Biochemical and Biophysical Research Communications **262,** 157–162 (1999)
Article ID bbrc.1999.1132, available online at http://www.idealibrary.com on IDEAL®

# Phosphorylation by p44 MAP Kinase/ERK1 Stimulates CBP Histone Acetyl Transferase Activity *in Vitro*

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Received July 5, 1999

**The transcriptional coactivator CBP displays an intrinsic histone acetyl transferase (HAT) activity which seems to participate in transcriptional activation through the destabilization of nucleosome structure. CBP is involved in the activity of several transcription factors that are nuclear endpoints of intracellular signal transduction pathways. In some instances, the transcription factors are phosphorylated upon cell activation, which induces their interaction with CBP. CBP itself is a phosphoprotein and can be phosphorylated by cycle-dependent kinases or by MAP kinases. Here we show that CBP phosphorylation by p44 MAP kinase/ERK1 results in the stimulation of its HAT enzymatic activity. The p44 MAP kinase/ERK1 phosphorylation sites are located in the C-terminal part of the protein, outside of the HAT domain. These sites are required for enzymatic stimulation, suggesting that phosphorylation by p44 MAP kinase/ERK1 induces a conformational change of the CBP molecule. Our data suggest that, in some instances, CBP itself might be a target for signal transduction pathways.** 

Intracellular signal transduction pathways induce the modification of gene expression during the course of cell responses to environmental changes. They modulate the activity of various transcription factors through post-translational modifications, among which phosphorylations play a major role. These modifications lead to appropriate changes in gene transcription. The transcriptional coactivator CBP (CREB binding protein) and the highly homologous E1A-associated p300 protein appear to play a major role in the activity of several transcription factors that are direct targets of these signal transduction pathways; examples include CREB, which is involved in the response to cAMP, or SRE-binding factors, involved in the response to serum (1–5).

CBP activates transcription by various mechanisms: it contains two transcriptional activation domains, one at each end of the molecule (2, 6), and is able to contact some of the basal transcription factors, the TATA Binding Protein (7), TFIIB (2) and RNA pol II itself (8), suggesting that it can function as a bridging factor. In addition, CBP has been shown to interact with two histone acetyl transferases (HATs), P/CAF (9) and SRC-1 (10). Finally, CBP displays an intrinsic HAT activity (11, 12).

HATs are able to acetylate the N-terminal tails of core nucleosomal histones (13, 14). This modification seems to weaken the interaction between the histone tails and the DNA, thereby creating an "open" chromatin structure, which would be more accessible to transcription factors. Accordingly, HATs are generally found within coactivator complexes (9–12, 15), whereas the "reverse" enzymes, the histone deacetylases (HDs) are associated with transcriptional repressors (16–19).

The mechanism through which the activating signal is transduced to CBP has been determined in some systems. For example, during the cyclic AMP response, the activation of protein kinase A (PKA) results in phosphorylation of the transcription factor CREB (cAMP Responsive Element Binding protein). Phosphorylated CREB, but not unphosphorylated CREB, will recruit CBP, which in turn mediates the transcriptional activation of CREB-dependent genes (1, 2). The situation is similar for nuclear hormone receptors, to which CBP is recruited upon ligand binding (20).

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Abbreviations used: CBP, CREB binding protein; HAT, histone acetyl transferase; HD, histone deacetylases; PKA, protein kinase; SRE, serum responsive element; SRF, serum response factor; TCF, ternary complex factor.



