

## Expression of the basic Helix-Loop-Helix ME1 E-protein during development and aging of the murine cerebellum

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### Abstract

Genesis of cerebellar granule cells is controlled by key transcription factors, such as the lineage-specific basic Helix-Loop-Helix (bHLH) transcription factor MATH-1, whose activity is dependent upon dimerization with bHLH E-proteins. In an effort to understand the molecular mechanisms of bHLH proteins orchestrating cerebellar development, we explored the spatio-temporal expression of the ME1 E-protein. Our results reveal that ME1 expression is first detected in the cerebellar primordium and then in the rhombic lip cells at E12.5. Its expression persists in the emerging external germinal layer during embryonic expansion. In adult cerebellum, prominent ME1 expression is detected in mature granule cells located in the internal granular layer. However, ME1 expression is not sustained in aged cerebellum. A similar declined pattern of expression is also observed in the aging hippocampus. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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The cerebellum is involved in two major functions, motor coordination and postural equilibrium, which are assumed by granule cell neurons [6]. The granule cell neurons are one of the two principle neurons and the most abundant in the cerebellum [21]. Cerebellar granule neurons display distinct patterns of gene expression during determination and differentiation processes [13]. Transcription factors such as the basic Helix-Loop-Helix (bHLH) proteins control the progressive restrictive steps leading to cerebellar development.

The bHLH transcription factors have been shown to control cell fate determination, differentiation, and maintenance during neurogenesis [1,16]. The bHLH proteins form dimeric complexes through the Helix-Loop-Helix (HLH) motif, and subsequently bind to DNA through the basic domains. They recognize a specific *cis*-acting element, called the E-box, which is characterized by a CANNTG consensus sequence [18]. The E-box-binding bHLH proteins have been organized in different classes, depending on their pattern of expression. Class A bHLH proteins or E-

proteins exhibit a broad pattern of expression, whereas class B bHLH proteins display a lineage-restricted pattern of expression. In many cases, tissue-specific bHLH proteins, such as NSCL-1, bind DNA as heterodimers by interacting with the required heterodimeric partners E-proteins [22]. The neuronal-specific MATH-1 bHLH protein, which is essential for cerebellar granule cell development, binds and acts as a transcriptional activator, only when heterodimerizing with an E-protein [2,7]. However, the E-protein pattern of expression has not been analyzed during cerebellar granule cell formation. The aim of the present study is to examine the expression pattern of the bHLH ME1 E-protein by *in situ* hybridization during development and aging of the cerebellum.

ME1 is a single copy gene, also referred as *tcf12* gene, located on the mouse chromosome 9, that encodes for an E-protein [19,20]. ME1 is highly conserved with its human counterpart HEB (92% identity), which maps on the chromosome 15q21 [15,23]. As a transcription factor, ME1 assumes a dual function: it can either behave as an activator or repressor depending on the promoter context [8–10].

The spatio-temporal expression pattern of ME1 was analyzed by *in situ* hybridization at various embryonic

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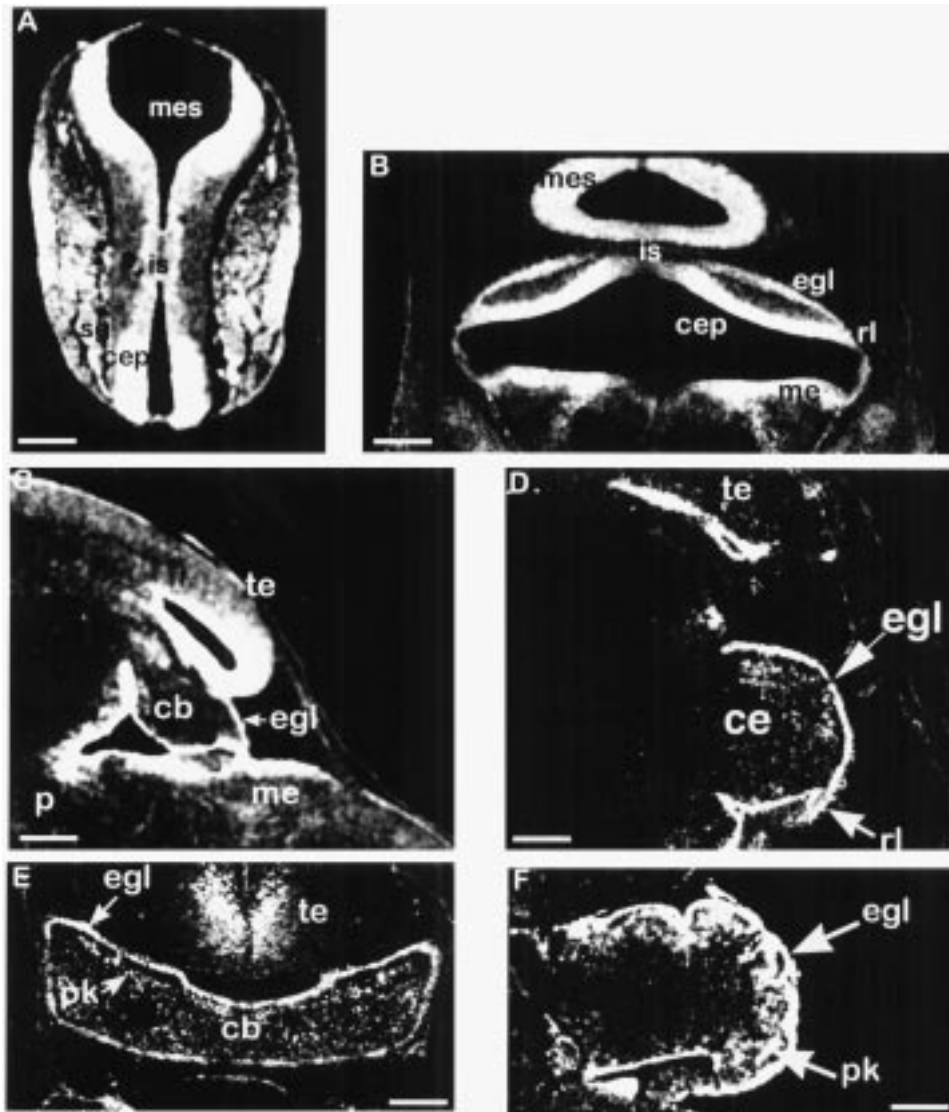


