Prions: What Are They Good For?

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Abstract

Prions, a self-templating amyloidogenic state of normal cellular proteins such as PrP, have been identified as the basis of a number of disease states, particularly diseases of the nervous system. This finding has led to the notion that protein aggregation, namely prionogenic aggregates and amyloids, is primarily harmful for the organism. However, identification of proteins in a prion-like state that are not harmful and may even be beneficial has begun to change this perception. This review discusses when and how a prion-based protein conformational switch may be utilized to generate a sustained physiological change in response to a transient stimulus.

Contents

INTRODUCTION

When you have eliminated the impossible, whatever remains, however improbable, must be the truth.
–Sir Arthur Conan Doyle, The Sign of the Four

Most proteins assume a single stable conformational state based on their primary amino acid sequence. Prions and prion-like proteins are an interesting exception to this broadly accepted norm in that the same primary amino acid sequence can give rise to at least two distinct, stable conformational states (Prusiner 1998, 2013). Equally remarkable is that one of the conformational states, an amyloidogenic oligomer, is self-sustaining in a dominant manner; once formed inside a cell, the oligomer can recruit monomers to itself and induce their conversion to maintain the oligomeric conformation over time as well as across cell divisions (Prusiner 1998, Prusiner et al. 1983). In mammals, this mechanism is the cause of numerous neurodegenerative diseases (Horwich & Weissman 1997, Kelly 1998, Prusiner 1991). However, in yeast, the same mechanism serves as the basis for specific, nonpathogenic epigenetic heritable phenotypes (Wickner et al. 2007). Recently, many proteins with amyloid and prion-like properties have been discovered in multicellular eukaryotes, from snail to human (Cai et al. 2014, Fowler et al. 2006, Heinrich & Lindquist 2011, Hou et al. 2011, Ishimaru et al. 2003, Maji et al. 2009, Majumdar et al. 2012, Si et al. 2003b, Tariq et al. 2013). The broad phylogenetic distribution of such proteins suggests that proteins with self-sustaining conformational states may be part of an evolutionarily conserved regulatory mechanism involved in normal physiological functions (Fowler et al. 2006, Shorter & Lindquist 2005, Soto 2012).

This review does not discuss the pathogenic or toxic aspects of prions, amyloids, misfolded protein aggregates, or the functional prion-like proteins in yeast and fungi, as many excellent reviews have already been written on this subject (Dobson 2004, Hartl et al. 2011, Lansbury 1997, Liebman & Chernoff 2012, Newby & Lindquist 2013, Wickner et al. 2007). Instead, it primarily focuses on the possibility and implications of prion-like proteins serving normal functions in multicellular eukaryotes. The number of functional prion-like proteins characterized to date is limited; therefore, this review is largely speculative in nature.

A BRIEF HISTORY OF THE DISCOVERY OF PRION AND PRION-LIKE PROTEINS

Belief gets in the way of learning. –Robert Heinlein, *Time Enough for Love*

From their inception, prion diseases have run contrary to established norms (Adams 1970, Liberski 2012). How often has the proposed etiology of a disease been tied to cannibalism, sexual overactivity, or thunderstorms? The prion diseases are a group of neurodegenerative diseases that include kuru, Creutzfeldt-Jakob disease (CJD), and fatal familial insomnia in humans. In other animals, prion diseases include transmissible spongiform encephalopathies, such as scrapie in sheep, chronic wasting disease in mule deer, and mad cow disease (Prusiner 1998). It was not the symptoms of these diseases but the nature of the causative agent that was most unusual: It lacked nucleic acid, which was deemed essential to copy and propagate biological information. Several putative causative agents were entertained, including a self-replicating membrane, a subvirus, a viroid, and spiroplasma (Liberski 2012). Eventually, in the 1980s, Stanley Prusiner and others established that prion diseases are caused by a dominant, self-templating amyloidogenic form of a normal cellular protein: PrP (Aguzzi & Weissmann 1998, Prusiner 1991). The PrP protein is necessary and sufficient for the disease state (Legname et al. 2004). The term prion was coined by Prusiner (1991) to capture the unusual proteinaceous nature of the infectious particle.

For a while, the term prion was associated with a very particular mode of protein-only infection by the PrP protein, and prions were thought to be an anomaly rather than the norm. In the early 1990s, the discovery of protein-based phenotypic inheritance in yeast by Reed Wickner (Wickner 1994, Wickner et al. 1995) changed the way we think about prions and their biological significance. The discovery of a large number of fungal prions serving various physiological functions suggested that prions are neither rare nor always bad (Caudron & Barral 2013, Coustou et al. 1997, Eaglestone et al. 1999, Halfmann et al. 2012, Holmes et al. 2013, Jarosz et al. 2014, Suzuki et al. 2012, True & Lindquist 2000, True et al. 2004). It also became evident that protein-based disease propagation is more prevalent than previously thought and that several disease-causing amyloids, such as Alzheimer aβ42, α-synuclein, and τ, can spread in a manner akin to prions (Cushman et al. 2010, Holmes & Diamond 2014, Polymenidou & Cleveland 2012).

At the heart of prion behavior is a protein's ability to inhabit at least two distinct physical states: a monomeric state and a self-templating, amyloidogenic, oligomeric aggregated state (Eisenberg & Jucker 2012). Solid-state nuclear magnetic resonance (NMR) data and microcrystals of small peptides from prion and prion-like proteins suggest that the oligomeric state has a common cross β-sheet structure. In addition to this structure, several additional criteria have been used to define amyloidogenic prions, such as binding to thioflavin or Congo red; resistance to detergent, chaotropic agents, or proteases; the formation of fibers of a certain dimension; and, importantly, the ability of the in vitro–formed oligomer to induce oligomerization in vivo and induce stable and heritable phenotypic change (Chien et al. 2004, Wiltzius et al. 2009). Some of these are operational definitions of a prion, involving the behavior of a protein in particular experimental conditions. Although operational definitions are commonly used in biology, it has been particularly tricky to settle on one for prion proteins because of their ability to adopt multiple conformational states with distinct biophysical and biochemical properties, both in vitro and in vivo. For example, resistance to the detergent sodium dodecyl sulfate (SDS), urea, high temperature, and proteases varies between prions and sometimes for the same protein under different experimental conditions, as does the size of the oligomers. Are these distinct molecular processes or variations of a common process? What are the similarities between various prion proteins, and how do they differ? Some of this confusion is no different from that which plagued the prion field until the discovery of the distinct physical states of PrPs, explaining both the cause of and variation in prion disease (Chien &

Weissman 2001, Collinge & Clarke 2007). In this review, I discuss examples of functional prions, proteins that fulfill several criteria associated with being a prion, though they do not necessarily fulfill all the criteria associated with being a disease-causing prion.

WHAT CONSTITUTES A FUNCTIONAL PRION?

