Proteostasis beyond chaperones

Auxiliary systems in the management of damaged proteins

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Till Oskar och Amelia

Fast jag är ganska välförsedd med styrka, mod och rättframhet,
kan jag någon gång bli rädd för allt det där som ingen vet

(from ”Bortom sol och måne”, by Benny Andersson and Björn Ulvaeus)

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ABSTRACT

Aging entails loss of functionality and increased risk of death, even for unicellular organisms, such as the yeast *Saccharomyces cerevisiae*. On a cellular level, aging is accompanied by an accumulation of harmful aging factors, e.g. damaged and aggregated proteins. Environmental stress can also cause a protein to misfold, leading to loss of function and aggregation. Protein misfolding is counteracted by systems of protein quality control. The temporal protein quality control system acts to refold or degrade the misfolded protein, while the spatial protein quality control system sorts damaged proteins to specific inclusion sites to prevent toxicity.

The proteostasis network is defined as the cellular machineries involved in protein synthesis, folding and degradation. Other systems affecting proteins and protein folding status are considered auxiliary systems to proteostasis. This thesis is based on genome-wide studies of *S. cerevisiae* aimed at finding new components in asymmetric inheritance of damaged proteins and the overall capacity of the cell to prevent protein aggregates. Vac17 was identified as a limiting factor for asymmetric inheritance, inclusion formation, endocytosis and lifespan. The effect of Vac17 on these processes requires endosomal components and fusion to the vacuole. In addition, I found that the GET pathway is a limiting factor in protein folding, as disruption causes massive aggregation of several proteins, not only substrates of the GET pathway. Given the major impact of these auxiliary systems on proteostatic processes, they should be considered part of the proteostasis network.

Keywords: Aging, proteostasis, protein quality control, asymmetric inheritance, vesicle trafficking

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SAMMANFATTNING PÅ SVENSKA

När vi åldras minskar kroppens funktionalitet och risken för att dö ökar. Även encelliga organismer, så som jästen *Saccharomyces cerevisiae*, åldras. Följer man en jästcell kan man se att den kommer dela sig ett givet antal gånger innan den dör. Åldrandet medför cellulära förändringar som brukar kallas åldrande faktorer. Dessa åldrande faktorer är extrakromosomala cirklar av ribosomalt DNA, icke-funktionella mitokondrier, ökat pH i vakuolen samt skadade proteiner. En viktig aspekt är att dessa faktorer selektivt hålls kvar i modercellen under celldelning, något som möjliggör föryngring av dottercellen.

För att ett protein ska vara funktionellt behöver det veckas till en given tredimensionell struktur. Skulle ett protein tappa sin struktur och bli felveckat kan det potentiellt bilda aggregat tillsammans med andra protein. För att undvika detta finns det proteinkvalitetskontrollsystem i cellen. Det temporala systemet verkar för att proteinet antingen ska återfå sin struktur eller om det inte är möjligt, brytas ner. Den spatiala proteinkvalitetskontrollen försöker begränsa skadan felveckade och aggregerade protein kan åstadkomma genom att sortera dem till särskilda platser i cellen, där risken att det skadade proteinet påverkar andra, funktionella protein, är mindre. Tillsammans utgör den temporala och den spatiala protienkvalitetskontollen det så kallade proteostasnätverket.

Den här avhandlingen baseras på studier där vi använt jästen *S. cerevisiae* för att hitta nya komponenter som påverkar proteinkvalitetskontroll och asymmetrisk nedärvning av skadade protein. Vac17 visade sig ha en tidigare okänd roll i asymmetrisk nedärvning, spatial proteinkvalitetskontroll och livslängd. Rollen som Vac17 har i proteinkvalitetskontroll är beroende av endosomala komponenter. Vidare binder Vac17 proteiner som är del av tidig endocytos och reglering av cellskelettet. Ytterligare en process utan tidigare känd roll i proteinkvalitetskontroll, GET-vägen för protein som ska sättas in i endoplasmatiska retikel-membranet, visade sig vara viktig för just proteinkvalitetskontroll. När man tar bort en av komponenterna och på så sätt stör GET-vägen påverkas veckningskapaciteten för hela cellen. Med tanke på den stora påverkan dessa processer som inte hör till proteostasnätverket, har på proteostas i helhet, föreslår vi att de bör inkluderas i begreppet proteostasnätverk

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Hill, S. M., Hao, X., Grönvall, J., Spikings-Nordby, S., Widlund, P. O., Amen, T., Jörhov, A., Josefson, R., Kaganovich, D., Liu, B., & Nyström, T. (2016). Asymmetric Inheritance of Aggregated Proteins and Age Reset in Yeast Are Regulated by Vac17-Dependent Vacuolar Functions. *Cell Rep, 16*, 1-13. doi:10.1016/j.celrep.2016.06.016

II. Josefson, R., Hao, X., Liu, B. & Nyström, T. (manuscript). The GET pathway is a major bottleneck for maintaining proteostasis in *Saccharomyces cerevisiae*.

III. Josefson, R., Hill, S. M., Hao, X. & Nyström, T. (manuscript). Vacuole inheritance-independent functions of Vac17 in spatial protein quality control and endocytosis.

Not included in the thesis.

Josefson, R., Andersson, R. & Nyström, T. (2017). How and why do toxic conformers of aberrant proteins accumulate during ageing? *Essays Biochem*, 61 (3):317-324. doi: https://doi.org/10.1042/EBC20160085

CONTENT

ABBREVIATIONS

Aβ	Amyloid β
AD	Alzheimer's Disease
AGG	Asymmetry Generating Gene
ALS	Amyotrophic Lateral Sclerosis
CR	Caloric Restriction
EGOC	EGO Complex
ERC	Extrachromosomal Ribosomal DNA Circle
GFP	Green Fluorescent Protein
GO	Gene Ontology
HD	Huntington's Disease
HSE	Heat Shock Element
HSP	Heat Shock Protein
Htt	Huntingtin (protein)
IB	Inclusion Body
INQ	IntraNuclear Quality control compartment
IPOD	Insoluble PrOtein Deposit
JUNQ	JuxtaNuclear Quality control compartment
NEF	Nucleotide Exchange Factor
PAS	Phagophore Assembly Site
PN	Proteostasis Network

PrD	Prion-determining Domain
PQC	Protein Quality Control
RLS	Replicative LifeSpan
ROS	Reactive Oxygen Species
RQC	Ribosome Quality Control complex
SAFE	Spatial Analysis of Functional Enrichment
sHSP	Small Heat Shock Protein
SNARE	Soluble *N*-ethylmaleimide-sensitive factor Attachment protein Receptor
STRE	Stress Responsive Element
TA	Tail-Anchored protein
UPS	Ubiquitin Proteasome System

AIM OF THE THESIS

Proteins are the tools by which the cell functions and they are only functional once they have reached their intended three-dimensional structure. This folded structure is under constant exposure to stresses and environmental changes and is sometimes lost. If this happens, the protein needs to refold or be properly disposed of. The failure to fold proteins has an adverse impact on cells and is a phenomenon associated with cellular aging and human disease.

The term protein homeostasis, or proteostasis, refers to how the cell controls the concentration, folding, binding and location of a protein by altering its intracellular components (Balch et al., 2008). Proteostasis and the connected proteostasis network (PN) that defines it, influence the cell's protein folding capacity and is of importance for protein conformational disease, viral infections, cancer and aging (Powers et al., 2009). The term proteostasis network has been specified as the coordination of protein synthesis, folding and degradation, specifically the translational machinery, molecular chaperones and cochaperones, the ubiquitin-proteasome system and autophagy (Labbadia and Morimoto, 2015).

The aim of this thesis was to use genome-wide screens in the yeast *Saccharomyces cerevisiae* to build a complete network of auxiliary components that affect different aspects of proteostasis, such as protein folding during normal, unstressed growth, asymmetrical inheritance of damaged proteins and formation of protein inclusions at defined sites in the cell.

1 AGING

1.1 REPLICATIVE AGING IN YEAST

Aging is an integral part of life, experienced by all organisms. Aging is associated with loss of functionality, while the risk of death increases. Several attempts have been made at explaining aging and several evolutionary theories have been put forth. The disposable soma theory, presented in 1977, draws upon the fact that very few animals live long enough for aging to become an issue, and that resources in the wild are limited (Kirkwood, 1977). This implies a tradeoff between reproduction and repair, where repair is disfavored as very few animals live beyond reproductive age (Kirkwood, 2005). As a consequence damage will accumulate as we pass reproductive age, ultimately overwhelming the cellular defense and causing age-associated cellular dysfunction (Kirkwood, 2005; Kirkwood and Austad, 2000).

The budding yeast *S. cerevisiae* is a much-used model organism in biology. Despite being unicellular, *S. cerevisiae* shares several important cellular functions with higher mammals including humans. Yeast, like mammals and humans, is eukaryotic and has distinct membrane-enclosed compartments, organelles, similar to higher eukaryotes. Moreover, 60% of yeast genes have a homologue or share a conserved domain with a human gene and 25% of human genes have a close yeast homologue. For instance, and of relevance for aging research, caloric restriction is known to extend life span in mammals as well as in yeast (Lin et al., 2000; Weindruch, 1996).

Another important technical feature of yeast is that its DNA is easily manipulated, as homologous recombination is very efficient, allowing for manipulation of yeast genes and introduction of newly designed constructs (reviewed in (Khurana and Lindquist, 2010). Impressively, there are now several libraries of different yeast strains, including libraries for gene deletions and reporter-tagged versions of almost all genes. Combining these libraries with crossing strategies has allowed for genome-wide screens to be constructed (papers I-III, Costanzo et al., 2010; Tong et al., 2001; Tong et al., 2004). This enables unbiased searches and descriptions of genetic interactions as well as the genetic foundation of several cellular processes.

While cultures of the yeast *S. cerevisiae* can be maintained indefinitely, single cell observations reveal that yeast cells have a finite capacity to generate daughter cells before they stop dividing and die (Barton, 1950). Additionally,

yeast cells display age-related changes, such as increased cell size, sterility, increased generation times and nuclear fragmentation, accompanied by an increased risk of death (Egilmez et al., 1990; Pohley, 1987; Smeal et al., 1996). The age of a yeast cell can be followed by micromanipulation techniques, whereby single cells are monitored and the newly generated daughter cells are removed, allowing for them to be counted (Mortimer and Johnston, 1959). The number of daughter cells produced is referred to as the replicative life span of a yeast cell and is considered to be comparable to the lifespan of other, asymmetrically dividing cells such as stem cells.

Early studies speculated that aging in yeast might be caused by a physical limitation in the cell wall of the mother cell (Johnston, 1966; Mortimer and Johnston, 1959). However other studies found that aging in yeast must be caused by intracellular factors and that there are genetic components affecting aging (Egilmez and Jazwinski, 1989). Since then several hallmarks of aging have been described and found to be of importance in several organisms (López-Otín et al., 2013). More specifically, in *S. cerevisiae*, the cytoplasmic aging factor predicted by Egilmez and colleagues have been specified as several aging factors. An aging factor is defined as a factor which has increased levels in old cells and low levels in young cells, is asymmetrically retained in the mother cell during cell division and artificially removing or introducing the factor will increase and decrease life span, respectively (Henderson and Gottschling, 2008; Lippuner et al., 2014). The acknowledged aging factors to date are extrachromosomal ribosomal DNA circles (ERCs), dysfunctional mitochondria, vacuolar pH (see below) and damaged proteins (chapter 1.2).

ERCs are formed as the ribosomal DNA repeats undergo homologous recombination, resulting in ERCs that each carry autonomously replicating sequences and thus can replicate and multiply independently of chromosomal replication (Guarente, 1997; Sinclair and Guarente, 1997). The mechanism through which ERC accumulation causes aging is not established. Nucleolar fragmentation associated with ERC accumulation (Guarente, 1997; Sinclair and Guarente, 1997; Steinkraus et al., 2008) and titration of DNA-binding factors (Sinclair and Guarente, 1997) as well as rDNA instability (Ganley et al., 2009; Merker Robert and Klein Hannah, 2002) have all been suggested as ERC aging mechanisms. Overexpression or deletion of the deacetylase *SIR2* in *S. cerevisiae* decreases and increases ERC levels respectively (Kaeberlein et al., 1999). The role of *SIR2* in aging has however been suggested to go beyond ERC accumulation (see chapter 4).

Mitochondria are the main energy producers of the cell. In aged yeast cells mitochondria are fragmented, with low ATP production yield and a more

oxidized redox state (Lai et al., 2002; Scheckhuber et al., 2007). Deletion of mitochondrial fission factors causes less mitochondrial fragmentation and extends yeast replicative life span (Scheckhuber et al., 2007). Moreover, daughter cells inherit the most fit (i.e. most reduced) mitochondria (Lai et al., 2002; McFaline-Figueroa et al., 2011), based on the faster movement of healthy mitochondria against the retrograde actin cable flow emanating from the daughter cell (Higuchi et al., 2013; McFaline-Figueroa et al., 2011).

The vacuole is a lysosome-like compartment found in yeast cells. This acidic organelle is involved in vesicle trafficking and storage and turnover of macromolecules. The vacuole undergoes fusion and fission events during the cell cycle and in response to environmental changes, such as hyper- and hypoosmotic stress (Ostrowicz et al., 2008; Wiemken et al., 1970; Zieger and Mayer, 2012). Early on during aging the vacuolar pH increases which contributes to later mitochondrial functional decline (Hughes and Gottschling, 2012). This can be counteracted by overexpressing the vacuolar ATPase component *VMA1*, which increases vacuole acidity and also increases life span (Hughes and Gottschling, 2012). The increased vacuolar pH was found to be reset in the daughter cell during cell division, a phenomenon that depends on asymmetric inheritance of the plasma membrane proton pump Pma1. Pma1 is retained in mother cells, where it pumps protons out of the cell, leaving fewer protons for acidification of the vacuole. By contrast, more protons are retained in the, at first, Pma1-less daughter cell, which can then be pumped into the vacuole, making it more acidic (Henderson et al., 2014; Thayer et al., 2014).

1.2 MISFOLDED AND DAMAGED PROTEINS

Proteins have diverse functions within the cell, both structural and enzymatic. In order to be fully functional, a protein needs its proper three-dimensional, folded structure, i.e. its native form. During aging misfolded and damaged proteins accumulate and are considered a hallmark of aging (David et al., 2010; Labbadia and Morimoto, 2015; López-Otín et al., 2013; Oliver et al., 1987). The misfolding of a protein is detrimental to the cell as (i) the activity of the protein is lost upon misfolding and (ii) the protein can interact erroneously with other proteins. Moreover, the presence of misfolded proteins has been shown to be a fitness cost in its own right (Geiler-Samerotte et al., 2011). During folding or as a consequence of stress exposure, proteins can enter misfolded, intermediate conformations other than their intended native conformation (figure 1). These states are a normal part of folding and will only cause distress if they persist for long time periods, increasing the risk for self-assembly into protein aggregates. Protein aggregates form as hydrophobic residues normally

buried within the protein, are exposed on the protein surface and partake in intermolecular interactions. Studies have found that the toxicity of a misfolded protein oligomer is determined by the surface hydrophobicity and the oligomer size (Mannini et al., 2014). The intermolecular interactions can lower the free energy of the assembly and can become the preferred state if the native conformation is compromised, e.g. by mutation or environmental changes (Clark, 2004; Powers and Gierasch, 2021). Studies in *Escherichia coli* (*E. coli*) found that aggregation prone sequences, generally found at the hydrophobic core of globular proteins or at protein-protein interaction surfaces, show little redundancy in the proteome (Khodaparast et al., 2018). This and findings from other studies indicate a selection for sequence divergence in aggregation-prone sequences, in order to avoid wide-spread aggregation (Ganesan et al., 2015; Khodaparast et al., 2018). Aggregation gatekeeper residues are found flanking aggregation-prone sequences. These gatekeepers are enriched for asparagine, glutamate, lysine, arginine and proline residues with different aggregation propensities being associated with different gatekeeper strength. For strongly aggregating sequences an enrichment is seen for arginine and lysine gatekeepers, related to their large and flexible side chains that impede aggregation (Rousseau et al., 2006). Gatekeeper residues affect cellular fitness as well as protein production, degradation and folding capacity of the cell (Beerten et al., 2012) and are under strong selective pressure, with higher gatekeeper conservation associated with higher aggregation propensity of the associated aggregation-prone sequence (De Baets et al., 2014).

Aggregated proteins can be either disordered (amorphous) or ordered (amyloid-like). Amorphous aggregates form as partially folded proteins self-associate driven by hydrophobic interactions between exposed hydrophobic stretches. In contrast, amyloid-like aggregates are formed through a nucleation-dependent process. Partially folded peptides act as seeds and will recruit other partially folded proteins, to form highly ordered arrays that eventually form amyloid-like fibers. The structure of an amyloid-like fiber is called cross-β-structure, with β-sheets running perpendicular to the aggregate axis (Hartl et al., 2011; Wolfe and Cyr, 2011).

One example of a parameter that will influence protein folding during aging, is reactive oxygen species (ROS). Accumulation of ROS is a consequence of organisms living in an aerobic environment. As the concentrations increase during aging there are detrimental effects to the cell, one of which is protein oxidation and inactivation (Oliver et al., 1987; Stadtman, 2006). In yeast, the age-dependent increase of protein carbonylation seems to be related to an increase in ROS production from aging mitochondria (Aguilaniu et al., 2001), however different causes of increased protein carbonylation have been found

in different systems (Nyström, 2005; Stadtman, 2006). Protein carbonylation is an irreversible modification that can cause protein inactivation. Molecular chaperones have been shown to be particularly susceptible to carbonylation (Dukan and Nyström, 1998; Erjavec et al., 2007). Moreover, highly carbonylated proteins tend to form aggregates and can greatly affect proteostasis as carbonylated proteins cannot be efficiently degraded by the proteasome (Grune et al., 2004; Nyström, 2005).

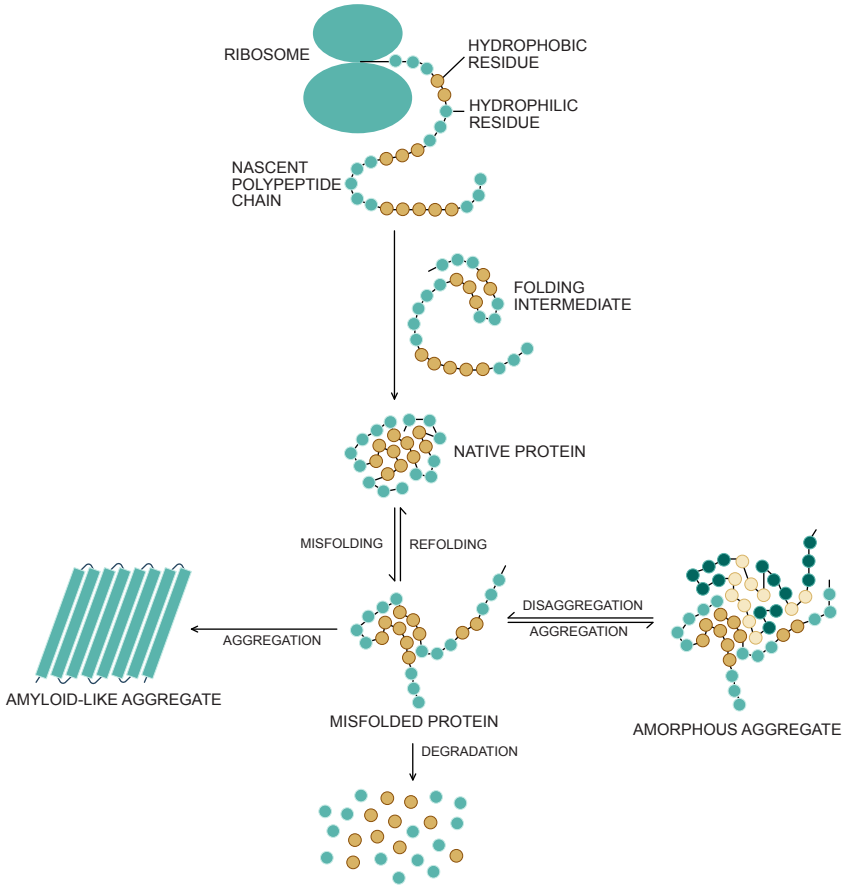

Figure 1. Protein folding and misfolding. As a newly translated polypeptide chain emerges from the ribosome, hydrophobic forces will drive its folding. The polypeptide chain will pass through intermediate folding states until it reaches its native folded state. The native protein can misfold upon stress or aging. A misfolded protein can either refold back into its native form, aggregate or be degraded.

1.3 MISFOLDED PROTEINS IN DISEASE

Aging is associated with neurodegenerative diseases, some of which are so-called protein conformational disorders. A protein conformational disorder is caused by a change in the three-dimensional structure of a protein, increasing its ability to bind to itself (Carrell and Lomas, 1997). There are known prion-related diseases as well as amyloid-like diseases. Amyloid-like diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), are characterized by the presence of insoluble fibrils or plaques (Chiti and Dobson, 2017). There is however little correlation between disease severity and plaques (see chapter 3).

A common mechanism is seen for amyloid-like protein folding states whereby misfolded species of the proteins go on to form amyloid-like inclusions that are capable of seeding further misfolding and fibril growth (Chiti and Dobson, 2017). While little cross reactivity has been described for amyloids, e.g. amyloid β (Aβ) of AD and islet amyloid peptide of type II diabetes will not influence each other in terms of aggregation (O'Nuallain et al., 2004), the phenomenon of supersaturation appears to contribute to neurodegenerative disease. A supersaturated protein is found at concentrations that surpass its solubility which leads to its aggregation. They are found throughout the proteome yet are enriched among proteins that tend to co-aggregate with disease proteins, suggesting that amyloid-like proteins can influence the folding state of other proteins, especially those that are supersaturated (Ciryam et al., 2015; Ciryam et al., 2013). Moreover, the introduction of an aggregation-prone protein has been found to trigger wide-spread protein aggregation (Gidalevitz et al., 2006). Introduction of a polyQ-expanded version of the HD-causing Huntingtin (Htt) exon 1 causes sequestration of the Hsp40 protein Sis1, which in turn influence the degradation of another misfolded protein in yeast (Park et al., 2013). Similar results were found in brains of a HD mouse model, where chaperones decreased in concentration over time and were selectively sequestered in nuclear aggregates (see chapter 2.1 for information on chaperones; Hay et al., 2004). Expression of polyQ-expanded Htt also makes cells more sensitive to heat shock and other stresses (Chafekar and Duennwald, 2012). Another study using a model protein designed to form β sheets, found that multiple proteins with essential roles in chromatin organization, transcription, translation and protein quality control were enriched in the aggregates (Olzscha et al., 2011). Expression of a mutant protein associated with amyotrophic lateral sclerosis (ALS), mutant SOD1, caused a change in proteins that bound Hsp70 and Hsc70, decreasing total interactions by around 40% and inducing new binding partners, characterized by increased protein size and internal disorder (Ryu et al., 2020). Thus,

aggregation-prone proteins can affect a wide range of cellular functions, which could explain the diverse pathogenic effects of aggregation-prone proteins in neurodegenerative disease.

Additionally, amyloid-like neurodegenerative disorders are associated with decreased clearance of the aggregation-prone species via the ubiquitin-proteasome system (UPS) (Mawuenyega et al., 2010). Another study also found that UPS function was abolished upon expression of aggregation-prone constructs (Bence et al., 2001). Likewise, multi-vesicular body sorting was found to be impaired in a mouse model of AD, a consequence of accumulation of the disease-causing Aβ which disrupts the UPS (Almeida et al., 2006).

The cells try to save themselves by refolding the misfolded aggregates. Indeed the molecular chaperone Hsp70 was found to rapidly associate and dissociate with Htt aggregates, indicative of it acting to refold the misfolded aggregate (Kim et al., 2002). Further indications of this come from a mouse HD model, where the authors found that blocking new synthesis of the aggregation-prone protein, caused the inclusions to disappear and the mouse recovered from the symptoms (Yamamoto et al., 2000).

