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Characterization of the Biological Functions of a Transcription Factor, c-*myc* Intron Binding Protein 1 (MIBP1)[1]

Shinji Fukuda,* Yuki Yamasaki,* Toru Iwaki,† Hiroaki Kawasaki,‡ Shizuka Akieda,* Naruhiko Fukuchi,* Tomoko Tahira,* and Kenshi Hayashi*,[2]

**Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, †Department of Neuropathology, Neurological Institute, Graduate School of Medical Sciences, and ‡Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582*

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The c-*myc* intron binding protein 1 (MIBP1) is a gigantic zinc finger protein comprising 2,437 amino acids and belonging to the MHC binding protein (MBP) family. MIBP1 is suggested to be a transcription factor involved in various biological functions. We show here that MIBP1 represses c-*myc* transcription from the major promoter, P2. Screening by the yeast two-hybrid system revealed that the MIBP1 protein interacts with the Ski-interacting protein (SKIP). *In vitro* pull-down assays and *in vivo* co-immunoprecipitation experiments confirmed this interaction. The acidic region of MIBP1 was found to be the site of interaction with the N-terminal half of SKIP. *In situ* hybridization analysis using developing rat embryos revealed that the MIBP1 mRNA is highly expressed in post-mitotic neurons, but the expression in immature neuroepithelium is low. The expression of MIBP1 in adult rat brain is also predominantly in neuronal cells, indicating that MIBP1 is involved in the physiology of mature neuronal cells.

Key words: c-*myc*, MIBP1, neural cell differentiation, protein–protein interaction, SKIP.

The cDNA of MIBP1 has been isolated by Southwestern screening using a 5′-portion of rat c-*myc* intron 1 as a probe (*1*). MIBP1, also called MHC binding protein–2, angiotensinogen gene–inducible enhancer-binding protein 1, human immunodeficiency virus type 1 enhancer binding protein 2, and Schnurri-2 (MBP-2/AGIE-BP1/HIV-EP2/Shn-2), has an acidic region, 12 SPKK repeats, and two widely separated pairs of C_2H_2-type zinc finger motifs that have been shown to bind to NF-κB–like elements (*2–4*). Recently, MIBP1 was found to regulate the transcription of somatostatin receptor type II (SSTR-2) by binding to a TC-rich element of the target gene (*5*).

In mammals, MIBP1 and two other structurally related proteins form the MBP family. These transcriptional modifiers have been shown to bind to NF-κB–like elements of various genes through their zinc finger motifs, which are highly conserved among family members. The second member of this family, MBP-1/HIV-EP1/PRDII-BF1/αA-CRYBP1/AT-BP2 has been characterized independently as a binding protein for the MHC class I enhancer (*6*), human immunodeficiency virus type I enhancer (*7*), interferon-β promoter (*8*), αA-crystallin promoter (*9*), and α_1-antitrypsin promoter (*10*). This protein has also been found to bind to an AG-rich motif in the enhancer of the type II collagen α1 gene, suppressing its expression (*11*). KBP1/HIV-EP3/KRC is the third member (*12–14*), and has been implicated in the transcriptional activation of a metastasis-associated gene, S100A4 (*15*).

Proteins of the MBP family are moderately homologous to *schnurri* (*shn*) in *Drosophila* (*16–18*). Shn acts as a downstream component of the decapentaplegic/transforming growth factor–β (Dpp/TGF-β) signaling pathway, by interacting directly with a signaling molecule, Mad, and regulates Dpp-responsive gene expression (*19, 20*).

Little is known about the biological function of MIBP1 in mammals. Recently, the MIBP1 (Shn-2)–knockout mouse was found to have defects in T cell maturation (*21*), but the role of MIBP1 in other tissues, especially the brain, which is known to be the major site of expression of the gene, is limited (*5*).

The aim of this study was to determine the molecular mechanism of transcriptional regulation by MIBP1, and to identify the biological processes with which the protein is associated. We first assessed the effect of MIBP1 on c-*myc* expression. We then used the yeast two-hybrid system to identify proteins that interact with MIBP1 and to characterize their interaction. We also surveyed the site of MIBP1 expression in adult brain by *in situ* hybridization, since previous reports showed that the MIBP1 mRNA is expressed at a high level in this organ (*1, 5, 10*). Embryos at different developmental stages were also examined to identify regions of MIBP1 expression.

MATERIALS AND METHODS

Plasmid Construction—For CAT assays, a full-length MIBP1 expression plasmid, pCAE-MIBP, was constructed by inserting a 7.3 kb rat cDNA fragment derived from p111 into pCAGGS (*1*). The mutant plasmid, pCAE-X, carrying a

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[2] To whom correspondence should be addressed. Tel: +81-92-642-6170, Fax: +81-92-632-2375, E-mail: khayashi@gen.kyushu-u.ac.jp

frameshift mutation at the 119th residue of MIBP1, was produced by a fill-in reaction of the *Xho*I site, followed by self-ligation. To construct the reporter plasmids, various fragments derived from pMP4, a rat c-*myc* genomic clone (*22*), were subcloned into pBLCAT30 [derived from pBLCAT3 (*23*)]. The region of c-*myc* inserted into each plasmid was: pBBitCAT, from −1302 to +1994; pBYCAT, from −1302 to +573; pBBCAT, from −1302 to +410; pBMCAT, from −159 to +410; where the first nucleotide of the c-*myc* transcript was numbered 1.

