

Review



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Understanding and exploiting interactions between cellular proteostasis pathways and infectious prion proteins for therapeutic benefit

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Several neurodegenerative diseases of humans and animals are caused by the misfolded prion protein (PrP^{Sc}), a self-propagating protein infectious agent that aggregates into oligomeric, fibrillar structures and leads to cell death by incompletely understood mechanisms. Work in multiple biological model systems, from simple baker's yeast to transgenic mouse lines, as well as *in vitro* studies, has illuminated molecular and cellular modifiers of prion disease. In this review, we focus on intersections between PrP and the proteostasis network, including unfolded protein stress response pathways and roles played by the powerful regulators of protein folding known as protein chaperones. We close with analysis of promising therapeutic avenues for treatment enabled by these studies.

1. Introduction

Transmissible spongiform encephalopathies (TSEs), or prion diseases, comprise a class of invariably fatal and usually zoonotic neurodegenerative disorders that affect mammalian species, including humans, livestock and wild animals (table 1) [51,57]. Hence, prion diseases are an important public health concern worldwide [58]. Thus far, prion diseases targeting humans include Creutzfeldt–Jakob disease (CJD) in their sporadic (sCJD), iatrogenic (iCJD), familial (fCJD) and variant (vCJD) forms, Gerstmann–Sträussler–Scheinker (GSS) syndrome, fatal familial insomnia (FFI), sporadic fatal insomnia (SFI), variably protease-sensitive prionopathy (VPSPr) and kuru [59–61]. Prion diseases also include scrapie in sheep and goats [62], bovine spongiform encephalopathy (BSE) in cattle [63], chronic wasting disease (CWD) in cervids [51], transmissible mink encephalopathy (TME) [64], feline spongiform encephalopathy (FSE) in domestic and larger captive *felidae* [65], exotic ungulate spongiform encephalopathy (EUE) in exotic zoo ruminants and camel prion disease [66]. Human and animal TSEs present a similar gross array of clinical features such as progressive motor dysfunction, cerebral ataxia and/or cognitive impairment. However, other disease phenotypes such as incubation period, histopathological lesions and clinical manifestation, among others, may vary considerably in some specific diseases [67,68]. TSEs are characterized by misfolding of the host-encoded, protease-sensitive prion protein (PrP^C) into a pathological, protease-resistant form (PrP^{Sc}) that self-aggregates into non-soluble, highly ordered, fibrillar deposits [69,70].

Table 1. Prion disorders and associated clinical presentations.

prion disorder	affected host	etiology	age of onset	clinical presentations
familial Creutzfeldt–Jakob disease (fCJD)	human	inherited	mean = 60 years (range, 31–87 years) [1–3]	rapidly progressive dementia with ataxia, persistent fatigue, weight loss without change in appetite, myoclonus [1,4–6]
sporadic Creutzfeldt–Jakob disease (sCJD)	human	sporadic	mean = 65 years (range, 42–91 years) [7]	limb ataxia, depression, anxiety, psychosis, cognitive and visual impairments [8–10]
variant Creutzfeldt–Jakob disease (vCJD)	human	infectious	mean age of death = 28 years [11–13]	rapidly progressive dementia with behavioural abnormalities, extrapyramidal features, ataxia, myoclonus [14,15]
iatrogenic Creutzfeldt–Jakob disease (iCJD)	human	infectious	variable (associated with cadaveric growth hormone treatment, dura grafts, neurosurgery) [16,17]	slow presentation of neurologic symptoms as well as behavioural abnormalities, extrapyramidal features, ataxia, myoclonus [16,17]
Gerstmann–Sträussler–Scheinker (GSS) syndrome	human	inherited	mean = 50 (range, 21–87 years) [3,18,19]	late-onset dementia and a slowly progressive ataxic or motoric disorder, absent reflexes in the legs [3,20,21]
fatal familial insomnia (FFI)	human	inherited	mean = 51 years (range, 19–83 years) [3,22]	progressive insomnia, dysautonomia such as tachycardia, hyperpyrexia and hyperhidrosis [23,24]
sporadic fatal insomnia (SFI)	human	sporadic	mean = 50 years (range, 13–70 years) [25–27]	progressive insomnia, motor abnormalities, dysautonomia, ataxia [27–29]
variably protease-sensitive prionopathy (VPSPr)	human	sporadic	mean = 64.5 years (range, 48–81 years) [30–32]	dementia, cognitive decline, mood/behavioural changes [30–32]
kuru	human	infectious	variable (≥ 5 years of age) [33]	progressive cerebellar ataxia, emotional changes such as compulsive laughter, apprehension, depression, inappropriate euphoria [33–35]
scrapie	sheep, goat and mouflon	infectious	variable (≥ 2 years of age) [36]	behavioural changes such as resistance to milking, aggression, gnashing of teeth and exaggerated response to external stimuli, hypokinaesia and even cannibalism [37]
bovine spongiform encephalopathy (BSE)	cattle	infectious	typically, 4–5 years [38]	gait ataxia, apprehension, hyperaesthesia, decreased milk production, loss of body weight despite continued appetite [39,40]
chronic wasting disease (CWD)	cevids	infectious	variable (> 1 year of age) [41]	excess salivation, teeth grinding, fever, rough or dry hair coat, aspiration pneumonia, dilute urine (if water is freely available) and emaciation [42,43]
transmissible mink encephalopathy (TME)	mink	infectious	variable (> 1 year of age) [44]	locomotor incoordination, difficulties swallowing, epileptic seizures, self-mutilation, progressively somnolent and debilitated behaviour [44,45]
feline spongiform encephalopathy (FSE)	domestic and wild felids	infectious	variable (> 2 years of age) [46–48]	hyperaesthesia, ataxia of gait with dysmetria and hypermetria of the extremities, loss of body weight with no change in appetite, behavioural changes such as timidity or aggressiveness; associated with sustained exposure to BSE-contaminated feed [48–50]
exotic ungulate spongiform encephalopathy	exotic zoo ruminants	infectious	variable [51,52]	severe ataxia, loss of condition characterized by a short, progressive clinical course; associated with sustained exposure to BSE-contaminated feed [53–55]
camel prion disease	camel	infectious	variable (> 8 years of age) [56]	weight loss, tremors, ataxia of the hind limbs, hesitant and uncertain gait, hyperreactivity, aggressiveness, occasional falls [56]

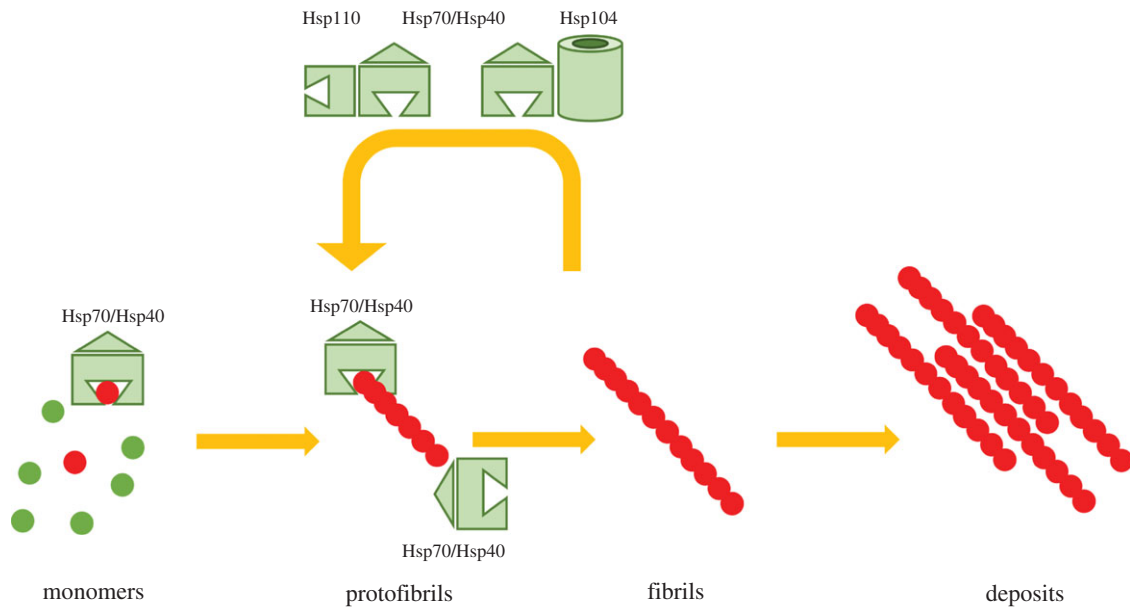


Figure 1. Prion templating and oligomerization is modulated by molecular chaperones. Soluble prion precursors (PrP^{C} , Sup35, Ure2) are recognized and converted by sub-stoichiometric prion forms of the same protein (PrP^{Sc} , [PSI⁺], [URE3]) that template addition to growing oligomers (protofibrils). Fibrils grow by end addition and self-associate to become large aggregates/insoluble plaques. Protein chaperones (Hsp40/Hsp70) interact at multiple points in the prion generation pathway, including recognition of prion monomers, capping of growing ends to slow fibrillization and cleavage of fibrils back to shorter protofibrils that exponentially amplify deposit formation. Cleavage is mediated by either additional interaction of the disaggregase Hsp104 (in yeast) or the Hsp70-like Hsp110 that generates weak disaggregase activity in concert with Hsp40/Hsp70 (yeast and humans).

