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AbdB-Like Hox Proteins Stabilize DNA Binding by the Meis1 Homeodomain Proteins

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Recent studies show that Hox homeodomain proteins from paralog groups 1 to 10 gain DNA binding specificity and affinity through cooperative binding with the divergent homeodomain protein Pbx1. However, the AbdB-like Hox proteins from paralogs 11, 12, and 13 do not interact with Pbx1a, raising the possibility of different protein partners. The *Meis1* homeobox gene has 44% identity to *Pbx* within the homeodomain and was identified as a common site of viral integration in myeloid leukemias arising in BXH-2 mice. These integrations result in constitutive activation of *Meis1*. Furthermore, the *Hoxa-9* gene is frequently activated by viral integration in the same BXH-2 leukemias, suggesting a biological synergy between these two distinct classes of homeodomain proteins in causing malignant transformation. We now show that the Hoxa-9 protein physically interacts with Meis1 proteins by forming heterodimeric binding complexes on a DNA target containing a Meis1 site (TGACAG) and an AbdB-like Hox site (TTTTACGAC). Hox proteins from the other AbdB-like paralogs, Hoxa-10, Hoxa-11, Hoxd-12, and Hoxb-13, also form DNA binding complexes with Meis1b, while Hox proteins from other paralogs do not appear to interact with Meis1 proteins. DNA binding complexes formed by Meis1 with Hox proteins dissociate much more slowly than DNA complexes with Meis1 alone, suggesting that Hox proteins stabilize the interactions of Meis1 proteins with their DNA targets.

Although the vertebrate homeobox genes play a critical role in embryonic development (30), there has been little progress in understanding how homeodomain proteins function. Despite the assumption that they are transcription factors (24), few natural regulatory targets have been identified (13, 16), and little data exist showing an in vivo transcriptional role for the mammalian homeodomain proteins. Vertebrate homeobox genes can be divided into the *Hox* family of clustered genes which contain homeobox sequences related to the *Drosophila HOM-C* homeobox genes and the numerous subfamilies of non-*Hox* genes which possess more distantly related homeobox sequences. The 39 *Hox* genes are arranged in four separate loci (A, B, C, and D), and the genes in different clusters can be aligned on the basis of homology within the homeobox to form so-called paralog groups, which have limited homology with the *Drosophila HOM-C* genes as well (1). A significant advance in understanding how Hox proteins function has been the demonstration that they form cooperative DNA binding complexes with the non-Hox homeodomain protein Pbx1a (10, 27, 42). We have recently reported that Hox proteins from paralog groups 1 through 10 gain DNA binding specificity through cooperative binding with Pbx1a, but proteins from the remaining three paralog groups, 11, 12, and 13, do not appear to interact with Pbx1a (8, 47). These findings suggest that the Hox proteins from paralog groups 11 through 13 might cooperatively bind to DNA with other non-Hox homeodomain protein partners.

In recent studies, we demonstrated that a novel homeobox gene, *Meis1*, is a common site of viral integration in myeloid leukemias occurring in BXH-2 mice (33). Two forms of the *Meis1* transcript, which differ in the amino acid sequence C-terminal to the homeodomain, were identified in all tissues examined (33). The homeodomain of Meis1 has homology with that of Pbx1, and both proteins contain a 3-amino-acid insertion which is a feature of the TALE class of homeobox genes (2). With the exception of the recently described Meis-related genes (34, 48), the N-terminal and C-terminal flanking regions of Meis1 have no homology to known homeodomain proteins. A biological synergy between Hox proteins and Meis1 proteins in leukemic transformation is suggested by the observation that in BXH-2 mice with leukemia, the malignant cells with insertional activation of *Meis1* frequently show retroviral activation of either the *Hoxa-9* or *Hoxa-7* locus (36).

The current study investigated the interactions between the Hox and Meis1 homeodomain proteins by use of DNA site selection, gel mobility shift assays, and immunoprecipitation experiments. We demonstrate that the AbdB-like subset of the Hox proteins (groups 9 to 13), including Hoxa-9, form DNA binding complexes with Meis1 proteins. Interactions of the Hox proteins with Meis1 do not require the conserved tryptophan which mediates interactions with the Pbx protein (8, 26, 37, 42, 45, 47) but rather appear to involve the N-terminal region of the Hox protein. In addition, Hox proteins appear to greatly stabilize Meis1-DNA interactions. Thus, Meis1 represents a second DNA binding partner for Hox proteins, interacting with a different, albeit overlapping, subset of Hox proteins than Pbx1a and interacting with different motifs on the Hox protein.

MATERIALS AND METHODS

Protein expression. cDNAs encoding representative full-length Hox proteins from paralog groups 4 and 6 through 13 as well as Meis1a and Meis1b were subcloned into either an sp65 vector containing an SP6 promoter (Promega, Madison, Wis.) engineered to express proteins containing an N-terminal FLAG epitope sequence (MDYKDDDDK; Meis1a and Meis1b and Hoxb-7 and Hoxa-10) or into a pET vector (Novagen, Madison, Wis.) containing a T7 promoter, which produces proteins with an N-terminal T7 epitope tag sequence (Meis1a, Meis1b, Hoxb-4, Hoxb-6, Hoxa-7, Hoxb-8, Hoxa-9, Hoxb-9, Hoxa-11, Hoxd-12,

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and Hoxb-13). cDNAs encoding the full-length Meis1a protein and the Meis1 homeodomain alone were also cloned into the pGEX vector (Piscataway, N.J.) in which they were expressed as glutathione *S*-transferase (GST) fusion proteins. The identity of each Hox protein was confirmed by Western blot (immunoblot) analysis of bacterially expressed proteins with specific polyclonal antisera, prepared under a cooperative NIH-funded program with the Berkeley Antibody Company, Inc. (Richmond, Calif.). For gel shift and DNA target selection assays, proteins containing the full-length homeodomain protein fused to the respective epitope tag were synthesized with the TNT-coupled in vitro transcription-translation system (Promega, Madison, Wis.) in parallel reactions in the presence and absence of [^{35}S]methionine. In each case, electrophoresis of the labeled proteins demonstrated synthesis of the appropriate full-length product (data not shown). By using autoradiography and densitometry of the ^{35}S-labeled proteins, and calculating the incorporation of labeled methionine of known specific activity into each protein, we estimated that the relative protein concentrations used were within a twofold range. Each of the epitope-tagged Meis1 and Hox proteins was shown to be functional in DNA site selection assays (see Results) and in previous studies (47).

Human *Hoxb-6*, *Hoxb-7*, and *Hoxa-10* cDNAs were cloned previously (25, 46). Full-length cDNAs for *Hoxa-7* and *Hoxd-12* were cloned from 12-day-old mouse embryo RNA by standard reverse transcription-PCR with primers from the published sequences (15, 19). The amplified *Hoxa-7* and *Hoxd-12* cDNAs were sequenced by conventional methods to confirm their identity and to ensure the fidelity of the PCRs. A full-length clone for human *Hoxb-13* was cloned from fetal fibroblast mRNA as described recently (49). The other full-length cDNA clones were as follows: human *Hoxb-4* (41), murine *Hoxb-8* (21), murine *Hoxa-9* (44), murine *Hoxb-9* (29), and murine *Hoxa-11* (14). The codon for the tryptophan residue located 6 amino acids N-terminal to the homeodomain of Hoxb-9 was changed to encode glutamine in the *Hoxb-9* cDNA with a Muta-gene M13 in vitro mutagenesis kit (Bio-Rad Laboratories, Richmond, Calif.). Plasmids encoding truncated Hoxa-9 and Meis1b proteins were generated by PCR and confirmed by DNA sequence analysis. The truncated proteins were expressed as T7 epitope fusion proteins by use of the pET system. Full-length clones for *Meis1a* and *Meis1b* have been described previously (33). The protein coding region of murine *Meis1a* was amplified with a 5′ oligonucleotide (5′-GATC GAATTCGATGGCGCAAAGGTACGAC-3′) and a 3′ oligonucleotide (5′-GA TCGAATTCGGTTACATGTAGTGCCACTG-3′) from cDNA clone SC-21 (33). The Meis1 homeodomain was amplified with a 5′ oligonucleotide (5′-GAT CGAATTCTTGGCTGACTGCTCGGTTG-3′) and a 3′ oligonucleotide (5′-G ATCGGATCCCCAGCACAGGTGACGATG-3′). Constructs were sequenced to ensure that no PCR mutations were introduced.

