genesis 32:184–186 (2002)
DOI 10.1002/gene.10039

Flp Recombinase Regulated *lacZ* Expression at the *ROSA26* Locus

Richard Possemato,[1,2] Kevin Eggan,[1,2] Benjamin J. Moeller,[1,2] Rudolf Jaenisch,[1,2] and Laurie Jackson-Grusby[1*]

[1]Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
[2]Massachusetts Institute of Technology, Cambridge, Massachusetts

Received 19 December 2001; Accepted 21 December 2001

Manipulation of gene expression patterns through gene targeting in mouse embryonic stem (ES) cells (Thomas and Capecchi, 1987) and the more recent advent of conditional mutagenesis has revolutionized mouse genetics. The recombinase system most commonly used for conditional alleles in mouse is Cre/*loxP* derived from bacteriophage P1 (Sauer and Henderson, 1988). While less utilized, FLP/FRT derived from *S. cerevisiae* (O'Gorman *et al.*, 1991) has been elegantly used by Martin and colleagues in combination with Cre to create a series of alleles for *Fgf8* of varying strength (Meyers *et al.*, 1998). The utility of Flp as a robust tool for mammalian genetics has been markedly improved by Stewart and colleagues who cleverly used cycling mutagenesis to select for variant alleles of Flp that carry out recombination at high efficiency in mammalian cells (Buchholz *et al.*, 1998). Experimental strategies to sequentially inactivate and reactivate a given gene, or to test epistatic relationships between genes in somatic cells, are now possible using a combination of Cre with the enhanced Flpe.

As Cre and Flp transgenes used to regulate conditional alleles may not recapitulate the expected pattern of expression (e.g., Tallquist and Soriano, 2000), it is essential to characterize the activity of these recombinase alleles to correctly interpret the phenotypes of conditionally mutant animals. Whereas several reporter lines for Cre have been described, we have found the allele created by Soriano in which Cre regulates lacZ expression at the ROSA26 locus to be especially useful (Soriano, 1999). ROSA26 insertions are ubiquitously expressed and show no overt phenotype when homozygous (Zambrowicz *et al.*, 1997). We therefore created an analogous ROSA26 allele, which we term R26FR, to monitor Flp activity in vivo.

The targeting vector contains an FRT-flanked neomycin resistance gene with four transcriptional stop signals placed upstream of a β-galactosidase-neo fusion (β-geo; Friedrich and Soriano, 1991) all inserted into ROSA26 genomic sequence (Fig. 1a). The vector was transfected into V6.5 ES cells (Eggan *et al.*, 2001), 48 clones were analyzed by Southern blotting, and 24 correctly targeted clones were identified (Fig. 1b). To demonstrate that the R26FR clones could activate β-geo, a CMV-FLPe expression plasmid (Buchholz *et al.*, 1998) was introduced by transient lipofection. One hundred ninety-two clones were picked and aliquots analyzed for β-galactosidase activity by X-gal staining. Both Flpe-recombined (Fig. 2a) and parental clones (Fig. 2d) were identified, and absence or presence of the stop cassette was confirmed by Southern blotting (Fig. 1b).

To create the reporter mouse strain, completely ES-derived mice (Eggan *et al.*, 2001; Nagy *et al.*, 1990) were made by injecting targeted clones into tetraploid blastocysts. Four viable pups were produced, and two were used to derive tail fibroblast cell lines. To demonstrate functionality of the reporter in somatic cells, Flpe was cloned into an MSCV-IRES-GFP retroviral vector (Van Parijs *et al.*, 1999) and used to infect the transformed reporter cell lines. Cells sorted for and against GFP expression were enriched by FACS and compared with those infected with an antisense Flpe retroviral control vector. Sorted cells were stained with X-gal and analyzed by Southern blotting, which demonstrated conditional recombination (Fig. 1b) and β-galactosidase activity (Fig. 2b) dependent upon Flpe. To confirm activity of the reporter allele in vivo, R26FR mice were crossed to a β-actin-Flpe transgenic mouse (Rodriguez *et al.*, 2000). Embryos dissected at day 10.5 pc were stained with X-gal and showed ubiquitous Flp-mediated activation (Fig. 2c).

The purpose of this FLP reporter allele is to specify the extent of FLP activity with a convenient and widely used enzymatic activity, β-galactosidase. Development of FLP/FRT tools such as our R26FR reporter allele and the recently described alkaline phosphatase reporter (Awatramani *et al.*, 2001) improve the ability to characterize Flpe alleles in vivo and therefore expand the opportunities for using precisely regulated dual recombinase experimental strategies.

