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Targeted expression of *Cre* recombinase to adipose tissue of transgenic mice directs adipose-specific excision of *loxP*-flanked gene segments

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ABSTRACT

Functional analysis of mammalian genes relies, in part, on targeted mutations generated by homologous recombination in mice. We have developed a strategy for adipose-specific inactivation of *loxP*-floxed gene segments. Transgenic mice have been established that express *Cre* recombinase under the control of the adipose-specific aP2 enhancer/promoter. Crossing of the aP2/*Cre* mice with any *loxP*-floxed gene will facilitate its functional analysis in adipose tissue.

A variety of strategies are being developed to gain insight into the function of mammalian genes. Many of these methods rely on the ability to successfully manipulate the mouse genome by introducing loss or gain of function mutations (1). Transgenic mouse lines are generated by either direct pronuclear injection of a recombinant DNA molecule or by injection of embryonal stem (ES) cells, which carry an engineered alteration in a gene of interest, into mouse blastocysts (2). The effect of the mutation can be studied once gene inactivation has been established in the germline and the mutated allele is universally established in all cells. Because many targeted inactivations of genes result in a pleiotropic phenotype that is sometimes difficult to analyze, it may be desirable to direct tissue-specific excision of the gene of interest to study the effects of the deletion in a tissue-specific manner. Therefore, tissue-specific inactivation of genes is a desirable feature for functional analysis of the role of a given gene in a distinct tissue.

Manipulation of transgenes can be accomplished *in vivo* by the use of the site-specific *Cre*/*loxP* recombination system of bacteriophage P1 (3,4). The *Cre* recombinase faithfully and efficiently directs both excision and insertion of DNA segments flanked by *loxP* sites in both bacterial and eukaryotic cells (4). Recombination occurs when two directly oriented *loxP* sites on a DNA substrate interact with the *Cre* recombinase. This interaction results in the excision of the intervening DNA molecule between the two *loxP* sites leaving a single *loxP* site in the genome.

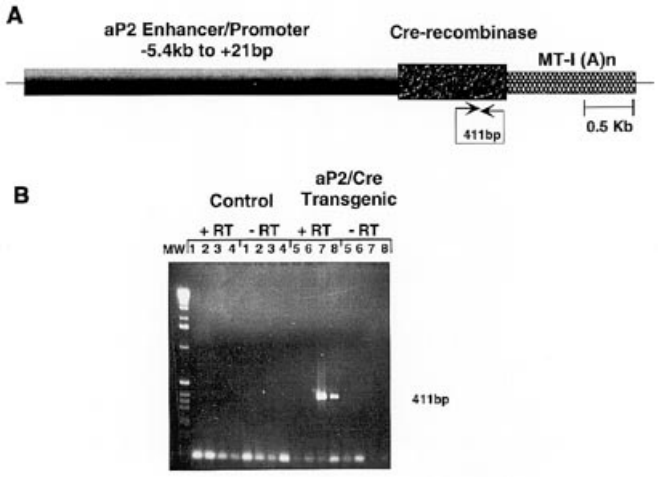

Figure 1. (**A**) Generation of the aP2/*Cre* transgenic lines. An aP2 enhancer/promoter (from –5.4 kb to +21 bp) was fused to the *Cre* coding sequence followed by the MT-I poly(A) tail, derived from plasmid pBS185. Not drawn to scale. (**B**) Tissue-specific expression of the *Cre* transgene. RT–PCR analysis with RNA extracted from a variety of tissues from a representative aP2/*Cre*-transgenic mouse line. Liver (lanes 1 and 5); kidney (lanes 2 and 6); brown adipose tissue (lanes 3 and 7); white adipose tissue (lanes 4 and 8). Control littermates are also shown. The presence of reverse transcriptase in the reaction is indicated by (+RT).

