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Tissue- and site-specific DNA recombination in transgenic mice

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ABSTRACT **We have developed a method of specifically modifying the mammalian genome *in vivo*. This procedure comprises heritable tissue-specific and site-specific DNA recombination as a function of recombinase expression in transgenic mice. Transgenes encoding the bacteriophage P1 Cre recombinase and the *loxP*-flanked β-galactosidase gene were used to generate transgenic mice. Genomic DNA from doubly transgenic mice exhibited tissue-specific DNA recombination as a result of Cre expression. Further characterization revealed that this process was highly efficient at distinct chromosomal integration sites. These studies also imply that Cre-mediated recombination provides a heritable marker for mitoses following the loss of Cre expression. This transgene-recombination system permits unique approaches to *in vivo* studies of gene function within experimentally defined spatial and temporal boundaries.**

Bacteriophage P1 encodes the 38-kDa Cre recombinase that catalyzes site-specific DNA recombination between 34-base-pair (bp) repeats termed *loxP* (1). Cre is a member of the integrase family of recombinases. These enzymes recognize specific nucleotide sequences and function through a transient DNA-protein covalent linkage (reviewed in refs. 2 and 3). Cre activity appears mechanistically identical to that of yeast FLP recombinase and can function *in vitro* in the absence of high-energy cofactors, topoisomerase activity, and DNA replication (4, 5). In Cre-mediated recombination, resultant DNA structures are dependent upon the orientation of *loxP* sites. Direct repeats of *loxP* dictate an excision of intervening sequences whereas inverted repeats specify inversion (4). Cre and FLP have been shown to mediate site-specific DNA recombination in tissue-cultured eukaryotic cells, *Drosophila*, and transgenic plants (6–12).

With the aim of applying Cre recombinase function to molecular studies of normal and abnormal mammalian physiology, we sought to generate a transgenic mouse system that would establish whether Cre could effectively mediate chromosomal DNA recombination. As a foundation for future applications, we devised a nondeleterious transgene strategy that would provide an assessment of the efficiency, position dependence, and heritability of Cre-mediated chromosomal DNA recombination in mammals.

MATERIALS AND METHODS

Transgene Construction and Transgenic Mouse Production. The Cre transgene expression vector was constructed by inserting the 1.5-kilobase (kb) *Xho* I–*Xba* I fragment of pBS31 (7), containing the Cre coding sequence, into the unique *Bam*HI site of p1017 (ref. 13 and Fig. 1*A*). A second transgene vector, in which the *Escherichia coli* β-galactosidase (β-gal) gene was flanked by *loxP* sites (Fig. 1*B*), was constructed as follows. The β-gal gene, obtained as a 3.5-kb *Not* I fragment from pCMVβ (14), was blunt-end ligated into the *Bam*HI site residing between two direct repeats of *loxP* in plasmid plox^2 (derived from pBS64, 7). In plox^2, two direct repeats of *loxP* are flanked by polylinker-derived *Eco*RI and *Hin*dIII restriction enzyme sites. Isolation of the *loxP*–β-gal–*loxP* fragment was followed by blunt-ended ligation into the *Bam*HI site of p1017. Transgene vectors were purified away from plasmid sequences following *Not* I digestion and are represented as those *Not* I fragments in Fig. 1 *A* and *B*. Transgenic mice were produced following harvest of ICR × ICR zygotes (albino outbred, Harlan–Sprague–Dawley), DNA microinjection, and implantation. The presence of transgene DNA was assessed by hybridizing mouse tail DNA with either Cre or β-gal DNA probes (depicted in Fig. 1). In surgical procedures, 0.5 ml of 2.5% avertin was used as an anesthetic.

