Diverse human aldolase C gene promoter regions are required to direct specific LacZ expression in the hippocampus and Purkinje cells of transgenic mice

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Abstract **Aldolase C is selectively expressed in the hippocampus and Purkinje cells in adult mammalian brain. The gene promoter regions governing cell-specific aldolase C expression are obscure. We show that aldolase C messenger expression in the hippocampus is restricted to CA3 neurons. The human distal promoter region (−200/−1200 bp) is essential for β-galactosidase (β-gal) expression in CA3 neurons and drives high stripe-like β-gal expression in Purkinje cells. The 200 bp proximal promoter region is sufficient to drive low brain-specific and stripe-like β-gal expression in Purkinje cells. Thus, the human aldolase C gene sequences studied drive endogenous-like expression in the brain.**

Keywords: Human aldolase C gene; Hippocampus; Purkinje cells; Transgenic mice; Zebrin

1. Introduction

The aldolase C gene encodes the brain-specific form of the glycolytic enzyme. The kinetic properties of the aldolase C enzyme, tested against the two major substrates fructose-1-phosphate and fructose-1,6-bisphosphate, are intermediate with respect to the A and B isoforms [1]. The gene is transcribed exclusively in the central nervous system (CNS), i.e., in the hippocampal neurons and in the Purkinje cells of the cerebellum [2–5]. Like other brain-specific genes, the aldolase C gene is endowed with properties characteristic of housekeeping genes: it lacks a normal TATA box, and a GC-rich region is present just upstream from the major transcriptional start site. Furthermore, a CAAT box is located much further upstream than expected [6–8]. Several tissue-specific methylation sites have been found around the transcription unit in the rat gene [9]. Aldolase C gene expression has been investigated in both in vivo and in vitro models. The proximal region of the rat promoter, spanning about 115 bp upstream from the major transcriptional start site, is able to direct CAT-reporter gene expression, albeit at low levels, in the brain of transgenic mice [10–12]. The upstream region binds Pax-6 transcriptional factor and is involved in the activation of rat aldolase C gene expression in the brain of transgenic mice [13]. We previously showed, in human-derived neuronal cells (SKNBE), that two regions (proximal and distal) are required for the complete transcriptional expression of the CAT-reporter gene of chimeric aldolase C [14]. We also characterized in the distal region a functional binding-site (element D) for the transcriptional activator NGFI-B. The binding of NGFI-B to element D increases transcription of the CAT-reporter gene in neuronal cell lines and mediates cAMP induced transcriptional activation of a chimeric aldolase C CAT gene in PC12 cells [15,16]. A peculiar cytohistological aldolase C pattern has been demonstrated in the mouse and rat cerebellum, where the zebrin II protein was identified as aldolase C [17,18]. Recently, we identified that pattern in the human brain [5].

In the present study, we compared the expression of endogenous aldolase C mRNA in adult mouse hippocampus and cerebellum to that of LacZ expression driven by diverse human aldolase C gene promoter regions. We found that the complete human promoter region (1200 bp) drives high LacZ expression in CA3 hippocampal neurons and its stripe-like expression in Purkinje cells of transgenic mice, thereby mimicking endogenous aldolase C expression. The 200-bp construct lacking the distal promoter region failed to direct LacZ expression in CA3 hippocampal neurons and conserved only the low stripe-like LacZ expression in Purkinje cells.

2. Materials and methods

2.1. Cloning and DNA preparation for microinjection

The transgenic construct (pAldC2500-LacZ) was obtained by cloning in the *Sal*I/*Bam*HI sites of the pBluescript SK+ vector (Stratagene) the human aldolase C promoter region up to the methionine residue in exon 2 (2500 bp) [19] that was fused in-frame with the methionine

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Abbreviations: CNS, central nervous system; β-gal, β-galactosidase

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residue of the bacterial LacZ cassette [20]. The mouse β-globin poly(A)$^+$ cassette (1500 bp) [21] was subcloned downstream from the LacZ cassette (see Figs. 1A and B). The Quick Change site-directed mutagenesis kit (Stratagene) was used to create pAldC2500*-LacZ lacking element D (Fig. 1B) and pAldC1500-LacZ containing only 36 bp of the distal region and element D (Fig. 1B). Recombinant mutated plasmids were purified over a Qiagen column and their sequences were verified by automated sequencing. After removal of vector sequences by *Sal*/*Not*I digestion, the microinjection fragments were purified from agarose gel by electroelution and phenol:chloroform extraction, resuspended in TE buffer at 2 ng/μl and microinjected into 1-cell fertilized eggs.

2.2. Generation and identification of transgenic mice

Single-cell embryos were harvested from the hybrid B6D2F1 mouse strain (Charles River). The purified Ald C/LacZ fragments were microinjected into the embryonic male pronuclei and surviving embryos were transferred into the oviducts of pseudopregnant CD-1 foster mothers (Charles River) essentially as described in Hogan et al. [22]. Transgenic founders and offspring were identified by Southern blot using a [α^{32}-P]ATP random-primed LacZ cDNA probe. Three mouse lines were obtained using the pAldC2500-LacZ construct; eight independent transgenic founders were obtained with pAldC2500*-LacZ and with pAldC1500-LacZ (see Fig. 1B). The Animal Experimentation Ethics Committee of the National Institute for Cancer Research of Genoa approved all the animal studies.

