In this paper we show that transcription factors Ets-1 and Ets-2 recruit transcription adapter proteins p300 and CBP (cAMP-responsive element-binding protein) during the transcriptional activation of the human stromelysin promoter, which contains palindromic Ets-binding sites. Ets-2 and p300/CBP exist as a complex in vivo. Two regions of p300/CBP between amino acids (a.a.) 328 and 596 and a.a. 1678 and 2370 independently can interact with Ets-1 and Ets-2 in vitro and in vivo. Both these regions of p300/CBP bind to the transactivation domain of Ets-2, whereas the C-terminal region binds only to the DNA binding domain of Ets-2. The N- and the C-terminal regions of CBP (a.a. 1–1097 and 1678–2442, respectively) which lack histone acetylation activity independently are capable of coactivating Ets-2. Other Ets family transcription factors failed to cooperate with p300/CBP in stimulating the stromelysin promoter. The LXXLL sequence, reported to be important in receptor-coactivator interactions, does not appear to play a role in the interaction of Ets-2 with p300/CBP. Previous studies have shown that the stimulation of transcriptional activation activity of Ets-2 requires phosphorylation of threonine 72 by the Ras/mitogen-activated protein kinase signaling pathway. We show that mutation of this site does not affect its capacity to bind to and to cooperate with p300/CBP.

The Ets family of transcription factors includes a large number of proteins that perform diverse functions in the cell including the serum stimulation of the c-fos promoter (Elk-1/SAP-1 (1)), activation of herpes simplex virus immediate early promoters (GA-binding protein α and β (2)), regulation of immunoglobulin light chain enhancers (Pu.1/Spi-1 (3)), erythroid differentiation (4), and Drosophila development (5). Constitutively active mutant Ets proteins (v-Ets-1 and -2) are involved in cellular transformation (6, 7). A characteristic feature of this class of proteins is a highly conserved 85-amino acid (a.a.) (2) DNA binding domain termed the Ets domain which contains a helix-turn-helix motif. The Ets domain binds to a GGA-rich core sequence found in the promoters and enhancers of viral and cellular genes. Outside the DNA binding domain, the Ets family proteins share limited homology (8–10).

Ets-1 and Ets-2 are ubiquitous proteins that share significant homology (11). The transactivation and the DNA binding (Ets) domains in both Ets-1 and -2 map to the N- and the C-terminal regions, respectively (12–14). The homologous regions include the Ets domain which is 95% conserved between Ets-1 and Ets-2 (11) and the Pointed domain located in the transactivation domain (15). The Pointed domain consists of approximately a 100-a.a. region that is conserved within a subgroup of Ets factors including Drosophila Ets factor Pointed P2 and Ets-1 and Ets-2 (15). The Pointed domain contains a MAP kinase phosphorylation site, and Pointed P2 is a target of Ras/MAP kinase signaling pathways in Drosophila (5). Ets-1 and Ets-2 are targets of the Ras signaling pathway (16), and Ras-mediated activation of Ets-1 and -2 transactivation activity requires phosphorylation of Ets-2 threonine 72 and the corresponding Ets-1 threonine 38, which are also conserved in Pointed P2 and Yan (a Drosophila repressor (17–19)). The conserved MAP kinase site (threonine 72) in Ets-2 is phosphorylated by MAP kinase (17). Ets-binding sites, as well as AP-1-binding sites, are often found in the promoters of Ras-induced genes (20), and Ets-2 and AP-1 cooperate in gene expression (21–23).

The abbreviations used are: a.a., amino acids; CBP, CREB-binding protein; CREB, cAMP-responsive element-binding protein; CMV, cytomegalovirus; HA, hemagglutinin; MAP, mitogen-activated protein; MEK, MAP kinase kinase; TBP, TATA box-binding protein; AP1, activator protein 1; CAT, chloramphenicol acetyltransferase; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; WT, wild type; bp, base pair; NLS, nuclear localization signal; MMP, matrix metalloproteases; TA, transactivation; DB, DNA binding.
as transcriptional coactivators by bridging a very large number of DNA-bound transcription factors with basal transcription complex (24–26). p300/CBP also binds to a number of proteins that are not transcription factors including viral oncoregion products (27, 28), SRC1 (29), Cdk2 (30), and a protein containing enzymatic activity as histone acetyltransferase, p300/CBP-associated factor (31). Recent studies show that p300/CBP also has enzyme activity as a histone acetyltransferase, linking chromatin remodeling with transcription (32). It has been suggested that the amount of p300/CBP in cells may be rate-limiting and that different transcription factors may compete for rate-limiting amounts of these coactivators and thus provide mechanisms for cross-talk in the regulation of gene expression (33, 34). Targeted gene disruption studies have confirmed that p300 function is essential for normal embryonic cellular proliferation, morphogenesis, and development with double knockouts resulting in 100% embryonic lethality (35). Normal levels of CBP in these mice did not substitute for the p300 functions suggesting that the double knock-out phenotype may be either due to gene dosage effect or the loss of specific functions provided by p300. Likewise, haploinsufficiency of CBP gives rise to severe developmental abnormalities characteristic of the Rubinstein-Taybi syndrome, including mental retardation, craniofacial abnormalities, skeletal abnormalities, and increased cancer incidence (36). These studies suggest that both proteins are required for embryonic development.

