



# Is RNA critical for the production of TSE infection?

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## A commentary on

### Recombinant prion protein induces a new transmissible prion disease in wild-type animals.

by Makarava, N., Kovacs, G. G., Bocharova, O., Savtchenko, R., Alexeeva, I., Budka, H., Rohwer, R. G., and Baskakov, I. V. (2010). *Acta Neuropathol.* 119, 177–187.

### Generating a prion with bacterially expressed recombinant prion protein.

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

### Mammalian prions generated from bacterially expressed prion protein in the absence of any mammalian cofactors.

by Kim, J., 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.

The prion hypothesis proposed by Prusiner (1998) stipulates that the causal agent of transmissible spongiform encephalopathies (TSEs) is composed exclusively of an infectious protein (PrP<sup>Sc</sup>) produced after structural conversion of a cellular protein (PrP<sup>C</sup>). Recently, three studies attempting to test predictions arising from this hypothesis have been published by Makarava et al. (2010), Wang et al. (2010), and Kim et al. (2010).

Paying particular attention to the molecular composition of inocula inducing TSE disease reported in these investigations, I plan to discuss here, how these novel findings complement our previous results (Simoneau et al., 2009). In a pioneer work, we substantiated the idea that the requirement of a cofactor may be indispensable for the acquisition of converted recombinant prion protein (recPrP) infectivity. Our results indicate that this crucial cofactor extracted from 263 K PrP<sup>Sc</sup> is composed of two populations of small RNA having an

average length of about 27 and 55 nucleotides. The association of these RNAs with recPrP reconstituted an infectious TSE agent showing in hamster brain strain characteristics slightly different from 263 K infectious agent (Simoneau et al., 2009).

As currently recognized, the demonstration of the prion hypothesis should be performed in wild-type (wt) animals. However I suggest that comprehensive proof of the causal agent of TSEs is composed exclusively of an infectious PrP<sup>Sc</sup> protein can only be obtained when experimentation meets four requirements. This tetrad of rules is equivalent to Koch's postulates for microbial agents, namely that:

1. *In vitro* conversion of pure (e.g., recombinant) PrP<sup>C</sup> to PrP<sup>Sc</sup> is required.
2. Experiments should be performed using wt animals.
3. Infected animals should develop fatal TSEs.
4. Transmissibility of the disease at the second passage should be demonstrated.

In the first study (Makarava et al., 2010) the authors inoculated different preparations of recPrP in hamsters. Though they use wt animals in accord with the second requirement, they fail to meet the first criterion. Indeed the key inoculum produced from a pure solution of recPrP fibrils remains innocuous, i.e., fails to cause disease. This recPrP is effective only if it is “activated” by an “annealing” process involving normal hamster brain homogenate (NHB). Other components may well have been incorporated from NHB. Moreover, the preparation induces a TSE-like disease only on second passage: the original inoculum did not have the ability to cause the TSE disease characterized at second pass; therefore the third condition is not met. Though a new scrapie agent has been created, the experimental context does not allow to know for sure that it is derived from converted recPrP alone.

Indeed, the inocula have had to undergo a long maturation step during the first passage (661 days). One could presume that converted recPrP, associated in a first step with endogenous RNA (present in NHB), is capable of inducing in the first brain the progressive synthesis of PrP<sup>Sc</sup> and small RNA. The association (PrP<sup>Sc</sup>–small RNA) we have reported (Simoneau et al., 2009) would form the infectious agent which inoculated in the second brain would induce overt TSE.

In a similar way to Makarava's work, the approach by Wang et al. (2010) of using wt animals to test the prion hypothesis is compromised by the incorporation of RNA into the inoculum. Infection is only achieved by adding some critical components to the recPrP solution, such as RNA (extracted from mouse liver) and synthetic phospholipid molecules, followed by a very extensive protein misfolding cyclic amplification (PMCA). The putative importance of the host RNA in this sophisticated preparation has not been explored or even debated. We must ask whether it is catalytic or not, imposing control on whether recPrP is associated or not with RNA. We can also ask what its molecular characteristics are: single or double stranded, short or long lengths and, eventually, what sequence? The role of RNA could be evaluated simply using inoculum containing recPrP without RNA.

The third study (Kim et al., 2010) used the classical PMCA reaction combined with PrP<sup>Sc</sup> to convert recombinant PrP. Again the solution destined to be injected is not as the authors believe, of absolute recPrP purity. They used PrP<sup>Sc</sup> partially purified from 263 K-infected hamster brain. In these conditions, native PrP<sup>Sc</sup> is known to be highly contaminated by nucleic acids particularly RNA a key factor for the acquisition of PMCA product infectivity (Geoghegan et al., 2007). On this basis, the crucial control would have been to assess the response of RNase treatment on the preparative solutions.

So the fundamental question as suggested by Supattapone (2010), is what phenomenon metamorphoses an innocuous recPrP preparation into an infectious one? It seems that a conversion step alone is not sufficient to obtain this objective. Rather, co-factors, RNA in some cases, appear to be necessary.

In conclusion, if one considers that the tetrad of rules that should be respected for a definitive demonstration of the prion hypothesis, ambiguities remain that should be clarified, with respect to the involvement of RNA.

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