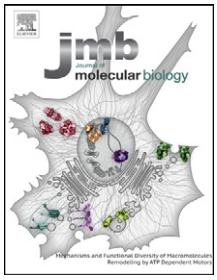
Review



Mechanistic and Structural Insights into the Prion-Disaggregase Activity of Hsp104

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Abstract

Hsp104 is a dynamic ring translocase and hexameric AAA+ protein found in yeast, which couples ATP hydrolysis to disassembly and reactivation of proteins trapped in soluble preamyloid oligomers, disordered protein aggregates, and stable amyloid or prion conformers. Here, we highlight advances in our structural understanding of Hsp104 and how Hsp104 deconstructs Sup35 prions. Although the atomic structure of Hsp104 hexamers remains uncertain, volumetric reconstruction of Hsp104 hexamers in ATPγS, ADP-AlF$_x$ (ATP hydrolysis transition-state mimic), and ADP via small-angle x-ray scattering has revealed a peristaltic pumping motion upon ATP hydrolysis. This pumping motion likely drives directional substrate translocation across the central Hsp104 channel. Hsp104 initially engages Sup35 prions immediately C-terminal to their cross-β structure. Directional pulling by Hsp104 then resolves N-terminal cross-β structure in a stepwise manner. First, Hsp104 fragments the prion. Second, Hsp104 unfolds cross-β structure. Third, Hsp104 releases soluble Sup35. Deletion of the Hsp104 N-terminal domain yields a hypomorphic disaggregase, Hsp104$^{\Delta N}$, with an altered pumping mechanism. Hsp104$^{\Delta N}$ fragments Sup35 prions without unfolding cross-β structure or releasing soluble Sup35. Moreover, Hsp104$^{\Delta N}$ activity cannot be enhanced by mutations in the middle domain that potentiate disaggregase activity. Thus, the N-terminal domain is critical for the full repertoire of Hsp104 activities.

Introduction

Protein disaggregases are potential therapeutic agents to reverse protein misfolding, aggregation, and amyloidogenesis that underpin several fatal human neurodegenerative disorders [1–15]. Moreover, amyloid fibrils highly abundant in human seminal fluid enhance HIV (human immunodeficiency virus) infection [16–18] and agents that reverse their assembly could help prevent HIV transmission [19–22]. The ability to reverse protein aggregation and recover pure, functional protein could also have important applications in basic research as well as in the purification and storage of valuable pharmaceutical proteins [23,24]. However, despite these potential basic, therapeutic, and pharmaceutical applications, we are still only beginning to understand the structural and mechanistic basis of protein disaggregases.

In yeast, a ring-shaped translocase and hexameric AAA+ protein, Hsp104, couples ATP hydrolysis to the disassembly and reactivation of disordered aggregates, preamyloid oligomers, amyloids, and prions [1,6,25–30]. Typically (but not always), Hsp104 disaggregase activity is optimal in the presence of the Hsp70 chaperone system [1,26,27,31,32]. Protein disaggregation is driven by Hsp104 coupling ATP hydrolysis to the partial or complete translocation of substrate across its central channel to solution by conserved tyrosine-bearing pore loops [33–37]. Hsp104 activity can be potentiated by mutations at specific positions in the coiled-coil middle domain (MD) or in nucleotide-binding domain 1 (NBD1) of Hsp104 (Fig. 1a) [7,38–41]. Potentiating mutations enable Hsp104 to dissolve fibrils formed by human neurodegenerative disease proteins such as TDP-43, FUS, and α-synuclein and mitigate neurodegeneration

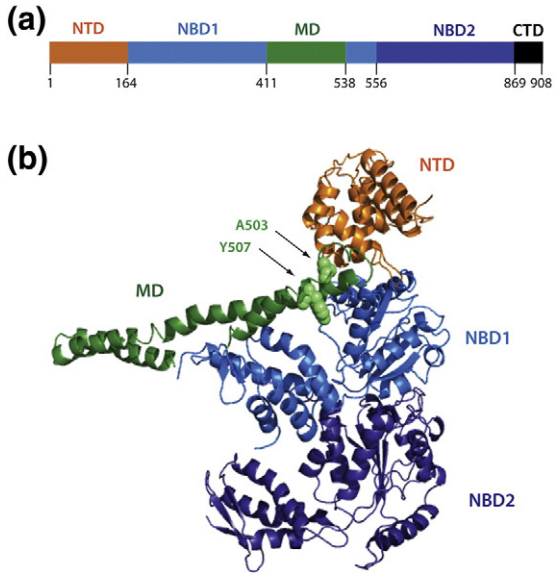

Fig. 1. Domain architecture and predicted monomeric structure of Hsp104. (a) Domain organization of the Hsp104 monomer (upper panel). NTD shown in orange, NBD1 shown in light blue, MD shown in green, NBD2 shown in dark blue, and CTD shown in black. (b) The separate domains of Hsp104 colorcoded as in (a) were homology modeled and fitted together. NTD is from PDB ID: 1khy and NBD1, MD, and NBD2 are from PDB ID: 1qvr. Note the positions of A503 and Y507 in helix 3 of the MD.

under conditions where wild-type Hsp104 is inactive [7]. Intriguingly, Hsp104 is absent from metazoa but is found in all other eukaryotes and in all eubacteria and some archaebacteria [42–45]. It has only recently been appreciated that metazoa rely upon the Hsp110, Hsp70, and Hsp40 chaperone system to disaggregate and reactivate proteins [5,14,46–51]. However, Hsp104 further stimulates the activity of this system [5,14,49]. Thus, Hsp104 could represent a disruptive technology to enhance metazoan proteostasis to counter fatal neurodegenerative disease or HIV infection [1–7,19,20].

Hsp104 confers several selective advantages in yeast. First, regulated amyloid remodeling by Hsp104 enables yeast to exploit prions for diverse adaptive purposes [52–57]. Second, Hsp104 confers tolerance to diverse thermal and chemical stresses by recovering natively folded protein from disordered aggregates harboring denatured proteins and by disassembling aggregated structures harboring functional proteins [25,26,58–60]. Third, Hsp104 promotes yeast longevity by regulating asymmetric partitioning of damaged proteins and aggregates during cell division [61–71].

In this review, we highlight recent advances in our structural and mechanistic understanding of Hsp104. In particular, we focus on how structural changes of Hsp104 hexamers during ATP hydrolysis promote substrate remodeling and how Hsp104 deconstructs Sup35 prions. Better structural and mechanistic understanding of Hsp104 will not only yield insight into its natural function but also open the door for directed evolution and protein design endeavors to tailor Hsp104 for specific remodeling of disease-associated aggregates and other therapeutic and research purposes.

Hsp104 Structure and Mechanism

An Hsp104 monomer is 908 residues and 102 kDa [72]. Hsp104 is composed of five domains: the N-terminal domain (NTD), two nucleotide-binding AAA+ domains (NBD1 and NBD2), a coiled-coil MD inserted within the small domain of NBD1, and a unique C-terminal extension (Fig. 1a and b) [72,73]. Like many AAA+ proteins [74], Hsp104 is only functional as a hexamer [25,75–78], but the precise structure of the ring-shaped hexamer remains unresolved and controversial [72,79–84]. Within the hexamer, each domain plays specific roles and allosterically communicates with adjacent domains and subunits [72,73]. However, the mechanistic and structural details of intraprotomer and interprotomer communication and precisely what roles the individual domains play in substrate remodeling are not fully resolved.

Crystal structures of the NTDs of the related bacterial Hsp100 proteins ClpA [85], ClpB [83,86], ClpV [87], and ClpC [88] have been solved. The NTDs are highly structurally conserved; they are very stable globular domains [85,86,88,89] made up of two imperfect repeats of four helical bundles [85,86,88] and are connected to the adjacent NBD1 by a highly mobile linker [83,90–92]. In ClpB, a bacterial Hsp104 orthologue, the NTD is involved in substrate binding [83,86,93–97,166] and casein-stimulated ATPase activity [93], and NTD mobility via the NTD–NBD1 linker is necessary for efficient translocation and disaggregation of substrate [98,99].

In contrast to the bacterial Hsp100 proteins, the function of the Hsp104 NTD has been less explored. *In vivo*, deletion of the NTD or specific point mutations (e.g., T160M) within the NTD has little effect on thermotolerance or prion propagation [30,100]. Therefore, Hsp104 NTD variants can still dissolve heat-denatured aggregates and fragment prions. However, overexpression of these mutants fails to cure [*PSI*$^{+}$] [30,100]. We have established that the ability of Hsp104 to dissolve amyloid conformers is selectively diminished by NTD deletion [41]. Indeed, Hsp104$^{\Delta N}$ can fragment but not dissolve Sup35 prions and is unable to dissolve amyloid forms of Ure2, α-synuclein, or polyglutamine [41]. Importantly, there is a fundamental difference in how amorphous and amyloid substrates are disaggregated by Hsp104 [1,101]. To disaggregate disordered aggregates, Hsp104 subunits within the

hexamer collaborate noncooperatively via probabilistic substrate binding and ATP hydrolysis [1]. By contrast, to resolve stable amyloid, several Hsp104 subunits within the hexamer must cooperatively engage substrate and hydrolyze ATP [1]. Thus, Hsp104 hexamers display operational plasticity [1]. Deletion of the NTD impairs this plasticity, which appears to be essential for the global cooperativity needed to resolve stable amyloid [1,41]. In addition, the Hsp104 NTD also participates in substrate binding and interactions with adapter proteins such as Hsp70 [41]. Indeed, the Hsp104 NTD regulates substrate binding and prevents nonproductive competition for substrate binding by pore loops [41].

The nucleotide-binding domains (NBDs) of AAA+ proteins are highly structurally conserved, consisting of a large α/β subdomain and a small α-helical subdomain [102]. Features that define these domains include regions involved in ATP binding and hydrolysis, namely, the Walker A and Walker B motifs, the arginine finger, sensor-1 and sensor-2 motifs, as well as tyrosine-bearing pore loops that engage substrate thereby coupling conformational changes of the AAA+ protein upon ATP hydrolysis to substrate remodeling [103]. In Hsp104, NBD1 and NBD2 are not simply duplicated NBDs, but differ significantly and originate from different clades of the AAA+ superfamily [103]. NBD1 belongs to clade 3 (members include the FtsH family, p97, NSF, and katanin) and NBD2 belongs to clade 5 (members include HslU/ClpX family, RuvB, and Lon family), indicating that Hsp104 arose from a gene fusion event [103]. Each NBD is able to bind and hydrolyze ATP, but they have very different catalytic properties [104]. By fitting the steady-state kinetics of ATP hydrolysis to two independent allosteric sites, as well as subsequent mutational analyses, the general properties of the two NBDs were determined [104]. NBD1 contains a low-affinity, high-turnover site and NBD2 contains a high-affinity, low-turnover site [104]. Nucleotide binding at NBD2 is critical for Hsp104 hexamerization [75–77], which is in contrast to ClpB, where nucleotide binding at NBD1 is critical for hexamerization [90]. Thus, in this regard, Hsp104 is more similar to its mitochondrial homolog, Hsp78, which also requires nucleotide binding at NBD2 (and not NBD1) for hexamerization [105]. Furthermore, while the majority of basal ATPase activity in Hsp104 is contributed by NBD1 [104], both NBDs in ClpB contribute to its basal ATPase rate [90]. Both Hsp104 NBDs display positive cooperativity and a high degree of allosteric communication between the two (e.g., hydrolysis in NBD1 depends upon the nucleotide state of NBD2) [104]. In addition to the conserved AAA+ motifs, NBD2 contains a nuclear localization signal [106]. Indeed, Hsp104 is active in both the cytoplasm and nucleus [25,106].