A functional prion protein shares biophysical properties with toxic prions and amyloids, but they differ in origin, regulation, and consequence. Disease-causing prions or amyloids arise from mutation, truncation, processing, or modification, leading to misfolding or unfolding (**Figure 1**). The misfolding or unfolding of proteins leads to oligomerization and aggregation in an uncontrolled manner. By contrast, the oligomeric, aggregated state of functional prions must arise from normal physiological processes, and therefore conversion to the prion state must be harnessed, particularly in multicellular eukaryotes (**Figure 1**). In unicellular organisms, conversion can be stochastic followed by selection by environmental conditions. However, in multicellular eukaryotes, the prion state must arise in response to an extra- or intracellular signaling event, which restricts its appearance both in space and time. In response to specific physiological signals, the preexisting monomeric nonprion form or the newly synthesized protein must be guided to assume the prion state. In folding energy landscapes, the amyloids occupy the lowest energy level and consequently are extremely stable. Although stability may be useful in certain biological contexts, a certain degree of reversibility is favorable for broader biological applications (Dobson 2004, Hartl et al. 2011). In the case of disease-causing prions, the biological response to such conversion is attempted degradation by the proteolytic or other protein surveillance machinery; failure to remove these proteins leads to the formation of large aggregates (Roth & Balch 2011). For disease-causing prions, the consequence of these physical events is inactivation of the protein and/or gain of toxic function. For functional prions, the prion-like state either evades protein surveillance or is perceived by the cell as normal. For them, the consequence of converting to the prion state need not be inactivation, and the conversion is certainly not toxic. To date, very few proteins fulfill all the criteria needed to qualify as bona fide functional prions.

EXAMPLES OF FUNCTIONAL PRIONS

At this point, it is important to discuss some of the inherent problems with studies of functional or good prions and protein aggregation in general. Many proteins can form aggregates under certain conditions (e.g., overexpression, expression in heterologous systems, expression of part of a protein, expression of a fusion protein, or absence of protein partners), chemical environments (redox state, salt concentration, pH), or temperature (Dobson 2004). In some cases, these protein aggregates have amyloid features (Wang et al. 2008). However, whether a protein can form amyloid or ordered aggregates is distinct from whether a protein can form a self-templating amyloid at physiological concentrations and conditions in relevant cell types. More important is whether a distinct functional consequence can be attributed to the prion-like state. Many cellular proteins form aggregates/oligomers to serve their normal functions (Brangwynne et al. 2009, Han et al. 2012, Kato et al. 2012, Kwon et al. 2013, Lee et al. 2013, Malinovska et al. 2013, Petrovska et al. 2014). Some of the oligomers are stable, though they are not amyloids, whereas other protein assemblies are labile but possess features of amyloids. In other words, various forms of protein assembly exist, some of which fall in the category of prion-like assembly (Harbi & Harrison 2014). Keeping these caveats in mind, I discuss examples in which proteins in a prion-like state serve normal physiological functions. The examples I discuss are primarily recent studies

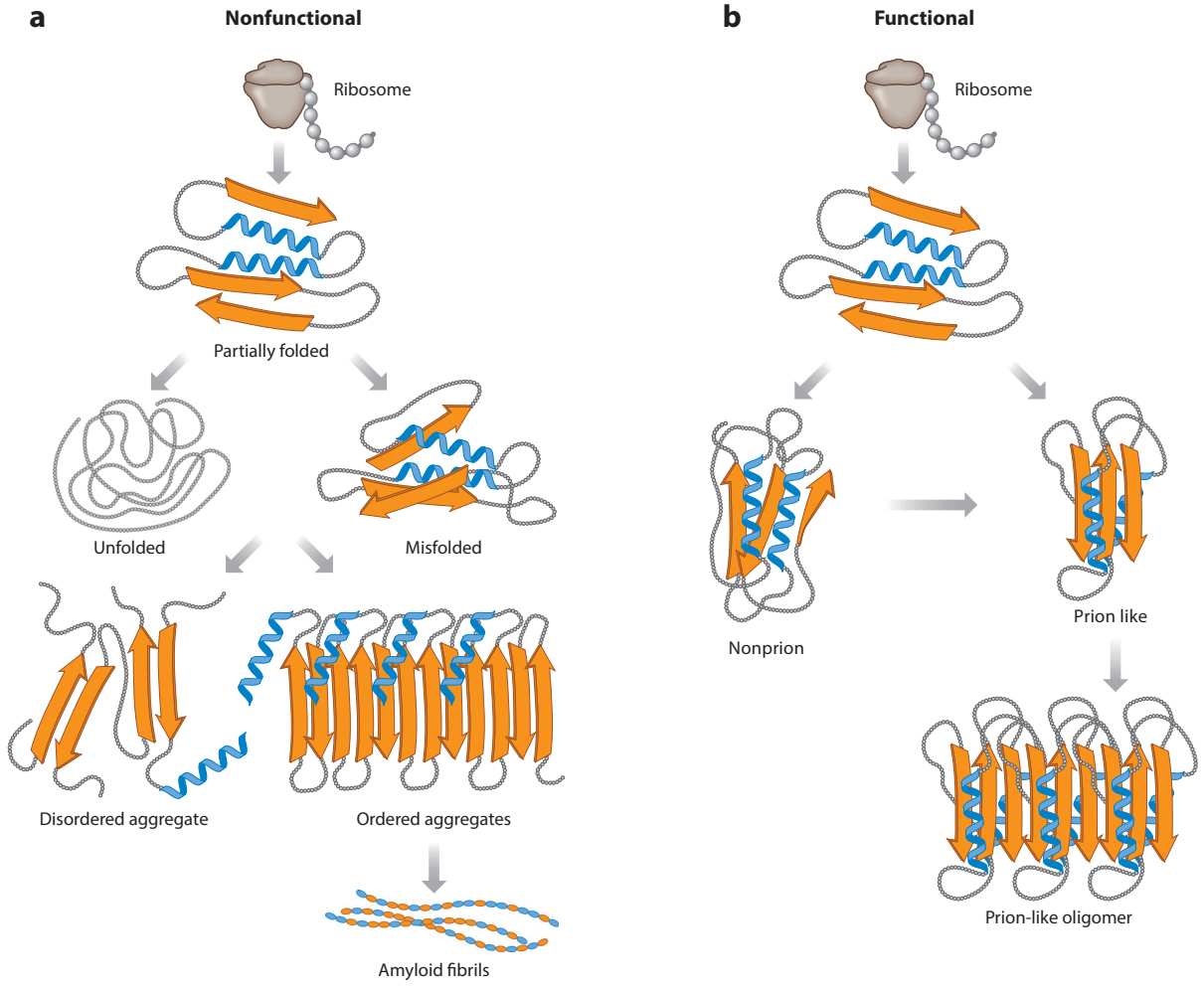

Figure 1

Formation of nonfunctional and functional prion-like aggregates/oligomers. Toxic/nonfunctional amyloids are formed in an unregulated manner. (*a*) If not rescued by chaperones, partially folded, unfolded, or misfolded proteins can take two pathways. In one pathway, they form disordered aggregates, which are often recognized by the proteasome and degraded. In the other pathway, they form ordered amyloid-like aggregates that are energetically extremely stable. (*b*) For functional prions or amyloids, the formation of the aggregated state is regulated or guided. Either the folded, mature nonprion form changes conformation to the prion form or the newly synthesized protein is guided to adopt the prion state. Once the prion conformation is attained, it interacts with the nonprion form to initiate the self-sustaining cycle.

in multicellular eukaryotes, as several excellent reviews have already been written about bacterial and fungal amyloids and prions.

