Thesis for the Degree of Doctor of Philosophy

Proteostasis and Aging in *Saccharomyces cerevisiae*
The role of a Peroxiredoxin

Sarah Hanzén

Department of Chemistry and Molecular Biology

Faculty of Science

UNIVERSITY OF GOTHENBURG

Cover picture:
Fluorescent microscopy images of yeast cells with peroxiredoxin Tsa1-GFP accumulating in protein aggregates induced by hydrogen peroxide.

Pictures taken and edited by: Sarah Hanzén

ISBN:
978-91-629-0239-1
http://hdl.handle.net/2077/52316

Printed by: Ineko AB, Kållered 2017.

To my family and friends

Abstract

Aging is characterized by a progressive decline in physiological functions that limits biological processes, increases the risk of disease, and ultimately leads to death. At the cellular level, aging is associated with accumulation of damaged components, including proteins, indicating that protein homeostasis (or proteostasis) fails to maintain the integrity and functionality of the proteome as cells age. Reduced caloric intake elevates proteostasis, counteracts the accumulation of damage during cellular aging, and prolongs lifespan in organisms ranging from yeast to primates. Caloric restriction is intimately linked to reduced signaling through nutrient sensing pathways, including the Target-Of-Rapamycin (TOR) and Protein Kinase A (PKA) pathways but which downstream targets of these nutrient-signaling pathways are most important for lifespan control is not known.

In this thesis, using the yeast *Saccharomyces cerevisiae* as a model organism, I found that the peroxiredoxin Tsa1, which belongs to a family of peroxide scavengers, is a downstream target of the PKA pathway and acts as a major modulator of aging. I found that Tsa1 is required for the resistance to hydrogen peroxide and lifespan extension induced by caloric restriction. Further, I traced the beneficial role of Tsa1 in longevity assurance to its involvement in proteostasis; an involvement linked to the hyperoxidized chaperone-like form of Tsa1. This function of Tsa1 in proteostasis entails recruitment of other molecular chaperones to misfolded and damaged proteins under hydrogen peroxide stress and in aged cells, as well as assistance in the clearance of protein aggregates. Our findings suggest that the cell utilizes distinct strategies for managing protein aggregates under different stress conditions, as Tsa1 is important for the management of protein aggregates under hydrogen peroxide stress but not upon elevated temperatures. The data also point to hydrogen peroxide and reduced proteasomal-dependent degradation as contributing factors for the accumulation of protein aggregates in aged cells.

Keywords: Aging, caloric restriction, oxidative stress, peroxiredoxins, proteostasis, protein aggregates, ubiquitin-proteasome system

Abbreviations

Prx	Peroxiredoxin
Trx	Thioredoxin
Grx	Glutaredoxin
Srx	Sulfiredoxin
CR	Caloric restriction
ROS	Reactive oxygen species
IGF-1	Insulin growth factor 1
TOR	Target of rapamycin
PKA	Protein kinase A
GPCR	G-protein coupled receptor
FRTA	Free radical theory of aging
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
ER	Endoplasmic reticulum
GCR	Gross chromosomal rearrangements
PN	Proteostasis network
ALS	Amyotrophic lateral sclerosis
HMW	High molecular weight
HSPs	Heat shock proteins
AZC	Azetidine-2-carboxylic acid
RLS	Replicative lifespan
CLS	Chronological lifespan
ERCs	Extra chromosomal rDNA circles
ARS	Autonomously replicating sequence
NPCs	Nuclear pore complexes
ISC	Iron-sulfur clusters
NEFs	Nucleotide exchange factors
UPS	Ubiquitin-proteasome system
DUBs	De-ubiquitinating proteins
SPQC	Spatial protein quality control
JUNQ	Juxtanuclear quality control compartment
IPOD	Insoluble protein deposit
INQ	Intranuclear quality control compartment

Papers included in this thesis:

I. Lifespan extension and H_2O_2 resistance elicited by caloric restriction require the peroxiredoxin Tsa1 in *Saccharomyces cerevisiae*

Molin M, Yang J, **Hanzén S**, Toledano MB, Labarre J, Nyström T

Mol Cell (2011) **43**:823-33

II. Lifespan control by redox-dependent recruitment of chaperones to misfolded proteins

Hanzén S, Vielfort K, Yang J, Roger F, Andersson V, Zamarbide-Forés S, Andersson R, Malm L, Palais G, Biteau B, Liu B, Toledano MB, Molin M, Nyström T

Cell (2016) **166**:140-51

III. Enhancing protein disaggregation restores proteasome activity in aged cells

Andersson V, **Hanzén S**, Liu B, Molin M, Nyström T.

Aging (2013) **5**:802-12

Other publications:

IV. Restricted access: spatial sequestration of damaged proteins during stress and aging

Hill SM, **Hanzén S**, Nyström T

EMBO rep (2017) **18**:377-391

V. Peroxiredoxin förlänger livet genom att guida chaperoner till skadade proteiner

Sarah Hanzén, Katarina Vielfort

Neurologi i Sverige (2016) nr 4-16

TABLE OF CONTENTS

1. Introduction

Peroxiredoxins (Prxs) are a family of highly conserved antioxidants that use redox-active cysteines to reduce hydrogen peroxide (H_2O_2), peroxinitrite, and organic hydroperoxides (Wood et al., 2003). Prxs were originally identified in yeast (Kim et al., 1988) and have since then been identified in organisms ranging from bacteria to humans (Wood et al., 2003). Prxs are divided into 1-Cys Prxs and 2-Cys Prxs based on the number of redox-active cysteines participating in the catalytic cycle. The basic catalytic mechanism of Prxs involves oxidation of a redox active cysteine thiol (Cys-SH) into a sulfenic acid (Cys-SOH) and a concomitant reduction of the peroxide substrate (Wood et al., 2003) (Fig. 1). This redox active cysteine is located near the N-terminal of the polypeptide and is referred to as the peroxidatic cysteine. 2-Cys Prxs have one additional active site cysteine, the resolving cysteine in the C-terminal of the protein, which is involved in the reduction of the enzyme. 2-Cys Prxs are further divided into typical and atypical based on the mechanism of reduction. Typical 2-Cys Prxs are homodimers and during their catalytic cycle the resolving cysteine from one subunit attacks the oxidized peroxidatic cysteine in the other subunit, forming a disulfide bond (Fig. 1). The disulfide bond is reduced by oxidoreductases e.g. the thioredoxin (Trx) system, which recycle the Prx to the reduced and native form (Fig. 1). Atypical 2-Cys Prxs have a similar mechanism of reduction, but as they are functionally monomeric the resolving cysteine is instead located within the same subunit. 1-Cys Prxs have only the peroxidatic cysteine, which is directly reduced in a process that is not completely understood, but has been suggested to include glutaredoxins (Grxs) and Trxs in yeast (Morano et al., 2012).

Typical 2-Cys Prxs can under certain conditions form high molecular weight (HMW) oligomers (Wood et al., 2003). Factors known to promote this oligomerization include low pH (Kristensen et al., 1999), high calcium levels (Plishker et al., 1992), heat (Jang et al., 2004), and oxidative stress (Schroder et al., 2000, Jang et al., 2004). Oxidatively induced oligomerization is associated with hyperoxidation of the peroxidatic cysteine, which occurs when the sulfenic acid (Cys-SOH) instead of being reduced by the resolving cysteine is further oxidized by a second molecule of H_2O_2 into a sulfinic acid (Cys-SOOH) (Fig. 1). The frequency of hyperoxidation is low under physiological conditions but increases upon elevated H_2O_2 concentrations. Hyperoxidized Prxs can be reduced by the action of sulfiredoxins (Srxs) in an ATP-dependent manner (Biteau et al., 2003) (Fig. 1).

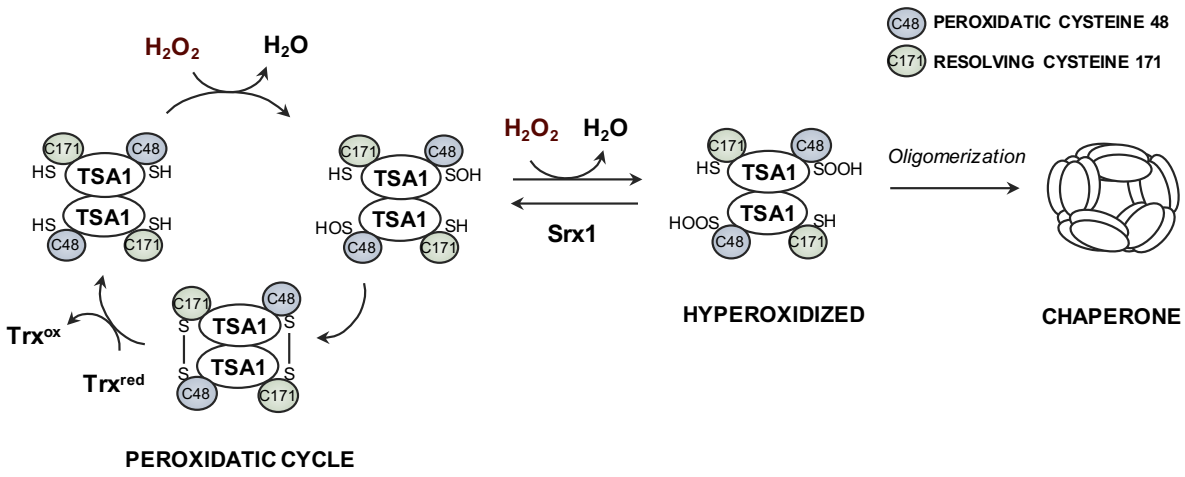

Figure 1. Catalytic cycle of the typical 2-Cys Prx Tsa1. H_2O_2 reduction by Tsa1 converts the peroxidatic cysteine (Cys48) to a sulfenic acid (SOH), which is followed by disulfide bond formation between Cys48 and the resolving cysteine (Cys171) on a second Tsa1 molecule, and subsequent reduction by the Trx system, thereby completing the catalytic cycle. The sulfenic acid (SOH) can also become further oxidized to a peroxide inactive, sulfinic acid (SOOH).

Prxs have, in addition to their role in peroxide scavenging, been implicated in other cellular processes including protein homeostasis (proteostasis), genome stability, and cellular signaling (Huang et al., 2003, Jang et al., 2004, Rhee and Woo, 2011). Hyperoxidation and oligomerization of 2-Cys Prxs is associated with a functional switch in which peroxidase activity is replaced by chaperone activity, an activity that is central to proteostasis (Jang et al., 2004). Moreover, deletion of the major yeast Prx *TSA1* leads to increased mutation rates and double strand breaks, indicating a function in genome maintenance (Huang et al., 2003). Prxs have also been suggested to function as mediators of H_2O_2-dependent signaling through different mechanisms (D'Autreaux and Toledano, 2007, Netto and Antunes, 2016). First, the hyperoxidation and subsequent peroxidase inactivation permits local accumulation of H_2O_2, which in turn could oxidize signaling proteins. Alternatively, oxidation of Prxs could be transferred to signaling proteins, and/or act through their reducing agent, the Trxs.

Intriguingly, studies have reveal a role for Prxs in aging. Prx-deficiency shortens the lifespan of yeast, worms, flies, and mice (Timmermann et al., 2010, Olahova et al., 2008, Lee et al., 2009, Neumann et al., 2003), and increased levels of a neuronal Prx extends lifespan of flies

(Lee et al., 2009). Prxs are also associated with various age-related diseases, including neurodegenerative diseases, inflammatory diseases, and cancer (Park et al., 2016). The role of Prxs in cancer remains complex, as they have been proposed to act as both suppressors and promoters of tumor development (Mishra et al., 2015, Park et al., 2016). Prx-deficiency leads to increased oncogenesis and overproduction can suppress the development of certain types of cancer (Neumann et al., 2003, Park et al., 2016). On the other hand, some cancer types, including breast cancer and lung cancer, display elevated levels of Prxs, which provides resistance to radiation and chemotherapy (Park et al., 2006, Chen et al., 2006, Mishra et al., 2015, Park et al., 2016). Srxs are also linked to cancer, with reports of both up-regulated and down-regulated Srx levels in different types of tumors (Mishra et al., 2015).

1.2 Aim of the thesis

Prxs are fundamental to the process of aging and disease, though the mechanism behind these effects is mostly unknown. Given the multifunctional nature of Prx, it remains to be elucidated which function is most critical for their role as modulators of aging and disease. The primary aim of my PhD studies has been to study Prxs and their role in aging. I have studied the yeast *Saccharomyces cerevisiae* and the major Prx Tsa1 to begin to approach questions regarding the different functions of Prxs and their contribution to the process of aging. Much of my work has been dedicated to the role of Tsa1 in proteostasis under acute stress, mainly oxidative stress, and in aging. Before going into the details of my results, I will describe aspects of aging relevant for my research.

2. Cellular mechanisms of aging

Aging is a multifactorial process characterized by a progressive decline of physiological functions that limits biological processes, increases the risk of disease, and ultimately leads to death. Aging is the major risk factor of numerous diseases including cardiovascular diseases, neurodegeneration, and most types of cancer (Lopez-Otin et al., 2013). Studies have identified cellular pathways central to the process of aging, many of which are connected to accumulation of damaged cellular components. Nutrient signaling, oxidative stress, genome stability, and proteostasis have all been linked to cellular aging (Lopez-Otin et al., 2013). I have summarized the main findings of these aging pathways and included known links to peroxiredoxins. Special attention has been given to the yeast *Saccharomyces cerevisiae,* and I have concluded with the specific nature of aging and aging factors in this model organism.

2.1 Gerontogenes

Studies of lifespan altering gene mutations have provided insights to cellular pathways linked to the process of aging. Genes that, when altered one way or the other, extend the lifespan of an organism are referred to as gerontogenes (Rattan, 1995, Nystrom et al., 2012). The first gerontogene was identified in a screen for long-lived mutants in the nematode *Caenorhabditis elegans* in the early 1980's (Klass, 1983). A mutation in the gene *age-1*, encoding a homolog to the mammalian phosphatidylinositol 3-kinase catalytic subunit, prolonged the lifespan of the nematode by 40% (Klass, 1983, Friedman and Johnson, 1988). AGE-1 was later found to be part of an insulin-like pathway involving the FOXO transcription factor DAF-16 (Ogg et al., 1997). The connection between aging and nutrient signaling proved to be substantial, since many gerontogenes discovered are involved in various nutrient signaling pathways (Kenyon, 2010), which will be discussed in subsequent sections.

Sirtuins represent another important class of gerontogenes, as these highly conserved deacetylases have been shown to influence longevity in several organisms and seem to be a key factor in many aging pathways (Guarente, 2007). Sirtuins were originally discovered in yeast due to their role in gene silencing. Sirtuins are NAD^+-dependent deacetylases that mediate genomic silencing of histone H3 and H4, resulting in a more tightly packed and transcriptionally repressed chromatin. In yeast, regions silenced by the Sirtuins include the mating type loci, the telomeres, and the repeated ribosomal DNA (rDNA) (Lin et al., 2000). The first evidence of an age-related function of Sirtuins included the observation that Sir2-

deficient yeast cells have a shortened lifespan, whereas Sir2 overproduction extends lifespan (Kaeberlein et al., 1999). Experiments from the same study connected this age-related function of Sir2 to genomic instability, although later studies indicate that this may not be the sole mechanism by which Sir2 regulates lifespan (discussed below). Sirtuins have been reported to exhibit anti-aging capabilities also in *C. elegans* (Tissenbaum and Guarente, 2001) and the fruit fly *Drosophila melanogaster* (Rogina and Helfand, 2004), but as in yeast, the mode of action is not entirely clear due to the connection to several aging pathways, including nutrient signaling, genome stability, and proteostasis (Guarente, 2007, Kenyon, 2010). The Prx family is a quite recent addition to the group of gerontogenes and their levels regulates the lifespan of several model organisms (Nystrom et al., 2012). Prxs are, similar to the Sir proteins, linked to several aging pathways (Nystrom et al., 2012), complicating the elucidation of their main function as aging regulators.

2.2 Nutrient signaling

One of the most important discoveries in the aging field came with the observation that life could be extended in rats by simply lowering the caloric intake without causing malnutrition (McCay et al., 1989), a phenomenon known as caloric restriction (CR). CR has since then been shown to extend lifespan of organisms ranging from yeast to primates and is one of the most well-studied lifespan-extending interventions (Colman et al., 2009, Kenyon, 2010). Remarkably, organisms subjected to CR do not only live longer, but they also experience delayed onset of age-related deterioration and diseases. In fact, CR has been associated with a wide variety of health benefits, including reduced risk of diseases such as cancers, neurodegenerative disorders, autoimmune diseases, cardiovascular disease, and diabetes (Fontana et al., 2010, Speakman and Mitchell, 2011).

Studies have established a clear connection between CR and reduced signaling through nutrient sensing pathways, including the insulin/insulin-like growth factor (IGF-1), the target-of-rapamycin (TOR), and the protein kinase A (PKA) (Kenyon, 2010, Fontana et al., 2010, Enns and Ladiges, 2010, Lin et al., 2000). Less is known about the downstream targets of these signaling pathways, although studies demonstrate that CR is associated with an altered gene expression program involving a switch from growth and reproduction, towards focus on maintenance and repair. Many organisms respond to CR with an increased resistance to oxidative stress, decreased production of reactive oxygen species (ROS), and reduced

accumulation of oxidative damage (Sohal et al., 1994b, Sohal et al., 1994a, Sohal and Weindruch, 1996, Fontana et al., 2010). Sir2 has been reported as one of the downstream targets of CR in yeast, worm, flies, and mice, supporting a role for Sirtuins as mediators of longevity (Lin et al., 2000, Guarente, 2007, Medvedik et al., 2007, Kenyon, 2010). However, Sir2-independent lifespan extension by CR has also been reported (Kaeberlein et al., 2004), suggesting that Sir2 and CR may extend lifespan by separate mechanisms, or possibly, that strain variations contribute to the distinct results seen between the different studies.

2.2.1 Insulin/insulin-like growth factor 1

Insulin and IGF-1 are two structurally similar growth factors involved in various cellular processes such as cell proliferation, differentiation, and glucose metabolism (Leroith et al., 2011). The insulin/IGF-1 pathway was first discovered to influence longevity in *C. elegans*, where decreased activity of a hormone receptor similar to the insulin/IGF-1 receptors lead to a prolonged lifespan (Kenyon, 2010). This receptor, called DAF-2, initiates a signaling cascade resulting in an altered gene expression through the transcription factors DAF-16, HSF-1, and SKN-1. As mentioned above, DAF-16 is a FOXO transcription factor, which constitutes a family of transcription factors that are central to signaling pathways regulating stress responses and longevity (Kenyon, 2010, Webb and Brunet, 2014). DAF-16, together with the heat shock transcription factor HSF-1 and the oxidative stress transcription factor SKN-1, up-regulates the expression of a wide range of genes, including stress-response genes, which ultimately contributes to lifespan extension. The importance of the insulin/IGF-1 pathway in longevity has proven to be conserved, as connections between the two have been found also in flies, mice, and humans (Kenyon, 2010). Moreover, CR seems to work in part by decreasing signaling through the insulin/IGF-1 pathway in at least worms, flies, and mice (Kenyon, 2010).

2.2.2 Target-of-rapamycin

TOR is a highly conserved protein kinase, originally discovered in the yeast *Saccharomyces cerevisiae* due to its role in rapamycin resistance (Wullschleger et al., 2006). TOR signaling is triggered by nutrients, growth factors, and stress. TOR signaling regulates cell growth by targeting both protein synthesis and protein degradation. Under normal, non-stressed conditions, protein synthesis is activated by TOR, while protein degradation by autophagy is inhibited (Kenyon, 2010). When nutrients are limited or stress levels increased, protein

synthesis is downregulated whereas protein turnover is increased. Reduced TOR activity by nutrient deprivation or genetic manipulation increases stress resistance and extends lifespan in many model organisms, including yeast, worms, and mice (Kenyon, 2010). The PHA-4/FOXO transcription factor is required for TOR-dependent lifespan extension in *C. elegans* and the Msn2/4 transcription factors in yeast. Translation is vital for growth and reducing the activity of this process may leave more resources for maintenance and repair. The potential importance of this tradeoff is supported by the fact that inhibition of translation by other means than nutrient deprivation also extends lifespan in yeast, worms, flies, and mice (Kenyon, 2010). One model for TOR-dependent longevity in yeast suggests that CR and reduced TOR signaling act through increased activity of Sir2 (Medvedik et al., 2007). In this view, reduced TOR signaling leads to increased production of the Msn2/4 target Pnc1, which is required for the degradation the Sir2 inhibitor nicotinamide.

2.2.3 Protein kinase A

The PKA signaling pathway regulates cellular processes such as cell growth, metabolism, and stress responses (Tamaki, 2007, Zaman et al., 2008). The PKA pathway is triggered by glucose, which is sensed by the guanine exchange factor Cdc25 and the G-protein coupled receptor (GPCR) Gpr1 (Fig. 2). Cdc25 and Gpr1 activates the small GTP binding proteins Ras and Gpa2 respectively. Both Gpa2 and Ras activate Adenylase Cyclase (AC), which catalyzes the conversion of ATP to cAMP. cAMP is a second messenger that in yeast binds the regulatory component (Bcy1) of PKA, thus releasing the catalytically active subunit (TPK). Active PKA forwards the signal by phosphorylation of targets proteins, two of which are the stress response transcription factors Msn2/4 that are inhibited by PKA phosphorylation (Zaman et al., 2008).

The PKA pathway has been linked to longevity in both yeast and mice. Lowering the glucose concentration in the growth medium, or reducing the activity of the pathway responsible for glucose sensing and import extends lifespan in yeast (Lin et al., 2000). Moreover, genetically reducing PKA signaling by removing components such as AC and TPK subunits also prolong lifespan and this extension is not further increased by also limiting glucose availability (Lin et al., 2000), suggesting that they act in the same pathway. In line with this, elevated PKA activity achieved by removal of Pde2, a phosphodiesterase that catalyzes the hydrolysis of cAMP to AMP, shortens yeast lifespan.

Sir2 has been implicated also in PKA-mediated lifespan extension with reports of CR acting through increased respiration and NAD^+ levels resulting in a boosted Sir2 activity (Lin et al., 2000, Lin et al., 2002, Lin et al., 2004). In mice, reduced PKA signaling leads to extended lifespan and increased health span, demonstrated by reduced body fat as well as an increased resistance to stress-induced cardiomyopathy (Enns and Ladiges, 2010).

