Defining the pathways involved in spatial protein quality control in Saccharomyces cerevisiae

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Cover illustration: “Saccharomyces cerevisiae brennt, weil sie so hice ist” by Nina-Melissa Seelinger

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Schließe für einen Moment
die Augen und du wirst sehen,
wofür all deine Mühen und
Anstrengungen gut waren.

Der Business-Lion

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ABSTRACT

Proteins need to be folded into specific three-dimensional conformations to be functional. An extensive network of factors, the protein quality control (PQC) machinery, ensures that the cell maintains a healthy proteome by coordinating their synthesis, folding, transport and degradation. A disruption in this machinery can lead to accumulation of protein aggregates, which is a hallmark of aging and many human pathologies. A key question in research on PQC is why and how certain aberrant proteins cause proteotoxicity, while others can be efficiently handled by the PQC. Sequestration of aggregates into larger inclusions and their deposition at distinct cellular sites have been suggested to be cytoprotective functions of spatial PQC.

The articles included in this thesis use budding yeast to study spatial PQC in heat-stressed cells. Expanding the toolbox by a set of misfolding reporters and comparing their behavior revealed that diverse protein species are cleared at differential rates, even when residing in shared, intermixed protein inclusions. Using genome-wide screens, we pinpointed Sed5 and Sec7, major regulators of vesicle trafficking, as key factors controlling spatial PQC and disease protein detoxification. Electron microscopy of heat-stressed cells showed that aggregates localize predominantly in proximity to both mitochondria and virus-like particles. Our findings contribute to an increased understanding of three major features of spatial PQC: aggregate clearance, sequestration and intracellular location.

Keywords: proteostasis, chaperones, aggregation, yeast, heat shock, stress, aging, mitochondria, virus-like particles

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SAMMANFATTNING PÅ SVENSKA

Definiering av processer involverade i rumslig proteinkvalitets-kontroll i Saccharomyces cerevisiae

Proteiner behöver veckas till en tredimensionell struktur för att vara funktionella. Ett stort nätverk av olika faktorer - tillsammans kallat proteinkvalitets-kontroll - säkerställer att celler vidmakthåller ett friskt 'proteome' (cellens kompletta arsenal av olika proteiner) genom att koordinera och balansera proteiners syntes, veckning, transport och nedbrytning. Många sjukdomar, samt åldrande, kännetecknas av störningar i denna balans och en ackumulering av proteinaggregat. En nyckelfråga inom forskningen kring proteinkvalitets-kontroll är varför och hur vissa fel-veckade proteiner är särskilt skadliga för cellen medan andra hanteras effektivt. Den rumsliga, eller spatiala, kvalitetskontrollen samlar proteinaggregat i stora konglomerat på avgränsade platser i cellen; en process som antas skydda cellen mot de toxiska effekter ett fel-veckat protein kan orsaka.

Denna avhandling har använt jäst för att studera rumslig kvalitetskontroll i värmestressade celler och en utvidgad repertoar av fluorescerande rapportörer för proteinveckning och aggregering. Analysen av dessa rapportörer visar att olika fel-veckade proteiner rensas med olika hastighet i cellen även när de återfinns i samma protein-konglomerat. Med hjälp av en genom-vid undersökning fann vi att Sed5 och Sec7, nyckelfaktorer i cellens vesikel-transport, kontrollerar rumslig kvalitetskontroll och skyddar mot sjukdomsalstrande proteiner. Elektronmikroskopi visade att konglomerat av fel-veckade proteiner i övervägande grad ansamlas vid mitokondrier och virus-lika partiklar. Våra resultat medverkar till en ökad förståelse för hur och var fel-veckade proteiner ansamlas vid speciella lokus och avlägsnas från cellen.

LIST OF PAPERS

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

I. Schneider KL, Wollman AJM, Nyström T, Shashkova S. Comparison of endogenously expressed fluorescent protein fusions behaviour for protein quality control and cellular ageing research. Sci Rep. 2021 Jun 17;11(1):12819.

II. Schneider KL, Ahmadpour D, Keuenhof KS, Eisele-Bürger AM, Berglund LL, Eisele F, Babazadeh R, Höög JL, Nyström T, Widlund PO. Using reporters of different misfolded proteins reveals differential strategies in processing protein aggregates. J Biol Chem. 2022 Sep 9:102476. Epub ahead of print.

III. Babazadeh R*, Ahmadpour D*, Jia S, Hao X, Widlund P, Schneider K, Eisele F, Edo LD, Smits GJ, Liu B, Nystrom T. Syntaxin 5 Is Required for the Formation and Clearance of Protein Inclusions during Proteostatic Stress. Cell Rep. 2019 Aug 20;28(8):2096-2110.e8.

IV. Babazadeh R*, Schneider KL*, Fischbach A, Hao X, Liu B, Nystrom T. The yeast guanine nucleotide exchange factor Sec7 is a bottleneck in spatial protein quality control and detoxifies neurological disease proteins. Manuscript.

V. Schneider KL, Hao X, Keuenhof KS, Berglund LL, Goméz P, Ahmadpour D, Höög JL, Nyström T, Widlund PO. Heat-induced protein aggregates co-localize with mitochondria and virus-like particles. Manuscript.

* indicates equal authorship contribution

The following articles are not included in this thesis.

Schneider KL, Nyström T, Widlund PO. Studying Spatial Protein Quality Control, Proteopathies, and Aging Using Different Model Misfolding Proteins in S. cerevisiae. Front Mol Neurosci. 2018 Jul 23;11:249. Review.

Keuenhof KS, Larsson Berglund L, Malmgren Hill S, Schneider KL, Widlund PO, Nyström T, Höög JL. Large organellar changes occur during mild heat shock in yeast. J Cell Sci. 2022 Mar 1;135(5):jcs258325. Epub 2021 Aug 11.

Schneider KL, Reibenspies LE, Nyström T, Shashkova S. Growth Rate Evaluation of the Budding Yeast Saccharomyces cerevisiae Cells Carrying Endogenously Expressed Fluorescent Protein Fusions. Methods Mol Biol. 2023;2564:213-222. Methods chapter.

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ABBREVIATIONS

3D-SIM	3-dimensional structured illumination microscopy
ALP	Alkaline phosphatase
APOD	Age-associated protein deposit
ATP	Adenosine triphosphate
CCT	Chaperonin Containing TCP-1
COG	Conserved oligomeric Golgi
COP	Coat protein complex
CORVET	Class C core vacuole/endosome tethering
EM	Electron microscopy
END	Endocytosis
ERAD	ER-associated degradation
GFP	Green fluorescent protein
GO	Gene ontology
HOPS	Homotypic fusion and protein sorting
HSP	Heat shock protein
IMiQ	Intramitochondrial protein quality control compartment
INQ	Intranuclear quality control compartment
IPOD	Insoluble protein deposit
JUNQ	Juxta nuclear quality control compartment
kDa	Kilodalton
LM	Light microscopy
LTR	Long-terminal repeat
MAGIC	Mitochondria as guardian in cytosol
mRNA	Messenger RNA
MTOC	Microtubule organizing center
MVB	Multivesicular bodies
PAS	Pre-autophagosomal structure
PKA	Protein kinase A
PQC	Protein quality control
RAC	Ribosome-associated complex
RCY	Recycling
RQC	Ribosome-associated quality control
SAFE	Spatial analysis of functional enrichment
SEC	Secretory
SGA	Synthetic genetic array
sHsp	Small heat shock protein
SNARE	Soluble *N*-ethylmaleimide-sensitive-factor attachment protein
TRiC	T-complex protein Ring Complex
ts	Temperature-sensitive
tss	Temperature-sensitive synthesis
UPR	Unfolded protein response
vCLAMP	Vacuole and Mitochondria Patch
VLP	Virus-like particle
VPS	Vacuolar protein sorting

AIM OF THE THESIS

The aim of this thesis was to elucidate the pathways of spatial PQC (sPQC) of cytosolic proteins upon heat stress and replicative aging using *Saccharomyces cerevisiae*. The articles included in this thesis focus on three major features of cytosolic PQC: the clearance of visible protein aggregates, the sequestration of aggregates into larger inclusions and their intracellular location.

For this, we expanded the proteostasis toolbox with non-toxic misfolding reporter proteins that are handled by the PQC machinery. I sought out to characterize fluorescent protein fusions of Hsp104, a commonly used marker for endogenous protein aggregates (Paper I), and guk1-7, gus1-3 and pro3-1, three yeast proteins that misfold upon elevated temperature (Paper II). We aimed to identify common and distinct PQC pathways among these reporters and found that the removal of different protein species out of the cells occurred at differential rates, even when they resided in intermixed inclusions (Paper II).

To complement candidate-based approaches that identified sorting factors of sPQC during heat stress, we employed a fluorescent protein fusion of Hsp104 for genome-wide screens using high-content microscopy (Paper III, IV). This approach led to the identification of new factors of sPQC, which are not part of the canonical proteostasis network (PN), specifically Sed5 and Sec7 of the vesicle trafficking machinery. We aimed to test the relevance of this pathway in a broader sense of proteotoxicity and found that it affects fitness upon other proteotoxic stress and acts in disease protein detoxification (Paper III, IV).

Previous studies showed that heat-induced protein aggregates localize to mitochondria and we intended to examine this intracellular localization in more detail (Paper III). We performed a genome-wide screen with high-content microscopy for factors required for formation of inclusions at this site and found that the machineries involved in this sPQC pathway differ depending on the protein species (Paper V). Imaging heat-stressed yeast cells at ultra-resolution allowed us to identify another cellular component that is in proximity to inclusions at mitochondria, the virus-like particles, which may provide novel insights into the potential significance of the localization of aggregates (Paper V).

1 PROTEIN QUALITY CONTROL

Proteins perform numerous essential functions in the cell; they act as enzymes, provide structure and carry out signaling tasks. They are ubiquitous in all forms of life and are encoded by the genome. Proteins have a certain timespan in which they are functional and active before being removed from the cell. Consequently, cells constantly produce, fold and degrade proteins (Figure 1). At all times and especially when environmental conditions change, these processes need to be in a functional balance in the cell, called proteostasis (protein homeostasis) (Balch et al., 2008). As proteins are critical for survival, a well-buffered machinery, the proteostasis network (PN), ensures their function (Hartl et al., 2011; Hipp et al., 2014). The PN consists of the translational machinery, molecular chaperones and co-chaperones and the two main proteolytic pathways, the ubiquitin-proteasome system (UPS) and autophagy (Labbadia and Morimoto, 2015a).

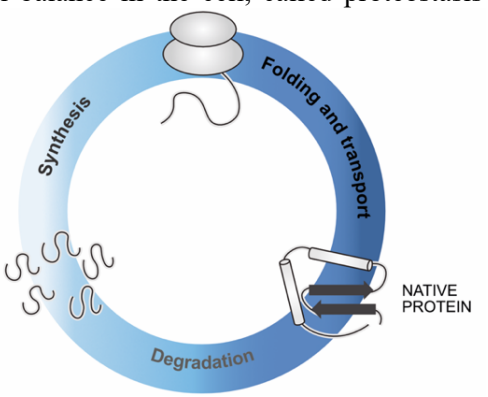

Figure 1: Protein homeostasis is the balanced synthesis, folding and degradation of proteins to maintain a functional proteome.

Proteins are diverse macromolecules, made up of amino acids that form polypeptide chains. The amino acids are determined by the genetic code, which is first transcribed into mRNA and then translated into polypeptide chains by ribosomes. The information to reach their functional three-dimensional structure is found within the amino acid sequence and folding is driven by hydrophobic forces, which cause proteins to hide their hydrophobic regions (non-polar amino acids) within a protein core to maintain a stable native state within the polar cytosol. In addition, molecular chaperones facilitate the folding process.

Changes in intracellular and environmental conditions challenge proteostasis as proteins can get damaged and un- or misfold, causing them to lose their stable structure (Figure 2). Stressors are, for example, a change in pH or temperature, exposure to cell-damaging agents such as UV, or oxidative stress. Aberrant proteins can also be generated due to mutations or erroneous translation. In addition, the cellular concentration of proteins above a certain threshold depending on their solubility causes them to be particularly

vulnerable to misfolding, as they adopt metastable, aggregation-prone states (supersaturation) (Ciryam et al., 2013, 2015). When protein function and stability are not maintained, proteostasis is at risk, posing an immediate threat to survival not only due to loss-of-function of essential proteins but also due to consequences of the crowded milieu that the cell provides, since aberrant proteins can enter toxic gain-of-function interactions with other proteins (Gidalevitz et al., 2006; Olzscha et al., 2011; Park et al., 2013; Watanabe et al., 2001; Yu et al., 2014). They can also enter an unfolded or intermediate folding state, either directly during/after translation or by leaving their once stable native fold. Misfolded proteins disturb proteostasis by exposing their hydrophobic sequences that are normally buried within the protein core, provoking further misfolding of other proteins in the cell and protein

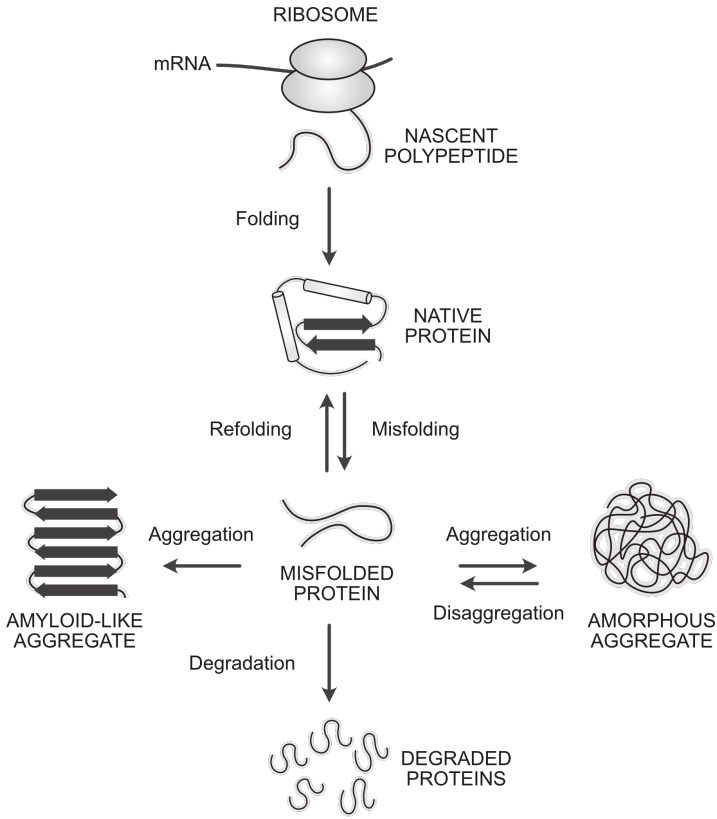

Figure 2: Protein fates in the cell. Nascent polypeptide chains are generated through translation and adopt a functional three-dimensional state. Proteins can misfold and pose a risk to proteostasis. Misfolded proteins can aggregate into amorphous or amyloid-like aggregates or be degraded. Disaggregation of amorphous aggregates allows recovery of the protein for subsequent refolding or degradation.

aggregation. The aggregates themselves can interfere with cellular function and sequester functional proteins or factors of protein quality control (PQC), which are consequently not available to facilitate folding of nascent polypeptide chains. Exposure of hydrophobic residues that seed aggregation can also lead to proteins forming highly structured, fibrillar aggregates called amyloids, which are hallmark protein accumulations of various pathologies. It is thus crucial that the cell targets aberrant proteins for refolding, degradation or sequestration into larger inclusions at protective sites to evade potential proteotoxicity (Balchin et al., 2016; Hartl et al., 2011; Hartl and Hayer-Hartl, 2009; Hipp et al., 2014; Sontag et al., 2014, 2017).

Denatured proteins can adopt different types of nonfunctional, aggregated forms and are generally categorized into amorphous aggregates and amyloids or amyloid-like aggregates. Amorphous aggregates are unstructured and expose hydrophobic patches, while amyloid-like aggregates are densely packed β-sheet-containing fibrils. Amyloidogenic/amyloid-like aggregates are common of prion and other neurological diseases. The categorization into these two types of protein aggregates is a simplification, since even the same protein species can adopt different aggregation structures with varying properties and effects on the cell and the aggregate structure is not always known (Caron et al., 2014; Gruber et al., 2018; Wang et al., 2010).

2 MISFOLDED PROTEINS IN DISEASE AND AGING

The accumulation of aberrant or damaged proteins is found in numerous human pathologies, often incurable and many of them associated with aging, including neurodegenerative diseases, amyotrophic lateral sclerosis, prion diseases, diabetes and cancer. They commonly entail protein conformation changes and aggregation and pose a challenge to understanding the exact mechanisms of toxicity due to the widespread effects of the proteinaceous accumulations. It is often unclear whether the aggregation of a protein is toxic in itself or cytoprotective. To make the study of such diseases even more complicated, the proteotoxicity is often not the only observed phenotype in models and patients. For example, many neurodegenerative diseases accumulate aggregates in the nucleus and cytoplasm of neurons but are concomitantly associated with mitochondrial dysfunction (Borsche et al., 2021; Ocampo et al., 2010; Papsdorf et al., 2015; Solans et al., 2006; Sorrentino et al., 2017). The following two sections highlight the significance of studying cellular PQC for human health by giving examples of protein conformational diseases and describing the deterioration of the proteostasis system during aging. Established yeast models are available for research addressing misfolded proteins in disease and aging and several will be discussed in more detail throughout the thesis.

2.1 PRION DISEASES

Prions are proteins that have adopted an alternative conformation and are self-propagating and transmissible. The proteins contain prion-domains that are intrinsically disordered and QN-rich. These characteristics of the amino acid sequence suffice for prion and amyloid formation. The hereditary element is the alternative protein isoform itself. Such a concept of transmission has first been shown using a yeast prion (Kraus et al., 2013; Wickner, 1994; Wickner et al., 2015) and is of major interest due to known mammalian prion diseases such as sheep scrapie, human Creutzfeldt-Jacob and bovine spongiform encephalopathy. These prion diseases are characterized by specific regular cellular proteins adopting alternative folds that cause the formation of insoluble cross-β sheet aggregates, called amyloids (Shewmaker et al., 2006; Wickner et al., 2008). These aggregates recruit the PQC machinery for their propagation: several chaperones are required to shear the fibrils into smaller fragments, which then act as “seeds” that nucleate further protein aggregation and prion segregation. While the articles included in this thesis do not cover

prions, they serve as a valuable example for the role of PQC in human disease and share many PQC factors with the machinery that handles other types of protein aggregates and protects the cells from proteotoxicity.

Much work on cellular mechanisms of prions has been done using budding yeast. An early study was able to demonstrate the link between the Sup35 prion, [PSI+] and the disaggregase Hsp104 in prion propagation, which is the general disaggregase in budding yeast and part of a tri-chaperone machinery that targets not only β-sheet rich amyloidogenic but also amorphous aggregates and attempts to re-solubilize them. Hsp104 is unable to aid in removal of prions as it can only fragment the fibrillar prion accumulations into smaller parts, which ends up promoting their propagation. Prions thus hijack the cellular PQC machinery for proliferation (Chernova et al., 2017; Liebman and Chernoff, 2012). However, other parts of the proteostasis machinery are capable of curing cells from prions (Kryndushkin et al., 2008). Consequently, the proteostasis machinery is intimately linked to prions and in-depth understanding of mechanisms of the PQC is necessary to advance research on prion diseases (Chernova et al., 2017).

2.2 OTHER NEURODEGENERATIVE DISEASES

A certain type of neurodegenerative disease manifests in the accumulation of polyglutamine-extended proteins, which form fibrillar amyloids. Such polyQ-expansions are hereditary, mostly in an autosomal-dominant fashion, due to an abnormal increase in number of CAG repeats within the coding region of a gene. The most prominent example of a neurological disorder based on an expanded polyQ tract within a regular cellular protein is Huntington's Disease (HD) (Sampaio-Marques et al., 2019). Similar to prions, the aggregates formed by mutant Huntingtin are mainly insoluble β-sheet-rich aggregates with a high tendency to aggregate with themselves. However, also other types of mutant Huntingtin aggregates have been described, such as soluble oligomers, which makes it challenging to understand the exact mechanism of toxicity in HD. Despite much effort and many model systems, consensus on what lies behind toxicity and distinguishes it from other types of protein aggregates has not been reached (Tenreiro and Outeiro, 2010). In Alzheimer's Disease (AD), two proteins have been found to form extracellular fibrils: amyloid beta forms extracellular plaques and tau captures microtubules and creates neurofibrillary tangles. The central idea for toxicity is the same as for other amyloid diseases in that the presence of amyloid accumulations induces neuronal loss. Parkinson's Disease (PD) and Dementia with Lewy Bodies both display

aggregating alpha-synuclein, (Franssens et al., 2010; Tenreiro et al., 2017), however, it is unclear whether soluble precursors or large inclusions or a combination of both are causative of neurotoxicity. Interestingly, much support for a role of alpha-synuclein in endocytosis has accumulated over recent years, which makes this neurological disease protein especially interesting in the context of the connection between PQC and vesicle trafficking (Ahmadpour et al., 2020; Treusch et al., 2011). Strikingly, it is evident that only certain protein species are associated with neurological disease etiology and even though there is no obvious sequence similarity among these known disease proteins, they all form amyloidogenic aggregates (Labbadia and Morimoto, 2015a).

2.3 PROTEIN QUALITY CONTROL IN AGING

Aging is a universal process that affects nearly all living organisms and is characterized by a gradual, progressive decline of cellular functions. The lifespan of organisms differs greatly between species, indicating that while aging overall is inevitable, the rate of aging may be modifiable. Thus, research focuses largely on aging-interventions and therapeutic means to tackle age-associated pathologies that imply higher mortality, such as cardiovascular diseases, atherosclerosis, inflammation, neurodegeneration and cancer (López-Otín et al., 2013). Since aging is complex and multifactorial, the fundamentals of aging need to be studied in simplified systems. Mechanisms of aging within the cell are intertwined, remarkably conserved across species and most importantly, still unsolved.

Aging is characterized by the intracellular accumulation of damage. One of the defined hallmarks of aging is the deterioration of proteostasis (López-Otín et al., 2013). The proteostasis machinery maintains a functional proteome during regular growth conditions and during stress. And while the PN is outstandingly robust and adaptable to various stress conditions, during aging it eventually loses its ability to manage aberrant and damaged proteins and overall declines in function. The link between aging and neurodegenerative disorders signifies how the proteostasis collapse during aging contributes to human pathology. Notably, modifying the capacity of the PQC machinery enables longer life- and healthspan of various organisms. The PN is thus considered a potential target for age-related disease therapeutics and aging interventions. It is certain that proteostasis needs to be investigated in multicellular systems, across tissues and organs to ultimately understand human aging. However, aging is currently far from fully understood on the cellular level, especially regarding the temporal organization of the cellular and proteostasis decline during aging

and the interconnectivity of aging (Gottschling and Nyström, 2017; Hill et al., 2017).

The decrease and ultimate collapse in cellular functions is connected to the focus of cells to allocate their resources to reproductivity (Labbadia and Morimoto, 2015b; Shai et al., 2014; Shemesh et al., 2013). This principle can be applied, for example, to the asymmetric inheritance of aging factors when cells divide, which facilitates rejuvenation of progeny at the cost of aging of the progenitor cell due to damage accumulation. The decline in proteostasis during aging is known to manifest in numerous ways and disturbs several parts of the PN. Indeed, the entire proteome undergoes remodeling during aging, including changes in the PN itself, and culminates in age-dependent protein aggregation. The ubiquitin-proteasome system (UPS), which oversees disposal of functional proteins for regular turnover and aberrant proteins, decreases in activity when cells age in various model systems (Torres et al., 2006; Vilchez et al., 2012; Walther et al., 2015). This can result in a detrimental feedback loop, since aggregated proteins can sequester proteasomes and impair their function further (Bence et al., 2001; Holmberg et al., 2004). Boosting proteasome capacity improves proteostasis in old cells and extends lifespan to some extent (Andersson et al., 2013; Nguyen et al., 2019). In fact, increasing levels of numerous PN pathways at once by overproducing master regulators of stress-adaptive responses has been shown to restore proteostasis and health upon aging (Ben-Zvi et al., 2009). Another major contributor to proteostasis, the molecular chaperone system, has also been found to change substantially upon aging, in that certain parts of the network become in- or decreased in their expression (Brehme et al., 2014). Specifically, small Hsps, ATP-independent chaperones (Section 3.1.3), are induced upon aging (Walther et al., 2015), while ATP-dependent chaperone families are repressed. Such changes in chaperone expression levels were observed in human brain datasets, and thus present a direct link between proteostasis, aging and age-associated neurological diseases (Brehme et al., 2014).

3 THE PROTEOSTASIS NETWORK

A healthy proteome is crucial for cell function and survival, as exemplified by the deterioration of proteostasis in many diseases and upon aging. Cells contain numerous dedicated factors and mechanisms, which operate in a concerted manner to maintain proteostasis by ensuring and regulating proper synthesis, folding, transport and degradation of proteins, and to prevent proteotoxicity. Molecular chaperones play key roles within all these PQC pathways and thus constitute a major part of the proteostasis network (PN).