Southern and Northern Analysis. Thymocytes and splenocytes were harvested as single-cell suspensions by gently teasing freshly removed tissue. DNA was prepared from tissues and cells by incubation with proteinase K (100 μg/ml; Bethesda Research Laboratories) in 100 mM Tris, pH 8.0/50 mM EDTA for 24 hr at 55°C followed by extraction with phenol/chloroform and ethanol precipitation. Purified DNA was subjected to restriction enzyme digestion and Southern blot analyses (15). For Northern analyses, RNA was prepared from freshly homogenized tissue or single-cell suspensions in 6 M guanidinium isothiocyanate and fractionated through a CsCl cushion (16). The RNA was denatured with formamide/formaldehyde for agarose gel electrophoresis, blotting, and hybridization (15). DNA fragments for use as probes were nick-translated in the presence of random oligonucleotides to a specific activity of 10^8 cpm/μg. Densitometric analyses were performed with a Molecular Dynamics scanning densitometer (Sunnyvale, CA).

Flow Cytometry. Spleen-derived T cells were stained for surface expression of CD4 or CD8 antigens with phycoerythrin-conjugated anti-CD4 and fluorescein-conjugated anti-CD8 (anti-L3T4 and anti-Lyt-2, respectively; Becton Dickinson). Sorted populations were obtained by using a Becton Dickinson FACStar Plus flow cytometer (Becton Dickinson). The sorted population was >97% pure as judged by subsequent FACScan analysis.

RESULTS

Creation of Transgenic Lines. In transgene vector constructs derived from p1017, expression is regulated by the mouse proximal *lck* promoter, while the hGH gene contributes exon–intron splicing and polyadenylylation signals. Transgene expression from this vector is generally obtained in a thymocyte-specific manner (13, 17–19). Following thymocyte development in the thymus, emigration of mature T cells to peripheral compartments, including the spleen, coincides with loss of proximal *lck* promoter activity.

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Abbreviations: β-gal, β-galactosidase; hGH, human growth hormone.

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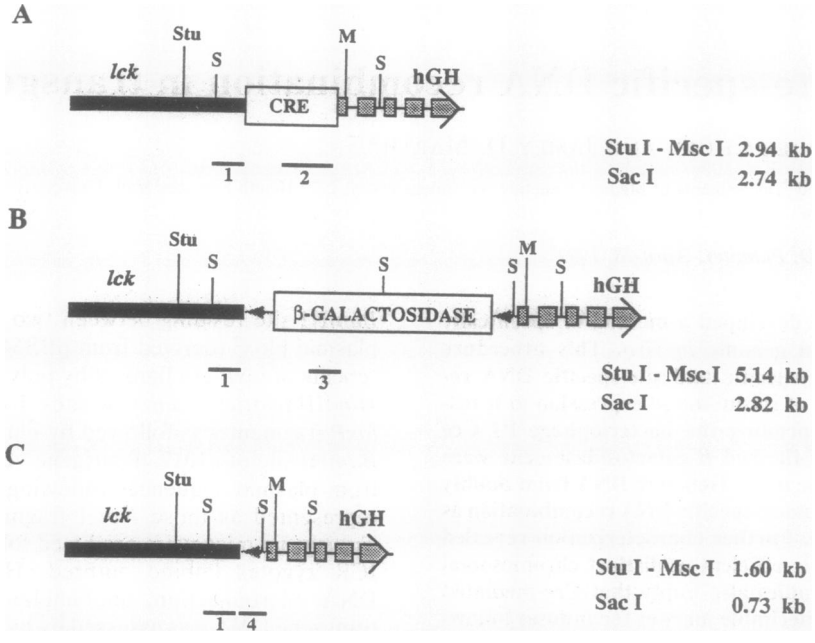

