The Role of Human MBF1 as a Transcriptional Coactivator

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Multiprotein bridging factor 1 (MBF1) is a coactivator which mediates transcriptional activation by interconnecting the general transcription factor TATA element-binding protein and gene-specific activators such as the Drosophila nuclear receptor FTZ-F1 or the yeast basic leucine zipper protein GCN4. The human homolog of MBF1 (hMBF1) has been identified but its function, especially in transcription, remains unclear. Here we report the cDNA cloning and functional analysis of hMBF1. Two isoforms, which we term hMBF1α and hMBF1β, have been identified. hMBF1α mRNA was detected in a number of tissues, whereas hMBF1β exhibited tissue-specific expression. Both isoforms bound to TBP and Ad4BP/SF-1, a mammalian counterpart of FTZ-F1, and mediated Ad4BP/SF-1-dependent transcriptional activation. While hMBF1 was detected in the cytoplasm by immunostaining, coexpression of the nuclear protein Ad4BP/SF-1 with hMBF1 induced accumulation of hMBF1 in the nucleus, suggesting that hMBF1 is localized in the nucleus through its binding to Ad4BP/SF-1. hMBF1 also bound to ATF1, a member of the basic leucine zipper protein family, and mediated its activity as a transcriptional activator. These data establish that the coactivator MBF1 is functionally conserved in eukaryotes.

Transcription factors are roughly divided into two categories, general and gene-specific, on the basis of their function in a transcription reaction. The general transcription factor is essential for constructing an active transcription initiation complex including RNA polymerase II (1–3). The initiation complex is formed around a transcription start site and yields a basal level of transcription (4, 5). In contrast, the gene-specific transcription factor binds to a specific DNA sequence and is not required for basal level of transcription, but can up- or downregulate the basal activity. Recently, a third category of proteins which influence transcription, termed coactivator or mediator proteins, has been identified (6, 7). Coactivators enhance transcription by exerting their effect on both general transcription factors and gene-specific transcription factors. Multiprotein bridging factor (MBF)1 has been purified from extracts of the silkworm Bombyx mori and identified as a transcriptional coactivator (8, 9). Insect MBF1 stimulates transcription in vitro from the fushi tarazu promoter through its binding to the general transcription factor TBP and a nuclear receptor FTZ-F1. It has been shown that a yeast MBF1 homolog plays an essential role in GCN4-dependent transcriptional activation (10). In addition, a human MBF1 homolog has been identified as a factor involved in repression of differentiation of human endothelial cells (11), but its function in transcription remained obscure.

FTZ-F1 is a positive regulator of the fushi tarazu gene in blastoderm embryo of Drosophila (12). Ad4BP/SF-1 is thought to be the mammalian homolog of FTZ-F1 because of the marked similarity of their amino acid sequences (13, 14). Despite their structural similarity, the functions and distributions of FTZ-F1 and Ad4BP/SF-1 appear to be different. Expression of Ad4BP/SF-1 is detectable at high levels in the steroidogenic adrenal gland and gonads and, at lower levels, in the nonsteroidogenic pituitary, hypothalamus, and spleen (15, 16). With respect to function, it is known that Ad4BP/SF-1 regulates the production of corticosteroids and sex steroids by controlling expression of the steroidogenic P450s (17–19). Ad4BP/SF-1 is also implicated in sex differentiation of the gonads, since it has been shown to act as a crucial transcriptional activator of the Müllerian inhibitory substance gene, the product of which is an essential factor for the development of male reproductive tracts (20, 21). Therefore, it appears that Ad4BP/SF-1 plays an important role in the establishment of reproductive function in addition to its role in the production of steroid hormones (22).

To test a possible functional relationship between MBF1 and Ad4BP, we cloned a cDNA encoding human MBF1 (hMBF1). Two isoforms, which we term hMBF1α and hMBF1β, were identified. They share an identical amino-terminal region, but the carboxyl-terminal amino acid sequence of hMBF1β is different from that of hMBF1α. hMBF1 bound to both TBP and Ad4BP/SF-1 and stimulated transcription in vivo in an Ad4BP-dependent fashion, suggesting that hMBF1 acts as a coactivator in a similar fashion to its insect and yeast counterparts. hMBF1 was mainly present in the cytoplasm when expressed in the absence of other factors, but when co-expressed with Ad4BP/SF-1, the majority of hMBF1 was detected in the nu...

