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Meander tail reveals a discrete developmental unit in the mouse cerebellum

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ABSTRACT **Analyses of phenotypic mutations with altered patterns of cellular organization in invertebrate systems have lead to the identification of genes important to histogenesis. Efforts to identify genes defining segments or compartments in mammalian systems and demonstrate a role for such genes in the establishment of the cellular architectonics of the brain have been hampered by the absence of phenotypic mutations that reveal compartments. Evidence is presented that in the cerebellum of the mutant mouse, meander tail, there is an abrupt transition from the normal cytoarchitecture seen in the posterior lobes to a severely disorganized cellular pattern. Thus, in the anterior lobes the Purkinje cells are positioned randomly throughout the cortex, and other cellular layers, including the internal granule cell layer, do not form. In addition, radial Bergmann glial processes are virtually absent. One explanation for the discrete boundary of the affected area in the anterior portion of this mutant cerebellar cortex is that the meander tail gene influences compartmental cellular organization in mammalian brain.**

Characterization of mutations that disturb cellular organization in the mouse cerebellum (1, 2) offers the promise of yielding insight into the molecular mechanisms governing morphogenesis in the mammalian brain. Although study of the neurologic mutant mouse strains has illuminated fundamental issues in brain histogenesis, including the importance of cell–cell interactions in the establishment of cellular form (3–6), most mutations are not suggestive of aberrations in pattern formation. Our interest is to identify and clone gene mutations that affect early organizational events in the central nervous system. Such mutations could be identified by a very early onset, involvement of numerous cell types, and a highly regional localization of the defect.

This report describes the cerebellar morphology of one such mutant, meander tail (*mea*). This mouse strain harbors an autosomal recessive mutation whose phenotype is characterized by mild ataxia and a skeletal abnormality resulting in a kinked tail (7). Meander tail displays a profound disorganization of the cerebellar cytoarchitecture that is striking because it is confined to the anterior lobes. This mutation is unusual, as it genetically defines a discrete region of the mammalian cerebellum that is not recognizable on the basis of any specific structural or functional attribute. The singular morphology of this mutant has lead us to suggest that the *mea* gene may perturb compartmental cellular organization in the mouse cerebellum.

METHODS

Adult (3–4 wk) *mea/mea* mice (5) and aged matched controls (+/+) were anesthetized with ketamine/xylazine (50 mg/kg) i.p. and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer. Sagittal sections were cut at 65 μm with a vibratome. Rabbit antisera against a vitamin D-dependent calcium-binding protein, calbindin (28 kDa) were a generous gift of S. Christakos (8). Rabbit antisera against gel-purified glial filament protein (51 kDa) were a generous gift of R. Liem (9). Immunostaining was carried out using the peroxidase-antiperoxidase method as described (10). In addition to the five *mea/mea* mice studied with this battery of antibodies and stain, several hundred cerebella were examined grossly during the course of developing our phenotypic cross.

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RESULTS AND DISCUSSION

The organization of the folia and features of three resident cell types in normal and *mea/mea* cerebella were compared. On thick vibratome sections granule, Purkinje, and astroglial cells were visualized with a Nissl stain (cresyl violet) or by immunostaining with one of two cell-specific markers: calbindin, a 28-kDA calcium-binding protein, which in the cerebellum is present only in Purkinje cells (8), or glial fibrillary acidic protein (GFAP), the major intermediate filament protein of astroglial cells (9). The adult *mea/mea* cerebellum displays normal foliation and cytoarchitecture from the posterior folia (X–VII) to the declive (the most dorsal-reaching lobe, which includes folia VI and VII). In these sagittal sections, a line can be drawn up through the white matter of the declive at the boundary of folia VI and VII, which demarcates an abrupt morphologic transition. Normal cellular architecture is apparent in the lobes posterior to this line, whereas anteriorly, a number of cellular defects are evident.

In the Nissl-stained sections (Fig. 1 *B–D* and Fig. 2*A*), three general cellular defects are obvious. (*i*) The three most anterior lobes, the lingula cerebelli, lobulus centralis, and culmen, representing folia I–V, are fused into two lobes. (*ii*) The internal granule cell layer thins abruptly at the transition line and rapidly becomes indiscernible more anteriorly. (*iii*) The few granule cells present in the anterior lobe are scattered randomly among disorganized Purkinje cells that no longer form a monolayer.

Calbindin-immunolabeled tissue sections (Fig. 1 *A* and *E–G* and Fig. 2*B*) from the *mea/mea* animals reveal a normal Purkinje cell monolayer in the posterior folia with its characteristic highly branched dendrites and axon collaterals. However, starting at the same transition line that was evident in Nissl-stained sections, Purkinje cells rapidly become disorganized. The monolayer disintegrates and cell bodies fill the anterior folia from the outer cortical rim to the white matter. Moreover, Purkinje cells in this region are oriented on their side or inverted, with dendrites projecting in all directions, including into the white matter (Fig. 2*B*).

