ELSEVIER

Brain Research 727 (1996) 196–204

BRAIN RESEARCH

Research report

A transgene insertion at *perinatal lethality* (*ple*) is associated with abnormalities of the cortex

David R. Beier [a,*], Holly Dushkin [a], Lisa V. Stone [b], Gordon F. Sherman [b]

[a] *Genetics Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA*
[b] *Neurology Department, Beth Israel Hospital, Boston, MA 02115, USA*

Accepted 19 March 1996

Abstract

In an inbred genetic background, mice homozygous for a transgene insertion at *perinatal lethality* (*ple*) were found to be significantly smaller than their heterozygous or wild-type siblings at birth, and rarely survived for more than 48 h. Homozygous progeny of *ple* mice obtained from a cross with a different strain were viable, but were still not obtained in the expected numbers, demonstrating some deleterious affect of this mutation even in a hybrid genetic background. Homozygous mice demonstrate variable expression of abnormalities in brain development. These usually appear as focal cortical ectopias, but also include other abnormalities, such as polymicrogyria. The genomic sequences corresponding to the region disrupted by the transgene were cloned by isolating a junction fragment between transgenic and wild-type sequences, which was then used to obtain the corresponding region of wild-type genomic DNA. Since some probes from this region do not hybridize with genomic DNA from homozygous *ple* mice, it appears likely that a deletion event coincided with the transgene insertion.

Keywords: Transgenic insertion mutation; Neuronal migration; Cortical ectopia; Mouse; *Perinatal lethality*; Microgyria; Dysgenesis; Cerebral cortex

1. Introduction

The intricacies of mammalian cortical development represent a formidable challenge for biological investigation. A variety of strategies have recently been devised that should serve to increase our understanding of this subject, and, as in other areas of developmental analysis, it is likely that mutations which result in tissue-specific abnormalities will contribute substantially to the elucidation of the genetic and cellular interactions that play a role in brain maturation. There are a number of human heritable disorders that include apparent defects in the migration of cortical neurons as an important component of their disease phenotype. The lissencephalies, including Miller–Diecker lissencephaly, represent one extreme; there are also disorders with more focal defects, such as the periventricular and cortical heterotopias found in Zellweger's cerebrohaptorenal syndrome [1]. An elevated incidence of focal abnormalities of the cortex has been also been found in patients with developmental dyslexia [11], and intractable epilepsy [10]. In the mouse, there are a small number of well-described disorders of neuronal migration, including the *reeler* mutation, in which the developing cortical neurons fail to migrate past existing neuronal layers as they normally do in wild-type mice [4,22]; and the New Zealand Black (NZB) and BXSB autoimmune mice, in which neurons migrate through a breach in the external limiting membrane to aberrant positions superficial to cortical layer II [25,26].

We have been studying a transgenic line of mice that has an apparent recessive mutation as a consequence of a transgene insertion event. This putative insertion mutation was originally suggested by our inability to maintain a line of mice that was homozygous for the transgene. As the transgene was bred into an inbred genetic background, the effect on viability of the putative mutation was more apparent, and most homozygous mice were found to survive less than 48 h after birth. The locus disrupted in this transgenic line was therefore designated *perinatal lethality* (*ple*) [2].

Genetic analysis of these mice revealed that the *ple* transgene is very tightly linked to the mutations *microcytic*

* Corresponding author. Fax: (1) (617) 232-4623; e-mail: beier@rascal.med.harvard.edu

0006-8993/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
PII S0006-8993(96)00380-0

anemia (*mk*) (1.2 ± 0.8 cM) and *Caracul* (*Ca*) (0.7 ± 0.7 cM) on mouse chromosome 15 [2]. To insure that the apparent linkage was not due to recombination suppression, recombination between *ple* and the more proximal genetic markers *Dominant megacolon* (*Dom*) and *belted* (*bt*) was also tested, and was found to be entirely consistent with a map position for *ple* near *Ca*. Additionally, no evidence of chromosome rearrangement associated with the transgene insertion was evident from physical analysis by in situ chromosome hybridization.

The present paper describes the further characterization of the *ple* mutation using genetic, molecular, and histological analyses. This work confirms the deleterious effect of the homozygous transgene insertion in both inbred and hybrid genetic backgrounds. Moreover, we show that homozygous *ple* mice show variable expression of neuroanatomical abnormalities that suggest defects in cortical neuronal migration.

2. Methods

2.1. Mice

The construction of the transgenic line and its dominant phenotype have been previously described [16,30]. Briefly, fertilized ova of a CD1 × C57BL/6J cross were microinjected with a fusion gene carrying an MMTV promoter placed approximately 1 kb 5′ to a mouse *c-myc* genomic fragment. A founder animal was identified, bred against CD1 animals, and maintained in this outbred background. The transgene was introduced into a C57BL/6J inbred background using mice obtained from The Jackson Laboratory. Analysis in a hybrid genetic background was done using progeny of crosses with C3HeB/FeJLe *a/a Ca/+* mice obtained from The Jackson Laboratory (Bar Harbor, ME).

2.2. Histology

Adult and neonatal mice were anesthetized with ethyl ether and perfused transcardially with 0.9% saline, then 10% formalin for 5 min. Brains were removed and stored in 10% formalin for two weeks. They were then dehydrated in 80%, 95%, 100% ethanol and ethanol/ether. The brains were then embedded in 3% celloidin for 3–4 days followed by 12% celloidin for 2–3 days. The embedded brains were cut on a sliding microtome at 30 μm and every fifth section was stained with cresyl violet for Nissl bodies and mounted on glass slides. In order to process embryos, pregnant mice were anesthetized with ethyl ether, the pups removed, and their heads placed into 10% formalin. The mothers were perfused transcardially and the brains of both the mothers and pups were processed as above. The slides were examined under a light microscope and the presence of cortical microgyria, ectopias, dysplasias, and other types of brain abnormalities were noted. Ectopias were judged to be either large, moderately-sized, or small. Large ectopias are characterized by a mushroom-like extrusion of cells into the molecular layer, containing more than 50 neurons; moderately sized ectopias present as collections of neurons in the molecular layer containing between 20 and 50 cells; small ectopias contain less than 20 neurons clustered in layer I. The architectonic and hemispheric location of the anomalies were recorded.

