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Review

Protein Misfolding in Prion and Prion-Like Diseases: Reconsidering a Required Role for Protein Loss-of-Function

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Abstract. Prion disease research has contributed much toward understanding other neurodegenerative diseases, including recent demonstrations that Alzheimer's disease (AD) and other neurodegenerative diseases are prion-like. Prion-like diseases involve the spread of degeneration between individuals and/or among cells or tissues via template directed misfolding, wherein misfolded protein conformers propagate disease by causing normal proteins to misfold. Here we use the premise that AD, amyotrophic lateral sclerosis, Huntington's disease, and other similar diseases are prion-like and ask: Can we apply knowledge gained from studies of these prion-like diseases to resolve debates about classical prion diseases? We focus on controversies about what role(s) protein loss-of-function might have in prion diseases because this has therapeutic implications, including for AD. We examine which loss-of-function events are recognizable in prion-like diseases by considering the normal functions of the proteins before their misfolding and aggregation. We then delineate scenarios wherein gain-of-function and/or loss-of-function would be necessary or sufficient for neurodegeneration. We consider roles of PrP^{C} loss-of-function in prion diseases and in AD, and conclude that the conventional wisdom that prion diseases are 'toxic gain-of-function diseases' has limitations. While prion diseases certainly have required gain-of-function components, we propose that disease phenotypes are predominantly caused by deficits in the normal physiology of PrP^{C} and its interaction partners as PrP^{C} converts to PrP^{Sc}. In this model, gain-of-function serves mainly to spread disease, and loss-of-function directly mediates neuron dysfunction. We propose experiments and predictions to assess our conclusion. Further study on the normal physiological roles of these key proteins is warranted.

Keywords: Alzheimer's disease, amyloid-β protein precursor, amyotrophic lateral sclerosis, huntingtin protein, Huntington's disease, prion diseases, protein misfolding diseases, superoxide dismutase 1, tau protein, tauopathies

INTRODUCTION

Prion diseases are incurable neurological diseases that produce a wide range of devastating symptoms in several mammalian species including humans (Creutzfeldt-Jakob Disease or CJD, Fatal Familial Insomnia or FFI, Kuru, etc.), cattle (bovine

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spongiform encephalopathy or BSE), and cervids (chronic wasting disease or CWD). Prion diseases are a unique and fascinating disease class because a normal protein (Cellular prion protein, or PrP^{C}) becomes misfolded and gain-of-function mechanisms associated with this misfolding not only propagate further PrP^{C} misfolding in neighboring cells and tissues, but can also infect other organisms. Not surprisingly, then, the principal focus of prion research has typically centered on the mechanisms of this infamous gain-of-function. Further, this concept of propagated misfolding has very recently inspired novel re-consideration of similar neurodegenerative diseases, such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), which are now considered to be prion-like in their etiology (defined below). Given that prion diseases have taught/inspired the field about important disease mechanisms, we speculated that the reverse ought to now be possible. We turned to prion-like diseases (especially HD, ALS, and AD) for inspiration to further our understanding of classic prion diseases and to seek out commonalities that might inform or inspire new therapeutic strategies for this class of devastating neurodegenerative diseases. Given this context, and our consideration of other recent developments in the prion field, it seemed timely to reconsider the issue of protein loss-of-function in prion diseases; thus we examine herein the balance of loss- versus gain-of-function in prion diseases and prion-like diseases.

We were prompted to evaluate the contribution of loss of PrP^{C} function in prion diseases in part because very little is known about the role of PrP^{C} in healthy brains and such knowledge is critical for implementing appropriate disease management strategies. Intriguingly, PrP^{C} has recently been implicated in AD, e.g., by serving as a receptor for the neurotoxic species of amyloid β oligomers [1]. Because PrP^{C} has been highly conserved through hundreds of millions of years of evolution, and is robustly expressed in the CNS, it undoubtedly plays an important part in organism physiology. It follows then, that conversion of PrP^{C} into a misfolded form lacking its normal function (typically denoted 'PrP^{Sc}', after Scrapie, the prototypical prion disease of sheep) ought to disrupt normal organism physiology at some point(s) during the disease course. Indeed, PrP^{C} has several neuroprotective functions that may be lost during disease progression (reviewed in [2, 3]). PrP^{Sc} could also interact with PrP^{C} in a dominant negative fashion to obscure its normal function [4]. Further, there is potential for haploinsufficiency in individuals who are heterozygous for familial *PRNP* mutations. From an alternative perspective, disrupting normally folded PrP^{C} itself has been proposed as a promising therapeutic strategy [5, 6], and thus it is critical to question whether such strategies would unintentionally potentiate loss-of-function aspects of etiology and thereby accelerate neurodegenerative disease or produce treatment side effects. If this outcome were anticipated, strategies to mitigate PrP^{C} misfolding or the toxicity of PrP^{Sc} might be advisable as more viable therapeutic strategies. Knowledge of when during prion disease progression PrP^{C} loss-of-function is a major contributor will also influence disease management strategies.

The dominant hypothesis in the field is that toxicity in prion diseases is mediated primarily through a gain-of-toxic-function (i.e., a neomorphic prion protein conformation is causal to disease. The neomorphic protein conformation may be encoded by *PRNP* mutation or induced by infection (reviewed in [7])). Gain-of-function and loss-of-function are often intertwined, as is the case in HD and ALS, which we outline later. As loss-of-function remains largely overlooked in prion diseases (although see [2, 3]), we examine where/when loss-of-function contributes to prion-like diseases with the aim of inspiring future studies into the role of loss-of-function in prion diseases. Thus we explore a family of alternate hypotheses, schematized in Fig. 1, including:

1) Prion diseases are gain-of-function diseases. New functions gained by prion protein misfolding are sufficient to produce disease phenotypes, i.e., loss-of-function is not required;
2) Gain-of-function initiates disease and is required for spread to new sites and/or individuals, but loss-of-function is both required and sufficient at end stages (e.g., is directly causal of neuron death, from which it follows that gain-of-function is not required at end stages of the etiology); and
3) Gain-of-function initiates disease spread, and a combination of gain- and loss-of-function occurs at many stages of disease.

UNTANGLING GAIN-OF-FUNCTION VERSUS LOSS-OF-FUNCTION IN PRION-LIKE DISEASES

Prion diseases have served as inspiration for untangling the mechanisms involved in other disparate

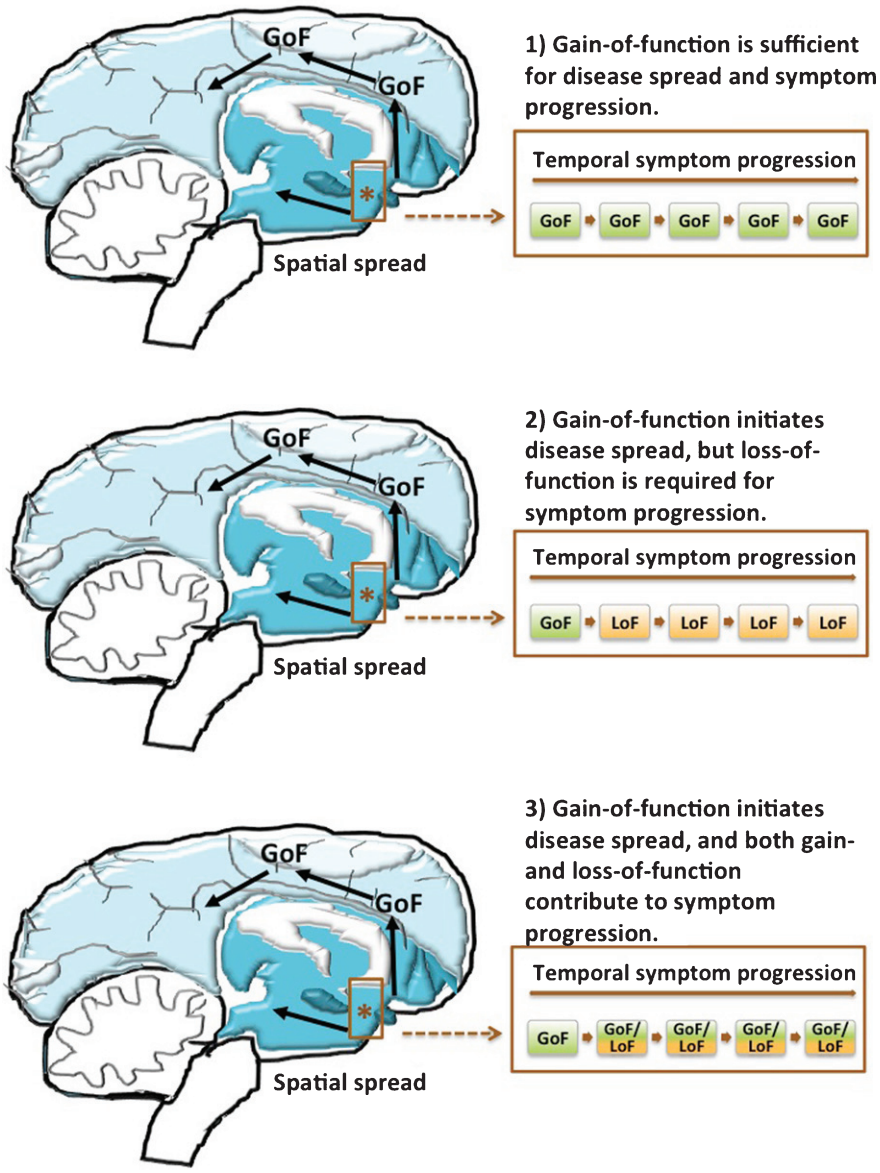

Fig. 1. Potential causes of prion and prion-like disease spread between brain regions and of temporal symptom progression at nucleation sites. In all models we propose that gain-of-function initiates spread between brain regions. Model 1. Gain-of-function produces neuron cell death and disease symptoms at all stages of disease. Model 2. Gain-of-function initiates disease at the nucleation site, but loss-of-function produces symptoms at subsequent disease stages. Model 3. Gain-of-function initiates disease at the nucleation site, and gain- and loss-of-function work in concert to produce symptoms at subsequent disease stages. We hypothesize that loss-of-function has an early role in prion diseases and prion-like diseases as illustrated in models 2 or 3. The example given is the regional spread of AD pathology through the brain. Darker areas of the brain represent more severely affected regions (Partially modeled after Fig. 3 in [16]).

