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Hsp104: A Weapon to Combat Diverse Neurodegenerative Disorders

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Abstract
Many of the fatal neurodegenerative disorders that plague humankind, including Alzheimer's and Parkinson's disease, are connected with the misfolding of specific proteins into a surprisingly generic fibrous conformation termed amyloid. Prior to amyloid fiber assembly, many proteins populate a common oligomeric conformation, which may be severely cytotoxic. Therapeutic innovations are desperately sought to safely reverse this aberrant protein aggregation and return proteins to normal function. Whether mammalian cells possess any such endogenous activity remains unclear. By contrast, fungi, plants and bacteria all express Hsp104, a protein-remodeling factor, which synergizes with the Hsp70 chaperone system to resolve aggregated proteins and restore their functionality. Surprisingly, amyloids can also be adaptive. In yeast, Hsp104 directly regulates the amyloidogenesis of several prion proteins, which can confer selective advantages. Here, I review the modus operandi of Hsp104 and showcase efforts to unleash Hsp104 on the protein-misfolding events connected to disparate neurodegenerative amyloidoses.

Introduction

Cellular existence demands that proteins fold into a multitude of diverse and elaborate structures to perform the vast majority of essential functions. Accordingly, vitiation of protein folding can have deleterious consequences [1–5]. Much of the information required for successful protein folding is encoded in the primary sequence of the protein itself [6]. However, in the arena of the extraordinarily crowded cellular milieu, nascent proteins may fail to fold and mature proteins may fail to remain propitiously folded [7]. Indeed, it has long been appreciated that proteins are dynamic systems of startling complexity [8]. Proteins repeatedly unfold, both partially and completely, even under native conditions [9, 10]. This protein folding problem is as ancient as life itself, and so are the solutions that have evolved to alleviate it. Thus, sophisticated molecular chaperones bind to and prevent misfolded proteins from aggregating [11], osmolytes operate as chemical chaperones [12], protein-remodeling factors disassemble protein aggregates [13–15], and degradation systems eliminate misfolded proteins [16].

Even so, these safeguards can be breached, especially as the aging process takes its toll [1, 2]. Elderly individuals will have encountered more environmental stress (such as head trauma, pesticide or heavy metal exposure) that can promote lethal protein misfolding [17, 18]. Moreover,

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toxic protein misfolding that occurs late in life has little effect on reproductive success. Thus, polymorphisms that might engender such misfolding events have not been purged from the population by natural selection [1]. Of particular concern are the inexorably lethal neurodegenerative or systemic disorders associated with the misfolding of specific proteins into a shared and surprisingly generic fold termed amyloid [1–5]. These disorders include Alzheimer's disease, Parkinson's disease (PD), Huntington's disease (HD), tauopathies, cardiac amyloidoses, cerebral amyloid angiopathy, type II diabetes and prion diseases [1–5]. A major risk factor for many of these diseases is aging. Thus, a consequence of increased life expectancy and shifting population demographics to older age groups is the increasing prevalence of these disorders [1–5].

Amyloid is an extremely stable, β-sheet-rich fibrillar conformation characterized by a common 'cross-β' structure in which the strands of the β-sheets run approximately perpendicular to the fiber axis [19–21]. Diverse polypeptides (perhaps even all polypeptides) can access the amyloid fold [22, 23], but natural selection has acted such that few do so under physiological conditions prior to postreproductive age [1]. Once initiated, amyloidogenesis can cascade out of control. This is because amyloid fibers are self-perpetuating aggregates. They self-template their specific cross-β conformation at their growing ends by converting other copies of the same protein to the cross-β amyloid form [21, 24]. Thus, once formed, amyloids can potentially convert all copies of a given protein to the amyloid state [24], and accumulate to debilitating, and even tissue-displacing, levels as in the systemic amyloidoses [25]. The exceptional stability of amyloids (including resistance to proteases, temperatures of 98°C, denaturants and even 2% SDS) makes them extraordinarily difficult to clear [1, 21, 26, 27]. Indeed, they are widely perceived as intractable. However, in many amyloidoses the quantities of deposited fibers can be extremely small [1, 25]. This, together with other data, has led to suggestions that the soluble oligomers that form during the characteristic lag phase of fiber assembly are actually the most cytotoxic species, while the fibers themselves may be relatively inert [1, 3, 28–30]. Moreover, sequestration of specific proteins into fiber inclusions at the expense of oligomers can sometimes be cytoprotective in disease settings [29, 31, 32]. Remarkably, the soluble oligomers that accumulate prior to fibers frequently possess a generic conformation, distinct from fibers, which is shared by many amyloidogenic proteins [29, 33]. These commonalities of amyloidogenesis suggest that successful therapeutics could have broad applicability [3]. However, despite intense investigation, it remains unclear how the processes of amyloid fiber and oligomer formation elicit the selective cell death that distinguishes various amyloid disorders [1–3]. Tragically, there are no effective remedies for any of these conditions.

