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A *cre*-transgenic mouse strain for the ubiquitous deletion of *loxP*-flanked gene segments including deletion in germ cells

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The gene targeting approach to study gene function in mice has been recently refined using the bacteriophage P1 Cre–*loxP* recombination system. Cre recombinase catalyses site-specific DNA recombination between 34 bp recognition (*loxP*) sites (1,2). If two *loxP* sites are introduced in the same orientation into a genomic locus, expression of Cre results in the deletion of the *loxP*-flanked DNA sequence. Cre-mediated recombination can be applied to various types of gene manipulation in embryonic stem (ES) cells including the removal of a selectable marker gene accompanied by the introduction of subtle mutations (3), gene replacement (4) and the deletion of large genomic regions to inactivate genes or gene clusters (5). However, Cre-mediated gene manipulation requires transient transfection of the mutant, *loxP*-containing ES cells with a vector encoding Cre recombinase and an additional round of selection to screen for ES cell clones carrying the desired deletion. This additional manipulation of the cells in culture is unpleasing since it might affect the capacity of ES cells to contribute to the generation of germ cells in chimeric mice, which is a serious problem in gene targeting technology.

The Cre–*loxP* recombination system has also been used to manipulate genes in a cell type-specific (6–8) or inducible (9) manner. If two *loxP* sites are introduced into the genome such that they are flanking an essential part of a target gene without affecting its function, Cre-mediated deletion of the *loxP*-flanked segment will lead to the inactivation of the gene. Gene inactivation *in vivo* can be restricted to a particular cell type or time point by crossing mice with a *loxP*-flanked target gene to transgenic mouse strains expressing *cre* under the control of a cell type-specific or inducible promoter. Such experiments often involve the generation of two independent mouse strains, one harbouring a *loxP*-flanked gene, suitable for conditional inactivation, and the other with a deletion of the respective gene to study the gene's function during mouse development. However, this requires the injection of different mutant ES cell clones into blastocysts to generate two independent mouse strains, which is expensive and time consuming.

These problems of Cre–*loxP* mediated gene targeting can be largely overcome with the help of a *cre*-transgenic mouse strain which we have generated. This strain mediates deletion of *loxP*-flanked genes in all tissues, including germ cells (Fig. 1). The strain was produced by injection of a DNA fragment containing a Cre gene under the transcriptional control of a human cytomegalovirus minimal promoter (P_{Bi-2}; ref. 10) into pronuclei of fertilized eggs. The Cre gene had been modified by adding a nuclear localisation signal (3) and the human growth hormone gene providing splicing and polyadenylation signals (11). Seven *cre*-transgenic founders were crossed to mice with a *loxP*-flanked allele of the DNA polymerase β gene (polβ^{flox}; ref. 8) to test for Cre-mediated deletion. Southern blot analysis showed that in all offspring derived from one founder carrying several copies of the transgene, transmission of *cre* was accompanied by the complete deletion of the polβ^{flox} allele in all organs. Back-crossing of the F_1 generation to wild-type mice revealed that the Cre-mediated deletion was also present in germ cells since the mutation was transmitted to the offspring (Fig. 2). The Cre gene appears to be X-linked since transgene transmission through males is restricted to female offspring. X-chromosome inactivation in females starts approximately at the time of implantation of the embryo (reviewed in 12) and occurs randomly with respect to the parental origin of the chromosome, resulting in cellular mosaicism. Since the Cre-mediated deletion is present in all cells, the Cre gene in this strain is likely to be expressed before implantation during early embryogenesis. The expression

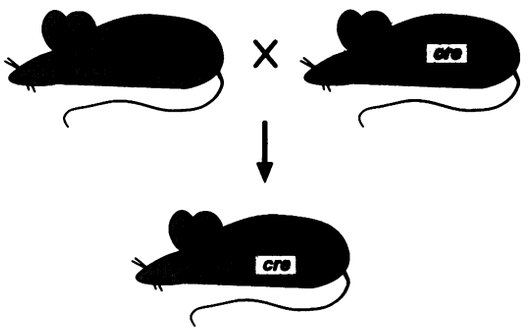

Figure 1. Deletion of *loxP*-flanked gene segments *in vivo* and transmission of the acquired mutation through the germline. Upper left mouse: a mouse carrying the target gene (black bar) flanked by two *loxP*-sites (triangles). Upper right mouse: *cre*-transgenic mouse. By crossing the mouse carrying the *loxP*-flanked locus to the *cre*-transgenic mouse, animals in which the target gene is deleted in all cells of the body, including germ cells, are obtained (lower animal).

