CREB-binding protein and p300 in Transcriptional Regulation*

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CREB-binding protein (CBP) and p300 are believed to participate in the activities of hundreds of different transcription factors (see Fig. 1). Current models suggest that the binding of these coactivators to transcription factor activation domains positions histone acetyltransferases (HATs) near specific nucleosomes in target gene promoter regions (for review, see Ref. 1). Interactions with components of the general transcriptional machinery, such as TFII D, TFII B, and the RNA polymerase II holoenzyme (RNAPII) have also been suggested to contribute to CBP/p300 function. The simultaneous interaction of multiple transcription factors with CBP/p300 has been proposed to contribute to transcriptional synergy. Conversely, competition for CBP/p300 binding has been suggested to mediate some examples of signal-induced transcriptional repression. An overview of CBP/p300 in cellular growth and differentiation has recently been published (2), but many questions regarding their role in transcriptional regulation remain unanswered. This review deals with some of the more controversial aspects of CBP/p300 function. In particular, we will ask whether CBP and p300 have distinct functions, review the evidence for their regulation by phosphorylation, and ask whether they function primarily by acetylating histones or other proteins. We will also revisit the evidence for the role of CBP/p300 as transcriptional "integrandators." Finally, we will attempt to localize CBP/p300 function within the complex series of processes involved in transcriptional activation.

Are CBP and p300 Redundant?
Although CBP and p300 are highly related and share many functional properties, there is evidence that these factors are not really interchangeable. Subtle differences in the expression of CBP and p300 during development (3) may explain why knockouts of the two coactivators in mice result in somewhat distinct phenotypes. For example, heterozygosity for CBP causes certain hematological defects and a predisposition to cancer that is not seen in mice lacking one allele of p300 (4). Studies of specific transcription factor pathways provide additional evidence of differences between the functions of CBP and p300. For example, fibroblasts derived from homozygous p300 knockouts are defective for retinoic acid receptor but not CREB signaling (5). Similarly, riboxygen-mediated ablation of p300, but not CBP, blocks the retinoic acid receptor response (6). Other differential functions of CBP and p300 have been revealed by their distinct interactions with viral transforming proteins. For example, the Kaposi sarcoma-associated herpesvirus protein vIRF has been reported to be stimulated by CBP and repressed by p300 (7). Distinct roles for CBP and p300 have also been suggested in the differentiation of muscle and F9 teratocarcinoma cells (8, 9). On the other hand, homozygous mutations in CBP and p300 both result in lethality and a similar constellation of phenotypic defects (4, 5). Moreover, CBP/p300 double heterozygotes are invariably lethal, suggesting that functions of CBP and p300 must overlap, at least to some degree. This complexity is not shared by simpler metazoans, such as Drosophila and Caenorhabditis elegans, which express only a single isoform of CBP/p300 (reviewed in Ref. 2).

How Does Phosphorylation Regulate CBP/p300 Function?
Although cell cycle-dependent phosphorylation of p300 was reported almost a decade ago (10), it is still not entirely clear how phosphorylation regulates CBP/p300 function. In large part, this lack of understanding is because of the fact that the specific phosphorylation sites in CBP/p300 have never been precisely identified. Phosphorylation of p300 and CBP by cyclin E/Cdk2 was reported by Perkins et al. (11) and Ait-Si-Ali et al. (12), respectively. In the case of p300, cyclin E/Cdk2 was shown to negatively regulate coactivator function in a manner that can be blocked by the cyclin-dependent kinase inhibitor, p21. In this model, p21 was proposed to participate in a positive feedback loop, whereby activators such as p53, which depend upon p300 for function, induce p21, which then alleviates the block in p300 action mediated by cyclin E/Cdk2. In contrast, cyclin E/Cdk2 was reported to increase the intrinsic HAT activity of CBP, potentially activating expression of S-phase genes that are repressed in early G1 (12). Because the phosphorylation sites in p300 and CBP have not been mapped, however, it may be premature to conclude that the two coactivators are differentially regulated by cyclin-dependent kinases.