Surprisingly, amyloids are not always a problem. It is increasingly clear that several amyloids have been harnessed during evolution for adaptive, beneficial purposes [34, 35]. For example, amyloids formed by yeast prions function as stable, protein-based genetic elements that can encipher beneficial traits [35, 36]. Certain CPEB amyloids may function in long-term memory formation [37] and Pmel17 amyloids may drive melanosome biogenesis [38]. Thus, amyloids can be tightly regulated, and need not be invariably deleterious. Understanding how nature has managed to control and even exploit these conformations may provide invaluable insights into how to attack pathogenic amyloid.

A colossal challenge lies in the development of methods to safely solubilize toxic amyloid conformers and intermediates, and return the misfolded protein to normal function. While this is simple to state, and may be an oversimplification in particular cases, it is undoubtedly extraordinarily difficult to achieve, and will require some revolutionary innovations. However, I propose that a candidate has emerged from a most unlikely source, the yeast *Saccharomyces cerevisiae*, which could help realize these challenges. A protein-remodeling factor from yeast, Hsp104, can rapidly resolve protein aggregates and amyloid conformers [33, 39, 40], and may hold considerable potential for our fight against multiple diseases associated with aberrant protein aggregation.

Hsp104 in Yeast

An AAA+ Protein with Disaggregation Activity

Hsp104 is a class 1 AAA+ (ATPases associated with diverse activities) protein [13]. Typically, members of this superfamily couple energy from ATP hydrolysis to the remodeling of a bewildering variety of protein, DNA and RNA substrates [41]. Hsp104 possesses a truly extraordinary biochemical activity, even for an AAA+ protein. Hsp104 solubilizes and reactivates proteins from denatured aggregates, usually with the assistance of Hsp70 and Hsp40 chaperones (fig. 1a) [39, 42–44]. Only a decade heretofore, biochemists thought this was highly improbable if not impossible. Protein aggregation was widely held to be an irreversible process. Dogma endured, sug-

