Three-amino acid extension loop (TALE) homeodomain proteins are highly conserved transcription regulators. We report that two members of this family, Meis2 and TGIF, which frequently have overlapping consensus binding sites on complementary DNA strands in opposite orientations, can function competitively. For example, in the $D_{IA}$ gene, which encodes the predominant dopamine receptor in the striatum, Meis2 and TGIF bind to the activator sequence ACT ($-1174$ to $-1154$) and regulate transcription differentially in a cell type-specific manner. Among the five cloned splice variants of Meis2, isoforms Meis2a–d activate the $D_{IA}$ promoter in most cell types tested, whereas TGIF competes with Meis2 binding to DNA and represses Meis2-induced transcription activation. Consequently, Meis2 cannot activate the $D_{IA}$ promoter in a cell that has abundant TGIF expression. The Meis2 message is highly co-localized with the $D_{IA}$ message in adult striatal neurons, whereas TGIF is barely detectable in the adult brain. Our observations provide in vitro and in vivo evidence that Meis2 and TGIF differentially regulate their target genes. Thus, the delicate ratio between Meis2 and TGIF expression in a given cell type determines the cell-specific expression of the $D_{IA}$ gene. We also found that splice variant Meis2e, which has a truncated homeodomain, cannot bind to the $D_{IA}$ ACT sequence or activate transcription. However, Meis2e is an effective dominant negative regulator by blocking Meis2d-induced transcription activation. Thus, truncated homeoproteins with no DNA binding domains can have important regulatory functions.

The three-amino acid loop extension (TALE) superclass of homeobox proteins is characterized by an extension of three amino acids between o-helices 1 and 2 within the homeodomain. The genes encoding these proteins are highly conserved and are present in the common ancestor of plants, fungi, and animals. Members of this family of homeoproteins include Meis, Pbx, and TGIF (1). Cooperative function among TALE family members is critical for transcription regulation (2–4), and several members have been shown to function as essential contributors to Hox-mediated developmental programs (5–7).

Human Meis1 (myeloid ecotropic viral integration site 1) has been cloned from BHX-2 myeloid tumors as a novel common oncogenic site of proviral integration (8). Murine Meis2 was isolated by DNA cross-hybridization with a murine Meis1 cDNA (9) or with a partial human Meis2 cDNA (10) and was found to be inducible by retinoic acid during differentiation of P19 embryonal carcinoma cells (11). Murine Meis2 is highly expressed in the lateral ganglionic eminence and in the developing striatum (12).

Human TGIF (5′-TG-3′-interacting factor) is known to bind to the retinoic acid receptor (RXR)-responsive element in the cellular retinol-binding protein II promoter, which contains an unusual DNA target for homeobox proteins. The interactions of TGIF and RXRα with this element occur on overlapping areas and generate a mutually exclusive binding. In addition, TGIF inhibits 9-cis-retinoic acid-dependent RXRα-induced transactivation of this promoter (13).

The interplay of a variety of transcription factors like the homeoproteins plays an important role in the regulated, tissue-specific, and developmental expression of eukaryotic genes. These factors exert their effects on target genes by both activating and repressing transcription. Such complex regulation is particularly evident in the brain, where a large number of genes are expressed with unique regional distributions. For example, specificity of the biological effects of dopamine is determined at least in part by the intricate spatial and temporal regulation of genes encoding its receptors. To date, five G protein-coupled dopamine receptors encoded by different genes have been cloned. Based on their homology and pharmacological criteria, they are classified into two subtypes, D1-like ($D_{IA}$ and $D_7$ or $D_{1B}$) and D2-like ($D_2$, D₃, and $D_4$) receptors (14, 15). Among these, the $D_{IA}$ subtype is the predominant dopamine receptor in the striatum, the brain region that is innervated by dopaminergic neurons of the substantia nigra which degenerate in Parkinson’s disease (16). Alterations in the functional state of dopamine receptors in neuropsychiatric disorders and with chronic dopaminergic therapy are thought to underlie long term complications such as parkinsonian motor response.
fluctuations and tardive dyskinesias (17, 18).