FIG. 1. Structures of transgene vector constructs and the Cre-mediated recombination event. (*A*) The Cre transgene represented as the microinjected *Not* I restriction fragment. The black bar represents 3.0 kb and contains the mouse thymus-specific proximal *lck* promoter. Human growth hormone (hGH) gene sequence (2.1 kb) is denoted by boxes (exons) and lines (introns) and contributes the polyadenylylation signal. Restriction enzyme sites used in Southern blotting analyses are depicted: S, *Sac* I; Stu, *Stu* I; M, *Msc* I. Probes used in Southern analyses are represented by numbered lines. Probe 1 is the 0.6-kb *Sac* I–*Bam*HI fragment of the *lck* promoter region. Probe 2 is a 900-bp *Bam*HI fragment of Cre. (*B*) The *loxP*-flanked β-gal transgene represented as the microinjected *Not* I restriction enzyme fragment. *loxP* sites are denoted by small arrows (represented at 10× relative construct size) flanking the β-gal gene as direct repeats. Probe 3 is a 600-bp *Hpa* I fragment of the β-gal gene. (*C*) Cre-mediated recombination structure resulting from excision of the *loxP*-flanked β-gal gene. A single *loxP* site is resolved following recombination. Probe 4 consists of the 180-bp *Hin*dIII–*Eco*RI fragment of plox^2 containing two *loxP* sites and a small contribution of plasmid polylinker sequence. Expected Southern blot fragment sizes are indicated.

A Cre transgenic line (I-57) was derived from zygotes injected with the Cre expression vector depicted in Fig. 1*A*. Two independent transgenic lines (I-86 and I-87) were derived from zygotes injected with the *loxP*–β-gal–*loxP* vector (Fig. 1*B*). As the β-gal gene is flanked by direct repeats of *loxP*, a Cre-mediated recombination event would be predicted to excise the β-gal gene, generating the structure depicted in Fig. 1*C*. These Cre and *loxP*–β-gal–*loxP* transgenic mouse lines bred as expected for unique single-site integration events and harbored the specific transgenes oriented as "head-to-tail" tandem arrays (data not shown), as is common for most transgene integration structures (reviewed in refs. 20–22). Transgenic mice from the Cre and *loxP*–β-gal–*loxP* lines were then bred to generate doubly transgenic progeny.

Genomic DNA was prepared from thymocytes and tails of Cre transgenic mice, *loxP*–β-gal–*loxP* transgenic mice, and doubly transgenic mice for Southern blot analyses. The Cre gene was visualized as the expected 2.94-kb *Stu* I–*Msc* I restriction fragment within the parental Cre transgenic mouse and a doubly transgenic offspring (Fig. 2*A*, lanes 3 and 5, respectively). With a β-gal-specific probe and the same thymocyte DNAs, the expected 2.82-kb *Sac* I fragment was observed in both the I-86 and the I-87 line (Fig. 2*B* and data not shown). Densitometric analysis of tail DNA (see below) revealed that the I-57 Cre line harbored the transgene at high copy number relative to the levels in the I-86 and I-87 *loxP*–β-gal–*loxP* transgenic lines (Fig. 2 *A* and *B* and see below).

Cre-Mediated Recombination Occurs *in Vivo* in Transgenic Mice. Southern analysis of thymocyte DNA from double transgenic mice revealed that the β-gal gene hybridization signal was eliminated (Fig. 2*B*, lane 5). This is the expected result following Cre-mediated recombination resulting in an excision of the β-gal gene situated between direct repeats of *loxP* (Fig. 1*C*). Evidence for the predicted Cre-mediated recombination structure was obtained by using a *loxP*-specific DNA probe (Fig. 2*C*). Hybridization was observed to a novel 1.60-kb *Stu* I–*Msc* I DNA fragment in the doubly transgenic thymocyte sample. Recombination was not detected in tail DNA derived from the same doubly transgenic mouse (Fig. 2*C*, lane 5). This new DNA structure was not present in parental thymocyte DNA derived from either the Cre or the *loxP*–β-gal–*loxP* transgenic mice (Fig. 2*C*, lanes 2 and 3). As would also be expected, there was a loss of the 5.14-kb DNA fragment (the double *loxP*-containing β-gal transgene).