Stromelysin is an important member of a family of matrix metalloproteases (MMPs) which degrade extracellular matrix during a variety of normal and pathological processes. In this paper, we show that two important members of the Ets family, Ets-1 and Ets-2, recruit p300/CBP in the activation of the stromelysin promoter, and this recruiting involves multiple protein-protein interactions. Consistent with these multiple interactions, the N- and the C-terminal halves of p300/CBP independently can coactivate Ets-2 to stimulate the stromelysin promoter. Other Ets family transcription factors do not cooperate with p300/CBP in the stimulation of the stromelysin promoter. We also show that mutation of the Ets-2 MAP kinase phosphorylation site, important for Ras-mediated regulation of Ets-2, does not affect its ability to bind to p300/CBP or its ability to cooperate with p300/CBP in the transcriptional activation.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—pSK200 is a promoter-reporter plasmid that contains 542 bp upstream of the cap site of the human stromelysin gene fused to the CAT reporter gene. pSK201 and pSK202 are two mutant versions of pSK200 in which the AP-1 site, located between −64 and −71, is mutated (pSK201) or the two palindromic Ets sites deleted (pSK202) (37). E18pal is a promoter-reporter plasmid in which two palindromic Ets sites were cloned upstream of a c-fos minimal promoter followed by the CAT gene. c-fos minimal promoter contains 56 bp upstream of the cap site of the c-fos gene (16). Plasmids pFNETs-1 and pFNETs-2 are two expression plasmids that express mouse e1s and e2s, respectively (17). The E1s and E2s protein coding sequences in these plasmids are tagged with FLAG epitope followed by the SV40 large T nuclear localization signal (NLS) at their N-terminal end. These plasmids are in pcDNA3 background. Plasmid pFNETs-2AT72 is a derivative of pFNETs-2 in which the MAP kinase substrate Thr at 72 residue is changed to Ala (TT2A mutation (17)). Plasmid p300CHA expresses hemagglutinin epitope-tagged p300 from the CMV promoter (27). Plasmids GST-p300N, GST-p300M, and GST-p300C contain a.a. 1–596, 744–1571, and 1572–2370, respectively, fused in frame with glutathione S-transferase coding sequences and kind gifts of Dr. D. Livingston of Harvard Medical School (27). Plasmids GST-p300N, GST-p300M, and GST-p300C are plasmids in which p300 cDNA sequences corresponding a.a. 1–746, 743–1572, and 1572–2414 respectively, cloned downstream of the T7 phage promoter in Bluescript SK. Full-length and truncated murine CBP coding sequences are expressed from a CMV promoter-based expression plasmid (pCMV23N-T (38)) in which the coding sequences are tagged at their N-terminal end with two copies of SV40 nuclear localization signals followed by three copies of HA epitope (38). These plasmids are kind gifts of Dr. A. Harel-Bellan (CNRS, Villejuif, France).

**GSTM Pull-down and Yeast Two-hybrid Assays**—To identify the Ets-2/CBP complex in live cells, proliferating human 293 cells were pelleted and lysed on ice in a lysis buffer (27) for 40 min, and the protein was quantitated by the Bradford method (39). The cell lysate, equivalent to 2 mg of protein, was concentrated by the addition of 5 volumes of ice-cold acetone for 30 min on ice and then centrifuged for 30 min at 15,000 rpm. The pellet was dissolved in 90 μl of the lysis buffer containing 0.5% Nonidet P-40, and it was then incubated with 40 μl of the anti-Ets-2 rabbit polyclonal antibody (Santa Cruz Biotechnology, SC-351) overnight at 4 °C. 50 μl of the protein A-agarose beads were then added, and the incubation was continued for another 2 h at 4 °C. The beads were then pelleted, washed three times with lysis buffer containing 0.3% Nonidet P-40, subjected to SDS–8% PAGE, and electrophoresed in Tris glycine buffer for 5 h as described (40). The immunoprecipitates were then assayed in Western blots using an anti-CBP rabbit polyclonal antibody (Santa Cruz Biotechnology, SC-583). For the negative control experiment, 2 mg of the protein from the above lysate was immunoprecipitated using an anti-PKR polyclonal antibody raised in our laboratory. For direct immunoprecipitation, cell lysate corresponding to 500 μg of the protein was passed through anti-CBP antibody, and the immunoprecipitated polypeptides were subjected to SDS–PAGE followed by Western blot as described above. For the detection of the complexes containing various forms of Ets and the truncated forms of CBP, 100-cm dishes of 293 cells were transfected with 15 μg of each of the plasmids indicated in the figure legends using the calcium phosphate precipitation method, and cell extracts were subjected to immunoprecipitations followed by Western blots as described above with appropriate antibodies.

**RESULTS**

**p300/CBP Cooperates with Ets-1 and Ets-2 in the Transcriptional Activation of the Human Stromelysin Promoter and a Synthetic Promoter Containing Palindromic Ets-binding Sites**

Transcription of the human stromelysin gene is driven by AP1/Fos and Ets-2 transcription factors and regulated by a variety of factors including 12-O-tetradecanoylphorbol-13-acetate, cytokines, growth factors, and protooncogenes (37, 43–46). The AP1/Fos and Ets elements of the stromelysin promoter map between −64 and −71 and −201 and −218 with respect to cap site, respectively (Fig. 1A) (37, 46). The Ets site in this promoter consists of two copies of a motif similar to the polyoma virus enhancer A-binding protein-3 (PEA-3) site that binds to the Ets family transcription factors Ets-1 and -2 and stimulates transcription (44). To determine whether p300 would cooperate with Ets-2 in the activation of the stromelysin promoter, HeLa cells were cotransfected with a stromelysin pro-

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2 C. A. Hauser, unpublished results.

3 E. Ferreira and G. Butticé, manuscript in preparation.
Ets-1 and Ets-2 Interactions with p300/CBP

To study further the cooperative activity of Ets-2 and p300, we tested a synthetic promoter-reporter construct in which two palindromic Ets sites were placed upstream of the c-fos minimal promoter containing −56 to +109 bp of the c-fos promoter fused to CAT reporter gene (Fig. 1A, E18pal) (16). By itself, p300 did not stimulate E18pal significantly (about 2-fold), whereas Ets-2 stimulated this construct by about 6-fold. In contrast, the two effectors together stimulated E18pal by about 17-fold (Fig. 1B, E18pal). These results provide evidence that Ets-2 and p300 can cooperate in the transcriptional activation of Ets-2 site containing promoters even in the absence of other upstream elements. CBP also cooperated with Ets-2 and stimulated the E18pal and the stromelysin promoter by about 8–10-fold (data not shown; also see Fig. 5).

We considered it possible that the dramatic increase in the reporter activity by p300 and Ets-1/Ets-2 proteins together in the promoter-reporter assays, described above, may be due to a significant increase in the levels of Ets-1/Ets-2 as a result of stimulation of the CMV promoter of the Ets expression vectors by p300/CBP. We found that in human cells, using identical transfection assay conditions described above, p300 increased the levels of Ets-1 and Ets-2 only marginally (1.5-fold, Fig. 1C; data for Ets-1 not shown). U2OS cells were used in this experiment because of their higher transfection efficiency; p300/CBP cooperated with Ets-1/Ets2 in the stimulation of the stromelysin promoter in these cells as efficiently as HeLa cells (data not shown). Thus we conclude that p300/CBP increases the transcriptional activation activity of Ets-1 and Ets-2 and stimulates the Ets-2 site containing promoters.