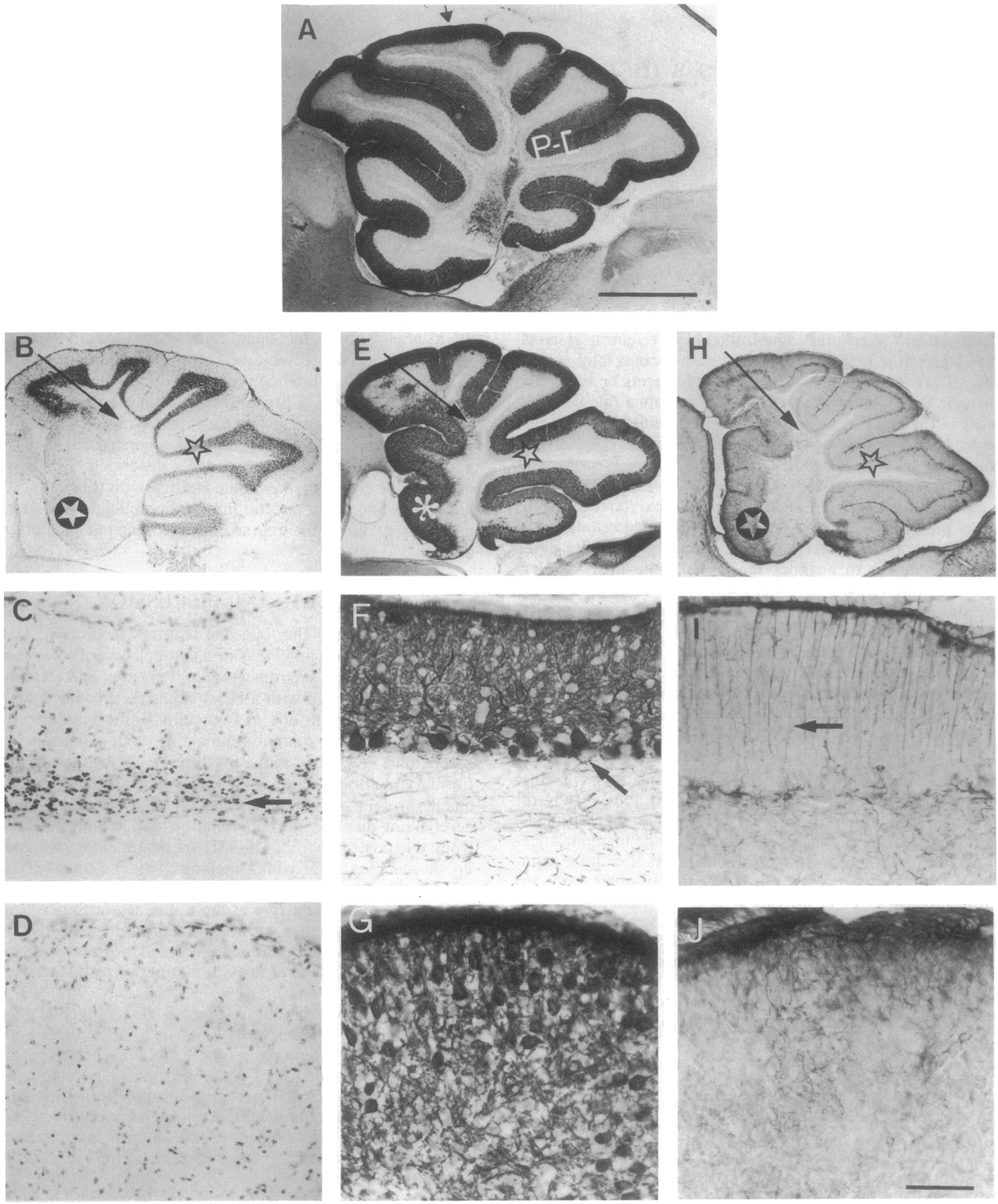

FIG. 1. Organization of granule cells (*B–D*), Purkinje cells (*A* and *E–G*), and astroglia (*H–J*) in normal (*A*) and meander tail (*B–J*) mature cerebellum. (*A*) Normal cerebellar cortex immunostained with antisera to calbindin (28-kDa calcium-binding protein) for comparison to *B–J*. Note the regularity of thickness of the Purkinje cell monolayer in every folium. Purkinje cell dendrites are oriented toward the pia and fill the molecular layer (white bracket). The thin arrow in *B*, *E*, and *H* indicates point of transition zone in *mea/mea* cerebellum, separating posterior (to right) from anterior (to left) poles. In *B*, *E*, and *H*, normal posterior lobe folia are indicated by an open star corresponding to the high-power views in *C*, *F*, and *I*. Defective anterior lobe folia are indicated by filled circle/star, corresponding to high-power views shown in *D*, *G*, and *J*. (*B–D*) Nissl-stained *mea/mea* cerebellum showing granule neurons. The internal granule cell layer, normally filled with numerous small darkly stained cells (*C*, arrow), rapidly disintegrates from the middle of the declive (left of arrow, *B*) to the anterior-most folium (filled circle/star in *B* and *D*). (*E–G*) Purkinje cells immunostained with antisera to calbindin are normal to the right of the line at the declive (arrow, *E*) and are in a monolayer (arrow, *F*), and their dendrites project upward to fill the molecular layer (compare *A* to *E* and *F* at open star). In the most affected folia (white asterisk, *E* and *G*), Purkinje cell dendrites and cell bodies are disoriented, filling the merged folia. (*H–J*) Astroglia immunostained

A particularly striking defect is apparent in the glial fibrillary acidic protein-immunolabeled sections (Fig. 1 *H–J* and Fig. 2*C*). As one proceeds anteriorly from the boundary noted above, there is total loss of radial organization of the distinctive Bergmann glia fibers, such that stained astroglial processes are seen only as small fragments with a "scrambled" appearance in the anterior lobe. In the most severely affected anterior folium, the glial processes appear greatly diminished in size and number. Such extreme disorganization of radial glia has not been described in other mutant mice.

The distribution of cellular defects in the *mea/mea* cerebellum extends from the medial to lateral poles of the structure. This is in contrast to other mouse mutants such as weaver (11–13), in which the area of the midline vermis is affected to a greater extent than the lateral poles of the cerebellum. It is striking that in the several hundred cerebella that we have grossly examined in the course of developing our phenotypic cross, the boundary between normal and abnormal morphology reproducibly occurs at the same position within the declive. Moreover, the full spectrum of abnormalities described above is evident in *mea/mea* animals as early as postnatal day 8, the youngest age examined in this study, suggesting that an early step in cellular organization is affected. Finally, the morphology of the heterozygote (*mea/+*) cerebellum is entirely normal, indicating that this is a true recessive mutation.

