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Genetics

Meis1 and pKnox1 bind DNA cooperatively with Pbx1 utilizing an interaction surface disrupted in oncoprotein E2a-Pbx1

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ABSTRACT **E2a-Pbx1 is a chimeric transcription factor oncoprotein produced by the *t*(1;19) translocation in human pre-B cell leukemia. Class I Hox proteins bind DNA cooperatively with both Pbx proteins and oncoprotein E2a-Pbx1, suggesting that leukemogenesis by E2a-Pbx1 and Hox proteins may alter transcription of cellular genes regulated by Pbx–Hox motifs. Likewise, in murine myeloid leukemia, transcriptional coactivation of Meis1 with HoxA7/A9 suggests that Meis1–HoxA7/9 heterodimers may evoke aberrant gene transcription. Here, we demonstrate that both Meis1 and its relative, pKnox1, dimerize with Pbx1 on the same TGATTGAC motif selected by dimers of Pbx proteins and unidentified partner(s) in nuclear extracts, including those from *t*(1;19) pre-B cells. Outside their homeodomains, Meis1 and pKnox1 were highly conserved only in two motifs required for cooperativity with Pbx1. Like the unidentified endogenous partner(s), both Meis1 and pKnox1 failed to dimerize significantly with E2a-Pbx1. The Meis1/pKnox1-interaction domain in Pbx1 resided predominantly in a conserved N-terminal Pbx domain deleted in E2a-Pbx1. Thus, the leukemic potential of E2a-Pbx1 may require abrogation of its interaction with members of the Meis and pKnox families of transcription factors, permitting selective targeting of genes regulated by Pbx–Hox complexes. In addition, because most motifs bound by Pbx–Meis1/pKnox1 were not bound by Pbx1–Hox complexes, the leukemic potential of Meis1 in myeloid leukemias may involve shifting Pbx proteins from promoters containing Pbx–Hox motifs to those containing Pbx–Meis motifs.**

The eukaryotic genome encodes multiple families of homeodomain (HD) proteins, which function as sequence-specific transcription factors that direct regional embryonic development, control anterior–posterior axial patterning, and regulate tissue-specific gene transcription (1, 2). In mice and humans, class I Hox proteins are encoded by tandomly arranged genes of the HoxA–HoxD loci, and their inappropriate expression can lead to homeotic, as well as oncogenic, transformation (3–6). Most Hox proteins, as well as Engrailed HD proteins, can bind DNA cooperatively with Pbx proteins, which are members of the TALE (three amino acid loop extension) HD family that contain a three-residue extension in the turn between helices one and two of the HD (7–12). *In vivo*, three widely expressed PBX genes, PBX1, PBX2, and PBX3, encode the common partners for a host of differentially expressed Hox proteins (13). In pediatric pre-B cell leukemia, the *t*(1;19) translocation forms a chimeric protein, E2a-Pbx1, containing the transactivation domain of E2a and the majority of Pbx1 (14, 15). E2a-Pbx1 induces myeloid and T-lymphoid leukemia in mice, blocks myeloid differentiation in marrow cultures, and activates transcription of reporter genes driven by canonical Pbx–Hox DNA motifs, suggesting that its mechanism of leukemogenesis may, in part, be accomplished by activating transcription of cellular genes regulated by Pbx–Hox heterodimers (16–22). The ability of DNA-binding mutants of E2a-Pbx1 to retain their transforming potential in fibroblasts indicates that some forms of transformation may also result from a dominant negative mechanism or from DNA-binding independent promoter targeting by direct protein–protein interactions (19).

Pbx and Hox HDs bind adjacent, unspaced half-sites, having the general structure TGATTAAT, upon which the Pbx protein contacts the 5′ TGAT, and the Hox protein binds the 3′ TAAT on the opposing face of the DNA helix (23, 24). In both Hox and Engrailed proteins, an unstructured tryptophan-containing motif N terminal to the HD is required for cooperative DNA binding with Pbx proteins (10, 12, 25–28). In Hox proteins, interaction of this motif with Pbx1 alters the DNA-binding specificity of the heterodimer at position 2 of the Hox core motif, shifting specificity from TAAT to either TGAT or TTAT (29, 30). In this configuration, the Hox protein retains its inherent specificity for a GG, GA, TG, or TA dinucleotide 3′ to its core (24). The minimal domain of Pbx proteins required for dimerization with Hox proteins includes the HD and a 17-residue predicted α-helix just C terminal to the HD (10, 23). This minimal domain is highly conserved among Pbx family members.

