Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 3932–3936, April 1996
Neurobiology

Targeted DNA recombination *in vivo* using an adenovirus carrying the *cre* recombinase gene

Y. WANG*, L. A. KRUSHEL*†, AND G. M. EDELMAN*†

*Department of Neurobiology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037; and †The Neurosciences Institute, 10640 John Jay Hopkins Road, San Diego, CA 92121

Contributed by G. M. Edelman, December 26, 1995

ABSTRACT **Conditional gene expression and gene deletion are important experimental approaches for examining the functions of particular gene products in development and disease. The *cre–loxP* system from bacteriophage P1 has been used in transgenic animals to induce site-specific DNA recombination leading to gene activation or deletion. To regulate the recombination in a spatiotemporally controlled manner, we constructed a recombinant adenoviral vector, Adv/cre, that contained the *cre* recombinase gene under regulation of the herpes simplex virus thymidine kinase promoter. The efficacy and target specificity of this vector in mediating *loxP*-dependent recombination were analyzed in mice that had been genetically engineered to contain *loxP* sites in their genome. After intravenous injection of the Adv/cre vector into adult animals, the liver and spleen showed the highest infectivity of the adenovirus as well as the highest levels of recombination, whereas other tissues such as kidney, lung, and heart had lower levels of infection and recombination. Only trace levels of recombination were detected in the brain. However, when the Adv/cre vector was injected directly into specific regions of the adult brain, including the cerebral cortex, hippocampus, and cerebellum, recombination was detectable at the injection site. Furthermore, when the Adv/cre vector was injected into the forebrains of neonatal mice, the rearranged *loxP* locus from recombination could be detected in the injected regions for at least 8 weeks. Taken together, these results demonstrate that the Adv/cre vector expressing a functional cre protein is capable of mediating *loxP*-dependent recombination in various tissues and the recombined gene locus may in some cases be maintained for an extended period. The use of the adenovirus vector expressing *cre* combined with localized delivery to specific tissues may provide an efficient means to achieve conditional gene expression or knockout with precise spatiotemporal control.**

The analysis of gene function *in vivo* requires the ability to manipulate genes in a precise spatiotemporal manner. Current methods use germ-line transgenic techniques in which a foreign gene is introduced or an endogenous gene is deleted in the host genome throughout the life of the organism (1, 2). The phenotypes that result from the gain or loss of function have provided insights into the physiological functions of particular genes in development and disease (3). For genes that are expressed in multiple areas and times during development and in the adult (for example, see ref. 4), the analysis of their function would be facilitated by the ability to alter gene expression at restricted times and in highly localized areas. One approach to achieve such conditional gene manipulation is the use of the newly developed *cre–loxP* system in transgenic animals (5, 6).

Cre is a 38-kDa DNA recombinase that is both necessary and sufficient for sequence-specific recombination in bacteriophage P1. The recombination occurs between two *loxP* sequences, each of which is 34 bp in length (7, 8). The intramolecular recombination of two *loxP* sites (oriented either head-to-head or head-to-tail) results in an inversion or deletion of the intervening DNA sequences, and an intermolecular recombination causes integration or reciprocal translocation at the *loxP* site. The *cre–loxP* system has been tested in various eukaryotic organisms and shown to function efficiently in mammalian cells (9–13). It has been used in transgenic animals to achieve conditional gene activation or deletion (knockout) of targeted genes in specific tissues (5, 6, 14). Generally, the system requires cross-breeding of two lines of transgenic animals. One line carries an allele with the locus of interest flanked at the 5′ and 3′ ends by *loxP* sequences. The other line has a *cre* transgene in which the expression of the cre protein is controlled by a heterologous promoter. Recombination between the *loxP* elements in the offspring is dependent upon the expression pattern of the *cre* transgene, which is defined by the specificity of the heterologous promoter (5, 6, 15). Ideally, the promoter used for directing *cre* gene expression should have tissue and developmental stage specificity, or should be inducible by a signaling molecule such as a hormone or cytokine (16). At present, however, such promoters are not generally available for each tissue or developmental stage of interest, particularly for applications to specific regions of the central nervous system (CNS).

An alternative approach for the delivery of transgenes into specific target tissues involves the use of recombinant adenovirus. This vector can infect a range of cell types with high efficiency, including epithelial cells, endothelial cells, myocytes, and neurons, making it a powerful vehicle for delivery of foreign gene products to various tissues (17–19). After injection of the virus into tissues, the high levels of infectivity and the limited spread of the virus results in a localized expression pattern of the transgene in various somatic tissues, including the brain, liver, lung, heart, and muscle (17–19).