* Correspondence to: Laurie Jackson-Grusby, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.
E-mail: jackson@wi.mit.edu

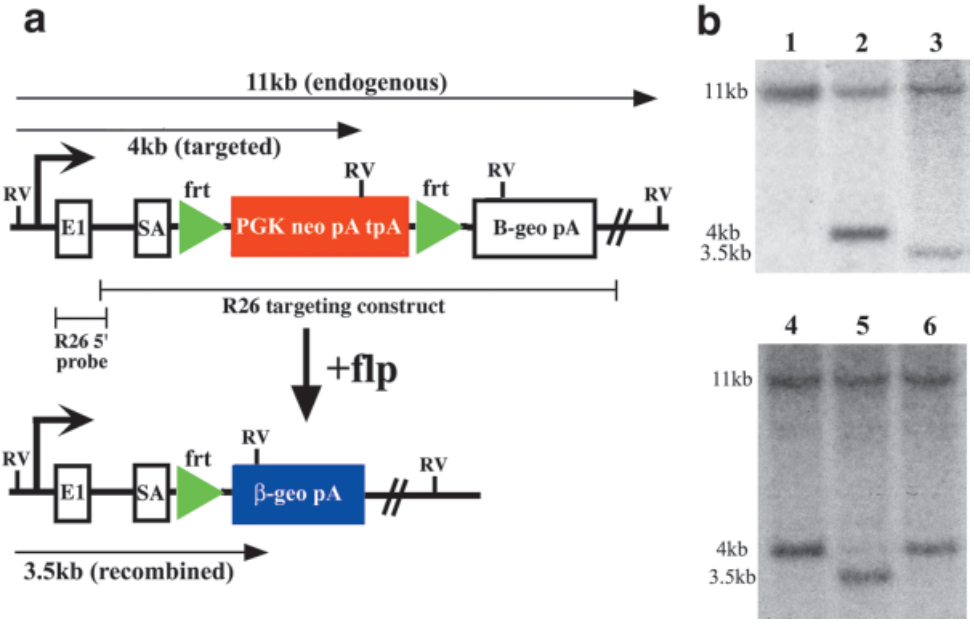

FIG. 1. (**a**) R26FR targeted allele and activation of β-geo by Flp. RV, EcoRV; SA, Splice acceptor; pA, polyadenylation sites; tpA, triple polyadenlyation sites. (**b**) Southern blot to confirm targeting of the ROSA26 locus with the R26FR vector and deletion of the stop sequence upon Flp-mediated recombination. Genotypes by lane: (1) wild-type V6.5 ES cells; (2) R26FR/+; (3) R26FR/+ after Flp-mediated recombination; (4) R26FR/+ tail fibroblasts; (5) R26FR/+ tail fibroblasts infected with Flpe retrovirus (MFIG); (6) R26FR/+ tail fibroblasts infected with antisense Flpe retrovirus. Methods: The R26FR targeting construct was made by creating an FRT flanked neo selectable marker (pFNtpa) that was inserted along with a splice acceptor-β-geo-polyA cassette into the ROSA26 targeting vector (kindly provided by P. Soriano). The vector was linearized with SacII, introduced into V6.5 ES cells by electroporation, and cells were selected with 200 μg/mL G418. Clone DNAs were analyzed for targeting using the R26 5′ probe as described (Soriano, 1999). Targeted clones were lipofected with CMV-Flpe (kindly provided by F. Stewart) using Cytofectene (Biorad). Mice were generated by tetraploid embryo complementation as described (Eggan *et al.*, 2001). Primary tail fibroblasts were isolated from newborn pups and transformed by infection with an SV40 large T antigen retrovirus (Jat *et al.*, 1986). Flpe sequences were subcloned into MSCV-IRES-GFP (Van Parijs *et al.*, 1999), and Flpe retrovirus (MFIG) was produced by transfection into 293 cells as described (Pear *et al.*, 1993).

