p300 Protein as a Coactivator of GATA-5 in the Transcription of Cardiac-restricted Atrial Natriuretic Factor Gene*

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A cellular target of adenovirus E1A oncoprotein, p300 is a transcriptional coactivator and a negative regulator of cellular proliferation. A previous study suggests that the p300 family is also involved in cell type-specific transcription in cardiac myocytes. However, nothing is known about which cardiac transcription factor(s) interact with and transactivate through these proteins. The transcription factors GATA-4/5/6 have been implicated as key regulators of cardiogenesis, and they participate in the transcription of many cardiac-specific genes. Here we show that E1A represses the GATA-5-dependent transactivation of a promoter derived from the cardiac-restricted atrial natriuretic factor gene. This repression is correlated with the interaction of E1A with p300, indicating that p300 participates in GATA-5-dependent transactivation. E1A markedly down-regulates endogenous atrial natriuretic factor expression, as well as disrupts the interaction between p300 and GATA-5. A small fragment of p300 containing the carboxyl-terminal cysteine/histidine-rich domain, sufficient to interact with GATA-5, prevents transcriptional activation by GATA-5 as a dominant-negative mutant. Consistent with its role as a coactivator, p300 markedly potentiates GATA-5-activated transcription. These results implicate p300 as an important component of myocardial cell differentiation and provide an insight into the relationship between mechanisms that mediate cell type-specific transcription and cell cycle regulation during cardiogenesis.

Cardiac myocytes are highly differentiated cells, the structural and functional properties of which are especially suited to maintain normal blood flow. The differentiated status of these cells is maintained by the expression of muscle-specific genes, the formation of sarcomeres, and rhythmic contractions (reviewed in Ref. 1). In contrast to skeletal myogenesis, a subset of differentiated cardiac myocytes continues to undergo cell division (2). However, during late gestation, increasing proportions of these cells arrest irreversibly in the G1 phase of the cell cycle (3, 4). Therefore, adult cardiac myocytes no longer have the ability to proliferate, and further growth is limited to hypertrophy, i.e. an increase in cell volume but not in number. As such, the identification of the signaling pathways that mediate cardiac myocyte differentiation is critical to the ultimate elucidation of the molecular basis of cardiac muscle hypertrophy and failure.

Clues regarding cellular proteins that regulate aspects of cardiac muscle differentiation have been provided by the effects of the adenovirus E1A oncoprotein in these cells. When E1A is expressed in already differentiated cardiac muscle cells, it stimulates cellular DNA synthesis and represses muscle-specific gene expression (5–7). E1A interacts with at least two classes of cellular factors, namely, the p300 and Rb1 families (reviewed in Ref. 8). As for the Rb pathway, adenovirus E1A targets the “pocket” domain of Rb and its relatives p107 and p130/Rb2, thereby perturbing normal cellular interaction with the transcription factor E2F/DP. Nevertheless, studies with E1A mutants indicate that interference with the p300 pathway is sufficient to stimulate DNA synthesis and repress muscle-specific gene expression in cardiac myocytes (5–7). These experiments suggest that p300 proteins are involved in maintaining both cell type-specific gene expression and cell cycle arrest in cardiac myocytes.

p300 proteins are classified as the transcriptional coactivator that communicates between transactivators binding on enhancer DNA and the basal transcriptional complex formed on the promoter near the transcription initiation site (9). This communication is required for transcriptional activation by the transactivators. Consistent with this view, the p300 family of transcriptional coactivators has been noted to modulate many examples of enhancer-mediated transcription (9–27). The members of this family, which, thus far, include p300 and the cAMP-response element binding protein binding protein (CBP), interact directly with components of the basal transcriptional apparatus (e.g. TFIIB and TATA box-binding protein (11, 18, 28)) and diverse enhancer-binding proteins. A p300 protein forms a specific complex with p53, providing a mechanism by which p300 modulates the checkpoint function in the G1 phase of the cell cycle (29, 30). In skeletal muscle, p300/CBP are transcriptional adaptors for MyoD and MEF-2, and these interactions are required for myogenesis (18–21). In addition to their possible “bridging function,” p300 proteins possess intrinsic histone acetyl transferase activity (31, 32), which is hypothesized to promote a locally “open” and transcriptionally active chromatin configuration.

