On Somatic Recombination in the Central Nervous System of Transgenic Mice

Complete immunoglobulin (Ig) and T cell receptor (TCR) genes are generated by developmentally controlled DNA rearrangement referred to as V(D)J recombination or V(D)J joining (*1*). V(D)J recombination has been thought to be restricted to lymphoid cells and, until recently, there had been no evidence that recombination signal sequence (RSS)–mediated recombination of non-Ig or non-TCR genes occurred during normal vertebrate development. However, it had been speculated that this or similar types of somatic DNA rearrangement might play a role in mammalian cell differentiation (*2*). Interest in this hypothesis was refueled by the report that *RAG-1* transcripts were present, albeit in low numbers, in the central nervous system (*3*). Furthermore, it was reported that somatic recombination was detectable in the brains (*4*) of Tg mice that harbored a V(D)J recombination substrate. A second study (*5*) reported a small amount of V(D)J recombination in the brain, but this was attributed to lymphocyte contamination. We had independently constructed similar Tg mice whose initial analysis (*6*) generated data consistent with the occurrence of V(D)J recombination in the brain. However, further analysis has led us to a different interpretation of that data.

In order to prepare a Tg V(D)J recombination substrate, we constructed a plasmid pSPH-1 (*7*). This plasmid contained the transcriptional promoter and enhancer of the mouse phosphoglycerate kinase-1 gene (*pgk-1*) (*8*), a pair of RSSs derived from the Ig $V_{\kappa 21C}$ and $J_{\kappa 1}$ gene segments (*9*), and a reporter gene *lacZ* encoding bacterial β-galactosidase. The reporter gene *lacZ* was placed in an orientation opposite to that of the promoter so that RSS-mediated inversional recombination would activate its expression (Fig. 1). Cells expressing *lacZ* can be detected histochemically after staining with X-gal. Alternatively, inversional recombination can be detected more directly at the molecular level by the polymerase chain reaction (PCR) method (*10*) with the use of appropriate primers. We used both of these methods.

We generated Tg mice by injecting the pSPH1 insert into C57BL/6J zygotes (*11*). We analyzed the heterozygous progeny of five Tg lines (1-7, 1-20, 1-21, 1-28, and 1-39) for expression of β-galactosidase activity by histochemistry (*12*). Liver sections of all Tg lines were negative for β-galactosidase activity. We observed considerable enzyme activity in kidney, spleen, and thymus sections, but also in the non-Tg littermates (presumably because of endogenous β-galactosidase activity). We observed β-galactosidase activity in the brain of two of the five Tg lines, 1-7 (Fig. 2) and 1-20; we saw no such activity in non-Tg littermates. Regions of the brain in which we saw intense β-galactosidase activity in the Tg line 1-7 (Fig. 2) include the hippocampus (the dentate gyrus and the CA1 and CA3 fields), the cerebral cortex (especially the superficial layers), the superior colliculus (upper layers), nuclei of the dorsal tegmentum, and the cerebellum (especially its molecular and Purkinje cell layers). Sparse β-galactosidase activity also appeared in other sites (Fig. 2A). There was low expression in the striatum and in much of the thalamus. In Tg line 1-20, we observed a roughly similar pattern of X-gal staining, but the staining was weak. In both lines, the X-gal staining was region-specific. Cells with β-galactosidase activity appeared to be neurons in both Tg lines. The staining appeared to be limited to a small (2 to 5 μm), eccentrically located compartment of the cytoplasm (Fig. 2, C and E). Both we and Matsuoka *et al.* detected X-gal staining in the cerebral cortex and the hippocampus, but the patterns differed considerably in other regions, such as the cerebellum, where Matsuoka *et al.* saw X-gal staining in the granule and Purkinje cell layers and we saw staining primarily in the molecular cell layer.

In order to test whether RSS-mediated inversion had occurred in some tissues of the Tg mice, we analyzed genomic DNA by PCR using primers 1 and 2 (Fig. 1). If the Tg RSSs underwent an inversional V(D)J recombination, a 328-bp DNA fragment containing the joined RSSs would be generated by PCR (Fig. 1). The predicted 328-bp product was observed with the DNA isolated from the thymus of the Tg line 1-7, but not with that from non-Tg mice or from any of the other four Tg lines (1-20, 1-21, 1-28, and 1-39) (Fig. 3A). We cloned the 328-bp fragment and determined its nucleotide sequence. It contained the precisely head-to-head joined Tg RSSs. We analyzed DNA isolated from additional tissues of each Tg line, but none of the tissues derived from any Tg lines other than line 1-7 produced the 328-bp DNA fragment. In line 1-7, only spleen and thymus produced the 328-bp DNA fragment; and liver, cerebral cortex, ovary, muscle, kidney, and lung were negative (Figs. 3, B and C). We also analyzed DNA isolated from cerebral cortex and hippocampus of line 1-7, regions in which β-galactosidase–positive cells were abundant (Fig. 2) and DNA isolated from the striatum, where those cells were rare (Fig. 2). We did not detect the 328-bp DNA fragment in these tissues (Figs. 3, B and C).