As mentioned above, there are many human genes, including disease-related genes with homologues in yeast, enabling study of basic cellular functions and mechanisms of toxicity (reviewed in Khurana and Lindquist, 2010). Additionally, human disease-related genes that lack a yeast homologue can be exogenously expressed in yeast and studies using this approach have greatly added to the knowledge of the molecular mechanisms underlying, for example, HD. Firstly, poly Q-expanded Htt exon 1 will aggregate upon expression in yeast (Krobitsch and Lindquist, 2000; Meriin et al., 2002). Secondly, yeast Hsp40 was found to modulate polyQ-expanded Htt aggregation and toxicity, something which was later confirmed for human and mouse Hsp40 (Gokhale et al., 2005; Hageman et al., 2010; Kakkar et al., 2016; Muchowski et al., 2000). Lastly, different high throughput screens using yeast have identified potential therapeutic targets as well as small-molecule inhibitors, with previously described links to human pathophysiology as well as shown effects on an HD model in *Drosophila melanogaster* (Giorgini et al., 2005; Zhang et al., 2005).

2 TEMPORAL PROTEIN QUALITY CONTROL

Proteins in the cell are exposed to a hydrophilic environment and this drives protein folding. Hydrophilic residues will tend to be exposed to the solvent on the outside of the protein, while hydrophobic residues are buried within the protein, explaining why the internal residues seem to be of greater importance for proper folding (Anfinsen, 1973). A simplified model states that the native form of a protein is that of least relative free energy, hence the most stable form (Haber and Anfinsen, 1962). Small proteins, e.g. RNase A, are able to fold on their own, suggesting that the amino acid order in itself, together with the hydrophobic forces, provide enough information for successful folding (Anfinsen et al., 1961).

There are however infinitely more complex proteins than RNase A that need to be folded, e.g. proteins with β-sheet folds such as collagen, and that are unable to attain their native form in solution (Harrington and Rao, 1970). Additionally, the time scale for folding in solution is not compatible with the folding rate demanded by the cell (Anfinsen, 1973; Anfinsen et al., 1961). Moreover, increasing the concentration of RNase A to physiological level decreased the folding efficiency yielding much less native RNase A (Anfinsen and Haber, 1961). Another important aspect that differs between the folding environment of a test tube and that of a cell, is that there are other molecules taking up space, i.e. macromolecular crowding. The concentration of molecules in a cell is estimated to 200-300 mg/mL. As an unfolded polypeptide chain emerges from the ribosome or as a protein misfolds, there is therefore a high risk of erroneous interactions with the surrounding environment (Ellis, 2001). This suggests that protein folding (and misfolding) is a complex process affected by many parameters.

Another important parameter to consider, is that protein folding is sensitive to environmental changes. One widely used and well-studied environmental change and stressor in yeast, is heat shock. Even a mild heat shock at 38°C (from 30°C) causes drastic changes in the organellar structure to occur (Keuenhof et al., 2022). In addition, cells respond to environmental changes, including heat shock, by altering their transcriptional programming. Besides the environmental stress response which alters transcription in response to several different environmental stressors, there is a conserved transcriptional program called the heat shock response (HSR) (Gasch et al., 2000; Pincus, 2020). In yeast the HSR is driven by the transcription factor Hsf1, which was found to bind heat shock elements (HSE) in promoters of genes known to be

induced upon heat stress (Sorger and Pelham, 1987). Further studies found that Hsf1 binds promoters and is essential for yeast even in unstressed conditions (Jakobsen and Pelham, 1988; Sorger and Pelham, 1988). Moreover, HSR induction will occur not only as response to heat stress, but will also be induced by the presence of misfolded proteins, oxidative stress and ribosome biogenesis problems (Trotter et al., 2002; Tye et al., 2019; Yamamoto et al., 2007). Hsf1 is under basal conditions bound by Hsp90 and Hsp70 (see chapter 2.1 and 2.1.1), that are themselves transcriptional targets of Hsf1. As a consequence of stress, Hsp70 and Hsp90 are titrated away from Hsf1, allowing Hsf1 binding to its target promoters. As Hsp70 and Hsp90 both are induced by Hsf1, this allows for a negative feedback loop that will abrogate the HSR once enough Hsp70 and Hsp90 is available and thus can bind Hsf1 again (Krakowiak et al., 2018; Masser et al., 2019; Zheng et al., 2016). Other, upstream negative regulators of Hsf1 activity are involved in proteasomal degradation, organelle targeting and the ribosome quality control complex (RQC; Brandman et al., 2012).

The transcriptional changes upon heat stress are vast and the numbers of genes with changed expression are in the thousands. Genes that become upregulated are involved in e.g. protein folding and protein catabolic processes, while genes related to translation and ribosome biogenesis are downregulated (Mühlhofer et al., 2019). Generally it seems that the genes regulated by Hsf1 under basal conditions are also bound strongly by Hsf1 during heat shock and therefore become overexpressed, while there is another set of Hsf1 target genes that are only bound during stress conditions (Pincus et al., 2018). Despite its name, studies have found that only a minority of the promoters regulated during heat shock are in fact bound and regulated by Hsf1 while the majority of transcriptional changes upon heat shock are instead regulated by Msn2 and Msn4 (Solís et al., 2016).

Just as chronic inflammation in humans will be detrimental, chronic activation of HSR will adversely affect the cells in what is called a maladaptive response. The presence of a misfolded protein causes chronic activation of the HSR, which has a negative impact on the folding capacity of the cells. In an AD *Caenorhabditis elegans* (*C. elegans*) model, stopping the maladaptive response by silencing of Hsp70 or HSF1, resulted in significantly fewer worms developing disease-related paralysis (Roth et al., 2014). Furthermore, deletion *of Hsf1* in mice protected the animals from carcinogenesis, even with p53 mutations predisposing the animals for cancer development (Dai et al., 2007). Similarly, overactivation of Hsf1 in yeast will cause cell cycle arrest and suspend growth (Zheng et al., 2016). Instead, a study of yeast adaptation to high temperatures found that after around 15 generations, the acute stress

response genes were back to baseline expression and instead other changes in the proteome were evident. One such change was the reduced expression of many proteins that had previously been identified as aggregation-prone (Domnauer et al., 2021)

The temporal protein quality control system acts to fold, refold or degrade proteins (figure 2). This applies to folding of nascent polypeptide chains that emerge from the ribosome, normal protein turnover as well as to proteins that misfold upon stress exposure or aging. Classically, molecular chaperones, the ubiquitin-proteasome system (UPS) and autophagy are said to be part of temporal PQC and proteostasis.

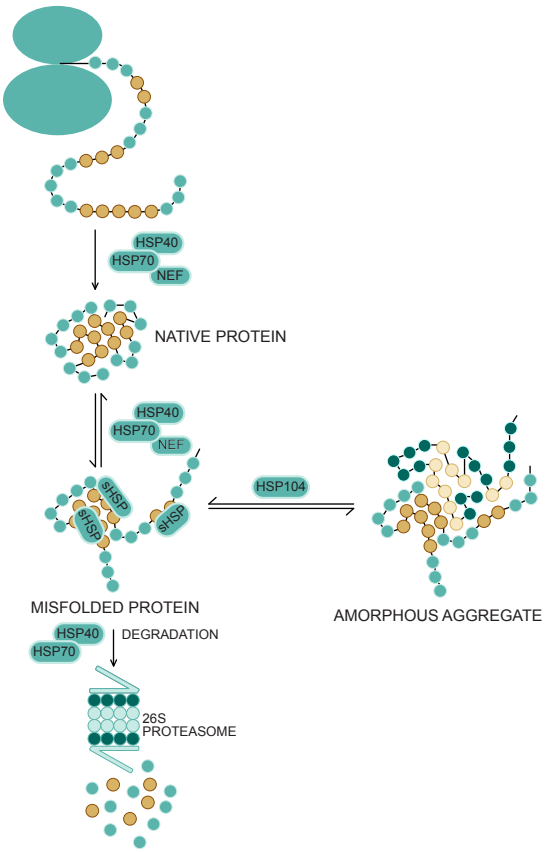

Figure 2. Temporal protein quality control. Molecular chaperones govern the disaggregation and refolding of misfolded proteins. Hsp70 and Hsp40 are part of folding, refolding and targeting proteins for degradation by the 26S proteasome. Hsp104 is the only known disaggregase, that can pull out misfolded proteins from aggregates, making them available substrates for Hsp70-Hsp40 refolding. Small HSPs bind in an ATP-independent fashion, preventing their aggregation and aiding in refolding by Hsp70-Hsp40.

2.1 CHAPERONES

Molecular chaperones are defined as molecules that aid proteins in reaching their final native fold, without being part of the final protein. Molecular chaperones fold newly synthesized proteins, refold misfolded proteins, disaggregate aggregated proteins, aid in oligomeric assembly and in protein degradation (Hartl et al., 2011). A lot of the known chaperones are known as different classes of heat shock proteins (HSP; Landry et al., 1982), which reflects their stress-inducible nature, although some chaperones are constitutively expressed and function in house-keeping processes. The chaperone substrate preference is for hydrophobic stretches and positively charged residues (Rüdiger et al., 1997). As aggregation-prone, hydrophobic stretches are flanked by positively charged gatekeepers it was suggested that chaperone binding to misfolding or aggregating sequences has evolved from the primary structure of gatekeeper-hydrophobic stretch-gatekeeper (Rousseau et al., 2006).

Molecular chaperones are divided into several groups; Hsp70, Hsp100, small HSPs (see 2.1.1-2.1.3), Hsp90 and chaperonins. Hsp90, though being essential has remained somewhat elusive as to what it specifically does. It has been shown that while not being required for folding of the majority of proteins in yeast, Hsp90 substrates are normally partially folded and involved in processes relating to vesicle trafficking, secretory pathway and signaling transduction (McClellan et al., 2007; Nathan et al., 1997). The function of Hsp90 is also greatly affected by its many cochaperones (reviewed in Li et al., 2012). Chaperonins are a class of oligomeric, high molecular weight complexes that in yeast are divided into two groups, Hsp60 and CCT (Hartl et al., 2011). CCT specifically folds monomeric actin and tubulin and the role of chaperonins appears to be in folding of substrates that are not readily folded by other chaperones (Llorca et al., 2000; Tian et al., 1995).

2.1.1 HSP70

The Hsp70 family of chaperones acts to promote protein folding in both protein synthesis and during stress that causes protein misfolding. These proteins consist of an ATPase domain, a substrate-binding domain and a C-terminal domain (Zhu et al., 1996). When Hsp70 is in an ATP-bound state it is in an open conformation, capable of substrate binding. ATP hydrolysis closes Hsp70, trapping the substrate, which is then released upon ADP exchange for ATP. Folding is promoted as Hsp70 binds exposed hydrophobic patches, thereby preventing aggregation. The substrate is then released and allowed to continue folding. This cycle can be repeated several times until folding is

completed (Mayer and Bukau, 2005). The consensus binding site for Hsp70 proteins is a hydrophobic stretch with flanking positively charged residues. These sequences are normally hidden within the protein core (Rüdiger et al., 1997). This corresponds very well to sequences identified as aggregation-prone (see chapter 1.2).

Two steps in the Hsp70 cycle are rate-limiting, the ATP hydrolysis and the nucleotide exchange (ADP is exchanged for ATP). Two classes of cochaperones are present and act to accelerate these steps. Hsp40s, or J proteins, aid in substrate recruitment and will promote ATP hydrolysis. There are multiple J proteins, something that adds to the broad number of processes and clients that are Hsp70-dependent (Kampinga and Craig, 2010). For example, the Hsp40 Sis1 in concert with Hsp70 has been shown to regulate induction of the HSR (Feder et al., 2020; Klaips et al., 2020). Interestingly, overexpression of *SIS1* was able to alter the nature of Htt97Q aggregates, making them soluble and enabling Hsp70 localization to the aggregate core (Klaips et al., 2020). The Hsp40 Apj1 promotes disaggregation together with Hsp70 inside the nucleus, independently of Hsp104 (den Brave et al., 2020). Hsp70-independent roles for Hsp40s have been described as well, e.g. Ydj1 is required for Rsp5-mediated ubiquitination of misfolded proteins upon heat stress (Fang et al., 2014; Lee et al., 1996). Hsp70 binding to nucleotide exchange factors (NEFs) accelerates Hsp70 nucleotide exchange, which causes release of the substrate protein (Mayer and Bukau, 2005). There are several classes of NEFs for Hsp70 in yeast, represented by Fes1 and the Hsp110 family member Sse1. Sse1 appears to be essential for Hsp70 refolding activities, while Fes1 acts in other Hsp70-dependent processes (Abrams et al., 2014). Sse1 has also been described in Hsp70-dependent targeting of substrates to the proteasome (Kandasamy and Andréasson, 2018).

The ribosome-associated Hsp70 chaperones Ssz1, Ssb1 and Ssb2 together with the J-domain-containing protein Zuo1 aid in the folding of nascent proteins, both while attached to the ribosome and just after finished translation (Hallstrom and Moye-Rowley, 2000; Nelson et al., 1992; Willmund et al., 2013; Yan et al., 1998). Proteins bound and aided by Ssb1 and Ssb2 are enriched for intrinsically disordered domains, short hydrophobic stretches and other conditions that increase risk of misfolding, e.g. domain length, translational rate and aggregation propensity (Willmund et al., 2013). Loss of Ssb function causes widespread aggregation of Ssb substrates, suggesting that Ssb function is essential for maintaining these substrates in a soluble state (Willmund et al., 2013). The SSA family of Hsp70s in the cytosol consists of the constitutively expressed members, Ssa1 and Ssa2, and two stress-inducible ones, Ssa3 and Ssa4 (Werner-Washburne et al., 1987). There is high homology

between the *SSA* members and deletion of a single *SSA* gene will not affect cell viability. Deletion of both the constitutively active Hsp70s, *SSA1* and *SSA2,* induces increased expression of *SSA4* (Boorstein and Craig, 1990), but this compensatory mechanism is not fully adequate as the *ssa1 ssa2* double mutant is heat sensitive, has several defects relating to PQC and a shortened life span (Andersson et al., 2021; Craig and Jacobsen, 1984; Öling et al., 2014).

The yeast Hsp70s have been implicated in processes such as nascent peptide chain folding, stress-related folding (see above), stress foci formation (see chapter 3) as well as translocation across organelle membranes and folding within organelles (Kang et al., 1990; Mayer and Bukau, 2005; Ungermann et al., 1994).

2.1.2 HSP100

Exposure of yeast to a mild heat shock of 37°C before an extreme heat shock of 50°C, gives the yeast acquired thermotolerance and allows for survival at 50°C. This acquired thermotolerance was found to be dependent on Hsp104 (Parsell et al., 1993; Sanchez and Lindquist Susan, 1990) and reactivation of aggregated proteins is essential for thermotolerance development (Weibezahn et al., 2004). The bacterial homolog ClpB is similarly required for protection at elevated temperatures (Squires et al., 1991). Hsp104 and ClpB are both part of the Hsp100/AAA+ protein family. Other family members are ClpA, ClpC and ClpX that bind peptidase proteins and carry out protein degradation in bacteria (Olivares et al., 2018). ClpB, ClpG and Hsp104 are Hsp100 members that do not engage peptidases and instead act in disaggregation and reactivation of aggregated proteins (Katikaridis et al., 2021). These proteins form hexamers and disaggregate aggregated proteins by threading them through the central pore of the hexamer (Mogk et al., 2018; Tessarz et al., 2008). It seems that only partial translocation through the pore is required for efficient protein disaggregation (Haslberger et al., 2008). Hsp100 proteins have conserved AAA domains, required for ATP hydrolysis and oligomerization, which is needed to form the active, hexameric forms (Katikaridis et al., 2021). The Hsp100 protein N-terminal domain is required for initial substrate interaction (Barnett et al., 2005; Rosenzweig et al., 2015). Additionally, the M domain is essential in regulating disaggregation activity (Heuck et al., 2016; Oguchi et al., 2012; Seyffer et al., 2012).

In order to efficiently disaggregate aggregated proteins, Hsp104 and ClpB needs to interact with Hsp70 (Glover and Lindquist, 1998; Mogk et al., 1999; Parsell et al., 1994). Hsp70 interaction with ClpB/Hsp104 occurs at the M domain, which depending on whether the state of ClpB/Hsp104 is active or

not, will be accessible for Hsp70 to bind (Carroni et al., 2014; Heuck et al., 2016; Seyffer et al., 2012). Hsp70 can only bind and activate ClpB/Hsp104 if bound by a substrate and the substrate is then transferred onto ClpB/Hsp104 upon recognition of hydrophobic regions by ClpB/Hsp104 (Haslberger et al., 2007; Hayashi et al., 2017; Rosenzweig et al., 2013). The rate-limiting step in reactivation of aggregated proteins seems to be the initial interaction of Hsp40 and Hsp70, which is then required for Hsp104 to bind the aggregate and the Hsp70-Hsp40-dependent refolding that follows (Schlieker et al., 2004; Winkler et al., 2012; Ziętkiewicz et al., 2004). However, Hsp70 alone fails to recruit Hsp104 to aggregated proteins under oxidative stress and requires the added action of the peroxiredoxin Tsa1 (Hanzén et al., 2016). The NEF Hsp110 has also been found to have a role in Hsp104 recruitment as well as Hsp104 disaggregation (Kaimal et al., 2017).

There is no HSP104 homologue in higher eukaryotes, begging the question as to how protein aggregates become disaggregated in these organisms. Instead, the Hsp70 cochaperones Hsp40/J proteins and Hsp110 disaggregate proteins in higher eukaryotes (Mattoo et al., 2013; Nillegoda et al., 2015; Rampelt et al., 2012; Shorter, 2011). Knocking down Hsp110 in *C. elegans* further confirmed a role in disaggregation as the animals displayed compromised dissolution of heat-induced aggregates and shortened lifespan after heat shock (Rampelt et al., 2012). Another intriguing aspect of disaggregating proteins is that ClpB homologues are essential for reactivation of dormant, resilient bacilli, including the tuberculosis-causing pathogen *Mycobacterium tubercolsis* (Alam et al., 2021; Tripathi et al., 2020). Moreover, the ClpG disaggregase is considered a virulence factor of bacteria, as it provides the bacterium with extreme heat resistance, making it hard to kill during normal sterilization procedures, and is present on plasmids that can be transferred to other bacteria (Katikaridis et al., 2021). Together this makes the issue of disaggregases and their regulation of clinical importance.

2.1.3 SMALL HEAT SHOCK PROTEINS

One class of molecular chaperones that is driven by ATP-independent mechanisms, is the small HSPs (sHSPs; (Jakob et al., 1993). These proteins are found in all kingdoms of life and though quite diverse in structure they share features such as small size, presence of an α-crystallin domain and the ability to form large oligomeric complexes (Bentley et al., 1992; de Jong et al., 1993). In yeast there are two sHSPs, the constitutively expressed Hsp42 and the heat shock-induced Hsp26 (Haslbeck et al., 2004; Haslbeck et al., 1999). The sHSPs bind many different substrates and act to prevent their aggregation and terminal misfolding in an ATP-independent manner (Ehrnsperger et al.,

1997; Haslbeck et al., 2004; Jakob et al., 1993). These substrates are kept in sHSP:substrate oligomeric complexes and can then be refolded with the aid of Hsp70 and Hsp104 (Cashikar et al., 2005; Ehrnsperger et al., 1997; Haslbeck et al., 2005; Mogk et al., 2003). Hsp70 competes with sHsp for binding at the outer edge of sHsp:substrate assemblies and then recruits Hsp104 for further disaggregation and reactivation (Żwirowski et al., 2017). Hsp42 has been found to drive the formation of aggregates, both under heat stress and during early aging in yeast, using its N-terminal prion-like domain (Escusa-Toret et al., 2013; Grousl et al., 2018; Saarikangas and Barral, 2015; Specht et al., 2011). Interestingly *in vitro* Hsp42 was shown to have aggregase function and also to give a fitness advantage to yeast growing at 37°C (Escusa-Toret et al., 2013; Ungelenk et al., 2016). Hsp26 on the other hand was found to be more effective in post-stress reactivation of substrates (Ungelenk et al., 2016).

2.2 THE UBIQUITIN-PROTEASOME SYSTEM

Protein levels can be regulated both by adjusting transcription and translation, but also by adjusting the degradation rate. Degradation of a protein is part of the normal turnover, but can also degrade misfolded proteins as part of the temporal PQC. The 26S proteasome is the proteolytic machinery responsible for the majority of protein degradation in the cell and consists of the 19S regulatory particle, responsible for recognition and partial refolding of substrates, and the 20S catalytic core, which proteolytically degrades substrates, ultimately releasing amino acids (Beck et al., 2012; Finley, 2009; Rock et al., 1994). Substrates are targeted to the 26S proteasome by addition of a polyubiquitin tag to the substrate, which is recognized by the 19S regulatory particle (Chau et al., 1989; Lam et al., 2002). These two processes are referred to as the ubiquitin-proteasome system (UPS). The process of ubiquitination starts as a ubiquitin molecule is activated by an E1 enzyme, then becomes transferred to an E2 conjugating enzyme which binds to an E3 ubiquitin ligase that transfers the activated ubiquitin onto the substrate (Finley, 2009; Haas et al., 1982). After initial ubiquitination an E4 enzyme is required to extend the ubiquitin chain, as a minimum of four linked ubiquitin molecules are required for proteasomal degradation (Hwang et al., 2010a; Koegl et al., 1999; Thrower et al., 2000).

In yeast there are 43 E3 ubiquitin ligases which contribute to the broad substrate specificity of UPS (Lee et al., 2008). For cytosolic misfolded substrates, Rsp5, Hul5, Ubr1 and San1 all contribute to proteasomal targeting (Eisele and Wolf, 2008; Fang et al., 2014; Fang et al., 2011; Heck et al., 2010). Substrate recognition can be done directly by E3 ligases exemplified by Rsp5

and heat-denatured proteins and by Doa10 and exposed acetylated N-terminal methionine residues (Fang et al., 2014; Hwang et al., 2010b). There is also cooperation between cytoplasmic chaperones Ydj1, Ssa1, Ssa2 and E3 ligases for recognition and ubiquitination of substrates (Fang et al., 2014; Heck et al., 2010; Park et al., 2007).

Deubiquitinating enzymes are also part of the UPS. Rpn11 and Ubp6 are present in the 19S regulatory particle where Rpn11 acts to promote degradation by removing the ubiquitin chain, while Ubp6 delays proteasomal degradation, allowing removal of the polyubiquitin chain (Hanna et al., 2006; Verma et al., 2002). Overproduction of another deubiquitinating enzyme, Ubp3, can suppress temperature-sensitivity in a yeast strain lacking both cytosolic Hsp70, Ssa1 and Ssa2, by rescuing misfolded proteins from proteasomal degradation and allowing them to refold instead (Öling et al., 2014). Ubp3 together with Ubp2 also modulate the action of E3 ligase Rsp5, allowing for preferential formation of K48-linked ubiquitin chains, rather than K63-linked chains, thus promoting proteasomal degradation over endocytosis (Fang et al., 2016).

As mentioned above, the proteasome is part of normal protein turnover as well as degradation of misfolded proteins. Nascent chains exiting the ribosome are constantly monitored and some peptides are even ubiquitinated co-translationally by the 60S ribosome-associated E3 ligase Hel2. These peptides are associated with inefficient co-translational folding (Duttler et al., 2013). The other 60S ribosome-associated E3 ligase Ltn1 is part of RQC and is important for ubiquitination of nonstop polypeptides (from mRNA lacking polyA tails), and polypeptides arising from stalled or arrested translation events (Brandman et al., 2012; Matsuda et al., 2014). The importance of RQC is evident as deleting *LTN1* causes accumulation of aggregates (Choe et al., 2016). Protein turnover by the proteasome has also been found to be important during acute starvation as this process produces free amino acids for sustained translation before the lysosomal degradation system is activated (Müller et al., 2015; Vabulas Ramunas and Hartl, 2005).

The proteasome system in yeast is adversely affected by aging. Proteasomes isolated from old yeast cells are as efficient *in vitro* as proteasomes isolated from young yeast cells. However, the proteasome activity *in vivo* in old cells, is less than that of proteasomes in young cells (Andersson et al., 2013). This suggests that there are factors present in the cell but not part of the proteasome, that affect proteasome activity adversely upon yeast cell aging. Inhibiting or further activating proteasome was found to increase or decrease age-related protein aggregation respectively. Additionally, overproduction of Hsp104 both rescued the *in vivo* proteasome activity and lessened the protein aggregate

burden in old cells (Andersson et al., 2013). Misfolded proteins themselves also negatively affect proteasome capacity. This has been shown both in mammalian cell culture and in the brains of AD patients (Bence et al., 2001; Keck et al., 2003).

