

Amplified Molecular Binding of Prion Protein Homologues in Self-progressive Injury of Neuronal Membranes and Trafficking Systems

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Prion particles might be viewed as essential sources of cyclical coupling and uncoupling of depolarization and repolarization of the neuronal plasmalemma. In terms of different parametric functions, the cellular PrPc, both as a source and also a mediated means of induced replication of the Prion particle PrPsc, would be considered in terms of agent amplification as induced by the bovine host. One might view bovine spongiform disease as a source of variant Creutzfeldt-Jakob disease in the human that would involve such amplified uncoupling of membrane-based phenomena of ion-determined polarization events. It would, in addition, appear that a spongiform change of neurons that progresses in conjunction with neuropil alterations and as a primarily self-amplified system of spreading progression in the brain would necessarily constitute a reactive series of cell injury. It is indeed in terms of a neuronal reactivity linked, for example, to mitochondrial overactivity and also to perhaps nucleotide inserts within neurons that one might consider species specificity of prion particle transmission a host-based series of events giving rise to and constituting also a final expression of disease process events.

It might be realistic to consider interspecies transmissibility, and once this occurs, a fundamental pathway of evolutionary adaptability that arises and progresses simply in terms of progressively amplified reactivity.

Reactivity to neuronal injury would appear an integral system of self-progression based on a primary and also a series of secondary changes that evolve in terms of a PrPc particle that itself promotes self-conversion to PrPsc. Indeed, conformational changes to PrPsc might be defined as a system disorder of neuronal reactivity that self-progresses as amplified progressiveness of the neuronal injury.

Keywords: Vacuolization; Molecular binding; Prion protein; Trafficking systems

ACCUMULATION OF PrPc AND PrPsc BEYOND SIMPLE TRANSFORMATIONAL EVENTS IN MEMBRANE ANCHORAGE-RELATED ASPIRATION INTO MYOFIBERS AND NEURONS IN THE VACUOLIZATION/ SPONGIFORM PHENOMENON

An essential increase in PrPc, as seen for example in skeletal myofibers of patients suffering from sporadic or hereditary forms of inclusion body myositis (Zanusso *et al.*, 2001), would prove a potentiating source of evolving effects that

directly implicate the myofiber vacuolization as analogous to the neuronal spongiform change in prion disease. However, the vacuoles in inclusion body myositis tend to be distinctly pleomorphic and vary from 3 to 10 µm in diameter; in addition, they tend to be rimmed by basophilic material, unlike spongiform vacuoles in prion disease of the central nervous system, and they contain complex membranous whorls and filamentous structures. Indeed, membrane-based pathogenesis in prion disease may possibly relate to a vacuolization that is self-progressive. Certainly, a fundamental aspect of the transformation of

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cellular PrPc to pathogenic PrPsc would necessarily appear to involve an increased production of PrPc in the context itself of evolving transformation perhaps, strictly based on such increase of PrPc content in case of prion disease.

It is indeed in this sense that one might recognize the presence of PrPsc fundamentally as disturbance related, not simply to the transformation of the PrPc but to an actual dynamic evolution involving increased expression of PrPc, that in turn potentiates such transformation to PrPsc.

This would appear, however, to involve binding interaction with an auxiliary factor designated protein X (Ryon *et al.*, 2003). In general terms, binding conformational transformation of PrPc protein to various molecular species including homologue Doppel (Dpl) (Behrens and Aguzzi, 2002) would indicate a basic peculiarity of PrPsc as a variability of the essential functionality of PrPc primarily related to neuronal and axonal trafficking. Inherent to any accumulative process, transformational events themselves would constitute a series of events subsequent to both accumulation of PrPc and transformation to PrPsc. Such mutually transformational events would sequentially tend to potentiate each other.

Essential transformation of the normal isoform of the PrPc as either a phenomenon of progression or of nonprogression in prion disease pathogenesis would not depend on just neuronal or myofiber content levels of the pathologic PrPsc isoform (Gibson, 2001).

It would appear that structural determinants in cooperative binding of prion particles to nucleic acid involving catalysis or chaperone functionality would implicate a subsequent transitional step towards non-genetic transformation (Grossman *et al.*, 2003).