Our previous analysis of the human D1A receptor gene revealed that the region between nucleotides −1173 and −1154 (ACT) may function for its neural cell-specific expression (19). In addition, the ACT sequence is highly conserved between human, pig, and rat D1A genes (20). We now sought to identify cell- or tissue-specific transcription factors that bind to this sequence using the yeast one-hybrid screen. We found that human Meis2 and TGIF differentially regulate transcription of the D1A gene by binding to complementary DNA sequences within its ACT region.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The murine neuroblastoma cell line N20Y was a gift from Dr. Marshall Nirenberg (NLHBI, National Institutes of Health, Bethesda, MD). Human neuroblastoma SK-N-MC and SH-SY5Y, murine neuroblastoma NB41A3, human hepatoblastoma HepG2, and opossum kidney OK cells were purchased from ATCC. All cells were grown in Dulbecco’s modified Eagle’s medium (Mediatech) supplemented with 10% fetal bovine serum (BioWhittaker) at 37 °C in a humidified atmosphere of 10% CO2.

**Yeast One-hybrid Screening for cDNAs Encoding ACT-binding Proteins**—The MATCHMAKER One-Hybrid System (CLONTECH) was used according to the supplier’s protocol. Three tandem repeats of the bp −1173 to −1154 sequence (ACT) from the human D1A promoter (20) were ligated into pHiSI and pLacZ to generate pHISI-ACT and pLacZ-ACT, respectively, for screening a human brain cDNA library (CLONTECH). The plasmid from the single positive blue clone was sequenced, and its homology was analyzed using BLAST.

**Cloning Human Meis2a–e and TGIF cDNAs and in Vitro Translaction**—The Meis2b cDNA isolated from the yeast one-hybrid screening was sequenced and oligonucleotide primers were synthesized to screen a SuperScript™ human brain cDNA library using GeneTrapper system (Life Technologies). These PCR primers were: forward, 5′-AGATGGGACGCCGGTCCTC-3′; reverse, 5′-GAGCTGCCGTCTCTTTCATC-3′. All positive clones were sequenced, and five different Meis2 splice variants were identified and designated pCMV-Meis2a–e, respectively.

**Cloning of TGIF cDNA**—The coding region of TGIF cDNA was cloned using RT-PCR with total RNA from human brain (CLONTECH) using primers: forward, 5′-AGATGGGACGCCGGTCCTC-3′; reverse, 5′-AGGCTGGCTCGGTGAGG-3′. The amplified PCR product was digested with BamHI and XhoI and subcloned into the respective sites of pcDNA3.1 (Invitrogen) to generate pcDNA-TGIF.

**Generation of GST-Meis2 Fusion Proteins and Gel Mobility Shift Assays**—The coding region of Meis2a–e cDNAs was amplified by PCR using pCMV-Meis2a–e as templates with primers containing either EcoRI or XhoI site underlined, respectively. The amplified TGIF cDNA was digested with BamHI and XhoI and subcloned into the respective sites of pcDNA3.1 (Invitrogen) to generate pcDNA-TGIF.

**Transfections for Transient Expression of CAT Constructs**—Transfections were carried out by the standard calcium phosphate co-precipitation method (Invitrogen). Briefly, HepG2, OK, SK-N-MC, and SH-SY5Y cells were plated in 100-mm dishes at a concentration of 1 × 106 cells/dish and cultured overnight before transfection. Various plasmids at the indicated concentrations in each figure were used. Total amount of DNA was kept constant by adding pcDNA3.1. After transfection periods of 6 h (OK) or 18 h (SK-N-MC, SH-SY5Y, and HepG2), cells were incubated in fresh medium for an additional 48 h. CAT protein was quantified by the CAT ELISA kit (Roche Molecular Biochemicals). Each experiment was carried out in triplicate.

**Generation of GST-Meis2a–e Fusion Proteins and Gel Mobility Shift Assays**—The coding regions of Meis2a–e were amplified by PCR using pCMV-Meis2a–e as templates with primers containing either EcoRI or XhoI sites, and sequences were confirmed. All five Meis2a–e cDNAs were subcloned into pGEX4T-1 to produce GST fusion proteins and designated pGEX-Meis2a–e, respectively. The resultant plasmids were used to transform Escherichia coli BL21. Fusion proteins of Meis2 isoforms were induced by 0.1 mM isopropyl-1-thio-β-D-galactopyranoside and purified using glutathione-conjugated Sepharose beads (Amersham Pharmacia Biotech).

