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Cloning and characterization of mouse CCAAT binding factor

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ABSTRACT

Isolation of cDNA clones for the mouse CCAAT binding factor (mCBF) has revealed the expression of two distinct forms of mCBF that are generated by alternative splicing of a single primary transcript from a gene that maps to chromosome 17. The mCBF1 mRNA encodes a protein of 997 amino acids, whereas the mCBF2 protein is predicted to be only 461 amino acids in length; mCBF1 and human CBF (hCBF) share >80% amino acid sequence identity. Analysis of adult mouse tissue RNAs has revealed that the mCBF1 and mCBF2 mRNAs are ubiquitously expressed, but that mCBF1 mRNA is 5- to 10-fold more abundant than mCBF2 mRNA. Similarly, mCBF mRNA was detected throughout the placenta and in all tissues of the developing embryo from day 8 to day 18 of gestation. Overexpression of the two forms of mCBF in mammalian cells has demonstrated that the mCBF1 and mCBF2 proteins localize to different cellular compartments, with mCBF1 found predominantly in the nucleus and mCBF2 restricted to the cytoplasm. Co-expression of these two forms influences their localization, however, indicating that CBF activity can be regulated by the relative amounts of the two forms expressed in a cell.

INTRODUCTION

Previous investigations of human heat shock protein 70 gene (*hsp70*) expression led to the identification of a CCAAT box at –70 as a promoter element critical for serum-inducible transcription (1–3) and the protein CBF as the transcription factor that acts through this element (4). Although several CCAAT factors have been identified, some of which have been shown to be able to bind to the CCAAT element of the *hsp70* gene promoter (4–8), CBF appears to be unique in its ability to activate transcription from this promoter (4). In addition, CBF mediates activation of the *hsp70* gene promoter by the adenovirus E1a oncoprotein (9) and repression of this promoter by the p53 tumor suppressor protein (10). The effects of E1a and p53 on this promoter are apparently due to the ability of these regulatory factors to form protein–protein complexes with CBF (9,10). Thus CBF appears to represent a critical node in mammalian cells for both growth promoting and growth repressing signaling pathways.

To date the analysis of CBF has been restricted to the human factor. To expand these studies we sought to isolate the mouse homolog of hCBF and to use this cDNA clone to characterize the forms of CBF synthesized in the mouse, to compare the sequences of the mouse and human proteins to reveal conserved domains, to map the chromosomal location of the *Cbf* gene in the mouse and to analyze the developmental expression and the tissue distribution of CBF mRNA. The results reported here identify a previously undetected form of CBF and demonstrate that the ability of this transcription factor to move into the nucleus depends on the relative amounts of the different CBF forms present in the cell.

MATERIALS AND METHODS

Cell culture and transfections

BALB/c 3T3 and COS cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL) supplemented with 10% fetal calf serum (Gibco-BRL), glutamine, penicillin and streptomycin. Cell cultures were starved by incubation in medium containing 0.5% serum for 48 h and then stimulated by addition of fresh medium containing 15% serum for varying lengths of time. DNA transfections of COS cells were performed using DEAE–dextran (11). For these transfections 5×10^5 COS cells were transferred into each 10 cm dish 24 h before addition of 5–20 μg plasmid DNA. Cells were harvested 48 h post-transfection.

cDNA isolation and plasmid construction

Total RNA was purified from BALB/c 3T3 cells that had been stimulated with serum for 2, 4, 6 and 8 h by centrifugation of guanidinium thiocyanate lysates through CsCl cushions (12). Equal amounts of RNA from these four time points were combined, selected by oligo(dT)–cellulose chromatography for poly(A)$^+$ RNA and reverse transcribed into single-stranded

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cDNA. RNA–DNA duplexes were converted into double-stranded DNA using RNase H and DNA polymerase I (12) and inserted into the λZAP vector (Stratagene), resulting in a library of 1.3×10^6 independent clones. A random primed probe was prepared (12) from the hCBF cDNA (4) to screen the library. The mCBF1 and mCBF2 cDNAs were recovered from the λZAP clones by phagemid excision and transferred into the pSP72 bacterial plasmid (Promega) and into the pMT2 mammalian expression vector (13). Dideoxy sequencing of the cDNA clones was performed using Sequenase 2.0 (United States Biochemicals); the sequences have been submitted to GenBank (U19891 and U19892).

