Identification of a Novel Transcriptional Activator, BSAC, by a Functional Cloning to Inhibit Tumor Necrosis Factor-induced Cell Death*

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Tumor necrosis factor (TNF) is a multifunctional cytokine, which induces proliferation or death in a cell type-dependent manner. We previously showed that murine embryonic fibroblasts (MEFs) from TNF receptor-associated factor 2 (Traf2) and Traf5 double-deficient (double knockout (DKO)) mice were highly susceptible to TNF-induced cell death. By functional cloning to rescue DKO MEFs from TNF-induced cell death, we have identified a novel gene, Bsac. BSAC is composed of N-terminal basic, SAP (SAF-A/B, Acinus, PIA5), and coiled-coil domains. BSAC is a nuclear protein, and overexpression of BSAC potently activates promoters containing A + T-rich sequences named CARG boxes. Domain mapping analysis revealed that both N-terminal basic and C-terminal proline-rich sequence are required for the transcriptional activity. Overexpression of BSAC in DKO MEFs partially inhibited TNF-induced cell death by suppressing activation of caspases. Interestingly, inhibition of TNF-induced cell death was not observed in DKO MEFs transfected with either N-terminal or C-terminal deletion mutant of BSAC, revealing an intimate correlation between transcriptional activity and antiapoptotic function. Recently, a human homologue of BSAC named MAL/MKL1 (megalakaryocytic acute leukemia/megakaryoblastic leukemia-1) was identified as a fusion transcript generated by t(1,22) translocation in acute megakaryoblastic leukemia. Collectively, BSAC is a novel transcriptional activator with antiapoptotic function, which may be involved in the leukemogenesis.

Cell death by apoptosis is an essential event not only for developmental process but also for elimination of transformed cells (1). Dysregulation of this process causes various pathological disorders such as autoimmune diseases, neurodegenerative diseases, and malignant tumors (2). Among various stimuli inducing apoptosis, two death receptors, Fas and tumor necrosis factor (TNF) receptor 1 (TNF-R1) receptor 1, have been extensively characterized. Upon ligand binding, these receptors activate an initiator caspase, caspase-8, via recruitment of TNF receptor 1-associated death domain protein and/or Fas-associated death domain protein, which in turn activates downstream effector caspases, such as caspase-3 and caspase-7, finally resulting in apoptosis (3). TNF receptor-associated factors (TRAFs) emerged as signal transducers for members of the TNF receptor superfamily. Traf2, Traf5, and Traf6 mediate NF-κB activation and c-Jun N-terminal kinase activation (4). We recently showed that Traf2−/− Traf5−/− (DKO) murine embryonic fibroblasts (MEFs) had a severe defect in TNF-induced NF-κB activation and were highly susceptible to TNF-induced cell death (5).

Serum response factor (SRF) is a MADS (MCM1, Agamous, Deficiens, and SRF) box-containing transcription factor that controls gene expression in response to growth and differentiation signals (6). SRF was first identified by its ability to mediate serum responsiveness of c-fos promoter (7). Activation of the c-fos promoter is generally mediated by the association of SRF with members of the ternary complex factor family of Esr domain transcription factors, which serves as targets for mitogen-activated protein kinase signaling (8–10). Association of ternary complex factor with the MADS box of SRF is dependent on the binding of SRF to a consensus A + T-rich sequence (CC(A/T)6GG) named the CARG box. CARG boxes are also required for expression of various muscle-specific genes including α-skeletal, α-cardiac, and γ-smooth muscle actin (11). Since the expression of SRF is not restricted to the heart or muscles, several myogenic accessory factors that selectively activate muscle target genes in cooperation with SRF have been identified (12–14). Among them, a heart-specific transcriptional activator named myocardin

* This work was supported in part by grants-in-aid for Scientific Research on Priority Areas C from the Ministry of Education, Culture, Sports, Science, and Technology, a grant-in-aid for Scientific Research C from the Japan Society for the Promotion of Science, Japan, and by a grant from the Human Frontier Science Program (HFSP). 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) AF385582.

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Received for publication, April 3, 2002, and in revised form, May 14, 2002
Published, JBC Papers in Press, May 17, 2002, DOI 10.1074/jbc.M203190200

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was recently identified by a bioinformatics approach (14). Myocardin belongs to the SAP (SAF-A/B, Acinus, PIAS) domain family of nuclear proteins. Overexpression of myocardin potently activated muscle-specific promoters containing multiple, but not single, CArG boxes. Moreover, myocardin directly bound to SRF and formed a ternary complex with CArG boxes in the presence of SRF (14).

