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The Homeodomain Proteins PBX and MEIS1 Are Accessory Factors That Enhance Thyroid Hormone Regulation of the Malic Enzyme Gene in Hepatocytes*

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Triiodothyronine (T3) stimulates a robust increase (>40-fold) in transcription of the malic enzyme gene in chick embryo hepatocytes. Previous work has shown that optimal T3 regulation of malic enzyme transcription is dependent on the presence of an accessory element (designated as region E) that immediately flanks a cluster of five T3 response elements in the malic enzyme gene. Here, we have analyzed the binding of nuclear proteins to region E and investigated the mechanism by which region E enhances T3 responsiveness. In nuclear extracts from hepatocytes, region E binds heterodimeric complexes consisting of the homeodomain proteins PBX and MEIS1. Region E contains four consecutive PBX/MEIS1 half-sites. PBX-MEIS1 heterodimers bind the first and second half-sites, the third and fourth half-sites, and the first and fourth half-sites. The configuration conferring the greatest increase in T3 responsiveness consists of the first and fourth half-sites that are separated by 7 nucleotides. Stimulation of T3 response element functions by region E does not require the presence of additional malic enzyme sequences. In pull-down experiments, PBX1a and PBX1b specifically bind the nuclear T3 receptor-α, and this interaction is enhanced by the presence of T3. A T3 receptor-α region containing the DNA binding domain plus flanking sequences (amino acids 21–157) is necessary and sufficient for binding to PBX1a and PBX1b. These results indicate that PBX-MEIS1 complexes interact with nuclear T3 receptors to enhance T3 regulation of malic enzyme transcription in hepatocytes.

Malic enzyme catalyzes the oxidative decarboxylation of malate to pyruvate and CO_2, simultaneously generating NADPH from $NADP^+$. This reaction is the primary source of reducing equivalents for *de novo* synthesis of long chain fatty acids in avian liver (1). Regulation of malic enzyme activity is typical of that of other lipogenic enzymes. Malic enzyme activity increases by 70-fold when previously starved chicks are fed a high carbohydrate, low fat diet and dramatically decreases when animals are starved (2). Nutritional regulation of malic enzyme activity is quantitatively mimicked in primary cultures of chick embryo hepatocytes by manipulating the concentrations of hormones and metabolic fuels in the culture medium. Insulin, glucose, and 3,5,3′-triiodothyronine (T3),[1] humoral factors that are elevated during consumption of a high carbohydrate, low fat diet, increase malic enzyme activity in chick embryo hepatocytes (3, 4). T3 alone stimulates malic enzyme activity; insulin and glucose amplify the action of T3 but have little effect when added by themselves. Glucagon and fatty acids, humoral factors that are elevated during starvation, inhibit the stimulation of malic enzyme activity caused by T3 (3, 5). Alterations in malic enzyme activity caused by nutritional manipulation *in vivo* and nutrients and hormones in culture are mediated primarily by changes in the rate of transcription of the malic enzyme gene (4–7).

In addition to regulation by nutrients and hormones, malic enzyme transcription is controlled in a tissue-specific or cell type-specific manner. In chicks fed a high carbohydrate, low fat diet, malic enzyme transcription is high in liver and low in heart, kidney, and brain (6). Nutritional manipulation has no effect on malic enzyme transcription in heart, kidney, and brain. Cell type-dependent differences in the regulation of malic enzyme are also observed in cells in culture. T3 stimulates a 40-fold or greater increase in malic enzyme transcription in chick embryo hepatocytes, whereas in chick embryo fibroblasts and quail QT6 cells, T3 has little or no effect on malic enzyme transcription (8).

The robust effects of T3 on malic enzyme transcription in chick embryo hepatocytes makes this an excellent system to study the molecular mechanisms by which T3 regulates transcription. T3 activates gene transcription by interacting with nuclear T3 receptors (TR) (9, 10). TRs bind T3 response elements (T3RE) of target genes as homodimers or heterodimers with the retinoid X receptor (RXR). T3REs consist of multiple copies of a hexameric sequence related to a consensus RGGWMA arranged as inverted repeats, everted repeats, direct repeats, or as extended single copies of the hexamer (11). The T3-induced increase in malic enzyme transcription in chick embryo hepatocytes is mediated by at least six T3REs (12–14). One strongly active and four weakly active T3REs are clustered in a 109-bp region located at −3878/−3769 bp relative to the transcription start site. This cluster of T3REs is referred to as a T3 response unit (T3RU). Another weakly active T3RE is located about 700 bp downstream of the T3RU. All of the malic enzyme T3REs consist of directly repeated hexameric half-sites separated by a 4-bp spacer and bind TR-RXR heterodimers in hepatic nuclear extracts.

In addition to T3REs, accessory elements that bind nonre-

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[1] The abbreviations used are: T3, 3,5,3′-triiodo-L-thyronine; TR, nuclear T3 receptor; RXR, retinoid X receptor; CAT, chloramphenicol acetyltransferase; T3RE, triiodothyronine response element; T3RU, triiodothyronine response unit; GST, glutathione *S*-transferase; LBD, ligand binding domain; PCE, PBX-cooperativity element; MCD, MEIS cooperativity domain; C/EBP, CCAAT/enhancer-binding protein; AP-1, activator protein-1; PAGE, polyacrylamide gel electrophoresis; bp, base pair.

ceptor proteins play an important role in mediating the effects of T3 on malic enzyme transcription. We have identified four accessory elements in the malic enzyme gene that confer differences in T3 regulation of malic enzyme transcription between chick embryo hepatocytes and chick embryo fibroblasts (15). Each element enhances T3 responsiveness of the malic enzyme promoter in chick embryo hepatocytes but has no effect on T3 responsiveness in chick embryo fibroblasts. Three of the accessory elements immediately flank the T3RU and are designated regions A, E, and F. The T3RU and accessory elements A, E, and F overlap with a hepatocyte-specific and T3-inducible region of DNase I hypersensitivity in chromatin. Region F (−3703/−3686 bp) binds the liver-enriched factor, CCAAT/enhancer-binding protein-α (C/EBPα). The identities of the proteins that bind region A (−3895/−3890 bp) and region E (−3761/−3744 bp) have not yet been determined. The nature of the proteins that bind region E is of particular interest because mutation of this element causes the largest decrease in T3-induced malic enzyme transcription of the four accessory elements identified.

PBX proteins are members of the 3-amino acid loop extension superclass of homeodomain proteins (16). The *pbx1* gene was first identified as a site of t(1,19) chromosomal translocation leading to the production of an E2a-PBX1 fusion protein in a subset of pre-B cell acute lymphoblastic leukemias in humans (17, 18). Highly conserved homologs of PBX have been identified in *Drosophila melanogaster* (19), *Caenorhabditis elegans* (16), and zebrafish (20). Genetic and biochemical analyses have shown that PBX proteins regulate developmental pathways by serving as cofactors for other homeodomain transcription factors. For example, PBX potentiates the effects of HOX proteins on segmentation along the anterior-posterior axis (21). This effect is mediated by heterodimeric interactions between PBX and HOX. PBX-HOX complexes bind DNA with enhanced specificity and affinity compared with complexes containing PBX or HOX alone (22–24). PBX also binds cooperatively to DNA with other 3-amino acid loop extension class homeodomain proteins including MEIS1 and the closely related factor, PREP1 (also referred to as pKnox) (25–28). Complexes containing PBX-MEIS1 or PBX-PREP1 are components of the transcriptional network controlling cell fate and segmental patterning during embryonic development (29–31).

Recent studies have shown that PBX, MEIS1, and PREP1 also function in maintaining cellular differentiation in adult tissues. For example, PBX-PREP1 heterodimers mediate the pancreatic-specific transcription of the genes for somatostatin and glucagon (32, 33). In the case of the somatostatin gene, PBX-PREP1 stimulates transcription in pancreatic cells by potentiating the transcriptional activity of the pancreatic-specific homeodomain factor, PDX1 (32). In the case of the glucagon gene, PBX-PREP1 binds to a cis-acting element that represses glucagon transcription in non-pancreatic cells without affecting glucagon transcription in pancreatic cells (33). Other work (34) has shown that a PBX-MEIS-PDX1 trimeric complex activates transcription of the elastase I gene in pancreatic acinar cells via interactions with the acinar cell-specific factor, PTF1. Still other studies have shown that PBX-PREP1 heterodimers interact with a cis-acting element that confers cell type-specific transcription of the human α2(V) collagen gene (35). In addition to the pancreas and tissues that express α2(V) collagen, PBX and MEIS1/PREP1 proteins are expressed at significant levels in other adult tissues, including liver (36–40). The physiological role of these proteins in liver has not been established.