CBP and p300 both contain a consensus protein kinase A (PKA) site adjacent to their third zinc finger domains, and several groups have proposed that phosphorylation by PKA may contribute to CBP/p300 regulation. For example, Xu et al. (13) have argued that phosphorylation of CBP is responsible for the PKA-mediated augmentation of the transcription factor Pit-1. This is an intriguing model because Pit-1 itself cannot be phosphorylated by PKA. Using microinjection assays, these workers demonstrated that the activation of Pit-1 by PKA was lost in the presence of CBP containing a point mutation at the consensus PKA site. This model was not confirmed by Zanger et al. (14), however, and Swope et al. (15) have suggested that the PKA-responsive domain in CBP resides near its amino terminus. At this point, the mechanism of PKA activation of CBP/p300 remains enigmatic.

Other kinases proposed to regulate CBP/p300 function include calcium/calmodulin (CaM) kinase IV, MAPK, and pp90Rsk. Although several reports suggested that CBP cannot mediate its transcriptional functions in the absence of CaM kinase IV stimulation (16–18), later studies showed that recruitment of CBP by itself was sufficient for transcriptional activation (19, 20). It remains possible, however, that phosphorylation of CBP/p300 by CaM kinase IV could contribute to signaling by augmenting the transcriptional response.

Phosphorylation and activation of CBP by MAPK was first reported by Janknecht and Nordheim (21). Activation of MAPK through the Ras pathway by insulin or nerve growth factor was reported to recruit pp90Rsk to the third zinc finger domain of CBP in a manner that prevents the binding of essential CBP effectors such as RNAPII (22). Interestingly, modulation of CBP by pp90Rsk does not appear to require its catalytic kinase activity. This inhibitory effect of pp90Rsk has not been seen by other investigators, however, so it is possible that it is cell type-specific (23). Clearly, the understanding of CBP/p300 regulation by phosphorylation remains a major topic for future study.

Are CBP and p300 Primarily HATs or FATs?
In addition to their intrinsic acetyltransferase functions, CBP and p300 are known to associate with additional HATs, including...
PCAF, SRC-1, and p/CIP. Why so many different HATs are required for transcriptional regulation is unknown, but the answer may lie in the differing preferences of these enzymes for free histones as compared with nucleosomes and their distinct targets within the histone substrates (for review, see Ref. 24). Although it has been suggested that the HAT domains in CBP/p300 are highly related to those in P/CAF and GCN5 (25), the primary sequences of these domains are actually quite different. Moreover, these differences are significant enough to allow the development of specific inhibitors of the P/CAF and CBP/p300 enzymatic activities (26). Kraus et al. (27) have shown that the p300-mediated activation of estrogen receptor (ER) function on reconstituted chromatin depends upon the intrinsic acetyltransferase activity of the coactivator, demonstrating that this enzymatic function is essential in the context of chromatin. Because histone acetylation is not required for transcription of naked DNA templates, these results imply that some component of chromatin is the acetylation target. These conclusions are supported by the results of Ludlam et al.,2 which show that flies containing an acetyltransferase-deficient form of CBP are incapable of activating specific target genes in vivo. Although confirming the importance of the CBP enzymatic function, these studies do not identify the acetylation target. Recent studies have shown that the HAT activity of CBP/p300 is directed toward nucleosomes through interactions with the histone chaperone, RbAp 48 (28). Moreover, Ito et al. (29) have found that histone acetylation by p300 facilitates the transfer of H2A-H2B from nucleosomes to the chaperone protein NAP-1. In this model, the recruitment of p300 and the subsequent histone acetylation follow a chromatin remodeling step mediated by ATP-dependent proteins in the ISWI family. These results are consistent with in vivo chromatin immunoprecipitation experiments in yeast showing that the association of SWI/SNF components on the HO promoter is required for the subsequent HAT recruitment (30, 31). Whether the release of H2A-H2B results from the acetylation of these proteins directly or whether other nucleosomal components are the primary targets of the acetyltransferases remains to be determined.