In the current study, we combined the specificity of *loxP*-dependent gene recombination with the ability of adenoviruses to effect localized gene delivery *in vivo*. We constructed a recombinant adenovirus expressing *cre* (Adv/cre) and demonstrated that intravenous or intracerebral injection of the Adv/cre vector into *loxP* transgenic mice results in recombination at the *loxP* locus in a variety of tissues. The efficacy of recombination in Adv/cre-treated animals and the target specificities of different delivery routes suggest a number of useful applications for this methodology.

MATERIALS AND METHODS

Construction of Adv/cre Recombinant Adenovirus. The pAdv/cre plasmid was generated using the herpes simplex virus (HSV)–cre minigene (including the HSV thymidine kinase gene enhancer/promoter, the *cre* gene coding region,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CNS, central nervous system; HSV, herpes simplex virus; pfu, plaque-forming units; RT, reverse transcriptase; β-gal, β-galactosidase.

and a polyadenylylation sequence) removed from the plC-cre plasmid (a generous gift from H. Gu, National Institutes of Health) by *Xho* I–*Sal* I digestion and then inserted into the *Xho* I site of the pXCJL.1 shuttle plasmid (a generous gift from Y. Dai, Baxter Health Care, Mundelein, IL). The Adv/cre adenovirus was generated through homologous recombination between cotransfected pJM17 plasmid (20) (a generous gift from F. L. Graham, McMaster University, Hamilton, ON, Canada) and the pAdv/cre plasmid in 293 cells as previously described (see Fig. 1; ref. 21). The structure of the Adv/cre recombinant adenovirus was confirmed both by restriction digestion of the viral DNA and by Southern blot analysis using the *cre* gene fragment as the labeled probe. The Adv/β-gal virus used as a control is an E1a deletion recombinant adenovirus containing a β-galactosidase (β-gal) gene with nuclear localization signal sequences driven by a human cytomegalovirus promoter (21). The presence of the cytomegalovirus promoter rather than the HSV promoter was not expected to lead to major differences in infectivity or expression. Both viruses were prepared as described (21). Titers of the purified viral stock were determined by measuring OD_{260} with each reading of 1.0 equivalent to 1×10^{12} plaque-forming units (pfu)/ml.

Administration of the Recombinant Adenovirus. The mαA-Stop-Tag transgenic mice (referred to as loxP mice; ref. 5) were generously provided by G. Martin (University of California, San Francisco) with permission from H. Westphal (National Institutes of Health). These animals are homozygous for the transgene with *loxP* elements flanking a 1-kb *Stop* sequence on a CB6 background (5; and G. Martin, personal communication; see Fig. 1). For intravenous injection, the purified adenovirus solution was injected through the tail veins of adult transgenic mice. For administration of the virus into the brain, adult animals were anesthetized with ketamine and xylazine. One-half microliter of Adv/cre or Adv/β-gal solution was infused using a stereotaxic apparatus (Stoelting) at a rate of 0.2 μl/min into the cerebral cortex, hippocampus, striatum, and cerebellum on one side of the brain. For adenovirus delivery into the neonatal mouse brain, newborn mice were anesthetized by hypothermia and injected on one side of the forebrain with 0.3 μl of the Adv/cre or Adv/β-gal solutions.

Analysis of the Transgenic Animals. After administration of adenovirus as described above, the treated animals were killed and genomic DNA from various tissues was extracted (QiAmp Tissue Kit; Qiagen, Chatsworth, CA) and analyzed for the presence of recombination products by PCR as described (5). For Southern blot analysis of the *loxP* locus, the genomic DNA from the mouse tissues was digested with *Eco*RI and *Hin*dIII and blotted with the 1652-bp ^{32}P-labeled *Eco*RI to *Hin*dIII fragment from the pBS241 plasmid (5) containing the *Stop* sequences. For reverse transcriptase-PCR analysis of the *cre* mRNA, total RNA was isolated from mouse tissues (Qiagen). The first strand cDNA was synthesized using avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) in the presence of oligo-dT primers followed by PCR amplification using primers specific for *cre* coding sequences as described (5). All experiments were performed on a minimum of three animals and the PCR analyses were repeated a minimum of three times.

Histochemical Analysis. Adult mice infused with Adv/β-gal that were to be used for histochemical analysis ($n = 6$) were perfused, and the tissue was removed, sectioned, and stained for β-gal activity as previously described (22).