In the present study, we have analyzed the binding of nuclear proteins to malic enzyme region E in hepatocytes. We show that this TR accessory element binds PBX-MEIS1 heterodimers in multiple configurations. In addition, we have developed data suggesting that the stimulation of T3-induced malic enzyme transcription by region E is mediated by direct interactions between PBX and TR.

EXPERIMENTAL PROCEDURES

Plasmids—The DNA fragments used to construct reporter plasmids were named by designating the 5′ and 3′ ends of each fragment relative to the start site of transcription of the malic enzyme gene. p[ME−3921/−3631]ME−147/+31CAT has been described previously (15). This reporter plasmid contains the malic enzyme T3RU and flanking sequences (−3921 to −3631 bp) linked to the minimal promoter of the malic enzyme gene (−147 to +31 bp). Site-directed mutations were introduced into p[ME−3921/−3631]ME−147/+31CAT using a polymerase chain reaction-based strategy (41). p[T3RE-2]ME−147/+31CAT was made by subcloning the major malic enzyme T3RE between −3883 and −3858 bp into the *Bam*HI/*Sac*I site upstream of the minimal promoter in ME−147/+31CAT. p[T3RE-2+Region E]ME−147/+31CAT was constructed by inserting region E (ME−3767/−3742) into the *Spe*I/*Sma*I site immediately downstream of the of T3RE-2 in p[T3RE-2]ME−147/+31CAT. Dr. M. Kamps (University of California, San Diego) provided the cDNAs for human PBX1a and PBX1b. The cDNA for mouse MEIS1 was obtained by Dr. N. Copeland (NCI-Frederick Cancer Research and Development Center). Drs. H. Chen and S. Antonarakis (University of Geneva) supplied the cDNA for human PREP1. cDNAs for full-length PBX1a (PBX1a-(1–430)), PBX1b (PBX1b-(1–347)), MEIS1 (MEIS1-(1–390)), and PREP1 (PREP1-(1–436)) and human RXRα (RXRα-(1–462)) were subcloned into pSV-SPORT1 (Life Technologies, Inc.). Expression plasmids containing N-terminal truncations of PBX1a (PBX1a-(80–430) and PBX1a-(119–430)) were provided by Dr. C. Murre (University of California, San Diego). The full-length cDNA of chicken TRα corresponding to amino acids 1–408 (TRα-(1–408)) was subcloned into pGEM-3Zf(−) (Promega). N-terminal and C-terminal deletion derivatives of chicken TRα were generated by polymerase chain reaction. Polymerase chain reaction products encoding TRα polypeptides containing amino acids 1–118, 1–157, 21–408, 51–408, and 120–408 were subcloned into pGEM-3Zf(−). To generate plasmids that express fusion proteins containing glutathione *S*-transferase (GST) linked to PBX1a, PBX1b, or TRα, cDNAs containing the full-length coding region of PBX1a, PBX1b, or TRα were subcloned into pGEX-2T (Amersham Pharmacia Biotech). Structures of reporter plasmids and expression plasmids were confirmed by restriction enzyme mapping and nucleotide sequence analyses.

Cell Culture and Transient Transfection—Primary cultures of chick embryo hepatocytes were prepared as described previously (42) and maintained in serum-free Waymouth medium MD705/1 containing 50 nM insulin (gift from Lilly) and corticosterone (1 μM). Hepatocytes were incubated at 40 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were transfected using a modification of the method of Baillie *et al.* (43). Briefly, chick embryo hepatocytes were isolated as described above and plated on 35-mm dishes. At 18 h of incubation, the medium was replaced with one containing 20 μg of LipofectACE (Life Technologies, Inc.), 3.0 μg of p[ME−3921/−3631]ME−147/+31CAT, or an equimolar amount of another reporter plasmid and 0.05 μg of pCMV-β-galactosidase. At 42 h of incubation, the transfection medium was replaced with fresh medium with or without T3 (1.5 μM). At 90 h of incubation, chick embryo hepatocytes were harvested, and cell extracts were prepared for CAT (44) and β-galactosidase (45) measurements. CAT activity was expressed relative to β-galactosidase activity to correct for differences in transfection efficiency between samples. All DNAs used in transfection experiments were purified using the Qiagen endotoxin-free kit.

Gel Mobility Shift Analysis—Nuclear extracts were prepared from chick embryo hepatocytes incubated with or without T3 for 24 h. Nuclei and nuclear extracts were prepared as described (46) except that the protease inhibitors leupeptin (0.25 μg/ml), benzamidine (10 mM), and PMSF (0.5 mM) were added to the extraction and dialysis buffers at the indicated concentrations. PBX1a, PBX1b, MEIS1, PREP1, RXRα, and TRα were translated *in vitro* using the TNT SP6-coupled reticulocyte lysate system (Promega). Incorporation of [^{35}S]methionine into PBX1a, PBX1b, MEIS1, PREP1, RXRα, and TRα was measured in parallel reactions in order to assess the relative efficiency of synthesis of the different transcription factors.

Double-stranded oligonucleotides were prepared by combining equal amounts of the complementary single-stranded DNA in a solution con-

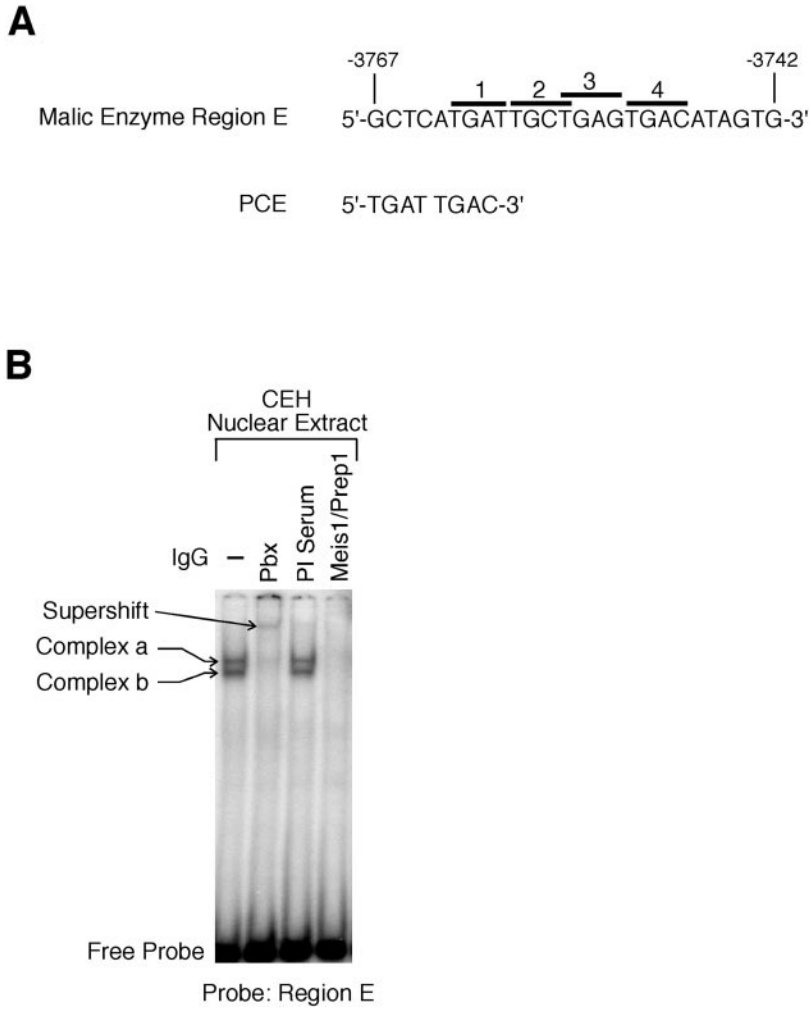

FIG. 1. **Characterization of proteins that bind to region E using the gel mobility shift assay.** *A,* comparison of the sequence of malic enzyme region E with the PBX cooperativity sequence (*PCE*). The four potential tetrameric half-sites are *overlined*. *B,* gel mobility shift experiments were performed using nuclear extracts prepared from chick embryo hepatocytes (*CEH*). A double-stranded DNA fragment containing region E (−3767 to −3742 bp) was labeled with [α-^{32}P]dCTP using the Klenow fragment of *E. coli* DNA polymerase. The radiolabeled probe was incubated with nuclear extract as described under "Experimental Procedures." DNA and DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels. Nuclear extracts were incubated with antibodies against PBX and MEIS1/PREP1 prior to the addition of the probe. Positions of the specific DNA-protein complexes and supershifted complexes are indicated by *arrows*. These data are representative of three different experiments employing independent preparations of nuclear extract. *PI serum,* preimmune serum.

