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Review

Cellular Handling of Protein Aggregates by Disaggregation Machines

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Both acute proteotoxic stresses that unfold proteins and expression of disease-causing mutant proteins that expose aggregation-prone regions can promote protein aggregation. Protein aggregates can interfere with cellular processes and deplete factors crucial for protein homeostasis. To cope with these challenges, cells are equipped with diverse folding and degradation activities to rescue or eliminate aggregated proteins. Here, we review the different chaperone disaggregation machines and their mechanisms of action. In all these machines, the coating of protein aggregates by Hsp70 chaperones represents the conserved, initializing step. In bacteria, fungi, and plants, Hsp70 recruits and activates Hsp100 disaggregases to extract aggregated proteins. In the cytosol of metazoa, Hsp70 is empowered by a specific cast of J-protein and Hsp110 co-chaperones allowing for standalone disaggregation activity. Both types of disaggregation machines are supported by small Hsps that sequester misfolded proteins.

Introduction

Definition and Features of Protein Aggregates

A protein can adopt many different conformational states within the cellular environment. Its conformation is determined not only by its intrinsic amino acid sequence, but also by interactions with other proteins, including partner proteins of oligomeric assemblies and molecular chaperones that guide in (co-translational) folding to the native state (Balchin et al., 2016; Chiti and Dobson, 2017). Failure of these guiding interactions, mutations, and proteotoxic stresses such as heat shock can cause proteins to adopt conformers that expose interactive surfaces including aggregation-prone hydrophobic regions. Newly synthesized proteins seem particularly vulnerable toward environmental stress, but also imbalances in the stoichiometry of subunits of protein complexes or the disassembly of such complexes can lead to aggregation of the un-complexed subunits (Oromendia et al., 2012; Zhou et al., 2014). Further, proteins are temporarily at increased risk of aggregation during transport over membranes or through pores.

Important considerations of protein aggregation are how it is triggered and whether it is solely driven by hydrophobic interactions. At the one extreme, acute global protein unfolding stresses can cause rather broad aggregation driven by hydrophobic interactions of multiple unfolded proteins in otherwise healthy cells. At the other extreme, as in most chronic protein conformation diseases, a single protein species initiates aggregation that, depending on the protein, may be based on hydrophobic associations (typically leading to amorphic aggregates) or β sheet or β-hairpin associations (typically found in amyloids) in often also otherwise compromised, aged cells. In the latter, aggregation initiation often requires external triggers that increase the fragility of the cells (Kampinga and Bergink, 2016) (Figure 1).

In general terms, a protein aggregate can be considered as a non-functional assembly of single or multiple types of proteins. As stated above, aggregates can adopt a wide variety of different protein conformers, varying from highly disordered to near-native states and structured amyloids (Chiti and Dobson, 2017; Wallace et al., 2015). Aggregates can also gradually change in structure and composition over time, especially in cells where the initial aggregate-inducing protein species can sequester and sometimes irreversibly trap other proteins, either as part of the organized inner structure of the initial aggregate or by binding to amino acid side chains at the surface of the (growing) aggregate (Figure 1).

Protein Aggregates and Cellular Toxicity

The importance of aggregation in protein conformational diseases is evidenced by the fact that genetically inherited forms of such diseases nearly always correlate with the aggregation of the affected protein (Chiti and Dobson, 2017). This is probably best illustrated by diseases caused by CAG-repeat expansions, where the size of the expansion is directly linked not only to the age at onset of the disease (Gusella and MacDonald, 2000; Kuiper et al., 2017), but also with the aggregation propensity of the corresponding polyglutamine protein (Morley et al., 2002). Moreover, for many of these chronic diseases, boosting of protein quality control activities (chaperones and proteases) has disease ameliorating effects in experimental disease models (Balch et al., 2008; Kakkar et al., 2014).

However, the toxic nature of protein aggregates has been a matter of extensive debate, especially in the area of neurodegenerative diseases. Part of this debate is owed to confusion between the biochemically defined detection as detergent-soluble material in cell fractionation experiments or their microscopic analysis as foci or differentially defined protein sequestrations or inclusions that not only differ in size and composition but also in cellular localization. We wish to emphasize that protein aggregates, that is, non-native interactions between proteins, are mostly below detection level in microscopic examinations

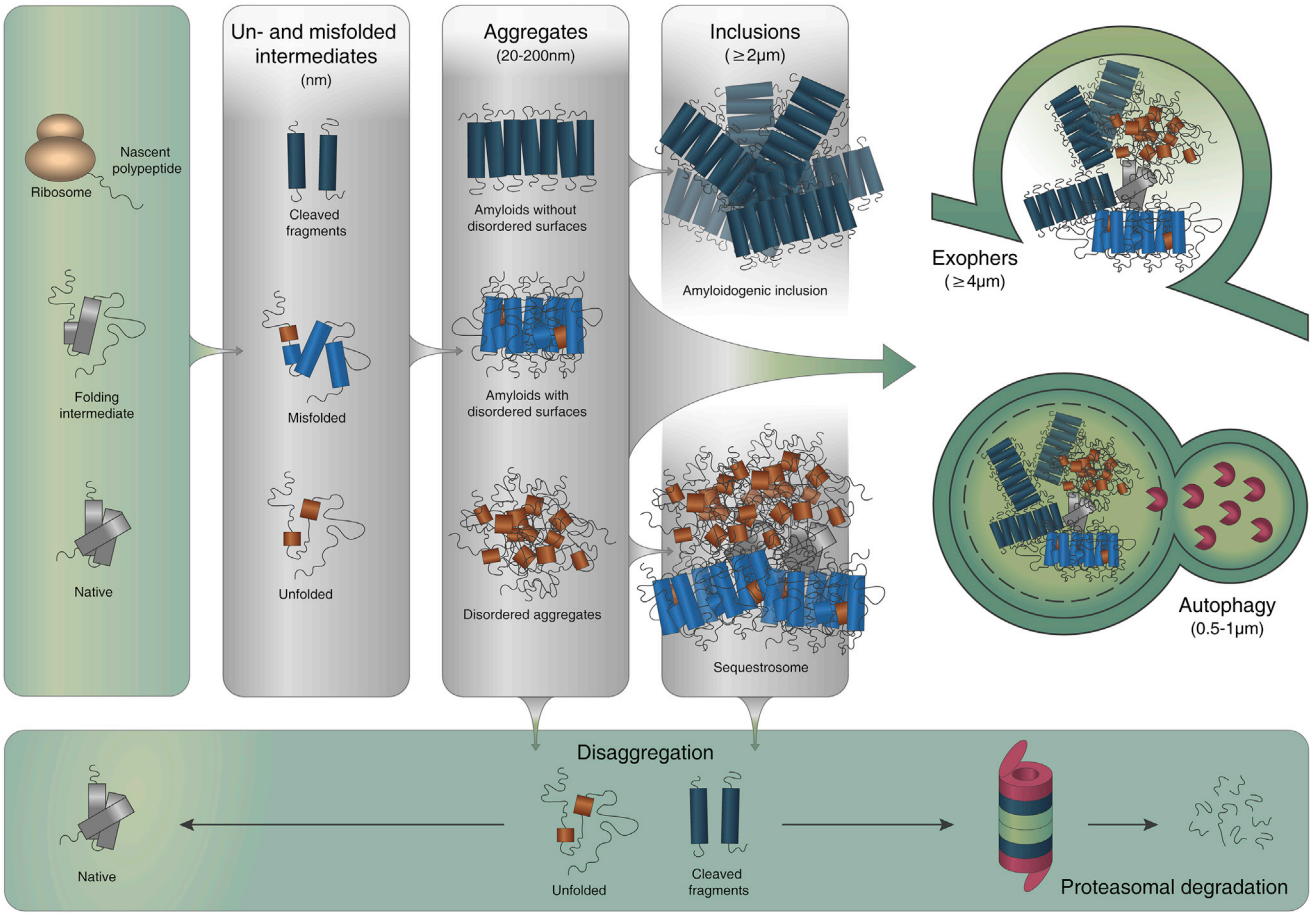

Figure 1. Fate of Un- or Misfolded Proteins in Cells

Nascent polypeptides form folding intermediates that are prone to aggregation when not properly chaperone-assisted toward to their native state. Unfolded intermediates may also accumulate in the case of genetic mutations that impair proper folding, under stress conditions due to unfolding, or upon post-translational modification or cleavage by proteases. These intermediates are aggregation prone and, if not dealt with properly by chaperones and/or proteasomal degradation, can form different types of aggregates. Depending on the surface-exposed regions, proteins may exclusively self-aggregate or co-aggregate with other unfolded proteins or even fully folded proteins (not indicated) in the cell and interact with other cellular constituents. To deal with these aggregates, cells have evolved several different options, which include temporal active sequestration into macromolecular assemblies (sequestrosomes), autophagic clearance, extracellular ejection via exophers, or asymmetric segregation in replicating cells (not indicated). Inclusion formation may also be self-driven, which could be the case for amyloidogenic proteins. Finally, proteins can be recovered from aggregates via disaggregation machines either to be refolded (likely the predominant path) or for degradation through the proteasome. The disaggregation process may be facilitated by prior sequestration reactions and/or modulation of the aggregates by sHsps (see text for further details).

(nm size) and are always potentially toxic (Figure 1). They should not be confused with the much larger (μm size) microscopically detectable inclusions that either may result from self-assembly of amyloidogenic fibers or reflect a regulated sequestration of proteins into various organized macromolecular assemblies (hereafter referred to as "sequestrosomes") as a mechanism to deal with them and prevent or reduce toxicity (Figure 1). However, these sequestrosomes may also (1) have disordered surfaces that could trap other cellular proteins, leading to secondary loss of function; (2) be a source for generation of smaller aggregates (by, e.g., shedding) that are more cytotoxic or generate new aggregates; or (3) be toxic through physical hindrance of cellular processes (see below). Moreover, confusions related to the toxicity of different aggregates also are due to the usage of different endpoints that were used to score toxicity (e.g., cell death versus singular functional deficits) and experimental issues related to whether aggregates arose intra- or extracellularly (Kampinga and Bergink, 2016).