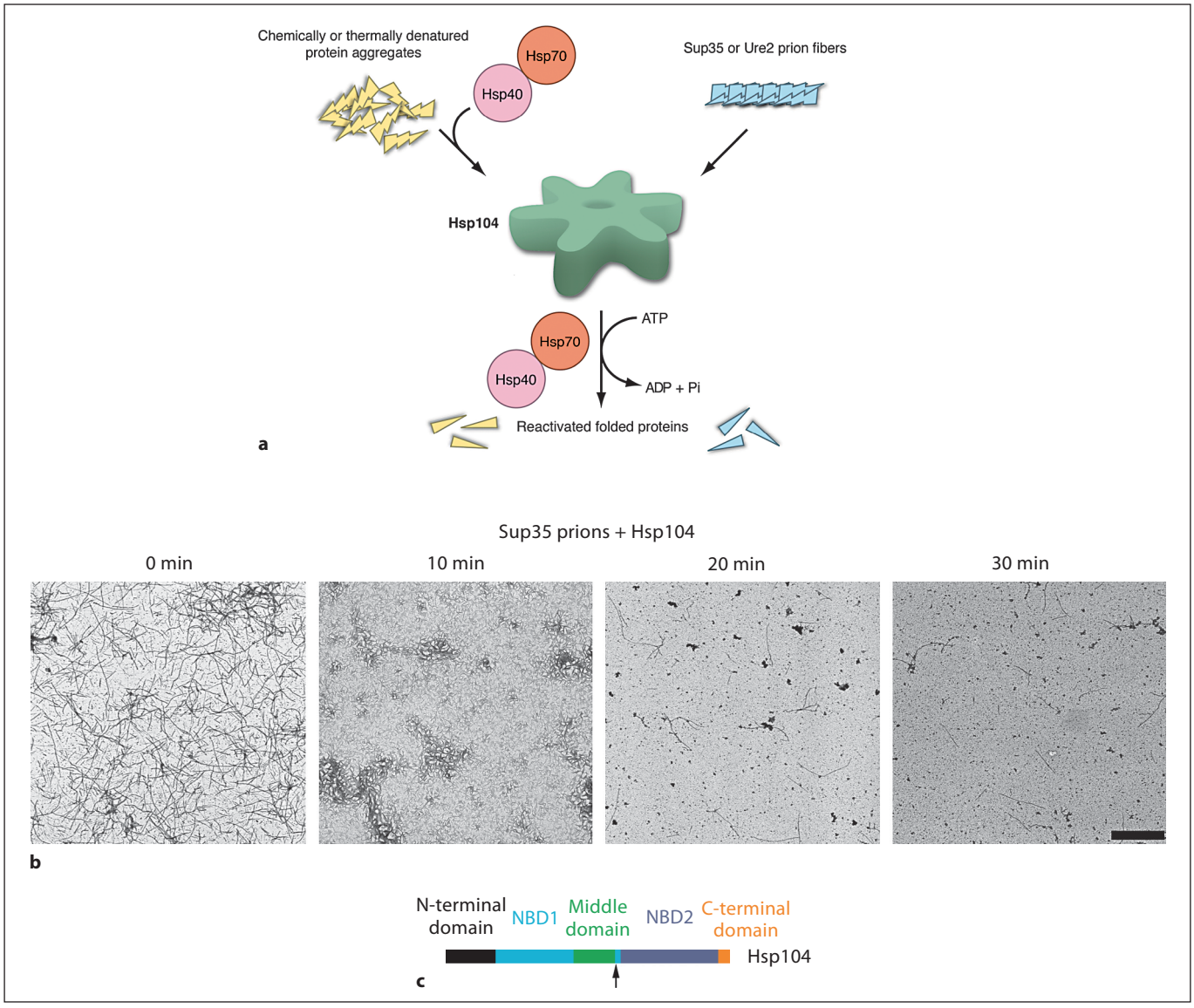

Fig. 1. Protein remodeling by Hsp104. **a** Hsp104 collaborates with Hsp70 and Hsp40 to promote the disaggregation of denatured aggregates. By contrast, Hsp70 and Hsp40 are not required for the disaggregation of Sup35 or Ure2 prion fibers. **b** Hsp104 rapidly disassembles Sup35 prions. Sup35 prions (2.5 μM) were incubated with Hsp104 (2 μM) plus ATP (5 mM) for 0–30 min at 25°C. At various times reactions were processed for EM. Scale bar = 2 μm. **c** Domain architecture of Hsp104. The arrow indicates where ClpB from *Thermus thermophilus* can be split into 2 pieces and still operate as a disaggregase.

gesting that even if any disaggregation was possible then the resolubilized proteins must be immediately degraded. However, seminal work by Glover and Lindquist [39] refuted these widely held views in one fell blow, and demonstrated that Hsp104 in collaboration with Hsp70 and Hsp40 can retrieve enzymatically active proteins from large chemically denatured aggregates (fig. 1a). A flurry of subsequent studies confirmed that the bacterial homolog, ClpB, possesses similar activity [45–47].

Role in Stress Tolerance

Protein aggregation becomes a severe issue in cells suddenly exposed to environmental stresses, such as elevated temperatures, which can overwhelm the safe-

guards for successful protein folding. Hsp104 functions to dissolve and renature thousands of diverse substrates during reactivation of the aggregated proteome after multifarious stresses [42, 48–51]. Importantly, Hsp104, which is a nonessential gene, can increase survival of yeast exposed to environmental stress by 10,000-fold [48–50]. Similar increases in stress tolerance are conferred in plants and bacteria by their Hsp104 homologs, Hsp101 and ClpB [52, 53]. In yeast, it has long been appreciated that reactivation rather than degradation of aggregated protein is essential for thermotolerance because Hsp104 does not associate with proteolytic systems and substrates are reactivated rather than degraded after heat shock [48, 54]. Further, if the C-terminal surface of ClpB (the *Escherichia coli* homolog of Hsp104) is modified to interact with the chambered ClpP protease, disaggregated proteins are degraded and, in the presence of high ClpP levels, recovery from thermal stress is abolished [55]. Thus, degradation instead of reactivation can actually be deleterious.

The ability to restore a previously aggregated protein to native structure and function obviates the huge energetic cost of degrading and resynthesizing them. Further, this simultaneously eliminates 3 malicious problems associated with protein aggregation: (1) the toxic gain of function of aggregated conformers; (2) the loss of function of the aggregated protein; (3) the sequestration of other essential proteins that coprecipitate with the aggregated protein. These 3 issues very likely synergize to varying extents in the etiology of several disparate neurodegenerative amyloidoses [1–5]. However, denatured protein aggregates that form in response to environmental stress are likely to differ considerably in structure from amyloid fibers and oligomers. Although little structural information is available for denatured aggregates, it is likely that they are maintained largely by hydrophobic contacts that only become exposed upon protein denaturation [7, 13]. By contrast, an extensive hydrogen bond network between adjacent β-strands maintains the highly ordered cross-β structure of amyloid fibers [21, 56–58]. Yet, can Hsp104 remodel the distinctive features of amyloid? This question is addressed by considering the role of Hsp104 in yeast prion regulation.

Role in Prion Regulation

Prions are a specialized class of amyloids that efficiently disseminate and transmit their self-templating activity from one cell to another, and even from one organism to another [35, 59]. They are infectious [40, 60–65]. In mammals, prions transmit debilitating neurodegenerative disorders, like the variant Creutzfeldt-Jakob disease in humans [66]. In striking contrast to this, however, in yeast prions can have beneficial adaptive effects [35]. For example, the yeast prion state [*PSI*$^{+}$], which is comprised of Sup35 prions, is a source of phenotypic diversity and confers selective advantages in diverse environmental settings [36]. Two other yeast proteins, Ure2 and Rnq1, form prions in yeast [67, 68]. Intriguingly, their formation and inheritance depend absolutely on Hsp104. Deletion of Hsp104 fully eliminates Sup35, Ure2 and Rnq1 prions from yeast cells [67, 69, 70]. By contrast, overexpression of Hsp104 eliminates Sup35 prions, but not Ure2 or Rnq1 prions [69–71]. These genetic data raise the following question: can Hsp104 deconstruct amyloid fibers?