Previous studies have shown that Ets-2 activates the human stromelysin promoter (37), whereas the rat stromelysin promoter is activated by both Ets-1 and Ets-2 (44). Both promoters contain palindromic Ets binding sites. Therefore, it was of interest to determine whether p300/CBP would cooperate with Ets-1 to activate the human stromelysin promoter. Ets-1 did not stimulate the stromelysin promoter significantly. In contrast, Ets-1 and p300 together stimulated the stromelysin promoter by about 25-fold (Fig. 1B). In the case of E18pal, p300 did not stimulate the promoter significantly, whereas it was stimulated by Ets-1 by about 5-fold. When the E18pal was tested in the combined presence of Ets-1 and p300, it was superactivated by about 38-fold (data not shown).
Ets-1 and Ets-2 Interactions with p300/CBP

Ets-2 and p300/CBP Exist as a Complex in Vivo

Because p300/CBP can cooperate with Ets-2 in the transcriptional activation of Ets-binding site containing promoters, we reasoned that Ets-2 and p300/CBP might interact and exist as a complex in vivo. To determine this, proliferating human 293 cells were lysed in a lysis buffer, and the cell lysate equivalent to 2 mg of protein was first immunoprecipitated with a polyclonal antibody directed against the C-terminal region of Ets-2. The immunoprecipitated proteins were then separated on an SDS-8% polyacrylamide gel (Fig. 2, lane 2). As a negative control, an equal amount of protein from the cell lysate was immunoprecipitated with a polyclonal antibody directed against the cellular double-stranded RNA-activated protein kinase (eIF-2α kinase, also called PKR (47)), and the immunoprecipitated proteins were loaded on the same gel (lane 3). Five hundred μg of protein from the same lysate was also immunoprecipitated with a polyclonal antibody which recognizes the N-terminal region of human CBP and the immunoprecipitated proteins were separated as above (lane 4). In lane 1, 60 μg of the protein from the same lysate was loaded directly. The gel was electrophoretically transferred to a nitrocellulose membrane and probed with an anti-CBP antibody. As shown in Fig. 2, anti-CBP antibody recognizes a band in all lanes except in lane 4, 500 μg of the protein was loaded directly. In lane 4, 500 μg of the protein was first immunoprecipitated with anti-CBP antibody. The protein band reacted with anti-CBP antibody is shown by an arrow.

Ets-1 and Ets-2 Bind to Two Regions in p300/CBP in Vitro and in Vivo

To determine the regions of p300/CBP that bind to Ets-2 and to determine whether Ets-1 also binds to p300/CBP in similar manner, we employed GST pull-down, coimmunoprecipitation, and yeast two-hybrid assays. Fragments of p300 from a.a. 1 to 596 (GST-p300N), 744 to 1571 (GST-p300M), and 1572 to 2370 (GST-p300C, Fig. 3A) were expressed as GST fusion proteins. Fig. 3B shows a Coomassie Blue-stained gel of the affinity purified GST-p300 fusion proteins; all three fusion proteins were overexpressed in Escherichia coli. Ets-1 and Ets-2 were labeled with [35S]methionine in a coupled transcription/translation system and were then incubated with equal quantities of GST fusion proteins immobilized on agarose beads. Agarose beads containing GST and luciferase labeled with [35S]methionine in vitro, as described above, were used as the negative controls. Quantitation of the radiolabeled bands indicated that about 6 and 8% of the input Ets-2 were bound to the N- and the C-terminal regions of p300, respectively (Fig. 3C). Similarly, about 4% of the Ets-1 bound to the N- and the C-terminal regions of p300. In contrast, binding of the radiolabeled luciferase to GST-p300M was negligible. Similarly, negligible amounts of Ets-1 and Ets-2 bound to beads containing only GST.

The above experiments were repeated in which full-length Ets-1 and Ets-2 proteins were expressed in E. coli as GST fusion proteins. Fig. 3D shows the Coomassie Blue-stained gel of the affinity purified GST-Ets-1 and GST-Ets-2 fusion proteins. Fragments of p300 corresponding to a.a. 1–746 (p300N), 743–1572 (p300M), and 1572–2414 (p300C) were radiolabeled in vitro and assayed with equal quantities of GST fusion proteins in GST pull-down assays. Considerable amounts of p300N (10%) and p300C (15%) were recovered when GST fusion proteins containing Ets-1 or Ets-2 were used (Fig. 3E). In contrast, less than 2% of the input p300M was detected for Ets-1 and Ets-2. Negligible amounts of p300 bound to GST beads without attached proteins.

To determine whether Ets-2 binds to the N- and the C-terminal regions of p300/CBP in vivo, we cotransfected human 293 cells with expression vectors encoding mouse ets-2 (pFN-ets-2), the N-terminal a.a. 1–1097 (pCBP1–1097), and the C-terminal a.a. 1678–2442 (pCBP1678–2442) regions of CBP. CBP expressed from these expression vectors contain a nuclear localization signal (NLS) followed by three copies of influenza HA epitope at their N-terminal ends (38). As a control, ets-2 expression plasmid was cotransfected with the empty vector containing only the NLS and the three copies of HA epitope (pCMV2N3-T). The cell extracts containing equal quantities of protein were immunoprecipitated with an anti-Ets-2 polyclonal antibody, and the immunoprecipitates were analyzed by Western blot using an anti-HA monoclonal antibody (12CA5). Strong signals were detected in lanes corresponding to cell extracts prepared from cells cotransfected with pFN-Ets-2 and CBP expression vectors expressing a.a. 1–1097 (Fig. 3F) and 1678–2442 (Fig. 3G). This band was not detected in cell extracts prepared from cells cotransfected with pFN-Ets-2 and the empty vector.

Also, in yeast two-hybrid assays, ets-2 cloned into yeast activation domain plasmid pACT-2 interacted with a region of p300 from a.a. 328 to 1000 but not from a.a. 962 to 1575 (data not shown; also see Fig. 7C). Amino acids 1–328 and 1575–2414 could not be tested in yeast two-hybrid assays as these two regions displayed significant transcriptional activation activities when cloned into DNA binding domain plasmids (48) (49). Taken together, the above data suggest that a.a. 338–452 and 1678–2370 of p300/CBP independently are able to form complexes with Ets-1/Ets-2.

