



# Challenging Proteostasis: Role of the Chaperone Network to Control Aggregation-Prone Proteins in Human Disease

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## Abstract

Protein homeostasis (Proteostasis) is essential for correct and efficient protein function within the living cell. Among the critical components of the Proteostasis Network (PN) are molecular chaperones that serve widely in protein biogenesis under physiological conditions, and prevent protein misfolding and aggregation enhanced by conditions of cellular stress. For Alzheimer's, Parkinson's, Huntington's diseases and ALS, multiple classes of molecular chaperones interact with the highly aggregation-prone proteins amyloid- $\beta$ , tau,  $\alpha$ -synuclein, huntingtin and SOD1 to influence the course of proteotoxicity associated with these neurodegenerative diseases. Accordingly, overexpression of molecular chaperones and induction of the heat shock response have been shown to be protective in a wide range of animal models of these diseases. In contrast, for cancer cells the upregulation of chaperones has the undesirable effect of promoting cellular survival and tumor growth by stabilizing mutant oncoproteins. In both situations, physiological levels of molecular chaperones eventually become

functionally compromised by the persistence of misfolded substrates, leading to a decline in global protein homeostasis and the dysregulation of diverse cellular pathways. The phenomenon of chaperone competition may underlie the broad pathology observed in aging and neurodegenerative diseases, and restoration of physiological protein homeostasis may be a suitable therapeutic avenue for neurodegeneration as well as for cancer.

## Keywords

Protein misfolding · Molecular chaperones · Neurodegenerative diseases · Proteostasis

## 4.1 Introduction

Protein homeostasis is regulated by the proteostasis network (PN) to control protein synthesis, folding and macromolecular assembly, localization, and degradation, processes that are essential for all living cells and organisms. An imbalance in the PN enhances the properties of destabilized mutant proteins that take advantage of the capacity of molecular chaperones to escape unfolding and degradation, leading to malignant phenotypes in cancer (see other chapters in this collection). The opposite scenario of failure of protein homeostasis is associated with aging and a plethora of

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protein misfolding diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). In this chapter, we discuss how interactions between molecular chaperones and neurodegenerative disease-associated substrates amyloid- $\beta$  (A $\beta$ ), tau,  $\alpha$ -synuclein, polyglutamine-expansion proteins and SOD1 are exploited *in vivo* to counteract toxic protein aggregation, whereas these same interactions can lead to the sequestration of molecular chaperones and the collapse of proteostasis.

The main classes of molecular chaperones are also known as heat shock proteins (Hsps) after the discovery in *Drosophila* that the expression of Hsps is induced by heat shock, a sudden increase in temperature. However, many of the genes encoding these chaperones are also constitutively expressed to ensure the proper balance of protein synthesis, folding, trafficking and translocation of a wide variety of proteins under physiological conditions. Under conditions of cellular stress such as heat shock, molecular chaperones such as Hsp70 and the J-domain protein Hdj-1 are titrated by misfolded client proteins from association with heat shock transcription factor 1 (HSF1) with which they interact under normal growth conditions (Zheng et al. 2016; Abravaya et al. 1992; Shi et al. 1998). Upon release from chaperones, HSF1 forms functional trimers that bind to heat shock elements in the promoters of genes encoding molecular chaperones and other components of the PN. This results in the release of the paused RNA polymerase II, post-translational modifications of HSF1 and rapid inducible transcription of the heat shock genes. The heat shock transcriptional response can attenuate either during prolonged exposure to heat shock stress as translation is arrested at the heat shock temperature, or upon return to ambient conditions, in both scenarios reducing the requirement for chaperones to prevent further misfolding (Abravaya et al. 1992; Shi et al. 1998; Krakowiak et al. 2018).

Among the most ubiquitous chaperones are the Hsp70 family that is essential for protein synthesis and folding of a wide range of client proteins, via a mechanism that involves cycles of

substrate binding and release driven by ATP hydrolysis. Hsp70 and its constitutively expressed counterpart Hsc70 function in a molecular machine that involves co-chaperones including Hsp40 proteins, which recruit substrates and regulate ATP hydrolysis of Hsp70, and Hsp110 and BAG proteins, which serve as nucleotide exchange factors (NEFs) (Kampinga and Craig 2010; Kim et al. 2013). Depending on the nucleotide binding and co-chaperone interactions, Hsp70 can function to hold non-native clients in a folding competent state or direct folding to a functional state (Freeman et al. 1995; Freeman and Morimoto 1996). Furthermore, specific combinations of Hsp70, Hsp40 and Hsp110 proteins form disaggregase machineries that can resolve luciferase aggregates (Nillegoda et al. 2015) and  $\alpha$ -synuclein fibrils (Gao et al. 2015). The Hsp70 chaperone system also interacts with Hsp90, which is another ATP-dependent chaperone. Hsp90 interacts with specific co-chaperones downstream of Hsp70 to aid the folding of a wide array of clients including kinases, phosphatases, transcription factors and other signalling molecules (Morán Luengo et al. 2019). The chaperonins of the Hsp60 family, corresponding to GroEL in bacteria and TRiC/CCT in eukaryotes, exist as multimeric assemblies that form a cage in which substrates are allowed to obtain their native fold dependent upon ATP hydrolysis. This machinery is critical to fold large filamentous proteins such as actin and tubulin (Hayer-Hartl et al. 2016; Gestaut et al. 2019). The small Hsps (sHsps) are ATP-independent chaperones and can function in holding denatured or non-native protein conformations to prevent their misfolding and aggregation (Treweek et al. 2015).

