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Primary spermatocyte-specific Cre recombinase activity in transgenic mice

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Abstract

We have evaluated the specificity of Cre recombinase activity in transgenic mice expressing Cre under the control of the synatonemal complex protein 1 (*Sycp1*) gene promoter. *Sycp1Cre* mice were crossed with the ROSA26 reporter line *R26R*, to monitor the male germ cell stage-specificity of Cre activity as well as to verify that Cre was not active previously during development of other tissues. X-gal staining detected Cre-mediated recombination only in testis. Detailed histological examination indicated that weak Cre-mediated recombination occurred as early as in zygotene spermatocytes at stage XI of the cycle of the seminiferous epithelium. Robust expression of X-gal was detected in early to mid-late spermatocytes at stages V–VIII. We conclude that this transgenic line is a powerful tool for deleting genes of interest specifically during male meiosis.

Keywords

Cre recombinase; *Sycp1Cre*; ROSA26; primary spermatocytes

Introduction

Spermatogenesis is a complex developmental process involving mitotic proliferation, meiosis, and differentiation of spermatids to spermatozoa. Meiosis includes the reduction of ploidy, introduction of diversity by homologous recombination, and maintenance of genomic integrity. Although meiosis plays a crucial role in sexual reproduction, the molecular mechanisms that control the entry and progression of germ cells into their differentiation pathway are poorly defined, particularly in higher organisms. Targeted mutagenesis in embryonic stem cells has identified genes required for spermatogenesis

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(reviewed in Eddy, 1999), however, unequivocal evaluation of their function has frequently been impeded due to embryonic lethality or other physiological consequences of the null mutation.

The Cre/loxP approach has been used to overcome these limitations in a variety of developmental systems (reviewed in Nagy, 2000). Insertion of loxP sites into a gene of interest and targeting Cre recombinase expression to specific cell types using a cell/tissue-specific promoter makes it possible to introduce cell/tissue-restricted or conditional mutations. Conditional gene disruption only in the testis or at particular stages during spermatogenic differentiation will be very useful to dissect the molecular pathways involved in spermatogenesis.

Promoters of several genes active in early male meiotic cells, such as *Hsp70-2* (Dix et al., 1996), *Pdha-2* (Iannello et al., 1997), *Spo11* (Baudat et al., 2000), and *Sycp1* (Vidal et al., 1998; Sage et al., 1999), have been characterized in transgenic mice.

Sycp1 encodes synaptonemal complex protein 1, a major component of the central element of the synaptonemal complex. It is present exclusively from the beginning of the zygotene up to the diplotene stage, in both male and female gonads (Heyting et al., 1989; Offenberg et al., 1991; Dietrich et al., 1992; Meuwissen et al., 1992; Moens et al., 1992; Dobson et al., 1994). Sequences within the 5′ proximal 260 bp of mouse *Sycp1* are sufficient to direct the expression of a *lacZ* transgene (*Sycp1lacZ*) in the testis, but are not active in the ovary (Sage et al., 1999). Transgenic mice expressing Cre recombinase driven by this *Sycp1* promoter have been generated and crossed with mice carrying a *RXR*α gene containing loxP sites (Vidal et al., 1998). RT-PCR analysis of the putatively recombined eighth intron of the *RXR*α gene revealed efficient and spermatocyte-specific Cre-mediated excision (Vidal et al., 1998). However, expression was neither examined in tissues other than testis nor was the cellular specificity of expression in the testis examined in detail (Vidal et al., 1998; Rassoulzadegan et al., 2002). In the present study, the reporter transgenic lines *R26R* was used for monitoring the tissue, cellular and temporal specificity of transgenic mice carrying the *Sycp1Cre* construct.

Methods, results and discussion

To determine the tissue and cellular specificity of *Sycp1*-driven Cre recombinase activity *in vivo*, the *Sycp1Cre* transgenic line (Vidal et al., 1998) was crossed with the reporter line *R26R* mice (Soriano, 1999). *R26R* carries a copy of *lacZ* into which loxP sites have been inserted such that no functional β-galactosidase (β-gal) is made; excision of the loxP sites by Cre restores β-gal production (Zambrowicz et al., 1997; Soriano, 1999). The reporter is ubiquitously expressed, including in male germ cells, which makes it a useful model system for examining the specificity of the *Sycp1Cre* transgene. β-Gal activity resulting from Cre-mediated recombination of the *R26R* locus was monitored by X-gal staining. Male progeny from *Sycp1Cre* and *R26R* cross-breedings were collected, tail DNA was prepared using the DNeasy Tissue Kit (Qiagen, CA), and subjected to PCR analysis for the presence of Cre according to Vidal and colleagues (Vidal et al., 1998) or for *lacZ* using primers ROSA26-I, II, III (Soriano, 1999).

