biosynthetic and energy-generating process (Epple et al., 2001; Reggiori & Klionsky, 2013a).

1.5 Regulation of autophagy in Saccharomyces cerevisiae

Autophagy is tightly regulated to ensure that it is activated in response to appropriate physiological conditions and only occurs at basal levels under favorable growth conditions (Wen & Klionsky, 2016). The pathway is vital for maintaining cellular homeostasis and ensuring survival during nutrient deprivation, as it provides the cell with essential building blocks like amino acids (Feng et al., 2014). While basal levels of autophagy occur constitutively, the pathway is robustly induced by various stress signals, most notably the limitation of nitrogen or carbon sources (Wen & Klionsky, 2016).

Upstream Signaling Pathways and Nutrient Sensing

The master regulator of autophagy in yeast is the Target of Rapamycin Complex 1 (TORC1), a conserved serine/threonine kinase that coordinates cell growth with nutrient availability (T. Noda & Ohsumi, 1998). Under nutrient-rich conditions, active TORC1 suppresses autophagy by phosphorylating core components of the Atg1 kinase complex, specifically Atg13 (Kamada et al., 2000). The phosphorylation of Atg13 prevents its stable association with Atg1, thereby inhibiting the

assembly of the Atg1-Atg13-Atg17-Atg29-Atg31 scaffold needed to initiate autophagosome formation (He & Klionsky, 2009). Upon starvation or treatment with rapamycin, TORC1 is inactivated, leading to the rapid dephosphorylation of Atg13 and the activation of the Atg1 kinase (Díaz-Troya et al., 2008).

In addition to TORC1, the Ras/PKA (Protein Kinase A) and Sch9 signaling pathways also sense the presence of nutrients, particularly glucose, to negatively regulate autophagy (Budovskaya et al., 2004). These pathways integrate environmental signals and converge on the Rim15 kinase, which acts as a positive regulator of the autophagic response (Pedruzzi et al., 2003). Inactivation of these nutrient-sensing kinases during starvation allows Rim15 to activate downstream effectors that promote the transcription of *ATG* genes (Bernard et al., 2015). This hierarchical signaling network ensures that autophagy is only fully induced when the cell faces a significant reduction in available energy (Jin & Klionsky, 2014).

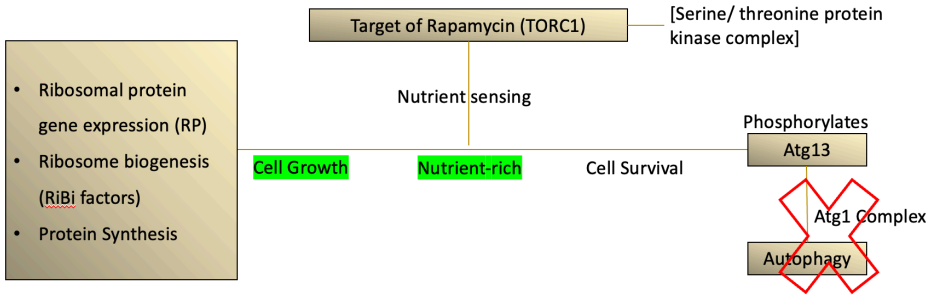

Figure 1.1 TORC1 regulation of cell growth under nutrient-rich conditions. TORC1, a serine/threonine protein kinase complex, functions as a central

nutrient sensor in cells. Under nutrient-rich conditions, TORC1 promotes cells growth by stimulating ribosomal protein (RP) gene expression, ribosome biogenesis (RiBi factors) and protein synthesis. At the same time TORC1 suppresses autophagy by phosphorylating components of the autophagy initiation machinery such as, Atg13, thereby inhibiting the Atg1 complex. As a result, autophagy remains inactive while cellular resources are redirected toward growth and biosynthetic processes.

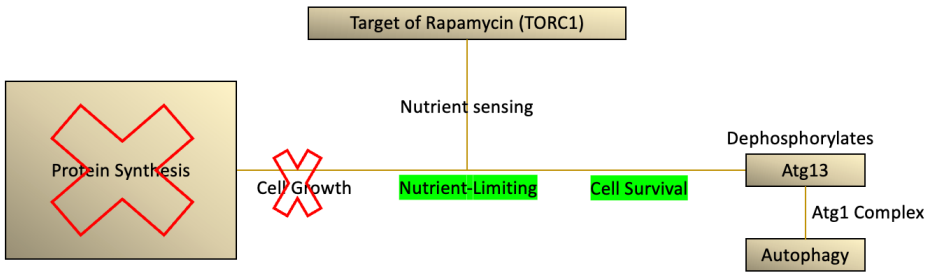

Figure: 1.2 TORC1 regulation of cell growth under nutrient-limiting conditions. Under nutrient-limiting conditions, TORC1 activity is reduced, leading to suppression of cellular growth and protein synthesis. Inactivation of TORC1 leads to dephosphorylation of Atg13 by protein phosphatases, which promotes activation of the Atg1 kinase complex. Activation of the Atg1 complex initiates autophagy, allowing cells to recycle intracellular components and promote survival during nutrient stress.

Transcriptional Regulation of *ATG* Genes

The intensity and duration of the autophagic response are significantly influenced by the expression levels of *ATG* genes, which dictate the size and frequency of autophagosome formation (Jin &

Klionsky, 2014). For instance, the concentration of Atg8 protein is a key determinant of autophagosome size, while the abundance of Atg9 influences the number of vesicles produced (Xie et al., 2008; Jin & Klionsky, 2014). Under nutrient-replete conditions, the transcription of many *ATG* genes is maintained at a basal level by several transcriptional repressors (Bernard et al., 2015).

Rph1 is a master repressor that binds directly to the promoters of genes such as *ATG7*. Upon nitrogen starvation, Rim15 phosphorylates Rph1, causing its dissociation from chromatin and allowing for gene induction (Bernard et al., 2015). Ume6 acts as a repressor of *ATG8* expression; its deletion results in larger autophagosomes and enhanced autophagic flux (Bartholomew et al., 2012). Pho23 primarily represses *ATG9*, and its absence leads to an increased frequency of autophagosome formation (Jin et al., 2014; Jin & Klionsky, 2014). Stb5 has been identified as a negative regulator that modulates cellular metabolism, and its expression is itself regulated by the Pho23-Rpd3 histone deacetylase complex (Delorme-Axford et al., 2023; Delorme-Axford et al., 2023).

When nutrients are limited, transcriptional activators like Gcn4, Gln3, and Gat1 bind to *ATG* promoters to drive robust gene expression (Delorme-Axford & Klionsky, 2018). Gcn4, the primary activator of the general amino acid control pathway, is required for the transcriptional upregulation of *ATG1* during amino acid starvation (Natarajan et al., 2001; Cebollero & Reggiori, 2009). This complex interplay between repressors

and activators allows the cell to finely tune its autophagic potential according to the severity of the stress (Jin & Klionsky, 2014).

Post-Transcriptional and Epigenetic Control

Recent studies have revealed that the regulation of autophagy extends beyond transcription to include post-transcriptional mechanisms. The 5' to 3' exoribonuclease Xrn1 acts as a negative regulator of autophagy by promoting the degradation of *ATG* mRNAs, such as *ATG1, ATG5,* and *ATG8*, under nutrient-rich conditions (Delorme-Axford et al., 2023). Similarly, the RNA decapping factor Dcp2 and the helicase Dhh1 facilitate the degradation of *ATG* transcripts when TORC1 is active (Hu et al., 2015; Delorme-Axford & Klionsky, 2019). Furthermore, the Rad53 kinase has been shown to regulate *ATG1* post-transcriptionally, highlighting a critical node that modulates autophagy during distinct nutrient stresses (Lahiri et al., 2022)

Epigenetic regulation, including histone modifications, also plays a crucial role in controlling the autophagic response (Baek & Kim, 2017). Global levels of histone H4 lysine 16 acetylation (H4K16ac) are reduced during starvation, and this hypoacetylation is associated with the repression of specific *ATG* genes to conserve resources (Shu et al., 2020). The balance between histone acetyltransferases like Esa1 and deacetylases like Rpd3 ensures that the autophagic machinery is appropriately adjusted to the cell's metabolic status (Baek & Kim, 2017). In summary, the regulation of autophagy in yeast is an intricately layered process that

integrates upstream signaling with transcriptional and post-transcriptional controls to maintain cellular viability (Reggiori & Klionsky, 2013a).

1.6 Ribosomal RNA processing 8

Ribosome biogenesis is a central and highly energy-demanding process in all eukaryotic cells, accounting for approximately 60% of total transcriptional activity in *Saccharomyces cerevisiae* (Warner, 1999; Mayer & Grummt, 2006). This complex orchestration occurs primarily within the nucleolus, where the transcription of ribosomal DNA (rDNA) by RNA polymerase I generates a large 35S pre-rRNA precursor to form functional 80S ribosomes (Bousquet-Antonelli et al., 2000; Jiao et al., 2023). This precursor must undergo a sophisticated series of endonucleolytic and exonucleolytic cleavages, coordinated with the assembly of 79 ribosomal proteins and over 200 non-ribosomal assembly factors (Peifer et al., 2013; Sharma et al., 2018). Among these factors, Rrp8 has emerged as a critical bifunctional protein essential for the accurate maturation of both the small (40S) and large (60S) subunits (Peifer et al., 2013).

Rrp8 is a highly conserved nucleolar protein in *Saccharomyces cerevisiae* that was originally identified through a synthetic lethal screen as a functional partner of Gar1p, an essential component of H/ACA small nucleolar ribonucleoproteins (snoRNPs) (Bousquet-Antonelli et al., 2000). While the deletion of *RRP8* is not lethal at optimal temperatures, it results in a pronounced cold-sensitive growth phenotype (Bousquet-Antonelli et al., 2000; Peifer et al., 2013). Rrp8 is found to have a human homolog,

nucleomethylin and the *C. elegans* ortholog RRP-8 (Sharma et al., 2018; Wu et al., 2018).

Structurally, Rrp8 is characterized as a Class I S-adenosyl-L-methionine (SAM)-dependent methyltransferase, containing a classic Rossmann-like fold (Kim et al., 2023; Peifer et al., 2013). Its primary enzymatic function is the N1-methylation of adenine 645 (m1A645) within the helix 25.1 of the 25S ribosomal RNA, which resides in the large ribosome subunit (Peifer et al., 2013; Sharma et al., 2018). This specific modification is highly conserved and is thought to optimize the topological framework required for the interaction between 5.8S and 25S rRNA (Peifer et al., 2013).

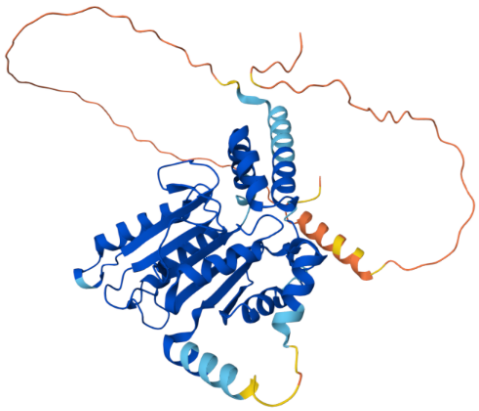

Figure: 1.3 The AlphaFold structural prediction of Rrp8 was analyzed using the per-residue confidence metric, predicted Local Distance Difference Test (pLDDT), which ranges from 0 to 100. Regions shown in dark blue indicate very high confidence (pLDDT > 90); whereas light blue regions represent high confidence. Yellow regions indicate low confidence, while orange

regions correspond to very low confidence scores (pLDDT < 50). Regions with low pLDDT values often correspond to flexible or intrinsically disordered segments when the protein is analyzed in isolation. Structural information for Rrp8 was obtained from AlphaFold Structure Protein Database (https://alphafold.ebi.ac.uk/entry/AF-P38961-F1).

Dual Mechanism in Ribosome Biogenesis

Rrp8 is unique in its dual functionality, participating in both rRNA processing and chemical modification. Its mechanism involves:

1. Pre-rRNA Processing: Rrp8 is required for the efficient endonucleolytic cleavage of pre-rRNA at site A2, a critical step that separates the biogenesis pathways of the small (40S) and large (60S) ribosomal subunits (Bousquet-Antonelli et al., 2000). This cleavage event is critical because it separates the maturation pathways of the 18S rRNA (small subunit) and the 25S/5.8S rRNAs (large subunit) (Bousquet-Antonelli et al., 2000; Peifer et al., 2013). In the absence of Rrp8, cleavage at site A2 site is strongly inhibited, leading to the accumulation of an aberrant 21S pre-rRNA species and a subsequent delay in the production of mature 18S rRNA (Bousquet-Antonelli et al., 2000; Peifer et al., 2013).

2. rRNA Modification: Independent of its role in site A2 cleavage, Rrp8 serves as the methyltransferase responsible for the m1A645 base modification (Peifer et al., 2013). Interestingly, point mutations in the SAM-binding motif of Rrp8 abolish its methyltransferase activity without affecting A2 cleavage (Peifer et al., 2013). This loss of methylation leads

to local structural perturbations in the 25S rRNA, which results in translation initiation defects and the formation of "half-mer" polysomes, indicating an inability of the 60S subunit to properly join the 43S pre-initiation complex (Sharma et al., 2018).

