

# Prion protein in myelin maintenance: what does the goat say?

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The cellular prion protein PrP<sup>C</sup> has been extensively studied because it can adopt a pathogenic three-dimensional conformation that causes rare, but invariably fatal, neurodegenerative prion diseases in humans and other mammals. The disease-causing conformer of the protein is called PrP<sup>Sc</sup>, of which oligomeric aggregates constitute prion agents that can bind to, and convert further, PrP<sup>C</sup> molecules into PrP<sup>Sc</sup> (Prusiner, 1998). Thus, in the poorly understood process of prion propagation, there is transfer of biological information encoded solely by protein conformation. Prions can spread within a tissue secondary to a spontaneous conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> or upon transmission of prion agents between individuals.

A major focus of the prion research community has been to prevent human and livestock prion diseases and to understand the nature of prion agents, rather than investigating the cellular functions of PrP<sup>C</sup>.

However, mice with ablation of *Prnp* (the gene encoding PrP<sup>C</sup>) were completely resistant towards prion disease, providing strong support to the original prion hypothesis. The mice appeared healthy and had normal life expectancies. The paucity of data concerning PrP<sup>C</sup> functions was rapidly alleviated, and today a bewildering variety of cellular functions has been attributed to PrP<sup>C</sup>. As in most areas of biomedical research, increasingly sophisticated and precise cellular and transgenic animal models have been instrumental in improving our understanding of PrP<sup>C</sup> physiology. In this process, some phenotypes that were initially attributed to PrP<sup>C</sup> were readdressed and shown to be mouse-line specific and/or caused by neighboring genes rather than PrP<sup>C</sup> itself. Nevertheless, the physiological relevance of most proposed roles for PrP<sup>C</sup> remains unclarified.

In one area, however, there has been notable progress, not least driven by Bremer et al. (2010). By generating new lines of mice lacking PrP<sup>C</sup>, they re-investigated a demyelinating phenotype that had been observed in *Prnp*

knockout mice in the late 1990s (Nishida et al., 1999). This work confirmed that, in the absence of PrP<sup>C</sup>, mice develop a chronic demyelinating polyneuropathy of varying clinical severity (Bremer et al., 2010). Moreover, the studies indicated that a peptide cleaved off from PrP<sup>C</sup> on the axonal surface diffuses to a receptor on the Schwann cell membrane where it elicits a myelin-maintenance signal (Kuffer et al., 2016). Within the framework of mouse models, this demyelinating neuropathy is one of most thoroughly documented results associated with loss of PrP<sup>C</sup> function. However, studies of transgenic goats (Yu et al., 2009) and cattle (Richt et al., 2007) without PrP did not observe or investigate this phenotype, leaving the myelin protective role of PrP unexplored in non-rodent mammalian models.

In 2012, we reported the finding of a nonsense mutation affecting the *PRNP*-gene in Norwegian dairy goats and described a 30-month-old goat that was homozygous for this mutation (Benestad et al., 2012). No abnormalities were recorded in this animal, which was completely devoid of PrP<sup>C</sup>. Flock mates with the same *PRNP* genotype were also healthy and behaved normally. It was immediately evident that this line of goats could be a powerful tool for investigations of PrP<sup>C</sup> biology and that the goats could be used in complementary studies to genetically engineered rodent models. This would enable critical cross-validation of data derived from these models in a mammal that is much closer to humans from an evolutionary perspective.

Besides analysis of peripheral nerves, which is the topic here, two peculiar features have been observed in these goats, in addition to resistance to prion infection (Salvesen et al., 2020). Firstly, a mild, but distinct, hematological phenotype was seen, with slight elevation in red blood cell numbers, accompanied by reduced red blood cell volumes (Reiten et al., 2015). This is worth mentioning because it mimics a phenotype observed in transgenic cattle with knockout of *PRNP* (Richt et al., 2007). Thus, in two PrP-deficient ruminant species an easily

recognizable hematological phenotype occurs. This reflects a functional role for PrP<sup>C</sup> in hematopoietic stem-cell microenvironments, in which non-myelinating Schwann cells have important roles. Mice without PrP have severely reduced capacity for bone-marrow renewal upon sub-lethal irradiation, underlining the importance of PrP<sup>C</sup> for maintenance of these specialized cellular niches.

Secondly, exposure of goats without PrP<sup>C</sup> to intravenous challenge with bacterial endotoxin resulted in significantly stronger and more pronounced sickness behavior than seen in PrP<sup>C</sup>-expressing flock-mates (Salvesen et al., 2015). Although not clarified in detail, this phenotype probably results from an increased sensitivity and/or intensity in inflammatory signals reaching the brain in the absence of PrP<sup>C</sup>. Thus, in this regard, the goat model echoes observations from murine *Prnp*-knockout models in which increased vulnerability towards pro-inflammatory signaling has been demonstrated in various experiments.