For the yeast two-hybrid system, a portion of the cDNA of MIBP1 (encoding amino acid residues 1721 to 2155) carrying the C-terminal Zinc finger motif–Acidic region–SPKK repeat (hereafter called MZAS) was amplified from the template plasmid p111, and inserted into pGBT9 (CLONTECH, Palo Alto, CA) to construct a plasmid, pGBT-MZAS. MZAS deletion constructs were made by inserting the following cDNA fragments into pGBT9; *Sma*I–*Bcl*I fragment (1721 to 1960 residues), *Sma*I–*Msp*I fragment (1721 to 1880 residues), *Msp*I–*Msp*I fragment (1881 to 2035 residues), *Bcl*I–*Pst*I fragment (1960 to 2155 residues). These inserts were confirmed to produce in-frame constructs with a GAL4 DNA-binding domain, by nucleotide sequencing.

For immunoprecipitation experiments, pCI-MZAS was constructed by inserting a fragment encoding the MZAS region tagged with a FLAG epitope into expression vector pCI (Promega, Madison, WI). This fragment was produced by PCR amplification from p111 using primers, one of which contained the FLAG coding sequence at the 5′ terminus. A SKIP expression plasmid, pCI-B49, was constructed by subcloning the insert of clone B49, which contains a HA epitope sequence, into pCI.

CAT Assay—COS-1 cells (a gift from Dr. Nakabeppu of this Institute) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. A total of 4×10^5 cells were seeded into a 60 mm Petri dish, 24 h prior to transfection. CAT reporter plasmids (0.5 μg) and pSV-β-galactosidase (0.5 μg) were co-transfected with either pCAE-MIBP1 or pCAE-X (1 μg) using the LipofectAmine reagent (Life Technologies, Rockville, MD). After 2 days of incubation, the cells were solubilized in reporter lysis buffer (Promega). Cell extracts (10 μl) were incubated at 37°C for 2 h in 30 μl of CAT reaction mixture containing 2.96 kBq of [^{14}C]1-deoxychloramphenicol (Amersham, Amersham, UK) and 0.47 mg/ml acetyl CoA. The percentage acetylation of 1-deoxychloramphenicol was determined with a BAS 2000 PhosphorImager (Fuji Photo Film, Tokyo).

Yeast Two-Hybrid Screening and Interaction Analysis—The MATCHMAKER Two-Hybrid System (CLONTECH) was used according to the manufacturer's instructions. The yeast strain Y190 was sequentially transformed with pGBT-MZAS and a pACT2-based mouse cDNA library, which was constructed using RNA extracted from IxN/2b pre-B cells (*24*). Transformants were selected on synthetic dropout (SD) medium lacking tryptophan, leucine, and histidine, supplemented with 25 mM 3-amino-triazole. After 7 days of incubation, colonies were picked up and restreaked on Whatman No. 5 filter papers wetted with the same medium and incubated for a further 3 days for the colony-lift filter assay. pACT2-based plasmids were isolated from His$^+$, LacZ$^+$ yeast colonies by utilizing the *leu2* complementation of the *Escherichia coli* strain, HB101. To quantify β-galactosidase activity, a liquid culture assay was performed using ONPG as a substrate.

Northern Blot Analysis—The Rat Multiple Tissue Northern Blot was purchased from CLONTECH. A fragment of MIBP1 spanning nucleotides 852 to 1294 (when the first nucleotide of the initiation methionine is numbered 1) was subcloned into pGEM2 (Promega) and amplified with T7 and SP6 primers. This PCR fragment was labeled with [α-^{32}P]dCTP (3,000 Ci/mmol, Amersham) by the Multiprime DNA Labeling System (Amersham). A fragment of SKIP (nucleotides 249 to 921) was amplified from the template clone B49, and labeled with [α-^{32}P]dCTP by PCR using a method essentially as described (*25*). Hybridization of the probes was performed according to the manufacturer's instruction.

Pull-Down Assay—The MZAS region tagged with a FLAG epitope, derived from pCI-MZAS, was inserted into the vector pAcGHLT-C, transferred to the baculoviral genome and expressed as a glutathione-S-transferase (GST) fusion protein in the insect cell line Sf21, following the instructions of the Baculovirus GST Expression and Purification Kit (PharMingen, San Diego, CA). Successful production of the expected protein was confirmed by Western blot analysis using the anti-FLAG monoclonal antibody, M2 (SIGMA, St. Louis, MO). The GST-XylE expression vector included in the kit was used as a control. Recombinant virus clones were plaque-purified, amplified and infected at a multiplicity of infection of five to obtain the proteins.

To produce proteins encoded by the plasmids isolated in the two-hybrid screen, inserts were amplified with specific primers, one of which was tagged with the T7 promoter sequence, and used as templates in an *in vitro* transcription/translation reaction in the presence of L-[^{35}S]methionine (Amersham) using the TNT Coupled Reticulocyte Lysate System (Promega).

GST fusion proteins bound to glutathione Sepharose were incubated with *in vitro* translated proteins in binding buffer (PBS containing 0.5% Triton X-100) at 4°C for 4 h. The beads were successively washed with binding buffer. Proteins eluted from the precipitates were separated on 10% SDS-PAGE and visualized by autoradiography and Coomassie Brilliant Blue staining.

