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Prion protein trafficking and the development of neurodegeneration

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The prion protein (PrP) is involved in causing a group of diverse transmissible, heritable and sporadically occurring neurodegenerative diseases. Although the identity, nature and replication of the transmissible agent have been intensely studied for decades, the cellular events underlying neuronal dysfunction and death have received comparatively little attention. Recent studies examining the occurrence and consequences of inappropriate cytoplasmic expression of the normally cell-surface PrP underscore an emerging role for PrP trafficking in prion disease pathogenesis.

Infectious diseases can be conceptually divided into two phases: (1) transmission, which encompasses replication, propagation and transfer of transmissible agent, and (2) pathogenesis, which describes the processes by which infection causes physiological dysfunction in the affected organism. Not surprisingly, the relationship between the transmissible agent and cellular pathology is not always direct or obvious. And so it is with prion diseases – neurodegenerative disorders that have in common the involvement of prion protein (PrP), a cell surface glycoprotein of unknown function [1]. In the transmissible forms of these diseases, a particular misfolded conformation of PrP (PrP^{Sc}) leads to the conversion of host-encoded PrP into additional copies of the insoluble and aggregation-prone PrP^{Sc}. Although this scheme provides a framework for understanding transmissibility, little is known about how PrP^{Sc} causes neurodegeneration.

Previous studies in mice suggest that neurons not expressing PrP are immune to the toxic effects of PrP^{Sc} accumulation and deposition [2]. In addition some, but not all, PrP mutations cause neurodegeneration without PrP^{Sc} formation [3–5]. Such observations illustrate that a disparate collection of inciting events, ranging from PrP^{Sc} accumulation in some cases to numerous seemingly unrelated PrP mutations in others, all somehow lead to neurodegeneration [1]. Yet, all heritable and transmissible prion diseases seem to share certain characteristic pathological changes and an obligate requirement for PrP expression, suggesting at least some common aspects to their pathogenesis. What might this putative point of convergence be? One provocative idea, suggested by recent studies from Ma *et al.* [6,7], is that the unifying event might be the access of PrP to the cytoplasm of neurons.

A venue for PrP toxicity: the cytoplasm

Because genetic prion diseases are autosomal dominant and mice lacking PrP show no overt phenotype [1], it is presumed that a gain-of-function mechanism underlies PrP-mediated disease. In a dramatic demonstration of the potential toxicity of PrP, Ma *et al.* showed that forced cytoplasmic expression of PrP in transgenic mice leads to severe and rapid onset of neurodegeneration [7]. The toxicity of cytoplasmic PrP (cyPrP) appears to be selective to neurons because other tissues expressing cyPrP (e.g. heart and skeletal muscle) did not show pathology. In addition, the cell-type-specific toxicity was recapitulated in culture, where a neuroblastoma cell line, but not non-neuronal cells, was particularly susceptible to cyPrP-mediated apoptosis. Thus, PrP can wreak havoc in the cytoplasm of neurons. But how and under what circumstances could PrP ever reside in this compartment? A satisfactory answer to this quandary is crucial in linking the neurotoxicity of cyPrP to the events that naturally occur during the pathogenesis of various prion diseases.

Like most cell-surface proteins, PrP enters the endoplasmic reticulum (ER) concurrent with its synthesis by membrane-bound ribosomes, such that most regions of nascent PrP cannot normally access the cytoplasm [8]. During or shortly after its entry into the ER, PrP undergoes numerous folding and maturation events that include signal sequence cleavage, N-linked glycosylation, formation of a disulfide bridge and addition of a glycolipid anchor. Given these complexities, it is plausible that some proportion of nascent PrP would fail to mature properly,

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making it a target for ER quality-control systems [9]. These are ubiquitous pathways in which misfolded proteins are recognized and triaged for ER-associated degradation, a process that involves retrograde translocation of substrates into the cytoplasm for destruction by proteasomes [10]. Thus, the normal, physiological processes of cellular quality control might act on a fraction of newly synthesized PrP to provide a potential route from the ER to the cytoplasm.

Evidence for this idea has recently been provided by studies in which proteasomal degradation is inhibited [6,11,12] or PrP misfolding is increased [12,13]. Under either of these conditions, a predominantly non-glycosylated form of PrP is observed to accumulate in the cytoplasm. One study estimated that at steady state, ~10% of newly synthesized PrP is constantly fluxing through the cytoplasm *en route* to its degradation [11]. Although the precise source or sources of this flux remain to be carefully investigated, initial analyses suggest that cyPrP has undergone processing events specific to the ER lumen [6], pointing to this as its site of origin. Thus, the presence of PrP in the cytoplasm, at least transiently, appears to be a normal feature of its metabolism. Other demonstrated or hypothetical trafficking pathways in which small amounts of PrP or regions of PrP access the cytoplasm are outlined in Figure 1.

Cytoplasmic PrP in prion diseases?

