Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2615–2620, March 1998
Neurobiology

Genomic sequences of aldolase C (Zebrin II) direct *lacZ* expression exclusively in non-neuronal cells of transgenic mice

ERNST U. WALTHER*†, MARTIN DICHGANS*†, STEPHEN M. MARICICH†‡, RITA R. ROMITO‡, FEI YANG‡, SUZAN DZIENNIS‡§, SAUL ZACKSON¶, RICHARD HAWKES¶, AND KARL HERRUP‡‖

*Neurologische Klinik, Marchioninstrasse 15, 81377 Munich, Germany; ‡Alzheimer Research Laboratory, Case Western Reserve Medical School, 10900 Euclid Avenue, Cleveland, OH 44106; and ¶Department of Anatomy, University of Calgary, Calgary, Alberta T2N 4N1, Canada

Communicated by Richard L. Sidman, Harvard Medical School, Southborough, MA, December 23, 1997 (received for review May 9, 1997)

ABSTRACT **Aldolase C is regarded as the brain-specific form of fructose-1,6-bisphosphate aldolase whereas aldolase A is regarded as muscle-specific. *In situ* hybridization of mouse central nervous system using isozyme-specific probes revealed that aldolase A and C are expressed in complementary cell types. With the exception of cerebellar Purkinje cells, aldolase A mRNA is found in neurons; aldolase C message is detected in astrocytes, some cells of the pia mater, and Purkinje cells. We isolated aldolase C genomic clones that span the entire protein coding region from 1.5 kb 5′ to the transcription start site to 0.5 kb 3′ to the end of the last exon. The bacterial gene, *lacZ*, was inserted in two different locations and the constructs tested in transgenic mice. When the protein coding sequences were replaced with *lacZ*, three of five transgenic lines expressed β-galactosidase only in cells of the pia mater; one line also expressed in astrocyte-like cells. When *lacZ* was inserted into the final exon (and all structural gene sequences were retained) transgene expression was observed in astrocytes in all regions of the central nervous system as well as in pial cells. Thus, with the exception of Purkinje cell expression, the behavior of the full-length transgene mimics the endogenous aldolase C gene. The results with the shorter transgene suggest that additional enhancer elements exist within the intragenic sequences. The absence of Purkinje cell staining suggests that the *cis* elements required for this expression must be located outside of the sequences used in this study.**

The temporal and spatial regulation of the expression of aldolase (fructose-1,6-bisphosphate aldolase) represents a classic problem in developmental genetics. Three isozymes of this phylogenetically ancient enzyme exist: aldolases A, B, and C, each of which is encoded by a separate gene. The kinetics of aldolase A favor the cleavage of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, and its gene is found on human chromosome 16 (1). Generally considered to be a muscle enzyme, the transcriptional regulation of this gene is unusually complex with three alternative promoters (2–5) that result in three different mRNA species. Curiously, the three transcripts differ only in their 5′-untranslated regions; each mRNA encodes the same structural enzyme. Of the three promoters, the 5′-most and 3′-most are expressed broadly in fetal tissues as well as in adult muscle and red blood cells. The central promoter (M-type or H-type) is highly active in skeletal muscle, particularly in fast-twitch fibers (6, 7). The 5′-most promoter is active, albeit at lower levels, in a range of other tissues, including brain (4). In the nervous system, the exact cellular location of the aldolase A isozyme is uncertain; Northern analysis of cultured cells suggests that it is present in both neurons and glia (8).

Aldolase B, found on human chromosome 9 (9), generally is considered to be a liver enzyme, although it also is found in kidney proximal tubule cells and enterocytes. This form of aldolase favors gluconeogenesis, befitting its higher concentration in the liver. During hepatic development, aldolase A and C are expressed earlier, but aldolase B soon takes over. Unlike the other isozymes, aldolase B is transcriptionally activated by signals from hormones and dietary factors (e.g., ref. 10). A proximal promoter region, approximately 200 bp in length, has been identified that contains many of the elements (including overlapping hepatic nuclear factor binding sites) necessary for properly regulated expression if it is paired with sequences in the first intron (11–13).

Aldolase C, found on human chromosome 17 (1), has kinetic properties that are intermediate between those of the A and B isoforms. In the adult, aldolase C is considered to be the brain-specific isozyme, with low but detectable activity in fetal tissues and hepatocarcinomas (8, 14–17). The exact cellular location of aldolase C is controversial. Studies of mRNA by Northern blot analysis, *in situ* hybridization, or transgene expression studies indicate that the gene is transcribed mostly in neurons (8, 14, 18). On the other hand, earlier studies using isozyme-specific antibodies report its location in gray matter astrocytes and cells of the pia mater (16, 19). All reports agree that Purkinje cells of the cerebellum contain by far the highest levels of the enzyme. By sequence analysis, the aldolase C promoter region is typical of a ''housekeeping'' type of promoter. A normal TATA box is missing; instead a GC-rich region can be found just upstream of the transcription start site (20). A CAAT box is present, but it is located far upstream from its expected position (21). Northern analysis using a probe specific for aldolase C mRNA demonstrates a 1.6-kb band; there is no evidence of alternative splicing. Two DNase I-sensitive sites flank the transcription unit: one immediately 5′ and the other about 1.5 kb 3′ to the nine exons of the gene. Several tissue-specific methylation sites have been identified in the immediate vicinity of the transcription unit (22). In transgenic mice (18, 22) large rat genomic fragments (or fragments with short marker tags inserted) have been shown to direct central nervous system (CNS)-specific expression. Expression outside of the CNS is suppressed. Even a short, 115-bp promoter element contains sufficient *cis*-active sequences to drive marker gene expression solely in the CNS (23).

