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Commentary

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Underglycosylated prion protein modulates plaque formation in the brain

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Protein-only infectious agents

Prions are protein-only infectious agents that subvert the normal form of the host cellular prion protein, PrP^{C}, to the self-templating scrapie prion form, PrP^{Sc} (1, 2). Due to the unique nature of prions, the properties of both the host PrP^{C} and agent PrP^{Sc} contribute to the pathogenesis of disease. Specifically, strains of prions result in distinct patterns of PrP^{Sc} deposition and neurodegeneration in the CNS that are encoded by strain-specific conformations of PrP^{Sc} (3, 4). Interestingly, changes in the host PrP^{C} can result in alteration of the phenotype of disease, including neuropathology, in the absence of altering the prion strain. For example, murine PrP^{C} is polymorphic with two alleles, and inoculation of ME7 from one PrP^{C} genetic background to the other resulted in a change in both the incubation period of disease and the distribution of neurodegeneration (5). Importantly, back passage of ME7 to the original PrP^{C} genetic background resulted in an incubation period and neuropathology indistinguishable from the original PrP^{C} allele (i.e., class 1 strain) (5). This seminal work indicated that differences in the amino acid sequence of PrP^{C} contribute to the pathogenesis of disease independently of the prion strain.

Altering prion pathogenesis

Posttranslational modifications of PrP^{C} can alter prion pathogenesis. PrP^{C} is tethered to the cell membrane by a glycophosphatidylinositol (GPI) anchor (6). In transgenic mice, deleting the PrP^{C} GPI anchor results in PrP^{C} retrafficking into the intracellular space. Subsequent prion inoculation resulted in PrP^{Sc} formation, indicating that prion conversion is independent of the GPI anchor (7). Importantly, these mice showed substantial PrP^{Sc} distribution differences in the whole body as well as differences in the CNS. In the CNS, the pattern of PrP^{Sc} deposition was characterized by dense plaque-like formations, compared with the more diffuse synaptic pattern of PrP^{Sc} deposition in nontransgenic WT animals. While prion infection of PrP^{C} GPI anchorless mice resulted in prion formation, these animals did not develop clinical signs of prion disease. It is unclear if their healthy phenotype is due to a failure of PrP^{Sc} to gain access to the areas of the CNS responsible for the onset of clinical signs, if the PrP^{Sc} generated was less neurotoxic, or if the absence of symptoms was a property of the strain tested.

In addition to a GPI anchor, PrP^{C} contains two N-linked glycosylation sites that have variable occupancy (8, 9). Transgenic mice in which the PrP^{C} lacked both N-linked glycosylation sites still supported prion formation. PrP^{Sc} from these mice transmits prion disease to WT mice, indicating that glycosylation is not required for prion formation or transmission (10). In the strains tested, however, the absence of PrP^{C} glycosylation resulted in an increase in incubation period and PrP^{Sc} amyloid plaque deposits compared with WT animals that have a full repertoire of PrP^{C} glycosylation. Shifting of PrP^{Sc} to a more amyloid plaque structure can reduce the pathogenicity of PrP^{Sc} by extraneuronal routes of infection, consistent with the hypothesis that small, soluble PrP^{Sc} oligomers are the preferred species of PrP^{Sc} for retrograde transport in the peripheral nervous system that facilitates neuroinvasion (11, 12). Both GPI anchorless PrP^{C} and underglycosylated PrP^{C} share a common neuropathological feature of parenchymal PrP^{Sc} plaques. These studies indicate that alteration of PrP^{C} posttranslational modifications can result in profound changes in the neuropathology of prion disease. The mechanism responsible for these observations is, however, unclear.

How PrP^{C} posttranslational modifications may alter neuropathology

The work of Sevillano et al. in this issue of the *JCI* examines the mechanism of how PrP^{C} posttranslational modifications alter