2. PrP structure and function

2.1. The cellular prion protein (PrP^{C})

The cellular prion protein is ubiquitously expressed; however, higher expression is found in the brain and the lymphoreticular system [69]. The mature PrP^{C} (~210 amino acids in length) is largely localized in lipid rafts (detergent-resistant subdomains) within the outer leaflet of the plasma membrane via a C-terminal glycosylphosphatidylinositol (GPI) anchor [57,67]. PrP^{C} is encoded by the *PRNP* gene, which is present in all mammals and is highly conserved [57]. High-resolution NMR studies using bacterially expressed recombinant prion protein (recPrP), a model for PrP^{C} that lacks post-translational modifications, have revealed a largely unstructured, flexible N-terminal domain and a folded C-terminus [71]. Depending on the animal species, at least four glycine-rich octapeptide repeats comprise the N-terminus and display a strong affinity for Cu^{2+} [72] and weaker binding to other divalent cations such as Zn^{2+} , Fe^{2+} , Ni^{2+} and Mn^{2+} [73]. The degree of conservation of the PrP globular domain, which consists of two short β -strands and three α -helices, with a disulfide bond bridging helices 2 and 3, varies among animal species. As further detailed below, this domain also contains two potential sites for N-linked glycosylation that appear to underlie distinct biochemical properties associated with PrP^{Sc} aggregates [67,68,74]. The primary function of PrP^{C} is still unclear, albeit many potential biological functions including pro-apoptotic [75,76] and anti-apoptotic [77,78] roles, receptor for toxic amyloid- β (A β) oligomers [79–82], neuronal differentiation [83], and others have been described for it. Nevertheless, transgenic mice lacking this protein are viable, have a normal lifespan and do not show gross abnormalities [84]. This suggests that any potential activity exerted by this protein may be redundant. To date, the only clear function of the prion protein is to facilitate TSE transmission and progression.

The phenomenon of prion transmission is mechanistically well explained by the seeding nucleation–polymerization hypothesis (figure 1). In summary, this model suggests that pre-formed PrP^{Sc} aggregates (spontaneously formed or exogenously incorporated) serve as aggregation templates or ‘seeds’ by recruiting normally folded proteins into growing aggregates [85–87]. Considering that PrP^{Sc} polymers grow solely at their ends, fragmentation of the aggregates into smaller units generates free ‘active’ ends that facilitate the conversion of ‘normal’ PrP^{C} into disease-associated isoforms. The resulting exponential $\text{PrP}^{\text{C}} \rightarrow \text{PrP}^{\text{Sc}}$ conversion finally leads to the deposition of the toxic protein isoform in specific brain regions that will eventually lead to the death of the affected individual.

2.2. Prion strains

As explained above, the seeding nucleation–polymerization model proposes that PrP^{C} monomers misfold using pre-existing PrP^{Sc} aggregates as a template. In that sense, the newly generated PrP^{Sc} particles are expected to adopt the conformation of the original PrP^{Sc} ‘seeds’. However, the amino acid sequence of PrP^{C} has been shown to strongly dictate the conformation that nascent PrP^{Sc} units will adopt [88]. PrP^{Sc} aggregates can acquire multiple conformations known as ‘strains’, each causing distinctive disease phenotypes (e.g. incubation times, region-specific histopathological lesions, etc.) [67,68]. Compelling evidence suggests that the specific conformation of a certain PrP^{Sc} strain depends on both the PrP sequence and the conformation of the original template [67,68,89–91]. Experiments in animal models and *in vitro* systems show that the prion strain whose conformation is most compatible with that of the host PrP^{C} will be preferred above others and impose its particular pathological profile on the infected host (reviewed in [91]).

Prion strains can be differentiated by characterizing their particular biochemical and pathological features. *In vivo* and *in vitro* data show that the prion protein exists in three main glycosylation states (glycoforms): di-, mono- and unglycosylated [68,92]. The ratios of PrP^{Sc} glycoforms may differ across prion strains, a property that facilitates their classification [68,89,93]. PrP^{Sc} strains may also differ in the degree to which their quaternary structure exposes proteolytic cleavage sites, resulting in distinct electrophoretic mobilities following proteinase-K (PK) digestion (known as the PK-resistant core) [89,90,94–96]. Pathological features allowing differentiation of prion strains include incubation periods, susceptibility to infection by different routes of administration, the extent of the clinical phase, clinical signs, anatomical distribution of pathological lesions in the brain (PrP^{Sc} deposition and spongiform degeneration), among many others [89,91,97,98]. Biochemical and pathological prion strain-specific phenotypes often persist upon serial transmission within the same animal species and validate the notion that characteristics of the infecting agent are significantly influenced by both the host PrP^C and PrP^{Sc} input [68,70]. Nevertheless, protein misfolding is a multi-step process and the generation of infectious prions or selection of specific prion strains may be strongly facilitated by the presence of other cofactors such as lipids and nucleic acids [99,100].

2.3. Mechanisms associated with amyloid toxicity

Misfolded proteins are not restricted to TSEs, but are at the core of several other pathological conditions collectively termed as protein misfolding disorders (PMDs) [87,101,102]. PMDs include several neurodegenerative and peripheral diseases such as Parkinson's, Huntington's and Alzheimer's diseases, type 2 diabetes, and many others [87,101,103]. Due to their protein-centric commonalities, conserved mechanisms of toxicity have been described for many of them.

Misfolded proteins form structurally similar aggregates, typically known as amyloids due to similarities to starch-rich structures in diagnostic histological staining procedures. These proteinacious entities are associated with synaptic alterations and cell death in different systems including cell cultures and animal models [104–106]. Misfolded proteins exist as a continuum of aggregates of different size, ranging from small oligomers to large fibrils. The distribution of aggregated units depends on the protein type and the specific conformation (strain) that is adopted [107–109]. Among them, misfolded protein oligomers (small molecular weight and aqueous-soluble aggregates [110]) are thought to be essential for pathological progression [111–113]. Although the role of oligomers in disease progression is clear, the specific mechanisms by which they exert toxicity is still debatable. Due to their increased hydrophobicity, misfolded protein oligomers are thought to bind and stabilize within the cellular lipid bilayer, forming pores [114–118]. Other reports suggest that toxic oligomers bind specific extracellular receptors that will trigger deleterious cascades leading to cell death [80,81]. Additionally, it has been proposed that these low molecular weight structures can be internalized by the cell, causing stress within the endomembrane/endoplasmic reticulum (ER) system and thereby triggering conserved responses such as the unfolded protein response (UPR).

3. Roles of chaperones in prion protein misfolding propagation and clearance

3.1. The unfolded protein response in the endoplasmic reticulum

Physiological stressors such as metabolic imbalance, calcium deprivation, oxidative stress or heat shock impact multiple cellular processes, with an immediate consequence being a disruption in protein homeostasis, or 'proteostasis' [119]. Protein misfolding and aggregation can occur during translation, as nascent chains emerge from the ribosome in the cytoplasm, or after translocation into the ER or mitochondria as unfolded polypeptide chains [119–121]. Misfolded or aggregated proteins recruit different classes of cytoprotective proteins known as molecular chaperones that play primary roles in preventing further aggregation, resolving aggregates and either refolding proteins or helping to facilitate their degradation. Coincident with the rise in misfolded protein substrates, cellular unfolded protein response systems are engaged and activated to ultimately increase chaperone activity to combat protein misfolding. These largely transcriptional programs are highly conserved in all eukaryotic cells and are best known as the UPR in the ER and the heat shock response (HSR) in the nucleus and cytoplasm [122–124]. Due to the high degree of conservation, lessons learned from the study of proteostasis in the budding yeast *Saccharomyces cerevisiae* are informative to complement work done in human and other animal cells.