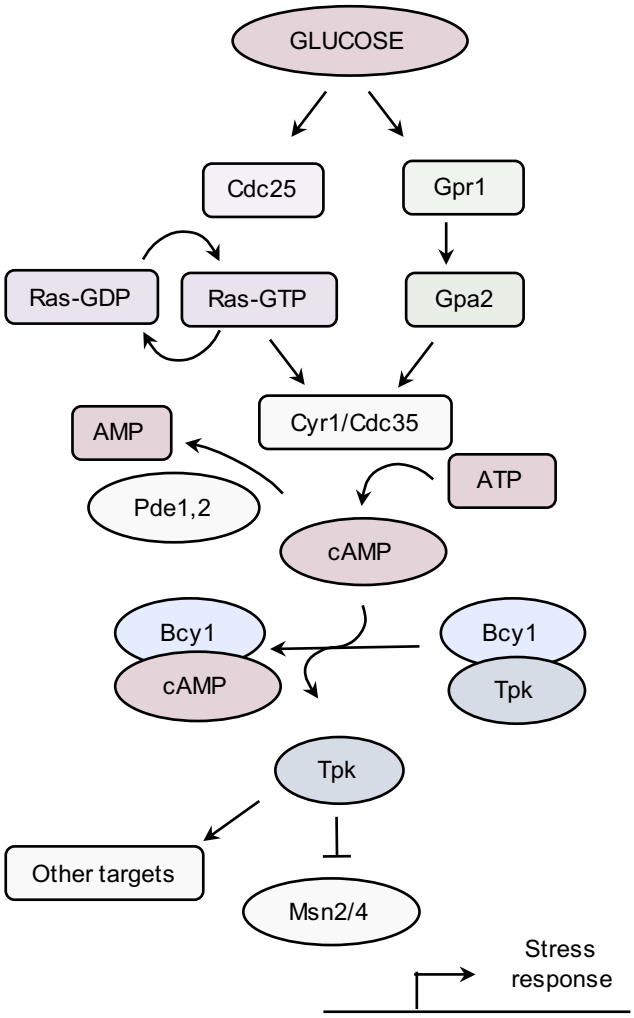

Figure 2. The PKA signaling pathway. Glucose triggers Cdc25 and Gpr1, which activates the small G-proteins Ras and Gpa2 respectively. Ras and Gpa2 activates adenylase cyclase (Cyr1/Cdc35) leading to production of the second messenger cAMP. cAMP binds the inhibitory component of PKA, thereby releasing the catalytically active component, Tpk, which conveys the signal by phosphorylation of targets proteins. The transcription factors Msn2/4 are two targets that are negatively regulated by PKA.

2.2.4 Nutrient signaling and peroxiredoxins

Studies in *Drosophila* have identified a Prx as one important effector of FOXO-mediated lifespan extension (Lee et al., 2009). Elevated neuronal levels of the 2-Cys Prx, Jafrac1, increased the resistance to the superoxide-generating chemical paraquat, reduced the levels of ROS, and extended lifespan, in a JNK/FOXO-mediated manner. Moreover, the classical 2-Cys Prx PRDX-2 is required for lifespan extension associated with the insulin/IGF-1 pathway in *C. elegans* (Olahova and Veal, 2015). Additionally, metformin, an antidiabetic drug with CR mimetic effects has been shown extend lifespan in *C. elegans* in a Prx-dependent manner (De Haes et al., 2014). The study demonstrated that PRDX-2 is required for metformin induced lifespan extension and in contrast to the reduced production of ROS typically seen in CR-treated organisms, an increased generation of H_2O_2 was observed in long-lived, metformin-treated worms. This type of positive effect of ROS will be discussed in the subsequent section.

2.3 Oxidative stress

Oxidative stress refers to a state of elevated oxidative damage, resulting from an imbalance between generation of ROS and the cellular defences against such species (Halliwell, 2007). Accumulation of oxidatively damaged molecules can be a consequence of increased generation of ROS, a decreased ability of the antioxidants system to detoxify ROS, and/or a reduced capacity to repair/remove oxidatively damaged molecules. Oxidative stress has been associated with numerous diseases, including cardiovascular diseases, neurodegenerative diseases, and cancer, and is one of the hallmarks of aging (Sohal and Weindruch, 1996, Finkel and Holbrook, 2000, Brieger et al., 2012).

2.3.1 Reactive oxygen species

ROS are highly reactive molecules produced during normal oxygen metabolism and include the superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2). ROS is mainly generated during normal oxidative phosphorylation at the mitochondrial electron transport chain, although NADPH oxidases and peroxisomes have been identified as other important sources (Finkel and Holbrook, 2000). The $O_2^{\bullet-}$ is produced by electron reduction of oxygen, typically at complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome *c* reductase) in the mitochondrial electron transport chain, whereas H_2O_2 and $HO^{\bullet}$ are produced by secondary reactions (Turrens, 1997). $O_2^{\bullet-}$ and $HO^{\bullet}$ are exceptionally unstable,

while H_2O_2 is comparatively long-lived and freely diffusible (Finkel and Holbrook, 2000). ROS can react with and oxidize all three of the major cellular constituents; DNA, lipids, and proteins, often with deleterious outcomes (Halliwell and Gutteridge, 1990, Sohal and Weindruch, 1996, Finkel and Holbrook, 2000).

The toxicity of ROS and its implication in aging were highlighted already in the 1950s when Denham Harman proposed the free radical theory of aging (FRTA), in which he proposed that ROS reacting with cellular macromolecules initiates the deterioration associated with aging (Harman, 1956). When the mitochondria later were identified as the main source of ROS, the theory was extended to the mitochondrial theory of aging (Harman, 1972). The FRTA has since then been subjected to extensive studies and an age-dependent increased mitochondrial production of both $O_2^{\bullet-}$ and H_2O_2 has been established in several model organisms (Farmer and Sohal, 1989, Sohal and Sohal, 1991, Laun et al., 2001, Sohal, 2002, Sasaki et al., 2010). In line with this, oxidatively damaged DNA, proteins, and lipids have all been found to increase with age (Fraga et al., 1990, Oliver et al., 1987, Roberts and Reckelhoff, 2001, Bokov et al., 2004). As mentioned above, lifespan extension by caloric restriction is in many organisms accompanied with a decreased production of ROS and oxidatively damaged macromolecules, further supporting the connection between oxidative stress and longevity (Sohal and Weindruch, 1996). However, experiments with overproduction of antioxidants have failed to consistently correlate with longevity under standard conditions and upon CR (Sohal and Orr, 2012). Together with increasing evidence of beneficial effects of ROS (D'Autreaux and Toledano, 2007, Sohal and Orr, 2012), there is an uncertainty regarding the validity the free radical theory of aging. However, the extensive correlation between oxidative stress and aging/age-related diseases suggest that high levels of ROS is detrimental to the cell and most likely contributes to the process of aging (Finkel and Holbrook, 2000).

2.3.2 Oxidative protein modifications

Proteins are one of the major targets of oxidation and protein oxidation reactions can cleave the peptide backbone, modify amino acid side chains, or cross-link amino acids to yield high molecular weight products (Stadtman, 2006). Some oxidative modifications are reversible and implicated in redox-regulated signaling pathways, conversely, most irreversible oxidations typically disrupt protein structure leading to loss of protein function (Dahl et al., 2015). Sulphur-containing amino acids such as cysteine and methionine are particularly susceptible

to oxidation. Oxidation of cysteine thiols (-SH) leads to the formation of sulfenic acids (-SOH), sulfinic acids ($-SO_2H$) or sulfonic acids ($-SO_3H$) (Cremers and Jakob, 2013). A sulfenic acid (SOH) can also react with a thiol (-SH) forming a disulfide bond (S-S), which can be reduced by the Trx system and/or the Grx system. The formation of a disulfide bond is an example of a reversible modification that can have beneficial outcomes. Disulfide formation in the endoplasmic reticulum (ER) can for example be important for protein folding and regulation of activity, and disulfide bonds formed from oxidative stress have been shown to be important for signaling pathways activating stress defenses such as increased antioxidant production (Cremers and Jakob, 2013).

Protein carbonylation is a type of irreversible oxidation that occurs through a metal ion-catalyzed oxidation and results in a reactive carbonyl group (aldehyde or ketone) (Stadtman and Levine, 2000). Carbonyl groups are composed of a carbon atom double-bonded to an oxygen atom and can be formed on several amino acids including lysine, histidine, arginine, and proline. Protein carbonylation typically disrupts protein structure and function, leading to formation of protein aggregates, and is highly connected to oxidative stress and aging (Levine, 1983, Stadtman and Levine, 2000, Nystrom, 2005, Erjavec et al., 2007).

2.3.3 Oxidative stress and peroxiredoxins

Prxs are part of the cell's defences against oxidative stress and upregulated together with other antioxidants such as catalases, superoxide dismutase (SOD), and glutathione peroxides (GPxs) in response to increased ROS levels. SODs are enzymes that specifically convert $O_2^{\bullet-}$ into H_2O_2, whereas catalases reduce H_2O_2 to H_2O and O_2 (Morano et al., 2012). Many organisms have several isoforms of antioxidants, often specifically localized to different cellular compartments. For example, mammals have six isoforms of Prxs, including four typical 2-Cys Prxs in the cytosol, mitochondria, and endoplasmic reticulum (ER), one atypical 2-Cys Prx, and one 1-Cys Prx (Fourquet et al., 2008). The five yeast Prxs include two typical 2-Cys Prxs and one atypical 2-Cys Prx in the cytosol, one nuclear atypical 2-Cys Prx, and the 1-Cys Prx, localized in the mitochondria. The major Prx in yeast is the typical 2-Cys Prx Tsa1, which is highly specific for the reduction of H_2O_2 (Chae et al., 1994, Lee et al., 1999b) and has been shown to act both freely in the cytosol as well as associated with translating ribosomes (Trotter et al., 2008). Prx activity is not essential in yeast, though cells lacking all five variants, together with the three GPxs, are unable to activate gene expression in response

to H_2O_2 exposure (Fomenko et al., 2011). The main transcriptional activator of the oxidative stress response in yeast is the transcription factor Yap1. Yap1 accumulates in the nucleus in response to oxidative exposure where it activates the expression of around 30 genes, some of which require the additional activation assistance of Skn7 (Lee et al., 1999a). *TSA1* is one of the genes regulated by the cooperative action of Yap1/Skn7. As a typical 2-Cys Prx, Tsa1 have 2 redox active cysteines; the N-terminal peroxidatic cysteine at position 48 and the C-terminal resolving cysteine at position 171 (Fig. 1). Although the catalytic efficiency of Prxs is low compared to antioxidants such as catalases and GPxs, their high abundance and conservation have established them as significant members of the cellular peroxide defense system (Wood et al., 2003).

Increasing evidence points to beneficial effects of ROS in cellular signaling, gene regulation, and redox regulation (Sohal and Orr, 2012). This type of positive effect of an otherwise toxic agent is referred to as hormesis and is characterized by a dose-dependent response, with low doses increasing survival, while higher doses are detrimental (Calabrese et al., 2015). Low doses of ROS have been suggested to boost the defense against oxidative stress and thereby promote cell growth, development, and lifespan extension (D'Autreaux and Toledano, 2007, Ristow and Zarse, 2010, Dahl et al., 2015, Goulev et al., 2017). Intriguingly, 2-Cys Prxs have been proposed to act as major mediators of H_2O_2 signaling (D'Autreaux and Toledano, 2007). A recent study demonstrated that low levels of H_2O_2 extended the lifespan of yeast in a Prx Tsa1-mediated manner, whereas higher levels proved harmful for the cells and instead shortened their lifespan (Goulev et al., 2017).

Three mechanisms have been described to explain the Prx contribution to H_2O_2 signaling (Netto and Antunes, 2016). In the first scenario, Prx hyperoxidation and subsequent peroxidase inactivation leads to a transient and local build-up in H_2O_2 concentrations. In this view, H_2O_2 reacts directly with signaling proteins such as phosphatases and/or transcription factors. In a second scenario, the Prx oxidation is selectively transferred to signaling proteins through protein-protein interactions and disulfide exchange reactions (Netto and Antunes, 2016). This was suggested to be the case for the metformin-induced lifespan extension in worms, mentioned above, in which PRDX-2 was proposed to activate the MAP-kinase signaling pathway involved in oxidative stress defense (De Haes et al., 2014). The third scenario centers around the reducing agents of Prxs; the Trxs. Following Prx reduction, the Trxs, in their oxidized state, can transfer their oxidative modification to downstream

regulatory proteins (Netto and Antunes, 2016). Alternatively, hyperoxidized Prxs are no longer substrates for the Trxs, which render them in their reduced form, free to act on other substrates (Day et al., 2012, Netto and Antunes, 2016). Altogether, the intracellular H_2O_2 availability seems vital, as both sub-physiological levels and elevated levels will result in cellular health defects.

2.4 Genome stability

Being central to life, DNA has since its discovery been linked to the process of aging. DNA is constantly at risk of becoming damaged by various endogenous and external sources, such as oxidative stress, radiation, and external mutagens (Vijg and Suh, 2013). A typical cell is subjected to tens of thousands of lesions every day, including backbone breaks (single and double stranded), base deletions, and DNA modifications. If DNA damage is not correctly repaired, lesions can lead to permanent alterations such as mutations (deletions, additions, and substitutions) and chromosomal rearrangements. For example, replication past the oxidatively induced modification 8-hydroxyguanine results in incorporation of the wrong base (Vijg and Suh, 2013). Additionally, mutations can arise as a cause of errors during replication, since the polymerase occasionally incorporates the incorrect base even though the template is undamaged. Notably, DNA damage is reversible as it can be restored by the DNA repair system, whereas mutations are not discovered by this system and thus become permanent changes in the genome.

Studies of radiation demonstrated the first link between genome stability and aging as it was shown that low doses of radiation are associated with mutation formation as well as reduced lifespan (Vijg and Suh, 2013). These radiation studies, together with the discovery of DNA, led to the hypothesis that aging is caused by the accumulation of somatic DNA mutations (Szilard, 1959). Numerous studies have since then verified an age-dependent accumulation of mutations in organisms ranging from yeast to humans (Vijg and Suh, 2013). Additionally, insufficient DNA repair accelerates aging in mice and is linked to several human age-related disorders (Vijg and Suh, 2013, Lopez-Otin et al., 2013). For example, the premature aging syndrome, Werner's syndrome, is caused by a mutation in the *WRN* gene, which encodes a DNA repair protein.

An additional drawback with the replication process is the so called “end replication problem”, which refers to the problem of replicating the ends of chromosomes (Kim Sh et al., 2002). The ends of chromosomes are called telomeres and consists of a DNA-protein stretch that allows the replication machinery to distinguish between the end of chromosomes and double-strand breaks. Due to the replication process, telomeres are shortened with each cell division resulting in progressively shorter chromosomes and eventually genome instability, cell senescence, and/or cell death (Kim Sh et al., 2002). This phenomenon has been suggested to function as an internal clock in which the telomere length is a direct measurement of how many divisions a cell can accomplish. The lifespan of human somatic cells correlates with telomere length, however other cells, such as germline cells and early embryonic cells avoid the problem of telomere shorting by carrying a telomere-elongating enzyme called telomerase (Kim Sh et al., 2002).

2.4.1 Genome stability and peroxiredoxins

Prxs have been implicated in genome stability in various model organisms. A genome wide screen in yeast identified the Prx Tsa1 as an important suppressor of both mutations and gross chromosomal rearrangements (GCRs) (Huang et al., 2003). The significance of Prxs as protectors of the genome in yeast was also supported by a study demonstrating that Tsa1 is maintaining genome stability in cooperation with DNA repair and checkpoint proteins, and that the human 2-Cys Prxs, PrxI and PrxII, can complement the genome defects of a *TSA1* mutant (Iraqui et al., 2008, Iraqui et al., 2009). Moreover, accelerated aging and tumor development in Prx-deficient mice are accompanied by an increased concentration of oxidative DNA damage in the form of 8-oxoguanine modifications (Neumann et al., 2003). Further characterization of Prx-deficient mice revealed a tissue specific accumulation of diverse DNA modifications and subcellular localization of ROS (Egler et al., 2005). As no well-defined connection between DNA oxidation and tumor susceptibility of the different tissues could be made it is not clear if DNA oxidation is the cause of tumor development in Prx-deficient mice, although the findings clearly pinpoint a protective role of Prx. Prxs are also linked to telomere homeostasis as Tsa1-deficient yeast display altered telomere length, which is connected the levels of oxidative stress (Lu et al., 2013).

2.5 Proteostasis

Proteins are responsible for the majority of cellular processes and maintaining the integrity of the proteome, protein homeostasis or proteostasis, is therefore essential for cell function (Tyedmers et al., 2010, Hartl et al., 2011, Labbadia and Morimoto, 2015). Most proteins must fold into a specific three-dimensional structure, or native form, which is crucial for their biological function and needs to be maintained throughout their cellular lifetime (Fig. 3). The native form of a protein is determined by the amino acid sequence and the process of folding is driven by the hydrophobic effect, in which hydrophobic regions is buried inside the structure (Hartl and Hayer-Hartl, 2002). Small proteins typically acquire their native form easily, whereas the folding process of larger proteins with multiple domains tend to include partly folded intermediates which are highly susceptible to misfolding. Some proteins contain intrinsically disordered regions and are particularly prone to misfolding (Dunker et al., 2008, Hipp et al., 2014). Additionally, proteins are constantly challenged by factors that accelerates misfolding such as mutations, translational errors, and environmental stress (Tyedmers et al., 2010, Hartl et al., 2011, Labbadia and Morimoto, 2015). Changes in temperature, pH, and oxidative stress can affect protein structures and induce misfolding. Thus, the misfolding propensity of a protein relies intrinsically on its folding kinetics as well as on its folding environment. Consequences of protein misfolding include loss of function and/or formation of potentially toxic aggregates (Fig. 3). Misfolded and aggregated proteins can engage in inaccurate interactions and disrupt cellular processes. Some proteins form unstructured disordered aggregates whereas others, glutamine/asparagine rich proteins, form highly structured aggregates called amyloids characterized by tightly packed β-sheets (Chiti and Dobson, 2006, Tyedmers et al., 2010, Hartl et al., 2011, Labbadia and Morimoto, 2015).

The system responsible for maintaining proteins intact and functional is collectively referred to as the proteostasis network (PN) and ensures proper folding, transport, and clearance of aberrant proteins (Tyedmers et al., 2010, Mitchell Sontag et al., 2017). Upon protein misfolding, the PN seems to employ three main strategies; refolding/reactivation, degradation, and/or sequestration into protective inclusions. Molecular chaperones are central to the PN as they have functions in many, if not all, branches of the system. Chaperones promote folding of newly synthesized polypeptides, reactivate misfolded/aggregated proteins, assist in the degradation of aberrant proteins as well as support inclusion formation of misfolded and aggregated proteins.

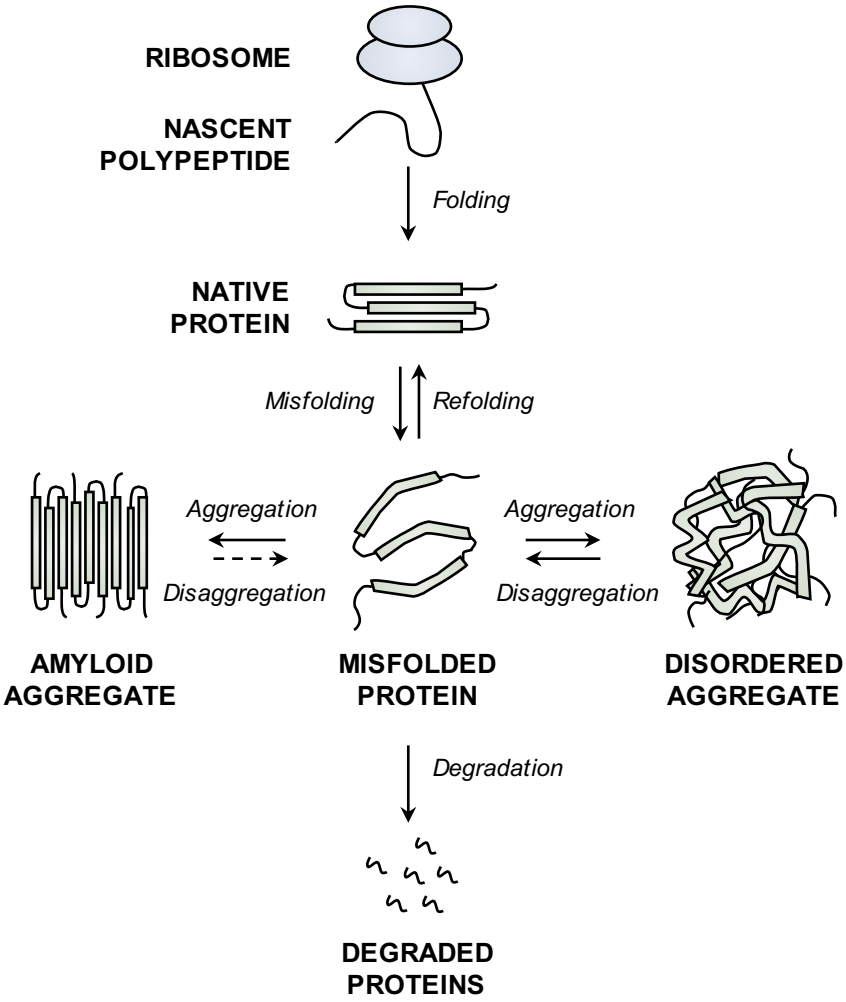

Figure 3. Management of misfolded and aggregated proteins. Newly synthesized polypeptides emerging from the ribosome are folded into their native state. Misfolded protein can be refolded, degraded or form aggregates of disordered or ordered (amyloid) nature.

The presence of damaged proteins and protein aggregates indicates proteostasis imbalance and is considered a hallmark of aging and several aging-related diseases (Oliver et al., 1987, Stadtman and Levine, 2000, Aguilaniu et al., 2003, David et al., 2010, Lopez-Otin et al., 2013, Kaushik and Cuervo, 2015, Labbadia and Morimoto, 2015). In fact, numerous human diseases are associated with the aggregation of a certain protein, often with amyloid-like characteristics. This includes neurodegenerative diseases such as Alzheimer's diseases, Huntington's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS) (Chiti and Dobson, 2006, Labbadia and Morimoto, 2015).

Notably, α-synuclein in Parkinson's disease and aβ/Tau in Alzheimer's diseases are proteins with intrinsically disordered regions that form particularly toxic aggregates (Dunker et al., 2008, Hipp et al., 2014). Additionally, there are many diseases linked to mutations of the PN, such as chaperones and components of protein degradation systems. ALS can for example be caused by a mutation in the gene encoding Ubiquilin-2, a ubiquitin-like protein involved in protein degradation, and mutations in genes encoding small heat shock proteins (sHsps) have been linked to the development of Charcot-Marie-Tooth disease (Labbadia and Morimoto, 2015).