Given the potential role played by p300 protein(s) in cardiac-specific transcription as coactivator(s), the question arises as to which transcription factor(s) interact with and transactivate...
through these proteins in cardiac myocytes. Because E1A can repress the transcription of many cardiac genes (5–7), p300 may interact with key developmental regulators expressed in these cells. The following evidence suggests the importance of GATA-4/5/6, a subclass of zinc finger GATA transcription factors, in cardiogenesis. First, the expression of GATA-4/5/6 is restricted to the heart and gut (33–37). All three genes are transcribed at very early stages of Xenopus, avian, and mouse cardiac development (35–40). Second, this subclass of GATA factors specifically binds the consensus motif conserved among many cardiac-specific promoters, where they function as positive regulators (34, 41, 42). Third, embryos that lack GATA-4 fail to develop a fusion of the two heart-forming primordia and foregut (43, 44). Despite these data supporting critical roles of GATA factors in cardiogenesis, the ectopic expression of GATA-4 is not sufficient to initiate cardiac differentiation, suggesting that co-factors are required (37, 45, 46).

These findings prompted us to investigate whether p300 and GATA-4/5/6/6 interact with each other and regulate a subset of cardiac muscle-specific genes. As a cardiac-specific promoter, we used a promoter derived from the atrial natriuretic factor (ANF) gene, which is expressed very early in embryonic development at the stage when cells are committed to the cardiac phenotype. Throughout embryonic and fetal development, ANF expression characterizes both atrial and ventricular but not skeletal or smooth muscle cells, suggesting that the ANF gene provides a good model system to identify cardiac determinant factors. We present data showing that p300 and GATA-5 cooperate in activating the cardiac-restricted ANF promoter and that these proteins physically interact in vivo. This molecular interaction provides further insights into the relationship between mechanisms that mediate cell type-specific transcription and cell cycle regulation during cardiogenesis.

MATERIALS AND METHODS

Plasmid Constructs—The plasmid constructs pANFluc and β-actinc-luc consist of the firefly luciferase (luc) cDNA driven by 3412 of rat ANF or 433 bp of avian cytoplasmic β-actin promoter sequences (7). Site-directed mutagenesis of the proximal (ANF) promoter was performed by amplifying a part of ANF promoter sequences with polymerase chain reaction (p131mutGATA-ANF). Wild type ANF promoter with correspondent length was also generated by polymerase chain reaction (p131wtANFluc). pRSVcat and pRSVluc contain the bacterial chloramphenicol acetyltransferase (CAT) and the luc gene driven by Rous sarcoma virus (RSV) long terminal repeat sequences, respectively (7, 47). The murine GATA-5 and GATA-6 expression plasmids, pCDNAG5 (40) and pCDNAG6 (39), respectively, were gifts from Dr. Michael S. Parmacek (University of Chicago, Chicago, IL) and are described elsewhere. The murine GATA-4 expression plasmid, pCDNA4, was subcloned by digesting pMT22-GATA-4 (33, 41) (provided by Dr. David Wilson, Washington University, St. Louis, MO) with EcoRI to isolate a 1.9-kilobase pair insert and by subcloning the resultant cDNA fragment encoding the murine GATA-4 into the EcoRI site of the eukaryotic expression plasmid, pcDNA3 (Invitrogen, Carlsbad, CA). pwtE1A is an expression vector for wild type 12 S. E. 1A. pde2–36E1A is derived from pwtE1A bearing an amino-terminal deletion of E1A 12 S between amino acid residues 2 and 36 (13). pCMVβ-gal, CMVwtp300, pCMVde2sp300, and pCMV1514–1922p300 contain the cytomegalovirus promoter/enhancer fused to β-galactosidase cDNA, a full-length human p300 cDNA (9), p300 cDNA with an internal deletion in the E1A binding domain (9), or a portion of p300 cDNA encoding amino acid residues 1514–1922 (18), respectively. pwtE1A, pde2–36E1A, pCMVwtp300, and pCMVde2sp300 were gifts from Drs. Richard Eckner and David M. Livingston (Harvard Medical School, Boston, MA), and pCMV1514–1922p300 was provided by Dr. Anton Thomas (Jefferson University, Philadelphia, PA). Plasmids were purified by anion exchange chromatography (Qiagen, Hilden, Germany), quantified by the measurement of A260, and examined on agarose gels stained with ethidium bromide prior to use.