For gel mobility shift assays, wild-type and mutant ACT probes (Fig. 1) were synthesized using an ABI DNA synthesizer. One strand was labeled with [γ-32P]ATP and annealed with the cold complementary strand. Double-stranded, end-labeled DNA probe (20 kmol/binding reaction, 5 fmol) was incubated with 200 ng of GST-Meis2 fusion proteins in a final volume of 20 μl at room temperature for 30 min. In some experiments, a polyclonal antibody to GST was co-incubated with GST-Meis2 fusion proteins for 30 min at room temperature prior to adding the probe. The reaction mixtures were electrophoresed in 4% polyacrylamide nondenaturing gel in 1× Tris/glycine buffer as described previously (19).

**In Situ Hybridization**—pCMV-Meis2a was digested with EcoRI and XhoI, and the Meis2a insertion was subcloned in pGEM-3Zf(+) to generate pGEM-Meis2a. Antisense probe was transcribed from the SP6 promoter after linearizing with EcoRI, and the sense probe was transcribed from the T7 promoter after linearizing with HindIII. Digoxigenin-labeled RNA probes were transcribed according to the protocol of the kit’s supplier (Roche Molecular Biochemicals) and hydrolyzed to approximately 200-mer fragments by alkaline hydrolysis. [35S]UTP-labeled D1A

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**Fig. 1. ACT sequence of the human D1A promoter.** An oligonucleotide with three tandem copies of the wild-type sequence was used as bait in the yeast one-hybrid screening. Consensus sequences for Meis2 and TGIF binding sites are shown in bold, and their respective orientations are represented by arrows. Bold nucleotides indicate that this ACT replace the Meis2/TGIF binding sites. Wild-type and mutant sequences were used in gel shift assays. Nucleotide numbering is relative to the first ATG codon (41).

**Mutant ACT sequence**

5′-ACTCTGCCAAATCAAGCCGG-3′

**TGIF**

5′-ACTCTGCCGTCAAGCCGG-3′

TGGACGAGAAGTCCGTCCTC

Meis2
probes were transcribed from pGEM-mD1A that had been linearized with EcoRI for generation of sense probe from the SP6 promoter or with HindIII for generation of antisense probe from the T7 promoter using the SP6/T7 transcription kit (Roche Molecular Biochemicals). Fourteen-μm-thick sections of the mouse striatum were subjected to simultaneous hybridization with two probes as described (21).

RESULTS

Isolation of Human Meis2a–e That Interact with the ACT Sequence in the D1A Promoter—To identify transcription factors that bind to the D1A ACT region, the yeast one-hybrid screen was performed. A double-stranded oligonucleotide having three tandem repeats of the ACT sequence (Fig. 1) was subcloned into pHISi and introduced into yeast cells. The resultant strain was transformed with a human brain cDNA library. Plasmids prepared from seven positive colonies that grew on selection medium were transformed into yeast strain containing the pLacZ-bait construct. One of these seven clones was found to be a true positive after the lacZ expression test. Sequence analysis of the positive clone indicated that it is the human homologue of murine Meis2b. The Meis2b cDNA insert in the library plasmid was fused in-frame with the GAL4 activation domain.

To obtain full-length Meis2 clones, PCRs were carried out using a SuperScript™ human brain cDNA library (Life Technologies) and five splice variants were isolated (Fig. 2A). These clones were designated Meis2a to Meis2e, analogous to the nomenclature of murine Meis2 isoforms (11). Human Meis2a and Meis2b lack exon 3(A), encode isoforms that end at the first termination codon. On the other hand, Meis2c and Meis2d lack both exons 3(A) and 4, encode isoforms that end at the second termination codon. Comparison of human and mouse Meis2a and Meis2b sequences revealed 100% amino acid identity. In Meis2c, the two species differ by a single amino acid at position 439, whereas in Meis2d they differ by two amino acids at positions 394 and 432. Meis2e, which had not been identified previously, has the same start codon as that of Meis2a–d, but an early termination codon TAA within the homeodomain due to alternative splicing resulting in deletion of exon 1 and a frameshift. Thus, the Meis2e protein has a truncated homeodomain (Fig. 2, B and C). To exclude the possibility that Meis2e could be an artifact of library construction, 3′ genomic walking was performed with gene-specific primers to determine the exon-intron boundary in the deleted
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Meis2 and D1A mRNAs Are Co-localized in the Striatum—

