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Spatio-temporal expression of *Pbx3* during mouse organogenesis

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Abstract

Pbx3 is a member of the Pbx family of TALE (three amino acid loop extension) class homeodomain transcription factors. These transcription factors are implicated in developmental and transcriptional gene regulation in numerous cell types through their abilities to form hetero-oligomeric DNA-binding complexes. *Pbx3* was found to be expressed at high levels in the developing central nervous system (CNS), including a region of the medulla oblongata which is implicated in the control of respiration. Furthermore, as reported, *Pbx3*-deficient mice develop to term but die within a few hours of birth from central respiratory failure. In this study, we have characterized *Pbx3* expression patterns during organogenesis in numerous tissues and organ systems other than the CNS, as a first step toward understanding the potentially overlapping functions of *Pbx3* with other *Pbx* family members during vertebrate development. We have performed in situ hybridization on whole mount and sectioned mouse embryos from gestational day (E) 9 to E16.5. During early organogenesis, until E12.5, *Pbx3* expression is found mostly in the embryonic head, forelimbs, and septum transversum, unlike *Pbx1* and *Pbx2* expression which is more widespread. Conversely, later in organogenesis, *Pbx3* expression becomes more widely detectable throughout the developing embryo. Epithelial and mesenchymal tissues, as well as the CNS, represent major sites of *Pbx3* expression. The enteric nervous system also expresses high levels of *Pbx3*, distinctively in the cells of the ganglia of Auerbach's myenteric nerve plexus, that also express *Dlx2* and *Notch1*. Cartilage is also a site of *Pbx3* expression. Interestingly, like *Pbx1*, *Pbx3* is highly expressed in proliferating chondrocytes but is lost as chondrocytes become hypertrophic during endochondral ossification. Finally, *Pbx3* is expressed only in the forelimb buds during early limb development, while the hindlimb bud is devoid of *Pbx3*. This finding leads us to add *Pbx3* to the sparse list of early forelimb-specific molecular markers.
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Keywords: Pbx3; TALE class homeodomain; Expression patterns; Organogenesis; Enteric nervous system; Forelimb-specific markers

1. Results and discussion

Pbx genes encode a family of highly conserved homeodomain proteins of the TALE (three amino acid loop extension) class that participate in multiprotein complexes to regulate developmental gene expression (Mann and Affolter, 1998; Moens and Selleri, 2006). *Pbx1* was originally identified in pre-B acute lymphoblastic leukemias (Kamps et al., 1990; Nourse et al., 1990). The highly related *Pbx2*, *Pbx3*, and *Pbx4* genes were later identified on the basis of sequence conservation with *Pbx1* (Monica et al., 1991; Wagner et al., 2001). *Pbx* orthologs were also characterized in *Caenorhabditis elegans* (*Ceh-20*), *Drosophila* (*Exd*), and *Danio rerio* (*lazarus*) (Burglin and Ruvkun, 1992; Rauskolb et al., 1993; Pöpperl et al., 2000; Vlachakis et al., 2000). In *Drosophila*, Exd acts as a co-factor to direct homeotic selector proteins to target genes (Rauskolb and Wieschaus, 1994; Wilson and Desplan, 1995). In zebrafish, *lazarus* was shown to globally mediate *Hox* gene function, while orchestrating the segmentation of the hindbrain and pharyngeal arches (Pöpperl et al., 2000; Waskiewicz et al., 2002).

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Pbx proteins share remarkable sequence identity within and flanking their DNA-binding homeodomains. Various isoforms of mammalian Pbx proteins arise from differential splicing of *Pbx* transcripts to yield high molecular weight (mw) (Pbx1a, Pbx2, Pbx3a, and Pbx4) and low mw (Pbx1b, Pbx3b) forms (Monica et al., 1991; Waskiewicz et al., 2002). Although the DNA-binding properties of Pbx proteins appear similar in vitro, the transcriptional effector properties of different isoforms can be distinguished on the basis of their differential recruitment of transcriptional co-factors (Asahara et al., 1999). Biochemical studies have established that Pbx proteins interact with a subset of Hox proteins to enhance their DNA-binding affinities and specificities (Chan et al., 1994; van Dijk and Murre, 1994; Chang et al., 1995; Knoepfler and Kamps, 1995; Lu et al., 1995; Peers et al., 1995; Phelan et al., 1995; Pöpperl et al., 1995; Chang et al., 1996; Peltenburg and Murre, 1996; Chang et al., 1997; Vlachakis et al., 2000). Pbx proteins also hetero-dimerize with the Meinox subfamily of TALE class proteins (Chang et al., 1997; Berthelsen et al., 1998; Kilstrup-Nielsen et al., 2003) to form trimeric complexes with Hox proteins (Berthelsen et al., 1998, 1999; Jacobs et al., 1999; Ferretti et al., 2000).