Cell Culture, Transfection, and Luciferase/CAT Assays—NIH3T3 cells (mouse fibroblasts) or COS7 cells (African green monkey kidney cells) were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. The cells were washed twice with serum-free medium and then transfected with a total of less than 5 μg of DNA in 60-mm plates using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s recommendation. After a 5-h incubation with DNA-LipofectAMINE complex, the cells were washed twice with serum-free medium and further incubated in the medium with 10% fetal bovine serum for 48 h. The cells were then washed twice with ice-cold phosphate-buffered saline and lysed as described (7, 47). Luc and CAT activities were determined in the same cell lysate as described previously (7, 47). The relative luc activity was calculated from the ratio of luc-background:CAT-background and expressed as the mean ± S.E. Groups were compared using the unpaired, two-tailed t test, and differences were considered significant when p < 0.05.

Electrophoretic Mobility Shift Assays (EMSAs)—A nuclear extract was prepared from COST cells transfected with pCDNAG5. Double-stranded oligonucleotides were designed that contained GATA motifs from the ANF promoter. The sequences of the sense strand of these oligonucleotides were as follows: ANF-GATA, 5′-CCCGAGCTCCGTCTGGAGCTGATGTAACCTTTAAAGGGCCATCTTC-3′ (45); mutANF-GATA, 5′-CCCGGATCCGCTCCCTGGAGCTGATGTAACCTTTAAAGGGCCATCTTC-3′; CEF-1, (GATA element in the cF4 promoter) (41), 5′-CCGGATCCCGCTGGAGCTGATGTAACCTTTAAAGGGCCATCTTC-3′; non-specific oligonucleotide, 5′-GGGCGATGTCGCGCGATGC-3′. Oligonucleotides were synthesized by Greiner Inc. (Tokyo, Japan) and purified by S.polyacrylamide gel electrophoresis. EMSAs were performed as described previously (47) at 4 °C for 20 min in 15-μl reaction mixtures containing 10 μg of nuclear extract, 0.25 ng (>20,000 cpm) of radiolabeled double-stranded oligonucleotide, 500 ng of poly(dI-dC), 5 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.5 mM dithiothreitol, 37.5 mM KCl, and 4% Ficoll 400. For cold competition experiments, a 100-fold molar excess of unlabeled competitor oligonucleotide was included in the binding reaction mixture. Protein-DNA complexes were separated by electrophoresis on 4% nondenaturing polyacrylamide gels in 0.25× TBE (1× TBE, 100 mM Tris, 1 mM EDTA, and 2 mM EDTA) at 4 °C. Anti-GATA-5 antibody for supershift experiment was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Immunoprecipitation and Western Blotting—Nuclear extracts were prepared from COST cells transfected with 2 μg of pCDNAG5 and 10 μg of either pCMVβ-gal or pCMV1514–1922p300. One mg of the extract was immunoprecipitated using an anti-mouse p300-polycyclonal antibody (Santa Cruz Biotechnology) or normal goat serum in low stringency buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 10 μg/ml aprotinin and leupeptin, and 0.5 mM phenylmethylsulfonyl fluoride) for 16 h at 4 °C and incubated with protein G agarose beads for 1 h at 4 °C. The precipitate was washed four times in the same buffer, resuspended in 50 μl of SDS-lysis buffer (20 mM Tris, pH 7.5, 50 mM NaCl, 0.5% SDS, 1 mM dithiothreitol), heated to 95 °C for 2 min, electrophoresed by a SDS-polyacrylamide gel (4%), transferred to an Immobilon membranes, and reacted with monoclonal antibodies against human p300 (a mixture of RW125, RW105, and RW109, Upstate Biotechnology, Lake Placid, NY), which was subsequently detected by horseradish peroxidase-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove PA). Signals were detected using the enhanced chemiluminescence Western blotting detection system (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