Budding yeast has two lines of defense to evade proteotoxicity, the temporal and the spatial PQC. Temporal PQC is considered to consist of chaperones, the 26S proteasome and autophagy pathways and controls folding and re-folding of proteins, degradation of misfolded proteins and of functional proteins as a regular turnover mechanism. The spatial PQC machinery handles oligomers and aggregated proteins by sequestration into larger inclusions at protective locations and acts in parallel to the temporal PQC (Hill et al., 2017; Josefson et al., 2017).

3.1 MOLECULAR CHAPERONES

Molecular chaperones act through binding and releasing client proteins and are thus not part of the client itself but aid in the protein folding process and other proteostasis pathways. Chaperones were initially discovered in *Drosophila melanogaster* upon heat shock and were thus named heat shock proteins (Hsps) (Ritossa, 1962) but are actually induced upon exposure to various stresses to ensure proteostasis in adverse conditions. As cells have to maintain proteostasis during standard growth as well, various Hsps are constitutively expressed. Molecular chaperones are categorized into families based on their molecular weight in kilodalton and are highly conserved from yeast to human. Budding yeast has 63 chaperones, which are categorized into small heat shock proteins (sHsps), Hsp100/AAA+, Hsp70, Hsp90, Hsp40, CCT/TRiC, Prefoldin/GimC, Hsp60 and Hsp10 (Gong et al., 2009). Some of these act specifically in/at organelles, the ER and mitochondria, while others function in the cytoplasm and nucleus. Strikingly, chaperones are capable of handling vastly different protein clients, which is exemplified by the ability of yeast chaperones to target human disease proteins without yeast homologs. Additionally, nucleotide exchange factors (NEFs), chaperonins and non-canonical chaperones contribute to proteostasis. The PN is further extended by recent genome-wide analyses, which revealed unforeseen pathways to be limiting for PQC. For example, vesicle trafficking and components within the

machinery were pinpointed as factors required for functional PQC, even though these factors are not part of the cytosolic PN network themselves (Hill et al., 2016, Paper III, IV).

The PQC system is well-buffered, mainly on account of pathway interconnectivity and of (partial) redundancy between chaperones and associated PN factors (Gong et al., 2009). For example, yeast encodes four cytosolic Hsp70s (Ssa1-4), which are >80% identical and of which only one needs to be functional to ensure survival. Furthermore, ubiquitin ligases in several cellular compartments function redundantly to facilitate degradation of substrates (Breckel and Hochstrasser, 2021, Section 3.2). Most single deletions of PQC genes do not result in fitness defects, even when global misfolding is induced by continuous 37°C heat stress or exposure to azetidine-2-carboxylic acid (Trotter et al., 2002). Though proteostasis imbalance occurs in such conditions, cells evade proteotoxicity by activating other cellular pathways that compensate fitness defects (Ghosh et al., 2019).

3.1.1 HSP70S AND HSP40S

The Hsp70 family of chaperones is highly conserved and ubiquitous. They are central to the PN as they assist in all processes of proteostasis, both in basal and stress-related functions, including *de novo* polypeptide folding, translocation of polypeptides into mitochondria and ER, protein refolding, degradation and sequestration of proteins (Rosenzweig et al., 2019). They act in an ATP-dependent manner and cooperate with several other PN factors, such as Hsp90, Hsp40s (J-domain proteins) and NEFs. As they perform central functions of PQC, Hsp70 family members handle substrates not only found in the cytosol but also exist as specialized Hsp70s in organelles, for example within mitochondria.

Hsp70s contain an N-terminal ATP-binding domain, a peptide binding domain and an unstructured C-terminal region and generally bind substrates that expose short hydrophobic motifs due to (partial) unfolding (Rüdiger et al., 1997). Hsp70s act through perpetual cycles of substrate binding and release, which continue until the substrate reaches its functional, native conformation. ATP binding causes Hsp70 to adopt an open state, allowing the substrate to bind. Subsequent ATP hydrolysis, often facilitated by Hsp40s, leads to a high affinity state of Hsp70 for the substrate, causing a closed structure of the chaperone, which thereby traps the substrate. In a final step, which can be accelerated by NEFs, the exchange of ADP to ATP releases the substrate. As Hsp70 function is rate-limited by ATP hydrolysis and nucleotide exchange, cooperation with Hsp40s and NEFs increase Hsp70 efficiency. In yeast, there

are seven Hsp70s in the cytosol. Ssb1 and Ssb2 function with Ssz1 and its Hsp40 co-chaperone Zuo1 as co-translational chaperones, forming the ribosome-associated complex (RAC), and Ssa1-4 are active with and without stress and carry out basal functions of proteostasis. The four Ssa isoforms exhibit high homology with different expression patterns. While presence of one Ssa suffices to ensure cellular survival, their functions are not entirely redundant, which manifests in several PQC defects in *SSA* mutant strains (Andersson et al., 2021; Sharma and Masison, 2008; Werner-Washburne et al., 1987).

The diversity of cellular functions of Hsp70s in basal and stress conditions implies a vast substrate recognition ability, which may be expanded by cooperation with other factors of the PN. In fact, apart from variations in the sequences of Hsp70s, which determine substrate specificity and function to some extent (Sharma and Masison, 2011), a major contributor to the recognition of clients are the diverse Hsp40s (Misselwitz et al., 1998). These Hsp70 co-chaperones contain a characteristic J-domain, which facilitates their ATPase stimulating activity. Hsp40s bind substrates for handover and can introduce conformational changes within them to facilitate Hsp70 client binding and downstream processing (Kellner et al., 2014). Hsp40s are versatile co-chaperones of Hsp70 and other chaperone families (Reidy et al., 2014), for example capable of determining the fate of aberrant proteins arising upon heat stress (den Brave et al., 2020) and of tail-anchored membrane proteins on their way to the ER (Cho et al., 2021).

3.1.2 HSP100

Another major class of chaperones that operates together with Hsp70s is the conserved Hsp100 AAA+ ATPases (ATPase associated with diverse cellular activities), which are found in bacteria and eukaryotes, but not in metazoans, and are mainly in charge of protein reactivation and disaggregation after proteostasis perturbance. The ATP-dependent Hsp100s in *E. coli* (ClpB) and *S. cerevisiae* (Hsp104) are known to provide thermotolerance to the cells and to reactivate proteins to regain a functional proteome upon protein folding stress (Parsell et al., 1994). The ClpB/Hsp104-type Hsp100s contain two nucleotide binding domains, an M-domain that regulates protein disaggregation and an N-terminal domain that initiates substrate interaction. Hsp100 monomers assemble into a homohexameric ring with a small central pore. Assembled Hsp100 likely performs its disaggregation activity by extracting polypeptides from protein aggregates via a threading mechanism, which translocates them through the central pore of the hexamer and thus enables Hsp70-Hsp40 to access the substrate for refolding. Partial threading of

the substrate through the pore is sufficient for effective aggregate solubilization (Haslberger et al., 2008). Hsp70 is also directly required for Hsp104 function as Hsp70 triggers substrate threading through the inner cavity of the disaggregase. As such, Hsp70-Hsp100 are commonly viewed as a bi-chaperone system and can even be extended to an Hsp40-Hsp70-Hsp100 tri-chaperone system based on the co-operation between Hsp70s and Hsp40s (Winkler et al., 2012). During heat stress, the co-operation of small Hsps becomes relevant as well, which likely act to stabilize and sequester aggregates (Ehrnsperger et al., 1997; Ratajczak et al., 2009). As Hsp70s are versatile, they target various substrates for disaggregation, including stress granules, heat-induced protein aggregates and amyloidogenic inclusions (Cherkasov et al., 2013; Gao et al., 2015; Walters et al., 2015; Winkler et al., 2012, Paper II).

The general disaggregase Hsp104 in yeast is a key chaperone to understand mechanisms of stress recovery and protein disaggregation. Specifically in sPQC research, fluorescently tagged Hsp104 is commonly used as a marker for endogenous protein aggregates (Paper I). Hsp104 has low basal expression levels as its function is not required during non-stress conditions but becomes strongly upregulated when cells experience proteotoxic stress and localizes to small protein aggregates and distinct intracellular inclusions. Loss of Hsp104 manifests in several defective PQC pathways under stress conditions, including loss of thermotolerance, impaired inclusion formation and aggregate clearance during physiological heat stress, symmetric inheritance of aberrant proteins, accumulation of age-associated protein deposits and shortened replicative lifespan (Escusa-Toret et al., 2013; Hill et al., 2016; Sanchez and Lindquist, 1990; Specht et al., 2011).

In metazoans, Hsp100s exist exclusively in the mitochondria, likely due to the costly maintenance of ClpB/Hsp104 in unstressed cells (Escusa-Toret et al., 2013). However, disaggregation activity was found also in the cytosol and nucleus of metazoans, which is promoted via unclear mechanisms by Hsp70, Hsp40 and NEFs, such as Hsp110 (Rampelt et al., 2012).

3.1.3 SMALL HSPS

The chaperone class small Hsps (sHsps) is rather diverse and promiscuous Nevertheless, sHsps share common features, including a small molecular mass, an ATP-independent mode of action, a distinctive α-crystallin domain flanked by diverse unstructured N- and C-termini, and the ability to dynamically form oligomers (Haslbeck and Vierling, 2015).

In yeast, there are two cytosolic sHsps, Hsp42 and Hsp26, and both are non-essential. While Hsp26 is only produced upon stress through activation by Hsf1 and Msn2/4 transcription factors (Amorós and Estruch, 2001), Hsp42 is both constitutively active and involved in the post-stress reactivation of proteins (Haslbeck et al., 2004; Susek and Lindquist, 1990; Ungelenk et al., 2016). The function of sHsps that was determined first was their binding to early unfolding intermediates to keep them in a near-native conformation. This prevents unhinged aggregation and terminal misfolding by keeping substrates in complexes for Hsp104-Hsp70 to access. sHsps thus present as a first line of defense upon exposure to protein folding stress. Additionally, Hsp42 performs aggregase function in that it sequesters substrates for deposition at distinct cellular sites. Specifically, Hsp42 promotes CytoQ formation, sorting to peripheral aggregates and formation of age-associated aggregate depositions (Jakob et al., 1993; Saarikangas and Barral, 2015; Ungelenk et al., 2016). The sHsps cooperate with other molecular chaperones, e.g. in Hsp100-Hsp70 recruitment and in aggregate clearance by degradation. The function of sHsps in sPQC is further discussed in 3.3 Spatial PQC.

3.1.4 OTHER CHAPERONES AND AUXILIARY FACTORS OF THE PN

Hsp90 is an ATP-dependent family of chaperones mainly involved in folding of nascent polypeptides, acting downstream of Hsp70. In budding yeast, the two cytosolic Hsp90 isoforms together (the cognate Hsc82 and the stress-induced Hsp82) are essential, as they act in crucial functions such as signaling, but are only required for *de novo* folding of a subset of proteins (Borkovich et al., 1989; Nathan et al., 1997; Schopf et al., 2017). Hsp90 is regulated by its vast number of co-chaperones, which for example execute handover of clients between Hsp70 and Hsp90 (McClellan et al., 2007).

Chaperonins, or Hsp60, are commonly categorized into subfamilies to distinguish, among others, the GroEL/GroES-type complexes found in bacteria and endosymbiotic organelles and the TCP-1-type complexes in archaea and the eukaryotic cytosol. The essential eukaryotic chaperonin CCT (Chaperonin Containing TCP-1), also named TRiC (T-complex protein Ring Complex), oligomerizes into a ring-shaped complex with a central cavity and facilitates folding of clients, including the cytoskeletal components actin (trough cooperation with the chaperone family Prefoldin/GimC) and tubulin, in an ATP-dependent manner (Gestaut et al., 2019). It has also been implicated as a modulator of protein aggregation (Behrends et al., 2006; Nollen et al., 2004; Tam et al., 2006).

An example of a non-canonical chaperone is the peroxiredoxin Tsa1 in yeast, which acts as a peroxidase and can exert chaperone function to combat protein damage in the cytosol upon oxidative stress or during translation. When cells are exposed to H_2O_2, Tsa1 oligomerizes, recognizes misfolded and aggregated proteins, and recruits Hsp70 with its Hsp40 Sis1 (in contrast to Ydj1 during heat stress) and Hsp104. Tsa1 thus exerts an unusual oxidative stress-specific chaperone function, which is dispensable during heat stress (Hanzén et al., 2016; Trotter et al., 2008).

Nucleotide exchange factors (NEFs) catalyze the substitution of ADP with ATP during Hsp70 cycles. In budding yeast, there are three classes of Hsp70 NEFs: the two Hsp110 family proteins Sse1 and Sse2, and Fes1 and Snl1. They interact with Hsp70 in several chaperone functions. For example, NEFs promote Hsp70 efficiency and recruitment of Hsp70 with its client to the UPS for degradation, thereby ultimately determining the fate of the substrate and preventing aggregation (Dragovic et al., 2006; Gowda et al., 2013; Kandasamy and Andréasson, 2018; Mayer and Bukau, 2005).

3.2 TEMPORAL PQC

The temporal PQC machinery and its components are highly conserved (Figure 3). The correct native fold of a nascent polypeptide chain is often reached with the help of several chaperone families, especially transient binding of Hsp70 and Hsp40, co-chaperones and nucleotide exchange factors. Some proteins require other or additional chaperones for maturation, e.g. Hsp90. During the folding process, hydrophobic residues are buried within the protein core by binding of chaperones. This ultimately ensures a stable fold of *de novo* proteins or during their trafficking and hinders any improper interactions of hydrophobic side chains of the polypeptide emerging at the ribosome. When proteins (partially) un- or misfold, either due to intracellular (e.g. changes in expression level, erroneous translation, aging) or environmental causes (e.g. heat, oxidative stress), they often expose hydrophobic residues to the aqueous cytosol. They risk the loss-of-function and aggregation of the protein itself and of other proteins, improper protein-protein interactions and may interfere with other biological functions and membranes (Balchin et al., 2016; Gonzalez-Garcia et al., 2021). Notably, not only protein synthesis and folding are costly for the cell but misfolding is as well: it may cause a loss-of-function of the misfolded protein itself or a toxic gain-of-function, and the futile production of an aberrant protein uses resources of the PN that are needed for essential processes. Under non-stress conditions, protein folding in yeast is likely highly efficient, as protein misfolding, even at

low levels, comes at a high dosage-dependent fitness cost (Geiler-Samerotte et al., 2011).

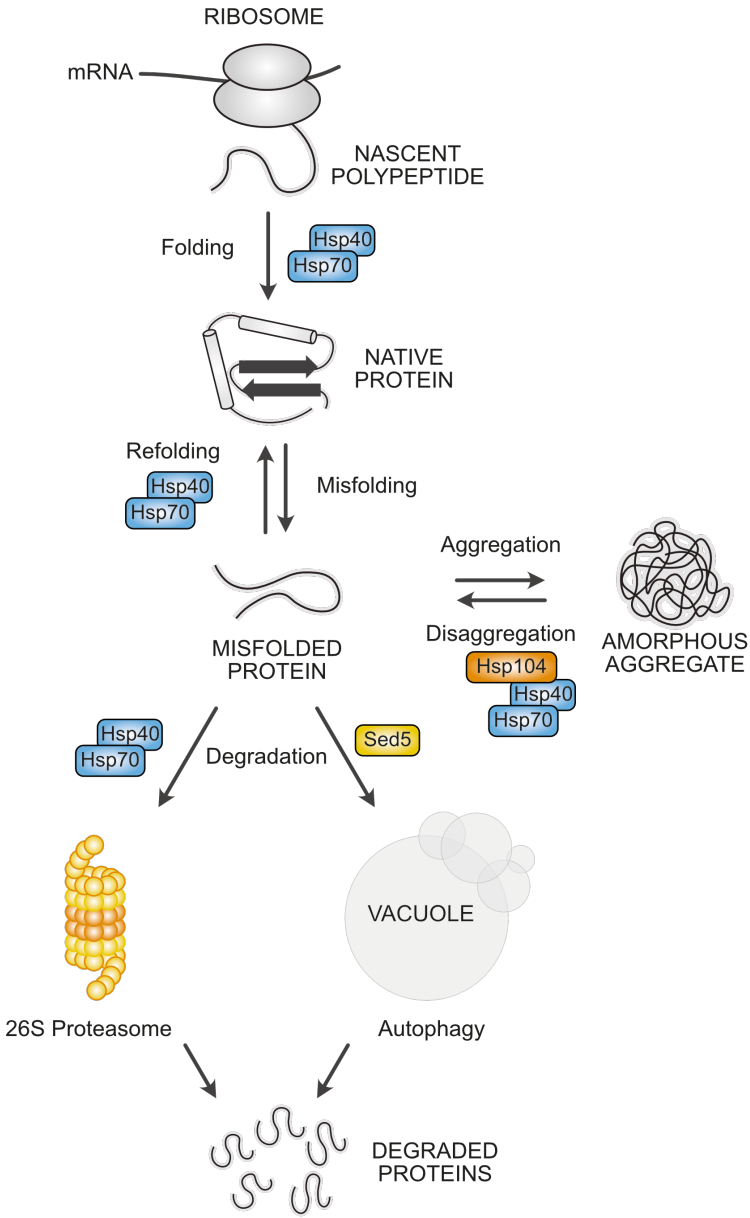

Figure 3: Overview of temporal PQC. The temporal PQC handles folding, refolding and degradation of proteins. Most processes are facilitated through the Hsp70-Hsp40 system. The main proteolytic pathways are the ubiquitin-proteasome system and autophagy at the vacuole. When proteins misfold, they can assemble into amorphous aggregates, which the disaggregase Hsp104 can entangle to allow their subsequent processing; reactivation by Hsp70-Hsp40 or degradation.

3.2.1 REGULATION OF THE PN

Upon an excess of aberrant proteins, eukaryotes induce genes required to maintain proteostasis by activating the master regulator HSF1 of a conserved transcriptional program, named heat shock response (HSR). In non-stress conditions, Hsf1 is required at basal expression levels to prevent protein misfolding in the cell and its stress response causes considerable up-regulation of expression of PN factors (Solís et al., 2016). Hsf1 is regulated by chaperone titration: once an excess of protein aggregates and other chaperone clients accumulate in the cytoplasm, Hsp70 is competed away from Hsf1 towards those substrates so that Hsf1 is free to induce the HSR in the nucleus. Hsf1 DNA-binding and transcription regulation also targets Hsp70 production, creating a negative feedback loop (Krakowiak et al., 2018). Once an excess of PN factors is reached in the cytosol again, Hsp70 resumes its function as a negative regulator of Hsf1 and HSR activation ceases (Masser et al., 2019). The negative Hsf1 regulation once stress ends appears to mostly rely on Hsp70 but may involve additional PN factors (Kmiecik et al., 2020). This elegant regulation of the HSR is relevant for numerous potentially proteotoxic stresses, relies on active protein translation for its function and mainly targets newly synthesized proteins for degradation (Medicherla and Goldberg, 2008). This mechanism is reasonable as newly synthesized proteins are also most susceptible to misfolding and aggregation (Xu et al., 2016). Only extreme proteotoxic stress caused by high ethanol concentration was found to activate the HSR even when translation was inhibited (Tye and Churchman, 2021). Recently, the chaperone titration model of HSR regulation was connected to the spatial aspect of PQC during heat stress (Feder et al., 2021); the Hsp40 co-chaperone Sis1 is diffuse in nucleus and cytosol and represses the Hsp70 Ssa1 in the nucleus when stress is absent. Upon exposure to heat stress, Sis1 re-localizes to the periphery of the nucleolus and the ER surface, possibly to aid in processing of misfolded proteins. Consequently, Hsp70 dissociates from Hsf1, which then induces the HSR. The HSR causes the production of many factors of the PN network to ensure proteome stability, such as Hsp104. Notably, many of the heat-shock responsive elements in the genome are actually activated by the general stress response transcription factors Msn2 and Msn4, (Solís et al., 2016). The redundant Msn2/4, in turn, are regulated through their phosphorylation status controlled by protein kinase A (PKA), which activates the environmental stress response.

3.2.2 UBIQUITIN-PROTEASOME SYSTEM

A crucial part of the PN is the ubiquitin-proteasome system in the cytosol and nucleus (Finley, 2009), a highly conserved machinery that consists of the proteasome and additional factors and is responsible for protein turnover.

Carefully regulated protein breakdown is important for many essential processes, including cell cycle progression and signaling, and thus crucial for survival. For example, proteasomal degradation is required to remove short-lived proteins of regulatory function, adjust protein levels according to need and to process aberrant or damaged proteins, especially upon erroneous targeting and translocation of proteins or during stress.

The cylindrical 26S proteasome consists of the 20S core forming a central pore and 19S regulatory particles on one or both ends and catalyzes the irreversible degradation of proteins through chymotrypsin-, trypsin- and caspase-like proteolytic functions to ultimately yield amino acids. The protease-function itself is buried within the 20S core, shielding the proteome from improper degradation. Substrate degradation by the UPS involves three sequential steps. First, ubiquitin is attached to the ubiquitin-activating enzyme (E1) in an ATP-dependent reaction. Second, the ubiquitin is transferred to ubiquitin conjugases (E2). Finally, ubiquitin ligases (E3) function in substrate recognition, often aided by chaperones, and transfer the ubiquitin onto lysine side chains of the substrate to target it for degradation by the proteasome. After a first ubiquitin monomer has been added, polyubiquitin chains are generated by addition of more ubiquitin. Some conjugates require additional E4 ligases for multiubiquitylation (Hoppe, 2005; Koegl et al., 1999). Once the substrate is bound to the proteasome, deubiquitylation enzymes (DUBs) remove the ubiquitin to recycle it back into the cellular ubiquitin pool. DUBs can also rescue substrates from degradation by acting before the proteasome (Crosas et al., 2006) and function in polyubiquitin chain modification required for proteostasis during heat stress (Fang et al., 2016). The substrate is translocated towards proteolytic sites of the proteasome, degradation is initiated at a disordered region within the substrate (Davis et al., 2021) and releases small peptides, which are broken down by aminopeptidases into amino acids for recycling. Every step of the proteasomal degradation cycle relies on conformational changes of the proteasome and consumes energy via ATP hydrolysis (Sahu and Glickman, 2021).

Ubiquitylation is a major targeting signal for protein degradation. Importantly, the mode of ubiquitylation determines the fate of the protein – not all ubiquitylation leads to proteasomal degradation. This posttranslational modification is involved in various signaling processes in the cell (Oh et al., 2018). The type of ubiquitylation is defined by the linkage of the ubiquitin chains and largely determines the conjugate's fate. There are numerous different linkage types; K48 is the linkage type that is responsible for bulk protein degradation, sometimes combined with K11 and other linkage types, while K63 generally does not target proteins to the proteasome.

Substrate specificity is controlled, chiefly, by E3 ligases, to allow the degradation of various proteins while avoiding promiscuity of the proteasome. Budding yeast has roughly 100 E3 ligases that confer the ability to target a wide range of substrates (Breckel and Hochstrasser, 2021). These ubiquitin ligases localize to several cellular sites, including nucleus, ER, cytosol and ribosomes, illustrating that aberrant proteins within the entire cell can be recognized by different E3 ligases within compartments. E3 ligases, aided by chaperones, can function both compartment-specific or work redundantly over several compartments (Samant et al., 2018). For example, the ubiquitin ligases San1 and Ubr1 function partially redundantly for numerous proteasome substrates, while San1 resides in the nucleus and Ubr1 is nuclear and cytosolic. Their targeting of misfolded proteins in the cytosol for degradation in the nucleus involves the Hsp40-Hsp70-Hsp110 chaperone system (Gowda et al., 2013; Khosrow-Khavar et al., 2012; Prasad et al., 2010, 2018).

Chaperones are intimately involved with the UPS at many steps during the actions of the UPS, e.g. to solubilize proteins prior to degradation (den Brave et al., 2020), and even in ensuring assembly of the functional 26S proteasome itself (Nahar et al., 2022). Protein synthesis does not occur in the yeast nucleus, where approximately 80% of proteasomes reside, so that nuclear proteasomes do not handle nascent polypeptides but degrade unstable, damaged, or misfolded nuclear proteins. A major ubiquitin ligase in the nucleus is San1, which recognizes unaggregated proteins with a certain amount of exposed hydrophobicity and can receive cytoplasmic proteins for nuclear degradation via their translocation into the nucleus mediated by the Hsp70-40-110 chaperones. Another E3 ligase in the nucleus is Doa10, which also localizes to the ER and participates in an organellar PQC pathway, the ER-associated degradation (ERAD). Doa10 is thus a ligase that handles substrates from cytosol and nucleus and membrane proteins from the ER. The fact that some ubiquitin ligases operate over several cellular compartments and can sometimes even recognize substrates redundantly, highlights the interconnectivity of PQC pathways across organelles.

The UPS is a critical component of the PN in the cytosol since it is the site of protein translation at ribosomes and newly created proteins are especially susceptible to aberrant folding and targeting. A major ubiquitin ligase of the yeast cytosol is Ubr1. It targets proteins that are part of the N-degron pathway, which involves Ubr1 ubiquitylating certain unstable substrates at their N-termini, and has also been shown to process various other cytosolic proteins destined for degradation, e.g. misfolded and temperature-sensitive proteins (Eisele and Wolf, 2008; Khosrow-Khavar et al., 2012). Such ubiquitylation by Ubr1 independent of the N-degron pathway relies on Hsp70 and Hsp40 co-

chaperones. Hul5 and Rsp5 are ubiquitin ligases, which participate in PQC in the cytosol by targeting un-/misfolded proteins specifically during heat stress (Fang et al., 2011, 2014, 2016). Another major UPS pathway in the cytosol is the degradation of nascent polypeptides at the ribosome, which involves the E3 ligase Ltn1. This process is linked to the ribosome-associated quality control (RQC) complex, which becomes crucial upon translation arrests, for example due to faulty mRNA or damaged ribosomes (Brandman et al., 2012). RQC is also connected to spatial PQC, as impaired RQC causes protein aggregation of nascent polypeptides, which, in turn, titrates molecular chaperones out from regular PQC processes (Choe et al., 2016; Yonashiro et al., 2016).