Chromosomal mapping

Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*) F_1 females and C57BL/6J males as described (14). A total of 205 N_2 mice were used to map the *Cbf* locus (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, transfer to Zetabind nylon membranes (AMF-Cuno) and filter hybridization were performed essentially as described (15). The probe, a 2.2 kb *Xho*I fragment of the mCBF1 cDNA, was labeled with [α-^{32}P]dCTP using a nick-translation labeling kit (Boehringer-Mannheim) and following hybridization the filters were washed at a final stringency of 0.5× SSCP (75 mM NaCl, 7.5 mM sodium citrate, 2 mM sodium phosphate), 0.1% SDS at 65°C. Fragments of 19.5 and 7.8 kb were detected in *Eco*RV-digested C57BL/6J DNA and fragments of 12.5 and 5.7 kb were detected in *Eco*RV-digested *M.spretus* DNA. The presence or absence of the 12.5 and 5.7 kb *M.spretus*-specific *Eco*RV fragments, which co-segregated, was followed in backcross mice.

The probes and restriction fragment length polymorphisms (RFLPs) for the loci linked to *Cbf*, including laminin A subunit (*Lama*), mouse homolog-1 of Sos (*Msos1*) and antiphosphotyrosine immunoreactive kinase (*Tik*), have been described previously (16,17). Recombination distances were calculated as described (18) using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

RNA analysis

Reverse transcription polymerase chain reaction (RT/PCR) assays were performed as described previously (19). In brief, 5 µg total RNA were reverse transcribed using random hexamer primers (Pharmacia Biotech) and the resulting cDNA was subjected to PCR in the presence of [α-^{32}P]dATP with the mCBF-specific oligonucleotide primers 5′-TAAGCTGGGAG-ATCCTCAGAACAG-3′ and 5′-GGCGGCATCTGTGTGCAG-GTGACC-3′; ribosomal L19 oligonucleotide primers were included as an internal control (19). Products were extracted with phenol/chloroform, precipitated with ethanol, resolved by polyacrylamide gel electrophoresis and visualized by autoradiography.

For *in situ* hybridization mouse fetuses were collected from pregnant Swiss-Webster mice (Harlan Breeding Laboratory) at days 8, 10, 12, 14, 16 and 18 of gestation and frozen at –80°C. Hybridizations were performed as described previously (19) with antisense and sense riboprobes generated by *in vitro* transcription of the linearized pSP72-mCBF1 construct in the presence of [α-^{33}P]UTP (DuPont-New England Nuclear).

Immunological detection of proteins

For immunofluorescence detection of CBF COS cells were grown on glass coverslips and transfected with CBF expression constructs or vector alone. Cells were fixed with 2% paraformaldehyde at room temperature for 10 min, followed by permeabilization with ice-cold methanol for 5 min. Cells were then treated with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 h at room temperature. Purified IgG from a rabbit antiserum raised against recombinant hCBF was diluted to a final concentration of 50 µg/ml (the polyclonal antiserum recognizes both the human and the mouse proteins). The secondary antiserum, Texas red-conjugated goat-anti-rabbit IgG, was purchased from Vector Laboratories and was visualized with a Zeiss Axiophot microscope.