Pure protein biochemistry has clarified these genetic data in relation to Sup35 and Ure2, and simultaneously revealed that Hsp104 possesses an unusually powerful amyloid-remodeling activity [33, 40]. Deciphering these genetic data proved to be extremely challenging. In retrospect, it is understood why. Hsp104 affects yeast prion protein conformations in several different ways, and with distinct reaction mechanisms, that depend upon: (1) the conformation of the prion protein (amyloid or nonamyloid), (2) the concentration of prion protein relative to Hsp104 and (3) the available adenine nucleotides.

When Hsp104 concentrations are high and Sup35 or Ure2 are in the non-prion-soluble state, Hsp104 blocks prion assembly [33, 40]. In this situation, Hsp104 couples ATP hydrolysis to the elimination of the critical oligomeric intermediates that nucleate Sup35 fiber assembly [33, 40]. Hsp104 also couples ATP hydrolysis to the rapid disassembly of amyloid fibers of both Sup35 and Ure2 (fig. 1b) [33, 40]. Note that no other protein is known to exhibit such devastating effects on structures that are otherwise so intractable. For example, like other amyloids, prions lie at the extremes of structural stability and resist dissolution by anionic surfactants (such as 2% SDS) and chaotropes (such as 3M guanidium chloride, GdmCl), which are typically powerful structural dissolution agents. The prion disassembly activity of Hsp104 is highly dependent upon Hsp104 concentration. No disassembly occurs until a threshold Hsp104 concentration is breached, indicating a highly cooperative reaction mechanism, perhaps even necessitating cooperation between Hsp104 hexamers [33, 40]. Surprisingly, these specific Hsp104 activities do not require the Hsp70 chaperone system (fig. 1b) [33, 40].

In contrast to the above, when Hsp104 concentrations are low and Sup35 or Ure2 are in the soluble state, then

Hsp104 catalyzes prion assembly [33, 40]. It does so in 2 ways: by reducing the lag phase for prion assembly as well as accelerating the assembly phase. Notably, these activities are mechanistically distinct but the same for both prions. Hsp104 reduces the lag phase by catalyzing the formation of critical oligomeric intermediates that nucleate fiber assembly. This activity requires only ATP binding. By contrast, accelerating assembly requires ATP hydrolysis, and involves the occasional severing of nascent fibers to expose new fiber ends, which in turn provide new seeds for further conformational replication [33, 40].

An initially puzzling observation was that GdmCl cures cells of prions [68, 72] at concentrations that are too low to dissolve the prions directly [26]. Surprisingly, millimolar GdmCl is a remarkably specific inhibitor of Hsp104 ATPase activity and diminishes the off-rate of ADP [39, 73]. In vitro, GdmCl inhibits the Sup35 and Ure2 prion assembly and fragmentation activities of Hsp104 [40, 74]. Thus, GdmCl may lock Hsp104 in an ADP-bound state that interacts with Sup35 and Ure2 with lower affinity, and is therefore incompetent for either fiber assembly or disassembly [33, 40, 73]. The elimination of both these Hsp104 activities by GdmCl explains how it can cure cells of Sup35 and Ure2 prions at low concentrations [75–77].

How then does deletion of Hsp104 cure cells of prions? When Hsp104 concentrations are low in vivo, the extreme concentration dependence of fiber severing observed in vitro [33, 40] suggests that fiber severing would be rare, perhaps occurring only once per prion fiber per cell cycle. This infrequent fiber-severing activity would promote prion transmission to progeny by dividing fibers for inheritance. Deletion of Hsp104 would eliminate fiber fragmentation activity and consequently block the formation of new active sites for conformation replication (that is, fiber ends). In cells lacking Hsp104, prion growth and division would lose synchrony with cell division, and eventually the rate of conformational replication would fall below that required to sustain the prion. Further, any effects of assisting de novo prion assembly will also be lost.

How does overexpression of Hsp104 affect prions? When Hsp104 is upregulated by overexpression, the strongly cooperative nature of prion remodeling would greatly increase severing and disassembly. Extended exposure of Sup35 prions to Hsp104 in vitro results in an ensemble of noninfectious amyloid-like aggregates and soluble protein [40]. By contrast, Hsp104 converts Ure2 fibers to a mixture of short, highly infectious prion fibers and soluble protein [40]. Importantly, these findings were verified in vivo, by transforming Hsp104-generated reaction products into prion-minus yeast cells [40]. Thus, the in vitro experimental findings were validated by a critically important biological assay that had not been employed in this manner before. These findings explain why overexpression of Hsp104 cures yeast cells of Sup35 prions, but not Ure2 prions [40].