Using the polymerase chain reaction (PCR), we have cloned the recombination structure residing within the 5′ *lck* untranslated sequence and the second exon of hGH. Analyses with this DNA revealed the expected structural attributes, including the exact size predicted, presence of a single *loxP* sequence, and the expected unique polylinker-derived restriction enzyme sites flanking this novel Cre-generated *loxP* site (data not shown).

The β-gal gene was incorporated as a second marker to ascertain Cre-mediated recombination by loss of β-gal enzymatic activity, but neither the I-86 nor the I-87 *loxP*–β-gal–*loxP* transgene allele was sufficiently active to allow protein detection (data not shown). However, RNA expression was observed following quantitative PCR assays (data not shown). These studies revealed, on average, three to five molecules of transgene-derived RNA per cell. Lack of high-level RNA expression in low-transgene-copy-number p1017-derived transgenic mice is routinely observed (unpublished observations) and does not reflect a specific inhibition mediated by *loxP* sites. Furthermore, studies in eukaryotic cells *in vitro* have shown substantial *loxP*-flanked gene expression at the level of both RNA and protein (6, 8, 12).

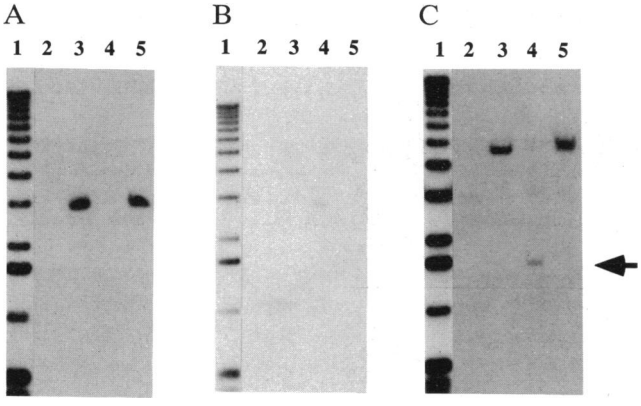

FIG. 2. Evidence of Cre-mediated recombination in genomic thymocyte DNA derived from doubly transgenic mice. (*A*) Southern blot analysis of *Stu* I/*Msc* I-digested thymocyte DNAs probed with ^{32}P-labeled Cre DNA (probe 2). Lane 1, ^{32}P-labeled 1-kb ladder (Bethesda Research Laboratories); lane 2, nontransgenic; lane 3, Cre parental transgenic; lane 4, *loxP*–β-gal–*loxP* parental transgenic; lane 5, doubly transgenic offspring 57/86-2. (*B*) Southern analysis of *Sac* I-digested thymocyte DNAs (identical to those used above) probed with ^{32}P-labeled β-gal DNA (probe 3). Lane 1, 1-kb ladder; lane 2, nontransgenic; lane 3, Cre parental transgenic; lane 4, *loxP*–β-gal–*loxP* parental transgenic; lane 5, doubly transgenic. (*C*) Southern blot analysis of *Stu* I/*Msc* I-digested genomic DNA (identical to those used above) hybridized with ^{32}P-labeled *loxP* fragment (probe 4). Lane 1, 1-kb ladder; lane 2, Cre parental thymocyte DNA; lane 3, *loxP*–β-gal–*loxP* parental thymocyte DNA; lane 4, doubly transgenic thymocyte DNA; lane 5, doubly transgenic tail DNA. Arrow, Cre-mediated recombination-derived fragment. A low level of hybridization to polylinker DNA sequence is seen in Cre-transgene-containing samples (*C*, lanes 2, 4, and 5), representing limited polylinker sequence residing within the Cre transgene fragment and the *loxP* probe. Five micrograms of DNA was loaded per lane.