2.3 AUTOPHAGY

The cell has other means than the UPS system to degrade material, both selectively or indiscriminately, via autophagy. The autophagic process entails the formation of a double membrane vesicle called an autophagosome, around either random cytoplasm or select targets. The autophagosomes then fuse with the vacuole, releasing an autophagic body into the vacuole lumen, where it is degraded by resident proteases. Autophagy can be induced by starvation in yeast and autophagosomes will start to accumulate in the cytosol and their degradation by the vacuolar proteinases contributes to cell survival during starvation (Baba et al., 1994; Takeshige et al., 1992; Teichert et al., 1989). Formation of the autophagosome starts at a site called the phagophore assembly site (PAS) which has been found to localize close to ER and vacuoles (Graef et al., 2013). Initiation of phagophore expansion and autophagosome biogenesis is supported by Atg9-vesicles and COPII vesicles emerging from ER exit sites (Graef et al., 2013; Ishihara et al., 2001; Lemus et al., 2016; Mari et al., 2010; Shima et al., 2019; Suzuki et al., 2013; Yamamoto et al., 2012). Intriguingly, the organelles lipid droplets have been dismissed as membrane source for phagophore expansion, but rather influence autophagy by affecting ER homeostasis (Velázquez et al., 2016). The acyl-CoA synthetase Faa1 drives phagophore expansion by shifting activated fatty acids into *de novo* phospholipid synthesis (Schütter et al., 2020). Later on the phagophore membrane will close in a membrane scission event mediated by the ESCRT machinery which is recruited to the phagophore membrane, forming the sealed autophagosome (Zhou et al., 2019). The outer membrane of the autophagosome will then fuse with the vacuole, a process which requires the HOPS tethering complex as well as the SNARE (Soluble *N*-ethylmaleimide-sensitive factor Attachment protein Receptor) proteins Ykt6, Vam3, Vam7 and Vti1 (Bas et al., 2018).

Regulation of autophagy induction impinges on TORC1 signaling; treating cells with the TORC1-specific inhibitor rapamycin induces autophagy, even though the cells are not experiencing starvation (Noda and Ohsumi, 1998). Later studies found that TORC1-mediated phosphorylation of the autophagy component Atg13 prevents its association with Atg1, a serine/threonine kinase that increases its activity under starvation and rapamycin treatment and is

required for autophagy induction (Kamada et al., 2000; Kamada et al., 2010; Matsuura et al., 1997). TORC1 signaling is regulated by input from amino acid availability via the vacuole-associated complex EGO (EGOC). EGOC consists of Ego3, Ego2, Ego1, Gtr1 and Gtr2 and activates TORC1 signaling once activated itself by Vam6 (a component of the tethering complex HOPS; Binda et al., 2009; Dubouloz et al., 2005; Gao and Kaiser, 2006; Hirose et al., 1998; Kira et al., 2015; Sekiguchi et al., 2001). EGOC in turn is regulated by the SEA complex, made up of SEACIT and SEACAT. SEACIT acts as a sensor of amino acid levels as well as an inhibitor of EGOC (Neklesa and Davis, 2009; Panchaud et al., 2013a). The other part of the SEA complex, SEACAT consists of Sea2, Sea3, Sea4, Seh1 and Sec13 and acts as an antagonist of SEACIT, thereby positively influencing TORC1 signaling (Panchaud et al., 2013b). TORC1 and EGOC components Gtr1 and Ego1 are found both at the vacuolar membrane as well as at endosomes (Hatakeyama et al., 2019; Sturgill et al., 2008). The localization of Tor1 to either the vacuole or endosomes was found to be affected by whether Gtr1 was bound by GTP or GDP, suggesting that its localization can also be part of TORC1 activation (Kira et al., 2015).

Autophagy appears to have a role in PQC as well. Studies have found a significant contribution of autophagy in the clearance of both heat-induced, aggregated proteins, expanded polyQ proteins and the protein implicated in Parkinson's disease, α-synuclein (Lu et al., 2014; Petroi et al., 2012). There is also a connection between the UPS and autophagy. The yeast protein Cue5 can recognize ubiquitinated proteins that are then tagged with Atg8, targeting them for destruction via autophagy. The effect of Cue5 on autophagic clearance was particularly strong for high molecular weight protein assemblies such as aggregated proteins (Lu et al., 2014).

2.4 LIMITING FACTORS IN PROTEIN FOLDING UNRELATED TO CHAPERONES

One conclusion that can be drawn from the above description of the temporal protein quality control system, is that there should be redundancy in the system. The yeast cytosolic Hsp70 genes, *SSA1*-4, have the capacity to broadly compensate for each other and it was established early on that while the entire family is essential for viability, the individual members are not (Werner-Washburne et al., 1987). A large-scale screen of gene duplications resulting from both whole-genome duplication and small-scale duplication events, found that while there are examples of preserved redundancy, there are also duplications where redundancy is a transient mode as most paralogs have lost their functional redundancy (Li et al., 2010). Thus there are mechanisms both

for decelerating drift from redundancy as well as for loss of redundancy, both of which can be seen for the *SSA* family (Andersson et al., 2021). Functional redundancy is also found in the regulation of stress-induced transcription. The promoters of several stress-responsive genes have the binding elements for both Hsf1 (heat shock elements, HSE) and Msn2 and Msn4 (stress-responsive elements, STRE; (Treger et al., 1998). Intriguingly, Msn2 and Msn4 are the result of a gene duplication where partial redundancy has been kept, while their role in gene regulation has been further refined. Studies have found that fast induction of genes can be done by either Msn2 or Msn4, while others that are induced more slowly require binding of both (AkhavanAghdam et al., 2016). Further refinement is achieved by Msn2 being constitutively expressed, while Msn4 is stress-induced, allowing for fine-tuned expression of Msn2/4 target genes (Chapal et al., 2019). There is also a redundancy in the Hsp40 cochaperone family. Introduction of several different J domain constructs were able to rescue the growth defect of *ydj1* mutant cells. However, similar to the partial redundancy seen in the *SSA* family, some of the Hsp40s, e.g. Sis1, could not be rescued in a similar fashion, suggesting a unique function for *SIS1* in the cell (Sahi and Craig, 2007).

One of the major benefits of research in yeast, is the feasibility of doing genome-wide screens. Screens have been performed to identify components important for and also affecting PQC aspects, such as protein folding during normal growth, inclusion formation and asymmetric inheritance and modulation of Htt25QP toxicity (paper I, paper II, Babazadeh et al., 2019; Berglund et al., 2017). Despite addressing processes central to PQC, no hits or enrichment of hits are of the above-mentioned chaperone systems, UPS or autophagy. Moreover, it was found that deletion of single chaperones did not cause an increase in Hsf1 activity (Brandman et al., 2012). Similarly, a genome-wide screen for degradation of misfolded proteins (cytosolic CPY with a point mutation, and VHL) showed that no single chaperone or cochaperone deletion (with the exception of *SGT1*) influenced degradation of the substrates (Eisele et al., 2021). Together, these studies indicate strong redundancy in the temporal protein quality system. However, it also suggests that the hits that were in fact identified should be considered a part of protein quality control.

Tail-anchored (TA) proteins have a transmembrane domain (TMD) in their C terminus and around 50 TA proteins can be found in yeast (Beilharz et al., 2003). The C-terminal TMD acts as a ER membrane sorting signal and these proteins are targeted for insertion into the ER membrane via a SRP/Sec61-independent mechanism (Yabal et al., 2003). Instead, another mechanism where Arr4/Get3 binds and shuttles TA proteins to the ER membrane receptors

Get1/Mdm39 and Get2/Rmd7 for insertion, has been described (Schuldiner et al., 2008). As TA proteins are released from the ribosome, they are captured by Ssa1 together with Ydj1. The TA protein is then passed on to Sgt2, aided by Ydj1 and Sis1, and later on transferred to Get3/Arr4, with the aid of the GET components Get4 and Get5 (Cho and Shan, 2018; Cho et al., 2021; Wang et al., 2010). Get3/Arr4 has previously been shown to act as an ATP-independent holdase during glucose starvation (Powis et al., 2013). During glucose starvation Get3-GFP colocalizes with several known chaperones including Hsp42, Hsp104. Sis1 and Ssa2 (Powis et al., 2013). In Paper II we identified the GET pathway components *GET1*/*MDM39*, *GET2*/*RMD7* and *GET3*/*ARR4* as limiting factors for protein folding in cells growing in rich medium at normal growth temperatures, as their deletion caused accumulation of Hsp104-GFP foci. We found that the proteins forming foci were not only TA proteins, suggesting an overall effect on the folding capacity of the cell. Moreover, deleting the identified GET components made yeast cells more sensitive to expression of the human disease-causing proteins Aβ42 and Htt103Q (paper II). Thus, pathways that act beyond protein synthesis and protein folding, e.g. the GET pathway, need to be considered as they will have a great impact on the protein status of the cell without being implicitly involved in protein folding per se.

There are several TA proteins in yeast and they include the majority of SNARE proteins (Beilharz et al., 2003; Borgese et al., 2003). SNARE proteins are central in vesicle trafficking as they mediate the final fusion step between vesicles and target membranes. One such SNARE is the Golgi-related Sed5, which mediates transport between ER and Golgi and intra-Golgi transport (Banfield et al., 1995; Hardwick and Pelham, 1992; Mollard et al., 1997; Parlati et al., 2002; Sacher et al., 1997; Søgaard et al., 1994). Sed5 was found to be limiting in clearance of heat shock-induced protein aggregates (Babazadeh et al., 2019). When *SED5* was overexpressed aggregates were cleared faster, despite no general increase in chaperone levels. This clearance was dependent on functional Hsp104 and Pep4, suggesting a role for the vacuole in this yeast strain. The authors also found that mutants of vesicle trafficking components were more sensitive to treatment with AZC, a proline analog that induces protein misfolding as it is incorporated in growing peptide chains (Babazadeh et al., 2019). These studies suggest that there are indeed processes in the cell which have a great impact on temporal PQC, without being directly related to chaperones or degradation systems (figure 3).

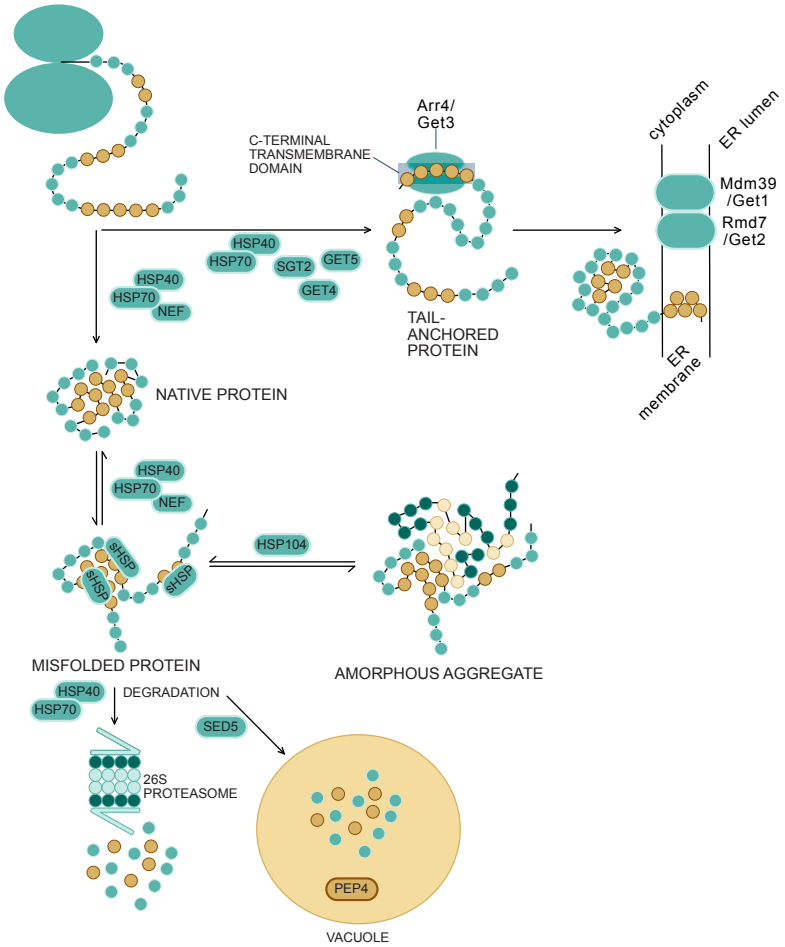

Figure 3. Temporal protein quality control extended to include the GET pathway which is a bottle neck in protein folding in basal conditions and Sed5, shown to promote protein degradation dependent on Pep4.

3 SPATIAL PROTEIN QUALITY CONTROL

Whether as a part of normal protein production and life, or as the result of disease or environmental stress, misfolded and aggregated proteins will be present in the cell. As described above, there are mechanisms that act to prevent aggregate formation, by disaggregation, refolding or degradation. When these systems do not manage to keep the proteome properly folded, during aging, disease or stress, spatial protein quality control acts to neutralize the damaged proteins, preventing further damage.

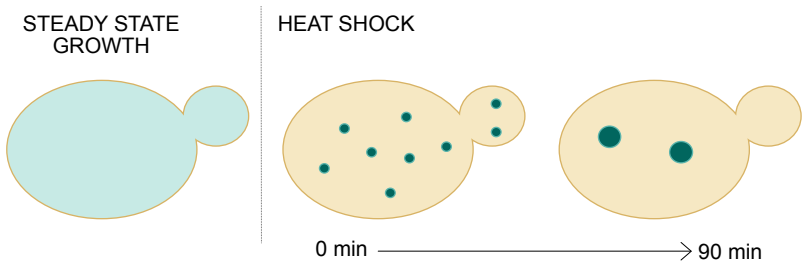

Figure 4. Inclusion formation upon heat shock can be tracked using Hsp104-GFP (green) as a reporter of aggregated protein. Hsp104-GFP is evenly distributed in the cytosol in unstressed cells. Immediately upon heat shock, Hsp104-GFP will redistribute to several cytosolic foci. After 90 min of heat shock, the foci will have fused to 1-2 inclusions.

As described above, protein misfolding can be triggered by exposing yeast cells to heat stress. During such stress, misfolded proteins first appear as small cytoplasmic foci (referred to as stress foci in this thesis) that are then sorted to inclusion sites in the cell (Escusa-Toret et al., 2013; Miller et al., 2015; Specht et al., 2011; Spokoini et al., 2012). Inclusion formation can be tracked using Hsp104-GFP as a reporter of aggregated proteins (figure 4). In an unstressed cell Hsp104-GFP is homogenously distributed in the cytosol. As the cells are exposed to a continuous heat shock of 38°C, Hsp104-GFP will first form several small foci throughout the cytosol, but after 90 min these foci will have coalesced into 1-2 inclusions (Babazadeh et al., 2019; Hill et al., 2016).

Two inclusion sites, localizing at or in the nucleus (juxtanuclear/intranuclear quality control compartment; JUNQ/INQ) as well as at the vacuole (insoluble protein deposit; IPOD) for damaged proteins have been described in yeast (Kaganovich et al., 2008; Miller et al., 2015). Aggregate sorting to the surface of mitochondria has also been seen (paper II, Babazadeh et al., 2019; Ruan et al., 2017; Zhou et al., 2014). Additionally, an age-specific inclusion site forms

early on in replicatively aged yeast cells, separate from the above-mentioned inclusion sites (Saarikangas and Barral, 2015). Amyloidogenic proteins are also sorted to a peripheral site that overlaps with the IPOD described for non-amyloidogenic substrates (Kaganovich et al., 2008). However, amyloidogenic sorting does not rely on the same mechanisms, as there is no reliance on stress foci formation or the same sorting factors as seen for non-amyloidogenic proteins (Escusa-Toret et al., 2013; Song et al., 2014; Specht et al., 2011) Inclusion sites or inclusion bodies (IBs) are also found in higher organisms, e.g. the pericentriolar aggresome which was first described in human embryonic kidney cells and Chinese hamster ovary cells (Johnston et al., 1998). Moreover, extracellular IBs are characteristic of human protein conformational disease (Chiti and Dobson, 2017), showing that IB formation is a human phenomenon as well.

Misfolded proteins can cause a debilitating loss of function, but of equal importance is the toxic gain of function, seen for example in neurodegenerative disease (Winklhofer et al., 2008). Misfolded proteins can also partake in aberrant interactions, both with other proteins as well as with cellular membranes (Bode et al., 2017; Fusco et al., 2017; Olzscha et al., 2011). Experimental evidence supports a cytoprotective role for inclusions and IBs in both experimental set ups and in disease. Indeed, the presence of IBs in neurodegenerative disease does not necessarily correlate with disease severity. Presence of extracellular plaques in patients with AD did not correlate with disease severity and there are elderly people with AD-related lesions in their brain, but who do not show signs of dementia or cognitive impairment (Bennett et al., 2006; Terry et al., 1991). The same was found for IB formation as the HD-related protein Htt was expressed in neurons. Arrasate and colleagues found no correlation between IB load and neuronal death, but instead a significant negative correlation between levels of soluble polyQ-expanded exon 1 of Huntingtin ($Htt^{ex1}Q_{47}$) and life span (Arrasate et al., 2004). Also, studies have shown that neuronal toxicity is strongly associated with an epitope, specifically found in monomeric and small oligomers of mutant Htt and not in higher molecular weight forms (Miller et al., 2011). Looking specifically at soluble compared to insoluble Htt oligomers, soluble Htt oligomers erroneously interact with more proteins than its insoluble Htt inclusion counterpart, indicating a mechanism for the toxicity of soluble Htt oligomers (Kim et al., 2016). However, insoluble Htt inclusion interactions are enriched for PN components, e.g. chaperones and ubiquitin-proteasome system (UPS) components (Kim et al., 2016). Similarly, toxicity of Aβ in *C. elegans* did not correlate with high molecular weight aggregates, but rather with small oligomeric species (Cohen et al., 2006). Inhibiting aggresome formation in mammalian cells was found to increase toxicity of polyQ-expanded proteins

and aggresome formation increased turnover of the misfolded protein (Taylor et al., 2003). Studies have also found that amyloid filaments (the species expected to be sorted to IBs) are in fact incapable of seeding further misfolding of native proteins, while soluble, oligomeric species were capable of such seeding (Mulaj et al., 2014). Fibril formation is, however, not conclusively beneficial. Studies making use of a model substrate capable of forming amyloid-like fibrils, found that the same peptide could form two very similar fibril species, one cytotoxic and the other one not. The cytotoxic species was found to expose more hydrophobic patches and could penetrate cellular membranes (Campioni et al., 2010). Likewise, α-synuclein fibrils can exert toxicity in neuronal cells, both by causing intercellular spread of the toxicity and by releasing oligomeric species that in turn strongly compromise membrane function (Cascella et al., 2021). Thus, these data suggest that IBs could be beneficial and part of the cellular response to misfolded proteins, but also that high molecular weight species are capable of causing toxicity in certain disease settings.

Evidence from yeast further support an active and beneficial role of formation of stress foci and inclusion formation. In cells lacking cytosolic Hsp70, misfolded proteins are ubiquitinated in a Doa10-dependent manner and their sorting to IBs prevents proteostasis collapse in Hsp70-deficient cells (Ho et al., 2019; Shiber et al., 2013). Limiting the Hsp40 Sis1 in yeast caused stabilization and foci formation of a short-lived GFP protein, which could still be degraded by the proteasome upon prolonged incubation (Summers et al., 2013). The toxicity of amyloidogenic model proteins, e.g. Htt103Q in yeast can be limited by sorting into IBs (Wolfe et al., 2013). Similarly, Sis1 counteracts yeast prion Rnq1 toxicity by promoting its assembly into [RNQ^{+}] amyloids (Douglas et al., 2008). Sorting of misfolded protein into IBs for later refolding also promotes cell survival by limiting protein degradation and energy waste in stressful conditions (Öling et al., 2014).

A study of aggregation-prone sequences in *E. coli* found that introduction of aggregation-prone peptide sequences could be bactericidal by inducing IB formation. IB formation in *E. coli* is not always followed by cell death and a comparison between lethal and non-lethal IBs found that lethal IBs consisted of a greater variety of different protein species, while non-lethal IBs generally contained few other proteins than the “core set” implicated in IB formation. This indicates that lethal IBs induce widespread protein aggregation. In contrast to prolonged antibiotic treatment which elicits resistance in the bacteria, the bacteria did not develop resistance to these aggregation-prone sequences. The aggregation-prone sequences were successfully used as

bactericidal agent in cocultures with HeLa cells and in a mouse bladder infection model, with high efficiency (Khodaparast et al., 2018).

Studies in the unicellular *S. cerevisiae* have been very useful in describing intracellular proteostatic processes and networks. However, an inherent limitation of this system is that no extracellular or intercellular processes can be studied. Instead, studies in the nematode *C.elegans* have proven useful in describing extracellular events. For example, it was found that neurons are capable of extruding a vesicle, termed exopher, containing misfolded and aggregated proteins, as well as dysfunctional mitochondria and lysosomes. The neurons that extruded exophers were healthier than those neurons that had not extruded any exophers in the same animal. Exopher extrusion could be induced by deleting *hsf-1* or by inhibiting the proteasome and autophagy. This indicates that exophers might be part of a proteostatic process (Melentijevic et al., 2017). Prions and oligomeric Aβ can also spread to other cells via vesicles and direct connections between cells (Nath et al., 2012; Nussbaum-Krammer et al., 2013). This suggests that there are important aspects of proteostasis that involve both extracellular and intercellular processes.

Another important aspect of inclusion formation is that it is necessary for the proper asymmetric inheritance of damaged proteins, i.e. when inclusions are not formed properly, the daughter cell will inherit damaged proteins (Aguilaniu et al., 2003; Erjavec et al., 2007; Hill et al., 2016; Spokoini et al., 2012; Zhou et al., 2014). This is of importance for cellular rejuvenation and lifespan and will be covered in chapter 4.

3.1 SORTING TO INCLUSION SITES

The sorting of aggregated proteins is summarized in figure 5. Initial sorting of heat-denatured proteins to stress foci upon mild heat stress seems to happen in close proximity of the cortical ER, with the aid of Hsp70 and Hsp42 (Escusa-Toret et al., 2013; Specht et al., 2011). A more severe heat shock can induce transient formation of foci containing Hsp82 and its cochaperone Sgt1, that are not dependent on Hsp42 for their formation (Eisele et al., 2021). Stress foci formation seems to be a balancing act between Hsp42 acting as an aggregase and promoting stress foci formation, and Hsp104 acting in stress foci resolution (Escusa-Toret et al., 2013). Cytosolic Hsp70s Ssa1 and Ssa2 are also important in stress foci formation, as *ssa1/2* double mutants cannot form stress foci, but rather display persistent IBs (Andersson et al., 2021; Öling et al., 2014; Shiber et al., 2013). The cytosolic Hsp70s are also crucial for recruitment of Hsp104 to the misfolded protein or aggregate, hence it is not surprising that the IBs formed in this double mutant persist (Glover and Lindquist, 1998;

Winkler et al., 2012). Stress foci formation with subsequent IB formation can be rescued in the *ssa1 ssa2* double mutant by overexpressing another Hsp70, *SSA4* (Andersson et al., 2021).

