Histone Acetylation and Recruitment of Serum Responsive Factor and CREB-Binding Protein Onto SM22 Promoter During SM22 Gene Expression

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Abstract—Chromatin acetylation and deacetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) are closely related to eukaryotic gene transcription. Although the binding of serum response factor (SRF) to the CArG boxes in the promoter region is necessary for SM22 expression, it has never been examined whether the local chromatin modification is involved in SM22 gene regulation. In this study, we used the SM22 gene as a model to address whether transcriptional activation of the gene can be manipulated through adjusting histone acetylation of the chromatin template and whether SRF- and HAT-containing coactivators can be recruited onto the SM22 promoter region during gene activation. Here, we showed that the stimulation of the SM22 promoter by the coactivator CREB-binding protein (CBP) was dependent on HAT activity. Overexpression of HDACs decreased SM22 promoter activity, whereas trichostatin A, an HDAC inhibitor, stimulated SM22 promoter activity in a CArG box–dependent manner and induced endogenous SM22 gene expression. Chromatin immunoprecipitation assays showed that trichostatin A treatment in 10T1/2 cells induces chromatin hyperacetylation in the SM22 gene. Although histone hyperacetylation of the SM22 gene occurred during SM22 gene expression and SRF and CBP immunocomplexes possess HAT activities in smooth muscle cells, both SRF and CBP were recruited to the CArG box–containing region of the promoter. This study provides evidence that chromatin acetylation is involved in smooth muscle cell–specific gene regulation. (Circ Res. 2002;90:858-865.)

Key Words: SM22 ■ serum response factor ■ CBP/p300 ■ histone acetylation ■ chromatin immunoprecipitation

Recently, an increasing number of nuclear proteins have been shown to possess histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities, and the coexistence of activators with HATs or of repressors with HDACs has been frequently found in the complexes of transcriptional machinery.1,2 In addition to modifying chromatin structure, HATs and HDACs interact with additional factors in a variety of cellular processes and function as coordinators and integrators during cell proliferation, differentiation, and apoptosis.2,3

The phenotypic plasticity of smooth muscle cells (SMCs) plays a critical role in physiological and pathological responses to cellular functional requirements and to environmental stimuli. Identifying the regulatory mechanisms that control the expression of these SMC genes will lead to better understanding of the phenotypic changes in SMCs during biological and pathological processes. During the past decade, much effort has gone into exploring the mechanisms that control the expression of SMC-specific markers.4 One of the most prominent elements associated with promoter activation is the CArG box. The CArG box is a cis-acting element that contains the CC(A/T)6GG consensus sequences in the regulatory region of almost all examined SM marker genes, which suggests the necessity of CArG box–mediated regulatory complexes during SMC differentiation.5 The serum response factor (SRF) binds to the CArG box.6 Previously, we and others found direct evidence that the CArG boxes in the promoter regions of SMC genes are required for promoter activation in SMCs in vitro and in vivo.7,13

In studying the SMC-specific regulatory network, several key observations drew our interest in designing experiments for the examination of the role of chromatin modification in SMC gene regulation. First, evidence links SRF-directed functions to the recruitment of HAT-containing coactivators. For example, the physical association among the transcription factor SRF, the coactivator CREB-binding protein (CBP)/p300, and the ternary complex factor Elk-1 contributes to CArG box–dependent transactivation of the c-fos gene promoter.13a,13b Second, experiments demonstrate that coactivators, such as CBP/p300 and PCAF, and HAT activities directly interact and collaborate with the myogenic basic helix-loop-helix transcriptional factors MyoD, Twist,14 and

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MADS-containing factor, MEF-2C,15,16 in skeletal myocyte differentiation. The interaction of p300 with zinc finger factors GATA517 and GATA618 and with the myogenic factor MEF-2D19 have also been reported in the transcription of cardiac- and SMC-specific genes. Third, small GTPase/RhoA-mediated signaling to activate the chromatin-embedded c-fos promoter functions only when local chromatin has been hyperacetylated.20 Evidence suggests that the SRF-directed expression of SM-specific genes is a downstream target of GT-Pase/RhoA-mediated signal transduction21 and that hypermethylated acetylation occurs in the activated vascular SMCs.22 Recently, it has been reported that histone hyperacetylation and the recruitment of SRF are involved in the regulation of SMC-specific genes, such as SM myosin heavy chain and SM α-actin.23

We selected the SM22 gene as a model to address the following questions: (1) Can adjusting the histone acetylation status of the chromatin template manipulate the transcriptional activation of the SM22 gene? (2) Can SRF- and HAT-containing coactivators be recruited into the SM22 promoter region during SM22 gene activation? Our findings directly demonstrate the recruitment of SRF and CBP to the chromatin-embodied SM22 promoter in vivo and provide evidence to establish the link of chromatin acetylation and SMC-specific gene regulation.