0006-291X/99 $30.00

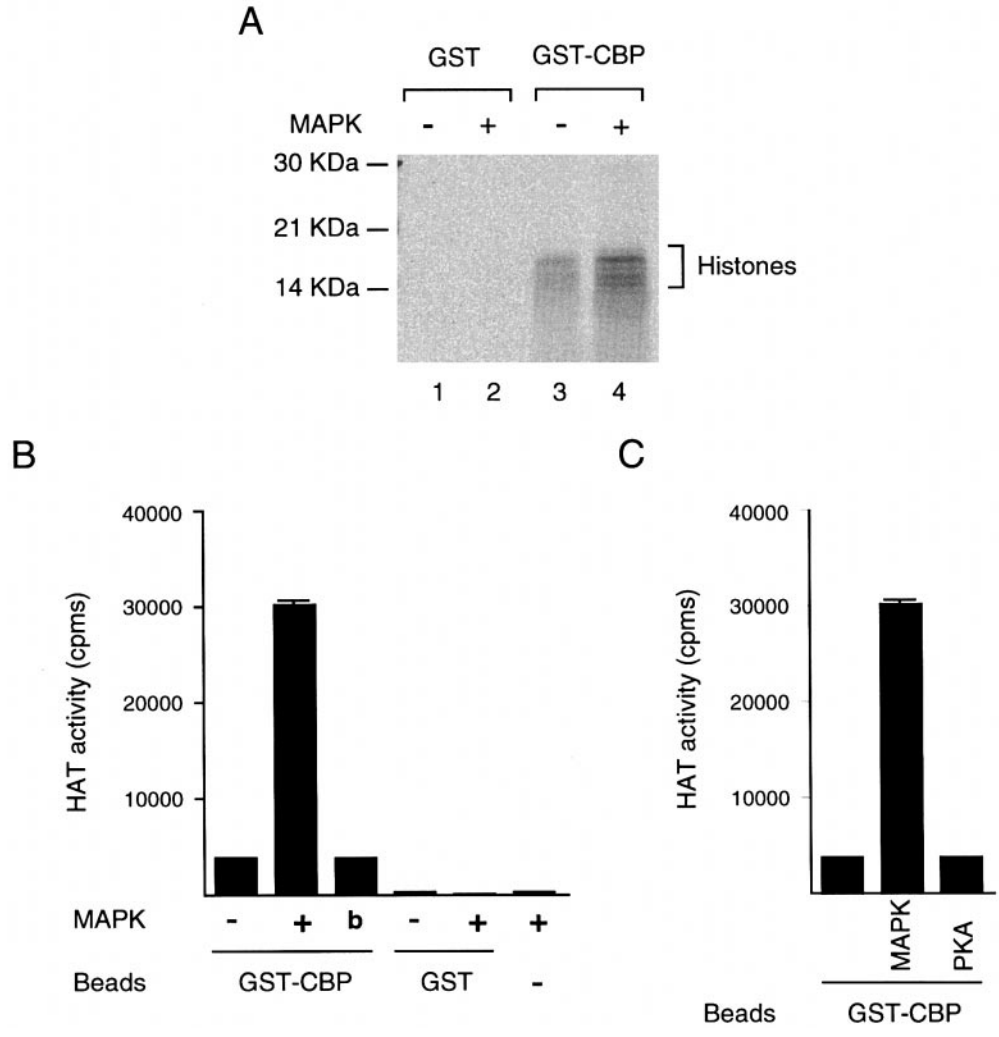


**FIG. 1.** p44 MAP kinase/ERK1 treatment of CBP stimulates its HAT activity. (A) GST-CBP or GST beads pretreated with p44 MAPK/ERK1 in the presence of ATP were used to acetylate purified histones (Sigma) in presence of $^{14}$C-Acetyl-Coenzyme A. Histones were analyzed by SDS-PAGE and autoradiography. (B) GST-CBP beads were either untreated, treated with native p44 MAP kinase/ERK1 or mock-treated with boiled p44 MAP kinase/ERK1 (b) in presence of ATP, prior to being assayed for HAT activity using the H4 peptide assay; the figure shows the results of a typical experiment. (C) p44 MAP kinase/ERK1, but not PKA, stimulates CBP HAT activity. GST-CBP beads were treated with p44 MAP kinase/ERK1 or PKA as indicated, and assayed for HAT activity.

