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AMYLOID REMODELING BY Hsp104

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7.1 PROTEIN MISFOLDING IS A UNIVERSAL PROBLEM

Life demands that proteins fold into very precise functional structures. Functional native structure is enciphered by primary sequence (Anfinsen, 1973; Englander *et al.*, 2007). However, native structures are dynamic systems composed of sophisticated networks of weak, mutually supportive contacts that are difficult to establish simultaneously during folding (Bartlett and Radford, 2009; Englander *et al.*, 2007). Thus, folding energy landscapes are often rugged and create challenges for successful folding (Bartlett and Radford, 2009). Polypeptides can become trapped in non-native intermediate states or become diverted into off-pathway states. Even after the completion of folding, cooperative units of native structure, termed *foldons*, repeatedly unfold and refold (Englander *et al.*, 2007). Moreover, mutation or errors in transcription or translation can yield polypeptides that are less able to form functional structures (Dobson, 2003; Lee *et al.*, 2006). Environmental stress can also disrupt protein folding (Parsell and Lindquist, 1993). Consequently, proteins can fail to fold or fail to remain correctly folded. These failures increase the risk of aggregation. The highly crowded macromolecular environment that cells are forced to maintain to function optimally further accentuates this risk (Dobson, 2003; Ellis and Minton,

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2006). Therefore, sophisticated protein homeostasis (proteostasis) systems have evolved, which ensure that polypeptides can effectively acquire, maintain, and reacquire their functional native structure or be eliminated should folding become too improbable (Balch *et al.*, 2008; Powers *et al.*, 2009).

To ensure a folding-competent state, molecular chaperones contact the nascent polypeptide even before translation is complete (Kramer *et al.*, 2009). Following translation, molecular chaperones prevent aggregation and assist polypeptides in acquiring their native form (Young *et al.*, 2004). The ubiquitin–proteasome system degrades any terminally misfolded forms (Varshavsky, 2005; Vembar and Brodsky, 2008). However, aggregated proteins resist proteasomal degradation (Bence *et al.*, 2001) but can be catabolized by autophagy (Cuervo, 2008). Finally, sophisticated disaggregases reverse protein aggregation. Disaggregation can be coupled to degradation (Bieschke *et al.*, 2009; Cohen *et al.*, 2006; Murray *et al.*, 2010) or renaturation (Doyle and Wickner, 2009; Glover and Lum, 2009; Shorter, 2008; Weibezahn *et al.*, 2005).

Once individuals reach postreproductive age, these proteostatic safeguards decline inexorably, and errors in protein folding can arise with devastating sequelae (Cohen *et al.*, 2006; Cuervo, 2008; Morimoto, 2006; Skovronsky *et al.*, 2006). A pernicious and recurring problem is that the functional native structure is not always the lowest free energy form (Englander *et al.*, 2007). Rather, many proteins, irrespective of primary sequence, can spontaneously form generic, cross-β polymers of even lower free energy, termed *amyloid* (Dobson, 2003; Englander *et al.*, 2007).

7.2 AMYLOID CONFORMERS CAN BE PATHOGENIC, PROTECTIVE, OR BENEFICIAL

Amyloidogenesis of various specific proteins is linked with a legion of devastating disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), type II diabetes, prion diseases, and various cardiovascular and systemic amyloidoses (Caughey and Lansbury, 2003; Cushman *et al.*, 2010; Kholova and Niessen, 2005; Skovronsky *et al.*, 2006; Taylor *et al.*, 2002). There are no effective treatments for any of these conditions. Furthermore, a severe risk factor for these diseases is aging (Morimoto, 2006). Indeed, because natural selection acts less powerfully on genetic variation expressed at postreproductive age, many genes may harbor "late-expressing" harmful mutations (Medawar, 1952). Some of these mutations may predispose proteins to forming amyloids or prions (infectious amyloids) in the environment of an aging individual where the proteostasis network is in decline. Several examples are found in the mammalian prion protein (PrP) (Kong *et al.*, 2004). As life spans are extended through improvements in medicine and public health, these disorders will inevitably increase in prevalence. Indeed, they threaten to become one among the most intractable barriers to living longer, more fulfilling lives.

Amyloids possess a "cross-β" form in which the strands of the β-sheets align perpendicular to the fiber axis (Nelson and Eisenberg, 2006; Sunde and

Blake, 1997; Sunde *et al.*, 1997). The amyloid fold is extremely stable and resists disruption by various denaturing conditions, including protease digestion, detergents, chaotropes, and high temperatures (Eisenberg *et al.*, 2006; Knowles *et al.*, 2007; Smith *et al.*, 2006). This extreme stability makes amyloid difficult to eliminate. Indeed, in systemic amyloidoses, amyloid accumulation can be so severe that tissue architecture becomes mechanically disrupted (Merlini and Westermark, 2004). The ends of amyloid fibers capture other copies of the same protein and convert them to the cross-β structure. Once initiated, this self-templating or "seeding" process can convert all the copies of a given protein to the amyloid fold (Lansbury and Caughey, 1995; Nelson and Eisenberg, 2006). Steric effects usually cause proteins to lose functionality in the amyloid state (Baxa *et al.*, 2002). This "loss-of-function" contributes to pathogenesis in some disorders (Forman *et al.*, 2004). Furthermore, by depleting other cellular components that coprecipitate, amyloids can also cause other proteins to lose functionality (Chen *et al.*, 2005).