The two most studied inclusion sites in yeast are the nuclear JUNQ/INQ and the peripheral IPOD. JUNQ/INQ is associated with a high concentration of proteasomes and is dynamic, while IPOD, as the name suggests, appears to be a site for disposal of terminally damaged and misfolded proteins. The non-amyloidogenic misfolding reporter VHL was found at INQ/JUNQ, but could be partitioned to IPOD as well if the cells were exposed to heat stress in addition to VHL expression. Ubiquitination seems to be part of targeting misfolded proteins to either compartment, as inhibition of ubiquitination promoted IPOD deposition, while tagging a preferential IPOD substrate with ubiquitin was sufficient to deposit it at JUNQ/INQ (Kaganovich et al., 2008). Ubiquitination is however not an absolute indicator of sorting of non-amyloidogenic proteins to either INQ/JUNQ or IPOD, as ubiquitinated and non-ubiquitinated non-amyloidogenic misfolded proteins can be found at both INQ/JUNQ and IPOD (Miller et al., 2015; Shiber et al., 2013). Instead Hsp42, Btn2 and Sis1 appear to determine deposition to the different inclusion sites. Hsp42 and Btn2 together target misfolded proteins to peripheral sites (i.e. IPOD), while Btn2 and Sis1 together promote nuclear deposition (i.e. JUNQ/INQ) (Malinovska et al., 2012; Specht et al., 2011). The sorting is regulated by Cur1 binding to Sis1, which mediates its nuclear sequestration, leaving Btn2 free to bind Hsp42 and promote IPOD sorting (Malinovska et al., 2012). Another model of inclusion site sorting suggests that Hsp42 and Btn2 act as aggregases that promote IB formation in the cytosol and in the nucleus respectively (Miller et al., 2015). Aggregase function has also been described for Hsp17 in *C. elegans*, suggesting that aggregation and sequestration of protein aggregates are conserved mechanisms (Iburg et al., 2020).

Amyloidogenic proteins are targeted solely to IPOD (Escusa-Toret et al., 2013; Kaganovich et al., 2008; Specht et al., 2011). Amyloidogenic proteins do not form stress foci or rely on Hsp42 for their sorting to IPOD (Escusa-Toret et al., 2013; Song et al., 2014; Specht et al., 2011). Instead Hsp70 together with Sis1 as well as the Hsp90 cochaperone Sti1, are involved in amyloidogenic protein sorting to IPOD (Park et al., 2013; Wolfe et al., 2013). Also, cells that are deficient in RQC fail to sort Htt103QP to IBs (Yang et al., 2016).

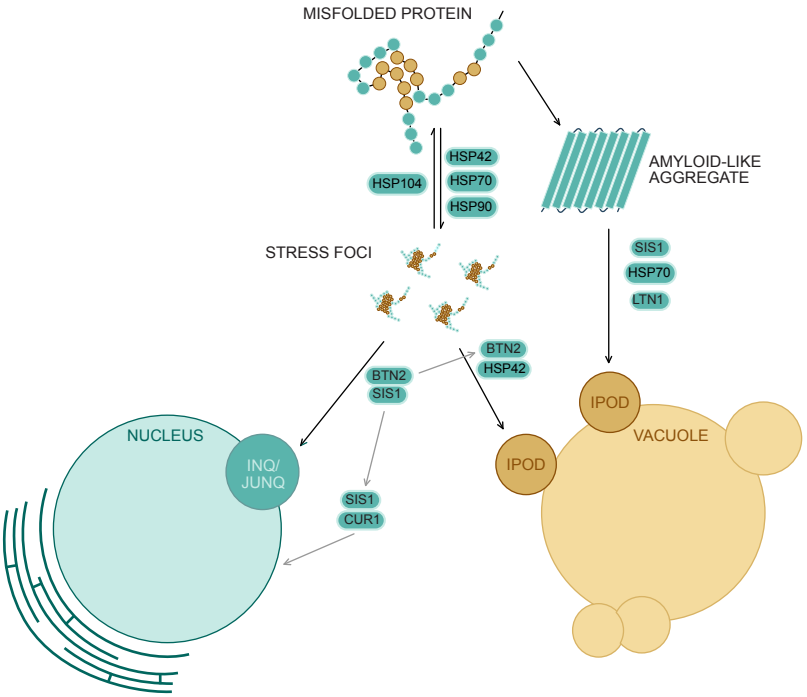

Figure 5. Spatial protein quality control. Immediately following heat shock misfolded proteins will form stress foci, a process mediated by Hsp90, Hsp70 and Hsp42. These stress foci can be resolved by Hsp104 or further sorted, either to the nuclear inclusion site INQ/JUNQ or the peripheral inclusion site, Btn2 and Sis1 are implicated in sorting to INQ/JUNQ, while Hsp42 is required for sorting to IPOD. Amyloidogenic misfolded proteins sort exclusively to IPOD but rely on different factors than non-amyloidogenic proteins

3.2 RESOLUTION OF INCLUSIONS

As mentioned above, it seems that sorting to INQ/JUNQ is a step towards degradation, as the site is enriched for proteasomes, while IPOD is less dynamic and misfolded proteins that go there are not processed further, as far as is known. It was noted that while INQ/JUNQ appeared as the cell experienced misfolding from expressing a single misfolding reporter, IPOD started to appear when the misfolding stress was increased by heat shock, or when amyloidogenic proteins were present (Kaganovich et al., 2008). This suggests that IPOD is a site used when the INQ/JUNQ capacity is overwhelmed, or when amyloidogenic proteins are present. Other studies have found that there is only very slow turnover of an amyloidogenic misfolding reporter as it is sorted to IPOD. This turnover was found to depend on Hsp104-mediated extraction of the misfolded protein from IPOD and subsequent

proteasomal degradation (Kumar et al., 2016). This might seem backwards as the location of IPOD next to the vacuole makes one intuitively think about autophagic degradation within the vacuole rather than proteasomal degradation. Moreover, proteins within the mammalian aggresome cannot be degraded by the proteasome and aggresome disposal occurs solely via autophagy (Fortun et al., 2003; Holmberg et al., 2004; Iwata et al., 2005; Taylor et al., 2003). The degradation of the ectopically expressed disease protein α-synuclein in yeast was found to rely on autophagy (Petroi et al., 2012). Autophagy has also been found to degrade ubiquitinated substrates via Cue5, suggesting that autophagy can be used when UPS is overwhelmed (Lu et al., 2014). Another interesting point is that the yeast vacuole displays multiple vacuolar invaginations after chronic heat stress, that are not dependent on autophagy components, but do require endosomal sorting proteins (Ishii et al., 2018). The consequences of these vacuolar invaginations were not further examined in this study, but it does show that the vacuole and endosomal sorting can be part of a response to heat shock, perhaps even for degradation of misfolded proteins.

Several temporal PQC components have been shown to be important for INQ/JUNQ resolution. The deubiquitinating protease Ubp3 was shown to be required for efficient JUNQ clearance (Öling et al., 2014). The E3 ubiquitin ligase San1 is required for degradation of cytosolic proteins that are transported into the nucleus in a Sse1-dependent manner (Heck et al., 2010). Studies using electron microscopy have found that exposing yeast cells to heat stress, oxidative stress and protein misfolding stress, induces nuclear envelope budding (NEB) associated with misfolded proteins (Panagaki et al., 2021). These events increased in frequency upon inhibition of proteasome activity, suggesting that they are part of a response to limited proteasome capacity (Panagaki et al., 2021). Other systems promote inclusion resolution in the nucleus, as Btn2-dependent inclusions can either be refolded by a Hsp70-Sis1-Hsp104 system or degraded by a Hsp70-Apj1-Sse1 system (den Brave et al., 2020). Several studies have placed Sse1 in IB resolution as well as in stress foci clearance and disaggregation (den Brave et al., 2020; Escusa-Toret et al., 2013; Kaimal et al., 2017). These results are interesting as the other disaggregating component found in yeast, Hsp104, is not conserved in higher eukaryotes. This role for Sse1/Hsp110 in disaggregation and IB resolution in yeast shows that there might be a conserved mechanism for disaggregation in yeast and higher eukaryotes that is Hsp104-independent.

3.3 EXPANDING SPATIAL PQC TO INCLUDE VESICLE TRAFFICKING

VAC17 was found to be an asymmetry-generating gene (AGG) and also to be limiting in asymmetric inheritance (paper I, see chapter 4 for further discussion on asymmetric inheritance). The only previously acknowledged role for Vac17 is as part of a vacuole-binding complex together with Myo2 and Vac8, in vacuole inheritance (Ishikawa et al., 2003; Tang et al., 2003). Intriguingly, deletion of *VAC17* also caused an inclusion formation deficiency. The opposite was found when *VAC17* was overexpressed as inclusion formation, specifically to IPOD, was boosted. The effect of *VAC17* deletion and overexpression on inclusion formation was true for aggregates induced by both heat shock and aging. This inclusion formation required Vac17-Myo2 interaction as well as the trafficking components Vps1 (endosomal dynamin-like component) and Vps16 (a component of the two tethering complexes HOPS and CORVET). Thus, inclusion formation to IPOD appears to be influenced by *VAC17* through vesicle trafficking (paper I).

Deletion of all GET pathway components causes an inclusion formation deficiency (paper II). As mentioned above, *VAC17* overexpression can boost inclusion formation, so also in *get2*/*rmd7* mutant cells (paper III). This was used as a tool to further elucidate the role of *VAC17* in inclusion formation, by performing a genome-wide microscopy screen. The endosomal components Vps21 and Vps9 were absolutely required for *VAC17* overexpression rescue of *get2*/*rmd7Δ* inclusion formation and the vacuolar SNARE Vam3 was also required. This shows that *VAC17* acts through endosomal-vacuolar trafficking. Examination of Vac17 physical interactors found that endocytosis and actin-nucleating components bind to Vac17 (paper III). This could explain how Vac17 overproduction can increase endocytosis rate (paper I). In addition, prevalence of actin-regulating proteins and early endocytosis components at the vacuole is increased in cells that overproduce Vac17, further supporting a role for Vac17 in endocytosis and vesicle trafficking towards the vacuole (paper III). Further tests are however needed to mechanistically connect Vac17, endocytosis/actin nucleation regulation and inclusion formation. Interestingly, cells that are deficient in RQC, are deficient in Htt103Q sorting to IPOD, but are also suffering from slowed endocytosis rate (Yang et al., 2016).

Sorting of amyloid-like proteins to IPOD seems likewise to rely on the actin cytoskeleton and vesicle trafficking. By screening for proteins that bind the model amyloid-like substrate prion determining domain (PrD)-GFP, Kumar and colleagues found that the SNARE-related protein Sec18 as well as the actin

cable-binding tropomyosins, Tpm1 and Tpm2, all bound to PrD-GFP. Furthermore, Tpm1 and Tpm2 were specifically needed for PrD-GFP sorting to IPOD (Kumar et al., 2016). Tropomyosins enhance the movement of myosin V motor protein, e.g. Myo2, along actin cables (Hodges et al., 2012). Similar to Tpm1 and Tpm2, Myo2 was also found to be needed for PrD-GFP sorting to IPOD. PreApe1 recruitment to the phagophore assembly site (PAS), which is localized close to IPOD, was found to rely on the same factors, namely Tpm1, Tpm2, and Myo2 (Kumar et al., 2016). The spatial sorting of another amyloid-like protein, Htt103QP, also requires genes involved in Golgi-vesicle trafficking. The ability to sort the normally non-toxic Htt103QP into IBs was lost upon yeast aging (Yang et al., 2016).

Further evidence for vesicle trafficking in inclusion formation came from a genome-wide microscopy screen searching for genes required for proper inclusion formation upon heat shock (Babazadeh et al., 2019). Indeed, spatial analysis of functional enrichment (SAFE) showed a strong bias for genes involved in vesicle trafficking and a requirement of the COG complex for inclusion formation was identified. It was found the COG complex acts via the Golgi t-SNARE gene *SED5* and promotes inclusion formation. However, the inclusion formation was targeted at mitochondria rather than the nucleus or vacuole. *SED5* overexpression also affected the movement of Hsp104 in the cell, suggesting a possible link between Hsp104, which binds misfolded proteins (see above), and Sed5, a Golgi vesicle resident (Babazadeh et al., 2019).

The physical interactors of Hsp104 lend further strength to a role for vesicle trafficking in inclusion formation. The interactors are functionally enriched for gene ontology (GO) terms ER to Golgi transport, transport vesicle, vesicle, membrane-bound vesicle and cytoplasmic membrane-bound vesicle (paper I). These interactors were analyzed using SAFE (Babazadeh et al., 2019). Hsp104 was found to interact with components of the 26S proteasome as well as vesicle trafficking, specifically ER-Golgi and COPI/II vesicles while the cells were growing in no stress conditions. After shifting the cells to heat shock conditions, Hsp104 interacted with protein folding machinery in addition to the 26S proteasome and vesicle-trafficking components (Babazadeh et al., 2019). These studies suggest that the network commandeering inclusion formation should be extended to include Myo2-trafficking along actin cables, endosomal vesicle trafficking to the vacuole as well as Sed5 promotion of inclusion body formation at mitochondria (figure 6).

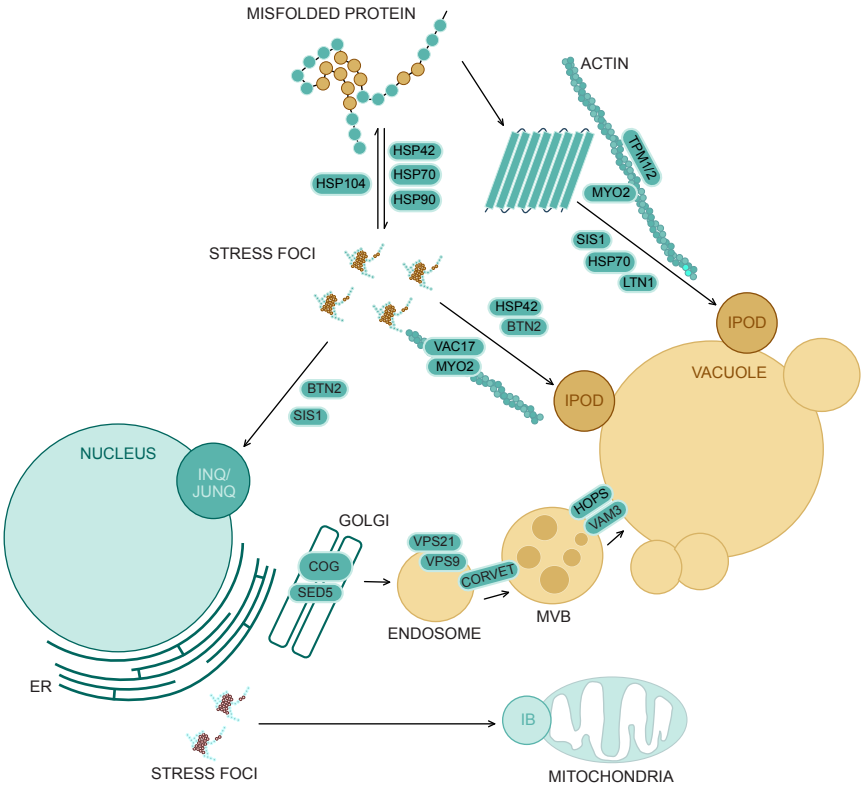

Figure 6. Spatial protein quality control extended to include Vac17-Myo2 transport along actin cables for non-amyloidogenic substrates to IPOD and Myo2- and Tpm1/2-mediated transport along actin cables for amyloidogenic substrates to IPOD. Vac17-mediated inclusion formation depends on the endosomal GTPase Vps21 and its activator Vps9 and the vacuolar SNARE Vam3. The COG tethering complex and the Golgi SNARE Sed5 promote inclusion formation at the mitochondria.

4 ASYMMETRIC INHERITANCE

As mentioned previously, early work found that a cytoplasmic factor that is unevenly distributed between yeast mother and daughter cells, contributes to aging (Egilmez and Jazwinski, 1989). No matter the age of the mother cell, mechanisms act to asymmetrically retain the cytoplasmic aging factor(s) within the mother, yielding daughter cells of full replicative capacity. It is not until very late in the mother cell life span that the asymmetrical retention breaks down, consequently shortening daughter cell life span (Kennedy et al., 1994). Later work identified several asymmetrically inherited aging factors (see chapter 1), including misfolded and damaged proteins, and asymmetric inheritance was also found to be essential for population fitness (Aguilaniu et al., 2003; Erjavec et al., 2008).

Sir2, in addition to its role in ERC production and lifespan, was also established as an essential component in generating asymmetry of damaged proteins, together with actin and Hsp104 (Aguilaniu et al., 2003; Erjavec et al., 2007; Tessarz et al., 2009). As damage asymmetry influences cellular fitness and *sir2Δ* cells have symmetrical inheritance of damaged proteins, the negative genetic interactions of *SIR2* were examined (Liu et al., 2010; Song et al., 2014). A negative genetic interaction is a fitness defect in a double mutant that is more severe than what is expected from each of the two single mutants. The negative genetic interactions of *SIR2* among non-essential genes, were found in actin-related processes, e.g. in networks concerning the polarisome (Liu et al., 2010). Aggregate retention was also shown to require the action of the polarisome, working through mechanisms involving actin, actin binding to Myo2, and tropomyosin Tpm1. In support of this view Hsp104-associated aggregates were found to associate with actin in cells. Daughter cells were also found to be able to clear themselves of protein aggregates via a retrograde aggregate transport back to the mother cell (Liu et al., 2010).

This description of aggregate retention and asymmetric inheritance as an active and factor-dependent process was disputed by researchers that claimed aggregate movement was best described by factor-independent movements with little confinement, which together with the yeast cell geometry best explained aggregate retention (Zhou et al., 2011). However, multiple points argue against random movement of aggregates (Liu et al., 2011). Firstly, protein aggregates associate with several cellular constituents, i.e. actin (Liu et al., 2010; Liu et al., 2011), the nucleus and the vacuole (Kaganovich et al., 2008; Spokoini et al., 2012), suggesting that random movement should be limited. These associations have all been shown to be factor-dependent (Liu et

al., 2011). Another point against random aggregate diffusion and cell geometry as aggregate retention mechanisms, was described for a yeast strain with deficient retention. The retention deficiency could only be explained by random movements and cell geometry if the bud neck was larger in these cells. However, the bud neck size was equal to that of wild type cells (Song et al., 2014). In addition, later publications from the authors of the random diffusion theory have provided evidence for a factor-dependent retention of aggregates, by showing that protein aggregate association to mitochondria is key to asymmetrical inheritance (Zhou et al., 2014).

The formation of inclusions is important for yeast proteostasis in detoxifying potentially dangerous protein conformers (see chapter 3). In addition, inclusion formation is important in establishment of aggregate retention and damage asymmetry (figure 7). One of the known asymmetry-generating genes, *HSP104*, was found to be required for stress foci to coalesce into IBs (Erjavec et al., 2007; Spokoini et al., 2012). When *HSP104* was deleted, stress foci would persist and were then symmetrically divided between the mother and daughter cell upon cell division (Spokoini et al., 2012). Hsp104-associated protein aggregates have also been found in close proximity to mitochondria (paper II, Babazadeh et al., 2019; Zhou et al., 2014). Disruption of this association caused more aggregates to leak into the daughter cell, mainly those that were unbound to mitochondria (Zhou et al., 2014). The interaction between mitochondria and protein aggregates was found to decrease with age (Zhou et al., 2014). This could indicate that part of the breakdown of asymmetry observed as yeast cells age, could be explained by reduced interaction between aggregates and cellular structures, such as mitochondria. As mentioned above, heat-induced protein aggregates (Liu et al., 2010; Liu et al., 2011) as well as the amyloid-like aggregates of Htt103Q and Htt103QP (Song et al., 2014; Yang et al., 2016) have been shown to associate with the actin cytoskeleton, likely contributing to both inclusion formation and aggregate retention. The actin cytoskeleton is also affected by aging and studies in *C. elegans* found that it loses integrity and structure as worms age (Higuchi-Sanabria et al., 2018). In addition, boosting the stability of actin cables prolonged the lifespan of worms (Higuchi-Sanabria et al., 2018).

Asymmetric inheritance and retention of misfolded proteins have been shown in multicellular eukaryotes as well. Aggresome formation in hamster lung cells and human embryonic kidney (HEK293) cells does not affect mitosis and allows for asymmetric inheritance of polyQ-expanded Htt protein by only one daughter cell. The daughter cell that inherited the damaged proteins underwent morphological changes and did not continue to divide, while the damage-free daughter cell went on to divide again (Rujano et al., 2006). Asymmetric

distribution of oxidatively damaged proteins upon cell division has also been seen for three stem cell types in *Drosophila melanogaster*, as damage was retained by the cell predicted to have the shortest chronological life span (Bufalino and van der Kooy, 2014).

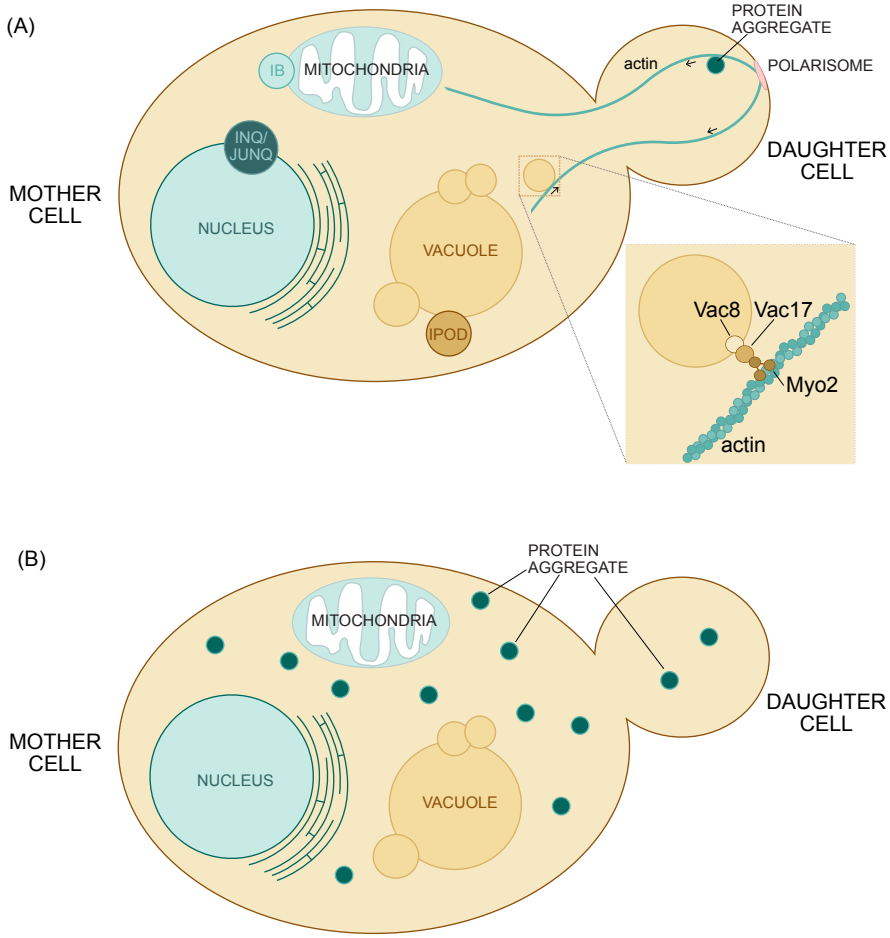

Figure 7. (A) Asymmetric inheritance of damaged proteins is established with proper inclusion formation and depends on Sir2, Hsp104 as well as actin, Myo2 and Tpm1. Also, daughter cells have the capacity to clear themselves of aggregates via retrograde actin cable movement emerging from the polarisome, redistributing protein aggregates back to the mother cell. (B) If inclusion formation is disturbed aggregated proteins are inherited symmetrically by both the mother and the daughter cell.

4.1 ASYMMETRY REQUIRES VESICLE TRAFFICKING AND CYTOSKELETON REGULATION

In paper I we set out to describe the genes that are involved in establishing asymmetry upon cell division in yeast, so-called AGGs. The biological processes identified were organelle inheritance, endosome transport, autophagy and vesicle-mediated transport. Moreover, *VAC17* was identified as an AGG and was found to be limiting for damage asymmetry. Similar to its role in spatial PQC, Vac17 acts through vesicle trafficking, as deletion of the genes *VPS1* and *VPS16* both caused asymmetry defects and *VAC17* overexpression could not rescue this (paper I).