Although Pbx proteins and E2a-Pbx1 behave alike in their ability to dimerize with Hox proteins, we demonstrated that the major Pbx partner in nuclear extracts (designated NFPP; nuclear factor Pbx partner) fails to dimerize detectably with E2a-Pbx1 and recognizes the sequence TGATTGAC (designated PCE: Pbx cooperativity element) in conjunction with endogenous Pbx proteins. This element was not bound by Pbx–Hox complexes (31). Because Pbx1 binds the 5′ TGAT in Pbx–Hox motifs, we proposed that NFPP bound the 3′ TGACAG half-site, that NFPP was distinct from class I Hox proteins, and that the oncogenic mechanism of E2a-Pbx1 might require abrogation of heterodimerization with NFPP to selectively target Pbx–Hox motifs. Here we demonstrate that both Meis1 and pKnox1, which prototype two other broadly expressed families of TALE HD proteins, behave biochemically in a manner similar to NFPP. Because MEIS1 is transcriptionally activated with HOXA7 or HOXA9 in myeloid leukemia of BXH-2 mice, MEIS1 and HOXA7/9 have been suggested to function as cooperating oncogenes, potentially

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Abbreviations: HD, homeodomain; TALE, three amino acid loop extension; NFPP, nuclear factor Pbx partner; PCE, Pbx cooperativity element; HA, hemagglutinin; EMSA, electrophoretic mobility shift assay; PCM, Pbx1 cooperativity motif.
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through heterodimer formation (32, 33). Dimerization of Meis1 and Pbx1 suggests that overexpression of Meis1 in myeloid leukemia could also alter the targeting of Pbx proteins from promoters containing Pbx–Hox motifs to those containing Pbx–Meis motifs, thus interfering with differentiation.

MATERIALS AND METHODS

Identification of Meis1c. A Meis1 cDNA predicted to contain nucleotides 422–1,654 was amplified by PCR by using day 11 embryonic mouse cDNA, forward primer GAAGTAGGAAGGGAGCCAGAGAG and reverse primer CTGGCATACTTTGCAGCCTTCC and cloned in pGEM3zf-. The PCR-amplified sequence varied from that of the reported Meis1 sequence by excision of nucleotides encoding Val-162 to Gln-210 of Meis1 (Fig. 1*A*) and was designated Meis1c. Sequences missing in Meis1c could result from alternative mRNA splicing in day 11 embryonic mice. Both the 5′ (AAG-GUA) and 3′ (CAG-C) junctions flanking the excised region in Meis1C are reasonable matches for the consensus 5′ [(C/A)AG-GU] and 3′ [(C/U)AG-G/A] splice donor and acceptor sites. Full-length cDNAs for Meis1 and TGIF were generous gifts of Takuro Nakamura (PRESTO JST, The Cancer Institute, Tokyo, Japan) and Roger Clerc (Roche Research Labs, Basil, Switzerland), respectively.

Epitope Tagging of Meis1c. Hemagglutinin antigen (HA)-tagged Meis1c was made by ligation of DNA encoding YPYDVPDYA into a *Mlu*I site introduced in frame at codons 2 and 3 of Meis1c. Thus HA-tagged Meis1c protein begins MTRYPYDVPDYATR, followed by codon 4. Supershift analysis was performed by using antibody 12CA5 (Boehringer Mannheim).

Mutagenesis. cDNAs were mutated by using the Muta-gene phagemid *in vitro* mutagenesis kit (Bio-Rad) according to the manufacturer's protocol. All mutations were verified by sequence analysis. Deletions were created by excision of sequences between two introduced Mlu1 sites, resulting in derivatives that contain the TR dipeptide at each excision junction. N-terminal deletion mutants of Pbx1 were made by PCR.

Electrophoretic Mobility Shift Assays (EMSAs). EMSA was performed as described (31), with the exception that formation of Meis1c–Pbx1 complexes was performed by using 20,000–40,000 cpm of probe, 3–6 μl of *in vitro*-translated proteins, and 0.2 μg of poly(dI:dC) in a buffer containing 10 mM Tris (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 3 mM $MgCl_2$, and 12% glycerol for 30 min at room temperature. The abundance of mutant and wild-type proteins was normalized by quantitation of [^{35}S]methionine-labeled proteins by using a PhosphorImager (Bio-Rad).

RESULTS

Pbx1, But Not Oncoprotein E2a-Pbx1, Cooperates Efficiently with Meis1c, Meis1, and pKnox1 in Binding TGAT-TGACAG. A search for widely expressed HD proteins that bound TGACAG half-sites suggested that three related TALE HD proteins, TGIF, Meis1, and pKnox1 (7, 33, 34), were candidates for NFPP, and all three proteins were tested for dimerization potential with Pbx1 and E2a-Pbx1 (Fig. 2). Although TGIF bound the PCE as a monomer, it failed to heterodimerize with Pbx1 (data not shown). In stark contrast, Meis1, Meis1c (a shorter Meis1 variant isolated by PCR, see *Materials and Methods* and Fig. 1*A*), and pKnox1 dimerized with Pbx1 on the PCE as efficiently as did Pbx1 plus HoxA5 on their optimal TGAT-TAAT motif (lanes 7, 8, 11, 12, 15, and 16 vs. 19). Surprisingly, unlike class I Hox proteins, Meis1, Meis1c, and pKnox1 dimerized 20- to 50-fold less efficiently with E2a-Pbx1 than with Pbx1 (lanes 9, 10, 13, 14, 17, and 18 vs. 20). Like Pbx1, Meis1c, Meis1, and pKnox1 did not bind DNA detectably as monomers. Therefore, the DNA-binding and heterodimerization properties of Meis1c, Meis1, and pKnox1 were similar to those of endogenous NFPP.

