

The prion protein family

Looking outside the central nervous system

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Although the pivotal implication of the host-encoded Prion protein, PrP, in the neuropathology of transmissible spongiform encephalopathy is known for decades, its biological role remains mostly elusive. Genetic inactivation is one way to assess such issue but, so far, PrP-knockout mice did not help much. However, recent reports involving (1) further studies of these mice during embryogenesis, (2) knockdown experiments in zebrafish and (3) knockdown of Shadoo, a protein with PrP-like functional domains, in PrP-knockout mice, all suggested a role of the Prion protein family in early embryogenesis. This view is challenged by the recent report that PrP/Shadoo knockout mice are healthy and fertile. Although puzzling, these apparently contradictory data may on the contrary help at deciphering the prion protein family role through focusing scientific attention outside the central nervous system and by helping the identification of other loci involved in the genetic robustness associated with PrP.

Prion diseases or transmissible spongiform encephalopathies (TSE) are a related group of rare, fatal brain-neurodegenerative diseases that affect animals and humans.¹ The isolation of the disease-associated protein, PrP^{Sc}, found to be the major, if not the sole, component of the infectious agent, led to the protein only hypothesis.² This protease resistant protein was later found to result from the conformational conversion of a cellular glycosylphosphatidylinositol-anchored host glycoprotein, PrP^C. PrP^C is encoded by the *Prnp* gene.

PrP primary and tertiary structures are well conserved among mammals. This protein is expressed in a broad range of vertebrate tissues and most abundantly in the central nervous system (CNS).³ Thus, among other approaches, many studies have focused on the analysis of the potential biological role of PrP in the CNS, indirectly helping deciphering the neurotoxicity associated with PrP^{Sc}.

PrP Biological Enigma

It was highly anticipated that the genetic ablation of the *Prnp* gene will allow the identification of the PrP^C biological function. It came as a surprise that its knockout in mice,^{4,5} cattle⁶ and goat⁷ were obtained with no drastic developmental phenotype. Its post-natal depletion in adult neurons resulted in similar observations.^{8,9} The only clear phenotype that knockout mice developed was their absolute resistance to TSE and to the neurotoxicity associated with PrP^{Sc}. However, PrP^C was reported to be involved in various physiological and developmental functions (see refs. 10 and 11 for recent reviews). To explain these apparent discrepancies, it was hypothesized that a host-encoded gene could compensate for the lack of PrP.¹² Such a host-encoded gene should thus share with PrP at least two characteristics: (1) an overlapping pattern of expression at a developing time and in cells where PrP has a crucial role and (2) a related biological function so that one can compensate the absence of the other either through strict redundancy or by modulating parallel pathways.

Keywords: Shadoo, doppel, prion, PrP, embryo, placenta, trophoblast, stem cell

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Table 1. Survey of the phenotypic results associated with PrP and/or Shadoo genetic manipulation

Mouse genotype	Trophectoderm phenotype?	Litter phenotype?
PrP knockout ²³	Decreased placental weights compaction of the labyrinth	Lower body weights
Shadoo knockout ¹⁶	Not tested	Lower body weights
PrP Knockout, Shadoo Knockdown ^{19,20}	Ectoplacental cone defect	Embryonic lethality
PrP and Shadoo Knockout ¹⁶	Not tested	Not reported

PrP Paralogs

In the last decade, two mammalian paralogs of PrP, Doppel and Shadoo, were discovered (see ref. 13 for review). These three loci probably derived by retro-transposition of an ancestral ZIP metal ion transporter gene.¹⁴ Doppel and Shadoo were demonstrated to be of neurological relevance. Doppel is normally mainly expressed in the testis of adult mammals and its ectopic expression can cause neuro-degeneration in the central nervous system (CNS). Shadoo is expressed in the CNS and both Shadoo and PrP share neuro-protective properties, notably against Doppel and N-terminally-truncated PrP neurotoxicities.¹⁵ However, as mentioned above, Doppel is not normally expressed in the adult CNS and careful examination of the pattern of expression of Shadoo in the adult brain revealed that it did not fully overlap that of PrP.¹⁶ These observations suggested that should Doppel and/or Shadoo be the putative host-encoded protein that compensate for the lack of PrP, such an effect should be searched elsewhere than in the adult brain.

Genetic Depletion of Several Members of the Prion Protein Family

Genetic depletion in transgenic mice of PrP and Shadoo¹⁶ and of PrP and Doppel^{17,18} have been published. The co-depletion of PrP and Doppel produced a phenotype identical to that of Doppel single knockout. The co-inactivation of PrP and Shadoo produced viable and fertile mice, although the output of crosses between *Sprn*^{KO}*Prnp*^{KO} × *Sprn*^{KO}*Prnp*^{KO} mice was not apparently assessed (see Table S1 in ref. 16). Shadoo knockout mice only suffered from a subtle alteration of the body weight which was not documented in the double-knockout mice¹⁶

(Table 1). Altogether, these observations lent support to the view that PrP and Shadoo on one side, Doppel and PrP on the other, have no-overlapping/redundant roles.

The outcome of the Shadoo-PrP double gene inactivation is discrepant with our previously published data that described early embryonic lethality in mice knockout for PrP and knockdown by RNA interference for Shadoo¹⁹ (Table 1). Origin of this lethality was recently found to result from a developmental failure of the trophoctoderm-derived compartment.²⁰ RNA interference is known to potentially induce knockdown of off-targets and thus false phenotypes. However, since it was observed with two independent ShRNAs targeting the Shadoo mRNA, it seems unlikely that this is at the origin of the observed lethality. Furthermore, an attractive alternative hypothesis, that the ShRNA were also targeting the *Mtg1* transcript that overlaps the 3'-end of the Shadoo transcript,^{16,21} could be dismissed by (1) the locations of the ShRNA targeted sequences outside the overlapping region¹⁹ and (2) the absence of differential expression of the *Mtg1* transcript in the Shadoo knockdown embryos.²⁰ Instead, recent data from the literature appear to be in frame with the induced embryonic lethal phenotype and to indirectly comfort this observation.