While proteasomes in both yeast and mammalian cells are normally concentrated in the nucleus, their location is adjusted when cells are exposed to certain stresses. A prominent example is the localization of the proteasome into the membrane-less JUNQ compartment upon proteasome inhibition in both yeast and mammalian cells, which is one of many pathways that connect the temporal with the spatial PQC machinery (Kaganovich et al., 2008). As the UPS is directly involved in the processing of aberrant proteins, it is considered an interesting target for therapeutic approaches against proteopathies (Dantuma and Bott, 2014; Galvin et al., 2022).

3.2.3 OTHER PATHWAYS OF CYTOSOLIC PROTEIN DEGRADATION

In addition to the nucleus and the cytoplasm, there are other organelles competent of processing cytosolic proteins by ubiquitylation and degradation. As mentioned above, the ER as a hub of protein synthesis has its own designated pathways to evade protein stress, the unfolded protein response (UPR) and ERAD. These pathways are important both to maintain homeostasis within the organelle and the cell as a whole. They comprise many chaperones and ubiquitin ligases, such as Doa10, to achieve ubiquitylation of cytosolic substrates destined for degradation.

Mitochondria have been attributed to function in degradation of misfolded cytosolic proteins in yeast (Ruan et al., 2017). This pathway directly ties temporal to spatial PQC since mitochondria are also appreciated as deposition sites of heat shock-induced protein aggregates (Babazadeh et al., 2019; Böckler et al., 2017; Zhou et al., 2014). The mitochondrial degradation of cytosolic proteins is termed MAGIC (mitochondria as guardian in cytosol) and may address the protein aggregates that associate with mitochondria during heat shock. Specifically designed misfolding reporter proteins and TDP-43 are

transported into mitochondria for subsequent degradation, while Hsp104 does not enter mitochondria, as anticipated. Biochemical data show that endogenous heat shock-induced misfolded proteins become degraded in mitochondria by resident proteases such as Pim1. The import of substrate proteins does not rely on the cytosolic Hsp70s but involves Hsp104, conceivably to disaggregate the inclusion to enable protein import into mitochondria, which remains to be demonstrated.

The other major pathway of the PN designated for protein degradation is autophagy. The autophagy system is highly conserved from yeast to humans and contributes to proteostasis through engulfment of cellular material, both selectively and non-selectively, including entire organelles and protein inclusions to target them to the vacuole/lysosome for degradation. This process involves the formation of the autophagosome, a double layer membrane structure that encloses the cellular content destined for degradation via vacuolar/lysosomal proteases, and is chiefly active during nutrient depletion stress (Takeshige et al., 1992). In yeast, autophagy is initiated through assembly of the pre-autophagosomal structure (PAS) at the vacuole, supported by vesicles, which forms a double membrane that invaginates cargo, thereby forming the autophagosome. This structure is then transported to the vacuole, involving many components of the vesicle trafficking machinery, and fused to the vacuole, which is mediated by the HOPS (homotypic fusion and protein sorting) complex and several SNARE (Soluble N-ethylmaleimide-sensitive-factor attachment) proteins (Reggiori and Klionsky, 2013).

Autophagy is intimately linked to the other major system handling cytosolic protein degradation, the UPS. For example, a decline in one of the degradation systems causes a compensatory upregulation of the other (Korolchuk et al., 2009b, 2009a; Pandey et al., 2007). Furthermore, ubiquitylation itself regulates several parts of the autophagy process (Chen et al., 2019). In line with the critical importance of functional protein degradation in proteostasis maintenance, there are also numerous connections between autophagy and protein aggregation. For example, a defining study of sPQC found the distinct inclusion IPOD at the vacuole to overlap with autophagy markers (Kaganovich et al., 2008). Additional reports show aggregate clearance/degradation of neurodegenerative disease proteins such as alpha-synuclein and huntingtin and of cytosolic aggregation-prone proteins via autophagy (Lu et al., 2014; Pankiv et al., 2007; Petroi et al., 2012).

3.3 SPATIAL PQC

The spatial PQC sequesters aberrant or damaged proteins and smaller aggregates into larger, membrane-less inclusions located at distinct sites within the cell (Figure 4). This process facilitates their clearance and serves a cytoprotective function, making sPQC a crucial system to maintain proteostasis. Spatial PQC is active in parallel to the temporal PQC, yet its outcomes become most noticeable when the temporal PQC is defective or overwhelmed, for example when protein misfolding is enhanced due to exposure to heat stress.

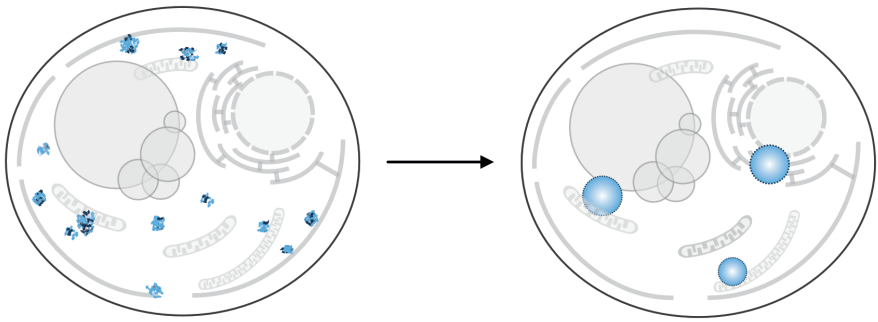

Figure 4: Spatial sequestration of protein aggregates (blue) into larger inclusions is the main feature of spatial PQC. Small aggregates, or CytoQ, (left) coalesce into larger inclusions at distinct intracellular sites (right) through the action of the spatial PQC machinery.

The sequestration into larger depositions of aberrant proteins is a highly conserved process from bacteria (Kirstein et al., 2008; Rokney et al., 2009; Winkler et al., 2010) to humans. The first report of a distinct protein deposition site in human cells, termed aggresome, found that it resides in proximity to the nucleus and becomes apparent after proteasome inhibition (Johnston et al., 1998). Aggresome formation involves microtubule and vimentin filaments and contains known PQC factors such as chaperones and proteasomes (Wigley et al., 1999). It is also a location of various disease proteins, including huntingtin and alpha-synuclein. Other types of protein inclusions were identified using substrates of specific organelles, such as substrates of the secretory pathway/ER (Huyer et al., 2004; Kamhi-Nesher et al., 2001; Kruse et al., 2006) or mitochondrial proteins (intramitochondrial protein quality control compartment, IMiQ (Bruderek et al., 2018)). A report in 2008, using budding yeast and mammalian cells, defined the partitioning of cytosolic misfolding proteins in two protein deposition sites with distinct cellular locations, namely the JUNQ (juxta nuclear quality control compartment) and IPOD (insoluble protein deposit) (Kaganovich et al., 2008). The data indicated that protein species are sorted to the respective inclusions according to specific features, such as ubiquitylation or amyloidogenicity. This finding was in line with data

highlighting that the properties of protein aggregates depend on the protein species involved (Matsumoto et al., 2006). The definition of two distinct PQC sites in yeast was central for further studies focused on sPQC and lead to the discovery of other, potentially additional quality compartments of cytosolic proteins, such as the intranuclear INQ (Miller et al., 2015a) and a site close to mitochondria (Zhou et al., 2014). It also generated and answered several key questions in the proteostasis field: all proteins harbor the potential to misfold, how come only a minor subset of them appears relevant in the pathology of protein conformational diseases? How do sPQC mechanisms manage to evade proteotoxicity of only certain proteins and fail to handle disease proteins? What determines the fate of different protein species? In recent years, a novel model relevant for biological processes such as proteostasis and stress responses was uncovered, which proposes that aggregation of soluble proteins is driven by liquid-liquid phase separation (LLPS), a de-mixing process that facilitates the formation of biomolecular condensates and membrane-less organelles in aqueous solution (Brangwynne et al., 2009; Riback et al., 2017). Such condensates were predominantly characterized with *in vitro* studies. *In vivo*, LLPS plays a role in several regulatory processes, for example, it is an established mechanism of translation regulation upon severe to sublethal heat shock (Mühlhofer et al., 2019; Riback et al., 2017). Such LLPS aggregates are generally most common for proteins with low-complexity regions and proteins that interact with nucleic acids, including numerous neurodegenerative disease proteins (Zbinden et al., 2020). The concept of LLPS is applicable to spatial PQC and will benefit from further studies *in vivo* to tie this aggregation model to previous work in the sPQC field, especially regarding the cellular response to physiological (mild) heat shock.

3.3.1 AGGREGATE DEPOSITION SITES

The inclusions that constitute the known sPQC compartments in budding yeast were identified using various reporter systems, e.g. by monitoring endogenous aggregate components and by expressing exogenous disease model proteins. The visualization of sPQC sites also heavily relied on the induction of stress, such as heat or oxidative stress, proteasome inhibition or on lack of factors central to the PN. These altering conditions entail that differential PQC responses by the cell may be active depending on the specific experimental context. Importantly, the formation of protein aggregates and their sorted sequestration to distinct deposition sites corroborate sPQC in itself as a non-random, active, factor-dependent process. While it is true that the response of the sPQC varies, several aggregate deposition sites for cytosolic proteins have been observed in many conditions and for various protein species, revealing an oftentimes exercised general sPQC response to protein stress. This common pathway begins with the emergence of many small foci throughout the cytosol,

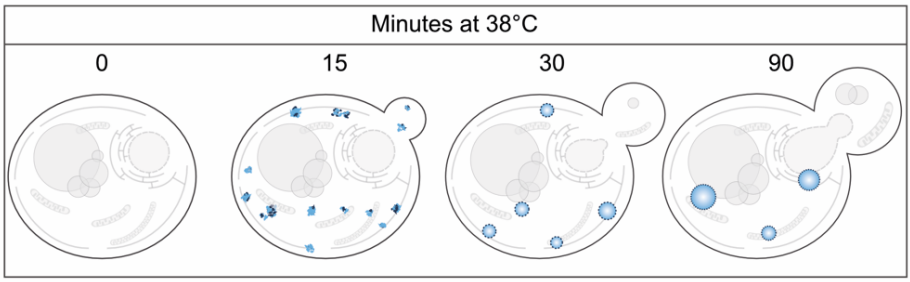

Figure 5: Spatial PQC during mild heat stress. Cells do not form visible aggregates before heat stress. At early time points of thermal insult, many small protein aggregates (blue) appear throughout the cytosol and in the vicinity of organelles. The aggregates become gradually sequestered into larger inclusions throughout the time course and end up in 1-3 inclusions per cell. The number of inclusions per cell and their intracellular location differ depending on the monitored protein species. Generally, large deposition sites exist at the nucleus, vacuole and mitochondria.

which are gradually sequestered into larger inclusions and are retained in the mother cell during division (Figure 5).

CytoQ

The small peripheral foci that become visible in the initial response to protein folding stress are called CytoQ, Q-bodies or stress foci (Escusa-Toret et al., 2013; Malinovska et al., 2012; Miller et al., 2015a; Specht et al., 2011; Spokoini et al., 2012). They emerge as the first visible protein aggregates formed during stress exposure, dispersed through the cytosol and at surfaces of organelles, including mitochondria, ER and vacuole, and coalesce into larger inclusions at later times if proteostasis stress continues (Zhou et al., 2014, Paper II, V). CytoQ formation depends on the ER and PN factors such as Hsp42 and enhances cellular fitness during stress (Escusa-Toret et al., 2013), however, during standard growth, the lack of Hsp42 increases lifespan, suggesting that Hsp42 function can be cytoprotective or costly, depending on the context of the sHsp's action. Recent studies point at a crucial role of the sHsp Hsp42 in CytoQ formation and coalescence, which mainly targets newly synthesized proteins during stress and keeps them in near-to-native conformation to allow refolding by the Hsp70-Hsp100 bichaperone system (Grousl et al., 2018). Hsp42 can actively sequester the CytoQ in an aggregase function and CytoQ formation is required for deposition of proteins in the JUNQ/INQ. These ATP-dependent fusion events of CytoQ have been proposed to be sequestration of aggregates into the JUNQ, which would thus define CytoQ as JUNQ precursors (Escusa-Toret et al., 2013; Sontag et al., 2017). The emergence of CytoQ as a first response of the general PQC pathway has been observed typically for non-amyloidogenic (Escusa-Toret et al., 2013)

misfolding protein reporters and various conditions, e.g. for Ubc9-ts (ts, temperature-sensitive) and guk1-7, gus1-3 and pro3-1 upon heat shock (Paper II) or VHL upon proteasome inhibition (Specht et al., 2011). Upon loss of Hsp42, reporters become visible as nucleus-associated inclusions (Malinovska et al., 2012; Miller et al., 2015a; Specht et al., 2011), pinpointing the sorting function of Hsp42 to peripheral aggregates. Upon severe heat shock (42°C), cells also form transient foci of Hsp90 and co-chaperones, which do not coalesce into larger inclusions but co-localize with CytoQ at certain times. This response requires Hsp70s, Hsp90, Hsp104 and proteasomal activity but is independent of Hsp42 (Eisele et al., 2021; Saarikangas and Barral, 2015). Another chaperone system required for proper CytoQ formation is the Hsp70s. Loss of Ssa1 and Ssa2 causes various proteostasis defects (Andersson et al., 2021) including formation of few, large inclusions, even before stress (Andersson et al., 2021; Shiber et al., 2013; Paper II). Instead of CytoQ fusing into larger inclusions, Hsp70-Hsp104 can also (additionally) resolve CytoQ. While the formation and subsequent fusion of CytoQ appears to be a standard PQC pathway for amorphous protein aggregates during stress, amyloidogenic proteins often localize in few inclusions per cell without exhibiting a preceding CytoQ formation step (Escusa-Toret et al., 2013; Specht et al., 2011). CytoQs are likely conserved structures, as numerous small foci of a misfolding reporter protein appear in the cytosol of human cells upon heat shock (Hageman et al., 2007).

JUNQ/INQ

One of the evolutionary conserved, large protein deposition sites in the cell is the perinuclear JUNQ (Kaganovich et al., 2008) (Figure 6). Formation of the JUNQ has been observed for several protein species, such as Ubc9-ts and VHL under heat shock and proteasome inhibition (Kaganovich et al., 2008). The initial study defining the JUNQ elucidated several important characteristics: the JUNQ is dynamic, the substrate sequestration is reversible, it mainly contains soluble misfolding proteins and one sorting signal towards the JUNQ is likely ubiquitylation (Kaganovich et al., 2008).

A report from 2015 redefined the JUNQ as the INQ (intranuclear quality control) compartment based mainly on electron microscopy of the sPQC sites in yeast, which revealed that the JUNQ actually localizes adjacent to the nucleolus and is thus not cytosolic (Miller et al., 2015a). Interestingly, the INQ contains misfolded proteins both of nuclear and cytosolic origin, with the latter being imported through nuclear pores. This translocation can be executed with the help of the Hsp70 co-chaperone Sis1 and likely involves additional or other PN factors depending on the protein species (Park et al., 2013). It is not

conclusively determined whether the INQ is actually the JUNQ for all misfolded protein species in general and how this relates to other visualized deposits. Recent studies reported INQ formation upon genotoxic stress without concomitant JUNQ formation and found certain proteins to localize exclusively to INQ and not JUNQ, indicating that they might represent two separate quality control compartments. Under both genotoxic and heat stress, similar PQC factors are involved in INQ formation and localize to the deposition site, such as Hsp104, Sis1 and the sHsp-like Btn2 (Gallina et al., 2015; Ho et al., 2019). However, it is still unclear whether JUNQ and INQ may be distinct sites (at least in some cases), which could form simultaneously, co-exist or are not always visible and distinguishable as such e.g. due to limitations in imaging resolutions (Kumar et al., 2022; Samant et al., 2018). Therefore, I refer to this inclusion site at and/or in the nucleus as JUNQ/INQ.

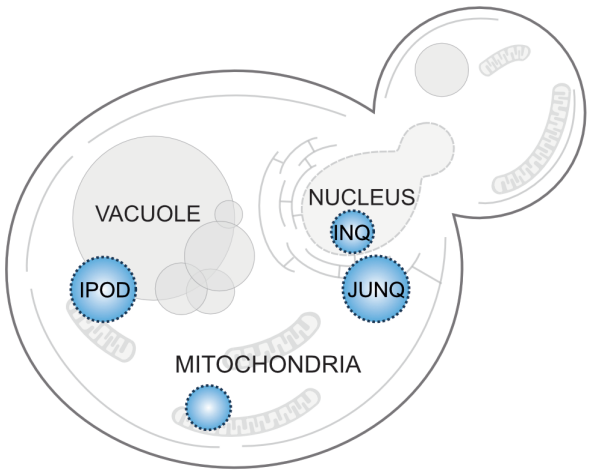

Figure 6: Inclusion sites of protein aggregates in budding yeast. At least four distinct inclusions sites exist in yeast, but their sorting factors and resident misfolded protein species differ. Two nuclear sites were described but may represent the same quality control site, the intranuclear INQ and juxtanuclear JUNQ. The IPOD is adjacent to the vacuole and may, at times, overlap with mitochondria and the vacuole-mitochondria contact site. Additionally, aggregates are sequestered to mitochondria.

As previously mentioned, the JUNQ/INQ is conserved, as a similar compartment forms upon stress exposure close to the nucleus at the MTOC (microtubule organizing center) and vimentin in mammalian cells (Kaganovich et al., 2008; Ogrodnik et al., 2014; Weisberg et al., 2012). These features were also described by the first report of protein inclusions, when the aggresome was defined, which forms via dynein-mediated transport on microtubules (Johnston et al., 1998). It is not yet clear how these entities differ

from one another and whether one might represent the precursor to the other (Ogrodnik et al., 2014).

IPOD

The second early identified intracellular protein deposit is the IPOD, which localizes to the vacuole. It was found adjacent to the autophagy marker Atg8, which is part of the PAS. Compared to the JUNQ/INQ, which is dynamic in turnover of soluble misfolded protein, the IPOD does not show such rapid exchange with the environment, suggesting that the IPOD harbors mainly terminally misfolded proteins (Kaganovich et al., 2008). Based on the misfolding protein reporters used in sPQC studies, it seems that the IPOD is the exclusive quality control compartment for amyloidogenic proteins, often even forming under non-stress conditions if the reporter protein is sufficiently highly expressed. The IPOD can also harbor amorphous protein aggregates formed by reporters such as Ubc9-ts and other temperature sensitive proteins upon physiological heat stress (Hill et al., 2016; Kaganovich et al., 2008, Paper II) and even JUNQ/INQ-resident proteins if they are targeted there (Hill et al., 2017; Kaganovich et al., 2008; Miller et al., 2015a; Sontag et al., 2014). It is unclear how the IPOD containing amorphous and the IPOD containing amyloidogenic proteins differ and when they co-aggregate. For example, co-expression of Ubc9-ts and Htt103QP leads to formation of the known Ubc9-ts inclusions, JUNQ and IPOD, and a single huntingtin inclusion adjacent to the Ubc9-ts-marked IPOD, indicating that two distinct IPOD-like compartments can co-exist in close proximity without protein species mixing (Yang et al., 2016). Some chaperones localize to all described IPODs, such as Hsp104 but formation requirements and sorting factors towards the IPOD types differ, suggesting that the sPQC pathway for IPOD formation depends on the protein species in question (Escusa-Toret et al., 2013; Song et al., 2014; Specht et al., 2011). The non-amyloidogenic disease model protein optineurin was also found to partially aggregate within the IPOD (Kryndushkin et al., 2012). IPOD formation was also reported for prions, e.g. Ure2 or Rnq1 and may serve as their nucleation site (Escusa-Toret et al., 2013; Kaganovich et al., 2008; Specht et al., 2011; Tyedmers et al., 2010; Winkler et al., 2012).

The IPOD is a conserved quality control compartment, as IPOD-like aggregates have been described in more complex eukaryotes. These sites share characteristics with the IPOD identified in yeast, such as a dense structure and immobile contents that do not exchange with the surrounding cytosol (Hipp et al., 2012; Kaganovich et al., 2008; Weisberg et al., 2012).

Deposition site at mitochondria

The first report of distinct protein inclusion sites in the cell described the two sites, JUNQ and IPOD at nucleus and vacuole, respectively (Kaganovich et al., 2008). These quality control compartments were expanded when a third site was defined in proximity to mitochondria, often adjacent to the organelle (Zhou et al., 2014). Aggregate deposition at this site is required for functional asymmetric inheritance of protein aggregates (Böckler et al., 2017; Zhou et al., 2014), boosts aggregate clearance (Paper III) and may facilitate import of cytosolic misfolded proteins into mitochondria for subsequent proteolysis (Ruan et al., 2017). Aggregate dissolution is not hampered by blocking the electron transport chain but by disrupting glycolysis, indicating that mitochondria themselves are not the main ATP source for disaggregation processes. A disruption in mitochondrial membrane potential, which impairs mitochondrial import, inhibits aggregate clearance (Zhou et al., 2014). In a follow-up study, Ruan et al. propose that cytosolic aggregates at mitochondria are disaggregated through Hsp104 action (without Hsp70), translocated via the mitochondrial import machinery and degraded within mitochondria with the help of mitochondrial proteases. In line with their previous work, disruption of membrane potential leads to slowed aggregate dissolution within mitochondria (Ruan et al., 2017). Recruitment of Hsp104 to heat shock-induced aggregates without the action of the Hsp70s Ssa1 and Ssa2 contradicts other reports (Andersson et al., 2021; Glover and Lindquist, 1998; Winkler et al., 2012).

Aggregate deposition at mitochondria has been reported for several stress conditions and numerous misfolding protein reporters: endogenous proteins (Zhou et al., 2014, Paper V), Ubc9-ts (Zhou et al., 2014) and the three ts reporters guk1-7, gus1-3 and pro3-1 (Paper V). Therefore, this PQC pathway appears to be common to numerous amorphous protein aggregate species. Interestingly, heat-induced Ubc9-ts protein inclusions, which define the JUNQ and IPOD sites (Kaganovich et al., 2008), localize in proximity to mitochondria, highlighting that discernment of the inclusion sites is an unresolved issue (Zhou et al., 2014).

It is overall unclear whether a deposition site at mitochondria is also relevant for localization of amyloidogenic protein species. As postulated in the first report of the IPOD, this inclusion site for aggregates of many disease proteins is perivacuolar (Kaganovich et al., 2008). It may concomitantly overlap with mitochondria as well due to organelle contacts but the IPOD and the deposition site at mitochondria may also be distinct sequestration sites. The mutant huntingtin protein illustrates how ambiguous observations regarding co-localization of aggregates with other cellular structures can be. Huntingtin

heterologously expressed in yeast was commonly observed cytosolic and close to different organelles, including mitochondria, ER and the vacuole. The numerous, small foci partially localize to the JUNQ outside the nucleus but are mainly distributed in the cytoplasm. They do not co-localize with IPOD markers but can be targeted to the immobile IPOD through overexpression of modifiers of toxicity, which cause the formation of fewer, larger inclusions (Kayatekin et al., 2014). Generally, the huntingtin model proteins differ due to expression levels, length of polyQ tract, flanking regions and experimental conditions, which influence toxicity and aggregation behavior. The aggregate localization clearly depends on these factors and suggest that fewer, large inclusions of Htt localize to the IPOD, while other aggregate types are often found at JUNQ, ER and mitochondria (Gruber et al., 2018; Kaganovich et al., 2008; Schlagowski et al., 2021; Solans et al., 2006). To make this situation even more complex, Htt can accumulate in both types of aggregate species, amorphous and amyloidogenic, which do co-exist but not necessarily co-localize (Caron et al., 2014; Gruber et al., 2018). Based on the well-established localization of the IPOD adjacent to the vacuole for numerous amyloidogenic reporter proteins and the recently established localization of amorphous aggregating reporter proteins to mitochondria (Böckler et al., 2017; Zhou et al., 2014, Paper III, V), it is tempting to speculate that there are, in fact, both the vacuolar IPOD and the mitochondrial deposition site and that they harbor mainly amyloidogenic or amorphous aggregating protein species, respectively. Strikingly, the disaggregase Hsp104 is able to recognize all those inclusions independent of their subcellular location and aggregate morphology. How the different sorting to inclusions based on protein species may work mechanistically or how it is potentially beneficial for the cell, remains to be investigated.

Additional proteinaceous inclusion types have been described for yeast and other cells. These have been observed in solitary instances for specific unusual protein substrates, in more complex eukaryotic cells or do not follow the generalized PQC pathway described here (Bjørkøy et al., 2005; Farrawell et al., 2015; Szeto et al., 2006; Tenreiro et al., 2014; Wang et al., 2009). These inclusions will not be described further to focus on the conserved quality control sites formed in budding yeast upon heat stress.