Prion-Like Proteins as a Substrate for Memory

One of the earliest examples of a functional prion-like protein in multicellular eukaryotes is the chance discovery of a neuronal RNA-binding protein involved in long-term synaptic plasticity and memory (Si et al. 2003b). Like other physiological processes, a biochemical basis for long-lasting memory exists (Bailey et al. 2004, Dudai 2002, Lynch & Baudry 1984, Roberts & Flexner

1969). The simplest notion is that external experience creates an internal biochemical trace of that experience in relevant neurons, and this altered biochemical state leads to altered neuronal properties and behavioral output. Such a biochemical trace of long-lasting memory would need to have several distinct properties: (*a*) be engaged by a temporally defined physiological stimulus, (*b*) form in response to some but not all physiological stimuli, (*c*) produce a change in the neuronal properties that elicit appropriate behavioral responses, and (*d*) overcome the natural turnover of individual proteins to enable a persistent change in neuronal properties and behavioral output (Crick 1984).

Protein synthesis in the synapses has emerged as one of the mechanisms to create enduring changes in protein composition and synaptic activity in a stimulus-specific manner (Martin et al. 2000, Richter & Klann 2009, Steward & Schuman 2001). The search for an activity-dependent regulator of synaptic protein synthesis led to the identification of the cytoplasmic polyadenylation element-binding protein (CPEB), a sequence-specific RNA-binding protein (Richter 2007). Surprisingly, the brain-specific form of CPEB has sequence features and conformational properties that resemble prions. Both the neuronal CPEB of *Aplysia*, the sea hare, and its *Drosophila* homolog, Orb2, exist in two states in their respective neuronal contexts: a monomeric state and an amyloidogenic oligomeric state (Majumdar et al. 2012; Si et al. 2003a, 2010; White-Grindley et al. 2014). The oligomeric state of each protein is generated by self-assembly of the monomeric form, and once formed the oligomeric state can stably propagate in a dominant manner.

However, the prion-like state of *Aplysia* CPEB and *Drosophila* Orb2 has features that distinguish it from both pathological prions and nonpathological, nonfunctioning prions. First, in most cases, the prion state is the inactive state of a protein. In the case of *Drosophila* Orb2 and *Aplysia* CPEB, however, the prion-like states retain their biochemical activities, such as the ability to bind mRNA (Majumdar et al. 2012, Raveendra et al. 2013). Second, for most known prions, conversion to the prion state appears to be spontaneous. By contrast, conversion of both *Aplysia* CPEB and *Drosophila* Orb2 to the oligomeric state is regulated by physiological signals: Repeated stimulation with the neurotransmitter serotonin in *Aplysia* or with dopamine and octopamine in *Drosophila* induces oligomerization (Majumdar et al. 2012, Si et al. 2010, White-Grindley et al. 2014). More importantly, inhibition of the amyloidogenic oligomeric state in *Aplysia* blocks persistence of synaptic facilitation and in *Drosophila* blocks the persistence of memory beyond a day (Keleman et al. 2007, Kruttner et al. 2012, Majumdar et al. 2012, Si et al. 2010).

Although most experiences elicit an immediate behavioral response, only a subset is stored as long-lasting memories to guide future behavior. Therefore, to serve as a substrate specifically for long-lasting memory, the prion-like conversion of neuronal CPEB must be regulated in space, time, and a stimulus-specific manner. What regulates the engagement of the prion-like state of neuronal CPEB, and how? The *Drosophila* Orb2 protein provides some clues to how this process may be regulated (White-Grindley et al. 2014). The *Orb2* gene produces two protein isoforms: Orb2A and Orb2B. Although both isoforms carry the prion-like domain, the extremely rare Orb2A protein has a very high propensity to form amyloids and acts as a seed to induce oligomerization of Orb2B. This seeding mechanism suggests that the amount and location of Orb2A are key determinants of when and where prion-like conversion occurs, and phosphorylation-dephosphorylation via protein-phosphatase 2A and Lim kinase are known to regulate the stability and abundance of Orb2A (White-Grindley et al. 2014). In mammals, the CPEB family member CPEB3, a potential prion-like protein, is sumoylated (Pavlopoulos et al. 2011). This raises the possibility that other protein translation modifications can also regulate the activity and/or conformational switch of CPEB.

The prion-like properties of neuronal CPEB in *Aplysia* and *Drosophila* provide a plausible biochemical mechanism to allow perpetuation of long-term synapse-specific changes and persistence of memory. According to this model, CPEB can assume at least two conformational

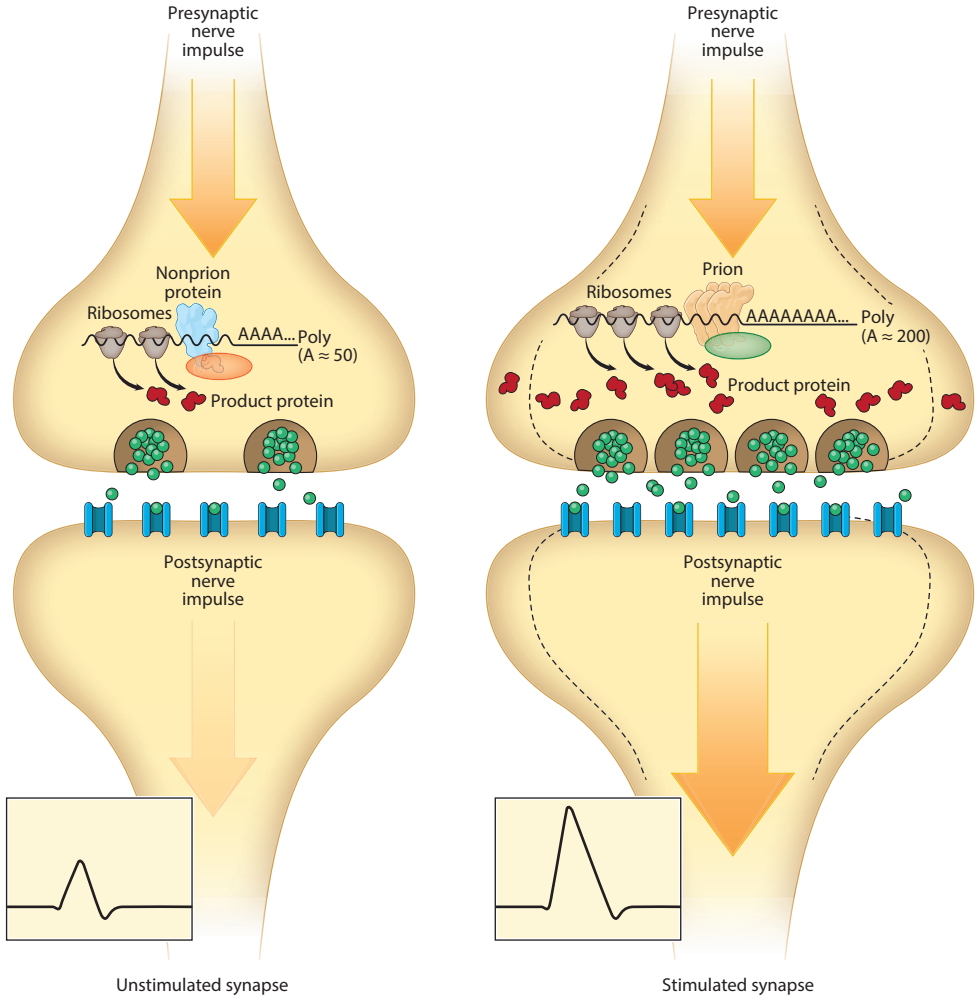

Figure 2

Prion-like state of neuronal cytoplasmic polyadenylation element binding protein (CPEB) as a potential stable and self-sustaining mark of the activated synapse. In this model, in the unstimulated synapse (*left*), the RNA-binding protein CPEB in its nonprion form binds to the target mRNA and keeps it in a translationally repressed state. In response to specific stimuli, the CPEB is converted to the self-sustaining aggregated state. In the aggregated state (*right*), it either lacks the inhibitory function or becomes a translation activator. Because one of the major functions of CPEB proteins is to regulate the polyA tail length of mRNA, it is feasible that translation repression and activation is carried out by altering the mRNA's polyA tail length. The enhanced translation can lead to an increase in synaptic transmission and synaptic growth. The model depicted here is based primarily on the work in *Aplysia* and *Drosophila*.

