

Temporal Regulation of Autophagy- and Mitophagy-related Genes in *Saccharomyces cerevisiae* under Gradual Glucose Restriction

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ABSTRACT

Nutrient availability is widely recognized as a critical regulator of cellular homeostasis and metabolic adaptation. In this study, we investigated the transcriptional dynamics of autophagy- and mitophagy-related genes in *Saccharomyces cerevisiae* under graded glucose restriction (1%, 0.5%, and 0.2%), using a 2% glucose environment as the control. Growth kinetics, metabolic vitality, and qualitative survival profiles were systematically evaluated on days 5 and 7, corresponding to critical chronological transition phases. Additionally, relative mRNA expression levels of *ATG8*, *ATG11*, and *ATG32* were quantified via qRT-PCR. On day 5, a pronounced, time-dependent metabolic surge was observed through MTT assays across all restricted environments, correlating with enhanced colony-forming efficiency in spotting assays. Concurrently, transcriptional analyses revealed that severe glucose restriction (0.2%) induced significant up-regulation of the mitophagy receptor *ATG32* and the scaffold protein *ATG11*. Furthermore, a substantial down-regulation of the core autophagy marker *ATG8* was simultaneously detected across all restricted groups, suggesting that decreased *ATG8* expression may reflect an overall reduction in general autophagic activity, while the concurrent induction of *ATG32* and *ATG11* indicates a localized retention or activation of selective mitochondrial quality control under extreme restriction. This selective transcriptional response suggests that *S. cerevisiae* possesses an acute, temporary adaptive capacity. By day 7, however, metabolic activity and transcriptional profiles had returned toward a baseline maintenance levels, with expression differences becoming statistically comparable to the control. Notably, overall cellular viability was successfully preserved throughout these nutritional adjustments. Consequently, this study demonstrates that *S. cerevisiae* has an acute, temporary adaptive capacity, whereby its autophagic machinery can be dynamically recalibrated to optimize energy expenditure and selectively manage organelle quality under prolonged nutritional stress.

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Introduction

Yeast, *Saccharomyces cerevisiae*, has long been used as a model organism in aging research, in which lifespan is classically assessed by replicative or chronological lifespan assays. The present study does not directly measure lifespan, and instead focuses on cellular and transcriptional

responses to graded glucose restriction (Longo, 2019; Mortimer and Johnston, 1959; Schulze *et al.*, 2024). In yeast culture, glucose serves as the primary carbon source and was experimentally modulated in this study to assess cellular responses under different nutritional conditions. All experiments were performed in YPD-based



media with defined glucose concentrations (Schwartz and Puhl, 2003).

High glucose availability alters yeast metabolism and has been associated with cellular stress responses. In this study, calorie restriction refers to a controlled reduction in glucose concentration to examine how nutrient availability modulates cellular and transcriptional responses. More precisely, we focus on changes in the expression of autophagy-related genes as indicators of metabolic adaptation under glucose-limited conditions (Kohli and Roth, 2010).

Among its specialized forms, mitophagy selectively targets damaged or dysfunctional mitochondria. In *S. cerevisiae*, impaired mitochondria are known to influence cellular metabolic balance and stress responses. Several autophagy-related genes, including *ATG32* as a mitophagy receptor and *ATG11* as an adaptor protein, are required for the selective recognition of mitochondria, while *ATG8* participates more broadly in autophagosome formation. Changes in the expression of these genes may therefore reflect alterations in mitochondrial quality control under different metabolic conditions (Camougrand *et al.*, 2020; Hoshyar-Chamanarae and Moghaddam, 2024; Innokentev and Kanki, 2021; Osellame *et al.*, 2012).

In complex multicellular systems, excessive caloric intake has been associated with metabolic imbalance and adverse health outcomes, as reported in epidemiological and clinical studies. Such observations highlight the importance of understanding how nutrient availability influences cellular metabolism. However, these conditions arise from multifactorial processes at the organismal level. Therefore, simplified eukaryotic models, such as *S. cerevisiae*, offer a controlled framework to investigate cellular responses to defined changes in nutrient availability without addressing disease-related outcomes (Boutelle *et al.*, 2014; Hossain *et al.*, 2024; Vasile *et al.*, 2023).

In higher eukaryotic systems, altered glucose homeostasis has been associated with a range of physiological and pathological conditions, as reported in previous studies. However, such associations arise from complex, multicellular contexts. In contrast, the present study uses *S. cerevisiae* as a simplified experimental model to examine how defined changes in glucose

availability influence cellular responses at the transcriptional level, without addressing organismal aging or disease outcomes (Madhusudhanan *et al.*, 2020).

These observations highlight the importance of understanding how changes in nutrient availability influence cellular processes. *S. cerevisiae* provides a well-established and experimentally tractable eukaryotic model to investigate cellular responses to defined nutritional conditions, owing to its genetic accessibility and suitability for controlled molecular analyses (Engel *et al.*, 2014; Jarolim *et al.*, 2004). This study examines how controlled reduction of glucose availability influences the transcriptional expression of selected autophagy- and mitophagy-related genes in *S. cerevisiae*. By focusing on defined molecular readouts under regulated nutritional conditions, this work aims to provide a cellular-level framework for future studies investigating nutrient-responsive pathways in eukaryotic systems, without addressing organismal lifespan or disease-related outcomes.

Materials and Methods

Yeast growth curve

S. cerevisiae strain ATCC 9763 was utilized throughout this study. The primary culture medium was Yeast Extract-Peptone-Dextrose (YPD). The standard control medium was formulated with 2% glucose, 2% peptone, and 1% yeast extract, while experimental media were adjusted to glucose concentrations of 1%, 0.5%, and 0.2% to investigate the effects of nutrient restriction without altering other components. For solid cultures, 1.5% to 2% agar was incorporated (Hill *et al.*, 2024).

