Mechanisms of Development 124 (2007) 364–376

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Identification of a new type of PBX1 partner that contains zinc finger motifs and inhibits the binding of HOXA9-PBX1 to DNA

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Received 22 December 2006; received in revised form 30 January 2007; accepted 31 January 2007
Available online 8 February 2007

Abstract

PBX1 belongs to the TALE-class of homeodomain protein and has a wide functional diversity during development. Indeed, PBX1 is required for haematopoiesis as well as for multiple developmental processes such as skeletal patterning and organogenesis. It has furthermore been shown that PBX1 functions as a HOX cofactor during development. More recent data suggest that PBX1 may act even more broadly by modulating the activity of non-homeodomain transcription factors. To better understand molecular mechanisms triggered by PBX1 during female genital tract development, we searched for additional PBX1 partners that might be involved in this process. Using a two hybrid screen, we identified a new PBX1 interacting protein containing several zinc finger motifs that we called ZFPIP for Zinc Finger PBX1 Interacting Protein. We demonstrated that ZFPIP is expressed in embryonic female genital tract but also in other PBX1 expression domains such as the developing head and the limb buds. We further showed that ZFPIP is able to bind physically and *in vivo* to PBX1 and moreover, that it prevents the binding of HOXA9/PBX complexes to their consensus DNA site. We suggest that ZFPIP is a new type of PBX1 partner that could participate in PBX1 function during several developmental pathways.

Keywords: PBX1; HOX; Cofactor; Genital tract

1. Introduction

PBX1 (pre-B-cell leukemia transcription factor 1) was initially isolated as a proto-oncogene in human leukaemia induced by the expression of the oncogenic fusion E2a-PBX1 protein (Kamps et al., 1990; Nourse et al., 1990). PBX1 belongs to the PBC group of the TALE class of homeodomain proteins that comprises PBX1, PBX2, PBX3 (Monica et al., 1991), PBX4 (Wagner et al., 2001), zebrafish Lazarus or lzr (Popperl et al., 2000; Waskiewicz et al., 2001), Drosophila Extradenticle or Exd and Caenorhabditis elegans Ceh-20 (Shanmugam et al., 1999; Shen et al., 1999). Several studies have demonstrated that PBC proteins were able to interact with a subset of HOX proteins and as such been considered as essential HOX cofactors involved in developmental gene regulation (reviewed in (Moens and Selleri, 2006).

Inactivation of *PBC* genes in different models have demonstrated that PBC proteins make critical contributions to cell fate and segmental patterning during development (reviewed in Moens and Selleri, 2006). In particular, loss-of-function studies have demonstrated a critical role for PBX1 in cellular proliferation and patterning and suggest its involvement in numerous regulatory pathways (Selleri et al., 2001). Indeed, *PBX1* mutants die at embryonic day 15/16 with severe hypoplasia or aplasia of multiple organs and widespread patterning defects of the axial and appendicular skeleton. Amongst abnormal organogenesis

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doi:10.1016/j.mod.2007.01.008

processes observed in these mutants, the urogenital system is severely affected with a markedly reduced urogenital ridge outgrowth and impaired differentiation of the mesonephros and kidneys. In addition, the Müllerian ducts which represent the antecedent of the female genital tract (i.e. oviducts, uteri and vagina) and are normally formed in E12.0 wild-type embryos, are lacking in both male and female mutants while the anlagen of the male genital tract, the Wölffian ducts are present (Schnabel et al., 2001).

Although some of the *PBX1* mutant phenotypes are similar to *HOX* mutants and can be thus attributed to effects on HOX function, some other aspects of the *PBX1*$^{-/-}$ mouse suggest that PBX1 may act more broadly in non HOX expressing embryonic area and/or in HOX independent pathways. In particular, the implication of *HOX* genes during female genital tract development differs quite significantly from that of *PBX1*. The Müllerian ducts do not form in *PBX1* mutant animals (Schnabel et al., 2003) whereas inactivation of the *HOXA10*, *HOXA11*, *HOXA13* or *HOXD13* gene provokes morphological defects along the proximodistal axis of the female reproductive tract that could initially differentiate (reviewed in Taylor, 2000). The absence of Müllerian ducts in *PBX1* mutants indicates the early involvement of the gene in this organogenesis. In addition, the gene seems to be required throughout female genital tract development as suggested by its constant expression during this process (Schnabel et al., 2001) and until puberty (Dintilhac et al., 2005).

