



FEMS Yeast Research, 18, 2018, foy043

doi: 10.1093/femsyr/foy043

Advance Access Publication Date: 14 April 2018

Minireview

MINIREVIEW

Linking cellular proteostasis to yeast longevity

Belém Sampaio-Marques^{1,2} and Paula Ludovico^{1,2,*},[†]¹Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal and ²ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal*Corresponding author: Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Tel: +00351 253604812; Fax: +00351 253604809; E-mail: pludovico@med.uminho.pt**One sentence summary:** This mini-review overviews the main mechanisms contributing for the maintenance of *Saccharomyces cerevisiae* proteostasis network. Particularly, the age-dependent changes and the multidimensional nature of the proteostasis network are highlighted.

Editor: Sergio Giannattasio

[†]Paula Ludovico, <http://orcid.org/0000-0003-4130-7167>

ABSTRACT

Proteostasis is a cellular housekeeping process that refers to the healthy maintenance of the cellular proteome that governs the fate of proteins from synthesis to degradation. Perturbations of proteostasis might result in protein dysfunction with consequent deleterious effects that can culminate in cell death. To deal with the loss of proteostasis, cells are supplied with a highly sophisticated and interconnected network that integrates as major players the molecular chaperones and the protein degradation pathways. It is well recognized that the ability of cells to maintain proteostasis declines during ageing, although the precise mechanisms are still elusive. Indeed, genetic or pharmacological enhancement of the proteostasis network has been shown to extend lifespan in a variety of ageing models. Therefore, an improved understanding of the interventions/mechanisms that contribute to cellular protein quality control will have a huge impact on the ageing field. This mini-review centers on the current knowledge about the major pathways that contribute for the maintenance of *Saccharomyces cerevisiae* proteostasis, with particular emphasis on the developments that highlight the multidimensional nature of the proteostasis network in the maintenance of proteostasis, as well as the age-dependent changes on this network.

Keywords: proteostasis; ageing; *Saccharomyces cerevisiae*; molecular chaperones; ubiquitin-proteasome system; autophagy

INTRODUCTION

Protein homeostasis or 'Proteostasis' refers to the healthy maintenance of the cellular proteome and involves highly complex and interconnected pathways that govern the fate of proteins from synthesis to degradation. Proteome fidelity is preserved through a multi-compartmental system that has the ability to coordinate protein synthesis, processing, trafficking, folding, localization, assembly/disassembly and degradation.

Proteins are permanently challenged by intrinsic (such as mutations and translational errors) and extrinsic/environmental stressors that can impair proteostasis over time. The first line of defence against loss of proteostasis relies mainly on molecular chaperones and degradation pathways

that attempt to solubilize, refold or degrade accumulated misfolded proteins. Nevertheless, age compromises the protein quality control components and promotes a general deterioration of proteostasis (Morimoto 2008; Douglas and Dillin 2010). Thus, aged cells show an enhanced incapability to maintain metastable proteins in properly folded states in the face of deteriorating proteostasis networks, initiating a cascade of protein destabilization and accumulation of misfolded protein, prone to form potentially toxic aggregates (Gidalevitz et al. 2006; Ben-Zvi, Miller and Morimoto 2009). The yeast *Saccharomyces cerevisiae* is a well-recognized ageing model that provides two distinct paradigms for ageing studies, the replicative lifespan (RLS), which represents the number of divisions that each individual cell can undergo, and the chronological lifespan

Received: 14 February 2018; Accepted: 12 April 2018

© FEMS 2018. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

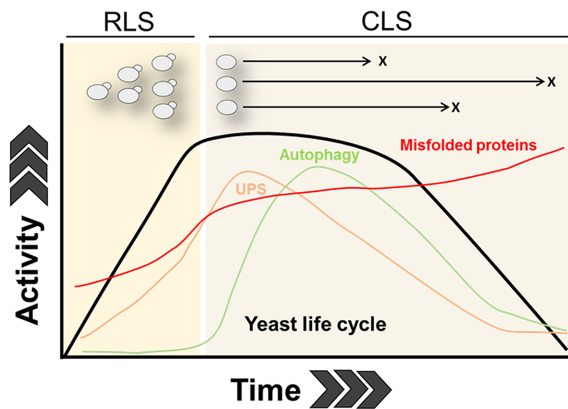


Figure 1. Temporal activity of the degradation pathways during yeast life cycle. The yeast *Saccharomyces cerevisiae* provides two distinct paradigms for ageing, the RLS and the CLS. These two distinct ageing stages are related with pronounced changes in the degradation pathways, particularly the UPS (orange line) and autophagy (green line), and the onset of protein aggregation (red line). See text for more details.

(CLS), which determines the time that cells can remain viable in a non-dividing state (Fig. 1), reviewed in (Longo et al. 2012; Sampaio-Marques, Burhans and Ludovico 2014). Herein, we present a mini-review on the molecular mechanisms contributing to maintenance of *S. cerevisiae* proteostasis network and how these processes are affected by age and contribute to the global breakdown of proteostasis during ageing.

THE YEAST PROTEOSTASIS NETWORK

Cells take advantage of three main processes to face proteotoxic stress: refolding, degradation or sequestration into spatially organized protective inclusions (Tyedmers, Mogk and Bukau 2010; Sontag, Samant and Frydman 2017). A brief overview of these three main processes operating in yeast as well as of the specific protein quality control of different organelles is presented.

Molecular chaperones

Molecular chaperones are crucial components of proteostasis network having multiple functions in different branches of the system. Molecular chaperones promote folding of newly synthesized proteins, refold of misfolded proteins and help in the degradation of aberrant proteins as well as on the formation of protective inclusions of misfolded and aggregated proteins (Fig. 2). Together with degradation pathways, molecular chaperones are the first line of defence to solubilize, refold or degrade misfolded proteins.

Many molecular chaperones are constitutively expressed, whereas others can be predominantly induced under different conditions of stress. Inducible molecular chaperones have originally been described in response to heat shock stress and are thus called heat shock proteins (HSPs), the name still given to different classes of molecular chaperones. Yet, it is currently known that molecular chaperones can also be induced as a consequence of other stressors (pH, osmotic conditions, energy depletion, ion concentration, etc.). Transcriptional regulation of molecular chaperones in yeast is accomplished by three transcription factors, Hsf1 (heat shock factor 1), Msn2 and the partially redundant Msn4 (Trott and Morano 2003).

Molecular chaperones generally recognize exposed hydrophobic regions, normally hidden in a properly folded protein, and prevent aggregation with other denatured proteins, but the mode of action varies between the different classes. Molecular chaperones also control denatured proteins by assisting on the delivering of unrepairable unfolded proteins to the proteasome for degradation (Fig. 2). The chaperone repertoire in the yeast *S. cerevisiae* proteome consists of 69 molecular chaperones and their co-chaperones that commonly assist chaperones on folding or unfolding of proteins (Voos and Rottgers 2002; Young et al. 2004; Kampinga and Craig 2010). They are classified into different families based on the molecular weight of monomers (Hsp40, Hsp60, Hsp70, Hsp90, Hsp100 and small Hsp) (Diaz-Villanueva, Diaz-Molina and Garcia-Gonzalez 2015).

