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GATA-6: A Zinc Finger Transcription Factor That Is Expressed in Multiple Cardiac Cell Lineages and Derived from Lateral Mesoderm

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Members of the GATA family of zinc finger transcription factors play important roles in the development of several mesodermally derived cell lineages. In the studies described in this report, we have isolated and functionally characterized the murine GATA-6 cDNA and protein and defined the temporal and spatial patterns of GATA-6 gene expression during mammalian development. The GATA-6 and -4 proteins share high-level amino acid sequence identity over a proline-rich region at the amino terminus of the protein that is not conserved in other GATA family members. GATA-6 binds to a functionally important nuclear protein binding site within the cardiac-specific cardiac troponin C (cTnC) transcriptional enhancer. Moreover, the cTnC promoter enhancer can be transactivated by overexpression of GATA-6 in noncardiac muscle cells. During early murine embryonic development, the patterns of GATA-6 and -4 gene expression are similar, with expression of GATA-6 restricted to the precardiac mesoderm, the embryonic heart tube, and the primitive gut. However, coincident with the onset of vasculogenesis and development of the respiratory and urogenital tracts, only the GATA-6 gene is expressed in arterial smooth muscle cells, the developing bronchi, and the urogenital ridge and bladder. These data are consistent with a model in which GATA-6 functions in concert with GATA-4 to direct tissue-specific gene expression during formation of the mammalian heart and gastrointestinal tract, but performs a unique function in programming lineage-restricted gene expression in the arterial system, the bladder, and the embryonic lung.

INTRODUCTION

The GATA family of zinc finger transcription factors play key roles in transducing nuclear events that modulate cell lineage differentiation during development (for review see Orkin, 1992; Simon, 1995; Weiss and Orkin, 1995). Six family members have been identified in vertebrate species, each of which is expressed in a developmentally regulated lineage-restricted pattern (Arceci *et al.*, 1993; Dorfman *et al.*, 1992; Evans *et al.*, 1988; Heikinheimo *et al.*, 1994; Ho *et al.*, 1991; Joulin *et al.*, 1991; Kelley *et al.*, 1993; Ko *et al.*, 1991; Laverriere *et al.*, 1994; Lee *et al.*, 1990; Tsai *et al.*, 1989; Wilson *et al.*, 1990; Yamamoto *et al.*, 1990; Zon *et al.*, 1991). Each family member contains a conserved Cys-X_2-Cys-X_{17}-Cys-X_2-Cys zinc finger DNA binding domain that recognizes and binds to a consensus motif (A/TGATAA/G), located within transcriptional regulatory elements that control the expression of sets of lineage-specific genes (Ko and Engel, 1993; Merika and Orkin, 1993; Omichinski *et al.*, 1993; Yang *et al.*, 1994). The GATA-1/2/3 subfamily of transcription factors is expressed in an overlapping pattern in the hematopoietic cell lineages. However, mice harboring targeted mutations in the genes encoding each of these family members exhibit distinct phenotypes (Pandolfi *et al.*, 1995; Pevny *et al.*, 1991; Simon *et al.*, 1992; Tsai *et al.*, 1994; Weiss *et al.*, 1994). Thus, each of these GATA family members directly, or indirectly, regulates a unique lineage-specific developmental program.

Recently, three novel GATA family members, designated GATA-4, -5, and -6, have been identified in avian and amphibian species (Jiang and Evans, 1996; Kelley *et al.*, 1993; Laverriere *et al.*, 1994). These three proteins display distinct but overlapping patterns of expression in the heart and gastrointestinal tract. Structurally, these GATA subfamily members are more closely related to each other than to the previously characterized family members and have therefore been designated the GATA-4/5/6 subfamily of transcription factors. However, in mammalian species, only the pattern of GATA-4 expression has been described, and a GATA-5 homologue has not, as yet, been identified (Arceci *et al.*, 1993; Heikinheimo *et al.*, 1994; Ip *et al.*, 1994; Kelley *et al.*, 1993; Tamura *et al.*, 1993). GATA-4 is first expressed in the visceral endoderm and precardiac mesoderm of the early primitive streak mouse embryo. During later embry-

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onic and postnatal development, GATA-4 expression is restricted exclusively to the myocardium, gut epithelium, and the Sertoli cells in the testes and ovarian stroma. Interestingly, targeted mutation of the GATA-4 gene in embryonic stem (ES) cells results in gross defects in the formation of the visceral endoderm and Reichert's membrane during differentiation of these cells into cystic embryoid bodies (CEBs) (Soudais *et al.*, 1995).

Recent studies have suggested that GATA-4 functions to direct tissue-specific gene expression during cardiac development (Grepin *et al.*, 1994, 1995; Ip *et al.*, 1994; Molkentin *et al.*, 1994). Functionally important GATA binding sites have been identified in multiple cardiac-specific transcriptional regulatory elements, and each of these regulatory elements is transactivated by overexpression of GATA-4 in noncardiac muscle cell lines (Grepin *et al.*, 1994; Ip *et al.*, 1994; Molkentin *et al.*, 1994). Moreover, expression of GATA-4 antisense transcripts in pluripotent P19 embryonal carcinoma cells blocks the development of beating cardiac myocytes and interferes with expression of contractile proteins (Grepin *et al.*, 1995). Given their overlapping patterns of expression in avian and amphibian species and their role in regulating gene expression within the heart and possibly visceral endoderm, it will be of interest to determine whether, like the GATA-1/2/3 subfamily of transcription factors, each family member serves a unique developmental function or alternatively whether partial or complete functional redundancy exists within the GATA-4/5/6 subfamily.

In the studies described in this report, we have isolated the murine GATA-6 cDNA and compared the structure of the deduced GATA-6 protein to other previously characterized GATA family members. We have demonstrated that forced expression of GATA-6 transactivates a cardiac-specific transcriptional regulatory element in noncardiac muscle cells. In addition, the GATA-6 cDNA was used as a molecular probe to define the tissue distribution and cell specificity of GATA-6 gene expression during murine embryogenesis. Taken together, our data suggest that GATA-6 and GATA-4 may serve partially, or completely, redundant functions in cardiac development, but that GATA-6 may subserve a unique function(s) during the development of the arterial and urogenital systems as well as within the developing lungs.

MATERIALS AND METHODS

Isolation of Murine GATA-6 cDNA Clones

To isolate the murine GATA-6 cDNA a Day 11 mouse embryonic heart λgt11 cDNA library (Clontech) was screened with a radiolabeled rat GATA-GT1 (Tamura *et al.*, 1993) cDNA probe (bp 992–1444) as described previously (Parmacek and Leiden, 1989). Two positively hybridizing clones (mG6-2 and mG6-7) were isolated and both the sense and antisense strands sequenced.

Southern and Northern Blot Analyses

Southern and Northern blot analyses were performed as described previously (Solway *et al.*, 1995). The 1169-bp *Eco*RI/*Xho*I (bp 1466–2635) GATA-6 cDNA probe was used for Southern blot analyses. Northern blot probes included the 521-bp *Acc*I/*Sac*I GATA-6 cDNA, the 329-bp (bp 1678–1907) GATA-4 3′ UTR cDNA (which do not cross-hybridize), and the 504-bp murine cardiac troponin C (cTnC) cDNA (Parmacek and Leiden, 1989). Quantitative image analyses were performed using a Molecular Dynamics PhosphorImager (Sunnyvale, CA).

Plasmids

The murine GATA-6 expression plasmid, pcDNAG6, and the GATA-4 expression plasmid, pcDNAG4, were cloned by subcloning the 2.6-kb murine GATA-6 cDNA (bp 1–2641) or the 1.7-kb murine GATA-4 cDNA (Arceci *et al.*, 1993), respectively, into pcDNA3 (Invitrogen). The p-124cTnCGH and p-124cTnCGHμCEF-1 growth hormone (GH) reporter plasmids contain the 124-bp cTnC cardiac-specific promoter enhancer and the cTnC enhancer with a 5-nucleotide mutation within the CEF-1 nuclear protein binding site, respectively, subcloned into the p0GH human GH reporter plasmid (Nichol's Institute). The pmα1GH (Wilson *et al.*, 1990) and the pμmα1globinGH reporter plasmids contain one copy of the consensus GATA site derived from the mouse α1-globin gene (5′ GGGCAACTGATAAGGATTCC 3′) or a mutated copy of the consensus GATA site derived from the mouse α1-globin gene (5′ GGGCAACTGGGGCCCATTCC 3′), respectively, subcloned immediately 5′ of the minimal rabbit β-globin promoter (5′ CGTCCTTGGGCATAAAAGGCAGTGCACTGCAGCTGCTGCTTA 3′) in the p0GH plasmid. Similarly, the pCEF-1GH and pμCEF-1GH plasmids contain a single copy of the murine cTnC CEF-1 nuclear protein binding site (5′ TCCAGCCTGAGATTAGAGGGAG 3′) or a copy of the cTnC CEF-1 nuclear protein binding site containing a mutation in the GATA binding site (5′ TCCAGCCTGGGGCCCCAGGGAG 3′), respectively, subcloned immediately 5′ of the minimal rabbit β-globin promoter in the p0GH plasmid. The pMSVβgal β-galactosidase (Parmacek *et al.*, 1994) reference plasmid has been described previously.