Hsp104 and ClpB each contain an ~85-Å-long coiled-coil MD inserted into the small α-helical subdomain of NBD1 (Fig. 1a and b) [72,83]. The MD consists of four helices that make up an antiparallel, broken coiled coil [72,83]. Helix 1 and half of helix 2 are designated motif 1, with the second half of helix 2 along with helices 3 and 4 designated motif 2 [72,83]. In ClpB, helix 3 appears to undergo conformational changes in response to nucleotide, possibly transitioning between loop and helix [83,107]. MDs of ClpB and Hsp104 are essential for disaggregation activity [90,108,109] and are the sites of interaction with Hsp70 [107,108,110–112,167]. The MD has been shown to interact with NBD1 and NBD2 in an autoinhibitory fashion, repressing activity of the hexamer [84,107,111,113,114]. Hsp70 binds directly and transiently to the MD, specifically in the region of helix 2 and 3 in motif 2 [110–112,167]. This interaction appears to relieve the autoinhibitory interactions between the MD and NBD1 [110–112,167]. Thus, it appears that the MD is a highly dynamic domain involved in regulation of hexamer activity. Indeed, point mutations in the MD can lead to inactive variants with stabilized MD–NBD1 or MD–NBD2 interactions [84,113] or hyperactive variants with enhanced unfoldase activity [7,38–40,109,113]. Interestingly, deletion of motif 1 (helix 1 and a portion of helix 2), motif 2 (a portion of helix 2, helix 3, and helix 4), or the entire MD confers a hyperactive state in ClpB [79,113]. By contrast, deletion of the MD or motif 1 inactivates Hsp104, whereas deletion of motif 2, helix 3, or helix 4 potentiates Hsp104 [109]. Thus, the requirements for Hsp104 potentiation are strikingly different from the requirements for generating hyperactive ClpB variants, indicating profound differences between Hsp104 and ClpB [1,19,109]. Regardless, many of the details of the placement, dynamics and the mechanism of MD-mediated regulation of Hsp104 and ClpB remain unresolved.

Hsp104 contains a unique C-terminal domain (CTD) of 40 residues (amino acids 869–908), which is not found in ClpB [43,72]. The region is enriched in acidic residues (42.5%), and the last four residues are a conserved DDLD motif that allows binding to the Hsp90 cochaperones Cpr7, Cns1, and Sti1 [115,116], although the importance of this interaction remains unknown as deletion of the DDLD motif does not affect thermotolerance [115]. The CTD was initially thought to be a key site of substrate interaction [117], and CTD binding to lysine-rich polypeptides stimulates ATPase activity in NBD1 via a conformational change in the MD [117]. However, subsequent work supported a model in which substrates are translocated N- to C-terminally by tyrosine-bearing pore loops [35–37], which leaves poly-Lys binding at the CTD of mysterious function. More recently, the CTD was shown to be essential for hexamerization [115,118], and though

the sequence indicates that it is highly disordered, this role implies that may not necessarily be the case. This requirement for hexamerization is surprising as the domain is absent in ClpB, indicating another difference between Hsp104 and ClpB.

Our structural and mechanistic understanding of Hsp104-mediated substrate remodeling activities remains incomplete [72,119,120]. Hsp104 assembles into dynamic ring-shaped hexamers with a central channel [1,75,81]. Hsp104 hexamers form in the presence of ADP or ATP [75–77] or in the absence of nucleotide at low salt concentrations [104]. ADP or ATP binding to NBD2 is critical for hexamerization of Hsp104, whereas ADP or ATP binding to NBD1 is dispensable for hexamerization [75–77]. Hsp104 hexamers exchange subunits on the minute timescale [1]. The hexamer undergoes large conformational changes upon ATP hydrolysis [73,82], but the details of these changes and how they are coupled to substrate remodeling remain unclear. Protein disaggregation is driven by Hsp104 coupling ATP hydrolysis to the partial or complete translocation of substrate across the central channel to solution via interaction with conserved tyrosine-bearing pore loops in NBD1 and NBD2 [33–37,41]. However, recent studies suggest that ClpB is a nonprocessive translocase, which takes only one to two translocation steps prior to releasing substrate, which appears insufficient for complete translocation across the central channel [121]. Thus, ClpB may dissociate protein aggregates by pulling and releasing exposed tails or loops [121]. In light of these studies [121], it will be important to determine the processivity of Hsp104.

Intriguingly, Hsp104 and ClpB use profoundly different mechanisms of intersubunit collaboration to disaggregate substrates. ClpB subunits couple cooperative ATP hydrolysis to probabilistic substrate binding [1]. By contrast, Hsp104 subunits display operational plasticity and can employ noncooperative substrate binding and ATP hydrolysis or cooperative substrate binding and hydrolysis depending upon the stability of the substrate [1]. Thus, more stable substrates elicit a switch in Hsp104 operating mode, such that more subunits are recruited to participate in the substrate binding and ATP hydrolysis needed for protein disaggregation [1]. This operational plasticity enables Hsp104 to adapt different mechanochemical coupling mechanisms that are tailored to the specific physical demands of the aggregated substrate [1]. Thus, if two subunits are sufficient to rapidly disaggregate a substrate, then only two will be used [1]. These differences between Hsp104 and ClpB have biological consequences. For example, ClpB has enhanced ability to dissolve disordered aggregates but extremely limited ability to dissolve amyloid [1,19,27,84]. By contrast, Hsp104 can readily dissolve both disordered aggregates and amyloid [1]. Indeed, even when ClpB is hyperactivated by mutations in the MD, it is unable to dissolve amyloid [19]. By contrast, the equivalent mutations in Hsp104 (e.g., Y507D; Fig. 1b) potentiate the ability of Hsp104 to antagonize the aggregation and toxicity of various human neurodegenerative disease proteins, including TDP-43, FUS, or α-synuclein [7].

Interestingly, despite this limited ability to dissolve amyloid, ClpB has been reported to have a weak ability to propagate Sup35 prions under artificial circumstances in yeast [122,123]. ClpB is unable to propagate [*PSI*$^{+}$] (Sup35 prions) in yeast [124], unless *Escherichia coli* DnaK (Hsp70) and GrpE (the DnaK nucleotide exchange factor) are also expressed [122,123]. However, even then, [*PSI*$^{+}$] propagation is considerably weaker than usual [122], indicating that ClpB has limited ability to fragment Sup35 prions even with DnaK and GrpE. Moreover, even with DnaK and GrpE expression, ClpB was unable to propagate [*URE3*] (Ure2 prions) [123], confirming a profound difference in Hsp104 and ClpB activity against Ure2 prions [1]. It has also been reported that *E. coli* can weakly propagate amyloid forms of NM (the N and M domains of Sup35) tagged with mCherry in a manner that requires ClpB [125]. However, here too, propagation was inefficient and NM-mCherry amyloid was rapidly lost from 50% of the original *E. coli* colonies [125]. This instability stands in stark contrast to the loss of strong [*PSI*$^{+}$] in 1 in every 10^{6} colonies in *Saccharomyces cerevisiae* [57,125]. Thus, even in its natural *E. coli* environment, the ability of ClpB to promote amyloid propagation is remarkably limited. Indeed, ClpB is not required for propagation of a RepA-WH1 prionoid in *E. coli* [126]. Finally, it is important to note that prion propagation in yeast can be driven solely by prion fragmentation and does not require prion dissolution per se [41,100,127,128]. Indeed, prion fragmentation and dissolution are separable phenomena [41]. For example, Hsp104$^{\Delta N}$ can fragment Sup35 prions without releasing soluble Sup35 and is unable to dissolve Sup35 prions and other amyloids [41]. We suggest that, in the presence of DnaK, DnaJ, and GrpE, ClpB has a very weak ability to fragment some amyloids [122,123,125] but not others [123,126]. Indeed, even very low activity of select Hsp104 mutants can propagate unstable prion variants in yeast [129,130]. Nevertheless, ClpB has diminished ability to dissolve amyloid in comparison to Hsp104, which stems from an altered mechanism of intersubunit collaboration [1,19,27,84].

The hexameric structure of Hsp104 remains unresolved. In fact, two radically distinct models were initially advanced [72]. Both were based on cryo-electron microscopy (cryo-EM) reconstructions with rigid-body fit domains from the crystal structure of the *Thermus thermophilus* homolog, tClpB [83]. Unfortunately, tClpB was not resolved in the functional, hexameric state [83]. Using assumptions based on other AAA+ proteins, an initial hexameric

structure with an external placement of the coiled-coil MD was proposed [83]. However, this structure was incompatible with the dimensions of Hsp104 hexamers observed by another cryo-EM study [81]. This incompatibility led to a second model with the coiled-coil MD intercalated between the two nucleotide-binding domains [81,82]. However, this new model did not preserve the typical intersubunit AAA+ nucleotide-binding sites, and the debate over the hexameric structure has continued [72,131].

More recently, a revised cryo-EM model has been proposed suggesting that the coiled-coil MD is on the surface of the hexamer, but can adopt diverse horizontally tilted positions [79]. This new placement of the MD preserves more canonical AAA+ architecture and is supported by site-resolved fluorescence, site-resolved cross-linking, site-resolved biotin labeling, and hydrogen–deuterium exchange studies [79,113]. Moreover, x-ray footprinting, site-resolved cross-linking, and site-resolved fluorescence studies reveal that the Hsp104 MD cannot project out into solution as initially proposed [80,83], but rather, it adopts a position that contacts NBD2 [84]. Thus, it appears that the MD can adopt diverse positions on the exterior of the hexamer [79,84,113].

Conformational Changes of Hsp104 Hexamers during ATP Hydrolysis

Hsp104 is a large, dynamic, multidomain hexamer that couples large conformational changes upon ATP hydrolysis to exertion of mechanical force on myriad substrates [73]. However, the nature of these conformational changes and how they promote substrate remodeling have remained unclear. To address this issue and enable an independent and complementary view, we have utilized small-angle x-ray scattering (SAXS) and wide-angle x-ray scattering (WAXS), which has provided important insights into the conformational changes of several other AAA+ proteins [132–136] and complex chaperonins [137] in solution. In previous SAXS studies of AAA+ proteins, crystal structures of domains fit well into the *ab initio* volumes [132–135], which allowed for hexameric models to be built from monomeric crystal structures and changes in conformation to be modeled based on the volumetric reconstructions. Additionally, changes in physical properties such as R_g and D_{max} and conformational changes of the reconstructed volumes in the presence of different nucleotides allowed for novel mechanistic understanding of wild-type and mutant proteins [132,133,135]. Cryo-EM reconstructions of Hsp104 have not yet yielded a unified picture of how Hsp104 catalyzes protein disaggregation [72,79–82,131]. Moreover, only dysfunctional variants of Hsp104 (e.g., HAP plus ClpP, $Hsp104^{N728A}$, $Hsp104^{\Delta N}$, and $Hsp104^{E285A:E687A}$) have been studied in a limited number of nucleotide states (only ATPγS, ATP, and ADP have been explored) [79–82]. Consequently, it remains difficult to extrapolate these findings to understand wild-type Hsp104. Importantly, SAXS/WAXS is performed in solution, under conditions where Hsp104 is active. Indeed, SAXS has emerged as powerful method for visualizing the workings of biological machines in solution [138] and avoids any potential issues caused by freezing or fixation in cryo-EM.