With the distinction between aggregates and microscopically detectable inclusions in mind, the evidence suggests that aggregate toxicity will depend on a number of aspects including the reactive surface, localization, compartmentalization, and stability of the aggregates. A detailed evaluation is beyond the scope of this review, but mechanisms of aggregate toxicity include physical damage to membranes (Lashuel et al., 2002), hindrance of transport processes including axonal transport (De Vos et al., 2008) and nucleo-cytoplasmic trafficking (Grima et al., 2017), translation impairment or stress-regulated translational shutdown (Smith and Mallucci, 2016), and co-aggregation of essential proteins like transcription factors (Chu et al., 2007), all leading to secondary loss of functions. In addition, trapping of chaperones and other components of the protein quality

Table 1. Chaperones Involved in Protein Disaggregation

	Escherichia coli	*Saccharomyces cerevisiae*	*Caenorhabditis elegans*	*Homo sapiens*
Hsp70	DnaK	Ssa1–4	HSP-1 and HSP-70	HSPA8 and HSPA1A
J-protein	DnaJ and CbpA	Ydj1 and Sis1	DNJ-12, DNJ-19, and DNJ-13	DNAJA2 and DNAJB1
Hsp110	–	Sse1/2	HSP110	HSPH1/2
Hsp100 disaggregase	ClpB	Hsp104	–	–
sHsps	IbpA and IbpB	Hsp26 and Hsp42	HSP-16.2, HSP-16.1, and SIP-1	HSPB1, HSPB3, HSPB4, and HSPB5

Representative members of indicated chaperone families, which have been demonstrated to assist in protein disaggregation. Note that for DNAJ proteins, this list may be incomplete as so far only few (combinations of) members have been tested. For sHsps, only members that have been shown to have promiscuous anti-aggregation activities have been listed (Mymrikov et al., 2017).

control network may occur and lead to a collapse of cellular proteostasis (Hipp et al., 2014), which can be a large contributor to the self-perpetuating progressive nature of aggregate toxicity.

Quality Control of Protein Aggregates

The primary aim of the chaperone network in cells is to promote native folding and prevent aggregation of misfolded proteins (Mayer and Bukau, 2005). The capacity of this network is, however, limited. Acute and chronic stress conditions can lead to its exhaustion, which results in accumulation of misfolded proteins that become sequestered into cellular deposits. Therefore, cells are equipped with specific machineries targeting aggregating proteins.

To respond to protein aggregation, cells employ a number of inter-connected strategies. These include (1) disaggregation followed by either refolding or proteasomal degradation, (2) autophagic clearance of aggregated proteins (also termed aggrephagy; Yamamoto and Simonsen, 2011), and (3) secretion of aggregates as exophers into the extracellular environment (Melentijevic et al., 2017) (Figure 1). As an intermediate to these processes, and as storage forms, cells can actively sequester non-native proteins into different (membrane-less) sequestrosomes (Johnston et al., 1998; Kaganovich et al., 2008; Miller et al., 2015) (Figure 1). Finally, in dividing cells inclusions can be asymmetrically segregated during cell division to ensure that at least one daughter cell is free of accumulated protein damage (Aguilaniu et al., 2003; Rujano et al., 2006). Although it seems that there is some hierarchical use of these strategies, there is yet little understanding of how fate decisions for aggregates are made. In this review, we will focus on the capacities and mechanisms of chaperones to assist in and execute protein disaggregation. Table 1 lists members of chaperone families linked to disaggregation processes (as described in more detail below) in diverse model organisms from bacteria to human.

Protein Disaggregation

Physiological Relevance of Protein Disaggregation

The importance of protein disaggregation came from the seminal work of the Lindquist laboratory on the Hsp104 disaggregase in yeast. Strains lacking functional Hsp104 are unable to develop tolerance to severe heat (Sanchez and Lindquist, 1990). Inversely, Hsp104 upregulation alone is sufficient to induce thermotolerance (Lindquist and Kim, 1996). The activity of Hsp104 responsible for this resistance was shown to be its ability to disassemble aggregated proteins (Parsell et al., 1994) and could be reconstituted *in vitro* (Glover and Lindquist, 1998). Disaggregation activities and physiological functions are conserved for bacterial and plant counterparts, ClpB and Hsp101, respectively (Mogk et al., 1999; Queitsch et al., 2000).

Metazoans lack an Hsp104 disaggregase; however, they also exhibit disaggregation activity: e.g., model proteins like firefly luciferase can be reactivated from a heat-aggregated state (Pinto et al., 1991). Also in metazoans, disaggregation is linked to stress resistance in cellular (Kampinga et al., 1987, 1994) and organismal models (Kirstein et al., 2017; Rampelt et al., 2012), documenting the essential need to reverse protein aggregation for cellular survival. Here, we will first describe the non-metazoan disaggregation machinery before reporting on the metazoan system.

Hsp100 Disaggregases: ATP-Driven Threading Machines

Hsp104/ClpB are Hsp100 members of the AAA+ (ATPase associated with diverse cellular activities) protein family and use energy derived from ATP hydrolysis to unfold bound substrates or remodel and dissociate protein complexes. Conserved AAA domains mediate ATP binding and hydrolysis, but also oligomerization, typically into homohexameric assemblies with a central pore. The mechanical work is executed by pore-located loop segments harboring conserved aromatic residues for substrate interaction (Figure 2A, middle panel). These loops are mobile and change their position at the central channel depending on the nucleotide state of the respective subunit. A downward movement upon ATP hydrolysis transforms into a pulling force on the bound substrate, ultimately leading to its threading through the AAA+ ring. Hsp104/ClpB can bind internal segments of substrates and simultaneously thread looped substrate structures consisting of two peptide strands (Haslberger et al., 2008). Notably, the cryoelectron microscopy (cryo-EM) structures of Hsp104/ClpB hexamers are asymmetric and pore loops are not aligned but separated by 6 Å along the central channel, implying that bound substrates are threaded in discrete steps of two amino acids (Figure 2A, left and middle panel) (Deville et al., 2017; Gates et al., 2017). Not all pore loops are in direct contact with the bound model substrate (casein), suggesting functionally distinct states of the pore loops during substrate threading (Figure 2A, middle panel). The pulling and holding of bound substrate by pore loops mediate threading and prevent backsliding of the substrate. Pore loop positions and substrate contacts are suggested to change in a sequential manner across