Regulation by TOR and Metabolic Coordination

Cellular growth and ribosome biogenesis are tightly regulated by nutrient-sensing pathways, primarily through TOR signaling pathways (Wullschleger et al., 2006; Loewith & Hall, 2011). As ribosome biogenesis is one of the most energy-intensive processes in the cell and TORC1 coordinates this activity with nutrient availability to maintain cellular homeostasis (Díaz-Troya et al., 2008). Given that Rrp8 functions as a ribosome biogenesis factor, it is possible that its regulation may also be coordinated with nutrient-responsive pathways although this relationship has not been previously established. Under favorable growth conditions, TORC1 remains active, promoting the transcription of ribosome biogenesis genes and ribosomal proteins to drive growth while, inhibiting catabolic processes (Jiao et al., 2023). However, when nutrients are limited or the cell experiences stress, TOR signaling is downregulated, leading to a rapid downregulation of rRNA processing factors and the induction stress responses (Díaz-Troya et al., 2008; Mayer & Grummt, 2006).

Beyond its role in biogenesis, Rrp8 has been linked to the regulation of primary metabolism (Sharma et al., 2018). Quantitative

proteomic analysis of *rrp8*Δ mutants has revealed significant alterations in the steady-state levels of enzymes involved in glycolysis and the pentose phosphate pathway, such as the 7-fold upregulation of Sol3p (Sharma et al., 2018). In the *C. elegans*, the inactivation of RRP-8 triggers nucleolar stress, which leads to transcription factor PHA-4/FoxA (Wu et al., 2018). Because ribosome biogenesis, translational control, and cellular energy balance are closely interconnected, Rrp8 may play roles in stress-responsive pathways beyond its canonical nucleolar functions. Notably, previous studies suggest that *RRP8* expression may be regulated by the TORC1-dependent transcription factor Sfp1, placing Rrp8 downstream of a central nutrient-sensing signaling pathway that also governs autophagy (Cipollina et al., 2008a).

1.7 Sfp1 transcription factor

While nucleolar factors like Rrp8 ensure the structural fidelity of individual ribosomes, the global availability of the biogenesis machinery is governed by master transcriptional regulators like Sfp1 (Split-finger protein 1) (Albert et al., 2019a; Fingerman et al., 2003). Sfp1 is a zinc-finger transcription factor that serves as a central regulator of ribosome biogenesis and cell growth in yeast (Fingerman et al., 2003; Marion et al., 2004). Sfp1 primarily controls the expression of ribosomal protein genes and RiBi genes transcribed by RNA polymerase II, thereby coordinating ribosome production with nutrient availability and cellular growth rate (Albert et al., 2019b; Fingerman et al., 2003; Jorgensen et al., 2004;

Marion et al., 2004). Because ribosome biogenesis represents one of the most energy-intensive processes in the cell, Sfp1 functions as a critical node linking metabolic signaling to translational capacity.

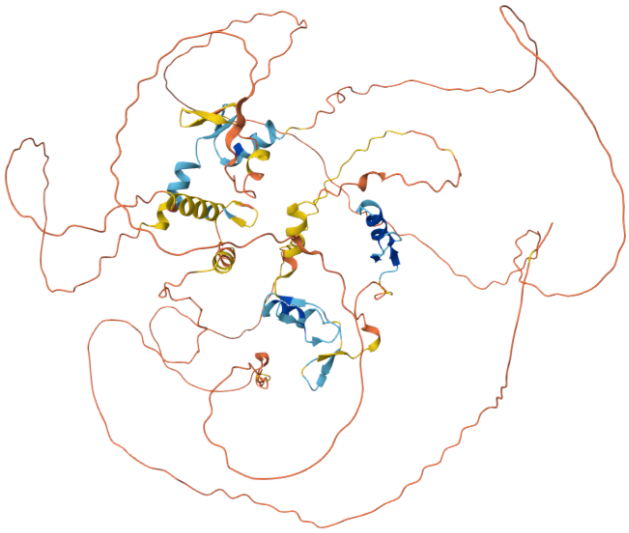

Figure: 1.4 AlphaFold prediction for Sfp1. The predicted three-dimensional structure of Sfp1 from Saccharomyces cerevisiae was obtained from the AlphaFold protein structure database. Structural confidence is indicated by the pre-residue pLDDT score, which ranges from 0-100. Dark blue regions indicate very high confidence predictions (pLDDT > 90), light blue indicates high confidence (70-90), yellow represents low confidence (50-70), and orange corresponds to very low confidence (<50). Regions with low pDLLT scores may represent flexible or intrinsically disordered regions of the protein. Structural information was retrieved from AlphaFold protein structure database (https://alphafold.ebi.ac.uk/entry/AF-P32432-F1).

Sfp1 activity is tightly regulated by nutrient-responsive signaling pathways, particularly the TORC1 (Lempiäinen et al., 2009). TORC1 functions as a master regulator of growth in response to amino acids and other nutrients (Jorgensen et al., 2004). Under nutrient-rich conditions, active TORC1 promotes nuclear localization and transcriptional activity of Sfp1, enabling high-level expression of RP and RiBi genes (Lempiäinen et al., 2009; Marion et al., 2004) (Figure 1.3). Upon nutrient limitation or rapamycin treatment, Sfp1 rapidly relocalizes from the nucleus to cytoplasm, resulting in repression of ribosomal gene transcription (Lempiäinen et al., 2009; Marion et al., 2004). This dynamic localization allows cells to rapidly downregulate ribosome production in response to environmental stress (Marion et al., 2004).

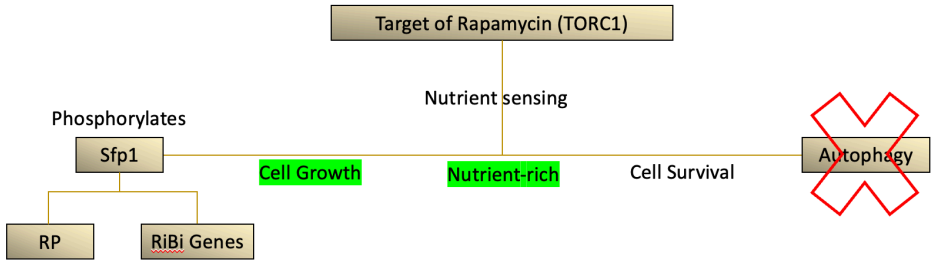

Figure: 1.5 TORC1-Sfp1 regulation of ribosome biogenesis and cell growth under nutrient-rich conditions. Under nutrient-rich conditions, TORC1 acts as a nutrient-sensor and promotes cellular growth. TORC1 phosphorylates the transcription factor, Sfp1, which activates the expression of RP genes and RiBi genes. This promotes ribosome production.

In addition to TORC1-dependent localization control, Sfp1 is subject to post-translational regulation that modulate its activity in response to nutrient availability (Marion et al., 2004; Albert et al., 2019a). Nutrient stress leads to repression of ribosome biogenesis programs through regulation of Sfp1 activity, providing an additional layer of control over ribosome production (Lempiäinen et al., 2009). These regulatory mechanisms ensure that ribosome synthesis is closely matched to metabolic capacity, preventing unnecessary energy expenditure under unfavorable conditions (Warner, 1999).

The role of Sfp1 must also be understood within the broader context of cellular resource allocation. Nutrient limitation triggers a shift from anabolic processes such as ribosome biogenesis toward catabolic pathways that recycles cellular components (Jorgensen et al., 2004). One such pathway is autophagy, that is induced upon TORC1 inactivation. In yeast, TORC1 negatively regulates autophagy and its inhibition under nitrogen starvation simultaneously represses ribosomal gene transcription and activates the autophagic flux (T. Noda & Ohsumi, 1998; Reggiori & Klionsky, 2013a). Thus, Sfp1-mediated transcriptional repression of ribosome biogenesis occurs in parallel with autophagy induction, reflecting coordinated regulation of growth-promoting and recycling pathways (Marion et al., 2004; Reggiori & Klionsky, 2013a).

Given that many ribosome biogenesis factors are transcriptionally regulated downstream of Sfp1, this regulatory axis may have broader

implications for cellular stress adaptation (Marion et al., 2004). For example, genes encoding nucleolar processing and rRNA modification and pre-rRNA processing (Rudra et al., 2007). As ribosome assembly and function influence cellular metabolism and stress responses, modulation of Sfp1 activity could indirectly affect processes such as autophagy through altered ribosome biogenesis (Warner, 1999; Reggiori & Klionsky, 2013a).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Yeast Strains, Media and Culture Conditions

All yeast strains used in this study are summarized in Table 5.1. Gene modifications were performed using one step PCR-based homologous recombination method as previously described (Longtine et al., 1998; Gueldener, 2002). Yeast cultures were inoculated in 3 mL of YPD medium and incubated overnight at 30℃ (Gibco™ YPD Broth containing 1% yeast extract, 2% peptone, and 2% glucose; ThermoFisher Scientific, Catalog # A1374501). The next day, overnight cultures were diluted to an OD_{600} of 0.2 and allowed cells to grow to mid-log phase (0.8 to 1.0 OD_{600}) prior to further experimentation.

2.2 SD-N (Starvation media)

Autophagy was induced using the nitrogen starvation media (SD-N; 0.17% yeast nitrogen base without ammonium sulfate or amino acids and 2% glucose). When cells reached mid-log phase during their subculturing, 1.0 OD_{600} of cells were shifted from rich-medium to the starvation media for the indicated time points. Cells were harvested by centrifuging at 3,500-3,700 rpm for 4 minutes at room temperature. Cells were then washed in sterile type 2 water, and frozen at -20℃ until further used in experiments.

2.3 Yeast Transformation

Yeast transformations were performed using the Frozen-EZ Yeast Transformation kit (Zymo Research Catalog #T2001) according to manufacturer's instruction. Briefly, yeast cells were grown overnight in 3 mL YPD medium at 30°C. The overnight culture was diluted to an OD_{600} of 0.2 in fresh YPD and grown to mid-log phase (OD_{600} of 0.8-1.0).

Cells equivalent to 1 OD_{600} unit were harvested by centrifugation at 3,500 rpm for 4 minutes, and the supernatant was discarded. The cell pellet was washed with 1000 µL Frozen-EZ Yeast Solution 1 by resuspension followed by centrifugation at 3500 rpm for 4 minutes, after which the supernatant was discarded. The pellet was then resuspended in 100 µL Frozen-EZ Yeast Solution 2 to prepare competent cells. About 30 µL transforming DNA (PCR product used for gene deletion or tagging) and 500 µL Frozen-EZ Yeast Solution 3 were added to the competent cells.

The transformation mixture was incubated at 30°C for 45-60 minutes to allow DNA uptake. Cells were then pelleted by centrifugation at 3,500 rpm for 4 minutes, and the supernatant was removed. The transformed cells were resuspended in 100 µL sterile water and plated onto appropriate selective media using sterile glass beads. Plates were incubated at 30°C for 2-3 days until the transformant colonies appeared.

2.4 TCA Precipitation

The cell lysates were stored at -20°C. Proteins from these cell lysates are precipitated using 1 mL 10% trichloroacetic acid (TCA). Cell lysates are treated with chilled 10% TCA and incubated on ice for 20 minutes. The cells are then centrifuged at 12,000 rpm for 5 minutes at 4°C. The pellets are then washed with ice-cold acetone and allowed to air dry on bench for about 10 minutes. The TCA-precipitated cell lysates can be frozen at -20°C at this point.

2.5 SDS PAGE

The TCA precipitated samples were resuspended in 50 µL loading buffer (Tasmi et al., 2026). Approximately 25 µL acid-washed glass beads were added, and the cells were vortexed for 5 minutes at 4°C. The lysed samples were then incubated for 15 minutes at 55°C on a heat block, vortexed by hand for a brief 4 seconds, and centrifuged at 10,000 rpm for 1 minute at room temperature. 10 µL of the supernatant containing the total proteins were loaded onto 10-12% Bio-Rad or MP Biomedicals pre-cast SDS-PAGE gels to separate the proteins via gel electrophoresis 5 µL Proteintech pre-stained (Catalog #PL00001) or GoldBio BLUEstain™ (Catalog # P007-500) were used as standard protein markers for referencing molecular weights.

2.6 Western Blotting

Next, the segregated proteins were transferred from the gel to the PVDF membrane by using the semi-dry transfer method with the Bio-Rad

TransBlot SD Semi-Dry Transfer Cell (Catalog #1703848) for about 50-60 minutes at 25 V. Once the transfer was completed, the PVDF membrane was blocked with 5% milk TTBS solution.

2.7 Antibodies

Different antibodies were used to recognize different protein antigens. Mouse monoclonal antibody that recognizes GFP was purchased from Clontech (JL-8; 63281). Peroxidase anti-Peroxidase (PAP) antibody used to detect PA tag was purchased from Jackson Immunoresearch (Catalog #323-005-024); GAPDH mouse monoclonal antibody (Proteintech, Catalog #60004-1-Ig) and Pgk1 (phosphoglycerate kinase) monoclonal antibody (Invitrogen, Catalog #459250) were used as loading controls. HRP-conjugated goat anti-mouse IgG secondary antibody was purchased from Proteintech (Catalog #SA00001-1).

