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Targeting of *cre* to the *Foxg1* (*BF-1*) Locus Mediates *loxP* Recombination in the Telencephalon and Other Developing Head Structures

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The use of genetics to study the development of the telencephalon and derivatives such as the cerebral cortex has been limited. The telencephalon begins to form midway through gestation, and targeted mutations in genes suspected of playing roles in its development often lead to early phenotypes that preclude analysis of their role at later stages. This problem can be circumvented using a Cre/*loxP* recombination system. A mouse line was produced in which *cre* was targeted to the *Foxg1* (*BF-1*) locus, a gene expressed specifically in the telencephalon and discrete head structures. Crosses between Foxg1–Cre mice and three separate *loxP* reporter mice generated embryos with recombination patterns matching that expected from the normal pattern of *Foxg1* expression. Recombination occurs invariably in the telencephalon, anterior optic vesicle, otic vesicle, facial and head ectoderm, olfactory epithelium, mid–hindbrain junction, and pharyngeal pouches. Recombination in some animals also occurs less efficiently in tissues not known to express *Foxg1*. We show that the genetic background of the parental mice and the *loxP* target allele can each contribute to differences in the exact pattern of recombination. Collectively, these data show that Foxg1–Cre mice should be useful in the deletion or ectopic expression of any floxed target gene in a *Foxg1*-like pattern. © 2000 Academic Press

Key Words: BF-1; Foxg1; Cre/loxP; **Foxg1-Cre; telencephalon; cerebral cortex; hippocampus; striatum; R26R; Z/AP.**

INTRODUCTION

During embryonic development, neuroepithelial cells in the anterior neural tube proliferate and evaginate to form the two telencephalic hemispheres. The ventral telencephalon generates the striatum, pallidum, septum, and amygdala, while the dorsal telencephalon develops primarily into the cerebral cortex, hippocampus, and cingulate cortex. Although the anatomy of telencephalic development has been described in much detail, the molecular mechanisms that generate these structures remain largely a mystery.

Genetic approaches to studying telencephalic development have been limited, in part because many genes suspected of playing a role in brain development are required for the formation of other tissues at earlier stages. The telencephalon emerges relatively late in development, at around embryonic day 10 (E10) in the mouse; disruption of genes using standard gene-targeting methods can prove uninformative if phenotypes at earlier stages of embryogenesis cause lethality or disrupt the formation of the telencephalon indirectly. For example, *Bmp4, Fgf8, Notch1,* and *Otx2* are required for normal gastrulation or early patterning (Swiatek *et al.*, 1994; Acampora *et al.*, 1995; Winnier *et al.*, 1995; Ang *et al.*, 1996; Meyers *et al.*, 1998; Sun *et al.*, 1999). Although these genes, as well as others, are suspected of playing roles in telencephalic development (Chenn and McConnell, 1995; Furuta *et al.*, 1997; Shimamura and Rubenstein, 1997; Zhong *et al.*, 1997; Meyers *et al.*, 1998; Rhinn *et al.*, 1998), studies of their function in the brains of standard knockout mice have been precluded by the onset of severe malformations at earlier developmental stages.

To circumvent this problem, Gu *et al.* (1994) have demonstrated the feasibility of knocking out a gene only in specific cell types or tissues, rather than a whole mouse, using the bacteriophage P1 Cre/*loxP* recombination system. Two mouse lines are used: in one, the Cre recombinase is expressed in a tissue of interest, and in the second, essential components of a particular gene are flanked by *loxP* sites ("floxed"). When the two lines are crossed, the floxed sequence is deleted exclusively in the tissue of interest. The Cre/*loxP* system can also be used to overexpress or ectopically express a gene in a specific tissue. A transgene can be carried silently as part of a *loxP* construct in which one or

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more floxed transcriptional stop sites separate a promoter from the transgene open reading frame (Lakso, 1992). Compared to traditional transgenic approaches using pronuclear injection, the Cre/*loxP* method enables the creation of stable mouse lines even when transgene expression results in lethality or sterility.

Here we attempt to facilitate genetic studies of telencephalic development by generating a mouse line, Foxg1–Cre, in which the sequence encoding the Cre recombinase is targeted to a locus expressed specifically in the telencephalon and discrete head structures. Forkhead box G1 [*Foxg1;* new name for brain factor-1 (*BF-1*); Mouse Genome Database Nomenclature Committee, 1999] encodes a putative transcription factor of the winged-helix family that is required for telencephalic development (Tao and Lai, 1992; Xuan *et al.*, 1995). *Foxg1* expression is first detected at the three-somite stage (~E8.0) in the anterior neural ridge, a region of ectoderm underlying the anterior neural plate (Shimamura *et al.*, 1995). At the five- to eight-somite stage (E8.0–8.5), *Foxg1* is expressed in the anterior neural plate itself. By E10.5, expression is found in most of the telencephalon, where it persists into adulthood in derivatives including the cerebral cortex, caudate putamen, and hippocampus (Tao and Lai, 1992). The otic vesicle, olfactory placode, pharyngeal pouches, and anterior half of the optic vesicle also express *Foxg1* (Tao and Lai, 1992; Hatini *et al.*, 1994). Mice in which *lacZ* was targeted to the *Foxg1* locus (Xuan *et al.*, 1995) show a *lacZ* expression pattern matching that observed by *in situ* analysis for *Foxg1* mRNA, with additional expression of *lacZ* detected at the mid–hindbrain junction between E9.0 and at least E12.5 (Dou *et al.*, 1999; Eseng Lai, personal communication).

The present study reveals that the recombination pattern obtained with Foxg1–Cre mice matches the normal expression pattern of *Foxg1*, thereby demonstrating the utility of this mouse for inducing tissue-specific recombination. Using two *loxP* mouse reporter lines, ROSA26 Reporters (R26R; Soriano, 1999) and Z/AP (Lobe *et al.*, 1999), we address the issue of whether the tissue specificity of recombination can be affected by the target *loxP* locus. Although both *loxP* reporter loci used here provide a common baseline pattern of recombination, we observe a higher incidence of recombination in the ectoderm of Z/AP embryos. Finally, we provide evidence that strain background can influence the pattern of Cre-mediated recombination.

