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Phosphatidylethanolamine as a prion cofactor

Potential implications for disease pathogenesis

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Mammalian prions with significant levels of specific infectivity can be formed in vitro from mixtures of prion protein (PrP) and cofactor molecules, but not from PrP alone. We recently isolated and identified the essential membrane phospholipid phosphatidylethanolamine (PE) as an endogenous cofactor for prion propagation in vitro.[1] In this article, we discuss the potential role of PE and other essential cofactor molecules as a molecular link between the processes of prion formation and prion-induced neurodegeneration.

Keywords: phosphatidylethanolamine, phospholipid, prion, cofactor, pathogenesis, flippase, aging, neurotoxicity

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The mechanisms by which mammalian prions propagate and cause disease in the brains of infected animals are currently unknown, and the relationship between the two processes is also unclear. Our recent work identifying the membrane phospholipid phosphatidylethanolamine (PE) as an endogenous cofactor for prion propagation in vitro[1] suggests the possibility of a unifying mechanism in which prion formation and disease pathogenesis may be interconnected through the involvement of essential membrane lipids such as PE.

There has been significant interest in studying the mechanism by which mammalian prions replicate infectivity because prions do not adhere the central dogma of molecular biology (i.e., genetic information being encoded exclusively by replicating nucleic acids) despite their remarkable abilities to infect normal hosts and exhibit phenotypic strain variation in a manner reminiscent of viruses. Critical early studies showed that prion propagation requires the cellular conformer of the prion protein (PrP^C)[2,3] and that during propagation PrP^C undergoes conformational change into a misfolded conformer termed PrP^{Sc}.[4,5] Later, a number of in vitro PrP^{Sc} propagation studies indirectly suggested that additional molecules might also be required for mammalian prion propagation, since PrP^{Sc} levels and/or prion infectivity could be amplified from crude brain homogenate but not a preparation of pure PrP^C or recombinant (rec) PrP molecules.[6-15] Employing a reductionist biochemical approach, we subsequently produced chemically defined hamster prions by using a substrate cocktail containing PrP^C, copurified lipid molecules and synthetic RNA molecules.[16] Remarkably, we found that hamster prions with moderate levels of specific infectivity could be produced from these components de novo, showing for the first time that infectious prions could be produced spontaneously in a rigorously prion-free environment, an event that must occur in sporadic Creutzfeldt-Jakob disease.[16] Subsequent studies showed that while RNA and other polyanionic molecules facilitate the propagation of hamster prions, they appear to play no role in the propagation of various strains of mouse or vole prions.[17] This surprising species specificity led us to seek the endogenous cofactor responsible for prion propagation in other animal species by using a variety of biochemical characterization and enrichment methods, resulting in the isolation and identification of PE as the essential molecule.[1] PE has a polar head group, is found in all living cells and is particularly enriched in nervous tissue. Within membranes, PE creates a more

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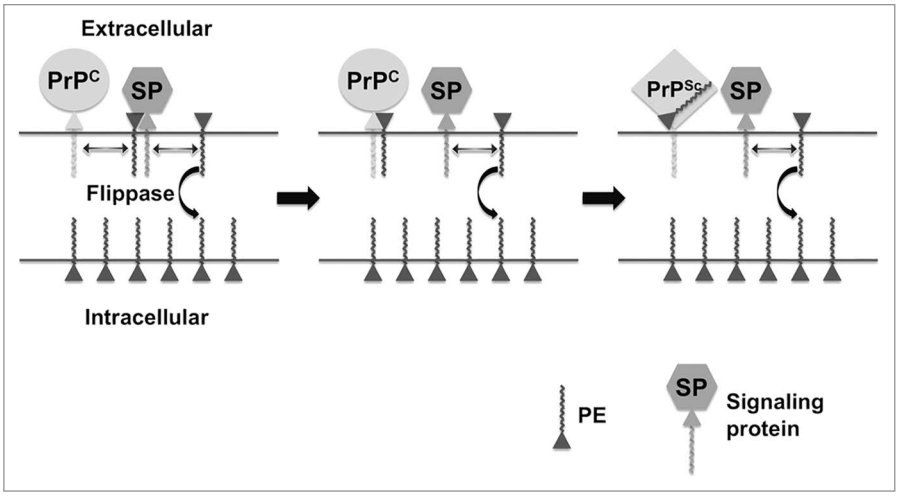