RNA Analysis—Primary ventricular cardiac myocytes were prepared from hearts of 1–2-day-old Harlan Sprague-Dawley rats as described (47). Twenty-four hours after plating, cells were washed twice with serum-free media and then transfected with 2 μg of pCMVβ-gal, pwtE1A or pde2–36E1A using LipofectAMINE Plus (Life Technologies, Inc.) according to the manufacturer’s recommendation. After a 2-h incubation with DNA-LipofectAMINE complex, the cells were washed twice with serum-free media and further incubated for 48 h. Northern blot analysis of total RNA from these cells was performed as described previously (7, 47). A DNA probe consisting of the PstI fragment of pANF-1 (47), which encompasses rat ANF coding region (nucleotides 1–580), was radiolabeled by random priming and used to detect ANF mRNA as described previously (7, 47). Abundance of mRNAs were quantified by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA). Values of ANF mRNA were normalized to those of GAPDH mRNA.

RESULTS

GATA-5 Is a Potent Activator of the ANF Promoter—Among members of the GATA transcription factor family, GATA-4, -5, and -6 are expressed in the heart (33–37). The 3412-bp se-
Fig. 1. Specific transactivation of a 3412-bp rat ANF promoter by GATA-4/5/6 in cultured neonatal cardiac myocytes. NIH3T3 cells were transfected with 2.5 µg of GATA-4/5/6 expression vector, 1.5 µg of a reporter plasmid (β-actinLuc, pANFluc, p31wtANFluc, or p31mutGATA-ANFluc) and 0.5 µg of pRSVCAT. Forty-eight hours later, the relative luciferase activities (luc/CAT) were determined. The results are expressed as fold activation of the normalized luciferase activity (luc/CAT) relative to co-transfection with the control β-galactosidase expression vector. The data shown are the mean ± S.E. of two independent experiments, each carried out in duplicate.

Co-activation of ANF by GATA-5 and p300

p300 Protein Is Involved in GATA-5-dependent Transactivation—To test the hypothesis that p300 is involved in GATA-5-activated transcription in NIH3T3 cells, we transfected an ANF reporter plasmid (pANFluc) together with an expression vector encoding wild type E1A (pwtE1A) or an E1A 12 S mutant (pdel2–36E1A) bearing an amino-terminal deletion that loses its ability to bind to p300, but not to Rb family proteins (49–50). pwtE1A completely repressed the GATA-dependent ANF promoter activity (Fig. 3, column 2). When we used pdel2–36E1A, it repressed the reporter activity much more weakly compared with the wild type (column 3), indicating that the complete repression by E1A requires its binding to p300. To test whether a causal connection exists between the repression by E1A of GATA-5-dependent transcription and the binding of E1A to p300 proteins, we assessed the effect of the overexpression of p300 on this transcriptional repression. To augment p300 levels in the cell, we overexpressed either full-
GATA-5 antibody (Fig. 4)ern blotting using the anti-p300 antibody shows that the anti-GATA-5 is correlated with these functional data. COS7 cells

Then, we addressed whether the interaction of p300 with repressed the GATA-5-dependent ANF promoter activity. Nuclear extracts expressing either GATA-5 alone (Fig. 4)GATA5 antibody did not coprecipitate p300 protein from precipitated p300 protein, even after extensive washing. The anti-GATA5 antibody as a positive control (Fig. 4)control (lane 4). West-

nuclear extracts expressing either GATA-5 alone (Fig. 4)luc and CAT were determined. The results are expressed as fold activation by GATA-5 of the normalized luc activity (luc/CAT) relative to co-transfection with the control β-galactosidase expression vector. The data shown are from two independent experiments, each carried out in duplicate.

