The mammalian cerebellum is an ideal model system in which to study the generation of spatially repeated patterns in a developing organism. How do the individuals of a seemingly homogeneous population of cells become different, one from the other, such that the differences respect (and/or define) a large scale spatial pattern? From the standpoint of its microarchitecture, the cerebellum is a highly repetitive structure; the dimensions of its three layers and its neurophysiological connectivity vary little from region to region. Unlike the mammalian neocortex there are no cytoarchitectonic boundaries which delineate its functional compartments. From the anatomical, physiological and biochemical point of view, however, the adult cerebellum is highly compartmentalized, divided into a series of slabs, stacked in the sagittal plane (see Hawkes and Gravel, 1991 for review).

These sagittal stacks or 'bands' are visible anatomically, in the climbing fiber axons that project from the inferior olive to the Purkinje cells of the cerebellar cortex. Small injections of tracer dye into the olive labels sagittally organized bands of axons in the cerebellar cortex. The receptive fields of cerebellar cortex to sensory stimuli are also sagittally oriented. At a biochemical level, histochemical stains for enzymes such as acetylcholine esterase (Boegman et al., 1988), 5′-nucleotidase (Eisenman and Hawkes, 1989) and cytochrome oxidase (Leclerc et al., 1990) or immunocytochemical stainings using any of several monoclonal antibodies also reveal a pattern of sagittal bands.

In addition to the enzymes mentioned above, several proteins exhibit a transient banding pattern during cerebellar development. In the developing anlage of Purkinje cells, these other gene products appear asynchronously along the sulci. As development progresses, their expression becomes generalized to all Purkinje cells, obliterating the banded pattern. Genes in this class include rhombotin, L7/pcp-2 and calbindin (Greenberg et al., 1990; Wassef and Sotelo, 1984; Wassef et al., 1985; Varghese et al., 1988; Oberdick et al., 1990; Smeyne et al., 1991; Vandaele et al., 2081).

The sagittal organization of the mammalian cerebellum can be observed at the anatomical, physiological and biochemical level. Previous screening of monoclonal antibodies produced in our laboratory has identified two intra-cellular antigens, zebrin I and II, that occur exclusively in adult cerebellar Purkinje cells. As their name suggests, the zebrin antibody staining of the Purkinje cell population is not uniform. Rather, zebrin-positive Purkinje cells are organized in stripes or bands that run from anterior to posterior across most of the cerebellum; interposed between the zebrin-positive cells are bands of Purkinje cells that are zebrin-negative. Comparison of the position of the antigenic bands with the anatomy of afferent projections suggests that the bands are congruent with the basic developmental and functional ‘compartments’ of the cerebellum. We report the isolation of cDNA clones of the 36×10^3 Mr antigen, zebrin II, by screening of a mouse cerebellum cDNA expression library. Sequence analysis reveals a 98% identity between our clone and the glycolytic isozyme, aldolase C. In order to more rigorously demonstrate the identity of the two proteins, we stained adult cerebellum with an independent monoclonal antibody raised against aldolase C. Anti-aldolase staining occurs in a previously unreported pattern of sagittal bands of Purkinje cells; the pattern is identical to that revealed by the zebrin II monoclonal.

SUMMARY

The cloning of zebrin II reveals its identity with aldolase C. In order to more rigorously demonstrate the identity of the two proteins, we stained adult cerebellum with an independent monoclonal antibody raised against aldolase C. Anti-aldolase staining occurs in a previously unreported pattern of sagittal bands of Purkinje cells; the pattern is identical to that revealed by the zebrin II monoclonal. Further, in situ hybridization of antisense aldolase C riboprobe shows that the accumulation of zebrin II/aldolase C mRNA corresponds to the pattern of the zebrin antigen in Purkinje cells. Zebrin II/aldolase C gene expression is thus regulated at the level of transcription (or mRNA stability). In light of previous work that has demonstrated the cell-autonomous and developmentally regimented expression of zebrin II, further studies of the regulation of this gene may lead to insights about the determination of cerebellar compartmentation.