By functional screening to identify molecules that inhibit TNF-induced cell death of DKO MEFs, here we identified a novel gene named *Bsac*. BSAC belongs to the SAP domain family of nuclear proteins and is composed of N-terminal basic, Q (glutamine-rich), SAP, and CC (coiled-coil) domains. Given that the domain structure of BASC is highly homologous to that of myocardin, these two proteins constitute a novel family. BSAC is ubiqui-
uitously expressed in various murine tissues, and overexpression of BSAC potently activated promoters containing CArG boxes. In contrast to myocardin, BSAC could activate c-fos promoter that contains a single CArG box. Moreover, BSAC inhibited TNF-induced cell death of DKO MEFs by suppressing activation of caspase-3 and -8. Interestingly, a human homologue of Bsac named megakaryocytic acute leukemia (MAL)/megakaryoblastic leukemia-1 (MKL1) has recently been identified as a fusion transcript with an OTT/RBM15 (one twenty-two/RNA-binding motif protein-15)
generated by t(1;22) translocation in acute megakaryoblastic leukemia (15, 16). Thus, the transcriptional activity and antiapoptotic function of BSAC may be involved in the development of acute leukemias.

**EXPERIMENTAL PROCEDURES**

**Library Screening—** cDNA library construction using a retroviral vector, transient transfection, and production of viral stock were performed essentially as previously described (17). Phoenix-Eco cells (1.5 × 10^6 cells) (kindly provided by Gary P. Nolan) were transiently transfected with 3 μg of a library DNA by LipofectAMINE (Invitrogen). Approximately 5 × 10^6 MEFs were infected with retroviral library for 8 h in the presence of 10 μg/ml of polybrene (Sigma). Then the cells were cultured in the presence of TNF (10 ng/ml; BD PharMingen). After 2 weeks, surviving clones were isolated and expanded. To sequence integrated cDNAs, genomic DNAs isolated from each clone were amplified using retroviral vector primers by PCR. A full-length BSAC cDNA was obtained by screening a murine spleen cDNA library (Stratagene) by standard methods.

**Plasmids and Antibodies—** A retroviral vector, pMX-FLAG-puro, was generated by inserting an N-terminal FLAG tag sequence into pMX-puro containing puromycin-resistant gene for drug selection (18). Full-length and truncated mutants of BSac were generated by PCR and subcloned into pMX-FLAG-puro, pcDNA3-Myc (kindly provided by Ko-hei Miyazono), or pFA-CMV containing the DNA binding domain of yeast GAL4 (Stratagene). pcDNA3-Myc-myocardin was generated by subcloning the reverse transcriptase-PCR product of myocardin into pcDNA3-Myc. pT7-ATG-SRF was provided by Ron Prywes (Columbia University). The PCR product of Bel-2 from pSFFV-Bel-2 (kindly provided by Nohoiro Inohara) was subcloned into pMX-FLAG-puro, designated pMX-FLAG-Bcl-2. Anti-GAL4 (Santa Cruz Biotechnology), anti-Myc (9E10, Santa Cruz Biotechnology), anti-FLAG (M2, Sigma), and anti-SRF (Santa Cruz Biotechnology) antibodies (Abs) were obtained from the indicated sources. Ab specific for BSAC was raised in rabbits against the E. coli-expressed N-terminal fragment of BSAC (glutathione S-transferase-BSAC-(46–147)) and affinity-purified on the glutathione S-transferase-BSAC column.

**Northern Blot Analysis—** Northern blot analysis was performed as described (19).

**Immunostaining—** HeLa cells were plated on glass slides and cultured for 12 h. Then the cells were transiently transfected with Myc-tagged deletion mutants of BSAC by using Metafectene (Biontex) according to the manufacturer’s instructions. The cells were washed with PBS, fixed with 2% paraformaldehyde, and then incubated with anti-

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**FIG. 4.** Mapping of BSAC domains responsible for transcriptional activity and nuclear localization. A and B, mapping of BSAC domains required for transcriptional activation with c-fos promoter. HEK293 cells were transiently transfected with 0.5 μg of Myc-tagged expression vectors for BSAC and its mutants, along with 0.5 μg of c-fos-luciferase and 0.05 μg of EF-LacZ. Cell lysates were analyzed by Western blotting with anti-Myc Ab. Positions of each protein are indicated by asterisks. Luciferase activity is expressed as -fold activation compared with mock (B). Data are represented as the mean ± S.D. of triplicate samples. C, D, E, and F, N-terminal 373 amino acids are essential for nuclear localization of BSAC. HeLa cells were transiently transfected with the indicated expression vectors for Myc-tagged BSACΔN-(1–516) (C), ΔN-(1–516) (D), ΔC-(543–964) (E), or ΔC-(601–964) (F) and stained with anti-Myc monoclonal antibody (green).
BSAC Is a Novel Transcriptional Activator

