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Specification of Physiologic and Disease States by Distinct Proteins and Protein Conformations

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Protein conformational states—from intrinsically disordered ensembles to amyloids that underlie the self-templating, infectious properties of prion-like proteins—have attracted much attention. Here, we highlight the diversity, including differences in biophysical properties, that drive distinct biological functions and pathologies among self-templating proteins. Advances in chemical genomics, gene editing, and model systems now permit deconstruction of the complex interplay between these protein states and the host factors that react to them. These methods reveal that conformational switches modulate normal and abnormal information transfer and that intimate relationships exist between the intrinsic function of proteins and the deleterious consequences of their misfolding.

On both the macroscopic and microscopic scale, form is indelibly linked to function. Proteins are synthesized as linear chains of amino acids and need to fold into precise three-dimensional architectures to perform their specific biological functions (Figure 1). This process impinges upon virtually every biological phenotype. Native folds are often only marginally stable (Fersht, 1998) and must be achieved in a crowded intracellular milieu (Ellis and Hartl, 1999). Such precarious folding landscapes pose a fundamental problem for phenotypic stability. Many proteins thus achieve their active conformations with the help of other proteins, called molecular chaperones (Kim et al., 2013; Lindquist and Craig, 1988). Although some proteins assume relatively static structures, others adopt multiple conformations to exert their functions and ensuing biological phenotypes. Examples include kinases, which oscillate between closed (inactive) and open (active) states (Morgan, 1996), and steroid hormone receptors, which are held in an inactive form by the heat shock protein (Hsp)90 chaperone until an activating ligand binds and reorders the protein, releasing it from the chaperone (Howard et al., 1990; Nathan and Lindquist, 1995; Pratt et al., 2006).

Many proteins also assemble into one or more higher-order conformational states. Perhaps none are so striking as amyloid—a highly ordered cross-beta-sheet fibrillary structure. This Review begins by considering a set of oligomeric and aggregating proteins—many of them amyloids—with a remarkable folding landscape (Figure 1) that enables them to exist in multiple conformations, at least one of which self-templates (Glover et al., 1997; Patino et al., 1996; Prusiner, 1982; Shorter and Lindquist, 2005; Wickner, 1994). These protein conformations can thus be considered "infectious" and are known as prions. Prions can spread through a cell, across a tissue, and even throughout an entire organism. In the most striking cases, conformational conversion can even be transmitted from one individual to another (Aguzzi and Calella, 2009; Griffith, 1967; Prusiner, 1982; Prusiner et al., 1983; Watts et al., 2006).

The prion concept was initially conceived to explain a spongiform encephalopathy with a baffling pattern of transmission (Prusiner, 1982). Our understanding of prions' importance in biology has exploded in the past three decades (Aguzzi and Calella, 2009; Byers and Jarosz, 2014; Prusiner, 1982; Saupe, 2007; Shorter and Lindquist, 2005; Wickner et al., 2007). Distinct higher-order conformations of the same protein can be tied to distinct protein functions and disease phenotypes. This concept, originally articulated in the context of prion "strains" (Carlson et al., 1989; Tanaka et al., 2004; Toyama et al., 2007), is now emerging as a general principle.

Higher-order protein conformers and amyloids participate in an astonishing array of cellular functions and pathologies, from driving memory loss in the most common devastating neurodegenerative diseases (Laurén et al., 2009; Walsh et al., 2002) to promoting long-term memory formation at the synapse (Si et al., 2003; Fioriti et al., 2015; Majumdar et al., 2012). Many reviews have covered principles of protein folding, misfolding and aggregation, and associated cellular responses (e.g., Dobson, 2003; Labbadia and Morimoto, 2015; Selkoe, 2003; Walker and Jucker, 2015; Wang and Kaufman, 2016; Wolff et al., 2014). Here, we focus on the ways in which individual (mis)folded proteins and their different folding states dictate distinct cellular function and pathology, instances in which this form of

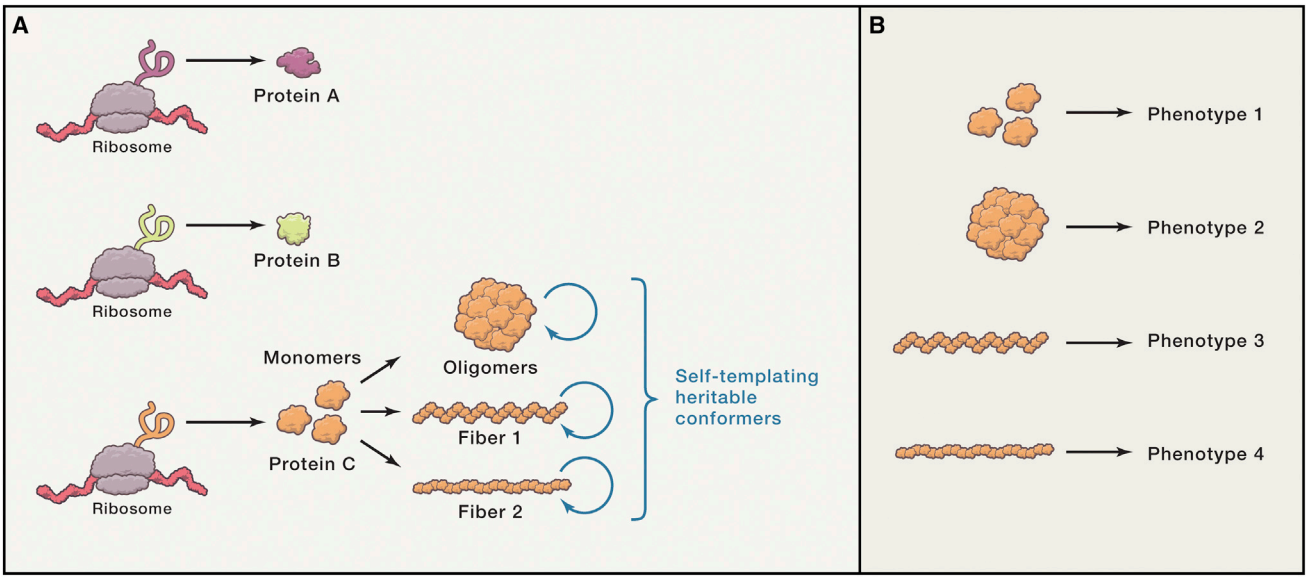

Figure 1. Relationship between Protein Conformation and Phenotype

(A) Many proteins (e.g., proteins A and B in this schematic) fold into a single native fold after they are synthesized by the ribosome. These protein sequences thus drive a single biological function and phenotype. Prion-like proteins (e.g., protein C) can adopt multiple conformations. Some such conformations (oligomers or fibers) are self-templating and are thus heritable.

(B) These different conformers of the same protein can each be associated with unique phenotypes.

information transfer occurs in nature, and the principles by which it operates.

Prion versus Prionoid and Transcellular Spread versus "Infectivity"

The protein-folding field abounds with confusing terms, often used interchangeably. Some clarification is in order (Table 1), particularly the term "prion" itself. The original definition of the term "prion" is sufficiently broad to encompass many different biochemical mechanisms. However, in practice, this definition is often limited to proteins that adopt an amyloid conformation, because prion protein (PrP) and many other prions adopt this structure. Likewise, infection between individuals (and even between organisms, subject to species barriers)—as can be seen for some PrP^{Sc}-driven diseases (Aguzzi and Calella, 2009; Gajdusek and Zigas, 1957; Prusiner, 1982) and fungal prions (Chernoff et al., 1995; Patino et al., 1996; Wickner, 1994)—is an important defining characteristic. Some have used terms like "prionoid" or "prion-like" to describe self-templating proteins that fall slightly short of these goalposts. The distinctions become fuzzier when applied to both single-celled and multicellular organisms. Here, we describe proteins as prion-like if they have the intrinsic capacity to self-template.

A classification of self-propagating proteins that may be helpful is presented in Table 1. Bona fide prions—in particular, certain intrinsically disordered proteins and amyloids—are both proteinaceous and infectious, with conformational conversion that spreads between cells and individuals and even across generations. In single-celled organisms, this can occur during mating. In contrast, type I prionoids spread between cells, but not between individuals. Because expression of the prion protein is required to propagate the infectious conformation, transmission cannot occur if the recipient tissue does not express the prion protein. Such protein conformers could in principle be infectious but are not in their endogenous biological context. Finally, type II prionoids self-template but are confined to the cell in which they arise. Examples include CPEB/ORB2 in animals (Khan et al., 2015; Majumdar et al., 2012; Si et al., 2010; Si et al., 2003) and the Whi3 "mnemon" in fungi (Caudron and Barral, 2013). As with any classification, there are shades of gray. Some prionoids spread from cell to cell but in a relatively limited way. These include the innate immunity protein MAVS (mitochondrial antiviral signaling) (Hou et al., 2011) and mutants of the p53 tumor suppressor (Soragni et al., 2016), although the nature of this biochemistry has been debated (Wang and Fersht, 2015).

How do prions and type I prionoids spread transcellularly? Many potential mechanisms have been described (Figure 2). The field is nascent, and it remains unclear how mechanisms described in cultured cellular systems or model organisms with specific proteins relate to operational mechanisms that might occur *in vivo* or in the context of human disease.

From Fungi to Metazoa: Protein Conformational States as Conduits of Information Transfer

The budding yeast *Saccharomyces cerevisiae* has been critically important for our understanding of prion biology. The prion hypothesis was first advanced in yeast to explain two phenotypes with unusual patterns of inheritance: [*URE3*] (Aigle and Lacroute, 1975; Lacroute, 1971; Wickner, 1994) and [PSI^+] (Chernoff et al., 1995; Cox et al., 1980; Patino et al., 1996; Wickner, 1994). Two features distinguished these traits from those encoded in the genome. First, they could be permanently eliminated when cells were transiently passaged on medium containing low

Table 1. Definitions of Terms Describing Protein-Folding Features and Consequences

Protein Conformers
Monomer: a single protein molecule; may bind to other proteins to create a polymer
Oligomer: a protein complex made of two or more subunits, often referring to ensembles with a range of stoichiometries
Multimer: interchangeable with oligomer although often associated with a defined physiologically relevant stoichiometry
Amyloid: a fibrous protein quaternary structure with a cross-beta fold, often used interchangeably with "amyloid fibril"
Amyloidogenic: proteins or protein sequences that produce or tend to produce amyloid deposits
Native state: a protein's properly folded, operational, and functional fold
Intrinsically disordered region: protein regions that do not adopt a single stable tertiary structure
Intrinsically disordered protein: a protein that is largely or entirely composed of intrinsically disordered regions
Prion and Prion-Like States
Prion: a proteinaceous and infectious particle most commonly amyloidogenic but sometimes an intrinsically disordered protein that does not form amyloid
Type I prionoid: a self-templating protein conformation that spreads between cells, but not between individuals
Type II prionoid: a self-templating protein conformation that spreads within a cell, but not between cells
Dynamic Transition States
Liquid droplet: dynamic liquid-like assemblies formed by proteins, many of which are intrinsically disordered, that often include RNA; sometimes used interchangeably with the term "membraneless organelle"
Solid: a protein conformation such as amyloid that is stable and far less dynamic than diffuse molecules or liquid assemblies
Protein-Folding Descriptors
Proteinaceous: relating to or containing protein
Proteostasis: the concept that integrated biological networks control the genesis, folding, trafficking, and degradation of all proteins
Protein misfolding: deviation from a protein's native three-dimensional structure
Proteinopathy: a human disease associated with protein misfolding
Proteotoxicity: the cellular pathologies (cytotoxicity) associated with protein misfolding

concentrations of guanidine hydrochloride. Second, they were transmitted to all progeny of meiosis in genetic crosses in defiance of Mendel's laws. [*PRION*$^+$] nomenclature derives from these behaviors (capitals for dominance; brackets for non-Mendelian inheritance). For nearly two decades, geneticists characterized the biology of these traits and related suppressor phenotypes. However, a decisive breakthrough emerged in the mid-1990s (Chernoff et al., 1995; Patino et al., 1996; Ter-Avanesyan et al., 1994; Wickner, 1994): the [*URE3*] and [*PSI*$^+$] phenotypes were driven by self-templating aggregation of Ure2 and Sup35—that is, Sup35 and Ure2 are prions.