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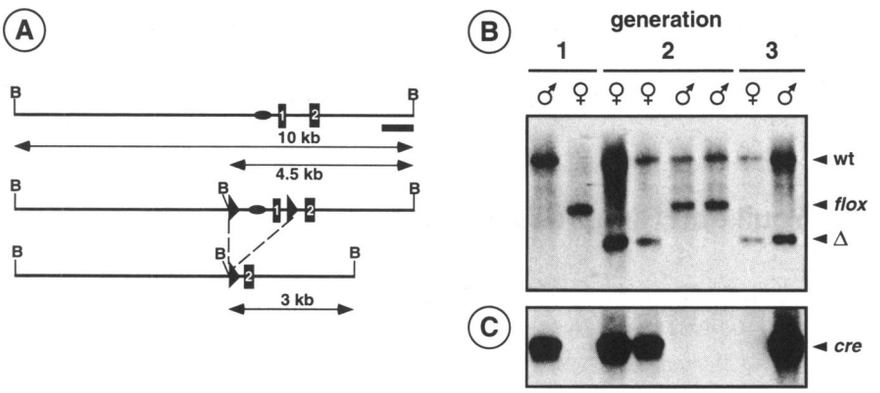

Figure 2. Southern blot analysis of tail DNA to test for Cre-mediated deletion. (**A**) Strategy to distinguish the polβ wild-type (upper line), *loxP*-flanked (middle line), and deleted (lower line) allele. The promoter region (ellipses), the first two exons of the polβ gene (numbered rectangles), and an adjacent probe used for hybridisation (bar) are shown. In the polβ^{flox} allele, a 1.5 kb gene segment has been flanked by two *loxP* sites (triangles). The wild-type, *loxP*-flanked, and deleted polβ alleles are represented by *Bam*HI (B)-fragments of 10, 4.5 and 3 kb, respectively. (**B** and **C**) Southern blot analysis of tail DNA from a male *cre*-transgenic and a female mouse homozygous for the polβ^{flox} allele (generation 1), four offspring derived from the cross of the two generation 1 mice (generation 2), and two mice derived from a back-cross of a *cre*-transgenic mouse of generation 2 carrying a deleted polβ allele to a wild-type mouse (generation 3). Genomic DNA was digested with *Bam*HI (B) or *Eco*RV (C) and hybridized with the polβ-specific probe shown in (A) and a *cre*-specific probe respectively. The positions of the fragments derived from the polβ wild-type (WT), *loxP*-flanked (*flox*) and deleted (Δ) alleles are indicated. Transmission of the *cre*-transgene through the male of generation 1 is restricted to females and accompanied by the presence of the deleted polβ allele. Presence of the deleted polβ allele in mice of generation 3 is independent of *cre*.

pattern of *cre* is attributed to the particular site of transgene integration in these mice as other lines, some of which carry even a higher number of transgenes, did not mediate deletion of the *loxP*-flanked locus in any tissue.

Cre-mediated gene deletion was confirmed in several independent experiments including an additional mouse strain which carries a *loxP*-flanked neomycin resistance gene located at the immunoglobulin κ chain locus (R. Pelanda, unpublished) and *cre*-transgenic mice of the third generation. These results suggest that the level of Cre expression in this transgenic strain is stable and sufficient to mediate deletion of any *loxP*-flanked locus in all cells of the body. Size limits of deletion remain to be determined. Due to this property we named the *cre*-transgenic mouse strain *deleter*.

The *deleter* strain is useful to introduce Cre-mediated gene modifications in mice rather than in ES cells (Fig. 1). This lowers the risk that the ES cells lose their pluripotency due to manipulation during tissue culture. In addition, deletion mutants can be derived by crossing *deleter* mice to mice harbouring a *loxP*-flanked gene in their genome. This reduces the time, the cost and the number of animals in comparison with what is required to generate, separately, a mouse mutant allowing conditional gene inactivation (6–9) and a mutant carrying a deletion of the respective locus in all cells of the body.

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