RESULTS
Identification of BSAC by a Functional Screening to Inhibit TNF-induced Cell Death—To perform functional screening, we first immortalized DKO MEFs by a 3T3 protocol and established several independent cell lines from primary DKO MEFs. TNF-induced cell death of immortalized DKO MEFs was completely inhibited by a broad caspase inhibitor, benzoyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-fmk) (data not shown), indicating that the cell death was caspase-dependent. After confirming that almost all DKO MEFs died within 2 weeks of TNF treatment (data not shown), we introduced a retroviral cDNA library derived from mouse bone marrow stromal cells to DKO MEFs and selected surviving clones. Through this functional screening, we identified a novel gene, named \textit{Bsac} (basic, SAP, and coiled-coil domain). \textit{Bsac} cDNA encodes 964 amino acids with a predicted molecular mass of 102.6 kDa. BSAC is composed of N-terminal basic, glutamine-rich, central SAP and C-terminal coiled-coil domains (Fig. 1A). Whereas the basic domain contains a putative bipartite nuclear localization signal, the SAP DNA binding domain is supposed to mediate the binding of DNA to nuclear scaffold or matrix and be involved in chromatin remodeling and transcriptional control (23). BSAC also contains proline-rich sequence at the C terminus, which is reminiscent of a transcriptional activation domain (24). A BLAST search revealed a high homology of Bsac to human MAL/MKL1, suggesting that Bsac is a murine homologue of human MAL/MKL1 (15, 16) (Fig. 1A). BSAC also showed a structural homology to a heart-specific transcription factor, myocardin (14), suggesting that these proteins consti-

Fig. 5. BSAC does not form a complex with CARG box even in the presence of SRF. A. EMSAs were performed with \textsuperscript{32}P-labeled oligonucleotides containing c-fos CARG box and in \textit{vitro} translation products of Myc-tagged myocardin in the presence or absence of SRF. Abs against SRF or the Myc epitope were included as indicated. The arrows indicate the Ab-supershifted complexes and the SRF-containing complexes. The asterisk indicates a ternary complex formed by myocardin and SRF. B, EMSA were performed as in A, with Myc-tagged BSAC instead of Myc-tagged myocardin.

|       | SRF | myc-myo | anti-SRF | anti-myc |
|-------|-----|---------|----------|----------|
| A     | +   | +       | -        | -        |
| myc-myo | -   | -       | +        | +        |
| anti-SRF | -   | -       | -        | +        |
| anti-myc | -   | -       | -        | -        |

Myc Ab. To detect endogenous BSAC, the fixed cells were stained with anti-BSAC Ab. The primary antibodies were detected by secondary antibodies conjugated with Alexa 488 (Molecular Probes, Inc., Eugene, OR). Stained cells were mounted in SlowFade (Molecular Probes) and analyzed on a laser-scanning confocal microscope (Bio-Rad).

Immunoprecipitation—Immunoprecipitation and Western blotting were performed as described previously (5).

Reporter Assay—Transfection and luciferase assay were performed as described previously (20). The pFR-luciferase contains five repeats of GAL4 binding elements upstream of the luciferase gene (Stratagene). The pSRF-luciferase reporter contains five tandem copies of CARG boxes (Stratagene). The c-fos-luciferase reporter contains a c-fos promoter region, 3'-700/+53 (kindly provided by Yuriko Suzuki). The c-fos CARG nt-luciferase contains CARG box mutations, which were introduced by changing the consensus sequence as follows: CCATATTAGG to CAATATTAGC (mutated residues are underlined).

Electrophoretic Mobility Shift Assay (EMSA)—SRF, Myc-tagged myocardin, and Myc-tagged BSAC were translated \textit{in vitro} with a TNT T7-coupled reticulocyte lysate system (Promega). The oligonucleotides containing c-fos CARG box were 5'-TGCTGACCAATGCTTATATTGGACATCTGCGTC-3' and 5'-CCCTACACAGGATGCCTACATTTAGGGACATCTGGCTG-3'. EMSA was performed as described previously (21).