3.3.2 SORTING TO SPQC SITES

sPQC pathways do not occur at random but are active, energy- and factor-dependent processes. While some protein species, mainly amyloidogenic proteins, localize to the perivacuolar IPOD, other proteins, depending on the stress condition and reporter, may localize to JUNQ/INQ. Some misfolding

reporter proteins are sequestered into all described compartments (e.g. Ubc9-ts). This indicates that sorting factors to distinct sites may determine the location of aberrant proteins, depending on properties of the aberrant protein species and the stress that the cell experiences. In fact, no unifying sorting determinants have been identified yet but protein sequestration was found to depend on several factors, including molecular chaperones and the cytoskeleton. Major factors involved in sPQC sorting are described in the following.

Ubiquitylation of JUNQ/INQ substrates

The pioneering sPQC study proposed ubiquitylation as a general sorting signal for proteins specifically to JUNQ/INQ: amyloidogenic Rnq1 could be re-routed from IPOD to JUNQ/INQ when ubiquitylated, additionally, impaired ubiquitylation of VHL and Ubc9-ts abrogated their sequestration into JUNQ/INQ (Kaganovich et al., 2008). However, following articles revealed that the reporter tGnd1-GFP localizes to CytoQ and JUNQ/INQ even when not ubiquitylated (Miller et al., 2015a) and that another reporter, DegAB-GFP, while ubiquitylated, is sorted to CytoQ but not JUNQ/INQ (although the DegAB-GFP aggregates may represent precursors of the JUNQ/INQ, as mentioned previously) (Shiber et al., 2013). Both results somewhat argue against ubiquitylation as an essential and common sorting signal for aberrant proteins to JUNQ/INQ and, at least regarding INQ specifically, no other clear commonality between protein species deposited to this site has been identified so far, e.g. upon comparison of protein domains and physical features of the INQ proteome (Kumar et al., 2022). Ubiquitylation aside, a direct link between the targeting of ubiquitylated proteins to the JUNQ/INQ was proposed based on the concurrent enrichment of proteasomes at the inclusion site and in the nucleus, since the two may present a convenient connection between inclusion formation and substrate degradation (Gallina et al., 2015; Kaganovich et al., 2008). Additionally, JUNQ/INQ substrates are mostly degraded by the UPS, which is promoted by the deubiquitinase Ubp3 (Oling et al., 2014). However, it was proposed that the proteasomal degradation can only occur after extraction from the inclusion through disaggregation and there are no obvious differences in degradation rates of substrates within CytoQ or JUNQ/INQ. This suggests that the sequestration of aberrant proteins to JUNQ/INQ does not serve the specific spatial link of misfolded proteins and the UPS (Miller et al., 2015a, 2015b). While this is true for misfolded proteins, the thermosensitivity of cells deficient in Hsp70s (*ssa1*Δ *ssa2*Δ) can be suppressed by Ubp3 overproduction, independent of Hsp104 due to the deubiquitinase activity of Ubp3 rescuing ubiquitylated substrates before proteasomal destruction. The

JUNQ/INQ may thus serve the spatial sequestration of misfolding proteins that can be either saved or removed (Oling et al., 2014).

Molecular chaperones

Hsp42 is required for formation of CytoQ and does not co-localize with the JUNQ/INQ, it is thus a specific sorting factor for peripheral aggregates (Specht et al., 2011) (Figure 7). Upon loss of Hsp42, cells form only the JUNQ/INQ (Escusa-Toret et al., 2013; Specht et al., 2011), which argues against CytoQ as an essential precursor of JUNQ/INQ. The unstructured N-terminal region of Hsp42 contributes to substrate specificity and contains a prion-like domain, which is crucial for its protein sorting action into CytoQ (Grousl et al., 2018; Miller et al., 2015a; Specht et al., 2011). A second unstructured N-terminal subdomain acts as an important regulator of Hsp42 sequestrase function and controls CytoQ number and stability (Grousl et al., 2018). The other yeast sHsp in the cytosol, Hsp26, is not required for formation of CytoQ or JUNQ/INQ and cannot compensate the sPQC functions of Hsp42 but localizes unspecifically to both quality control compartments (Specht et al., 2011).

Fusion and clearance events of CytoQ are also managed by chaperones and are likely two connected processes that are regulated by a balancing act between Hsp42 and the disaggregase Hsp104: CytoQ coalesce by Hsp104 action to re-solubilize the proteins, which are then added into one existing CytoQ by Hsp42 (Escusa-Toret et al., 2013). This model explains why simultaneous lack of Hsp104 and Hsp42 does not affect CytoQ formation or degradation but disrupts CytoQ fusion (Escusa-Toret et al., 2013). Interestingly, single deletions of *HSP104* or *HSP42* do not affect Ubc9-ts degradation, even though CytoQ fusion or CytoQ formation is impaired, respectively, indicating that following the general sPQC pathway through CytoQ formation and coalescence is not essential for Ubc9-ts degradation. Hsp42 is dispensable for IPOD formation, in contrast to Hsp104 (Escusa-Toret et al., 2013). Additional major factors involved in CytoQ sorting are the cytosolic Hsp70s, as CytoQ formation is abolished in *ssa1*Δ *ssa2*Δ cells. The large inclusions formed in this Hsp70 mutant are also not properly targeted by Hsp104 for disaggregation and following degradation of the substrate proteins so that the substantial inclusions persist in the cells (Andersson et al., 2021; Escusa-Toret et al., 2013; Oling et al., 2014; Shiber et al., 2013, Paper II).

Protein disaggregation and subsequent handover to other PN machineries are intricate parts of sPQC and affect aggregate formation, sequestration into protein deposits, aggregate clearance and retention in the mother cell. As such, the tri-chaperone system Hsp104-Hsp70-Hsp40 controls aggregate number

and location. The Hsp40s determine substrate specificity by recruiting Hsp70 via Ydj1 to stress-induced or Sis1 to amyloidogenic substrates (Reidy et al., 2014). All known cytosolic protein deposition sites are recognized and targeted by Hsp104, however, the recognition by the disaggregase does not result in the same dynamics of their clearance (Escusa-Toret et al., 2013; Kaganovich et al., 2008). The machinery is supported by NEFs (Hsp110); for example, Sse1 and its interaction with Hsp70 are required for proper CytoQ resolution and substrate degradation (den Brave et al., 2020; Escusa-Toret et al., 2013). Lack of Hsp104 results in defective CytoQ and JUNQ/INQ resolution (Escusa-Toret et al., 2013; Miller et al., 2015a) and client refolding or degradation can only occur after disaggregation through Hsp104. IPOD formation is dependent on Hsp104, which is also relevant to its function in prion fragmentation for propagation.

Hsp90 is a chaperone that is not essential to CytoQ formation but affects their dynamics and removal (Escusa-Toret et al., 2013). It is, however, required for the recently described formation of conserved Hsp82 (Hsp90) and Sgt1 foci upon heat shock (42°C), whose formation depends on Hsp70-Hsp90-Hsp104 and a functional proteasome but not on Hsp42 (Eisele et al., 2021). These foci partially overlap with CytoQ marked by Hsp42 but do no coalesce, and disappear rapidly, indicating that their co-localization is transient and upstream of later fusion events of CytoQ into larger inclusions. The Hsp70-Hsp90-Hsp104 foci interestingly co-localize with synphilin-1 of the Parkinson's disease and defects in this pathway hinder synphilin-1 clearance (Eisele et al., 2021).

Another chaperone system that is involved in sPQC is the conserved RQC consisting of Rqc1, Rqc2, Ltn1 and Cdc48. The RQC targets aberrant translation products, which are usually generated due to ribosome stalling, for proteasomal degradation (Brandman et al., 2012; Defenouillère et al., 2013). If this PQC pathway fails and aberrant polypeptides accumulate, oligomers and insoluble cytosolic inclusions are formed and cause proteotoxicity in yeast. This is accompanied by widespread aggregation of other constituents of the proteome, including factors of the PN network (Choe et al., 2016). The RQC is also required for inclusion formation and detoxification of a huntingtin model protein in yeast and thus functions as a sorting factor of this protein (Yang et al., 2016). In another huntingtin model protein in yeast, the RQC controls the location of huntingtin inclusions and directs them to the cytosolic IPOD. Loss of Ltn1 or Rqc1 causes faulty sorting leading to inclusion formation in the nucleus, which is associated with exacerbated toxicity compared to cytosolic inclusions (Zheng et al., 2017).

Cytoskeleton and intracellular trafficking

Over recent years, the sPQC machinery has been expanded, unexpectedly, by the intracellular vesicle trafficking system. The trafficking system, including endocytosis and endomembrane trafficking, has been implied in numerous neurodegenerative diseases and aging but its specific connection to sPQC was unclear (Berglund et al., 2017; Bettayeb et al., 2016a, 2016b; Meriin et al., 2003; Singh and Muqit, 2020).

Intracellular vesicle trafficking consists of essential and conserved cellular pathways that govern and ensure the transport of cargo, mostly proteins, to their proper destination within the cell. These processes are facilitated by membrane material enclosing the cargo, transport of the vesicle by the cytoskeleton and finally fusion with its membranous destination site to release the cargo at the correct location. The major function of vesicle trafficking is to direct membrane and soluble organellar proteins, which are co-translationally transferred into the ER, further along the secretory pathway.

Yeast vesicle trafficking is well-conserved and consists of several interconnected pathways, whose concerted actions rely on material exchange between components and organellar proximity (Feyder et al., 2015). The trafficking routes are the exocytosis/secretory (SEC) pathway, directing cargo to the plasma membrane or out of the cell, the vacuolar protein sorting (VPS) pathway and the more direct alkaline phosphatase (ALP) pathway, which both manage transport of cargo towards the vacuole. The internalization of plasma membrane proteins or extracellular medium components is executed by endocytosis (END pathway), which sends cargo-engulfing endosomes either for vacuolar degradation or to the Golgi for recycling (RCY pathway). These pathways require intricate sorting of cargo and numerous factors handling the transport itself, such as SNARE (soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor) proteins, COP (coat protein complex)-coated vesicles, Rab GTPases and other specialized protein complexes (Ahmadpour et al., 2020; Feyder et al., 2015).

While intracellular trafficking entails several direct functions critical to maintain proteostasis, such as correct folding and membrane insertion of ER proteins, which rely on classic molecular chaperones such as the Hsp70 Ssa1, I will focus on the pathways involved in sPQC of aberrant cytosolic and disease proteins in *S. cerevisiae*. Several lines of evidence suggest an involvement of vesicle trafficking in sPQC of amorphous, amyloidogenic and age-associated protein aggregates. A genome-wide screen of the non-toxic Htt103QP variant of the Huntington's Disease revealed that the formation of large huntingtin

inclusions depends not only on RQC factors, as mentioned previously, but also the Golgi-vesicle trafficking machinery, pinpointing vesicle trafficking as a sorting factor to IPOD. The study demonstrated a close association of the aggregates with the actin cytoskeleton, which is also known for the Htt103Q variant (Song et al., 2014), and found that mutation of actin-related genes causes toxicity of the otherwise non-toxic Htt103QP variant. In accordance, expression of Htt103QP in wild type cells caused defects in endocytosis (Yang et al., 2016). A specific sorting factor for IPOD during heat stress and aging is Vac17, which acts as the vacuolar adapter protein for Myo2-dependent vacuolar inheritance during cell division (Hill et al., 2016). Interestingly, its function in sPQC is vacuole-independent and instead requires components of the vesicle trafficking machinery. Vac17 controls the formation of IPOD, which requires its interaction with Myo2 and additionally the endosome-component Vps1 and the HOPS/CORVET-subunit Vps16. The article also includes an unbiased screen for Hsp104 heat stress-specific physical interactors, performed by Hsp104-GFP immunoprecipitation followed by mass spectrometry. This approach revealed numerous intracellular trafficking components as general Hsp104 interactors, including the essential guanine exchange factor Sec7 and the SNARE-related Sec18. Strikingly, the bias towards vesicle trafficking components among Hsp104 interactors was even more pronounced when analyzing only heat stress-specific interactors, indicating that the trafficking machinery and endogenous protein aggregates increasingly engage upon heat stress (Hill et al., 2016). Hill et al. mainly investigated Vac17-mediated inclusion formation of heat-induced endogenous and Ubc9-ts aggregates, but also report that loss of Vac17 does not disturb Htt103QP inclusion formation. Kumar et al. identified a similar involvement of Myo2-actin-dependent vesicle trafficking for sequestration of an amyloid-like, prion-domain containing model protein into IPOD (Kumar et al., 2016). Specifically, factors required for proper IPOD formation are tropomyosin, which interacts with actin cables, Myo2 and Sec18. The key role of Myo2 is emphasized by the fact that it is required for functional IPOD targeting of several amyloid model proteins (Kumar et al., 2016). Paper III contains a screen of the deletion mutant collection for factors required for proper and timely aggregate sequestration of endogenous proteins during heat stress and reveals enrichments for several components of the intracellular trafficking machinery as well. These results are expanded by Paper IV, which contains the same screening procedure performed for the essential gene mutant collection. Several proteins of the vesicle trafficking machinery are among the factors, including Sed5 and Sec7 and again, Myo2, which is also required for proper aggregate sequestration at the mitochondrial deposit site (Paper III). As mentioned previously, this site may overlap with the IPOD that can contain amorphous proteins at the vacuole. This idea is strengthened by the partial co-

localization of aggregates with vacuole-mitochondria contact site components, which, additionally, are required for correct localization of the deposit. The results from Paper III and IV are discussed in more detail in section 5 but demonstrate that vesicle trafficking and its endomembrane system are required for sPQC of several reporter proteins and under various conditions. The precise mechanisms behind each described pathway overlap to some extent, as they converge on the essential Myo2, and may highlight variations in a general sPQC pathway depending on the misfolding protein species and the inclusion type to which it is targeted.

Additional factors

Two well-characterized sorting factors of sPQC in yeast are the sHsp-like Btn2 and its paralog Cur1. Both have been implicated in prion propagation and curing and in sorting of amorphous misfolding proteins to inclusions (Kryndushkin et al., 2012; Malinovska et al., 2012). Prior to its identification as a sorting factor to PQC sites, Btn2 was known to function in trafficking and recycling of certain late endosome-Golgi substrates. *BTN2* expression in standard growth conditions is low but heat shock or other stress on the proteome induces a striking up-regulation and localization of the protein into the nucleus (Malinovska et al., 2012; Miller et al., 2015a). This mechanism relates to its specific requirement for JUNQ/INQ formation, which may be explained by two models of Btn2 function. One model focuses on the compartment-specific aggregase/sequestrase function of Btn2 and Hsp42, which facilitate inclusion formation in the nucleus and cytosol, respectively (Miller et al., 2015a). Another model includes different Btn2 binding partners that sort substrates to distinct inclusions. Hsp42 and Btn2 sort towards peripheral aggregates, while Btn2 binding Sis1 allows the import of cytosolic misfolded proteins through nuclear pores to the INQ. This process is, in turn, regulated through Cur1 binding Sis1, thereby sequestering it to the nucleus, which allows Btn2 to act in the cytosol with Hsp42. This is in line with Cur1 being dispensable for targeting of misfolded proteins to CytoQ and JUNQ/INQ (Malinovska et al., 2012; Specht et al., 2011).

The sorting factors of misfolding proteins to inclusions at mitochondria are not well-understood. The sequestration of aggregates at mitochondria occurs under diverse stress conditions for various misfolding proteins and is preceded by CytoQ formation mainly at mitochondria and ER, often simultaneously (Zhou et al., 2014), which is in line with our observations (Paper V). Deposition at mitochondria is successful independent of a functional actin cytoskeleton but several sorting factors to mitochondrial deposition sites were identified: based on the assumption that the sorting may depend on a tether to mitochondria,

Zhou et al. performed a small genetic screen of mitochondrial outer membrane protein mutants and we expanded their data with a candidate-based approach and by performing a genome-wide screen (Zhou et al., 2014, Paper III, V). Contributors to successful deposition of aggregates in proximity to mitochondria are, for example Fis1, Mdm12, Mmm1, Clu1, Myo2 and vCLAMP (organelle contact site named vacuole and mitochondria patch)

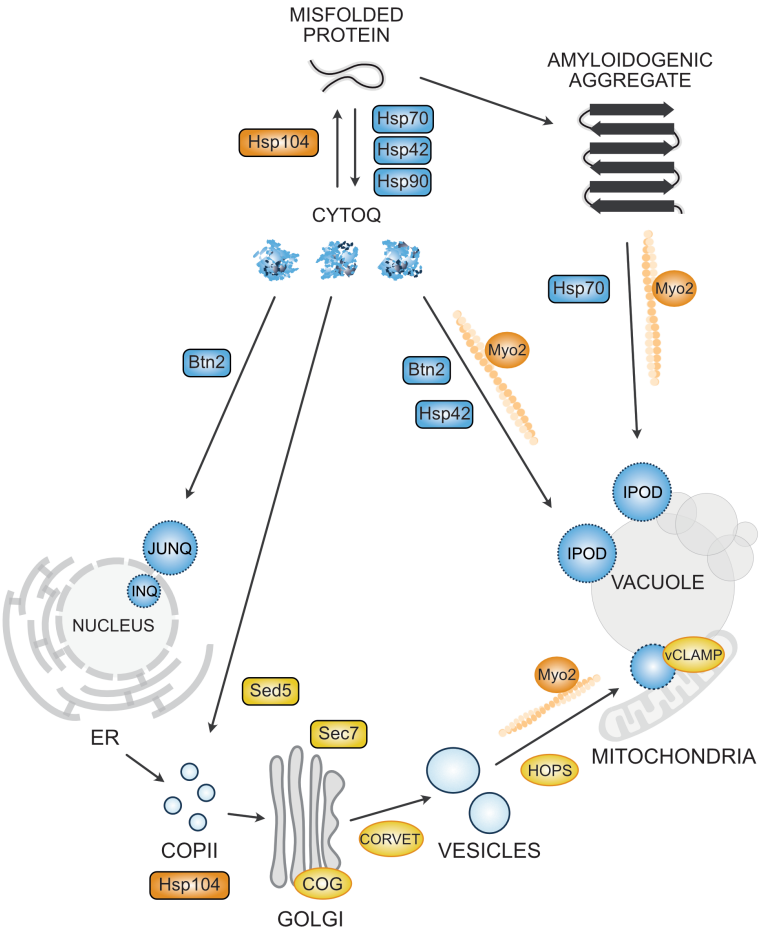

Figure 7: Spatial sorting factors and pathways involved in sPQC. Misfolded proteins assemble into small, amorphous aggregates, CytoQ, which are sequestered into larger inclusions. Pathways requiring Btn2 target CytoQ to the nucleus to JUNQ/INQ, or, involving Hsp42, to the vacuole into IPOD. The latter pathway may operate together or in parallel with Vac17-Myo2-actin transport to the vacuole. Another pathway sorting CytoQ largely relies on Sed5 and ER-Golgi anterograde trafficking and additional components of the vesicle trafficking machinery, including Sec7. This pathway also requires Myo2-actin transport and the vacuole-mitochondria contact site for inclusion deposition proximal to mitochondria. Amyloidogenic aggregates become deposited at the IPOD via Myo2-actin transport.

components, which all contribute to mitochondrial functions. Additionally, many mutants with no known connection to the organelle have been identified to be required for this sPQC pathway. It is notable that for most mutants, the disruption in mitochondrial association of aggregates is subtle, which can be due to the random association that is expected to occur in the crowded cellular environment or due to robust sPQC pathway buffering. While mitochondria are known to play a role in neurodegenerative disease aggregate localization and toxicity (Camilleri et al., 2013; Hashimoto et al., 2003; Pasinelli et al., 2004), the sorting of amorphously aggregating reporters to mitochondria in yeast and the potential sorting of amyloidogenic proteins towards a mitochondria-adjacent site are unclear.

3.3.3 FUNCTIONS OF SPQC

The fact that sPQC is an energy-intensive and factor-dependent, organized process is indicative of its key function in maintaining a healthy proteome. Numerous specific functions of sPQC have been identified. While they may be context-dependent, much data indicates an overall positive effect of functional sPQC upon protein misfolding.

Fitness and survival

Early studies of heat shock proteins revealed their essential function in thermotolerance, i.e. survival and recovery after heat insult, which implies a direct function of molecular chaperones in stress resistance. It is the disaggregase function of Hsp104 that is essential for thermotolerance as it re-activates proteins sequestered into inclusions (Glover and Lindquist, 1998, Paper I). The formation of CytoQ and of larger inclusions contributes to stress resistance. For example, yeast cells that cannot form CytoQ due to loss of Hsp42 have a reduced fitness at 37°C (Escusa-Toret et al., 2013). Another report emphasizing the important function of protein aggregate formation for stress resistance found that many mature, native proteins undergo reversible foci formation during stress, often not misfolding or being damaged in the process, which is also not followed by their degradation but actual recovery of proteins upon stress removal (Wallace et al., 2015). Even irreversible formation of the age-associated protein deposit in yeast cells does not impair their PQC capacity and does not correlate with proteostasis imbalance, suggesting that sequestration during aging may also be an initially protective, rather than deleterious fitness and survival strategy (Saarikangas and Barral, 2015).

Healthy proteome

Aberrant proteins cause cytotoxicity due to a loss-of-function, i.e. when factors essential for PQC and survival become misfolded and nonfunctional or essential factors become sequestered into pre-existing aggregates, or due to a much more difficult to predict gain-of-function, which may be based on faulty interactions of the misfolded protein. Conformers can also become toxic due to sequestration of unrelated proteins into aggregates and interference with cellular structures such as membranes (Balchin et al., 2016; Bode et al., 2017; Fusco et al., 2017; Gruber et al., 2018; Hartl et al., 2011; Hartl and Hayer-Hartl, 2009; Olzscha et al., 2011; Park et al., 2013). These potentially harmful processes caused by protein misfolding in the crowded cellular environment can be avoided or mitigated by sequestration into aggregates and organized deposition at protective sites. sPQC can thus save essential proteins from further aggregation, restrict aberrant interactions and shield indispensable cellular processes and components from toxic conformers.

sPQC also serves important purposes for the PN components upon stress. When cells are exposed to heat stress or other protein misfolding conditions, they become vulnerable to widespread protein aggregation of previously folded proteins and especially of nascent polypeptides during translation. The controlled aggregation of damaged or misfolded proteins and their deposition into protective inclusions relieves PN factors from this burden and facilitates to prioritize essential tasks upon immediate stress. To gather proteins for refolding after stress relief can also be superior to the destruction of proteins when it comes to energy costs (Oling et al., 2014). Additionally, orderly sequestration of protein aggregates into inclusions is beneficial for their clearance through repair or degradation, likely due to the more efficient temporal and spatial accumulation of PQC components at such sites, which facilitates downstream processing. This becomes evident when taking into account that the JUNQ/INQ is a dynamic compartment, while the IPOD is rather immobile and may harbor terminally misfolded protein species (depending on the definition of IPOD).

While protein deposits are known features of numerous diseases, mounting evidence indicates that aggregates themselves are not toxic to the cell and that sequestration of misfolded proteins into larger inclusions is actually a regulated, protective mechanism to evade proteotoxicity (Arrasate et al., 2004; Miller et al., 2015b; Sontag et al., 2017; Wolfe et al., 2013). This observation holds true for cells, animal models and patient data (Arrasate et al., 2004; Gutekunst et al., 1999; Kuemmerle et al., 1999), suggesting that further sPQC research is crucial to understand the differences in toxic versus benign

aggregates. A useful explanation is that several smaller aggregates and misfolded oligomers have a higher aggregate surface area available for aberrant interactions compared to larger inclusions. Such a restriction in exposed aggregate surface may explain why fewer, larger inclusions mitigate toxicity of diverse disease proteins. However, surface area alone cannot account for toxicity (Duennwald et al., 2006b). This model for inclusion formation as a beneficial strategy to combat aberrant proteins is especially applicable for amyloid and prion proteins. Such protein conformers are known to seed aggregation by functioning as templates, gradually trapping more proteins within aggregates, and to then propagate within the cell into further small aggregates for inheritance of these structures (Douglas et al., 2008).