states: a monomer and a self-sustaining, stable, amyloidogenic oligomer. In a naïve synapse, the monomeric form represses protein synthesis (**Figure 2**). Synaptic activation leads to the conversion of CPEB to the dominant oligomeric state. Unlike conventional prions, the dominant state of CPEB is either active or devoid of the inhibitory function of the monomeric form. This dominant state creates a self-sustaining active state of protein synthesis only at the activated synapse, allowing for the maintenance of synaptic changes and memory over time.

The possibility of a biochemical switch (Crick 1984, Lisman 1994, Lynch & Baudry 1984, Roberts & Flexner 1969), including a prion switch (Tompa & Friedrich 1998), underlying long-term memory has been anticipated for some time. What remains unclear is the exact nature of such a switch. Is the prion-like state of neuronal CPEB a plausible biochemical switch for long-lasting memory? First, it can be engaged by behavioral training that produces long-term memory. Second, regulated prion-like conversion can confer the specificity and selectivity of long-term memory. Third, CPEB-dependent repression and activation of synaptic mRNAs can alter the protein composition of the synapse, thereby altering synaptic properties and neuronal output. Finally, once triggered, the stable and dominant self-perpetuating capacity of the prion-like oligomer should outlast protein turnover to sustain memory over long periods of time. If this assumption is correct, it also implies that the long-term stabilization of memory requires a specific and unique biochemical process and therefore should be amenable to selective manipulation.

However, examining the biochemical basis of memory is challenging. Memory is a higher-order process, and stored information must be accessed and retrieved in an appropriate context to elicit proper behavioral responses. Therefore, memory must involve several processes, including multiple biochemical events serving functions distinct from memory storage. Not surprisingly, perturbations of a large number of biochemical processes affect memory, rendering it difficult to delineate exactly which step of memory depends on a particular biochemical event. Therefore, some outstanding questions remain with regard to prion-like CPEB serving as a substrate for long-lasting memory: Does persistence of memory require the continued presence of the prion-like state? Is the presence of the prion-like state predictive of long-lasting memory? Does decay of memory coincide with the disappearance of the prion-like state, and can a transient memory be stabilized by artificial recruitment of the prion-like state?

Prions in Immune Response

Another clear example of a functional prion-like protein in multicellular eukaryotes is the mitochondrial transmembrane antiviral signaling (MAVS) protein (Cai & Chen 2014, Cai et al. 2014, Hou et al. 2011). Viral infection triggers an innate antiviral response. Part of this response is initiated by the RIG-1 protein, a cytoplasmic RNA helicase that recognizes viral double-stranded RNA (dsRNA). MAVS, present in the mitochondrial membrane, links RIG-1 to downstream signaling molecules, which in turn activate the transcription factors eRF3 and NF-κB. Activation of eRF3 and NF-κB induces expression of interferons (**Figure 3**). Intriguingly, viral infection or in vitro activation of RIG-1 causes aggregation of MAVS into a detergent- and protease-resistant form. More importantly, MAVS aggregates, formed in vitro by a truncated form, are sufficient to induce aggregation of the full-length endogenous MAVS, and aggregation of MAVS, in turn, activates downstream transcription factors. In other words, MAVS, in response to viral infection, undergoes a conformational conversion, and this new form of the protein has the capacity to self-propagate both its own conformational state and the functions associated with it.

The prion-like properties of MAVS also illustrate how conventional thinking about prions does not capture the whole picture. Although electron microscopy (EM) shows the prion-like domain of MAVS forms amyloid-like fibers, and the domain can functionally substitute for a canonical prion-like domain, the self-templating state of MAVS does not stain with Congo red or thioflavin T, properties often associated with prions (Hou et al. 2011, Xu et al. 2014). In addition, the prion-forming caspase activation and recruitment domain (CARD) does not have structural features that are normally thought to be associated with prion-like proteins (e.g., a bias for certain amino acids, such as Q, N, and G, or low complexity). These observations highlight a few things. First, functional prion-like behavior may arise from seemingly different structural states. Second,

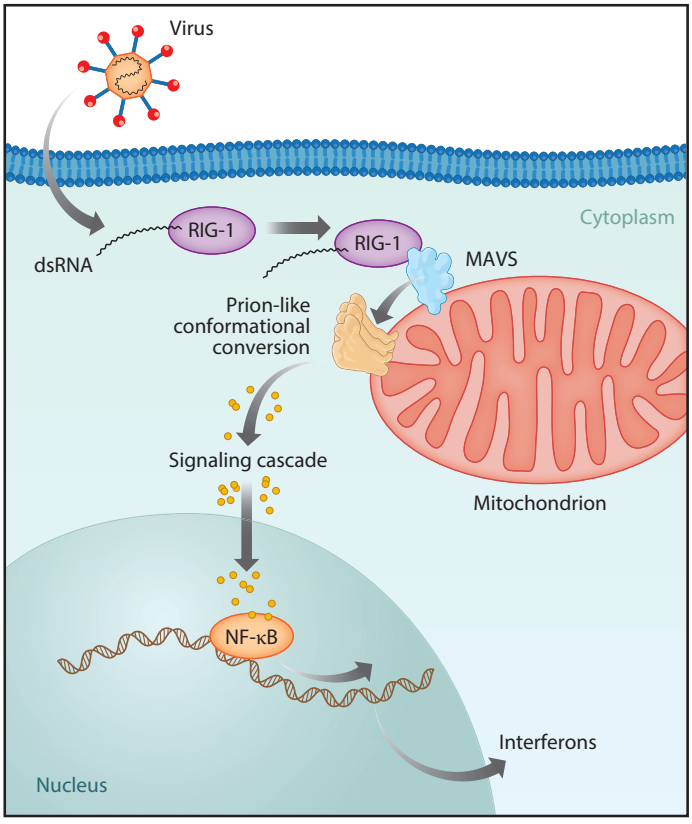

Figure 3

Prion-like conversion of mitochondrial transmembrane antiviral signaling (MAVS) protein activates antiviral signaling. MAVS is a mitochondrial membrane protein that mediates expression of antiviral genes. The presence of double-stranded RNA (dsRNA) in the cell initiates the prion-like aggregation of MAVS, and aggregation of MAVS renders the protein active.

it may be difficult to identify which proteins can assume a functional prion-like state from amino acid sequences alone. Finally, we lack knowledge regarding the structural features that confer self-propagation.