Cell Culture and Transfections

Primary cultures of adult rat aortic smooth muscle cells, murine NIH 3T3 cells, C3H10T1/2 cells, C2C12 and Sol8 myoblasts and myotubes, Hep G2, EL4, and rat A7r5 cells were grown as described previously (Parmacek and Leiden, 1989; Solway *et al.*, 1995). For transient cotransfection experiments, 10^6 NIH 3T3 cells were cotransfected with 25 μg of the appropriate GATA expression plasmid, 1 μg of pMSVβgal reference plasmid, 2.5 μg of the appropriate GH reporter plasmid, and 20 μl of Lipofectin reagent (Gibco). Forty-eight hours following transfection, cell supernatants were assayed for growth hormone by radioimmunoassay (Nichol's Institute), and cell lysates were prepared and analyzed for β-galactosidase activity and protein content as described previously (Ip *et al.*, 1994). Data are expressed as normalized growth hormone production ± SEM. All experiments were repeated at least three times to ensure reproducibility.

Electrophoretic Mobility Shift Assays (EMSAs)

Nuclear extracts were prepared from 3T3 cells 48 hr following transient transfection with the pcDNAG6, pcDNAG4, or pcDNA3 expression plasmids as described (Ip *et al.*, 1994). The following complementary oligonucleotides were synthesized with *Bam*HI/

*Bgl*II overhanging ends: cTnC CEF-1, 5′ GATCTCCAGCCTG-AGATTACAGGGAG 3′; cTnC μCEF-1, 5′ GATCTCCAGCCT-GGGGCCCCAGGGAG 3′; mα-globin, 5′ GATCTCCGGCAACT-GATAAGGATTCCCTG 3′. The native and mutated GATA sites are underlined. EMSAs were performed as described previously (Ip *et al.*, 1994).

In Situ Hybridization of Staged Murine Embryos

In situ hybridization was performed essentially as described by Eichele and co-workers (Kuratani *et al.*, 1994). The murine GATA-6 and GATA-4 cDNAs subcloned into pGEM7Z and pGEM11Z, respectively, were *in vitro* transcribed using T7 or SP6 polymerase in the presence of ^{35}S-labeled UTP to generate sense and antisense cRNA probes, respectively. Sections were processed for emulsion autoradiography and were poststained with Hoechst 33258 and visualized by epifluorescence and dark-field microscopy on a Zeiss Axiophot microscope. To detect nonspecific background, hybridizations were performed with the radiolabeled sense riboprobe to alternate sections under identical conditions.

RESULTS

Isolation and Structural Characterization of Murine GATA-6 cDNA Clones

To isolate novel GATA family members expressed in the mammalian heart, a Day 11 mouse embryonic heart cDNA library was screened by low-stringency hybridization with a radiolabeled cDNA probe derived from the rat GATA-GT1 cDNA. As shown in Fig. 1, the murine GATA-6 cDNA includes a 1332-bp open reading frame encoding a 444-amino-acid polypeptide with a calculated molecular mass of 45.0 kDa. Southern blot analyses confirmed that GATA-6 is encoded by a single-copy gene in the murine genome (data not shown). Comparison of the deduced amino acid sequence of murine GATA-6 with the previously isolated chicken GATA-6 cDNA revealed that the two GATA-6 homologues are 65% identical at the amino acid level. Of note, the chicken GATA-6 protein is 387 amino acids in length, while the murine protein is 444 amino acids in length. Comparison of the deduced murine GATA-6 and GATA-4 proteins revealed high-level sequence identity across the conserved zinc finger and adjacent C-terminal basic domains (Fig. 1B, shaded gray). In addition, a region of high-level amino acid sequence identity (57% identity) was identified in the amino terminus (aa 1–65) of these two GATA subfamily members (Fig. 1B), a region of the proteins that was not conserved in the previously characterized GATA-1/2/3 subfamily members. Interestingly, this region of the GATA-4 protein encodes an independent transcriptional activation domain (E. Morrisey *et al.*, submitted for publication). Of note, the GATA-6 cDNA encodes six proline residues within this region, four of which are conserved in the GATA-4 protein. Outside of the amino terminus, zinc fingers, and adjacent C-terminal basic domain, the amino acid sequences of the GATA-6 and GATA-4 proteins have diverged significantly (Fig. 1B).

Tissue and Cellular Specificity of GATA-6 Gene Expression

To determine the *in vivo* tissue specificity of GATA-6 gene expression in mammalian species during postnatal development, the GATA-6 cDNA was radiolabeled and used to probe Northern blots containing RNA obtained from adult murine tissues. As shown in Fig. 2, the GATA-6 cDNA probe hybridized to a single species of mRNA of approximately 3.2 kb, which is expressed in the heart, aorta, stomach, small intestine, and bladder (Fig. 2, arrow, lanes 4, 5, 7, 8, and 13). In addition, prolonged autoradiographic exposures revealed low, but detectable, expression in the liver, kidney, uterus, and lung (Fig. 2, lanes 1, 6, 9, and 10). No hybridization to brain, skeletal muscle, spleen, or thymus RNA was detected (Fig. 2, lanes 2, 3, 11, and 12). These data demonstrate that GATA-6 is expressed in a tissue-restricted pattern during adult development with high-level expression restricted to the heart, intestine, stomach, aorta, and bladder.

In order to determine the cellular specificity of GATA-6 gene expression, the GATA-6 cDNA probe was hybridized to Northern blots containing RNA from primary cultures of rat aortic SMCs (VSMCs), the immortalized rat aortic SMC line, A7r5, NIH 3T3 and C3H10T1/2 fibroblasts, the SV-40 transformed kidney cell line COS-7, murine C2C12 and Sol8 myoblasts and myotubes, the human hepatocellular carcinoma cell line, HepG2, and the murine lymphoid cell line, EL-4 (Fig. 3). High levels of GATA-6 mRNA were detected solely in primary rat aortic SMCs (Fig. 3, lane 1). In addition, low levels of GATA-6 mRNA were present in the immortalized rat vascular smooth muscle cell line, A7r5, and NIH3T3 and C3H10T1/2 fibroblasts (Fig. 3, lanes 2–4). Prolonged (2–3 week) autoradiographic exposures also revealed a faint 3.2-kb signal in RNA prepared from HepG2 cells (Fig. 3, lane 10). In contrast, GATA-6 mRNA was not detected in COS-7 cells, C2C12 or Sol8 myoblasts or myotubes, or EL-4 cells even after prolonged autoradiographic exposures (Fig. 3, lanes 5–9 and 11). To determine whether the lineage-restricted patterns of GATA-4 and -6 gene expression could be distinguished, the radiolabeled murine GATA-4 cDNA probe was hybridized to a Northern blot containing RNA prepared from primary cultures of rat aortic SMCs, 3T3 and 10T1/2 fibroblasts, and C2C12 myoblasts and myotubes. In contrast to the high levels of GATA-6 mRNA that were detected in RNA prepared from primary rat aortic SMCs (Fig. 3, lane 1), the GATA-4 probe failed to detect expression of GATA-4 mRNA in this muscle cell lineage or in RNA prepared from any of the other cell lines on this blot (data not shown). Taken together, these studies revealed that GATA-6 is expressed in a tissue- and cell lineage-restricted pattern and that the GATA-6 and GATA-4 genes are differentially expressed in the aorta, bladder, and primary vascular SMCs.

