Proteostasis during arsenite stress in *Saccharomyces cerevisiae*

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To my family

ABSTRACT

Around 200 million people are exposed to high levels of arsenic through contaminated soil and, consequently, contaminated food and drinking water. Arsenic exposure is associated with several malignancies, from different types of cancer to neurodegenerative disorders. At a cellular level, arsenic exerts its toxicity by affecting a variety of protein functions. Arsenic increases production of reactive oxygen species, interferes with protein function and cellular pathways, and induces aggregation of newly synthesized polypeptides. This thesis focusses on arsenite-induced protein aggregation and aims to provide new insights on how cells regulate the formation and degradation of protein aggregates and the role of protein quality control systems in this process, using *Saccharomyces cerevisiae* as a model organism. A genome-wide study revealed that protein aggregation induced by arsenite can be influenced by a series of different cellular processes. Particularly, strict transcriptional and translational control is essential to alleviate protein aggregation and sensitivity during arsenite stress. A systematic evaluation of protein quality control and protein degradation systems revealed that co-translational folding aids in mitigating protein aggregation. Furthermore, the protein aggregates formed in the presence of arsenite are polyubiquitinated and mainly degraded by the ubiquitin-proteasome system, while disaggregation by Hsp104 and the autophagy pathway play minor roles. Our data also suggest that arsenite influences aggregate structure making it less accessible for chaperone-mediated disaggregation. In summary, our findings reveal that an interplay between different quality control systems is crucial to maintain proteostasis during arsenic stress.

Keywords: proteostasis, protein aggregation, toxicity, arsenite, *S. cerevisiae*

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SAMMANFATTNING

Omkring 200 miljoner människor exponeras mot höga halter av arsenik genom förorenad jord och, följaktligen, förorenad mat och dricksvatten. Långvarig exponering mot arsenik är förknippad med flera sjukdomar såsom olika typer av cancer och neurodegenerativa sjukdomar. På cellnivå påverkar arsenik en mängd olika cellfunktioner. Arsenik ökar produktionen av syreradikaler, stör proteinfunktioner och cellulära processer och inducerar aggregering av nybildade proteiner. Denna avhandling fokuserar på arsenik-inducerad proteinaggregering och syftar till att ge nya insikter om hur celler reglerar bildning och nedbrytning av proteinaggregat samt vilken roll cellens olika kvalitetskontrollsystem spelar för proteinhomeostas, med jäst som modellorganism. Våra resultat visar att korrekt kontroll av genuttryck och proteinsyntes krävs för att minska proteinaggregering vid arsenikexponering. Även cellens proteinveckningssystem är viktiga för att upprätthålla proteinhomeostas i närvaro av arsenik. Vidare visar vi att arsenik-inducerade proteinaggregat huvudsakligen bryts ned via ubiquitin-proteasomsystemet, medan autofagi och disaggregeringschaperoner spelar en mindre viktig roll. Vi visar även att arsenik kan påverka proteinaggregatens struktur vilket hindrar effektiv inbindning av chaperoner till dessa. Sammantaget visar våra resultat att ett samspel mellan olika kvalitetskontrollsystem är avgörande för att upprätthålla proteinhomeostas under arsenikexponering.

LIST OF PAPERS

This thesis is based on the following studies:

I. **Genome-wide imaging screen uncovers molecular determinants of arsenite-induced protein aggregation and toxicity**
Stefanie Andersson, Antonia Romero, Joana Isabel Rodrigues, Sansan Hua, Xinxin Hao, Therese Jacobson, Vivien Karl, Natalie Becker, Arghavan Ashouri, Sebastien Rauch, Thomas Nyström, Beidong Liu and Markus J. Tamás. *Journal of Cell Science (2021) 134(11):jcs258338 doi: 10.1242/jcs.258338*

II. **Ribosomal and non-ribosomal protein quality control during arsenite stress in *Saccharomyces cerevisiae***
Joana Isabel Rodrigues, Emma Lorentzon, Sansan Hua, Andrew Boucher and Markus J. Tamás. *Manuscript*

III. **Differential contributions of the proteasome, autophagy, and chaperones to the clearance of arsenite-induced protein aggregates in yeast**
Sansan Hua, Agnieszka Kłosowska, Joana Isabel Rodrigues, Gabriel Petelski, Lídia A. Esquembre, Emma Lorentzon, Lars F. Olsen, Krysztof Liberek and Markus J. Tamás. *Under revision in Journal of Biological Chemistry*

PAPER NOT INCLUDED IN THE THESIS:

Effects of the Toxic Metals Arsenite and Cadmium on α-synuclein Aggregation *In Vitro* and in Cells
Emma Lorentzon, Istvan Horvath, Ranjeet Kumar, Joana Isabel Rodrigues, Markus J. Tamás and Pernilla Wittung-Stafshede. *International Journal of Molecular Sciences (2021) 22(21):1145 doi: 10.3390/ijms222111455*

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LIST OF ABBREVIATIONS

CHX	Cycloheximide
CLIPS	Chaperones linked to protein synthesis
CP	Core particle
CVT	Cytoplasm to vacuole targeting pathway
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
GSH	Glutathione
GTF	General transcription factor
HSP	Heat-shock protein
INQ	Intranuclear quality control compartment
IPOD	Insoluble protein deposit
JUNQ	Juxtanuclear quality control compartment
LLPS	Liquid-liquid phase separation
RNP	Ribonuclearprotein
mRNPs	Messenger ribonuclearprotein complexes
NAC	Nascent associated complex
NGD	No-Go decay
ORF	Open reading frame
PB(s)	Processing body(ies)
PIC	Pre-initiation complex
PQC	Protein quality control
PTM	Post-translational modification
ROS	Reactive oxygen species
RP	Regulatory particle
RQC	Ribosomal quality control
RTC	RQC trigger complex
SG(s)	Stress granule(s)
SSB-RAC	SSB-ribosome associated complex
UPS	Ubiquitin proteasome system
UTR	Untranslated region
WHO	World Health Organization

AIM OF THE THESIS

Arsenic is a non-essential metalloid present in the Earth's crust. Chronic exposure to arsenic has been linked to a series of disorders, ranging from neuropathologies to various forms of cancer. The combination of arsenic's abundance in the environment, its toxicity and the availability for human exposure, makes arsenic the most dangerous naturally-occurring substance. Thus, it is crucial to better understand arsenic toxicity.

Arsenic is known to interfere with several cellular processes and functions. One of these effects, induction of protein aggregation, has also been associated with several neurodegenerative disorders. While it was previously known that arsenic induces widespread aggregation in *Saccharomyces cerevisiae* in a concentration-dependent manner, much is still unknown regarding the regulation of aggregate formation and aggregate clearance. This thesis compiles three distinct papers that aim to answer these unanswered questions.

In **paper I**, a genome-wide imaging screen was performed in search of cellular processes and functions implicated in proteostasis during arsenite, As(III), stress. Proteins involved in both transcriptional and translational control were identified having crucial roles in regulating As(III)-induced aggregation and follow-up experiments addressed how impairment of these systems influenced protein aggregation caused by the metalloid.

In **paper II**, we sought to understand how selected protein quality control systems contribute to proteostasis under As(III) exposure and their importance to alleviate As(III)-induced protein aggregation and toxicity.

Lastly, in **paper III**, we explored which protein degradation mechanisms mediate the degradation of As(III)-induced protein aggregates. In addition, we investigated the effects of As(III) on chaperone binding and activity.

INTRODUCTION

1. PROTEIN SYNTHESIS AND FOLDING

Proteostasis, the perfect balance between protein synthesis, folding and degradation, is of paramount importance for proper cell function (Figure 1). An imbalance in this chain of cellular processes leads to an accumulation of protein aggregates which, when the cell is unable to degrade or resolubilize said aggregates, causes cell death. Several human disorders have been associated with protein aggregation, mostly neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, amongst others.

Protein synthesis is performed based on mRNA by ribosomes in the cytoplasm or at the ER surface, in a process termed translation. In eukaryotes, the 80S eukaryotic ribosome is composed of two subunits, the 40S small subunit and the 60S large subunit. Succinctly, translation can be divided into four steps: initiation, elongation, termination, and ribosome recycling. Translation initiation begins with the identification of the start codon by the 43S preinitiation complex on the small subunit. After joining the small and large subunits, elongation commences by sequentially adding amino acids, supplied by tRNA molecules, to the polypeptide chain. Once the end of the stop codon is reached, translation is terminated, the ribosomes are recycled after dissociation of the two subunits and all the translational components are recovered for reuse in later rounds of translation (Deuerling et al., 2019; Dever and Green, 2012; Hinnebusch, 2011; Jackson et al., 2010; Pestova et al., 2001; Schmeing and Ramakrishnan, 2009; Shoemaker and Green, 2011; Zhouravleva et al., 1995).

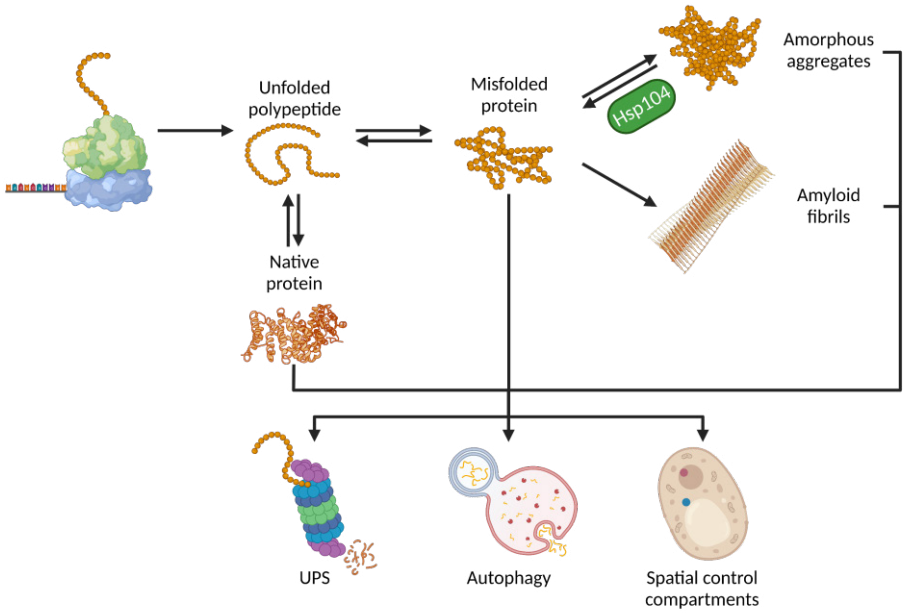

Figure 1| Proteostasis is the balance between protein synthesis, folding, refolding and degradation. During synthesis, an unfolded polypeptide is folded into its native conformation. The polypeptide can misfold when proper folding is not achieved and, if not refolded, the protein can form protein aggregates or amyloid fibrils. In yeast cells, aggregated proteins can be disaggregated by the Hsp104 chaperone. Distinct degradation pathways, UPS and autophagy, are responsible for the degradation of misfolded or aggregated proteins, as well as degradation of folded proteins that are no longer viable or necessary. When the degradation pathways are overwhelmed, cells store the misfolded or aggregated proteins in spatial control compartments. (Created with BioRender.com)

Since proteins are the basis of almost every biological process, a correct folding and a stable three-dimensional structure are necessary for proper cellular function. *In vivo*, proteins start folding on the ribosome during protein synthesis, i.e., co-translationally. There is a factor-binding platform around the ribosomal tunnel exit where various ribosome-associated factors bind to act on nascent peptides and aid on protein folding. However, folding of one third of newly synthesize proteins occurs in specific compartments, such as the mitochondria or the endoplasmic reticulum (ER) (Cabrita et al., 2010; Deuerling et al., 2019; Dobson, 2003; Hartl and Hayer-Hartl, 2009; Komar,

2018). Studies have shown that folding proceeds from the N-terminal to the C-terminal end of the protein, resulting in sequential folding of distinct regions of the nascent polypeptide (Hartl and Hayer-Hartl, 2009; Komar, 2018; Kramer et al., 2009). The folding process passes through intermediate structures and ends with a defined functional structure, known as the 'native fold' or 'native state', which corresponds to the protein's lowest energy state. The different structures formed along the folding process are stabilized by hydrogen bonds between carbonyl and amide groups (Cabrita et al., 2010; Dobson, 2003).

Any protein that aids in the conformational folding and unfolding of another protein is termed as a molecular chaperone. Chaperones can either interact with nascent chains as they emerge from the ribosomal tunnel or by guiding the folding process at later stages (Cabrita et al., 2010; Dobson, 2003). Eukaryotes present two different chaperone networks: one to aid in translation and folding, also known as chaperones linked to protein synthesis (CLIPS) and another to aid in protein rescue after stress. The CLIPS network in yeast includes the nascent polypeptide associated complex (NAC), the SSB-ribosome associated complex (SSB-RAC), the Prefoldin complex, the TriC/CCT complex, and the Sse1 and Ssa1 chaperones, amongst others (Albanèse et al., 2006).

1.1 The Nascent polypeptide Associated Complex

The NAC was firstly identified by Wiedmann and colleagues as a complex bound to ribosomal nascent chains (Wiedmann et al., 1994). In yeast, NAC is a highly stable complex composed of three proteins: Egd2 (α-NAC), and Egd1 (β_1-NAC) or Btt1 (β_3-NAC). While all proteins interact with the nascent chain, only the β-subunits bind to the ribosome. Ribosome binding is essential for the entire NAC complex to interact with the nascent chains (Beatrix et al., 2000; Reimann et al., 1999; Wegrzyn et al., 2006).

Initially, NAC was thought to be only a negative regulator for protein translocation to the ER and to the mitochondria (George et al., 1998; Lauring et al., 1995a; Lauring et al., 1995b; Powers and Walter, 1996; Wiedmann and Prehn, 1999). However, since then, NAC was shown to be one of the first cytosolic factors that nascent chains encounter upon exit of the ribosomal tunnel, responsible for creating a protective environment and impeding premature degradation of newly formed polypeptides. It binds to a broad array of substrates, such as unfolded, folded or intrinsically disordered proteins. NAC gives aggregation-prone proteins the chance for proper folding before degradation (Duttler et al., 2013; Martin et al., 2018; Wang et al., 1995). Furthermore, upon proteostasis imbalance, the complex dissociates from the ribosome, binds to protein aggregates and assists in the clearance of aggregated proteins, preventing accumulation of protein aggregates. Additionally, release of NAC from the ribosome decreases the levels of translating ribosomes, probably in an attempt to prevent further synthesis of aggregation-prone proteins (Alamo et al., 2011; Kirstein-Miles et al., 2013; Koplin et al., 2010). Interestingly, in the absence of NAC together with the heat shock protein (HSP) 70 chaperones SSB, the levels of insoluble proteins increase, but not when only NAC is absent. Among the protein aggregates formed in the absence of NAC and SSB are ribosomal proteins and ribosomal biogenesis factors, indicating that NAC and SSB have a role in ribosomal biogenesis (Koplin et al., 2010).

1.2 The Hsp70, Hsp40 and Hsp110 chaperones

The role of Hsp70, Hsp40 and Hsp110 chaperones in protein folding has been extensively studied. The Hsp70 chaperones interact with polypeptides via their C-terminal substrate-binding domain, depending on the ATP binding state. Initially, Hsp40 co-chaperones interact with the polypeptide, recruiting them to an ATP-bound Hsp70 (which presents an open conformation) and stimulating ATP hydrolysis, altering the Hsp70 conformation into a closed state and ensnaring the polypeptide. This way, Hsp70 binds to unfolded

polypeptides and prevents misfolding and aggregation. Hsp110 chaperones, acting as Nucleotide Exchange Factors (NEF), exchange the ADP bound to the Hsp70 for an ATP, opening the Hsp70 substrate-binding domain and releasing the substrate. The polypeptide is now free to fold properly; when accurate folding is not achieved or folding does not occur fast enough, the process restarts (Figure 2). Hsp70 chaperones have a broad range of substrates, from nascent polypeptides to folding intermediates and misfolded proteins. In *S. cerevisiae* there are several Hsp70-Hsp40 chaperone systems, such as the SSB-RAC complex and the SSA proteins (Beckmann et al., 1990; Buchberger et al., 1994; Gething and Sambrook, 1992; Lewis and Pelham, 1985; McCarty et al., 1995).

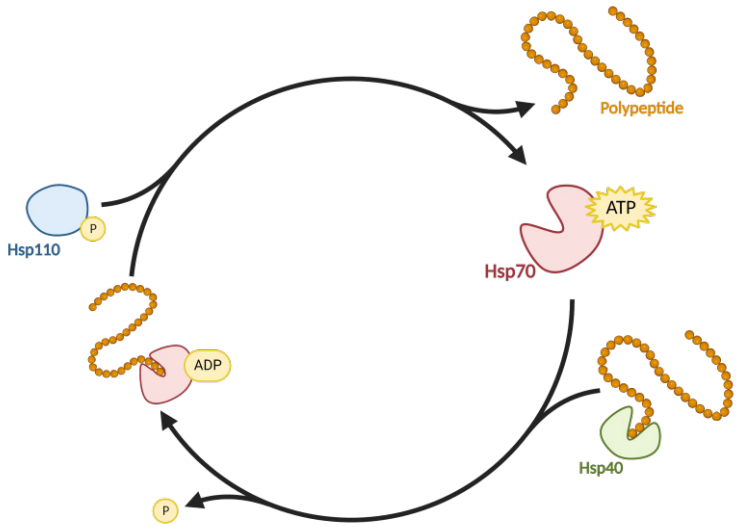

Figure 2| The Hsp70, Hsp40 and Hsp110 cycle. Initially, an Hsp40 chaperone interacts with the unfolded polypeptide and directs the polypeptide to an ATP-bound Hsp70, which presents an open conformation. The Hsp40 stimulates ATP hydrolysis, inducing a conformational change in Hsp70, which adapts a closed conformation and entraps the polypeptide, preventing misfolding. The Hsp110 NEF returns the Hsp70 to its open conformation by exchanging the ADP for an ATP molecule. The polypeptide is now free for folding; if folding is not achieved, the cycle resumes. (Created with BioRender.com)

The SSB-Ribosome Associated Complex

The SSB-RAC in *S. cerevisiae* is composed of the Ssb1 and Ssb2 Hsp70 chaperones (from now on named as SSB), and the Zuo1 and Ssz1 chaperones. SSB was first discovered to be associated with translating ribosomes and nascent peptide chains, followed by identification of Zuo1 as a ribosome-associated Hsp40. The Hsp70 Ssz1 was then identified as a Zuo1 co-chaperone and as a component of the RAC heterodimer (Gautschi et al., 2001; Nelson et al., 1992; Pfund et al., 1998; Yan et al., 1998).

RAC binds to ribosomes via Zuo1 at three different contact sites: one on the 40S ribosomal subunit and the other two on the 60S subunit, near the exit tunnel (Gautschi et al., 2001; Leidig et al., 2013; Zhang et al., 2014). RAC binding to both ribosomal subunits limits peptide elongation during translation (Zhang et al., 2014). Meanwhile, SSB binding to the ribosome, which is dependent on Zuo1, promotes faster translation (Döring et al., 2017; Gautschi et al., 2001; Yan et al., 1998). Furthermore, deletion of *SSB* decreases the number of translating ribosomes (Nelson et al., 1992). Taken together, these studies implicate the SSB-RAC in translational control.

SSB-RAC also aids in co-translational protein folding. RAC binds initially to nascent chains, followed by transfer of the nascent polypeptide from Zuo1 to SSB (Nelson et al., 1992; Pfund et al., 1998; Zhang et al., 2020). Ssz1 then facilitates Zuo1 in stimulating the ATPase activity of SSB, enforcing binding of SSB to the nascent chains (Gautschi et al., 2002; Huang et al., 2005; Hundley et al., 2002). Wilmund and colleagues found that absence of SSB-RAC leads to widespread aggregation of newly synthesized peptides, and that the complex has a wide range of substrates, but prefers aggregation prone, hydrophobic or intrinsically disordered proteins. Furthermore, in the absence of Zuo1, co-translational misfolding enhances, consolidating the hypothesis that the SSB-RAC aids in protein folding (Amor et al., 2015; Willmund et al., 2013).

Besides a role in folding, SSB-RAC has also been linked to protein translocation to both the mitochondria and the ER (Acosta-Sampson et al., 2017; Döring et al., 2017; Gautschi et al., 2001).

SSA chaperones

The cytosolic SSA Hsp70 chaperone family in yeast consists of four functionally similar SSA proteins (Ssa1-4), where at least one SSA protein is required for cell viability (Werner-Washburne et al., 1987). SSA chaperones have been shown to aid in the folding of newly synthesized polypeptides with the help of the Hsp110 Sse1 (Crombie et al., 1994; Jung et al., 2000; Kim et al., 1998; Melville et al., 2003; Roberts et al., 2004; Yam et al., 2005). Although functionally similar, each SSA chaperone has distinct roles in protein folding and refolding (Andersson et al., 2021; Hasin et al., 2014). Besides folding, SSA proteins are also involved in protein translocation to the ER and the mitochondria, protein delivery to the proteasome, as well as in ubiquitin-dependent degradation of short-lived proteins (Deshaies et al., 1988; Lee do et al., 2016; Ngosuwan et al., 2003; Park et al., 2007).

1.3 The TriC/CCT chaperonin and the Prefoldin co-chaperones

TriC/CCT is a chaperonin assembled in a two-ringed barrel structure containing eight individual subunits, creating a central cavity. TriC/CCT acts together with the Prefoldin multi-subunit co-chaperone complex, and both are required for the folding of newly synthesized actin, tubulin and other folding substrates, such as huntingtin and α-synuclein (Behrends et al., 2006; Brehme et al., 2014; Grantham et al., 2002; Nollen et al., 2004; Shahmoradian et al., 2013; Sot et al., 2017; Sternlicht et al., 1993; Tam et al., 2006). Furthermore, TriC/CCT has also been implicated in the assembly of mTOR by aiding in the folding of two proteins involved in the complex (Cuéllar et al., 2019). Prefoldin is responsible for transferring target polypeptides to TriC/CCT for proper folding (Siegers et al., 1999; Vainberg et al., 1998). The polypeptide chains bind to TriC/CCT and folding occurs inside the central cavity via an ATP-driven reaction cycle that alternates the conformational states until the polypeptide chain has acquired its tertiary structure (Anfinsen, 1973; Martin et al., 1993). Besides binding to the target polypeptide, Prefoldin also associates with TriC/CCT and aligns the Prefoldin and TriC/CCT substrate

binding chambers and, consequently, enhancing the yield and rate of the folding process and preventing premature release of the protein from TriC/CCT (Gestaut et al., 2019; Siegers et al., 1999).

