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Yeast chronological lifespan and proteotoxic stress: autophagy good or bad?

Q1 **Belém Sampaio-Marques, C. Felgueiras, Alexandra Silva, Fernando Rodrigues and Paula Ludovico[1]**

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Abstract

Autophagy, a highly conserved proteolytic mechanism of quality control, is essential for the maintenance of metabolic and cellular homoeostasis and for an efficient cellular response to stress. Autophagy declines with aging and is believed to contribute to different aspects of the aging phenotype. The nutrient-sensing pathways PKA (protein kinase A), Sch9 and TOR (target of rapamycin), involved in the regulation of yeast lifespan, also converge on a common targeted process, autophagy. The molecular mechanisms underlying the regulation of autophagy and aging by these signalling pathways in yeast, with special attention to the TOR pathway, will be discussed here. The question of whether or not autophagy could contribute to yeast cell death occurring during CLS (chronological lifespan) will be discussed in light of our findings obtained after autophagy activation promoted by proteotoxic stress. Autophagy progressively increases in cells expressing the aggregated-prone protein α-synuclein and seems to participate in the early cell death and shortening of CLS under these conditions, highlighting that autophagy activity should be maintained under physiological levels to exert its promising anti-aging effects.

Introduction

Macroautophagy, hereafter autophagy, is a main proteolytic cellular system that guarantees the quality of proteins and organelles through their sequestration within double-membrane autophagosomes that are delivered to lysosomes for digestion [1]. As a result, the indispensable recycled components can be reprocessed not only for the synthesis of new macromolecules, but also as a cellular energy source attributing an imperative role to autophagy in response to nutrient starvation or under specific physiological conditions requiring extensive cellular remodelling such as differentiation, development and homoeostasis (reviewed in [1–3]). However, an excessive activation of autophagy might result in the abolishment of its cytoprotective function and its enrolment in a self-destructive process [4].

Accumulating evidence suggests that different signalling pathways regulating aging converge on autophagy, and recent evidence obtained in multiple species ranging from invertebrates to mouse models reveals an interplay between autophagy activity and long-lived phenotypes (reviewed in [3,5]). However, several pieces of the interplay between autophagy and aging are poorly enlightened, and more detailed studies are needed in order to answer several key questions and to avoid generalization and oversimplifications made in light of findings obtained in particular contexts. The yeast *Saccharomyces cerevisiae* CLS (chronological lifespan) has emerged as a powerful tool to study the connections between signalling pathways, autophagy and aging [5–7], in post-mitotic cells where failure of quality control systems such as autophagy is particularly detrimental [8,9]. The present review focuses on the regulation of autophagy by nutrient-sensing pathways in yeast, especially the most explored one, the TOR (target of rapamycin) pathway. The role of the signalling pathways in autophagy and aging will be discussed and the interventions known to promote longevity extension dependent on autophagy will be highlighted. The pro-survival and pro-death effects of autophagy will be addressed by data obtained during yeast CLS under proteotoxic stress, a condition known to induce activation of autophagy.

Regulation of autophagy and aging

Aging is associated with a general and progressive decline of cellular processes that ultimately culminate in cell death. The age-dependent decrease in the activity of quality control mechanisms such as autophagy is believed to be the main cause of the accumulation of dysfunctional organelles and damaged molecules, particularly critical in post-mitotic cells [8,9]. The genetic and pharmacological inhibition or enhancement of autophagy is respectively correlated to decreased or increased longevity. Moreover, the vast majority of interventions found to increase longevity are autophagy dependent, supporting the crucial cytoprotective role of autophagy and the close relation between autophagy and aging.

In yeast, abrogation of the conserved TOR, Ras/ cAMP-dependent protein kinase [or PKA (protein kinase A)] or Sch9 proteins, which integrate the network of

nutrient-sensing pathways, is known to promote longevity [6,10–12]. These signalling pathways are negative regulators of autophagy, reinforcing that autophagy and aging are co-ordinately regulated by a complex network of different signalling pathways, with partial overlapping branches and not yet disclosed hierarchic connections.