In Vitro Binding Studies Identify Two p300-binding Sites in Ets-2

To determine whether the transactivation (TA) or the DNA binding (DB) domains of both bind to p300/CBP, GST pull-down assays were carried out using GST-Ets fusion proteins containing the TA (GST-Ets-2-(1–290)) or the DB domains (GST-Ets-2-(289–469); Fig. 4B). Stable GST-Ets fusion proteins containing the TA and the DB domains were overexpressed in E. coli as evidenced by SDS-PAGE analysis of the affinity purified proteins (data not shown). GST-Ets-2 fusion proteins containing full-length a.a. 1–290 (TA domain) and 289–469 (DB domain) were assayed with in vitro radiolabeled
Ets-1 and Ets-2 Interactions with p300/CBP

Other studies have shown that the N- and the C-terminal regions of p300/CBP contain strong transcriptional activation activity (48). Because our studies suggest the N- and the C-terminal regions independently can bind to Ets-2, and because these two regions also contain the transcriptional activation domains, it seemed possible that the N-terminal and C-terminal regions of p300/CBP independently can cooperate with Ets-2 in the transcriptional activation of the stromelysin promoter. To test this possibility, HeLa cells were cotransfected with pSK200 containing the stromelysin promoter-reporter plasmid, the expression vectors expressing ets-2, and the full-length CBP, and the N- and the C-terminal regions of CBP (a.a. 1–1097 and 1678–2442, respectively). CAT activities in the transfected cell lysates were then determined. As shown in Fig. 5, in the presence of Ets-2, the N- and the C-terminal halves of CBP independently stimulated the stromelysin promoter by about 8–10-fold which was comparable to that observed for the full-length CBP. The N- and the C-terminal regions of p300 also behaved similarly in these assays (data not shown). These results suggest that in transient assays, the N- and the C-terminal halves of p300/CBP independently are able to cooperate with Ets-2 in the stimulation of the stromelysin promoter as efficiently as the full-length p300/CBP, and only one of the two Ets-binding sites of p300/CBP is sufficient to coactivate Ets-2.

Other Ets Family Transcription Factors Do Not Cooperate with p300/CBP in the Transcriptional Activation of the Stromelysin Promoter

Although Ets family transcription factors consist of a large number of transcription factors related by virtue of their Ets domain, the target genes that are activated by these proteins are dependent on the specificity of interaction between these proteins and their binding sequences. Ets family proteins bind to a 10-bp DNA sequence with an invariant core motif (C/A)GGA(A/T). The specificity of the interaction is determined by the flanking sequences surrounding this core motif (8–10). We determined whether other Ets family transcription factors such as PEA3 and Erg would cooperate with p300/CBP in the transcriptional activation of the stromelysin promoter. HeLa cells were cotransfected with the stromelysin or the E18pal promoters on the transcriptional activation of the stromelysin promoter. HeLa cells were cotransfected with the stromelysin or the E18pal promoters, and the full-length CBP. The N- and the C-terminal regions of CBP independently stimulated the stromelysin promoter by about 8–10-fold which was comparable to that observed for the full-length CBP. The N- and the C-terminal regions of p300 also behaved similarly in these assays (data not shown). These results suggest that in transient assays, the N- and the C-terminal halves of p300/CBP independently are able to cooperate with Ets-2 in the stimulation of the stromelysin promoter as efficiently as the full-length p300/CBP, and only one of the two Ets-binding sites of p300/CBP is sufficient to coactivate Ets-2.

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stimulate the stromelysin promoter. Consistent with previous results (Fig. 1B), the stromelysin promoter activity increased by about 20-fold in the presence of Ets-2 and p300. Thus, it appears that in the context of the stromelysin promoter, the Ets-2-binding sites show strict specificity with respect to transcriptional cooperativity between p300/CBP and the Ets family transcription factors.

Role of LXXLL Sequence in Ets-1 and Ets-2 in Their Interactions with p300/CBP

Recent studies suggest that protein-protein interactions between steroid receptor family members such as SRC-1/p160 and p300/CBP are mediated by a short sequence motif LXXLL (where L is leucine and X is any amino acid) (50). A sequence, LLELL, that fits the consensus LXXLL sequence is conserved in loop 1 of the Ets domain in all known Ets family transcription factors with the exception of PEA-3 (8), raising the possibility that this sequence may mediate interaction of Ets family proteins with p300/CBP. In Ets-2, this sequence motif maps in the DB domain between a.a. 368 and 374. Therefore, we determined whether a mutation of this sequence affects its interaction with p300/CBP. Because both the TA and the DB domains of Ets-2 independently interact with p300/CBP, we compared the ability of the WT DB domain and the DB domain containing mutations in LLELL sequence to interact with the p300 C-terminal region in vitro in GST pull-down assays.

The LLELL motif in an expression plasmid coding for the WT human Ets-2 was changed to AAAAA (Ets-2AAAAA; see “Experimental Procedures”). The GST fusion constructs coding for the DNA binding domain (a.a. 334–469) of the WT or the AAAA mutant were then overexpressed in E. coli. Data presented in Fig. 7A (Coomassie Blue-stained gel) indicate that all three fusion proteins were overexpressed in E. coli. Equal amounts of GST-fusion proteins were then assayed for their capacity to bind to in vitro synthesized radiolabeled p300N and the p300C proteins. In agreement with data shown in Fig. 3,
the GST fusion protein containing the WT Ets-2 interacted with both the p300N and the p300C proteins. The WT DB domain interacted only with the C-terminal region of p300. The mutant DB domain, in which the LLELL motif was mutated to AAAAA, also interacted with p300C as efficiently as the WT DB domain. Thus we conclude that the LLELL motif does not play a role in Ets-2 interaction with p300.

We also compared the p300/CBP-binding capacities of the WT and the Ets-2 AAAAA mutant in yeast and human cells. Ets-2 cDNA with AAAAA mutation was cloned in frame with 1890 of CBP. The Ets proteins were tagged with FLAG epitope at their N-terminal ends, whereas CBP was tagged with three copies of the HA epitope at its C-terminal end. The lysates were immunoprecipitated from transfected cells using anti-FLAG monoclonal antibody, transferred to a nitrocellulose membrane, and probed with anti-HA antibody 12CA5. The vector lane corresponds to an experiment in which the Ets expression vectors were cotransfected with a plasmid in which the FLAG epitope is expressed from the CMV promoter.