The accumulation of protein aggregates in aged cells is most likely a combined effect of elevated levels of protein damaging agents and a decreased capacity to remove damage. Aging is characterized by, for example, increased mutations, ROS, and vacuolar pH, all of which could affect protein stability and lead to the formation of aggregates (Vijg and Suh, 2013, Laun et al., 2001, Erjavec and Nystrom, 2007, Hughes and Gottschling, 2012). Age-associated changes could also affect components of the PN and thereby initiate a gradual loss of proteostasis (Taylor and Dillin, 2011, Kaushik and Cuervo, 2015). In support of this, protein degradation by autophagy and the ubiquitin-proteasome system have both been reported to decline with age (Cuervo and Dice, 2000, Tonoki et al., 2009). Additionally, once protein aggregates start to accumulate they are likely to themselves disrupt the PN by titrating out important PN components, further contributing to the loss of proteostasis associated with aging and disease (Hipp et al., 2014). The significance of proteostasis in aging is strengthened by the fact that a diminished PN is associated with accelerated aging and, conversely, an enhanced PN delays aging (Taylor and Dillin, 2011, Nystrom and Liu, 2014, Kaushik and Cuervo, 2015).

2.5.1 Proteostasis and peroxiredoxins

The chaperone activity of Prxs has been connected to the formation of high molecular weight (HMW) structures (Jang et al., 2004, Saccoccia et al., 2012, Rhee and Woo, 2011). The oligomerization of Prxs was first observed in human erythrocytes, where the crystal structure the 2-Cys Prx TPx-B revealed a ring-shaped HMW structure composed of five hyperoxidized dimers (Schroder et al., 2000). This type of oligomerization is similar to that of sHsps, ATP-independent chaperone holdases that form a variety of sphere-like HMW structures upon different stresses (Haslbeck and Vierling, 2015).

Studies in yeast demonstrated a heat shock sensitivity of cells lacking the 2-Cys Prxs Tsa1 and Tsa2 (Jang et al., 2004). The heat sensitivity of Prx-deficient yeast cells could partly be rescued with a peroxidase-inactive Tsa1. Furthermore, electron micrography of Tsa1 and Tsa2 supported the existence of (Prx) HMW structures and that formation of such structures is associated with a functional switch, in which the *in vitro* enzyme activity is altered from peroxidase to chaperone activity (Jang et al., 2004). Low molecular weight (LMW) structures of the Prx mainly displayed peroxidase activity, whereas chaperone activity dominated in the HMW form. Both H_2O_2 and heat shock were shown to trigger Tsa1 oligomerization *in vivo*. Further, the peroxidatic Cys48 was essential for H_2O_2 induced oligomerization, while the resolving Cys171 was not. In fact, Prxs lacking active Cys171 did not revert to their LMW form upon H_2O_2 removal, an observation that was true also for Prxs from cells lacking Srx1. Taken together, these data suggest that oxidation of Cys48 is a prerequisite for oligomerization and chaperone activity under peroxide stress *in vivo*.

Further characterization of Prxs have identified the C-terminal as critical for hyperoxidation. It has been reported that a part of the C-terminal, referred to as the YF motif, forms an α-helix above the active site thereby stabilizing its structure. As local unfolding of the active site is necessary for the resolution reaction, this stabilization of the active site by the YF motif enables further oxidation of the active site cysteine (Wood et al., 2003). Indeed, Prxs with truncated C-terminals have been shown resistant to hyperoxidation (Koo et al., 2002, Wood et al., 2003). The C-terminal was highlighted also in a structural study of a 2-Cys Prx in the parasite worm *Schistosoma mansoni*, in which the HMW structure was found to be a stacked double decamer (dodecamer) with an unstructured and disordered C-terminal in comparison to the LMW form, which could be responsible for the chaperone activity (Saccoccia et al., 2012).

Prx-related chaperone activity has now been reported for several organisms (Noichri et al., 2015). For example, the human 2-Cys Prxs, PrxI and PrxII, have been shown to display chaperone activity associated with structural changes of the enzyme (Moon et al., 2005, Jang et al., 2006, Park et al., 2011, Pan et al., 2014). Most of the described cases have been consistent with previous reports of hyperoxidation as a prerequisite for oligomerization. A recent *in vivo* study could confirm that the HMW structures of Tsa1 indeed consist of hyperoxidized Tsa1, assembled into a dodecamer (Noichri et al., 2015).

An exception from these findings is a mitochondrial Prx from the parasite *Leishmania infantum*. This Prx undergoes structural changes in the center of its decameric ring, which leads to increased surface hydrophobicity and binding/protection of client proteins from thermal aggregation (Teixeira et al., 2015). In this case, the decamer is formed in a reduced state during elevated temperature and may therefore represent a chaperone activity distinct from the one induced by hyperoxidation.

In yeast, Tsa1 is required for protection against protein aggregation induced by the reducing agent dithiothreitol (DTT) and the protein misfolding-inducing proline analog azetidine-2-carboxylic acid (AZC) (Rand and Grant, 2006, Weids and Grant, 2014). Additionally, the peroxidatic Cys48 of Tsa1, but not the resolving Cys171, is important for survival under zinc-deficiency (MacDiarmid et al., 2013). Zinc is a biological cofactor important for protein folding and as the peroxidatic cysteine is required for chaperone activity, while the resolving cysteine is not (Jang et al., 2004), it was suggested that Tsa1 counteract protein misfolding under conditions of low zinc. This was further supported by the fact that the growth defect of Tsa1-deficient cells cultivated at low zinc concentrations could be suppressed by overexpression of the sHsps, Hsp26 and Hsp42.

3. Aging in *Saccharomyces cerevisiae*

Saccharomyces cerevisiae, or baker's yeast, is a unicellular organism that divides asymmetrically by budding off a daughter cell of a smaller size (Hartwell and Unger, 1977). Yeast can proliferate in culture indefinitely and was long thought of as immortal and unusable as a model for aging. However, studies in the 1950's utilized the nature of asymmetrical division to distinguish and remove the daughter cell from each budding event, allowing the division of individual yeast cells to be monitored for the first time. These experiments lead to the discovery that yeast has a finite lifespan and initiated studies of yeast as a model for cellular aging (Barton, 1950, Mortimer and Johnston, 1959). Two models are now used to study the effects of aging in yeast; replicative lifespan (RLS) and chronological lifespan (CLS). The RLS is defined as the number of daughters generated by each mother cell and is usable as a model for aging in dividing cells, such as stem cells (Denoth Lippuner et al., 2014). CLS is in contrast based on the survival of yeast cells in a post-replicative state, which is thought of as model for aging in non-dividing cells such as neurons (Longo and Fabrizio, 2012). The RLS model has been used exclusively in the works of this thesis and will therefore be the main focus hereafter.

The mean RLS of yeast is around 25 generations and increased replicative age is associated with morphological and physiological changes such as increased cell size, altered cell shape, and an extended generation time (Jazwinski, 1999, Denoth Lippuner et al., 2014). Remarkably, the daughters of aging mothers are born with a full replicative lifespan potential without signs of age-associated changes, indicating that aging/senescence factors are retained in the mother cell during division (Mortimer and Johnston, 1959, Jazwinski et al., 1989, Kennedy et al., 1994, Aguilaniu et al., 2003, Henderson and Gottschling, 2008). An exception to this rejuvenation phenomenon is daughters of old cells, which typically inherit some of the mother's aging factors and thus have a shorter lifespan.

3.1 Aging and asymmetric division

Asymmetric division in yeast is tightly linked to the polarity machinery (Higuchi-Sanabria et al., 2014). The polarity machinery localizes to the bud site before cell division and is responsible for polarized growth. The polarity machinery includes a large number of components whose function is to establish a septin ring and a polarized actin cytoskeleton at the bud site. The polarisome is part of the polarity machinery and constitutes a protein complex that includes scaffolding proteins and proteins responsible for actin polymerization (Higuchi-Sanabria et al., 2014, Nystrom and Liu, 2014). The actin cytoskeleton is polymerizing from the bud tip, generating linearized actin cables spanning from the bud into the mother during cells division (Fig. 4). These actin cables serve as tracks for transporting cellular components needed for cell polarity and bud growth. Actin cable-flow is directed towards the mother, due to actin nucleation at the polarisome, whereas ATP-dependent motor proteins (myosins) move against the actin cable flow enabling transport into the bud. Studies suggest that the cell utilizes actin movement in both directions to prevent damaged components from entering the bud during cell division, discussed further below (Higuchi-Sanabria et al., 2014, Nystrom and Liu, 2014).

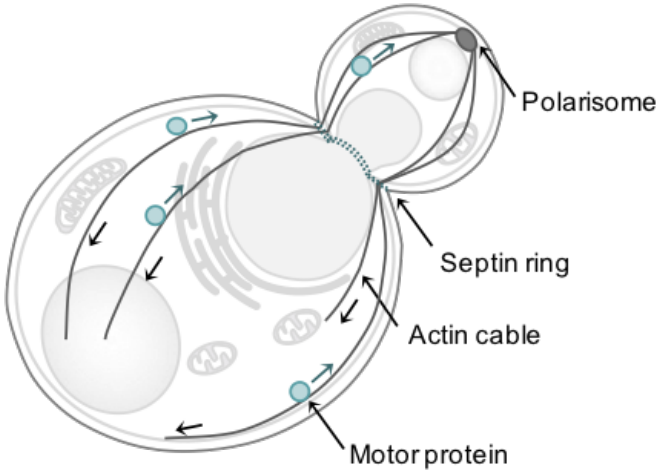

Figure 4. Actin dynamics during cell division. The polarity machinery is responsible for polymerized growth and establishment of a septin ring at the bud neck. The polarisome located at the bud tip ensures polymerization of actin cables toward the mother cell. Myosins moving against the cable flow enable transport of cellular constituent into the bud.

3.2 Yeast aging factors

Four criteria have been described to define aging factors in yeast; an aging factor should (1) increase with age, (2) be retained in the mother cell during mitosis, (3) accelerate aging when overproduced, and finally, (4) extend lifespan when its accumulation is reduced (Henderson and Gottschling, 2008, Denoth Lippuner et al., 2014). Although these definitions might require modifications as new research emerges, there are currently four factors meeting these criteria, involving changes to the nucleus, mitochondria, vacuole, and cytoplasm (Denoth Lippuner et al., 2014, Nystrom and Liu, 2014) (Fig. 5).

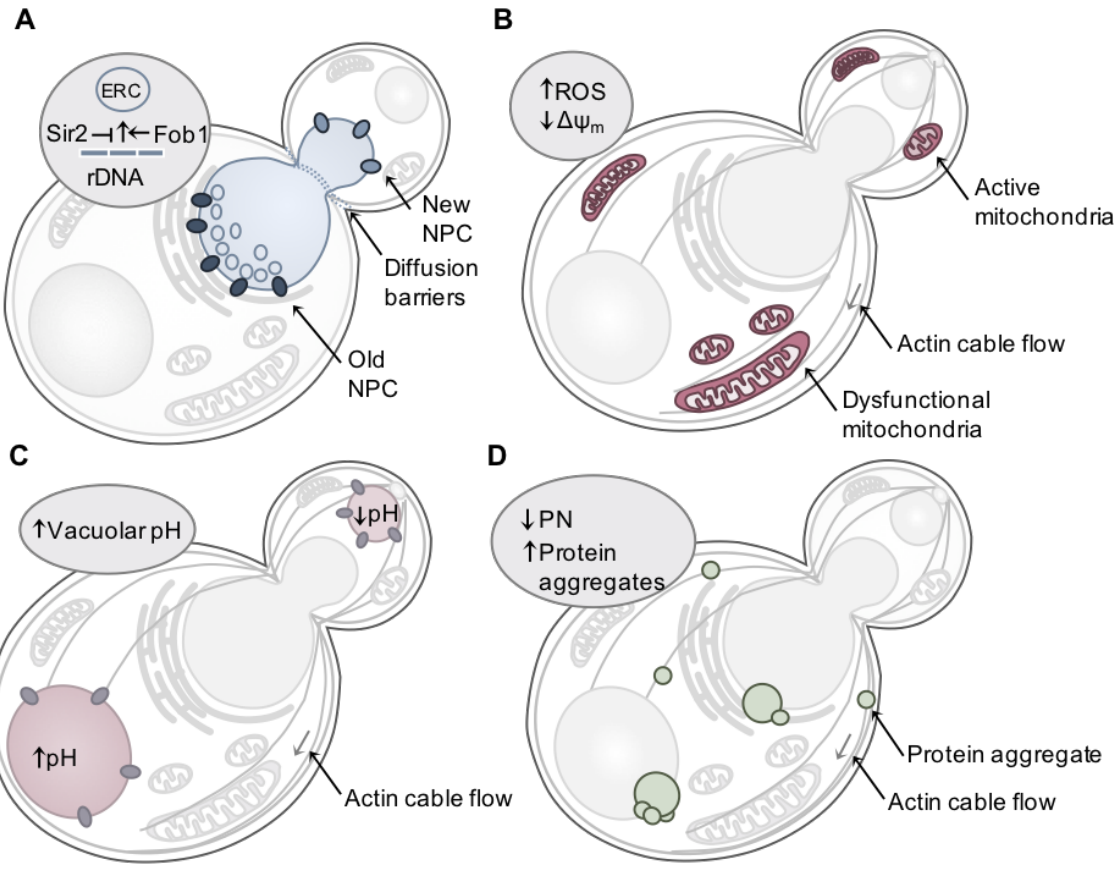

Figure 5. Yeast aging factors and their asymmetric inheritance. **A.** Sir2-dependent ERC formation in the nucleus is subjected to asymmetrical inheritance, possibly by association to nuclear pore complexes. **B.** Dysfunctional mitochondria, display decreased membrane potential and increased production of ROS. Mitochondria are associated with actin cables during cell division, and healthy mitochondria move faster against the cable flow, compared to dysfunctional ones, increasing their concentration in the daughter cell. **C.** Vacuolar pH increases early upon replicative aging, disrupting the storage of amino acids as well as mitochondrial membrane potential. Vacuoles are associated with actin during cell division and daughters have the capacity to restore vacuolar pH of newly inherited vacuoles **D.** Protein aggregates accumulate in aging cells and associate with actin cables, directly and/or tethered to organelles, and are thus prevented from entering the bud.

3.2.1 Extrachromosomal ribosomal DNA circles

One of the first aging factors identified in yeast were nuclear extrachromosomal rDNA circles (ERCs) (Sinclair and Guarente, 1997) (Fig. 5A). The rDNA locus is located on chromosome XII and consist of an array of 100-200 copies of a 9.1kb repeat encoding the 35S rRNA and 5S rRNA. Replication of the rDNA repeats is unidirectional due to a Fob1-dependent replication fork block. This block can cause double-strand breaks and initiation of a reparation process by homologous recombination which sometimes results in excision of a single repeat or multiple adjacent repeats of rDNA, resulting in the formation of an ERC. ERCs replicate autonomously due to the presence of an ARS (autonomously replicating sequence) and accumulation of ERCs is associated with the fragmented nucleolus seen in aged yeast cells (Guarente, 1997, Steinkraus et al., 2008). ERCs have been shown to accumulate exponentially in yeast mother cells, segregate to the mother during cell division, shorten lifespan when introduced artificially, and extend lifespan when eliminated by the removal of Fob1 (Sinclair and Guarente, 1997, Defossez et al., 1999).

The deacetylase Sir2 is responsible for silencing of the rDNA locus and Sir2-deficiency leads to increased recombination and formation of ERCs. As previously described, Sir2 levels are limiting for yeast RLS, an effect that has been linked to the formation of ERCs (Kaeberlein et al., 1999). However, *sir2Δfob1Δ* and *fob1Δ* cells have equally low levels of ERCs, yet the Sir2 deficient cells have a shorter lifespan, indicating an additional anti-aging function of Sir2 that is independent of ERCs (Kaeberlein et al., 1999, Erjavec et al., 2007, Kaeberlein, 2010). The mechanism behind asymmetric division of ERCs is not entirely clear, though one model suggests that it involves association between the ERCs and the nuclear pore complexes (NPCs). In this model, the pre-existing NPCs are retained in the mother and the associated ERCs are therefore prevented from entering the daughter cell. This asymmetry involves a mechanism based on a diffusion barrier in the outer nuclear membrane (Shcheprova et al., 2008, Nystrom and Liu, 2014). However, a later study reported that NPCs fail to efficiently segregate to the mother cell during cell division (Khmelinskii et al., 2010), questioning if binding to NPCs can fully account for the asymmetric segregation of ERCs.

3.2.2 Dysfunctional mitochondria

Mitochondria are tubular organelles operating as the main energy supplier in the cell. Studies in yeast have revealed that replicatively aged cells accumulate fragmented mitochondria with impaired membrane potential, alterations of the mitochondrial DNA, reduced ATP production, increased ROS production, and a more oxidized redox state (Scheckhuber et al., 2007, Higuchi-Sanabria et al., 2014) (Fig. 5B). Mitochondrial dysfunction leads to nuclear genome instability, which is linked to mitochondrial biogenesis of iron-sulfur clusters (ISC) that are important co-factors for many proteins involved in DNA repair and maintenance (Veatch et al., 2009). In agreement with the definitions of yeast aging factors, counteracting age-related mitochondrial fragmentation by deletion of a gene involved in mitochondrial fission extends lifespan of yeast mother cells (Scheckhuber et al., 2007). Moreover, daughters of old yeast cells predominantly inherit higher functioning mitochondria, (Lai et al., 2002, McFaline-Figueroa et al., 2011). Additionally, cells with impaired mitochondrial F1-ATP synthase fail to segregate active mitochondria to the daughter cells and this is accompanied with lost rejuvenation of these daughters, indicating that the process of mitochondrial segregation is required for age asymmetry in yeast (Lai et al., 2002).

The segregation of mitochondria involves anchorage to motor proteins on actin cables and subsequent transport towards the bud during cell division (Higuchi et al., 2013). It has been shown that reduced, healthy mitochondria move faster against the actin cable flow compared to oxidized, dysfunctional mitochondria, resulting in an accumulation of the latter in the mother cell (McFaline-Figueroa et al., 2011, Higuchi et al., 2013). Accordingly, modulating the rate of the actin cable flow alters the balance of active and dysfunctional mitochondria in the daughter and influences lifespan (McFaline-Figueroa et al., 2011, Higuchi et al., 2013). The significance of mitochondrial segregation is further supported by the fact that this has been observed also in mammalian stem cells, where segregation of dysfunctional mitochondria is essential to maintain stem cell properties (Katajisto et al., 2015).

3.2.3 Increased vacuolar pH

The yeast vacuole is an acidic, membrane-bound organelle that is involved in amino acid storage and degradation/recycling of cellular components through autophagy. Autophagy entails sequestration of cytoplasmic material in double-membrane vesicles followed by transport and fusion of such vesicles to the vacuole, in which the material is degraded (Abeliovich and Klionsky, 2001). This type of autophagy is called macroautophagy and is the most commonly observed. Two other types of autophagy include microautophagy, where material is directly engulfed into the vacuole, and chaperone-mediated autophagy. Autophagy is believed to have anti-aging effects as enhanced autophagy is associated with lifespan extension in worms and flies (Rubinsztein et al., 2011). Similar to mitochondria, yeast vacuoles deteriorate with age, demonstrated by morphological changes and a failure to maintain a low pH (Hughes and Gottschling, 2012) (Fig. 5C). Such a declined acidity is observed early during replicative aging and is contributing to the impaired mitochondrial membrane potential seen in aged cells (Hughes and Gottschling, 2012). Amino acid storage is the suggested link between the two organelles, as an increased pH in aging vacuoles disrupts import of neutral amino acids, which are instead redirected to the mitochondria where they are normally catabolized. The increased load of amino acids in the mitochondria affects membrane potential and initiate an age-related decline in mitochondrial function.

Vacuoles are transported via the actin cytoskeleton to the daughter cell during division, however there does not seem to be a selective process in which pristine vacuoles are segregated to the daughter (Nystrom and Liu, 2014). Instead, the restored vacuolar pH control in daughter cells is believed to depend on their intracellular environment: the increased vacuolar pH was traced back to an asymmetric inheritance of a proton pump in the plasma membrane, Pma1 (Thayer et al., 2014, Henderson et al., 2014). Pma1 is responsible for pumping protons from the cytoplasm over the plasma membrane, thus competing with the vacuolar proton pump, Vma1. The majority of the Pma1 proton pumps are retained in the mother during cell division, resulting in an increased proton flow out of the cell and depletion of the cytoplasmic proton pool available for import into the vacuole. As the daughter receives less Pma1, more protons are accessible for vacuole import and the vacuolar pH is restored. Increasing vacuolar acidity by overexpressing a vacuolar proton pump suppress mitochondrial dysfunction and extends lifespan, confirming the role of vacuoles as aging factors (Hughes and Gottschling, 2012, Nystrom and Liu, 2014).

3.2.4 Protein aggregates

As described previously, the native form of a protein is essential for proper function and if lost, misfolded and aggregated proteins can interact with and disrupt cellular processes. Oxidatively damaged proteins and protein aggregates have been shown to accumulate and segregate to the mother during replicative aging (Aguilaniu et al., 2003, Erjavec et al., 2007, Tessarz et al., 2009, Liu et al., 2010, Hill et al., 2014) (Fig. 5D). The asymmetric segregation of protein aggregates requires the actin cytoskeleton, the deacetylase Sir2, and the protein disaggregase Hsp104 (Aguilaniu et al., 2003, Erjavec et al., 2007, Tessarz et al., 2009, Liu et al., 2010). The mechanism involving these three actors is not entirely elucidated, though it has been suggested to involve protein aggregates interacting with the actin cytoskeleton. The Sir2 contribution to such a process could be through regulation of chaperonin-mediated folding of actin (Liu et al., 2010, Erjavec et al., 2007), whereas Hsp104 could be mediating the interaction between protein aggregates and actin cables (Liu et al., 2011). Hsp104 could also affect asymmetry through the polarisome complex, as one of its members (Spa2) have been reported as a Hsp104 substrate (Tessarz et al., 2009).

Another mechanism of protein aggregate retention is linked to the coalescence of misfolded and aggregated proteins into protective inclusions, which are tethered to organelles that are themselves subjected to asymmetric segregation (Kaganovich et al., 2008, Spokoini et al., 2012, Zhou et al., 2014). In addition to being able to retain damaged proteins in the mother, Sir2-dependent asymmetry can also preferentially deliver more active catalase to the bud, thus enhancing the daughter cells capacity to combat oxidative stress and contributing to its rejuvenation (Erjavec and Nystrom, 2007).