Fungal prions can confer deleterious phenotypes. For example, [*PSI*$^+$] imparts a fitness detriment of ~1% in standard laboratory conditions; some variants are more toxic (McGlinchey et al., 2011; True and Lindquist, 2000). But fungal prions can also confer adaptive benefit (Griswold and Masel, 2009; Hou et al., 2011; Jarosz et al., 2014; Si et al., 2003; True and Lindquist, 2000). [*PSI*$^+$] elicits growth advantages in about 25% of conditions tested. These traits depend strongly on genetic background (Halfmann et al., 2012; True and Lindquist, 2000), likely because aggregation of Sup35, a translation termination factor, causes regions downstream of stop codons to be translated, unleashing the phenotypic consequences of variation that has accumulated in those regions (True et al., 2004). The extent to which prions might provide evolutionary benefit has been highly controversial. Some argue that most fungal prions are diseases. Others suggest that prions can serve adaptive functions, for example, in fluctuating environments as sophisticated bet-hedging devices.

Briefly, the bet-hedging hypothesis posits that high switching rates between [*prion*$^-$] and [*PRION*$^+$] cells (~1 in 100,000 to ~1 in 1,000) mean that within a large population, a small number will always harbor the prion state. This creates sub-populations that express different traits than the majority. If those traits are detrimental, only a few individuals are lost. But if they are beneficial, the sub-population can ensure that the population survives when it would otherwise perish. [*PRION*$^+$] cells also revert to a [*prion*$^-$] state at similar frequencies, providing a complementary survival advantage. In fluctuating environments, this type of reversible epigenetic switching can be more adaptive than mutations, which arise more rarely and can create a phenotypic "lock-in." Models for [*PSI*$^+$] suggest that the prion's bet-hedging function might be sufficient for its evolutionary retention (Griswold and Masel, 2009). These arguments are even stronger for some other prions, such as [*GAR*$^+$] (Brown and Lindquist, 2009; Jarosz et al., 2014; Garcia et al., 2016). For further discussion, we point the reader to a number of reviews on the topic (Byers and Jarosz, 2014; Halfmann et al., 2012; Wickner et al., 2007).

The number of prions and prion-like proteins has expanded considerably in the last 15 years (Alberti et al., 2009; Brown and Lindquist, 2009; Chakrabortee et al., 2016; Derkatch et al., 2001; Du et al., 2008; Garcia and Jarosz, 2014; Hou et al., 2011; Si et al., 2003). The first were discovered largely serendipitously. More recently, efforts to identify new prions have been guided by searches for modular N-/Q-rich domains akin to those that typify the first prions to be discovered (Alberti et al., 2009; King et al., 1997; Masison and Wickner, 1995; Osherovich and Weissman, 2001; Ross et al., 2004). These efforts have identified two dozen prion domains in the yeast proteome (Alberti et al., 2009) that can replace the N-/Q-rich region of Sup35 and support conformational conversion. Some, such as [*MOT3*$^+$], have been characterized as bona fide prions in their own right (Alberti et al., 2009).

Indeed, a comprehensive survey of ~700 diverse yeast strains established that prions are relatively common in fungi (Halfmann et al., 2012). In addition to numerous strains containing well-known prions such as [*PSI*$^+$], hundreds that did not harbor these elements nonetheless expressed heritable traits with the hallmarks of prion biology. 40% of these phenotypes were beneficial, suggesting that fungal prions govern heritable traits in nature in a manner that can expand adaptive opportunities.

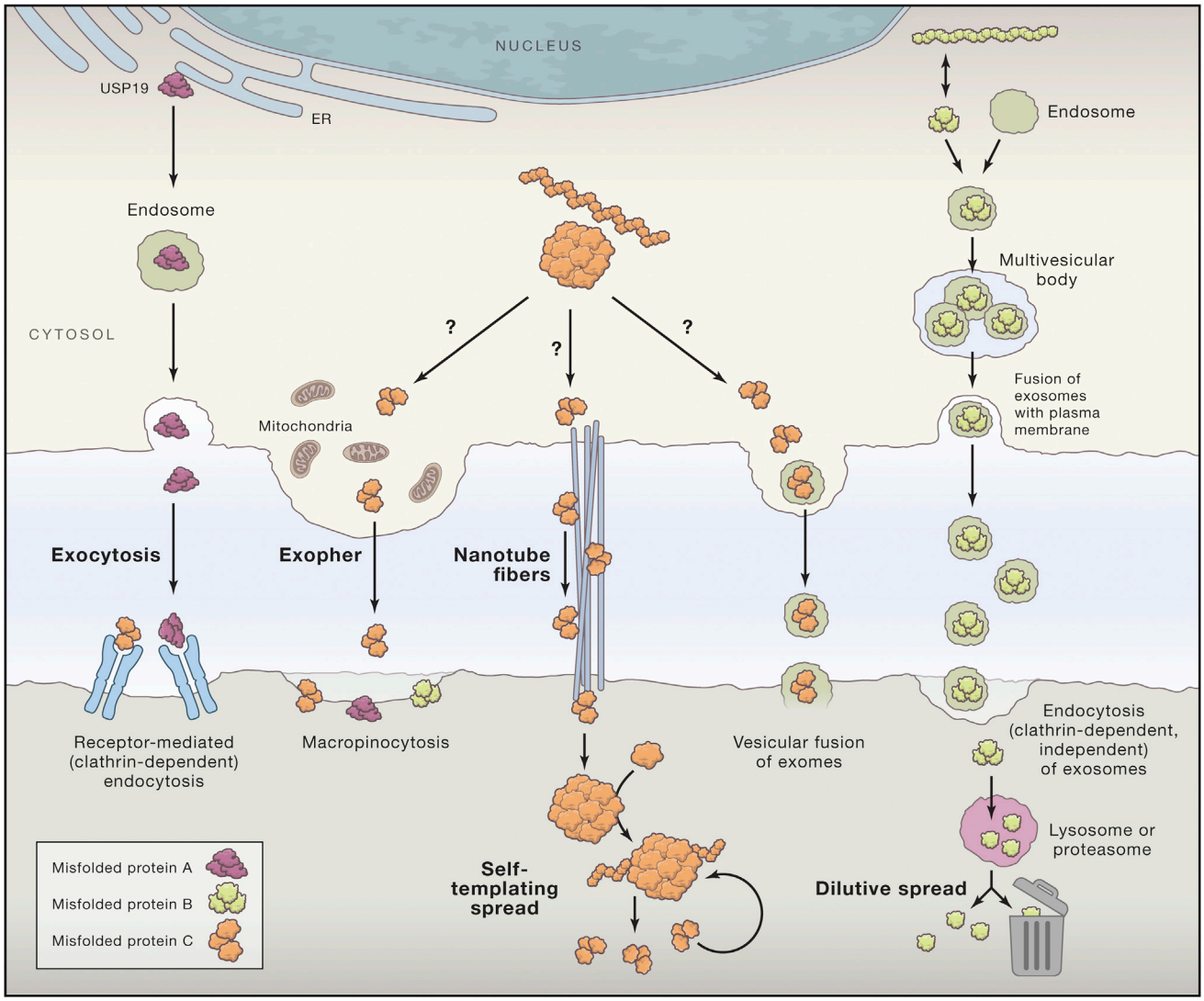

Figure 2. Potential Mechanisms of Transcellular Spread of Proteins.
Experimental evidence supports proteinacious spread between cells through (1) unconventional secretion and exocytosis from the plasma membrane (Rabouille, 2017), including USP19-dependent "MAPS" (misfolding-associated protein unconventional secretion) that involves translocation of endoplasmic reticulum (ER) proteins into the ER lumen during proteasomal stress (Lee et al., 2016); (2) exosome release through small budding events or fusion of multivesicular bodies to plasma membrane (Fevrier et al., 2004); (3) extrusion of proteins and mitochondria through large buds (exophers; Melentijevic et al., 2017); and (4) physical tunneling nanotubes directly connecting cells (Gousset et al., 2009). Mechanisms through which recipient cells take up misfolded proteins include (1) tunneling nanotubes, as above, and (2) any of a multitude of mechanisms described for uptake of extracellular material, including events that require specific binding to the membrane (e.g., receptor-/clathrin-dependent endocytosis and caveolin- or lipid-raft-mediated endocytosis) and others that do not (e.g., pinocytosis or fluid-phase endocytosis, which involve constant uptake of extracellular material) (Guo and Lee, 2014). In the nervous system, prions spread along nerve fiber tracts via microtubule-associated motors, transsynaptically in both anterograde and retrograde directions, and bi-directionally between nerve fibers and tissues that they innervate, including muscles and gland. The mechanisms of transsynaptic spread remain unclear, although different endocytic mechanisms have been implicated (Mao et al., 2016; Shearin and Bessen, 2014). It is important to understand the difference between spread that is dilutive (that is, the transfer of a protein that diminishes over time) versus spread that is truly self-templating and amplifying. Many experiments do not explicitly distinguish between the two—for instance, spread is often demonstrated without biochemical evidence of self-templating or amyloid formation.

In a variety of model systems ranging from *Aplysia* (Si et al., 2010; Si et al., 2003) to *Drosophila* (Khan et al., 2015; Majumdar et al., 2012) and more recently in mice (Fioriti et al., 2015), long-term memory has been linked to the conformational conversion of a protein known as CPEB/ORB2. In response to serotonin, CPEB/Orb2 engages in a prion-like conformational conversion in the stimulated neuron. This is associated with facilitation at the synapse and long-term memory of courtship behavior in flies (Keleman et al., 2007; Khan et al., 2015; Krüttner et al., 2012; Majumdar et al., 2012). Other non-amyloid but still relatively static structures, such as signalosomes, formed by self-templating caspase activation and recruitment domains (CARDs) are important in innate immunity (Wu and Fuxreiter, 2016). Yet other self-assembling complexes can be more dynamic. For example,

multivalent signaling condensates can form from SH3 domains (Li et al., 2012), and ribonucleoprotein (RNP) granules can be formed by low-complexity motifs and their interactions with RNA (Brangwynne et al., 2009).