FIG. 3. Meis2 proteins bind to the D1A gene ACT sequence. GST-Meis2a–e fusion proteins were used in gel shift assays with ACT probe containing the Meis2 consensus site. GST-Meis2a–d fusion proteins interacted with the ACT probe (lanes 2–5), whereas GST-Meis2e or GST did not (lanes 6 and 1, respectively). Anti-GST polyclonal antibody supershifted GST-Meis2a–d fusion proteins and ACT complexes (lanes 7–10). Cold competitor incubated with GST-Meis2a–d fusion proteins before adding ACT probe inhibited all DNA-protein interactions (lanes 11–14). Mutant ACT probe used instead of wild-type ACT failed to bind with GST-Meis2a–d fusion proteins (lanes 15–18). Ab, antibody.

To verify the endogenous expression of the Meis2e splice variant in biological tissues, PCR was carried out on cDNA obtained from multiple adult mouse tissues as well as from different embryonic stages (CLONTECH) using a primer pair designed to separate Meis2e from the other four Meis2 isoforms (Fig. 2C). In addition to a doublet band representing 454 bp for variants “a” and “c” and 433 bp for variants “b” and “d,” a 355-bp fragment was amplified and cloned. Upon sequencing, the latter was confirmed to represent Meis2e. The relative abundance of Meis2e in different tissues was quite variable with highest level in muscle and lowest levels in brain, heart, and kidney.

The ability of all five Meis2 cDNA isoforms to encode proteins was verified by in vitro translation and the products were analyzed on SDS-PAGE (Fig. 2D). All Meis2a–e isoforms were expressed and electrophoresed slower than expected from their calculated molecular weights, probably because of the abundance of prolines and stretches of acidic residues (9).

GST-Meis2a–d Fusion Proteins Bind Specifically to the D1A ACT Sequence in Vitro—To confirm whether Meis2 can indeed bind to the ACT sequence, gel mobility shift assays were carried out using GST fusion proteins of each human Meis2 isoform (Fig. 3). All GST-Meis2 fusion proteins were able to shift the target ACT oligonucleotide probe except GST-Meis2e, which has a truncated homeodomain. This finding indicates that the homeodomain in Meis2 is essential for consensus sequence recognition and binding. The specificity of this DNA-protein interaction was verified by complete inhibition in the presence of cold wild-type ACT oligonucleotide and by lack of Meis2a–d binding to a mutant ACT probe. Furthermore, the shifted Meis2a–d bands were supershifted with a GST polyclonal antibody, indicating that they represent GST-Meis2 fusion proteins. Interestingly, Meis2a–d showed two shifted bands (Fig. 3, lanes 2–5), although purified GST-Meis2a–d fusion proteins ran as single major bands of the expected sizes on SDS-PAGE (data not shown). The latter observations suggest that Meis2 proteins could form homodimers similar to murine Meis1 (23).

Meis2a–d Activate Transcription through the D1A ACT Sequence—We had previously demonstrated that the D1A promoter in pCATD1–1197 harboring the ACT region is expressed in a neuronal cell-specific manner (19). To study this observation further, Meis2 Northern analysis was initially carried out using RNA from various cell lines in order to select appropriate cells for Meis2 co-transfection with pCATD1–1197. All neuronal cell lines tested (NS20Y, SK-N-MC, NB41A3, SH-SY5Y) had endogenous Meis2 expression, whereas non-neuronal cells HepG2 and OK did not (Fig. 4).

To determine whether Meis2 isoforms could increase the activity of pCATD1–1197 in non-neuronal cells, OK and HepG2 cells were co-transfected with Meis2a–e expression constructs and with pCATD1–1197 template plasmid. All co-transfected Meis2 isoforms were able to increase CAT expression significantly in both cell lines, except Meis2e, which lacks the homeodomain necessary for DNA binding (Fig. 5A). These results indicate that Meis2 proteins can function as activators of D1A gene transcription even in non-neuronal cells. Meis2b could increase CAT activity in HepG2, but not in OK cells, a finding that remains of unclear significance.