2.8 The Pho8Δ60 Assay (Alkaline Phosphatase /ALP Assay)

The ALP assay provides a quantitative way to measure nonselective autophagic activity using the Pho8Δ60 reporter (Torggler et al., 2017a). Yeast cells were cultured in YPD media until reaching mid-log phase (OD600 0.8–1.0). 1.0 OD_{600} units of cells were harvested by centrifugation (4,000 rpm, 5 min), washed once with sterile water, and centrifuged again under the same conditions. The washed cell pellets were then stored at -20°C. Autophagy was induced by shifting the remaining cells to nitrogen starvation medium (SD-N) for 4 hr. Following starvation, cells were washed and resuspended in ice-cold lysis buffer (20 mM

PIPES-KOH, pH 6.8, 50 mM KCl, 100 mM KOAc, 10 mM $MgSO_4$, 10 µM $ZnSO_4$, 0.5% Triton X-100) supplemented with 1 mM PMSF. Samples were lysed by vortexing and cleared via centrifugation (16,000 x g for 5 minutes at 4°C). Cleared lysates (20 µL) were combined with 80 µL reaction buffer (250 mM Tris-HCl, pH 8.5, 10 mM $MgSO_4$, 10 µM $ZnSO_4$, 0.4% Triton X-100) containing the substrate 4-nitrophenyl phosphate (pNPP) were loaded into each sample well in a 96-well plate. Reaction buffer was added at regular intervals of 10 seconds. After 30-45 minutes of incubation at 30°C, the reaction was terminated using stop solution (1 M glycine-NaOH, pH 11) at 10 second of regular interval. The plate contents were then gently mixed on a rocker for approximately 2 minutes to ensure uniform mixing. Finally, the absorbance was recorded at 405 nm using the SMARTREADER 96 (ACCURIS instruments). Sample values were subtracted from the blank (lysis buffer only).

2.9 Bicinchoninic Acid Assay (BCA assay)

The enzymatic activity measured in the ALP assay must be adjusted based on the total amount of protein in each sample. This is achieved through the bicinchoninic acid (BCA) protein assay using the Pierce BCA Protein Assay Reagents A and B (Catalog #23223 and 23224) from Thermo Fisher Scientific. Pierce Bovine Serum Albumin Standard Ampules (Catalog 23209, Lot# KG134878) from Thermo Fisher Scientific were prepared according to manufacturer's protocol. A standard curve was established using known concentrations of 25 µL bovine serum albumin

(BSA) standard, 25 µL of lysis buffer/PMSF only, or 25 µL of sample to provide a baseline for comparison. The experimental samples were treated with a working reagent, incubated to allow for color development, and then analyzed using the SMARTREADER 96 (ACCURIS instruments) at 562 nm. For each sample, ALP activity is normalized to BCA. Results are reported as a percentage of the activity seen in starved wild-type cells, which is set at 100%.

2.10 Yeast Survival Assay

Yeast survival assays were performed as previously described (Yin et al., 2023) with minor modifications. Yeast cells were grown in nutrient-rich (YPD) media to mid-log phase and subsequently transferred to nitrogen starvation (SD-N) medium for the indicated time points. At each time point, an aliquot corresponding to 1 OD_{600} unit of cells was collected and serially diluted. A volume of 5 µL from each dilution was spotted onto YPD plates. The plates were incubated at 30°C of 2 days, and cell growth was recorded using a ChemiDoc Touch imaging system (Bio-Rad).

2.11 RNA extraction

RNA extraction protocol was published previously (Hu et al., 2015). Yeast cells were cultured in YPD starting from 0.2 OD_{600} to mid-log phase and then shifted to SD-N (1 hour) for autophagy induction. Cells were collected ($1OD_{600}$) and then promptly frozen using liquid nitrogen. Total RNA was extracted using a NucleoSpin RNA extraction kit (Takara

Bio USA, Inc., Catalog #740955.50). RNA concentration was measured using EzDrop (BLUE-RAY BIOTECH).

2.12 Real-time quantitative PCR (RT-qPCR)

cDNA synthesis was carried out using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems/Thermo Fisher Scientific, Catalog #4368814) using 1 µg of total RNA per reaction. Quantitative real-time PCR (RT-qPCR) analysis was subsequently performed using Power SYBR™ Green PCR Master Mix (Applied Biosystems/Thermo Fisher Scientific, Catalog #4367659). Reactions were prepared in 96-well clear PCR plates (Bio-Rad, Catalog #HSP9901) and run on a Bio-Rad Opus 96 Real-Time PCR detection system. Each PCR reaction was prepared in a total volume of 15 µL, containing 7.5µL SYBR Green master mix, 1µL primer, 1.5 µL nuclease-free water and 5 µL of cDNA template (diluted 1:10). Following amplification, melt curve analysis was performed to confirm primer specificity. Relative transcript levels were calculated using the comparative Ct ($2^{-\Delta\Delta CT}$) method with normalization performed as specified. Primer sequences used in this study are listed in Table 5.2.

2.13 Quantification and Statistical Analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software, USA). For all the experiments, statistical significance was determined from at least 3 independent experiments using two-tailed unpaired student's t-test unless otherwise indicated. One-

Way ANOVA with Tukey's multiple comparisons test was used to analyze GFP-Atg8 assay results. A p value of <0.05 was considered significant. The number of independent experiments and p values are indicated in the figure legends.

CHAPTER THREE

RESULTS

3.1 Loss of *RRP8* decreases cell survival under prolonged nitrogen starvation

3.1.1 Survival Assay

In this assay, serial dilutions of exponentially growing cultures are spotted onto the solid YPD media and incubated to allow colony formation. Differences in growth across the dilution series provide a semi-quantitative comparison of cellular fitness and stress sensitivity. Due to its simplicity and reproducibility, and ability to compare multiple strains simultaneously, the spot assay remains a standard tool in yeast genetics.

To further assess long-term survival during nitrogen starvation, we performed a spot assay using WT, *rrp8*Δ, and *atg8*Δ strains. Cultures were subjected to nitrogen starvation in SD-N medium for 7 days, after which equal cell densities were serially diluted and spotted onto YPD plates to evaluate viability. The *atg8*Δ strain served as a negative control, as Atg8 is an essential component of autophagosome formation and its deletion abolishes autophagy (Ichimura et al., 2000; Xie et al., 2008). Following 7 days of nitrogen starvation, WT cells retained robust viability, whereas *rrp8*Δ strains showed reduced survival compared to WT. As expected, *atg8*Δ strain exhibited a more severe survival defect, with little to no colony formation observed across the dilution series. The intermediate survival phenotype observed in and *rrp8*Δ cells suggests that loss of *RRP8* compromises the ability of cells to survive prolonged nutrient stress but

does not result in a complete loss of viability as observed in autophagy-deficient mutants such as *atg8*Δ.

This survival defect in the absence of *RRP8* represents the first indication that Rrp8 may play an important role during nutrient stress conditions. Because prolonged survival under nitrogen starvation depends heavily on efficient metabolic adaptation and intracellular recycling pathways such as autophagy, these findings led us to hypothesize that Rrp8 may influence autophagy regulation.

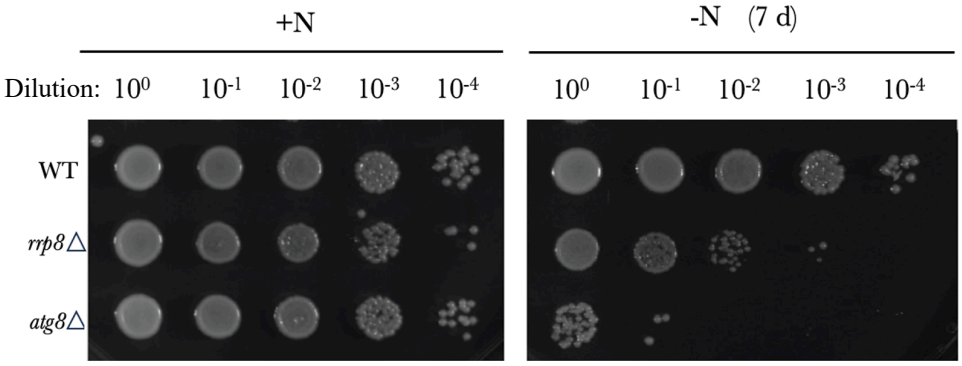

Figure: 3.1.1 Loss of *RRP8* decreases cell survival under prolonged nitrogen starvation. WT (BY4742), *rrp8*Δ (BY4742, *rrp8*Δ*::KAN*), and *atg8*Δ (YAB369) were grown in nutrient rich medium (+N) until mid-log phase and then starved for nitrogen for 7 days (-N). Cells were serially diluted and spotted onto YPD plates, incubated for 2 days, and imaged. The image shown is representative of n=4 independent experiments.

3.2 Rrp8 is positively regulates autophagy flux

3.2.1 GFP-Atg8 Assay

Based on this finding, we next examined whether autophagy was impaired in *rrp8*Δ cells by performing the GFP-Atg8 processing assay. GFP-Atg8 is a fundamental biochemical method used to monitor the autophagic flux by observing the vacuolar delivery and subsequent proteolysis of the GFP-Atg8 chimera (Torggler et al., 2017b). This assay relies on the N-terminal tagging of Atg8, a ubiquitin-like protein that is conjugated to phosphatidylethanolamine (PE) and localized to the autophagosomal membrane. Upon induction of autophagy, the inner-membrane-bound GFP-Atg8 is delivered to the vacuole, where the Atg8 is degraded by the resident hydrolases (Nair, Thumm, et al., 2011; Torggler et al., 2017b). Because the GFP moiety is relatively resistant to vacuolar proteolysis, it accumulates as an intact fragment in the vacuolar lumen (Kirisako et al., 1999). The ratio of free GFP to total GFP-Atg8 were detected via Western blot using anti-GFP antibodies, thus serves as a semi-quantitative readout for the autophagic rate.

GFP-Atg8 processing assay was performed to assess bulk autophagy, accompanied with analysis through western blotting. Autophagy was induced through nitrogen starvation in WT and *rrp8*Δ cells expressing GFP-Atg8. Samples were collected at specific time intervals as mentioned and analyzed by SDS-PAGE and western blotting to detect GFP-Atg8 and the accumulation of free GFP moiety. GFP signals

were detected through an antibody that recognizes GFP, with Pgk1 used as a loading control. We observed a significant decrease in the release of free GFP in the *rrp8*Δ cells compared to the WT. This reduction in autophagic flux indicates that Rrp8 may act as a positive regulator of autophagy flux.

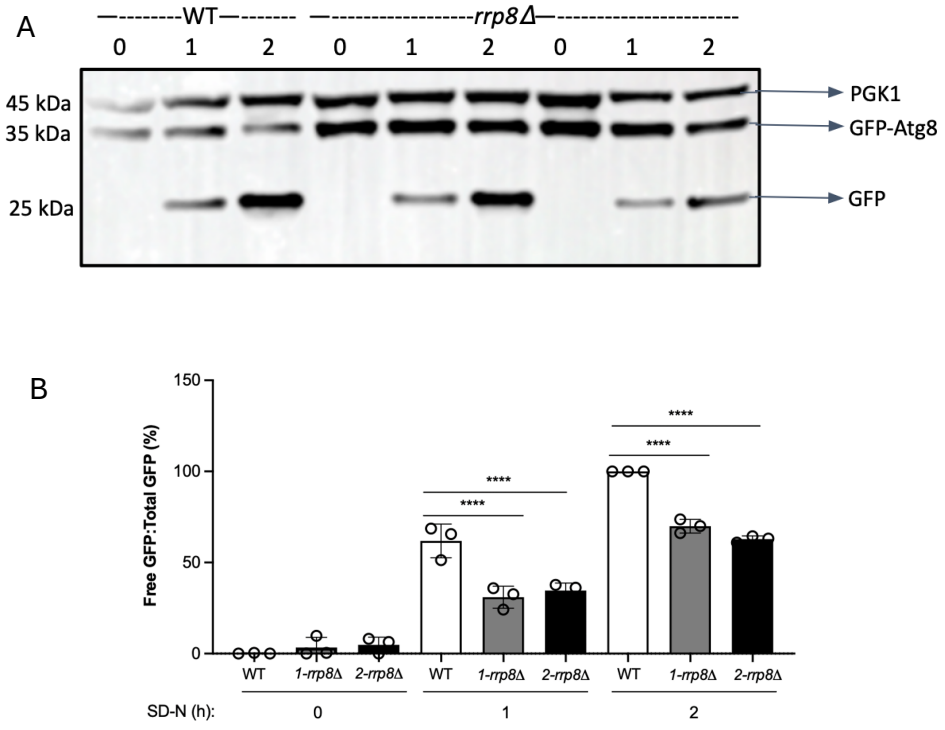

Figure: 3.2.1 Rrp8 positively regulates non-selective autophagy flux as measured by the GFP-Atg8 assay.

