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Generation of *Sprn*-regulated reporter mice reveals gonadic spatial expression of the prion-like protein Shadoo in mice

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ABSTRACT

The protein Shadoo (Sho) is a paralogue of prion protein, and encoded by the gene *Sprn*. Like prion protein it is primarily expressed in central nervous system, and has been shown to have a similar expression pattern in certain regions of the brain. We have generated reporter mice carrying a transgene encompassing the *Sprn* promoter, exon 1, intron 1 and the 5′-end of exon 2 driving expression of either the LacZ or GFP reporter gene to study the expression profile of Shadoo in mice. Expression of the reporter genes was analysed in brains of these transgenic mice and was shown to mimic that of the endogenous gene expression, previously described by Watts et al. [1]. Consequently, the *Sprn*-LacZ mice were used to study the spatial expression of Sho in other tissues of the adult mouse. Several tissues were collected and stained for β-gal activity, including the thymus, heart, lung, liver, kidney, spleen, intestine, muscle, and gonads. From this array of tissues, the transgene was consistently expressed only in specific cell types of the testicle and ovary, suggesting a role for Shadoo in fertility and reproduction. These mice may serve as a useful tool in deciphering the regulation of the prion-like gene *Sprn* and thus, indirectly, of the Shadoo protein.

1. Introduction

The prion protein family consists of three proteins; Prion, Doppel, and Shadoo. Expression profiles of both Prion and Doppel have been characterized in several species including mouse, human, goat, and sheep, however little has been reported on the expression of *Sprn*. Prion (PrP^c) is a ubiquitously expressed protein, with a high expression level in the brain. The function of this GPI-anchored protein is not yet clear, as *Prnp*-knockout mice [2,3], cattle [4] and goat [5] suffer from no drastic phenotype. An abnormal form of the prion protein, PrP^{Sc}, is the infectious agent in prion diseases such as Bovine Spongiform Encephalopathy, Creutzfeldt–Jakob Disease, Scrapie and Chronic Wasting Disease. Various roles have been proposed for PrP in neuroprotection, cellular homeostasis, response to oxidative stress, cell proliferation and differentiation, synaptic function and signal transduction [6–9].

Doppel is highly expressed in the testis, and to a lesser extent the ovaries and spleen. Knockout of the gene encoding Doppel (*Prnd*) resulted in male sterility [10,11], both studies performed resulted in phenotypes suggesting a role for Doppel in spermatogenesis and/or male fertility. A role for Doppel in early sex differentiation in goats was also suggested, due to its expression pattern in testis and ovary at various developmental stages [12].

Shadoo expression has been less well characterized than that of Doppel and PrP. The *Sprn* gene was identified in 2003 by Premzl et al. [13]. Since then homology shared between the N-terminal of the Prion and Shadoo protein structures [14], and overlapping expression pattern in the brain [1] has raised the question of its ability to compensate for lack of PrP^c in *Prnp*-knockout animals [15].

Here we describe transgenic reporter mice generated using *Sprn*-regulatory sequences and either a green fluorescent protein (GFP) or LacZ reporter gene. These mice were used to analyse the expression profile of *Sprn* in the adult mouse.

2. Materials and methods

All animal manipulations were done according to the "French Commission de Génie Génétique" recommendations.

2.1. Generation of Sprn-GFP and Sprn-LacZ transgenic mice

A 5.3 kb genomic fragment including exon 1, intron 1 and the 5′-end of exon 2 of the mouse *Sprn* gene was amplified using the following primers: sense 5′-CAAGAGGATCTCTATAAACTCAAGGCTA-3′ and antisense 5′-AATCTGCGAGAAGAGGGTGGAA-3′. It was

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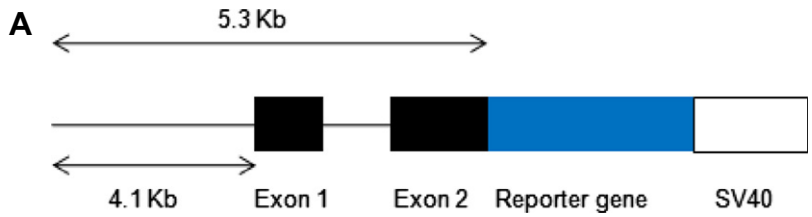
Table 1
Sequences of primers used for genotyping and RT-PCR analysis.