Cre Recombinase Activity Can Be Tissue-Specific and Heritable in Transgenic Mice. Evidence of the tissue specificity of Cre-mediated recombination was obtained following RNA and Southern blot analyses of multiple tissue samples. Cre-specific RNA was detected in the thymus but not in the brain, kidney, liver, spleen, or tail (Fig. 3*A* and data not shown). The predominant Cre RNA species migrated with an apparent molecular size of ≈3.9 kb. Analyses of these RNA samples with hGH probes, and the obvious presence of multiple Cre RNA species, provided evidence that the 3.9-kb transcripts reflected incomplete splicing of the hGH gene (data not shown). More completely spliced Cre transgene RNA was observed migrating slightly larger than 18S (≈2.4 kb; Fig. 3*A*, lane 2). In immunoblotting experiments using a polyclonal anti-Cre antibody (provided by Brian Sauer, DuPont) with total cellular protein derived from these tissues, we observed the predicted 38-kDa Cre recombinase specifically expressed within the thymocyte population (data not shown). This Cre expression profile is identical to that obtained from all transgenic descendents of the Cre transgenic founder animal. DNA samples from these tissues were also subjected to Southern blot analysis. In brain, kidney, liver, and tail DNAs, β-gal gene hybridization remained constant and the 1.60-kb recombination structure was not present (Fig. 3*B*).

Although Cre expression was undetectable in splenocyte RNA (Fig. 3*A*, lane 4), evidence of the recombination event was apparent in splenocyte DNA (Fig. 3*C*, lane 2). Southern analyses of the purified splenic T-cell subpopulation (≈20% of total splenocytes) revealed an enrichment for the recombination structure at the expense of the nonrecombined *loxP*–β-gal–*loxP* transgene allele (Fig. 3*C*, lane 3). The expected *loxP*-hybridizing recombination product, as displayed in Fig. 2*C*, was also observed in this and other blotting analyses (see below). As Cre expression was not detected in either the total splenocyte or the splenic T-cell subpopulation (Fig. 3*A* and data not shown), the high degree of recombination within the *loxP*–β-gal–*loxP* transgene array (a loss of ≈95% of β-gal DNA; see below) implies that this Cre-mediated recombination occurred in the thymus, the natural site of T-cell ontogeny. In experiments that expanded the number of spleen-derived T cells by using the polyclonal lectin concanavalin A and interleukin 2, the recombined transgene allele frequency was retained following multiple mitoses (over at least 14 cell division cycles) in the absence of detectable Cre RNA (data not shown). These data indicate that Cre-mediated recombination can be a stable and heritable chromosomal alteration *in vivo* but do not rule out the possibility that undetected levels of splenic T-cell Cre recombinase are mediating *loxP*-flanked DNA recombination.

Transgenic Cre-Mediated Recombination Is Highly Efficient. For assessments of transgene copy number and recombination efficiency, Southern blot analyses were performed on *Stu* I/*Msc* I-digested DNAs hybridized to *lck* promoter sequence (probe 1) in a strategy that provided stoichiometric