The majority of proteins destined for secretion or retention within the secretory pathway are synthesized on the ER membrane and translocated into the lumen or inserted into the ER membrane [125]. Maturation of many such proteins, including PrP^C, requires further processing by enzymes localized in the ER or Golgi apparatus [126]. However, proteins that fail to properly fold are retained in the ER or recovered from post-ER compartments and re-enter the protein folding cycle, where they will be successfully folded or targeted for ER-associated degradation (ERAD). An ER-specific network of soluble luminal chaperones is responsible for overseeing the disposition of the pool of folding-compromised proteins, and it is therefore no surprise that the majority of sensing for the activation of the UPR occurs in the ER lumen [127–131]. The presence of misfolded proteins in the ER results in de-repression of the multi-pronged UPR, allowing the synthesis and translocation of specific transcription factors to the nucleus where gene expression for ER-specific molecular chaperones is initiated. Three ER-specific signalling pathways are each negatively regulated by the Hsp70 chaperone BiP (discussed in detail below) as a component of negative regulation circuits that hold these systems in check until stress in the form of misfolded proteins is detected (figure 2).

Inositol-requiring kinase one (IRE1) is a transmembrane serine/threonine kinase and RNase highly conserved between yeast and humans. IRE1 remains a monomer in the ER membrane until it is activated by UPR stress as signalled by the increase in unfolded proteins within the organelle. BiP prevents IRE1 multimerization in the absence of misfolded proteins. Upon proteotoxic stress resulting in accumulation of misfolded proteins in the ER lumen, BiP dissociates and binds the unfolded polypeptides, allowing IRE1

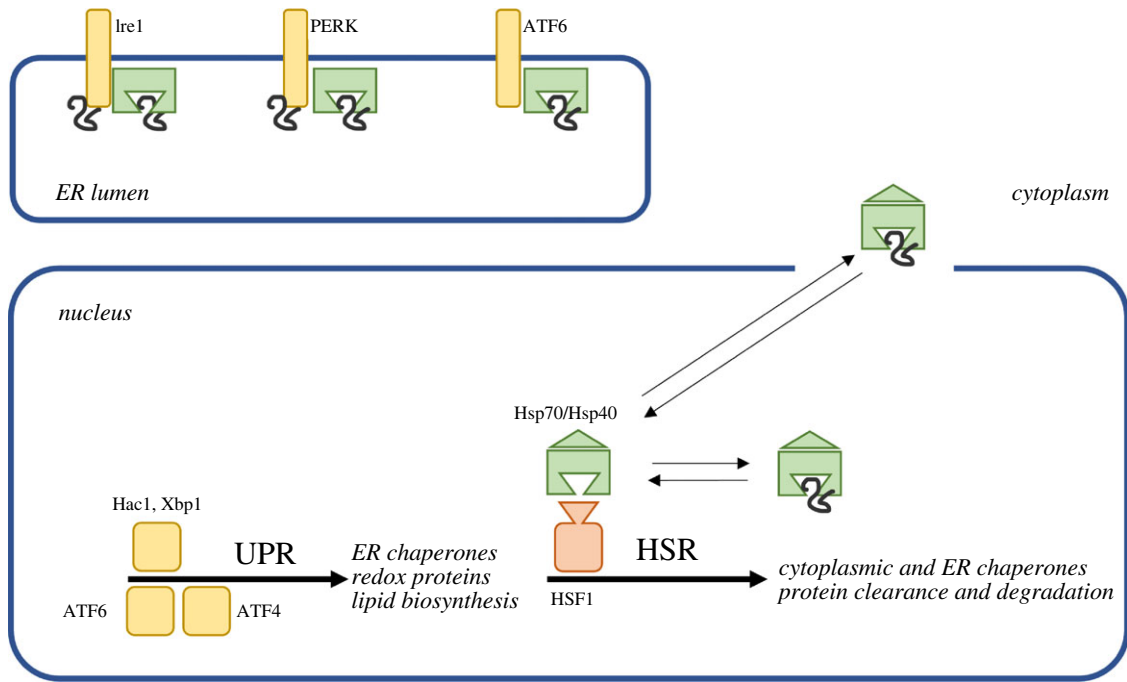


Figure 2. Key unfolded protein stress response pathways in humans and yeast. The ER unfolded protein response (UPR) recognizes misfolded proteins within the ER lumen and membrane and activates downstream transcriptional responses to restore ER proteostasis. The yeast UPR is governed solely by Ire1, while humans possess three parallel pathways: IRE1, PERK and ATF6. The cytosolic heat shock response operates through Hsp70-mediated recognition of misfolded proteins in the cytoplasm and nucleoplasm and activates downstream gene expression through HSF1 to rebalance proteostasis in those compartments, as well as many ER-resident chaperones. Hsp70 chaperones play a common role in sensing and transducing the misfolded protein signal.

to multimerize and trans-autophosphorylate adjacent monomers via the cytoplasmic-localized serine/threonine kinase domain. One of the consequences of this activation is the splicing out of a 26-bp mRNA intron of the X-box-binding protein (XBP1) to form the trans-acting leucine-zipper transcription factor XBP1. XBP1 translocates into the nucleus and activates the transcription of ER genes required for the UPR [132]. A nearly identical process occurs in yeast, with activation of the Hac1 transcription factor [133]. Unlike yeast, human cells express two variants of IRE1; IRE1 α is ubiquitously expressed while IRE1 β is selectively expressed in intestinal and pulmonary tissues [134,135]. A second trigger for activation of the IRE1 arm of the UPR is mediated by direct recognition and binding of unfolded polypeptides to the luminal domain. Each monomer possesses ‘half’ of a major histocompatibility complex (MHC)-like binding site, and peptide binding across adjacent luminal domains stabilizes the dimer structure, promoting kinase and endonuclease IRE1 activities [136]. Different misfolded proteins may activate IRE1 in distinct ways, as yeast studies using the model misfolded protein carboxypeptidase Y (CPY⁺) demonstrated direct binding to IRE1, but not to BiP [137]. However, in a study using purified human IRE1 α , peptide binding was not required for IRE1 α dimerization [138]. The role of the IRE1 branch in mediating prion and non-prion amyloid propagation remains inconclusive (see §3.5).

The PKR-like ER kinase (PERK) pathway is activated when BiP dissociates from PERK monomers and binds misfolded proteins that enter and accumulate in the ER. PERK dimerizes and phosphorylates the eukaryotic translation factor eukaryotic initiation factor 2 α (eIF2 α), rendering it inactive. eIF2 α is responsible for the majority of translation initiation in the cell and its inactivation results in global repression of protein synthesis [139,140]. Gcn2 is the PERK

homologue in yeast, acting as an eIF2 α kinase and regulator of translation during ER stress [141–143]. Evidence suggests that the PERK pathway, unlike IRE1, is responsive to prion accumulation and stress in the ER. In a 2014 study, the fusion of PrP^{Sc} to the ER membrane induced a strong unfolded protein response through activation of the PERK pathway [144].

Activating transcription factor 6 (ATF6) is a 90 KDa protein that is maintained in the ER membrane through interactions with BiP at its luminal tail. However, when misfolded proteins accumulate, BiP dissociates, leaving the Golgi localization sequence exposed and allowing ATF6 to translocate to the Golgi apparatus [127]. In the Golgi, ATF6 undergoes cleavage by two Golgi-specific proteases, S1P and S2P, to form a 50 KDa transcription-activating fragment. Once generated, the ATF6 fragment enters the cytosol and translocates to the nucleus where it interacts with the transcription factor NF-Y to form the ER stress response factor (ERSF). ERSF activates the transcription of ER-specific genes by binding to the ER stress element in the promoters of UPR target genes, including Grp78/BiP and Grp94/Hsp90 [145,146].

