Catalytic Subunit of Protein Kinase A Is an Interacting Partner of the Inflammation-Responsive Transcription Factor Serum Amyloid A-Activating Factor-1

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Catalytic Subunit of Protein Kinase A Is an Interacting Partner of the Inflammation-Responsive Transcription Factor Serum Amyloid A-Activating Factor-1

Bimal K. Ray, Jing Chen, and Alpana Ray

Serum amyloid A-activating factor-1 (SAF-1) is a zinc finger transcription factor that is activated by many mediators of inflammation including IL-1, IL-6, and bacterial LPS. However, the mechanism of activation is not fully understood. To identify possible activation partners for SAF-1, we used a yeast two-hybrid system that detected interaction between the catalytic subunit of cyclic AMP-dependent protein kinase (PKA-Cα) and SAF-1. Immunofluorescence and combined immunoprecipitation-Western blot analyses revealed colocalization and interaction between SAF-1 and PKA-Cα. In vivo evidence of SAF-1 and PKA-Cα interaction was further revealed by coimmunoprecipitation of these two proteins in cAMP-activated liver cells. We further show that SAF-1 is phosphorylated in vitro by PKA-Cα and that addition of cAMP markedly induces in vivo phosphorylation of SAF-1 and transcription of SAF-regulated reporter genes. These results showed that SAF1-PKA-Cα interaction is involved in functional activation of SAF-1. The Journal of Immunology, 2001, 167: 2343–2348.

Serum amyloid A (SAA), a family of plasma proteins, is linked with the pathophysiology of several inflammatory diseases, including secondary amyloidosis, rheumatoid arthritis, and atherosclerosis. In response to inflammation, expression of some SAA isoforms is induced by as much as 1000-fold due to transcriptional induction (reviewed in Refs. 1 and 2). Proinflammatory cytokines, IL-1, IL-6, and TNF-α, either alone or in combination and inflammatory mediators like PMA can increase transcription of SAA. Regulation of SAA gene expression has been studied and characterized by different groups, including ours (3–9). Multiple cis-acting elements have been defined to be important for transcriptional induction of SAA genes, which include CCAAT enhancer-binding protein (C/EBP), NF-κB, YY-1, Sp1, and SAA-activating factor (SAF) transcription factor DNA-binding elements. Nonhepatic expression of rabbit SAA2 is primarily regulated by the SAF DNA-binding element given that mutation of this region abolishes 60–80% of the SAA2 promoter activity in many nonhepatic cells (9). Because nonhepatic expression of SAA seems to play a critical role in the pathogenesis of SAA-linked inflammatory diseases, SAF might have a significant role in the disease outcome.

SAF is a family of zinc finger transcription factors containing multiple Cys2-His2-type zinc finger motifs at their C terminus (10). DNA-binding activity of SAF is induced in response to LPS (11) or IL-1 and IL-6 treatment of cells (12). Structural analysis showed that one member of this family, SAF-1, is a homologue of human myc-associated zinc finger protein (MAZ) (13) and murine Pur-1 (14). To date, SAF-1/MAZ/Pur-1 is identified as a regulator of c-myc, insulin, serotonin 1A receptor, CD4, γ-fibrinogen, phe- nylethanolamine N-methyltransferase, and CLC-K1 genes (13–19). These findings suggest that the SAF-1/MAZ/Pur-1 family of proteins have a wide regulatory role in governing many cellular processes.

Protein-protein interaction between transcription factors and other accessory proteins is a unique mechanism through which strict cellular control of gene expression is maintained. We hypothesized that association of other proteins is necessary for SAF-1/MAZ/Pur-1 to regulate a variety of genes in a cell-type or stimulus-specific manner. The yeast two-hybrid system is a powerful tool for identifying protein-protein interactions that occur in vivo in the course of regulating many physiological activities including signal transduction process. By two-hybrid assay, we detected interaction between SAF-1 protein and the catalytic subunit of cAMP-dependent protein kinase (PKA-Cα). The interaction between these two proteins was subsequently demonstrated by both in vitro and in vivo analyses. Further studies showed that activation of endogenous PKA by intercellular elevation of cAMP leads to increased expression of SAF-1-regulated promoters.