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1 The abbreviations used are: MBF1, multiprotein bridging factor 1; TBP, TATA element-binding protein; UZIF, basic leucine zipper; GST, glutathione S-transferase; EDF-1, endothelial differentiation-related factor 1; DBD, DNA-binding domain; aa, amino acids; PCR, polymerase chain reaction; ATF, activating transcription factor; CREB, cAMP response element-binding protein; CREB1, CREB protein 1.
cles in COS-1 cells. We also found that hMBF1 bound to a basic leucine zipper (bZIP) protein, ATF1, c-Jun, and c-Fos, and stimulated transcription from a promoter containing ATF1-binding sites when ATF1 was coexpressed with hMBF1 in COS-1 cells. FTZ-F1, Ad4BP/SF-1, GCN4, ATF1, c-Jun, and c-Fos contain a conserved basic region in their DNA-binding domains. These findings suggest that the coactivator hMBF1 mediates transcriptional activation through its interaction with a DNA binding regulator carrying the conserved basic region.

**MATERIALS AND METHODS**

**cDNA Cloning**

A PCR product of approximately 140 base pairs was amplified from total genomic DNA (100 ng) prepared from HeLa cells, using primers designed to match the cDNA sequence of *B. mori* MBF1. Using this PCR product as a probe, we screened a HeLa cell cDNA library and obtained full-length cDNAs encoding the two hMBF1 isoforms hMBF1α and hMBF1β. The cDNAs were sequenced by the dideoxy chain termination method using a 373A DNA sequencer (ABI).

**Plasmids**

The plasmids pET-hMBF1α and pET-hMBF1β were constructed by using PCR to introduce *Nco*I and *Bam*HI sites at the 5′ and 3′ ends, respectively, of the cDNA clones encoding hMBF1α and hMBF1β. These PCR products were then cloned between the *Nco*I and *Bam*HI sites of the vector pET3d (NOVAGEN). In order to express GST-hMBF1 fusion proteins, PCR-generated *Bam*HI-*Eco*RI fragments containing various portions of the hMBF1α and hMBF1β sequences were cloned into the *Bam*HI-*Eco*RI sites of the vector pGEX-2T (Amersham Pharmacia Biotech). The plasmids pGST-hMBF1α and pGST-hMBF1β, and derivatives thereof, were constructed in this manner.

The plasmids pCA-hMBF1α and pCA-hMBF1β were constructed by digesting pET-hMBF1α or pET-hMBF1β with *Bgl*II and *Bam*HI and subcloning the resulting hMBF1-encoding fragments into the *Bam*HI site of the pCAGGS mammalian expression vector. The SV40 origin was removed from pCA-hMBF1α and pCA-hMBF1β by digesting with *Bam*HI to yield pCA-hMBF1α(−) and pCA-hMBF1β(−).

pET-Ad4BP and pET-Ad4BP-DBD were constructed as follows. The DNA fragments encoding bovine Ad4BP/SF-1 and its DNA-binding domain were prepared by digesting pSVL-Ad4BP (14) with *Sac*I and *Bam*HI, and *Sac*I and *Sal*I, respectively. These DNA sequences were end-filled using the Klenow fragment of DNA polymerase I (Klenow) and subcloned into the vector pET14bk (Novagen), which had been digested with *Nde*I and end-filled with Klenow fragment. The plasmids pGEX-Ad4BP-WT, -mut1, -mut2, and -mut3 were constructed by using PCR to introduce *Bam*HI and *Sac*I sites at the 5′ and 3′ ends, respectively, of the cDNA clone encoding Ad4BP/SF-1, which was then subcloned into pGEX-2T (Amersham Pharmacia Biotech). pGEX-ATF1-WT, -AcZIP, -bZIP, and -BR, pGEX-cJun-bZIP, and pGEX-cFos-bZIP were constructed as described elsewhere (23). pM-FLAG-Ad4BP was constructed by digesting pUC-Ad4BP as described above and subcloning the resulting DNA fragment encoding bovine Ad4BP/SF-1 into the vector pM-FLAG, which had been cleaved with *Mlu*I and end-filled with Klenow fragment. pG4-Ad4 contained four tandemly repeated Ad4 sequences inserted into the *Bgl*II site of the pGV-P (TOYO INK co., Tokyo, Japan) as described elsewhere (14).