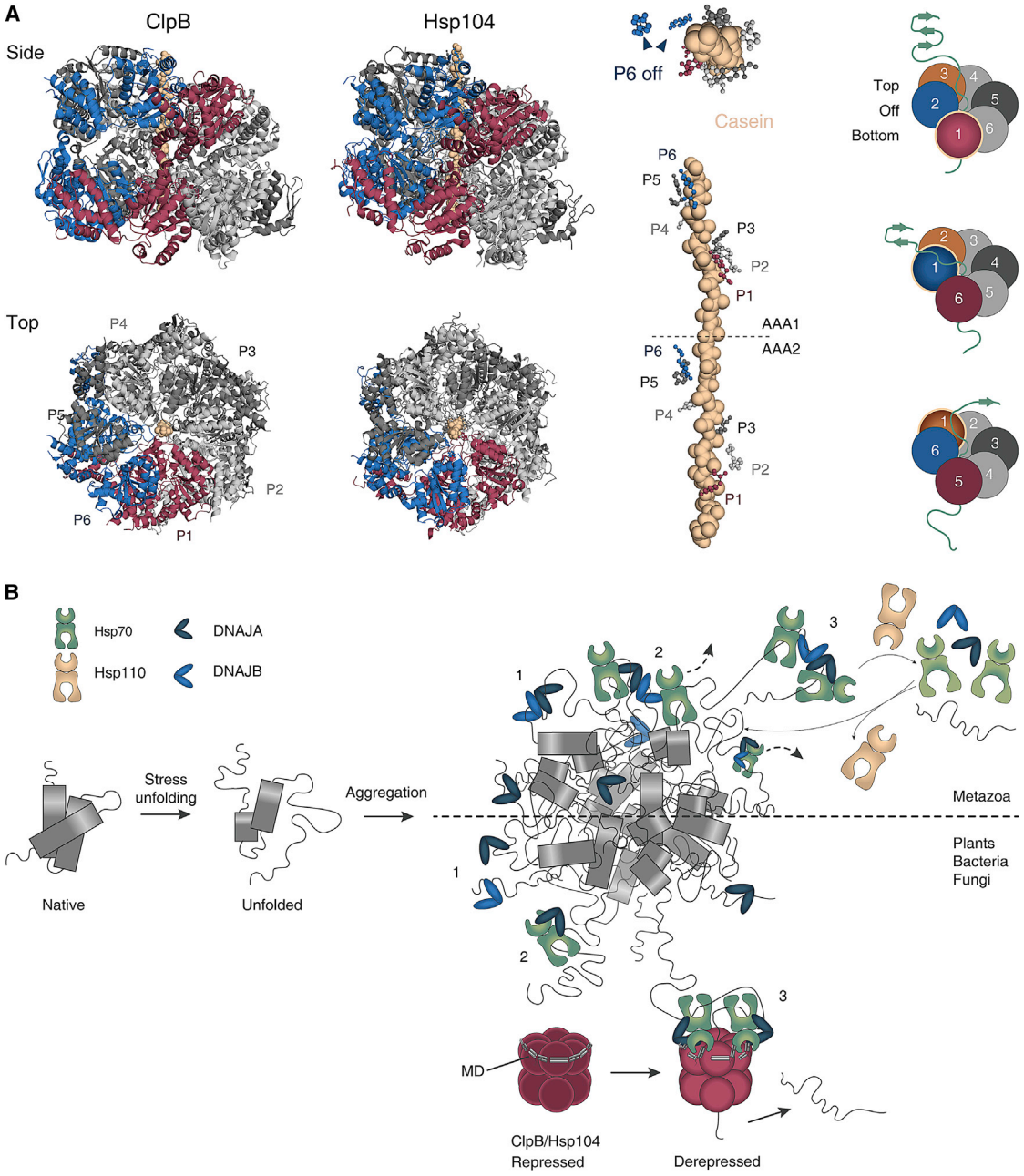

Figure 2. Mechanisms of Disaggregation Machines

(A) Structural basis for substrate binding and threading by Hsp104/ClpB. Left: structure of ClpB and Hsp104 with bound model substrate casein shown as extended poly-Ala strand model. N-terminal (N) and middle domains (MD) were removed to increase clarity. Side and top views of the complex are given, Hsp104/ClpB subunits are numbered (P1–P6), and the two layers of the Hsp104/ClpB ring (AAA-1 and AAA-2) are indicated. Middle: interactions of casein with conserved, pore-located tyrosine residues of Hsp104, which form a spiral staircase. All tyrosines except two (both from subunit P6) contact casein. Right: putative model of substrate translocation by AAA+ proteins. Alternating switching of pore loop positions of individual subunits is suggested to drive threading of extended or looped polypeptides through an AAA+ hexamer. A detached pore loop (blue subunit) rebinds substrate in the top position (orange) upon activation of the respective subunit and moves down a spiral staircase upon subsequent cycles before becoming displaced again. The movements of one pore loop/AAA+ subunit (yellow circle) are indicated.
(B) Unfolding stress conditions like heat shock can cause protein unfolding and the formation of amorphous aggregates. A bi-chaperone system formed by Hsp70 and Hsp104/ClpB in bacteria, fungi, and plants solubilizes aggregated proteins. Cooperating chaperones bind in a hierarchical order, involving J-protein binding first (1), followed by Hsp70 targeting (2) that finally recruits Hsp104/ClpB to the aggregate surface (3). Hsp70-Hsp104/ClpB cooperation and Hsp104/ClpB activity are controlled by MDs, forming an inhibitory belt surrounding the ClpB/Hsp104 hexamer in the repressed state. Partial detachment of the MD from the ring causes derepression and allows for Hsp70 binding, which stabilizes the derepressed state. Subsequent substrate engagement leads to full activation of Hsp104/ClpB ATPase activities and threading of the aggregated substrate. In metazoa, Hsp70 exhibits a standalone disaggregation activity if supported by a specific set of co-chaperones. Cooperation between class A and B-type DNAJ-proteins increases loading of Hsp70 at the aggregate surface. Movements of Hsp70s away from the aggregate might apply pulling forces that disentangle trapped polypeptides. Binding of Hsp110 recycles Hsp70 to initiate new binding and pulling events.

the Hsp104/ClpB ring, pointing to a rotary translocation mechanism (Figure 2A, right panel) (Gates et al., 2017).

Hsp100 Disaggregases Require Hsp70 for Protein Disaggregation

Although historically the process of protein disaggregation is connected to the Hsp104/ClpB disaggregase, this machine does not exhibit disaggregation activity on its own but instead strictly requires cooperation with cognate Hsp70 chaperone systems (Glover and Lindquist, 1998; Mogk et al., 1999). It is the Hsp70 chaperone that forms the initializing core of the Hsp70/Hsp104 bi-chaperone machinery (Weibezahn et al., 2004; Zietkiewicz et al., 2004) (Figure 2B). Hsp70 chaperones are highly versatile and involved in a multiplicity of cellular processes including folding of newly synthesized proteins, protein transport and degradation, and prevention of protein aggregation (Mayer and Bukau, 2005). This broad set of activities is made possible by co-chaperones of the DNAJ protein family that target Hsp70 to specific cellular sites including protein aggregates (see below). Hsp70s promiscuously bind to different kinds of protein aggregates, including bacterial inclusion bodies formed upon overproduction of heterologous proteins, stress granules composed of translation factors and translationally repressed mRNA, heat stress-generated protein aggregates composed of misfolded proteins, and amyloid fibrils formed by disease proteins (Carrió and Villaverde, 2005; Gao et al., 2015; Walters and Parker, 2015). This underlines the central role of Hsp70 in aggregate handling. The disaggregation potential of Hsp70 is, however, limited, and aggregate-bound Hsp70 typically requires assistance to be most effective (Diamant et al., 2000). In this sense, Hsp104/ClpB represent specialized Hsp70 partner chaperones that increase the potential of the Hsp70 machinery in protein disaggregation (Figure 2B). Protein disaggregation is thus initiated by Hsp70 binding to the surface of protein aggregates followed by subsequent recruitment of the cooperating Hsp100 disaggregase (Acebrón et al., 2009; Winkler et al., 2012).

Hsp70s Activate the Hsp100 Threading Motor

Next to targeting Hsp100 disaggregases to protein aggregates, Hsp70 activity is also required for activation of the substrate threading motor of Hsp104/ClpB. This Hsp70-mediated activity control prevents Hsp104/ClpB from nonspecific and therefore deleterious threading of a large spectrum of proteins (Lipińska et al., 2013; Oguchi et al., 2012). The Hsp104/ClpB N-terminal domains located on top of the AAA rings control substrate access to the pore site (Deville et al., 2017; Rosenzweig et al., 2015). The coiled-coil middle domains (MDs) form a repressing belt surrounding the upper AAA ring that reduces Hsp104/ClpB ATPase activity, which is lower as compared to other AAA+ proteins (Carroni et al., 2014; Heuck et al., 2016; Oguchi et al., 2012) (Figure 2B). This repression is mediated via head-to-tail interactions of neighboring MDs that stabilize them in a horizontal position around the ring, thereby tightly interacting with and repressing the ATPase subunits. Next to their regulatory function, the MDs also mediate Hsp70 coupling by directly contacting the Hsp70 ATPase domain in a transient manner (Rosenzweig et al., 2013; Seyffer et al., 2012) (Figure 2B). Hsp70 interaction requires partial dissociation of MDs from the AAA ring and upward bending, thus rendering the Hsp70 binding site accessible. Hsp70 binding therefore stabilizes an MD in a displaced conformation and disrupts the repressing MD belt, shifting Hsp104/ClpB to a de-repressed state exhibiting high ATPase activities (Figure 2B).

How is the Hsp70 interaction with Hsp104/ClpB, the key step for activation of the disaggregase, precisely controlled? MD positions in ClpB hexamers are asymmetric. While three MDs adopt a repressing conformation on one side of the ring, the three MDs on the opposite side are detached from the ring and primed for Hsp70 binding and activation (Deville et al., 2017). The detached MDs of Hsp104/ClpB can selectively interact with Hsp70 in the ADP-bound state. Interaction with the ATP-bound state of Hsp70 is prevented by steric clashes of the MD binding site and the substrate binding domain docked onto the Hsp70 ATPase domain (Hayashi et al., 2017). This selectivity ensures that Hsp104/ClpB only associates with substrate-bound Hsp70. Furthermore, a single Hsp70 protein is not sufficient for Hsp104/ClpB activation, but simultaneous binding of two or more Hsp70 partners is required (Oguchi et al., 2012). This condition is fulfilled at an aggregate surface to which multiple Hsp70s associate in close vicinity. A high Hsp70 density therefore functions as specificity determinant for Hsp104/ClpB recruitment and activation (Figure 2B). This avidity effect restricts Hsp104/ClpB activation to aggregate-Hsp70 complexes and prevents detrimental Hsp104/ClpB recruitment to other Hsp70 targets, like newly synthesized polypeptides (Mayer and Bukau, 2005).

Regulated Substrate Transfer from Hsp70 to Hsp100 Disaggregases

How is substrate transfer from Hsp70 to Hsp104/ClpB organized? Recent results obtained with ClpB suggest the following model. The Hsp104/ClpB oligomer likely recognizes a hydrophobic stretch of the aggregated substrate and actively displaces the substrate from Hsp70 by applying a pulling force (Rosenzweig et al., 2013). After substrate transfer, all ClpB MDs switch back to a repressing horizontal conformation, thereby obscuring the Hsp70 binding site (Deville et al., 2017). This results in Hsp70 dissociation upon substrate transfer and restricts the high Hsp104/ClpB activity to initial power strokes. In agreement with this model, Hsp104/ClpB exhibits lower unfolding activity and processivity as compared to other AAA+ family members, with the consequence that some protein substrates are only partially threaded (Duran et al., 2017; Haslberger et al., 2008). Partial substrate threading demands a mechanism to allow the engaged polypeptide to become released from Hsp104/ClpB. This may be accomplished by either partial or complete opening of the ClpB ring, which was reported to occur on the second-to-minute timescale and may allow for dissociation of partially threaded polypeptides (Haslberger et al., 2008; Werbeck et al., 2008).

The partial threading feature might enable Hsp104/ClpB to sense the conformational state of an aggregated substrate and halt threading when encountering tightly folded domains. This could increase refolding efficiencies of solubilized substrates as it avoids complete exposure of hydrophobic cores and unfavorable interactions between unfolded peptide stretches originating from different domains. We consider this a physiologically relevant feature of the disaggregase since protein aggregates

can include proteins with native-like structures (Kampinga et al., 1985; Rinas et al., 2017) or entire protein complexes that become trapped by unfolding of one component (Wallace et al., 2015). Partial substrate threading by Hsp104/ClpB may have evolved to match the nature of protein aggregates formed in cells during common stresses, such as heat stress.