Materials and Methods

Cell Culture

Murine 10T1/2 cells and rat pulmonary arterial SMCs (PAC1 cells) were maintained in DMEM (GIBCO-BRL) with 10% FBS at 37°C with 5% CO2. Confluent cells were growth-arrested for 24 hours in 0.5% FBS and then incubated for 8 to 24 hours with the same medium containing 200 nmol/L trichostatin A (TSA, Sigma Chemscintillation counter.

Plasmids and Transient Transfection Studies

All SM22 promoter/luciferase reporter vectors have been described previously.24 The expression vectors pCl-p300 and pCDNA3-PCAF, pCDNA3-Twist, pCDNA3-TwistΔ1-60, and pCDNA3-TwistΔ1-112 were generous gifts from Drs Yoshihiro Nakatani (Harvard Medical School, Boston, Mass) and Yasuo Hamamori (University of Southern California, Los Angeles, Calif). pRc/RSV-CBP was obtained from Marc R. Montminy (The Salk Institute, La Jolla, Calif). pRc/RSV-CBPΔ1-60 (referred to as CBP-HAT-mut), in which the Phe1541 essential for HAT activity in CBP was substituted with alanine,25 was obtained from Dr Tony Kouzarides. The expression vectors for HDAC1-6 were from Dr Stuart Schreiber (Harvard University, Cambridge, Mass).

Reporter plasmid (300 ng, 12-plate well) and equal amounts of effector plasmid were transiently cotransfected by using Lipofectamine Plus (GIBCO-BRL). Cell lysate was used for luciferase assay with the Luciferase Reporter Assay System (Promega). Each transfection was performed in duplicate or triplicate and was repeated at least 3 times. Luciferase activity was normalized by using the protein concentration of cell lysate, determined by Bradford assay.

Immunostaining

To determine whether transiently transfected HDACs are expressed in the nuclei of 10T1/2 cells, fusion proteins of HDAC3-HA and HDAC5-flag were detected by incubating fixed cells first with anti–HA-biotin (Roche Diagnostics, Co) and anti–flag-biotin (Sigma) antibody for 1 hour at room temperature and then with horseradish peroxidase–streptavidin conjugate (ABC system, Vector Laboratories) for 30 minutes. Positive cells were visualized by diaminobenzidine staining for 30 minutes at room temperature (Roche).

RNase Protection Assay

Total RNA was isolated from 10T1/2 cells with the use of Trizol (GIBCO-BRL). The SM22 riboprobe has been described previously.25 Riboprobe was prepared by using MAXscript (Ambion) and was mixed with 5 to 10 μg RNA in each RNase protection assay (RPA) reaction by using the 18S rRNA riboprobe as an internal control (RPAII kits were from Ambion).

Immunoprecipitation-HAT Assay

Briefly, ~1.0 to 1.25×106 of PAC1 cells were collected in 5 mL ice-cold buffer (1× PBS plus 1 mmol/L AEBSF, 1 μg/mL antipain, 1 μg/mL aprotinin, and 1 μg/mL pepstatin) and pelleted by centrifugation at 750g for 10 minutes at 4°C. The cell pellet was resuspended in 1 mL of lysis buffer (20 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 2.5 mmol/L EDTA, 2.5 mmol/L EGTA, 1 mmol/L AEBSF, 10 μg/mL antipain, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 10 μg/mL pepstatin) and incubated at 4°C for 15 minutes. Debris was eliminated by centrifugation at 12 000g for 10 minutes at 4°C. The extracts were precleared by incubation with 4 μg of rabbit IgG and 30 μL of protein A/G agarose (a 50/50 mix from Santa Cruz Biotech) for 30 minutes at 4°C and centrifugation at 7000 rpm for 5 minutes in a microcentrifuge. Cleared extracts were separately mixed with 4 μg of antibodies against SRF and CBP (Santa Cruz Biotech) and Flag-tag (Sigma). After rotation at 4°C for 2 hours, 50 μL of protein A/G agarose was added for further incubation overnight. The immune complexes were pelleted at 7000 rpm in a microcentrifuge for 5 minutes and washed 3 times with lysis buffer and twice with 1× HAT buffer (Pierce). The HAT activities of the immune complexes were detected with a HAT-Check activity assay kit (Pierce), which was used in accordance with the manufacturer’s manual, with the use of [3H]acetate coenzyme A and a synthetic peptide corresponding to the first 23 amino acids of histone H4 as substrates. The HAT activities in the complexes were measured by the incorporation of [3H]acetate in the substrates with the use of scintillation counter.