The interest of our laboratories in the aldolase isozymes grew out of the discovery that aldolase C is the antigen identified by the Zebrin II mAb (24). Zebrin II has long been

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

Abbreviations: CNS, central nervous system; GFAP, glial fibrillary acidic protein; X-Gal, 5-bromo-4-chloro-3-indolyl-β-D-galactoside.

†E.U.W., M.D., and S.M.M. contributed equally to the work. Their names are listed in the order in which they began work on the project.

§Present address: Beth Israel Hospital, 99 Brookline Avenue, Boston, MA 02115.

‖To whom reprint requests should be addressed. e-mail: kxh26@po.cwru.edu.

recognized as a Purkinje cell biochemical marker of the sagittal compartments of the adult cerebellar cortex (see for review refs. 25–29). As part of our effort to define the *cis*-acting elements that regulate compartment-limited cerebellar gene expression, we wished to learn more about the aldolase C promoter and its cell type-specific features. We report here a detailed *in situ* hybridization study of the endogenous pattern of aldolase A and C expression, and the behavior of two transgene constructs in which sequences from mouse aldolase C are joined with the histochemical marker gene, β-galactosidase, in various configurations. The expression pattern of these constructs in multiple independent transgenic lines reveals significant information on the spatiotemporal regulation of this intriguing gene.

MATERIALS AND METHODS

***In Situ* Hybridization.** Animals were perfused with 4% paraformaldehyde, and their brains were postfixed, equilibrated with 18% sucrose in PBS, embedded in OCT compound, and cryo-sectioned at 10 μm. Cerebellar RNA was reverse-transcribed, then amplified by 35 cycles of PCR using these primers containing T3 or T7 polymerase sites (underlined) at their 5′ ends:

Aldolase C (258 bp)—(T3): 5′-AATTAACCCTCACTAA-AgggggCAgAgATgAACgggCT-3′; and (T7): 5′-TgTAATA-CgACTCACTATAgggCgAATCCTgTggTCTCgggTA-3′.

Aldolase A (297 bp)—(T3): 5′-AATTAACCCTCACTAA-AgggTgAAggCAgCCCAggAgg-3′; and (T7): 5′-TgTAATA-CgACTCACTATAggggCgATTggCAgTgggCTggAAg-3′.

^{35}S-labeled riboprobes were generated by transcribing from either the T7 (antisense) or T3 (sense) strand. Hybridization was carried out at 48°C overnight in 50% formamide. After rinsing, slides first were exposed to x-ray film to obtain a low-resolution image, after which they were dipped in Kodak NTB-3 emulsion and stored in the dark at 4°C for 4 weeks. After developing in Dektol 1:1, the slides were lightly counterstained with cresyl violet and coverslipped.

Cloning Strategy. A mouse 129/SvJ genomic library (complexity: 1.2×10^6) constructed in LambdaFix II (Stratagene) was screened by using a mouse aldolase C cDNA clone covering the first 387 bp (exons 1, 2 and part of 3; ref. 24). The presence of genomic sequences between exons 1–2 and 8–9 (as defined by the rat sequence) was confirmed by PCR using exon specific primers. One clone was isolated from plaque-purified phage and subcloned into pBluescript II SK+ (Stratagene). A 6.2-kb *Hin*dIII fragment containing 1.5 kb of 5′-untranslated region, the complete coding region, plus 500 bp of 3′-untranslated region including the poly(A) signal was subcloned into pTZ19U. An *Xho*I site (underlined) was introduced into exon 2, exon 9, or into both exons by oligonucleotide-mediated site-directed mutagenesis (SDM). The primers used for SDM were: 5′-ggCAgggCATTCTCgAggCAgATgAgTCC (exon 2) and 5′-gCAgCACAgTCCCTCgAgATCgCCAACCAT (exon 9).

A 3-kb *Sal*I fragment from vector pKK42 (36) (kindly provided by Kevin Kelley, Mount Sinai School of Medicine, New York) containing the gene for bacterial β-galactosidase (*lacZ*), was inserted in-frame into the *Xho*I site in exon 9 generating the MACZ9 construct. A second construct (MACZ2Δ9) was made in which the entire genomic region between the new *Xho*I sites was removed and replaced with the 3-kb *lacZ* sequence (see Fig. 2*B*).

Generation and Identification of Transgenic Mice. Linearized fragments were microinjected into recently fertilized B6SJLF2 eggs. Surviving embryos were transferred to the oviducts of 0.5-day pseudopregnant CD1 females. Genomic DNA was isolated from tail biopsies, and the presence of the transgene was detected by PCR with primers specific for the *lacZ* gene. Primers were: up, 5′-ATACgCCgAACgATCgCC-AgTTCT-3′; and down, 5′-CACTACgCgTACTgTgAgCCA-gAg-3′.

Lines were established from DNA-positive founders and maintained by continued backcrossing to the C57BL/6J strain. The copy number of the transgene was determined by Southern blot using a radiolabeled probe against *lacZ*. Each line harbors 10 or fewer copies of the transgene.

