Starvation-Induced Autophagy in Saccharomyces cerevisiae in Metabolomics Perspectives

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The application of metabolomics has extended the scope of autophagy and provided newer intervention targets against cancer as well as neurodegenerative diseases in which autophagy is implicated.

Keywords: autophagy ; starvation ; metabolomic ; metabolites ; Saccharomyces cerevisiae ; yeast ; nutritional stress ; nitrogen starvation ; glucose starvation ; self-degradation

1. Introduction

The maintenance and preservation of life are constantly under threat of fluctuations or limited availability of nutrient supply [1]. Fasting, an act of refraining oneself from drinking and eating within a specified amount of time, either voluntarily or obligatorily [2][3], showed beneficial outcomes. In humans, fasting and fasting-mimicking diets (FMDs) give many beneficial effects, such as improving cognitive function in older adults [4], lowering blood pressure, reducing total body fat, weight, and trunk, and decreasing insulin-like growth factors 1 (IGF-1) [5]. In fruit flies [6] and cell cultures [7], fasting extends lifespan, while in mice, it up-regulates the longevity Sirt1 gene [8] and preserves skeletal muscle with aging [9].

One of the effects of fasting or nutrient deprivation is autophagy [10]. Autophagy is a self-degradation process by which the cell consumes its damaged or mutant proteins and organelles to maintain cellular homeostasis [11]. Cells have evolved to elaborate metabolic responses to nutritional stressors that would allow them to break down and recycle endogenous macromolecules and reuse them as building blocks for the synthesis of other macromolecules to maintain the production of energy [12]. The three tracks of autophagy are macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). In macroautophagy, autophagosome engulfs targeted cargo and merge with lysosomes, whereby the lysosomal enzymes proceed to degrade the content [13]. In contrast, microautophagy involves direct lysosomal invagination of the cargo [14]. In CMA, the process of transporting the chaperone-targeted protein into the lysosome is aided by lysosomal membrane-associated protein [15]. Impaired autophagy has been linked with the accumulation of damaged mitochondria [16], West syndrome [17], inducing parenteral nutrition-associated lung injury [18], and the worsening of Alzheimer's disease [19]. In unicellular organisms such as the yeast *Saccharomyces cerevisiae*, fasting is simulated in research by nutrient deprivation (or nutrient starvation) [20] which also resulted in extended lifespan [21].

Baker's yeast *Saccharomyces cerevisiae* has been used as research models for diseases such as neurodegenerative disorders [22], aging [23], lifespan [24] oxidative stress [25], and autophagy [26]. The genes in *S. cerevisiae* are highly homologous to those in humans [27][28], and the simplicity of manipulating the genes [14][29][30] and nutrition [31][32] of this short-lived yeast make it a favorable eukaryotic representative. Autophagy defective mutant, *atg1* was first discovered in *S. cerevisiae*, and through this led to the discoveries of other ATG related genes that are involved in the autophagy following starvation [33].

Autophagosome biogenesis in *S. cerevisiae* involves at least 13 vital ATG genes that encode the respective Atg proteins. Atg13 has a vital role in initiating the process, by stimulating Atg1, a serine/threonine kinase in the formation of pre-autophagosomal structure [34]. *Atg1* and *Atg2* encode Atg1 (serine/threonine kinase) and Atg2 proteins, respectively. Both are necessary for autophagy vesicle production and the cytoplasm-to-vacuole targeting (Cvt) pathway [35]. Atg3, encoded by *Atg3*, is an enzyme that catalyzes the formation of Atg8-phosphatidylethanolamine conjugates, which is also mediated by Atg7, a vital step for Atg8 lipidation [36][37]. Atg4 is a protease that cleaves Atg8 to form autophagosomes and vesicles [36]. Atg5 conjugates with Atg12 (mediated by Atg10) [36] and Atg16 to bind to the membrane to efficiently promote Atg8 lipidation [36][38]. Atg6 (encoded by *Atg6*) is an essential subunit of phosphatidylinositol 3-kinase complexes I and II, required for the localization of Atg8 and Atg5-Atg12-Atg16 complex to the phagosome assembly site [39]. Another protein that facilitates the attachment of Atg12 to Atg5p and Atg8 is Atg7, which is encoded by *Atg7* [36]. At the membrane, a

transmembrane protein Atg9 plays a big role in autophagic vesicles biogenesis [35]. Atg11 acts as a protein scaffold directing the receptor-bound cargo to the phagophore assembly sites [40].