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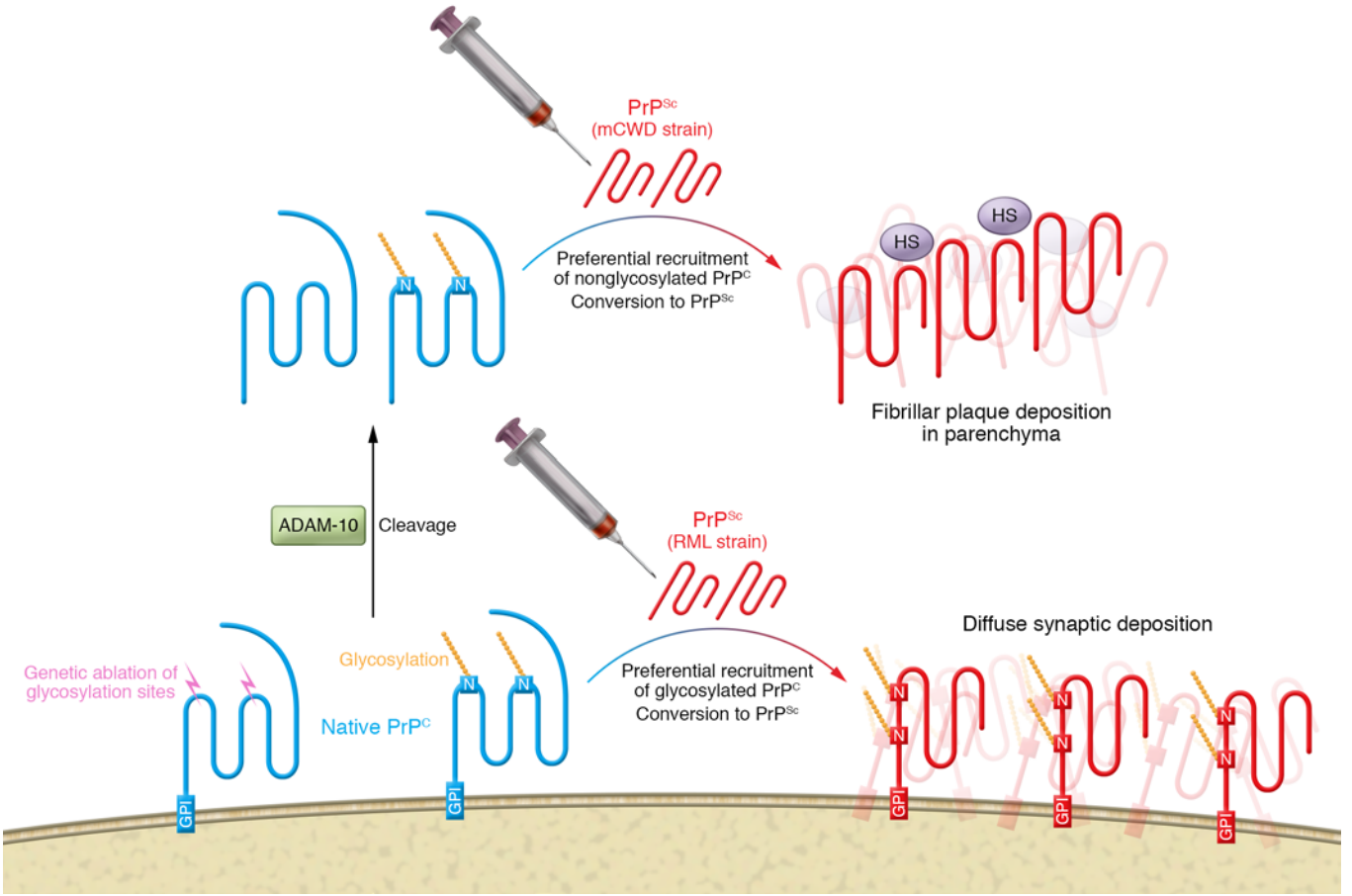

Figure 1. Role of posttranslational processing of PrP^C in the formation of prion aggregates. Fibrillar prion strains such as mCWD preferentially incorporate underglycosylated PrP^C (blue) that is released from the cell surface by ADAM-10 into plaques that form in association with HS in the parenchyma. Subfibrillar prion stains, such as RML, preferentially incorporate glycosylated PrP^C into diffuse synaptic deposits of PrP^{Sc} (red) without a HS scaffold. Genetic ablation of N-linked glycosylation sites (N) can switch in nonfibrillar strain pathology from synaptic diffuse deposition to plaque-like deposition of PrP^{Sc} in the parenchyma.

neuropathology (13). Mice expressing unglycosylated PrP^C were generated (*Prnp*$^{180Q/196Q}$) and were shown to have normal PrP^C expression patterns that, importantly, failed to spontaneously develop neurodegeneration. The *Prnp*$^{180Q/196Q}$ mice were challenged with four murine-adapted prion strains, three of which (RML, 22L, and ME7) are categorized as subfibrillar strains with a predominately diffuse synaptic pattern of PrP^{Sc} deposition in the CNS, and one of which (mCWD) is a fibrillar strain that is characterized by large plaques of PrP^{Sc} in the CNS. All of the strains tested resulted in the *Prnp*$^{180Q/196Q}$ animals developing prion disease. Notably, the mice inoculated with the subfibrillar strains developed plaque-like deposits. This altered neuropathology is consistent with previous studies indicating that reduced PrP^C glycosylation results in an increase in PrP^{Sc} plaque formation (10). To examine the composition of these plaque-like deposits in the *Prnp*$^{180Q/196Q}$ mice, the authors used an anti-PrP antibody that specifically recognizes PrP that has been cleaved from the cell surface by the metalloproteinase ADAM-10 (14). It was determined that the PrP^{Sc} plaques were enriched for ADAM-10–cleaved PrP. Next, the authors identified that heparan sulfate (HS) colocalized to the plaques but was restricted from areas with diffuse aggregates of PrP^{Sc}. Consistently, HS specifically bound to PrP that had been cleaved by ADAM-10. Further, the HS/ADAM-10–cleaved PrP interaction was influenced by the glycosylation state of PrP where a progressive reduction in the glycosylation of PrP resulted in a corresponding increase in affinity of PrP to bind HS. These data suggest that underglycosylated PrP^C that is cleaved from the cell surface in combination with HS produces large plaque-like structures.