A

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                                                                       AAAAGTCAGGTT
GGAGTAGCGCCGGACAGTGGATGGCCTTGACGGACTGCGGCGGTGCCTGCCGAAGCGTTTCGGGGCTGCTGCTGCG
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CGCGACCGCCAGCAAGCTGTTGTGGTCCAGCCGGGGCGCCAAACTGAGCCCCTTCGCGGCCGAGCAGCCGGAGGAA

ATG TAC CAG ACC CTC GCC GCC CTG TCC AGC CAG GGG CCC GCC GCT TAC GAC GGC GCG
 M   Y   Q   T   L   A   A   L   S   S   Q   G   P   A   A   Y   D   G   A

CCC GGC GGC TTC GTG CAC TCC GCA GCG GCG GCG GCC GCT GCC GCC GCG GCA GCC AGC
 P   G   G   F   V   H   S   A   A   A   A   A   A   A   A   A   A   A   S

TCC CCG GTC TAC GTG CCC ACC ACG CGC GTG GGC TCC ATG CTG TCC GGC CTG CCC TAC
 S   P   V   Y   V   P   T   T   R   V   G   S   M   L   S   G   L   P   Y

CTT CAA GGG GCG GGC AGC GGG CCC AGC AAT CAC GCG GGC GGA GCG GGT GCC CAC CCA
 L   Q   G   A   G   S   G   P   S   N   H   A   G   G   A   G   A   H   P

GGC TGG TCC CAG GCC TCC GCC GAC AGC CCC CCG TAT GGC GGG GGT GGC GCA GCC GGC
 G   W   S   Q   A   S   A   D   S   P   P   Y   G   G   G   G   A   A   G

GGC GGC GCG GCC GGA CCT GGA GGT GCG GGA TCG GCT ACG GCC CAC GCC TCT GCA CGC
 G   G   A   A   G   P   G   G   A   G   S   A   T   A   H   A   S   A   R

TTT CCC TAC TCG CCC AGC CCG CCC ATG GCC AAC GGC GCC GCG CGA GAC CCC GGG GGC
 F   P   Y   S   P   S   P   P   M   A   N   G   A   A   R   D   P   G   G

TAC GTG GCT GCG GGC GGC ACG GGC GCA GGC AGT GTG AGT GGA GGT GGC GGC ACG CTG
 Y   V   A   A   G   G   T   G   A   G   S   V   S   G   G   G   G   T   L

GCG GCC ATG GGT GGC CGG GAG CAC CAG TAC AGC TCG CTG TCC GCA GCT CGG CCG CTG
 A   A   M   G   G   R   E   H   Q   Y   S   S   L   S   A   A   R   P   L

AAC GGA ACG TAC CAC CAC CAC CAT CAC CAT CAC CCG ACC TAC TCG CCC TAC ATG GCC
 N   G   T   Y   H   H   H   H   H   H   H   P   T   Y   S   P   Y   M   A

GCA CCG CTG ACT CCT GCC TGG CCA GCA GGA CCC TTC GAA ACG CCG GTG CTC CAC AGC
 A   P   L   T   P   A   W   P   A   G   P   F   E   T   P   V   L   H   S

TTA CAG GGC CGC GGG CGG GAG CTC CAC TCC CGG TGC CAC GGG GGC CCC AGC ACA GAC
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CTG TTG GAG GAC CTG TCG GAG AGC CGC GAG TGC GTG AAC TGC GGC TCC ATC CAG ACG
 L   L   E   D   L   S   E   S   R   E   C   V   N   C   G   S   I   Q   T

CCA CTG TGG AGA CGA GAC GGC ACC GGT CAT TAC CTG TGC AAT GCA TGC GGT CTC TAC
 P   L   W   R   R   D   G   T   G   H   Y   L   C   N   A   C   G   L   Y

AGC AAG ATG AAT GGC CTC AGC AGG CCC CTC ATC AAG CCA CAG AAG CGC GTG CCT TCA
 S   K   M   N   G   L   S   R   P   L   I   K   P   Q   K   R   V   P   S

TCA CGG CGG CTT GGA CTG TCC TGT GCC AAC TGT CAC ACC ACA ACC ACT ACC TTA TGG
 S   R   R   L   G   L   S   C   A   N   C   H   T   T   T   T   T   L   W

CGT AGA AAT GCT GAG GGT GAG CCT GTG TGC AAT GCT TGC GGG CTC TAT ATG AAA CTC
 R   R   N   A   E   G   E   P   V   C   N   A   C   G   L   Y   M   K   L

CAT GGG GTG CCT CGA CCA CTT GCT ATG AAA AAA GAA GGA ATT CAA ACC AGG AAA CGA
 H   G   V   P   R   P   L   A   M   K   K   E   G   I   Q   T   R   K   R

AAA CCT AAA AAT ATA AAT AAG TCA AAA GCT TGC TCC GGT AAC AGC AGT GGC TCT GTC
 K   P   K   N   I   N   K   S   K   A   C   S   G   N   S   S   G   S   V

CCT ATG ACT CCT ACT TCC TCT TCT TCT AAT TCA GAT GAC TGC ACC AAA AAT ACT TCT
 P   M   T   P   T   S   S   S   S   N   S   D   D   C   T   K   N   T   S

CCT TCT ACA CAA GCG ACC ACC TCA GGG GTA GGG GCA TCA GTG ATG TCT GCA GTG GGA
 P   S   T   Q   A   T   T   S   G   V   G   A   S   V   M   S   A   V   G

GAA AAC GCC AAC CCC GAG AAC AGT GAC CTC AAG TAT TCA GGT CAA GAC GGC CTC TAC
 E   N   A   N   P   E   N   S   D   L   K   Y   S   G   Q   D   G   L   Y

ATA GGT GTC AGT CTG TCC TCC CCT GCC GAA GTC ACA TCC TCC GTG CGA CAG GAT TCT
 I   G   V   S   L   S   S   P   A   E   V   T   S   S   V   R   Q   D   S

TGG TGT GCT CTG GCC CTG GTC TGA
 W   C   A   L   A   L   V

GCTGGTGCTACCAAGAGGCAAGGAGGGCTCTGAAGGCCTCATACCACTTGTGTCTGATATTGTCCAGCAGTCCAGAT
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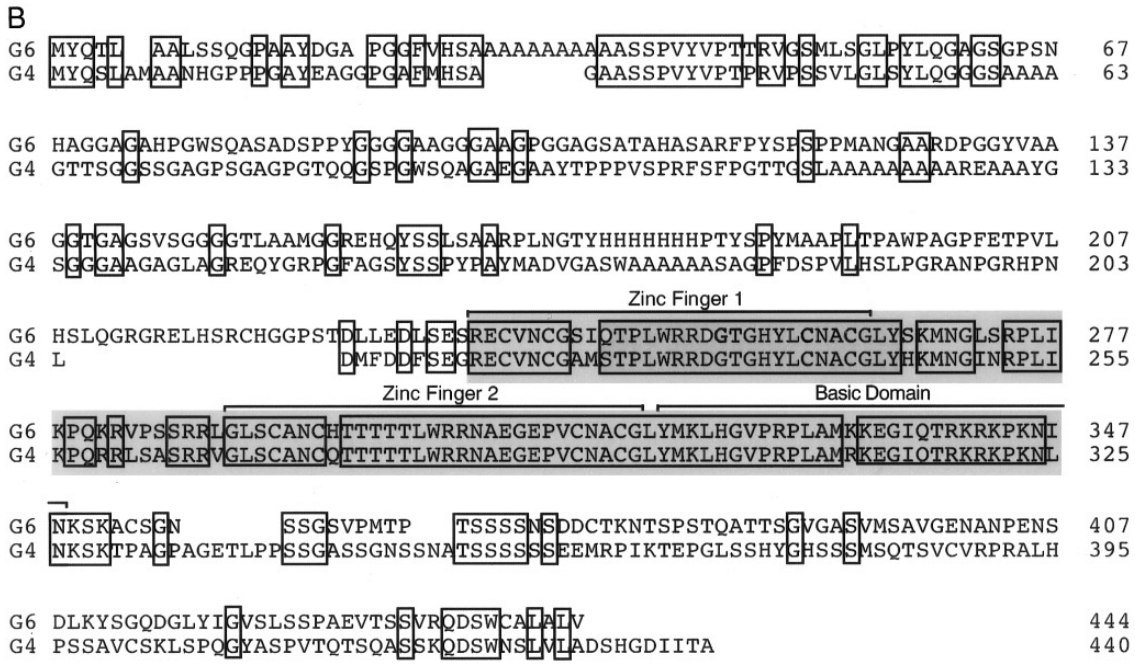

FIG. 1. The structure of the murine GATA-6 cDNA. (A) The nucleotide and deduced amino acid sequence of the murine GATA-6 cDNA. (B) Comparison of the amino acid sequences of the murine GATA-6 (G6) and GATA-4 (G4) proteins. Identical amino acid residues are boxed. The conserved zinc fingers and basic domain are shown in gray. Amino acid residue numbers are shown to the right of each sequence.