One must also wonder about the advantage of employing a prion-like mechanism to mount an innate immune response. As Cai et al. (2014) have pointed out, perhaps such a mechanism ensures sensitivity and acts as a signaling platform: Even a very low level of MAVS activation would ensure rapid conversion and a full response or the spread of the response to neighboring cells. However, such a mechanism raises further questions. The persistent self-assembled form of MAVS would continue to signal even after the clearance of infection. Therefore, the signal must presumably be terminated following viral clearance. How is the aggregated MAVS removed?

POSSIBILITIES AND IMPLICATIONS

How many times must a man look up before he can see the sky? –Bob Dylan, "Blowin' in the Wind"

Perhaps one of the most influential papers on prions is by Griffith (1967, p. 1,043). In this paper, he suggests how a polypeptide could self-replicate without causing the "whole theoretical structure of molecular biology to come tumbling down." He proposes the somewhat heretical model that

proteins can self-replicate based on the pathophysiology of the scrapie disease and almost nonexistent molecular analysis. Although in hindsight speculation can be considered either prescient or meaningless, it is nonetheless important to bind empirical observations to conceptual frameworks and to generate experimentally verifiable hypotheses.

Consider for a moment what new mechanistic features have emerged from the limited but increasing number of prion-like proteins identified and whether we can use these insights to envision biological contexts in which such features may be useful. First, the self-sustaining conformational state of prions is a novel mechanism for how altered activity states of proteins can be maintained within and across cell division. Second, prion-like properties allow proteins to adopt different stable conformations without chemical modification and to switch between distinct activity states, effectively serving as a mechanism of posttranslational control of protein activity. Because conformational conversion is an intrinsic property of prion-like proteins, once the self-sustaining conformation is attained, additional factors are no longer required to maintain the altered activity state. Third, given that these proteins can assume multiple conformational states, they can, in theory, also produce multiple activity states, therefore generating variation in activity (**Figure 4**). Fourth, because it is a protein conformation–based mechanism, any change in the environment, external or internal, can rapidly induce functional change (**Figure 5**). Therefore,

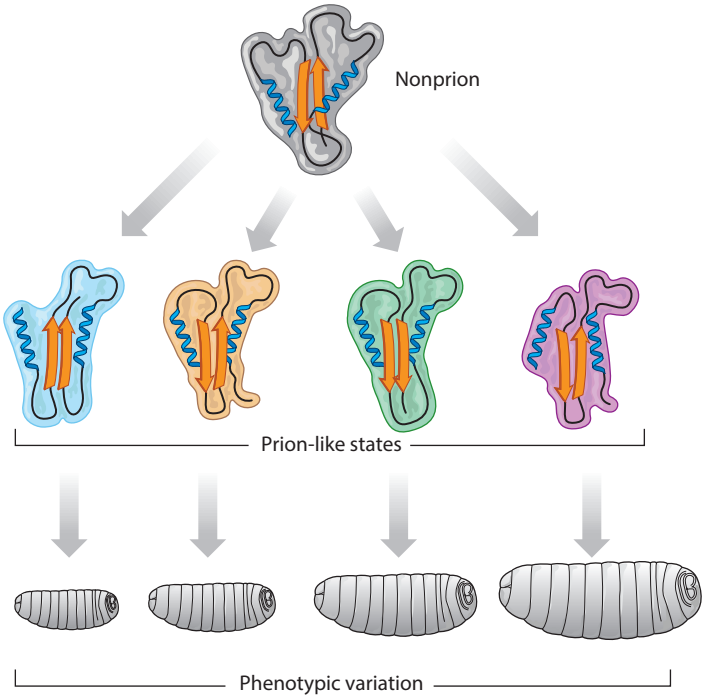

Figure 4

Prion-like conversion can create functional and phenotypic diversity. One of the most intriguing features of prions and prion-like proteins is that the same primary amino acid sequence can give rise to aggregated states that are conformationally distinct. These distinct states are defined as prion strains. Conformational variants have a common cross-β sheet structure, but they are divergent enough to not cross templates. In the case of functional prions, such structural variation can cause changes in functional output. Because each functional state is self-sustaining, variants can also create phenotypic diversity, thereby increasing the coding capacity of a fixed genome.

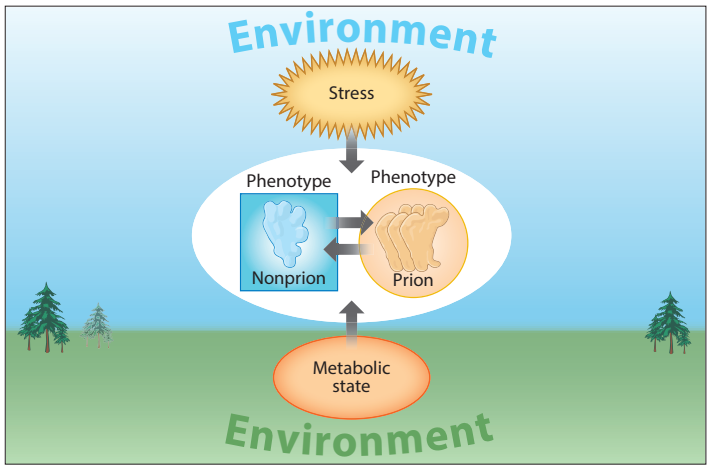

Figure 5

Prion-like conversion can act as a sensor for environmental change. Proteins are poised to respond to environmental change. Change in protein activity causes changes in gene expression and cellular processes. Therefore, it is conceivable that prion-based conformational switches can be rapidly engaged to directly respond to environmental change.

such proteins are poised to immediately respond to and integrate environmental cues to produce a sustained response. Prion properties are driven entirely by protein-based mechanisms. Finally, the prion-like state could be reversible either by altering or removing the self-templating form or the monomeric substrate. Under what circumstances would these features of a protein-based switch be useful? How would a physiological process benefit from utilizing an amyloid-like state, one of the most stable protein structural states? Broadly, such a mechanism would be useful when a transient stimulus needs to result in a sustained and/or heritable response or when it is necessary to attain metastable functional states.

Prions as an Epigenetic Mechanism

One of the most profound insights in biology is that natural selection leads to the preservation of favorable variation and the rejection of injurious variation; however, as Darwin [2003 (1859), p. 157] pointed out, "our ignorance of the laws of variation is profound." Mendel's laws of inheritance, the discovery of DNA as genetic material, and the characterization of its structure provided a mechanism for how genetic information can be copied and maintained and for how phenotypic variability can be produced. Although alteration in the DNA sequence is the primary driver for heritable phenotypic change, increasing evidence shows that heritable changes can also be achieved in an epigenetic manner, without changing the nucleotide sequence. Epigenetic mechanisms are important for adaptive changes within the lifetime of an organism and, in some cases, across generations. Unlike Darwin, Lamarck has traditionally been vilified in textbooks, but he was the first naturalist to envision adaptive evolutionary changes. Such adaptive heritable changes can be seen in unicellular fungi and more complex multicellular organisms and encompass alterations in the size, shape, and function of various organs; metabolism; and animal behavior (Franklin et al. 2010, Herb et al. 2012, Lim & Brunet 2013, Seong et al. 2011, Zeybel et al. 2012). Some of these changes persist for several generations but are not necessarily permanent. However, the molecular basis for adaptable changes in traits and their inheritance remains unclear. Among numerous

epigenetic mechanisms, DNA methylation and noncoding mRNA have emerged as primary mediators of such heritable phenotypes. In yeast, prions underlie adaptive and heritable change in traits (Eaglestone et al. 1999, Halfmann et al. 2012, Jarosz et al. 2014, True et al. 2004); here, I discuss such a possibility in multicellular eukaryotes.

