Functional Interaction between Coactivators CBP/p300, PCAF, and Transcription Factor FKLF2*

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The Sp1/KLF family of factors regulates diverse cellular processes, including growth and differentiation, and is essential for early embryonic development (1–3). This family of proteins is characterized by the presence of three highly homologous C2H2 type zinc fingers near the C terminus that bind GC/GT boxes. Amino acid sequences in the transcription activation domain of PCGF2 not only bind GC/GT boxes but also stimulate FKLF2 transcriptional activity. The integrity of the acetyltransferase domain of PCAF but not that of CBP/p300 is required for stimulating FKLF2 transcriptional activity. Further show that p300 and PCAF act cooperatively in stimulating FKLF2 transcriptional activation. FKLF2 interacts with both CBP and PCAF through specific domains, and CBP and PCAF acetylate FKLF2. Both CBP/p300 and PCAF stimulate FKLF2 DNA binding activity. The integrity of the acetyltransferase domain of PCAF but not that of CBP/p300 is required for stimulating FKLF2 DNA binding activity. These results demonstrate that CBP/p300 and PCAF stimulate FKLF2 transcriptional activity at least by enhancing its DNA binding. The acetyltransferase activities of CBP/p300 and PCAF play a distinct role in stimulating FKLF2 transcription and DNA binding.

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by FKLF2 in K562 cells. The acetyltransferase activity of PCAF but not that of CBP/p300 is required for the stimulation of FKLF2 activity, indicating that PCAF and CBP/p300 may play different roles in coacivation with FKLF2. We further show that p300 and PCAF act cooperatively in stimulating FKLF2-mediated transcription. FKLF2 interacts with both CBP and PCAF through its zinc finger domain. FKLF2 interacts with specific regions of CBP and PCAF. Both CBP and PCAF acetylate FKLF2 in the zinc finger domain in vitro. The binding of FKLF2 to the γ CACC box was strongly stimulated by CBP and PCAF. The histone acetyltransferase (HAT) activity of PCAF but not of CBP/p300 is required for stimulating FKLF2 binding to the CACC box of the γ promoter. Therefore, the functional HAT domain of PCAF but not that of CBP/p300 is required both for stimulation of FKLF2 DNA binding and for coactivation of the γ promoter. Together with other studies, these results demonstrate that FKLF2 and other members of this family (such as EKLF) interact differentially with CBP/p300 or PCAF and that the activities of these family members are regulated by these coactivators through distinct mechanisms. The differential utilization of and regulation by CBP/p300 and PCAF may play important roles in the specific activation of target genes by members of this highly conserved family of transcription factors.

EXPERIMENTAL PROCEDURES

Plasmids—The plasmids pSG5DD and pSG5/FKLF2, which express the murine FKLF2 and the γ- and β-subunit bovine reporter plasmids were as described (21). Expression plasmids for wild-type and HAT-defective PCAF were provided by Y. Nakatani, T. Kouzarides, and I. Talianidis (20, 24, 25). Expression plasmids for wild-type p300 and HAT-defective p300 (DI485/A1468L) were provided by A. Hecht (28). Expression plasmids for wild-type CBP and HAT-defective CBP (L1690K/C1691L) were provided by I. Talianidis (25). FKLF2-Myc plasmids that express the full-length or a series of mutants of FKLF2 with a Myc tag at their C termini were constructed by inserting FFLK2 or its mutants and a Myc tag into a mammalian expression vector. FLAG-CBP1 containing C termini were constructed by inserting FKLF2—Protein Acetyltransferase Assays—Protein acetyltransferase assays were carried out in reaction mixtures (30 μl) containing 50 mM HEPES (pH 8.0), 10% glycerol, 50 mM KCl, 2 mM dithiothreitol, 10 mM sodium butyrate, 1 μl of [3H]acetyl-CoA, 1 μg of purified GST-FKLF2 fusion protein on beads, and 50 ng of purified GST-CBP-HAT containing the HAT domain of CBP (residues 1186–1718), GST-PCA wild-type, or the GST-PCA/HAT-defective mutant. After incubating at 30 °C for 1 h with gentle mixing, the reaction mixtures were subjected to SDS-PAGE electrophoresis and analyzed using a phosphorimagery.