(A) Wild-type (EDA291) and *rrp8*Δ (SBN03) strains expressing GFP-Atg8 were grown in nutrient-rich media and then shifted to SD-N medium for 0, 1, and 2 hours. GFP-Atg8 and free GFP are detectable by western blotting using an antibody that recognizes GFP. Pgk1 is the loading control. The blot shown is representation of 3 independent experiments.

(B) Densitometry of blots represented in A. The relative percent processed GFP-Atg8 was calculated by determining the ratio of free GFP: total GFP (sum of full-length GFP-Atg8 and free GFP). Error bars indicate SD (n=3; **** $p<0.0001$).

3.2.2 The Pho8Δ60 Assay (ALP Assay)

The Pho8Δ60 (Alkaline phosphatase or ALP) assay serves as a sensitive, quantitative measure of nonselective bulk autophagy (Kirisako et al., 1999). This employs a truncated version of the alkaline phosphatase, Pho8Δ60, which lacks the amino-terminal signal sequence and remains localized in the cytosol in an inactive form instead of entering the secretory pathway. Upon the induction of autophagy by nitrogen starvation or rapamycin, Pho8Δ60 is randomly sequestered into autophagosomes and delivered to the vacuole (Kirisako et al., 1999; Torggler et al., 2017b). Once inside the vacuole, its pro-peptide is cleaved through proteases, converting the inactive enzyme into its active form. The resulting phosphatase activity is measured in cell lysates by monitoring the dephosphorylation of substrates such as para-nitrophenyl phosphate (pNPP) (Torggler et al., 2017b).

To further quantitatively evaluate bulk autophagy activity, we performed the Pho8Δ60 (ALP) assay using this system. We compared the autophagic activity of WT, *rrp8*Δ, and *atg13*Δ strains after inducing autophagy via nitrogen starvation in SD-N medium for 4 hours. The *atg13*Δ strain served as a negative control because Atg13 is a critical

component of the early autophagic machinery, and its deletion effectively blocks the sequestration of the inactive Pho8Δ60 precursor from the cytosol into autophagosomes (Kamada et al., 2000; N. N. Noda et al., 2008). While starved WT cells exhibited a robust increase in ALP activity, we observed a significant decrease in phosphatase activity of the *rrp8*Δ mutant strains compared to the wild type. This reduction in autophagic magnitude supports the conclusion that Rrp8 acts as a positive regulator of autophagy flux.

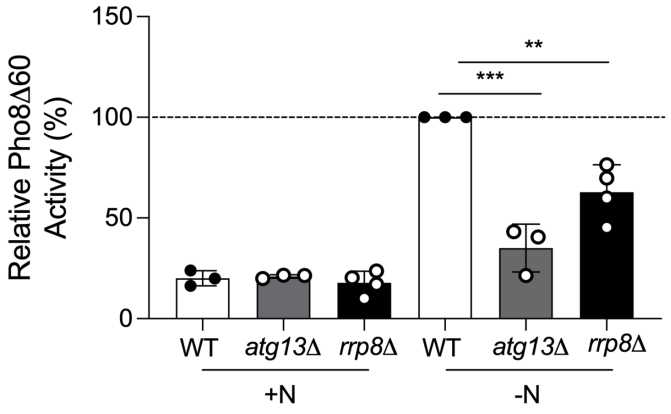

Figure: 3.2.2: Rrp8 positively regulates non-selective autophagy flux as measured by Pho8Δ60 assay. Wild-type (JMY347), *atg13*Δ (EDA328), and *rrp8*Δ (SBN08) strains expressing Pho8Δ60 were grown in YPD (rich-medium) and shifted to SD-N medium for 4 hours. Pho8Δ60 activity was measured and normalized to the activity of the WT cells at 4 hours, which was set at 100%. Results are shown as the means of at least 3 independent experiments. Error bars indicate SD (*p* values are as follows: ** $p < 0.01$; *** $p < 0.001$).

3.3 Rrp8-PA fusion protein expression decreases with nitrogen starvation and autophagy induction

3.3.1 Autophagy alters Rrp8 protein levels

To determine whether Rrp8 protein levels are modulated during autophagy-inducing conditions, we examined Rrp8 expression during nitrogen starvation. Because nitrogen starvation suppresses growth-associated processes and induces autophagy, we investigated whether Rrp8 protein abundance changes under these conditions.

To monitor Rrp8 levels, *RRP8* was chromosomally tagged at the C-terminus with Protein A (PA), allowing detection of the endogenous Rrp8-PA fusion protein by western blot analysis. Cells expressing Rrp8-PA were grown in nutrient-rich YPD medium to mid-log phase and then shifted to SD-N medium to induce autophagy for defined time points. Western blot analysis revealed a noticeable decrease in Rrp8-PA protein levels during nitrogen starvation compared to nutrient-rich conditions suggests that Rrp8 is downregulated during nutrient stress, consistent with its role in ribosome biogenesis and rRNA modification (Bousquet-Antonelli et al., 2000; Peifer et al., 2013). This observation further supports a potential link between Rrp8 and autophagy regulation and indicates that Rrp8 expression is responsive to metabolic cues that activate autophagy.

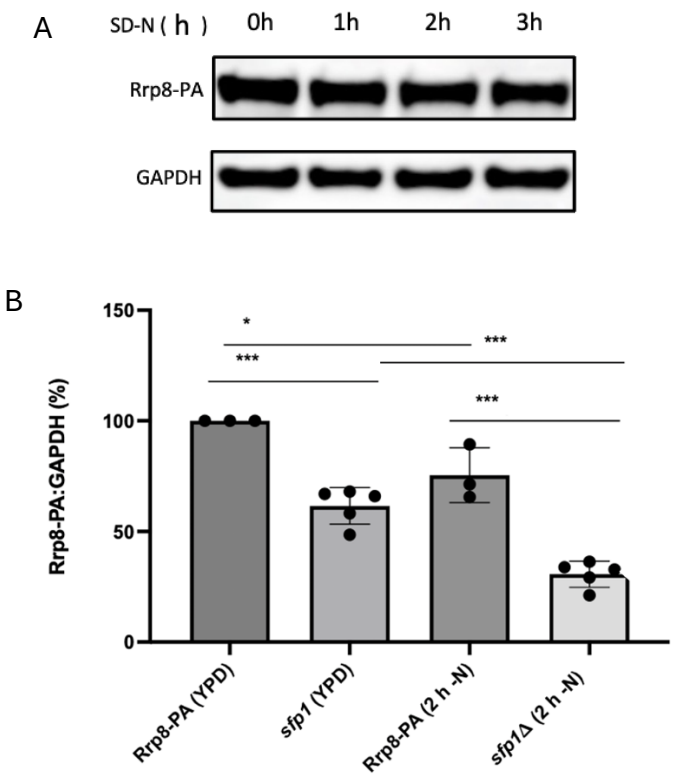

Figure: 3.3.1: Rrp8-PA fusion protein levels decrease with nitrogen starvation.

(A) Cells (SBN15) endogenously expressing Rrp8-PA fusion protein were grown in rich-medium and then shifted to SD-N medium for 0, 1, 2, and 3 hours. Protein extracts were resolved by SDS-PAGE and blotted with anti-PA antibody. GAPDH is the loading control. The blot shown is representation of 4 independent experiments.

(B) Densitometry of blots represented in A. The percentage of Rrp8-PA: GAPDH was quantified. Error bars indicate SD for 4 independent experiments (** p<0.01; **** p<0.0001).

3.3.2 Autophagy alters RRP8 mRNA levels

To determine whether *RRP8* expression changes during autophagy-inducing conditions, we measured *RRP8* transcript levels using RT-qPCR. Two independent primer sets (*1-RRP8* and *2-RRP8*) were used to ensure the reliability of the measurements. Following nitrogen starvation (-N), both *RRP8* primer sets showed a significant reduction in relative transcript levels. As a control, *SLD3* expression was measured and did not show a significant change between nutrient-rich and starvation conditions, indicating that the observed decrease in *RRP8* expression is specific and not due to global transcriptional changes. These results indicate that *RRP8* expression decreases during nitrogen starvation. Consistent with our western blot results, this suggests that the reduction in Rrp8 protein levels during autophagy-inducing conditions may be partially regulated at the transcriptional level.

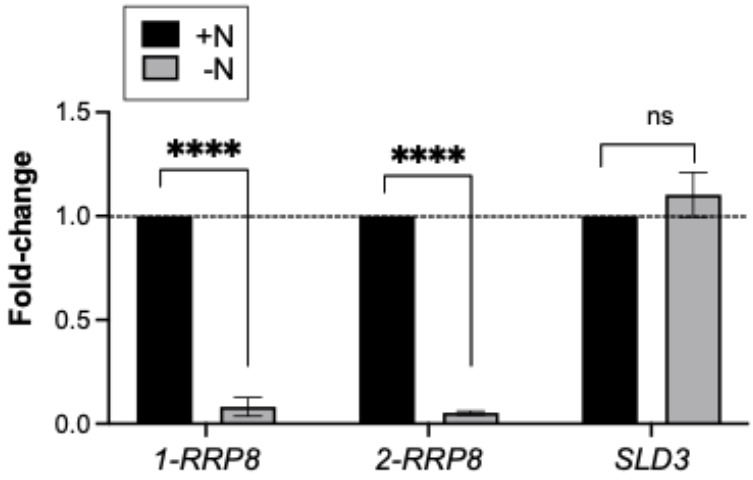

Figure: 3.3.2 *RRP8* expression decreases after autophagy induction. WT (BY4741) cells were grown to mid-log phase in YPD (+N) and then shifted to nitrogen starved (-N) media for 1 hour. Total RNA was extracted, and RT-qPCR was performed. Results shown are relative to the level of WT cells in +N conditions, which were set to 1. The housekeeping gene *UBC6* was used for normalization of relative expression levels. Error bars indicate SD (n=3; **** p<0.0001; ns indicates not significant).

3.4 SFP1 positively regulates Rrp8-PA fusion protein

3.4.1 Western blot

To investigate whether the nutrient-responsive transcription factor Sfp1 contributes to the regulation of Rrp8, we compared Rrp8-PA protein levels in WT and *sfp1*Δ cells. Sfp1 is a positive regulator of ribosomal protein and ribosome biogenesis gene expression, that functions downstream of nutrient-sensing pathways, including TORC1, and its activity is sensitive to changes in nutrient availability and cellular stress (Marion et al., 2004; Lempiäinen et al., 2009).

Using strains expressing Rrp8-PA fusion protein, we performed western blot analysis to assess Rrp8-PA abundance in the presence and absence of *SFP1* under both nutrient-rich and starvation conditions. Under nutrient-rich conditions, WT cells exhibited robust Rrp8-PA expression. In contrast, *sfp1*Δ in YPD showed a significant reduction in Rrp8-PA levels compared to WT cells. Following 2 hours of nitrogen starvation, Rrp8-PA levels decreased in WT cells compared to their rich media counterparts,

reflecting nutrient-dependent downregulation. Notably in *sfp1*Δ cells, Rrp8-PA levels were even lower during starvation. These results suggest that Sfp1 helps maintain normal Rrp8 protein levels and acts as a positive regulator of Rrp8 expression.

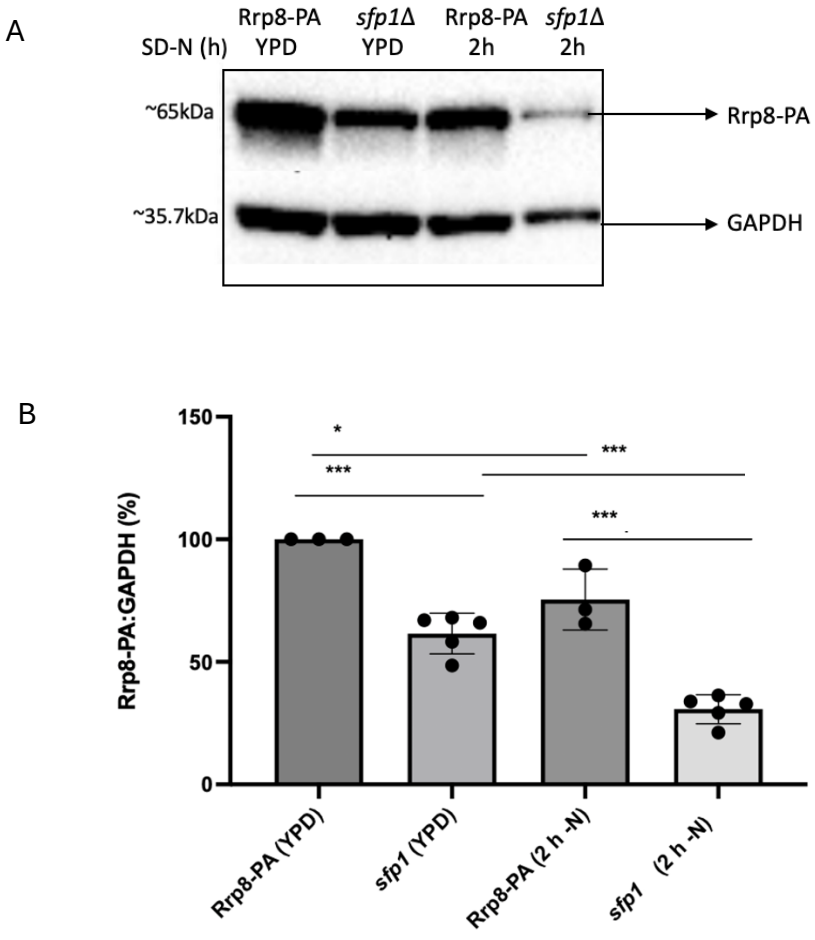

Figure: 3.4.1 Loss of *SFP1* decreases Rrp8-PA fusion protein levels. (A) WT (SBN15) and *sfp1*Δ (SBN21) were grown in rich-medium and then shifted to SD-N medium for 2 hours. Protein extracts were resolved by SDS-PAGE and blotted with anti-PA antibody. GAPDH is the loading control. The blot shown is representation of 3 independent experiments. (B)

Densitometry of blots represented in A. The percentage of Rrp8-PA: GAPDH was quantified. Error bars indicate SD for 3 independent experiments (* $p<0.05$; *** $p<0.001$).

