MEIS proteins form heteromeric DNA-binding complexes with PBX monomers and PBX/HOX heterodimers. We have shown previously that transcriptional activation by PBX/HOX is augmented by either protein kinase A (PKA) or the histone deacetylase inhibitor trichostatin A (TSA). To examine the contribution of MEIS proteins to this response, we used the chromatin immunoprecipitation assay to show that MEIS-1 in addition to PBX1, HOXA1, and HOXB1 was recruited to a known PBX/HOX target, the Hoxb1 autoregulatory element following Hoxb1 transcriptional activation in P19 cells. Subsequent to TSA treatment, MEIS1 recruitment lagged behind that of HOX and PBX partners. MEIS1 also enhanced the transcriptional activation of a reporter construct bearing the Hoxb1 autoregulatory element after treatment with TSA. The MEIS1 homeodomain and protein-protein interaction with PBX contributed to this activity. We further mapped TSA-responsive and CREB-binding protein-dependent PKA-responsive transactivation domains to the MEIS1A and MEIS1B C termini. Fine mutation of the 56-residue MEIS1A C terminus revealed four discrete regions required for transcriptional activation function. All of the mutations impairing the response to TSA likewise reduced activation by PKA, implying a common mechanistic basis. C-terminal deletion of MEIS1 impaired transactivation without disrupting DNA binding or complex formation with HOX and PBX. Despite sequence similarity to MEIS and a shared ability to form heteromeric complexes with PBX and HOX partners, the PREP1 C terminus does not respond to TSA or PKA. Thus, MEIS C termini possess transcriptional regulatory domains that respond to cell signaling and confer functional differences between MEIS and PREP proteins.

Meis11 (myeloid ecotropic viral insertion site 1) was identified at one of the common viral integration sites in myeloid leukemic cells of BXH-2 mice (1) and has been shown to promote oncogenic transformation in several contexts (2, 3). Meis1 and its fly homolog homothorax (hth) encode homeoproteins of the three-amino acid loop extension class and therefore are related to the vertebrate pre-B cell transformation (Pbx) genes and their fly homolog extradenticle (exd) (4, 5). Three genes constitute the mammalian Meis family (6–10) with Meis1 transcripts alternatively spliced to yield multiple isoforms (1, 11). In addition, the Meis-related genes Prep1 and Prep2 (for PBX regulatory protein) have also been identified (12–14).

MEIS/PREP/HTH proteins form stable heterodimers with PBX/EXD partners. In addition, PBX or MEIS family members bind DNA cooperatively with subsets of HOX partners (Ref. 15 and references therein), thus permitting the formation of DNA-bound heterotrimERIC MEIS-PBX-HOX complexes. These heterodimeric and trimeric complexes modulate the functional specificity of HOX proteins, perhaps by increasing DNA binding affinity and selectivity (16–20) and/or by modulating co-ordinating regulators (21). PBX/EXD and MEIS/PREP/HTH are mutually dependent for accumulation in the nucleus (22–24), but PKA and non-muscle myosin heavy chain II B can independently modulate PBX subcellular localization (25, 26).

At least in some contexts, MEIS, PREP, or HTH proteins are required for PBX-HOX complexes to exert positive or negative transcriptional control (27–36). Consistent with this observation, MEIS family proteins cooperate with PBX and HOX for hind brain patterning in Xenopus and zebrafish (10, 37, 38). Recent studies in both of these vertebrates as well as in Dro sophila suggest a transcriptional activation function for MEIS/HTH (38–40), but activation domains have not been mapped. The Hoxb1 ARE is a PBX-HOX target that directs expression in rhombomere (r) 4 of the developing hind brain (41). It contains binding sites for three PBX-HOX complexes (41–44), MEIS/PREP1 (28–30), SOX, and OCT transcription factors (45), although only the PBX-HOX sites are required for r4 enhancer function. The Hoxb1 ARE drives the expression of a lacZ reporter in P19 cells induced to differentiate down the neural pathway by aggregation in the presence of retinoic acid (RA) (46). However, P19 cell monolayers fail to activate the ARE following RA treatment, despite the presence of Hox, Pbx, and Meis products. By contrast, the HDAC inhibitor TSA induces ARE-driven transgene expression in monolayers, consistent with evidence implicating PBX in HDAC recruitment (46, 47, 50).
In addition, both TSA and PKA strongly activate expression through PBX-HOX binding sites in HEK293 cells. Again this can be explained in part by the inhibition of PBX-associated HDACs but also increased association of the HOXD4 transcriptional activation domain with CBP, a coactivator with histone acetyltransferase activity (46).