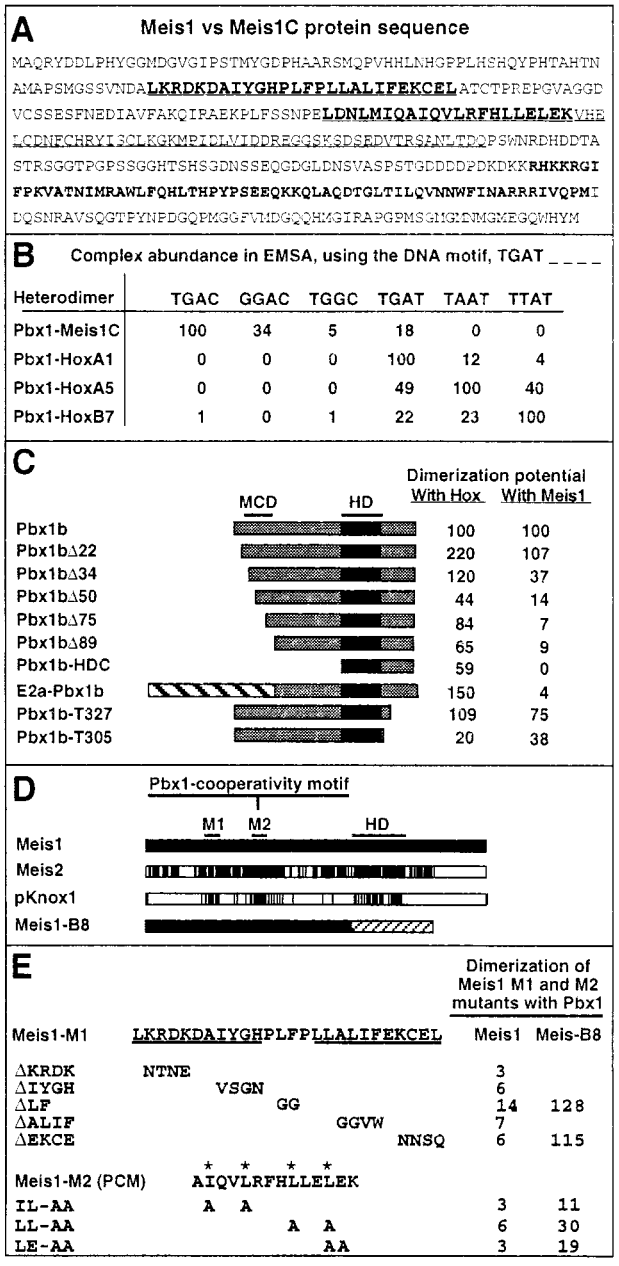

FIG. 1. Heterodimer specificity of wild-type and mutant Pbx1, Meis1, and Hox proteins. (*A*) Amino acid sequence differences between Meis1 and Meis1c. The underlined, unbolded sequence represents Meis1 residues absent from Meis1c. The bolded, underlined, central sequences represent M1 and M2, respectively. The bold C-terminal sequence represents the HD. (*B*) Quantitation of the relative DNA-binding specificities of Pbx1–Meis1 and Pbx1–Hox heterodimer complexes. The degree of binding to the highest affinity site in each case defined as 100. (*C*) Pbx1 and E2a-Pbx1 proteins utilized in this study and their relative abilities to bind DNA cooperatively with Hox proteins and Meis1. Wild-type Pbx1b binding is defined as 100%. (*D*) Sequence identity map between Meis1, Meis2, pKnox1, and the chimera MB8. (*E*) Site-directed mutant forms of Meis1 motifs M1 and M2, and their relative abilities to bind DNA cooperatively with Pbx1 when introduced within Meis1 and MB8.

Meis1 Exhibits a Half-Site Specificity Different from That of Hox Proteins, and Similar to That of NFPP. The DNA-binding specificity of Pbx1–Meis1c and Pbx1–Hox complexes were compared on Pbx1 motifs that bind optimally to Pbx–Hox complexes (TGAT-TGAT, TGAT-TAAT, and TGAT-TTAT)