Based on the sort of interaction with client proteins, chaperones are also classified as holdases, foldases and disaggregases (Diaz-Villanueva, Diaz-Molina and Garcia-Gonzalez 2015). Some chaperones, as Hsp90 and Hsp104, play relevant functions during ageing (Leak 2014). *S. cerevisiae* genome encodes for two Hsp90 homologs, Hsp82 and Hsc82, which shares almost 90% sequence identity (Borkovich et al. 1989). While HSC82 is constitutively expressed, expression of HSP82 is usually induced under stress conditions (Borkovich et al. 1989). The two Hsp90 isoforms have also been shown to have distinct and opposite roles on the control of selective translation during stress and cell death (Silva et al. 2013). The Hsp90 chaperone activity is particularly relevant, since it acts on nascent, partially folded proteins (Li, Soroka and Buchner 2012). Nevertheless, it is not able to refold denatured proteins, instead it needs the participation of other chaperones to complete the task, particularly Hsp40, which binds to unfolded proteins, and Hsp70 that under stress conditions solubilizes protein aggregates and refolds aggregated proteins (Ran, Bali and Michels 2008; Kampinga and Craig 2010; Sakurai and Ota 2011). Importantly, HSP90 genes are under control of Hsf1, and the essential roles of this transcriptional factor in response to various conditions including proteotoxic stress and ageing are partially mediated by Hsp90 together with five additional mitochondrial HSP genes (Sakurai and Ota 2011). Several observations have implicated Hsf1 in the regulation of longevity in yeast (Harris et al. 2001; Cheng et al. 2007) as well as in other ageing models as *Caenorhabditis elegans* (Morley and Morimoto 2004). Besides Hsp90, the yeast molecular chaperone Hsp104, a member of the Hsp100 family, is also considered a chaperone with a role in the modulation of chronological ageing (Speldewinde and Grant 2017). HSP104 expression is induced by a variety of environmental stresses and when cells enter stationary phase or sporulate (Sanchez and Lindquist 1990; Sanchez et al. 1992). In contrast to Hsp90, Hsp104 cannot prevent aggregation of denatured proteins but acts by re-solubilizing aggregated proteins in cooperation with the Hsp70 family of chaperones and co-chaperones (Parsell et al. 1994; Glover and Lindquist 1998). Hsp104 can also interact with Hsp26, along with other small HSPs, such as Hsp42, which assist in the refolding of the aggregation-prone proteins (Cashikar, Duennwald and Lindquist 2005). The complex constituted by Hsp104 and Hsp70 was recently found to be recruited by the peroxiredoxin Tsa1 and to mediate the disaggregation of the H₂O₂-generated aggregates that are formed during ageing, leading to ageing delay (Hanzen et al. 2016). Furthermore, Hsp104 seems to have a role on the asymmetric inheritance of aggregates by mother cells during division and thus, its deletion results in a shortened RLS (Erjavec et al. 2007). In the yeast models of Huntington's disease, in contrast to the transgenic mice model, Hsp104 has a paradoxical function, promoting the aggregation

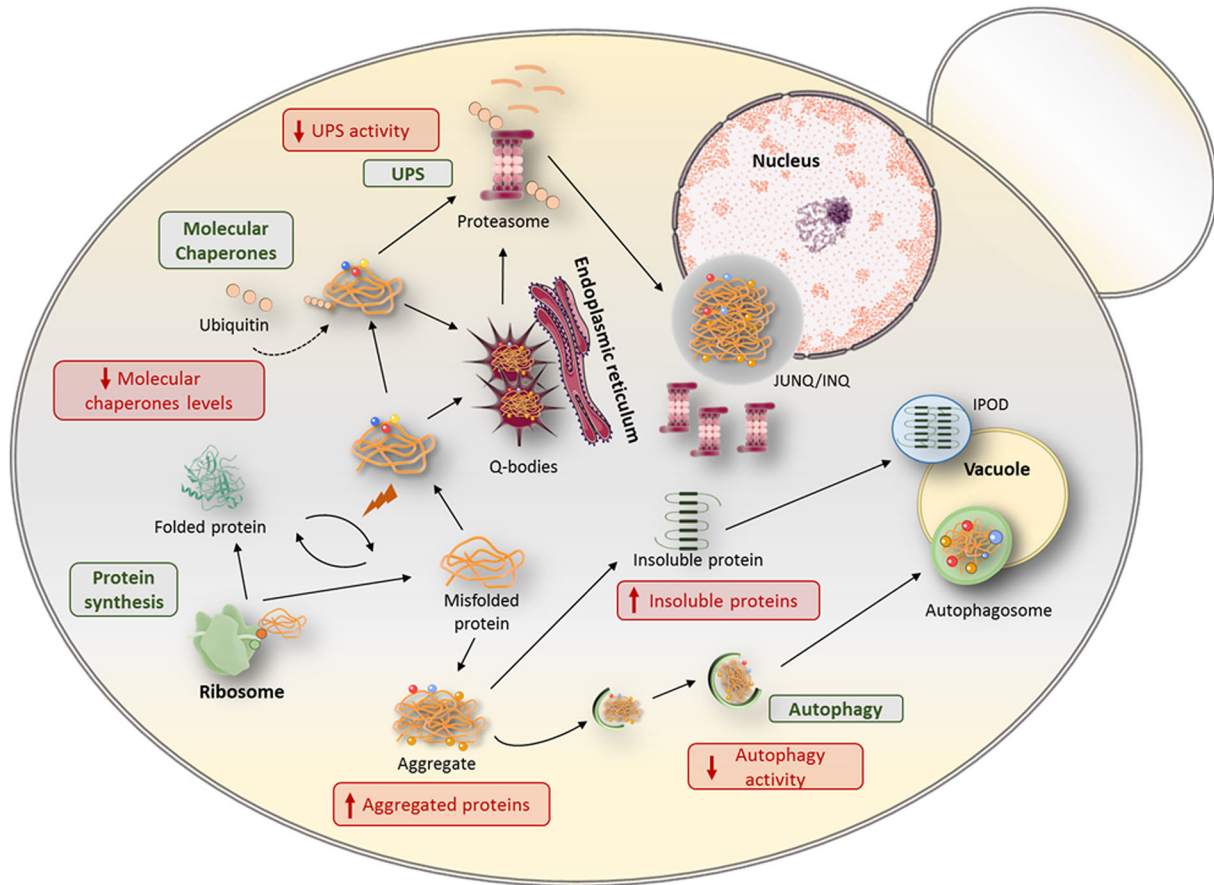


Figure 2. Impact of ageing in the yeast proteostasis network. Proteostasis is maintained essentially by the action of molecular chaperones and the two degradation pathways, the UPS and autophagy. Molecular chaperones assist de novo protein synthesis and promote protein refolding or prevent aggregation of misfolded or damaged proteins. In the presence of misfolded proteins, molecular chaperones associate with them to promote their refolding. When protein refolding is not possible, molecular chaperones target the unfolded protein for degradation by the proteasome, after ubiquitination, or by vacuoles. Once misfolded proteins organize into insoluble aggregates, they are target to be degraded by autophagy. Misfolded and aggregated proteins can also be sequestered into larger spatially organized protective inclusions: the JUNQ/the INQ and the peripheral vacuole-associated, IPOD. Red boxes indicate the changes in the proteostasis network machinery promoted by ageing. See text for more details.

and toxicity of polyglutamine (polyQ) (Krobitsch and Lindquist 2000).

In summary, the observations described above support the important functions of molecular chaperones on the maintenance of protein folding and cellular homeostasis required for longevity.

Degradation pathways

Molecular chaperones are crucial players to refold and/or to redirect non-native proteins toward degradation pathways, the ubiquitin-proteasome system (UPS) and autophagy. Protein degradation is a ubiquitous process critical for cell repair and survival, regulating several processes such as cell cycle, signaling, DNA transcription, repair and translation, among others. Protein degradation is particularly relevant during ageing, a scenario of cellular waste accumulation, where degradation pathways have to assist on the cleaning of ageing-accelerating factors.

The UPS mainly serves to target individual proteins to the proteasome, while autophagy clears larger aggregates or membrane-associated proteins (Menzies, Fleming and Rubinsztein 2015; Kwon and Ciechanover 2017). Both degra-

dition pathways allow the recycling of macromolecules and energy, being central in the regulation and maintenance of cellular homeostasis.