Extending the genetic interaction network of *SIR2* to include essential genes, it was found that *SIR2* has negative genetic interactions with genes in actin polarity, actin folding and actin nucleation (Song et al., 2014), similar to what was described for the non-essential genes (see above; Liu et al., 2010). When these negative genetic interactors were screened for asymmetry defects, 40% were found to be less efficient in maintaining mother-daughter asymmetry. Specifically, *CMD1*, *MYO2*, and *SEC18* were all found to genetically interact with *SIR2* and affect damage asymmetry both for heat-induced and amyloidogenic aggregates. Colocalization experiments show that Htt103Q colocalized to a great degree with Cmd1 and Myo2, suggesting a physical interaction between these factors and aggregates which could explain their role in upholding mother-daughter asymmetry (Song et al., 2014). These results show that, similar to inclusion formation, actin, Myo2 and vesicle trafficking components are of importance in establishing mother-daughter asymmetry.

5 PQC, AGING AND LIFESPAN

Aging affects the PQC system and protein folding, causing damaged and misfolded proteins (see chapter 1.1). This is problematic for the cells, not only in terms of loss of protein function and production of aberrant protein-protein interactions, but also as the chronically induced stress response elicited by constitutive presence of a misfolded protein, is detrimental for the cell (Roth et al., 2014). Looking at the *C. elegans* proteome quantitatively one can see that approximately a third of the proteome is altered during aging. Specifically, 19S and 20S proteasome subunits and other UPS components were upregulated, which was reflected in increased proteasome activity *in vitro*. The Hsp70 and Hsp90 systems including their co-chaperones were largely unchanged, while sHSPs were forcefully induced (Walther et al., 2015). Studies of proteins that are insoluble in young and old worms showed that a lot of proteins become insoluble in the aging process, with an overrepresentation of proteins involved in proteasomal degradation, ribosomes, chaperones, sHSPs and chaperonins (David et al., 2010). PQC components can be sequestered in protein aggregates, e.g. the essential Hsp40 Sis1 becomes sequestered both in aggregates resulting from faulty RQC as well as in aggregates caused by ectopic expression of polyQ aggregation prone proteins (Choe et al., 2016; Olzscha et al., 2011; Park et al., 2013; Yu et al., 2014). The Hsp70 Ssa1 and the Hsp40 Ydj1 were shown to be less mobile in aged yeast cells, which was linked to increased cell cycle length and entry into senescence prior to cell death (Moreno et al., 2019). Aspects of spatial PQC are also affected by aging, as aged yeast cells fail to form inclusions of polyQ-expanded Htt103QP (Yang et al., 2016). An inducible mouse model of the protein conformational disease spinocerebellar ataxia 17 (SCA17), showed that disease symptoms developed faster in aged mice, concomitant with a decrease in chaperone activity and Hsc70 levels (Yang et al., 2014). Surprisingly, proteostasis capacity, detected as increased misfolding of temperature sensitive reporter alleles, changes early on in *C. elegans* adulthood. This happens at the same time as the capacity to mount strong anti-stress responses declines (Ben-Zvi et al., 2009).

Other examples of the impact of aging on PQC include rat and rhesus monkey splenic lymphocytes: the ability to induce Hsp70 expression upon heat shock and HSE binding capacity were both reduced with age in these model systems (Pahlavani et al., 1995). Age affects proteostasis in human cells as well. Studies in mononuclear blood cells, found that the ability to mount a HSR was decreased in cells isolated from middle-aged persons (Singh et al., 2006). The same was found for fibroblasts from aged humans as well as for senescent *in*

vitro cultured cells (Gutsmann-Conrad et al., 1998). Basically the reversed situation is reported for healthy centenarians; Epstein-Barr virus-transformed B-lymphocytes isolated from centenarians were capable of inducing Hsp70 expression to the same degree as those isolated from young persons (Ambra et al., 2004). Studies in yeast found that the stress response amplitude to osmotic stress decreased in yeast cells as they aged. Moreover, the stress response amplitude together with the cell cycle time could accurately predict cell senescence rate (Chen et al., 2020)

These effects of aging on protein folding status, PQC and the fact that damaged and misfolded proteins are an established aging factor, suggest that manipulation of PQC components could have an impact on the lifespan of yeast cells. Deletion of the cytosolic Hsp70s and Hsp104 reduces the median lifespan (Andersson et al., 2021; Erjavec et al., 2007). Interestingly, Hsp104 is not limiting lifespan, as Hsp104 overproduction did not extend lifespan (Andersson et al., 2013). In contrast, the peroxiredoxin Tsa1 which recruits Hsp104 to protein aggregates formed upon oxidative stress and aging, was found to be limiting for replicative lifespan (Hanzén et al., 2016). This lifespan extension was independent of Msn2 and Msn4, which are known to be required for lifespan extension induced by caloric restriction (CR) and rapamycin treatment (Hanzén et al., 2016; Medvedik et al., 2007).

CR is a classic means of extending the lifespan in yeast as well as in nematodes, flies, rats and monkeys (Colman Ricki et al., 2009; Kenyon, 2010; McCay et al., 1989). In yeast CR lifespan extension has been linked to nutrient signaling via both PKA and TOR, as deletion of signaling components in these pathways extend lifespan in a manner that cannot be further extended by CR (Kaeberlein et al., 2005; Lin et al., 2000). Deletion of ribosomal proteins can extend lifespan and, similar to CR and TOR inhibition, results in translation repression which depends on the transcription factor Gcn4 (Mittal et al., 2017; Steffen et al., 2008). Interestingly, while CR affects TOR signaling, CR induced by glucose starvation has been reported to have little effect on autophagy (measured as Cvt pathway maturation of Ape1). Moreover, most autophagy-related genes were found not to be required for glucose starvation CR-induced lifespan extension in yeast (Tang et al., 2008). This is in contrast to CR in *C. elegans* which extends lifespan in an autophagy-dependent manner (Hansen et al., 2008; Jia and Levine, 2007). Using another form of CR, induced by reducing amino acid concentration, also elicited a robust lifespan extension (Jiang et al., 2000). This indicates that the specific medium used for CR studies in yeast is of great importance, specifically the rich YPD medium used contains nitrogen sources that can have an effect on yeast metabolism despite containing CR concentrations of glucose. CR causes changes in gene expression and

ultimately the proteome is changed. Looking at the proteomic readout of CR in yeast, it is evident that CR induced by limiting glucose causes a shift towards mitochondrial respiration. CR also induces expression of the sHSP Hsp26 and overproduction of Hsp26 induced RLS extension in cells growing in normal, non-CR medium (Campion et al., 2021).

5.1 AUXILIARY SYSTEMS AFFECT LIFESPAN

As described above, Vac17 was identified as a limiting factor in asymmetric inheritance of damaged proteins while also influencing inclusion formation after heat stress and during aging (paper I, see chapter 3 and 4). Remarkably, overproduction of Vac17 also increased the replicative lifespan. This extension of lifespan was dependent on the cytosolic Hsp70s Ssa1 and Ssa2, Vac17 binding to Myo2 as well as the HOPS and CORVET subunit Vps16 (paper I). The role of Vac17 in lifespan extension was independent of its previously described role as a binding partner for Vac8, suggesting that the role of Vac17 in lifespan extension is independent of its role in vacuole inheritance (paper III).

Apart from the effects on inclusion formation, asymmetric inheritance and lifespan, Vac17 overproduction was also found to counteract age-induced vacuole fragmentation and decrease in endocytosis rate (paper I, Hughes and Gottschling, 2012; Tang et al., 2008). The increase in endocytosis rate induced by Vac17 overproduction was similarly independent of Vac8 and dependent on Vac17 binding to Myo2 (paper III). Moreover, Myo5 was found to physically interact with Vac17 and also to be required for the increased endocytosis rate induced by Vac17 overproduction. Another intriguing finding is that the vacuolar proteome changes upon Vac17 overproduction and several TORC1-regulating proteins are affected (paper III). Given the role of TORC1 in lifespan and CR-mediated extension of lifespan, a link between Vac17, TORC1 activity and lifespan deserves future attention.

Disruption of GET pathway components caused protein aggregation in almost 100% of the cells. However, despite this very strong aggregation phenotype, an effect on lifespan was only found for *arr4Δ* cells and not *rmd7Δ* cells. This suggests that the presence of protein aggregates is not the ultimate cause of lifespan reduction in *arr4Δ* cells, but is rather due to a different phenotype resulting from deletion of *ARR4* specifically (paper II).

6 MAIN FINDINGS

In this thesis I have argued for an expansion of what is generally considered to be components or processes involved in protein quality control, to include processes such a protein targeting (specifically via the GET pathway) and vesicle trafficking. This thesis is based on the following main findings.

Paper I

- Asymmetry Generating Genes (AGGs) are enriched in biological processes concerned with vesicle transport and cytoskeleton organization.
- Hsp104, which binds to aggregated proteins, also binds proteins in vesicle trafficking in heat shock conditions
- The vacuole inheritance component Vac17 is an AGG and limiting for protein aggregate inheritance.
- Vac17 binding to Myo2 is essential for asymmetric aggregate inheritance.
- Overproduction of Vac17 improves aggregate inheritance and inclusion formation, mainly at the vacuole.
- Overproduction of Vac17 robustly extends the replicative life span.

Paper II

- The GET pathway components *GET3*/*ARR4, GET1/MDM39* and *GET2/RMD7* are limiting factors for protein folding in unstressed conditions.
- Misfolding reporters Ubc9ts-GFP and gus1-3-GFP misfold to a greater extent in *GET3*/*ARR4* and *GET2/RMD7* mutant cells.
- Mutations in the *GET3*/*ARR4, GET1/MDM39* and *GET2/RMD7* genes render the cells more sensitive to expression of the disease-causing proteins Htt103Q and Aβ42.
- Increasing chaperone levels does not alleviate the aggregate load in *get2Δ/rmd7Δ* cells.
- The GET pathway efficiency is unchanged as cells age.

Paper III

- Overexpression of *VAC17* rescues inclusion formation in *rmd7Δ* cells.
- The rescue requires endosomal component genes *VPS21* and *VPS9*, as well as vacuolar SNARE gene *VAM3.*
- Vac17 physically interacts with components in early endocytosis and actin cytoskeleton regulation.
- Overexpression of *VAC17* changes the vacuolar proteome
- Endocytosis components and actin are found at higher concentrations at the vacuole when *VAC17* is overexpressed.
- TORC1 regulating components are less prevalent at the vacuole when *VAC17* is overexpressed.

7 FUTURE PERSPECTIVES

The aim of this thesis is to identify systems which despite not being considered a part of the established PN definition, have been found to greatly impact cellular management of damaged proteins. Vesicle trafficking has been identified by us and others as a system of particular importance in proteostasis, specifically in asymmetric inheritance and inclusion formation for both amyloidogenic and non-amyloidogenic misfolded proteins. Particularly Vac17 was defined as a limiting factor for inclusion formation, asymmetric inheritance and lifespan. The only previously described role for Vac17 is in vacuole inheritance during cell division, prompting the question as to how Vac17 is able to impact proteostasis and aging. Indeed, paper III has started exploring the role of Vac17 in inclusion formation and endocytosis, but several questions need to be addressed further. Given the finding that TORC1-regulating components are affected by the overexpression of *VAC17*, and the established role for TORC1 in regulation of lifespan, is the robust lifespan extension induced by *VAC17* overexpression caused by effects on TORC1 signaling? Alternatively, can the effects seen on endocytosis and inclusion formation account for the life span extension? Another interesting point is the new physical interactors found for Vac17, where several components of actin polymerization and early endocytosis were found. This could indicate a role for Vac17 in actin polymerization and regulation of the actin cytoskeleton, which in turn has been found to be important for both asymmetric inheritance and IB formation.

The GET pathway, previously only known for insertion of TA proteins into the ER membrane, was identified as a major limiting factor in protein quality control. Deletion of one of the three most downstream GET components caused extensive protein aggregation in nearly all cells. However, this protein folding bottleneck does not seem to contribute to protein misfolding and aggregate formation during aging, as GET pathway efficiency does not decrease as cells grow older. However, GET deletion mutants are more sensitive to stress and display increased Hsf1 activity, suggesting that the protein aggregates are indeed sensed by the cell and that the cell tries to dissolve them. These mutants are also more sensitive to the presence of disease-causing proteins, e.g. Aβ42 and Htt103Q. In chapter 2 I describe a chronic activation of the HSR called the maladaptive response, which renders the cells with less protein folding capacity. Could a similar phenomenon be seen in yeast GET mutants, causing them to be more sensitive to the constant presence of misfolded proteins? Another intriguing aspect is that lifespan is only affected in one of the two tested GET mutants, despite their shared

phenotype of prevalent protein aggregates. Indeed, while *arr4* mutant cells have a shorter lifespan than wildtype cells, *rmd7* mutants display the same lifespan as wildtype cells. This suggests that the protein aggregates caused by deleting either *ARR4* or *RMD7* are not the sole cause of the compromised lifespan seen in *ARR4* deletion cells. This suggests that there is something else resulting from *ARR4* deletion that is detrimental to aging and also that the misfolded proteins resulting from mutations in the GET pathway are not always proteotoxic.

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(from "Proud Corazón" by Adrian Molina and Germaine Franco with Swedish lyrics by Robert Cronholt for the movie "Coco")

REFERENCES

Abrams, JL, Verghese, J, Gibney, PA, and Morano, KA (2014). Hierarchical Functional Specificity of Cytosolic Heat Shock Protein 70 (Hsp70) Nucleotide Exchange Factors in Yeast*. Journal of Biological Chemistry, 289(19), 13155-13167.

Aguilaniu, H, Gustafsson, L, Rigoulet, M, and Nyström, T (2001). Protein Oxidation in G0 Cells of Saccharomyces cerevisiae Depends on the State Rather than Rate of Respiration and Is Enhanced in pos9 but Notyap1 Mutants*. Journal of Biological Chemistry, 276(38), 35396-35404.

Aguilaniu, H, Gustafsson, L, Rigoulet, M, and Nyström, T (2003). Asymmetric Inheritance of Oxidatively Damaged Proteins During Cytokinesis. Science, 299(5613), 1751-1753.

AkhavanAghdam, Z, Sinha, J, Tabbaa, OP, and Hao, N (2016). Dynamic control of gene regulatory logic by seemingly redundant transcription factors. eLife, 5, e18458.

Alam, A, Bröms, JE, Kumar, R, and Sjöstedt, A (2021). The Role of ClpB in Bacterial Stress Responses and Virulence. Frontiers in Molecular Biosciences, 8(283).

Almeida, CG, Takahashi, RH, and Gouras, GK (2006). β-Amyloid Accumulation Impairs Multivesicular Body Sorting by Inhibiting the Ubiquitin-Proteasome System. The Journal of Neuroscience, 26(16), 4277-4288.

Ambra, R, Mocchegiani, E, Giacconi, R, Canali, R, Rinna, A, Malavolta, M, and Virgili, F (2004). Characterization of the HSP70 response in lymphoblasts from aged and centenarian subjects and differential effects of in vitro zinc supplementation. Experimental Gerontology, 39(10), 1475-1484.

Andersson, R, Eisele-Bürger, AM, Hanzén, S, Vielfort, K, Öling, D, Eisele, F, Johansson, G, Gustafsson, T, Kvint, K, and Nyström, T (2021). Differential role of cytosolic Hsp70s in longevity assurance and protein quality control. PLOS Genetics, 17(1), e1008951.

Andersson, V, Hanzén, S, Liu, B, Molin, M, and Nyström, T (2013). Enhancing protein disaggregation restores proteasome activity in aged cells. Aging (Albany NY), 5(11), 802-812.

Anfinsen, CB (1973). Principles that Govern the Folding of Protein Chains. Science, 181(4096), 223-230.

Anfinsen, CB, and Haber, E (1961). Studies on the Reduction and Re-formation of Protein Disulfide Bonds. Journal of Biological Chemistry, 236(5), 1361-1363.

Anfinsen, CB, Haber, E, Sela, M, and White, FH (1961). The kinetics of formation of native ribonuclease during oxidation of the reduced polypeptide chain. Proceedings of the National Academy of Sciences, 47(9), 1309.

Arrasate, M, Mitra, S, Schweitzer, ES, Segal, MR, and Finkbeiner, S (2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature, 431(7010), 805-810.

Baba, M, Takeshige, K, Baba, N, and Ohsumi, Y (1994). Ultrastructural analysis of the autophagic process in yeast: detection of autophagosomes and their characterization. Journal of Cell Biology, 124(6), 903-913.

Babazadeh, R, Ahmadpour, D, Jia, S, Hao, X, Widlund, P, Schneider, K, Eisele, F, Edo, LD, Smits, GJ, Liu, B, and Nyström, T (2019). Syntaxin 5 Is Required for the Formation and Clearance of Protein Inclusions during Proteostatic Stress. Cell Rep, 28(8), 2096-2110 e2098.

Balch, WE, Morimoto, RI, Dillin, A, and Kelly, JW (2008). Adapting Proteostasis for Disease Intervention. Science, 319(5865), 916-919.

Banfield, DK, Lewis, MJ, and Pelham, HRB (1995). A SNARE-like protein required for traffic through the Golgi complex. Nature, 375(6534), 806-809.

Barnett, ME, Nagy, M, Kedzierska, S, and Zolkiewski, M (2005). The Amino-terminal Domain of ClpB Supports Binding to Strongly Aggregated Proteins *. Journal of Biological Chemistry, 280(41), 34940-34945.

Barton, AA (1950). Some Aspects of Cell Division in Saccharomyces cerevisiae. Microbiology, 4(1), 84-86.

Bas, L, Papinski, D, Licheva, M, Torggler, R, Rohringer, S, Schuschnig, M, and Kraft, C (2018). Reconstitution reveals Ykt6 as the autophagosomal SNARE in autophagosome-vacuole fusion. The Journal of cell biology, 217(10), 3656-3669.

Beck, F, Unverdorben, P, Bohn, S, Schweitzer, A, Pfeifer, G, Sakata, E, Nickell, S, Plitzko, JM, Villa, E, Baumeister, W, and Förster, F (2012). Near-atomic resolution structural model of the yeast 26S proteasome. Proceedings of the National Academy of Sciences, 109(37), 14870-14875.

Beerten, J, Jonckheere, W, Rudyak, S, Xu, J, Wilkinson, H, De Smet, F, Schymkowitz, J, and Rousseau, F (2012). Aggregation gatekeepers modulate protein homeostasis of aggregating sequences and affect bacterial fitness. Protein Engineering, Design and Selection, 25(7), 357-366.

Beilharz, T, Egan, B, Silver, PA, Hofmann, K, and Lithgow, T (2003). Bipartite Signals Mediate Subcellular Targeting of Tail-anchored Membrane Proteins in Saccharomyces cerevisiae *. Journal of Biological Chemistry, 278(10), 8219-8223.

Ben-Zvi, A, Miller, EA, and Morimoto, RI (2009). Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proceedings of the National Academy of Sciences of the United States of America, 106(35), 14914--14919.

Bence, NF, Sampat, RM, and Kopito, RR (2001). Impairment of the Ubiquitin-Proteasome System by Protein Aggregation. Science, 292(5521), 1552-1555.

Bennett, DA, Schneider, JA, Arvanitakis, Z, Kelly, JF, Aggarwal, NT, Shah, RC, and Wilson, RS (2006). Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology, 66(12), 1837.

Bentley, NJ, Fitch, IT, and Tuite, MF (1992). The small heat-shock protein Hsp26 of Saccharomyces cerevisiae assembles into a high molecular weight aggregate. Yeast, 8(2), 95-106.

Berglund, LL, Hao, X, Liu, B, Grantham, J, and Nyström, T (2017). Differential effects of soluble and aggregating polyQ proteins on cytotoxicity and type-1 myosin-dependent endocytosis in yeast. Scientific Reports, 7(1), 11328.

Binda, M, Péli-Gulli, M-P, Bonfils, G, Panchaud, N, Urban, J, Sturgill, TW, Loewith, R, and De Virgilio, C (2009). The Vam6 GEF Controls TORC1 by Activating the EGO Complex. Molecular Cell, 35(5), 563-573.

Bode, DC, Baker, MD, and Viles, JH (2017). Ion Channel Formation by Amyloid-β42 Oligomers but Not Amyloid-β40 in Cellular Membranes. Journal of Biological Chemistry, 292(4), 1404-1413.

Boorstein, WR, and Craig, EA (1990). Structure and regulation of the SSA4 HSP70 gene of Saccharomyces cerevisiae. Journal of Biological Chemistry, 265(31), 18912-18921.

Borgese , N, Colombo , S, and Pedrazzini , E (2003). The tale of tail-anchored proteins : coming from the cytosol and looking for a membrane. Journal of Cell Biology, 161(6), 1013-1019.

Brandman, O, Stewart-Ornstein, J, Wong, D, Larson, A, Williams, Christopher C, Li, G-W, Zhou, S, King, D, Shen, Peter S, Weibezahn, J, Dunn, Joshua G, Rouskin, S, Inada, T, Frost, A, and Weissman, Jonathan S (2012). A Ribosome-Bound Quality Control Complex Triggers Degradation of Nascent Peptides and Signals Translation Stress. Cell, 151(5), 1042-1054.

Bufalino, MR, and van der Kooy, D (2014). The aggregation and inheritance of damaged proteins determines cell fate during mitosis. Cell Cycle, 13(7), 1201-1207.

Campion, R, Bloxam, L, Burrow, K, Brownridge, P, Pentland, D, Thomas, P, Gourlay, C, Eyers, CE, Barclay, J, and Morgan, A (2021). Proteomic analysis of dietary restriction in yeast reveals a role for Hsp26 in lifespan extension. Biochemical Journal https://doi.org/10.1042/BCJ20210432.

Campioni, S, Mannini, B, Zampagni, M, Pensalfini, A, Parrini, C, Evangelisti, E, Relini, A, Stefani, M, Dobson, CM, Cecchi, C, and Chiti, F (2010). A causative link between the structure of aberrant protein oligomers and their toxicity. Nature Chemical Biology, 6(2), 140-147.

Carrell, RW, and Lomas, DA (1997). Conformational disease. The Lancet, 350(9071), 134-138.

Carroni, M, Kummer, E, Oguchi, Y, Wendler, P, Clare, DK, Sinning, I, Kopp, J, Mogk, A, Bukau, B, and Saibil, HR (2014). Head-to-tail interactions of the coiled-coil domains regulate ClpB activity and cooperation with Hsp70 in protein disaggregation. eLife, 3, e02481.

Cascella, R, Chen, SW, Bigi, A, Camino, JD, Xu, CK, Dobson, CM, Chiti, F, Cremades, N, and Cecchi, C (2021). The release of toxic oligomers from α-synuclein fibrils induces dysfunction in neuronal cells. Nature Communications, 12(1), 1814.

Cashikar, AG, Duennwald, M, and Lindquist, SL (2005). A Chaperone Pathway in Protein Disaggregation: Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. Journal of Biological Chemistry, 280(25), 23869-23875.

Chafekar, SM, and Duennwald, ML (2012). Impaired Heat Shock Response in Cells Expressing Full-Length Polyglutamine-Expanded Huntingtin. PLOS ONE, 7(5), e37929.

Chapal, M, Mintzer, S, Brodsky, S, Carmi, M, and Barkai, N (2019). Resolving noise–control conflict by gene duplication. PLOS Biology, 17(11), e3000289.

Chau, V, Tobias John, W, Bachmair, A, Marriott, D, Ecker David, J, Gonda David, K, and Varshavsky, A (1989). A Multiubiquitin Chain Is Confined to Specific Lysine in a Targeted Short-Lived Protein. Science, 243(4898), 1576-1583.

Chen, K, Shen, W, Zhang, Z, Xiong, F, Ouyang, Q, and Luo, C (2020). Age-dependent decline in stress response capacity revealed by proteins dynamics analysis. Scientific Reports, 10(1), 15211.

Chiti, F, and Dobson, CM (2017). Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. Annual Review of Biochemistry, 86(1), 27-68.

Cho, H, and Shan, S-o (2018). Substrate relay in an Hsp70-cochaperone cascade safeguards tail-anchored membrane protein targeting. The EMBO Journal, 37(16), e99264.

Cho, H, Shim, WJ, Liu, Y, and Shan, S-o (2021). J-domain proteins promote client relay from Hsp70 during tail-anchored membrane protein targeting. Journal of Biological Chemistry https://doi.org/10.1016/j.jbc.2021.100546, 100546.