We estimated the sensitivity of this PCR assay for the detection of V(D)J recombination by analyzing a fixed amount of non-Tg thymus DNA mixed with different amounts of Tg (line 1-7) thymus DNA (Fig. 3C). The 328-bp DNA fragment was detectable in DNA samples in which the Tg thymus DNA constituted only 1 part in 1000 of the total DNA. As only a fraction of the thymus cells from the Tg mice would have undergone RSS-mediated recombination, these data indicate that the sensitivity of the PCR method is at least 1 in 1000 cells. In some parts of the brain the proportion of β-galactosidase–positive cells among total nucleated cells far exceeded the sensitivity of the PCR method. For instance, we estimated their proportion in the cerebral cortex to be 1 to 10% (*13*), a figure at least one to two orders of magnitude greater than the PCR detection limit. We therefore conclude that the majority of β-galactosidase–positive cells observed did not result from RSS-mediated inversion. We also analyzed the absolute sensitivity of this PCR assay by carrying out a reconstitution experiment (*14*) in which different amounts of plasmid DNA containing *pgk-1* promoter

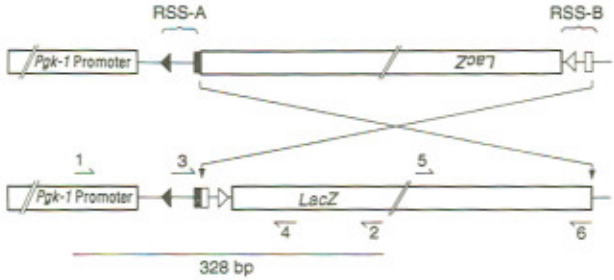

Fig. 1. Schematic representations of the Tg V(D)J recombination substrate (top) and the predicted product (bottom) of a V(D)J recombination event. (Top) In the substrate the bacterial β-galactosidase gene, *lacZ*, is oriented inversely to the *pgk-1* promoter. Recombination signal sequences (RSS-A and RSS-B) flank *lacZ* and are comprised of heptamer (rectangle), nonamer (triangle), and spacer elements. (Bottom) V(D)J recombination of the Tg substrate is expected to join the two RSSs precisely and to invert *lacZ*, thereby activating its transcription by the *pgk-1* promoter. Oligonucleotide primers used for PCR amplification (small arrows) are numbered. PCR amplification of the predicted product of a V(D)J recombination event with primers 1 and 2 results in the 328-bp product indicated at bottom.