CHAPTER FOUR

CONCLUSION

This study investigated the role of Rrp8, a nucleolar ribosome biogenesis factor, in autophagy regulation during nitrogen starvation in *Saccharomyces cerevisiae*. Ribosome biogenesis is one of the most energetically demanding processes in rapidly growing cells and is tightly coordinated with nutrient availability and metabolic state (Jorgensen et al., 2004). During nutrient limitation, cells suppress ribosome production while activating autophagy to recycle intracellular components and maintain energy homeostasis (T. Noda & Ohsumi, 1998; Reggiori & Klionsky, 2013a). Given this reciprocal regulation of anabolic and catabolic pathways, we hypothesized that Rrp8 may contribute to starvation adaptation.

Our initial survival assay demonstrated that *rrp8*Δ cells exhibit significantly reduced viability after prolonged nitrogen starvation compared to wild-type cells. Because long-term survival under starvation relies heavily on efficient autophagic recycling (T. Noda & Ohsumi, 1998; Wen & Klionsky, 2016), this phenotype suggested that Rrp8 may influence autophagy.

To directly test this possibility, we performed two independent assays of autophagic flux: GFP-Atg8 processing and the Pho8Δ60 alkaline phosphatase (ALP) assay. Both assays revealed reduced autophagic flux in *rrp8*Δ cells relative to wild type, indicating that loss of Rrp8

compromises autophagy progression during nitrogen starvation. These findings suggest that Rrp8 functions as a positive modulator of autophagy flux under nutrient stress conditions. Although Rrp8 is not a core autophagy protein, defects in ribosome biogenesis can influence metabolic balance and stress adaptation (Jorgensen et al., 2004), providing a potential indirect mechanism for this phenotype.

We next examined whether Rrp8 itself is regulated during autophagy-inducing conditions. Both RT-qPCR and Rrp8-PA western blot analyses revealed a decrease in *RRP8* mRNA and protein levels during nitrogen starvation. This coordinated downregulation is consistent with the well-established repression of ribosome biogenesis during nutrient limitation (Jorgensen et al., 2004; Marion et al., 2004). Because ribosome production is energetically costly, its suppression during starvation likely reflects cellular efforts to conserve energy while activating catabolic processes such as autophagy (Reggiori & Klionsky, 2013a).

Given that Rrp8 expression declines during starvation, we investigated whether the nutrient-responsive transcription factor Sfp1 contributes to its regulation. Sfp1 is a central regulator of ribosomal protein and ribosome biogenesis gene expression downstream of *TORC1* signaling (Lempiäinen et al., 2009; Marion et al., 2004). Loss of *SFP1* resulted in reduced Rrp8 protein levels, suggesting that Sfp1 acts as a positive regulator of Rrp8 expression. While these findings do not yet demonstrate direct promoter binding, they support a model in which the

TORC1–Sfp1 axis may coordinate Rrp8 expression with nutrient status. Notably, TORC1 is also a major negative regulator of autophagy (T. Noda & Ohsumi, 1998), placing Rrp8 within a signaling network that may influence autophagy regulation.

Collectively, these findings suggest that Rrp8 contributes to cellular adaptation during nutrient stress and may influence autophagic efficiency. Although Rrp8 is known to function in ribosome biogenesis, ribosome biogenesis itself was not directly examined in this study. Therefore, the reduced autophagic flux observed in *rrp8*Δ cells may arise through multiple possible mechanisms. These may include indirect effects on cellular metabolism, altered translational capacity, nucleolar stress responses, and other nutrient-responsive regulatory pathways rather than direct defects in ribosome assembly.

Alternatively, Rrp8 may influence autophagy through its role as a SAM-dependent rRNA methyltransferase, potentially linking ribosome modification to metabolic regulation (Sharma et al., 2018). Because TORC1 regulates ribosome biogenesis partly through phosphorylation and regulation of Sfp1 (Marion et al., 2004; Lempiäinen et al., 2009), and further analyses suggest a regulatory relationship between Sfp1 and *RRP8*, it is possible that Rrp8 functions downstream of TORC1 via Sfp1 as part of a nutrient-responsive regulatory network (Cipollina et al., 2008b; Jorgensen et al., 2004). This proposed TORC1-Sfp1-Rrp8 relationship may therefore represent a point of crosstalk between metabolism and

autophagy. However, these possibilities remain speculative and will require future investigation.

Future work should also determine whether Rrp8 participates in selective ribosome turnover through ribophagy. Ribophagy is a selective form of autophagy that targets ribosomes during nutrient stress (Kraft et al., 2009; Ossareh-Nazari et al., 2010). Determining whether Rrp8 is subject to ribophagic turnover or instead indirectly affected by ribosome remodeling will further clarify its role in coordinating ribosome homeostasis and autophagy.

Future studies will focus on clarifying the regulatory mechanisms governing *RRP8* expression and function. Quantitative analysis of *RRP8* transcript levels in *sfp1*Δ cells will determine whether Sfp1 regulates *RRP8* levels. Chromatin immunoprecipitation (ChIP) experiments could establish direct binding of Sfp1 to the *RRP8* promoter. In addition, ribophagy-specific assays will be required to distinguish whether the autophagy defects observed in *rrp8*Δ cells reflect defects in selective ribosome turnover or broader effects on macroautophagy.

In summary, this work identifies Rrp8 as a previously uncharacterized contributor to autophagy regulation during nitrogen starvation. By linking nutrient signaling, metabolic adaptation, and autophagic flux, these findings provide new insight into how cells coordinate anabolic and catabolic pathways to maintain homeostasis during stress.

CHAPTER FIVE

TABLES

Table 5.1. Strains used in this study

Strains	Genotype	Reference
BY4741	MATα *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Horizon Discovery
BY4742	MATα *his3*Δ1 *leu2*Δ0 *lys2*Δ0 *ura3*Δ0	Horizon Discovery
BY4742, *rrp8Δ::KANMX*	BY4742, *rrp8Δ::KANMX*	Horizon Discovery
SEY6210	MATα *leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 suc2-Δ9 lys2-801; GAL*	(Robinson et al., 1988)
JMY347	SEY6210, *pho13Δ ZEO1p-pho8Δ60*, *CUP1p-GFP - ATG8(405)*::*LEU2*	(Wen & Klionsky, 2016)
EDA291	WLY176, *pRS405-GFP-ATG8::LEU2* (*CUP1*pr)	This study
EDA328	JMY347, *atg13*Δ::*HIS5*	(Tasmi et al., 2026)
SBN03	EDA291, *rrp8Δ::URA3*	This study
SBN08	JMY347, *rrp8*Δ::*HIS5*	This study
SBN15	BY4742, *RRP8-PA::HIS3*	This study
SBN21	SBN15, *sfp1*Δ::*URA3*	This study
YAB369	YTS158, *atg8*Δ::*HIS5*	(Delorme-Axford et al., 2018)

Table 5.2. RT-qPCR primers used in this study

Primer	Sequence (5' to 3')	Reference
1-RRP8-qPCR-F	ACGCTTTGAAGCTGATGGGA	This study
1-RRP8-qPCR-R	TAATCTCCGCAGGTGGCTTG	This study
2-RRP8-qPCR-F	TTTAGCGCCAAGGGGTGAAT	This study
2-RRP8-qPCR-R	CCCATCAGCTTCAAAGCGTC	This study
SLD3-qPCR-F	CGCAACTTCAAAGCATCATTGAATCGC	(Bernard et al., 2015)
SLD3-qPCR-R	GGGGCTTATTAGTGGGAGTAGAGG	(Bernard et al., 2015)
UBC6-qPCR-F	GATACTTGGAATCCTGGCTGGTCTGTCTC	(Teste et al., 2009)
UBC6-qPCR-R	AAAGGGTCTTCTGTTTCATCACCTGTATTTGC	(Teste et al., 2009)

APPENDIX

A. IBC APPROVAL

cayuse
Hazard Safety

Reference: IBC Protocol # IBC-00001151

Protocol Title: EXEMPT - RAM protocol 4575 - Molecular mechanisms regulating autophagy in the yeast Saccharomyces cerevisiae

Principal Investigator: Delorme-Axford, Elizabeth

Approval Period: 09/14/2022 - 09/14/2025

Dear Professor Delorme-Axford, Elizabeth:

The Institutional Biosafety Committee (IBC), responsible for the review of research involving materials of human origin, recombinant DNA, and biohazardous, infectious or toxic materials, has reviewed the original proposal, noted the revisions provided by you upon committee's request (if applicable), and approved the project referenced above. This is your formal approval notice; please save it for your records.

The proposed activities are approved for a period of three years, starting 09/14/2022 and ending 09/14/2025, and assigned the approval # IBC-00001151. Please be aware that protocols expire at 12:01 AM on the expiration date. The highest BSL level approved for the protocol can be found on the Overview page of the protocol form.

Any unanticipated problems, changes in, or halting of the activities proposed in the project, as described, must be promptly reported to the IBC or the biosafety officer.

In addition, all faculty, staff, and students involved in the project must be trained and qualified to conduct the project in a responsible manner. Please note, in this regard, that you, the Principal Investigator, are fully responsible at all times for limiting or restricting access to laboratories and for assessing, in each circumstance, who may enter or work in a laboratory when biohazardous materials are in use.

Prior to initiating any work on this approved project, please contact me by email (ghallau@oakland.edu) to schedule a startup Biosafety Audit. This audit will verify that all biosafety controls described in your application have been implemented.

Please let me know if you have any questions. Thank you.

Sincerely,

Janell Hallauer
Biosafety Officer
Laboratory Safety and Compliance

REFERENCES

Alberghina, L., Smeraldi, C., Ranzi, B. M., & Porro, D. (1998). Control by Nutrients of Growth and Cell Cycle Progression in Budding Yeast, Analyzed by Double-Tag Flow Cytometry. *Journal of Bacteriology*, *180*(15), 3864–3872. https://doi.org/10.1128/JB.180.15.3864-3872.1998

Albert, B., Tomassetti, S., Gloor, Y., Dilg, D., Mattarocci, S., Kubik, S., Hafner, L., & Shore, D. (2019). Sfp1 regulates transcriptional networks driving cell growth and division through multiple promoter-binding modes. *Genes & Development*, *33*(5–6), 288–293. https://doi.org/10.1101/gad.322040.118

ATG8—An overview | ScienceDirect Topics. (2025). Retrieved January 16, 2025, from https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/atg8

Avogo, E. W., Burlingame, N. A., Narasimhaiah, S. B., & Delorme-Axford, E. (2025). Mot2 is a potential regulator of autophagy in yeast identified by bioinformatics analysis. *microPublication Biology.*

Baek, S. H., & Kim, K. I. (2017). Epigenetic Control of Autophagy: Nuclear Events Gain More Attention. *Molecular Cell*, *65*(5), 781–785. https://doi.org/10.1016/j.molcel.2016.12.027

Bartholomew, C. R., Suzuki, T., Du, Z., Backues, S. K., Jin, M., Lynch-Day, M. A., Umekawa, M., Kamath, A., Zhao, M., Xie, Z., Inoki, K., & Klionsky, D. J. (2012). Ume6 transcription factor is part of a signaling cascade that

regulates autophagy. *Proceedings of the National Academy of Sciences*, *109*(28), 11206–11210. https://doi.org/10.1073/pnas.1200313109

Bejarano, E., & Cuervo, A. M. (2010). Chaperone-Mediated Autophagy. *Proceedings of the American Thoracic Society*, *7*(1), 29–39. https://doi.org/10.1513/pats.200909-102JS

Bergman, A., Vitay, D., Hellgren, J., Chen, Y., Nielsen, J., & Siewers, V. (2019). Effects of overexpression of *STB5* in *Saccharomyces cerevisiae* on fatty acid biosynthesis, physiology and transcriptome. *FEMS Yeast Research*, *19*(3). https://doi.org/10.1093/femsyr/foz027

Bernard, A., Jin, M., González-Rodríguez, P., Füllgrabe, J., Delorme-Axford, E., Backues, S. K., Joseph, B., & Klionsky, D. J. (2015). Rph1/KDM4 Mediates Nutrient-Limitation Signaling that Leads to the Transcriptional Induction of Autophagy. *Current Biology*, *25*(5), 546–555. https://doi.org/10.1016/j.cub.2014.12.049

