A p300 Protein as a Coactivator of GATA-6 in the Transcription of the Smooth Muscle-Myosin Heavy Chain Gene*

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The mechanisms that regulate smooth muscle development and differentiation are poorly understood. Although recent studies have suggested the possible role of a zinc finger transcription factor, GATA-6, in the differentiation of vascular smooth muscle cells (VSMCs), the downstream gene targeted by GATA-6 is unknown. The expression of smooth muscle-myosin heavy chain (Sm-MHC) provides a highly specific marker for the differentiated phenotype of VSMCs as well as the smooth muscle cell lineage. Here, we show that GATA-6 bound to a GATA-like motif (~810/~805) within the rat Sm-MHC promoter in a sequence-specific manner and activated this promoter through this site. In addition, we show that the transcriptional coactivator p300 associated with GATA-6 during the transcription of the Sm-MHC gene. A p300/GATA-6 complex in VSMCs was up-regulated by induction of the quiescent phenotype. A wild-type E1A, which interferes with endogenous p300, but not a mutant E1A defective for p300 binding, markedly down-regulated the expression of endogenous Sm-MHC in quiescent-phenotype VSMCs. These studies provide the first identification of a functionally important GATA-6 binding site within a smooth muscle-specific promoter and suggest a role for p300 in the maintenance of the differentiated phenotype in VSMCs as a coactivator of GATA-6.

The intimal proliferation of vascular smooth muscle cells (VSMCs)* is known to play an integral role in development of atherosclerotic disease and restenosis after angioplasty (1–7). The proliferating SMCs within thickened arterial intima are phenotypically altered compared with normal medial SMCs (3, 8, 9). These alterations include decreased expression of proteins characteristic of differentiated SMCs, including the smooth muscle isoforms of contractile proteins (reviewed in Ref. 3). Because the expression of these isoforms is closely correlated with the phenotypic state of VSMCs and the growth properties of the cells, it is of fundamental importance to elucidate the mechanisms that regulate the expression of smooth muscle-specific proteins in order to understand vascular pathology.

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Our understanding of muscle gene expression has been greatly advanced in recent years by the identification of several key cis elements and trans factors that regulate cardiac and skeletal muscle-specific transcription (10–12). However, little is known about the myogenic program that controls smooth muscle-specific gene expression. Recently, a zinc finger protein, GATA-6, which is expressed in VSMCs, was cloned (13). GATA-6 is a member of the GATA family of transcription factors which comprises six members. The GATA-1/2/3 subfamily is expressed in the hematopoietic cell lineage, and each of these regulates a unique lineage-specific developmental program (14). On the other hand, GATA-4/5/6 genes encode a subfamily of factors that are expressed in the cardiovascular system and endodermal derivatives (14, 15, 16). Among these, GATA-6 is the only member of the GATA family that is expressed in VSMCs (17–19), and its expression in VSMCs is rapidly down-regulated upon mitogen stimulation (15). In addition, overexpression of GATA-6 in proliferating VSMCs induces cell cycle arrest (20). These data suggest that GATA-6 plays a role in the maintenance of the differentiated phenotype in VSMCs. However, the downstream smooth muscle-specific gene targeted by GATA-6 has yet to be identified.

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. Recently, we reported that adenovirus E1A oncoprotein represses the GATA-5-dependent transactivation of a promoter derived from the cardiac-restricted atrial natriuretic factor gene. This repression was correlated with the interaction of E1A with p300, indicating that p300 participates in GATA-5-dependent transactivation (21). p300 proteins are transcriptional coactivators that communicate between transactivators binding on enhancer DNA and the basal transcriptional complex formed on the promoter near the transcription initiation site (22). 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-binding protein (23–25)) and diverse enhancer-binding proteins. The interaction of p300 and GATA proteins and a possible role of GATA-6 in VSMC differentiation suggest the hypothesis that p300 mediates smooth muscle-specific gene expression.