Asymmetric inheritance

The concentration of damaged or potentially toxic protein products into inclusions serves an evolutionary conserved mechanism with great importance for cellular aging, the asymmetric inheritance that facilitates rejuvenation of progeny (Figure 8). Functional inclusion formation is crucial for the mother-biased retention that keeps daughter cells free of damage. This asymmetry is known from bacteria to yeast and stem cells and ultimately contributes to aggregate clearance on the cell-population level, since daughter cells will arise damage-free and with a reset replicative potential (Aguilaniu, 2003; Erjavec et al., 2007). Accordingly, mutant cells with symmetric inheritance phenotypes, such as *sir2*Δ or *vac17*Δ, have a shorter replicative lifespan (Erjavec et al., 2007; Hill et al., 2016). Importantly, this mechanism is not limited to the budding divisions of yeast but also exists in symmetrically dividing cells (Bufalino et al., 2013; Fuentealba et al., 2008; Hernebring et al., 2006; Rujano et al., 2006). Asymmetric inheritance of faulty proteins and aggregates in budding yeast is governed by numerous factors and cellular structures. Several

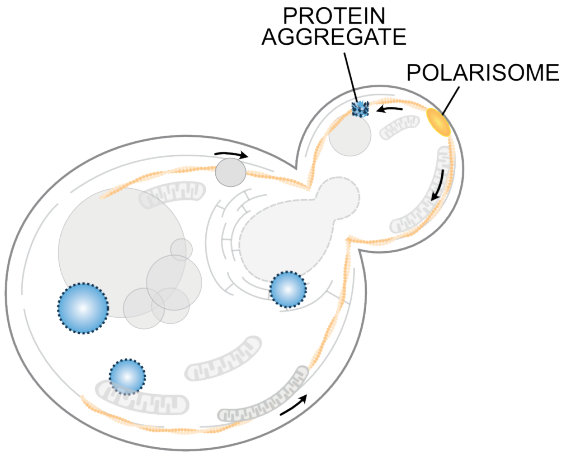

Figure 8: Asymmetric inheritance of protein aggregates in budding yeast depends on Hsp104, Myo2, actin and additional factors and may be facilitated through confinement of inclusions to organelles and the controlled inheritance of organelles. Protein aggregates can be transported into the mother cell through retrograde actin cable flow originating at the polarisome.

models of asymmetry establishment in yeast have been proposed and are not necessarily mutually exclusive, especially since they may differ depending on the inclusion species of interest. One model is largely based on attachment of aggregates to the actin cytoskeleton (Liu et al., 2010; Song et al., 2014), which prevents aggregates from leaking to the mother cell due to the retrograde flow of actin cables from the polarisome during budding. Additionally, on rare occasions, aggregates may escape this system and are transported back from the daughter into the mother cell via active retrograde transport (Erjavec et al., 2007; Liu et al., 2010; Song et al., 2014). It was proposed that the important role of Hsp104 in asymmetric inheritance is based on its crucial attachment of the aggregates to actin, which would explain why *hsp104*Δ cells display perturbed asymmetry (Erjavec et al., 2007; Liu et al., 2011; Spokoini et al., 2012; Tessarz et al., 2009). The other models are linked to organelle association of protein aggregates, which possibly relates to the proposed function of actin in asymmetric inheritance as actin is required for organelle inheritance and in direct contact with organelles. One report builds on the confinement of JUNQ/INQ and IPOD to nucleus and vacuole, respectively, and suggests that this attachment to organelles restricts aggregates from entering the bud (Kaganovich et al., 2008; Spokoini et al., 2012). The asymmetry of inclusions at the vacuole, shown for the misfolded protein-containing IPOD, is established through the previously mentioned inclusion sorting factor Vac17 and its interaction with Myo2 and actin in vesicle trafficking (Hill et al., 2016). The last model is based on the finding that aggregates are captured and sequestered into larger deposits at mitochondria in a Fis1-dependent process, which reduces their mobility and confines them to the mother cell during budding and Myo2-mediated mitochondrial inheritance (Böckler et al., 2017; Zhou et al., 2014).

3.3.4 SPQC DURING AGING

As mentioned in section 2, damaged and misfolded proteins are known to accumulate during aging and a decline in proteostasis is a hallmark of aging (López-Otín et al., 2013). In yeast, spatial PQC is affected by aging and exhibits signs of deterioration, and protein aggregates and misfolded proteins are determined aging factors. A clear manifestation of this is the age-dependent, gradual formation of typically a single inclusion visualized with Hsp104-GFP, termed age-associated protein deposit (APOD), which appears early during aging, i.e. in >80% of cells older than 6 generations (Aguilaniu, 2003; Erjavec et al., 2007; Saarikangas and Barral, 2015). This protein deposit co-localizes with the Hsp70s Ssa1 and Ssa2, Hsp42, the J-domain protein Ydj1 and, to a low extent, with Hsp26 but not with the PN factors Sis1 or Hsp82. The deposit is distinct from other known protein inclusions. The formation

occurs from precursors confined to the ER diffusion barrier of the mother cell and is Hsp42-dependent. Its mother-biased retention during budding is likely facilitated through actin cables and/or association to mitochondria, which is in line with the major models of asymmetric inheritance of protein aggregates in yeast developed mainly using stressed young cells (Saarikangas et al., 2017; Saarikangas and Barral, 2015; Zhou et al., 2014). The formation of this deposit is not necessarily the manifestation of a failing PQC system itself but may be part of a response to damage accumulating during cellular aging and the sPQC attempting to maintain proteostasis as well as possible. In fact, old yeast cells are capable of handling certain proteotoxic stresses like young cells, exemplified by the successful clearance of heat-induced aggregates. The presence of an age-related deposit even promotes degradation of cytosolic substrates by the UPS (Saarikangas and Barral, 2015). Overall, the data suggest that the formation of an inclusion of damaged/misfolded proteins during age is a protective mechanism that attempts to ensure functional PQC while systems gradually decline rather than the manifestation of the deteriorating sPQC system itself. This is consistent with the general notion that inclusion formation by sPQC likely serves a cytoprotective function in many cellular contexts (section 3.3.3). Of course, the PQC enters a battle that is eventually lost, causing deterioration and cell death. For example, aged cells are no longer able to sequester Htt103QP into large inclusions (Yang et al., 2016) and Hsp70 accumulates in small peripheral aggregates with increasing age (Oling et al., 2014). They also exhibit symmetric inheritance of protein aggregates, which interfere with daughter cell rejuvenation and their replicative lifespan potential reset (Aguilaniu, 2003; Erjavec et al., 2007; Zhou et al., 2014).

How yeast lifespan and PQC are mechanistically related and the sequence of events during aging are still under investigation, but recent articles have found specific pathways that clearly link the two. For example, the non-canonical chaperone Tsa1, which is required for proper sPQC of aggregates induced by oxidative stress, is also important for management of deposits associated with aging. The loss of Tsa1 causes a reduced replicative lifespan, while overproduction increases lifespan, with the lifespan extension partially depending on certain PN factors (Hanzén et al., 2016). Similarly, the role of Vac17 in sPQC via its vacuole inheritance-independent functions controls replicative lifespan. Overproducing Vac17 even results in lifespan extension (Hill et al., 2016). The reduction in lifespan caused by loss of different chaperones also speaks for the critical function of proteostasis maintenance when cells age (Andersson et al., 2021; Erjavec et al., 2007; Oling et al., 2014) but is intimately tied to the inherent energy cost that comes with chaperone function, requiring a careful balance to impact lifespan positively (Andersson et al., 2013; Escusa-Toret et al., 2013). Additionally, it may be specific PQC

functions of chaperones that are crucial to reach a normal lifespan, while others may be dispensable (Andersson et al., 2021). The above described APOD is not formed upon loss of Hsp42 and arises at younger age in *hsp104*Δ cells, which is similar to the proposed mechanism of Hsp104-Hsp42 balance for CytoQ formation. Loss of Hsp42 causes an increased lifespan, while loss of Hsp104 shortens it, which correlates with the APOD formation and is in line with another report for extended lifespan when counteracting protein aggregation (Hill et al., 2014). This argues for the APOD being a consequence and a cause for aging, even though it may also serve cytoprotective functions as APOD formation does not impair proteostasis, even ensures its functionality (Saarikangas and Barral, 2015). These examples show that the impact of sPQC on aging and vice-versa are not well understood. Overall, it is clear that cells lose the ability to properly sequester damaged/misfolded proteins and that the formation of large inclusions may be important to maintain regular lifespan. The progressive deterioration of sPQC is likely connected to other systems accumulating damage, such as organellar dysfunctions and failing co-translational PQC (Hill et al., 2017; Stein et al., 2022) and it will be difficult to identify and manipulate a single starting point for cellular aging. The accumulation of aggregates of certain proteins is a hallmark of aging (López-Otín et al., 2013) and may cause a feedback loop by sequestering essential PN factors, exacerbating a proteostasis decline into collapse (Moreno et al., 2019; Moreno and Aldea, 2020), even though the sequestration process itself may be an attempt at a cytoprotective response by the cells (Walther et al., 2015). A recent report determined that aged cells often undergo an arrest in the G1 phase of the cell cycle directly before cell death/lysis and accumulate key regulators of the start of the cell cycle in the nucleus (Moreno et al., 2019). The authors propose a pathway for aging, integrating several aging phenotypes, that is based on the gradual decrease in chaperone availability during aging due to accumulation of damage thanks to the mother-biased retention of protein aggregates, which impairs the processing of key cell cycle regulators. The damages that arise in multiple systems converge in this gradual collapse in proteostasis, which ultimately leads to a final cell cycle arrest and cell death (Moreno and Aldea, 2020).

4 STUDYING SPQC IN S. CEREVISIAE

Budding yeast is a single-celled eukaryote known for its feasibility, a wide range of available resources and the relative ease of genetic manipulation of the cells. Much of our current knowledge within the proteostasis field is based on studies performed with *S. cerevisiae*. Yeast has several important features as a model for this work: pathways are highly conserved, such as the heat shock response and the molecular chaperones maintaining proteostasis. The cells have a short and finite replicative lifespan, which makes them useful for studies of PQC in the context of cellular aging. Their asymmetrical divisions entail the mother-biased retention of aging factors, including protein aggregates, which allows daughter cell rejuvenation and is thus relevant to research on aging and stem cells. Numerous genome-wide screens in yeast helped identify parts of the PQC involved in management of neurodegenerative disease proteins in other eukaryotes. The pioneering report in cytosolic sPQC, which showed that inclusions are confined to specific sites in the cell depending on protein species, relied on yeast for initial work and expanded the findings to more complex cells (Kaganovich et al., 2008). Consequently, budding yeast has been, from the beginning, a proven model system to study spatial PQC.

4.1 HEAT STRESS AND REPLICATIVE AGING

Exposing cells to stress that causes protein un- and misfolding is a common strategy to induce and study the response of the PQC machinery. Elevated temperature is known to destabilize proteins and induces the highly conserved cellular heat shock response (HSR). How yeast cells attempt to maintain a healthy proteome upon this protein folding stress varies depending on the applied temperature. The optimal growth temperature for wild type yeast cells is 30°C and a shift to 37-38°C is considered a mild/moderate heat shock, also called heat stress, which causes a notable adjustment of the cells to the environment, while their growth rate and fitness are not perturbed, as translation continues. A severe to sublethal heat shock is often performed by exposing cells to 42-46°C and results in a different response to this more severe stress with ultimately slowed growth due to decreased/stalled translation. A heat shock at 50°C can only be applied for short timespans followed by recovery at lower temperature, since the cells would otherwise die upon prolonged exposure (Mühlhofer et al., 2019). Heat-shocking yeast cells at different temperatures was instrumental to the discovery of heat shock proteins and the concept of thermotolerance (Sanchez and Lindquist, 1990; Verghese Jacob et al., 2012). In the articles included in this thesis, we mostly perform a mild heat shock at 38°C, occasionally followed by a period of recovery at

30°C. It is important to be aware of how the cell reacts to these conditions when interpreting data from PQC assays.

Mild heat stress and severe heat shock are established methods to study the cytosolic PQC response and both come with distinct cellular adaptations. A recent study acquired transcriptomics, translatomics and proteomics data from yeast cells exposed to 37, 42 and 46°C and the results show clear variations in the cellular response depending on the severity of stress (Mühlhofer et al., 2019). Mild heat stress conditions cause a rapid response of the transcriptome with the induction of ca. 1,800 genes after 15 minutes, which is recapitulated in translatomics but not in proteomics, as previously observed (Jarnuczak et al., 2018). This initial sharp rise in transcriptomal kinetics upon mild heat stress is followed by a slow recovery when heat stress continues, indicative of the adaptation of the cell to elevated temperature. Such reprogramming is not sufficient when the temperature is higher and the transcriptome thus answers with a continuous increase in upregulation of certain genes. Global protein production is strongly decreased at severe heat shock (Cherkasov et al., 2013; Shalgi et al., 2013), indicative of the cellular adjustment away from adaptation towards a halt in growth until recovery. Importantly, the actual protein levels do not correlate well with the gene expression data that indicate such a sharp induction of certain genes at early time points. In fact, at 37 and 42°C, chaperones and other molecules involved in stress resistance and UPS components were found to be increased in abundance, while ribosomal proteins and other translational components were decreased, which is in line with known physiological adaptations to such stress, but not with the transcriptomal readout (Mühlhofer et al., 2019). Mild heat stress leads to increased levels of especially sHsps, which indicates that the cellular PQC focuses first on preventing aggregation before it addresses protein re-activation and degradation (Jarnuczak et al., 2018). In accordance with increased UPS components, ubiquitylation of global proteins is elevated after 30 min at 37/42°C and decreased after 1 hour of recovery (Mühlhofer et al., 2019). It appears that the increase in protein degradation mainly affects newly synthesized proteins and not the global proteome (Medicherla and Goldberg, 2008). This effect ceases after 90 min at 38°C, indicating that the proteome is now stably adjusted to the new temperature (Medicherla and Goldberg, 2008). Proteasomal clearance is thus an adaptation during mild heat shock that continues during stress relief and may play a role in ensuring solubility of certain proteins, as some identified proteins are constantly translated and degraded during heat shock, keeping total levels unchanged (Mühlhofer et al., 2019). The authors claim that aggregation does not play an important role in the HSR at 37 and 42°C but only at sublethal temperatures, when known phase separating proteins form condensates, which is in agreement with such

aggregation mostly being observed when cells are under severe stress conditions. The authors conclude that, while some factors known to undergo LLPS aggregate even at mild heat shock, LLPS does not play a key regulatory role during mild heat stress (Cherkasov et al., 2013; Mühlhofer et al., 2019; Wallace et al., 2015), as important regulators, such as Pab1, remain soluble. Importantly, these biochemical approaches to heat shock highlight the differences between fluorescent foci detectable with the microscope ("visible aggregates") and those that are part of the pelletable fraction in the cell but do not form foci (Wallace et al., 2015). If UPS is disrupted upon mild heat stress and cannot degrade properly during early time points as it should, aggregation occurs, indicating that the degraded proteins are removed due to thermal damage (Medicherla and Goldberg, 2008).

An important detail in their report is that, while transcriptional changes that take place upon thermal stress (37/42°C) seem to be reverted when cells are returned to standard growth temperature (25°C), the proteome does not respond as fast and remains in its stress configuration until the 1h recovery measurement (Mühlhofer et al., 2019) or even longer (Jarnuczak et al., 2018). This likely aids in recovery processes that gradually allow the cell to adapt back to non-stress growth conditions. Specifically at 37°C, the stress response leads to an efficient adaptation without perturbing growth, which links to the concept of thermotolerance, in that elevated temperature causes cells to prepare for more strenuous conditions (Mühlhofer et al., 2019).

In addition to the above-described changes in gene expression and proteostasis, the yeast cell undergoes large structural changes during continuous mild heat shock. Cells increase in size and concomitantly, many organelles and cellular components enlarge as well, all in a rapid response already after 15 min of heat stress. For example, mitochondria and vacuoles expand, which also impacts organelle contact sites. The nucleus first reduces in size at the early 15 min time point and then recovers to its size before stress (Keuenhof et al., 2022). During proteotoxic stress, nuclear envelope budding is increased, which functions in exchange of material between nucleus and cytoplasm. The authors suggest that this stress response may contribute to PQC by facilitating nucleocytoplasmic communication (Panagaki et al., 2021).

Another condition that causes proteostasis imbalance in yeast is replicative aging, which occurs when yeast cells produce daughter cells. The replicative lifespan potential of single yeast cells is limited and wild type laboratory strains generate a median of 25 daughter cells. The finite replicative potential differs strongly among each cell within a population and cells can reach an age of 50-60 divisions. As aging is accompanied by the accumulation of

intracellular damage, including protein aggregates, cells ultimately divide a final time and eventually lyse, often after being arrested in G1 of the cell cycle. The daughter cells that arise do not inherit the cellular damage from the mother and are thus rejuvenated with a full replicative potential (Egilmez and Jazwinski, 1989; Jazwinski et al., 1989).

The first characterizations of the budding yeast replicative lifespan were performed using dissection microscopes for manual dissection of mother and daughter cells (Mortimer and Johnston, 1959). Such an assessment of lifespan is still considered a standard method in yeast aging but is more and more replaced by less labor-intensive assays that allow a higher throughput of cells, such as microfluidics. Aged yeast cells can be enriched in cultures via a biotin-streptavidin affinity purification method with magnetic bead-sorting to study the effects of cellular aging (Smeal et al., 1996). The cell walls of young cells from a mid-logarithmic culture are biotinylated and allowed to divide under regular growth conditions. The cells are sorted with streptavidin-coated magnetic beads, so that newly generated unlabeled daughter cells are removed through washes, while old biotinylated cells are collected. These cycles of growth and sorting can be performed multiple times depending on the desired average age of the collected cells. The enrichment of old cells can also be performed through genetic manipulation (mother enrichment program, MEP) (Lindstrom and Gottschling, 2009) or by using a chemostat that continuously removes and resupplies media (Hendrickson et al., 2018; Miller et al., 2013; Yang and Pon, 2022). The age of yeast cells can be determined by staining and counting bud scars, since each division event leaves the cell with a round scar, where the daughter cell budded off.

4.2 REPORTERS TO STUDY SPQC

To monitor the process of spatial sequestration of protein aggregates, the usage of different misfolding proteins has been key. The method of choice is commonly fluorescence microscopy, which is why the reporter proteins often contain fusions to fluorescent proteins. Pathways of sPQC have been studied using protein fusions of molecular chaperones that associate with protein aggregates, mostly by tagging the general disaggregase Hsp104 (Fujita et al., 1998; Lum et al., 2004). Additionally, various reporters were employed to model endogenous misfolding proteins, e.g. Ubc9-ts (Kaganovich et al., 2008). Both strategies serve to understand the response of the budding yeast PQC in different conditions. The cell is capable of handling numerous challenges to proteostasis without any obvious defects in cellular fitness, e.g. when overproducing the misfolding reporter proteins guk1-7, gus1-3 and pro3-1

(Paper II), which provokes the questions why a small subset of misfolding protein species leads to toxicity and which protein attributes constitute proteotoxicity (Kaganovich et al., 2008). Unraveling the cellular pathways that fail to handle such proteins is crucial to understand disease etiology and to find therapeutic targets.

Numerous models of protein conformational disorders have been developed for budding yeast, including several prions, huntingtin (HD), alpha-synuclein (PD) and amyloid-beta (AD). Since *S. cerevisiae* lacks homologs of neurodegenerative disease proteins, they are commonly ectopically expressed, often with inducible promoters to control toxicity. The use of such disease proteins in budding yeast has considerably contributed to the understanding of cellular mechanisms that handle such aggregates. For example, expression of the huntingtin exon-1 in yeast and mammalian cells results in the same aggregation behavior in those cell models in that the length of the polyQ tract determines the severity of aggregation (Krobitsch and Lindquist, 2000). The yeast model helped unravel the complex mechanisms behind the cellular toxicity of mutant huntingtin. It was found that amino acid sequences flanking the polyQ exon determine toxicity, even when expressed in trans in other polypeptides, and that other parts of the proteome, such as presence and conformation of certain other Q-rich proteins can modulate toxicity (Duennwald et al., 2006b, 2006a). Moreover, the yeast Hsp40s Ydj1 and Sis1 were found to modulate toxicity of mutant huntingtin (Gokhale et al., 2005) and later reports confirmed such a role also for human Hsp40 in a mouse model (Kakkar et al., 2016b) and for other neurological disease proteins (Kakkar et al., 2016a). The expression of disease proteins in yeast also indicated that metastable and disease proteins competing for the same PQC resources, i.e. limited chaperone availability and proteasome capacity, contributes to toxicity (Park et al., 2013). Similar results were obtained in a mouse model of HD, where the progressive decline of several chaperone levels in the brain correlated with disease pathogenesis (Hay et al., 2004). This concept would also account for the age association that is observed for such neurodegenerative diseases, since the PQC capacity progressively declines with age. We employed the neurological disease proteins of HD, PD and AD to assess how the function of vesicle trafficking in sPQC of endogenous and misfolding reporter proteins may translate when exposing the cells to neurological disease proteins (Paper III, IV). We monitored growth rate and fitness as phenotypic readouts of toxicity and found a potential function in mitigating toxicity for both Sed5 and Sec7 of the vesicle trafficking machinery.

4.3 GENOME-WIDE SCREENS

A major benefit of working with budding yeast is that genetic manipulations are easy and fast compared to more complex eukaryotes. Additionally, mutant collections are available for the entire yeast genome. Such collections are commercially available for various genetic manipulations such as overexpression of a gene or titratable shutoff of expression and for fusions of gene products with different tags, e.g. GFP or HA, allowing for microscopic and biochemical applications.

The collections are not only convenient to attain certain modified strains but also to perform entire genome-wide screens. For this, the collections of interest are crossed with a query strain according to synthetic genetic array (SGA) methodology. The SGA query strain contains certain genetic elements that allow plate-based selection of the haploid double mutants of interest (Tong et al., 2001). Genome-wide screens are an explorative approach that allows to pinpoint, for example, pathways, complexes or biological functions that are relevant for the phenotype in question. The resulting genes or "hits" can be applied to GO (gene ontology) term enrichment analysis, facilitating the grouping into biological and molecular functions, cellular location of the gene product, metabolic pathway etc. based on known GO term annotations. Another analysis available for yeast is SAFE (Spatial analysis of functional enrichment) (Baryshnikova, 2016), which reveals enrichments for genetic profile similarity to annotate data. This analysis is based on the genetic interaction data of *S. cerevisiae* that has been acquired by the research groups that designed the SGA methodology and performs an analysis based on the similarity between genetic interaction profiles of genes; it is thus different from GO term enrichment analysis. A simplified version of SAFE analysis is available as an in-browser online tool (Usaj et al., 2017).

Yeast collections have been used extensively in studies on proteostasis (Hill et al., 2016; Liu et al., 2010; Popova et al., 2021; Song et al., 2014; Willingham et al., 2003) as they allow to explore questions on a genome-wide basis. The SGA methodology can be used to create custom genome-wide libraries with different follow-up applications. We employed genome-wide screens to uncover vesicle trafficking as a major pathway of sPQC by assessing mutant fitness on AZC plates and by monitoring inclusion formation with high-content microscopy (Paper III, IV). In another microscopy screen we uncover sorting factors required for the subcellular localization of protein aggregates (Paper V). Other proteostasis research based on SGA methodology in yeast defined the RQC and endocytosis as pathways involved in managing mutant huntingtin (Berglund et al., 2017; Yang et al., 2016; Zheng et al., 2017), found

modifiers of toxicity of amyloid-beta (AD) (Chen et al., 2020; Treusch et al., 2011), alpha-synuclein (PD) (Höllerhage et al., 2019; Willingham et al., 2003) and mutant huntingtin (HD) (Willingham et al., 2003) and identified asymmetry-generating genes for heat-induced protein aggregates (Hill et al., 2016). The unbiased screens performed in our studies and others helped unravel a potential mechanism of CytoQ to IPOD inclusion formation via vesicle trafficking components, which emphasizes that our knowledge about the PQC machinery is not exhaustive and novel factors can still be identified (Hill et al., 2016; Rössler et al., 2019, Paper III).

4.4 MICROSCOPY

Studies of sPQC heavily rely on microscopy to visualize protein aggregates and how they are sequestered into larger inclusions at certain sites in the cell. Light microscopy (LM) allows the visualization of fluorescent protein fusions in live cells, which is especially useful for timelapse microscopy to monitor e.g. aggregate inheritance, disaggregation behavior of aggregates or localization of protein aggregates throughout the recovery process at standard growth temperature after heat shock (Paper I, II, III). Fluorescence microscopy also enables co-localization studies with other protein fusions (Paper IV) or organelles (Paper V). For most sPQC questions, a standard microscope suffices but it is possible to increase resolution to visualize details, e.g. with SIM (structured illumination microscopy, Paper II). The visualization of aggregates in live cells also allows application of advanced imaging methods such as FCS (fluorescence correlation spectroscopy, Paper III, IV), which we used to quantify Hsp104-GFP dynamics. When live dynamics are not desired, it is possible to fix the cells at different time points during stress treatments (Papers I-V). We found fixation especially useful for the high content-microscopy screen because it allows us to fix the cells on the entire 96-well plate at the same time. Live cells could erroneously display certain sPQC phenotypes due to the image acquisition time across a 96-well plate. The addition of tags on proteins comes with certain risks (Paper I), it is thus advantageous to confirm that the fluorescent protein fusion is a valid reporter on the behavior of the protein itself. One option to confirm functionality of a protein fusion is to perform immunofluorescence against the untagged protein of interest and compare to the tagged version (Paper V).

Another option to investigate sPQC is electron microscopy (EM), which visualizes protein aggregates as ribosome-excluding, electron-dense structures. It is thus possible to omit immunolabeling when endogenous protein aggregates are of interest (Paper II). In this context, yeast cells can be imaged

at ultra-resolution without employing any fluorescent fusions for protein aggregates. Additionally, the images show organelles and most cellular structures simultaneously without the need of fluorescent stains or protein fusions, which are limited due to compatibility of fluorescent tags. The ultra-resolution of yeast cells allowed us to discover the clustering of virus-like particles (VLPs) in proximity to protein aggregates during heat stress, which could have gone unnoticed when only relying on LM (Paper V). Immunolabeling can be performed to visualize specific proteins or structures using antibodies with colloidal gold. We were able to expand the method through double-immunolabeling and determined the distances between two protein species mixed within common inclusions (Paper II).

5 RESULTS AND DISCUSSION

5.1 MAIN FINDINGS

The following highlights findings from each article, which are discussed and put into context of proteostasis research in the sections thereafter.