Among the tissues examined, Cre activity in the *R26RSycp1Cre* mice was restricted to testis. Figure 1 shows a typical X-gal staining pattern in various selected organs (Figures 1A–J), including brain, heart, lung, liver, intestine, thymus, kidney, spleen, thyroid, and testis. Slight X-gal staining was detected in the thyroid (Figure 1I), likely because of endogeneous β-gal activity. Histological testicular sections were counterstained with neutral red and tubules were staged to determine the developmental and cellular specificity of appearance of Cre recombinase activity (Figure 1K–N). Very weak Cre-mediated

recombination occurred as early as in zygotene spermatocytes at stage XI (Figure 1N). As meiotic prophase progressed, β-gal was detected robustly in early to mid-late spermatocytes at stage V–VIII (Figures 1K and L, respectively). The recombined β-gal protein was apparently not turned over, since X-gal staining was detected in stages as late as step 16 spermatids (Figure 1K–N). In contrast, β-gal expression was not detected in any other testicular cell type within tubules or interstitial regions.

As presented in Table 1, several examples of Cre expression targeted to germ cells have been reported. However, ectopic Cre expression was observed in many of these mouse strains. Cre driven by 450-bp of the *Pgk2* promoter, which in adult mice accurately targets Cre recombinase to male germ cells, is subject to ectopic expression during embryonic development (Bhullar et al., 2001). A larger 1.4-k bp *Pgk2* fragment was reported to be specific to spermatocytes and spermatids (Ando et al., 2000), although only liver, kidney and brain of the *Pgk2CreCagCatZ* mice were examined. In *c-kitCre* transgenic mice, recombination of a floxed *Neo* gene was found in several tissues in which *c-kit* is not known to be expressed, likely resulting from an excision event in embryogenesis (Bergqvist et al., 1998). Ectopic Cre recombination had not been examined in detail for S*ycp1Cre* mice, but it had been noted that the endogenous *Sycp1* gene is transiently active at the 2- to 4-cell stage during embryogenesis (Vidal et al., 1998). This raised the possibility that the lack of evidence for recombined alleles in various tissues in *Sycp1Cre* mice may be due to the method used to detect recombination events (genotyping of remaining floxed alleles) or the limited number of tissues examined (Vidal et al., 1998; Rassoulzadegan et al., 2002). Our current detailed examination of the various selected organs in *R26RSycp1Cre* mice by X-gal staining clearly illustrate the cellular and tissue specificity of Cre recombinase activity driven by *Sycp1* promoter as well as a lack of effect of any Cre activity in early embryos, which would have been expected to donate recombined alleles to virtually all tissues.

The induction of Cre expression in mouse cells is clearly an artificial situation, but is generally believed not to affect gene expression other than that of the floxed gene of interest. However, illegitimate, Cre-dependent chromosome arrangements in transgenic mouse spermatids and resulting sterility have been reported in mice carrying a transgene consisting of 4.1 kb promoter fragment of *Prm1* driving *Cre* (Schmidt et al., 2000). Whether this reflects the vulnerable nature of DNA in spermatids to Cre-mediated reactions is not clear (Schmidt et al., 2000). However, mice generated from a truncated form of the *Prm1* promoter (652 bp) driving Cre recombinase gene were fully fertile (O'Gorman et al., 1997). Further, *PrmCre* males can efficiently recombine a floxed RNA polymerase II locus *P2Bc* (Pol II, β-gal, conditional) in the male germ line of mice (O'Gorman et al., 1997).

In *R26RSycp1Cre* mice, loxP recombination fell to very low levels in the second generation of mice (Rassoulzadegan et al., 2002). Although Cre was still expressed during meiosis, recombination of remaining loxP sites was inhibited, putatively due to methylation of cytosines initiated within the loxP sequence and the surrounding chromosomal region (Rassoulzadegan et al., 2002). As such, *Sycp1Cre* males cannot be propagated with genomic loxP sites for successive generations without a rapid loss in recombination efficiency. The double transgenic *Sycp1Cre/loxP* genotypes could be maintained through the female germ line since the *Sycp1* promoter sequence from −722 to +102 relative to the transcription start is not active during female meiosis (Sage et al., 1999; Rassoulzadegan et al., 2002). Accordingly, loxP sites would remain intact when transferred through the female germ line. Alternatively, transgenic mice expressing the tamoxifen-inducible Cre-ER^{T} recombinase under the control of *Sycp1* promoter, in which the *Cre* transgene is not active unless in the presence of tamoxifen, could be generated. As the *Sycp1Cre* mouse line is currently the only available strain that targets Cre recombinase to male meiotic prophase cells, they provide

means for generating mutations during meiosis in spermatocytes, and thus, to define the role of specific genes in spermatogenesis at the zygotene to pachytene and later stages.