In this context, we were very interested to compare Hsp104 to $Hsp104^{\Delta N}$, as $Hsp104^{\Delta N}$ is a hypomorphic disaggregase that is unable to dissolve amyloid conformers but can dissolve disordered aggregates [41]. Thus, we examined Hsp104 and $Hsp104^{\Delta N}$ in AMP-PNP (a nonhydrolyzable ATP analog), ATPγS (a slowly hydrolyzable ATP analog), ATP, ADP-AlF_x (hydrolysis transition-state mimic), ADP, and apo states by SAXS/WAXS to a nominal resolution limit of ~7.6–8.4 Å [41]. By studying Hsp104 in these various nucleotide states, we can elucidate hexameric states that are likely to be populated during its natural reaction cycle [41]. Volumetric reconstructions reveal Hsp104 and $Hsp104^{\Delta N}$ hexamers that can readily accommodate six monomers via rudimentary domain fitting (Fig. 2) [41]. Moreover, Hsp104 and $Hsp104^{\Delta N}$ hexamers exhibit large structural differences between these different nucleotide conditions (Fig. 3a and b) [41]. Indeed, our findings indicate that Hsp104 and $Hsp104^{\Delta N}$ hexamers contract upon ATP hydrolysis and expand upon ATP binding, suggesting a pumping mechanism to drive substrate translocation [41]. Interestingly, SAXS reconstructions of another AAA+ protein, p97, revealed that the p97 hexamer also appears to contract upon ATP hydrolysis (ADP-AlF_x state) [133]. Thus, cycles of hexamer expansion and contraction coupled to ATP binding and hydrolysis could be a general feature of how certain AAA+ proteins remodel substrates.

One striking feature of the Hsp104 and $Hsp104^{\Delta N}$ hexamers was the positioning of a projection of density on the hexamer exterior, along the plane of the largest dimension [41]. Hsp104 and $Hsp104^{\Delta N}$ have external projections that occupy a more N-terminal position (in no nucleotide, AMP-PNP, ATPγS, and ATP) to a more C-terminal position (in ADP-AlF_x and ADP) upon ATP hydrolysis (Fig. 3a and b, arrows) [41]. It is probable that the external projection corresponds to the MD [41], which is consistent with cryo-EM reconstructions of HAP plus ATPγS and ClpP [79]. The SAXS reconstruction of Hsp104 in ATPγS and cryo-EM reconstruction of HAP plus ATPγS and ClpP reveal particles of similar dimensions (Fig. 4) [41,79]. We suggest that our SAXS reconstructions help to clarify MD location [41,79–82,131]. The shift

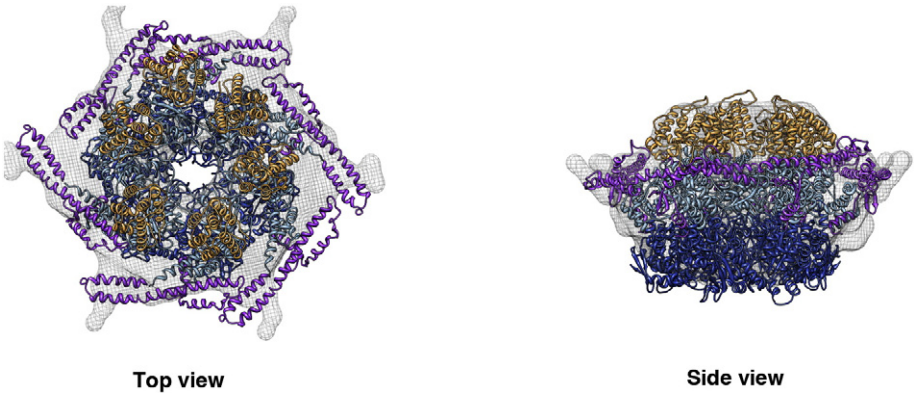

Fig. 2. Rigid-body fitting individual Hsp104 domains into SAXS reconstructions of the Hsp104 hexamer in the presence of ATP. Individual Hsp104 domains were homology modeled based on the ClpB crystal structure PDB ID: 1khy (NTD) and PDB ID: 1qvr (for NBD1, MD, and NBD2). The domains were then rigid-body fit into the volume envelope generated from SAXS/WAXS data of the Hsp104 hexamer in the presence of ATP. Top and side views are shown. The individual domains are color coded: NTD (orange), NBD1 (light blue), MD (purple), and NBD2 (dark blue). Adapted from Ref. [41].

in positioning of the external projection (Fig. 3a and b, arrows) suggests that the MD might move from an N-terminal horizontal position to a C-terminally tilted position upon ATP hydrolysis [41,79,84].

To disaggregate substrates, Hsp104 is thought to translocate proteins either partially or completely through its central channel [19,33,37]. Additional striking features of Hsp104 and Hsp104$^{\Delta N}$ hexamers were changes in the diameter and contours of the central channel in the various nucleotide states (Fig. 5a and b) [41]. The motions of the Hsp104 channel are highly reminiscent of a peristaltic wave [41]. In a peristaltic wave, there is a relaxation at the site of substrate entrance, followed by a wave of constriction that travels in the direction the substrate is being pumped. Substrate enters through the N-terminal opening and can be fully translocated out the C-terminal end of the channel [19,37]. In the ATPγS or ATP states, when the hexamer is primed to engage substrate, the extreme N-terminal side is open (Fig. 5a). After the opening, there is a region of constriction, still N-terminal (Fig. 5a, arrow). As we step through the simulated ATPase cycle, the channel first constricts fully, correlating with the most contracted state, in the ADP-AlF$_x$ transition-state mimic, and then the point of constriction moves toward the C-terminus in the ADP state (Fig. 5a). This peristaltic pumping motion explains how the Hsp104 hexamer is able to transduce energy from ATP hydrolysis to conformational change and substrate remodeling using physical force.

The role of the NTD in facilitating the peristaltic pump motion is both simple and significant. In the absence of the NTD, the channel is unable to contract; rather, the entire hexamer is smaller in the N- to C-terminal direction, and the channel appears to have a uniformly large diameter throughout (Fig. 5b). As the Hsp104$^{\Delta N}$ channel progresses through the ATPase cycle, it is clear that the missing domain is essential for the proper movements of the channel. This defect is most obvious in the transition-state mimic ADP-AlF$_x$, which is constricted in the center but open on both sides (Fig. 5b). While there still appears to be an area of contraction that shifts N- to C-terminally, it is clear that the peristaltic motion is greatly perturbed in the Hsp104$^{\Delta N}$ hexamer (Fig. 5b). Specifically, it appears that substrate could more readily diffuse out of the N-terminal opening of the channel of the Hsp104$^{\Delta N}$ hexamer.

Our SAXS findings indicate that deletion of the NTD results in expansion of the ends of the central cavity (similar to a cryo-EM study of the Hsp104$^{\Delta N}$ hexamer [81]), as well as abnormal changes of the central channel during the ATPase cycle (Fig. 5b). Hsp104$^{\Delta N}$ is unable to undergo nucleotide-dependent changes in the central channel similar to Hsp104, which helps explain the reduced ability of Hsp104$^{\Delta N}$ to translocate substrate through the central channel [41].

How Hsp104 Deconstructs Sup35 Prions

We have also recently elucidated the mechanism of Sup35 prion fragmentation and dissolution by Hsp104 [41]. Sup35 is an essential translation termination factor that can switch to various prion conformations that encode [*PSI*$^+$] phenotypes [57]. Sup35 contains a C-terminal GTPase domain (amino acids 254–685, black), a highly charged MD (M, amino acids 124–253, dark gray), and a

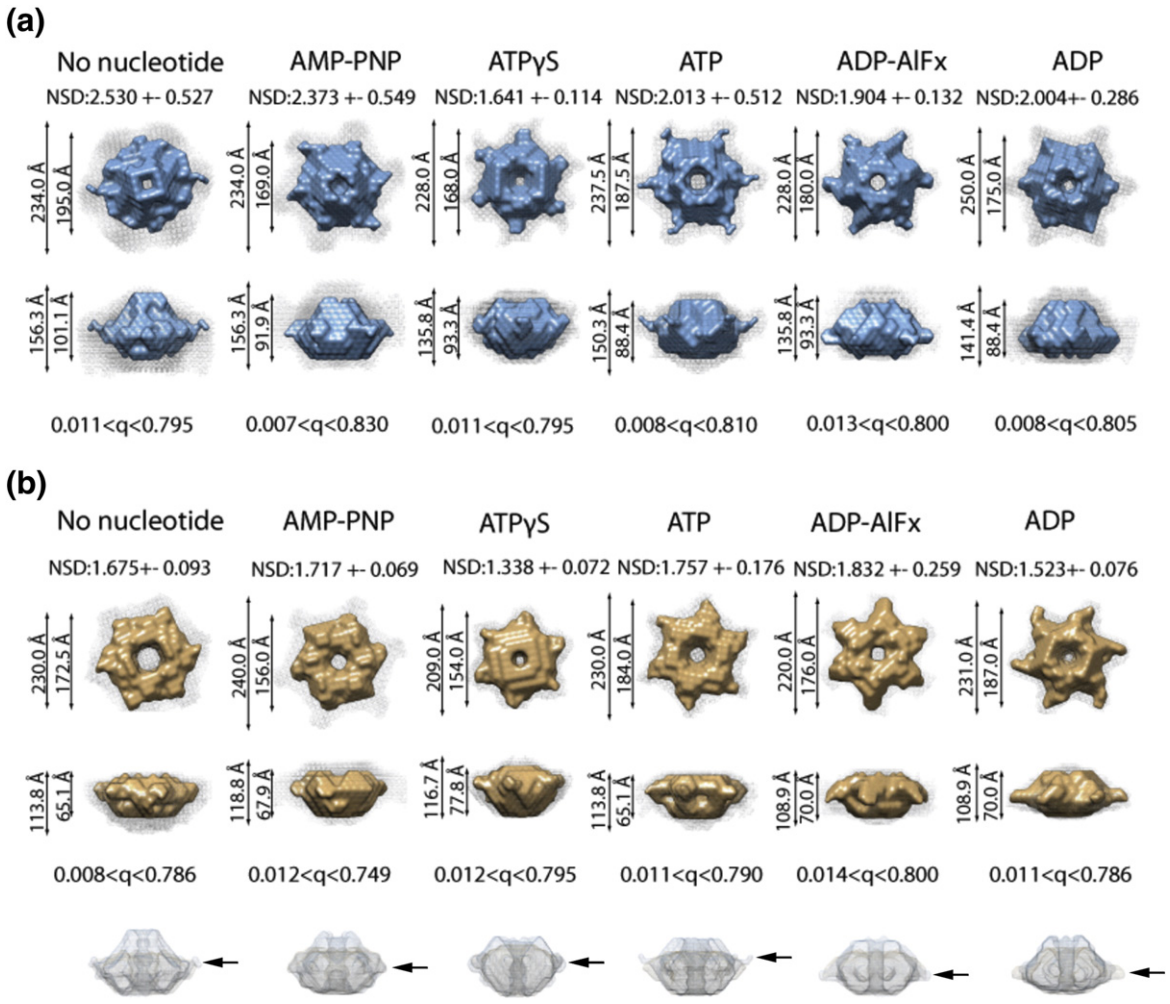

Fig. 3. NTD deletion alters ATPase-driven conformational changes of Hsp104 hexamers. Volumetric envelope reconstructions of Hsp104 and Hsp104$^{\Delta N}$ derived from SAXS/WAXS data. Averaged *ab initio* GASBOR [164] volume reconstructions of Hsp104 (a) and Hsp104$^{\Delta N}$ (b) from the SAXS/WAXS data. Filtered density is filled blue (Hsp104) or orange (Hsp104$^{\Delta N}$) overlaid with the unfiltered average shown in gray mesh. Normalized spatial discrepancy (NSD) of the averaged models and the *q* range used for the reconstructions are shown along with the average particle dimensions. Overlay of the Hsp104 and Hsp104$^{\Delta N}$ average reconstructions for each state, which were used to orient the particles, is shown at the bottom of (b). The Hsp104 and Hsp104$^{\Delta N}$ hexamers undergo large changes in shape that depend upon the identity of the nucleotide. Reconstructions are oriented with the N-terminus pointing toward the top of the page. Arrows denote position of external projection, likely corresponding to the MD, in each state. Adapted from Ref. [41].

prionogenic NTD (N, amino acids 1–123, light gray) enriched in glutamine, asparagine, tyrosine, and glycine residues (Fig. 6a) [57]. Within N, prion recognition elements establish homotypic intermolecular contacts such that alternating head-to-head (red) and tail-to-tail (green) contacts hold adjacent Sup35 monomers together within the assembled prion (Fig. 6a) [41,139–146]. A central core (blue) in between these prion recognition elements is sequestered by intramolecular cross-β contacts (Fig. 6a) [41,139–146]. The residues that comprise the head, the central core, and the tail vary depending on the Sup35 prion strain that assembles [41,139–146].