Paper I

- Comparative analysis of a suite of fluorescent proteins fused to endogenous Hsp104 reveals that the tags affect several PQC functions of the disaggregase to varying degrees
- Hsp104 fusions cause decreased Hsp104 levels
- Hsp104-mScI displays rapid aggregate clearance and full thermotolerance, which were both impaired in other Hsp104 fusions

Paper II

- guk1-7, gus1-3 and pro3-1 form heat-induced non-toxic protein aggregates that are subjected to sPQC
- The reporters mainly follow common PQC pathways but significant differences exist in their processing
- pro3-1 is cleared faster than other protein aggregates even from inclusions shared with slowly resolved protein
- Rapid pro3-1 clearance from shared inclusions requires Hsp104 and is not facilitated through spatial compartmentalization of different protein species

Paper III

- Screening the non-essential gene mutant collection for factors required for inclusion formation reveals vesicle trafficking components such as the COG (conserved oligomeric Golgi) complex
- COG acts through Sed5 and COPII-dependent anterograde trafficking in inclusion formation and clearance
- Hsp104 associates with COP vesicles
- Sed5 mitigates proteotoxicity
- Sed5 sorts misfolded proteins to mitochondria, thereby boosting aggregate clearance

Paper IV

- Screening the essential gene mutant collection for factors required for inclusion formation reveals vesicle trafficking components including Sec7
- Sec7 is a limiting factor for inclusion formation during heat stress and aging
- Sec7 has a role in mitigating proteotoxicity

Paper V

- Endogenous protein aggregates and misfolding proteins localize to both mitochondria and virus-like particles (VLPs) upon heat stress
- Factors required for sorting towards this aggregate deposition site differ depending on the misfolding protein species
- Lack of Clu1 causes partial mis-localization of aggregates but only subtle effects on proteostasis
- VLPs may affect PQC and vice-versa

5.2 FLUORESCENT PROTEIN FUSIONS AND MISFOLDING REPORTER PROTEINS

Several fluorescently tagged misfolding reporters have been developed for studies of sPQC, as they allow either tracking components of the PN in the cell or directly monitoring misfolding proteins. Such reporters exist both for endogenous proteins (e.g. fluorescently tagged Hsp104, Hsp42, Ubc9-ts) and for proteins of conformational protein diseases (e.g. mutant Huntingtin, alpha-synuclein). They come with potential caveats that may affect the physiology of the cell. For example, tagging Hsp104 influences various PQC phenotypes (Paper I), Ubc9-ts is toxic (Oling et al., 2014), VHL does not have a properly folding control reporter (Kaganovich et al., 2008), and expression of neurodegenerative disease proteins is often controlled with inducible promoters to evade toxicity during growth and entails a shift in cell physiology to induce expression, e.g. a change in carbon source. Therefore, we aimed to extend the available endogenous fluorescent protein fusions of Hsp104 and test their behavior in common PQC assays (Paper I) and additionally, characterize a set of misfolding proteins, which are subjected to sPQC and not toxic (Paper II).

We tagged Hsp104 with mGFP, mNeonGreen and mSc-I, and compared the newly generated fluorescent fusions to the Hsp104-GFP strain from the GFP-

tagged collection (Huh et al., 2003), to untagged Hsp104 (BY4741) and to a strain lacking the disaggregase (*hsp104*Δ), in several standard proteostasis assays (Paper I). The fluorescent proteins did not cause any toxic gain-of-function detectable in growth rate or fitness, since all tagged strains behaved the same as the untagged strain and the strain lacking the gene. In several assays, however, we noted phenotypic differences not only due to presence of a tag but differing in severity depending on which fluorescent protein was fused to Hsp104. All protein fusions were affected in their protein levels and in the induction of Hsp104 production upon heat shock compared to the untagged disaggregase. However, the GFP fusion performed poorly in aggregate clearance after heat shock and in thermotolerance. While mGFP and mNeonGreen displayed intermediate phenotypes, the mSc-I fusion was markedly more efficient at aggregate removal and thermotolerance than the other fluorescent protein fusions.

The comparative analysis of these different endogenous Hsp104 fusions clearly shows that fluorescent tagging affects Hsp104 function and, more importantly, that the extent of such effects differs depending on the respective fluorescent protein. Consequently, fluorescent tagging can alter and impair chaperone function tremendously. It is thus important to consider that phenotypic readouts may differ depending on the fluorescent tag, especially when comparing Hsp104 behavior across studies. The results strengthen the idea that it is favorable to include several reporters in sPQC studies and to combine fluorescently tagged chaperones with monitoring misfolding protein reporters (e.g. Paper III, V).

The misfolding reporter proteins guk1-7, gus1-3 and pro3-1 allowed us to study the response of the PQC machinery towards the same reporter constructs of different protein species (Paper II). gus1-3 requires partially different PQC machineries than the other two reporters, which emphasizes the necessity of different misfolding reporter proteins to identify general versus context-specific PQC mechanisms and that this has the potential to reveal novel PQC pathways. Additionally, our data indicates that even when sorting of aggregates follows a common pathway, i.e. to the inclusion site at mitochondria, the sorting factors differ depending on the protein species (Paper V). Going forward with research on these reporter proteins, it would be exciting to find the PQC pathways responsible for gus1-3 degradation, since the proteasome does not successfully degrade gus1-3 (Khosrow-Khavar et al., 2012, Paper II). Overall, the PN components that handle the three reporter proteins during heat stress require further characterization. We found that aggregate formation before stress, disaggregation behavior of guk1-7 and gus1-3 during continuous mild heat shock and inclusion formation efficiency

were similarly impaired in both *hsp104*Δ and *ssa1*Δ *ssa2*Δ mutant cells, which is plausible given the functional interdependency of the Hsp100-Hsp70 bi-chaperone system (Winkler et al., 2012) and is in line with previous reports using these mutants (Andersson et al., 2021). The behavior of pro3-1 during the time course, however, is notably different between the two PQC mutants. In contrast to *hsp104*Δ cells, pro3-1 forms aggregates at standard growth temperature in the Hsp70 double mutant. We have not observed pro3-1 to aggregate before stress in any other condition or mutant, it would thus be interesting to elucidate the mechanism of Hsp70 function or related pathways that maintain pro3-1 stable at standard growth.

The reporter proteins will also prove useful to investigate PQC under regular growth conditions and during replicative aging, since guk1-7 and gus1-3 misfold to some extent at 30°C. This allows performing experiments in the absence of stress, in contrast to the conditions required for other reporters of sPQC, such as proteasome inhibition and heat shock. While they do not affect replicative lifespan or fitness (Paper II), it would be interesting to elucidate the machineries handling these proteins successfully during aging and, in comparison to toxic disease proteins, whether any of these PQC pathways fail for other proteins. The reporters could also be used to define the PQC pathways required to maintain their proper processing and if they can become toxic when such genes or pathways are incapacitated. For this, genome-wide SGA screens could be performed using mutant collections containing the ts versions (guk1-7-GFP, gus1-3-GFP, pro3-1-GFP) versus corresponding properly folding wild type versions (Guk1-GFP, Gus1-GFP, Pro3-GFP). A fitness decrease when comparing double mutants of the ts and the wild type reporters would reveal components that are required to prevent their cytotoxicity.

5.3 DIFFERENTIAL PROCESSING OF MISFOLDING PROTEINS

Deciphering the PQC response to different misfolding proteins is crucial to gain insight into detrimental characteristics of misfolded proteins that cause the PQC machinery to fail and result in toxicity. We sought out to compare the processing of three misfolding proteins to determine if the PQC machinery handles them similarly. None of the reporters were able to evade PQC, as they are all processed successfully and thus non-toxic (Paper II). In fact, gus1-3 seemed to pose a bigger challenge to the cell to be recognized and removed by the PQC machinery, yet we detected no perturbation in cell growth and fitness, even at higher temperatures, when misfolding of gus1-3 is heavily induced. gus1-3 overall requires additional pathways of PQC for its management

compared to guk1-7 and pro3-1, as its degradation is not proteasome-dependent, gus1-3 aggregates are partially deficient in recruitment of the Hsp104 disaggregase and form a small fraction of "additional" aggregates that do not co-localize with the other two reporters in pairwise combinations. Even when increasing temperature of the thermal insult, gus1-3 behaved somewhat different from the other reporters, since guk1-7 and pro3-1 accumulated into nucleolar rings at 42°C in addition to cytosolic aggregates, while gus1-3 did not (Paper II). Interestingly, despite these differences, all reporters were ultimately sequestered to an inclusion site in proximity to mitochondria during mild heat shock, even though they likely rely on differential sorting factors for this localization (Paper V). Despite mainly co-aggregating at the same deposit site, pro3-1 was more rapidly cleared out from cells than the other reporters and its clearance rate was not decelerated when combined in the same inclusion with guk1-7 or gus1-3, which are both slowly resolved. The differential processing of pro3-1 in pairwise combination strains requires action of Hsp104, but the mechanism is not resolved. Since pro3-1 aggregate clearance is barely affected in *ssa1*Δ *ssa2*Δ cells but depends on Hsp104, a different cytosolic Hsp70 could be part of the PQC targeting pro3-1. pro3-1 misfolds during active translation at the ribosome (temperature-sensitive synthesis, tss protein) and could thus be a client of the RAC-Ssb system. For future work on this project, it would be interesting to pinpoint the factors involved in such differential clearance and whether PQC factors targeting the proteins differ, or this occurs based exclusively on properties of the protein species involved (e.g. due to higher stability of pro3-1 at 30°C than of guk1-7). It is conceivable that mechanisms of differential processing of certain protein species may also play a role in the handling of disease proteins. To understand potential implications for toxicity, it would thus be useful to assess clearance of combinations of non-toxic and disease protein reporters and determine the operating parts of the PQC machinery. Overall, the article highlights that each protein species comes with its own characteristics and depends on differential processing factors (Paper II).

5.4 VESICLE TRAFFICKING IN SPQC AND DISEASE PROTEIN DETOXIFICATION

We employed unbiased genome-wide screens combined with high content-microscopy to identify factors required for functional inclusion formation of heat-induced endogenous protein aggregates (Paper III, IV). We found, in both the non-essential and the essential mutant collections, strong enrichments for vesicle trafficking pathways and components. These findings are in accordance with previous work on sorting endogenous and amyloid-like misfolding

proteins to IPOD inclusions (Hill et al., 2016; Kumar et al., 2016). Our screens revealed specific machineries of late endocytosis required for inclusion formation, including the inositide-3,5-bisphosphate kinase complex, the COG complex, the HOPS and CORVET complexes and the Mon2-Arl1 Golgi network. We pinpointed that the COG complex acts through the essential yeast cis-Golgi t-SNARE syntaxin, Sed5, in inclusion formation and aggregate clearance and found Sed5 to specifically boost disaggregation without simply increasing levels of known PN factors. Sed5 function in sPQC depended on COPII-dependent anterograde transport from ER to Golgi, which defines this part of vesicle trafficking as a component of the PN. The connection between endogenous protein aggregates and anterograde trafficking is further strengthened by biochemical data from Hill et al., which identified vesicle trafficking components, including COPII vesicle components, as physical interactors of Hsp104 (Hill et al., 2016). We also found that Hsp104 co-localizes and physically interacts with the trans Golgi network component Sec7 (Paper III, Hill et al., 2016), which we defined as limiting in sPQC in a similar manner as Sed5, acting in an Hsp70-dependent PQC pathway (Paper IV). The inclusion formation boosted by Sed5 leads to deposition of protein aggregates in proximity to mitochondria, not JUNQ/INQ, and thus identifies the Sed5-mediated inclusion formation as a sorting pathway of endogenous heat-induced protein aggregates to mitochondria. The sorting towards mitochondria is important for Sed5-mediated aggregate clearance, and depends on Myo2-dependent transport, mitochondrial function and vacuole-mitochondria contact sites (vCLAMP). This newly defined vesicle trafficking route as a component of the PN was not only relevant in sPQC but also in detoxification of misfolded proteins (genome-wide screen with AZC, Paper III) and neurological disease proteins (Paper III, IV). We expanded our understanding of vesicle trafficking in sPQC during heat shock to replicatively aged cells and found that, while overproduction of Sec7 does not extend replicative lifespan, it does improve age-associated aggregation (Paper IV). It is currently unclear if the Vac17, Sed5 and Sec7 pathways for inclusion deposition are targeting misfolded proteins to the same, common inclusion site. It will be important to put these findings into context, for example to determine whether the Sed5 PQC pathway converges with the Sec7 pathway downstream in the vesicle trafficking route and how Sed5 and Sec7 relate to the Vac17-Myo2-dependent inclusion formation at the vacuole.

A part of the vesicle trafficking route in sPQC that is rather understudied is the involvement of the actin cytoskeleton. Assuming that aggregates associated with vesicles and potentially hitchhiked on them to inclusion sites, their transport would demand a functional actin cytoskeleton. However, the involvement of the cytoskeleton in sorting to inclusion sites has been

ambiguous (Escusa-Toret et al., 2013; Kaganovich et al., 2008; Specht et al., 2011), in contrast to its clear function in establishing asymmetry (Liu et al., 2011). While we and others were able to identify the role of various vesicle trafficking components and Myo2-based transport in IPOD inclusion formation (Hill et al., 2016; Kumar et al., 2016, Paper III), and know aggregates of several protein species to localize to actin cables (Liu et al., 2011; Song et al., 2014; Yang et al., 2016), the presumed direct involvement of actin and its transport function in this sPQC pathway remains to be demonstrated.

5.5 INCLUSION FORMATION AT MITOCHONDRIA AND VLPS

The inclusion formation of aggregates at mitochondria was previously proposed for certain heat shock conditions and reporter proteins (Böckler et al., 2017; Zhou et al., 2014, Paper III), which we confirmed by EM of endogenous protein aggregates resolved temporally over a continuous mild heat shock (Paper V). We found the misfolded reporter proteins guk1-7, gus1-3 and pro3-1 to form inclusions at the same site (Paper II), indicating that sequestration of aggregates at mitochondria is a common pathway of various protein species, even when they partially rely on different PQC machineries for their processing (Paper II). High content-microscopy screens identified differential sorting factors for gus1-3 and Hsp104 into inclusions near mitochondria, however, no major defects in fitness and proteostasis were found when disrupting the inclusion formation at mitochondria by genetics (*clu1*Δ). It is possible that any defects would be moderate and not detected by our assays since the localization of aggregates to mitochondria can only be disrupted to a limited extent due to random association that accounts for factors such as cell size and the space that mitochondria and aggregates occupy within the cell. Zhou et al. predicted this random association to be around 50% in wild type cells and the largest disruption they observed was from ca. 90% of aggregates at mitochondria in wild type to ca. 55% in *fis1*Δ cells, emphasizing that a substantial number of protein aggregates still localize to mitochondria. The random

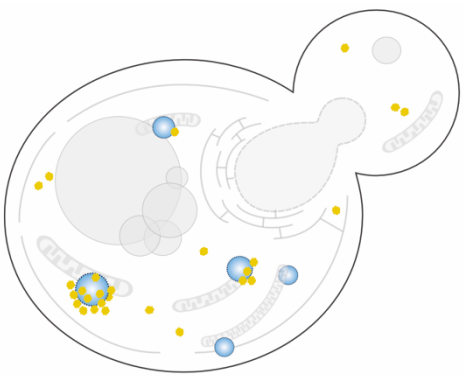

Figure 9: Virus-like particles (yellow) cluster at protein aggregates (blue) near mitochondria at intermediate times of mild heat shock.

association could explain the somewhat subtle effects of distal aggregate localization relative to mitochondria on asymmetric inheritance (Zhou et al., 2014) and clearance of aggregates (Paper III).

We identified another cellular component in proximity of aggregates and mitochondria, the virus-like particles (VLPs) (Paper V, Figure 9). VLPs are small, membrane-less bodies, similar to retroviral nucleocapsids, and are part of the retrotransposition cycle of the Ty transposable elements (Garfinkel et al., 1985). Budding yeast harbors five families of Ty retrotransposons, Ty1-Ty5, which are long-terminal repeat (LTR) RNA transposons scattered over the genome. They make up ca. 3.5% of the S288C yeast genome (Maxwell, 2020). These retrotransposons are evolutionary conserved and play a role in adaptation to environmental cues and contribute to evolution (Garfinkel, 2005). They proliferate in a similar way to retroviruses but are not infectious.

Ty1 is the most abundant and best characterized of the five retrotransposon families in *S. cerevisiae* (Curcio M. Joan et al., 2015). The Ty1 genetic element is flanked by LTRs at each end and consists of two partially overlapping open reading frames, *GAG* and *POL* (Figure 10). *GAG* encodes the structural capsid protein, while *POL*, with a small overlap into *GAG*, encodes the Gag-Pol polyprotein, which is cleaved into three enzymes required for retrotransposition: protease, reverse transcriptase and integrase. The intracellular replication cycle of Ty retrotransposons (Figure 11) begins with transcription of the Ty genomic element and its subsequent export from the nucleus into the cytoplasm. The RNA becomes translated into p49-Gag and p199-Gag-Pol. These three components assemble into cytoplasmic retrosomes or T-bodies, which are sites of VLP nucleation. The RNA becomes packaged as dimers into VLPs, in which the maturation into p45-Gag, protease, reverse transcriptase and integrase occurs. Consequently, all components are assembled for retrotransposition itself to occur, i.e. reverse transcription of Ty RNA into cDNA, which becomes integrated into the nuclear genome.

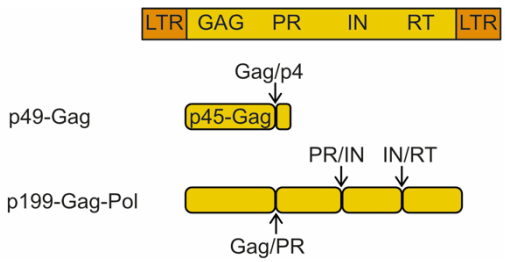

Figure 10: Ty1 genetic element and its translation products p49-Gag and p199-Gag-Pol. Arrows indicate processing sites within the products encoded by the Ty1 element.

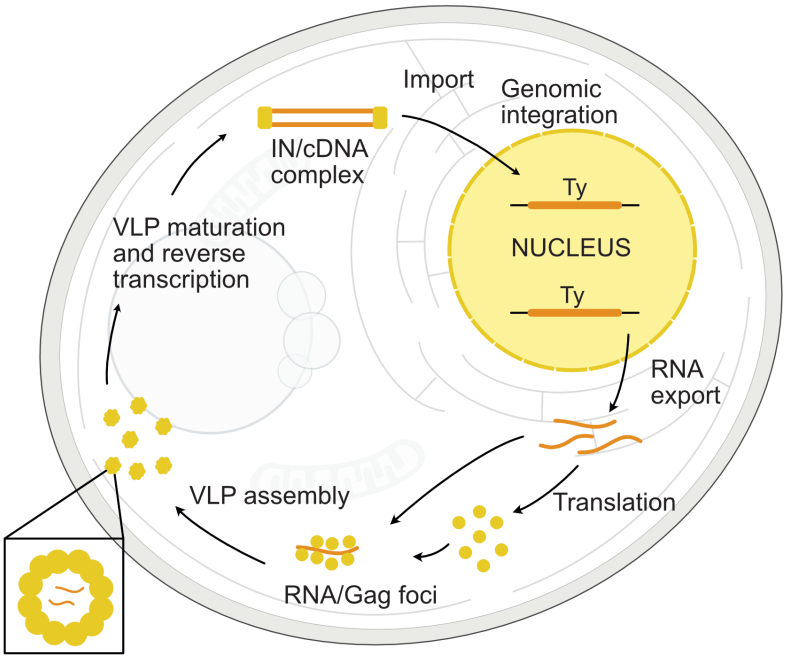

Figure 11: Ty1 retrotransposition cycle. RNA transcribed from the Ty1 element within a chromosome is exported into the cytoplasm for translation. RNA and Gag accumulate into foci, which become assembled into VLPs, which are Gag capsids containing RNA dimers (inset). Ty1 cDNA is generated by reverse transcription and finally imported into the nucleus for genomic integration.

Retrotransposition is the only established function of VLPs but does not occur at 37°C (Lawler Joseph F. et al., 2002; Menees and Sandmeyer, 1996; Paquin and Williamson, 1984). Retrotransposition frequency is optimal at 20°C and thus studied at lower temperature. It is known that VLPs at 37°C do not contain cDNA due to a cDNA synthesis defect and a reduction in RT and PR activities, potentially due to conformational changes of the Gag-Pol polyprotein. Visualization by EM indicates that the structures we observe are assembled VLPs. The constructs we used in LM, Gag1-GFP and anti-Gag, are the Gag protein of Ty1 tagged with GFP and an antibody recognizing the Gag proteins of all Ty families, respectively. At this point, we cannot exclude that we are visualizing partially unassembled/immature VLPs or potentially misfolding components of them using these methods and thus observe the co-localization with protein aggregates, which could imply that PQC affects VLPs. In turn, it is also possible that VLPs affect sPQC, i.e. that their presence at aggregates has a role in protein aggregation, clearance or sorting into inclusions. We will

follow up by using a VLP-less strain and monitoring sPQC in these cells upon heat shock. Whether there is any physiological relevance to the localization of aggregates to mitochondria and/or VLPs remains to be determined. Similarly, since this co-localization was not reported before, nothing is known about the clustering of VLPs at mitochondria during heat shock, regardless of protein aggregation. It will be interesting to test if mutants with mis-localization of protein aggregates distal to mitochondria display altered VLP localization. Our preliminary data using GBP-Pea2 constructs indicates that aggregates and VLPs can localize somewhat separately from one another (Paper V) but we have not visualized mitochondria in this regard.

Retrotransposition is increased with age in eukaryotes from budding yeast and *C. elegans* to mice and human cells (De Cecco et al., 2013b, 2013a; Dennis et al., 2012; Hu et al., 2014; Li et al., 2013; Van Meter et al., 2014). This increase correlates with genomic instability (Maxwell et al., 2011; Patterson et al., 2015; Umezu et al., 2002), one of the hallmarks of aging (López-Otín et al., 2013). It was previously shown that the increase in Ty1 retrotransposition in yeast mothers relative to daughters was not due to asymmetry between Ty1 mRNA levels or Gag accumulation in mother cells but was correlated specifically with a Ty1 cDNA increase in mother cells. The researchers also tested mutants of the ER diffusion barrier and efficient retention of protein aggregates in mother cells (*hsp104*Δ) and found that these known asymmetry establishing factors are not required for the mother-biased Ty1 retromobility (Patterson et al., 2015). In this context of aging, we plan to monitor VLP-less cells during aging, their replicative lifespan and the capacity of their sPQC machinery.

An important question that I would like to address in future work is which functional, physiological relevance the intracellular location of inclusions has, which is intimately connected to further our understanding about aggregate features that constitute toxicity. At this point, some reports argue for the actual site of sequestration influencing fitness, while others suggest that the intracellular location itself is not relevant for cytotoxicity, as long as smaller aggregates are sequestered into larger, protective inclusions. For example, cells lacking the NEF Fes1 and Hsp104 are temperature-sensitive due to stabilization of Hsp70-client interactions, but their fitness can be maintained or even restored by expressing a nucleus-targeted Hsp42 version or by expressing a Btn2 variant restricted to the cytosol. This indicates that the sequestrase itself (Hsp42 vs. Btn2) and the site of inclusions (nucleus vs. cytosol), are interchangeable and that cells maintain fitness as long as sequestration occurs (Ho et al., 2019). This concept would be in line with the lack of toxicity in proteostasis assays observed in a mutant of the mitochondrial

deposition pathway of endogenous aggregates (*clu1*Δ, Paper V). In contrast to these findings, using amyloid-like aggregate reporters in human cells, it was found that inclusion formation in the perinuclear region is more cytoprotective than cytosolic aggregation, as the inclusions in the cytosol co-aggregated with unrelated cellular proteins and thereby disrupted secondary processes (Woerner et al., 2016). Similarly, it was shown that toxicity is determined by aggregation within JUNQ vs. aggregation in cytosolic foci (Weisberg et al., 2012). Additionally, a study using budding yeast found the Hsp70/90 co-chaperone Sti1 to suppress cytotoxicity of mutant Huntingtin by promoting the formation of juxtanuclear inclusions instead of cytosolic aggregation (Wolfe et al., 2013). The ambiguity of the significance of subcellular inclusion location for proteotoxicity might be related to the different characteristics that the protein species of interest entails, for example whether it forms amorphous or amyloid-like aggregates.

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REFERENCES

Aguilaniu H. 2003. Asymmetric Inheritance of Oxidatively Damaged Proteins During Cytokinesis. Science 299:1751–1753.

Ahmadpour D, Babazadeh R, Nystrom T. 2020. Hitchhiking on vesicles: a way to harness age-related proteopathies? FEBS J 287(23):5068–5079.

Amorós M, Estruch F. 2001. Hsf1p and Msn2/4p cooperate in the expression of Saccharomyces cerevisiae genes HSP26 and HSP104 in a gene- and stress type-dependent manner. Mol Microbiol 39(6):1523–1532.

Andersson R, Eisele-Bürger AM, Hanzén S, Vielfort K, Oling D, Eisele F, Johansson G, Gustafsson T, Kvint K, Nyström T. 2021. Differential role of cytosolic Hsp70s in longevity assurance and protein quality control. PLoS Genet 17:1008951.

Andersson V, Hanzén S, Liu B, Molin M, Nyström T. 2013. Enhancing protein disaggregation restores proteasome activity in aged cells. Aging 5:802–812.