1.4 Hsp90 chaperones

Hsp90 chaperones interact with different substrates, from viral proteins to actin and tubulin (Nishida et al., 1986; Oppermann et al., 1981; Sanchez et al., 1987; Sanchez et al., 1988; Shaknovich et al., 1992; Xu and Lindquist, 1993). This interaction occurs during or immediately after protein synthesis via the disordered regions of Hsp90 (Pursell et al., 2012). The chaperones are capable of suppressing protein aggregation by binding to early unfolded intermediates, mediating folding and preventing unproductive molecular interactions (Jakob et al., 1995; Schneider et al., 1996; Shue and Kohtz, 1994; Wiech et al., 1992). Furthermore, Hsp90 has been shown to also dissociate protein aggregates and aid in folding (Miyata and Yahara, 1992). Hsp90 is necessary for a variety of cellular functions, such as cellular transport, secretory pathway, cell cycle, cytokinesis and meiosis; however, this Hsp90 requirement for different functions might be rooted in its role during folding and assembly of multi-subunit complexes (Johnson and Toft, 1995; McClellan et al., 2007).

S. cerevisiae possesses two Hsp90 chaperones: Hsc82, which is constitutively expressed, and Hsp82 which is stress inducible. Despite a 97% sequence similarity between the two isoforms and the identical interactomes, Hsp82 has higher stability and refolding rate (Girstmair et al., 2019).

1.5 Small heat shock proteins

As the name suggests, small heat shock proteins (sHSP) have a low molecular mass of 15 to 30kDa. These chaperones bind to denaturing proteins and either prevent protein aggregation or promote refolding in an ATP-

independent manner (Jakob et al., 1993). When folding is not achieved, sHSPs deliver the polypeptides to one of the degradation systems (Vendredy et al., 2020).

sHSPs are sensitive to changes in the cellular environment, such as temperature and pH changes, phosphorylation, oxidative stress and metals. The complex formation between the sHSPs and their substrate is dependent on several factors such as the sHSP itself, the substrate, the type of aggregate or the presence and type of stress (Janowska et al., 2019). The same substrate can be complexed with different sHSPs through distinct mechanisms, however, the ability of the sHSP to delay aggregation or induce refolding depends on the strength of the complex formed (Mymrikov et al., 2017).

Yeast cells possess two sHSPs: Hsp26 and Hsp42. Hsp26 suppresses aggregation of its substrates and, for proper chaperone function, it disassembles into dimers when under heat shock (Haslbeck et al., 1999). Hsp42, on the other hand, does not require stress, as it is active under all conditions. In the presence of heat shock, both sHSPs supress aggregation of one-third of all cytosolic proteins (Haslbeck et al., 2004).

1.6 Ribosome Stalling and Ribosomal Quality Control

The presence of faulty mRNAs that stall ribosomes during translation elongation is a common source for defective proteins. Ribosomes tend to stall due to translation of an mRNA molecule past its open reading frame (ORF) into the 3'-untranslated region (UTR), leading to translation of the poly(A) tail or due to translation of twelve consecutive basic residues. Indeed, Dimitrova and colleagues propose that positively charged side chains interact with the ribosome tunnel (Crowder et al., 2015; Dimitrova et al., 2009; Ito-Harashima et al., 2007).

Stalling of ribosomes can be toxic because ribosomal subunits need to be recycled and the aberrant proteins formed from stalled ribosomes are usually

misfolded and, consequently, often form toxic aggregates (Defenouillère and Fromont-Racine, 2017).

In *S.* cerevisiae, the E3 ubiquitin ligase Hel2 (also known as "RQC-trigger factor 1; Rqt1), identifies stalled ribosomes and induces two quality control pathways:

(i) No-go decay, where stalled mRNAs are endonucleolytic cleaved and posteriorly degraded (Doma and Parker, 2006);
(ii) Ribosomal Quality Control (RQC), where the stalled polypeptides are ubiquitinated and degraded via the proteasome (Figure 3).

In fact, Hel2 mutants fail to trigger RQC and only initiate an alternative NGD (Ikeuchi et al., 2019).

Together with Ubc4, an E2 ubiquitin-conjugating enzyme, Hel2 mediates K63-linked poly-ubiquitination of the ribosomal protein uS10. This ubiquitination triggers the RQC trigger complex (RTC), responsible for the splicing of the ribosomal subunits, in a still unknown mechanism. The RTC is composed of Slh1/Rqt2, an RNA helicase-family protein; Cue3/Rqt3 ubiquitin-binding protein and Rqt4 (Ikeuchi et al., 2019; Matsuo et al., 2017). The ubiquitin-binding activity of Cue3 and the ATPase activity of Slh1 is crucial to elicit RQC (Matsuo et al., 2017).

After the RTC recognizes the stalled ribosomes, the Hbs1-Dom34 protein complex induces ribosome splitting into large and small subunits together with the ATPase Rli1. Hbs1 is a GTPase with homology to translation release factors, while Dom34 adopts a conformation that mimics tRNA structure. GTP hydrolysis by Hbs1, together with a conformational change in Dom34, recruits Rli1, whose ATPase activity stimulates ribosome dissociation (Shoemaker et al., 2010; Shoemaker and Green, 2011; Tsuboi et al., 2012).

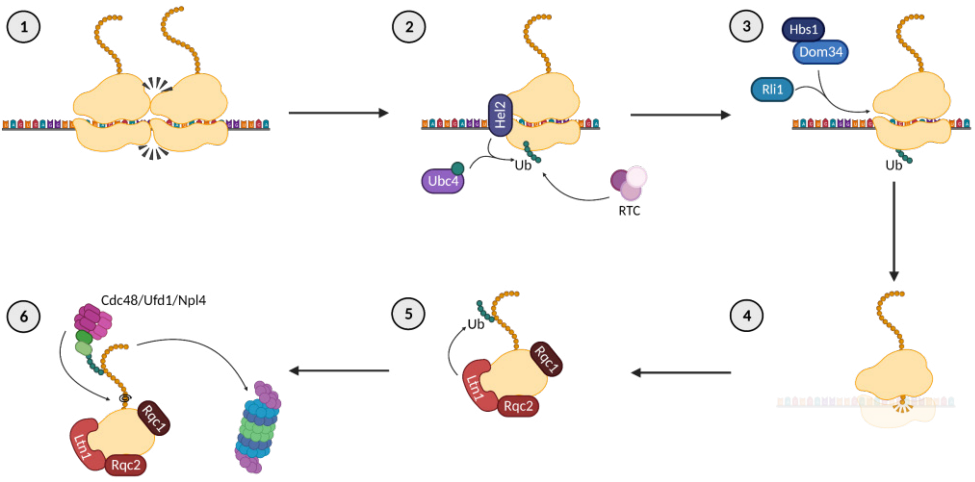

Figure 3| Ribosomal collisions trigger the RQC for degradation of the stalled polypeptide in *S. cerevisiae*. ① Ribosomes can stall and collide for a variety of reasons during translation rounds. ② Hel2 recognizes the stalled ribosomes and mediates K63-linked polyubiquitination of the small ribosomal subunit together with Ubc4. RTC then triggers the start of RQC. ③ Hbs1, Dom34 and Rli1 stimulate ribosome dissociation. ④ Both the mRNA and the small ribosomal subunit are degraded. ⑤ Ltn1 binds to the large ribosomal subunit, stabilized by Rqc2, and poly-ubiquitinates the nascent polypeptide. Ltn1 and Rqc1 recruit the Cdc48 ATPase and its cofactors. ⑥ Cdc48, together with Ufd1 and Npl4, hydrolysis ATP, creating a force capable of extracting the polypeptide from the ribosomal tunnel. The polypeptide is then degraded via UPS. (Created with BioRender.com)

Following ribosomal dissociation, the RQC complex, composed of Ltn1, Rqc1 and Rqc2, binds to the 60S subunit of the ribosome. Ltn1, an E3 ubiquitin ligase, poly-ubiquitinates the defective nascent peptide. Rqc2 (also known as Tae2) stabilizes the binding of Ltn1 to the 60s subunit and adds a CAT tail to the nascent polypeptide. The CAT tail addition acts as a fail-safe mechanism for efficient ubiquitination by Ltn1, since it extracts the lysine residues that might be sequestered in the ribosomal tunnel (Bengtson and Joazeiro, 2010; Brandman et al., 2012; Matsuo et al., 2017; Osuna et al., 2017). It is worth

noting that Defenouillère *et al.* have demonstrated that ubiquitination of the nascent polypeptide might start on the stalled 80S ribosome and continues on to the 60S subunit after ribosome splitting (Defenouillère et al., 2013). Recently, a link between the SSB-RAC system and Ltn1 was found. SSB-RAC promotes Ltn1-dependent ubiquitination and facilitates the binding of Ltn1 to ribosomes (Ghosh and Shcherbik, 2020). Importantly, recent studies revealed that addition of CAT tails to the stalled polypeptide can direct the peptide for proteasomal degradation off the ribosome (Sitron and Brandman, 2019).

Ltn1 and Rqc1 recruit the Cdc48 ATPase and its cofactors, Ufd1 and Npl4, which hydrolyze ATP creating a mechanical force capable of extracting the poly-ubiquitinated peptide from the ribosomal exit tunnel. The RQC components deliver the defective peptide to the proteasome for degradation (Defenouillère and Fromont-Racine, 2017).

1.7 The Ccr4-Not complex in translational control

Besides involvement in transcriptional control, the yeast Ccr4-Not complex also plays a role in translational control. Albert *et al.* first discovered that Not4 is an E3 ubiquitin ligase (Albert et al., 2002). Not4 is associated with ribosomes carrying nascent peptides and mRNA; it interacts with Ecm29, a protein responsible for proteasome stability, and ubiquitinates the small ribosomal proteins Rsp7A and Rsp7B (Dimitrova et al., 2009; Panasenko and Collart, 2011; Preissler et al., 2015). The proteasome is defective in the absence of a functional Not4 (Halter et al., 2014).

Deletion of *NOT4*, together with *LTN1* deletion, enhances ribosome stalling and production of arrest products thus releasing truncated polypeptides that escape quality control by Ltn1 and Not4. During stalling, Not4 is required for translational repression at the mRNA level (Preissler et al., 2015). The Not4 subunit is also necessary for degradation of translational arrest products via the proteasomal pathway, as deletion of *NOT4* leads to an accumulation of

polyubiquitinated proteins and overall protein aggregation (Dimitrova et al., 2009; Halter et al., 2014; Panasenko and Collart, 2011; Panasenko and Collart, 2012). Both deletion of *NOT2* and *NOT5* also induced an increase in protein aggregation (Panasenko and Collart, 2012).

Two other subunits of the Ccr4-Not complex, Ccr4 and Caf1, can also interact with the proteasome. Moreover, together with Not4, the three subunits can bind to ubiquitin conjugates and lead certain ubiquitin-dependent proteasomal substrates to degradation by the 26S proteasome (Kandasamy et al., 2021).

2. PROTEIN AGGREGATION

Due to the high complexity of protein folding, protein misfolding is expected. Both folding intermediates and misfolded proteins have exposed hydrophobic amino acids and unstructured polypeptide backbone regions that, otherwise, would be buried in the native structure (Eichner et al., 2011; Hartl et al., 2011; Hartl and Hayer-Hartl, 2009; Tyedmers et al., 2010). Hydrophobic forces between exposed regions causes misfolding of proteins, which can later lead to protein aggregation. By definition, a protein aggregate is any association of two or more non-native proteins, which can occur due to a variety of factors, such as ageing, environmental stress, mutations, or chemical modifications (Hipp et al., 2014; Tyedmers et al., 2010; Weids et al., 2016).

Protein aggregates can be classified based on their structure. Since aggregation is a step-by-step process, it is possible that the same proteins acquire different structures before its final aggregated form (Vetri and Foderà, 2015):

- **Amorphous aggregates** do not have a defined structure and are often the result of non-specific interactions;
- **Oligomers** are small round-shape aggregates formed by a small number of unfolded proteins. Oligomer intermediates are usually formed in the pathway for amyloid fibrillation and are hypothesized to be more toxic that amyloid fibrils (Zamotin et al., 2006);
- **Amyloid fibrils** have a highly organized hydrogen-bound structure, with a β-strand across the long fibril axis that allows for the aggregates to linger for long periods of time (Sunde and Blake, 1997);
- **Protofibrils** are thin structures rich in β-sheet structures. It is hypothesized that protofibrils are only an intermediate structure before fibril formation (Modler et al., 2003);
- **Spherulites** have a spherical amyloid-like structure and usually form at low pH and high temperatures (Smith et al., 2012);

› **Protein particulates** also present a spherical structure, but are smaller than spherulites (Krebs et al., 2007).

Interestingly, the same protein can aggregate in different morphologies, implying that the final structure of a protein aggregate does not depend on the protein sequence alone (Dobson, 2003; Hartl and Hayer-Hartl, 2009; Hipp et al., 2014).

2.1 Phase separation

Although phase separation and protein aggregation are completely distinct processes, improper phase separation and liquid-to-solid transition might give rise to protein aggregation.

Dispersed protein bound-RNA in the cytoplasm or nucleoplasm coalesce into a concentrated state, creating a membrane-less droplet or ribonucleoprotein (RNP) granule, by liquid-liquid phase separation (LLPS). RNP granules, which encompasses the nucleolus, stress granules (SGs) and processing bodies (PBs), are characterized by having a higher protein concentration than the surrounding environment. In fact, granule assembly occurs promptly once protein saturation is reached. Proteins present in RNP granules often contain intrinsically disordered regions with specific amino acid types that can facilitate phase separation (Aguzzi and Altmeyer, 2016; Alberti and Dormann, 2019; Babinchak and Surewicz, 2020; Nedelsky and Taylor, 2019; Wang et al., 2018).

Heat-induced misfolded proteins in the nucleus are often assembled into liquid-like granules by LLPS, preventing protein aggregation and enabling refolding (Frottin et al., 2019). Besides, a connection between phase separation and protein aggregation has been observed in several neurodegenerative disorder-associated proteins. Distinct studies on FUS, TDP43 and Htt, have demonstrated assembly of granules containing these proteins by LLPS, followed by protein aggregation when phase-separation or

liquid-to-solid transition is impaired (Conicella et al., 2016; Murakami et al., 2015; Patel et al., 2015; Peskett et al., 2018).

Stress granules and processing bodies

Proteins that coalesce with non-translated mRNAs can form two types of granules: PBs and SGs. Both types of foci are formed due to an accumulation of mRNA molecules stalled in translation initiation (Buchan and Parker, 2009). PBs are cytoplasmic foci containing messenger RNP complexes (mRNPs), mRNA degradation intermediates, mRNA decay factors, translational repressors, and might contain translation initiation factors (Brengues and Parker, 2007; Cougot et al., 2004; Sheth and Parker, 2003). However, mRNA molecules present in PBs can return to translation when nutrients are provided, indicating that PBs are not where degradation of mRNA occurs but only a storage for mRNA and mRNA decay factors (Brengues et al., 2005; Sheth and Parker, 2003). SGs and PBs share several protein components and harbor the same species of mRNA, however, SGs do not contain mRNA decay factors, unlike PBs. SGs contain mRNPs, RNA-binding proteins, 40S ribosomal subunits and translation initiation factors (Buchan and Parker, 2009; Gallardo et al., 2021; Kedersha et al., 2005).

Both the assembly and the composition of SGs is influenced by different stresses (Grousl et al., 2009). Although some stresses induce formation of PBs and not SGs, SGs form from pre-existing PBs, where mRNA is transferred from the PBs to the SGs. The mRNA molecules are within the SGs only for a moment and then reenter the process of translation (Buchan et al., 2008; Mollet et al., 2008). The formation of SGs decreases the amount of its components dispersed in the cytosol, probably preventing mRNA degradation, promoting easy assembly of translation initiation complexes and translation of specific mRNA molecules that are important in stress conditions (Buchan et al., 2008).

3. PROTEIN DISAGGREGATION AND DEGRADATION

To counteract the toxic effects of protein misfolding and aggregation and maintain a balance between protein synthesis and degradation, cells use several protein quality control (PQC) mechanisms. These include: (i) chaperones that aid during refolding or disaggregation; (ii) spatial sequestration of misfolded and aggregated proteins into intracellular deposition sites; (iii) degradation pathways like the ubiquitin-proteasome system (UPS) and autophagy (Cuervo, 2004; Glover and Lindquist, 1998; Hill et al., 2017).

3.1 The Hsp104 disaggregase

Hsp104 is a non-metazoan cytosolic chaperone from the Hsp100 family. Hsp100 chaperones protect cells from extreme stress conditions, such as high temperatures (Parsell et al., 1991). Initially thought to be only a stress tolerance factor (Parsell et al., 1994a; Sanchez and Lindquist, 1990; Sanchez et al., 1992), Hsp104 was later found to resolubilize protein aggregates (Parsell et al., 1994b). Parsell and colleagues attributed this disaggregase function as the reason for Hsp104 thermotolerance activity. This was shown by deletion of *HSP104*, which caused an increase in heat-induced protein aggregates (Parsell et al., 1994b). Hsp104 does not act alone, the Ydj1 Hsp40 first associates with protein aggregates and recruits the Ssa1 Hsp70 which, in turn, recruits Hsp104 (Glover and Lindquist, 1998). Hsp104 forms a hexameric ring structure and disaggregates protein aggregates using an unfolding/threading mechanism in the axial channel of the chaperone that requires ATP hydrolysis (Lee et al., 2003; Lum et al., 2004; Parsell et al., 1994a).

3.2 Spatial quality control compartments

Misfolded proteins can be sequestered to spatial control compartments as an intergenerational protection and degradation strategy. Kaganovich *et al.* discovered two spatial control compartments in yeast cells, the Insoluble Protein Deposit (IPOD) and the Juxtanuclear Quality control compartment (JUNQ) (Kaganovich et al., 2008). Initially, the JUNQ was thought to be located in an indentation of the nucleus; however, a later study revealed that this compartment was localized inside the nucleus, hence the redefinition to Intranuclear Quality control compartment (INQ) (Miller et al., 2015b). INQ functions as a deposit for both cytosolic and misfolded proteins and is highly dynamic, with a rapid exchange of proteins between the compartment and the cytosol. The INQ forms first upon stress and is enriched for proteasomes, suggesting that it might be the preferred control compartment for misfolded proteins. The IPOD, on the other hand, is localized next to the vacuole and forms later and remains longer after stress; it has little to no exchange of proteins with the cytosol, indicating that it harbors terminally insoluble proteins (Kaganovich et al., 2008; Miller et al., 2015a; Miller et al., 2015b). The misfolded proteins are sorted into the two compartments based on their ubiquitination state and solubility. Kaganovich and colleagues showed that only ubiquitinated misfolded proteins are directed to the INQ. Furthermore, they showed that blocking ubiquitination reduced solubility of the misfolded protein, supporting the hypothesis that the IPOD harbors insoluble proteins. Importantly, proteins localized to the INQ can be refolded by the Hsp104 chaperone, while the proteins deposited in the IPOD are terminally sequestered (Kaganovich et al., 2008).

This spatial control seems to prevent daughter cells from inheriting aggregated proteins from the mother cell. In fact, a study by Spokoini and colleagues revealed that misfolded proteins originally aggregate in proteostatic stress foci and then develop into inclusions prior to the budding event, being retained in the mother cell (Spokoini et al., 2012).

3.3 ER-associated degradation

As mentioned before, one-third of all proteins are folded in compartments, such as the ER. Newly synthesized proteins enter the ER lumen through the translocon, where chaperones aid in protein folding and assembly of multiprotein complexes. Once the native conformation is achieved, the proteins are released from the ER. However, when the proteins fail to acquire a native conformation in the ER, they become substrates for ER-associated degradation (ERAD) (Ruggiano et al., 2014).

Proteins present in the ER must pass through two checkpoints. The first checkpoint, specific for ER membrane proteins, examines the cytoplasmic domain of the membrane proteins; the second checkpoint, for both membrane and soluble proteins, examines the domains localized in the ER lumen. Proteins that fail to pass one or both checkpoints are targeted for ERAD (Vashist and Ng, 2004). Misfolded or unfolded polypeptides are recognized by the ERAD system through recognition factors, which are part of the E3 ligase complex present in the ER membrane. Furthermore, certain post-translational modifications (PTM) can also target proteins for ERAD, such as *O*-mannosylation (Goder and Melero, 2011; Xu et al., 2013).

Following recognition, the substrate is retrotranslocated, i.e., transported across the ER membrane into the cytoplasm, where it is ubiquitinated by a membrane-associated E3 ligase. With the help of the Cdc48 ATPase complex, the substrate is extracted from the membrane in an ATP-dependent manner, released into the cytosol and then subjected to the UPS for protein degradation (Carvalho et al., 2006; Ruggiano et al., 2014; Xie and Ng, 2010).

3.4 The ubiquitin-proteasome system

The UPS is the primary system for degradation and removal of defective or aggregated proteins in eukaryotic cells. Succinctly, proteins are

polyubiquitinated with K48-linked chains and transported into the 26S proteasome for degradation (Figure 4).

Ubiquitination is a PTM essential for protein degradation. Free ubiquitin is activated by an E1 ubiquitin-activating enzyme in an ATP-dependent manner and then transferred to an E2 ubiquitin-conjugating enzyme. The E2 enzyme associates with an E3 ubiquitin ligase and transfers the ubiquitin molecule. The E3 ligase may or may not be bound to a substrate protein before the ubiquitin transfer (Weissman, 2001). The ubiquitination process differs depending on the E3 enzyme: RING (really interesting new gene) E3 enzymes promote direct transfer of the ubiquitin from an E2 to the substrate, while HECT (homologous to the E6AP carboxyl-terminus) E3 enzymes form an intermediate with ubiquitin prior to the transfer to the substrate (Zheng and Shabek, 2017). A protein can be ubiquitinated at a lysine residue in a ubiquitin-binding domain or at the amino terminus (Breitschopf et al., 1998).