taining 10 mM Tris, pH 8.0, 1 mM EDTA followed by heating to 95 °C for 2 min and then cooling to room temperature. The annealed oligonucleotides were labeled by filling in overhanging 5′ ends using the Klenow fragment of *Escherichia coli* DNA polymerase in the presence of [α-^{32}P]dCTP. Binding reactions were carried out in 20 μl containing 18 mM HEPES, pH 7.9, 90 mM KCl, 0.18 mM EDTA, 0.45 mM dithiothreitol, 18% glycerol (v/v), 0.3 mg/ml bovine serum albumin, and 2 μg of poly(dI-dC). A typical reaction contained 20,000 cpm of labeled DNA and 10 μg of nuclear extract or 2 μg of *in vitro* translated proteins. The reaction was carried out on ice for 60 min. DNA and DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels at 4 °C in 0.5× TBE (45 mM Tris, pH 8.3, 45 mM boric acid, 1 mM EDTA). Following electrophoresis, the gels were dried and subject to storage phosphor autoradiography. For competition experiments, unlabeled competitor DNA was mixed with radiolabeled oligomer prior to addition of nuclear extract. For gel supershift experiments, nuclear extracts or *in vitro* translated proteins were incubated with antibodies for 1 h at 0 °C prior to addition of the oligonucleotide probe. An antiserum that reacts with all isoforms of PBX (PBX1, -2, and -3) (47) was generously provided by Dr. M. Kamps (University of California, San Diego). A polyclonal antibody that reacts with MEIS1 and PREP1 (sc-6245) was purchased from Santa Cruz Biotechnology. Synthetic oligonucleotides that were used as probes or competitors in gel mobility shift assays are listed in Fig. 3.

Protein-Protein Interactions—GST or GST fusion proteins were expressed in *E. coli* (BL21, pLysS) and purified using standard techniques (48). Briefly, bacteria were transformed with pGEX-2T or recombinant pGEX-2T plasmids expressing GST fusion proteins. Overnight bacterial cultures in ampicillin (250 μg/ml) were diluted 1:100 into 250 ml of Luria broth and grown at 37 °C to an A_{600} = 1.0 before induction with 1 mM isopropylthiogalactopyranoside for 60 min. Cells were pelleted and resuspended in 5 ml of buffer A (50 mM KCl, 25 mM HEPES, pH 7.9, 6% glycerol, 5 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.05% Triton X-100). Cells were lysed on ice by sonication and centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was mixed for 1 h at 4 °C on a rotator with 0.5–1 ml of 50% glutathione-Sepharose beads (Amersham Pharmacia Biotech) that were preswollen in buffer A. After absorption, beads were collected by centrifugation at 4 °C and washed three times with 1 ml of buffer A. Fusion proteins coupled to the glutathione-Sepharose beads were stored at 4 °C as 50% (v/v) slurry in buffer A. The concentrations and sizes of GST and GST fusion proteins were estimated by SDS-PAGE, using a known quantity of molecular weight standards.

L-[^{35}S]Cysteine- or L-[^{35}S]methionine-labeled proteins were prepared by using TNT reticulocyte lysates (Promega). Approximately 2.5 × 10^4 to 5 × 10^4 cpm of ^{35}S-labeled protein were incubated with 100 ng of GST fusion protein immobilized on glutathione-Sepharose beads in 300 μl of buffer A for 1 h at 4 °C on a rotator. Beads were collected by centrifugation at 4 °C and washed three times with 1 ml of buffer A. The bound proteins were eluted with SDS-gel loading buffer and analyzed by SDS-PAGE followed by storage phosphor autoradiography. Where indicated 1 μM T3 or 9-*cis*-retinoic acid was included in the binding reaction mixture. ^{35}S-Labeled proteins were analyzed by electrophoresis and storage phosphor autoradiography to ensure that similar levels of input radioactivity of the labeled protein were used in the GST binding assays.

RESULTS

PBX/MEIS1 Heterodimers Bind Region E of the Malic Enzyme Gene—Previous work (25–28) has shown that PBX binds DNA as heterodimers with other homeodomain factors such as MEIS1 and PREP1 in terminally differentiated cells. PBX, MEIS1, and PREP1 interact with 4-bp sequence elements referred to as half-sites. Selection studies with degenerate oligonucleotides have shown that PBX-MEIS1 and PBX-PREP1 heterodimers bind two contiguous half-sites with the consensus sequence, 5′-TGAT-TGAC-3′, in which PBX contacts the upstream half-site and MEIS1/PREP1 contacts the downstream half-site (Fig. 1*A*) (27, 47). This sequence is referred to as the

PBX-cooperativity element (PCE). Analysis of the sequence in region E indicated the presence of four tetrameric sequence elements that are identical or strongly resemble binding sites for PBX, MEIS1, and PREP1. These half-sites are designated 1, 2, 3, and 4. To investigate whether PBX heterodimers interacted with region E, gel mobility shift experiments were performed using antibodies against PBX and MEIS1/PREP1. Incubation of a ^{32}P-labeled oligonucleotide probe containing region E with nuclear extract from chick embryo hepatocytes resulted in the formation of two protein-DNA complexes that were designated a and b in the order of increasing mobility (Fig. 1*B*). The pattern of protein binding to region E was similar in hepatocytes incubated in the absence and presence of T3 (data not shown) (15). Incubation of hepatocyte nuclear extracts with antiserum against all isoforms of PBX completely disrupted the formation of complex a and b, indicating that these complexes contained PBX or a highly related factor. Incubation of nuclear extracts with an antibody that reacts with MEIS1 and PREP1 also completely disrupted the formation of complex a and complex b. These data indicate that complex a and complex b contain PBX-MEIS1 and/or PBX-PREP1 heterodimers. Further evidence that PBX-MEIS1 and/or PBX-PREP1 bound region E in hepatocytes was obtained from competition experiments. An unlabeled oligonucleotide containing the PCE was as effective as an unlabeled oligonucleotide containing region E in competing for the binding of complex a and complex b (Fig. 3*B*).

The ability of region E to bind heterodimers containing PBX-MEIS1 and PBX-PREP1 was confirmed by gel mobility shift experiments employing *in vitro* synthesized transcription factors. At least 5 isoforms of PBX have been identified that are derived from three different genes (17, 18, 36). PBX1a, PBX2, and PBX3a are products of the *pbx1*, *pbx2*, and *pbx3* genes, respectively. PBX1b and PBX3b are variants that arise from alternative processing. As PBX1a and PBX1b are expressed in hepatic cells (36–38), we investigated whether these proteins bound region E in the absence or presence of MEIS1 and PREP1. Incubation of *in vitro* translated human PBX1a, human PBX1b, mouse MEIS1, or human PREP1 with the region E probe resulted in no specific binding activity (Fig. 2). Inclusion of MEIS1 in the binding reactions with PBX1a and PBX1b stimulated the formation of high affinity protein-DNA complexes containing PBX1a-MEIS1 and PBX1b-MEIS1, respectively. Inclusion of PREP1 in the binding reactions with PBX1a and PBX1b also stimulated the formation of high affinity heterodimeric complexes. Thus, PBX1a and PBX1b bind region E cooperatively with MEIS1 or PREP1. Cooperative interactions between PBX1 and MEIS1 and between PBX1 and PREP1 have been reported for other PBX-binding sites (25–28, 32, 33). The mobilities of the protein-DNA complexes containing PBX1a-PREP1 and PBX1b-PREP1 were slower than the mobilities of corresponding complexes containing PBX1a-MEIS1 and PBX1b-MEIS1. This was due to the larger size of PREP1 (436 amino acids) relative to MEIS1 (390 amino acids). Interestingly, the mobility of complex a in hepatocyte nuclear extracts was identical to that of PBX1a-MEIS1 and the mobility of complex b was identical to that of PBX1b-MEIS1. Assuming that the sizes of chicken PBX1a, PBX 1b, MEIS1, and PREP1 are similar to their corresponding mammalian homologs, we propose that complex a and complex b contain PBX1a-MEIS1 and PBX1b-MEIS1, respectively. Complex a may also contain PBX2-MEIS1 heterodimers, as PBX2 is approximately the same size of PBX1a, is expressed in liver, and binds DNA cooperatively with MEIS1 (25, 27, 36). Because expression of PBX3a and PBX3b in liver is very low compared with that of PBX1 and PBX2 (36), the former proteins are not likely to be present in complex a and complex b.

