Short Communication

A Mutation of the *Prdm9* Mouse Hybrid Sterility Gene Carried by a Transgene

(*Prdm9* / transgene / meiosis)

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Abstract. PRDM9 is a protein with histone-3-methyltransferase activity, which specifies the sites of meiotic recombination in mammals. Deficiency of the *Prdm9* gene in the laboratory mouse results in complete arrest of the meiotic prophase of both sexes. Moreover, the combination of certain PRDM9 alleles from different mouse subspecies causes hybrid sterility, e.g., the male-specific meiotic arrest found in the (PWD/Ph × C57BL/6J)F1 animals. The fertility of all these mice can be rescued using a *Prdm9*-containing transgene. Here we characterized a transgene made from the clone RP24-346I22 that was expected to encompass the entire *Prdm9* gene. Both (PWD/Ph × C57BL/6J)F1 intersubspecific hybrid males and *Prdm9*-deficient laboratory mice of both sexes carrying this transgene remained sterile, suggesting that *Prdm9* inactivation occurred in the Tg(RP24-346I22) transgenics. Indeed, comparative qRT-PCR analysis of testicular RNAs from transgene-positive versus negative animals revealed similar expression levels of *Prdm9* mRNAs from the exons encoding the C-terminal part of the protein but elevated expression from the regions coding for the N-terminus of PRDM9, indicating that the transgenic carries a new null *Prdm9* allele. Two naturally occurring alternative *Prdm9* mRNA isoforms were overexpressed in Tg(RP24-346I22), one formed via splicing to a 3'-terminal exon consisting of short interspersed element B2 and one isoform including an alternative internal exon of 28 base pairs. However, the overexpression of these alternative transcripts was apparently insufficient for *Prdm9* function or for increasing the fertility of the hybrid males.

Introduction

Mammalian meiosis cannot be accomplished without the repair of programmed double-stranded DNA breaks by homologous recombination (Bolcun-Filas and Schimenti, 2012). The sites of these breaks are specified by histone-3-lysine-4-methyltransferase PRDM9 in the mouse, cattle, and man (Baudat et al., 2010; Parvanov et al., 2010; Pratto et al., 2014; Ma et al., 2015). The mouse *Prdm9* gene is necessary for successful meiosis in the laboratory mice, as their *Prdm9*$^{-/-}$ germ cells arrest in the meiotic prophase (Hayashi et al., 2005). *Prdm9* is haploinsufficient and its product may act as a multimer (Baker et al., 2015). *Prdm9* was identified with the Hybrid sterility 1 gene (*Hst1*) by positional cloning (Trachtulec and Forejt, 1999; Mihola et al., 2007, 2009; Trachtulec et al., 1994, 2008). *Hst1* causes meiotic arrest of spermatogenesis in the offspring of males of *Mus m. domesticus* origin (such as of the laboratory strain C57BL/6J, henceforth B6) crossed to females coming from *M. m. musculus* (e.g., of the PWD/Ph strain; Forejt and Ivanyi, 1974). The fertility of these hybrids can be rescued by transgenes carrying *Prdm9* (Flachs et al., 2012, 2014; Mihola et al., 2009). Curiously, variation in the *Prdm9* dosage is not accompanied by changes in global meiotic recombination rate (Balcova et al., 2016).

PRDM9 carries C-terminal DNA-binding C_2H_2 zinc-fingers that are variable and also differ among the *Hst1* alleles (Mihola et al., 2009; Forejt et al., 2012). The PRDM9 protein also harbours the N-terminal KRAB domain of unknown function and the internal PR/SET histone-3-methyltransferase domain. Mouse *Prdm9* is alternatively spliced (Hayashi et al., 2005; Mihola et al., 2009), but the function of the alternative isoforms has been unknown. Two C-terminus-truncating alleles of

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Abbreviations: B6 – C57BL/6J, BW – body weight, *Hst1* – Hybrid sterility 1 gene, PRDM9 – PR-domain 9, PWD – PWD/Ph, qRT-PCR – quantitative reverse-transcription polymerase chain reaction, SC – sperm count, SPF – specific pathogen-free, TW – testis weight.