Materials and Methods

Yeast two-hybrid screen and strains

A truncated form of SAF-1 (aa 187–477), hereafter designated as SAF-1187–477, was cloned into the pAS2-1 vector (CLONTECH Laboratories, Palo Alto, CA), and the resultant pAS2-1-SAF1187–477 plasmid was used as a bait to screen a mouse brain MATCHMAKER cDNA library (CLONTECH Laboratories) following the manufacturer’s protocol. CG1945 yeast cells were transformed with pAS2-1-SAF1187–477 plasmid DNA. A 200-µg sample of the cDNA library cloned in pACT2 plasmid was then transformed into CG1945 cells containing pAS2-1-SAF1187–477. Transformed cells were plated onto medium lacking tryptophan, leucine and histidine but containing 15 mM 3-amino-1,2,4-triazole. Growth on His+ plates indicated an interaction between SAF1187–477 and the protein coded by an unknown cDNA. Selected colonies were further assayed for β-galactosidase activity. After 3 rounds of selection, 20 colonies were chosen for further analysis by DNA sequencing.

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Cell culture and transfection

BNL CL2 liver cells, a normal embryonic liver cell line derived from a BALB/c mouse, were obtained from American Type Culture Collection (Manassas, VA) and cultured in DMEM containing high glucose (4.5 g/L) supplemented with 7% FCS. These cells retained many of the features of normal liver cells. Transient transfections were conducted by the calcium phosphate precipitation method (20). Reporter plasmids and expression vectors containing SAF1 cDNA were transfected in duplicate, along with 1 μg pSV-β-gal (Promega, Madison, WI). The pSV-β-gal plasmid DNA was used as an internal control for measuring transfection efficiency. The amount of DNA in each transfection assay was kept same by using carrier DNA. Some cells were treated with 8-bromo (8-Br)-cAMP (0.4 mM), myristoylated cell-permeable PKA inhibitor 14-22 amide, (PKI) (5 μM), and H89 (5 μM). Cells were harvested 48 h posttransfection, and cell extracts containing equivalent amounts of β-galactosidase activity were used for chloramphenicol acetyl transferase (CAT) assay as described previously (9–11). β-Galactosidase activity was assayed with the substrate 0-nitrophenyl-β-D-galactopyranoside as described (20). All transfection experiments were performed at least three times.

Plasmid constructs

The CAT reporter plasmids wtSAF-CAT and mtSAF-CAT were constructed by ligating three copies of wild-type or mutant SAF DNA-binding element, −254 to −226 bp, of the SAA promoter (9) into pBLCAT2 plasmid. The sequences of the wild-type and mutant SAF DNA-binding elements are 5′-CCCTCCTCCTCACCACGCCCCATG-3′ and 5′-CCCTACTGTGACTGACAGCTACCATGG-3′. Underlined bases represent altered sequences. The pCMV-SAF1 expression plasmid was prepared by inserting a full length SAF-1 cDNA (10) under the control of CMV promoter in pCDNA3 vector (Invitrogen, San Diego, CA). Bacterially expressed SAF1-1 protein was obtained by using FLAG-SAF1 plasmid constructed as described earlier (21) and affinity puriﬁed from anti-FLAG-agarose gel as described (21).

Immunoprecipitation, communoprecipitation, and Western blotting

Bacterially expressed FLAG-SAF1 protein (0.5 μg) was incubated with 1.0 U (1.3 μg) of a purified preparation of catalytic subunit of PKA (Calbiochem-Novabiochem, La Jolla, CA) in 20 μl 10 mM HEPES (pH 7.9), 10 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT, 0.1 mM ZnCl₂, 0.5 mM sodium orthovanadate, 10 mM NaF, 1 mM PMSF, 0.5 mg/ml benzamidine buffer at 25° C for 1 h. After incubation, 0.5 ml immunoprecipitation buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2.5 mM PMSF, 1% Nonidet P-40, 0.1% SDS, 0.5 mg/ml benzamidine was added to the mixture, and proteins were immunoprecipitated with anti-PKA Ab (Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C for 16 h. Next, 50 μl protein G-agarose slurry was added to the reaction mixture and incubated for 2 h at 4°C, and bound proteins were eluted and separated by SDS-PAGE. The proteins were transferred to nitrocellulose membrane and probed with anti-FLAG Ab (Sigma, St. Louis, MO). Chemiluminescence reaction was performed with ECL detection kit using the manufacturer’s protocol (Amersham, Arlington Heights, IL).