To test the hypothesis that glycosylation influences plaque-like pathology, Sevillano and colleagues generated novel transgenic mice that expressed PrP^C with three N-linked glycosylation sites (*Prnp*^{187N}) (13). Inoculation of the *Prnp*^{187N} mice with the plaque-forming strain mCWD resulted in a neuropathology that lacked plaques and instead was comprised of diffuse PrP^{Sc} deposits compared with WT mice that contain 2 N-linked PrP^C glycosylation sites. This observation is consistent with the premise that PrP glycosylation inhibits PrP^{Sc} fibril formation by interfering with binding to HS. Overall, a model for prion formation suggests that underglycosylated PrP released from the cell surface associates with HS to form large parenchymal deposits, whereas cell-bound fully glycosylated PrP^{Sc} preferentially forms small diffuse aggregates (Figure 1 and ref. 13).

The role of the prion strain in the preponderance to form plaques versus diffuse deposits of PrP^{Sc} is only partially understood. Transmission of all four prion strains to *Prnp*$^{180Q/196Q}$ mice resulted in an increase in plaque-like PrP^{Sc} deposits in the CNS.

However, the degree to which plaque-like deposits increased was not uniform between the strains tested. The mechanism behind this observation remains unclear (13). It is possible that the strain-specific differences in the preferred PrP^C substrate for conversion, as has been identified using in vitro studies, may play a role in this observation (15). In addition, strain-specific rates of formation and clearance may favor specific patterns of prion accumulation (16).

Changing the incubation period of prion disease

Strain-specific responses in the alteration of the incubation period of disease in the *Prnp*$^{180Q/196Q}$ mice were observed. Specifically, the incubation period of RML-infected WT or *Prnp*$^{180Q/196Q}$ mice was similar. This was in contrast to that of 22L-infected animals, where the incubation period of *Prnp*$^{180Q/196Q}$–infected mice was longer compared with WT mice. Importantly, the extended incubation period of RML-infected *Prnp*$^{180Q/196Q}$ mice was preserved upon second passage. One potential explanation for this observation is that differences in the PrP amino acid sequence between the WT and *Prnp*$^{180Q/196Q}$ mice may cause a species barrier–like phenomenon that can alter strain properties. Alternatively, while evidence suggests that glycosylation is not required on the inoculum PrP^{Sc} to maintain strain properties (17), it is possible the host PrP^C glycosylation directly or indirectly via interactions with cellular cofactors plays a role in maintenance of strain properties during prion conversion (18). The small differences in incubation period between WT and *Prnp*$^{180Q/196Q}$ mice infected with RML suggest that glycosylation may not substantially affect prion formation in contrast to what has been observed in vitro where glycosylation inhibits prion formation (19, 20). Several scenarios could explain this discrepancy. It is possible that the strains tested in this study respond differently to changes in glycosylation compared with strains that were used in the in vitro studies (13, 19, 20). Many factors in addition to the kinetics of PrP^{Sc} accumulation can influence the incubation period of disease. The relative rate of PrP^{Sc} formation, clearance, and transport to populations of neurons that are susceptible to PrP^{Sc} neurotoxicity are only a few of the factors that contribute to the incubation period of disease.

When Sevillano and colleagues introduced a third glycosylation site in PrP^C, the incubation period shortened. Further, there was a corresponding increase in diffuse PrP^{Sc} aggregates and an absence of PrP^{Sc} plaques (13). These findings suggest that a more rapid disease course corresponds with diffuse PrP^{Sc} deposits. While more work is needed to further characterize the contributions of posttranslational modifications of PrP^C and the prion strain to prion pathogenesis, the Sevillano et al. study illustrates that shifting diffuse synaptic PrP^{Sc} deposits to plaques is a viable therapeutic modality to slow the progression of prion disease.

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