Finally, protein aggregates leaking in to the daughter are efficiently removed, either by actin dependent transportation back to the mother or by disaggregation/degradation by the daughter proteostasis machinery (Hill et al., 2014). Consistent with the aging factor criteria, deletion of key PN components leads to accumulation of protein aggregates and accelerates aging (Erjavec et al., 2007, Kruegel et al., 2011, Oling et al., 2014), and overproduction counteracts age-dependent protein aggregate formation and extends lifespan (Erjavec et al., 2007, Kruegel et al., 2011, Hill et al., 2014).

The process of asymmetric segregation of protein aggregates has been reported in organisms ranging from bacteria to humans (Ackermann et al., 2003, Nyström, 2007, Rujano et al., 2006, Bufalino et al., 2013, Ogrodnik et al., 2014). Interestingly, in *Drosophila* stem cells, the segregation of protein aggregates correlated with longevity, as the cell with the shortest lifecycle inherits most of the protein damage whereas the cell destined for a long life inherits less damage and is thus rejuvenated (Bufalino et al., 2013).

3.2.5 The interconnectivity of aging pathways

The aging pathways described so far are not to be viewed as competing mechanisms of aging, but rather highly connected processes that are part of a large network. This is evident when considering the aging factors in yeast; the asymmetric segregation of a plasma membrane proton pump leads to increased vacuolar pH, which in turn disrupts mitochondrial membrane potential resulting in increased production of ROS and nuclear genome instability (Veatch et al., 2009, Hughes and Gottschling, 2012, Higuchi-Sanabria et al., 2014, Thayer et al., 2014, Henderson et al., 2014, Gottschling and Nystrom, 2017). ROS can affect both DNA and protein stability and can thus promote formation of both mutations and protein aggregates (Sohal and Weindruch, 1996, Finkel and Holbrook, 2000). Furthermore, overproduction of Vac17, an adaptor protein involved in vacuole-actin interactions, improves segregation of protein aggregates (Hill et al., 2016). Additionally, products of both cytoplasmic and mitochondrial translation contribute to the assembly of the electron transport chain, thus cytoplasmic proteostasis affects mitochondrial activity (Couvillion et al., 2016). Finally, caloric restriction extends lifespan in part by counteracting the lost vacuolar acidity and mitochondrial dysfunction seen in aging cells (Hughes and Gottschling, 2012). Caloric restriction is a great example of interconnectivity between aging pathways, as many of the targets of the signaling pathways described above are involved in proteostasis and the defense against oxidative stress, including chaperones, translational factors, and antioxidants (Kenyon, 2010, Fontana et al., 2010).

The interconnectivity of different subsystems may seem counteractive, as the breakdown of one system could affect the function of another, but it could also create a back-up situation in which the failure of one system is compensated by another (Gottschling and Nystrom, 2017). Elucidating the interconnectivity of aging pathways is a key aspect of understanding the process of aging.

3.3 The yeast proteostasis network

The major task of the proteostasis network (PN) is to maintain a functional proteome and prevent the accumulation of toxic protein conformers. The fate of a misfolded protein involves three main routes; (1) refolding/reactivation, (2) degradation, and (3) sequestration into protective inclusion (Tyedmers et al., 2010, Mitchell Sontag et al., 2017) (Fig. 6). Molecular chaperones are central to the PN and operate within all three routes.

3.3.1 Molecular chaperones

Molecular chaperones work to prevent protein misfolding during normal growth as well as under stress conditions (Hartl and Hayer-Hartl, 2002). Accordingly, some chaperones are constitutively expressed while others are mainly induced under times of stress. The expression of many chaperones is up-regulated during increased temperature and they are therefore termed heat shock proteins (Hsps), although their production is elevated during other types of stress as well. Hsps generally recognize hydrophobic regions, yet the mode of action varies greatly between different classes.

Small Hsps (sHsps) are ATP-independent chaperones that bind and merge misfolded proteins and are sometimes referred to as holdases or aggregases (Haslbeck and Vierling, 2015, Mogk and Bukau, 2017). The sequence and size of sHsps is quite variable, though a few characteristics are shared within this group; a small molecular mass, a conserved C-terminal α-crystallin domain, and the ability to form high molecular weight oligomers (Haslbeck and Vierling, 2015). sHsps are dynamic and shift between a monomeric/dimeric state and an oligomeric state, typically composed of 12-42 subunits.

The Hsp40 family of chaperones are co-chaperones that bind unfolded proteins and recruit and stimulate the ATPase activity of Hsp70 chaperones (Kampinga and Craig, 2010). Hsp40s are often referred to as J-proteins, owing to their signature J-domain which is responsible for their ATPase stimulating ability. J-proteins are further divided into class I, II and III based on the presence of certain motifs and domains. Organisms usually have multiple J-proteins and 22 have been identified in yeast. The great number of J-proteins is thought to provide diversity, in turn, to the assignments of the Hsp70 chaperones (Kampinga and Craig, 2010).

The Hsp70 chaperones are fundamental participants of the PN, as they are involved in all divisions of the system. Hsp70's are ATP-dependent chaperones that can promote *de novo* folding, refolding, degradation, and sequestration of their substrates (Erbse et al., 2004, Mayer and Bukau, 2005, Park et al., 2007, Malinovska et al., 2012, Escusa-Toret et al., 2013). Hsp70 have an ATP-binding domain and a substrate binding domain, and their mode of action involves cycles of substrate binding and release until the substrate has acquired its native fold (Mayer and Bukau, 2005). ATP binding leads to an open Hsp70 state and enables substrate binding, ATP hydrolysis to ADP induces a closed state in which the substrate is locked, and finally ADP to ATP exchange supports substrate release. Hsp40 chaperones accelerate ATP hydrolysis whereas nucleotide exchange factors promote ADP to ATP exchange, both increasing Hsp70 efficiency (Mayer and Bukau, 2005). Hsp110 are structurally related to the Hsp70 and act as nucleotide exchange factors (NEFs), but have also been shown to prevent protein aggregation by a chaperone holdase function (Kim et al., 2013).

The disaggregase Hsp104 belongs to the family of Hsp100 AAA+ ATPases and appears to act primarily on previously aggregated proteins (Glover and Lindquist, 1998, Bosl et al., 2006, Mogk et al., 2015). Hsp104 contains two nucleotide binding domains and assembles into a double ring-shaped hexamer. Two models have been proposed to explain the mechanism of Hsp104 protein disaggregation (Bosl et al., 2006). In the threading model, polypeptides are extracted from an aggregate followed by ATP-driven translocation through the central channel. In the crowbar model, an ATP-dependent mechanical force is thought to disrupt the protein aggregate. In both models, the refolding of polypeptides is achieved by the Hsp40/Hsp70 chaperones.

The chaperonins are large protein complexes composed of several subunits assembled in two rings surrounding a central cavity, in which substrate folding take place in an ATP-dependent manner (Hartl et al., 2011). The chaperonins are divided into two structurally distinct classes, which in yeast are the Hsp60 and the chaperonin-containing CCT (TCP1). The essential role of CCT is linked to its folding of certain essential substrates such as actin and tubulin (Spiess et al., 2004). The Hsp90 family of chaperones promotes *de novo* folding of many substrates and while the Hsp70 system act early in the folding process, the Hsp90 are typically active later (Hartl et al., 2011). Hsp90 recognizes substrates with the help of co-chaperones and is important for the folding of many proteins involved in signaling pathways, such as kinases and transcription factors (Taipale et al., 2010).

Chaperones cooperate to manage misfolded and aggregated proteins (Glover and Lindquist, 1998, Tyedmers et al., 2010, Mogk et al., 2015) (Fig. 6). sHsps bind and merge misfolded proteins immediately after stress, maintaining substrates in a folding competent state as well as preventing unspecific aggregation (Mogk and Bukau, 2017). The co-chaperone Hsp40 together with Hsp70 and NEFs bind misfolded proteins in aggregates and target the substrate to Hsp104 (Tyedmers et al., 2010). Hsp70 recruit and stimulate Hsp104 activity through a direct physical interaction (Mogk et al., 2015). Moreover, the NEF Hsp110 has recently been shown to assist in both Hsp70/Hsp104 recruitment to heat-induced protein aggregates as well as the Hsp104-dependent disaggregation (Kaimal et al., 2017). A protein disaggregation function of Hsp110, in cooperation with Hsp40 and Hsp70, has been reported also in mammalian cells, which lack a homolog of Hsp104 (Shorter, 2011). Disaggregated proteins can either be refolded, degraded, or sequestered into larger inclusions, all of which are chaperone-mediated processes (Tyedmers et al., 2010, Mitchell Sontag et al., 2017). The cytosolic J-protein Ydj1 and Sis1 have been suggested to determine the fate of Hsp70 substrates; Ydj1 is thought to support substrate refolding whereas Sis1 directs substrates to the nucleus for degradation (Lu and Cyr, 1998, Park et al., 2013, Shiber et al., 2013).

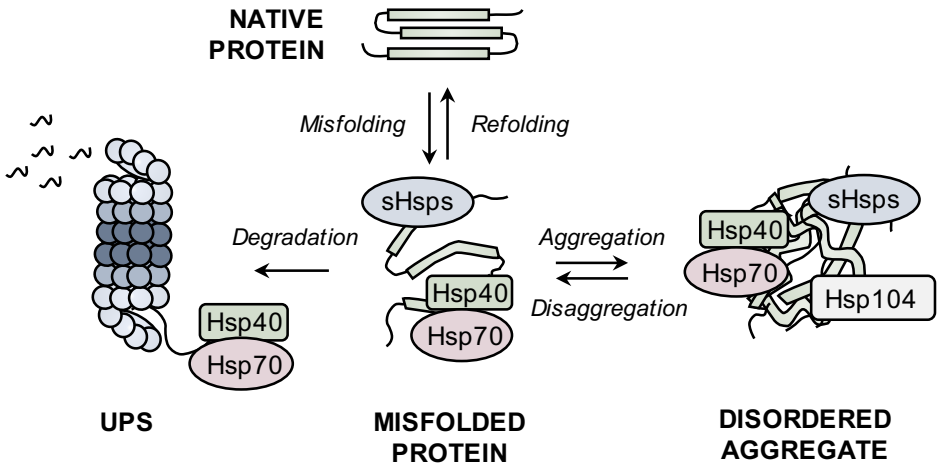

Figure 6. Chaperone-mediated management of misfolded and aggregated proteins. sHsps bind misfolded proteins, maintaining them in a folding competent state and supporting coalescence into larger inclusions. Hsp40/Hsp70 and NEFs (not shown) localization to misfolded proteins enable refolding or UPS degradation. Hsp104 performs protein disaggregation after which Hsp70-mediated refolding or UPS degradation can take place. Misfolded and aggregated proteins are merged into larger inclusions with the assistance of sHsps, Hsp40, Hsp70, and Hsp104.

3.3.2 The ubiquitin-proteasome system

The ubiquitin-proteasome system (UPS), together with autophagy, represent two principle modes of protein degradation in the cell. The proteasome is a large protein complex made up of a 20S catalytic core particle and one or two 19S regulatory particles (Tanaka, 2009) (Fig. 7). The 19S subunit bound to either one or both ends of the 20S core constitute the 2.4MDa protein complex referred to as the 26S proteasome. The core particle is arranged in a barrel-like structure with the proteolytic active sites localized within the internal space of the core particle (Tanaka, 2009). The 19S consists of a ring-shaped base and a lid, and regulates substrate entry by opening up a translocation channel through the proteolytic chamber of the 20S core. Proteasome degradation is tightly linked to the ubiquitin system, a cascade of enzymes that targets proteins in need of degradation with ubiquitin (Finley et al., 2012). Ubiquitin-targeting is also involved in regulating the activity of various proteins. The fate of a ubiquitinated protein appears to be determined by the number of ubiquitin modifications linked to the protein as a polyubiquitin chain typically is required for proteasomal degradation.

Ubiquitin is covalently linked to proteins in a multi-step process involving four enzymes; E1-E4 (Finley et al., 2012) (Fig. 7). The reaction is initiated by the ubiquitin-activating enzyme E1, which binds and activates a ubiquitin (Ub) molecule in an ATP-dependent reaction. The activated Ub is transferred to the ubiquitin-conjugating enzyme E2, and the E2-Ub complex is recognized by a substrate-specific ubiquitin ligase E3 which transfers Ub to the substrate. Multiple rounds of the ubiquitin-conjugation process lead to a polyubiquitin-marked substrate. E4 are a class of ubiquitin ligases that act by extending the polyubiquitin chain, and thus exclusively recognize previously ubiquitinated substrates (Finley et al., 2012). Yeast contains one essential E1 activating enzyme, eleven E2 conjugating enzymes, and 42 E3 ligases thought to confer substrate specificity to the system (Lee et al., 2008). The major E3 ligases in the cytosol appears to be Ubr1, Ubr2, Hul1, and Ltn1, which promote degradation of proteins in cooperation with the Hsp40/Hsp70 chaperone machinery (Esser et al., 2004, Park et al., 2007, Eisele and Wolf, 2008, Finley et al., 2012).

Polyubiquitin-tagged substrates are recognized by the 19S proteasome subunit which removes the ubiquitin molecules and partly unfolds the substrates, enabling entry and proteolysis within the 20S core particle (Tanaka, 2009). Ubiquitin can also be removed by

deubiquitinating enzymes (DUBs) that are not part of the 19S proteasome. The action of DUBs is important for recycling of ubiquitin but also provides a regulatory mechanism where proteins can be rescued from degradation and reactivated (Finley et al., 2012, Oling et al., 2014).

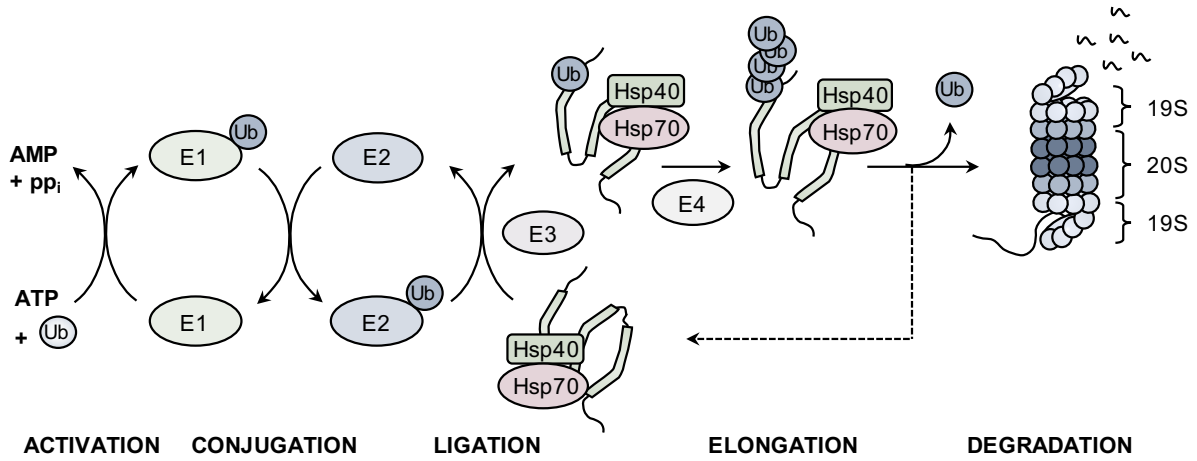

Figure 7. Protein degradation by the Ubiquitin Proteasome System (UPS). The ubiquitin-activating enzyme, E1, initiates the ubiquitination process by activating a ubiquitin molecule (Ub) in a ATP-dependent manner. The ubiquitin-conjugating enzyme, E2, receives the Ub and is subsequently recognized by the ubiquitin ligase, E3, which transfers Ub to a substrate. E4 ubiquitin ligases extends the ubiquitin chain. Multiple rounds of this ubiquitination process leads to a polyubiquitin marked substrate that is recognized by the 19S proteasome subunit. The Hsp40/Hsp70 system works in cooperation with the ubiquitin system to target substrates for proteasomal degradation. Deubiquitination is achieved by the 19S proteasome subunit or DUBs (not shown), and is required for substrate entry and proteolytic degradation within the 20S core particle.

3.3.3 Spatial protein quality control

In addition to the temporal proteostasis network described above, yeast has developed a spatial protein quality control (SPQC) system where misfolded and aggregated proteins are sequestered into larger inclusions (Fig. 8). Much of the work describing SPQC has been performed using a set of misfolding proteins as reporters of protein aggregation, including a destabilized version of the sumo-conjugating enzyme Ubc9, the unassembled von Hippel-Lindau tumor suppressor (VHL), and a thermolabile luciferase. Immediately upon stress, all of the above substrates misfold and accumulate in aggregates throughout the cytosol, referred to as stress foci (also called CytoQ or Q bodies) (Kaganovich et al., 2008, Specht et al., 2011, Malinovska et al., 2012, Escusa-Toret et al., 2013, Miller et al., 2015). These initial stress foci coalesce into larger inclusions upon prolonged stress and eventually end up in (at least) two distinct locations; the juxtanuclear quality control site (JUNQ) and the vacuole-associated, insoluble protein deposit (IPOD) (Kaganovich et al., 2008, Tyedmers et al., 2010, Chen et al., 2011). Reports of cytosolic proteins located inside the nucleus lead to the suggestion of re-naming JUNQ to the intranuclear quality control (INQ) (Miller et al., 2015). As the matter of localization is not entirely clear, I hereafter refer to this site as JUNQ/INQ.

The sequestration of misfolded and aggregated proteins limits toxic interactions as well as aids in their clearance. Heat-denatured proteins have been shown to localize to both JUNQ/INQ and IPOD, whereas amyloidogenic proteins typically are targeted to the IPOD (Kaganovich et al., 2008, Specht et al., 2011, Escusa-Toret et al., 2013). The initial stress foci formation and coalescence seems to involve the ER, as well as chaperones, such as sHsps, Hsp40, Hsp70, and Hsp104 (Kaganovich et al., 2008, Specht et al., 2011, Escusa-Toret et al., 2013). The JUNQ/INQ deposit is highly dynamic, with rapid protein turnover due to the high concentration of proteasomes (Kaganovich et al., 2008). The IPOD is in contrast less dynamic, formed upon prolonged stress, and remains longer after stress relief. The sorting of proteins to IPOD and JUNQ/INQ is not fully understood. Ubiquitin was originally proposed as a sorting mechanism, based on the high levels of ubiquitin-tagged proteins at JUNQ and ubiquitin-tagged IPOD substrates being re-directed to JUNQ (Kaganovich et al., 2008). However, ubiquitin-independent sorting was later reported, indicating that ubiquitin may not be a universal sorting mechanism to JUNQ (Miller et al., 2015). The formation of IPOD and JUNQ/INQ has been found to involve the actin cytoskeleton and a network of chaperones and sorting factors (Kaganovich et al., 2008, Specht et al., 2011, Malinovska et al., 2012, Escusa-

Toret et al., 2013). The sHsp Hsp42 is required for sorting proteins into peripheral inclusions and IPOD, whereas the J-protein Sis1 has been shown to deliver misfolded proteins to the nucleus for degradation (Specht et al., 2011, Park et al., 2013). Moreover, the v-SNARE binding protein Btn2 and its paralog Cur1 have also been reported to regulate protein sorting to inclusions (Malinovska et al., 2012, Miller et al., 2015). It has been suggested that Btn2 binding to Hsp42 directs substrates to IPOD, whereas Btn2 binding to Sis1 promotes sorting to JUNQ/INQ (Malinovska et al., 2012). In this model, Btn2 and Hsp42 are working as aggregases, supporting inclusion formation of misfolded substrates in the nucleus and cytoplasm respectively. The role of Cur1 appears to be regulation of Sis1 availability in the cytosol; Cur1 binds and recruits Sis1 to the nucleus without substrates, thereby leaving substrates free to interact with Btn2/Hsp42. As mentioned above, inclusion formation is linked to asymmetric segregation of damaged proteins and factors such as Hsp40s, Hsp70s, and Hsp104s are therefore essential for damage asymmetry (Erjavec et al., 2007, Spokoini et al., 2012, Zhou et al., 2014, Coelho et al., 2014).

Studies of SPQC so far involve a limited number of substrates, thus the subcellular locations, dynamics, and factor dependence may differ for other substrates. Indeed, the formation of amyloid containing inclusions seem to skip the stress foci formation step and cut directly to the larger IPOD deposit in a Hsp42-independent manner (Specht et al., 2011, Escusa-Toret et al., 2013). Additionally, the conditions used are typically heat stress and/or proteasome inhibition, and it remains to be elucidated how these results translate to the SPQC taking place during other conditions, such as oxidative stress and aging. A clue to age-related SPQC came with the notion of a Hsp104-containing deposit formed early during replicative aging (Saarikangas and Barral, 2015). This age-associated deposit was formed in a Hsp42-dependent manner and co-localized with Hsp42/Hsp40/Hsp70, but not with Btn2 and Sis1.

Protein inclusion formation has been seen also in multicellular organisms. Mammalian inclusions or aggresomes, are formed in a microtubule-dependent manner surrounded by vimentin filaments and enriched for chaperones and proteasomes (Kopito, 2000). Furthermore, studies of the VHL substrate in mammalian cell lines revealed a vimentin enclosed JUNQ with active proteasomes that were asymmetrically segregated during division (Ogrodnik et al., 2014).

