CHAPTER 2

Introduction

Barcoding heat shock proteins to human diseases: looking beyond the heat shock response


* Equal contribution
Abstract

There are numerous human diseases that are associated with the formation of toxic protein aggregates. Activating the heat shock response (HSR) -and thus generally restoring the disturbed protein homeostasis in such diseases- has often been suggested as a therapeutic target. Yet, most data on activating the HSR or its downstream targets in mouse models of diseases associated with aggregate-formation have been rather disappointing. However, the human chaperonome consists of many more heat shock proteins (HSPs) that are not regulated by the HSR. In this review, we summarize the existing literature on a set of aggregation diseases and discovered that each of them can be ‘barcoded’ by a different set of HSPs, each of which can rescue specific aggregation types. Many of these effective HSPs are not regulated by the HSR and some have now also demonstrated effectiveness in mouse models. Interestingly, several of these ‘non-canonical’ HSPs also cause diseases when mutated (so-called chaperonopathies), which are also discussed in this review.
Introduction

Many heat shock protein (HSP) family members are known to function as molecular chaperones, meaning that they stabilize and assist in the correct folding of nascent polypeptides (Ellis & Hartl, 1999). In addition to their role in de novo protein folding, HSPs are involved in various aspects of proteome maintenance, including macromolecular complex assembly, protein transport and degradation, as well as aggregate dissociation and refolding of stress-denatured proteins. Under normal cellular conditions, HSP levels match the overall level of protein synthesis. Under stress-induced conditions, mature proteins unfold and exceed the capacity of chaperone networks to prevent aggregation. This type of acute proteotoxic stress induces a regulated response resulting in increased expression of some HSPs to rebalance protein homeostasis.

The human genome encodes for more than 100 different HSPs in 7 different families: HSPH (Hsp110), HSPC (Hsp90), HSPA (Hsp70), DNAJ (Hsp40), HSPB (small Hsp), the human chaperonin families HSPD/E (HSP60/HSP10) and CCT (TRiC), plus several regulatory co-factors (Kampinga et al., 2009). In terms of their regulation, the HSP family members can also be categorized into three groups: 1) constitutively expressed, but not induced by stress; 2) constitutively expressed and induced upon stress; and 3) induced only upon stress (Morimoto, 2008). Besides their differential regulation, the various HSPs also show a large degree of functional diversities with respect to client specificity and client processing (Kampinga & Craig 2010). These functional differences may be very important when investigating their potential relevance for diseases in which cells are chronically exposed to proteins that are prone to form toxic protein aggregates. Examples of such diseases are polyglutamine diseases, Parkinson’s disease, amyotrophic lateral sclerosis and Alzheimer’s disease. This review discusses how these diseases can actually be ‘barcoded’ by a different set of HSPs that can rescue their disease-specific aggregations.

The cellular functions of HSPs

*HSPs and de novo protein folding*

The general organization of co-translational folding is highly conserved throughout evolution. Ribosome binding chaperones (e.g., nascent-chain-associated complex and specialized Hsp70/HSPAs) first interact with the nascent polypeptide, followed by a second set of HSPs that do not have a direct affinity for the ribosome (the classical Hsp70/HSPA system). The Hsp70/HSPA family is the central component of the cellular network of molecular chaperones and folding catalysts (Fig. 1A). Hsp70/HSPA proteins are involved in a wide range of protein quality control functions (PQC), including de novo protein folding, refolding of stress-denatured proteins,
protein transport, membrane translocation and protein degradation. Hsp70/HSPAs never function alone; they require Hsp40/DNAJ proteins and nucleotide exchange factors (NEFs) as partners. DNAJ proteins bind and deliver client proteins to the Hsp70/HSPA system, upon which the client protein and DNAJ function together thereby stimulating HSPA to hydrolyze ATP, leading to high substrate affinity of HSPA. Following ATP-hydrolysis, NEFs such as BAG-1, HSPBP1 and HSPH, bind HSPA and induce ADP-ATP exchange, leading to substrate release. DNAJs thus mainly confer client specificity to the Hsp70/HSPA machine, but can also affect the fate of HSPA clients (Fig. 1A). The Hsp40/DNAJ proteins constitute a large family consisting of 50 members in humans. All of them contain a J domain, which can bind to the N-terminal ATPase domain of Hsp70 and the adjacent linker region. DNAJs have been categorized in 3 classes (Fig. 2). Class I and class II J proteins have an N-terminal J-domain contain Gly and Phe-rich regions following the 1st 25 amino acids carboxy-terminal to the J domain. In C terminal domain 1 (CTD I), canonical class I members have a zinc finger-like region (ZFLR) that class II members lack. However, class II members often do have Cys-rich stretches. Class II J proteins are more heterologous and the J-domain is not always N-terminal. DNAJs members of classes I and II are thought to function as promiscuous chaperones independently and recruit HSPA to non-native substrate proteins. Other DNAJs (class III) are more diverse and combine the J domain with a variety of functional modules (Bukau et al., 2000; Kampinga & Craig, 2010). While NEFs seem to be mainly involved in client fate (Bukau et al., 2000; Kampinga & Craig, 2010) (Fig. 1A). The NEFs BAG1 and BAG3 can, like all the NEF, associate with the ATPase domain of HSPAs and they can also interact with CHIP (C terminus of Hsc70-interacting protein) (Fig. 2). BAG1 has been suggested to target proteins for degradation by the UPS, whereas BAG3 was suggested to be involved in protein degradation by autophagy [9,25–27]. The latter has led to the interesting suggestion that BAG1 and BAG3 may be important factors to maintain protein homeostasis [25], a question that will be intensively addressed in this thesis.

The DNAJ/HSPA system might also receive clients from small Hsp/HSPB proteins. The HSPB proteins were first described as a set of proteins of small molecular weight (15–30 kDa). Some of them have the ability to oligomerize and the oligomerization state can modulate the affinity for their different partners and the consequent function. HSPB chaperone activity does not need ATP. However, direct interaction with ATP-dependent chaperones like HSPA promotes the release of the bound substrate and subsequent refolding (Boncoraglio et al., 2012; Garrido et al., 2012). Interestingly, interactions between small HSPs (in particular HSPB8) and BAG proteins (in particular BAG3) have been reported [12,28] (Fig. 2) further pointing to intricate interactions within the cellular chaperone network systems.
Figure 1. Model of actions and interactions of the HSP network required for normal protein folding and refolding upon (A) acute stress or (B) during chronic stress.

HSP families constitute a large group of chaperones that interact with non-native proteins, assisting their correct protein folding. HSPs are constitutively expressed, but their expression level can increase under conditions of stress. They are mainly divided into groups: sHsp/HSPBs, Hsp70/HSPAs, Hsp90/HSPCs and members of the chaperonin family (see main text for details).