Many of these protein assemblies arise from nucleated conformational conversion. Such assemblies may first form de-mixed liquids that progress to become more gel-like (Patel et al., 2015; Riback et al., 2017; Wallace et al., 2015). The degree to which these phase transitions are related to one another or whether they are intermediates to amyloid formation (Murray et al., 2017; Figure 3) is fiercely debated. However, it is remarkable that nearly 30% of the human proteome is intrinsically disordered. The ubiquity of these protein sequences suggests that self-assembly or some other feature that they encode may be biologically useful. Many questions remain, however, such as how specificity is determined, which factors govern differences in templating, and whether such domains might help to orchestrate biochemistry and gene regulation in time and space.

Protein-Based Genetic Elements with Distinct Conformational "Alleles"

Prions such as [*PSI*$^+$] can assemble into different self-templating conformations (Diaz-Avalos et al., 2005; King and Diaz-Avalos, 2004; Tanaka et al., 2004; Toyama et al., 2007; Uptain et al., 2001). This results in distinct and stable activity states of the prion called strains—structural variants that can be thought of as "conformational alleles." Importantly, these strains are formed by the same polypeptide sequence; they are not the same as genetic alleles. However, genetic variants can favor the formation of certain prion strains. This type of allelic variation also occurs for PrP and most other prions that have been tested (Carlson et al., 1989; Chien et al., 2004). It is now clear that prion strains derive from different fiber structures that correlate with distinct heritable phenotypes (Tanaka et al., 2004; Tanaka et al., 2006; Uptain et al., 2001). Structural differences can manifest in the dynamics of the fibers, patterns of twist and turn, and even mesoscopic flexibility and strength (Bradley et al., 2002; Derkatch et al., 1996; Diaz-Avalos et al., 2005; Dong et al., 2010; Krishnan and Lindquist, 2005; Tanaka et al., 2004).

Gold-standard evidence that prions are genetic elements—and that structural polymorphs can precipitate distinct phenotypes—comes from experiments using fibers assembled from purified protein to heritably transform naive cells. This was originally shown for [*PSI*$^+$] variants (Tanaka et al., 2004) and, more recently, for PrP^{Sc} (Wang et al., 2010) and [*MOT3*$^+$] (Alberti et al., 2009). These experiments directly linked the physical properties of amyloid fibers to the different stable phenotypes associated with them, thus providing a compelling explanation for prion strains.

Between nascent polypeptide and the amyloid fiber, prion proteins can adopt a wide range of conformers (Knowles et al., 2014). Some are on pathway to the formation of amyloid aggregates; others are not (Figure 1). Progress has been accelerated by conformation-specific antibodies (Kayed et al., 2003). Some of these, such as A11, appear to recognize obligate intermediates that correlate with toxicity in a variety of cellular and organismal models. Kinetic models of Sup35 assembly suggest that formation of oligomeric "seeds" is critical for rapid fiber assembly (Glover et al., 1997; Serio et al., 2000; Serio and Lindquist, 2000). It is also possible to isolate Sup35 variants that only form soluble oligomers (Dulle et al., 2013) but are competent to propagate the prion.

Broadening the Prion Concept: How Form Dictates Function

As revelatory as screens for N-/Q-rich prion-like proteins have been, several known prion-like elements would not have been found by these approaches. One striking example is [*GAR*$^+$] (Brown and Lindquist, 2009; Jarosz et al., 2014; Garcia et al., 2016), which reverses glucose-associated repression in fungi (Brown and Lindquist, 2009) and is adaptive in complex carbohydrates. Both strong and weak strains of the prion exist and can be induced through cross-kingdom chemical communication with a variety of lactic-acid-producing bacteria (Garcia et al., 2016; Jarosz et al., 2014), benefitting fungi and bacteria alike. [*GAR*$^+$] and its induction by bacteria have been conserved for hundreds of millions of years. This prion would have been missed by efforts to identify self-templating proteins because it is not rich in asparagine or glutamine residues, nor does it form amyloid aggregates or depend on Hsp104 to propagate from one generation to the next (Brown and Lindquist, 2009).

These observations motivated a proteome-wide screen to identify prions in a way that is agnostic to primary sequence or amyloid formation (Chakrabortee et al., 2016). Transient overexpression of prion proteins increases the likelihood that they will adopt their self-templating fold. Once prion conversion occurs, all other protein within the cell converts. Thus, ensuing biological traits remain stable even when protein levels return to normal. Transient induction of ~80 proteins created permanent, protein-specific changes in growth. Many had defining genetic properties of prion-based traits: non-Mendelian inheritance patterns and extreme sensitivity to the protein-folding environment in the cell. They could also be transmitted from one cell to another using protein alone.

All but three of the prions uncovered in this screen were previously unknown. Most were not rich in N/Q residues or low-complexity sequences, and none formed amyloid fibers. The proteins did share one striking property: "flexible" intrinsic disorder, which does not arise from a conserved primary sequence. Rather, the disorder itself is conserved over evolutionary time despite drift in protein sequence. These disordered domains were necessary for the propagation of prion phenotypes and conserved from yeast to humans. Indeed, several human homologs that were tested retained the capacity to self-template. Although it remains to be established under what biological circumstances these states might be engaged, these findings suggest that non-amyloid prion biology may be common in nature.

Diversity among Proteinopathies: Proteins and Strains

Many proteins that misfold in neurodegenerative diseases are capable of self-templating and transcellular spread, if falling short of being bona fide infectious prions. A substantial body of work has defined common mechanisms of toxicity and cellular "proteostasis" responses (Labbadia and Morimoto, 2015) and how these processes are affected by aging, the major risk factor for neurodegeneration (Cohen et al., 2006). But it is also

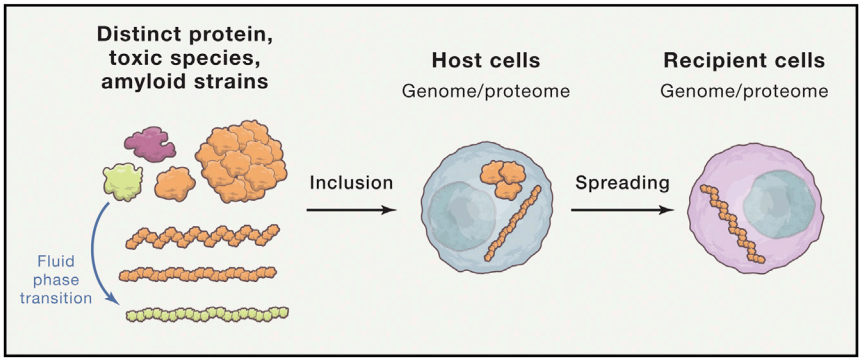

Figure 3. The Many Layers of Heterogeneity in the Relationship between Protein Conformer and Host

The simple path of aggregation, inclusion formation, and spread depicted here hides many layers of heterogeneity, beginning with the distinct protein that misfolds and the species that it misfolds into. Each host and recipient cell, with their unique proteomes, responds in unique ways to a proteotoxicity: many different types of inclusions may form, and spread can occur through multiple mechanisms (see Figure 2). Arrow: Fluid-phase transitions ordinarily allow cells to organize cytoplasmic contents rich in intrinsically disordered proteins into membrane-less compartments. Although not the subject of this review, in some circumstances, amyloid structures can also form when these transitions go awry.

becoming clear that there is specificity and heterogeneity among different proteinopathies—at the level of protein, mechanisms of spread, and cellular response, and ultimately, at the organismal level (Figure 3). Notably, much fundamental work in the field has utilized proteins that do not give rise to human disease or has explored biology in the context of exaggerated cellular stress. However, a "one-size-fits-all" strategy may not hold true for many aspects of proteinopathy, and studies in one system or with one particular protein may not be generalizable.

The diversity of cellular responses to protein misfolding is well illustrated by variants in the PrP protein that lead to distinct diseases (Aguzzi and Calella, 2009). For example, the D178N mutation can lead to familial Creutzfeldt-Jakob disease (CJD), a rapidly progressive degenerative disorder characterized by dementia and imbalance. Yet the same mutation, when coupled to methionine instead of valine at amino acid position 129, leads to fatal familial insomnia (FFI), a disease characterized by insomnia, psychological disturbances, and hallucinosis. Wild-type PrP can also misfold into different strains, leading to distinct patterns of degeneration. For example, iatrogenic CJD contracted from infected medical instrumentation differs from variant CJD contracted from infected meat, or from sporadic fatal insomnia, and so forth. These differences underscore the complex interplay between strain and host. Put another way, if strain switches of the same protein can make the difference between whether a cell type succumbs to a misfolded protein or survives untouched, then cellular responses to misfolding are likely to be highly distinct and potentially exploitable therapeutically.

Emerging data suggest that the host-variant and host-strain phenomena may extend beyond prion diseases to more common degenerative proteinopathies. For example, aggregation of the wild-type tau protein leads to Pick's disease, progressive supranuclear palsy, and corticobasal degeneration. Each disease exhibits distinct ultrastructural features of tau fibers, cellular and circuit pathologies, and clinical presentations (Stopschinski and Diamond, 2017). Similarly, synucleinopathies—including Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA)—result from misfolding and mislocalization of the same protein, alpha-synuclein (α-syn), with predilection for distinct cell types and circuits in the nervous system. Point mutations or multiplication at the α-syn-encoding *SNCA* locus lead to highly penetrant forms of neurodegenerative diseases. Some mutations predispose to motor symptoms (parkinsonism) followed by later-onset dementia (Golbe et al., 1990) and others to earlier dementia (Zarranz et al., 2004).

In 2006, Jucker and colleagues demonstrated that transgenic amyloid-precursor-protein (APP)-overexpressing mice could be induced to seed beta-amyloid in distinct patterns when injected with Aβ-containing brain extracts derived from different hosts (Meyer-Luehmann et al., 2006). Likewise, different conformers of tau and α-syn prepared from synthetic monomer lead to highly distinct yet stereotyped patterns of neurodegeneration when seeded directly into mouse brain (Peelaerts et al., 2015; Stopschinski and Diamond, 2017). Moreover, postmortem brain material from MSA patients is far more effective at seeding α-syn in transgenic mice and cell lines than material from PD or DLB patients (Prusiner et al., 2015). These findings have collectively raised the possibility that, just as with PrP, distinct conformer strains of tau and α-syn exist and that the distinct clinical patterns of neurodegenerative proteinopathies may relate to tropism of these strains for distinct cells and circuits within the brain.