ChIP Assay

The chromatin immunoprecipitation (ChIP) assay was modified according to Boyd and Farnham.26 The antibodies used were anti–acetyl histone H3 and anti–acetyl-histone H4 (Upstate Biotech), anti-SRF (G20), and anti-CBP (A-22, Santa Cruz Biotech). The immunoprecipitated DNA and input chromatin were analyzed by using polymerase chain reaction (PCR) with primers (Figure 1) to the SM22 promoter CArG-containing region (5'-GGTCTGCGCATAAAGGTTT-3' and 5'-TGCCCCATGGAG-TCTGCTTG-3') and exon 5 region (5'-AAGCCCCAGGACAGATA-GAGGGACT-3' and 5'-GAAGGAGACATGGTGCCACTAG-3'). An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results

HAT Activities of Coactivators Are Required to Stimulate SM22 Promoter

Previously, we have reported that the 445-bp upstream SM22 promoter sequence containing two conserved CArG boxes is sufficient to drive reporter gene expression in arterial SMCs in vitro and in vivo27 (Figure 1). To determine whether HAT-containing coactivators are involved in activating the SM22 promoter, we tested the potentials of CBP/p300 and PCAF to stimulate the activity of transfected SM22-445 in...
mouse fibroblast 10T1/2 cells. As shown in Figure 2A, all coactivators, including CBP/p300 and PCAF, increased SM22 promoter activity at least 2-fold. To test whether this increase was caused by their intrinsic HAT activity, a HAT-deficient CBP mutant was used for cotransfection. This mutant lost most of its capacity to stimulate SM22 promoter activity, suggesting that CBP-mediated transactivation of the SM22 promoter is HAT-activity dependent.

To assess whether the coactivators function through direct participation in the transcriptional regulatory network, we tested the effects of a mammalian basic helix-loop-helix, Twist, on SM22 promoter activity. Twist is a potent transcriptional inhibitor that competitively binds to the HAT domain of coactivators, such as CBP/p300 or PCAF, and displaces them from transcriptional regulatory complexes.14,16,27,28 The overexpression of Twist strongly inhibited SM22 promoter activity in both 10T1/2 cells and PAC1 cells (Figure 2B). When the CBP/p300-binding domain (amino acids 1 to 60) or CBP/p300- and PCAF-binding domain (amino acids 1 to 110) in Twist was deleted,14 the mutants TwistΔ1-60 and TwistΔ1-120 completely lost their capacities to repress SM22 promoter activity in both cell lines (Figure 2B). Therefore, we conclude that the coactivators CBP/p300 and PCAF may potentiate SM22 promoter activity in a HAT-activity–dependent manner, suggesting that the acetylation of the target proteins (histones and/or transcription factors) plays an important role during SM22 promoter activation.

**Inhibitory Effect of Twist on Activation of SM22 Promoter Can Be Relieved by Treating 10T1/2 Cells With TSA**

To determine whether the overall increase of acetylation could affect the SM22 promoter activity, we used TSA, a specific inhibitor for deacetylases, to treat cells after cotransfection with SM22-445. TSA treatment was able to stimulate the SM22 promoter activity >7-fold (Figure 3). Similarly, it also stimulated the SM22 promoter activities to a comparable extent in the presence of Twist mutants (TwistΔ1-60 and TwistΔ1-120). TSA treatment completely relieved Twist-mediated suppression of SM22 promoter activity (Figure 3). The fact that TSA was able to completely alleviate the inhibitory effect of Twist and further stimulated the SM22 promoter was not surprising, inasmuch as TSA may have the capacity to open up the whole chromosome and episome structures by increasing the overall acetylation of histone and transcription factors. This opens up the possibility of applying TSA to other cellular systems where acetylation plays a critical role in gene expression.
critical transcription factors involved in SM22 gene transcription. Therefore, these results suggest that the inhibitory effect of Twist on the SM22 promoter activity is involved in the acetylation states of histone and/or transcription factors. Whether TSA also affects the interaction of Twist with E12 and MEF2 to repress the SM22 promoter remains to be determined.