The initial cell density across four different glucose environments was standardized using OD measurements at 600 nm. Yeast cultures were grown in sterilized vessels and incubated in a shaker incubator at a constant temperature of 25°C with continuous orbital shaking at 180 rpm. To monitor growth dynamics, the absorbance at 600 nm was measured at regular 24-hour intervals over a 16-day period. To maintain experimental consistency and minimize the risk of cell clumping or sedimentation, all samples were serially diluted (up to 10⁻⁶) in sterile media before

OD600 recording and downstream assays. This normalization procedure ensured that changes in optical density were accurately reflected and solely attributable to *S. cerevisiae* growth kinetics, while visual inspections confirmed the absence of microbial contamination throughout the study (Olivares-Marin *et al.*, 2018).

Monitoring of growth kinetics continued for 16 days to ensure the cultures reached and maintained the stationary phase. Based on the generated growth curves, the 5th and 7th days were selected for subsequent physiological and transcriptional analyses. These time points represent critical stages in the yeast chronological transition, allowing for the assessment of cellular adaptation to prolonged glucose restriction (Parrella and Longo, 2008).

MTT assay

To assess the metabolic vitality of *S. cerevisiae* under varying glucose concentrations, an MTT assay was performed on days 5 and 7. The cell density for each treatment group was standardized using a hemocytometer and an optical microscope to ensure a consistent initial population of 10^6 cells per well. Each treatment was analyzed in triplicate to ensure reproducibility. The cells were suspended in 100 μ l of the specific culture media, followed by the addition of 10 μ l of MTT solution (5 mg/ml). The plates were incubated at 25°C for 6 hours under dark conditions to prevent reagent photodegradation. After incubation, the plates were centrifuged at 800 rpm for 10 minutes, and the supernatant was carefully discarded. To solubilize the resulting formazan crystals, 100 μ l of dimethyl sulfoxide (DMSO) was added to each well. After a 5-minute stabilization period, the absorbance was measured at 570 nm using a microplate reader (Biotek Power Wave XS2). The results were expressed as a percentage of cellular metabolic activity relative to the control. Data processing and statistical analysis were performed using GraphPad Prism software.

Spotting Assay

To evaluate the re-growth capacity and qualitative survival of cells, a spotting assay was performed using cultures harvested on days 5 and 7. The cells, which had been previously cultivated in their respective liquid media, were prepared for transition to solid YPD agar plates. These plates

were formulated with 2% agar and adjusted to four distinct glucose concentrations (2%, 1%, 0.5%, and 0.2%).

To ensure experimental consistency and reliable comparison between treatments, 1 ml of each culture was extracted and subjected to a serial dilution process. Based on preliminary optimization tests to ensure the robustness of the results, a final dilution of 10^{-6} was selected. This high dilution factor was essential to achieve a normalized cell density across all groups and to allow for the observation of distinct re-growth patterns without excessive cell clumping.

For the spotting procedure, a custom-designed template and a physical stencil were utilized for the 10-cm plates to ensure the precise and reproducible positioning of each sample. Using a high-precision sampler, exactly 5 μ l of each normalized treatment was carefully extracted and spotted onto the designated locations on the agar surface (Fig. 1). To minimize potential technical errors, five technical replicates were performed for each treatment across all glucose environments. The plates were then incubated at a constant temperature of 25°C. Following an optimized incubation period of 18 hours—which allowed for the initial capture of colony formation—digital images were recorded to document the presence or absence of re-growth. Although the visual presentation was intended for qualitative confirmation, the consistent growth patterns observed across five replicates provided sufficient evidence for the comparative analysis of yeast survival under glucose restriction.

qRT-PCR assay

Quantitative real-time PCR (qRT-PCR) was employed to precisely quantify the relative expression levels of *ATG32*, *ATG11*, and *ATG8* under different glucose concentrations on days 5 and 7. *ACT1* was utilized as the internal reference (housekeeping) gene for normalization.

Specific primers for the target genes were designed using Gene Runner, NCBI Primer-BLAST, and PerlPrimer. Design parameters were strictly controlled, including a primer length of 18-24 nucleotides, a melting temperature (T_m) between 55 and 60°C (with a maximum difference of 2°C between pairs), and an amplicon length of 100- 200 bp. To ensure optimal binding and prevent non-specific interactions or

secondary structures (such as hairpins and dimers), the GC content was maintained between 40- 55%. Primer specificity was verified via NCBI Primer-BLAST prior to synthesis by Sinaclon.

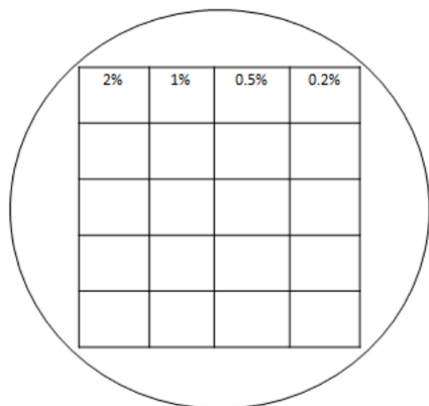


Fig. 1. Spot positioning stencil: The stencil shown in the picture was designed by the authors to ensure the correct placement of the spots and was placed behind the pages.

Total RNA was extracted using the RNX-PLUS kit (Sinaclon, Iran) according to the manufacturer's instructions. To ensure the

integrity of the transcriptional data, all extraction procedures were conducted under RNase-free conditions. The concentration and purity of the extracted RNA were evaluated using a NanoDrop 1000 spectrophotometer (Thermo Scientific). To eliminate any potential genomic DNA (gDNA) contamination, samples were treated with DNase I (Sinaclon, Iran). Post-treatment, RNA samples were re-evaluated via NanoDrop to confirm the absence of impurities and the preservation of RNA integrity.

Subsequently, cDNA synthesis was performed using a specialized kit for yeast cells (Denazist, Iran). The resulting cDNA was quantified and then subjected to qRT-PCR using the SYBR Green Master Mix (Parstous, Iran). Reactions were carried out in a Rotor-Gene 3000 thermal cycler (Corbett Research). Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method across three biological replicates, with the 2% glucose group serving as the calibrator (control= 1.0). Prior to statistical evaluation, data normality assumptions were verified. Group differences were analyzed using one-way ANOVA followed by Dunnett's test, and data were visualized using GraphPad Prism software.

Table 1. Primer specifications for *S. cerevisiae*.