2.3. Immunohistochemistry

The monoclonal antibody Rat-401 (Developmental Studies Hybridoma Bank, NIH, Bethesda, MD) was used to stain radial glial fibers in the embryonic and neonatal brains. Adult tissue was stained for neurofilament fibers using a monoclonal antibody to neurofilament protein (68 kDa; ICN Immunobiologicals, Costa Mesa, CA). For both procedures free floating sections were rinsed twice in 0.5 M PBS (pH 7.4) for 5 min. Sections were incubated in 0.6% H_2O_2 for 20 min to block endogenous peroxidase, and rinsed twice in PBS. Rat-401 was diluted 1:4 in 3% normal rabbit serum in PBS with 0.3% Triton X-100, and the neurofilament antibody was diluted 1:250 in 3% normal horse serum in PBS with 0.3% Triton X-100. Sections were incubated overnight on a shaker table at 4°C. The following day, tissue was rinsed twice in PBS. The Rat-401 tissue placed into rabbit anti-mouse immunoglobulins (Dakopatts, Dako Corporation, Carpinteria, CA) diluted 1:20, the neurofilament tissue placed into biotinylated anti-mouse IgG (Vector Labs, Burlingame, CA) diluted 1:60, and both incubated on a shaker table for 2 h at room temperature. Sections were rinsed twice. Rat-401 tissue placed into mouse PAP (Dakopatts, Dako Corporation, Carpinteria, CA) diluted 1:250, neurofilament tissue placed into ABC (Vector Labs, Burlingame, CA) made in PBS and incubated on a shaker table at room temperature for 2 h. Sections were rinsed twice in PBS, and twice in 0.5 M Tris (pH 7.6) for 5 min. All tissue was developed in the substrate 0.05% diaminobenzedine (DAB), with 0.01% H_2O_2, in 0.5 M Tris (pH 7.6) for about 5 min or until proper staining was visualized. Sections were rinsed in Tris, mounted onto chrom–alum subbed slides and dried overnight. Slides were rehydrated and intensified in 0.5% cupric sulfate in 0.9% saline for 5 min, counterstained with methyl green/alcian blue, dehydrated and coverslipped using Permount.

2.4. Cloning

Southern analysis, colony hybridization, restriction analysis and plasmid subcloning experiments were done essentially as described in [24]. DNA sequencing was done using Sequenase (USB, Cleveland, OH) according to the manufacturer's recommendations. The cloning of the junctional fragment containing the transgene and its flanking

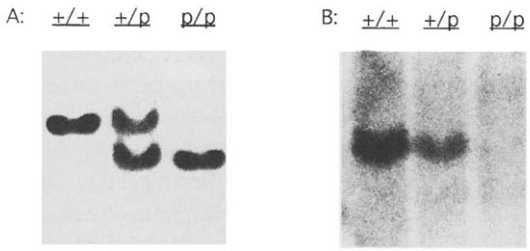

Fig. 1. Southern analysis of DNA probes from the *ple* region. (A) Hybridization of the *D15Bei1* probe (derived from a transgene junction fragment) to *Pst*1-digested genomic DNA from wild-type (+/+), heterozygous (+/*p*), and homozygous (*p*/*p*) transgenic mice. The polymorphic band correlates with the presence of the transgene. (B) Hybridization of an amplified PCR fragment (A63ss) from a bacteriophage clone that is contiguous with sequence of the transgene insertion site to *Taq*1-digested genomic DNA from wild-type, heterozygous, and homozygous transgenic mice. These sequences are apparently deleted in the transgenic mice (see Section 3).

genomic sequences was complicated by the fact that the transgene is present in 5–6 copies and is comprised of MMTV sequences, which are moderately repetitive in the mouse genome, and murine *c-myc*. Since the MMTV/*c-myc* transgene does not contain an *Eco*R1 site and is tandemly repeated, unique bands found in Southern analyses of transgenic animals using *Eco*R1 in combination with other infrequent cutting enzymes should identify junction fragments, with the *Eco*R1 site being contributed by genomic sequence. Hybridization of a transgene-specific probe to *Eco*R1/*Xho*1-digested DNA identifies an 8 kb band not seen in either transgenic or wildtype DNA digested with *Eco*R1 or *Xho*1 alone (data not shown). DNA approximately 6–9 kb in size was isolated from an agarose gel and cloned into the Lambda FIX genomic cloning vector (Stratagene). Positive clones were identified by colony hybridization using a transgene specific probe and found to have a 8 kb *Eco*R1–*Xho*1 insert, which was subcloned. Both the transgene-specific probe and total mouse DNA hybridized with this clone, suggesting it carried a junction fragment between the transgene and a region of presumptive genomic DNA. Sequencing of the subcloned *Eco*R1/*Xho*1 fragment using a primer derived from MMTV LTR sequence identifies the predicted transgene sequence (with only a few bases deleted from its end) and adjacent sequence that contains mouse repetitive elements. Bacteriophage clones carrying sequences corresponding to the wild-type *ple* locus were identified in a genomic library cloned in EMBL3. The primers which amplify A63ss, the 239 bp PCR-based clone used in Fig. 1 to demonstrate the apparent deletion of wild-type sequences in *ple* mice are:

Forward: TTCTTTCTCGACAGCTCTCCC
Reverse: TTAAGGAGGGGAGTTGTTGGA

3. Results

3.1. Genetic analysis of the ple mutation

Two intercrosses were performed to test the affect on viability of the *ple* transgenic insertion. Heterozygous C57BL/6J *ple*/+ mice of either the N11 and N13 backcross generations were intercrossed. Determination of the genotype was done using the probe *D15Bei1* (described below) derived from one junction between genomic and transgenic sequences. This permits the discrimination between homozygous transgenic, heterozygous transgenic, and wild-type mice (Fig. 1). Genotype analysis was done on progeny found to be either dead or clearly morbid within two days of birth. The remainder of the litter was genotyped after weaning at three to four weeks. In both crosses there is a marked deficiency of homozygous mice that survive to weaning age; the total of 8 found is about 10% of what would be expected, since an intercross should generate a 2:1 ratio of heterozygous to homozygous offspring (Table 1). Homozygous *ple* mice are over-represented in the cohort of mice found dead or morbid within