In our previous study, we focused on two of the homeoproteins predicted to act through PBX-HOX-response elements. Here, we investigate the contribution of a third partner, MEIS1, to transcriptional regulation by MEIS-PBX-HOX heterotrimers through the Hoxb1 ARE, both at the endogenous locus in P19 cells and with transfected reporters. We correlate transcriptional activation of Hoxb1 with histone acetylation and MEIS1, PBX1, and HOX recruitment to the ARE and map TSA- and PKA-responsive transcriptional activation domains in the MEIS1A and MEIS1B C termini. In addition, we present evidence suggesting that differences in their C termini distinguish the functions of MEIS and PREP family proteins.

MATERIALS AND METHODS

Plasmids Construction—Expression vectors for HOXA1, MEIS1A, MEIS1A (N51S), PBX1A, and PBX1A-(31–89) have been described previously (28, 48). The entire coding region of MEIS1B and PREP1 were subcloned into the pCS2+ vector. pML, pML(2xPBXMEIS), and pML5xUAS are luciferase reporters driven by adenovirus major late promoter alone, two copies of PBX-MEIS (TGATTGAGCAG) binding sites, or five copies of GAL4 binding sites, respectively (48, 49). pMLHoxb1ARE was constructed by subcloning the 150-bp ARE of Hoxb1 gene into HindIII-Xhol sites of pML. GAL4 DNA binding domain (GAL-DDB) fusion proteins were generated by subcloning corresponding coding sequences into pGal-O45 (49) as described below. A Small-Smalg fragment and a Small-EcoRV fragment encoding murine MEIS1A residues 1–231 and 232–390 were cloned into pGal-O45 to produce GAL-MEIS1A-1–231 and GAL-MEIS1A-232–390. To construct GAL-MEIS1A-(1–371), GAL-MEIS1A-(335–372), GAL-MEIS1A-(372–390), GAL-MEIS1B-(1335–465), and GAL-MEIS1B-(372–465), corresponding DNA fragments were amplified by PCR and cloned 3′ to the GAL-DDB binding region. An EcoRI-Xhol fragment from pBshPREP1 was used to construct GAL-PREP1 (316–465). Coding sequences for MEIS1A-(1–334) and MEIS1A-(1–371) were amplified by PCR using pfu polymerase and cloned into pCS2+. Protein expression was verified by Western blot using a rabbit polyclonal anti-MEIS1 antibody (26). Expression vectors for CBP, PKA catalytic domain, and E1A have been described previously (46).

Antibodies—Rabbit polyclonal antibodies against murine MEIS1A (26) and HOXB1 were raised by immunization with fusion proteins containing MEIS1A-(1–231) and MEIS1A residues 14–161 of HOXB1. Affinity purification was performed over a column presenting covalently bound GST that was fused to the same MEIS1A or HOXB1 residues (50). The anti-HOXA1 (sc-17146) and anti-PBX1 (sc-889) antibodies were purchased from Santa Cruz Biotechnology.

RT-PCR and Chromatin Immunoprecipitation (CHIP) Assay—P19 cells were plated at 106 cells/ml and aggregated in the presence of 0.3 μM RA or cultured overnight in monolayer and treated with 200 nM TSA for indicated times. For RT-PCR, first strand cDNAs were generated by Superscript II reverse transcriptase (Invitrogen) using total RNA from P19 cells. ChIP assays were performed according to the protocol from Upstate Biotechnology and as described previously (51). Cells were washed twice using ice-cold phosphate-buffered saline containing proteinase inhibitors (10752800, Roche Applied Science). Chromatinized DNA was cross-linked in 1% formaldehyde for 10 min at 37 °C. The lysates were diluted 10-fold in ChIP dilution buffer (0.01% SDS, 1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, and 167 mM NaCl) and precleared with sperm DNA-protein A-agarose beads (Upstate Biotechnology) at 4 °C for 1 h. Following incubation with 5 μg of anti-acetyl (K9,K14,K18) (063599, Upstate Biotechnology), 20 μg of anti-MEIS1, anti-HOXA1, or anti-HOXB1, or 10 μg of anti-PBX1 antibodies, immune complexes were immobilized by sperm DNA-protein A-agarose beads. After extensive washing and elution with 1% SDS and 0.1 M NaHCO3, cross-links were reversed by incubation at 65 °C for 4 h in the presence of 0.2 M NaCl. The released DNA was phenol-chloroform-purified, and the Hoxb1 ARE and gapdh sequences were detected by real-time PCR and agarose gel visualization. 5% (by volume) of the immunoprecipitated material was used as a template for real-time PCR by using a SYBR Green Taq ReadyMix kit for quantitative PCR from Sigma with a Roche LightCycler.