Gene name	Oligomer sequences (5'→3')	Length (bp)	Tm (°C)	Position
ATG32	F: 5'- GATGATGGTGATGAAGATGAG	21	56.38	528
	R: 5'- TGTGTTAGTGATAGTTTGGG	20	55.14	634
ATG11	F: 5'- CCAGCAAGAAATAACACCA	19	55.83	35
	R: 5'- CATAGGTAGTAGAATCAGCAG	22	56	204
ATG8	F: 5'- GTTCCTGCTGACCTTACC	18	57.40	150
	R: 5'- ACCCGTCCTTATCCTTGTG	19	59.17	309
ACT1	F: 5'- GTTACTCTTCTCCACCAC	19	55.27	589
	R: 5'- GCTCTGAATCTTTCGTTACC	20	56.7	769

Results

Yeast lifespan curves

The growth dynamics of *S. cerevisiae* were monitored across four glucose concentrations, with the 2% glucose environment serving as the control. Spectrophotometric analysis revealed that all cultures exhibited a rapid exponential growth phase during the first 72 hours. Starting from the 3rd day, a distinct transition from the exponential to the stationary phase was observed across all media. In the control group (2% glucose), this transition was marked by a

significant reduction in growth rate between days 3 and 5 (Fig. 2).

In contrast, while the 1%, 0.5%, and 0.2% glucose groups also showed a decrease in growth kinetics after the 3rd day, the slope of this decline was markedly more gradual compared to the control. By the 5th day, the growth curves of the glucose-restricted groups maintained a higher relative density compared to the sharper decline observed in the 2% environment. By the 7th day, the growth profiles of all treatment groups converged, reaching a plateau where optical density measurements became statistically comparable across all environments (Fig. 3).

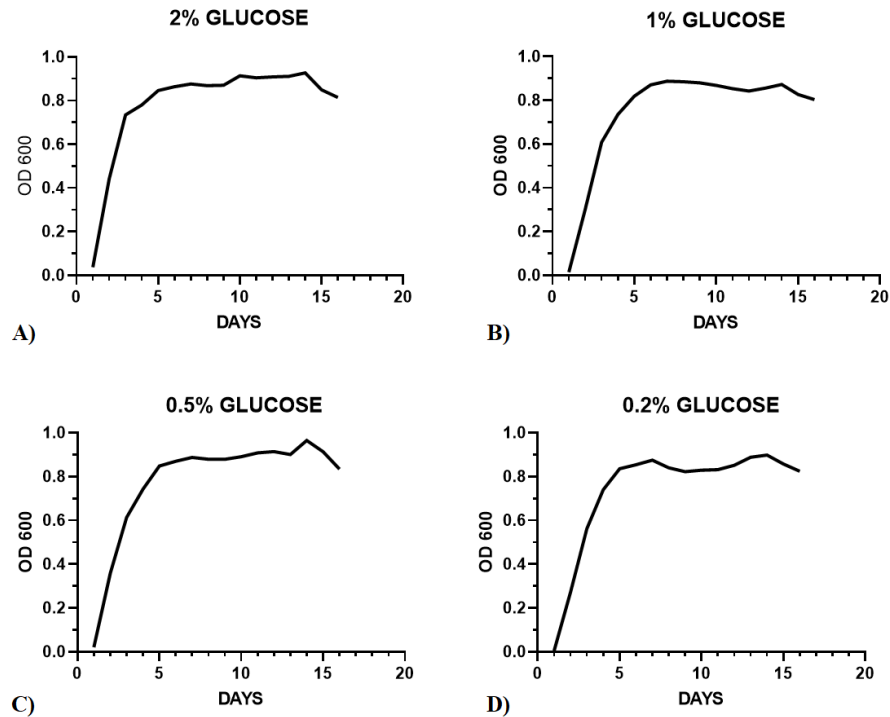


Fig. 2. Yeast growth kinetics under different glucose concentrations: A) 2% glucose; B) 1% glucose; C) 0.5% glucose; D) 0.2% glucose. Growth kinetics of *S. cerevisiae* were monitored over 16 days based on optical density (OD_{600 nm}) measurements.

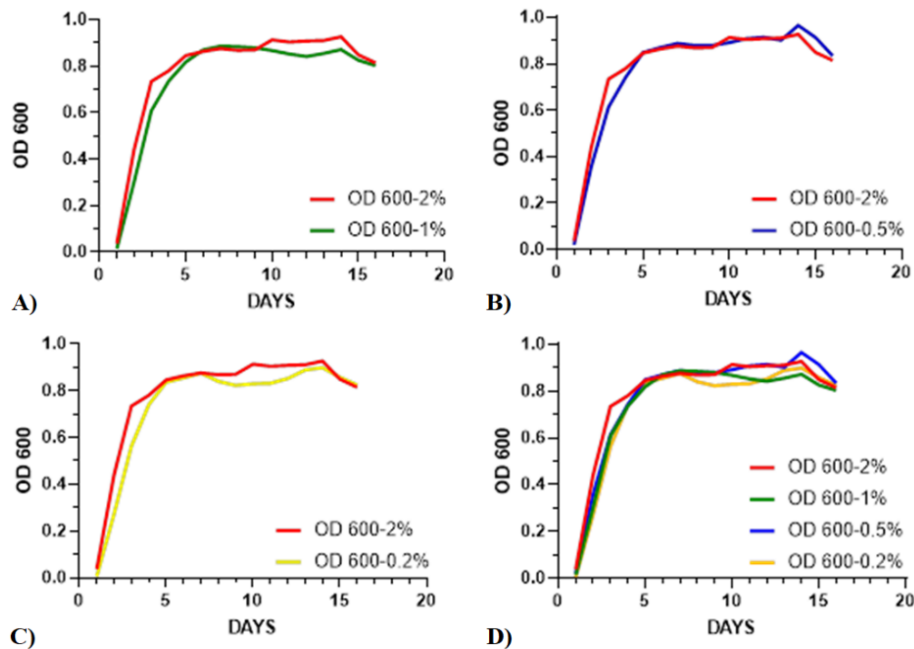


Fig. 3. Glucose restriction growth curves illustrating the transition from exponential to stationary phase: A) Comparison of control (OD_{600 nm} -2%, red) and 1% glucose restriction (green); B) Comparison of control (OD_{600 nm} -2%, red) and 0.5% glucose restriction (blue); C) Comparison of control (OD_{600 nm} -2%, red) and 0.2% glucose restriction (yellow); D) Combined curves showing a notable divergence from day 3 to day 5 where restricted groups exhibit a more gradual decline in growth rate, indicating an altered metabolic transition in response to caloric restriction.