Our functional studies demonstrated that E1A completely repressed the GATA-5-dependent ANF promoter activity. Then, we addressed whether the interaction of p300 with GATA-5 is correlated with these functional data. COS7 cells

were transfected with an expression plasmid encoding either β-galactosidase (Fig. 4B, lanes 1–3) or E1A (Fig. 4B, lanes 4–6), in addition to the templates for p300 and GATA-5. Nuclear extracts from these cells were subjected to immunoprecipitation with an anti-p300 antibody (Fig. 4B, lanes 1 and 4), IgG (lanes 2 and 5), or an anti-GATA5 antibody (lanes 3 and 6) and subjected to Western blot by the anti-p300 antibody.

**Interaction between p300 and GATA-5 Proteins**—Based on the proposed coactivator role of p300, its involvement in GATA-5-dependent transcription activation suggested that p300 might interact with GATA-5. To test this possibility, we performed immunoprecipitations, followed by Western blots. COS7 cells were transfected with either an expression plasmid encoding GATA-5 (pcDNA5G5) (Fig. 4A, lane 3), that encoding p300 (pCMVwt300) (lane 4), or both (lanes 1, 2, and 5). Forty-eight hours later, nuclear extracts derived from the transfected cells were subjected to immunoprecipitation with an anti-p300 antibody as a positive control (Fig. 4A, lane 1), IgG as a negative control (lane 2) or an anti-GATA-5-antibody (lanes 3–5). Western blotting using the anti-p300 antibody shows that the anti-GATA-5 antibody (Fig. 4A, lane 5), but not IgG (lane 2) coprecipitated p300 protein even after extensive washing. The anti-GATA-5 antibody did not coprecipitate p300 protein from nuclear extracts expressing either GATA-5 alone (Fig. 4A, lane 3) or p300 alone (lane 4). Thus, p300 interacted with GATA-5.

**Co-activation of ANF by GATA-5 and p300**

**FIG. 4.**  p300 interacts with GATA-5. A, COS7 cells were co-transfected with 10 µg of pCMVwtp300 and/or 2 µg of pcDNA5G5 as indicated. Nuclear extracts derived from these cells were immunoprecipitated with anti-p300 antibody (lane 1), with IgG (lane 2), or with anti-GATA5 antibody (lanes 3–5). After electrophoresis and electroblotting, the membranes containing immobilized immunocomplexes were subjected to Western blot by the anti-p300 antibody. B, COS7 cells were co-transfected with 0.45 µg of pCMVβ-gal (lanes 1–3) or pwtE1A (lanes 3–6) in addition to 10 µg of pCMVwt300 and 2 µg of pcDNA5G5. Nuclear extracts from these cells were immunoprecipitated with the anti-p300 antibody (lanes 1 and 4), with IgG (lanes 2 and 5), or with the anti-GATA5 antibody (lanes 3 and 6) and subjected to Western blot by the anti-p300 antibody.

**E1A Represses Endogenous ANF Expression in Cardiac Myocytes**—To assess the function of E1A in the context of differentiated cardiac myocytes, we examined the effects of wild type and a mutant form of E1A on the expression of the endogenous ANF gene in primary cardiac myocytes from neonatal rats. As shown in Fig. 5A, wild type and mutant E1A were abundantly and similarly expressed in cardiac myocytes transfected with a correspondent expression vector. There was no E1A expression detectable in cardiac myocytes transfected with a β-galactosidase expression vector. As shown in Fig. 5B, the expression of the cardiac-specific ANF gene was markedly reduced in these cells transfected with an E1A expression vector (lane 2) compared with that in cells transfected with a β-galactosidase expression vector (lane 1). However, transfection with E1Adel2–36, a mutant defective for p300 binding (lane 3), mildly affected the expression of the ANF gene. Quantitative
performed using anti-E1A antibody as described previously (7). The transfection of the GATA-5-expressing vector produced a marked stimulation of transcription in COS7 cells. We cotransfected the ANF reporter plasmid together with GATA-5 expression vector alone or in combination with a vector encoding p300. Forty-eight hours later, we measured the ANF reporter activity. The transfection of the GATA-5-expressing vector produced a marked stimulation of transcription, whereas a mutant E1A defective for p300 binding only mildly repressed it. These findings suggest that endogenous p300 in these cells plays a role in the maintenance of ANF gene transcription.