Generation of Stable Transfectants—Phoenix-Eco cells (1.5 x 10\textsuperscript{5} cells) were transiently transfected with 3 \mu g of the indicated vectors using LipofectAMINE. For infection, DKO MEFs (1 x 10\textsuperscript{5} cells) were seeded in six-well plates and incubated with 2 ml of the viral supernatants for 8 h. After changing the medium, the cells were cultured in fresh 10% fetal calf serum/Dulbecco's modified Eagle’s medium for 16 h and then selected with 2.5 \mu g/ml puromycin (Sigma) to isolate stable transfectants. Puromycin-resistant pools were used for the experiments.

Cell Death Assay—MEFs (5 x 10\textsuperscript{5} cells) were plated in 96-well plates and incubated with various amounts of murine TNF for 24 h. Cell viability was determined by the WST (2-(4-iodophenyl)-3-(4-nitophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) assay using a cell counting kit (Dojindo). Statistical analysis was performed using Student's t test. \( p < 0.05 \) was considered to be significant.

Caspase Activity—Caspase activity was measured by the fluorometric assay as previously described (22). Briefly, the cell lysates were incubated with acetyl-Asp-Glu-Val-Ala-Asp-aminomethylcoumarin (Ac-DEVD-MCA) (for caspase-3) and acetyl-Ile-Glu-Thr-Asp-7-amido-4-(trifluoromethyl)coumarin (Ac-IETD-MCA) (for caspase-8). Then the release of fluorescent 7-amino-4-methylcoumarin was measured on a fluorometer (Labystems).
tute a novel family (Fig. 1B). We also found related proteins in Drosophila and Caenorhabditis elegans (Fig. 1B).

Expression and Subcellular Localization of BSAC—Northern blot analysis showed that the Bsac transcripts of 5.1, 2.7, and 1.5 kb are ubiquitously expressed in various adult murine tissues (Fig. 2A). The 2.7- and 1.5-kb transcripts might represent alternative splicing products of Bsac.

A polyclonal Ab against BSAC was raised in rabbits by immunizing a glutathione S-transferase-BSAC-(46–147) fusion protein prepared in Escherichia coli. Western blot analysis of the lysate from wild-type MEFs demonstrated that the anti-BSAC Ab specifically recognized a protein of ~150 kDa (Fig. 2B). The apparent molecular mass of BSAC was significantly larger than the predicted molecular mass (102.6 kDa). This discrepancy might be due to the C-terminal proline-rich sequence of BSAC. The molecular mass of endogenous BSAC in human cell lines (HeLa and 293) appeared to be slightly smaller than that in murine MEFs. This difference may be due to the fact that murine BSAC contains 35 amino acids in addition to the N terminus of human BSAC/MAL/MKL1 (Fig. 1A).

Immunofluorescent analysis using HeLa cells showed that BSAC is a nuclear protein (Fig. 2C).

BSAC Is a Transcriptional Activator—Because BSAC contains a SAP DNA binding motif and is localized in the nuclei, we next tested whether BSAC possesses transcriptional activity by fusing it to the DNA binding domain of yeast GAL4. Expression of GAL4 fusion protein containing full-length BSAC and its deletion mutants was verified by Western blotting using anti-GAL4 Ab (data not shown). Overexpression of BSAC potentially activated GAL4-dependent reporter activity (Fig. 3A). Serial deletion mutants revealed that deletion of the N-terminal 779 amino acids, ΔN-(1–779), did not reduce but significantly enhanced its transcriptional activity, suggesting that the N-terminal portion of BSAC suppresses transcriptional activity of the C-terminal portion. On the other hand, a deletion mutant lacking the C-terminal 364 amino acids, ΔC-(601–964), did not exhibit transcriptional activity (Fig. 3A). Collectively, these results indicated that BSAC is a transcriptional activator, and the C-terminal portion (residues 780–964) mediates this activity.
Given that BSAC has transcriptional activity, we next tried to identify a target promoter of BSAC. A previous study showed that myocardin activated promoters containing multiple, but not single, CArG boxes that serve as the binding site for SRF (6, 14). Since BSAC showed homology to myocardin (Fig. 1B), we tested whether BSAC also activates promoters containing the CArG boxes. As expected, BSAC potently activated a reporter containing five tandem copies of the CArG boxes (pSRF-luciferase) (Fig. 3B). More importantly, in contrast to myocardin, BSAC also activated the c-fos promoter that contained a single CArG box (Fig. 3C). Mutation of the CArG box in the c-fos promoter significantly reduced the activation by BSAC (Fig. 3C), indicating that the transcriptional activity of BSAC is mediated by the canonical CArG box sequence.