Our examination of the meander tail mouse reveals that the *mea* gene product is required for normal development and positioning of at least three cell types in a discrete region of the anterior lobe of the cerebellum. Although the sharp boundary of the affected area is reminiscent of invertebrate "compartments," we have no data regarding cell lineage to formally support that designation as it is applied in *Drosophila* genetics. However, our analysis demonstrates a regional definition of the cerebellum in discrete developmental units. Such discrete localization has not been recognized in other mouse neurological mutants, since they typically display abnormalities that are widespread in the cerebellum. Nevertheless, at least one other mouse mutant may lend support to the notion of a specific developmental unit in the anterior cerebellum. The leaner mutant (14) displays striking degeneration of Purkinje, granule, and Golgi cells primarily in the anterior lobe while preserving the position and topographical relationships of cells in that region. Though the leaner mutation affects the same region of the cerebellum, it results in an entirely different phenotype in which neuronal degeneration occurs late in postnatal development and cytoarchitecture is normal. That two independent mutations affect the same region of the mouse cerebellum supports the hypothesis that this developmental unit may be a true compartment. Alternatively, the regional defect in the *mea* cerebellum could conceivably result from aberrant afferent or efferent connections specifically localized to the anterior cerebellar cortex. It will be important to examine the morphology of the *mea* cerebellum and its connections over a broad developmental time course.

Striking features of *mea* are the disarray of astroglial cells and the absence of a trilaminar organization of cerebellar cortex in the anterior lobe. The complete disorganization of the *mea* cerebellum suggests that this mutation disrupts an earlier step in cerebellar ontogeny than does the leaner mutation. Since other mutations have revealed the importance of the relationship between neurons and glia in cellular positioning in the developing brain (3–6) it is possible that the defect seen here arises from abnormal neuron–glia interactions. For example, the *mea* gene may affect astroglial differentiation as a primary event with subsequent disruption of neuronal positioning. Alternatively, the primary defect may be neuronal, as previous studies of the weaver mouse (6, 15) have shown that mutation of genes acting in neurons can perturb a cascade of events, eventually leading to defects in glial cell function and cell positioning. *In vitro* analyses and construction of chimeric mice will be required to establish the site of action of the *mea* gene product and to understand its effects on cell–cell interactions and morphogenesis.