neurodegenerative diseases. After S.B. Prusiner's discovery that misfolded PrP was the infectious agent in prion diseases [8], protein aggregates began to be viewed as a cause rather than simply as signs and symptoms of disease. Experimental prion diseases in animals faithfully recapitulated natural disease course and symptom onset, and led scientists to question whether similar disease processes (e.g., self-propagation of protein misfolding) could be occurring in other neurodegenerative diseases. Experimental methods were subsequently borrowed from the prion field, leading other diseases to be classified as being prion-like (Table 1). If prion-like diseases have important loss-of-function components, they may provide unique insight on how to experimentally separate the loss- and gain-of-

Table 1
Characteristics common to prion disease and prion-like diseases

Disease	Protein: Native form, Misfolded form/aggregated form	Nature of misfolded/ aggregated form	Gene encoding the aggregated protein, mode of inheritance	Location of intracellular aggregates	Extracellular aggregate seed site; spreads to	Experimentally Transmissible?
Prion	PrP^{C}, PrP^{Sc}	-β -sheet rich	*PRNP*, Autosomal dominant or sporadic (reviewed in [2]	Cell surface, endosomes, lysosomes, associated with lipid rafts (reviewed in [7])	Various; Various	Yes (reviewed in [159]
AD	AβPP, Aβ aggregates	-β-sheet rich	*APP,* Autosomal dominant or sporadic (reviewed in [2])	Early endosomes and/or TGN [160]	Various; Anatomically connected regions	Yes (For example [85, 86])
	Tau, hyper-phosphorylated tau	-β-sheets [11, 161]	*MAPT,* Autosomal dominant or sporadic (reviewed in [2])	Cytosol [11]	Entorhinal cortex; Dentate gyrus, other axonally connected regions (reviewed in [162]).	Yes (For example [87–93])
HD	Huntingtin, Mutant huntingtin with ≥35–40 poly-Q repeats [17]	-β-sheet rich -Stable [17]	*HTT,* Autosomal dominant [21]	Nucleus and cytosol [17]	Cortical areas; Striatum [17]	Yes* Spread of transgenic huntingtin in *Drosophila* [26]
ALS	SOD1, misfolded wild type or mutant SOD1	-SOD1 aggregates -Less stable than wild type [15, 68]	*SOD1*, Depends on mutation	Cytosol, ER/Golgi, outer membrane of extracellular vesicles [15]	NMJ (possibly originates in skeletal muscle [163]	Yes [44, 45]

function components of other diseases, including classical prion diseases that continue to threaten the health, ecology and socioeconomic well-being of many regions internationally. There has been spirited debate regarding which of the diseases qualify as being 'prion-like' [9–13], though the list is now broadly accepted to include AD, PD, other tauopathies, HD, and ALS. For the purposes of this review, we consider a disease to be prion-like if it includes the following main features: 1) intramolecular conversion of a native protein into a misfolded form; 2) the misfolded conformer causes misfolding of the normal protein via either template directed misfolding or nucleated polymerization (reviewed in [14]); 3) secretion of misfolded protein and uptake/ interaction with neighboring cells leads to toxicity (Fig. 2); and 4) the propensity to experimentally seed the transmission of misfolded protein from one site to a distant site [9–13, 15–19]. We compare several diseases in our analysis, and our logic assumes that prion-like mechanisms are at play in each. We also note that the proteins that become misfolded and/or aggregated in these diseases (huntingtin, SOD1, Aβ, and tau) have many putative functions that are expected to be lost when they become misfolded (Table 2). Although familial cases of these diseases are generally autosomal dominant (Table 1), loss-of-function may occur through haploinsufficiency or dominant negative mechanisms. In the following sections we discuss the balance of gain- versus loss-of-function in HD, ALS, and AD.

We begin by considering HD because it represents an example where experiments have unambiguously categorized it as being primarily a gain-of-function disease, and this serves as a good context from which to contrast the remaining disease comparators. We then discuss familial cases of ALS associated with SOD1 misfolding, wherein experiments demonstrate a substantial role for SOD1 loss-of-function early during disease progression. We end the section with a consideration of AD to illustrate that there is much to be learned about the intertwined roles of gain- versus loss-of-function in other prion-like diseases. We will also discuss AD as a topical case study in how protein misfolding can instigate loss-of-function by inducing disruptions to the protein-protein interactions that underpin healthy neurons as well as normal learning and memory.

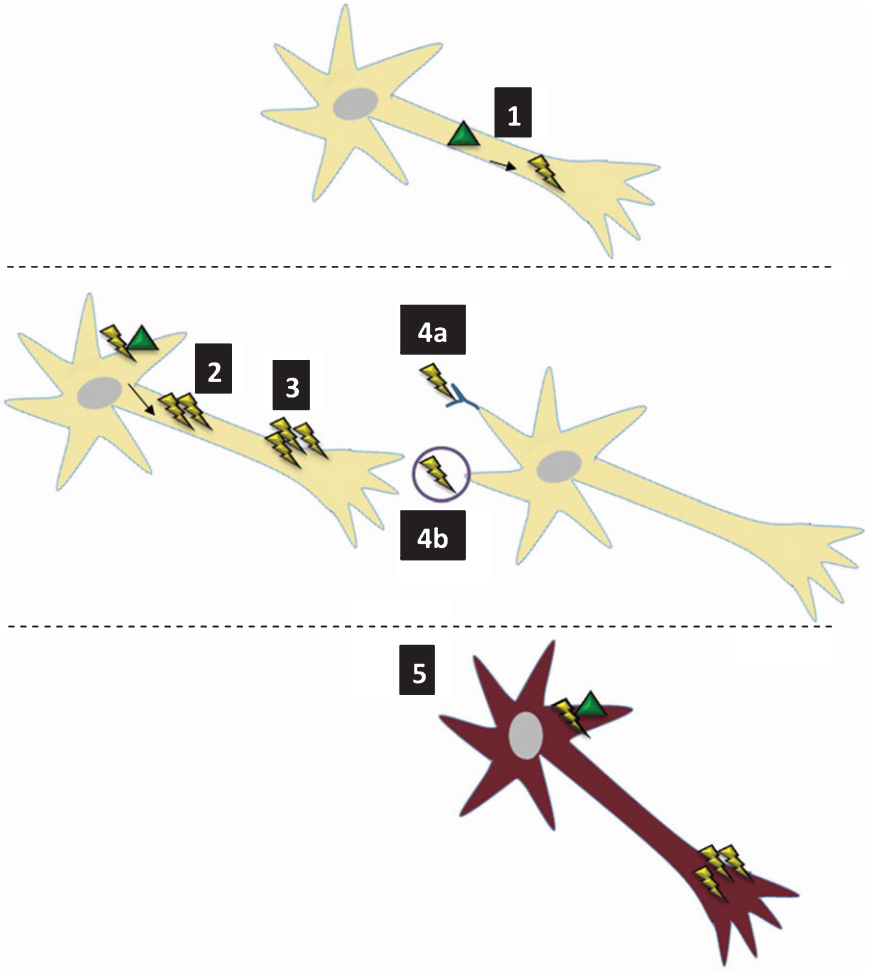

Fig. 2. Features of prion-like disease. 1) Induction of native protein into a misfolded protein (may be spontaneous or driven by altered kinetics of a mutant version of the protein); 2) Misfolded protein propagates misfolding of the native form into the misfolded form within a cell (e.g., within a neuron); 3) Aggregation of the misfolded form into oligomers and fibrils; 4) The misfolded protein exits the cell (e.g., through exosomes) and interacts with surface receptors of a neighboring cell or is taken up by neighboring cells; 5) Misfolded protein propagates in the neighboring cell leading to cell death.

Huntington's disease etiology is dominated by gain-of-function outcomes during huntingtin misfolding, but loss-of-function cannot be excluded as a contributor

HD is a classic example of an autosomal dominant disease dominated by its toxic gain-of-function component, the latter having been repeatedly demonstrated experimentally; yet even in this disease some aspects of etiology appear to be due to the loss of normally folded huntingtin. HD is a fatal neurodegenerative disease characterized by motor deficits including chorea and loss of coordination, cognitive decline (especially deficits in executive function), and psychiatric and behavioral symptoms [20]. The disease is caused by an excess of CAG (poly-glutamine) repeat expansions in one copy of the gene *HTT* [21]. Huntingtin is required for embryogenesis [22] and is ubiquitously expressed, with expression enriched most highly in the brain and testes [23]. Huntingtin is proteolytically cleaved to produce N-terminal fragments. N-terminal fragments with polyglutamine tract expansions aggregate into inclusions and cause cytoplasmic and/or nuclear pathogenesis [23]. While huntingtin is typically excluded from the nucleus, it was recently found that disruption of the N17 domain (the nuclear exclusion signal) in small N-terminal fragments causes them to accumulate and aggregate in the nucleus [24].