For the preceding reasons, in the present study we investigated the roles of p300 and GATA-6 in smooth muscle-specific transcription. For this purpose, we utilized a promoter derived from a gene encoding smooth muscle-myosin heavy chain (Sm-MHC), a well characterized smooth muscle-specific contractile protein (3). In addition to the usefulness of this protein as a highly specific marker for the smooth muscle lineage, the ex-
pression of Sm-MHC is decreased or absent in proliferating, dedifferentiated VSMCs (8, 26, 27). Once SMCs in the neointima of atherosclerotic vessels cease proliferating, they re-express the SM1 Sm-MHC isofrom (28). Therefore, the Sm-MHC gene provides a potentially useful promoter for studying the regulation of the SMC differentiation state and the factors involved in specifying the proliferative or quiescent/differentiated SMC phenotype.

We present data showing that GATA-6 binds a conserved GATA-like motif within the rat Sm-MHC promoter in a sequence-specific manner and that p300 and GATA-6 cooperate in activating the Sm-MHC promoter. The involvement of p300 in Sm-MHC gene transcription as a coactivator of GATA-6 provides further insights into the transcriptional program that directs phenotype modulation of VSMCs.

**MATERIALS AND METHODS**

**Plasmid Constructs**—The plasmid construct pSm-MHC-CAT, consisting of the bacterial chloramphenicol acetyltransferase (CAT) driven by the 1346 bp of the rat Sm-MHC gene promoter (29) was a generous gift from Dr. Gary K. Owens (University of Virginia, Charlottesville). Site-directed mutagenesis of the distal (~810/~805) GATA element in the context of the 836-bp rat Sm-MHC promoter was performed by amplifying part of the Sm-MHC sequence with polymerase chain reaction (pmutSm-MHClcuc). The corresponding wild type Sm-MHC promoter with the same length was also generated by polymerase chain reaction (pwtSm-MHClcuc). The cat gene driven by Rous sarcoma virus (RSV) long terminal repeat sequences, respectively (30, 31). The human GATA-6-expressing plasmid, phGATA-6, was kindly donated by Dr. Kenneth Walsh (Tufts University, Boston, MA) (31). pwtE1A is an expression vector for wild type E1A 12S. pdel2-36E1A is derivative of expression vector for wild type E1A 12S. pdel2-36E1A, pdel2h36E1A, and pCMVwtp300 were and a portion of p300 cDNA encoding amino acid residues 1514–1922 of pCMV1514-1922p300 carry the cytomegalvirus promoter/enhancer.

**VSMC Culture and Transfection**—Primary human aortic VSMCs were obtained from Kurabo Industries Ltd. and cultured in medium prepared from the manufacturer’s recommendations. After a 3-h incubation with DNA-PLUS-Lipofectamine complex, VSMCs were cultured in medium containing 15% fetal bovine serum (FBS) for 18 h. The total amount of DNA was washed twice with serum-free media and then transfected with 2 μg of DNA in 60-mm plates using lipofectamine PLUS (Life Technologies, Inc.) according to the manufacturer’s recommendations. After a 3-h incubation with DNA-PLUS-Lipofectamine complex, VSMCs were cultured in serum-free medium (10% FBS) in the absence of growth factors to induce the differentiated phenotype. 48 h later, these cells were harvested with lysis buffer (10 mM HEPES-KOH, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 1.5 μM aprotinin) for subsequent Western blot analysis.

**Immunoprecipitation and Western Blotting**—Nuclear extracts were prepared from COS7 cells transfected with 10 μg of pCMVwtp300 and 2 μg of phGATA-6. Aliquots of the extracts containing 1 mg of protein were immunoprecipitated using anti-mouse GATA-6 polyclonal antibodies (Santa Cruz Biotechnology) or a corresponding amount of normal rabbit IgG in low stringency buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.5% SDS, Nonidet P-40, 1 mM EDTA, 10 μg/ml aprotinin and leupeptin, and 0.5 mg phenylmethylsulfonyl fluoride) for 16 h at 4 °C in 2% Normal Goat Serum (Sigma) beads for 1 h at 4 °C. The IgG was washed four times in the same buffer, resuspended in 20 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 on an SDS-polyacrylamide gel (6%), transferred to an Immobilon membrane, reacted with monoclonal antibodies against human p300 (a mixture of RW128, RW105, and RW109 antibodies, Upstate Biotechnology), and then detected using horseradish peroxidase-conjugated anti-mouse IgG (Jackson Immunoresearch Laboratories). Signals were detected using the enhanced chemiluminescence Western blotting detection system (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

Western blots for Sm-MHC and β-actin was performed using aliquots (10 μg of protein) of cell lysates prepared from VSMCs transfected with pwtE1A, pdel2-36E1A, or CMVβgal. Both primary antibodies were obtained from Dako Laboratories. Western blotting for E1A was performed as described previously (21, 30).

