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Mini-Review

Prion proteins: Upcoming actors of early embryonic development

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ABSTRACT

The PrP prion protein is well known for its crucial role in transmissible encephalopathies and its implication in other amyloid-based neuro-degenerative diseases has recently been suggested. However, its physiological function remains poorly understood. The prion protein family is composed of three related genes. Both PrP and Shadoo share neuroprotective properties and are expressed in overlapping adult tissues. Doppel appears to be mostly involved in late spermatogenesis. Prion proteins have been shown to be involved in various adult stem cell self-renewal and/or differentiation, suggesting a role of these proteins in adult stem cell homeostasis. Recent data highlighted the crucial role of PrP1 and PrP2 in early embryogenesis of Zebrafish and we recently discovered that of PrP, in conjunction with Shadoo, in early mouse embryonic development. Transcriptomic analyses revealed that in these distant species similar pathways might be affected by the disruption of these genes although the resulting phenotypes differed both in terms of developmental stage and location. This short review summarizes the recent data obtained on the prion protein implications in early embryonic animal development, highlighting species specificities and biological convergences.

KEYWORDS: prion, embryogenesis, mouse, zebrafish, placenta

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INTRODUCTION

The discovery of the Prion protein PrP and of its encoding gene Prnp is associated with its involvement in neurological diseases called Transmissible Spongiform Encephalopathies (TSE) [1, 2]. PrP is an evolutionary conserved glycosyl-phosphatidyl-inositol (GPI)-anchored cell surface protein with two sites of Asn-linked glycosylation, which is expressed in a broad range of vertebrate tissues and most abundantly in the central nervous system [3]. Genetic invalidation of *Prnp* in mice clearly demonstrated that this gene is indeed required for TSE to occur [4, 5]. *Prnp*-knockout (PrP^{KO}) mice were found to be resistant to TSE infection, while this gene invalidation has no major visible phenotypic consequences [6, 7]. However, subtle biological alterations were noticed in these animals as, for example, in their neurotransmission and synaptic plasticity, their memory, circadian rythms and immune responses [8-11 for recent reviews]. Similarly, PrP^{KO} cattle [12] and goat [13] were obtained with no drastic developmental phenotype. A similar observation was made when this gene was invalidated in adult mouse neurons [14, 15].

Thus despite its evolutionary conservation in Mammals which suggested an important role, the lack of obvious phenotypic response to *Prnp* gene invalidation complicated the search for the PrP physiological function that remains unclear even though its implication in neuroprotection, response to oxidative stress, cell proliferation and differentiation, synaptic function and signal transduction has been proposed [8, 9,11 for recent reviews]. PrP temporal regulation also led to

suspect an implication of this protein in early embryogenesis [16-19]. Several recent data link PrP with stem cells proliferation and/or differentiation in various lineages such as embryonic [20-22], hematopoietic [23, 24], erythroid [25], cardio- and skeletal-myogenic [26, 27], neuronal [28-31], ameloblastic and odontoblastic [32, 33], ovarian follicular and spermatogenic stem cells [34, 35]. These properties can also be related to the observed potential implication of PrP in various cancers [36, 37 for recent reviews]. To explain this apparent discrepancy between potential PrP involvement in various stem cell homeostasis suggesting a role unlikely to be dispensable and lack of drastic phenotype in *Prnp* invalidated mammals, it was hypothesized that another host-encoded protein is able to compensate for the lack of PrP [38]. It could thus come as a surprise that the first direct evidence for PrP implication in early embryogenesis was reported with the knockdown of either of the two orthologous PrP genes in Zebrafish.

Evidence for PrP involvement in early embryogenesis of zebrafish

Zebrafish is a teleost model intensively used for studying various aspects of vertebrate development [39 for review]. Its genome has duplicated, resulting in the presence of two functional PrP-encoding genes, called PrP1 and PrP2. The expression pattern of PrP1 and PrP2 was shown to differ. PrP1 is highly and ubiquitously expressed in early mid blastula and its expression decreases after gastrulation to become more spatially restricted, being only detected in forebrain and eyes at 30 hours post-fertilization (hpf) [40]. PrP2 transcripts are detected only at somitogenesis and are highly expressed by 30 hpf in the brain and in some neurons of the central nervous system [40].

Expression of both genes was recently knockdown using morpholino oligonucleotides, an antisense strategy classically used in Zebrafish [41 for review].