A Dedicated Set of Co-chaperones Empowers Hsp70-Only Based Disaggregation in Metazoa

As stated above, Hsp104 homologs are absent from metazoan (with the exception of mitochondria), an event eventually caused by Hsp104/ClpB-associated fitness costs in unstressed cells (Escusa-Toret et al., 2013). This raised the question of which cellular machinery compensates for Hsp104 loss. Disaggregation activities in mammalian cells could be linked to the level of expression of Hsp70 (Michels et al., 1997; Nollen et al., 1999), in line with its evolutionary conserved aggregate binding capacity. But to what extent and how did Hsp70 evolve to function independently from Hsp104/ClpB in metazoa? Recent findings indicate that a specific set of Hsp70 co-chaperones empowers Hsp70 to exhibit potent, standalone disaggregation activity (Figure 2B). To understand how such Hsp70 complexes can work on protein aggregates, it is first needed to recapitulate the mechanistic basis of Hsp70 chaperone activity.

The Hsp70 ATPase and Chaperone Cycle

Hsp70 chaperones consist of a nucleotide-binding domain (NBD) with ATPase activity and a substrate-binding domain (SBD) composed of a β sandwich pocket and an α-helical lid domain (Mayer and Bukau, 2005). The β sandwich pocket of the SBD typically interacts with short peptide stretches of substrates enriched in aliphatic side chains. The nucleotide-binding state of the NBD dictates substrate-binding affinities, which are low in the ATP and high in the ADP-bound state. More precisely, association and dissociation rates of substrates are high in the ATP state whereas the substrate becomes trapped in the ADP state. Extensive allosteric interactions between NBD and SBD in the ATP state, including docking of the α-helical lid domain onto the ATPase domain, are the structural basis for the control of substrate affinities. Dissociation of the SBD upon ATP hydrolysis is accompanied by locking of substrates and partial or complete lid closure (Marcinowski et al., 2011; Schlecht et al., 2011).

Basal ATPase activities of Hsp70 proteins are generally low, impeding coupling of substrate binding to an open SBD and subsequent SBD closure triggered by ATP hydrolysis. This barrier is overcome by DNAJ protein (Hsp40) co-chaperones that target substrates to Hsp70, while concurrently stimulating Hsp70 ATPase activities, resulting in efficient substrate locking (Mayer and Bukau, 2005). Hsp40 function is reflected in its domain organization, which includes a conserved DNAJ domain for Hsp70 interaction and ATPase stimulation and a variable SBD responsible for targeting Hsp70 to distinct substrates, driving diversity of Hsp70 functions (Kampinga and Craig, 2010). The number of DNAJ proteins has increased during evolution from seven in *E. coli* and 22 in *S. cerevisiae* to at least 45 in humans (Kampinga and Craig, 2010), indicating increased exploration and specialization of Hsp70 functions in higher eukaryotes. The substrate-binding specificities of DNAJ proteins can be very different, spanning from highly specific interactions to rather broad ones with the latter typically mediating binding to a variety of un- or misfolded conformational states.

While substrate trapping by Hsp70 prevents unfavorable, hydrophobic interactions of the bound substrate stretch, the substrate also has to be released, allowing (re)folding to complete. Substrate dissociation requires ADP dissociation, which is accelerated by another set of Hsp70 co-chaperones acting as nucleotide exchange factors (NEFs). Metazoa harbor different types of NEFs, including Bag and HspBP1 types and Hsp110, which can determine the fate of released substrates by targeting them to refolding or degradation pathways.

HSP110: The NEF Crucial for Protein Disaggregation by Hsp70-Only Machines

Hsp110 has attracted particular interest as it can boost Hsp70 disaggregation activity *in vitro* more potently as compared to other NEFs (Gao et al., 2015; Rampelt et al., 2012; Shorter, 2011). Consistently, Hsp110 is crucial for protein disaggregation in nematodes and mammalian cells, underlining its unique contribution to an Hsp70-only based disaggregation activity (Hjerpe et al., 2016; Rampelt et al., 2012) (Figure 2B).

Hsp110s constitute a specific branch of the Hsp70 superfamily, also composed of NBD and SBD. However, they differ from Hsp70 by several functionally critical features. First, like *E. coli* DnaJ (Rüdiger et al., 2001) but unlike DnaK (*E. coli* Hsp70), Hsp110 preferentially binds peptides enriched in aromatic side chains (Xu et al., 2012), which may affect the range of substrates recognized by Hsp110. Hsp110 on its own exhibits holdase activity, i.e., it can prevent aggregation of model substrates, and its upregulation can make cells heat resistant (Oh et al., 1997; Xu et al., 2012). Second, a charged linker sequence (instead of the conserved hydrophobic linker of Hsp70) connects the SBD and NBD, which should affect the allosteric coupling between the domains. Third, for yeast Sse1, the *in vivo* activity required for cell viability, and also its function in boosting Hsp70-dependent disaggregation activities *in vitro*, does not require ATP hydrolysis (Rampelt et al., 2012; Shaner et al., 2004). This indicates that Hsp110 does not need to cycle between different nucleotide states, in contrast to Hsp70, where substrate binding/release is allosterically controlled by nucleotide.

The similarity in domain organization to Hsp70 and the identification of an autonomous substrate-binding capacity led to the suggestion that Hsp110 directly contributes to protein disaggregation by contacting aggregated proteins at different positions and as part of a ternary complex with Hsp70 (Gao et al., 2015; Shorter, 2011). However, boosting of Hsp70 activity in disaggregation of model substrates solely relies on Hsp110 NEF function and does not require Hsp110 ATPase or substrate-binding activities (Garcia et al., 2017; Rampelt et al., 2012). Furthermore, substoichiometric ratios of Hsp110 to Hsp70 are optimal for disaggregation, while stoichiometric Hsp110 levels even inhibit Hsp70-dependent refolding of aggregated proteins, supporting a mere catalytic function of Hsp110 as NEF (Rampelt et al., 2012) (Figure 2B). The precise importance of substrate binding of Hsp110 remains to be elucidated. One could speculate that it helps, together with DNAJs, in tethering Hsp70s to the aggregates in cells; this could then also explain why other NEFs, such as Fes1 and BAGs (Kaimal et al., 2017; Rampelt et al., 2012),

may not be able to substitute for Hsp110 in efficient protein disaggregation. Although attractive, however, data to substantiate this speculation are yet entirely lacking. In addition or alternatively, as the most potent and most abundant cytosolic NEF, Hsp110 may serve *in vivo* to release substrates from Hsp70, thereby preventing the depletion of the functional pool of Hsp70 available for aggregate binding and disaggregation (Kaimal et al., 2017).

Cooperation of DNAJ Proteins Increases Hsp70 Disaggregation Activity

Crucial components of the metazoan Hsp70 co-chaperone machines enabling autonomous disaggregation are specific DNAJ proteins (Figure 2B). Synergistic networking of distinct DNAJ protein classes has been shown to confer higher disaggregation potential to eukaryotic Hsp70 (Nillegoda et al., 2015, 2017). Cooperating DNAJ proteins of classes A and B (e.g., class A-class B pairs of human DNAJA2/DNAJB1 and yeast Ydj1/Sis1) harbor a double β-barrel structure for substrate binding and differ by the presence of a zinc-binding domain (DNAJA2 and Ydj1) (Kampinga and Craig, 2010). Class A and class B DNAJ protein members are crucial players in general protein quality control but exhibit differences in aggregate binding, though it remains largely unclear which particular structural states are recognized by separate DNAJ proteins at the aggregate surface. DNAJ protein cooperation involves reciprocal transient electrostatic interactions between the DNAJ domain of one of the homodimers with the SBD of the other DNAJ homodimer and is suggested to nucleate the formation of larger oligomeric chaperone complexes upon Hsp70 recruitment (Nillegoda et al., 2015). Additionally, specific recognition of diverse surface patterns of protein aggregates by cooperating DNAJ proteins might increase Hsp70 loading beyond a critical level required to initiate disaggregation.

The disaggregation activity of metazoan Hsp70 machines is not restricted to stress-induced amorphous aggregates but also includes amyloid fibrils of the Parkinson disease protein α-synuclein (Gao et al., 2015; Shorter, 2011). Notably, efficient disaggregation of ordered amyloid aggregates by human Hsp70 does not require DNAJ protein cooperation but specific targeting of Hsp70 by DNAJB1 (Gao et al., 2015). The Hsp70-Hsp110-DNAJB1-based disaggregation machinery promotes the release of monomers by end depolymerization as well as fibril fragmentation. It is possible that these two outcomes result from different sites of action of the machinery (at fibril ends versus internal sites). It is also possible that pulling of α-synuclein molecules at fibril-internal sites eventually generates short oligomeric protofilament fragments, which are more unstable and facilitate detachment of α-synuclein molecules from the fibril ends (Gao et al., 2015). The biological implications of the generated products might be very different. Monomeric α-synuclein is non-toxic while fibrillar fragments might even act as seeds to stimulate amyloid formation, thereby eventually increasing toxicity (Gao et al., 2015). In analogy in *S. cerevisiae*, the fragmentation activity of Hsp70/Hsp104 toward prion fibrils is crucial for prion propagation by generating propagons that are transmitted to daughter cells (Chernova et al., 2017). To what extent fibril disaggregation is beneficial, therefore, remains to be investigated.