In Vivo Interaction Study—COS-1 cells were co-transfected with pCI-MZAS (1 μg) and pCI-B49 (1 μg) as described above. After 2 days of incubation, the cells were solubilized in lysis buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100), and the lysates were incubated with anti-FLAG M2 affinity gel (SIGMA) overnight at 4°C. Immunoprecipitates were washed with washing buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Triton X-100), resolved by 8% SDS-PAGE and blotted onto an Immobilon membrane (MILLIPORE, Bedford, MA). Proteins on the membrane were detected by the anti-FLAG antibody, M2, or the anti-HA polyclonal antibody, Y-11 (Santa Cruz, Santa Cruz, CA), as the primary antibodies. Anti-mouse or anti–rabbit IgG antibody conjugated with horseradish peroxidase (Amersham) were used as the secondary antibodies. Detection was by the chemiluminescence method (ECL Western Blotting Detection Reagents, Amersham).

In Situ Hybridization—*In situ* hybridization was performed using Hybrid-Ready Tissues (Rat Adult Tissues and Rat Embryo Tissues, Novagen, Madison, WI) according to

the SureSite II System Manual (Novagen). The probes were prepared by an *in vitro* transcription reaction in the presence of [α-^{33}P]UTP (2,500 Ci/mmol, Amersham) using Riboprobe Combination Systems (Promega), and purified by 5% acrylamide gel electrophoresis. The PCR fragment of MIBP1 used in the Northern blot analysis served as a template for making sense and antisense RNA probes. After hybridization, the slides were exposed to X-ray film (Bio-Max MR-1, Kodak), and then dipped in emulsion (NTB-2, Kodak). The slides were counterstained with hematoxylin.

RESULTS

Downregulation of c-myc Gene Expression by MIBP1—We examined the potential role of MIBP1 in c-*myc* regulation, since MIBP1 was originally isolated as a binding protein to the c-*myc* intron 1 region (*1*). A full-length MIBP1 expression plasmid was transiently transfected into COS-1 cells together with the various CAT reporter plasmids shown in Fig. 1A. The inhibitory effect by MIBP1 was observed when pBBitCAT (–1302 to +1994) was used as a reporter plasmid, whereas no such inhibition was observed with the control reporter plasmid, pBLCAT20, which has the minimal promoter of HSV-*tk* and no enhancer elements (Fig. 1B, BBit and control). Consistent repression by MIBP1 was observed on other target plasmids, pBYCAT (–1302 to +573) and pBBCAT (–1302 to +410) (Fig. 1B, BY and BB). Thus, although the binding site of MIBP1 was initially identified in the c-*myc* intron 1, other sites seem to be present within exon 1 and its upstream region where MIBP1 exerts its inhibitory action, as discussed later.

A similar level of inhibition by MIBP1 was observed with pBMCAT, which contains exon 1 and 159 bp upstream, but lacks most of the upstream region, including the P1 promoter (Fig. 1B, BM). Thus, MIBP1 inhibits transcription from the major promoter of c-*myc*, P2. Within exon 1, there are three segments that are similar to the reported binding sites of MIBP1 (*2, 5, 12*), *i.e.*, one NF-κB–like motif and two TC-rich motifs. MIBP1 may exert its inhibitory effect by using some of these sequence motifs, although we have not identified which of the motifs are responsible for the repression.

Two-Hybrid Screening for MIBP1 Binding Proteins—To characterize the molecular mechanism of transcriptional regulation by MIBP1, we searched for its binding proteins by the yeast two-hybrid system. A fusion protein consisting of the yeast GAL4 DNA-binding domain and a portion of MIBP1 carrying the C-terminal Zinc finger motif–Acidic region–SPKK repeat (MZAS) was used as bait (Fig. 2). These motifs are conserved among MBP family members and are thought to play an important role in transcriptional regulation. The bait expression plasmid and the pACT2-based mouse pre-B cell library were used for sequential transformation of the yeast strain Y190. Among the 10^6 yeast transformants, 50 clones showed His$^+$ and LacZ$^+$ phenotypes. cDNA clones were recovered from these colonies and sorted into 18 independent groups according to the results of sequencing or restriction mapping.

The strengths of interactions were compared by liquid β-galactosidase assay and colony-lift filter assay, using yeast transformed with the bait plasmid and several representative plasmids of each group (data not shown). Clones of one group (B20, B33, and B49) demonstrated strong β-galactosidase activity specifically in the presence of the bait. In-frame fusion with the GAL4 transactivation domain was confirmed by sequencing these three clones. A BLAST

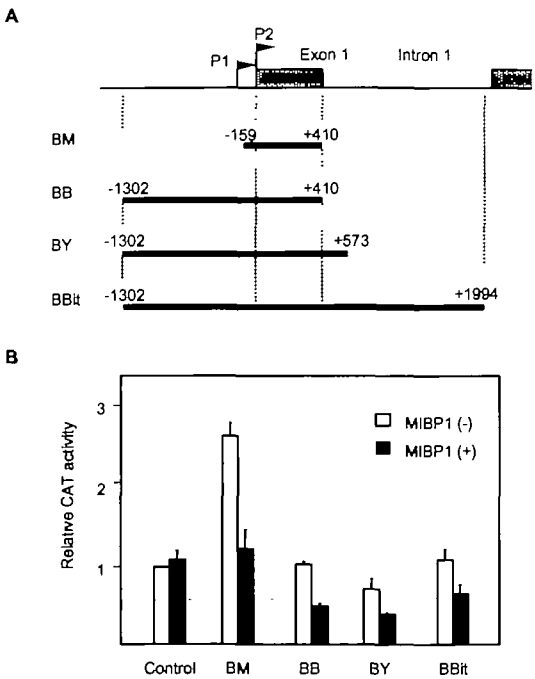

Fig. 1. **Transcriptional repression of c-*myc* by MIBP1.** (A) Various rat c-*myc* genomic fragments used in the CAT assay. Reporter plasmids used were: BM, pBMCAT; BB, pBBCAT; BY, pBYCAT; BBit, pBBitCAT. (B) The effect of MIBP1 on various CAT reporter plasmids. CAT activity was derived from at least two independent experiments, each performed in duplicate, and normalized to co-transfected β-galactosidase activity. The values represent relative CAT activity as determined by comparing the CAT activity of COS-1 cells co-transfected with the control plasmid pBLCAT20 and pCAE-X.