MTT assay

Mitochondrial function was assessed using the MTT assay, in which cellular metabolic activity reflects the enzymatic capacity of mitochondrial dehydrogenases to convert MTT into formazan. On the fifth day of cultivation, cells grown under glucose-restricted conditions (1%, 0.5%, and 0.2%) displayed a marked elevation in cellular metabolic activity compared to the 2% glucose control (Fig. 4). The most prominent increase was observed in the 1% glucose group ($p = 0.0098$), followed by significant increases in the 0.5% ($p =$

0.0385) and 0.2% ($p = 0.0462$) groups. This suggests that moderate to extreme glucose restriction initially stimulates mitochondrial efficiency or biogenesis as cells enter the stationary phase.

By the seventh day, this metabolic surge reached a plateau. The activity levels across all restricted groups declined and became statistically comparable to the control. This transition indicates that the initial mitochondrial stimulation observed on day 5 is a transient adaptive response that stabilizes as the culture matures toward the late stationary phase.

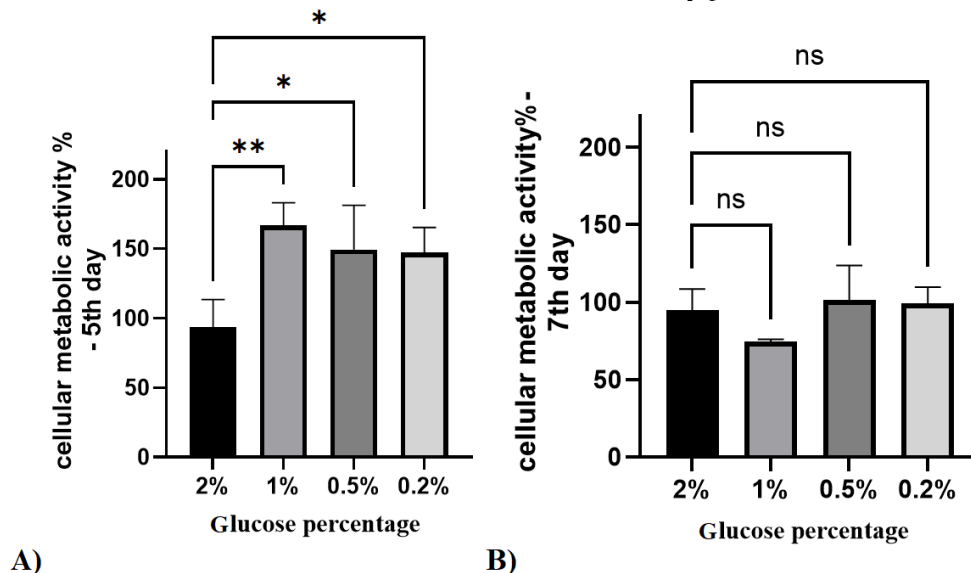


Fig. 4. Metabolic activity under different glucose concentrations: A) Cellular metabolic activity on the 5th day assessed using the MTT assay; B) Cellular metabolic activity on the 7th day. Data were analyzed via One-way ANOVA followed by Dunnett's multiple comparisons test. Error bars represent the standard error of the mean (SEM). Asterisks indicate significant differences compared to the control (* $p < 0.05$, ** $p < 0.01$). N/A: Not applicable.

Spotting assay

The spotting assay provided a qualitative evaluation of the re-growth capacity and survival resilience of *S. cerevisiae* following exposure to varying glucose concentrations. On the fifth day of cultivation, cells previously grown in the 2% glucose control medium displayed a diminished re-growth capacity when transferred to enriched YPDA plates. In contrast, cells harvested from glucose-restricted environments (1%, 0.5%, and 0.2%) exhibited enhanced re-growth potential. Among the restricted groups, the 1% glucose environment yielded the most robust colony formation, suggesting that moderate caloric restriction preserves cellular viability during the mid-stationary phase (Fig. 5).

By the seventh day, a distinct shift in growth patterns was observed. The 2% glucose group, which had shown limited re-growth on day 5, exhibited a relative recovery in its ability to re-establish colonies. However, cells from the 1% glucose-restricted environment visually displayed a consistently denser colony-forming pattern, qualitatively reflecting enhanced survival compared to all other groups (Fig. 6). These results indicate that while standard glucose levels (2%) lead to an earlier decline in immediate re-growth potential, glucose restriction—particularly at the 1% level—effectively maintains higher qualitative survival and cellular resilience during the late stationary phase.

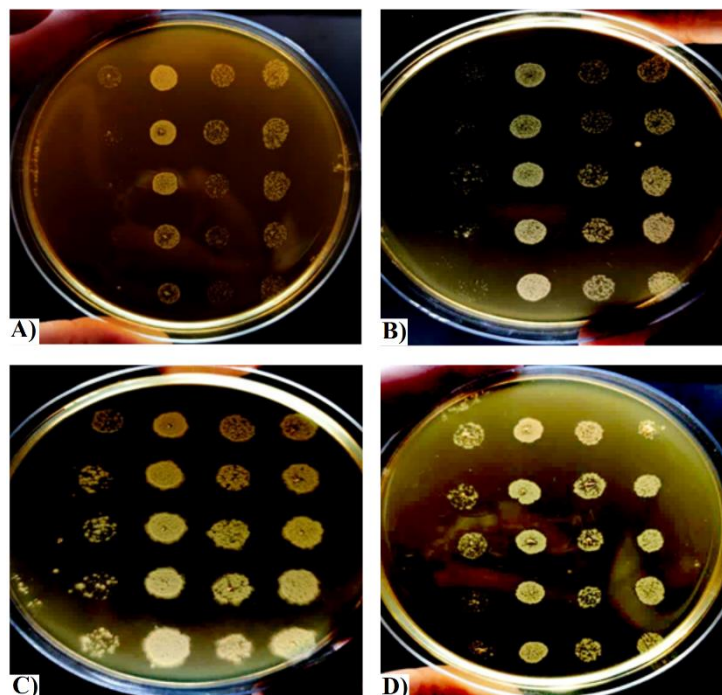


Fig. 5. Qualitative regrowth capacity of *S. cerevisiae* on different YPD agar plates by spotting assay on day 5: A) Plate with 2% glucose agar (Control); B) Plate with 1% glucose agar; C) Plate with 0.5% glucose agar; D) Plate with 0.2% glucose agar. Each spot reflects the colony-forming ability of the normalized 10^{-6} dilution, with columns corresponding to cells previously grown in 2%, 1%, 0.5%, and 0.2% glucose liquid media, respectively.