Key words: cerebellum, Purkinje cell, zebrin, compartmentation, aldolase C

INTRODUCTION

The mammalian cerebellum is an ideal model system in which to study the generation of spatially repeated patterns in a developing organism. How do the individuals of a seemingly homogeneous population of cells become different, one from the other, such that the differences respect (and/or define) a large scale spatial pattern? From the standpoint of its microarchitecture, the cerebellum is a highly repetitive structure; the dimensions of its three layers and its neurophysiological connectivity vary little from region to region. Unlike the mammalian neocortex there are no cytoarchitectonic boundaries which delineate its functional compartments. From the anatomical, physiological and biochemical point of view, however, the adult cerebellum is highly compartmentalized, divided into a series of slabs, stacked in the sagittal plane (see Hawkes and Gravel, 1991 for review).

These sagittal stacks or 'bands' are visible anatomically, in the climbing fiber axons that project from the inferior olive to the Purkinje cells of the cerebellar cortex. Small injections of tracer dye into the olive labels sagittally organized bands of axons in the cerebellar cortex. The receptive fields of cerebellar cortex to sensory stimuli are also sagittally oriented. At a biochemical level, histochemical stains for enzymes such as acetylcholine esterase (Boegman et al., 1988), 5′-nucleotidase (Eisenman and Hawkes, 1989) and cytochrome oxidase (Leclerc et al., 1990) or immunocytochemical stainings using any of several monoclonal antibodies also reveal a pattern of sagittal bands.

In addition to the enzymes mentioned above, several proteins exhibit a transient banding pattern during cerebellar development. In the developing anlage of Purkinje cells, these other gene products appear asynchronously in parasagittal bands alongside morphologically identical cells with no detectable expression. As development progresses, their expression becomes generalized to all Purkinje cells, obliterating the banded pattern. Genes in this class include rhombotin, L7/pcp-2 and calbindin (Greenberg et al., 1990; Wassef and Sotelo, 1984; Wassef et al., 1985; Varghese et al., 1988; Oberdick et al., 1990; Smeyne et al., 1991; Vandaele et al., 2081).
Zebrins are antigens with no previously known function, found within a subset of adult Purkinje cells, the output cells of the cerebellum. Immunocytochemical staining with monoclonal antibodies reveal, as their name suggests, a series of stripes that are continuous across most folia of the vermis (the middle third of the cerebellum) and are present, albeit with less precise registration, in the hemispheres (Fig. 1). Zebrin I is a 120×10^3 M_r intracellular antigen found throughout the Purkinje cell from dendritic spine to axon tip (Hawkes et al., 1985; Hawkes and Leclerc, 1986, 1987). Zebrin II is a 36×10^3 M_r species (Doré et al., 1990) with a cellular distribution identical to zebrin I (Brochu et al., 1990). The compartmentalized expression of zebrin II is preserved through evolution, as demonstrated in opossum, rat, mouse and teleost fishes (Brochu et al., 1990; Doré et al., 1990; Lannoo et al., 1991; Meek et al., 1992).

Zebrin bands may serve as developmental boundaries

The stripes revealed by antibody staining are congruent with functionally defined sagittal bands. For example, the olivary climbing fibers respect the boundaries defined by zebrin I (Gravel et al., 1987). The spinocerebellar projection, the mossy fibers, indirectly interact with Purkinje cells via the granule cells, but are also organized in a similar manner (Gravel and Hawkes, 1990). Thus zebrin staining serves as an immunocytochemical marker for the ‘compartment’ structure of the adult cerebellum. In addition, the expression pattern of this antigen undergoes complex changes during the most significant phases of Purkinje cell arborization and connectivity. In rat, zebrin-positive (zebrin⁺) staining first appears during postnatal day 6 (P6) in a stereotyped and saltatory manner, first in the posterior lobes of the vermis, then in the entire vermis and finally in the hemispheres (Leclerc et al., 1988; Tano et al., 1992). By P12, the antigen appears in all Purkinje cells. The adult banding pattern emerges from around P20 through the suppression of this antigen in the Purkinje cells that form the zebrin-negative (zebrin⁻) interbands.

Bands, blobs or stripes are not uncommon in the organization of the CNS (such as ocular dominance columns in the visual cortex), but many of these are established by functional and environmental cues, requiring an appropriate connectivity. In contrast, zebrin bands seem to define compartments that are more rigidly specified. The positive or negative zebrin phenotype may represent one aspect of a developmental genetic division of the Purkinje cell population into two basic types. Three lines of evidence suggest that zebrin expression is cell autonomous and defines a functional identity among Purkinje cells. While the emergence of zebrin banding is coincident with the maturation of the climbing fiber afferent system (already sagittally organized (Sotelo et al., 1984)), the zebrin stripes, unlike ocular dominance columns, are unaffected by deafferentation (Leclerc et al., 1988). Furthermore, transplantation of the embryonic cerebellar anlage to the anterior chamber of the eye, or to a cavity in cerebral cortex (Wassef et al., 1990), or to kainate-depleted adult cerebellar cortex (Rouse and Sotelo, 1990) has no effect on the development of Purkinje cells that are both zebrin⁺ and zebrin⁻.