DNA site selection protocol. Site selection with the in vitro-synthesized Meis1 and Hox proteins was performed by the basic protocol described by Blackwell and Weintraub (3). The T7 epitope-tagged Hox fusion protein of interest and T7-tagged Meis1a or Meis1b were synthesized in vitro and incubated at 4°C for 30 min with a 59-mer containing a random 20-mer core flanked by arms which contained cloning sites (5′-GCTCGAATTCAAGCTTCTN$_{20}$CATGGATCCTG CAGAATTCAGT-3′). DNA site selection by Hoxa-10 with Meis1b was performed with FLAG-tagged proteins. Bound DNA was immunoprecipitated with an antiserum to the T7 or FLAG tag sequences fused to both the Hox and Meis1 proteins. Following extensive washing steps, the DNA was amplified by 15 to 20 cycles of PCR (94°C, 1 min; 54°C, 1 min; 72°C, 1 min) with primers designed against the flanking arms. After five cycles of selection, the amplified DNA was subcloned and sequenced by standard methods. Consensus sequences were determined by visual alignment of sequences from unique clones.

DNA site selection with GST fusion proteins was performed by use of a binding site selection technique (5). A double-stranded oligonucleotide (5′-AGACGGATCCATTGCAN$_{14}$CTGTAGGAATTCGGA-3′) which contained 14 random bases flanked by specific sequences was synthesized. This oligonucleotide was PCR amplified with two oligonucleotides (5′-AGACGGATCCATTG CA-3′ and 5′-TCCGAATTCCTACAG-3′) which are identical and complementary, respectively, to the nonrandom flanking regions. Binding assays were performed with the PCR-amplified selection oligonucleotide and each of the glutathione-Sepharose-bound pGEX-Meis1a and pGEX-Meis1 homeodomain proteins. The binding reaction mixture consisted of the selection oligonucleotide, protein-Sepharose mix, and binding buffer [10 mM Tris-Cl (pH 7.5), 50 mM NaCl, 0.5 mM dithiothreitol (DTT), 10% glycerol, 0.5% Nonidet P-40, 50 ng of poly(dI-dC) per ml, 5 mM $MgCl_2$] in a 20-μl volume. Binding was performed at room temperature for 30 min. The glutathione-Sepharose beads were washed with binding buffer. Water was added, and the beads were boiled for 5 min to release the bound oligonucleotide and then centrifuged. Aliquots of the boiled binding reaction mixture were subjected to PCR to amplify the bound DNA. This amplified DNA was then used in a fresh binding reaction mixture with fresh protein. Three cycles of selection were performed with both the pGEX-Meis1a and pGEX-Meis1 homeodomain. After the final round of selection, the selected binding sites were subcloned into BSII (Stratagene, La Jolla, Calif.) with *Eco*RI and *Bam*HI sites engineered into the selection oligonucleotide. The subcloned selected binding sites were then sequenced and analyzed for the presence of common sequences.

Electrophoretic mobility shift assays (EMSA). Complementary oligonucleotides (upper strands shown in parentheses) containing the site identified for Meis1 by site selection (in capital letters) (see Fig. 1) (5′-ccagatcTGACAGttgg gggacagatctcc-3′), an oligonucleotide containing the consensus inverse palindrome binding site (in capital letters) (5′-ccagatctgtTGACAGCTGTCAaca-3′), and two versions of the consensus binding site determined for Hoxa-9 with Meis, which contain either a TTAT or a TTAC core Hox recognition site (underlined) (see Fig. 3) (5′-ccagatcTGACAGTTTTACGACagatctcc-3′, and 5′-ccagatcTGA CAGTTTTATGACagatctcc-3′), were synthesized (Operon Technologies, Alameda, Calif.). The conditions used were similar to those described previously (8). Briefly, double-stranded, end-labeled DNA (50,000 cpm/binding reaction; 10 nM) was incubated with 2 μl of reticulocyte lysate reaction mixture containing the test Hox protein (1 nM) in the presence of either 2 μl of reticulocyte lysate reaction mixture containing Meis1 (1 nM) or 2 μl of the lysate control and 75 mM NaCl, 1 mM EDTA, 1 mM DTT, 10 mM Tris-HCl (pH 7.5), 6% glycerol, 2 μg of bovine serum albumin (BSA), and 16 ng of dI-dC plus 0.1 μg of single-stranded salmon sperm DNA as nonspecific competitors, in a final reaction volume of 15 μl. Experiments designed to detect DNA-protein complex formation were performed with a 30-min incubation at 4°C. Reaction mixtures were run on a 6% polyacrylamide gel to visualize complex formation by retardation of the ^{32}P-labeled target DNA. In some experiments, polyclonal antisera to the appropriate epitope tags were incubated with aliquots of the reaction mixture for an additional 30 min. The Hox protein was fused to one epitope tag, while the Meis1 molecule was fused to a different epitope tag, such that it was possible to use specific antisera to identify the presence of the Hox protein or the Meis1 protein in the complex by supershifting the retarded complex band. Gel electrophoresis was performed in 0.25× Tris-borate-EDTA buffer as described previously (45). For each gel shift reaction, a control containing the reticulocyte lysate and appropriate viral polymerase was used to detect possible DNA binding by endogenous lysate factors.

Calculation of complex half-lives. EMSA gels were autoradiographed for densitometric quantitation of complex bands with a MacIntosh 8500 power PC computer and the NIH Image software program. Each gel was autoradiographed for various times to ensure that the densities measured were within the linear range of the scanner and software program. A dissociation rate was calculated for each Hox-Meis1-DNA complex from the slope of the regression line generated by plotting the log of the complex band intensities versus time. For each dissociation experiment, the correlation coefficient for the line was >0.96. For each complex, the half-life was calculated with the equation $t_{1/2} = -\log(0.5)/K_d$.

Protein coimmunoprecipitation assays. The appropriate Hox and Meis1 proteins were synthesized as T7- or FLAG-tagged fusion proteins in the presence or absence of [^{35}S]methionine, as required, as described above. Proteins were incubated at 4°C in binding buffer (50 mM NaCl, 1 mM EDTA, 1 mM DTT, 10 mM Tris-HCl [pH 7.5], 6% glycerol), in the presence of either an oligonucleotide containing a Meis1-Hox binding site (TGACAGTTTTACGAC), a random oligonucleotide (TTGGAATTCGGTTGGTGCAATTCTCAATAGAG), or DNase 1 (20 U), for 60 min prior to the addition of anti-FLAG antiserum. Following an additional overnight incubation, protein G beads which had been preblocked in a solution of 1% BSA in binding buffer were added to bind antibodies and associated proteins. Following centrifugation and extensive washing (75 mM NaCl, 15 mM Tris-HCl [pH 7.5], 1% BSA, 0.15% Triton X-100), proteins were solubilized in Laemmli buffer and subjected to polyacrylamide gel electrophoresis.

RESULTS

Meis1 proteins bind to a TGACAG core recognition sequence. Two alternatively spliced forms of *Meis1* cDNAs were previously identified in murine tissues (33). The two cDNAs encode proteins, Meis1a and Meis1b, which are identical in their N-terminal flanking regions and homeodomains but different in their C-terminal regions, with the last 18 amino acids of Meis1a being replaced by a unique 93-amino-acid sequence in Meis1b. DNA site selection experiments were employed to identify putative DNA binding sites for the Meis1a and Meis1b proteins. When site selection experiments were performed with the Meis1b protein fused to a T7 N-terminal epitope tag, a consensus sequence, TGACAG, was detected, usually as multiple copies in an array of orientations and spacings, including a substantial number of inverted palindrome sequences (Fig. 1). Full-length Meis1a protein and the Meis1 homeodomain alone fused to GST yielded similar results (data not shown).

Meis1 proteins form EMSA complexes in the absence of Hox proteins. In previous studies, Pbx, the canonical member of the vertebrate TALE proteins, appeared to require a Hox partner to exhibit DNA binding by gel shift analysis (10, 45). EMSA analysis using reticulocyte lysate-synthesized Meis1 proteins and a target DNA containing the TGACAG consensus se-

Direct repeats:

1. TGACAGTTCTGACAG
2. TGACACCTCTGACAC
3. TGTCAGTTTATGACAG
4. TGACAAGGATGACAG
5. TGTCACAACTTGACAG
6. TGTCAGAGGAGTGACAG
7. TGACAGGTGACA
8. TGTCACGCAATGACAG
9. TCACAGTGACAG

Inverted repeats:

10. CTGTCTGGTCCTGACA
11. CTGTCACTAAGATGACA
12. CTGTCACGGTCTGAC
13. CTGTCCTATTCTGACA
14. ATGTCAAGGTAGTGACAG

Inverted palindrome:

15. TGACACCTGTCA
16. TGACAGCTGTCA
17. TGGCAGCTGTCA
18. TGACAGGTGTCA
19. TGACAGCTCCCA
20. TGACAGCTGTCA

CONSENSUS

T G A C A G

96 98 90 98 100 82

FIG. 1. DNA site selection with Meis1b protein. Numbers beneath consensus sequence indicate percentages of occurrence at that site.