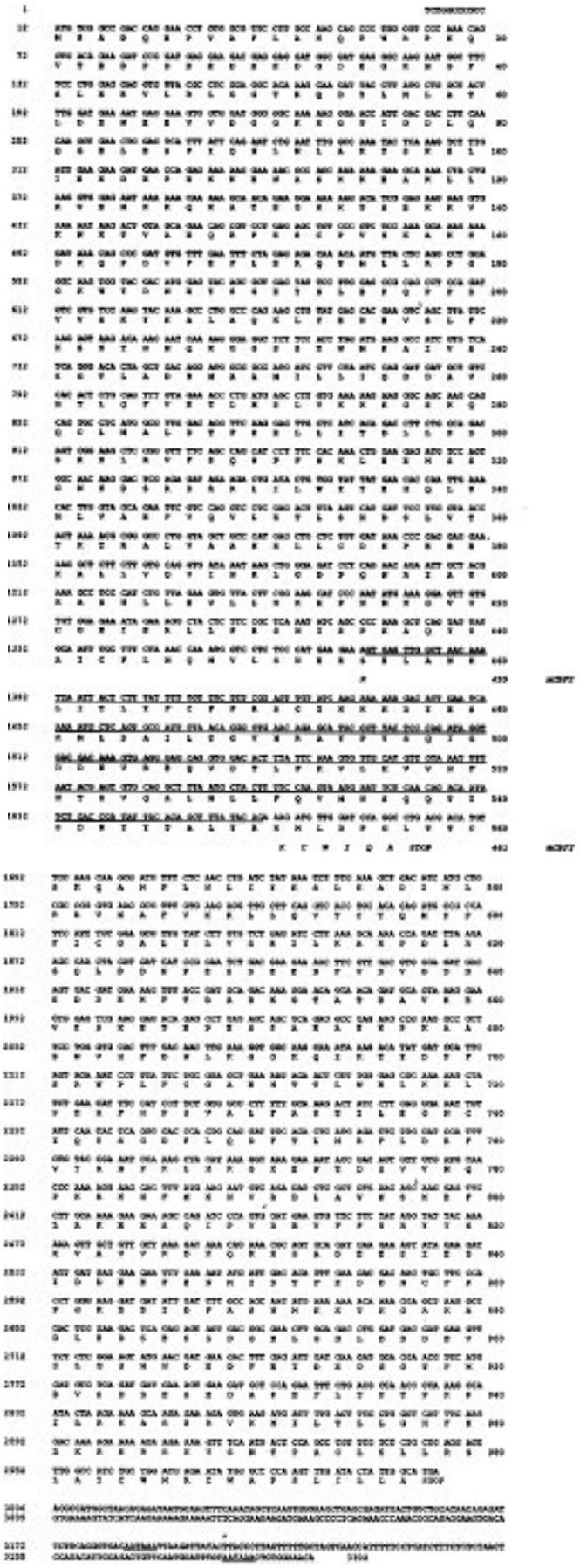
To detect CBF by immunoblotting, extracts were prepared from transfected COS cells as described (20) and 100 µg protein were fractionated by SDS–PAGE and transferred to nitrocellulose (Biotrace). Filters were incubated with 5% non-fat milk in low salt buffer (20 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.05% Triton X-100) before addition of 2 µg/ml rabbit polyclonal anti-CBF IgG. Filters were washed in low salt buffer and then in high salt buffer (20 mM Tris–HCl, pH 7.6, 1 M NaCl, 0.4% *N*-lauryl sarcosine). Goat-anti-rabbit IgG coupled to alkaline phosphatase (Cappel) was added and detected with an alkaline phosphatase conjugate kit (BioRad).

Epitope-tagged forms of CBF were generated by inserting double-stranded oligonucleotides encoding either a single copy of the influenza hemagglutinin (HA) tag (YPYDVPDYA) or the FLAG tag (DYKDDDDK) immediately after the translation initiation ATG codon in the mCBF1 and mCBF2 cDNAs. The tagged proteins were detected with anti-HA monoclonal antibody 12CA5 (Berkeley Antibody) or anti-FLAG monoclonal antibody M2 (IBI-Eastman Kodak). In transfections with one tagged construct binding of the primary antibody was detected with FITC-conjugated horse anti-mouse IgG (Vector Laboratories). In co-transfection experiments with both tagged constructs FITC-conjugated rat anti-mouse IgG1 and biotinylated rat anti-mouse IgG2b (both from Zymed Laboratories) were used to detect anti-FLAG M2 antibody and anti-HA 12CA5 antibody respectively. Texas red avidin D (Vector Laboratories) was used to detect the presence of biotinylated secondary antibody. The secondary antibodies were found to be specific, such that rat anti-mouse IgG1 did not recognize the 12CA5 antibody and the rat anti-mouse IgG2b failed to interact with the M2 antibody. Immunoblots of HA- and FLAG-tagged proteins were incubated with the 12CA5 and M2 antibodies and then developed with alkaline phosphatase-conjugated goat anti-mouse IgG.