**HDAC Members Inhibit Activation of SM22 Promoter, and Such Inhibitory Effects Can Be Relieved by Treating 10T1/2 Cells With TSA**

To determine whether deacetylation by overexpression of HDACs could exert an inhibitory effect on the SM22 promoter, we cotransfected SM22-445 with either class I (hHDAC1-3) or class II (hHDAC 4-6) expression vectors into 10T1/2 cells. As expected, overexpression of HDACs decreased the SM22 promoter activities (Figure 4A). To determine whether it was the deacetylase activities in HDACs that suppressed the SM22 promoter activity, we showed that TSA treatments could rescue the SM22 promoter from the inhibition of HDACs (Figure 4A). HDAC members can be present in either nuclei or cytoplasm; we showed that class I HDAC3 and class II HDAC5 were nuclearly localized in this assay (Figure 4B). Taken together, SM22 promoter activity was regulated by overall acetylation status mediated through the balance of HAT and HDAC activities.

**TSA Stimulates Transfected SM22 Promoter Activity in a CArG Box–Dependent Manner and Enhances Transcription of the Endogenous SM22 Gene**

In searching for the potential TSA response elements in the SM22 promoter region, we used a series of SM22 promoters. We found that TSA treatment enhanced reporter activities 8-to 10-fold in all constructs containing either one or two CArG boxes (Figure 5A). However, both CArG box–less promoters (SM22-104 and SM22-144) and the 1344-bp promoter with the CArG box–null mutation (SM22-1344null), which did not contain SRF-binding sites, failed to respond to TSA stimulation (Figure 5A). The same effect was observed after treatment with another HDAC inhibitor, sodium butyrate (data not shown). To confirm that the CArG box is involved in the response to TSA treatment, we linked the CArG-near box to a heterologous heat shock protein (hsp) promoter. Because TSA has been shown to activate a number of genes, it is not surprising that it also stimulated the activity of the hsp promoter (Figure 5B). Although one copy of the CArG-near box increased the hsp promoter activity 1.5-fold, its response to TSA treatment was only slightly higher than that of the hsp promoter alone, suggesting that the CArG-near box might require cooperation with other cis elements in the SM218 promoter to achieve a high level of transcriptional activity (Figure 5B). Three copies of the CArG-near box increased the hsp promoter activity 3-fold, and this promoter exhibited a >8-fold response to TSA stimulation (Figure 5B). We concluded that the transcriptional network containing the CArG box is regulated by TSA treatment. Such response requires either the cooperation of the CArG box with other cis elements or multiple copies of the CArG box.

To test whether the endogenous SM22 gene is among those genes induced by TSA treatment, we performed an RPA. TSA could stimulate transcription of the endogenous SM22 gene (Figure 5C), suggesting that an overall increase in chromatin acetylation stimulates SM22 gene transcription in vivo. We also observed the induction of another SM marker, the SMα-actin gene (Figure 5C). However, the kinetics for its response was different from that for SM22 gene, suggesting that TSA could regulate different genes via different mechanisms. These results suggest that TSA also induces endogenous SMC gene expression as well as other non-SM genes.

**TSA Treatment Induces Histone Acetylation in the SM22 Gene–Embedded Chromatin Region**

The ChIP assay is an efficient and widely used method to identify the interaction between nuclear proteins and targeted DNA sequences in the chromatin template. To determine whether TSA-induced endogenous SM22 gene transcription was accompanied by the induction of chro-
mation acetylation in the SM22 gene locus in vivo, we performed a ChIP assay by using formaldehyde-fixed 10T1/2 cells with or without TSA treatment. As shown in Figure 6, acetylated histones H3 and H4 were both identified in the open reading frame of the SM22 gene in response to TSA stimulation. These results demonstrated that TSA did induce histone acetyl modification in the SM22 gene locus in vivo and provided further evidence that a close relationship exists between SM22 gene activation and acetylated histone accumulation.