Metabolites or small molecules entities (metabolomes) (<1 kDa) represent the downstream products of the complex interactions that define biological processes and functions expressed by the genes (genomes), transcripts (transcriptome), and proteins (proteomes) [41][42]. They are highly influenced by the interaction of both genetic as well as the external and/or internal environments, and as such, the flux that brought about the changes of their levels offers a very close measure of the organisms' physiology [43][44]. Due to this, the study of metabolites has become an important tool in predicting and/or profiling key metabolic biomarkers involved in maintaining homeostasis or dealing with stressful conditions. Metabolomics is a study that systematically identifies and quantifies these small molecules through high-throughput detection methods within a specific time frame [43]. The study of yeast metabolites has only been widely conducted through traditional analytical means [45]. Therefore, a comprehensive view of the organism's metabolome is still inadequate. As mentioned previously the process or induction of autophagy is tightly dependent on the cellular stress status. As such, autophagy-related metabolomes will be subjected to change according to the nature of the stresses occurring in the cells [46][47].

2. Nucleosides and Nucleobases

2.1. Nitrogen Starvation

The application of mass spectrometry as reported in this review has led to the discovery of the temporal changes of intracellular RNA-derived metabolites following nitrogen starvation. Nitrogen starved yeast cells exhibited a transient increase in the relative levels of nucleosides followed by the increase in the levels of purine and pyrimidine nucleobases in the organism following starvation [48][49]. This was made possible through the breakdown and hydrolyzation of sequestered RNAs (3′NMPs) in the vacuole with the help of T2-type RNase and phosphatase; Rny1 and Pho8 [49]. Similar depletion of RNAs has recently been reported in *Ure2*Δ strains as compared to wild-type [50]. Deletion of the *URE2* gene induces a similar physiological state as nitrogen starvation and autophagy in yeast was consistent with the bulk-autophagy pathway [50].

To date, the question of whether that autophagy-induced RNA degradation occurs preferentially or non-selectively is yet to be fully understood [51]. While it was previously suggested that ribosomes are selectively degraded via ribophagy in a Ubp3 (deubiquitinase) -Bre5 (its co-factor) dependent manner [52], findings from Huang and colleagues reported here [49], have shown only temporal delay in RNA degradation for individual *Bre5*Δ and *Ubp3*Δ strains relative to wild-type cells. The deletion of these genes did not for the most part block autophagy-induced RNA breakdown as RNAs were still being delivered into the vacuoles [49]. This is key and interesting to note for future consideration as the need to identify and investigate whether the amount and/or types of RNA play a role in determining the type or preference specific autophagic pathways [53]. The previous study by Kraft and colleagues indicated the role of Ubp3 for the non-selective uptake of mature ribosomes for bulk autophagy and selective uptake of the 40S and 60S ribosomal subunits [52]. Further study on Ubp3 demonstrated its mediation on selective degradation of translation and RNA turnover factors under nitrogen-starved conditions [54]. A most recent study has reported that a subset of mRNAs that encodes for amino acid biosynthesis and ribosomal proteins were preferentially delivered to the vacuole by in rapamycin-induced autophagy in yeast for subsequent Rny-1 mediated degradation in the vacuole [51].

Our metabolomics review also reported the fate of RNA catabolism products namely purines and pyrimidines, as they were eventually excreted rather than salvaged by yeast cells under nitrogen starvation conditions [49]. While it is unclear as to this paradoxical endpoint, one recent study has pointed that the plausible reason for such occurrence could be to compensate for the short supply of available nucleobases [55]. The study has noted that the induction of autophagy following purine and pyrimidine starvation has resulted in the reuptake and salvage of extracellular nucleobases to rescue cells [55]. Additionally, it is also reported that following the drop in nucleotide pools, survival during starvation in autophagy-deficient Kras-driven lung tumor cells was fully rescued by glutamine, glutamate, or nucleosides [56].

2.2. Carbon Starvation

Moreover, targeted metabolomics analyses have allowed for the elucidation of the nucleotide degradation pathways in glucose-starved yeast cells [48]. We saw a considerable overlap with regards to the autophagy-induced breakdown of RNA metabolites as seen in nitrogen starvation conditions. Starvation of either through glucose or nitrogen in yeast cells has caused an accumulation of nucleosides, nucleobases, as well as sedoheptulose species (only for glucose starvation). Autophagy in the study was found to be induced by the activation of SNF1, an AMP-activated protein kinase, as well as the inactivation of cyclic-AMP dependent protein kinase (PKA). The activities of these two molecules were confirmed in a