(A) During *de novo* protein folding and for the refolding of acute stress-denatured, unfolded proteins, the functional cooperation of different HSPs is primarily aimed at the structural stabilization of native proteins for (re)folding. However, in case of failure of protein folding HSPs can also assist client degradation through the ubiquitin-proteasome system (UPS) or the autophagy-lysosome pathway. The central component of the chaperone network and folding catalysts is the Hsp70/HSPA family. DNAJs hydrolyze ATP (bound to HSP70/HSPA) to ADP increasing the affinity of its substrate-binding domain for unfolded proteins. Nucleotide exchange factor (NEF) proteins remove ADP and substitute ATP, reducing HSP70/HSPA’s substrate binding affinity, allowing release of the folded protein. Proteins that are unable to utilize HSPAs for complete folding are transferred to the chaperonin or the HSPC system. HSPC proteins require HOP as a co-chaperone in order to transfer substrates from HSPA to HSPC. Under acute stress conditions, HSPB oligomers dissociate into dimers to bind unfolded substrates, thereby avoiding irreversible aggregation of client proteins. This process allows ATP-dependent chaperones to assist in the substrates refolding when normal physiological conditions are restored.

(B) In the presence of chronic stress, which triggers protein-misfolding, re-folding attempts might be particularly unsuccessful. Under such conditions, a HSP network, which rather functions in protein unfolding, disaggregation, and specific targeting of the misfolded or even aggregated proteins for degradation, is required. Members of each HSP family are shown to interact with mutated/misfolded proteins and to reverse the formation of aggregates. However, whether different HSPs functionally cooperate with each other in order to modulate mutated protein toxicity is not completely clear yet.
Proteins that cannot be completely folded by Hsp70/HSPA machines are transferred to, or handled independently by Hsp90/HSPC system (Buchner, 1999; Yam et al., 2008) (Fig. 1A). Substrate transfer to Hsp90/HSPC protein is mediated by the HSP organizing protein (HOP) (Fig. 2), which uses multiple tetratricopeptide repeat domains to form a bridge between HSPA and HSPC (Buchner, 1999; Young et al., 2001). Hsp90/HSPC is one of the most abundant proteins in eukaryotes and it has a central role in cell regulation. Although the mechanism by which client protein folding is coordinated with Hsp90/HSPC’s reaction cycle is largely unclear [29,30].

Finally, Hsp70 machines can transfer clients to the chaperonins (Fig. 1A). Chaperonins are a class of oligomeric, high-molecular-weight chaperones. They consist of two-ring assemblies with a central cavity, in which substrate folding occurs in an ATP-dependent manner. Chaperonins are structurally classified into group I and group II. Group I chaperonins comprises HSPD1 in mitochondria and Cpn60 in chloroplasts. They cooperate with lid-shaped co-chaperones (HSPE1 and Cpn10/20) to encapsulate substrates. The group II chaperonins includes the archaeal thermosome and its eukaryotic homolog tailess complex polypeptide-1 (TCP-1) ring complex (TRiC), also known as chaperonin-containing TCP-1 (CCT), which have a built-in lid [31]. While the handoff from Hsp70 to chaperonins remains unclear in mammals, work in the prokaryotic system has begun to reveal some interesting possibilities. For example, it has been shown that Hsp70/DnaK binds the M domain of ClpB to recruit DnaK-bound substrates to the chaperonin (Seyffer et al., 2012).

To guarantee the correct folding of newly made proteins, a complex chaperone network is required in all cells. The Hsp70/HSPA members are the central organizer of the chaperone network, through the physical interaction with members of each of HSP families, can orchestrate the fate of newly made proteins. Hsp70/HSPAs can receive subset of proteins from chaperones that act upstream, such as DNAJ proteins, and when necessary, distribute them to chaperones that act downstream, such as Hsp90/HSPC or chaperonin proteins (Fig. 1-2). When the folding is unsuccessful, Hsp70/HSPAs throughout the interaction with its NEF BAG1 or BAG3 is able to degrade the misfolded proteins.

**HSPs and acute proteotoxic stress conditions**

Cells are constantly challenged by a variety of changes to their environment. Acute stress conditions, such as heat shock, cause many proteins to become unfolded. The accumulation of stress-denatured proteins increases the risk of aggregate formation. In addition to their role in co-translational folding, the constitutively expressed HSP members might also assist in aggregate protection and refolding of stress unfolded proteins (Fig. 1A). However, it has been shown in yeast that the
stress-inducible cytosolic members of the families, which are strongly upregulated by heat shock factor-1 (HSF-1), become more important under such conditions (Albanèse et al., 2006). Next to this transcriptional response, HSPB proteins represent an even more rapid response to environmental stresses (Fig. 1A). Several HSPB-members are rapidly and transiently phosphorylated, whereby their oligomeric state is dynamically altered and their protective activities are activated. These protective activities include prevention of cytoskeletal collapse and chaperoning of soluble proteins, which can enhance protein refolding or support client degradation (Garrido et al., 2012).

In parallel to the HSF-1 regulated heat shock response (HSR) in the cytosol, interconnected pathways in different cellular compartments also respond to acute cellular stress, including the unfolded protein response (UPR) in the endoplasmic reticulum and the mitochondria (Haynes & Ron, 2010; Morimoto, 2011; Walter & Ron, 2011). Each pathway not only induces the transcriptional up-regulation of genes that enhance refolding capacity but also the expression of HSP members assisting in degradation of unfolded proteins through the proteasome and lysosome-mediated pathways, together protecting cells from stress (Parsell & Lindquist, 1993; Haynes & Ron, 2010; Morimoto, 2011; Walter & Ron, 2011).
HSPs and chronic stress conditions

Protein aggregation hallmarks a high number of chronic diseases (Balch et al., 2008) that can either be loss-of-function or toxic gain-of-function disorders. Loss-of-function diseases, including cystic fibrosis and Gaucher’s disease, are typically caused by recessive mutations leading to inefficient folding of the mutated proteins and their consequent degradation or dysfunction (Fan et al., 1999) (Fig. 1B). Of note, in recessive diseases, the HSF-1-regulated HSPs can promote some refolding of (metastable) mutant proteins, thereby displaying disease-rescuing potential (Yang et al., 2013). In addition, chaperone inhibition, resulting in less recognition of the mutant peptides and their degradation, has been shown to be protective in such diseases (Chanoux & Rubenstein, 2012). Toxic gain-of-function diseases, on the other hand, usually manifest with the formation of intracellular and/or extracellular deposits of aggregated proteins, as will be further discussed below (Chiti & Dobson, 2006; Balch et al., 2008; Morimoto, 2008). These aggregates are often fundamentally different from those formed during acute stress as they initially are formed without being sensed by the (acute) stress responses in the cells. Moreover, unlike in response to acute stress where proteins are unfolded, proteins in chronic stress are intrinsically misfolded and can generally not be refolded but must be disposed of (Fig. 1B). This could imply that different HSPs might be crucial –or rate-limiting- to provide protection in chronic protein aggregation diseases than for acute stress. Below, we will focus on toxic gain-of-function diseases and provide an overview of the literature on HSPs that could prevent aggregation or/and toxicity of the disease-associated proteins. Because we aim to identify HSPs that might be rate-limiting factors for aggregate prevention and thus targets for intervention in these diseases, we will mainly discuss effects of HSP-overexpression and not include studies on down-regulation of HSPs. Interestingly, HSP down-regulation is often associated with toxicity and lethality and can result in disease itself (Table 1). Therefore, this review will furthermore provide an overview of aggregation diseases, known as chaperonopathies, which are caused by mutations in HSPs. In this way, we aim to recapitulate the role of HSPs in chronic aggregation diseases from two angles: the prevention of toxic gain-of-function diseases and their role in causing disease themselves.