Choe, Y-J, Park, S-H, Hassemer, T, Körner, R, Vincenz-Donnelly, L, Hayer-Hartl, M, and Hartl, FU (2016). Failure of RQC machinery causes protein aggregation and proteotoxic stress. Nature, 531(7593), 191-195.

Ciryam, P, Kundra, R, Morimoto, RI, Dobson, CM, and Vendruscolo, M (2015). Supersaturation is a major driving force for protein aggregation in neurodegenerative diseases. Trends in Pharmacological Sciences, 36(2), 72-77.

Ciryam, P, Tartaglia, Gian G, Morimoto, Richard I, Dobson, Christopher M, and Vendruscolo, M (2013). Widespread Aggregation and Neurodegenerative Diseases Are Associated with Supersaturated Proteins. Cell Reports, 5(3), 781-790.

Clark, PL (2004). Protein folding in the cell: reshaping the folding funnel. Trends in Biochemical Sciences, 29(10), 527-534.

Cohen, E, Bieschke, J, Perciavalle Rhonda, M, Kelly Jeffery, W, and Dillin, A (2006). Opposing Activities Protect Against Age-Onset Proteotoxicity. Science, 313(5793), 1604-1610.

Colman Ricki, J, Anderson Rozalyn, M, Johnson Sterling, C, Kastman Erik, K, Kosmatka Kristopher, J, Beasley, TM, Allison David, B, Cruzen, C, Simmons Heather, A, Kemnitz Joseph, W, and Weindruch, R (2009). Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys. Science, 325(5937), 201-204.

Costanzo, M, Baryshnikova, A, Bellay, J, Kim, Y, Spear, ED, Sevier, CS, Ding, H, Koh, JLY, Toufighi, K, Mostafavi, S, Prinz, J, St. Onge, RP, VanderSluis, B, Makhnevych, T, Vizeacoumar, FJ, Alizadeh, S, Bahr, S, Brost, RL, Chen, Y, Cokol, M, Deshpande, R, Li, Z, Lin, Z-Y, Liang, W, Marback, M, Paw, J, San Luis, B-J, Shuteriqi, E, Tong, AHY, van Dyk, N, Wallace, IM, Whitney, JA, Weirauch, MT, Zhong, G, Zhu, H, Houry, WA, Brudno, M, Ragibizadeh, S, Papp, B, Pál, C, Roth, FP, Giaever, G, Nislow, C, Troyanskaya, OG, Bussey, H, Bader, GD, Gingras, A-C, Morris, QD, Kim, PM, Kaiser, CA, Myers, CL, Andrews, BJ, and Boone, C (2010). The Genetic Landscape of a Cell. Science, 327(5964), 425-431.

Craig, EA, and Jacobsen, K (1984). Mutations of the heat inducible 70 kilodalton genes of yeast confer temperature sensitive growth. Cell, 38(3), 841-849.

Dai, C, Whitesell, L, Rogers, AB, and Lindquist, S (2007). Heat Shock Factor 1 Is a Powerful Multifaceted Modifier of Carcinogenesis. Cell, 130(6), 1005-1018.

David, DC, Ollikainen, N, Trinidad, JC, Cary, MP, Burlingame, AL, and Kenyon, C (2010). Widespread Protein Aggregation as an Inherent Part of Aging in C. elegans. PLOS Biology, 8(8), e1000450.

De Baets, G, Van Durme, J, Rousseau, F, and Schymkowitz, J (2014). A Genome-Wide Sequence–Structure Analysis Suggests Aggregation Gatekeepers Constitute an Evolutionary Constrained Functional Class. Journal of Molecular Biology, 426(12), 2405-2412.

de Jong, WW, Leunissen, JA, and Voorter, CE (1993). Evolution of the alpha-crystallin/small heat-shock protein family. Molecular Biology and Evolution, 10(1), 103-126.

den Brave, F, Cairo, LV, Jagadeesan, C, Ruger-Herreros, C, Mogk, A, Bukau, B, and Jentsch, S (2020). Chaperone-Mediated Protein Disaggregation Triggers Proteolytic Clearance of Intra-nuclear Protein Inclusions. Cell Reports, 31(9), 107680.

Domnauer, M, Zheng, F, Li, L, Zhang, Y, Chang, CE, Unruh, JR, Conkright-Fincham, J, McCroskey, S, Florens, L, Zhang, Y, Seidel, C, Fong, B, Schilling, B, Sharma, R, Ramanathan, A, Si, K, and Zhou, C (2021). Proteome plasticity in response to persistent environmental change. Molecular Cell, 81(16), 3294-3309.e3212.

Douglas, PM, Treusch, S, Ren, H-Y, Halfmann, R, Duennwald, ML, Lindquist, S, and Cyr, DM (2008). Chaperone-dependent amyloid assembly protects cells from prion toxicity. Proceedings of the National Academy of Sciences of the United States of America, 105(20), 7206-7211.

Dubouloz, F, Deloche, O, Wanke, V, Cameroni, E, and De Virgilio, C (2005). The TOR and EGO Protein Complexes Orchestrate Microautophagy in Yeast. Molecular Cell, 19(1), 15-26.

Dukan, S, and Nyström, T (1998). Bacterial senescence: stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon. Genes & development, 12(21), 3431-3441.

Duttler, S, Pechmann, S, and Frydman, J (2013). Principles of Cotranslational Ubiquitination and Quality Control at the Ribosome. Molecular Cell, 50(3), 379-393.

Egilmez, NK, Chen, JB, and Jazwinski, SM (1990). Preparation and partial characterization of old yeast cells. J Gerontol, 45(1), B9-17.

Egilmez, NK, and Jazwinski, SM (1989). Evidence for the involvement of a cytoplasmic factor in the aging of the yeast Saccharomyces cerevisiae. Journal of Bacteriology, 171(1), 37-42.

Ehrnsperger, M, Gräber, S, Gaestel, M, and Buchner, J (1997). Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. The EMBO Journal, 16(2), 221-229.

Eisele, F, Eisele-Bürger, AM, Hao, X, Berglund, LL, Höög, JL, Liu, B, and Nyström, T (2021). An Hsp90 co-chaperone links protein folding and degradation and is part of a conserved protein quality control. Cell Reports, 35(13), 109328.

Eisele, F, and Wolf, DH (2008). Degradation of misfolded protein in the cytoplasm is mediated by the ubiquitin ligase Ubr1. FEBS Letters, 582(30), 4143-4146.

Ellis, RJ (2001). Macromolecular crowding: obvious but underappreciated. Trends in Biochemical Sciences, 26(10), 597-604.

Erjavec, N, Cvijovic, M, Klipp, E, and Nyström, T (2008). Selective benefits of damage partitioning in unicellular systems and its effects on aging. Proceedings of the National Academy of Sciences, 105(48), 18764.

Erjavec, N, Larsson, L, Grantham, J, and Nyström, 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 & Development, 21(19), 2410-2421.

Escusa-Toret, S, Vonk, WI, and Frydman, J (2013). Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. Nature Cell Biology, 15(10), 1231-1243.

Fang, NN, Chan, GT, Zhu, M, Comyn, SA, Persaud, A, Deshaies, RJ, Rotin, D, Gsponer, J, and Mayor, T (2014). Rsp5/Nedd4 is the main ubiquitin ligase that targets cytosolic misfolded proteins following heat stress. Nature Cell Biology, 16(12), 1227-1237.

Fang, NN, Ng, AHM, Measday, V, and Mayor, T (2011). Hul5 HECT ubiquitin ligase plays a major role in the ubiquitylation and turnover of cytosolic misfolded proteins. Nature Cell Biology, 13(11), 1344-1352.

Fang, NN, Zhu, M, Rose, A, Wu, K-P, and Mayor, T (2016). Deubiquitinase activity is required for the proteasomal degradation of misfolded cytosolic proteins upon heat-stress. Nature Communications, 7(1), 12907.

Feder, ZA, Ali, A, Singh, A, Krakowiak, J, Zheng, X, Bindokas, VP, Wolfgeher, D, Kron, SJ, and Pincus, D (2020). Subcellular localization of the J-protein Sis1 regulates the heat shock response. Journal of Cell Biology, 220(1).

Finley, D (2009). Recognition and Processing of Ubiquitin-Protein Conjugates by the Proteasome. Annual Review of Biochemistry, 78, 477-513.

Fortun, J, Dunn, WA, Joy, S, Li, J, and Notterpek, L (2003). Emerging Role for Autophagy in the Removal of Aggresomes in Schwann Cells. The Journal of Neuroscience, 23(33), 10672-10680.

Fusco, G, Chen Serene, W, Williamson Philip, TF, Cascella, R, Perni, M, Jarvis James, A, Cecchi, C, Vendruscolo, M, Chiti, F, Cremades, N, Ying, L, Dobson Christopher, M, and De Simone, A (2017). Structural basis of membrane disruption and cellular toxicity by α-synuclein oligomers. Science, 358(6369), 1440-1443.

Ganesan, A, Debulpaep, M, Wilkinson, H, Van Durme, J, De Baets, G, Jonckheere, W, Ramakers, M, Ivarsson, Y, Zimmermann, P, Van Eldere, J, Schymkowitz, J, and Rousseau, F (2015). Selectivity of Aggregation-Determining Interactions. Journal of Molecular Biology, 427(2), 236-247.

Ganley, ARD, Ide, S, Saka, K, and Kobayashi, T (2009). The Effect of Replication Initiation on Gene Amplification in the rDNA and Its Relationship to Aging. Molecular Cell, 35(5), 683-693.

Gao, M, and Kaiser, CA (2006). A conserved GTPase-containing complex is required for intracellular sorting of the general amino-acid permease in yeast. Nature Cell Biology, 8(7), 657-667.

Gasch, AP, Spellman, PT, Kao, CM, Carmel-Harel, O, Eisen, MB, Storz, G, Botstein, D, and Brown, PO (2000). Genomic Expression Programs in the Response of Yeast Cells to Environmental Changes. Molecular Biology of the Cell, 11(12), 4241-4257.

Geiler-Samerotte, KA, Dion, MF, Budnik, BA, Wang, SM, Hartl, DL, and Drummond, DA (2011). Misfolded proteins impose a dosage-dependent fitness cost and trigger a cytosolic unfolded protein response in yeast. Proceedings of the National Academy of Sciences, 108(2), 680-685.

Gidalevitz, T, Ben-Zvi, A, Ho Kim, H, Brignull Heather, R, and Morimoto Richard, I (2006). Progressive Disruption of Cellular Protein Folding in Models of Polyglutamine Diseases. Science, 311(5766), 1471-1474.

Giorgini, F, Guidetti, P, Nguyen, Q, Bennett, SC, and Muchowski, PJ (2005). A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. Nature Genetics, 37(5), 526-531.

Glover, JR, and Lindquist, S (1998). Hsp104, Hsp70, and Hsp40: A Novel Chaperone System that Rescues Previously Aggregated Proteins. Cell, 94(1), 73-82.

Gokhale, KC, Newnam, GP, Sherman, MY, and Chernoff, YO (2005). Modulation of Prion-dependent Polyglutamine Aggregation and Toxicity by Chaperone Proteins in the Yeast Model*. Journal of Biological Chemistry, 280(24), 22809-22818.

Graef, M, Friedman, JR, Graham, C, Babu, M, and Nunnari, J (2013). ER exit sites are physical and functional core autophagosome biogenesis components. Molecular Biology of the Cell, 24(18), 2918-2931.

Grousl, T, Ungelenk, S, Miller, S, Ho, C-T, Khokhrina, M, Mayer, MP, Bukau, B, and Mogk, A (2018). A prion-like domain in Hsp42 drives chaperone-facilitated aggregation of misfolded proteins. The Journal of Cell Biology 10.1083/jcb.201708116.

Grune, T, Jung, T, Merker, K, and Davies, KJA (2004). Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes' during oxidative stress, aging, and disease. The International Journal of Biochemistry & Cell Biology, 36(12), 2519-2530.

Guarente, L (1997). Link between aging and the nucleolus. Genes & Development, 11(19), 2449-2455.

Gutsmann-Conrad, A, Heydari, AR, You, S, and Richardson, A (1998). The Expression of Heat Shock Protein 70 Decreases with Cellular Senescencein Vitroand in Cells Derived from Young and Old Human Subjects. Experimental Cell Research, 241(2), 404-413.

Haas, AL, Warms, JV, Hershko, A, and Rose, IA (1982). Ubiquitin-activating enzyme. Mechanism and role in protein-ubiquitin conjugation. Journal of Biological Chemistry, 257(5), 2543-2548.

Haber, E, and Anfinsen, CB (1962). Side-chain Interactions Governing the Pairing of Half-cystine Residues in Ribonuclease. Journal of Biological Chemistry, 237(6), 1839-1844.

Hageman, J, Rujano, MA, van Waarde, MAWH, Kakkar, V, Dirks, RP, Govorukhina, N, Oosterveld-Hut, HMJ, Lubsen, NH, and Kampinga, HH (2010). A DNAJB Chaperone Subfamily with HDAC-Dependent Activities Suppresses Toxic Protein Aggregation. Molecular Cell, 37(3), 355-369.

Hallstrom, TC, and Moye-Rowley, WS (2000). Hyperactive forms of the Pdr1p transcription factor fail to respond to positive regulation by the Hsp70 protein Pdr13p. Molecular Microbiology, 36(2), 402-413.

Hanna, J, Hathaway, NA, Tone, Y, Crosas, B, Elsasser, S, Kirkpatrick, Donald S, Leggett, DS, Gygi, SP, King, RW, and Finley, D (2006). Deubiquitinating Enzyme Ubp6 Functions Noncatalytically to Delay Proteasomal Degradation. Cell, 127(1), 99-111.

Hansen, M, Chandra, A, Mitic, LL, Onken, B, Driscoll, M, and Kenyon, C (2008). A role for autophagy in the extension of lifespan by dietary restriction in C. elegans. PLoS genetics, 4(2), e24-e24.

Hanzén, S, Vielfort, K, Yang, J, Roger, F, Andersson, V, Zamarbide-Forés, S, Andersson, R, Malm, L, Palais, G, Biteau, B, Liu, B, Toledano, Michel B, Molin, M, and Nyström, T (2016). Lifespan Control by Redox-Dependent Recruitment of Chaperones to Misfolded Proteins. Cell, 166(1), 140-151.

Hardwick, KG, and Pelham, HR (1992). SED5 encodes a 39-kD integral membrane protein required for vesicular transport between the ER and the Golgi complex. Journal of Cell Biology, 119(3), 513-521.

Harrington, WF, and Rao, NV (1970). Collagen structure in solution. I. Kinetics of helix regeneration in single-chain gelatins. Biochemistry, 9(19), 3714-3724.

Hartl, FU, Bracher, A, and Hayer-Hartl, M (2011). Molecular chaperones in protein folding and proteostasis. Nature, 475(7356), 324-332.

Haslbeck, M, Braun, N, Stromer, T, Richter, B, Model, N, Weinkauf, S, and Buchner, J (2004). Hsp42 is the general small heat shock protein in the cytosol of Saccharomyces cerevisiae. The EMBO journal, 23(3), 638-649.

Haslbeck, M, Miess, A, Stromer, T, Walter, S, and Buchner, J (2005). Disassembling Protein Aggregates in the Yeast Cytosol: the cooperation of Hsp26 with Ssa1 and Hsp104. Journal of Biological Chemistry, 280(25), 23861-23868.

Haslbeck, M, Walke, S, Stromer, T, Ehrnsperger, M, White, HE, Chen, S, Saibil, HR, and Buchner, J (1999). Hsp26: a temperature-regulated chaperone. The EMBO Journal, 18(23), 6744-6751.

Haslberger, T, Weibezahn, J, Zahn, R, Lee, S, Tsai, FTF, Bukau, B, and Mogk, A (2007). M Domains Couple the ClpB Threading Motor with the DnaK Chaperone Activity. Molecular Cell, 25(2), 247-260.

Haslberger, T, Zdanowicz, A, Brand, I, Kirstein, J, Turgay, K, Mogk, A, and Bukau, B (2008). Protein disaggregation by the AAA+ chaperone ClpB involves partial threading of looped polypeptide segments. Nature Structural & Molecular Biology, 15(6), 641-650.

Hatakeyama, R, Péli-Gulli, M-P, Hu, Z, Jaquenoud, M, Garcia Osuna, GM, Sardu, A, Dengjel, J, and De Virgilio, C (2019). Spatially Distinct Pools of TORC1 Balance Protein Homeostasis. Molecular Cell, 73(2), 325-338.e328.

Hay, DG, Sathasivam, K, Tobaben, S, Stahl, B, Marber, M, Mestril, R, Mahal, A, Smith, DL, Woodman, B, and Bates, GP (2004). Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. Human Molecular Genetics, 13(13), 1389-1405.

Hayashi, S, Nakazaki, Y, Kagii, K, Imamura, H, and Watanabe, Y-h (2017). Fusion protein analysis reveals the precise regulation between Hsp70 and Hsp100 during protein disaggregation. Scientific Reports, 7(1), 8648.

Heck, JW, Cheung, SK, and Hampton, RY (2010). Cytoplasmic protein quality control degradation mediated by parallel actions of the E3 ubiquitin ligases Ubr1 and San1. Proceedings of the National Academy of Sciences, 107(3), 1106-1111.

Henderson, KA, and Gottschling, DE (2008). A mother's sacrifice: what is she keeping for herself? Current Opinion in Cell Biology, 20(6), 723-728.

Henderson, KA, Hughes, AL, and Gottschling, DE (2014). Mother-daughter asymmetry of pH underlies aging and rejuvenation in yeast. eLife, 3, e03504.

Heuck, A, Schitter-Sollner, S, Suskiewicz, MJ, Kurzbauer, R, Kley, J, Schleiffer, A, Rombaut, P, Herzog, F, and Clausen, T (2016). Structural basis for the disaggregase activity and regulation of Hsp104. eLife, 5, e21516.

Higuchi, R, Vevea, Jason D, Swayne, Theresa C, Chojnowski, R, Hill, V, Boldogh, Istvan R, and Pon, Liza A (2013). Actin Dynamics Affect Mitochondrial Quality Control and Aging in Budding Yeast. Current Biology, 23(23), 2417-2422.

Higuchi-Sanabria, R, Paul, rJW, Durieux, J, Benitez, C, Frankino, PA, Tronnes, SU, Garcia, G, Daniele, JR, Monshietehadi, S, and Dillin, A (2018). Spatial regulation of the actin cytoskeleton by HSF-1 during aging. Molecular Biology of the Cell, 29(21), 2522-2527.

Hill, SM, Hao, X, Grönvall, J, Spikings-Nordby, S, Widlund, PO, Amen, T, Jörhov, A, Josefson, R, Kaganovich, D, Liu, B, and Nyström, T (2016). Asymmetric Inheritance of Aggregated Proteins and Age Reset in Yeast Are Regulated by Vac17-Dependent Vacuolar Functions. Cell Reports, 16, 1-13.

Hirose, E, Nakashima, N, Sekiguchi, T, and Nishimoto, T (1998). RagA is a functional homologue of S. cerevisiae Gtr1p involved in the Ran/Gsp1-GTPase pathway. Journal of Cell Science, 111(1), 11-21.

Ho, CT, Grousl, T, Shatz, O, Jawed, A, Ruger-Herreros, C, Semmelink, M, Zahn, R, Richter, K, Bukau, B, and Mogk, A (2019). Cellular sequestrases maintain basal Hsp70 capacity ensuring balanced proteostasis. Nature Communications, 10(1), 4851.

Hodges, Alex R, Krementsova, Elena B, Bookwalter, Carol S, Fagnant, Patricia M, Sladewski, Thomas E, and Trybus, Kathleen M (2012). Tropomyosin Is Essential for Processive Movement of a Class V Myosin from Budding Yeast. Current Biology, 22(15), 1410-1416.

Holmberg, CI, Staniszewski, KE, Mensah, KN, Matouschek, A, and Morimoto, RI (2004). Inefficient degradation of truncated polyglutamine proteins by the proteasome. The EMBO journal, 23(21), 4307--4318.

Hughes, AL, and Gottschling, DE (2012). An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. Nature, 492(7428), 261-265.

Hwang, C-S, Shemorry, A, Auerbach, D, and Varshavsky, A (2010a). The N-end rule pathway is mediated by a complex of the RING-type Ubr1 and HECT-type Ufd4 ubiquitin ligases. Nature Cell Biology, 12(12), 1177-1185.

Hwang, C-S, Shemorry, A, and Varshavsky, A (2010b). N-Terminal Acetylation of Cellular Proteins Creates Specific Degradation Signals. Science, 327(5968), 973-977.

Iburg, M, Puchkov, D, Rosas-Brugada, IU, Bergemann, L, Rieprecht, U, and Kirstein, J (2020). The noncanonical small heat shock protein HSP-17 from Caenorhabditis elegans is a selective protein aggregase. Journal of Biological Chemistry, 295(10), 3064-3079.

Ishihara, N, Hamasaki, M, Yokota, S, Suzuki, K, Kamada, Y, Kihara, A, Yoshimori, T, Noda, T, and Ohsumi, Y (2001). Autophagosome Requires Specific Early Sec Proteins for Its Formation and NSF/SNARE for Vacuolar Fusion. Molecular Biology of the Cell, 12(11), 3690-3702.

Ishii, A, Kawai, M, Noda, H, Kato, H, Takeda, K, Asakawa, K, Ichikawa, Y, Sasanami, T, Tanaka, K, and Kimura, Y (2018). Accelerated invagination of vacuoles as a stress response in chronically heat-stressed yeasts. Scientific Reports, 8(1), 2644.

Ishikawa, K, Catlett, NL, Novak, JL, Tang, F, Nau, JJ, and Weisman, LS (2003). Identification of an organelle-specific myosin V receptor. Journal of Cell Biology, 160(6), 887-897.

Iwata, A, Riley, BE, Johnston, JA, and Kopito, RR (2005). HDAC6 and Microtubules Are Required for Autophagic Degradation of Aggregated Huntingtin. Journal of Biological Chemistry, 280(48), 40282-40292.

Jakob, U, Gaestel, M, Engel, K, and Buchner, J (1993). Small heat shock proteins are molecular chaperones. Journal of Biological Chemistry, 268(3), 1517-1520.

Jakobsen, BK, and Pelham, HR (1988). Constitutive binding of yeast heat shock factor to DNA in vivo. Molecular and Cellular Biology, 8(11), 5040-5042.

Jia, K, and Levine, B (2007). Autophagy is Required for Dietary Restriction-Mediated Life Span Extension in C. elegans. Autophagy, 3(6), 597-599.

Jiang, JC, Jaruga, E, Repnevskaya, MV, and Jazwinski, SM (2000). An intervention resembling caloric restriction prolongs life span and retards aging in yeast. The FASEB Journal, 14(14), 2135-2137.

Johnston, JA, Ward, CL, and Kopito, RR (1998). Aggresomes: A Cellular Response to Misfolded Proteins. The Journal of Cell Biology, 143(7), 1883-1898.

Johnston, JR (1966). Reproductive capacity and mode of death of yeast cells. Antonie van Leeuwenhoek, 32(1), 94-98.

Kaeberlein, M, McVey, M, and Guarente, L (1999). The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes & development, 13(19), 2570-2580.

Kaeberlein, M, Powers, RW, Steffen Kristan, K, Westman Eric, A, Hu, D, Dang, N, Kerr Emily, O, Kirkland Kathryn, T, Fields, S, and Kennedy Brian, K (2005). Regulation of Yeast Replicative Life Span by TOR and Sch9 in Response to Nutrients. Science, 310(5751), 1193-1196.

Kaganovich, D, Kopito, R, and Frydman, J (2008). Misfolded proteins partition between two distinct quality control compartments. Nature, 454(7208), 1088--1095.

Kaimal, JM, Kandasamy, G, Gasser, F, and Andréasson, C (2017). Coordinated Hsp110 and Hsp104 Activities Power Protein Disaggregation in Saccharomyces cerevisiae. Molecular and Cellular Biology, 37(11), e00027-00017.