**Expression of Proteins in E. coli**

Expression plasmids other than pET-Ad4BP or pET-Ad4BP-DBD were introduced into *E. coli* BL21 (DE3) and protein expression was induced by addition of 0.4 mM isopropyl-1-thio-β-D-galactopyranoside followed by shaking at 37 °C for 4 h. pET-Ad4BP and pET-Ad4BP-DBD were introduced into BL21 (DE3) carrying a thioredoxin expression plasmid as described elsewhere (24) and protein expression was induced by addition of 0.4 mM isopropyl-1-thio-β-D-galactopyranoside followed by shaking at 30 °C for 3 h. Purification of the histidine-tagged or GST-tagged proteins was performed according to the manufacturer’s instructions (Qiagen) or (Amersham Pharmacia Biotech).

**Northern Blot Analyses**

Northern blot analyses of a human multiple tissue blot (CLONTECH) were performed according to the manufacturer’s instructions. A cDNA fragment encoding the NH2-terminal region of hMBF1 (amino acids 1–108) and oligonucleotides complementary to the specific carboxyl termini of hMBF1α or hMBF1β were used as DNA probes (Fig. 1A).

**Gel Mobility Shift Assay**

**TBPs—**The TATA element of the adenovirus E4 promoter was primered from pUCE4–64 (26). The EcoRI-HindIII fragment containing the TATA element was radiolabeled using Klenow fragment and [γ-32P]ATP. Binding reactions and electrophoresis were performed as described elsewhere (8).

**Ad4BP/SF-1—**Preparation of the DNA probe containing the Ad4BP/SF-1 binding element, binding reactions and electrophoresis were performed as described elsewhere (14). The binding reaction mixture contained 15 mM Hepes (pH 7.9), 1 mM EDTA, 12% glycerol, 1 mM dithiothreitol, 4 mg/ml bovine serum albumin, 500 mM KCl, and purified recombinant His-hMBF1 as a Transcriptional Coactivator

Recombinant His-TBP and His-Ad4BP were labeled in vitro with [γ-32P]ATP using the catalytic subunit of protein kinase. Glutathione S-transferase (GST) or GST-hMBF1 (400 ng) were mixed with the labeled proteins in 500 μl of GST binding buffer (50 mM Tris-HCl (pH 7.9), 20% glycerol, 1 mM EDTA, 0.1% Nonidet P-40, 10 mM MgCl2, 0.1 mM CaCl2, 1 mM dithiothreitol) supplemented with 50 mM KCl for TBP and 500 mM KCl for Ad4BP/SF-1 and incubated at 30 °C (TBP) or 4 °C (TBP) for 1 h. The mixtures were then rocked for 1 h at 4 °C with 5 μl of glutathione-Sepharose beads (Amersham Pharmacia Biotech). The beads were washed three times with 1 ml of binding buffer and suspended in 10 μl of SDS-polyacrylamide gel electrophoresis sample buffer. The bound proteins were eluted by boiling and detected on a 12.5% SDS-polyacrylamide gel followed by autoradiography.

Recombinant His-hMBF1α (30 ng) was also incubated at 30 °C with 2 μg of GST-Ad4BP, GST-TAF1, GST-Jun, or GST-c-Fos protein immobilized on glutathione-Sepharose beads. After 1 h incubation, the beads were washed and the bound proteins were eluted as described above. His-hMBF1α was detected on a 12.5% SDS-polyacrylamide gel, transferred to polyvinylidene difluoride membrane (Millipore), and visualized using the anti-hMBF1α antibody and ECL (Amersham Pharmacia Biotech).