Table 1
Genetic analysis of crosses using *ple* mice

			Viable progeny			Non-viable progeny		
Expt	Cross	Generation	+/+	*ple*/+	*ple*/*ple*	+/+	*ple*/+	*ple*/*ple*
1	*ple*/+ × *ple*/+	N11	29	65	3	0	2	13
2	*ple*/+ × *ple*/+	N13	40	78	5	4	16	10
Total			69	143	8	4	18	23
				ple/+	*ple*/*ple*			
3	*Ca* +/+ *ple* × *ple*/*ple*	N8		46	21			
				+/+ and *ple*/+	*ple*/*ple*			
4	*Ca* +/+ *ple* × *Ca* +/+ *ple*	N17		98	11			

Expts 1 and 2 are intercrosses between C57BL/6J *ple*/+ mice. Viable progeny were scored at 3–4 weeks. Non-viable progeny were either found dead or morbid within 48 h post-birth. Genotype analysis was done using the probe *D15Bei1* (Fig. 1). Expts 3 and 4 are crosses using B6/C3H hybrid mice carrying the tightly linked marker *Ca* in repulsion with *ple*. Genotypes were inferred based upon phenotype at *Ca* (see Section 3).

two days of birth (Table 1). The total of homozygous *ple* mice identified in these populations remains significantly less than the one-half of heterozygotes that would be expected from an intercross; this could be due either to incomplete ascertainment after birth due to maternal consumption of pups, or an in utero effect on viability. Genotype analysis of 35 mice harvested between embryonic day 17 and 19 shows no significant deficiency of homozygous mice, suggesting the effect is post-natal (although the statistical power of this sample to detect a deviation is small).

In contrast, heterozygosity for *ple* has no apparent effect on viability. This has been evident during the ongoing introduction of *ple* into the C57BL/6J genetic background, which has been done by serial backcrosses. For example, for mice of generations N14 to N17, backcrosses of *ple*/+ mice to wild-type females produced a total of 51 *ple*/+ and 57 +/+ mice ($\chi^2 = 0.33$, P = not significant).

Since viable adult homozygous mice had been identified for the *ple* transgenic line when it was derived and maintained in an outbred genetic background [16] we tested whether we could 'rescue' homozygous mice by outcrossing *ple* with a different strain of inbred mice. This was first done by crossing N8 C57BL/6J *ple*/+ mice with C3HeB/FeJLe *a*/*a* *Ca*/+ mice. *Ca* is a dominantly acting coat texture mutation that is very tightly linked to *ple* (0.7 ± 0.7 cM) [2]. It was used as a convenient genetic marker to identify homozygous *ple* mice, since non-*Ca* progeny of an intercross between *Ca* +/+ *ple* mice are homozygous for *ple* (in the absence of recombination). Numerous viable non-*Ca* mice were found in the progeny of this cross. Genotype analysis confirmed these mice to be homozygous for the *ple* transgene. However, there is a significant relative decrease in viability even in a hybrid genetic background. This was evident when doubly heterozygous *Ca* +/+ *ple* mice were backcrossed with *ple*/*ple* homozygous mice. While the ratio of heterozygotes to homozygotes expected from such a cross is 1:1 (based on Mendelian inheritance), less than half the number of homozygous mice compared to heterozygous mice were found at weaning age ($\chi^2 = 13.6$, $P < 0.001$, Table 1). A similar experiment has been repeated with the same result. N17 C57BL/6J *ple*/+ mice were crossed with C3HeB/FeJLe *a*/*a* *Ca*/+ mice and their doubly heterozygous *Ca* +/+ *ple* progeny were intercrossed. 98 *Ca* progeny were found. If the *Ca*-appearing mice include both *Ca* +/+ *Ca* and *Ca* +/+ *ple* mice in a ratio of 1:2 as expected for an intercross, then 33 homozygous mice should also have been obtained. As shown in Table 1 only 11 were recovered ($\chi^2 = 14.7$, $P < 0.001$).

The effect on viability of the homozygous *ple* transgene is also demonstrated by the fact that we have been able to maintain a line of homozygous mice only with great difficulty. While both male and female homozygous mice from the hybrid C57BL/6J-C3HeB/FeJLe (B6/C3H) genetic background are fertile, their reproductive performance is poor, and females generally have few and small litters. Furthermore, homozygous mice rarely survive more than a year, and appear predisposed to develop a variety of tumors.

3.2. Phenotype of the ple mutation

In an inbred genetic background, mice homozygous for the transgene insertion at *ple* can usually be recognized at birth by their small size. The results in Table 2 show that the average weight in homozygous mice is significantly less than their heterozygous or wild-type sibs. These mice will only rarely have an evident milk spot and frequently appear morbid. Our initial histological analysis revealed that homozygous newborn mice often have variable accumulation of hepatic microvesicular fat. However, this finding proved inconsistent, and could occasionally be seen in heterozygous sibs. The origin of this finding is unclear, and may represent the incomplete mobilization of hepatic fat stores after birth.

Examination of the brain revealed striking abnormalities including ectopic collections of neurons in neocortical layer I, cortical microgyria, focal gliotic areas in the cortex, and partial agenesis of the callosum. These anomalies were almost exclusively present in homozygous mice (Table 3). The most common anomaly was neuronal ectopias in the molecular layer of the cortex. Twenty-seven of 116 (23%) *ple* homozygotes had large ectopic collections of neurons in layer I of the neocortex. The ectopias were characterized by mushroom-shaped protrusions of neurons into the molecular layer and overlying subarachnoid space (Fig. 2). Cortical layers II–VI underlying the ectopias also were distorted. Usually one or two ectopias were seen per affected brain and most were present in motor areas (Fr1, 2, or 3; architectonic locations according to Zilles [32]). The next most affected architectonic area

Table 2
Weight of newborn mice from *ple* crosses

+/+	*n*	*ple*/+	*n*	*ple*/*ple*	*n*
1.28 ± 0.14	17	1.29 ± 0.15	33	1.16 ± .09	14

Progeny of intercrosses between C57BL/6J *ple*/+ mice were identified on the day of birth. Mean weight ± standard deviation is shown in grams. Genotype analysis was done as described in Fig. 1. There is no significant difference between the means of +/+ and *ple*/+ mice using a *t*-test. The mean weight of the *ple*/*ple* mice is significantly less ($P < 0.004$).