In regulating yeast prions, Hsp104 likely specifically recognizes and deconstructs elements of the generic cross-β structure of amyloid fibers as well as the distinct generic structure of their cytotoxic oligomeric precursors [33, 40, 78, 79], which are common to disparate disease-associated amyloids [29]. The unprecedented alacrity with which Hsp104 remodels these structures (fig. 1b) might have therapeutic applications for many of the deadly and intractable disorders associated with amyloidogenesis. Furthermore, under other circumstances, Hsp104 can actually promote amyloid fiber formation, at the expense of potentially toxic oligomeric conformers [33, 40, 80]. Therefore, Hsp104 could be useful in different ways against various amyloidoses, in either promoting or reversing amyloidogenesis. Yet, how does the molecular structure of Hsp104 facilitate this remarkable set of activities?

Structure and Mechanism of Action

Hsp104 forms hexamers of ~155 Å in diameter in the presence of ADP or ATP [81]. Each protomer contains 2 AAA+ ATPase domains (nucleotide-binding domains 1 and 2, NBD1 and NBD2), which are separated by a coiled-coil middle domain and flanked by N-terminal and C-terminal domains (fig. 1c) [82]. The crystal structure of ClpB reveals that the 2 AAA+ domains are stacked head to tail, and the middle region comprises 2 antiparallel coiled-coil motifs that resemble a 2-bladed propeller [83]. Unfortunately, in the crystal, ClpB is not hexameric, but is resolved as a spiral of 3 ClpB monomers with distinct conformations [83]. EM and single-particle reconstruction of Hsp104 and ClpB reveal an axial channel, with a narrow 10- to 15-Å N-terminal entrance, which spans the length of the hexamer [81, 83]. Fitting the crystal structure of ClpB into cryo-EM reconstructions of glutaraldehyde-fixed ClpB hexamers yielded a model where the coiled-coil middle domains were proposed to emanate out laterally from the surface of the hexamer, even though they were not completely visible in this vicinity [83]. Despite such uncertainties, a picture is gradually emerging about how the specialized architecture of Hsp104 hexamers might enable the resolution of aggregated sub-

strates. By contrast, little is known about precisely how Hsp104 promotes the formation of the amyloidogenic oligomers that nucleate prion assembly, which requires ATP binding but not hydrolysis.

In the ATP-bound conformation, Hsp104/ClpB hexamers engage substrates [84, 85]. Both NBDs of Hsp104 exhibit cooperative ATPase activity, and allosteric communication occurs within and between the 2 domains [86–88]. Elimination of ATPase activity compromises full protein-remodeling activity [33, 43, 89]. Yet, how is protein remodeling elicited? The powerful remodeling activities of Hsp104 are highly selective and strictly regulated. Even very high levels of expression are not toxic. That is, despite being able to remodel diverse substrates, Hsp104 does not dissociate large functional protein complexes or polymers [90]. Recent advances have clarified that to elicit remodeling of diverse substrates, ranging from soluble GFP aggregates to RepA dimers, ATPase activity must be decelerated asymmetrically (that is, at NBD1 but not NBD2 or vice versa) at a subset of the 12 AAA+ domains of the hexamer [43, 91, 92]. This can be achieved by providing mixtures of ATP and ATPγS, a slowly hydrolyzable ATP analog, or by selectively impairing ATP hydrolysis at one NBD by mutation of particular conserved AAA+ motifs [43, 91, 92]. This suggests that a division of labor among the 12 AAA+ domains of the hexamer is essential for successful protein remodeling. One subset must slowly hydrolyze ATP to facilitate substrate binding, while another subset must rapidly hydrolyze ATP to promote substrate unfolding.

Remarkably, artificially inducing this division of labor by mutating conserved AAA+ motifs or by supplying mixtures of ATP and ATPγS, allows Hsp104 and ClpB to remodel some substrates without any need for the Hsp70 chaperone system [43]. This suggests that one role of the Hsp70 chaperone system is to help coordinate, directly or indirectly, the requisite mode of Hsp104/ClpB ATPase activity. However, the autonomous ClpB activity induced by ATP and ATPγS fails to synergize with the Hsp70 chaperone system and is unable to operate on larger aggregated species [91]. This is likely because the Hsp70 chaperone system performs several other key functions during disaggregation, including presenting aggregated polypeptides to Hsp104/ClpB and refolding newly solubilized polypeptides that are released in an unfolded state [39, 45, 55].