RESULTS

CBP/p300 Function as Transcriptional Coactivators of FKLF2—To understand the molecular mechanisms by which FKLF2 activates transcription, we determined whether CBP/p300 acts as an FKLF2 coactivator and potentiates transcriptional activity in K562 cells. As expected from previous studies (21), FKLF2 activated the γ globin promoter. FKLF2 activity was further enhanced by CBP (Fig. 1). CBP by itself showed no effect on the γ globin promoter-driven luciferase expression, indicating a functional interaction between CBP and FKLF2 at the γ globin promoter in vivo. These results demonstrate that CBP functions as a coactivator for FKLF2 (Fig 1).

CBP and FKLF2 Interact in Vivo and in Vitro—We next determined whether FKLF2 physically interacts with full-length CBP in vivo by a coimmunoprecipitation assay using whole cell extracts from COS cells expressing FLAG-tagged full-length CBP and Myc-tagged FKLF2. As shown in Fig. 2A, immunoprecipitation with the anti-Myc antibody revealed the complex formation between CBP and FKLF2 in vivo. Reciprocal immunoprecipitation using anti-FLAG antibody also revealed the specific interaction between FKLF2 and CBP (Fig. 2B). C and D show that the FLAG-tagged CBP and Myc-tagged FKLF2 are expressed at similar levels. These data demonstrate that FKLF2 associates with full-length CBP in vivo (Fig. 2).

CBP contains distinct domains that interact with transcription factors. The CBP domains and some of their interacting proteins are illustrated in Fig. 3A. GST fusion proteins containing different regions of CBP were tested for their ability to interact with FKLF2. A GST pull-down assay was performed.
on GST-CBP fusion proteins and whole cell extracts prepared from COS cells expressing Myc-tagged FKLF2. As shown in Fig. 3A, this assay revealed that FKLF2 interacts specifically with CBP2, which is also essential for interactions with factors including E1a, c-Fos, PCAF, MyoD, GATA-1, and TFIIB (17). The observed specific interaction of FKLF2 with CBP2 but not CBP1, CBP3, and GST is not due to a difference in the amount of these fusion proteins used in the assay, because the same amount of each protein was included in the reaction as determined by SDS-PAGE and Coomassie Blue staining (data not shown). To determine the in vivo association between FKLF2 and CBP2, coimmunoprecipitation assays were carried out using whole cell extracts from COS cells expressing FLAG-tagged CBP1 or CBP2 and Myc-tagged FKLF2. As shown in Fig. 3B, coimmunoprecipitation with the anti-Myc antibody revealed the complex formation between CBP2 and FKLF2 in vivo. Consistent with the results from the GST pull-down assay shown in (Fig. 3A), no in vivo association between CBP1 and FKLF2 was detected (Fig. 3B). Reciprocal immunoprecipitation using the anti-FLAG antibody also revealed the specific interaction between FKLF2 and CBP2 (Fig. 3B). The FLAG-tagged CBP1, CBP2, and Myc-tagged FKLF2 are expressed at similar levels (Fig. 3B).

Like other members of the Sp1/KLF family, FKLF2 contains distinct domains including a proline-rich potential transactivation domain in the N-terminal portion and three highly conserved zinc fingers (DNA-binding domain) in the C-terminal portion (Fig. 3C). To determine which region of FKLF2 interacts with CBP, GST pull-down assays were carried out by incubating whole cell extracts prepared from COS cells expressing Myc-tagged wild-type or deletion mutants of FKLF2 proteins with a purified GST-CBP2 fusion protein immobilized on glutathione-agarose beads. As shown in Fig. 3C, the GST pull-down assay demonstrates that CBP2 interacts specifically with the zinc finger region of FKLF2. The Myc-tagged FKLF2 and its mutants used in this assay are expressed at similar levels (Fig. 3C). To further establish the interaction of the FKLF2 zinc finger region with CBP2, GST pull-down assays were carried out using whole cell extracts from COS cells expressing Myc-tagged FKLF2-(149–289) and GST-CBP1 or GST-CBP2. This assay revealed a specific interaction of FKLF2-(149–289) with CBP2 but not with CBP1 (Fig. 3C). By establishing the specific interaction of the FKLF2 zinc finger region with CBP2 but not CBP1, this result rules out the possibility that the observed interaction of FKLF2-(149–289) with CBP2 is due to a nonspecific interaction of the cysteine rich zinc finger region (Fig. 3).