Ubiquitin can be added to the protein as a single ubiquitin molecule, termed monoubiquitination, or in a ubiquitin chain. A fourth ubiquitin enzyme, E4, was found to aid in the formation of ubiquitin chains. In the absence of E4, ubiquitin chains are only comprised of a few ubiquitin molecules, which is insufficient for targeting to the proteasome (Koegl et al., 1999) .

Ubiquitin contains seven lysine residues (K6, K11, K27, K29, K33, K48 and K63), but only four ubiquitin lysines can form ubiquitin-ubiquitin linkages (K11, K29, K48 and K63). Chains formed from different lysine residues have different roles; for example, while K48 chains target proteins for proteolytic degradation, K63 chains are important for DNA repair, endocytosis and vacuolar degradation (Hicke, 2001; Spence et al., 1995; Weissman, 2001). Interestingly, a recent study revealed that branched K48/K63-linked ubiquitin chains can target a protein for proteasomal degradation (Ohtake et al., 2018).

Most ubiquitinated proteins have K48-linked chains and are recognized by the 26S proteasome, the main protease in eukaryotic cells. Ubiquitinated proteins are targeted to the proteasome by interacting with ubiquitin receptors, either intrinsic or transiently bound (Bard et al., 2018).

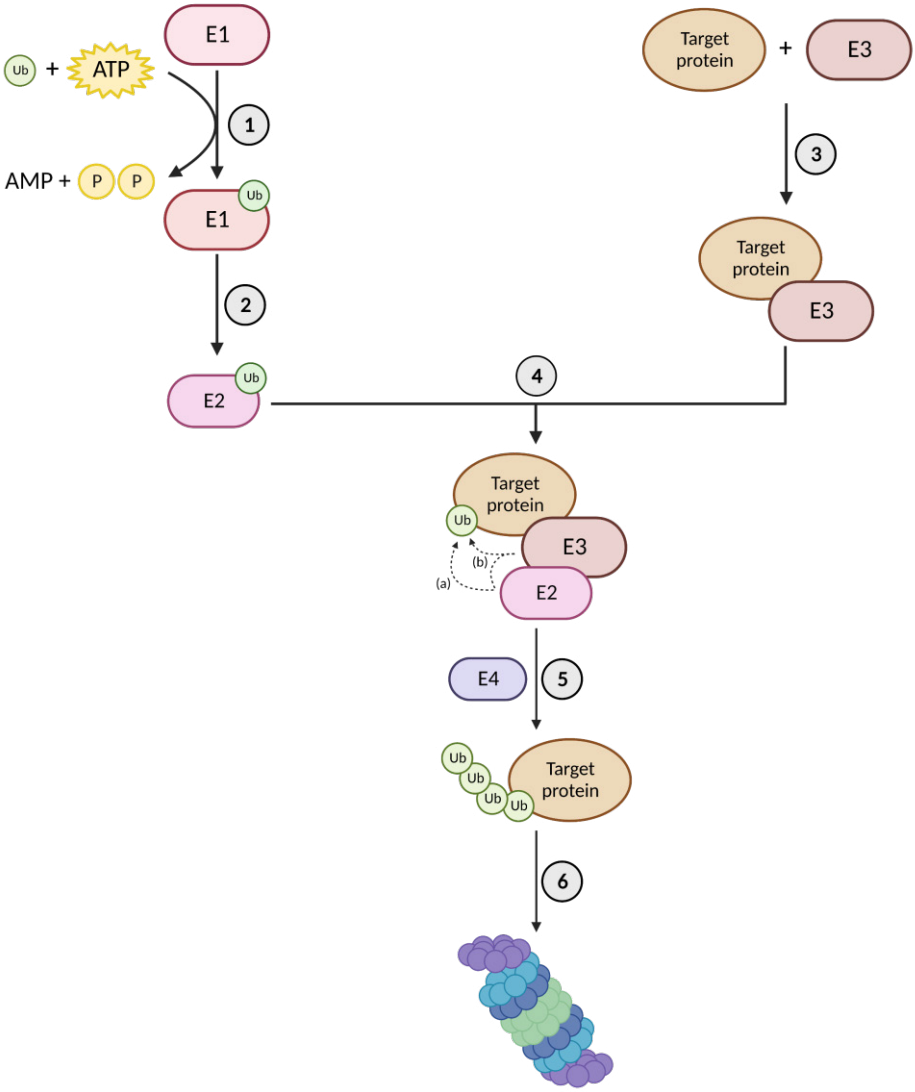

Figure 4| Addition of K48-linked ubiquitin chains, targets proteins for proteasomal degradation. ① E1 ubiquitin-activating enzymes activate ubiquitin using ATP. ② E1 then transfers the ubiquitin molecule to an E2 ubiquitin-conjugating enzyme. ③ Simultaneously, an E3 ubiquitin-ligase may bind to a substrate protein. ④ The E2 enzyme associates with a RING E3 ligase and transfers the ubiquitin molecule to the target protein (a), or with a HECT E3 ligase, which forms an intermediate with ubiquitin prior to transferring the ubiquitin to the substrate protein. ⑤ An E4 ubiquitin enzyme forms ubiquitin chains. ⑥ If the ubiquitin chain is K48-linked, the protein is delivered to the proteasome for degradation. (Created with BioRender.com)

While monoubiquitination is not enough to target the protein for proteasomal degradation, proteins that lack an ubiquitin chain can still be targeted for degradation when in association with an ubiquitinated partner, which acts as a proteasome adaptor (Prakash et al., 2009).

The 26S proteasome is composed of the 20S core particle (CP) and the 19S regulatory particle (RP). The proteolytic active sites localize within the barrel-shaped 20S CP, which is gated by 19S RP in one or both sides of the CP. The ATPases present in the RP trigger the opening of the gate upon ATP binding, allowing entrance of ubiquitinated proteins into the degradation chamber in the CP for proteolytic cleavage. The ATPases are also responsible for disrupting higher-order structures via ATP hydrolysis, unfolding the substrate prior to entrance in the CP (Glickman et al., 1998; Groll et al., 2000; Rabl et al., 2008; Smith et al., 2007; Tian et al., 2011). Furthermore, the 19S RP contains deubiquitinases that, as the name suggests, remove ubiquitin chains from the substrates for recycling of ubiquitin molecules (Glickman et al., 1998).

The proteasome has a large range of substrates, however, all proteasomal substrates must possess a targeting signal (the ubiquitin chain) or ubiquitinated partner, and an unstructured region. Proteolytic cleavage at the CP begins at the unstructured region of the protein, which serves as an initiation site for degradation, and the other regions are digested sequentially (Prakash et al., 2004; Takeuchi et al., 2007).

3.5 Autophagy

Autophagy comprises different processes that degrade a range of intracellular constituents by the action of vacuolar/lysosomal enzymes. Autophagic bodies were found to carry misfolded and unfolded proteins, mitochondria, rough ER, ribosomes, cytosolic enzymes, and lipid and glycogen granules (Baba et al., 1994; Takeshige et al., 1992). Autophagy can be classified depending on the process or the substrates being degraded, with macroautophagy and

microautophagy being the two main autophagic processes (Figure 5). Additionally, in a process similar to autophagy, yeast cells deliver vacuolar enzymes from the cytoplasm to the vacuole via the cytoplasm to the vacuole targeting pathway (CVT) (Baba et al., 1997).

Briefly, during macroautophagy, the elements to be degraded are engulfed by a double membrane, forming an autophagosome. The autophagosome outer membrane fuses with the vacuolar/lysosomal membrane, and the autophagic body, composed of the substrates surrounded by the autophagosome inner membrane, enters the vacuolar/lysosomal lumen. The autophagic body membrane is then disintegrated, allowing vacuolar/lysosomal enzymes to degrade the substrates (Baba et al., 1995; Baba et al., 1994; Fengsrud et al., 2000; Takeshige et al., 1992). The cellular elements to be degraded can also be engulfed by the lysosomal/vacuolar membrane directly, without the formation of an autophagosome, in a process termed microautophagy (Cuervo, 2004; Müller et al., 2000).

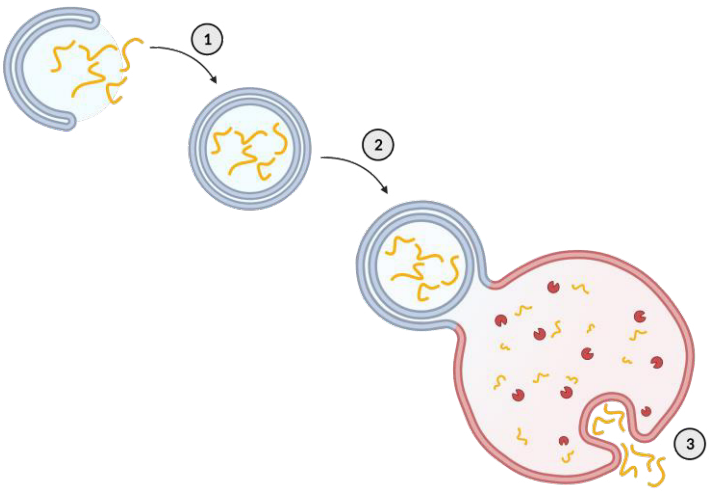

Figure 5| Proteins can be degraded via macro- and microautophagy. ① During macroautophagy, the substrates are engulfed by a double membrane, forming the autophagosome. ② The autophagosome then fuses its outer membrane with the vacuolar membrane (in yeast) or the lysosomal membrane (in animal cells), while the inner membrane is dissolved. The substrates are now degraded in the vacuolar/lysosomal lumen. ③ During microautophagy, there is no formation of autophagosomes; instead, the substrates are directly engulfed by the vacuolar/lysosomal membrane. (Created with BioRender.com)

As mentioned before, autophagy can be categorized based on the degradation substrates. Pexophagy is the autophagic degradation of peroxisomes (Sakai et al., 1998), while mitophagy is the autophagic degradation of mitochondria (Kanki and Klionsky, 2008). Portions of the nucleus can also be degraded by piecemeal microautophagy of the nucleus, where portions of the nucleus are released into the vacuole, via nucleus-vacuole junctions, for degradation (Roberts et al., 2003). Studies in human cell lines have revealed an autophagic degradation of insoluble cytoplasmic protein aggregates, termed aggrephagy; a portion of the rough ER, free of ribosomes, engulfs the protein aggregates and delivers the aggregates to the lysosome for degradation (Park et al., 2020). In addition, autophagy efficiently degrades misfolded or aggregated proteins that are resistant to ERAD (Houck et al., 2014).

4. HEAVY METALS AND ARSENIC

Heavy metals are metallic elements with an increased density when compared to water and are found naturally in the Earth's crust. The heavy metal element group includes both metalloids and transition metals.

Some heavy metals are essential to the human body, such as zinc, copper, manganese, cobalt, and iron: insufficient intake of these metals may lead to deficiency diseases and syndromes. However, these metals can be toxic at high concentrations. On the other hand, some heavy metals, like mercury, arsenic, cadmium, and lead, are xenobiotic and deleterious to the human health if accumulated in the body. Heavy metals have been shown to accumulate in the liver, kidneys, heart, and brain, and to perturb the normal function of these organs. At a cellular level, heavy metals affect organelles and cellular components, such as the membrane, ER, mitochondria, lysosome, nuclei and many enzymes involved in, for example, damage repair, metabolism and detoxification (Lentini et al., 2017; Rehman et al., 2018; Tamás et al., 2014; Tchounwou et al., 2012; Wang and Shi, 2001).

Arsenic is classified as a metalloid since it shares properties with metals and non-metals. It is considered to be the most hazardous substance due to its abundance in the environment, its toxicity, and the potential for human exposure (Hettick et al., 2015; Martinez et al., 2011; Rehman et al., 2018; Wysocki and Tamás, 2010). Arsenic can occur in four different valence states: As(0), metallic arsenic; As(III), known as arsenite and considered the most toxic state, and more rapidly absorbed; As(V), known as arsenate; and, lastly, arsine gas (Vahter, 2002).

4.1 Arsenic exposure

Arsenic occurs naturally in the atmosphere, soils, rocks, and natural waters. In water, arsenic is mostly found in inorganic form as oxyanions of As(III) and As(V). Despite the fact that arsenic exposure is mainly due to natural sources,

part of the human exposure also results from anthropogenic activities. In the past, arsenic has been used as a pesticide in agriculture, wood preservation, as an additive to livestock feed and as a drug to treat several diseases (Basu et al., 2013; Smedley and Kinniburgh, 2002).

Humans are exposed to arsenic mainly through drinking contaminated water and consumption of contaminated food. The latest data from the World Health Organization (WHO) reveals that almost 200 million people in 50 countries drink water containing arsenic at levels above the guideline value (10µg/L), with more than 45 million people belonging to developing Asian countries drinking water with 50µg/L of arsenic (Brammer and Ravenscroft, 2009; WHO, 2018).

At the moment, the WHO considers the arsenic contamination of groundwater in Bangladesh *"the largest mass poisoning of a population in history"*. Around 28% of the public water wells that service the population exhibit arsenic concentrations that exceed the recommended values. It is estimated that between 35 to 77 million Bangladeshis are exposed to arsenic-contaminated drinking-water, which corresponds to 28% - 62% of the entire population of Bangladesh (Joseph et al., 2015; Smith et al., 2000). Several arsenic contamination episodes have occurred over time in different countries: Argentina, Australia, Bulgaria, Canada, Chile, China, England, Germany, Ghana, Greece, Hungary, India, Japan, Mexico, New Zealand, Philippines, Poland, Scotland, Spain, Sri Lanka, Sweden, Taiwan, Thailand, USA, and Vietnam (reviewed in Mandal and Suzuki, 2002).

4.2 Cellular effects of arsenic

Arsenic causes a series of effects at the cellular level, from production of reactive oxygen species (ROS) to protein aggregation.

ROS are the product of a reaction between electrons and oxygen and its main sources are cellular respiration and metabolic processes. ROS are involved in several cellular functions, such as host defense, cell signaling and biosynthetic

processes. However, at high concentrations, ROS and reactive nitrogen species (RNS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and nitric oxide (NO), react with proteins, lipids, nucleic acids and carbohydrates, altering their functions or even leading to their destruction (Brieger et al., 2012; Dröge, 2002; Shi et al., 2004). Interaction of ROS with DNA can lead to DNA oxidation and, consequently, can cause mutations and gene expression changes (Wiseman and Halliwell, 1996), while interaction with proteins may lead to protein aggregation (Brieger et al., 2012).

In distinct model organisms, As(III) has been shown to induce ROS production and disrupt the redox equilibrium (Du et al., 2007; Liu et al., 2001; Menezes et al., 2008; Samikkannu et al., 2003; Shi et al., 2004; Wu et al., 2013). Generation of free radicals such as $O_2^{\cdot-}$ and $^{\cdot}OH$ occurs during reduction of pentavalent arsenic to trivalent arsenic. In addition, all arsenic forms release iron from ferritin, inducing iron-dependent formation of ROS (Ahmad et al., 2000; Kitchin and Wallace, 2008). $O_2^{\cdot-}$ is the primary species formed under As(III) stress, due to activation of the NADH oxidase in the mitochondria, which later promotes a cascade of production of other ROS, mostly H_2O_2 and $^{\cdot}OH$ (Shi et al., 2004). In fact, arsenic treated cells increase their rate of oxygen consumption, implying enhanced metabolism and production of ROS (Barchowsky et al., 1999).

The imbalance in the redox equilibrium arising from As(III) exposure is accompanied by peroxidation of several cellular structures, leading to suppression of metabolic activity and, consequently, apoptosis (Du et al., 2007; Samokhvalov et al., 2003; Wang et al., 1996). Indeed, hydrogen peroxide is a mediator in cytochrome c release to the cytosol, degradation of PARP and activation of the CPP32 protease, all hallmarks of apoptosis (Chen et al., 1998).

Indirect activation of Met4 by As(III) in yeast leads to the biosynthesis of the antioxidant glutathione (GSH). It is worth noting that, besides having antioxidant properties, GSH is also involved in detoxification of As(III) (Yen et al., 2005). Studies in yeast revealed an upregulation of the sulfate assimilation

and GSH biosynthesis pathways in the presence of As(III), indicating an increase in antioxidant defenses (Haugen et al., 2004; Thorsen et al., 2007). Furthermore, deletion mutants of genes involved in protection against oxidative stress are sensitive to As(III), confirming that oxidative stress is an important toxicity process involved in As(III)-induced cell death (Vujcic et al., 2007).

This surge in ROS production and oxidative stress stemming from arsenic exposure has been linked to chromosomal and DNA damage in distinct cellular models. Formation of micronuclei, a hallmark of chromosome instability, is observed in cells exposed to As(III) as an aftermath of oxidative stress (Gurr et al., 1998; Lynn et al., 1998; Wang et al., 1997). Chromosome breaks were observed in cells treated with both As(V) and As(III) (Nakamuro and Sayato, 1981). Besides, ROS production also induces DNA strand breaks, fragmentation and deletions, NAD depletion and poly(ADP-ribosylation); with the later implicated in DNA repair, apoptosis and signal transduction (Du et al., 2007; Lee-Chen et al., 1993; Litwin et al., 2013; Lynn et al., 2000; Lynn et al., 1998; Wang and Shi, 2001).

Alternatively, DNA damage accumulates under As(III) stress due to the ability of As(III) to inhibit DNA repair. Specifically, As(III) inhibits the incision and ligation steps of the nucleotide excision repair and the incorporation of dNTPs into the damaged DNA (Hartwig et al., 1997; Lee-Chen et al., 1993; Li and Rossman, 1989; Lynn et al., 1997; Takahashi et al., 2000). A yeast deletion collection screen performed in the presence of As(III) oxide revealed that deletion of DNA repair genes decreased cell growth (Dilda et al., 2008), suggesting that DNA repair is required in this condition. However, in another genome-wide screen performed, deletion mutants of genes involved in DNA repair were not found to be sensitive to As(III) (Thorsen et al., 2009).

Activation of the DNA damage checkpoint leads to phosphorylation of Rad53, which, in turn, can lead to cycle arrest via the Mec1/Rad53 pathway (Paulovich and Hartwell, 1995). As(III) stress promotes Rad53 phosphorylation, confirming that As(III) causes DNA damage in yeast cells. Besides activation of the Mec1/Rad53 pathway, the Met4/Met32 can also

lead to cell cycle arrest; indeed, As(III) was also shown to activate Met4 by blocking ubiquitination (Yen et al., 2005). Indeed, yeast triggers G1 and G2 checkpoint delays in the presence of As(III) via the Hog1 pathway (Migdal et al., 2008). Additionally, cell division is impaired in As(III) conditions due to interaction between As(III) and β-tubulin, which disrupts tubulin polymerization. Cell division interference can lead to aneuploidy, polyploidy and mitotic arrest (Hoffman and Lan, 1992; Kitchin and Wallace, 2008; Menzel et al., 1999; Pan et al., 2010; Zhang et al., 2007).

Besides tubulin, As(III) has also been shown to interact with several other proteins namely actin, pyruvate kinase, pyruvic acid dehydrogenase, the transcription factor Yap8, thioredoxin reductase, PML-RARα oncoprotein, PARP-1, estrogen receptor-α, arsenic methyltransferase and keap-1 (Kitchin and Wallace, 2008; Kumar et al., 2015; Lallemand-Breitenbach et al., 2008; Lu et al., 2007; Zhang et al., 2007; Zhang et al., 2010). However, As(III) does not always interfere with the function of the protein it is bound to, as shown by Zhang *et al.*, who proposes that the function of the protein is only altered if As(III) can cause conformational changes or block active sites (Zhang et al., 2007). Besides direct interaction, As(III) can also interfere with protein function by inducing protein oxidation (Samikkannu et al., 2003).

Binding of As(III) to unfolded reduced proteins interferes with the folding pathway and induces fibril formation. It is worth noting that the principal target of As(III) seems to be the target unfolded protein, and not the enzymatic machinery behind folding (Ramadan et al., 2009). However, As(III) was shown to inhibit chaperones involved in protein folding, namely CCT (Pan et al., 2010). A proteome-wide analysis of protein aggregation performed on *S. cerevisiae* under arsenite stress revealed that proteins that aggregate during arsenite stress were involved in protein synthesis, folding, translation, metabolism, and the unfolded protein response. Interestingly, the proteins that aggregated due to As(III) were characterized by high translation rates as well as extensive physical interactions. Most aggregation prone proteins were substrates of ribosome-associated Hsp70 chaperones (Ibstedt et al., 2014). Our group found that As(III) triggers protein aggregation in a concentration-

dependent manner, by interfering with the folding of only newly-synthesized proteins (Figure 6). Furthermore, As(III)-induced protein aggregates can act as seeds and promote the aggregation of other proteins even when As(III) is no longer present (Jacobson et al., 2012).

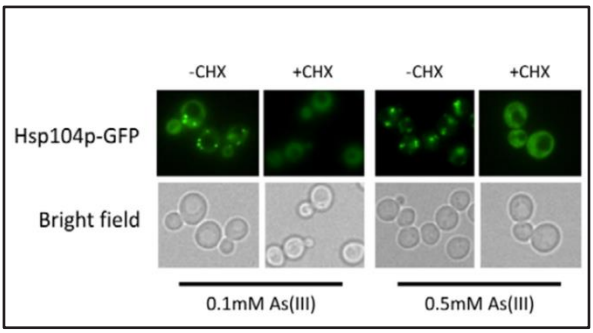

Figure 6| As(III) only induces aggregation of newly-synthesized polypeptides. As(III) induces protein aggregation, as visualized through Hsp104-GFP, in *Saccharomyces cerevisiae*. When cells are treated with the translation inhibitor cycloheximide (CHX), As(III)-induced protein aggregation is abolished, indicating that only newly-synthesized polypeptides aggregate during As(III) stress in yeast. (Jacobson et al., 2012)

Besides widespread misfolding of unfolded polypeptides, As(III) also triggers the formation of SGs and PBs (Frydrýšková et al., 2020; Ibstedt et al., 2014; Kedersha et al., 1999). Interestingly, although As(III) did not change ubiquitination levels of SGs' proteins, As(V)-induced SGs are devoid of poly-ubiquitinated proteins (Markmiller et al., 2019; Mateju et al., 2017).