RESULTS

Adv/cre-Mediated Recombination in Liver. To investigate whether the adenovirus vector could express functional cre protein *in vivo*, we constructed the Adv/cre recombinant adenovirus as illustrated in Fig. 1 and used CB6 mice carrying

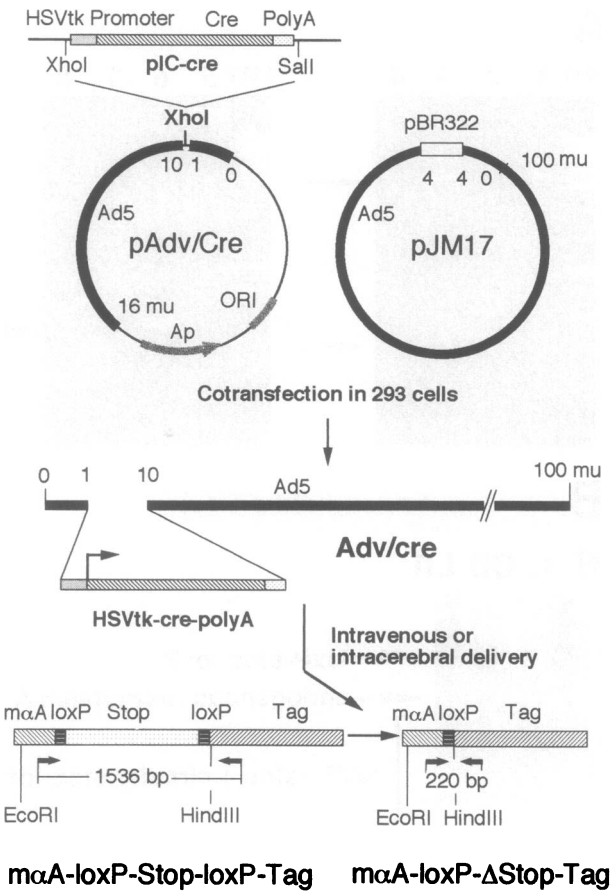

FIG. 1. Generation of the Adv/cre vector. The HSV thymidine kinase *cre* minigene was excised from the pIC-cre plasmid and cloned into the shuttle plasmid pXCJ.1. As a result of homologous recombination between pAdv/cre and pJM17 DNA, the recombinant adenovirus, Adv/cre, has the E1a region replaced by the HSV thymidine kinase-*cre*-poly(A) sequences. The schematic structure of the *loxP* transgene shown at the bottom is adopted from Lakso *et al.* (5). Upon *cre* expression, the 1318-bp *Stop* sequence is excised as a result of recombination between the two flanking *loxP* elements. The restriction enzymes used in Southern blot hybridization and the primers specific for the *loxP* locus used in PCR amplification (small arrows) and the expected sizes of the PCR products are indicated. mαA, Mouse α-crystallin A gene promoter; Tag, simian virus 40 large T antigen; mu, map unit; and Ad5, human adenovirus type 5.

homozygous mαA-Stop-Tag transgene (hereafter referred to as loxP mice) as our *in vivo* system (5). Approximately 10^{10} pfu of the Adv/cre or Adv/β-gal were infused into adult loxP mice by tail vein injection. Five days after the injection of the adenovirus vector, the livers and brains of the animals were collected and genomic DNA and total RNA were prepared from these tissues as described in *Materials and Methods*. The RNA samples were subjected to reverse transcription and amplification of first-strand cDNA by PCR using primers specific for the *cre* coding sequences (5). Genomic DNA samples were analyzed by PCR using primers outside of the two *loxP* sequences. The PCR analysis should yield a 1536-bp product from the intact *loxP* locus in the transgene or give a 220-bp product as the result of *cre*-induced recombination between the two *loxP* sites and the subsequent deletion of the 1.3-kb *Stop* sequences (5; see Fig. 1).

After intravenous injections of Adv/cre, *cre* mRNA was detected by reverse transcriptase (RT)-PCR in the liver (Fig. 2*A*, lane 6) but not in the brain (Fig. 2*A*, lane 5). This was consistent with previous studies using other adenoviruses injected through the tail vein (23). As a control, mice receiving tail vein injections of Adv/β-gal showed no detectable *cre*