The Acetyltransferase Activity of CBP/p300 Is Dispensable for Its Function as Coactivator for FKLF2—In addition to the ability to function as an intermediary molecule by direct interaction with both transcription activators and the general transcription machinery (14), CBP/p300 has intrinsic HAT activity (18, 19). Nucleosome acetylation has been associated with chromatin remodeling and gene regulation (31–35). The acetylation of transcription factors by CBP/p300 has also been shown to modulate the activity of these proteins at multiple levels, including DNA binding, protein-protein interactions, stability, and nucleocytoplasmic shuttling (24, 25, 36). The acetyltransferase function of CBP/p300 is required for the superactivation of EKLF (36). However, several studies have shown that the HAT activity of p300 is not required for coactivation with a number of transcription factors, including E2F, MyoD, β-catenin, and the HIV Tat protein (24, 28, 37, and 38). Therefore, the exact role of the acetylase activity of CBP/p300 in the coactivation of these two proteins with transcription activators remains unknown. We examined whether the HAT activity of CBP/p300 is required for the potentiation of FKLF2 transcriptional activity using a HAT-defective p300 (p300 HAT−), which contains a DI to AL exchange of p300 residues 1485 and 1486 and abolishes its HAT activity (Refs. 28 and 39 and data not shown). Transient assays were carried out by cotransfection of a reporter containing the γ globin promoter and the FKLF2 expression plasmid together with expression vectors for either wild-type p300 or p300 HAT− in K562 cells. As shown in Fig. 4A, p300 and FKLF2 coactivated the γ globin promoter. P300 alone showed no effect on the γ globin promoter activity, indicating that its recruitment to the γ globin promoter is mediated through functional interaction with FKLF2. Both the wild-type and the HAT-defective p300 are capable of stimulating FKLF2 transcriptional activation of the γ globin promoter. A higher degree of stimulation of FKLF2 activity by the HAT-defective p300 was also observed. To further establish the requirement of CBP/p300-HAT activity for the stimulation of FKLF2 transcriptional activity, the wild-type CBP and the mutant CBP (CBP-HAT−, L1690K/C1691L), which lack HAT activity (25, 40 and our data not shown), were also tested for the ability to stimulate FKLF2 activation of the pγLuc reporter in K562 cells. These assays also showed that the HAT activity of CBP is not required for its coactivation of FKLF2 transcriptional activation (data not shown). Protein assays demonstrated that the wild-type and mutant p300 are expressed at similar levels (data not shown). These results demonstrate that CBP/p300 functions as a coactivator for FKLF2 in the transcriptional activation of the γ globin promoter and that the HAT activity of CBP/p300 is dispensable for its function as a coactivator for FKLF2.

