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Previews

Lipids, a Missing Link in Prion Propagation

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Prion protein is considered to have an infectious ability by itself. However, in order to explain the main features of prion diseases, additional cofactors would be required. Sanghera et al. (in this issue of *Chemistry and Biology*) have found evidence that a ganglioside, GM1, is a ligand for the C-terminal region of prion protein.

The "protein only" hypothesis attributes infectious ability to a single protein and has been harshly debated for decades (Soto and Castilla, 2004). Although there was compelling evidence that supported this idea, studies based on cell-free conversion (Kocisko et al., 1994), protein misfolding cyclic amplification (PMCA) (Castilla et al., 2005), and generation of synthetic (Legname et al., 2004) and recombinant prions (Wang et al., 2010) were required to confirm the hypothesis. While the key protagonist role of prion protein (PrP) has not been challenged, the failure to explain the principal features of prion diseases has motivated the search for additional cofactors. First, studies to determine the minimal components for in vitro prion replication showed how enriched membrane subsets and detergent-solubilized membrane fractions were enough to support prion replication in vitro (Nishina et al., 2004). Later, studies based on PMCA successfully replicated prions using only cellular PrP (PrP^C), co-purified lipid molecules, and a synthetic polyanion (Deleault et al., 2005). In a similar way, Wang et al. (2010), using mouse recombinant PrP, lipids, and RNA as cofactors for the PMCA substrate, obtained the first highly infectious recombinant prions. At that time, it seemed that lipids were an inevitable requirement for in vitro prion replication. However, Kim et al. (2010), using a PMCA variation, showed that prions causing transmissible spongiform encephalopathy in wild-type hamsters could be generated solely from highly-purified, bacterially-expressed recombinant hamster PrP without any mammalian or synthetic cofactors (other than buffer, salts, and detergent). Despite these studies demonstrating that no other cofactors were necessary for in vitro prion replication, the fact that recovered infectivity was low, led some researchers to consider lipids not just as helpers for prion replication but as triggers for in vivo prion propagation. Moreover, none of the previous studies, with the exception of the standard PMCA (Castilla et al., 2005), were able to maintain the strain phenomenon (different conformations of the PrP showing distinct biological and physicochemical properties), thus after every in vitro process a new prion strain was generated.

In this in vitro situation and focusing on lipids as PrP ligands, it could be suggested that they might act by clustering the PrP^C and PrP^{Sc} (misfolded form of PrP^C) molecules, thus propitiating protein-protein interaction. In a similar way but in an in vivo environment, Sanghera et al. (2011, in this issue of *Chemistry & Biology*) propose that GM1 ganglioside is a ligand for the C-terminal region of PrP. The authors show that subtle structural changes in PrP occur as a consequence of GM1 binding. In particular, GM1 lipid chains appear to have a role in modulating PrP structure. In contrast, PrP does not bind 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (zwitterionic) bilayers, and it does bind 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phospho-*rac*-[1-glycerol] (negatively charged) bilayers, but then extensive protein aggregation on the membrane surface occurs. Sanghera et al. (2011) have shown how GM1, a lipid very abundant in raft-like domains, binds a specific region of PrP.

Two aspects of observed PrP binding to the cell membranes deserve some commentary. One is the apparent involvement of both electrostatic and hydrophobic forces in GM1-PrP interaction, which is common with many other proteins operating at the lipid-water interface in membranes. Even more interesting is the fact that PrP is membrane-anchored through a glycosylphosphatidylinositol (GPI), covalently linked to the protein C terminus. The authors show that the GM1 binding site is also close to the C terminus, an area suggested to modulate PrP misfolding. Thus, the immediate question is raised about the influence of the GPI moiety on ganglioside binding in vivo, an obvious object for further investigation.