For communoprecipitation analysis, BNL CL2 liver cells were grown in duplicate in 100-mm dishes. One set of cells was not treated with any reagents, and the other set of cells was treated with 8-Br-cAMP (0.4 mM, final concentration) for 4 h. After incubation, cells were harvested, washed in PBS, and resuspended in a lysis buffer containing 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.5% Nonidet P-40, 2.5 mM PMSF, 0.5 mg/ml benzamidine, 5 μg/ml leupeptin, 10 μg/ml aprotinin, 0.2 mM sodium orthovanadate, and 100 mM NaF. Clarified cell extracts were further immunoprecipitated with either anti-SAF1 Ab or anti-PKA Ab (Santa Cruz Biotechnology). Immunoprecipitated proteins were fractionated by SDS-PAGE, transferred onto nitrocellulose membrane, and further probed with anti-PKA or anti-SAF1 Ab, as indicated in Figs. 2 and 3. The migration position of PKA or SAF-1 in the immunoblot was verified by fractionating a sample of puriﬁed catalytic subunit of PKA (Calbiochem-Novabiochem) or bacterially expressed pure SAF-1 protein.

Immunofluorescence analysis

BNL CL2 cells were transfected in duplicate, with pCMV-FLAG-SAF1 plasmid DNA. After transfection, one set of cells was stimulated with 8-Br-cAMP (0.4 mM). The cells were ﬁxed with acetone-chloroform (1:1), incubated for 10 min, and permeabilized in PBS-0.2% Triton X-100 for 5 min at room temperature. After a washing in PBS, cells were blocked for 1 h in a blocking buffer (PBS-1% BSA-1% goat serum) and further incubated for another hour with anti-FLAG mouse IgG plus anti-PAK rabbit IgG in the blocking buffer. After three washings in PBS, cells were incubated with a mixture of goat anti-mouse IgG Alexa Fluor 546, goat anti-rabbit IgG Alexa Fluor 488, and DAPI (all three reagents were obtained from Molecular Probes, Eugene, OR) in the blocking buffer for 30 min in the dark and then washed three times in PBS. A few drops of Fluoromount-G (Fisher Scientific, Pittsburgh, PA) were added, the coverglass was placed, and the edges of the coverglass were sealed with clear nail polish. Fluorescent-labeled cells were visualized in an epifluorescence microscope (Axioskop; Zeiss, Oberkochen, Germany).

In vivo phosphorylation of SAF-1

BNL CL2 liver cells were transfected with pCMV-SAF1-1 expression plasmid. The cells were metabolically labeled with 13C3]orthophosphate (3P; 0.3 μCi/ml) in phosphate free DMEM for 6 h in the absence or presence of 8-Br-cAMP (0.4 μM). Before the labeling, cells were grown in DMEM plus 5% FCS for 24 h. The 3P-labelled cells were harvested, washed quickly in phosphate-free DMEM, and lysed by adding hot lysis buffer (50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.5% Nonidet P-40, 2.5 mM PMSF, 0.5 mg/ml benzamidine, 5 μg/ml leupeptin, 10 μg/ml aprotinin, 0.2 mM sodium orthovanadate, 100 mM NaF plus 1% SDS). After lysis, SDS concentration was reduced to 0.1% by adding lysis buffer without SDS. The 3P-labelled SAF-1 protein was isolated by immunoprecipitation with anti-SAF1 Ab, fractionated in an SDS-11% polyacrylamide gel, and detected by autoradiography.

In vitro phosphorylation and immunoprecipitation of SAF-1

The phosphorylation reaction was performed in 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 100 μM unlabeled ATP, 10 μCi [γ-32P]ATP (6000 Ci/ mmol), 0.5 μg puriﬁed FLAG-SAF1 protein, 1.0 U (1.3 μg) of puriﬁed catalytic subunit of PKA (Calbiochem-Novabiochem) at 30°C for 30 min in a total volume of 50 μl. In some reactions, PKI inhibitor peptide (5 μM) was added (Calbiochem-Novabiochem). After incubation, one-half of in vitro phosphorylated reaction was immunoprecipitated with anti-FLAG Ab (Sigma).