Proteinacious Spread versus Differential Host Cellular Responses

Compatible explanations for the strikingly distinct pathology induced by distinct protein conformers include intrinsic cellular vulnerability and transcellular spread. Until recently, the consensus in the field was that innate biological properties of specific cell types dictate their vulnerability to pathologic insults. For example, a body of literature has highlighted differences between vulnerable and resistant dopaminergic neurons that can alter relative vulnerability to PD-related toxicities (Chung et al., 2005; Surmeier et al., 2017). Recently, there have been challenges to this view. First, detailed neuropathologic analysis of different neurodegenerative diseases, including Alzheimer's disease (AD) (Braak and Braak, 1991) and PD (Braak et al., 2003), has revealed stereotypic stages of disease progression between areas that are connected through defined neuroanatomical pathways and synaptic connections. Second, studies of transplanted fetal grafts in PD patients (in an effort to restore dopaminergic transmission) revealed the presence of Lewy bodies in

transplanted tissue more than a decade after grafting, raising the possibility that α-syn pathology had been transmitted in a prion-like fashion from host to graft (Li et al., 2008). Third, functional connectivity fMRI studies have identified stereotyped patterns of dysfunction that correlate with clinical neurodegenerative syndromes and correspond to neuroanatomical circuits (Seeley et al., 2009).

A wide variety of amyloidogenic proteins—from Aβ (Morales et al., 2012) to tau (de Calignon et al., 2012) to α-syn (Luk et al., 2012) to polyglutamine (polyQ)-expanded huntingtin (He et al., 2017)—can spread between neurons transsynaptically or between neurons and glia (Reyes et al., 2014). These data were generated *in vivo* or in cultured cells after either inoculation of exogenous brain extracts or purified protein conformers, or through selective expression of an amyloidogenic protein in a subset of cells. This literature has recently been extensively reviewed (Walker and Jucker, 2015; Stopschinski and Diamond, 2017). The conclusion has been that many neurodegenerative proteins can form distinct strains that exhibit self-templating and transcellular spread (type I prionoids in the classification we suggest above; Table 1).

Many questions remain about how significant the spread phenomenon is to disease progression at physiologic protein concentrations (Walsh and Selkoe, 2016). As noted above in Figure 2, transcellular spread can involve "dilutive" mechanisms that do not require self-templating, particularly in the context of the inter-connected circuitry of the nervous system. These mechanisms have not generally been ruled out in work to date, and even if spread is a fundamental driver of neurodegenerative disease progression, underlying mechanisms may be distinct for different amyloids. For example, the USP-19-dependent mechanism of unconventional secretion (misfolding-associated protein unconventional secretion, MAPS; Figure 2) is apparently relevant to α-syn secretion, but not to tau (Lee et al., 2016). Indeed, within whole organisms, genetic analysis has revealed that mechanisms of proteinacious spread are demonstrably different for distinct proteins in different model systems (Pearce, 2017).

In our view, the positing of contiguous spread versus innate cellular vulnerability as competing theories to explain distinct neuropathologies is unnecessary. Both mechanisms must be at play. For example, it is difficult for the contiguous spread hypothesis to convincingly explain why patients harboring dominant mutations (in whom all neurons express the mutant and wild-type protein) exhibit diseases in which only a small subset of cells succumbs. The initial location of seed formation and the pattern of spread in these circumstances are likely to be dictated by intrinsic neuronal factors (Walsh and Selkoe, 2016). Moreover, differential vulnerability of specific cell types occurs in the absence of amyloid pathology. For example, many forms of parkinsonism, genetic and environmental, exhibit the same pattern of dopaminergic neuronal vulnerability as PD but without Lewy α-syn pathology (Surmeier et al., 2017).

Heterogeneity among Proteotoxic Species and Inclusions: α-Synuclein as Case in Point

Beyond distinct amyloid strains, many potential protein species (amyloid fiber, oligomeric intermediates, and so forth) have been postulated to be "proteotoxic" in degenerative proteinopathies (Treusch et al., 2009), and these may differ among different proteins and cellular contexts. As noted above, the term proteotoxicity is a general one that refers to a cytotoxicity associated with protein misfolding, regardless of the specific conformer that forms or whether self-templating occurs. Alpha-synucleinopathies provide a case in point. Lewy bodies are composed of beta-sheet-rich fibrillar α-syn surrounded by clustered vesicles (Duffy and Tennyson, 1965), but in patient brain, transgenic animal models, and *in vitro*, α-syn can exist in a wide variety of oligomeric and fibrillar forms (Lashuel et al., 2013) variously associated with membrane components and vesicles (Gitler et al., 2008; Volpicelli-Daley et al., 2014). Some lines of evidence suggest that pre-fibrillar α-syn may be a relevant toxic species. *In vitro*, α-syn oligomers abnormally increase permeability of membranes, an effect that has been linked to perturbed calcium homeostasis and organelle damage. In mouse models, progressive neurodegeneration correlates with the formation of α-syn oligomers (reviewed in Lashuel et al., 2013). Recently, the adage that all α-syn oligomers are detrimental has been turned on its head with evidence mounting that α-syn, long thought to exist physiologically as an intrinsically disordered monomer, may exist in physiologic multimeric states (Bartels et al., 2011; Dettmer et al., 2016). Stabilization of such forms may offer therapeutic benefit to patients, as has been shown for other amyloids (Bulawa et al., 2012).

Pre-fibrillar forms of α-syn are clearly toxic in genetically tractable model organisms. For example, yeast cells succumb to α-syn overexpression. Prominent inclusions form in this model, but they are not beta-sheet-rich aggregates. They instead consist of α-syn oligomers associated with stalled transport vesicles (Gitler et al., 2008). Despite the absence of amyloid pathology, genome-wide screens against α-syn toxicity in this model have recovered many known PD genetic risk factors (Khurana et al., 2017) and led to the discover of cellular pathologies in PD patient-derived neurons (Chung et al., 2013), suggesting relevance to human disease. Likewise, in a genetically tractable fly model of α-syn toxicity, there is an inverse correlation between toxicity and inclusion formation (Chen and Feany, 2005) reminiscent of the situation for polyQ-expansion toxicity in this organism (Warrick et al., 1999).

In contrast, in mammalian cells and primary neurons, overexpression of α-syn achieves only modest toxicity in the absence of amyloid-rich inclusions. Indeed, this toxicity can be greatly exacerbated when neurons are exposed in culture or *in vivo* to sonicated pre-formed fibrils (PFFs), a process that better emulates the amyloid pathology and post-translational modification of α-syn observed in patient brain (Luk et al., 2012; Volpicelli-Daley et al., 2014). *In vivo*, after injection of PFFs into transgenic mice, formation of inclusions is associated with subsequent neuronal demise (Osterberg et al., 2015), although it remains unclear whether amyloid or some toxic species on or off pathway to amyloid is responsible for that toxicity. There are emerging experimental data supporting the disease-relevance of PFFs, including the enhanced formation of inclusions by known human genetic risk factors for PD (Volpicelli-Daley et al., 2016). Nevertheless, the profound disconnection between extent of Lewy pathology and severity of clinical phenotype keeps open the question of

how significant macro-inclusions are to disease pathogenesis (Burke et al., 2008; Jellinger, 2008). This has similarly been questioned for other proteinopathies, including Huntington's disease (HD) (Gutekunst et al., 1999; Kuemmerle et al., 1999) and AD (Haass and Selkoe, 2007). The controversies over whether inclusions are protective or detrimental might of course be related to heterogeneity of intracellular inclusions. A bewildering number of inclusions have been described in different cell types under different conditions, many forming through energy-dependent processes in the cell. Primary inclusions that result from different perturbations of proteostasis (as opposed to a secondary process like stalled vesicle transport or aberrant fluid-phase transitions) include aggresomes, insoluble protein deposits (IPODs), and juxtanuclear quality control (JUNQ) compartments (Wolff et al., 2014). Indeed, the same aggregating protein can be rendered nontoxic when redirected from JUNQ to IPOD compartments, providing strong support for the functional differences between these inclusion types (Weisberg et al., 2012).

Genetic Analysis Reveals that Specific Mechanisms Underlie Distinct Proteotoxicities

Over the last three decades, the discovery that point mutations or multiplication of genes encoding misfolding proteins lead to dominantly inherited neurodegenerative diseases has tied these proteins to disease etiology beyond any reasonable doubt. These advances have also enabled the generation of cellular and whole organism models through overexpression of wild-type or mutant proteins or knockin approaches at endogenous loci. These models thus recapitulate proteotoxicities but are "blind" to specific protein conformation states.

Three genetically tractable organisms—Baker's yeast *S. cerevisiae*, the fruit fly *D. melanogaster*, and the roundworm *Caenorhabditis elegans*—have enabled systematic genetic dissection of mechanisms underlying proteotoxicity. For some models, genetic screening has covered almost every gene (that is, the organisms have been screened "to saturation"). These data have now begun to be cataloged in publicly available databases (for example, http://www.chibi.ubc.ca/neurogem/) and provide an aerial view of genetic architecture underlying protein misfolding, albeit in primitive organisms. Perhaps the most striking finding is how little overlap there is between genetic modifiers of different proteotoxicities. Comprehensive (98% coverage) genetic screens against SOD1 (amyotrophic lateral sclerosis [ALS]), α-syn (PD), and tau (AD/tauopathy) toxicities in worms recovered 165, 290, and 75 genetic modifiers of toxicity, respectively. Yet only three overlapped between tau and SOD1, two between tau and α-syn, and four between SOD1 and α-syn. Some modifiers implicate common arms of the proteostasis network (e.g., heat-shock factor-1 [HSF1] and protein trafficking machinery), but overall, the message from such screens is that cellular responses are highly specific to each proteotoxicity.

One way to validate genetic modifiers recovered in model systems has been through cross-correlation with known human genetic risk factors. This overlap has been best explored for models of proteotoxicity in Baker's yeast models. Inducible overexpression of α-syn, TDP-43, Aβ, or polyQ-expanded proteins creates robust growth toxicities in this organism that can easily be screened in high-throughput assays. Systematic genome-wide deletion and overexpression of modifier genes have given the broadest view yet of the comparative genetic landscape of protein-misfolding pathologies (Khurana and Lindquist, 2010; Khurana et al., 2017). For example, arrayed screens of most of the ~6,000 genes that make up the yeast genome have identified genes that, when overexpressed, exacerbate or rescue from toxicity. Modifiers in each of these screens correlate well with known human genetic risk factors in the counterpart diseases—for example, unbiased α-syn toxicity screens have recovered known Mendelian parkinsonism genes, and Aβ toxicity screens have recovered AD genome-wide association study hits (Figure 4A). The Ataxin-2 encoding gene (*ATXN2*) was identified as a novel ALS gene through a yeast screen for TDP-43 toxicity modifiers (Elden et al., 2010). Notably, even recovery of common modifiers between proteotoxicities does not necessarily indicate similar mechanisms of toxicity. For example, Ataxin-2 upregulation suppresses α-syn toxicity (Khurana et al., 2017) but is an enhancer of TDP-43 toxicity (Elden et al., 2010; Khurana et al., 2017).

Surprisingly, the proteostasis network is not uniformly implicated in these screens (Figure 4A; Khurana and Lindquist, 2010; Khurana et al., 2017). Contrary to expectations, this is also true of yeast prions. An unbiased screen to identify suppressors of [*RNQ*$^+$] toxicity pinpointed the cause as a single, highly specific cell-cycle defect rather than a profound disturbance of proteostasis (Treusch and Lindquist, 2012).