SRF- and CBP-Containing Complexes, Possessing HAT Activities, Are Recruited to the SM22 Promoter In Vivo, and the Chromatin Region Embedded Within the SM22 Gene Locus Is Highly Acetylated in Cultured Arterial SMCs

The dynamic balance of histone acetylation and deacetylation can regulate SM22 gene transcription in 10T1/2 cells. Therefore, we wanted to examine whether critical factors, such as SRF and coactivator CBP, could be recruited to the SM22 promoter in vivo. We were also interested in confirming that histone hyperacetylation occurred in the noncoding region, such as the SM22 promoter, and the coding region in cultured SMCs, where the SM22 gene is highly expressed. By using primers corresponding to the SM22 promoter region containing the critical CArG boxes, we detected that a 200-bp DNA fragment by PCR in the complexes was immunoprecipitated by using antibodies against SRF, CBP, and acetylated histone H4 (Figure 7A). This result indicated that both SRF and CBP were recruited onto the CArG box–containing chromatin region of the SM22 promoter in SMCs, where histone H4 in the nucleosome of this region was hyperacetylated. In addition, a 450-bp DNA fragment identified with a pair of primers corresponding to exon 5 was detected only in the anti-acetyl histone H3–associated and anti-acetyl histone H4–associated complexes but not in the complexes including antibody SRF or CBP (Figure 7A).
promoter region in vivo. and CBP were recruited to the CArG box–containing factors in SMCs. The immunocomplexes of SRF and CBP possessed HAT activities, and SRF gene locus during SM22 gene expression. The immunocomplexes were examined by incubating [3H]acetyl coenzyme A with a synthetic peptide corresponding to the first 23 amino acids of histone H4. The HAT activities in the complexes were immunoprecipitated by the anti-SRF antibody. The immunoprecipitation-HAT activity assay demonstrated that the isolated SRF complex possessed HAT activity 7-fold higher than that of the complex immunoprecipitated by the anti-CBP antibody as a positive control (Figure 7C). This result indicated that exon 5 of the SM22 gene, where there are no DNA binding sites for SRF and CBP, was also hyperacetylated.

The presence of a 200-bp DNA fragment containing CArG boxes in the SM22 promoter region (A) and a 450-bp DNA fragment in the exon 5 region (B) were identified by PCR with the use of primers as indicated in Figure 1. C, SRF and CBP complexes were immunoprecipitated from PAC1 cells by using anti-SRF and anti-CBP antibodies. The HAT activities in these complexes were examined by incubating [3H]acetyl coenzyme A with a synthetic peptide corresponding to the first 23 amino acids of histone H4. The HAT activities in the complexes were measured by the incorporation of [3H]acetyl in the substrates.

Discussion

The SM marker SM22 serves as an excellent model for studying the regulatory mechanisms of SM-specific genes. Previous studies, including ours, have established the central role of CArG boxes in controlling the expression of SM22 in vitro and in transgenic mice. However, whether chromatin modification and remodeling are involved in SM22 promoter activation has not yet been examined. In the present study, we focused on the role of chromatin acetylation in SM22 gene regulation. We found that the dynamic balance of histone acetylation/deacetylation is involved in the regulation of both the episomal SM22 promoter and the endogenous SM22 locus.

The mammalian-cell transient transfection system is the primary tool for identifying and characterizing the interaction of cis-acting elements with their corresponding trans-acting factors and associated cofactors. This system has been accepted as a model to examine whether acetylation is involved in the regulation of gene transcription. As early as 12 to 16 hours after being introduced into mammalian cells, episomal plasmid DNA assembles into minichromosomes in which nucleosome spacing is similar to that in counterparts integrated in the mammalian genome. Using this system, we compared SM22 promoter activity by manipulating histone acetylation levels of the SM22 promoter embodied in minichromosomes in 10T1/2 cells. The 10T1/2 cells are multipotential fibroblast cells that have been successfully induced to differentiate into smooth muscle–like cells either with transforming growth factor-β treatment or with the overexpressing homeodomain factor HOXB7. We found that SM22 promoter activity was enhanced with the accumulation of histone acetylation on minichromosomes either by the overexpression of HAT-containing coactivators by or by treating cells with HDAC-specific inhibitors, such as TSA and sodium acetate. Consistently, deacetylation of the minichromosome through overexpression of HDAC or Twist, an inhibitor of HAT activity in CBP/p300, presented an antagonistic effect on SM22 promoter activity. Although HDAC5 has the capacity to be shuffled from nuclei to the cytoplasm, HDAC5 was predominantly present in nuclei in our cotransfection experiments.

