Transcriptional Activation of the Human Manganese Superoxide Dismutase Gene Mediated by Tetradecanoylphorbol Acetate*

(Hyong-Pyo Kim‡§, Jung-Hye Roe§, P. Boon Chock‡, and Moon B. Yim‡¶)

From the §Laboratory of Biochemistry, NHLBI, National Institutes of Health, Bethesda, Maryland 20892-0342 and the ¶Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University, Seoul 151-742, Korea

Transcriptional activation of human manganese superoxide dismutase (MnSOD) mRNA induced by a phor- bol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), was examined to identify the responsive transcriptional regulator. The effect of various deletions and mutations within the 5′-flanking region of the human MnSOD gene promoter was evaluated using the luciferase reporter system in A549 human lung carcinoma cells. Deletion of a region between −1292 and −1202 nucleotides upstream of the transcription start site abolished TPA-responsive induction, whereas deletion of the putative binding sequence for NF-κB or AP-1 did not. The region between −1292 and −1202 contains a cAMP-responsive element-like sequence, TGACGTCT, which we identified as the manganese superoxide dismutase TPA-responsive element, MSTRE. Site-specific mutation of the MSTRE abolished the TPA-responsive induction, validating the critical role of this sequence. We detected specific MSTRE activity from nuclear extracts and demonstrated by antibody supershift assay that this activity is closely related to CREB-1/ATF-1. TPA treatment rapidly induced phosphorylation of the CREB-1/ATF-1-like factor via the protein kinase C pathway. These results led us to conclude that the human MnSOD gene having the promoter construct used in this study is induced by TPA via activation of a CREB-1/ATF-1-like factor and not via either NF-κB or AP-1. In addition, we found that this induction was blocked by inhibitors of flavoproteins and NADPH oxidases, indicating involvement of enhanced generation of superoxide radical anion as an upstream signal.

Superoxide dismutase catalyzes the disproportionation reaction of superoxide radical anion (O2−) to hydrogen peroxide (1).

In eukaryotes, manganese superoxide dismutase (MnSOD) is located in the mitochondrial matrix, which is encoded by a nuclear gene. Mitochondria are particularly prone to oxidative DNA damage because they metabolize over 95% of the oxygen (2), but they lack histones and have a poor ability for DNA repair (3). Thus, MnSOD is regarded as the primary defensive enzyme against oxidative stress within mitochondria. Loss of MnSOD is implicated in causing various human tumors (4–6), whereas its overexpression suppresses tumorigenicity, presumably by decreasing the concentration of O2−, which promotes cancer (7–10).

A number of studies demonstrated the induction of MnSOD in various cell lines and tissues following oxidative stress, such as treatments with TNF-α (11, 12), interleukin-1 (12), lipopolysaccharide (13), interferon-γ (14), 12-O-tetradecanoylphorbol-13-acetate (TPA) (15–17), and x-irradiation or hyperoxia (18, 19). Most of these treatments could generate reactive oxygen species in cells that, in turn, could activate transcription factors, mainly NF-κB and AP-1 (20, 21), to allow their nuclear translocation and allow them to bind to genes involved in the stress responses. In accordance, the involvement of NF-κB (22–24) and AP-1 (25) in activating MnSOD genes in response to TNF-α, TPA, H2O2, or thiol-reducing agents has been suggested in human and rat cells. These studies correlated the conditions that changed the DNA binding activity of either NF-κB or AP-1 with the induction of MnSOD, and thus concluded that the activation of the MnSOD gene is mediated by either of these factors. However, the responsive cis-acting elements, as well as the changes in or modification of the binding factor, have not been directly demonstrated. Recently, Jones et al. (26) have shown that the mouse MnSOD gene is regulated by TNF-α and interleukin-1 through a complex intronic enhancer located in intron 2 involving binding of C/EBP and NF-κB. They also found that the 5′-flanking promoter region of the MnSOD gene is not involved in the TNF-mediated MnSOD mRNA induction. However, relatively little information is available regarding the molecular mechanism for modulating the expression of the MnSOD gene, SOD2, by TPA.

In this study, we present evidence that the TPA-responsive element in the induction of MnSOD gene expression is a cAMP-responsive element (CREB)-like element bound to a CREB-1/ATF-1-like factor, which is located in the 5′-flanking DNA sequence. The activation of the CREB-1/ATF-1-like factor is caused by rapid phosphorylation via the protein kinase C (PKC) pathway. In addition, NADPH oxidase or similar flavoproteins are also involved in the induction of the MnSOD gene, which may indicate that superoxide radical anion is an upstream signal for this induction.