The ubiquitin-proteasome system

The primary route for the degradation of numerous short-lived proteins is the ubiquitination-dependent degradation by the proteasomal machinery, UPS, which consists of both substrate-recruiting and substrate-degrading machinery (Low 2011). Proteasome is organized into the 19S regulatory subunit, which recognizes substrates to be degraded, and the 20S core subunit that contains the proteolytic active sites (Groll et al. 1997). The mechanism of degradation assured by the proteasome is linked to the ubiquitin system, which consists of a cascade of enzymes that targets the proteins to be degraded with ubiquitin (Finley et al. 2012) (Fig. 2). Covalent attachment of ubiquitin to a substrate is catalyzed by the E1-E2-E3 enzyme cascade, reviewed in Finley et al. (2012). The reaction is initiated by the ubiquitin-activating enzyme E1 (Uba1) and activated ubiquitin is transferred to one of the ubiquitin-conjugating enzymes (E2 or Ubc enzymes). Finally, E3 enzymes (ubiquitin ligases) catalyze the formation of isopeptide bonds between lysine residues in substrate proteins and the activated carboxyl group of ubiquitin. The conjugation

machinery shows hierarchical organization and in yeast is composed by one E1, 11 E2s and 60–100 E3s enzymes reviewed in Finley *et al.* (2012).

Multiple repetitions of the ubiquitin-conjugation process results in the polyubiquitination of the targeted substrate (Finley *et al.* 2012). The polyubiquitin-tagged substrates are recognized by the 19S proteasome subunit that removes the molecules of ubiquitin (Ub) and partially unfolds the substrates, allowing the substrates entrance and proteolysis within the 20S core (Tanaka 2009) (Fig. 2). Alongside this process, Ub can also be removed by deubiquitinating enzymes (DUBs), which do not integrate the 19S proteasome. Besides recycling Ub, these DUBs also function as a regulatory mechanism where proteins can be rescued from degradation and reactivated (Finkel, Deng and Mostoslavsky 2009; Oling *et al.* 2014). Several reports on different human tissues, senescent primary cultures and whole organisms suggest that the maintenance of proteasome activity is directly correlated with longevity, reviewed in Saez and Vilchez (2014). In *S. cerevisiae*, an enhanced preservation of proteasome-mediated protein degradation promoted by the overexpression of Ump1, a protein needed for proteasome assembly, increased viability during chronological ageing (Chen *et al.* 2006). In another study, proteasome activity was enhanced by increasing the levels of the transcription factor Rpn4, which regulates the levels of proteasome subunits (Dohmen, Willers and Marques 2007). This modulation resulted in increased RLS and resistance to proteotoxic stress (Kruegel *et al.* 2011). Calorie restriction, an intervention known to extend the maximum lifespan of a variety of species including *S. cerevisiae* (Lee and Longo 2016), was found associated with increased proteasome activity and maintenance of UPS activity (da Cunha, Demasi and Kowaltowski 2011). Accumulating evidence indicates that the UPS, particularly the proteasome, is a key component of the ageing process.

Autophagy

Autophagy refers to the vacuolar/lysosomal degradation of intracellular macromolecular components. Autophagy was initially viewed as a non-selective bulk process induced by starvation. However, this process is now recognized as a central regulator of cellular homeostasis with intricate links to cell metabolism and growth control. Autophagy is a determinant factor on cell survival and death decisions with a major impact on ageing (Reggiori and Klionsky 2013).

In yeast, autophagy can be divided into two main types, microautophagy and macroautophagy, hereafter named autophagy, and both of them include nonselective and selective processes (Shintani and Klionsky 2004). In microautophagy, the cargo is sequestered directly by the vacuole membrane, and the following invagination leads to its delivery into the vacuole lumen. In contrast, during autophagy the cargo is sequestered within cytosolic double-membrane vesicles that subsequently fuse with the vacuole, allowing breakdown of the cargo and recycling of the resulting macromolecules (Eskelinen *et al.* 2011). The budding yeast *S. cerevisiae* has also another type of selective autophagy—the cytoplasm to vacuole targeting (Cvt) pathway—that operates in nutrient-rich conditions. Cvt pathway is usually responsible for constitutive and selective transport of vacuolar hydrolases such as α -mannosidase (Ams1) and aminopeptidase I (Ape1) to the vacuoles using many of the same molecular components as autophagy. Therefore, this pathway is considered a biosynthetic rather than a degradative pathway (Scott *et al.* 1997; Lynch-Day and Klionsky 2010).

A hallmark of autophagy is the formation of an autophagosome, a double-membrane vesicle that sequesters cytosol and

organelles, that fuses with the vacuole releasing the content to be degraded. This process is supported by two main groups of genes, autophagy-related genes (ATG) and vacuolar protein sorting genes. Initiation of autophagy is tightly regulated by Atg1 complex that responds to nutrient starvation by recruiting multiple Atg proteins to the pre-autophagosomal structure (Reggiori and Klionsky 2013; Tyler and Johnson 2018). After initiation, autophagy proceeds with vesicle nucleation, expansion and completion ending in its fusion with the vacuole (Fig. 2).

Selective autophagy encompasses the degradation of specific cargos such as peroxisomes by pexophagy, mitochondria by mitophagy or endoplasmic reticulum (ER) by reticulophagy, reviewed in (Galluzzi *et al.* 2017). The selective degradation of yeast peroxisomes (micropexophagy) or even nuclear regions (piecemeal microautophagy) can be also performed by microautophagy (Klionsky *et al.* 2007). Overall, selective autophagy, including the Cvt pathway, is largely similar to non-selective autophagy but relies on cargo-specific receptors that interact with Atg8.

Mounting evidence indicates that functional autophagy is essential for longevity in several scenarios, reviewed in Sampaio-Marques, Burhans and Ludovico (2014), Madeo *et al.* (2015), and Tyler and Johnson (2018). Importantly, lifespan and autophagy share as common regulators the major nutrient-sensing pathways. In fact, many of the interventions that extend lifespan of diverse organisms decrease activity of nutrient-signaling pathways. These pathways, thus, well-recognized as 'longevity pathways', are important regulators of autophagy that in turn are essential for the maintenance of the proteome and a healthy cellular ageing (Sampaio-Marques, Burhans and Ludovico 2014). This has recently been nicely reviewed by Tyler and Johnson (2018).

Besides the genetic abrogation (Powers *et al.* 2006) and the inhibition by rapamycin (Alvers *et al.* 2009) of the TOR signaling, other interventions known to extend yeast lifespan, such as calorie restriction or treatment with spermidine or resveratrol, are dependent on autophagy induction (Eisenberg *et al.* 2009; Morselli *et al.* 2011). Autophagy also plays a role in the extension of CLS by methionine restriction, a dietary intervention that has been demonstrated to extend the lifespan of a variety of multicellular model organisms, including yeast (Johnson and Johnson 2014; Ruckenstuhl *et al.* 2014). Consistently, a large-scale screen for genes that regulate longevity in *S. cerevisiae* identified several autophagy gene mutants with reduced lifespan (Matecic *et al.* 2010). Epigenetic regulation of yeast lifespan has also been described as dependent on autophagy. In this context, we have shown that Sir2 is an important regulator of autophagy during ageing of alpha-synuclein expressing cells (Sampaio-Marques *et al.* 2012; Guedes, Ludovico and Sampaio-Marques 2017). Studies by Eisenberg *et al.* (2014) showed that accumulation of acetyl-coenzyme A downregulates the transcription of different ATG genes during ageing due to histone hyperacetylation.

The contribution of autophagy to lifespan extension is associated with maintenance and/or improvement of several cellular processes. Autophagy is involved in the turnover and clearance of organelles and aberrant damaged or toxic macromolecules; enhances the resistance to oxidative stress, through regulation of ROS production/accumulation; modulates the DNA damage response by the regulation of several processes including cell cycle checkpoints and DNA repair, among others, reviewed in Tyler and Johnson (2018). This brief review demonstrates that autophagy has a deep impact on ageing modulation and longevity extension.

Spatial protein quality control

As described above, cells have evolved an elaborated proteostasis network to preserve the functionality of their proteomes. Nevertheless, while the chaperones and degradation systems are the first line of defence for repair or elimination of misfolded proteins providing a temporal quality control, cells also possess a spatial protein quality control system. Accumulating evidence suggests that damaged proteins are not randomly distributed inside the cells—instead, misfolded and aggregated proteins are sequestered into larger protective inclusions that are spatially organized (Alberti 2012). This spatial protein quality control limits the toxicity of damaged and misfolded/aggregated proteins and guarantees that the damage is not inherited by succeeding cell generations (Hill, Hanzen and Nystrom 2017).