Acetylation of transcription factors (through FAT, factor acetyltransferase activities) by CBP/p300 may provide an equally important mode of regulation. First identified in the context of the tumor suppressor p53 (32), acetylation of transcription factors has been increasingly recognized as a mechanism of gene regulation. In some instances, acetylation has clearly been shown to increase the binding of transcription factors to DNA (32). In most cases, however, the mechanism of activation is unknown. Recent evidence suggests that coactivator acetyltransferases might also serve to disrupt activator and repressor complexes. For example, Evans and co-workers (33) have shown that the recruitment of p300 to the ligand-activated ER leads to the acetylation of ACTR (an associated acetyltransferase), disruption of the ACTR-p300-ER complex, and the termination of transcription. Another possibility, consistent with the multistep model of transcription proposed by Roeder (reviewed in Refs. 34 and 35), is that CBP/p300-mediated acetylation of the complex may promote the transition from a CBP/p300-dependent to a mediator-dependent stage of transcription (see below).

A converse mechanism was proposed by Zhang et al.3 In these studies, interaction of the histone deacetylase-binding corepressor, CtBP (carboxy-terminal binding protein), to a variety of transcriptional repressors was shown to be blocked by acetylation of the CtBP interaction sites. In this instance, as in the classical histone acetylation model, acetylation is proposed to activate transcription by disrupting protein complexes involved in repression. Paradoxically, acetylation by CBP can also cause transcriptional repression in some systems. For example, in flies, CBP has been shown to inhibit wingless signaling by acetylating the Drosophila homologue of the high mobility group protein, LEP/TCF-1 (36). Acetylation of a specific residue in LEP/TCF-1 is believed to block the binding of the coactivator β-catenin/Armadillo, one of the intermediates in the wingless signaling pathway. In support of this model, CBP loss-of-function mutants have been found to suppress the effects of an Armadillo mutation.

Does CBP/p300 Function as a Transcriptional Integrator?

It is somewhat surprising that CBP and p300, which mediate the activities of so many different transcription factors, might be present in the cell at limiting concentrations. Nonetheless, there is considerable evidence that this is the case. Even discounting experiments involving transcription factor overexpression, which would perhaps be expected to exceed the capacity of the endogenous CBP/p300, studies have shown that relatively small decreases in the concentrations of coactivator are deleterious. For example, in the human Rubinstein-Taybi syndrome, loss of a single CBP allele results in severe developmental defects (37). The idea that CBP/p300 levels are limiting is also supported by tissue culture experiments, as exemplified by the studies of Hottiger et al. (38), which examined the ability of interferon-α (IFN-α) to inhibit tumor necrosis factor-α-stimulated human immunodeficiency virus gene expression. This inhibition was shown to be mediated by competition between STAT-2 (stimulated by IFN-α) and the p65 subunit of NF-κB (stimulated by tumor necrosis factor-α) for a shared binding site within the first zinc finger domain of CBP/p300. It is not

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2 W. Ludlam, R. H. Goodman, and S. Smolik, submitted for publication.

3 Zhang, Q., Yao, H., Vo, N., and Goodman, R. H. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14323–14328.
FIG. 2. The IFN-β enhanceosome complex. Assembly of the IFN-β enhanceosome creates a stereospecific interaction surface for recruitment of CBP/p300 and the basal transcription machinery to allow multiple rounds of transcription. GTFs indicate general transcription factors. p160 refers to the SRC/TIF/cIP family of coactivators.

FIG. 3. The multistep model of transcription. First, ATP-dependent remodeling complexes alter the structure of chromatin. Second, coactivator HATs facilitate the formation of enhanceosomes and permit the actions of mediator complexes. CBP/p300 may facilitate the recruitment of the mediator complex to active sites of transcription. Mediator, in turn, regulates transcription through interactions with components of the basal transcription machinery. The dashed line indicates an unknown association with DNA. GTFs, general transcription factors.

certain that two transcription factors must compete for the same binding site to be mutually antagonistic, however. If CBP/p300 levels are truly limiting, it is possible that they could be directed toward specific genes to the exclusion of others. Testing this hypothesis will require the use of experimental paradigms that do not involve the overexpression of exogenous transcription factors.