In hereditary and sporadic forms of inclusion body myositis, high levels of PrPc increased expression of prion protein in spite of unchanged levels of PrPmRNA in prion diseases might actually constitute a mechanism in the production of prion disease as a generic category in pathogenesis. Systems of progression would involve transformation of PrPc as an accumulation of cell damage causing vacuolization of the skeletal myofibers. The spongiform change in neurons and neural tissues might reflect an anchorage-related dysfunction arising from both accumulation of PrPc and transformation of such accumulated PrPc to PrPsc.

Indeed, such accumulation would constitute an essential pathogenesis for prion disease whereby inclusion body myositis involves an analogous though variant cytologic effect. Such cytopathology would result from an invasive phenomenon involving the myofiber directly consequent to membrane anchorage of the PrPc and PrPsc and subsequent disturbed ionic or membrane transport dynamics.

In a global context, intracellular trafficking systems together with axonal transporting and internalization events would relate to neurotrophins as a pathogenic evolution implicating a wide range of prion and neurodegenerative disorders (Butowt and Von Bartheld, 2003).

THE BOVINE HOST AS AN AMPLIFYING MECHANISM OF PRION DISEASE PROGRESSION IN VARIANT CREUTZFELDT-JAKOB DISEASE IN HUMANS

Prion disease might actually constitute a powerful stimulus for the generation of oxidative stress (Guentchev *et al.*, 2000) in a manner particularly related to neuronal injury. This might at least partly arise from depletion of the normal PrPc protein isoform through transformation to PrPsc. Such neuronal pathology might be suggestive of an accumulative phenomenon resulting in spongiform change. Massive accumulation of 4-hydroximononeal adducts in an astrocytic population would constitute an active source of oxidative stress linked especially to primary and secondary failure of neuronal antioxidant activities (Andreolletti *et al.*, 2002). Also, at times, possible toxic copper toxicity and even homocysteine-induced effects might be implicated (White *et al.*, 2001).

In terms suggestive particularly of injury to mitochondria in neurons involved by the disease process, physical conformation of the normal cellular isoform of PrPc might reflect ongoing events arising primarily from the neuronal spongiform change. Such a process would perhaps operate as systemic participating roles on the part of the immune system (Drisko, 2002). This would perhaps apply in spite of a general lack of inflammatory activity in prion diseases. Mitochondrial pathology would be expected to potentially contribute to a significant loss of neuronal viability that would be self-progressive.

A high degree of progressiveness of the dementia in prion disease within a context of high selectivity for neurons and for specific neuronal subpopulations might indicate that the PrPsc isoform fulfills a function in the ongoing activity of the prion disease that is reflected in a neuronal apoptosis (Jesionek-Kupnicka *et al.*, 2001) that is specifically self-programmed (Offeu *et al.*, 2000).

Active axonal transport of PrPc as a fundamental attribute of subsequent possible conversion to a neuro-invasive PrPsc isoform would inherently evolve as defects of the axonal transport mechanisms themselves (Kunzi *et al.*, 2002). Therefore, PrPsc might reflect effects of a disease process rather than constitute a cause for the spongiform encephalopathy.

In this sense, the long incubation period of prion encephalopathies (Fraser, 2002) would contrast sharply with a very rapid dementing disease course, once this is clinically established.

Active trafficking systems of neuronal and axonal spread would operate as neuroinvasion on the part of the prion protein in terms of fundamental indices of pathologic involvement in these disorders. In fact, PrPsc as a physical conformational change involving the generation of beta-sheets and amyloid generation might relate to variant Creutzfeldt-Jakob disease as a disease process with highly progressive attributes in the human (Irani, 2003).

Native bovine systems of participation in the generation of the PrP^{sc} would help to account for the florid amyloid plaque, characteristic of the human variant of a bovine spongiform encephalopathy that also incorporates oxidative stress as induced progressiveness. In this regard, it might be significant to consider clusterin (apolipoprotein J), that is also implicated in amyloid production in Alzheimer brains, in terms of an integrating molecular pathway involving colocalization in plaque PrP deposits (Sasaki *et al.*, 2002). In fact, a series of molecular conformation changes appears implicated in the creation of various protein homologues including Doppel (Behrens *et al.*, 2001).