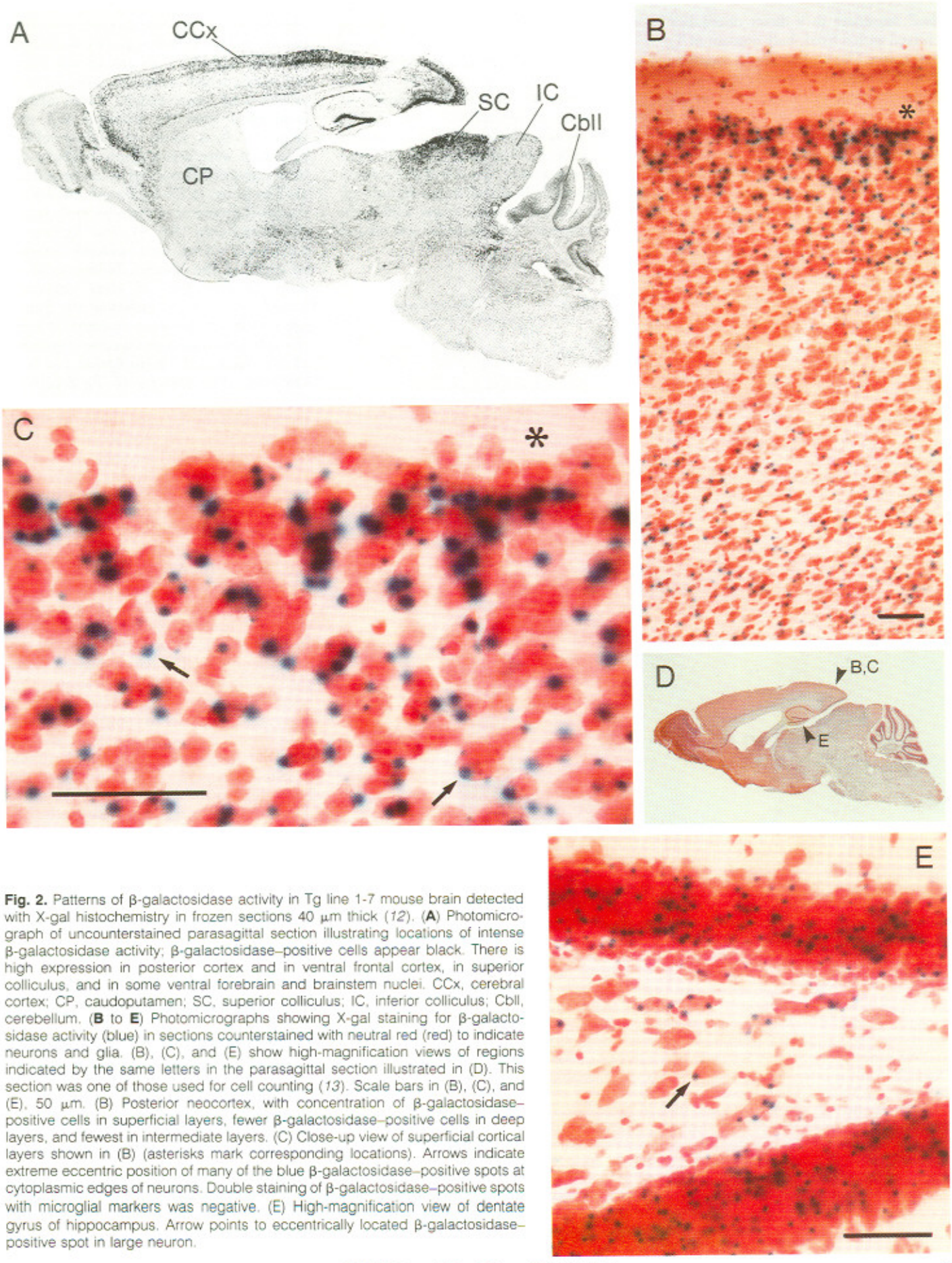

Fig. 2. Patterns of β-galactosidase activity in Tg line 1-7 mouse brain detected with X-gal histochemistry in frozen sections 40 μm thick (*12*). (**A**) Photomicrograph of uncounterstained parasagittal section illustrating locations of intense β-galactosidase activity; β-galactosidase–positive cells appear black. There is high expression in posterior cortex and in ventral frontal cortex, in superior colliculus, and in some ventral forebrain and brainstem nuclei. CCx, cerebral cortex; CP, caudoputamen; SC, superior colliculus; IC, inferior colliculus; CbII, cerebellum. (**B** to **E**) Photomicrographs showing X-gal staining for β-galactosidase activity (blue) in sections counterstained with neutral red (red) to indicate neurons and glia. (B), (C), and (E) show high-magnification views of regions indicated by the same letters in the parasagittal section illustrated in (D). This section was one of those used for cell counting (*13*). Scale bars in (B), (C), and (E), 50 μm. (B) Posterior neocortex, with concentration of β-galactosidase–positive cells in superficial layers, fewer β-galactosidase–positive cells in deep layers, and fewest in intermediate layers. (C) Close-up view of superficial cortical layers shown in (B) (asterisks mark corresponding locations). Arrows indicate extreme eccentric position of many of the blue β-galactosidase–positive spots at cytoplasmic edges of neurons. Double staining of β-galactosidase–positive spots with microglial markers was negative. (E) High-magnification view of dentate gyrus of hippocampus. Arrow points to eccentrically located β-galactosidase–positive spot in large neuron.

and *lacZ* sequences in a direct orientation were mixed with a fixed, bulk amount of Tg cerebral cortex DNA. The results suggested that our PCR conditions would detect as few as one to ten recombination events among 10^5 cells. These results confirmed that, if V(D)J recombination occurs in the nonlymphoid tissues that we examined, it is rare.