Table 3
Incidence of cerebral anomalies in *ple* mice

	Homozygous *ple*					Heterozygous *ple*				
Age	ectop	microgyr	NFZ	agenesis	*n*	ectop	microgyr	NFZ	agenesis	*n*
adult	11	0	1	3	51	2	0	0	0	27
nb	8	2	0	0	25	2	0	1	0	25
E17–19	7	0	0	0	19	0	0	0	0	13
E13–16	1	0	0	0	21	0	0	0	0	16
all	27	2	1	3	116	4	0	1	0	81

Abbreviations: ectop, ectopias; microgyr, microgyria: NFZ, neuron-free zone; agenesis, agenesis of the corpus callosum; nb, newborn; E, embryonic day.

was Par1. A small number of ectopias also were seen in Cg1, FL, and Par2.

When the *ple* homozygous mice were analyzed by age, it was seen that 22% of the adults ($n = 51$), 32% of the newborns ($n = 25$) and 37% of the E17–19 fetuses ($n = 19$) had cortical ectopias. In addition, one ectopia was seen in a E15 fetus. As in previous studies of NZB mice [27], staining of radial glial fibers in newborns using the Rat-401 antibody revealed a breach in the pial-glial boundary underlying the ectopia (Fig. 3). Neurofilament positive fiber bundles also were present in the subjacent layers of ectopias in adults (Fig. 3). In addition, 2 cases of microgyria were present in the frontal/motor cortex on the right side (Fig. 4) and one adult had a focal gliotic region in the cortex. Three adult cases of partial agenesis of the corpus collosum also were seen, two of which were in mice with ectopias.

Wild-type litter mates had no signs of brain anomalies and only 4 of 81 (5%) heterozygous mice had cortical ectopias. In addition, one newborn heterozygote had a

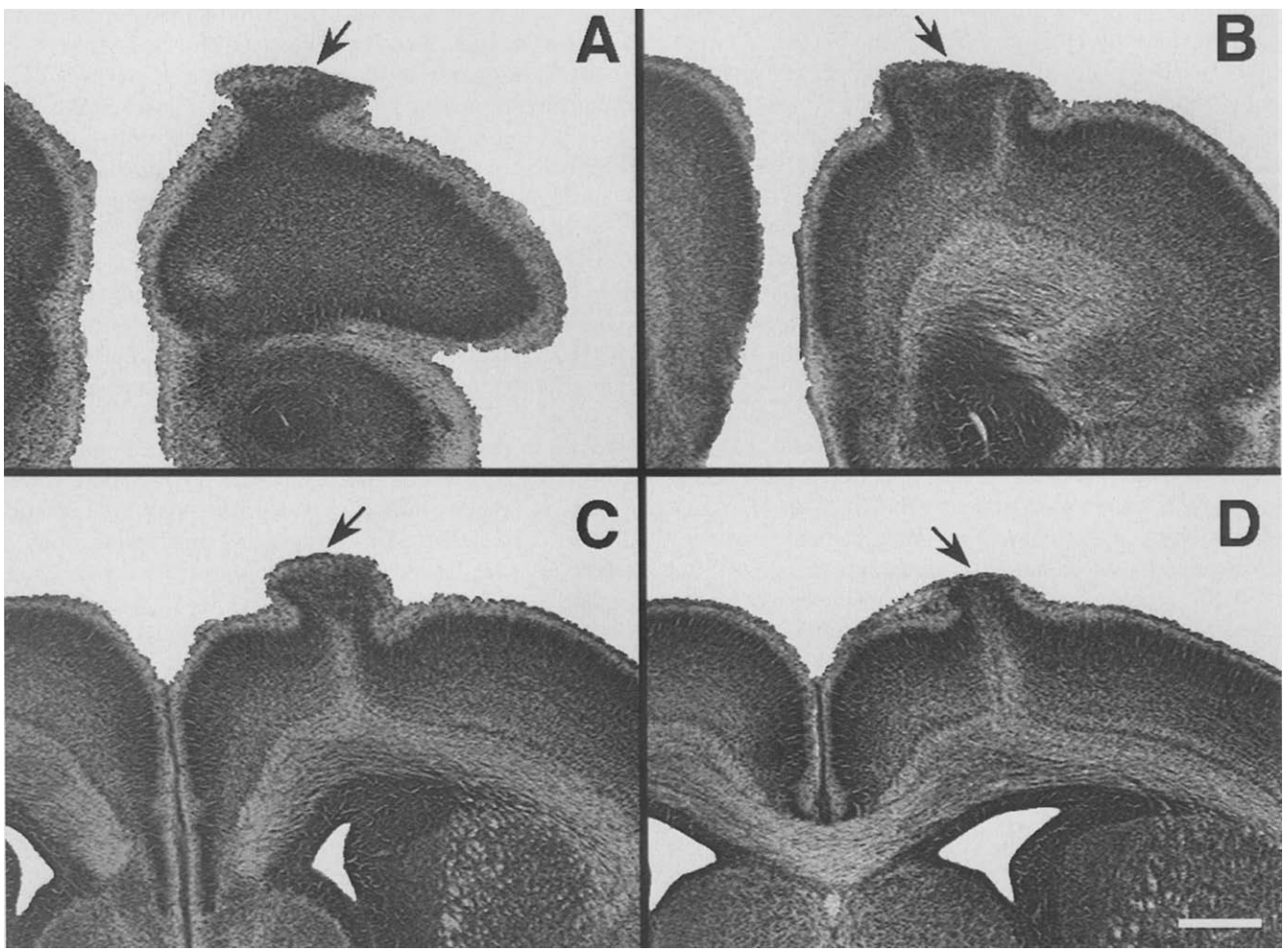

Fig. 2. (A–D) Digitized composite (in Adobe Photoshop) showing Nissl staining of a large ectopia (arrows) in the left hemisphere of a fetal *ple* homozygous mouse. Panels are arranged from rostral to caudal with 150 μm between sections. Processing artifacts were digitally excised. Bar = 250 μm.

focal gliotic region in the cortex. No instances of microgyria or agenesis were seen. A comparison of the homozygous and heterozygous *ple* mice revealed that the ectopia distributions were significantly different from each other ($\chi^2 = 11.04$, df = 1, $P < 0.001$).