HD has recently been classified as a prion-like disease (Table 1). While it has been shown to be prion-like in cell culture, evidence of *in vivo* prion-

Table 2
Putative functions of prion-like proteins

Disease	Gene	Loss-of-function Animal Models		
		Drosophila	Zebrafish	Mice
AD	*MAPT*	*Mapt* **knockdown** -3% viable to adulthood -Photoreceptor defects -Neuronal degeneration [165]		*Mapt*$^{-/-}$ -No overt phenotype [164, 166–167] -Axon defects [164, 166, 168] -Axonal transport defects (e.g., iron transport [169]) -Motor deficits [112, 113, 169] -Cognitive deficits [113, 114] (age and strain dependent)
	APP	*Appl*$^{-/-}$ -Defective locomotion [170] -Memory impairments [171]	*Appa* **knockdown** -Developmental defects [145] -CNS apoptosis [145] *Appb knockdown* -Developmental defects [145, 172] -Defects in motor axon outgrowth [157, 173] -Locomotion defects and defects in NMJ synapse formation [173] -CNS apoptosis [145]	*APP* **KO** -No overt phenotype -Reduced brain and/or body mass [108, 109, 174, 175] -Reduced grip strength [108, 175] -Increased seizure susceptibility [176] -Age-dependent cognitive deficits [109, 110] **Conditional *APP* KO in muscle & motor neurons** -Aberrant patterning of NMJ synapses -Mislocalization of high affinity choline transporter affects choline vesicle release [177]
	APP, *APLP1*, *APLP2*			**APP**$^{-/-}$; **APLP2**$^{-/-}$ -Postnatal lethality [178] -Defects in NMJ structure and synaptic transmission [179] **APP**$^{-/-}$; **APLP1**$^{-/-}$; **APLP2**$^{-/-}$ Postnatal lethality, brain structure defects [180]
ALS	*SOD1*			*Sod1*$^{-/-}$ **mice** -Develop normally [56] -Mitochondrial oxidative stress [59] -More sensitive to neuronal injury [56] -Disruption of neuromuscular junction [reviewed in [38]] -Accelerated skeletal muscle denervation [reviewed in [38]]
HD	*HTT*			*Htt*$^{-/-}$ **mice** -Perinatal lethality and defects in brain development -Apoptotic cell death in the testes [reviewed in [22]]

like spread remains sparse. Mutant huntingtin was taken up by cells in culture and could seed the conversion of labeled huntingtin (reviewed in [17]). Further, mutant huntingtin aggregates are transferred between cultured cells by direct contact and the aggregates are likely spread through tunneling nanotubes [25]. It was recently found that aggregates of fluorescently labeled mutant huntingtin in *Drosophila* olfactory receptor neurons could seed the conversion of wild type huntingtin expressed in adjacent phagocytic glia [26].

Experimental evidence demonstrates that toxic gain-of-function is undoubtedly important in HD. A humanized mouse model of HD (mice that have one copy of the human mutant *HTT* gene, one copy of the human wild type *HTT* gene, and lack the mouse *Htt* gene) recapitulates features of HD neuropathology including forebrain atrophy, reductions in cortical and striatal volume and further displays psychiatric, motor learning, object recognition, and spatial learning deficits [27]. Jacobsen et al. [21] experimentally demonstrated that gain-of-function is at play in HD by assessing gene expression profiles in *Htt*$^{-/-}$ cells compared to similar cells expressing an allelic series of mouse *Htt* with increasing CAG length (an aspect of huntingtin known to be causal of increased disease severity). Because the CAG repeat expressing cells affected a largely distinct set of genes and biological

pathways from the $Htt^{-/-}$ cells, it can be concluded that HD follows a simple toxic gain-of-function mechanism that does not involve a detectable loss-of-function component [21]. Thus gain-of-function is clear in HD because 1) it has a prion-like mechanism for disease spread; 2) mice expressing mutant human *HTT* recapitulate features of HD; and 3) expression of mutant *Htt* in cell lines affects different cellular pathways compared to when the *Htt* gene is knocked out.

Despite unambiguous evidence for gain-of-function mechanisms dominating HD progression, perhaps more so than for any other of the prion-like diseases, several other lines of inquiry show that loss-of-function might also play important roles in the disease. First, neurodegeneration is observed when the huntingtin protein is disrupted. For example, neurodegeneration occurs in mouse adult forebrain neurons when *Htt* is conditionally ablated [28]. Further, transgenically expressed mutant human *HTT* induces apoptotic death in the testes of $Htt^{-/-}$ mice, and this apoptosis is reduced in transgenic mice with an $Htt^{+/-}$ background and absent in transgenic mice with an $Htt^{+/+}$ background [23]. This indicates that wild type *Htt* is protective, and loss of normally folded huntingtin in the disease state induces cell death (i.e., Haploinsufficiency/gene ablation induces a cell death phenotype): thus loss-of-function appears detrimental to disease outcomes.

Mechanistically, effects of huntingtin loss-of-function may be related to the roles of wild type huntingtin in transcription and trafficking of brain derived neurotrophic factor (BDNF). BDNF promotes survival and differentiation of striatal neurons and protects against glutamate excitoxicity [29]. BDNF levels are reduced in both mouse $Htt^{-/-}$ neural stem cells and in mouse neural stem cells with knock-in of mutant mouse *Htt* (knock-in of one copy of *Htt* with glutamine expansion), compared to BDNF levels in mouse $Htt^{+/+}$ neural stem cells [30]. Reduced BDNF levels are also observed in patients with HD [31]. Huntingtin is further involved in the intracellular trafficking of BDNF, and mutant huntingtin is unable to perform this function, likely contributing to neuronal apoptosis observed in HD [32]. Wild type huntingtin also associates with PSD-95 to regulate NMDA receptors. As mutant huntingtin is unable to bind PSD-95, NMDA receptors in mutant *HTT* expressing cells become sensitized leading to excitoxicity [33].

In sum, loss-of-function is likely occurring in HD as indicated by 1) the induction of neuron death upon conditional ablation of *Htt* [28]; 2) the ability of wild type huntingtin to reverse phenotypes imparted by a mutant *HTT* allele [23]; 3) the shared reduction in BDNF levels in both *Htt* loss-of-function models [30, 31] and HD patients; and 4) the inability of mutant huntingtin to perform functions inherent of wild type huntingtin [32, 33].

While it is clear that polyQ expansion is a requirement for HD pathology (e.g., loss of one copy of the gene is insufficient to cause disease) [34], subtle aspects of HD, such as reduction of BDNF levels are phenocopied in loss-of-function models [30]. Hence careful comparison between diseased animals and loss-of-function animal models can provide insight into where/when loss-of-function may be occurring in the diseased state.

We selected HD as the exemplar among prion- and prion-like disease wherein gain-of-function mechanisms are most dominant, unambiguous, and most thoroughly demonstrated by experimental evidence. Even in this extreme case, however, we cannot conclude that gain-of-function is sufficient for disease, as loss-of-function appears itself to recapitulate many symptoms and cellular/molecular events in HD progression.

We next consider an opposing example in this spectrum of prion-like diseases, ALS, wherein loss-of-function is clearly occurring early in disease progression.

Etiology of ALS unambiguously acts through loss-of-function during SOD1 misfolding, however gain-of-function is also required

ALS is a devastating neuromuscular disease caused by prion-like spread of misfolded proteins in the neuromuscular system. Familial mutations have been identified in the *SOD1, TARDBP, C9ORF72,* and *FUS* genes, and bone morphogenetic protein modifier genes affect susceptibility (reviewed in [35]). While familial genetics of ALS involve various loci, SOD1 appears central to disease progression regardless of genetic source or sporadic incidence. Wild type SOD1 misfolds and causes disease if overexpressed [36] and misfolded SOD1 is present in sporadic ALS and other familial forms not associated with SOD1 mutation [37]. The disease is characterized by muscle weakness and paralysis due to loss of upper and lower motor neurons and defects at neuromuscular junctions. Death usually occurs within 3–5 years of disease onset due to loss of respiratory muscle activity. Misfolding of SOD1 triggers disease in

prominent forms of ALS, but loss of SOD1 function, via dominant negative mechanisms, also contributes to pathology early in the disease course (reviewed in [38]).

ALS has been classified as a prion-like disease in cell culture models [15, 39–43], and *in vivo* [44, 45]. In the former *in vivo* study SOD1 was fused to a fluorescent protein [44], but it has recently been shown that untagged SOD1 can also propagate aggregates and disease in transgenic mice expressing human $SOD1^{G85R}$ [45]. Mutant SOD1 causes wild type SOD1 to misfold through nucleation dependent polymerization [46, 47]. ALS phenotypes were propagated between cells in mice expressing $SOD1^{G85R}$ fused to an YFP reporter [44]. When postnatal mice heterozygous for the $SOD1^{G85R}$-YFP transgene were injected with inoculum from terminal stage SOD1 mice, they developed hind-limb paralysis coincident with inclusion-like structures containing YFP accumulated in their spinal cord, brainstem and thalamus. Mice expressing untagged versions of $SOD1^{G85R}$ were less vulnerable to motor neuron degeneration than the YFP-tagged versions [44]. Two other strains of untagged SOD1 (human $SOD1^{G85R}$ and human $SOD1^{D90A}$), however, were recently found to cause motor neuron degeneration and ALS-like symptoms in hemizygous transgenic mice expressing human $SOD1^{G85R}$ [45]. Thus toxicity is likely dependent on the strain of the inoculum.

Several lines of evidence suggest that neurodegeneration and physiological phenotypes in ALS can be caused by a toxic-gain-of function mechanism associated with misfolded SOD1. Mouse models overexpressing SOD1 with various familial mutations exhibit neurodegeneration in similar patterns to what is seen in human ALS cases (for examples see [48–50]; for review see [51]). Similar results are observed when familial mutants of SOD1 are expressed in rats (reviewed in [52]), zebrafish [35, 53, 54], or invertebrate models (reviewed in [52]). Mice expressing human $SOD1^{G93A}$ also have changes in their motor system physiology that are similar to what is seen in ALS patients including reduction in motor unit function [55]. Zebrafish expressing mutant human *SOD1* also recapitulate features of ALS including defects at the neuromuscular junction [35, 53, 54], decreased muscular endurance in a swim tunnel test [35, 53], and paralysis at end of life stages [53]. Disease is not caused by SOD1 loss-of-function because when that function is replaced, symptoms are not alleviated: e.g., addition of wild type human SOD1 either has no effect or reduces the survival of transgenic mouse models expressing mutant SOD1 (reviewed in [51]). $Sod1^{-/-}$ mice do not have motor neuron degeneration [56], arguing against a simple loss of SOD1 function underlying this disease phenotype. Along this same line of reasoning, reducing levels of murine SOD1 did not significantly change survival time or axon survival in transgenic mice expressing $SOD1^{G85R}$ [57]. In sum, gain-of-function in ALS is evidenced by 1) the ability of mutant human SOD1 to recapitulate ALS phenotypes [53–55]; 2) the inability of wild type murine *Sod1* to modulate survival time and axon phenotypes in mutant human *SOD1* transgenic mice [57]; and 3) the inability of *Sod1* knockout to induce key features of ALS etiology such as motor neuron degeneration [56] (though the latter point is debated below).