An early cellular event of spatial protein quality control, occurring immediately upon misfolding, seems to be the active and chaperone dependent (such as sHsps, Hsp40, Hsp70 and Hsp104; Kaganovich et al. 2008; Specht et al. 2011; Escusa-Toret, Vonk and Frydman 2013) transport of damaged proteins to dynamic compartments called Q-bodies, which are attached to the ER en route to clearance by the UPS (Escusa-Toret, Vonk and Frydman 2013). The damaged proteins inside of these Q-bodies are rapidly cleared through the UPS, but if clearance is impaired, these misfolded proteins concentrate in a protein quality control compartment (Kaganovich et al. 2008; Escusa-Toret, Vonk and Frydman 2013) (Fig. 2). Two major protein quality control compartments sequester misfolded cytosolic proteins, the juxta nuclear quality control site (JUNQ) and the peripheral vacuole-associated, insoluble protein deposit (IPOD) (Kaganovich et al. 2008; Tyedmers et al. 2010; Chen et al. 2011). A ubiquitin-based sorting mechanism targets misfolded proteins to JUNQ, while non-ubiquitinated proteins are sent to IPOD (Kaganovich et al. 2008). However, the disaggregase Hsp104-Hsp70 complex is also associated with this JUNQ (Specht et al. 2011), raising the hypothesis that sequestration of misfolded protein precedes the determination of their proteasomal degradation or chaperone-assisted refolding. Damaged proteins can travel in and out of the JUNQ; thus, the JUNQ is considered a highly dynamic deposit, with rapid protein turnover due to the high concentration of proteasomes (Kaganovich et al. 2008). The IPOD is in contrast less dynamic, and is typically formed upon prolonged stress, and remains longer after stress relief. More recently, JUNQ was found inside the nucleus defining a new intranuclear quality control compartment (INQ) for the deposition of both nuclear and cytosolic misfolded proteins, irrespective of ubiquitination (Miller et al. 2015) (Fig. 2). It is now well established that chaperones and co-chaperones assist the sorting of proteins to quality control compartments. Hsp42 is required for sorting proteins into peripheral inclusions and IPOD, while the co-chaperone Sis1 delivers misfolded proteins to the nucleus for degradation (Specht et al. 2011; Park et al. 2013). The v-SNARE binding protein Btn2 and its paralog Cur1 are crucial to regulate protein sorting to inclusions (Malinowska et al. 2012; Miller et al. 2015). Btn2 can either directly interact with Hsp42 directing the substrates to IPOD or bind to Sis1 promoting the sorting to JUNQ (Malinowska et al. 2012). In these events, Btn2 and Hsp42 work as aggregases, assisting inclusion formation of misfolded substrates in the nucleus and cytoplasm, respectively. Cur1 in turn regulates Sis1 availability in the cytosol. It binds and recruits Sis1 to the nucleus without substrates, allowing the interaction between the free substrates and Btn2/Hsp42 in cytosol (Alberti 2012; Nystrom and Liu 2014).

The molecular principles governing the sorting and distribution of misfolded, damaged and aggregation prone-proteins are not fully resolved. Yet, it is evident that the cell uses specific factors in a dynamic fashion to be as efficient as possible on the management of misfolded proteins and in maintaining cellular homeostasis.

Organelle protein quality control

A communication between the cytoplasm proteostasis network and the specific protein quality control of different organelles seems to be relevant for the maintenance of proteostasis. Nevertheless, little is known about the factors involved in this communication as well as about the hierarchical order whereby preservation of proteostasis in some compartments is prioritized.

The specific protein quality control system of ER and mitochondria are among the most well studied and explored. The ER is an organelle hosting protein synthesis and thus needs a tightly protein-quality surveillance (Walter and Ron 2011). Newly synthesized secretory and membrane proteins that do not pass ER quality control are usually retained by ER resident chaperones and routed to ER-associated degradation (ERAD) pathways (Hegde and Ploegh 2010). However, when nascent unfolded and misfolded proteins increase and overcome ER processing capacity, a process known as the unfolding protein response (UPR), a signaling pathway that transcriptionally up-regulates genes involved in expanding the ER protein folding capacity, is activated. In yeast, UPR signaling pathway is triggered by the ER-located transmembrane protein Ire1, which promotes splicing of the HAC1 premRNA (HAC1u) to produce the translatable transcription factor mRNA (HAC1i) (Kimata et al. 2006). This transcriptional factor induces the up-regulation of the expression of several genes involved in protein processing and maturation in an attempt to maintain homeostasis. Yet, if the amount of ER unfolded proteins exceeds the ability of the UPR to restore homeostasis, unfolded proteins will be exported to the cytosol (Brodsky 2012). A novel mechanism to eliminate misfolded glycosylphosphatidylinositol (GPI)-anchored proteins via the secretory pathway, termed 'rapid ER stress-induced export' (RESET) has been described (Satpute-Krishnan et al. 2014). RESET involves the export of misfolded GPI proteins to the plasma membrane for subsequent degradation by the lysosome. In yeast, this secretory pathway is dependent on two GPI-linked aspartyl proteases (Yps1/2) and components of the UPR (Ire1 and Hac1) but is independent of ERAD elements (Miller, DiDone and Krysan 2010).

The maintenance of mitochondrial proteostasis is particularly challenging due to the oxidative environment of this organelle. The first line defence against loss of mitochondria proteostasis involves molecular chaperones, which have been conserved from bacteria to higher eukaryotic cells, as well as various proteolytic enzymes, reviewed in Baker, Tatsuta and Langer (2011). The vast majority of the mitochondrial proteome is synthesized in the cytosol and imported, in a relatively unfolded state, with guidance by members of the Hsp70 and Hsp90 family, into the organelle. Once inside mitochondria, additional chaperones aid the correct folding of imported proteins. There are two major matrix mitochondrial chaperone systems—mtHsp70, part of pre-sequence translocase-associated import-motor (PAM) complex, which directly folds the incoming proteins, and multimeric HSP60–HSP10 chaperonins that also assist on folding of proteins to their native state (reviewed in Chacinska et al. 2009).

Complementary to the chaperones' action to maintain mitochondria proteostasis, specific mitochondrial proteases recognize and degrade proteins that fail to fold or assemble properly (Tatsuta and Langer 2008). The Lon protease family resides in the mitochondrial matrix and is required for the removal of oxidatively damaged proteins (Suzuki et al. 1994; Bota and Davies 2002). Importantly, Lon seems to impact fungal ageing given that its overexpression in *Podospora anserina* results in an increased lifespan (Luce and Osiewacz 2009). The mitochondrial inner membrane has different classes of proteases being the two AAA + metalloprotease complexes highly relevant to ensure protein quality control (Koppen and Langer 2007).

The mitochondrial protein quality control is also influenced by the UPS and includes a mitochondria-specific unfolded protein response (UPRmt) (Heo et al. 2010; Braun et al. 2015). The UPRmt senses the perturbations that overload mitochondria quality control network capacities, and activates the transcription of nuclear-encoded protective genes in order to re-establish mitochondrial homeostasis (Haynes and Ron 2010).

Another level of mitochondrial protein quality control is provided by the dynamic nature of this organelle and the fusion and fission phenomenon. Mitochondrial fusion allows dysfunctional damaged mitochondria to fuse with healthy neighbor mitochondria facilitating quality control and diluting the toxic effects of the accumulation of misfolded/aggregated proteins. In contrast, fission allows for the separation of damaged mitochondria and to promote their turnover through mitophagy (Wang and Klionsky 2011). Mitophagy, which is the selective degradation of mitochondria by autophagy, is indeed the most common route for degradation of dysfunctional mitochondria (Lemasters 2014). Several studies reported that the impairment of mitophagy and consequently mitochondrial clearance, in aged cells, is the basis of several neurodegenerative disorders. Finally, a last level of quality control occurs when extensive mitochondrial damage promotes the release of death factors that result in turnover of the entire cell (Baker, Tatsuta and Langer 2011).

