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Inducible Proteopathies

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Abstract

Numerous degenerative diseases are characterized by the aberrant polymerization and accumulation of specific proteins. These proteopathies include neurological disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and the prion diseases, in addition to diverse systemic disorders, particularly the amyloidoses. The prion diseases have been shown to be transmissible by an alternative conformation of the normal cellular prion protein. Other proteopathies have been thought to be non-transmissible, but there is growing evidence that some systemic and cerebral amyloidoses can be induced by exposure of susceptible hosts to cognate molecular templates. The mechanistic similarities among these diseases provide unprecedented opportunities for elucidating the induction of protein misfolding and assembly *in vivo*, and for developing an integrated therapeutic approach to degenerative proteopathies.

Introduction

In a remarkable variety of neurological and systemic disorders, specific proteins accumulate within cells and tissues, usually as a result of a change in protein conformation that renders the molecules prone to self-aggregation and resistant to clearance. These conformational diseases, or 'proteopathies', comprise systemic amyloidoses in addition to neurodegenerative conditions that are marked by the buildup of characteristic proteins in the brain, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and the prion diseases [1–4]. In this article, we consider the mechanistic commonalities among seemingly distinct protein-based diseases, and in particular emerging evidence that some proteopathies can be induced in animal models by exposure to exogenous material. We argue that an understanding of the earliest events that induce protein misconformation and aggregation *in vivo* will yield more focused strategies for discovering treatments for these devastating diseases.

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Induction of prion diseases

The prion diseases, although rare, have attracted special attention because of their lethality and unorthodox transmissibility. They include Creutzfeldt-Jakob disease, kuru, fatal familial insomnia and Gerstmann-Straussler-Scheinker Syndrome in humans, and several diseases in nonhuman species, the best known being scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, transmissible mink encephalopathy, and chronic wasting disease in deer and elk [5]. The prion diseases are typified pathologically by spongiform degeneration, astrocytosis, neuron loss, and the accumulation of aberrantly folded forms of the prion protein (PrP) in specific brain regions [2,6] (Figure 1).

According to the prion hypothesis of infectivity, normal PrP (PrP cellular, or PrP^{C}) assumes an anomalous, β-sheet-rich conformation (PrP scrapie, or PrP^{Sc}) that initiates and sustains the replication of the pathogenic molecule *in vivo* [7,8] by a mechanism termed *permissive templating* [9] (Figure 2). Unlike conventional infectious illnesses, which require the initial invasion of a microorganism, prion diseases also can arise *de novo* in both hereditary and idiopathic forms. In these instances, a mutation or a stochastic event, respectively, is thought to trigger the misfolding and polymerization of endogenously produced PrP^{C} [2,7,9], although the participation of an exogenous factor in the induction of idiopathic prion disease has not been ruled out. Indeed, the unequivocal identification of the infectious agent in the prion diseases has been a fascinating and contentious area of research for many decades [10]. The experimental transmission of mammalian prionosis typically is accomplished by exposing the recipient to material from prion-laden tissue. The efficiency of disease induction is governed by route of administration, dose, and various host-specific and donor-specific factors [6–8,10–15] (Figure 3), all of which must be considered when assessing the inducibility of proteopathies [16].

Applying the prion model of induction to other proteopathies

In the mid-1800's, Rudolf Virchow first employed the term 'amyloid', meaning 'starch-like', to describe accumulations of an unusual substance in animal organs that stained in a similar way to some constituents of plants. Today, 'amyloid' is generally used to describe fibrillar aggregates of particular proteins that have assumed a non-native, β-sheet-rich configuration. More than 20 proteins are known to form disease-related amyloid deposits *in vivo*, each having a unique amino acid sequence and yielding a characteristic disease phenotype [17]. Under permissive conditions, vulnerable proteins misfold and aggregate into polymeric fibrils and soluble oligomers. The appearance and secondary structure of each form are highly similar among proteins, regardless of the primary amino acid sequence of the starting protein [15,18,19]. Just as the probability of spontaneous misfolding varies among proteins [15], the susceptibility of proteins to permissive templating is likely to differ, such that some native polypeptides are more readily converted by misfolded cognate proteins acting as seeds, and thus are more apt to be transmissible.