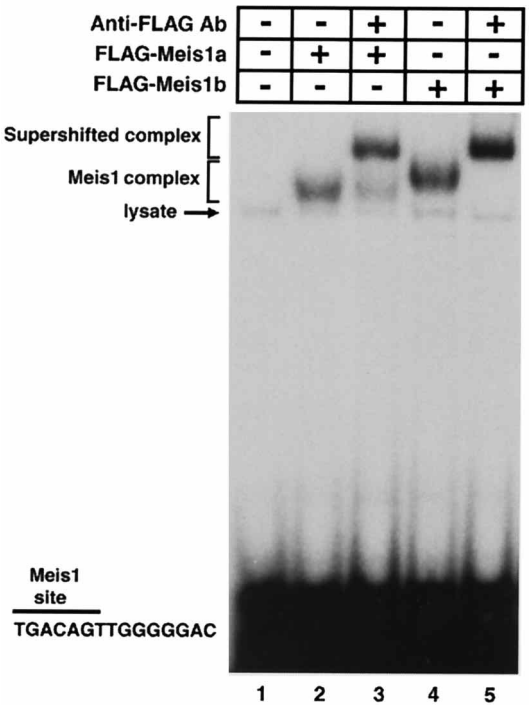

FIG. 2. Meis1 proteins form DNA binding complexes. EMSA analysis of Meis1a and Meis1b proteins, synthesized as FLAG epitope-tagged fusion proteins by in vitro transcription-translation, was performed with an oligonucleotide target containing the consensus Meis1 sequence (TGACAG) in Fig. 1. Tag-specific antiserum was used in supershift experiments to demonstrate the presence of Meis1 proteins in the gel shift bands (lanes 3 and 5). A variable-intensity band observed in the absence of exogenous Meis1 proteins represents nonspecific DNA binding by lysate proteins (lane 1). Ab, antibody.

quence was used to investigate whether the Meis1 proteins also require Hox partners for formation of gel shift complexes. Both Meis1a and Meis1b formed a DNA binding complex (Fig. 2, lanes 2 and 4), which could be supershifted with antisera to the FLAG epitope tag incorporated into the respective Meis fusion proteins (lanes 3 and 5).

Meis1 proteins cooperatively bind DNA with Hoxa-9. Since *Meis1* and *Hoxa-9* are frequently coactivated in leukemias arising in BXH-2 mice (36), we initially examined the possible interactions between these proteins by use of a DNA site selection protocol with N-terminal T7 epitope-tagged Meis1 and Hoxa-9 proteins in conjunction with antisera to the T7 epitope tag. With this approach, it should be possible to precipitate DNA molecules bound by either the Meis1 or Hox proteins alone as well as targets reflecting cooperative binding. We have recently identified the sequence (T)TTTA(T/C)GAC (Hox core recognition site underlined here and below) as a consensus DNA binding site for the AbdB-like Hox proteins, including those of the Hox-9 paralog (47). DNA site selection with Hoxa-9 in the presence of either Meis1a or Meis1b yielded a consensus sequence of TGACAGTTTTA(T/C)G(G/A), which contains a Meis1 binding site (TGACAG) adjacent to an AbdB-like Hox binding site (Fig. 3). In this experiment, there were no instances of individual Meis1 or Hox sites being selected, suggesting that cooperative binding by the Meis1 proteins with Hoxa-9 was favored over binding of the individual proteins.

To further investigate the DNA binding reactions of Meis1 proteins with Hox homeodomain proteins, FLAG epitope-tagged Meis1 proteins and T7 epitope-tagged Hoxa-9 were used in EMSA with an oligonucleotide probe containing a combined Meis1-Hox binding site (TGACAGTTTACGAC) (Fig. 4). Meis1a was able to weakly shift the target DNA in the absence of Hox protein (Fig. 4A, lanes 2 and 3). Hoxa-9 alone formed a strong, faster-migrating complex (Fig. 4A, lanes 4 and 5). When Hoxa-9 was incubated with Meis1a and the DNA target, a strong gel shift band which migrated slightly faster than the complex formed by Meis1 alone was observed (Fig. 4A, lane 6, upper band). Antisera to specific epitope tags on the Meis1 and Hoxa-9 proteins were used to demonstrate that this band consisted of a heterodimeric Meis1a–Hoxa-9 complex (compare lanes 7 and 8 with lane 6). An identical experiment using Meis1b demonstrated that this protein was also capable of forming heterodimeric DNA binding complexes with Hoxa-9 (Fig. 4B). Similar experiments using an oligonucleotide containing a Meis1 site adjacent to a TTAT core Hox protein binding site gave essentially identical results (data not shown).

Meis1 proteins form complexes with Hoxa-9 in the absence of DNA. To further demonstrate interactions between Hoxa-9 and Meis1 proteins, we performed coimmunoprecipitation experiments with antibodies specific for the FLAG epitope fused to Meis1 proteins to precipitate ^{35}S-labeled T7-tagged Hoxa-9 (Fig. 5). As anticipated from the EMSA results, immunoprecipitation of Meis1 in the presence of an oligonucleotide containing the Meis1-Hox consensus site (TGACAGTTTTACGAC) brought down the Hoxa-9 protein (lanes 3 and 7). Since this result might have been due to binding of the two proteins to a bridging oligonucleotide, experiments were performed in the presence of a random oligonucleotide as well as in the presence of DNase to remove residual DNA from the reaction. Strong Meis1–Hoxa-9 binding was also observed in both the

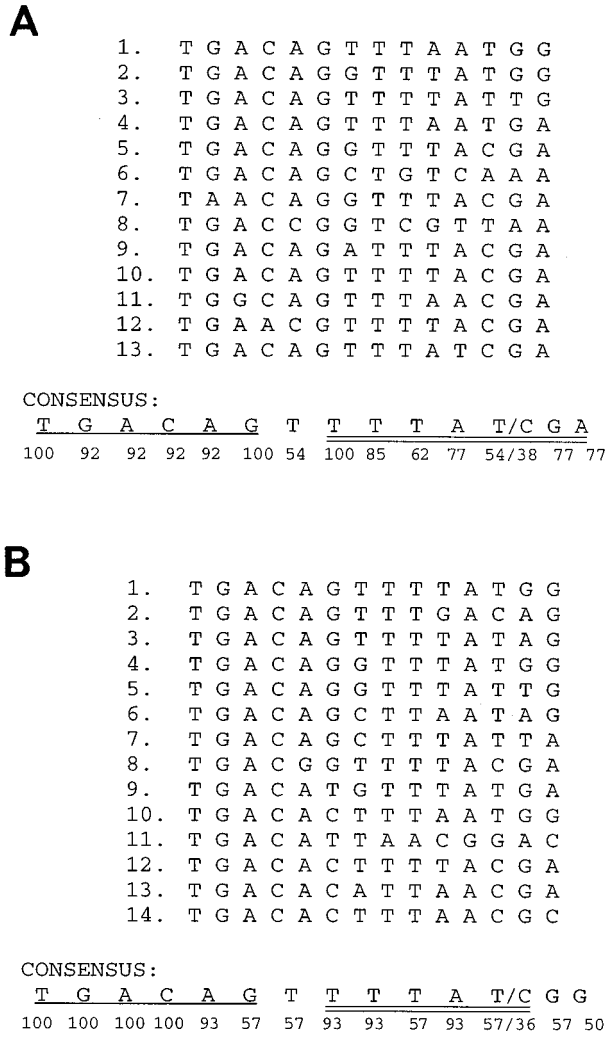

FIG. 3. DNA site selection of Meis1a (A) and Meis1b (B) proteins with Hoxa-9. Numbers beneath consensus sequences indicate percentages of occurrence at that site.

presence of a random oligonucleotide (lanes 4 and 8) and in the absence of DNA (lanes 5 and 9). In a control experiment to demonstrate the lack of nonspecific coimmunoprecipitation, a T7 epitope-tagged, 200-amino-acid N-terminal fragment of Hoxa-7 was not precipitated by antisera to the FLAG epitope in the presence of FLAG-tagged Meis1 proteins (data not shown).