2.3. β-Galactosidase assay

β-Galactosidase (β-gal) activity was assayed in intact brains and body parts of adult transgenic mice obtained from the three constructs; coronal plate sections were identified according to Altman and Bayer [23]. After fixation in 2% paraformaldehyde for 1 h, the organs were rinsed and incubated overnight at 30 °C in phosphate-buffered saline (PBS) containing 400 μg/ml X-Gal substrate (5-bromo-4-chloro-3-indolyl-β-D-galactoside), 4 mM potassium ferricyanide, 4 mM $MgCl_2$, and 0.1% Nonidet P-40 as described in Smeyne et al. [24] and in Oberdick et al. [25]. Cryostat sections from adult transgenic mice were obtained as described in Oberdick et al. [25]. After β-gal assay, the brains were equilibrated with 30% sucrose overnight, embedded in OCT compound (VWR), cut into 10-μm serial sections on a cryostat or in 200-μm sections on a vibratome and collected on coated glass slides (VWR).

2.4. Northern blot analysis

Total RNA was isolated from mouse adult brain, liver, kidney and heart using the CsCl method [26]. For the Northern blot procedure, we used 20 μg of total RNA as previously described [15]. A 1600-bp *Bam*HI fragment from the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA [27], a 3100-bp fragment containing the coding region for bacterial β-gal [20] and a 560-bp fragment containing the 3′-region of mouse cDNA specific for the aldolase C isoform [7] (i.e., the same probes used in in situ hybridization) served as probes. The fragments were labelled according to a random priming kit procedure (Takara). Endogenous aldolase C messenger expression and heterologous aldolase C-β-gal expression were determined by Phosphor Imager membrane analysis (Storm 840, Molecular Dynamics, Amersham Biosciences). The relative abundance, normalized for GAPDH expression, was calculated using the Image Quant v 1.1 software and is expressed as the mean values of two experiments.

2.5. In situ hybridization

Animals were killed by cervical dislocation; brains were removed, washed in PBS and fixed in phosphate-buffered 4% paraformaldehyde. The brains were then embedded in paraffin, cut in 7-μm microtome sections and collected on coated glass slides. To generate RNA probes with which to detect the endogenous messenger a 560-bp *Pst*I fragment of the 3′-mouse aldolase C cDNA [7] was subcloned in the *Pst*I site in the pBluescript KS+ vector. Sense and antisense cRNA probes were generated using T3 and T7 RNA polymerases in the presence of digoxigenin-11-UTP (Roche). In situ hybridization was essentially as described in Boehm et al. [28].