To counteract the effect of As(III) on protein aggregation, yeast cells enhance their proteasomal activity, as well as enhance expression of the disaggregase Hsp104 and other chaperones (Jacobson et al., 2012; Sanchez et al., 1992; Thorsen et al., 2007). In fact, cells where proteasomal activity is diminished are more sensitive to As(III) (Di and Tamás, 2007; Haugen et al., 2004; Thorsen et al., 2009). Meanwhile, to prevent aggregation, the expression of proteins known to aggregate due to As(III) is downregulated (Ibstedt et al., 2014).

In addition to proteins involved in folding and proteolysis, the expression of several other proteins is also altered in the presence of As(III). Genes whose expression is upregulated encode protein involved in metal ion transport and homeostasis, response and detox of ROS, RNA polymerase II transcription, energy production, stress response, and sulfur metabolism/GSH biosynthesis, among others. Meanwhile, downregulated genes encode proteins that participate in protein biosynthesis, target and transport, ribosome biogenesis and assembly, and transcription elongation and termination, amongst others (Jin et al., 2008; Thorsen et al., 2007).

4.3 Arsenic-linked complications in humans

Humans can be exposed to As(III) via contaminated ground water and, consequently, contaminated food. Acute exposure to arsenic leads to a variety of symptoms, starting with burning lips, dry mouth, and dysphagia culminating in vomiting and diarrhea (Civantos et al., 1995).

Continuous exposure to arsenic-containing waters has led to thousands of cases of skin lesions, such as hyperkeratosis, melanosis, and hypopigmentation, in people of varying ages. Skin lesions induced by arsenic can progress to malignancies, such as squamous cell carcinoma, basal-cell carcinoma, and Bowen's disease (Kadono et al., 2002; Tseng, 1977).

Both inhalation and dermal exposure to arsenic cause lung cancer and non-melanoma skin cancers, respectively (Hettick et al., 2015). In the lungs, arsenic has been shown to be both cytotoxic and genotoxic to human lung cells (Xie et al., 2014). Different studies have implicated arsenic in other cancers, such as bladder, kidney, liver, and prostate cancer. The carcinogenicity of arsenic is most likely due to the ability of arsenic to induce chromosomal and DNA damage, inhibit DNA repair, stimulate angiogenesis, and to disrupt cell cycle (Singh et al., 2011; Xie et al., 2014). It is estimated that 1 in 10 Bangladeshi dies from arsenic-related cancers (Joseph et al., 2015; Smith et al., 2000).

Arsenic is known to cause several cardiovascular disorders, such as atherosclerosis, ischemic heart diseases, ventricular arrhythmias, and hypertension. Arsenic phosphorylates myosin light-chain kinase, which leads to vasoconstriction of the blood vessels, and increases the synthesis of mediators of blood vessel inflammation (Bunderson et al., 2004; Chen et al., 2007; Cifuentes et al., 2009; Lee et al., 2005). Another vascular disorder, known as Blackfoot disease, has been associated with arsenic. This disorder is characterized by arterial occlusion in the lower extremities, followed by ulceration, gangrene and posterior amputation, either spontaneous or surgical (Engel et al., 1994; Tseng, 2005).

Diabetes mellitus is also linked to arsenic exposure. In pancreatic cells, insulin production, secretion and activity are reduced in the presence of arsenic (Díaz-Villaseñor et al., 2006; Izquierdo-Vega et al., 2006). Arsenite inhibits insulin-stimulated glucose uptake in adipocytes by suppressing insulin-dependent phosphorylation of protein kinase B and reduces the production of an insulin-responsive glucose transporter (Paul et al., 2007; Walton et al., 2004). Lastly, sodium arsenite impairs adipocytic differentiation and adipogenic signaling, which has been linked to insulin resistant and type II diabetes (Guilherme et al., 2008; Wauson et al., 2002).

At a neurological level, arsenic can induce apoptosis of cerebral neurons via different pathways, such as p38 mitogen-activated kinase, the JNK3 pathway or through mitochondrial impairment and production of ROS (Bashir et al., 2006; Kaler et al., 2013; Namgung and Xia, 2001; Prakash et al., 2016; Singh et al., 2011). Destabilization of cytoskeletal framework occurs during chronic arsenic exposure, causing degeneration and morphological changes in axons and impairing the peripheral nervous system (Vahidnia et al., 2008). The metabolism of various neurotransmitters is altered during long exposure to arsenite. Production of dopamine, acetylcholine, GABA, and glutamate are increased, while synthesis of norepinephrine, epinephrine and serotonin is reduced (Shila et al., 2005; Yadav et al., 2011). Several studies have correlated arsenic exposure with compromised cognitive development, particularly

learning and memory loss (Baum et al., 2010; Chin-Chan et al., 2015; O'Bryant et al., 2011; Rosado et al., 2007; Tyler and Allan, 2014).

Since arsenic induces protein aggregation, it is unsurprising that the metalloid is linked to neurodegenerative disorders, such as Alzheimer's and Parkinson's disease. In neurodegenerative disorders, arsenic's impact on protein aggregation is amplified by the declining efficiency of chaperones and proteolytic mechanisms that occurs with age (Hinault et al., 2011). High concentration of arsenic in both topsoil and pesticides correlates with a prevalence of Alzheimer's and Parkinson's disease (Dani, 2010; Elbaz et al., 2009).

Alzheimer's disease is characterized by aggregation of distinct proteins, such as amyloid precursor protein (APP), whose cleavage leads to β-amyloid formation, and tau protein. Sodium arsenite enhances transcription of APP, but does not increase levels of β-amyloid, suggesting an inhibition of the γ-cleavage step. However, dimethylarsinic species enhanced production of β-amyloid, indicating a differential effect on β-amyloid depending on inorganic or methylated arsenic (Dewji et al., 1995; Gong and O'Bryant, 2010; Zarazúa et al., 2011). Regarding tau, arsenic induces hyperphosphorylation of several amino acids, as observed in pathological conditions (Giasson et al., 2002; Gong and O'Bryant, 2010).

Parkinson's disease is characterized by an accumulation of Lewy bodies, composed of αSyn. Arsenic promotes aggregation of αSyn in human cells, mice, and yeast. *In vitro* analysis has revealed that As(III) influences the fiber pitch of αSyn and is incorporated into the fibers. Arsenic also hindered autophagic flux and consequent clearance of αSyn aggregates in yeast, aggravating αSyn toxicity (Cholanians et al., 2016; Lin et al., 2007; Lorentzon et al., 2021).

4.4 Arsenic as a therapeutic agent

Throughout history, different forms of arsenic have been used to treat a range of diseases, such as eczema, psoriasis, asthma, heartburn, rheumatism, malaria, sleeping sickness, amongst others (Hoonjan et al., 2018; Soignet et al., 1998; Waxman and Anderson, 2001; Zhu et al., 2017). Arsenic trioxide has shown potential as an anti-cancer drug in gastric cancer, malignant melanoma, pancreatic carcinoma, and hepatocellular carcinoma cell lines (Ding et al., 2015). More specifically, arsenic trioxide inhibits mammalian thioredoxin reductase, allowing for thioredoxin system-mediated apoptosis (Lu et al., 2007). In tumors were p53 is mutated, arsenic trioxide induces degradation of mutated p53 through the proteasome (Yan et al., 2011).

Arsenic trioxide is currently used as an effective drug against acute promyelocytic leukemia and can be administered in patients either intravenously or orally (Shen et al., 1997; Soignet et al., 1998; Zhu et al., 2019). Furthermore, arsenic-containing Qinghuang Powder has proved efficient in treating myelodysplastic syndrome, a blood disorder that can later develop into acute promyelocytic leukemia (Zhu et al., 2017). Acute promyelocytic leukemia is characterized by a PML/RARα fusion protein, capable of blocking the expression of genes involved in myeloid cells differentiation through interaction with RARα. The fusion protein further hinders the normal function of both PML and RARα and inhibits apoptosis. Arsenic trioxide interacts with cysteine-rich PML and induces SUMOylation of the fusion protein. A SUMO-dependent ubiquitin ligase adds K48-linked chains to PML, leading to proteasomal degradation of PML/RARα (Hai et al., 2019; Lallemand-Breitenbach et al., 2008; Yousefnia, 2021; Zhang et al., 2010). Additionally, arsenic trioxide-induced apoptosis in an acute promyelocytic leukemia cell line, is caused by production of ROS (Chen et al., 1996; Gurr et al., 1999).

5. *SACCHAROMYCES CEREVISIAE* AS A CELLULAR MODEL FOR THE STUDY OF ARSENITE TOXICITY AND DETOXIFICATION MECHANISMS

Saccharomyces cerevisiae is a single-cell eukaryote with membrane-bound organelles, such as the nucleus, mitochondria, and the endomembrane system (Duina et al., 2014). The unique features of the budding yeast granted its establishment as a robust model organism in biology. *S. cerevisiae* is non-pathogenic, inexpensive due to its short division time and simple growth conditions, and has high amenability for genetic manipulation (Duina et al., 2014; Mattiazzi et al., 2012; Menezes et al., 2015). Moreover, the budding yeast shares fundamental aspects with mammalian and human cells, namely the mechanisms of protein folding, quality control and degradation, the components involved in the secretory pathway and vesicular trafficking, mitochondrial dysfunction and oxidative stress, and cell death and survival mechanisms, allowing for extrapolation with higher organisms (Menezes et al., 2015). *S. cerevisiae* was the first organism to have its genome completely sequenced (Goffeau et al., 1996). Around 60% of the budding yeast genes display sequence homology to a human orthologue and, of human disease-related genes, over 30% have a homologue in yeast, making it possible to use budding yeast as a cellular model to study human diseases (Foury, 1997; Menezes et al., 2015).

In this work, we use the budding yeast, *Saccharomyces cerevisiae*, as a cellular model to study the cellular effects of arsenite and the cellular responses to this stress. *S. cerevisiae* has different mechanisms of uptake and detoxification depending on the oxidation state of arsenic (Figure 7), explained further.

5.1 The ACR cluster

The ACR cluster, composed of *ACR1/YAP8*, *ACR2* and *ACR3*, was identified in a yeast genomic library overexpression screen, conferring resistance to

arsenic compounds. The first member of the identified cluster, *ACR1*/*YAP8* encodes a positive transcriptional regulator of both *ACR2* and ACR3 (Bobrowicz et al., 1997). Yap8 associates with the promoter shared between *ACR2* and *ACR3* in both normal and stress conditions (Bobrowicz et al., 1997; Wysocki et al., 2004). As(III) binds to cysteine residues in Yap8 and induces conformational changes, leading to its activation (Kumar et al., 2015). A deletion mutant of *YAP8* is hypersensitive to both arsenate and arsenite, indicating that it is involved in the resistance to both arsenic valence states. Further studies revealed that the main function of Yap8 is to control expression of both *ACR2* and *ACR3* as a positive transcription factor, and its activation is dependent on arsenic (Bobrowicz et al., 1997; Menezes et al., 2004; Wysocki et al., 1997; Wysocki et al., 2004).

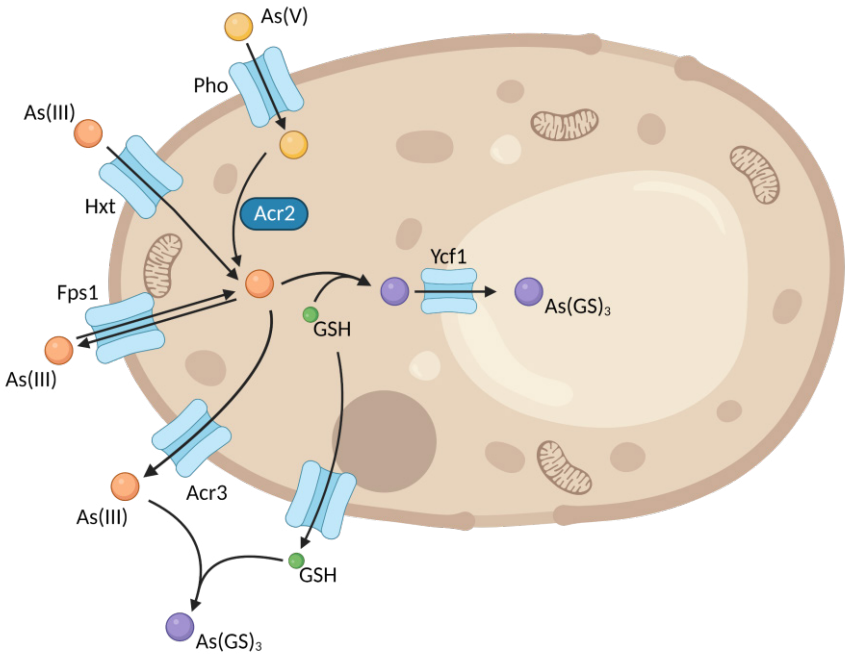

Figure 7 | As(III) and As(V) uptake and detoxification mechanisms in *Saccharomyces cerevisiae*. As(V) can enter the cell via phosphate transporters, while As(III) enters the cell via Fps1 or hexose transporters. Inside the cell, As(V) is reduced to As(III) by Acr2. During detoxification, As(III) is either exported outside the cell by Acr3, or sequestered into the vacuole after conjugation with GSH. Additionally, GSH can be exported to the extracellular medium, where it chelates extracellular As(III), impeding its entry in the cell. (Created with BioRender.com)

Meanwhile, *ACR2* encodes an arsenate reductase that reduces As(V) to As(III). Since deletion of *ACR2* conferred sensitivity to As(V) only, and not As(III), a role in As(III) detoxification was discarded. Later, Acr2 was found to exhibit arsenate-reductase activity, which corroborated the notion that Acr2 was not involved in As(III) detoxification but, instead, reduced As(V) into the more toxic form, As(III) (Bobrowicz et al., 1997; Mukhopadhyay and Rosen, 1998). Interestingly, GSH and glutaredoxins have been associated with reduction of As(V), as GSH has been shown to reduce As(V) via GSH oxidation (Csanaky and Gregus, 2005; Delnomdedieu et al., 1994). Besides, Acr2 uses GSH and glutaredoxin as a hydrogen donor and forms complexes with GSH (Rosen, 2002).

In yeast, after reduction of As(V), As(III) can be exported outside the cell by the Acr3 metalloid/H^+ antiporter (Maciaszczyk-Dziubinska et al., 2011). Corroborating the role of Acr3 as an arsenite exporter, disruption of *ACR3* leads to an accumulation of As(III) inside the cell and increases cell sensitivity (Ghosh et al., 1999). As expected, overexpression of *ACR3* confers resistance to As(III) but not to As(V). However, overexpression of both *ACR2* and *ACR3* confers resistance to both As(III) and As(V) (Bobrowicz et al., 1997).

5.2 Uptake of arsenate by phosphate transporters

As(V) is a chemical analogue of inorganic phosphate, thus it can enter the cell via phosphate transporters. Bun-ya *et al.* revealed that the phosphate transporters Pho84 and Pho87 can import As(V) into the cell. Indeed, deletion of *PHO84* and *PHO87* increased As(V) tolerance (Bun-ya et al., 1996). On the other hand, overexpression of *PHO84* enhanced As(V) uptake, confirming Pho84 as an important phosphate transporter for As(V) (Shen et al., 2012). Furthermore, disruption of *PHO86*, which encodes a protein involved in Pho84 trafficking, and *PHO88*, that encodes a membrane protein similar to Pho86, did not affect cell viability. Even double deletion of *PHO86/PHO87* and *PHO86/PHO88* had no effect in cell viability. Yet, a triple deletion mutant of *PHO86, PHO87* and *PHO88* showed arsenate resistance similar to the single

deletion mutant of *PHO84*. Disruption of *PHO86* and *PHO88* lead to reduced uptake of As(V), confirming that Pho86 and Pho88 are required for As(V) uptake (Yompakdee et al., 1996a; Yompakdee et al., 1996b).

5.3 Uptake of arsenite by aquaglyceroporins

Studies in different model organisms have shown that As(III) is transported into the cell by aquaglyceroporins when in its most common form, $As(OH)_3$, which resembles glycerol. Aquaglyceroporins, together with aquaporins, are part of the major intrinsic proteins, a family of membrane proteins known as diffusion facilitators of water and other small solutes, such as glycerol (Bienert et al., 2007).

In yeast, Fps1 was first identified as a glycerol transporter, whose main role is to export, rather than import glycerol (Tamás et al., 1999). Later, Fps1 was shown to be an important transporter of As(III) into the cell, as deletion of *FPS1* induced As(III) tolerance as a consequence of reduced uptake of As(III) (Wysocki et al., 2001). Furthermore, Fps1 can also mediate the efflux of As(III) down the concentration gradient when the intracellular concentration exceeds the extracellular concentration (Maciaszczyk-Dziubinska et al., 2010). Indeed, overexpression of *FPS1* enhances accumulation of As(III) inside the cell, but also increases export of As(III) (Shah et al., 2010).

Opening and closing of the Fps1 channel is dependent on mitogen-activated protein kinase Hog1, Acr3 and the valence state of arsenic. Hog1 is activated by methylarsenite, a product of As(III) metabolism, which inhibits the phosphatases that normally maintain Hog1 in its inactive state, increasing Hog1 phosphorylation levels. Active Hog1 closes the Fps1 channel, blocking import of As(III) to the cell. Contrarily, activation of Hog1 by As(V), which occurs through a different mechanism, does not close the Fps1 channel, allowing the efflux of the As(III) resultant from reduction of As(V) (Lee and Levin, 2018; Sotelo and Rodríguez-Gabriel, 2006).

Additionally, four cysteine residues of Hog1 are arsenylated in the presence of both As(V) and As(III). Arsenylated Hog1 prevents phosphorylation of the Fps1 regulator Rgc2 by an Acr3 loop that interacts with Rgc2, maintaining the channel open. However, this is only advantageous for cells exposed to As(V). Interestingly, in the presence of methylarsenite, three cysteine residues within the Acr3 loop are methylarsenylated, relieving the arsenylated Hog1-induced blocking of Fps1 closure (Lee and Levin, 2022). Accordingly, As(III)-induced Hog1 phosphorylation triggers an upregulation of Acr3 (Sotelo and Rodríguez-Gabriel, 2006). Moreover, cells with impaired Hog1 have elevated intracellular As(III) levels and are sensitive to metalloids, while cells with enhanced Hog1 activity present increased tolerance (Thorsen et al., 2006).

5.4 Uptake of arsenite by hexose permeases

Three molecules of $As(OH)_3$ can form a ring structure similar to hexose sugars, thus it is not surprising that As(III) can be imported into the cell via hexose permeases. Liu and colleagues demonstrated that As(III) is transported into yeast cells via Hxt7 and Hxt9. As(III) and other hexose sugars compete for transport via these hexose permeases, as glucose can inhibit As(III) uptake and, conversely, As(III) can also inhibit the uptake of glucose (Liu et al., 2004). Deletion of all hexose permeases reduces the uptake of As(III), while overexpression of *HXT7* increases As(III) accumulation in the cell (Liu et al., 2004; Shah et al., 2010).

5.5 Chelation and sequestration of As(III) into the vacuole

To counteract the effects of As(III) in the cell, As(III) can also be chelated and sequestered to the vacuole. As(III) forms a complex with GSH, $As(GS)_3$, spontaneously in normal conditions. GSH can conjugate with a variety of lipophilic compounds by the action of glutathione *S*-transferases (Grant, 2001). GSH binds to cysteine residues of proteins via disulfide bonds in a process termed *S*-glutathionylation (Pompella et al., 2003).

As(III) enhances production of GSH by stimulating the sulfur pathway. Indeed, cysteines can be obtained from methionines via the transsulfuration pathway, and later used for GSH synthesis (Lu, 2013; Thorsen et al., 2007). Production of GSH starts with formation of γ-glutamylcysteine from glutamate and cysteine by γ-glutamylcysteine synthetase, Gsh1. GSH synthetase then produces GSH from γ-glutamylcysteine and glycine (Lu, 2013).

Ycf1 (yeast cadmium factor 1), a MgATP-dependent vacuolar GSH *S*-conjugate pump, has a wide range of substrate specificity and transports the $As(GS)_3$ conjugates into the vacuole (Delnomdedieu et al., 1994; Ghosh et al., 1999; Li et al., 1996). The importance of Ycf1 under arsenic stress was confirmed by disruption of *YCF1*, which leads to cell sensitivity to both As(III) and As(V) (Ghosh et al., 1999). This sequestration into the vacuole protects other proteins and cellular components against As(III). For example, binding of GSH to As(III) protects against interactions with nascent chains and arsenite-induced ubiquitination of proteins (Habib et al., 2007; Talemi et al., 2014). However, Talemi *et al.* revealed that As(III) is capable of leaving the vacuole by an unknown export mechanism (Talemi et al., 2014).

Expression of both *YCF1* and *GSH1* is regulated by the transcription factor Yap1 (Grant, 2001; Wemmie et al., 1994; Wu and Moye-Rowley, 1994). Yap1 stimulates the expression of several genes in the presence of As(III), namely genes that encode proteins involved in GSH synthesis and the *YCF1* gene (Grant, 2001; Haugen et al., 2004; Thorsen et al., 2007). As mentioned before, yeast cells exposed to As(III) increase expression of genes in the GSH biosynthesis pathway (Jin et al., 2008; Thorsen et al., 2007).

Besides Ycf1 and Acr3, there are other transport systems in *S. cerevisiae* that contribute to detoxification of As(III). GSH can be exported outside the cell by different transporters and then chelate extracellular As(III). Since $As(GS)_3$ cannot enter yeast cells, extracellular GSH inhibits As(III) uptake (Thorsen et al., 2012).

MAIN FINDINGS

Paper I

GENOME-WIDE IMAGING SCREEN UNCOVERS MOLECULAR DETERMINANTS OF ARSENITE-INDUCED PROTEIN AGGREGATION AND TOXICITY

To uncover cellular processes and functions that influence As(III)-induced protein aggregation, we performed a genome-wide imaging screen. The screen was based on the synthetic genetic array technique, where the yeast single deletion collection was mated with a strain containing an Hsp104-GFP fusion protein, allowing for detection of protein aggregates. The resulting mutants were exposed to As(III) and the percentage of cells with protein aggregates was scored. Two sets of hits were obtained: 202 deletion mutants had enhanced aggregation, while 198 deletion mutants had decreased aggregation when compared to wild type cells after As(III) exposure.