In various diseases, however, the quantity of amyloid deposits can be minimal and their presence can correlate with cell survival (Arrasate *et al.*, 2004; Cohen *et al.*, 2006; de Calignon *et al.*, 2010; Dobson, 2003). These findings have generated proposals that amyloid forms may be relatively benign and reflect a cellular defense mechanism that sequesters toxic soluble species (Bucciantini *et al.*, 2002; Kayed *et al.*, 2003). This benefit might outweigh the cost of these space-occupying lesions. Indeed, the soluble oligomeric species that assemble during the distinctive lag phase of amyloid formation can be highly toxic and share a generic conformation, which is distinct from fibers and independent of primary sequence (Bucciantini *et al.*, 2002; Haass and Selkoe, 2007; Kayed *et al.*, 2003; Lashuel *et al.*, 2002; Lesne *et al.*, 2006). These shared features of amyloidogenesis indicate that effective therapeutics might have broad applicability (Skovronsky *et al.*, 2006). Despite these similarities, however, a major unresolved issue concerns how the amyloidogenesis of different proteins can confer the selective neuronal cell death that distinguishes various neurodegenerative disorders (Cushman *et al.*, 2010; Skovronsky *et al.*, 2006).

Recent studies, however, suggest that the amyloid state is unlikely to be invariably benign. In a mouse AD model, β-amyloid (Aβ) plaques can form rapidly and mediate pathology (Meyer-Luehmann *et al.*, 2008). Amyloid might also slowly release toxic misfolded species. For example, natural lipids can destabilize amyloid fibers and liberate toxic oligomers (Martins *et al.*, 2008). Another issue concerns the ability of amyloidogenic proteins to fold into multiple structurally distinct amyloid forms or "strains," which confer distinct phenotypes (Legname *et al.*, 2006; Safar *et al.*, 1998; Tanaka *et al.*, 2006). Beyond sharing the cross-β amyloid form, little is known about the underlying atomic structures of these distinct strains or how structural polymorphism enciphers distinct phenotypes or disease states (Wiltzius *et al.*, 2009). Distinct ensembles of strains form depending on the environment (e.g., pH, temperature). Strains are distinguished by distinct intermolecular contacts between fiber protomers and different lengths of primary sequence sequestered in cross-β structure (Krishnan and Lindquist, 2005; Roberts

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et al., 2009; Tessier and Lindquist, 2007; Toyama *et al.*, 2007). Different strains of Aβ40 fibers, which are connected with AD, and polyglutamine (polyQ), which are connected to HD, confer different levels of toxicity (Nekooki-Machida *et al.*, 2009; Petkova *et al.*, 2005). Some strains are relatively benign, whereas others are highly toxic (Nekooki-Machida *et al.*, 2009; Petkova *et al.*, 2005). Intriguingly, toxic strains are more abundant in brain regions with the most neurodegeneration in mouse models of HD (Nekooki-Machida *et al.*, 2009). These data suggest that local proteostatic buffers or expression levels might create strain biases *in situ*. It will be critical to determine how strain variation correlates with affected brain regions in various neurodegenerative amyloidoses. Identifying which strains are toxic and which are benign will help inform potential targeted therapies.

Thus, amyloid can be either detrimental or benign, depending on the precise strain. It is therefore not surprising that benign amyloids have been captured during evolution for functional, adaptive purposes (Fowler *et al.*, 2007; Shorter and Lindquist, 2005b). For example, Pmel17 amyloids function in melanosome formation (Berson *et al.*, 2003; Fowler *et al.*, 2006; Watt *et al.*, 2009). Amyloid forms of cytoplasmic polyadenylation element-binding protein (CPEB) might function in synapse stabilization, which promotes long-term memory formation (Si *et al.*, 2003, 2010). In yeast, many proteins can form infectious amyloids, termed *prions*, which provide a vast reservoir of heritable phenotypic variation that can be advantageous under diverse environmental conditions (Alberti *et al.*, 2009; Griswold and Masel, 2009; King and Masel, 2007; Shorter and Lindquist, 2005b; True and Lindquist, 2000; Tyedmers *et al.*, 2008). In these cases, the proteostasis network ensures that benign amyloid conformers assemble instead of toxic intermediates or strains (Douglas *et al.*, 2008; Shorter and Lindquist, 2004; Treusch *et al.*, 2009). An accurate understanding of how amyloids have been exploited for beneficial purposes will likely yield important insights into how to safely eliminate toxic amyloid fibers and preamyloid oligomers.

There are no cures or effective treatments for any of the neurodegenerative amyloidoses confronting humankind. Therapies remain palliative in nature and do not antagonize the underlying causative continuum of amyloid forms or cytotoxic oligomers. A seminal therapeutic advance will come with the ability to enhance proteostasis to eliminate entire spectra of toxic amyloid strains and preamyloid oligomers, while leaving beneficial amyloid structures unperturbed. Here, we discuss the notion of enhancing mammalian proteostasis with a protein disaggregase from yeast, heat shock protein (Hsp104), which can rapidly resolve amyloid conformers and preamyloid oligomers (Shorter, 2008). First, however, I will introduce Hsp104 and consider the mechanistic basis of its activity.