The Acetyltransferase Activity of PCAF Is Required for Its Function as a Coactivator for FKLF2—PCAF is another member of the transcription coactivators with acetylase activity (20). Both CBP/p300 and PCAF have been shown to function as coactivators for a number of transcription factors, including p53, E2F, and the estrogen receptor (24, 41–43). In contrast, CBP/p300 but not PCAF is required for coactivation with the erythroid-specific transcription factors GATA-1 and EKLF (8, 44, 45). The results from these studies suggest that transcription activators may have a differential requirement for CBP/p300 or PCAF coactivators. We therefore determined whether PCAF also functions as a coactivator for FKLF2 in transcriptional activation of the γ globin promoter in K562 cells. Fig. 4B
interacts specifically with CBP2. The top panel shows the schematics of CBP, some of its interacting proteins, and its protein interaction domains used in this study. The bottom panel shows the GST pull-down results. GST pull-down assays were carried out by incubating 10 μl of whole cell extracts prepared from COS cells expressing the Myc-tagged FKLF2 protein with 2 μg of purified GST-CBP fusion proteins immobilized on glutathione-agarose beads as indicated. The presence of FKLF2 was detected by immunoblotting using the anti-Myc 9E10 monoclonal antibody and chemiluminescence. B, FKLF2 interacts with CBP2 in vivo. Coimmunoprecipitation assays were performed using whole cell extracts from COS cells expressing FLAG-tagged CBP1 (CBP1-F) or FLAG-tagged CBP2 (CBP2-F) and Myc-tagged FKLF2 (FKLF2-M). Top left, immunoprecipitation with an anti-Myc antibody revealed the presence of CBP2 but not CBP1 in the immunoprecipitates. Top right, reciprocal immunoprecipitation using an anti-FLAG antibody also revealed that FKLF2 specifically interacts with CBP2. Bottom left and right, FLAG-tagged CBP1, FLAG-tagged CBP2, and Myc-tagged FKLF2 are expressed at similar levels. The asterisks indicate immunoglobulin chains. C, FKLF2 interacts with CBP through its zinc finger domain. a, a schematic presentation of the domain structure of FKLF2 and a series of Myc-tagged FKLF2 proteins used in this study. b, GST pull-down assays were performed using whole cell extracts from COS cells expressing Myc-tagged FKLF2 proteins as indicated and a purified GST-CBP2 fusion protein immobilized on glutathione-agarose beads. Bound proteins were identified by immunoblotting using an anti-Myc 9E10 monoclonal antibody and chemiluminescence. c, Myc-tagged FKLF2 proteins used in this assay are expressed at similar levels. d, a GST pull-down assay was carried out using whole cell extracts from COS cells expressing Myc-tagged FKLF2-(149–289) as shown in c and GST-CBP1 or GST-CBP2 as in b. This assay revealed the specific interaction of FKLF2 with CBP2 but not with CBP1.
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GST/PCAF were used. These results demonstrated that FKLF2 physically contacts PCAF (Fig. 5).

PCAF and p300 Act Synergistically in Stimulating FKLF2-driven Transcription—Having established that both CBP/p300 and PCAF stimulated FKLF2 activity when individually co-expressed with FKLF2, we sought to investigate whether PCAF and CBP/p300 act cooperatively in stimulating FKLF2 transcription. Cotransfection experiments were carried out in K562 cells using a γ globin promoter-driven luciferase reporter and the expression plasmids for FKLF2, PCAF, and p300 or their respective empty vectors. This assay revealed that the inclusion of PCAF further stimulated transcription driven by FKLF2 and p300 (Fig. 6). This cooperative activation also requires the acetylase activity of PCAF, because no stimulation was observed when the acetylase-defective PCAF was used (Fig. 6). Instead, a slight decrease in luciferase activity was found, indicating an inhibitory effect on FKLF2 and p300 co-activation by an acetylase-defective PCAF (Fig. 6).

The Acetylation of FKLF2 by PCAF and CBP—FKLF2 is an acetylated protein in vivo when expressed in COS cells (data not shown). To test whether PCAF and CBP acetylate FKLF2, acetylation assays were performed using purified GST fusion proteins. These results demonstrated that FKLF2 physically contacts PCAF (Fig. 5).

Fig. 4. The histone acetyltransferase activity of PCAF but not of p300 is required for its function as coactivator of FKLF2. A, the acetyltransferase activity of p300 is not required for its function as a coactivator of FKLF2. K562 cells cultured in 12-well plates were transfected with 500 ng of the human γ globin promoter containing reporter plvuc, 20 ng of empty vector (FKLF2 −) or FKLF2 expression vector (FKLF2 +), and 100 ng of empty vector (empty), wild-type p300 (p300wt), or HAT-defective mutant p300 (p300 HAT −) as indicated. The results are presented as the mean ± S.D. (n = 3) of the relative light unit (RLU). B, the acetyltransferase activity of PCAF is required for its function as coactivator of FKLF2. Similar assays were carried out as described in A except that 0.05 μg, 0.1 μg, or 0.2 μg of expression plasmid for wild-type (PCAFwt) or HAT-defective mutant PCAF (PCAF-HAT −) was used. The results are presented as the mean ± S.D. (n = 3) of the RLU.