Non-prion cerebral amyloidoses have been considered to be non-transmissible [20], but paradigms similar to those developed to study the transmission of prion diseases suggest that diverse conformational disorders can be induced in animal models by seeding-like

mechanisms [21,22]. The inducible proteopathies include systemic amyloid A (AA) amyloidosis, systemic senile (apolipoprotein AII) amyloidosis, and cerebral amyloid-β (Aβ) amyloidosis. Although these proteopathies differ clinically and pathologically from the prionoses, the model systems used to investigate protein seeding are illuminating both the requisite host factors and the properties of the inducing material that are important for this unconventional mode of infectivity. They also provide a test-bed for potential therapeutic interventions. Analysis of the mechanisms by which heterologous proteins are impelled to aggregate in living organisms thus could yield fruitful insights into this surprisingly wide-ranging pathogenic process.

Induction of amyloid A amyloidosis

Under chronic inflammatory conditions that increase the hepatic production of amyloid A protein, the levels of amyloid A rise dramatically in blood, and this protein accumulates as amyloid fibrils in systemic organs, including the kidneys, liver and spleen [23]. With time, the burgeoning amyloid load triggers the impairment or failure of organ function. In animal models, administration of a systemic inflammatory stimulus (such as silver nitrate) eventually causes amyloid A deposition, but the process is slow. If an extract from organs rich in amyloid A protein – termed amyloid enhancing factor (AEF) - is administered along with the inflammatory agent, amyloidogenesis is strikingly accelerated [23–25]. The identity of this ‘factor’ remained uncertain until recently, when a purified fraction of splenic extract corresponding to aggregated amyloid A protein was shown to seed amyloid A deposition *in* vivo [25]. Although the isolated fraction probably contained small amounts of other, unidentified material [21], a compelling case can be made that aggregated amyloid A protein *per se* is the active component of AEF. Interestingly, amyloid A amyloidosis also can be elicited, although less potently, by diverse exogenous substances that are high in amyloid-type β-sheet content, including silk fibrils, the yeast prion Sup35, and bacterial curli fimbriae [26]. All of these proteins possess elements of amyloid structure. The corruption of certain normal proteins by heterologous ‘seeds’ suggests a tantalizing link to environmental factors, an issue that warrants further study.

Induction of apolipoprotein AII amyloidosis

Apolipoprotein AII (ApoAII) is an abundant, yet poorly understood, apolipoprotein [27] that can deposit spontaneously as amyloid fibrils in aged mice [28] and in a hereditary human disease caused by a stop-codon mutation in the apoAII gene [29]. Mouse senile amyloidosis entails the accumulation of ApoAII in systemic organs, a process that can be stimulated by peripheral injection of ApoAII fibrils isolated from affected liver [28]. ApoAII also induces amyloid disease when introduced into the gastrointestinal tract via gavage or in drinking water, and might even be transmitted to cage-mates by the ingestion of feces containing ApoAII fibrils [30]. When fibrils of a strongly amyloidogenic sequence-variant of the protein (ApoAII[C]) are injected into mice expressing a more resistant protein subtype (ApoAII[B]), the normally refractory mice produce conformationally altered ApoAII[B]-amyloid that has become highly amyloidogenic [31]. Denaturation of the ApoAII fibrillar extracts abolishes their ability to initiate disease [28,32], implicating ApoAII conformation in the induction of ApoAII amyloidosis. Like amyloid A amyloidosis, ApoAII amyloidosis

is most potently stimulated by the same protein, though it also can be seeded *in vivo* by other substances. For example, in ApoAII[C]-expressing mice, ApoAII[C] fibrils are the most effective seed, but β-sheet rich fibrils from heterogeneous sources (including amyloid A and synthetic Aβ) also promote ApoAII deposition, albeit with reduced efficacy [33].