A large body of evidence argues for a disease-modifying role of SOD1 loss-of-function early in the ALS disease process. SOD1's most notable role is its antioxidant activity through catalyzing the conversion of superoxide free radicals to oxygen and water [58]. Indeed the overall activity of SOD1 is reduced in patients with most fALS mutations, due to a combination of reduced intrinsic protein activity and the reduced half-life of mutant SOD1 in the tissues compared to wild type SOD1 (even mutant forms that retain their intrinsic activity *in vitro* have reduced activity *in vivo*) [38]. Axon outgrowth defects of $SOD1^{-/-}$ primary motor neuron cultures can be rescued by addition of an antioxidant, which supports a role for SOD1 loss-of-function playing a role in disease [59]. Wild type SOD1 co-aggregates with mutant SOD1 in mouse models [60]; thus normal functions of properly folded SOD1 might be lost in a dominant negative fashion when the protein is misfolded. Additional functions of SOD1 are listed in Table 2.

A role for loss of SOD1 function in ALS is supported by observations that loss-of-function is sufficient to mimic several ALS symptoms. $Sod1^{-/-}$ knockout mice display features that are similar to those in ALS and/or ALS mouse models including a reduction in motor neuron units [55], muscle denervation, selective damage to the distal-most part of motor neuron axons [59], and disruption of mitochondrial function [61]. As in mouse models of familial ALS, fast muscle fibers are more vulnerable to denervation than slow twitch muscle fibers in $Sod1^{-/-}$ knockout mice [59, 62]. Further, glutamate transport is disrupted in ALS, and *Sod1* deficient mice are more susceptible to glutamate-induced excitotoxicity [63, 64]. While normally developing (uninjured) $Sod1^{-/-}$ mice do not display motor neuron degen-

eration, motor neurons in *Sod1*$^{-/-}$ mice are more vulnerable to tissue injury [56].

Further exemplifying the intertwined gain- and loss-of-function components of ALS, one *SOD1* familial mutation (D83G) has been observed in mice that both phenocopies aspects of other transgenic mouse ALS models and also phenocopies features of *Sod1*$^{-/-}$ mice particularly well. *Sod1*^{D83G} is a point mutation that was induced by N-ethyl-N-nitrosourea in mouse *Sod1*. This mutation renders the protein dismutase inactive due to a disruption in the zinc-binding site. This mutated Sod1 protein is present at lower levels than wild type Sod1 [65]. A gain-of-function phenotype induced by this allele (and typically seen in ALS patients and human *SOD1* transgenic mouse models) includes loss of upper and lower motor neurons. Loss-of-function phenotypes in *Sod1*^{D83G}, which are also seen in *Sod1*$^{-/-}$ mice, include peripheral axonopathy, mitochondrial defects, neuromuscular junction damage, loss of motor force, and the development of liver cancer [65]. Thus the *SOD1*^{D83G} mutation clearly produces loss-of-function phenotypes.

In addition to causing subtle defects in ALS pathology as described above, loss of normally folded SOD1 during the ALS disease course may also exacerbate gain-of-toxic-function mechanisms. Misfolded SOD1 molecules may lose their enzymatic activities contributing to oxidative stress. As oxidation is known to dissociate dimers of both mutant and wild type SOD1 into monomers [66], which are more prone to misfolding than dimers, this may lead to increased misfolding and production of SOD1 aggregates (reviewed in [38]).

Most strikingly, loss of SOD1 function is conclusively prominent in ALS etiology because mutant and misfolded SOD1 are inherently unstable. This ironic inversion of events relative to prion protein misfolding biology (wherein misfolded protein is notoriously stable and difficult to eradicate, including from surgical tools or from the environment) is typified by normally folded SOD1 being extremely stable relative to most proteins [67]. Since mutant SOD1 is infamously unstable [68–73], individual molecules likely don't persist for long to cause tissue damage, but newly misfolded SOD1 takes its place.

We conclude that prion-like propagation of SOD1 misfolding in ALS has an unambiguous and substantial loss-of-function component in its etiology. However, even in this extreme example of loss-of-function in a prion-like disease, both gain- and loss-of-function mechanisms work together to propagate disease. The disease is not entirely loss-of-function because *Sod1*$^{-/-}$ mice do not exhibit all features of ALS, while animal models expressing human *SOD1* with familial mutations exhibit motor neuron degeneration and other classic symptoms. Further, phenotypes in these transgenic mice cannot be reversed by addition of wild type SOD1. Thus the gain-of-function component minimally exists insofar that misfolded proteins can operate to propagate misfolding (and spread disease). Dominant negative mechanisms are at work early in the disease to disrupt the normal functions of SOD1 (including protection of tissues from oxidative stress), leading to the multitude of phenotypes shared by ALS patients and *Sod1*$^{-/-}$ mice.

Complex roles for gain- and loss-of-function in AD Etiology

AD is a prion-like disease wherein the balance of toxic gain-of-function versus loss-of-function remains ambiguous. This ambiguity can be largely attributed to the many putative functions of its keystone protein culprits: amyloid β protein precursor (AβPP), the various cleavage products of AβPP, and the isoforms of microtubule associated protein, tau (MAPT). Amyloid β (Aβ) peptides are formed by sequential cleavage of AβPP by β and γ secretases (Fig. 3). The prevailing toxic gain-of-function hypothesis in the field is the Amyloid Cascade Hypothesis, which proposes that Aβ has an early role in disease and induces tau pathology [74]. Thus AβPP, Aβ peptides and tau have been proposed as targets for AD drug development [75–77]. It is important to consider how loss of the normal roles of these proteins may impact disease progression so that appropriate therapeutic interventions can be developed.

Aβ oligomers and misfolded tau have prion-like properties. It has long been established that Aβ forms fibrillar and oligomeric intermediates *in vitro* (for examples, see [78, 79]). Further, $A\beta_{42}$ (which is more abundant in AD patients than in healthy individuals) is more prone to aggregation than $A\beta_{40}$ (reviewed in [80]). Some familial mutations in AβPP (e.g., the Arctic mutation) result in Aβ strains that are more prone to aggregation than wild type Aβ (reviewed in [81]). More recently it has been shown that pyroglutamylated Aβ can seed the conversion of Aβ into oligomers that are toxic to cells in culture [82]. $A\beta_{42}$ oligomers can also seed the oligomerization of tau *in vitro* [83]. Polymerization of wild

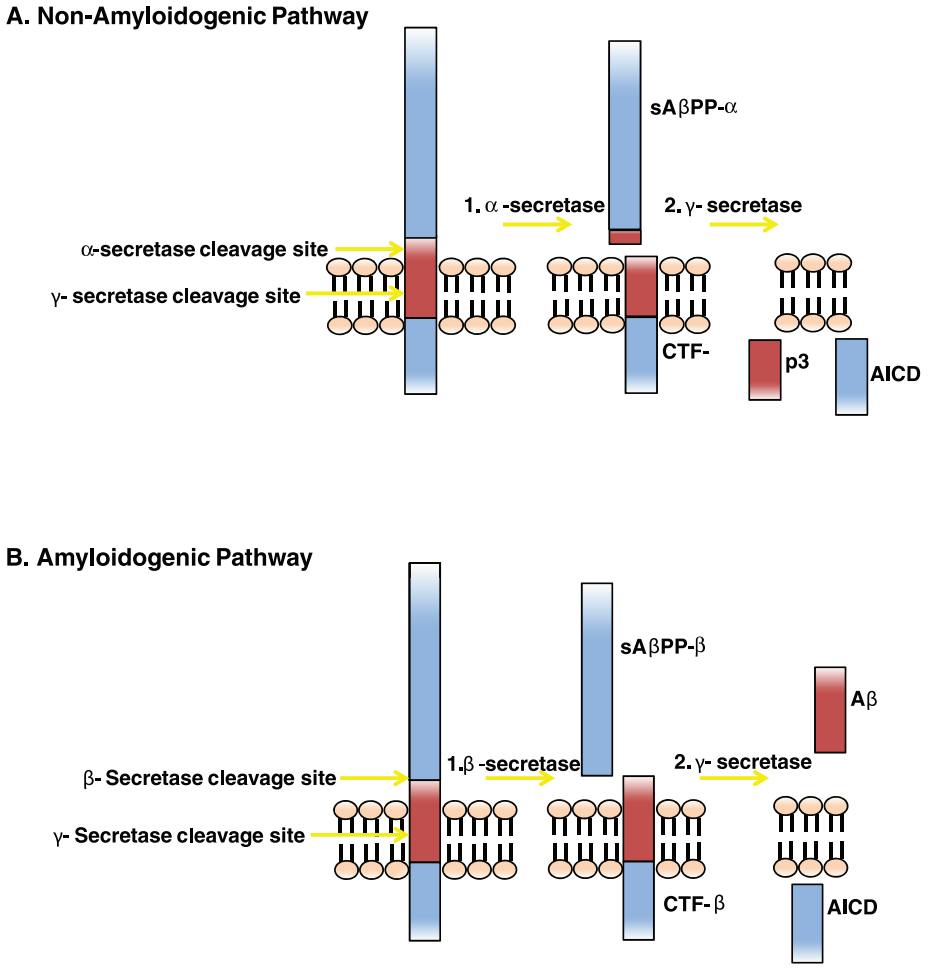

Fig. 3. AβPP is processed through the non-amyloidogenic pathway (A) and the amyloidogenic pathway (B). A) Cleavage by α-secretase yields sAβPP-α and CTF-α (1). CTF-α is then processed by the γ-secretase complex to produce p3 and AICD (2). B) Cleavage by β-secretase yields sAβPP-β and CTF-β (1). CTF-β is then processed by the γ-secretase complex to produce Aβ peptides of varying lengths (which aggregate into neurotoxic oligomers) and the AICD (2).

type tau can be induced *in vitro* by polyanionic compounds, and mutations in the tau gene (*MAPT*) enhance tau's ability to polymerize (for review see [84]). Of special relevance to the prion-like aspects of AD are the findings that both Aβ and tau aggregates from exogenous sources can propagate protein misfolding/aggregation in mouse models of AD and tauopathies. Brain homogenates from AD patients and AβPP transgenic mice, when appropriately delivered, can seed the misfolding and spread of Aβ in AβPP transgenic mice [85, 86]. Likewise, tau pathology can be seeded by exogenous tau aggregates in mouse models of tauopathy [87–92] and in wild type mice [93]. In some tauopathy models, the spread of tau pathology is associated with neurodegeneration [90, 92]. In sum, both Aβ and tau exhibit prion-like mechanisms *in vitro* and *in vivo*.