Bieganowski, P., Seidle, H. F., Wojcik, M., & Brenner, C. (2006). Synthetic Lethal and Biochemical Analyses of NAD and NADH Kinases in Saccharomyces cerevisiae Establish Separation of Cellular Functions. *Journal of Biological Chemistry*, *281*(32), 22439–22445. https://doi.org/10.1074/jbc.m513919200

Bousquet-Antonelli, C., Vanrobays, E., Gélugne, J.-P., Caizergues-Ferrer, M., & Henry, Y. (2000). Rrp8p is a yeast nucleolar protein functionally linked to Gar1p and involved in pre-rRNA cleavage at site A2. *RNA*, *6*(6), 826–843. https://doi.org/10.1017/S1355838200992288

Budovskaya, Y. V., Stephan, J. S., Reggiori, F., Klionsky, D. J., & Herman, P. K. (2004). The Ras/cAMP-dependent Protein Kinase Signaling Pathway Regulates an Early Step of the Autophagy Process in Saccharomyces cerevisiae. *Journal of Biological Chemistry*, *279*(20), 20663–20671. https://doi.org/10.1074/jbc.M400272200

Cebollero, E., & Reggiori, F. (2009). Regulation of autophagy in yeast Saccharomyces cerevisiae. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1793*(9), 1413–1421. https://doi.org/10.1016/j.bbamcr.2009.01.008

Chen, Y., Hu, J., Zhao, P., Fang, J., Kuang, Y., Liu, Z., Dong, S., Yao, W., Ding, Y., Wang, X., Pan, Y., Wu, J., Zhao, J., Yang, J., Xu, Z., Liu, X., Zhang, Y., Wu, C., Zhang, L., … Yi, C. (2025). Rpl12 is a conserved ribophagy receptor. *Nature Cell Biology*. https://doi.org/10.1038/s41556-024-01598-2

Cheong, H., & Klionsky, D. J. (2008). Chapter 1 Biochemical Methods to Monitor Autophagy-Related Processes in Yeast. In *Methods in Enzymology* (Vol. 451, pp. 1–26). Elsevier. https://doi.org/10.1016/S0076-6879(08)03201-1

Cipollina, C., Alberghina, L., Porro, D., & Vai, M. (2005). *SFP1* is involved in cell size modulation in respiro-fermentative growth conditions. *Yeast*, *22*(5), 385–399. https://doi.org/10.1002/yea.1218

Cipollina, C., Van Den Brink, J., Daran-Lapujade, P., Pronk, J. T., Porro, D., & De Winde, J. H. (2008a). Saccharomyces cerevisiae SFP1: At the

crossroads of central metabolism and ribosome biogenesis. *Microbiology*, *154*(6), 1686–1699. https://doi.org/10.1099/mic.0.2008/017392-0

Cipollina, C., Van Den Brink, J., Daran-Lapujade, P., Pronk, J. T., Porro, D., & De Winde, J. H. (2008b). Saccharomyces cerevisiae SFP1: At the crossroads of central metabolism and ribosome biogenesis. *Microbiology*, *154*(6), 1686–1699. https://doi.org/10.1099/mic.0.2008/017392-0

Delorme-Axford, E., Abernathy, E., Lennemann, N. J., Bernard, A., Ariosa, A., Coyne, C. B., Kirkegaard, K., & Klionsky, D. J. (2018). The exoribonuclease Xrn1 is a post-transcriptional negative regulator of autophagy. *Autophagy*, *14*(5), 898–912. https://doi.org/10.1080/15548627.2018.1441648

Delorme-Axford, E., Guimaraes, R. S., Reggiori, F., & Klionsky, D. J. (2015). The yeast Saccharomyces cerevisiae: An overview of methods to study autophagy progression. *Methods*, *75*, 3–12. https://doi.org/10.1016/j.ymeth.2014.12.008

Delorme-Axford, E., & Klionsky, D. J. (2018). Transcriptional and post-transcriptional regulation of autophagy in the yeast Saccharomyces cerevisiae. *Journal of Biological Chemistry*, *293*(15), 5396–5403. https://doi.org/10.1074/jbc.R117.804641

Delorme-Axford, E., & Klionsky, D. J. (2019). On the edge of degradation: Autophagy regulation by RNA decay. *WIREs RNA*, *10*(3), e1522. https://doi.org/10.1002/wrna.1522

Delorme-Axford, E., & Klionsky, D. J. (2020). The LC3-conjugation machinery specifies cargo loading and secretion of extracellular vesicles. *Autophagy*, *16*(7), 1169–1171. https://doi.org/10.1080/15548627.2020.1760057

Delorme-Axford, E., Tasmi, T. A., Klionsky, D. J. (2023). The Pho23-Rpd3 histone deacetylase complex regulates the yeast metabolic transcription factor Stb5. *microPublication Biology.*

Delorme-Axford, E., Wen, X., & Klionsky, D. J. (2023). The yeast transcription factor Stb5 acts as a negative regulator of autophagy by modulating cellular metabolism. *Autophagy*, *19*(10), 2719–2732. https://doi.org/10.1080/15548627.2023.2228533

Díaz-Troya, S., Pérez-Pérez, M. E., Florencio, F. J., & Crespo, J. L. (2008). The role of TOR in autophagy regulation from yeast to plants and mammals. *Autophagy*, *4*(7), 851–865. https://doi.org/10.4161/auto.6555

Dice, J. F. (2007). Chaperone-Mediated Autophagy. *Autophagy*, *3*(4), 295–299. https://doi.org/10.4161/auto.4144

Epple, U. D., Suriapranata, I., Eskelinen, E.-L., & Thumm, M. (2001). Aut5/Cvt17p, a Putative Lipase Essential for Disintegration of Autophagic Bodies inside the Vacuole. *Journal of Bacteriology*, *183*(20), 5942–5955. https://doi.org/10.1128/JB.183.20.5942-5955.2001

Feng, Y., He, D., Yao, Z., & Klionsky, D. J. (2014). The machinery of macroautophagy. *Cell Research*, *24*(1), 24–41. https://doi.org/10.1038/cr.2013.168

Fingerman, I., Nagaraj, V., Norris, D., & Vershon, A. K. (2003). Sfp1 Plays a Key Role in Yeast Ribosome Biogenesis. *Eukaryotic Cell*, *2*(5), 1061–1068. https://doi.org/10.1128/EC.2.5.1061-1068.2003

Furukawa, K., Ginevskaia, T., & Kanki, T. (2024). Re-exploration of all *ATG* genes. *Autophagy Reports*, *3*(1), 2386194. https://doi.org/10.1080/27694127.2024.2386194

Gade, P., & Kalvakolanu, D. V. (2012). Chromatin Immunoprecipitation Assay as a Tool for Analyzing Transcription Factor Activity. In A. Vancura (Ed.), *Transcriptional Regulation* (Vol. 809, pp. 85–104). Springer New York. https://doi.org/10.1007/978-1-61779-376-9_6

Gasch, A. P., & Werner-Washburne, M. (2002). The genomics of yeast responses to environmental stress and starvation. *Functional & Integrative Genomics*, *2*(4–5), 181–192. https://doi.org/10.1007/s10142-002-0058-2

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: Cellular and molecular mechanisms. *The Journal of Pathology*, *221*(1), 3–12. https://doi.org/10.1002/path.2697

Gómez-Virgilio, L., Silva-Lucero, M.-C., Flores-Morelos, D.-S., Gallardo-Nieto, J., Lopez-Toledo, G., Abarca-Fernandez, A.-M., Zacapala-Gómez, A.-E., Luna-Muñoz, J., Montiel-Sosa, F., Soto-Rojas, L. O., Pacheco-Herrero, M., & Cardenas-Aguayo, M.-C. (2022). Autophagy: A Key Regulator of Homeostasis and Disease: An Overview of Molecular Mechanisms and Modulators. *Cells*, *11*(15), 2262. https://doi.org/10.3390/cells11152262

Grummt, I., & Ladurner, A. G. (2008). A Metabolic Throttle Regulates the Epigenetic State of rDNA. *Cell*, *133*(4), 577–580. https://doi.org/10.1016/j.cell.2008.04.026

Gubas, A., & Dikic, I. (2022). A guide to the regulation of selective autophagy receptors. *The FEBS Journal*, *289*(1), 75–89. https://doi.org/10.1111/febs.15824

Gueldener, U. (2002). A second set of loxP marker cassettes for Cre-mediated multiple gene knockouts in budding yeast. *Nucleic Acids Research*, *30*(6), 23e–223. https://doi.org/10.1093/nar/30.6.e23

He, C., & Klionsky, D. J. (2009). Regulation Mechanisms and Signaling Pathways of Autophagy. *Annual Review of Genetics*, *43*(1), 67–93. https://doi.org/10.1146/annurev-genet-102808-114910

Hu, G., McQuiston, T., Bernard, A., Park, Y.-D., Qiu, J., Vural, A., Zhang, N., Waterman, S. R., Blewett, N. H., Myers, T. G., Kehrl, J. H., Uzel, G., Klionsky, D. J., & Williamson, P. R. (2016). Tor-dependent post-transcriptional regulation of autophagy: Implications for cancer therapeutics. *Molecular & Cellular Oncology*, *3*(5), e1078923. https://doi.org/10.1080/23723556.2015.1078923

Hu, G., McQuiston, T., Bernard, A., Park, Y.-D., Qiu, J., Vural, A., Zhang, N., Waterman, S. R., Blewett, N. H., Myers, T. G., Maraia, R. J., Kehrl, J. H., Uzel, G., Klionsky, D. J., & Williamson, P. R. (2015). TOR-dependent post-transcriptional regulation of autophagy. *Autophagy*, *11*(12), 2390–2392. https://doi.org/10.1080/15548627.2015.1091142

Hu, L.-F. (2019). Epigenetic Regulation of Autophagy. In *Autophagy: Biology and Diseases* (pp. 221–236). Springer, Singapore. https://doi.org/10.1007/978-981-15-0602-4_11

Iadevaia, V., Liu, R., & Proud, C. G. (2014a). mTORC1 signaling controls multiple steps in ribosome biogenesis. *Seminars in Cell & Developmental Biology, Development of the Urogenital System & mTOR Signalling & Tight Junctions in Health and Disease*, *36*, 113–120. https://doi.org/10.1016/j.semcdb.2014.08.004

Iadevaia, V., Liu, R., & Proud, C. G. (2014b). mTORC1 signaling controls multiple steps in ribosome biogenesis. *Seminars in Cell & Developmental Biology, Development of the Urogenital System & mTOR Signalling & Tight Junctions in Health and Disease*, *36*, 113–120. https://doi.org/10.1016/j.semcdb.2014.08.004

Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Kominami, E., Ohsumi, M., Noda, T., & Ohsumi, Y. (2000). A ubiquitin-like system mediates protein lipidation. *Nature*, *408*(6811), 488–492. https://doi.org/10.1038/35044114

Jiao, L., Liu, Y., Yu, X.-Y., Pan, X., Zhang, Y., Tu, J., Song, Y.-H., & Li, Y. (2023). Ribosome biogenesis in disease: New players and therapeutic targets. *Signal Transduction and Targeted Therapy*, *8*(1), 1–22. https://doi.org/10.1038/s41392-022-01285-4

Jin, M., He, D., Backues, S. K., Freeberg, M. A., Liu, X., Kim, J. K., & Klionsky, D. J. (2014). Transcriptional Regulation by Pho23 Modulates the

Frequency of Autophagosome Formation. *Current Biology*, *24*(12), 1314–1322. https://doi.org/10.1016/j.cub.2014.04.048

Jin, M., & Klionsky, D. J. (2014). Regulation of autophagy: Modulation of the size and number of autophagosomes. *FEBS Letters*, *588*(15), 2457–2463. https://doi.org/10.1016/j.febslet.2014.06.015

Jorgensen, P., Rupeš, I., Sharom, J. R., Schneper, L., Broach, J. R., & Tyers, M. (2004). A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. *Genes & Development*, *18*(20), 2491–2505. https://doi.org/10.1101/gad.1228804

Ju, H.-Q., Lin, J.-F., Tian, T., Xie, D., & Xu, R.-H. (2020). NADPH homeostasis in cancer: Functions, mechanisms and therapeutic implications. *Signal Transduction and Targeted Therapy*, *5*(1). https://doi.org/10.1038/s41392-020-00326-0

Kamada, Y., Funakoshi, T., Shintani, T., Nagano, K., Ohsumi, M., & Ohsumi, Y. (2000). Tor-Mediated Induction of Autophagy via an Apg1 Protein Kinase Complex. *The Journal of Cell Biology*, *150*(6), 1507–1513. https://doi.org/10.1083/jcb.150.6.1507

Kamada, Y., Yoshino, K., Kondo, C., Kawamata, T., Oshiro, N., Yonezawa, K., & Ohsumi, Y. (2010). Tor Directly Controls the Atg1 Kinase Complex To Regulate Autophagy. *Molecular and Cellular Biology*, *30*(4), 1049–1058. https://doi.org/10.1128/MCB.01344-09