IS DISTURBED NEURONAL MEMBRANE BIOPHYSICS THE CENTRAL MECHANISM OF CELL DEATH IN SPONGIFORM ENCEPHALOPATHY THROUGH THE DYSFUNCTION OF IONIC/WATER BALANCE?

Reactive inhibition of calcium entry within neurons would appear to be a mechanism that not only depletes neurons of calcium, but also promotes a progression of events involving membrane-based polarization and sequential ionic and water interactions (Thellung *et al.*, 2000).

Strict spongiform change as a phenomenon that affects neurons both in terms of extracellular and intracellular dimensions related to the neuronal cell membrane would appear to progress largely as an ionic channel dysfunction affecting especially water transfer in and out of the cells. Perhaps water, as a process of accumulation intracellularly and also extracellularly, would concern the neuronal plasmalemma as the main target in prion disease.

Water accumulation, with strict reference to ionic channel dysfunction of the neuronal plasmalemma, might entail a full series of subsequent biophysical phenomena directly damaging the plasmalemma and also giving rise to abnormal distribution of various ions within such neurons. It is perhaps a water/ionic disturbance affecting the neuronal membrane potential and other membrane biophysical attributes of neurons that would eventually account for widespread neuronal cell death as central to spongiform encephalopathy.

AMYLOID PLAQUE DEPOSITION AND NUCLEOTIDE REPEAT INCLUSIONS IN DEMENTIA PROGRESSION IN PRION DISEASE

Homozygous versus heterozygous polymorphisms of the PrP^c gene within a hereditary trait disorder involving modified protein conformational change (Kretzschmar, 1999) would perhaps implicate amyloid plaque formation as cortical deposits. A phenomenon of nucleotide repeats within the coding region of the PrP^c gene (OMIM 176640) is perhaps a fundamental aspect of Prion disease giving rise to dynamics of a template-based replication of

the prion particle as an amplification of translational or post-translational events.

Converted or transformed cell biologic processes might be subverted to abnormal alternate modes of protein utilization and deposition. In such a context, progression of Prion disease might evolve as cell tropism, virulence and rate of replication of the prion particle (Stumpf and Krakauer, 2000).

Prion protein utilization would constitute a function of prion deposition that constitutes a homozygous polymorphism affecting functional prion protein utilization.

Any concept of template replication of the prion protein within strict systems of altered conformation of protein molecular fibrillogenesis might form a unique translational disturbance. This would reflect not simply Prion gene disease progression but especially an evolutionary history of polymorphic modifications ultimately targeting protein molecular biology. Altered protein conformation would specifically implicate heavy prion protein deposition that is progressively modified to subsequently induce prion protein utilization.

Upstream AUG codons would appear to control ribosomal entry in terms of subsequent modulation of prion protein translation as a progression step in prion encephalopathy (Schroder *et al.*, 2002). Downregulation of PrP^c mRNA translation develops with protein synthesis, also apparently in terms of plasma membrane binding (Rybner *et al.*, 2002).

Florid beta-pleated amyloid plaques, as seen in variant Creutzfeldt-Jakob disease of bovine spongiform encephalopathy type, would constitute a function of modes of handling of the involved prion particle due to passage handling first in the bovine brain and subsequently, in the human brain.

In addition, it would appear that specific molecular dynamics of binding interaction between prion protein and amyloid protein would operate in a milieu of active inflammation that in turn determines overall disease activity (Eikelembroom *et al.*, 2002).

A strict concept of template replication of the prion particle might invoke a system operability that is characterized by modes of utilization of prion proteins mutually influencing each other in dynamic progression.

Such operative systems would perhaps implicate intrinsically binding molecular interactions as homologue variants of a full range of membrane and trafficking systems in neurons and axons (Trojanowski and Lee, 2003). Ultrastructurally, abnormal configurations of plasma membranes are seen due to excess production of a membrane constituent and referable possibly to membrane rafts or clathrin-coated pits containing prion particles that convert to PrP^{sc}.

Certainly, an evolutionary history of prion particle utilization and disposal would concern PrP^c and especially, conversion of PrP^c to PrP^{sc} that disturbs various neuronal systems of viability. Insertion of nucleotide repeat would evolve as a progressive tendency for progression of the clinical dementia. Such a conceptual

framework of primarily binding characteristics of the prion protein would relate especially to neurons as a phenomenon of neuroinvasion rather than simply as spread in the cerebrospinal fluid (Wong *et al.*, 2001).