**Statistical Analysis**—Data are presented as means ± S.E. Statistical comparisons were performed using unpaired two-tailed Student’s t tests or analysis of variance with Scheffe’s test where appropriate, with a probability value of less than 0.05 taken to indicate significance.
proximal GATA-like motif (−709/−704) (data not shown). To further confirm that the retarded band represents an interaction of the probe with GATA-6, we performed supershift experiments. The retarded band was strongly supershifted by anti-GATA-6 antibody (Fig. 1, lane 6) but not by either anti-GATA-4 antibody (Fig. 1, lane 7) or normal rabbit IgG (Fig. 1, lane 5). Thus, GATA-6 can bind to the GATA-like motif (−810/−805) within the rat Sm-MHC promoter in a sequence-specific manner.

The Role of GATA-6 for Sm-MHC Transcription in Differentiated VSMCs—To determine whether a forced expression of GATA-6 can transactivate the rat Sm-MHC promoter via the distal GATA site, we performed transient transfection experiments using COS7 cells. We co-transfected a luciferase expression vector driven by the 836-bp wild type Sm-MHC promoter (pwtSm-MHC-luc) or the Sm-MHC promoter with a mutation in the distal GATA site (pmutSm-MHC-luc) with eukaryotic expression plasmids encoding 0.5 or 2.0 μg of GATA-6 expression vector (phGATA-6). The total amount of DNA was kept constant by co-transflecting pCMVβ-gal. The transfection efficiency was monitored by co-transfected pRSVCAT activity. As shown in Fig. 2, the expression of GATA-6 resulted in the activation of the wild type Sm-MHC promoter, and this activation was significantly reduced by the distal GATA site mutation that ablates GATA-6 binding. These findings demonstrate that GATA-6 transactivation of the Sm-MHC promoter is dependent on an intact GATA sequence.

To examine the role of the GATA site in VSMCs of proliferative and differentiated phenotypes, VSMCs were co-transfected with 3 μg of pwtSm-MHC-luc or pmutSm-MHC-luc and 0.6 μg of pRSVCAT. Then, these cells were cultured in 5% fetal bovine serum with growth factors to maintain the proliferative phenotype or in a low serum (1%) medium in the absence of growth factors to induce differentiated phenotype. 48 h later, the cells were collected and luciferase activities normalized with co-transfected pRSVCAT activity were determined. As shown in Fig. 2B, in the proliferative phenotype of VSMCs, mutation of the GATA element within the Sm-MHC promoter decreased the promoter activity by only 12%. In the differentiated phenotype, however, the mutation decreased the activity more than 40%. Thus, the GATA element may play a more important role in the maintenance of the transcriptional activity of the Sm-MHC promoter in the differentiated phenotype of VSMCs than in the proliferative phenotype.

**p300 Interacts with GATA-6**—To determine whether GATA-6 and p300 associate in vivo, we performed immunoprecipitations followed by Western blotting. COS7 cells were transfected with an expression plasmid encoding p300 (pCMV-wtp300) and one encoding GATA-6 (phGATA-6) or β-galactosidase as a control (pCMVβ-gal) (Fig. 3). 48 h later, nuclear

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**Fig. 1.** Analysis of interactions between the Sm-MHC GATA site and GATA-6. EMSA studies in nuclear extracts from COS7 cells overexpressing GATA-6 (lanes 2–7) or β-galactosidase (lane 1) were performed with a radiolabeled oligonucleotide containing the Sm-MHC GATA site. Unlabeled competitor DNAs were present at a 100-fold molar excess as indicated: wild-type Sm-MHC GATA (wt) in lane 3; Sm-MHC GATA with a mutation (mut) in lane 4. Supershift experiments were performed in the presence of 2 μl of anti-GATA-6 antibody (lane 6), anti-GATA-4 antibody (lane 7), or normal rabbit IgG (lane 5) as indicated. The arrow indicates the complex corresponding to the interaction between the Sm-MHC GATA site and GATA-6, and the arrowhead indicates the position of the GATA-6-dependent supershifted band (lane 6).