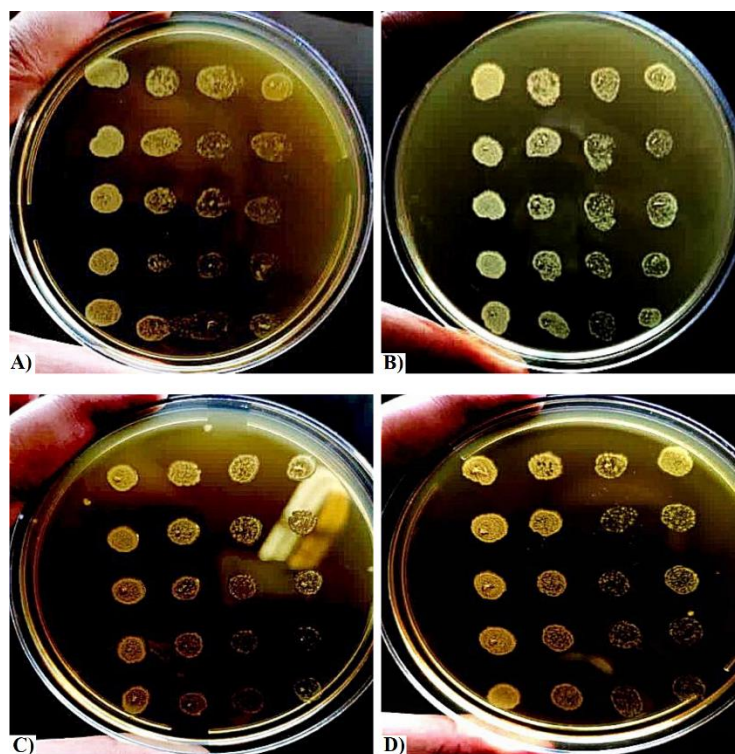


Fig. 6. Qualitative regrowth capacity of *S. cerevisiae* on different YPD agar plates by spotting assay on day 7: A) Plate with 2% glucose agar (Control); B) Plate with 1% glucose agar; C) Plate with 0.5% glucose agar; D) Plate with 0.2% glucose agar. Each spot reflects the colony-forming ability of the normalized 10^{-6} dilution, with columns corresponding to cells previously grown in 2%, 1%, 0.5%, and 0.2% glucose liquid media, respectively.

qRT-PCR assay

The transcriptional expression of the mitophagy-specific receptors (*ATG32* and *ATG11*) and the core autophagy marker (*ATG8*) was analyzed to identify the molecular shifts under various glucose restriction regimes. On the fifth day of cultivation, mRNA expression levels revealed a distinct regulatory response to nutrient limitation. Under extreme glucose restriction (0.2%), a significant up-regulation was observed for both *ATG32* ($p < 0.05$) and *ATG11* ($p < 0.0001$) compared to the 2% glucose control. This suggests an initial activation of the mitophagy machinery when glucose availability is critically low. In contrast, moderate restriction (1% and 0.5%) resulted in a significant down-regulation of *ATG32* and *ATG11*, or showed no significant difference. Regarding *ATG8*, a significant decrease in expression was observed across all restricted groups (1%, 0.5%, and 0.2%) compared to the control ($p < 0.001$), suggesting that decreased *ATG8* expression may reflect an overall reduction in general autophagic activity, while the concurrent induction of *ATG32* and *ATG11* points to a localized retention or activation of selective mitochondrial quality control under extreme restriction (Fig. 7).

By the seventh day, the expression profiles exhibited gene-specific patterns rather than a

uniform down-regulation. While *ATG32* and *ATG11* remained significantly reduced, *ATG8* expression in the 0.5% and 0.2% groups returned to control levels. *ATG32* expression was significantly reduced in all restricted environments (1%, 0.5%, and 0.2%) compared to the control ($p < 0.0001$), with the lowest expression noted in the 0.2% glucose group. Similarly, *ATG11* levels were significantly lower in the 0.5% and 0.2% groups ($p < 0.01$). While *ATG8* expression remained significantly lower in the 1% group ($p < 0.01$), it showed a relative stabilization in the 0.5% and 0.2% groups, becoming statistically comparable to the control. This overall decline in transcriptional activity suggests that the initial molecular response observed on day 5 settles into a basal maintenance state as the yeast culture matures in the late stationary phase (Fig. 8).

Discussion

Nutrient availability is a key regulator of cellular homeostasis and metabolic adaptation. In *S. cerevisiae*, controlled reduction of glucose provides a robust framework to investigate how eukaryotic cells adjust their metabolic priorities and organelle quality control mechanisms under stress.