Secondly, zebrin⁺ Purkinje cells are also positively defined, by the presence of a separate antigen, P-path, a cell surface 9-O-acetylated glycolipid recognized by a different monoclonal antibody (Leclerc et al., 1992). This observation suggests that the zebrin⁻ cells are not a catch-basin of many different Purkinje cell types and underscores the compartment analogy.

Finally, zebrin expression in cerebellar mutants seems to reflect the developmental identity of their Purkinje cells. In the lurcher (Lc/+ ) mouse, the development of the Purkinje cells is arrested at P7, followed by their death during postnatal weeks 2-5. Zebrin staining in these animals is frozen at the posterior lobe vermis stage (Tano et al., 1992). In the staggerer (Xg/Xg) mutant mouse, Purkinje cells appear developmentally arrested at a maturational age equivalent to approximately P4. In

Fig. 1. Immunohistochemical staining of adult mouse brain with monoclonal antibody to zebrin II. The horizontal plane of section permits the visualization of zebrin banding across the folds of the vermis. Peroxidase reaction products darkly stain the Purkinje cell bodies, and the dendrites above them produce the striped fields.
agreement with the developmental sequence described above, sg/sg Purkinje cells never develop zebrin staining. Whatever organizes the postnatal differentiation of Purkinje cells must also affect zebrin, so understanding the control of zebrin expression may also provide insights into Purkinje-cell-specific gene expression and differentiation.

The sagittal organization of cerebellum in general and the development of zebrin bands in particular presents a classic problem of pattern formation: how does a population of seemingly homogeneous cells, the Purkinje cells, differentiate into two distinct phenotypes. And further, what processes are at work that allow phenotypes to be assigned based on a large-scale pattern, subtending the entire cerebellum, that is recognizable as stripes or bands. The results reported in this paper are part of a longer term strategy to answer this question at the molecular level. To begin this process, we have constructed an expression library from P20 mouse cerebellum and isolated clones containing the cDNA for zebrin II. The questions that we wished to ask through this approach were: (i) would the gene’s identity provide any information about the function of zebrin II, and (ii) is the striping pattern of the antigen regulated at the level of its mRNA. Our positive findings in both of these areas suggests a path for further investigation.

MATERIALS AND METHODS

Immunohistochemistry

Horizontal sections of adult mouse cerebellum were prepared and stained as described previously (Leclerc et al., 1988). All animals were handled using approved protocols. Briefly, adult mice were deeply anesthetized with sodium pentobarbital and fixed by perfusion through the ascending aorta. 100 ml of 0.9% saline solution was followed with 200 ml of 4% paraformaldehyde in 0.1 M phosphate buffer and 0.9% NaCl. The cerebellum and adjacent tissue was cut from the gel prior to transfer, stained and compared to the western blot signals to estimate molecular weight. The scanned image of the western blot is represented as a percentage of the density of the zebrin II peak.

Library construction

Poly(A)+ RNA from forty P20 cerebella (from the C57BL/6 inbred strain) was extracted by homogenization, proteolysis and adsorption to oligo(dT) matrix as per the Fast Track Kit (Invitrogen), yielding 20 µg RNA, enough for all subsequent procedures. RNA from whole adult brain was isolated in the same way. 5 µg cerebellum RNA was used to construct a cDNA library using the Lambda Zap expression library system (Stratagene). This procedure permitted the directional cloning of the cDNA into the expression vector. Packaged library was titrated and estimated to be 98% recombinant by X-gal selection. 24-100 mm plates, each with 50,000-60,000 pfu, were plated and eluted individually and titered. A normalized aliquot of each dilution was pooled and titered again for expression screening. We estimate the complexity of this library to be 1-2x10^6.