RESULTS

Sequence of mCBF

Nine clones of mCBF were identified in a screen of 10^6 plaques from a serum-stimulated BALB/c 3T3 cDNA library using hCBF cDNA as a probe. Six clones were characterized, with five found to correspond to a mRNA that is very similar in overall structure to the cloned hCBF mRNA, whereas the sixth clone apparently represents an alternatively spliced mRNA that deletes 286 nt in



the coding region. The longest cDNA in the group of five clones is 3302 bp, with an open reading frame predicted to encode a protein of 997 amino acids with a molecular weight of 116 kDa (Fig. 1); this protein is designated mCBF1. The sixth clone lacks mCBF1 nt 1375–1660, resulting in the internal deletion of 95 codons and the creation of a new reading frame for only seven amino acids before a termination codon is reached (Fig. 1); the predicted product from this clone, designated mCBF2, is 461 amino acids with a molecular weight of 50 kDa.

Comparison of the predicted mCBF1 and hCBF proteins revealed that they are 80% identical in amino acid sequence (Fig. 2). This degree of identity is maintained along the length of the proteins except near the C-terminus, where the two proteins diverge considerably in sequence (11% identity for the final 63 amino acid residues).

Chromosomal mapping of the *Cbf* gene

The mouse chromosomal location of *Cbf* was determined by interspecific backcross analysis using progeny derived from matings of (C57BL/6J × *M.spretus*) F_1 × C57BL/6J mice. This interspecific backcross mapping panel has been typed for over 1800 loci that are well distributed among all the autosomes as well as the X chromosome (14). C57BL/6J and *M.spretus* DNAs were digested with several enzymes and analyzed by filter hybridization for informative RFLPs using the mCBF1 cDNA (see Materials and Methods). The mapping results indicated that *Cbf* is located in the distal region of mouse chromosome 17, linked to *Lama*, *Tik* and *Msos1*. Although 136 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 3), up to 180 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: *–Lama*–9/176–*Tik*–0/180–*Cbf*–1/149–*Msos1*. The recombination frequencies (expressed as genetic distances in cM ± SE) are: *–Lama*–5.1 ± 1.7–[*Tik*, *Cbf*]–0.7 ± 0.7–*Msos1*. No recombinants were detected between *Tik* and *Cbf* in 180 animals typed in common, suggesting that the two loci are within 1.7 cM of each other (upper 95% confidence limit).

Distribution of CBF mRNAs in adult and fetal mouse tissues

The levels of expression of mCBF1 and mCBF2 mRNAs in adult tissue and in the developing conceptus at different stages of gestation were determined by RT/PCR analysis. To detect both forms of mCBF mRNA primers were utilized that flank the splice site, so that amplification of mCBF1 and mCBF2 would yield fragments of 634 and 347 bp respectively. As an internal control

Figure 1. Nucleotide and deduced amino acid sequence of mCBF1 and mCBF2. Nucleotide residues are numbered on the left and amino acid residues on the right. The underlined sequence from nt 1375 to 1660 of mCBF1 is not present in mCBF2. Shown in italics is the predicted protein sequence of mCBF2, which diverges from mCBF1 beginning at amino acid 455 and terminating at residue 461. The mCBF2 cDNA also has a shorter 3′-untranslated region that terminates at the nucleotide denoted by an asterisk. The two consensus polyadenylation signals in the 3′-untranslated region have been underlined.