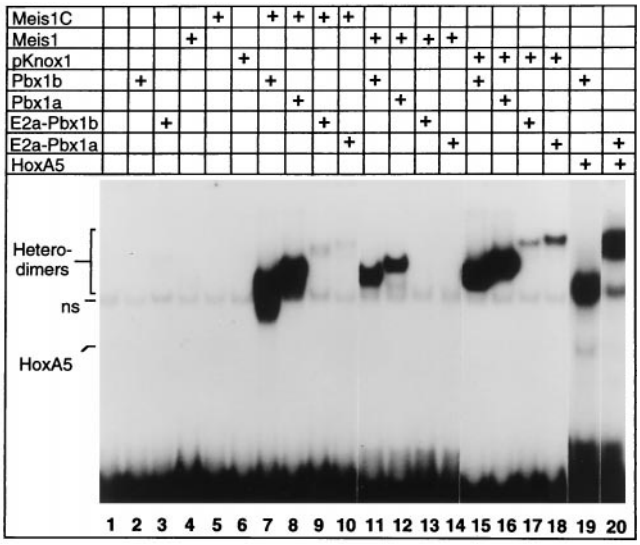

FIG. 2. Pbx1, but not oncoprotein E2a-Pbx1, cooperates efficiently with Meis1c, Meis1, and pKnox1 in binding TGAT-TGACAG. Lanes 1–6 contain reticulocyte lysate, Pbx1b, E2a-Pbx1b, Meis1, Meis1c, and pKnox1 alone, respectively. Complex formation between Pbx1 or E2a-Pbx1 and Meis1c (lanes 7–10, respectively), Meis1 (unspliced; lanes 11–14), or pKnox1 (lanes 15–18) on a TGATTGAC element (lanes 1–18) was compared with that of HoxB7 and Pbx1 or E2a-Pbx1 on a TGATTTAT element (lanes 19 and 20) by using EMSA. Equimolar amounts of Pbx1 and E2a-Pbx1 proteins were used. Additions to the gel-shift reactions are indicated above each lane by plus signs. Pbx1a and E2a-Pbx1a differ from Pbx1b and E2a-Pbx1b at their C termini because of alternative mRNA splicing.

or Pbx–NFPP complexes (TGAT-TGAC, TGAT-GGAC, TGAT-TGGC; Fig. 1*B*). The DNA-binding specificity of Pbx1–Meis1c heterodimers mirrored that of Pbx–NFPP in its strong preference for a 3′ TGAC half-site, in its ability to also bind TGAT, GGAC, or TGGC 3′ half-sites, and in its failure to bind two of the best Hox half-sites, TAAT and TTAT (Fig. 1*B*). Thus, the DNA-binding specificity of Pbx1–Meis1c heterodimers is very different from that of Pbx–Hox heterodimers, overlapping only on TGAT-TGAT motifs.

Within the Pbx1-Meis1-TGATTGAC Complex, Both Pbx1 and Meis1c Bind DNA, and Meis1c Is Positioned 3′ to Pbx1. To demonstrate that both Pbx1 and Meis1 bind DNA within the Pbx1–Meis1–DNA complex, DNA-binding mutants of each protein were made and tested for their ability to form heterodimers. In each case, mutants were created by converting Asp-51 of the HD to serine (N51S), as this mutation destroys monomeric DNA binding for both Hox and Pbx proteins (21). Inclusion of N51S versions of Meis1c or Pbx1 in binding reactions abolished complex formation (Fig. 3*A*, lanes 2–4). Polyclonal anti-Pbx1 serum completely disrupted the Pbx1–Meis1 complex (lane 5). Pbx1 and Meis1c were also fused to EE or HA epitopes, respectively, and mAbs to the EE epitope supershifted exclusively complexes containing EE-Pbx1 (lane 9 vs. 6), whereas mAbs to HA supershifted some and disrupted most of the HA–Meis1c–Pbx1 complex (lane 11 vs. 7).

The fact that Pbx1 binds TGAT suggests that Pbx1 binds the 5′ TGAT half-site in the TGAT-TGAC Pbx–Meis1c recognition element. Likewise, the fact that the third "recognition" helix of the Meis1 HD is nearly identical to that of TGIF, which binds a TGAC core, suggests that Meis1c binds the 3′ TGAC core. If Meis1, like Hox proteins, occupies the 3′ half-site, then Meis1 might also contain a Pbx1 interaction motif N-terminal to its HD, similar to the pentapeptide motif of Hox proteins, whose elimination strongly suppresses cooperative DNA binding with Pbx1 (pentapeptide point mutant HoxB8WF in Fig. 3*B*, lane 5 vs. 3). We reasoned that both the hypothesis that