Although the experiments have been performed using recombinant PrP, these studies should be the prelude for others in which PrP^{Sc} or rec-PrP^{Sc} [respectively infectious and misfolded forms of PrP(23-231)] would be involved as a further approach toward understanding prion propagation in vivo. The fact that the authors have observed a direct association between PrP and the GM1 oligosaccharide moiety suggests that lipids might have a role for propagating prions through PrP^C-PrP^{Sc} conversion within raft domains. Because this association causes some structural changes in PrP, and assuming that identical changes take place when PrP is GPI-anchored to a membrane, a trigger role for sporadic cases of prion diseases might be attributed to the lipid. In order to support this idea, it would be crucial to know how the interaction of GM1 and PrP^{Sc} occurs and how such interaction would mediate in the entire propagation process.

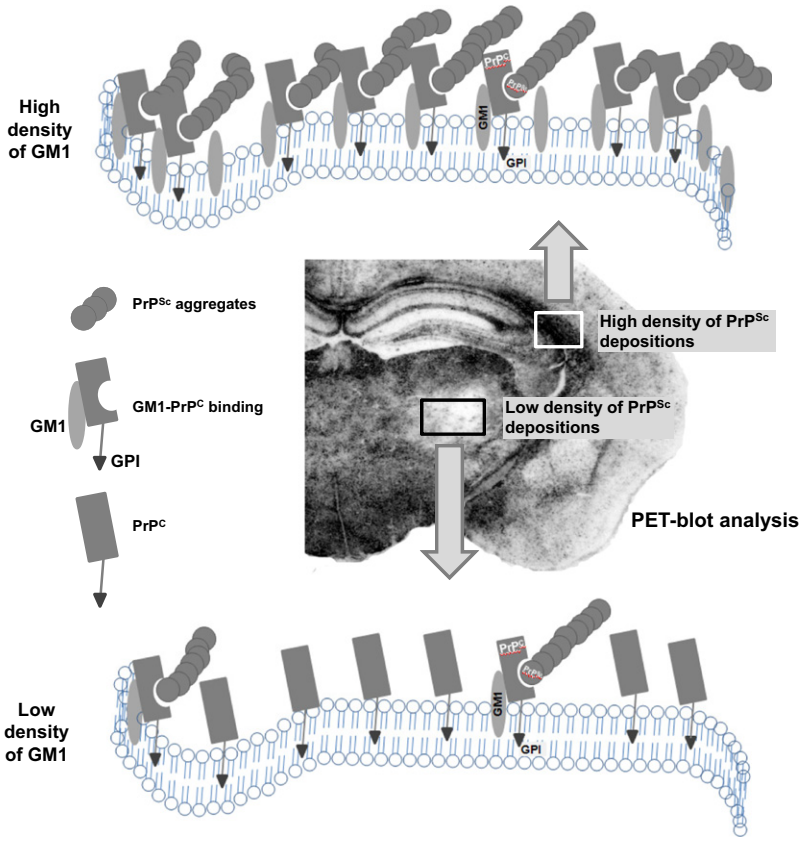

Figure 1. PET-Blot Analysis Shows a Characteristic Pattern of PrP^{Sc} Deposition
PrP^{Sc} depositions are very specific for each prion strain and might signify a differential PrP^{Sc} interaction and/or replication depending on the amount of PrP^{C} ready to be converted. The figure shows a plausible model where this convertibility might be depending on the GM1 binding that would slightly modify the PrP^{C} structure, making it more suitable for conversion. The differences in GM1 density might be determinant and would explain the different PrP^{Sc} deposition patterns observed in PET-blot analysis.