Fig. 5. FKLF2 interacts with PCAF in vivo and in vitro. A, FKLF2 interacts with PCAF in vivo. Coimmunoprecipitation assays were performed using whole cell extracts from COS cells expressing FLAG-tagged PCAF (PCAF-F) and Myc-tagged FKLF2 (FKLF2-M). Top left, immunoprecipitation with an anti-Myc antibody revealed the presence of PCAF in the immunoprecipitates. Top right, reciprocal immunoprecipitation using an anti-FLAG antibody also revealed that FKLF2 specifically interacts with PCAF. Bottom left and right, FLAG-tagged PCAF and Myc-tagged FKLF2 are expressed at similar levels. B, FKLF2 interacts with PCAF through its zinc finger domain. GST pull-down assays were performed as described in the Fig. 3C legend using whole cell extracts from COS cells expressing Myc-tagged FKLF2 proteins as indicated and a purified GST/PCAF fusion protein immobilized on glutathione-agarose beads. The interaction between CBP2 and FKLF2(149–289) was included in the assay as positive control. The asterisk indicates nonspecific signals.

Fig. 6. The cooperative activation of the human γ globin promoter by FKLF2, PCAF, and p300. K562 cells cultured in 12-well plates were transfected with 500 ng of the human γ globin promoter containing reporter plvuc, 20 ng of empty vector (FKLF2 −) or FKLF2 expression vector (FKLF2 +), and 100 ng of empty vector, the expression vector for p300, PCAF, or HAT-defective mutant PCAF (PCAF-HAT −) as indicated. The results are presented as the mean ± S.D. (n = 3) of the relative light unit (RLU).
proteins of FKLF2, PCAF-HAT, and CBP-HAT. FKLF2 contains 13 lysines with 11 of them located in the zinc finger DNA-binding domain. The N-terminal portion of FKLF2 (amino acids 1–160, containing two lysine residues) was not detectably acetylated by CBP and was acetylated very weakly by PCAF (data not shown). To further define the sequences in the zinc fingers that are acetylated, we divided the zinc fingers of FKLF2 into two portions, and each was subjected to an acetylation assay. The N-terminal half from amino acids 49 to 206 containing five lysine residues was acetylated by CBP but not by PCAF. The C-terminal half from amino acids 200 to 289 containing six lysines residues was acetylated by both CBP and PCAF (Fig. 7). These results indicate that FKLF2 is a possible target of CBP/p300 and PCAF acetylation (Fig. 7).

CBP/p300 and PCAF Stimulate FKLF2 Binding to the CACCC Box of the γ Promoter, and the Integrity of the HAT Domain of CBP/p300 Is Required for the Stimulation of FKLF2 DNA Binding.—To study the mechanisms by which the CBP/p300 and PCAF coactivators stimulate FKLF2 transcription activity, we first determined whether they increase the DNA binding activity of FKLF2 using quantitative EMSA assays. As shown in Fig. 8A, the DNA binding activity of FKLF2 was strongly enhanced by both GST-CBP-HAT and GST-PCAF but not by GST alone. We next determined whether the HAT activity of CBP and PCAF is required for stimulating FKLF2 binding to the γ CACCC box. As shown in Fig. 8B, GST-CBP-HAT+ stimulated FKLF2 DNA binding activity as well as the wild-type CBP-HAT. In contrast, the HAT-defective mutant of PCAF stimulated FKLF2 DNA binding very weakly (4-fold) compared with the nearly 20–30-fold stimulation by the wild-type PCAF, wild-type CBP, or CBP-HAT+). The requirement of the integrity of the HAT domain of PCAF but not that of CBP for stimulating FKLF2 DNA binding as shown by EMSA assays is consistent with the co-transfection assays, which also showed the requirement of the HAT activity of PCAF but not CBP/p300 for coactivation with FKLF2 (Fig. 4, A and B). GST-CBP-HAT, GST-CBP-HAT+, GST-PCAF wild-type, or GST-PCAF-HAT+ alone showed no binding to the γ CACCC box-containing probe (data not shown). In summary, these results demonstrate that the co-activators CBP/p300 and PCAF coactivate FKLF2 transcriptional activation of the γ promoter, at least in part by stimulating its binding to the CACCC box of the γ promoter and that the integrity of the HAT domain of PCAF but not that of CBP/p300 is required for stimulating FKLF2 DNA binding and transcriptional activity (Fig. 8).