Meis1b selectively interacts with the AbdB-like Hox homeodomain proteins. *Hox* genes are arrayed in mammalian chromosomal loci in a 3′ (*Hox-1*) to 5′ (*Hox-13*) orientation (1). While proteins from paralog groups 1 to 8 have a conserved YPWM motif, the proteins from the five 5′ genes (Hox-9 through Hox-13) lack this motif, exhibit equal homology between their homeodomains and the homeodomain of the *Drosophila* AbdB protein, and are collectively grouped as the AbdB-like Hox proteins (15). We previously reported that the AbdB-like Hox proteins can be functionally divided by the fact that Pbx1a cooperatively binds DNA targets with Hox-9 and Hox-10 proteins but does not appear to interact with the Hox proteins from paralog groups 11, 12, and 13 (8, 47). Since Hoxa-9 is a member of the AbdB-like subgroup of Hox homeodomain proteins, we examined whether Meis1 proteins could be DNA binding partners for representative members from the remaining AbdB-like paralogs, i.e., Hoxa-10, Hoxa-11, Hoxd-12, and Hoxb-13. In addition, we also examined the possible interactions of Meis1b with proteins from several of the other Hox paralogs.

Since all of the AbdB-like Hox proteins preferentially select DNA targets containing a TTAC core recognition sequence (47), we used a probe containing a Meis1 site and a Hoxa-9 site (TGACAGTTTACGAC) to test for cooperative DNA binding by the AbdB-like Hox proteins with Meis1b. As was the case for Hoxa-9, the analysis of these experiments was complicated by the fact that DNA gel shift complexes formed with Meis1 alone migrate with a mobility similar to that of the putative Meis1-Hox heterodimers. To gain evidence for heterodimer formation, antibodies to the distinct epitope tags fused to the Hox or Meis1b proteins were used to supershift the complex detected when the two proteins were incubated with DNA, using the strategy employed for Meis1b with Hoxa-9 described above. These experiments demonstrated that the three AbdB-like Hox proteins which were incapable of forming DNA binding complexes with Pbx1a (Hoxa-11, Hoxd-12, and Hoxb-13) were capable of forming complexes with Meis1b (Fig. 6A). The Hoxa-11, Hoxd-12, and Hoxb-13 proteins are all capable of forming very strong gel shift complexes with the TTAC-containing target in the absence of Meis1b (Fig. 6A, lanes 7, 11, and 15). For each of these proteins, the gel shift band formed from the addition of the Hox protein with Meis1b was much stronger than that observed for the DNA binding complex of Meis1b alone (Fig. 6A, compare lanes 8, 12, or 16 with lane 2). In each case, the band ascribed to the Meis1b-Hox complex could be supershifted with the appropriate antisera to the different epitope tags incorporated into the Meis1b and Hox proteins. Complexes formed between Hoxa-10 and this oligonucleotide target were much weaker (Fig. 6A, lanes 3 to 6), possibly reflecting the equal preference of this Hox protein for a TTAT site as well as a TTAC site (8, 11). However, an oligonucleotide containing a Meis1 site and a TTTTATGAC Hox binding site gave similar results (data not shown). The weak complex formed when Meis1b and Hoxa-10 were incubated with the TTAC-containing oligonucleotide (Fig. 6A, lane 4) was not supershifted by the antisera directed against the FLAG epitope tag incorporated into the Hoxa-10 protein (Fig. 6A, lane 6). It is possible that the epitope tag on the Hoxa-10 protein is not readily accessible to the antisera when this protein is complexed with Meis1b.

DNA site selection experiments were performed to further investigate the possible cooperative DNA binding of the AbdB-like Hox proteins with Meis1. When DNA site selection was performed with FLAG-tagged Hoxa-10 and T7-tagged Meis1b, the majority of selected clones contained Meis1 sites in various orientations, and only a single Meis1–Hoxa-10 site, identical to that used in Fig. 6, was detected (data not shown). In contrast to experiments with Hoxa-10, approximately 50% of the clones obtained in site selection experiments with Hoxa-11 or Hoxd-12 in the presence of Meis1b contained sequences with the combined Meis1 and AbdB-like Hox binding sites (TGACAGTTTTACGAC) initially observed for Hoxa-9 with Meis1 proteins (data not shown). Thus, the gel shift and site selection data suggest that the interactions of Hoxa-10 with Meis1 proteins are much weaker than that observed for the other AbdB-like Hox proteins. However, as described below, it was still possible to detect Hoxa-10 interactions with Meis1b by a dissociation assay.

Non-AbdB-like Hox proteins do not form DNA binding complexes with Meis1 proteins. In addition to *Hoxa-9*, *Hoxa-7* was

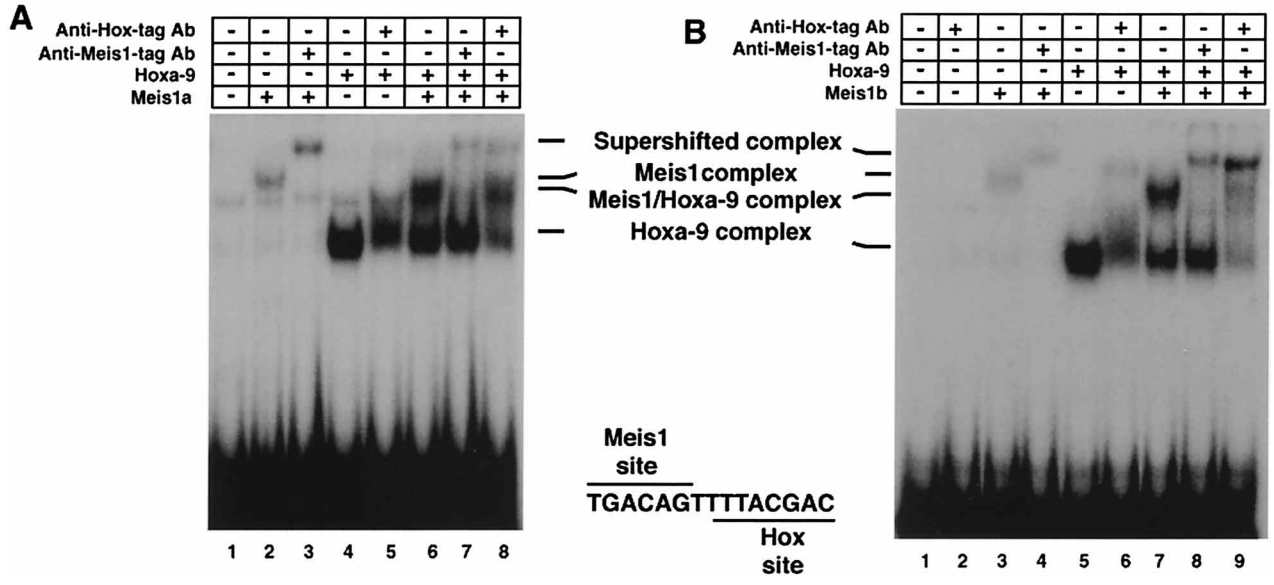

FIG. 4. Meis1 proteins form DNA binding complexes with Hoxa-9. EMSA was performed with in vitro-translated proteins and an oligonucleotide containing a consensus Meis1–Hoxa-9 binding site (TGACAGTTTTACGAC) identified by site selection as shown in Fig. 3. The Meis1 proteins were expressed as FLAG epitope-tagged fusion proteins, and Hoxa-9 was expressed as a T7 epitope-tagged fusion protein. Antisera to the respective epitope tags were used to supershift protein-DNA complexes. (A) Meis1a forms DNA binding complexes with Hoxa-9. Meis1a formed a weak complex with this DNA target (lane 2), while Hoxa-9 bound this target well in the absence of Meis1b (lane 4). A band which migrated slightly faster than the Meis1a complex was observed when Meis1a and Hoxa-9 were incubated together with the DNA target (lane 6, upper band). This band could be supershifted by antisera to either epitope tag, indicating the presence of Meis1a and Hoxa-9 in the complex (lanes 7 and 8). (B) Meis1b forms DNA binding complexes with Hoxa-9. Similar assays to those described for panel A were performed with Meis1b in place of Meis1a. Essentially identical results were obtained, demonstrating that Meis1b formed a heterodimeric complex with Hoxa-9 on this DNA target and that Meis1b was capable of DNA binding in the absence of Hox proteins. Ab, antibody.

also identified as being upregulated with *Meis1* in some cases of leukemia from BXH-2 mice (36). We were thus interested in examining the potential interactions of Hoxa-7 with Meis1 proteins as well as establishing the range of Hox paralog proteins which interact with Meis1 proteins. Since the Hox proteins in paralog groups 4 through 8 appear to bind well to a TTAT core recognition sequence in the presence of Pbx1a (8),