AβPP cleavage products contribute to AD pathology through toxic gain-of-function mechanisms. AβPP can be cleaved through several different pathways (Fig. 3). There is some evidence that Aβ oligomers disrupt synaptic plasticity *in vivo* [94]. $A\beta_{42}$ promotes glial cell formation [95], and this could exacerbate disease by increasing astrogliosis (reviewed in [96]). Numerous mouse models have been developed that overexpress mutant forms of human AβPP and these models recapitulate some aspects of AD in humans, though typically do not exhibit detectable neuron loss (reviewed in [97]). For example, PDAPP mice have dystrophic neuritis,

gliosis, reduced synapse number, and extracellular plaque pathology with regional spread mimicking that of AD [98], but do not exhibit neuron loss in the entorhinal cortex or CA1 region [99]. PDAPP mice and TgCRND8 mice also present with memory deficits as assessed by the Morris water maze [100, 101].

Tau also contributes to AD pathology through toxic gain-of-function. Tau that is aberrantly phosphorylated inhibits association of normal tau with tubulin, causing breakdown of axon microtubules [102]. Several lines of tau overexpressing mice have been generated and some of these phenocopy aspects of AD (reviewed in [97]). For example JNPL3 mice, which express P301L mutant tau (mutation found in patients with FTDP-17) under the prion protein promoter, display neurofibrillary tangles, cell loss, and memory impairment [103, 104].

The biochemical basis for Aβ and tau toxic gain-of-function is still under exploration, but there is evidence that Aβ and tau toxicity are linked. The longstanding Amyloid Cascade Hypothesis postulates that Aβ induces tau hyperphosphorylation, though perhaps indirectly [74]. Support for this hypothesis comes from mouse models combining amyloid and tau pathology. Crosses of Tg2576 AD mice with JNPL3 tau mice had enhanced tau pathology compared to JNPL3 mice [105], whereas in 3xTg-AD mice (expressing $A\beta PP^{Swe}$, Presenilin 1 M146V, and Tau P301L), amyloid deposits precede neurofibrillary tangles [106]. In sum, both Aβ and tau contribute to AD pathology through toxic gain-of-function mechanisms. However, as current mouse models of AD do not recapitulate all features of AD including hallmarks such as progressive neuron loss (reviewed in [97]), it is both reasonable and important to question whether toxic gain-of-function is the only process underlying AD phenotypes.

The complexity and diversity of AD etiology makes it difficult to identify phenotypes that are unambiguously the result of either AβPP or tau loss-of-function, but there are many putative functions of these proteins that, when lost during the disease course, might be ascribed to the observed symptomology (Table 2). The amyloidogenic AβPP cleavage pathway is favored over the non-amyloidogenic pathway in the AD state (Fig. 3), which means that there may be insufficient sAβPPα and AICD. sAβPPα promotes proliferation of neural progenitors, facilitates neurite outgrowth, and is neuroprotective (reviewed in [107]). Aβ peptides also appear to have important physiological functions at low concentrations. For example, $A\beta_{40}$ promotes neural stem cell proliferation and neurogenesis [95]. Aβ monomers also stimulate neurite outgrowth (reviewed in [107]). AβPP knockout mice exhibit gliosis [108] and age-dependent memory impairments [109, 110] as seen in AD. Similarly, $Mapt^{-/-}$ mice share some features of mouse tauopathy models including axonal dystrophy and microtubule defects that can be reversed by the microtubule stabilizer, Epothiolone D [111]. Muscle weakness and hyperkinesis are also shared features of $Mapt^{-/-}$ mice and tauopathy models [112, 113]. It is noteworthy, though, that AD-like cognitive deficits in $Mapt^{-/-}$ mice are controversial and may be dependent on mouse background strain [112–114]. In fact, memory-impairing effects of a human AβPP transgene (with familial Aβ inducing mutations) were reduced in $Mapt^{+/-}$ mice and blocked in $Mapt^{-/-}$ mice [115]. Speculatively, this may be because tau increases the animals' sensitivity to excitotoxic insult [115]. To summarize, AβPP, Aβ, and tau all have functions in healthy brains that can be expected to be disrupted as a result of protein misfolding, and knockout mice lacking AβPP or tau display some (though importantly not all) symptoms of AD and other tauopathies. In sum, it is clear that Aβ and tau contribute to AD through toxic gain-of-function mechanisms, but studies from knockout mice suggest that loss of normal functions of AβPP and tau may contribute to disease progression.

Both gain- and loss-of-function mechanisms are at play in prion-like diseases to varying degrees. A comparison of the extent of gain- versus loss-of-function in prion-like diseases is schematized in Fig. 4.

UNPACKING THE EVIDENCE FOR LOSS-OF-FUNCTION IN PRION DISEASES

Gain-of-function is required but not sufficient for prion disease etiology: Evidence from classical prion disease studies

There exists strong consensus in the field that toxic gain-of-function is required for prion diseases, consistent with a large body of supporting experimental evidence (reviewed in [2, 7]). Thus for prion diseases gain-of-function is accepted to be required—but is it sufficient? After considering the role of loss-of-function in other diseases, we suggest that protein loss-of-function is a causal contributor to prion disease pathology, rather than just a consequence. Prion diseases are slow and have multifaceted complex

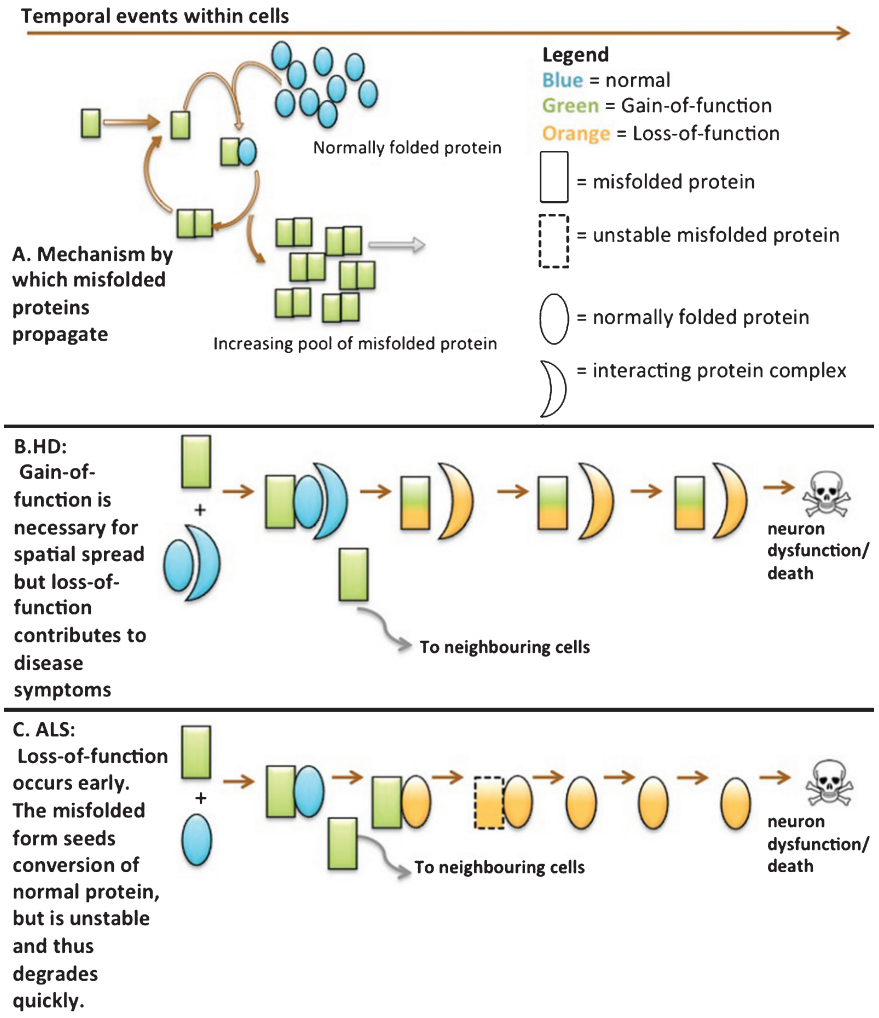

Fig. 4. Both loss- and gain-of-function contribute to the etiology of prion-like diseases. A) In prion-like diseases misfolded proteins seed the misfolding and/or aggregation of normal proteins and are required for spatial spread of the diseases. B) In HD, misfolded huntingtin associates with normally folded huntingtin and converts it to the misfolded form. Mutant huntingtin is unable to properly interact with other proteins such as PSD-95 and BDNF, thus neither mutant huntingtin nor huntingtin's interactors are able to perform their normal function (i.e., loss-of-function mechanisms are at work). Other disease phenotypes, however, can only be attributed to gain-of-toxic function. C) In ALS, misfolded SOD1 associates with normally folded SOD1 and converts it to the misfolded form. We hypothesize that since misfolded SOD1 is less stable than normally folded SOD1, misfolded SOD1 does not persist to cause damage inside the cell. Instead, loss of normally folded SOD1 has a strong influence on cell death and disease phenotypes at later temporal stages. Misfolded SOD1 also interacts with wild type SOD1 producing loss-of-function phenotypes through dominant negative mechanisms.

etiology, thus gain- and loss-of-function could be involved at different steps and in different brain regions (Fig. 5). In this section we consider how PrP^C levels correlate with disease progression and severity, and we later highlight the putative functions of PrP^C (Table 3) and potential physiological consequences of its loss relative to the symptoms observed during the course of prion disease.