3.3. Cloning of the transgene insertion site

The cloning of a junction fragment containing both transgene and wild-type genomic sequences is described in Section 2. Characterization of the genomic region on this junctional clone was complicated by the presence of abundant mouse repetitive sequence. DNA sequencing was utilized to identify a 300 bp region that, when used as a probe, hybridizes to a single band on a Southern blot that is polymorphic between the transgene and wild-type DNA. Since this probe, called *D15Bei1* in accordance with the rules for the nomenclature of anonymous DNA clones [18], can unambiguously distinguish wild-type, heterozygous, and homozygous transgenic animals, it has been useful for genotyping mice (Fig. 1).

The *D15Bei1* probe was used to identify hybridizing clones in a genomic library prepared in EMBL3. Positive clones were identified, and one (λ4) was proven to contain sequences corresponding to *D15Bei1* by sequence analysis. Subcloned fragments and PCR-based probes derived from a region of this clone that lies 'downstream' from the transgene junction site fail to hybridize to genomic DNA prepared from homozygous *ple* mice, demonstrating that the *ple* transgene insertion is associated with a deletion of wild-type genomic DNA (Fig. 1). Probes from the deleted region were used to extend the region of cloned sequences from wild-type DNA; the sequences contained on one contiguous bacteriophage clone (λ3) are apparently entirely deleted from *ple* mice. The extent of this deletion is as yet undefined, since we have not obtained mouse sequence corresponding to the second junction of the transgene and wild-type sequence. It appears at least greater

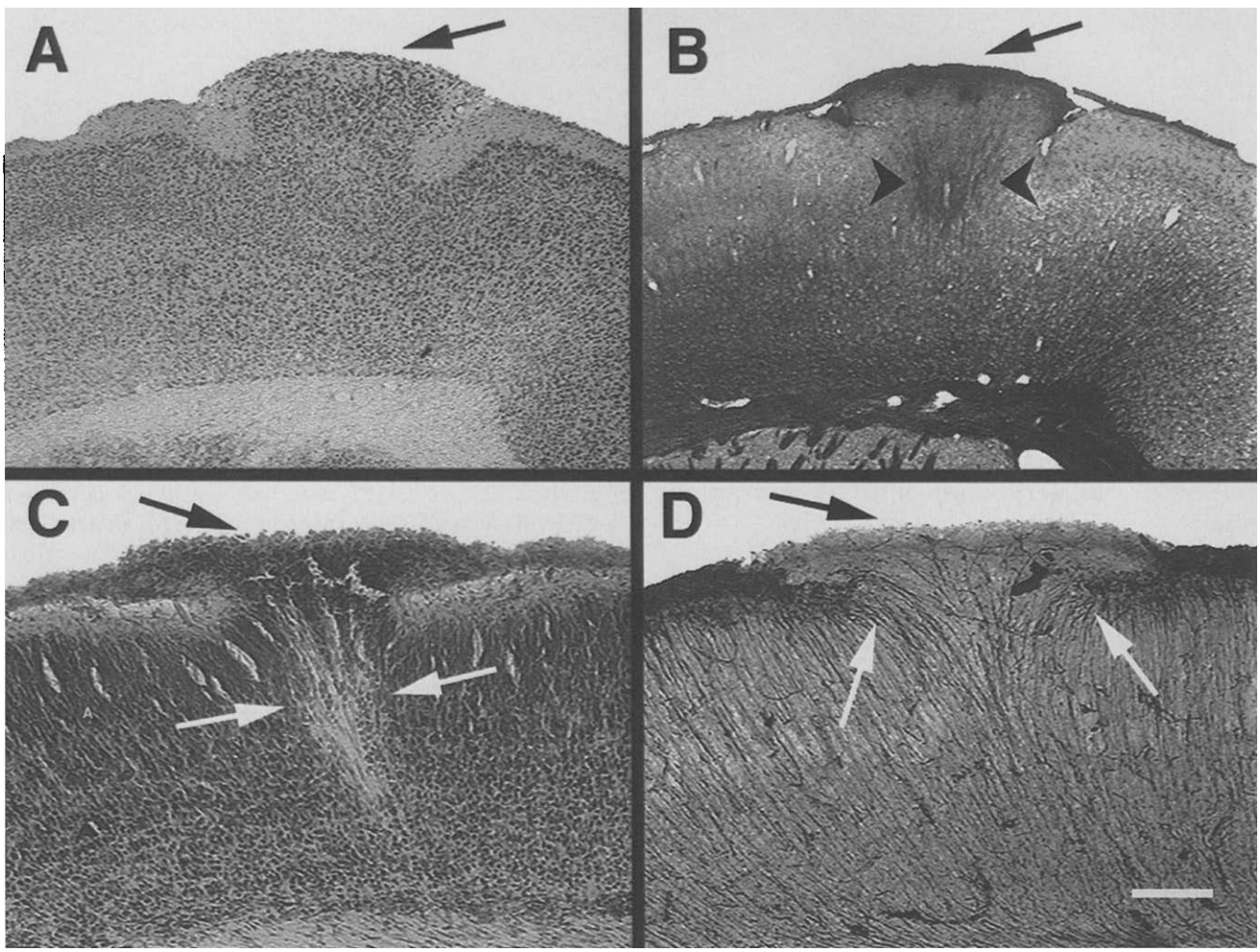

Fig. 3. (A–D) Digitized composite (in Adobe Photoshop) showing (A) Nissl staining of a large ectopia (arrow) in adult *ple* homozygous mouse; (B) adjacent section stained with a monoclonal antibody directed against neurofilament protein (68 kDa). Note the increased density of the neurofilament fibers (black arrowheads) underlying the ectopia. (C) Nissl stain of a large ectopia (arrow) in a fetal *ple* homozygous mouse. Note the underlying distortion of cortical layers (white arrows); (D) adjacent section to panel C stained with a monoclonal antibody (Rat-401) directed against radial glial fibers. Note the breach in the glial-pial border (white arrows). Processing artifacts were digitally excised. Bar = 250 μm for A/B and 65 μm for C/D.