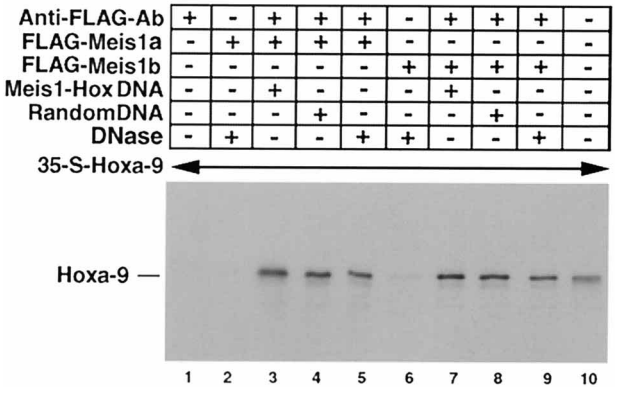

FIG. 5. Meis1–Hoxa-9 binding in the absence of DNA. Immunoprecipitation assays were performed with a FLAG epitope-tagged Meis1 protein in conjunction with a T7 epitope-tagged, ^{35}S-labeled Hoxa-9 protein to detect protein-protein interactions. As anticipated from gel shift experiments, antiserum to the FLAG epitope was capable of precipitating Hoxa-9 associated with the FLAG-tagged Meis1 proteins in the presence of an oligonucleotide containing a Meis1-Hox binding site (TGACAGTTTTACGAC) (lanes 3 and 7). Specific protein-protein interactions were demonstrated by coprecipitation performed in the presence of a random oligonucleotide (lanes 4 and 8) as well as in the presence of DNase to ensure the absence of contaminating DNA (lanes 5 and 9). Lane 10 contains reticulocyte lysate-synthesized Hoxa-9 (1/20 of that used for immunoprecipitation) as a migration standard. Ab, antibody.

we initially used a probe containing a Meis1 site and a Hox site containing this sequence (TGACAGTTTTATGAC) to examine DNA binding by Meis1b with Hoxb-4, Hoxb-6, Hoxa-7, and Hoxb-8. In contrast to the AbdB-like Hox proteins, none of these Hox proteins alone exhibited strong DNA binding (Fig. 6B, lanes 4, 8, 12, and 16). In each case, the addition of the Hox protein to Meis1 produced a gel shift complex which was indistinguishable from that observed for Meis1b alone (Fig. 6B, compare lanes 5, 9, 13, or 17 with lane 2). The lack of DNA binding by these Hox proteins in the presence of Meis1b was reflected by the inability of the antisera to the epitope tag on the respective Hox proteins to supershift the bands containing the putative Meis1-Hox heterodimers (Fig. 6B, lanes 7, 11, 14, and 18). Given the apparent biological synergy between *Hoxa-7* and *Meis1*, we considered the possibility that although we had sequenced the *Hoxa-7* cDNA clone and had shown that it made an immunoreactive protein of the correct size, this protein was somehow compromised. We therefore tested the ability of Hoxb-7, the paralog protein of Hoxa-7, to interact with Meis1 proteins. These experiments showed that this Hox-7 protein was also incapable of interacting with Meis1 on a DNA target containing the TTAT core Hox binding site (data not shown).

To ensure that the lack of apparent interaction of Hoxa-7 with Meis1b was not due to an inappropriate DNA target, we repeated the gel shift experiments with the TTAC-containing oligonucleotide. Again, neither supershift nor dissociation experiments showed evidence of protein-protein interactions (data not shown). To further confirm that the Hox-7 paralog proteins do not cooperatively bind DNA with Meis1b, we performed a DNA site selection assay with T7 epitope-tagged Hoxb-7 and T7 epitope-tagged Meis1b. In this case, the majority of sites selected were mixtures of Meis1b single sites in various orientations ($N = 10$), with Meis1 inverted palindrome

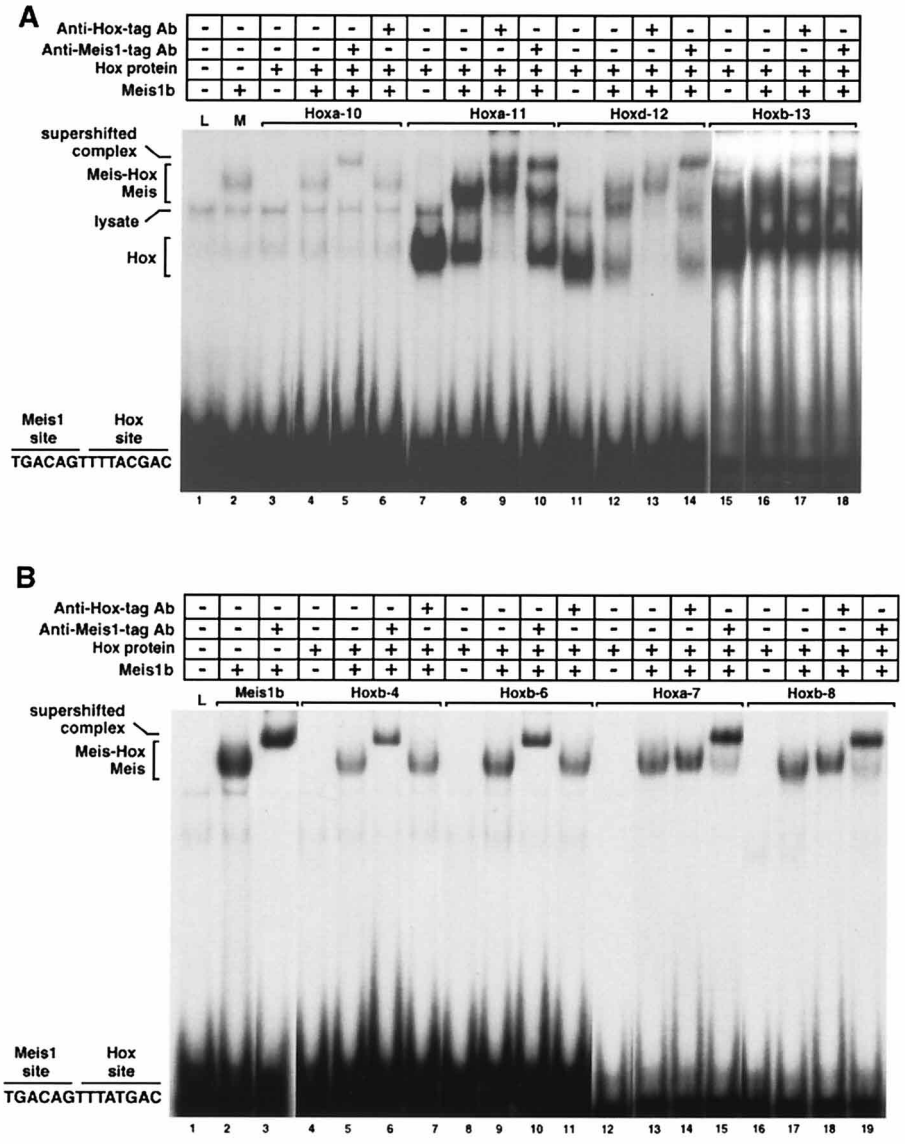

FIG. 6. AbdB-like Hox proteins but not other Hox proteins form DNA binding complexes with Meis1 proteins. (A) AbdB-like Hox proteins bind DNA with Meis1b. EMSA was used to demonstrate that representative members of the Hox-11, Hox-12, and Hox-13 paralogs form DNA binding complexes in the presence of Meis1b, which can be supershifted by antisera to specific epitope tags on either protein. The antisera to the FLAG epitope on the Hoxa-10 protein was not capable of supershifting the Meis1b–Hoxa-10–DNA complex (lane 6), but dissociation data indicated that Hoxa-10 was also capable of cooperatively binding DNA with Meis1b (see Fig. 7D). The oligonucleotide used contained a Meis1 binding site adjacent to a consensus binding site for the AbdB-like Hox proteins (TGACAGTTTTACGAC). L (lane 1), lysate control; M (lane 2), Meis1b alone. (B) Hox proteins from other paralog groups do not bind DNA with Meis1b. Representative Hox proteins from the Hox-4, Hox-6, Hox-7, and Hox-8 paralog groups were unable to bind with Meis1b to a DNA target containing a Hox binding site previously shown to be optimal for the cooperative binding of these Hox proteins with Pbx1a (TGACAGTTTTATGAC). In addition, these Hox proteins, in contrast to the AbdB-like Hox proteins, were unable to bind the oligonucleotide alone (lanes 4, 8, 12, and 16).

sites ($N = 2$) and Hoxb-7 sites ($N = 3$) also being detected. No sequences containing a Meis1–Hoxb-7 site were observed. These data, taken together with that for site selections using Hoxa-9, Hoxa-11, or Hoxd-12 with Meis1 proteins, demonstrate that the site selection protocol is capable of detecting cooperative Meis1-Hox binding to a DNA target and that the Hox-7 paralog proteins do not cooperatively bind to DNA with Meis1 proteins.