Results

Yeast two-hybrid assay detected interaction between SAF-1 and PKA-Cα

To identify proteins that might in vivo interact with SAF-1 and therefore be involved in regulating its activity, we used the yeast two-hybrid system. We generated a bait that produced a fusion protein containing the GAL4 DNA-binding domain (GAL4-DBD) and a truncated form of SAF1 (GAL4-477) protein using pAS2-1 vector. This bait construct did not autoactivate the HIS3 and lacZ reporter genes on transformation in CG1945 yeast cells. The GAL4DBD-SAF1 477 bait (i.e., pAS2-1-SAF1 187–477 plasmid) was used for screening a mouse brain MATCHMAKER cDNA library constructed using pACT2 vector that produces a fusion protein containing the GAL4 activation domain (GAL4-AD) and proteins coded by the cDNA molecules in the library. Of the 1 × 106 colonies screened, 20 clones displayed growth on His− medium and β-galactosidase activity indicating interaction between SAF1 187–477 and the selective cloned proteins. For further conﬁrmation, DNA was isolated from these yeast colonies, transformed into bacteria, and reintroduced into freshly grown yeast with pAS2-1-SAF1 187–477 and tested for growth on His− medium. Ten clones passed this final round of selection and were used for DNA sequence analysis. Four independent cDNAs were identiﬁed as coding for the catalytic subunit of PKA-Cα (22). Two clones contained the full length sequence, and the other clones contained partial sequences of PKA-Cα, 21–350 and 33–350 aa, respectively. As shown in Fig. 1, yeast containing both GAL4-DBD: SAF1 187–477 and pACT2- PKA-Cα grew on plate lacking histidine. As a negative control, pAS2-1-SAF1 187–477 plasmid was cotransformed with pACT2 empty vector, which did not grow on histidine-lacking plates. This data suggested that PKA-Cα has afﬁnity for SAF-1 that results in a protein-protein interaction between these two proteins.
SAF-1 interacts in vitro with PKA-Cα

To test whether SAF-1 and PKA proteins might interact in vitro as well, we used a combined immunoprecipitation/Western blot analysis. Bacterially expressed FLAG-SAF1 protein was incubated with purified preparation of the catalytic subunit of PKA (Calbiochem-Novabiochem). After incubation, proteins were immunoprecipitated with anti-PKA Ab and Western blotted with anti-FLAG Ab. As seen in Fig. 2, lane 2, FLAG-SAF1 was immunoprecipitated with anti-PKA and further detected by anti-FLAG Ab. In the absence of PKA (Fig. 2, lane 3), SAF-1 could not be immunoprecipitated and thus remained absent in Western blot analysis. We show that anti-FLAG Ab is specific because it detects only FLAG-SAF1 protein (lane 4) but not PKA (lane 5). These results showed that SAF-1 interacts with PKA under in vitro conditions.

PKA interacts with SAF in vivo

To test whether PKA-Cα and SAF interact in vivo with each other, we used coimmunoprecipitation assay. Cellular proteins of BNL CL2 liver cells were treated with anti-SAF1 Ab to selectively immunoprecipitate intracellular SAF-1 (Fig. 3). When anti-SAF1 immunoprecipitated proteins were probed with anti-PKA Ab, a band (Fig. 3, lane 2) was preferentially detected in cAMP-treated cells that comigrated with the catalytic subunit of PKA (Fig. 3, lane 3). This result suggested that in vivo SAF and PKA interact with each other. Similar to our finding, a recent report showed interaction between PKA-Cα and SOX-9, a transcription factor involved in chondrocyte differentiation (23). Surprisingly, the level of PKA coimmunoprecipitated with SAF-1 protein was much less in untreated BNL cells (Fig. 3, lane 1) indicating that cAMP treatment of the cells is necessary either for optimal release of the catalytic subunit of PKA from its native form or for optimal interaction of SAF-1 with PKA. In a reciprocal experiment, immunoprecipitation of cellular proteins with anti-PKA-Cα Ab pulled down SAF-1 protein at a favorable level when cAMP was added to the medium (Fig. 3, compare lanes 4 and 5). The role of cAMP in SAF-PKA interaction in vivo is interesting and suggested that cAMP-mediated actions facilitate interaction between these two proteins.