**Fig. 2.** GATA-6 is involved in Sm-MHC transcription. A, COS7 cells were transfected with 0.5 or 2.5 μg of phGATA-6, 2.0 μg of a reporter plasmid (pwtSm-MHC-luc or pmutSm-MHC-luc), and 0.2 μg of pRSVCAT. 48 h later, the relative luciferase activities (luc/CAT) were determined. The results are expressed as fold activation of the normalized luciferase activities (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. B, VSMCs were co-transfected with 3 μg of pwtSm-MHC-luc or pmutSm-MHC-luc and 0.6 μg of pRSVCAT. These cells then were cultured in 5% fetal bovine serum with growth factors to maintain the proliferative phenotype or in a low-serum (1%) medium in the absence of growth factors to induce differentiated phenotype. 48 h later, the cells were collected and luciferase activities normalized with co-transfected pRSVCAT activity were determined. The activity of the wild type Sm-MHC promoter was set to 100% in each experiment. The data shown are the mean ± S.E. of two independent experiments, each carried out in duplicate.
extracts derived from the transfected cells were subjected to immunoprecipitation with an anti-p300 antibody as a positive control (Fig. 3, lane 1), normal rabbit IgG (lane 2) as a negative control, or an anti-GATA-6 antibody (lanes 3 and 4). Western blotting using the anti-p300 antibody showed that the anti-GATA-6 antibody did not coprecipitate p300 protein from nuclear extracts expressing p300 alone (lane 3). However, the anti-GATA-6 antibody (lane 4) but not normal rabbit IgG (lane 2) coprecipitated p300 protein from nuclear extracts expressing both p300 and GATA-6, even after extensive washing. Thus, p300 interacted with GATA-6.

*p300 Is Involved in GATA-6-dependent Sm-MHC Transcription*—To examine possible functional cooperation between GATA-6 and p300, we performed transient transfection assays in COS7 cells. We cotransfected a CAT expression vector driven by the 1346-bp Sm-MHC promoter region together with a eukaryotic expression plasmid encoding GATA-6 alone or in combination with a vector encoding p300. The transfection efficiency was monitored by co-transfected pRSV

![Fig. 3. p300 interacts with GATA-6. COS7 cells were co-transfected with 10 μg of pCMVwtp300 and 2 μg of phGATA-6 or pCMVβgal as indicated. Nuclear extracts derived from these cells were immunoprecipitated (IP) with the anti-p300 antibody (lane 1), with IgG (lane 2), or with the anti-GATA-6 antibody (lanes 3 and 4). After electrophoresis and electrolabting, the membranes containing immobeld immunocomplexes were subjected to Western blot by the anti-p300 antibody. kd, kilodaltons.](image)

![Fig. 4. p300 is involved in the GATA-6-dependent Sm-MHC transcription. A, COS7 cells were co-transfected with 2 μg of a Sm-MHC CAT reporter, 0.1 μg of pRSVluc, 1.5 μg of phGATA-6 or pCMVβgal, and 1.5 μg of pCMVwtp300 or pCMVβgal, as indicated. The data shown are from two independent experiments, each carried out in duplicate. B, COS7 cells were co-transfected with: 2 μg of a Sm-MHC CAT reporter, 0.1 μg of pRSVluc, 0.5 μg of phGATA-6 or pCMVβgal, 2.0 μg of an expression plasmid encoding 1514–1922p300 (p300 DN mutant) containing the regions required for the interaction with GATA factors. The total DNA content was equalized in each sample with pCMVβgal. Samples were assayed for luciferase and CAT activities 48 h after transfection. The results are expressed as -fold activation by GATA-6 of the normalized luciferase activity (luc/CAT). The data shown are the mean ± S.E. of two independent experiments, each carried out in duplicate.](image)

A small fragment of p300 (1514–1922) lacks the transactivation ability shown by Gal4 reporter assays (24) but retains the ability to interact with GATA factors (21). Thus, this small fragment acts as a dominant negative mutant (21, 24). To clarify the role of p300 in the GATA-6-mediated transactivation of the Sm-MHC promoter, we cotransfected a Sm-MHC reporter plasmid into COS7 cells together with the GATA-6 expression vector alone, or in combination with a vector encoding the dominant negative mutant of p300 (pCMV1514–1922p300). 48 h later, we measured the Sm-MHC reporter activity. As shown in Fig. 4, the coexpression of p300 and GATA-6 induced a marked (22-fold) stimulation of expression of the reporter above the levels observed with either GATA-6 or p300 alone (2.9- and 6.6-fold, respectively). Therefore, the transcription regulatory activity of GATA-6 is markedly enhanced by p300.