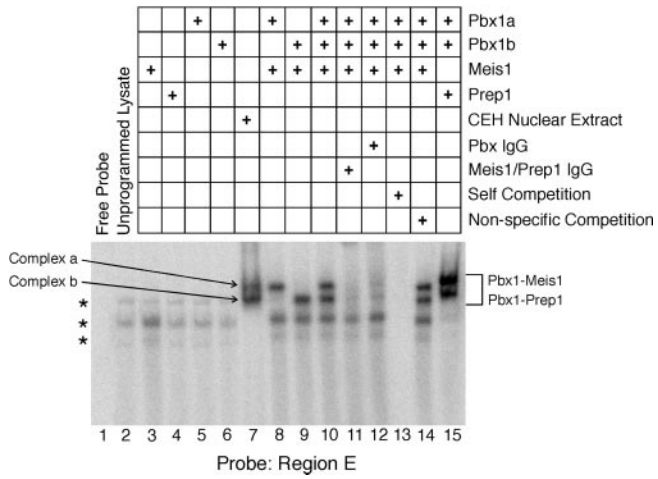

FIG. 2. **PBX1a and PBX1b bind region E cooperatively with MEIS1 or PREP1.** Gel mobility shift assays were performed as described under "Experimental Procedures" using ^{32}P-labeled region E (−3767 to −3742 bp) as the probe. In *lanes 3–6* and *lanes 8–15*, equimolar amounts of *in vitro* translated PBX1a or PBX1b were incubated in the absence or presence of *in vitro* translated MEIS1 or PREP1 as indicated. In *lane 7*, the ^{32}P-labeled probe was incubated with nuclear extract from chick embryo hepatocytes (*CEH*). In *lanes 11* and *12*, *in vitro* synthesized proteins were incubated with antibodies against PBX or MEIS1/PREP1 prior to the addition of the probe. In *lanes 13* and *14*, competition analysis was performed with a 100-fold molar excess of unlabeled competitor DNA. *Arrows* indicate the binding of complex a and complex b in chick embryo hepatocytes nuclear extracts. The *bracket* indicates the binding of specific complexes formed from *in vitro* translated proteins. *Asterisks* indicate the binding of endogenous complexes in lysates.

Identification of Sequences in Region E That Bind PBX/MEIS1 Heterodimers in Nuclear Extracts from Hepatocytes—In the bovine *cyp17* gene, PBX-MEIS1 interacts with a cAMP-responsive sequence (CRS-1) that consists of tandem 4-bp half-sites with no spacer between the half-sites (28). Studies with artificial sequence elements have shown that PBX1-MEIS1 can also bind tandem half-sites that are separated by 3 or 6 nucleotides (49). The sequence in region E contains five possible binding configurations for PBX-MEIS1 heterodimers (Fig. 3*A*). Two of the binding configurations are composed of two half-sites with no spacer (*i.e.* sites 1 and 2 and sites 3 and 4), another two binding configurations are composed of two half-sites separated by a 3-bp spacer (*i.e.* sites 1 and 3 and sites 2 and 4), and one binding configuration contains two half-sites separated by a 7-bp spacer (*i.e.* sites 1 and 4). To determine which of these configurations bind complex a and complex b, competition experiments were performed with oligonucleotide competitors containing mutations in specific half-sites. Mutation of site 1 (mut E 1), site 2 (mut E 2), or site 3 (mut E 3) had little or no effect on the binding affinity of complex a and complex b, whereas mutation of site 4 (mut E 4) decreased the binding affinity of complex a and complex b (Fig. 3*B*). These results suggest that complex a and complex b bind region E in two or more configurations involving site 4. To delineate further the region E binding configurations, competition analyses were conducted with oligonucleotides containing specific mutations in two of the four half-sites. Mutation of sites 1 and 3 (mut E 1/3), sites 1 and 4 (mut E 1/4), and sites 2 and 4 (mut E 2/4) abolished protein binding to region E. Mutation of sites 3 and 4 (mut E 3/4) inhibited but did not abolish the binding of complex a and complex b to region E. Mutation of sites 1 and 2 and sites 2 and 3 had little or no effect on protein binding activity. Collectively, these data indicate that complex a and complex b

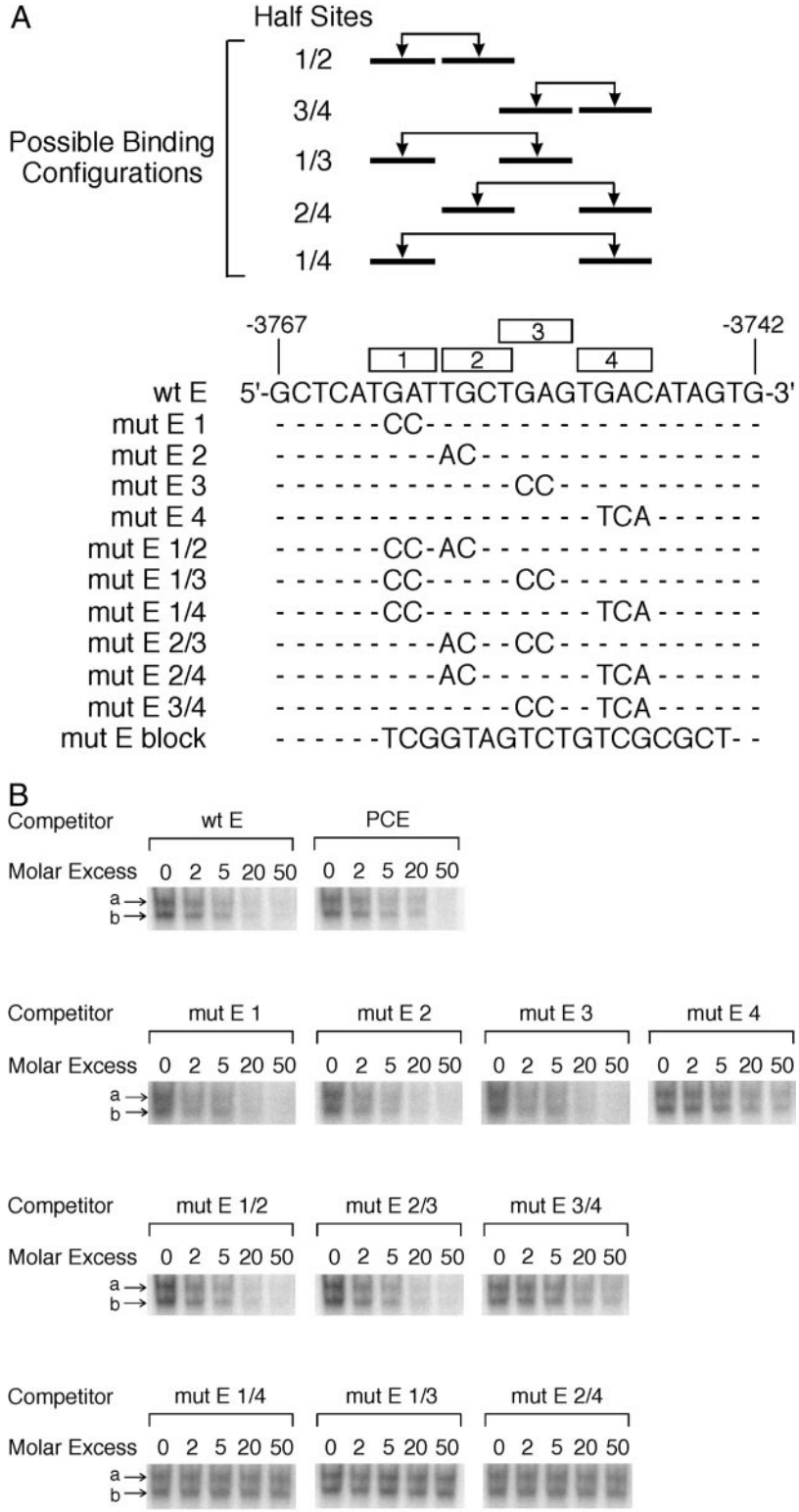

FIG. 3. **Complex a and complex b bind region E in multiple configurations.** Gel mobility shift experiments were performed as described under "Experimental Procedures" using ^{32}P-labeled region E (−3767 to −3742 bp) as a probe and nuclear extracts prepared from chick embryo hepatocytes. Nuclear extracts were incubated with the region E probe in the presence of different concentrations of unlabeled competitor DNAs. Each reaction contained 10 μg of nuclear protein. DNA and DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels. *A,* possible binding configurations of PBX-MEIS1 on region E. The position of putative tetrameric half-sites in region E are indicated by the *boxes above* the wild-type sequence for region E. The half-sites are labeled *1–4*. The sequence of oligonucleotide competitors containing mutations of one or more half-sites is also shown. Mut 1 to mut 4 refer to the number of the half-site that was mutated in each oligonucleotide. The sequence of the PCE is shown in Fig. 1*A*. *B,* competition analysis with native and mutant forms of region E. Unlabeled competitor DNAs (2-, 5-, 20-, or 50-fold molar excess) were mixed with the radiolabeled probe prior to the addition of nuclear extract. Complex a and complex b are indicated by *arrows*. These data are representative of four experiments employing independent preparations of nuclear extract.

bind region E in the following configurations: half-sites 1 and 2, half-sites 1 and 4, and half-sites 3 and 4. The binding affinity of complex a and complex b for half-sites 1 and 2 is lower than that for half-sites 1 and 4 and half-sites 3 and 4. Competition analysis with region E mutants indicated that *in vitro* synthesized PBX1a-MEIS1 and PBX1b-MEIS1 bound region E in a manner identical to that of complex a and complex b (data not shown). These data are consistent with the proposal that complex a and complex b contain PBX1a-MEIS1 and PBX1b-MEIS1, respectively.