Gangliosides are abundant in the brain and are found in virtually all mammalian cell membranes, yet their specific role remains largely unknown. A defect in ganglioside synthesis has been related to a form of familiar epilepsy (Simpson et al., 2004). Gangliosides are effective targets for a number of bacteria and bacterial toxins, while also providing resistance to phospholipase attack (Urbina et al., 2011). Now GM1 has been found to bind a specific region of PrP likely involved in disease susceptibility. Given the high concentrations of gangliosides in neurons and their localization in specific raft domains, the authors suggest that the association of PrP with GM1 can play a role in modulating disease susceptibility. This study is especially relevant due to our lack of knowledge on how the transmission barriers are controlled in vivo and provides new insights for its understanding. In the same direction, the specific lipid-protein interaction might be used to explain another important feature of prion diseases related to prion strains, namely the erratic behavior of different prion strains when infecting different culture cells. It could also help to understand why prion strains show specific PrP^{Sc} accumulation patterns in different regions of the brain. Prion strains could have specific affinity for certain neurons depending on the different lipid composition in their membranes (Figure 1).

Although the first thought that comes to mind when PrP is mentioned is prion diseases, PrP likely has a specific physiological function, unfortunately as yet unknown. Despite this lack of understanding, PrP presence in raft-like domains would have important implications. Thus, the authors propose that lipid-PrP interactions might be involved in cell signaling and cell recognition, processes at the cell membrane in which protein segregation into specific domains and lipid-protein interactions could be essential.

The very interesting observation of GM1-PrP interaction in vitro demands an investigation in the in vivo situation. Equally important is the exploration of GM1-PrP^{Sc} interaction, i.e., whether or not the interaction is protein conformation dependent. Also, the nature of the ganglioside may be important. Are other gangliosides binding PrP with similar affinities? Prions are known to replicate easily in the lymphoreticular tissue; what are the gangliosides found in that tissue, and what is their interaction with PrP? A screening of gangliosides appears as a prime line of research. Finally, the pathogenesis of prion diseases remains largely unknown. PrP^{Sc} does not appear to be toxic in itself, thus the process leading to neuronal death remains an enigma. A putative binding of PrP^{Sc} to ganglioside(s) would open a new avenue for research in this area.

REFERENCES

Castilla, J., Saá, P., Hetz, C., and Soto, C. (2005). Cell *121*, 195–206.

Deleault, N.R., Geoghegan, J.C., Nishina, K., Kascsak, R., Williamson, R.A., and Supattapone, S. (2005). J. Biol. Chem. *280*, 26873–26879.

Kim, J.I., Cali, I., Surewicz, K., Kong, Q., Raymond, G.J., Atarashi, R., Race, B., Qing, L., Gambetti, P., Caughey, B., and Surewicz, W.K. (2010). J. Biol. Chem. *285*, 14083–14087.

Kocisko, D.A., Come, J.H., Priola, S.A., Chesebro, B., Raymond, G.J., Lansbury, P.T., and Caughey, B. (1994). Nature *370*, 471–474.

Legname, G., Baskakov, I.V., Nguyen, H.O., Riesner, D., Cohen, F.E., DeArmond, S.J., and Prusiner, S.B. (2004). Science *305*, 673–676.

Nishina, K., Deleault, N.R., Lucassen, R.W., and Supattapone, S. (2004). Biochemistry *43*, 2613–2621.

Sanghera, N., Correia, B.E.F.S., Correia, J.R.S., Ludwig, C., Agarwal, S., Nakamura, H.K., Kuwata, K., Samain, E., Gill, A.C., Bonev, B.B., and Pinheiro, T.J.T. (2011). Chem. Biol. *18*, this issue, 1422–1431.

Simpson, M.A., Cross, H., Proukakis, C., Priestman, D.A., Neville, D.C., Reinkensmeier, G., Wang, H., Wiznitzer, M., Gurtz, K., Verganelaki, A., et al. (2004). Nat. Genet. *36*, 1225–1229.

Soto, C., and Castilla, J. (2004). Nat. Med. Suppl. *10*, S63–S67.

Urbina, P., Flores-Díaz, M., Alape-Girón, A., Alonso, A., and Goñi, F.M. (2011). Biochim. Biophys. Acta *1808*, 279–286.

Wang, F., Wang, X., Yuan, C.G., and Ma, J. (2010). Science *327*, 1132–1135.