Using site-resolved cross-linking, we have established that Hsp104 initially engages assembled Sup35 prions by binding to a region spanning amino acids 96–151 of the N and M domains (Fig. 6b, purple regions) [41]. This observation is consistent with previous peptide array binding studies [147]. After this initial engagement, Hsp104 then exerts a directed pulling force that selectively unfolds cross-β structure N-terminal to this initial binding site (Fig. 6b) [41]. Remarkably, Hsp104 does not unfold regions C-terminal to this binding site (Fig. 6b) [41]. This specific partial translocation mechanism enables Hsp104 to dissolve Sup35 prions without unfolding the C-terminal

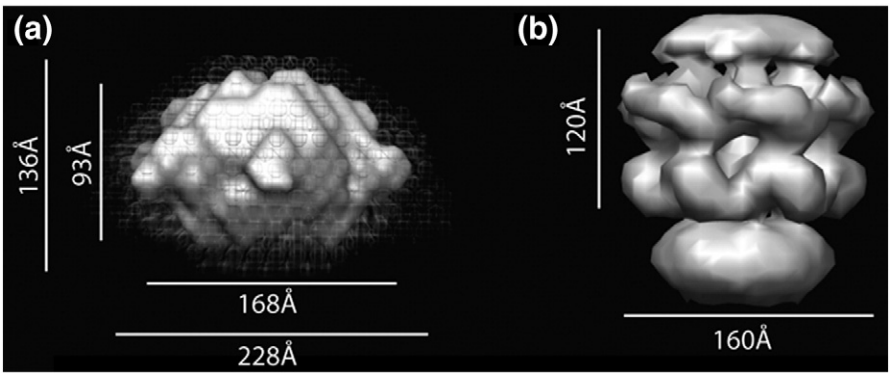

Fig. 4. SAXS reconstruction of Hsp104 in ATPγS and the cryo-EM reconstruction of HAP plus ClpP in ATPγS. (a) Side view of SAXS reconstruction of Hsp104 in ATPγS. Filtered (filled) and unfiltered density (caged) plus dimensions are indicated [41]. (b) Cryo-EM reconstruction of HAP plus ClpP in ATPγS [79]. Dimensions are indicated. Note the density of ClpP below the HAP envelope.

GTPase domain (Fig. 6b) [41]. Thus, Hsp104 rapidly releases functional, folded Sup35 from the assembled prion and can rapidly eliminate the loss-of-function [*PSI*$^+$] prion phenotype [27,30,32,148,149]. This type of partial translocation mechanism may also enable Hsp104 to rapidly

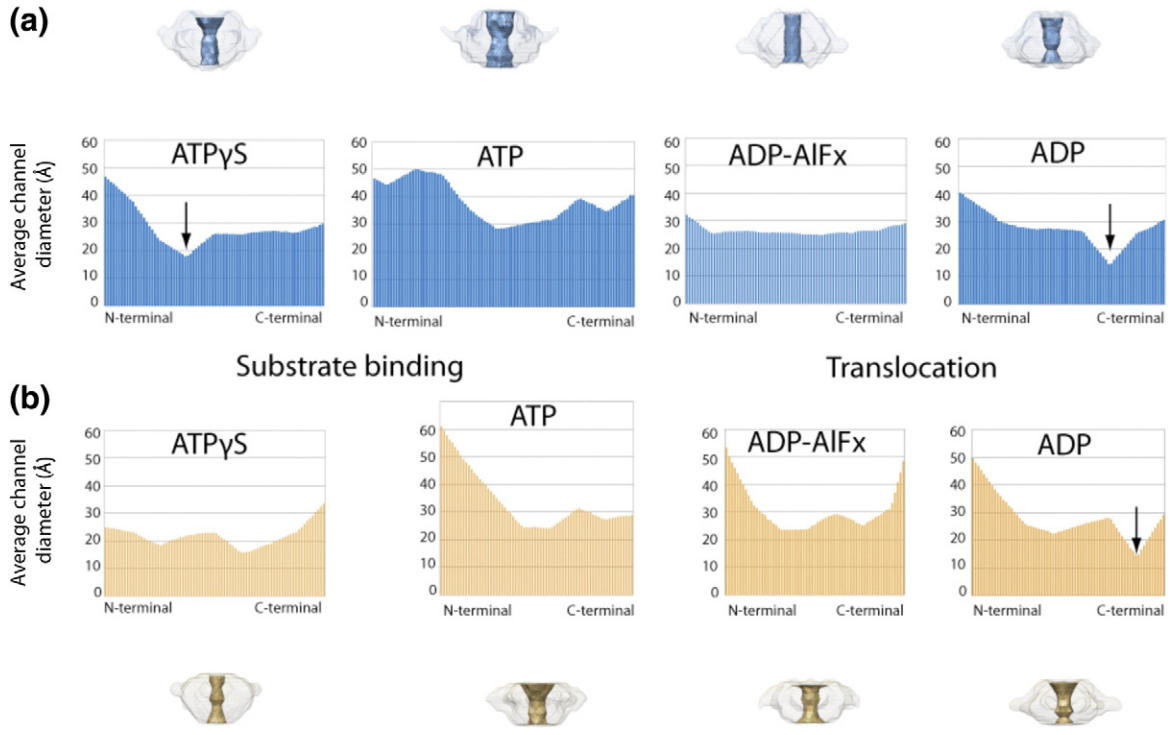

Fig. 5. Channel motions of the Hsp104 and Hsp104$^{\Delta N}$ hexamers. (a and b) Mapping of the Hsp104 (a) and Hsp104$^{\Delta N}$ (b) hexamer channels. Cut-away side views of the Hsp104 (a) and Hsp104$^{\Delta N}$ (b) hexamers in the ATPγS, ATP, ADP-AlF$_x$, and ADP state are shown. The volume of the channel was reconstructed in Chimera [165] using the filtered average volumes for each nucleotide state. The bar graphs display the average diameter of each 1 Å z slice of the channel volume starting from the N-terminus as calculated using Matlab. Substrate binds in the ATPγS state of the hexamer and is translocated from the N-terminal entrance to the C-terminal exit. The Hsp104 channel exhibits a peristaltic wave motion; relaxation at the N-terminal entrance followed by a contraction of the N-terminal end of the channel and finally a shift in the location of a constriction from the N-terminal to the C-terminal region (arrow). The Hsp104$^{\Delta N}$ hexamer displays defects in the peristalsis motion, especially in the N-terminal entrance to the channel, which fails to contract in the ADP-AlF$_x$ and ADP states. This defect is most noticeable in the ADP-AlF$_x$ state. Adapted from Ref. [41].

