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Gene expression pattern

Expression of *Meis* and *Pbx* genes and their protein products in the developing telencephalon: implications for regional differentiation

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Abstract

The *Meis* and *Pbx* genes encode for homeodomain proteins of the TALE class and have been shown to act as co-factors for other homeodomain transcription factors (Mann and Affolter, 1998. Curr. Opin. Genet. Dev. 8, 423–429). We have studied the expression of these genes in the mouse telencephalon and found that *Meis1* and *Meis2* display region-specific patterns of expression from embryonic day (E)10.5 until birth, defining distinct subterritories in the developing telencephalon. The expression of the *Meis* genes and their proteins is highest in the subventricular zone (SVZ) and mantle regions of the ventral telencephalon. Compared to the *Meis* genes, *Pbx* genes show a broader expression within the telencephalon. However, as is the case in *Drosophila* (Rieckhof et al., 1997. Cell 91, 171–183; Kurrant et al., 1998. Development 125, 1037–1048; Pai et al., 1998. Genes Dev. 12, 435–446), nuclear localized PBX proteins were found to correlate highly with *Meis* expression. In addition, DLX proteins co-localize with nuclear PBX in distinct regions of the ventral telencephalon.

Keywords: DLX; Ganglionic eminence; MEIS; Mouse; PBX; Telencephalon

1. Results

The *Meis* gene family in vertebrates consists of *Meis1-3* (Nakamura et al., 1996) and the related *Prep1/Pknox* (Berthelsen et al., 1998). We have only detected high level expression of two of these genes within the telencephalon; *Meis1* and *Meis2*. Telencephalic *Meis1* and *Meis2* expression is first detected around E10.5 in the ventrolateral telencephalon. At this time, *Meis1* transcripts are expressed at low levels in the ventricular zone (VZ) while *Meis2* is expressed at high levels in cells underlying the VZ lateral to the medial ganglionic emeinence (MGE) (i.e. the presumptive lateral ganglionic eminence (LGE)) (Fig. 1A,B). From E11.5, when the MGE and LGE are morphologically distinct, *Meis1* is expressed at high levels in the caudal ganglionic eminence (CGE) and developing amygdala and weaker in the LGE and MGE (Fig. 1E). At E12.5, *Meis1* transcripts are found in the lateral edges of both the MGE and LGE (Fig. 1G). *Meis1* continues to be expressed in the ventro-lateral regions of the striatum and cortex with higher expression in the ventral pallidum and medial septum at later stages (Fig. 1K). The expression pattern of *Meis2* in midgestation embryos and onwards has recently been described (Cecconi et al., 1997; Oulad-Abdelghani et al., 1997; Toresson et al., 1999), however, for comparison purposes we have included this expression here. Unlike *Meis1*, *Meis2* is found at moderate levels throughout the VZ of the entire telencephalon with the exception of the ventro- and dorso-medial regions (Fig. 1H). The highest expression is detected in the SVZ of the LGE and developing striatum (Fig. 1F,H,L). By E16.5, *Meis2* transcripts are also found in the cortical plate (Fig. 1L). As was the case for *Meis1*, the CGE and amygdala (Fig. 1F; Cecconi et al., 1997) also express high levels of *Meis2*. *Meis* gene expression in the telencephalon remains unchanged at birth (data not shown) and even into adulthood (data not shown; Toresson et al., 1999). At all stages examined, MEIS protein is found where the respective gene is expressed (Fig. 2A,B). Confocal microscopy of MEIS1 and MEIS2 stainings, shows an area of overlap in the ventro-lateral LGE (Fig. 2C).

The *Drosophila* homologue of the *Meis* genes, *homothorax*, has previously been shown to be required for the nuclear localization of the PBX homologue, extradenticle (Rieckhof et al., 1997; Kurrant et al., 1998; Pai et al., 1998).