HSPs and proteinopathies

There are numerous human diseases that are associated with the aggregation of a single dominant peptide or protein. Examples of such diseases, known as proteinopathies, include polyglutamine diseases (polyQ), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), and Alzheimer’s disease (AD). The monogenic forms of neurodegenerative proteinopathies are rare, and
are generally histopathologically indistinguishable from their corresponding sporadic forms, making it likely that both forms share a final common pathway. Protein aggregates are either found inside neurons (e.g. tau tangles in AD) or outside neurons, in the extracellular space (e.g. amyloid-β-plaques in AD). Aggregates are generated when proteins become destabilized, either by mutations changing their native state (e.g. SOD1 in ALS) or quantity (e.g. α-synuclein in PD), by the elongation of a certain domain (e.g. huntingtin in Huntington’s disease, HD), or by domain truncations (e.g. TDP-43 in ALS). Aggregates range from extremely dense amyloidogenic aggregates with β-sheet cores (huntingtin, ataxin-3, amyloid-β) to more amorphous aggregates (α-synuclein, SOD1, TDP-43). Although it is still debated whether the small oligomers or the large inclusions are more toxic, the overall evidence from model systems strongly suggests that aggregate prevention generally results in disease-amelioration. Therefore, this review focuses on aggregate prevention by HSPs and will reveal that each of these proteinopathies is associated with a different pattern or ‘barcode’ of rescue depending either on the HSR or individual HSPs. The elucidation of these barcodes provides a platform for a rational design of disease-specific therapeutic strategies. For each disease, evidence was categorized into 4 levels (Fig. 3): in vitro (lowest level), cell studies, non-mammalian model systems and mammals (highest level). Furthermore, evidence in Fig. 3 was graded according to their specific effects: prevention of aggregate formation (black), buffering of toxic effects caused by diseased protein (gray), and absence of protective effects (white).

**Polyglutamine diseases (polyQ)**

In polyQ diseases, the polyglutamine tract is elongated beyond a certain threshold. The transcribed polyQ peptide fragments are thought to be the initiators of amyloid fibrils and have a strong propensity to assemble into highly ordered polymers that are extremely rich in β-sheet structure, thereby creating SDS-insoluble aggregates (Wellington et al., 2000; Chiti and Dobson 2009). PolyQ expansions in huntingtin (Htt), ataxins, and in the androgen receptor have been associated with dominant, late onset, toxic gain-of-function diseases; HD, spinocerebellar ataxias (SCA) and spinal bulbar muscular atrophy (SBMA), respectively. All these diseases are associated with severe motor problems and/or muscle atrophy (Chiti & Dobson, 2009; Banno et al., 2012; Seidel et al., 2012). Both age of onset and protein aggregation propensity are strongly associated with the length of the polyQ expansion, further suggesting that aggregate formation forms the basis of disease (Gusella & MacDonald, 2000; Wellington et al., 2000).
Figure 3. Heat shock protein barcodes associated with diverse proteinopathies

Summary of literature pertaining to the effects of activating either the cytosolic heat shock response (HSR/HSF-1), using HSF-1 activators or HSP90 inhibitors, or overexpressing specific HSPs from the different families (HSPC, HSPA, HSPD/CCT, DNAJ, etc.) on each disease, evidence was categorized into 4 levels according to the system/organism in which the effect was examined: *In vitro* (A), cell studies (B), non-mammalian model systems (C) and mammals (D). Evidence was further graded according to the specific effects of the heat shock protein(s) on the disease: prevention of aggregate formation (black), buffering of toxic effects caused by diseased protein (gray), and absence of effects (white). See main text for further explanation.

Abbreviations: ref–articles with general information used for the figure; polyQ – polyglutamine diseases; Htt – huntingtin; SCA – spinocerebellar ataxia; AR – androgen receptor; PD – Parkinson’s disease; α-syn - α-synuclein; ALS – amyotrophic lateral sclerosis; AD – Alzheimer’s disease; Aβ - Amyloid-β; 990 – HSP990; AMCL – arimoclomol; GA – geldanamycin; CLST – celastrol; RA – radicicol; PU – PU-H71.
In cells and non-mammalian model organisms, activation of the acute HSR pathways has been shown to reduce the extent of polyQ aggregation. Overexpression of HSF-1 led to fewer but larger polyQ aggregates in cells (Pierce et al., 2010). In agreement with this finding, chemical up-regulation of the HSR in cells and non-mammalian animal organisms reduced a number of dysfunctions caused by polyQ overexpression (see Fig. 3 for associated references). Although overexpression of HSF-1 in muscle tissue of the R6/2 mouse model for HD increased life span, there were only small effects on aggregates (Fujimoto et al., 2005), implying that effects were compensatory and did not affect the underlying toxic gain-of-function. Chemical up-regulation of the HSR by the use of Hsp90/HSPC-inhibitors in the R6/2 mouse model led to transient beneficial effects, which disappeared during disease progression (Labbadia et al., 2011). Moreover, Hsp90/HSPC-inhibition led to accelerated degradation of soluble polyQ-Htt, which was apparently independent of HSR activation; however, this was most likely due to pleiotropic effects associated with the inhibition of Hsp90/HSPC instead (Baldo et al., 2012; Yam et al., 2008; Buchner, 1999).

Whereas injection of HSF-1 into an SBMA-mouse model resulted in only small effects in neurons, Hsp90/HSPC inhibition by 17-AAG, GGA and geldanamycin (GA) not only substantially increased life span, but also diminished aggregates (Fig. 2). Hsp90s/HSPCs are required for the degradation, regulation, ligand-binding affinity and stabilization of the androgen receptor, as well as for its trafficking (Peterson & Blagg, 2009). The strong effects of Hsp90/HSPC-inhibitors on SBMA -but not HD- suggest that HSF-1-activation and the resulting up-regulation of the HSR is insufficient to modulate polyQ diseases in general. Protective effects of Hsp90/HSPC-inhibitors, if found, are therefore most likely due to HSF-1 unrelated effects (Baldo et al., 2012).

Up-regulation of individual members from HSF-1-regulated HSP families (e.g. HSPA1A, DNAJB1, HSPB1) was effective in preventing polyQ aggregation or the associated toxicity in vitro and in cellular models (Fig. 2). However, in comparative screens involving larger polyQ expansions, the HSR-regulated HSPs were usually rather ineffective compared to non-canonical HSPs (Vos et al., 2010; Hageman et al., 2011; Hageman et al., 2012). Some effects of Hsp70/HSPA overexpression on polyQ toxicity were reported in Drosophila melanogaster (Fig. 3). However, these effects were not associated with aggregate reduction, suggesting that the observed protection was due to compensatory effects downstream of aggregate formation; for instance, the loss of normal protein quality control functions due to entrapment of key chaperones, such as DNAJB1 (Park et al., 2013). Yet, this loss of protein quality control is apparently not at the heart of disease in mammals, as restoration of protein quality control by HSP70 overexpression did not delay disease-progression in the
R6/2 HD mouse model (Hansson et al., 2003; Hay et al., 2004). The same is true for the canonical small Hsp HSPB1. Opposing an earlier report which suggested that HSPB1 overexpression led to a small delay of Htt-toxicity in rats (Perrin et al., 2007), studies in a mouse model for HD and cells proved HSPB1 to be rather inefficient in delaying polyQ-aggregation (Zourlidou et al, 2007; Fig. 3). Combined these results suggest that HSPB1 might have some compensatory effects that initially slightly delay disease but are not affecting aggregates directly, thereby eventually proving to be insufficient to rescue the disease in mammals.