The main findings of this paper are:

a) There are several cellular processes and functions that influence protein aggregation under As(III) stress.
b) Although a stable transcriptional control is required for the management of As(III)-induced protein aggregates, these protein aggregates are not a result of transcriptional errors.
c) Accurate transcriptional control is required to mitigate protein aggregation in As(III) stress.
d) Transcription levels correlate with global translation levels and, consequently, protein aggregation in the presence of As(III).
e) In the presence of As(III), cells reduced global translation as means to mitigate protein aggregation.

Paper II

RIBOSOMAL AND NON-RIBOSOMAL PROTEIN QUALITY CONTROL DURING ARSENITE STRESS IN *SACCHAROMYCES CEREVISIAE*

To maintain proteostasis, cell impose a series of protein quality control systems to aid in folding and degradation of proteins. Since As(III) interferes with proteostasis, we sought to uncover the significance of different protein quality control systems. The importance of different co-translational folding systems, molecular chaperones, ribosomal quality control system, and ubiquitin ligases was studied in the presence of As(III).

This study showed that:

a) Loss of the ribosome-associated folding system SSB-RAC leads to decreased protein aggregation and As(III) resistance, likely due to low global protein translational levels.
b) Protein aggregates formed in the presence of As(III) are not due to increased ribosome stalling or impairment in RQC.
c) Although the Prefoldin complex is involved in maintaining protein homeostasis during As(III) stress, impairment of TRiC/CCT does not have an effect on As(III)-induced protein aggregation.
d) As(III)-induced protein aggregates have both K48- and K63-linked ubiquitin chains, and K63-linked chains are important for the clearance of aggregates and growth in the presence of As(III).

Paper III

DIFFERENTIAL CONTRIBUTIONS OF THE PROTEASOME, AUTOPHAGY, AND CHAPERONES TO THE CLEARANCE OF ARSENITE-INDUCED PROTEIN AGGREGATES IN YEAST

Degradation of protein aggregates is crucial to maintain protein homeostasis. In this paper we investigated the contribution of different PQC pathways in clearing As(III)-induced protein aggregates, namely the ubiquitin proteasome system, autophagy, vacuolar degradation pathway, and disaggregating chaperones.

This paper revealed that:

a) The UPS is the major protein degradation system for clearance of As(III)-induced aggregates.
b) Although not as important, autophagy and the vacuolar pathways contribute to the clearance of aggregates formed due to As(III).
c) Chaperone disaggregation activity contributes to clearance of As(III)-induced aggregates.
d) A(III) impairs chaperone binding to protein aggregates by, most likely, interfering with the substrate.

DISCUSSION AND CONCLUDING REMARKS

At a cellular level, As(III) exposure interferes with a range of functions and processes. Our group has previously shown that As(III) induces aggregation of newly-synthesized polypeptides in *S. cerevisiae,* impairing cell growth (Jacobson et al., 2012). Nonetheless, to better understand how As(III) exerts its toxicity through protein aggregation, a deeper knowledge of how As(III) affects protein homeostasis was vital.

This thesis focuses on elucidating how protein folding and aggregation are regulated under As(III) stress, which protein quality control systems are required to maintain proteostasis in the presence of As(III), and how As(III)-induced protein aggregates are cleared.

In **paper I**, a genome-wide imaging study was performed to identify genes, and consequently cellular functions, important to maintain proteostasis in the presence of As(III). The imaging screen revealed two distinct set of mutants with either enhanced or reduced aggregation induced by As(III), in comparison with wild-type cells. Both sets comprised different cellular processes, suggesting that protein aggregation during As(III) stress is regulated by a variety of cellular machineries. Genes involved in transcriptional control were identified in both sets of mutants; more specifically, deletion of positive regulators of transcription led to reduced aggregation and increased resistance to As(III), whereas loss of negative transcriptional regulators led to enhanced aggregation and increased sensitivity to As(III). In fact, dysregulation of transcription was found to be linked to deviations in translational levels, explaining the changes in protein aggregation. Additionally, the imaging screen also revealed that deletion of genes encoding proteins involved in ribosome biogenesis decreased protein aggregation, supporting that translation levels regulate protein aggregation under As(III) stress. Moreover, cells were shown to decrease global translational levels in the presence of As(III), potentially as a protective mechanism. Ultimately, proper transcriptional and translational control are required to maintain proteostasis in the presence of As(III).

Maintaining proteostasis requires a balance between protein synthesis, folding and degradation. In fact, the screen performed in **paper I** revealed that deletion of genes that encode proteins involved in protein folding altered protein aggregation levels under As(III) stress. In **papers II** and **III**, the contribution of different protein folding and clearance systems was evaluated during As(III) stress.

Unexpectedly, **paper II** revealed that impairment of co-translational folding systems decreased protein aggregation and increased resistance to As(III); however, a decrease in global translation levels was also observed upon impairment of ribosomal folding systems, corroborating the importance of translational control during As(III) stress, as stated in **paper I**. In contrast, non-ribosomal chaperone systems appear to contribute primarily to aggregate clearance. These results imply that networks that mediate folding co-translationally are more important to maintain protein homeostasis in the presence of As(III), than non-ribosomal chaperone systems.

The ubiquitin-proteasome system was identified as the major degradation pathway for As(III)-induced protein aggregates in **paper III**. In fact, the paper shows that proteins that aggregate during As(III) stress are marked with K48-linked ubiquitin chains, targeting the proteins for proteasomal degradation. Meanwhile, in **paper II**, we revealed that K63-linked ubiquitin chains are also required for degradation of proteins that aggregate in the presence of As(III). Interestingly, recent studies revealed that branched ubiquitin chains containing both K48 and K63 linkages can play a role in proteasomal degradation (Ohtake et al., 2018). The requirement of K48- and K63-linked ubiquitin chains for degradation of protein aggregates, corroborates the major role of the ubiquitin-proteasome pathway during degradation of As(III)-induced protein aggregates.

Additionally, **paper III** also revealed that autophagy and the vacuolar degradation pathway contribute to the degradation of As(III)-induced protein aggregates, but to a lesser extent than the UPS. The disaggregase Hsp104, as well as its co-chaperones, are also required for degradation of protein aggregates formed in the presence of As(III). Although, As(III) was shown to

impair the binding of chaperones to protein aggregates, it does not alter chaperone activity.

The findings in this thesis give new insights on the regulation of protein aggregation, through transcriptional, translational and protein folding control, as well as on the clearance of protein aggregates in the presence of As(III). Besides transcription, translation, folding and protein degradation, several other cellular mechanisms were identified in the screen as important for the regulation of As(III)-induced protein aggregation. In conclusion, an interplay of distinct cellular mechanisms is required to maintain protein homeostasis during As(III) stress in *S. cerevisiae*.

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REFERENCES

Acosta-Sampson, L., K. Döring, Y. Lin, V.Y. Yu, B. Bukau, G. Kramer, and J.H.D. Cate. 2017. Role for ribosome-associated complex and stress-seventy subfamily B (RAC-Ssb) in integral membrane protein translation. *Journal of Biological Chemistry*. 292:19610–19627.

Aguzzi, A., and M. Altmeyer. 2016. Phase Separation: Linking Cellular Compartmentalization to Disease. *Trends Cell Biol*. 26:547-558.

Ahmad, S., K.T. Kitchin, and W.R. Cullen. 2000. Arsenic species that cause release of iron from ferritin and generation of activated oxygen. *Arch Biochem Biophys*. 382:195-202.

Alamo, M.d., D.J. Hogan, S. Pechmann, V. Albanèse, P.O. Brown, and J. Frydman. 2011. Defining the Specificity of Cotranslationally Acting Chaperones by Systematic Analysis of mRNAs Associated with Ribosome-Nascent Chain Complexes. *PLoS Biology*. 9:e1001100.

Albanèse, V., A.Y.-W. Yam, J. Baughman, C. Parnot, and J. Frydman. 2006. Systems Analyses Reveal Two Chaperone Networks with Distinct Functions in Eukaryotic Cells. *Cell*. 124:75-88.

Albert, T.K., H. Hanzawa, Y.I.A. Legtenberg, M.J.d. Ruwe, F.A.J.v.d. Heuvel, M.A. Collart, R. Boelens, and H.T.M. Timmers. 2002. Identifcation of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. *The EMBO Journal*. 21:355-364.

Alberti, S., and D. Dormann. 2019. Liquid-Liquid Phase Separation in Disease. *Annu Rev Genet*. 53:171-194.

Amor, A.J., D.T. Castanzo, S.P. Delany, D.M. Selechnik, A.v. Ooy, and D.M. Cameron. 2015. The ribosome-associated complex antagonizes prion formation in yeast. *Prion*. 9:144-164.

Andersson, R., A.M. Eisele-Bürger, S. Hanzén, K. Vielfort, D. Öling, F. Eisele, G. Johansson, T. Gustafsson, K. Kvint, and T. Nyström. 2021. Differential role of cytosolic Hsp70s in longevity assurance and protein quality control. *PLoS Genet*. 17:e1008951.

Anfinsen, C.B. 1973. Principles that govern the folding of protein chains. *Science*. 181:223-230.

Baba, M., M. Osumi, and Y. Ohsumi. 1995. Analysis of the membrane structures involved in autophagy in yeast by freeze-replica method. *Cell Struct Funct*. 20:465-471.

Baba, M., M. Osumi, S.V. Scott, D.J. Klionsky, and Y. Ohsumi. 1997. Two distinct pathways for targeting proteins from the cytoplasm to the vacuole/lysosome. *J Cell Biol*. 139:1687-1695.

Baba, M., K. Takeshige, N. Baba, and Y. Ohsumi. 1994. Ultrastructural analysis of the autophagic process in yeast: detection of autophagosomes and their characterization. *J Cell Biol*. 124:903-913.

Babinchak, W.M., and W.K. Surewicz. 2020. Liquid-Liquid Phase Separation and Its Mechanistic Role in Pathological Protein Aggregation. *J Mol Biol*. 432:1910-1925.

Barchowsky, A., L.R. Klei, E.J. Dudek, H.M. Swartz, and P.E. James. 1999. Stimulation of reactive oxygen, but not reactive nitrogen species, in vascular endothelial cells exposed to low levels of arsenite. *Free Radic Biol Med*. 27:1405-1412.

Bard, J.A.M., E.A. Goodall, E.R. Greene, E. Jonsson, K.C. Dong, and A. Martin. 2018. Structure and Function of the 26S Proteasome. *Annu Rev Biochem*. 87:697-724.

Bashir, S., Y. Sharma, M. Irshad, S.D. Gupta, and T.D. Dogra. 2006. Arsenic-induced cell death in liver and brain of experimental rats. *Basic Clin Pharmacol Toxicol*. 98:38-43.

Basu, A., D. Saha, R. Saha, T. Ghosh, and B. Saha. 2013. A review on sources, toxicity and remediation technologies for removing arsenic from drinking water. *Research on Chemical Remediates*. 40:447-485.

Baum, L., I.H. Chan, S.K. Cheung, W.B. Goggins, V. Mok, L. Lam, V. Leung, E. Hui, C. Ng, J. Woo, H.F. Chiu, B.C. Zee, W. Cheng, M.H. Chan, S. Szeto, V. Lui, J. Tsoh, A.I. Bush, C.W. Lam, and T. Kwok. 2010. Serum zinc is decreased in Alzheimer's disease and serum arsenic correlates positively with cognitive ability. *Biometals*. 23:173-179.

Beatrix, B., H. Sakai, and M. Wiedmann. 2000. The α and β Subunit of the Nascent Polypeptide-associated Complex Have Distinct Functions. *The Journal of Biological Chemistry*:37838–37845.

Beckmann, R.P., L.A. Mizzen, and W.J. Welch. 1990. Interaction of Hsp 70 with Newly Synthesized Proteins: Implications for Protein Folding and Assembly. *Science*. 248.

Behrends, C., C.A. Langer, R. Boteva, U.M. Böttcher, M.J. Stemp, G. Schaffar, B.V. Rao, A. Giese, H. Kretzschmar, K. Siegers, and F.U. Hartl. 2006. Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. *Mol Cell*. 23:887-897.

Bengtson, M.H., and C.A.P. Joazeiro. 2010. Role of a ribosome-associated E3 ubiquitin ligase in protein quality control. *Nature*. 467:470-473.

Bienert, G.P., M.D. Schüssler, and T.P. Jahn. 2007. Metalloids: essential, beneficial or toxic? Major intrinsic proteins sort it out. *Trends in Biochemical Sciences*. 33:20-26.

Bobrowicz, P., R. Wysocki, G. Owsianik, A. Goffeau, and S. Ułaszewski. 1997. Isolation of three contiguous genes, ACR1, ACR2 and ACR3, involved in resistance to arsenic compounds in the yeast Saccharomyces cerevisiae. *Yeast*. 13:819-828.

Brammer, H., and P. Ravenscroft. 2009. Arsenic in groundwater: A threat to sustainable agriculture in South and South-east Asia. *Environment International*. 35:647–654.

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

Brehme, M., C. Voisine, T. Rolland, S. Wachi, J.H. Soper, Y. Zhu, K. Orton, A. Villella, D. Garza, M. Vidal, H. Ge, and R.I. Morimoto. 2014. A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep*. 9:1135-1150.

Breitschopf, K., E. Bengal, T. Ziv, A. Admon, and A. Ciechanover. 1998. A novel site for ubiquitination: the N-terminal residue, and not internal lysines of MyoD, is essential for conjugation and degradation of the protein. *Embo j*. 17:5964-5973.

Brengues, M., and R. Parker. 2007. Accumulation of polyadenylated mRNA, Pab1p, eIF4E, and eIF4G with P-bodies in Saccharomyces cerevisiae. *Mol Biol Cell*. 18:2592-2602.

Brengues, M., D. Teixeira, and R. Parker. 2005. Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. *Science*. 310:486-489.

Brieger, K., S. Schiavone, F.J. Miller, Jr., and K.H. Krause. 2012. Reactive oxygen species: from health to disease. *Swiss Med Wkly*. 142:w13659.

Buchan, J.R., D. Muhlrad, and R. Parker. 2008. P bodies promote stress granule assembly in Saccharomyces cerevisiae. *J Cell Biol*. 183:441-455.

Buchan, J.R., and R. Parker. 2009. Eukaryotic stress granules: the ins and outs of translation. *Mol Cell*. 36:932-941.

Buchberger, A., A. Valencia, R. McMacken, C. Sander, and B. Bukau. 1994. The chaperone function of DnaK requires the coupling of ATPase activity

with substrate binding through residue E171. *The EMBO Journal*. 13:1687 - 1695.

Bun-ya, M., K. Shikata, S. Nakade, C. Yompakdee, S. Harashima, and Y. Oshima. 1996. Two new genes, PHO86and PHO87, involved in inorganic phosphate uptake in Saccharomyces cerevisiae. *Current Genetics*. 29:344—351.

Bunderson, M., D.M. Brooks, D.L. Walker, M.E. Rosenfeld, J.D. Coffin, and H.D. Beall. 2004. Arsenic exposure exacerbates atherosclerotic plaque formation and increases nitrotyrosine and leukotriene biosynthesis. *Toxicol Appl Pharmacol*. 201:32-39.

Cabrita, L.D., C.M. Dobson, and J. Christodoulou. 2010. Protein folding on the ribosome. *Current Opinion Structural Biology*. 20:33-45.

Carvalho, P., V. Goder, and T.A. Rapoport. 2006. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. *Cell*. 126:361-373.

Chen, G.Q., J. Zhu, X.G. Shi, J.H. Ni, H.J. Zhong, G.Y. Si, X.L. Jin, W. Tang, X.S. Li, S.M. Xong, Z.X. Shen, G.L. Sun, J. Ma, P. Zhang, T.D. Zhang, C. Gazin, T. Naoe, S.J. Chen, Z.Y. Wang, and Z. Chen. 1996. In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia: As2O3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. *Blood*. 88:1052-1061.

Chen, S.C., M.H. Tsai, H.J. Wang, H.S. Yu, and L.W. Chang. 2007. Involvement of substance P and neurogenic inflammation in arsenic-induced early vascular dysfunction. *Toxicol Sci*. 95:82-88.

Chen, Y.C., S.Y. Lin-Shiau, and J.K. Lin. 1998. Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. *J Cell Physiol*. 177:324-333.

Chin-Chan, M., J. Navarro-Yepes, and B. Quintanilla-Vega. 2015. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front Cell Neurosci*. 9:124.

Cholanians, A.B., A.V. Phan, E.J. Ditzel, T.D. Camenisch, S.S. Lau, and T.J. Monks. 2016. Arsenic induces accumulation of a-synuclein: Implications for synucleinopathies and neurodegeneration. *Toxicological Sciences*. 153:271–281.

Cifuentes, F., J. Bravo, M. Norambuena, S. Stegen, A. Ayavire, and J. Palacios. 2009. Chronic exposure to arsenic in tap water reduces acetylcholine-induced relaxation in the aorta and increases oxidative stress in female rats. *Int J Toxicol*. 28:534-541.

Civantos, D.P., A.L. Rodriguez, J.M. Aguado-Borruey, and J.A.J. Narvaez. 1995. Fulminant malignant arrythmia and multiorgan failure in acute arsenic poisoning. *Chest*. 108:1774-1775.

Conicella, A.E., G.H. Zerze, J. Mittal, and N.L. Fawzi. 2016. ALS Mutations Disrupt Phase Separation Mediated by α-Helical Structure in the TDP-43 Low-Complexity C-Terminal Domain. *Structure*. 24:1537-1549.

Cougot, N., S. Babajko, and B. Séraphin. 2004. Cytoplasmic foci are sites of mRNA decay in human cells. *J Cell Biol*. 165:31-40.

Crombie, T., J.P. Boyle, J.R. Coggins, and A.J.P. Brown. 1994. The folding of the bifunctional TRP3 protein in yeast is influenced by a translational pause which lies in a region of structural divergence with Escherichia coli indoleglycerol-phosphate synthase. *European Journal of Biochemistry*. 226:657-664.

Crowder, J.J., A. Schink, M. Geigges, S.G. Kreft, R.T. Gibson, E.S. Fults, B.W. Buchanan, N. Sachs, and E.M. Rubenstein. 2015. Rkr1/ltn1 ubiquitin ligase-mediated degradation of translationally stalled endoplasmic reticulum proteins. *The Journal of Biological Chemistry*. 290:18454–18466.

Csanaky, I., and Z. Gregus. 2005. Role of glutathione in reduction of arsenate and of gamma-glutamyltranspeptidase in disposition of arsenite in rats. *Toxicology*. 207:91-104.

Cuéllar, J., W.G. Ludlam, N.C. Tensmeyer, T. Aoba, M. Dhavale, C. Santiago, M.T. Bueno-Carrasco, M.J. Mann, R.L. Plimpton, A. Makaju, S. Franklin, B.M. Willardson, and J.M. Valpuesta. 2019. Structural and functional analysis of the role of the chaperonin CCT in mTOR complex assembly. *Nat Commun*. 10:2865.

Cuervo, A.M. 2004. Autophagy: in sickness and in health. *Trends in Cell Biology*. 14:70-77.

Dani, S.U. 2010. Arsenic for the fool: an exponential connection. *Sci Total Environ*. 408:1842-1846.

Defenouillère, Q., and M. Fromont-Racine. 2017. The ribosome-bound quality control complex: from aberrant peptide clearance to proteostasis maintenance. *Current Genetics*. 63.

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

Delnomdedieu, M., M.M. Basti, J.D. Otvos, and D.J. Thomas. 1994. Reduction and binding of arsenate and dimethylarsinate by glutathione: a magnetic resonance study. *Chem Biol Interact*. 90:139-155.

Deshaies, R.J., B.D. Koch, M. Werner-Washburne, E.A. Craig, and R. Schekman. 1988. A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. *Nature*. 332:800-805.

Deuerling, E., M. Gamerdinger, and S.G. Kreft. 2019. Chaperone Interactions at the Ribosome. *Cold Spring Harb Perspect Biol*. 11.

Dever, T.E., and R. Green. 2012. The Elongation, Termination, and Recycling Phases of Translation in Eukaryotes. *Cold Spring Harb Perspect Biol*. 4.

Dewji, N.N., C. Do, and R.M. Bayney. 1995. Transcriptional activation of Alzheimer's beta-amyloid precursor protein gene by stress. *Brain Res Mol Brain Res*. 33:245-253.

Di, Y., and M.J. Tamás. 2007. Regulation of the arsenic-responsive transcription factor Yap8p involves the ubiquitin-proteasome pathway. *J Cell Sci*. 120:256-264.

Díaz-Villaseñor, A., M.C. Sánchez-Soto, M.E. Cebrián, P. Ostrosky-Wegman, and M. Hiriart. 2006. Sodium arsenite impairs insulin secretion and transcription in pancreatic beta-cells. *Toxicol Appl Pharmacol*. 214:30-34.

Dilda, P.J., G.G. Perrone, A. Philp, R.B. Lock, I.W. Dawes, and P.J. Hogg. 2008. Insight into the selectivity of arsenic trioxide for acute promyelocytic leukemia cells by characterizing *Saccharomyces cerevisiae* deletion strains that are sensitive or resistant to the metalloid. *The International Journal of Biochemistry & Cell Biology*. 40:1016–1029.

Dimitrova, L.N., K. Kuroha, T. Tatematsu, and T. Inada. 2009. Nascent peptide-dependent translation arrest leads to not4p-mediated protein degradation by the proteasome. *The Journal of Biological Chemistry*. 284:10343–10352.

Ding, W., L. Zhang, S. Kim, W. Tian, Y. Tong, J. Liu, Y. Ma, and S. Chen. 2015. Arsenic sulfide as a potential anti-cancer drug. *Molecular Medicine Reports*. 11:968-974.

Dobson, C.M. 2003. Protein folding and misfolding. *Nature*. 426:884-890.

Doma, M.K., and R. Parker. 2006. Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation. *Nature*. 440:561-564.