Force Generation by Metazoan Hsp70 Disaggregation Machine

How oligomeric DNAJ protein/Hsp70 complexes exactly exert disaggregation forces largely remains a black box, but can be described by several models (Goloubinoff and De Los Rios, 2007; Sousa, 2014) (Figure 2B). First, a power-stroke model may apply in which ATP-driven conformational changes in Hsp70 are transmitted to bound substrate, causing its local unfolding. Second, in a Brownian ratchet model Hsp70 could capture spontaneous structural fluctuations of substrates and stabilize the more detached, labile structures or prevent back-association of substrate segments with aggregate surfaces. Third, an entropic pulling model can be envisioned in which Hsp70 binding to a peptide stretch of an aggregated protein will restrict its mobility, thus lowering its entropic state. Movement of substrate-bound Hsp70 away from the aggregate surface would re-increase entropy, thereby applying a directional pulling force of 10–20 pN to the associated substrate (Goloubinoff and De Los Rios, 2007) (Figure 2B). In this scenario, Hsp70 harnesses the energy of DnaJ protein-stimulated ATP hydrolysis to tightly lock substrates at the aggregate surface and apply force upon random movements away from the aggregate. This entropic pulling model is in concordance with the mechanism proposed for Hsp70-mediated disassembly of clathrin complexes (here termed collision-pressure model), a reaction mechanistically similar to protein disaggregation (Sousa et al., 2016). Coordinated recruitment of large Hsp70 complexes by DnaJ protein networking and concerted complex dissociation by Hsp110-triggered nucleotide release might lead to increased pulling forces.

The disaggregation activity of metazoan Hsp70 is lower as compared to the Hsp70/Hsp100 disaggregation system. However, maybe metazoan might not normally require very high disaggregation activities given that the risk and degree of protein aggregation are likely lower in mobile, multicellular organisms than in unicellular eukaryotes and sessile plants, which are more exposed to severe environmental stress, causing more massive protein aggregation. This might also explain why the yeast Hsp70 system, despite the contributions of DnaJ and Hsp110, still relies on cooperation with the Hsp104 disaggregase for survival of severe stress conditions (Nillegoda et al., 2017). Specific differences between yeast and human Hsp70 chaperones as well as in the availability of DnaJ proteins might additionally contribute to diverse disaggregation powers. Notably, human Hsp70 and its constitutive isoform Hsc70 differ substantially in their disaggregation potentials, indicating that mechanistic differences also exist between metazoan Hsp70 chaperones (Gao et al., 2015). Whether changes in Hsp70 allostery or co-chaperone interactions are responsible for diverging Hsp70 disaggregation activities requires further investigation.

Fate of Aggregated Proteins

The products of the disaggregation reaction are soluble yet still unfolded proteins, raising the question of triage decision: is the disaggregated substrate targeted to refolding or degrading pathways? Proteostasis networks do not impose a categorical guideline. Decisions seem to be based on intrinsic substrate features, including refolding kinetics and affinities toward chaperones and

proteolytic components. However, for the Hsp100-Hsp70 bi-chaperone system, evidence indicates that substrate refolding is favored over degradation in bacteria and yeast. The efficient coating of the aggregate surface by Hsp70 conceivably restricts access of other protein quality control components (Haslberger et al., 2008). In bacteria, this restriction includes AAA+ chaperones of the Hsp100 family that associate with proteases, exhibit disaggregation activities *in vitro*, and directly target aggregated proteins to proteolysis (Kirstein et al., 2009). The Hsp104/ClpB disaggregases instead do not associate with proteases in contrast to the majority of Hsp100 family members. In direct competition *in vitro* and *in vivo*, the bacterial DnaK-ClpB system has prioritized access to aggregated substrates, as compared to proteolytic systems. This implies that for cell survival under stress conditions (i.e., thermotolerance), refolding of aggregated proteins, including those with essential cellular functions, is of critical importance. Concordantly, engineered Hsp104/ClpB disaggregases that are tailored to thread substrates into associated peptidases do not confer thermotolerance to *E. coli* and *S. cerevisiae* cells (Tessarz et al., 2008; Weibezahn et al., 2004). A recent proteomic study furthermore showed that heat-aggregated yeast proteins are quantitatively refolded without degradation occurring, underlining that refolding rather than destruction is the primary cellular strategy to cope with thermal stress (Wallace et al., 2015). This strategy will save cellular resources, will allow for faster recovery upon stress relief, and can even become essential for cell growth as central factors of transcription and translation machineries are depleted upon heat-induced aggregation (Mogk et al., 1999; Wallace et al., 2015). The rapid kinetics and high refolding efficiencies of heat-aggregated luciferase in mammalian cells suggest this strategy is evolutionarily conserved. However, the fraction that can be recovered from the aggregates strongly depends on the severity of the heat shock conceived (Michels et al., 1997; Nollen et al., 1999). Furthermore, aggregated proteins can also be targeted to the 26S proteasome after Hsp70-mediated disaggregation. In this pathway, UBQLN2 serves as bridging factor linking Hsp70 and bound substrates to the proteasome (Hjerpe et al., 2016). Polyubiquitination of the disaggregated substrate is required for substrate targeting, providing direct information for pathway decision (Hjerpe et al., 2016).

sHsps Facilitate Protein Disaggregation and Impact Fate Decisions

Whereas the machines described above suffice to disentangle protein aggregates, their efficiency can be increased substantially if cellular factors were present during the aggregation-initiating event. Small heat shock proteins (sHsps) represent key components that modulate protein aggregation and impact on downstream disaggregation processes. sHsps have been most extensively studied for their role as "holdases," hereby changing aggregate structure and size (Figure 3). Some sHsps also assist to sequester bound substrates into large inclusions (sequestrosomes) (Escusa-Toret et al., 2013; Specht et al., 2011) (Figures 1 and 3). Yeast cells lacking such "sequestrase activity" exhibit reduced fitness during stress conditions, implying that protein aggregation, if organized and controlled by cellular sequestrases, can be cytoprotective (Escusa-Toret et al., 2013; Ungelenk et al., 2016). A protective function might be explained by confining the sticky interactive surface of soluble misfolded proteins, thereby preventing aberrant interactions with other cellular factors but also reducing chaperone load. sHsp sequestrase function seems to become particularly important in aged organisms and has indeed been genetically linked to longevity in fruit flies (Morrow et al., 2004; Vos et al., 2016) and nematodes (Hsu et al., 2003; Walther et al., 2015). In agreement with such a role, long-lived nematodes accumulate specific sHsps in the insoluble cell fraction during aging, implying a cytoprotective aggregation response (Walther et al., 2015). In addition, the organized sequestration of misfolded proteins by sHsps is part of a mechanism to facilitate protein disaggregation (see below).

sHsps associate with misfolded proteins, preventing their uncontrolled aggregation, yet they do not display refolding activities. sHsps consist of a conserved α-crystallin domain, which is flanked by variable N- and C-terminal extensions (Haslbeck and Vierling, 2015) (Figure 3A). Most sHsps form dynamic oligomeric assemblies of diverse subunit numbers (12 to >48) and oligomer dynamics positively correlates with chaperone activity (Haslbeck and Vierling, 2015). All sHsp domains/extensions contribute to substrate binding; however, the intrinsically disordered N-terminal region provides most substrate contacts. The N-terminal extension is frequently enriched in aromatic residues, likely providing affinity for misfolded proteins. Many sHsps interact with early-unfolding intermediates of substrates and assemble with them into large oligomers. This rapid association preserves a native-like state of bound substrates and protects them from further unfolding (Cheng et al., 2008; Ungelenk et al., 2016) (Figure 3B). sHsps thereby act as a first line of cellular defense against unfolding stress and create a reservoir of proteins competent for disaggregation and refolding upon stress relief (Ehrnsperger et al., 1997; Kampinga et al., 1994; Lee et al., 1997). Of note, however, a holdase activity may not be shared among all members of the sHsp family, and in humans not all members share the same anti-aggregation capacity (Mymrikov et al., 2017; Vos et al., 2008).

Protein aggregates that include sHsps are altered in size, composition, and architecture (Figure 3B). The majority of data suggests that sHsp/substrate complexes are rather stable entities and substrates are not (or only rarely) spontaneously released. Substrate displacement thus requires force application by Hsp70 and Hsp100 disaggregases to disrupt sHsp-substrate interactions, similar to disaggregation of protein aggregates generated in their absence. However, the formation of mixed sHsp-unfolded substrate complexes facilitates post-stress disaggregation of substrates *in vivo* (Kampinga et al., 1994; Mogk et al., 2003a; Ungelenk et al., 2016) and *in vitro* (Haslbeck et al., 2005; Mogk et al., 2003b; Nillegoda et al., 2015) as compared to substrates aggregated in isolation (Figure 3B). sHsps seem to prevent the formation of more tight interactions between the unfolded proteins. In addition, even though sHsps are ATP independent, they may provide some dynamics to the trapped proteins as sHsps reside mobile in sHsp/substrate complexes via their oligomerization dynamics. Together, these features of sHsp-substrate complexes may facilitate the untangling of aggregated proteins by the disaggregation machines.