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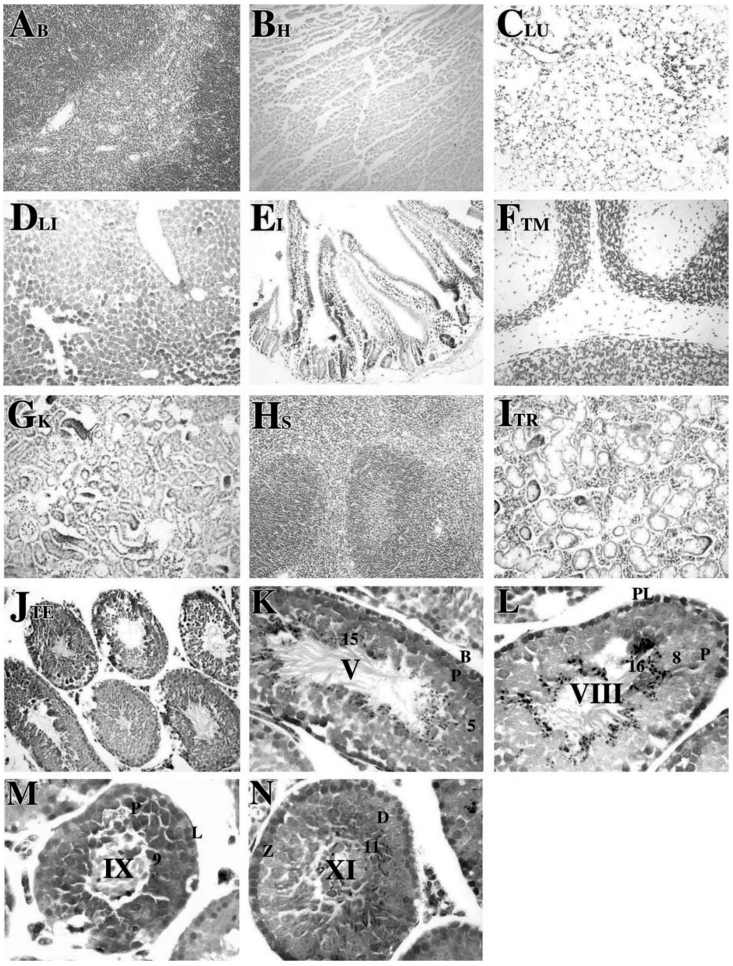

Figure 1.

Tissue-specific and spermatogenic cell-specific expression of β-gal following Cre recombination. Transgenic and non-transgenic control mice were euthanized, perfused with fixatives, and tissues were removed. The tissues were fixed again for 1 h at room temperature in 100 mM sodium phosphate, pH 7.3, containing 2% paraformaldehyde, 0.2% glutaraldehyde, 0.1% sodium deoxycholate, 0.2% Nonidet P-40, 5 mM EGTA and 2 mM $MgCl_2$. The tissues were incubated overnight at 30 °C in 100 mM sodium phosphate, pH 7.3, 1.3 mM $MgCl_2$, 3 mM $K_3Fe(CN)_6$, 3 mM $K_4Fe(CN)_6$ and 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) as described previously (Langford et al., 1991; Fire, 1992). The tissues were embedded in paraffin, sectioned at 5 μm, and mounted on Superfrost slides (Fisher Scientific, NJ). After X-gal staining, histological sections of brain (A), heart (B), lung (C), liver (D), intestine (E), thymus (F), kidney (G) spleen (H), thyroid (I), and testis (J) were obtained and counterstained with neutral red (Catalogue number N129, Fisher Scientific, NJ) according to standard procedures and viewed on a Nikon photomicroscope under bright-field optics. Photomicrographs were taken using a digital camera (Spot advanced software, Diagnostic Instruments, Inc.). Specific expression of β-galactosidase following Cre recombination was observed only in testis (J). Magnification: ×20. Histological testicular sections from *R26RSycp1Cre* males were examined at higher magnification (K–N, ×60) to determine cell-specific expression of Cre as detected by X-gal staining. β-gal activity was detected in zygotene spermatocytes at stage XI (N) as very weak signals. The expression was detected continuously from early primary spermatocytes to mid-late pachytene spermatocytes at stage V, VIII (K and L, respectively)

to step 1–16 spermatids (K–N). β-gal activity was not detected in Sertoli cells nor in interstitial cells. Abbreviations: B, type B spermatogonia; PL, preleptotene spermatocytes; L, leptotene spermatocytes; Z, zygotene spermatocytes; D, diplotene spermatocytes; P, pachytene spermatocytes; arabic numerals, the step of elongated spermatid; Roman numerals indicated the stage of the seminiferous tubules, staged as described by Russell et al. (1990).

Table 1
Summary of mouse strains carrying Cre expression targeted to male germ cells

Mouse strain	Target cells	References
Prm1-Cre	Haploid spermatid	O'Gorman et al. (1997)
	Haploid spermatid, sterile	Schmidt et al. (2000)
c-Kit-Cre	Germ cells; ubiquitous deletion during embryogenesis	Bergqvist et al. (1998)
Sycp1-Cre	Primary spermatocytes	Vidal et al. (1998)
Pgk2-Cre	Spermatocytes	Ando et al. (2000)
	Spermatocytes; Ectopic expression during embryogenesis	Bhullar et al. (2001)
TNAP-Cre	Primordial germ cells	Lomeli et al. (2000)
PGK-1-Cre	Diploid primordial germ cells	Lallemand et al. (1998)