**Cell Culture and Reporter Gene Assay**

COS-1 and Y-1 cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum. For the reporter gene assay, 0.5 μg of pGV-Ad4, varying amounts of pCA-hMBF1α or pCA-hMBF1β, and 0.5 μg of pSV-β-gal (Promega) were transfected into Y-1 cells (1 × 10^6 cells/35-mm dish) using a calcium phosphate method (26). The cells were incubated for 24 h and then transfected with 1 μl of fresh medium. The cells were harvested 36 h later and luciferase and β-galactosidase activities were analyzed.

**Immunofluorescence Staining**

COS-1 cells (3 × 10^6 cells/35-mm dish) were transfected by the calcium phosphate method (26) with 1 μg of pCA-hMBF1(−) together with 10 μg of either pM-FLAG-Ad4BP or an empty vector. After 48 h, the cells were washed twice with cold phosphate-buffered saline (−) and fixed with 3.7% formaldehyde in phosphate-buffered saline (−) for 30 min. Immunostaining was performed with an anti-hMBF1 rabbit polyclonal antibody and an anti-FLAG mouse monoclonal antibody (Kodak), followed by detection with a rhodamine-conjugated anti-rabbit antibody (Chemicon) and an fluorescein isothiocyanate-conjugated anti-mouse antibody (Cappel). The rabbit anti-hMBF1α antibody was raised against His-hMBF1α using standard procedures. IgGs against hMBF1α were purified on a protein A-Sepharose column. DNA was stained with DAPI (SIGMA). The stained samples were observed with a Carl Zeiss Axiosphoto microscope.

**Immunoprecipitation Assay**

COS-1 cells (1 × 10^6) cells in five 100-mm dishes) were co-transfected with pCA-hMBF1α (10 μg) and pM-FLAG-Ad4BP (3 μg) as described above. After 48 h, the cells were lysed with 2 ml of a lysis buffer (50 mM Tris (pH 7.6), 100 mM NaCl, 1% Nonidet P-40). The total cell lysate was incubated with 20 μl of anti-FLAG M2 antibody-conjugated resin (Kodak) for 2 h at 4 °C. The immunoprecipitate was washed three times with the lysis buffer and eluted twice with 30 μl of 180 μM FLAG peptide (Kodak). The eluates (15 μl) were analyzed by Western blotting.
**RESULTS**

Cloning of hMBF1α and hMBF1β—Using primers based on the cDNA sequence of B. mori MBF1, we amplified a small section of the hMBF1 gene by PCR. We then used this fragment as a probe to isolate full-length cDNA clones of hMBF1 from a HeLa cell cDNA library. Three positive clones were obtained by using primers based on the hMBF1 sequence. The DNA region indicated by stippling (Fig. 1) showed 62.8% similarity, representing regions of identity. B. mori and hMBF1 differ in their carboxyl-terminal sequences (129 aa) but shared identical amino acid sequences of cDNAs encoding MBF1α and MBF1β. The DNA region indicated by stippling (Fig. 1A) showed 62.8 and 62.6% similarity, respectively, to that of B. mori MBF1 (Fig. 1B). On the basis of these similarities, we concluded that the cDNA clones represent bona fide human homologs of MBF1. Recently, a yeast MBF1 homolog has also been isolated (10). Together with our results, this indicates that MBF1 is a highly conserved protein across species from yeast to human. During the course of this work, Dragoni et al. (11) independently isolated a clone encoding a human protein homologous to B. mori MBF1, which they named endothelial differentiation-related factor 1 (EDF-1). We found that hMBF1α was identical to EDF-1.

Tissue Distribution of hMBF1α and hMBF1β—Northern blot analysis with a DNA probe containing most of the sequence common to hMBF1α and hMBF1β (Fig. 1A, underlined) detected a single 0.9-kilobase mRNA, a length consistent with that of the cDNA clones, in all tissues tested, although the intensity of the hybridizing bands differed among tissues (Fig. 2A). High levels of hMBF1 expression were observed in heart and pancreas. In contrast, lung and kidney expressed hMBF1 weakly (Fig. 2A). An hMBF1-specific DNA probe (Fig. 1A, dashed underline) revealed the same expression pattern as the probe which detected both isoforms (Fig. 2B). However, hMBF1 mRNA was detected with a specific probe (Fig. 1A, dashed underline) only in the pancreas (Fig. 2C). These results indicate that hMBF1α is a ubiquitous protein, whereas hMBF1β is a tissue-specific variant.