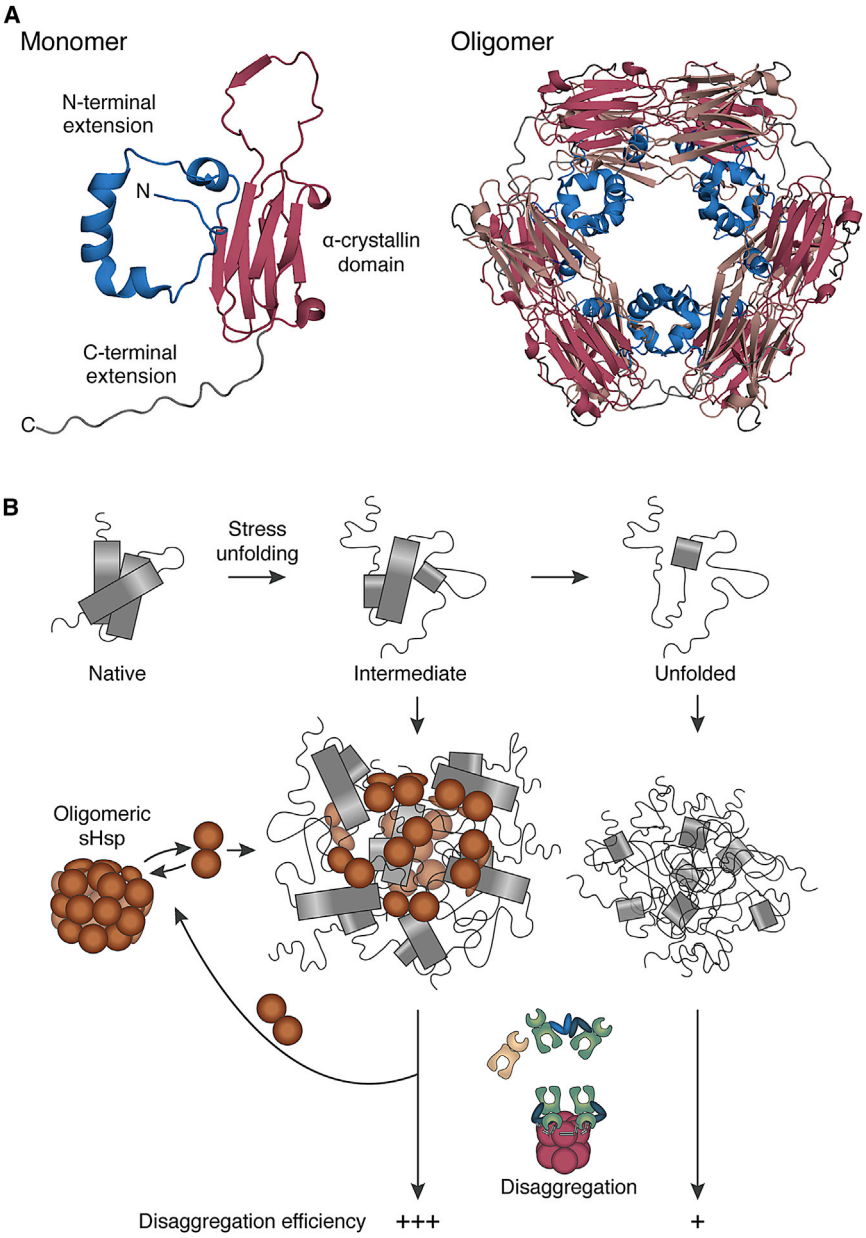

Figure 3. Modulation of Aggregate Structure and Disaggregation Efficiency by sHsps

(A) Domain organization and structure of sHsps. sHsps are composed of a conserved, central α-crystallin domain (ACD), which is flanked by disordered and variable N-terminal and C-terminal extensions (NTE and CTE). The structure of wheat Hsp16.9 monomer (left) and oligomer (right) is shown. The ACD is the building block for dimer and oligomer formation. The CTE contributes to oligomerization by interacting with adjacent ACD dimer.

(B) sHsps sequester substrates in near-native states to form sHsp/substrate complexes, leading to higher disaggregation efficiencies. Without sHsps, protein substrates unfold upon stress application to form tight aggregates largely devoid of residual tertiary structure. sHsps bind early unfolding intermediates retaining substantial tertiary structure and form sHsp/substrate complexes, which differ in composition, size, and architecture as compared to protein aggregates formed without sHsps. sHsp oligomers can dissociate into dimeric species that interact with substrate proteins. Some sHsp molecules dynamically bind and dissociate from the sHsp/substrate complex. Hsp70 can displace surface-located sHsps and initiate protein disaggregation. For both Hsp70/Hsp100 and Hsp70-only disaggregation machineries, the disaggregation of sHsp-substrate complexes is faster and more efficient as compared to protein aggregates that are not complexed with sHsp.

sHsps additionally can provide selectivity toward triage decisions in that they guide bound substrates preferentially to refolding pathways (Żwirowski et al., 2017). sHsps cooperate with Hsp70 chaperones originating from diverse species, arguing against direct physical contacts as the basis for the sHsp-Hsp70 interconnection. Recent data suggest that the Hsp70 preference is based on the molecular architecture of sHsp/substrate complexes, which are suggested to be composed of an immobile core largely consisting of substrates and an outer shell built of dynamic sHsps (Żwirowski et al., 2017). The outer sHsps shield the sticky aggregate surface and prevent access by diverse cellular components including proteases (Figure 3B). Only Hsp70 is capable of displacing the outer dynamic sHsps and getting access to the inner core structure presumably caused by tighter binding to the aggregated proteins. Efficient sHsp displacement demands elevated Hsp70 levels and might sensitize substrate release toward cellular Hsp70 capacity. Since this capacity is low during stress conditions, substrate dissociation and subsequent refolding will be delayed till favorable growth and folding conditions are available (Żwirowski et al., 2017).

In summary, sHsps modulate protein aggregation and facilitate downstream disaggregation processes by modifying the conformational state of misfolded proteins and changing aggregate size and architecture.

Perspectives and Future Directions

Protein aggregation can be detrimental to cellular homeostasis as it causes loss of viability during extreme stress conditions and is linked to various disease states including neuro- and (cardio)muscular degeneration. Even when aggregation is executed in an organized manner to reduce toxicity, sequestered proteins need to be disaggregated to regain functionality. All cells are equipped with disaggregation machineries; however, their capacities are limited and they also decline in aged organisms, which may be one of the factors contributing to the

late onset of these degenerative diseases. Increasing the disaggregation potential of cells to prevent or revert protein aggregation is therefore an attractive strategy to prevent or delay outbreak of aggregation-linked diseases. Increasing disaggregation potential might also confer superior stress resistance to cells and organisms, an approach that seems particularly attractive for cultivated plants.

In a proof-of-principle study, potentiated Hsp104 disaggregase variants exhibiting increased disaggregation power were shown to revert toxic protein aggregation of disease proteins in yeast cells and in a *C. elegans* α-synuclein model system (Jackrel et al., 2014). While these findings are encouraging, empowered disaggregases also raise issues that need to be considered. Potentiated Hsp104 mutants cause severe cellular damage, which likely results from uncontrolled protein unfolding events (Lipińska et al., 2013; Schirmer et al., 2004). This indicates a delicate balance between beneficial and toxic effects of increased disaggregation potential. Enhanced disaggregation might also generate more short fibrils and oligomeric species from amyloid aggregates, thereby eventually increasing amyloid seeding capacity and enhancing prion-like spreading of disease conformers. Hsp70/Hsp100-based disaggregation therefore has Janus head features, and its activity and substrate selectivity require careful control mechanisms. Increasing substrate specificity of potentiated Hsp104 could prevent off-target binding to circumvent toxic side effects. Other strategies could employ the search for novel disaggregase systems. For example, the novel, standalone AAA+ disaggregase ClpG/ClpK confers superior heat tolerance to some bacterial species (Lee et al., 2017).

In conclusion, a bit over 25 years after the groundbreaking discoveries of Susan Lindquist's laboratory on the eminent importance of protein disaggregation for stress resistance in yeast, we are now beginning to understand how these processes are executed at the molecular level by diverse machines and how they impact cellular physiology and disease development. Future insights into the specification specificities and mechanisms of these aggregate disentangling machines will be needed to translate them into amenable targets for increasing cellular and organismal resistance to stress and disease. A major open question concerns the molecular basis of the pathway decisions directing misfolded proteins to sequestration, refolding, or degradation by UPS and autophagy, and how various disease and stress states impact these decisions. The latter includes the unknown nature of the sorting principles for misfolded proteins, which must rely on biochemical features of the substrates and target selectivity of the interacting quality control components. With respect to the protein disaggregation machinery, it will be important to dissect the regulatory framework of the Hsp70s and their plethora of co-chaperones, which exhibit unexpectedly high complexity and versatility. All protein disaggregation machines, from the bacterial/fungal Hsp100-Hsp70 bi-chaperone system to the metazoan Hsp70 disaggregase, are potentially harmful to cells and hence require tight regulation of their activities. The underlying mechanisms of repression and target-specific activation of these machineries need to be elucidated further, as they are at the heart of the cellular strategies fighting stress and disease states.

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REFERENCES

Acebrón, S.P., Martín, I., del Castillo, U., Moro, F., and Muga, A. (2009). DnaK-mediated association of ClpB to protein aggregates. A bichaperone network at the aggregate surface. FEBS Lett. *583*, 2991–2996.

Aguilaniu, H., Gustafsson, L., Rigoulet, M., and Nyström, T. (2003). Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science *299*, 1751–1753.

Balch, W.E., Morimoto, R.I., Dillin, A., and Kelly, J.W. (2008). Adapting proteostasis for disease intervention. Science *319*, 916–919.

Balchin, D., Hayer-Hartl, M., and Hartl, F.U. (2016). In vivo aspects of protein folding and quality control. Science *353*, aac4354.

Carrió, M.M., and Villaverde, A. (2005). Localization of chaperones DnaK and GroEL in bacterial inclusion bodies. J. Bacteriol. *187*, 3599–3601.

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

Cheng, G., Basha, E., Wysocki, V.H., and Vierling, E. (2008). Insights into small heat shock protein and substrate structure during chaperone action derived from hydrogen/deuterium exchange and mass spectrometry. J. Biol. Chem. *283*, 26634–26642.

Chernova, T.A., Wilkinson, K.D., and Chernoff, Y.O. (2017). Prions, chaperones, and proteostasis in yeast. Cold Spring Harb. Perspect. Biol. *9*, https://doi.org/10.1101/cshperspect.a023663.

Chiti, F., and Dobson, C.M. (2017). Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. Annu. Rev. Biochem. *86*, 27–68.