MATERIALS AND METHODS

Construction of the Foxg1–Cre targeting vector. Xuan *et al.* (1995) have previously targeted *lacZ* to the *Foxg1* locus. Using their targeting construct, the *lacZ* open reading frame with its intron and polyadenylation [poly(A)] sequences was replaced with a *cre* coding sequence, along with an SV40 intron and poly(A) sequence. The cloning strategy is as follows. First, the *Foxg1* genomic sequences from the 5′ *Sal*I site to the downstream ATG start site were amplified using the following primers: 5′cagacta*gtcgacggctagccag*3′ and 5′agcggtaccagatctgaacgaattccaaggggc*ccatcacccaggcgtcg*3′ (*Foxg1* sequences are italicized, the remaining sequences comprise restriction sites used in the following cloning steps). The amplified fragment was cut with *Spe*I and *Kpn*I and inserted into the *Spe*I–*Kpn*I sites of pBluescript (Stratagene). A *cre* open reading frame, with a nuclear localization sequence and a consensus Kozak sequence, flanked by *Apa*I and *Eco*RI sites from plasmid pML78 (gift from Mark Lewandoski) was then inserted into the *Apa*I–*Eco*RI sites at the 3′ end of the cloned *Foxg1* fragment above. An *Eco*RI–*Bam*HI SV40 intron–poly(A) fragment from pSKCMVSV40 (gift from Yi-Hsin Liu) was then inserted into the *Eco*RI–*Bgl*II sites. Finally, the *Foxg1–cre*–intron–poly(A) fragment was isolated with *Sal*I and *Kpn*I and inserted into the *Foxg1* targeting construct from Xuan *et al.* (1995) cut with a complete *Sal*I digest and a partial *Kpn*I digest that cuts at the 3′ end of the *lacZ* poly(A) sequence (Fig. 1A).

Identification of homologous recombinant cell clones and production of mice. The Foxg1–Cre targeting vector was linearized with *Sfi*I and electroporated into HM1 ES cells. ES cells were maintained and selected as described previously (Hébert *et al.*, 1994), except that 300 μg/ml G418 (Gibco BRL) in the absence of FIAU was used to select for stable DNA integration on 0.1% gelatin-coated plates without feeder cells. Homologous recombinant cell clones were identified by PCR using one primer that hybridizes to *Pgk1* sequences (5′agtattgttttgccaagttctaat3′) and one to *Foxg1* sequences (5′tcctataagttgaatggtattttg3′) downstream of the 3′ *Foxg1* sequence present in the targeting vector. The frequency of G418-resistant clones in which homologous recombination occurred was 1:100, identical to that previously obtained with a similar *Foxg1* targeting construct (Xuan *et al.*, 1995). Two ES cell clones that underwent homologous recombination were injected into blastocysts and gave rise to chimeric mice (University of Cincinnati, Gene-Targeted Mouse Service). Germ-line transmission of the Foxg1–Cre allele was confirmed by genomic Southern blot analysis as described (Xuan *et al.*, 1995). In initial studies, the pattern of Cre activity was assessed in both Foxg1–Cre mouse lines. As expected from integration of the construct into the same genetic locus in both lines, the patterns observed were identical (data not shown). Therefore only one of the two Foxg1–Cre lines was used subsequently for the studies described here. This line is designated TgH(Foxg1-Cre)1Skm in accordance with the International Committee on Standardized Genetic Nomenclature for Mice.

Maintenance of mouse lines. All four mouse lines used in this study, Foxg1–Cre, R26R (Soriano, 1999), Z/AP (Lobe *et al.*, 1999), and P2Bc (generous gift from Stephen O'Gorman), were maintained as heterozygotes (Foxg1–Cre, Z/AP, and P2Bc mice are not viable as homozygotes). Foxg1–Cre, R26R, and Z/AP mice were backcrossed to several strains to assess the potential influence of genetic background on the Cre-mediated recombination pattern (see Fig. 4 and Table 1). Pups were genotyped by PCR with primers to *cre* (Foxg1–Cre), to *lacZ* (R26R and Z/AP), or to RNA pol II and PGK (P2Bc).

RNA in situ hybridization analyses. E10.5, E12.5, and E16.5 embryos and brains from adults generated from crosses between Foxg1–Cre heterozygous mice and wild-type mice from mixed strain backgrounds were collected. Mice were genotyped by genomic Southern blots to determine which carried the Foxg1–Cre allele. Frozen sections were prepared and hybridized as previously described (Frantz *et al.*, 1994). Either the entire *cre* open reading frame or a *Foxg1* 3′ untranslated sequence (provided by Michael Depew) was used to generate an antisense probe. Emulsion-dipped slides were exposed for 3 days and developed. A littermate not carrying the allele was used as a negative control for each age and

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showed no hybridization with the antisense *cre* probe (data not shown).

Whole-mount staining for lacZ and human placental alkaline phosphatase (hPLAP). Embryos from E7.5 to E13.5 and brains from E12.5 to E14.5 were dissected in PBS for whole-mount staining. For embryos E9.5 and older, holes were punctured throughout the embryos with 30- to 22-gauge needles, depending on the age, to allow for quicker and more even penetration of staining substrates as well as to avoid product trapping. For both *lacZ* and hPLAP staining, samples were fixed in PBS with 4% paraformaldehyde, 5 mM EGTA, and 2 mM $MgCl_2$ at 4°C for 30 min to 3 h depending on the age of the embryos. For *lacZ* staining, samples were then rinsed three times in 0.1 M phosphate buffer (pH 7.3) with 2 mM $MgCl_2$, 0.01% sodium deoxycholate, and 0.02% NP-40 and then stained at 37°C for 4 h in this same phosphate buffer supplemented with 1 mg/ml Xgal and 5 mM each potassium ferricyanide and potassium ferrocyanide. For hPLAP staining, after fixation, samples were rinsed twice in PBS, incubated in PBS at 74°C for 40 min to inactivate endogenous phosphatases, rinsed twice in 100 mM Tris–Cl (pH 9.5) with 100 mM NaCl and 10 mM $MgCl_2$, and stained for 4 h at room temperature with the BM purple substrate (Boehringer Mannheim No. 1442074) supplemented with 0.1% Tween. To stop staining, *lacZ* and hPLAP samples were rinsed in 20 mM EDTA in phosphate buffer, postfixed for several hours in 2% glutaraldehyde in phosphate buffer, then stored at 4°C in phosphate buffer. Samples to be sectioned were dehydrated and embedded in plastic (JB-4 Embedding Kit; Polysciences, Inc., No. 00226). Ten-micrometer sections were cut and counterstained with Nuclear Fast red. For initial experiments, all embryos were genotyped by Southern blot analysis or PCR to determine which embryos inherited both the Foxg1–Cre allele and the *loxP* reporter allele. However, since no background staining was ever observed in embryos that did not inherit both the Foxg1–Cre and the *loxP* reporter alleles, and since embryos older than E8.5 that inherited both alleles always showed staining, genotyping was omitted for subsequent experiments on embryos E8.75 and older. For embryos between E7.5 and E8.5, the yolk sac and amnion were collected for genotyping.

RESULTS

Cre Targeted to the Foxg1 Locus Is Expressed in a Foxg1 Pattern

To achieve conditional gene expression in the telencephalon using the Cre/*loxP* system, the sequence encoding the Cre recombinase was targeted to a gene with telencephalon-specific expression, *Foxg1* (also known as *BF-1*), to generate a mouse line with a Foxg1–Cre allele (Fig. 1A). To determine whether the expression pattern of *cre* in Foxg1–Cre embryos matches the pattern of *Foxg1* expression, RNA *in situ* hybridization analysis was performed on serial sections of Foxg1–Cre embryos and adult brains. At all ages the pattern of *cre* mRNA expression matched that of *Foxg1* (Figs. 1B, 1D, and 1E). For example, at E12.5, *cre* mRNA is detected in the dorsal and ventral telencephalon, developing ear, olfactory epithelium, and foregut (Figs. 1B and 1E), as is *Foxg1* (Fig. 1D and Xuan *et al.*, 1995).