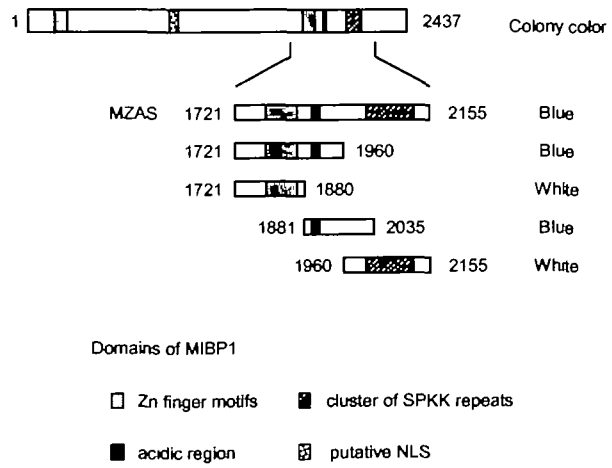

Fig. 2. **Schematic representation of MIBP1 and various constructs used in the yeast two-hybrid system.** The structure of MIBP1 is shown with those of MIBP1 Zinc finger motifs–Acidic region–SPKK repeats (MZAS) and its derivatives. The results of the colony-lift filter assay, showing interactions between various MZAS deletion constructs and SKIP 5–313 amino acid residues (encoded by clone B49), is indicated to the right.

search against non-redundant nucleotide databases in Genbank revealed that the three clones encode parts of a mouse homolog of the human nuclear protein, SKIP, which was originally isolated as a protein that binds to Ski oncoprotein (*26*).

To characterize the full coding region of the mouse SKIP cDNA, we searched the mouse EST database in Genbank and identified a cDNA clone, MNCb-2916 (accession no.