Döring, K., N. Ahmed, T. Riemer, H.G. Suresh, Y. Vainshtein, M. Habich, J. Riemer, M.P. Mayer, E.P. O'Brien, G. Kramer, and B. Bukau. 2017.

Profiling Ssb-Nascent Chain Interactions Reveals Principles of Hsp70-Assisted Folding. *Cell*. 170:298–311.

Dröge, W. 2002. Free radicals in the physiological control of cell function. *Physiol Rev*. 82:47-95.

Du, L., Y. Yu, J. Chen, Y. Liu, Y. Xia, Q. Chen, and X. Liu. 2007. Arsenic induces caspase- and mitochondria-mediated apoptosis in Saccharomyces cerevisiae. *FEMS Yeast Res*. 7:860-865.

Duina, A.A., M.E. Miller, and J.B. Keeney. 2014. Budding yeast for budding geneticists: A primer on the saccharomyces cerevisiae model system. *Genetics*. 197:33-48.

Duttler, S., S. Pechmann, and J. Frydman. 2013. Principles of cotranslational ubiquitination and quality control at the ribosome. *Molecular Cell*. 50:379–393.

Eichner, T., A.P. Kalverda, G.S. Thompson, S.W. Homans, and S.E. Radford. 2011. Conformational conversion during amyloid formation at atomic resolution. *Molecular Cell*. 41:161–172.

Elbaz, A., J. Clavel, P.J. Rathouz, F. Moisan, J.P. Galanaud, B. Delemotte, A. Alpérovitch, and C. Tzourio. 2009. Professional exposure to pesticides and Parkinson disease. *Ann Neurol*. 66:494-504.

Engel, R.R., C. Hopenhayn-Rich, O. Receveur, and A.H. Smith. 1994. Vascular effects of chronic exposure: a review. *Epidemiologic Reviews*. 16:184-209.

Fengsrud, M., E.S. Erichsen, T.O. Berg, C. Raiborg, and P.O. Seglen. 2000. Ultrastructural characterization of the delimiting membranes of isolated autophagosomes and amphisomes by freeze-fracture electron microscopy. *Eur J Cell Biol*. 79:871-882.

Foury, F. 1997. Human genetic diseases: a cross-talk between man and yeast. *Genetics*. 195:1-10.

Frottin, F., F. Schueder, S. Tiwary, R. Gupta, R. Körner, T. Schlichthaerle, J. Cox, R. Jungmann, F.U. Hartl, and M.S. Hipp. 2019. The nucleolus functions as a phase-separated protein quality control compartment. *Science*. 365:342-347.

Frydrýšková, K., T. Mašek, and M. Pospíšek. 2020. Changing faces of stress: Impact of heat and arsenite treatment on the composition of stress granules. *Wiley Interdiscip Rev RNA*. 11:e1596.

Gallardo, P., S. Salas-Pino, and R.R. Daga. 2021. Reversible protein aggregation as cytoprotective mechanism against heat stress. *Curr Genet*. 67:849-855.

Gautschi, M., H. Lilie, U. Fünfschilling, A. Mun, S. Ross, T. Lithgow, P. Rücknagel, and S. Rospert. 2001. RAC, a stable ribosome-associated

complex in yeast formed by the DnaK-DnaJ homologs Ssz1p and zuotin. *PNAS*. 98:3762–3767.

Gautschi, M., A. Mun, S. Ross, and S. Rospert. 2002. A functional chaperone triad on the yeast ribosome. *PNAS*. 99:4209–4214.

George, R., T. Beddoe, K. Landl, and T. Lithgow. 1998. The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria in vivo. *PNAS*. 95:2296–2301.

Gestaut, D., S.H. Roh, B. Ma, G. Pintilie, L.A. Joachimiak, A. Leitner, T. Walzthoeni, R. Aebersold, W. Chiu, and J. Frydman. 2019. The Chaperonin TRiC/CCT Associates with Prefoldin through a Conserved Electrostatic Interface Essential for Cellular Proteostasis. *Cell*. 177:751-765.e715.

Gething, M.-J., and J. Sambrook. 1992. Protein folding in the cell. *Nature*. 355:33-45.

Ghosh, A., and N. Shcherbik. 2020. Cooperativity between the Ribosome-Associated Chaperone Ssb/RAC and the Ubiquitin Ligase Ltn1 in Ubiquitination of Nascent Polypeptides. *Int J Mol Sci*. 21.

Ghosh, M., J. Shen, and B.P. Rosen. 1999. Pathways of As(III) detoxification in Saccharomyces cerevisiae. *PNAS*. 96:5001–5006.

Giasson, B.I., D.M. Sampathu, C.A. Wilson, V. Vogelsberg-Ragaglia, W.E. Mushynski, and V.M. Lee. 2002. The environmental toxin arsenite induces tau hyperphosphorylation. *Biochemistry*. 41:15376-15387.

Girstmair, H., F. Tippel, A. Lopez, K. Tych, F. Stein, P. Haberkant, P.W.N. Schmid, D. Helm, M. Rief, M. Sattler, and J. Buchner. 2019. The Hsp90 isoforms from S. cerevisiae differ in structure, function and client range. *Nat Commun*. 10:3626.

Glickman, M.H., D.M. Rubin, V.A. Fried, and D. Finley. 1998. The regulatory particle of the Saccharomyces cerevisiae proteasome. *Mol Cell Biol*. 18:3149-3162.

Glover, J.R., and S. Lindquist. 1998. Hsp104, Hsp70, and Hsp40: A novel chaperone system that rescues previously aggregated proteins. *Cell*. 94:73-82.

Goder, V., and A. Melero. 2011. Protein O-mannosyltransferases participate in ER protein quality control. *J Cell Sci*. 124:144-153.

Goffeau, A., B.G. Barrell, H. Bussey, R.W. Davis, B. Dujon, H. Feldmann, F. Galibert, J.D. Hoheisel, C. Jacq, M. Johnston, E.J. Louis, H.W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin, and S.G. Oliver. 1996. Life with 6000 Genes. *Science*. 274:546-567.

Gong, G., and S.E. O'Bryant. 2010. The arsenic exposure hypothesis for Alzheimer disease. *Alzheimer Dis Assoc Disord*. 24:311-316.

Grant, C.M. 2001. Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions. *Mol Microbiol*. 39:533-541.

Grantham, J., L.W. Ruddock, A. Roobol, and M.J. Carden. 2002. Eukaryotic chaperonin containing T-complex polypeptide 1 interacts with filamentous actin and reduces the initial rate of actin polymerization in vitro. *Cell Stress Chaperones*. 7:235-242.

Groll, M., M. Bajorek, A. Köhler, L. Moroder, D.M. Rubin, R. Huber, M.H. Glickman, and D. Finley. 2000. A gated channel into the proteasome core particle. *Nat Struct Biol*. 7:1062-1067.

Grousl, T., P. Ivanov, I. Frýdlová, P. Vasicová, F. Janda, J. Vojtová, K. Malínská, I. Malcová, L. Nováková, D. Janosková, L. Valásek, and J. Hasek. 2009. Robust heat shock induces eIF2alpha-phosphorylation-independent assembly of stress granules containing eIF3 and 40S ribosomal subunits in budding yeast, Saccharomyces cerevisiae. *J Cell Sci*. 122:2078-2088.

Guilherme, A., J.V. Virbasius, V. Puri, and M.P. Czech. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol*. 9:367-377.

Gurr, J.R., D.T. Bau, F. Liu, S. Lynn, and K.Y. Jan. 1999. Dithiothreitol enhances arsenic trioxide-induced apoptosis in NB4 cells. *Mol Pharmacol*. 56:102-109.

Gurr, J.R., F. Liu, S. Lynn, and K.Y. Jan. 1998. Calcium-dependent nitric oxide production is involved in arsenite-induced micronuclei. *Mutat Res*. 416:137-148.

Habib, G.M., Z.Z. Shi, and M.W. Lieberman. 2007. Glutathione protects cells against arsenite-induced toxicity. *Free Radic Biol Med*. 42:191-201.

Hai, Y., X. Wang, P. Song, J.Y. Li, L.H. Zhao, F. Xie, X.M. Tan, Q.J. Xie, L. Yu, Y. Li, Z.R. Wu, and H.Y. Li. 2019. Realgar transforming solution-induced differentiation of NB4 cell by the degradation of PML/RARα partially through the ubiquitin-proteasome pathway. *Arch Pharm Res*. 42:684-694.

Halter, D., M.A. Collart, and O.O. Panasenko. 2014. The Not4 E3 Ligase and CCR4 Deadenylase Play Distinct Roles in Protein Quality Control. *PLoS ONE*. 9:e86218.

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

Hartl, F.U., and M. Hayer-Hartl. 2009. Converging concepts of protein folding in vitro and in vivo. *Nature Structural & Molecular Biology*. 16:574-581.

Hartwig, A., U.D. Gröblinghoff, D. Beyersmann, A.T. Natarajan, R. Filon, and L.H.F. Mullenders. 1997. Interaction of arsenic(III) with nucleotide excision repair in UV-irradiated human fibroblasts. *Carcinogenesis*. 18:399-405.

Hasin, N., S.A. Cusack, S.S. Ali, D.A. Fitzpatrick, and G.W. Jones. 2014. Global transcript and phenotypic analysis of yeast cells expressing Ssa1, Ssa2, Ssa3 or Ssa4 as sole source of cytosolic Hsp70-Ssa chaperone activity. *BMC Genomics*. 15:194.

Haslbeck, M., N. Braun, T. Stromer, B. Richter, N. Model, S. Weinkauf, and J. Buchner. 2004. Hsp42 is the general small heat shock protein in the cytosol of Saccharomyces cerevisiae. *Embo j*. 23:638-649.

Haslbeck, M., S. Walke, T. Stromer, M. Ehrnsperger, H.E. White, S. Chen, H.R. Saibil, and J. Buchner. 1999. Hsp26: a temperature-regulated chaperone. *Embo j*. 18:6744-6751.

Haugen, A.C., R. Kelley, J.B. Collins, C.J. Tucker, C. Deng, C.A. Afshari, J.M. Brown, T. Ideker, and B.V. Houten. 2004. Integrating phenotypic and expression profiles to map arsenic-response networks. *Genome Biology*. 5:R95.91-R95.18.

Hettick, B.E., J.E. Cañas-Carrell, A.D. French, and D.M. Klein. 2015. Arsenic: A Review of the Element's Toxicity, Plant Interactions, and Potential Methods of Remediation. *Journal of Agricultural and Food Chemistry*. 63:7097-7107.

Hicke, L. 2001. Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol*. 2:195-201.

Hill, S.M., S. Hanzén, and T. Nyström. 2017. Restricted access: spatial sequestration of damaged proteins during stress and aging. *EMBO Reports*. 18:377-391.

Hinault, M.P., A. Farina-Henriquez-Cuendet, and P. Goloubinoff. 2011. Molecular chaperones and associated cellular clearance mechanisms against toxic protein conformers in Parkinson's disease. *Neurodegener Dis*. 8:397-412.

Hinnebusch, A.G. 2011. Molecular Mechanism of Scanning and Start Codon Selection in Eukaryotes. *Microbiology and Molecular Biology Reviews*. 75:434–467.

Hipp, M.S., S.-H. Park, and F.U. Hartl. 2014. Proteostasis impairment in protein misfolding and aggregation diseases. *Trends in Cell Biology*. 24:506-514.

Hoffman, R.D., and M.D. Lan. 1992. Iodophenylarsine oxide and arsenical affinity chromatography: New probes for dithiol proteins. *The Journal of Biological Chemistry*. 267:14005-14011.

Hoonjan, M., V. Jadhav, and P. Bhatt. 2018. Arsenic trioxide: insights into its evolution to an anticancer agent. *Journal of Biological Inorganic Chemistry* 23:313-329.

Houck, S.A., H.Y. Ren, V.J. Madden, J.N. Bonner, M.P. Conlin, J.A. Janovick, P.M. Conn, and D.M. Cyr. 2014. Quality control autophagy degrades soluble ERAD-resistant conformers of the misfolded membrane protein GnRHR. *Mol Cell*. 54:166-179.

Huang, P., M. Gautschi, W. Walter, S. Rospert, and E.A. Craig. 2005. The Hsp70 Ssz1 modulates the function of the ribosomeassociated J-protein Zuo1. *Nature Structural & Molecular Biology*. 12:497-504.

Hundley, H., H. Eisenman, W. Walter, T. Evans, Y. Hotokezaka, M. Wiedmann, and E. Craig. 2002. The *in vivo* function of the ribosome-associated Hsp70, Ssz1, does not require its putative peptide-binding domain. *PNAS*. 99:4203–4208.

Ibstedt, S., T.C. Sideri, C.M. Grant, and M.J. Tamás. 2014. Global analysis of protein aggregation in yeast during physiological conditions and arsenite stress. *Biology Open*. 3:913–923

Ikeuchi, K., P. Tesina, Y. Matsuo, T. Sugiyama, J. Cheng, YasushiSaeki, K. Tanaka, T. Becker, R. Beckmann, and T. Inada. 2019. Collided ribosomes form a unique structural interface to induce Hel2-driven quality control pathways. *The EMBO Journal*. 38:e100276.

Ito-Harashima, S., K. Kuroha, T. Tatematsu, and T. Inada. 2007. Translation of the poly(A) tail plays crucial roles in nonstop mRNA surveillance via translation repression and protein destabilization by proteasome in yeast. *Genes & Development*. 21:519-524.

Izquierdo-Vega, J.A., C.A. Soto, L.C. Sanchez-Peña, A. De Vizcaya-Ruiz, and L.M. Del Razo. 2006. Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite. *Toxicol Lett*. 160:135-142.

Jackson, R.J., C.U.T. Hellen, and T.V. Pestova. 2010. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol*. 11:137-127.

Jacobson, T., C. Navarrete, S.K. Sharma, T.C. Sideri, S. Ibstedt, S. Priya, C.M. Grant, P. Christen, P. Goloubinoff, and M.J. Tamás. 2012. Arsenite interferes with protein folding and triggers formation of protein aggregates in yeast. *Journal of Cell Science*. 125:5073-5083.

Jakob, U., M. Gaestel, K. Engel, and J. Buchner. 1993. Small heat shock proteins are molecular chaperones. *J Biol Chem*. 268:1517-1520.

Jakob, U., H. Lilie, I. Meyer, and J. Buchner. 1995. Transient interaction of Hsp90 with early unfolding intermediates of citrate synthase. Implications for heat shock in vivo. *J Biol Chem*. 270:7288-7294.

Janowska, M.K., H.E.R. Baughman, C.N. Woods, and R.E. Klevit. 2019. Mechanisms of Small Heat Shock Proteins. *Cold Spring Harb Perspect Biol*. 11.

Jin, Y.H., P.E. Dunlap, S.J. McBride, H. Al-Refai, P.R. Bushel, and J.H. Freedman. 2008. Global transcriptome and deletome profiles of yeast exposed to transition metals. *PLoS Genet*. 4:e1000053.

Johnson, J.L., and D.O. Toft. 1995. Binding of p23 and hsp90 during assembly with the progesterone receptor. *Mol Endocrinol*. 9:670-678.

Joseph, T., B. Dubey, and E.A. McBean. 2015. A critical review of arsenic exposures for Bangladeshi adults. *Sci Total Environ*. 527-528:540-551.

Jung, G., G. Jones, R.D. Wegrzyn, and D.C. Masison. 2000. A Role for Cytosolic Hsp70 in Yeast [PSI+] Prion Propagation and [PSI+] as a Cellular Stress. *Genetics*. 156:559–570

Kadono, T., T. Inaoka, N. Murayama, K. Ushijima, M. Nagano, S. Nakamura, C. Watanabe, K. Tamaki, and R. Ohtsuka. 2002. Skin manifestations of arsenicosis in two villages of Bangladesh. *International Journal of Dermatology*. 41:841-846.

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

Kaler, S., P. Dhar, A. Bhattacharya, and R.D. Mehra. 2013. Preliminary morphological and immunohistochemical changes in rat hippocampus following postnatal exposure to sodium arsenite. *Toxicol Int*. 20:160-169.

Kandasamy, G., A.K. Pradhan, and R. Palanimurugan. 2021. Ccr4-Not complex subunits Ccr4, Caf1, and Not4 are novel proteolysis factors promoting the degradation of ubiquitin-dependent substrates by the 26S proteasome. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 1868:1-15.

Kanki, T., and D.J. Klionsky. 2008. Mitophagy in yeast occurs through a selective mechanism. *J Biol Chem*. 283:32386-32393.

Kedersha, N., G. Stoecklin, M. Ayodele, P. Yacono, J. Lykke-Andersen, M.J. Fritzler, D. Scheuner, R.J. Kaufman, D.E. Golan, and P. Anderson. 2005. Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J Cell Biol*. 169:871-884.

Kedersha, N.L., M. Gupta, W. Li, I. Miller, and P. Anderson. 1999. RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. *J Cell Biol*. 147:1431-1442.

Kim, S., B. Schilke, E.A. Craig, and A.L. Horwich. 1998. Folding in vivo of a newly translated yeast cytosolic enzyme is mediated by the SSA class of cytosolic yeast Hsp70 proteins. *PNAS*. 95:12860-12865.

Kirstein-Miles, J., A. Scior, E. Deuerling, and R.I. Morimoto. 2013. The nascent polypeptide-associated complex is a key regulator of proteostasis. *The EMBO Journal*. 32:1451–1468.

Kitchin, K.T., and K. Wallace. 2008. The role of protein binding of trivalent arsenicals in arsenic carcinogenesis and toxicity. *Journal of Inorganic Biochemistry*. 102:532-539.

Koegl, M., T. Hoppe, S. Schlenker, H.D. Ulrich, T.U. Mayer, and S. Jentsch. 1999. A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell*. 96:635-644.

Komar, A.A. 2018. Unraveling co-translational protein folding: concepts and methods. *Methods*. 137:71-81.

Koplin, A., S. Preissler, Y. Ilina, M. Koch, A. Scior, M. Erhardt, and E. Deuerling. 2010. A dual function for chaperones SSB–RAC and the NAC nascent polypeptide–associated complex on ribosomes. *The Journal of Cell Biology*. 189:57–68.

Kramer, G., D. Boehringer, N. Ban, and B. Bukau. 2009. The ribosome as a platform for co-translational processing, folding and targeting of newly synthesized proteins. *Nature Structural & Molecular Biology*. 16:589-597.

Krebs, M.R., G.L. Devlin, and A.M. Donald. 2007. Protein particulates: another generic form of protein aggregation? *Biophys J*. 92:1336-1342.

Kumar, N.V., J. Yang, J.K. Pillai, S. Rawat, C. Solano, A. Kumar, M. Grøtli, T.L. Stemmler, B.P. Rosen, and M.J. Tamás. 2015. Arsenic Directly Binds to and Activates the Yeast AP-1-Like Transcription Factor Yap8. *Mol Cell Biol*. 36:913-922.

Lallemand-Breitenbach, V., M. Jeanne, S. Benhenda, R. Nasr, M. Lei, L. Peres, J. Zhou, J. Zhu, B. Raught, and H. de Thé. 2008. Arsenic degrades PML or PML-RARalpha through a SUMO-triggered RNF4/ubiquitin-mediated pathway. *Nat Cell Biol*. 10:547-555.

Lauring, B., G. Kreibich, and M. Wiedmann. 1995a. The intrinsic ability of ribosomes to bind to endoplasmic reticulum membranes is regulated by signal recognition particle and nascent-polypeptide-associated complex. *PNAS*. 92:9435-9439.

Lauring, B., H. Sakai, G. Kreibich, and M. Wiedmann. 1995b. Nascent polypeptide-associated complex protein prevents mistargeting of nascent chains to the endoplasmic reticulum. *PNAS*. 92:5411-5415.

Lee-Chen, S.F., J.R. Gurr, I.B. Lin, and K.Y. Jan. 1993. Arsenite enhances DNA double-strand breaks and cell killing of methyl methanesulfonate-treated cells by inhibiting the excision of alkali-labile sites. *Mutat Res*. 294:21-28.

Lee do, H., M.Y. Sherman, and A.L. Goldberg. 2016. The requirements of yeast Hsp70 of SSA family for the ubiquitin-dependent degradation of short-lived and abnormal proteins. *Biochem Biophys Res Commun*. 475:100-106.

Lee, J., and D.E. Levin. 2018. Intracellular mechanism by which arsenite activates the yeast stress MAPK Hog1. *Mol Biol Cell*. 29:1904-1915.

Lee, J., and D.E. Levin. 2022. Differential metabolism of arsenicals regulates Fps1-mediated arsenite transport. *J Cell Biol*. 221.

Lee, M.Y., Y.H. Lee, K.M. Lim, S.M. Chung, O.N. Bae, H. Kim, C.R. Lee, J.D. Park, and J.H. Chung. 2005. Inorganic arsenite potentiates vasoconstriction through calcium sensitization in vascular smooth muscle. *Environ Health Perspect*. 113:1330-1335.

Lee, S., M.E. Sowa, Y.H. Watanabe, P.B. Sigler, W. Chiu, M. Yoshida, and F.T. Tsai. 2003. The structure of ClpB: a molecular chaperone that rescues proteins from an aggregated state. *Cell*. 115:229-240.

Leidig, C., G. Bange, J. Kopp, S. Amlacher, A. Aravind, S. Wickles, G. Witte, E. Hurt, R. Beckmann, and I. Sinning. 2013. Structural characterization of a eukaryotic chaperone - the ribosome-associated complex. *Nature Structural & Molecular Biology*. 20:23-30.

Lentini, P., L. Zanoli, A. Granata, S.S. Signorelli, P. Castellino, and R. Dell'Aquila. 2017. Kidney and heavy metals - The role of environmental exposure (Review). *Molecular Medicine Reports*. 15:3413-3419.

Lewis, M.J., and H.R.B. Pelham. 1985. Involvement of ATP in the nuclear and nucleolar functions of the 70 kd heat shock protein. *The EMBO Journal*. 4:3137-3143.

Li, J.-H., and T.G. Rossman. 1989. Mechanism of comutagenesis of sodium arsenite with n-methyl-n-nitrosourea. *Biological Trace Element Research*. 21:373-381.