PrP and Shadoo in the Trophoblastic Cell Lineages

Expression of PrP during embryonic development, including in the extra-embryonic tissue, had already being described.^{22,23} Knockout of the PrP gene was recently shown to induce intrauterine growth retardation²⁴ (Table 1). This phenotype, which is also associated with significant lower body weights in the adulthood, was attributed to an impairment of the trophoblast angiogenic function in PrP-deficient

embryos and suggested a role for PrP in the development of the placental zones associated with invasion of the trophoblast cells into the maternal deciduas. Such an induced phenotype could appear congruent with the regulatory role on embryonic cell adhesion attributed to PrP1 in zebrafish²⁵ and with the biological pathways identified by comparative transcriptomic analysis of PrP and PrP-knockout early mouse embryos: cell mobility and angiogenesis.²⁵

Shadoo was also recently found to be expressed in the trophoblast cells of the placenta.^{20,27} Furthermore, comparative transcriptomic analyses performed between E6.5 and E7.5 Shadoo-knockdown embryos and their wild-type counterparts suggested that Shadoo has functions complementary, not necessary overlapping, with those of PrP, associated with cellular movement and hematological system development and differentiation.²⁰ In the knockout experiment, it was reported that a relationship probably exists between levels of Shadoo expression and body mass¹⁶ (Table 1). It was suggested that this relationship could be associated with the observed natural expression of Shadoo in hypothalamic neurons since this neuro-anatomical structure contains nuclei that control feeding behavior.²¹ An alternative explanation, yet to be substantiated, would be that this relationship is a consequence of a placental developmental defect, as observed for PrP. The reported difference observed in the mean litter size of Shadoo-knockout mice (7 vs. 7.4; see Table S1 in ref. 16) could indirectly support this latter hypothesis.

Potential Future Directions

What then could explain the difference in the outcome between the double knockout and the knockout/knockdown approaches? A classical explanation for such differences is the use of different

genetic backgrounds. Although, as previously stated,²¹ the two used genetic backgrounds (FVB/N for the knockdown¹⁹ vs. FVB/NCr × 129Pas for the knockout¹⁶) are not very different, it should be noted that neurological signs were observed in aged FVB/NCr × 129Pas but not in aged FVB/N PrP-knockout mice.¹⁶ Thus, although similar, these two genetic backgrounds differ. An experimental approach to solve this question would be to invalidate the Shadoo locus specifically in the FVB/N genetic background. We are currently performing such an approach using zinc-finger-nucleases.²⁸ If it confirms that the double genetic depletion of PrP and Shadoo is lethal in the FVB/N genetic background, its comparison with the FVB/NCr × 129Pas one would be of great interest to identify partners of PrP and Shadoo in controlling the development and differentiation of the trophoblastic cell lineages. Furthermore, a trophoblastic-restricted rescue of Shadoo expression would then also allow to assess whether the development and/or differentiation of embryonic lineage(s) is (are) also affected by the double-gene knockout in FVB/N mice.

Should the double gene knockout turn out to be viable in the FVB/N genetic background, alternative hypotheses could be tested. A potential compensation phenomenon by other genes has already been discussed and will not be further detailed here.²¹ Another possibility is that the double-knockout mice are

more sensitive to biological stress. PrP was recently shown to interact with Argonaute and to be involved in the regulation of the microRNA-induced silencing complexes (RISCs).²⁹ It was noted that Shadoo might compensate for the lack of PrP and that the observed age of lethality of the PrP-knockout/Shadoo-knockdown embryos is reminiscent of that of Dicer knockout embryos.²⁸ The knockdown experiment induces high level of expression of ShRNA that have to be incorporated into RISCs. Such a perturbation might be detrimental in transgenic mice harboring altered RISCs due to the absence of both PrP and Shadoo. It would be easy to test this hypothesis by challenging the double PrP and Shadoo knockout mice with various ShRNA expressing cassettes. Alternatively, lentiviral infection by itself might cause a biological stress.

Although we could exclude that a knockdown of Mtg1 was at the origin of the lethal phenotype as suggested^{16,21} (see discussion above), it remains possible that the two ShRNA were indeed targeting other transcript(s). Because of their location and sequence specificity, an attractive hypothesis would be that they also target a yet unknown transcript that overlaps the last exon of the Shadoo gene, such as for instance a long non-coding RNA.³⁰ Such transcripts are implicated in various biological functions and often overlap gene's transcription units. Its sequence, but not necessarily its expression, would be partially affected by the

knockout process, leaving the possibility that it remains active. Whatever the ShRNA target transcript beside Shadoo is, its identification would be of great interest as its depletion induces a phenotype only in a PrP-knockout genetic background. Its identification would be facilitated by comparative transcriptomic analysis of ShRNA treated or not Shadoo-knockout embryos, which would allow to get rid of the perturbations associated with Shadoo depletion itself.

Thus although the phenotypic difference between the PrP/Shadoo knockout and knockdown mice is puzzling, it raises fascinating biological questions on the potential involvement of these two genes in early mammalian embryogenesis alongside a yet unknown locus. Elucidating the role of this protein family could have medical implication since expression of PrP was noticed to be deregulated in human placental pathologies.²⁹ It would also be tempting to similarly study the role of Doppel, which is also expressed at these early developmental stages in mouse (our own unpublished observation). We are currently assessing this point using again a ZFN approach.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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