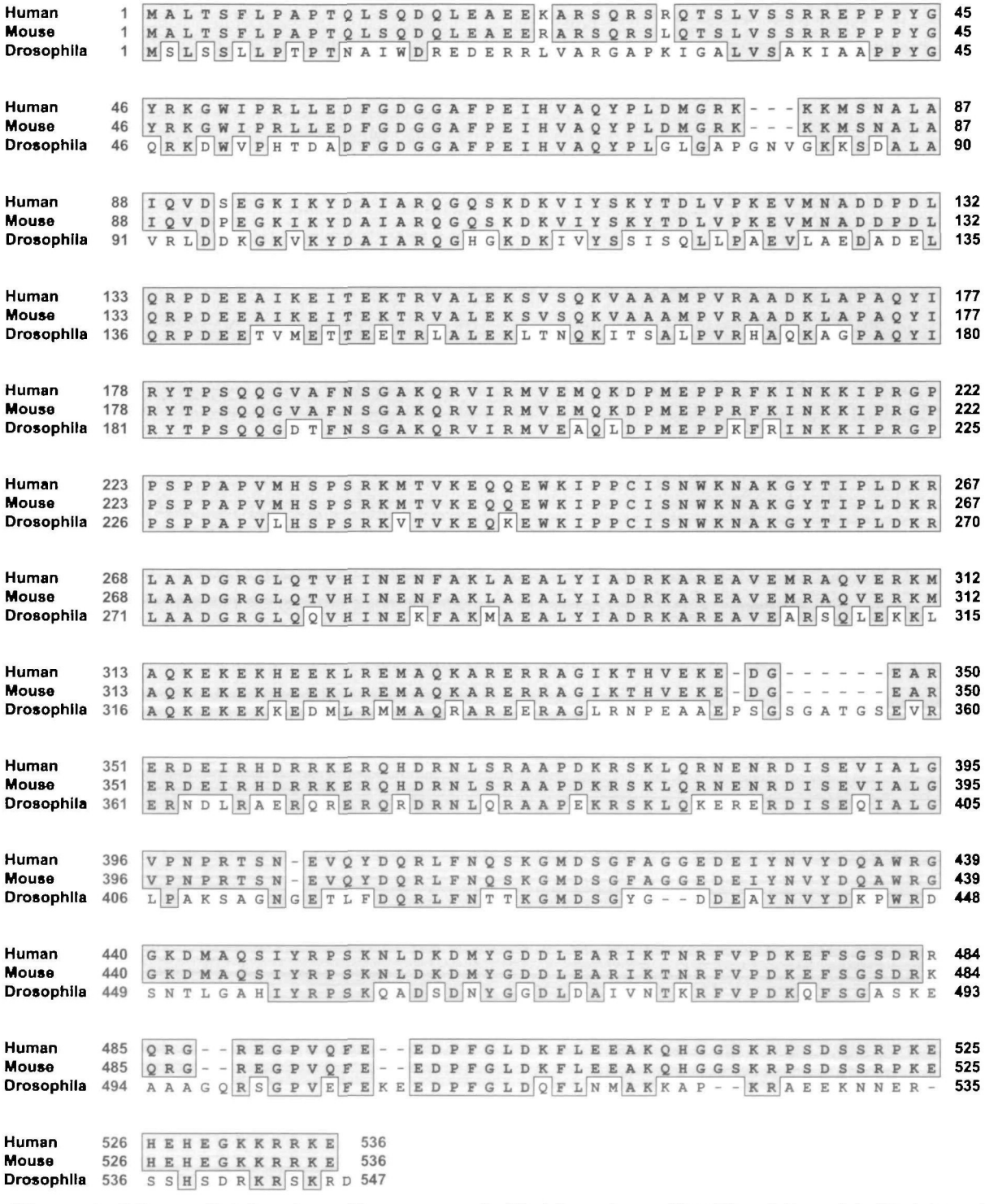

Fig. 3. **Alignment of the predicted amino acid sequences of SKIP homologs from human, mouse, and *Drosophila*.** The amino acid sequence of mouse SKIP was derived from a cDNA clone MNCb-2916, except for two residues, which were corrected according to other EST sequences of mouse SKIP. The sequences of human SKIP (accession no. U51432) and *Drosophila* SKIP (accession no. X64536) were sourced from Genbank. Shaded boxes indicate identical amino acids. The alignment was generated using MacVector (Oxford Molecular group).

AU067071). Sequence determination of this clone revealed it to contain the full coding region of mouse SKIP (536 amino acid residues) (Fig. 3). B20 and B33 encode the region from 2 to 313 amino acids and B49 encodes 5 to 313 amino acids of mouse SKIP. This indicates that the region in SKIP that is involved in the interaction with MIBP1 is located in the N-terminal half.

Alignment of the amino acid sequence of mouse SKIP with that of its human counterpart revealed extremely high homology between them. Among the 536 amino acid residues, 532 are identical and two are similar (Fig. 3). We also noted a remarkable conservation between the mammalian SKIP and its *Drosophila* homolog Bx42 (61% identity and 13% similarity), which was originally identified as a chromatin binding protein. The middle region of the SKIP protein is especially conserved between mouse and *Drosophila* (approximately 95% of residues 170 to 340 in mouse), indicating its role in the fundamental process of chromatin-mediated transcriptional regulation.

SKIP-Interacting Domain within MZAS—To identify the region of MIBP1 that interacts with SKIP, various MZAS deletion plasmids were constructed and examined by colony-lift filter assay in a yeast two-hybrid system, using the N-terminal half of the SKIP, encoded by B49, as a binding protein (Fig. 2). The results indicate that the segment of MIBP1 that includes the acidic region, but not the zinc-finger or SPKK repeat regions, is responsible for the interaction. Since the N-terminal half of SKIP contains many positively charged amino acids residues (pI = 9.6), it may be reasonable to assume that ionic interactions are involved in the binding.

In Vitro and In Vivo Interactions—To confirm the interaction observed in the yeast two-hybrid system, *in vitro* pull-down assays were performed using the MZAS region of MIBP1, expressed in the insect cell line Sf21 as a GST fusion protein, and the N-terminal half of SKIP prepared by *in vitro* transcription/translation of clone B49. As shown in Fig. 4A, the *in vitro* translated N-terminal half of SKIP coprecipitated with GST-MZAS. This binding was not observed between GST-MZAS and *in vitro* translated luci-

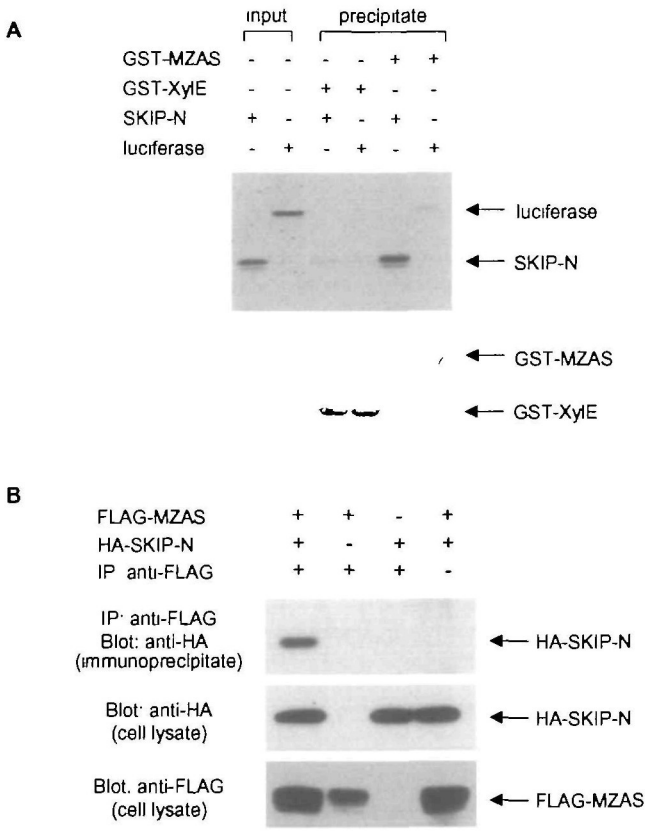

Fig. 4. ***In vitro* and *in vivo* interaction between the MZAS region of MIBP1 and the N-terminal half of SKIP.** (A) *In vitro* interaction as examined by the GST pull-down assay is shown. The interaction between the N-terminal half of SKIP (SKIP-N) and GST-MZAS was tested as indicated on top. Luciferase and GST-XylE fusion protein served as negative controls. *In vitro* translated proteins were visualized by autoradiography (upper panel). The expression of SKIP-N and luciferase are shown in the input lanes (5% of total lysate). Proteins in the precipitates were visualized by Coomassie Brilliant Blue staining (lower panel). (B) *In vivo* interaction examined by immunoprecipitation is shown. COS-1 cells were transfected with the FLAG-tagged MZAS region (FLAG-MZAS) and the HA-tagged N-terminal half of SKIP (HA-SKIP-N) as indicated on top. FLAG-MZAS and its associated proteins were immunoprecipitated from the lysates with anti-FLAG agarose, and analyzed by Western blot analysis using anti-HA antibody (upper panel). Protein G Sepharose was used as a negative control (fourth lane). The expression of each protein in the lysates was confirmed using anti-HA (middle panel) or anti-FLAG antibodies (lower panel).