AGE-RELATED EFFECTS ON PROTEOME INTEGRITY AND PROTEOSTASIS NETWORK

Ageing is a complex and multifactorial physiological process that results in the progressive accumulation of molecular alterations associated with the decline of different cellular functions. Loss of proteostasis, promoted among other factors by the accumulation of misfolded and damaged proteins, is proposed to be one of the primary hallmarks of ageing (Lopez-Otin et al. 2013) observed in different ageing models (David et al. 2010; Reis-Rodrigues et al. 2012; Sampaio-Marques, Burhans and Ludovico 2014). Accumulation of these aberrant proteins predisposes to the formation of aggregates that are potentially toxic to the cell. Protein aggregates are associated with several neurodegenerative diseases but their role in physiological ageing remains largely unexplored. Nevertheless, studies in *C. elegans* suggest that the decline in proteome integrity may not be caused by a random and gradual accumulation of molecular damage—instead, it can be the result of early programmed events (Benzvi, Miller and Morimoto 2009). Further contributing to loss of proteostasis during ageing is the decline of the protein quality control machinery (Fig. 1). The functioning of molecular chaperones, the first line of defence of the proteostasis network, is impaired by ageing. Particularly the chaperones with ATP-dependent activity are repressed due to the limited amounts of ATP available in aged cells (Stroo et al. 2017). The activity of

molecular chaperones could also be affected by their availability, which does not meet the needs of aged cells, and by the lack of substrate recognition due to undesired ageing protein alterations (Csermely 2001; Nardi et al. 2006; Kumar, Kumar and Reddy 2007). Consistently, recent studies in *C. elegans*, demonstrated that during ageing a more prominent function for sHSPs, ATP-independent, is observed in the preservation of proteostasis, instead of the HSP70 and HSP90 families (Walther et al. 2015).

The age-associated alterations on proteostasis are not restricted to molecular chaperones—the degradation pathways, UPS and autophagy, are also affected by age. Aged cells display decreased proteasome subunit expression associated with alterations and/or replacement of proteasome subunits culminating in decreased UPS activity throughout lifespan (Carrard et al. 2002; Vernace, Schmidt-Glenewinkel and Figueiredo-Pereira 2007). It is also suggested that the factors required for UPS activity, such as Ub ligases/proteases or proteasome activators, might be sequestered in aggregates during ageing (Andersson et al. 2013). Consistently, our experimental results showed an accumulation of ubiquitinated proteins associated with an increase in levels of RPN4 along chronological ageing, reflecting an impairment of proteasome activity (Figs 1 and 3). The relationship between UPS and ageing is nicely shown by the genetic studies performed in *S. cerevisiae* demonstrating that abrogation of RPN4 proteasome assembly factor promotes a shortening of RLS (Kruegel et al. 2011), while overexpression of proteasome-related genes, like the UMP1, increases longevity (Chen et al. 2006). Caloric restriction has been described as an inducer of UPS activity and enhanced UPS activity is linked to increased lifespan in yeast, worms, rodents and humans (Carrano et al. 2009; da Cunha, Demasi and Kowaltowski 2011; Kruegel et al. 2011; Vilchez et al. 2012). In aged yeast cells, the proteasome activity is decreased and the ubiquitination process is impaired but caloric restriction is able to prevent/delay these effects that occur during ageing (da Cunha, Demasi and Kowaltowski 2011).

As mentioned above, the other main degradation pathway, autophagy, is also affected during ageing. Similar to UPS, decreased autophagy during ageing has been reported in different model systems, reviewed in Rubinsztein, Marino and Kroemer (2011). However, due to the autophagy process complexity and the stochastic nature of ageing, the mechanisms underlying the decreased autophagy remain largely unclear. Nevertheless, down-regulation of the autophagy genes ATG5 and ATG7 was reported in human brain during normal ageing (Lipinski et al. 2010; Martinez-Lopez, Athonvarangkul and Singh 2015) and decreased autophagic activity have been detected in rodent models (Bergamini et al. 1993; Donati et al. 2001). The mechanisms responsible for the decrease in ATG levels remain to be elucidated. Besides the molecular alterations observed in autophagy, an expansion of lysosomal compartments, accumulation of autophagic vacuoles, as well as the deposition of autofluorescent pigment lipofuscin was observed in aged cells of different tissues (brain, heart, muscle and kidney) (Donati et al. 2001; Del Roso et al. 2003). These alterations in the lysosome led to a decreased lysosomal activity due to a dysfunctional proteolytic activity, which resulted in the decline of autophagic activity (Donati et al. 2001; Del Roso et al. 2003). In *S. cerevisiae*, vacuolar homeostasis is also affected during ageing due to increased vacuolar pH that limits the activities of vacuolar proteases, resulting in impaired autophagy (Nakamura et al. 1997) (Fig. 1). Since pharmacologic or genetic inhibition of autophagy induces changes that resemble the phenotype associated with ageing (Simonsen et al. 2008; Toth et al. 2008; Matecic et al. 2010), it is assumed that decline of autophagy promoted by ageing will impact on

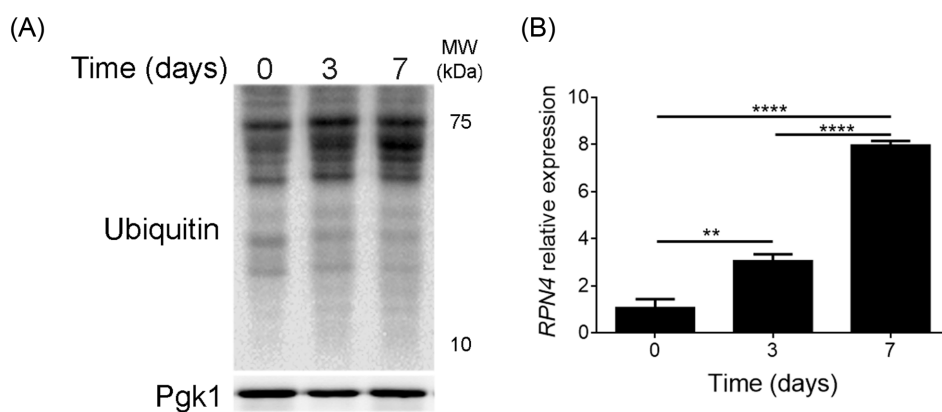


Figure 3. Proteasome activity is decreased during yeast ageing. (A) Ubiquitination profile and (B) RPN4 mRNA relative expression levels of wild type BY4742 yeast cells during CLS. Three reference genes (*ACT1*-actin, *PDA1*-alpha subunit of pyruvate dehydrogenase and *TDH2*-isoform 2 of glyceraldehyde-3-phosphate dehydrogenase) were used as internal standards and for the normalization of mRNA expression levels. Data represent mean \pm SEM of three biological independent replicates. The error bars represent the standard error of the mean (SEM). Significance of the data was determined by one-way ANOVA (**** $P < 0.0001$; ** $P < 0.01$).

the normal cell functioning converging in the different aspects of the ageing phenotype. Thus, this age-dependent decline of autophagy may cause the build-up of severely accumulation of aberrant proteins/aggregates, further causing additional molecular and cellular damage with age, as a vicious cycle.

Deregulation of autophagy in old cells can also be a consequence of persistent activity stimulation. Although increased autophagy might initially have a favorable outcome, its maintained activation can lead to the depletion of functional organelles/protein and essential autophagic molecules contributing to cell death and shortening of lifespan (Meijer and Codogno 2007; Sampaio-Marques et al. 2012).