Reverse Transcription–PCR. To analyze transgene expression, RNA was isolated from adult brain, liver, or muscle using the guanidinium thiocyanate method. After DNase I digestion to remove contaminating genomic DNA, reverse transcription was performed under standard conditions, and PCR was done by using the specific *lacZ* primers listed previously.

Histology. Postnatal transgenic mice were anesthetized and perfused transcardially with ice-cold 3% paraformaldehyde in 0.1 M Pipes (pH 6.9). Embryonic mice were decapitated and immersion-fixed in the same solution. Tissues were cryosectioned at 10 μm and stained at 37°C overnight for *lacZ* activity by using 1 mg/ml X-Gal substrate (5-bromo-4-chloro-3-indolyl-β-D-galactoside) in 35 mM $K_3Fe(CN)_6$, 35 mM $K_4Fe(CN)_6{\cdot}3H_2O$, 1.0 ml 1 M $MgCl_2$, 0.01% desoxycholic acid, 0.02% Nonidet P-40. For immunocytochemistry, mice were perfused and brains postfixed in 4% paraformaldehyde/0.125 M phosphate buffer. Polyclonal rabbit anti-β-galactosidase (5′→3′, 1:400 dilution) and monoclonal mouse anti-glial fibrillary acidic protein (GFAP; Chemicon, 1:400 dilution) were used either singly or in combination. Goat anti-rabbit fluorescein isothiocyanate-conjugated and goat anti-mouse Texas red-conjugated secondary antibodies (Jackson, 1:200 dilution) were used for visualization.

For Zebrin II immunocytochemistry, nontransgenic brains were fixed in Bouin's fixative, then paraffin-embedded. Sections (15 μm) were immunoperoxidase-stained by using diaminobenzidine (30). To emphasize the glial immunoreactivity, anti-Zebrin II was used directly as spent culture medium without dilution, or diluted 1:1 in various blocking solutions; none reduced the Zebrin II/aldolase C immunoreactivity. When anti-Zebrin II was omitted or replaced by myeloma-conditioned medium, all staining was abolished.

RESULTS

***In Situ* Hybridization.** Isozyme-specific riboprobes were hybridized to sections of adult mouse brain. In cerebellum, the previously reported bands of aldolase C-positive Purkinje cells were seen, confirming that this pattern is controlled at the level of gene transcription (not shown). Other cells were clearly positive in the Purkinje cell layer, however, and their size and location suggested that they were Bergmann glial cells. Examination of other brain regions revealed a pattern of dispersed gray matter cells as well as cells at the pial surface, particularly in the hippocampal region (Fig. 1 *A* and *B*), suggesting that a low level of aldolase C mRNA is found in gray matter astrocytes and pial cells but not neurons. By contrast, aldolase A antisense probe labels virtually all neurons in telencephalon, diencephalon, midbrain, and spinal cord (Fig. 1*C*). In no CNS region examined could the pattern of aldolase A message be attributed to expression in glial cells. Curiously, in cerebellum, only granule cells and deep nuclear neurons were aldolase A-positive; Purkinje cells showed little or no message. Sense strand probes showed no signal (not shown). To determine whether the genetic regulatory elements that controlled this dichotomous expression pattern were present in *cis*-sequences proximal to the structural genes, we created transgenic mice by using different configurations of the aldolase C gene.

The MACZ2Δ9 Lines. To create the MACZ2Δ9 construct, two *Xho*I sites were engineered into the genomic sequences: the first 90 bp from the translation start site in the second exon, and the second 24 bp from the translation stop codon in exon 9. As the *lacZ* sequences used lack their own promoter and

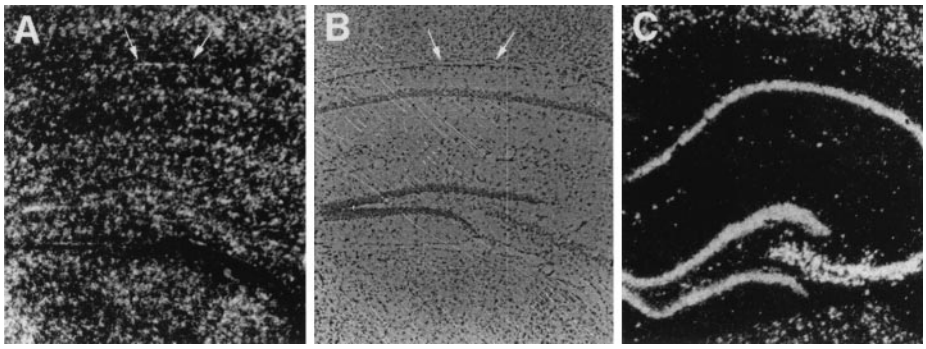

FIG. 1. *In situ* hybridization of aldolase A and C in adult CNS. (*A*) Dark-field image of aldolase C mRNA in non-neuronal cells in hippocampus. (*B*) Bright-field image of the same section shown in *A*. Arrows show positive pial cells. (*C*) Dark-field image of aldolase A hybridization in neurons of hippocampus and cortex. (×100.)

have no independent translation start site, the predicted product of this fusion gene is a peptide consisting of a short stretch of N-terminal amino acids from the aldolase C protein joined to full-length bacterial β-galactosidase (Fig. 2*B*, bottom line). Expression of the transgene in brain was confirmed by reverse transcription–PCR (Fig. 2*C*).