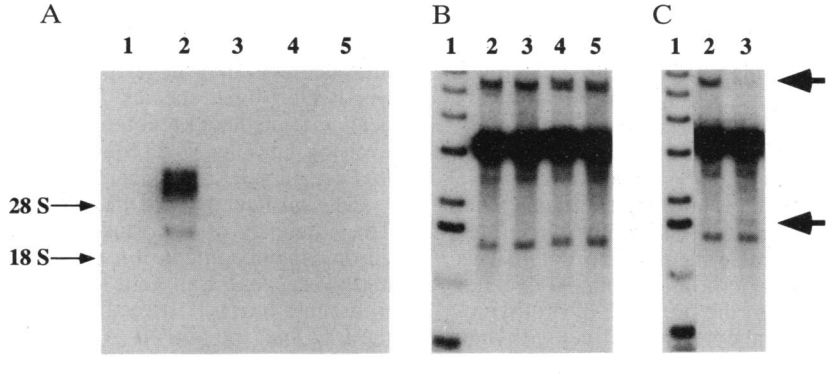

FIG. 3. Distribution of Cre RNA expression and *loxP*–β-gal–*loxP* transgene structure provides evidence of tissue-specific and heritable Cre-mediated recombination *in vivo*. (*A*) RNA blot analysis of Cre expression among normal tissues in a doubly transgenic offspring (57/86-4). Lane 1, brain; lane 2, thymus; lane 3, kidney; lane 4, spleen; lane 5, liver. RNA preparation and blotting procedures were performed as described (9). Blot was probed with ^{32}P-labeled Cre DNA (probe 2). Positions of 28S and 18S rRNAs are indicated. (*B* and *C*) Southern blot analyses of *Stu* I/*Msc* I-digested tissue DNAs, from the same animal analyzed in *A*, hybridized to *lck* promoter sequence (probe 1). In *B*: lane 1, 1-kb ladder; lane 2, tail; lane 3, liver; lane 4, brain; lane 5, kidney. In *C*: lane 1, 1-kb ladder; lane 2, spleen; lane 3, spleen-derived T cells. The Cre transgene is observed as the high-copy 2.9-kb fragment. The 2.5-kb fragment represents an aberrant integration event derived from p1017 sequence contained within the I-57 Cre transgene array (data not shown). Upper arrow, nonrecombined β-gal transgene fragment; lower arrow, 1.60-kb Cre-mediated recombination-derived fragment. The 1.40-kb fragment represents the endogenous *lck* gene. In the experiments above, 5 μg of either total cellular RNA or genomic DNA was loaded per lane.

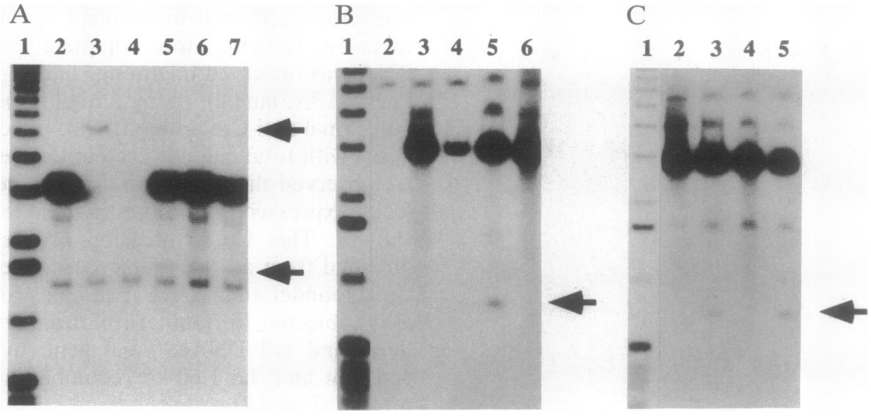

FIG. 4. Transgene-encoded Cre recombinase is highly efficient and can function at distinct chromosomal sites. These Southern blot analyses used the *lck* promoter fragment (probe 1). (*A*) *Stu* I/*Msc* I-digested thymocyte DNA. Lane 1, 1-kb ladder; lane 2, Cre parental transgenic; lane 3, *loxP*–β-gal–*loxP* parental transgenic; lane 4, nontransgenic; lanes 5–7, individual doubly transgenic progeny (57/86-8, 57/86-10, and 57/86-18, respectively). (*B*) *Sac* I-digested thymocyte DNA. Lane 1, 1-kb ladder; lane 2, nontransgenic; lane 3, Cre parental; lane 4, *loxP*–β-gal–*loxP* parental transgenic; lane 5, doubly transgenic thymocytes (57/86-10); lane 6, doubly transgenic tail from the same animal as in lane 5. An additional *lck*-hybridizing fragment at 4.2 kb represents an aberrant integration event derived from p1017 sequence contained within the I-57 Cre transgene array (data not shown). (*C*) *Sac* I-digested DNA derived from doubly transgenic mice produced by a cross between Cre transgenic line I-57 and the *loxP*–β-gal–*loxP* transgenic line I-87. Lane 1, 1-kb ladder; lane 2, tail DNA from offspring 57/87-3; lane 3, thymocyte DNA from 57/87-3; lane 4, tail DNA from offspring 57/87-7; lane 5, thymocyte DNA from 57/87-7. In the *Sac* I digest, the endogenous *lck* gene is apparent as a 5.3-kb fragment. Upper arrow, intact β-gal transgene fragment; lower arrow, Cre-mediated recombination fragment. An additional *lck*-hybridizing band at 1.7 kb reflects an aberrant integration event derived from truncated p1017 sequence contained within the I-87 *loxP*–β-gal–*loxP* transgene array (data not shown).

