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Designer protein disaggregases to counter neurodegenerative disease

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Protein misfolding and aggregation unify several devastating neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. There are no effective therapeutics for these disorders and none that target the reversal of the aberrant protein misfolding and aggregation that cause disease. Here, I showcase important advances to define, engineer, and apply protein disaggregases to mitigate deleterious protein misfolding and counter neurodegeneration. I focus on two exogenous protein disaggregases, Hsp104 from yeast and gene 3 protein from bacteriophages, as well as endogenous human protein disaggregases, including: (a) Hsp110, Hsp70, Hsp40, and small heat-shock proteins; (b) HtrA1; and (c) NMNAT2 and Hsp90. I suggest that protein-disaggregase modalities can be channeled to treat numerous fatal and presently incurable neurodegenerative diseases.

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Introduction

Deleterious protein misfolding and aggregation underpin several invariably fatal and age-related neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [1]. Typically, in each disease specific proteins misfold, aggregate, and wreak havoc on the nervous system [1]. In AD, amyloid-β (Aβ) peptides form extracellular, neuritic plaques and tau forms intracellular neurofibrillary tangles in afflicted neurons [1]. By contrast, in PD, α-synuclein (α-syn) forms cytoplasmic Lewy bodies in degenerating dopaminergic neurons [1]. In most ALS cases, RNA-binding proteins with prion-like domains, such as TDP-43 or FUS, mislocalize from the nucleus to cytoplasmic aggregates in degenerating motor neurons and glia [1,2]. Current treatments for these disorders are palliative and ineffective. No therapeutics exist that reverse the aberrant protein misfolding and aggregation that underlie disease. The lack of effective therapies is a cause of immense angst as these diseases are increasing in prevalence as our population ages.

Complexity of protein misfolding

Protein misfolding is a complex, multistate process [3,4]. The specific proteins that misfold in neurodegenerative disease are often intrinsically disordered, harbor an intrinsically disordered domain, or passage through partially unfolded states that enables them to morph into an eclectic menagerie of misfolded structures with variable toxicities [1–5]. These structures include self-templating amyloid fibrils with cross-β architecture, disordered aggregates, and small soluble oligomers [1,3]. For example, in PD, a small intrinsically-disordered protein, α-syn, forms amyloid fibrils that self-template or 'seed' their own assembly via recruiting soluble forms of α-syn to their elongating ends where α-syn is conformationally converted to the cross-β structure [1,6–8,9•,10•]. α-Syn amyloid can spread from cell to cell, thereby propagating pathology [1,6,8,11,12••,13,14]. Indeed, amyloid fibrils formed by recombinant α-syn in the test tube can induce a PD-like disease when injected into the brain of a mouse [6,11,13]. This transforming principle establishes that the self-replicating structure of α-syn amyloid can encode the PD phenotype, which develops via the ongoing conversion of endogenous α-syn to the amyloid state as α-syn fibrils spread through the brain [1,8,13,15]. Moreover, α-syn can form fibrils with different cross-β structures, termed 'strains', which encode distinct neurodegenerative phenotypes [9•,10•,16,17•,18,19]. The lateral face of α-syn amyloid provides a surface where α-syn oligomers can nucleate [20]. α-Syn populates diverse soluble, oligomeric species before, during, and after α-syn amyloidogenesis, which can be on or off pathway for amyloid formation [7,21–25,26•]. α-Syn oligomers are typically more toxic than mature fibrils [7,23]. Small soluble oligomers or short, fragmented amyloid fibrils are more toxic than very large aggregated species, which due to their low surface-area-to-volume-ratio shield damaging surfaces inside the aggregate [7,23,27]. A major challenge for any therapeutic aimed at mitigating protein misfolding is the ability to remodel diverse, toxic misfolded conformers, including soluble oligomers and amyloid fibrils into benign species [28,29].

Protein disaggregases as potential therapeutics

I have postulated that protein disaggregases could be uniquely suited to meet this challenge as they can safely deconstruct self-templating amyloid and toxic soluble oligomers, and recover soluble protein with restored functionality from these structures [28,29]. Thus, protein disaggregases could mitigate any toxic gain-of-function or toxic loss-of-function connected with protein misfolding, and simultaneously could eradicate self-templating species that propagate disease [28,29]. Protein disaggregation might also be coupled to protein degradation, which could also be beneficial to eliminate toxic and self-templating conformers, and subsequent translation of new protein could antagonize any toxic loss-of-function [29]. However, protein disaggregases remain among the least understood components of the proteostasis network, and we are only at the inception of realizing their existence and potential [28,29]. Here, I highlight recent advances to define, engineer, and apply protein disaggregases to reverse deleterious protein misfolding in neurodegenerative disease.