A p300/GATA-6 Complex Is Up-regulated in the Differentiated Phenotype of VSMCs—To determine whether Sm-MHC GATA binding activity is modulated depending on the phenotype of VSMCs, EMSAs were performed. Nuclear extracts prepared from VSMCs with either proliferating (Fig. 5A, lane 1) or differentiated (Fig. 5, A, lanes 2–6 and B, lanes 1–4) phenotype were probed with a radiolabeled oligonucleotide containing the Sm-MHC GATA site in the presence or absence of competitor DNAs. The induction of the differentiated phenotype was confirmed by immunostaining with anti-smooth muscle-specific actin antibody. As shown in Fig. 5A, competition EMSAs revealed that a retarded band represented specific binding, as evidenced by the fact that it was competed out by an excess of
Differentiated VSMCs were transfected with 2 μg of pCMV/β-gal (β-gal), pwtE1A (E1A), or pdel2-36E1A (E1A del2-36). Whole cell lysates from these cells were subjected to Western blot by the anti-Sm-MHC antibody, anti-β-actin antibody, or anti-E1A antibody. M.W., molecular weight.

unlabeled Sm-MHC GATA oligonucleotide (lanes 3 and 4) but not by the same amount of an oligonucleotide containing the Sm-MHC GATA site with a mutation (lanes 5 and 6). To further confirm that the retarded band represents an interaction of the probe with GATA-6, we performed supershift experiments. As shown in Fig. 5B, the retarded band was supershifted by anti-GATA-6 antibody (lane 3) but not by anti-GATA-4 antibody (lane 2). Furthermore, the retarded band was supershifted by anti-p300 antibody (lane 4), suggesting that GATA-6 formed a complex with p300 in VSMCs with the differentiated phenotype. Notably, the amount of the specific complex containing p300 and GATA-6 markedly increased in nuclear extracts from VSMCs with the differentiated phenotype (Fig. 5A, lane 2) as compared with those from VSMCs with the proliferating phenotype (Fig. 5A, lane 1).

Endogenous Expression of Sm-MHC in VSMCs Is Down-regulated by E1A—To investigate the role of endogenous p300 in differentiated-phenotype VSMCs, we performed Western blotting after expressing adenovirus E1A 12S oncoprotein or an E1A 12S mutant protein with an amino-terminal deletion resulting in loss of its ability to bind to p300. Differentiated-phenotype VSMCs were transfected with pwtE1A, pdel2-36E1A, or pCMV/β-gal as a control. Whole cell extracts were obtained from these cells and subjected to Western blotting using an anti-Sm-MHC antibody. As shown in Fig. 6, the expression of Sm-MHC was markedly reduced in VSMCs transfected with a wild type E1A expression vector (lane 2) as compared with cells transfected with a β-galactosidase expression vector (lane 1). However, transfection with E1Adel2-36, encoding a mutant defective for p300 binding, minimally affected the expression of the Sm-MHCz (lane 3). In contrast, VSMC expression of ubiquitously expressed β-actin was altered by neither wild type E1A nor mutant E1A. We checked the expression of E1A in VSMCs transfected with an expression vector encoding wild type or mutant E1A, or that encoding β-galactosidase as a control. As shown in Fig. 6, wild type and mutant E1A were abundantly and similarly expressed in VSMCs transfected with a correspondent expression vector. There was no E1A expression detectable in VSMCs transfected with a β-galactosidase expression vector. These findings suggest that endogenous p300 in differentiated-phenotype VSMCs plays a role in the maintenance of smooth muscle-specific Sm-MHC expression.