To ascertain whether this expression pattern results in a tissue-specific pattern of Cre-mediated recombination, Foxg1–Cre mice were crossed to three mouse lines that each carry a different *loxP* target allele. The first line, P2Bc, carries *loxP* sites at the locus encoding the large subunit of RNA Pol II (gift from Steven O'Gorman). On genomic Southern blots, the recombined allele at this locus is easily distinguished from the unrecombined and the wild-type alleles using an RNA Pol II genomic DNA fragment as a probe (plasmid pOG257A, gift from S.O'G.). Southern analysis of genomic DNA from body parts of embryonic and postnatal Foxg1–Cre;P2Bc mice revealed the extent of recombination in each sample. For example, in the E14.5 embryo shown in Fig. 1C, DNA from the cerebral cortex appeared to be recombined completely, whereas in most other body parts little recombination was detected. Because *Foxg1* is expressed outside of the telencephalon in small, localized areas of the face, head, and neck (Tao and Lai, 1992), it is not surprising that the DNA from these regions exhibited a low level of recombination. Little recombination was detected in the body and tail (Fig. 1C), consistent with the reported pattern of *Foxg1* expression; however, in other animals, recombination levels were variable, ranging from undetectable to highly recombined (data not shown). Experiments described below suggest that this variability may be due in part to the mixed genetic background of the mice used in this experiment.

The Recombination Pattern in Foxg1–Cre Mice Closely Matches the Expression Pattern of Foxg1

To obtain a better histological representation of Cre-mediated recombination, Foxg1–Cre mice were crossed to two reporter lines, ROSA26 Reporter mice and Z/AP mice. Z/AP mice ubiquitously express *lacZ* (βgeo) prior to Cre-mediated recombination and the human placental alkaline phosphatase after recombination (Lobe *et al.*, 1999); results using Z/AP mice are described later. R26R mice express *lacZ* ubiquitously upon recombination (Soriano, 1999). After Foxg1–Cre mice were crossed to R26R mice, the patterns of recombination observed by *lacZ* staining in embryos between E7.5 and E13.5 were compared to the previously described *Foxg1* expression pattern.

Tissue-specific recombination is first detected at E8.5 in the anterior tip of the head folds and the immediately underlying ectoderm (the anterior neural ridge, or ANR; Fig. 2A). Scattered stained cells are also detected in the prospective otic vesicle. Within a few hours, staining extends posteriorly into the budding telencephalon and ventrally along the ectoderm that eventually covers the proximal part of the branchial arches (Fig. 2B). At this stage, staining is apparent in the otic vesicle. By E9.5, most of the telencephalon expresses *lacZ*, as does the anterior half of the optic vesicle (Fig. 2C). In all these cases, the staining pattern matches the developmental pattern of *Foxg1* expression (Tao and Lai, 1992; Shimamura *et al.*, 1995).

The ectoderm surrounding the branchial arches and the anterior half of the head is also stained at E9.5. One day later, at E10.5, the precursor to the lens of the eye, the

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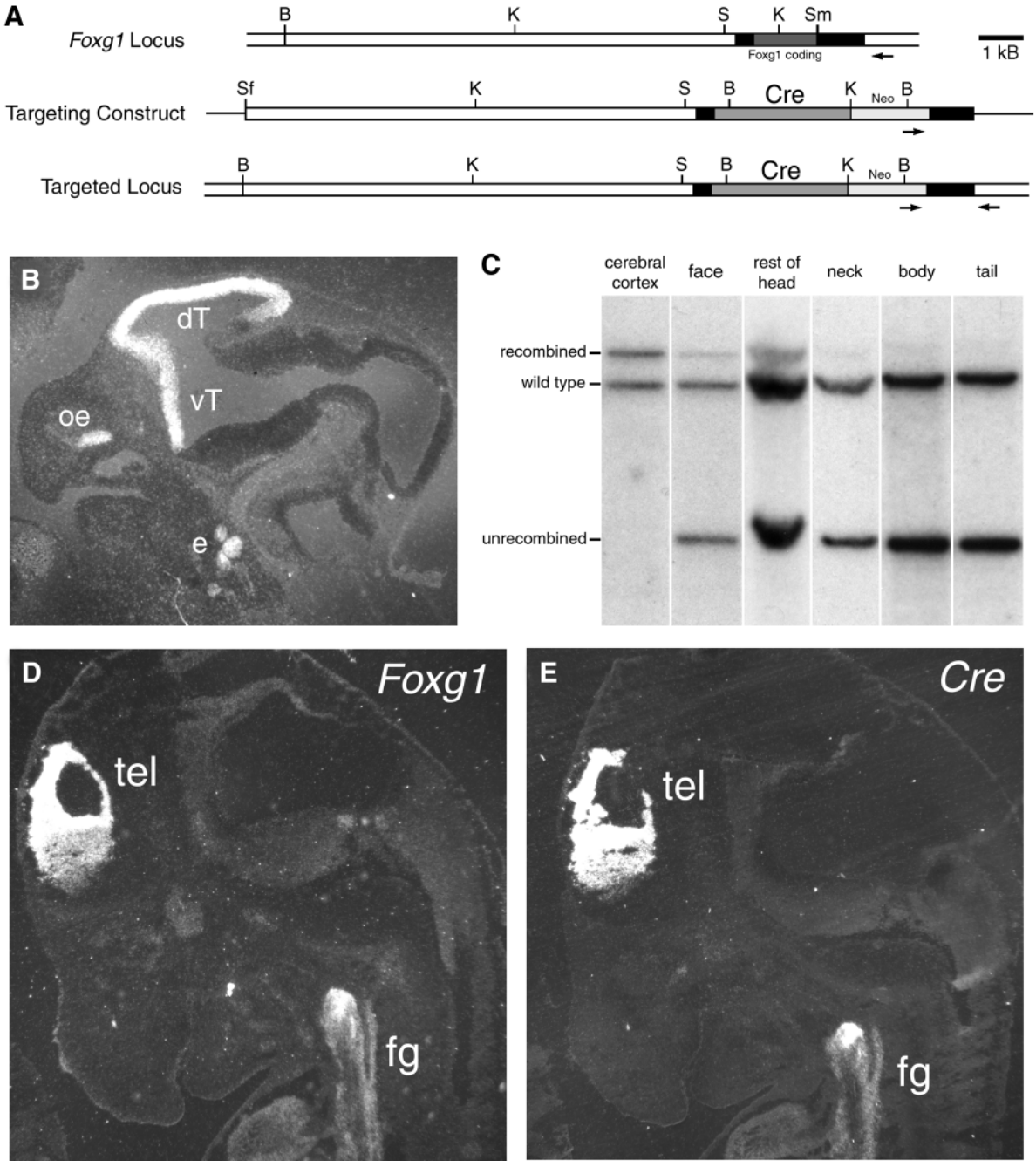