As in various species [13–17], in yeast, accumulating evidence has disclosed mechanisms that establish the direct regulation of autophagy by the TOR pathway, especially TORC1 (TOR complex 1). TORC1 negatively regulates autophagy by a direct targeting of ATG (autophagy-related gene) proteins or indirectly by still elusive mechanisms, such as transcriptional and translational control or through interactions with proteins that further regulate autophagy players (Figure 1; reviewed in [18]). In yeast, the transcriptional control of autophagy relies mostly on the regulation of the autophagy-related genes *ATG8* and *ATG14* [19–21]. The levels of *ATG14*, controlled by Gln3, could reach more than 20-fold in nitrogen starvation conditions [21]. The Atg8 protein increases 10–20-fold after induction of autophagy by starvation conditions [22]. Nevertheless, the transcription factor(s) that regulate the expression levels of the *ATG8* gene remain elusive. The transcriptional regulation of other *ATG* genes by starvation conditions was also described in [23], but to reveal the relevance of autophagy genetic control, further studies are still needed. On the other hand, the induction of autophagy in yeast is initiated by the activation of Atg1 kinase complex that includes Atg13 and the sub-complex formed by Atg17, Atg31 and Atg29 [24]. TORC1 affects the formation of the Atg1 complex by inducing hyperphosphorylation of Atg13, decreasing its affinity for Atg1 and consequently hampering the activation of the first step of the autophagy pathway. In contrast, TORC1 signalling inhibition results in partial dephosphorylation of Atg13, which allows its binding to Atg1 [25]. Atg13 phosphorylation is believed to be a crucial step in the activation of Atg1 complexes given the ability that a non-phosphorylable Atg13 form has to induce autophagy independent of TORC1 regulation [26]. In yeast, TORC1 also inhibits autophagy by regulating the phosphorylation of several proteins required for autophagy via Tap42 and PP2A (protein phosphatase 2A) [27].

Modulators of TOR pathway have also been used as forefront evidence supporting the relation between signalling of autophagy and aging. Rapamycin and the so-called rapalogues are the most effective inducers of autophagy dependent on the TOR pathway emerging as potential enhancers of health and lifespan. TOR signalling inhibition by rapamycin was shown to up-regulate autophagy in multiple species (reviewed in [3,6]) and functional autophagy has proved to be necessary for the lifespan extension by rapamycin-mediated TOR inhibition in yeast [15]. TOR pathway inhibition mimics the physiological characteristics of starvation, including activation of autophagy (Figure 1) and yeast CLS extension dependent on autophagy [15]. On the other hand, the *S. cerevisiae TOR1* mutant cells present increased CLS associated with increased autophagy activity as detected by *ATG8–GFP* (green fluorescent protein; Figure 2).

Figure 1 | Regulation of autophagy and its implications on yeast longevity

TORC1 directly or indirectly regulates autophagy. Direct mechanism of negative autophagy regulation by TORC1 includes hyperphosphorylation of Atg13 that reduces the activation of the Atg1 complex. Starvation conditions or rapamycin results in autophagy induction through TORC1 inactivation. PKA and Sch9 pathways also negatively regulate autophagy through the involvement of Atg1 complex assembly or through the downstream targets Msn2/Msn4 and the protein kinase Rim15. Sch9 is a substrate of TORC1 and acts on PKA further, highlighting the complex network of these nutrient-sensing pathways. Inactivation of these pathways is also associated with long-lived phenotypes that require autophagy indication. See the text for details.
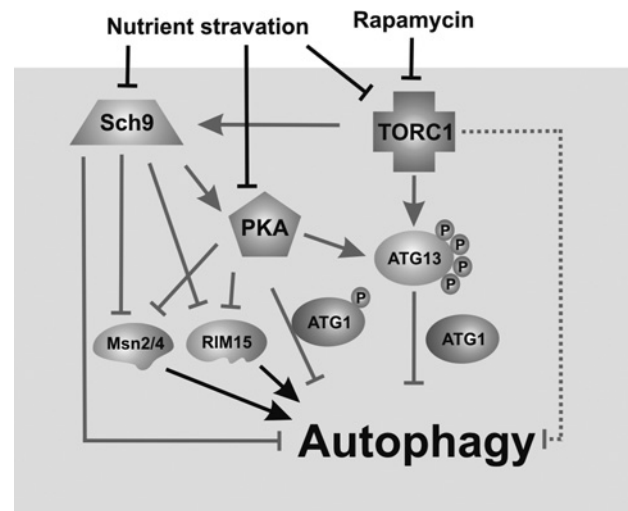

Although rapamycin is considered an inducer of yeast CLS extension and ameliorate neurodegenerative proteinopathies via activation of autophagy, opinion concerning its effective role is still controversial given the pleiotropic impact of the TOR pathway on various downstream targets besides autophagy and on the cross-talk of TOR with other signalling pathways.