GATA-6 Binds to the Cardiac-Specific cTnC Transcriptional Enhancer

To determine whether GATA-6 binds to the functionally important CEF-1 nuclear protein binding site located within the murine cTnC promoter enhancer (Parmacek *et al.*, 1992), nuclear extracts prepared from 3T3 cells transiently transfected with the GATA-6 expression plasmid, pcDNAG6, were incubated with a double-stranded radiolabeled oligonucleotide corresponding to the cTnC CEF-1 nuclear protein binding site, and EMSAs were performed. As shown in Fig. 4, nuclear extracts containing recombinant GATA-6 bound a single nuclear protein complex (Fig. 4, lane 7) that was abolished by the addition of unlabeled CEF-1 or mα1-globin oligonucleotide (Fig. 4, lanes 8 and 9), but not by the μCEF-1 oligonucleotide containing a 5-nucleotide substitution in the CEF-1 GATA binding site (data not shown). Interestingly, the GATA-6 binding activity was partially ablated and supershifted by preincubation with polyclonal antiserum raised against recombinant GATA-4 protein (Fig. 4, dashed arrows), but not by preimmune serum (Fig. 4, lanes 10 and 11). In addition, the recombinant GATA-6 protein bound to the radiolabeled mα1 probe that contains a consensus GATA motif from the mouse α1-globin locus control region (Fig. 4, lane 13) (Wilson *et al.*, 1990; Yamamoto *et al.*, 1990). Similar binding activities were demonstrated with nuclear extracts containing recombinant GATA-4 protein (Fig. 4, lanes 2–6 and 12). These data demonstrate that GATA-6 can bind to a functionally important nuclear protein binding site in the cardiac-specific cTnC promoter enhancer.

GATA-6 Transactivates the cTnC Cardiac-Specific Transcriptional Enhancer in Noncardiac Muscle Cells

To determine whether GATA-6 can transactivate the cTnC promoter enhancer in noncardiac muscle cells, 3T3 cells were cotransfected with a GH reporter plasmid containing the intact cTnC promoter enhancer and an expression plasmid encoding either GATA-6 or GATA-4. Consistent with previous reports (Ip *et al.*, 1994), the p-124GH reporter construct was inactive in NIH 3T3 cells (Fig. 5A, column 1). However, cotransfection of either the pcDNAG6 expression plasmid (Fig. 5, column 2) or pcDNAG4 (Fig. 5, column 4) resulted in a 75- to 90-fold increase in GH reporter activity. To determine whether this increase in activity was dependent on the intact CEF-1 GATA binding site, we tested the effects of forced expression of GATA-6 and GATA-4 on the p-124GHμCEF-1 reporter plasmid containing a mutation in the CEF-1 nuclear protein binding site. In each case there was a 70% reduction in the relative GH production (Fig. 5A, columns 3 and 5).

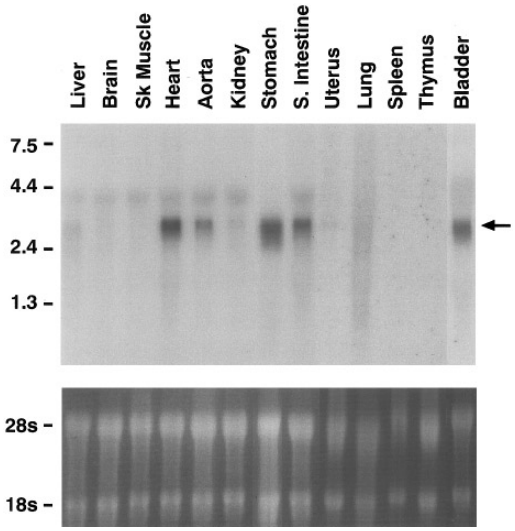

FIG. 2. The *in vivo* tissue distribution of GATA-6 gene expression. The top panel shows a Northern blot analysis of RNA samples isolated from adult murine tissues hybridized to the radiolabeled GATA-6 cDNA probe (24 hr exposure). RNA markers are shown in kilobases to the left of the blot. The GATA-6 probe hybridized to a single 3.2-kb species of mRNA which was present at high levels in the heart, aorta, stomach, small intestine, and bladder (arrow). The bottom panel shows the ethidium-stained gel prior to membrane transfer of RNA. The locations of the 28S and 18S ribosomal RNA bands are indicated to the left of the gel.

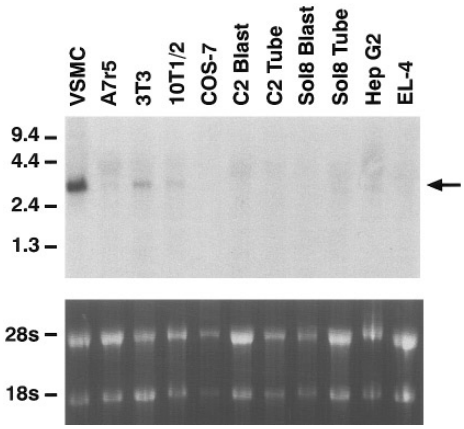

FIG. 3. The cellular specificity of GATA-6 gene expression. Northern blot analysis of RNA samples isolated from primary rat aortic smooth muscle cells (VSMC), A7r5 (A7r5), C3H10T1/2 (10T1/2), NIH 3T3 (3T3), and COS-7 cells, C2C12 myoblasts (C2 Blasts) and C2C12 myotubes (C2 Tubes), Sol8 myoblasts (Sol8 Blasts) and myotubes (Sol8 Tubes), and HepG2 and EL-4 cells hybridized to the radiolabeled GATA-6 cDNA probe. The GATA-6 cDNA probe hybridized to a 3.2-kb species of mRNA (arrow) that was present at high levels in VSMCs and at low levels in A7r5 immortalized rat aortic smooth muscle cells and 10T1/2 and 3T3 fibroblasts. The bottom panel shows the ethidium-stained gel prior to transfer of RNA.

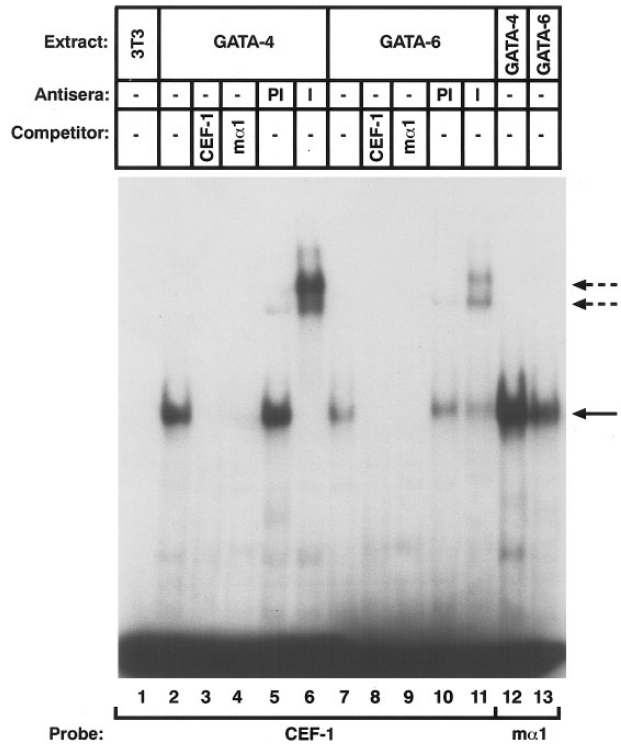

FIG. 4. Binding of GATA-4 and GATA-6 to the CEF-1 site of the cTnC enhancer and the GATA site in the mouse α1-globin (mα1) enhancer. Radiolabeled CEF-1 and mα1 oligonucleotides were used in EMSAs with nuclear extracts prepared from NIH 3T3 (3T3) cells transiently transfected with either GATA-4 or GATA-6 expression vectors, pcDNAG6 or pcDNAG4, respectively. Where indicated, binding reactions were preincubated with either control preimmune rabbit serum (PI), a rabbit antiserum raised against a recombinant GATA-4 protein (I), or 100 ng of unlabeled competitor oligonucleotides. The bands corresponding to the GATA-6 and GATA-4 nuclear protein complexes and the antibody-supershifted complexes are indicated with solid and dashed arrows, respectively.

Several recent studies have demonstrated that GATA family members can activate transcription of target genes by binding to accessory factors, including Sp1, which bind to their cognate binding sites in a fashion that does not require DNA binding by the GATA family member (Crossley *et al.*, 1995; Osada *et al.*, 1995). The finding that overexpression of GATA-4 or -6 transactivated the mutant p-124GHμCEF-1 reporter plasmid above levels obtained with the pcDNA3 negative control plasmid suggested that both GATA-4 and GATA-6 might be capable of indirectly activating the cTnC cardiac-specific promoter enhancer. Therefore, to examine how overexpression of GATA-6 activated transcription from the cTnC promoter enhancer, the GATA-6 expression plasmid was transiently cotransfected with either the pCEF-1GH reporter plasmid, containing a single copy of the cTnC CEF-1 motif upstream of the minimal rabbit β-globin promoter, or the pμCEF-1GH reporter