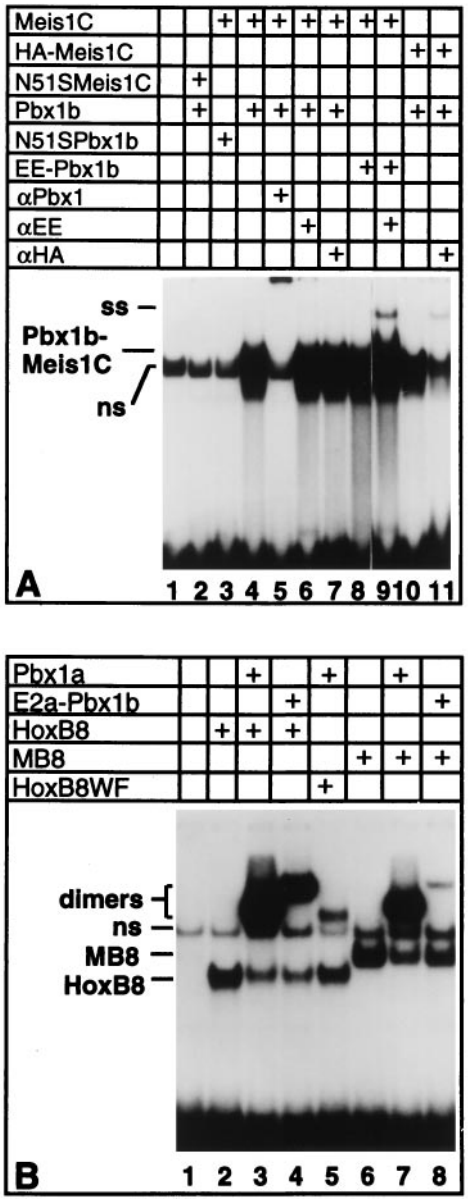

FIG. 3. Pbx1 and Meis1c are positioned on DNA in the same order as Pbx and Hox proteins. (*A*) DNA-binding mutants, designated N51S Meis and N51S Pbx1, and epitope-tagged versions of Pbx1 and Meis1 were tested for complex formation potential and their ability to be supershifted or disrupted by anti-epitope antibodies. Supershift analysis was performed by addition of 200 ng of αEE or αHA mAbs. (*B*) Conversion of the DNA-binding specificity of Meis1 into that of HoxB8 alters its dimerization specificity with Pbx1 at the 3′ half-site. Equimolar amounts of wild-type and mutant proteins were used. Additions to the gel-shift reactions are indicated above each lane by plus signs.

Meis1 contained an N-terminal Pbx1 interaction motif, as well as that Meis1 bound at the 3′ half-site could be tested by determining whether a chimeric protein containing Meis1 sequences N terminal to its HD tethered to the HD and C terminus of HoxB8 could dimerize with Pbx1 on TGAT-TTAT, a consensus Pbx–HoxB8 binding site. If this chimera (designated MB8) dimerized with Pbx1 on this element, it would suggest that Meis1 is positioned 3′ to Pbx1 and contains an N-terminal Pbx1 interaction domain. Indeed, the chimera efficiently dimerized with Pbx1 on TGAT-TTAT (Fig. 3*B*, lane 7). Like WT Meis1, MB8 could not dimerize effectively with E2a-Pbx1 (lane 8 vs. 4).

The potential transcriptional properties of Pbx1–Meis1 dimers were examined by cotransfecting HeLa cells with

expression vectors encoding Pbx1 and Meis1 together with a chloramphenicol acetyltransferase (CAT) reporter gene driven by four TGAT-TGACAG motifs. Coexpression of Meis1 with either Pbx1 or E2a-Pbx1 failed to activate reporter gene transcription whereas E2a-Pbx1 activated transcription of a CAT reporter driven by four TGAT-TGAT motifs greated than 15-fold (data not shown).

A 3,4-Hydrophobic Heptad Repeat Within a Putative Alpha Helix of Meis1 Is Essential for Dimer Formation with Pbx1. To localize Meis1 sequences required for dimerization with Pbx1, systematic substitution mutagenesis within elements conserved in both Meis1 and pKnox1 was performed (Figs. 1 *D* and *E* and 4). Meis1 and pKnox1 contain 30% sequence identity, which is concentrated in two N-terminal motifs (designated M1 and M2 in Fig. 1*D*) and in the HD. Failure of Meis1c to bind DNA as a monomer necessitated introduction of all mutants into MB8 so that mutants in Meis1 sequences that abrogate cooperativity without affecting monomeric DNA binding could be identified. M1 (L71–L96, Fig. 1*E*) contains a predicted helix-turn-helix structure. Although all mutations within this motif disrupted dimerization of Meis1c with Pbx1 (Fig. 4*A*, lanes 1–8), none significantly disrupted dimerization of MB8 with Pbx1 (lanes 9–11), suggesting this motif either does not contact Pbx1 directly or is important for interaction with Pbx1 only in the context of native Meis1. M2 (L143-K161, Fig. 1*E*) has a predicted alpha helical structure containing a 3,4-hydrophobic heptad repeat of leucine and isoleucine residues (3,4-repeat underlined: LMIQA**I**QV**L**RF-H**L**LE**L**EK), and double alanine mutations at either the first two or last two hydrophobic positions of this repeat disrupted complex formation of both Meis1 and MB8 with Pbx1 without altering monomeric DNA binding by MB8 (Fig. 4*A*, lanes 13–21). Therefore, M2 behaves like a Pbx1 cooperativity motif (PCM). Such 3,4-hydrophobic heptad repeats are also required for interaction of the Pbx1 relatives, the yeast a1 and α2 transcription factors (35).