CBP is also involved in transcriptional activation by serum responsive elements (SREs) (4, 5). These elements control immediate early genes such as *c-fos*. They bind two proteins: SRF (Serum Response Factor), which binds as a homodimer, and TCF (Ternary Complex Factor), a protein of Ets family (21). The signal delivered to the cell membrane by growth factors is transduced to the SRE, at least in part, through a pathway involving the mitogen activated protein kinases (MAP kinases) ERK-1 or ERK-2 (22, 23). MAP kinases phosphorylate the TCF, thereby activating transcription through an unknown mechanism (23). The CBP coactivator is involved in SRE activity through physical interactions with the SRF/TCF complex (4, 5, 24). However, these interactions appear to be independent of both TCF phosphorylation and the transcriptional activity of the promoter (4). The modification induced by MAP kinases which results in immediate early gene activation by CBP through SREs is unknown.

CBP is phosphorylated *in vitro* by MAP kinases/ERKs (24), suggesting that it could be an important target of this signal transduction pathway. We show here that this phosphorylation stimulates CBP HAT activity, and thus could be involved in the activation of immediate early gene expression.

## MATERIALS AND METHODS

*Vectors and GST fusion proteins.* cDNAs of interest were cloned into the relevant pGEX-2T vector (Pharmacia). Details of constructions are available upon request.

*Cell culture and immunoprecipitations.* NIH3T3 cells were maintained in DMEM supplemented with 10% fetal calf serum (Sigma). Immunoprecipitations were performed, using standard procedures, with the A-22 anti-CBP antibody (Santa Cruz) or with anti-myogenin antibody as an irrelevant control. Beads were washed twice with RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, and a cocktail of protease inhibitors (Boehringer)), and three times with HAT buffer (50 mM Tris pH 7.5, 1 mM EDTA, 10 mM Na-Butyrate and protease inhibitors) prior to HAT assay.

*HAT assay.* HAT assays were performed as previously described (25). Briefly, a synthetic peptide (Chiron), corresponding to the first 24 amino acids of histone H4 (sequence SGRGKGGKGLGKGGAKRHRKVLR) coupled through a GSGS linker sequence to a biotin molecule, was used as a substrate. Beads covered with either GST-CBP or immunoprecipitated cellular CBP were washed three times with HAT buffer and mixed with the H4 biotinylated peptide (30 mM final) in 30 $\mu$l of HAT buffer supplemented with 100 nCi of $^{14}$C-acetyl-CoA (2.3 GBq/mmol, ICN). Samples were incubated at 30°C for 45 min. After centrifugation for 5 min at 14000 rpm, supernatants were incubated in HAT buffer (500 $\mu$l) with 10 $\mu$l of pre-washed streptavidin-agarose beads (Sigma) for 20 min at 4°C on a rotating wheel. Bound radioactivity was counted using a liquid scintillation counter (LKB).

Acetylation of purified histones was performed as described in (12).

*In vitro phosphorylation and phosphatase treatment.* GST fusion proteins were purified as described previously (29).

For *in vitro* phosphorylation, the recombinant proteins were incubated with 20 ng of MAPK (p44-ERK-1) (Euromedex), 100 $\mu$M ATP (and $\gamma$ $^{32}$P-ATP to a final specific activity of 100 mCi/mmol, where indicated), for 45 min at 30°C in 30 $\mu$l of 25 mM Tris, pH 7.5, 0.1 mM $NaVO_4$, 0.1 mM EGTA, 10 mM Mg acetate, 0.04 mM DTT, 0.1 mM $ZnSO_4$, supplemented with protease inhibitors.

Phosphatase treatment was performed using 400 U of the Lambda Protein Phosphatase (Biolabs), 30 min at 30°C.