Each of the PBX1-MEIS1 binding configurations on region E overlaps with each other suggesting that complex a and complex b are dimeric rather than tetrameric complexes. To confirm this supposition, gel mobility shift experiments were performed with region E probes that contained mutations in half-sites 1 and 2 (mut E 1/2) or half-sites 2 and 3 (mut E 2/3). Hepatic nuclear proteins and *in vitro* synthesized PBX1a, PBX1b, and MEIS1 bound both of these probes in a pattern identical to that of the wild-type region E probe (data not shown). Thus, protein binding to region E represents dimers of

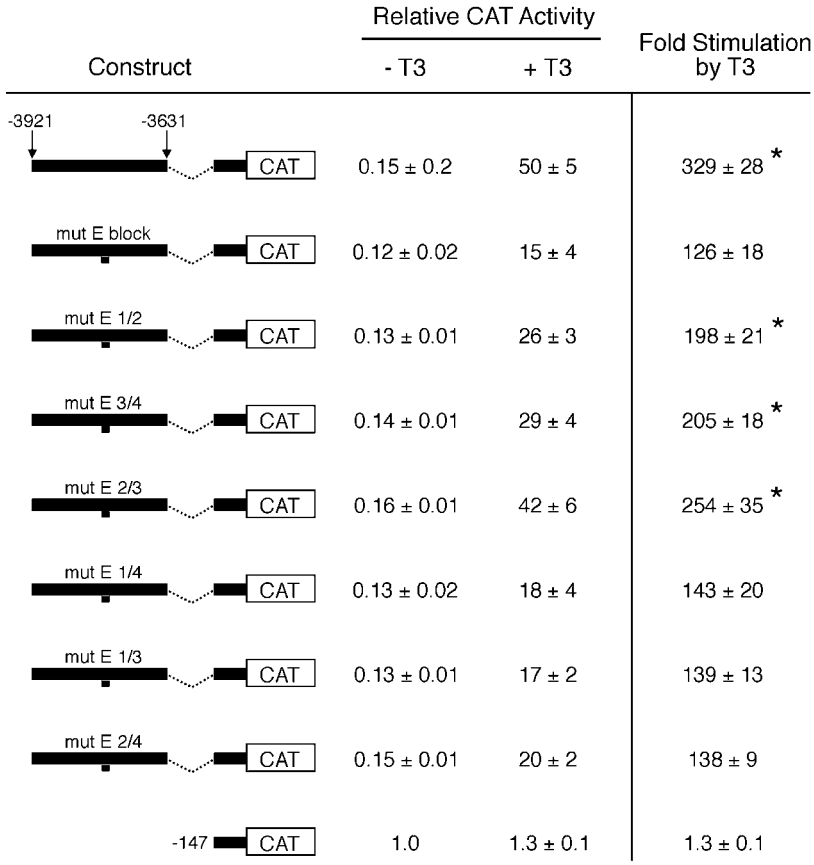

FIG. 4. **Stimulation of T3 responsiveness by region E is correlated with complex a and complex b binding activity.** p[ME−3921/−3631]ME−147/+31CAT or constructs containing mutations of region E in the context of p[ME−3921/−3631]ME−147/+31CAT were transiently transfected into chick embryo hepatocytes as described under "Experimental Procedures." Transfections also contained CMV-β-galactosidase as a control for transfection efficiency. After the transfection, cells were treated with or without T3 for 48 h. Cells were then harvested, extracts prepared, and CAT and β-galactosidase assays performed. *Left,* the constructs used in these experiments. Numbers indicate the 5′ or 3′ boundaries of malic enzyme DNA in nucleotides relative to the start site for transcription. The mutated sequences in region E are shown in Fig. 3*A*. *Right,* CAT activity was expressed relative to β-galactosidase activity to correct for differences in transfection efficiency. CAT activity in cells transfected with pME−147/+31CAT and incubated without T3 was set at 1, and the other activities were adjusted proportionately. The -fold stimulation by T3 was calculated by dividing CAT activity in hepatocytes treated with T3 (+*T3*) by that for hepatocytes not treated with T3 (−*T3*). The -fold responses were calculated for individual experiments and then averaged. The results are the means ± S.E. of 5 experiments. *, the -fold stimulation by T3 was significantly higher than that of cells transfected with p[ME−3921/−3631] ME−147/+31CAT containing mut E block ($p < 0.05$).

PBX1a, PBX1b, and MEIS1 and not higher order structures of these proteins.

Next, we investigated the effects of site-specific mutations of region E on T3 responsiveness in chick embryo hepatocytes. Hepatocytes were transiently transfected with reporter constructs containing mutations of two of the four half-sites of region E in the context of p[ME−3921/−3631]ME−147/+31CAT. A block mutation of region E extending from −3761 to −3744 (mut E block, Fig. 3*A*) inhibited T3 responsiveness by 58% (Fig. 4). This effect was due to a decrease in promoter activity in the presence of T3. Site-specific mutations that abolished protein binding to region E (*i.e.* mut E 1/3, mut E 1/4, and mut E 2/4) also inhibited T3 responsiveness, and the extent of this inhibition was similar to that of the block mutation of region E. Thus, site-specific mutations that blocked protein binding to region E also blocked the ability of region E to stimulate T3 responsiveness. Site-specific mutations that had no effect (*i.e.* mut E 1/2 and mut E 2/3) or partially inhibited (*i.e.* mut E 3/4) protein binding to region E conferred a higher level of T3 responsiveness than mutations that abolished protein binding to region E (*i.e.* mut E block, mut E 1/3, mut E 1/4, and mut E 2/4). Mut E 2/3 conferred the highest level of T3 responsiveness of all of the mutations examined. These data indicate that all three PBX-MEIS1 binding configurations on region E are capable of enhancing T3 responsiveness directed by the upstream region of the malic enzyme gene and that binding to half-sites 1 and 4 results in the greatest stimulation of T3 responsiveness. Interestingly, the latter configuration perfectly matches the consensus binding sequence for PBX1-MEIS1 heterodimers (*i.e.* TGATTGAC) (26, 27) except that the tetrameric half-sites are separated by a 7-bp spacer. This is the first description of a native PBX1-MEIS1-binding sequence with this arrangement of half-sites.

Region E Alone Can Enhance T3 Regulation Directed by a T3RE—PBX-MEIS1 bound to region E (−3762/−3748 bp) may enhance T3 regulation of malic enzyme transcription by interacting with TR-RXR heterodimers bound to the T3RU (−3878/−3769 bp). Alternatively, PBX-MEIS1 may enhance T3 responsiveness by interacting with other TR accessory factors bound to sequences flanking the T3RU. For example, PBX-MEIS1 may interact with C/EBPα bound to region F (−3703/−3686 bp). To assess the role of these possible mechanisms of PBX-MEIS1 action, transient transfection experiments were conducted to determine whether region E alone could enhance T3 regulation of transcription conferred by a T3RE. Within the malic enzyme T3RU is a strongly active T3RE designated as T3RE-2 (−3878/−3863 bp). When hepatocytes were transfected with a reporter plasmid containing T3RE-2 linked to the minimal promoter of the malic enzyme gene (p[T3RE-2]ME−147/+31CAT), T3 stimulated a 10-fold increase in promoter activity

Construct	Relative CAT Activity - T3	Relative CAT Activity + T3	Fold Stimulation by T3
T3RE-2 | ME-147/+31 | CAT	1.0	10 ± 0.3	10 ± 0.3
T3RE-2 | Region E | ME-147/+31 | CAT	1.6 ± 0.2	75 ± 14	47 ± 5 *
T3RE-2 | mut E block | ME-147/+31 | CAT	0.9 ± 0.1	9 ± 2	10 ± 0.6

FIG. 5. **Region E alone can enhance T3 regulation directed by a T3RE.** Oligonucleotides containing region E (−3767 to −3742 bp) or a block mutation of region E (mut E block) were inserted downstream of malic enzyme T3RE-2 in p[T3RE-2]ME−147/+31CAT. Hepatocytes were transiently transfected with these constructs and treated with or without T3 as described in the legend to Fig. 4 and under "Experimental Procedures." *Left*, constructs used in these experiments. The sequence for region E and mut E block are shown in Fig. 3*A*. *Right*, CAT activity in cells transfected with p[T3RE-2]ME−147/+31CAT and treated without T3 was set at 1, and the other activities were adjusted proportionately. The -fold stimulation by T3 was calculated as described in the legend in Fig. 4. The results are the means ± S.E. of 5 experiments. *, the -fold stimulation by T3 for p[T3RE-2+Region E]ME−147/+31CAT was significantly higher than that of any other construct ($p < 0.05$).