FIG. 1. Targeting of *cre* to the *Foxg1* locus and expression of Cre activity. (A) Illustration of the *Foxg1* locus and the DNA construct used to target *cre* to it by homologous recombination. Arrows indicate the locations of the primers used in screening for homologous recombinants. Abbreviations: Neo, neomycin resistance selection cassette; B, *Bam*HI; K, *Kpn*I; S, *Sal*I; Sf, *Sfi*I. (B) *In situ* RNA hybridization using an antisense *cre* probe on a section of an E12.5 heterozygous Foxg1–Cre embryo from a mixed strain background. Only the head is shown. Abbreviations: dT, dorsal telencephalon; vT, ventral telencephalon; e, ear; oe, olfactory epithelium. (C) Genomic Southern blot analysis of DNA extracted from the body parts of an E14.5 Foxg1–Cre;P2Bc embryo probed with an RNA Pol II fragment (P2Bc mice, provided by S. O'Gorman, have two *loxP* sites at the large subunit of RNA Pol II locus). The sample labeled "rest of head" lacks telencephalic and face DNA. (D, E) *In situ* RNA hybridization with a *Foxg1* probe (D) and a *cre* probe (E) on serial sections of an E12.5 heterozygous Foxg1–Cre embryo. Abbreviations: tel, telencephalon; fg, foregut.

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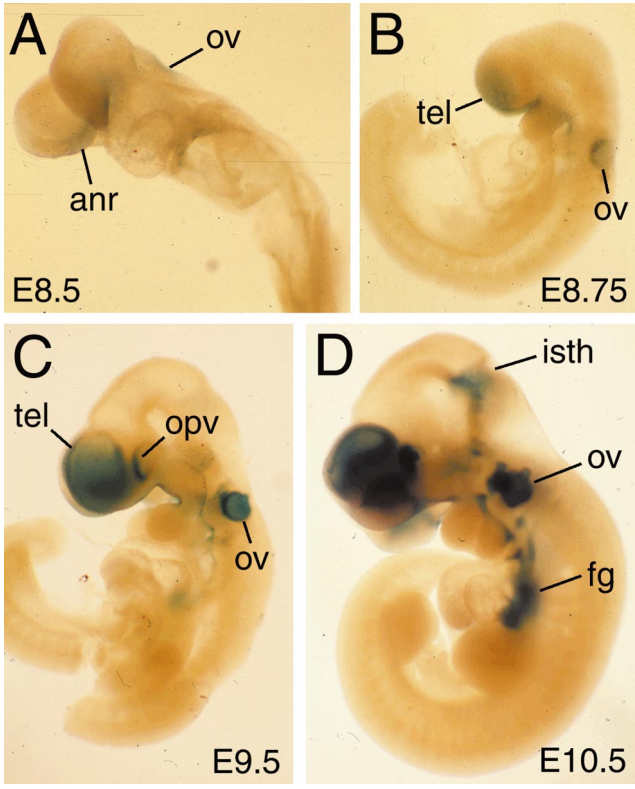

FIG. 2. Developmental onset and time course of Cre recombination in Foxg1–Cre;R26R embryos. Foxg1–Cre;R26R embryos were derived from parents that were backcrossed to Swiss Webster mice for at least three generations. Whole-mount embryos were then stained for *lacZ* expression. (A) At E8.5, the first signs of *lacZ* staining are visible at the anterior neural ridge (anr) and at the presumptive otic vesicle (ov). (B) Shortly thereafter, by E8.75, staining is apparent in the telencephalon (tel) and otic vesicle. (C) In E9.5 embryos, staining is stronger and similar in pattern to that seen earlier, with additional staining now present in the anterior region of the optic vesicle (opv). (D) At E10.5, strong *lacZ* expression is visible in the regions described above, as well as at the mid–hindbrain junction or isthmus (isth) and in the foregut (fg).

olfactory epithelium, the mid–hindbrain junction (isthmus), and the foregut are stained (Fig. 2D). This pattern is maintained with little change at E11.5 (Fig. 3A), with additional staining in the optic stalk. All the tissues that are stained for *lacZ* at E11.5, except the lens and head ectoderm, express *Foxg1* (Tao and Lai, 1992; Hatini *et al.*, 1994; Dou *et al.*, 1999; Eseng Lai, personal communication). Staining in lens and head ectoderm may have resulted from inheritance of the recombined R26R allele from precursor cells that did express *Foxg1* and *cre*. Fate mapping of the ANR, which expresses *Foxg1* at E8.0, suggests that ANR derivatives contribute in part to the formation of the facial and anterior head ectoderm (Couly and Le Douarin, 1988; Osumi-Yamashita *et al.*, 1994). Cells of the developing lens may inherit the recombined locus from the surface ectoderm, which is induced to form the lens by the optic vesicle.

In sections through the head of an E11.5 Foxg1–Cre;R26R embryo, the telencephalon and otic vesicle display extensive *lacZ* staining (Fig. 3E). Staining is also detected in the ectoderm surrounding the forebrain and in scattered cells in the diencephalon and hindbrain. Recombination at the R26R locus occurs in all telencephalic derivatives and appears efficient and uniform, as observed in sections through the E13.5 cerebral cortex (Fig. 3F), hippocampus (Fig. 3G), and basal telencephalon (Fig. 3J). The vast majority of cells in both the neuroepithelial progenitor cell layers and the differentiated cell layers are stained for *lacZ*. Staining was also observed in scattered cells in the E13.5 thalamus and hypothalamus (data not shown), where *Foxg1* is not known to be expressed.

The E11.5 dorsal telencephalic midline and the E13.5 choroid plexus, tissues that do not express *Foxg1* (Tao and Lai, 1992), are also stained to varying degrees (Figs. 3H and 3K). These tissues are derived from the medial telencephalon, where *Foxg1* is expressed prior to the formation of the superior bridge that comes to separate the lateral ventricles (Tao and Lai, 1992). Cells of the dorsal midline and choroid plexus are therefore likely to have inherited the recombined R26R allele from earlier medial telencephalic precursor cells.

Finally, a close match between Foxg1–Cre recombination and *Foxg1* expression is apparent in the E11.5 optic cup. Uniform *lacZ* staining occurs only in the anterior (nasal) half of the developing retina (Fig. 3I), the region to which *Foxg1* expression is restricted (Hatini *et al.*, 1994). The optic stalk, which also expresses *Foxg1*, shows some staining at E11.5 (data not shown), as does the anterior half of the retinal pigmented epithelium (RPE; Fig. 3I). It is not known if the RPE itself expresses *Foxg1*, but its immediate precursor, the medial anterior (nasal) quadrant of the optic vesicle, does express *Foxg1* (Hatini *et al.*, 1994).