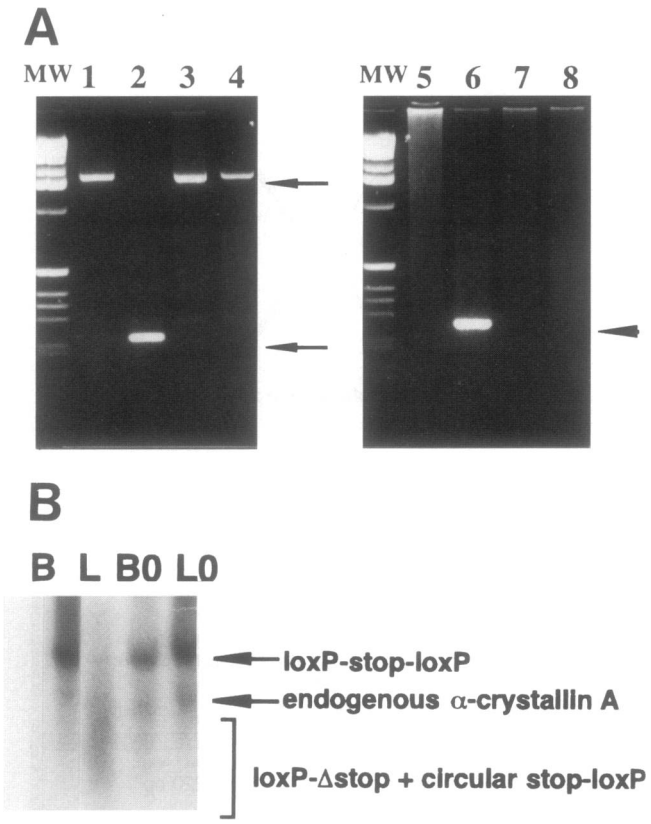

FIG. 2. Site-specific recombination *in vivo* mediated by the Adv/cre vector. (*A Left*) Analysis of genomic DNA by PCR using specific primers for the *loxP* locus. DNA samples were from the brain (lane 1) and liver (lane 2) of an Adv/cre-treated mouse and from the brain (lane 3) and liver (lane 4) of an Adv/β-gal-treated mouse. (*Right*) RNA analysis by RT-PCR using the *cre*-specific primers. RNA samples were from the brain (lane 5) and liver (lane 6) of an Adv/cre-treated mouse and from the brain (lane 7) and the liver (lane 8) of an Adv/β-gal-treated mouse. Arrows indicate the specific PCR products of 1316-bp (*Upper*) and 220-bp (*Lower*) lengths. The arrowhead on the right indicates the predicted 276-bp RT-PCR product from the *cre* mRNA. MW, 1-kb molecular weight marker (GIBCO). (*B*) Southern blot analysis of the *loxP* transgene in Adv/cre-treated animals. Genomic DNA samples were digested with *Eco*RI and *Hin*dIII enzymes and hybridized with the 1764-bp *Eco*RI and *Hin*dIII fragment from the pBS241 plasmid (5). The DNA samples were prepared from the brain (lane B) and liver (lane L) of an Adv/cre-treated mouse or from the brain (lane B0) and liver (L0) of an Adv/β-gal-treated mouse. The hybridization signals were quantitated on a PhosphorImager (Molecular Dynamics) using the IMAGEQUANT software. The percentage of recombination was estimated based on the reduction of the signal intensity of the 1764-bp bands in each lane using the mouse α-crystallin A gene (see Fig. 1 *Lower*, mαA) as the internal control for normalization.

expression in either tissue (Fig. 2*A*, lanes 7 and 8). PCR examination of genomic DNA using specific primers for the *loxP* locus produced a 220-bp PCR product only in the livers of the Adv/cre-treated animals (Fig. 2*A*, lane 2) but not in the brains (Fig. 2*A*, lane 1). In control animals injected intravenously with the Adv/β-gal vector, the 1536-bp PCR product from the intact *loxP* transgene (but not the 220-bp fragment) was detected in both the liver and the brain (Fig. 2*A*, lanes 3 and 4). The identities of these PCR products were confirmed by restriction enzyme analysis (data not shown).

Southern blot analysis was performed to assess the efficiency of the Adv/cre-mediated recombination at the *loxP* locus. The intact *loxP* site in the transgene was detected as a prominent 1764-bp hybridization signal in DNA samples from the brain of the Adv/cre-treated mice or from the brain or the liver of the Adv/β-gal-treated control animals (Fig. 2*B*, lanes B, B0, and L0 respectively). However, the hybridization signal was markedly reduced in the liver of the Adv/cre-treated animals (Fig. 2*B*, lane L). Instead, a low molecular weight smear was observed representing heterogeneous extrachromosomal recombination products resulting from the random pairing between any of the *loxP* sites in the approximately 50 tandomly repeated copies of the transgene (5; J. G. Pichel, personal communication). The extent of the recombination at the *loxP* locus measured by the reduction of the 1764-bp signal was estimated to be >99% in the liver (Fig. 2*B*, lane L).

cre mRNA expression as detected by RT-PCR correlated with the recombination events as detected by PCR analysis and quantitated by Southern blot analysis in the Adv/cre-treated animals. These results suggested that the Adv/cre vector produced a functional *cre* gene protein capable of mediating efficient recombination between the *loxP* sites *in vivo*.