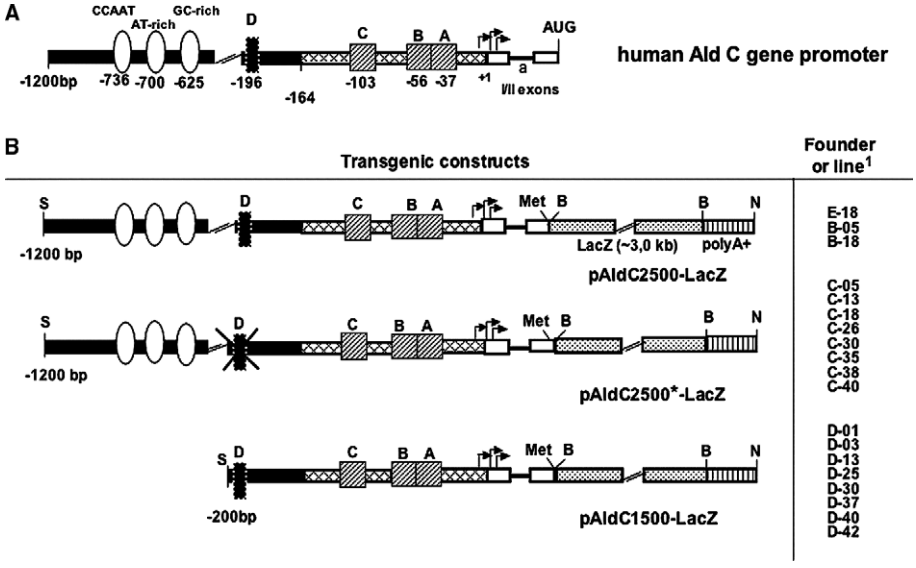

Fig. 1. Structure of the human aldolase C gene promoter and human transgenic constructs. (A) Human aldolase C promoter structure. +1 is the major transcription start site. *cis*-acting elements in the proximal region (A, B and C) are shown. The black rectangle in the distal region (heavy black bar) is element D [15]; the transcription factor-binding sites in the distal region (GC-rich, AT-rich and CAAT motif) are circled. The hatched rectangles represent the proximal promoter region that is conserved (more than 75% homology) among the mouse, rat and human genes [11,14,15]; the empty rectangles represent exons 1 and 2 up to the AUG; the intron 1 is indicated by (a). (B) The transgenic construct pAldC2500-LacZ contains 1200 bp of the human promoter region with the proximal and the distal region including element D, exon 1 and intron 1, up to the methionine (Met) in exon 2. The LacZ cassette is indicated by the dotted box; the poly(A)$^+$ cassette is indicated by a rectangle with vertical lines; the transgenic construct pAldC2500*-LacZ is identical to the pAldC2500-LacZ construct but lacks element D; transgenic construct pAldC1500-LacZ contains the proximal promoter region and only 36 bp of the distal region including element D. E-18 is the not stabilized transgenic mouse line, B-05 and B-18 are the stabilized transgenic mouse lines obtained from the pAldC2500-LacZ construct; C-05, C-13, C-18, C-26, C-30, C-35, C-38 and C-40 are the transgenic founders obtained using the pAldC2500*-LacZ construct; D-01, D-03, D-13, D-25, D-30, D-37, D-40 and D-42 are the transgenic founders obtained using construct pAldC1500-LacZ. Restriction sites: B = *Bam*HI, N = *Not*I, S = *Sal*I. [1]Copy numbers ranged from 5 to 24. There was no correlation between copy number and β-gal activity.

2.6. Immunohistochemistry

Paraffin sections from brain were rinsed in PBS with 0.2% Triton X-100 (PBST) and incubated 14 h at 4 °C with anti-calbindin (Swant), anti-glial fibrillary acidic protein (GFAP) and with anti-β-gal polyclonal antibodies (Chemicon) in 1.5% goat serum in PBST. The sections were then rinsed and incubated for 1 h in biotinylated secondary antibody. Immunocomplexes were visualized with an ABC Elite kit and the 3,3′-diaminobenzidine method (Vector Laboratories). Different developing times were used for the two constructs: 15–20 s for pAldC2500-LacZ and pAldC2500*-LacZ and 50 s for pAldC1500-LacZ.

3. Results

3.1. Brain-specific expression of the fusion human gene in transgenic mice

To investigate the promoter region(s) of the human aldolase C gene involved in its brain-specific expression, we produced transgenic mice with three different constructs (Figs. 1A and B). Plasmid pAldC2500-LacZ containing the complete promoter region of the human gene (1200 bp), plasmid pAldC2500*-LacZ carrying deletion (10 bp) of distal element D (Fig. 1B, second line), and plasmid pAldC1500-LacZ carrying the proximal region (164 bp) plus 36 bp of the distal region, and intact element D (Fig. 1B, third line) were microinjected in embryonic male pronuclei to generate transgenic founders. We obtained three pAldC2500-LacZ mouse lines (E18, B-05 and B-18), two of which (B-05 and B-18) were established. Eight independent transgenic founders were obtained from plasmids pAldC2500*-LacZ and pAldC1500-LacZ, (Fig. 1B). Brains from three B-05 and three B-18 adult animals and from three founders (C-05, C-13 and C-18) obtained with pAldC2500*-LacZ and three founders (D-01, D-03 and D-13) obtained with pAldC1500-LacZ were dissected from the bodies, and both brains and bodies were examined for β-gal activity in a whole-mount experiment. No β-gal activity was detected in the three brains from the transgenic founders D-01, D-03 and D-13 (Fig. 2A). β-gal activity was detected only in the brains of B-05 and B-18 mice and of pAldC2500*-LacZ founders (Fig. 2C). Activity was similar in the brains of these mice; it was highest in the cerebellum, and low in the midbrain and in the brain hemispheres (Fig. 2C, cb, mid and h, respectively). A coronal vibratome section of plate 25 [23] from a B-18 transgenic mouse shows in greater detail the intense β-gal activity in the cerebellum (Fig. 2D, cb) and the low activity in the pons and medulla (Fig. 2D, po and m). A coronal cryostat section from the same transgenic mouse cerebellum shows β-gal activity in clusters of Purkinje cells alternating with clusters of β-gal-negative Purkinje cells (Fig. 2E), thereby resulting in the characteristic zebrin-like distribution described for the rat endogenous aldolase C mRNA [17]. Apart from brain, no β-gal activity was found in any organ (not shown).