The specificity of the Meis2d effect on pCATD1–1197 was investigated by co-transfecting OK cells with two additional D1A promoter-CAT constructs, pCATD1–1154 and pCATD1–1120, which lack the ACT region (Fig. 5B). This experiment demonstrated that the Meis2d effect is specific to pCATD1–1197, which harbors the ACT sequence. Interestingly, although Meis2e cannot bind to the ACT sequence (Fig. 3), it potently inhibits Meis2d-induced pCATD1–1197 activation in OK cells (Fig. 5C). The latter finding indicates that the NH2-terminal region of Meis2 proteins could play a role in regulating D1A gene transcription in addition to their homeodomains, which recognize target DNA sequences.

The physiological significance of Meis2-induced activation of pCATD1–1197 transcription was addressed next by investigating changes in endogenous D1A mRNA levels in OK cells. This cell line was chosen because of the absence of Meis2 mRNA (Fig. 4) to allow interpretation of reconstitution experiments. In addition, these cells had no detectable D1A mRNA in our Northern analysis (Fig. 4), they have previously been shown to express this dopamine receptor (24), indicating that they have the basal transcription machinery to express this gene. Total RNA was prepared from cells transfected with pCMV-Meis2d or with control vector (pcDNA3.1) and Northern analysis was performed (Fig. 5D). Meis2d significantly increased the endogenous D1A message compared with control vector-transfected cells.
measured by ELISA. Meis2a–d significantly increased CAT expression in both cell lines, whereas Meis2e did not. Data shown are means ± S.E. for triplicate samples. B, Meis2d-induced transcriptional activation of the D1A promoter is dependent on the ACT sequence. 5′ Deletion mutants of the D1A gene 5′-flanking region shown on the left were co-transfected with pCMV-Meis2d into OK cells. *, p < 0.05. Data shown are means ± S.E. for triplicate samples. C, Meis2e inhibits Meis2d-induced transactivation of pCATD1–1197 in a concentration-dependent manner. Rising amounts of pCMV-Meis2e were used to co-transfect OK cells with fixed amounts of pCMV-Meis2d and pCATD1–1197. ANOVA, p = 0.03. Data shown are means ± S.E. for triplicate samples. D, Meis2d activates the endogenous D1A gene in OK cells. Total RNA was prepared from OK cells that were transfected with pCMV-Meis2d expression vector, control vector alone (pcDNA3.1), or with no DNA (Mock). A 32P-labeled mouse D1A fragment was used to detect the D1A message, which is seen only in Meis2d-transfected cells. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used to demonstrate uniformity of the RNA amount loaded in each lane.

The functional relevance of our findings in cell lines was further addressed by investigating the cellular co-localization of Meis2 and D1A messages within the adult striatum, the brain region in which both genes are highly expressed (12, 25). Simultaneous hybridization of a digoxigenin-labeled Meis2 probe and a radiolabeled D1A probe revealed considerable overlap between the two signals (Fig. 6). While some cells express Meis2 alone, very few neurons express D1A alone. This observation could reflect the important activating function of this nuclear protein on the D1A dopamine receptor gene.

**TGIF Binds to the D1A Promoter ACT Sequence and Competes with Meis2-induced Transactivation.** To study the effect of Meis2 overexpression on the D1A gene in human neuronal cell lines, SK-N-MC and SH-SY5Y cells expressing different amounts of D1A mRNA (Fig. 4) were co-transfected with all five Meis2 expression constructs and pCATD1–1197. Compared with the results in SK-N-MC cells, Meis2a–d induced robust increases of CAT activity in SH-SY5Y cells (Fig. 7A). The minimal changes with Meis2c and Meis2d in SK-N-MC cells are of unclear biologic significance. The decrease with Meis2e in both cell lines could be explained by repression of endogenous Meis2 activity.

Since Meis2e is a potent repressor of Meis2d-induced pCATD1–1197 activity (Fig. 5C), the expression levels of Meis2e were checked in SH-SY5 and SK-N-MC cells looking for a clue for the notable inability of Meis2a–d to activate the D1A promoter in the latter cell line. RT-PCR with primer pairs P1 and P2, designed to amplify a 355-bp Meis2e cDNA, failed to detect the expected fragment in either cell line but could amplify a complex band corresponding to Meis2a–d (Fig. 7B). In addition, RT-PCR using primers P1 and P3 could amplify each of the Meis2a–d cDNAs in both cell lines but not Meis2e (Fig. 7C). Thus, the difference between these two cell lines with respect to Meis2a–d-induced activation of pCATD1–1197 (Fig. 7A) could not be explained by differences in Meis2e expression since neither cell had this truncated isoform.