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

Babazadeh R, Ahmadpour D, Jia S, Hao X, Widlund P, Schneider K, Eisele F, Edo LD, Smits GJ, Liu B. 2019. Syntaxin 5 Is Required for the Formation and Clearance of Protein Inclusions during Proteostatic Stress. CellReports 28:2096-2110 2098.

Balch WE, Morimoto RI, Dillin A, Kelly JW. 2008. Adapting Proteostasis for Disease Intervention. Science 319:916–919.

Balchin D, Hayer-Hartl M, Hartl FU. 2016. In vivo aspects of protein folding and quality control. Science 353:4354–4354.

Baryshnikova A. 2016. Systematic Functional Annotation and Visualization of Biological Networks. Cell Syst 2(6):412–421.

Behrends C, Langer CA, Boteva R, Böttcher UM, Stemp MJ, Schaffar G, Rao BV, Giese A, Kretzschmar H, Siegers K, Hartl FU. 2006. Chaperonin TRiC Promotes the Assembly of polyQ Expansion Proteins into Nontoxic Oligomers. Mol Cell 23(6):887–897.

Bence NF, Sampat RM, Kopito RR. 2001. Impairment of the ubiquitin-proteasome system by protein aggregation. Science 292(5521):1552–1555.

Ben-Zvi A, Miller EA, Morimoto RI. 2009. Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proc Natl Acad Sci 106(35):14914–14919.

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

Bettayeb K, Chang JC, Luo W, Aryal S, Varotsis D, Randolph L, Netzer WJ, Greengard P, Flajolet M. 2016a. δ-COP modulates Aβ peptide formation via retrograde trafficking of APP. Proc Natl Acad Sci U S A 113(19):5412–5417.

Bettayeb K, Hooli BV, Parrado AR, Randolph L, Varotsis D, Aryal S, Gresack J, Tanzi RE, Greengard P, Flajolet M. 2016b. Relevance of the COPI complex for Alzheimer's disease progression in vivo. Proc Natl Acad Sci U S A 113(19):5418–5423.

Bjørkøy G, Lamark T, Brech A, Outzen H, Perander M, Øvervatn A, Stenmark H, Johansen T. 2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol 171(4):603–614.

Böckler S, Chelius X, Hock N, Klecker T, Wolter M, Weiss M, Braun RJ, Westermann B. 2017. Fusion, fission, and transport control asymmetric inheritance of mitochondria and protein aggregates. J Cell Biol 216:2481–2498.

Bode DC, Baker MD, Viles JH. 2017. Ion Channel Formation by Amyloid-β42 Oligomers but Not Amyloid-β40 in Cellular Membranes. J Biol Chem 292(4):1404–1413.

Borkovich KA, Farrelly FW, Finkelstein DB, Taulien J, Lindquist S. 1989. hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol Cell Biol 9(9):3919–3930.

Borsche M, Pereira SL, Klein C, Grünewald A. 2021. Mitochondria and Parkinson's Disease: Clinical, Molecular, and Translational Aspects. J Park Dis 11(1):45–60.

Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li G-W, Zhou S, King D, Shen PS, Weibezahn J, Dunn JG, Rouskin S, Inada T, Frost A, Weissman JS. 2012. A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. Cell 151(5):1042–1054.

Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, Jülicher F, Hyman AA. 2009. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 324(5935):1729–1732.

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

Breckel CA, Hochstrasser M. 2021. Ubiquitin Ligase Redundancy and Nuclear-Cytoplasmic Localization in Yeast Protein Quality Control. Biomolecules 11(12).

Brehme M, Voisine C, Rolland T, Wachi S, Soper JH, Zhu Y, Orton K, Villella A, Garza D, Vidal M, Ge H, Morimoto RI. 2014. A Chaperome Subnetwork Safeguards Proteostasis in Aging and Neurodegenerative Disease. Cell Rep 9(3):1135–1150.

Bruderek M, Jaworek W, Wilkening A, Rüb C, Cenini G, Förtsch A, Sylvester M, Voos W. 2018. IMiQ: a novel protein quality control compartment protecting mitochondrial functional integrity. Mol Biol Cell 29(3):256–269.

Bufalino MR, DeVeale B, Kooy D. 2013. The asymmetric segregation of damaged proteins is stem cell–type dependent. J Cell Biol 201:523–530.

Camilleri A, Zarb C, Caruana M, Ostermeier U, Ghio S, Högen T, Schmidt F, Giese A, Vassallo N. 2013. Mitochondrial membrane permeabilisation by amyloid aggregates and protection by polyphenols. Biochim Biophys Acta 1828(11):2532–2543.

Caron NS, Hung CL, Atwal RS, Truant R. 2014. Live cell imaging and biophotonic methods reveal two types of mutant huntingtin inclusions. Hum Mol Genet 23(9):2324–2338.

Chen R-H, Chen Y-H, Huang T-Y. 2019. Ubiquitin-mediated regulation of autophagy. J Biomed Sci 26(1):80.

Chen X, Ji B, Hao X, Li X, Eisele F, Nyström T, Petranovic D. 2020. FMN reduces Amyloid-β toxicity in yeast by regulating redox status and cellular metabolism. Nat Commun 11(1):867.

Cherkasov V, Hofmann S, Druffel-Augustin S, Mogk A, Tyedmers J, Stoecklin G, Bukau B. 2013. Coordination of translational control and protein homeostasis during severe heat stress. Curr Biol CB 23(24):2452–2462.

Chernova TA, Wilkinson KD, Chernoff YO. 2017. Prions, Chaperones, and Proteostasis in Yeast. Cold Spring Harb Perspect Biol 9(2).

Cho H, Shim WJ, Liu Y, Shan S-O. 2021. J-domain proteins promote client relay from Hsp70 during tail-anchored membrane protein targeting. J Biol Chem 296:100546.

Choe Y-J, Park S-H, Hassemer T, Körner R, Vincenz-Donnelly L, Hayer-Hartl M, 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, Vendruscolo M. 2015. Supersaturation is a major driving force for protein aggregation in neurodegenerative diseases. Trends Pharmacol Sci 36(2):72–77.

Ciryam P, Tartaglia GG, Morimoto RI, Dobson CM, Vendruscolo M. 2013. Widespread aggregation and neurodegenerative diseases are associated with supersaturated proteins. Cell Rep 5(3):781–790.

Crosas B, Hanna J, Kirkpatrick DS, Zhang DP, Tone Y, Hathaway NA, Buecker C, Leggett DS, Schmidt M, King RW, Gygi SP, Finley D. 2006. Ubiquitin chains are remodeled at the proteasome by opposing ubiquitin ligase and deubiquitinating activities. Cell 127(7):1401–1413.

Curcio M. Joan, Lutz Sheila, Lesage Pascale. 2015. The Ty1 LTR-Retrotransposon of Budding Yeast, Saccharomyces cerevisiae. Microbiol Spectr 3(2):3.2.19.

Dantuma NP, Bott LC. 2014. The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. Front Mol Neurosci 7.

Davis C, Spaller BL, Matouschek A. 2021. Mechanisms of substrate recognition by the 26S proteasome. Curr Opin Struct Biol 67:161–169.

De Cecco M, Criscione SW, Peckham EJ, Hillenmeyer S, Hamm EA, Manivannan J, Peterson AL, Kreiling JA, Neretti N, Sedivy JM. 2013a. Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. Aging Cell 12(2):247–256.

De Cecco M, Criscione SW, Peterson AL, Neretti N, Sedivy JM, Kreiling JA. 2013b. Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. Aging 5(12):867–883.

Defenouillère Q, Yao Y, Mouaikel J, Namane A, Galopier A, Decourty L, Doyen A, Malabat C, Saveanu C, Jacquier A, Fromont-Racine M. 2013. Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products. Proc Natl Acad Sci U S A 110(13):5046–5051.

Dennis S, Sheth U, Feldman JL, English KA, Priess JR. 2012. C. elegans germ cells show temperature and age-dependent expression of Cer1, a Gypsy/Ty3-related retrotransposon. PLoS Pathog 8(3):e1002591.

Douglas PM, Treusch S, Ren H-Y, Halfmann R, Duennwald ML, Lindquist S, Cyr DM. 2008. Chaperone-dependent amyloid assembly protects cells from prion toxicity. Proc Natl Acad Sci U S A 105(20):7206–7211.

Dragovic Z, Broadley SA, Shomura Y, Bracher A, Hartl FU. 2006. Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. EMBO J 25(11):2519–2528.

Duennwald ML, Jagadish S, Giorgini F, Muchowski PJ, Lindquist S. 2006a. A network of protein interactions determines polyglutamine toxicity. Proc Natl Acad Sci U S A 103(29):11051–11056.

Duennwald ML, Jagadish S, Muchowski PJ, Lindquist S. 2006b. Flanking sequences profoundly alter polyglutamine toxicity in yeast. Proc Natl Acad Sci U S A 103(29):11045–11050.

Egilmez NK, Jazwinski SM. 1989. Evidence for the involvement of a cytoplasmic factor in the aging of the yeast Saccharomyces cerevisiae. J Bacteriol 171(1):37–42.

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

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

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

Erjavec N, Larsson L, Grantham J, 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 Dev 21:2410–2421.

Escusa-Toret S, Vonk WIM, Frydman J. 2013. Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. Nat Cell Biol 15:1231–1243.

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

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

Fang NN, Zhu M, Rose A, Wu K-P, Mayor T. 2016. Deubiquitinase activity is required for the proteasomal degradation of misfolded cytosolic proteins upon heat-stress. Nat Commun 7:12907.

Farrawell NE, Lambert-Smith IA, Warraich ST, Blair IP, Saunders DN, Hatters DM, Yerbury JJ. 2015. Distinct partitioning of ALS associated TDP-43, FUS and SOD1 mutants into cellular inclusions. Sci Rep 5:13416.

Feder ZA, Ali A, Singh A, Krakowiak J, Zheng X, Bindokas VP, Wolfgeher D, Kron SJ, Pincus D. 2021. Subcellular localization of the J-protein Sis1 regulates the heat shock response. J Cell Biol 220(1).

Feyder S, De Craene J-O, Bär S, Bertazzi DL, Friant S. 2015. Membrane trafficking in the yeast Saccharomyces cerevisiae model. Int J Mol Sci 16(1):1509–1525.

Finley D. 2009. Recognition and processing of ubiquitin-protein conjugates by the proteasome. Annu Rev Biochem 78:477–513.

Franssens V, Boelen E, Anandhakumar J, Vanhelmont T, Büttner S, Winderickx J. 2010. Yeast unfolds the road map toward α-synuclein-induced cell death. Cell Death Differ 17(5):746–753.

Fuentealba LC, Eivers E, Geissert D, Taelman V, De Robertis EM. 2008. Asymmetric mitosis: Unequal segregation of proteins destined for degradation. Proc Natl Acad Sci U S A 105(22):7732–7737.

Fujita K, Kawai R, Iwahashi H, Komatsu Y. 1998. Hsp104 Responds to Heat and Oxidative Stress with Different Intracellular Localization inSaccharomyces cerevisiae. Biochem Biophys Res Commun 248(3):542–547.

Fusco G, Chen SW, Williamson PTF, Cascella R, Perni M, Jarvis JA, Cecchi C, Vendruscolo M, Chiti F, Cremades N, Ying L, Dobson CM, De Simone A. 2017. Structural basis of membrane disruption and cellular toxicity by α-synuclein oligomers. Science 358(6369):1440–1443.

Gallina I, Colding C, Henriksen P, Beli P, Nakamura K, Offman J, Mathiasen DP, Silva S, Hoffmann E, Groth A, Choudhary C, Lisby M. 2015. Cmr1/WDR76 defines a nuclear genotoxic stress body linking genome integrity and protein quality control. Nat Commun 6(1):6533.

Galvin J, Curran E, Arteaga F, Goossens A, Aubuchon-Endsley N, McMurray MA, Moore J, Hansen KC, Chial HJ, Potter H, Brodsky JL, Coughlan CM. 2022. Proteasome activity modulates amyloid toxicity. FEMS Yeast Res 22(1).

Gao X, Carroni M, Nussbaum-Krammer C, Mogk A, Nillegoda NB, Szlachcic A, Guilbride DL, Saibil HR, Mayer MP, Bukau B. 2015. Human Hsp70 Disaggregase Reverses Parkinson's-Linked α-Synuclein Amyloid Fibrils. Mol Cell 59(5):781–793.

Garfinkel DJ. 2005. Genome evolution mediated by Ty elements in Saccharomyces. Cytogenet Genome Res 110(1–4):63–69.

Garfinkel DJ, Boeke JD, Fink GR. 1985. Ty element transposition: reverse transcriptase and virus-like particles. Cell 42(2):507–517.

Geiler-Samerotte KA, Dion MF, Budnik BA, Wang SM, Hartl DL, Drummond DA. 2011. Misfolded proteins impose a dosage-dependent fitness cost and trigger a cytosolic unfolded protein response in yeast. Proc Natl Acad Sci 108(2):680–685.

Gestaut D, Limatola A, Joachimiak L, Frydman J. 2019. The ATP-powered gymnastics of TRiC/CCT: an asymmetric protein folding machine with a symmetric origin story. Curr Opin Struct Biol 55:50–58.

Ghosh A, Gangadharan A, Verma M, Das S, Matai L, Dash DP, Dash D, Mapa K, Chakraborty K. 2019. Cellular responses to proteostasis perturbations reveal non-optimal feedback in chaperone networks. Cell Mol Life Sci CMLS 76(8):1605–1621.

Gidalevitz T, Ben-Zvi A, Ho KH, Brignull HR, Morimoto RI. 2006. Progressive disruption of cellular protein folding in models of polyglutamine diseases. Science 311(5766):1471–1474.

Glover JR, 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, Chernoff YO. 2005. Modulation of prion-dependent polyglutamine aggregation and toxicity by chaperone proteins in the yeast model. J Biol Chem 280(24):22809–22818.

Gong Y, Kakihara Y, Krogan N, Greenblatt J, Emili A, Zhang Z, Houry WA. 2009. An atlas of chaperone-protein interactions in Saccharomyces cerevisiae: implications to protein folding pathways in the cell. Mol Syst Biol 5:275.

Gonzalez-Garcia M, Fusco G, De Simone A. 2021. Membrane Interactions and Toxicity by Misfolded Protein Oligomers. Front Cell Dev Biol 9.

Gottschling DE, Nyström T. 2017. The Upsides and Downsides of Organelle Interconnectivity. Cell 169(1):24–34.

Gowda NKC, Kandasamy G, Froehlich MS, Dohmen RJ, Andréasson C. 2013. Hsp70 nucleotide exchange factor Fes1 is essential for ubiquitin-dependent degradation of misfolded cytosolic proteins. Proc Natl Acad Sci U S A 110(15):5975–5980.

Grousl T, Ungelenk S, Miller S, Ho C-T, Khokhrina M, Mayer MP, Bukau B, Mogk A. 2018. A prion-like domain in Hsp42 drives chaperone-facilitated aggregation of misfolded proteins. J Cell Biol 217(4):1269–1285.

Gruber A, Hornburg D, Antonin M, Krahmer N, Collado J, Schaffer M, Zubaite G, Lüchtenborg C, Sachsenheimer T, Brügger B, Mann M, Baumeister W, Hartl FU, Hipp MS, Fernández-Busnadiego R. 2018. Molecular and structural architecture of polyQ aggregates in yeast. Proc Natl Acad Sci 115(15):E3446–E3453.

Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, Li XJ. 1999. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci Off J Soc Neurosci 19(7):2522–2534.

Hageman J, Vos MJ, Waarde MAWH van, Kampinga HH. 2007. Comparison of intra-organellar chaperone capacity for dealing with stress-induced protein unfolding. J Biol Chem 282(47):34334–34345.

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

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

Hartl FU, Hayer-Hartl M. 2009. Converging concepts of protein folding in vitro and in vivo. Nat Struct Mol Biol 16:574–581.

Hashimoto M, Rockenstein E, Crews L, Masliah E. 2003. Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. Neuromolecular Med 4(1–2):21–36.

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

Haslbeck M, Vierling E. 2015. A first line of stress defense: small heat shock proteins and their function in protein homeostasis. J Mol Biol 427(7):1537–1548.

Haslberger T, Zdanowicz A, Brand I, Kirstein J, Turgay K, Mogk A, Bukau B. 2008. Protein disaggregation by the AAA+ chaperone ClpB involves partial threading of looped polypeptide segments. Nat Struct Mol Biol 15(6):641–650.

Hay DG, Sathasivam K, Tobaben S, Stahl B, Marber M, Mestril R, Mahal A, Smith DL, Woodman B, 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. Hum Mol Genet 13(13):1389–1405.

Hendrickson DG, Soifer I, Wranik BJ, Kim G, Robles M, Gibney PA, McIsaac RS. 2018. A new experimental platform facilitates assessment of the transcriptional and chromatin landscapes of aging yeast. eLife 7.

Hernebring M, Brolén G, Aguilaniu H, Semb H, Nyström T. 2006. Elimination of damaged proteins during differentiation of embryonic stem cells. Proc Natl Acad Sci U S A 103(20):7700–7705.

Hill SM, Hanzén S, Nyström T. 2017. Restricted access: spatial sequestration of damaged proteins during stress and aging. EMBO Rep 18:377–391.

Hill SM, Hao X, Grönvall J, Spikings-Nordby S, Widlund PO, 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(3):826–838.

Hill SM, Hao X, Liu B, Nystrom T. 2014. Life-span extension by a metacaspase in the yeast Saccharomyces cerevisiae. Science 344:1389–1392.

Hipp MS, Park S-H, Hartl FU. 2014. Proteostasis impairment in protein-misfolding and -aggregation diseases. Trends Cell Biol 24:506–514.

Hipp MS, Patel CN, Bersuker K, Riley BE, Kaiser SE, Shaler TA, Brandeis M, Kopito RR. 2012. Indirect inhibition of 26S proteasome activity in a cellular model of Huntington's disease. J Cell Biol 196(5):573–587.

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

Höllerhage M, Bickle M, Höglinger GU. 2019. Unbiased Screens for Modifiers of Alpha-Synuclein Toxicity. Curr Neurol Neurosci Rep 19(2):8.

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

Hoppe T. 2005. Multiubiquitylation by E4 enzymes: "one size" doesn't fit all. Trends Biochem Sci 30(4):183–187.

Hu Z, Chen K, Xia Z, Chavez M, Pal S, Seol J-H, Chen C-C, Li W, Tyler JK. 2014. Nucleosome loss leads to global transcriptional up-regulation and genomic instability during yeast aging. Genes Dev 28(4):396–408.

Huh W-K, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK. 2003. Global analysis of protein localization in budding yeast. Nature 425(6959):686–691.

Huyer G, Longsworth GL, Mason DL, Mallampalli MP, McCaffery JM, Wright RL, Michaelis S. 2004. A striking quality control subcompartment in Saccharomyces cerevisiae: the endoplasmic reticulum-associated compartment. Mol Biol Cell 15(2):908–921.

Jakob U, Gaestel M, Engel K, Buchner J. 1993. Small heat shock proteins are molecular chaperones. J Biol Chem 268(3):1517–1520.

Jarnuczak AF, Albornoz MG, Eyers CE, Grant CM, Hubbard SJ. 2018. A quantitative and temporal map of proteostasis during heat shock in Saccharomyces cerevisiae. Mol Omics 14(1):37–52.

Jazwinski SM, Egilmez NK, Chen JB. 1989. Replication control and cellular life span. Exp Gerontol 24(5–6):423–436.

Johnston JA, Ward CL, Kopito RR. 1998. Aggresomes: A Cellular Response to Misfolded Proteins. J Cell Biol 143:1883–1898.

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.

Kaganovich D, Kopito R, Frydman J. 2008. Misfolded proteins partition between two distinct quality control compartments. Nature 454:1088–1095.

Kakkar V, Kuiper EFE, Pandey A, Braakman I, Kampinga HH. 2016a. Versatile members of the DNAJ family show Hsp70 dependent anti-aggregation activity on RING1 mutant parkin C289G. Sci Rep 6:34830.

Kakkar V, Månsson C, Mattos EP de, Bergink S, Zwaag M van der, Waarde MAWH van, Kloosterhuis NJ, Melki R, Cruchten RTP van, Al-Karadaghi S, Arosio P, Dobson CM, Knowles TPJ, Bates GP, Deursen JM van, Linse S, Sluis B van de, Emanuelsson C, Kampinga HH. 2016b. The S/T-Rich Motif in the DNAJB6 Chaperone Delays Polyglutamine Aggregation and the Onset of Disease in a Mouse Model. Mol Cell 62(2):272–283.

Kamhi-Nesher S, Shenkman M, Tolchinsky S, Fromm SV, Ehrlich R, Lederkremer GZ. 2001. A novel quality control compartment derived from the endoplasmic reticulum. Mol Biol Cell 12(6):1711–1723.

Kandasamy G, Andréasson C. 2018. Hsp70–Hsp110 chaperones deliver ubiquitin-dependent and -independent substrates to the 26S proteasome for proteolysis in yeast. J Cell Sci 131(6):jcs210948.

Kayatekin C, Matlack KES, Hesse WR, Guan Y, Chakrabortee S, Russ J, Wanker EE, Shah JV, Lindquist S. 2014. Prion-like proteins sequester and suppress the toxicity of huntingtin exon 1. Proc Natl Acad Sci 111(33):12085–12090.

Kellner R, Hofmann H, Barducci A, Wunderlich B, Nettels D, Schuler B. 2014. Single-molecule spectroscopy reveals chaperone-mediated expansion of substrate protein. Proc Natl Acad Sci U S A 111(37):13355–13360.

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

Khosrow-Khavar F, Fang NN, Ng AHM, Winget JM, Comyn SA, Mayor T. 2012.

Kirstein J, Strahl H, Molière N, Hamoen LW, Turgay K. 2008. Localization of general and regulatory proteolysis in Bacillus subtilis cells. Mol Microbiol 70(3):682–694.

Kmiecik SW, Le Breton L, Mayer MP. 2020. Feedback regulation of heat shock factor 1 (Hsf1) activity by Hsp70-mediated trimer unzipping and dissociation from DNA. EMBO J 39(14):e104096.

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

Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC. 2009a. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Mol Cell 33(4):517–527.

Korolchuk VI, Menzies FM, Rubinsztein DC. 2009b. A novel link between autophagy and the ubiquitin-proteasome system. Autophagy 5(6):862–863.

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

Kraus A, Groveman BR, Caughey B. 2013. Prions and the potential transmissibility of protein misfolding diseases. Annu Rev Microbiol 67:543–564.

Krobitsch S, Lindquist S. 2000. Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. Proc Natl Acad Sci U S A 97(4):1589–1594.

Kruse KB, Brodsky JL, McCracken AA. 2006. Characterization of an ERAD gene as VPS30/ATG6 reveals two alternative and functionally distinct protein quality control pathways: one for soluble Z variant of human alpha-1 proteinase inhibitor (A1PiZ) and another for aggregates of A1PiZ. Mol Biol Cell 17(1):203–212.

Kryndushkin D, Ihrke G, Piermartiri TC, Shewmaker F. 2012. A yeast model of optineurin proteinopathy reveals a unique aggregation pattern associated with cellular toxicity. Mol Microbiol 86(6):1531–1547.

Kryndushkin DS, Shewmaker F, Wickner RB. 2008. Curing of the [URE3] prion by Btn2p, a Batten disease-related protein. EMBO J 27(20):2725–2735.

Kuemmerle S, Gutekunst CA, Klein AM, Li XJ, Li SH, Beal MF, Hersch SM, Ferrante RJ. 1999. Huntington aggregates may not predict neuronal death in Huntington's disease. Ann Neurol 46(6):842–849.

Kumar A, Mathew V, Stirling PC. 2022. Nuclear protein quality control in yeast: The latest INQuiries. J Biol Chem 298(8):102199.

Kumar R, Nawroth PP, 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, Morimoto RI. 2015a. The biology of proteostasis in aging and disease. Annu Rev Biochem 84:435–464.

Labbadia J, Morimoto RI. 2015b. Repression of the Heat Shock Response Is a Programmed Event at the Onset of Reproduction. Mol Cell 59(4):639–650.

Lawler Joseph F., Haeusser Daniel P., Dull Angie, Boeke Jef D., Keeney Jill B. 2002. Ty1 Defect in Proteolysis at High Temperature. J Virol 76(9):4233–4240.

Li W, Prazak L, Chatterjee N, Grüninger S, Krug L, Theodorou D, Dubnau J. 2013. Activation of transposable elements during aging and neuronal decline in Drosophila. Nat Neurosci 16(5):529–531.

Liebman SW, Chernoff YO. 2012. Prions in Yeast. Genetics 191(4):1041–1072.

Lindstrom DL, Gottschling DE. 2009. The mother enrichment program: a genetic system for facile replicative life span analysis in Saccharomyces cerevisiae. Genetics 183(2):413–422, 1SI-13SI.

Liu B, Larsson L, Caballero A, Hao X, Oling D, Grantham J, 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 SM, Andersson V, Höglund D, Song J, Yang X, Öling D, Grantham J, Winderickx J, Nyström T. 2011. Segregation of protein aggregates involves actin and the polarity machinery. Cell 147(5):959–961.

López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. Cell 153(6):1194–1217.

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

Lum R, Tkach JM, Vierling E, Glover JR. 2004. Evidence for an Unfolding/Threading Mechanism for Protein Disaggregation by Saccharomyces cerevisiae Hsp104 *. J Biol Chem 279(28):29139–29146.