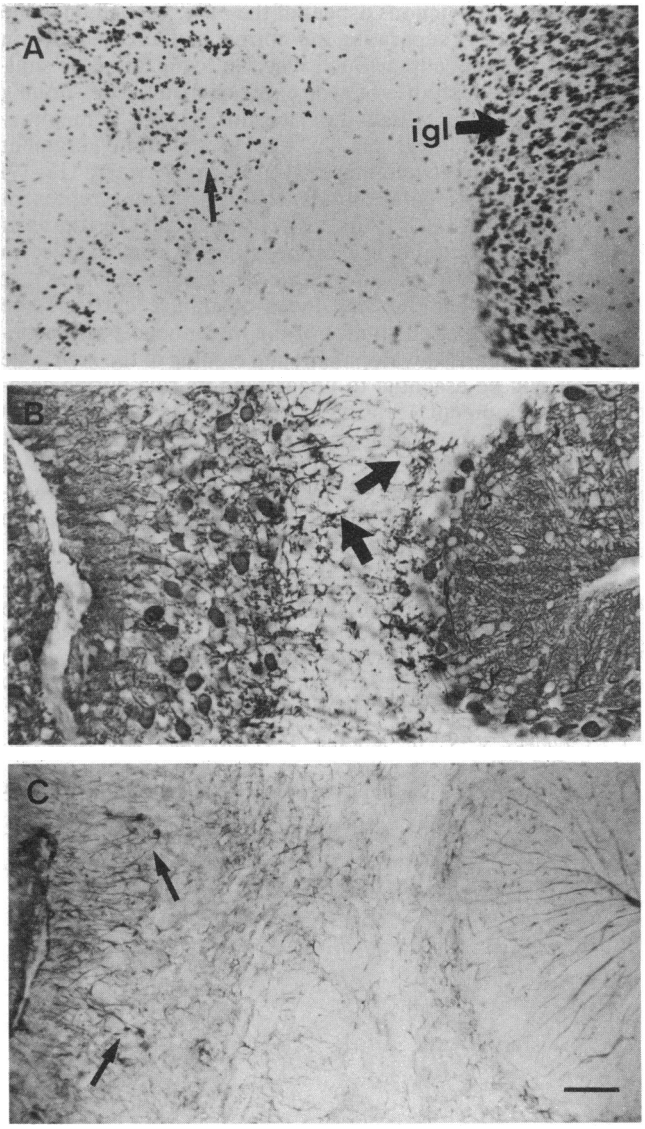

FIG. 2. Higher magnification views of the base of the declive in *mea/mea* cerebellum. Arrowheads in Fig. 1 *B*, *E*, and *H* point to areas enlarged in *A*, *B*, and *C*, respectively. (*A–C*) Cortical layers are deranged on the left (anterior aspect), yet, because of the infolding of the foliation pattern, they face normal cortical regions on the right (base of a folium in the posterior cerebellum). Granule cells are diminished in number (arrow, *A*) compared to the normal granule layer (*A*, igl). Purkinje cells are disoriented and project dendrites into the white matter tracts (arrows, *B*). Bergmann glial fibers are disoriented (arrows, *C*). (Bar in *C* for *A–C* = 50 μm.)

with antisera to glial filament protein. Bergmann glia are normally regularly spaced, span the Purkinje and molecular layers, and project fibers radially, perpendicular to the pia (open star, *H* and *I*). In the most severely affected folia (star/circle, *H* and *J*), glial fibers are short, disoriented, and reduced in density (*J*) and, like the Purkinje cells in this area (*E* and *G*), fill the folium. (Bar in *A* for *A*, *B*, *E*, and *H* = 1 mm; bar in *J* for *C*, *D*, *F*, *G*, *I*, and *J* = 50 μm.) All are midline sagittal sections.

The most conspicuous aspect of the *mea* cerebellum is the precise boundary separating the normal and affected area in the anterior cerebellum. Such discrete localization is reminiscent of recent studies of gene expression in the developing mouse hindbrain, which indicate that Hox-2 homeobox-containing genes may play a role in specifying segment phenotype in the developing central nervous system (16–19) and that developmental units such as these may represent compartments (20, 21). Although a similar role for the *mea* gene product in cerebellar morphogenesis is conceivable, no known homeobox- or zinc finger-containing gene has yet been mapped near the *mea* locus on mouse chromosome four. Further detailed studies of the *mea* phenotype, particularly during early development, and cloning of the *mea* gene product will be essential to understand its role during cerebellar development. Studies of the *mea* gene, and other interesting mouse neurological mutations, will complement the information presently being generated by analysis of mammalian homologues to *Drosophila* developmental regulators and may allow identification of gene products specific to mammalian neurogenesis.

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