The idea that CBP/p300 contributes to transcriptional synergy is probably best supported by studies of the IFN-β enhanceosome (see below), but other complex promoters have also been shown to contain binding sites for multiple CBP/p300-interacting transcription factors. Indeed, given the large number of factors that bind CBP/p300, it is difficult to imagine a promoter where this would not be the case.) Nonetheless, although transcriptional synergy through CBP/p300 is an appealing model, it has not been shown conclusively that these coactivators interact with multiple transcription factors simultaneously. In addition, although the recruitment of coactivators to the enhanceosome appears to be required for synergistic activation, tethering CBP/p300 to the promoter through a heterologous DNA-binding domain is not sufficient. As suggested by Merika et al. (39), the activation domains of the individual transcription factors comprising the enhanceosome may contribute critical interactions with basal factors. Alternatively, CBP/p300 may only participate in a transient (albeit required) step in the transcriptional process. It may be equally important for CBP/p300 to be replaced by other factors, such as the mediator complex, for transcription to proceed. In support of this idea, Kraus and Kadonaga (40) have demonstrated that although both the ER and p300 are necessary for transcriptional initiation from chromatin templates, only the ER is required for reinitiation.

How Does CBP/p300 Fit into the Complex Series of Events That Mediate Transcriptional Activation?

Transcriptional processes are regulated through the sequential interactions of a large number of modulatory multiprotein complexes. Assembly of basal transcription factors at the promoter represents the end result of these interactions. Regulation is imparted by additional components such as enhanceosomes and mediator complexes which, along with coactivators, integrate specific extracellular events and intracellular signals.

Enhanceosomes are stable multiprotein complexes that promote the cooperative recruitment of coactivators and the RNAPII complex to active sites of transcription. In one well-characterized example, formation of the enhanceosome involves recruitment of NFκB, ATF-2/c-Jun, interferon regulatory factors, and HMG1(Y) to enhancer elements in the IFN-β promoter to create stereospecific interaction surfaces between the enhancer binding proteins and the CBP/p300-associated RNAPII complex (Fig. 2) (41, 42). The critical role of CBP/p300 in this context is to promote the rapid formation of the preinitiation and reinitiation complex to facilitate multiple rounds of transcription (43). Depletion of CBP/p300 from this complex decelerates the rate of transcription (44). CBP/p300 may also participate in terminating IFN-β gene transcription by acetylating HMG1(Y), decreasing its affinity for DNA and disrupting the enhanceosome (43, 45, 46). Whether enhanceosomes actually rely on the HAT activity of CBP/p300 has not been determined, however. Recent evidence demonstrating an intrinsic, phosphorylation-dependent HAT activity in ATF-2, one of the DNA-binding proteins found in the IFN-β enhanceosome, suggests that the CBP/p300 HAT function could be redundant (47).

Mediator complexes provide the penultimate step in the activation process, leading to the recruitment of the general transcription machinery (Fig. 3). These mediators, ARC/DRIP/TRAP/SMCC, NAT, CRSP, SRB/Med, and mouse Mediator, share a subset of common components (reviewed in Refs. 48 and 35). The relationships between these complexes and CBP/p300 have not been entirely resolved, however. TRAP, the first of the mammalian mediators to be characterized, does not contain CBP/p300 and lacks detectable HAT activity (49, 50). Consistent with its absence of associated HAT activities and with its relationship to the yeast Mediator, TRAP shows potent coactivator functions with diverse activators on naked DNA templates, whereas additional functions with chromatin templates remain to be tested. In contrast, ARC- and DRIP-mediated transcription has been observed on chromatin templates, possibly reflecting the presence of some TRAP components in the assays with DNA templates and/or the loose association or copurification of CBP/p300 (or other HATs) with ARC and DRIP complexes (51, 52). Might TRAP components also exhibit additional essential (or enhanced) functions with chromatin templates, indicating a potential need for additional protein-protein interactions for formation of the preinitiation complex in this context? What is the role of CBP/p300 in ARC and DRIP? Does CBP/p300 bridge transcriptional activators to the mediator complex, or is it its role to alter nucleosome structure in a manner that allows the mediators to function at a subsequent stage of transcription?