In addition to TORC1, the nutrient sensory kinases PKA and Sch9 also play a role of negative regulators of autophagy, and the inactivation of both PKA and Sch9 release autophagy inhibition [28]. Hyperactivation of Sch9 or constitutive activation of PKA results in the suppression of autophagy even when TOR is inhibited by rapamycin or nutrient starvation [28] respectively. These data indicate that TORC1, PKA and Sch9 signalling pathways have a co-ordinated and parallel action on the regulation of autophagy. The common downstream targets of PKA and Sch9 pathways, the stress resistance transcription factors Msn2/Msn4 and the protein kinase Rim15, are required for autophagy induced by inactivation of these pathways, but not for autophagy induced by inactivation of TORC1 [28]. On the other hand, autophagy induction by Sch9 or PKA inhibition is also dependent on Atg1 kinase complex [28]. Atg1 [29] and Atg13 [30], as well as other Atg proteins, have also been assigned as PKA, Sch9 and TORC1 substrates [30,31] (Figure 1). Besides

Figure 2 | **Evaluation of the autophagy activity in yeast control cells or cells expressing wild-type α-synuclein, during CLS**

(**A**) Survival, determined by colony-forming units. (**B**) Autophagy activity measured by the ALP assay. (**C**) *ATG8* mRNA levels, expressed as the ratio between the *ATG8* gene and the reference gene, *ACT1*. (**D**) Images of wild-type and Δ*tor1* cells expressing Atg8–GFP and stained with the fluorescent vacuole marked FM4–64. The images reflect the co-localization of Atg8-GFP in vacuolar membranes. Results represent means±S.E.M. for three independent experiments. Statistical significance (***$P < 0.001$) was determined by two-way ANOVA.

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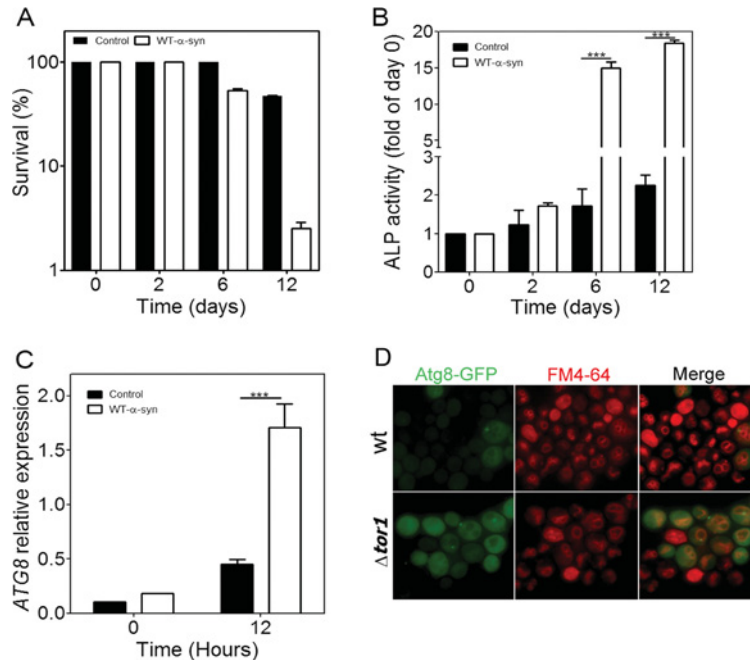
the convergence of TORC1, PKA and Sch9 pathways on common downstream targets, they also cross-talk between each other. The originally proposed yeast Akt (also known as protein kinase B) homologue, Sch9, now believed to be the S6K1 (S6 kinase 1) homologue [32], is a substrate of TORC1 and an intermediate of the signalling from TORC1 to PKA [33] (Figure 1). Nevertheless, it is still not known whether or not PKA is also a substrate of TORC1 [33].

Another important aspect to consider when analysing the regulation of autophagy and aging by nutrient-sensing pathways is the dietary intervention, CR (caloric restriction). CR has been shown to promote longevity in various species, including yeast, and is known to inactivate TOR, PKA and Sch9 signalling pathways (reviewed in [11]). Although there is no direct evidence in yeast, it can be expected that increased autophagy underlies part of the effects of CR on promoting longevity as demonstrated in other species (reviewed in [3,34]).

Although TORC1 is the most studied regulator of autophagy, it is controlled by a complex network of different pathways also implicated in aging. Molecules such as the acetylase inhibitor spermidine and the deacetylase (Sirtuin1) activator resveratrol prolong the lifespan of multiple species in a TOR-independent autophagy-dependent fashion [35]. Interestingly, in yeast the longevity effects of CR are also associated with sirtuin activity (reviewed in [3,34,36,37]) pointing to a complex and elaborated network regulating autophagy and aging.

Regulation of autophagy is a complex puzzle in which several pieces have already been entrenched in the network; nevertheless, the picture is far from being complete, since numerous components have a yet unknown place. For example, an issue that merits concern is whether the change in Atg13 phosphorylation is a crucial step in the activation of Atg1 complex given the ability a non-phosphorylable Atg13 form has to induce autophagy independent of TORC1 regulation [26]. Additionally, although the strategies to enhance longevity using autophagy as an anti-aging intervention are appealing, some crucial aspects have to be taken into consideration. The mechanisms to modulate and to keep autophagy activity at physiological levels after its induction and the effects that persistent activation of autophagy might have in aging progress remain unknown. The study of these aspects could be facilitated by using a condition of constant autophagy activation such as proteotoxic stress.