In dedicated screens for members of the HSP families that might be better suppressors of polyQ aggregation, a number of very effective HSPs were identified, including DNAJB2, DNAJB6, DNAJB8, HSPB6, HSPB7, HSPB8, HSPB9. Interestingly, most of them were not, or were only marginally regulated by HSF-1 and were not effective in stimulating substrate-refolding after acute stress (Vos et al., 2010; Hageman et al., 2011; Hageman et al., 2012; Kakkar et al., 2013). Instead, these HSPs were associated with degradation of clients through the proteasomal and autophagic degradation routes. Moreover, whereas DNAJB6, HSPB7, and HSPB8 delayed aggregation in Drosophila, DNAJB2 was the first HSP that demonstrated a protective effect on aggregate formation, functional endpoints, and survival in mice (Fig. 3). Interestingly, our preliminary data regarding transgenic overexpression of DNAJB6 indicate even larger protective effects in the R6/2 mice (Kakkar et al, in preparation). The effectiveness of these non-canonical HSPs in cells, non-mammalian model organisms and mice might be related to their ability to prevent initiation of aggregate formation or to their ability to assist aggregate clearance through autophagy, a finding that would be consistent with the important role autophagy plays in proteinopathies (Vos et al., 2010; Boncoraglio et al., 2012; Rubinsztein et al., 2012; Gillis et al., 2013; Mansson et al., 2013).

In a nutshell, the HSR and individual HSF-1-regulated HSP members have marginal and mainly compensatory effects in polyQ diseases. In contrast, other members of the HSP families that can prevent aggregate initiation or dispose of aggregates might have potential as targets for therapy in polyQ diseases.

**Parkinson’s disease (PD)**

About 5-10% of PD cases are monogenic and are caused by either loss-of-function or toxic gain-of-function mutations. The most commonly occurring disease-causing mutations are in the mitochondria-associated genes Parkin (PARK2), PINK1(PARK6), and DJ-1 (PARK7) (Lesage & Brice, 2009; Shapira & Tolosa, 2010; Martin et al., 2011; Klein & Westenberger, 2012). Mutations in these genes are recessively inherited and usually result in a loss-of-function effect, mainly impeding mitochondrial function
and turnover. By contrast, a toxic gain-of-function phenotype resulting in PD is caused by rare dominantly inherited mutations and multiplications in the SNCA (PARK1, PARK4) and LRRK2 (PARK8) (Lesage & Brice, 2009; Shapira & Tolosa, 2010; Klein & Westenberger, 2012). This review will focus on these rare toxic gain-of-function mutations.

Mutations in or multiplications of SNCA lead to increased oligomerization of the gene product α-synuclein, which is an intrinsically disordered protein. This enhanced oligomerization increases the tendency of α-synuclein to form β-sheet structures and eventually fibrous amyloidogenic inclusions, called Lewy bodies and Lewy neurites (Lesage & Brice, 2009; Martin et al., 2011; Roostaee et al., 2013). LRRK2 is a kinase that is involved in the phosphorylation of α-synuclein. Mutations in LRRK2 are thought to promote α-synuclein expression, aggregation, and toxicity, thereby increasing the propensity of α-synuclein to self-aggregate (Shapira & Tolosa, 2010; Martin et al., 2011).

As in polyQ diseases, genetic or chemical activation of HSF-1 can temporarily compensate for LRRK2 and α-synuclein toxicity in cells and Drosophila (Fig. 3).

Although individual HSPs such as DNAJA1, DNAJB2, HSPB2/HSPB3, HSPB6 and HSPB8 inhibited α-synuclein aggregation in vitro, none of them have proven to be effective in cells thus far (Fig. 3). HSPB1 and HSPB5 were effective in preventing α-synuclein aggregation in vitro, in cells and in Drosophila; however, there is currently no evidence of success in mouse models. Overexpression of HSPA1 was also shown to be able to inhibit α-synuclein aggregation in vitro, and decrease α-synuclein toxicity in cells and in Drosophila. Moreover, HSPA1 overexpression in mice did show some protective effects, although the data are still disputed (Klucken et al., 2004; Shimshek et al., 2010) (Fig. 3). As is the case for polyQ diseases, neither Hsp70/HSPA1, nor any other canonical HSP could prevent aggregate formation or reduce aggregate size and quantity.

These data taken together would suggest that compensation for loss of normal protein quality control by sequestration of HSPs into aggregates plays a more important role in PD than it does in polyQ diseases. In line with this notion, a study of α-synuclein in mice showed that transgenic overexpression of HSPA5 delayed disease onset without affecting cytosolic protein aggregation. Because HSPA5 is an ER-resident Hsp70/HSPA and is not expressed in the cytoplasm of the cell, its mode of action must be indirect. Instead of directly affecting aggregate formation, HSPA5 most likely compensates for downstream consequences of aggregation and thereby delays disease onset (Gorbatyuk et al., 2012).

To conclude, the biophysical nature and intracellular localization of α-synuclein aggregates are clearly different from aggregates in polyQ diseases (Ciechanover & Brundin, 2003). Expression of (mutant) α-synuclein rapidly activates HSF-1, whereas polyQ expression either does not activate HSF-1 at all, or only transiently
and very late in disease (Ciechanover & Brundin, 2003; Seidel et al., 2012). The potential HSP suppressors of PD thus seem to differ from that of polyQ diseases, thereby resulting in a different HSP barcode of potential treatment targets (Fig. 3).

Amyotrophic lateral sclerosis (ALS)

About 5% of ALS cases are currently categorized as dominant monogenic ALS; the most commonly occurring mutations being in SOD1, TDP-43, and FUS (Anderson & Al-Chalabi, 2011; Al-Chalabi et al., 2012). Clinically, sporadic and monogenic ALS are nearly indistinguishable, as SOD1 and TDP-43 positive inclusions are present in both forms of disease, thereby implying a final common pathway (Turnder et al., 2013). About 166 mutations in SOD1 have been associated with monogenic ALS. Although SOD1 mutations were initially thought to cause disease via a loss of wild-type SOD1 function, SOD1-knockout mice displayed no phenotype (Saccon et al., 2013). Instead, the overexpression of mutant SOD1 leads to disease, implying that the mutant gained a toxic function (Siddique & Deng, 1996; Anderson & Al-Chalabi, 2011). Mutations in SOD1 indeed structurally destabilize the protein, thereby increasing its aggregation propensity, which eventually results in amyloid fibril-formation (Luheshi & Dobson, 2009). Mutations in TDP-43, a RNA- processing protein that usually shuttles between the nucleus and cytoplasm of the cell, render the protein aggregation-prone, which leads to the formation of dense round or filamentous aggregates in the cytoplasm alongside stress granules (Luheshi & Dobson, 2009; Anderson & Al-Chalabi, 2011; Al-Chalabi et al., 2012). Mutations in FUS, another protein involved in RNA metabolism, also result in large globular and elongated cytoplasmic inclusions (Anderson & Al-Chalabi, 2011; Al-Chalabi et al., 2012). Nevertheless, FUS-related ALS is defined as an atypical form because TDP-43 positive aggregates are not part of the pathology, which is why this review will not discuss this FUS-related ALS.