In this chapter we will discuss the detailed modes of interaction between these classes of molecular chaperones and aggregation-prone proteins and peptides as characterised *in vitro*, and summarize evidence that chaperone activity is beneficial to combat protein aggregation in *in vivo* models of neurodegenerative diseases. Under these disease conditions, molecular chaperones may eventually become overwhelmed by these misfolding-prone proteins, leading to impaired protein homeostasis of physiological

processes in the cell. The concept of chaperone competition not only serves as an explanation for the collapse of protein homeostasis during aging and neurodegenerative diseases, but also provides opportunities for therapeutic intervention. These approaches are also relevant to cancer in which it has been shown that the downregulation of certain molecular chaperones can be exploited to induce chaperone competition, promoting aggregation or degradation of oncoproteins and halting further proliferation of tumor cells (see other chapters in this collection).

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## 4.2 Molecular Chaperones and Protein Aggregation in Neurodegenerative Diseases

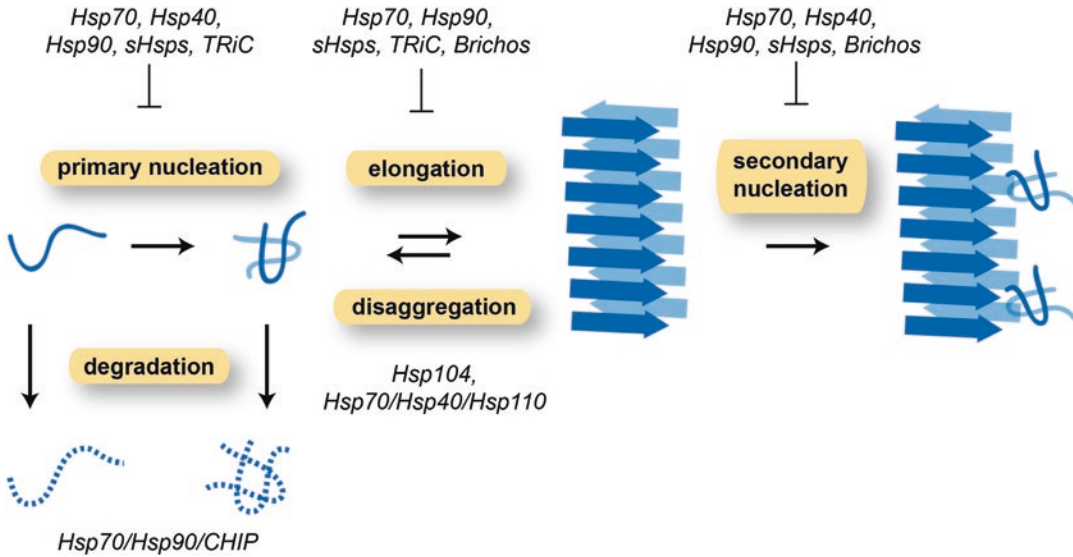
### 4.2.1 Amyloid- $\beta$

A $\beta$  is the main component of extracellular plaques that accumulate in the brains of AD patients. Processing of the amyloid precursor protein (APP) generates multiple isoforms of the A $\beta$  peptide of which the most abundant species, the 42-residue A $\beta$  peptide (A $\beta$ 42) is more aggregation-prone than the 40-residue form. *In vitro*, the aggregation of A $\beta$ 42 is delayed by the intracellular chaperones Hsp70 and Hsp90 at sub-stoichiometric ratios of ~1:50 (Evans et al. 2006). This effect is dependent on ATP, and the potency of Hsp70 is increased by the co-chaperone Hsp40 (family member DNAJB1), which stimulates ATPase activity, suggesting that to prevent aggregation the chaperones employ the same catalytic cycle that aids protein folding (Evans et al. 2006). Suppression of A $\beta$ 42 aggregation can also be achieved by sub-stoichiometric levels of DNAJB6, a member of the Hsp40 family, and quantitative analysis has revealed that DNAJB6 blocks both primary and secondary nucleation (Fig. 4.1) by preferentially binding to oligomeric species (Månsson et al. 2014a). The protective effects of Hsp70 against A $\beta$ -associated toxicity have also been observed *in vivo* in the fruit fly *Drosophila melanogaster*. Overexpression of Hsp70 effectively suppresses

neurotoxicity associated with the extracellular deposition of A $\beta$ 42 in the fly disease model, irrespective of whether the chaperone is expressed intracellularly or targeted to the extracellular space (Fernandez-Funez et al. 2016; Martín-Peña et al. 2018). The beneficial effect of intracellular Hsp70 may occur by a general enhancement of global proteostasis, whereas in the extracellular space it likely depends on its ‘holding’ activity in the absence of ATP.

Another class of chaperone activities is represented by the Brichos domains of ProSP-C and Bri2 that inhibit A $\beta$ 42 aggregation *in vitro*. Whereas the Brichos domain of ProSP-C blocks secondary nucleation of A $\beta$ 42 by binding to the fibrillar surface (Cohen et al. 2015), the Brichos domain derived from Bri2 prevents elongation of A $\beta$ 42 in addition to secondary nucleation by binding both to the fibril surface and to fibril ends (Arosio et al. 2016) (Fig. 4.1). Brichos domains physiologically function in the extracellular space, and expression of Brichos domains from ProSP-C and from Bri2 has been shown to be protective in *Drosophila* A $\beta$ 42 models by improving motor function and lifespan (Hermansson et al. 2014; Poska et al. 2016). Overexpression of the Brichos domain of ProSP-C increases the levels of soluble versus insoluble A $\beta$ 42 and delays its deposition during aging of the flies, suggesting that this Brichos domain could act by preventing the formation of toxic oligomeric species (Hermansson et al. 2014). In further support, ProSP-C Brichos rescued the toxicity of a mixture of A $\beta$ 42 monomers and fibrils on mouse brain slices using an electrophysiology assay (Cohen et al. 2015).

