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Minireview

Traffic of prion protein between different compartments on the neuronal surface, and the propagation of prion disease

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Abstract **The key mechanism in prion disease is the conversion of cellular prion protein into an altered, pathogenic conformation, in which cellular mechanisms play a poorly understood role. Both forms of prion protein are lipid-anchored and reside in rafts that appear to protect the native conformation against conversion. Neurons rapidly traffic their cellular prion protein out of its lipid rafts to be endocytosed via coated pits before recycling back to the cell surface. It is argued in this review that understanding the mechanism of this trafficking holds the key to understanding the cellular role in the conformational conversion of prion protein.**

Keywords: Amyloid; Endocytosis; Lipid domain; Neuron; Neurodegeneration; Prion protein

1. Introduction

The prion diseases[1] are caused by a normal cell surface protein, prion protein (PrP^{C}; superscript C denotes the normal cellular form) adopting an alternative conformation with markedly increased β-pleated sheet content that aggregates to form amyloid fibrils and plaques within the brain, with accompanying fatal neurodegeneration [1].

This conversion occurs spontaneously at an extremely low rate. In man, spontaneous CJD kills people at a frequency of 1 in a million of the population, and then only in old age. The low spontaneous frequency can be greatly increased by mutations within PrP, with some invariably causing fatal disease in homozygotic carriers [1]. Some of these disease-causing mutations have been shown to destabilize the normal conformation of PrP^{C}, but others, particularly in the unstructured N-terminal domain, do not discernibly affect PrP^{C} itself and so probably influence the protein's interaction with other proteins, either with infectious PrP^{Res} or with normal cellular proteins such as those involved in its trafficking [2].

Conversion can also be triggered, very efficiently, by the introduction of even minute quantities of infectious PrP^{Res} via food, wounds, grafting or contamination on surgical instruments.[2] The bovine spongiform encephalopathy epidemic (BSE, or mad cow disease) in the UK dramatically demonstrated the high efficiency of infection within a single species, and its capacity to cross species to infect man, albeit at low incidence.

This combination of aetiology by spontaneous, inherited and infectious routes is unique for a disease dependent upon the product of a single gene, and gives the prion diseases a special place in investigating the role of protein misfolding in neurodegeneration. Unlike, for instance, Alzheimer's disease, for which the cumulative effect of triple gene knockouts is required to produce a reasonable experimental model, genetically normal mice can be infected with mouse adapted scrapie. It is not a model system, it is the disease. Questions such as whether neurodegeneration begins by damage to pre-synaptic axon terminals (a candidate cause of neurodegeneration in a range of misfolding diseases) can be examined with precision in scrapie-infected mice [3].

2. Prion protein misfolding and amyloid formation

Since the key interaction in prion disease is between the cellular and pathogenic conformations of a single protein, the simplest mechanism is to posit a direct interaction between PrP^{C} and the infectious PrP^{Res} form. This 'protein chemistry only' view can be represented by schemes such as that shown in Fig. 1.

Studies of prion infectivity show that monomers and small oligomers of PrP^{Res} are not infectious, it is mid-sized oligomers

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[1] The prion diseases, classed overall as transmissible spongiform encephalopathies (TSE), are grouped in man as Creutzfeldt–Jacob Disease (CJD), Gerstmann–Straussler–Scheinker disease (GSS), fatal familial insomnia (FFI) and kuru; in cattle and mink, bovine/mink spongiform encephalopathy; in North American deer as chronic wasting disease; and in sheep and goats, or in TSE from another species transferred to mice, as scrapie.

[2] The altered, infectious conformation of PrP is relatively resistant to proteolysis, a property often used to identify this form which is designated PrP^{Res}. Where the infectious form is identified by infectious titre, it is usually designated PrP^{Sc} for the Scrapie form. PrP^{Res} is a subset of, but not identical to, PrP^{Sc}, probably because a single standard set of conditions are used to define protease resistance, but different strains of prion disease show different degrees of resistance. Infectious PrP^{Sc} of some prion strains can be destroyed by the stringent proteolysis that detects most of the PrP^{Sc} as PrP^{Res} in other strains. Here we use PrP^{Res} to denote protease-resistant infectious prions.

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Fig. 1. Scheme of prion amyloid formation, emphasizing that until stable mid-sized oligomers of PrP^{Res} (represented as squares) are formed, equilibria (or kinetic barriers) favour the normal conformation of monomer PrP^{C} (represented as spheres), with small oligomers (<hexomer; rectangles) being metastable or otherwise less effective in seeding further conversion of PrP^{C}. The initial step in spontaneous and familial disease is the conversion of PrP^{C} to the metastable monomer, which with repeated cycles builds up and converts to the seeding oligomer. Infectious disease short-cuts this process by introducing seeding oligomer.