Transient transfection of a *Cre*-expressing plasmid in ES cells targeted for segments of the IgH locus has demonstrated that this approach is feasible in ES cells (5). However, the additional *ex vivo* manipulation required for the screening of the desired deletion may compromise the ability of the ES cells to maintain their pluripotent phenotype. Recently, two strategies for the ubiquitous

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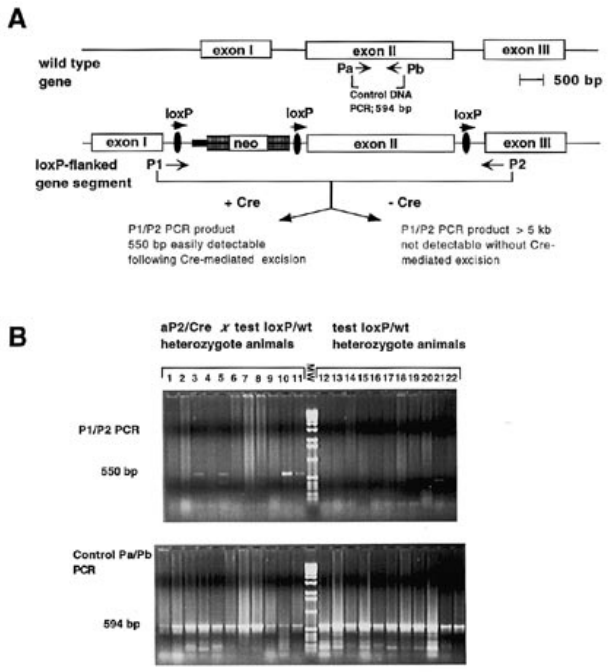

Figure 2. (**A**) Schematic representation of the loxP-flanked gene segment used to detect *Cre* activity. The position of the primers and the expected fragments before and after site-specific recombination are indicated. The hemizygous aP2/*Cre* transgenic line was crossed with a test strain carrying a *loxP*-flanked test gene. Progeny were examined that are either doubly transgenic for aP2/*Cre* and the *loxP*-flanked test gene or are singly transgenic for the *loxP*-flanked test gene segment. (**B**) PCR assays using genomic DNA extracted from a variety of mouse tissues derived from crossings between aP2/*Cre* and a *loxP*-flanked gene segment reporter transgenic mice. Template DNA from animals doubly transgenic for aP2/*Cre* and a *loxP*-flanked test gene (lanes 1–11) or heterozygous for a *loxP*-flanked test gene only (lanes 12–22). MW, molecular weight markers. Brain (lanes 1 and 12); liver (lanes 2 and 13); heart (lanes 3 and 14); lung (lanes 4 and 15); muscle (lanes 5 and 16); kidney (lanes 6 and 17); spleen (lanes 7 and 18); thymus (lanes 8 and 19); testes (lanes 9 and 20); white adipose tissue (lanes 10 and 21); brown adipose tissue (lanes 11 and 22).

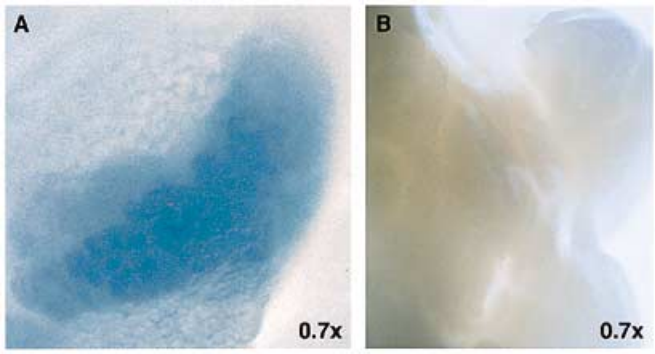

Figure 3. (**A**) *Cre*/*loxP*-mediated recombination in adipose tissue of animals doubly transgenic for aP2/*Cre* and loxP-βgal. The targeted cells were visualized by X-Gal blue cytostaining. (**B**) White adipose tissue from loxP-βgal mice does not stain with X-Gal.

deletion of *loxP*-flanked DNA segments were described that use either a *Cre* transgenic mouse strain expressing the recombinase under the control of human cytomegalovirus promoter (6) or under the control of the adenovirus EIIa promoter (7). In addition, tissue-specific expression of *Cre* in transgenic mice has been reported in, for example, mouse lenses (8), thymocytes (9) and mouse brain (10). Here we describe a transgenic mouse strain that targets expression of the *Cre* recombinase to adipose cells only. This strain is engineered to direct deletion of *loxP*-flanked DNA segments exclusively in white adipose and brown adipose tissue.