Kakkar, V, Månsson, C, de Mattos, Eduardo P, Bergink, S, van der Zwaag, M, van Waarde, Maria AWH, Kloosterhuis, Niels J, Melki, R, van Cruchten, Remco TP, Al-Karadaghi, S, Arosio, P, Dobson, Christopher M, Knowles, Tuomas PJ, Bates, Gillian P, van Deursen, JM, Linse, S, van de Sluis, B, Emanuelsson, C, and Kampinga, Harm H (2016). The S/T-Rich Motif in the DNAJB6 Chaperone Delays Polyglutamine Aggregation and the Onset of Disease in a Mouse Model. Molecular Cell, 62(2), 272-283.

Kamada, Y, Funakoshi, T, Shintani, T, Nagano, K, Ohsumi, M, and Ohsumi, Y (2000). Tor-Mediated Induction of Autophagy via an Apg1 Protein Kinase Complex. Journal of Cell Biology, 150(6), 1507-1513.

Kamada, Y, Yoshino, K-i, Kondo, C, Kawamata, T, Oshiro, N, Yonezawa, K, and Ohsumi, Y (2010). Tor Directly Controls the Atg1 Kinase Complex To Regulate Autophagy. Molecular and Cellular Biology, 30(4), 1049-1058.

Kampinga, HH, and Craig, EA (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol, 11(8), 579-592.

Kandasamy, G, and Andréasson, C (2018). Hsp70–Hsp110 chaperones deliver ubiquitin-dependent and -independent substrates to the 26S proteasome for proteolysis in yeast. Journal of Cell Science, 131(6).

Kang, P-J, Ostermann, J, Shilling, J, Neupert, W, Craig, EA, and Pfanner, N (1990). Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. Nature, 348(6297), 137-143.

Katikaridis, P, Bohl, V, and Mogk, A (2021). Resisting the Heat: Bacterial Disaggregases Rescue Cells From Devastating Protein Aggregation. Frontiers in Molecular Biosciences, 8(320).

Keck, S, Nitsch, R, Grune, T, and Ullrich, O (2003). Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. Journal of Neurochemistry, 85(1), 115-122.

Kennedy, BK, Austriaco, NR, Jr., and Guarente, L (1994). Daughter cells of Saccharomyces cerevisiae from old mothers display a reduced life span. The Journal of cell biology, 127(6 Pt 2), 1985-1993.

Kenyon, CJ (2010). The genetics of ageing. Nature, 464(7288), 504-512.

Keuenhof, KS, Larsson Berglund, L, Malmgren Hill, S, Schneider, KL, Widlund, PO, Nyström, T, and Höög, JL (2022). Large organellar changes occur during mild heat shock in yeast. Journal of Cell Science, 135(5), jcs258325.

Khodaparast, L, Khodaparast, L, Gallardo, R, Louros, NN, Michiels, E, Ramakrishnan, R, Ramakers, M, Claes, F, Young, L, Shahrooei, M, Wilkinson, H, Desager, M, Mengistu Tadesse, W, Nilsson, KPR, Hammarström, P, Aertsen, A, Carpentier, S, Van Eldere, J, Rousseau, F, and Schymkowitz, J (2018). Aggregating sequences that occur in many proteins constitute weak spots of bacterial proteostasis. Nature Communications, 9(1), 866.

Khurana, V, and Lindquist, S (2010). Modelling neurodegeneration in Saccharomyces cerevisiae: why cook with baker's yeast? Nature Reviews Neuroscience, 11(6), 436-449.

Kim, S, Nollen, EAA, Kitagawa, K, Bindokas, VP, and Morimoto, RI (2002). Polyglutamine protein aggregates are dynamic. Nature Cell Biology, 4(10), 826-831.

Kim, Yujin E, Hosp, F, Frottin, F, Ge, H, Mann, M, Hayer-Hartl, M, and Hartl, FU (2016). Soluble Oligomers of PolyQ-Expanded Huntingtin Target a Multiplicity of Key Cellular Factors. Molecular Cell, 63(6), 951-964.

Kira, S, Kumano, Y, Ukai, H, Takeda, E, Matsuura, A, and Noda, T (2015). Dynamic relocation of the TORC1–Gtr1/2–Ego1/2/3 complex is regulated by Gtr1 and Gtr2. Molecular Biology of the Cell, 27(2), 382-396.

Kirkwood, TBL (1977). Evolution of ageing. Nature, 270(5635), 301-304.

Kirkwood, TBL (2005). Understanding the Odd Science of Aging. Cell, 120(4), 437-447.

Kirkwood, TBL, and Austad, SN (2000). Why do we age? Nature, 408(6809), 233-238.

Klaips, CL, Gropp, MHM, Hipp, MS, and Hartl, FU (2020). Sis1 potentiates the stress response to protein aggregation and elevated temperature. Nature Communications, 11(1), 6271.

Koegl, M, Hoppe, T, Schlenker, S, Ulrich, HD, Mayer, TU, and Jentsch, S (1999). A Novel Ubiquitination Factor, E4, Is Involved in Multiubiquitin Chain Assembly. Cell, 96(5), 635-644.

Krakowiak, J, Zheng, X, Patel, N, Feder, ZA, Anandhakumar, J, Valerius, K, Gross, DS, Khalil, AS, and Pincus, D (2018). Hsf1 and Hsp70 constitute a two-component feedback loop that regulates the yeast heat shock response. eLife, 7, e31668.

Krobitsch, S, and Lindquist, S (2000). Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. Proceedings of the National Academy of Sciences, 97(4), 1589-1594.

Kumar, R, Nawroth, PP, and Tyedmers, J (2016). Prion Aggregates Are Recruited to the Insoluble Protein Deposit (IPOD) via Myosin 2-Based Vesicular Transport. PLoS Genet, 12(9), e1006324.

Labbadia, J, and Morimoto, RI (2015). The Biology of Proteostasis in Aging and Disease. Annual Review of Biochemistry, 84(1), 435-464.

Lai, C-Y, Jaruga, E, Borghouts, C, and Jazwinski, SM (2002). A mutation in the ATP2 gene abrogates the age asymmetry between mother and daughter cells of the yeast Saccharomyces cerevisiae. Genetics, 162(1), 73-87.

Lam, YA, Lawson, TG, Velayutham, M, Zweier, JL, and Pickart, CM (2002). A proteasomal ATPase subunit recognizes the polyubiquitin degradation signal. Nature, 416(6882), 763-767.

Landry, J, Bernier, D, Chrétien, P, Nicole, LM, Tanguay, RM, and Marceau, N (1982). Synthesis and Degradation of Heat Shock Proteins during Development and Decay of Thermotolerance. Cancer Research, 42(6), 2457.

Lee, DH, Sherman, MY, and Goldberg, AL (1996). Involvement of the molecular chaperone Ydj1 in the ubiquitin-dependent degradation of short-lived and abnormal proteins in Saccharomyces cerevisiae. Molecular and Cellular Biology, 16(9), 4773-4781.

Lee, W-C, Lee, M, Jung, JW, Kim, KP, and Kim, D (2008). SCUD: Saccharomyces Cerevisiae Ubiquitination Database. BMC Genomics, 9(1), 440.

Lemus, L, Ribas, JL, Sikorska, N, and Goder, V (2016). An ER-Localized SNARE Protein Is Exported in Specific COPII Vesicles for Autophagosome Biogenesis. Cell Reports, 14(7), 1710-1722.

Li, J, Soroka, J, and Buchner, J (2012). The Hsp90 chaperone machinery: Conformational dynamics and regulation by co-chaperones. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1823(3), 624-635.

Li, J, Yuan, Z, and Zhang, Z (2010). The Cellular Robustness by Genetic Redundancy in Budding Yeast. PLOS Genetics, 6(11), e1001187.

Lin, S-J, Defossez, P-A, and Guarente, L (2000). Requirement of NAD and SIR2 for Life-Span Extension by Calorie Restriction in Saccharomyces cerevisiae. Science, 289(5487), 2126-2128.

Lippuner, AD, Julou, T, and Barral, Y (2014). Budding yeast as a model organism to study the effects of age. FEMS Microbiology Reviews, 38(2), 300-325.

Liu, B, Larsson, L, Caballero, A, Hao, X, Oling, D, Grantham, J, and Nyström, T (2010). The polarisome is required for segregation and retrograde transport of protein aggregates. Cell, 140(2), 257-267.

Liu, B, Larsson, L, Franssens, V, Hao, X, Hill, Sandra M, Andersson, V, Höglund, D, Song, J, Yang, X, Öling, D, Grantham, J, Winderickx, J, and Nyström, T (2011). Segregation of Protein Aggregates Involves Actin and the Polarity Machinery. Cell, 147(5), 959-961.

Llorca, O, Martín-Benito, J, Ritco-Vonsovici, M, Grantham, J, Hynes, GM, Willison, KR, Carrascosa, JL, and Valpuesta, JM (2000). Eukaryotic chaperonin CCT stabilizes actin and tubulin folding intermediates in open quasi-native conformations. The EMBO journal, 19(22), 5971-5979.

López-Otín, C, Blasco, MA, Partridge, L, Serrano, M, and Kroemer, G (2013). The Hallmarks of Aging. Cell, 153(6), 1194-1217.

Lu, K, Psakhye, I, and Jentsch, S (2014). Autophagic Clearance of PolyQ Proteins Mediated by Ubiquitin-Atg8 Adaptors of the Conserved CUET Protein Family. Cell, 158(3), 549-563.

Malinovska, L, Kroschwald, S, Munder, MC, Richter, D, and Alberti, S (2012). Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. Molecular Biology of the Cell, 23(16), 3041-3056.

Mannini, B, Mulvihill, E, Sgromo, C, Cascella, R, Khodarahmi, R, Ramazzotti, M, Dobson, CM, Cecchi, C, and Chiti, F (2014). Toxicity of Protein Oligomers Is Rationalized by a Function Combining Size and Surface Hydrophobicity. ACS Chemical Biology, 9(10), 2309-2317.

Mari, M, Griffith, J, Rieter, E, Krishnappa, L, Klionsky, DJ, and Reggiori, F (2010). An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis. Journal of Cell Biology, 190(6), 1005-1022.

Masser, AE, Kang, W, Roy, J, Kaimal, JM, Quintana-Cordero, J, Friedländer, MR, and Andréasson, C (2019). Cytoplasmic protein misfolding titrates Hsp70 to activate nuclear Hsf1. eLife, 8, e47791.

Matsuda, R, Ikeuchi, K, Nomura, S, and Inada, T (2014). Protein quality control systems associated with no-go and nonstop mRNA surveillance in yeast. Genes to Cells, 19(1), 1-12.

Matsuura, A, Tsukada, M, Wada, Y, and Ohsumi, Y (1997). Apg1p, a novel protein kinase required for the autophagic process in Saccharomyces cerevisiae. Gene, 192(2), 245-250.

Mattoo, RUH, Sharma, SK, Priya, S, Finka, A, and Goloubinoff, P (2013). Hsp110 Is a Bona Fide Chaperone Using ATP to Unfold Stable Misfolded Polypeptides and Reciprocally Collaborate with Hsp70 to Solubilize Protein Aggregates*. Journal of Biological Chemistry, 288(29), 21399-21411.

Mawuenyega, KG, Sigurdson, W, Ovod, V, Munsell, L, Kasten, T, Morris, JC, Yarasheski, KE, and Bateman, RJ (2010). Decreased Clearance of CNS β-Amyloid in Alzheimer's Disease. Science, 330(6012), 1774-1774.

Mayer, MP, and Bukau, B (2005). Hsp70 chaperones: Cellular functions and molecular mechanism. Cellular and Molecular Life Sciences, 62(6), 670.

McCay, CM, Crowell, MF, and Maynard, LA (1989). The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. Nutrition, 5(3), 155-171; discussion 172.

McClellan, AJ, Xia, Y, Deutschbauer, AM, Davis, RW, Gerstein, M, and Frydman, J (2007). Diverse Cellular Functions of the Hsp90 Molecular Chaperone Uncovered Using Systems Approaches. Cell, 131(1), 121-135.

McFaline-Figueroa, JR, Vevea, J, Swayne, TC, Zhou, C, Liu, C, Leung, G, Boldogh, IR, and Pon, LA (2011). Mitochondrial quality control during inheritance is associated with lifespan and mother–daughter age asymmetry in budding yeast. Aging Cell, 10(5), 885-895.

Medvedik, O, Lamming, DW, Kim, KD, and Sinclair, DA (2007). MSN2 and MSN4 Link Calorie Restriction and TOR to Sirtuin-Mediated Lifespan Extension in Saccharomyces cerevisiae. PLOS Biology, 5(10), e261.

Melentijevic, I, Toth, ML, Arnold, ML, Guasp, RJ, Harinath, G, Nguyen, KC, Taub, D, Parker, JA, Neri, C, Gabel, CV, Hall, DH, and Driscoll, M (2017). C. elegans neurons jettison protein aggregates and mitochondria under neurotoxic stress. Nature, 542(7641), 367-371.

Meriin, AB, Zhang, X, He, X, Newnam, GP, Chernoff, YO, and Sherman, MY (2002). Huntingtin toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. The Journal of Cell Biology, 157(6), 997-1004.

Merker Robert, J, and Klein Hannah, L (2002). hpr1Δ Affects Ribosomal DNA Recombination and Cell Life Span in Saccharomyces cerevisiae. Molecular and Cellular Biology, 22(2), 421-429.

Miller, J, Arrasate, M, Brooks, E, Libeu, CP, Legleiter, J, Hatters, D, Curtis, J, Cheung, K, Krishnan, P, Mitra, S, Widjaja, K, Shaby, BA, Lotz, GP, Newhouse, Y, Mitchell, EJ, Osmand, A, Gray, M, Thulasiramin, V, Saudou, F, Segal, M, Yang, XW, Masliah, E, Thompson, LM, Muchowski, PJ, Weisgraber, KH, and Finkbeiner, S (2011). Identifying polyglutamine protein species in situ that best predict neurodegeneration. Nature Chemical Biology, 7(12), 925-934.

Miller, SB, Ho, CT, Winkler, J, Khokhrina, M, Neuner, A, Mohamed, MY, Guilbride, DL, Richter, K, Lisby, M, Schiebel, E, Mogk, A, and Bukau, B (2015). Compartment-specific aggregases direct distinct nuclear and cytoplasmic aggregate deposition. EMBO J, 34(6), 778-797.

Mittal, N, Guimaraes, JC, Gross, T, Schmidt, A, Vina-Vilaseca, A, Nedialkova, DD, Aeschimann, F, Leidel, SA, Spang, A, and Zavolan, M (2017). The Gcn4 transcription factor reduces protein synthesis capacity and extends yeast lifespan. Nature Communications, 8(1), 457.

Mogk, A, Bukau, B, and Kampinga, HH (2018). Cellular Handling of Protein Aggregates by Disaggregation Machines. Molecular Cell, 69(2), 214-226.

Mogk, A, Schlieker, C, Friedrich, KL, Schönfeld, H-J, Vierling, E, and Bukau, B (2003). Refolding of Substrates Bound to Small Hsps Relies on a Disaggregation Reaction Mediated Most Efficiently by ClpB/DnaK*. Journal of Biological Chemistry, 278(33), 31033-31042.

Mogk, A, Tomoyasu, T, Goloubinoff, P, Rüdiger, S, Röder, D, Langen, H, and Bukau, B (1999). Identification of thermolabile Escherichia coli proteins: prevention and reversion of aggregation by DnaK and ClpB. The EMBO Journal, 18(24), 6934-6949.

Mollard, GFv, Nothwehr, SF, and Stevens, TH (1997). The Yeast v-SNARE Vti1p Mediates Two Vesicle Transport Pathways through Interactions with the t-SNAREs Sed5p and Pep12p. Journal of Cell Biology, 137(7), 1511-1524.

Moreno, DF, Jenkins, K, Morlot, S, Charvin, G, Csikasz-Nagy, A, and Aldea, M (2019). Proteostasis collapse, a hallmark of aging, hinders the chaperone-Start network and arrests cells in G1. eLife, 8, e48240.

Mortimer, RK, and Johnston, JR (1959). Life Span of Individual Yeast Cells. Nature, 183(4677), 1751-1752.

Muchowski, PJ, Schaffar, G, Sittler, A, Wanker, EE, Hayer-Hartl, MK, and Hartl, FU (2000). Hsp70 and Hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. Proceedings of the National Academy of Sciences, 97(14), 7841.

Mühlhofer, M, Berchtold, E, Stratil, CG, Csaba, G, Kunold, E, Bach, NC, Sieber, SA, Haslbeck, M, Zimmer, R, and Buchner, J (2019). The Heat Shock Response in Yeast Maintains Protein Homeostasis by Chaperoning and Replenishing Proteins. Cell Reports, 29(13), 4593-4607.e4598.

Mulaj, M, Foley, J, and Muschol, M (2014). Amyloid Oligomers and Protofibrils, but Not Filaments, Self-Replicate from Native Lysozyme. Journal of the American Chemical Society, 136(25), 8947-8956.

Müller, M, Schmidt, O, Angelova, M, Faserl, K, Weys, S, Kremser, L, Pfaffenwimmer, T, Dalik, T, Kraft, C, Trajanoski, Z, Lindner, H, and Teis, D (2015). The coordinated action of the MVB pathway and autophagy ensures cell survival during starvation. eLife, 4, e07736.

Nath, S, Agholme, L, Kurudenkandy, FR, Granseth, B, Marcusson, J, and Hallbeck, M (2012). Spreading of Neurodegenerative Pathology via Neuron-to-Neuron Transmission of β-Amyloid. The Journal of Neuroscience, 32(26), 8767.

Nathan, DF, Vos, MH, and Lindquist, S (1997). *In vivo* functions of the *Saccharomyces cerevisiae* Hsp90 chaperone. Proceedings of the National Academy of Sciences, 94(24), 12949-12956.

Neklesa, TK, and Davis, RW (2009). A Genome-Wide Screen for Regulators of TORC1 in Response to Amino Acid Starvation Reveals a Conserved Npr2/3 Complex. PLOS Genetics, 5(6), e1000515.

Nelson, RJ, Ziegelhoffer, T, Nicolet, C, Werner-Washburne, M, and Craig, EA (1992). The translation machinery and 70 kd heat shock protein cooperate in protein synthesis. Cell, 71(1), 97-105.

Nillegoda, NB, Kirstein, J, Szlachcic, A, Berynskyy, M, Stank, A, Stengel, F, Arnsburg, K, Gao, X, Scior, A, Aebersold, R, Guilbride, DL, Wade, RC, Morimoto, RI, Mayer, MP, and Bukau, B (2015). Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. Nature, 524(7564), 247-251.

Noda, T, and Ohsumi, Y (1998). Tor, a Phosphatidylinositol Kinase Homologue, Controls Autophagy in Yeast*. Journal of Biological Chemistry, 273(7), 3963-3966.

Nussbaum-Krammer, CI, Park, K-W, Li, L, Melki, R, and Morimoto, RI (2013). Spreading of a Prion Domain from Cell-to-Cell by Vesicular Transport in Caenorhabditis elegans. PLOS Genetics, 9(3), e1003351.

Nyström, T (2005). Role of oxidative carbonylation in protein quality control and senescence. The EMBO Journal, 24(7), 1311-1317.

O'Nuallain, B, Williams, AD, Westermark, P, and Wetzel, R (2004). Seeding Specificity in Amyloid Growth Induced by Heterologous Fibrils*. Journal of Biological Chemistry, 279(17), 17490-17499.

Oguchi, Y, Kummer, E, Seyffer, F, Berynskyy, M, Anstett, B, Zahn, R, Wade, RC, Mogk, A, and Bukau, B (2012). A tightly regulated molecular toggle controls AAA+ disaggregase. Nature Structural & Molecular Biology, 19(12), 1338-1346.

Öling, D, Eisele, F, Kvint, K, and Nyström, T (2014). Opposing roles of Ubp3-dependent deubiquitination regulate replicative life span and heat resistance. The EMBO Journal, 33(7), 747-761.

Olivares, AO, Baker, TA, and Sauer, RT (2018). Mechanical Protein Unfolding and Degradation. Annual Review of Physiology, 80(1), 413-429.

Oliver, CN, Ahn, BW, Moerman, EJ, Goldstein, S, and Stadtman, ER (1987). Age-related changes in oxidized proteins. Journal of Biological Chemistry, 262(12), 5488-5491.

Olzscha, H, Schermann, SM, Woerner, AC, Pinkert, S, Hecht, MH, Tartaglia, GG, Vendruscolo, M, Hayer-Hartl, M, Hartl, FU, and Vabulas, RM (2011). Amyloid-like Aggregates Sequester Numerous Metastable Proteins with Essential Cellular Functions. Cell, 144(1), 67-78.

Ostrowicz, CW, Meiringer, CTA, and Ungermann, C (2008). Yeast vacuole fusion: A model system for eukaryotic endomembrane dynamics. Autophagy, 4(1), 5-19.

Pahlavani, MA, Harris, MD, Moore, SA, Weindruch, R, and Richardson, A (1995). The Expression of Heat Shock Protein 70 Decreases with Age in Lymphocytes from Rats and Rhesus Monkeys. Experimental Cell Research, 218(1), 310-318.

Panagaki, D, Croft, JT, Keuenhof, K, Larsson Berglund, L, Andersson, S, Kohler, V, Büttner, S, Tamás, MJ, Nyström, T, Neutze, R, and Höög, JL (2021). Nuclear envelope budding is a response to cellular stress. Proceedings of the National Academy of Sciences, 118(30), e2020997118.

Panchaud, N, Péli-Gulli, M-P, and De Virgilio, C (2013a). Amino Acid Deprivation Inhibits TORC1 Through a GTPase-Activating Protein Complex for the Rag Family GTPase Gtr1. Science Signaling, 6(277), ra42-ra42.

Panchaud, N, Péli-Gulli, M-P, and De Virgilio, C (2013b). SEACing the GAP that nEGOCiates TORC1 activation. Cell Cycle, 12(18), 2948-2952.

Park, S-H, Bolender, N, Eisele, F, Kostova, Z, Takeuchi, J, Coffino, P, and Wolf, DH (2007). The cytoplasmic Hsp70 chaperone machinery subjects misfolded and endoplasmic reticulum import-incompetent proteins to degradation via the ubiquitin-proteasome system. Molecular Biology of the Cell, 18(1), 153-165.

Park, S-H, Kukushkin, Y, Gupta, R, Chen, T, Konagai, A, Hipp, Mark S, Hayer-Hartl, M, and Hartl, FU (2013). PolyQ Proteins Interfere with Nuclear Degradation of Cytosolic Proteins by Sequestering the Sis1p Chaperone. Cell, 154(1), 134-145.

Parlati, F, Varlamov, O, Paz, K, McNew, JA, Hurtado, D, Söllner, TH, and Rothman, JE (2002). Distinct SNARE complexes mediating membrane fusion in Golgi transport based on combinatorial specificity. Proceedings of the National Academy of Sciences, 99(8), 5424.

Parsell, DA, Kowal, AS, Singer, MA, and Lindquist, S (1994). Protein disaggregation mediated by heat-shock protein Hspl04. Nature, 372(6505), 475-478.

Parsell, DA, Taulien, J, Lindquist, SL, Viitanen, P, Jaenicke, R, Horwich, A, Hartl, FU, Ellis, RJ, Welch, WJ, Ellis, RJ, Laskey, RA, and Lorimer, GH (1993). The role of heat-shock proteins in thermotolerance. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, 339(1289), 279-286.

Petroi, D, Popova, B, Taheri-Talesh, N, Irniger, S, Shahpasandzadeh, H, Zweckstetter, M, Outeiro, TF, and Braus, GH (2012). Aggregate clearance of alpha-synuclein in Saccharomyces cerevisiae depends more on autophagosome and vacuole function than on the proteasome. J Biol Chem, 287(33), 27567-27579.

Pincus, D (2020). Regulation of Hsf1 and the Heat Shock Response. In HSF1 and Molecular Chaperones in Biology and Cancer, M.L. Mendillo, D. Pincus, and R. Scherz-Shouval, eds. (Cham: Springer International Publishing), pp. 41-50.

Pincus, D, Anandhakumar, J, Thiru, P, Guertin, MJ, Erkine, AM, and Gross, DS (2018). Genetic and epigenetic determinants establish a continuum of Hsf1 occupancy and activity across the yeast genome. Molecular Biology of the Cell, 29(26), 3168-3182.

Pohley, H-J (1987). A formal mortality analysis for populations of unicellular organisms (Saccharomyces cerevisiae). Mechanisms of Ageing and Development, 38(3), 231-243.