The ChIP primers for gapdh are 5′-AACGACCCCTTTAGGAC-3′ (forward) and 5′-TCACGACATCTGACAC-3′ (reverse). The Hoxb1 ARE primers are 5′-CTCTGCTTCCTTTCCC-3′ (forward) and 5′-GCCGAGCTTGTGGACTG-3′ (reverse). The RT-PCR primers for gapdh are the same as those for ChIP, Meis1a, 5′-ACAAGCGCCGCTGTATTGGA-3′ (forward) and 5′-GCTACTGTCGAGTCCGAGC-3′ (reverse).

Cell Culture and Luciferase Assay—HEK293 and P19 mouse embryonal carcinoma cells were cultured in α-minimum essential medium supplemented with 1-glutamine, 10% fetal calf serum, and penicillin/streptomycin (Invitrogen). A total of 5–106 DNA containing 1–3 μg of luciferase reporter construct and 0.5–1 μg expression vector were transfected into HEK293 cells by the calcium phosphate coprecipitation method. A cotransfected lucZ reporter was used to normalize transfection efficiency. Luciferase assays were performed as described previously by Phelan et al. (48).

Western Blot Analysis—HEK293 and P19 cells were harvested and lysed in IBC lysis buffer (500 mM NaCl, 50 mM Tris-Cl, pH 8.0, 0.5% Nonidet P-40, and protease inhibitors) followed by brief sonication. Whole cell extracts were separated by SDS-PAGE, and antibodies against MEIS1A, ACTIN (Sigma), and GAL-DDB (Santa Cruz Biotechnology) were used to detect the presence of the corresponding proteins. Secondary antibodies were conjugated with horseradish peroxidase and detected by enhanced chemiluminescence (PerkinElmer Life Sciences).

Transcription and Electrophoretic Mobility Shift Assay—HOXA1, PBX1A, MEIS1A, and MEIS1A-(1–371) were generated with an Sp6 T7 coupled in vitro transcription-translation kit (Promega). Reactions containing [35S]methionine were in parallel to verify translational efficiency. Electrophoretic mobility shift assay was performed as described previously (48). Radioactively labeled DNA probes containing PBX-MEIS (5′-TCAGCTAGATTTGCAGATG-3′) or PBX-HOX (5′-AGGGTTCTGTCAGGCGCCAGGCTACTGTCGAGTCCGAGC-3′) binding sites (Fig. 4C) were used.

Results

MEIS Proteins Contribute to the TSA Responsiveness of the Hoxb1 ARE through the Homeodomain and by Interaction with PBX—To assess the contribution of MEIS1 to the TSA responsiveness of PBX-HOX complexes, we examined the activity of a luciferase reporter driven by the Hoxb1 ARE in transiently transfected HEK293 cells (Fig. 1, upper panel).

The Hoxb1 ARE is only weakly TSA-responsive 24 h following drug treatment (Fig. 1, lane 1). However, as noted previously (46), cotransfection with expression vectors for HOXA1 and PBX1A (lane 2) or an N-terminally truncated PBX1A (lane 3) greatly increases TSA responsiveness, an effect dependent on PBX-HOX binding sites. Importantly, wild-type MEIS1A also increased the TSA response (lane 4), whereas the DNA-binding impaired MEIS1A(N51S) was inactive (lane 5). Coexpression of MEIS1A with HOXA1 and PBX1A provoked a synergistic increase in TSA responsiveness (lane 6), an effect abrogated either by mutation of the MEIS1A HD (lanes 7 and 9) or an N-terminal deletion of PBX1A that abolishes MEIS recruitment (lanes 8 and 9). Together, these results strongly suggest that MEIS1A does indeed contribute to the TSA responsiveness of PBX-HOX complexes.