7.3 Hsp104 IS AN AAA+ ATPase WITH PROTEIN DISAGGREGASE ACTIVITY

Hsp104 is an *A*TPase *A*ssociated with diverse *A*ctivities (AAA+) protein (Erzberger and Berger, 2006; Neuwald *et al.*, 1999), which enhances yeast

survival several 1000-fold after a variety of environmental stresses that induce protein aggregation, including heat and chemical shock (Sanchez and Lindquist, 1990; Sanchez *et al.*, 1992). Orthologs in bacteria (ClpB) and plants (Hsp101) provide selective advantages of a similar magnitude (Queitsch *et al.*, 2000; Squires *et al.*, 1991). This adaptive benefit of Hsp104 lies in its ability to rescue proteins from denatured aggregates and restore them to native structure and function. This extraordinary activity is coordinated by the Hsp70 chaperone system (Cashikar *et al.*, 2005; Glover and Lindquist, 1998; Goloubinoff *et al.*, 1999; Mogk *et al.*, 1999; Parsell *et al.*, 1993, 1994b; Weibezahn *et al.*, 2004). The Hsp70 chaperone system helps deliver aggregated substrates to Hsp104 (Glover and Lindquist, 1998; Tessarz *et al.*, 2008). Once an unfolded substrate is released from the aggregate, the Hsp70 chaperone system also promotes refolding (Doyle *et al.*, 2007b; Glover and Lindquist, 1998). This salvage and rapid renaturation of proteins obviates the severe energetic costs of protein degradation and *de novo* biosynthesis that would otherwise be required to eliminate and replace the aggregated protein. Thus, cells can recover rapidly from environmental stresses that induce protein aggregation.

Hsp104 can be divided into five domains: an N-terminal domain, a first AAA+ nucleotide-binding domain (NBD1), a coiled-coil middle domain, a second AAA+ nucleotide-binding domain (NBD2), and a short C-terminal domain (Doyle and Wickner, 2009). Like many AAA+ proteins, Hsp104 is only active as a hexamer, which forms upon adenosine diphosphate (ADP) or adenosine triphosphate (ATP) binding to NBD2 (Parsell *et al.*, 1994a; Schirmer *et al.*, 1998, 2001). Unfortunately, there is no atomic resolution structure of the Hsp104 hexamer. However, cryo-electron microscopy and single particle reconstruction have revealed that the Hsp104 hexamer is a three-tiered ring structure that envelops a large central cavity or channel (Wendler and Saibil, 2010; Wendler *et al.*, 2007, 2009).

The monomeric structure of the *T. thermophilus* ortholog, tClpB, has been solved (Lee *et al.*, 2003). Using this structure, the Hsp104 monomer has been homology modeled and fitted as rigid bodies into electron density envelopes (Wendler and Saibil, 2010; Wendler *et al.*, 2007, 2009). These studies have revealed that a small ring of N-terminal domains forms the top tier of the hexamer, whereas expanded rings of NBD1 and NBD2 form the middle and lower tiers, respectively (Wendler *et al.*, 2007, 2009). The distinctive middle domain, which is composed of two antiparallel coiled-coil motifs reminiscent of a two-bladed propeller (Lee *et al.*, 2003), intercalates between NBD1 and NBD2 in the wall of the hexamer (Wendler *et al.*, 2007, 2009). This hexameric model of Hsp104 differs markedly from a hexameric model advanced for tClpB, where the coiled-coil domains protrude laterally from the surface of the hexameric ring (Lee *et al.*, 2003, 2007). There are several potential explanations for these differences, which are discussed in detail elsewhere (Wendler and Saibil, 2010).

Hsp104 and orthologs use energy from ATP binding and hydrolysis to translocate polypeptides from the aggregate surface across the central channel to solution (Lum *et al.*, 2004, 2008; Schlieker *et al.*, 2004; Shorter and Lindquist,

2005a; Weibezahn *et al.*, 2004). However, we are only beginning to understand the mechanistic and structural basis of this activity. Simultaneous elimination of ATPase activity at *both* NBDs abolishes disaggregase activity (Doyle *et al.*, 2007b; Parsell *et al.*, 1991; Shorter and Lindquist, 2004). Hsp104 initially engages misfolded substrates when NBD1 is in an ATP-bound conformation (Bosl *et al.*, 2005; Schaupp *et al.*, 2007). Both NBDs catalyze ATP hydrolysis cooperatively, and allosteric communication occurs within and between NBD1 and NBD2 (Cashikar *et al.*, 2002; Doyle *et al.*, 2007b; Hattendorf and Lindquist, 2002; Schaupp *et al.*, 2007; Schirmer *et al.*, 2001). In the absence of substrate, NBD1 makes the major contribution to ATPase activity ($k_{\text{cat}} \sim 76$ min^{-1}, $K_M \sim 170$ μM, $n_h = 2.3$) but has a lower affinity for nucleotide compared to NBD2 ($k_{\text{cat}} \sim 0.27$ min^{-1}, $K_M \sim 4.7$ μM, $n_h = 1.6$) (Hattendorf and Lindquist, 2002). Despite these advances, little is known about precisely how allosteric regulation of ATP hydrolysis within and between NBD1 and NBD2 is coupled to the substrate binding, unfolding, and translocation that are required for disaggregation. Indeed, how individual subunits within the hexamer collaborate to coordinate protein disaggregation remains obscure.

Several insights have been afforded by artificially inducing disaggregation activity in ClpB and Hsp104 in the absence of Hsp70 and Hsp40. For example, dissolution of denatured aggregates by ClpB and Hsp104 can be triggered with specific mixtures of ATP and ATPγS, a slowly hydrolyzable ATP analog (Doyle *et al.*, 2007a, 2007b; Hoskins *et al.*, 2009). Alternatively, mutation of conserved AAA+ motifs (Walker A, Walker B, or sensor-1) at one nucleotide-binding domain (NBD) to slow ATP hydrolysis at that site can also elicit disaggregase or substrate unfolding activity in the absence of Hsp70 and Hsp40 (Doyle *et al.*, 2007a, 2007b; Hoskins *et al.*, 2009; Schaupp *et al.*, 2007). ClpB hexamers exchange protomers rapidly, which might enable recycling of monomers should disaggregation stall or fail (Haslberger *et al.*, 2008; Werbeck *et al.*, 2008). This rapid exchange of monomers facilitates titration experiments with mutant subunits to assess how easily a hexamer can be inactivated, for example, by one or two mutant subunits (Crampton *et al.*, 2006). A particularly useful mutant bears Walker B mutations in both NBDs (Weibezahn *et al.*, 2003). This mutant can engage substrate, bind nucleotide, and form hexamers, and is solely defective in ATP hydrolysis (Weibezahn *et al.*, 2003). In situations where ClpB is activated in the absence of Hsp70 and Hsp40, ClpB hexamers are relatively insensitive to this mutant, suggesting that ClpB subunits can act via a probabilistic mechanism to promote some disaggregation events (Hoskins *et al.*, 2009).