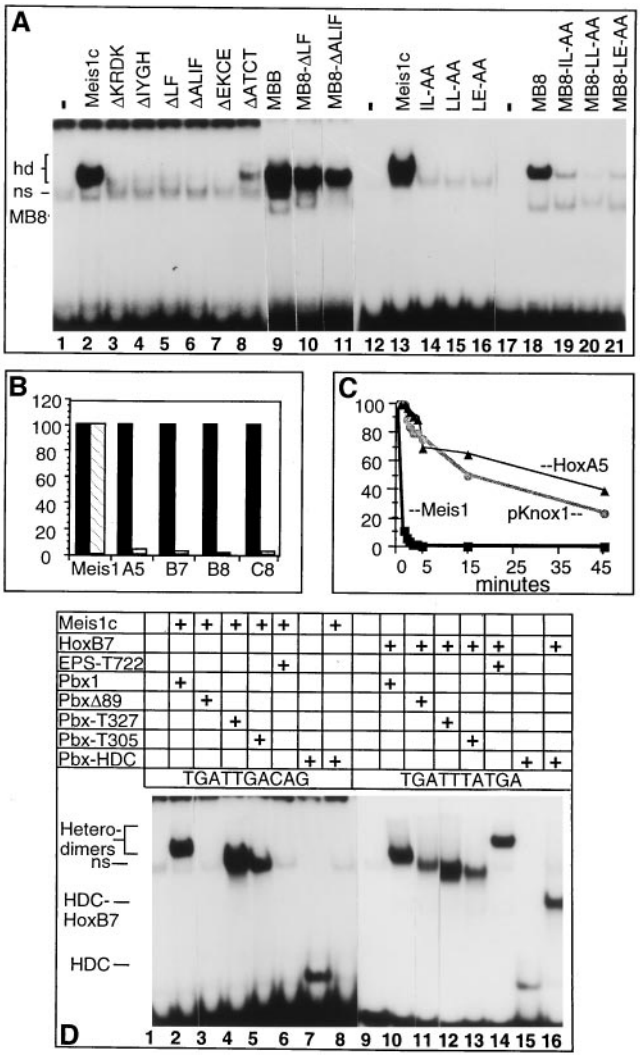

FIG. 4. Comparative properties of Pbx1–Meis1 and Pbx1–Hox complexes. (*A*) Wild-type and mutant forms of Meis1c and MB8 were tested for their ability to form cooperative heterodimers with Pbx1 on a TGATTGAC probe by using EMSA. Proteins added to the binding reactions are indicated at the top of each lane and correspond to the mutants depicted in Fig. 1*E*. (*B*) Disruption of Pbx1–Hox and Pbx1–Meis1c complexes by inclusion of a synthetic peptide containing the pentapeptide motif of HoxA5 was quantitated by EMSA. Filled and open bars represent complex abundance in reactions without and with peptide, respectively. (*C*) Off-rate analysis was performed by using cold double-stranded competitors (TGATTGAC for Pbx1–Meis1 and pKnox1 and TGATTTAT for Pbx1–HoxB7). Squares, circles, and triangles represent dissociation rates of Pbx1–Meis1, Pbx1–pKnox1, and Pbx1–HoxA5, respectively. (*D*) Complex formation by wild-type and mutant forms of Pbx1 with Meis1 and HoxB7 on TGATTGAC (lanes 1–8) or TGATTTAT elements (lanes 9–16), respectively, by using EMSA. Additions to the gel-shift reactions are indicated above each lane by plus signs and described in the text.

Identification of M2 as a PCM suggested that Meis1 uses an interaction mechanism unlike that of the tryptophan-containing Hox pentapeptide, which we have suggested binds the Pbx1 HD itself (23). Although Meis1 and pKnox1 do not contain a conserved tryptophan motif, a single tryptophan residue (W164) is positioned N terminal to the Meis1 HD. This residue was not essential for cooperativity, as its mutation to glycine diminished cooperativity less than 25% (data not shown). In addition, a synthetic peptide containing the pentapeptide motif of HoxA5 disrupted Pbx1 complexes containing HoxA5, HoxB7, HoxB8, or HoxC8 but had no effect on that containing Meis1 (Fig. 4*B*). Failure of the peptide to disrupt Pbx–Meis1 complexes was not caused by their intrinsic higher stability; in fact, Pbx complexes containing either Meis1 or Meis1c had off-rates of less than 1 min whereas that of a Pbx–HoxA5 heterodimer was approximately 30 min and that of a Pbx1–pKnox1 heterodimer was 15 min (Fig. 4*C*). These experiments indicated that Meis1 contains a unique interaction motif that does not bind the same surface of Pbx1 that is bound by the tryptophan-containing motif of Hox proteins.

Fusion with E2a Eliminates Pbx1 Sequences Required for Cooperative DNA Binding with Meis1. Fusion with E2a eliminates the first 89 residues of Pbx1 in oncoprotein E2a-Pbx1. Because E2a-Pbx1 exhibits a 20- to 50-fold reduction in cooperative binding with Meis1 and pKnox1 proteins, we hypothesized that the Meis1 cooperativity domain (MCD) in Pbx1 was either deleted or inaccessible in E2a-Pbx1. To determine whether the MCD resided within the first 89 residues of Pbx1, a mutant of Pbx1 lacking the first 89 residues was tested for dimerization with Meis1 and Hox proteins. Although Pbx1Δ89 dimerized with HoxB7 (Fig. 4*D*, lane 11), its ability to dimerize with Meis1c was barely detected (lane 3). The Pbx1 MCD was further defined by using mutations eliminating the N-terminal 22, 34, 50, and 75 residues of Pbx1 (Fig. 1*C*). Although cooperative binding of PbxΔ22 with Meis1c was unchanged, that of PbxΔ34, PbxΔ50, and PbxΔ75 were reduced 3-, 8-, and 14-fold, respectively (Fig. 1*C*), indicating that the first 89 residues of Pbx1 contains the majority of the MCD. Within this domain of Pbx1, residues 44–54 encode a 3–4 isoleucine heptad repeat (**I**GD**I**LQQ**I**MT**I**) conserved in all Pbx family members that could interact in a coiled-coil manner with the cognate 3–4 repeat of the Meis1 PCM. The fact that both PbxΔ89 and E2a-Pbx1 retain a small yet consistent ability to cooperate Meis1c and pKnox1 suggests that a portion of the MCD extends C terminal to Pbx residue 89.