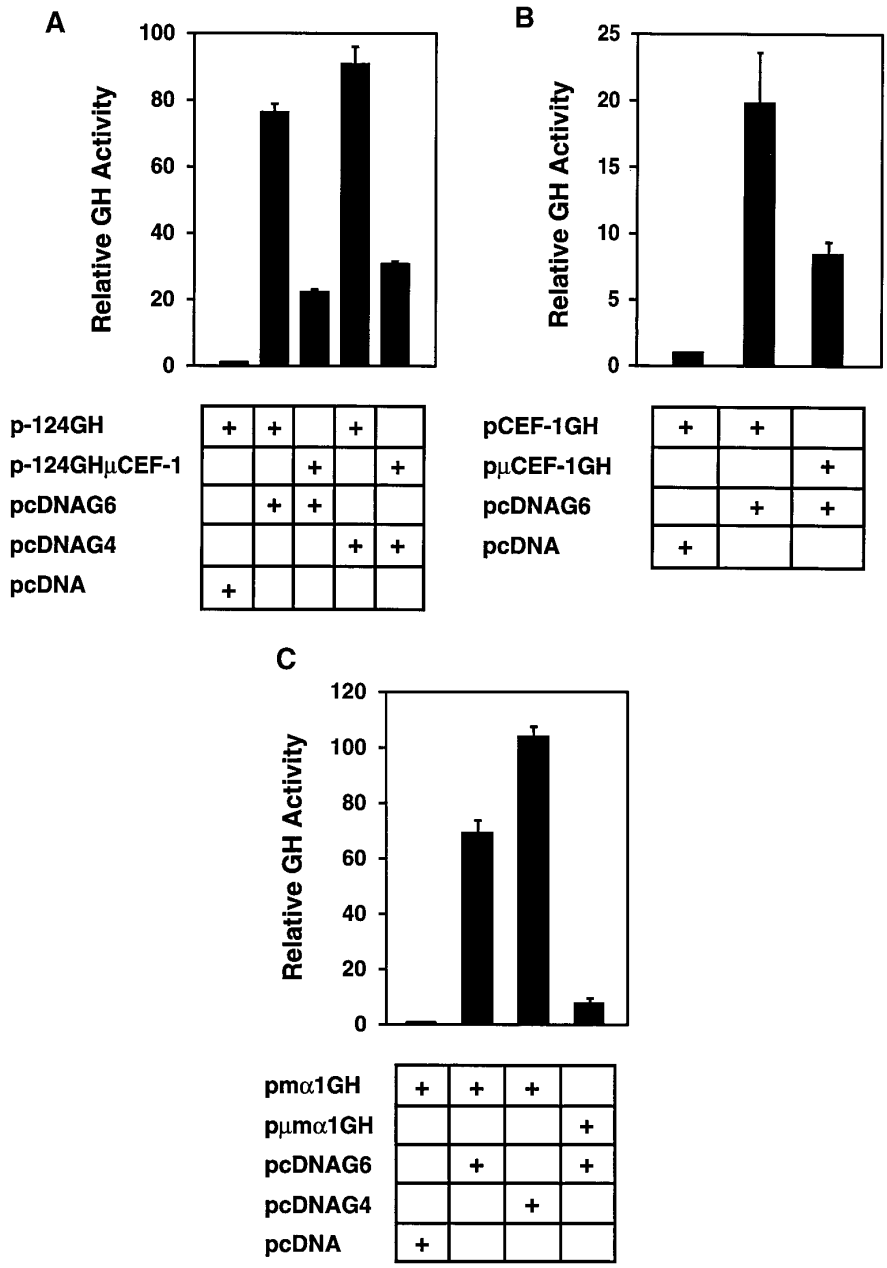

FIG. 5. GATA-6-modulated transactivation of the cTnC promoter enhancer and a minimal GATA-containing promoter in noncardiac muscle cells. (A) NIH 3T3 cells were transfected with 2.5 μg of either the p-124GH (p124GH +) or the p-124GHμCEF-1 (p-124GHμCEF-1 +) GH reporter plasmids and 25 μg of the control expression plasmid (pcDNA +), the GATA-6 expression plasmid (pcDNAG6 +), or the GATA-4 expression plasmid (pcDNAG4 +). All transfections contained 1 μg of the pMSVβgal reference plasmid. Forty-eight hours following transfection, the amount of secreted growth hormone and the β-galactosidase activities were determined. Relative GH activity was determined by correcting for differences in transfection efficiencies and normalizing to the amount of growth hormone obtained following transfection of the p-124GH plasmid with the pcDNA3 plasmid. The data are presented as relative GH activity ± SEM. (B) Transient cotransfections were performed as described above with the exception that the pCEF-1GH or pμCEF-1GH reporter plasmids, which contain a single CEF-1 nuclear protein binding site or a CEF-1 mutant nuclear protein binding site subcloned upstream of the minimal rabbit β-globin promoter, were utilized. (C) Transient cotransfections were performed as described above with the exception that the pmα1GH reporter plasmid, containing a single GATA binding site upstream of the rabbit β-globin promoter or the pμmα1GH reporter plasmid, which contains a mutant GATA site upstream of the β-globin promoter, were utilized.

plasmid, containing a mutated copy of the CEF-1 motif subcloned upstream of the minimal β-globin promoter, into 3T3 cells. Of note, in these experiments the minimal rabbit β-globin promoter was utilized because it has been demonstrated previously not to be transactivated by overexpression of GATA family members (Martin and Orkin, 1990). In addition, these reporter plasmids served to isolate the functional activity of the cTnC CEF-1 nuclear protein binding site away from the three other transcriptional regulatory elements within the 124-bp murine cTnC promoter (Parmacek *et al.*, 1992). As shown in Fig. 5B, forced expression of GATA-6 in 3T3 cells resulted in a 20-fold induction in activity of the pCEF-1GH reporter plasmid (Fig. 5B, column 2). In contrast, only a 7-fold induction in transcriptional activity was demonstrated with the pμCEF-1GH expression plasmid. Interestingly, this percentage of reduction in relative GH activity (70%) was nearly identical the percentage of reduction when the transcriptional activities of the p-124GH and p-124GHμCEF-1 plasmids were compared (Fig. 5A, compare columns 2 and 3). Taken together, these data suggest that: (i) the cTnC CEF-1 nuclear protein site either within the context of the native cTnC promoter enhancer or when fused to a heterologous promoter can be transactivated by overexpression of GATA-6 in noncardiac muscle cells; (ii) the majority (70%) of the transcriptional activity induced upon overexpression of GATA-4 or -6 is dependent upon an intact GATA motif within the cTnC CEF-1 nuclear protein binding site; and (iii) the residual induction in transcriptional activity demonstrated by the mutant CEF-1 reporter constructs results from the indirect activation of the cTnC CEF-1 element.

To determine whether overexpression of GATA-6 could activate transcription of a noncardiac transcriptional regulatory element, NIH 3T3 cells were cotransfected with a growth hormone reporter plasmid containing one copy of the consensus GATA motif derived from the α1-globin gene located immediately upstream of a minimal rabbit β-globin promoter and either the pcDNAG6 or pcDNAG4 expression plasmids. As shown in Fig. 5C, forced expression of GATA-6 increased GH reporter activity approximately 70-fold (Fig. 5C, column 2). This level of transcriptional activation was similar to that obtained following cotransfection of the GATA-4 expression plasmid with the GH reporter plasmid (Fig. 5C, column 3). To determine whether this increase in activity was dependent on the consensus GATA motif within the α1-globin promoter, we compared the effects of forced expression of GATA-6 on the pmα1GH and pμmα1GH reporter plasmids. As shown in Fig. 5C (compare columns 2 and 4), mutation of the consensus GATA motif resulted in a 90% reduction in transcriptional activity from this reporter plasmid, demonstrating that most of the induction in transcriptional activation is dependent upon an intact consensus GATA binding motif. Thus, both GATA-4 and -6 are potent transcriptional activators of GATA motifs located within both cardiac-specific transcriptional regulatory elements and other consensus GATA motifs.

GATA-6 Has a Unique Lineage-Restricted Pattern of Expression during Murine Embryogenesis

To determine whether differences in the temporal and/or spatial patterns of expression might distinguish the functional role(s) of GATA-6 and -4 during mammalian development, *in situ* hybridization experiments were performed. Of note, as reported previously, the patterns of GATA-4 gene and protein expression correlate closely within the developing heart tube (Heikenheimo *et al.*, 1994). To define the patterns of GATA-6 and -4 gene expression prior to the formation of the heart tube, gene-specific riboprobes were hybridized to sections of E7.0 mouse embryos. The GATA-6 probe hybridized to the embryonic mesoderm in the primitive streak (PS) region which gives rise to the heart tube (Fig. 6B, white arrow), as well as the embryonic mesoderm subjacent to the future headfold (HF) region of the embryo, which contains the cardiogenic plate (Fig. 6B, white arrow). Extraembryonic hybridization of the GATA-6 probe to the allantois (A) (Fig. 6B, white arrow) and Reichert's membrane (RM) (Fig. 6B, white arrow) was also observed. Similarly, the GATA-4 riboprobe hybridized to the embryonic mesoderm of the primitive streak and future headfold regions, as well as to the allantois and Reichert's membrane (compare Figs. 6B and 6C). In addition, significant levels of hybridization of the GATA-4 probe to the visceral endoderm (VE) was observed in multiple sections (Fig. 6C, white arrow). Thus, in the primitive streak embryo, GATA-6 and GATA-4 are coexpressed in regions of the embryonic mesoderm involved in formation of the embryonic heart. In addition, both genes are expressed in Reichert's membrane and the allantois, while GATA-4 mRNA appears to be more abundant than GATA-6 mRNA in the visceral endoderm.