Autophagy and aging under proteotoxic stress

During CLS yeast cells do not remain tight; in contrast, they are metabolically and biosynthetically active, responding to alterations in the nutrients availability [10,17]. Accumulating evidence suggests that autophagy is required in order to regulate this phase, since during CLS the non-dividing cells are unable to dilute or to get rid of 'metabolic waste' by cell

division [8,9]. The relationship between these two processes, CLS and autophagy, is extremely complex and not fully understood and one of the most important questions relies on whether persistent autophagy induction would be an ideal anti-aging intervention [3]. Although autophagy is a well-established cytoprotective process that promotes survival of cells in metabolic distress, autophagy can also be a self-destructive process by its contribution and participation in cell death [4]. To further analyse the interplay between CLS and autophagy and to elucidate if the persistent autophagy induction indeed encloses a harmful effect, we assessed the autophagy activity during CLS of yeast cells under proteotoxic stress. The heterologous expression of proteins such as α-synuclein, an aggregation-prone protein, involved in the pathogenesis of Parkinson's disease (reviewed in [38]) that is known to induce autophagy could be helpful in clarifying some aspects of the role of autophagy in CLS. Our data show that during CLS of wild-type cells the autophagy activity (evaluated by alkaline phosphatase, ALP assay and *ATG8* mRNA expression levels) is maintained at low levels until day 12 of CLS (Figure 2). However, the heterologous expression of α-synuclein-induced toxicity and a reduction of yeast longevity associated with a pronounced increase in the autophagy activity and of the *ATG8* mRNA levels over time (Figure 2). This increased autophagy activity was coincident with the early appearance, at days 6 and 12, of cell death, described to be of an apoptotic as well as necrotic nature during CLS [39,40]. These results seem to indicate that the excessive and persistent activation of autophagy due to proteotoxic stress might lead to autophagy participation in the cell death occurring under these conditions in CLS, revealing the other face of the coin and the sinister nature of autophagy.

Conclusions

Evidence gathered from different genetic and pharmacological manipulations reveals that different signalling pathways converge on autophagy to regulate longevity. Pharmacological manipulation of autophagy appears as an attractive strategy to delay aging and has been motivating the development and search for drugs that directly or indirectly, through nutrient signalling pathways, regulate autophagy activity. Nevertheless, the pleiotropic nature of nutrient signalling pathways regulating autophagy and the dual and opposite functions, cytoprotective or detrimental according to the context, of autophagy generate serious limitations. Our data on yeast CLS under proteotoxic stress claim that the cytoprotective role of autophagy could not be generalized and that, although autophagy activity could be initially beneficial, its maintained activation is associated with cell death and shortening of lifespan (Figure 3). Among the multiple unanswered questions regarding the interplay between autophagy and aging, one that is particularly important and that requires further studies is the level of autophagy activity and the phase of CLS that should be targeted by anti-aging strategies.

Figure 3 | Role of autophagy in cell survival and death

Impaired or low autophagy activity induces cellular damage, failing the clearance of damage proteins and organelles and the energy supply for essential cell functions contributing to the aging phenotypes. Physiological levels of autophagy act as a cytoprotective mechanism and maintain cellular homoeostasis, allowing protein and organelles turnover and sustaining the energetic levels. Excessive levels of autophagy culminate in cell death.
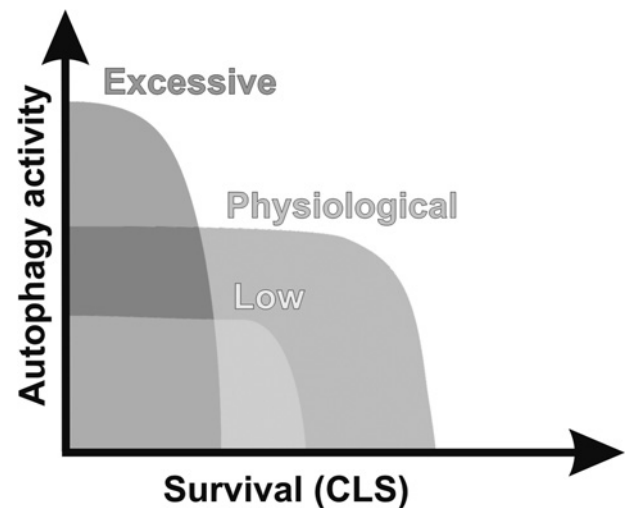
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