The prion-remodeling activity of Hsp104 has several unique characteristics. Surprisingly, Hsp104 does not require Hsp70 and Hsp40 to remodel either amyloid fibers or oligomers comprised of yeast prion proteins [33, 40, 78, 79]. Further, mixtures of ATP and ATPγS actually inhibit this activity [43], suggesting that prion fibers may induce the requisite remodeling mode of Hsp104 ATPase activity, which promotes their disassembly. Alternatively, the extreme stability of amyloid fibers may require that all the AAA+ domains bind and hydrolyze ATP in a sequential or concerted manner. Genetic data also suggest that prion fragmentation requires maximal Hsp104 activity. A broad spectrum of single missense mutations that span the Hsp104 open reading frame permit recovery from thermal stress, but preclude prion propagation [93, 94]. Hence, perturbations of Hsp104 that can be tolerated for the disaggregation of denatured aggregates cannot be tolerated for prion fragmentation. Deletion of the N-terminal domain of Hsp104, which hinders the disaggregation of very stable aggregates by ClpB [95], has no significant effect on thermotolerance and prion propagation, but eliminates the ability of Hsp104 to cure cells of Sup35 prions by overexpression [96]. This suggests that the N-terminal domain of Hsp104 contributes to the powerful amyloid-remodeling activity that converts Sup35 prions to a mixture of soluble protein and noninfectious amyloid-like aggregates [40].

How does Hsp104/ClpB extract polypeptides from aggregates? The N- and C-terminal domains may help engage substrates and cofactors [86, 95]. Also, in each NBD, conserved tyrosines in axial channel loops change conformation in response to ATP binding to make direct contact with substrates [55, 97, 98]. The uncharged polar and aromatic properties of tyrosine are well suited for productive interactions with the diverse substrates handled by Hsp104. Tyrosine can mediate electrostatic interactions with cationic groups, hydrophobic interactions and hydrogen bonds. Further, its median hydrophilicity confers adaptability to hydrophilic and hydrophobic environments, while its uncharged character obviates electrostatic repulsion effects. Remarkably, tyrosine also appears to play a critical role in many antigen-binding sites of antibodies [99, 100]. Rearranging tyrosines in these regions facilitates a plethora of diverse antigen-binding interfaces with distinct specificities [99, 100]. By analogy, in Hsp104 and ClpB, dynamic rearrangements of channel loop tyrosines synchronized with the appropriate ATPase events may facilitate the transient handling of diverse substrates [43, 91, 101]. These tyrosines, and particularly the NBD2 tyrosine, are crucial for disaggregation by ClpB and Hsp104, as well as prion maintenance by Hsp104 [55, 94, 96–98].

These observations have led to suggestions that ATP hydrolysis might elicit a ratcheting motion that extracts

polypeptides from aggregates by translocating them across the central channel [13, 14, 55, 97]. Often, such an activity is cast as alternative to a mechanism in which Hsp104 makes multivalent contacts with the aggregate to forcibly alter the positions of bound substrates (or distinct parts of the same substrate) relative to each other upon ATP hydrolysis [13, 39]. Whether such activities are, in actuality, so different remains unclear. Support for a translocation mechanism was provided by modifying the C-terminal surface of ClpB to interact with the chambered ClpP protease, which facilitates the degradation of aggregated substrates by ClpP [55]. These data suggest that disaggregating polypeptides are conducted across the ClpB channel and passed directly to the ClpP entry portal, as occurs in ClpA and ClpX (with soluble substrates), the AAA+ proteins that naturally interface with ClpP [55]. However, it remains unclear whether a modified ClpB tethered to ClpP accurately recreates all events catalyzed by ClpB [82]. Further, whether all aggregated substrates are dissolved by a translocation mechanism, or whether other disaggregation mechanisms contribute is not known. Finally, it is also not clear how the narrow ClpB channel is well suited for handling large aggregated substrates [83].

The unique coiled-coil middle domain distinguishes Hsp104 and its immediate homologs from all other AAA+ proteins, and presumably helps confer the powerful disaggregation activity. The middle domain undergoes large motions during the Hsp104 ATPase cycle and coordinates communication between NBD1 and NBD2 [86]. Restriction of this mobility or deletion of the domain eliminates ClpB disaggregation activity [83, 89, 102]. Specific point mutations in the middle domain of Hsp104 also eliminate thermotolerance and prion propagation [90, 94]. Thus, the middle domain is clearly important, but its precise location and function in the hexameric structure remains uncertain. Hence, whether it adds a mechanical feature to any translocation process or confers an additional remodeling activity awaits structural clarification. Another AAA+ protein, ClpA, lacks the coiled-coil domain and is able to disaggregate some substrates, but is inhibited by the Hsp70 chaperone system [43, 91, 103]. This suggests that the middle domain may help coordinate disaggregation with the Hsp70 chaperone system, and helix 3 of the middle domain appears to facilitate the transfer of aggregated polypeptides from the Hsp70 chaperone system to the ClpB channel [102]. Regardless of precisely how Hsp104 operates, its application to maladies of protein aggregation holds considerable promise.