Fig. 1. ME1 expression during cerebellum development. Sections of brains at E10.5 (A), E12.5 (B), E14.5 (C), E16.5 (D), E17.5 (E), newborn (F). (A,B,E) Show frontal sections, whereas (C,D,F) display sagittal sections. ME1 transcripts are first detected in the cerebellar primordium and then in the rhombic lip cells. Its expression persists in the developing EGL. Purkinje cells also express ME1. cb, cerebellum; cep, cerebellar primordium; egl, external germinal layer; is, isthmus; me, medulla; mes, mesencephalon; p, pons; pk, purkinje cells; rl, rhombic lip; sg, spinal ganglion, te, tectum. Scale bar, 250  $\mu\text{m}$  (A); 120  $\mu\text{m}$  (B–D); 20  $\mu\text{m}$  (E,F).

and postnatal stages of cerebellar development. Embryos and postnatal brains were generated by mating C57B1/6 mice, dissected and immediately frozen on dry ice. Eight micron thick sections were cut at different stages of the mouse developing nervous system and treated as described [11]. To generate ME1-specific in situ probe, the 677 base-pair long BamHI-EcoRI fragment obtained from the pcDNA3.1/FL-ME1a plasmid was subcloned into pBlue-script SK vector. This fragment does not carry the bHLH motif, which is highly conserved between bHLH proteins. The pBS/ME1a construct was linearized with either the EcoRI enzyme or BamHI enzyme to generate antisense and sense probes respectively. Single-stranded sense and antisense mRNA probes (677 bp) were transcribed in vitro

using 100  $\mu\text{Ci}$  [ $^{35}\text{S}$ ]-UTP (ICN) and T7 or T3 RNA polymerase, respectively. The antisense and sense probes were then diluted to approximately  $5 \times 10^4$  cpm/ $\mu\text{l}$  in a buffer containing 50% formamide, 0.3 M NaCl, 10 mM Tris, 10 mM NaPO<sub>4</sub> (pH 6.8), 5 mM EDTA, 1 $\times$  Denhardt's, 10% dextran sulphate, 10 mM DTT, and 1 mg/ml tRNA. Hybridization was performed as described [11].

ME1 transcripts were first abundantly detected at E10.5 in the cerebellar primordium as well as in the mesencephalon, the isthmus, and the spinal ganglion (Fig. 1A). No signal was detected by a sense probe (data not shown). At this stage, the neuroepithelium of the cerebellar primordium gives rise to the precursors of the deep nuclei and the Purkinje cells [5]. At E12.5, ME1 started being expressed