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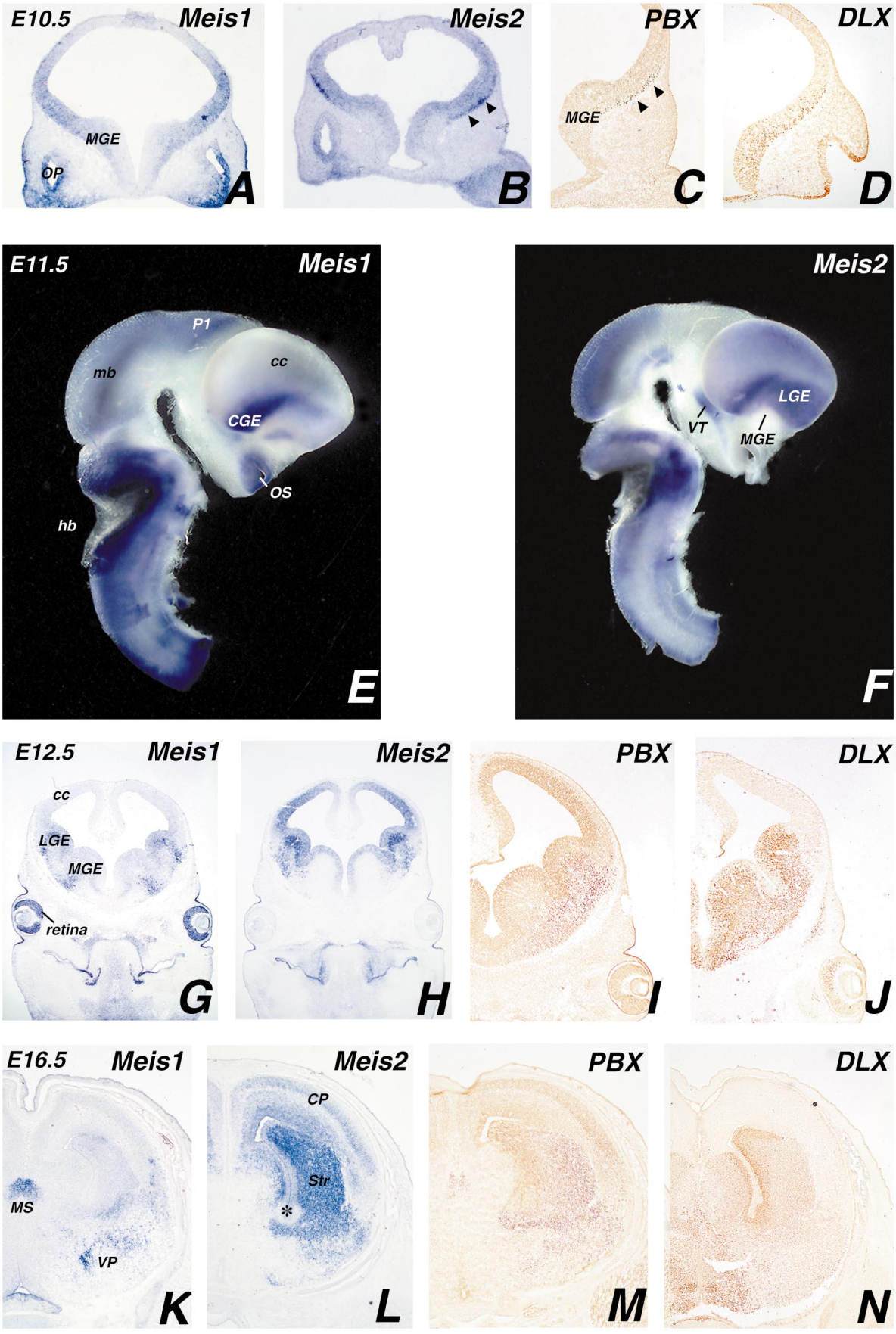
E10.5
Meis1
MGE
OP
A
Meis2
B
PBX
MGE
C
DLX
D
E11.5
Meis1
P1
mb
cc
CGE
OS
hb
E
Meis2
LGE
VT
MGE
F
E12.5
Meis1
cc
LGE
MGE
retina
G
Meis2
H
PBX
I
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E16.5
Meis1
MS
VP
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Meis2
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*
L
PBX
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Interestingly, in the mouse there is a strong correlation between *Meis* gene expression and nuclear localized PBX proteins (including PBX1, 2 and 3) (Fig. 1A–C,G–I,K–M). In fact, double in situ hybridization-immunohistochemistry reveals co-localization of PBX proteins and *Meis2* transcripts (Fig. 2D). This protein localization does not completely parallel that of *Pbx* gene expression. While *Pbx1* expression is rather broad (Roberts et al., 1995; Fig. 2E), *Pbx3* transcripts show a similar localization to *Meis1*and *Meis2* (Fig. 2F). Thus, interactions between MEIS and PBX molecules may have been conserved from *Drosophila* to mouse.

MEIS and PBX proteins form DNA binding complexes with a number of homeodomain transcription factors, including each other (Mann and Affolter, 1998). The *Dlx* family of homeobox genes is interesting in this respect, since they are expressed in both the LGE and MGE (Liu et al., 1997) which are known to give rise to distinct neuronal phenotypes in the ventral telencephalon (Olsson et al., 1995, 1998). The LGE represents the principal source of striatal projection neurons. In fact, both *Dlx1* and *Dlx2* are required for normal striatal differentation (Anderson et al., 1997). We show here, that DLX protein expression overlaps extensively with the domains of MEIS and PBX expression in the telencephalon (Fig. 1A–D,G–N). Indeed, within the SVZ of the LGE (Fig. 2H) but not the MGE (Fig. 2G), cells expressing DLX proteins were also found to co-express nuclear PBX proteins.