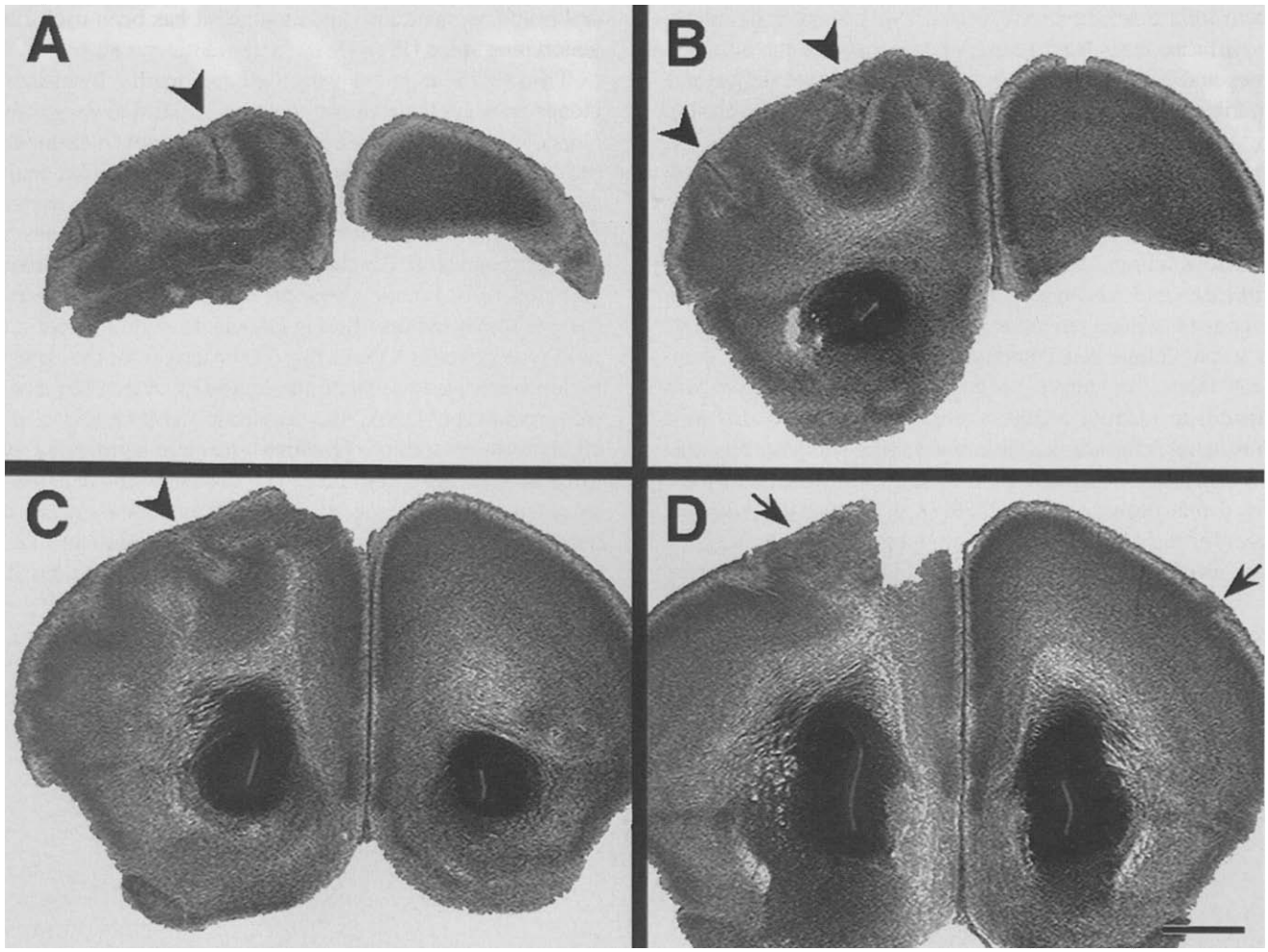

Fig. 4. (A–D) Digitized composite (in Adobe Photoshop) showing Nissl stained coronal sections containing 4 layered microgyria (arrowheads), ectopia (arrow) and related gross distortion of the right hemisphere (right is left side) of a newborn *ple* homozygous/*ple* mouse. Note the presence in panel D of the small ectopic collection of neurons (arrow) in layer I of the opposite hemisphere. Panels are arranged rostral to caudal with approximately 0.1 mm between sections. Processing artifacts were digitally excised. Bar = 250 μm.

than 16 kb, which is the length of the λ3 bacteriophage clone.

4. Discussion

In an inbred genetic background, mice homozygous for a transgene insertion at *ple* were found to be significantly smaller than their heterozygous or wild-type siblings at birth, and rarely survived for more than 48 h. When C57BL/6J *ple* mice were crossed with mice from the strain C3Heb/FeJLe, homozygous progeny of the F1 mice were viable, but were still not obtained in the expected numbers, demonstrating some deleterious effect of this presumptive mutation even in the hybrid genetic background. Examination of these homozygous mice revealed variable expression of abnormalities in brain development. These usually appeared as focal cerebrocortical ectopias, but also included other related abnormalities, such as microgyria and gross distortion of cortical layering. The ectopias are similar to those that prenatally develop in NZB/B1NJ and BXSB/MpJ mice [25,26], although they were generally larger in the *ple* mutants. Similar ectopias and microgyria can also be induced at birth in rats [9,15,23].