The cell's capacity to maintain the spatial protein quality control and to form the above described JUNQ/INQ or IPOD inclusions declines with ageing (Yang et al. 2016). Furthermore, the nature of the inclusions formed during ageing is apparently distinct from JUNQ/INQ or IPOD, representing another form of quality control site (Saarikangas and Barral 2015). The cause of the impairment of the spatial protein quality control is not well disclosed, but apparently it could be the result of the collapse of multiple systems that also fails during ageing (Dillin, Gottschling and Nystrom 2014). Particularly, an enhancement of aggregated proteins and the decline of the proteostasis network could underlie the age-associated loss of spatial protein quality control (Shiber et al. 2013). In addition, the homeostasis changes that some organelles experience could be also linked. The increased cytosolic acidification, due to the alteration on the vacuolar pH, might impact on the protein stability, ultimately causing the collapse of the spatial protein quality control. The loss of vacuole acidification detected during ageing can lead to the breakdown of the vesicle trafficking and fusion with the vacuole (Baars et al. 2007), central processes for spatial sequestration of aggregated proteins (Kumar, Nawroth and Tyedmers 2016). Furthermore, the disruption of a functional actin cytoskeleton that occurs during ageing can also impair the trafficking and fusion of vesicles, promoting the inefficient inclusion formation that is observed in ageing cells (Gourlay et al. 2004).

CONCLUSIONS

Mounting evidence suggests that maintenance of proteostasis is fundamental to delay ageing. Loss of proteostasis accelerates ageing—thus it is expected that genetic or pharmacological

enhancement of the proteostasis network might extend lifespan and suppress age-related phenotypes. Indeed, the genetic enhancement of the expression or activity of individual proteostasis components is associated with the suppression or the delays of the ageing and age-associated diseases onset. The pharmacological development and use of small molecules that can enhance the expression of molecules or pathways of proteostasis network might provide effective strategies for promoting proteostasis. One of the previously employed strategies is the activation of Hsf1, which induces the simultaneous expression of multiple molecular chaperones. This approach is usually accomplished through the pharmacologic inhibition of Hsp90 with radicicol, geldanamycin, or 17-AAG (Sittler et al. 2001; Hay et al. 2004; Waza et al. 2005; Fujikake et al. 2008). In fact, this strategy was previously shown to assist in the suppression of polyQ aggregation and toxicity several models, including *Drosophila melanogaster*, *C. elegans* and mouse models of disease (Sittler et al. 2001; Auluck and Bonini 2002; Fujikake et al. 2008; Labbadia et al. 2011). Nevertheless, inhibition of Hsp90 is not a viable option due to the central role of Hsp90 in numerous essential cellular processes (Taipale, Jarosz and Lindquist 2010). Therefore, new strategies target to the suppression or the delay of ageing and age-associated diseases are urgently needed. Part of the molecular mechanisms responsible for proteostasis network remodeling and the signals that promote the age-associated changes remain unknown. Therefore, a more complete understanding of the age-dependent repertoire of proteostasis network alterations as well as the signaling pathways responsible for those alterations could have a tremendous impact on our ability to modulate the ageing process and allow the development of new strategies to successfully improve health ageing.

FUNDING

This work was developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER). BSM is supported by the fellowship SFRH/BPD/90 533/2012 funded by the Fundação para a Ciência e Tecnologia (FCT, Portugal).

Conflict of interest. None declared.

REFERENCES

- Alberti S. Molecular mechanisms of spatial protein quality control. *Prion* 2012;**6**:437–42.
- Alvers AL, Wood MS, Hu D et al. Autophagy is required for extension of yeast chronological life span by rapamycin. *Autophagy* 2009;**5**:847–9.
- Andersson V, Hanzen S, Liu B et al. Enhancing protein disaggregation restores proteasome activity in aged cells. *Aging* 2013;**5**:802–12.
- Auluck PK, Bonini NM. Pharmacological prevention of Parkinson disease in *Drosophila*. *Nat Med* 2002;**8**:1185–6.
- Baars TL, Petri S, Peters C et al. Role of the V-ATPase in regulation of the vacuolar fission fusion equilibrium. *Mol Biol Cell* 2007;**18**:3873–82.
- Baker MJ, Tatsuta T, Langer T. Quality control of mitochondrial proteostasis. *Cold Spring Harb Perspect Biol* 2011;**3**:a007559.
- Ben-Zvi A, Miller EA, Morimoto RI. Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc Natl Acad Sci* 2009;**106**:14914–9.
- Bergamini E, Del Roso A, Fierabracci V et al. A new method for the investigation of endocrine-regulated autophagy and protein degradation in rat liver. *Exp Mol Pathol* 1993;**59**:13–26.
- Borkovich KA, Farrelly FW, Finkelstein DB et al. hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. *Mol Cell Biol* 1989;**9**:3919–30.
- Bota DA, Davies KJ. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. *Nat Cell Biol* 2002;**4**:674–80.
- Braun RJ, Sommer C, Leibiger C et al. Accumulation of basic amino acids at mitochondria dictates the cytotoxicity of aberrant ubiquitin. *Cell Rep* 2015;**10**:1557–71.
- Brodsky JL. Cleaning up: ER-associated degradation to the rescue. *Cell* 2012;**151**:1163–7.
- Carrano AC, Liu Z, Dillin A et al. A conserved ubiquitination pathway determines longevity in response to diet restriction. *Nature* 2009;**460**:396–9.
- Carrard G, Bulteau AL, Petropoulos I et al. Impairment of proteasome structure and function in aging. *Int J Biochem Cell Biol* 2002;**34**:1461–74.
- Cashikar AG, Duennwald M, Lindquist SL. A chaperone pathway in protein disaggregation. *J Biol Chem* 2005;**280**:23869–75.
- Chacinska A, Koehler CM, Milenkovic D et al. Importing mitochondrial proteins: machineries and mechanisms. *Cell* 2009;**138**:628–44.
- Chen B, Retzlaff M, Roos T et al. Cellular strategies of protein quality control. *Cold Spring Harb Perspect Biol* 2011;**3**:a004374.
- Chen Q, Thorpe J, Dohmen JR et al. Ump1 extends yeast lifespan and enhances viability during oxidative stress: central role for the proteasome? *Free Radic Biol Med* 2006;**40**:120–6.
- Cheng C, Fabrizio P, Ge H et al. Inference of transcription modification in long-live yeast strains from their expression profiles. *BMC Genomics* 2007;**8**:219.
- Csermely P. Water and cellular folding processes. *Cell Mol Biol (Noisy-le-grand)* 2001;**47**:791–800.
- da Cunha FM, Demasi M, Kowaltowski AJ. Aging and calorie restriction modulate yeast redox state, oxidized protein removal, and the ubiquitin–proteasome system. *Free Radic Biol Med* 2011;**51**:664–70.
- David DC, Ollikainen N, Trinidad JC et al. Widespread protein aggregation as an inherent part of aging in *C. elegans*. *PLoS Biol* 2010;**8**:e1000450.
- Del Roso A, Vittorini S, Cavallini G et al. Ageing-related changes in the in vivo function of rat liver macroautophagy and proteolysis. *Exp Gerontol* 2003;**38**:519–27.
- Diaz-Villanueva JF, Diaz-Molina R, Garcia-Gonzalez V. Protein folding and mechanisms of proteostasis. *Int J Mol Sci* 2015;**16**:17193–230.
- Dillin A, Gottschling DE, Nystrom T. The good and the bad of being connected: the integrons of aging. *Curr Opin Cell Biol* 2014;**26**:107–12.
- Dohmen RJ, Willers I, Marques AJ. Biting the hand that feeds: Rpn4-dependent feedback regulation of proteasome function. *Biochim Biophys Acta* 2007;**1773**:1599–604.
- Donati A, Cavallini G, Paradiso C et al. Age-related changes in the autophagic proteolysis of rat isolated liver cells: effects of antiaging dietary restrictions. *J Gerontol Ser A: Biol Sci Med Sci* 2001;**56**:B375–83.
- Donati A, Cavallini G, Paradiso C et al. Age-related changes in the regulation of autophagic proteolysis in rat isolated hepatocytes. *J Gerontol Ser A: Biol Sci Med Sci* 2001;**56**:B288–93.
- Douglas PM, Dillin A. Protein homeostasis and aging in neurodegeneration. *J Cell Biol* 2010;**190**:719–29.
- Eisenberg T, Knauer H, Schauer A et al. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* 2009;**11**:1305–14.
- Eisenberg T, Schroeder S, Andryushkova A et al. Nucleocytosolic depletion of the energy metabolite acetyl-coenzyme a stimulates autophagy and prolongs lifespan. *Cell Metabolism* 2014;**19**:431–44.
- Erjavec N, Larsson L, Grantham J et al. Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. *Genes Develop* 2007;**21**:2410–21.
- Escusa-Toret S, Vonk WI, Frydman J. Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. *Nat Cell Biol* 2013;**15**:1231–43.
- Eskelinen EL, Reggiori F, Baba M et al. Seeing is believing: the impact of electron microscopy on autophagy research. *Autophagy* 2011;**7**:935–56.
- Finkel T, Deng CX, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. *Nature* 2009;**460**:587–91.
- Finley D, Ulrich HD, Sommer T et al. The ubiquitin-proteasome system of *Saccharomyces cerevisiae*. *Genetics* 2012;**192**:319–60.
- Fujikake N, Nagai Y, Popiel HA et al. Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. *J Biol Chem* 2008;**283**:26188–97.
- Galluzzi L, Baehrecke EH, Ballabio A et al. Molecular definitions of autophagy and related processes. *EMBO J* 2017;**36**:1811–36.
- Gidalevitz T, Ben-Zvi A, Ho KH et al. Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* 2006;**311**:1471–4.
- Glover JR, Lindquist S. Hsp104, Hsp70, and Hsp40. *Cell* 1998;**94**:73–82.
- Gourlay CW, Carpp LN, Timpson P et al. A role for the actin cytoskeleton in cell death and aging in yeast. *J Cell Biol* 2004;**164**:803–9.
- Groll M, Ditzel L, Lowe J et al. Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature* 1997;**386**:463–71.
- Guedes A, Ludovico P, Sampaio-Marques B. Caloric restriction alleviates alpha-synuclein toxicity in aged yeast cells by controlling the opposite roles of Tor1 and Sir2 on autophagy. *Mech Ageing Dev* 2017;**161**:270–6.