Chu, C.T., Plowey, E.D., Wang, Y., Patel, V., and Jordan-Sciutto, K.L. (2007). Location, location, location: altered transcription factor trafficking in neurodegeneration. J. Neuropathol. Exp. Neurol. *66*, 873–883.

De Vos, K.J., Grierson, A.J., Ackerley, S., and Miller, C.C. (2008). Role of axonal transport in neurodegenerative diseases. Annu. Rev. Neurosci. *31*, 151–173.

Deville, C., Carroni, M., Franke, K.B., Topf, M., Bukau, B., Mogk, A., and Saibil, H.R. (2017). Structural pathway of regulated substrate transfer and threading through an Hsp100 disaggregase. Sci. Adv. *3*, e1701726.

Diamant, S., Ben-Zvi, A.P., Bukau, B., and Goloubinoff, P. (2000). Size-dependent disaggregation of stable protein aggregates by the DnaK chaperone machinery. J. Biol. Chem. *275*, 21107–21113.

Duran, E.C., Weaver, C.L., and Lucius, A.L. (2017). Comparative analysis of the structure and function of AAA+ motors ClpA, ClpB, and Hsp104: common threads and disparate functions. Front. Mol. Biosci. *4*, 54.

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

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

Gao, X., Carroni, M., Nussbaum-Krammer, C., Mogk, A., Nillegoda, N.B., Szlachcic, A., Guilbride, D.L., Saibil, H.R., Mayer, M.P., and Bukau, B. (2015). Human Hsp70 disaggregase reverses Parkinson's-linked α-synuclein amyloid fibrils. Mol. Cell *59*, 781–793.

Garcia, V.M., Nillegoda, N.B., Bukau, B., and Morano, K.A. (2017). Substrate binding by the yeast Hsp110 nucleotide exchange factor and molecular

chaperone Sse1 is not obligate for its biological activities. Mol. Biol. Cell *28*, 2066–2075.

Gates, S.N., Yokom, A.L., Lin, J., Jackrel, M.E., Rizo, A.N., Kendsersky, N.M., Buell, C.E., Sweeny, E.A., Mack, K.L., Chuang, E., et al. (2017). Ratchet-like polypeptide translocation mechanism of the AAA+ disaggregase Hsp104. Science *357*, 273–279.

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

Goloubinoff, P., and De Los Rios, P. (2007). The mechanism of Hsp70 chaperones: (entropic) pulling the models together. Trends Biochem. Sci. *32*, 372–380.

Grima, J.C., Daigle, J.G., Arbez, N., Cunningham, K.C., Zhang, K., Ochaba, J., Geater, C., Morozko, E., Stocksdale, J., Glatzer, J.C., et al. (2017). Mutant Huntingtin disrupts the nuclear pore complex. Neuron *94*, 93–107.e6.

Gusella, J.F., and MacDonald, M.E. (2000). Molecular genetics: unmasking polyglutamine triggers in neurodegenerative disease. Nat. Rev. Neurosci. *1*, 109–115.

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

Haslbeck, M., Miess, A., Stromer, T., Walter, S., and Buchner, J. (2005). Disassembling protein aggregates in the yeast cytosol. The cooperation of Hsp26 with Ssa1 and Hsp104. J. Biol. Chem. *280*, 23861–23868.

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

Hayashi, S., Nakazaki, Y., Kagii, K., Imamura, H., and Watanabe, Y.H. (2017). Fusion protein analysis reveals the precise regulation between Hsp70 and Hsp100 during protein disaggregation. Sci. Rep. *7*, 8648.

Heuck, A., Schitter-Sollner, S., Suskiewicz, M.J., Kurzbauer, R., Kley, J., Schleiffer, A., Rombaut, P., Herzog, F., and Clausen, T. (2016). Structural basis for the disaggregase activity and regulation of Hsp104. eLife *5*, https://doi.org/10.7554/eLife.21516.

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

Hjerpe, R., Bett, J.S., Keuss, M.J., Solovyova, A., McWilliams, T.G., Johnson, C., Sahu, I., Varghese, J., Wood, N., Wightman, M., et al. (2016). UBQLN2 mediates autophagy-independent protein aggregate clearance by the proteasome. Cell *166*, 935–949.

Hsu, A.L., Murphy, C.T., and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science *300*, 1142–1145.

Jackrel, M.E., DeSantis, M.E., Martinez, B.A., Castellano, L.M., Stewart, R.M., Caldwell, K.A., Caldwell, G.A., and Shorter, J. (2014). Potentiated Hsp104 variants antagonize diverse proteotoxic misfolding events. Cell *156*, 170–182.

Johnston, J.A., Ward, C.L., and Kopito, R.R. (1998). Aggresomes: a cellular response to misfolded proteins. J. Cell Biol. *143*, 1883–1898.

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

Kaimal, J.M., Kandasamy, G., Gasser, F., and Andréasson, C. (2017). Coordinated Hsp110 and Hsp104 activities power protein disaggregation in Saccharomyces cerevisiae. Mol. Cell. Biol. *37*, https://doi.org/10.1128/MCB.00027-17.

Kakkar, V., Meister-Broekema, M., Minoia, M., Carra, S., and Kampinga, H.H. (2014). Barcoding heat shock proteins to human diseases: looking beyond the heat shock response. Dis. Model. Mech. *7*, 421–434.

Kampinga, H.H., and Bergink, S. (2016). Heat shock proteins as potential targets for protective strategies in neurodegeneration. Lancet Neurol. *15*, 748–759.

Kampinga, H.H., and Craig, E.A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat. Rev. Mol. Cell Biol. *11*, 579–592.

Kampinga, H.H., Jorritsma, J.B., and Konings, A.W. (1985). Heat-induced alterations in DNA polymerase activity of HeLa cells and of isolated nuclei. Relation to cell survival. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. *47*, 29–40.

Kampinga, H.H., Luppes, J.G., and Konings, A.W. (1987). Heat-induced nuclear protein binding and its relation to thermal cytotoxicity. Int. J. Hyperthermia *3*, 459–465.

Kampinga, H.H., Brunsting, J.F., Stege, G.J., Konings, A.W., and Landry, J. (1994). Cells overexpressing Hsp27 show accelerated recovery from heat-induced nuclear protein aggregation. Biochem. Biophys. Res. Commun. *204*, 1170–1177.

Kirstein, J., Molière, N., Dougan, D.A., and Turgay, K. (2009). Adapting the machine: adaptor proteins for Hsp100/Clp and AAA+ proteases. Nat. Rev. Microbiol. *7*, 589–599.

Kirstein, J., Arnsburg, K., Scior, A., Szlachcic, A., Guilbride, D.L., Morimoto, R.I., Bukau, B., and Nillegoda, N.B. (2017). In vivo properties of the disaggregase function of J-proteins and Hsc70 in Caenorhabditis elegans stress and aging. Aging Cell *16*, 1414–1424.

Kuiper, E.F., de Mattos, E.P., Jardim, L.B., Kampinga, H.H., and Bergink, S. (2017). Chaperones in polyglutamine aggregation: beyond the Q-stretch. Front. Neurosci. *11*, 145.

Lashuel, H.A., Hartley, D., Petre, B.M., Walz, T., and Lansbury, P.T., Jr. (2002). Neurodegenerative disease: amyloid pores from pathogenic mutations. Nature *418*, 291.

Lee, G.J., Roseman, A.M., Saibil, H.R., and Vierling, E. (1997). A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J. *16*, 659–671.

Lee, C., Franke, K.B., Kamal, S.M., Kim, H., Lünsdorf, H., Jäger, J., Nimtz, M., Trček, J., Jänsch, L., Bukau, B., et al. (2017). Stand-alone ClpG disaggregase confers superior heat tolerance to bacteria. Proc. Natl. Acad. Sci. USA. Published online December 20, 2017. https://doi.org/10.1073/pnas.1712051115.

Lindquist, S., and Kim, G. (1996). Heat-shock protein 104 expression is sufficient for thermotolerance in yeast. Proc. Natl. Acad. Sci. USA *93*, 5301–5306.

Lipińska, N., Ziętkiewicz, S., Sobczak, A., Jurczyk, A., Potocki, W., Morawiec, E., Wawrzycka, A., Gumowski, K., Slusarz, M., Rodziewicz-Motowidło, S., et al. (2013). Disruption of ionic interactions between the nucleotide binding domain 1 (NBD1) and middle (M) domain in Hsp100 disaggregase unleashes toxic hyperactivity and partial independence from Hsp70. J. Biol. Chem. *288*, 2857–2869.

Marcinowski, M., Höller, M., Feige, M.J., Baerend, D., Lamb, D.C., and Buchner, J. (2011). Substrate discrimination of the chaperone BiP by autonomous and cochaperone-regulated conformational transitions. Nat. Struct. Mol. Biol. *18*, 150–158.

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

Melentijevic, I., Toth, M.L., Arnold, M.L., Guasp, R.J., Harinath, G., Nguyen, K.C., Taub, D., Parker, J.A., Neri, C., Gabel, C.V., et al. (2017). C. elegans neurons jettison protein aggregates and mitochondria under neurotoxic stress. Nature *542*, 367–371.

Michels, A.A., Kanon, B., Konings, A.W., Ohtsuka, K., Bensaude, O., and Kampinga, H.H. (1997). Hsp70 and Hsp40 chaperone activities in the cytoplasm and the nucleus of mammalian cells. J. Biol. Chem. *272*, 33283–33289.

Miller, S.B., Ho, C.T., Winkler, J., Khokhrina, M., Neuner, A., Mohamed, M.Y., Guilbride, D.L., Richter, K., Lisby, M., Schiebel, E., et al. (2015). Compartment-specific aggregases direct distinct nuclear and cytoplasmic aggregate deposition. EMBO J. *34*, 778–797.