An interesting puzzle has recently emerged in the literature regarding the abundance of PrP^C and disease incubation period and severity. It was newly found that PrP^C is dramatically reduced in abundance during the preclinical disease stage, and this was suggested to occur through proteostatic mechanisms [116]. Arguably, the decrease in PrP^C abundance might be viewed as a protective response undertaken by cells, as it extends the incubation time of the disease [116]. At the same time, however, this response may sensitize cells to PrP^{Sc} by diminishing the neuroprotective properties of PrP^C. Regardless of

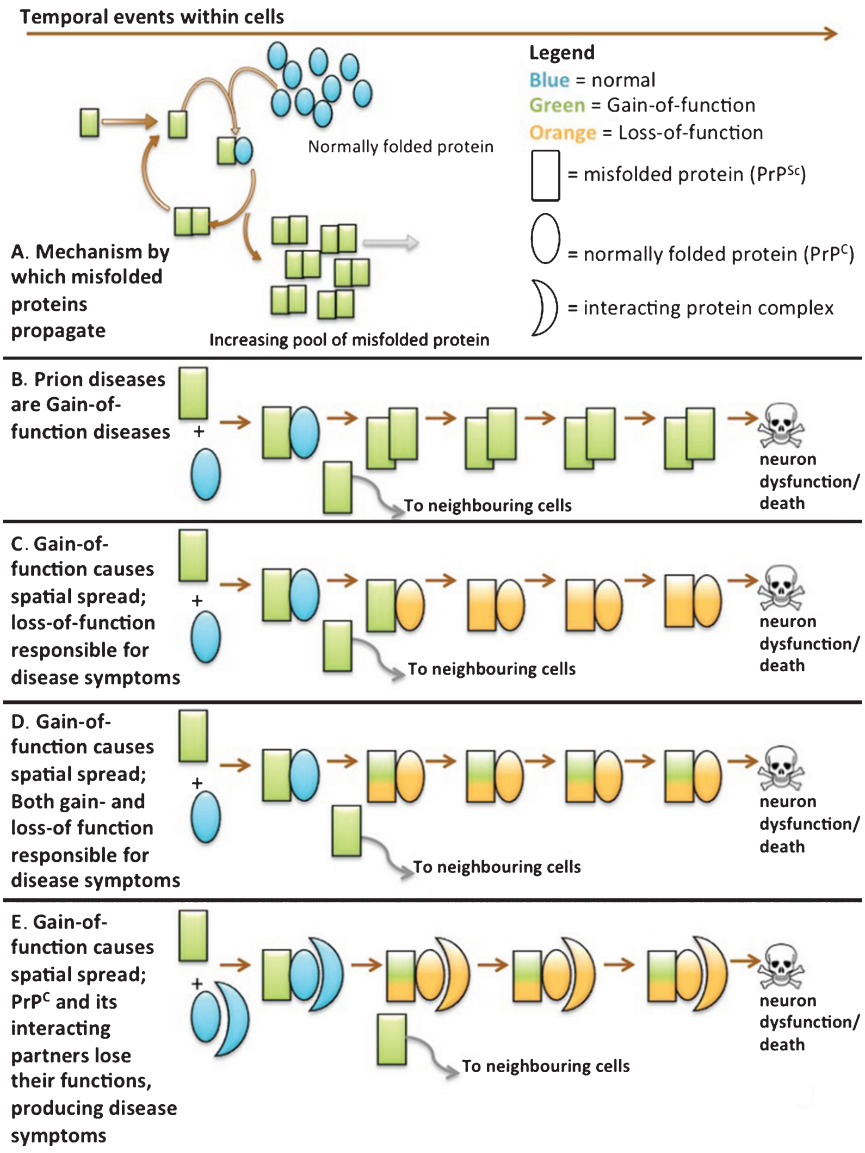

Fig. 5. Imagined and simplified hypothetical events in prion disease (and related protein misfolding diseases) with respect to the role of protein gain-of-function vs. protein loss-of-function. A) Simplified mechanism describing how misfolded proteins propagate. B–E) In each scenario we assume the process begins with a gain-of-function (GOF, green) event via the misfolded protein infecting a new cell (left side) and ends with cell death (right side). Normally functioning protein is represented in blue, whereas protein with some loss of function is represented in orange. The presented hypotheses (rows B–E) differ in the propensity for GOF to play a role, and whether any such role occurs throughout disease or early in disease. The two most extreme views are represented first, for emphasis that some mixture of GOF and LOF is likely. B) Prion disease etiology is solely via a toxic gain-of-function, and toxic misfolded prion protein kills cells. Newly misfolded protein goes on to nucleate additional events in adjacent cells (curved arrow). C) At the opposite extreme to schema B, Prion disease might be GOF only during spread of misfolded protein to an uninfected cell (either an adjacent cell or a cell in a distant tissue). Considering the biochemical stability of misfolded prion protein, this option may be unlikely. D) Hypothetically, misfolding of the prion protein may exert its effects through both GOF and LOF at different phases of disease, including the assumed GOF required for spread to a new cell inducing loss of function(s) both early and late in etiology. E) The reality of such diseases are complex, and we conclude that both GOF and LOF are required for prion & prion-like disease progression; this invokes questions regarding if both aspects are likely occurring in several stages of disease. Certainly protein-protein interactions are occurring amongst pools of proteins, in various states of oligomerization and with differential kinetics of protein turnover, thereby complicating the formation of testable hypotheses. PrP^{Sc} and PrP^{C} interact with each other in a complex environment of other proteins and macromolecules, and misfolding is reasonably expected to disrupt interacting proteins leading to toxic effects. Firstly, interacting proteins (complexes) may lose their own function when unable to interact normally with PrP^{C}. Further, accumulation of PrP^{Sc} is found to be coincident with an apparent degradation of PrP^{C} (Similar to Fig. 4C, see text), and as such interacting proteins would be expected to lose their function. If, for example, the functions of interacting proteins were neuroprotective, then cell death would be favored.

Table 3
Putative functions of PrP^C and its interactome

Broad Function	Specific Function	Interacting proteins	Downstream pathway
Cell adhesion	Embryonic cell adhesion [181, 182]	Src kinases [181, 182]	Ca^{2+}-independent homophilic interactions [181]
			Ca^{2+}-dependent, Trafficking of E-cadherin, β-catenin, F-actin to plasma membrane [181, 182]
	Zebrafish lateral line [183]		E-cadherin, β-catenin [183]
	Neuritogenesis (growth cone formation) [184]	Reggie, Fyn/MAP kinase [184]	N-cadherin [184]
Neurite outgrowth	Neurite outgrowth [185]	Integrins/caveolin 1/Fyn [185]	Raf/Ras→Erk1/2 [185]
	Neurite outgrowth [186]	NCAM→Fyn [186]	TBD
	Neurite outgrowth [187]	Lnγ1→mGluRI/MgluR5 [187]	Phospholipase C, Ca^{2+} mobilization, protein-kinase C (PKC), extracellular signal-regulated kinase (ERK1/2) [187]
Regulation of neurotransmission	Regulation of NMDA receptors [127, 129]		
	Regulation of Kv4.2 channels [126]	DPP6 [126]	
Metal homeostasis	Zinc uptake at the synapse [188]	AMPA receptors (GluR2 and/or GluR1) [188]	Inhibits tyrosine phosphatase activity [188]
Regulation of protein processing	APP processing [146, 149]	BACE [146, 149]	sTBD

its debated consequence, the observation that PrP^C is dramatically less abundant early in preclinical phases of prion disease starkly underscores the likelihood that PrP^C loss-of-function is a substantial and pervasive contributor to disease etiology.

Studies where PrP^C is knocked out or reduced during experimentally induced prion infection have produced mixed results, highlighting the need for further study into the contexts in which PrP^C is neuroprotective versus when it is instead detrimental to the disease course. On one hand, *Prnp* expression [117] and the presence of the GPI anchor [118] are requirements to infect mice with mouse prion strains, and halving the *Prnp* dosage (i.e., in $Prnp^{+/-}$ mice) is also protective [117]. On the other hand, murine PrP^C appears to be protective when prions from other species are present. Exemplifying the former, $Prnp^{+/-}$ mice had delayed onset of gliosis and spongiosis compared to wild type mice [119]. Suppression of *TgPrnp* expression prevents CNS dysfunction, neuronal loss, vacuolation, and gliosis [120]. Further, specific ablation of neuronal *TgPrnp* with a Cre-Lox system prevented neuronal loss, gliosis, and spongiosis despite accumulation of extraneuronal PrP^{Sc} [121]. On the other hand, *Tg(HuPRNP)* mice with a $Prnp^{+/+}$ background were resistant to inoculum with human prions, but *Tg(HuPRNP)* mice with a $Prnp^{-/-}$ background were susceptible to prion disease (i.e., displayed clinical symptoms). Chimeric Tg (Mhu2M) mice with a $Prnp^{-/-}$ background were also more susceptible to prion infection (i.e., less time to symptom onset) than chimeric mice with a $Prnp^{+/+}$ background, but to a lesser extent. The authors hypothesized that the endogenous murine PrP^C had a greater affinity to the hypothetical murine conversion cofactor, termed 'Protein X', than human or chimeric PrP^C, and thus hindered conversion of human or chimeric prions (by outcompeting the human or chimeric PrP^C for access to murine 'Protein X') [122]. The 'Protein X' hypothesis has largely been discounted since a cofactor for the conversion of PrP^C to PrP^{Sc} has not been identified. Instead, PrP^C molecules encoded by different alleles are thought to compete for nascent prion seeds [123]. An alternate hypothesis to explain the phenomenon observed by Telling et al. [122] is that endogenous PrP^C has neuroprotective functions. Supporting this hypothesis, *Tg(MoPrnp P101L)* mice with a *Prnp* null background succumbed to disease faster than those with a murine $Prnp^{+/+}$ background and had more prion protein plaques and spongiform degeneration [124]. *PRNP* P101L is a familial mutation in humans that underlies Gerstmann–Sträussler–Scheinker syndrome (GSS) and causes spontaneous misfolding of PrP^C into PrP^{Sc}. Additionally, when brain homogenate from sick *Tg(MoPrnp P101L)* mice were used to inoculate *Tg(MoPrnp-101L)/*$Prnp^{-/-}$ and *Tg(MoPrnp-101L)/*$PrP^{+/+}$ mice, the *Tg(MoPrnp-*

101L)/PrP$^{-/-}$ mice presented disease symptoms sooner than mice expressing *Prnp* [124]. Overall these studies suggest that GPI-anchored PrP^C is a requirement for prion disease progression and reducing PrP expression slows disease. In some instances, however, if there is a sufficient 'species barrier' or 'strain barrier', other versions of PrP^C can be protective.