Amyloid A and ApoAII amyloidoses affect systemic organs that are supplied by fenestrated capillaries, which facilitate the entry of large molecules such as proteins into the tissues. By contrast, the most salient clinical manifestations of prion disease result from the proliferation of prions within the central nervous system, where the blood-brain barrier helps to protect the brain from many exogenous agents. Prions, probably with the aid of immune cells, are able to circumvent this obstacle [11]. Even so, the most effective way to transmit prion disease is by direct inoculation of the agent into the brain. As we will now review, emerging data suggest that Aβ-amyloidosis, perhaps the most common age-associated cerebral proteopathy, also can be stimulated in experimental models by the intracerebral injection of diseased tissue extracts.

Induction of Aβ proteopathy

Aβ is a minor proteolytic cleavage product of the Aβ-precursor protein (βAPP), a ubiquitous, type-1 transmembrane protein that is abundant in brain. Aβ, like other proteopathic molecules, is liable to misconformation and aggregation into macromolecular assemblies such as oligomers and amyloid fibrils. Aggregated Aβ constitutes the cores of senile plaques, and forms deposits in the walls of brain blood vessels known as cerebral β-amyloid angiopathy. In humans and several other mammalian species, the probability of developing Aβ lesions in the brain increases considerably in old age [34,35].

Substantial genetic, biochemical and pathologic evidence supports a primary role of aberrant Aβ in the genesis of Alzheimer's disease (AD) [4], although some cognitively normal humans and all nonhuman species fail to acquire the full phenotype of AD, despite sometimes copious Aβ in the brain. The reasons for the apparent resistance to AD in animals that generate the identical, human-type Aβ-sequence remain uncertain. It is possible that large, extracellular Aβ aggregates (i.e. senile plaques) are relatively benign, and that, instead, cytotoxicity is mediated mainly by disease-specific oligomeric assemblies of Aβ; another possibility, which is not necessarily exclusive, is that the intracellular milieu is in some way more conducive to cytopathology in AD than in resistant organisms (Box 1). Interestingly, Aβ also is implicated in inclusion body myopathy, a degenerative muscle disorder characterized by the intracellular buildup of Aβ and βAPP fragments in myocytes [36].

To date, there is no evidence that AD *per se* is transmissible, but several laboratories have begun to explore the possibility that Aβ deposition, one pathological hallmark of the disease, can be induced by exogenous seeding in animal models. The first attempts to transmit cerebral Aβ-amyloidosis employed nonhuman primates [37], which have a human-like Aβ sequence and naturally develop β-amyloid deposits (but not AD) in old age. In these experiments, senile plaques and Aβ-angiopathy were induced in young marmosets by the intracerebral inoculation of Aβ-rich brain homogenates, but a limitation of this paradigm

is that the lesions do not materialize for several years in marmosets [37–39]. Mice have a much shorter life-span than do primates, and although wild-type mice do not manifest Aβ-amyloidosis due to idiosyncrasies in the murine Aβ sequence, several lines of mice that are transgenic for human βAPP develop plaques and β-amyloid angiopathy with age (e.g., [40,41]). In young βAPP-transgenic mice, cerebral Aβ-amyloidosis can be seeded by dilute cortical extracts from autopsied AD patients within the span of only a few months [22,42]. Interestingly, Aβ-rich brain extracts from βAPP-transgenic mice produce Aβ-seeding similar to that achieved using cortical material from humans [43], indicating that the inducing agent is not uniquely present in the human brain. Extracts from young murine or human brains that are devoid of Aβ-lesions have no effect in transgenic mice, and seeding does not occur when Aβ-rich extract is injected into wild-type mice, which produce a non-polymerogenic form of Aβ [42,43].