* This work was supported in part by the International Collaborative Research Program of the National Heart, Lung, and Blood Institute, National Institutes of Health (United States) and the Korea Science and Engineering Foundation (Korea). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF059197.

§ To whom all correspondence should be addressed: Laboratory of Biochemistry, NHLBI, National Institutes of Health, Bldg. 3, Rm. 202, 3 Center Dr., MSC-0342, Bethesda, MD 20892-0342. Tel.: 301-496-9494; Fax: 301-496-0599; E-mail: yimm@gwgate.nhlbi.nih.gov.

¶ The abbreviations used are: MnSOD, manganese superoxide dismutase; TPA, 12-O-tetradecanoylphorbol-13-acetate; CRE, cAMP-responsive element; TBE, TPA-responsive element; MSTRE, MnSOD TPA-responsive element; CREB, CAMP-responsive element-binding protein; ATF, activating transcription factor; C/EBP, CCAAT/enhancer binding protein; BIM, bisindolylmaleimide; DPI, diphenylene iodonium; AEBSF, 4-(2-aminomethyl)-benzenesulfonyl fluoride; PKC, protein kinase C; PKA, protein kinase A.
Regulation of Human MnSOD Gene by TPA

EXPERIMENTAL PROCEDURES

Cell Lines—The human lung adenocarcinoma cell line A549 was purchased from American Type Culture Collection (Rockville, MD). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Life Technologies, Inc.) at 37 °C under 5% CO₂. Northern Analysis—Total cellular RNA was prepared from A549 cells using Trizol reagent (Life Technologies, Inc.) (30). The total RNA (10–20 μg) were run on 1% agarose gel containing 2.2% formaldehyde. Polyadenylated RNA was transferred to nylon membranes (Schleicher & Schuell). After hybridization with ³²P-labeled probe at 68 °C in the presence of Quickhyb hybridization solution (Stratagene, La Jolla, CA), the membrane was washed twice at room temperature in 2× saline sodium citrate (SSC) and 0.1% SDS for 10 min each, and then washed at 60 °C in 0.1× SSC and 0.1% SDS for 15 min. The Kodak X-Omat AR films were exposed for 2–12 h with an intensifying screen at −70 °C. The intensity of a band was quantitated by the PhosphorImager Storm 860 (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Nucleotide Sequence Analysis of the Promoter Region—The newly determined 5'-flanking region sequence of the human MnSOD gene was deposited in GenBank/EMBL data base under accession number AF059197. This promoter region of the human MnSOD gene up to −3340 residues was searched against the transcription factor data base for putative transcriptional regulatory elements using SIGSCAN programs (version 4.05) (31). The analysis did not reveal any TATA or CAAT boxes consistent with previous observations with bovine, rat, and human MnSOD genes (32–36). It contains a GC-rich (75%) region within 500 base pairs of the transcription start site, which is preceded by nine potential binding sites for Sp1 and six sites for AP-2. Several other potential regulatory elements were also detected. Two copies of the AP-1 binding sequence were found at positions −2883 and −820, two copies of the NF-κB binding sequence were found at positions −3215 and −1551, and one copy of the CREB/ATF family-binding sequence was found at position −1258.

The TPA-induced MnSOD Promoter Activity—In order to identify the cis-acting elements responsible for TPA-induced transcription of the MnSOD gene, a series of 5' deletions of the promoter fragment from −3340 were subcloned to the pGL3-Basic vector containing the coding sequence of the firefly luciferase gene (Fig. 1) as described under “Experimental Procedures.” The resultant pSODLUC plasmids were transfected into A549 cells and the promoter activities were measured by luciferase assay. The results depicted in Fig. 1A show that cells transfected with pSODLUC-3340 induced MnSOD gene expression by 2-fold upon treatment with TPA (20 ng/ml). However, similar treatment of these cells with TNF-α (50 ng/ml) did not show any increase in the luciferase activity. This result indicates that the regulation of the human MnSOD gene by TNF-α involves parts of the gene other than the −3340 base pairs 5’-flanking region. This finding is in agreement with previous results obtained in the mouse MnSOD gene (26), demonstrating that the gene is regulated by TNF-α through intronic enhancer, but not through the 5’-flanking region. Deletion of sequences between −3340 and −1396 (Fig. 1B), which includes two NF-κB and one AP-1 binding sequences, did not significantly affect the basal activity of the MnSOD promoter (Fig. 1B). The promoter activity of the MnSOD gene is significantly influenced by TPA induction.