Prdm9 have been obtained (Fairfield et al., 2011; Weiss et al., 2012), but have not been tested for their effect on the fertility of hybrids. Here we show that overexpression of a C-terminus-truncating allele and two alternative isoforms of *Prdm9* does not affect the fertility parameters of *Prdm9*-deficient laboratory mice or (PWD × B6)F1 intersubspecific hybrid males.

Ethical guidelines statement

The European Community Council Directive 86/609/EEC, Appendix A of the Council of Europe Convention ETS123, and the Czech Republic Act for Experimental Work with Animals (Decree No. 207/2004 Sb, and the Acts Nos. 246/92 Sb and 77/2004 Sb) were applied to laboratory animal care and experiments.

Material and Methods

Mice

The PWD/Ph strain was established in Prague and reviewed in Gregorová and Forejt (2000); the C57BL/6J strain originated from The Jackson Laboratory (stock 000664). The transgenic Tg(RP24-346I22) was described by Howell et al. (2005). The null allele *Prdm9*^{tm1Ymat} (abbreviated *Prdm9*^{tm}) was generated by replacement of the first five coding exons with LacZ (Hayashi et al., 2005). All mice were kept in the specific pathogen-free (SPF) barrier facility of the Institute of Molecular Genetics ASCR in Prague in described conditions (Flachs et al., 2014).

Genotyping and expression analysis

The genotyping conditions have been published (Howell et al., 2005; Mihola et al., 2009; Flachs et al., 2012, 2014). Total testicular RNAs were used to quantify mRNA expression levels by real-time qRT-PCR exactly as done previously (Mihola et al., 2009; Flachs et al., 2012).

Results and Discussion

To refine the effect of the increased number of *Prdm9* copies on hybrid sterility, the fertility parameters of 9-week-old (PWD × B6)F1 males carrying bacterial artificial chromosome RP24-346I22 were analysed. This transgenic was previously used to rescue the *t* haplolethal 1 (*Thl1*) gene (Howell et al., 2005) and overlaps the *Prdm9* gene. No increase of fertility was observed when transgenics were compared to (PWD × B6)F1 littermates (Table 1, lines 1, 2). These results contrast with the fertility of (PWD × B6)F1 males harbouring *Prdm9* transgenes characterized previously (Mihola et al., 2009), e.g., Tg(CHORI34-255E14) also shown in Table 1 (line 3). Because some intersubspecific hybrids display delayed fertility (Campbell and Nachmann 2014; Flachs et al., 2014), aged (PWD × B6)F1-Tg(RP24-346I22) males were also phenotyped (Table 1, lines 4, 5); however, no fertility rescue was found.

To find out whether the transgenic received an intact *Prdm9*, a complementation test was performed. To this end, 52 offspring from the intercrosses of laboratory mice carrying both a *Prdm9* null allele (*Prdm9*^{tm}) and Tg(RP24-346I22) were generated and genotyped. Five *Prdm9*$^{tm/tm}$ animals carrying the transgene were obtained, three males and two females. The three *Prdm9*$^{tm/tm}$ transgenic males displayed very small testicles and no sperm, similarly as the non-transgenic controls (Table 1, lines 6, 7). In addition, none of the two *Prdm9*$^{tm/tm}$ females with RP24-346I22 produced offspring during 70 days of mating. The combined fertility data suggest that *Prdm9* was inactivated during the preparation of this transgenic.