A role for the TRiC/CCT chaperonin on A $\beta$ 42 phenotypes was demonstrated in a genetic screen of the chaperome in *C. elegans* expressing A $\beta$ 42 in the body wall muscle cells. Individual knock-down of each of the eight subunits of TRiC/CCT decreased the motility of the worms, and the screen also identified Hsc70, Hsp40, Hsp90 and its co-chaperones Cdc37 and Sti1 (Brehme et al. 2014). Many of the same chaperones were shown to be protective against toxicity in a *C. elegans* model expressing expanded polyglutamine, suggesting that this subset of



**Fig. 4.1** Schematic reaction of amyloid formation indicating where chaperones act to prevent protein misfolding in the case of amyloid- $\beta$ , tau,  $\alpha$ -synuclein, polyglutamine expansion proteins and/or SOD1. Inhibition of primary

nucleation is inferred from the binding of chaperones to the monomeric proteins. Hsp90 alone and with its co-chaperone Aha1 has also been reported to promote aggregation in the case of tau

chaperones may have a general beneficial effect on proteostasis, rather than or in addition to making direct interactions with A $\beta$ 42. The relevance of TRiC/CCT was furthermore underlined by several of its subunits being downregulated in the aging human brain and in patients with AD (Brehme et al. 2014).

sHsps, a class of ATP-independent chaperones, have also been linked to A $\beta$  aggregation, as it was shown that expression of human A $\beta$  in *C. elegans* body wall muscle cells induces Hsp16 (Link et al. 1999), the overexpression of which completely restores the paralysis phenotype of the A $\beta$  worms (Fonte et al. 2008). This effect appears to result from a direct interaction that modulates aggregation, not only because Hsp16 co-localises with the deposits, but also because its overexpression reduces the amyloid plaque load in the worms, leaving total A $\beta$  levels unaltered. *In vitro*, the sHsp  $\alpha$ B-crystallin binds along the sides and at the ends of A $\beta$ 42 fibrils, suggesting it can inhibit secondary nucleation and elongation of the fibrils (Shammas et al. 2011).

An important aspect of chaperone biology is that not all chaperones are protective against protein aggregation. The extracellular chaperone

clusterin, which is a risk factor for late-onset AD, was reported to have more complex effects in mouse models of A $\beta$  aggregation, presumably because it shifts the clearance pathways of A $\beta$ , thus complicating the interpretation of its potential anti-aggregation effect (DeMattos et al. 2002; Wojtas et al. 2017). Extracellular chaperones may also alter A $\beta$  toxicity by modulating targets that interact with toxic A $\beta$  species. Sti1, which is a co-chaperone of Hsp90, but observed to be secreted by astrocytes, was shown to bind to the PrP<sup>C</sup> receptor and thereby block its interaction with A $\beta$  oligomers *in vitro* and in cell culture (Ostapchenko et al. 2013). Increased levels of Sti1 have been observed in aged AD mice, as well as in human AD patients compared to control brains, consistent with a protective mechanism (Ostapchenko et al. 2013).

#### 4.2.2 Tau

Tau is a microtubule-associated protein that forms intracellular aggregates in AD brains, and in patients suffering from various types of frontotemporal dementia (FTD) and ALS, collec-

tively known as Tauopathies. Both Hsp70 and Hsp90 chaperones have been shown to directly interact with tau *in vitro*, which may seem surprising given the disordered and highly hydrophilic nature of tau. However, the sequence motifs that have  $\beta$ -strand propensity, and are involved in the formation of cross- $\beta$  fibrils, contain hydrophobic residues that mediate binding to the constitutively expressed Hsc70 chaperone (Mitul et al. 2008). Hsp70 also interacts with tau monomers and oligomers to inhibit its nucleation as well as elongation in *in vitro* aggregation assays (Kundel et al. 2018). In *C. elegans* models expressing human tau in mechanosensory neurons, co-expression of human Hsp70 has modest beneficial effects on restoration of the touch response (Miyasaka et al. 2005). In mice, overexpression of Hsp70 reduces endogenous tau levels in aged animals, and especially reduces the insoluble high-molecular weight species (Petrucci et al. 2004). Similar results have been observed using small molecule inhibitors of the ATPase activity of Hsp70 that lead to reduced levels of total and phosphorylated tau in tau transgenic mice. This suggests that both overexpression and inhibition of the folding cycle of Hsp70 may converge to promote tau degradation by the ubiquitin-proteasome system (Jinwal et al. 2009). Both Hsc70/Hsp70 and Hsp90 are co-localized with tau tangles in a transgenic mouse model and in human AD brains; moreover upregulation of these chaperones suppresses the formation of tau aggregation in cellular models by partitioning tau into a productive folding pathway that restores tau binding to microtubules (Luo et al. 2007).