In E9.5 embryos, the embryonic patterns of GATA-6 (Fig. 7B) and GATA-4 (Fig. 7C) gene expression continue to be very similar. Within the two-chambered heart, both genes are expressed within the single ventricle (V) and atria (A). In addition, high levels of hybridization of both probes were detected in the septum transversum (ST) which gives rise to cardiac precursor cells and to cells of the embryonic liver (white arrows, Figs. 7B and 7C), and to the cells lining the midgut and hindgut regions (Figs. 7B and 7C, open arrows). Taken together, through Day 9.5 of murine embryonic development, GATA-6 and GATA-4 have virtually identical embryonic patterns of expression in structures that give rise to the heart and gut.

By Day 13.5 of development significant differences in the tissue-restricted patterns of GATA-6 and GATA-4 gene expression have evolved. As shown in Figs. 7E (GATA-6) and 7F (GATA-4), both genes continue to be expressed throughout the heart and in the epithelial layer of the stomach, small intestine, and large intestine (see also Fig. 8C, open arrow). However, only the GATA-6 gene is expressed in the dorsal aorta (Fig. 7E, open arrows), within the lung buds, and in the urogenital ridge (compare Figs. 7E and 7F). High levels of hybridization of the GATA-6 riboprobe were visualized in the four-chambered heart throughout the left ven-

tricle (Fig. 8A, LV), left atrium (Fig. 8A, LA), and cardiac outflow tract including the aorta (Fig. 8D, Ao). In addition, hybridization of the GATA-6 riboprobe to the major arteries including the dorsal aorta (Fig. 8A, Ao) and major veins including the right and left superior vena cavae (Fig. 8D, SVC) was demonstrated. Moreover, the GATA-6 mRNA was expressed in the developing bronchi (Br) (Fig. 8B and 8D, open arrows), within the developing lung buds, and within the urogenital ridge (UG) (Fig. 8C, solid arrows). Conversely, a high level of hybridization of the GATA-4 riboprobe to the E13.5 ovary (Fig. 7F, white arrow) and a low level of hybridization to the embryonic liver were detected (Fig. 7F). However, no hybridization of the GATA-4 probe to either the dorsal aorta (Fig. 7F, open arrows) or urogenital ridge was observed.

During late fetal development (E20.0), GATA-6 and GATA-4 maintain overlapping but distinct tissue-restricted patterns of expression. As in earlier developmental stages (and during postnatal development), both the GATA-6 and -4 genes are expressed at high levels throughout the myocardium and endocardium and in the epithelial layer of the stomach, small intestine, and large intestine. Interestingly, though high levels of GATA-6 were detected in primary aortic SMCs and within the developing arterial system, GATA-6 mRNA was not detected within the medial layer of the gastrointestinal tract (compare Figs. 7H (GATA-6) and 7I (GATA-4); see also Figs. 8C and 8E, open arrows). At this late developmental stage, only the GATA-6 riboprobe hybridized to the major arteries including the aorta (Fig. 8F), the smaller branch arteries (compare Figs. 7H and 7I, thin white arrow) and within the mucosal, but not the epithelial, layer of the bladder (Fig. 8E and compare Figs. 7H and 7I, thick white arrow). In addition, some hybridization of the GATA-6 riboprobe to the developing airways within the fetal lung was visualized (compare Figs. 7H and 7I). Conversely, the GATA-4 riboprobe hybridized to the liver as well as the testes and ovaries (Fig. 7I and data not shown).

In summary, together with the Northern blot data (see Figs. 2 and 3), these analyses revealed that the GATA-6 gene is expressed in a lineage-restricted, developmentally regulated pattern which through Day 9.5 of murine development is very similar, if not identical, to that of GATA-4. High levels of expression of both the GATA-6 and -4 genes persists throughout the heart and within the gut epithelium during postnatal development. However, beginning in the midembryonic period (Postcoital Day 9.5–13.5), coinciding with the onset of vasculogenesis and the development of the respiratory and urogenital tracts, only the GATA-6 gene is expressed in the arteries (within the medial layer) and veins, within the urogenital ridge which gives rise to the mucosal layer of the bladder, and in the embryonic and fetal bronchi and bronchioles within the lungs.

DISCUSSION

The evolutionarily conserved GATA family of zinc finger transcription factors play key roles in regulating lineage-specific gene expression during development (for review see Orkin, 1992; Simon, 1995; Weiss and Orkin, 1995). In vertebrates, a great deal is understood about the function of the GATA-1/2/3 subfamily of proteins in the hematopoietic cell lineages. In contrast, relatively little is understood about the recently identified GATA-4/5/6 subfamily of transcription factors. In this report, we have isolated and structurally and functionally characterized the mouse GATA-6 protein and determined the temporal and spatial pattern of GATA-6 gene expression during murine development. Structural comparison of GATA-6 and -4 proteins revealed significant sequence identity within a novel amino-terminal activation domain that is not shared by other members of the GATA family. In addition, both GATA-4 and -6 are expressed within the heart and precardiac mesoderm, and overexpression of either transcription factor transactivates a cardiac-specific promoter enhancer in noncardiac muscle cells. Moreover, coincident with the onset of vasculogenesis and development of the urinary and respiratory tracts, the GATA-6 gene is expressed in arterial smooth muscle cells, the bladder, and the developing bronchi, tissues in which the GATA-4 gene is not expressed. These findings are consistent with a model in which GATA-6 and -4 function in concert to regulate tissue-specific gene expression within the developing heart, while GATA-6 may independently program lineage-specific gene expression in the arterial system, the bladder, and the developing lungs.

Several groups have reported previously that the GATA-6 gene is expressed at high levels within the heart, lung, stomach, intestine, and ovary in avian and amphibian species in a pattern that temporally and spatially overlaps with that of GATA-4 (Laverriere *et al.*, 1994; Jiang and Evans, 1996). In addition, Tamura and co-workers reported that the rat GATA-6 gene (designated GATA-GT1) is expressed exclusively in the stomach and intestine, but not in the heart, lung, or testes (Tamura *et al.*, 1993). To finely map the pattern of GATA-6 gene expression throughout mammalian development, we performed a series of *in situ* hybridization experiments on staged murine embryos. These analyses demonstrated that, as in lower vertebrate species, GATA-6 is expressed within the myocardium, endocardium, gut epithelium, and lung, suggesting that the function(s) of GATA-6 in these tissues has been conserved through vertebrate evolution (Figs. 2, 7, and 8). Given the close evolutionary relationship between mice and rats, it is unclear why Tamura and co-workers failed to detect expression of GATA-6 (GATA-GT1) in the rat heart by Northern blot analyses, while we detected abundant levels of GATA-6 mRNA in the embryonic and adult murine heart by both *in situ* hybridization and Northern blot analyses. Moreover, in contrast to each of these earlier reports, our analyses revealed that the murine GATA-6 gene is expressed at high levels and is differentially expressed from GATA-4 in vascular SMCs, the developing bronchi, the urogenital ridge, and within the muscular wall of the bladder (Figs. 3, 7, and 8). As previous analyses of both the chicken and the *Xenopus* GATA-6 genes focused primarily on the role of GATA-6 in