A sequence just C terminal to the Pbx1 HD (K297–G320) that is predicted to fold as an alpha helix promotes cooperative DNA binding by class I Hox proteins and Pbx1 (23). As with Hox partners, partial truncation of this helix reduced the efficiency of heterodimerization by Meis1 and Pbx1 (Fig. 4*D*, lanes 4, 5, 12, and 13). Unlike Hox proteins, however, the Pbx1 HD and C-terminal helix (HDC) were not sufficient determinants to form stable heterodimers with Meis1c (Fig. 4*D*, lanes 7, 8, 15, and 16), reitterating a dimerization model in which the Pbx1 interaction motif of Meis1, unlike the pentapeptide motif of Hox proteins, contacts Pbx1 sequences N terminal to the HD (model in Fig. 5).

DISCUSSION

HD proteins represent a large family of developmental regulators that, when misexpressed, represent an equally large pool of potential oncoproteins. Hox HD oncoproteins, such as HoxB8, dimerize on DNA with Pbx1, whose transcriptionally activated form, E2a-Pbx1, is also an oncoprotein. Thus, both members of this transcription factor complex can cause oncogenesis, a paradigm also observed for the Fos and Jun constituents of AP1 (36). As in the case of Fos and Jun, the mechanism of leukemogenesis by certain class I Hox proteins and E2a-Pbx1 may proceed through transcriptional targeting of a common subset of genes, Hox proteins acting in concert with endogenous Pbx proteins, and E2a-Pbx1 acting in concert with endogenous Hox proteins. The implication that Meis1 and HoxA7 or HoxA9 are involved in myeloid transformation

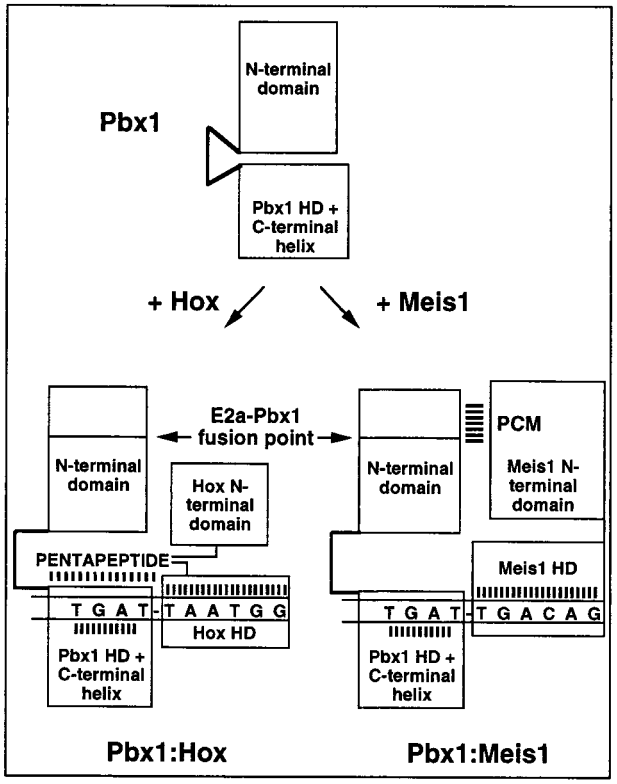

FIG. 5. Model for differential DNA binding by Pbx1–Hox and Pbx1–Meis1 heterodimers. Monomeric Pbx1 contains an N-terminal domain preventing DNA binding by the HD. The monomeric configuration is indicated by the closed-hinge conformation. Dimerization with Hox proteins converts Pbx1 to the open DNA-binding conformation, requiring contact of the Hox pentapeptide with the Pbx1 HD/C-terminal helix domain. Dimerization with Meis1 is proposed to convert Pbx1 to the open DNA-binding conformation through contacts between Pbx1 sequences removed by translocation with E2a and Meis1 sequences that include the M2 Pbx1 cooperativity motif.

presents another instance of potential cooperativity of oncoproteins (32); however, although the simplest hypothesis is that Meis1 and HoxA7 or HoxA9 themselves heterodimerize, demonstration that Meis1 heterodimerizes with Pbx1 includes Pbx-regulated genes as potential targets for normal Meis proteins in development as well as for overexpressed Meis proteins in leukemia. Failure of E2a-Pbx1 to dimerize strongly with Meis1 indicates that E2a-Pbx1 does not target genes regulated by Pbx–Meis1 dimers but rather those regulated by Pbx–Hox dimers, and suggests that part of the oncogenic requirements of E2a-Pbx1 may be abrogation of interaction with Meis proteins.