Hoxa-9 protein stabilizes Meis1 binding to DNA. Since the addition of Hoxa-9 appeared to increase Meis1 binding to DNA (Fig. 4A, lane 6, and Fig. 4B, lane 7), we next asked if this was a result of an increase in the stability of a Meis-Hox heterodimer. We used dissociation experiments to examine the stability of heterodimeric Meis1-Hox complexes and complexes of Meis1 alone on DNA. To study the stability of protein-DNA complexes, preformed complexes of Meis1 proteins with Hoxa-9 on a labeled DNA target containing a Meis1–Hoxa-9 site (TGACAGTTTTACGAC) were incubated with a 100-fold excess of the unlabeled oligonucleotide as a competitor to reduce complex reformation with labeled DNA to background levels. As shown in Fig. 7A, a preformed complex of Meis1a and Hoxa-9 (lane 1, top band) dissociated relatively slowly, with a calculated half-life of 2.2 min (Table 1). By comparison, although Meis1a alone was capable of forming a DNA binding complex with the oligonucleotide (Fig. 7A, lane 7), this complex was completely dissociated by the first time

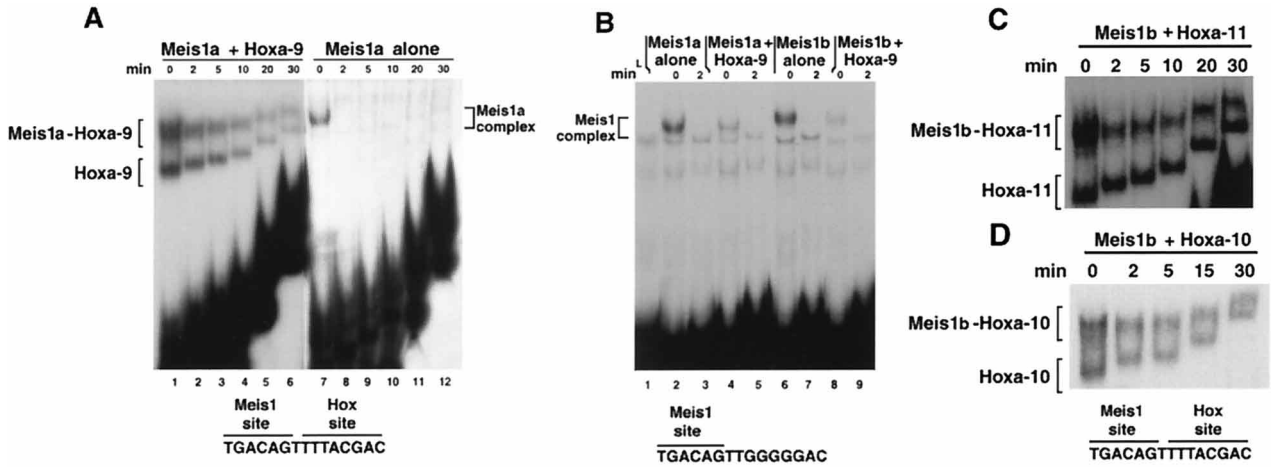

FIG. 7. AbdB-like Hox proteins stabilize Meis1-DNA binding. Dissociation experiments were performed by adding a 100-fold excess of unlabeled oligonucleotide to preformed DNA-protein complexes following the removal of a zero time sample. Samples of the incubation mixtures were removed and applied to the running gels at the times indicated. (A) Hoxa-9 stabilizes Meis1a binding to DNA. Meis1a and Hoxa-9 formed a complex on an oligonucleotide containing a Meis1 site and a TTAC-containing Hox-9 site (lane 1, upper band), which dissociated with a half-life of 2.2 min, and the complex was still clearly detectable at 30 min after the addition of cold competitor DNA (lane 6). The dissociation rate was similar to that observed for the complex formed by Hoxa-9 alone with the DNA target (lower band). In contrast, Meis1a alone formed a gel shift complex (lane 7) which was completely dissociated by the 2-min time point (lane 8). (B) A Hox binding site is required for stabilization of Meis1 DNA binding. When an oligonucleotide which contained a Meis1 binding site but lacked a Hox-9 binding site was used, neither Meis1a nor Meis1b formed stable DNA binding complexes, either in the presence or absence of Hoxa-9. In this experiment, the complex was so unstable that it was not detected at the earliest time point measured following the addition of cold competitor DNA, and the later time points have been deleted from the data presented. L (lane 1) denotes lysate control. (C) Hoxa-11 forms a very stable DNA binding complex with Meis1b. The gel shift complex formed by Hoxa-11 and Meis1b (upper band) on an oligonucleotide containing the Meis1-Hox site was extremely stable under the conditions used to measure the dissociation rates for Meis1–Hoxa-9–DNA complexes. The dissociation rate was similar to that observed for the complex formed by Hoxa-11 alone with the DNA target (lower band). (D) Hoxa-10 stabilizes Meis1b DNA binding. Hoxa-10 and Meis1b formed a gel shift complex which was much more stable than that formed by Meis1b alone. Again, the dissociation rate was similar to that observed for the complex formed by Hoxa-10 alone with the DNA target (lower band).

point (2 min) following the addition of competitor (lane 8), and the half-life was too short to determine. We note that in this instance, as well as in the case of other Meis1-Hox-DNA complexes (see below), the half-life of the Meis1a–Hoxa-9 complex is slightly shorter than that of Hoxa-9 alone (Fig. 7A, lanes 1 to 6, lower bands; Table 1). Essentially identical data were obtained for the relative dissociation rates for a Meis1b–Hoxa-9 complex versus a Meis1b complex from a DNA target containing Meis1 and Hox sites (Table 1). These data suggest that the Hoxa-9 protein exhibits inherent stability on the DNA target and assists the stabilization of the inherently unstable Meis1-DNA interactions. The resulting stability for the Meis1-Hox-DNA complexes appears to be somewhat lower than that of the Hox protein-DNA complexes alone.

Meis1-DNA complexes require Hox protein-DNA binding for stability. To further demonstrate the requirement for Hox protein-DNA binding for stabilization of Meis1-DNA binding, we performed dissociation experiments with an oligonucleotide target containing a mutated Hox binding site (in capital letters) (5′-ccagatcTGACAGttgggggacagatctcc-3′). Complexes of both Meis1 proteins on this target exhibited extremely rapid dissociation (Fig. 7B, lanes 1 and 2 or 5 and 6). In addition, Hoxa-9 was not capable of stabilizing Meis1 binding to the target containing a single Meis1 site (Fig. 7B, lanes 3 and 4 or 7 and 8) or to an oligonucleotide containing an inverted palindrome Meis1 site (data not shown). These data demonstrate that Hox protein binding to DNA is a prerequisite for stabilization of Meis1-Hox-DNA interactions. In fact, Hoxa-9 appeared to diminish Meis1-DNA binding to the target containing a single Meis1 site (Fig. 7B, compare lanes 3 and 4 or 6 and 8). This finding may reflect the fact that Hoxa-9 can bind Meis1 proteins in the absence of DNA (Fig. 5) and thus perhaps alter the conformation of the Meis1 protein.

The AbdB-like Hox proteins stabilize Meis1-DNA interactions, but other Hox proteins do not stabilize Meis1-DNA binding. To confirm the presence of Meis1b-Hox protein-DNA binding complexes in the gel shift bands shown in Fig. 6A, we examined the dissociation rates for the putative complexes formed between Meis1b and the AbdB-like Hox proteins on the TTAC-containing target. The complexes for Meis1b with Hoxa-11 (Fig. 7C) and with Hoxd-12 and Hoxb-13 (Table 1) were shown to have half-lives which were much longer than that of Meis1b alone and were substantially longer than those observed for Meis1 with Hoxa-9. In addition, Hoxa-10 formed

TABLE 1. Half-lives of Hox-Meis1 protein-DNA complexes

Partner protein	Half-life[a] of complex with:						
	Hoxa-9	Hoxb-9	Hoxb-9 W→Q	Hoxa-10	Hoxa-11	Hoxd-12	Hoxb-13
Meis1a	2.2	ND[b]	ND	ND	ND	ND	ND
Meis1b	3.0	2.7	2.0	3.0	>60	>60	>60
ΔN-term Meis1b	9.1	ND	ND	ND	ND	ND	ND
None	5.2	4.2	3.5	4.6	>60	>60	>60

[a] Half-lives (minutes) were calculated from the equation $t_{1/2} = -\log(0.5/K_d)$.
[b] ND, not done.