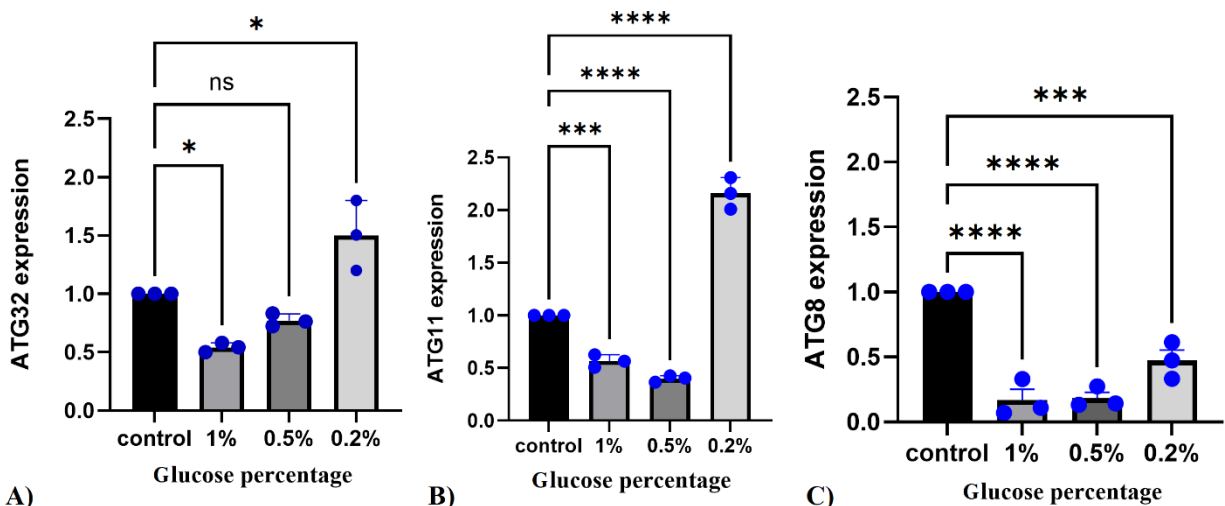


Fig. 7. Relative mRNA expression levels of autophagy- and mitophagy-related genes on day 5: A) *ATG32* expression; B) *ATG11* expression; C) *ATG8* expression. All data were normalized to the housekeeping gene *ACT1* and calculated using the $2^{-\Delta\Delta CT}$ method, with the 2% glucose group serving as the calibrator (control= 1.0). Data were analyzed via One-way ANOVA followed by Dunnett's multiple comparisons test. Error bars represent the standard error of the mean (SEM). Asterisks indicate significant differences compared to the control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$). N/A: Not applicable.

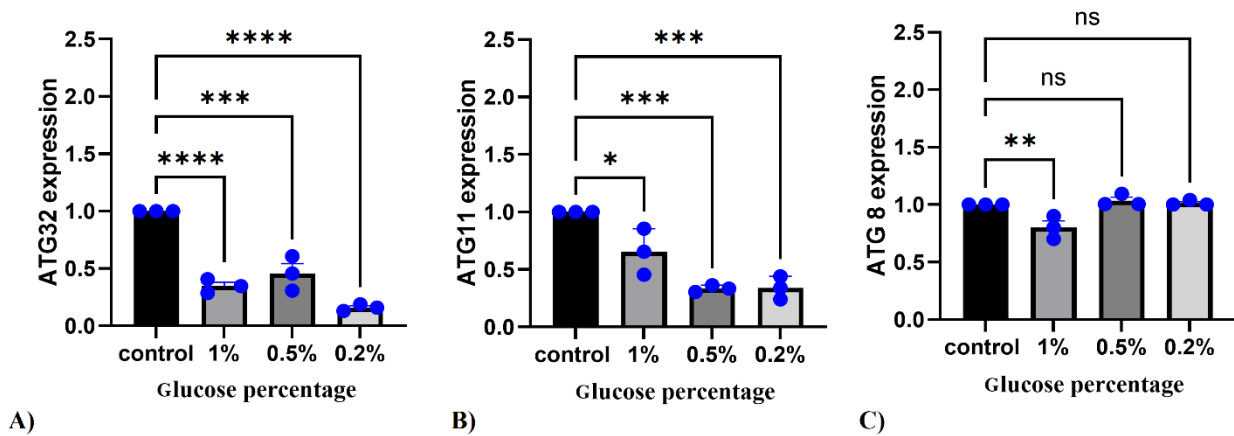


Fig. 8. Relative mRNA expression levels of autophagy- and mitophagy-related genes on day 7: A) *ATG32* expression; B) *ATG11* expression; C) *ATG8* expression. All data were normalized to the housekeeping gene *ACT1* and calculated using the $2^{-\Delta\Delta CT}$ method, with the 2% glucose group serving as the calibrator (control= 1.0). Data were analyzed via One-way ANOVA followed by Dunnett's multiple comparisons test. Error bars represent the standard error of the mean (SEM). Asterisks indicate significant differences compared to the control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$). N/A: Not applicable.

Table 2. Relative expression of *ATG* genes under glucose restriction.

Days	Target genes	Glucose group	Relative expression	Standard Error	p-value (vs. Control)	Significance
Day 5	<i>ATG32</i>	2% (Control)	1	N/A	-	-
		1%	0.541	± 0.125	0.0162	*
		0.50%	0.772	± 0.125	0.2387	ns
		0.20%	1.502	± 0.125	0.0101	*
	<i>ATG11</i>	2% (Control)	1	N/A	-	-
		1%	0.566	± 0.067	0.0005	****
		0.50%	0.398	± 0.067	< 0.0001	****
		0.20%	2.158	± 0.067	< 0.0001	****
	<i>ATG8</i>	2% (Control)	1	N/A	-	-
		1%	0.169	± 0.086	< 0.0001	****
		0.50%	0.183	± 0.086	< 0.0001	****
		0.20%	0.473	± 0.086	0.0008	***
Day 7	<i>ATG32</i>	2% (Control)	1	N/A	-	-
		1%	0.346	± 0.067	< 0.0001	****
		0.50%	0.456	± 0.067	0.0001	****
		0.20%	0.159	± 0.067	< 0.0001	****
	<i>ATG11</i>	2% (Control)	1	N/A	-	-
		1%	0.655	± 0.092	0.0143	*
		0.50%	0.334	± 0.092	0.0002	****
		0.20%	0.341	± 0.092	0.0003	****
	<i>ATG8</i>	2% (Control)	1	N/A	-	-
		1%	0.801	± 0.046	0.0072	**
		0.50%	1.035	± 0.046	0.7968	ns
		0.20%	1.014	± 0.046	0.9811	ns

Data represent relative fold change (the $2^{-\Delta\Delta CT}$) on days 5 and 7 of growth. Values are expressed as Mean $\pm$ SEM (n= 3 biological replicates). Statistical analysis was performed using One-way ANOVA followed by Dunnett's multiple comparisons test. The 2% glucose group served as the calibrator (control= 1.000). Asterisks indicate significant differences compared to the control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). N/A: Not Applicable.