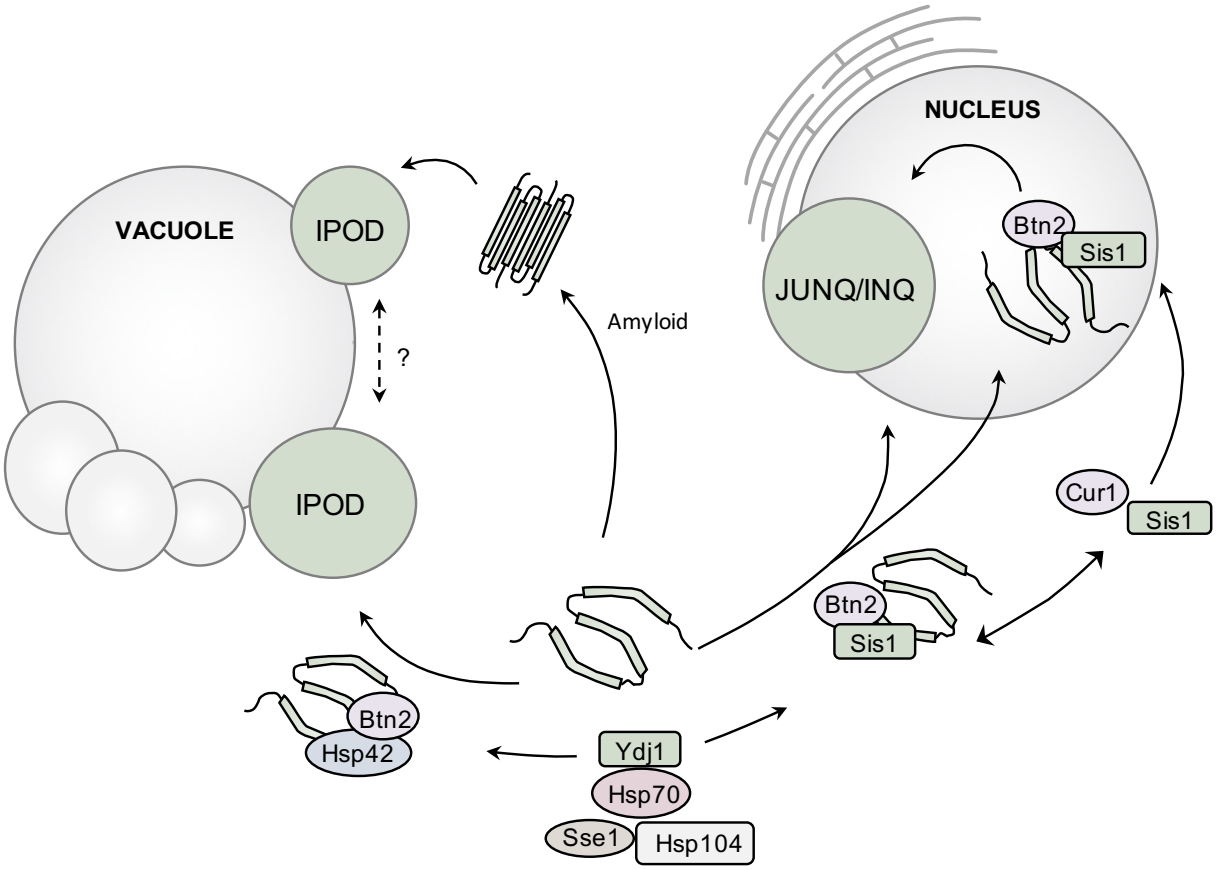

Figure 8. Spatial quality control of misfolded and aggregated proteins. Hsp40 (Ydj1 and Sis1), Hsp70, and the disaggregase Hsp104 bind misfolded proteins and are required for sequestration into quality control sites. The sHsp Hsp42 together with the sorting factor Btn2 are thought to direct proteins into the IPOD compartment, whereas Btn2 and Sis1 deliver misfolded proteins into the nuclear JUNQ/INQ site. Sis1 levels in the cytosol are regulated through competitive binding by Cur1, which translocate Sis1 into the nucleus without misfolded substrates, thus directing Btn2 to assist in Hsp42-mediated IPOD delivery. Amyloid proteins are believed to localize to IPOD in an Hsp42 independent manner, and it is currently unclear if this site corresponds to the same IPOD inhabited by non-amyloid proteins. Figure adapted from (Hill et al., 2017).

4. Results and discussion

4.1 Paper I: Lifespan extension and H_2O_2 resistance elicited by caloric restriction require the peroxiredoxin Tsa1 in *Saccharomyces cerevisiae*

Caloric restriction (CR) is one of the most conserved lifespan extending interventions (Kenyon, 2010, Fontana et al., 2010). In our first study, we sought to investigate the effects and downstream targets of CR in yeast.

CR induce H_2O_2 resistance through the PKA signaling pathway

The resistance to oxidative stress seen in many caloric-restricted organisms (Sohal and Weindruch, 1996, Fontana et al., 2010), has been reported to be absent in yeast (Lin et al., 2002). In order to test this carefully, we subjected yeast cells to CR by reducing the glucose concentration in the medium from 2% to 0.5%, grew cells to different cell densities and scored survival on plates with increasing H_2O_2 concentrations. We found that CR provided resistance to H_2O_2, although this was only effective at lower cellular densities (OD), since cells reaching mid-exponential phase acquired resistance independent on CR. H_2O_2 resistance by CR at low cell densities or growth to mid-exponential phase were both dependent on the cAMP/PKA signaling pathway and we were able to establish a resistance to H_2O_2 of cells at low cell density by reducing PKA signaling genetically; deletion of *RAS2*, *GPA2*, *GRP1*, as well as overexpression of *PDE2* (converts cAMP to AMP) all lead to reduced PKA activity and resulted in H_2O_2 resistance of cells at low cell densities. Conversely, deletion of *PDE2* made cells sensitive to H_2O_2 also in mid-exponential phase. The transcription factors Msn2/4 are inhibited by PKA (Zaman et al., 2008) and the synthesis rate of some of their targets confirmed that PKA activity correlates negatively with H_2O_2 resistance. Reduced TOR activity did not affect H_2O_2 sensitivity regardless of cell density, demonstrating that resistance to H_2O_2 is specifically mediated by the PKA pathway.

The redox pair Tsa1/Srx1 are downstream targets of PKA-dependent H_2O_2 resistance

We next searched for downstream targets of PKA signaling and identified the transcription factors Yap1/Skn7 as mediators of H_2O_2 resistance development by low PKA signaling. Yap1/Skn7 regulate gene expression in response to oxidative stress (Lee et al., 1999a), and among their targets are the major peroxiredoxin *TSA1*. The rate of Tsa1 synthesis increased at low PKA activity, suggesting that Tsa1 is a target of reduced PKA signaling. As described

previously, Tsa1 belongs to the 2-Cys group of Prxs, which are sensitive to hyperoxidation and oligomerization upon increased H_2O_2 levels (Wood et al., 2003). Our results revealed that Tsa1 accumulated in a hyperoxidized (Tsa1-SOOH) form in cells exposed to H_2O_2 with high PKA activity and, conversely, reduced PKA activity counteracted the accumulation of Tsa1-SOOH upon H_2O_2 exposure. Furthermore, high PKA resulted in a diminished ability to reduce Tsa1-SOOH during H_2O_2 treatment. CR, growth to mid-exponential phase, and reduced PKA signaling by genetic interventions all induced H_2O_2 resistance in a Tsa1-dependent manner.

The peroxidase activity of Tsa1 involves both the peroxidatic cysteine and the resolving cysteine, whereas the chaperone activity of Tsa1 is primarily dependent on the peroxidatic cysteine (Wood et al., 2003, Jang et al., 2004). We found that CR-induced H_2O_2 resistance is dependent on both Tsa1 redox-active cysteines, indicating that this response is mediated by Tsa1 peroxidase activity. Cells lacking Srx1 were unable to develop H_2O_2 resistance, suggesting that reduced levels of Tsa1-SOOH achieved by Srx1 are critical for resistance. We found that PKA inhibited *SRX1* translation through Gcn2, a regulator of translation initiation factor 2 (elF2), and lowering PKA activity relieved this translational block of *SRX1* thereby increasing the reduction of Tsa1-SOOH.

Tsa1/Srx1 regulates aging

Next, we asked if the functions of Tsa1 and Srx1 are important for the regulation of replicative aging. Removal of *TSA1* resulted in a shortened lifespan, confirming that Tsa1-deficiency accelerates aging (Timmermann et al., 2010). We observed that hyperoxidized Tsa1 accumulated in aged cells, something that has been observed also in rats (Musicco et al., 2009). Overproducing Srx1 2-fold counteracted this age-associated Tsa1 hyperoxidation and extended lifespan by 20%. Furthermore, Tsa1 and Srx1 were required for lifespan extension by CR as well as by genetically reduced PKA signaling, supporting a role for Tsa1/Srx1 as regulators of aging. Lifespan extension by Srx1 overproduction were independent of Sir2, suggesting that this longevity pathway is parallel to Sir2 function.

Our results demonstrate that the budding yeast *Saccharomyces cerevisiae* responds to CR by increasing their resistance to oxidative stress, thereby supporting the significance of oxidative stress in the process of aging and as well as verifying the conservation of the CR response in yeast. We furthermore present two new targets of CR- and PKA-dependent H_2O_2 resistance

and lifespan extension; the Tsa1/Srx1 redox pair. The peroxidatic cycle of Tsa1 is required for PKA-induced H_2O_2 resistance and Tsa1-SOOH reduction is regulated by Gcn2-mediated translation of *SRX1*. We propose that PKA, by inhibiting Gcn2, blocks *SRX1* translation and subsequent Tsa1-SOOH reduction (Fig. 9). Increased H_2O_2 levels lead to hyperoxidized Tsa1 and activate Yap1/Skn7-mediated *SRX1* transcription, but since *SRX1* translation is inhibited, Tsa1 accumulates in the hyperoxidized form. CR reduces PKA signaling leading to elevated *SRX1* translation and a reduction of Tsa1-SOOH, thus promoting longevity.

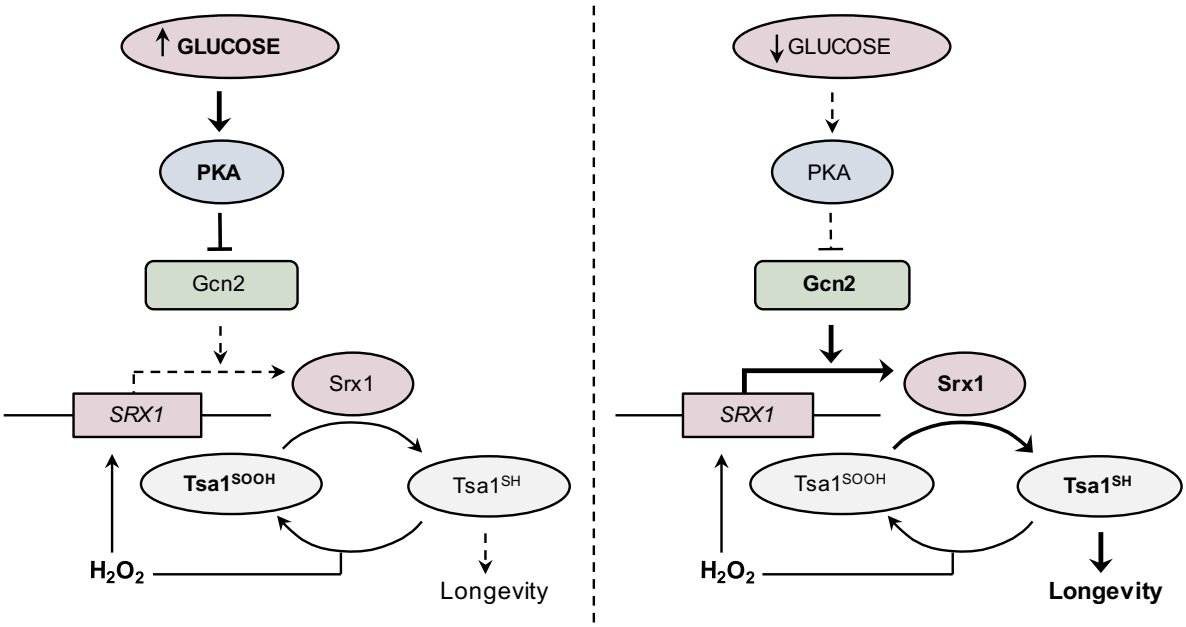

Figure 9. PKA dependent regulation of Srx1/Tsa1 under high and low glucose levels. Under high glucose levels (left), PKA inhibits the regulator of the translational initiation factor, Gcn2. H_2O_2 activates *SRX1* transcription and triggers Tsa1-hyperoxidation. As *SRX1* mRNA translation is under Gcn2 regulation and thus blocked by PKA, this results in accumulation of Tsa1-SOOH. At low glucose levels (right), PKA activity is reduced, relieving the translational block of *SRX1* and increasing reduction of hyperoxidized Tsa1.

4.2 Paper II: Lifespan control by redox-dependent recruitment of chaperones to misfolded proteins

The data obtained in **paper I** supports a role for Tsa1 in replicative aging and we elaborated on these findings in our second study to understand how Tsa1 levels influence aging and which of its many functions is most critical for this role.

Overproduction of Tsa1 extends lifespan

Remarkably, overproducing Tsa1 twofold extended replicative lifespan by 40-50%, in a partially Srx1-dependent manner. The Msn2/4 transcription factors that are linked to TOR-associated lifespan extension (Medvedik et al., 2007) were not needed for lifespan extension by Tsa1 overproduction. Given the many functions of Prxs, we pursued to explore which of these functions are most critical for their role in longevity. As H_2O_2 consumption, mutation rates, and double-strand breaks were unaltered by elevated levels of Tsa1 we concluded that peroxide scavenging and protection against DNA damage are most likely not the main contributors of lifespan extension by Tsa1 overproduction. Furthermore, CR reduced mutation rates in cells lacking Tsa1 and Srx1 (**paper II**), yet failed to extend lifespan in these strains (**paper I**), indicating that accumulation of mutations is not a determinant for yeast longevity, which is consistent with recent reports of a poor correlation between mutation rates and aging patterns (Kaya et al., 2015).

Tsa1 recruits Hsp70 chaperones and Hsp104 disaggregase to H_2O_2-induced protein aggregates

To address the question of which Tsa1 function that is responsible for its role in longevity, a genome wide screen searching for genetic interactions of *TSA1* was performed. The results confirmed the role of Tsa1 in protecting the genome, as several of the identified hits represented enzymes involved in DNA repair and maintenance. The screen also revealed connections to functions in stress responses, signaling, vacuolar function, and proteostasis. Among the interactors were genes encoding PN components, including the ubiquitin ligase complex Ubp3 and Bre5, the Hsp90 regulator Hch1, and the organelle specific Hsp70s Lhs1 (ER) and Ecm10 (mitochondria). Intriguingly, nine of the J-protein family members were found among the synthetic sick interactions.

Given the fact that 2-Cys Prxs have *in vitro* chaperone activity (Jang et al., 2004), we decided to investigate a potential PN role that could account for the anti-aging capacity of Tsa1 in yeast. We used green fluorescent protein (GFP) fusions to explore the proteostasis phenotype under the microscope. The Hsp70s, Ssa1/Ssa2, and the Hsp104 disaggregase localized to protein aggregates upon heat shock and H_2O_2 exposure in wild type yeast cells, visualized as distinct GFP foci. However, in *tsa1Δ* cells, both Hsp104 and Ssa1/2 failed to efficiently associate with protein aggregates induced by H_2O_2. The misfolding substrates ΔssCPY*-GFP (Stolz and Wolf, 2012) and Cdc19-GFP (Narayanaswamy et al., 2009) both accumulated into aggregates, suggesting that the lack of Ssa1/2 and Hsp104 at such aggregates reflects a reduced recruitment mediated by Tsa1 and not an absence of aggregation as such. In line with this, the insoluble protein aggregate fraction induced by H_2O_2 exposure was equal in wild type and *tsa1Δ* cells. Ssa1/2 and Hsp104 localized to heat-induced protein aggregates in a Tsa1-independent manner, demonstrating that Tsa1-dependent recruitment is specific to protein aggregates produced by H_2O_2. Furthermore, Tsa1 localized to Hsp104-associated protein aggregates under both H_2O_2 and heat stress and in agreement with Tsa1-dependent recruitment of chaperones to H_2O_2-induced protein aggregates, Hsp104 failed to associate with ΔssCPY*-GFP foci in *tsa1Δ* cells only under H_2O_2 treatment.

Imaging revealed a sequential recruitment of chaperones to protein aggregates under H_2O_2 exposure: Tsa1 appears to associate with protein aggregates independently of Ssa1/Ssa2 and Hsp104, Ssa1/2 required Tsa1 but not Hsp104 for their sequestration, and Hsp104 was dependent on both Tsa1 and either Ssa1 or Ssa2 for proper sequestration to protein aggregates. Taken together, Tsa1 appears to localize to protein aggregates first. This was also supported by the observation that Tsa1-GFP foci formation peaked rapidly upon H_2O_2 stress, within 30min, whereas Hsp104 foci formation typically peaked later, after 1-2hrs. Examination of Tsa1 and Hsp104 in the same cell confirmed that Tsa1 was sequestered to aggregated proteins first, as the Tsa1 foci emerged before the Hsp104 foci in almost all studied cells. In contrast, Tsa1 and Ssa1 seem to localize to protein aggregates simultaneously, since the appearance of both foci occurred within the same timeframe. The co-recruitment of Tsa1 and Ssa1 was further supported by a physically interaction, which was demonstrated biochemically with immunoprecipitation and co-purification of the two proteins.

In view of the known spatial quality control 'compartments' described before, we decided to determine if the protein aggregates formed under H_2O_2 stress is similar to the IPOD/JUNQ deposits generated upon heat stress (Kaganovich et al., 2008). However, as none of the substrates previously used to study IPOD/JUNQ formation ($Ubc9^{ts}$, VHL, Luciferase) accumulated into aggregates under H_2O_2 stress, a sequential stress protocol including both H_2O_2 and heat treatment was applied. Although the spatial quality control may be altered by this type of dual stress conditions, the localization of Tsa1 to protein aggregates that also harbored $Ubc9^{ts}$ indicates that protein aggregates under H_2O_2 stress is sorted into a SPQC similar to heat-induced aggregates.

Tsa1-mediated chaperone recruitment requires hyperoxidation of cysteine 48

In vivo oligomerization and chaperone activity of Tsa1 have been reported to be dependent on the peroxidatic Cys48 whereas the peroxidase activity involves both Cys48 and the resolving Cys171 (Jang et al., 2004, Wood et al., 2003). Our results revealed that Cys48 but not Cys171 was required for Hsp104 and Ssa1 localization to protein aggregates under H_2O_2 stress, indicating that the peroxidase activity of Tsa1 is dispensable for this function. Furthermore, we could confirm that removal of the C-terminal YF motif significantly diminished the hyperoxidation of Cys48 (Koo et al., 2002, Wood et al., 2003) and also decreased Hsp104 recruitment to protein aggregates. As Cys48 hyperoxidation is tightly linked to oligomerization and chaperone activity (Wood et al., 2003, Noichri et al., 2015), these results support a role for Tsa1 chaperone activity in the recruitment of chaperones to misfolded and aggregated proteins.

Tsa1 supports prevention of protein aggregate accumulation

We noted that that Tsa1-deficient cells accumulated ΔssCPY*-GFP aggregates upon H_2O_2 exposure at a faster rate compared to wild type cells. As the Hsp70 chaperones have functions in preventing protein aggregation; refolding/reactivation and UPS degradation of misfolded proteins, it seemed reasonable that Tsa1 could aid in such activities which might account for the delayed accumulation of protein aggregates in cells containing Tsa1. The ΔssCPY*-LEU construct, which is stabilized in cells with impaired proteasomal degradation and thus confers increased growth on medium lacking leucine, has been used as a read-out for UPS activity (Eisele and Wolf, 2008, Oling et al., 2014). As previously reported, cells lacking Ssa1/Ssa2 and the E3 ubiquitin ligase Ubr1 grows better on plates without leucine and we found that

growth of *tsa1Δ* cells was similar to the *ssa1Δssa2Δ* strain. Furthermore, *tsa1Δ* cells suffered an increased load of ubiquitinated proteins under H_2O_2 stress, and Tsa1 overproduction reduced this accumulation to some extent. Taken together, the data indicate that Tsa1, by aiding Hsp70-mediated proteasomal degradation, contribute to the prevention of aggregate accumulation under H_2O_2 stress. It remains to be elucidated if Tsa1 can assist also in Hsp70 mediated folding/refolding.

Resolution of protein aggregates is accelerated by Srx1-dependent reduction of Tsa1-SOOH

Although the recruitment of Hsp104 to H_2O_2-induced protein aggregates was independent on Srx1, Tsa1-SOOH reduction appears important for efficient resolution of such aggregates. Resolution of aggregates was impaired in *srx1Δ* cells and this correlated with Tsa1-SOOH reduction. The J-protein Ydj1 is required for the resolution of heat-induced protein aggregates (Glover and Lindquist, 1998), this was confirmed by our data which also revealed that its presence was dispensable for protein aggregate resolution under H_2O_2 stress. In contrast, the Hsp40 Sis1 was essential for the resolution of H_2O_2 produced protein aggregates, demonstrating differences between these two classes of Hsp40s. Ydj1 and Sis1 are members of Class I and Class II respectively, and one of their distinguishing features is the presence of a cysteine-rich zinc-finger domains only in Class I J proteins (Kampinga and Craig, 2010). In the human J-protein, Hdj2, chaperone activity has been reported to be inactivated upon H_2O_2 treatment due to the oxidization of zinc-finger cysteines (Choi et al., 2006), and such oxidative inactivation could be a possible explanation for the failure of Ydj1 to assist in disaggregation under H_2O_2 exposure.

Tsa1 has a role in proteostasis in aged cells

We next sought to determine if Tsa1 is important also for the management of aged-induced protein aggregates. Interestingly, as for H_2O_2 stress, aged-induced protein aggregates required Cys48 for the recruitment of Hsp104. Furthermore, aged *tsa1Δ* cells accumulated more protein aggregates and ubiquitinated proteins, and overproducing Tsa1 mitigated protein aggregate accumulation in aged cells, similar to what was observed during H_2O_2 exposure. The proteostasis function of Tsa1 in aged cells prompted us to examine if this was linked to the lifespan-extending capability of Tsa1.

Lifespan extension by Tsa1 overproduction is linked to proteostasis

We analyzed the requirements of certain key components of the proteostasis network and found that overproduction of Tsa1 completely failed to extend lifespan in *ssa1Δssa2Δ* cells, as well as in cells lacking the transcription factor Rpn4; which is responsible for upregulation of ubiquitin-proteasome components (Mannhaupt et al., 1999). The disaggregase Hsp104 was only partly required for lifespan extension mediated by Tsa1 overproduction. This is possibly due to Hsp70 functions beyond Hsp104-dependent disaggregation, for example refolding and degradation, in which Tsa1 could be participating. Sir2 was also required for lifespan extension by Tsa1 overproduction, suggesting that Tsa1 overproduction extends lifespan in a pathway linked to Sir2 function. Taken together, lifespan extension by Tsa1 overproduction appears to be linked to proteostasis.

We propose a model for the management of protein aggregates produced by H_2O_2 stress (Fig. 10). We envision that Tsa1, by recruiting Hsp70 to misfolded proteins, contributes to the prevention of protein aggregation by Hsp70 mediated refolding/reactivation and by proteasomal degradation. Additionally, the recruitment of Hsp104 promotes protein aggregate disaggregation and resolution. The redox state of Tsa1 seems to be central to its proteostasis functions, as Cys48 hyperoxidation is required for recruitment of chaperones to protein aggregates and, conversely, Srx1-dependent reduction of Cys48 hyperoxidation is needed for efficient resolution such species.