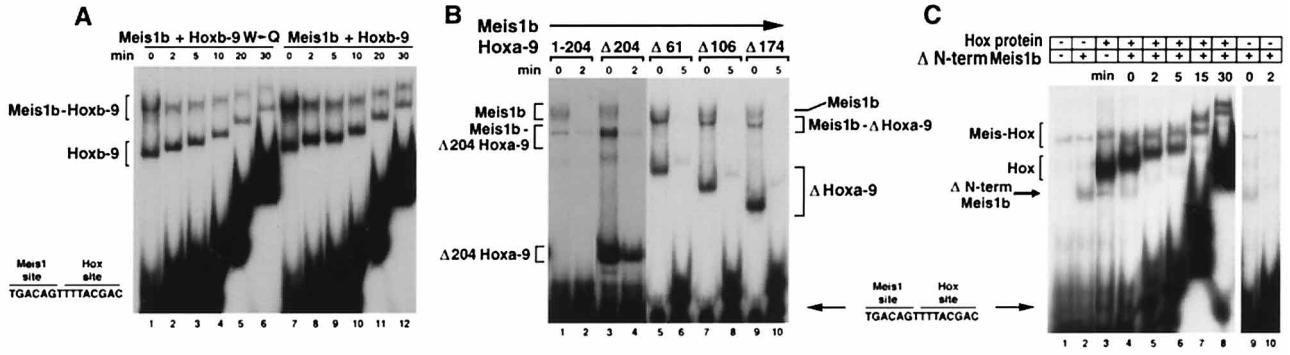

FIG. 8. Defining interaction domains between Hoxa-9 and Meis proteins. (A) A conserved tryptophan is not required for interactions between Hox proteins and Meis1b. The tryptophan previously shown to mediate Hox-9 and Hox-10 interactions with Pbx1a was mutated to glutamine (Hoxb-9, W→Q). The dissociation rate of the complex formed between the mutant Hoxb-9 with Meis1b (lanes 1 to 6) was essentially identical to that formed between Hoxb-9 and Meis1b (lanes 7 to 12). (B) The N-terminal and homeodomain regions of Hoxa-9 are required for stabilization of Meis1b binding to DNA. An N-terminal fragment of Hoxa-9 lacking the homeodomain (Hoxa-9, amino acids 1 to 204) was unable to bind DNA or stabilize Meis1 binding (lanes 1 and 2), while a C-terminal Hoxa-9 fragment containing the homeodomain but lacking the N-terminal 204 amino acids (Δ204) was capable of binding to DNA but was not capable of stabilizing Meis1 DNA interactions (lanes 3 and 4). Progressively shorter Hoxa-9 proteins missing the N-terminal 61 amino acids (Δ61), 106 amino acids (Δ106), or 174 amino acids (Δ174) were all capable of binding DNA (lanes 5, 7, and 9) but were not capable of stabilizing Meis1-DNA binding (lanes 6, 8, and 10). (C) The homeodomain-containing C-terminal region of Meis1b is sufficient for interactions with Hoxa-9. Meis1b lacking the first 270 N-terminal amino acids but containing the homeodomain and 131 C-terminal flanking residues (ΔN-term Meis1b) is capable of forming a cooperative DNA binding complex with Hoxa-9, as reflected by the relatively long half-life of the heterodimeric complex (lanes 4 to 8). In contrast, the ΔN-term Meis1b protein alone forms an unstable complex with a Meis1-Hox DNA target (lanes 9 and 10). In this experiment, when Hoxa-9 alone was incubated with the DNA target, an additional, slowly migrating, variable-intensity band due to proteins in the reticulocyte lysate was observed (lane 3); this band disappeared at the 2-min time point following the addition of cold competitor (lane 5).

a relatively stable complex with Meis1b compared to that of Meis1b alone (compare Fig. 7D and B). In each case, the stability of the Meis1b-Hox-DNA complex was roughly similar to the relative stability of the Hox protein alone on the DNA target (Fig. 7A, C, and D, compare the upper and lower bands). These data, which are summarized in Table 1, suggest that (i) the AbdB Hox proteins alone form relatively stable DNA binding complexes compared to the Hox proteins from other paralog groups (see above), (ii) the AbdB-like Hox proteins stabilize Meis1 protein binding to the target oligonucleotide, and (iii) the three proteins from the 5′ end of the locus, Hox-11, Hox-12, and Hox-13, form extremely stable DNA binding complexes with Meis1 proteins.

We interpreted the lack of supershifting of putative complexes shown in Fig. 6B to reflect a lack of interaction between the non-AbdB-like Hox proteins with Meis1. However, as observed above for Hoxa-10, it was possible that the lack of a supershifted complex was due to the inability of the antibody to recognize the tag sequence within a putative Meis1-Hox-DNA complex. We therefore confirmed the absence of Meis1-Hox interactions by measuring the dissociation rates for the complexes detected when the Meis1b protein was incubated with the respective Hox protein and the TTAT-containing target. In each case, the complex dissociated by the time the first time point sample could be loaded on the gel (data not shown), indicating that these complexes represent Meis1 binding to DNA alone and that the non-AbdB-like Hox proteins do not interact with or stabilize Meis1-DNA interactions.

Meis1-Hox interactions are not mediated by a conserved tryptophan. We next attempted to define the portion of the Hox protein which mediates interactions with Meis1 proteins. Since previous studies showed that tryptophan residues within the highly conserved YPWM motif in paralogs 1 through 8 and in a conserved ANW sequence in paralogs 9 and 10 are required for interactions with Pbx1a (8, 26, 45, 47), we examined the possible role of this residue in mediating Hox-9 interactions with Meis1b. As shown in Fig. 8A, lanes 7 to 12, Hoxb-9 forms a DNA binding complex with Meis1b which exhibits similar stability to that observed for Hoxa-9 (compare to Fig. 7A and Table 1). A Hoxb-9 protein containing a W-to-Q mutation in the conserved ANW sequence was able to form a complex with Meis1b which showed a half-life similar to that of the native Hox-9 proteins (Fig. 8A, lanes 1 to 6; Table 1). Thus, the interaction of Meis1b protein with Hox-9 proteins does not appear to have the mechanism previously described for the related Pbx1a homeodomain protein. The observation that Hoxa-11 forms a complex with Meis1 confirms the finding that the interaction of the Hox proteins with Meis1 proteins is not mediated by an N-terminal tryptophan residue, since the Hoxa-11 molecule does not contain a tryptophan in the 200 amino acids N-terminal to its homeodomain (14).

The N-terminal region of Hoxa-9 appears to mediate interactions with Meis1b. To dissect which portion of the Hox protein is responsible for interactions with Meis1 proteins, we first subcloned the 5′ and 3′ portions of the *Hoxa-9* cDNA into the pET fusion vector to produce T7 epitope-tagged N-terminal and C-terminal Hox proteins. The C-terminal Hoxa-9, which is missing the first 204 amino acids but contains the homeodomain and a short C-terminal tail, is capable of binding to DNA in the presence of Meis1b to form a gel shift band which migrates in a position consistent with a heterodimeric complex (Fig. 8B, lane 3). However, this complex is very unstable (Fig. 8B, lane 4), suggesting that the C-terminal region of the Hoxa-9 protein is not sufficient for stable protein-protein interactions with Meis1. In addition, the N-terminal fragment (residues 1 to 204, lacking the homeodomain) is not capable of binding DNA nor of stabilizing Meis1b binding to DNA (Fig. 8B, lanes 1 and 2). These data suggest that the homeodomain of the Hox protein is necessary but not sufficient for stabilization of Meis1-DNA interactions. To further identify the region of Hoxa-9 which confers DNA binding stability to Meis1b, a series of N-terminal deletion mutants were created and used in gel shift dissociation experiments. As shown in Fig. 8B, T7-tagged fusion proteins missing the first 174, 106, or 61 amino acids of Hoxa-9 were all capable of binding to DNA in the presence of Meis1b to form a trimolecular complex (lanes 5, 7, and 9). However, in each case, the complex was very unstable, suggesting that these mutant Hoxa-9 proteins were incapable

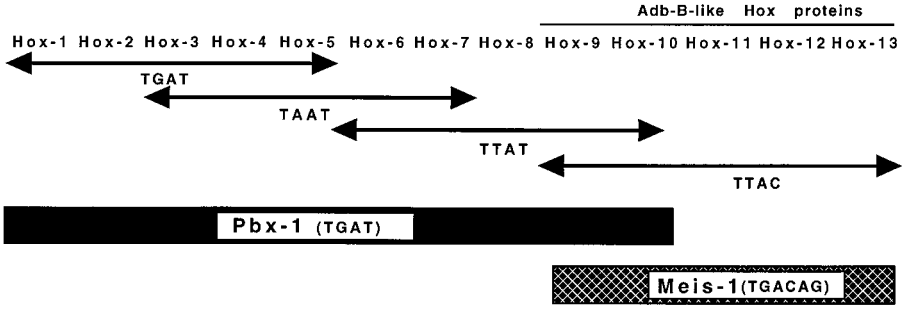

FIG. 9. Hox proteins from each paralog group cooperatively bind DNA with a non-Hox homeodomain partner protein. Hox proteins from paralog groups 1 through 10 gain DNA binding specificity through cooperative interactions with Pbx1a (10). The preferred core recognition site is shown below each set of Hox genes, but substantial overlap exists between paralog groups for DNA site preferences. The AbdB-like Hox proteins from paralog groups 9 through 13 all bind to the same DNA sequence in the presence or absence of Meis1 but greatly stabilize Meis1 binding to DNA. Hox-9 and Hox-10 proteins overlap and interact with both partners. The half-lives of the Meis1-Hox-DNA complexes formed by paralog group 11 to 13 members appear to be much longer than those formed by Hox-9 and Hox-10.

of binding Meis1b in a manner leading to stabilization of the Meis1b-DNA interactions. These data suggest that the capacity to stably interact with Meis1 protein resides within the first 61 amino acids of the Hoxa-9 protein. However, when the first 89 amino acids of Hoxb-8 were replaced with the N-terminal 61 amino acids of Hoxa-9, the resulting fusion protein did not stabilize Meis1-DNA interactions (data not shown).