3.2. Protein molecular chaperones govern protein homeostasis

As introduced earlier, molecular chaperones play a major role in maintaining proteostasis by protecting nascent proteins and helping to maintain mature proteins in their native states. The predominant chaperone class is generally considered to be the Hsp70 superfamily, characterized by an amino-terminal nucleotide-binding/ATPase domain and a carboxyl-terminal substrate-binding domain connected by a flexible linker [147]. Hsp70s bind to nascent polypeptides

Table 2. Protein molecular chaperone homologues in humans and yeast.

class	human	yeast	localization	function
Hsp110	Apg-1/2, Hsp105 α	Sse1,2	cytosolic	nucleotide exchange factor for Hsp70; <i>in vitro</i> anti-aggregation [124,158]
Hsp100	—	Hsp104	cytosolic	disaggregase [159,160]
Hsp90	Hsp90 α	Hsc82, Hsp82	cytosolic	maturation of cell cycle and signal transduction proteins [161]
	Grp94	—	ER – lumen	Proper folding of secreted and membrane proteins [162]
Hsp70	Hsc70/Hsp70	Ssa1,2,3,4	cytosolic	protein folding [119]
	—	Ssb1,2	ribosomal	co-translational protein folding [124]
	BiP/Grp78	Kar2	ER	protein folding; UPR activation [163]
	Hsp70L1	Ssz	ribosomal	co-translational protein folding [164]
Hsp60	Hsp60	Hsp60	mitochondrial	chaperonin; promotes folding of imported polypeptides [165]
Hsp40	Hdj2/DnaJA1	Ydj1	cytosolic	ATPase activator; recognition of misfolded polypeptides [166,167]
	Hdj1/DnaJB1	Sis1	cytosolic	ATPase activator; delivers misfolded substrates for degradation [166,168]
	DnaJC2	Zuo1	ribosomal	ATPase activator [164,166]
ER-specific	calnexin	Cne1	ER	refolding of mono-glycosylated polypeptides [169]
	calreticulin	—	ER	re-glycosylation and refolding [169]
	Grp58/ERp57	Pdi1	ER	lectin interacting; folding of glycoproteins [170]

as they are emerging from the ribosome to ensure their proper folding in the cytosol or on the trans-side or organelles such as the ER or mitochondria. This binding helps shield hydrophobic regions of immature proteins from aggregating and promotes proper folding through iterative cycles of substrate binding and release that are in turn controlled by the Hsp70 ATPase rate. In the Hsp70-mediated folding cycle, co-chaperones both interact with substrates and regulate Hsp70 ATPase activity. For example, the Hsp40 co-chaperone helps deliver substrates to Hsp70 and potently stimulates Hsp70 ATPase activity [148,149]. Hsp110 co-chaperones are nucleotide exchange factors that allow for the rapid dissociation of ADP from the Hsp70 nucleotide-binding site and replacement with ATP to continue the Hsp70 folding cycle.

The HSR is a transcriptional program carried out in nearly all cells in response to stress. Activation of the HSR results in an upregulation of molecular chaperones that safeguard the proteome from proteotoxic damage by preventing the misfolding of proteins critical for cell survival. Mitigating proteotoxic stress ensures crucial proteins are available to conduct essential functions and also prevents the formation of toxic aggregates which occur when a critical mass of proteins unfold. HSR induction also results in the down-regulation of other cellular processes such as ribosome biosynthesis and the cell cycle to divert energy toward recovery [150,151]. Chaperone induction and the accompanying repression of other cellular processes are executed by depression of the transcription factor and master regulator of the HSR, HSF1 (Hsf1 in yeast) [152–154]. Under non-stress conditions, Hsf1 resides in the cytosol in an inactive monomeric form. During heat shock, Hsf1 relocates from the cytosol to the nucleus where it trimerizes and binds to the heat shock element (HSE) in the promoters of heat shock protein (HSP) encoding cytoprotective genes [119,155–157] (figure 2). HSF1 is regulated by several post-translational modifications, including phosphorylation and acetylation, but recent work has conclusively demonstrated

that feedback inhibition by direct binding of Hsp70 to transcriptional activation domains within the protein is the primary control mechanism for the HSR. This is best shown in yeast, where four distinct cytosolically localized soluble Hsp70s are encoded by the *SSA1*, *SSA2*, *SSA3* and *SSA4* genes with largely but incompletely overlapping physiological roles (table 2). Genetic or pharmacological disruption of Hsp70 activity corresponds with an increase of Hsf1 activity [171].

When unfolded proteins accumulate in the cytosol and nucleus, Hsp70 dissociates from Hsf1 to preferentially bind polypeptides with exposed hydrophobic residues [172,173]. Hsp70 mediates refolding of these substrates in an ATP-dependent manner until a native conformation is reached. In concert with Hsp40 co-chaperones and Hsp110 nucleotide exchange factors, Hsp70 interacts with a multitude of substrates to promote proper folding in singular or iterative cycles [147]. In yeast, dismantling of aggregates formed by unfolded or misfolded polypeptides requires the Hsp100 disaggregase Hsp104. Hsp104, with the assistance of Hsp70 and Hsp40, localizes to aggregates and resolubilizes single polypeptides (figure 1) [159]. The mechanisms by which Hsp70–40–110 and Hsp104 chaperones counteract stress-induced protein misfolding will be discussed below. When proteins cannot be folded into a native state, they are sequestered within cytoplasmic or nuclear protein assemblies (juxtannuclear quality control (JUNQ); insoluble protein deposits (IPOD) or aggresomes) until they are eventually degraded [174].

3.3. Roles of protein chaperones in yeast prion propagation

Prions are formed by the misfolding and structured aggregation of specific proteins and propagate in part by exploiting the protein chaperone network; for example, chaperone availability determines whether prions are further seeded and

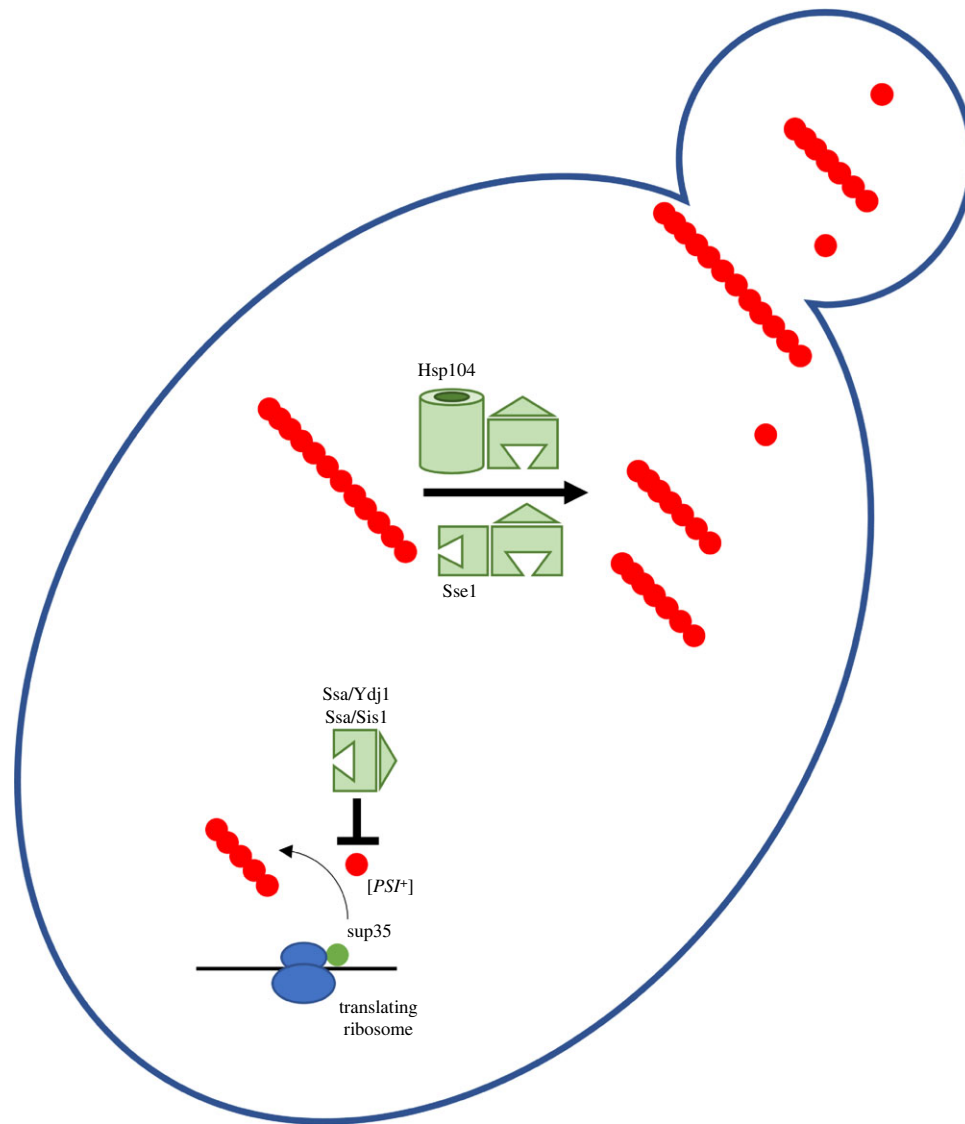


Figure 3. *[PSI⁺]* prion biogenesis and chaperone interactions in yeast. The Sup35 protein is a critical translation termination factor in yeast that can be converted to the prion form *[PSI⁺]* via templated conversion. The yeast Hsp70/Hsp40 chaperone pair retards the conversion and formation of protofibrils. Fibrils can be disassembled via the action of either the Hsp104 disaggregase partnering with Hsp40/Hsp70, or the recently described chaperone triad disaggregase formed by Hsp70/Hsp40/Hsp110. Yeast protein names are shown in the figure and are further detailed in table 2. Small oligomers and *[PSI⁺]* monomers are capable of passing through the bud neck while larger fibrils are not, leading to *[PSI⁺]* curing in experimental models lacking disaggregase activity. Similar chaperone/prion dynamics are observed for *[URE3]*.