Fig. 7. Effect of mutation of the LLELL sequence in Ets-2 (a.a. 369–373) in the interaction with p300/CBP and in the transcriptional activation of E18pal. A, Coomassie Blue-stained gel showing the overexpression of the GST fusion proteins expressing the WT Ets-2 (open arrow) and the DB domain (a.a. 334–469) of the WT and Ets-2 AAAAA mutant (solid arrow). The LLELL sequence was mutated to AAAAA in the context of full-length Ets-2 using a site-directed mutagenesis procedure, and the DNA sequences corresponding to a.a. 334–469 were then cloned in frame into a GST plasmid. GST fusion proteins were overexpressed as described under “Experimental Procedures” and fractionated on a SDS-10% PAGE. Note that WT Ets-2 in this gel comigrates with an E. coli protein. B, GST pull-down assays showing the interaction between the N- and the C-terminal regions of p300 and the full-length Ets-2, Ets-2 DB domain (a.a. 334–469) from WT, and the Ets-2 mutant containing AAAAA mutation. Lanes Input contain 1/10 of the radiolabeled proteins used in binding experiments. Binding assays were repeated three times, and the results of a typical experiment are shown. C, yeast two-hybrid assays showing interaction of full-length WT Ets-2 (human) and full-length Ets-2 containing AAAAA mutation with p300 fragments from a.a. 328 to 1000 (p300-1) and 962 to 1575 (p300-2). Also included in this experiment is an Ets-2 mutant in which the MAP kinase substrate Thr-72 mutated to Ala (see Fig. 8). Yeast cells were cotransformed with Gal-4 fusion plasmids containing p300 fragments, and the activation domain plasmids containing various Ets mutants and the β-galactosidase activity in the transformants were quantitated (42). Average values and error bars from three independent experiments are shown. D, transactivation of E18pal by the human WT Ets-2 and Ets-2 containing AAAAA mutation in the presence and absence of p300. Transactivation assays were carried out exactly as described in legend to Fig. 1 except that the Ets plasmids contained human Ets-2 tagged with FLAG epitope similar to that described for mouse Ets-2. Yeast cells were cotransformed with the activation domain plasmid containing WT or the mutant forms of Ets-2 genes (Ets-2 AAAAA), the DNA binding domain plasmids Gal4-p300-1 and Gal4-p300-2 containing a.a. 328–1000 and a.a. 962–1575 of p300, respectively. The β-galactosidase activities generated in the transformants were quantitated (42). As shown in Fig. 7C, plasmid coding for the Ets-2AAAAA mutant protein generated about 80% of WT β-galactosidase activity (Ets-2A72 data in Fig. 7C relate to the experiments shown in Fig. 8 and will be discussed below). Coimmunopre-
p300/CBP binds to and coactivates a mutant Ets-2

Nonphosphorylatable by MAP Kinase

Interaction of Ets-2 T72A Mutant with p300/CBP—Previous studies have shown that the efficient activation of Ets-2 transcriptional activity by Ras requires phosphorylation of the conserved MAP kinase site threonine 72, and mutant Ets-2 in which Thr-72 is mutated to Ala is not efficiently activated by Ras (17). It was of interest to determine whether p300/CBP would bind to and coactivate this Ets-2 mutant. Western blot data presented in Fig. 1C show that in transient assays, the Ets-2 T72A mutant is as stable as the WT Ets-2 protein.

Equal quantities of GST fusion proteins corresponding to p300 fragments a.a. 1–596 (GST-p300N) and 743–1572 (GST-p300M; see Fig. 3B) were assayed in GST pull-down assays with in vitro radiolabeled WT and T72A Ets proteins. As shown in Fig. 8A, the amounts of radiolabeled WT and the T72A mutant Ets proteins recovered from the agarose beads containing GST-p300N were comparable. In agreement with previous data, GST-p300M did not bind to WT or the mutant Ets proteins. Our data presented in Figs. 4 and 7 have indicated that the DB (Ets) domain does not bind to the N-terminal region of p300/CBP. Thus, the binding of the Ets-2 observed in this experiment is sole due to an interaction between the TA domain of Ets-2 and the N-terminal region of p300/CBP. These results indicate that the phosphorylation of Thr-72 in the Pointed domain is not critical for the interaction of the TA domain with the N-terminal region of p300/CBP. We have not investigated whether T72A mutation of Ets-2 affects its interaction with the C-terminal region of p300/CBP.

We also compared the p300/CBP binding capacities of the WT and the Ets-2 T72A mutant in yeast cells. Human Ets-2 cDNA with T72A mutation was cloned in frame with activation domain plasmid of the yeast two-hybrid system. Yeast cells cotransformed with activation domain fusion plasmid containing WT or T72A forms of Ets-2 genes and the Gal4-p300-1 fusion plasmids (p300-1; a.a. 328–1000), and the β-galactosidase activity was quantitated. As shown in Fig. 7C, the plasmid coding for the T72A Ets-2 mutant protein (Ets-2A72) generated about 80% of WT β-galactosidase activity. These results are in agreement with results obtained in GST pull-down assays (Fig. 8A) and confirm that the T72A mutation of Ets-2 did not significantly affect its capacity to bind to the N-terminal region of p300/CBP.

To determine whether interaction of Ets-2 with p300 is influenced by the phosphorylation of Ets-2 at Thr-72 in human cells, we carried out the coimmunoprecipitation assays followed by Western blots similar to those described in Fig. 3F. Data presented in Fig. 8F indicated that both WT and the mutant Ets-2 proteins coimmunoprecipitated the epitope-tagged CBP fragment a.a. 1–1890 from transfected 293 cell lysates at comparable levels. Because the CBP expression vector used in this experiment contained most of the CBP coding sequences, these results did not allow us to separate the Ets-2 interaction with N-terminal region of p300/CBP from the Ets-2 interaction with the C-terminal region. Nevertheless, they suggest that the mutation of the conserved MAP kinase phosphorylation site did not affect its overall interaction with p300/CBP in human cells.

Coactivation of T72A Ets-2 Mutant by p300/CBP—Next, we determined whether coactivation of Ets-2 by p300 is dependent on the phosphorylation of Ets-2 at Thr-72 by MAP kinase. To ensure that the Thr-72 of WT Ets-2 is phosphorylated by MAP kinase, we included a mutant of MAP kinase kinase (MEK-1) which constitutively phosphorylates and activates MAP kinase (51). An expression plasmid encoding this MEK-1 mutant when included in transfection assays is expected to express the mutant MEK protein and phosphorylate the Thr-72 of the WT Ets-2 but not the Ala-72 of the mutant Ets-2 expressed from the transiently transfected Ets-2 expression plasmids.