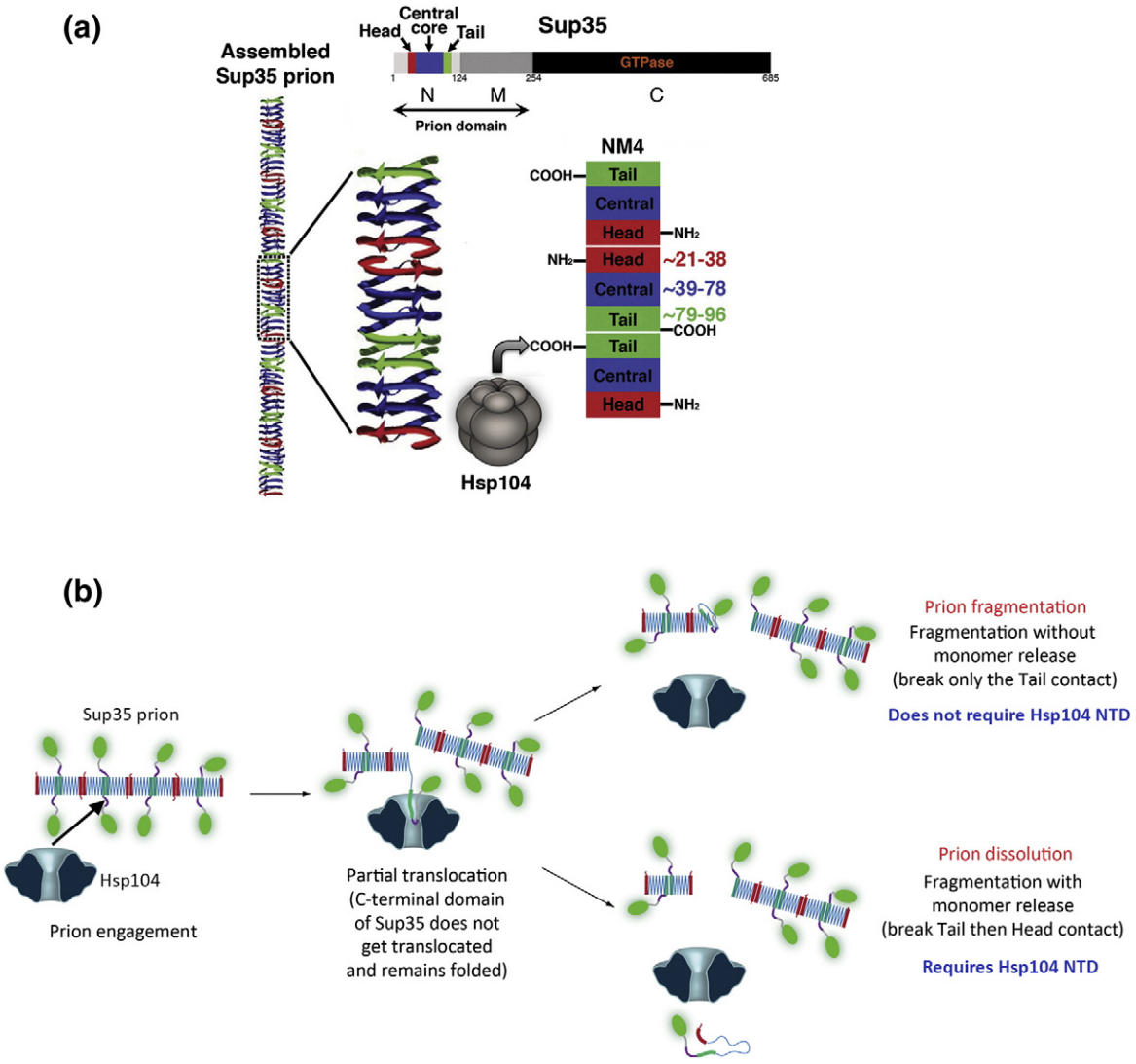

Fig. 6. How Hsp104 deconstructs Sup35 prions. (a) Sup35 is composed of a C-terminal GTPase domain (amino acids 254–685, black) that confers translation termination activity, a highly charged MD (M, amino acids 124–253, dark gray), and a prionogenic NTD (N, amino acids 1–123, light gray) enriched in glutamine, asparagine, tyrosine, and glycine. Within N, prion recognition elements within the NTD of Sup35 make homotypic intermolecular contacts such that Sup35 prions are maintained by an alternating sequence of head-to-head (red) and tail-to-tail (green) contacts. The central core (blue) is sequestered by intramolecular contacts. The position of the head contact, central core, and tail contact is shown for NM4 prions. Hsp104 is depicted engaging Sup35 prions C-terminal to the tail contact. (b) Model of Sup35 prion fragmentation *versus* dissolution by Hsp104. Hsp104 initially engages Sup35 prions in a region (amino acids 96–151; purple) C-terminal to the tail contact (dark green). Directional pulling on the N-terminal cross-β structure leads to partial translocation and breakage of the tail contact and Sup35 prion fragmentation. Further translocation breaks central core contacts (blue) and the head contacts (red) resulting in monomer release. In this way, the Sup35 prion is fragmented with or without monomer release, and the CTD of Sup35 remains folded throughout this process. $Hsp104^{\Delta N}$ retains the ability to break the tail but not the central core or head contacts. Therefore, $Hsp104^{\Delta N}$ is able to fragment Sup35 prions but not resolubilize Sup35 monomers. Adapted from Ref. [41].

disassemble heat-induced assemblies of functional proteins and immediately release active, soluble protein [33,60]. By contrast, remodeling of seminal amyloid fibrils formed by PAP248-286 and PAP85-120 appears to involve complete translocation of the peptides across the Hsp104 channel [19] as with other model substrates [37]. Thus, Hsp104 can exhibit plasticity in the translocation mechanism employed to resolve different aggregated structures.

After binding to the Sup35 prion, Hsp104 selectively remodels cross-β structure N-terminal to its binding site in three steps [41]:

(1) Hsp104 severs the tail-to-tail contact to fragment the prion (Fig. 6b, dark-green regions) [41].
(2) Hsp104 unfolds the central cross-β core (Fig. 6b, blue regions) [41].
(3) Hsp104 breaks the head-to-head contact (Fig. 6b, red regions) to release a soluble, folded Sup35 monomer [41].

Intriguingly, Hsp104$^{\Delta N}$ is dysfunctional in the second and third steps of this stepwise prion-dissolution process [41]. Hsp104$^{\Delta N}$ binds assembled Sup35 prions at the same site as Hsp104 and proceeds to rupture the tail-to-tail contact [41]. However, Hsp104$^{\Delta N}$ is subsequently unable to remodel the central core or separate the head-to-head contact [41]. Thus, Hsp104$^{\Delta N}$ is a hypomorphic disaggregase that fragments but does not dissolve Sup35 prions (Fig. 6b) [41]. This finding helps explain why Hsp104$^{\Delta N}$ can propagate [*PSI*$^+$] but is much less able to cure [*PSI*$^+$] at high concentrations *in vivo* [30,100]. Indeed, deleting the NTD restricts Hsp104 activity to a mode where it preferentially stimulates Sup35 prionogenesis and amplifies prions that confer strong [*PSI*$^+$] [41,145].

Several *in vivo* studies suggest that elevated Hsp104 concentrations can cure yeast of [*PSI*$^+$] (Sup35 prions) via direct dissolution of Sup35 prions by Hsp104 [29,30,148,150]. It has also been suggested that Sup35 prion remodeling by excess Hsp104 yields noninfectious Sup35 amyloid-like conformers with reduced seeding activity [32,145,147,151]. Indeed, remodeling of pure Sup35 prions by Hsp104 yields a mixture of soluble Sup35 and nontemplating, amyloid-like Sup35 *in vitro* [31,32,145]. Moreover, overexpression of Hsp104 in yeast cures [*PSI*$^+$] and yields a mixture of soluble Sup35 and SDS-resistant, amyloid-like forms of Sup35 [152]. However, mechanisms distinct from prion remodeling or dissolution have been proposed to explain [*PSI*$^+$] curing by Hsp104 overexpression. For example, it has been proposed that an indirect inhibition of Sup35 prion fragmentation due to displacement of Ssa1 from Sup35 prions by excess Hsp104 cures [*PSI*$^+$] [153]. However, these experiments were performed exclusively with fluorescently tagged proteins and remain uncorroborated with native, untagged proteins and native prions *in vivo* [153]. Indeed, it remains unclear if the fluorescence and colocalization techniques employed in this study truly measure prion fragmentation or even direct prion-chaperone binding events [153]. Finally, it is important to note that [*PSI*$^+$] curing kinetics by Hsp104 overexpression are simply too rapid to be explained solely by an inhibition of prion fragmentation [30,154].

Essential Role for the NTD in Potentiated Hsp104 Activity

We have established that the NTD is essential for potentiation of Hsp104 activity by mutations in the MD [41]. Unlike their full-length equivalents, neither Hsp104$^{\Delta N\text{-}A503V}$ nor Hsp104$^{\Delta N\text{-}A503S}$ rescued TDP-43, FUS, or α-synuclein aggregation or toxicity in yeast [41]. Potentiating mutations at the A503 position of the MD (Fig. 1b) likely destabilize a fragilely constrained autoinhibited state that restricts Hsp104 activity or mimic a critical allosteric activation step [7,38–40]. Consequently, these mutations enhance Hsp104 ATPase activity, substrate translocation rate, unfoldase activity, and disaggregase activity [7]. These hyperactivating sequelae are nixed by NTD deletion [41], which suggests that future designs of potentiated Hsp104 variants should include the NTD. Further studies will clarify any direct role for the NTD in potentiated Hsp104 activity. However, potentiation may simply require the critical role of the NTD in nucleotide-dependent conformational changes that enable productive hexamer cooperativity [41]. It will be interesting to determine whether mutations in the NTD can potentiate Hsp104 activity in the absence of MD mutations or whether novel NTD mutations can further enhance the activity of potentiated Hsp104 variants.

Closing Remarks

Our recent advances highlight the importance of the Hsp104 NTD in enabling the full repertoire of Hsp104 activities [41], whereas previously, the NTD has been considered to be more dispensable [35,90,94,100,153]. Indeed, the Hsp104 NTD enables globally cooperative substrate translocation, which is critical for prion dissolution and potentiated disaggregase activity [41]. In other AAA+ proteins, such as Lon, the NTD is more stringently integral to enzyme function [155]. By contrast, other AAA+ proteins harbor NTDs that can appear at least partially dispensable as with ClpA or ClpX [89,156–159] or even negatively regulate activity, as with Cdc48 and PAN [160–163]. However, we suggest that optimal functionality for ring-shaped, hexameric AAA+ proteins that must unfold structurally diverse repertoires of misfolded proteins or disrupt divergent protein complexes depends on NTDs that enable hexamer plasticity and potentiation [41].

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Abbreviations used:
NTD, N-terminal domain; MD, middle domain; CTD, C-terminal domain; cryo-EM, cryo-electron microscopy; SAXS, small-angle x-ray scattering; WAXS, wide-angle x-ray scattering; NIH, National Institutes of Health; NBD, nucleotide-binding domain.

References

[1] M.E. DeSantis, E.H. Leung, E.A. Sweeny, M.E. Jackrel, M. Cushman-Nick, A. Neuhaus-Follini, et al., Operational plasticity enables Hsp104 to disaggregate diverse amyloid and nonamyloid clients, Cell 151 (2012) 778–793.

[2] J. Shorter, Hsp104: A weapon to combat diverse neurodegenerative disorders, Neurosignals 16 (2008) 63–74.

[3] S. Vashist, M. Cushman, J. Shorter, Applying Hsp104 to protein-misfolding disorders, Biochem. Cell Biol. 88 (2010) 1–13.

[4] M. Cushman-Nick, N.M. Bonini, J. Shorter, Hsp104 suppresses polyglutamine-induced degeneration post onset in a *Drosophila* MJD/SCA3 model, PLoS Genet. 9 (2013) e1003781.

[5] M.L. Duennwald, A. Echeverria, J. Shorter, Small heat shock proteins potentiate amyloid dissolution by protein disaggregases from yeast and humans, PLoS Biol. 10 (2012) e1001346.

[6] C. Lo Bianco, J. Shorter, E. Regulier, H. Lashuel, T. Iwatsubo, S. Lindquist, et al., Hsp104 antagonizes alpha-synuclein aggregation and reduces dopaminergic degeneration in a rat model of Parkinson disease, J. Clin. Invest. 118 (2008) 3087–3097.

[7] M.E. Jackrel, M.E. Desantis, B.A. Martinez, L.M. Castellano, R.M. Stewart, K.A. Caldwell, et al., Potentiated hsp104 variants antagonize diverse proteotoxic misfolding events, Cell 156 (2014) 170–182.

[8] J. Carmichael, J. Chatellier, A. Woolfson, C. Milstein, A.R. Fersht, D.C. Rubinsztein, Bacterial and yeast chaperones reduce both aggregate formation and cell death in mammalian cell models of Huntington's disease, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 9701–9705.

[9] C. Vacher, L. Garcia-Oroz, D.C. Rubinsztein, Overexpression of yeast Hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease, Hum. Mol. Genet. 14 (2005) 3425–3433.

[10] S.H. Satyal, E. Schmidt, K. Kitagawa, N. Sondheimer, S. Lindquist, J.M. Kramer, et al., Polyglutamine aggregates alter protein folding homeostasis in *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5750–5755.

[11] Y.H. Liu, Y.L. Han, J. Song, Y. Wang, Y.Y. Jing, Q. Shi, et al., Heat shock protein 104 inhibited the fibrillization of prion peptide 106-126 and disassembled prion peptide 106-126 fibrils *in vitro*, Int. J. Biochem. Cell Biol. 43 (2011) 768–774.

[12] Y. Kim, J.H. Park, J.Y. Jang, H. Rhim, S. Kang, Characterization and Hsp104-induced artificial clearance of familial ALS-related SOD1 aggregates, Biochem. Biophys. Res. Commun. 434 (2013) 521–526.

[13] V. Perrin, E. Regulier, T. Abbas-Terki, R. Hassig, E. Brouillet, P. Aebischer, et al., Neuroprotection by Hsp104 and Hsp27 in lentiviral-based rat models of Huntington's disease, Mol. Ther. 15 (2007) 903–911.

[14] M.P. Torrente, J. Shorter, The metazoan protein disaggregase and amyloid depolymerase system: Hsp110, Hsp70, Hsp40, and small heat shock proteins, Prion 7 (2013) 457–463.