Because cyPrP is neurotoxic [7] and there are multiple potential routes for its generation (Fig. 1), it is conceivable that the access of PrP to the cytoplasm is the neurodegenerative trigger in at least some naturally occurring prion diseases. Support for this idea remains fragmentary but comes from two sources. First, a mutated form of PrP (D177N) that causes heritable prion disease was shown to access the cytoplasm to a greater degree than did wild-type PrP [6,12]. This might be because of inefficient or incorrect maturation of this mutant, as suggested by its different pattern of glycosylation, decreased trafficking to the cell surface and increased ER retention [12,14]. Second, mutations in a central hydrophobic region of PrP that lead to increased generation of transmembrane forms of PrP cause neurodegeneration in transgenic mice and in some heritable prion diseases [5,15]. This is noteworthy with respect to cyPrP, as nearly half of the PrP molecule is exposed to the cytoplasm in the transmembrane

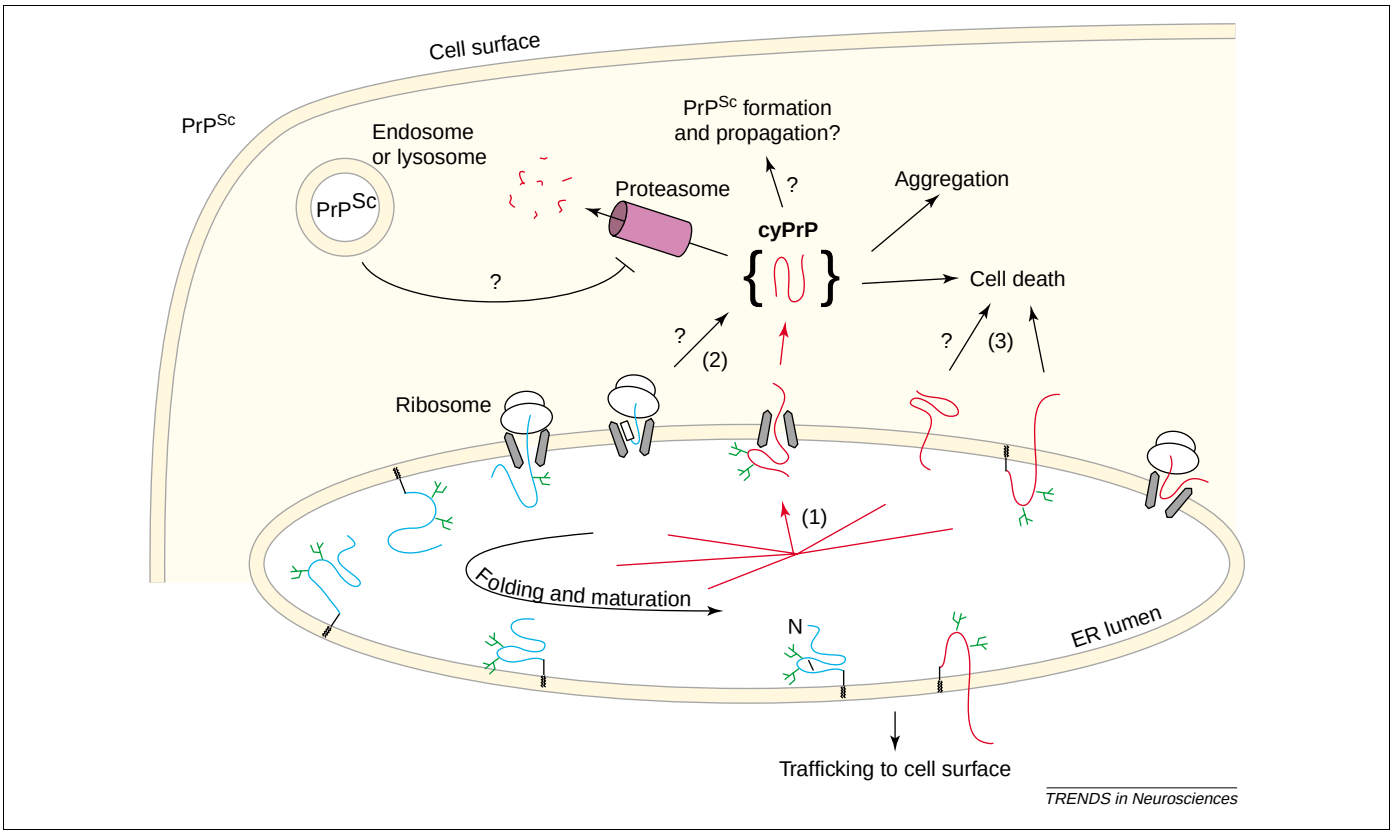

Fig. 1. Is cytoplasmic PrP (cyPrP) at the center of all prion diseases? This diagram outlines a speculative model that relates a recently described cytoplasmic form of prion protein (PrP) to various aspects of prion disease pathogenesis and transmission. In this scheme, the vast majority of newly synthesized prion protein (blue peptide) is translocated into the endoplasmic reticulum (ER), where it is subjected to several post-translation modifications [for example, addition of glycosyl groups (green)] and chaperone-assisted folding events, before further trafficking along the secretory pathway to the cell surface. However, minor populations of PrP (red peptide) could have access to the cytoplasm by following alternative routes that might include (1) reverse translocation of improperly matured PrP out of the ER (red arrows) [11–13], (2) aborted translocation into the ER [8,17] or (3) generation of transmembrane forms of PrP [5,8,15]. Under normal conditions, the residence time of PrP in the cytoplasm would be extremely short (indicated by brackets), owing to its rapid degradation by the proteasome [11,12]. However, if PrP is allowed to remain in the cytoplasm for a significant length of time, it is capable of inducing cell death in neurons [7], aggregating with itself (and perhaps other proteins) [6,11–13] and potentially misfolding into a self-propagating form that can resemble the transmissible form of PrP, PrP^{Sc} [6]. Conditions that would favor elevated levels or prolonged exposure time of PrP in the cytoplasm might include mutations of PrP that interfere with its proper folding [6,12,14] or favor generation of the transmembrane forms, and cellular conditions that compromise ER function or proteasome activity [11–13]. PrP^{Sc} accumulation (either deposited extracellularly or in the endosomal–lysosomal system) might incite neuronal death by leading to inhibition of cell degradation pathways, thereby indirectly leading to an increased residence time of PrP in the cytoplasm. Pathways or relationships in this model that remain to be examined experimentally are indicated with question marks.

configuration and because transmembrane forms can also be subject to degradation by cytoplasmic proteasomes. Thus, heritable mutations might, via multiple mechanisms, converge to a final pathway involving access of at least a portion of PrP to the cytoplasm, where it could inappropriately interact with cellular pathways that eventually lead to apoptosis.