Deletion of sequences between −3340 to −263 did not significantly affect the basal activity of the MnSOD promoter in A549 cells. Further 5’ deletions to nucleotides −106 and −1 resulted in a 50% and 90% decrease, respectively, in the basal activity of the MnSOD promoter. This result may indicate the presence of a minimal promoter between −262 and −1, the region that contains six SP1 and three AP2 binding sites.
and TNF-α for 12 h was analyzed as described in "Experimental Procedures." The A549 cells were transiently transfected with plasmid containing the luciferase reporter under the pSODLUC-3340 and with the plasmid pRL-TK as a transfection efficiency control. The cells were treated with medium only, TPA (20 ng/ml), or TNF-α (50 ng/ml) for 12 h. The relative light units correspond to the activity of firefly luciferase corrected for transfection efficiency by normalizing with the activity of Renilla luciferase from pRL-TK. The data shown are from one set of representative experiments with three to four independent measurements. B, deletion analysis of the 5′-flanking region of the MnSOD gene. The pSODLUC constructs containing different lengths of the 5′-flanking region of human MnSOD gene were numbered according to the position of the 5′ deletion end point. Existence of putative binding sites for NF-κB, AP-1, and CREB are indicated. Luciferase activity from A549 cells transiently transfected with the indicated reporter plasmids and treated with or without 20 ng/ml TPA for 12 h was analyzed as described in A and under “Experimental Procedures.”

Identification of a TPA-responsive Element in the MnSOD Promoter—To test whether this CRE-like sequence is responsible for the induction by TPA treatment, two nucleotides of this motif were mutated in the pSODLUC-1517 to create the plasmid pSODLUC-1517mt. Expression from this construct was analyzed by luciferase assay (Fig. 2). The mutation resulted in a substantial decrease in TPA-promoted induction with little effect on basal expression, indicating that this CRE-like element is a TPA-responsive element in MnSOD gene (MSTRE).

The binding activity of the MSTRE in a nuclear extract of A549 cells was examined by electrophoretic mobility shift assay using the oligonucleotide MSTRE. Formation of several prominent complexes was observed (Fig. 3A, lane 1), among which complexes I, II, and III were suggested to arise from specific binding to the MSTRE motif, since they were not competed off by the mutated MSTRE probe (Fig. 3A, lane 3). Competition with the CREB-binding consensus sequence (TGACGTCA) abolished these complexes (Fig. 3A, lane 4). The AP-1-binding consensus sequence (TGAGTCA) competed partially (lane 5), whereas the Oct-1-binding sequence (TGCAAT) did not (lane 6). These results suggest that the specific binding to MSTRE is due to a CREB-like factor, which can also recognize the AP-1 consensus sequence with lower affinity.

In order to identify the specific transcription factor that binds to MSTRE, we performed antibody supershift analysis. As shown in Fig. 3B, the antibody against both CREB-1 and ATF-1 specifically abolished complexes I and II, decreased the intensity of complex III, and created slower-moving supershifted complexes (lane 2). More specific antibody that recognized CREB-1 but not ATF-1 completely abolished complex I and II (lane 3), while the antibody against ATF-1 abolished complex II and decreased the intensity of complex III (lane 4). A longer exposure of the gel also showed the supershifted bands in lanes 3 and 4, although they are much weaker than that in lane 2 (data not shown). In contrast, antibodies against ATF-2, c-Jun, and c-Fos, did not affect the binding pattern (lanes 5–7). Therefore, it is most likely that the MSTRE-specific binding factors are the homodimers of CREB-1 and ATF-1 (for complex I and complex III, respectively), heterodimer of CREB-1/ATF-1

(continued on the next page)
The total amount of CREB protein in the same nuclear extracts did not change, as judged by blotting with antibody that recognizes CREB regardless of phosphorylation status. As shown in Fig. 4A (upper panel), little if any phospho-CREB is present in untreated A549 cells (lane 2). However, treatment with TPA (20 ng/ml) increased CREB phosphorylation at Ser\(^{133}\) within 15 min (lane 3), which slowly disappeared afterward. The antibody against CREB phosphorylated at Ser\(^{133}\) also reacted with a 58-kDa protein (marked as p-ATF-1 in Fig. 4B) that co-migrates with ATF-1, a related transcription factor that shares amino acid sequence identity surrounding Ser\(^{133}\) and cross-reacts with the antibody used. The total amount of CREB protein in the same nuclear extracts did not change, as judged by blotting with antibody that recognizes CREB, regardless of their phosphorylation status at Ser\(^{133}\) (Fig. 4B, lower panel).