In many ways, human genetic analysis corroborates model organism data by indicating highly distinct candidate genetic loci for different proteinopathies. In fact, there is not a single common genetic risk factor recovered from conventional genome-wide association studies for PD, AD, or ALS. Some of this, however, may relate to incompleteness, because most of the factors underlying heritability of neurodegenerative disorders remain unknown. Consistent with this, overlap between distinct diseases has been found through detailed analysis of specific genetic risk factors. For example, ApoE4, the best validated risk factor for late-onset AD, has emerged in this way as a risk factor for other neurodegenerative proteinopathies, including DLB (Keogh et al., 2016).

Consequences of Protein Misfolding Are Tied to Protein Identity and Native Interactions

Increasing evidence suggests that the distinct mechanisms of proteotoxicities implicated by genetic analyses may have a good deal to do with the intrinsic function of the protein that is misfolding and its native interactions. These data thus bring us back from a conformation-centric view of proteotoxicity to the unique biology of the native protein itself (Figure 1). Some of the best evidence for this has emerged from studies of polyQ-expanded proteinopathies. In these diseases, the same fundamental mutation within different protein backbones leads to highly distinct diseases. It is clear that the modulation of native protein interactions and physiologic functions must be important for pathogenesis (Orr, 2012). Indeed, in yeast cells, single changes in the proteome or minor changes in backbone sequence can dramatically change the toxic outcome of polyQ overexpression (Duennwald et al., 2006). In mammalian models,

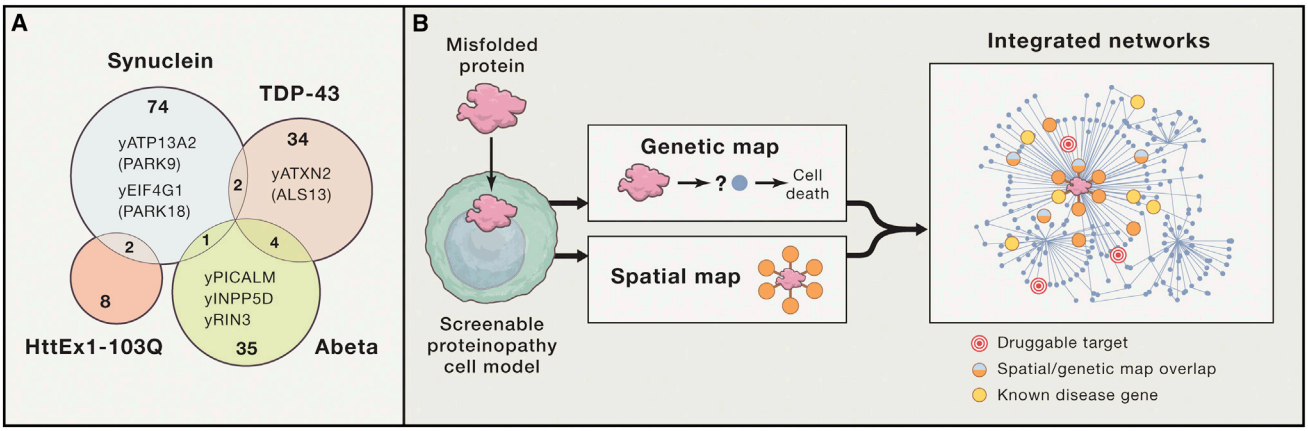

Figure 4. Proteotoxicities Are Distinct and Tied to the Intrinsic Function of the Toxic Protein

(A) Unbiased overexpression screens against four different proteotoxicities in yeast reveal minimal overlap (Kayatekin et al., 2014; Khurana et al., 2017). Cross-correlation with human genetic datasets affirmed the validity of these datasets with parkinsonism (PARK) genes identified in the alpha-synuclein (α-syn) screen, AD risk factors (PICALM, INPP5D, RIN3) emerging in the beta-amyloid (Abeta) screen, an ALS risk factor (Ataxin-2) in the TDP-43 screen.

(B) A schematic diagram illustrating the concept of genetic and spatial mapping of proteotoxicity. A genetic map is a molecular network encompassing genes that impact a proteotoxicity when overexpressed or deleted. A spatial map comprises proteins that are in the immediate vicinity of a protein of interest. A schematic diagram of an integrated network is shown at right. Recently, such maps were generated for α-syn proteoxicity (a genetic map in yeast and a spatial map in neurons; Khurana et al., 2017; Chung et al., 2017), revealing a connection between this toxicity and 12 known parkinsonism genes. The significant overlap of genetic and spatial maps revealed an intimate relationship of α-syn toxicity to its functional interactions and location.

the importance of native protein function and interactions has perhaps best been shown for Ataxin-1, polyQ expansion of which lead to spinocerebellar ataxia (SCA) type 1. Ataxin-1 toxicity is distinct from the toxicity of overexpressing an expanded polyQ tract, and the perturbation of multiple native interactions between Ataxin-1 and other proteins contributes to this difference (Lim et al., 2008). Specific domains and post-translational modifications of Ataxin-1 that mediate these interactions have been defined (Tsuda et al., 2005). Thus, beyond the dominant "toxic gain-of-function" mechanism of toxicity that one would expect from an aggregating protein, polyQ-dependent loss of endogenous Ataxin-1 function also contributes to neurodegeneration. Accordingly, deletion of *ATXN1* exacerbates pathology in a SCA-1 mouse model (Crespo-Barreto et al., 2010).

Over the last decade, perturbation of endogenous function has also emerged as a common theme in other polyQ proteotoxicites. For example, patients with SCA type 7, caused by polyQ expansion of Ataxin-7, uniquely suffer from blindness due to photoreceptor loss. Ataxin-7 is a component of the SAGA histone acetyltransferease complex. PolyQ expansion of Ataxin-7 leads to increased recruitment of the SAGA complex to promoters and hyperacetylation of certain photoreceptor-specific genes (La Spada et al., 2001). This hyperacetylation leads in turn to severe chromatin decondensation and downregulation of gene transcription in photoreceptors (Helmlinger et al., 2006), tying perturbed intrinsic function of the Ataxin-7 protein to differential cellular vulnerability in SCA-7.

The relationship between native interactions of a protein within a cell and the mechanism of its toxicity has very recently been explored in yeast and neurons for the protein α-syn (schematized in Figure 4B). 332 genetic modifiers of α-syn toxicity emerged from unbiased yeast screens (Khurana et al., 2017). In these screens, most genes of the yeast genome were systematically overexpressed or deleted and the effects on α-syn toxicity assessed. A cross-species computational approach (TransposeNet) assembled these genes into a coherent "humanized" molecular network. This "genetic" map was cross-compared with a "spatial" map generated by proximity biotin labeling to uncover the local α-syn proteome at < 10 nm resolution under close-to-physiologic expression levels (Chung et al., 2017). The spatial and genetic maps significantly overlapped at the level of protein classes and specific proteins. Thus, the intrinsic location and protein interactions of α-syn are directly related to its mechanism of toxicity when it misfolds. This may turn out to be a general theme, explaining in part the exquisite specificity of protein-misfolding pathologies.

Future Directions

In the last two decades, the field of protein misfolding has coalesced on broadly common themes: that amyloidogenic proteins proceed through a set of definable conformation states from monomer to amyloid, and that an increase in steady-state levels (whether through proteostasis stress, aging, or gene mutation) can push these proteins along this aggregation pathway. This process now provides a biological framework in which to understand aggregated protein species that give cells the capacity to survive and thrive in stressful environments, provide paradigm-shifting modes of information transfer and, perhaps most remarkably, drive the maintenance of long-term memories. And of course it is within this framework that we better understand how pathologic protein conformers and amyloids contribute to devastating diseases, from degenerative proteinopathies to production of antibiotic-resistant bacterial biofilms.

For obvious reasons, the most intense research efforts in this field have been geared toward alleviating human proteinopathies. There is a growing belief that common mechanisms of toxicity and cellular response will lead to common therapeutic

measures, none garnering more attention than the reduction of protein levels through small-molecule interventions or immunotherapy (Stopschinski and Diamond, 2017). We are entering a period in which clinical trials can be executed earlier in better-stratified patient cohorts. For example, stratification that favored earlier-stage patients with definitive evidence of Aβ plaques on positron emission tomography (PET) imaging may explain a recent vaunted success in an early clinical trial for the Aβ-directed antibody aducanamab (Sevigny et al., 2016). A slew of such trials are underway or being prepared for a number of protein-misfolding pathologies, including anti-sense oligonucleotide treatments to lower protein levels. Other strategies aim to augment or exploit the proteostasis and protein quality-control machineries. These include small-molecule inhibitors of the deubiquitinase USP14 that can enhance degradation of misfolding proteins that are proteasome substrates (Lee et al., 2010).

Early therapeutic success is likely to be achieved for genetically well-defined subsets of these diseases. For others with complex genetic inheritance, stratification at the level of genetic risk or biomarkers will be critical to match subsets of patients to appropriate therapies. In our view, this will require a deeper biological understanding of the uniqueness of proteins and protein conformers in the specific context of a cellular and organismal host, the central theme of this review. Although the complexity is staggering, technological breakthroughs have emerged that will empower us to unravel the myriad ways in which protein states affect biological function and give rise to human disease. For example, stem-cell technologies enable growth of specific cell types and organoid tissues in a patient-specific genetic background from induced pluripotent stem cells (iPScs). These cells can in turn be seeded by exposure to patient-specific amyloid strains and conformers.

The advent of CRISPR/Cas9-based genome-editing technologies will enable unprecedented dissection of host-strain relationships in these models in a way that was unthinkable just a few years ago. Sophisticated unbiased genetic approaches to identify druggable targets, currently tenable in tractable model systems such as yeast, will soon become available in human cellular models (Khurana et al., 2015). Moreover, beyond investigating single cells, powerful chemogenetic and optogenetic tools will enable precise control of neural circuits *in vitro* and *in vivo* in subsets of living neurons under different activity and protein aggregation states. Such manipulations have already begun to reveal connections between neuronal activity and proteinopathy *in vivo* (Musacchio et al., 2017; Qi et al., 2017). Genetic manipulations in these sophisticated models will be able to parse differences between distinct mechanisms of transcellular spread in tractable neural circuits. For example, the emergence of radioligands that bind specific amyloids will be useful to distinguish self-templating from dilutive mechanisms of transcellular spread and so forth.

From this field's inception, the interplay between disease biology and fundamental biology has been constant and mutually enriching. Careful observations of devastating fatal conditions spawned a field that shed light on how protein states encode and transmit information and how protein homeostatic responses keep these processes in check (or in some cases, fuel them). Even our current incomplete understanding of the scope of such processes in health and disease vastly exceeds what might originally have been predicted by pioneering researchers studying spongiform encephalopathies half a century ago. Fortunately, the explosion of new tools available to connect the behavior of individual molecules to biological phenotype means that the time is now ripe to fully understand these complex mechanisms in health and disease alike.