**A Dominant-negative Form of p300 Prevents Transcriptional Activation by GATA-5**—Cys/His residues (C/H domains) in p300 are well conserved among many species and serve as binding sites for sequence-specific transcription factors. To test whether a region (1514–1922p300) containing the carboxy-terminal C/H domains (C/H-3) is sufficient to mediate binding of GATA-5, COS7 cells were transfected with pCMV1514–1922p300 and pcDNA5. Nuclear extracts derived from the transfected cells were subjected to immunoprecipitation with an anti-GATA-5 antibody. Western blot analysis of these precipitates revealed the presence of a discrete fraction of 1514–1922p300 associated with GATA-5 (Fig. 6A, lane 3). These findings suggest that C/H-3 domain of p300 is sufficient to interact with GATA-5.

A small fragment of p300 (1514–1922) lacks the transactivation ability shown by Gal4 reporter assays (18) but retains the ability to interact with GATA-5. These findings suggest that this fragment might impair the ability of endogenous p300 to associate with GATA-5, thereby inactivating the GATA-5-dependent transcription. To test this hypothesis, we cotransfected an ANF reporter plasmid into NIH3T3 cells together with GATA-5 expression vector alone or in combination with a vector encoding 1514–1922p300. Forty-eight hours later, we measured the ANF reporter activity. The transfection of the GATA-5-expressing vector produced only a minimal stimulation of transcription in COS7 cells (Fig. 7, column 2). This may be explained by the fact that these cells express SV40 large T antigen, which disrupts the function of endogenous p300. The coexpression of full-length p300 and GATA-5 induced a marked stimulation of the reporter above the levels observed with GATA-5 alone (Fig. 7, column 4). Therefore, the transcriptional activity of GATA-5 is markedly enhanced by p300.

**DISCUSSION**

Cell type-specific gene expression is established through the combinatorial action of restricted and more widely expressed transcription factors. The architecture of a regulatory element directs the assembly of these factors into higher-order structures that ultimately determine the transcriptional readout. Our present findings add GATA-5 to the increasing set of factors of which the function is regulated by the ubiquitously expressed transcriptional coactivator p300. We showed that p300 markedly augments the GATA-5-dependent transcription of the ANF promoter. The coexpression of wild type E1A completely abrogated GATA-5-mediated transcription, whereas a mutant E1A unable to bind p300 did not significantly repress it. Because GATA-5 is one of the earliest markers of precardiac cells (40) and may play critical roles in cardiogenesis, GATA-5 is likely to be an important target of p300 in cardiac myocytes.

To date, six related zinc finger-containing proteins have been described that recognize and bind the GATA motif (36, 39, 40). These proteins fall into two subgroups; those containing GATA-1, -2, and -3 and those with GATA-4, -5, and -6. The subgroups are defined by both sequence homology and expression pattern, with GATA-1, -2, -3 predominating in blood and endodermal derivatives, and GATA-4, -5, and -6 predominating in heart and endodermal derivatives. Interestingly, the genes encoding GATA-4 and -6 are expressed in the heart throughout embryonic and postnatal development, whereas the murine GATA-5 gene is normally expressed in a temporally and spatially restricted pattern within the embryonic heart (39, 40). The present findings demonstrated that GATA-5 has striking transactivation ability for the ANF promoter, whereas GATA-4/6 has modest activation ability. We observed that p300 can associate with not only GATA-5 but also GATA-4 and GATA-6 (data not shown). These results are consistent with a recent report that in the erythroid cell lineage, CBP associates with GATA-1 through its zinc-finger region (51), which is conserved among GATA-1/2/3 and GATA-4/5/6. Possible mechanisms for the difference in the transactivation potentials among GATA-4/5/6 should be further investigated.