Since the transgene is an active MMTV/murine *c-myc* chimeric gene, containing an intact *c-myc* promoter, it is not yet possible to prove that the phenotype in homozygous transgenic mice is not due to transgene dosage. However, the early onset of mortality and the absence of similar phenotype in numerous transgenic lines carrying MMTV/*c-myc* argues against a transgene dosage effect. In addition, the identification of a significant deletion of genomic sequences at the transgene insertion site supports the likelihood that this insertion is associated with a disruption of an endogenous locus. The phenotype of *ple* is not similar to other mutations mapped near the *Ca* locus and therefore probably represents a new mutation.

While the exact nature of the mechanism of neuronal migration is not entirely known, there is considerable support for the hypothesis that many neurons achieve their

final position in the cortical plate by migrating out of the ventricular zone along a fascicle of radial fibers [13,21]. In some cases of disordered neuronal migration, such as the *weaver* and *reeler* mutations, the defect appears to be intrinsic to the neuron [12,22]. Analysis of the expression of reelin (the *reeler* gene product) by in situ hybridization [8] or antibody analysis [19] support a neuronal-specific effect. How this molecule, which has motifs suggesting that it is either secreted or bound to the external cell surface, specifies neuronal localization remains to be determined. In the *ple* mutation described here, however, and in the NZB and BXSB mouse strains, it is perhaps more likely that the defect is in the external glial limiting membrane where the endfeet of the radial glia are attached. This superficial cortical boundary must be intact for normal neuronal migration to take place. It has been suggested that damage to this border could result in the production of focal cortical layer I ectopias seen in human autopsy specimens [5–7] and in NZB and BXSB mice [27].

There are a variety of potential mechanisms by which the hypothesized defect in the external glial limiting membrane may occur. One possibility would be as a consequence of a local injury, such as bleeding or vascular occlusion, that results in ischemic damage to the cortical boundary. This model is consistent with the usually focal nature of the abnormality in *ple* mice, its variable expression, and the evidence that similar lesions can be induced in the developing rat brain by cryogenic or puncture injury [9,15,23]. Depending on the size of the ischemic injury and timing of the insult, the resultant damage to the developing cerebral cortex could be either gross distortion of layering, microgyria or molecular layer ectopias. An alternative model would be that there is a primary defect in the development of an intact cortical boundary. Another possibility is suggested by the evidence that *reeler*, whose phenotype includes the abnormal localization of neurons in the marginal zone of the fore-brain cortex [22], is a defect in a neuronal-specific gene. And, while the focal nature of the abnormalities found in *ple* mice might argue against the hypothesis that its defect is neuron-specific, it has recently been shown in a transgenic system that selective gene expression in specific cortical radial units may occur, suggesting there can be molecular heterogeneity of cortical progenitors [29]. The identification of the gene disrupted by the *ple* transgene insertion, and characterization of its pattern of expression and function, should facilitate a more specific examination of these questions.

It should be noted that, while the ectopias in the *ple* and NZB mice appear similar, it is unlikely that they are due to an identical molecular abnormality. This is suggested by the fact that, in an inbred genetic background, *ple* mice are runted and usually do not survive the perinatal period; that is, the cortical ectopias seen in these mice are likely part of a more global defect. The presence of ectopias is not itself incompatible with survival, as the incidence of ectopias in NZB mice is, at 40%, higher than that found in *ple* mice, and the incidence in the NXSM recombinant inbred subline D is higher yet, at 80% [28]. In addition, NZB mice rarely have microgyria. Finally, an analysis of NZB mice showed no evidence of genetic linkage of the region of chromosome 15 identified by the transgene with the ectopia phenotype (unpublished results).

In summary, we have found that a transgenic insertional mutation that results in perinatal lethality in inbred mice is associated with a focal abnormality of cortical neuronal migration. Transgenic insertions have proved extremely useful as molecular 'tags' that can be utilized for the cloning of disrupted loci; examples that have been successfully characterized include insertional mutations in *limb-deformity* (*ld*) [31], *micro-opthalmia* (*mi*) [14], and *reeler* [8]. We have cloned a junction fragment containing the *ple* transgene and the adjacent mouse genomic sequences, and used this fragment to clone the locus of the transgene insertion from wild-type mouse DNA (data not shown). It should be feasible to apply recently developed strategies for the identification of transcribed sequences in cloned genomic DNA, such as cDNA selection [17,20] and exon-trapping [3], for the purpose of identifying the gene disrupted by the insertion at *ple* that is apparently required for normal cortical development.

Acknowledgements

We are grateful for the encouragement and support of Dr. Philip Leder, in whose laboratory this work was initiated. We thank Dr. Robert D. Cardiff and the University of California at Davis Transgenic Histophatology Laboratory for their help. The Rat-401 antibody was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205 and the Department of Biological Sciences, University of Iowa, Iowa City, IA 52242, under contract NO1-HD-2-3144 from the NICHD. This work was supported by grant RO1-HD29028 from the National Institute of Child Health and Development to D.R.B. and NICHD 20806 to G.F.S.

References

[1] Barth, P.G., Disorders of neuronal migration, *Can. J. Neurol. Sci.*, 114 (1987) 1–16.

[2] Beier, D.R., Morton, C.C., Leder, A., Wallace, R. and Leder, P., Perinatal lethality (ple): a mutation caused by integration of a transgene into distal mouse chromosome 15, *Genomics*, 4 (1989) 498–504.

[3] Buckler, A.J., Chang, D.D., Graw, S.L., Brook, J.D., Haber, D.A., Sharp, P.A. and Housman, D.E., Exon amplification: a strategy to isolate mammalian genes based on RNA splicing, *Proc. Natl. Acad. Sci. USA*, 88 (1991) 4005–4009.

[4] Caviness, V.S., Neocortical histogenesis and reeler mice: a developmental study based upon (^{3}H)thymidine autoradiography, *Dev. Brain Res.*, 4 (1982) 293–302.

[5] Caviness, V.S., Evrard, P. and Lyon, G., Radial neuronal assemblies, ectopia and necrosis of developing cortex: a case analysis, *Acta Neuropathol.*, 41 (1978) 67–72.