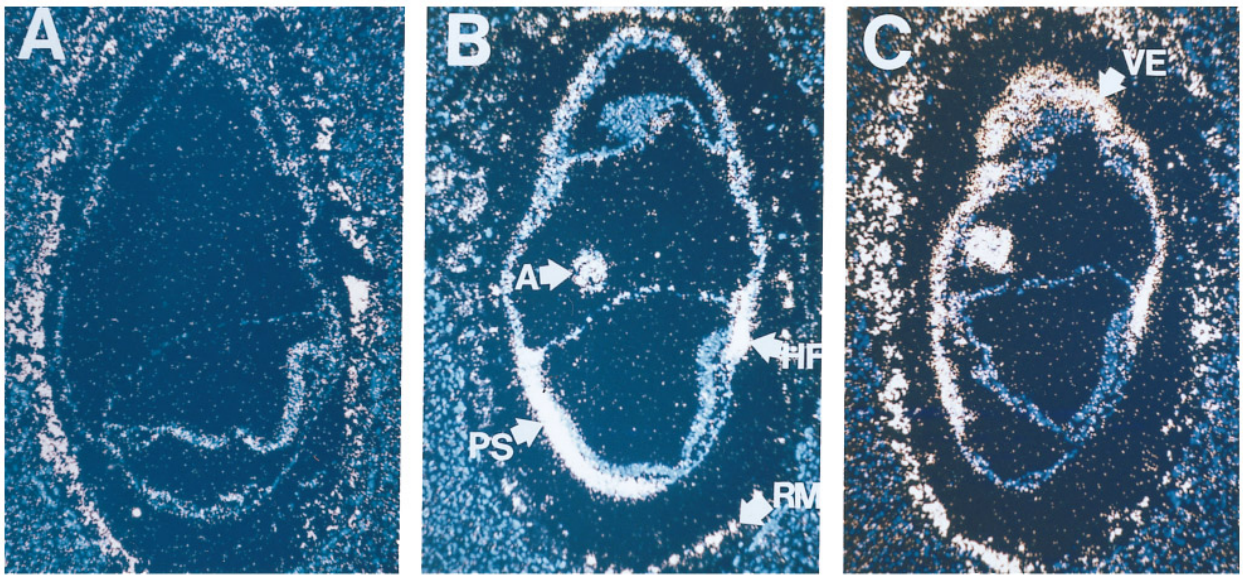

FIG. 6. Expression of the GATA-6 gene in the early primitive streak embryo. *In situ* hybridization analyses were performed using radiolabeled antisense GATA-6 (B) or GATA-4 riboprobes (C) or the control GATA-6 sense riboprobe (A) on E7.0 staged murine embryos. (A) Autofluorescence from erythrocytes was observed in the embryonic and surrounding maternal tissues when sections were hybridized to the control GATA-6 sense riboprobe. (B) Specific hybridization (white) of the GATA-6 antisense riboprobe to the mesoderm in the primitive streak (PS) region (arrow) and subjacent to the headfold (HF) region (arrow) was visualized. In addition, hybridization of the GATA-6 riboprobe to the allantois (A) and Reichert's membrane (RM) was demonstrated. (C) Specific hybridization of the GATA-4 antisense riboprobe to embryonic mesoderm of the primitive streak and subjacent to the headfold regions was observed. In addition, extraembryonic hybridization to the allantois, Reichert's membrane, and visceral endoderm (VE) was observed. Magnification, 25×.

cardiac development and neither study examined the expression (or lack of expression) of GATA-6 within the vasculature and/or urinary tract, it will be of interest to determine whether a novel function for GATA-6 in these tissues has evolved in mammalian species, or, alternatively, whether GATA-6 is also expressed in avian and amphibian arterial SMCs, developing airways and the bladder.

The finding that GATA-6 is expressed in the lateral mesoderm and subsequently expressed in the myocardium, endocardium, arterial smooth muscle cells, and within the bladder wall in mammalian species is consistent with the hypothesis that GATA-6 coordinates the differentiation of diverse cell lineages from undifferentiated lateral mesoderm. When viewed in this context, the expression of GATA-6 in the myocardium, which is derived from the splanchnic mesoderm, as well as the endocardium, which is derived from hemangioblastic tissue (a derivative of the lateral mesoderm), may be understood. Furthermore, the differential expression of GATA-4 and -6 genes may direct the undifferentiated mesenchyme toward ovarian or testicular stroma (in the case of GATA-4) versus SMCs that make up the arterial media and tissues such as the bladder wall (in the case of GATA-6). Interestingly, the related family members, GATA-1, -2 and -3, have been shown to differentially restrict the developmental potential of pluripotent stem cells derived from the splanchnic mesoderm into distinct mature hematopoietic cell lineages (Weiss and Orkin, 1995). However, the finding that overexpression of GATA-4 or GATA-6 in NIH 3T3 cells fails to activate expression of endogenous cardiac-specific genes, including cTnC (H. Ip and M. Parmacek, unpublished observation), suggests that expression of GATA-4 or -6 may be necessary, but is not sufficient, to drive uncommitted mesodermal cells toward the cardiac muscle cell lineage. A similar role has been postulated for GATA-1 during differentiation of the erythroid lineage (Orkin, 1992; and Weiss and Orkin, 1995). Taken together, these data suggest that the GATA family of zinc finger transcription factors may, in concert with other transcription factors, coordinate cellular differentiation along a developmental axis originating with precursor cells in the lateral mesoderm.

The coexpression of GATA-4 and GATA-6 within the precardiac mesoderm and heart raises the question of whether these two proteins subserve partially or completely redundant functions during cardiac development. In this regard, it is noteworthy that gene targeting experiments have demonstrated that the myogenic basic-helix–loop–helix family members, MyoD and Myf-5, serve a redundant function with respect to programming skeletal muscle lineage specification (Rudnicki *et al.*, 1992, 1993). The findings that: (i) the GATA-4 and -6 proteins share significant sequence homology across putative DNA binding and tran-

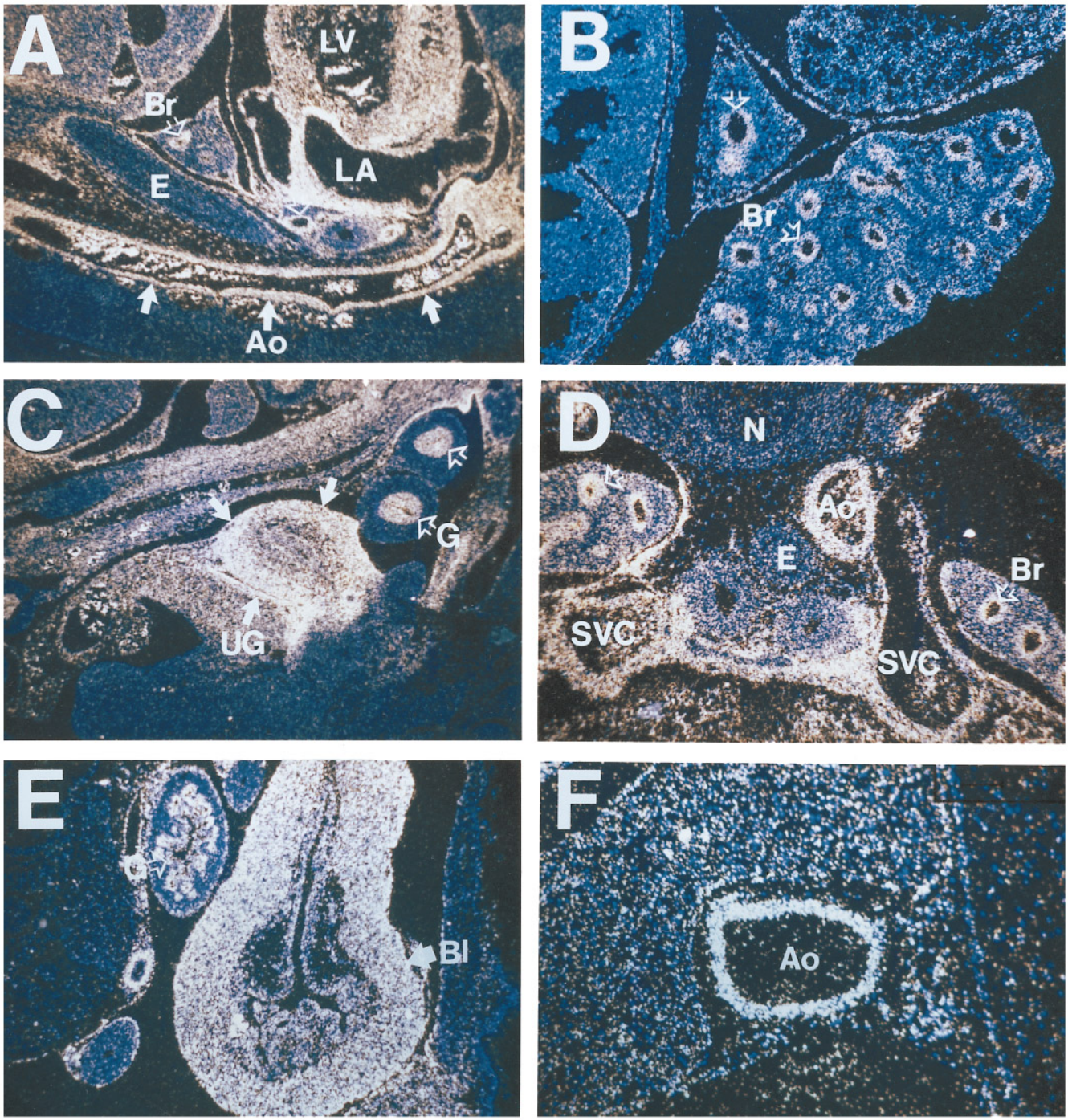

FIG. 8. The GATA-6 gene is expressed in the vasculature, bladder, and embryonic bronchi. *In situ* hybridization analyses were performed using a GATA-6 antisense riboprobe and sections through staged murine E13.5 (A–D) and E20.0 (E and F) embryos. (A) Hybridization to the left ventricular (LV) myocardium and endocardium, the left atria (LA), cardiac outflow tract, dorsal aorta (Ao, solid arrows) and bronchi (Br) within the lung bud. No hybridization to the esophageal (E) epithelium was observed. Original magnification, 12.5×. (B) Hybridization to the bronchi (Br) within the developing lung bud. Original magnification, 25×. (C) Hybridization to the intestinal epithelium (G, open arrows) and urogenital ridge (UG, solid arrow). Original magnification, 12.5×. (D) Hybridization to the right and left superior vena cavae (SVC), the aortic outflow tract (Ao) and the bronchi (Br) within the lung bud. No hybridization to the esophagus (E) was observed. Original magnification, 25×. (E) Hybridization to the bladder mucosa (Bl) and gut epithelium (G). Original magnification, 12.5×. (F) Hybridization across the media of the descending aorta (Ao). Original magnification, 25×.