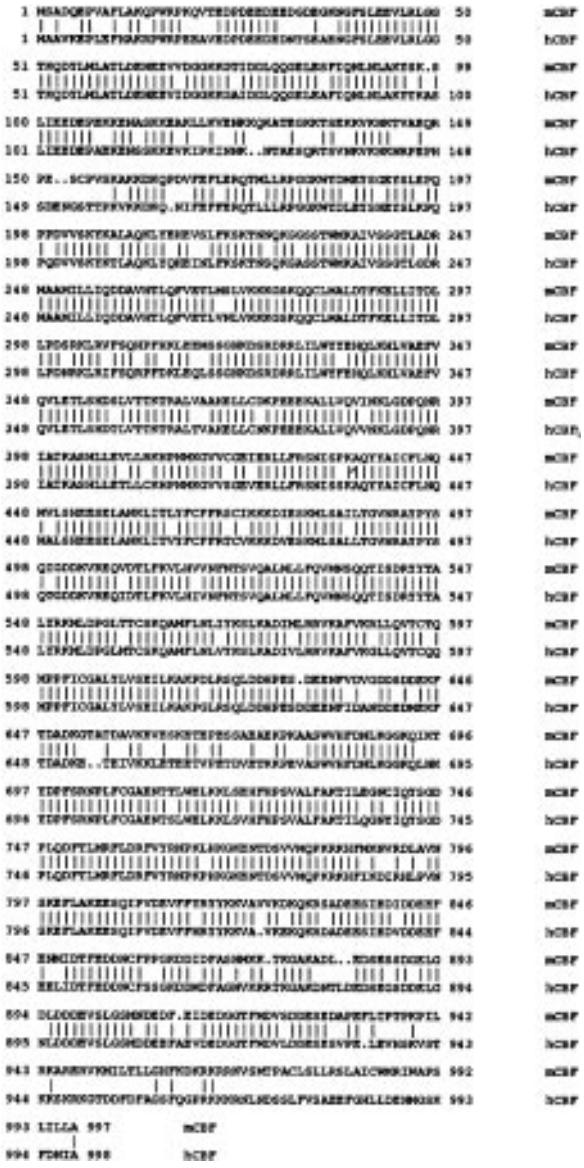


Figure 2. Amino acid sequence similarity between mCBF1 and hCBF. The predicted mCBF1 and hCBF proteins were aligned using the Gap program (Genetics Computer Group) to maximize sequence identity.

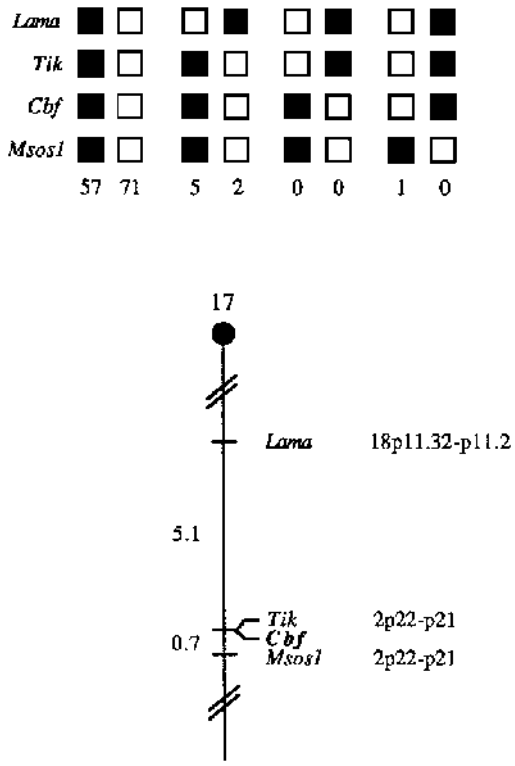


Figure 3. Chromosomal mapping of the *Cbf* gene. *Cbf* was placed on mouse chromosome 17 by interspecific backcross analysis. The segregation patterns of *Cbf* and flanking genes in 136 backcross animals that were typed for all loci are shown at the top of the figure. For individual pairs of loci >136 animals were typed (see text). Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M.spretus*) F_1 parent. The shaded boxes represent the presence of a C57BL/6J allele and white boxes represent the presence of a *M.spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 17 linkage map showing the location of *Cbf* in relation to linked genes is shown at the bottom of the figure. Recombination distances between loci in cM are shown to the left of the chromosome and the positions of loci in human chromosomes, where known, are shown to the right. References for the human map positions of loci mapped in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H.Welch Medical Library of The Johns Hopkins University School of Medicine (Baltimore, MD).