detection of the Cre transgene, the *loxP*–β-gal–*loxP* transgene, the endogenous *lck* gene, and the recombination structure (as in Fig. 3 *B* and *C*). In comparison to the single-copy (per haploid genome) *lck* gene (15), the I-57 Cre line was estimated to contain 110 copies of the transgene while both the I-86 and I-87 *loxP*–β-gal–*loxP* transgenic lines harbored ≈8 copies each (Fig. 4*A*). Southern analyses of doubly transgenic mice from multiple matings revealed that thymocyte Cre-mediated recombination produced the expected fragment sizes in all animals surveyed, as exemplified in Fig. 4 *A* and *B*. However, variation in the degree of Cre-mediated recombination among doubly transgenic mice was observed.

To assess this variation in Cre-mediated recombination among doubly transgenic progeny, densitometric comparisons were performed using the endogenous *lck* gene hybridization levels as internal controls. In these experiments, the extent of recombination was approximately 87%, 99%, and 97% in three doubly transgenic mice, as measured by loss of β-gal DNA (Fig. 4*A*, lanes 5–7, respectively). In spleen-derived T-cell DNA analyses, a >95% deletion of the β-gal gene was routinely observed (Fig. 3*C*, lane 3, and data not shown). In identical analyses using hGH sequence as a probe, similar results were obtained (data not shown). These results represent an average of analyses undertaken on >20 doubly transgenic mice generated from seven litters.

As the *loxP*–β-gal–*loxP* lines harbor 8 copies of transgene per cell, removal of >88% of the total β-gal transgene level would indicate that some cells have lost all copies of the β-gal gene. The efficacy of transgenic Cre-mediated recombination may be greater than evaluated here, as remaining levels of β-gal hybridization may indicate the presence of prothymocytes or contamination of thymocytes with B cells, thymic epithelial cells, and dendritic cells during cell preparation. These cells do not express *lck* and hence presumably would not express the Cre recombinase.

Cre-Mediated Recombination Excises the Transgene Array. The length of DNA between two *loxP* sites is a variable that may influence the efficiency of transgenic Cre-mediated recombination. In thymocyte DNA of doubly transgenic mice, the copy number of the resultant Cre-mediated DNA recombination structure is approximately half that of the endogenous *lck* gene (Figs. 3 and 4). Combined with the observations that β-gal and hGH DNA elimination can be virtually complete, and must be totally complete in some cells (perhaps those expressing the highest levels of Cre), these data imply that Cre-mediated recombination reduces the eight copies of the *loxP*–β-gal–*loxP* transgene array to a recombined allele consisting of a single copy of the *lck* promoter, the residual *loxP* site, and the hGH gene (Fig. 1*C*). This requires recombination between *lck* and hGH sequences within the transgene array, as may be expected since a "head-to-tail" transgene structure would contain direct repeats of *loxP* flanking the 3.0-kb *lck* promoter and the 2.1-kb hGH minigene. Thus at least 5.1 kb of chromosomal DNA can be excised in a single event. However, the exact distance between specific recombination events could be in integrals of 3.5 and 5.1 kb up to ≈70 kb, the estimated length of the transgene array.

The excised β-gal DNA is degraded, as hybridization to β-gal sequences is not observed at other chromosomal sites. Further, we were unable to detect the presence of intermediates that would reveal a sequential recombination process in transgene array reduction (data not shown). While those intermediates may nevertheless exist, they would be relatively short-lived species. These findings show that transgenic Cre-mediated recombination can be highly efficient in excising multiple DNA sequences flanked by direct repeats of *loxP* at a single chromosomal integration site.