Part of the ambiguity regarding the association of CBP/p300 with mediator complexes may stem from the different methods used to purify these mediators. TRAP was purified using a functional assay (49), whereas ARC and DRIP were identified through their binding to activated transcription factors (52). It is likely that the fusion proteins used to purify ARC and DRIP interact with CBP/p300 and the mediators in a mutually exclusive manner. This explanation would be consistent with the multistep interaction model proposed by Roeder (35). In this model, activated transcription factors have the capacity to interact with both CBP/p300 and the mediator complexes, but the mediator interactions might be nonfunctional until appropriate nucleosomal modifications have been induced by CBP/p300.

Nonetheless, it may be premature to conclude that mediators do not contain HATs. Lorch et al. (53) have determined that the yeast Mediator forms direct interactions with nucleosomes and contains a subunit, Nut1, that specifically acetylates nucleosomal histone H3. Therefore, at least in yeast (which does not contain CBP/p300), mediator complexes do have intrinsic HAT activity. These studies
might also contain loosely associated HATs or whether this activity reopen the issue of whether the mammalian mediator complexes to mediate specific genetic responses to diverse cellular functions will shed light on how cells use common transcriptional complexes to mediate specific genetic responses to diverse cellular signals.

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REFERENCES

1. Sterner, D. E., and Berger, S. L. (2000) Microbiol. Mol. Biol. Rev. 64, 435–459
2. Goodman, R. H., and Smolik, S. (2000) Genes Dev. 14, 1553–1577
3. Partanen, A., Motoyama, J., and Hui, C. C. (1999) Int. J. Dev. Biol. 43, 487–494
4. Kung, A. L., Rebel, V. I., Bronson, R. T., Ch’ng, L. E., Sieff, C. A., Livingston, D. M., and Yao, T. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11566–11571
5. Kawasaki, H., Eckner, R., Yao, T. P., Taira, K., Nakatani, Y., and Yokoyama, K. K. (1998) Nature 393, 284–289
6. Jayachandra, S., Low, K. G., Thlick, A. E., Yu, J., Ling, P. D., Chang, Y., and Moore, P. S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11566–11571
7. Cuervo, A., Saitou, K., and Harel-Bellan, A. (1998) Nature 396, 523–527
8. Kawasaki, H., Eckner, R., Yao, T. P., Taiara, K., Chin, R., Livingston, D. M., and Yokoyama, K. K. (1998) Mol. Cell. Biol. 18, 13108–13113
9. Yie, J., Merika, M., Munshi, N., Chen, G., and Thanos, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13108–13113
10. Yie, J., Merika, M., Munshi, N., Chen, G., and Thanos, D. (1999) EMBO J. 18, 3074–3089
11. Yie, J., Senger, K., and Thanos, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13108–13113
12. Nakajima, T., Fukamizu, A., Takahashi, J., Gage, F. H., Fisher, T., Blenis, J., and Montminy, M. R. (1996) Cell 86, 465–474
13. Xiong, W. L., Manning, E. T., and Kadonaga, J. T. (1999) Genes Dev. 13, 1412–1421
14. Zhang, Q., Yu, N., and Goodman, R. H. (2000) Mol. Cell. Biol. 20, 4970–4978
15. Ait-Si-Ali, S., Ramirez, S., Barre, F. X., Dkhissi, F., Magnaghi-Jaulin, L., Zanger, K., Cohen, L. E., Hashimoto, K., Radovick, S., and Wondisford, F. E. (1996) Cell 85, 457–467
16. Kawasaki, H., Schlitz, L. G., Ikehara, T., Nakagawa, T., Kraus, W. L., and Muramatsu, M. (2000) Mol. Cell. Biol. 20, 1546–1552