Studies on Hsp90 have shown that the site of Hsp90 binding on tau includes a broad region encompassing the hydrophobic motifs, generating an extended interaction surface held together by a combination of weak hydrophobic and electrostatic interactions (Karagöz et al. 2014). The levels of phosphorylated tau but not total tau levels in a transgenic mouse model are reduced when the ATPase activity of Hsp90 is inhibited by small molecules (Dickey et al. 2007). Hsp90 inhibition also leads to activation of the heat shock response and the subsequent increase in expression of chaperones including Hsp70, but

drug treatment may have a stronger effect than only inducing the heat shock response. Reduction in phospho-tau levels upon the inhibition of Hsp90 may depend on increased activity of its co-chaperone CHIP, which mediates ubiquitination and proteasomal degradation (Dickey et al. 2007). Another small molecule inhibitor of Hsp90 also reduces levels of phosphorylated tau as well as total tau, while increasing Hsp70 levels in mouse models expressing wild-type tau or the FTD-associated tau mutant P301L (Luo et al. 2007). In these studies, Hsp90 interacts directly with the mutant but not wild-type tau, suggesting that the mechanisms promoting tau clearance may differ between the two models, and thus potentially between AD and other types of (familial) dementia (Luo et al. 2007).

Modulation of Hsp90 activity involves co-chaperones such as Aha1, and together these can promote tau aggregation both *in vitro* and in a tau transgenic mouse model. Moreover, a small molecule that blocks the Hsp90-Aha1 interaction reduces this effect, demonstrating the power of combining mechanistic insights from *in vitro* experiments with *in vivo* models in developing therapeutic avenues (Shelton et al. 2017). Co-chaperones may also affect protein aggregation independently, as noted above for A $\beta$ . For tau, it has been shown that the Hsp40 protein DnaJA2 is a potent inhibitor of its aggregation *in vitro* (Mok et al. 2018). DnaJA2 binds to monomeric tau, and also reduces seeded aggregation in cells, suggesting that DnaJA2 may have an effect on multiple steps of the aggregation process. In AD patient neurons with tau pathology, DnaJA2 is highly abundant and is localised with aggregated tau, perhaps by being upregulated as a protective, but insufficient cellular response (Mok et al. 2018). In contrast, FKBP51, another co-chaperone of Hsp90 that co-localises with tau pathology in AD brains may have a role in promoting tau misfolding (Blair et al. 2013). Overexpression of FKBP51 in tau transgenic mice results in increased overall tau levels and neuronal loss, whereas the numbers of tau tangles are decreased. Consistent with these results, *in vitro* experiments have suggested that FKBP51 in complex with Hsp90 may especially increase the

formation of oligomeric tau species (Blair et al. 2013).

A role for the sHsp Hsp27 in modulating tau was shown in transgenic mice overexpressing Hsp27 that reduced tau levels and ameliorated the defects in long-term potentiation. This effect was shown to depend on the oligomerisation of Hsp27, since a phosphorylation mutant of Hsp27 that cannot undergo this cycle did not affect tau pathology or other phenotypes of the mouse (Abisambra et al. 2010).

### 4.2.3 $\alpha$ -Synuclein

The 140-residue, largely disordered protein  $\alpha$ -synuclein is found in intracellular inclusions termed Lewy Bodies, which form primarily in dopaminergic neurons of the substantia nigra in PD patients. Several Hsps have been identified as components of Lewy Bodies (Auluck et al. 2002; McLean et al. 2002; Outeiro et al. 2006), and sub-stoichiometric concentrations of Hsp70 are sufficient *in vitro* to suppress the formation of  $\alpha$ -synuclein fibrils in the absence of ATP (Dedmon et al. 2005; Luk et al. 2008; Roodveldt et al. 2009; Aprile et al. 2017). This effect is dependent on the interaction of Hsp70 with the hydrophobic NAC region of  $\alpha$ -synuclein, which is essential for fibril formation (Luk et al. 2008). Upon addition of ATP, Hsp70 delays fibril formation primarily by binding to the fibril ends, thus inhibiting elongation (Aprile et al. 2017). Furthermore, a complex of the constitutively expressed Hsc70 together with specific Hsp40 co-chaperones and a Hsp110 NEF can dissociate preformed  $\alpha$ -synuclein fibrils (Gao et al. 2015). The effect of Hsp70 on  $\alpha$ -synuclein aggregation *in vivo* is thus likely to depend on the relative levels of specific co-chaperones and ATP.

Similarly, in yeast expressing human  $\alpha$ -synuclein, induction of Hsp70 expression by a brief heat shock is protective against  $\alpha$ -synuclein-induced apoptosis and the generation of reactive oxygen species. Similar effects have been obtained by direct overexpression of the yeast Hsp70 orthologue Ssa3 or by inhibiting Hsp90

using geldanamycin, which also induces the heat shock response (Flower et al. 2005). On the other hand, in *C. elegans*, knock-down of Hsp70 does not affect  $\alpha$ -synuclein inclusion formation in muscle cells, suggesting that this chaperone does not have a beneficial effect in this model system (Van Ham et al. 2008). However, knock-down of Hip, an Hsp70 co-chaperone, significantly increases the number of inclusions in this *C. elegans* model, suggesting that the Hsp70-Hip complex acts against inclusion formation (Roodveldt et al. 2009).

In a *Drosophila* model of  $\alpha$ -synuclein, co-expression of human Hsp70 with wild-type  $\alpha$ -synuclein or the familial mutants A53T or A30P in dopaminergic neurons restores locomotion and lifespan without affecting the number or size of Lewy Body-like inclusions (Auluck et al. 2002). This is further supported by geldanamycin treatment which induces the heat shock response and similarly protects against neurodegeneration (Auluck and Bonini 2002; Auluck et al. 2005), whereas LB-like pathology is not affected and the levels of insoluble  $\alpha$ -synuclein are even increased (Auluck et al. 2005), suggesting that Hsp70 may reduce the presence of toxic oligomeric species.