transmitted to progeny cells or retained within the mother cell in yeast [175]. Like human PrP^{Sc}, yeast prions are templated from the misfolding of previously soluble and functional cellular proteins [176]. While multiple yeast prion or prion-like proteins have been described, we will focus on two exemplars in this review: the *[PSI⁺]* prion (that derives from the misfolding of Sup35, a translation termination factor) and *[URE3]* (an isoform of Ure2, a transcriptional repressor in the nitrogen catabolism pathway) [177,178]. Both Sup35 and Ure2 contain a flexible N-terminal prion domain (PrD) that forms structured β -sheet amyloids that recruit natively folded monomers [179]. Similar to human prions, yeast prions also exist in a variety of strains that are distinguishable by their associated phenotypes and how stably they propagate across generations of proliferating yeast cultures [180]. However, unlike PrP^{Sc}, the phenotypes associated with yeast prions can confer a selective advantage to the host (e.g. *[PSI⁺]* has been shown to suppress nonsense mutations during translation [181]). While there is no direct

yeast homologue of PrP^C, the study of yeast prions has provided tremendous insight into the mechanisms behind the biochemistry, cellular biology, inheritance patterns and progression of human prion diseases [182,183].

The most extensively studied yeast prions *[PSI⁺]* and *[URE3]* give insight into the role of the Hsp70 machinery with regard to prion maintenance in the cell. Ssa was shown to act with Ydj1 to block Sup35 polymerization and *[PSI⁺]* propagation (figure 3) [184]. Interestingly, another study showed singular overexpression of Ssa1 or Ssa2 had no effect on *[PSI⁺]* propagation, but Ssa1 overexpression did have a curing effect on *[URE3]* status [185]. The introduction of homologous mutations into Ssa1 (Ssa1–21) and Ssa2 (Ssa2–21) both resulted in a weakening of *[URE3]*, but only the Ssa2–21 strain showed a weakening of *[PSI⁺]* [186]. Another study revealed Hsp104, Sis1 and Sse1 preferentially bound *[PSI⁺]* prions but not Sup35 monomers [187]. Together, these investigations highlight the general importance of Hsp70 chaperones and co-chaperones in mediating

prion maintenance but also reveal substrate-specific differences that are not understood.

The Ssa proteins are responsible for the propagation of certain yeast prions, with Ssa1 and Ssa2 being responsible for $[PSI^+]$ and $[URE3]$, respectively [186,188,189]. Overexpression of Ssa has been shown to have antagonistic effects for Hsp104, obstructing its disaggregase activity and allowing the propagation of $[PSI^+]$ [190]. The Ssb class of Hsp70s is encoded by two genes in budding yeast, *SSB1* and *SSB2*, which together are necessary for cell survival [191]. Ssb is part of the ribosome-associated complex (RAC) that facilitates folding of nascent chains as they emerge from the ribosome. Because of its association with the ribosome, Ssb is thought to have anti-prion effects. In a 1999 study, Ssb depletion resulted in an increase in $[PSI^+]$ conversion when compared with wild-type cells [192]. In the same study, the overexpression of Ssb rescued cells from $[PSI^+]$ by mediating Hsp104-dependent curing, a finding corroborated by other studies [193]. This is in contrast with Ssa overexpression, which seems to protect prions from Hsp104 activity [190]. In another study, Ssa was shown to interact with Sup35 in $[PSI^+]$ cells but not in $[psi^-]$ backgrounds, in contrast with Ssb, which interacted with Sup35 in both $[PSI^+]$ and $[psi^-]$ cells [187]. RAC inactivation also rescues yeast cells from $[PSI^+]$ prion-associated toxicity. It is hypothesized this happens by freeing the ribosome-associated Hsp40, Ssz from the ribosome, allowing for the improved protein folding of Sup35 [194]. These studies highlight the dynamic interactions of cytosolic Hsp70s with yeast prions, specifically the distinct roles of Hsp70 subclasses in maintenance and propagation of the $[PSI^+]$ variant.

Hsp40s promote disaggregation of prions by recruiting Hsp70 to aggregates, and in turn Hsp70 recruits the yeast disaggregase Hsp104 to resolve aggregates [195–197]. The two major cytosolic Hsp40s (Ydj1 and Sis1) influence prion propagation [198,199]. Ydj1 suppresses aggregation and toxicity of another yeast prion, $[RNQ^+]$, by recognizing and binding to glutamine- and asparagine-rich motifs through its CAAX domain [200]. In a screen of Ssa co-chaperones, it was revealed that only the expression of Ydj1 results in a curing effect on $[URE3]$, inhibiting $[URE3]$ prion formation and directly interacting with the Ure2 protein [201,202]. Sis1 is essential and required for the stabilization and propagation of $[PSI^+]$, $[RNQ^+]$ and $[URE3]$ [198]. The glycine/phenylalanine domain of Sis1 is required for the propagation of $[RNQ^+]$ despite being expendable for other cellular processes [203]. Overexpression of Sis1 promoted $[PSI^+]$ curing and suppressed the conversion of Sup35 to $[PSI^+]$ [204].

Similar to Hsp70, the nucleotide exchange factor (NEF) Hsp110 chaperone is composed of an N-terminal nucleotide-binding domain and a C-terminal substrate-binding domain connected by a flexible linker. In addition to this family, two other NEF proteins are conserved in eukaryotic cells, Fes1/HspBP1 and BAG/Snl1 [205]. The expression of human Huntingtin in yeast missing Sse1 and other NEFs results in impaired degradation of aggregated proteins, prion aggregates and fibrils [206]. Sse1 plays a role in modulating the formation of yeast prions *in vivo* in coordination with other Hsp70 chaperones, for example, promoting $[PSI^+]$ propagation by accelerating Ssa and Ssb activity through nucleotide exchange [207]. Furthermore, Sse1 independently promotes $[PSI^+]$ propagation by stabilizing the intermediate form of a Sup35 fragment (Sup35NM) containing only the N and M

sub-domains but lacking the GTPase region, the minimal prion-forming elements of Sup35 in yeast, and allowing its nucleation *in vitro* [207]. These results are supported by another study in which Sse1 overexpression was found to promote Sup35NM aggregation and $[PSI^+]$ formation [208]. Sse1 and Hsp104 both localize to $[PSI^+]$ prions in the absence of Ssa, indicating a direct role for Sse1 in modulating prion states [209]. Specifically, the loss of Sse1 results in the formation of longer $[PSI^+]$ fibrils [209]. Sse1 expression also promotes the formation of $[URE3]$, as overexpression of Sse1 increases the solubility of Ure2, presumably due to Sse1 NEF activity [202]. Although Sse1 possesses a conserved substrate-binding domain similar to that of Ssa, *in vivo* studies suggest the SBD function may be expendable [210,211].

In yeast cells, solubilization of misfolded and aggregated proteins is primarily accomplished by the cytosolic disaggregase Hsp104, a member of the AAA+ ATPase superfamily. Hsp104 is a hexamer consisting of monomers each comprised an N-terminal domain, N-terminal nucleotide-binding domain (NBD1), a middle domain (MD), C-terminal NBD2 and a C-terminal domain [212]. Hsp104 couples ATP hydrolysis with the translocation of unfolded polypeptides through its central pore to allow for aggregate disassembly and re-solubilization [213,214]. In order to resolubilize proteins, Hsp104 must be recruited to aggregates with the help of Hsp40 and Hsp70. Hsp104 recognizes cytosolic aggregates with its N-terminal domain and resolubilizes polypeptides by extraction out of the aggregates in an iterative, ATP-dependent manner [215]. The resulting unfolded polypeptides are then redirected into the Hsp70-mediated protein folding cycle.