The capacity of the WT and T72A mutant Ets-2 to cooperate with p300 in the transcriptional activation of E18pal in the presence and absence of MEK-1 was assayed as described above. HeLa cells were cotransfected with E18pal and expression vectors encoding WT mouse ets-2 (pFNEts-2) or Ets-2 T72A mutant (pFNEts-2A72), p300, and MEK-1 mutant in appropriate combinations, and the CAT activities obtained from the lysates are presented in a bar diagram shown in Fig. 8C. Western blot data presented in Fig. 1C showed that the T72A Ets-2 produced from pFNEts-2A72 is stable in transfection assays and that p300 does not increase the levels of this mutant Ets-2 significantly. Because we used a constitutively active MEK-1 mutant in these assays, which may have some general transcriptional stimulatory effect in the cell, we also assayed in parallel the empty vector (dFosCAT (16)) and subtracted these values from those obtained for E18pal, i.e. each transfection was carried out in duplicate with one set containing E18pal reporter plasmid and the second set containing dFosCAT reporter plasmid. The value obtained for the empty vector was subtracted from the value obtained for E18pal in a matching set, and the fold increase was then determined. The results show that in the presence of MEK-1, the WT Ets-2 and p300 together stimulated the activity of the E18pal by about 85-fold when compared with basal activity (E18pal without any activator; Fig. 6C). This activity was dramatically higher than that observed for the E18pal reporter in the presence of p300, MEK-1, and without Ets-2 (22-fold, lane 7). Basal activity of the E18pal was increased by about 5-fold by the WT Ets-2 (lane 4), whereas the T72A mutant Ets-2 did not increase the basal activity of E18pal significantly (1.5-fold, lane 9). This is consistent with previous reports showing that T72A Ets-2 mutant is severely defective for transcription activation activity (17). MEK-1 increased the Ets-2 Ala-72-mediated stimulation of E18pal only marginally (compare lane 10 with lane 9). Together, p300 and Ets-2A72 increased the E18pal activity by about 7-fold (compare lane 11 with lane 9). A combination of
Ets-2 A72, p300, and MEK-1 together increased the E18pal activity by about 63-fold (lane 12). This fold increase in activity is much higher than all the controls, including that obtained for the E18pal in the presence of p300 and MEK-1 (22-fold; lane 7). Thus, we conclude that in the presence of MEK-1, p300/CBP was able to coactivate both the WT and the mutant Ets-2 in which the MAP kinase substrate Thr-72 is mutated to Ala.

**DISCUSSION**

In this paper, we have shown that Ets-2 and p300/CBP exist as a complex in human cells under physiological conditions (Fig. 3). By using a variety of approaches, we have identified two Ets-1/Ets-2-binding sites on p300/CBP. One binding region maps between a.a. 328 and 596, and the second region maps between a.a. 1678 and 2370 (Figs. 3 and 7C). These two regions contain cysteine/histidine-rich regions I and III, respectively. These results are consistent with the recent data published by Yang et al. (52) who showed that Ets-1 interacts with a.a. 313 to 452 and 1449 to 1892 of p300/CBP. Surprisingly, we did not detect interactions between Ets-1/Ets-2 and p300/CBP in a region between a.a. 744 and 1571 which contains the bromo-domain and the second C/H region. It is interesting to note that the Ets-1/Ets-2-binding regions on p300/CBP interact with many cellular transcription factors and viral oncogenes. For example, the N-terminal Ets-1/Ets-2-binding regions of p300/CBP interact with a large number of transcription factors including CREB/activating transcription factor (53), c-Jun (34, 54), c-Myc (55), Stat1 (56), Stat2 (57), hypoxia-inducible factor-1a (58), and sterol regulatory element-binding protein-2 (59). The C-terminal Ets-1/Ets-2-binding region also over laps with binding sites for several transcription factors including c-Jun and JunB (60), YY1 (61), MyoD (48, 62), and p53 (63).

Currently, we do not know whether two different transcription factors compete for a single site on p300/CBP. If such a competition exists, one can speculate the interaction of Ets-1/2 with p300/CBP may exclude the interaction of other transcription factors that compete for the same site. Thus, Ets-1 or Ets-2 when present in high concentrations may interfere in p300/CBP interacting with other transcription factors that bind to the same region. Also, availability of two Ets-1/Ets-2-binding sites in p300/CBP may allow these proteins to compete more efficiently for limiting amounts of p300/CBP and divert this coactivator to pathways in which Ets proteins are intimately involved. Recent studies suggest that certain cellular activators compete for the limiting amounts of p300/CBP resulting in the inhibition of activity of one transcription factor by another. Such a competition is believed to be a molecular basis of reciprocal antagonism between AP1 and (i) retinoic acid receptors (34) and (ii) p53 (63) and signal transducers and activators and transcriptions (57). It is likely that several other reciprocal antagonisms may be discovered in the future.

We attempted to address the question of whether both p300/CBP binding regions are essential for coactivation of Ets-1/Ets-2. Surprisingly, we found that the N- and the C-terminal regions independently are capable of coactivating Ets-2 in the transcriptional stimulation of the stromelysin promoter. This suggests that only one of the two Ets-1/Ets-2-binding sites is adequate for the transcriptional coactivation of Ets-2. These results are consistent with the properties of p300/CBP. Both the N- and the C-terminal regions of p300/CBP contain strong transcriptional activation domains, and both these regions bind to TBP ((48, 64) and transcription factor IIB (65)). Thus, it is conceivable that these truncated p300/CBP molecules are capable of bridging the promoter-bound Ets-1/Ets-2 and the basal transcription complex and stimulating transcriptional initiation. Based on these data, it would appear that the Ets-1/Ets-2 interacting regions on p300/CBP are functionally redundant. However, these studies need to be extended to cellular genes in the chromosomal context to understand the significance of multiple interactions between Ets-1/Ets-2 and p300/CBP.

Our observations that the TA and the DB domains of Ets-2 can independently interact with the N- and the C-terminal regions of p300/CBP further increase the complexity of Ets-1/Ets-2 interactions with this family of coactivators. The Ets-2 TA domain (a.a. 1–290) is able to bind to both the N- and the C-terminal regions of p300. However, the Ets domain binds only to the C-terminal region. It is possible that the nature of the Ets binding domains in the N- and the C-terminal regions of p300/CBP are significantly different. A detailed mutational analysis of Ets-2-binding sites on p300/CBP as well as the a.a. sequences of Ets-2 that interact with different domains in p300/CBP will be necessary to understand further the Ets-1/Ets-2-p300/CBP interactions.