Recombination in Somatic Tissues After Different Routes of Adv/cre Administration. We examined *loxP*-specific recombination in different organs 5 days after intravenous or intracerebral infusion of the Adv/cre vector. Genomic DNA was prepared from different tissues, including liver, kidney, spleen, lung, heart, and brain, and the samples were analyzed by PCR for *loxP*-specific recombination. When the Adv/cre vector was delivered (10^{10} pfu) via intravenous infusion, high levels of recombination were reproducibly detected in the liver (Fig. 3, lane 1) and spleen (lane 3). Lower levels were seen in the kidney (lane 2), heart (lane 4), and lung (lane 5). Only trace levels of recombination were found in the brain (lane 6), presumably because the brain–blood barrier blocked the viral particles from reaching and infecting brain tissues (24). Control animals injected with Adv/β-gal showed no *cre*-mediated recombination (Fig. 3, lanes C1–C6).

To demonstrate recombination mediated by Adv/cre in the brain, ≈1.5 × 10^8 pfu was stereotaxically infused into the

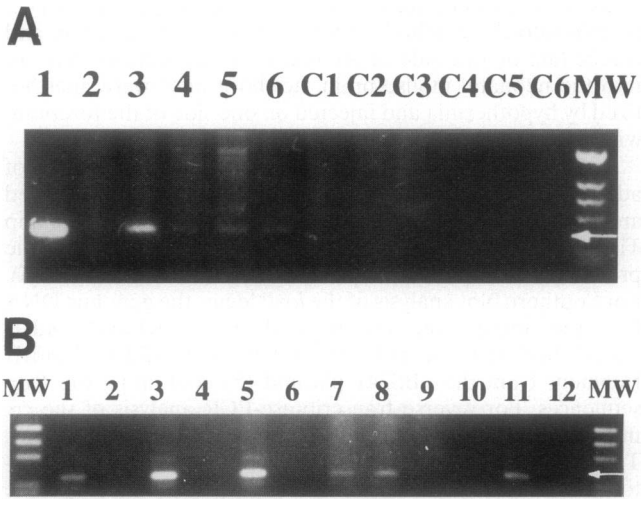

FIG. 3. Recombination in somatic tissues of Adv/cre-treated loxP mice. (*A*) PCR analysis of the genomic DNA was performed as described in Fig. 2. Samples are from the liver (lanes 1 and C1), kidney (lanes 2 and C2), spleen (lanes 3 and C3), heart (lanes 4 and C4), lung (lanes 5 and C5), and brain (lanes 6 and C6). The DNA samples were prepared from mice that had received intravenous injections of Adv/cre (lanes 1–6) or Adv/β-gal (lanes C1–C6). The arrow denotes the 220-bp product expected by PCR analysis if recombination had occurred. (*B*) PCR analysis of genomic DNA samples from Adv/cre-treated loxP mice after stereotaxic injection into different brain regions. The DNA was extracted from different brain regions that had received Adv/cre injections: cerebral cortex (lane 1), hippocampus (lane 3), cerebellum (lane 5), and the corresponding uninjected contralateral regions (lanes 2, 4, and 6). Other tissues analyzed were liver (lane 7), kidney (lane 8), heart (lane 9), lung (lane 10), spleen (lane 11), and skeletal muscle (lane 12). The arrow denotes the 220-bp product expected from PCR analysis if recombination had occurred.

cerebral cortex, hippocampus, and cerebellum on one side. Five days after the infusion, the brain tissues were dissected and genomic DNA was isolated for PCR analysis. *loxP*-dependent recombination was evident in the injected brain regions (Fig. 3*B*, lanes 1, 3, and 5) and was either detected at lower levels or not detectable in corresponding regions of the contralateral side (Fig. 3*B*, lanes 2, 4, and 6). Some recombination was also observed in the liver and kidney (lanes 7 and 8, respectively), presumably due to leakage of the virus into the circulatory system.

To examine the distribution of virally infected cells within different tissues, the Adv/β-gal vector was used. Histochemical analysis for β-galactosidase in mice that received the Adv/β-gal virus (10^{10} pfu) intravenously revealed numerous positively stained cells throughout the liver (Fig. 4*A*). In the kidney, β-gal staining was seen in the tubular epithelial cells of the cortex (Fig. 4*B*) but was absent in the medulla (data not shown). In the spleen, cells outside of the germinal zone were positively stained for β-gal (Fig. 4*C*). β-Gal positive cells were seen throughout the lung and heart (Fig. 4 *D* and *E*) but at a lower density as compared to the liver, spleen, and kidney. Only a very few cells were positive for β-gal in the brain; many of these had the appearance of perivascular microglia (Fig. 4*F*). When Adv/β-gal vector (2.2×10^8 pfu) was injected intracerebrally, numerous virally infected cells were observed at the injection sites (Fig. 4 *G* and *H*) and some weakly stained cells could be detected in other brain areas (data not shown) presumably because of retrograde transport of the viral particles by axons projecting to the injection areas (25, 26). In addition, some β-gal positive cells were seen in the liver and kidney (data not shown) presumably due to leakage of the viral particles into circulation during the procedure. The distribution patterns of Adv/β-gal were in good agreement with previous reports (23) and were qualitatively correlated with the observed distribution of recombination events after intravenous and intracerebral injection of Adv/cre.