Li, Z.-S., M. Szczypka, Y.-P. Lu, D.J. Thiele, and P.A. Rea. 1996. The Yeast Cadmium Factor Protein (YCF1) Is a Vacuolar Glutathione S-Conjugate Pump. *The Journal Of Biological Chemistry*. 27:6509–6517.

Lin, A.M., P.L. Chao, S.F. Fang, C.W. Chi, and C.H. Yang. 2007. Endoplasmic reticulum stress is involved in arsenite-induced oxidative injury in rat brain. *Toxicol Appl Pharmacol*. 224:138-146.

Litwin, I., T. Bocer, D. Dziadkowiec, and R. Wysocki. 2013. Oxidative stress and replication-independent DNA breakage induced by arsenic in Saccharomyces cerevisiae. *PLoS Genet*. 9:e1003640.

Liu, S.X., M. Athar, I. Lippai, C. Waldren, and T.K. Hei. 2001. Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. *PNAS*. 98:1643-1648.

Liu, Z., E. Boles, and B.P. Rosen. 2004. Arsenic trioxide uptake by hexose permeases in *Saccharomyces cerevisiae*. *The Journal of Biological Chemistry*. 279:17312–17318.

Lorentzon, E., I. Horvath, R. Kumar, J.I. Rodrigues, M.J. Tamás, and P. Wittung-Stafshede. 2021. Effects of the Toxic Metals Arsenite and Cadmium on α-Synuclein Aggregation In Vitro and in Cells. *International Journal of Molecular Sciences*. 22.

Lu, J., E.H. Chew, and A. Holmgren. 2007. Targeting thioredoxin reductase is a basis for cancer therapy by arsenic trioxide. *Proc Natl Acad Sci U S A*. 104:12288-12293.

Lu, S.C. 2013. Glutathione synthesis. *Biochim Biophys Acta*. 1830:3143-3153.

Lum, R., J.M. Tkach, E. Vierling, and J.R. Glover. 2004. Evidence for an unfolding/threading mechanism for protein disaggregation by Saccharomyces cerevisiae Hsp104. *J Biol Chem*. 279:29139-29146.

Lynn, S., J.-R. Gurr, H.-T. Lai, and K.-Y. Jan. 2000. NADH oxidase activation is involved in arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. *Circulation Research*. 86:514–519.

Lynn, S., H.-T. Lai, J.-R. Gurr, and K-YJan. 1997. Arsenite retards DNA break rejoining by inhibiting DNA ligation. *Mutagenesis*. 12:353-358.

Lynn, S., J.N. Shiung, J.R. Gurr, and K.Y. Jan. 1998. Arsenite stimulates poly(ADP-ribosylation) by generation of nitric oxide. *Free Radic Biol Med*. 24:442-449.

Maciaszczyk-Dziubinska, E., I. Migdal, M. Migocka, T. Bocer, and R. Wysocki. 2010. The yeast aquaglyceroporin Fps1p is a bidirectional arsenite channel. *FEBS Letters*. 584:726–732.

Maciaszczyk-Dziubinska, E., M. Migocka, and R. Wysocki. 2011. Acr3p is a plasma membrane antiporter that catalyzes As(III)/H(+) and Sb(III)/H(+) exchange in Saccharomyces cerevisiae. *Biochim Biophys Acta*. 1808:1855-1859.

Mandal, B.K., and K.T. Suzuki. 2002. Arsenic round the world: a review. *Talanta*. 58:201-235.

Markmiller, S., A. Fulzele, R. Higgins, M. Leonard, G.W. Yeo, and E.J. Bennett. 2019. Active Protein Neddylation or Ubiquitylation Is Dispensable for Stress Granule Dynamics. *Cell Rep*. 27:1356-1363.e1353.

Martin, E.M., M.P. Jackson, M. Gamerdinger, K. Gense, T.K. Karamonos, J.R. Humes, E. Deuerling, A.E. Ashcroft, and S.E. Radford. 2018. Conformational flexibility within the nascent polypeptide– associated complex enables its interactions with structurally diverse client proteins. *Journal of Biological Chemistry*. 293:8554-8568.

Martin, J., M. Mayhew, T. Langer, and F.U. Hartl. 1993. The reaction cycle of GroEL and GroES in chaperonin-assisted protein folding. *Nature*. 366:228-233.

Martinez, V.D., E.A. Vucic, D.D. Becker-Santos, L. Gil, and W.L. Lam. 2011. Arsenic Exposure and the Induction ofHuman Cancers. *Journal of Toxicology*. 2011:1-13.

Mateju, D., T.M. Franzmann, A. Patel, A. Kopach, E.E. Boczek, S. Maharana, H.O. Lee, S. Carra, A.A. Hyman, and S. Alberti. 2017. An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *Embo j*. 36:1669-1687.

Matsuo, Y., K. Ikeuchi, Y. Saeki, S. Iwasaki, C. Schmidt, T. Udagawa, F. Sato, H. Tsuchiya, T. Becker, K. Tanaka, N.T. Ingolia, R. Beckmann, and T. Inada. 2017. Ubiquitination of stalled ribosome triggers ribosome-associated quality control. *Nature Communications*. 8:1-14.

Mattiazzi, M., U. Petrovic, and I. Krizaj. 2012. Yeast as a model eukaryote in toxinology: A functional genomics approach to studying the molecular basis of action of pharmacologically active molecules. *Toxicon*. 60:558–571.

McCarty, J.S., A. Buchberger, J. Reinstein, and B. Bukau. 1995. The Role of ATP in the Functional Cycle of the DnaK Chaperone System. *Journal of Molecular Biology*. 249:126-137.

McClellan, A.J., Y. Xia, A.M. Deutschbauer, R.W. Davis, M. Gerstein, and J. Frydman. 2007. Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. *Cell*. 131:121-135.

Melville, M.W., A.J. McClellan, A.S. Meyer, A. Darveau, and J. Frydman. 2003. The Hsp70 and TRiC/CCT chaperone systems cooperate in vivo to assemble the von Hippel-Lindau tumor suppressor complex. *Mol Cell Biol*. 23:3141-3151.

Menezes, R.A., C. Amaral, L. Batista-Nascimento, C. Santos, R.B. Ferreira, F. Devaux, E.C.A. Eleutherio, and C. Rodrigues-Pousada. 2008. Contribution of Yap1 towards *Saccharomyces cerevisiae* adaptation

to arsenic-mediated oxidative stress. *Biochemistry Journal*. 414:301-311.

Menezes, R.A., C. Amaral, A. Delaunay, M. Toledano, and C. Rodrigues-Pousada. 2004. Yap8p activation in Saccharomyces cerevisiae under arsenic conditions. *FEBS Letters*. 566:Yap8p activation in Saccharomyces cerevisiae under arsenic conditions.

Menezes, R.A., S. Tenreiro, D. Macedo, C.N. Santos, and T.F. Outeiro. 2015. From the baker to the bedside: yeast models of Parkinson's disease. *Microbial Cell*. 2:262-279.

Menzel, D.B., H.K. Hamadeh, V. Said, R.E. Rasmussen, H. Greene, E. Lee, D.M. Meacher, and R.N. Roth. 1999. Arsenic binding proteins from human lymphoblastoid cells. *Toxicology Letters*. 105:89–101.

Migdal, I., Y. Ilina, M.J. Tamás, and R. Wysocki. 2008. Mitogen-Activated Protein Kinase Hog1 Mediates Adaptation to G1 Checkpoint Arrest during Arsenite and Hyperosmotic Stress. *Eukaryotic Cell*. 7:1309–1317.

Miller, S.B.M., C.-T. Ho, J. Winkler, M. Khokhrina, A. Neuner, M.Y.H. Mohamed, D.L. Guilbride, K. Richter, M. Lisby, E. Schiebel, A. Mogk, and B. Bukau. 2015a. Compartment-specific aggregases direct distinct nuclear and cytoplasmic aggregate deposition. *The EMBO Journal*. 34:778-797.

Miller, S.B.M., A. Mogk, and B. Bukau. 2015b. Spatially Organized Aggregation of Misfolded Proteins as Cellular Stress Defense Strategy. *Journal of Molecular Biology*. 427.

Miyata, Y., and I. Yahara. 1992. The 90-kDa heat shock protein, HSP90, binds and protects casein kinase II from self-aggregation and enhances its kinase activity. *J Biol Chem*. 267:7042-7047.

Modler, A.J., K. Gast, G. Lutsch, and G. Damaschun. 2003. Assembly of amyloid protofibrils via critical oligomers--a novel pathway of amyloid formation. *J Mol Biol*. 325:135-148.

Mollet, S., N. Cougot, A. Wilczynska, F. Dautry, M. Kress, E. Bertrand, and D. Weil. 2008. Translationally repressed mRNA transiently cycles through stress granules during stress. *Mol Biol Cell*. 19:4469-4479.

Mukhopadhyay, R., and B.P. Rosen. 1998. *Saccharomyces cerevisiae ACR2* gene encodes an arsenate reductase. *FEMS Microbiology Letters*. 168:127^136.

Müller, O., T. Sattler, M. Flötenmeyer, H. Schwarz, H. Plattner, and A. Mayer. 2000. Autophagic tubes: vacuolar invaginations involved in lateral membrane sorting and inverse vesicle budding. *J Cell Biol*. 151:519-528.

Murakami, T., S. Qamar, J.Q. Lin, G.S. Schierle, E. Rees, A. Miyashita, A.R. Costa, R.B. Dodd, F.T. Chan, C.H. Michel, D. Kronenberg-Versteeg, Y. Li, S.P. Yang, Y. Wakutani, W. Meadows, R.R. Ferry, L. Dong, G.G. Tartaglia, G. Favrin, W.L. Lin, D.W. Dickson, M. Zhen, D. Ron, G. Schmitt-Ulms, P.E. Fraser, N.A. Shneider, C. Holt, M. Vendruscolo, C.F. Kaminski, and P. St George-Hyslop. 2015. ALS/FTD Mutation-Induced Phase Transition of FUS Liquid Droplets and Reversible Hydrogels into Irreversible Hydrogels Impairs RNP Granule Function. *Neuron*. 88:678-690.

Mymrikov, E.V., M. Daake, B. Richter, M. Haslbeck, and J. Buchner. 2017. The Chaperone Activity and Substrate Spectrum of Human Small Heat Shock Proteins. *J Biol Chem*. 292:672-684.

Nakamuro, K., and Y. Sayato. 1981. Comparative studies of chromosomal aberration induced by trivalent and pentavalent arsenic. *Mutation Research*. 88:73--80.

Namgung, U., and Z. Xia. 2001. Arsenic induces apoptosis in rat cerebellar neurons via activation of JNK3 and p38 MAP kinases. *Toxicol Appl Pharmacol*. 174:130-138.

Nedelsky, N.B., and J.P. Taylor. 2019. Bridging biophysics and neurology: aberrant phase transitions in neurodegenerative disease. *Nat Rev Neurol*. 15:272-286.

Nelson, R.J., T. Ziegelhoffer, C. Nicolet, M. Werner-Washburne, and E.A. Craig. 1992. The Translation Machinery and 70 kd Heat Shock Protein cooperate in Protein Synthesis. *Cell*. 71:97-105.

Ngosuwan, J., N.M. Wang, K.L. Fung, and W.J. Chirico. 2003. Roles of cytosolic Hsp70 and Hsp40 molecular chaperones in post-translational translocation of presecretory proteins into the endoplasmic reticulum. *J Biol Chem*. 278:7034-7042.

Nishida, E., S. Koyasu, H. Sakai, and I. Yahara. 1986. Calmodulin-regulated binding of the 90-kDa heat shock protein to actin filaments. *J Biol Chem*. 261:16033-16036.

Nollen, E.A., S.M. Garcia, G. van Haaften, S. Kim, A. Chavez, R.I. Morimoto, and R.H. Plasterk. 2004. Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. *Proc Natl Acad Sci U S A*. 101:6403-6408.

O'Bryant, S.E., M. Edwards, C.V. Menon, G. Gong, and R. Barber. 2011. Long-term low-level arsenic exposure is associated with poorer neuropsychological functioning: a Project FRONTIER study. *Int J Environ Res Public Health*. 8:861-874.

Ohtake, F., H. Tsuchiya, Y. Saeki, and K. Tanaka. 2018. K63 ubiquitylation triggers proteasomal degradation by seeding branched ubiquitin chains. *Proc Natl Acad Sci U S A*. 115:E1401-e1408.

Oppermann, H., W. Levinson, and J.M. Bishop. 1981. A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat-shock protein. *Proc Natl Acad Sci U S A*. 78:1067-1071.

Osuna, B.A., C.J. Howard, S. Kc, A. Frost, and D.E. Weinberg. 2017. In vitro analysis of RQC activities provides insights into the mechanism and function of CAT tailing. *Elife*. 6.

Pan, X., S. Reissman, N.R. Douglas, Z. Huang, D.S. Yuan, X. Wang, J.M. McCaffery, Z. Huang, J. Frydman, and J.D. Boeke. 2010. Trivalent Arsenic Inhibits the Functions of Chaperonin Complex. *Genetics*. 186:725–734

Panasenko, O.O., and M.A. Collart. 2011. Not4 E3 Ligase Contributes to Proteasome Assembly and Functional Integrity in Part through Ecm29. *Molecular and Cellular Biology*. 31:1610–1623.

Panasenko, O.O., and M.A. Collart. 2012. Presence of Not5 and ubiquitinated Rps7A in polysome fractions depends upon the Not4 E3 ligase. *Molecular Microbiology*. 83:640–653.

Park, S., C. Zuber, and J. Roth. 2020. Selective autophagy of cytosolic protein aggregates involves ribosome-free rough endoplasmic reticulum. *Histochem Cell Biol*. 153:89-99.

Park, S.H., N. Bolender, F. Eisele, Z. Kostova, J. Takeuchi, P. Coffino, and D.H. Wolf. 2007. The cytoplasmic Hsp70 chaperone machinery subjects misfolded and endoplasmic reticulum import-incompetent proteins to degradation via the ubiquitin-proteasome system. *Mol Biol Cell*. 18:153-165.

Parsell, D.A., A.S. Kowal, and S. Lindquist. 1994a. Saccharomyces cerevisiae Hsp104 protein. Purification and characterization of ATP-induced structural changes. *J Biol Chem*. 269:4480-4487.

Parsell, D.A., A.S. Kowal, M.A. Singer, and S. Lindquist. 1994b. Protein disaggregation mediated by heat-shock protein Hsp104. *Nature*. 372:475-478.

Parsell, D.A., Y. Sanchez, J.D. Stitzel, and S. Lindquist. 1991. Hsp104 is a highly conserved protein with two essential nucleotide-binding sites. *Nature*. 353:270-273.

Patel, A., H.O. Lee, L. Jawerth, S. Maharana, M. Jahnel, M.Y. Hein, S. Stoynov, J. Mahamid, S. Saha, T.M. Franzmann, A. Pozniakovski, I. Poser, N. Maghelli, L.A. Royer, M. Weigert, E.W. Myers, S. Grill, D. Drechsel, A.A. Hyman, and S. Alberti. 2015. A Liquid-to-Solid Phase Transition

of the ALS Protein FUS Accelerated by Disease Mutation. *Cell*. 162:1066-1077.

Paul, D.S., A.W. Harmon, V. Devesa, D.J. Thomas, and M. Stýblo. 2007. Molecular mechanisms of the diabetogenic effects of arsenic: inhibition of insulin signaling by arsenite and methylarsonous acid. *Environ Health Perspect*. 115:734-742.

Paulovich, A.G., and L.H. Hartwell. 1995. A checkpoint regulates the rate of progression through S phase in *S. cerevisiae* in response to DNA damage. *Cell*. 82:841-847.

Peskett, T.R., F. Rau, J. O'Driscoll, R. Patani, A.R. Lowe, and H.R. Saibil. 2018. A Liquid to Solid Phase Transition Underlying Pathological Huntingtin Exon1 Aggregation. *Mol Cell*. 70:588-601.e586.

Pestova, T.V., V.G. Kolupaeva, I.B. Lomakin, E.V. Pilipenko, I.N. Shatsky, V.I. Agol, and C.U.T. Hellen. 2001. Molecular mechanisms of translation initiation in eukaryotes. *PNAS*. 98:7029–7036.

Pfund, C., N. Lopez-Hoyo, T. Ziegelhoffer, B.A. Schilke, P. Lopez-Buesa, W. A.Walter, M. Wiedmann, and E. A.Craig. 1998. The molecular chaperone Ssb from Saccharomyces cerevisiae is a component of the ribosome–nascent chain complex. *The EMBO Journal*. 17:3981–3989.

Pompella, A., A. Visvikis, A. Paolicchi, V. De Tata, and A.F. Casini. 2003. The changing faces of glutathione, a cellular protagonist. *Biochem Pharmacol*. 66:1499-1503.

Powers, T., and P. Walter. 1996. The nascent polypeptide-associated complex modulates interactions between the signal recognition particle and the ribosome. *Current Biology*. 6:331–338.

Prakash, C., M. Soni, and V. Kumar. 2016. Mitochondrial oxidative stress and dysfunction in arsenic neurotoxicity: A review. *J Appl Toxicol*. 36:179-188.

Prakash, S., T. Inobe, A.J. Hatch, and A. Matouschek. 2009. Substrate selection by the proteasome during degradation of protein complexes. *Nat Chem Biol*. 5:29-36.

Prakash, S., L. Tian, K.S. Ratliff, R.E. Lehotzky, and A. Matouschek. 2004. An unstructured initiation site is required for efficient proteasome-mediated degradation. *Nat Struct Mol Biol*. 11:830-837.

Preissler, S., J. Reuther, M. Koch, A. Scior, M. Bruderek, T. Frickey, and E. Deuerling. 2015. Not4-dependent translational repression is important for cellular protein homeostasis in yeast. *The EMBO Journal*. 34:1905–1924.

Pursell, N.W., P. Mishra, and D.N. Bolon. 2012. Solubility-promoting function of Hsp90 contributes to client maturation and robust cell growth. *Eukaryot Cell*. 11:1033-1041.

Rabl, J., D.M. Smith, Y. Yu, S.C. Chang, A.L. Goldberg, and Y. Cheng. 2008. Mechanism of gate opening in the 20S proteasome by the proteasomal ATPases. *Mol Cell*. 30:360-368.

Ramadan, D., P.C. Rancy, R.P. Nagarkar, J.P. Schneider, and C. Thorpe. 2009. Arsenic(III) Species Inhibit Oxidative Protein Folding in Vitro. *Biochemistry*. 48:424–432.

Rehman, K., F. Fatima, I. Waheed, and M.S.H. Akash. 2018. Prevalence of exposure of heavy metals and their impact on health consequences. *J Cell Biochem*. 119:157-184.

Reimann, B., J. Bradsher, J. Franke, E. Hartmann, M. Wiedmann, S. Prehn, and B. Wiedmann. 1999. Initial Characterization of the Nascent Polypeptide-Associated Complex in Yeast. *Yeast*. 15:397-407.

Roberts, B.T., H. Moriyama, and R.B. Wickner. 2004. [URE3] prion propagation is abolished by a mutation of the primary cytosolic Hsp70 of budding yeast. *Yeast*. 21:107-117.

Roberts, P., S. Moshitch-Moshkovitz, E. Kvam, E. O'Toole, M. Winey, and D.S. Goldfarb. 2003. Piecemeal microautophagy of nucleus in Saccharomyces cerevisiae. *Mol Biol Cell*. 14:129-141.

Rosado, J.L., D. Ronquillo, K. Kordas, O. Rojas, J. Alatorre, P. Lopez, G. Garcia-Vargas, M. Del Carmen Caamaño, M.E. Cebrián, and R.J. Stoltzfus. 2007. Arsenic exposure and cognitive performance in Mexican schoolchildren. *Environ Health Perspect*. 115:1371-1375.

Rosen, B.P. 2002. Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. *Comp Biochem Physiol A Mol Integr Physiol*. 133:689-693.

Ruggiano, A., O. Foresti, and P. Carvalho. 2014. Quality control: ER-associated degradation: protein quality control and beyond. *J Cell Biol*. 204:869-879.

Sakai, Y., A. Koller, L.K. Rangell, G.A. Keller, and S. Subramani. 1998. Peroxisome degradation by microautophagy in Pichia pastoris: identification of specific steps and morphological intermediates. *J Cell Biol*. 141:625-636.

Samikkannu, T., C.H. Chen, L.H. Yih, A.S. Wang, S.Y. Lin, T.C. Chen, and K.Y. Jan. 2003. Reactive oxygen species are involved in arsenic trioxide inhibition of pyruvate dehydrogenase activity. *Chem Res Toxicol*. 16:409-414.

Samokhvalov, V.A., N.Y. Museikina, G.V. Mel'nikov, and V.V. Ignatov. 2003. Arsenite as an inducer of lipid peroxidation in *Saccharomyces cerevisiae* cells. *Microbiology*. 72:266–269.

Sanchez, E.R., S. Meshinchi, M.J. Schlesinger, and W.B. Pratt. 1987. Demonstration that the 90-kilodalton heat shock protein is bound to the glucocorticoid receptor in its 9S nondeoxynucleic acid binding form. *Mol Endocrinol*. 1:908-912.

Sanchez, E.R., T. Redmond, L.C. Scherrer, E.H. Bresnick, M.J. Welsh, and W.B. Pratt. 1988. Evidence that the 90-kilodalton heat shock protein is associated with tubulin-containing complexes in L cell cytosol and in intact PtK cells. *Mol Endocrinol*. 2:756-760.

Sanchez, Y., and S.L. Lindquist. 1990. HSP104 required for induced thermotolerance. *Science*. 248:1112-1115.

Sanchez, Y., J. Taulien, K.A. Borkovich, and S. Lindquist. 1992. Hsp104 is required for tolerance to many forms of stress. *Embo j*. 11:2357-2364.

Schmeing, T.M., and V. Ramakrishnan. 2009. What recent ribosome structures have revealed about the mechanism of translation. *Nature Reviews*. 461:1234-1242.

Schneider, C., L. Sepp-Lorenzino, E. Nimmesgern, O. Ouerfelli, S. Danishefsky, N. Rosen, and F.U. Hartl. 1996. Pharmacologic shifting of a balance between protein refolding and degradation mediated by Hsp90. *Proc Natl Acad Sci U S A*. 93:14536-14541.