Interaction of hMBF1 with TBP—Both insect and yeast MBF1 have been shown to bind to TBP (9, 10). To determine whether hMBF1α also binds to human TBP, electrophoretic mobility shift assays were carried out using a DNA probe containing the TATA element derived from the adenovirus E4 promoter together with histidine-tagged TBP and GST-tagged MBF1. TBP bound to the probe, but neither GST nor GST-hMBF1 showed any binding (Fig. 3A). When GST-hMBF1α was added to the mixture containing TBP and the DNA probe, the migration of the band corresponding to probe-bound TBP was retarded (lane 5). This band shift was not observed when GST alone (lane 4) was added and disappeared when specific antibody against hMBF1α (lane 6) was added to the mixture, suggesting that GST-hMBF1α can bind to TBP on the TATA element. To confirm the interaction, GST pull-down assays were performed using in vitro 32P-labeled TBP and GST-hMBF1α or GST-hMBF1β (Fig. 3C). 32P-labeled TBP was recovered from beads fused to GST-hMBF1α or GST-hMBF1β but not from those carrying GST alone. Deletion analysis revealed that mutant 1, which lacked residues 1–68 of hMBF1, did not bind to TBP. In contrast, mutant 4, which lacked residues 1–108 of hMBF1, retained the ability to bind to TBP. Taken together, these results demonstrate that hMBF1α can bind to human TBP and suggest that the central region (aa 69–108) of hMBF1α is required for this binding.

Interaction of hMBF1 with Ad4BP/SF-1—Both insect and yeast MBF1 have been shown to bind to TBP (9, 10). To determine whether hMBF1α also binds to human TBP, electrophoretic mobility shift assays were carried out using a DNA probe containing the TATA element derived from the adenovirus E4 promoter together with histidine-tagged TBP and GST-tagged MBF1. TBP bound to the probe, but neither GST nor GST-hMBF1 showed any binding (Fig. 3A). When GST-hMBF1α was added to the mixture containing TBP and the DNA probe, the migration of the band corresponding to probe-bound TBP was retarded (lane 5). This band shift was not observed when GST alone (lane 4) was added and disappeared when specific antibody against hMBF1α (lane 6) was added to the mixture, suggesting that GST-hMBF1α can bind to TBP on the TATA element. To confirm the interaction, GST pull-down assays were performed using in vitro 32P-labeled TBP and GST-hMBF1α or GST-hMBF1β (Fig. 3C). 32P-labeled TBP was recovered from beads fused to GST-hMBF1α or GST-hMBF1β but not from those carrying GST alone. Deletion analysis revealed that mutant 1, which lacked residues 1–68 of hMBF1, did not bind to TBP. In contrast, mutant 4, which lacked residues 1–108 of hMBF1, retained the ability to bind to TBP. Taken together, these results demonstrate that hMBF1α can bind to human TBP and suggest that the central region (aa 69–108) of hMBF1α is required for this binding.
ing activity of Ad4BP/SF-1 through interaction with the DNA-binding domain of Ad4BP/SF-1.

To test for a direct interaction between hMBF1 and Ad4BP/SF-1, we performed GST pull-down assays using purified recombinant proteins. 32P-Labeled Ad4BP/SF-1 was recovered from glutathione-Sepharose beads carrying GST-hMBF1α and GST-hMBF1β but not from beads carrying GST alone (Fig. 4 C). We then tested a series of GST-tagged deletion mutants of hMBF1α. Neither mutant 1 (aa 1–68) nor mutant 5 (aa 109–148) produced a detectable band. In contrast, 32P-labeled Ad4BP/SF-1 was recovered from beads carrying either mutant 3 or mutant 4, both of which contained the central region of hMBF1 (aa 69–108). These results suggest that the central region of hMBF1, which also contains the TBP-binding domain described above, is essential for interaction with Ad4BP/SF-1.