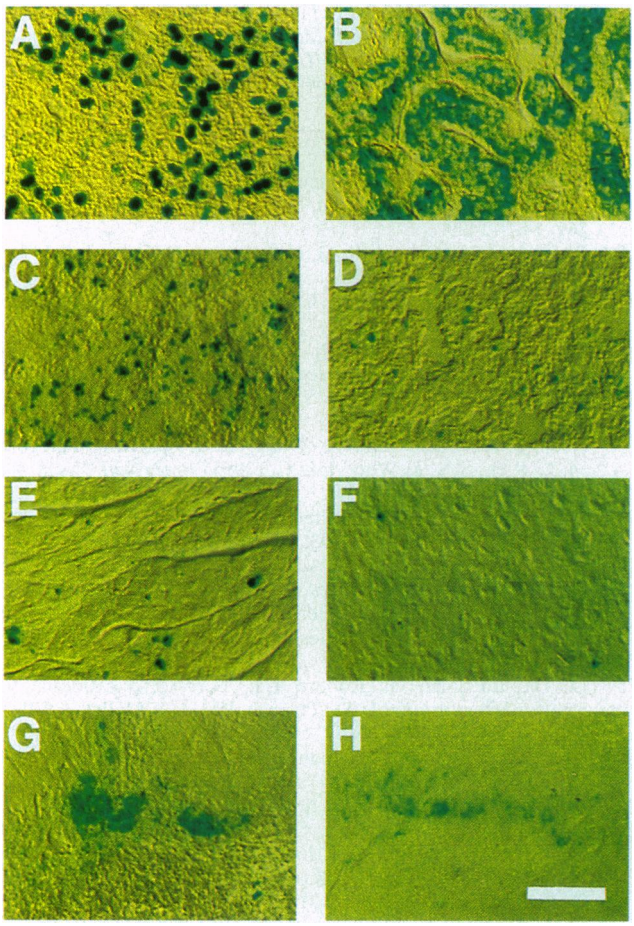

FIG. 4. Histochemical staining for β-gal after intravenous and intercerebral injections of adv/β-gal. Following a tail vein injection of adv/β-gal, numerous cells were positive for β-gal in the liver (*A*), cortex of the kidney (*B*), and in the spleen (*C*). Fewer positively stained cells were observed in the lung (*D*) and heart (*E*), and only an occasional cell was seen in the brain (*F*). After Adv/β-gal injections into different brain regions including the cerebellum (*G*) and hippocampus (*H*), numerous positively stained cells were seen at the injection site. (Bar = 85 μm.)

These results demonstrate that using different delivery routes, Adv/cre virus could induce *loxP*-dependent recombination in different tissues including the liver, spleen, and specified local regions of the brain.

Long-Term Stability of Recombination at *loxP* Site in Neonatal Mouse Brains. The ability to induce and maintain gene rearrangement would be important for the analysis of phenotypic changes over a sustained periods of time during development. We therefore examined whether *loxP*-dependent recombination could be achieved and recombination products could be maintained throughout neonatal brain development. Adv/cre (0.3 μl, ≈1.0×10^8 pfu) was injected into one hemisphere of newborn *loxP* mice. Two to 8 weeks after injection, genomic DNA was isolated from each hemisphere and subjected to PCR analysis. *loxP*-dependent recombination products were detected in the injected hemispheres at all the time points during the entire experimental period (Fig. 5). Lower levels of recombination products were also occasionally seen in the contralateral hemisphere, presumably due to retrograde transport (see Fig. 5, lanes 8, 10, and 12, for examples). These results demonstrate that the Adv/cre vector can induce *loxP*-dependent recombination in the neonatal brain and suggest that a mosaic of the *loxP* transgene may be established and maintained in selected regions of the developing nervous system.