Prions in Cellular Memory and Animal Development

One can safely assume that processes that affect animal development greatly influence the emergence of new forms and functions (Reik 2007). A key event in animal development is cellular differentiation and the acquisition of new cell fate. Both of these processes require the coordinated activation and inactivation of genes to yield a unique transcriptional profile, ultimately leading to a new cellular identity. Once a particular cell fate is achieved, the unique transcriptional profile associated with that cell fate is believed to be maintained epigenetically (Ringrose & Paro 2007). These epigenetic states are not only inherited across cell division but in some cases can be inherited across generations via germ cells (Lim & Brunet 2013). What are the molecular mechanisms that allow this cell type–specific, heritable change in gene expression over a long period?

Presently, epigenetic mechanisms for lineage-specific expression are thought to occur in three steps: (*a*) initiation of transcription factor–dependent gene expression; (*b*) transformation of this gene expression state into a heritable form, to establish cellular memory; and (*c*) maintenance of this cellular memory over time. Consequently, epigenetic factors should themselves fulfill a specific set of criteria: They should sense the altered transcription state, mark specific loci, and perpetuate the mark in newly synthesized DNA to recreate the active or repressed state (Sarge & Park-Sarge 2009, Zaidi et al. 2010).

To date, the epigenetic mechanisms for marking specific loci for cellular memory include locus-specific recruitment of transcription factors, modification of histones (such as methylation, acetylation, and ubiquitination), modification of DNA (such as methylation), and small RNAs (Ringrose & Paro 2007). However, currently identified epigenetic mechanisms do not fully address how, after nucleosome dispersal during DNA replication along with the attendant loss of DNA modifications, specific patterns of DNA or nucleosome modifications are re-established. Moreover, how is the activity of a transcription factor altered during the initial differentiation event and subsequently maintained as the cell divides and new transcription factors are synthesized (Ringrose & Paro 2007)?

Many prion-like proteins, including Ure2, Swi1, Spf1, Cyc8, and Mot3 in yeast (Alberti et al. 2009, Du et al. 2008, Patel et al. 2009, Rogoza et al. 2010, Wickner 1994) and *Drosophila* GAGA factor (Tariq et al. 2013), are transcriptional regulators. Could transcription regulators with prion-like properties serve as marking mechanisms to establish and maintain cellular memory? By attaining multiple stable conformational states, can they create enough functional diversity to create phenotypic diversity? By virtue of their dominant self-sustaining properties, such transcriptional regulators can, once engaged, maintain the altered transcriptional state following cell division in an epigenetic manner (**Figure 6**). Given that this is a protein-based mechanism, it is also conceivable that under certain circumstances it can be reversed, resulting in a differentiated cell reverting to its earlier fate or to an entirely new cell fate. Such transcriptional regulators may include sequence-specific DNA-binding proteins, proteins that are recruited to the transcriptional complex via protein-protein interactions, or enzymes that can modify transcription factor activity.

In the prion-like state, such transcriptional regulators may alter gene expression in several ways. Because the prion-like state is a stable oligomer, it may create diffusion barriers, restrict a protein to a subcellular compartment, reduce the effective concentration of the freely available pool of protein, or, conversely, increase the efficiency of a reaction by increasing the effective concentration

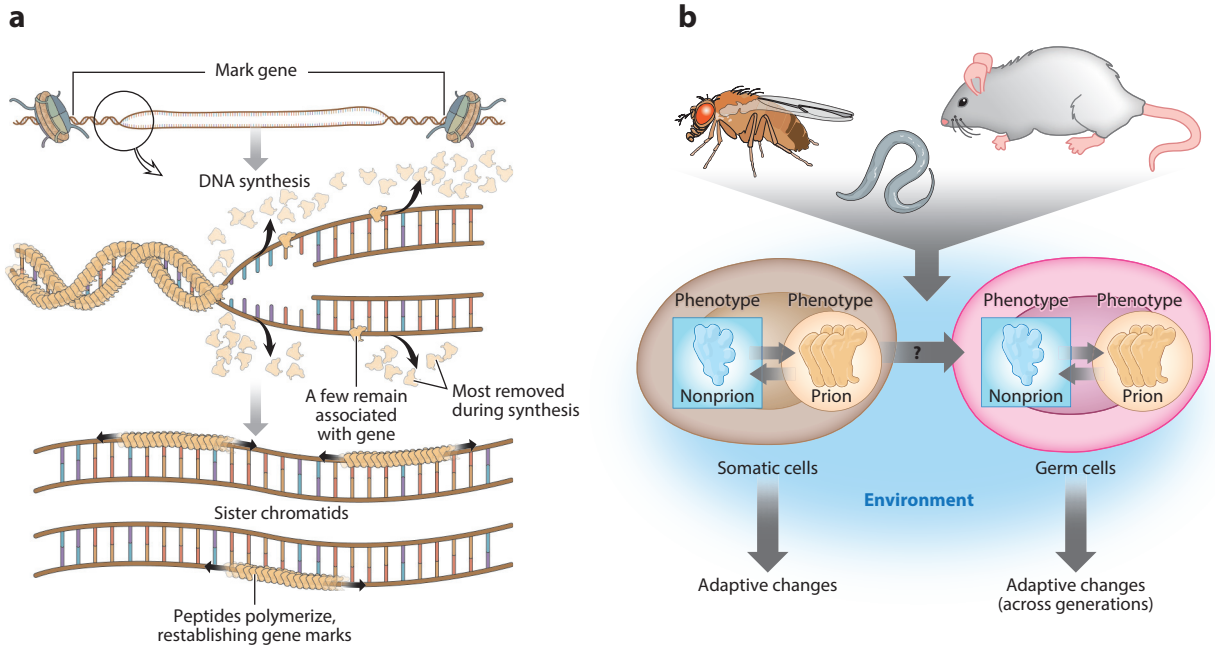

Figure 6

(*a*) Prion proteins can create a self-assembled genomic mark and serve as a conduit for adaptive change. In any given cell type, some genes are turned on, whereas others are turned off. Sequence-specific DNA-binding proteins lead to the assembly of transcription activator or repressor complexes. Following DNA replication, the transcriptional complexes are reconstituted on the newly synthesized strand of DNA, and the active or repressed state of transcription is re-established. A prion-like state of a transcription factor can recreate a mark from just a few bound molecules by virtue of its self-assembling property. (*b*) If engaged in a somatic cell, this self-sustaining transcriptional marking event can serve as a cellular memory. If such a marking event is triggered in a germ cell, it can be a conduit for heritable phenotypic change.

of a protein. The prion-like conversion is also associated with significant conformational change: It can change the activity state of the protein and also lead to the formation of distinct protein complexes. Finally, it can act as a self-templating genomic bookmark, allowing reconstitution of the repressor or activator complex on newly synthesized DNA (**Figure 6**). However, to act in this manner, the protein must stay bound to a chromosome during replication. Such proteins may act in concert with other epigenetic mechanisms, such as sequence-specific DNA-binding proteins, recruiting histone-modifying enzymes or DNA-methylating enzymes to establish or maintain active or repressed chromatin states.