primers were included to amplify the mouse ribosomal L19 mRNA (19). Both the mCBF1 and mCBF2 mRNAs were present in all tissues examined (Fig. 4). The identities of the PCR fragments were confirmed by cloning and sequencing, thereby demonstrating that the mCBF2 cDNA clone represents a naturally occurring mRNA. Although the level of each of these mRNAs was found to be approximately constant among all of these tissues, the concentration of the mCBF2 mRNA was significantly lower than that of the mCBF1 mRNA in each sample (Fig. 4), consistent with their representation in the cDNA library. The intensities of the mCBF1 and mCBF2 RT/PCR products were determined by phosphorimager analysis and normalized to the amount of the L19 product. In all tissues the amount of the mCBF1 mRNA was ~5- to 10-fold greater than the mCBF2 mRNA; for the BALB/c 3T3 cell line the ratio of mCBF1 to mCBF2 mRNA was ~20:1 (Fig. 4).

To examine the pattern of mCBF expression during mouse development sections through concepti isolated from day 8 to day 18 of gestation were hybridized with an mCBF riboprobe; this probe detects both mCBF1 and mCBF2. As shown in Figure 5, mCBF mRNA was found to be distributed uniformly throughout the placenta (shown for days 8, 10 and 12) and the embryo (days 8–18). Thus mCBF is a ubiquitous factor that is expressed throughout development.

Cellular localization of mCBF1 and mCBF2 proteins

Both mCBF1 and hCBF contain a potential nuclear localization signal near their C-termini (residues 942–946, Leu–Arg–Lys–Ala–Arg in mCBF1 and 943–947, Thr–Lys–Lys–Ser–Lys in hCBF) which is not present in mCBF2. To determine if the mCBF1 and mCBF2 proteins localize to distinct cellular

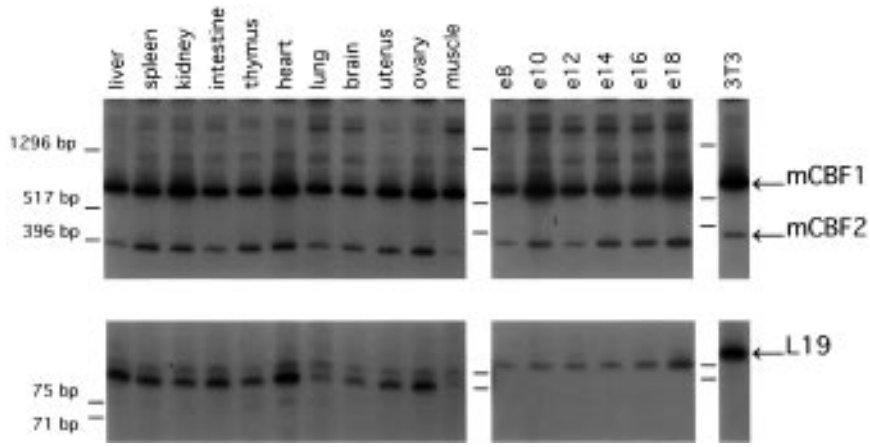


Figure 4. Expression of mCBF mRNA in mouse tissues. Total RNA (5µg) from various adult mouse tissues (left panel), from embryos at different stages of gestation (center panel) or actively growing BALB/c 3T3 cells (right panel) was analyzed by RT/PCR. Arrows identify the PCR products of mCBF1 (634 bp), mCBF2 (347 bp) and internal control ribosomal L19 mRNA (90 bp). Higher molecular weight products may represent mCBF pre-mRNAs. No products were detected in control reactions lacking either RNA or reverse transcriptase (data not shown).

compartments expression constructs containing the mCBF1 and mCBF2 cDNAs were transfected into COS cells. By immunofluorescence staining mCBF1 was found to translocate efficiently to the nucleus, whereas mCBF2 remained in the cytoplasm and accumulated in the perinuclear region (Fig. 6). A low level of fluorescence in both the nucleus and in perinuclear structures was detected in untransfected cells (Fig. 6), probably from endogenous CBF1 and CBF2 proteins.