Induction of Hoxb1 Correlates with MEIS1 Recruitment to the ARE—The potentiation of the TSA response by MEIS1A could be caused by drug-induced recruitment to the Hoxb1 ARE or, non-exclusively, the function of a MEIS1A TSA-responsive transcriptional activation domain. To assess whether TSA can augment the amount of MEIS1A in the cell and at the Hoxb1 ARE, we performed RT-PCR, Western, and ChIP analyses on endogenous Meis1a products in P19 embryonal carcinoma cells.

We have previously shown that both RA and TSA induce the production of HOXB1 and the activity of the Hoxb1 ARE in P19
also clearly evident (Fig. 2). A more striking RA-induced H3 diacetylation at 24 h was rapid (1 h) acetylation of histone H3 that was maintained at higher levels 24 h post-treatment. Therefore, ChIP assays were performed to determine whether domains of MEIS1A could confer the PKA response on the GAL-DDB. As it did for TSA, the MEIS1A C terminus responded strongly to PKA signaling (Fig. 3C). Coexpression of E1A reduced the PKA-induced activity, implicating a CBP-dependent mechanism. However, simple overexpression of CBP did not significantly induce transcriptional activation by the MEIS1A C terminus (Fig. 3C).

C-terminal Deletion of MEIS1 Impairs Transactivation without Disrupting DNA Binding or Ternary Complex Formation—The above results indicated that the MEIS1A C terminus harbors a TSA- and PKA-responsive transactivation domain. If so, deletion of this region should impair the ability of MEIS to facilitate transcriptional activation by MEIS/PBX-HOX complexes. To investigate this possibility, we generated two mutants lacking the C-terminal transactivation domain, MEIS1A-(1–334) and MEIS1A-(1–371). Results showed that mutants lost the ability to cooperate with PBX-HOX for activation through the Hoxb1 ARE (Fig. 4A). Full-length and C terminally deleted proteins accumulated to similar levels (data not shown).

PKA has been shown to influence the activity of PBX-containing complexes at a natural CAMP-responsive enhancer (61–64). Therefore, we tested the role of the MEIS C terminus in mediating PKA responsiveness of PBX-MEIS heterodimers. Although wild-type PBX-MEIS is highly PKA-responsive on an appropriate promoter, the loss of the MEIS C terminus abolishes this activity (Fig. 4B). These results confirm that the MEIS1A C terminus mediates the responsiveness of both TSA and PKA.

To exclude the possibility that C-terminal deletion of MEIS1 affects its ability to bind DNA cooperatively with PBX and HOX, we performed electrophoretic mobility shift assays using in vitro translated PBX1A, HOX1A, MEIS1A, and MEIS1A-(1–371). MEIS1A and MEIS1A-(1–371) cooperatively bound DNA with PBX1A and HOX1A with similar efficiencies (Fig. 4C, left panel, compare lanes 4 and 5). Wild-type and mutant MEIS1A also formed heterodimers with PBX1A on a PBX-MEIS binding site (Fig. 4C, right panel, compare lanes 4 and 6). Thus, the loss of transactivation function by MEIS1A C-terminal mutants is due to the deletion of a transactivation domain and not an impairment of DNA binding or PBX interaction.

MEIS and PREP/C Termini Have Different Transcriptional Activities—The vertebrate MEIS/PREP family comprises
MEIS1, MEIS2, and MEIS3 (including several splicing isoforms) and PREP1 and PREP2. Although some domains are well conserved between family members, the C termini are more diverged. In addition, the MEIS1A and MEIS1B isoforms differ at their C termini. To determine whether these differences affect function, we generated GAL-DBD fusion proteins using corresponding C-terminal regions of MEIS1A, MEIS1B, and PREP1 (Fig. 5, A and B). Transcriptional activation was compared in the absence and presence of TSA and PKA signaling (Fig. 5B). The PREP1 C terminus was non-responsive in both assays. By contrast, the MEIS1B C terminus displayed a strong constitutive transactivation function that was potenti-
activated by TSA treatment or PKA signaling (Fig. 5B). These differences were reflected in the relative abilities of full-length MEIS1A, MEIS1B, and PREP1 to mediate TSA responsiveness at the Hoxb1 ARE (Fig. 5D).

Further division of the MEIS1A and MEIS1B C termini into regions common and unique to the two isoforms abolished (MEIS1A) or reduced (MEIS1B) the transactivation functions of GAL4 fusions (Fig. 5B). Nonetheless, the extreme C-terminal region unique to MEIS1B displayed a robust response to both TSA and PKA, confirming functional differences between the MEIS1 isoforms. Whereas transactivation by construct GAL-MEIS1A-(372–390) may have been compromised by low expression levels (Fig. 5C), experiments described below confirm the importance of extreme C-terminal residues unique to MEIS1A versus MEIS1B.