(Fig. 5). When a DNA fragment containing region E (−3767/−3742 bp) was inserted downstream of T3RE-2 in p[T3RE-2]ME−147/+31CAT, the T3-induced stimulation of promoter activity was increased to 47-fold. The increase in T3 responsiveness caused by region E was due to an increase in promoter activity in the presence of T3. Insertion of DNA fragment containing mut E block downstream of T3RE-2 in p[T3RE-2]ME−147/+31CAT had no effect on T3 responsiveness, indicating that the increase in T3 responsiveness caused by region E was not due to changes in the spacing between T3RE-2 and the minimal malic enzyme promoter. Region E also enhanced T3 responsiveness when it was ligated downstream to the malic enzyme T3RU in p[ME−3921/−3768]ME−147/+31CAT (data not shown). These data indicate that the stimulation of T3-induced malic enzyme transcription caused by region E is mediated, at least in part, by interactions between PBX-MEIS1 and TR-RXR.

PBX1 Physically Interacts with TR—Previous studies have demonstrated that PBX-MEIS1 heterodimers function as cofactors for other transcription factors (50). For example, PBX1-MEIS1 bound to a PCE strongly augments transcriptional activation by MyoD or myogenin containing complexes bound to an adjacent E box motif. This effect of PBX1-MEIS1 is mediated, in part, by direct interactions between the PBX1 homeodomain and a highly conserved tryptophan motif flanking the basic helix-loop-helix domain of MyoD and myogenin. These findings coupled with the close proximity of region E to the T3RU led us to hypothesize that PBX1-MEIS1 enhanced T3 responsiveness of malic enzyme transcription by directly interacting with TR-RXR. To investigate this hypothesis, we determined whether PBX1 or MEIS1 physically interacted with TR and RXR using a pull-down assay. In our initial experiments, we investigated the ability of *in vitro* synthesized TRα and RXRα to bind a bacterially expressed fusion protein containing glutathione *S*-transferase (GST) linked to PBX1a. ^{35}S-Labeled TRα bound to GST-PBX1a, and the presence of T3 enhanced this interaction (Fig. 6*B*). ^{35}S-Labeled TRα also interacted with GST-PBX1b in a T3-regulated manner (Fig. 6*C*). Inclusion of unlabeled RXRα in the binding reaction had no effect on the interaction of ^{35}S-labeled TRα with GST-PBX1a and GST-PBX1b (data not shown). No interaction was observed between ^{35}S-labeled TRα and GST. In contrast to the results for TRα, little or no interaction was observed between ^{35}S-labeled RXRα and GST-PBX1a (Fig. 6*B*) and between ^{35}S-labeled RXRα and GST-PBX1b (Fig. 6*C*). The lack of interaction between RXRα and PBX1 proteins was confirmed by pull-down experiments employing GST-RXRα as the bait and ^{35}S-labeled PBX1a and PBX1b synthesized *in vitro* (data not shown). These data indicate that PBX1a and PBX1b selectively interact with TR of the TR-RXR heterodimeric complex.

Pull-down assays were also carried out using GST-TRα as the bait protein. Consistent with results of experiments employing GST-PBX1a and GST-PBX1b as bait proteins, ^{35}S-labeled PBX1a and PBX1b strongly interacted with GST-TRα (Fig. 7). In contrast, a very weak interaction was observed between ^{35}S-labeled MEIS1 and GST-TRα in the absence or presence of unlabeled PBX1a. These data further demonstrate that interactions between PBX1 proteins and TRα are specific. In contrast to results of experiments analyzing interactions between GST-PBX1 proteins and ^{35}S-labeled TRα, interactions between GST-TRα and ^{35}S-labeled PBX1 proteins were not affected by the presence of T3. The latter observation suggests that appending GST to the N terminus of TRα blocks ligand-induced conformational changes that facilitate interactions between TRα and PBX1 proteins.

To map the motifs in TRα that interact with PBX1a, pull-down experiments were conducted using various truncations of TRα labeled with ^{35}S *in vitro*. Deletion of the first 20 amino acids from the N terminus of TRα had little or no effect on the binding of TRα to GST-PBX1a (Fig. 8). Further deletion to amino acid 51 decreased the binding of TRα to GST-PBX1a. When deletion of the N terminus of TRα was extended to amino acid 120, binding of TRα to GST-PBX1a was abolished. To analyze further the interaction between TRα and PBX1a, TRα polypeptides containing amino acids 1–157, amino acids 1–118, and amino acids 51–157 were tested for their ability to interact with GST-PBX1a. A strong interaction was observed between GST-PBX1a and a TRα polypeptide containing amino acids 1–157. Markedly weaker but detectable interactions were observed between GST-PBX1a and TRα polypeptides containing amino acids 1–118 and amino acids 51–157. GST-PBX1b bound to TRα truncations in a manner similar to that of GST-PBX1a (data not shown). Collectively, these data suggest that a TRα region containing the DNA binding domain plus flanking sequences (amino acids 21–157) is required for optimal binding to PBX1a and PBX1b. Interestingly, interactions between GST-PBX1 fusion proteins and TRα truncations lacking the ligand binding domain were not enhanced by the presence of T3. This observation is consistent with the scenario that T3 binding to the ligand binding domain of TRα causes a conformational

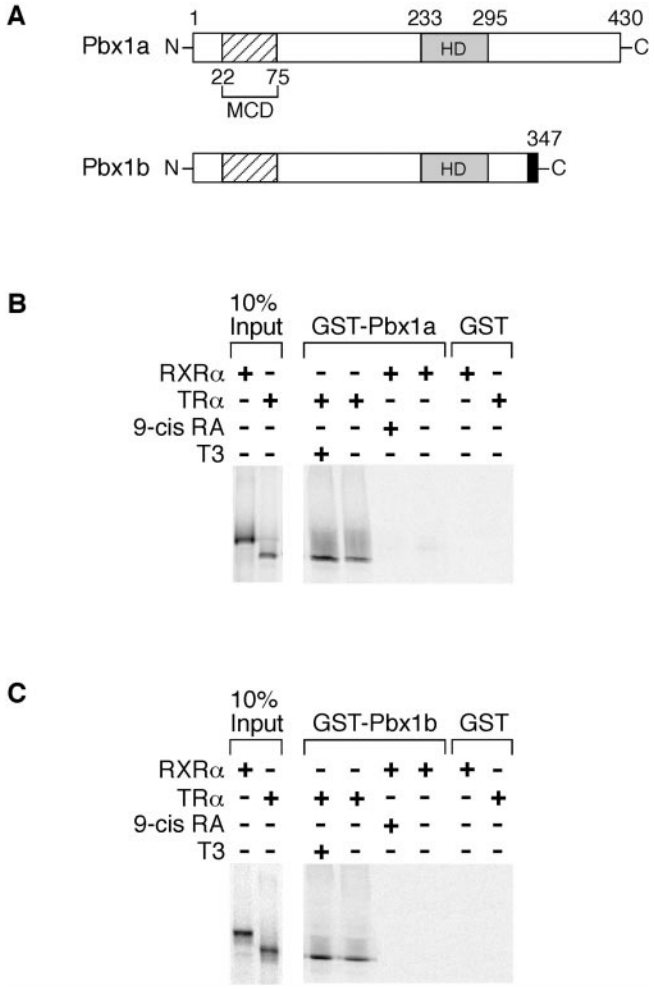

FIG. 6. **TRα interacts with PBX1a and PBX1b in a T3-regulated manner.** *A,* schematic representation of PBX1a and PBX1b. The MEIS cooperativity domain (*MCD*) and homeodomain (*HD*) region are indicated. *B,* TRα interacts with GST-PBX1a. Bacterially produced GST or GST-PBX1a immobilized on glutathione-Sepharose beads was incubated with *in vitro* translated and ^{35}S-labeled TRα or RXRα as described under "Experimental Procedures." Incubations were performed in the absence and presence of 1 μM T3 or 1 μM 9-*cis*-retinoic acid (*9-cis RA*) as indicated. After the matrix was extensively washed, labeled proteins retained on the beads were eluted, resolved by SDS-PAGE, and visualized by storage phosphor autoradiography together with 10% of the total radiolabeled receptor input used in each binding reaction. *C,* incubations were performed as described in *B* except that GST-PBX1b was used as the bait protein. These data are representative of four experiments.

change that enhances the ability of the N-terminal region of TRα to interact with PBX1a and PBX1b.