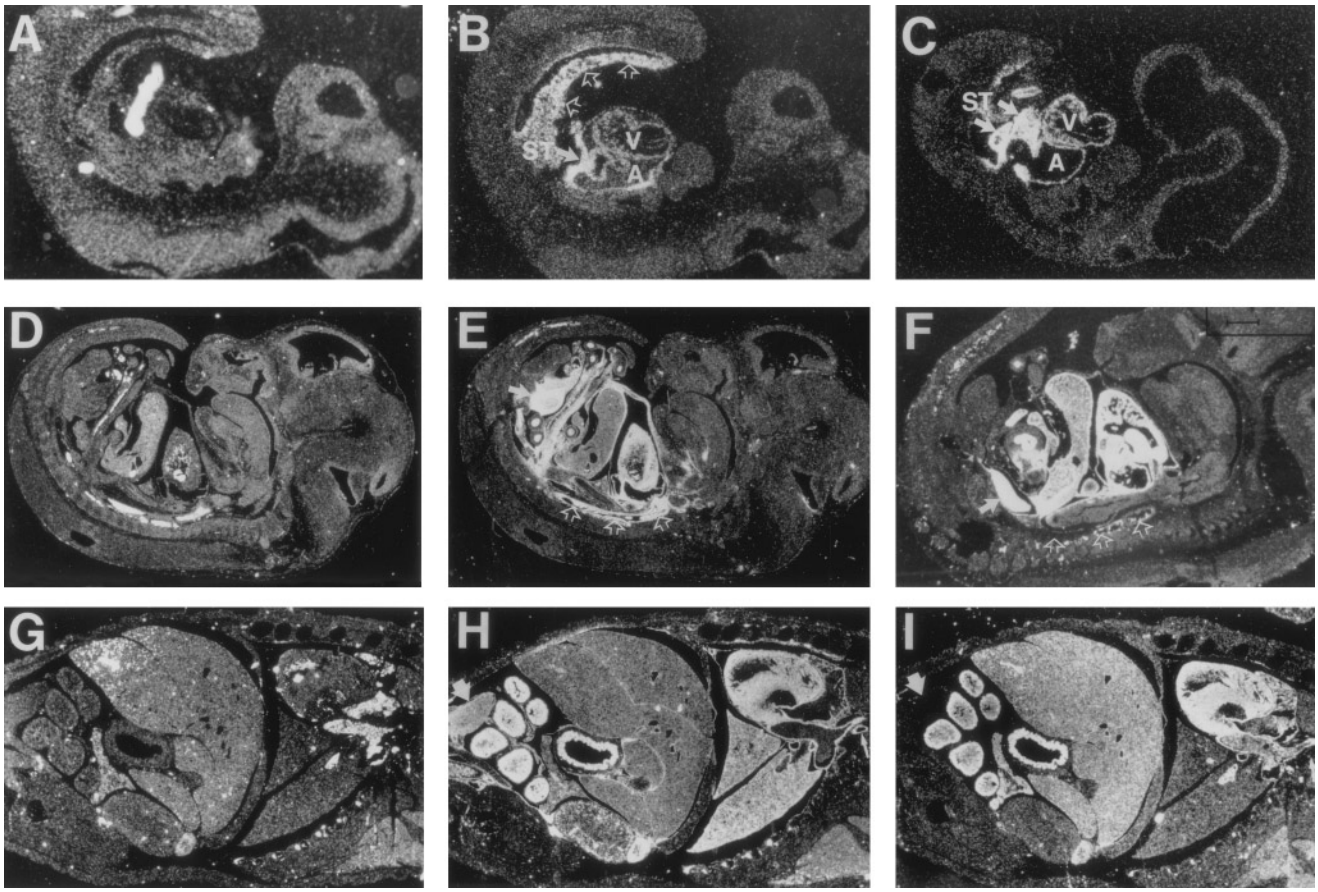

FIG. 7. The temporal and spatial patterns of GATA-6 and GATA-4 gene expression during murine embryonic development. *In situ* hybridization analyses were performed using the control GATA-6 sense riboprobe (A, D, and G), the GATA-6 antisense riboprobe (B, E, and H), or the GATA-4 antisense riboprobe (C, F, and I) on staged E9.5 (A–C), E13.5 (D–F), and E20.0 (G–H) embryos. (A–C) In E9.5 embryos, both the GATA-6 (B) and GATA-4 (C) riboprobes hybridized (white staining) to the atria (A) and ventricle (V) of the primitive heart, the septum transversum (ST), and the cells lining the midgut and hindgut regions (open arrows). Original magnification, 12.5× (D–F) In E13.5 embryos, both the GATA-6 (E) and GATA-4 (F) riboprobes hybridized to the heart (see also Fig. 8A) the stomach, and the small intestinal and large intestinal epithelium (see also Fig. 8C). Only the GATA-6 riboprobe hybridized to the dorsal aorta (compare E and F, open arrows), the lung buds (see also Figs. 8B and 8D), and the urogenital ridge (E, white arrow). High levels of GATA-4 gene expression were demonstrated in the embryonic ovary (F, white arrow) and liver. Original magnification, 3.1–3.5× (G–I). In E20.0 embryos, both the GATA-6 (H) and GATA-4 (I) riboprobes hybridized to the heart, stomach, and intestinal epithelium. Only the GATA-6 riboprobe hybridized to the aorta (compare H and I, thin white arrows) (see also Fig. 8F), bladder (compare H and I, white arrows) (see also Fig. 8E), and developing lungs. The GATA-4 riboprobe hybridized to the liver, testes, and ovaries (I and sections not shown). Original magnification, 3.1×.

scriptional activation domains, (ii) GATA-4 and -6 exhibit virtually identical temporal and spatial patterns of gene expression in the precardiac mesoderm and within the developing heart, and (iii) GATA-4 and -6 each can bind to the same element within the cTnC cardiac-specific transcriptional enhancer and that overexpression of either protein transactivates the cTnC promoter enhancer in noncardiac muscle cell lineages strongly suggest that these genes serve at least partially redundant functions in the developing heart. However, alternative pathways of activation and/or differential binding of accessory factors (either positive or negative regulatory) could still allow these two proteins to differentially activate cardiac-specific transcription. Interestingly, preliminary experiments suggest that in GATA-4-/-ES cells (Soudais *et al.*, 1995), GATA-6 gene expression is up-regulated three- to fivefold above levels expressed in wild-type ES cells during differentiation into cystic embryoid bodies, suggesting that an autoregulatory loop between GATA-4 and -6 may exist (C. Soudais, E. Morrisey, M. Parmacek and J. Leiden, unpublished observation).

The demonstration that GATA-6 is a potent transcriptional activator and the observation that the GATA-6 gene

is expressed in tissues where GATA-4 is not expressed, such as the arteries, veins, bladder, and developing airways, suggests that GATA-6 may play a unique role in the development of these tissues. Our studies demonstrate that within the vasculature and bladder, and possibly within the developing airways, the GATA-6 gene is expressed abundantly in SMCs. Relatively little is currently understood about the molecular mechanisms that control SMC lineage specification and differentiation (for review see Owens, 1995). As such, our studies serve to identify GATA-6 as a potential key developmental regulator of this muscle cell lineage. However, in contrast to previously described markers of the SMC lineage which are expressed in SMCs in tissues throughout the body, the GATA-6 gene was expressed only in the vasculature and bladder, but not in uterine or gastrointestinal smooth muscle cells. These data suggest that a previously unrecognized molecular program may distinguish subsets of the SMCs. In support of this hypothesis, recent studies examining the molecular mechanisms that control expression of the SMC-specific SM22α gene have served to identify a vascular SMC-specific transcriptional regulatory element that functions in transgenic mice (Solway *et al.*, 1995; Li *et al.*, 1996). Moreover, these data are consistent with a model in which GATA-6 functions downstream of a more generalized SMC determination factor. The elucidation of transcriptional targets for GATA-6 in SMCs as well as the factors that regulate GATA-6 expression in this lineage should increase our understanding of the transcriptional program that directs the differentiation of this important muscle cell lineage.

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