To generate transgenic lines, pronuclei of FVB/N fertilized eggs were injected with the aP2/*Cre* transgene (Fig. 1). The aP2/*Cre* construct was developed as follows. A 2.7 kb *Xho*I–*Hin*dII fragment from plasmid pBS185 (11) containing the *Cre* gene with modified translation initiation codon and the mouse metallothionin I (MT-I) polyadenylation signal, was blunt-ended and ligated to the *Sma*I site of the 5.4 aP2/SK+ plasmid (12). The plasmid was linearized by *Not*I and was purified prior to injection. The 5.4 kb regulatory element of the adipose protein 2 (aP2) gene, from –5.4 kb to +21 bp, is sufficient to direct high level expression of a heterologous gene in transgenic mice exclusively in adipose tissue (13).

Transgenic mice were produced and identified by PCR and Southern blot analysis. Eight *Cre* transgenic founders were characterized and transgene copy numbers varied between 1 and 50. Once established, several animals from each line were sacrificed for RNA analysis. RNA from liver, kidney, brown adipose and white adipose tissue was isolated by guanidine thiocyanate using the Ultraspec RNA extraction kit (Biotecx, Houston, TX). RT–PCR analysis to determine the levels of *Cre* expression in all lines was performed (Fig. 1B). A correctly amplified 411 bp *Cre* segment was detected in white and brown adipose tissue and only in the presence of reverse transcriptase (+RT; Fig. 1B). Similar results, that confirmed the presence of a *Cre* transgene transcript in white and brown adipose tissue, were obtained by Northern blot analysis using a *Cre*-specific probe (data not shown). These experiments demonstrate that the *Cre* transgene was properly expressed and was restricted to white and brown adipose tissue in all eight lines. To test for proper function of the *Cre* recombinase *in vivo*, three aP2/*Cre* transgenic lines that expressed the highest levels of *Cre* recombinase were crossed to mice with a *loxP*-flanked allele of a reporter test gene generated by homologous recombination in ES cells (Fig. 2A). Tissue-specific deletion of the reporter gene segment was demonstrated by PCR analysis using genomic DNA isolated from a variety of tissues in the offspring of these crossings. As shown in Figure 2B, a 550 bp fragment was successfully amplified in adipose tissue of *loxP*/wt heterozygote animals that were crossed with an aP2/*Cre* transgenic line (Fig. 2B, lanes 10 and 11). This fragment is only detectable following excision of the *loxP*-flanked test gene segment by *Cre* (Fig. 2A). The presence of a correctly amplified product in heart and muscle is a result of adipose cells present in these two tissues (Fig. 2, lanes 3–5). Thus, transmission of the *Cre* transgene and targeted expression in adipose tissue correlated with the deletion of the reporter loxP-flanked gene segment in adipose cells only. A combination of primers Pa and Pb was used as a DNA control reaction to amplify a 594 bp fragment from the wt gene segment (Fig. 2A and B). Furthermore, aP2/*Cre* transgenics were crossed with reporter loxP-βgal mice (14). Successful *Cre*/*loxP*-mediated recombination in the offspring of these crosses results in excision that is demonstrated by lacZ activation and visualization of the targeted cells by X-Gal blue cytostaining.

Figure 3 shows specific staining of adipose tissue in animals doubly transgenic for aP2/*Cre* and loxP-βgal. In contrast, no X-Gal staining was detected in heart, skeletal muscle, kidney or brain cells (data not shown).

As reported earlier, *Cre* expression is stably transmitted in transgenic mice and high levels of *Cre* do not appear to be toxic to the mice (8). Thus, a colony of adipose-expressing *Cre* mice can be easily maintained. The targeted expression of the *Cre* recombinase in adipose tissue will facilitate the analysis of the function of a variety of genes in adipocytes. Mice with deletion of *loxP*-flanked genes in an adipose-specific manner can be generated by crossing the aP2/*Cre* transgenic line to mice with any *loxP*-flanked locus of interest. These animals will be helpful in the analysis of the function of a variety of genes in the adipose tissue.

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