Five MACZ2Δ9 lines were established, and the tissues of various adult progeny were examined for β-galactosidase activity. Two lines revealed no trace of transgene expression in any tissue. The remaining three lines had significant staining in a small number of cells (Table 1). All three showed X-Gal staining in epithelial cells of the pia mater, the innermost of the three linings of the brain, primarily in posterior cerebral cortex (occipital and hippocampal; Fig. 3*E*). Although the intensity of staining varied from line to line (MACZ2Δ9/6 > MACZ2Δ9/4 > MACZ2Δ9/8), the location of the stained cells never varied. No staining was observed outside of the nervous system, nor was any neuronal staining found. MACZ2Δ9/6 also had transgene activity in a modest number of cells that appeared from their morphology to be gray matter astrocytes.

The MACZ9 Lines. To create the MACZ9 construct, the parent plasmid was opened at the engineered *Xho*I site in exon 9 (no modification in exon 2) and *lacZ* was inserted in-frame (Fig. 2*B*, first line). Note that this construct contains all of the endogenous genomic sequences. The predicted product of this fusion gene is a peptide consisting of nearly the entire aldolase C enzyme joined at its C terminus to full-length bacterial β-galactosidase. Four lines of transgenic mice were produced from this construct, and all four show robust β-galactosidase staining throughout the CNS, but never in neurons (Fig. 3 *A–D* and *F*). Two lines (MACZ9/2 and MACZ9/4) show such high expression of the transgene that X-Gal reaction product forms extracellularly (Fig. 3*C*); close inspection reveals that neuronal cell bodies do not express β-galactosidase. Rather, X-Gal reaction product is localized in small highly branched astrocyte-like cells in the gray matter of all brain regions of all four transgenic lines. Curiously, the area with the least staining activity, both in terms of the number of cells and the intensity of labeling within any one cell, is the cerebellum (Fig. 3*A*). Astrocyte-like cells are stained, primarily in the internal granule cell layer, as are a number of Bergmann glial cells (Fig. 3 *B* and *D*). Staining is never observed in Purkinje cells. Apart from the brain, no transgene activity is found in any other organ either by X-Gal staining (not shown) or by reverse transcription–PCR (Fig. 2*C*).

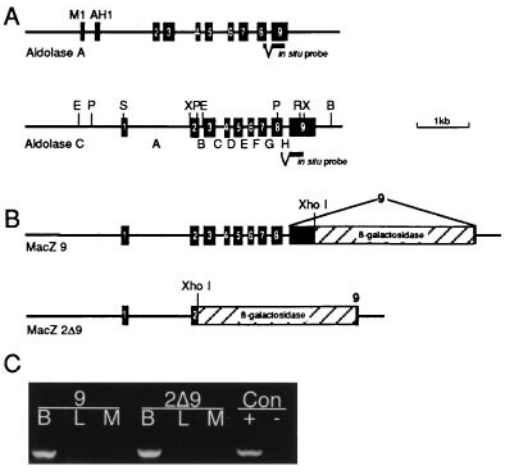

FIG. 2. Genomic organization of aldolase A and C, transgenic constructs, and analysis of transgene expression in transgenic mice. (*A*) Genomic organization of aldolase A and aldolase C. *In situ* hybridization probes are indicated below the eighth and ninth exons. Restriction sites: B = *Bam*HI, E = *Eco*RI, *P* = *Pst*I, S = *Sac*II, X = *Xba*I. (*B*) Transgenic constructs. (*C*) Reverse transcription–PCR of DNase I-treated RNA isolated from brain (B), liver (L), and muscle (M) of MACZ9 (indicated by 9) and MACZ2Δ9 (2Δ9) mice. Control (Con): tail DNA from a MACZ9 mouse (+) and a nontransgenic littermate (−).

To confirm the identity of the X-Gal-positive cells, immunocytochemistry using anti-GFAP and anti-β-galactosidase antibodies was performed on MACZ9/1 and MACZ 9/2 brains. Colocalization of signal was found in cortex (Fig. 3 *G–I*) and Bergmann glia of the cerebellum (data not shown). Cells of the pia mater, which are GFAP-negative, expressed β-galactosidase only (not shown). Thus, this staining cannot be attributed to glial end feet, but rather is intrinsic to the pial cell population.

Aldolase C/Zebrin II Antibody Staining. We reinvestigated the specificity of Zebrin II immunostaining by using high concentrations of primary antiserum. As predicted by the molecular studies, a significant level of immunoreactivity was found in glial cells throughout the brain. The typical pattern of bands of Zebrin II/aldolase C-positive Purkinje cells was

Table 1. Summary of expression patterns in MACZ2Δ9 and MACZ9 lines

Area	MACZ 9	MACZ 2Δ9
CNS		
Cerebellum	4/4	1/5
Hippocampus	4/4	0/5
Brainstem	4/4	1/5
Cerebral cortex	4/4	1/5
Midbrain	4/4	1/5
Spinal cord	4/4	0/5
Pia	4/4	3/5
Outside CNS	0/4	0/5

Each entry represents the number of lines (or founders) that expressed the transgene/total number of lines examined.