Knockdown of PrP1 resulted in an early developmental defect with a gastrulation failure and the inability to carry out epiboly (Fig. 1) [40]. This phenotype was morphologically characterized by a loss of embryonic cell adhesion and an abnormal intracellular processing and/or transport of E-cadherin. PrP1 was also shown to modulate the accumulation of Fyn tyrosine kinase and tyrosine phosphorylated proteins at cell contacts, suggesting that this protein action on the stability of the E-cadherin/β-catenin adhesive complexes

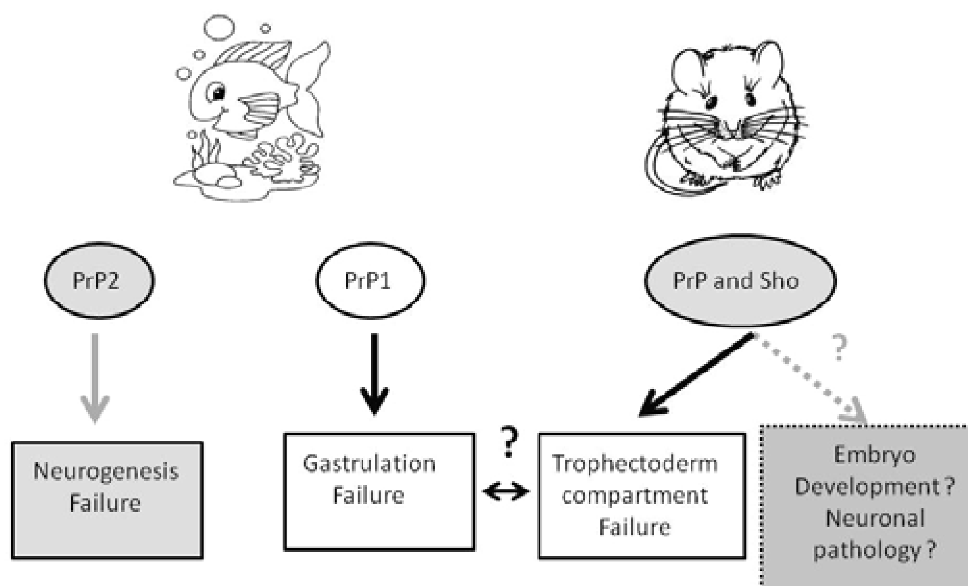

Fig. 1. Genetic invalidation in zebrafish and mouse.

could involve such a signal transduction pathway [40, 42]. Such a link between PrP and Fyn activation was already highlighted in murine neuronal differentiation cellular models [43]. Interestingly, both PrP2 and mouse PrP could partially rescue the PrP1-knockdown phenotype suggesting that some biological properties of PrP1 were conserved in its ortholog PrP2 and in distantly related, homologous proteins.

Consistent with its different spatio-temporal expression pattern, the knockdown of PrP2 resulted in a later phenotype with morphological defects in the head midbrain and hindbrain regions, leading to lethality that occurs between 24 hpf and 7 dpf according to the morpholino that was used and to its concentration (Fig. 1) [40, 44]. This phenotype was further characterized by microarray studies that revealed the differential expression of 249 genes [44]. Clustering analysis highlighted functions associated with embryonic and central nervous system developments, further suggesting that the knockdown of PrP2 impaired neurogenesis. Such a role of PrP was also proposed in mammals [28-31]. Similarly, this transcriptomic analysis suggested an anti-apoptosis action of PrP2, that recalled the reported protection associated with mammalian PrP against Bax-induced cell death [45, 46].

Interestingly, although no lethal phenotype is associated with the genetic invalidation of PrP in mouse, comparative transcriptional analysis of wild-type and PrP^{KO} embryos at 6.5 and 7.5 dpc by RNAseq revealed the differential expression of 73 and 263 genes, respectively [47]. It highlighted a striking biological convergence between the mouse PrP^{KO} induced deregulation and that described in PrP1-knockdown zebrafish and alterations of specific networks involved in nervous system development reminiscent of PrP2-knockdown. Overall, these data alongside the above-mentioned rescue experiment, suggest that biological functions of PrP were conserved among orthologous proteins and during evolution. The difference of phenotype between the PrP1 and the PrP2 knockdown zebrafish might simply reflect the differential expression pattern of the two genes. It also suggests that in Mammals, another host-encoded gene induces a sufficient compensatory mechanism to avoid lethality to occur. Recent experiments point out *Sprn* as the potential hypothetical gene.

Evidence for prion protein family involvement in early embryogenesis of mouse

Two other members of the mammalian prion family have been described during the last two decades, Shadoo (Sho), encoded by *Sprn*, and Doppel (Dpl), encoded by *Prnd* [48 for review]. The three genes probably derived from an ancestral ZIP metal ion transporter gene following a retrotransposition event [49]. These two proteins are GPI-anchored at the cell surface, and while Dpl resembles to the carboxy-terminal globular domain of PrP, Sho bears similarity with its hydrophobic central region and contains tandem repeats with charged residues, thus more resembling to the unstructured N-terminal and central region of PrP. Dpl is mainly expressed in the adult in the male gonads but also, at lower levels, in several other tissues and its gene invalidation resulted in male infertility associated with either the sperm inability to perform acrosome reaction or a failure of late spermatogenesis [50, 51]. The co-invalidation of *Prnd and Prnp* did not appear to modify the observed phenotype [51]. Although *Prnd* was reported to be expressed in embryonic stem cells [21], no embryonic developmental abnormality was reported in those single or double knockout mice.