2. Materials and methods

2.1. In situ hybridization

In situ hybridization was carried out as described previously using digoxygenin-labeled cRNA probes (Toresson et al., 1999). The Meis1 (Moskow et al., 1995) and Pbx1–3 (Swift et al., 1998) probes were generated from full-length cDNAs. Whole-mount in situ hybridization was carried out essentially as described by Wilkinson (1992). Double in situ-immunohistochemistry stainings were performed by first detecting the PBX antigen (as described below) and subsequently performing in situ hybridization (as above).

2.2. Immunohistochemistry

Immunohistochemistry was carried out as previously described (Toresson et al., 1999). The rabbit MEIS1 and MEIS2 antisera were used at 1:5000 (provided by A. Buchberg). The rabbit anti-panPBX antibody (Santa Cruz) was used at a 1:400 dilution. Rabbit anti-Distal-less (DLX) was used at 1:300 (provided by G. Panganiban). Double stainings (MEIS1/MEIS2 and PBX/DLX) were done by detecting the first antigen using an elevated concentration of secondary antibody (1:50) or at a normal concentration (i.e. 1:200) followed by an excess of unconjugated anti-rabbit Fab fragments (Jackson ImmunoResearch) in order to block the subsequent secondary antibody from binding to the first primary antibody. The second primary antibody was detected using a normal concentration of secondary antibody (1:200). As an additional control, stainings were performed where the second primary antibody was omitted.

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Fig. 1. (A–D) Coronal sections of E10.5 telencephalon. (A) *Meis1* expression is detected in the lateral portions of the VZ. (B) Low levels of *Meis2* transcripts are also seen in the lateral VZ, however, cells underlying the VZ in the presumptive LGE, express high levels of *Meis2* (arrowheads). (C) PBX proteins (including PBX1, 2 and 3) are nuclear in the same region that *Meis2* is strongly expressed (arrowheads). (D) DLX proteins (including DLX1, 2, 5 and 6) are expressed in many nuclei of the ventral telencephalon including both the MGE and presumptive LGE. (E,F) Whole-mounts of E11.5 brains. (E) *Meis1* expression within the telencephalon in the caudal ganglionic eminence (CGE) with two stripes extending into in the LGE and MGE. In the diencephalon, *Meis1* expression is seen in the optic stalk (OS) and prosomere 1 (P1). Transcripts are also detected at weak to moderate levels in the midbrain (mb) and at high levels in the hindbrain (hb). (F) *Meis2* expression in the E11.5 telencephalon is highest in the LGE and CGE. Diencephalic expression is confined to the ventral thalamus (VT). *Meis2* transcripts are found highly expressed in both the midbrain and hindbrain (Cecconi et al., 1997; Oulad-Abdelghani et al., 1997). (G–J) Coronal sections of E12.5 embryos at the level of the MGE and LGE. (G) At E12.5, *Meis1* is expressed in the lateral parts of the LGE and MGE as well as in the developing retina. (H) *Meis2* expression, at this stage, is found at high levels in the LGE SVZ and the forming striatal mantle. Moderate levels are observed in the VZ. (I) Nuclear PBX protein expression overlaps completely with *Meis1* and *2* gene expression in the E12.5 telencephalon. (J) DLX proteins are expressed in the vast majority of ventral telencephalic cells at E12.5 showing extensive overlap with *Meis* and PBX expression. (K–N) Coronal sections of E16.5 telencephalon. (K) *Meis1* expression is seen at moderate levels in the ventro-lateral part of the striatum (Str) and cortex and at higher levels in the medial septum (MS) and ventral pallidum (VP). (L) Apart from the striatal expression, *Meis2* transcripts are found in the cortical plate (CP) and lateral septum. Note that even at this stage, the remnant of the MGE (asterisk) does not express *Meis2*. (M) Similar to earlier stages, PBX proteins are nuclear in areas where *Meis1* and/or *Meis2* is expressed. (N) DLX proteins are expressed predominantly in ventral telencephalic regions including the striatum, pallidum and septum. cc, cerebral cortex; op, olfactory pit.