Autophagy in *S. cerevisiae* operates through two primary modalities: non-selective (bulk) macro autophagy and cargo-specific (selective) autophagy. Bulk autophagy mediates the random sequestration of cytoplasmic constituents for recycling during nutrient deprivation, whereas selective autophagy pathways provide targeted quality control, such as the elimination of

damaged mitochondria (mitophagy). Within this framework, Atg8 serves as an essential structural component for general autophagosome formation, Atg32 functions specifically as a mitophagy receptor, and Atg11 acts as a crucial scaffold protein for selective cargo recognition. Consequently, alterations in *ATG8* expression serve as a reliable proxy for global autophagic

capacity, whereas transcriptional shifts in *ATG32* and *ATG11* provide mechanistic insights into the activation of selective degradation pathways.

Under nutrient-limited conditions, cells often undergo structural and functional adjustments that are associated with aging-related phenotypes, such as altered cell size, accumulation of metabolic by-products, increased reactive oxygen species (ROS), and reduced mitochondrial efficiency. These changes are not considered direct causes of aging but are commonly observed features accompanying cellular aging (Mirisola and Longo, 2022; Pignatti *et al.*, 2020; Sampaio-Marques *et al.*, 2014; Swinnen *et al.*, 2014).

In *S. cerevisiae*, cellular aging is defined by measurable physiological parameters, including reduced proliferative capacity, metabolic imbalance, and altered stress responses. Intracellular nutrient-sensing pathways play a central role in coordinating these processes. Therefore, the experimental modulation of nutrient availability provides a robust framework for investigating how cellular systems regulate aging-associated pathways to maintain cellular homeostasis (Arlia-Ciommo *et al.*, 2014; Kyryakov *et al.*, 2012; Longo and Fabrizio, 2012; McCleary and Rine, 2017).

In yeast, cellular aging is commonly characterized using two well-established models: replicative lifespan and chronological lifespan. Replicative aging refers to the limited number of divisions a single cell can undergo, while chronological aging describes the survival capacity of non-dividing cells over time. Both models are associated with measurable physiological changes, including reduced proliferative potential, metabolic imbalance, altered stress resistance, and progressive decline in organelle function. In addition, aging in yeast is accompanied by changes in the expression of genes involved in cellular maintenance processes, specifically those related to autophagy (Burtner *et al.*, 2009; Longo *et al.*, 2012; Mirisola and Longo, 2022; Sampaio-Marques *et al.*, 2014). Although the present investigation does not empirically record longevity kinetics or chronological lifespan extension, quantifying the transcriptional response of core autophagic machinery under glucose restriction provides a functional proxy for early cellular preservation. Consequently, citing established aging models serves exclusively to

contextualize the organelle quality control processes that underpin cellular homeostasis under nutritional stress.

Crucially, chronological senescence is not a strictly immutable process at the single-cell level. Instead, several evolutionarily conserved mechanisms have been shown to delay or attenuate aging-associated phenotypes. These mechanisms include the preservation of genomic stability, maintenance of mitochondrial reticular function, and activation of autophagy-mediated quality control pathways. Autophagy plays a central role in removing damaged proteins and organelles, thereby supporting cellular homeostasis under stress or nutrient-limited conditions. Rather than reversing aging, these processes contribute to slowing the progression of functional decline and promoting cellular adaptation to environmental changes (Aris *et al.*, 2013; Delaney *et al.*, 2013; Sampaio-Marques *et al.*, 2011).

Autophagy in *S. cerevisiae* operates through two primary modalities: non-selective (bulk) macro autophagy and cargo-specific (selective) autophagy. Bulk autophagy mediates the random sequestration of cytoplasmic constituents, including cytosolic proteins and redundant organelles, into the vacuole for degradation and recycling, primarily serving as a survival mechanism during nutrient deprivation. Conversely, selective autophagy pathways provide a targeted quality control mechanism, precisely eliminating damaged mitochondria (mitophagy), peroxisomes (pexophagy), or aberrant protein aggregates. Within this regulatory framework, certain autophagy-related (*ATG*) genes serve as functional indicators of these distinct pathways: Atg8 is an essential structural component required for autophagosome formation in both bulk and selective processes; Atg32 functions specifically as a mitophagy receptor to target mitochondria; and Atg11 acts as a critical scaffold protein explicitly required for selective cargo recognition rather than bulk autophagic degradation (Klionsky *et al.*, 2021; Mizushima and Komatsu, 2011). Within this regulatory hierarchy, evaluating the expression patterns of these specific markers provides targeted mechanistic insight into how *S. cerevisiae* orchestrates organelle maintenance prior to the onset of metabolic decline, linking

transcriptional dynamics to the broader principles of cellular preservation described in foundational literature.

Mitochondria are central to cellular adaptation under nutrient limitation, not only as energy producers but also as critical hubs for maintaining organelle quality and metabolic balance. In yeast, selective removal of dysfunctional mitochondria through mitophagy is a key mechanism for preserving mitochondrial integrity and supporting cellular homeostasis under stress. The mitophagy receptor Atg32 interacts with the autophagy machinery via Atg11 and Atg8, which allows targeted degradation of damaged mitochondria while leaving the remainder of the cytoplasm intact. Such selective clearance optimizes cellular energy use without resorting to broad autophagic degradation. Recent studies have highlighted the importance of coordinating mitochondrial turnover with organelle biogenesis and general metabolic regulation to maintain overall mitochondrial homeostasis. In higher eukaryotic systems, altered glucose homeostasis has been associated with a range of physiological and pathological conditions, as reported in previous studies. However, such associations arise from complex, multicellular contexts. In contrast, the present study uses *S. cerevisiae* as a simplified experimental model to examine how defined changes in glucose availability influence cellular responses at the transcriptional level, without addressing organismal aging or disease outcomes (Madhusudhanan *et al.*, 2020). Under conditions of calorie restriction and nutrient stress. These mechanisms contribute to adaptation by ensuring that mitochondria with the highest functional capacity are retained, which supports metabolic stability during the initial stationary phase (Abeliovich, 2023; Liu *et al.*, 2023).