If not an RSS-mediated inversion, what mechanism allows β-galactosidase to be expressed in the brain? To answer this question, we synthesized cDNA of *lacZ* mRNA isolated from the brain of Tg line 1-7, cloned the amplified cDNA (*15*), and determined the nucleotide sequences of the clones. Among nine randomly selected clones (Fig. 4), one (1-2) produced sequences that started at RSS-B (Fig. 1). This cDNA did not indicate whether the reporter *lacZ* gene was rearranged. On the other hand, the sequence of each of the remaining eight cDNA clones began farther upstream, and six of these began within the *pgk-1* promoter. The promoter was used in the orientation opposite to the conventional one, and no RSS-A sequence was present on these cDNA's adjacent to the RSS-B sequences, which would be expected if the cDNA had been derived from mRNAs transcribed from the inverted *lacZ* gene (Fig. 1). It is likely that these cDNA sequences were derived from mRNAs that were transcribed from the unrearranged *lacZ* gene by using, in the backward orientation, the *pgk-1* promoter of the adjacently inserted plasmid copy (Fig. 4). The Tg line 1-7 contains about ten such tandemly integrated copies of the plasmid. Bidirectional activity of promoters of some eukaryotic housekeeping genes, including the human *pgk-1* promoter, has been reported (*16*), but the cell type and tissue-specific regulation of the "backward" transcription has been unknown. The patterns of Tg β-galactosidase (*lacZ*) expression that we found in the brains of the Tg mice appear to reflect, at least in part, such regulation.