Previous studies have shown that heterodimeric interactions between PBX1 and MEIS1 are mediated by a PBX1 region containing a 3,4-isoleucine heptad repeat (26). This region is designated as the MEIS cooperativity domain (MCD) and extends from amino acids 22 to 75 (Fig. 6*A*). To investigate whether TRα interacted with a PBX1 region that was separate from the MCD, we determined the ability of N-terminal truncations of PBX1a to interact with GST-TRα. Deletion of the first 79 or 118 amino acids of PBX1a had no effect on the binding of PBX1a to GST-TRα (data not shown). These data indicate that TRα interacts with a PBX1 region that is distinct from that mediating heterodimeric interactions with MEIS1.

DISCUSSION

Protein complexes containing PBX and MEIS1/PREP1 play a critical role in embryonic development in both invertebrates and vertebrates (29–31). Although less is known about the functions of PBX-MEIS1/PREP1 in adult tissues, data from recent studies suggest that these complexes are involved in controlling endocrine function. For example, PBX-MEIS1 complexes mediate the stimulatory effects of cAMP on transcription of 17α-hydroxylase cytochrome P450, a steroid hydroxylase involved in the production of steroid hormones from cholesterol (28, 37, 51). Other studies have shown that PBX-PREP1 complexes are involved in mediating the pancreatic-specific expression of the genes for somatostatin (32) and glucagon (33). Still other studies have demonstrated that interactions between PBX complexes and the glucocorticoid receptor mediate the inhibitory effect of glucocorticoids on prolactin transcription in non-pituitary cell lines (38). In the present study, we show that PBX-MEIS1 complexes interact with TR-RXR complexes to enhance T3 regulation of malic enzyme transcription in avian hepatocytes. These studies are the first to establish a role for PBX-MEIS1 in the control of thyroid hormone action.

Data from transfection (Figs. 4 and 5) and protein binding assays (Figs. 6 and 7) suggest that stimulation of T3-induced malic enzyme transcription by region E is mediated by direct interactions between PBX-MEIS1 and TR-RXR. Due to the close proximity of region E to the T3RU, PBX-MEIS1 may bind the malic enzyme gene as a tetrameric complex with TR-RXR. In support of this possibility, peptide sequences mediating the interaction between PBX1a/b and TRα are distinct from those mediating interactions between PBX1a/b and MEIS1 (data not shown) and between TRα and RXRα (Fig. 8). Furthermore, in gel mobility shift assays, *in vitro* synthesized TRα, RXRα, PBX1a/b, and MEIS1 form a complex containing all four proteins on an oligonucleotide probe consisting of T3RE-2 linked to region E (data not shown). Because the formation of this tetrameric complex is not associated with any changes in the total binding of TRα-RXRα and PBX1a/b-MEIS1 to the probe, the mechanism by which region E modulates T3 responsiveness does not appear to involve alterations in DNA binding. Previous studies have shown that the stimulatory effects of T3 on transcription are mediated by the recruitment of coactivator proteins to the TR (10, 52). Coactivators of TR may regulate transcription by directly interacting with the basal transcriptional machinery, by modulating interactions between TR and the basal transcriptional machinery, and by modifying chromatin structure. Complex formation between TR-RXR and PBX-MEIS1 may augment T3-induced malic enzyme transcription by facilitating the recruitment of coactivators to the malic enzyme promoter.

A role for PBX-MEIS1 complexes in modulating the T3 responsiveness of the malic enzyme gene may be conserved in humans. Sequence comparison analysis of the human malic enzyme gene indicates the presence of a putative PCE at −140/−124 bp. This PCE is located immediately upstream of a previously described T3RE at −105/−87 bp (53) and is composed of consecutive tetrameric half-sites (TGAT and TGAC) separated by a 9-bp spacer. González-Manchón *et al.* (53) have shown that the region containing the putative PCE enhances T3 responsiveness conferred by the T3RE at −105/−87.

A role for accessory DNA binding factors in modulating T3 responsiveness has been described for other genes. For example, the T3-induced stimulation of the phosphoenolpyruvate carboxykinase promoter in hepatoma cells is mediated by a T3RE and a sequence that binds the liver-enriched factors, C/EBPα and C/EBPβ (54). In primary hepatocyte cultures, T3 stimulation of the *S14* gene requires the presence of T3REs located between −2700 and −2500 bp and a nuclear factor Y-binding site near the transcription start site (55). Increased

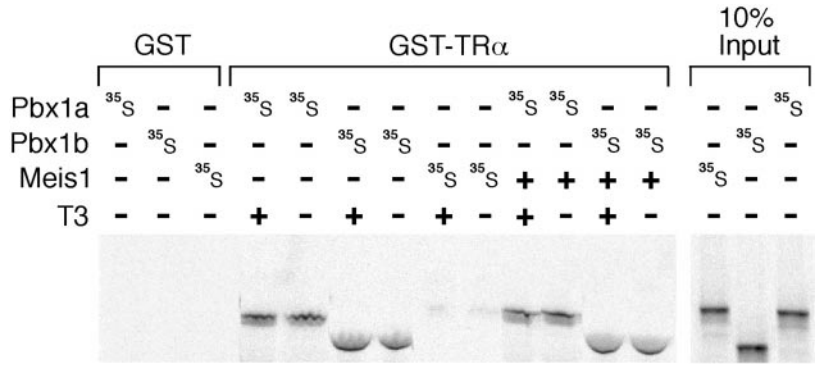

FIG. 7. **TRα strongly interacts with PBX1a and PBX1b but not MEIS1.** GST or GST-TRα immobilized on glutathione-Sepharose beads was incubated with *in vitro* translated and ^{35}S-labeled PBX1a, PBX1b, or MEIS1 in the absence and presence of T3. An equimolar amount of unlabeled MEIS1 was included in some incubations containing ^{35}S-labeled PBX1a and PBX1b. After the matrix was extensively washed, labeled proteins retained on the beads were eluted, resolved by SDS-PAGE, and visualized by storage phosphor autoradiography together with 10% of the total radiolabeled protein input used in each binding reaction. Additional experimental details are described under "Experimental Procedures."