The evidence increasingly implicates exogenous Aβ itself as a crucial element in the seeding phenomenon. However, many important questions remain to be addressed, and the precise nature of the agent remains to be defined. To date, synthetic Aβ fibrils, in concentrations similar to those in brain extracts, have not been demonstrated to stimulate the endogenous generation of β-amyloid pathology in transgenic mice, suggesting that intrinsic properties of the Aβ peptide, or brain-specific cofactors in the extract, are needed. In this regard, it is useful to note that most attempts to transmit prion disease by *in vitro*-generated, recombinant prion protein have failed [15,44]. Even when successful, recombinant PrP is inefficient compared to prionotic brain extracts [12]. One possibility is that multimeric proteins can assume different 3D configurations, or ‘strains’, depending on the conditions under which they are formed. Indeed, Aβ, like PrP and other proteins, can form distinct strains that differ both in their structure and cytotoxicity [45–49]. As with prions, conformational strain differences also might influence the efficiency (and possibly the eventual phenotype) of Aβ-seeding. This question can be addressed experimentally using suitable animal models, in the context of newly emerging tools for analyzing disease-related conformations of Aβ in Alzheimer’s disease [50–52].

Inducible proteopathies: some caveats

The transmission of prion diseases is relatively unambiguous because the clinical manifestations (ultimately death) are particularly obvious [2,5]. By contrast, the neurologic consequences of cerebral Aβ-amyloidosis, especially in non-human species, often are more subtle and variable than those of the prionoses [22,38,39,42]. As a result, the effects of ‘infection’ might be relatively difficult to discern in some proteopathies, at least from a functional standpoint. This matter is complicated by the fact that monkeys and βAPP-transgenic mice spontaneously generate Aβ-pathology with age; hence, it is likely that Aβ seed-rich tissue extracts accelerate amyloidogenesis by supplementing (or anticipating) endogenously generated Aβ-seeds [22]. Analogously, AEF greatly accelerates amyloid A amyloidogenesis, even though systemic inflammation alone eventually results in amyloid disease [23].

For several reasons, then, the non-prion proteopathies might not be communicable in exactly the same sense as are prionoses. However, we contend that the ability of diseased

tissue extracts to augment the pathogenesis of diverse proteopathies in vulnerable hosts indicates that, at the molecular level, the prion model of permissive templating has parallels in other protein conformational disorders. The concept of inducible proteopathies also accommodates the heterologous induction of a conformational change in a susceptible protein by different molecules that share critical structural features [19,21,33,53–55]. In this regard, the potential for cross-seeding by nanoscale-organized structures such as amyloid fibrils [56] might require careful toxicological assessment for some applications of nanotechnology [57] (Box 2). A fuller understanding of proteopathic induction *in vivo*, and of the common cellular and molecular mechanisms by which aggregation-prone proteins propagate misfolding and exert their toxicity, might lead to unified strategies for deciphering the ontogeny of a number of seemingly disparate disorders.

Concluding remarks

The weight of evidence now supports the concept that exogenous, structurally complementary molecules can induce specific diseases of protein conformation and assembly in animals. Key objectives for future research are to define, at the molecular level, how disease originates *de novo* in both the sporadic and the hereditary proteopathies, to establish the structural idiosyncrasies of agents that act as corruptive protein templates, and to elucidate the cytotoxic mechanisms of protein aggregates. Finally, it is essential to determine the genetic, biochemical and physiological characteristics of the host that regulate the permissiveness of templating in protein deposition disorders.

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Box 1.

Cytotoxic mechanisms of pathogenic proteins

Increasing evidence suggests that different pathogenic proteins damage and kill cells by similar mechanisms. In each case, oligomeric assemblies recently have emerged as prime suspects [50,58,59], and cell membranes are the sites where much of the molecular mischief occurs. Although attention initially focused on the plasma membrane, these proteins also can aggregate intracellularly, resulting in impaired organelle function and cell death [60,61]. Aβ, PrP and other aggregation-prone proteins possess amphipathic properties that facilitate their interactions with lipid membranes (particularly within lipid rafts [62–64]), where hydrogen peroxide production, lipid peroxidation [65–67] and disruption of cellular ion homeostasis [68–71] might be important steps in the neurotoxic cascade. In addition, other chemical changes might occur during the aggregation of the misfolded peptide [72,73]. Specific metal ions such as iron, copper and zinc can promote the multimerization of pathogenic proteins and the generation of reactive oxygen species in neurodegenerative diseases [74–78]. The age-associated increase in oxidative stress [62,79,80] and protein accumulation [81–83] might explain why spontaneous proteopathies are typically age-related, often with incubation periods of many years.