Malinovska L, Kroschwald S, Munder MC, Richter D, Alberti S. 2012. Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. Mol Biol Cell 23:3041–3056.

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

Matsumoto G, Kim S, Morimoto RI. 2006. Huntingtin and mutant SOD1 form aggregate structures with distinct molecular properties in human cells. J Biol Chem 281(7):4477–4485.

Maxwell PH. 2020. Diverse transposable element landscapes in pathogenic and nonpathogenic yeast models: the value of a comparative perspective. Mob DNA 11(1):16.

Maxwell PH, Burhans WC, Curcio MJ. 2011. Retrotransposition is associated with genome instability during chronological aging. Proc Natl Acad Sci U S A 108(51):20376–20381.

Mayer MP, Bukau B. 2005. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci CMLS 62(6):670–684.

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

Medicherla B, Goldberg AL. 2008. Heat shock and oxygen radicals stimulate ubiquitin-dependent degradation mainly of newly synthesized proteins. J Cell Biol 182(4):663–673.

Menees TM, Sandmeyer SB. 1996. Cellular stress inhibits transposition of the yeast retrovirus-like element Ty3 by a ubiquitin-dependent block of virus-like particle formation. Proc Natl Acad Sci U S A 93(11):5629–5634.

Meriin AB, Zhang X, Miliaras NB, Kazantsev A, Chernoff YO, McCaffery JM, Wendland B, Sherman MY. 2003. Aggregation of expanded polyglutamine domain in yeast leads to defects in endocytosis. Mol Cell Biol 23(21):7554–7565.

Miller AW, Befort C, Kerr EO, Dunham MJ. 2013. Design and use of multiplexed chemostat arrays. (72). https://doi.org/10.3791/50262.

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

Miller SBM, Mogk A, Bukau B. 2015b. Spatially Organized Aggregation of Misfolded Proteins as Cellular Stress Defense Strategy. Mol Chaperones Protein Qual Control Part I 427(7):1564–1574.

Misselwitz B, Staeck O, Rapoport TA. 1998. J proteins catalytically activate Hsp70 molecules to trap a wide range of peptide sequences. Mol Cell 2(5):593–603.

Moreno DF, Aldea M. 2020. Proteostatic stress as a nodal hallmark of replicative aging. Exp Cell Res 394(2):112163.

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

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

Mühlhofer M, Berchtold E, Stratil CG, Csaba G, Kunold E, Bach NC, Sieber SA, Haslbeck M, Zimmer R, Buchner J. 2019. The Heat Shock Response in Yeast Maintains Protein Homeostasis by Chaperoning and Replenishing Proteins. Cell Rep 29(13):4593-4607.e8.

Nahar A, Sokolova V, Sekaran S, Orth JD, Park S. 2022. Assembly checkpoint of the proteasome regulatory particle is activated by coordinated actions of proteasomal ATPase chaperones. Cell Rep 39(10).

Nathan DF, Vos MH, Lindquist S. 1997. In vivo functions of the Saccharomyces cerevisiae Hsp90 chaperone. Proc Natl Acad Sci U S A 94(24):12949–12956.

Nguyen NN, Rana A, Goldman C, Moore R, Tai J, Hong Y, Shen J, Walker DW, Hur JH. 2019. Proteasome β5 subunit overexpression improves proteostasis during aging and extends lifespan in Drosophila melanogaster. Sci Rep 9(1):3170.

Nollen EAA, Garcia SM, Haaften G van, Kim S, Chavez A, Morimoto RI, Plasterk RHA. 2004. Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. Proc Natl Acad Sci U S A 101(17):6403–6408.

Ocampo A, Zambrano A, Barrientos A. 2010. Suppression of polyglutamine-induced cytotoxicity in Saccharomyces cerevisiae by enhancement of mitochondrial biogenesis. FASEB J Off Publ Fed Am Soc Exp Biol 24(5):1431–1441.

Ogrodnik M, Salmonowicz H, Brown R, Turkowska J, Średniawa W, Pattabiraman S, Amen T, Abraham A-C, Eichler N, Lyakhovetsky R. 2014. Dynamic JUNQ inclusion bodies are asymmetrically inherited in mammalian cell lines through the asymmetric partitioning of vimentin. Proc Natl Acad Sci USA 111:8049–8054.

Oh E, Akopian D, Rape M. 2018. Principles of Ubiquitin-Dependent Signaling. Annu Rev Cell Dev Biol 34:137–162.

Oling D, Eisele F, Kvint K, Nystrom T. 2014. Opposing roles of Ubp3-dependent deubiquitination regulate replicative life span and heat resistance. EMBO J 33:747–761.

Olzscha H, Schermann SM, Woerner AC, Pinkert S, Hecht MH, Tartaglia GG, Vendruscolo M, Hayer-Hartl M, Hartl FU, Vabulas RM. 2011. Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. Cell 144(1):67–78.

Panagaki D, Croft JT, Keuenhof K, Larsson Berglund L, Andersson S, Kohler V, Büttner S, Tamás MJ, Nyström T, Neutze R, Höög JL. 2021. Nuclear envelope budding is a response to cellular stress. Proc Natl Acad Sci U S A 118(30).

Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, Schwartz SL, DiProspero NA, Knight MA, Schuldiner O, Padmanabhan R, Hild M, Berry DL, Garza D, Hubbert CC, Yao T-P, Baehrecke EH, Taylor JP. 2007. HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 447(7146):859–863.

Pankiv S, Clausen TH, Lamark T, Brech A, Bruun J-A, Outzen H, Øvervatn A, Bjørkøy G, Johansen T. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 282(33):24131–24145.

Papsdorf K, Kaiser CJO, Drazic A, Grötzinger SW, Haeßner C, Eisenreich W, Richter K. 2015. Polyglutamine toxicity in yeast induces metabolic alterations and mitochondrial defects. BMC Genomics 16(1):662.

Paquin CE, Williamson VM. 1984. Temperature effects on the rate of ty transposition. Science 226(4670):53–55.

Park S-H, Kukushkin Y, Gupta R, Chen T, Konagai A, Hipp MS, Hayer-Hartl M, Hartl FU. 2013. PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. Cell 154(1):134–145.

Parsell DA, Kowal AS, Singer MA, Lindquist S. 1994. Protein disaggregation mediated by heat-shock protein Hsp104. Nature 372(6505):475–478.

Pasinelli P, Belford ME, Lennon N, Bacskai BJ, Hyman BT, Trotti D, Brown RHJ. 2004. Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. Neuron 43(1):19–30.

Patterson MN, Scannapieco AE, Au PH, Dorsey S, Royer CA, Maxwell PH. 2015. Preferential retrotransposition in aging yeast mother cells is correlated with increased genome instability. DNA Repair 34:18–27.

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

Popova B, Wang D, Pätz C, Akkermann D, Lázaro DF, Galka D, Kolog Gulko M, Bohnsack MT, Möbius W, Bohnsack KE, Outeiro TF, Braus GH. 2021. DEAD-box RNA helicase Dbp4/DDX10 is an enhancer of α-synuclein toxicity and oligomerization. PLoS Genet 17(3):e1009407.

Prasad R, Kawaguchi S, Ng DTW. 2010. A Nucleus-based Quality Control Mechanism for Cytosolic Proteins. Mol Biol Cell 21(13):2117–2127.

Prasad R, Xu C, Ng DTW. 2018. Hsp40/70/110 chaperones adapt nuclear protein quality control to serve cytosolic clients. J Cell Biol 217(6):2019–2032.

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

Ratajczak E, Zietkiewicz S, Liberek K. 2009. Distinct activities of Escherichia coli small heat shock proteins IbpA and IbpB promote efficient protein disaggregation. J Mol Biol 386(1):178–189.

Reggiori F, Klionsky DJ. 2013. Autophagic processes in yeast: mechanism, machinery and regulation. Genetics 194(2):341–361.

Reidy M, Sharma R, Shastry S, Roberts B-L, Albino-Flores I, Wickner S, Masison DC. 2014. Hsp40s specify functions of Hsp104 and Hsp90 protein chaperone machines. PLoS Genet 10(10):e1004720.

Riback JA, Katanski CD, Kear-Scott JL, Pilipenko EV, Rojek AE, Sosnick TR, Drummond DA. 2017. Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response. Cell 168(6):1028-1040.e19.

Ritossa F. 1962. A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia 18(12):571–573.

Rokney A, Shagan M, Kessel M, Smith Y, Rosenshine I, Oppenheim AB. 2009. E. coli transports aggregated proteins to the poles by a specific and energy-dependent process. J Mol Biol 392(3):589–601.

Rosenzweig R, Nillegoda NB, Mayer MP, Bukau B. 2019. The Hsp70 chaperone network. Nat Rev Mol Cell Biol 20(11):665–680.

Rössler I, Embacher J, Pillet B, Murat G, Liesinger L, Hafner J, Unterluggauer JJ, Birner-Gruenberger R, Kressler D, Pertschy B. 2019. Tsr4 and Nap1, two novel members of the ribosomal protein chaperOME. Nucleic Acids Res 47(13):6984–7002.

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

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

Rujano MA, Bosveld F, Salomons FA, Dijk F, Waarde MAWH, Want JJL, Vos RAI, Brunt ER, Sibon OCM, Kampinga HH. 2006. Polarised Asymmetric Inheritance of Accumulated Protein Damage in Higher Eukaryotes. PLOS Biol 4:417.

Saarikangas J, Barral Y. 2015.

Saarikangas J, Caudron F, Prasad R, Moreno DF, Bolognesi A, Aldea M, Barral Y. 2017. Compartmentalization of ER-Bound Chaperone Confines Protein Deposit Formation to the Aging Yeast Cell. Curr Biol CB 27(6):773–783.

Sahu I, Glickman MH. 2021. Proteasome in action: substrate degradation by the 26S proteasome. Biochem Soc Trans 49(2):629–644.

Samant RS, Livingston CM, Sontag EM, Frydman J. 2018. Distinct proteostasis circuits cooperate in nuclear and cytoplasmic protein quality control. Nature 563(7731):407–411.

Sampaio-Marques B, Burhans WC, Ludovico P. 2019. Yeast at the Forefront of Research on Ageing and Age-Related Diseases. Prog Mol Subcell Biol 58:217–242.

Sanchez Y, Lindquist SL. 1990. HSP104 required for induced thermotolerance. Science 248(4959):1112–1115.

Schlagowski AM, Knöringer K, Morlot S, Sánchez Vicente A, Flohr T, Krämer L, Boos F, Khalid N, Ahmed S, Schramm J, Murschall LM, Haberkant P, Stein F, Riemer J, Westermann B, Braun RJ, Winklhofer KF, Charvin G, Herrmann JM. 2021. Increased levels of mitochondrial import factor Mia40 prevent the aggregation of polyQ proteins in the cytosol. EMBO J 40(16):e107913.

Schopf FH, Biebl MM, Buchner J. 2017. The HSP90 chaperone machinery. Nat Rev Mol Cell Biol 18(6):345–360.

Shai N, Shemesh N, Ben-Zvi A. 2014. Remodeling of Proteostasis Upon Transition to Adulthood is Linked to Reproduction Onset. Get Free Open Access 15(2):122–129.

Shalgi R, Hurt JA, Krykbaeva I, Taipale M, Lindquist S, Burge CB. 2013. Widespread regulation of translation by elongation pausing in heat shock. Mol Cell 49(3):439–452.
Sharma D, Masison DC. 2008. Functionally redundant isoforms of a yeast Hsp70 chaperone subfamily have different antiprion effects. Genetics 179(3):1301–1311.
Sharma D, Masison DC. 2011. Single methyl group determines prion propagation and protein degradation activities of yeast heat shock protein (Hsp)-70 chaperones Ssa1p and Ssa2p. Proc Natl Acad Sci U S A 108(33):13665–13670.
Shemesh N, Shai N, Ben-Zvi A. 2013. Germline stem cell arrest inhibits the collapse of somatic proteostasis early in Caenorhabditis elegans adulthood. Aging Cell 12(5):814–822.
Shewmaker F, Wickner RB, Tycko R. 2006. Amyloid of the prion domain of Sup35p has an in-register parallel beta-sheet structure. Proc Natl Acad Sci U S A 103(52):19754–19759.
Shiber A, Breuer W, Brandeis M, Ravid T. 2013. Ubiquitin conjugation triggers misfolded protein sequestration into quality control foci when Hsp70 chaperone levels are limiting. Mol Biol Cell 24(13):2076–2087.
Singh PK, Muqit MMK. 2020. Parkinson's: A Disease of Aberrant Vesicle Trafficking. Annu Rev Cell Dev Biol 36:237–264.
Smeal T, Claus J, Kennedy B, Cole F, Guarente L. 1996. Loss of transcriptional silencing causes sterility in old mother cells of S. cerevisiae. Cell 84(4):633–642.
Solans A, Zambrano A, Rodríguez M, Barrientos A. 2006. Cytotoxicity of a mutant huntingtin fragment in yeast involves early alterations in mitochondrial OXPHOS complexes II and III. Hum Mol Genet 15(20):3063–3081.
Solís EJ, Pandey JP, Zheng X, Jin DX, Gupta PB, Airoldi EM, Pincus D, Denic V. 2016. Defining the Essential Function of Yeast Hsf1 Reveals a Compact Transcriptional Program for Maintaining Eukaryotic Proteostasis. Mol 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, Nyström T. 2014. Essential genetic interactors of SIR2 required for spatial sequestration and asymmetrical inheritance of protein aggregates. PLoS Genet 10(7):e1004539.
Sontag EM, Samant RS, Frydman J. 2017. Mechanisms and Functions of Spatial Protein Quality Control. Annu Rev Biochem 86:97–122.
Sontag EM, Vonk WI, Frydman J. 2014. Sorting out the trash: the spatial nature of eukaryotic protein quality control. Cell Archit 26:139–146.
Sorrentino V, Romani M, Mouchiroud L, Beck JS, Zhang H, D'Amico D, Moullan N, Potenza F, Schmid AW, Rietsch S, Counts SE, Auwerx J. 2017. Enhancing mitochondrial proteostasis reduces amyloid-β proteotoxicity. Nature 552(7684):187–193.
Specht S, Miller SBM, Mogk A, Bukau B. 2011. Hsp42 is required for sequestration of protein aggregates into deposition sites in Saccharomyces cerevisiae. J Cell Biol 195:617–629.
Spokoini R, Moldavski O, Nahmias Y, England JL, Schuldiner M, Kaganovich D. 2012. Confinement to Organelle-Associated Inclusion Structures Mediates Asymmetric Inheritance of Aggregated Protein in Budding Yeast. Cell Rep 2:738–747.
Stein KC, Morales-Polanco F, Lienden J van der, Rainbolt TK, Frydman J. 2022. Ageing exacerbates ribosome pausing to disrupt cotranslational proteostasis. Nature 601(7894):637–642.
Susek RE, Lindquist S. 1990. Transcriptional derepression of the Saccharomyces cerevisiae HSP26 gene during heat shock. Mol Cell Biol 10(12):6362–6373.
Szeto J, Kaniuk NA, Canadien V, Nisman R, Mizushima N, Yoshimori T, Bazett-Jones DP, Brumell JH. 2006. ALIS are Stress-Induced Protein Storage Compartments for Substrates of the Proteasome and Autophagy. Autophagy 2(3):189–199.
Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y. 1992. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. J Cell Biol 119(2):301–311.

Tam S, Geller R, Spiess C, Frydman J. 2006. The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. Nat Cell Biol 8(10):1155–1162.

Tenreiro S, Franssens V, Winderickx J, Outeiro TF. 2017. Yeast models of Parkinson's disease-associated molecular pathologies. Curr Opin Genet Dev 44:74–83.

Tenreiro S, Outeiro TF. 2010. Simple is good: yeast models of neurodegeneration. FEMS Yeast Res 10(8):970–979.

Tenreiro S, Reimão-Pinto MM, Antas P, Rino J, Wawrzycka D, Macedo D, Rosado-Ramos R, Amen T, Waiss M, Magalhães F, Gomes A, Santos CN, Kaganovich D, Outeiro TF. 2014. Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease. PLoS Genet 10(5):e1004302.

Tessarz P, Schwarz M, Mogk A, Bukau B. 2009. The yeast AAA+ chaperone Hsp104 is part of a network that links the actin cytoskeleton with the inheritance of damaged proteins. Mol Cell Biol 29(13):3738–3745.

Tong AH, Evangelista M, Parsons AB, Xu H, Bader GD, Pagé N, Robinson M, Raghibizadeh S, Hogue CW, Bussey H, Andrews B, Tyers M, Boone C. 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294(5550):2364–2368.

Torres C, Lewis L, Cristofalo VJ. 2006. Proteasome inhibitors shorten replicative life span and induce a senescent-like phenotype of human fibroblasts. J Cell Physiol 207(3):845–853.

Treusch S, Hamamichi S, Goodman JL, Matlack KES, Chung CY, Baru V, Shulman JM, Parrado A, Bevis BJ, Valastyan JS, Han H, Lindhagen-Persson M, Reiman EM, Evans DA, Bennett DA, Olofsson A, DeJager PL, Tanzi RE, Caldwell KA, Caldwell GA, Lindquist S. 2011. Functional links between Aβ toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast. Science 334(6060):1241–1245.

Trotter EW, Kao CMF, Berenfeld L, Botstein D, Petsko GA, Gray JV. 2002. Misfolded Proteins Are Competent to Mediate a Subset of the Responses to Heat Shock in Saccharomyces cerevisiae. J Biol Chem 277:44817–44825.

Trotter EW, Rand JD, Vickerstaff J, Grant CM. 2008. The yeast Tsa1 peroxiredoxin is a ribosome-associated antioxidant. Biochem J 412(1):73–80.

Tye BW, Churchman LS. 2021. Hsf1 activation by proteotoxic stress requires concurrent protein synthesis. Mol Biol Cell 32(19):1800–1806.

Tyedmers J, Treusch S, Dong J, McCaffery JM, Bevis B, Lindquist S. 2010. Prion induction involves an ancient system for the sequestration of aggregated proteins and heritable changes in prion fragmentation. Proc Natl Acad Sci U S A 107(19):8633–8638.

Umezu K, Hiraoka M, Mori M, Maki H. 2002. Structural analysis of aberrant chromosomes that occur spontaneously in diploid Saccharomyces cerevisiae: retrotransposon Ty1 plays a crucial role in chromosomal rearrangements. Genetics 160(1):97–110.

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

Usaj M, Tan Y, Wang W, VanderSluis B, Zou A, Myers CL, Costanzo M, Andrews B, Boone C. 2017. TheCellMap.org: A Web-Accessible Database for Visualizing and Mining the Global Yeast Genetic Interaction Network. G3 GenesGenomesGenetics 7(5):1539–1549.

Van Meter M, Kashyap M, Rezazadeh S, Geneva AJ, Morello TD, Seluanov A, Gorbunova V. 2014. SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age. Nat Commun 5:5011.

Verghese Jacob, Abrams Jennifer, Wang Yanyu, Morano Kevin A. 2012. Biology of the Heat Shock Response and Protein Chaperones: Budding Yeast (Saccharomyces cerevisiae) as a Model System. Microbiol Mol Biol Rev 76(2):115–158.

Vilchez D, Morantte I, Liu Z, Douglas PM, Merkwirth C, Rodrigues APC, Manning G, Dillin A. 2012. RPN-6 determines C. elegans longevity under proteotoxic stress conditions. Nature 489(7415):263–268.

Wallace EWJ, Kear-Scott JL, Pilipenko EV, Schwartz MH, Laskowski PR, Rojek AE, Katanski CD, Riback JA, Dion MF, Franks AM, Airoldi EM, Pan T, Budnik BA, Drummond DA. 2015. Reversible, Specific, Active Aggregates of Endogenous Proteins Assemble upon Heat Stress. Cell 162(6):1286–1298.

Walters RW, Muhlrad D, Garcia J, Parker R. 2015. Differential effects of Ydj1 and Sis1 on Hsp70-mediated clearance of stress granules in Saccharomyces cerevisiae. RNA N Y N 21(9):1660–1671.

Walther DM, Kasturi P, Zheng M, Pinkert S, Vecchi G, Ciryam P, Morimoto RI, Dobson CM, Vendruscolo M, Mann M, Hartl FU. 2015. Widespread Proteome Remodeling and Aggregation in Aging C. elegans. Cell 161(4):919–932.

Wang L, Schubert D, Sawaya MR, Eisenberg D, Riek R. 2010. Multidimensional structure-activity relationship of a protein in its aggregated states. Angew Chem Int Ed Engl 49(23):3904–3908.

Wang Y, Meriin AB, Zaarur N, Romanova NV, Chernoff YO, Costello CE, Sherman MY. 2009. Abnormal proteins can form aggresome in yeast: aggresome-targeting signals and components of the machinery. FASEB J 23(2):451–463.

Watanabe M, Dykes-Hoberg M, Culotta VC, Price DL, Wong PC, Rothstein JD. 2001. Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. Neurobiol Dis 8(6):933–941.

Weisberg SJ, Lyakhovetsky R, Werdiger A-C, Gitler AD, Soen Y, Kaganovich D. 2012. Compartmentalization of superoxide dismutase 1 (SOD1G93A) aggregates determines their toxicity. Proc Natl Acad Sci USA 109:15811.

Werner-Washburne M, Stone DE, Craig EA. 1987. Complex interactions among members of an essential subfamily of hsp70 genes in Saccharomyces cerevisiae. Mol Cell Biol 7(7):2568–2577.

Wickner RB. 1994. [URE3] as an altered URE2 protein: evidence for a prion analog in Saccharomyces cerevisiae. Science 264(5158):566–569.

Wickner RB, Dyda F, Tycko R. 2008. Amyloid of Rnq1p, the basis of the [PIN+] prion, has a parallel in-register beta-sheet structure. Proc Natl Acad Sci U S A 105(7):2403–2408.

Wickner RB, Shewmaker FP, Bateman DA, Edskes HK, Gorkovskiy A, Dayani Y, Bezsonov EE. 2015. Yeast prions: structure, biology, and prion-handling systems. Microbiol Mol Biol Rev MMBR 79(1):1–17.

Wigley WC, Fabunmi RP, Lee MG, Marino CR, Muallem S, DeMartino GN, Thomas PJ. 1999. Dynamic association of proteasomal machinery with the centrosome. J Cell Biol 145(3):481–490.

Willingham S, Outeiro TF, DeVit MJ, Lindquist SL, Muchowski PJ. 2003. Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. Science 302(5651):1769–1772.

Winkler J, Seybert A, König L, Pruggnaller S, Haselmann U, Sourjik V, Weiss M, Frangakis AS, Mogk A, Bukau B. 2010. Quantitative and spatio-temporal features of protein aggregation in Escherichia coli and consequences on protein quality control and cellular ageing. EMBO J 29(5):910–923.

Winkler J, Tyedmers J, Bukau B, Mogk A. 2012. Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation. J Cell Biol 198(3):387–404.

Woerner AC, Frottin F, Hornburg D, Feng LR, Meissner F, Patra M, Tatzelt J, Mann M, Winklhofer KF, Hartl FU, Hipp MS. 2016. Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and RNA. Science 351(6269):173–176.

Wolfe KJ, Ren HY, Trepte P, Cyr DM. 2013. The Hsp70/90 cochaperone, Sti1, suppresses proteotoxicity by regulating spatial quality control of amyloid-like proteins. Mol Biol Cell 24:3588–3602.

Xu G, Pattamatta A, Hildago R, Pace MC, Brown H, Borchelt DR. 2016. Vulnerability of newly synthesized proteins to proteostasis stress. J Cell Sci 129(9):1892–1901.

Yang EJ, Pon LA. 2022. Enrichment of aging yeast cells and budding polarity assay in Saccharomyces cerevisiae. STAR Protoc 3(3):101599.

Yang J, Hao X, Cao X, Liu B, Nyström T. 2016. Spatial sequestration and detoxification of Huntingtin by the ribosome quality control complex. eLife 5:11792.

Yonashiro R, Tahara EB, Bengtson MH, Khokhrina M, Lorenz H, Chen K-C, Kigoshi-Tansho Y, Savas JN, Yates JR, Kay SA, Craig EA, Mogk A, Bukau B, Joazeiro CAP. 2016. The Rqc2/Tae2 subunit of the ribosome-associated quality control (RQC) complex marks ribosome-stalled nascent polypeptide chains for aggregation. eLife 5:e11794.

Yu A, Shibata Y, Shah B, Calamini B, Lo DC, Morimoto RI. 2014. Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition. Proc Natl Acad Sci U S A 111(15):E1481-1490.

Zbinden A, Pérez-Berlanga M, De Rossi P, Polymenidou M. 2020. Phase Separation and Neurodegenerative Diseases: A Disturbance in the Force. Dev Cell 55(1):45–68.

Zheng J, Yang J, Choe Y-J, Hao X, Cao X, Zhao Q, Zhang Y, Franssens V, Hartl FU, Nyström T, Winderickx J, Liu B. 2017. Role of the ribosomal quality control machinery in nucleocytoplasmic translocation of polyQ-expanded huntingtin exon-1. Biochem Biophys Res Commun 493(1):708–717.

Zhou C, Slaughter BD, Unruh JR, Guo F, Yu Z, Mickey K, Narkar A, Ross RT, McClain M, Li R. 2014. Organelle-Based Aggregation and Retention of Damaged Proteins in Asymmetrically Dividing Cells. Cell 159:530–542.