Further study is needed to ascertain when and where PrP^C is neuroprotective and when it becomes detrimental to cell/tissue health. It may be that PrP^C is protective early in, and prior to, the disease (when PrP^{Sc} levels are still relatively low), but that the presence of PrP^C accelerates disease at later stages. It is also possible that PrP^C is protective when expressed in some cell types and detrimental when expressed in other cell types. Regardless, reduced PrP^C abundance early in disease, accompanied by the myriad functions of PrP^C (reviewed immediately below) that are lost when PrP^C disappears and/or misfolds, combine to compel the argument that loss-of- function may be a substantial contributor to prion disease progression. In the next section we consider the putative functions of PrP^C and how these functions may be disrupted during disease.

Putative functions of PrP^C *may be lost during the course of prion disease infection*

PrP^C has a number of putative functions (Table 3) that are reasonably expected to be disrupted as a result of its decreased abundance early during disease, its conversion to PrP^{Sc}, and/or through dominant negative interactions between PrP^C and PrP^{Sc} in prion diseases. There is solid support for loss-of-function being a component of prion disease etiology because many prion disease symptoms can be mimicked by PrP^C loss-of-function (Table 4). Conversely, no disease symptom can be exclusively attributed to gain-of-function in transgenic overexpression models, as loss-of-function may also be occurring in these models. Thus it is difficult (if not impossible) to experimentally disentangle gain- from loss-of-function in transgenic overexpression models (Table 4). Efforts to study the normal physiology of PrP^C have typically been thwarted by inconsistency of phenotypes between different *Prnp*$^{-/-}$ mouse lines (Table 5, reviewed in [125]). Some functions of PrP^C have been verified in other species including rats and zebrafish, and further research in these alternative animal models will help to establish which PrP^C functions are the most important/relevant to disease etiology. Currently, solid evidence exists for roles of PrP^C in neuroprotection and learning. While disparate lines of evidence support a role for PrP^C in neuroprotection, molecular mechanisms that underlie this outcome remain mysterious. Thus failure to acknowledge that we have much to learn about the role of PrP^C in normal physiology would severely limit progress on uncovering the pathophysiology of prion diseases and novel therapeutic strategies. Here we will examine what is known about the role of PrP in neuroprotection and learning.

PrP^C *regulates neurotransmission and provides protection from seizures*

PrP^C promotes the survival of neurons in healthy brains by regulating neurotransmission, thus protecting against excitoxicity. PrP^C has been shown to regulate potassium currents in rat neurons, and this function is lost in PrP^C with a GSS mutation (insertion of an extra octarepeat) [126]. PrP^C also regulates NMDA receptors in brain slice cultures [127, 128]. Further, we have shown that zebrafish PrP2 (a zebrafish homlog of PrP^C) regulates NMDA receptors [129]. Loss of normally folded PrP likely causes neuron death at some stages of prion disease courses because there is less PrP^C available to regulate neurotransmission.

PrP^C loss-of-function may also account for seizures and seizure-like symptoms in prion diseases, and support for this comes from PrP^C loss-of-function animal models that have increased susceptibility to convulsants. Seizures occur in 15% of patients with sporadic CJD and some patients with genetic prion diseases ($\sim$10% of patients with familial CJD, <10% of patients with GSS or FFI) [130]. Tremors also occur in some cases of BSE [131]. Several studies have found that *Prnp* knockout mice are more susceptible to seizure-inducing drugs than wild type mice [132–135]. Controversy has existed regarding whether these seizures observed in *Prnp* knockout mice are a result of PrP^C loss-of-function or strain differences. A recent study, however, clarified this issue when the authors reported that increased seizure susceptibility in *Prnp* knockout mice exists when *Prnp* knockout mice are compared to wild type mice of the same strain [135] (Table 5). Strikingly, we also found that the zebrafish homolog of PrP^C, PrP2, reduces the susceptibility of zebrafish larvae to pentylenetetrazole-induced seizures, pointing to an ancient and conserved (i.e., important) role of PrP^C in modulating seizures and neuronal activity [129].

Table 4
Disease symptoms observed in *Prnp* knockout animals

	Loss-of-function?	Defects observed in:		
		KO or KD fish	KO mice/ KO cell lines	Gain-of-function models
Neuroprotection from ischemia/traumatic brain injury	✓	TBD	Increased susceptibility to ischemia [189, 190]	?
Neuroprotection against excitoxicity/seizures	✓	Increased susceptibility to PTZ convulsant [129]	Increased susceptibility to convulsants [132–135]	?
Regulation of NMDA receptor	✓	Altered NMDA receptor regulation [129]	Altered NMDA receptor regulation [127, 191–193]	?
Metal homeostasis	✓	Preliminary/TBD	Altered distribution of iron, copper and zinc [194]	?
Cell Adhesion	✓	Disrupted cell adhesion in early development [145, 181, 195]	TBD	?
Synaptogenesis	✓	TBD	TBD	? Decreased Purkinje cell dendritic spine density during infection [196]
Adult Neurogenesis	✓	TBD	Reduced neural precursor proliferation [197]	? Prion-infected NSC have defective neuronal differentiation [198]
Learning	✓	Leighton et al. unpublished	Decline in spatial learning [137], age-dependent decline in learning [138, 139]	?

Table 5
Phenotypes of $Prnp^{-/-}$ mouse lines (modified from Striebel et al. 2013 [125])

$Prnp^{-/-}$ Line	Genetic Background	Overt/anatomical phenotypes	Seizure susceptibility /learning deficits	Caveats
Edin 129/Ola [199]	129/Ola	No overt phenotype	Seizure susceptibility [135], learning deficits [137]	
Edin 129/Ola [200]	C57BL/10SnJ	None reported	Learning deficits [137]	Flanking genes
ZrchI 129/Sv [201]	Various	No overt phenotype [201], demyelinating polyneuropathy [202]	Seizure susceptibility [132–135, 203], Learning deficits [138, 139]	Flanking genes
Ngsk 129/Sv [204]	C57BL/6	No overt phenotype [204], demyelinating polyneuropathy [202]	Not tested	Increased Doppel Expression [205, 206], Flanking genes
Rikn 129/Ola [207]	C57BL/6	Tremor and ataxia in aged mice [207]	Not tested	Likely has increased Doppel Expression (not tested to our knowledge), Flanking genes
ZrchII 129/Ola [208]	C57BL/6	Ataxia and Purkinje cell death [208]	Not tested	Increased Doppel Expression [208], Flanking genes
Rcm0 129/Ola [209]	Unknown	Ataxia and purkinje cell death	Not tested	Increased Doppel Expression [205], Flanking genes

PrP^C facilitates learning and memory

Cognitive deficits observed in prion disease patients may be due to the loss of normal PrP^C function. Prion disease patients (sporadic, iatrogenic, and familial CJD) have cognitive dysfunction beginning early in the presentation of disease course. While the most prevalent symptoms are executive dysfunction and language impairments, some patients have memory impairments that are related to visuospatial problems [136]. $Prnp^{-/-}$ mice have learning deficits supporting a role for PrP^C loss-of-function in prion diseases [137–139]. $Prnp^{-/-}$ mice had reduced hippocampal dependent spatial learning compared to wild type mice (which was rescued by a neuron-specific human *PRNP* transgene) [137], and aged $Prnp^{-/-}$ mice performed poorly in the novel object recognition [138] and inhibitory avoidance tasks [139] compared to aged wild type mice. Aged rats treated with α-PrP^C antibody also performed poorly

in an inhibitory avoidance test relative to aged rats treated with a control antibody [139]. It is possible, however, that the α-PrP^C antibody induced a gain-of toxic function through PrP^C as some α-PrP^C antibodies induce toxicity and trigger the unfolded protein response [140]. We have also found that loss of the PrP2 paralog in zebrafish reduces the ability of aged zebrafish to recognize a novel object (Leighton et al., unpublished), supporting a conserved, ancient, and important role for PrP^C as a mediator of learning and memory.

Gain-of-function is required for initiation and spread of prion diseases, but loss-of-function is also an important disease contributor

In sum, while toxic gain-of-function is necessary to initiate and spread prion disease (as evidenced by lack of disease in $Prnp^{-/-}$ mice [117] and lack of infectivity in $Prnp^{-/-}$ tissue surrounding tissue grafts that express PrP^C [141]), loss of PrP^C undoubtedly has detrimental effects for neuron health at some, if not all, stages of neurodegenerative disease. PrP^C levels are reduced through various mechanisms during the disease course (e.g., by conversion to PrP^{Sc} and by proteostatic mechanisms reducing its abundance [116]). Thus normal/healthy physiological processes are expected to be disrupted, including neuron survival signaling, regulation of neurotransmission, and synaptic plasticity underlying cognition. Many prion disease symptoms are observed in PrP loss-of-function animal models, and loss-of-function may be induced in gain-of-function animal models. We conclude that gain-of-function and loss-of-function occur in (a cacophonous) concert in classical prion diseases. Future work is needed to determine *when* loss-of-function is most important for disease etiology because this will inform disease management strategies.

PrP^C INFLUENCES THE FUNCTION AND DYSFUNCTION OF OTHER PROTEINS: A CASE STUDY ON THE LOSS OF PrP^C FUNCTION DURING AD

PrP^C interacts with numerous CNS proteins that have keystone roles in neurodegenerative diseases, including AβPP and tau. Thus loss of PrP^C function may have a role in other diseases (e.g., AD, frontotemporal dementia, and PD). Likewise, loss of AβPP and tau function may impact classical prion disease course. Since AD and prion diseases have similar disease pathology and AβPP and PrP^C interact both physically and genetically (see below), we selected AβPP as a candidate for the case study below, as an exemplar of how loss of PrP^C might impact upon the normal physiology of other proteins and the progression of other neurodegenerative diseases.