[6] Caviness, V.S., Misson, J.-P. and Gadisseux, J.-F., Abnormal neuronal patterns and disorders of neortical development. In A.M. Galaburda (Eds.), *From Reading to Neurons*, MIT Press/Bradford Books, Cambridge, MA, 1978, pp. 405–442.

[7] Dambska, M., Wisniewski, K.E. and Sher J.H., Marginal glioneuronal heterotopias in nine cases with and without cortical abnormalities, *J. Child Neurol.*, 1 (1986) 149–157.

[8] D'Arcangelo, G., Miao, G.G., Chen, S.-C., Soares, H.D., Morgan, J.I. and Curran, T., A protein related to extracellular matrix proteins deleted in the mouse mutant *reeler*, *Nature*, 374 (1995) 719–723.

[9] Dvorak, K. and Feit, J., Migration of neuroblasts through partial necrosis of the cerebral cortex in newborn rats - contribution to the problems of morphological development and developmental period of cerebral microgyria, *Acta Neuropathol.*, 38 (1977) 203–212.

[10] Farrell, M.A., DeRosa, M.J., Curran, J.G., Secor, D.L., Cornford, M.E., Comair, Y.G., Peacock, W.J., Shields, W.D. and Vinters, H.V., Neuropathologic findings in cortical resections (including hemispherectomies) performed for the treatment of intactable childhood epilepsy, *Acta Neuropathol.*, 83 (1992) 246–259.

[11] Galaburda, A.M., Sherman, G.F., Rosen, G.D., Aboitiz, F. and Geschwind, N., Developmental dyslexia: four consecutive patients with cortical anomalies, *Ann. Neurol.*, 18 (1985) 222–233.

[12] Gao, W.Q. and Hatten, M.E., Neuronal differentiation rescued by implantation of Weaver granule cell precursors into wild-type cerebellar cortex, *Science*, 260 (1993) 367–369.

[13] Gressens, P. and Evrard, P., The glial fascicle: an ontogenic and phylogenetic unit guiding, supplying and distributing mammalian cortical neurons, *Dev. Brain Res.*, 76 (1993) 272–277.

[14] Hodgkinson, C.A., Moore, K.J., Nakayama, A., Steingrimsson, E., Copeland, N.G., Jenkins, N.A. and Arnheiter, H., Mutations at the mouse micropthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein, *Cell*, 74 (1993) 395–404.

[15] Humphreys, P., Rosen, G.D., Press, D.M., Sherman, G.F. and Galaburda, A.M., Freezing lesions of the newborn rat brain: a model for cerebrocortical microgyria, *J. Neuropathol. Exp. Neurol.*, 50 (1991) 145–160.

[16] Leder, A., Pattengale, P.K., Kuo, A., Stewart, T.A. and Leder, P., Consequences of widespread deregulation of the c-myc gene in transgenic mice: multiple neoplasms and normal development, *Cell*, 45 (1986) 485–495.

[17] Lovett, M., Kere, J. and Hinton, L.M., Direct selection: a method for the isolation of cDNAs encoded by large genomic regions, *Proc. Natl. Acad. Sci. USA*, 88 (1991) 9628–9632.

[18] Lyon, M.F. and Searle, A.G. (Ed.), *Genetic Variants and Strains of the Laboratory Mouse* (2nd ed.), Oxford University Press, Oxford, 1989.

[19] Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H. and Mikoshiba K. The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons, *Neuron*, 14 (1995) 899–912.

[20] Parimoo, S., Patajal, S.R., Shukla, H., Chaplin, D.D. and Weissman, S.M., cDNA selection: efficient PCR approach for the selection of cDNAs encoded in large chromosomal DNA fragments, *Proc. Natl. Acad. Sci. USA*, 88 (1991) 9623–9627.

[21] Rakic, P., Radial versus tangential migration of neuronal clones in the developing cerebral cortex, *Proc. Natl. Acad. Sci. USA*, 92 (1995) 11323–11327.

[22] Rakic, P. and Caviness, V.S., Cortical development: view from neurological mutants two decades later, *Cell*, 14 (1995) 1101–1104.

[23] Rosen, G.D., Sherman, G.F., Richman, J.M., Stone, L.V. and Galaburda, A.M., Induction of molecular layer ectopias by puncture wounds in newborn rats and mice, *Dev. Brain Res.*, 67 (1992) 285–291.

[24] Sambrook, J., Fritsch, E.F. and Maniatis, T., *Molecular Cloning* (2 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[25] Sherman, G.F., Galaburda, A.M., Behan, P.O. and Rosen, G.D., Neuroanatomical anomalies in autoimmune mice, *Acta Neuropathol.*, 74 (1987) 239–242.

[26] Sherman, G.F., Galaburda, A.M. and Geschwind, N., Cortical anomalies in brains of New Zealand mice: a neuropathologic model of dyslexia? *Proc. Natl. Acad. Sci. USA*, 82 (1985) 8072–8074.

[27] Sherman, G.F., Rosen, G.D., Stone, L.V., Press, D.M. and Galaburda, A.M., The organization of radial glial fibers in spontaneous neocortical ectopias of newborn New Zealand Black mice, *Dev. Brain Res.*, 67 (1992) 279–283.

[28] Sherman, G.F., Stone, L.V., Denenberg, V.H. and Beier, D.R., A genetic analysis of neocrotical ectopias in New Zealand Black autoimmune mice, *Neuroreport*, 5 (1994) 721–724.

[29] Soriano, E., Dumesnil, N., Auladell, C., Cohen-Tannoudji M., Sotelo, C., Molecular heterogeneity of progenitors and radial migration in the developing cerebral cortex revealed by transgene expression, *Proc. Natl. Acad. Sci. USA*, 92 (1995) 11676–80.

[30] Stewart, T.A., Pattengale, P.K. and Leder, P., Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes, *Cell*, 38 (1984) 627–637.

[31] Woychik, R.P., Stewart, T.A., Davis, L.G., D'eustachio, P. and Leder, P., An inherited limb deformity created by insertional mutagenesis in a transgenic mouse, *Nature*, 318 (1985) 36–40.

[32] Zilles, K., *The Cortex of the Rat*, Springer-Verlag, Berlin, 1985.