Powers, ET, and Gierasch, LM (2021). The Proteome Folding Problem and Cellular Proteostasis. Journal of Molecular Biology https://doi.org/10.1016/j.jmb.2021.167197, 167197.

Powers, ET, Morimoto, RI, Dillin, A, Kelly, JW, and Balch, WE (2009). Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem, 78, 959-991.

Powis, K, Schrul, B, Tienson, H, Gostimskaya, I, Breker, M, High, S, Schuldiner, M, Jakob, U, and Schwappach, B (2013). Get3 is a holdase chaperone and moves to deposition sites for aggregated proteins when membrane targeting is blocked. Journal of Cell Science, 126(2), 473-483.

Rampelt, H, Kirstein-Miles, J, Nillegoda, NB, Chi, K, Scholz, SR, Morimoto, RI, and Bukau, B (2012). Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. The EMBO Journal, 31(21), 4221-4235.

Rock, KL, Gramm, C, Rothstein, L, Clark, K, Stein, R, Dick, L, Hwang, D, and Goldberg, AL (1994). Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell, 78(5), 761-771.

Rosenzweig, R, Farber, P, Velyvis, A, Rennella, E, Latham, MP, and Kay, LE (2015). ClpB N-terminal domain plays a regulatory role in protein disaggregation. Proceedings of the National Academy of Sciences, 112(50), E6872.

Rosenzweig, R, Moradi, S, Zarrine-Afsar, A, Glover John, R, and Kay Lewis, E (2013). Unraveling the Mechanism of Protein Disaggregation Through a ClpB-DnaK Interaction. Science, 339(6123), 1080-1083.

Roth, DM, Hutt, DM, Tong, J, Bouchecareilh, M, Wang, N, Seeley, T, Dekkers, JF, Beekman, JM, Garza, D, Drew, L, Masliah, E, Morimoto, RI, and Balch, WE (2014). Modulation of the Maladaptive Stress Response to Manage Diseases of Protein Folding. PLOS Biology, 12(11), e1001998.

Rousseau, F, Serrano, L, and Schymkowitz, JWH (2006). How Evolutionary Pressure Against Protein Aggregation Shaped Chaperone Specificity. Journal of Molecular Biology, 355(5), 1037-1047.

Ruan, L, Zhou, C, Jin, E, Kucharavy, A, Zhang, Y, Wen, Z, Florens, L, and Li, R (2017). Cytosolic proteostasis through importing of misfolded proteins into mitochondria. Nature, 543(7645), 443-446.

Rüdiger, S, Germeroth, L, Schneider-Mergener, J, and Bukau, B (1997). Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. The EMBO Journal, 16(7), 1501-1507.

Rujano, MA, Bosveld, F, Salomons, FA, Dijk, F, van Waarde, MAWH, van der Want, JJL, de Vos, RAI, Brunt, ER, Sibon, OCM, and Kampinga, HH (2006). Polarised Asymmetric Inheritance of Accumulated Protein Damage in Higher Eukaryotes. PLoS Biol, 4(12), e417.

Ryu, SW, Stewart, R, Pectol, DC, Ender, NA, Wimalarathne, O, Lee, J-H, Zanini, CP, Harvey, A, Huibregtse, JM, Mueller, P, and Paull, TT (2020). Proteome-wide identification of HSP70/HSC70 chaperone clients in human cells. PLOS Biology, 18(7), e3000606.

Saarikangas, J, and Barral, Y (2015). Protein aggregates are associated with replicative aging without compromising protein quality control. eLife, 4, e06197.

Sacher, M, Stone, S, and Ferro-Novick, S (1997). The Synaptobrevin-related Domains of Bos1p and Sec22p Bind to the Syntaxin-like Region of Sed5p*. Journal of Biological Chemistry, 272(27), 17134-17138.

Sahi, C, and Craig, EA (2007). Network of general and specialty J protein chaperones of the yeast cytosol. Proceedings of the National Academy of Sciences, 104(17), 7163.

Sanchez, Y, and Lindquist Susan, L (1990). HSP104 Required for Induced Thermotolerance. Science, 248(4959), 1112-1115.

Scheckhuber, CQ, Erjavec, N, Tinazli, A, Hamann, A, Nyström, T, and Osiewacz, HD (2007). Reducing mitochondrial fission results in increased life span and fitness of two fungal ageing models. Nature Cell Biology, 9(1), 99-105.

Schlieker, C, Tews, I, Bukau, B, and Mogk, A (2004). Solubilization of aggregated proteins by ClpB/DnaK relies on the continuous extraction of unfolded polypeptides. FEBS Letters, 578(3), 351-356.

Schuldiner, M, Metz, J, Schmid, V, Denic, V, Rakwalska, M, Schmitt, HD, Schwappach, B, and Weissman, JS (2008). The GET Complex Mediates Insertion of Tail-Anchored Proteins into the ER Membrane. Cell, 134(4), 634-645.

Schütter, M, Giavalisco, P, Brodesser, S, and Graef, M (2020). Local Fatty Acid Channeling into Phospholipid Synthesis Drives Phagophore Expansion during Autophagy. Cell, 180(1), 135-149.e114.

Sekiguchi, T, Hirose, E, Nakashima, N, Ii, M, and Nishimoto, T (2001). Novel G Proteins, Rag C and Rag D, Interact with GTP-binding Proteins, Rag A and Rag B*. Journal of Biological Chemistry, 276(10), 7246-7257.

Seyffer, F, Kummer, E, Oguchi, Y, Winkler, J, Kumar, M, Zahn, R, Sourjik, V, Bukau, B, and Mogk, A (2012). Hsp70 proteins bind Hsp100 regulatory M domains to activate AAA+

disaggregase at aggregate surfaces. Nature Structural & Molecular Biology, 19(12), 1347-1355.

Shiber, A, Breuer, W, Brandeis, M, and Ravid, T (2013). Ubiquitin conjugation triggers misfolded protein sequestration into quality control foci when Hsp70 chaperone levels are limiting. Molecular Biology of the Cell, 24(13), 2076-2087.

Shima, T, Kirisako, H, and Nakatogawa, H (2019). COPII vesicles contribute to autophagosomal membranes. Journal of Cell Biology, 218(5), 1503-1510.

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(10), e26319.

Sinclair, DA, and Guarente, L (1997). Extrachromosomal rDNA Circles— A Cause of Aging in Yeast. Cell, 91(7), 1033-1042.

Singh, R, Kølvraa, S, Bross, P, Jensen, UB, Gregersen, N, Tan, Q, Knudsen, C, and Rattan, SIS (2006). Reduced heat shock response in human mononuclear cells during aging and its association with polymorphisms in HSP70 genes. Cell stress & chaperones, 11(3), 208-215.

Smeal, T, Claus, J, Kennedy, B, Cole, F, and Guarente, L (1996). Loss of Transcriptional Silencing Causes Sterility in Old Mother Cells of S. cerevisiae. Cell, 84(4), 633-642.

Søgaard, M, Tani, K, Ye, RR, Geromanos, S, Tempst, P, Kirchhausen, T, Rothman, JE, and Söllner, T (1994). A rab protein is required for the assembly of SNARE complexes in the docking of transport vesicles. Cell, 78(6), 937-948.

Solís, Eric J, Pandey, Jai P, Zheng, X, Jin, Dexter X, Gupta, Piyush B, Airoldi, Edoardo M, Pincus, D, and Denic, V (2016). Defining the Essential Function of Yeast Hsf1 Reveals a Compact Transcriptional Program for Maintaining Eukaryotic Proteostasis. Molecular Cell, 63(1), 60-71.

Song, J, Yang, Q, Yang, J, Larsson, L, Hao, X, Zhu, X, Malmgren-Hill, S, Cvijovic, M, Fernandez-Rodriguez, J, Grantham, J, Gustafsson, CM, Liu, B, and Nyström, T (2014). Essential genetic interactors of SIR2 required for spatial sequestration and asymmetrical inheritance of protein aggregates. PLoS genetics, 10(7), e1004539-e1004539.

Sorger, PK, and Pelham, HR (1987). Purification and characterization of a heat-shock element binding protein from yeast. The EMBO Journal, 6(10), 3035-3041.

Sorger, PK, and Pelham, HRB (1988). Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell, 54(6), 855-864.

Specht, S, Miller, SBM, Mogk, A, and Bukau, B (2011). Hsp42 is required for sequestration of protein aggregates into deposition sites in Saccharomyces cerevisiae. Journal of Cell Biology, 195(4), 617--629.

Spokoini, R, Moldavski, O, Nahmias, Y, England, JL, Schuldiner, M, and Kaganovich, D (2012). Confinement to organelle-associated inclusion structures mediates asymmetric inheritance of aggregated protein in budding yeast. Cell Reports, 2(4), 738--747.

Squires, CL, Pedersen, S, Ross, BM, and Squires, C (1991). ClpB is the Escherichia coli heat shock protein F84.1. Journal of Bacteriology, 173(14), 4254-4262.

Stadtman, ER (2006). Protein oxidation and aging. Free Radical Research, 40(12), 1250-1258.

Steffen, KK, MacKay, VL, Kerr, EO, Tsuchiya, M, Hu, D, Fox, LA, Dang, N, Johnston, ED, Oakes, JA, Tchao, BN, Pak, DN, Fields, S, Kennedy, BK, and Kaeberlein, M (2008). Yeast Life Span Extension by Depletion of 60S Ribosomal Subunits Is Mediated by Gcn4. Cell, 133(2), 292-302.

Steinkraus, KA, Kaeberlein, M, and Kennedy, BK (2008). Replicative Aging in Yeast: The Means to the End. Annual Review of Cell and Developmental Biology, 24(1), 29-54.

Sturgill, TW, Cohen, A, Diefenbacher, M, Trautwein, M, Martin Dietmar, E, and Hall Michael, N (2008). TOR1 and TOR2 Have Distinct Locations in Live Cells. Eukaryotic Cell, 7(10), 1819-1830.

Summers, DW, Wolfe, KJ, Ren, HY, and Cyr, DM (2013). The Type II Hsp40 Sis1 Cooperates with Hsp70 and the E3 Ligase Ubr1 to Promote Degradation of Terminally Misfolded Cytosolic Protein. PLOS ONE, 8(1), e52099.

Suzuki, K, Akioka, M, Kondo-Kakuta, C, Yamamoto, H, and Ohsumi, Y (2013). Fine mapping of autophagy-related proteins during autophagosome formation in Saccharomyces cerevisiae. Journal of Cell Science, 126(11), 2534-2544.

Takeshige, K, Baba, M, Tsuboi, S, Noda, T, and Ohsumi, Y (1992). Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. Journal of Cell Biology, 119(2), 301-311.

Tang, F, Kauffman, EJ, Novak, JL, Nau, JJ, Catlett, NL, and Weisman, LS (2003). Regulated degradation of a class V myosin receptor directs movement of the yeast vacuole. Nature, 422(6927), 87-92.

Tang, F, Watkins, JW, Bermudez, M, Gray, R, Gaban, A, Portie, K, Grace, S, Kleve, M, and Craciun, G (2008). A lifespan-extending form of autophagy employs the vacuole-vacuole fusion machinery. Autophagy, 4(7), 874-886.

Taylor, JP, Tanaka, F, Robitschek, J, Sandoval, CM, Taye, A, Markovic-Plese, S, and Fischbeck, KH (2003). Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. Human Molecular Genetics, 12(7), 749-757.

Teichert, U, Mechler, B, Müller, H, and Wolf, DH (1989). Lysosomal (Vacuolar) Proteinases of Yeast Are Essential Catalysts for Protein Degradation, Differentiation, and Cell Survival. Journal of Biological Chemistry, 264(27), 16037-16045.

Terry, RD, Masliah, E, Salmon, DP, Butters, N, DeTeresa, R, Hill, R, Hansen, LA, and Katzman, R (1991). Physical basis of cognitive alterations in alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Annals of Neurology, 30(4), 572-580.

Tessarz, P, Mogk, A, and Bukau, B (2008). Substrate threading through the central pore of the Hsp104 chaperone as a common mechanism for protein disaggregation and prion propagation. Molecular Microbiology, 68(1), 87-97.

Tessarz, P, Schwarz, M, Mogk, A, and Bukau, B (2009). The Yeast AAA+ Chaperone Hsp104 Is Part of a Network That Links the Actin Cytoskeleton with the Inheritance of Damaged Proteins. Molecular and Cellular Biology, 29(13), 3738-3745.

Thayer, NH, Leverich, CK, Fitzgibbon, MP, Nelson, ZW, Henderson, KA, Gafken, PR, Hsu, JJ, and Gottschling, DE (2014). Identification of long-lived proteins retained in cells undergoing repeated asymmetric divisions. Proceedings of the National Academy of Sciences, 111(39), 14019-14026.

Thrower, JS, Hoffman, L, Rechsteiner, M, and Pickart, CM (2000). Recognition of the polyubiquitin proteolytic signal. The EMBO Journal, 19(1), 94-102.

Tian, G, Vainberg, IE, Tap, WD, Lewis, SA, and Cowan, NJ (1995). Specificity in chaperonin-mediated protein folding. Nature, 375(6528), 250-253.

Tong, AHY, Evangelista, M, Parsons, AB, Xu, H, Bader, GD, Pagé, N, Robinson, M, Raghibizadeh, S, Hogue, CWV, Bussey, H, Andrews, B, Tyers, M, and Boone, C (2001).

Systematic Genetic Analysis with Ordered Arrays of Yeast Deletion Mutants. Science, 294(5550), 2364-2368.

Tong, AHY, Lesage, G, Bader, GD, Ding, H, Xu, H, Xin, X, Young, J, Berriz, GF, Brost, RL, Chang, M, Chen, Y, Cheng, X, Chua, G, Friesen, H, Goldberg, DS, Haynes, J, Humphries, C, He, G, Hussein, S, Ke, L, Krogan, N, Li, Z, Levinson, JN, Lu, H, Ménard, P, Munyana, C, Parsons, AB, Ryan, O, Tonikian, R, Roberts, T, Sdicu, A-M, Shapiro, J, Sheikh, B, Suter, B, Wong, SL, Zhang, LV, Zhu, H, Burd, CG, Munro, S, Sander, C, Rine, J, Greenblatt, J, Peter, M, Bretscher, A, Bell, G, Roth, FP, Brown, GW, Andrews, B, Bussey, H, and Boone, C (2004). Global Mapping of the Yeast Genetic Interaction Network. Science, 303(5659), 808-813.

Treger, JM, Schmitt, AP, Simon, JR, and McEntee, K (1998). Transcriptional Factor Mutations Reveal Regulatory Complexities of Heat Shock and Newly Identified Stress Genes in Saccharomyces cerevisiae *. Journal of Biological Chemistry, 273(41), 26875-26879.

Tripathi, P, Singh, LK, Kumari, S, Hakiem, OR, and Batra, JK (2020). ClpB is an essential stress regulator of Mycobacterium tuberculosis and endows survival advantage to dormant bacilli. International Journal of Medical Microbiology, 310(3), 151402.

Trotter, EW, Kao, CMF, Berenfeld, L, Botstein, D, Petsko, GA, and Gray, JV (2002). Misfolded Proteins Are Competent to Mediate a Subset of the Responses to Heat Shock in Saccharomyces cerevisiae *. Journal of Biological Chemistry, 277(47), 44817-44825.

Tye, BW, Commins, N, Ryazanova, LV, Wühr, M, Springer, M, Pincus, D, and Churchman, LS (2019). Proteotoxicity from aberrant ribosome biogenesis compromises cell fitness. eLife, 8, e43002.

Ungelenk, S, Moayed, F, Ho, C-T, Grousl, T, Scharf, A, Mashaghi, A, Tans, S, Mayer, MP, Mogk, A, and Bukau, B (2016). Small heat shock proteins sequester misfolding proteins in near-native conformation for cellular protection and efficient refolding. Nature Communications, 7, 13673.

Ungermann, C, Neupert, W, and Cyr Douglas, M (1994). The Role of Hsp70 in Conferring Unidirectionality on Protein Translocation into Mitochondria. Science, 266(5188), 1250-1253.

Vabulas Ramunas, M, and Hartl, FU (2005). Protein Synthesis upon Acute Nutrient Restriction Relies on Proteasome Function. Science, 310(5756), 1960-1963.

Velázquez, AP, Tatsuta, T, Ghillebert, R, Drescher, I, and Graef, M (2016). Lipid droplet–mediated ER homeostasis regulates autophagy and cell survival during starvation. Journal of Cell Biology, 212(6), 621-631.

Verma, R, Aravind, L, Oania, R, McDonald, WH, Yates John, R, Koonin Eugene, V, and Deshaies Raymond, J (2002). Role of Rpn11 Metalloprotease in Deubiquitination and Degradation by the 26S Proteasome. Science, 298(5593), 611-615.

Walther, Dirk M, Kasturi, P, Zheng, M, Pinkert, S, Vecchi, G, Ciryam, P, Morimoto, Richard I, Dobson, Christopher M, Vendruscolo, M, Mann, M, and Hartl, FU (2015). Widespread Proteome Remodeling and Aggregation in Aging C. elegans. Cell, 161(4), 919-932.

Wang, F, Brown, EC, Mak, G, Zhuang, J, and Denic, V (2010). A Chaperone Cascade Sorts Proteins for Posttranslational Membrane Insertion into the Endoplasmic Reticulum. Molecular Cell, 40(1), 159-171.

Weibezahn, J, Tessarz, P, Schlieker, C, Zahn, R, Maglica, Z, Lee, S, Zentgraf, H, Weber-Ban, EU, Dougan, DA, Tsai, FTF, Mogk, A, and Bukau, B (2004). Thermotolerance Requires

Refolding of Aggregated Proteins by Substrate Translocation through the Central Pore of ClpB. Cell, 119(5), 653-665.
Weindruch, R (1996). The Retardation of Aging by Caloric Restriction: Studies in Rodents and Primates. Toxicologic Pathology, 24(6), 742-745.
Werner-Washburne, M, Stone, DE, and Craig, EA (1987). Complex interactions among members of an essential subfamily of hsp70 genes in Saccharomyces cerevisiae. Molecular and Cellular Biology, 7(7), 2568.
Wiemken, A, Matile, P, and Moor, H (1970). Vacuolar dynamics in synchronously budding yeast. Archiv für Mikrobiologie, 70(2), 89-103.
Willmund, F, del Alamo, M, Pechmann, S, Chen, T, Albanèse, V, Dammer, Eric B, Peng, J, and Frydman, J (2013). The Cotranslational Function of Ribosome-Associated Hsp70 in Eukaryotic Protein Homeostasis. Cell, 152(1–2), 196-209.
Winkler, J, Tyedmers, J, Bukau, B, and Mogk, A (2012). Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation. Journal of Cell Biology, 198(3), 387-404.
Winklhofer, KF, Tatzelt, J, and Haass, C (2008). The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. The EMBO Journal, 27(2), 336-349.
Wolfe, KJ, and Cyr, DM (2011). Amyloid in neurodegenerative diseases: Friend or foe? Seminars in Cell & Developmental Biology, 22(5), 476-481.
Wolfe, KJ, Ren, HY, Trepte, P, and Cyr, DM (2013). The Hsp70/90 cochaperone, Sti1, suppresses proteotoxicity by regulating spatial quality control of amyloid-like proteins. Molecular Biology of the Cell, 24(23), 3588-3602.
Yabal, M, Brambillasca, S, Soffientini, P, Pedrazzini, E, Borgese, N, and Makarow, M (2003). Translocation of the C Terminus of a Tail-anchored Protein across the Endoplasmic Reticulum Membrane in Yeast Mutants Defective in Signal Peptide-driven Translocation. Journal of Biological Chemistry, 278(5), 3489-3496.
Yamamoto, A, Lucas, JJ, and Hen, R (2000). Reversal of Neuropathology and Motor Dysfunction in a Conditional Model of Huntington's Disease. Cell, 101(1), 57-66.
Yamamoto, A, Ueda, J, Yamamoto, N, Hashikawa, N, and Sakurai, H (2007). Role of Heat Shock Transcription Factor in Saccharomyces cerevisiae Oxidative Stress Response. Eukaryotic Cell, 6(8), 1373-1379.
Yamamoto, H, Kakuta, S, Watanabe, TM, Kitamura, A, Sekito, T, Kondo-Kakuta, C, Ichikawa, R, Kinjo, M, and Ohsumi, Y (2012). Atg9 vesicles are an important membrane source during early steps of autophagosome formation. Journal of Cell Biology, 198(2), 219-233.
Yan, W, Schilke, B, Pfund, C, Walter, W, Kim, S, and Craig, EA (1998). Zuotin, a ribosome-associated DnaJ molecular chaperone. The EMBO journal, 17(16), 4809-4817.
Yang, J, Hao, X, Cao, X, Liu, B, and Nyström, T (2016). Spatial sequestration and detoxification of Huntingtin by the ribosome quality control complex. eLife, 5, e11792.
Yang, S, Huang, S, Gaertig, Marta A, Li, X-J, and Li, S (2014). Age-Dependent Decrease in Chaperone Activity Impairs MANF Expression, Leading to Purkinje Cell Degeneration in Inducible SCA17 Mice. Neuron, 81(2), 349-365.
Yu, A, Shibata, Y, Shah, B, Calamini, B, Lo, DC, and Morimoto, RI (2014). Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition. Proceedings of the National Academy of Sciences, 111(15), E1481-E1490.

Zhang, X, Smith, DL, Meriin, AB, Engemann, S, Russel, DE, Roark, M, Washington, SL, Maxwell, MM, Marsh, JL, Thompson, LM, Wanker, EE, Young, AB, Housman, DE, Bates, GP, Sherman, MY, and Kazantsev, AG (2005). A potent small molecule inhibits polyglutamine aggregation in Huntington's disease neurons and suppresses neurodegeneration *in vivo*. Proceedings of the National Academy of Sciences of the United States of America, 102(3), 892.

Zheng, X, Krakowiak, J, Patel, N, Beyzavi, A, Ezike, J, Khalil, AS, and Pincus, D (2016). Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. eLife, 5, e18638.

Zhou, C, Slaughter, Brian D, Unruh, Jay R, Eldakak, A, Rubinstein, B, and Li, R (2011). Motility and Segregation of Hsp104-Associated Protein Aggregates in Budding Yeast. Cell, 147(5), 1186-1196.

Zhou, C, Slaughter, Brian D, Unruh, Jay R, Guo, F, Yu, Z, Mickey, K, Narkar, A, Ross, Rhonda T, McClain, M, and Li, R (2014). Organelle-Based Aggregation and Retention of Damaged Proteins in Asymmetrically Dividing Cells. Cell, 159(3), 530-542.

Zhou, F, Wu, Z, Zhao, M, Murtazina, R, Cai, J, Zhang, A, Li, R, Sun, D, Li, W, Zhao, L, Li, Q, Zhu, J, Cong, X, Zhou, Y, Xie, Z, Gyurkovska, V, Li, L, Huang, X, Xue, Y, Chen, L, Xu, H, Xu, H, Liang, Y, and Segev, N (2019). Rab5-dependent autophagosome closure by ESCRT. Journal of Cell Biology, 218(6), 1908-1927.

Zhu, X, Zhao, X, Burkholder William, F, Gragerov, A, Ogata Craig, M, Gottesman Max, E, and Hendrickson Wayne, A (1996). Structural Analysis of Substrate Binding by the Molecular Chaperone DnaK. Science, 272(5268), 1606-1614.

Zieger, M, and Mayer, A (2012). Yeast vacuoles fragment in an asymmetrical two-phase process with distinct protein requirements. Molecular biology of the cell, 23(17), 3438-3449.

Ziętkiewicz, S, Krzewska, J, and Liberek, K (2004). Successive and Synergistic Action of the Hsp70 and Hsp100 Chaperones in Protein Disaggregation*. Journal of Biological Chemistry, 279(43), 44376-44383.

Żwirowski, S, Kłosowska, A, Obuchowski, I, Nillegoda, NB, Piróg, A, Ziętkiewicz, S, Bukau, B, Mogk, A, and Liberek, K (2017). Hsp70 displaces small heat shock proteins from aggregates to initiate protein refolding. The EMBO Journal, 36(6), 783-796.