much recent study by Adachi and colleagues [57] and are key for the induction of autophagy during glucose starvation. Phm8 as shown in the current study was found to be a crucial nucleotidase in yeast. Moreover, similar to nitrogen conditions, purines and pyrimidine were broken down with the help of Pnp1, and Urh1 proteins. *These proteins are essential in ribosome salvage as individual deletion of PNP1, URH1, and PHM8 exhibited a reduction in sedoheptulose species (S7P and SBP); key ribose-derived carbon species that are central in the conversion of ribose into glycolytic intermediates* [48]. In addition, long-term starvation in these mutant strains caused a decreased viability in comparison to wild-type, which shows that nucleotide degradation and ribose salvage are essential for survival under stress [48]. Ribose salvage during glucose starvation in yeast requires intact non-oxidative pentose-phosphate pathways and the increase in the levels of sedoheptulose species as the author suggested provides nutrient reserves of carbon and phosphate for the cells [48]. The previous study has indicated that autophagy is closely linked to the metabolic state of the cell [57]. Gene expression for the utilization of alternative carbon sources and respiration is strictly repressed in the presence of sufficient glucose [57]. This carbon catabolic repression negatively regulates autophagy under carbon starvation and is positively correlated with yeast's respiratory metabolism [57].

3. Amino Acid

3.1. Nitrogen Starvation

Interestingly, through mass spectrometry, it was also demonstrated that autophagy is a crucial stress response in maintaining the amino acid pools to sustain the survival of yeast [14][58][59]. In this review, studies have reported that nitrogen-starved yeast cells are capable of synthesizing amino acids at varying degrees with much emphasis on glutamate and aspartate [14]. Amassing glutamate is needed as it is a major cellular nitrogen donor that replenishes nitrogen levels to support macromolecule synthesis [14][60]. The study also found that ammonium from degraded proteins is an important source to assimilate via the GS-GOGAT pathway to synthesize glutamine, and subsequently glutamate and aspartate [14]. These nitrogen starved cells invested more glutamate in aspartate synthesis to produce derivative amino acids as well as providing the building blocks for proteins and nucleic acids [14]. Aspartate from this study was found to be significantly being incorporated into proteins [14]. Maintaining pools of amino acids as the author suggested, is key during starvation as it helps the cells to sustain protein synthesis [58], recycle and replenish nucleotide pools as previously mentioned, and is critical to complete its cell cycle [61]. A very recent study has shown that amino acid starvation inhibits autophagy in lipid-droplet deficient yeast [62]. Lipid droplets act as storage sites for neutral lipids. In response to nitrogen starvation, lipid droplets are required for autophagy to proceed and to maintain endoplasmic reticulum (ER) homeostasis [62].

3.2. Glucose Starvation

With regards to glucose starvation, a global metabolome and lipidome profiling of yeast were successfully undertaken [32]. The study demonstrated that respiration is crucial for the survival of yeast cells, as the dramatic reduction of ATP was observed in *CBP2* deleted yeast as compared to isogenic wild-type [32]. The result was replicated in a previous study where an immediate drop in the levels of intracellular ATP was observed and persisted for 3 h following the onset of glucose starvation in wild-type yeast [57]. Further depletion of intracellular metabolite pools that are central in glycolysis and citric acid cycle were exhibited in this cell, with several metabolites including glutamate and acetyl-CoA were found to be completely depleted. While rapid depletion of extracellular amino acids, namely aspartate and methionine, was observed, it did not affect the ATP levels nor offer a long-term survival advantage to the cells in glucose and amino acid-deprived conditions as compared to glucose-only [32].

There is a need for a comprehensive look into the complex interplay between autophagy-derived amino acids and ATP/cellular energy requirements in health and diseased cells. A recent study on the mammary carcinoma cancer cell line has suggested that the maintenance of an intracellular pool of amino acids in MDAMB231 cells is critical for its homeostasis and preservation of ATP following acute amino acid starvation [63]. *Preferential utilization of autophagy-derived amino acids can help mitigate the reduced levels of ATP following amino acid starvation. However, this reliance on autophagy-produced substrates to maintain homeostasis is only transient.* [63]. Moreover, glucose starvation without supplementation of amino acids in gastric cancer cells causes inhibition of growth and cellular apoptosis [64]. By supplying non-essential amino acids to these cells, growth inhibition and apoptosis were rescued [64]. This shows that amino acids under the context of autophagy are crucial as an alternative energy source from other cells. Starvation-induced autophagy in the current study however has caused no decrease in the levels of ATP to the same extent as in *CBP2* deleted yeast implying an alternative pathway of generating short-term ATP during glucose starvation [32].