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The interplay between disease and fundamental biology addressed in this review is personal to us. Almost a decade ago, we joined the laboratory of Susan Lindquist as postdoctoral fellows, one of us (D.F.J.) a biochemist searching for answers to fundamental questions in cellular and organismal evolution and the other (V.K.) a physician-scientist looking to alter dire outcomes for patients with neurodegenerative disease. While we found ourselves compartmentalized in Lindquist laboratory subgroups that communicated little, the connections were undoubtedly seamless in the mind of a mentor who had made seminal contributions to both sides of the protein-folding equation. Susan died in October 2016 and left a gaping hole in the world of biology and in our personal world, too. She had participated in creating a breathtaking new cellular view in which dimensionality of function at every level was tied to plurality of protein identity, conformation, and higher-order assemblies. She knew (and took great delight in knowing) that the surface of this field may barely have been scratched but that transformative tools had arrived to change that. We wrote this review to commemorate her and bring those subgroups together at last.

REFERENCES

Aguzzi, A., and Calella, A.M. (2009). Prions: protein aggregation and infectious diseases. Physiol. Rev. *89*, 1105–1152.

Aigle, M., and Lacroute, F. (1975). Genetical aspects of [URE3], a non-mitochondrial, cytoplasmically inherited mutation in yeast. Mol. Gen. Genet. *136*, 327–335.

Alberti, S., Halfmann, R., King, O., Kapila, A., and Lindquist, S. (2009). A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. Cell *137*, 146–158.

Bartels, T., Choi, J.G., and Selkoe, D.J. (2011). α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature *477*, 107–110.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. *82*, 239–259.

Braak, H., Del Tredici, K., Rüb, U., de Vos, R.A.I., Jansen Steur, E.N.H., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging *24*, 197–211.

Bradley, M.E., Edskes, H.K., Hong, J.Y., Wickner, R.B., and Liebman, S.W. (2002). Interactions among prions and prion "strains" in yeast. Proc. Natl. Acad. Sci. USA *99* (*Suppl 4*), 16392–16399.

Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science *324*, 1729–1732.

Brown, J.C., and Lindquist, S. (2009). A heritable switch in carbon source utilization driven by an unusual yeast prion. Genes Dev. *23*, 2320–2332.

Bulawa, C.E., Connelly, S., Devit, M., Wang, L., Weigel, C., Fleming, J.A., Packman, J., Powers, E.T., Wiseman, R.L., Foss, T.R., et al. (2012). Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. Proc. Natl. Acad. Sci. USA *109*, 9629–9634.

Burke, R.E., Dauer, W.T., and Vonsattel, J.P.G. (2008). A critical evaluation of the Braak staging scheme for Parkinson's disease. Ann. Neurol. *64*, 485–491.

Byers, J.S., and Jarosz, D.F. (2014). Pernicious pathogens or expedient elements of inheritance: the significance of yeast prions. PLoS Pathog. *10*, e1003992.

Carlson, G.A., Westaway, D., DeArmond, S.J., Peterson-Torchia, M., and Prusiner, S.B. (1989). Primary structure of prion protein may modify scrapie isolate properties. Proc. Natl. Acad. Sci. USA *86*, 7475–7479.

Caudron, F., and Barral, Y. (2013). A super-assembly of Whi3 encodes memory of deceptive encounters by single cells during yeast courtship. Cell *155*, 1244–1257.

Chakrabortee, S., Byers, J.S., Jones, S., Garcia, D.M., Bhullar, B., Chang, A., She, R., Lee, L., Fremin, B., Lindquist, S., and Jarosz, D.F. (2016). Intrinsically Disordered Proteins Drive Emergence and Inheritance of Biological Traits. Cell *167*, 369–381.e12.

Chen, L., and Feany, M.B. (2005). Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nat. Neurosci. *8*, 657–663.

Chernoff, Y.O., Lindquist, S.L., Ono, B., Inge-Vechtomov, S.G., and Liebman, S.W. (1995). Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi+]. Science *268*, 880–884.

Chien, P., Weissman, J.S., and DePace, A.H. (2004). Emerging principles of conformation-based prion inheritance. Annu. Rev. Biochem. *73*, 617–656.

Chung, C.Y., Seo, H., Sonntag, K.C., Brooks, A., Lin, L., and Isacson, O. (2005). Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. Hum. Mol. Genet. *14*, 1709–1725.

Chung, C.Y., Khurana, V., Auluck, P.K., Tardiff, D.F., Mazzulli, J.R., Soldner, F., Baru, V., Lou, Y., Freyzon, Y., Cho, S., et al. (2013). Identification and rescue of α-synuclein toxicity in Parkinson patient-derived neurons. Science *342*, 983–987.

Chung, C.Y., Khurana, V., Yi, S., Sahni, N., Loh, K.H., Auluck, P.K., Baru, V., Udeshi, N.D., Freyzon, Y., Carr, S.A., et al. (2017). In Situ Peroxidase Labeling and Mass-Spectrometry Connects Alpha-Synuclein Directly to Endocytic Trafficking and mRNA Metabolism in Neurons. Cell Syst. *4*, 242–250.e4.

Cohen, E., Bieschke, J., Perciavalle, R.M., Kelly, J.W., and Dillin, A. (2006). Opposing activities protect against age-onset proteotoxicity. Science *313*, 1604–1610.

Cox, B.S., Tuite, M.F., and Mundy, C.J. (1980). Reversion from suppression to nonsuppression in SUQ5 [psi+] strains of yeast: the classificaion of mutations. Genetics *95*, 589–609.

Crespo-Barreto, J., Fryer, J.D., Shaw, C.A., Orr, H.T., and Zoghbi, H.Y. (2010). Partial loss of ataxin-1 function contributes to transcriptional dysregulation in spinocerebellar ataxia type 1 pathogenesis. PLoS Genet. *6*, e1001021.

de Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, D.H., Kopeikina, K.J., Pitstick, R., Sahara, N., Ashe, K.H., Carlson, G.A., et al. (2012). Propagation of tau pathology in a model of early Alzheimer's disease. Neuron *73*, 685–697.

Derkatch, I.L., Chernoff, Y.O., Kushnirov, V.V., Inge-Vechtomov, S.G., and Liebman, S.W. (1996). Genesis and variability of [PSI] prion factors in Saccharomyces cerevisiae. Genetics *144*, 1375–1386.

Derkatch, I.L., Bradley, M.E., Hong, J.Y., and Liebman, S.W. (2001). Prions affect the appearance of other prions: the story of [PIN(+)]. Cell *106*, 171–182.

Dettmer, U., Selkoe, D., and Bartels, T. (2016). New insights into cellular α-synuclein homeostasis in health and disease. Curr. Opin. Neurobiol. *36*, 15–22.

Diaz-Avalos, R., King, C.Y., Wall, J., Simon, M., and Caspar, D.L. (2005). Strain-specific morphologies of yeast prion amyloid fibrils. Proc. Natl. Acad. Sci. U S A *102*, 10165–10170.

Dobson, C.M. (2003). Protein folding and misfolding. Nature *426*, 884–890.

Dong, J., Castro, C.E., Boyce, M.C., Lang, M.J., and Lindquist, S. (2010). Optical trapping with high forces reveals unexpected behaviors of prion fibrils. Nat. Struct. Mol. Biol. *17*, 1422–1430.

Du, Z., Park, K.W., Yu, H., Fan, Q., and Li, L. (2008). Newly identified prion linked to the chromatin-remodeling factor Swi1 in Saccharomyces cerevisiae. Nat. Genet. *40*, 460–465.

Duennwald, M.L., Jagadish, S., Giorgini, F., Muchowski, P.J., and Lindquist, S. (2006). A network of protein interactions determines polyglutamine toxicity. Proc. Natl. Acad. Sci. USA *103*, 11051–11056.

Duffy, P.E., and Tennyson, V.M. (1965). Phase and electron microscopic observations of Lewy bodies and melanin granules in substantia nigra and locus coeruleus in Parkinson's disease. J. Neuropathol. Exp. Neurol. *24*, 398–414.

Dulle, J.E., Bouttenot, R.E., Underwood, L.A., and True, H.L. (2013). Soluble oligomers are sufficient for transmission of a yeast prion but do not confer phenotype. J. Cell Biol. *203*, 197–204.

Elden, A.C., Kim, H.-J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M.M., et al. (2010). Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature *466*, 1069–1075.

Ellis, R.J., and Hartl, F.U. (1999). Principles of protein folding in the cellular environment. Curr. Opin. Struct. Biol. *9*, 102–110.

Fersht, A.R. (1998). Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding, First Edition (W.H. Freedman).

Fevrier, B., Vilette, D., Archer, F., Loew, D., Faigle, W., Vidal, M., Laude, H., and Raposo, G. (2004). Cells release prions in association with exosomes. Proc. Natl. Acad. Sci. USA *101*, 9683–9688.

Fioriti, L., Myers, C., Huang, Y.-Y., Li, X., Stephan, J.S., Trifilieff, P., Colnaghi, L., Kosmidis, S., Drisaldi, B., Pavlopoulos, E., and Kandel, E.R. (2015). The Persistence of Hippocampal-Based Memory Requires Protein Synthesis Mediated by the Prion-like Protein CPEB3. Neuron *86*, 1433–1448.

Gajdusek, D.C., and Zigas, V. (1957). Degenerative disease of the central nervous system in New Guinea; the endemic occurrence of kuru in the native population. N. Engl. J. Med. *257*, 974–978.

Garcia, D.M., and Jarosz, D.F. (2014). Rebels with a cause: molecular features and physiological consequences of yeast prions. FEMS Yeast Res. *14*, 136–147.

Garcia, D.M., Dietrich, D., Clardy, J., and Jarosz, D.F. (2016). A common bacterial metabolite elicits prion-based bypass of glucose repression. Elife *5*.

Gitler, A.D., Bevis, B.J., Shorter, J., Strathearn, K.E., Hamamichi, S., Su, L.J., Caldwell, K.A., Caldwell, G.A., Rochet, J.-C., McCaffery, J.M., et al. (2008). The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis. Proc. Natl. Acad. Sci. USA *105*, 145–150.

Glover, J.R., Kowal, A.S., Schirmer, E.C., Patino, M.M., Liu, J.J., and Lindquist, S. (1997). Self-seeded fibers formed by Sup35, the protein determinant of [PSI+], a heritable prion-like factor of S. cerevisiae. Cell *89*, 811–819.

Golbe, L.I., Di Iorio, G., Bonavita, V., Miller, D.C., and Duvoisin, R.C. (1990). A large kindred with autosomal dominant Parkinson's disease. Ann. Neurol. *27*, 276–282.

Gousset, K., Schiff, E., Langevin, C., Marijanovic, Z., Caputo, A., Browman, D.T., Chenouard, N., de Chaumont, F., Martino, A., Enninga, J., et al. (2009). Prions hijack tunnelling nanotubes for intercellular spread. Nat. Cell Biol. *11*, 328–336.

Griffith, J.S. (1967). Self-replication and scrapie. Nature *215*, 1043–1044.

Griswold, C.K., and Masel, J. (2009). Complex adaptations can drive the evolution of the capacitor [PSI], even with realistic rates of yeast sex. PLoS Genet. *5*, e1000517.

Guo, J.L., and Lee, V.M.-Y. (2014). Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. Nat. Med. *20*, 130–138.