We next measured endogenous aldolase C mRNA expression in the brain, liver, kidney and heart of two B-18 mice, two pAldC2500*(C-26 and C-30) and two pAldC1500-LacZ founders (D-25 and D-30) in a Northern blot experiment. Endogenous aldolase C mRNA expression occurred only in brains (Fig. 3, dotted bar). Similarly, β-gal expression was detected only in the brains of the two pAldC2500-LacZ, pAldC2500*-LacZ and pAldC1500-LacZ mice brains examined (Fig. 3, white and black bars). This experiment confirms that β-gal expression is restricted to the brain and that it coincides with the expression of the endogenous messenger. The

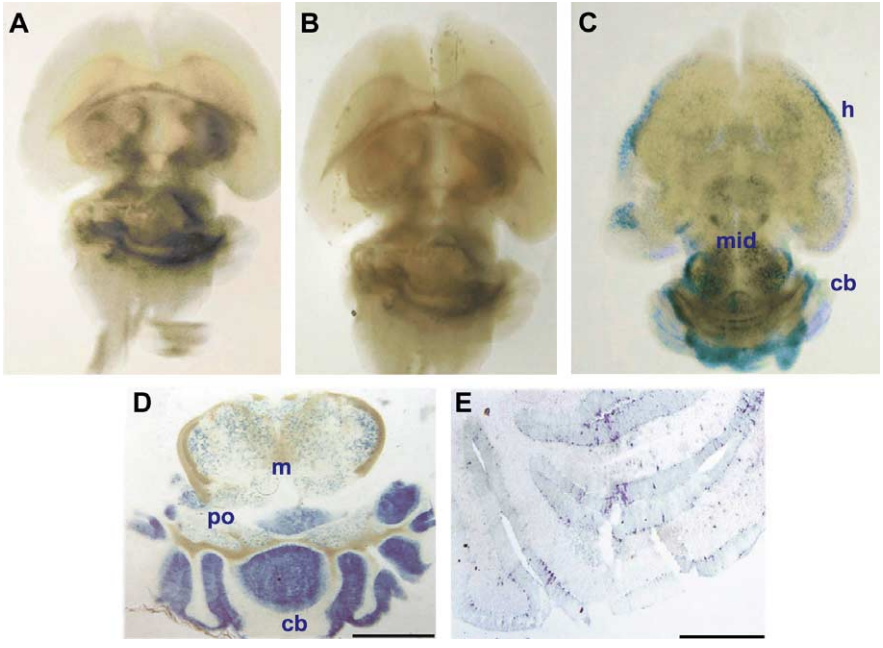

Fig. 2. β-gal activity in whole-mount brain and in cryo-sections brain from transgenic mice. (A) β-gal whole-mount staining of D-03 transgenic mouse brain obtained with the pAldC1500-LacZ construct; note the absence of β-gal activity. (B) Whole-mount staining of non-transgenic mouse brain. (C) β-gal whole-mount activity of a mouse brain from the B-18 line; β-gal expression is scattered, more or less sparsely, over the cerebral hemispheres (h) and midbrain (mid); staining is highest in the cerebellum (cb). (D) Coronal vibratome brain section from the same brain shown in (C) taken at the coronal plate 25 [23] in which β-gal activity is strong in the cerebellum (cb), and much lower in the pons (po) and medulla (m). (E) Coronal cryostat section of the same brain shown in (C) with β-gal activity restricted to clusters of Purkinje cells within the cerebellum cortex. (A–C) the images are magnified 5 times with respect to the slides. Scale bars: (D) 1000 μm; (E) 500 μm.

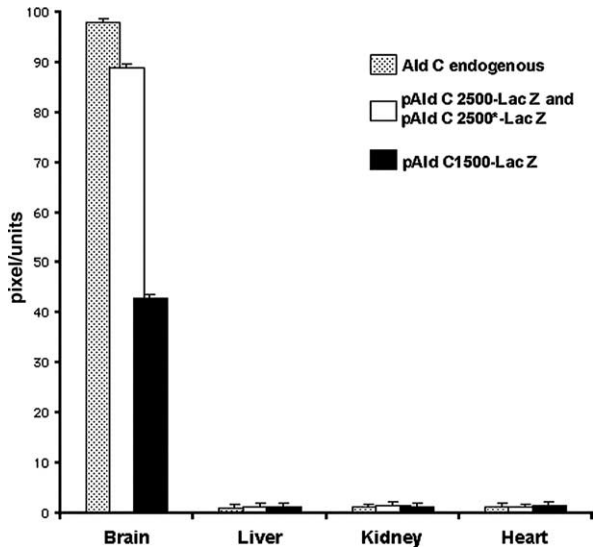

Fig. 3. Phosphor Imager results of Northern blot experiments with aldolase C and aldolase-C-LacZ mRNA expression in different tissues of transgenic mice. Total RNA from brain, liver, kidney and heart obtained from two B-18 transgenic mice and from two pAldC2500*-LacZ founders (C-26 and C-30) and two pAldC1500-LacZ founders (D-25 and D-30) was analyzed. The dotted bar shows endogenous aldolase C mRNA expression, the white bar shows messenger aldolase C-β-gal expression obtained with the pAldC2500-LacZ and pAldC2500*-LacZ constructs; the black bar shows messenger aldolase C-β-gal expression obtained with the pAldC1500-LacZ construct. The values represent means ± S.D. of two experiments.