4. ATP and Lipids

Glucose Starvation

Further observation demonstrated that μ-lipophagy, the alternative degradative pathways that involve direct engulfment of lipid droplets into the yeast vacuole as opposed to the autophagosome-based lipophagy provides a long-term energy maintenance in glucose-starved [32]. The finding from this study is consistent with the previous report whereby acute glucose reduction in yeast led to the survival deficiency of the cells after 7 days [65]. AMP-activated protein kinase (AMPK) and ATG14 protein (a kinase) in yeast cells orchestrated lipophagy to extend yeast's lifespan [65]. The author of the current work also suggested that AMPK may have been activated early during starvation [32]. In addition, it was also noted that the consumption of lipids through β-oxidation in the peroxisomes contributes to the intracellular levels of ATP within several hours of glucose starvation. Double deletion of β-oxidation and autophagy exhibited lower levels of intracellular ATP than the single-deletion mutants [32]. While it was previously argued that autophagy is dispensable in glucose-starved yeast cells and that vacuolar hydrolysis is key in replenishing cells with energy [66], the metabolome and lipidome analyses showed in the current study indicated that it is essential for cell survival and that ATP levels depend on autophagy within the first 24 h [32]. In addition, previous work has demonstrated alterations of lipid metabolism following starvation. The study on serum and amino acid-starved mouse embryonic fibroblast (MEF) demonstrated autophagy-induced changes of the cellular lipidome with significant alteration occurring to the free fatty acid, glycerophospholipid as well as sphingolipid metabolisms [67]. Lipids were consumed and protected these cells from death during starvation [67]. Cellular lipidome analysis in the current study exhibited no major changes in lipid levels between control and glucose starvation conditions [32]. While it was initially hypothesized that major remodeling of the membrane and or the liberation of lipids for energy synthesis would occur following starvation, the findings show that cellular membranes did not undergo significant changes and only specific classes of lipids were affected (polyketides and dolichols) [32].

5. General Application of Metabolomics in the Study of Starvation

In recent years, there is a rising interest in the application of metabolomics in the general study of starvation in healthy or diseased organisms other than yeasts. Fasting in humans or mice has resulted in major alterations of the plasma metabolome, with relatively minor changes occurring in the intracellular metabolome of circulating leukocytes [68]. Alteration of free fatty acids and/or different acylcarnitine species in the plasma as observed in the study is suggested to reflect the breakdown of lipids of endogenous storage under nutritional stress [69]. Moreover, in a different study, LC-MS analyses on mouse embryonic fibroblast cells (MEFs), had seen a similar alteration in amino acid, energy, carbohydrate, and lipid metabolism in response to nutrient stress [70]. Amino acid metabolism was found to be affected following acute starvation. Among the many affected pathways are glutamine and glutamate metabolism as well as aspartate and glutamate metabolism [70]. Induction of autophagy with subsequent upregulation of lipid metabolism offered protection to MEFs and delayed cell death [70]. Furthermore, subsequent GC-MS analyses on these cells provided additional altered metabolic pathways and products that were previously unaccounted for including valine, leucine, and isoleucine, as well new targets of glucose metabolism products following starvation [71]. In addition, in the study of cancer, a comprehensive metabolome analysis of glutamine-deprived cancer cells demonstrated an accumulation of phosphoethanolamine metabolite that protects the cancer cells through the downregulation of rate-limiting enzyme of phosphatidylethanolamine biosynthesis, PCYT2 [72]. Reduced expression of PCYT2 was correlated with decreased survival in cancer patients [72]. In a different study, metabolic pathways, including, amino acid, pyrimidine, glycerophospholipid metabolism, and the TCA cycle, were found to be most affected by arginine starvation in breast cancer cells xenograft models [73]. Mitochondrial dysfunction and aspartate exhaustion through arginine starvation allow cancer cells to be killed [73].

References

1. Ohsumi, Y. Historical landmarks of autophagy research. Cell Res. 2013, 24, 9–23.
2. Nasaruddin, M.; Halim, S.S.A.; Kamaruzzaman, M. Studying the Relationship of Intermittent Fasting and β-Amyloid in Animal Model of Alzheimer's Disease: A Scoping Review. Nutrients 2020, 12, 3215.
3. Harun, C.S.C.; Abu Zahrin, S.N.; Mastor, K.A. The Six Days of Voluntary Fasting in Syawal and Its Significance to the Personality of Muslims. MALIM J. Pengaj. Umum ASIA TENGGARA (SEA J. Gen. Stud.) 2019, 20, 74–85.
4. Ooi, T.C.; Meramat, A.; Rajab, N.F.; Shahar, S.; Ismail, I.S.; Azam, A.A.; Sharif, R. Intermittent Fasting Enhanced the Cognitive Function in Older Adults with Mild Cognitive Impairment by Inducing Biochemical and Metabolic changes: A 3-Year Progressive Study. Nutrients 2020, 12, 2644.