Box 2.

Nanotoxic?

The repeating molecular structure of amyloid fibrils is attracting interest in the rapidly expanding field of nanotechnology. Engineering of nanomaterials and nanomachines in sizes ranging from smaller than antibodies up to viruses (1 – 100 nm) is a feasible objective. A variety of medical applications take advantage of the ability of nanoparticles to deliver drug cargoes across biological membranes or to control the release of their contents [84]. Early nanomaterials were spawned by the microelectronics industry, which built small devices on a massive scale. Organic polymers soon were adapted to take advantage of their variable structures and easily accessible chemistry for customizing properties. The quasi-crystalline and controllable self-assembly of amyloidogenic proteins has suggested that amyloid fibrils and other higher-order protein assemblies might be useful as nanomaterials.

The rush to nanotechnology has raised concerns about the largely untested toxicological and environmental hazards of nanomaterials. Little is known about how they interact with biological materials and how they are transported, modified, or degraded. The templating nature of amyloid-like assemblies discussed in this article is an example of a nanoscale property that could have health-related implications. The cross-seeding observed for a variety of amyloids [26,33] and the ability of materials such as silk to accelerate amyloid fibril formation by the amyloid A protein [26,85] suggest a need to evaluate the toxicology of nanoscale assemblies. A natural substrate candidate for seeding by amyloid-like structures is the amyloid A protein, which increases dramatically as a normal physiological response to routine inflammatory stimuli and in certain medical conditions [23]. Other misfolding proteins, such as those involved in the chronic neurodegenerative diseases, theoretically could be templated by exposure to amyloid-like materials that penetrate the blood-brain barrier. It is also worth noting that nanomaterials, such as fullerene (buckyballs), might be employed therapeutically to *inhibit* abnormal protein assembly [86]. In any case, nanotoxicological issues deserve a prominent place in current and future nanomedicine initiatives.