Comparison between Recombination Patterns Observed with R26R and Z/AP Mice

To ascertain whether different *loxP* target alleles result in distinct patterns of recombination, we compared staining in Foxg1–Cre;Z/AP and Foxg1–Cre;R26R embryos. The pattern of staining in Foxg1–Cre;Z/AP mice was similar to that obtained with R26R mice, with hPLAP expression observed primarily in the telencephalon, otic vesicle, foregut, mid–hindbrain junction, anterior retina, and olfactory epithelium (Figs. 4A, 4E, and 4F and data not shown). When staining encompassed more widespread patterns (as observed in some strain backgrounds; see section below), these patterns matched those observed in Foxg1–Cre;R26R embryos, with more extensive recombination primarily in the CNS and the digestive system (Figs .4E, 4F, 5C, and 5D). Two striking differences, however, were observed when the Foxg1–Cre;R26R and Foxg1–Cre;Z/AP recombination patterns were compared.

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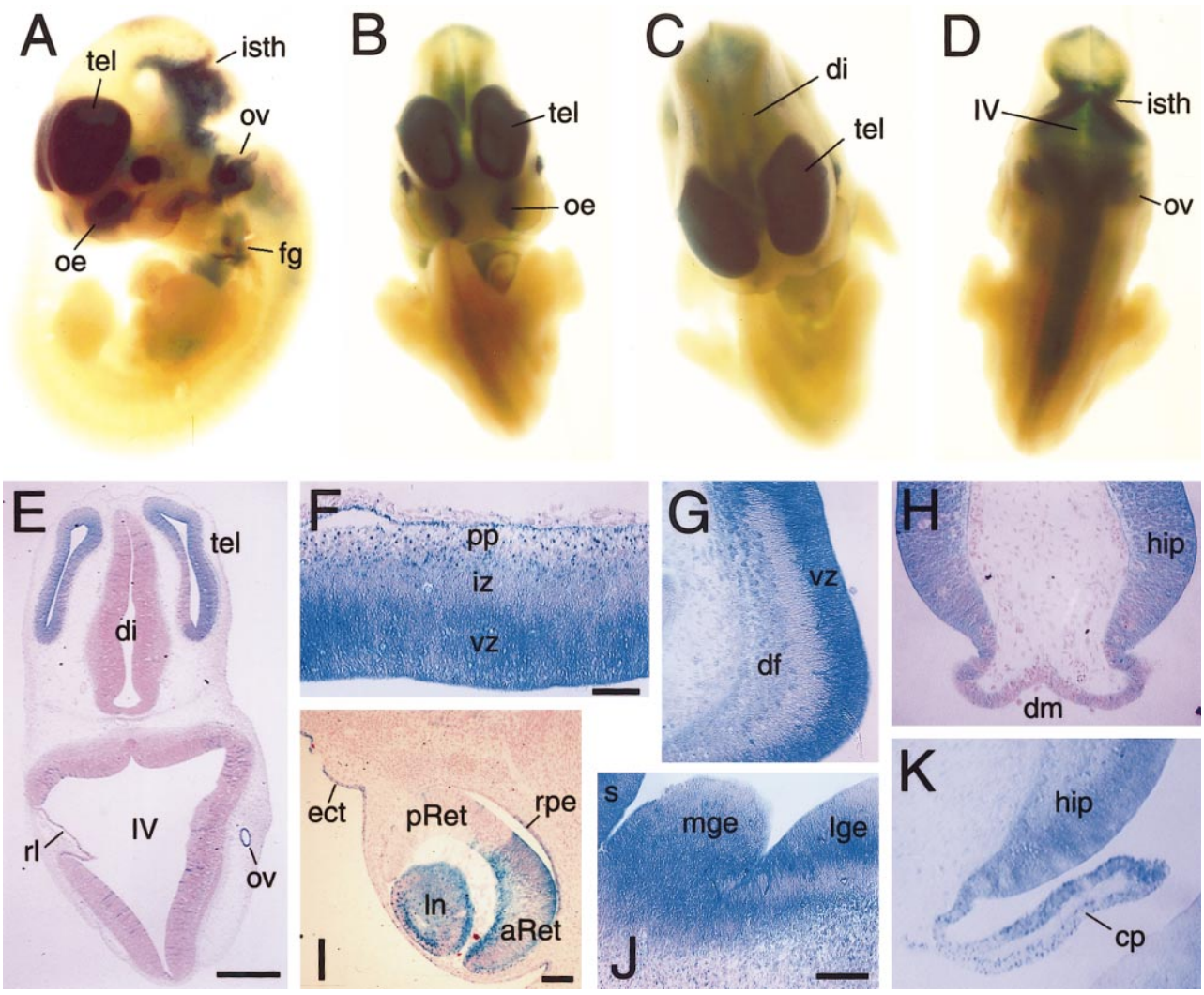

FIG. 3. Pattern of Cre-mediated recombination in Foxg1–Cre;R26R embryos. All embryos are derived from parents that were backcrossed for at least three generations to either 129SvJ or Swiss Webster mice. Whole-mounted embryos (A–D) and sections (E–K) were stained to reveal *lacZ* expression (blue); sections were also counterstained with Nuclear Fast red. (A) E11.5 embryo, showing robust *lacZ* expression in the telencephalon (tel), olfactory epithelium (oe), mid–hindbrain region or isthmus (isth), otic vesicle (ov), and foregut (fg). (B–D) Ventral, top, and dorsal views of an E11.5 embryo with a *lacZ* expression pattern similar to that in (A). (E) Cross section through an E11.5 Foxg1–Cre;R26R head. Staining is apparent in the telencephalon (tel), with scattered cells visible in the diencephalon (di) and in tissue surrounding the fourth ventricle (IV) and rhombic lip (rl). The otic vesicle (ov) shows strong *lacZ* expression. Scale bar, 50 μm. (F) Coronal section through the E13.5 cerebral wall, suggesting that all or nearly all cells express *lacZ*. Abbreviations: pp, preplate; iz, intermediate zone; vz, ventricular zone. Scale bar, 50 μm. (G) Coronal section through the E13.5 hippocampus, with *lacZ* staining apparent in the ventricular zone (vz) and in the differentiating field (df). Scale bar, 100 μm. (H) Cross section through the dorsomedial telencephalon at E11.5 showing scattered staining in the dorsal midline (dm) and abundant staining in the hippocampus (hip). (I) Horizontal section through an E11.5 eye. While *lacZ* staining is apparent in the anterior (nasal) retina (aRet) and lens (ln), little recombination is observed in posterior (temporal) retina (pRet). Staining is also present in the anterior retinal pigmented epithelium (rpe) and in the facial ectoderm (ect). Scale bar, 100 μm. (J) Coronal section through the E13.5 septum (s), medial ganglionic eminence (mge), and lateral ganglionic eminence (lge), telencephalic derivatives that show robust Cre-mediated recombination. Scale bar, 100 μm. (K) Coronal section through the E13.5 hippocampus (hip) and choroid plexus (cp) showing extensive staining in both tissues.