[15] Y.S. Eisele, C. Monteiro, C. Fearns, S.E. Encalada, R.L. Wiseman, E.T. Powers, et al., Targeting protein aggregation for the treatment of degenerative diseases, Nat. Rev. Drug Discov. 14 (2015) 759–780.

[16] F. Arnold, J. Schnell, O. Zirafi, C. Sturzel, C. Meier, T. Weil, et al., Naturally occurring fragments from two distinct regions of the prostatic acid phosphatase form amyloidogenic enhancers of HIV infection, J. Virol. 86 (2012) 1244–1249.

[17] J. Munch, E. Rucker, L. Standker, K. Adermann, C. Goffinet, M. Schindler, et al., Semen-derived amyloid fibrils drastically enhance HIV infection, Cell 131 (2007) 1059–1071.

[18] N.R. Roan, J. Munch, N. Arhel, W. Mothes, J. Neidleman, A. Kobayashi, et al., The cationic properties of SEVI underlie its ability to enhance human immunodeficiency virus infection, J. Virol. 83 (2009) 73–80.

[19] L.M. Castellano, S.M. Bart, V.M. Holmes, D. Weissman, J. Shorter, Repurposing Hsp104 to antagonize seminal amyloid and counter HIV infection, Chem. Biol. 22 (2015) 1074–1086.

[20] L.M. Castellano, J. Shorter, The surprising role of amyloid fibrils in HIV infection, Biology 1 (2012) 58–80.

[21] L.M. Castellano, R.M. Hammond, V.M. Holmes, D. Weissman, J. Shorter, Epigallocatechin-3-gallate rapidly remodels pAP85-120, SEM1(45-107), and SEM2(49-107) seminal amyloid fibrils, Biol. Open 4 (2015) 1206–1212.

[22] E. Lump, L.M. Castellano, C. Meier, J. Seeliger, N. Erwin, B. Sperlich, et al., A molecular tweezer antagonizes seminal amyloids and HIV infection, Elife (2015), http://dx.doi.org/10.7554/eLife.05397.

[23] S.J. Shire, Formulation and manufacturability of biologics, Curr. Opin. Biotechnol. 20 (2009) 708–714.

[24] M. Lebendiker, T. Danieli, Production of prone-to-aggregate proteins, FEBS Lett. 588 (2014) 236–246.
[25] D.A. Parsell, A.S. Kowal, M.A. Singer, S. Lindquist, Protein disaggregation mediated by heat-shock protein Hsp104, Nature 372 (1994) 475–478.
[26] J.R. Glover, S. Lindquist, Hsp104, Hsp70, and Hsp40: A novel chaperone system that rescues previously aggregated proteins, Cell 94 (1998) 73–82.
[27] J. Shorter, S. Lindquist, Hsp104 catalyzes formation and elimination of self-replicating Sup35 prion conformers, Science 304 (2004) 1793–1797.
[28] S. DiSalvo, A. Derdowski, J.A. Pezza, T.R. Serio, Dominant prion mutants induce curing through pathways that promote chaperone-mediated disaggregation, Nat. Struct. Mol. Biol. 18 (2011) 486–492.
[29] C.L. Klaips, M.L. Hochstrasser, C.R. Langlois, T.R. Serio, Spatial quality control bypasses cell-based limitations on proteostasis to promote prion curing, Elife (2014), http://dx.doi.org/10.7554/eLife.04288.
[30] Y.N. Park, X. Zhao, Y.I. Yim, H. Todor, R. Ellerbrock, M. Reidy, et al., Hsp104 overexpression cures *Saccharomyces cerevisiae* [*PSI*$^{+}$] by causing dissolution of the prion seeds, Eukaryot. Cell 13 (2014) 635–647.
[31] J. Shorter, S. Lindquist, Hsp104, Hsp70 and Hsp40 interplay regulates formation, growth and elimination of Sup35 prions, EMBO J. 27 (2008) 2712–2724.
[32] J. Shorter, S. Lindquist, Destruction or potentiation of different prions catalyzed by similar Hsp104 remodeling activities, Mol. Cell 23 (2006) 425–438.
[33] T. Haslberger, A. Zdanowicz, I. Brand, J. Kirstein, K. Turgay, A. Mogk, et al., Protein disaggregation by the AAA+ chaperone ClpB involves partial threading of looped polypeptide segments, Nat. Struct. Mol. Biol. 15 (2008) 641–650.
[34] J. Weibezahn, P. Tessarz, C. Schlieker, R. Zahn, Z. Maglica, S. Lee, et al., Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB, Cell 119 (2004) 653–665.
[35] R. Lum, M. Niggemann, J.R. Glover, Peptide and protein binding in the axial channel of Hsp104. Insights into the mechanism of protein unfolding, J. Biol. Chem. 283 (2008) 30139–30150.
[36] R. Lum, J.M. Tkach, E. Vierling, J.R. Glover, Evidence for an unfolding/threading mechanism for protein disaggregation by *Saccharomyces cerevisiae* Hsp104, J. Biol. Chem. 279 (2004) 29139–29146.
[37] P. Tessarz, A. Mogk, B. Bukau, Substrate threading through the central pore of the Hsp104 chaperone as a common mechanism for protein disaggregation and prion propagation, Mol. Microbiol. 68 (2008) 87–97.
[38] M.E. Jackrel, J. Shorter, Potentiated Hsp104 variants suppress toxicity of diverse neurodegenerative disease-linked proteins, Dis. Model. Mech. 7 (2014) 1175–1184.
[39] M.E. Jackrel, J. Shorter, Reversing deleterious protein aggregation with re-engineered protein disaggregases, Cell Cycle 13 (2014) 1379–1383.
[40] M.E. Jackrel, J. Shorter, Engineering enhanced protein disaggregases for neurodegenerative disease, Prion 9 (2015) 90–109.
[41] E.A. Sweeny, M.E. Jackrel, M.S. Go, M.A. Sochor, B.M. Razzo, M.E. DeSantis, et al., The Hsp104 N-terminal domain enables disaggregase plasticity and potentiation, Mol. Cell 57 (2015) 836–849.
[42] A.J. Erives, J.S. Fassler, Metabolic and chaperone gene loss marks the origin of animals: Evidence for Hsp104 and Hsp78 chaperones sharing mitochondrial enzymes as clients, PLoS One 10 (2015) e0117192.
[43] E.C. Schirmer, J.R. Glover, M.A. Singer, S. Lindquist, HSP100/Clp proteins: A common mechanism explains diverse functions, Trends Biochem. Sci. 21 (1996) 289–296.
[44] C.J. Shih, M.C. Lai, Analysis of the AAA+ chaperone clpB gene and stress-response expression in the halophilic methanogenic archaeon *Methanohalophilus portucalensis*, Microbiology 153 (2007) 2572–2583.
[45] C.J. Shih, M.C. Lai, Differentially expressed genes after hyper- and hypo-salt stress in the halophilic archaeon *Methanohalophilus portucalensis*, Can. J. Microbiol. 56 (2010) 295–307.
[46] A. Finka, S.K. Sharma, P. Goloubinoff, Multi-layered molecular mechanisms of polypeptide holding, unfolding and disaggregation by HSP70/HSP110 chaperones, Front. Mol. Biosci. 2 (2015) 29.
[47] R.U. Mattoo, S.K. Sharma, S. Priya, A. Finka, P. Goloubinoff, Hsp110 is a bona fide chaperone using ATP to unfold stable misfolded polypeptides and reciprocally collaborate with Hsp70 to solubilize protein aggregates, J. Biol. Chem. 288 (2013) 21399–21411.
[48] H. Rampelt, J. Kirstein-Miles, N.B. Nillegoda, K. Chi, S.R. Scholz, R.I. Morimoto, et al., Metazoan Hsp70 machines use Hsp110 to power protein disaggregation, EMBO J. 31 (2012) 4221–4235.
[49] J. Shorter, The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system, PLoS One 6 (2011) e26319.
[50] X. Gao, M. Carroni, C. Nussbaum-Krammer, A. Mogk, N.B. Nillegoda, A. Szlachcic, et al., Human Hsp70 disaggregase reverses Parkinson's-linked alpha-synuclein amyloid fibrils, Mol. Cell 59 (2015) 781–793.
[51] N.B. Nillegoda, J. Kirstein, A. Szlachcic, M. Berynskyy, A. Stank, F. Stengel, et al., Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation, Nature 524 (2015) 247–251.
[52] G.A. Newby, S. Lindquist, Blessings in disguise: Biological benefits of prion-like mechanisms, Trends Cell Biol. 23 (2013) 251–259.
[53] S. Alberti, R. Halfmann, O. King, A. Kapila, S. Lindquist, A systematic survey identifies prions and illuminates sequence features of prionogenic proteins, Cell 137 (2009) 146–158.
[54] J.S. Byers, D.F. Jarosz, Pernicious pathogens or expedient elements of inheritance: The significance of yeast prions, PLoS Pathog. 10 (2014) e1003992.
[55] D.M. Garcia, D.F. Jarosz, Rebels with a cause: Molecular features and physiological consequences of yeast prions, FEMS Yeast Res. 14 (2014) 136–147.
[56] R. Halfmann, D.F. Jarosz, S.K. Jones, A. Chang, A.K. Lancaster, S. Lindquist, Prions are a common mechanism for phenotypic inheritance in wild yeasts, Nature 482 (2012) 363–368.
[57] J. Shorter, S. Lindquist, Prions as adaptive conduits of memory and inheritance, Nat. Rev. Genet. 6 (2005) 435–450.
[58] Y. Sanchez, S.L. Lindquist, HSP104 required for induced thermotolerance, Science 248 (1990) 1112–1115.