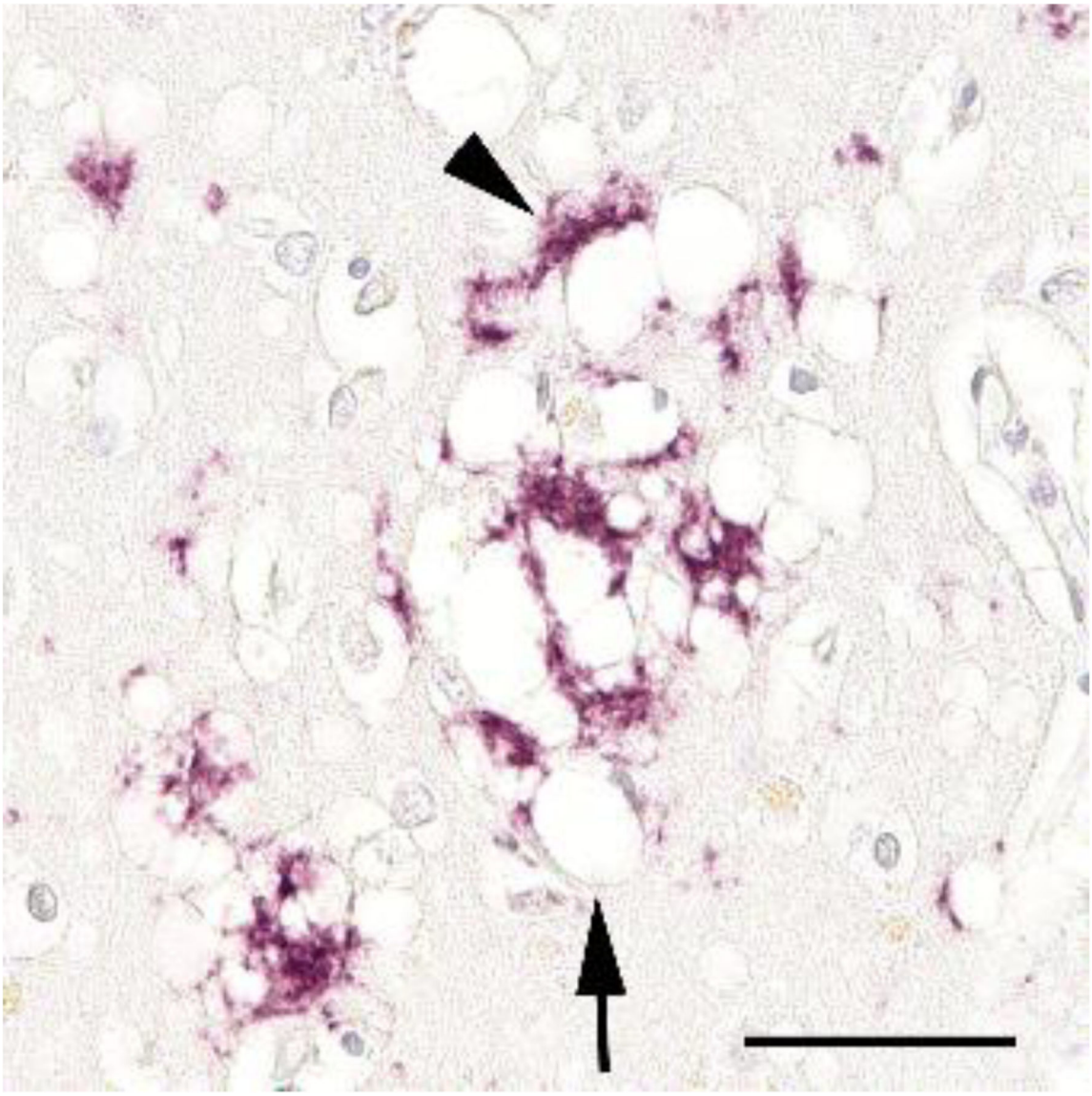

Figure 1.

Spongiform degeneration (arrow) and the accumulation of prion protein (arrowhead) are consistent pathological features of prion disease, along with astrocytosis and neuronal degeneration. Shown is a section of neocortex from a patient who died of idiopathic Creutzfeldt-Jakob disease, labeled using the anti-PrP antibody 3F4 (Nissl counterstain). Bar = 50μm.

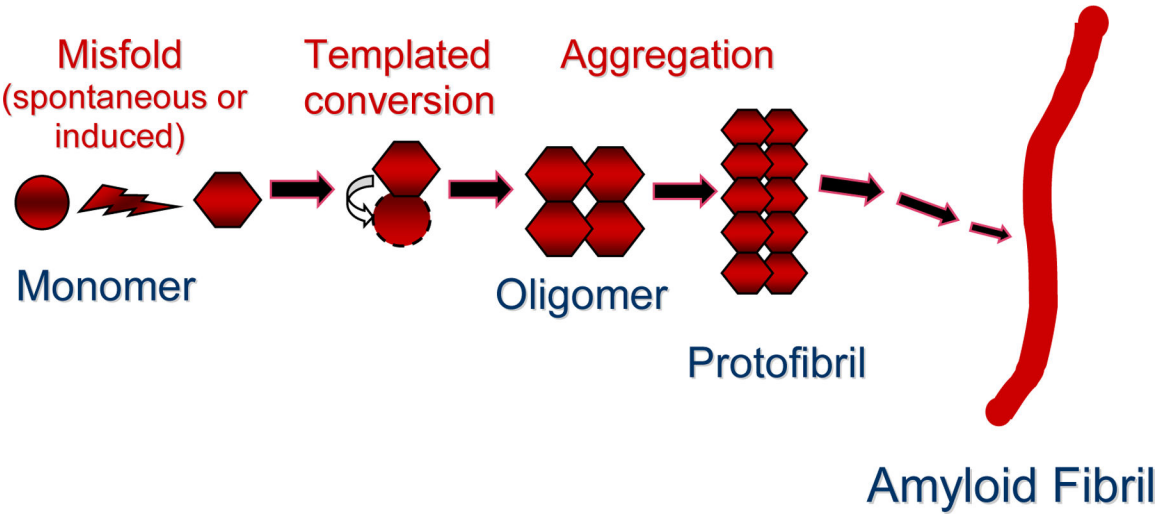

Figure 2.