Gutekunst, C.A., Li, S.H., Yi, H., Mulroy, J.S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R.J., Hersch, S.M., and Li, X.-J. (1999). Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J. Neurosci. *19*, 2522–2534.

Haass, C., and Selkoe, D.J. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat. Rev. Mol. Cell Biol. *8*, 101–112.

Halfmann, R., Jarosz, D.F., Jones, S.K., Chang, A., Lancaster, A.K., and Lindquist, S. (2012). Prions are a common mechanism for phenotypic inheritance in wild yeasts. Nature *482*, 363–368.

He, B., Zheng, M., Liu, Q., Shi, Z., Long, S., Lu, X., Pei, Z., Yuan, T.-F., Su, H., and Yao, X. (2017). Injected Amyloid Beta in the Olfactory Bulb Transfers to Other Brain Regions via Neural Connections in Mice. Mol. Neurobiol. *1854*, 769.

Helmlinger, D., Hardy, S., Abou-Sleymane, G., Eberlin, A., Bowman, A.B., Gansmüller, A., Picaud, S., Zoghbi, H.Y., Trottier, Y., Tora, L., and Devys, D. (2006). Glutamine-expanded ataxin-7 alters TFTC/STAGA recruitment and chromatin structure leading to photoreceptor dysfunction. PLoS Biol. *4*, e67.

Hou, F., Sun, L., Zheng, H., Skaug, B., Jiang, Q.X., and Chen, Z.J. (2011). MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. Cell *146*, 448–461.

Howard, K.J., Holley, S.J., Yamamoto, K.R., and Distelhorst, C.W. (1990). Mapping the HSP90 binding region of the glucocorticoid receptor. J. Biol. Chem. *265*, 11928–11935.

Jarosz, D.F., Brown, J.C.S., Walker, G.A., Datta, M.S., Ung, W.L., Lancaster, A.K., Rotem, A., Chang, A., Newby, G.A., Weitz, D.A., et al. (2014). Cross-kingdom chemical communication drives a heritable, mutually beneficial prion-based transformation of metabolism. Cell *158*, 1083–1093.

Jellinger, K.A. (2008). A critical reappraisal of current staging of Lewy-related pathology in human brain. Acta Neuropathol. *116*, 1–16.

Kayatekin, C., Matlack, K.E., Hesse, W.R., Guan, Y., Chakrabortee, S., Russ, J., Wanker, E.E., Shah, J.V., and Lindquist, S. (2014). Prion-like proteins sequester and suppress the toxicity of huntingtin exon 1. Proc. Natl. Acad. Sci. USA. *111*, 12085–12090.

Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W., and Glabe, C.G. (2003). Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science *300*, 486–489.

Keleman, K., Krüttner, S., Alenius, M., and Dickson, B.J. (2007). Function of the Drosophila CPEB protein Orb2 in long-term courtship memory. Nat. Neurosci. *10*, 1587–1593.

Keogh, M.J., Kurzawa-Akanbi, M., Griffin, H., Douroudis, K., Ayers, K.L., Hussein, R.I., Hudson, G., Pyle, A., Cordell, H.J., Attems, J., et al. (2016). Exome sequencing in dementia with Lewy bodies. Transl. Psychiatry. *6*, e728.

Khan, M.R., Li, L., Pérez-Sánchez, C., Saraf, A., Florens, L., Slaughter, B.D., Unruh, J.R., and Si, K. (2015). Amyloidogenic Oligomerization Transforms Drosophila Orb2 from a Translation Repressor to an Activator. Cell *163*, 1468–1483.

Khurana, V., and Lindquist, S. (2010). Modelling neurodegeneration in Saccharomyces cerevisiae: why cook with baker's yeast? Nat. Rev. Neurosci. *11*, 436–449.

Khurana, V., Tardiff, D.F., Chung, C.Y., and Lindquist, S. (2015). Toward stem cell-based phenotypic screens for neurodegenerative diseases. Nat. Rev. Neurol. *11*, 339–350.

Khurana, V., Peng, J., Chung, C.Y., Auluck, P.K., Fanning, S., Tardiff, D.F., Bartels, T., Koeva, M., Eichhorn, S.W., Benyamini, H., et al. (2017). Genome-Scale Networks Link Neurodegenerative Disease Genes to α-Synuclein through Specific Molecular Pathways. Cell Syst. *4*, 157–170.e14.

Kim, Y.E., Hipp, M.S., Bracher, A., Hayer-Hartl, M., and Hartl, F.U. (2013). Molecular chaperone functions in protein folding and proteostasis. Annu. Rev. Biochem. *82*, 323–355.

King, C.Y., and Diaz-Avalos, R. (2004). Protein-only transmission of three yeast prion strains. Nature *428*, 319–323.

King, C.Y., Tittmann, P., Gross, H., Gebert, R., Aebi, M., and Wüthrich, K. (1997). Prion-inducing domain 2-114 of yeast Sup35 protein transforms in vitro into amyloid-like filaments. Proc. Natl. Acad. Sci. USA *94*, 6618–6622.

Knowles, T.P., Vendruscolo, M., and Dobson, C.M. (2014). The amyloid state and its association with protein misfolding diseases. Nat. Rev. Mol. Cell Biol. *15*, 384–396.

Krishnan, R., and Lindquist, S.L. (2005). Structural insights into a yeast prion illuminate nucleation and strain diversity. Nature *435*, 765–772.

Krüttner, S., Stepien, B., Noordermeer, J.N., Mommaas, M.A., Mechtler, K., Dickson, B.J., and Keleman, K. (2012). Drosophila CPEB Orb2A mediates memory independent of Its RNA-binding domain. Neuron *76*, 383–395.

Kuemmerle, S., Gutekunst, C.A., Klein, A.M., Li, X.-J., Li, S.H., Beal, M.F., Hersch, S.M., and Ferrante, R.J. (1999). Huntington aggregates may not predict neuronal death in Huntington's disease. Ann. Neurol. *46*, 842–849.

La Spada, A.R., Fu, Y.H., Sopher, B.L., Libby, R.T., Wang, X., Li, L.Y., Einum, D.D., Huang, J., Possin, D.E., Smith, A.C., et al. (2001). Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. Neuron *31*, 913–927.

Labbadia, J., and Morimoto, R.I. (2015). The biology of proteostasis in aging and disease. Annu. Rev. Biochem. *84*, 435–464.

Lacroute, F. (1971). Non-Mendelian mutation allowing ureidosuccinic acid uptake in yeast. J. Bacteriol. *106*, 519–522.

Lashuel, H.A., Overk, C.R., Oueslati, A., and Masliah, E. (2013). The many faces of α-synuclein: from structure and toxicity to therapeutic target. Nat. Rev. Neurosci. *14*, 38–48.

Laurén, J., Gimbel, D.A., Nygaard, H.B., Gilbert, J.W., and Strittmatter, S.M. (2009). Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. Nature *457*, 1128–1132.

Lee, B.-H., Lee, M.J., Park, S., Oh, D.-C., Elsasser, S., Chen, P.-C., Gartner, C., Dimova, N., Hanna, J., Gygi, S.P., et al. (2010). Enhancement of proteasome activity by a small-molecule inhibitor of USP14. Nature *467*, 179–184.

Lee, J.-G., Takahama, S., Zhang, G., Tomarev, S.I., and Ye, Y. (2016). Unconventional secretion of misfolded proteins promotes adaptation to proteasome dysfunction in mammalian cells. Nat. Cell Biol. *18*, 765–776.

Li, J.-Y., Englund, E., Holton, J.L., Soulet, D., Hagell, P., Lees, A.J., Lashley, T., Quinn, N.P., Rehncrona, S., Björklund, A., et al. (2008). Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat. Med. *14*, 501–503.

Li, P., Banjade, S., Cheng, H.C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J.V., King, D.S., Banani, S.F., et al. (2012). Phase transitions in the assembly of multivalent signalling proteins. Nature *483*, 336–340.

Lim, J., Crespo-Barreto, J., Jafar-Nejad, P., Bowman, A.B., Richman, R., Hill, D.E., Orr, H.T., and Zoghbi, H.Y. (2008). Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature *452*, 713–718.

Lindquist, S., and Craig, E.A. (1988). The heat-shock proteins. Annu. Rev. Genet. *22*, 631–677.

Luk, K.C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J.Q., and Lee, V.M.-Y. (2012). Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science *338*, 949–953.

Majumdar, A., Cesario, W.C., White-Grindley, E., Jiang, H., Ren, F., Khan, M.R., Li, L., Choi, E.M.-L., Kannan, K., Guo, F., et al. (2012). Critical role of amyloid-like oligomers of Drosophila Orb2 in the persistence of memory. Cell *148*, 515–529.

Mao, X., Ou, M.T., Karuppagounder, S.S., Kam, T.-I., Yin, X., Xiong, Y., Ge, P., Umanah, G.E., Brahmachari, S., Shin, J.-H., et al. (2016). Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3. Science *353*, aah3374.

Masison, D.C., and Wickner, R.B. (1995). Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells. Science *270*, 93–95.

McGlinchey, R.P., Kryndushkin, D., and Wickner, R.B. (2011). Suicidal [PSI+] is a lethal yeast prion. Proc. Natl. Acad. Sci. USA *108*, 5337–5341.

Melentijevic, I., Toth, M.L., Arnold, M.L., Guasp, R.J., Harinath, G., Nguyen, K.C., Taub, D., Parker, J.A., Neri, C., Gabel, C.V., et al. (2017). C. elegans neurons jettison protein aggregates and mitochondria under neurotoxic stress. Nature *542*, 367–371.

Meyer-Luehmann, M., Coomaraswamy, J., Bolmont, T., Kaeser, S., Schaefer, C., Kilger, E., Neuenschwander, A., Abramowski, D., Frey, P., Jaton, A.L., et al. (2006). Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science *313*, 1781–1784.

Morales, R., Duran-Aniotz, C., Castilla, J., Estrada, L.D., and Soto, C. (2012). De novo induction of amyloid-β deposition in vivo. Mol. Psychiatry *17*, 1347–1353.

Morgan, D.O. (1996). The dynamics of cyclin dependent kinase structure. Curr. Opin. Cell Biol. *8*, 767–772.

Murray, D.T., Kato, M., Lin, Y., Thurber, K.R., Hung, I., McKnight, S.L., and Tycko, R. (2017). Structure of FUS Protein Fibrils and Its Relevance to Self-Assembly and Phase Separation of Low-Complexity Domains. Cell *171*, 615–627.

Musacchio, T., Rebenstorff, M., Fluri, F., Brotchie, J.M., Volkmann, J., Koprich, J.B., and Ip, C.W. (2017). Subthalamic nucleus deep brain stimulation is neuroprotective in the A53T α-synuclein Parkinson's disease rat model. Ann. Neurol. *81*, 825–836.

Nathan, D.F., and Lindquist, S. (1995). Mutational analysis of Hsp90 function: interactions with a steroid receptor and a protein kinase. Mol. Cell. Biol. *15*, 3917–3925.

Orr, H.T. (2012). Polyglutamine neurodegeneration: expanded glutamines enhance native functions. Curr. Opin. Genet. Dev. *22*, 251–255.