To gain further insight into the inability of Meis2a–d to activate transcription in SK-N-MC cells, careful examination of the ACT sequence led to the observation that a TGIF consensus sequence actually overlaps with the Meis2 consensus sequence with the opposite orientation (Fig. 1). Furthermore, TGIF and Meis2 mRNAs are expressed at different levels in the cell lines tested, with relatively high TGIF in cells that have low or no D1A mRNA (Fig. 4). Therefore, to investigate the role of TGIF in regulating Meis2 effects on the D1A gene, the TGIF cDNA was cloned by RT-PCR from a human brain cDNA library and subcloned into pcDNA3.1 expression vector. The ability of TGIF to bind to the ACT region was tested by gel mobility shift assay using in vitro translated protein (Fig. 5D). TGIF indeed bound to the wild-type ACT probe but not to the mutant ACT probe. Cold competitor significantly competed off TGIF binding.

The overlapping TGIF and Meis2 binding sites within the D1A ACT sequence raised the possibility that the two proteins could compete for binding to the same target. In addition, TGIF has been known as a transcriptional repressor (13). To address this hypothesis, first, competitive gel shift assay was performed. In vitro translated TGIF and GST-Meis2a fusion protein were added to the gel shift reaction mixture and electrophoresed in a 4% native polyacrylamide gel (Fig. 7E). Addition of rising amounts of Meis2a with a constant amount of TGIF diminished TGIF binding to DNA. At the same time, presence of TGIF suppressed the relative intensity of the band shifted by
rising amounts of Meis2a. Second, to investigate whether the in vitro competition of TGIF for the Meis2 recognition site has a functional impact within cells, SH-SY5Y cells were co-transfected with expression vectors for TGIF and for Meis2a and with pCATD1–1197. Co-expression of increasing amounts of TGIF resulted in a dramatic inhibition of Meis2a-induced pCATD1–1197 activity in a concentration-dependent manner (Fig. 7F). Since TGIF has its own repressor domain (26), the possibility that it blocks Meis2a-induced activation of the D1A promoter without necessarily displacing Meis2a was entertained next. pTGIF-HD expressing only the homeodomain of TGIF fused to a nuclear localization signal significantly re-
pressed Meis2a-induced activation of pCATD1–1197 in SH-SYSY cells in a dose-dependent manner (Fig. 7G), indicating that TGIF competes with Meis2 function by displacing it from its target DNA. However, full-length TGIF appeared more potent than its homeodomain alone in repressing the Meis2 effect.

DISCUSSION

In our search for transcription factors that regulate the human DIA dopamine receptor gene, we have identified two TALE superclass homeobox genes, Meis2 and TGIF, which share overlapping and complementary common binding sites in the activator ACT sequence of the DIA promoter. The yeast one-hybrid screen employed with the ACT sequence as bait led to the isolation of Meis2 only but not TGIF. The very low expression of TGIF in the adult brain could explain the latter observation.

The consensus DNA sequence for the murine Meis1 binding site has been previously characterized (23) and the third helix of the homeodomain has been shown to be important for its DNA binding (27, 28). Since murine Meis1 and Meis2 as well as human Meis2 have an identical amino acid sequence within their homeodomains (11), human Meis2 likely binds to the same consensus DNA sequence as murine Meis1. Thus, it was not surprising that we cloned Meis2 with the yeast one-hybrid screen since the DIA ACT bait contains a consensus sequence for Meis1 binding, TGACAG, albeit in the opposite orientation relative to the promoter (Fig. 1).