The first was that patches of ectodermal cells located along the entire body were frequently stained in Foxg1–Cre; Z/AP embryos, on all strain backgrounds examined (Table 1), but not in Foxg1–Cre;R26R embryos on any strain background. Labeled cells were often found in patches or clusters (Fig. 4D, asterisk) that resembled clones, which

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TABLE 1

Effect of Strain Background on the Recombination Pattern Observed in Foxgl–Cre;R26R and Foxgl–Cre;Z/AP Embryos

Strain:[a]	129SvJ	Swiss Webster	FVB/N	C57BL/6[b]	CD-1	BALB/c
Total No. embryos examined:	19	14	13	9	31	16
No. embryos (R26R, Z/AP) scored as						
I	12 (9,3)	4 (3,1)	1 (1,0)	1 (0,1)	1 (1,0)	1 (1,0)
II	4 (2,2)	6 (5,1)	5 (4,1)	3 (0,3)	6 (4,2)	0 (0,0)
III	1 (1,0)	4 (4,0)	5 (4,1)	2 (0,2)	11 (10,1)	2 (2,0)
IV	2 (2,0)	0 (0,0)	1 (0,1)	2 (0,2)	10 (8,2)	10 (10,0)
V	0 (0,0)	0 (0,0)	1 (0,1)	1 (0,1)	3 (2,1)	3 (3,0)

[a] Foxgl–Cre, R26R, and Z/AP mice were backcrossed for 3 to 5 generations to the 6 strains indicated. Foxgl–Cre mice of 1 strain background were crossed to R26R or Z/AP mice of the same strain background. E11.5 embryos were collected and stained in whole mount for either *lacZ* (R26R) or hPLAP (Z/AP) and classified using the scheme exemplified in Fig. 5, which shows Foxgl–Cre;R26R embryos. The classification scheme was similar for Foxgl–Cre;Z/AP embryos (not shown).

[b] We observed a discrepancy between the staining patterns reported here for C57BL/6 mice, and the pattern of DNA recombination revealed by Southern blot analysis (cf. Fig. 1). Mice carrying the Foxgl–Cre allele and a *loxP* target transgene were each backcrossed for 5 generations to C57BL/6 mice, and the 2 lines were then crossed. In 8/8 E14.5 embryos from 4 separate litters derived from these crosses, high levels of recombination were observed in the tail and hindlimbs by Southern blot analysis (data not shown). In contrast, only 3/9 mice from the same crosses were scored as IV or V when recombination was assessed by staining whole mounts for hPLAP.

could arise if a single ectodermal progenitor cell at a younger developmental age recombined at random and proliferated to give rise to a patch of progeny cells. The size and number of ectodermal patches varied greatly, from a few small patches to abundant staining (Figs. 4A–4C). The second difference between the Foxg1–Cre;Z/AP and the Foxg1–Cre;R26R embryos was in the level of expression or ease of detection of the two reporter loci in particular cell types. The Z/AP locus, when recombined in cells of the anterior head ectoderm, invariably led to a much more intense hPLAP staining relative to other hPLAP-stained tissues than did the recombined R26R locus, which led to relatively weak *lacZ* staining in the anterior head ectoderm (Figs. 3A–3D and 4A–4D). This more intense hPLAP staining was independent of strain background.

Strain Background May Influence the Recombination Pattern Obtained with Foxg1–Cre Mice

Evidence from Southern blot analysis suggested that the strain background might influence the pattern of recombination. When Foxg1–Cre mice on a mixed genetic background were crossed to P2Bc mice, also on a mixed background, the resulting embryos exhibited variable levels of recombination outside the regions of *Foxg1* expression (data not shown). Variable patterns of recombination were also observed when the same Foxg1–Cre mice were crossed to R26R and Z/AP mice on mixed strain backgrounds. To examine the possible influence of genetic background on the pattern of recombination, Foxg1–Cre, R26R, and Z/AP mice were backcrossed for three to five generations to four inbred (129SvJ, FVB/N, C57BL/6, BALB/c) and two outbred (Swiss Webster, CD-1) strains (Table 1). E11.5 embryos were collected from crosses between Foxg1–Cre mice and either R26R or Z/AP mice and were stained for *lacZ* expression (R26R) or hPLAP (Z/AP). Because embryos in 129SvJ and Swiss Webster backgrounds displayed the most consistent *Foxg1*-like patterns of recombination, animals in these two backgrounds were used in the experiments described above in Figs. 2 and 3.

With both R26R and Z/AP reporters, staining outside of regions that express *Foxg1* did not occur at random, but rather was observed repeatedly in the same set of tissues. Based on the extent to which these tissues were stained, embryos were grouped in one of five categories (I through V, Fig. 5). I: These embryos show a pattern of staining closest to that of *Foxg1* expression (Table 1, Fig. 5A). II: These embryos show a *Foxg1* pattern of staining with low levels of additional recombination in the CNS (Fig. 5B), with staining extending from the mid–hindbrain junction caudally along the developing spinal cord, rostrally into the midbrain, and from the telencephalon into the diencephalon. III: These embryos showed more extensive staining along the length of the CNS (Fig. 5C) and into the posterior optic cup. IV: In these embryos, staining occurred ventrally and outside of the CNS, along the developing gut (Fig. 5D). V: In rare cases, staining was observed to varying degrees in most tissues (Fig. 5E). The extensive recombination in these embryos might be explained by early random recombination events, which were detected in some embryos between E7.75 and E8.5 in cells not known to express *Foxg1* (data not shown).

Embryos from 129SvJ or Swiss Webster backgrounds were the most likely to show a restricted, *Foxg1*-like pattern of recombination (Table 1). The BALB/c and CD-1 backgrounds, on the other hand, were least likely to restrict recombination in a tissue-specific pattern. These results

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suggest that genetic background can influence the recombination pattern obtained when using Foxg1–Cre mice, perhaps by modifying the basal level of *cre* expression at the *Foxg1* locus (see Discussion).

DISCUSSION

We have generated and characterized mice that express the bacteriophage recombinase Cre under control of regulatory elements that normally direct the expression of the winged-helix transcription factor Foxg1. Foxg1–Cre mice confer efficient and consistent recombination of *loxP* target loci in the telencephalon and in other head structures, including the developing lens, retina, ear, olfactory epithelium, mid–hindbrain junction (and its derivative cerebellum), facial and head ectoderm, and foregut. That three different *loxP* targets, P2Bc, R26R, and Z/AP, are each recombined in a common tissue-specific pattern suggests that the Foxg1–Cre mice will be generally useful in recombining any *loxP* target locus in this pattern. We did, however, observe two important indications of variability in the precise pattern of Cre-mediated recombination. First, we observed a marked influence of background strain on the overall extent of recombination, with some strains (129SvJ and Swiss Webster) showing patterns matching closely that of *Foxg1* expression and others (CD-1 and BALB/c) exhibiting more extensive recombination in tissues not known to express *Foxg1*. Second, one target allele (Z/AP) showed a variable incidence of what appeared to be a random pattern of recombination in the ectoderm of embryos from all strain backgrounds, suggesting that the *loxP* target locus can influence the recombination pattern obtained with Cre mice. In the present experiments we have employed Foxg1–Cre mice to activate the expression of the marker genes *lacZ* (R26R mice) and hPLAP (Z/AP mice). Foxg1–Cre mice should prove useful not only in overexpressing or ectopically expressing genes, but also in inactivating floxed genes in the telencephalon and other tissues in which *loxP* targets are recombined consistently.