However, the disaggregation of denatured aggregates is most effective when coordinated by Hsp70 and Hsp40. Hsp70 and Hsp40 are required to present denatured aggregated substrates to Hsp104 (Glover and Lindquist, 1998; Tessarz *et al.*, 2008; Weibezahn *et al.*, 2004) and likely play some role in coordinating Hsp104 ATPase cycling (Doyle *et al.*, 2007a, 2007b; Hoskins *et al.*, 2009). In some cases, the substrate itself (e.g., amyloid) can impose the requisite changes (Doyle *et al.*, 2007b; Shorter and Lindquist, 2004). A small fraction of double Walker B mutant subunits, perhaps as little as one per hexamer, poisons ClpB-mediated

disaggregation of denatured aggregates, which is coordinated by the Hsp70 chaperone system (Haslberger *et al.*, 2008; Hoskins *et al.*, 2009; Werbeck *et al.*, 2008).

Collectively, these data suggest that a cooperative division of labor among the 12 AAA+ domains drives protein disaggregation. One subset slowly hydrolyzes ATP to facilitate substrate binding, whereas another subset rapidly hydrolyzes ATP to promote substrate unfolding and translocation. This division of labor is adaptable, and precisely how it is established can vary depending on the substrate and the presence of the Hsp70 system. For example, under the conditions where disaggregation is artificially elicited by mutation or by mixture of ATP and ATPγS, the subunits that hydrolyze ATP are determined on some probabilistic basis (Hoskins *et al.*, 2009). By contrast, in the presence of Hsp70 and Hsp40, the division of labor is coordinated such that individual subunits must hydrolyze ATP in a concerted or sequential manner to drive disaggregation (Haslberger *et al.*, 2008; Hoskins *et al.*, 2009; Werbeck *et al.*, 2008). Such concerted or sequential intersubunit collaboration is considerably more effective in driving disaggregation than the probabilistic mode (Doyle *et al.*, 2007a, b; Hoskins *et al.*, 2009).

How does this cooperative division of labor promote substrate translocation across the central channel? The N- and C-terminal domains may help bind substrates and cofactors (Barnett *et al.*, 2005; Cashikar *et al.*, 2002; Mackay *et al.*, 2008). However, critical substrate interactions are mediated by an α-helical insertion in NBD1 and a β-hairpin insertion in NBD2, located before helix α 2 in the αβ subdomain in both NBDs (Lum *et al.*, 2004, 2008; Schlieker *et al.*, 2004; Tessarz *et al.*, 2008; Weibezahn *et al.*, 2004). Short, highly conserved loops, KYKG in NBD1 and GYVG in NBD2, project into the channel (Wendler *et al.*, 2009). Of particular importance is the tyrosine residue in these loops, as mutation of this residue to alanine disrupts substrate interactions and disaggregation activity *in vitro* and *in vivo* (Lum *et al.*, 2004, 2008; Tessarz *et al.*, 2008). Mutation of the NBD2 loop tyrosine confers the most drastic effects *in vivo* and phenocopies deletion of Hsp104 (Lum *et al.*, 2004). More conservative substitutions of the NBD1 or NBD2 loop tyrosines, such as phenylalanine and tryptophan, maintain partial functionality (Cashikar *et al.*, 2002; Hung and Masison, 2006). Dynamic rearrangements of channel loop tyrosines, which are proposed to "grip" the substrate, synchronized with ATPase cycling likely provide a series of motions that translocate substrates across the channel.

Cryo-electron microscopy reconstructions of Hsp104 hexamers in the presence of ATPγS, ATP, and ADP have provided structural insight into the conformational changes that facilitate substrate translocation (Wendler *et al.*, 2009). This study employed the NBD2 sensor-1 mutant, $Hsp104^{N728A}$, which is able to disaggregate denatured aggregates without Hsp70 or Hsp40 (Doyle *et al.*, 2007b), but is defective in prion disaggregation and provides only limited thermotolerance *in vivo* (Hattendorf and Lindquist, 2002; Shorter and Lindquist, 2004). Reconstructions with imposed sixfold symmetry reveal that ATP binding and hydrolysis induce large domain movements in NBD1 that impart a peristaltic mechanism for substrate translocation. The extremely large size of the Hsp104