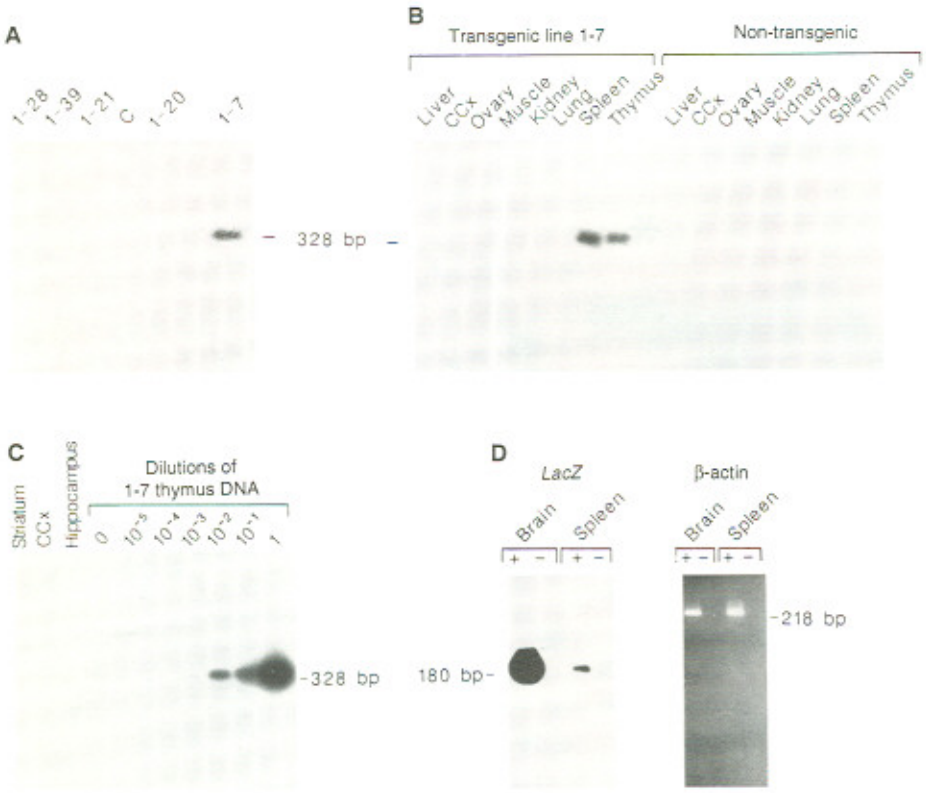

Fig. 3. (**A**) V(D)J recombination of the Tg substrate detected in the thymuses of mice from Tg line 1-7 but not in the thymuses of mice from other Tg lines (1-28, 1-39, 1-21, 1-20) or in the thymus of non-Tg control mice (C). One microgram of genomic DNA was digested with the restriction enzyme Eco RI, precipitated by ethanol, and then subjected to 30 cycles of PCR (*24*) with primers 1 and 2 (Fig. 1). Ten percent of the PCR products was then separated on a 2% agarose gel and examined by DNA (Southern) blotting. ^{32}P-labeled oligonucleotide 29, a 32-bp oligonucleotide that includes RSS-B, was used as a hybridization probe. (**B**) V(D)J recombination of the Tg substrate, which is limited to the lymphoid organs in mice from Tg line 1-7. Assays performed as in (A). CCx, cerebral cortex. (**C**) Sensitivity of the PCR assay. To establish the detection limit of this assay, thymus DNA from strain 1-7 Tg mice was mixed with thymus DNA from non-Tg littermates. The proportion of the Tg DNA in each sample is indicated. Each sample consisted of 1 μg of DNA. Assays were performed as described in (A) except that 40 cycles of PCR were performed instead of 30. A very faint band was visible in the 10^{-5} dilution sample which appeared at very low dilutions of transgene thymus DNA; we presume it is a PCR artifact. CCx, cerebral cortex. (**D**) Abundant transciption of the *lacZ* transgene in the brain. Poly(A)$^+$–positive RNA was isolated (*25*) from mice that had been perfused with 1% sodium nitrate in phosphate-buffered saline and then with deoxyribonuclease I (*26*). One microgram of poly(A)$^+$ RNA was reverse-transcribed (+), or the reverse transcriptase was omitted (−), to establish that PCR products were derived from RNA and were not contaminating genomic DNA. Samples were subjected to 30 cycles of PCR (*24*) with primers 5 and 6 (Fig. 1), and then 10% of the PCR products was separated and examined by Southern blotting as in (A). ^{32}P-labeled oligonucleotide 13 (5′-GTCCAAACTCATCAATGTATCTT-3′) was used as a hybridization probe. PCR analysis was also performed with β-actin–specific primers 11 (5′-GGATGCAGAAGGAGAT TACT-3′) and 12 (5′-AAAACGCAGCTCAGTAACAG-3′) under the same conditions in order to establish that equivalent amounts of cDNA were present in the two samples (right). An agarose gel stained with ethidium bromide is illustrated.

The lack of V(D)J recombination of the Tg substrate in the brain may have resulted from either a lack of recombinase activity or an inaccessibility of the substrate. The latter condition seems to be correlated with the absence of transcripts (*17*). Our ability to clone the cDNA of *lacZ* mRNA from the brain of the Tg line 1-7 suggested that the Tg substrate is accessible in this organ, but there nevertheless remained the possibility that the level of *lacZ* transcription was insufficient for V(D)J recombination to occur. We therefore used a PCR assay with a relatively low number of reaction cycles (that is, 30) in order to compare the levels of *lacZ* transcription in the brain and the spleen of Tg line 1-7. We found the transgene to be transcribed more strongly in the brain than in the spleen (Fig. 3D). These data reinforce the argument that the lack of V(D)J recombination in the brain does not result from the inaccessibility of substrate, although we cannot rule out the possibility that the transgene is inaccessible in restricted regions or during developmental stages of the nervous system where or when V(D)J recombinase is available.

Although we focus on a single transgenic line in this study, we believe our conclusions are valid because in this line V(D)J recombination does take place in the lymphoid organs, and therefore the transgene present in this line is fully capable of undergoing V(D)J recombination. We, of course, do not rule out the possibility that a small fraction of the β-galactosidase–positive cells did undergo V(D)J recombination at a frequency that was smaller than the detection limit of the PCR assay. However, this does not change our conclusion that evidence for somatic V(D)J recombination in the brain, if any indeed occurs, is yet to be obtained.

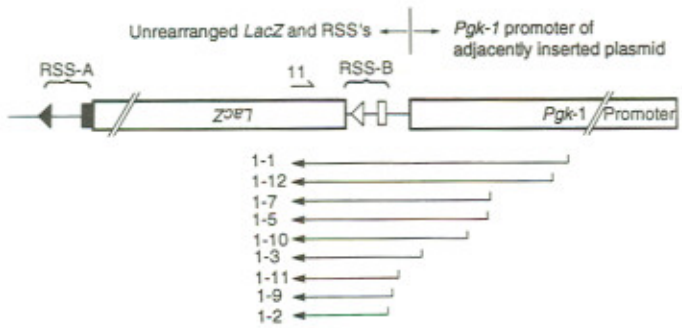

Fig. 4. β-galactosidase mRNA detected in the brains of Tg mice from line 1-7 that are not derived from a recombined transgene. Complementary DNA of mRNA from the brains of line 1-7 Tg mice was amplified by using anchored PCR (*16*). PCR products were then cloned into the vector Bluescript and sequenced as described. The *pgk-1* promoter of an adjacent transgene was present at the 5′ end of all clones that extended beyond the 5′ end of the *lacZ* gene and the adjacent RSS-B. This *pgk-1* promoter, however, is inverted relative to the *lacZ* gene. Individual PCR products are depicted by arrows and are numbered.