Use of Foxg1–Cre Mice for the Generation of Conditional Mutations

A caveat must be considered in using Foxg1–Cre mice to produce conditional mutations. The first is that the Foxg1–Cre allele is predicted to be a null allele for FOXG1 function, due to replacement of *Foxg1* coding sequences with the *cre* gene. Although animals heterozygous for a null allele at the *Foxg1* locus are wild type in appearance (Xuan *et al.*, 1995), it remains possible (albeit unlikely) that any phenotype resulting from a Cre-mediated conditional mutation could result in part from heterozygosity at the *Foxg1* locus.

Despite this caveat, preliminary evidence suggests that the Foxg1–Cre mice will indeed be useful in studying the function of genes in *Foxg1*-expressing tissues. In these experiments, Foxg1–Cre mice were crossed to mice carrying a floxed *Fgf8* gene. The complete *Fgf8* knockout results in lethality at the time of gastrulation; however, Foxg1–Cre; floxedFgf8 mice survive in Mendelian ratios up to shortly after birth, when they die due to breathing problems. In these animals, *Fgf8* expression is abolished in tissues in which recombination occurs (anterior forebrain, mid–hindbrain isthmus, and pharyngeal region), whereas expression is maintained in the somites and in the AER of the limb (A. Neubüser, personal communication). The conditional *Fgf8* knockout is thus enabling an analysis of *Fgf8* function at later stages of development than was afforded by the traditional knockout and in a specific subset of *Fgf8* expression domains.

Lineal Inheritance of a Recombined Allele

In embryos in which the pattern of Foxg1–Cre-mediated recombination displayed the highest degree of tissue specificity, the bulk of the pattern matched that predicted by the normal expression of *Foxg1*. However, not every tissue that showed *loxP* reporter gene activity is known to express *Foxg1*. One possibility is that such tissues, which included the choroid plexus, anterior head ectoderm, and lens, express low levels of *cre* (although we note that the levels must have been so low as to escape detection by mRNA *in situ* hybridization; Figs. 1B and 1E, and data not shown). However, a likely alternative explanation is that these tissues inherited the recombined alleles from precursor tissues that did express *Foxg1*. For example, *lacZ* and hPLAP staining in the choroid plexus of Foxg1–Cre;R26R and Foxg1–Cre;Z/AP embryos were probably inherited from the E10 medial telencephalon, which expresses *Foxg1* (Tao and Lai, 1992). Similarly, cells of the anterior head ectoderm and lens may have inherited a recombined allele from cells in and around the ANR, in which recombination occurs quite early, by E8.5 (Fig. 2A). The ANR is known both to express *Foxg1* and to generate ectoderm covering the facial and oral region (Couly and Le Douarin, 1988; Serbedzija *et al.*, 1992; Osumi-Yamashita *et al.*, 1994). Other tissues such as the pharyngeal pouches, in which some cells consistently express the recombined *loxP* loci, may be formed, at least in part, by the movement or migration of cells. Interestingly, potentially migratory mesenchymal-like cells expressing the *loxP* reporters were found scattered through E9.5 heads (see Figs. 4E and 4F), but were not usually observed in older E11.5 heads when migration is likely to have ceased (Fig. 3E).

Variability in the Pattern of Foxg1–Cre-Mediated Recombination

Recombination was observed with variable incidence in tissues that do not express *Foxg1* and that are not lineal descendants of cells that do, such as the spinal cord and the mid- and hindgut (Figs. 4E, 4F, 5C, and 5D). In rare cases, recombination even occurred in most tissues of the embryo

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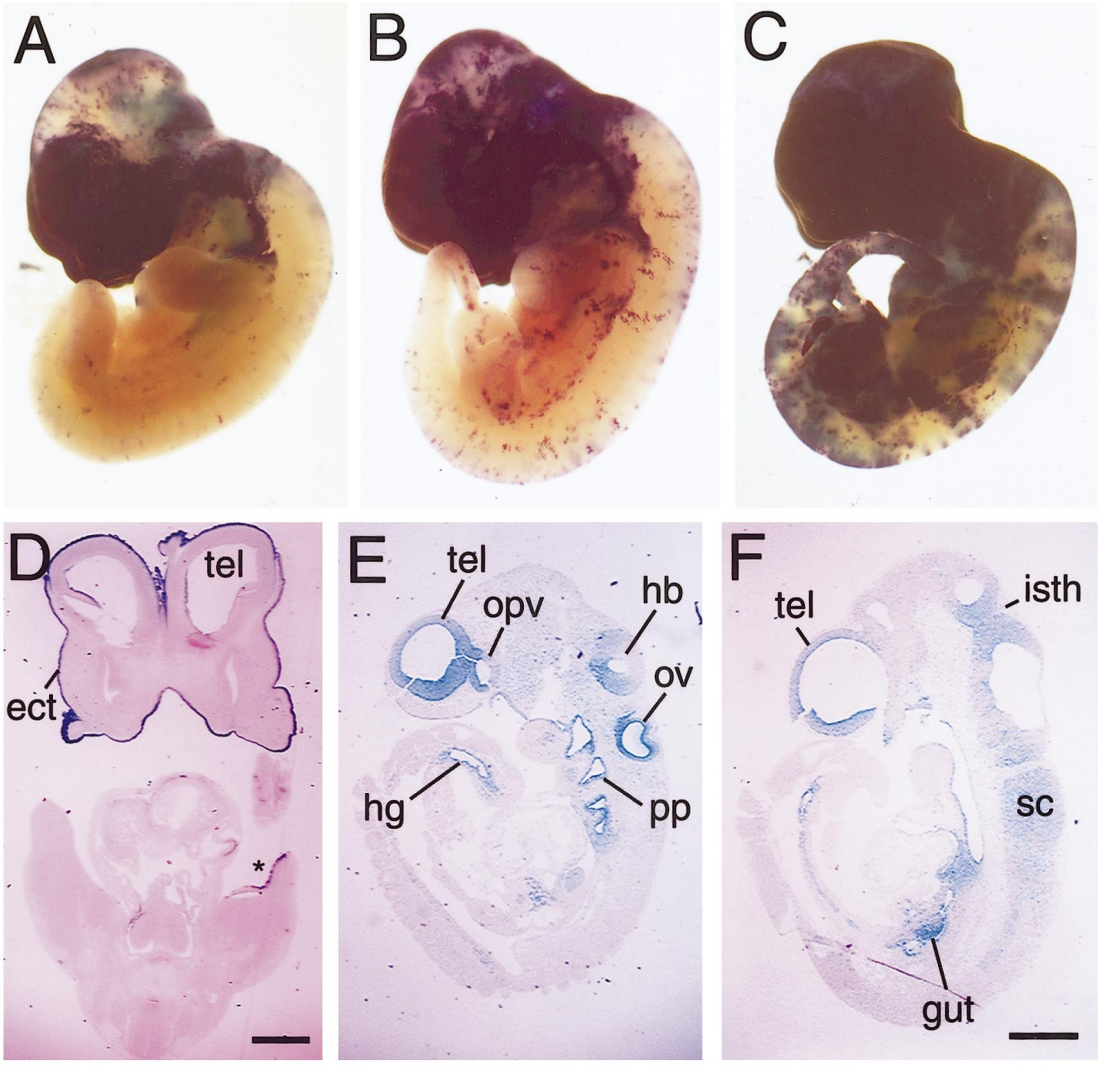