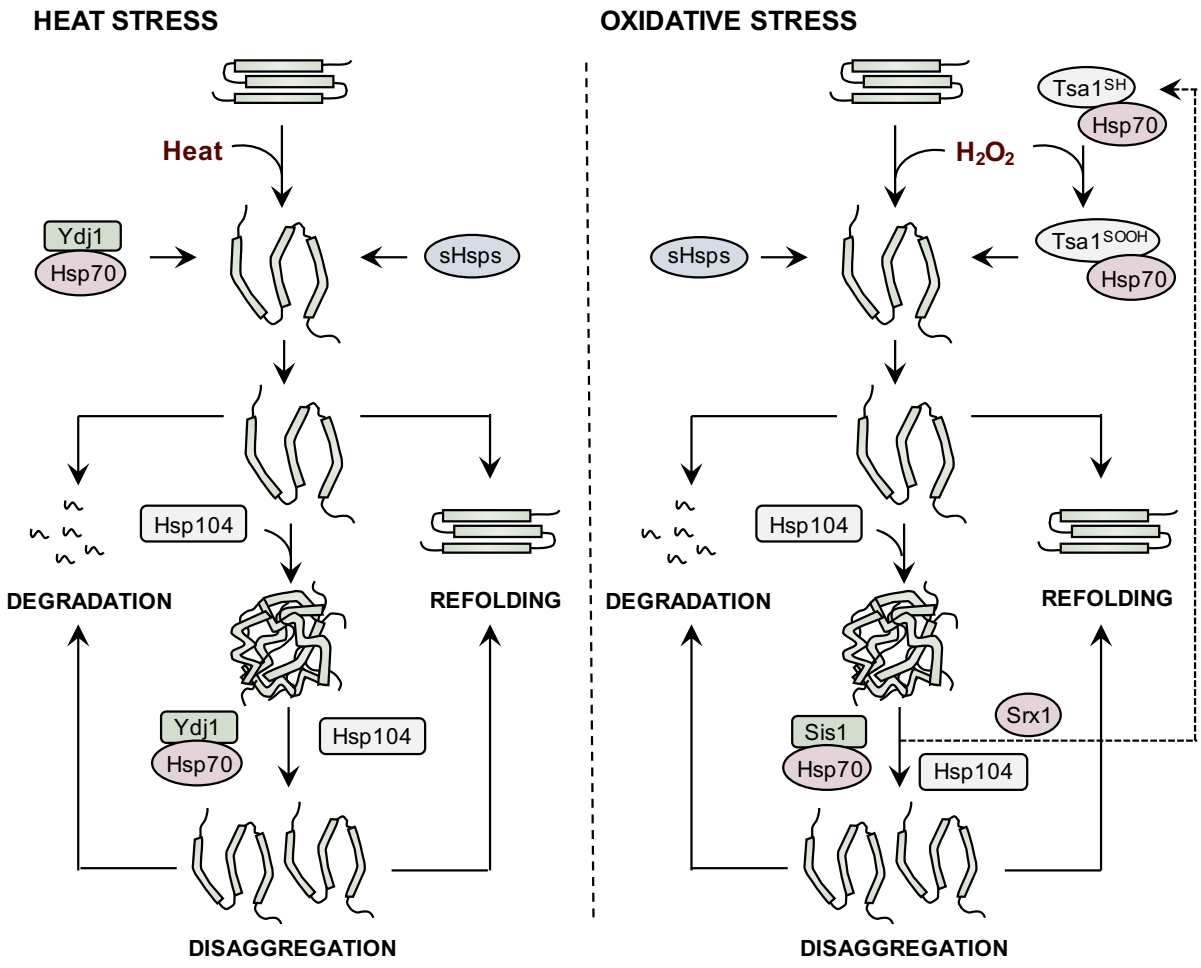

Figure 10. Differential management of misfolded proteins under heat and H_2O_2 exposure. Small Hsps, Hsp40/Hsp70, and Hsp104 are sequentially recruited to misfolded proteins and protein aggregates. Misfolded proteins can be refolded, degraded, or aggregated. Heat-induced protein aggregates (left) are disaggregated by the action of Hsp70 and Hsp104 together with the Hsp40 protein Ydj1, which binds to protein aggregates and enables refolding or degradation of the extracted misfolded protein species. Upon H_2O_2 exposure (right), Tsa1 is hyperoxidized and recruited to misfolded proteins and protein aggregates along with Hsp70 and Hsp104. Proteins misfolded upon oxidative stress can be degraded or refolded in a manner supposedly similar to the pathway for heat-denatured proteins. However, disaggregation of H_2O_2-induced protein aggregates depends on the core Hsp70/Hsp104 machinery together with the Hsp40 Sis1, while the heat-related Ydj1 is not required. Furthermore, disaggregation upon oxidative stress is facilitated by Srx1-dependent reduction of hyperoxidized Tsa1. As for heat stress, these disaggregated proteins can either be refolded or degraded. Figure adapted from (Hill et al., 2017).

The fact that Tsa1 is required for the management of protein aggregates under H_2O_2 exposure and not under heat stress reflects significant differences between protein aggregates produced by these two stressors. This is supported by our notion that Tsa1-GFP-associated protein aggregates induced by H_2O_2 stress displayed increased mobility compared to the protein aggregates formed by heat. A specific, but similar, subset of the proteome has been shown to aggregate under three distinct stresses (H_2O_2, arsenite and AZC) (Weids et al., 2016), indicating that protein species is not what distinguish protein aggregates under different stressors. Instead, reports of proteins forming different types of aggregates depending on the denaturing conditions (Ben-Zvi and Goloubinoff, 2002, Wang et al., 2010) suggest that the stressor could determine the structural nature of the misfolded proteins destined for aggregation.

H_2O_2 is the only factor known to cause hyperoxidation of Tsa1-Cys48 and considering that Cys48 is required for Hsp104 recruitment to protein aggregates in aged cells, it seems likely that H_2O_2 contributes to the formation of these protein aggregates. This is consistent with H_2O_2 accumulating in aged mother cells (Laun et al., 2001, Erjavec and Nystrom, 2007). Intriguingly, the mobility of Tsa1-GFP associated protein aggregates seen in aged mother cells contained at least two subpopulations, of which one displayed higher mobility and the other was more stationary. The more mobile protein aggregates could represent H_2O_2 induced aggregates, in line with H_2O_2 as a contributing factor to age-related protein aggregate formation. Bearing in mind the different aging pathways in yeast, it seems reasonable that the collapse of different systems could give rise to different types of protein aggregates. For example, increased oxidative stress could result in one type of protein aggregate, whereas for example mutations and changed pH could give rise to distinct types of protein aggregates. Analysis of protein content, biochemical properties, dynamics, and involvement of PN components could shed light on the nature of protein aggregation in aged cells.

4.3 Paper III: Enhancing protein disaggregation restores proteasome activity in aged cells

Data from **paper II** suggests that protein aggregates are form in aged cells, in part, as a result of oxidative damage. The accumulation of protein aggregates in aged cells could thus be a consequence of increased oxidative damage or diminished activity of the PN. Certainly, the activity of the ubiquitin-proteasome system (UPS) is connected to replicative aging in yeast. Reducing the expression of proteasome components by deletion of *RPN4* accelerates replicative aging and, conversely, Rpn4 stabilization by deletion of the E3 ligase *UBR2* elevates proteasome activity and extends lifespan (Kruegel et al., 2011). In our third study, we wanted to explore the possibility that an age-related decline in UPS function represents one of the sources of the accumulation of protein aggregates in aged cells.

Proteasome activity regulate the accumulation of protein aggregates

Using GFP-tagged Hsp104 and the UPS substrate ΔssCPY* (Eisele and Wolf, 2008), we could confirm that aged cells accumulate protein aggregates (Aguilaniu et al., 2003, Erjavec et al., 2007, Tessarz et al., 2009, Liu et al., 2010, Hill et al., 2014). To further explore the UPS and its implication in protein aggregate formation, proteasome activity was reduced by blocking the proteolytic activity of the 26S proteasome complex or by using a temperature sensitive version of one of the ATPases in the 19S subunit (Rpt4). Both methods increased the load of ubiquitinated proteins and/or protein aggregation in young cells, as well as disrupted the clearance of such aggregates. Moreover, elevated proteasome activity by *UBR2* deletion counteracted the accumulation of protein aggregates under H_2O_2 exposure and in aged cells.

Proteasome activity, but not capacity, declines with replicative age

The accumulation of a UPS substrate in aged cells and the fact that elevated proteasome activity mitigates age-related protein accumulation suggest that UPS activity affects protein aggregate accumulation in aged cells. To test this hypothesis, a ubiquitinated β-galactosidase UPS substrate was used to analyze proteasome activity *in vivo*. Although young cells efficiently degraded ubiquitinated β-galactosidase (Ub-Pro-βgal), aged cells displayed impaired degradation. However, this reduced proteasome activity was not a result of decreased capacity, as the *in vitro* degradation of aged proteasomes was similar to proteasomes from young cells.

Increased protein disaggregation enhances proteasome activity in aged cells

As the proteasome capacity was unaltered in aged cells, we envisioned that protein aggregates could be disrupting the UPS and thus contributing to age-related UPS decline. Enhancing protein disaggregation by overproducing Hsp104 significantly accelerated the clearance of H_2O_2 induced protein aggregates and reduced the accumulation of protein aggregates in aged cells. Additionally, Hsp104 overproduction increased proteasome activity almost to the same degree as young cells without altering proteasome levels or proteasome capacity. However, Hsp104 overproduction did not extend lifespan, suggesting that its effect on proteasome activity did not reach that obtained in *ubr2Δ* cells, which leads to a robust lifespan extension (Kruegel et al., 2011). This is supported by the extremely rapid *in vivo* degradation activity (**paper III**) and enhanced *in vitro* degradation capacity (Kruegel et al., 2011) of *ubr2Δ* cells. The failure of Hsp104 overproduction to prolong lifespan also indicate that protein disaggregation may not be the most critical event in proteostasis during aging, or possibly that other factors such as the Hsp70 system or Tsa1 are limiting Hsp104 function. Taken together, we propose that protein aggregates disrupt proteasomal degradation in aged cells, and thus contribute to the declining UPS activity (Fig. 11).

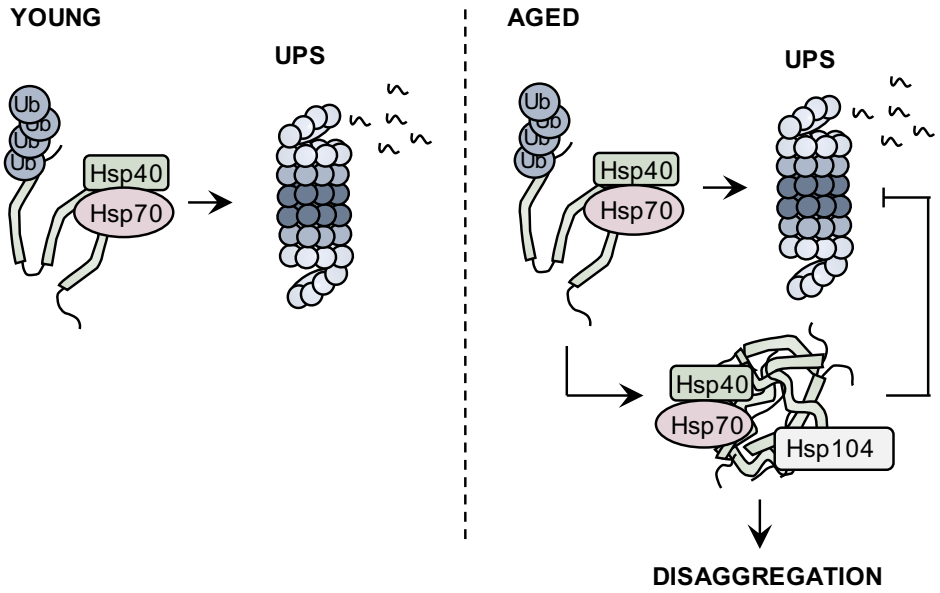

Figure 11. Protein degradation by the UPS in young versus aged cells. In young cells (left), the UPS degrade polyubiquitin marked proteins in cooperation with Hsp40/Hsp70 chaperones. In aged cells (right), the increased levels of protein aggregate could interfere with the UPS and thus contribute to its age-dependent decline. Increased protein disaggregation, by overproducing Hsp104, counteract protein aggregate accumulation and restores proteasome activity in aged cells.

4.4 Main findings

Paper I

- CR increases cellular H_2O_2 resistance in yeast grown by lowering PKA activity.
- Tsa1 and Srx1 are required for CR-induced H_2O_2 resistance and lifespan extension.
- H_2O_2 resistance by CR is mediated by reduced PKA signaling, leading to increased Gcn2-dependent translation of *SRX1* and subsequent reduction of hyperoxidized Tsa1.
- Counteracting age-dependent Tsa1 hyperoxidation, by overproduction of Srx1, extends lifespan.

Paper II

- Tsa1 overproduction reduces protein aggregate accumulation in aged cells and extends lifespan in a H_2O_2-scavenging independent but proteostasis network-dependent manner.
- Tsa1 recruits chaperones to misfolded and aggregated proteins upon H_2O_2 stress and in aged cells, as well as aids in their clearance.
- Tsa1-Cys48 hyperoxidation is required for the recruitment of molecular chaperones to aggregated proteins and its reduction accelerates protein aggregate resolution.
- The requirement of Tsa1-Cys48 hyperoxidation for chaperone recruitment to protein aggregates in aged cells suggest that H_2O_2 contributes to age-related protein misfolding and aggregation.

Paper III

- Protein degradation by the ubiquitin-proteasome system declines during replicative aging of yeast cells.
- Enhanced protein disaggregation counteracts protein aggregate accumulation in aged cells and restores proteasome activity.

5. Concluding remarks

The multifaceted nature of aging is reflected by its vast connections to various cellular pathways. Caloric restriction (CR) is one of the most well conserved anti-aging pathways, although the downstream targets of CR and how it affects longevity mechanistically is not completely understood. Proteostasis has emerged as one fundamental process responding to CR in longevity assurance and the proteostasis network is constantly expanding with the discovery of new components and auxiliary processes being involved in maintaining a functional proteome. Unraveling the aging network and elucidating the function and interconnectivity of its components, including the proteostasis network, is essential for determining their contribution to the process of aging and age-related diseases. With the data in this thesis, we provide new insight into the aging process by showing that the redox pair Tsa1/Srx1 is a member of the yeast proteostasis network acting as a key regulator of replicative aging.

We found that Tsa1 and Srx1 are downstream targets of CR and provide a mechanism for their PKA-mediated regulation; a regulation that bestows the cells with an increased resistance to hydrogen peroxide (**paper I**). Furthermore, both Srx1 and Tsa1 are required for CR-induced lifespan extension and overproduction of either protein extends lifespan in the absence of CR (**paper I and II**). Lifespan extension by Tsa1 overproduction requires Sir2 (**paper II**), while Srx1 overproduction extended lifespan independently of Sir2 (**paper I**), suggesting that Tsa1 and Srx1 may mediate lifespan extension partly through different mechanisms (let's discuss). Life span extension achieved by Tsa1 overproduction was linked to proteostasis (**paper II**), which may explain its requirement for Sir2, previously connected to proteostasis (Erjavec et al., 2007, Liu et al., 2010). Srx1 overproduction counteracted age-dependent hyperoxidation of Tsa1 (**paper I**) and it is possible that Tsa1 is reduced to such a degree that it is no longer efficient as a chaperone (as this requires hyperoxidation). In this scenario, H_2O_2 scavenging and/or intracellular signaling could account for the lifespan extension activities of Tsa1 upon Srx1 overproduction, which under these circumstances are Sir2-independent. As Srx1 is important also for protein disaggregation (**paper II**) it would be interesting to see if overproducing both Srx1 and Tsa1 would extend replicative lifespan even further. Determining the requirement of the redox active cysteines and YF motif for lifespan extension by the Srx1 and Tsa1 overproducing strains could clarify the question of different Tsa1-mediated longevity pathways.

We define an *in vivo* function of Tsa1 in proteostasis under oxidative stress and in aged cells. Tsa1 recruits molecular chaperones to misfolded and aggregated proteins as well as aid in their clearance (**paper II**). This proteostasis function of Tsa1 centers around its redox state, as hyperoxidation of redox active Cys48 is required for chaperone recruitment to misfolded and aggregated proteins and, conversely, Srx1-dependent Cys48-SOOH reduction accelerate protein disaggregation (**paper II**). Recruitment of Hsp70 chaperones to misfolded proteins support prevention of protein aggregate formation by the action of refolding and/or UPS degradation, whereas Hsp70/Hsp104 recruitment enable resolution of already aggregated proteins. The broader range of proteostasis functions of Hsp70 compared to Hsp104 could explain why lifespan extension by Tsa1 overproduction is only partly dependent on Hsp104, but completely reliant on the Hsp70s (**paper II**). Hsp104 and Srx1 are both involved in protein disaggregation and the fact that neither is essential lifespan extension by Tsa1 overproduction (**paper II**) indicates that prevention of protein aggregation is of more significance in the context of longevity. This is further supported by the failure of Hsp104 overproduction to extend lifespan (**paper III**). Our data suggest that H_2O_2 contribute to protein misfolding in aging cells (**paper II**) and that protein aggregates accumulate, in part, due to a reduced proteasome activity (**paper III**). Additionally, lifespan extension by Tsa1 overproduction is accompanied by reduced protein aggregate accumulation in aged cells (**paper II**), supporting the significance of protein aggregates as true aging factors.

Moving forward with the role of Tsa1 in proteostasis, it would be interesting to elucidate the involvement of Tsa1 in Hsp70 functions such as folding/refolding and degradation by the UPS. It would also be interesting to see if Tsa1 has a role in SPQC and asymmetric inheritance of protein aggregates under peroxide exposure and in aged cells, which could contribute to its longevity promoting function. Considering the interconnectivity of aging pathways, Tsa1 function in proteostasis may very well affect other aging factors as well, and thus investigating the role of Tsa1 in for example vacuole and mitochondrial function may also be relevant. Ultimately, it would be exciting to explore the possibility of Prxs assisting in protein aggregate management also in multicellular organisms and if this function is linked to their role in aging and disease. Similar to Prxs, overexpression of *HSP70* chaperones has been observed in several human tumors, providing cells with increased resistance to hypoxia and oxidative stress (Morano, 2007). It is possible that Prxs and Hsp70 chaperones act in concert in proteostasis to promote tumor development and chemotherapy resistance, a relationship worth pursuing in future studies.

6. Acknowledgements

It's been a few years and there are many people who in different ways have contributed in making those the best so far, this section is for you.

First of all,
My supervisor **Thomas,** thank you for letting me be part of your group, these years have been the best in so many ways. Thank you for your support and guidance, your immense knowledge has truly been inspiring. My co-supervisor **Mikael,** thank you for (trying to) teach me everything there is to know about yeast and peroxiredoxins, for letting me try every experiment that came to mind, and for having answers to my endless stream of questions.

I have had the fortune of working with amazing people who are also my close friends. Thank you for all support, conversations, all the laughs, and for the very exciting "friday-freezings".

Sandra, my roomie, we have done everything together from the start and I have been so lucky to have you by my side. You are such an inspiration, thank you for your friendship, support, and for being my own personal google. And for updating me on things going on outside our window with nature and stuff.

Lisa, you are so kind (there it is again), encouraging, and altogether awesome. Thank you for your unconditional support, scientific and non-scientific, not the least during the last few months. **Rebecca J,** I am happy I found the one person in the world who share my exact sense of humor (which is awesome). Thank you for all support and for always being on my side =) **Anna,** thank you for sharing my interest of talking for hours about anything and everything, I hope we will keep doing so for a long, long time =) **Veronica**, thank you for answering all of my question during my first time in the lab and for being the enthusiastic and fun person you always are. **Andrea,** you were my first partner in crime, thank you for making those undergraduate years so much fun. **Katarina**, you are one of the most hard-working people I have ever met and always happy, positive, and eager to talk science (as well as mushrooms, comedians, and movies no one has ever heard of). I am so happy you joined the Tsa1 project and I think we made a great team, thank you for everything. **Rebecca A,** I certainly picked the best student, thank you for all the hard work and interesting discussions.

Thank you to all (present and past) members of the TN group:

Per, **Fredrik, Anna-Maria, Doryaneh, Roja, Kanika,** thank you for all support, your knowledge and assistance has been invaluable. Per, you have been a very welcomed addition to our breakfast club =) **Xinxin, Beidong, Junsheng**, thank you for your kindness and for always helping in any way you can. **David, Stephanie, Johan G,** you are awesome people and I wished we still worked together. Stephanie, David, you made lifespans suck a little less. Johan G, I miss having you next to me in the lab at as we shared everything, and of course, we all miss your great acting skills. **Anne**, you are easily the best teacher I have ever had, and a true inspiration, thank you for introducing me to science. **Laurence**, I am forever grateful for your support during my first time in the lab and I wish we could have worked together longer. **Malin, Bertil, Kristian, Antonio, Ana, Örjan, Åsa, Friedi, Ken, Rehan, Zheng Ju, Lihua, Qian Liu, Xiuling, Zhaolei, Song Jia, Xiaoxue**, thank you all for creating such as supportive and friendly environment. Thanks to students and visiting researchers who have at some point added to the atmosphere: **Karin**, **Johanna**, **Sara S-F, Chikako, Banu, Johan, Froste, Kara.**

Ingrid, Ellinor, Agneta, Ann, Helena, Lars, Bruno, Andreas, thank you for all practical and technical support.

My family and friends. My parents, **Joanna** and **Janne**, thank you for supporting me in everything and for taking such good care of my kids. My brothers, **Daniel, Rasmus**, and **Sebastian**, thank you for making me the way one can only become by growing up with three brothers, and for sharing my interest in discussing movies/series/actors etc etc. **Therese** and **Magnus**, you are two of my absolute favorite people, thank you for reminding me of the real world on a regular basis.

Jesper, you are my best friend and the love of my life. This last few years has been quite challenging, but I think we make a great team and I feel like we could do anything together. I know I could not have done this without you, thank you for always having my back.

Adrian and **Ofelia**, you have certainly added to the challenge of completing this thesis =) But you have also given me perspective and constantly reminded me of the real world, you are amazing little people and I cannot wait to see what you will do with your lives.