A hypothetical pathway leading from normally folded, monomeric protein to multimeric assemblies such as small oligomers, protofibrils and amyloid fibrils. In this instance, a particular monomeric protein (circle) assumes an atypical β-sheet-rich fold (hexagon), either as a stochastic or seeded event. This corrupted protein then impels the templated misconformation and consequent self-assembly of endogenously produced, cognate proteins. Multimeric protein aggregates can exist in multiple 3D forms consisting of various numbers of monomers; it is likely that multimers can themselves feed back into the proteopathic cascade as seeds. ‘Strain’ differences in inducibility appear to be coded in subtle conformational variations in proteins. There might be several pathways leading to different higher order assemblies. The biological activity of specific multimers, and the conditions that favor each step in the pathogenic sequence *in vivo*, remain incompletely understood.

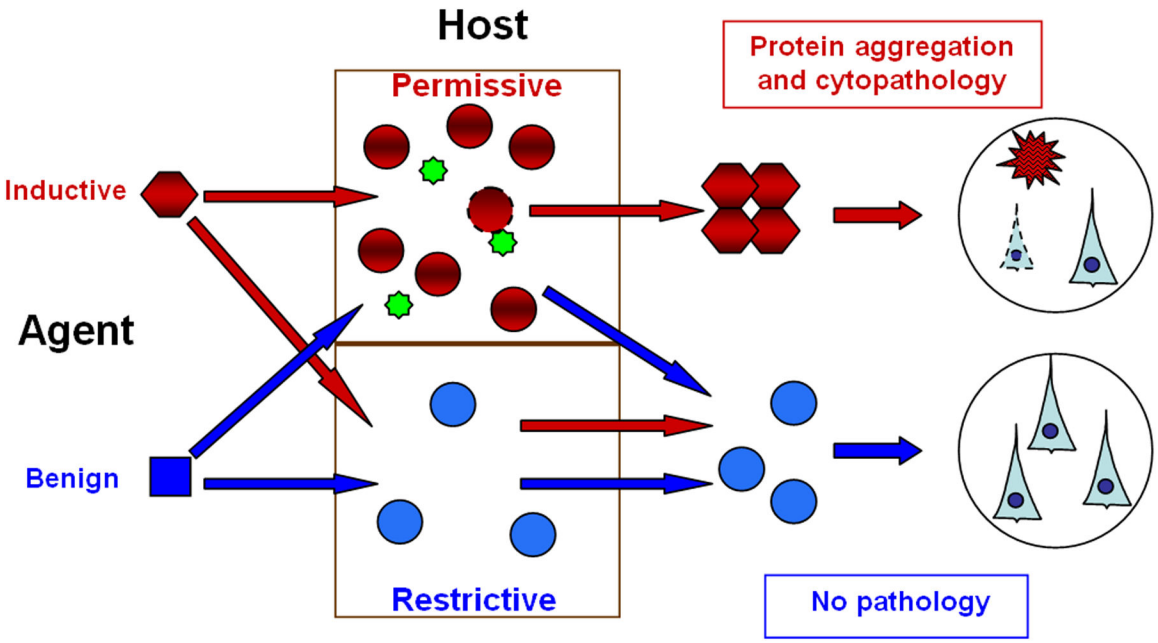

Figure 3.

The interaction between the agent and host in the induction of disease by a proteopathic agent. By definition, a benign protein (blue square) is incapable of transmitting disease. An inductive agent (red hexagon) is pathogenic *only* in the context of a permissive environment (top). Such environments might include: A configuration of the cognate protein that is conducive to templating (red circles); increased production or sequestration of the cognate protein by the host; and/or the presence of essential cofactor(s) (green stars). A restrictive host environment, by contrast, might consist of a protein configuration that is resistant to templating (blue circles, bottom), sub-optimal protein levels, and/or the absence of cofactors. The role of soluble oligomeric species in seeding-induced aggregation and cytopathology remains to be defined.