(~14–28-omers) that are maximally infective [4]. Realization that it is an oligomer, not the monomer, that seeds amyloid formation simplifies a long-standing problem in prion research: how to explain the existence of many different 'strains' of prion disease. 'Strain' (used here in analogy to viral strains) refers to the fact that different types of prion disease can vary so markedly in the type of pathology (±plaques, ±spongiform degeneration), the areas of the brain attacked, the major cells affected (neurons vs astrocytes or even vascular endothelia) and the timing of disease progression (from months to many years), that they can appear to be entirely different diseases. It is clear, from relative protease sensitivity and exposure of epitopes to antibodies, that PrP^{Res} also differs molecularly in different prion strains [5]. But how could a single protein encode this range of multiple disease types as different conformations? The problem becomes much more understandable if the information is encoded, not in the conformation of a monomer, but in that of oligomers which could be assembled in multiple ways.

3. Conversion of PrP^{C} is chaperoned by cells

Prion disease cannot be analysed purely as a problem of protein chemistry. As with other protein folding diseases, how the target protein PrP^{C} is folded, trafficked and degraded within the cell plays a central role in the disease. Evidence for this ranges from the difficulty of producing infectious amyloid that mimics *in vivo* disease in cell-free conditions [6] to studies of the effect of mutations that control PrP^{C} trafficking or membrane anchorage upon prion infection [7,8]. And since PrP^{C} and PrP^{Res} are tethered to the surface membrane by glycosylphosphatidylinositol (GPI) anchors, their partitioning into lipid rafts is a key component.

The influence of membrane rafts upon PrP^{C} trafficking and its conversion to PrP^{Res}, and wider issues of PrP^{C} function and dysfunction in disease, are the subject of some excellent recent reviews [9–12] that tackle a formidable and often conflicting array of data. Major problems (not restricted to PrP) that impede decisive experimentation include the lack of specific means to alter rafts within cells; doubt concerning the use of detergent resistant membranes (DRMs) as isolates of rafts; and the difficulty of visualizing compartmentalization of raft proteins on living cells.

Here, we focus upon a single question – how does the trafficking of PrP^{C} on neurons affect its interaction with PrP^{Res}? From this vantage point, we will also comment upon some wider methodological problems.

4. Endocytic trafficking of a GPI-anchored protein

Endocytic trafficking is not an autonomous property of a protein, but rather requires it to interact with other molecules in its environment. The textbook example is endocytosis via coated pits, for which endocytic adaptor proteins (e.g. AP2, β-arrestin) bind to trafficking motifs present on the cytoplasmic domain of transmembrane proteins. GPI-anchored proteins, lacking any cytoplasmic domain, can be endocytosed through coated pits by binding extracellularly to a transmembrane receptor that has the requisite endocytic motifs on its cytoplasmic domain. The prototypical example is the uPA receptor-PA1 inhibitor complex that binds extracellularly to the LDL receptor-related protein 1 (LRP1), which has the requisite cytoplasmic trafficking motifs and is rapidly endocytosed via coated pits [13]. Aggregate properties of rafts are suggested to also drive alternative, non-coated pit endocytosis, by mechanisms that remain to be clarified.

Since clathrin and its adaptors are ubiquitously and very highly expressed, the endocytosis of a transmembrane protein can be studied by transfecting it into any convenient cell. However, for a GPI-anchored protein such as PrP^{C}, there can be no guarantee that its transfection into a non-expressing cell, or even into a native expressing cell at excess levels, will result in its interaction with the full range of molecules that normally internalize the protein. If the native mechanism is not available, other mechanisms will take over, the default being bulk flow into endocytic organelles resulting in the 16-18h internalization times reported for PrP^{C} in a number of studies (see reviews [11,12]).

Caveolae are irrelevant to prion trafficking on neurons as cavealae do not occur on adult mammalian neurons. No membrane in biology has been examined ultrastructurally as minutely and repeatedly as the neuronal surface for the past 50 years, without to our knowledge caveolae ever being reported on adult mammalian neurons. Caveolae are seen on accessory and glial cells (in some cases, in abundance, such as on perineurial cells and Schwann cell myelin in peripheral nerve), which explains the presence of caveolin proteins in neural homogenates.

5. PrP^{C} trafficking on the neuronal surface

We have studied the endocytic trafficking of endogenously expressed PrP^{C} on primary cultured adult sensory neurons, by labelling the protein with fluorochrome- or gold-coupled Fab antibody fragment (i.e. a monovalent ligand) at a sub-endocytic temperature (10–15 °C), and then raising the temperature to 37 °C to allow endocytosis (Figs. 2A, B and 3). We found that PrP^{C} leaves its rafts to recycle every few minutes via clathrin coated pits between the cell surface and recycling endosomes [14]. PrP^{C} on the neural cell line N2a traffics similarly [14,15], although with ten times slower kinetics [14] as does transfected PrP^{C} on SH-SY5Y neural cells (although here the process of leaving rafts requires the presence of Cu^{2+} [16] which is not the case with sensory neurons).

The distinctive feature of this trafficking is that, while still on the cell surface, PrP^{C} leaves its raft environment to cross non-raft membrane and then enter coated pits, where (still not in rafts) it is endocytosed and returned to the surface (Fig. 2).