Hsp104, a protein disaggregase from yeast

Hsp104 is an asymmetric ring-shaped translocase and hexameric AAA+ protein found in yeast [30,31••]. Hsp104 couples ATP hydrolysis to the rapid dissolution and reactivation of diverse proteins trapped in disordered aggregates, ordered stress-induced assemblies, preamyloid oligomers, amyloids, and prions [32–37,38••]. Optimal Hsp104 disaggregase activity can require collaboration with the Hsp70 chaperone system [30]. In yeast, Hsp104 performs critical functions in stress tolerance, prion inheritance, asymmetric partitioning of aggregates during cell division, and promoting longevity [30]. Hsp104 rapidly disaggregates Sup35 prions within a few minutes [35–37,39]. Moreover, Hsp104 effectively dissolves amyloids formed by diverse human degenerative disease proteins, including: Aβ42, tau, polyglutamine, α-syn, prion protein (PrP), and amylin [34,40–42]. Hsp104 also rapidly remodels amyloid fibrils formed by fragments of prostatic acid phosphatase (PAP248-286 and PAP85-120) [43••], which are abundant in human seminal fluid and promote HIV infection [44]. This rapid and broad-spectrum amyloid-disaggregase activity of Hsp104 is unusual and might represent a therapeutic opportunity [28].

Intriguingly, Hsp104 is absent from metazoa, but is found in all non-metazoan eukaryotes, all eubacteria, and some archaebacteria [45]. Thus, Hsp104 could be developed into a vital disruptive technology that retools proteostasis to combat neurodegenerative disease and HIV infection [28,43••,46]. Indeed, we have established Hsp104 as the only factor known to dissociate α-syn oligomers and amyloids connected with PD *and* rescue α-syn-induced neurodegeneration in the substantia nigra of a rat PD model [34,40,41]. Moreover, Hsp104 rescues polyglutamine toxicity and neurodegeneration in *Caenorhabditis elegans*, fly, mouse, and rat [28,47]. Hsp104 even rescues polyglutamine toxicity after degeneration has begun [47]. Hsp104 expression is not detrimental in metazoa and can be broadly and safely expressed in worm, fly, mouse, and rat, as well as in mammalian cell and neuronal cultures [28,34,47]. These findings make it difficult to understand why Hsp104 was lost from metazoa, but also emphasize that Hsp104 might be safely introduced and developed as a therapeutic agent [28,43••,46].

Despite these encouraging activities, very high Hsp104 concentrations are needed for optimal disaggregation of human disease proteins, such as α-syn, which may restrict efficacy [34,40,41]. Thus, we have engineered potentiated Hsp104 variants, which rescue aggregation and toxicity of proteins associated with neurodegenerative disease such as TDP-43, FUS, TAF15, and α-syn, and mitigate neurodegeneration in the metazoan nervous system at concentrations where Hsp104 is ineffective [46,48••,49,50,51••,52]. Hsp104 activity can be potentiated by single missense mutations at specific positions in the middle domain or nucleotide-binding domain 1 of Hsp104 [46]. Potentiating mutations reconfigure how Hsp104 subunits collaborate, alter substrate discrimination, alleviate any stringent requirements for Hsp70, and enhance Hsp104's ATPase, translocase (rate at which substrates are translocated across the central channel of Hsp104), unfoldase, and disaggregase activity [48••,49,50]. These combined properties enable potentiated Hsp104 variants to outperform Hsp104 under conditions where an aggregation-prone protein such as TDP-43, FUS, or α-syn has exceeded proteostatic buffers and is undergoing widespread misfolding and aggregation [46]. Potentiated Hsp104 variants can have off-target effects [48••,49], and may require further engineering to minimize these via increasing substrate selectivity [46]. Importantly, substoichiometric concentrations of potentiated Hsp104 variants can remodel amyloid [43••]. For example, nanomolar concentrations of an enhanced Hsp104 variant, $Hsp104^{A503V}$, can remodel micromolar concentrations of PAP248-296 sequestered in SEVI fibrils [43••]. The challenge ahead is to determine whether Hsp104 or enhanced variants can confer increased therapeutic benefits in mammalian cells, patient-derived neurons, and additional animal models of neurodegeneration.

An issue that is often raised about introducing any exogenous protein as a therapeutic is whether the patient might mount a deleterious immune response against the therapeutic protein. However, it is important to note that the central nervous system (CNS) exhibits immune privilege [53]. Thus, immune responses to CNS antigens are very slow to develop [53], which could provide a therapeutic window for exogenous agents, such as Hsp104 [29], tetanus and botulinum toxin variants [54], or even