channel compared to other AAA+ proteins might enable the translocation of exposed loops or more than one polypeptide, rather than having to search for exposed N- or C-termini of individual polypeptides (Haslberger *et al.*, 2008; Wendler *et al.*, 2007). Upon ATP binding, the NBD1 substrate-binding KYKG motifs move up toward the N-terminal end of the channel and are poised to receive the substrate. Upon ATP hydrolysis, NBD1 generates a large motion that displaces the KYKG motif from the N-terminal end to the center of the channel. Simultaneously, the NBD2 substrate-binding GYVG motifs rotate into the center of the channel to receive the substrate translocated by NBD1. Subsequent ATP binding to NBD1 then moves the NBD1 KYKG motifs back up toward the N-terminal entrance, while simultaneously moving the NBD2 GYVG motifs down toward the C-terminal end of the channel. Thus, the NBD2 GYVG motif is able to exert a pulling force without ATP hydrolysis by NBD2. The ADP state of the hexamer suggests that ATP hydrolysis at NBD2 might induce a dramatic rotation of this domain that would eject substrate. These interdependent motions of NBD1 and NBD2 ensure continuous substrate handling during disaggregation (Wendler *et al.*, 2009). Throughout the Hsp104 ATPase cycle, the coiled-coil middle domain, which distinguishes Hsp104 and orthologs from all other AAA+ proteins, appears to play a critical structural role that facilitates the dramatic rotations of NBD1 and NBD2 that forcibly drive substrate translocation (Wendler and Saibil, 2010; Wendler *et al.*, 2007, 2009).

7.4 Hsp104 HAS A POWERFUL AMYLOID-REMODELING ACTIVITY

Hsp104 possesses an unusually powerful amyloid-remodeling activity and couples ATP hydrolysis to the rapid deconstruction of amyloid forms of Sup35 and Ure2, two yeast PrPs (Narayanan *et al.*, 2006; Savistchenko *et al.*, 2008; Shorter and Lindquist, 2004, 2006, 2008). Curiously, bacterial homologs appear to lack the ability to remodel amyloid (Shorter and Lindquist, 2004; Tipton *et al.*, 2008). Importantly, even a brief overexpression of Hsp104 is sufficient to eliminate Sup35 prions (Chernoff *et al.*, 1995). At lower concentrations, Hsp104 fragments yeast prions, which ensures their inheritance through successive generations (Chernoff *et al.*, 1995; Kryndushkin *et al.*, 2003; Patino *et al.*, 1996; Paushkin *et al.*, 1996; Shorter and Lindquist, 2004, 2006). This ability to tightly regulate amyloid conformers endows yeast with another massive selective advantage: the ability to employ prions as metastable switches in protein function (Alberti *et al.*, 2009; Halfmann *et al.*, 2010; Shorter and Lindquist, 2005b). Indeed, yeast exploits prions as a vast reservoir of heritable phenotypic variation, which can be advantageous in diverse environments (Alberti *et al.*, 2009; Griswold and Masel, 2009; King and Masel, 2007; Shorter and Lindquist, 2005b; True and Lindquist, 2000; Tyedmers *et al.*, 2008).

Dissolution of amyloid structure by Hsp104 does not require Hsp70 and Hsp40 (Narayanan *et al.*, 2006; Savistchenko *et al.*, 2008; Shorter and Lindquist, 2004, 2006). However, the presence of the Hsp70 chaperone system can ameliorate

Hsp104 activity against various amyloids *in vitro* (Lo Bianco *et al.*, 2008; Shorter and Lindquist, 2008; Sweeny and Shorter, 2008) and *in vivo* (Chernoff *et al.*, 1999; Higurashi *et al.*, 2008; Tipton *et al.*, 2008). Hsp104 also resolves preamyloid oligomers of Sup35 (Shorter and Lindquist, 2004, 2006), which adopt a generic conformation shared by many disease-associated amyloidogenic proteins (Kayed *et al.*, 2003).

Perplexingly, no clear metazoan homolog or analog of Hsp104 has been identified. Moreover, no activity that couples protein disaggregation to renaturation has been identified in metazoa. Initial attempts to isolate an analogous disaggregase by biochemical fractionation of mammalian cytosol have been unsuccessful (Mosser *et al.*, 2004). Crude homogenates from *C. elegans* and mouse are able to slowly disaggregate Aβ40 and Aβ42 fibers (Bieschke *et al.*, 2009; Cohen *et al.*, 2006; Murray *et al.*, 2010). However, disaggregation is invariably coupled to degradation unless protease inhibitors are added (Bieschke *et al.*, 2009; Cohen *et al.*, 2006; Murray *et al.*, 2010). Compared to Hsp104, this disaggregation activity is relatively slow. Moreover, it displays an unusual resistance to inactivation by high temperature and pH (Bieschke *et al.*, 2009; Murray *et al.*, 2010). Identification of the metazoan factor(s) that promote disaggregation will be extremely illuminating and might enable therapeutic manipulations (Bieschke *et al.*, 2009; Cohen *et al.*, 2006; Murray *et al.*, 2010).

Regardless of the identity of these putative metazoan disaggregases, the ability of Hsp104 to rapidly disassemble the generic cross-β forms of various yeast prions as well as the shared generic structure of preamyloid oligomers raises the possibility of unleashing Hsp104 on metazoan systems to prevent or reverse various amyloidoses (Shorter, 2008). An agent that reverses the formation of amyloid fibers and preamyloid oligomers would antagonize multiple recalcitrant pathological events that likely synergize to various degrees in the etiology of diverse amyloid disorders: (i) the toxic gain-of-function of amyloid or preamyloid oligomers; (ii) the loss-of-function of the protein sequestered in misfolded forms; and (iii) the depletion of various essential proteins that might coaggregate with the disease-associated polypeptide.