This conclusion is at variance with the interpretations drawn by Matsuoka *et al.* (*4*), who used Tg mice that were constructed independently but with a similar strategy. They also found abundant and region-specific expression of *lacZ* in the brain. However, in contrast to our findings with PCR, their PCR assay detected sequences that were apparently produced by inversional recombination of the reporter gene that took place 9 to 138 bp away from the head of the RSSs. Matsuoka *et al.* concluded that "somatic gene rearrangement may be involved in neonatal development" (*18*). Although it is possible that our conclusion differs from that of Matsuoka *et al.* because of variations in the experimental protocols, including differences in the composition of the Tg plasmids, we believe it is more likely that the different conclusions arise from different interpretations of data.

First, in light of our analysis of β-galactosidase cDNA clones, we concluded that the β-galactosidase expression observed in the brains of our Tg mice is most probably due to backward transcription from the promoter of an adjacent transgene rather than RSS-mediated inversion. We suspect that the same may be true for the Tg mice reported by Matsuoka *et al.* because the arrangement of various sequence motifs in the chicken cytoplasmic β-actin promoter that Matsuoka *et al.* used is similar to that in the chicken skeletal α-actin promoter, a demonstrated bidirectional promoter (*19*).

Second, we did not detect any evidence of V(D)J recombination with brain DNA using PCR. In contrast, Matsuoka *et al.* cloned PCR products that they interpret as having been derived from "imprecise" V(D)J recombination that had occurred at sites 9 to 138 bp away from the heads of the RSSs. There are at least two other interpretations of this finding. Recombination may have occurred in vitro during PCR amplification by "PCR mediated recombination" (*20*). The two parental sequences involved in each recombination event reported by Matsuoka *et al.* carry 10-, 3-, and 2-bp homologies, respectively, at the recombination sites. Short homology is expected at the site of PCR recombination, whereas it is more an exception than a rule in the noncoding joints of V(D)J recombination. In one published case 12 nucleotides (*21*), and in another study 7 and 5 nucleotides (*22*), have been shown to be sufficient for PCR recombination. If PCR recombination accounted for the sequences that Matsuoka *et al.* observed, the apparent tissue specificity of the PCR products (*5*, figure 2) may have resulted from sample-to-sample variation, which is commonly encountered with PCR artifacts. Another possibility is that the observed joints resulted from rare illegitimate recombination events that took place among the 15 copies of the integrated plasmid that would be unrelated to developmentally meaningful somatic recombination. Transgenes are generally unstable genetic elements, and short stretches of homology of one to five nucleotides at the junction are generally observed in illegitimate recombination (*23*).

In summary, we suggest that it is premature to conclude whether or not developmentally meaningful somatic recombination occurs in the brain. However, a positive and interesting finding that emerged from our study (and possibly also from that of Matsuoka *et al.*) is that backward transcription in the brain can occur in a highly region- and neuron-specific manner. The physiological role of backward transcription is unknown, but in light of its remarkable tissue or organ specificity, it is possible that backward transcription may participate in the regulation of genes associated with a bidirectional promoter, including genes in the central nervous system.