**DISCUSSION**

CBP/p300 functions as a coactivator for many transcription activators (15). CBP/p300 also has intrinsic acetyltransferase activities (18, 19). The acetylation of histone and transcription factors by CBP/p300 has been implicated in the regulation of gene expression (15, 31). At present, however, the exact role of the HAT activity of CBP/p300 in coactivation with transcription activators remains to be established. For example, the HAT activity of p300 is not required for coactivation of the siamois promoter by p300 and β-catenin in 293 cells (28). The HAT activity of p300 is also dispensable for its function as a coactivator for HIV Tat under integrated and unintegrated conditions (37). Studies on the transcriptional activation by hepatocyte
nuclear factor 1 (HNF-1) showed that the HAT activity of PCAF but not that of CBP/p300 is required for the stimulation of HNF-1 transcription under a transient transfection assay. However, the HAT activities of both CBP/p300 and PCAF are important on a genome-integrated promoter (25). Studies on KLF1/EKLF, the founding member of the family, demonstrated that the HAT activity of CBP/p300 is required for transcriptional superactivation with KLF/EKLF (36). We therefore tested whether the HAT activity of p300 is required for its function as a coactivator for FKLF2 using a HAT-defective CBP/p300 (25, 28, 39). Our results showed that the HAT activity of CBP/p300 is not required for its coactivation of the human γ globin promoter with FKLF2. It was also noted that the HAT-defective p300 showed a slightly higher stimulation of FKLF2 transcription activity. Together, these studies indicate that different factors may have distinct requirements of the HAT activity of CBP/p300 for their coactivation. The data presented here and in studies by Zhang et al. (36) suggest that even members of the same family of transcription factors have different requirements for the HAT activity of CBP/p300 and differential coactivation by CBP/p300 and PCAF (see below). Further studies are needed to establish the requirement for the HAT activity of CBP/p300 in the coactivation of the γ globin promoter with FKLF2 under chromosomal context.

PCAF is a member of a family of acetylases (48). PCAF exists in a complex of more than 20 polypeptides (49) and functions as a coactivator for a number of transcription factors (15). Studies have shown that transcription factors may have selectivity in coactivation with coactivators. For example, CBP but not PCAF stimulates KLF1/EKLF transcriptional activity. We therefore determined whether PCAF functions as a FKLF2 coactivator. FKLF2 transcriptional activation of the γ globin promoter was further stimulated by co-expression of PCAF. By the use of PCAF harboring a deletion of residues 497–526 that abolishes its HAT activity (24), we demonstrated that the HAT activity of PCAF is required for coactivation with FKLF2. An inhibition of FKLF2 activation by HAT-defective PCAF was observed at a higher expression level, indicating a possible dominant negative effect of the HAT-defective PCAF on FKLF2 transactivation. It has been shown that PCAF induces erythroid cell differentiation, and the expression of the β globin gene was stimulated by wild-type PCAF and inhibited by HAT-defective PCAF in erythroleukemia cells (50). The HAT activity of PCAF was also required for coactivation with E2F, MyoD, and the Tat protein (18, 24, 38).