Two kinds of specific protein kinase inhibitors were used to examine whether the phosphorylation of the CREB-1/ATF-1-like factor is directly related to the TPA-mediated MnSOD mRNA induction. The H-89 (K\(_i\) for PKA = 48 nM; K\(_i\) for PKC = 32 \(\mu\)M) and bisindolylmaleimide (BIM) (K\(_i\) for PKC = 10 nM; K\(_i\) for PKA = 2 \(\mu\)M) are highly selective and cell-permeable inhibitors of protein kinase A and C, respectively (39–41). Pre-incubation of A549 cells with BIM (1 \(\mu\)M) inhibited the TPA-mediated MnSOD mRNA induction by 80% (Fig. 5A). In contrast, H-89 (up to 4 \(\mu\)M) had little effect on the induction (Fig. 5B). These results suggest that TPA treatment induces MnSOD mRNA through the PKC rather than the PKA pathway.

**DISCUSSION**

The results obtained in this study allowed us to identify a responsive element for TPA-induced MnSOD mRNA, the MSTRE, at the 5’-flanking region of MnSOD gene (Figs. 1 and 2). The TPA-responsive induction of MnSOD mRNA requires binding of the MSTRE to the CREB-1/ATF-1-like transcription factor (Fig. 3), which is activated by PKC-catalyzed phosphorylation (Figs. 4 and 5). This induction was blocked by inhibi-
Regulation of Human MnSOD Gene by TPA

Regulation of Human MnSOD Gene by TPA

Fig. 6. Effect of NADPH oxidase inhibitors on MnSOD mRNA induction by TPA. A, effect of DPI. A549 cells were incubated in the absence (lanes 1 and 4) or presence of DPI (lanes 2, 3, 5, and 6) for 1 h. Total RNA was prepared from cells after incubation with medium only (lanes 1–3) or 20 ng/ml TPA (lanes 4–6) for 4 h. Northern analysis of the RNA was carried out using 32P-labeled MnSOD or glyceraldehyde-3-phosphate dehydrogenase probes. B, effect of AEBSF. A549 cells were incubated in the absence (lanes 1 and 4) or presence of AEBSF (lanes 2, 3, 5, and 6) for 1 h. Other procedures are identical to those in A.

The ubiquitously expressed CRED protein is a well characterized transcription factor regulated by phosphorylation, in most cases, via the PKA and PKC pathways (52–54). Phosphorylation in response to cAMP stimulation has no effect on the ability of CRED to bind CRE, but it enhances transcriptional efficiency 20-fold in vitro (51, 55). In addition, it has been shown that the CREB binding site of the p53 gene is bound to CREB in the absence of cAMP and PKA, but it is not bound in the presence of PKA (52, 54). These results suggest that CREB is a good candidate for the role of the enzyme against O2.

REFERENCES
1. Fridovich, I. (1995) Annu. Rev. Biochem. 64, 97–112
2. Guidot, D. M., McCord, J. M., Wright, R. M., and Repine, J. E. (1993) J. Biol. Chem. 268, 26708–26713
3. Richter, C. J., Park, W. J., and Ames, B. N. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6465–6467
4. Church, S. L., Farmer, D. R., and Nelson, D. M. (1992) Dev. Biol. 149, 177–184
5. Lee, J. H., Kavanagh, J. J., Wildrick, D. M., Wharton, J. T., and Blick, M. D. (1999) Cancer Res. 59, 151–172
6. Trent, J. M., Stanbridge, E. J., McBride, H. L., Meese, E. M., and Nagle, R. B. (1990) Science 247, 587–588
7. St. Clair, D. K., Turner, J. W., Ridnour, L. A., Oberley, L. W., Swanson, P. E., Meltzer, P. S., and Trent, J. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3113–3117
8. Oberley, L. W., and Buresh, G. R. (1979) Cancer Res. 39, 1141–1149
9. Cerutti, P. A. (1988) Science 227, 379–381
10. Wong, G. H., Elwell, J. H., Oberley, L. W., and Goeddel, D. V. (1989) Cell 58, 923–931
11. Wong, G. H., and Goeddel, D. V. (1988) Science 242, 941–944