Colocalization of SAF-1 and PKA in the cells after cAMP stimulation

For an interaction between two proteins to have a functional and physiological consequence, they must be expressed in the same cell. To address this, BNL CL2 cells were transfected with pCMV-FLAG-SAF1 plasmid and grown on coverslips. Next, one set of cells was stimulated with 8-Br-cAMP and processed for immunofluorescence assay using both anti-FLAG and anti-PKA Abs. As seen in Fig. 4A, SAF protein was seen in the cytoplasm but also at a lower level in the nucleus of unstimulated cells. In contrast, PKA

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**FIGURE 1.** SAF-1 interacts with the catalytic subunit of PKA in yeast. A, Bait plasmid used for screening. Sequences containing amino acids from 187 to 477 of SAF-1 were cloned into pAS2-1 vector (CLONTECH Laboratories) in frame with the GAL4-DBD. B, Yeast colonies that were transformed with pAS2-1-SAF1187–477 and pACT2 alone or pAS2-1-SAF1187–477 and pACT2-PAKACα were streaked on selective plates with (His) histidine.

**FIGURE 2.** Bacterially expressed SAF-1 protein interacts with catalytic subunit of PKA in vitro. FLAG-SAF1 fusion protein was incubated with the catalytic subunit of PKA (Calbiochem-Novabiochem). *Lanes 1 and 4* contain 0.5 μg FLAG-SAF1 protein, *lane 2* contains 0.5 μg FLAG-SAF1 protein plus 1.0 U (1.3 μg) PKA, and *lanes 3 and 5* contain 1.0 U (1.3 μg) PKA. Proteins in *lanes 1–3* were immunoprecipitated with anti-PKA Ab (Santa Cruz Biotechnology) before their separation by 10% SDS-PAGE. After transfer to a nitrocellulose membrane, all proteins were probed with anti-FLAG Ab.

**FIGURE 3.** Cellular SAF-1 interacts with the catalytic subunit of PKA in vivo. BNL CL2 liver cells were treated with 8-Br-cAMP (0.4 mM) for 4 h. Cells were harvested and lysed as described in *Materials and Methods*. Lysates from both control and cAMP-treated cells were used in the immunoprecipitation reaction. As a control, the catalytic subunit of PKA (Calbiochem-Novabiochem) was used in lane 7 that was not immunoprecipitated. In a reciprocal experiment, purified SAF1 protein was used in lane 6 as a control. Proteins in *lanes 1 and 2* were immunoprecipitated with anti-SAF1 Ab before their separation in 10% SDS-PAGE. Proteins in *lanes 4 and 5* were immunoprecipitated with anti-PKA Ab (Santa Cruz Biotechnology) before their separation in a 10% SDS-PAGE. After transfer to a nitrocellulose membrane, proteins were probed with either anti-PKA (lanes 1–3) or anti-SAF (lanes 4–6) Abs.
cAMP increases and inhibitors of PKA block expression of an SAF-regulated reporter gene

Interaction of SAF-1 with PKA provided a clue for the involvement of the cAMP/PKA signaling pathway in modulating SAF function. To that end, we sought to determine whether intracellular elevation of cAMP, an inducer of PKA activity in cells, has any effect on SAF activity. A CAT gene containing three tandem copies of SAF-binding elements present in the SAA gene was chosen as a reporter gene system to monitor the function of activated SAF. BNL CL2 liver cells were transfected in duplicate with SAF-CAT reporter constructs either alone or with 1.0 μg pCMV-SAF1 plasmid DNA. After transfection, one set of cells were incubated with 8-Br-cAMP (0.4 mM) or H89 (5 μM) or myristoylated PKI peptide [14–22] amide (1 μM) (Calbiochem-Novabiochem). CAT activity was determined as described in Materials and Methods. The results represent averages of three separate experiments.

FIGURE 5. Regulators of PKA affect SAF-mediated reporter gene expression. BNL CL2 liver cells were cotransfected in duplicate, with 1.0 μg wild-type SAF-CAT reporter constructs either alone or with 1.0 μg pCMV-SAF1 plasmid DNA. After transfection, one set of cells were incubated with 8-Br-cAMP (0.4 mM) or H89 (5 μM) or myristoylated PK peptide [14–22] amide (1 μM) (Calbiochem-Novabiochem). CAT activity was determined as described in Materials and Methods. The results represent averages of three separate experiments.

were not due to a change in the expression of SAF1 protein because the level of SAF-1 protein remained same during these different treatments, as determined by Western immunoblot analysis (data not shown). This suggested that although SAF-1 is present in the cells, its activity is regulated by PKA on activation by cAMP.