Expression screening

20-100 mm plates with 50,000 pfu were expression screened by standard methods (Sambrook et al., 1989) using the monoclonal antibody to zebrin II. HRP-linked anti-mouse secondary antibody was detected with DAB. 19 replicating positive signals were plaque-purified by subsequent rounds of screening, and the insert was excision rescued as per the Lambda Zap protocol. The resulting phagemid is the Bluescript SK vector, into which the insert is cloned within the EcoRI and Xhol restriction sites. DNA restriction mapping was performed by agarose electrophoresis following treatment with restriction endonucleases.

Sequencing and sequence comparisons

Since all 19 clones mapped identically by restriction analysis (Sambrook et al., 1989), 4 clones were chosen at random for sequence analysis using the dideoxy chain termination method with a modified T7 DNA polymerase (USB) or with Taq polymerase (ABI, Perkin Elmer). Sequence analysis, comparison and BLAST comparison to the GenBank database was performed on GCC software (Altschul et al., 1990).

Northern blot analysis

RNAs from adult mouse brain and P20 cerebellum were analyzed by standard methods (Sambrook et al., 1989). Briefly, 2 µg poly(A)+ RNA were separated by electrophoresis on a 1% agarose/formaldehyde gel and transferred to a nylon membrane (NEN Hybond N*). The RNA was cross-linked to the membrane with 1200 µCi UV light (Stratagene Stratatalinker) and hybridized to 32P-dCTP oligo-labeled probe (Boehringer Mannheim, Amersham). Autoradiography was performed on a Molecular Dynamics PhosphorImager and further imaged on Adobe Photoshop.

Production of zebrin II riboprobe

Antisense and sense control riboprobe was respectively transcribed from the T7 and T3 RNA polymerase promoters flanking the insert using standard methods (Sambrook et al., 1989). 35S-CTP was incorporated into the transcript, and quantitated by TCA precipitation.

In situ hybridization of mouse brain

Adult mouse was anesthetized and perfused with 4% paraformaldehyde, cryoprotected in 18% sucrose and cryostat sectioned into 16 µm sections in the horizontal plane onto gelatin-subbed slides. Sample pretreatment, hybridization, RNAse treatment, washing, autoradiography and emulsion dipping was performed by standard methods as described previously (Wilson and Higgins, 1990). Slides were

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RESULTS

Quantitative time course of zebrin II expression

Immunohistochemical staining reveals zebrin II as an intracellular antigen that begins to appear in all Purkinje cells during the first week of postnatal life, becoming confined to a subset of those cells, organized into sagittal bands, during the third postnatal week (Leclerc et al., 1988). In order to quantitatively determine the period of maximal zebrin II expression by western blot analysis, as described in Materials and Methods. The zebrin II antibody detects a single 36kDa antigen from P10, coming to its maximal levels by P20 (data not shown). Based on these data, we chose P20 mouse (C57BL/6J) cerebellum as the source of the RNA for our cDNA library.

Library construction and screening

We isolated poly(A)+ RNA from P20 mice and constructed a cDNA library as described in Materials and Methods. In order
to minimize the unbalanced amplification of cDNA, the library was packaged and plated onto 24 separate plates, eluted and titered individually before pooling the amplified phage. We estimate that 98% of the primary plating was recombinant, with a complexity of $1.2 \times 10^6$. $10^6$ phage were replicated onto duplicate nitrocellulose filters and screened with the zebrin II mAb. 19 duplicating signals were plaque-purified by subsequent rounds of screening. The insert was excision-rescued from the Lambda Zap phage within the Bluescript SK phagemid. Restriction mapping of these inserts showed that they were all identical (data not shown).

**DNA sequencing and determination of zebrin II identity**

We chose 4 clones at random for sequence analysis. Sequence from the flanking vector showed that these clones contained the same class of insert, with small differences in length at the cloning sites of all four clones. These differences are consistent with the interpretation that these clones arose from 4 independent cloning events. Comparison of this partial sequence to the GenBank database identified its similarity to the previously reported human and rat aldolase C genes (Rottmann et al., 1987; Kukita et al., 1988).

In order to be sure that our cDNA was indeed the mouse aldolase C gene, and that zebrin II was not an alternatively spliced form of aldolase C, we completed the sequence determination of one of the clones. The partial mouse cDNA for aldolase C in the GenBank database (Paolella et al., 1986) matched nearly identically at the nucleotide level within the open reading frame and exactly in its deduced amino acid sequence. Fig. 2A shows the cDNA sequence and the deduced amino-acid sequence of the open reading frame from the first ATG initiation codon, which lies within the context of a good Kozak consensus. At the 3' end of the clone, a polyadenylation signal is followed by a poly(A) stretch.