## RESULTS AND DISCUSSION

### *p44 MAP Kinase/ERK1 Treatment Stimulates CBP HAT Activity*

MAP kinases are able to phosphorylate CBP *in vitro* (24). We tested the effect of CBP phosphorylation on its enzymatic activity using radiolabeled Acetyl-CoA and nucleosomal histones as substrates (Fig. 1A). Pretreatment of GST-CBP with purified p44 MAP kinase/ERK1 and ATP resulted in a higher level of histone acetylation than that observed with control untreated CBP (compare lanes 3 and 4). HAT activity was also assayed using a more quantitative assay, in which the transfer of radiolabeled acetyl groups to a synthetic peptide substrate can be directly detected (Fig. 1B) (25). p44 MAP kinase/ERK1 treatment stimulated CBP HAT activity more than 7 fold. The GST negative control similarly treated by purified p44 MAP kinase/ERK1, or purified p44 MAP kinase/ERK1 alone, did not show any detectable HAT activity (Fig. 1B). Furthermore, this stimulation was dependent upon an active p44 MAP kinase/ERK1, as shown by the lack of stimulation using inactivated enzyme (Fig. 1B).

MAP kinases belong to the superfamily of the proline-dependent serine/threonine kinases. Among members of this family, we found that cyclin E/cdk2 could also stimulate CBP HAT activity *in vitro* (26). This stimulating effect is restricted to a limited number of kinases: treatment of CBP with PKA, a kinase involved in the cAMP pathway and which is able to phosphorylate CBP *in vitro* (4) and data not shown), did not result in the stimulation of CBP HAT activity (Fig. 1C). Thus kinases involved in distinct signal transduction pathways, all involving CBP, have distinct effects on CBP HAT activity.

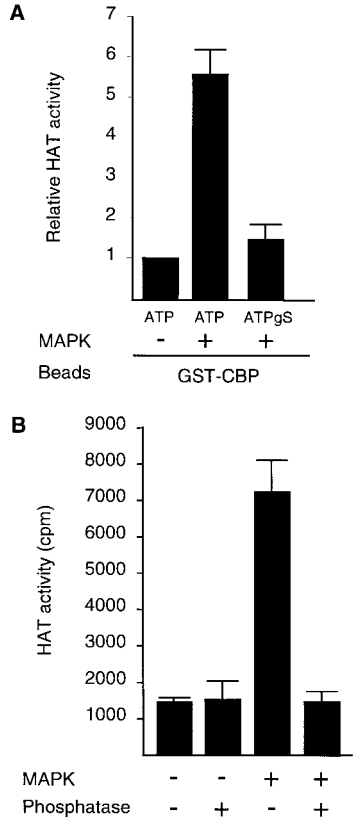


**FIG. 2.** p44 MAP kinase/ERK1 stimulation of CBP HAT activity is due to phosphorylation of CBP. (A) GST-CBP beads were treated with p44 MAP kinase/ERK1 in the presence of ATP or [$\gamma$-S]ATP as indicated, and samples were then tested for HAT activity. (B) GST-CBP beads were treated first with p44 MAP kinase/ERK1 and then with Lambda phosphatase, as indicated, before the HAT assay.

### *CBP Phosphorylation by p44 MAP Kinase/ERK1 Is Required for the Stimulation*

To test whether CBP HAT stimulation requires CBP phosphorylation, we used a nonhydrolyzable form of ATP, [$\gamma$-S]ATP, in the phosphorylation assay. Under these conditions, no stimulation of CBP HAT activity could be detected (Fig. 2A), indicating that a phosphorylation event is indeed required for the stimulation.