Multiple Regions in the MEIS1A C Terminus Mediate TSA and PKA Responsiveness—The experiments described above suggested that at least two domains within the 56 residues of the MEIS1A C terminus were required for transcriptional activation function. To refine this observation and to explore the extent to which TSA and PKA responses were coextensive, we scanned the MEIS1A C terminus by conversion of 5- or 6-residue blocks to alanine (Fig. 6). Confirming the results of the broad deletion analysis above, we found that both the proximal C-terminus common between MEIS1A and MEIS1B (residues 334–372), as well as the distal 18 residues unique to MEIS1A-(372–390), harbored sequences required for transactivation function. Thus, the conversion of the extreme C-terminal six residues to alanine abolished the response to both TSA and PKA. Likewise, conversion to alanine at positions 345–349, 355–359, and 365–369 abolished or greatly reduced activation by both inducers. Although alanine substitution did affect the steady-state levels of the GAL-MEIS fusion proteins, all of the mutants accumulated to higher levels than the wild-type parent. Thus, differences in the amount of protein produced do not account for changes in transcriptional activation function (data not shown).

Transcriptional Activation by MEIS C Termini

The experiments described above suggested that at least two domains within the 56 residues of the MEIS1A C terminus were required for transcriptional activation function. To refine this observation and to explore the extent to which TSA and PKA responses were coextensive, we scanned the MEIS1A C terminus by conversion of 5- or 6-residue blocks to alanine (Fig. 6). Confirming the results of the broad deletion analysis above, we found that both the proximal C-terminus common between MEIS1A and MEIS1B (residues 334–372), as well as the distal 18 residues unique to MEIS1A-(372–390), harbored sequences required for transactivation function. Thus, the conversion of the extreme C-terminal six residues to alanine abolished the response to both TSA and PKA. Likewise, conversion to alanine at positions 345–349, 355–359, and 365–369 abolished or greatly reduced activation by both inducers. Although alanine substitution did affect the steady-state levels of the GAL-MEIS fusion proteins, all of the mutants accumulated to higher levels than the wild-type parent. Thus, differences in the amount of protein produced do not account for changes in transcriptional activation function (data not shown). The coincident loss of both TSA and PKA responses via multiple distinct mutations strongly suggests a common underlying mechanism. A similar analysis of the MEIS1B C terminus did not identify any one subdomain critical for activation function (data not shown).

DISCUSSION

We had previously established that PBX-HOX complexes mediate gene activation in response to TSA and PKA. Knowing that MEIS or PREP partners form trimers with PBX-HOX, we asked whether MEIS contributed to the TSA and PKA response at the Hoxb1 ARE. We found that MEIS partners were important for the full response to TSA and PKA and that this effect was mediated via the MEIS1A and MEIS1B C termini. In the course of this study, we also used affinity-purified anti-MEIS1 antibodies in the ChIP assay to detect MEIS1 at the Hoxb1 ARE in P19 cells. This provided biochemical evidence for the presence of MEIS1 at a PBX-HOX regulatory element in the cell and allowed us to correlate changes in chromatin with MEIS1 recruitment and the activation state of Hoxb1.

Hoxb1 Activation, Histone Acetylation, and MEIS Recruitment—Consistent with a role for MEIS proteins in mediating responses to TSA and PKA, MEIS1 is recruited to the Hoxb1 ARE of P19 cells within 24 h of TSA exposure. RA, the physiological inducer of Hoxb1 in the central nervous system, likewise induces MEIS1 binding at the endogenous ARE. Just 1 h of TSA treatment is sufficient to increase levels of acetylated H3 and to recruit HOXA1, HOXB1, and PBX. Such rapid recruitment argues against an effect of increased protein levels and in favor of greater accessibility to the ARE either through direct modification of the homeoproteins, chromatin opening, or both. This rapid initial recruitment of HOX and PBX, in conjunction with increased H3 acetylation and further chromatin opening, may be sufficient for the rapid induction of Hoxb1 transcripts in response to TSA. We also cannot exclude that some of the increase in Hoxb1 message may be due to effects at the level of mRNA stability.