Initial efforts to introduce Hsp104 into metazoan systems have been extremely encouraging. Despite being a yeast protein, Hsp104 is well tolerated in metazoan systems and confers no noticeable toxicity. Indeed, expression of Hsp104 in several mammalian cell lines increases their resistance to stresses that promote protein aggregation (Dandoy-Dron *et al.*, 2006; Mosser *et al.*, 2004). Furthermore, Hsp104 synergizes with the mammalian Hsp70 system to resolve denatured aggregates (Glover and Lindquist, 1998; Mosser *et al.*, 2004; Schaupp *et al.*, 2007). Remarkably, Hsp104 protects mammalian cells from several diverse protein-misfolding events, including polyQ aggregation associated with HD (Carmichael *et al.*, 2000; Perrin *et al.*, 2007) and poly (A)-binding protein 2 misfolding associated with oculopharyngeal muscular dystrophy (Bao *et al.*, 2002). Expression of Hsp104 in *C. elegans* or rodents counters polyQ toxicity (Dandoy-Dron *et al.*, 2006; Satyal *et al.*, 2000; Vacher *et al.*, 2005). Transgenic mice that express Hsp104 are grossly normal (Dandoy-Dron *et al.*, 2006;

Vacher *et al.*, 2005). Moreover, Hsp104 expression reduced polyQ aggregation and prolonged the life span of an HD mouse model by ∼20% (Vacher *et al.*, 2005). These studies suggest that Hsp104 can enhance metazoan proteostasis and counter protein aggregation and amyloidogenesis. In the remainder of this chapter, I consider two recent applications of Hsp104 to the amyloidogenic events that distinguish AD and PD.

7.5 Hsp104 AND AD

Beyond minor symptomatic relief, there are no effective treatments (Roberson and Mucke, 2006) for AD, the most common fatal neurodegenerative disorder, which afflicts ∼35 million people (Prince *et al.*, 2009). AD is characterized by gross diffuse atrophy of the brain and neurodegeneration in the cerebral cortex and certain subcortical regions (Wenk, 2003). The defining pathological lesions are intracellular neurofibrillary tangles composed of amyloid forms of the microtubule-binding protein tau (Skovronsky *et al.*, 2006) and extracellular neuritic plaques composed primarily of amyloid forms of the Aβ peptides: Aβ42 and Aβ40 (Glenner and Wong, 1984; Iwatsubo *et al.*, 1994; Masters *et al.*, 1985). Several potential treatments are in clinical trials (Roberson and Mucke, 2006) and several small molecules have been isolated that inhibit (Gestwicki *et al.*, 2004) or even reverse Aβ42 fibrillization (Wang *et al.*, 2008). The effects of Hsp104 on Aβ42 amyloidogenesis have recently been tested (Arimon *et al.*, 2008).

Aβ40 and Aβ42 interact with Hsp104 directly and modulate its ATPase activity (Arimon *et al.*, 2008; Cashikar *et al.*, 2002; Schirmer and Lindquist, 1997). The interaction between Hsp104 and Aβ42 very potently inhibited *de novo* Aβ42 fibrillization, even when Hsp104 was at concentrations 1000-fold lower than Aβ42 (Arimon *et al.*, 2008). This substoichiometric inhibition indicates that Hsp104 might selectively antagonize an obligate intermediate that nucleates Aβ42 fibrillization. In support of this concept, Hsp104 antagonized the conversion of Aβ42 oligomers into fibers and interacted directly with Aβ42 oligomers (Arimon *et al.*, 2008). These inhibitory activities were observed in the presence of ATPγS, a slowly hydrolyzable ATP analog and in the presence of the double Walker B mutant $Hsp104^{E285Q:E687Q}$, which is able to bind, but not hydrolyze, ATP. Therefore, it would appear that this potent inhibition does not require ATP hydrolysis. ATP-restricted Hsp104 is likely to bind to Aβ42 conformers and passively inhibit amyloidogenesis. Indeed, similar observations have been made with Sup35. Inhibition of *de novo* Sup35 fibrillization by high concentrations of Hsp104 can occur without ATP hydrolysis (Shorter and Lindquist, 2004, 2006). In the presence of AMP-PNP (adenosine monophosphate-phosphoneuroprotein) or AMP-PCP, two nonhydrolyzable ATP analogs, Hsp104 inhibits the maturation of molten Sup35 oligomers and thereby prevents fiber nucleation (Shorter and Lindquist, 2004, 2006). Importantly, Hsp104 also potently inhibited fibrillization that was seeded by preformed Aβ42 fibers (Arimon *et al.*, 2008). Consistent with these data, Hsp104 inhibited Aβ42 fibrillization when added during lag phase

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or assembly phase (Arimon *et al.*, 2008). The ability to inhibit seeded assembly is a key finding, because from a therapeutic standpoint, any treatment is only likely to be applied after substantial accumulation of seeding-competent amyloid forms.

Intriguingly, despite the apparent interaction between Hsp104 and Aβ42, Hsp104 did not disassemble Aβ42 fibers or oligomers (Arimon *et al.*, 2008). This might reflect a requirement for the Hsp70 system, which can improve Hsp104-mediated amyloid disassembly (Higurashi *et al.*, 2008; Lo Bianco *et al.*, 2008; Shorter and Lindquist, 2008; Sweeny and Shorter, 2008; Tipton *et al.*, 2008). Alternatively, the Aβ42 fiber strain that formed under the conditions employed might be resistant to Hsp104. Indeed, it is conceivable that all strains of Aβ42 fibers are refractory to Hsp104, since they are substrates that Hsp104 never ordinarily encounters. Nevertheless, the ability of Hsp104 to bind Aβ42 monomers and inhibit seeded assembly, coupled to the fact that amyloids exchange monomers very slowly via a soluble pool (Carulla *et al.*, 2005), might enable Hsp104 to slowly shift the equilibrium away from the assembled fibrous state. Thus, Hsp104 might slowly resolve Aβ42 fibers over a time frame longer than those thus far explored (Arimon *et al.*, 2008).