The deduced amino acid sequence of the single large open reading frame predicts a $36 \times 10^3$ M$_r$ polypeptide, which agrees with the estimated size of both zebrin II and aldolase C. Comparison of the deduced amino acid sequence from mouse zebrin II (clone z11) with the complete rat aldolase C gene shows that they are 98% identical (Fig. 2B). By comparison, mouse zebrin II is 96% identical to rat aldolase A and 70% identical to rat aldolase B (Fig. 2C).

**Northern blot analysis of zebrin II mRNA**

Northern blot analysis of zebrin II expression in whole brain and cerebellum is shown in Fig. 3. When probed under high stringency, the ~1.6 kb transcript is seen in whole brain and cerebellum. This result is in agreement with the previously reported tissue distribution of this aldolase isoform (Lebherz and Rutter, 1969).

**Independent confirmation that zebrin II is aldolase C**

The aldolase C literature fails to note its banded appearance in the cerebellum. Histochemical localization of aldolase C to Purkinje cells of the cerebellum does not note this pattern (Royds et al., 1987; Ironside et al., 1988). Two other studies note a specific but discontinuous distribution of aldolase C mRNA in Purkinje cells by in situ hybridization but do not note the characteristic zebrin pattern (Popovici et al., 1990; Mukai et al., 1991). To address the concern that the zebrin II antibody recognizes an epitope that is also, coincidentally, present on aldolase C (i.e., our colony screen led to the isolation of a separate protein from the one that we set out to identify), we obtained an independently derived antibody, raised against aldolase C (Ironside et al., 1988).

Fig. 4 shows that both antibodies detect a single band on western blot of identical apparent molecular weight. Preincu-
bution of a lane with rabbit anti-aldolase C antiserum greatly diminishes the ability of the zebrin II monoclonal antibody to detect its epitope, showing that zebrin II antigen shares its epitope with those found in isolated aldolase C (Fig. 4). Densitometry of four separate determinations showed that preincubation consistently diminished the zebrin II signal by 28%. Separate control experiments showed that anti-aldolase C preincubation had no effect on three monoclonal antibodies to other proteins, and that the signal remaining after preincubation is at least in part due to the secondary antibody cross-reacting to the rabbit anti-aldolase (data not shown). Fig. 5 shows near-adjacent sections of adult mouse cerebellum stained with the two independent antibodies. The identical pattern of staining confirms that these earlier studies failed to notice the banded pattern of aldolase C expression.

Zebrin II is regulated at the level of mRNA
There remained the formal possibility that zebrin II protein may be differentially stabilized. Perhaps the banding pattern is due to a post-translational modification of a uniformly expressed aldolase gene product. The zebrin II immunohistochemical pattern would then be regulated at the level of this

![Fig. 5. Immunohistochemistry of serial sections of adult mouse cerebellum stained with anti-zebrin II antibody (A,C,E) and with an independent anti-aldolase C antibody (B,D,F) show an identical staining pattern. (A,B) Low power view of the posterior lobe. Scale bar, 2 mm. (C,D) Same section as in A and B, showing lobules VII and VIII of the vermis. Scale bar, 1 mm. (E,F) The anterior lobules of the vermis. The P1+ and P2+ bands are marked on lobule III. Scale bar, 500 µm.](image-url)
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other modifying enzyme. Indeed, such a system is likely to operate for the P-path antigen, since chemical deacylation of the tissue section before immunohistochemistry effectively destroys the epitope, and the striping pattern, while exposing the 7C7 antigen (a deacylated form of the glycoconjugate) (Leclerc et al., 1992).

We prepared an in situ hybridization of horizontal sections of adult mouse brain using antisense riboprobe from the entire zebrin cDNA (clone z11). Over all regions of the brain, the level of signal from the sense control probe was very low or nearly absent (data not shown). These observations are consistent over three separate determinations. As shown in Figs 6 and 7, high levels of zebrin/aldolase C autoradiographic signal is seen over the Purkinje cells of the cerebellum. Although there is some signal over the entire Purkinje cell layer, the intensity of hybridization is not uniform. A much more intense signal forms a clear pattern of sagittal bands identical to the pattern of zebrin II antibody staining. A series of five, bilaterally symmetric bands is found in the anterior vermis. These are the P3*, P2* and the fused midline P1* bands defined by Hawkes and colleagues (Hawkes et al., 1985; Hawkes and Leclerc, 1987). In the posterior vermis, the zebrin+ bands widen such that the zebrin− interbands (P1− and P2−) are barely visible. In the hemispheres, a bilaterally symmetric heterogeneity of high and low message levels is evident in the Purkinje cell layer, but the anterior-posterior registration of ‘bands’ is lost (as is the case in the zebrin II antibody stain).