The idea that transmissible prion diseases, in which the inciting event is PrP^{Sc} formation and accumulation, could also work through the increased generation of cytoplasmic PrP remains totally unexplored. However, one speculative possibility is that PrP^{Sc} accumulation leads to inhibition of the cellular degradation machinery that normally disposes of cyPrP. Interestingly, proteasome activity has been demonstrated to decrease upon accumulation of certain aggregated proteins [16]. Whether PrP^{Sc} could also mediate such an effect from its poorly characterized sites of accumulation merits further study. Clearly, establishing any relationship between PrP^{Sc} and cyPrP represents a formidable challenge and will necessitate the development of new tools to readily detect and modulate cyPrP formation in precise and selective ways.

Toxicity, aggregation and transmissibility of cyPrP

A striking consequence of inappropriate and prolonged exposure of PrP to the cytoplasmic environment is an increased propensity to misfold and aggregate [6,11–13]. Whether this aggregation is a protective mechanism or a contributing cause of cell death is not known. However, the aggregates seem to be 'self-perpetuating': a brief exposure to a reversible proteasome inhibitor results not only in the initial accumulation of cyPrP aggregates but also in continued accumulation even after removal of the inhibitor [6]. This rapid accumulation from an initial 'seed' was not seen to the same extent for the cystic fibrosis transmembrane-conductance regulator (CFTR), another aggregation-prone proteasome substrate that was coexpressed in the same cells. Interestingly, some of the accumulated cyPrP is relatively insoluble and partially resistant to protease digestion. These biochemical characteristics, together with its propagating phenotype and its apparent specificity for PrP, are all reminiscent of properties ascribed to PrP^{Sc}, the transmissible agent of prion diseases.

Thus, it is tempting to speculate, as the authors do, that *bona fide* transmissible PrP^{Sc} might be generated *de novo*. If indeed PrP can be misfolded into self-propagating forms in the cytoplasm, this could provide an explanation for where and how PrP^{Sc} arises during sporadic, and certain heritable, prion diseases. It will, therefore, be extremely interesting to see whether lysates from the cell-culture experiments [6] or transgenic mice expressing cyPrP [7] can in fact transmit disease to experimental animals. Such bioassay studies would greatly strengthen the case for a potential relationship between cyPrP and the *de novo* formation of transmissible prion protein.

Concluding remarks and outstanding questions

The recent studies discussed here have revealed a single situation, involving PrP in the cytoplasm, that can elicit tissue-specific cell death on the one hand and misfolding, aggregation and potential self-propagation on the other. The apparent requirement for only small amounts of cyPrP in inducing apoptosis, together with the multiple pathways by which it could potentially be generated, make this an attractive common pathway used by multiple prion diseases. The challenge ahead will be to establish mechanistic links, if they exist, between the newest observations and the currently established but seemingly disparate facets of the diverse prion diseases. Does PrP^{Sc} formation cause cell death by inducing cyPrP? Do all heritable PrP mutations result in increased cyPrP generation? Does the PrP^{Sc} of sporadic and certain heritable prion diseases originate in the cytoplasm? If so, how does it eventually recruit cell-surface PrP in its propagation? How does the presence of PrP in the cytoplasm lead to cell-type-specific apoptosis? Because any unifying principle behind all prion diseases requires an explanation of how events in multiple cellular compartments influence each other, it is becoming clear that deciphering the intricate relationships between the transmission, pathogenesis and cell biology of prion diseases will require a complete understanding of the complex intracellular trafficking and metabolism of PrP.

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