Furthermore, if CBP phosphorylated by p44 MAP kinase/ERK1 was treated with a phosphatase prior to the HAT assay, its HAT activity was reduced to non-induced levels (Fig. 2B), in parallel with the dis-

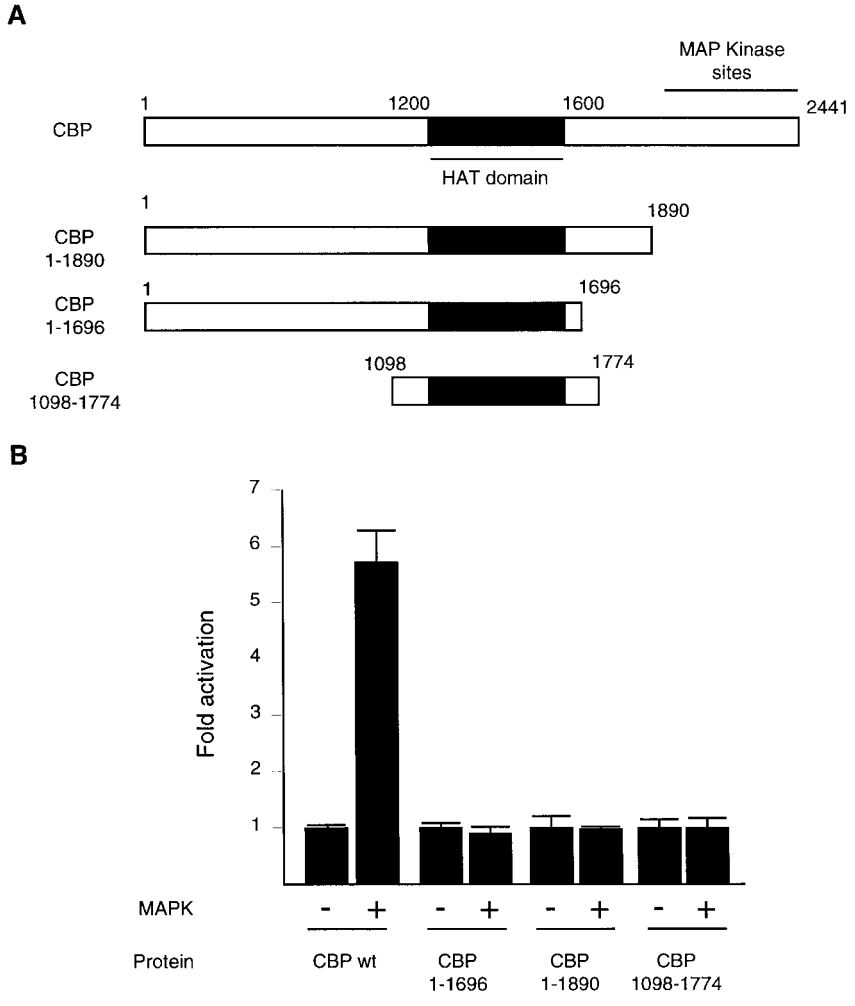


**FIG. 3.** CBP HAT activation by p44 MAP kinase/ERK1 requires the C-terminal region of CBP. (A) Schematic representation of the CBP molecule indicating the positions of the HAT domain, the previously described MAPK phosphorylation sites, and amino acids corresponding to the endpoints of the various deletion mutants. (B) Full length (wt) or deletion mutants of CBP were treated with p44 MAP kinase/ERK1 (or not) and assayed for HAT activity. Fold activation is shown, with reference to the non-activated control.

appearance of the $^{32}P$ labeling (data not shown). Taken together, these data indicate that *in vitro* phosphorylation of CBP by p44 MAP kinase/ERK1 results in the stimulation of CBP HAT activity. This stimulation could be reversed by phosphatase treatment, suggesting that a balance between the activating MAP kinase and an inactivating phosphatase could thus lead to a precise regulation of CBP HAT activity in live cells.

### *The Phosphorylation Site Is Located in the C-Terminus of the Protein*

CBP has been shown to be phosphorylated *in vitro* by MAP kinases at the far C-terminus of the protein (24), outside of the HAT domain. Accordingly, mutants (Fig. 3A) which have been deleted for the C-terminal region of the molecule could not be stimulated by p44 MAP kinase/ERK1 treatment. This result indicates that the phosphorylation sites which are required for the stimulation are located at the C-terminus of the protein. Since this region does not include the HAT domain, it seems likely that CBP phosphorylation by p44 MAP kinase/ERK1 induces an intramolecular conformational change that allows the HAT domain to be more active. Note that the same region of CBP was involved in CBP HAT activation upon phosphorylation by cycE-cdk2 (26).