β-gal expression in the brains of animals carrying the complete promoter sequence (pAldC2500-LacZ) or deletion of element D (pAldC2500*-LacZ) (Fig. 3, white bar) was as high as endogenous aldolase C mRNA expression, whereas it was about 50% lower in animals carrying the large deletion in the distal promoter (pAldC1500-LacZ, Fig. 3, black bar). This result indicates that 200 bp of the human promoter region (164 bp of the proximal region plus 36 bp of the distal region) is sufficient to induce brain-specific β-gal expression, confirming the results of in vitro experiments [14,15]. The apparent discrepancy between undetectable β-gal activity in whole mount pAldC1500-LacZ brains and the presence of β-gal-AldC hybrid mRNA in the brains of mice carrying the same construct can be ascribed to the greater sensitivity of the Northern blot method versus the histochemical procedure. However, the function of element D, which induces CAT reporter gene transcription 2.5-fold compared with the 164 bp construct in human neuroblastoma cells [15], seems to be exerted also by other elements present in the distal promoter region.

3.2. Differential expression of aldolase C-LacZ transgene in the hippocampus and cerebellum

To investigate the distribution of endogenous aldolase C mRNA within hippocampus and cerebellum we used a mouse aldolase C-specific antisense riboprobe to hybridize sagittal paraffin sections of adult transgenic mouse brain. Within the hippocampus, aldolase C was expressed only in the pyramidal neurons of hippocampus region CA3 (Fig. 4A). A sense strand

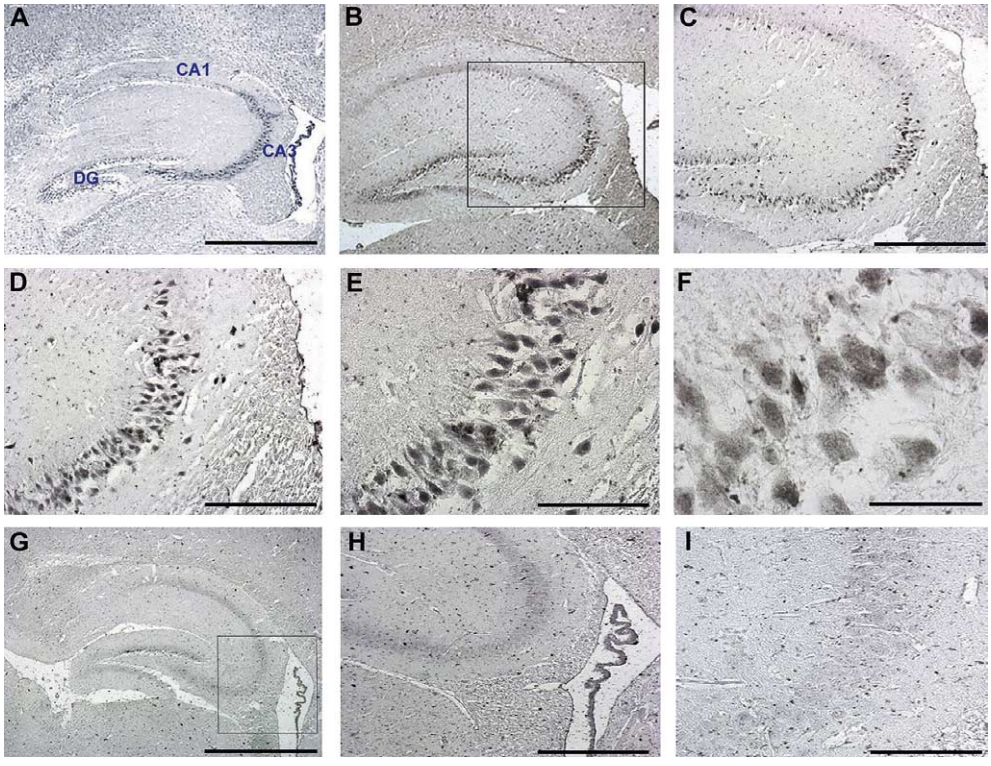

Fig. 4. In situ hybridization and anti-β-gal immunostaining on sagittal hippocampal paraffin sections of adult transgenic mice. (A) In situ hybridization with specific mouse aldolase C antisense riboprobe shows specific endogenous aldolase C mRNA expression restricted to the CA3 neurons in the hippocampus. (B) β-gal immunostaining of sagittal hippocampal sections from B-18 transgenic mice: specific labeling is evident in the same CA3 neuronal cells that express the endogenous messenger. (C–F) Magnifications of the boxed area indicated in (B). (G–I) β-gal immunostaining on a hippocampal section obtained from a D-40 transgenic founder obtained using pAldC1500-LacZ construct: no immunostaining is evident in CA3 neuronal cells even at the higher magnification shown in (H) and (I). DG = dentate gyrus, CA1 and CA3 = hippocampal subregions. Scale bars: (A, B, G) 1000 μm; (C, H) 500 μm; (D) 200 μm; (E, I) 100 μm; (F) 50 μm.