Rodent models for expression of human  $\alpha$ -synuclein have yielded inconsistent results. Overexpression of rat Hsp70 in a mouse model resulted in a strong decrease in both high molecular weight  $\alpha$ -synuclein species, and Triton X-100 insoluble protein (Klucken et al. 2004), which contrasts with another study in which human Hsp70 and  $\alpha$ -synuclein A53T were co-overexpressed and the levels of high molecular weight and insoluble  $\alpha$ -synuclein were unaffected (Shimshek et al. 2010). Another study found beneficial effects from co-expressing Hsp70 with  $\alpha$ -synuclein in the rat brain, showing a reduction in the number of dystrophic neurites which typically precede neurodegeneration (Moloney et al. 2014). The protective effects of Hsp70 may depend on the ratio of Hsp70, its co-chaperones and  $\alpha$ -synuclein in these models as mentioned above, and potential differences in the binding affinities between Hsp70 from different

species and wild-type and A53T  $\alpha$ -synuclein could furthermore affect the outcome.

In addition to Hsp70 and Hsp40, the expression of Hsp27 is increased upon viral expression of  $\alpha$ -synuclein in mouse brains (St Martin et al. 2007). Likewise, mice expressing  $\alpha$ -synuclein A53T had increased levels of Hsp25 and  $\alpha$ B-crystallin, Hsp25 being primarily increased in astrocytes rather than neurons.  $\alpha$ B-crystallin inhibits *in vitro* aggregation of  $\alpha$ -synuclein isolated from the mouse brain (Wang et al. 2008), which is consistent with another *in vitro* result that  $\alpha$ B-crystallin interacts directly with  $\alpha$ -synuclein fibrils to prevent fibril elongation from pre-formed seeds (Waudby et al. 2010). It has also been reported to interact with early intermediates in *in vitro* aggregation reactions (Rekas et al. 2007). In line with these findings, expression of  $\alpha$ B-crystallin in the fly eye ameliorates the rough eye phenotype induced by  $\alpha$ -synuclein expression (Tue et al. 2012).

Hsp90 can inhibit  $\alpha$ -synuclein aggregation *in vitro* in seeded aggregation assays of  $\alpha$ -synuclein A53T. This activity is ATP-independent, and relies on the interaction of Hsp90 with oligomeric  $\alpha$ -synuclein species (Daturpalli et al. 2013). The yeast disaggregase Hsp104 can inhibit  $\alpha$ -synuclein aggregation and remodel pre-formed  $\alpha$ -synuclein fibrils *in vitro*, and overexpressing it together with the A30P  $\alpha$ -synuclein mutant in rats reduces inclusion formation and neuronal loss (Lo Bianco et al. 2008).

#### 4.2.4 Polyglutamine Expansions

Nine human neurodegenerative diseases are associated with genetic expansions leading to the production of different proteins with expanded polyglutamine (polyQ) tracts. Irrespective of the protein, disease symptoms occur beyond a pathogenic threshold of ~35–40 glutamine residues, accompanied by the formation of cytoplasmic and nuclear inclusions in neuronal tissue (Lieberman et al. 2019). The onset of pathology and disease is correlated with the length of the

polyQ tract, with longer polyQ lengths having increasing aggregation propensity *in vitro* and in cellular models. HD is the most prevalent of these polyQ disorders, and is related to an expansion within the huntingtin gene *HTT*. In particular, a fragment of the huntingtin protein corresponding to the first exon of the gene in which the polyQ expansion is located is found to accumulate in disease, and this fragment is sufficient to drive neurodegeneration and inclusion formation in mouse models (Mangiarini et al. 1996; Scherzinger et al. 1997).

In the nematode worm *C. elegans*, expression of a construct comprising 40 glutamine residues (Q40) with a YFP-tag for visualisation in the body wall muscle cells is sufficient for protein aggregation and formation of toxic immobile inclusions (Morley et al. 2002). Expression of 35 residues (Q35) also leads to aggregation and toxicity, but later in adulthood, whereas shorter polyQ lengths of 19 or 24 glutamine residues remain diffuse. These polyQ lines were used for a genome-wide genetic screen to identify genetic modifiers of protein aggregation which identified components of the proteostasis network for transcription and splicing, translation, folding, transport and degradation including the chaperones Hsp70, Hsp40 and subunits of TRiC/CCT (Nollen et al. 2004). The mechanism by which TRiC/CCT inhibits polyQ aggregation has been further explored *in vitro*, and it was shown to interact with the tips of mutant huntingtin fibrils and to encapsulate smaller oligomers (Shahmoradian et al. 2013).

Other genetic screens in yeast and *Drosophila* have additionally identified multiple Hsps, including Hsp70, Hsp40, Hsp90 and sHsps, as well as Hsp104, the yeast disaggregase (Krobitsch and Lindquist 2000; Willingham et al. 2003; Kazemi-Esfarjani and Benzer 2000; Giorgini et al. 2005; Zhang et al. 2010; Jimenez-Sanchez et al. 2015). Overexpression of Hsp70 and Hsp40 ameliorates multiple phenotypes in *Drosophila* and mouse polyQ disease models, typically without altering the numbers of mature protein aggregates (Warrick et al. 1999; Chan et al. 2000;

Cummings et al. 2001; Hay et al. 2004; Labbadia et al. 2012). Consistent with these findings, Hsp70 has been shown to associate with huntingtin oligomers *in vitro*, but not with monomers or detergent-insoluble inclusions, and it is able to prevent further aggregation together with Hsp40 and in the presence of ATP (Lotz et al. 2010).