[59] Y. Sanchez, J. Taulien, K.A. Borkovich, S. Lindquist, Hsp104 is required for tolerance to many forms of stress, EMBO J. 11 (1992) 2357–2364.
[60] E.W.J. Wallace, J.L. Kear-Scott, E.V. Pilipenko, M.H. Schwartz, P.R. Laskowski, A.E. Rojek, et al., Reversible, specific, active aggregates of endogenous proteins assemble upon heat stress, Cell 162 (2015) 1286–1298.
[61] P. Tessarz, M. Schwarz, A. Mogk, B. Bukau, The yeast AAA+ chaperone Hsp104 is part of a network that links the actin cytoskeleton with the inheritance of damaged proteins, Mol. Cell. Biol. 29 (2009) 3738–3745.
[62] V. Andersson, S. Hanzen, B. Liu, M. Molin, T. Nystrom, Enhancing protein disaggregation restores proteasome activity in aged cells, Aging 5 (2013) 802–812.
[63] N. Erjavec, L. Larsson, J. Grantham, T. Nystrom, Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p, Genes Dev. 21 (2007) 2410–2421.
[64] S.M. Hill, X. Hao, B. Liu, T. Nystrom, Life-span extension by a metacaspase in the yeast *Saccharomyces cerevisiae*, Science 344 (2014) 1389–1392.
[65] B. Liu, L. Larsson, A. Caballero, X. Hao, D. Oling, J. Grantham, et al., The polarisome is required for segregation and retrograde transport of protein aggregates, Cell 140 (2010) 257–267.
[66] B. Liu, L. Larsson, V. Franssens, X. Hao, S.M. Hill, V. Andersson, et al., Segregation of protein aggregates involves actin and the polarity machinery, Cell 147 (2011) 959–961.
[67] C. Zhou, B.D. Slaughter, J.R. Unruh, A. Eldakak, B. Rubinstein, R. Li, Motility and segregation of Hsp104-associated protein aggregates in budding yeast, Cell 147 (2011) 1186–1196.
[68] R. Spokoini, O. Moldavski, Y. Nahmias, J.L. England, M. Schuldiner, D. Kaganovich, Confinement to organelle-associated inclusion structures mediates asymmetric inheritance of aggregated protein in budding yeast, Cell Rep. 2 (2012) 738–747.
[69] S. Pattabiraman, D. Kaganovich, Imperfect asymmetry: The mechanism governing asymmetric partitioning of damaged cellular components during mitosis, Bioarchitecture 4 (2014) 203–209.
[70] J. Saarikangas, Y. Barral, Protein aggregates are associated with replicative aging without compromising protein quality control, Elife (2015), http://dx.doi.org/10.7554/eLife.06197.
[71] M. Coelho, A. Dereli, A. Haese, S. Kuhn, L. Malinovska, M.E. DeSantis, et al., Fission yeast does not age under favorable conditions, but does so after stress, Curr. Biol. 23 (2013) 1844–1852.
[72] M.E. DeSantis, J. Shorter, The elusive middle domain of Hsp104 and ClpB: Location and function, Biochim. Biophys. Acta 2012 (1823) 29–39.
[73] V. Grimminger-Marquardt, H.A. Lashuel, Structure and function of the molecular chaperone Hsp104 from yeast, Biopolymers 93 (2010) 252–276.
[74] P.I. Hanson, S.W. Whiteheart, AAA+ proteins: Have engine, will work, Nat. Rev. Mol. Cell Biol. 6 (2005) 519–529.
[75] D.A. Parsell, A.S. Kowal, S. Lindquist, *Saccharomyces cerevisiae* Hsp104 protein. Purification and characterization of ATP-induced structural changes, J. Biol. Chem. 269 (1994) 4480–4487.
[76] E.C. Schirmer, C. Queitsch, A.S. Kowal, D.A. Parsell, S. Lindquist, The ATPase activity of Hsp104, effects of environmental conditions and mutations, J. Biol. Chem. 273 (1998) 15546–15552.
[77] E.C. Schirmer, D.M. Ware, C. Queitsch, A.S. Kowal, S.L. Lindquist, Subunit interactions influence the biochemical and biological properties of Hsp104, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 914–919.
[78] D.A. Parsell, Y. Sanchez, J.D. Stitzel, S. Lindquist, Hsp104 is a highly conserved protein with two essential nucleotide-binding sites, Nature 353 (1991) 270–273.
[79] M. Carroni, E. Kummer, Y. Oguchi, P. Wendler, D.K. Clare, I. Sinning, et al., Head-to-tail interactions of the coiled-coil domains regulate ClpB activity and cooperation with Hsp70 in protein disaggregation, Elife 3 (2014) e02481.
[80] S. Lee, B. Sielaff, J. Lee, F.T. Tsai, CryoEM structure of Hsp104 and its mechanistic implication for protein disaggregation, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 8135–8140.
[81] P. Wendler, J. Shorter, C. Plisson, A.G. Cashikar, S. Lindquist, H.R. Saibil, Atypical AAA+ subunit packing creates an expanded cavity for disaggregation by the protein-remodeling factor Hsp104, Cell 131 (2007) 1366–1377.
[82] P. Wendler, J. Shorter, D. Snead, C. Plisson, D.K. Clare, S. Lindquist, et al., Motor mechanism for protein threading through Hsp104, Mol. Cell 34 (2009) 81–92.
[83] S. Lee, M.E. Sowa, Y.H. Watanabe, P.B. Sigler, W. Chiu, M. Yoshida, et al., The structure of ClpB: A molecular chaperone that rescues proteins from an aggregated state, Cell 115 (2003) 229–240.
[84] M.E. DeSantis, E.A. Sweeny, D. Snead, E.H. Leung, M.S. Go, K. Gupta, et al., Conserved distal loop residues in the Hsp104 and ClpB middle domain contact nucleotide-binding domain 2 and enable Hsp70-dependent protein disaggregation, J. Biol. Chem. 289 (2014) 848–867.
[85] D. Xia, L. Esser, S.K. Singh, F. Guo, M.R. Maurizi, Crystallographic investigation of peptide binding sites in the N-domain of the ClpA chaperone, J. Struct. Biol. 146 (2004) 166–179.
[86] J. Li, B. Sha, Crystal structure of the *E. coli* Hsp100 ClpB N-terminal domain, Structure 11 (2003) 323–328.
[87] A. Pietrosiuk, E.D. Lenherr, S. Falk, G. Bonemann, J. Kopp, H. Zentgraf, et al., Molecular basis for the unique role of the AAA+ chaperone ClpV in type VI protein secretion, J. Biol. Chem. 286 (2011) 30010–30021.
[88] F. Wang, Z. Mei, Y. Qi, C. Yan, Q. Hu, J. Wang, et al., Structure and mechanism of the hexameric MecA-ClpC molecular machine, Nature 471 (2011) 331–335.
[89] J.H. Lo, T.A. Baker, R.T. Sauer, Characterization of the N-terminal repeat domain of *Escherichia coli* ClpA-A class I Clp/HSP100 ATPase, Protein Sci. 10 (2001) 551–559.
[90] A. Mogk, C. Schlieker, C. Strub, W. Rist, J. Weibezahn, B. Bukau, Roles of individual domains and conserved motifs of the AAA+ chaperone ClpB in oligomerization, ATP hydrolysis, and chaperone activity, J. Biol. Chem. 278 (2003) 17615–17624.
[91] T. Ishikawa, M.R. Maurizi, A.C. Steven, The N-terminal substrate-binding domain of ClpA unfoldase is highly mobile and extends axially from the distal surface of ClpAP protease, J. Struct. Biol. 146 (2004) 180–188.
[92] S. Lee, J.M. Choi, F.T. Tsai, Visualizing the ATPase cycle in a protein disaggregating machine: Structural basis for substrate binding by ClpB, Mol. Cell 25 (2007) 261–271.

[93] S.K. Park, K.I. Kim, K.M. Woo, J.H. Seol, K. Tanaka, A. Ichihara, et al., Site-directed mutagenesis of the dual translational initiation sites of the clpB gene of *Escherichia coli* and characterization of its gene products, J. Biol. Chem. 268 (1993) 20170–20174.
[94] P. Beinker, S. Schlee, Y. Groemping, R. Seidel, J. Reinstein, The N terminus of ClpB from *Thermus thermophilus* is not essential for the chaperone activity, J. Biol. Chem. 277 (2002) 47160–47166.
[95] M.E. Barnett, M. Nagy, S. Kedzierska, M. Zolkiewski, The amino-terminal domain of ClpB supports binding to strongly aggregated proteins, J. Biol. Chem. 280 (2005) 34940–34945.
[96] N. Tanaka, Y. Tani, H. Hattori, T. Tada, S. Kunugi, Interaction of the N-terminal domain of *Escherichia coli* heat-shock protein ClpB and protein aggregates during chaperone activity, Protein Sci. 13 (2004) 3214–3221.
[97] S.M. Doyle, J.R. Hoskins, S. Wickner, DnaK chaperone-dependent disaggregation by caseinolytic peptidase B (ClpB) mutants reveals functional overlap in the N-terminal domain and nucleotide-binding domain-1 pore tyrosine, J. Biol. Chem. 287 (2012) 28470–28479.
[98] S. Mizuno, Y. Nakazaki, M. Yoshida, Y.H. Watanabe, Orientation of the amino-terminal domain of ClpB affects the disaggregation of the protein, FEBS J. 279 (2012) 1474–1484.
[99] T. Zhang, E.A. Ploetz, M. Nagy, S.M. Doyle, S. Wickner, P.E. Smith, et al., Flexible connection of the N-terminal domain in ClpB modulates substrate binding and the aggregate reactivation efficiency, Proteins 80 (2012) 2758–2768.
[100] G.C. Hung, D.C. Masison, N-terminal domain of yeast Hsp104 chaperone is dispensable for thermotolerance and prion propagation but necessary for curing prions by Hsp104 overexpression, Genetics 173 (2006) 611–620.
[101] S.M. Doyle, J. Shorter, M. Zolkiewski, J.R. Hoskins, S. Lindquist, S. Wickner, Asymmetric deceleration of ClpB or Hsp104 ATPase activity unleashes protein-remodeling activity, Nat. Struct. Mol. Biol. 14 (2007) 114–122.
[102] L.M. Iyer, D.D. Leipe, E.V. Koonin, L. Aravind, Evolutionary history and higher order classification of AAA+ ATPases, J. Struct. Biol. 146 (2004) 11–31.
[103] J.P. Erzberger, J.M. Berger, Evolutionary relationships and structural mechanisms of AAA+ proteins, Annu. Rev. Biophys. Biomol. Struct. 35 (2006) 93–114.
[104] D.A. Hattendorf, S.L. Lindquist, Cooperative kinetics of both Hsp104 ATPase domains and interdomain communication revealed by AAA sensor-1 mutants, EMBO J. 21 (2002) 12–21.
[105] J. Krzewska, G. Konopa, K. Liberek, Importance of two ATP-binding sites for oligomerization, ATPase activity and chaperone function of mitochondrial Hsp78 protein, J. Mol. Biol. 314 (2001) 901–910.
[106] J.M. Tkach, J.R. Glover, Nucleocytoplasmic trafficking of the molecular chaperone Hsp104 in unstressed and heat-shocked cells, Traffic 9 (2008) 39–56.
[107] T. Haslberger, J. Weibezahn, R. Zahn, S. Lee, F.T. Tsai, B. Bukau, et al., M domains couple the ClpB threading motor with the DnaK chaperone activity, Mol. Cell 25 (2007) 247–260.
[108] B. Sielaff, F.T. Tsai, The M-domain controls Hsp104 protein remodeling activity in an Hsp70/Hsp40-dependent manner, J. Mol. Biol. 402 (2010) 30–37.
[109] M.E. Jackrel, K. Yee, A. Tariq, A.I. Chen, J. Shorter, Disparate mutations confer therapeutic gain of Hsp104 function, ACS Chem. Biol. (2015), http://dx.doi.org/10.1021/acschembio.5b00765.
[110] M. Miot, M. Reidy, S.M. Doyle, J.R. Hoskins, D.M. Johnston, O. Genest, et al., Species-specific collaboration of heat shock proteins (Hsp) 70 and 100 in thermotolerance and protein disaggregation, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 6915–6920.
[111] F. Seyffer, E. Kummer, Y. Oguchi, J. Winkler, M. Kumar, R. Zahn, et al., Hsp70 proteins bind Hsp100 regulatory M domains to activate AAA+ disaggregase at aggregate surfaces, Nat. Struct. Mol. Biol. 19 (2012) 1347–1355.
[112] J. Lee, J.H. Kim, A.B. Biter, B. Sielaff, S. Lee, F.T. Tsai, Heat shock protein (Hsp) 70 is an activator of the Hsp104 motor, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 8513–8518.
[113] Y. Oguchi, E. Kummer, F. Seyffer, M. Berynskyy, B. Anstett, R. Zahn, et al., A tightly regulated molecular toggle controls AAA+ disaggregase, Nat. Struct. Mol. Biol. 19 (2012) 1338–1346.
[114] J.E. Dulle, K.C. Stein, H.L. True, Regulation of the hsp104 middle domain activity is critical for yeast prion propagation, PLoS One 9 (2014) e87521.
[115] R.G. Mackay, C.W. Helsen, J.M. Tkach, J.R. Glover, The C-terminal extension of *Saccharomyces cerevisiae* Hsp104 plays a role in oligomer assembly, Biochemistry 47 (2008) 1918–1927.
[116] T. Abbas-Terki, O. Donze, P.A. Briand, D. Picard, Hsp104 interacts with Hsp90 cochaperones in respiring yeast, Mol. Cell. Biol. 21 (2001) 7569–7575.
[117] A.G. Cashikar, E.C. Schirmer, D.A. Hattendorf, J.R. Glover, M.S. Ramakrishnan, D.M. Ware, et al., Defining a pathway of communication from the C-terminal peptide binding domain to the N-terminal ATPase domain in a AAA protein, Mol. Cell 9 (2002) 751–760.
[118] J.M. Tkach, J.R. Glover, Amino acid substitutions in the C-terminal AAA+ module of Hsp104 prevent substrate recognition by disrupting oligomerization and cause high temperature inactivation, J. Biol. Chem. 279 (2004) 35692–35701.
[119] H. Saibil, Chaperone machines for protein folding, unfolding and disaggregation, Nat. Rev. Mol. Cell Biol. 14 (2013) 630–642.
[120] A. Mogk, E. Kummer, B. Bukau, Cooperation of Hsp70 and Hsp100 chaperone machines in protein disaggregation, Front. Mol. Biosci. 2 (2015) 22.
[121] T. Li, C.L. Weaver, J. Lin, E.C. Duran, J.M. Miller, A.L. Lucius, *Escherichia coli* ClpB is a non-processive polypeptide translocase, Biochem. J. 470 (2015) 39–52.
[122] M. Reidy, M. Miot, D.C. Masison, Prokaryotic chaperones support yeast prions and thermotolerance and define disaggregation machinery interactions, Genetics 192 (2012) 185–193.
[123] M. Reidy, R. Sharma, S. Shastry, B.L. Roberts, I. Albino-Flores, S. Wickner, et al., Hsp40s specify functions of Hsp104 and Hsp90 protein chaperone machines, PLoS Genet. 10 (2014) e1004720.
[124] K.A. Tipton, K.J. Verges, J.S. Weissman, *In vivo* monitoring of the prion replication cycle reveals a critical role for Sis1 in delivering substrates to Hsp104, Mol. Cell 32 (2008) 584–591.
[125] A.H. Yuan, S.J. Garrity, E. Nako, A. Hochschild, Prion propagation can occur in a prokaryote and requires the ClpB chaperone, Elife 3 (2014) e02949.
[126] F. Gasset-Rosa, A.S. Coquel, M. Moreno-Del Alamo, P. Chen, X. Song, A.M. Serrano, et al., Direct assessment in bacteria of prionoid propagation and phenotype selection by Hsp70 chaperone, Mol. Microbiol. 91 (2014) 1070–1087.