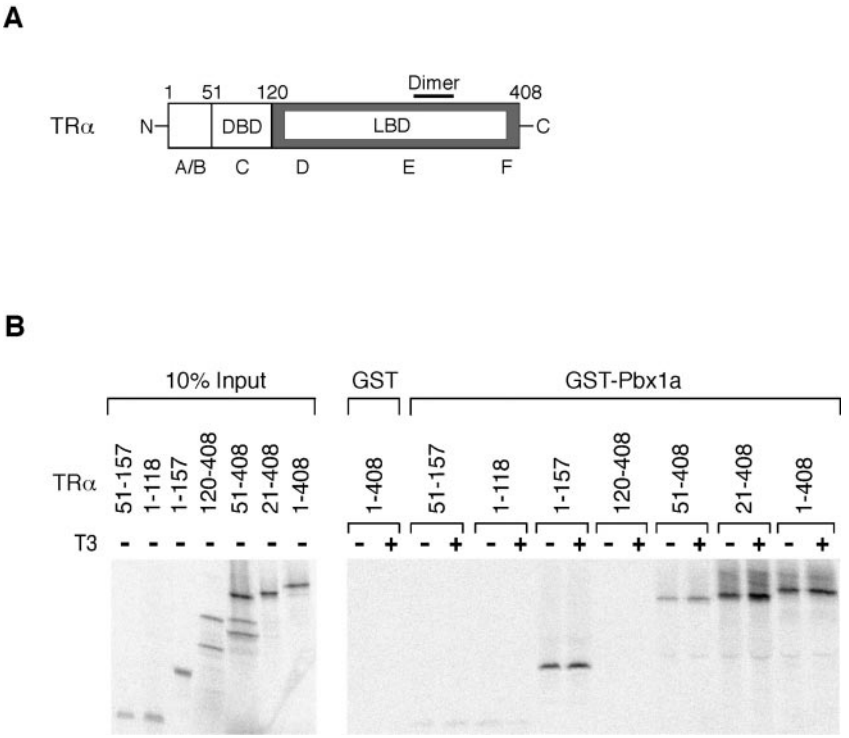

FIG. 8. **A TRα region containing amino acids 21–157 is required for optimal binding to PBX1a.** *A,* the schematic representation of TRα. The DNA binding domain (*DBD*) and the ligand binding domain (*LBD*) are indicated. Embedded within the LBD of TRα is a heptad repeat region involved in dimerization. A ligand-induced change in the conformation of the LBD is responsible for providing an interface for interaction with coactivator proteins. *B,* wild-type and truncated forms of TRα labeled with ^{35}S by *in vitro* translation were incubated with GST or GST-PBX1a bound to glutathione-Sepharose beads. After the matrix was extensively washed, labeled proteins retained on the beads were eluted, resolved by SDS-PAGE, and visualized by storage phosphor autoradiography together with 10% of the total radiolabeled protein input used in each binding reaction.

T3 responsiveness of the rat growth hormone promoter in pituitary cells relative to non-pituitary cells is mediated by a synergistic interaction between TR and the pituitary-specific DNA-binding protein, Pit-1 (also referred to as GHF-1) (56). Physical interactions between Pit-1 and TR enhance the binding of TR to the rat growth hormone promoter (57). In cardiac muscle cells, myocyte-specific enhancer factor 2 potentiates the ability of TR to stimulate α-cardiac myosin heavy chain transcription in the presence of T3 (58). Protein binding assays indicate that myocyte-specific enhancer factor 2 and TR specifically bind each other. Collectively, these findings and the results of the present study indicate that a wide variety of transcription factors can interact with TR on promoters of T3-responsive genes. Such factors expand the dynamic range of T3 responsiveness and constitute an important mechanism by which T3 regulates transcription in a tissue- or cell type-specific manner.

Complexes containing PBX-MEIS1/PREP1 have been shown to function as accessory proteins for other transcription factors besides TR. For example, on the urokinase plasminogen activator promoter, PBX-PREP1 binds a cooperation mediating element that is positioned between an activator protein-1 (AP-1) site and a combined PEA3/AP-1 site (25, 59). Binding of PBX-PREP1 to the cooperation mediating element is required for synergistic activation of urokinase plasminogen activator transcription by the AP-1 and PEA3/AP-1 sites. The elastase I promoter contains a PBX-binding site that consists of part of an enhancer element conferring cell type-specific regulation of transcription (34, 60). In pancreatic acinar cells, this PBX-binding site potentiates the transcriptional activity of an adjacent element that binds the pancreatic acinar cell-specific factor, PTF-1. The somatostatin promoter contains adjacent binding sites for PBX-PREP1 and the pancreatic cell-specific factor, PDX1 (32). PBX-PREP1 alone is devoid of transcriptional activity but interacts with PDX1 to stimulate a 10-fold increase in somatostatin promoter activity. This functional interaction between PBX-PREP1 and PDX1 is not mediated by alterations in the binding of PBX-PREP1 and PDX1 to the somatostatin promoter. E2a-MyoD heterodimers in muscle cell nuclear extracts bind to E box motifs that are flanked at their 5′ end by a PCE (50). In transfection assays, the binding of PBX-MEIS1 to the PCE enhances transcriptional activation by

E2a-MyoD bound to the adjacent E box motif. In contrast to the interaction between PBX-PREP1 and PDX1 on the somatostatin promoter and the interaction between PBX-MEIS1 and TR-RXR on the malic enzyme promoter, the interaction between PBX-MEIS1 and E2a-MyoD on the PCE/E-box element is mediated, in part, by alterations in protein binding to DNA. These studies indicate that PBX-MEIS1/PREP1 complexes functionally interact with a wide variety transcription factors and that the mechanisms mediating these interactions vary depending on the nature of the proteins that interact with PBX-MEIS1/PREP1.

The results of the present study are the first to establish a role for PBX and MEIS1 in the control of gene transcription in hepatocytes. PBX1-MEIS complexes may regulate the transcription of other genes in liver besides malic enzyme. For example, the AC element of the phosphoenolpyruvate carboxykinase promoter contains a sequence (−255 to −248 bp) that perfectly matches the consensus PCE, TGATTGAC. Mutation of the AC element abrogates the effects of cAMP and insulin on phosphoenolpyruvate carboxykinase transcription in H4IIe hepatoma cells (61). There are no reports addressing whether PBX-MEIS1 binds to the AC element. Additional experimentation will be needed to determine whether PBX-MEIS1 complexes play a broader role in the regulation of genes involved in carbohydrate and lipid metabolism in liver.

Several coactivators of TR have been identified that enhance T3-dependent transcriptional activation by physically interacting with the ligand binding domain (LBD) of TR. Examples of such proteins include CREB-binding protein (62) and steroid receptor coactivator 1 (63). T3-dependent transcriptional activation is also controlled by nuclear proteins that physically interact with TR regions distinct from the LBD. For example, the general transcription factor, TFIIB, facilitates T3-dependent transcriptional activation by interacting with a 10-amino acid sequence in the A/B region of TRα (64). Myocyte-specific enhancer factor 2 and the hematopoietic basic ZIP protein p45/NF-E2 enhance T3-dependent transcriptional activation by interacting with the DNA binding domain of TR (58, 65). Results from the present study demonstrating that PBX1a/b interacts with sequences in the A/B region and DNA binding domain of TRα provide further support for a role of TR regions distinct from the LBD in modulating T3-dependent transcriptional activation.

Results from DNA binding analyses (Fig. 3) indicate that region E contains a novel PCE that binds PBX-MEIS1 heterodimeric complexes in three different configurations. One configuration is composed of tandem half-sites separated by a 7-bp spacer (*i.e.* half-sites 1 and 4) and the other two configurations are composed of tandem half-sites with no spacer sequence (*i.e.* half-sites 1 and 2 and half-sites 3 and 4). Results from transfection experiments suggest that all three binding configurations contribute to the stimulation of T3 responsiveness by region E with binding to half-sites 1 and 4 making the greatest contribution. Interestingly, site-specific mutations of region E that restricted PBX-MEIS1 binding to half-sites 1 and 4 (mut E 2/3) or half-sites 3 and 4 (mut E 1/2) had no effect on the DNA binding affinity yet conferred a smaller increase in T3 responsiveness than wild-type region E. This observation suggests that sequences flanking the half-sites and/or the distance between the half-sites can modulate the functional activity of PBX-MEIS1 complexes without altering their ability to bind DNA.

We previously reported that region E was one of four cis-acting elements mediating differences in T3 regulation of malic enzyme transcription between chick embryo hepatocytes and chick embryo fibroblasts (15). This conclusion was based on the finding that mutation of region E markedly inhibited T3 regulation of malic enzyme transcription in chick embryo hepatocytes but had no effect on T3 regulation in chick embryo fibroblasts. Cell type-dependent differences in region E activity are probably not due to alterations in expression of PBX and MEIS1, as these proteins are expressed in a wide variety of tissues (17, 18, 36, 40). This supposition is supported by data from gel mobility shift assays demonstrating that the pattern and extent of protein binding to region E in chick embryo fibroblasts is similar to that observed in chick embryo hepatocytes (15). We postulate that the difference in region E activity between chick embryo hepatocytes and chick embryo fibroblasts is mediated by a post-translational mechanism. One possibility is that PBX-MEIS1 interacts with an activator or inhibitor whose activity varies in different cell types. In support of this possibility, Abramovich *et al.* (66) have recently identified a novel PBX-interacting protein that inhibits the ability of PBX complexes to bind DNA and activate transcription. Further studies are needed to determine the role of this protein in mediating differences in T3 responsiveness between chick embryo hepatocytes and chick embryo fibroblasts.

In summary, the present study establishes a role for PBX-MEIS1 complexes in conferring optimal T3 regulation of malic enzyme transcription in liver. Stimulation of T3-induced transcription by PBX-MEIS1 may by mediated by physical interactions between PBX and TR. The observation that PBX-MEIS1 heterodimers functionally and physically interact with TR provides further support for the proposal that PBX-MEIS1 heterodimers serve as accessory transcription factors in the developmental and hormonal regulation of gene expression (50).

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