More mechanistic studies on the activities of Hsp70, Hsp40 and Hsp110 against huntingtin exon 1 aggregation performed in cell culture have revealed that DNAJB6 and DNAJB8 members of the Hsp40 family are highly effective (Hageman et al. 2010). Subsequently, DNAJB6 was shown to inhibit protein aggregation in an HD mouse model, delaying the onset of symptoms and extending lifespan (Kakkar et al. 2016). *In vitro*, DNAJB6 inhibits the primary nucleation of polyQ peptides which depends on a serine-threonine rich region on its surface (Kakkar et al. 2016). This activity does not, however, depend on the presence of Hsp70 or ATP (Månsson et al. 2014b), providing an interesting example of independent chaperone activity of Hsp40 proteins.

#### 4.2.5 SOD1

Point mutations in superoxide dismutase 1 (SOD1) are one of the causes of familial forms and sporadic cases of ALS (Cook and Petrucelli 2019). In contrast to the proteins discussed above that are largely disordered, SOD1 is a well folded soluble globular protein that binds copper and zinc ions and is stabilized by a disulfide bond. Disease-associated mutations are thought to destabilize the native state of SOD1, rendering it more prone to aggregation (Lindberg et al. 2005; Prudencio et al. 2009).

In a genome-wide RNAi screen on a *C. elegans* strain expressing SOD1 with G85R mutation throughout the neurons, the majority of hits belonged to the category of protein quality control, including the regulator of the heat shock response HSF1, several chaperones and compo-

nents of the degradation machinery (Wang et al. 2009). Induction of the heat shock response is protective against SOD1 G93A aggregation and toxicity in mice, as demonstrated by the overexpression of SIRT1, which has HSF1 amongst its substrates. The beneficial effect in this case was found to be limited by the expression levels of inducible Hsp70, which were not sufficient to restore the phenotype of mice with higher levels of SOD1 (Watanabe et al. 2014).

A role for the Hsp70/Hsp40/Hsp110 machinery in aggregate disassembly as described above for  $\alpha$ -synuclein has also been suggested for SOD1. Overexpression of Hsp110 in mice expressing YFP-tagged SOD1 G85R in motorneurons extends their lifespan, and a reduction of aggregates has been observed in a subset of animals (Nagy et al. 2016). Addition of YFP-SOD1 G85R to isolated squid axoplasm inhibits axonal transport, and supplementing Hsc70, but more so Hsp110, was found to suppress these defects (Song et al. 2013). Likewise, overexpression of the Hsp40 family member DNAJB2 reduces aggregation of SOD1 G93A in late stages of disease progression in mice, and improves motorneuron survival and muscle strength. DNAJB2 has been found to associate with the SOD1 aggregates, providing evidence for a direct interaction which was suggested to reduce aggregate formation by promoting ubiquitination (Novoselov et al. 2013).

sHsps have also been proposed to modulate SOD1 aggregation and toxicity. Hsp25 and  $\alpha$ B-crystallin co-elute with insoluble SOD1 mutant protein from mice (Wang et al. 2003), and in *in vitro* studies using a brain homogenate,  $\alpha$ B-crystallin inhibits aggregation (Wang et al. 2005a). This is further supported by experiments with recombinant Hsp27 and  $\alpha$ B-crystallin that inhibit SOD1 G93A aggregation by interfering with aggregate growth, rather than the primary nucleation step (Yerbury et al. 2013). Mutations in Hsp27 have been identified in sporadic cases of ALS, which may be related to the inability to prevent SOD1 aggregation (Capponi et al. 2016).