DISCUSSION

Determining the molecular mechanisms of VSMC differentiation is important for the understanding of vascular disease, because VSMCs can undergo a dedifferentiation process referred to as phenotypic modulation in response to vascular injury. Phenotypically modulated VSMCs are proliferative and express low levels of contractile proteins. However, the precise mechanisms that mediate transcriptional regulation during this process are poorly understood. It has been shown that a zinc finger transcription factor, GATA-6, is expressed in VSMCs and is rapidly down-regulated when VSMCs are induced to proliferate (13). In addition, GATA-6 induces cell cycle arrest with a concomitant increase in the expression of cyclin-dependent kinase inhibitor (20). These findings demonstrate that GATA-6 is involved in the promotion of the quiescent phenotype in VSMCs. However, the downstream gene targeted by GATA-6 is unknown. Sm-MHC expression is highly restricted to differentiated phenotype VSMCs and provides a useful marker for this phenotype. By a computer search, we found two GATA-like motifs (−810/−805 and −709/−704) within the rat Sm-MHC promoter. These motifs are conserved in rats and humans, suggesting their functional importance. EMSAs in the present study demonstrated that GATA-6 bound the distal GATA-like motif (−810/−805) in a sequence-specific manner. In addition, activation of this promoter by GATA-6 was significantly reduced by the mutation of this element. Although these data do not rule out the possibility that GATA-6 binds other sequences within this promoter, they provide the first identification of a functionally important GATA-6 binding site within a smooth muscle-specific promoter.

Cell type-specific gene expression is established through the combinatorial actions of restricted and more widely expressed transcription factors. The p300 protein is a ubiquitously expressed transcriptional coactivator and is involved in cardiac and skeletal muscle differentiation (21, 22, 24). Previous studies, including ours (21), demonstrated that p300 interacts with a zinc finger domain of GATA transcription factors. Because zinc finger regions are highly conserved among members of the GATA family, it seemed likely that p300 might be able to interact with GATA-6 as well. We showed that p300 interacted with GATA-6 and markedly augmented the GATA-6-dependent transcription of the Sm-MHC promoter. The marked potentiation of GATA-6-dependent transactivation by p300 indicates that p300 participates in the transactivation mediated by the interaction between GATA-6 and the Sm-MHC GATA element. Because p300 is found in complexes containing TFIIB and TATA-binding protein, the interaction between p300 and GATA-6 suggests that p300 serves as a link between GATA-6 and the basal transcriptional complex during transactivation. This feature of p300 defines it as a transcriptional coactivator for GATA-6 in the transcription of the Sm-MHC gene. The involvement of p300 in VSMC differentiation was further suggested by experiments that used the adenovirus E1A oncoprotein to interfere with p300. A wild type E1A but not a mutant E1A, which had lost the ability to interact with p300, was able to inhibit the endogenous expression of Sm-MHC in differentiated-phenotype VSMCs. Taken together, these findings suggest a role for p300 in the maintenance of the differentiated phenotype in VSMCs as a coactivator of GATA-6.

EMSA experiments provide evidence, albeit indirect, that p300 interacts with GATA-6 in differentiated-phenotype VSMCs. A nuclear complex containing p300 and GATA-6 was markedly up-regulated during the induction of the differenti-
VSMC differentiation. However, neither the expression level of p300 nor of GATA-6 differed between the proliferating and differentiated-phenotype VSMCs (data not shown). These findings suggest that phenotype modulation regulates p300 and/or GATA-6 post-translationally and alters the interaction between these two proteins. At present, the precise mechanisms that regulate this interaction are unclear. Interestingly, Kitabayashi and colleagues (34) demonstrated that changes in the phosphorylation status of p300 are correlated with retinoic acid-induced terminal differentiation of embryonal carcinoma F9 cells. They found that p300 differentiation regulatory factor-containing complexes exist in F9 cells and bind their target DNA sequences. They speculated that differently phosphorylated forms of p300 might reflect a dual role for this protein in positive and negative regulation of transcription, with unphosphorylated p300 correlated with a repressive activity of the protein toward the c-jun promoter in undifferentiated cells. In this model, upon stimulation of cells with retinoic acid, p300 would become hyperphosphorylated and transcriptionally active. p300 hyperphosphorylation also occurs in calcium-induced keratinocyte differentiation (35). Although the regulation of the interaction between p300 and GATA-6 might involve a more complex scenario, it would be of particular interest to determine the possible role of p300 phosphorylation status in VSMC differentiation.

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