probe produced no signal (not shown). To examine the expression of transgene aldolase C, we used β-gal immunohistochemistry in brain sections from the mice carrying the complete or partially deleted promoter region of the human gene fused to the β-gal reporter gene. β-gal immunostaining driven by the pAldC2500-LacZ construct was restricted to the same CA3 hippocampal region that expressed the endogenous aldolase C (Figs. 4A and B). A higher magnification shows that β-gal expression was localized in the cytoplasm of these cells (Figs. 4C–F). Similar results were observed in C-35, C-38 and C-40 founders obtained with the pAldC2500*-LacZ construct that lacked element D, indicating that the binding of NGFI-B to this element is not involved in β-gal expression in the hippocampus in vivo (data not shown). β-gal was not evident in hippocampus sections from D-37, D-40 and D-42 mice that carry a large deletion in the distal region not involving element D (pAldC1500-LacZ plasmid) (Fig. 4G); this is more evident at higher magnification (Figs. 4H and I). The results of these experiments indicate that β-gal expression in the hippocampus is driven by upstream elements located upstream from element D.

We next measured endogenous aldolase C messenger expression on sagittal cerebellar sections of adult mouse brain using the same specific riboprobe we used for hippocampus sections. Fig. 5A shows the result of one experiment showing groups of aldolase C-positive Purkinje cells alternating to groups of aldolase C-negative Purkinje cells. The alternating positive-negative Purkinje cells and the cytoplasmic location of the messenger are more evident at higher magnification (Fig. 5B).

To identify the human aldolase C gene region that contains the elements sufficient to direct β-gal expression in the Purkinje cells of transgenic mice, we evaluated anti-β-gal antibody immunostaining in different cerebellar sections from the same transgenic mice analyzed for immunostaining in hippocampus regions. Immunoreactivity was intense in the Purkinje cell layer of mice carrying the pAldC2500-LacZ plasmid, which contains the entire promoter region (Fig. 5C). The pattern of β-gal expression in the Purkinje cell layer is similar to the stripe-like pattern of the endogenous aldolase C mRNA found by in situ hybridization (compare Figs. 5A and C). β-gal expression was localized in the cytoplasm of stained Purkinje cells (see higher magnification in Figs. 5D and E). Similar results were obtained with cerebellar sections from the transgenic mice obtained with the pAldC2500*-LacZ construct in which element D was deleted (data not shown).

Analysis of cerebellar sections from the same three pAldC1500-LacZ founders (used to detect immunostaining in the hippocampus) showed low β-gal immunoreactivity in the Purkinje cell layer (Fig. 5F), as indicated by the longer exposure time required to develop the specific signal (see Section 2). This result is in agreement with the reduced AldC-LacZ messenger expression in the brains of adult transgenic founders obtained with pAldC1500-LacZ (see Fig. 3, black bar). It also reinforces in vitro [14,15] and in vivo [10–13] results obtained with human and rat promoter sequences.

To evaluate whether the processing procedure damaged the cerebellar slices, we stained cerebellar sections adjacent to those shown in Figs. 5C–E and in Figs. 5F and G with anti-calbindin antibody, which is a specific Purkinje cell marker. The protein was distributed throughout the Purkinje cell layer indicating that the layer was not damaged during processing (Fig. 5H). This result also confirms the stripe-like distribution of aldolase C messenger in the Purkinje cell layer and demonstrates that 200 bp of the human aldolase C gene promoter region contains all the elements necessary to direct striped expression of aldolase C messenger in this layer (Figs. 5F and G).