Kaushik, S., & Cuervo, A. M. (2012). Chaperone-mediated autophagy: A unique way to enter the lysosome world. *Trends in Cell Biology*, *22*(8), 407–417. https://doi.org/10.1016/j.tcb.2012.05.006

Kaushik, S., & Cuervo, A. M. (2018). The coming of age of chaperone-mediated autophagy. *Nature Reviews Molecular Cell Biology*, *19*(6), 365–381. https://doi.org/10.1038/s41580-018-0001-6

Kiffin, R., Christian, C., Knecht, E., & Cuervo, A. M. (2004). Activation of chaperone-mediated autophagy during oxidative stress. Molecular biology of the cell, 15(11), 4829–4840. https://doi.org/10.1091/mbc.e04-06-0477

Kim, K. H., & Lee, M.-S. (2014). Autophagy—A key player in cellular and body metabolism. *Nature Reviews Endocrinology*, *10*(6), 322–337. https://doi.org/10.1038/nrendo.2014.35

Kim, M., Lee, S. H., & Jeon, J. (2023). A Nucleolar Protein, MoRRP8 Is Required for Development and Pathogenicity in the Rice Blast Fungus. *Mycobiology*, *51*(5), 273–280. https://doi.org/10.1080/12298093.2023.2257996

Kirisako, T., Baba, M., Ishihara, N., Miyazawa, K., Ohsumi, M., Yoshimori, T., Noda, T., & Ohsumi, Y. (1999). Formation Process of Autophagosome Is Traced with Apg8/Aut7p in Yeast. *The Journal of Cell Biology*, *147*(2), 435–446. https://doi.org/10.1083/jcb.147.2.435

Kitada, M., & Koya, D. (2021). Autophagy in metabolic disease and ageing. *Nature Reviews Endocrinology*, *17*(11), 647–661. https://doi.org/10.1038/s41574-021-00551-9

Klionsky, D. J., Abdel-Aziz, A. K., Abdelfatah, S., Abdellatif, M., Abdoli, A., Abel, S., Abeliovich, H., Abildgaard, M. H., Abudu, Y. P., Acevedo-Arozena, A., Adamopoulos, I. E., Adeli, K., Adolph, T. E., Adornetto, A., Aflaki, E., Agam, G., Agarwal, A., Aggarwal, B. B., Agnello, M., … Tong, C.-K. (2021). Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)[1]. *Autophagy*, *17*(1), 1–382. https://doi.org/10.1080/15548627.2020.1797280

Klionsky, D. J., Petroni, G., Amaravadi, R. K., Baehrecke, E. H., Ballabio, A., Boya, P., Bravo-San Pedro, J. M., Cadwell, K., Cecconi, F., Choi, A. M. K., Choi, M. E., Chu, C. T., Codogno, P., Colombo, M. I., Cuervo, A. M., Deretic, V., Dikic, I., Elazar, Z., Eskelinen, E., … Pietrocola, F. (2021). Autophagy in major human diseases. *The EMBO Journal*, *40*(19), e108863. https://doi.org/10.15252/embj.2021108863

Klionsky, D. J., & Schulman, B. A. (2014). Dynamic regulation of macroautophagy by distinctive ubiquitin-like proteins. *Nature Structural & Molecular Biology*, *21*(4), 336–345. https://doi.org/10.1038/nsmb.2787

Kraft, C., Deplazes, A., Sohrmann, M., & Peter, M. (2008). Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nature Cell Biology*, *10*(5), 602–610. https://doi.org/10.1038/ncb1723

Kraft, C., Reggiori, F., & Peter, M. (2009). Selective types of autophagy in yeast. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1793*(9), 1404–1412. https://doi.org/10.1016/j.bbamcr.2009.02.006

Lahiri, V., Metur, S. P., Hu, Z., Song, X., Mari, M., Hawkins, W. D., Bhattarai, J., Delorme-Axford, E., Reggiori, F., Tang, D., Dengjel, J., & Klionsky, D. J. (2022). Post-transcriptional regulation of *ATG1* is a critical node that modulates autophagy during distinct nutrient stresses. *Autophagy*, *18*(7), 1694–1714. https://doi.org/10.1080/15548627.2021.1997305

Lempiäinen, H., Uotila, A., Urban, J., Dohnal, I., Ammerer, G., Loewith, R., & Shore, D. (2009). Sfp1 Interaction with TORC1 and Mrs6 Reveals Feedback Regulation on TOR Signaling. *Molecular Cell*, *33*(6), 704–716. https://doi.org/10.1016/j.molcel.2009.01.034

Levine, B., & Kroemer, G. (2019). Biological Functions of Autophagy Genes: A Disease Perspective. *Cell*, *176*(1–2), 11–42. https://doi.org/10.1016/j.cell.2018.09.048

Li, J., Zhang, H., & Wang, H. (2022). N1-methyladenosine modification in cancer biology: Current status and future perspectives. *Computational and Structural Biotechnology Journal*, *20*, 6578–6585. https://doi.org/10.1016/j.csbj.2022.11.045

Li, Y. F., & Shi, F. (2006). Partial rescue of pos5 mutants by YEF1 and UTR1 genes in Saccharomyces cerevisiae. Acta biochimica et biophysica Sinica, 38(5), 293–298. https://doi.org/10.1111/j.1745-7270.2006.00162.x

Liang, Y. (2023). Phagophore-lysosome/vacuole fusion in mutant yeast and mammalian cells. *Autophagy*, *19*(9), 2595–2600. https://doi.org/10.1080/15548627.2023.2205272

Loewith, R., & Hall, M. N. (2011). Target of Rapamycin (TOR) in Nutrient Signaling and Growth Control. *Genetics*, *189*(4), 1177–1201. https://doi.org/10.1534/genetics.111.133363

Longtine, M. S., Mckenzie Iii, A., Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Philippsen, P., & Pringle, J. R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast*, *14*(10), 953–961. https://doi.org/10.1002/(SICI)1097-0061(199807)14:10%3C953::AID-YEA293%3E3.0.CO;2-U

Lopez, A. D., Tar, K., Krügel, U., Dange, T., Ros, I. G., & Schmidt, M. (2011). Proteasomal degradation of Sfp1 contributes to the repression of ribosome biogenesis during starvation and is mediated by the proteasome activator Blm10. *Molecular Biology of the Cell*, *22*(5), 528–540. https://doi.org/10.1091/mbc.e10-04-0352

Low, P. (2022). Recent advances in autophagy research. *Biologia Futura*, *73*(2), 133–136. https://doi.org/10.1007/s42977-022-00125-4

Marion, R. M., Regev, A., Segal, E., Barash, Y., Koller, D., Friedman, N., & O'Shea, E. K. (2004). Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. *Proceedings of the National Academy of Sciences*, *101*(40), 14315–14322. https://doi.org/10.1073/pnas.0405353101

Martens, S., & Fracchiolla, D. (2020). Activation and targeting of ATG8 protein lipidation. *Cell Discovery*, *6*(1), 1–11. https://doi.org/10.1038/s41421-020-0155-1

Mayer, C., & Grummt, I. (2006). Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. *Oncogene*, *25*(48), 6384–6391. https://doi.org/10.1038/sj.onc.1209883

Metur, S. P., & Klionsky, D. J. (2024). Nutrient-dependent signaling pathways that control autophagy in yeast. *FEBS Letters*, *598*(1), 32–47. https://doi.org/10.1002/1873-3468.14741

Mijaljica, D., Prescott, M., & Devenish, R. J. (2011). Microautophagy in mammalian cells: Revisiting a 40-year-old conundrum. *Autophagy*, *7*(7), 673–682. https://doi.org/10.4161/auto.7.7.14733

Mizushima, N., & Komatsu, M. (2011). Autophagy: Renovation of Cells and Tissues. *Cell*, *147*(4), 728–741. https://doi.org/10.1016/j.cell.2011.10.026

Mizushima, N., Sugita, H., Yoshimori, T., & Ohsumi, Y. (1998). A New Protein Conjugation System in Human. *Journal of Biological Chemistry*, *273*(51), 33889–33892. https://doi.org/10.1074/jbc.273.51.33889

Moreau, K., Luo, S., & Rubinsztein, D. C. (2010). Cytoprotective roles for autophagy. *Current Opinion in Cell Biology*, *22*(2), 206–211. https://doi.org/10.1016/j.ceb.2009.12.002

Morselli, E., Galluzzi, L., Kepp, O., Criollo, A., Maiuri, M. C., Tavernarakis, N., Madeo, F., & Kroemer, G. (2009). Autophagy mediates pharmacological

lifespan extension by spermidineand resveratrol. *Aging*, *1*(12), 961–970. https://doi.org/10.18632/aging.100110

Murayama, A., Ohmori, K., Fujimura, A., Minami, H., Yasuzawa-Tanaka, K., Kuroda, T., Oie, S., Daitoku, H., Okuwaki, M., Nagata, K., Fukamizu, A., Kimura, K., Shimizu, T., & Yanagisawa, J. (2008). Epigenetic Control of rDNA Loci in Response to Intracellular Energy Status. *Cell*, *133*(4), 627–639. https://doi.org/10.1016/j.cell.2008.03.030

Nair, U., Jotwani, A., Geng, J., Gammoh, N., Richerson, D., Yen, W.-L., Griffith, J., Nag, S., Wang, K., Moss, T., Baba, M., McNew, J. A., Jiang, X., Reggiori, F., Melia, T. J., & Klionsky, D. J. (2011). SNARE proteins are required for macroautophagy. *Cell*, *146*(2), 290–302. https://doi.org/10.1016/j.cell.2011.06.022

Nair, U., Thumm, M., Klionsky, D. J., & Krick, R. (2011). GFP-Atg8 protease protection as a tool to monitor autophagosome biogenesis. *Autophagy*, *7*(12), 1546–1550. https://doi.org/10.4161/auto.7.12.18424

Nakatogawa, H., Ichimura, Y., & Ohsumi, Y. (2007). Atg8, a Ubiquitin-like Protein Required for Autophagosome Formation, Mediates Membrane Tethering and Hemifusion. *Cell*, *130*(1), 165–178. https://doi.org/10.1016/j.cell.2007.05.021

Natarajan, K., Meyer, M. R., Jackson, B. M., Slade, D., Roberts, C., Hinnebusch, A. G., & Marton, M. J. (2001). Transcriptional Profiling Shows that Gcn4p Is a Master Regulator of Gene Expression during Amino Acid

Starvation in Yeast. *Molecular and Cellular Biology*, *21*(13), 4347–4368. https://doi.org/10.1128/MCB.21.13.4347-4368.2001

Noda, N. N., Kumeta, H., Nakatogawa, H., Satoo, K., Adachi, W., Ishii, J., Fujioka, Y., Ohsumi, Y., & Inagaki, F. (2008). Structural basis of target recognition by Atg8/LC3 during selective autophagy. *Genes to Cells*, *13*(12), 1211–1218. https://doi.org/10.1111/j.1365-2443.2008.01238.x

Noda, T., & Ohsumi, Y. (1998). Tor, a Phosphatidylinositol Kinase Homologue, Controls Autophagy in Yeast. *Journal of Biological Chemistry*, *273*(7), 3963–3966. https://doi.org/10.1074/jbc.273.7.3963

Oie, S., Matsuzaki, K., Yokoyama, W., Tokunaga, S., Waku, T., Han, S.-I., Iwasaki, N., Mikogai, A., Yasuzawa-Tanaka, K., Kishimoto, H., Hiyoshi, H., Nakajima, Y., Araki, T., Kimura, K., Yanagisawa, J., & Murayama, A. (2014). Hepatic rRNA Transcription Regulates High-Fat-Diet-Induced Obesity. *Cell Reports*, *7*(3), 807–820. https://doi.org/10.1016/j.celrep.2014.03.038

Ossareh-Nazari, B., Bonizec, M., Cohen, M., Dokudovskaya, S., Delalande, F., Schaeffer, C., Van Dorsselaer, A., & Dargemont, C. (2010). Cdc48 and Ufd3, new partners of the ubiquitin protease Ubp3, are required for ribophagy. *EMBO Reports*, *11*(7), 548–554. https://doi.org/10.1038/embor.2010.74

Ouyang, L., Holland, P., Lu, H., Bergenholm, D., & Nielsen, J. (2018). Integrated analysis of the yeast NADPH-regulator Stb5 reveals distinct differences in NADPH requirements and regulation in different states of yeast

metabolism. *FEMS Yeast Research*, *18*(8). https://doi.org/10.1093/femsyr/foy091

Padilla, C. A., Bárcena, J. A., López-Grueso, M. J., & Requejo-Aguilar, R. (2019). The regulation of TORC1 pathway by the yeast chaperones Hsp31 is mediated by SFP1 and affects proteasomal activity. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1863*(3), 534–546. https://doi.org/10.1016/j.bbagen.2018.12.011

Parzych, K. R., Ariosa, A., Mari, M., & Klionsky, D. J. (2018). A newly characterized vacuolar serine carboxypeptidase, Atg42/Ybr139w, is required for normal vacuole function and the terminal steps of autophagy in the yeast *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*, *29*(9), 1089–1099. https://doi.org/10.1091/mbc.E17-08-0516