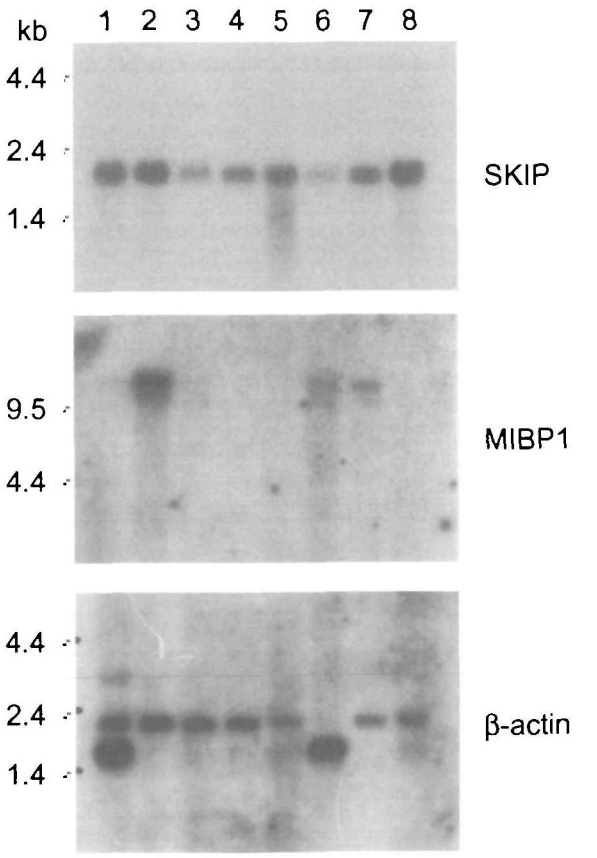

Fig. 5. **Expression of SKIP and MIBP1 mRNA in rat tissues.** MIBP1 was used as a probe in the Northern blot analysis of RNA from various tissues (middle panel). The filter was reprobed with the SKIP and β-actin probes (top and bottom panel, respectively). Lanes 1 through 8 contained 2 μg of polyA$^+$ RNA from heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis.

ferase, nor between GST-fused *Pseudomonas* XylE protein and SKIP. These results indicate that the MZAS region interacts specifically with the N-terminal half of SKIP, *in vitro*. We also tested possible *in vitro* interactions between MZAS and other proteins that are encoded by the remaining 17 cDNA groups isolated in the screening. The results show that the observed binding was negative or extremely weak (data not shown).

Having demonstrated the *in vitro* association, we sought to examine whether the MZAS region associates with the N-terminal half of SKIP *in vivo*. The MZAS region tagged with the FLAG epitope, and the N-terminal half of SKIP tagged with the HA epitope, were co-expressed in COS-1 cells, and the cell lysate was subjected to immunoprecipitation using anti-FLAG antibody (Fig. 4B). The results showed that the N-terminal half of SKIP was precipitated only in the presence of FLAG-tagged MZAS. Thus, we concluded that the MZAS region of MIBP1 interacts specifically with the N-terminal half of SKIP, both *in vitro* and *in vivo*.

SKIP mRNA Expression among Tissues—We then examined the expression of SKIP in various rat tissues and found that the mRNA was expressed at a high level in several organs including brain, where conspicuous MIBP1 expression was observed (Fig. 5). SKIP expression was also high in the heart, liver, and testis, but low in the spleen and skeletal muscle. The broad spectrum of expression of SKIP is in accordance with the previous observations from humans (*27*), and suggests that this transcriptional regulator is involved in a variety of biological processes.

In Situ Hybridization Analysis of MIBP1 in Adult Rat Brain—Our Northern blot analyses (Fig. 5), as well as previous reports, indicate that the brain is the major tissue in which MIBP1 is expressed at a high level (*1, 5, 10*). In an effort to determine which types of cells in the brain express the mRNA *in vivo*, we examined MIBP1 distribution in adult rat brain by *in situ* hybridization. MIBP1 mRNA was strongly expressed in the olfactory bulb, cerebral cortex, hippocampal pyramidal cell layer, granule cell layer of the dentate gyrus, and cerebellum (Fig. 6A). The signal intensity in the olfactory bulb was high and seemed different from the previous results obtained in mouse (*5*). In the cerebral cortex, a positive signal was detected strongly in neurons but not in glial cells (Fig. 6B). In the olfactory bulb, a positive signal was strongly detected in the granule cell layer (Fig. 6C). Only a faint signal was detected in the subependymal layer where neuronal progenitor cells proliferate, some of which migrate and form a granule cell layer (*28*). These results suggest that MIBP1 is expressed mainly in mature neurons, and that expression in immature neurons and glial cells is low in adult brain.