The results showing that both CBP/p300 and PCAF function as coactivators for FKLF2 whereas CBP but not PCAF functions as a coactivator for EKLF (8) suggest that there may be selective utilization of coactivators among different members of the Sp1/KLF family. To further establish whether CBP/p300 and PCAF selectively stimulate FKLF2 transcriptional activation of the γ promoter, we also tested the ability of these coactivators to stimulate EKLF transcriptional activation of the γ promoter. In agreement with our previous results (21), EKLF activated the γ promoter only marginally (2-fold), whereas FKLF2 activated the same reporter 100-fold in parallel transient transfection assays (Ref. 21 and data not shown). This marginal activation of the γ promoter by EKLF is not significantly stimulated by p300 or PCAF (data not shown). Consistent with previous studies, EKLF strongly activated the β promoter (8, 51), and this activation of the β promoter was further stimulated by p300 (Ref. 8 and data not shown). The differential interaction with coactivators may be one of the mechanisms by which members of the Sp1/KLF family of transcription factors accomplish their specificity. Given the differences in promoter and coactivator selectivity and in the mechanisms by which coactivators stimulate their transcriptional activity, FKLF2 and EKLF transcription factors may provide an excellent model for studying how coactivators modulate the activity of transcription factors of the same family. These studies may shed significant light on our understanding of tissue and developmental stage-specific expression of the globin genes.

Protein-protein interaction studies show that CBP and PCAF physically interact with FKLF2 through the zinc finger region. Functional studies suggest that that both CBP and PCAF act cooperatively in the coactivation of FKLF2 transcription. EMSA assays demonstrate that both CBP/p300 and PCAF strongly stimulated the DNA binding activity of FKLF2. The integrity of the HAT function of PCAF but not that of CBP/p300 is required for the stimulation of FKLF2 DNA binding. Consistent with the cooperative coactivation of FKLF2 transcription in transient assays (Fig. 6), our preliminary quantitative EMSA assays also showed that CBP and PCAF act cooperatively in stimulating FKLF2 binding to the CACCC box of the γ promoter, as demonstrated by the stronger stimulation of FKLF2 DNA binding by inclusion of both CBP-HAT and PCAF wild-type than either coactivator alone. The observed cooperative stimulation of FKLF2 DNA binding also requires the integrity of the HAT domain of PCAF, since it is not observed with PCAF-HAT− (data not shown), the same as with the cooperative stimulation of FKLF2 transcription.

An acetylation assay showed that CBP acetylates the zinc finger region of FKLF2. The acetylation of p53 by p300 has been reported to stimulate its DNA binding activity (52). However, our results demonstrate that the HAT activity of CBP is not required for stimulating FKLF2 DNA binding activity. Studies on EKLF also demonstrated that the acetylation of EKLF by CBP has no effect on its DNA binding (36). We therefore tested whether the HAT-defective CBP can stimulate p53 binding to its target site. Our results showed that the HAT-defective CBP stimulated p53 DNA binding as well as the HAT wild-type CBP, demonstrating that the HAT activity of CBP is not required for stimulating p53 DNA binding (data not shown). The study in this report and other studies (8, 52) demonstrate that FKLF2, p53, and EKLF are the targets of CBP acetylation. The acetylation activity of CBP is not required for stimulating FKLF2 and p53 DNA binding. Therefore, the biological significance of the acetylation of these factors by CBP remains to be determined. The acetylation of EKLF enhanced its interaction with the SWI/SNF complex rather than its DNA binding (36). Further studies will determine whether the acetylation of FKLF2 and p53 by CBP also regulates their interaction with other proteins. PCAF acetylates FKLF2 weakly in comparison with CBP. Nevertheless, the integrity of the HAT activity of PCAF is required for stimulating FKLF2 transcriptional and DNA binding activity. There are at least two possible explanations for the observed requirement of PCAF-HAT. First, the HAT-defective mutant may be defective in its interaction with FKLF2. Second, the weak acetylation is sufficient to stimulate FKLF2 DNA binding. Our protein-protein interaction studies using the wild-type and HAT-defective PCAF demonstrated that FKLF2 interacts with both the PCAF-(352–832) wild-type and the HAT-defective mutant equally well (data not shown). By ruling out the first possibility, our results suggest that the acetylation of FKLF2 by wild-type PCAF is necessary for stimulating its transcriptional and DNA binding activity.

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