In summary, the key features of the pattern are consistent and equivalent in both zebrin II immunocytochemistry and aldolase C in situ hybridization. In particular, note that the medial apex of the paramedian lobule is positive for both signals in a similar spatial pattern (compare Fig. 1 with Fig. 6B). Following exposure to film, the same slides were dipped in photographic emulsion to determine the localization of signal at the cellular level. After 4 days, the slides were developed to reveal the silver grains and the sections were counterstained with hematoxylin (Fig. 7). These images clearly demonstrate autoradiographic signal specifically over the Purkinje cells (Fig. 7A). Fig. 7B demonstrates a typical border between zebrin+ (light arrows) and zebrin− (dark arrowheads) Purkinje cells. Other neuronal and glial cell populations have background levels of message.

DISCUSSION

Several independent cDNA clones encoding the zebrin II antigen were isolated by antibody screening of an expression library made from P20 mouse cerebellum mRNA. Sequencing the inserts reveal an open reading frame whose deduced amino acid sequence is 98% identical to that previously reported for the rat aldolase C isozyme. The deduced and apparent molecular weight of aldolase C is indistinguishable from the apparent molecular weight of the zebrin II antigen. An independently generated antibody to aldolase C reveals an immunohistochemical pattern in the cerebellum that is indistinguishable from that of the zebrin II antibody. In situ hybridization of the cDNA to adult mouse brain shows an autoradiographic pattern of the mRNA identical to that of zebrin II immunohistochemistry. We conclude from these four distinct lines of evidence that the protein carrying the zebrin II epitope is aldolase C and that its expression is regulated at the level of transcription or mRNA stability.

The aldolase isozymes

Aldolase catalyzes a step of the glycolytic pathway, the aldol hydrolysis of fructose-1,6-bisphosphate into two three-carbon moieties — dihydroxyacetone phosphate and glycerol-3-phosphate. Three isoforms of the enzyme are known, aldolase A, B and C. Each is a product of a separate gene located, at least in human, on one of three separate chromosomes (Skala et al., 1987; Rocchi et al., 1989). The enzymes differ slightly in their substrate specificity. Aldolase A has catalytic properties that suggest it functions in the glycolytic pathway while aldolase B is more likely to function in gluconeogenesis (Blostein and Rutter, 1963; Rottmann et al., 1987). Aldolase C has properties intermediate between these two. In each case, however, the differences in substrate specificity of these electrophoretically similar but distinct isozymes are quantitative, not qualitative. Aldolase A is primarily a muscle enzyme; aldolase B is found predominantly in liver; aldolase C is a brain-specific enzyme with the bulk of the activity found in the cerebellum.
cerebellar Purkinje cells (Blostein and Rutter, 1963; Lebherz and Rutter, 1969; Royds et al., 1987; Popovici et al., 1990; Mukai et al., 1991). Comparison of these isozymes at the cDNA level have led to the hypothesis that the three genes arose by two distinct gene duplications, the first duplication produced A and B, while a second more recent event produced C from A (Kukita et al., 1988).

Identification of the zebrin II antigen
The genomic organization of the rat and human aldolase C genes is conserved (Rottmann et al., 1987; Buono et al., 1988; Mukai et al., 1991). In both species, 9 exons are spaced over 3 to 4 kb of genomic DNA. Given this relatively complex organization, we considered whether the zebrin II antigen could be accounted for by a novel, alternatively spliced form of the aldolase C gene. Sequencing of an entire cDNA which was expression screened with zebrin II antibody reveals no differences in exon utilization with the known rat aldolase C cDNA. Furthermore, the rat and human aldolase C genes also show great similarity to its isozyme relatives, A and B. Consistent with the idea that the zebrin II antigen is shared among the aldolases is the previous finding that muscle aldolase cross-reacts with zebrin II antibody on western blot (Hawkes, 1992). In addition, antibodies to aldolase A also show zebrin-like staining in the cerebellum (R. H., unpublished observations).