Prions and the Origin of the Ribonucleoprotein Complex

Several prion-like proteins bind to nucleic acids, particularly mRNA (King et al. 2012). As a posttranscriptional gene regulatory mechanism, formation of a ribonucleoprotein complex by prion-like proteins may serve to sequester mRNA, alter the activity state of mRNA, serve as an immediately available pool of the mRNA, enhance the efficiency of mRNA-dependent processes, or increase the local concentration of the mRNA (Keene & Tenenbaum 2002). Is this apparent abundance of mRNA-binding proteins assuming a prion-like state in various species just happenstance? Or does the abundance of RNA-binding proteins with prion-like properties reflect an ancient relationship between RNA and prion-like proteins (Chernoff 2004)?

Widely held views about the molecular origins of life include the RNA world, protein world, and iron-sulfur world theories. Because most life forms as we know them today do not utilize an iron-sulfur mechanism, this theory is not pertinent to our discussion. Although the relative contributions of RNA and protein are heavily debated (Bernhardt 2012), it is generally agreed that the formation of RNA-protein complexes was an important step in the molecular evolution of life (Cech 2009). The discovery of ribozymes, the identification of small RNAs with catalytic activity, and the presence of complex molecular machines, such as the spliceosome, ribosome, and telomerase, that use RNA in their catalytic core support the idea that RNA played an important role in the molecular evolution of life. However, as has been widely acknowledged, RNA has certain limitations as a self-replicating catalytic molecule (Robertson & Joyce 2012). First, RNA is unstable, particularly at high temperatures and basic pH, a condition prevalent in the hydrothermal vents where life is supposed to have started. Second, the catalytic repertoire of RNA is limited. Finally, catalytic mRNA is extremely rare; approximately 10^{14} to 10^{16} RNA molecules are required to isolate catalytically active RNA. However, most enzymes are proteins, and amino acids were prevalent in the prebiotic environment, raising the possibility of RNA-protein complex formation in the very early stages of molecular evolution.

Therefore, one can think about what sort of properties these early ribonucleoparticles would need to catalyze as well as self-replicate. Prion-like domains are modular in nature (Alberti et al. 2009, Wickner et al. 2000); various amino acid sequences can confer prion properties; the amyloid state is generally extremely stable; amino acids, such as glycine and serine, that enhance amyloid formation were supposedly abundant in the prebiotic soup (Johnson et al. 2008, Miller & Urey 1959); small peptides in isolation tend to adopt the amyloid state; and amyloids have been postulated to be the ancestral protein fold (Chernoff 2004, Dobson 2004). Therefore, it is conceivable that at some point in the molecular evolution of life, association between stable amyloidogenic peptides and unstable catalytic RNA could have created self-replicating catalytic ribonucleoprotein particles. If this association stabilized or influenced the function of the catalytic RNA, the particles would have been subjected to positive selection (**Figure 7**). If this hypothesis is correct, prion-like or amyloidogenic proteins would have been present very early in life, and bacteria or viruses with simple genomes may contain RNA- or nucleic acid–binding prion-like proteins.

Can Functional Prions Provide Insight into Prion and Amyloid Diseases?

In mammals, misfolded protein aggregates, including amyloids, are often associated with fatal neurodegenerative disorders and other disease states (Dobson 2004, Horwich & Weissman 1997, Roth & Balch 2011). However, the growing list of proteins that form the so-called functional prions and amyloids in many organisms raises the question: Why does the propensity to form amyloids exist in a biological system when it is primarily detrimental to the cell? In a similar vein, although disease-causing proteins are expressed in diverse cell types, why are only neurons, and in most cases specific neurons, susceptible to the disease state? One of the most surprising observations is that neuronal CPEB in its amyloidogenic state may support memory, although one of the early phenotypes in several amyloid-based neurodegenerative diseases is cognitive dysfunction, including memory impairment (Ashe & Zahs 2010, Walsh & Selkoe 2004). Do functional amyloids such as CPEB share common features of the amyloidogenic pathway with toxic amyloids involved in disease? And, if so, how do they manage to be functional instead of causing fatal disease? Do amyloid-based diseases originate from perturbation of functional amyloids or other proteins that rely on supramolecular assembly (Polymenidou & Cleveland 2012)?

Neurodegenerative diseases have roughly two stages: an early, restricted, and often cell type–specific stage and a late, more widespread stage, which leads to degeneration and loss of neurons

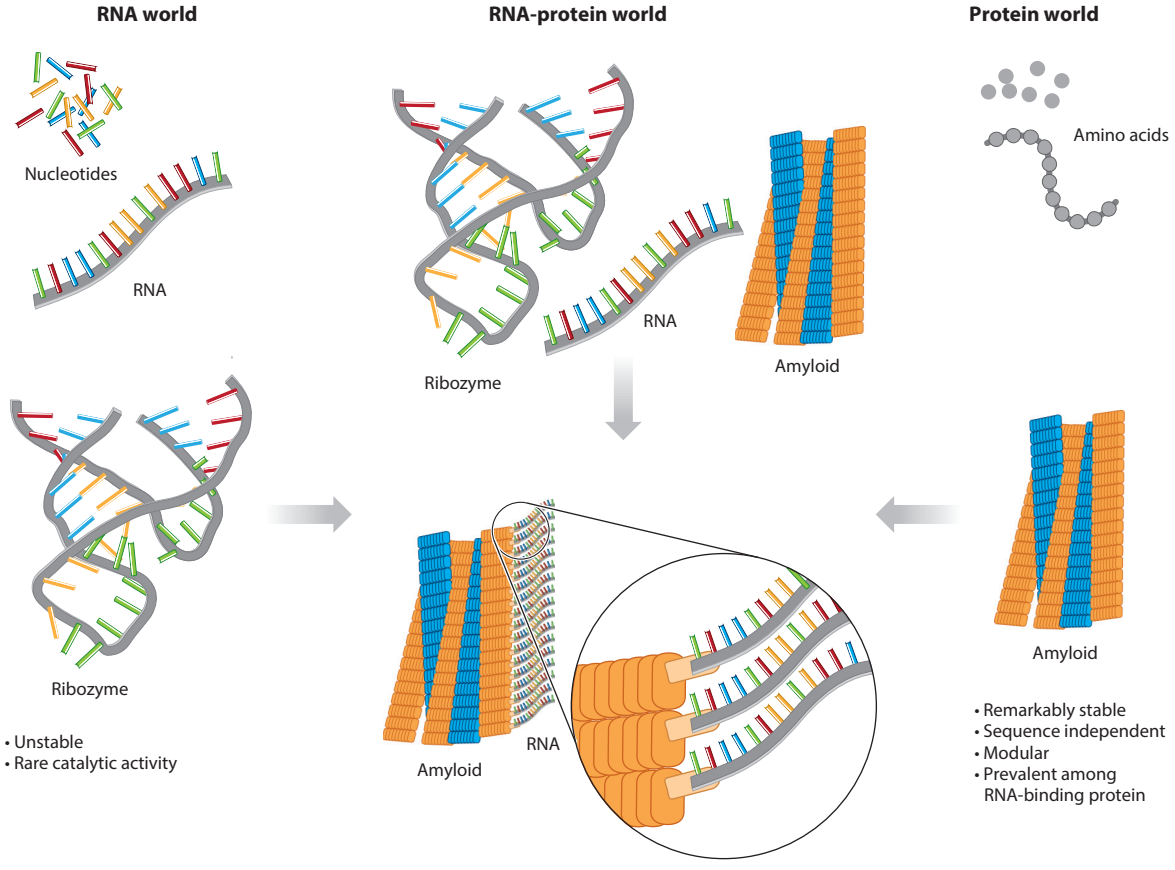

Figure 7

Prion-like proteins in early ribonucleoprotein particles. Several RNA-binding proteins appear to have prionogenic properties. This enrichment may reflect an early evolutionary relationship between RNA and RNA-binding proteins, in which the stable prion state may have contributed to the stability and function of catalytic RNA.