As recently reported, we addressed the proposed roles for PrP<sup>C</sup> in maintenance of peripheral-nerve myelin by using the goat model (Skedsmo et al., 2020). Clinical neurological examination and analysis of the nociceptive withdrawal response in goats without PrP<sup>C</sup> failed to reveal abnormalities. However, bearing in mind that thousands of mice without PrP<sup>C</sup> have been bred and used in experiments without neurological disturbances being noted, detailed analyses of the peripheral nerves of these apparently healthy goats were clearly warranted.

Our approach to investigate this was using teased nerve-fiber preparations, which are easily available in large-animal models, such as goats. This is a very powerful method for detection and quantification of segmental demyelination by allowing analysis of consecutive myelin internodes along individual nerve fibers. To our surprise, teased nerve-fiber preparations from goats lacking PrP<sup>C</sup> revealed histopathological changes characteristic of a demyelinating neuropathy validating the data already reported from transgenic mice (Bremer et al., 2010; Kuffer et al., 2016).

However, the teased nerve-fiber preparations also showed distinct abnormalities that were less prominent or unrecognized in the murine models.

Most striking were myelin outfoldings, up to several hundred micrometers in length and located adjacent to single intercalated internodes, resulting from previous episodes of paranodal demyelination. These observations suggest that PrP<sup>C</sup> deficiency can destabilize the paranode, which is an area of particularly intimate interactions between Schwann cells and axons. This area is characterized by extensive paranodal loops of the Schwann cell plasma membrane, interconnected with autotypic adherens junctions, and further junctions connecting the paranodal loops and the axon. Detailed investigation of PrP<sup>C</sup> in the paranodal region of myelinating Schwann cells might therefore provide valuable clues regarding the protein function and reveal the molecular pathogenesis of the polyneuropathy. It is conceivable that PrP<sup>C</sup>, being present on both the axon and Schwann cell surface, benefits the stability of these highly specialized membrane domains in the paranodal region.

The pathogenesis of the paranodal abnormalities could also be immune-mediated, as paranodal antigens are frequent targets in immune-mediated neuropathies and dysmorphic paranodes are a prominent feature in such diseases (Gross et al., 2016). This would imply that PrP<sup>C</sup>, which is highly expressed in immune-privileged tissues (such as brain and testicle), contributes to the neuro-immunological homeostasis of these cellular environments.

The numbers of endoneurial macrophages and T lymphocytes were elevated in the nerves from goats lacking PrP<sup>C</sup>. Thus, the immune system is clearly “aware” of the ongoing neuropathological processes. At the ultrastructural level, however, we did not see any signs of macrophage-mediated myelin stripping, which is a hallmark of inflammatory demyelinating neuropathy. This suggests that the macrophage invasion is secondary, neither initiating nor driving the pathology. Further studies are needed, however, to clarify the order of events more closely. For instance, we are currently investigating whether immune cells invade peripheral nerves in goat kids at stages before detectable pathology. While transgenic mice lacking PrP<sup>C</sup> also had increased macrophage numbers in the nerves, T lymphocytes were absent (Bremer et al., 2010).

These aspects, taken together, indicate that the demyelinating neuropathy

elicited by loss of PrP<sup>C</sup> function is a mild, but progressive, condition in which the immune system apparently plays a secondary role. Nevertheless, being conspicuously present at neuro-immunological interfaces, PrP<sup>C</sup> probably serves roles in neuro-immunological communication, integrating activity at the interface between the internal and external environments. The specialized myelinating Schwann cells could be particularly sensitive to chronic distortions of the neuro-immunological homeostasis, which possibly accompanies loss of PrP<sup>C</sup>, hence precipitating the observed neuropathy. Whether the communication between CNS neurons and myelinating oligodendroglia is also affected by the absence of PrP<sup>C</sup>, is another area for further investigations, although CNS demyelination is not prominent in *Prnp* knockout mice (Bremer et al., 2010).

This line of goats that is naturally devoid of PrP<sup>C</sup> is an excellent example of a spontaneously occurring animal model that allows critical testing of data derived from transgenic-model animals. It should be an important tool for improving our understanding of, not only PrP<sup>C</sup> functions and the expected consequences of therapeutic downregulation or silencing of the protein in human prion disease, but also fundamental processes; already we envisage a web of future investigations.

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