### 4.3 Chaperone Competition as a Basis for Proteostasis Collapse in Protein Misfolding Diseases

#### 4.3.1 The Chaperone Competition Hypothesis

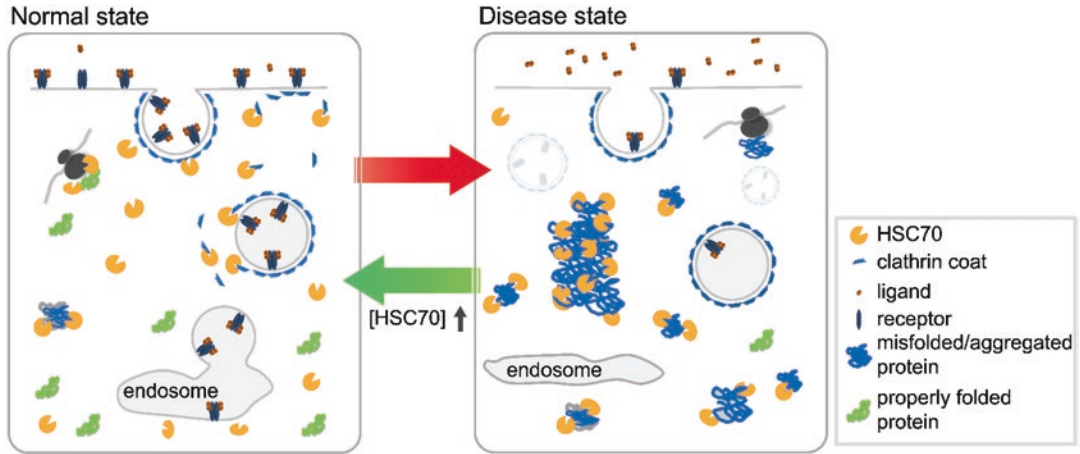
The interaction of molecular chaperones with intermediate states of highly aggregation-prone disease-related proteins is a finely tuned process, in which chaperones can either direct and maintain functional client interactions for cellular health or result in protein aggregation and proteome mismanagement in aging and stress. This imbalance can be enhanced by increased protein expression, coding mutations, posttranslational modifications or alterations in the composition and functional properties of the proteostasis network to shift the balance towards aggregation and proteotoxicity. While misfolded and aggregated proteins have been directly linked to cellular toxicity (Bucciantini et al. 2002; Baglioni et al. 2006; Marsh et al. 2000; Fath et al. 2002), the diverse protein misfolding diseases have very complex pathologies likely resulting from multiple molecular defects. The chaperone competition hypothesis provides an explanation why aggregation of a disease-associated protein can interfere with multiple cellular pathways (Yu et al. 2014; Yu et al. 2019). The multifaceted roles of chaperone networks raise the possibility that competition between misfolding proteins and endogenous clients for limited chaperone resources will have consequences on protein functionality. The higher localized concentration of misfolded proteins in aggregates results in a spatial redistribution of chaperones and other components of the proteostasis network. Kinetically, this will have negative effects on many chaperone-regulated processes resulting in multiple pathological symptoms, exacerbating disease progression. Chaperone sequestration initiated by intracellular accumulation of misfolded and aggregated proteins is common to all protein misfolding diseases, supporting the hypothesis that the loss of chaperone function

upon protein aggregation can accelerate cellular toxicity.

#### 4.3.2 Chaperone Sequestration During Protein Misfolding

Multiple families of chaperones and co-chaperones form extensive protein-protein interaction networks to assist in the folding of diverse clients. Chaperone-client interactions are transient in nature to allow reversible engagement with multiple substrates including nascent polypeptides, unfolded and misfolded proteins and folding intermediates (Kim et al. 2013; Hiller 2019; Koldewey et al. 2017). Compared with on-pathway substrates for Hsc70, misfolded species are more likely to expose hydrophobic regions to allow Hsc70 to bind with higher apparent avidity (Kundel et al. 2018; Pemberton et al. 2011). Consequently, Hsc70 is preferentially occupied by aberrantly folded protein substrates in stressed cells or upon expression of metastable proteins. When protein aggregates form after stable interaction of misfolded proteins, Hsc70 as well as other interacting partners become sequestered into the aggregates (Fig. 4.2).

Histochemical and biochemical studies have revealed that Hsc70 and other chaperones colocalize with a variety of protein inclusions in multiple cell and animal models of disease-associated protein aggregation and in patient-obtained brain tissues. Proteomic analysis of laser dissected amyloid plaques (Liao et al. 2004) and tau tangles (Wang et al. 2005b) from AD patient brains show that these inclusions sequester molecular chaperones, and other proteins that may be conformationally challenged. Hsc70 and the proteasome also co-localize with intracellular A $\beta$  aggregates in a cellular model (Bückig et al. 2002). A human cell system for seeding-dependent tau aggregation has shown that the chaperones Hsc70/Hsp70, Hsp90 and J-domain co-chaperones are sequestered by tau aggregates (Yu et al. 2019). Likewise in PD, immunohistochemistry and proteomics have identified major chaperones including Hsc70, Hsp90, Hsp40 and



**Fig. 4.2** Aggregate-driven chaperone competition explains the pathological complexity associated with disease-associated aggregation-prone proteins. Shown is a model depicting chaperone competition between protein aggregates and the protein folding and vesicular trafficking arms of the proteostasis network. Under normal conditions (left), Hsc70 is at sufficiently high levels to mediate

CME as well as basal protein client folding. Under disease conditions where protein aggregates have accumulated and the Hsc70 relocates to aggregates, both protein folding and CME are inhibited (right). This can be reversed by increasing the levels of Hsc70 by small molecule activation of HSF1 to restore chaperone function

Hsp27 as constituents of filamentous Lewy bodies, co-localizing with  $\alpha$ -synuclein (Uryu et al. 2006; Leverenz et al. 2007).

In the context of HD, Hsc70 binds specifically to the N-terminal flanking region of huntingtin exon 1. Using a conditional human cell system and immunofluorescence, the chaperones BiP/GRP78, Hsp70, Hsp40, proteasome subunits and other aggregation-prone proteins were shown to colocalize with the perinuclear inclusions of huntingtin exon 1 with an expanded polyQ (Waelter et al. 2001). Proteomic profiling of HD inclusions revealed widespread sequestration of proteins into the mutant huntingtin inclusion bodies (Hosp et al. 2017). Similarly, chaperones colocalize with ataxin 1 and ataxin 3 polyQ protein inclusions (Cummings et al. 1998; Chai et al. 1999). For ALS, mutant SOD1 is a substrate of interactions with Hsc70/Hsp70, and mice expressing mutant SOD1 form inclusions containing ubiquitin, the proteasome and Hsc70 (Zetterström et al. 2011). Hsc70-positive inclusions have also been detected in sporadic ALS cases (Watanabe et al. 2001). Chaperone association has also been detected in cells expressing an artificial aggregation-prone  $\beta$ -sheet protein

(Olzscha et al. 2011). Collectively, these observations provide strong evidence for sequestration of key components of the proteostasis network such as molecular chaperones and constituents of the protein degradation machinery as a unifying feature of protein misfolding diseases. The delicate balance between the levels of available molecular chaperones and client proteins is further underscored by the fact that many types of cancer cells depend on elevated levels of chaperones for their survival, accommodating for the increased demand by destabilized and misfolding-prone oncoproteins (see other chapters in this collection).