- Hanzen S, Vielfort K, Yang J et al. Lifespan control by redox-dependent recruitment of chaperones to misfolded proteins. *Cell* 2016;**166**:140–51.
- Harris N, MacLean M, Hatzianthis K et al. Increasing *Saccharomyces cerevisiae* stress resistance, through the overactivation of the heat shock response resulting from defects in the Hsp90 chaperone, does not extend replicative life span but can be associated with slower chronological ageing of nondividing cells. *Mol Genet Genomics* 2001;**265**:258–63.
- Hay DG, Sathasivam K, Tobaben S et al. Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. *Hum Mol Genet* 2004;**13**:1389–405.
- Haynes CM, Ron D. The mitochondrial UPR - protecting organelle protein homeostasis. *J Cell Sci* 2010;**123**:3849–55.
- Hegde RS, Ploegh HL. Quality and quantity control at the endoplasmic reticulum. *Curr Opin Cell Biol* 2010;**22**:437–46.
- Heo JM, Livnat-Levanon N, Taylor EB et al. A stress-responsive system for mitochondrial protein degradation. *Mol Cell* 2010;**40**:465–80.
- Hill SM, Hanzen S, Nystrom T. Restricted access: spatial sequestration of damaged proteins during stress and aging. *EMBO Rep* 2017;**18**:377–91.
- Johnson JE, Johnson FB. Methionine restriction activates the retrograde response and confers both stress tolerance and lifespan extension to yeast, mouse and human cells. *PLoS One* 2014;**9**:e97729.
- Kaganovich D, Kopito R, Frydman J. Misfolded proteins partition between two distinct quality control compartments. *Nature* 2008;**454**:1088–95.
- Kampinga HH, Craig EA. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* 2010;**11**:579–92.
- Kimata Y, Ishiwata-Kimata Y, Yamada S et al. Yeast unfolded protein response pathway regulates expression of genes for anti-oxidative stress and for cell surface proteins. *Genes Cells* 2006;**11**:59–69.
- Klionsky DJ, Cuervo AM, Dunn WA, Jr. et al. How shall I eat thee? *Autophagy* 2007;**3**:413–6.
- Koppen M, Langer T. Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases. *Crit Rev Biochem Mol Biol* 2007;**42**:221–42.
- Krobitsch S, Lindquist S. Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proc Natl Acad Sci* 2000;**97**:1589–94.
- Kruegel U, Robison B, Dange T et al. Elevated proteasome capacity extends replicative lifespan in *Saccharomyces cerevisiae*. *PLoS Genet* 2011;**7**:e1002253.
- Kumar PA, Kumar MS, Reddy GB. Effect of glycation on alpha-crystallin structure and chaperone-like function. *Biochem J* 2007;**408**:251–8.
- Kumar R, Nawroth PP, Tyedmers J. Prion aggregates are recruited to the Insoluble Protein Deposit (IPOD) via myosin 2-Based vesicular transport. *PLoS Genet* 2016;**12**:e1006324.
- Kwon YT, Ciechanover A. The ubiquitin code in the ubiquitin-proteasome system and autophagy. *Trends Biochem Sci* 2017;**42**:873–86.
- Labbadia J, Cunliffe H, Weiss A et al. Altered chromatin architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. *J Clin Invest* 2011;**121**:3306–19.
- Leak RK. Heat shock proteins in neurodegenerative disorders and aging. *J Cell Commun Signal* 2014;**8**:293–310.
- Lee C, Longo V. Dietary restriction with and without caloric restriction for healthy aging. *F1000Res* 2016;**5**(F1000 Faculty Rev):117.
- Lemasters JJ. Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3). *Redox Biol* 2014;**2**:749–54.
- Li J, Soroka J, Buchner J. The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. *Biochim Biophys Acta* 2012;**1823**:624–35.
- Lipinski MM, Zheng B, Lu T et al. Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease. *Proc Natl Acad Sci* 2010;**107**:14164–9.
- Longo VD, Shadel GS, Kaeberlein M et al. Replicative and chronological aging in *Saccharomyces cerevisiae*. *Cell Metabolism* 2012;**16**:18–31.
- Lopez-Otin C, Blasco MA, Partridge L et al. The hallmarks of aging. *Cell* 2013;**153**:1194–217.
- Low P. The role of ubiquitin-proteasome system in ageing. *Gen Comp Endocrinol* 2011;**172**:39–43.
- Luce K, Osiewacz HD. Increasing organismal healthspan by enhancing mitochondrial protein quality control. *Nat Cell Biol* 2009;**11**:852–8.
- Lynch-Day MA, Klionsky DJ. The Cvt pathway as a model for selective autophagy. *FEBS Lett* 2010;**584**:1359–66.
- Madeo F, Zimmermann A, Maiuri MC et al. Essential role for autophagy in life span extension. *J Clin Invest* 2015;**125**:85–93.
- Malinowska L, Kroschwald S, Munder MC et al. Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. *Mol Biol Cell* 2012;**23**:3041–56.
- Martinez-Lopez N, Athonvarangkul D, Singh R. Autophagy and aging. *Adv Exp Med Biol* 2015;**847**:73–87.
- Matecic M, Smith DL, Pan X et al. A microarray-based genetic screen for yeast chronological aging factors. *PLoS Genet* 2010;**6**:e1000921.
- Meijer AJ, Codogno P. Macroautophagy: protector in the diabetes drama? *Autophagy* 2007;**3**:522–5.
- Menzies FM, Fleming A, Rubinsztein DC. Compromised autophagy and neurodegenerative diseases. *Nat Rev Neurosci* 2015;**16**:345–57.
- Miller KA, DiDone L, Krysan DJ. Extracellular secretion of overexpressed glycosylphosphatidylinositol-linked cell wall protein Utr2/Crh2p as a novel protein quality control mechanism in *Saccharomyces cerevisiae*. *Eukaryotic Cell* 2010;**9**:1669–79.
- Miller SB, Ho CT, Winkler J et al. Compartment-specific aggregates direct distinct nuclear and cytoplasmic aggregate deposition. *EMBO J* 2015;**34**:778–97.
- Morimoto RI. Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Develop* 2008;**22**:1427–38.
- Morley JF, Morimoto RI. Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol Biol Cell* 2004;**15**:657–64.
- Morselli E, Marino G, Bennetzen MV et al. Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. *J Cell Biol* 2011;**192**:615–29.
- Nakamura N, Matsuura A, Wada Y et al. Acidification of vacuoles is required for autophagic degradation in the yeast, *Saccharomyces cerevisiae*. *J Biochem (Tokyo)* 1997;**121**:338–44.
- Nardi T, Carlot M, De Bortoli E et al. A rapid method for differentiating *Saccharomyces sensu stricto* strains from other yeast species in an enological environment. *FEMS Microbiol Lett* 2006;**264**:168–73.