Figure 1. Hypothetical model of PE in prion formation and pathogenesis. Prion formation: the relative paucity of PE on the extracellular leaflet of the plasma membrane of cells is actively maintained by a flippase enzyme, whose activity declines with age, allowing more PE to be localized on the extracellular surface where it is accessible to PrP^{C}. Pathogenesis: if extracellular PE becomes consumed during the process of prion formation by binding irreversibly to PrP^{Sc}, its depletion from the membrane surface will cause biophysical changes in the membrane itself and dysregulation of surface-expressed signaling proteins (SP) that normally bind PE, resulting in cell death.

viscous bilayer than phosphatidylcholine. The prion promoting ability of PE was definitively confirmed by using synthetic PE as a solitary cofactor to generate infectious recombinant prions.[1]

As an in vitro prion propagation cofactor, PE displays several unique properties. When reconstituted with either PrP^{C} or recPrP substrate alone (i.e., without RNA or any other cofactors), PE facilitates the propagation of prions from every animal species tested, including deer chronic wasting disease (CWD) and sheep scrapie.[1] The conversion of recPrP to $recPrP^{Sc}$ in vitro is ~10-fold higher in terms of percent conversion when facilitated by PE alone compared with the previously described combination of phosphatidylglycerol and RNA. Treatment of brain homogenate with phospholipase abolishes the in vitro propagation of both hamster and mouse prions, whereas RNase only inhibits hamster prion propagation.[1] Thus, PE appears to be an endogenous cofactor that can by itself efficiently facilitate the in vitro propagation of prions from multiple animal species. It is currently unknown whether any prion cofactor identified biochemically, including PE, plays a physiological role during the process prion replication in vivo. However, because PE facilitates (and phospholipase inhibits) the conversion of prions from multiple species in vitro, it seems reasonable to hypothesize that PE and other phospholipids may play a widespread role in prion formation in the brains of infected animals. A practical obstacle to testing the hypothesis that phospholipids participate in prion conversion in vivo is that membrane lipids are highly regulated, essential and difficult to manipulate. New approaches will need to be developed in order to manipulate phospholipid levels in living cells and animals.

PrP^{C} is located on the extracellular leaflet of the plasma membrane, and PrP^{Sc} formation is believed to occur either on the cell surface or within an endocytic compartment[18] (**Fig. 1**). PE is distributed asymmetrically across the plasma membrane; ~80% is located within the intracellular leaflet and ~20% is located in the extracellular leaflet of erythrocytes.[19] Phospholipid asymmetry is maintained by ATP-dependent flippase enzymes, and is disrupted by processes such as apoptosis and cellular aging.[20] It is interesting to speculate that age-dependent disruption of phospholipid distribution might play a role in the pathogenesis of prion disease and other neurodegenerative disorders by allowing cofactor molecules to become more accessible to surface-expressed pathogenic proteins such as PrP (**Fig. 1**). This hypothetical scenario offers an intriguing molecular explanation for the increased incidence of neurodegenerative diseases among the elderly.

It is also interesting to speculate that the distribution of phospholipids in the membrane of infected neurons might also become disrupted if specific surface phospholipid molecules such as PE were irreversibly bound to PrP^{Sc} during the prion conversion process (**Fig. 1**). In this scenario, the pathogenic process (i.e., the mechanism by which PrP^{Sc} formation leads to cell death) would be tied to the infectious process (i.e., the mechanism by which PrP^{Sc} molecules are formed from PrP^{C} molecules in cells) at a molecular level through the common involvement of an essential membrane phospholipid cofactor. Indeed, even minor changes in the distribution of PE across the plasma membrane are known to cause cellular dysfunction and degeneration, presumably by changing the biophysical properties of the membrane itself and by making surface PE less accessible to various signaling proteins that require it for activity (**Fig. 1**). This hypothetical model of prion pathogenesis provides an alternative mechanism that does not need to invoke the production of an unidentified toxic PrP species distinct from PrP^{Sc} during the process of prion formation.[21] The depletion of surface PE (or other phospholipids) would require membrane anchoring of PrP^{C} during the conversion process, in agreement with the observation that transgenic mice expressing only PrP^{C} molecules not anchored to the plasma membrane display attenuated pathological changes.[22] Also, the rate of depletion of surface phospholipid cofactors would be proportional to PrP^{C} expression levels, in agreement with the observation that the rate of pathogenic progression in prion-infected animals (and their incubation period) is proportional to PrP^{C} protein levels.[23]

In conclusion, we have recently isolated PE as an endogenous cofactor for mammalian prion formation in vitro. Although new systems will be needed to study whether PE or other phospholipids play a similar role during prion replication in vivo, their identification allows us to envision a new unified model of prion propagation and pathogenesis.

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