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REFERENCES AND NOTES

1. C. Brack, M. Hirama, R. Lenhard-Schuller, S. Tonegawa, *Cell* **15**, 1 (1978); S. Tonegawa, *Nature* **302**, 575 (1983).
2. N. K. Jerne, in *The Neurosciences*, G. C. Quarton, T. Melnechuk, F. O. Schmitt, Eds. (Rockefeller Univ. Press, New York, 1967), pp. 200–205.
3. J. J. Chun, D. G. Schatz, M. A. Oettinger, R. Jaenisch, D. Baltimore, *Cell* **64**, 189 (1991).
4. M. Matsuoka *et al.*, *Science* **254**, 81 (1991).
5. M. Kawaichi, C. Oka, R. Reeves, M. Kinoshita, T. Honjo, *J. Biol. Chem.* **266**, 18387 (1991).
6. S. Tonegawa, in *Immunology in the XXI Century*, F. W. Alt, Ed. (Sigma Chemical Co., St. Louis, MO, in press).
7. Plasmid pSPH1 was constructed as follows. Oligonucleotides 29 (5′-TCGAGCACAGTGCTCCAGGGCTAACAAAAACC-3′) and 30 (5′-AGCTTGGTTTTTGTTCAGCCCTGGAGCACTGTG-3′), which constitute RSS-A, and oligonucleotides 27 (5′-GATCCACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC-3′) and 28 (5′-TCGAGGGTTTTTGTACACCCAGACAGTGGAGTACTACCACAGTG-3′), which constitute RSS-B, were annealed and then ligated to a Bam HI–Hind III fragment of plasmid pCH110 (Pharmacia, Piscataway, NJ) that contains *lacZ*. The ligation product was ligated into the Sal I site of pUC19 to form the plasmid pAA14. A Sma I–Pst I fragment of pAA14 was treated with T4 polymerase and then ligated into Bam HI–Pst I–digested and Klenow enzyme–treated plasmid pKJ (containing the murine *pgk-1* promoter, a gift from M. Rudnicki and M. McBurney) to form the plasmid pK14, in which *lacZ* was inserted in an orientation opposite to the *pgk-1* promoter, and the plasmid p11, in which *lacZ* was inserted in the same orientation as the *pgk-1* promoter. An Eco RI fragment of pK14 that contains the *pgk-1* promoter was subsequently ligated into the Eco RI site of pAA14 to form pSPH1.
8. C. N. Adra, P. H. Boer, M. W. McBurney, *Gene* **60**, 65 (1987).
9. S. Akira, K. Okazaki, H. Sakano, *Science* **238**, 1134 (1987).
10. R. Saiki *et al.*, *ibid.* **239**, 487 (1988).
11. M. Bonneville *et al.*, *Nature* **342**, 931 (1989).
12. C. Walsh and C. L. Cepko, *Science* **241**, 1342 (1988). Mice were perfused with 2% paraformaldehyde in PIPES buffer under deep sodium pentobarbitol anesthesia (anesthesia procedure according to guidelines of the Massachusetts Institute of Technology Division of Comparative Medicine), and their brains were postfixed for up to 8 hours at 4°C and soaked overnight in phosphate buffer containing 30% sucrose and 2 mM $MgCl_2$ at 4°C. Parasagittal or transverse sections were cut at 30 or 40 μm on a sliding microtome and were stained for β-galactosidase with X-gal. Selected sections were counterstained with neutral red.