Treatment of Drosophila with the Hsp90/HSPC-inhibitor 17-AAG reduced the characteristic ALS eye-degeneration-phenotype in a TDP-43-model (Gregory et al., 2012). In addition, treatment of the SOD1-G93A mouse model with 17-AAG not only delayed age of symptom onset, but also increased lifespan (Kieran et al., 2004; Kiaei et al., 2005; Kalmar et al., 2008). However, these protective effects were not reproducible in the SOD1-G37R or the SOD1-G85R mouse model (Chiti & Dobson, 2009; Gifondorwa et al., 2012). These contradictory results indicate that protein aggregation and toxicity mechanisms might depend on the exact kind of mutation, and therefore result in a different barcode of HSPs for each SOD1-mutation.

Regarding the effects of individual HSPs on SOD1 aggregation and toxicity, data in cell lines expressing mutant SOD1 suggest protective effects of HSPA1, DNAJB1, DNAJB2, HSPB1 and HSPB8 (Fig. 3). Furthermore, HSPB8 alleviated TDP-
43 aggregation and toxicity in cells and HSPA1A reduced TDP43-associated eye degeneration in Drosophila (Fig. 3). The intracranial injection of SOD1-G93A mice with HSPA1 was also protective, whereas long-term effects of HSPB1 overexpression in mice were absent, although this awaits further investigation (Gifondorwa et al., 2007; Krishnan et al., 2008; Sharp et al., 2008; Gifondorwa et al., 2012).

To conclude, except for the aforementioned Hsp90/HSPC-inhibitors, none of the discussed HSPs resulted in long-term rescue or had direct effects on SOD1-aggregates (Fig. 3). Moreover, it is not clear whether the effects of the Hsp90/HSPC-inhibitors are due to the elevation of HSF-1-regulated HSPs, or whether they are due to the broad effects these inhibitors exert on cell homeostasis. In summary, the barcode of HSPs that protect against ALS is still very limited.

Alzheimer’s disease (AD)

The most commonly occurring form of AD is sporadic late onset AD. In contrast, only about 1-2% of AD cases display an early onset and are due to autosomal dominant mutations in amyloid precursor protein (APP), presenilin 1 (PSEN1), or presenilin 2 (PSEN2) (Guerreiro et al., 2012). Although intracellular tangles, consisting of hyperphosphorylated tau and extracellular amyloid-β (Aβ) plaques are present in both sporadic and monogenic AD, it is still unclear how toxicity in AD proceeds. It is disputed as to whether amyloid-β aggregation leads to cellular stress and results in tau-hyperphosphorylation and aggregation (described as the amyloid cascade hypothesis), or tau-hyperphosphorylation and aggregation precede amyloid-β accumulation (described as the tau-axis-hypothesis) (Goetz et al., 2011). Here, we will provide an unbiased summary on the effects of HSPs on both, Aβ- and tau-related aggregation.

Amyloid-β peptides are the result of APP-cleavage via one of two pathways: a non-amyloidogenic pathway that leads to the generation of the most common isoform, Aβ_{40}, or an amyloidogenic pathway that results in the generation of Aβ_{42} (Guerreiro et al., 2012). AD-related mutations in APP usually affect the ratio or properties of these different amyloid-β species (Guerreiro et al., 2012). Similarly, mutations in PSEN1 and PSEN2, which are rate-limiting components of the γ-secretase complex in the amyloidogenic pathway, result in increased generation of the more fibrillogenic Aβ_{42} (Goetz et al., 2011; Guerreiro et al., 2012).

HSF-1-injection into an APP rat model increased neuronal health and reduced Aβ plaque-load (Jiang et al., 2013). Similarly, in another study, genetic overexpression of HSF-1 in APP mice diminished soluble Aβ-levels (Pierce et al., 2013). In line with these findings, treatment of APP-mice with the HSF-1 activator celastrol slightly decreased Aβ-plaque load (Fig. 3).
In vitro, it was shown that HSPA1 and HSPA5, HSPC1, HSPA1/DNAJB1, as well as HSPB1, HSPB5, HSPB6 and HSPB8 slow down Aβ-aggregation (Fig. 3). Furthermore, purified DNAJB1, HSPB1, HSPB5 and HSPB8 protect cells from extracellular Aβ-toxicity when co-incubated (Wilhelmus et al., 2006; Carnini et al., 2012). However, considering that HSPs are intracellular proteins, whereas Aβ-plaques are generally considered to be extracellular, the relevance of such findings could be debated. Interestingly, evidence stating that intracellular Aβ-aggregation might precede extracellular plaque formation is accumulating, which increases the relevance of findings indicating that HSPs are able to prevent the initiation of aggregation in cells (Hu et al., 2009; Sakono & Zako, 2010). Although there is no cellular data available yet, these findings might explain why transgenic overexpression of HSPA1 and HSPB1 had protective effects in mouse models for Aβ (Hoshino et al., 2011; Toth et al., 2013). However, it is questionable whether Aβ-aggregation was directly affected by HSPA1 overexpression in transgenic mice, or whether the observed protective effects were due to more general compensatory effects of HSPA1 (Hoshino et al., 2011).

Another protein that has been associated with neuronal death in AD is tau. Tau is an unstructured and dynamic protein that is normally involved in stabilization of microtubules, but becomes hyperphosphorylated and detaches from microtubules under stress conditions. This detachment results in microtubular collapse and in the aggregation of tau into well-ordered and periodic protein deposits (Goetz et al., 2011; Mandelkow & Mandelkow, 2012).

In cells, the HSF-1 activator HSF1A reduced tau aggregation by increasing proteasomal degradation of tau (Oppatova et al., 2013). Likewise, Hsp90/HSPC-directed drugs, such as geldanamycin, enhanced clearance of tau from cells, thereby reducing its toxicity (Fig. 3). Moreover, the HSF-1 activator radicicol and the GA derivative 17-AAG alleviated tau toxicity in Drosophila larvae (Fig. 3).

Multiple HSPs alleviated tau toxicity in cells, including HSPA8, HSPA1, Hsp90s/HSPC, DNAJA1 and HSPB1 (Fig. 2). In addition, HSPB1 rescued behavioral defects in a mouse model for tauopathy (Abisambra et al., 2010). However, there are no studies investigating the role of the other HSPs in mammals available yet.