Share many structural properties with CREB (56). Our results demonstrated that ATF-1 as well as a CREB-1-like factor bound to MSTRF become phosphorylated upon TPA treatment and suggest that heterodimeric as well as homodimeric complexes of ATF-1 and CREB-1-like factors are involved in inducing the MnSOD gene. In addition, our results demonstrate that the specific inhibitor for PKC, BIM, rather than the PKA inhibitor, blocked the TPA-mediated induction of MnSOD mRNA (Fig. 5). The previous report (53), as well as our preliminary results, showed that BIM also inhibits TPA-responsive phosphorylation of CREB. These findings together suggest that the TPA-responsive MnSOD mRNA induction is mediated mainly by the phosphorylation of CREB-1/ATF-1-like factors via the PKC pathway.

Superoxide radical anions are generated by NADPH oxidase in phagocytes as a host defense. In a resting state, NADPH oxidase assembly in phagocytes consists of several proteins: p47phox, p67phox, and Ras-related GTP binding protein, in cytosol, and gp91phox and p20phox, which are two components of a flavoprotein cytochrome b558, located in plasma membrane. During activation, p47phox is phosphorylated and translocated together with other cytosolic components to the plasma membrane, where they interact with a flavoprotein cytochrome b558 to form an active complex that catalyzes the formation of O2− from oxygen (42, 57). In other type of cells, such as fibroblasts, the NADPH oxidase-like enzyme is also implicated in the generation of superoxide radical anions in a similar manner, which is probably involved in signaling processes (42, 43). Our results showed that both DPI, an inhibitor of flavoproteins, and AEBSF, an inhibitor for the interaction of p47phox and/or p67phox with cytochrome b558, block the induction of MnSOD mRNA. It suggests, therefore, that an upstream signal for TPA-mediated induction of MnSOD mRNA may be O2− generated by NADPH oxidase or similar oxidases activated by TPA.

MSTRE may not be the only TPA-responsive element mediating activation of human MnSOD gene in vivo. The level of MnSOD mRNA increases about 6-fold following treatment of A549 cells with TPA for 4 h. The TPA-induced luciferase expression from pSODLUC exhibits, however, only about 2–3-fold stimulation over the untreated cells. The difference in the extent of induction may imply that other TPA-responsive elements exist in the region outside of the promoter sequences examined in this work. Nevertheless, a mechanism for the TPA-mediated MnSOD induction is likely to include the activation of a NADPH oxidase-like enzyme to generate O2− as an upstream signal and phosphorylation of a CREB-1/ATF-1-like factor needed for MSTRE-mediated transcriptional activation of the MnSOD gene. This or a similar mechanism involving O2− as an upstream signal for the induction of MnSOD may be congruent with the role of the enzyme against O2− toxicity in vitro.
Regulation of Human MnSOD Gene by TPA