Taken together, our results show that phosphorylation of CBP *in vitro* by p44 MAP kinase/ERK1 results in the stimulation of CBP HAT activity. However, endogenous CBP HAT activity did not show any change at early time points: 10, 20, 30 and 60 minutes after cell stimulation by serum, although *c-fos* SRE activity was induced normally (data not shown). Furthermore, no change in the phosphorylation of endogenous CBP could be detected by metabolic labeling with $^{32}P$ inorganic phosphate upon MAP kinase activation (data not

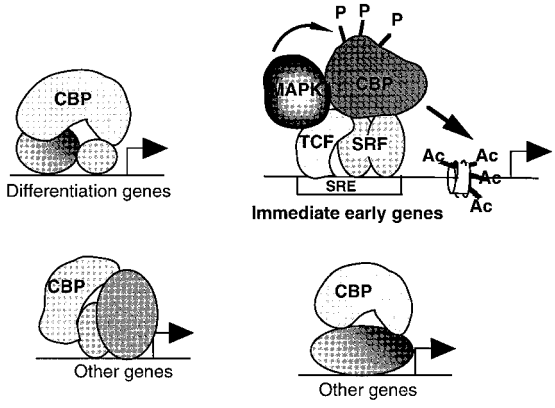


**FIG. 4.** Hypothetical model for SRE activation. An activated MAP kinase is recruited to the SRE by members of the TCF family, where it phosphorylates locally the CBP protein. This phosphorylation results in the stimulation of CBP HAT activity only for those molecules located in the vicinity of the SRE. It finally results in immediate early gene expression.

shown). In contrast, phosphorylation of CBP and stimulation of its HAT activity could both be detected approximately 9 hours after serum stimulation, concomitantly with cycE-cdk2 activation (26). These data indicate that the majority of CBP molecules are not targets for the MAP kinase-dependent signal transduction pathway, in contrast to what is observed for cyc E-cdk 2. Recently, it has been shown that, during stimulation of PC12 cells by NGF, CBP transcriptional activity increased with a kinetic consistent with MAP kinase activation (27). However, despite extensive searching, we could not detect any concomitant stimulation of CBP HAT activity, suggesting that other transcriptional activities of CBP could be targeted by intracellular signal transduction pathways. Indeed, we have previously shown that HAT activity is not absolutely required for SRE co-activation by CBP (5).

However, it is possible that some CBP molecules, which are recruited to the SRE element together with MAP kinases (24), are locally phosphorylated by these enzymes (Fig. 4).

Although the HAT activity does not seem to be absolutely required for SRE coactivation by CBP (5), it is reasonable to hypothesize that CBP uses both HAT-dependent and HAT-independent mechanisms to coactivate immediate early genes. Indeed, serum stimulation of the c-fos promoter requires a signal inducing histone acetylation (28). It is possible that some CBP molecules, recruited to SRE elements together with TCF-bound MAP kinases, are locally phosphorylated by these enzymes (Fig. 4), leading to CBP HAT activity stimulation.

## ACKNOWLEDGMENTS

We thank Dr. J. Pouysségur for the gift of materials. This work was supported by grants from the Comité des Yvelines and Comité du Val de Marne de la Ligue Contre le Cancer, from the Ligue Nationale Contre le Cancer, from the Association de Recherche sur le Cancer, and from the Groupement d'Entreprises Françaises pour la lutte contre le Cancer. S.A. was supported by a fellowship from the Ligue Nationale Contre le Cancer. S.R. and L.C.U. were recipients of travel awards from the Colombian Government (Colciencias).