Also interesting is the fact that the N- and the C-terminal regions (a.a. 1–1097 and 1678–2442, respectively) used in these studies do not contain the region responsible for the histone acetylation activity of p300/CBP as this enzyme activity maps between a.a. 1195 and 1673 on CBP (66). The mutant pCBP1-1097 also lacks the p300/CBP-associated factor and RNA helicase A binding regions (31) (67). RNA helicase is believed to bridge CBP and RNA polymerase II (67). Both p300/CBP and p300/CBP-associated factor are reported to acetylate histones and augment transcription (68). Other studies have shown that a truncated CBP containing only the N-terminal 1–714 a.a. can stimulate phospho-CREB and activating transcription factor activity (69). Similarly, the N-terminal half of CBP was shown to cooperate with p65^SEBP, a serum response element-binding protein, in the transcriptional stimulation through SRE (38). Currently, it is not known how a mutant of p300/CBP that lacks the ability to acetylate histones or to bind to RNA polymerase II is able to cooperate with various transcription factors and stimulate transcription. It is conceivable that transcription of transiently introduced templates does not require histone acetylation, whereas transcriptional activation of the chromosomal genes may be linked to p300/CBP-associated histone acetylation activity.

We have also shown that a single signature motif LLELL reported to be important in receptor-coactivator interactions, located in the DNA binding domain of Ets-2, does not appear to play in the interactions with p300/CBP. This motif is present in the first helix turn helix region (H1) of Ets-2 which is directly involved in DNA binding (10, 70). The fact that the N-terminal region of Ets-2 can interact with the C-terminal region of p300/CBP also supports this suggestion. Both the N-terminal region of Ets-2 and the C-terminal region of p300/CBP lack such sequences (the N-terminal region of p300/CBP contain two copies of this motif (50)). However, we have discovered that the Ets-2 mutant carrying this mutation is severely defective for transactivation consistent with an important role for this region in DNA binding (10, 70).

Surprisingly, we found that two other Ets family transcription factors PEA-3 and Erg-2 did not cooperate with p300 in the stimulation of the stromelysin promoter, although they cooperated with p300/CBP to superactivate E18pal. Other studies have shown that the PEA-3 expression vector used in this study synthesizes PEA-3 protein and binds to the E18pal (17), and our Erg-2 expression vector stimulates the minimal promoter containing the Erg-binding sites efficiently (71). Therefore, the lack of superactivation of the stromelysin promoter by these transcription factors in the presence of p300/CBP may not be due to their inability to interact with p300/CBP but may

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be due to other reasons. For example, the superactivation of the stromelysin promoter requires functional synergy between p300/CBP, Ets-1/Ets-2, and AP-1 during formation of active transcription complex. Although PEA3 and Erg may interact with p300/CBP, their interaction with Ets-binding sites on the stromelysin promoter may not provide appropriate protein-protein interactions with AP-1 and other stromelysin promotor-specific transcription factors to generate active transcription complex. For example, studies have shown that Erg can bind to Ets sites on the stromelysin promoter and competitively inhibit promoter activation by Ets-2 (72). Formation of an active transcription complex on a native promoter is a complex process in which promoter-specific transcription factors interact with selected members of TBP-associated factors and general transcription factors in an orderly fashion to initiate transcription (reviewed in Ref. 73). E18pal reporter construct used in this study consists of a synthetic promoter in which the palindromic Ets-binding sites are placed upstream of the c-fos minimal promoter. Thus, it is possible that E18pal would allow protein-protein interactions between PEA-3 and Erg-2 and p300 and basic transcription complex to stimulate transcription, whereas with the stromelysin promoter being a natural promoter such interactions would not occur. Clearly, further studies are needed to resolve these complex issues.

In these studies, we have also shown that the Ets-2 mutant in which the MAP kinase-phosphorylatable Thr residue mutated to Ala binds to p300/CBP as efficiently as the WT Ets-2. Thus interactions between the TA domain of Ets-2 and p300/ CBP is not dependent on the phosphorylation of the Thr-72 residue of the Pointed domain. It is strikingly in contrast to the interactions observed between CREB and p300/CBP. In the case of CREB, phosphorylation of Ser-133 is absolutely required for it to bind to CBP (53). It is also noteworthy that phosphorylation of Ets-2 at this site is not required for its coactivation by p300/CBP. Our transfection assays included a constitutively active MEK-1 mutant and thus, in the transfection assays, the phosphorylation of the Thr-72 residue of the WT Ets-2 would occur but not the Ala-72 residue of the mutant. Consistent with a previous report (17), the capacity of the T72A Ets-2 mutant to activate the E18pal is dramatically reduced. However, in the presence of MEK-1 and p300/CBP, the residual transactivation activity of the T72A Ets-2 mutant is dramatically increased. Thus our results suggest that p300/CBP may play a role in regulating the basal transcriptional activation activity of Ets-2. Such a role may be important in the regulation of expression of the Ets-2-driven genes under conditions where the transcriptional activation activity of Ets-2 may be low in the cell such as during serum starvation where Ets proteins are not phosphorylated by MAP kinase.

Stromelysin is an important member of a family of matrix metalloproteases (MMPs) which act on extracellular matrix during tissue remodeling, growth, and morphogenesis (74). For example, in chronic inflammation such as rheumatoid arthritis, macrophages produce stromelysin-1 (MMP3) and collagenase I (MMP1) and contribute directly to joint destruction (75). Both these MMPs require Ets-1/Ets-2 and AP1 for their activation. Stromelysin I has been shown to be important in the mammary gland development and branching morphogenesis (76). Metastatic tumor cells express matrix metalloproteases including stromelysin at high levels, a process that correlates with the metastatic potential of the tumor cells (74, 77). Studies have shown that in addition to stromelysin, Ets-2 is also important in the activation of the type I collagenase promoter (78). Thus, p300 may play a role in the regulated expression of these different MMP genes and contribute directly in processes where MMPs are implicated.