[127] V.V. Kushnirov, M.D. Ter-Avanesyan, Structure and replication of yeast prions, Cell 94 (1998) 13–16.
[128] P. Satpute-Krishnan, S.X. Langseth, T.R. Serio, Hsp104-dependent remodeling of prion complexes mediates protein-only inheritance, PLoS Biol. 5 (2007) e24.
[129] J.E. Dulle, R.E. Bouttenot, L.A. Underwood, H.L. True, Soluble oligomers are sufficient for transmission of a yeast prion but do not confer phenotype, J. Cell Biol. 203 (2013) 197–204.
[130] J.E. Dulle, H.L. True, Low activity of select Hsp104 mutants is sufficient to propagate unstable prion variants, Prion 7 (2013) 394–403.
[131] P. Wendler, H.R. Saibil, Cryo electron microscopy structures of Hsp100 proteins: Crowbars in or out? Biochem. Cell Biol. 88 (2010) 89–96.
[132] B. Chen, T.A. Sysoeva, S. Chowdhury, L. Guo, S. De Carlo, J.A. Hanson, et al., Engagement of arginine finger to ATP triggers large conformational changes in NtrC1 AAA+ ATPase for remodeling bacterial RNA polymerase, Structure 18 (2010) 1420–1430.
[133] J.M. Davies, H. Tsuruta, A.P. May, W.I. Weis, Conformational changes of p97 during nucleotide hydrolysis determined by small-angle x-ray scattering, Structure 13 (2005) 183–195.
[134] A. Roll-Mecak, R.D. Vale, Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin, Nature 451 (2008) 363–367.
[135] T.A. Sysoeva, S. Chowdhury, L. Guo, B.T. Nixon, Nucleotide-induced asymmetry within ATPase activator ring drives sigma54-RNAP interaction and ATP hydrolysis, Genes Dev. 27 (2013) 2500–2511.
[136] E. Arias-Palomo, V.L. O'Shea, I.V. Hood, J.M. Berger, The bacterial DnaC helicase loader is a DnaB ring breaker, Cell 153 (2013) 438–448.
[137] A.S. Meyer, J.R. Gillespie, D. Walther, I.S. Millet, S. Doniach, J. Frydman, Closing the folding chamber of the eukaryotic chaperonin requires the transition state of ATP hydrolysis, Cell 113 (2003) 369–381.
[138] B. Nagar, J. Kuriyan, SAXS and the working protein, Structure 13 (2005) 169–170.
[139] R. Krishnan, S.L. Lindquist, Structural insights into a yeast prion illuminate nucleation and strain diversity, Nature 435 (2005) 765–772.
[140] P.M. Tessier, S. Lindquist, Prion recognition elements govern nucleation, strain specificity and species barriers, Nature 447 (2007) 556–561.
[141] P.M. Tessier, S. Lindquist, Unraveling infectious structures, strain variants and species barriers for the yeast prion [*PSI*$^{+}$], Nat. Struct. Mol. Biol. 16 (2009) 598–605.
[142] H. Wang, M.L. Duennwald, B.E. Roberts, L.M. Rozeboom, Y.L. Zhang, A.D. Steele, et al., Direct and selective elimination of specific prions and amyloids by 4,5-dianilinophthalimide and analogs, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 7159–7164.
[143] B.E. Roberts, M.L. Duennwald, H. Wang, C. Chung, N.P. Lopreiato, E.A. Sweeny, et al., A synergistic small-molecule combination directly eradicates diverse prion strain structures, Nat. Chem. Biol. 5 (2009) 936–946.
[144] J. Shorter, Emergence and natural selection of drug-resistant prions, Mol. BioSyst. 6 (2010) 1115–1130.
[145] M.E. DeSantis, J. Shorter, Hsp104 drives "protein-only" positive selection of Sup35 prion strains encoding strong [PSI(+)], Chem. Biol. 19 (2012) 1400–1410.
[146] J. Dong, C.E. Castro, M.C. Boyce, M.J. Lang, S. Lindquist, Optical trapping with high forces reveals unexpected behaviors of prion fibrils, Nat. Struct. Mol. Biol. 17 (2010) 1422–1430.
[147] C.W. Helsen, J.R. Glover, Insight into molecular basis of curing of [*PSI*$^{+}$] prion by overexpression of 104-kDa heat shock protein (Hsp104), J. Biol. Chem. 287 (2012) 542–556.
[148] S.V. Paushkin, V.V. Kushnirov, V.N. Smirnov, M.D. Ter-Avanesyan, Propagation of the yeast prion-like [psi+] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor, EMBO J. 15 (1996) 3127–3134.
[149] Y.O. Chernoff, S.L. Lindquist, B. Ono, S.G. Inge-Vechtomov, S.W. Liebman, Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+], Science 268 (1995) 880–884.
[150] J. O'Driscoll, D. Clare, H. Saibil, Prion aggregate structure in yeast cells is determined by the Hsp104-Hsp110 disaggregase machinery, J. Cell Biol. 211 (2015) 145–158.
[151] C.W. Helsen, J.R. Glover, A new perspective on Hsp104-mediated propagation and curing of the yeast prion [PSI (+)], Prion 6 (2012) 234–239.
[152] D.S. Kryndushkin, I.M. Alexandrov, M.D. Ter-Avanesyan, V.V. Kushnirov, Yeast [*PSI*$^{+}$] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104, J. Biol. Chem. 278 (2003) 49636–49643.
[153] J. Winkler, J. Tyedmers, B. Bukau, A. Mogk, Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation, J. Cell Biol. 198 (2012) 387–404.
[154] M. Reidy, D.C. Masison, Yeast prions help identify and define chaperone interaction networks, Curr. Pharm. Biotechnol. 15 (2014) 1008–1018.
[155] M.L. Wohlever, T.A. Baker, R.T. Sauer, Roles of the N domain of the AAA+ Lon protease in substrate recognition, allosteric regulation and chaperone activity, Mol. Microbiol. 91 (2014) 66–78.
[156] R.T. Sauer, T.A. Baker, AAA+ proteases: ATP-fueled machines of protein destruction, Annu. Rev. Biochem. 80 (2011) 587–612.
[157] J. Hinnerwisch, B.G. Reid, W.A. Fenton, A.L. Horwich, Roles of the N-domains of the ClpA unfoldase in binding substrate proteins and in stable complex formation with the ClpP protease, J. Biol. Chem. 280 (2005) 40838–40844.
[158] S. Cranz-Mileva, F. Imkamp, K. Kolygo, Z. Maglica, W. Kress, E. Weber-Ban, The flexible attachment of the N-domains to the ClpA ring body allows their use on demand, J. Mol. Biol. 378 (2008) 412–424.
[159] G. Thibault, W.A. Houry, Role of the N-terminal domain of the chaperone ClpX in the recognition and degradation of lambda phage protein O, J. Phys. Chem. B 116 (2012) 6717–6724.
[160] D. Barthelme, J.Z. Chen, J. Grabenstatter, T.A. Baker, R.T. Sauer, Architecture and assembly of the archaeal Cdc48*20S proteasome, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) E1687–E1694.
[161] F. Zhang, Z. Wu, P. Zhang, G. Tian, D. Finley, Y. Shi, Mechanism of substrate unfolding and translocation by the regulatory particle of the proteasome from *Methanocaldococcus jannaschii*, Mol. Cell 34 (2009) 485–496.
[162] A. Gerega, B. Rockel, J. Peters, T. Tamura, W. Baumeister, P. Zwickl, VAT, the thermoplasma homolog of mammalian p97/VCP, is an N domain-regulated protein unfoldase, J. Biol. Chem. 280 (2005) 42856–42862.
[163] A. Rothballer, N. Tzvetkov, P. Zwickl, Mutations in p97/VCP induce unfolding activity, FEBS Lett. 581 (2007) 1197–1201.

[164] D.I. Svergun, M.V. Petoukhov, M.H. Koch, Determination of domain structure of proteins from x-ray solution scattering, Biophys. J. 80 (2001) 2946–2953.
[165] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, et al., UCSF Chimera—A visualization system for exploratory research and analysis, J. Comput. Chem. 25 (2004) 1605–1612.
[166] R. Rosenzweig, P. Farber, A. Velyvis, E. Rennella, M.P. Latham, L.E. Kay, ClpB N-terminal domain plays a regulatory role in protein disaggregation, Proc. Natl. Acad. Sci. U. S. A. (2015), http://dx.doi.org/10.1073/pnas.1512783112.
[167] R. Rosenzweig, S. Morad, A. Zarrine-Afsar, J.R. Glover, L.E. Kay, Unraveling the mechanism of protein disaggregation through a ClpB-DnaK interaction, Science 339 (2013) 1080–1083.