Alternatively spliced variants that lack the homeodomain have been recognized in several homeobox genes such as Drosophila bicoid (29), Xenopus XHbox 2 (30), and Xenopus Xhox 3β (31), but the precise functional role of these variants is unclear. In the present study, we identified five alternatively spliced Meis2 isoforms (Meis2a–e) in biological tissues, one of which, Meis2e, has a truncated homeodomain. In contrast to Meis2a–d, which transactivate the DIA promoter in pCATD1–1197 through the ACT sequence, Meis2e cannot because of its inability to bind to its target sequence. Furthermore, the homeodomain-lacking Meis2e inhibits Meis2d-induced transcriptional activation of pCATD1–1197. It, thus, appears that Meis2e acts as a natural dominant transcriptional repressor of Meis2 proteins. Another homeodomain-lacking protein, CSX1, has been reported to transactivate a reporter gene (32). These observations collectively suggest that homeodomain-lacking proteins can act either as dominant negative regulators or as positive regulators by a homeodomain-independent mechanism. One of these homeodomain-independent mechanisms could be protein-protein interactions. For example, the amino termini of Meis1 and Pbx1a are known to be necessary and sufficient for their dimerization in solution and their interaction enhances transcriptional activity of Hoxb2 (33). It is conceivable that the NH2 termini of Meis2 proteins bind to an unidentified factor, and that Meis2e could interrupt such interactions without binding to the target DNA sequence. Whether such an interacting factor is a DNA-binding protein or not, Meis2e could act as a dominant negative regulator.

The present study revealed that the consensus sequences for Meis2 and for TGIF binding sites usually overlap. These sequences are on complementary DNA strands and in opposite orientations. In the DIA promoter, for example, the target site for TGIF is in the same orientation as the promoter whereas that for Meis2 is in the reverse orientation (Fig. 1). Many genes, in fact, have similar overlapped sequences such as CRBPII (34), lactoferrin (35), complement factor H (36), and myosin heavy chain (37–39). Although the Meis2 consensus site is in the reverse orientation relative to the 5'-3' orientation of the DIA promoter, Meis2a–d have positive regulatory effects on pCATD1–1197. Our finding is consistent with the recent report that murine Meis1 can activate transcription even if its recognition site has an orientation opposite that of the promoter (33). The effect of sequence orientation of the TGIF consensus binding site remains to be investigated.

While Meis2a–d proteins activate transcription of the DIA gene, TGIF represses it by binding to complementary DNA sequences. Therefore, the ratio between TGIF and Meis2 concentrations would be a critical factor for the transcriptional regulation of their target genes. Our gel shift experiments revealed a competition for binding to the ACT sequence between Meis2 and TGIF (Fig. 7E). In addition, our co-transfection experiments indicated that TGIF or its homeodomain down-regulate Meis2-induced transactivation of the DIA promoter (Fig. 7, F and G), supporting the importance of Meis2 displacement from its target DNA. However, full-length TGIF was more potent than its homeodomain alone, suggesting an additional functional role of the TGIF repressor domain in modulating this effect. Thus, the inability of Meis2 to activate the DIA promoter in SK-N-MC cells (Fig. 7A) may well be due to the high steady-state levels of TGIF expression in this cell line demonstrated by Northern blot analysis (Fig. 4). On the other hand, the relatively low TGIF expression in HepG2 and OK cells could account for the ability of Meis2 to activate pCATD1–1197 in both cell lines (Fig. 5A), and account for the ability of Meis2 to increase endogenous DIA message levels in OK cells (Fig. 5D). Furthermore, comparison of mRNA levels in different cell lines revealed that the presence of more Meis2 relative to TGIF is a requisite for endogenous DIA expression (Fig. 4). Hence, our studies provide both in vitro and in vivo evidence that the balance between Meis2 and TGIF is involved in the intricate regulation of DIA gene transcription.

During development, Meis2 is highly expressed in the lateral ganglionic eminence of embryonic day 12.5 mouse telencephalon, suggesting that it can be a marker of striatal neuronal progenitors (12). Similar in situ hybridization studies have shown that the DIA dopamine receptor gene is also highly expressed in the striatum from day 14 embryos (40). Thus, Meis2 expression begins prior to that of the DIA gene prematurely supporting the ability of Meis2 to activate the DIA gene during striatal development. Furthermore, Meis2 and DIA mRNAs are highly co-localized within the same neurons of the adult striatum (Fig. 6), providing the anatomic requisite for their functional interaction.

In conclusion, the foregoing observations indicate that truncated homeoproteins such as Meis2e can function as dominant negative repressors of transcription. Detailed analysis of the spatial and temporal expression patterns of Meis2e compared with Meis2a–d can shed light on the mechanism of action of Meis2 proteins. In addition, since TGIF and Meis2 consensus sites usually overlap on complementary strands and in opposite orientations, and since TGIF represses Meis2-induced transactivation, their relative abundance in the cell determines the expression level of their target genes such as that encoding the DIA dopamine receptor.

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