These *in vitro* findings are promising (Arimon *et al.*, 2008). However, extension to cell culture and animal models is needed for validation. Neuroblastoma cell lines have been widely used to assess the toxicity of Aβ fibers and oligomers (Kayed *et al.*, 2003; Petkova *et al.*, 2005). This system might be readily adapted to test whether the Hsp104–Aβ42 interactions reduce toxicity to cultured neurons. Another issue is that the majority of Aβ42 fibers are extracellular in AD, which may make them challenging targets for Hsp104. However, intraneuronal Aβ42 is also found in AD and may contribute to disease progression (Grundke-Iqbal *et al.*, 1989; LaFerla *et al.*, 2007; Wertkin *et al.*, 1993). It is possible that Hsp104 might be efficacious against intraneuronal pools of misfolded Aβ42.

7.6 Hsp104 AND PD

There are no efficacious treatments for PD, the most common neurodegenerative movement disorder, which afflicts several million people worldwide (Dorsey *et al.*, 2007). PD is due to a severe and selective devastation of dopaminergic neurons from the substantia nigra pars compacta although neuropathology extends into other regions of the brain (Braak *et al.*, 2003). Intracellular inclusions termed *Lewy bodies* and *Lewy neurites*, which are composed of amyloid forms of the small presynaptic protein, α-synuclein (α-syn), are the signature lesion of PD (Spillantini *et al.*, 1997). Although PD is most frequently a sporadic disorder, mutations in α-syn (e.g., A30P, A53T, E46K) and duplication or triplication of the wild-type gene are linked with early-onset PD in rare familial forms of the disease (Moore *et al.*, 2005). α-Syn function is uncertain but may play a key regulatory role in dopamine release from synaptic vesicle pools (Abeliovich

et al., 2000; Gitler and Shorter, 2007; Larsen *et al.*, 2006). Pure α-syn readily accesses amyloid forms *in vitro*, which bear remarkable similarities to α-syn fibers isolated from synucleinopathy patients (Crowther *et al.*, 2000; Spillantini *et al.*, 1998).

In vitro, Hsp104 potently inhibited the fibrillization of α-syn and the early-onset PD-linked variants: A30P, A53T, and E46K (Lo Bianco *et al.*, 2008). Inhibition was most effective in the presence of ATP. In the presence of AMP-PNP, Hsp104 failed to inhibit α-syn fibrillization (Lo Bianco *et al.*, 2008). Furthermore, a double Walker A mutant, $Hsp104^{K218T:K620T}$, which cannot bind or hydrolyze ATP, also failed to inhibit assembly (Lo Bianco *et al.*, 2008). Hsp104 coupled ATP hydrolysis to the disassembly of toxic oligomers composed of α-syn A30P (Lo Bianco *et al.*, 2008). Hsp104 also coupled ATPase activity to the disassembly of α-syn fibers (Lo Bianco *et al.*, 2008). Disassembly was enhanced by the mammalian Hsp70 system, and in particular, by the specific combination of Hsc70 and Hdj2 (Lo Bianco *et al.*, 2008). All α-syn variant fibers were effectively disassembled, except for the E46K PD-linked mutant, which was highly resistant to Hsp104 (Lo Bianco *et al.*, 2008). This might indicate that α-syn E46K forms a different strain of amyloid. Indeed, α-syn E46K fibers tend to form compact bundles and meshwork arrays not observed with wild-type α-syn (Choi *et al.*, 2004; Greenbaum *et al.*, 2005). Nonetheless, this battery of remodeling activities suggested that Hsp104 might effectively buffer α-syn misfolding and toxicity *in vivo*.

Unfortunately, the development of PD therapies has been hindered by a paucity of animal models that successfully recreate the progressive and selective degeneration of dopaminergic neurons and formation of phosphorylated α-syn inclusions. However, a rat PD model based on the lentiviral-mediated expression of human α-syn A30P in the substantia nigra has successfully recapitulated these key phenotypes (Lo Bianco *et al.*, 2002, 2004). Thus, Hsp104 and α-syn A30P were expressed simultaneously in the rat substantia nigra using the lentiviral delivery system. Remarkably, Hsp104 reduced the formation of phosphorylated α-syn A30P inclusions and prevented nigrostriatal dopaminergic neurodegeneration (Lo Bianco *et al.*, 2008). Thus, Hsp104 is able to buffer α-syn A30P misfolding and toxicity in the physiological arena of the mammalian substantia nigra.

While these results are promising, several questions remain that must be addressed in subsequent studies. First, it has not yet been possible to express Hsp104 after α-syn has already aggregated, which is a situation that might mimic more closely any potential treatment. Thus, whether Hsp104 can reverse α-syn aggregation in the setting of the rat substantia nigra remains unclear. Another issue concerns the release of a large pulse of soluble α-syn from Lewy bodies in surviving neurons. Such a pulse might be detrimental since high levels of soluble α-syn can inhibit synaptic vesicle release and perturb other membrane trafficking events (Gitler and Shorter, 2007; Gitler *et al.*, 2008; Larsen *et al.*, 2006). However, this situation is likely to be preferable to the persistence of toxic α-syn conformers. Finally and most importantly, further study is needed to assess any dangers of long-term Hsp104 expression in the mammalian brain.