In addition to resolubilizing protein aggregates, Hsp104 also plays a role in prion propagation in yeast cells. Hsp104 activity must be tightly regulated—overactivity can dismantle prions but also allows prions to be broken into smaller sized seeds [157,178]. These seeds are heritable, transmitting into daughter cells during cell division, thereby allowing prions to be propagated over generations. This is specifically observed in Hsp104 interactions with $[PSI^+]$; however, Hsp104 hyperactivity does not result in antagonistic effects on $[URE3]$ or $[RNQ^+]$ despite Hsp104 being required for propagation [216–218]. Studies suggest the M-domain plays a role in seed propagation via its ability to couple and regulate ATPase activity and disaggregation. Specifically, the de-repression of the M-domain encourages prion propagation by allowing for a more rapid dismantling of prions into transmissible seeds [219]. Perhaps counterintuitively, genetic or chemical inactivation of Hsp104 also leads to prion curing by allowing the formation of prions so large they cannot be transmitted to daughter cells during division (figure 3) [220].

Unlike yeast, mammalian cells do not have a well characterized and dedicated chaperone for aggregate disassembly. An siRNA screen uncovered two AAA+ family proteins RuvB-like AAA ATPase (RUVBL1) and (RUVBL2), homologues of the bacterial helicase RuvB, that were shown to form hexameric structures and localize to protein aggregates in a manner similar to Hsp104 [221–224]. RUVBL1 was shown to promote the formation of aggresomes and facilitate the resolution of aggregates in an ATP-dependent manner. Interactions with unfolded proteins and fibrils also stimulate RUVBL1 ATPase activity, suggesting RUVBL1 directly acts on aggregates to somehow promote disaggregation. Interestingly, RUVBL1 expression is not upregulated during heat shock stress [221]. The eukaryotic Hsp70–110 chaperone

triad has recently been found to play a significant role in disassembling cytosolic aggregates, albeit in a slow manner relative to yeast Hsp104. *Ex vivo* studies using rat liver and kidney cell extracts show Hsp110 activates Hsp70 and Hsp40 to solubilize aggregates in an ATP-dependent manner; however, this activity is inefficient [225]. Because Hsp70–40–110 disaggregase activity can be accelerated by increasing the amount of Hsp110 in the reaction, it has been suggested that this chaperone may be a limiting factor [226–228]. Whether the mammalian disaggregase machines play any role in prion propagation and progression of TSEs remains to be determined.

3.4. *In vitro* analysis of protein chaperone interactions with mammalian infectious prions

Numerous *in vitro* experiments have been key to uncovering the mechanisms underlying interactions between chaperones and infectious prion proteins in animals. In a first approach, Edenhofer *et al.* [229] conducted yeast two-hybrid screen to search for proteins that interact specifically with the mature form of the Syrian golden hamster prion protein. In this study, glutathione *S*-transferase (GST) was fused to either the mature Syrian golden hamster PrP (encoding amino acids 23–231, GST-PrP^C23–231), or a recombinant PrP fragment consisting of amino acids 90–231 (termed GST-recPrP27–30, named after the molecular weight of the protease-resistant core associated with PrP^{Sc}). Subsequently, fusion proteins were immobilized and bound to glutathione-Sepharose beads followed by incubation with either Hsp60 or Hsp70. The authors noted that Hsp60, but not Hsp70, was detected in the presence of GST-PrP^C23–231 and GST-recPrP27–30, suggesting that the interaction of PrP^C23–231 and Hsp60 was specific. Subsequent analyses revealed that the prokaryotic Hsp60 homologue, GroEL, was similarly detected in the presence of GST-PrP^C23–231 and GST-recPrP27–30, but not GST. Moreover, different PrP fragments fused to GST and incubated with either Hsp60 or GroEL revealed that chaperones selectively bind to the region between amino acids 180–210 of PrP. This experiment indicated specific binding between PrP and Hsp60 and GroEL. Importantly, this study also demonstrated the direct interaction between PrP and molecular chaperones [229]. However, future experiments would define the specific affinity between normally folded and disease-associated PrP proteins and molecular chaperones.

A seminal work linking molecular chaperones and infectious prions was described by Hetz *et al.* [230] in 2003. In these experiments, mouse neuroblastoma N2a cells were treated with different doses of PrP^{Sc} purified from brains of mice infected with the 139A murine-adapted scrapie prion strain and levels of several chaperone proteins were measured during infection. The results showed that prion-infected cells were more sensitive to ER stress-mediated death compared with controls, as evidenced by pre-treating cultures with ER stress-inducers tunicamycin, thapsigargin, brefeldin A and the ionophore A23187. Importantly, such susceptibility was not observed when cells were treated with mitochondrial stress-inducers such as serum deprivation or staurosporine. Further analyses revealed that prion-contaminated cells expressed increased levels of the stress protein Grp58, suggesting a relationship between this

particular protein and prion-mediated neurotoxicity [230]. Later studies [231] further confirmed the role of this specific chaperone protein in prion infection. There, a PrP^{Sc} dose of 50 nM, which failed to induce cell death in Grp58-overexpressing N2a cells, resulted in a robust increase of death in cells pre-treated with siRNA against Grp58 (greater than 70%). Further, immunoprecipitation of PrP showed higher levels of associated Grp58 in chronically infected cells compared with non-infected controls (figure 4). This suggested that Grp58 either has a higher affinity for PrP^{Sc} and/or may be expressed at higher levels in infected cells. Of note, Grp58 expression levels did not influence the glycosylation state of PrP demonstrating that the protective role of Grp58 upregulation is not due to influencing the ability of PrP to bypass the ER-Golgi protein quality control [231].

Later *in vitro* experiments described the interaction of other chaperone proteins and PrP^{Sc}. Based on previously published data showing altered levels of Grp78 in prion-infected mice [232,233], Park *et al.* [234] studied the variation of chaperone protein expression in RML prion-infected CAD5 cells transfected with either Grp78 siRNA or a Grp78 overexpressing plasmid. In this experiment, a negative correlation between Grp78 levels and PrP^{Sc} accumulation was uncovered. In addition, western blot analysis of RML brain homogenates incubated with different concentrations of recombinant Grp78 for different time periods showed a dose- and time-dependent reduction of PrP^{Sc}. Co-immunoprecipitation experiments revealed that Grp78 interacts with PrP. Moreover, co-localization of anti-Grp78 and anti-PrP antibodies was also observed following immunocytochemistry analysis of primary cultures of wild-type, non-infected mouse fibroblasts. Specifically, co-localization analyses of confocal microscope-derived images were used to quantify the pixel co-distribution of PrP and Grp78, revealing co-localization between both proteins. This study also used the murine catecholaminergic CAD5 cell line chronically infected with mouse prions [235] and reported that siRNA-induced reduction of Grp78 led to significantly increased PrP^{Sc} accumulation. On the contrary, Grp78 overexpression was associated with decreased PrP^{Sc} levels. Notably, the authors revealed that PrP^C levels in non-infected cells remained unchanged under the aforementioned siRNA treatment conditions, confirming that the fluctuating levels of PrP^{Sc} accumulation due to the siRNA treatment was not due to changes in the expression of PrP^C. Overall, these data suggest that PrP^{Sc} propagation is susceptible to Grp78 expression. Further experiments exploring the mechanisms of Grp78-mediated toxicity in prion-infected cells showed that highly purified PrP^{Sc} (RML) aggregates incubated with purified recGrp78 showed a dose- and time-dependent reduction of protease-resistant PrP^{Sc}. The incubation of recGrp78 with PrP^{Sc} from two other murine prion strains (301C and 79A) resulted in a similar, albeit less pronounced, effect, suggesting that this chaperone targets misfolded proteins with distinct conformational arrangements. Together, these findings indicate that Grp78 modulates the biochemical/structural properties of PrP^{Sc} into relatively more protease-sensitive conformations, thereby permitting the direct inhibition of PrP^{Sc} propagation [234].