Absence of Metazoan Homologs of Hsp104

Perplexingly, Hsp104 is conserved in plants, bacteria and fungi, but for unknown reasons has been lost from metazoa. Whether animals even possess an analogous protein disaggregase that functions to restore protein functionality remains unclear [14], and initial attempts to uncover such activities in mammalian cells have been unsuccessful [104]. Remarkably, ClpB can function as a disaggregase if it is split into 2 pieces (N-NBD1-M and NBD2; fig. 1c) [105]. This raises the possibility that two class 2 AAA+ proteins (which contain only 1 AAA+ domain per monomer) may be able to cooperate to drive protein disaggregation. Intriguingly, *Caenorhabditis elegans* extracts possess some amyloid disaggregation and degradation activity [106]. However, the identity of the factors that promote disaggregation in *C. elegans* remains unknown. Furthermore, the clearance of Sup35 amyloids by Hsp104 is orders of magnitude more rapid than the amyloid β disaggregation activity uncovered in *C. elegans* extracts [40]. Yet, why Hsp104 has no metazoan homologs remains moot and unaddressed. In metazoa, the quality control machinery may be more geared towards preventing protein aggregation than reversing it. Switching off genes encoding aggregated proteins has revealed that animal cells do possess mechanisms to clear amyloid and even prion aggregates [107, 108], but whether this simply reflects autophagy and other degradation pathways [5, 16] as opposed to reactivation remains unknown. This inability to reactivate aggregated proteins may contribute to the lethality of excessive protein aggregation in animal cells.

That Hsp104 can disassemble yeast prions raises consciousness that amyloid fibers are not intractable, but can be cleared by a protein-remodeling factor [33, 40, 79]. In contrast to the highly individualistic structures of globular proteins, amyloids are proposed to share a generic cross-β structure, and although this is probably an oversimplification as local steric details may differ enormously [21, 56–58], it raises the hypothesis that agents that antagonize amyloid fibers of one protein may also be active against amyloid fibers composed of another. Furthermore, the cytotoxic oligomeric precursors of fibers are also proposed to share a distinct common structure [29]. Thus, can the reintroduction of Hsp104 into metazoan systems help prevent or reverse various amyloid disorders? A key point is that even transiently high levels of Hsp104 are sufficient to purge yeast cells of Sup35 prions [69]. Thus, perhaps even transient, targeted expression could help antagonize certain amyloid disorders. In the

remainder of this review, I highlight attempts to apply Hsp104 activity to protein-misfolding disorders.

Hsp104 in Yeast Models of Neurodegeneration

The yeast *S. cerevisiae* is increasingly employed as a versatile and powerful model system for several neurodegenerative diseases [109], and has been used as a venue to test the effects of Hsp104 on protein-misfolding events associated with neurodegenerative amyloidoses.

Huntington's Disease

HD is a neurodegenerative disorder characterized by a selective loss of neurons in the striatum and cortex [5, 110, 111]. Expanded polyglutamine (polyQ) tracts in the huntingtin protein cause HD [112]. Fragments of huntingtin that contain expanded polyQ stretches misfold and form aggregates with many of the characteristics of amyloid fibers [113]. Remarkably, in yeast polyQ-expanded huntingtin fragments can aggregate and cause toxicity [114, 115], but this depends on an imbroglio of intra- and intermolecular interactions [116, 117]. Rnq1 must be in its prion conformation for such huntingtin fragments to aggregate and be toxic [115, 116]. Accordingly, by curing cells of Rnq1 prions, deletion of Hsp104 eliminates polyQ aggregation and toxicity [67, 114, 115]. Importantly, overexpression of Hsp104 efficiently solubilized SDS-resistant aggregates formed by a polyQ-expanded huntingtin fragment and greatly reduced toxicity [42]. These effects were potentiated by the co-overexpression of the small heat shock proteins Hsp26 or Hsp42 [42]. This suggests that small heat shock proteins can be important cochaperones for protein disaggregation by Hsp104. Indeed, the coaggregation of Hsp26 with heat-denatured citrate synthase allows disaggregation and reactivation by Hsp104 without any requirement for the Hsp70 chaperone system [44].

Parkinson's Disease

PD results primarily from a severe and selective devastation of dopaminergic neurons in the substantia nigra [3, 4]. Overwhelming evidence implicates the presynaptic protein α-synuclein (α-syn) in the pathogenesis of PD [3, 4]. α-syn is the major component of Lewy bodies, intracellular inclusions comprised of abnormal α-syn filaments, which are pathognomonic for PD [3, 4]. Intriguingly, overexpression of human α-syn in yeast causes toxicity and the formation of cytoplasmic foci [118]. In contrast to the yeast HD model, overexpression of Hsp104 has no effect on α-syn toxicity [42]. However, the α-syn foci that form in the yeast cytosol may not be amyloid-like aggregates [119, 120]. Rather, these α-syn foci appear to result from the colocalization of α-syn with a membrane compartment, which may contribute to impairment of endoplasmic reticulum-Golgi transport [119, 120], just as high levels of α-syn perturb other transport events in neurons [121, 122]. Since α-syn does not undergo amyloidogenesis in yeast cells, it is perhaps not surprising that Hsp104 fails to rescue α-syn toxicity.