To further support this conclusion and to reveal the site of the rearrangement, qRT-PCR analysis of testicular RNAs from wild-type and RP24-346I22 positive animals as well as from *Prdm9*$^{tm/tm}$ males with and without the transgene was performed. Twelve primers from various *Prdm9* constitutive and alternative exons were used (Fig. 1). Primers from the KRAB-domain-encoding exons and from two alternative isoforms detected ele-

Table 1. The effect of RP24-346I22 transgene on male mouse fertility

Genotype	Age (w)	N	TW (mg)	TW/BW (mg/g)	SC (million)	OFM
(PWD × B6;129P2)F1	9 ± 0.2	9	60 ± 6	2.8 ± 0.2	0.0 ± 0.0	N. d.
(PWD × B6;129P2)F1-Tg(346I22)	9 ± 0.2	9	63 ± 6	2.6 ± 0.3	0.0 ± 0.0	N. d.
(PWD × B6;129P2)F1-Tg(255E14)	9 ± 0.2	5	171 ± 12	7.3 ± 0.4	2.9 ± 1.1	7 ± 1 (7)
(PWD × B6;129P2)F1	18 ± 1	5	62 ± 2	2.2 ± 0.2	0.0 ± 0.0	N. d.
(PWD × B6;129P2)F1-Tg(346I22)	18 ± 1	3	62 ± 2	1.9 ± 0.1	0.0 ± 0.0	0 ± 0 (3)
B6;129P2-*Prdm9*$^{tm/tm}$ Tg(346I22)	17 ± 2	3	61 ± 6	1.9 ± 0.1	0.0 ± 0.0	0 (2)
B6;129P2-*Prdm9*$^{tm/tm}$	17 ± 3	8	54 ± 4	2.1 ± 0.1	0.0 ± 0.0	N. d.

Prdm9^{tm}, the null allele *Prdm9*^{tm1Ymat}; Tg(255E14), transgenic Tg(CHORI34-255E14) that rescues hybrid fertility (Mihola et al., 2009); Tg(346I22), Tg(RP24-346I22) transgene; B6;129, genetic background mixed from B6 and 129P2/Ola (both carrying the same *Prdm9* allele).

W – weeks, N – number of males, TW – mean weight of paired testicles (mg ± standard deviation), TW/BW – relative testis weight in mg of TW per gram of body weight (BW), SC – sperm count (millions) in caput epididymis, OFM – offspring per female per month (for N males), N. d. – not determined.