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

Mogk, A., Deuerling, E., Vorderwülbecke, S., Vierling, E., and Bukau, B. (2003a). Small heat shock proteins, ClpB and the DnaK system form a functional triade in reversing protein aggregation. Mol. Microbiol. *50*, 585–595.

Mogk, A., Schlieker, C., Friedrich, K.L., Schönfeld, H.J., Vierling, E., and Bukau, B. (2003b). Refolding of substrates bound to small Hsps relies on a disaggregation reaction mediated most efficiently by ClpB/DnaK. J. Biol. Chem. *278*, 31033–31042.

Morley, J.F., Brignull, H.R., Weyers, J.J., and Morimoto, R.I. (2002). The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *99*, 10417–10422.

Morrow, G., Battistini, S., Zhang, P., and Tanguay, R.M. (2004). Decreased lifespan in the absence of expression of the mitochondrial small heat shock protein Hsp22 in Drosophila. J. Biol. Chem. *279*, 43382–43385.

Mymrikov, E.V., Daake, M., Richter, B., Haslbeck, M., and Buchner, J. (2017). The chaperone activity and substrate spectrum of human small heat shock proteins. J. Biol. Chem. *292*, 672–684.

Nillegoda, N.B., Kirstein, J., Szlachcic, A., Berynskyy, M., Stank, A., Stengel, F., Arnsburg, K., Gao, X., Scior, A., Aebersold, R., et al. (2015). Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. Nature *524*, 247–251.

Nillegoda, N.B., Stank, A., Malinverni, D., Alberts, N., Szlachcic, A., Barducci, A., De Los Rios, P., Wade, R.C., and Bukau, B. (2017). Evolution of an intricate J-protein network driving protein disaggregation in eukaryotes. eLife *6*, https://doi.org/10.7554/eLife.24560.

Nollen, E.A., Brunsting, J.F., Roelofsen, H., Weber, L.A., and Kampinga, H.H. (1999). In vivo chaperone activity of heat shock protein 70 and thermotolerance. Mol. Cell. Biol. *19*, 2069–2079.

Oguchi, Y., Kummer, E., Seyffer, F., Berynskyy, M., Anstett, B., Zahn, R., Wade, R.C., Mogk, A., and Bukau, B. (2012). A tightly regulated molecular toggle controls AAA+ disaggregase. Nat. Struct. Mol. Biol. *19*, 1338–1346.

Oh, H.J., Chen, X., and Subjeck, J.R. (1997). Hsp110 protects heat-denatured proteins and confers cellular thermoresistance. J. Biol. Chem. *272*, 31636–31640.

Oromendia, A.B., Dodgson, S.E., and Amon, A. (2012). Aneuploidy causes proteotoxic stress in yeast. Genes Dev. *26*, 2696–2708.

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

Pinto, M., Morange, M., and Bensaude, O. (1991). Denaturation of proteins during heat shock. In vivo recovery of solubility and activity of reporter enzymes. J. Biol. Chem. *266*, 13941–13946.

Queitsch, C., Hong, S.W., Vierling, E., and Lindquist, S. (2000). Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. Plant Cell *12*, 479–492.

Rampelt, H., Kirstein-Miles, J., Nillegoda, N.B., Chi, K., Scholz, S.R., Morimoto, R.I., and Bukau, B. (2012). Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. EMBO J. *31*, 4221–4235.

Rinas, U., Garcia-Fruitós, E., Corchero, J.L., Vázquez, E., Seras-Franzoso, J., and Villaverde, A. (2017). Bacterial inclusion bodies: discovering their better half. Trends Biochem. Sci. *42*, 726–737.

Rosenzweig, R., Moradi, S., Zarrine-Afsar, A., Glover, J.R., and Kay, L.E. (2013). Unraveling the mechanism of protein disaggregation through a ClpB-DnaK interaction. Science *339*, 1080–1083.

Rosenzweig, R., Farber, P., Velyvis, A., Rennella, E., Latham, M.P., and Kay, L.E. (2015). ClpB N-terminal domain plays a regulatory role in protein disaggregation. Proc. Natl. Acad. Sci. USA *112*, E6872–E6881.

Rüdiger, S., Schneider-Mergener, J., and Bukau, B. (2001). Its substrate specificity characterizes the DnaJ co-chaperone as a scanning factor for the DnaK chaperone. EMBO J. *20*, 1042–1050.

Rujano, M.A., Bosveld, F., Salomons, F.A., Dijk, F., van Waarde, M.A., van der Want, J.J., de Vos, R.A., Brunt, E.R., Sibon, O.C., and Kampinga, H.H. (2006). Polarised asymmetric inheritance of accumulated protein damage in higher eukaryotes. PLoS Biol. *4*, e417.

Sanchez, Y., and Lindquist, S.L. (1990). HSP104 required for induced thermotolerance. Science *248*, 1112–1115.

Schirmer, E.C., Homann, O.R., Kowal, A.S., and Lindquist, S. (2004). Dominant gain-of-function mutations in Hsp104p reveal crucial roles for the middle region. Mol. Biol. Cell *15*, 2061–2072.

Schlecht, R., Erbse, A.H., Bukau, B., and Mayer, M.P. (2011). Mechanics of Hsp70 chaperones enables differential interaction with client proteins. Nat. Struct. Mol. Biol. *18*, 345–351.

Seyffer, F., Kummer, E., Oguchi, Y., Winkler, J., Kumar, M., Zahn, R., Sourjik, V., Bukau, B., and Mogk, A. (2012). Hsp70 proteins bind Hsp100 regulatory M domains to activate AAA+ disaggregase at aggregate surfaces. Nat. Struct. Mol. Biol. *19*, 1347–1355.

Shaner, L., Trott, A., Goeckeler, J.L., Brodsky, J.L., and Morano, K.A. (2004). The function of the yeast molecular chaperone Sse1 is mechanistically distinct from the closely related hsp70 family. J. Biol. Chem. *279*, 21992–22001.

Shorter, J. (2011). The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. PLoS ONE *6*, e26319.

Smith, H.L., and Mallucci, G.R. (2016). The unfolded protein response: mechanisms and therapy of neurodegeneration. Brain *139*, 2113–2121.

Sousa, R. (2014). Structural mechanisms of chaperone mediated protein disaggregation. Front. Mol. Biosci. *1*, 12.

Sousa, R., Liao, H.S., Cuéllar, J., Jin, S., Valpuesta, J.M., Jin, A.J., and Lafer, E.M. (2016). Clathrin-coat disassembly illuminates the mechanisms of Hsp70 force generation. Nat. Struct. Mol. Biol. *23*, 821–829.

Specht, S., Miller, S.B., Mogk, A., and Bukau, B. (2011). Hsp42 is required for sequestration of protein aggregates into deposition sites in Saccharomyces cerevisiae. J. Cell Biol. *195*, 617–629.

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

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

Vos, M.J., Hageman, J., Carra, S., and Kampinga, H.H. (2008). Structural and functional diversities between members of the human HSPB, HSPH, HSPA, and DNAJ chaperone families. Biochemistry *47*, 7001–7011.

Vos, M.J., Carra, S., Kanon, B., Bosveld, F., Klauke, K., Sibon, O.C., and Kampinga, H.H. (2016). Specific protein homeostatic functions of small heat-shock proteins increase lifespan. Aging Cell *15*, 217–226.

Wallace, E.W., Kear-Scott, J.L., Pilipenko, E.V., Schwartz, M.H., Laskowski, P.R., Rojek, A.E., Katanski, C.D., Riback, J.A., Dion, M.F., Franks, A.M., et al. (2015). Reversible, specific, active aggregates of endogenous proteins assemble upon heat stress. Cell *162*, 1286–1298.

Walters, R.W., and Parker, R. (2015). Coupling of ribostasis and proteostasis: Hsp70 proteins in mRNA metabolism. Trends Biochem. Sci. *40*, 552–559.

Walther, D.M., Kasturi, P., Zheng, M., Pinkert, S., Vecchi, G., Ciryam, P., Morimoto, R.I., Dobson, C.M., Vendruscolo, M., Mann, M., and Hartl, F.U. (2015). Widespread proteome remodeling and aggregation in aging C. elegans. Cell *161*, 919–932.

Weibezahn, J., Tessarz, P., Schlieker, C., Zahn, R., Maglica, Z., Lee, S., Zentgraf, H., Weber-Ban, E.U., Dougan, D.A., Tsai, F.T., et al. (2004). Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. Cell *119*, 653–665.

Werbeck, N.D., Schlee, S., and Reinstein, J. (2008). Coupling and dynamics of subunits in the hexameric AAA+ chaperone ClpB. J. Mol. Biol. *378*, 178–190.

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

Xu, X., Sarbeng, E.B., Vorvis, C., Kumar, D.P., Zhou, L., and Liu, Q. (2012). Unique peptide substrate binding properties of 110-kDa heat-shock protein (Hsp110) determine its distinct chaperone activity. J. Biol. Chem. *287*, 5661–5672.

Yamamoto, A., and Simonsen, A. (2011). The elimination of accumulated and aggregated proteins: a role for aggrephagy in neurodegeneration. Neurobiol. Dis. *43*, 17–28.

Zhou, C., Slaughter, B.D., Unruh, J.R., Guo, F., Yu, Z., Mickey, K., Narkar, A., Ross, R.T., McClain, M., and Li, R. (2014). Organelle-based aggregation and retention of damaged proteins in asymmetrically dividing cells. Cell *159*, 530–542.

Zietkiewicz, S., Krzewska, J., and Liberek, K. (2004). Successive and synergistic action of the Hsp70 and Hsp100 chaperones in protein disaggregation. J. Biol. Chem. *279*, 44376–44383.

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