To conclude, interpreting the data about the effects of HSPs on AD needs to be done with great caution for two main reasons: Firstly, it is not yet known whether AD is initiated by intracellular (tau or Aβ) or extracellular (Aβ) aggregates. Secondly, although extracellularly added or leaked HSPs might affect toxicity of Aβ-plaques, it is still unclear how intracellular overexpression of HSPs can have direct effects on toxicity in AD, thereby limiting proper interpretation of the barcode of HSPs for AD.
Different aggregation diseases have a different HSP barcode

Although all diseases discussed here are toxic gain-of-function aggregation diseases, the barcode of HSPs with protective potential clearly differs depending on the disease (Fig. 3). This variability strongly suggests that the neurodegenerative proteinopathies discussed in this review are biochemically and biologically distinct. Because all proteinopathies presumably impede on overall protein homeostasis, simply rescuing the overall folding capacity by activation of the complete (acute) HSR (Fig. 1A), or expression of individual components thereof might lead to some protective effects. However, these effects are generally small and transient, and do not actually affect aggregate formation of the specific diseased proteins themselves. Instead, the effects of the HSR might compensate for entrapment of chaperones and/or other components into aggregates (e.g. certain crucial transcription factors). The underlying toxicity of the aggregates themselves is likely to go beyond these effects on protein homeostasis and will furthermore directly impair other functions, such as axonal transport, organelle dynamics (physical obstruction or cytoskeletal collapse) and membrane integrity. Assuming that aggregation indeed is the reason for toxicity in all these diseases, each proteinopathy requires specific HSPs that either directly prevent aggregation (initiation) or that recognize early aggregate intermediates and target these for degradation. These HSPs are likely to be found amongst the “non-canonical HSPs”, many of which have not been fully explored yet for each of these diseases.

Chaperonopathy: the case of “sick” HSPs

So far we have highlighted how HSPs might act as a first line of defense in preventing proteinopathies. Sometimes, however, HSPs themselves are mutated, leading to several pathological conditions, termed chaperonopathies (Macario & de Macario, 2002; Macario et al., 2005; Macario & de Macario, 2007). Although the term chaperonopathy was initially used to include any putative alteration in the expression, post-translational modifications or localization of chaperones, this review will only discuss genetically inherited mutations in the HSPs that are the direct causative factor to a specific disease (Table 1).

Genetic chaperonopathies described so far only concern members of the HSPB, DNAJ and chaperonin families, as well as some chaperone co-factors (Table 1). No genetic chaperonopathies are associated with the Hsp70/HSPA or Hsp90/HSPC family members, either because of functional redundancy within these families or because they are crucial to the central chaperone machinery such that functional mutations are incompatible with life.
Figure 4. Overview of chaperonopathies caused by mutations in heat shock proteins

Mutations that lead to either recessive (grey boxes) or dominant (black boxes) chaperonopathies have been described for six ‘families’ of heat shock protein. Each chaperonopathy is categorized as a neuropathy, myopathy or retina-related disease (cataracts). The mutations in HSPs involved in both recessive and dominant diseases have been shaded with both colors (grey and black). h-SP: Hereditary-spastic paraplegia; dHMN: Distal Hereditary Motor Neuropathy; MN: Motor Neuropathy; DCM: Dilated Cardiomyopathy; MFM: Myofibrillar Myopathy; LD: Leukodystrophy; MD: Muscular Dystrophy; CC: Congenital Cataract; DT: Dystrophy; CMT2: Charcot Marie Tooth Disease 2.

Disease-wise, genetic chaperonopathies can be categorized into neuropathies (hereditary spastic paraplegia, motor neuropathy, distal hereditary motor neuropathy), myopathies (dilated cardiomyopathy leukodystrophy, desmin related myopathy, mitochondrial myopathy, muscular dystrophy), or retina and eye lens related diseases (congenital cataracts) (Macario et al., 2005). Although some chaperonopathies are recessive (and thus probably related to loss-of-function of the chaperone), most were found to be dominant, as is especially the case for the HSPBs (Table 1). We have labeled or “barcoded” these HSP-associated chaperonopathies depending on their disease type and inheritance (Fig. 4).

**Hsp60/HSPD and TRiC/CCT-related chaperonopathies**

A mutation in the Hsp60/HSPD chaperone system has been linked to an autosomal dominant disease known as hereditary spastic paraplegia 13 (SPG13). The disease is characterized by spasticity of lower limbs due to massive degeneration of distal ends of long axons in the spinal cord. The mutation leads to reduced chaperonin
activity, which has been attributed to haploid insufficiency due to incorporation of functionally deficient Hsp60/HSPD subunits (Bross et al., 2008; Hansen et al., 2002). Another chaperonopathy involving Hsp60/HSPD is the recessive mitCHAP-60 disease, associated with psychomotor developmental delay, where mutations lead to entropic destabilization of the Hsp60/HSPD oligomer and cause its premature disassembly. This renders Hsp60/HSPD incapable of fulfilling its normal function and thus results in disease (Parnas et al., 2009).

The Hsp60/HSPD complex resides in mitochondria; however, a comparable eukaryotic chaperonin system known as TRiC/CCT is present in the cytosol and is mainly involved in tubulin and actin folding. Mutations in TRiC/CCT subunits might affect its complex formation and thereby its ability to bind and fold tubulin and actin. As cytoskeletal integrity is crucial in axonal transport, this might explain why such mutants primarily affect functionality of long axons thus leading to sensory neuropathies (Lee et al., 2003).

**DNAJ related chaperonopathies**

There are four recessive chaperonopathies associated with members of the DNAJ family (Table 1). The first one involves a DNAJB2 (splice) mutation that causes distal hereditary motor neuropathy (dHMN) due to progressive degeneration of motor neurons in the spinal cord characterized by muscle weakness of the extremities (Blumen et al., 2012). DNAJB2 has several clients and possesses degradation-related functions (Chapple et al., 2004; Westhoff et al., 2005). The DNAJB2 mutant is unable to handle its natural clients, which therefore eventually aggregate and form intracellular inclusions (Blumen et al., 2012).

The second recessive disease caused by mutations in DNAJ involves DNAJC29. Mutations in DNAJC29 lead to cerebellar ataxia with peripheral neuropathy, which is referred to as ARSACS. The disease is characterized by dysarthria, distal muscle wasting, foot deformities and truncal ataxia, including absence of sensory evoked potentials in the lower limbs (Bouchard et al., 1998). Although the normal function of DNAJC29 is not well understood, roles in mitochondrial dynamics and in recruitment of HSPA for the mediation of ataxin-1 degradation have been suggested (Parfitt et al., 2009; Girard et al., 2012). In turn, a recently found ARSACS causing mutation (T3702A) resides in the ubiquitin-binding domain of this protein (Gregianin et al., 2013). However, mutations outside this domain can also lead to disease, and it remains unclear if/how this could lead to loss-of-function.