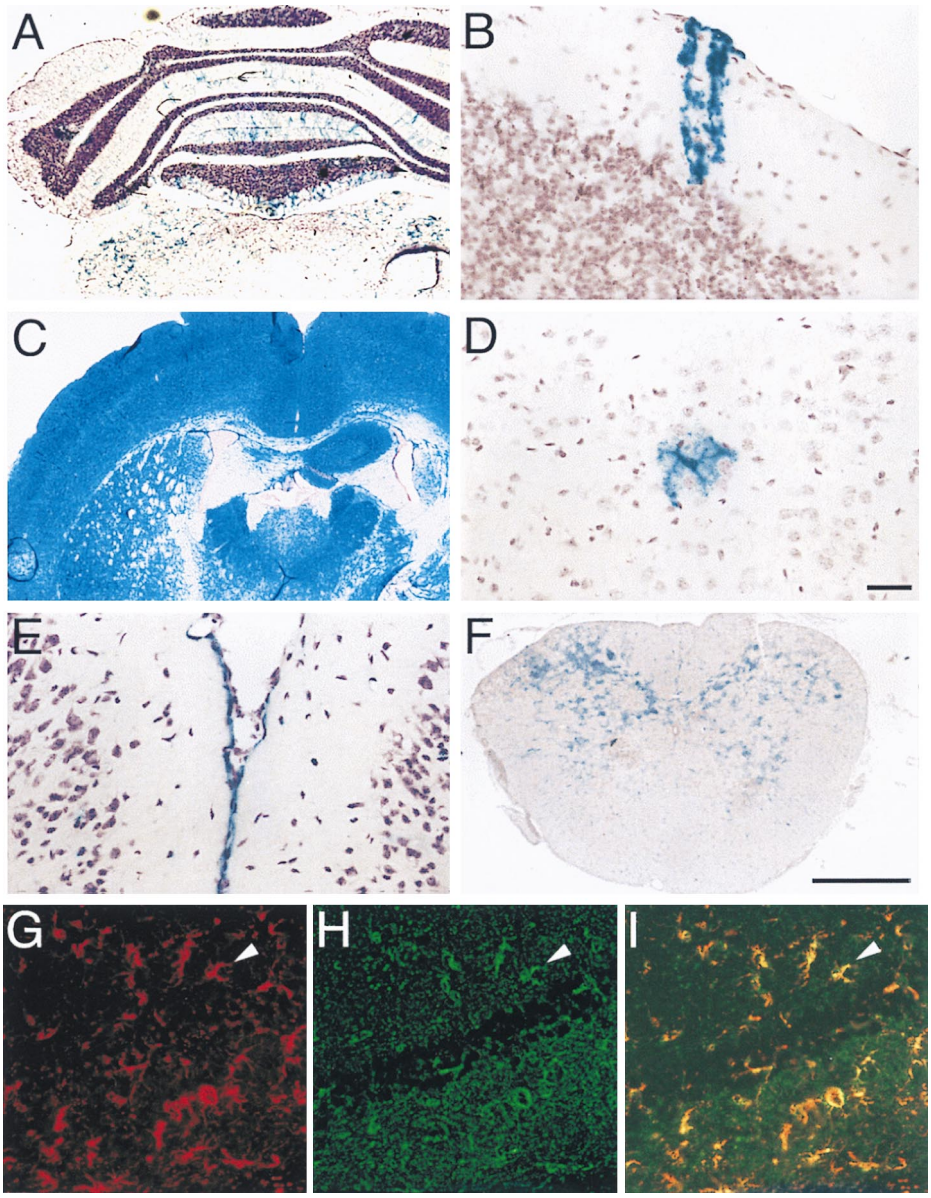

FIG. 3. Transgenic lines show expression in glial and pial cells. All sections are coronal. (*A–F*) X-Gal-reacted tissue counterstained with hematoxylin. (*A*) MACZ 9/1 cerebellum (×25). Staining is present in Bergmann glia, but absent in Purkinje cells. (*B*) Bergmann glial cell in cerebellar cortex (×400). (*C*) MACZ 9/2 cerebral cortex and hippocampus (×25). (*D*) MACZ9/3 cortical astrocyte (×400). (*E*) MACZ2Δ9 pial cells (×400). (*F*) Stained astrocytes in spinal cord gray matter of MACZ9/1 mouse (×25). (*G–I*) Hippocampal section of MACZ9/2 brain treated with both anti-β-galactosidase and anti-GFAP primary antibodies (×400). Arrowhead indicates same cell in each panel. (*G*) Anti-GFAP, visualized by Texas red-conjugated secondary antibody. (*H*) Anti-β-galactosidase staining visualized by fluorescein isothiocyanate-conjugated secondary antibody. (*I*) Double-exposure. [Bars = 25 μm (*F*) and 500 μm (*H*).]

found (Fig. 4*A*), but within both the Zebrin II-negative (Fig. 4 *B* and *C*) and -positive bands (Fig. 4 *B–D*), small cells with antigen positive perikarya were interspersed among the Purkinje cell somata. Their size and location make it likely that they are Bergmann glia. The radial Bergmann glial fibers are not immunoreactive (Fig. 4 *B* and *C*). Additional antibody-stained glia-like cells can be seen in the granule cell layer (Fig. 4 *B* and *C*). Thus, the glial location of the endogenous aldolase C message (as detected with *in situ* hybridization), the transgene (as detected with X-Gal staining and immunocytochemistry) and the protein (as detected with Zebrin II immunocytochemistry) are all in agreement. The only exception to this consistency is the cerebellar Purkinje cells that are mRNA- and protein-positive, but transgene-negative.