5. Wei, M.; Brandhorst, S.; Shelehchi, M.; Mirzaei, H.; Cheng, C.W.; Budniak, J.; Groshen, S.; Mack, W.J.; Guen, E.; Di Biase, S.; et al. Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. Sci. Transl. Med. 2017, 9.
6. Catterson, J.H.; Khericha, M.; Dyson, M.; Vincent, A.J.; Callard, R.; Haveron, S.M.; Rajasingam, A.; Ahmad, M.; Partridge, L. Short-Term, Intermittent Fasting Induces Long-Lasting Gut Health and TOR-Independent Lifespan Extension. Curr. Biol. 2018, 28, 1714–1724.e4.
7. Davidson, M.D.; Khetani, S.R. Intermittent Starvation Extends the Functional Lifetime of Primary Human Hepatocyte Cultures. Toxicol. Sci. 2020, 174, 266–277.
8. Qin, J.; Zhou, J.; Dai, X.; Zhou, H.; Pan, X.; Wang, X.; Zhang, F.; Rao, J.; Lu, L. Short-term starvation attenuates liver ischemia-reperfusion injury (IRI) by Sirt1-autophagy signaling in mice. Am. J. Transl. Res. 2016, 8, 3364–3375.
9. Wallace, M.A.; Aguirre, N.W.; Marcotte, G.R.; Marshall, A.G.; Baehr, L.M.; Hughes, D.C.; Hamilton, K.L.; Roberts, M.N.; Lopez-Dominguez, J.A.; Miller, B.F.; et al. The ketogenic diet preserves skeletal muscle with aging in mice. Aging Cell 2021, 20, e13322.
10. Bedu, M.; Marmagne, A.; Masclaux-Daubresse, C.; Chardon, F. Transcriptional Plasticity of Autophagy-Related Genes Correlates with the Genetic Response to Nitrate Star-vation in Arabidopsis Thaliana. Cells 2020, 9, 1021.
11. Rahman, M.; Cho, Y.; Nam, G.; Rhim, H. Antioxidant Compound, Oxyresveratrol, Inhibits APP Production through the AMPK/ULK1/mTOR-Mediated Autophagy Pathway in Mouse Cortical Astrocytes. Antioxidants 2021, 10, 408.
12. Kaur, J.; Debnath, J. Autophagy at the crossroads of catabolism and anabolism. Nat. Rev. Mol. Cell Biol. 2015, 16, 461–472.
13. He, H.-Y.; Deng, Y.-H.; Dong, L.-L.; Zhang, Y.-J.; Zhao, X.-M. Enriched environment boosts the post-stroke recovery of neurological function by promoting autophagy. Neural Regen. Res. 2021, 16, 813–819.
14. Liu, K.; Sutter, B.M.; Tu, B.P. Autophagy sustains glutamate and aspartate synthesis in Saccharomyces cerevisiae during nitrogen starvation. Nat. Commun. 2021, 12, 1–14.
15. Cao, D.; Shan, D.; Yan, W.; Zhang, Z.; Song, Q.; Jiang, Y.; Zhang, X.; Zhang, Z.; Wang, Z.; Wang, Y.; et al. Chaperone-mediated autophagy affects tumor cell proliferation and cisplatin resistance in esophageal squamous cell carcinoma. Thorac. Cancer 2021, 12, 1048–1057.
16. Nichenko, A.S.; Sorensen, J.R.; Southern, W.M.; Qualls, A.E.; Schifino, A.G.; McFaline-Figueroa, J.; Blum, J.E.; Tehrani, K.F.; Yin, H.; Mortensen, L.J.; et al. Lifelong Ulk1-Mediated Autophagy Deficiency in Muscle Induces Mitochondrial Dysfunction and Contractile Weakness. Int. J. Mol. Sci. 2021, 22, 1937.
17. He, H.; Cao, X.; Yin, F.; Wu, T.; Stauber, T.; Peng, J. West Syndrome Caused by a Chloride/Proton Exchange-Uncoupling CLCN6 Mutation Related to Autophag-ic-Lysosomal Dysfunction. Mol. Neurobiol. 2021, 58, 2990–2999.
18. Yang, J.; Sun, H.; Tian, F.; Wan, S.; Mamtawla, G.; Wang, P.; Gao, X.; Zhang, L.; Li, J.; Shen, Y.; et al. Autophagy suppression plays a role in parenteral nutrition-associated lung injury. Clin. Nutr. 2020, 40, 560–570.
19. Li, J.G.; Chu, J.; Praticò, D. Downregulation of autophagy by 12/15Lipoxygenase worsens the phenotype of an Alzheimer's disease mouse model with plaques, tangles, and memory impairments. Mol. Psychiatry 2021, 26, 604–613.
20. Kira, S.; Noguchi, M.; Araki, Y.; Oikawa, Y.; Yoshimori, T.; Miyahara, A.; Noda, T. Vacuolar protein Tag1 and Atg1–Atg13 regulate autophagy termination during persistent starvation in S. cerevisiae. J. Cell Sci. 2021, 134.
21. Kwong, M.M.Y.; Lee, J.W.; Samian, M.R.; Watanabe, N.; Osada, H.; Ong, E.B.B. Comparison of microplate- and bottle-based methods to age yeast for chronological life span assays. J. Microbiol. Methods 2019, 167, 105743.
22. Soczewka, P.; Tribouillard-Tanvier, D.; di Rago, J.P.; Zoladek, T.; Kaminska, J. Targeting Copper Homeostasis Improves Functioning of vps13Δ Yeast Mutant Cells, a Model of VPS13-Related Diseases. Int. J. Mol. Sci. 2021, 22, 2248.
23. Dahiya, R.; Mohammad, T.; Alajmi, M.F.; Rehman, T.; Hasan, G.M.; Hussain, A.; Hassan, I. Insights into the Conserved Regulatory Mechanisms of Human and Yeast Aging. Biomolecules 2020, 10, 882.
24. TA, K. Tocotrienol-Rich Fraction (TRF) Improves the Viability of Wild-Type Saccharomyces cerevisiae in the Initial Stationary Phase. Med. Health 2019, 14, 106–117.
25. Oliveira, G.; Radovanovic, N.; Nunes, M.C.D.N.; Fristedt, R.; Alminger, M.; Andlid, T. Extracts of Digested Berries Increase the Survival of Saccharomyces cerevisiae during H2O2 Induced Oxidative Stress. Molecules 2021, 26, 1057.
26. Takeshige, K.; Baba, M.; Tsuboi, S.; Noda, T.; Ohsumi, Y. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. J. Cell Biol. 1992, 119, 301–311.
27. Liu, W.; Li, L.; Ye, H.; Chen, H.; Shen, W.; Zhong, Y.; Tian, T.; He, H. From Saccharomyces cerevisiae to human: The important gene co-expression modules. Biomed. Rep. 2017, 7, 153–158.