13. The percentage of β-galactosidase–positive cells in the cerebral cortex of the transgenic line 1-7 mice was estimated as follows. First, to establish the total number of cells per unit volume, a Biocom computer (Les Ulis, France) was used to determine the numbers of neutral red–positive cells visible in cortical samples in parasagittal sections 40 μm thick. Stained cells were counted in rectangles (100 by 150 μm) stacked to cover the full depth of the cortex. Values were calculated as cells per 0.1 mm by 0.15 mm by 0.25 mm = 0.006 cubic millimeters. Counts were made for frontal (n = 2), mid-anteroposterior (n = 2), and posterior (n = 1) cortex in two parasagittal sections. Figure 2D illustrates one such section, in which counts were made of the cortex illustrated in B and C (lettered arrowhead in D). Blue (β-galactosidase–positive) spots (Fig. 2) were counted in the same sections and sample sites. For numbers of neutral red cells, we obtained values of 333,000 to 833,000 cells per cubic millimeter. In order to compare our estimates to figures in the literature, we used the estimate of three neurons to one glial cell for rat (*27*) and, multiplying by 0.75, obtained an estimate of 250,000 to 625,500 neurons per cubic millimeter. These values are slightly higher but in the same order of magnitude as those in the literature for mouse neocortex (87,000 to 214,000 per cubic millimeter) (*28*). We estimate that roughly 10% of the cortical cells were β-galactosidase–positive. Some neurons appeared to have more than one X-gal–stained spot associated with them (Fig. 2), and some neutral red–stained cells may have overlapped each other. However, even if we had reduced our estimate by an order of magnitude to take into account these potential sources of error, we still would have obtained a value of 1% of the cortical cells being β-galactosidase–positive.
14. To estimate the absolute sensitivity of the PCR assay used (Fig. 3, A and B), serial dilutions of plasmid p11 (*8*), which contains directly oriented *pgk-1* and *lacZ* sequences, were added to 1 μg of cerebral DNA, the equivalent of approximately 10^5 genomes. PCR amplification of plasmid p11 with primers 1 and 2 resulted in a 266-bp product that was slightly smaller than the 328-bp product expected after amplification of a V(D)J recombined Tg plasmid. Because of the similarity in size and structure of the 328-bp and 266-bp PCR products, it is likely that the sensitivity of this PCR assay is equivalent for the plasmid p11 and the V(D)J recombined Tg plasmid.
15. M. Frohman, in *PCR Protocols*, M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White, Eds. (Academic Press, San Diego, CA, 1990), pp. 28–38. Briefly, 1 μg of poly(A)$^+$ mRNA was primed with oligonucleotide 7 (5′-TTGCGAGTTTACGT-3′) and reverse-transcribed (or reverse transcriptase was omitted to demonstrate that subsequent products were derived from RNA and not from contaminating genomic DNA), and the cDNA product was precipitated by ethanol and then tailed with terminal deoxynucleotide transferase. The tailed cDNA was subjected to PCR amplification with oligonucleotides 8 (5′-GACTCGAGTCGAGTCGACATCGATTTTTTTTTTTTTTT-3′), 9 (5′-GACTCGAGTCGACATCG-3′), and 10 (5′-GGGCATGCGTGTCCCAGCCTGTTTA-3′). PCR parameters for five cycles were 60 s at 94°C, 90 s at 45°C, and 15 s at 72°C. For the subsequent 40 cycles, parameters were as described in (*25*). To clone the amplified cDNAs, an additional 30 cycles of PCR were performed on 1% of the initial reaction products, as described (*25*), with the use of oligonucleotides 9 and 11 (5′-GTTCGAGGGGAAAATAG-3′).
16. P. Johnson and T. Friedman, *Gene* **88**, 207 (1990).
17. F. W. Alt, T. K. Blackwell, G. D. Yancopoulos, *Science* **238**, 1079 (1987).
18. From the context of this sentence, we believe the word "neonatal" was inadvertently used instead of "neuronal."
19. J. M. Grichnik, B. A. French, R. J. Schwartz, *Mol. Cell. Biol.* **8**, 4587 (1988).
20. A. Meyerhans, J.-O. Vartanian, S. Wain-Hobson, *Nucleic Acids Res.* **18**, 1687 (1990); M. Frohman and G. Martin, in *PCR Protocols*, M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White, Eds. (Academic Press, San Diego, CA, 1990), pp. 228–236.
21. A. Yolov and Z. A. Shabarova, *Nucleic Acids Res.* **18**, 3983 (1990).
22. N. Nakanishi, personal communication.
23. D. B. Roth and J. H. Wilson, *Mol. Cell. Biol.* **26**, 4295 (1986).
24. PCR cycle parameters consisted of 60 s at 94°C, 60 s at 50°C, and 90 s at 72°C. Autofluorography was performed on the BioImage Analyzer (Fuji Film). Sequences of oligonucleotide primers used in this assay were as follows. 1, (5′-ATTCTGCACGCTTCAAAAAGCGCA-3′); 2, (5′-CATTGTTCAGAAGGCATCAGTCG-3′); 3, (5′-ACACCCAGACAGTGGAGTACTAC-3′); 4, (5′-AGTGTCCCAGCCTGTTTATCTAC-3′); 5, (5′-TCTCTGTTCTCGCTATT-3′); 6, (5′-AGACATGATAAGATACATTGATG-3′).
25. T. Maniatis, E. Frisch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
26. M. S. Schlissel and D. Baltimore, *Cell* **58**, 1001 (1989).
27. A. Peters, D. A. Kara, K. M. Harriman, *J. Comp. Neurol.* **238**, 263 (1985).
28. A. Peters, in *Cerebral Cortex*, E. G. Jones and A. Peters, Eds. (Plenum, New York, 1987), vol. 6, chap. 7.
29. We thank L. Van Kaer for oligonucleotide [illegible] thesis; M. Rudnicki and M. McBurney for plasmid pKJ; G. Holm and D. Major for help with the histology; and H. Hall for the photography. A.M.G. is supported by Javits Award NS25529 and the Human Frontiers Science Program. S.T. is supported by grants from the Howard Hughes Medical Institute and National Institutes of Health. A.A. is an NIH Medical Scientist Training Program predoctoral fellow.

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