The Temporal Regulation of Transgene Staining. Aldolase C/Zebrin II first appears in the mouse cerebellum at about postnatal day 6. Antigen levels then increase until postnatal day 15 when all Purkinje cells are positive. Between postnatal days 15 and 30, staining is lost from the cells in the Zebrin II negative bands, culminating in the familiar zebra-like staining pattern (31). Animals from one line of MACZ9 were sacrificed on the day of birth, on postnatal day 4 and on postnatal day 7. Little or no activity is observed in any brain region at birth. By postnatal day 4, staining has begun to appear in cerebellum, cortex and hippocampus (Fig. 5); staining increases at postnatal day 7 and by postnatal day 30 adult levels of staining are seen throughout the CNS. Thus, the levels of transgene activity are temporally well-regulated.

DISCUSSION

Previous studies of aldolase C transgenic mice (22, 23) report that a short promoter region located within 115 bp of the transcription start site is sufficient to restrict the expression of

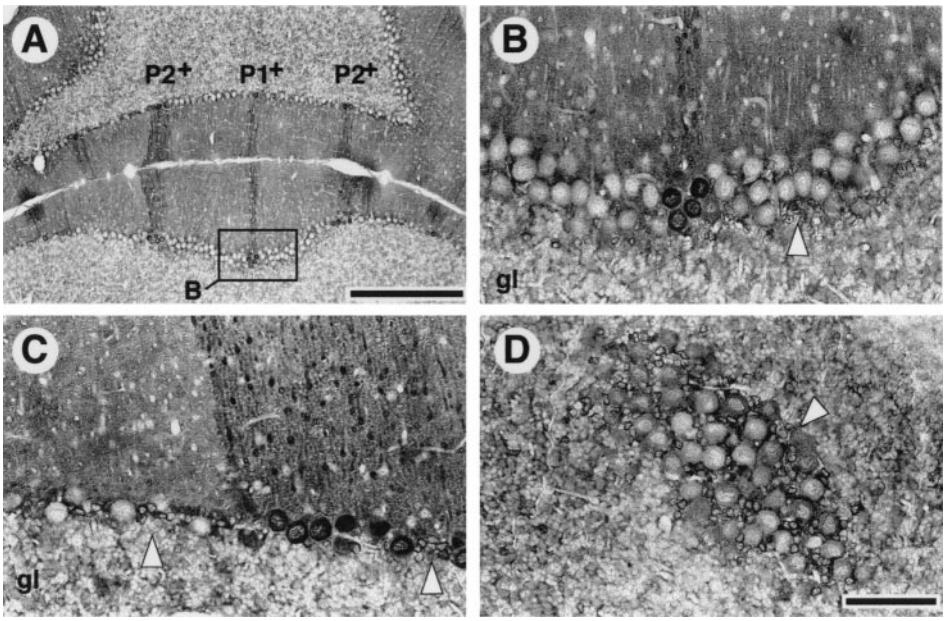

FIG. 4. Immunoperoxidase staining of the mouse cerebellar cortex with supra-normal concentrations of anti-Zebrin II/aldolase C. (*A*) The typical Purkinje cell bands ($P1^+$ and $P2^+$ are labeled). The area outlined by the rectangle is shown at higher magnification in *B*. (*B*) Small labeled profiles (arrowhead) among the stained Purkinje cell somata are Bergmann glia. The Bergmann glial fibers are not stained. (*C*) Glial immunoreactivity is not associated with a particular Purkinje cell phenotype: glial immunoreactivity is apparent in both $Zebrin^+$ and $Zebrin^-$ Purkinje cell domains (arrowheads). (*D*) Glancing section through the Purkinje cell layer where the Purkinje cell somata are unreactive but numerous Bergmann glial profiles are stained (arrowhead). [Bars = 500 μm (*A*) and 50 μm (*B–D*; in *D*).]

aldolase C to the CNS. This result is in agreement with our transgenic results. The comparison of the staining patterns observed in the MACZ9 lines with those in MACZ2Δ9 extends these findings by suggesting that significant enhancer elements must lie within the structural gene itself between exons 2 and 9. This suggestion is based on the fact that although only three of five MACZ2Δ9 lines show any detectable transgene activity, each of the four MACZ9 lines has strong levels of bacterial β-galactosidase activity in their brains. This is reminiscent of the aldolase B gene, in which elements located in the first intron are absolute requirements for transgene expression *in vivo* (13) and are capable of increasing the activity of the basal promoter by as much as 50-fold *in vitro* (12). Our results suggest a similar enhancer-like activity must exist in the aldolase C gene, although clearly not in the first intron. The finding of enhancer elements within the coding region of a gene is not new, but it emphasizes the importance of including these sequences as part of any detailed analysis of the regulation of the aldolase C transcriptional unit.