While the normal functions and molecular mechanisms of AβPP and PrP^C remain enigmatic (see sections "Complex roles for gain- and loss-of-function ..." and "Putative functions of PrP^C ..." above), numerous studies have demonstrated that AβPP and PrP^C have interacting roles in cell/organism physiology. It was recently established that PrP^C interacts biochemically with AβPP [142–146] and with Aβ (first reported in [1]). It has been proposed that PrP^C may contribute to AD by acting as a receptor/mediator of Aβ toxicity [1]. One question that has been asked less often is whether these protein interactions have relevance for prion diseases. As PrP^C and AβPP interact, loss of normally folded PrP in prion diseases may disrupt normal physiology of AβPP and contribute to prion disease progression. Prion diseases and AD share many similarities (Table 6) and we postulate that studying interactions between AβPP and PrP^C will synergistically lead to insights on the mechanisms and novel therapies for both prion diseases and AD.

PrP^C has the potential to modulate AD pathogenesis and prion disease through regulation of AβPP metabolism and other underexplored mechanisms. PrP^C levels are reduced in sporadic AD patients (intriguingly reminiscent of reduced PrP^C early in prion disease) suggesting that loss of PrP^C could play a role in sporadic AD progression [147, 148]. PrP^C also inhibits β-secretase cleavage of wild type AβPP [146, 149] such that loss of normally folded PrP may cause cells to favour the amyloidogenic pathway of AβPP cleavage. The PrP^C N1 cleavage product has also been reported to inhibit Aβ toxicity [150–153]. It has been shown that prion infection can enhance $Aβ_{42}$ production in mouse models of AD [154, 155], but a question that remains open is whether levels of Aβ peptides change during natural prion infection. Aβ oligomers can redistribute PrP^C to the cell surface, which may further propagate Aβ toxicity in AD or PrP^{Sc} toxicity in prion disease [156]. We have also found that zebrafish *APPa* and *PrP1* have a synergistic neuroprotective effect at the genetic level. Co-knockdown of zebrafish *APPa* and *PrP1* with morpholinos induces neuron death, but concerted knockdown of other zebrafish gene

Table 6
Similarities between Alzheimer's disease and prion diseases

Alzheimer's Disease (AD)	Prion diseases (prion diseases: BSE, CWD, CJD)
Protein misfolding → spreading amyloid plaques →neuron death → dementia	Protein misfolding → spreading amyloid plaques → neuron death → dementia
Experimentally transmissible [85, 86]	Experimentally transmissible (reviewed in [159]
Sporadic & Familial [*APP*, *PS1*, *PRNP*, etc] (reviewed in [210, 211])	Infectious, Sporadic & Familial [*PRNP*, *SPRN*] (reviewed in [159])
Amyloid β Protein Precursor (AβPP) → Aβ plaques [→Tau into neurofibrillary tangles]	Prion Protein (PrP^{C}) → Scrapie (PrP^{Sc}) plaques
AβPP is transmembrane multidomain protein in lipid rafts [212], processed by endoproteolysis to release fragments (reviewed in [213])	PrP is GPI-anchored multidomain protein in lipid rafts [214], processed by endoproteolysis to release fragments (reviewed in [215])
AβPP's biological function is enigmatic	PrP^{C}'s biological function is enigmatic
Homodimers of AβPP affect their processing towards pathogenesis [216]	Homodimers of PrP affect their aggregation towards pathogenesis [217]
$APP^{-/-}$ mouse is surprisingly normal [108, 109]	$Prnp^{-/-}$ mouse is surprisingly normal [199, 201]
Zebrafish with 2 copies: *appa* & *appb* [218]	Zebrafish with 2 copies: *prp1* & *prp2* [219]
Mammalian orthologues can replace zebrafish orthologues [145]	Mammalian orthologues can replace zebrafish prp1 orthologue [145]
Knockdown of *appa* or *appb* in zebrafish leads to a neurodevelopmental phenotype [145, 173]	Knockdown of *prp1* or *prp2* in zebrafish leads to a neurodevelopmental phenotype [145, 181]

paralogs did not induce such effects. This effect is conserved since either mouse PrP^{C} or human AβPP rescues the phenotype [145]. Thus a niche role for the interaction between mammalian AβPP and PrP^{C} was revealed, including modulating neuron survival [145]. The molecular mechanisms behind this synergistic neuroprotection remain to be resolved, but AβPP and PrP^{C} clearly have important overlapping roles and ancient important interactions in normal CNS physiology.

We have highlighted several roles for PrP^{C} in modulating AβPP physiology and have noted that PrP^{C} abundance is reduced in some AD cases [147, 148]. Since AβPP and PrP^{C} interact [145], and because they have overlapping roles in the CNS [145], loss of PrP^{C} and its normal functions must be expected to influence AD progression (and loss of AβPP's normal functions may contribute at some stages of classical prion disease progression). The mechanisms underlying the interactions between AβPP and PrP^{C} remain enigmatic, and further study in this area will importantly inform design of therapeutic strategies for both prion diseases and AD.

FUTURE PERSPECTIVES ON PRION AND PRION-LIKE DISEASES

Experimental approaches that have been used to unravel loss- and gain-of-function in prion-like diseases

The historic approach that has been used to identify evidence of loss-of-protein-function in disease has been to examine how and to what extent knockout animals can phenocopy the disease state. As described in the sections above, loss-of-function phenotypes are relevant in prion-like diseases but are often subtle and difficult to untangle from the overt phenotypes that are induced by misfolded/aggregated proteins. An important addition to knockdown/knockout experimental approaches is to rescue the phenotypes by genetic complementation (i.e., transgene rescue, or better yet, conditional knock-in of the targeted gene). These types of experiments can also be used to determine whether mutant versions of a protein have lost their normal physiological functions. If the mutant form of a protein can rescue a loss-of- function phenotype, it means that the mutant form performs the same function as the wild type form but has an additional gain-of-function toxic mechanism. If the mutant form cannot rescue the phenotype, the theoretical interpretation is that the mutant form also loses its normal function. This result, however, is not definitive as it is difficult to prove that the negative result is due to loss-of mutant protein function. To date, very few studies of this type have been done for prion-like diseases (Table 7). An experiment in zebrafish suggests that the Swedish mutation renders AβPP unable to maintain its normal function in motor axon maintenance [157], though further experiments in this area are warranted. Similarly, it has been shown that mutant huntingtin can rescue embryonic lethality in $Htt^{-/-}$ mice [22]. Conditional knock-ins of mutant hungtintin at later developmental stages could be used to determine whether mutant hungtintin loses the neuroprotective functions of wild type huntingtin. Future

Table 7
Rescue experiments can be used to determine whether mutant proteins retain their normal functions in prion-like disease

Disease	Gene	Mutation	Rescue?
AD	*MAPT*	This has not been done to our knowledge	N/A
	APP	APPswe (K670N, M671L)	Does not rescue convergent extension defects in zebrafish morphants [172] or motor axon deficits [157]
ALS	*SOD1*	This has not been done to our knowledge	N/A
HD	*HTT*	128 Poly-Q expansion	Rescues embryonic lethality in ko mice (reviewed in [22])
PD	*SNCA*	This has not been done to our knowledge	N/A

research in this area would provide crucial insights into the progression of prion-like diseases.

How can we determine if and when PrP^C loss-of-function is important?

For injury or for many diseases outside of neurodegeneration, protein loss-of-function is a well-accepted disease mechanism, often associated with mutation (e.g., tumor suppressors mutated in cancers, or *CTFR* mutated in cystic fibrosis). Here we have synthesized information from disparate diseases to argue that loss-of-function in template directed misfolding is prevalent and the norm rather than an exception. Prion disease is similar to HD in that the misfolded protein forms stable beta sheets and thus the toxic effects of the misfolded form are long lasting, masking the effects of PrP^C loss-of-function. To determine how PrP^C loss-of-function contributes to the disease it will be critical to identify subtle phenotypes in PrP^C null animals and determine whether these are phenocopied during prion infection. Part of this process will be to compare global gene expression in *Prnp* null mice to those with prion disease (a study of this nature has been done in the HD field [21]). The genetic background in such studies would of course need to be carefully controlled. Assessing the ability of mutant PrP^C to rescue phenotypes in PrP^C null animals will also shed light on whether mutant PrP^C can perform its normal functions. Adding and removing PrP^C at different stages of experimentally induced prion disease could be used to determine when PrP^C loss-of-function is important for disease progression. We propose that expanding this approach by deploying transgenic variants of PrP^C that are seemingly inert to protein misfolding, such as rabbit PrP^C [158], would allow one to assess outcomes when PrP^C is not reduced in abundance or misfolded (i.e., the transgene would rescue the loss-of-function) and yet presumably would not be contributing to further gain-of-function etiology. A potential caveat of the proposed experiment is that expression of rabbit PrP^C may interfere with the gain-of-function conversion of PrP^C to its misfolded form and its associated toxicity, as has previously been the case when heterologous pools of PrP^C are present [122].

Implications for disease prevention and management

Misfolded PrP^{Sc} is an obvious therapeutic target in prion disease, and much effort has been put forth to enable such strategies, but PrP^C has also been proposed as a therapeutic target for prion disease prophylaxis and treatment. Disruption of PrP^C through down-regulation or blocking interaction with PrP^{Sc} via antibodies or small molecules is proposed as a therapeutic strategy for prion diseases and AD. If PrP^C loss-of-function is an early disease mechanism, however, mitigation of treatment side effects will likely also require finding ways to normalize protective portions of PrP^C abundance or to target downstream pathways. Overall, further research is needed to understand the normal roles of PrP^C and prion-like proteins such as AβPP in healthy brains and their protective roles when AD or prion disease begin.

CONCLUSION

Prion disease research has contributed much toward understanding progression of other neurodegenerative diseases. Here we 'turned the tables' to argue that strategies used to study prion-like diseases should now be applied to prion diseases to unravel the complexity of gain- versus loss-of PrP^C function in prion diseases. Our proposed strategies include: 1) Careful comparison between diseased animals and loss-of-function animal models to identify relevant phenotypes; 2) Genetic complementation to rescue phenotypes in *Prnp* null animals, and 3) Evaluation of whether mutant versions of PrP can adequately rescue phenotypes in *Prnp* null animals. We have also noted that there are many

putative functions shared between PrP^C and its interaction partner, AβPP, which warrant continued investigation. Further research on the normal protective functions of both PrP^C and AβPP will be necessary to understand prion disease and AD etiology and to design novel and efficacious therapeutics.

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