During the life cycle of *S. cerevisiae*, mitochondrial function and dynamics change according to the growth phase and nutrient availability. In the logarithmic (log) phase, cells experience rapid growth and division, and mitochondria are highly active in oxidative phosphorylation to meet the demands for ATP and biosynthesis. Under these conditions, mitochondrial respiration is efficient and supports energy production and cellular proliferation. As cultures enter the stationary phase, nutrient depletion reduces cellular division and alters

metabolic priorities. In this phase, mitochondria maintain essential functions but with reduced respiratory activity; quality control mechanisms, including selective removal of damaged mitochondria through mitophagy, become more prominent to preserve mitochondrial integrity and support survival under stress. During prolonged stress or chronological aging, mitochondrial function further declines, resulting in decreased ATP production and reduced efficiency of quality control processes. Mitochondria in aged cells are often less capable of sustaining bioenergetic demands, and the reduced removal of dysfunctional organelles contributes to a decline in overall cellular viability. Therefore, understanding the functional transitions of mitochondria across these phases provides context for interpreting changes in selective autophagy and cellular fitness in response to calorie restriction (Jazwinski, 2005; Pan, 2011). Consequently, alterations in *ATG8* expression serve as a reliable proxy for global autophagic capacity, whereas transcriptional shifts in *ATG32* and *ATG11* provide mechanistic insights into the activation of cargo-specific degradation pathways. This molecular distinction serves as a fundamental baseline for interpreting the distinct cellular responses captured on day 5.

On day 5, graded glucose restriction induced specific phenotypic and transcriptional transitions without compromising overall cellular viability. The absence of marked deviations in growth kinetics and MTT-mediated metabolic profiles suggests that restricted cells successfully preserved their proliferative and bioenergetic capacities during the entry into the stationary phase. This homeostatic resilience is further supported by the spotting assays, which demonstrated a robust colony-forming capability. Conversely, the synchronized down-regulation of *ATG8* and *ATG11* in the moderate restriction groups (1% and 0.5%), contrasted by the pronounced up-regulation of *ATG32* under severe restriction (0.2%), points to a complex metabolic adjustment. While the down-regulation of *ATG8* may indicate reduced general autophagic flux, the pronounced up-regulation of *ATG32* under severe restriction highlights that cells selectively maintain specific mitochondrial quality control components rather than relying on indiscriminate macro autophagy to systematically clear

dysfunctional organelles, optimizing energy expenditure under specific nutritional constraints. By day 7, the yeast cultures reached a stable adaptation plateau. The metabolic activity profiles, growth kinetics, and transcriptional activities—particularly the normalization of *ATG8* alongside sustained reduction of mitophagy markers—exhibited a relative stabilization, becoming statistically comparable to the control environment. This chronological transition indicates that the pronounced mitochondrial stimulation and selective transcriptional shifts observed on day 5 represent a transient, acute adaptive phase. As the culture matures toward the late stationary phase, this active response subsides into a basal maintenance state, demonstrating that *S. cerevisiae* dynamically recalibrates its autophagic machinery to sustain long-term viability under prolonged nutritional constraints. Finally, while our transcriptional profiling provides granular kinetic insights, the reliance on a limited sample size ($n=3$ biological replicates) represents a methodological constraint that warrants consideration, and future high-throughput validations will be valuable to expand upon these foundational observations. Ultimately, these transcriptional shifts on days 5 and 7 demonstrate that calorie restriction activates localized, targeted autophagic adaptation rather than indiscriminate organelle clearance, establishing a mechanistic bridge between glucose availability and the molecular maintenance of cellular fitness. While the present study provides significant insights into the transcriptional regulation of mitophagy under glucose restriction, further investigations are required to bridge the gap between mRNA expression and functional protein activity. Future research should prioritize western blotting analysis of Atg32p and Atg11p to verify if protein stability correlates with the observed transcript levels. Additionally, employing fluorescence microscopy with mitochondrial markers (*e.g.*, MitoTracker or GFP-tagged mitochondria) would allow for the direct visualization of mitophagic flux and mitochondrial morphology changes. Investigating the role of ROS scavenging enzymes and the potential involvement of other selective autophagy pathways, such as pexophagy, could further elucidate the complex survival strategies

employed by *S. cerevisiae* during prolonged nutrient scarcity.

Conclusion

Overall, this study demonstrates that graded glucose restriction induces a dynamic, time-dependent transcriptional response in *S. cerevisiae*. On day 5, severe restriction (0.2%) triggered a pronounced up-regulation of the mitophagy markers *ATG32* and *ATG11*, whereas moderate restriction (1% and 0.5%) resulted in reduced or baseline expression of these markers, alongside a marked down-regulation of *ATG8* across all restricted groups. By day 7, this transcriptional profile stabilized; *ATG8* expression returned toward control levels in the 0.5% and 0.2% groups, while mitophagy markers exhibited attenuated shifts. These temporal dynamics point to an acute adaptive response during initial restriction that transitions into a stable maintenance state as cultures progress through stationary phase. These molecular adjustments were accompanied by physiological changes, as restricted cells showed elevated cellular metabolic activity on day 5 (via MTT assay) before returning to control levels by day 7. Visual evaluation through spotting assays further revealed enhanced regrowth capacity under 1% glucose. Notably, these physiological metrics reflect cellular metabolic activity and colony-forming potential rather than direct measurements of mitochondrial function, autophagic flux, or lifespan extension. Although these findings highlight distinct transcriptional regulation between general and selective autophagic pathways, changes in mRNA abundance alone do not confirm protein stability or functional degradation rates. Protein validation and direct flux assays will therefore be essential in future studies to confirm how these transcriptional shifts translate into organellar quality control. Ultimately, these findings offer a clearer framework for how *S. cerevisiae* coordinates organelle preservation under nutritional stress over time.

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Conflict of interest

The authors have no conflicts of interest to declare.

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