Mutations in DNAJC19 have been identified to cause an autosomal recessive cardiomyopathy (Davey et al., 2006; Ojala et al., 2012). DNAJC19 normally plays a crucial role in mitochondrial import (Mokranjac et al., 2003), implying that deficiency causes disease via mitochondrial defects.
### Table I : Chaperonopathies

<table>
<thead>
<tr>
<th>Family</th>
<th>Mutation</th>
<th>Inheritance</th>
<th>Disease</th>
<th>Chaperone Mediated rescue</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp60/HSPD</td>
<td>V72I in SPG13; Chromosome 2q33.1; c.292G &gt; A/p.V98I</td>
<td>Dominant</td>
<td>Hereditary Spastic Paraplegia</td>
<td>NV</td>
<td>Bross et al., 2008; Hansen et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypomyelinating leukodystrophies (HMLs); MitCHAP-60 disease</td>
<td>NV</td>
<td>Magen et al., 2008</td>
</tr>
<tr>
<td>mitHSP60</td>
<td>D29G</td>
<td>Recessive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hsp40/DNAJ</td>
<td>Splice mutation in HSJ1 gene</td>
<td>Recessive</td>
<td>Distal Hereditary Motor Neuropathy (dHMN)</td>
<td>NV</td>
<td>Blumen et al., 2012</td>
</tr>
<tr>
<td>DNAJB6</td>
<td>F93L, F89I, P96R</td>
<td>Dominant</td>
<td>Limb-Girdle Muscular Dystrophy type 1D (LGMD1D)</td>
<td>NV</td>
<td>Sarparanta et al., 2012; Harms et al., 2012</td>
</tr>
<tr>
<td>DNAJC5</td>
<td>c.346_348 delCTC, c.344T&gt;G; pLeu116del, pLeu115Arg</td>
<td>Dominant</td>
<td>Autosomal dominant-adult-onset neuronal ceroid lipofuscinosis (ANCL) or Kuf’s disease</td>
<td>NV</td>
<td>Noskova et al., 2011; Velinov et al., 2012; Cadieux-Dion et al., 2013</td>
</tr>
<tr>
<td>DNAJC6</td>
<td>p.Q734X; c.801-2 A→G</td>
<td>Recessive</td>
<td>juvenile Parkinsonism</td>
<td>NV</td>
<td>Edvardson et al., 2012; Köröglu et al., 2013</td>
</tr>
</tbody>
</table>

**CCT/TRiC**

| CCTγ/ MKKS | 20p12 in MKKS gene; H84Y, A242S               | Recessive   | McKusick-Kaufman Syndrome (MKS)             | NV                        | Stone et al., 2000               |
| CCT/ MKKS  | MKKS locus BBS6, BBS10, BBS12                | Recessive   | Bardet- Biedel Syndrome (BBS)               | NV                        | Stone et al., 2000; Katsanis et al., 2000; Slavotinek et al., 2000; Stoetzel et al., 2006 |
| CCT delta/ MKKS | C450Y in CCT delta                     | Recessive   | Hereditary Sensory Neuropathy (HSN): Charcot-Marie-Tooth (CMT), Hereditary Motor & sensory neuropathy (HMSN) | NV                        | Lee et al., 2003                  |

**Hsp40/DNAJ**

| DNAJB2 (HSJ1) | Splice mutation in HSJ1 gene | Recessive   | Distal Hereditary Motor Neuropathy (dHMN)   | NV                        | Blumen et al., 2012             |

Chapter 2
<table>
<thead>
<tr>
<th>Family</th>
<th>Mutation</th>
<th>Inheritance</th>
<th>Disease</th>
<th>Chaperone Mediated rescue</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNAJC19</td>
<td>IVS3-1GRC; c.300delA</td>
<td>Recessive</td>
<td>Dilated cardiomyopathy with ataxia (DCMA)</td>
<td>NV</td>
<td>Davey et al., 2006; Ojala et al., 2012</td>
</tr>
<tr>
<td></td>
<td>c.3484 G&gt;T, p.E1162X; c.11,707C&gt;T, p.R 3903X in SACS; T3702A</td>
<td>Recessive</td>
<td>Spastic ataxia of Charlevoix-Saguenay (ARSACS)</td>
<td>NV</td>
<td>Bouchard et al., 1998; Engert et al., 2000; Bouhlal et al. 2011; Grogianin et al., 2013</td>
</tr>
</tbody>
</table>

**Small Hsp/HSPB**

<table>
<thead>
<tr>
<th>HspB1</th>
<th>P39L, G34R, E41K, G84R, L99M, R127W, S135F, R136W, R140G, K141Q, T15115115115151151</th>
<th>Dominant; Recessive</th>
<th>Williams Syndrome; Charcot-Marie-Tooth Disease 2 (CMT2), Distal Hereditary Motor Neuropathy (dHMN)</th>
<th>HspB8 mediated rescue for P182L mutation*</th>
<th>*Carra et al., 2010; Boncoraglio et al., 2012; Datskevich et al., 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>HspB3</td>
<td>R7S</td>
<td>Recessive</td>
<td>Motor Neuropathy (MN)</td>
<td>NV</td>
<td>Kolb et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(W9X)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R56W)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HspB8</td>
<td>K141E, K141N, K141T</td>
<td>Dominant</td>
<td>Distal Hereditary Motor Neuropathy (dHMN), Charcot-Marie-Tooth Disease 2 (CMT2)</td>
<td>NV</td>
<td>Irobi et al., 2004; Tang et al., 2005; Nakhro et al., 2013</td>
</tr>
</tbody>
</table>

NV: Not verified
More recently, mutations in DNAJC6 were found to be associated with juvenile-onset Parkinsonism (Edvardson et al., 2012; Koroglu et al., 2013). DNAJC6/ Auxilin is a neuron-specific protein that assists Hsc70/HSPA8 in mediating clathrin-coated vesicle disassembly and thus plays a role in synaptic vesicle recycling (Ungewickell et al., 1995; Xing et al., 2010). Mutations in DNAJC6 are predicted to lead to a truncated version of the protein, which fails to support Hsc70/HSPA8 in its normal function.

Next to these recessive diseases, two DNAJ-related chaperonopathies are dominantly inherited (Table 1) and could either cause disease through haploinsufficiency, by dominant-negative effects or via a toxic gain-of-function. Mutations in DNAJB6, all of which are found in the glycine-phenylalanine region (G/F), are associated with limb-girdle muscular dystrophy type 1D (LGMD1D). The disease leads to progressive muscle weakness and muscle atrophy. The molecular mechanism underlying the disease has been suggested to involve loss-of-function, resulting in protein accumulations and autophagic pathology in muscle fibers (Harms et al., 2012; Sarparanta et al., 2012). This reduced chaperone function might be due to haploinsufficiency, but as DNAJB6 is present in cells as polydispersed complexes, mutants might also exert dominant negative effects on the wild-type protein.

Mutations in DNAJC5 cause an autosomal dominant neurodegenerative disease, named Kuf’s disease or adult onset neuronal ceroid lipofuscinosis. Clinical symptoms include a dementia, ataxia and speech disorder which worsens over time. Normally, DNAJC5 is found in synaptic vesicles and involved in polymerization of dynamin (Zhang et al., 2012). Dysfunction of DNAJC5 due to mutations in the crucial lysine position leads to its reduced palmitoylation and hence abnormal sorting and localization of DNAJC5 with ER and Golgi markers (Noskova et al., 2011). This leads to decreased levels of DNAJC5 in the brain of diseased individuals, meaning that the disease is most likely caused by haploinsufficiency.