(Skovronsky et al. 2006). Innumerable studies, using all available molecular and cellular analysis tools, have found that toxic aggregates either directly or indirectly affect the expression or function of proteins involved in transcription, translation, protein and mRNA stability, endocytosis, exocytosis, axonal transport, and mitochondrial function, to name a few of the processes involved (Bossy-Wetzel et al. 2004, Encalada & Goldstein 2014, Ramanan & Saykin 2013, Ramaswami et al. 2013). These findings indicate that pathogenic or toxic amyloid formation results in a global alteration of cellular physiology. Although such global dysregulation may explain the degeneration of neurons and broad phenotypic effects in later stages of disease, it does not provide a satisfactory explanation for the cell-type and phenotypic specificity in early stages of disease or for the incubation period, which is frequently long. The other possibility, which is not mutually exclusive, is that prions and amyloids have evolved in various cellular contexts to serve normal functions. As the nervous system employs all available posttranscriptional mechanisms to create structural and functional diversity, it is likely to enlist a prion-based protein switch. In this scenario, interactions between functional and toxic prions/amyloids affect cell type–specific function in the early stages

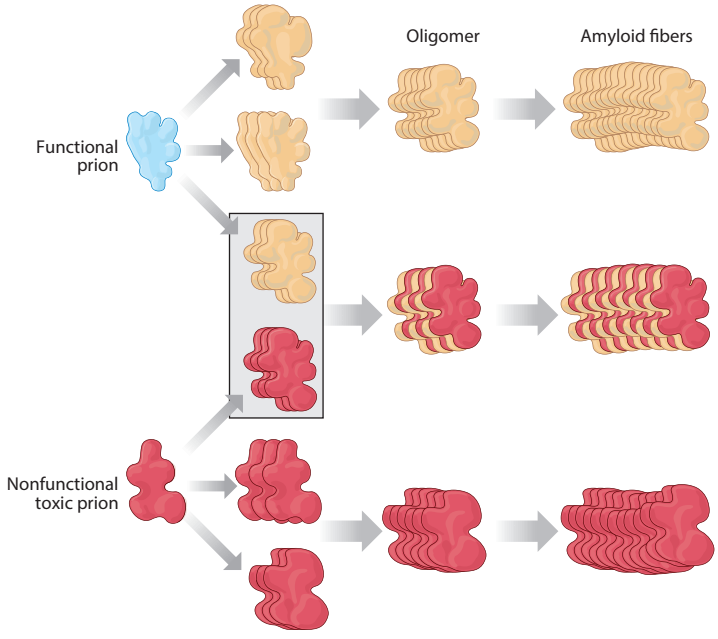

Figure 8

Cross-templating between functional and toxic prions may contribute to the early stages of neurodegenerative disease. Both functional and toxic prions adopt multiple conformational states in the process of aggregate formation, resulting in conformational states that are similar to each other. Therefore, it is conceivable that some conformational states can cross templates, resulting in heterologous protein aggregates. Such a process would eventually perturb the normal function of such proteins and could precipitate a disease state.

of disease, and a latter, broad phenotypic effect may emerge from a general decline in protein homeostasis.

Two obvious modes for such an interaction between pathological and functional amyloids/prions are indirect and direct. The formation of pathogenic amyloids may interfere with cellular factors, such as chaperones or signaling modules, which are critical for the formation and/or maintenance of functional prion-like proteins. Therefore, accumulation of toxic amyloids over time may affect functional amyloids and associated cellular functions. Alternatively, pathogenic amyloids may directly interact with functional amyloids, either by sequestering or perturbing their counterparts (**Figure 8**). Cross-seeding, although rare and inefficient, occurs among different amyloids and aggregate-prone proteins (Derkatch et al. 2004, Giasson et al. 2003, Kotzbauer et al. 2004, Ripaud et al. 2014). Therefore, although a slow event, the accumulation of toxic amyloids over time may guide functional amyloids to attain a nonfunctional or toxic state and lose functional capacity. In both scenarios, the specific function the functional amyloid is supposed to serve in a particular neuron or cell type would be disrupted, resulting in a more specific phenotype in the early stages of disease. As the disease progresses, dysfunction in the protein handling machinery may lead a large number of proteins that are inherently aggregation prone to aggregate.

The existence of functional prions, particularly in the nervous system, may also have a practical implication for disease treatment. If amyloid formation does indeed serve a normal function in neurons, we need to rethink how we approach and treat amyloid-based diseases.

The anti-amyloidogenic compounds used to treat neurodegenerative diseases, particularly those involving cognitive impairment (e.g., Alzheimer's, Huntington's, and Parkinson's), may interfere with other amyloid-related biological functions. Anti-amyloidogenic compounds may reduce amyloid loads without improving cognitive capacity, owing to the unintended consequence of inhibiting functional amyloids. A solution to this problem is a better understanding of not only the similarities between functional and toxic amyloids but also the differences. Differences may include the structural states or specific molecular programs they utilize. Therefore, understanding the basic biology of functional amyloids and a comparative analysis of functional and toxic amyloids may provide unanticipated insights.

OUTLOOK

Given that nonpathogenic prion-like proteins are broadly distributed across multiple phyla, proteins with self-sustaining conformational states may be part of an evolutionarily conserved regulatory mechanism involved in normal physiological function (Soto 2012). Identifying these proteins and defining their functions could open the door to new, unsuspected biological mechanisms. However, functional prion-like proteins in multicellular eukaryotes have thus far been discovered entirely by chance. To rigorously determine how broadly functional prion-like mechanisms are employed would require a systematic approach. Such a strategy must address: (*a*) how prevalent such proteins are in biological systems, (*b*) what specific functions they serve in the prion-like state, and (*c*) how they are regulated. Therefore, the search for novel, functional prion-like proteins should employ species amenable to genetic, cell biological, and biochemical analyses that possess a wide array of well-defined physiological processes, allowing any putative prion-like protein to be placed in its proper biological context.

The search for functional prions should extend beyond animals into plants. Plants must cope with short- and long-term environmental changes, and, being sessile, they must alter their physiological processes to accommodate environmental changes within their lifetime using an existing genetic repertoire. Moreover, unlike organogenesis in animals, which takes place during embryogenesis, organogenesis in plants continues throughout life. Therefore, plant development needs to coordinate with a changing environment. This coordination requires a certain time delay between the triggering event and initiation of differentiation; consequently, a prolonged memory of the trigger is needed (Iwasaki & Paszkowski 2014). An epigenetic protein conformation-based mechanism, such as the prion, which can rapidly respond to a changed environment and persistently alter cellular physiology, would be ideally suited. In summary, the beneficial forms of prions may not be the exception, rather the disease-causing forms are!

NOTE ADDED IN PROOF

Recently Kandel and colleagues (Fioriti et al. 2015, Stephan et al. 2015) have shown that the prion-like state of CPEB3 is involved in long-term memory in mammals.

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