Expression Patterns of MIBP1 in Developing Rat Embryos—The expression of MIBP1 mRNA was also examined using rat embryonic sections 10 to 18 days (E10 to E18) after the start of gestation. Before E15, expression was low in all tissues examined (data not shown). Expression became evident in the forebrain at E16, and was increasingly detected at E17 and E18 (Fig. 7A and data not shown). Expression was also detected in other tissues, such as the tongue (Fig. 7A), but the signal in the forebrain was far more conspicuous. Higher magnification of the forebrain revealed the expression to be restricted to the cortical plate, a region composed mainly of post-mitotic neurons, and no signals were detected in the neuroepithelium, subventricular zone or intermediate zone (Fig. 7B).

Our results indicate that the expression of MIBP1 becomes more prominent in the brain area as development proceeds. Perhaps, it is safe to say that the expression of MIBP1 is controlled spatially and temporally, and differentiating, post-mitotic neurons are the major site of MIBP1

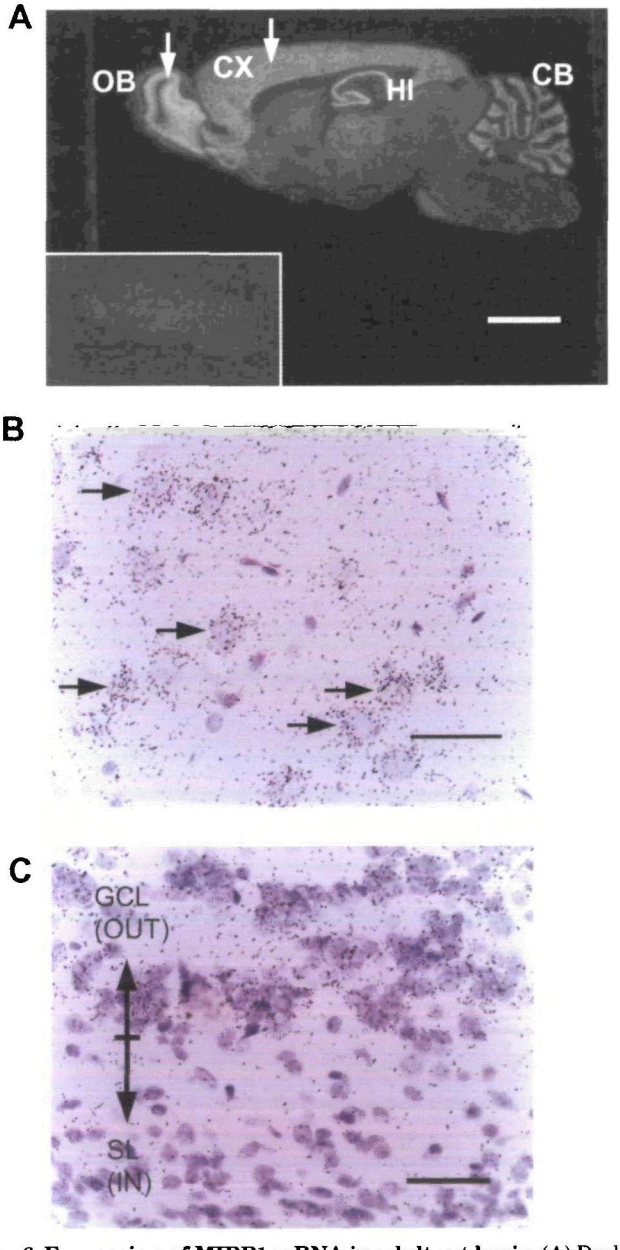

Fig. 6. **Expression of MIBP1 mRNA in adult rat brain.** (A) Dark field view of *in situ* hybridization. Sense or antisense probes of MIBP1 hybridized to a sagittal section of adult rat brain. Signals from the antisense probe were detected in the olfactory bulb (OB), cerebral cortex (CX), hippocampus (HI), and cerebellum (CB). The sense probe showed weak non-specific background signals (inset of A). White arrows indicate the positions of the high magnification views shown in B (cerebral cortex) and C (olfactory bulb). (B) A dense signal was localized to the periphery of large nuclei of neurons. (C) A positive signal was observed in the granule cell layer (GCL), but not in the subependymal layer (SL). Scale bars: 3 mm (A) and 30 μm (B and C).