### 4.3.3 Functional Consequence of Chaperone Sequestration

As described above, many cellular processes are affected by the sequestration of chaperones by protein aggregation. The functional consequences of chaperone competition were determined by measuring multiple Hsc70-mediated cellular processes (Yu et al. 2014, 2019). Clathrin-mediated endocytosis (CME), the main entry route of bio-

logical molecules into the cell (Kirchhausen et al. 2014) involves the self-assembly of trimeric clathrin units on the plasma membrane to cause membrane curvature and the formation of coated pits. The released vesicles rapidly lose their clathrin coats in a process catalyzed by Hsc70 together with the co-chaperone auxilin in order to fuse with intracellular endosomes (Massol et al. 2006). Reducing cellular levels of Hsc70 by siRNA inhibits CME, indicating the requirement of Hsc70 for the assembly and disassembly of clathrin-coated vesicles (Yu et al. 2014).

The kinetic correlation between cytosolic Hsc70 concentration and CME therefore provides a highly sensitive functional readout of chaperone competition in the presence of protein aggregation. By monitoring CME in prostate cancer PC-3 cells expressing different aggregation-prone proteins including polyglutamine, huntingtin, ataxin1 and SOD1, a reduction of CME due to the sequestration of Hsc70 by aggregates was observed (Yu et al. 2014). The sensitivity of CME to Hsc70 depletion suggests that chaperone abundance is rate-limiting in cells expressing aggregation-prone proteins. Moreover, suppression of CME by protein aggregation could be reversed by conditionally increasing Hsc70. These effects were also observed in neuronal cells showing that protein aggregation causes dysregulated internalization of the AMPA receptor, a neuron-specific CME cargo.

The observations of chaperone competition extend beyond Hsc70, as other chaperones, including Hsp90, Hsp40 and Hsp27, are also sequestered in tau inclusions. Single-cell analysis of protein folding and CME in a cell model of tau aggregation reveals that both chaperone-dependent activities are impaired by tau aggregation (Yu et al. 2019). These observations are further supported by the finding in yeast that sequestration of Sis1p, a low-abundant Hsp40 homolog chaperone, by polyQ aggregates interferes with nuclear degradation of misfolded proteins and leads to the formation of cytosolic inclusions (Park et al. 2013). Consequently, the decline in chaperone-dependent arms of the proteostasis network will have profound negative effects on the protein quality control capacity of

the cell. Besides cytosolic chaperones, various species of misfolded proteins and aggregates interact with and sequester components of the protein degradation machinery. This sequestration will further compromise the protein quality control capacity (Thibaudeau et al. 2018; Guo et al. 2018; Yang et al. 2015; Holmberg et al. 2004).

#### 4.3.4 Restoration of Chaperone Homeostasis as a Therapeutic Strategy

The functional dissection of chaperone competition will lead to a better understanding of the early events of protein aggregation, and may uncover novel strategies to intervene at early stages of protein misfolding diseases. All Hsc70-regulated processes will be negatively affected by a subcellular redistribution of Hsc70 among its clients, resulting in a decline in multiple chaperone-dependent processes leading to multiple pathological symptoms that exacerbate disease progression. Consequently, restoring Hsc70 homeostasis could be an effective strategy to battle age-related protein conformational diseases. The appearance of a misfolded conformational state of tau associated with CME failure can be detected before the appearance of mature tau inclusions and the stable sequestration of Hsc70 (Yu et al. 2019). The timing of these cellular events therefore suggests that the inhibition of CME is an early cellular event of tauopathy and precedes failure of other cellular processes such as chaperone-dependent protein folding. Moreover, both CME and protein folding can be restored by small molecule regulators of HSF1 resulting in expression of cytosolic chaperones (Yu et al. 2019), suggesting that enhancing chaperone expression could have beneficial consequences on chaperone function and cellular health without reversing tau aggregation. Similarly, overexpressing protein sequestered by polyQ aggregates has been shown to rescue loss of function phenotypes and relieve polyQ dependent toxicity (Park et al. 2013).

## 4.4 Outlook

Further studies will be required to bridge the gap between the kinetic studies of protein aggregation and their modulation by molecular chaperones *in vitro* and *in vivo* in healthy and disease states. Cellular probes capable of detecting aggregating protein species, especially in the early and oligomeric states, may allow real-time monitoring of chaperone engagement of disease-associated substrates. Furthermore, molecular chaperones do not operate in isolation and the coordination of chaperone networks to maintain all arms of the proteostasis network needs to be further characterized for each of the aggregation-prone proteins in disease-relevant tissues and cell types. The systems approach for proteostasis in neurodegenerative diseases likely will differ from cancer, although both classes of diseases are related by the consequence of aging on the robustness of cell stress responses and the functional capacity of the proteostasis network. The use of primary patient derived cells and live cell-based approaches to measure the cellular state of proteostasis during disease progression and in the context of aging will provide a basis to screen for small molecules that restore cellular proteostasis. Targeting the restoration of proteostasis, in particular chaperone homeostasis, can potentially serve as a major therapeutic avenue to treat many forms of protein misfolding disorders ranging from neurodegenerative diseases to cancer.

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