Hsp104 in *C. elegans* Models of Neurodegeneration

The ability to dissolve SDS-resistant aggregates formed by polyQ in yeast reinforces the potential of Hsp104 as an anti-amyloid therapy. However, can Hsp104 be transplanted into animal systems, away from its natural co-chaperone partners, and exert similar effects? This was first tested in a nematode HD model.

Huntington's Disease

Expression of a polyQ-expanded protein in body wall muscle cells of *C. elegans* causes cytoplasmic aggregates that appear to delay larval development [123]. Importantly, expression of Hsp104 reduced inclusion formation of the polyQ-expanded protein and alleviated the developmental delay [123]. This required ATPase activity by both NBDs, as an Hsp104 variant with both Walker A motifs mutated was ineffective [123]. These data establish that the potent protein-remodeling activity of Hsp104 can be successfully transplanted into a metazoan system to antagonize a protein-misfolding disorder.

Parkinson's Disease

Dopaminergic neurons degenerate after exposure to 6-hydroxydopamine (6-OHDA), and this can be used to model PD in *C. elegans* [124]. Hsp104 was unable to counteract 6-OHDA neurotoxicity unlike another AAA+ protein, torsinA [124]. However, 6-OHDA toxicity is primarily due to oxidative stress, which may not be antagonized by protein disaggregation and reactivation.

Hsp104 in Mammalian Cells

Thermotolerance

Ultimately, it will be critical to express Hsp104 in mammalian systems to antagonize protein-misfolding disorders. Importantly, Hsp104 is able to collaborate with

the mammalian Hsp70 chaperone system to promote protein disaggregation and reactivation [39, 92, 104]. Yet, a concern with expressing Hsp104 in metazoan systems is whether it might interfere with essential processes crucial for metazoan life. However, Hsp104 expression is tolerated extremely well in both tissue culture cells and even in transgenic rodents [104, 125–128]. This suggests that the tight regulation of Hsp104 activity may stem from the intrinsic properties of the hexamer itself [90]. Indeed, Hsp104 actually confers increased tolerance to thermal stress, and disaggregates and reactivates substrates, when it is expressed in various mammalian cell lines [104, 126].

Huntington's Disease

Building on experiments in yeast and *C. elegans*, Hsp104 was also found to antagonize polyQ aggregation and toxicity in mammalian neuronal cultures, as well as in rat and mouse models of HD [125, 127, 128]. Critically, Hsp104 prolonged the life span of HD mice by 20%, although it did not improve motor phenotypes or prevent the dramatic weight loss associated with this model [128]. In the rat model, reductions in toxicity conferred by Hsp104 were accompanied by modifications to the distribution and number of polyQ inclusions [127]. Hsp104 caused a significant reduction in large nuclear inclusions and a significant increase in small nonnuclear inclusions [127]. Hsp104 can shuttle in and out of the nucleus, allowing disaggregation and reactivation in both the nucleoplasm and cytoplasm [104].

Prion Diseases

Given the effects of Hsp104 on yeast prions, it might seem natural to test the effects of Hsp104 on mammalian prions. In vitro, Hsp104 assists the conversion of cellular prion protein (PrP^{C}) to the infectious scrapie PrP (PrP^{Sc}) form [129]. However, unlike the known yeast prion proteins, the mammalian prion protein (PrP) is not localized in the cytoplasm, but instead resides on the outer leaflet of the plasma membrane as a glycosylphosphatidylinositol-anchored protein [66]. This critical difference in localization makes it seem unlikely that cytoplasmic Hsp104 might affect PrP folding. Yet, aberrant PrP accumulation in the cytosol can be neurotoxic, although whether this contributes to cell death during prion infection remains unclear [130–132]. When mice expressing Hsp104 specifically in neurons were infected intracerebrally with ME7 prions, there was no significant difference in survival time or PrP^{Sc} accumulation relative to control mice [126].

Concluding Remarks

The studies outlined above point to the therapeutic potential of Hsp104, particularly for misfolding diseases where the aggregated protein resides in the cytoplasm or nucleus. However, it is likely that existing Hsp104 specificity represents an evolutionary compromise that is optimized for the disaggregation of multifarious types of substrates rather than any single class. Indeed, this versatility is likely essential for Hsp104 to disaggregate the entire aggregated proteome after environmental stress. Furthermore, Hsp104 may have coevolved with beneficial yeast prions to act on features specific to their amyloid form.

However, now comes the important point. A protein-remodeling factor (that is, Hsp104) *has evolved* to precisely regulate and even eliminate amyloid conformers. Therefore, even if Hsp104 activity is restricted to Sup35 and Ure2, it may be possible to engineer or evolve Hsp104 to be active against disease-associated amyloids, or indeed, other nonamyloid, disease-associated protein accumulations such as occur in the TDP-43-opathies [133]. An important consideration in generating any such arsenal of substrate-optimized Hsp104 variants will be to ensure that the final products of their remodeling activities are nontoxic conformers. In this way, a wide range of protein aggregation disorders may ultimately become treatable using designer, substrate-optimized Hsp104 variants that are specific to the particular aggregated protein and disease in question.

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