Gene	Forward primer	Reverse primer
Sprn-GFP	5′-ACATGAAGCAGCACGACTTCTT-3′	5′-TTCTGCTTGTCGGCCATGATATAGACGTT-3′
Sprn-LacZ	5′-GGTCCTAAACCACGCTCCAC-3′	5′-GTTTTCCCAGTCACGACGTTG-3′
Endogenous *Sprn*	5′-CAGTCGTGAGCTCTGCCTAA-3′	5′-GGAACAGCTGTCACAGAGGA-3′
Beta-actin	5′-CACCAGTCCGCCATGGATG-3′	5′-TCCCCACCATCACACCCTG-3′



B

	GFP	LacZ
Number of mice born	297	208
% Transgenic	35% (6)	33% (3)
% Lines expressing	100% (6/6)	100% (3/3)

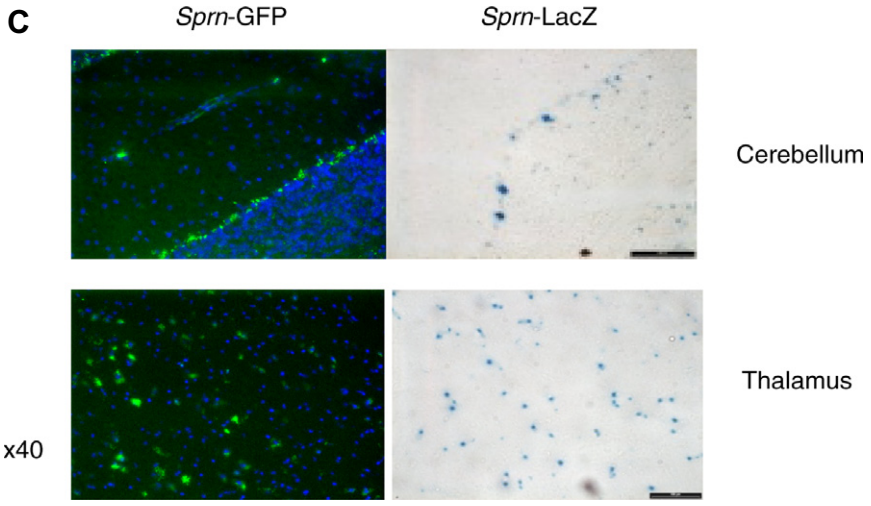


Fig. 1. Transgene structure, transmission and expression. (A) Two transgenes were made to report on *Sprn* gene expression in mice, one using a GFP reporter gene and the other using LacZ. In both cases 5.3 kb of the regulatory region of the *Sprn* gene was used. (B) The table above summarises the mouse lines established for both transgenes. (C) Brain expression profile of *Sprn*-GFP (green) and *Sprn*-LacZ (blue). Both transgenes gave the same profile of expression. Cells positive for the transgene resemble the Purkinje cells of the cerebellum and the neurons of the thalamus. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

inserted into either pEGFP-N1 vector or pCMVβ vector, to make the *Sprn*-GFP and *Sprn*-LacZ constructs respectively.

Recombinant vectors were digested with either *SalI-AflII* (*Sprn*-GFP) or *SalI* (*Sprn*-LacZ), gel-purified and micro-injected into FVB/N $Prnp^{0/0}$ oocytes [2,16]. Offspring were genotyped by PCR using the following primers: GFP sense 5′-ACATGAAGCAGCACGACTTCTT-3′, GFP antisense 5′-TTCTGCTTGTCGGCCATGATATAGACGTT-3′, or LacZ sense 5′-GGTCCTAAACCACGCTCCAC-3′, LacZ antisense 5′-GTTTTCCCAGTCACGACGTTG-3′.

2.2. Analysis of transgene expression

Three male and three female transgenic mice from each line (six GFP and three LacZ lines) were sacrificed by cervical dislocation. Three male and three female wild type mice were also used as negative controls. Tissues were collected for either cryosections or RNA extraction in PBS or liquid nitrogen respectively. *Sprn*-LacZ tissues were fixed in LacZ fix (0.2% gluteraldehyde, 5 mM EGTA, 100 mM $MgCl_2$, PBS) for 4 h then incubated overnight in 20% sucrose solution. *Sprn*-GFP tissues were incubated in 20% sucrose overnight without fixation. Tissues were mounted in Cryomount (Histolabs) at −25 °C in a cryostat, and then stored at −80 °C.

Ten micron cryosections of *Sprn*-GFP or *Sprn*-LacZ tissues were cut and collected on Superfrost slides (Thermo Scientific). *Sprn*-GFP sections were counterstained with DAPI using Vectorshield + DAPI (Vectorlabs). *Sprn*-LacZ sections were re-fixed for 10 min in LacZ fix, then washed in a detergent wash (2 mM $MgCl_2$, 0.01% sodium deoxycholate, 0.02% Igepal, PBS) 2 × 5 min, then