Phosphorylation of SAF-1 is increased by cAMP

To determine whether phosphorylation of SAF-1 in vivo is increased by cAMP, we labeled pCMV-SAF1-transfected BNL CL2 cells with 32P in the presence or absence of 8-Br-cAMP. The 32P-labeled cellular phosphoproteins were immunoprecipitated with anti-SAF-1 Ab and separated by SDS-PAGE. As seen in Fig. 6A, cells transfected with SAF1 expression plasmid revealed a phosphorylated protein band, the intensity of which increased by severalfold when cells were labeled in the presence of cAMP (Fig. 6A, lane 2) and reduced in the presence of H89 (Fig. 6A, lane 3). This increase of phosphorylation was not due to any increase in the expression of the transfected SAF1 gene, because the level of SAF-1 protein was the same during cAMP treatment (Fig. 6B, lanes 1–3).

For a more direct confirmation of whether SAF-1 is a substrate for PKA, we in vitro phosphorylated bacterially expressed FLAG-SAF1 protein with purified catalytic subunit of PKA and [γ-32P]ATP (Fig. 6C). After incubation, a portion of in vitro phosphorylated samples were immunoprecipitated with anti-FLAG Ab (Fig. 6C, lanes 2 and 3). Two bands, one comigrating with FLAG-SAF1 protein and another comigrating with the catalytic subunit of PKA at 38 kDa position were detected (Fig. 6C, lane 2). No radioactivity was seen in the lane that contained PKI inhibitor during in vitro phosphorylation (Fig. 6C, lane 3). These results clearly indicated that SAF-1 is a substrate of PKA, and immunoprecipitation of both SAF-1 and PKA with anti-FLAG Ab further confirmed their association. Taken together, these results indicated that cAMP/PKA mediate cellular event regulates functional activity of SAF-1 protein.

Discussion

Our data show for the first time that in vivo, SAF-1 transcription factor interacts with PKA-Cα and acts as a substrate for PKA.

FIGURE 4. Colocalization of SAF-1 and PKA-Cα in liver cells. BNL CL2 cells were transfected with FLAG-SAF-1 expression plasmid DNA in duplicate and grown on coverslips. One set of cells was incubated in the presence of 8-Br-cAMP (0.4 mM) for 4 h (B), and the other was left untreated (A). Fluorescent microscopy was performed as described in Materials and Methods. Cells were stained with 4′,6′-diamidino-2-phenylindole (DAPI) for nuclear staining and probed with anti-FLAG and anti-PKA Ab.

was seen predominantly in the cytoplasm of unstimulated cells. However, in response to cAMP stimulation (Fig. 4B), both PKA and SAF proteins were present at a higher level in the nuclei. These results demonstrated that SAF and PKA colocalize under some in vivo conditions.
SAF-1 protein is seen in nuclei of unstimulated cells. In contrast, PKA is predominantly present in the cytoplasm of unstimulated cells but migrates to the nucleus after 8-Br-cAMP treatment. Therefore, it seems that nuclear localization of SAF-1 is not dependent on its phosphorylation by PKA. These results are in agreement with previous observations, which detected SAF protein in nuclear extract of unstimulated cells (10) and migration of the PKA-Co in the nucleus on intracellular elevation of CAMP (26).

Several earlier studies speculated that function of SAF is modulated by serine/threonine phosphorylation (9, 10). Recently, we have shown that PKC plays a significant role in increasing the DNA-binding ability of SAF, which results in increasing SAF-regulated gene expression (21). Another study demonstrated increased level of DNA-binding activity of MAZ, a homologue of SAF-1, by casein kinase II (27). The results presented in this report strongly suggest a role of PKA in activating SAF and SAF-regulated metabolic processes. At present, although SAF is shown to be activated by PKC, casein kinase II, and PKA, it does not necessarily mean that this transcription factor is regulated only by these three protein kinases. In fact, because SAF is seen to be activated by different inflammatory signals, including LPS (11), cytokines (12), and minimally modified low density lipoprotein (28), we speculate that these diverse effects may be mediated by a combination of many signal transducing protein kinases. Further studies should reveal more about the regulatory mechanisms involving activation of SAF-1 protein.

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References