28. Zulkifli, M.; Neff, J.K.; Timbalia, S.A.; Garza, N.M.; Chen, Y.; Watrous, J.D.; Murgia, M.; Trivedi, P.P.; Anderson, S.K.; Tomar, D.; et al. Yeast homologs of human MCUR1 regulate mitochondrial proline metabolism. Nat. Commun. 2020, 11, 1–15.

29. Ramzi, A.B.; Bahaudin, K.N.A.K.; Baharum, S.N.; Me, M.L.C.; Goh, H.-H.; Hassan, M.; Noor, N.M. Rapid Assembly of Yeast Expression Cassettes for Phenylpropanoid Biosynthesis in Saccharomyces cerevisiae. Sains Malays. 2018, 47, 2969–2974.

30. Becares, E.; Pedersen, P.; Gourdon, P.; Gotfryd, K. Overproduction of Human Zip (SLC39) Zinc Transporters in Saccharomyces cerevisiae for Biophysical Characterization. Cells 2021, 10, 213.

31. Bermúdez-Moretti, M.; Gulias, F.; Valencia-Guillén, J.; Muñoz, S.A.; Forfar, I.; Correa-García, S. Monitoring changes in the cellular content of biomolecules during ageing with FTIR spectroscopy. Vib. Spectrosc. 2019, 105, 102972.

32. Weber, C.A.; Sekar, K.; Tang, J.H.; Warmer, P.; Sauer, U.; Weis, K. beta-Oxidation and autophagy are critical energy providers during acute glucose depletion in Saccharo-myces cerevisiae. Proc. Natl. Acad. Sci. USA 2020, 117, 12239–12248.

33. Tsukada, M.; Ohsumi, Y. Isolation and characterization of autophagy-defective mutants ofSaccharomyces cerevisiae. FEBS Lett. 1993, 333, 169–174.

34. Suzuki, S.W.; Yamamoto, H.; Oikawa, Y.; Kondo-Kakuta, C.; Kimura, Y.; Hirano, H.; Ohsumi, Y. Atg13 HORMA domain recruits Atg9 vesicles during autophagosome formation. Proc. Natl. Acad. Sci. USA 2015, 112, 3350–3355.

35. Reggiori, F.; Tucker, K.A.; Stromhaug, P.E.; Klionsky, D.J. The Atg1-Atg13 Complex Regulates Atg9 and Atg23 Retrieval Transport from the Pre-Autophagosomal Structure. Dev. Cell 2004, 6, 79–90.

36. Meijer, W.H.; van der Klei, I.J.; Veenhuis, M.; Kiel, J.A. ATGGenes Involved in Non-Selective Autophagy are Conserved from Yeast to Man, but the Selective Cvt and Pexophagy Pathways also Require Organism-Specific Genes. Autophagy 2007, 3, 106–116.

37. Wang, S.; Li, Y.; Ma, C. Atg3 promotes Atg8 lipidation via altering lipid diffusion and rearrangement. Protein Sci. 2020, 29, 1511–1523.

38. Walczak, M.; Martens, S. Dissecting the role of the Atg12–Atg5-Atg16 complex during autophagosome formation. Autophagy 2013, 9, 424–425.