**Interaction of hMBF1 with bZIP Family Proteins—** Although it has been shown that yeast MBF1 specifically binds to a basic region in the bZIP domain of GCN4 (10), neither FTZ-F1 nor Ad4BP/SF-1 contain such a domain. However, FTZ-F1 shares some homology with the basic region of GCN4, that is conserved among bZIP proteins (10). This led us to examine whether hMBF1 bound to certain bZIP proteins. Electrophoretic mobility shift assays were performed using a DNA probe containing the ATF-binding site of the adenovirus E4 promoter. Histidine-tagged ATF1, CREB, and CREBP1 were expressed in E. coli and purified. These proteins were then

**Fig. 2.** Northern blot analysis of hMBF1α and hMBF1β in various human tissues. RNA blots containing 2 μg of human poly(A)1 RNA per lane (CLONTECH) were hybridized to probes containing most of the region common to hMBF1α and hMBF1β (A), the hMBF1α-(B), or the hMBF1β-specific region (C) or β-actin (D). N.S. indicates a nonspecific band.

**Fig. 3.** Interactions between hMBF1 and human TBP. A, electrophoretic mobility shift assay of the binding of hMBF1 to TBP. A 32P-labeled double-stranded oligonucleotide containing the TATA element of the adenovirus E4 promoter was incubated with 400 ng of GST or GST-hMBF1α, 30 ng of His-TBP, and/or 1 μl of anti-hMBF1α antibody. N.S. indicates a nonspecific band. B, schematic representation of the amino acid sequences of the wild-type and truncated hMBF1 proteins. The shadowed portions indicate the region required for TBP binding. C, GST pull-down assay of the binding of GST-hMBF1 to TBP. 32P-TBP was incubated with 400 ng of GST (lane 2), GST-hMBF1α (lane 3), GST-MBF1β (lane 4), or the GST-tagged MBF1 deletion derivatives (lanes 5–8). Lane 9 shows the same amount 32P-TBP as was included in the binding reactions.
added to binding reactions in the absence or presence of hMBF1α and the products were analyzed on polyacrylamide gels (Fig. 5A). The DNA binding activities of ATF1, CREB, and CREBP1 were stimulated by addition of hMBF1 to the reactions, in a similar manner to that in which hMBF1 enhanced the DNA binding activity of Ad4BP/SF-1 (Fig. 4A).

We further examined the direct interaction between His-hMBF1α and the basic region of bZIP proteins using GST pull-down assays followed by Western blotting with the anti-hMBF1α antibody (Fig. 5C). His-hMBF1α was recovered from glutathione-Sepharose beads carrying GST-Ad4BP-WT but not from the beads bearing GST alone. We then tested a series of...
GST-tagged deletion mutants of Ad4BP. GST-Ad4BP mt1 (aa 1–147) and mt3 (aa 83–461) both produced detectable bands, but mt2 (aa 148–461) did not. These results indicate that hMBF1α interacts with the region containing aa 83–147 of Ad4BP, which contains the basic region (aa 83–114). Next, we tested the interaction of hMBF1α with the GST-tagged bZIP proteins ATF1 (WT, ΔbZIP, bZIP, and BR), c-Jun (bZIP) and c-Fos (bZIP). His-hMBF1α was recovered from glutathione-Sepharose beads carrying GST-ATF1 (WT, bZIP, and BR), c-Jun (bZIP), and c-Fos (bZIP), all of which contained the basic region, but not from beads carrying GST-ATF1ΔbZIP. These results indicate that hMBF1α interacts specifically with transcription factors containing the conserved basic region and increases their DNA binding activities.

Induction of Ad4BP/SF-1-dependent Transcriptional Activation by hMBF1—To examine the effects of hMBF1 on Ad4BP/SF-1-dependent transcriptional activation in vivo, a pGV-Ad4 reporter construct was co-transfected with plasmids encoding hMBF1 into mouse adrenal Y-1 cells constitutively expressing Ad4BP/SF-1. The reporter plasmid carries the SV40 core promoter-driven luciferase gene containing four tandemly repeated Ad4BP/SF-1-binding elements within the promoter region. The transcriptional activity from the reporter plasmid was increased approximately 3.5-fold by expression of hMBF1α (Fig. 6). Approximately 4-fold stimulation of the transcriptional activity was also observed upon expression of hMBF1β (Fig. 6). Transcription from the control reporter construct lacking the Ad4BP/SF-1-binding site, pGV-P, was not stimulated by hMBF1 expression. The ectopic expression of hMBF1 in Y-1 cells was confirmed by a Western blot analysis using the anti-hMBF1 polyclonal antibodies (data not shown). These results suggest that hMBF1 mediates Ad4BP/SF-1-dependent transcriptional activation in vivo. We also performed co-transfection assays using reporter constructs carrying ATF1, AP-1, NF-κB, or E4TF1-binding elements in 293 cells. hMBF1 activated transcription from the promoter containing the ATF1-binding element, but had no effect on the AP-1 binding element or the other constructs, although hMBF1 bound to c-Jun and c-Fos in vitro GST pull-down assays (Fig. 5C). hMBF1 therefore appears to specifically mediate transcriptional activation by Ad4BP/SF-1 and ATF1.