Zebrin II/aldolase C is regulated at the level of transcription or mRNA stability
The development of the bands, through the suppression of zebrin in the interbands (see Introduction), left open the possibility that the pattern was created by some post-translational process. If this were the case then zebrin banding would be regulated not by its own synthesis but rather by the levels of the enzyme (or enzymes) that were responsible for the modification. Our in situ hybridization studies clearly show a spatially heterogeneous pattern of message levels congruent in every way with zebrin immunocytochemistry. If there are indeed post-translational processes that further modify the levels of aldolase C mRNA, they are not needed to account for its banded appearance.

Because differential splicing does not play a role in the creation of the zebrin epitope and because the in situ hybridization of the cDNA to mouse brain is identical to zebrin II immunohistochemistry, we conclude that zebrin II is aldolase C and that its pattern of expression is regulated at the level of transcription or mRNA stability. The low level of aldolase mRNA seen in other brain regions may reflect low levels of transcript whose protein product is not detectable by immunohistochemistry. Previous in situ hybridization studies report aldolase C in subpopulations of neurons of the cerebral cortex, striatum, hippocampus, hypothalamic nuclei and primary olfactory cortex, though at much lower levels (Popovici et al., 1990).

Comparison and contrast with the L7 cerebellar compartmentation gene
Oberdick and colleagues have reported a transcript, L7, whose expression in Purkinje cells is also confined to sagittal bands (Smyney et al., 1991). Using a transgene into which a reporter has been inserted into the last exon of the genomic sequence, these authors report that expression of the reporter is regulated in a compartment-specific and developmentally stereotyped manner at the level of transcription or mRNA stability. Whereas L7 is transiently expressed in bands, giving way to a generalized expression, zebrin/aldolase C is generally expressed and then stably restricted to a banded pattern in the adult (see Introduction).

Although the timing and details of the banding patterns do not match exactly, the registration of the L7 and zebrin bands will be of great interest. We have isolated genomic clones for the mouse zebrin II gene and the construction of a reporter transgene is in progress. Previously published reports of the rat
and human genes describe the promoter region as an ‘unusual housekeeping’ promoter (Rottmann et al., 1987; Buono et al., 1988; Mukai et al., 1991). Does the regulation of transcription of both aldolase C and L7 transcripts reflect part of a single fundamental characteristic of cerebellar compartmentation? Such a theory would also have to account for the temporal difference in their expression.

**Why is aldolase C in bands in the cerebellum?**

Our findings raise the question of why the cerebellar Purkinje cell population is organized into alternating bands with high and low concentrations of a relatively unremarkable glycolytic enzyme. Perhaps the basal metabolic rate of the cells in the zebrin-/aldolase-positive cells is higher than in the negative bands. The distribution of cytochrome oxidase (CO) supports this notion, but only in a complex way. CO is a mitochondrial enzyme associated with increased rates of oxidative phosphorylation. In the rat, CO is found in a pattern of sagittal bands in both the molecular and granular layers in regions that correspond to the zebrin I pattern, whereas in the monkey, the banded appearance of CO shows higher activity in the zebrin I negative bands (Hess and Boogd, 1986; Leclerc et al., 1990). An alternative explanation for the banding of aldolase C is suggested by the work of Drager and colleagues (McCaflery et al., 1991). Using a monoclonal antibody that identifies a concentration gradient of an unknown embryonic retinal antigen, a cDNA was sequenced and identified as an isozyme of aldehyde dehydrogenase. While this enzyme, like aldolase C, is a ‘housekeeping’ metabolic enzyme, further study revealed that one of its preferred substrates was retinaldehyde. The isoyme thus catalyzes the transformation of retinaldehyde to retinoic acid, a morphogen whose gradient sets the anteroposterior axis of the developing limb bud (Eichele, 1989; Thaller and Eichele, 1990). Regrettably, no comparably interesting aldolase substrate suggests itself in our review of the literature. Nonetheless, the notion of a substrate with developmental activity remains an attractive alternative hypothesis to explain the aldolase C bands.

In keeping with the cerebellar ‘compartmentation’ analogies discussed in the Introduction, transcriptional activity of the aldolase C gene is responding to the pattern information in the cerebellum. This makes it analogous to what have been called ‘realization genes’ in Drosophila and, as such, it becomes an attractive candidate to help identify the molecular substrate of the pattern forming machinery of the mammalian cerebellum.

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