Sho shares with PrP some spatial regulation and properties, such as neuroprotective ones [52, review in 53]. Knockdown of *Sprn* was achieved in various genotype backgrounds using lentiviral vectors to deliver short-hairpin interfering RNAs (shRNA) [54]. A lethal phenotype, occurring at early embryonic stages (between E8 and E11), was reported when *Sprn* was targeted in FVB/N PrP^{KO} embryos and was not observed when similar experiments were performed on FVB/N embryos, which indirectly suggest that plain *Sprn* expression is not required for normal mouse development (Fig. 1) [54]. The PrP^{KO} specificity of this lethal phenotype was later confirmed by the use of a transgenic line expressing physiological level of ovine PrP under a FVB/N PrP^{KO} genetic background that behaved as FVB/N mice when infected by Sho-shRNA [55]. These data suggested that PrP or Sho is required for

early mouse embryogenesis, further strengthening the hypothesis that the lack of drastic phenotype following *Prnp* invalidation is the consequence of a biological redundancy with a related gene, *Sprn*. Restricting *Sprn* downregulation to the trophoblastic lineage by infecting eggs at the blastocyst stage [56] allowed circumscribing the origin of the lethal phenotype to a trophectoderm-derived compartment developmental failure, histologically found to more specifically affect the ectoplacental cone [55]. Because in mouse embryo, trophectoderm is the first differentiated tissue to form with cells needing complex adhesive structures, this phenotype is reminiscent of that of PrP1-knockdown Zebrafish. Unfortunately, it precludes to formally assess if such double-invalidated mouse embryos would also suffer from neuronal developmental defects, as suggested by the failure of closure of the cranial tube [54], similarly to PrP2-knockdown zebrafish (Fig. 1).

Transcriptomic analyses by RNAseq were performed on FVB/N *Sprn*-downregulated mouse embryos at E6.5 and E7.5. The differential expression of 58 and 54 transcripts, respectively, was reported [55]. Compared to their PrP^{KO} counterpart, it revealed that Sho and PrP are involved in similar biological functions and suggested a convergent molecular response of mouse embryos to the absence of Sho or PrP. Some of the differentially expressed genes emphasize the role of this protein in the development of the trophectoderm-derived compartment with, for example, the deregulation of prolactin-related genes known to be specifically expressed in cells of the ectoplacental cone [57] at locations compatible with that recently reported for *Sprn* [58].

Open questions

Comparative analysis of knockdown experiments in Zebrafish and Mammals (see above) suggests that the biological functions of the teleost PrP1 and PrP2 proteins are, at least in mice, shared by PrP and Sho. Because the spatial expression pattern of Sho appears to be less ubiquitous compared to that of PrP, it would suggest that in mammals this required biological redundancy between PrP and Sho to avoid the appearance of a lethal phenotype in PrP^{KO} animals, as observed in Zebrafish, is restricted to a subset of cells that could be identified by *Sprn* expression. It also opens several yet unanswered questions:

i) What is the expression profile and role of the two *Sprn* genes in Zebrafish?

ii) What is the precise expression pattern of *Sprn* in the developing mouse embryo?

iii) Can mouse or teleost *Sprn* rescue PrP1 or PrP2 knockdown in Zebrafish?

iv) When did the biological redundancy between PrP and Sho started at an evolutionary scale? Is it related to the acquisition of biological properties and/or to some modifications of the expression profiles?

Currently, the analysis of a potential PrP and Sho complementary action on embryonic development in mice has not been assessed due to the lethal phenotype associated with the trophectoderm developmental failure. Several strategies could be used to circumvent it, such as a specific expression of a recombinant Sho in the trophectoderm either by classical transgenesis or lentiviral specific-delivery. Such experiments will allow verifying whether such double knockout embryos can survive or will suffer from lethal developmental defects as observed in PrP2-knockdown Zebrafish.

Dpl is not expressed in Zebrafish. The biological properties of this protein have been studied so far mainly in the male mammalian gonads, a tissue where *Prnd* is the most highly expressed in adults, and in the central nervous system due to its neurotoxic effects following its ectopic deregulation in some PrP^{KO} mouse lines. As mentioned above, *Prnd* is expressed in embryonic stem cells [21] and we have evidences for its expression in both embryonic and extra-embryonic tissues at early developmental stages (our unpublished observation). Although the double knockout of *Prnp* and *Prnd* in mice did not apparently affect the development of the embryos, the expression pattern of these gene remains largely unknown as is the potential impact of its invalidation in the embryonic development of *Sprn*- and *Sprn*/*Prnp* knockout embryos. We are currently investigating some of these points.

CONCLUSION

Early studies based on expression pattern analyses suggested the implication of PrP in embryonic development [16-18]. However, the gene invalidation in several mammalian species did not confirm it. Only recently, invalidation of PrP in Zebrafish and of PrP and Sho in mice revealed that this protein family is indeed crucial for early embryogenesis [40, 54]. These data open a new investigation field aiming at deciphering the precise role of these proteins at these early embryonic stages, their biological redundancy and its evolutionary appearance. It will benefit from the use of distantly related animal models and the emergence of new tools to specifically target genomic modifications. It may contribute also to better understand the role PrP in several pathologies that include not only TSE but also potentially other neurological diseases [59-61], placental defects [62] and carcinomas [36, 37 for recent reviews].

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