Whereas HOXA1, HOXB1, and PBX are recruited to the Hoxb1 ARE by 1 h of TSA treatment, MEIS is only recruited later (observed at 24 h), despite the fact that MEIS1 is present in the cells from the beginning. This finding suggests that

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3 S.-L. Goh, unpublished observations.
FIG. 4. C-terminal deletion of MEIS1 impairs transcriptional activity without disrupting cooperative DNA binding with PBX and HOX. A, MEIS1A C-terminal deletions cannot potentiate HOX-PBX transcriptional activity on the Hoxb1ARE. Expression vectors for HOXA1, PBX1A, and MEIS1A derivatives were transfected into HEK293 cells as indicated. Transcriptional assays were performed on the pMLHoxb1ARE reporter in untreated or TSA-treated cells (control or TSA). Error bars give the mean ± S.D. over three experiments. B, C-terminal deletion of MEIS1A abrogates stimulation by PKA. Transcriptional assays were conducted with a pML(2XPBX-MEIS) reporter bearing two PBX-MEIS heterodimer binding sites in the absence or presence of the PKA catalytic domain (control or PKA). Error bars represent the mean ± S.D. over three experiments. C, MEIS1A-(1–371) cooperatively binds to DNA with PBX1A and HOXA1 at levels similar to wild-type MEIS1A (left panel, compare lanes 4 and 5). It can also form heterodimers with PBX1A on a PBX-MEIS binding site (right panel, compare lanes 4 and 6). Arrows point to dimeric or trimeric complexes.
MEIS is not recruited as part of a preformed trimeric complex with HOX and PBX in response to TSA but independently and later. This implies that other events, such as modification of HOX, PBX, or MEIS, or nuclear accumulation, may be required for MEIS recruitment. Moreover, whatever events lead to MEIS recruitment, they seem likely to require the synthesis of new gene products given the 24-h lag time observed.

We note that RA treatment consistently provokes higher levels of H3 acetylation and HOX, PBX, and MEIS recruitment than does TSA. This may be due to the broader effects of RA, not just on the recruitment/release of a variety of histone acetyltransferases and HDACs but also on the activity of multiple RA-responsive genes whose products impinge on events at the Hoxb1 ARE.

The Role of MEIS in Heterotrimeric Complexes—In addition to TSA-induced recruitment, we determined that the MEIS1A and MEIS1B C termini contained an activation domain responding to TSA and PKA in HEK293 cells. Whereas the MEIS1A C terminus appeared inert in the absence of TSA and PKA, the MEIS1B C terminus displayed a constitutive 30-fold activation function when fused to the GAL-DBD. The activities of both C termini were further stimulated another 30–50-fold by TSA and PKA, although MEIS1A remained less active than MEIS1B.

The more distal of two cAMP-responsive regions of the bovine CYP17 promoter binds PBX-MEIS heterodimers, and overexpression of PBX potentiates the CYP17 response to cAMP (62). Because PKA is activated through cAMP second messengers, our results suggest that MEIS C termini contribute to the cAMP response at this locus. Within MEIS-PBX-HOX heterotrimers, both HOX- and MEIS-derived activation domains respond to PKA (and TSA), providing at least two levels at which the response can be modulated to counterbalance the negative effects contributed by PBX repression domains (46, 47).

Our mapping studies suggest that MEIS activation domains are bipartite with the region common to MEIS1A and MEIS1B (residues 334–372) required to potentiate or reveal the transactivation function of the isoform-specific domains C-terminal to residue 372. Alternatively, the common region may augment transactivation function by displacing the unique regions away from the GAL-DBD, thereby rendering them more accessible.