- Nystrom T, Liu B. Protein quality control in time and space - links to cellular aging. *FEMS Yeast Res* 2014;**14**:40–48.
- Oling D, Masoom R, Kvint K. Loss of Ubp3 increases silencing, decreases unequal recombination in rDNA, and shortens the replicative life span in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2014;**25**:1916–24.
- Park SH, Kukushkin Y, Gupta R et al. PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell* 2013;**154**:134–45.
- Parsell DA, Kowal AS, Singer MA et al. Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* 1994;**372**:475–8.
- Powers RW, 3rd, Kaeberlein M, Caldwell SD et al. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Develop* 2006;**20**:174–84.
- Ran F, Bali M, Michels CA. Hsp90/Hsp70 chaperone machine regulation of the *Saccharomyces MAL*-activator as determined in vivo using noninducible and constitutive mutant alleles. *Genetics* 2008;**179**:331–43.
- Reggiori F, Klionsky DJ. Autophagic processes in yeast: mechanism, machinery and regulation. *Genetics* 2013;**194**:341–61.
- Reis-Rodrigues P, Czerwieniec G, Peters TW et al. Proteomic analysis of age-dependent changes in protein solubility identifies genes that modulate lifespan. *Aging Cell* 2012;**11**:120–7.
- Rubinsztein DC, Marino G, Kroemer G. Autophagy and aging. *Cell* 2011;**146**:682–95.
- Ruckenstuhl C, Netzberger C, Entfellner I et al. Lifespan extension by methionine restriction requires autophagy-dependent vacuolar acidification. *PLoS Genet* 2014;**10**:e1004347.
- Saarikangas J, Barral Y. Protein aggregates are associated with replicative aging without compromising protein quality control. *Elife* 2015;**4**:e06197.
- Saez I, Vilchez D. The mechanistic links between proteasome activity, aging and age-related diseases. *Curr Genomics* 2014;**15**:38–51.
- Sakurai H, Ota A. Regulation of chaperone gene expression by heat shock transcription factor in *Saccharomyces cerevisiae*: importance in normal cell growth, stress resistance, and longevity. *FEBS Lett* 2011;**585**:2744–8.
- Sampaio-Marques B, Burhans WC, Ludovico P. Longevity pathways and maintenance of the proteome: the role of autophagy and mitophagy during yeast ageing. *Microb Cell* 2014;**1**:118–27.
- Sampaio-Marques B, Felgueiras C, Silva A et al. SNCA (alpha-synuclein)-induced toxicity in yeast cells is dependent on siruin 2 (Sir2)-mediated mitophagy. *Autophagy* 2012;**8**:1494–509.
- Sanchez Y, Lindquist SL. HSP104 required for induced thermotolerance. *Science* 1990;**248**:1112–5.
- Sanchez Y, Taulien J, Borkovich KA et al. Hsp104 is required for tolerance to many forms of stress. *EMBO J* 1992;**11**:2357–64.
- Satpute-Krishnan P, Ajinkya M, Bhat S et al. ER stress-induced clearance of misfolded GPI-anchored proteins via the secretory pathway. *Cell* 2014;**158**:522–33.
- Scott SV, Baba M, Ohsumi Y et al. Aminopeptidase I is targeted to the vacuole by a nonclassical vesicular mechanism. *J Cell Biol* 1997;**138**:37–44.
- Shiber A, Breuer W, Brandeis M et al. Ubiquitin conjugation triggers misfolded protein sequestration into quality control foci when Hsp70 chaperone levels are limiting. *Mol Biol Cell* 2013;**24**:2076–87.
- Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science* 2004;**306**:990–5.
- Silva A, Sampaio-Marques B, Fernandes A et al. Involvement of yeast HSP90 isoforms in response to stress and cell death induced by acetic acid. *PLoS One* 2013;**8**:e71294.
- Simonsen A, Cumming RC, Brech A et al. Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult *Drosophila*. *Autophagy* 2008;**4**:176–84.
- Sittler A, Lurz R, Lueder G et al. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum Mol Genet* 2001;**10**:1307–15.
- Sontag EM, Samant RS, Frydman J. Mechanisms and functions of spatial protein quality control. *Annu Rev Biochem* 2017;**86**:97–122.
- Specht S, Miller SB, Mogk A et al. Hsp42 is required for sequestration of protein aggregates into deposition sites in *Saccharomyces cerevisiae*. *J Cell Biol* 2011;**195**:617–29.
- Speldewinde SH, Grant CM. The frequency of yeast [PSI⁺] prion formation is increased during chronological ageing. *Microb Cell* 2017;**4**:127–32.
- Stroo E, Koopman M, Nollen EA et al. Cellular regulation of amyloid formation in aging and disease. *Front Neurosci* 2017;**11**:64.
- Suzuki CK, Suda K, Wang N et al. Requirement for the yeast gene LON in intramitochondrial proteolysis and maintenance of respiration. *Science* 1994;**264**:273–6.
- Taipale M, Jarosz DF, Lindquist S. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nat Rev Mol Cell Biol* 2010;**11**:515–28.
- Tanaka K. The proteasome: overview of structure and functions. *Proc Jpn Acad, Ser B* 2009;**85**:12–36.
- Tatsuta T, Langer T. Quality control of mitochondria: protection against neurodegeneration and ageing. *EMBO J* 2008;**27**:306–14.
- Toth ML, Sigmond T, Borsos E et al. Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. *Autophagy* 2008;**4**:330–8.
- Trott A, Morano KA. The yeast response to heat shock. in: Hohmann S, Mager PWH (eds). *Yeast Stress Responses*. Heidelberg, Germany: Springer-Verlag, 2003;71–119.
- Tyedmers J, Mogk A, Bukau B. Cellular strategies for controlling protein aggregation. *Nat Rev Mol Cell Biol* 2010;**11**:777–88.
- Tyedmers J, Treusch S, Dong J et al. Prion induction involves an ancient system for the sequestration of aggregated proteins and heritable changes in prion fragmentation. *Proc Natl Acad Sci* 2010;**107**:8633–8.
- Tyler JK, Johnson JE. The role of autophagy in the regulation of yeast life span. *Ann NY Acad Sci* 2018; DOI: 10.1111/nyas.13549.
- Vernace VA, Schmidt-Glenewinkel T, Figueiredo-Pereira ME. Aging and regulated protein degradation: who has the UPPER hand? *Aging Cell* 2007;**6**:599–606.
- Vilchez D, Morante I, Liu Z et al. RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions. *Nature* 2012;**489**:263–8.
- Voos W, Rottgers K. Molecular chaperones as essential mediators of mitochondrial biogenesis. *Biochim Biophys Acta* 2002;**1592**:51–62.
- Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 2011;**334**:1081–6.

- Walther DM, Kasturi P, Zheng M et al. Widespread proteome remodeling and aggregation in Aging *C. elegans*. *Cell* 2015;161:919–32.
- Wang K, Klionsky DJ. Mitochondria removal by autophagy. *Autophagy* 2011;7:297–300.
- Waza M, Adachi H, Katsuno M et al. 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. *Nat Med* 2005;11:1088–95.
- Yang J, Hao X, Cao X et al. Spatial sequestration and detoxification of Huntingtin by the ribosome quality control complex. *Elife* 2016;5:e11792.
- Young JC, Agashe VR, Siegers K et al. Pathways of chaperone-mediated protein folding in the cytosol. *Nat Rev Mol Cell Biol* 2004;5:781–91.