The architecture of the Pbx1–Meis1–DNA complex was both similar and different to that of Pbx–Hox–DNA complexes. Based on the binding specificity of MB8 to a 3′ TTAT half-site, Meis1, like Hox proteins, is suggested to bind 3′ of the TGAT Pbx1 half-site. In this conformation, both Meis1 and Hox proteins contact Pbx1 by using sequences N terminal to their HDs, class I Hox and Engrailed proteins utilizing an unstructured tryptophan-containing sequence that binds the Pbx1 HD/C-terminal helix and Meis1 utilizing an unrelated structure containing a 3,4-hydrophobic heptad repeat. It remains to be demonstrated whether this repeat motif contacts the 3,4-isoleucine heptad repeat (residues 44–54) in Pbx1. The 64 residues between this leucine repeat and the HD in Meis1c contain numerous glycine residues, perhaps endowing Meis1 with sufficient flexibility to permit interaction of the leucine repeat with the Pbx1 N-terminal domain. The Meis1 M2 motif is highly conserved in Meis2 and Meis3, suggesting that all member of the Meis1 gene family may use this same protein surface to interact with Pbx1.

The Meis1 cooperativity domain of Pbx1 lay principally within a region of high sequence identity with Pbx2 and Pbx3 (residues 37–89 of Pbx1). Therefore, all members of the Pbx and Meis protein families may form heterodimers. Differential interactions based on divergent sequences between family members could contribute to differential DNA-binding stability or sequence specificity and should certainly be investigated.

The DNA-binding specificity and stability properties of Pbx–Meis complexes were very different from those of Pbx–Hox complexes. Although both Pbx1–Meis1 and Pbx–NFPP bind TGAT-TGAC, they do not bind either of the predominant Pbx–Hox motifs–TGAT-TAAT or TGAT-TTAT. TGAT-TGAT was recognized by both Pbx–Meis and Pbx–Hox complexes. Therefore, Pbx–Meis and Pbx–Hox complexes could regulate both distinct and overlapping subsets of genes. If Pbx–Meis complexes are as unstable *in vivo* as they are when formed with proteins synthesized *in vitro*, their instability could permit a rapid reconfiguration of gene transcription in response to up-regulation of Hox proteins, such as by retinoic acid (37). Alternatively, TGATTGACAG may not represent the optimal Pbx1–Meis1 site. DNA site selection experiments using recombinant Pbx and Meis proteins should resolve this issue, as well as that of possible differences in specificity of heterodimers containing different members of the Pbx and Meis protein families.

Because Pbx1, Meis1, and pKnox1 belong to the divergent TALE family of HD proteins and overlap substantially in their expression patterns, their ability to bind DNA as heterodimers provides yet another level of combinatorial complexity to models of normal gene regulation by HD proteins in development, and abnormal gene transcription by oncogenic HD proteins in leukemia. Differential splicing of Meis1—potentially the mechanism accounting for the production of Meis1c, which lacks residues just N terminal to the M2 Pbx interaction motif—could also produce forms of Meis1 capable of differential interaction with adjacent transcription factor complexes and thus of differential gene targeting during embryogenesis. In BXH-2 mouse leukemias, coactivation of

Meis1 with HoxA7 or HoxA9 could produce at least four fundamental alterations in the abundance of factors controlling myeloid differentiation: (*i*) up-regulation of Meis1 could shift the distribution of Pbx proteins from promoters containing Pbx–Hox motifs to those containing Pbx–Meis motifs, (*ii*) up-regulation of HoxA7 or HoxA9 could titrate out other endogenous Hox partners of Pbx proteins, forming predominantly Pbx–HoxA7 or Pbx–HoxA9 heterodimers, (*iii*) HoxA7 or HoxA9 could replace other endogenous Hox proteins on promoters that bind Hox monomers, and (*iv*) heterodimers consisting of Meis–HoxA7 or Meis–HoxA9 could target DNA elements, if, in fact, Meis1–Hox complexes form. Other forms of developmental abnormalities, such as Down syndrome, Knobloch syndrome, and holoprosencephaly have also been linked to the pKnox1 locus at 21q22.3 (34). If any of these phenotypes are indeed mediated by mutation of pKnox1, then Pbx proteins could also contribute to the mutant phenotype through their role as HD partners.

The function of Pbx–Meis and Pbx–pKnox1 heterodimers remains unclear, and their target genes unidentified. Of the known target genes regulated by Pbx proteins, several contain elements that bind Pbx complexes whose heterodimer partner could be Meis or pKnox1 proteins. These included repeats 2 (TGATTGAAG) and 3 (TGATGGATGG) of the HoxB1 promoter (38), the TGATGGACAG motif of the bovine cytochrome P450 17 gene (39), and the TTGATTGATT motif found in the somatostatin promoter (40). In all cases, using *t*(1;19) cell nuclear extracts Pbx-containing complexes can form on these elements whereas those containing E2a-Pbx1 cannot (31), suggesting that the nuclear partner(s) behave biochemically similar to Meis1 and pKnox1. In the case of the somatostatin promoter, the unidentified complex in pancreatic cells that binds TTGATTGATT augments transcription activated by the binding of the STF-1 HD protein to an adjacent motif but itself possesses no intrinsic ability to activate transcription (40). In light of the failure of Pbx1 plus Meis1 to mediate transcriptional activation in reporter assays, its is possible that Pbx–Meis and Pbx–pKnox complexes cooperate with other cell type-specific activators or repressors to differentially regulate transcription. A clearer understanding of the function of Pbx1–Meis1 and Pbx1–pKnox1 heterodimers, as well as of Pbx1–Hox heterodimers, awaits further identification of their biochemical functions as well as their genetic targets.

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