39. Yue, J.; Sun, H.; Zhang, W.; Pei, D.; He, Y.; Wang, H. Wheat homologs of yeast ATG6 function in autophagy and are implicated in powdery mildew immunity. BMC Plant Biol. 2015, 15, 1–15.

40. Matscheko, N.; Mayrhofer, P.; Rao, Y.; Beier, V.; Wollert, T. Atg11 tethers Atg9 vesicles to initiate selective autophagy. PLoS Biol. 2019, 17, e3000377.

41. Jendoubi, T. Approaches to Integrating Metabolomics and Multi-Omics Data: A Primer. Metabolites 2021, 11, 184.

42. Pinu, F.R.; Beale, D.J.; Paten, A.M.; Kouremenos, K.; Swarup, S.; Schirra, H.J.; Wishart, D. Systems Biology and Multi-Omics Integration: Viewpoints from the Metabolomics Research Community. Metabolites 2019, 9, 76.

43. Ashrafian, H.; Sounderajah, V.; Glen, R.; Ebbels, T.; Blaise, B.J.; Kalra, D.; Kultima, K.; Spjuth, O.; Tenori, L.; Salek, R.M.; et al. Metabolomics: The Stethoscope for the Twenty-First Century. Med Princ. Pract. 2020, 30, 301–310.

44. Reed, L.K.; Baer, C.F.; Edison, A.S. Considerations when choosing a genetic model organism for metabolomics studies. Curr. Opin. Chem. Biol. 2016, 36, 7–14.

45. Sailwal, M.; Das, A.J.; Gazara, R.K.; Dasgupta, D.; Bhaskar, T.; Hazra, S.; Ghosh, D. Connecting the dots: Advances in modern metabolomics and its application in yeast system. Biotechnol. Adv. 2020, 44, 107616.

46. Jacomin, A.-C.; Gul, L.; Sudhakar, P.; Korcsmaros, T.; Nezis, I.P. What We Learned from Big Data for Autophagy Research. Front. Cell Dev. Biol. 2018, 6, 92.

47. Stryeck, S.; Birner-Gruenberger, R.; Madl, T. Integrative metabolomics as emerging tool to study autophagy regulation. Microb. Cell 2017, 4, 240–258.

48. Xu, Y.-F.; Létisse, F.; Absalan, F.; Lu, W.; Kuznetsova, E.; Brown, G.; Caudy, A.; Yakunin, A.; Broach, J.; Rabinowitz, J.D. Nucleotide degradation and ribose salvage in yeast. Mol. Syst. Biol. 2013, 9, 665.

49. Huang, H.; Kawamata, T.; Horie, T.; Tsugawa, H.; Nakayama, Y.; Ohsumi, Y.; Fukusaki, E. Bulk RNA degradation by nitrogen starvation-induced autophagy in yeast. EMBO J. 2014, 34, 154–168.

50. Liu, J.-J.; Woodruff, W.; Deewan, A.; Jagtap, S.S.; Yun, E.J.; Walukiewicz, H.E.; Jin, Y.-S.; Rao, C.V. Investigating the role of the transcriptional regulator Ure2 on the metabolism of Saccharomyces cerevisiae: A multi-omics approach. Appl. Microbiol. Biotechnol. 2021, 105, 5103–5112.

51. Makino, S.; Kawamata, T.; Iwasaki, S.; Ohsumi, Y. Selectivity of mRNA degradation by autophagy in yeast. Nat. Commun. 2021, 12, 1–10.

52. Kraft, C.; Deplazes, A.; Sohrmann, M.; Peter, M. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nature 2008, 10, 602–610.

53. Frankel, L.B.; Lubas, M.; Lund, A. Emerging connections between RNA and autophagy. Autophagy 2016, 13, 3–23.

54. Kelly, S.P.; Bedwell, D.M. Both the autophagy and proteasomal pathways facilitate the Ubp3p-dependent depletion of a subset of translation and RNA turnover factors during nitrogen starvation inSaccharomyces cerevisiae. RNA 2015, 21, 898–910.

55. Mimura, K.; Sakamaki, J.-I.; Morishita, H.; Kawazu, M.; Mano, H.; Mizushima, N. Genome-wide CRISPR screening reveals nucleotide synthesis negatively regulates autophagy. J. Biol. Chem. 2021, 296, 100780.

56. Guo, J.Y.; Teng, X.; Laddha, S.V.; Ma, S.; Van Nostrand, S.C.; Yang, Y.; Khor, S.; Chan, C.S.; Rabinowitz, J.D.; White, E. Autophagy provides metabolic substrates to maintain energy charge and nucleotide pools in Ras-driven lung cancer cells. Genes Dev. 2016, 30, 1704–1717.