Cellular Localization of hMBF1—To observe the distribution of hMBF1 in cells, specific polyclonal antibodies against hMBF1α were used to carry out indirect immunofluorescence staining. Western blot analysis showed that the polyclonal antibodies reacted with a 19- or 18-kDa protein when hMBF1α or hMBF1β, respectively, were expressed in COS-1 cells (data not shown). When the pCA-hMBF1α(−) construct, which does not contain an SV40 replication origin, was used for expression of hMBF1α in COS-1 cells, hMBF1α was localized in the cytoplasm (Fig. 7A). In contrast, the distribution of Ad4BP/SF-1 fused to the FLAG epitope tag (FLAG-Ad4BP) was restricted to the nucleus (Fig. 7B). When hMBF1α and FLAG-Ad4BP were coexpressed, the majority of hMBF1α and all of the FLAG-tagged Ad4BP/SF-1 were detected in the nucleus in 20% of the

**Fig. 6. Transcriptional activation by hMBF1 and Ad4BP/SF-1.** The expression plasmids pCA-hMBF1α or pCA-hMBF1β, or the mock plasmid, pCAGGS, were co-transfected with pSV-β-gal and the reporter plasmids, pGV-Ad4, or pGV-P, into Y-1 cells. The luciferase activities of the transfected cells (normalized to the β-galactosidase activities) are shown in the *lower panel*. The *upper panel* shows the construction of the reporter plasmids. The results shown are the averages (± S.E.) of five experiments.

**Fig. 7. Cellular localization of hMBF1α.** COS-1 cells were transfected with the expression plasmids pCA-hMBF1α(−) and pM-FLAG-Ad4BP either separately (A and B, respectively) or together (C). The cellular localization of hMBF1α and FLAG-Ad4BP was examined by immunofluorescence staining using anti-hMBF1α and anti-FLAG antibodies, respectively. DNA was stained with DAPI. D, immunoprecipitation assay of the binding of hMBF1α and FLAG-Ad4BP. pCA-hMBF1α and pM-FLAG-Ad4BP were co-transfected into COS-1 cells and FLAG-Ad4BP was immunoprecipitated with anti-FLAG antibody from the total cell lysate. Western blotting was performed using the anti-Ad4BP or the anti-hMBF1α antibody. Input indicates 10% of the amount of the total cell lysate. *IP* indicates an immunoprecipitate.
cells expressing both hMBF1α and FLAG-Ad4BP proteins (Fig. 7C). We also carried out the same experiments using the plasmid pCA-hMBF1α, which contains an SV40 replication origin and expressed a 20-fold higher level of hMBF1α protein than pCA-hMBF1α (−). Under these conditions, the hMBF1α protein was detected in the cytoplasm regardless of whether or not FLAG-Ad4BP was coexpressed (data not shown). It is likely that the molar excess of hMBF1α over Ad4BP/SP-1 increased the proportion of hMBF1α molecules in the cytoplasm. These results suggest that hMBF1α is transported into the nucleus through its binding to Ad4BP/SP-1. Next, to confirm the interaction between hMBF1α and Ad4BP/SP-1 in vitro, we carried out immunoprecipitation assays. hMBF1α was transfected into COS-1 cells with or without FLAG-Ad4BP and the cell lysate was subjected to immunoprecipitation using anti-FLAG M2 antibody-conjugated resin. Immunoprecipitates were analyzed by Western blotting using the anti-Ad4BP and anti-hMBF1α antibodies. When hMBF1α or FLAG-Ad4BP were transfected independently, hMBF1α was not detected in the immunoprecipitate (Fig. 7D). In contrast, when hMBF1α and FLAG-Ad4BP were co-transfected, hMBF1α was coimmunoprecipitated with FLAG-Ad4BP. This result, in conjunction with the results of GST pull-down assays (Fig. 5), indicates that hMBF1α interacts with Ad4BP/SP-1 both in vivo and in vitro.