Osherovich, L.Z., and Weissman, J.S. (2001). Multiple Gln/Asn-rich prion domains confer susceptibility to induction of the yeast [PSI(+)] prion. Cell *106*, 183–194.

Osterberg, V.R., Spinelli, K.J., Weston, L.J., Luk, K.C., Woltjer, R.L., and Unni, V.K. (2015). Progressive aggregation of alpha-synuclein and selective degeneration of lewy inclusion-bearing neurons in a mouse model of parkinsonism. Cell Rep. *10*, 1252–1260.

Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., Stoynov, S., Mahamid, J., Saha, S., Franzmann, T.M., et al. (2015). A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell *162*, 1066–1077.

Patino, M.M., Liu, J.J., Glover, J.R., and Lindquist, S. (1996). Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. Science *273*, 622–626.

Pearce, M.M. (2017). Prion-like transmission of pathogenic protein aggregates in genetic models of neurodegenerative disease. Curr. Opin. Genet. Dev. *44*, 149–155.

Peelaerts, W., Bousset, L., Van der Perren, A., Moskalyuk, A., Pulizzi, R., Giugliano, M., Van den Haute, C., Melki, R., and Baekelandt, V. (2015). α-Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature *522*, 340–344.

Pratt, W.B., Morishima, Y., Murphy, M., and Harrell, M. (2006). Chaperoning of glucocorticoid receptors. Handb. Exp. Pharmacol. (172), 111–138.

Prusiner, S.B. (1982). Novel proteinaceous infectious particles cause scrapie. Science *216*, 136–144.

Prusiner, S.B., McKinley, M.P., Bowman, K.A., Bolton, D.C., Bendheim, P.E., Groth, D.F., and Glenner, G.G. (1983). Scrapie prions aggregate to form amyloid-like birefringent rods. Cell *35*, 349–358.

Prusiner, S.B., Woerman, A.L., Mordes, D.A., Watts, J.C., Rampersaud, R., Berry, D.B., Patel, S., Oehler, A., Lowe, J.K., Kravitz, S.N., et al. (2015). Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism. Proc. Natl. Acad. Sci. USA *112*, E5308–E5317.

Qi, C., Varga, S., Oh, S.J., Lee, C.J., and Lee, D. (2017). Optogenetic Rescue of Locomotor Dysfunction and Dopaminergic Degeneration Caused by Alpha-Synuclein and EKO Genes. Exp. Neurobiol. *26*, 97–103.

Rabouille, C. (2017). Pathways of Unconventional Protein Secretion. Trends Cell Biol. *27*, 230–240.

Reyes, J.F., Rey, N.L., Bousset, L., Melki, R., Brundin, P., and Angot, E. (2014). Alpha-synuclein transfers from neurons to oligodendrocytes. Glia *62*, 387–398.

Riback, J.A., Katanski, C.D., Kear-Scott, J.L., Pilipenko, E.V., Rojek, A.E., Sosnick, T.R., and Drummond, D.A. (2017). Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response. Cell *168*, 1028–1040.e19.

Ross, E.D., Baxa, U., and Wickner, R.B. (2004). Scrambled prion domains form prions and amyloid. Mol. Cell. Biol. *24*, 7206–7213.

Saupe, S.J. (2007). A short history of small s: a prion of the fungus Podospora anserina. Prion *1*, 110–115.

Seeley, W.W., Crawford, R.K., Zhou, J., Miller, B.L., and Greicius, M.D. (2009). Neurodegenerative diseases target large-scale human brain networks. Neuron *62*, 42–52.

Selkoe, D.J. (2003). Folding proteins in fatal ways. Nature *426*, 900–904.

Serio, T.R., and Lindquist, S.L. (2000). Protein-only inheritance in yeast: something to get [PSI+]-ched about. Trends Cell Biol. *10*, 98–105.

Serio, T.R., Cashikar, A.G., Kowal, A.S., Sawicki, G.J., Moslehi, J.J., Serpell, L., Arnsdorf, M.F., and Lindquist, S.L. (2000). Nucleated conformational conversion and the replication of conformational information by a prion determinant. Science *289*, 1317–1321.

Sevigny, J., Chiao, P., Bussière, T., Weinreb, P.H., Williams, L., Maier, M., Dunstan, R., Salloway, S., Chen, T., Ling, Y., et al. (2016). The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature *537*, 50–56.

Shearin, H., and Bessen, R.A. (2014). Axonal and transynaptic spread of prions. J. Virol. *88*, 8640–8655.

Shorter, J., and Lindquist, S. (2005). Prions as adaptive conduits of memory and inheritance. Nat. Rev. Genet. *6*, 435–450.

Si, K., Lindquist, S., and Kandel, E.R. (2003). A neuronal isoform of the aplysia CPEB has prion-like properties. Cell *115*, 879–891.

Si, K., Choi, Y.B., White-Grindley, E., Majumdar, A., and Kandel, E.R. (2010). Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. Cell *140*, 421–435.

Soragni, A., Janzen, D.M., Johnson, L.M., Lindgren, A.G., Thai-Quynh Nguyen, A., Tiourin, E., Soriaga, A.B., Lu, J., Jiang, L., Faull, K.F., et al. (2016). A Designed Inhibitor of p53 Aggregation Rescues p53 Tumor Suppression in Ovarian Carcinomas. Cancer Cell *29*, 90–103.

Stopschinski, B.E., and Diamond, M.I. (2017). The prion model for progression and diversity of neurodegenerative diseases. Lancet Neurol. *16*, 323–332.

Surmeier, D.J., Obeso, J.A., and Halliday, G.M. (2017). Selective neuronal vulnerability in Parkinson disease. Nat. Rev. Neurosci. *18*, 101–113.

Tanaka, M., Chien, P., Naber, N., Cooke, R., and Weissman, J.S. (2004). Conformational variations in an infectious protein determine prion strain differences. Nature *428*, 323–328.

Tanaka, M., Collins, S.R., Toyama, B.H., and Weissman, J.S. (2006). The physical basis of how prion conformations determine strain phenotypes. Nature *442*, 585–589.

Ter-Avanesyan, M.D., Dagkesamanskaya, A.R., Kushnirov, V.V., and Smirnov, V.N. (1994). The SUP35 omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [psi+] in the yeast Saccharomyces cerevisiae. Genetics *137*, 671–676.

Toyama, B.H., Kelly, M.J., Gross, J.D., and Weissman, J.S. (2007). The structural basis of yeast prion strain variants. Nature *449*, 233–237.

Treusch, S., and Lindquist, S. (2012). An intrinsically disordered yeast prion arrests the cell cycle by sequestering a spindle pole body component. J. Cell Biol. *197*, 369–379.

Treusch, S., Cyr, D.M., and Lindquist, S. (2009). Amyloid deposits: protection against toxic protein species? Cell Cycle *8*, 1668–1674.

True, H.L., and Lindquist, S.L. (2000). A yeast prion provides a mechanism for genetic variation and phenotypic diversity. Nature *407*, 477–483.

True, H.L., Berlin, I., and Lindquist, S.L. (2004). Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits. Nature *431*, 184–187.

Tsuda, H., Jafar-Nejad, H., Patel, A.J., Sun, Y., Chen, H.-K., Rose, M.F., Venken, K.J.T., Botas, J., Orr, H.T., Bellen, H.J., and Zoghbi, H.Y. (2005). The AXH domain of Ataxin-1 mediates neurodegeneration through its interaction with Gfi-1/Senseless proteins. Cell *122*, 633–644.

Uptain, S.M., Sawicki, G.J., Caughey, B., and Lindquist, S. (2001). Strains of [PSI(+)] are distinguished by their efficiencies of prion-mediated conformational conversion. EMBO J. *20*, 6236–6245.

Volpicelli-Daley, L.A., Gamble, K.L., Schultheiss, C.E., Riddle, D.M., West, A.B., and Lee, V.M.-Y. (2014). Formation of α-synuclein Lewy neurite-like aggregates in axons impedes the transport of distinct endosomes. Mol. Biol. Cell *25*, 4010–4023.

Volpicelli-Daley, L.A., Abdelmotilib, H., Liu, Z., Stoyka, L., Daher, J.P.L., Milnerwood, A.J., Unni, V.K., Hirst, W.D., Yue, Z., Zhao, H.T., et al. (2016). G2019S-LRRK2 Expression Augments α-Synuclein Sequestration into Inclusions in Neurons. J. Neurosci. *36*, 7415–7427.

Wallace, E.W., Kear-Scott, J.L., Pilipenko, E.V., Schwartz, M.H., Laskowski, P.R., Rojek, A.E., Katanski, C.D., Riback, J.A., Dion, M.F., Franks, A.M., et al. (2015). Reversible, Specific, Active Aggregates of Endogenous Proteins Assemble upon Heat Stress. Cell *162*, 1286–1298.

Walker, L.C., and Jucker, M. (2015). Neurodegenerative diseases: expanding the prion concept. Annu. Rev. Neurosci. *38*, 87–103.

Walsh, D.M., and Selkoe, D.J. (2016). A critical appraisal of the pathogenic protein spread hypothesis of neurodegeneration. Nat. Rev. Neurosci. *17*, 251–260.

Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., and Selkoe, D.J. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature *416*, 535–539.

Wang, G., and Fersht, A.R. (2015). Mechanism of initiation of aggregation of p53 revealed by Φ-value analysis. Proc. Natl. Acad. Sci. USA *112*, 2437–2442.

Wang, M., and Kaufman, R.J. (2016). Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature *529*, 326–335.

Wang, F., Wang, X., Yuan, C.G., and Ma, J. (2010). Generating a prion with bacterially expressed recombinant prion protein. Science *327*, 1132–1135.

Warrick, J.M., Chan, H.Y., Gray-Board, G.L., Chai, Y., Paulson, H.L., and Bonini, N.M. (1999). Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. Nat. Genet. *23*, 425–428.

Watts, J.C., Balachandran, A., and Westaway, D. (2006). The expanding universe of prion diseases. PLoS Pathog. *2*, e26.

Weisberg, S.J., Lyakhovetsky, R., Werdiger, A.-C., Gitler, A.D., Soen, Y., and Kaganovich, D. (2012). Compartmentalization of superoxide dismutase 1 (SOD1G93A) aggregates determines their toxicity. Proc. Natl. Acad. Sci. USA *109*, 15811–15816.

Wickner, R.B. (1994). [URE3] as an altered URE2 protein: evidence for a prion analog in Saccharomyces cerevisiae. Science *264*, 566–569.

Wickner, R.B., Edskes, H.K., Shewmaker, F., Nakayashiki, T., Engel, A., McCann, L., and Kryndushkin, D. (2007). Yeast prions: evolution of the prion concept. Prion *1*, 94–100.

Wolff, S., Weissman, J.S., and Dillin, A. (2014). Differential scales of protein quality control. Cell *157*, 52–64.

Wu, H., and Fuxreiter, M. (2016). The Structure and Dynamics of Higher-Order Assemblies: Amyloids, Signalosomes, and Granules. Cell *165*, 1055–1066.

Zarranz, J.J., Alegre, J., Gómez-Esteban, J.C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atarés, B., et al. (2004). The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann. Neurol. *55*, 164–173.