DISCUSSION

We have found that a replication-defective adenovirus expressing *cre* recombinase is capable of mediating efficient *loxP*-dependent recombination *in vivo*. This extends previous work, which has shown that adenovirus vectors expressing the cre protein can mediate *loxP*-dependent DNA recombination in tissue culture cells (27). After systemic delivery, the *cre* adenoviral vector was able to induce recombination in a broad range of nonneural tissues, including the liver, spleen, and kidney. Other tissues, such as brain and heart, had low levels of recombination at the *loxP* sites. However, injections of Adv/cre directly into adult brains produced *loxP*-specified recombination in targeted regions. Adv/cre injections into neonatal brains resulted in *loxP*-dependent recombination and the rearranged *loxP* locus was stable in the genome for at least 8 weeks. These experiments indicate that site-specific recom-

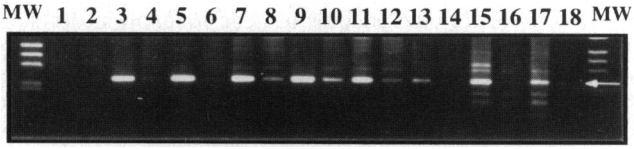

FIG. 5. Stability of *cre*-dependent recombination products in neonatal mice brains. DNA from neonatal mouse brains was analyzed by loxP locus-specific PCR. The samples were obtained from untreated animals (lanes 1 and 2) and mice 2 weeks (lanes 3–6), 3.5 weeks (lanes 7–10), 5 weeks (lanes 11–14), and 8 weeks (lanes 15–18) following Adv/cre injection. Hemispheres injected with the virus are shown in the odd-numbered lanes and the contralateral hemispheres are shown in the even-numbered lanes. The arrow denotes the 220-bp product expected from PCR analysis after recombination.

bination in somatic tissues of the *loxP* mice can be mediated by a recombinant adenovirus expressing *cre* and that a mosaic of the *loxP* locus can be established in the CNS for a extended period of time.

The adenoviral based *cre/loxP* system possesses certain distinct advantages over conventional transgenic approaches. Adenoviral vectors have high levels of infectivity in a broad range of cell types across many species (17). When introduced with timed local delivery techniques, these vectors can allow temporally and spatially specific expression of a foreign gene in discrete regions (19, 25). Presently, the precise temporal control of gene expression in specific tissues by conventional transgenic techniques is difficult. Recent developments of inducible and tissue-specific promoters may help overcome some of these limitations (16). In the CNS, however, there are only a few cell type-specific promoters that can be directed to distinct regions for the study of neuronal functions. With the Adv/cre system, one may be able to overcome these limitations by employing discrete injections into particular neural regions. Moreover, with this approach, only a single transgenic line with the *loxP* site in a particular location need be used to study different areas at multiple developmental time points.

While these advantages recommend the use of this methodology, there are certain limitations associated with adenoviral gene delivery. As shown in several of our experiments, the possibility of leakage during injections of the virus, or retrograde neuronal transport of viral particles, may compromise the local specificity of CNS infections. On the other hand, our results indicated that not all cell types within a tissue were equally infected with the adenovirus. For example, in the kidney numerous tubular epithelial cells in the cortex, but not in the medulla, were infected. This may be attributed to the route of adenoviral administration (23) and/or the lack of high affinity receptors for the virus (28). The use of the Adv/cre *in vivo* is therefore likely to be restricted to cell types that are accessible and particularly susceptible to adenovirus infection. In this situation, the specificity of adenoviral vectors could be further restricted by incorporating cell type specific promoters for the transgene. The use of neuronal or glial specific promoters in adenoviral vectors, for example, has been shown to target transgene expression in particular cell types in the brain (29).

Although E1A-deleted recombinant adenoviral vectors are replication defective in nonpermissive cells, high doses of infection can still result in cytopathology and even cell death. Adenoviruses can be antigenic and cause both humoral and cellular immune responses against viral particles and virally infected cells in adults and to a lesser degree in neonates (30–32). These effects can be magnified in immunologically privileged areas such as CNS when injections break both blood vessels and the blood–brain barrier. These side effects may affect host cell gene expression and compromise the analysis of phenotypes resulting from *cre*-induced gene rearrangement. The development of improved vectors may help to minimize these drawbacks (33).

Spatiotemporal coordination of specific gene rearrangement mediated by Adv/cre vectors *in vivo* opens several possible routes for the analysis of gene function in development and disease. Depending on whether the intervening sequence flanked by *loxP* elements is a coding region of an endogenous gene or a regulatory sequence for a transgene, such as a stop sequence, *loxP*-dependent recombination can be designed to generate targeted gene disruption (conditional knockout) or to achieve specific gene activation. When a gene encoding a toxin is expressed after *cre*-mediated recombination, physiological consequences of restricted cell ablation may be studied at specific time and place. When *loxP* elements are engineered into specific genomic loci, targeted chromosomal rearrangements can be induced in somatic tissues to establish and study genetic disease models. With further refinement, the *cre* expressing adenoviral vector may prove to be a powerful tool for *in vivo* studies of gene function.

We thank Dr. George Miklos of The Neurosciences Institute and our colleagues at the Department of Neurobiology for critical readings of the manuscript. This work was supported by U.S. Public Service Grants HD09635 and HD33576 and a grant from the G. Harold and Leila Y. Mathers Charitable Foundation (to G.M.E.). G.M.E. is a consultant to Becton Dickinson and Company.

1. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. (1983) *Science* **222,** 809–814.
2. Thomas, K. R. & Capecchi, M. R. (1987) *Cell* **51,** 503–512.
3. Chisaka, O. & Capecchi, M. R. (1991) *Nature (London)* **350,** 473–479.
4. Meyer, D. & Birchmeier, C. (1995) *Nature (London)* **378,** 386–390.
5. Lakso, M., Sauer, B., Mosinger, B., Jr., Lee, E. J., Manning, R. W., Yu, S. H., Mulder, K. L. & Westphal, H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6232–6236.
6. Gu, H., Marth, J. D., Orban, P. C., Mossmann, H. & Rajewsky, K. (1994) *Science* **265,** 103–106.
7. Sternberg, N. & Hamilton, D. (1981) *J. Mol. Biol.* **150,** 467–486.
8. Sternberg, N., Hamilton, D. & Hoess, R. (1981) *J. Mol. Biol.* **150,** 487–507.
9. Sauer, B. & Henderson, N. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5166–5170.
10. Araki, K., Araki, M., Miyazaki, J. & Vassalli, P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 160–164.
11. Smith, A. J., De Sousa, M. A., Kwabi-Addo, B., Heppell-Parton, A., Impey, H. & Rabbits, P. (1995) *Nat. Genet.* **9,** 376–385.
12. VanDeursen, J., Fornerod, M., Van Rees, B. & Grosveld, G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7376–7380.
13. Fukushige, S. & Sauer, B. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7905–7909.
14. Orban, P. C., Chui, D. & Marth, J. D. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6861–6865.
15. Sauer, B. (1993) *Methods Enzymol.* **225,** 890–900.
16. Kühn, R., Schwenk, F., Aguet, M. & Rajewsky, K. (1995) *Science* **269,** 1427–1429.
17. Brody, S. L. & Crystal, R. G. (1994) *Ann. N.Y. Acad. Sci.* **716,** 90–101.
18. Smith, L. C., Eisensmith, R. C. & Woo, S. L. (1995) *Adv. Exp. Med. Biol.* **369,** 79–88.
19. Davidson, B. L., Allen, E. D., Kozarsky, K. F., Wilson, J. M. & Roessler, B. J. (1993) *Nat. Genet.* **3,** 219–223.
20. McGrory, W. J., Bautista, D. S. & Graham, F. L. (1988) *Virology* **163,** 614–617.
21. Chen, S.-H., Chen, X. H. L., Wang, Y., Kosai, K.-I., Finegold, M. J., Rich, S. S. & Woo, S. L. C. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2577–2581.
22. Wang, Y., Jones, F. S., Krushel, L. A. & Edelman, G. M. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1892–1896.
23. Huard, J., Lochmuller, H., Acsadi, G., Jani, A., Massie, B. & Karpati, G. (1995) *Gene Ther.* **2,** 107–115.
24. Doran, S. E., Ren, X. D., Betz, A. L., Pagel, M. A., Neuwelt, E. A., Roessler, B. J. & Davidson, B. L. (1995) *Neurosurgery* **36,** 965–970.
25. Le Gal La Salle, G., Robert, J. J., Berrard, S., Ridoux, V., Stratford-Perricaudet, L. D., Perricaudet, M. & Mallet, J. (1993) *Science* **259,** 988–990.
26. Ridoux, V., Robert, J. J., Zhang, X., Perricaudet, M., Mallet, J. & Le Gal La Salle, G. (1994) *Brain Res.* **648,** 171–175.
27. Anton, M. & Graham, F. L. (1995) *J. Virol.* **69,** 4600–4606.
28. Wickham, T. J., Filardo, E. J., Cheresh, D. A. & Nemerow, G. R. (1994) *J. Cell Biol.* **127,** 257–264.
29. Hashimoto, M., Aruga, J., Kanegae, Y., Saito, I., Yoshida, S., Hosoya, Y., Yaginuma, H. & Mikoshiba, K. (1995) *Soc. Neurosci. Abstr.* **21,** 1800.
30. Kass-Eisler, A., Falck-Pedersen, E., Elfenbein, D. H., Alvira, M., Buttrick, P. M. & Leinwand, L. A. (1994) *Gene Ther.* **1,** 395–402.
31. Yang, Y., Nunes, F. A., Berencsi, K., Furth, E. E., Gönczöl, E. & Wilson, J. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4407–4411.
32. Byrnes, A. P., Rusby, J. E., Wood, M. J. & Charlton, H. M. (1995) *Neuroscience* **66,** 1015–1024.
33. Engelhardt, J. F., Ye, X., Doranz, B. & Wilson, J. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6196–6200.