**DISCUSSION**

We report here the cDNA cloning of a human counterpart of MBF1, which was originally identified as a mediator of the *Drosophila* transcriptional activator FTZ-F1 (9). We found two isoforms, hMBF1α and hMBF1β, which have a common NH2-terminal sequence and different COOH-terminal regions. Difference was also observed in their expression patterns. The mRNA encoding hMBF1α was found in all tissues tested whereas hMBF1β was only detected in pancreas and HeLa cells. Although we were unable to find a functional difference between hMBF1α and hMBF1β, it is possible that hMBF1β may play a unique role in the pancreas through its specific COOH-terminal region.

We showed here that both hMBF1α and hMBF1β can bind to TBP and to a mammalian homolog of FTZ-F1, Ad4BP/SP-1, as expected from the binding of insect MBF1 to TBP and FTZ-F1. These data support the notion that MBF1 serves as a coactivator by forming a bridge between DNA binding regulators and TBP (10). In addition, deletion analysis of hMBF1 showed that the central region (aa 69–108) of hMBF1, which is common to both isoforms, is necessary for the binding to TBP and Ad4BP/SP-1. It has been shown that the corresponding region of insect and yeast MBF1 proteins is also essential for protein-protein interactions (9, 10). Recent NMR studies provided evidence that the central region of eukaryotic MBF1 proteins consists of well conserved α helices and suggested that the four helices play an important role in protein–protein interactions.⁴

Previous studies have shown that insect and yeast MBF1 proteins interact with a conserved basic region in FTZ-F1 and GCN4, enhancing their DNA binding activities and thereby mediating transcriptional activation by these regulators (9, 10). In the present study, hMBF1 bound to the basic region of Ad4BP/SP-1, and also to ATF1, CREB, CREBP, c-Jun, and c-Fos. In addition, hMBF1 enhanced the DNA binding activity of Ad4BP/SP-1 and ATF1 family proteins. Transient expression assays revealed that hMBF1 mediated Ad4BP/SP-1- and ATF1-dependent transcriptional activation in transfected cells. In contrast, hMBF1 had no effect on AP-1-dependent transcriptional activation. Furthermore, hMBF1 had no effect on transcriptional activation by a regulator lacking for the conserved basic region such as NF-κB, SP-1, or E4TF1. These results illustrate the importance of the basic region conserved among certain DNA-binding regulators for their interactions with hMBF1. PC4 and several TBP-associated factors are known to mediate transcriptional activation by binding to the activation domain of regulators (28). In contrast, MBF1 is a unique coactivator that mediates transcriptional activation by forming a connection between the DNA-binding domain of regulators and TBP. In addition, MBF1 can enhance the DNA binding activity of regulators, and this property may contribute significantly to its coactivator function.

We also examined the distribution of hMBF1 in mammalian cells. When hMBF1 was ectopically expressed in COS-1 cells, the majority of the expressed protein localized in the cytoplasm. Coexpression of Ad4BP/SP-1 induced nuclear localization of hMBF1α and interaction with hMBF1 and Ad4BP/SP-1 as revealed by immunostaining and immunoprecipitation assays in COS-1 cells. On the basis of our results, we propose that hMBF1 interacts with newly synthesized regulators in the cytoplasm and then the complex moves to the nucleus to carry our transcriptional activation. This may allow temporal or tissue-specific regulation even though hMBF1α is a ubiquitous protein.

Recently, another group has independently cloned a clone identical to hMBF1α, as EDF-1 that is down-regulated upon differentiation of endothelial cells (11). hMBF1 therefore seems to be involved in the modulation of transcription during endothelial cell differentiation. However, the binding partner of hMBF1 in this process remains unknown.

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