7. References

ABELIOVICH, H. & KLIONSKY, D. J. 2001. Autophagy in yeast: mechanistic insights and physiological function. *Microbiol Mol Biol Rev,* 65**,** 463-79, table of contents.

ACKERMANN, M., STEARNS, S. C. & JENAL, U. 2003. Senescence in a bacterium with asymmetric division. *Science,* 300**,** 1920.

AGUILANIU, H., GUSTAFSSON, L., RIGOULET, M. & NYSTRÖM, T. 2003. Asymmetric Inheritance of Oxidatively Damaged Proteins During Cytokinesis. *Science,* 299**,** 1751-1753.

BARTON, A. A. 1950. Some aspects of cell division in saccharomyces cerevisiae. *J Gen Microbiol,* 4**,** 84-6.

BEN-ZVI, A. P. & GOLOUBINOFF, P. 2002. Proteinaceous infectious behavior in non-pathogenic proteins is controlled by molecular chaperones. *J Biol Chem,* 277**,** 49422-7.

BITEAU, B., LABARRE, J. & TOLEDANO, M. B. 2003. ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin. *Nature,* 425**,** 980-4.

BOKOV, A., CHAUDHURI, A. & RICHARDSON, A. 2004. The role of oxidative damage and stress in aging. *Mech Ageing Dev,* 125**,** 811-26.

BOSL, B., GRIMMINGER, V. & WALTER, S. 2006. The molecular chaperone Hsp104--a molecular machine for protein disaggregation. *J Struct Biol,* 156**,** 139-48.

BRIEGER, K., SCHIAVONE, S., MILLER, F. J., JR. & KRAUSE, K. H. 2012. Reactive oxygen species: from health to disease. *Swiss Med Wkly,* 142**,** w13659.

BUFALINO, M. R., DEVEALE, B. & VAN DER KOOY, D. 2013. The asymmetric segregation of damaged proteins is stem cell-type dependent. *J Cell Biol,* 201**,** 523-30.

CALABRESE, E. J., DHAWAN, G., KAPOOR, R., IAVICOLI, I. & CALABRESE, V. 2015. What is hormesis and its relevance to healthy aging and longevity? *Biogerontology,* 16**,** 693-707.

CHAE, H. Z., CHUNG, S. J. & RHEE, S. G. 1994. Thioredoxin-dependent peroxide reductase from yeast. *J Biol Chem,* 269**,** 27670-8.

CHEN, B., RETZLAFF, M., ROOS, T. & FRYDMAN, J. 2011. Cellular strategies of protein quality control. *Cold Spring Harb Perspect Biol,* 3**,** a004374.

CHEN, M. F., CHEN, W. C., WU, C. T., LIN, P. Y., SHAU, H., LIAO, S. K., YANG, C. T. & LEE, K. D. 2006. p53 status is a major determinant of effects of decreasing peroxiredoxin I expression on tumor growth and response of lung cancer cells to treatment. *Int J Radiat Oncol Biol Phys,* 66**,** 1461-72.

CHITI, F. & DOBSON, C. M. 2006. Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem,* 75**,** 333-66.

CHOI, H. I., LEE, S. P., KIM, K. S., HWANG, C. Y., LEE, Y. R., CHAE, S. K., KIM, Y. S., CHAE, H. Z. & KWON, K. S. 2006. Redox-regulated cochaperone activity of the human DnaJ homolog Hdj2. *Free Radic Biol Med,* 40**,** 651-9.

COELHO, M., LADE, S. J., ALBERTI, S., GROSS, T. & TOLIC, I. M. 2014. Fusion of protein aggregates facilitates asymmetric damage segregation. *PLoS Biol,* 12**,** e1001886.

COLMAN, R. J., ANDERSON, R. M., JOHNSON, S. C., KASTMAN, E. K., KOSMATKA, K. J., BEASLEY, T. M., ALLISON, D. B., CRUZEN, C., SIMMONS, H. A., KEMNITZ, J. W. & WEINDRUCH, R. 2009. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science,* 325**,** 201-4.

COUVILLION, M. T., SOTO, I. C., SHIPKOVENSKA, G. & CHURCHMAN, L. S. 2016. Synchronized mitochondrial and cytosolic translation programs. *Nature,* 533**,** 499-503.

CREMERS, C. M. & JAKOB, U. 2013. Oxidant sensing by reversible disulfide bond formation. *J Biol Chem,* 288**,** 26489-96.

CUERVO, A. M. & DICE, J. F. 2000. Age-related decline in chaperone-mediated autophagy. *J Biol Chem,* 275**,** 31505-13.

D'AUTREAUX, B. & TOLEDANO, M. B. 2007. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol,* 8**,** 813-24.

DAHL, J. U., GRAY, M. J. & JAKOB, U. 2015. Protein quality control under oxidative stress conditions. *J Mol Biol,* 427**,** 1549-63.

DAVID, D. C., OLLIKAINEN, N., TRINIDAD, J. C., CARY, M. P., BURLINGAME, A. L. & KENYON, C. 2010. Widespread protein aggregation as an inherent part of aging in C. elegans. *PLoS Biol,* 8**,** e1000450.

DAY, A. M., BROWN, J. D., TAYLOR, S. R., RAND, J. D., MORGAN, B. A. & VEAL, E. A. 2012. Inactivation of a peroxiredoxin by hydrogen peroxide is critical for thioredoxin-mediated repair of oxidized proteins and cell survival. *Mol Cell,* 45**,** 398-408.

DE HAES, W., FROONINCKX, L., VAN ASSCHE, R., SMOLDERS, A., DEPUYDT, G., BILLEN, J., BRAECKMAN, B. P., SCHOOFS, L. & TEMMERMAN, L. 2014. Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. *Proc Natl Acad Sci U S A,* 111**,** E2501-9.

DEFOSSEZ, P. A., PRUSTY, R., KAEBERLEIN, M., LIN, S. J., FERRIGNO, P., SILVER, P. A., KEIL, R. L. & GUARENTE, L. 1999. Elimination of replication block protein Fob1 extends the life span of yeast mother cells. *Mol Cell,* 3**,** 447-55.

DENOTH LIPPUNER, A., JULOU, T. & BARRAL, Y. 2014. Budding yeast as a model organism to study the effects of age. *FEMS Microbiology Reviews,* 38**,** 300-325.

DUNKER, A. K., SILMAN, I., UVERSKY, V. N. & SUSSMAN, J. L. 2008. Function and structure of inherently disordered proteins. *Curr Opin Struct Biol,* 18**,** 756-64.

EGLER, R. A., FERNANDES, E., ROTHERMUND, K., SEREIKA, S., DE SOUZA-PINTO, N., JARUGA, P., DIZDAROGLU, M. & PROCHOWNIK, E. V. 2005. Regulation of reactive oxygen species, DNA damage, and c-Myc function by peroxiredoxin 1. *Oncogene,* 24**,** 8038-50.

EISELE, F. & WOLF, D. H. 2008. Degradation of misfolded protein in the cytoplasm is mediated by the ubiquitin ligase Ubr1. *FEBS Lett,* 582**,** 4143-6.

ENNS, L. C. & LADIGES, W. 2010. Protein kinase A signaling as an anti-aging target. *Ageing Res Rev,* 9**,** 269-72.

ERBSE, A., MAYER, M. P. & BUKAU, B. 2004. Mechanism of substrate recognition by Hsp70 chaperones. *Biochem Soc Trans,* 32**,** 617-21.

ERJAVEC, N., LARSSON, L., GRANTHAM, J. & NYSTROM, T. 2007. Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. *Genes Dev,* 21**,** 2410-21.

ERJAVEC, N. & NYSTROM, T. 2007. Sir2p-dependent protein segregation gives rise to a superior reactive oxygen species management in the progeny of Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A,* 104**,** 10877-81.

ESCUSA-TORET, S., VONK, W. I. & FRYDMAN, J. 2013. Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. *Nat Cell Biol,* 15**,** 1231-43.

ESSER, C., ALBERTI, S. & HOHFELD, J. 2004. Cooperation of molecular chaperones with the ubiquitin/proteasome system. *Biochim Biophys Acta,* 1695**,** 171-88.
FARMER, K. J. & SOHAL, R. S. 1989. Relationship between superoxide anion radical generation and aging in the housefly, Musca domestica. *Free Radic Biol Med,* 7**,** 23-9.
FINKEL, T. & HOLBROOK, N. J. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature,* 408**,** 239-47.
FINLEY, D., ULRICH, H. D., SOMMER, T. & KAISER, P. 2012. The ubiquitin-proteasome system of Saccharomyces cerevisiae. *Genetics,* 192**,** 319-60.
FOMENKO, D. E., KOC, A., AGISHEVA, N., JACOBSEN, M., KAYA, A., MALINOUSKI, M., RUTHERFORD, J. C., SIU, K. L., JIN, D. Y., WINGE, D. R. & GLADYSHEV, V. N. 2011. Thiol peroxidases mediate specific genome-wide regulation of gene expression in response to hydrogen peroxide. *Proc Natl Acad Sci U S A,* 108**,** 2729-34.
FONTANA, L., PARTRIDGE, L. & LONGO, V. D. 2010. Extending healthy life span--from yeast to humans. *Science,* 328**,** 321-6.
FOURQUET, S., HUANG, M. E., D'AUTREAUX, B. & TOLEDANO, M. B. 2008. The dual functions of thiol-based peroxidases in H2O2 scavenging and signaling. *Antioxid Redox Signal,* 10**,** 1565-76.
FRAGA, C. G., SHIGENAGA, M. K., PARK, J. W., DEGAN, P. & AMES, B. N. 1990. Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci U S A,* 87**,** 4533-7.
FRIEDMAN, D. B. & JOHNSON, T. E. 1988. A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. *Genetics,* 118**,** 75-86.
GLOVER, J. R. & LINDQUIST, S. 1998. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell,* 94**,** 73-82.
GOTTSCHLING, D. E. & NYSTROM, T. 2017. The Upsides and Downsides of Organelle Interconnectivity. *Cell,* 169**,** 24-34.
GOULEV, Y., MORLOT, S., MATIFAS, A., HUANG, B., MOLIN, M., TOLEDANO, M. B. & CHARVIN, G. 2017. Nonlinear feedback drives homeostatic plasticity in H2O2 stress response. *Elife,* 6.
GUARENTE, L. 1997. Link between aging and the nucleolus. *Genes Dev,* 11**,** 2449-55.
GUARENTE, L. 2007. Sirtuins in aging and disease. *Cold Spring Harb Symp Quant Biol,* 72**,** 483-8.
HALLIWELL, B. 2007. Biochemistry of oxidative stress. *Biochem Soc Trans,* 35**,** 1147-50.
HALLIWELL, B. & GUTTERIDGE, J. M. 1990. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol,* 186**,** 1-85.
HARMAN, D. 1956. Aging: A Theory Based on Free Radical and Radiation Chemistry. *Journal of Gerontology,* 11**,** 298-300.
HARMAN, D. 1972. The biologic clock: the mitochondria? *J Am Geriatr Soc,* 20**,** 145-7.
HARTL, F. U., BRACHER, A. & HAYER-HARTL, M. 2011. Molecular chaperones in protein folding and proteostasis. *Nature,* 475**,** 324-32.
HARTL, F. U. & HAYER-HARTL, M. 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science,* 295**,** 1852-8.
HARTWELL, L. H. & UNGER, M. W. 1977. Unequal division in Saccharomyces cerevisiae and its implications for the control of cell division. *J Cell Biol,* 75**,** 422-35.
HASLBECK, M. & VIERLING, E. 2015. A first line of stress defense: small heat shock proteins and their function in protein homeostasis. *J Mol Biol,* 427**,** 1537-48.

HENDERSON, K. A. & GOTTSCHLING, D. E. 2008. A mother's sacrifice: what is she keeping for herself? *Curr Opin Cell Biol,* 20**,** 723-8.
HENDERSON, K. A., HUGHES, A. L. & GOTTSCHLING, D. E. 2014. Mother-daughter asymmetry of pH underlies aging and rejuvenation in yeast. *Elife,* 3**,** e03504.
HIGUCHI, R., VEVEA, J. D., SWAYNE, T. C., CHOJNOWSKI, R., HILL, V., BOLDOGH, I. R. & PON, L. A. 2013. Actin dynamics affect mitochondrial quality control and aging in budding yeast. *Curr Biol,* 23**,** 2417-22.
HIGUCHI-SANABRIA, R., PERNICE, W. M., VEVEA, J. D., ALESSI WOLKEN, D. M., BOLDOGH, I. R. & PON, L. A. 2014. Role of asymmetric cell division in lifespan control in Saccharomyces cerevisiae. *FEMS Yeast Res,* 14**,** 1133-46.
HILL, S. M., HANZEN, S. & NYSTROM, T. 2017. Restricted access: spatial sequestration of damaged proteins during stress and aging. *EMBO Rep,* 18**,** 377-391.
HILL, S. M., HAO, X., GRONVALL, J., SPIKINGS-NORDBY, S., WIDLUND, P. O., AMEN, T., JORHOV, A., JOSEFSON, R., KAGANOVICH, D., LIU, B. & NYSTROM, T. 2016. Asymmetric Inheritance of Aggregated Proteins and Age Reset in Yeast Are Regulated by Vac17-Dependent Vacuolar Functions. *Cell Rep,* 16**,** 826-38.
HILL, S. M., HAO, X., LIU, B. & NYSTROM, T. 2014. Life-span extension by a metacaspase in the yeast Saccharomyces cerevisiae. *Science,* 344**,** 1389-92.
HIPP, M. S., PARK, S. H. & HARTL, F. U. 2014. Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol,* 24**,** 506-14.
HUANG, M. E., RIO, A. G., NICOLAS, A. & KOLODNER, R. D. 2003. A genomewide screen in Saccharomyces cerevisiae for genes that suppress the accumulation of mutations. *Proc Natl Acad Sci U S A,* 100**,** 11529-34.
HUGHES, A. L. & GOTTSCHLING, D. E. 2012. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature,* 492**,** 261-5.
IRAQUI, I., FAYE, G., RAGU, S., MASUREL-HENEMAN, A., KOLODNER, R. D. & HUANG, M. E. 2008. Human peroxiredoxin PrxI is an orthologue of yeast Tsa1, capable of suppressing genome instability in Saccharomyces cerevisiae. *Cancer Res,* 68**,** 1055-63.
IRAQUI, I., KIENDA, G., SOEUR, J., FAYE, G., BALDACCI, G., KOLODNER, R. D. & HUANG, M. E. 2009. Peroxiredoxin Tsa1 is the key peroxidase suppressing genome instability and protecting against cell death in Saccharomyces cerevisiae. *PLoS Genet,* 5**,** e1000524.
JANG, H. H., KIM, S. Y., PARK, S. K., JEON, H. S., LEE, Y. M., JUNG, J. H., LEE, S. Y., CHAE, H. B., JUNG, Y. J., LEE, K. O., LIM, C. O., CHUNG, W. S., BAHK, J. D., YUN, D. J., CHO, M. J. & LEE, S. Y. 2006. Phosphorylation and concomitant structural changes in human 2-Cys peroxiredoxin isotype I differentially regulate its peroxidase and molecular chaperone functions. *FEBS Lett,* 580**,** 351-5.
JANG, H. H., LEE, K. O., CHI, Y. H., JUNG, B. G., PARK, S. K., PARK, J. H., LEE, J. R., LEE, S. S., MOON, J. C., YUN, J. W., CHOI, Y. O., KIM, W. Y., KANG, J. S., CHEONG, G. W., YUN, D. J., RHEE, S. G., CHO, M. J. & LEE, S. Y. 2004. Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. *Cell,* 117**,** 625-35.
JAZWINSKI, S. M. 1999. Molecular mechanisms of yeast longevity. *Trends Microbiol,* 7**,** 247-52.
JAZWINSKI, S. M., EGILMEZ, N. K. & CHEN, J. B. 1989. Replication control and cellular life span. *Exp Gerontol,* 24**,** 423-36.
KAEBERLEIN, M. 2010. Lessons on longevity from budding yeast. *Nature,* 464**,** 513-9.

KAEBERLEIN, M., KIRKLAND, K. T., FIELDS, S. & KENNEDY, B. K. 2004. Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol,* 2**,** E296.
KAEBERLEIN, M., MCVEY, M. & GUARENTE, L. 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev,* 13**,** 2570-80.
KAGANOVICH, D., KOPITO, R. & FRYDMAN, J. 2008. Misfolded proteins partition between two distinct quality control compartments. *Nature,* 454**,** 1088-95.
KAIMAL, J. M., KANDASAMY, G., GASSER, F. & ANDREASSON, C. 2017. Coordinated Hsp110 and Hsp104 activities power protein disaggregation in Saccharomyces cerevisiae. *Mol Cell Biol*.
KAMPINGA, H. H. & CRAIG, E. A. 2010. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol,* 11**,** 579-92.
KATAJISTO, P., DOHLA, J., CHAFFER, C. L., PENTINMIKKO, N., MARJANOVIC, N., IQBAL, S., ZONCU, R., CHEN, W., WEINBERG, R. A. & SABATINI, D. M. 2015. Stem cells. Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness. *Science,* 348**,** 340-3.
KAUSHIK, S. & CUERVO, A. M. 2015. Proteostasis and aging. *Nat Med,* 21**,** 1406-15.
KAYA, A., LOBANOV, A. V. & GLADYSHEV, V. N. 2015. Evidence that mutation accumulation does not cause aging in Saccharomyces cerevisiae. *Aging Cell,* 14**,** 366-71.
KENNEDY, B. K., AUSTRIACO, N. R., JR. & GUARENTE, L. 1994. Daughter cells of Saccharomyces cerevisiae from old mothers display a reduced life span. *J Cell Biol,* 127**,** 1985-93.
KENYON, C. J. 2010. The genetics of ageing. *Nature,* 464**,** 504-12.
KHMELINSKII, A., KELLER, P. J., LORENZ, H., SCHIEBEL, E. & KNOP, M. 2010. Segregation of yeast nuclear pores. *Nature,* 466**,** E1.
KIM, K., KIM, I. H., LEE, K. Y., RHEE, S. G. & STADTMAN, E. R. 1988. The isolation and purification of a specific "protector" protein which inhibits enzyme inactivation by a thiol/Fe(III)/O2 mixed-function oxidation system. *J Biol Chem,* 263**,** 4704-11.
KIM SH, S. H., KAMINKER, P. & CAMPISI, J. 2002. Telomeres, aging and cancer: in search of a happy ending. *Oncogene,* 21**,** 503-11.
KIM, Y. E., HIPP, M. S., BRACHER, A., HAYER-HARTL, M. & HARTL, F. U. 2013. Molecular chaperone functions in protein folding and proteostasis. *Annu Rev Biochem,* 82**,** 323-55.
KLASS, M. R. 1983. A method for the isolation of longevity mutants in the nematode Caenorhabditis elegans and initial results. *Mech Ageing Dev,* 22**,** 279-86.
KOO, K. H., LEE, S., JEONG, S. Y., KIM, E. T., KIM, H. J., KIM, K., SONG, K. & CHAE, H. Z. 2002. Regulation of thioredoxin peroxidase activity by C-terminal truncation. *Arch Biochem Biophys,* 397**,** 312-8.
KOPITO, R. R. 2000. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol,* 10**,** 524-30.
KRISTENSEN, P., RASMUSSEN, D. E. & KRISTENSEN, B. I. 1999. Properties of thiol-specific anti-oxidant protein or calpromotin in solution. *Biochem Biophys Res Commun,* 262**,** 127-31.
KRUEGEL, U., ROBISON, B., DANGE, T., KAHLERT, G., DELANEY, J. R., KOTIREDDY, S., TSUCHIYA, M., TSUCHIYAMA, S., MURAKAMI, C. J., SCHLEIT, J., SUTPHIN, G., CARR, D., TAR, K., DITTMAR, G., KAEBERLEIN, M., KENNEDY, B. K. & SCHMIDT, M. 2011.

Elevated proteasome capacity extends replicative lifespan in Saccharomyces cerevisiae. *PLoS Genet,* 7**,** e1002253.
LABBADIA, J. & MORIMOTO, R. I. 2015. The biology of proteostasis in aging and disease. *Annu Rev Biochem,* 84**,** 435-64.
LAI, C. Y., JARUGA, E., BORGHOUTS, C. & JAZWINSKI, S. M. 2002. A mutation in the ATP2 gene abrogates the age asymmetry between mother and daughter cells of the yeast Saccharomyces cerevisiae. *Genetics,* 162**,** 73-87.
LAUN, P., PICHOVA, A., MADEO, F., FUCHS, J., ELLINGER, A., KOHLWEIN, S., DAWES, I., FROHLICH, K. U. & BREITENBACH, M. 2001. Aged mother cells of Saccharomyces cerevisiae show markers of oxidative stress and apoptosis. *Mol Microbiol,* 39**,** 1166-73.
LEE, J., GODON, C., LAGNIEL, G., SPECTOR, D., GARIN, J., LABARRE, J. & TOLEDANO, M. B. 1999a. Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. *J Biol Chem,* 274**,** 16040-6.
LEE, J., SPECTOR, D., GODON, C., LABARRE, J. & TOLEDANO, M. B. 1999b. A new antioxidant with alkyl hydroperoxide defense properties in yeast. *J Biol Chem,* 274**,** 4537-44.
LEE, K. S., IIJIMA-ANDO, K., IIJIMA, K., LEE, W. J., LEE, J. H., YU, K. & LEE, D. S. 2009. JNK/FOXO-mediated neuronal expression of fly homologue of peroxiredoxin II reduces oxidative stress and extends life span. *J Biol Chem,* 284**,** 29454-61.
LEE, W. C., LEE, M., JUNG, J. W., KIM, K. P. & KIM, D. 2008. SCUD: Saccharomyces cerevisiae ubiquitination database. *BMC Genomics,* 9**,** 440.
LEROITH, D., SCHEINMAN, E. J. & BITTON-WORMS, K. 2011. The Role of Insulin and Insulin-like Growth Factors in the Increased Risk of Cancer in Diabetes. *Rambam Maimonides Med J,* 2**,** e0043.
LEVINE, R. L. 1983. Oxidative modification of glutamine synthetase. I. Inactivation is due to loss of one histidine residue. *J Biol Chem,* 258**,** 11823-7.
LIN, S. J., DEFOSSEZ, P. A. & GUARENTE, L. 2000. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae *Science,* 289**,** 2126-8.
LIN, S. J., FORD, E., HAIGIS, M., LISZT, G. & GUARENTE, L. 2004. Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev,* 18**,** 12-6.
LIN, S. J., KAEBERLEIN, M., ANDALIS, A. A., STURTZ, L. A., DEFOSSEZ, P. A., CULOTTA, V. C., FINK, G. R. & GUARENTE, L. 2002. Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. *Nature,* 418**,** 344-8.
LIU, B., LARSSON, L., CABALLERO, A., HAO, X., OLING, D., GRANTHAM, J. & NYSTROM, T. 2010. The polarisome is required for segregation and retrograde transport of protein aggregates. *Cell,* 140**,** 257-67.
LIU, B., LARSSON, L., FRANSSENS, V., HAO, X., HILL, S. M., ANDERSSON, V., HOGLUND, D., SONG, J., YANG, X., OLING, D., GRANTHAM, J., WINDERICKX, J. & NYSTROM, T. 2011. Segregation of protein aggregates involves actin and the polarity machinery. *Cell,* 147**,** 959-61.
LONGO, V. D. & FABRIZIO, P. 2012. Chronological aging in Saccharomyces cerevisiae. *Subcell Biochem,* 57**,** 101-21.
LOPEZ-OTIN, C., BLASCO, M. A., PARTRIDGE, L., SERRANO, M. & KROEMER, G. 2013. The hallmarks of aging. *Cell,* 153**,** 1194-217.
LU, J., VALLABHANENI, H., YIN, J. & LIU, Y. 2013. Deletion of the major peroxiredoxin Tsa1 alters telomere length homeostasis. *Aging Cell,* 12**,** 635-44.