7.7 DEVELOPMENT OF SUBSTRATE-OPTIMIZED Hsp104 VARIANTS AS POTENTIAL THERAPIES

The foregoing sections increase optimism that Hsp104 may have therapeutic potential for antagonizing or even reversing the specific amyloidogenic events connected with AD and PD. However, the amyloid-remodeling activity of Hsp104 might also have applications that extend beyond various neurodegenerative amyloidoses. For example, the peptide hormone, amylin, forms amyloid inclusions in the endocrine pancreas in 90% of patients with type II diabetes (Cooper *et al.*, 1987; Kahn *et al.*, 1999; Maloy *et al.*, 1981) and likely exacerbates β-cell failure (Janson *et al.*, 1999; Lorenzo *et al.*, 1994). Another potential amyloid target is provided by fragments of prostatic acidic phosphatase, an abundant component of semen. Amyloid forms of these peptides can drastically potentiate human immunodeficiency virus (HIV) infection by $\sim 10^5$-fold, whereas soluble forms of these peptides have no effect (Munch *et al.*, 2007). Thus, these amyloid species represent a novel target for preventing sexual transmission of HIV.

The ability of Hsp104 to prevent or reverse the formation of nonamyloid, disease-associated aggregates should also be considered. For example, two devastating neurodegenerative disorders: amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusions are connected with the formation of nonamyloid, aggregated species of a conserved hnRNP, transactive response DNA-binding protein-43 (TDP-43) (Johnson *et al.*, 2009; Kerman *et al.*, 2010; Kwong *et al.*, 2008; Neumann *et al.*, 2006). Other forms of ALS are associated with the nonamyloid aggregation of another hnRNP, FUS (Kwiatkowski *et al.*, 2009; Vance *et al.*, 2009). Both FUS and TDP-43 may represent promising targets for Hsp104 because they both contain a domain that resembles a yeast prion domain (Cushman *et al.*, 2010).

Nevertheless, a daunting array of issues must be addressed if Hsp104 is to be developed as a therapeutic agent. Not least is the issue that gene therapy would seem to be required to introduce Hsp104 as a therapeutic agent. (See Chapter 12 for more information on gene therapy.) Gene therapy has produced encouraging preclinical outcomes for several disorders including congenital blindness (Bainbridge *et al.*, 2008; Hacein-Bey-Abina *et al.*, 2002; Maguire *et al.*, 2008). However, technical and safety issues continue to restrict translation to the clinic. Indeed, gene therapy approaches to treat neurodegenerative amyloidoses remain in very early developmental stages, and considerable caution is needed at this time. However, initial studies suggest that gene therapy in the adult brain might be safe for various neurodegenerative disorders, including PD (Feigin *et al.*, 2007; Kaplitt *et al.*, 2007; Stoessl, 2007). Thus, even though we await several key advances in gene therapy before any Hsp104 gene therapy (or any other gene therapy) becomes feasible, it remains important to develop solutions to amyloid problems and to test these solutions both *in vitro* and in animal models.

Another issue concerns the fact that existing Hsp104 specificity or activity is unlikely to be optimal against substrates that it never ordinarily encounters, such as α-syn or Aβ42. Indeed, disassembly of α-syn fibers requires considerably

larger amounts of Hsp104 than disassembly of Sup35 or Ure2, two natural prion substrates (Lo Bianco *et al.*, 2008; Shorter and Lindquist, 2006). Even for the natural substrates Sup35 and Ure2, high concentrations of Hsp104 are required to reverse amyloid formation (Shorter and Lindquist, 2006). Acting at lower concentrations, Hsp104 fragments Sup35 and Ure2 prions, which generates more fiber ends that can convert soluble copies of the protein to the prion form (Shorter and Lindquist, 2006). Thus, an important therapeutic consideration is to express Hsp104 above a certain threshold that reduces and does not exacerbate the amyloid burden. Indeed, fragmenting fibers might initially be detrimental because short amyloid fibers can be more toxic than long fibers, at least in cell culture (Xue *et al.*, 2009, 2010).

Hsp104 is likely to be a generalist since it must disaggregate large portions of the yeast proteome after environmental stress. Regarding amyloid conformers, it seems likely that Hsp104 might be adapted to remodel cross-β structures composed of the uncharged polar residues that distinguish the prion domains of many proteins in yeast (Alberti *et al.*, 2009). However, Hsp104 is able to propagate HET-s prions in yeast, which harbor a prion domain that is very distinct to those of other yeast prions (Taneja *et al.*, 2007). Promiscuous disaggregation activity might also be undesirable in a therapeutic setting. Ideally, a therapeutic disaggregase would selectively eliminate toxic strains and misfolded species, and not eradicate benign strains or even beneficial amyloids such as CPEB prions, which might encode long-term memory (Shorter and Lindquist, 2005b; Si *et al.*, 2003, 2010). Thus, an important goal is to engineer or evolve Hsp104 variants with enhanced and selective ability to eradicate specific amyloid or aggregated conformers. Ultimately, designer disaggregases might be developed to annihilate purely toxic conformers unique to each particular disease. This might require a very different tailored Hsp104 variant for each disease. Nonetheless, the development of Hsp104-based disaggregases dedicated to the resolution of select proteins or protein conformations remains an important future goal that will simultaneously facilitate a deeper understanding of how this intriguing protein disaggregase operates.

• Q5

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Queries in Chapter 7

Q1. Can we change "β-amyloid" to "amyloid-β' in order to match the occurence in other chapters

Q2. Please provide the expansion of "AMP-PCP" in the sentence "In the presence of . . ."

Q3. Please confirm if the abbreviation "hnRNP" needs to be spelt out. If yes, please provide the expansion.

Q4. Please confirm if the abbreviation "FUS" needs to be spelt out. If yes, please provide the expansion.

Q5. Please confirm if the abbreviation "HET-s" needs to be spelt out. If yes, please provide the expansion.

Q6. Please confirm if "International AsD" in the reference "Prince *et al*, 2009" should be considered as "editor".