In the aim to better understand the molecular mechanisms triggered by PBX1 during female genital tract development, we searched for new partners that might be involved in higher-order molecular gene regulation complexes during this organogenesis. For this purpose, we performed a two hybrid screen using full length PBX1B as bait and a cDNA library constructed with RNAs extracted from differentiating Müllerian ducts of E16.5 to E18.5 embryos.

By this way, we identified a new PBX1 interacting protein containing several zinc finger motifs that we called ZFPIP for Zinc Finger PBX1 Interacting Protein. We demonstrated that ZFPIP is expressed in embryonic female genital tract but also in other PBX1 expression domains such as the developing head and the limb buds. We further showed that ZFPIP is able to bind physically and *in vivo* with PBX1 and moreover, that it prevents the binding of HOXA9/PBX complexes to their consensus DNA site. We therefore suggest that ZFPIP is a new type of PBX1 partner that could participate in PBX1 function during several developmental pathways.

2. Results

2.1. Identification of a novel PBX1B partner expressed during female genital tract development

To isolate cDNAs encoding proteins that associate with PBX1B during female genital tract development, we first created a randomly primed cDNA library from polyA + RNAs from Müllerian ducts and used full-length PBX1B as bait. Prior to library screening, we verified that PBX1B possessed no intrinsic transcriptional activation of the reporter genes (not shown). Out of 1.2×10^5 transformants screened, 50 positive clones were finally selected for sequencing analysis based on two nutritional markers as well as by *lacZ* and *MEL1* reporter expression. Amongst these 50 clones, only 20 cDNAs were in frame with the Gal4 ORF in the prey plasmids. Two of these clones were identified as independent, overlapping *PBX1* cDNAs. Since it has been demonstrated, using different approaches, that PBX1 can homodimerize (Calvo et al., 1999), the presence of these clones validated the screen.

One of the positive clones was further studied. Fig. 1A shows that the corresponding peptide is able to activate reporter genes in the two hybrid system whereas no such

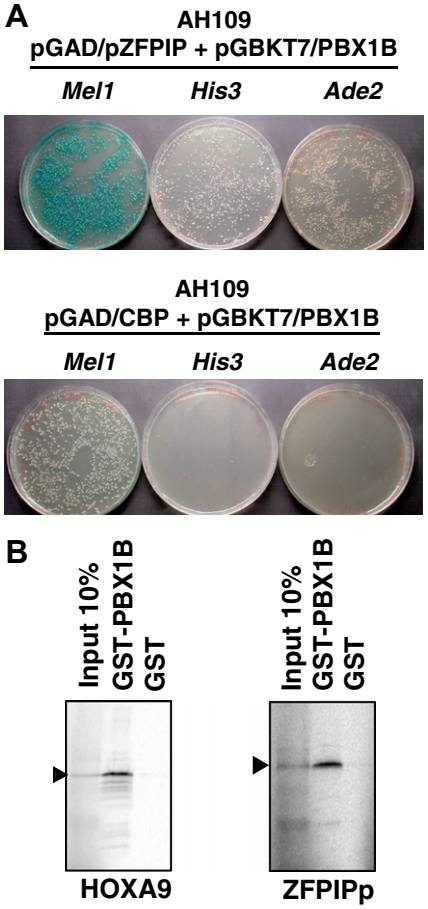

Fig. 1. Identification of a new putative PBX1 partner. (A) Positive clones identified by the two hybrid screen were isolated and tested by a secondary screen. Using the full length PBX1B protein as bait, one selected clone corresponding peptide was able to activate reporter genes (*Mel1*, *His3*, *Ade2*) in the two hybrid system whereas no such activation was obtained with CBP as a control protein. (B) This clone was further analyzed by GST-pull down. Radio-labelled *in vitro* translated putative partner was incubated with Glutathione–Sepharose beads loaded with either GST or GST-PBX1B. After several washes, proteins were eluted and analyzed on SDS–PAGE. Compared by GST pull down, the binding of the selected clone encoding peptide with PBX1B was similar to the binding of HOXA9.