13. Visner, G. A., Dougall, W. C., Wilson, J. M., Burr, I. A., and Nick, H. S. (1990) J. Biol. Chem. 265, 2856–2864
14. Harris, C. A., Derbin, K. S., Hunte-McDonough, B., Krauss, M. R., Chen, K. T., Smith, D. M., and Epstein, L. B. (1991) J. Immunol. 147, 149–154
15. Fujii, J., and Taniguchi, N. (1991) J. Biol. Chem. 266, 23142–23146
16. Whitsett, J. A., Clark, J. C., Wispe, J. R., and Fryhuber, G. S. (1992) Am. J. Physiol. 263, L688–L693
17. Fujii, J., Nakata, T., Miyoshi, E., Ikeda, Y., and Taniguchi, N. (1994) Biochem. J. 301, 31–34
18. Akashi, M., Hachiya, M., Paquette, R. L., Osawa, Y., Shimizu, S., and Suzuki, G. (1995) J. Biol. Chem. 270, 15864–15869
19. Oberley, L. W., St. Clair, D. K., Autor, A. P., and Oberley, T. D. (1987) Arch. Biochem. Biophys. 254, 69–80
20. Pahl, H. L., and Baueuerle, P. A. (1994) BioEssays 16, 497–502
21. Schulze-Osthoff, K., Les, M., and Baueuerle, P. A. (1995) Biochem. Pharmacol. 50, 733–741
22. Warner, B. B., Stuart, L., Gebb, S., and Wispe, J. R. (1996) Am. J. Physiol. 271, L150–L158
23. Das, K. C., Lewis-Molock, Y., and White, C. W. (1995) Am. J. Physiol. 269, L588–L602
24. Das, K. C., Lewis-Molock, Y., and White, C. W. (1995) Mol. Cell. Biochem. 148, 45–57
25. Borrello, S., and Demple, B. (1997) Arch. Biochem. Biophys. 348, 289–294
26. Jones, P. L., Ping, D., and Boss, J. M. (1997) Mol. Cell. Biol. 17, 6970–6981
27. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
28. Yim, H. S., Kang, J. H., Chock, P. B., Stadtman, E. R., and Yim, M. B. (1997) J. Biol. Chem. 272, 8861–8863
29. Stasiewicz, K., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
30. Chomczynski, P. (1993) BioTechniques 15, 532–534
31. Prestridge, D. S. (1996) Comput. Appl. Biosci. 12, 157–160
32. Church, S. L., Grant, J. W., Meese, E. U., and Trent, J. M. (1992) Genomics 14, 823–825
33. Wan, X. S., Devalaraja, M. N., and St. Clair, D. K. (1994) DNA Cell Biol. 13, 1127–1136
34. Zhang, N. (1996) Biochem. Biophys. Res. Commun. 220, 171–180
35. Meyrick, B., and Magnuson, M. A. (1994) Am. J. Respir. Cell. Mol. Biol. 10, 113–121
36. Ho, Y. S., Howard, A. J., and Crapo, J. D. (1991) Am. J. Respir. Cell. Mol. Biol. 4, 278–286
37. Hunter, T., and Karin, M. (1992) Cell 70, 375–387
38. Gonzalez, G. A., and Montminy, M. R. (1989) Cell 59, 675–680
39. Geelen, C. C., Wieprecht, M., Wieder, T., and Reutter, W. (1992) FEBS Lett. 299, 381–384
40. Fidler, D., Song, Q., Hidaka, H., and Lavin, M. (1995) J. Cell. Biochem. 57, 12–21
41. Toulele, D., Pianetti, P., Coste, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boisson, P., Boursier, E., Lorioile, F., Duhamel L., Charon D., and Kirilovsky, J. (1991) J. Biol. Chem. 266, 15771–15781
42. Chankock, S. J., el Benna, J., Smith, R. M., and Babior, B. M. (1994) J. Biol. Chem. 269, 24519–24522
43. Irani, K., Xia, Y., Zweier, J. L., Solfott, S. J., Der, C. J., Fearon, E., R., Sundaresan, M., Finkel, T., and Goldschmidt-Clermont, P. J. (1997) Science 275, 1649–1652
44. Meir, B., Cross, A. R., Hancock, J. T., Kauf, F. J., and Jones, O. T. G. (1991) Biochem. J. 275, 241–245
45. Nausier, J., and Vignais, P. V. (1992) Eur. J. Biochem. 208, 61–71
46. Diatchuk, V., Lotan, O., Koshkin, V., Wilkstroem, P., and Pick, E. (1997) J. Biol. Chem. 272, 13292–13301
47. Karin, M. (1992) FASEB J. 6, 2581–2590
48. Karin, M., and Smeal, T. (1992) Trends Biochem. Sci. 17, 418–422
49. Sassone-Corsi, P., Ransone, L. J., and Verma, I. M. (1990) Oncogene 5, 427–431
50. Maekawa, T., Sakura, H., Kame-Ishii, C., Sudo, T., Yoshinura, T., Fujisawa, J., Yoshida, M., and Ishii, S. (1989) EMBO J. 8, 2023–2028
51. Hoffeller, J. P., Deutsch, P. J., Lin, J., and Habener, J. F. (1989) Mol. Endocrinol. 3, 868–880
52. Montminy, M. (1997) Annu. Rev. Biochem. 66, 807–822
53. Muthusamy, N., and Leiden, J. M. (1998) J. Biol. Chem. 273, 22841–22847
54. Brindle, P., Nakajima, T., and Montminy, M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10521–10525
55. Yamamoto, K. K., Gonzalez, G. A., Biggs, W. H. D., and Montminy, M. R. (1988) Nature 334, 494–498
56. Hsu, T. W., Liu, F., Coukos, W. J., and Green, M. R. (1989) Genes Dev. 3, 2083–2090
57. Aegal, A. W., and Abo, A. (1993) Trends Biochem. Sci. 18, 43–47