17. Yie, J., Merika, M., Munshi, N., Chen, G., and Thanos, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13108–13113
18. Nakajima, T., Fukamizu, A., Takahashi, J., Gage, F. H., Fisher, T., Blenis, J., and Montminy, M. R. (1996) Cell 86, 465–474
19. Xiong, W. L., Manning, E. T., and Kadonaga, J. T. (1999) Genes Dev. 13, 1412–1421
20. Gu, W., and Roeder, R. G. (1997) Cell 90, 595–606
21. Chen, H., Lin, R. J., Xie, W., Wilpitz, D., and Evans, R. M. (1999) Cell 98, 675–686
22. Roeder, R. G. (1999) in Transcription Regulation in Eukaryotes (Champon, P., Fukasawa, T., Kornberg, R., and Coath, C., eds) pp. 106–121, Human Frontiers Science Program, Strasbourg, France
23. Malik, S., and Roeder, R. G. (2000) Trends Biochem. Sci. 25, 277–283
24. Melton, D. A., and Melton, D. A. (1998) Nature 395, 521–525
25. Petri, J., and Moller, M. (1998) Science 281, 28138–28145
26. Zhang, Q., Yu, N., and Goodman, R. H. (2000) Mol. Cell. Biol. 20, 4970–4978
27. Ito, T., Ikehara, T., Nakagawa, T., Kraus, W. L., and Muramatsu, M. (2000) Genes Dev. 14, 1899–1907
28. Ait-Si-Ali, S., Ramirez, S., Barre, F. X., Dkhissi, F., Magnaghi-Jaulin, L., Zanger, K., Cohen, L. E., Hashimoto, K., Radovick, S., and Wondisford, F. E. (1996) Cell 85, 457–467
29. Cochrane, C., and Green, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8284–8288
30. Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999) Cell 97, 299–311
31. Krebs, E. J., Kuo, M. H., Allis, C. D., and Petersen, C. L. (1999) Genes Dev. 13, 1412–1421
32. Roeder, R. G. (1999) in Transcription Regulation in Eukaryotes (Champon, P., Fukasawa, T., Kornberg, R., and Coath, C., eds) pp. 106–121, Human Frontiers Science Program, Strasbourg, France
33. Malik, S., and Roeder, R. G. (2000) Trends Biochem. Sci. 25, 277–283
34. Malach, S., and Roeder, R. G. (1999) Mol. Cell 2, 457–467
35. Sekhar, K., and Thanos, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13108–13113
36. Sekhar, K., and Thanos, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13108–13113
37. Sekhar, K., and Thanos, D. (1999) Mol. Cell 2, 457–467
38. Bergel, M., Herrera, J. E., Thatcher, B. J., Prymakowska-Bosak, M., Vassilev, A., Nakatani, Y., Martin, B., and Bustin, M. (2000) J. Biol. Chem. 275, 11514–11520
39. Kawasaki, H., Schlitz, L. G., Ikehara, T., Takahara, K., Taira, K., Nakatani, Y., and Yokoyama, K. K. (2000) Nature 405, 195–200
40. Kingston, R. E. (1999) Nature 399, 199–200
41. Fondell, J. D., Ge, H., and Roeder, R. G. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 8284–8288
42. Rachez, C., Lemon, B. D., Sudan, Z., Brumleve, V., Gamble, M., Naar, A. M., Erdjument-Bromage, H., Tempst, P., and Freedman, L. P. (1999) Nature 398, 824–828
43. Lorch, Y., Beve, J., Gustafsson, C. M., Myers, L. C., and Kornberg, R. D. (2000) Mol. Cell 6, 197–201
44. Hardingham, G. E., Chawla, S., Cruzalegui, F. H., and Bading, H. (1999) Science 281, 1505–1509
45. Hardingham, G. E., Chawla, S., Cruzalegui, F. H., and Bading, H. (1999) Neuron 22, 789–798
46. Chawla, S., Hardingham, G. E., Quinn, D. R., and Bading, H. (1998) Science 281, 1505–1509
47. Cardinaux, J. R., Notis, J. C., Zhang, Q., Yu, N., Craig, J. C., Daniel, P. M., Brennan, R. G., and Goodman, R. H. (1999) Mol. Cell. Biol. 20, 1546–1552
48. Gu, W., and Roeder, R. G. (1997) Cell 90, 595–606