## REFERENCES

1. Chrivia, J. C., Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R., and Goodman, R. H. (1993) *Nature* **365**(6449), 855–859.
2. 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**(6486), 223–226.
3. Arias, J., Alberts, A. S., Brindle, P., Claret, F. X., Smeal, T., Karin, M., Feramisco, J., and Montminy, M. (1994) *Nature* **370**(6486), 226–9.
4. Janknecht, R., and Nordheim, A. (1996) *Oncogene* **12**(9), 1961–1969.
5. Ramirez, S., Ait Si Ali, S., Robin, P., Trouche, D., and Harel-Bellan, A. (1997) *J. Biol. Chem.* **272**(49), 31016–31021.
6. Swope, D. L., Mueller, C. L., and Chrivia, J. C. (1996) *J. Biol. Chem.* **271**(45), 28138–28145.
7. Dallas, P. B., Yaciuk, P., and Moran, E. (1997) *J. Virol.* **71**(2), 1726–1731.
8. Kee, B. L., Arias, J., and Montminy, M. R. (1996) *J. Biol. Chem.* **271**(5), 2373–2375.
9. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) *Nature* **382,** 319–324.
10. Yao, T. P., Ku, G., Zhou, N., Scully, R., and Livingston, D. M. (1996) *Proc. Natl. Acad. Sci. USA* **93**(20), 10626–10631.
11. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87**(5), 953–959.
12. Bannister, A. J., and Kouzarides, T. (1996) *Nature* **384**(6610), 641–643.
13. Wade, P. A., and Wolffe, A. P. (1997) *Curr. Biol.* **7**(2), R82–R84.
14. Wade, P. A., Pruss, D., and Wolffe, A. P. (1997) *Trends Biochem. Sci,* **22**(4), 128–132.
15. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) *Cell* **84**(6), 843–851.
16. Brehm, A., Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J., and Kouzarides, T. (1998) *Nature* **391**(6667), 597–601.
17. Luo, R. X., Postigo, A. A., and Dean, D. C. (1998) *Cell* **92**(4), 463–473.
18. Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorain, S., Le Villain, J. P., Troalen, F., Trouche, D., and Harel-Bellan, A. (1998) *Nature* **391,** 601–605.
19. Ferreira, R., Magnaghi-Jaulin, L., Robin, P., Harel-Bellan, A., and Trouche, D. (1998) *Proc. Natl. Acad. Sci. USA* **95**(18), 10493–10498.
20. Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K., and Rosenfeld, M. G. (1998) *Science* **279**(5351), 703–707.
21. Treisman, R. (1995) *EMBO J.* **14**(20), 4905–4913.
22. Janknecht, R., Ernst, W. H., Pingoud, V., and Nordheim, A. (1993) *EMBO J.* **12**(13), 5097–5104.
23. Marais, R., Wynne, J., and Treisman, R. (1993) *Cell* **73,** 381–393.
24. Janknecht, R., and Nordheim, A. (1996) *Biochem. Biophys. Res. Commun.* **228**(3), 831–837.
25. Ait-Si-Ali, S., Ramirez, S., Robin, P., Trouche, D., and Harel-Bellan, A. (1998) *Nucleic Acids Res.* **26,** 3869–3870.
26. Ait-Si-Ali, S., Ramirez, S., Barre, F. X., Dkhissi, F., Magnaghi-

Jaulin, L., Girault, J. A., Robin, P., Knibiehler, M., Pritchard, L. L., Ducommun, B., Trouche, D., and Harel-Bellan, A. (1998) *Nature* **396**(6707), 184–186.

27. Liu, Y. Z., Chrivia, J. C., and Latchman, D. S. (1998) *J. Biol. Chem.* **273**(49), 32400–32407.
28. Alberts, A. S., Geneste, O., and Treisman, R. (1998) *Cell* **92**(4), 475–487.
29. Groisman, R., Masutani, H., Leibovitch, M. P., Robin, P., Soudant, I., Trouche, D., and Harel-Bellan, A. (1996) *J. Biol. Chem.* **271**(9), 5258–5264.