Cre Can Function at Different Chromosomal Sites *in Vivo*. The efficiency of Cre function may also depend upon the chromosomal context of *loxP* sites. In this regard, a second *loxP*–β-gal–*loxP* transgene line (I-87) was mated with the Cre transgenic line I-57. Breeding experiments revealed that in the I-87 line, the *loxP*–β-gal–*loxP* transgene is at a different chromosomal site from that of the I-86 transgenic line (data not shown). Tissue-specific Cre-mediated recombination was also detected in the progeny of the I-57 × I-87 cross (Fig. 4*C*). Thus, in two transgene integrations that occurred at distinct chromosomal sites, Cre recombinase was able to access the transgenic *loxP* sites and function in producing chromosomal DNA recombination. While there may be some mammalian chromosomal sites at which Cre is unable to generate recombination, further studies will be required to address this in a spatially and statistically significant manner.

DISCUSSION

These studies provide a basis for the use of DNA recombination strategies in transgenic animal technology. Although one experimental approach initially considered included generating specific developmental phenotypes, we reasoned that modifying cellular fate, proliferative status, or viability would alter the validity of recombinase efficacy measurements. We find that the Cre recombinase can function in a highly efficient manner in directing tissue-specific, site-specific, and heritable chromosomal DNA recombination events *in vivo* in transgenic mice. Although, like FLP, Cre does not contain a canonical nuclear localization signal, Cre could access chromatin either through diffusion or following the transient breakdown of the nuclear membrane during mitosis.

Cre activity can be observed at both chromosomal sites assayed in this study, suggesting that the majority of the mammalian genome may be accessible to Cre function. From the transgene constructs used in this study we conclude that Cre can effectively recombine at least 5.1 kb of DNA in a single event. However, the reduction of the entire transgene array described herein (≈70 kb) suggests that Cre may mediate larger recombination events. The ability of Cre recombinase to recombine DNA over large distances in the mammalian genome may provide an experimental method of directing specific chromosomal translocations *in vivo*. This activity can be inferred from studies of FLP in *Drosophila* (23).

This technological advance allows studies of various aspects of gene function *in vivo* that could not be addressed hitherto. For example, current methodologies for gene ablation in transgenic mice, following embryonic stem-cell manipulations, produce null alleles in all cells of animals bred to homozygosity (reviewed in ref. 24). However, with the recombinase approach described in this study, null alleles could be generated in a tissue-specific and developmentally regulated manner. *loxP* sites could be positioned, by homologous recombination, to flank the target gene/exon in a nondeleterious manner; alternatively, *loxP* sites could be inserted to flank a gene replacement vector for complementation of the null background. When bred into a Cre transgenic line with a predefined Cre expression profile, the exact spatial and temporal pattern of recombination would be known. This would permit reproducible studies of the immediate metabolic alterations taking place following recessive genetic lesions and also reveal tissue-specific functions for genes shown to be expressed in multiple spatial and temporal patterns. Additionally, this methodology can generate transgene ablation. A requirement for constitutive transgene expression in the evolution of developmental and pathologic phenotypes could be addressed.

The heritable nature of Cre-mediated recombination allows a significant advance in the methodology for cell fate determination in mammals. Expression of a marker gene could be engineered to be dependent upon a Cre-mediated recombination event that would excise a *loxP*-flanked "stop" sequence (for example a transcriptional stop and RNA splice donor site) placed between a pan-specific promoter and the marker gene. Following recombination, marker gene expression would commence. In this way daughter cells could be identified by virtue of the initial activity from the Cre transgene promoter, irrespective of subsequent Cre expression. We expect that these applications of this transgene-recombination system will greatly enhance the information gained in gene function and disease modeling research employing transgenic animals.

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