The forced expression of E1A blocked GATA-5-dependent
transcription, whereas that of a mutant E1A that lacks p300 binding but retains the potential to interact with Rb-related proteins had a minimal effect on transcriptional repression. The likely model envisaged from these findings is one in which E1A sequesters p300 (and/or a p300-related protein), making it unavailable to participate in the GATA-5-mediated transactivation. Although we cannot rule out the possibility that E1A sequesters GATA-5 through a binding site similar to that for p300, direct involvement of p300 in the transcription is strongly supported by the finding that p300del33, a mutant lacking residues required for binding by E1A, but not wild type p300, diminished the repression of the GATA-5-dependent transcription by E1A. In addition, the marked potentiation of GATA-5-dependent transactivation by p300 indicates that p300 participates in transactivation mediated by the interaction between GATA-5 and the ANF GATA element. Because p300 is found in complexes containing TFIIH and TATA box-binding protein, the interaction between p300 and GATA-5 proteins suggests that p300 serves as a link between GATA-5 and the basal transcriptional complex during transactivation. This feature of p300 defines it as a transcriptional coactivator for GATA-5. The present study also demonstrated that a small fragment of p300 (1514–1922p300) containing the carboxyl-terminal cysteine/histidine-rich domain inhibited endogenous p300 function in the GATA-5-dependent transactivation of the ANF promoter. Because this fragment is able to bind GATA-5 but has no transcriptional activity, its negative role may be associated with sequestering GATA-5 from the wild type p300 in cells (a dominant-negative effect). Taken together, these results strongly support the contention that p300 serves as a coactivator for GATA-5.

Several lines of evidence indicate that p300 and CBP are required for the maintenance of the G1 phase of the cell cycle in differenti-ated cardiac myocytes. The binding of p300/CBP by E1A stimulates quiescent primary cardiac myocytes to undergo DNA synthesis (5, 6). In keeping with this view, p300 and/or CBP can function as coactivators of p53 in the induction of cell cycle arrest. These functions of p300/CBP are abrogated by E1A (29, 30). The interaction of p300 with both p53 and cardiac-restricted GATA factors suggest that the p300 family of transcriptional coactivators provides a link between cell cycle arrest and cell type-specific transcription during cardiogenesis. However, differentiation and proliferation in the cardiac myocyte lineage are not mutually exclusive. At least, a subset of differentiated cardiac myocytes still undergo cell division, whereas increasing proportions of these cells arrest in G1 during late gestation (2–4). Considering this discrepancy, we speculate that p300 in cardiac myocytes interacts with either p53 or GATA-4/5/6 at specific time points during cardiogenesis. Further studies are needed on the interaction of p300 with these proteins in different developmental stages.

Mice lacking a functional p300 gene were recently generated (52). Animals nullizygous for p300 died between days 9 and 11.5 of gestation, exhibiting defects in heart development. The heart in the p300−/− embryos showed defects of cardiac muscle differentiation and in trabeculation. The expression of the myocardial contractile proteins was clearly reduced in the mutant hearts compared with the wild type hearts of littermates. These findings are evidence that p300 is required for proper heart development. GATA-4/5/6 have been implicated in the transcription of many cardiac muscle structural proteins (39–42). In addition, it has been reported that MEF-2 proteins,
which play important roles in cardiac and skeletal muscle development, also interact with p300 (20). There may be other p300-interacting proteins that are involved in cardiogenesis. Functional impairment of these factors in the absence of p300 may underlie the defects in the expression of myocardial contractile proteins in the p300−/− mouse embryos.

In addition to the critical roles of GATA-4/5/6 in cardiogenesis, recent studies, including ours, have demonstrated that cardiac GATA factors are involved in the regulated expression of cardiac genes during myocardial cell hypertrophy (47, 53). p300/CBP is present in the nucleus in limited concentration of cardiac genes during myocardial cell hypertrophy (47, 53). p300/CBP is present in the nucleus in limited concentration of cardiac genes during myocardial cell hypertrophy (47, 53).

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