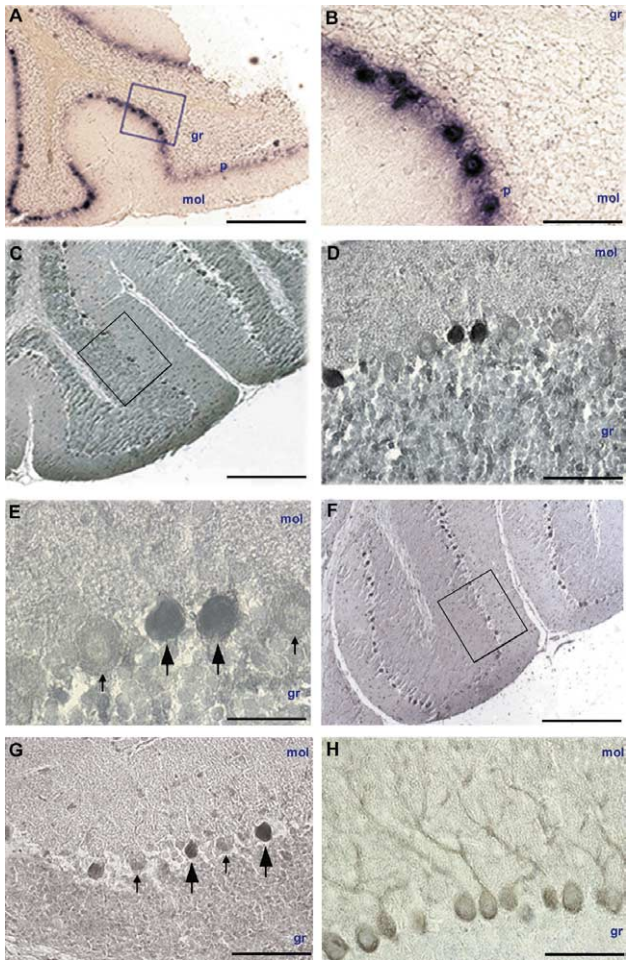

Fig. 5. In situ hybridization and β-gal immunostaining on paraffin sagittal cerebellar sections from transgenic mice. (A) In situ hybridization using mouse specific aldolase C antisense riboprobe: note the intense expression of the endogenous messenger throughout the Purkinje cell layer; aldolase C is localized in the perikaryon of Purkinje cells. (B) The boxed area in (A) at a higher magnification. (C) Anti-β-gal immunostaining on sections of B-18 transgenic mice: β-gal expression is evident in groups of Purkinje cells surrounded by negative Purkinje cells. (D and E) Part of the boxed area indicated in C is shown at a higher magnification in D and E: two positive Purkinje cells (thick arrows) surrounded by negative Purkinje cells (thin arrows) are indicated. (F) Anti-β-gal immunostaining on sections from a transgenic D-40 founder mouse: note the same β-gal expression pattern throughout the Purkinje cells as observed in the B-18 transgenic mouse; (G) part of the boxed area indicated in F is reported at a higher magnification. Note the clusters of Purkinje cells expressing β-gal (thick arrows) alternating with clusters of β-gal-negative Purkinje cells (thin arrows). (H) Anti-calbindin immunohistochemistry. Staining throughout the Purkinje cell layer shows the integrity of this layer; note the localization in the perikaryon of Purkinje cells. mol = molecular, p = Purkinje, gr = granular cell layers. Scale bars: (A, C, F) 200 μm; (B, D, G, H) 100 μm; (E) 50 μm.

To verify that the β-gal-positive cells were neurons and not glial cells, we examined transgenic brains using immunohistochemistry and anti-GFAP antibodies. Sagittal cerebellar

sections from an adult B-18 pAldC2500-LacZ mouse showed specific immunoreactivity in glial cells (Figs. 6A and B); this reactivity was absent from Purkinje cells expressing this transgene (Fig. 6C). Anti-GFAP immunohistochemistry of sagittal sections from hippocampus of the same pAldC2500-LacZ mouse confirmed specific immunoreactivity only in glial cells (Figs. 6D–F); there was no immunoreactivity in pyramidal CA3 neurons (Fig. 6D, rectangle) that express pAldC2500-LacZ and pAldC2500*-LacZ transgenic constructs.

4. Discussion

Notwithstanding numerous studies focusing on aldolase C in mammals [10–12,29], the human gene promoter region governing brain- and cell-specific aldolase C expression is not known. Our study conducted with three transgenic mice carrying the complete promoter region and a short and a long deletion in the distal promoter region, respectively, shows that diverse human aldolase C gene promoter regions are required to direct specific LacZ expression in different brain areas of these animals. Our investigation of the hippocampus shows that aldolase C is expressed exclusively in the pyramidal CA3 neurons, and that its expression is driven by the distal promoter region (−200/−1200 bp) of the human gene. This region shares a sequence homology of about 52% with the mouse and rat genes. A CAAT motif and an AT-rich region spanning from −669 to −735 bp in the human promoter are identical with the rat and mouse sequences [12]. In the rat gene, the AT-rich sequences in the fragment located −800 bp from the transcription start site bind POU proteins [12,13]. It has been suggested that the binding of these proteins to the rat promoter gene results in brain-specific *cis*-acting elements that direct the neuronal expression of this gene [12]. Hence, it is not inconceivable that the aldolase C expression in CA3 hippocampal neurons in man observed in our study may be driven by a modular structure constituted by an AT-rich motif in the human promoter distal region and cis-acting elements in the promoter proximal region.

Here, we also demonstrate that a fragment of the human gene sequence (1190 bp) that lacks element D was able to drive high brain-specific aldolase C expression in transgenic mice. This fragment is much shorter than the rat 6000- and 5500-bp promoter sequences used by Arai et al. [10] and by Skala et al. [12], respectively. Therefore, we have narrowed-down the region within which to search for the *cis*-element responsible for high aldolase C expression and its localization in hippocampal CA3 neurons. Our data also suggest that the human-specific element D is not essential for high aldolase expression in brain when the fragment of about 1200 bp is present, whereas it is essential when a much shorter segment (164 bp) [15] is allowed to direct the transcription of the human aldolase C gene. The discrepancy between β-gal activity detected by histochemical staining and β-gal expression detected with Northern blotting is due to the different sensitivity of the two techniques. In fact, β-gal activity reflects the promoter activity used to produce active β-gal protein molecules. The β-gal expression of pAldC1500-LacZ in transgenic mice brain is one-half that of pAldC2500-LacZ expression and results in a very low number of β-gal molecules. This is confirmed by the fact that it takes twice as long for anti-β-gal immunostaining to develop in pAldC1500-LacZ mice. Furthermore, the discrepancy between X-gal histochemical staining and anti-β-gal immunostaining detection have been yet reported [30].