The direct interaction of molecular chaperones and infectious prion proteins was further demonstrated by a study performed by Mays *et al.* [236]. RKM7-RML cells (RK13-derived prion culture cell model sensitive to RML mouse-adapted PrP^{Sc}) and their prion-free counterparts (RKM7)

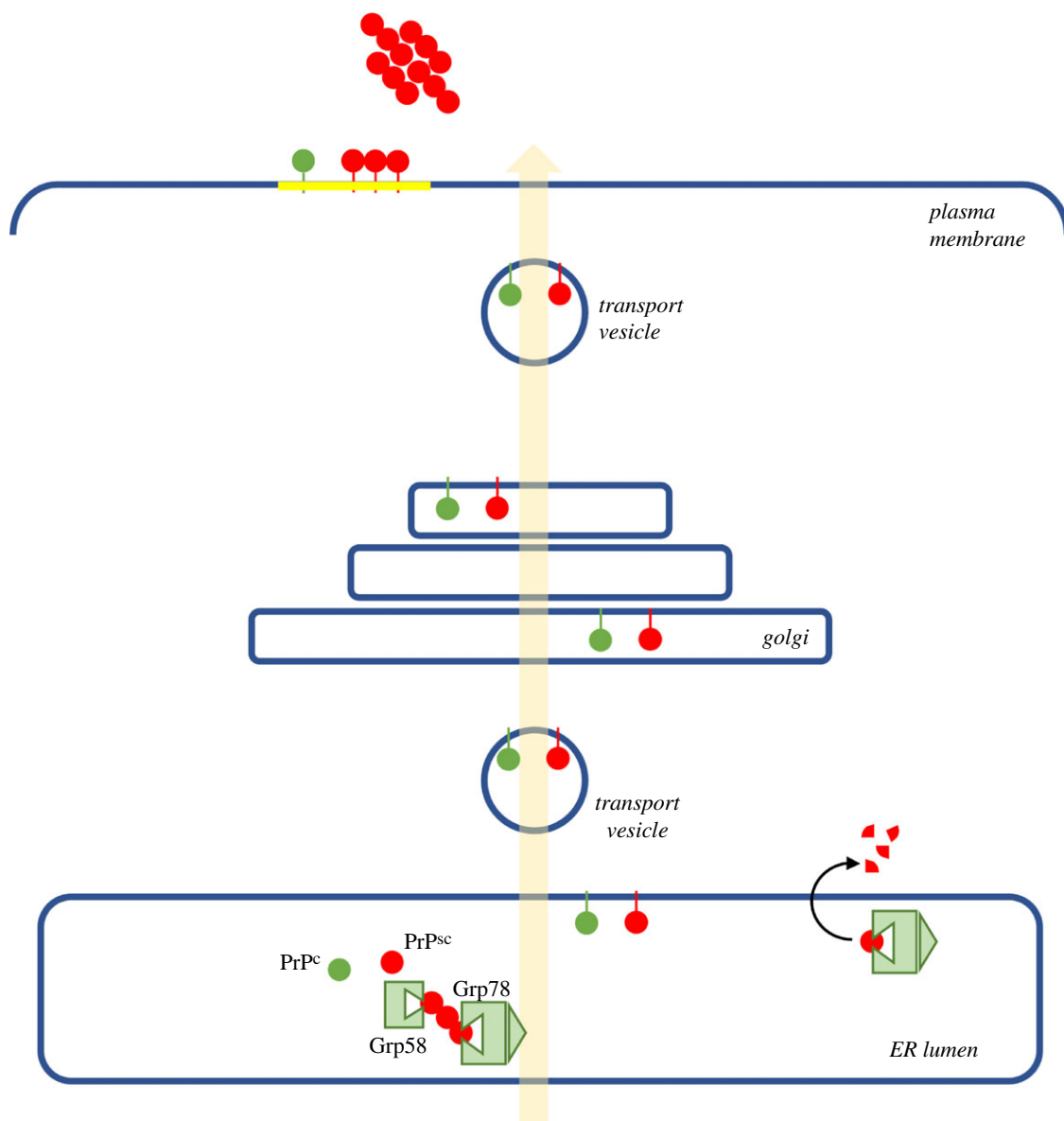


Figure 4. PrP-chaperone interactions during biogenesis. The cellular form of PrP is generated within the ER lumen and post-translationally modified by the addition of a GPI anchor (green dots). It is then transported through the secretory pathway (transparent arrow) for localization on the outer leaflet of the plasma membrane, where it exists in lipid raft sub-domains (yellow). Although it is unclear where precisely PrP^c to PrP^{Sc} (red dots) conversion occurs, available data are consistent with ER chaperones Grp58 (protein disulfide isomerase) and Grp78 (Hsp70; BiP) interacting with the PrP^{Sc} form within the lumen, targeting it for degradation through the ERAD pathway. This model does not exclude conversion at later points in the secretory pathway. PrP^{Sc} can also escape through the secretory pathway to localize to the plasma membrane and template conversion of PrP^c, ultimately adding to growing extracellular fibril chains.

were treated with different doses of 17-DMAG, a water-soluble geldanamycin derivative that activates an array of molecular chaperones, including Grp94 in the ER as well as cytosolic Hsp40, Hsp70, Hsp90 and Hsp105. This study reported that 17-DMAG treatment led to a 50% reduction of PrP^{Sc} in RKM7-RML while not altering PrP^c levels. As many chaperones were altered by 17-DMAG treatment, the effects of specific proteins on prion propagation were also explored. Hsp70 was particularly studied due to its master role in protein misfolding as described above. To understand the role of Hsp70 in the propagation of misfolded mammalian prions, the authors took advantage of protein misfolding cyclic amplification (PMCA) technology, an *in vitro* system able to replicate infectious prions in a cell-free context [237]. PMCA reactions were seeded with RML prions using brains from wild-type mice or animals lacking the Hsp70 protein (*Hsp70*^{-/-}) as substrates for the *in vitro*

amplification reactions. The results revealed that the presence of Hsp70 was associated with more efficient PrP^{Sc} replication compared with reactions using brains lacking this protein. Collectively, these studies and others have fuelled efforts to decipher the influence of chaperones to prion disease *in vivo*. This will be discussed in the following sections.

3.5. Altered levels of chaperone proteins in experimental and natural prion diseases

Invertebrate models of prion disease have been useful to understand the role of misfolded prion proteins in relation to altered levels of chaperone activity. Fernandez-Funez *et al.* [238] showed that 30-day-old transgenic *Drosophila melanogaster* expressing hamster PrP^c (Tg-PrP) exhibited several hallmarks of prion disease such as cytosolic

vacuolation, nuclear condensation, spongiform degeneration in the brain and optic lobes, and vacuole formation on the cortex and neuropiles. These pathological features were progressive and absent in relatively younger Tg-PrP flies. Interestingly, 30-day-old Tg-PrP flies co-expressing human Hsp70 exhibited normal nuclei and fewer vacuolated cells compared with their age-matched counterparts lacking Hsp70, which displayed condensed nuclei and a greater frequency of vacuolated cells. This indicated that Hsp70 was protective against the severe spongiform vacuolar degeneration observed in 30-day-old Tg-PrP flies. The neuroprotectivity of Hsp70 was further evidenced during locomotor activity, which was performed using a line of Tg-PrP flies expressing weaker levels of PrP that exhibited a steady decline in climbing ability (50% climbing activity at day 7). By contrast, when co-expressing Hsp70, these flies showed a steady, but less pronounced decline (50% climbing activity at day 13). In addition, the improved locomotor ability of flies co-expressing Hsp70 and PrP was attested by significantly higher climbing activity from day 5 to day 31, and ten times higher average speed at day 20 compared with Tg-PrP flies [238]. Importantly, the prion aggregates generated in the Tg-PrP flies were not infectious. However, due to the strong effect of Hsp70 in the phenotype of this invertebrate model of prion toxicity, experiments in animal models were warranted. In that line, Mays *et al.* [236] showed that RML-infected mice genetically deficient in Hsp70 (Hsp70^{-/-}) developed terminal prion disease significantly faster than Hsp70^{+/+} mice.