The C-terminal portion of Meis1b is capable of protein-protein binding with Hoxa-9. We used dissociation experiments to assess the capability of a C-terminal fragment of Meis1b to interact with Hoxa-9. As shown in Fig. 8C, this truncated C-terminal Meis1b containing the homeodomain but lacking the N-terminal 270 amino acids formed DNA complexes with Hoxa-9 (lanes 4 to 8) that were relatively stable compared to the truncated Meis1b protein alone on DNA (Fig. 8C, lanes 9 and 10; Table 1). Since both Meis1a and Meis1b interact with Hox proteins and the Meis1 proteins diverge 38 residues after the homeodomain, these data suggest that the homeodomain itself or the 38-amino-acid common C-terminal region may contain the Hox protein interaction motif. In this regard, protein-protein interactions mediated by the homeodomain have previously been demonstrated for the Hox proteins (53), while the region immediately C-terminal to the Pbx1 homeodomain appears to mediate interactions with Hox proteins (10, 26). However, there is no obvious sequence conservation between the regions C-terminal to the homeodomains of the Meis1 and Pbx1 proteins.

DISCUSSION

There is increasing evidence that homeodomain proteins function by binding DNA as heterodimers with partners from different homeodomain classes. Early studies demonstrated that the yeast mating homeodomain proteins a1 and α2 cooperatively bind DNA as heterodimers (28). The *Caenorhabditis elegans* POU-type homeodomain protein UNC-86 and the LIM-type homeodomain protein MEC-3 bind cooperatively as a heterodimer to the *mec-3* promoter (52). Following reports that certain *Drosophila* Hom-C proteins form DNA binding complexes with the Exd homeodomain protein (6, 51), a number of laboratories showed that Hox proteins from paralog groups 1 through 10 form heterodimeric DNA binding complexes with the Pbx1a homeodomain protein, the vertebrate homolog of Exd (10, 27, 37, 39, 42). We have now extended this pattern to show that a second non-Hox homeodomain protein, Meis1, forms cooperative DNA binding complexes with the AbdB-like subset of Hox proteins, including members of the three paralog groups which do not bind DNA with Pbx1a, Hox-11, Hox-12, and Hox-13 (47). As shown in Fig. 9, proteins from each of the Hox paralog groups form cooperative DNA binding complexes with at least one non-Hox homeodomain partner, and the protein products of *Hox* genes positioned at the interface between the *AbdB* and non-*AbdB* genes in the *Hox* cluster (e.g., *Hoxa-9* and *Hoxa-10*) can bind both Pbx1a and Meis1. It is gratifying that two of the first in vivo DNA targets for Hox proteins contain Pbx-Hox binding sites which follow the code shown in Fig. 9 (12, 43).

Interactions with Pbx1a appear to greatly increase the DNA binding affinity and specificity of the Hox proteins from paralog groups 1 to 8 (8). In contrast, the affinity and specificity of AbdB-like Hox proteins are not enhanced by their interactions with either Meis1 or Pbx1a. However, cooperative interactions between the Abd-B-like Hox proteins and Meis1 is reflected by the site selection data for Hoxa-9, Hoxa-11, and Hoxa-12, which show that in the presence of Meis1, these Hox proteins bind preferentially to DNA targets containing contiguous Meis1 and Hox protein sites in the same orientation as that observed for Pbx1a and Hox proteins. In addition, each of the AbdB-like Hox proteins greatly stabilized the inherently weak binding of Meis1 proteins to DNA. In the current study, there were substantial differences in the dissociation rates for complexes formed from the various AbdB-like Hox proteins with Meis1b on an appropriate DNA target, similar to the differences in stability previously observed between DNA complexes formed by the Hox proteins with Pbx1a (47). Thus, the proteins from the extreme 5′ end of the locus, Hox-11, Hox-12, and Hox-13, form DNA binding complexes with Meis1 which are much more stable than those formed with Hox-9 or Hox-10 proteins.

Pbx1a appears to enhance Hox-DNA interactions by inducing a conformational change which moves the hexapeptide region to facilitate DNA binding (7). In contrast, the AbdB-like Hox proteins exhibit very strong DNA binding in the absence of Meis1 protein, and, in fact, the Meis1-Hox-DNA complexes dissociate somewhat more rapidly than the respective Hox-DNA complexes. In contrast to Pbx1a, which does not appear to bind DNA strongly in the absence of Hox partners (10), the Meis1 proteins form detectable DNA binding complexes with targets containing a single TGACAG site. We note that the consensus Meis1 site is identical to a biologically relevant binding site identified for another member of the TALE class, TGIF (2). Our observation of a significant number of inverted palindrome sequences in the Meis1 DNA site selection experiment, together with the aberrant migration po-

sition of Meis1-DNA complexes, suggested that Meis1 is also capable of forming homodimers. In this regard, we have preliminary data demonstrating Meis-Meis interactions which are DNA independent and are currently investigating the nature of Meis1 protein dimerization. Taken together, our data suggest a complex competition for protein partners which would also be influenced by the availability of various target sites. Proteins from two Hox paralog groups, Hox-9 and Hox-10, should exhibit an especially complex set of potential interactions since they can form complexes with both Pbx and Meis1 proteins. Since both Meis1 (33) and at least one of the Pbx proteins (31) appear to be expressed in most or all cell types, these data suggest that competition for Hox proteins may play a role in the function of the Meis and Pbx molecules.

There is increasing evidence for a role of Hox proteins in leukemic transformation (23). The *Hoxb-8* gene was originally isolated as the site of a viral insertion in a murine leukemia cell line (20). Constitutive expression of a *Hoxb-8* cDNA (40) or a *Hoxa-10* cDNA (50) in murine bone marrow cells leads to the development of myeloid leukemias. In addition, a number of *HOX* genes are strongly expressed in human leukemic cell lines and in primary samples of human leukemia (reviewed in reference 22). The recent report that the t(7;11)(p15;15) translocation associated with myeloid leukemias results in a fusion of the *Hoxa-9* homeodomain to the N-terminal portion of the nucleoporin gene provided the first evidence of mutations of *HOX* genes in human leukemia (4, 35). Non-Hox homeobox proteins are also involved in leukemic transformation. Pbx1 was initially discovered as part of a fusion protein resulting from the t(1,19) chromosomal translocation in lymphoid leukemias (18, 38). The various fusion proteins, which consist of the Pbx homeodomain and the activation domain of E2A, were shown to be transforming (17, 32). Chang et al. have recently shown that the transforming activity of the E2A-Pbx1 fusion protein is retained in a small protein domain containing a Hox protein interaction motif, which may bring the E2A activation domain to a DNA target through the Hox protein bridge (9). In this setting, the observation that *Hoxa-9* and *Meis1* are jointly upregulated by viral insertions in the BXH-2 model of murine myeloid leukemia (36) was one driving force for the current studies. Our demonstration that these proteins physically interact suggests that this direct interaction is crucial to transform bone marrow cells. Studies on the synergistic transforming capacity of Hox and Meis1 proteins are currently in progress.

At present, it is unclear how the Meis1 and Pbx proteins function with the Hox proteins in nontransformed cells. Previous studies were unable to show transcriptional activity for native Pbx1a, and only the E2A-Pbx1a fusion molecule yielded transcriptional activity with Hox proteins in transient assays (8). In the current study, we were unable to detect either activation or repression of basal activity in transient transcription assays when plasmids encoding Meis1a or Meis1b were cotransfected with Hoxa-9 or Hoxa-10 expression plasmids with a reporter containing multiple copies of the Meis1–Hox-9 binding site in either F9 cells or NIH 3T3 cells (47a). We hypothesize that these proteins may act at higher levels of gene regulation, such as influencing chromatin folding or organization (50).

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