In vivo experiments in mouse reveal that the binding of MEIS or PREP1 is required for the activity of the Hoxb2 r4 enhancer (29, 30). Moreover, an ARE of the fly labial gene as well as a repressor element in a distalless enhancer depends on binding sites for HOX, EXD, and HTH (32–34). However, DNA binding by MEIS/PREP proteins is not necessary for trimer formation at all PBX/HOX binding sites (28) including the Hoxb1 ARE (29, 30). Why then does a mutation impairing DNA binding (MEIS1A(N51S)) also reduce the activational response to TSA? The homeodomain is known to undergo multiple intramolecular interactions. Given that direct and indirect intramolecular contacts to the HD of HOX and three-amino acid loop extension class proteins are known to regulate function (24, 65, 66), DNA binding by MEIS/PREP proteins may be required to expose the C-terminal activation domain and/or provide a favorable platform for recruiting transcriptional coactivators or components of the basal transcriptional machinery at some enhancers, such as Hoxb2, but not at the Hoxb1 ARE. Conceivably, the N51S homeodomain may have an increased affinity for the MEIS C terminus, thereby masking the activation domain and impairing function.
FIG. 6. Transactivation by mutants bearing alanine substitutions in the MEIS1A C terminus. The indicated GAL-MEIS1A fusions were tested for their ability to activate pML5xUAS in response to TSA (A) and PKA (B). Protein levels determined by Western blotting with an antibody against the GAL-DBD revealed that all of the mutants accumulated to higher levels than the wild-type GAL-MEIS1A-(335–390) fusion (data not shown). Thus, a mutant such as Gal-(385-GQWHYM) in which transactivation has been lost accumulates to higher levels than the fully active and wild-type parent. We did not recover mutants spanning residues 350–354 and 375–379.

Because the TSA response of both the HOXD4 and MEIS1 activation domains is dependent on CBP and HOX activation domains are known to recruit CBP (46, 67), it seems plausible that MEIS and HOX activation domains might synergize for the recruitment of this coactivator.

TSA and PKA Reveal the Function of MEIS1 C-terminal Activation Domains—Transcriptional activation by acidic, proline-rich, and glutamine-rich activation domains are potentiated by histone H4 acetylation (68). A similar effect may underlie the action of the HDAC inhibitor TSA on HOX and MEIS1 transcriptional activation domains used in this and other studies (46, 67). We find that the same mutations of the MEIS1A C terminus that lower TSA-induced activation also impinge on the PKA response, suggesting that both agents ultimately act on MEIS targets through a common mechanism. PKA also promotes increased histone acetylation through phosphorylation of serine 10 on histone H3, providing a common pathway for the effects of TSA and PKA on eukaryotic transcriptional activation domains. TSA and PKA may mimic changes to chromatin normally produced by adjacent bound enhancer factors, explaining the dependence of some PBX-HOX sites for nearby elements that are responsive to cell signaling cues (69–71). Within the context of the MEIS-PBX-HOX heterotrimer, TSA and possibly PKA would also act to inhibit HDAC activity recruited by PBX (46, 47), thereby allowing the activation functions of HOX and MEIS to predominate (Fig. 7).

TSA and PKA could also act directly on MEIS proteins. A presumptive PKA phosphorylation site (serine 343 in MEIS1) lies just C-terminal to the MEIS homeodomain in a region conserved among MEIS1A, MEIS1B, MEIS2A, MEIS2B, MEIS3A, and MEIS3B. However, the region containing this site (MEIS1A-(334–372)) is inactive on its own upon PKA stimulation (Figs. 5 and 6) and so would have to act in conjunction with unique residues C-terminal to this point. By contrast, an absence of lysine indicates that MEIS1A and MEIS1B C termini are not direct targets for acetylation.

C-terminal Regions of MEIS1 Isoforms and PREP1 Have Different Transcriptional Activities—Previous studies have reported functional similarities between MEIS1 and PREP1 in directing PBX/EXD nuclear localization (22–24) and forming trimeric complexes with HOX and PBX (27, 28, 31). Despite these similarities, MEIS1 and PREP1 differ in their oncogenic properties. MEIS1 can collaborate with several HOX proteins to induce acute myeloid leukemia from primary bone marrow cells (72, 73). By contrast, overexpression of PREP1 does not accelerate HOXA9-induced acute myelogenous leukemia (3). Functional differences between MEIS1 isoforms have also been shown, because XMeis1a and XMeis1b induce neural crest cell fate to different extents (74).

In contrast to MEIS1A and MEIS1B, the PREP1 C terminus was unresponsive to TSA and PKA in our assay, suggesting functional differences between MEIS and PREP proteins and between the MEIS isoforms. Thus, by regulating the extent to which MEIS1A, MEIS1B, or PREP1 form heterotrimers with PBX-HOX (or dimers with PBX), the cell could set high, intermediate, or low levels of response to specific cell signals. Thus, although MEIS and PREP are both present in r4 and are able to bind with HOX and PBX partners to the Hoxb2 r4 enhancer (29, 30), it seems likely that they differentially regulate target gene expression, consistent with the evolutionary selection of
these related but distinct families within the vertebrate genome.

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