LU, Z. & CYR, D. M. 1998. Protein folding activity of Hsp70 is modified differentially by the hsp40 co-chaperones Sis1 and Ydj1. *J Biol Chem,* 273**,** 27824-30.
MACDIARMID, C. W., TAGGART, J., KERDSOMBOON, K., KUBISIAK, M., PANASCHAROEN, S., SCHELBLE, K. & EIDE, D. J. 2013. Peroxiredoxin chaperone activity is critical for protein homeostasis in zinc-deficient yeast. *J Biol Chem,* 288**,** 31313-27.
MALINOVSKA, L., KROSCHWALD, S., MUNDER, M. C., RICHTER, D. & ALBERTI, S. 2012. Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. *Mol Biol Cell,* 23**,** 3041-56.
MANNHAUPT, G., SCHNALL, R., KARPOV, V., VETTER, I. & FELDMANN, H. 1999. Rpn4p acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast. *FEBS Lett,* 450**,** 27-34.
MAYER, M. P. & BUKAU, B. 2005. Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci,* 62**,** 670-84.
MCCAY, C. M., CROWELL, M. F. & MAYNARD, L. A. 1989. The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. *Nutrition,* 5**,** 155-71; discussion 172.
MCFALINE-FIGUEROA, J. R., VEVEA, J., SWAYNE, T. C., ZHOU, C., LIU, C., LEUNG, G., BOLDOGH, I. R. & PON, L. A. 2011. Mitochondrial quality control during inheritance is associated with lifespan and mother-daughter age asymmetry in budding yeast. *Aging Cell,* 10**,** 885-95.
MEDVEDIK, O., LAMMING, D. W., KIM, K. D. & SINCLAIR, D. A. 2007. MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in Saccharomyces cerevisiae. *PLoS Biol,* 5**,** e261.
MILLER, S. B., HO, C. T., WINKLER, J., KHOKHRINA, M., NEUNER, A., MOHAMED, M. Y., GUILBRIDE, D. L., RICHTER, K., LISBY, M., SCHIEBEL, E., MOGK, A. & BUKAU, B. 2015. Compartment-specific aggregases direct distinct nuclear and cytoplasmic aggregate deposition. *EMBO J*.
MISHRA, M., JIANG, H., WU, L., CHAWSHEEN, H. A. & WEI, Q. 2015. The sulfiredoxin-peroxiredoxin (Srx-Prx) axis in cell signal transduction and cancer development. *Cancer Lett,* 366**,** 150-9.
MITCHELL SONTAG, E., SAMANT, R. S. & FRYDMAN, J. 2017. Mechanisms and Functions of Spatial Protein Quality Control. *Annu Rev Biochem*.
MOGK, A. & BUKAU, B. 2017. Role of sHsps in organizing cytosolic protein aggregation and disaggregation. *Cell Stress Chaperones*.
MOGK, A., KUMMER, E. & BUKAU, B. 2015. Cooperation of Hsp70 and Hsp100 chaperone machines in protein disaggregation. *Front Mol Biosci,* 2**,** 22.
MOON, J. C., HAH, Y. S., KIM, W. Y., JUNG, B. G., JANG, H. H., LEE, J. R., KIM, S. Y., LEE, Y. M., JEON, M. G., KIM, C. W., CHO, M. J. & LEE, S. Y. 2005. Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H2O2-induced cell death. *J Biol Chem,* 280**,** 28775-84.
MORANO, K. A. 2007. New tricks for an old dog: the evolving world of Hsp70. *Ann N Y Acad Sci,* 1113**,** 1-14.
MORANO, K. A., GRANT, C. M. & MOYE-ROWLEY, W. S. 2012. The response to heat shock and oxidative stress in Saccharomyces cerevisiae. *Genetics,* 190**,** 1157-95.
MORTIMER, R. K. & JOHNSTON, J. R. 1959. Life span of individual yeast cells. *Nature,* 183**,** 1751-2.

MUSICCO, C., CAPELLI, V., PESCE, V., TIMPERIO, A. M., CALVANI, M., MOSCONI, L., ZOLLA, L., CANTATORE, P. & GADALETA, M. N. 2009. Accumulation of overoxidized Peroxiredoxin III in aged rat liver mitochondria. *Biochim Biophys Acta,* 1787**,** 890-6.

NARAYANASWAMY, R., LEVY, M., TSECHANSKY, M., STOVALL, G. M., O'CONNELL, J. D., MIRRIELEES, J., ELLINGTON, A. D. & MARCOTTE, E. M. 2009. Widespread reorganization of metabolic enzymes into reversible assemblies upon nutrient starvation. *Proc Natl Acad Sci U S A,* 106**,** 10147-52.

NETTO, L. E. & ANTUNES, F. 2016. The Roles of Peroxiredoxin and Thioredoxin in Hydrogen Peroxide Sensing and in Signal Transduction. *Mol Cells,* 39**,** 65-71.

NEUMANN, C. A., KRAUSE, D. S., CARMAN, C. V., DAS, S., DUBEY, D. P., ABRAHAM, J. L., BRONSON, R. T., FUJIWARA, Y., ORKIN, S. H. & VAN ETTEN, R. A. 2003. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature,* 424**,** 561-5.

NOICHRI, Y., PALAIS, G., RUBY, V., D'AUTREAUX, B., DELAUNAY-MOISAN, A., NYSTROM, T., MOLIN, M. & TOLEDANO, M. B. 2015. In vivo parameters influencing 2-Cys Prx oligomerization: The role of enzyme sulfinylation. *Redox Biol,* 6**,** 326-33.

NYSTROM, T. 2005. Role of oxidative carbonylation in protein quality control and senescence. *EMBO J,* 24**,** 1311-7.

NYSTRÖM, T. 2007. A Bacterial Kind of Aging. *PLoS Genet,* 3**,** e224.

NYSTROM, T. & LIU, B. 2014. The mystery of aging and rejuvenation - a budding topic. *Curr Opin Microbiol,* 18**,** 61-7.

NYSTROM, T., YANG, J. & MOLIN, M. 2012. Peroxiredoxins, gerontogenes linking aging to genome instability and cancer. *Genes Dev,* 26**,** 2001-8.

OGG, S., PARADIS, S., GOTTLIEB, S., PATTERSON, G. I., LEE, L., TISSENBAUM, H. A. & RUVKUN, G. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. *Nature,* 389**,** 994-9.

OGRODNIK, M., SALMONOWICZ, H., BROWN, R., TURKOWSKA, J., SREDNIAWA, W., PATTABIRAMAN, S., AMEN, T., ABRAHAM, A. C., EICHLER, N., LYAKHOVETSKY, R. & KAGANOVICH, D. 2014. Dynamic JUNQ inclusion bodies are asymmetrically inherited in mammalian cell lines through the asymmetric partitioning of vimentin. *Proc Natl Acad Sci U S A,* 111**,** 8049-54.

OLAHOVA, M., TAYLOR, S. R., KHAZAIPOUL, S., WANG, J., MORGAN, B. A., MATSUMOTO, K., BLACKWELL, T. K. & VEAL, E. A. 2008. A redox-sensitive peroxiredoxin that is important for longevity has tissue- and stress-specific roles in stress resistance. *Proc Natl Acad Sci U S A,* 105**,** 19839-44.

OLAHOVA, M. & VEAL, E. A. 2015. A peroxiredoxin, PRDX-2, is required for insulin secretion and insulin/IIS-dependent regulation of stress resistance and longevity. *Aging Cell,* 14**,** 558-68.

OLING, D., EISELE, F., KVINT, K. & NYSTROM, T. 2014. Opposing roles of Ubp3-dependent deubiquitination regulate replicative life span and heat resistance. *EMBO J,* 33**,** 747-61.

OLIVER, C. N., AHN, B. W., MOERMAN, E. J., GOLDSTEIN, S. & STADTMAN, E. R. 1987. Age-related changes in oxidized proteins. *J Biol Chem,* 262**,** 5488-91.

PAN, Y., JIN, J. H., YU, Y. & WANG, J. 2014. Significant enhancement of hPrx1 chaperone activity through lysine acetylation. *Chembiochem,* 15**,** 1773-6.

PARK, J. H., KIM, Y. S., LEE, H. L., SHIM, J. Y., LEE, K. S., OH, Y. J., SHIN, S. S., CHOI, Y. H., PARK, K. J., PARK, R. W. & HWANG, S. C. 2006. Expression of peroxiredoxin and thioredoxin in human lung cancer and paired normal lung. *Respirology,* 11**,** 269-75.

PARK, J. W., PISZCZEK, G., RHEE, S. G. & CHOCK, P. B. 2011. Glutathionylation of peroxiredoxin I induces decamer to dimers dissociation with concomitant loss of chaperone activity. *Biochemistry,* 50**,** 3204-10.

PARK, M. H., JO, M., KIM, Y. R., LEE, C. K. & HONG, J. T. 2016. Roles of peroxiredoxins in cancer, neurodegenerative diseases and inflammatory diseases. *Pharmacol Ther,* 163**,** 1-23.

PARK, S. H., BOLENDER, N., EISELE, F., KOSTOVA, Z., TAKEUCHI, J., COFFINO, P. & WOLF, D. H. 2007. The cytoplasmic Hsp70 chaperone machinery subjects misfolded and endoplasmic reticulum import-incompetent proteins to degradation via the ubiquitin-proteasome system. *Mol Biol Cell,* 18**,** 153-65.

PARK, S. H., KUKUSHKIN, Y., GUPTA, R., CHEN, T., KONAGAI, A., HIPP, M. S., HAYER-HARTL, M. & HARTL, F. U. 2013. PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell,* 154**,** 134-45.

PLISHKER, G. A., CHEVALIER, D., SEINSOTH, L. & MOORE, R. B. 1992. Calcium-activated potassium transport and high molecular weight forms of calpromotin. *J Biol Chem,* 267**,** 21839-43.

RAND, J. D. & GRANT, C. M. 2006. The thioredoxin system protects ribosomes against stress-induced aggregation. *Mol Biol Cell,* 17**,** 387-401.

RATTAN, S. I. 1995. Gerontogenes: real or virtual? *FASEB J,* 9**,** 284-6.

RHEE, S. G. & WOO, H. A. 2011. Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H(2)O(2), and protein chaperones. *Antioxid Redox Signal,* 15**,** 781-94.

RISTOW, M. & ZARSE, K. 2010. How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Exp Gerontol,* 45**,** 410-8.

ROBERTS, L. J., 2ND & RECKELHOFF, J. F. 2001. Measurement of F(2)-isoprostanes unveils profound oxidative stress in aged rats. *Biochem Biophys Res Commun,* 287**,** 254-6.

ROGINA, B. & HELFAND, S. L. 2004. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci U S A,* 101**,** 15998-6003.

RUBINSZTEIN, DAVID C., MARIÑO, G. & KROEMER, G. 2011. Autophagy and Aging. *Cell,* 146**,** 682-695.

RUJANO, M. A., BOSVELD, F., SALOMONS, F. A., DIJK, F., VAN WAARDE, M. A., VAN DER WANT, J. J., DE VOS, R. A., BRUNT, E. R., SIBON, O. C. & KAMPINGA, H. H. 2006. Polarised asymmetric inheritance of accumulated protein damage in higher eukaryotes. *PLoS Biol,* 4**,** e417.

SAARIKANGAS, J. & BARRAL, Y. 2015. Protein aggregates are associated with replicative aging without compromising protein quality control. *Elife,* 4.

SACCOCCIA, F., DI MICCO, P., BOUMIS, G., BRUNORI, M., KOUTRIS, I., MIELE, A. E., MOREA, V., SRIRATANA, P., WILLIAMS, D. L., BELLELLI, A. & ANGELUCCI, F. 2012. Moonlighting by different stressors: crystal structure of the chaperone species of a 2-Cys peroxiredoxin. *Structure,* 20**,** 429-39.

SASAKI, T., UNNO, K., TAHARA, S. & KANEKO, T. 2010. Age-related increase of reactive oxygen generation in the brains of mammals and birds: is reactive oxygen a signaling

molecule to determine the aging process and life span? *Geriatr Gerontol Int,* 10 Suppl 1**,** S10-24.
SCHECKHUBER, C. Q., ERJAVEC, N., TINAZLI, A., HAMANN, A., NYSTROM, T. & OSIEWACZ, H. D. 2007. Reducing mitochondrial fission results in increased life span and fitness of two fungal ageing models. *Nat Cell Biol,* 9**,** 99-105.
SCHRODER, E., LITTLECHILD, J. A., LEBEDEV, A. A., ERRINGTON, N., VAGIN, A. A. & ISUPOV, M. N. 2000. Crystal structure of decameric 2-Cys peroxiredoxin from human erythrocytes at 1.7 A resolution. *Structure,* 8**,** 605-15.
SHCHEPROVA, Z., BALDI, S., FREI, S. B., GONNET, G. & BARRAL, Y. 2008. A mechanism for asymmetric segregation of age during yeast budding. *Nature,* 454**,** 728-34.
SHIBER, A., BREUER, W., BRANDEIS, M. & RAVID, T. 2013. Ubiquitin conjugation triggers misfolded protein sequestration into quality control foci when Hsp70 chaperone levels are limiting. *Mol Biol Cell,* 24**,** 2076-87.
SHORTER, J. 2011. The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS One,* 6**,** e26319.
SINCLAIR, D. A. & GUARENTE, L. 1997. Extrachromosomal rDNA circles--a cause of aging in yeast. *Cell,* 91**,** 1033-42.
SOHAL, R. S. 2002. Role of oxidative stress and protein oxidation in the aging process. *Free Radic Biol Med,* 33**,** 37-44.
SOHAL, R. S., AGARWAL, S., CANDAS, M., FORSTER, M. J. & LAL, H. 1994a. Effect of age and caloric restriction on DNA oxidative damage in different tissues of C57BL/6 mice. *Mech Ageing Dev,* 76**,** 215-24.
SOHAL, R. S., KU, H. H., AGARWAL, S., FORSTER, M. J. & LAL, H. 1994b. Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech Ageing Dev,* 74**,** 121-33.
SOHAL, R. S. & ORR, W. C. 2012. The redox stress hypothesis of aging. *Free Radic Biol Med,* 52**,** 539-55.
SOHAL, R. S. & SOHAL, B. H. 1991. Hydrogen peroxide release by mitochondria increases during aging. *Mech Ageing Dev,* 57**,** 187-202.
SOHAL, R. S. & WEINDRUCH, R. 1996. Oxidative stress, caloric restriction, and aging. *Science,* 273**,** 59-63.
SPEAKMAN, J. R. & MITCHELL, S. E. 2011. Caloric restriction. *Mol Aspects Med,* 32**,** 159-221.
SPECHT, S., MILLER, S. B., MOGK, A. & BUKAU, B. 2011. Hsp42 is required for sequestration of protein aggregates into deposition sites in Saccharomyces cerevisiae. *J Cell Biol,* 195**,** 617-29.
SPIESS, C., MEYER, A. S., REISSMANN, S. & FRYDMAN, J. 2004. Mechanism of the eukaryotic chaperonin: protein folding in the chamber of secrets. *Trends Cell Biol,* 14**,** 598-604.
SPOKOINI, R., MOLDAVSKI, O., NAHMIAS, Y., ENGLAND, J. L., SCHULDINER, M. & KAGANOVICH, D. 2012. Confinement to organelle-associated inclusion structures mediates asymmetric inheritance of aggregated protein in budding yeast. *Cell Rep,* 2**,** 738-47.
STADTMAN, E. R. 2006. Protein oxidation and aging. *Free Radic Res,* 40**,** 1250-8.
STADTMAN, E. R. & LEVINE, R. L. 2000. Protein oxidation. *Ann N Y Acad Sci,* 899**,** 191-208.
STEINKRAUS, K. A., KAEBERLEIN, M. & KENNEDY, B. K. 2008. Replicative aging in yeast: the means to the end. *Annu Rev Cell Dev Biol,* 24**,** 29-54.

STOLZ, A. & WOLF, D. H. 2012. Use of CPY and its derivatives to study protein quality control in various cell compartments. *Methods Mol Biol,* 832**,** 489-504.
SZILARD, L. 1959. On the Nature of the Aging Process. *Proc Natl Acad Sci U S A,* 45**,** 30-45.
TAIPALE, M., JAROSZ, D. F. & LINDQUIST, S. 2010. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nat Rev Mol Cell Biol,* 11**,** 515-28.
TAMAKI, H. 2007. Glucose-stimulated cAMP-protein kinase A pathway in yeast Saccharomyces cerevisiae. *J Biosci Bioeng,* 104**,** 245-50.
TANAKA, K. 2009. The proteasome: overview of structure and functions. *Proc Jpn Acad Ser B Phys Biol Sci,* 85**,** 12-36.
TAYLOR, R. C. & DILLIN, A. 2011. Aging as an event of proteostasis collapse. *Cold Spring Harb Perspect Biol,* 3.
TEIXEIRA, F., CASTRO, H., CRUZ, T., TSE, E., KOLDEWEY, P., SOUTHWORTH, D. R., TOMAS, A. M. & JAKOB, U. 2015. Mitochondrial peroxiredoxin functions as crucial chaperone reservoir in Leishmania infantum. *Proc Natl Acad Sci U S A,* 112**,** E616-24.
TESSARZ, P., SCHWARZ, M., MOGK, A. & BUKAU, B. 2009. The yeast AAA+ chaperone Hsp104 is part of a network that links the actin cytoskeleton with the inheritance of damaged proteins. *Mol Cell Biol,* 29**,** 3738-45.
THAYER, N. H., LEVERICH, C. K., FITZGIBBON, M. P., NELSON, Z. W., HENDERSON, K. A., GAFKEN, P. R., HSU, J. J. & GOTTSCHLING, D. E. 2014. Identification of long-lived proteins retained in cells undergoing repeated asymmetric divisions. *Proc Natl Acad Sci U S A,* 111**,** 14019-26.
TIMMERMANN, B., JAROLIM, S., RUSSMAYER, H., KERICK, M., MICHEL, S., KRUGER, A., BLUEMLEIN, K., LAUN, P., GRILLARI, J., LEHRACH, H., BREITENBACH, M. & RALSER, M. 2010. A new dominant peroxiredoxin allele identified by whole-genome re-sequencing of random mutagenized yeast causes oxidant-resistance and premature aging. *Aging (Albany NY),* 2**,** 475-86.
TISSENBAUM, H. A. & GUARENTE, L. 2001. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature,* 410**,** 227-30.
TONOKI, A., KURANAGA, E., TOMIOKA, T., HAMAZAKI, J., MURATA, S., TANAKA, K. & MIURA, M. 2009. Genetic evidence linking age-dependent attenuation of the 26S proteasome with the aging process. *Mol Cell Biol,* 29**,** 1095-106.
TROTTER, E. W., RAND, J. D., VICKERSTAFF, J. & GRANT, C. M. 2008. The yeast Tsa1 peroxiredoxin is a ribosome-associated antioxidant. *Biochem J,* 412**,** 73-80.
TURRENS, J. F. 1997. Superoxide production by the mitochondrial respiratory chain. *Biosci Rep,* 17**,** 3-8.
TYEDMERS, J., MOGK, A. & BUKAU, B. 2010. Cellular strategies for controlling protein aggregation. *Nat Rev Mol Cell Biol,* 11**,** 777-88.
VEATCH, J. R., MCMURRAY, M. A., NELSON, Z. W. & GOTTSCHLING, D. E. 2009. Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. *Cell,* 137**,** 1247-58.
VIJG, J. & SUH, Y. 2013. Genome instability and aging. *Annu Rev Physiol,* 75**,** 645-68.
WANG, L., SCHUBERT, D., SAWAYA, M. R., EISENBERG, D. & RIEK, R. 2010. Multidimensional structure-activity relationship of a protein in its aggregated states. *Angew Chem Int Ed Engl,* 49**,** 3904-8.
WEBB, A. E. & BRUNET, A. 2014. FOXO transcription factors: key regulators of cellular quality control. *Trends Biochem Sci,* 39**,** 159-69.

WEIDS, A. J. & GRANT, C. M. 2014. The yeast peroxiredoxin Tsa1 protects against protein-aggregate-induced oxidative stress. *J Cell Sci,* 127**,** 1327-35.
WEIDS, A. J., IBSTEDT, S., TAMAS, M. J. & GRANT, C. M. 2016. Distinct stress conditions result in aggregation of proteins with similar properties. *Sci Rep,* 6**,** 24554.
WOOD, Z. A., SCHRODER, E., ROBIN HARRIS, J. & POOLE, L. B. 2003. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci,* 28**,** 32-40.
WULLSCHLEGER, S., LOEWITH, R. & HALL, M. N. 2006. TOR signaling in growth and metabolism. *Cell,* 124**,** 471-84.
ZAMAN, S., LIPPMAN, S. I., ZHAO, X. & BROACH, J. R. 2008. How Saccharomyces responds to nutrients. *Annu Rev Genet,* 42**,** 27-81.
ZHOU, C., SLAUGHTER, B. D., UNRUH, J. R., GUO, F., YU, Z., MICKEY, K., NARKAR, A., ROSS, R. T., MCCLAIN, M. & LI, R. 2014. Organelle-based aggregation and retention of damaged proteins in asymmetrically dividing cells. *Cell,* 159**,** 530-42.