Mapping of Domains Required for Transcriptional Activity and Nuclear Localization—To further investigate the mechanism by which BSAC exerts transcriptional activity, we next performed reporter assays using Myc-tagged deletion mutants of BSAC with the c-fos promoter. Expression of these mutants was veriﬁed by Western blotting using anti-Myc Ab (Fig. 4A). In contrast to the reporter assays using GAL4 fusion proteins (Fig. 3A), either BSACAN(1–373) or ΔN(601–964) did not activate the c-fos promoter (Fig. 4B). These results indicated that both the N- and C-terminal portions are required for the BSAC-mediated transcriptional activity. The inability of N-terminal deletion mutants to exhibit transcriptional activity in this system might be caused by a defect in nuclear localization. To address this possibility, we transiently transfected HeLa cells with Myc-tagged deletion mutants of BSAC and then examined the subcellular localization of BSAC by confocal microscopy. Whereas BSACAN(1–373) and ΔN(1–516) lost their abilities to localize in the nuclei (Fig. 4, C and D), ΔC-(543–964) and ΔC-(601–964) were predominantly localized in the nuclei (Fig. 4, E and F). Collectively, these data indicated that the N-terminal 373 amino acids containing basic and glutamine-rich domains is required for the nuclear localization of BSAC.

BSAC neither Directly Bound to CArG Box nor Formed a Ternary Complex with SRF—We next investigated whether BSAC directly binds to the CArG boxes by EMSA. Recombinant Myc-tagged BSAC, Myc-tagged myocardin, and SRF proteins were translated in vitro and subjected to EMSA using 32P-labeled oligonucleotides containing the CArG box derived from the c-fos promoter. SRF formed a complex with the CArG box, which was supershifted by anti-SRF Ab (Fig. 5A). As reported previously, myocardin alone did not form a complex with the CArG box, but the addition of Myc-tagged myocardin to SRF formed an additional upper complex (Fig. 5A, asterisk). The upper complex was removed by anti-Myc Ab, indicating that this complex contained the Myc-tagged myocardin. BSAC alone also did not form a complex with the CArG box. In contrast to myocardin, however, BSAC did not form a complex with the CArG box even in the presence of SRF (Fig. 5B). Moreover, we did not detect a complex of BSAC with the CArG box using a nuclear extract from 293 cells transiently transfected with both BSAC and SRF (data not shown).

BSAC Inhibits TNF-induced Cell Death—Since BSAC was initially identified by functional screening, we examined whether BSAC actually inhibits TNF-induced cell death of DKO MEFs. DKO MEFs were stably transfected with retroviral expression vectors for mock, FLAG-tagged BSAC, or FLAG-tagged Bcl-2. To avoid clonal variation, pooled cells after drug selection were used for experiments. The expression of transfected proteins was verified by Western blotting with anti-FLAG Ab (Fig. 6A). As shown in Fig. 6B, BSAC as well as Bcl-2 significantly protected the cells from TNF-induced cell death. However, such a protective effect of BSAC was not observed against cell death induced by staurosporin (Fig. 6C), which is primarily mediated by mitochondria-dependent pathway.

To explore the mechanism by which BSAC inhibits TNF-induced cell death, we examined whether BSAC inhibits TNF-induced caspase activation. In the mock transfectant, robust activation of caspase-3 and -8 was observed at 12 and 24 h after TNF treatment (Fig. 6, D and E). In contrast, activation of both caspase-3 and -8 was significantly inhibited in the BSAC and Bcl-2 transfectants (Fig. 6, D and E). Collectively, these results indicated that BSAC suppresses TNF-induced cell death by inhibiting activation of caspases. We also tested whether BSAC inhibits cell death induced by other death receptors such as Fas. However, we observed only a marginal inhibitory effect of BSAC on Fas-induced apoptosis of murine B cell lymphoma A20.2J (data not shown), suggesting that the antiapoptotic function of BSAC depends on cell type and proapoptotic stimuli.

Correlation of Transcriptional Activity to Antiapoptotic Function of BSAC—We finally determined the responsible domain of BSAC for inhibiting TNF-induced cell death of DKO MEFs. We stably transfected DKO MEFs with FLAG-tagged BSAC deletion mutants by retroviral vectors, and the expression of transfected proteins was verified by Western blotting with anti-FLAG Ab (Fig. 7A). DKO MEFs stably expressing either BSAC ΔN(1–373), ΔN(1–516), ΔC-(543–964), or ΔC-(601–964) were not significantly protected from TNF-induced cell death as compared with the mock transfectant (Fig. 7B). Given that BSAC lost its transcriptional activity when deleted of either N- or C-terminal domains (Fig. 4B), these results...
suggested that the transcriptional activity of BSAC is indispensable for the antiapoptotic function.