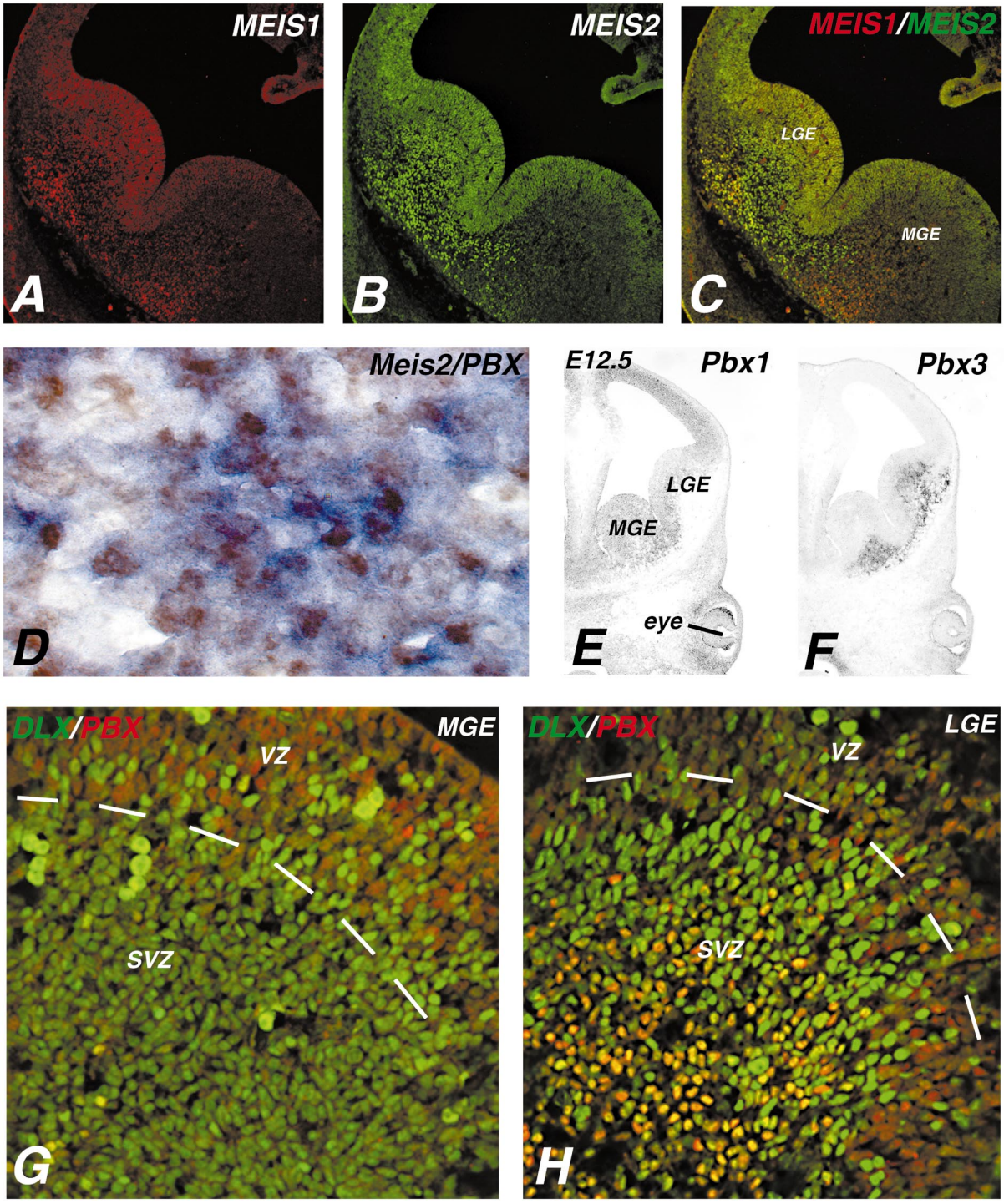

Fig. 2. (A,B) MEIS1 and MEIS2 expression is similar to their respective gene expression (see Fig. 1G,H). (C) Merged images of (A,B) showing cells co-expressing MEIS1 and MEIS2 (yellow nuclei) in the ventro-lateral portion of the LGE mantle while the majority of cells in the LGE SVZ predominantly express MEIS2 (green nuclei). A group of cells deep to the MGE express MEIS1 (red nuclei) only. (D) Double staining for PBX protein (brown nuclear staining) and *Meis2* transcripts (fuzzy blue staining) in the striatal mantle at E16.5. Note that many brown stained nuclei are surrounded by blue cytoplasmic staining, indicating co-localization of *Meis2* and PBX. (E,F) Coronal sections of E12.5 telencephalon. (E) *Pbx1* expression is seen throughout the telencephalic VZ and SVZ. (F) *Pbx3* transcripts are more discreetly localized, showing expression in the LGE SVZ as well as the mantle region of both the MGE and LGE. High power view of MGE (G) and LGE (H) from E12.5 coronal sections at a level similar to the that in Fig. 1G–J. Confocal micrographs of PBX (red) and DLX (green) immunostaining. No double-labeled nuclei are found in the MGE (G) while many nuclei co-express proteins of the two families in the SVZ of the LGE (i.e. yellow nuclei in H).

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