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REFERENCES

1. Treisman, R. (1996) Curr. Opin. Cell Biol. 8, 205–215
2. LaMarca, K., Thompson, C. C., Byers, B. P., Walton, E. M., and McKnight, S. L. (1991) Science 253, 789–792
3. Eisenbeis, C. F., Singh, H., and Sterb, U. (1995) Genes Dev. 9, 1377–1387
4. Schneikert, J., Lutz, Y., and Wasylyk, B. (1992) Oncogene 7, 249–256
5. Goldman, P. S., Tran, V. K., and Goodman, R. H. (1997) Curr. Biol. 7, 1407–1416
6. Somasundaram, K., Jayaraman, G., Williams, T., Moran, E., Frisch, S., and Potempa, J. (1996) Cell. Mol. Biol. Res. 1127–1134
7. Fuchs, S. M., and Ruley, H. E. (1997) J. Biol. Chem. 272, 3617–3622
8. Nomura, N., Osaka, I., and Ohtsubo, E. (1996) J. Biol. Chem. 271, 9791–9798
9. Seth, A., and Papas, T. S. (1990) Trends Genet. 6, 951–954
10. Gross, B. F., and Peters, J. M. (1998) Adv. Cancer Res. 75, 1–55
11. Watson, D. K., McWilliam, J. M., Lapis, P., Luitenberger, J. A., Schweinfest, C. W., and Papas, T. S. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7862–7866
12. Schneikert, J., Lutz, Y., and Wasylyk, B. (1992) Oncogene 7, 249–256
13. Goldman, P. S., Tran, V. K., and Goodman, R. H. (1997) Curr. Opin. Cell Biol. 8, 205–215
14. Frisch, S. M., and Ruley, H. E. (1987) Anal. Biochem. 163–176
15. Bhat, N. K., Fisher, R. J., Fujiwara, S., Ascione, R., and Papas, T. S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3161–3165
16. Schneikert, J., Lutz, Y., and Wasylyk, B. (1992) Oncogene 7, 249–256
17. Schneikert, J., Lutz, Y., and Wasylyk, B. (1992) Oncogene 7, 249–256
18. Alsafadi, A., and Cassatella, M. A. (2009) Trends Cell Biol. 19, 241–246
19. Alsafadi, A., and Cassatella, M. A. (2009) Trends Cell Biol. 19, 241–246
20. Alsafadi, A., and Cassatella, M. A. (2009) Trends Cell Biol. 19, 241–246
21. Alsafadi, A., and Cassatella, M. A. (2009) Trends Cell Biol. 19, 241–246
22. Alsafadi, A., and Cassatella, M. A. (2009) Trends Cell Biol. 19, 241–246
23. Alsafadi, A., and Cassatella, M. A. (2009) Trends Cell Biol. 19, 241–246
24. Alsafadi, A., and Cassatella, M. A. (2009) Trends Cell Biol. 19, 241–246
49. Sartorelli, V., Huang, J., Hamanori, Y., and Kedes, L. (1997) Mol. Cell. Biol. 17, 1010–1026
50. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1997) Nature 387, 733–736
51. Mansour, S. J., Matten, W. T., Hermann, A. S., Candia, J. M., Rong, S., Fukasawa, K., Vande Woude, G. F., and Ahn, N. G. (1994) Science 265, 966–970
52. Yang, C., Shapiro, L. H., Rivera, M., Kumar, A., and Brindle, P. (1997) Mol. Cell. Biol. 17, 2218–2229
53. Chrivia, J. C., Kwok, R. P. S., Lamb, N., Hagiwara, M., Montminy, M. R., and Goodman, R. H. (1993) Nature 365, 855–859
54. Bannister, A. J., Oehler, T., Wilhelm, D., Angel, P., and Kouzarides, T. (1995) Oncogene 11, 2509–2514
55. Dai, P., Akimaru, H., Tanak, Y., Hou, D. X., Yasukawa, T., Kanei-Ishii, C., Takahashi, T., and Ishii, S. (1996) Genes Dev. 10, 528–540
56. Zhang, J. J., Vinkemeier, U., Gu, W., Chakravarti, D., Horvath, C. M., and Darnell, J. E. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15092–15096
57. Bhattacharya, S., Eckner, R., Grossman, S., Oldread, E., Arany, Z., D’Andrea, A., and Livingston, D. M. (1996) Nature 383, 344–347
58. Arany, Z., Huang, L. E., Eckner, R., Bhattacharya, S., Jiang, C., Goldberg, M. A., Bunn, H. F., and Livingston, D. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12909–12973
59. Olifer, J. D., Andresen, J. M., Hansen, S. K., Zhou, S., and Tjian, R. (1996) Genes Dev. 10, 2903–2911
60. Lee, J. S., See, R. H., Deng, T., and Shi, Y. (1996) Mol. Cell. Biol. 16, 4312–4316
61. Lee, J. S., Galvin, K. M., See, R. H., Eckner, R., Livingston, D., Moran, E., and Shi, Y. (1995) Genes Dev. 9, 1188–1198
62. Eckner, R., Yan, T. P., Oldread, E., and Livingston, D. M. (1996) Genes Dev. 10, 2478–2490
63. Avantaggiati, M. L., Ogryzko, V., Gardner, K., Giordano, A., Levine, A. S., and Kelly, K. (1997) Cell 88, 1175–1184
64. Abraham, S. E., Lobo, S., Yaciuk, P., Wang, H. G., and Moran, E. (1993) Oncogene 8, 1639–1647
65. Kwok, R. P., Lundblad, J. R., Chrivia, J. C., Richards, J. P., Bachinger, H. P., Brennan, R. G., Roberts, S. G., Green, M. R., and Goodman, R. H. (1994) Nature 370, 223–226
66. Ogryzko, V. V., Sluitz, R. L., Russanov, V., Howard, B. H., and Nakatani, S. (1996) Cell 87, 953–959
67. Nakajima, T., Uchida, C., Anderson, S. F., Lee, C.-G., Hurvitz, J., Parvin, J. D., and Montminy, J. D. (1997) Cell 90, 1107–1112
68. Brownell, J. E., and Allis, C. D. (1996) Curr. Opin. Genet. & Dev. 6, 176–184
69. Swope, D. L., Mueller, C. L., and Chrivia, J. C. (1996) J. Biol. Chem. 271, 21936–21945
70. Kodandapani, R., Pio, F., Ni, C. Z., Piccialli, G., Klemaz, M., McKercher, S., Maki, R. A., and Ely, K. R. (1996) Nature 380, 456–460
71. Siddique, H. R., Rao, V. N., and Reddy, S. P. (1993) Oncogene 8, 1751–1755
72. Buttice, G., Duterque-Couillaud, M., Basu, B. C., Carrere, S., Kurkinen, M., and Stelhelin, D. (1996) Oncogene 13, 2297–2306
73. Burely, S. K., and Roeder, R. G. (1996) Annu. Rev. Biochem. 65, 769–799
74. Birkedel-Hanson, H., Moore, W. G., Barden, M. K., Windsor, L. J., Birkedel-Hansen, B., DeCaro, A., and Engler, J. A. (1996) Crit. Rev. Oral Biol. Med. 4, 197–250
75. Birnkerhoff, C. E. (1991) Arthritis Rheum. 34, 1073–1075
76. Symons, C. J., Talbourn, H. S., Alexander, C. M., Chir, S. M., Bissell, M. J., and Web, Z. (1994) J. Cell Biol. 125, 861–893
77. Liotta, L. A., Steeg, P. S., and Stetler-Stevenson, W. G. (1991) Cell 64, 327–336
78. Sato, H., and Seiki, M. (1993) Oncogene 8, 395–405