**DISCUSSION**

In the present study, we identified a novel transcriptional activator, BSAC, by functional cloning. BSAC is a potent transcriptional activator and suppresses TNF-induced cell death by inhibiting activation of caspases.

BSAC is a SAP domain-containing nuclear protein and shows a high structural similarity to a heart-specific transcription factor, myocardin. Although BSAC activates promoters containing the CArG boxes, we could not demonstrate a complex formation of BSAC with the CArG box even in the presence of SRF. Our preliminary data showed that BSAC did not interact with SRF, but overexpression of SRF inhibited BSAC-induced transcriptional activity with the c-fos promoter. Although the detailed mechanism by which BSAC activates CArG box-containing promoters remains to be determined by further study, one possible explanation is that BSAC may bind a molecule other than SRF to form a ternary complex with the CArG boxes. Alternatively, an additional molecule may be required for the complex formation of BSAC with SRF.

Domain mapping analysis of BSAC showed that deletion of the N-terminal 373 or C-terminal 364 amino acids almost completely abolished transcriptional activity with the c-fos promoter (Fig. 4B). Subcellular localization analysis showed that the deletion of the N-terminal 373 amino acids impaired nuclear localization of BSAC (Fig. 4C), suggesting that nuclear localization signal exists in this region. Since the basic domain contains the KKAKELKPKVKKLLK sequence, this may act as a bipartite nuclear localization signal (25). Taken that the C-terminal 185 amino acids containing proline-rich sequence was sufficient for BSAC-mediated transcriptional activation (Fig. 3A), the N-terminal basic region and the C-terminal proline-rich sequence of BSAC are required for nuclear localization and transcriptional activation, respectively. Functional roles of the SAP and coiled-coil domains of BSAC remain to be determined by further studies.

Because BSAC contains a SAP DNA binding motif and is localized in the nuclei, it seems unlikely that BSAC directly inhibits caspase activation. Indeed, we could not observe any interaction or co-localization of BSAC with caspase-3 and -8 (data not shown). A transcription factor, NF-κB, has been shown to exert antiapoptotic function by up-regulating anti-apoptotic genes, such as *Cash/Flip*, *Xiap*, or *Bcl-xL*. However, we could not observe up-regulation of *Cash/Flip*, *Xiap*, *Bcl-xL*, or *Bcl-xL* in the BSAC transfectants (data not shown), suggesting that BSAC may control some other antiapoptotic molecules yet to be determined.

Although both BSAC and myocardin activate promoters containing the CArG boxes, we observed significant differences between these two proteins. First, the expression of myocardin was restricted to the heart (14), but the expression of BSAC was ubiquitous. Second, in contrast to myocardin, BSAC did not form a ternary complex with the CArG boxes in the presence of SRF. Third, BSAC activated promoters containing either single or multiple CArG boxes, but myocardin could not activate the c-fos promoter containing a single CArG box. Collectively, BSAC and myocardin seem to differentially regulate target genes in a cell type- and promoter-dependent manner. Myocardin has been shown to be essential for heart development (14). Since BSAC is ubiquitously expressed in various tissues, it would be interesting to examine whether BSAC is involved in the development of various organs.

Recently, two groups independently identified the human homologue of BSac named MAL/MKL1 as a fusion transcript with OTT/RBM15 generated by t(1;22) translocation in acute megakaryocytic leukemia (15, 16). Although these studies suggested a critical role of OTT/RBM15-BSAC/MAL/MKL1 in the development of acute leukemias, the function of these proteins remains unknown. Accumulating data have demonstrated that the deregulation of c-fos is critically involved in oncogenesis (27). In this regard, the transcriptional function of BSAC to strongly activate c-fos promoter may represent a link of BSAC to the leukemogenesis. In another aspect, the antiapoptotic function of BSAC may also be involved in the development of leukemias. Further studies need to be undertaken in order to address these possibilities.

**Acknowledgments**—We thank Y. Suzuki, G. P. Nolan, N. Inohara, R. Pyryves, and R. K. Miyazono for reagents. We especially thank W-Y. C. and T. W. Mak for providing *Traf2−/−* mice.

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