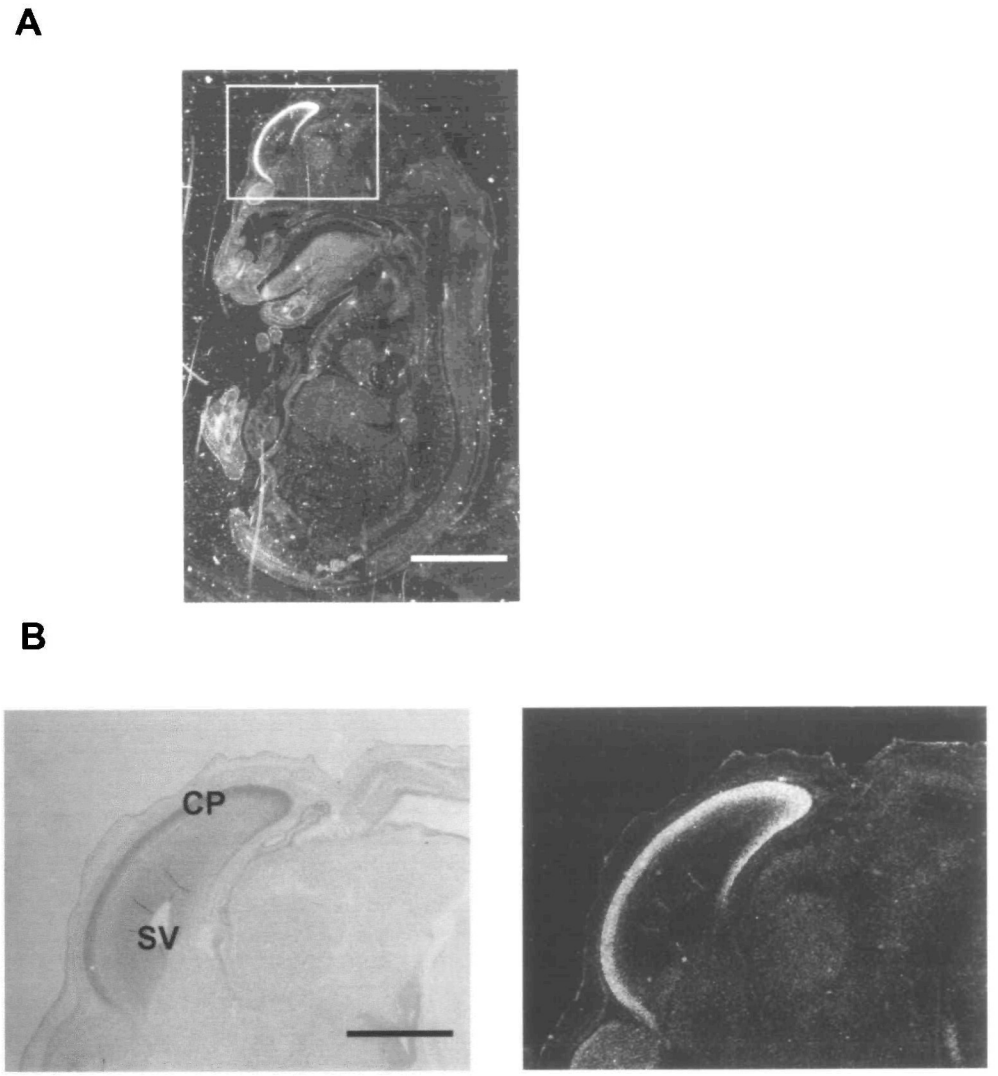

Fig. 7. **Expression of MIBP1 mRNA in rat embryos.** (A) Expression of MIBP1 mRNA at embryonic day 17 is shown. The MIBP1 probe was the same as used in Fig. 6. (B) Bright-field (left) and dark-field (right) photographs of the higher magnification of the boxed area in A are shown. A strong signal was detected in the cortical plate of the frontal cortex (CP). Note that no signal was detected in the subventricular zone (SV). Scale bar: 3 mm (A) and 1 mm (B).

expression. MIBP1 may be involved in the terminal differentiation or maintenance of neurons, although further refined experiments are needed to confirm this hypothesis.

DISCUSSION

Proteins of the MBP family have been shown to bind to the *cis*-elements of various genes involved in immune responses, angiogenesis, and neuroendocrine function. One such target gene is c-*myc*, which is involved in oncogenesis, immortalization and cell differentiation. In this study, we observed that MIBP1 represses c-*myc* transcription by about twofold. This result is consistent, with regard to repressor activity, with previous observations that a portion of MIBP1 (AGIE-BP1) shows transcriptional repression of the angiotensinogen gene (*3*). Recently, αA-CRYBP1/PRDII-BF1, another member of the MBP family, has also been shown to repress Col2α1 expression by twofold (*11*). In contrast, MIBP1 is reported to enhance transcription from the SSTR-2 promoter in cooperation with another transcription factor, SEF-2 (*5*). This apparent duplicity of MIBP1 in transcriptional regulation (*i.e.*, activation and repression) resembles the actions of Shn, which activates *Ultrabithorax* expression while repressing *brinker* expression (*19, 29*).

SKIP, identified here as an MIBP1 binding protein, has also been shown to be involved in transcriptional activation and repression. SKIP binds to the vitamin D receptor, MyoD, and Smad proteins and functions as a coactivator (*27, 30, 31*). In contrast, SKIP represses transcription when fused to the GAL4 DNA binding domain (*32*). Its interaction with SKI, Sin3A, and SMRT, which form a histone deacetylase complex, is implicated in coactivator/corepressor activity (*32, 33*). SKIP itself would be a ubiquitous factor rather than a determinant of tissue-specific transcriptional regulation as is indicated by its broad tissue distribution (Fig. 5).

The biological significance of the interaction between MIBP1 and SKIP has not been determined. In our preliminary study, we could not demonstrate that MIBP1 and SKIP act cooperatively to regulate c-*myc* expression (data not shown). Perhaps the system we adopted lacks other proteins needed for their cooperative action.

In previous extensive studies, members of MBP family were assumed to specifically recognize NF-κB–like sequences (*2, 8, 12*). However, a recent study by Dorflinger *et*

al. showed that MIBP1 also binds to a TC-rich motif in the SSTR-2 gene region (*5*), while Tanaka *et al.* showed that αA-CRYBP1 binds to an AG-rich sequence motif (*11*). Thus, a large variety of genes may be target genes.

We show here that differentiating, post-mitotic neurons are the major site of MIBP1 expression in the developing embryo and adult rat brain (Figs. 6 and 7). c-*myc* is expressed only in rapidly proliferating tissues of the murine embryo and is not expressed in non-proliferating tissues, including the cortical plate (*34, 35*), where MIBP1 is strongly expressed. It is conceivable that the physiological role of MIBP1 may be to downregulate c-*myc* expression or keep expression in a repressed state in post-mitotic neurons. However, there must be other target genes for MIBP1 involved in the homeostasis of neuronal cells. Further studies may reveal diverse functions of this gigantic transcription factor, MIBP1.

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