Shah, D., M.W.Y. Shen, W. Chen, and N.A.D. Silva. 2010. Enhanced arsenic accumulation in *Saccharomyces cerevisiae* overexpressing transporters Fps1p or Hxt7p. *Journal of Biotechnology*. 150:101-107.

Shahmoradian, S.H., J.G. Galaz-Montoya, M.F. Schmid, Y. Cong, B. Ma, C. Spiess, J. Frydman, S.J. Ludtke, and W. Chiu. 2013. TRiC's tricks inhibit huntingtin aggregation. *Elife*. 2:e00710.

Shaknovich, R., G. Shue, and D.S. Kohtz. 1992. Conformational activation of a basic helix-loop-helix protein (MyoD1) by the C-terminal region of murine HSP90 (HSP84). *Mol Cell Biol*. 12:5059-5068.

Shen, M.W., D. Shah, W. Chen, and N. Da Silva. 2012. Enhanced arsenate uptake in Saccharomyces cerevisiae overexpressing the Pho84 phosphate transporter. *Biotechnol Prog*. 28:654-661.

Shen, Z.X., G.Q. Chen, J.H. Ni, X.S. Li, S.M. Xiong, Q.Y. Qiu, J. Zhu, W. Tang, G.L. Sun, K.Q. Yang, Y. Chen, L. Zhou, Z.W. Fang, Y.T. Wang, J. Ma, P. Zhang, T.D. Zhang, S.J. Chen, Z. Chen, and Z.Y. Wang. 1997. Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood*. 89:3354-3360.

Sheth, U., and R. Parker. 2003. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science*. 300:805-808.

Shi, H., X. Shi, and K.J. Liu. 2004. Oxidative mechanism of arsenic toxicity and carcinogenesis. *Molecular and Cellular Biochemistry*. 255:67-78.

Shila, S., V. Kokilavani, M. Subathra, and C. Panneerselvam. 2005. Brain regional responses in antioxidant system to alpha-lipoic acid in arsenic intoxicated rat. *Toxicology*. 210:25-36.

Shoemaker, C.J., D.E. Eyler, and R. Green. 2010. Dom34:Hbs1 Promotes Subunit Dissociation and Peptidyl-tRNA Drop-Off to Initiate No-Go Decay. *Science*. 330:369-372.

Shoemaker, C.J., and R. Green. 2011. Kinetic analysis reveals the ordered coupling of translation termination and ribosome recycling in yeast. *PNAS*. 108:E1392–E1398.

Shue, G., and D.S. Kohtz. 1994. Structural and functional aspects of basic helix-loop-helix protein folding by heat-shock protein 90. *J Biol Chem*. 269:2707-2711.

Siegers, K., T. Waldmann, M.R. Leroux, K. Grein, A. Shevchenko, E. Schiebel, and F.U. Hartl. 1999. Compartmentation of protein folding in vivo: sequestration of non-native polypeptide by the chaperonin-GimC system. *Embo j*. 18:75-84.

Singh, A.P., R.K. Goel, and T. Kaur. 2011. Mechanisms pertaining to arsenic toxicity. *Toxicol Int*. 18:87-93.

Sitron, C.S., and O. Brandman. 2019. CAT tails drive degradation of stalled polypeptides on and off the ribosome. *Nature - Structure and Molecular Biology*. 26:450-459.

Smedley, P.L., and D.G. Kinniburgh. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry*. 17:517-568.

Smith, A.H., E.O. Lingas, and M. Rahman. 2000. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull World Health Organ*. 78:1093-1103.

Smith, D.M., S.C. Chang, S. Park, D. Finley, Y. Cheng, and A.L. Goldberg. 2007. Docking of the proteasomal ATPases' carboxyl termini in the 20S proteasome's alpha ring opens the gate for substrate entry. *Mol Cell*. 27:731-744.

Smith, M.I., V. Foderà, J.S. Sharp, C.J. Roberts, and A.M. Donald. 2012. Factors affecting the formation of insulin amyloid spherulites. *Colloids Surf B Biointerfaces*. 89:216-222.

Soignet, S.L., P. Maslak, Z.-G. Wang, S. Jhanwar, E. Calleja, L.J. Dardashti, D. Corso, A. DeBlasio, J. Gabrilove, D.A. Scheinberg, P.P. Pandolfi, and

R.P. Warrell. 1998. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *The New England Journal of Medicine*. 339:1341-1348.

Sot, B., A. Rubio-Muñoz, A. Leal-Quintero, J. Martínez-Sabando, M. Marcilla, C. Roodveldt, and J.M. Valpuesta. 2017. The chaperonin CCT inhibits assembly of α-synuclein amyloid fibrils by a specific, conformation-dependent interaction. *Sci Rep*. 7:40859.

Sotelo, J., and M.A. Rodríguez-Gabriel. 2006. Mitogen-activated protein kinase Hog1 is essential for the response to arsenite in Saccharomyces cerevisiae. *Eukaryot Cell*. 5:1826-1830.

Spence, J., S. Sadis, A.L. Haas, and D. Finley. 1995. A ubiquitin mutant with specific defects in DNA repair and multiubiquitination. *Mol Cell Biol*. 15:1265-1273.

Spokoini, R., O. Moldavski, Y. Nahmias, J.L. England, M. Schuldiner, and D. Kaganovich. 2012. Confinement to Organelle-Associated Inclusion Structures Mediates Asymmetric Inheritance of Aggregated Protein in Budding Yeast. *Cell Reports*. 2:738–747.

Sternlicht, H., G.W. Farr, M.L. Sternlicht, J.K. Driscoll, K. Willison, and M.B. Yaffe. 1993. The t-complex polypeptide 1 complex is a chaperonin for tubulin and actin in vivo. *Proc Natl Acad Sci U S A*. 90:9422-9426.

Sunde, M., and C. Blake. 1997. The structure of amyloid fibrils by electron microscopy and X-ray diffraction. *Adv Protein Chem*. 50:123-159.

Takahashi, S., E. Takeda, Y. Kubota, and R. Okayasu. 2000. Inhibition of repair of radiation-induced DNA double-strand breaks by nickel and arsenite. *Radiation Research*. 154:686-691.

Takeshige, K., M. Baba, S. Tsuboi, T. Noda, and Y. Ohsumi. 1992. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol*. 119:301-311.

Takeuchi, J., H. Chen, and P. Coffino. 2007. Proteasome substrate degradation requires association plus extended peptide. *Embo j*. 26:123-131.

Talemi, S.R., T. Jacobson, V. Garla, C. Navarrete, A. Wagner, M.J. Tamás, and J. Schaber. 2014. Mathematical modelling of arsenic transport, distribution and detoxification processes in yeast. *Mol Microbiol*. 92:1343-1356.

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

Tamás, M.J., K. Luyten, F.C. Sutherland, A. Hernandez, J. Albertyn, H. Valadi, H. Li, B.A. Prior, S.G. Kilian, J. Ramos, L. Gustafsson, J.M. Thevelein, and S. Hohmann. 1999. Fps1p controls the accumulation and release

of the compatible solute glycerol in yeast osmoregulation. *Mol Microbiol*. 31:1087-1104.

Tamás, M.J., S.K. Sharma, S. Ibstedt, T. Jacobson, and P. Christen. 2014. Heavy metals and metalloids as a cause for protein misfolding and aggregation. *Biomolecules*. 4:252-267.

Tchounwou, P.B., C.G. Yedjou, A.K. Patlolla, and D.J. Sutton. 2012. Heavy metal toxicity and the environment. *Exp Suppl*. 101:133-164.

Thorsen, M., Y. Di, C. Tängemo, M. Morillas, D. Ahmadpour, C. Van der Does, A. Wagner, E. Johansson, J. Boman, F. Posas, R. Wysocki, and M.J. Tamás. 2006. The MAPK Hog1p modulates Fps1p-dependent arsenite uptake and tolerance in yeast. *Mol Biol Cell*. 17:4400-4410.

Thorsen, M., T. Jacobson, R. Vooijs, C. Navarrete, T. Bliek, H. Schat, and M.J. Tamás. 2012. Glutathione serves an extracellular defence function to decrease arsenite accumulation and toxicity in yeast. *Mol Microbiol*. 84:1177-1188.

Thorsen, M., G. Lagniel, E. Kristiansson, C. Junot, O. Nerman, J. Labarre, and M.J. Tamás. 2007. Quantitative transcriptome, proteome, and sulfur metabolite profiling of the Saccharomyces cerevisiae response to arsenite. *Physiol Genomics*. 30:35-43.

Thorsen, M., G.G. Perrone, E. Kristiansson, M. Traini, T. Ye, I.W. Dawes, O. Nerman, and M.J. Tamás. 2009. Genetic basis of arsenite and cadmium tolerance in Saccharomyces cerevisiae. *BMC Genomics*. 10.

Tian, G., S. Park, M.J. Lee, B. Huck, F. McAllister, C.P. Hill, S.P. Gygi, and D. Finley. 2011. An asymmetric interface between the regulatory and core particles of the proteasome. *Nat Struct Mol Biol*. 18:1259-1267.

Tseng, C.H. 2005. Blackfoot disease and arsenic: a never-ending story. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 23:55-74.

Tseng, W.-P. 1977. Effects and dose response relationships of skin cancer and blackfoot disease with arsenic. *Environmental Health Perspectives*. 19:109-119.

Tsuboi, T., K. Kuroha, K. Kudo, S. Makino, E. Inoue, I. Kashima, and T. Inada. 2012. Dom34:Hbs1 Plays a General Role in QualityControl Systems by Dissociation of a Stalled Ribosome at the 30 End of Aberrant mRNA. *Molecular Cell*. 46:518-529.

Tyedmers, J., A. Mogk, and B. Bukau. 2010. Cellular strategies for controlling protein aggregation. *Nature Reviews Molecular Cell Biology*. 11:777-788.

Tyler, C.R., and A.M. Allan. 2014. The Effects of Arsenic Exposure on Neurological and Cognitive Dysfunction in Human and Rodent Studies: A Review. *Curr Environ Health Rep*. 1:132-147.

Vahidnia, A., F. Romijn, G.B. van der Voet, and F.A. de Wolff. 2008. Arsenic-induced neurotoxicity in relation to toxicokinetics: effects on sciatic nerve proteins. *Chem Biol Interact*. 176:188-195.

Vahter, M. 2002. Mechanisms of arsenic biotransformation. *Toxicology*. 181-182:211-217.

Vainberg, I.E., S.A. Lewis, H. Rommelaere, C. Ampe, J. Vandekerckhove, H.L. Klein, and N.J. Cowan. 1998. Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. *Cell*. 93:863-873.

Vashist, S., and D.T. Ng. 2004. Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. *J Cell Biol*. 165:41-52.

Vendredy, L., E. Adriaenssens, and V. Timmerman. 2020. Small heat shock proteins in neurodegenerative diseases. *Cell Stress Chaperones*. 25:679-699.

Vetri, V., and V. Foderà. 2015. The route to protein aggregate superstructures: Particulates and amyloid-like spherulites. *FEBS Lett*. 589:2448-2463.

Vujcic, M., M. Shroff, and K.K. Singh. 2007. Genetic determinants of mitochondrial response to arsenic in yeast Saccharomyces cerevisiae. *Cancer Res*. 67:9740-9749.

Walton, F.S., A.W. Harmon, D.S. Paul, Z. Drobná, Y.M. Patel, and M. Styblo. 2004. Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes. *Toxicol Appl Pharmacol*. 198:424-433.

Wang, J., J.M. Choi, A.S. Holehouse, H.O. Lee, X. Zhang, M. Jahnel, S. Maharana, R. Lemaitre, A. Pozniakovsky, D. Drechsel, I. Poser, R.V. Pappu, S. Alberti, and A.A. Hyman. 2018. A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. *Cell*. 174:688-699.e616.

Wang, S., H. Sakai, and M. Wiedmann. 1995. NAC Covers Ribosome-associated Nascent Chains Thereby Forming a Protective Environment for Regions of Nascent Chains Just Emerging from the Peptidyl Transferase Center. *The Journal of Cell Biology*. 130:519-528.

Wang, S., and X. Shi. 2001. Molecular mechanisms of metal toxicity and carcinogenesis. *Molecular and Cellular Biochemistry*. 222:3-9.

Wang, T.S., C.F. Kuo, K.Y. Jan, and H. Huang. 1996. Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. *J Cell Physiol*. 169:256-268.

Wang, T.S., Y.F. Shu, Y.C. Liu, K.Y. Jan, and H. Huang. 1997. Glutathione peroxidase and catalase modulate the genotoxicity of arsenite. *Toxicology*. 121:229-237.

Wauson, E.M., A.S. Langan, and R.L. Vorce. 2002. Sodium arsenite inhibits and reverses expression of adipogenic and fat cell-specific genes during in vitro adipogenesis. *Toxicol Sci*. 65:211-219.

Waxman, S., and K.C. Anderson. 2001. History of the Development of Arsenic Derivatives in Cancer Therapy. *The Oncologist*. 6:3-10.

Wegrzyn, R.D., D. Hofmann, F. Merz, R. Nikolay, T. Rauch, C. Graf, and E. Deuerling. 2006. A Conserved Motif Is Prerequisite for the Interaction of NAC with Ribosomal Protein L23 and Nascent Chains. *The Journal of Biological Chemistry*. 281:2847–2857.

Weids, A.J., S. Ibstedt, M.J. Tamás, and C.M. Grant. 2016. Distinct stress conditions result in aggregation of proteins with similar properties. *Scientific Reports*. 6.

Weissman, A.M. 2001. Themes and Variations on Ubiquitylation. *Nature Reviews Molecular Cell Biology*. 2:169-178.

Wemmie, J.A., M.S. Szczypka, D.J. Thiele, and W.S. Moye-Rowley. 1994. Cadmium tolerance mediated by the yeast AP-1 protein requires the presence of an ATP-binding cassette transporter-encoding gene, YCF1. *J Biol Chem*. 269:32592-32597.

Werner-Washburne, M., D.E. Stone, and E.A. Craig. 1987. Complex Interactions among Members of an Essential Subfamily of hsp70 Genes in Saccharomyces cerevisiae. *Molecular and Cellular Biology*. 7:2568-2577.

WHO. 2018. Arsenic. Vol. 2020.

Wiech, H., J. Buchner, R. Zimmermann, and U. Jakob. 1992. Hsp90 chaperones protein folding in vitro. *Nature*. 358:169-170.

Wiedmann, B., and S. Prehn. 1999. The nascent polypeptide-associated complex (NAC) of yeast functions in the targeting process of ribosomes to the ER membrane. *FASEB Letters*. 458:51-54.

Wiedmann, B., H. Sakai, T.A. Davis, and M. Wiedmann. 1994. A protein complex required for signal-sequence-specific sorting and translocation. *Nature*. 370:434-440.

Willmund, F., M.d. Alamo, S. Pechmann, T. Chen, V. Albanese, E.B. Dammer, J. Peng, and J. Frydman. 2013. The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. *Cell*. 152:196–209.

Wiseman, H., and B. Halliwell. 1996. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J*. 313 (Pt 1):17-29.

Wu, A.L., and W.S. Moye-Rowley. 1994. GSH1, which encodes gamma-glutamylcysteine synthetase, is a target gene for yAP-1 transcriptional regulation. *Mol Cell Biol*. 14:5832-5839.

Wu, L., H. Yi, and H. Zhang. 2013. Reactive oxygen species and Ca2+ are involved in sodium arsenite-induced cell killing in yeast cells. *FEMS Microbiology Letters*. 343:57–63.

Wysocki, R., P. Bobrowicz, and S. Ułaszewski. 1997. The Saccharomyces cerevisiae ACR3 Gene Encodes a Putative Membrane Protein Involved in Arsenite Transport. *The Journal of Biological Chemistry*. 272:30061–30066.

Wysocki, R., C.C. Chéry, D. Wawrzycka, M.V. Hulle, R. Cornelis, J.M. Thevelein, and M.J. Tamás. 2001. The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in Saccharomyces cerevisiae. *Molecular Microbiology*. 40:1391–1401.

Wysocki, R., P.K. Fortier, E. Maciaszczyk, M. Thorsen, A. Leduc, A. Odhagen, G. Owsianik, S. Ulaszewski, D. Ramotar, and M.J. Tamás. 2004. Transcriptional activation of metalloid tolerance genes in Saccharomyces cerevisiae requires the AP-1-like proteins Yap1p and Yap8p. *Mol Biol Cell*. 15:2049-2060.

Wysocki, R., and M.J. Tamás. 2010. How Saccharomyces cerevisiae copes with toxic metals and metalloids. *FEMS Microbiology Reviews*. 34:925–951.

Xie, H., S. Huang, S. Martin, and J.P.W. Sr. 2014. Arsenic is cytotoxic and genotoxic to primary human lung cells. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*. 760:33-41.

Xie, W., and D.T. Ng. 2010. ERAD substrate recognition in budding yeast. *Semin Cell Dev Biol*. 21:533-539.

Xu, C., S. Wang, G. Thibault, and D.T. Ng. 2013. Futile protein folding cycles in the ER are terminated by the unfolded protein O-mannosylation pathway. *Science*. 340:978-981.

Xu, Y., and S. Lindquist. 1993. Heat-shock protein hsp90 governs the activity of pp60v-src kinase. *Proc Natl Acad Sci U S A*. 90:7074-7078.

Yadav, R.S., L.P. Chandravanshi, R.K. Shukla, M.L. Sankhwar, R.W. Ansari, P.K. Shukla, A.B. Pant, and V.K. Khanna. 2011. Neuroprotective efficacy of curcumin in arsenic induced cholinergic dysfunctions in rats. *Neurotoxicology*. 32:760-768.

Yam, A.Y., V. Albanèse, H.T. Lin, and J. Frydman. 2005. Hsp110 cooperates with different cytosolic HSP70 systems in a pathway for de novo folding. *J Biol Chem*. 280:41252-41261.

Yan, W., B. Schilke, C. Pfund, W. Walter, S. Kim, and E. A.Craig. 1998. Zuotin, a ribosome-associated DnaJ molecular chaperone. *The EMBO Journal*. 17:4809–4817.

Yan, W., Y. Zhang, J. Zhang, S. Liu, S.J. Cho, and X. Chen. 2011. Mutant p53 protein is targeted by arsenic for degradation and plays a role in arsenic-mediated growth suppression. *J Biol Chem*. 286:17478-17486.

Yen, J.L., N.Y. Su, and P. Kaiser. 2005. The yeast ubiquitin ligase SCFMet30 regulates heavy metal response. *Mol Biol Cell*. 16:1872-1882.

Yompakdee, C., M. Bun-ya, K. Shikata, N. Ogawa, S. Harashima, and Y. Oshima. 1996a. A putative new membrane protein, Pho86p, in the inorganic phosphate uptake system of Saccharomyces cerevisiae. *Gene*. 171:41-47.

Yompakdee, C., N. Ogawa, S. Harashima, and Y. Oshima. 1996b. A putative membrane protein, Pho88p, involved in inorganic phosphate transport in *Saccharomyces cerevisiae*. *Molecular Genetics and Genomics*. 251:580-590.

Yousefnia, S. 2021. Mechanistic effects of arsenic trioxide on acute promyelocytic leukemia and other types of leukemias. *Cell Biol Int*. 45:1148-1157.

Zamotin, V., A. Gharibyan, N.V. Gibanova, M.A. Lavrikova, D.A. Dolgikh, M.P. Kirpichnikov, I.A. Kostanyan, and L.A. Morozova-Roche. 2006. Cytotoxicity of albebetin oligomers depends on cross-beta-sheet formation. *FEBS Lett*. 580:2451-2457.

Zarazúa, S., S. Bürger, J.M. Delgado, M.E. Jiménez-Capdeville, and R. Schliebs. 2011. Arsenic affects expression and processing of amyloid precursor protein (APP) in primary neuronal cells overexpressing the Swedish mutation of human APP. *Int J Dev Neurosci*. 29:389-396.

Zhang, X., F. Yang, J.-Y. Shim, K.L. Kirk, D.E. Anderson, and X. Chen. 2007. Identification of arsenic-binding proteins in human breast cancer cells. *Cancer Letters*. 255:95-106.

Zhang, X.W., X.J. Yan, Z.R. Zhou, F.F. Yang, Z.Y. Wu, H.B. Sun, W.X. Liang, A.X. Song, V. Lallemand-Breitenbach, M. Jeanne, Q.Y. Zhang, H.Y. Yang, Q.H. Huang, G.B. Zhou, J.H. Tong, Y. Zhang, J.H. Wu, H.Y. Hu, H. de Thé, S.J. Chen, and Z. Chen. 2010. Arsenic trioxide controls the fate of the PML-RARalpha oncoprotein by directly binding PML. *Science*. 328:240-243.

Zhang, Y., G.V. Gesé, C. Conz, K. Lapouge, J. Kopp, T. Wölfle, S. Rospert, and I. Sinning. 2020. The ribosome-associated complex RAC serves in a relay that directs nascent chains to Ssb. *Nature Communications*. 11:1-12.

Zhang, Y., C. Ma, Y. Yuan, J. Zhu, N. Li, C. Chen, S. Wu, L. Yu, J. Lei, and N. Gao. 2014. Structural basis for interaction of a cotranslational chaperone with the eukaryotic ribosome. *Nature Structural & Molecular Biology*. 21:1042-1046.

Zheng, N., and N. Shabek. 2017. Ubiquitin Ligases: Structure, Function, and Regulation. *Annu Rev Biochem*. 86:129-157.

Zhouravleva, G., L. Frolova, X.L. Goff, R.L. Guellec, S. Inge-Vechtomov, L. Kisselev, and M. Philippe. 1995. Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. *The EMBO Journal*. 14:4065-4072.

Zhu, H.H., J. Hu, F. Lo-Coco, and J. Jin. 2019. The simpler, the better: oral arsenic for acute promyelocytic leukemia. *Blood*. 134:597-605.

Zhu, Q., Z. Deng, S. Zhu, P. Zhao, M. Wang, and X. Hu. 2017. Study on the clinical safe and effective methods of arsenic-containing compound Qinghuang powder in the treatment of myelodysplastic syndrome. *Evidence-Based Complementary and Alternative Medicine*. 2017.