Cell Type Specificity. In their analysis of the behavior of a 13-kb rat aldolase C transgene containing 6 kb of 5′ flanking sequence and 3 kb of 3′ flanking sequence, Arai *et al.* (18) found transgene mRNA in neurons, including Purkinje cells. Our results differ significantly from theirs in that all of the

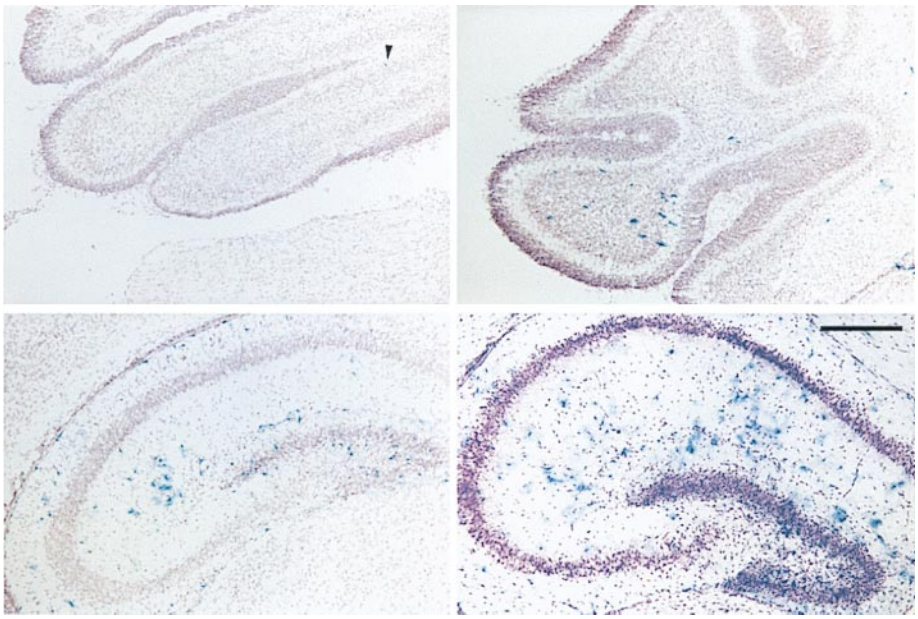

FIG. 5. Temporal regulation of expression in MACZ9/1 mice. Expression of the transgene is first seen at postnatal day 4 (*Left*) and by postnatal day 7 (*Right*) has increased in all regions examined. Expression is never seen outside of the CNS. (*Upper*) Cerebellum. (*Lower*) Hippocampus. All photographs are ×100. (Bar = 100 μm.)

MACZ constructs that we have analyzed are expressed only in gray matter astrocytes and pial cells. Our results also conflict with those of Popovici *et al.* (8) who found endogenous aldolase C message in neuronal cell cultures whereas aldolase A message was present in both neuronal and glial cell cultures. The reason for the discrepancies is not obvious. However, both our immunocytochemistry and *in situ* hybridization suggest that (with the exception of cerebellar Purkinje cells) the expression pattern of the endogenous aldolase C gene is identical to the pattern of transgene expression. Our results also agree with earlier immunocytochemical studies of the localization of aldolase C (16, 19). Interestingly, a distinct 120-kDa antigen defined by the Zebrin I antibody also has been reported in glial cells of cerebrum and cerebellum (32–34).

The precisely complementary patterns of expression of the endogenous aldolase A and C genes among the two major cell types of the brain is remarkable and unexpected. In every CNS region we have examined, aldolase A message is high in neurons and undetectable in glia. By contrast, aldolase C message is absent from neurons but found in astrocytes and cells of the pia. The only exception to this glial/neuronal split between the A and C isoforms is in cerebellar Purkinje cells where the complementarity is maintained, but aldolase C is neuronal whereas aldolase A is silent. This reciprocal pattern of expression is all the more intriguing given the hypothesis that the A and C isoforms diverged from a single ancestral gene several million years ago (1, 35).

The Purkinje Cells. Our findings demonstrate that the genetic element(s) that drive high levels of aldolase C expression in Purkinje cells are missing from the transgenes used in this study. It seems likely that the elements responsible for this precise cell type specificity must lie in the 4.5 kb of sequences that differ between the construct used by Arai *et al.* (18) and the 8.5-kb fragment that we have used. Likely candidates include the methylation-sensitive site at −3.8 kb (methylated in liver but not in brain) or the DNase I-sensitive site at 1.5 kb 3′ to the polyadenylation signal (22). Either of these sites would be plausible as a location for the neuronal enhancer as they are contained in the large genomic constructs of previous studies, but missing in all of the chimeric genes reported here. Still unknown is whether the missing Purkinje cell enhancer also will direct the banded expression pattern that is characteristic of Zebrin II/aldolase C.

This work was supported by grants from the National Institutes of Health (NS18381) to K.H. and the Medical Research Council (Canada) to R.H., E.U.W., and M.D. were supported by fellowships from the Jung-Stiftung für Wissenschaft und Forschung, awarded to R. B. Daroff.