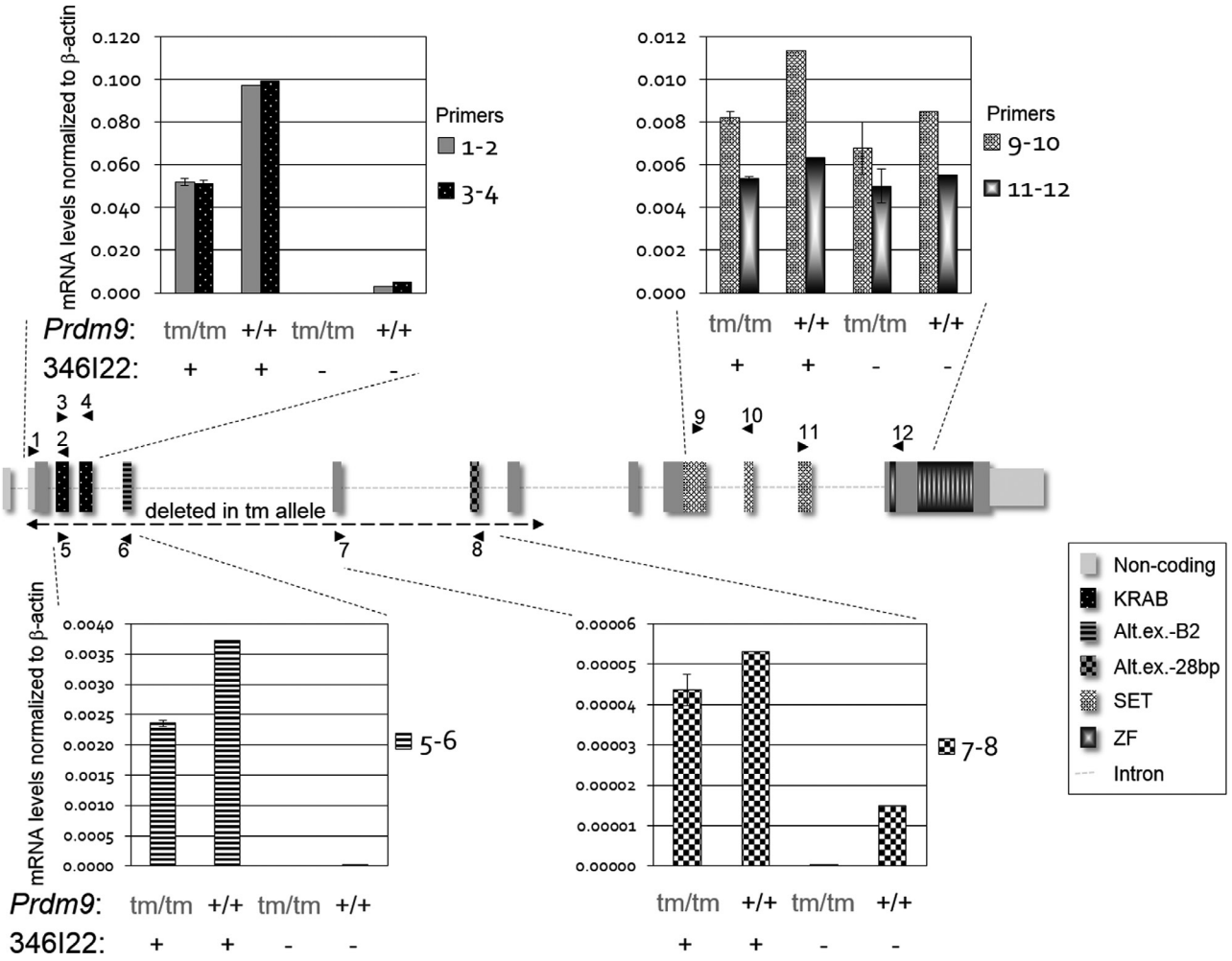

Fig. 1. The expression analysis of *Prdm9* transcripts in adult Tg(RP24-346I22) and control testes by qRT-PCR. Three transgenic and three control males were used. The middle part of the picture depicts the exon-intron structure of *Prdm9* (not to scale); the arrowheads symbolize qRT-PCR primers. The two upper graphs show expression from constitutive exons and the two lower graphs expression of natural alternative isoforms (all y-axes in arbitrary units). Alt.ex.-B2, alternative 3'-exon formed by short interspersed repeat element B2; Alt.ex.-28bp, alternative internal exon of 28 base pairs (putatively causing a frame-shift); KRAB, SET, ZF, exons encoding the KRAB, PR/SET, and zinc-finger domains, respectively; tm, the null allele (targeted mutation) *Prdm9*^{tm1Ymat}.

vated expression in Tg(RP24-346I22) animals, also showing the presence of the *Prdm9* regulatory sequences in the transgene. However, similar expression levels of *Prdm9* mRNA in non- and transgenic testes were uncovered using primers from the exons encoding the internal PR/SET and C-terminal Zn-finger domains (Fig. 1). The expression data thus support the conclusion that the Tg(RP24-346I22) mice carry a null *Prdm9* allele. This allele therefore allowed us to assay for the function of two alternative mRNA isoforms overexpressed in these animals (Fig. 1, Table 1). Their overexpression failed to rescue the fertility of hybrids and of *Prdm9*$^{-/-}$ laboratory mice. Our results, namely the requirement of the C-terminal part of PRDM9 for hybrid fertility rescue, are in agreement with the finding that humanization of the C-terminal Zn-finger domains affects F1 hybrid sterility (Davies et al., 2016).

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