Several lines of evidence demonstrate increased levels of other molecular chaperones in CJD human brains, including Grp58, Grp78 and Grp94 [234,236,239–241]. Hetz *et al.* [230] revealed that mice infected with the 139A prion strain exhibited increased expression of Grp58, but not other molecular chaperones (i.e. Grp94, calnexin, Hsp60 or Hsp70) in multiple brain regions such as the hippocampus, brain stem, thalamus, cerebellum, anterior cortex and posterior cortex. This finding indicates that upregulation of unique members of the ER proteostasis network is a characteristic of PrP^{Sc} accumulation and in turn, prompts consideration of the biomarker potential of the molecular chaperone profile. Further, these results underscore a critical need to expand knowledge on the role of chaperones in prion disease pathology. In a later study [231], Hetz and colleagues performed hippocampal injection of 139A prions in naïve mice to longitudinally examine the relationship between prion replication and induction of ER stress markers, including related molecular chaperones. Western blot and histological analyses of multiple brain regions, including hippocampus, cortex, thalamus and brainstem, showed that Grp58 expression levels appeared to be upregulated at pre-symptomatic stages of the disease and displayed a positive correlation with PrP^{Sc} accumulation. At the terminal stage of the disease, Grp58 expression levels appeared to decrease in both the thalamus and the hippocampus whereas in the cortex, the levels of this protein displayed a near ninefold increase. Further, at the beginning of the symptomatic phase, molecular chaperones such as Hsp60, Hsp70 and calnexin were not significantly upregulated and notably, only transient induction of Grp78/Grp94 was observed, which showed no correlation with PrP^{Sc} accumulation. This finding indicated that PrP^{Sc} may trigger a non-classical ER stress response that results in the specific induction of

Grp58. At the terminal stage of the disease, Grp58 down-regulation was associated with brain areas exhibiting neuronal death and caspase-12 activation [231]. Other experiments in animal models lacking chaperone proteins have also provided insight into the complicated relationship between PrP^{Sc} dynamics and the proteostatic network. One example involves Grp78, following evidence gathered from the brain of CJD patients. Specifically, Park *et al.* [234] observed indistinguishable lesions in the brains of RML-infected Grp78 heterozygous (Grp78^{+/-}) mice and homozygous (Grp78^{+/+}) mice, despite the significantly accelerated disease pathogenesis associated with Grp78^{+/-} mice compared with Grp78^{+/+} mice. These observations suggest that chaperones influence PrP^{Sc} kinetics rather than abolishing prion conversion.

Surprisingly, the UPR response regulator X-box-binding protein-1 (XBP-1) has no detectable role in the progression of prion disease and prion propagation in experimental animals [242]. Evidence collected using *in vitro* systems proposed this protein as an important player in the rate of prion misfolding under stress conditions [243,244]. However, animals lacking this protein specifically in the CNS showed no differences in incubation periods or pathological changes when compared with their non-transgenic counterparts [242]. Although the role of UPR in prion diseases is supported by several lines of evidence, the data discussed here suggest that some branches of the UPR response may not be as important for specific diseases, or they can be compensated by alternative pathways. Collectively, the experiments discussed above support the promising notion of chaperones as therapeutic targets against prion diseases [245–247]. These avenues have been poorly explored. Therapeutic development at this level has the potential to benefit not only prion diseases, but other protein misfolding disorders acting through similar mechanisms.

4. Targeting the proteostasis network as a therapeutic strategy against prion diseases

4.1. Regulation of chaperone production as a therapeutic target

The aforementioned efforts examining the links between prionopathies, UPR signalling and chaperones in yeast and mammals suggested that interventions at this level may have therapeutic potential. This prospect has been explored in different systems including cell cultures and animal models with variable success [248–250]. Due to the common involvement of the UPR in several protein misfolding disorders, modifications at this level are of interest as they could be applied to other protein misfolding disorders as described above. Below, we will discuss some promising therapeutic strategies explored on this front.

A potential UPR-related therapeutic target involves phosphorylated/activated PERK (PERK-P), a key sensor protein that can function as a molecular chaperone. The role of this protein in prion infection has been shown in Grp78^{+/-} mice where an approximately threefold increase in PERK-P levels was observed when compared with wild-type mice infected with the same prion agent [234]. Prion-infected tg37^{+/-}

mice (associated with a characteristic approximately threefold higher expression of PrP^C) treated orally with the PERK inhibitor GSK2606414 showed preserved hippocampal CA1 pyramidal neurons when evaluated after completing the treatment. These mice also displayed a reduction in both memory deficit, as measured by novel object recognition, and abnormal burrowing behaviour, a hippocampus-dependent measure of motivation [251]. However, the therapeutic application of GSK2606414 is questionable due to its toxicity to the pancreas, which relies on a robust ER stress response for normal function, leading to subsequent weight loss and mild hyperglycaemia [251]. By contrast, ISRIB (integrated stress response inhibitor), a PERK kinase inhibitor identified by Mallucci and colleagues, showed no toxicity to the pancreas in the same murine model [252]. In this study, prion-infected Tg37^{+/-} mice subjected to intra-peritoneal administration of ISRIB revealed decreased spongiform pathology and neuronal loss in the hippocampus, relative to vehicle-treated mice. Further, ISRIB-treated prion-infected mice displayed significantly increased survival compared with vehicle-treated mice [252].

Another target for therapeutic intervention involves eIF2 α . This protein, a downstream target of the UPR [253], is hyperphosphorylated in the brains of prion disease patients. The Mallucci group performed daily treatment of prion-infected tg37^{+/-} mice with either one of two eIF2 α -P signalling inhibitors, trazodone hydrochloride (a licensed antidepressant) or dibenzoylmethane (DBM), after prion infection. They showed that these compounds prevented the development of neurological disease without pancreatic toxicity [248]. Specifically, daily administration of this therapy led to differences in the number of mice showing prion confirmatory neurological signs after treatment with either trazodone (3/15), DBM (6/21) or vehicle (20/20). In addition, both drugs substantially reduced the loss of CA1–3 hippocampal cells at the time of sacrifice compared with vehicle-treated mice and rescued the loss of object recognition memory. DBM also inhibited the characteristic loss of burrowing behaviour. Lastly, this study showed significantly increased lifespan in trazodone-treated (12/15) and DBM-treated (15/21) mice [248].

To date, we are aware of no clinical trials against prion diseases involving the molecular chaperone pathway. However, the field of targeted chaperone modulation is in its infancy, with promising candidates that affect chaperone activity in a biological context. Some of these compounds may serve as leads for future drug development.

4.2. Chemical chaperones as potential modulators of prion diseases

An alternative therapeutic strategy on this front involves chemical chaperones. These molecules are low-molecular-weight compounds with a non-specific mode of action that directly influence protein folding and conformation and modulate the activity of molecular chaperones [254]. Like their molecular counterparts, chemical chaperones have also yielded promising results as therapeutic agents against protein misfolding diseases [246]. Shaked *et al.* [255] examined the effect of dimethyl sulfoxide (DMSO) on the incubation time and PrP^{Sc} accumulation rates of a 263 K hamster scrapie model. This study reported that infected

hamsters subjected to daily administrations of DMSO exhibited longer incubation periods and delayed accumulation of PrP^{Sc} compared with control animals. Notably, DMSO-treated and untreated hamsters showed no difference in the banding pattern of brain PrP^{Sc}, indicating that DMSO influenced disease kinetics and not prion strain compatibility with host PrP. DMSO treatment also increased levels of PrP^{Sc} in the urine of scrapie-infected hamsters compared with control hamsters, suggesting more efficient clearance of the infectious agent. However, prolonged DMSO treatment of infected hamsters resulted in significant weight loss at different time points compared with untreated hamsters. Thus, this highlights the potentially toxic effects associated with this treatment [255]. Rationally designed chemical chaperones have been recently shown to be useful in treating prion diseases in murine and non-human primate models of prion diseases [256].

Considering the increase in prion incubation periods, and associated mechanisms of action, exploring the chaperone avenue as a therapeutic target against prion diseases seems reasonable. However, additional studies targeting different strains of the prion agent, and combinatory therapy attacking upstream events in prion pathogenesis (e.g. protein misfolding) could result in a most needed therapy against this fatal group of diseases.

5. Summary and perspectives

At present, there is no disease-modifying treatment against prion diseases. However, years of research have revealed pathological cascades leading to prion misfolding, clinical manifestations and death. Based on these observations, pharmacological targeting of PrP^{Sc} formation looks to be a plausible therapeutic avenue, providing protection before extensive brain damage is caused. Due to the important role of chaperones in prion misfolding, their pharmacological modification is promising. However, the secondary effects that chaperone modulation might generate are still unknown. It is known that the UPR and associated chaperones do not only act as a response to disease, but also participate in multiple critical physiological processes [257]. In that sense, the adverse side effects of chaperone modulation must be carefully analysed. The particular branches of the UPR activated by different prion strains [234], the direct binding of chaperones to PrP^{Sc} (displaying strain variation) and the lack of strain-specific diagnostic methods remain challenges in exploiting this line of therapy. Nevertheless, the common mechanisms observed between TSEs and other diseases associated with protein misfolding (Alzheimer's, Parkinson's, Huntington's diseases) suggest that common therapies for several neurodegenerative disorders could be generated and be highly impactful at this level.

Data accessibility. This article has no additional data.

Authors' contributions. All authors planned and outlined the scope of the manuscript. U.M.Y. and C.S.G.C. wrote the initial drafts and were responsible for all revisions. R.M. and K.A.M. edited the manuscript. K.A.M. designed and created the figures.

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