**HSPB-related chaperonopathies**

Mutations in several members of the HSPB family, irrespective of the member involved, are found in highly conserved amino acid residues or in the α-crystallin domain, which is a characteristic feature of this family of HSPs (Boncoraglio et al., 2012). The α-crystallin domain is required for intra/inter-molecular interactions and the stabilization of homo- and hetero-oligomer formations of the HSPB members. As HSPBs are highly expressed in muscles and have a role in cytoskeleton stability (Tessier et al., 2003; Kampinga & Garrido, 2012), mutations usually affect the cells’ axonal transport (neurological and sensory disorders) and contractile functions (muscular disorders).
The presence of many of the dominant HSPB mutants in protein aggregates implies that they might have acquired a toxic gain-of-function similar to the proteins in proteinopathies (Fig. 3). There is indeed biochemical evidence that some mutants, such as the P182L mutant of HSPB1 (Ackerley et al., 2006), R49C and R116C of HSPB4 (Andley et al., 2002; Mackay et al., 2003), and R120G, Q151X and 464delCt of HSPB5 (Bova et al., 1999; Perng, 2004; Hayes et al., 2008) are intrinsically unstable and might thus cause disease by forming aggregates (toxic gain-of-function). However, it must be noted that the presence of HSPBs in aggregates could also be due to a loss-of-function, reflecting their failed attempt to handle a client with whom they subsequently co-aggregate.

Evidence for haploinsufficiency, at least for HSPB1 mutations, is suggested by findings implying that reduced levels of HSPB1 lead to damage in sensory and motor neurons, that can be rescued by ectopic expression of HSPB1 (Lewis et al., 1999; Boncoraglio et al., 2012). Partial evidence for haploinsufficiency has also been provided for HSPB8 mutants, which have lost the HSPB8 chaperone-like activity to deal with aggregation-prone polyQ proteins and cause Charcot Marie Tooth disease, characterized by distal muscle weakness and atrophy and sensory loss (Carra et al., 2008).

Moreover, considering that HSPBs are known to form oligomers with other members of the same family, it is possible that HSPB mutants could affect the function of other HSPBs via dominant negative effects. HSPB8 mutants for example have an abnormally high affinity for endogenous HSPB1, thus potentially impairing HSPB1 or HSPB1-HSPB8 complex function (Irobi et al., 2004). Similarly, certain HSPB1 mutants affect endogenous HSPB8, leading to loss of HSPB8-HSPB1 complex formation (Fonatine et al., 2006). Furthermore, abnormal interaction of the R116C-HSPB4 mutant with HSPB5 and HSPB1 has been reported (Fu and Liang, 2002).

Therefore, not only a toxic gain-of-function (aggregation) might be responsible for HSPB-related chaperonopathies, but also a loss-of-function, which could either be direct, due to the mutation, or indirect, due to sequestration of wild-type HSPBs by the mutated HSPB forms. In addition, alteration of HSPB-oligomerization properties and interactions with other HSPB members and/or haploinsufficiency might play a role in HSPB-related chaperonopathies.

**Chaperone intervention to rescue chaperonopathies**

Different HSPs have a role in anti-aggregation of various proteinopathies (Fig. 3). However, whether other HSPs might be able to rescue chaperonopathies has been scarcely studied. A few reports suggest this could indeed be possible: Firstly, aggregation caused by some HSPB5 mutants could be prevented by overexpression of wild-type HSPB1 (Zhang et al., 2010; Raju & Abraham, 2013), BAG3 (Hishiya et al.,
Chapter 2

2011) and wild-type HSPB8 (Zobel et al. 2003). Secondly, aggregation associated with the expression of the P182L-HSPB1 mutant in cell models was significantly reduced by the overexpression of wild-type HSPB8 (Carra et al., 2010). Whether such rescues are due to prevention of the formation of toxic aggregates containing mutant HSPB or whether they reflect a compensation for loss of (redundant) HSPB functions remains to be elucidated.

**Conclusions and future perspectives**

The existence of different ‘barcodes’ for the rescue of specific aggregation diseases suggests that, although loss of protein homeostasis with aging might contribute to disease initiation (e.g., by HSF-1 abrogation, restoring general protein homeostasis or components thereof), boosting HSF-1 activity is usually insufficient for long-term protection in most dominantly inherited proteinopathies. Chronic expression of these aggregation-prone proteins in fact often does not trigger activation of the HSR until late in disease. By then, aggregates might have already sequestered chaperones and thereby disturbed normal protein homeostasis, resulting in cell death. In earlier stages of disease, protein aggregates could already affect neuronal and muscular cell function (even without causing cell death) by altering functions such as axonal transport, organelle dynamics and plasma membrane (receptor) function, without directly impairing protein homeostasis. It has been shown in several mouse models for HD that reversible functional impairments precede neuronal cell loss (Yamamoto et al., 2000). However, in cellular and simple animal models, such functional defects could be missed and cell death-related effects (including disturbances in protein homeostasis) might prevail, which would explain the observed rescue by the activation of the HSR or by the overexpression of its individual components. However, these HSR-related effects usually do not coincide with aggregate prevention and therefore do not lead to significant, long-term, effects in mammalian animal models.

The human genome encodes many HSP members that are not regulated by the acute HSR. Although not yet studied intensively, our review clearly shows that some of these “non-canonical” members can specifically rescue aggregation caused by the distinct proteinopathies, some of which have now also been demonstrated to be effective in mouse models. Interestingly though, several of these non-canonical HSPs also cause chaperonopathies if mutated (DNAJB2, DNAJB6, HSPB8). This not only indicates that these HSPs have essential PQC functions, but furthermore suggests that their effects on proteinopathies might not be an artefact of their overexpression, but rather reflect an augmentation of their natural function.
A potential worry in all HSP overexpression or boosting studies is that it leads to network adaptations (which would annihilate long-term effectiveness) or to multiple side effects, including increasing carcinogenesis, as was demonstrated for the manipulation of HSF-1 activity (Mendillo et al., 2002). Although network adaptations are to be expected upon manipulation of the driving forces of chaperone machinery (e.g. Hsp90/HSPC or Hsp70/HSPA), such effects might be less likely for those components that only steer the specificity of these machines (e.g. HSPBs or DNAJs). Although we found no evidence for DNAJB6 effects on the chaperone network, it remains important to further investigate whether (long-term) overexpression of DNAJB6 or other proteinopathy-rescuing HSPs might have side effects.

Finally, only limited comparative data on the potential rescue of the non-HSR regulated HSPs and the various proteinopathies or chaperonopathies are available. There still might be many novel suppressors of specific diseases to be uncovered, which would further barcode these diseases. This would not only help to find therapeutic targets for intervention, but would also help with understanding differences and similarities between the toxic mechanisms underlying the various proteinopathies.

Acknowledgements

We are grateful to Peter Nagle for proofreading the manuscript. The authors were supported by grants from the Prinses Beatrix Fonds/Dutch Huntington Association (WAR09-23) awarded to S. Carra and H.H. Kampinga and from Senter Novem (IOP-IGE07004) awarded to H.H. Kampinga.
References:


Chapter 2