Because the regulatory mechanisms involved in chromosome changes using episomal and chromosomal DNA templates are not always consistent, we further addressed whether our finding in transient transfected templates functionally resembled that in the corresponding endogenous promoters. Using the RPA and ChIP assay, we showed that TSA-induced endogenous SM22 transcription was accompanied by the increase of histone acetylation in the chromatin region containing the endogenous SM22 gene locus in 10T1/2 cells. The SM22 gene locus in PAC1 cells, where the SM22 gene is actively transcribed, also consistently exhibited a high level of histone acetylation. Because of the high level of acetylation of the SM22 gene, TSA treatment could not further stimulate SM22 promoter activity in PAC1 cells (authors’ unpublished data, 2002). These findings validate the feasibility of using the episomal SM22 promoter as a template to study chromatin regulation of endogenous SM22 expression and clearly establish the link between SM22
promoter activation and histone acetylation. The fact that TSA can stimulate endogenous SM22 gene expression is intriguing, suggesting that TSA affects not only epichromosomal promoter activity but also the overall transcriptional regulatory machinery for SM22 gene expression. We would like to stress that TSA is an agent that increases the overall acetylation of proteins in the cells and, thus, selectively induces the expression of a variety of genes, including both SMC-specific and non–SMC-specific genes.29,30

In light of the critical role of the interaction between SRF and the CArG box during SMC differentiation, whether the effect of hyperacetylation on the SM22 promoter was dependent on SRF binding to the CArG box was further tested by comparing the responses to TSA stimulation in promoters with and without CArG boxes. We observed that only functional CArG box–containing promoters responded efficiently to TSA treatment, whereas deleting or mutating the CArG box in the SM22 promoter caused an inert response to TSA treatment. Consistently, multiple copies of the CArG box, when linked to a heterologous promoter, also responded to the treatment of TSA. The finding suggested that the dynamic balance of histone acetylation is involved in the activities of the SRF/CArG box–mediated regulatory network.

SRF can direct tissue-specific transcription by recruiting additional proteins to form a stable tissue-specific regulatory network. Some of these proteins are involved in the regulation of acetylation in histone/transcription factors. In the present study, we have shown that the SRF immuno-complex possesses HAT activity in SMCs, suggesting that SRF may associate with factors containing HAT activities. On the basis of the finding that SRF and CBP/p300 cooperate in the function of the c-fos promoter13a,13b and our observation that CBP depended on its HAT domain to stimulate the SM22 promoter activity, it is likely that SRF and CBP may form a complex in vivo to regulate SM22 gene expression. SRF may also be involved in the down-regulation of SM22 gene expression by associating with SMRT (silencing mediator for retinoid and thyroid-hormone receptors), which recruits HDAC3.13c We used the ChIP assay to confirm that both SRF and CBP were colocalized in the CArG box–containing promoter region of the endogenous SM22 gene in vivo. At the same time, the local histones H3 and H4, which are part of the core particle in nucleosomes, were highly acetylated. Our findings provide evidence demonstrating that SRF– and HAT-containing coactivators could stimulate SM22 gene expression by direct recruitment to the promoter region in the context of chromatin. The present study is consistent with a recent report on the recruitment of SRF onto the CArG box in the promoters of SM myosin heavy chain and SM α-actin in the retinoic acid–induced P19-derived SMC model.15 It remains to be determined whether SRF itself can serve as a substrate for acetylation to regulate its function during SM gene expression.

In summary, the present evidence supplies data for future studies to address whether SRF directly recruits CBP/p300 accessory coactivators to induce local chromatin remodeling and on-site assembly of transcriptional machinery. These studies establish the groundwork for future comprehensive investigation into the molecular mechanism of SM-specific gene regulation within the chromatin context.

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