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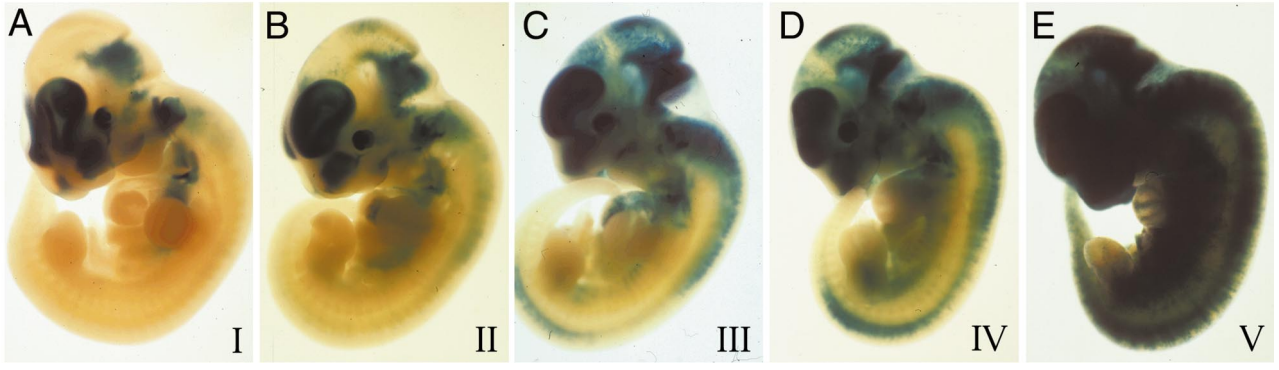

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(Fig. 5E; Table 1). It remains unclear why these more widespread recombination patterns were observed. However, here we have provided evidence that the detailed pattern of Cre-mediated recombination can be influenced by the strain background of the mice (Table 1), raising the question of how strain background could exert this effect. One possibility is that modifier loci, present as different alleles in the different strains, alter a low basal level of *Foxg1* expression. Alternatively, these modifier loci might not influence expression of the wild-type *Foxg1* locus, but instead alter that of the Foxg1–Cre allele, which contains sequences not normally found at this locus. A final possibility is that the activity of the Cre recombinase itself can be modulated by factors present in some strains of mice but not in others. Clearly, further studies using Cre/*loxP* mice are needed to resolve these issues.

To date there has been little evidence that *loxP* target loci themselves can influence the recombination pattern obtained with Cre mice. Evidence provided here suggests that this can indeed occur. Although both the R26R and the Z/AP reporter mice generated similar overall recombination patterns when crossed to Foxg1–Cre mice, patches of recombined ectodermal cells were present in Foxg1–Cre; Z/AP embryos, regardless of strain background. The patches occurred in various sizes, but the sizes were similar within any individual embryo. Presumably the size of a patch represents the time at which recombination occurred (and the subsequent period of clonal expansion), suggesting that recombination events within the ectoderm were correlated temporally within a given embryo. It is not clear why recombination throughout the ectoderm should occur specifically in crosses using the Z/AP reporter mice. It is unlikely that the Cre recombinase is generally more active in ectoderm, because no such recombination is observed in Foxg1–Cre;R26R embryos, and it is clear that the ROSA26 regulatory elements are capable of driving *lacZ* expression in skin (Soriano, 1999). An alternative explanation is that the Z/AP locus itself is somehow more susceptible than the R26R locus to recombination. Presumably, very low levels of Cre are present in ectoderm and are sufficient to occasionally trigger recombination of the Z/AP allele, but not of other *loxP* targets such as R26R. What could make one *loxP* target more susceptible to recombination than another? It is conceivable, although speculative, that local differences in chromatin structure can alter the efficiency of recombinase activity.

SUMMARY

The Foxg1–Cre mouse line should prove useful in facilitating genetic studies of the telencephalon and its derivatives, as well as the development of the other head structures described here. Collectively our studies suggest that the exact pattern of Cre-mediated recombination must be analyzed in a manner specific to the *loxP* locus and the genetic background of the mice involved in each study, but that with due caution such mice will provide enormously valuable tools for the targeted disruption of genetic loci hypothesized to play key roles in development or adult function.

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FIG. 4. Pattern of Cre-mediated recombination in Foxg1–Cre;Z/AP embryos. All embryos were stained for hPLAP expression (blue), and sections were counterstained with Nuclear Fast red. (A–C) E11.5 whole-mount embryos stained for equal amounts of time under identical conditions. The embryos display varying degrees of hPLAP staining in the ectoderm, with the embryo in (A) showing only a few clusters of hPLAP-labeled cells in the ectoderm, the embryo in (B) showing many such clusters, and the embryo in (C) showing the most hPLAP staining. (D) Transverse section through an E11.5 embryo comparable to that shown in (A), illustrating strong staining for hPLAP in the facial and anterior head ectoderm (ect). This embryo was stained in whole mount and then sectioned. The telencephalon (tel) appears only weakly stained due to poor penetration of the hPLAP substrate. The asterisk marks a patch of hPLAP-positive ectodermal cells on the limb bud. Scale bar, 0.5 mm. (E, F) Parasagittal sections through an E9.5 embryo from a mixed strain background showing hPLAP staining in a variety of structures: hb, hindbrain; hg, hindgut; isth, isthmus; opv, optic vesicle; ov, otic vesicle; pp, pharyngeal pouches; sc, spinal cord; tel, telencephalon. Scale bar, 0.5 mm.

FIG. 5. Range of recombination patterns obtained with Foxg1–Cre;R26R embryos. E11.5 embryos were stained for *lacZ* expression under identical conditions for equal amounts of time. These embryos serve as examples for each category of recombination pattern used in Table 1. Embryos were classified as: (A) "I" with a pattern of recombination most closely resembling that of the normal expression pattern of *Foxg1*, (B) "II" with additional recombination in the CNS, (C) "III" with more extensive recombination in the CNS, (D) "IV" with additional recombination in the mid- and hindguts, or (E) "V" with recombination detected in most tissues.

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