Parzych, K. R., & Klionsky, D. J. (2014). An Overview of Autophagy: Morphology, Mechanism, and Regulation. *Antioxidants & Redox Signaling*, *20*(3), 460–473. https://doi.org/10.1089/ars.2013.5371

Peifer, C., Sharma, S., Watzinger, P., Lamberth, S., Kötter, P., & Entian, K.-D. (2013). Yeast Rrp8p, a novel methyltransferase responsible for m1A 645 base modification of 25S rRNA. *Nucleic Acids Research*, *41*(2), 1151–1163. https://doi.org/10.1093/nar/gks1102

Pickart, C. M., & Eddins, M. J. (2004). Ubiquitin: Structures, functions, mechanisms. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, The Ubiquitin-Proteasome System*, *1695*(1), 55–72. https://doi.org/10.1016/j.bbamcr.2004.09.019

Powers, T., & Walter, P. (1999). Regulation of Ribosome Biogenesis by the Rapamycin-sensitive TOR-signaling Pathway in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*, *10*(4), 987–1000. https://doi.org/10.1091/mbc.10.4.987

Reggiori, F., & Klionsky, D. J. (2013a). Autophagic Processes in Yeast: Mechanism, Machinery and Regulation. *Genetics*, *194*(2), 341–361. https://doi.org/10.1534/genetics.112.149013

Reggiori, F., & Klionsky, D. J. (2013b). Autophagic Processes in Yeast: Mechanism, Machinery and Regulation. *Genetics*, *194*(2), 341–361. https://doi.org/10.1534/genetics.112.149013

Ribosome Biogenesis—An overview | ScienceDirect Topics. (2025). Retrieved January 4, 2025, from https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ribosome-biogenesis#

Robinson, J. S., Klionsky, D. J., Banta, L. M., & Emr, S. D. (1988). Protein Sorting in Saccharomyces cerevisiae: Isolation of Mutants Defective in the Delivery and Processing of Multiple Vacuolar Hydrolases. *MOL. CELL. BIOL.*, *8*.

Romanov, J., Walczak, M., Ibiricu, I., Schüchner, S., Ogris, E., Kraft, C., & Martens, S. (2012). Mechanism and functions of membrane binding by the Atg5–Atg12/Atg16 complex during autophagosome formation. *The EMBO Journal*, *31*(22), 4304–4317. https://doi.org/10.1038/emboj.2012.278

Rudra, D., Mallick, J., Zhao, Y., & Warner, J. R. (2007). Potential Interface between Ribosomal Protein Production and Pre-rRNA Processing. *Molecular and Cellular Biology*, *27*(13), 4815–4824. https://doi.org/10.1128/MCB.02062-06

Ryter, S. W., Cloonan, S. M., & Choi, A. M. K. (2013). Autophagy: A Critical Regulator of Cellular Metabolism and Homeostasis. *Molecules and Cells*, *36*(1), 7–16. https://doi.org/10.1007/s10059-013-0140-8

Sakoh-Nakatogawa, M., Matoba, K., Asai, E., Kirisako, H., Ishii, J., Noda, N. N., Inagaki, F., Nakatogawa, H., & Ohsumi, Y. (2013). Atg12–Atg5 conjugate enhances E2 activity of Atg3 by rearranging its catalytic site. *Nature Structural & Molecular Biology*, *20*(4), 433–439. https://doi.org/10.1038/nsmb.2527

Schilling, V., Peifer, C., Buchhaupt, M., Lamberth, S., Lioutikov, A., Rietschel, B., Kötter, P., & Entian, K. (2012). Genetic interactions of yeast *NEP1 (EMG1)* , encoding an essential factor in ribosome biogenesis. *Yeast*, *29*(5), 167–183. https://doi.org/10.1002/yea.2898

Sharma, S., Hartmann, J. D., Watzinger, P., Klepper, A., Peifer, C., Kötter, P., Lafontaine, D. L. J., & Entian, K.-D. (2018). A single N1-methyladenosine on the large ribosomal subunit rRNA impacts locally its structure and the translation of key metabolic enzymes. *Scientific Reports*, *8*(1), 11904. https://doi.org/10.1038/s41598-018-30383-z

Shi, F., Kawai, S., Mori, S., Kono, E., & Murata, K. (2005). Identification of ATP-NADH kinase isozymes and their contribution to supply of

NADP(H) in *Saccharomyces cerevisiae*. *The FEBS Journal*, *272*(13), 3337–3349. https://doi.org/10.1111/j.1742-4658.2005.04749.x

Shintani, T., Huang, W.-P., Stromhaug, P. E., & Klionsky, D. J. (2002). Mechanism of Cargo Selection in the Cytoplasm to Vacuole Targeting Pathway. *Developmental Cell*, *3*(6), 825–837. https://doi.org/10.1016/S1534-5807(02)00373-8

Shu, W.-J., Chen, R., Yin, Z.-H., Li, F., Zhang, H., & Du, H.-N. (2020). Rph1 coordinates transcription of ribosomal protein genes and ribosomal RNAs to control cell growth under nutrient stress conditions. *Nucleic Acids Research*, *48*(15), 8360–8373. https://doi.org/10.1093/nar/gkaa558

Suzuki, K. (2001). The pre-autophagosomal structure organized by concerted functions of APG genes is essential for autophagosome formation. *The EMBO Journal*, *20*(21), 5971–5981. https://doi.org/10.1093/emboj/20.21.5971

Tasmi, T. A., Solomon, E., Avogo, E. W., Badenahalli Narasimhaiah, S., & Delorme-Axford, E. (2026). The nonsense-mediated mRNA decay factor Upf3 negatively regulates bulk autophagy progression in *Saccharomyces cerevisiae*. *Autophagy Reports*, *5*(1), 2623730. https://doi.org/10.1080/27694127.2026.2623730

Teixeira, M. C., Monteiro, P. T., Palma, M., Costa, C., Godinho, C. P., Pais, P., Cavalheiro, M., Antunes, M., Lemos, A., Pedreira, T., & Sá-Correia, I. (2018). YEASTRACT: An upgraded database for the analysis of

transcription regulatory networks in Saccharomyces cerevisiae. *Nucleic Acids Research*, *46*(D1), D348–D353. https://doi.org/10.1093/nar/gkx842

Teste, M.-A., Duquenne, M., François, J. M., & Parrou, J.-L. (2009). Validation of reference genes for quantitative expression analysis by real-time RT-PCR in Saccharomyces cerevisiae. *BMC Molecular Biology*, *10*(1), 99. https://doi.org/10.1186/1471-2199-10-99

Tooze, S. A., & Dikic, I. (2016). Autophagy Captures the Nobel Prize. *Cell*, *167*(6), 1433–1435. https://doi.org/10.1016/j.cell.2016.11.023

Torggler, R., Papinski, D., & Kraft, C. (2017a). Assays to Monitor Autophagy in Saccharomyces cerevisiae. *Cells*, *6*(3), 23. https://doi.org/10.3390/cells6030023

Torggler, R., Papinski, D., & Kraft, C. (2017b). Assays to Monitor Autophagy in Saccharomyces cerevisiae. *Cells*, *6*(3), 23. https://doi.org/10.3390/cells6030023

Tsukada, M., & Ohsumi, Y. (1993). Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS letters, 333(1-2), 169–174. https://doi.org/10.1016/0014-5793(93)80398-e

Turi, Z., Lacey, M., Mistrik, M., & Moudry, P. (2019). Impaired ribosome biogenesis: Mechanisms and relevance to cancer and aging. Aging, 11(8), 2512–2540. https://doi.org/10.18632/aging.101922

Miyagi, H., Kawai, S., & Murata, K. (2009). Two sources of mitochondrial NADPH in the yeast Saccharomyces cerevisiae. The Journal of biological chemistry, 284(12), 7553–7560. https://doi.org/10.1074/jbc.M804100200

Ubiquitination—An overview | ScienceDirect Topics. (2021). Retrieved December 30, 2024, from https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ubiquitination

Waku, T., Nakajima, Y., Yokoyama, W., Nomura, N., Kako, K., Kobayashi, A., Shimizu, T., & Fukamizu, A. (2016). NML-mediated rRNA base methylation links ribosomal subunit formation to cell proliferation in a p53-dependent manner. *Journal of Cell Science*, *129*(12), 2382–2393. https://doi.org/10.1242/jcs.183723

Wang, J., & Chen, G. (2024). Engineering *Saccharomyces cerevisiae* for the Production of Punicic Acid-Rich Yeast Biomass. *Journal of Agricultural and Food Chemistry*, *72*(43), 23917–23927. https://doi.org/10.1021/acs.jafc.4c08252

Wang, L., Klionsky, D. J., & Shen, H.-M. (2023). The emerging mechanisms and functions of microautophagy. *Nature Reviews Molecular Cell Biology*, *24*(3), 186–203. https://doi.org/10.1038/s41580-022-00529-z

Warner J. R. (1999). The economics of ribosome biosynthesis in yeast. *Trends in biochemical sciences*, *24*(11), 437–440. https://doi.org/10.1016/s0968-0004(99)01460-7

Wen, X., Gatica, D., Yin, Z., Hu, Z., Dengjel, J., & Klionsky, D. J. (2020). The transcription factor Spt4-Spt5 complex regulates the expression of *ATG8* and *ATG41*. *Autophagy*, *16*(7), 1172–1185. https://doi.org/10.1080/15548627.2019.1659573

Wen, X., & Klionsky, D. J. (2016). An overview of macroautophagy in yeast. *Journal of Molecular Biology*, *428*(9), 1681–1699. https://doi.org/10.1016/j.jmb.2016.02.021

Wu, J., Jiang, X., Li, Y., Zhu, T., Zhang, J., Zhang, Z., Zhang, L., Zhang, Y., Wang, Y., Zou, X., & Liang, B. (2018). PHA-4/FoxA senses nucleolar stress to regulate lipid accumulation in Caenorhabditis elegans. *Nature Communications*, *9*(1), 1195. https://doi.org/10.1038/s41467-018-03531-2

Wullschleger, S., Loewith, R., & Hall, M. N. (2006). TOR Signaling in Growth and Metabolism. *Cell*, *124*(3), 471–484. https://doi.org/10.1016/j.cell.2006.01.016

Xiao, W., Wang, R.-S., Handy, D. E., & Loscalzo, J. (2018). NAD(H) and NADP(H) Redox Couples and Cellular Energy Metabolism. *Antioxidants & Redox Signaling*, *28*(3), 251–272. https://doi.org/10.1089/ars.2017.7216

Xie, Z. (Ed.). (2021). *Autophagy: Biology and Diseases: Technology and Methodology* (Vol. 1208). Springer Singapore. https://doi.org/10.1007/978-981-16-2830-6

Xie, Z., Nair, U., & Klionsky, D. J. (2008). Atg8 Controls Phagophore Expansion during Autophagosome Formation. *Molecular Biology of the Cell*, *19*(8), 3290–3298. https://doi.org/10.1091/mbc.e07-12-1292

Yang, L., Song, T., Chen, L., Kabra, N., Zheng, H., Koomen, J., Seto, E., & Chen, J. (2013). Regulation of SirT1-Nucleomethylin Binding by rRNA Coordinates Ribosome Biogenesis with Nutrient Availability. *Molecular*

and Cellular Biology, *33*(19), 3835–3848. https://doi.org/10.1128/MCB.00476-13

Yang, Z., & Klionsky, D. J. (2010). Eaten alive: A history of macroautophagy. *Nature Cell Biology*, *12*(9), 814–822. https://doi.org/10.1038/ncb0910-814

Yao, Z., Delorme-Axford, E., Backues, S. K., & Klionsky, D. J. (2015). Atg41/Icy2 regulates autophagosome formation. *Autophagy*, *11*(12), 2288–2299. https://doi.org/10.1080/15548627.2015.1107692

Ye, M., Chen, Y., Liu, Z., Wang, Y., & Yi, C. (2024). Detection of ribophagy in yeast and mammals. *Biophysics Reports*, *10*(2), 82. https://doi.org/10.52601/bpr.2024.240002

Yeh, Y.-Y., Shah, K. H., & Herman, P. K. (2011). An Atg13 Protein-mediated Self-association of the Atg1 Protein Kinase Is Important for the Induction of Autophagy. *Journal of Biological Chemistry*, *286*(33), 28931–28939. https://doi.org/10.1074/jbc.M111.250324

Yin, Z., Zhang, Z., Lei, Y., & Klionsky, D. J. (2023). Bidirectional roles of the Ccr4-Not complex in regulating autophagy before and after nitrogen starvation. *Autophagy*, *19*(2), 415–425. https://doi.org/10.1080/15548627.2022.2036476

Yu, L., Chen, Y., & Tooze, S. A. (2017). Autophagy pathway: Cellular and molecular mechanisms. *Autophagy*, *14*(2), 207–215. https://doi.org/10.1080/15548627.2017.1378838

Zhu, Y., Liu, F., Jian, F., & Rong, Y. (2024). Recent progresses in the late stages of autophagy. *Cell Insight*, *3*(2), 100152. https://doi.org/10.1016/j.cellin.2024.100152