We have recently established the differential contributions of Pbx1, Pbx2, and Pbx3 proteins to mammalian development (Selleri et al., 2001, 2004; Rhee et al., 2004; Moens and Selleri, 2006). Our studies have demonstrated that Pbx1 and Pbx3 have unique and essential functions in embryonic development and postnatal survival, respectively. *Pbx1*-deficient embryos die at gestational day (E) 15/16 with severe hypoplasia and/or aplasia of multiple organs (Selleri et al., 2001; Kim et al., 2002; Schnabel et al., 2003a,b; Manley et al., 2004; Brendolan et al., 2005), as well as homeotic transformation (Selleri et al., 2001) and hematopoietic abnormalities (Di Martino et al., 2001). Mice deficient for *Pbx3* develop to term, but die within a few hours of birth due to respiratory failure (Rhee et al., 2004). Conversely, despite its widespread embryonic expression, *Pbx2* is not an essential gene as, when it alone is lost, it does not affect normal organogenesis, fertility, hematopoiesis or immune function, likely due to compensation by related *Pbx* family members (Selleri et al., 2004). While spatio-temporal domains of expression for both *Pbx1* and *Pbx2* have been established (Selleri et al., 2001, 2004; Di Martino et al., 2001; Kim et al., 2002; Schnabel et al., 2003a,b; Manley et al., 2004; Brendolan et al., 2005), and *Pbx4* mRNAs have been found preponderantly in testes (Wagner et al., 2001), *Pbx3* gene expression patterns in early development are as yet unknown. As a first step toward assessing potential overlapping functions of *Pbx3* with *Pbx1* and *Pbx2* in the development of select organ systems, we have analyzed *Pbx3* expression during vertebrate organogenesis by in situ hybridization both on whole mount and sectioned mouse embryos (Wilkinson and Nieto, 1993; Selleri et al., 2001, 2004) from E9 to E16.5.

1.1. Pbx3 expression patterns in mouse organogenesis from E9 to E12.5

Whole mount in situ hybridizations with a 3′ UTR probe demonstrated that from E9 to E12.5 *Pbx3* expression was limited to only a few sites in the developing embryo. Indeed, *Pbx3* early signal was detectable mostly in the head and developing forelimb (FL), while the rest of the embryo appeared transparent and devoid of *Pbx3*, except for the presence of hybridization signal in the developing thoracic region and septum transversum (Fig. 1).

In the developing head, at E9.5 and E10.5, expression was detected distinctively in the frontonasal process (FnP), optic vesicle (OpV), maxilla (Mx), mandibular (Md) component of branchial arch 1 (BA1), and in BA2 (Figs. 1A and green inset and B). BA3 did not exhibit detectable hybridization signal at E9.5, although by E10.5 a weak signal could be detected by whole mount in situ hybridization (Figs. 1A and B; data not shown). At E10.5, *Pbx3* signal became highest in the Mx and in BA1, especially in their distal domains (Fig. 1B), unlike *Pbx1*, which is predominantly expressed in BA2 (Selleri et al., 2001). Staining in the otic vesicle (OtV) (Figs. 1A and B) was due to probe trapping inside this chamber, as it was also detectable with a *Pbx3* sense probe (data not shown). Furthermore, in situ hybridization on E10.5 sections showed that *Pbx3* expression is present both in the mesenchyme and ectoderm of the FnP and BA1 and BA2, while in BA3 only the mesenchyme shows *Pbx3* signal (Fig. 1C and data not shown). This BA3 pattern likely explains why whole mount hybridizations do not reveal strong *Pbx3* expression. At E11.5, the FnP as well as the Mx, BA1 and BA2 exhibited relatively unchanged *Pbx3* expression levels compared to E10.5 (data not shown). Interestingly, the expression domains of *Pbx1* (Selleri et al., 2001; Manley et al., 2004), *Pbx2* (unpublished results), and *Pbx3* in the developing head and, in particular, the BAs do not significantly overlap, presaging a scenario in which *Pbx* compound mutants may display complex and multifaceted craniofacial phenotypes.

Intriguingly, *Pbx3* expression was also detectable at E9 in the thoracic region of the developing embryo and, caudal to the bulbo-ventricular region of the primitive heart, in the septum transversum (Kaufman and Bard, 1999) (Fig. 1E). This expression pattern is worthy of note since the rostral part of the septum transversum gives rise to the diaphragm (Ackerman et al., 2005) and mice deficient for *Pbx3* die perinatally due to respiratory failure (Rhee et al., 2004).

During early limb development, from E9.5 to E11.0, *Pbx3* expression was observed only in the FL, while the hindlimb (HL) was devoid of hybridization signal (Fig. 2) even after extended overnight incubations in developing solution (see Section 2). Regarding the FL, *Pbx3* was expressed only anteriorly and proximally at E9.5 (Fig. 2A; white arrowhead). At E10.5, *Pbx3* signal remained localized approximately to this same FL domain,

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