PRION PARTICLE TEMPLATE REPLICATION AS AN INHERITED NEURONAL REACTIVITY IN DETERMINING TRANSMISSIBILITY

A template-driven replication of the prion particle that is inherent to post-translational conformational modifications of the PrPc to the PrPsc isoform would account for a hereditary predisposition to spongiform encephalopathy involving agent transmissibility (OMIM 123400). In a sense, perhaps, hereditary traits would predispose to template replication and also to specific post-translational conformation changes that subsequently induce encephalopathy. Indeed, pathologic effects might relate, for example, to damage linked directly to various functionally determined attributes of the PrPc on a hereditary basis. It would, in addition, appear that a concept of evolving molecular processing of the prion protein would implicate structural changes related to extraoctapeptide repeat insertions within the PrP gene. Such a mechanism would help explain a basic phenomenon of binding of prion protein across a potentially highly variable spectrum of protein molecular species (Yanagihara *et al.*, 2002).

In this sense, perhaps, distinguishing PrPc from PrPsc would concern a phenomenon of neuronal viability or nonviability as a series of reactivation events involving various cell types in the central nervous system.

Indeed, reactivation of microglia and inflammatory responses arising peripherally would potentially constitute specific systems of prion protein availability and of neuroinvasion (Combrink *et al.*, 2002).

A basic molecular transformation of prion particles to a neuroinvasive form might essentially concern, for example, dynamics of a defective axonal system of trafficking arising from such conversion of the PrPc to the PrPsc. Reactivity of constituent cell elements in the central nervous system might operatively constitute tangible traits that are both inherited and propagated in terms of a dichotomy of combined reactive processes, on the one hand, and of mechanisms of amplified replication of the prion particles, on the other.

In such fashion, inherited reactivity would perhaps constitute integral mechanisms of prion particle template replication. The immune response as related to prion protein presentation by dendritic cells and also to transmission of antigenic effect of such presented prion protein, might integrate cellular and humoral immune components as lymphoreticular participation in progression of the neuroinvasive disease (Aucouturier and Carnaud, 2002).

A pattern of characterized template replication would perhaps define transmissibility of the spongiform encephalopathy in terms of how reactive changes in

handling of the modified Prion PrPse somehow would induce subsequent endless cycles of modified template replication and also conformational changes of the prion particle.

In this sense, perhaps, one might explain the species-specificity in transmissibility of PrPsc in terms based on reactivity processes that are inherited and that strictly characterize the conformational modifications of the PrPc to PrPsc.

IS PRPSE A POSSIBLE CAUSE OF WIDE PORE CREATION IN THE POSTSYNAPTIC MEMBRANE INTERFERING WITH A MEMBRANE-BASED CREATION OF THE DEPOLARIZING STATE?

A long term potentiation phenomenon that implicates in some way the coupling of an afferent input impulse with a simultaneously occurring depolarizing event of the post-synaptic membrane (Johnston *et al.*, 1998) might characterize the synapse itself as a region in congruity with both the afferent and terminal target neuron. The synapse is an active phenomenon of integration in terms of both spatial and temporal dimensions.

Synapses would constitute organs integral to both the stimulus presynaptically, and to the response postsynaptically. Long-term potentiation (LTP) would, in particular, constitute a series of presynaptic and postsynaptic events in a manner that would materially influence each other in terms of progression.

An essential phenomenon of LTP would potentiate both the presynaptic afferent input and the postsynaptic depolarization event in terms of a summation coupling centered either on the dendritic spine or on the postsynaptic membrane. Synaptic potentiation of impulses as a phenomenon that integrates the presynaptic terminal with the postsynaptic membrane and dendritic spine would necessarily involve adhesion molecules and also the creation of effective unitary complexes that integrate ionic channeling with depolarization and impulse generation. The PrPsc would appear to constitute a breakdown of barriers preventing integration of an ion channeling. This would prevent unified, depolarizing events to develop. Instead, de-coupling of the presynaptic afferent input from the postsynaptic depolarizing event that would result in a phenomenon of wide pore creation that prevents membrane-based depolarizing events to reach a threshold marked scale of upregulation or down regulation of membrane excitability in generation of the action potential.

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