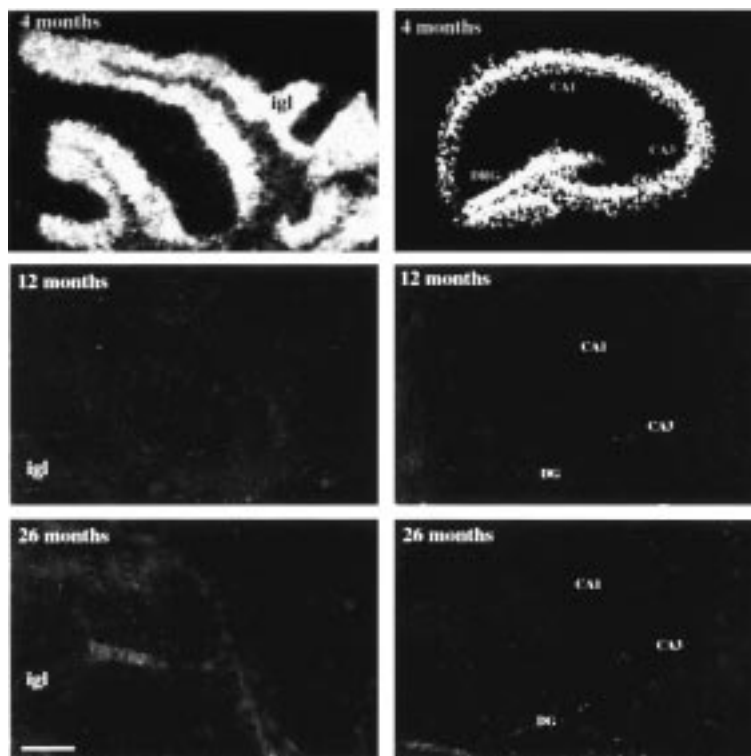


Fig. 2. ME1 expression in adult brain. Sagittal sections of adult (4 months), old (12 months) and senile (24 months) cerebellum (left) and hippocampus (right). ME1 is highly expressed in adult cerebellum and hippocampus, but ceases to be expressed in aged brain. CA1, Ammon's horn; dg, dentate gyrus; igl, internal granular layer. Scale bar, 250  $\mu$ m.

in the rhombic lip cells and remained expressed in the ventricular surface of the cerebellar anlage (Fig. 1B). The rhombic lip originates from the rostral portion of the metencephalon across the surface of the cerebellar anlage and gives rise to the precursors of the cerebellar granule neurons [4,12,17]. However, the proliferative rhombic lip cells are already specified to become granule neurons [3]. By E14.5, ME1 expression increased in the rhombic lip cells as they expand [12]. In addition, ME1 transcripts were located in proliferative progenitor cells migrating rostrally over the surface of the cerebellar anlage to form a displaced proliferative zone, called the external germinal layer (EGL) (Fig. 1C). ME1 remained expressed in the ventricular zone of the tectum, pons and medullary primordium (Fig. 1C). At E16.5, ME1 expression was still detected in the rhombic lip cells and the forming EGL, which undergoes an embryonic expansion and extends across the whole roof of the developing cerebellum as a single-cell layer (Fig. 1D). It is worth noting that ME1 expression was detected in a punctuated fashion in the developing cerebellum, suggesting expression in the Purkinje cells (Fig. 1D). At E17.5, ME1 expression strongly persisted in the single-cell layer EGL (Fig. 1E). At birth, ME1 transcripts remained detected the EGL, which undergoes a major postnatal expansion (Fig. 1F). In the adult brain, ME1 was strongly expressed in mature granular neurons located in the internal granular layer (IGL) of the

cerebellum, as well as the granule cells of the dentate gyrus of the hippocampus (Fig. 2). ME1 transcripts were also detected in the pyramidal cells in the CA1 to CA3 regions of the hippocampus (Fig. 2). Interestingly, ME1 expression was not sustained in aging cerebellum and hippocampus (Fig. 2).

The bHLH transcription factors have been classified into two groups based on the timing of their expression relative to the stage of neuronal development [16]: (1) those that are expressed in proliferating neural precursors, and (2) those that are expressed in postmitotic differentiating and mature neurons. Based on our results, ME1 is expressed in both actively proliferating cerebellar granule cell precursors, such as the rhombic lip cells, as well as postmitotic mature granule cells located in the IGL. This pattern of expression is consistent with a role for ME1 in the dynamic process of cerebellar granule cell development as the proliferating rhombic lip cells undergo differentiation. During the early steps of embryonic cerebellar development, ME1 and MATH-1 display overlapping expression domains. MATH-1 is expressed in the rhombic lip cells as well as in the embryonically expanding EGL [7]. However, in an adult brain, ME1 and MATH-1 are no longer coexpressed in mature granule neurons, since MATH-1 ceases to be expressed in postmitotic differentiating neurons before their migration to the IGL [14]. These results suggest that ME1 must heterodimerize with other partners, such as

NeuroD, to maintain the differentiated state during adulthood [16]. Thus, a network of interacting bHLH proteins controls cerebellar development as well as maintenance of the differentiated state in adult brain. However, ME1 ceases to be expressed in old and senile brains, raising the possibility that ME1 may be required for sustaining the maintenance of the fully functional state of neurons. Thus, these results support the idea that during brain aging there is a change in the dynamic network of transcription factors controlling the expression of structural and functional genes, leading to an overall functional decline of neurons. Therefore, it would be informative to unravel the molecular mechanisms controlling ME1 expression at different stages of development and aging.

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