Although the intriguing "on/off" pattern of aldolase C expression in the Purkinje cell layer was first reported over a decade ago [17], the gene region governing this pattern in the human gene was not known. Here we demonstrate that elements in the proximal promoter sequence (−200 bp) of the human aldolase C gene are able to drive the striped expression of the gene in Purkinje cells. We may infer that the stripe-like expression of the aldolase C gene in Purkinje cells is due to the presence in the proximal region and/or in the first intron of the human gene of a binding-site of a positive or negative

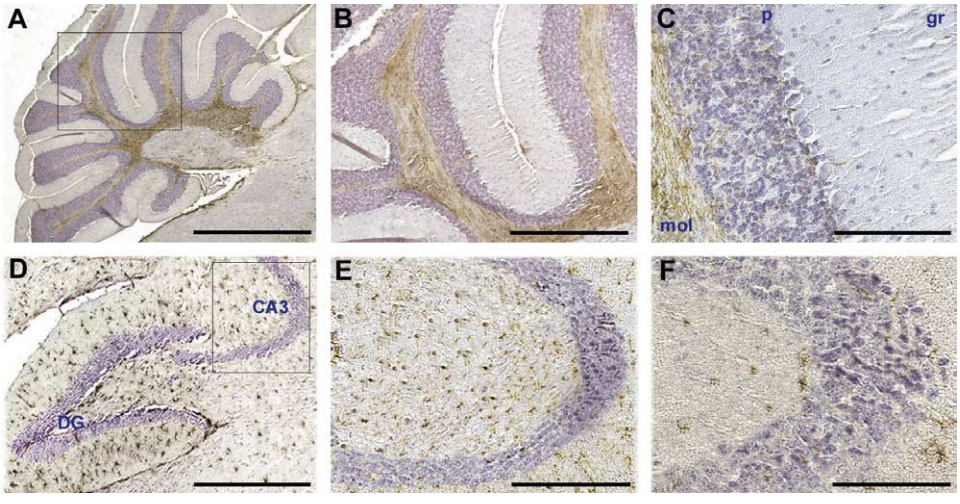

Fig. 6. GFAP-immunostaining on cerebellar and hippocampal paraffin sagittal sections of adult mice. (A) Intense immunoreactivity in the white matter of cerebellum is evident; (B and C) higher magnifications of the boxed area indicated in A show the absence of labeling in the Purkinje cell layer. (D) Scattered GFAP-labeling is evident in hippocampus glial cells; (E and F) the area outlined by the box is reported in E and F at higher magnifications; note the absence of immunostaining in CA3 pyramidal cells. All the sections were counterstained with hematoxylin. mol = molecular, p = Purkinje, gr = granular cell layers, DG = dentate gyrus, CA3 = hippocampal subregion. Scale bars: (A, D) 1000 μm; (B, E) 200 μm; (C, F) 100 μm.

element that would switch aldolase C expression on/off. Alternatively, the restricted expression of this gene in groups of Purkinje cells could be due to post-transcriptional events. Interestingly, the stripe-like expression of aldolase C that we identified in Purkinje cells exactly matches that of the cyclin-dependent kinase 5 activator, protein p39, identified in mouse cerebellum [31]; however, we found no sequence homology between human aldolase C and p39 promoters using the bioinformatic program EMBOSS TFSCAN. Like aldolase C, also the expression of the $GABA_B$ metabotropic receptor variant 1b in the rat hippocampus is restricted to the CA3 neurons [32]. Very recently, it has been reported that the expression in the hippocampus of the $GABA_B$ R1b gene is regulated by cAMP through a canonical CREB-binding site and by upstream stimulatory factor 1 (USF1) that overlaps the CREB site in the promoter [33]. We found a sequence homology of about 35% between the two promoters using the mVista bioinformatic program. Interestingly, the distal promoter region of the human aldolase C gene carries a canonical USF1-binding site just upstream from the NGFI-B binding site (−228/−233 bp). Furthermore, we recently observed that the binding of USF1 to the promoter region of the aldolase C gene is involved, together with NGFI-B binding, in the transcriptional regulation of the aldolase C gene by NGF in PC12 cells (personal observation).

In conclusion, this study shows that the −1200-bp human aldolase C gene promoter region contains all the elements required to drive specific LacZ expression in hippocampal CA3 neurons and its stripe-like expression in Purkinje cells. It also provides the groundwork for studies to identify the transcriptional factors responsible for aldolase C expression in the CA3 hippocampal neurons as well as the protein factors involved in the stripe-like expression of this gene in Purkinje cells. Lastly, it is tempting to infer that, besides its glycolytic function, human aldolase C plays another brain-specific role in cells like the CA3 neurons and in alternate clusters of Purkinje cells.

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