1. Tolan, D., Niclas, J., Bruce, B. & Lebo, R. (1987) *Am. J. Hum. Genet.* **41,** 907–924.
2. Maire, P., Gautron, S., Hakim, V., Grégori, C., Mennecier, F. & Kahn, A. (1987) *J. Mol. Biol.* **197,** 425–438.
3. Izzo, P., Costanzo, P., Lupo, A., Rippa, E., Paolella, G. & Salvatore, F. (1988) *Eur. J. Biochem.* **174,** 569–578.
4. Mukai, T., Joh, K., Arai, Y., Yatsuki, H. & Hori, K. (1986) *J. Biol. Chem.* **261,** 3347–3354.
5. Joh, K., Arai, Y., Mukai, T. & Hori, K. (1986) *J. Mol. Biol.* **190,** 401–410.
6. Salminen, M., López, S., Maire, P., Kahn, A. & Daegelen, D. (1996) *Mol. Cell. Biol.* **16,** 76–85.
7. Salminen, M., Maire, P., Concordet, J.-P., Moch, C., Porteu, A., Kahn, A. & Daegelen, D. (1994) *Mol. Cell. Biol.* **14,** 6797–6808.
8. Popovici, T., Berwald-Netter, Y., Vibert, M., Kahn, A. & Skala, H. (1990) *FEBS Lett.* **268,** 189–193.
9. Lebo, R., Tolan, D., Bruce, B., Cheung, M.-C. & Kan, Y. (1985) *Cytometry* **6,** 478–483.
10. Weber, A., Marie, J., Cottereau, D., Simon, M., Besmond, C., Dreyfus, J. & Kahn, A. (1984) *J. Biol. Chem* **259,** 1798–1802.
11. Tsutsumi, K., Ito, K. & Ishikawa, K. (1989) *Mol. Cell. Biol.* **9,** 4923–4931.
12. Grégori, C., Ginot, F., Decaux, J., Weber, A., Berbar, T., Kahn, A. & Pichard, A. (1991) *Biochem. Biophys. Res. Commun.* **176,** 722–729.
13. Sabourin, J.-C., Kern, A.-S., Grégori, C., Porteu, A., Cywiner, C., Châtelet, F.-P., Kahn, A. & Pichard, A.-L. (1996) *J. Biol. Chem.* **271,** 3469–3473.
14. Mukai, T., Yatsuki, H., Masuko, S., Arai, Y., Joh, K. & Hori, K. (1991) *Biochem. Biophys. Res. Commun.* **174,** 1035–1042.
15. Royds, J., Ironside, J., Warnaar, S., Taylor, C. & Timperley, W. (1987) *Neuropathol. Appl. Neurobiol.* **13,** 11–21.
16. Thompson, R., Kynoch, P. & Willson, V. (1982) *Brain Res.* **232,** 489–493.
17. Schapira, F., Reuber, M. & Hatzfeld, A. (1970) *Biochem. Biophys. Res. Commun.* **40,** 321–327.
18. Arai, Y., Kajihara, S., Masuda, J., Ohishi, S., Zen, K., Ogata, J. & Mukai, T. (1994) *Eur. J. Biochem.* **221,** 253–260.
19. Wachsmuth, E., Thorner, M. & Pfleiderer, G. (1975) *Histochemistry* **45,** 143–161.
20. Vibert, M., Henry, J., Kahn, A. & Skala, H. (1989) *Eur. J. Biochem.* **181,** 33–39.
21. Buono, P., de Conciliis, L., Olivetta, E., Izzo, P. & Salvatore, F. (1993) *FEBS Lett.* **328,** 243–249.
22. Makeh, I., Thomas, M., Hardelin, J.-P., Briand, P., Kahn, A. & Skala, H. (1994) *J. Biol. Chem.* **269,** 4194–4220.
23. Thomas, M., Skala, H., Kahn, A. & Tuy, F. (1995) *J. Biol. Chem.* **270,** 20316–20321.
24. Ahn, A., Dziennis, S., Hawkes, R. & Herrup, K. (1994) *Development (Cambridge, U.K.)* **120,** 2081–2090.
25. Hawkes, R. & Gravel, C. (1991) *Progr. Neurobiol.* **36,** 309–327.
26. Hawkes, R., Brochu, G., Dore, L., Gravel, C. & Leclerc, N. (1992) in *Zebrins: Molecular Markers of Compartmentation in the Cerebellum*, eds. Llinas, R. & Sotelo, C. (Springer, New York), pp. 22–55.
27. Hawkes, R., Blyth, S., Chockkan, V., Tano, D., Zhongqi, J. & Mascher, C. (1993) *Can. J. Neurol. Sci.* **20,** Suppl. 3, S29–S35.
28. Hawkes, R. & Herrup, K. (1996) *J. Mol. Neurosci.* **6,** 147–158.
29. Herrup, K. & Kuemerle, B. (1997) *Annu. Rev. Neurosci.* **20,** 61–90.
30. Eisenman, L. & Hawkes, R. (1993) *J. Comp. Neurol.* **335,** 586–605.
31. Leclerc, N., Gravel, C. & Hawkes, R. (1988) *J. Comp. Neurol.* **273,** 399–420.
32. Plioplys, A. & Hawkes, R. (1986) *Brain Res.* **375,** 1–12.
33. Plioplys, A. & Hawkes, R. (1987) *Brain Res. Bull.* **18,** 19–24.
34. Plioplys, A. & Hawkes, R. (1988) *J. Neurosci. Res.* **20,** 359–375.
35. Kukita, A., Mukai, T., Miyata, T. & Hori, K. (1986) *Eur. J. Biochem.* **171,** 471–478.
36. Kelley, K., Friedrich, V., Sonshine, A., Hu, Y., Lax, J., Li, J., Drinkwater, D., Dressler, H. & Herrup, K. (1994) *Mol. Brain Res.* **24,** 261–274.