57. Adachi, A.; Koizumi, M.; Ohsumi, Y. Autophagy induction under carbon starvation conditions is negatively regulated by carbon catabolite repression. J. Biol. Chem. 2017, 292, 19905–19918.

58. Onodera, J.; Ohsumi, Y. Autophagy Is Required for Maintenance of Amino Acid Levels and Protein Synthesis under Nitrogen Starvation. J. Biol. Chem. 2005, 280, 31582–31586.

59. Parzych, K.R.; Klionsky, D.J. Vacuolar hydrolysis and efflux: Current knowledge and unanswered questions. Autophagy 2018, 15, 212–227.

60. Magasanik, B. Ammonia Assimilation by Saccharomyces cerevisiae. Eukaryot. Cell 2003, 2, 827–829.

61. Matsui, A.; Kamada, Y.; Matsuura, A. The Role of Autophagy in Genome Stability through Suppression of Abnormal Mitosis under Starvation. PLoS Genet. 2013, 9, e1003245.

62. Voisin, P.; Bernard, M.; Bergès, T.; Regnacq, M. Amino acid starvation inhibits autophagy in lipid droplet-deficient cells through mitochondrial dysfunction. Biochem. J. 2020, 477.

63. Thomas, M.; Davis, T.; Loos, B.; Sishi, B.; Huisamen, B.; Strijdom, H.; Engelbrecht, A.M. Autophagy is essential for the maintenance of amino acids and ATP levels during acute amino acid star-vation in MDAMB231 cells. Cell Biochem. Funct. 2018, 36, 65–79.

64. Wang, G.; Dai, L.; Luo, L.; Xu, W.; Zhang, C.; Zhu, Y.; Chen, Z.; Hu, W.; Xu, X.; Pan, W.; et al. Non-essential amino acids attenuate apoptosis of gastric cancer cells induced by glucose starvation. Oncol. Rep. 2014, 32, 332–340.

65. Seo, A.Y.; Lau, P.W.; Feliciano, D.; Sengupta, P.; Le Gros, M.A.; Cinquin, B.; Larabell, C.A.; Lippincott-Schwartz, J. AMPK and vacuole-associated Atg14p orchestrate mu-lipophagy for energy production and long-term survival under glucose starvation. Elife 2017, 6, e21690.

66. Lang, M.; Martínez-Márquez, J.; Prosser, D.C.; Ganser, L.; Buelto, D.; Wendland, B.; Duncan, M.C. Glucose Starvation Inhibits Autophagy via Vacuolar Hydrolysis and Induces Plasma Membrane Internalization by Down-regulating Recycling. J. Biol. Chem. 2014, 289, 16736–16747.

67. Shen, S.; Yang, L.; Li, L.; Bai, Y.; Liu, H. Lipid metabolism in mouse embryonic fibroblast cells in response to autophagy induced by nutrient stress. Anal. Chim. Acta 2018, 1037, 75–86.

68. Pietrocola, F.; Demont, Y.; Castoldi, F.; Enot, D.; Durand, S.; Semeraro, M.; Baracco, E.E.; Pol, J.; Pedro, J.M.B.-S.; Bordenave, C.; et al. Metabolic effects of fasting on human and mouse blood in vivo. Autophagy 2017, 13, 567–578.

69. Finn, P.F.; Dice, J.F. Proteolytic and lipolytic responses to starvation. Nutrition 2006, 22, 830–844.

70. Shen, S.; Weng, R.; Li, L.; Xu, X.; Bai, Y.; Liu, H. Metabolomic Analysis of Mouse Embryonic Fibroblast Cells in Response to Autophagy Induced by Acute Starvation. Sci. Rep. 2016, 6, 34075.

71. Shen, S.; Li, L.; Song, S.; Bai, Y.; Liu, H. Metabolomic study of mouse embryonic fibroblast cells in response to autophagy based on high resolution gas chromatography–mass spectrometry. Int. J. Mass Spectrom. 2018, 434, 215–221.

72. Osawa, T.; Shimamura, T.; Saito, K.; Hasegawa, Y.; Ishii, N.; Nishida, M.; Ritsuko, A.; Ayano, K.; Muyassar Anwar Kodama, T. Phosphoethanolamine Accumulation Protects Cancer Cells under Glutamine Starvation through Downregulation of PCYT2. Cell Rep. 2019, 29, 89–103.e7.

73. Cheng, C.-T.; Qi, Y.; Wang, Y.-C.; Chi, K.; Chung, Y.; Ouyang, C.; Chen, Y.-R.; Oh, M.E.; Sheng, X.; Tang, Y.; et al. Arginine starvation kills tumor cells through aspartate exhaustion and mitochondrial dysfunction. Commun. Biol. 2018, 1, 1–15.