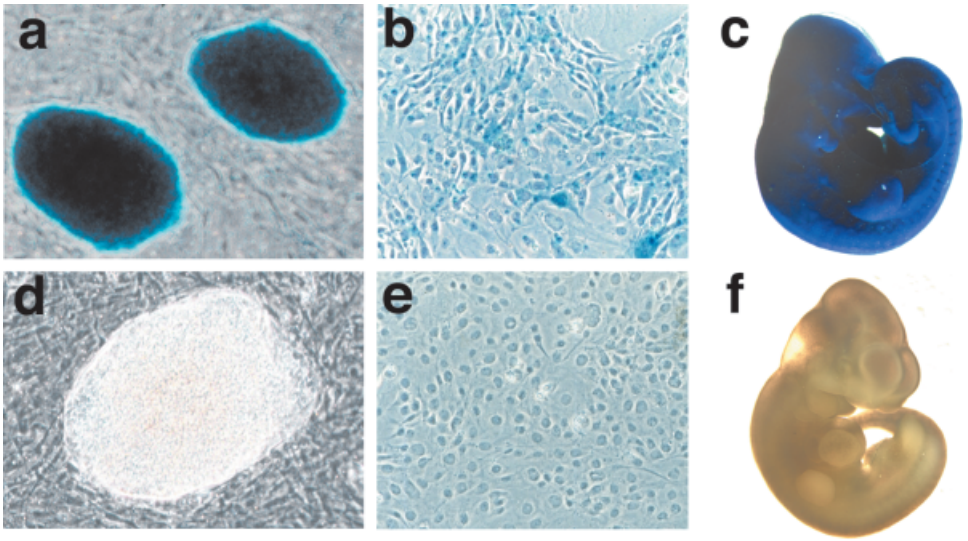

FIG. 2. Conditional β-galactosidase expression caused by Flpe-mediated recombination. (**a**) R26FR ES cells after transient expression of CMV-Flpe; (**b**) R26FR tail fibroblasts expressing MFIG; (**c**) R26FR day 10.5 embryo expressing β-actin-Flpe (kindly provided by S. Dymecki); (**d**) R26FR ES cells; (**e**) R26FR tail fibroblasts expressing antisense Flpe; (**f**) control R26FR day 10.5 embryo. Methods: Cells and embryos were fixed with 4% paraformaldehyde, washed with phosphate-buffered saline (PBS), and incubated in X-gal stain (PBS containing 5 mM ferricyanide, 5 mM ferrocyanide, 1 mg/mL X-gal, 1 mM $MgCl_2$) overnight at 37°C to monitor β-galactosidase expression.

LITERATURE CITED

Awatramani R, Soriano P, Mai JJ, Dymecki S. 2001. An Flp indicator mouse expressing alkaline phosphatase from the ROSA26 locus. Nat Genet 29:257–259.

Buchholz F, Angrand PO, Stewart AF. 1998. Improved properties of FLP recombinase evolved by cycling mutagenesis. Nat Biotechnol 16:657–662.

Eggan K, Akutsu H, Loring J, Jackson-Grusby L, Klemm M, Rideout WM III, Yanagimachi R, Jaenisch R. 2001. Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation. Proc Natl Acad Sci U S A 98:6209–6214.

Friedrich G, Soriano P. 1991. Promoter traps in embryonic stem cells: a genetic screen to identify and mutate developmental genes in mice. Genes Dev 5:1513–1523.

Jat PS, Cepko CL, Mulligan RC, Sharp PA. 1986. Recombinant retroviruses encoding simian virus 40 large T antigen and polyomavirus large and middle T antigens. Mol Cell Biol 6:1204–1217.

Meyers EN, Lewandoski M, Martin GR. 1998. An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. Nat Genet 18:136–141.

Nagy A, Gocza E, Diaz EM, Prideaux VR, Ivanyi E, Markkula M, Rossant J. 1990. Embryonic stem cells alone are able to support fetal development in the mouse. Development 110:815–821.

O'Gorman S, Fox DT, Wahl GM. 1991. Recombinase-mediated gene activation and site-specific integration in mammalian cells. Science 251:1351–1355.

Pear WS, Nolan GP, Scott ML, Baltimore D. 1993. Production of high-titer helper-free retroviruses by transient transfection. Proc Natl Acad Sci U S A 90:8392–8396.

Rodriguez CI, Buchholz F, Galloway J, Sequerra R, Kasper J, Ayala R, Stewart AF, Dymecki SM. 2000. High-efficiency deleter mice show that FLPe is an alternative to Cre- loxP [letter]. Nat Genet 25:139–140.

Sauer B, Henderson N. 1988. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. Proc Natl Acad Sci U S A 85:5166–5170.

Soriano P. 1999. Generalized lacZ expression with the ROSA26 Cre reporter strain [letter]. Nat Genet 21:70–71.

Tallquist MD, Soriano P. 2000. Epiblast-restricted Cre expression in MORE mice: a tool to distinguish embryonic vs. extra-embryonic gene function. genesis 26:113–115.

Thomas KR, Capecchi MR. 1987. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell 51:503–512.

Van Parijs L, Refaeli Y, Lord JD, Nelson BH, Abbas AK, Baltimore D. 1999. Uncoupling IL-2 signals that regulate T cell proliferation, survival, and Fas-mediated activation-induced cell death. Immunity 11:281–288.

Zambrowicz BP, Imamoto A, Fiering S, Herzenberg LA, Kerr WG, Soriano P. 1997. Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. Proc Natl Acad Sci U S A 94:3789–3794.