The different locations of mCBF1 and mCBF2 in the cell suggested that these two proteins have distinct actions. Co-expression of mCBF1 and mCBF2 in a cell might also provide a means of regulating the translocation of mCBF1 into the nucleus. To explore this latter possibility expression constructs were generated that encode mCBF1 and mCBF2 tagged with the HA or FLAG epitopes respectively. Immunoblot analysis of extracts from COS cells transfected with these constructs demonstrated that the tagged proteins were produced at equivalent levels and that recognition by anti-HA or anti-FLAG antibodies was specific (Fig. 7). Immunofluorescence staining of transfected cells detected mCBF1–FLAG primarily in the nucleus and mCBF2–HA in the cytoplasm, as was seen for the untagged proteins (Table 1). Identical results were found for mCBF1 tagged with HA and mCBF2 tagged with FLAG (data not shown). Significantly, some mCBF1 was detected in the cytoplasm of transfected cells, with some cells harboring primarily cytoplasmic mCBF1 (Table 1). Despite the apparent absence of a nuclear localization signal, some nuclear mCBF2 could be detected in a small percentage of cells (Table 1). The unexpected variation in the sites of mCBF1 and mCBF2 accumulation could not be attributed to the detection of endogenous CBF proteins, since COS cell CBF was not detected with the antibodies against the epitope tags.

In contrast to the results obtained upon transfection of one expression construct, co-transfection of the mCBF1 and mCBF2 expression constructs resulted in a markedly different localization pattern. Co-expression of mCBF1 and mCBF2 resulted in increased translocation of mCBF2 into the nucleus and retention of mCBF1 in the cytoplasm (Table 1). Thus each form of mCBF appears to influence the distribution of the other form in the cell, suggesting that the relative levels of these two proteins in the cell may be critical in regulating mCBF access to target genes.

Table 1. Distribution of mCBF1 and mCBF2 in transfected cells

Construct	Percentage of cells[a] with CBF		
	In nucleus	In nucleus and cytoplasm	In cytoplasm
(A) Transfection with one construct			
mCBF1–FLAG	95.2 ± 0.2	3.5 ± 0.2	1.2 ± 0.1
mCBF2–HA	3.1 ± 0.6	3.6 ± 1.2	93.3 ± 1.7
(B) Co-transfection			
mCBF1–FLAG + mCBF2–HA	54.7 ± 5.5 / 3.0 ± 1.0	44.3 ± 1.8 / 67.7 ± 0.7	1.0 ± 0.6 / 29.7 ± 1.5

[a]Three independent transfections were conducted to generate the mean ± SE. Cells were scored for the compartment in which most of the immunostaining was detected. The total number of immunofluorescent cells counted for each condition ranged from 300 (for co-transfections) to 2800 (for individual transfections).

DISCUSSION

In a screen for the mouse homolog of hCBF cDNA we have identified two mRNA forms, mCBF1 and mCBF2, that arise by alternative splicing of a single transcript. The N-terminal 454 amino acids of mCBF1 and mCBF2 are identical. This region includes residues 1–192, which in hCBF are sufficient to mediate specific interactions with both the adenovirus E1a oncoprotein and the p53 tumor supressor protein (9,10). Thus mCBF1 and mCBF2 may overlap in the set of proteins with which they interact, including mouse p53.

The mCBF2 protein lacks the C-terminal half of mCBF1, which includes the putative nuclear localization signal. Consistent with this difference, mCBF1 was detected predominantly in the nucleus, whereas mCBF2 was found to localize primarily in the cytoplasm. The detection of some mCBF1 in the cytoplasm (and some cells with mainly cytoplasmic mCBF1) and some mCBF2 in the nucleus (with some cells containing mostly nuclear

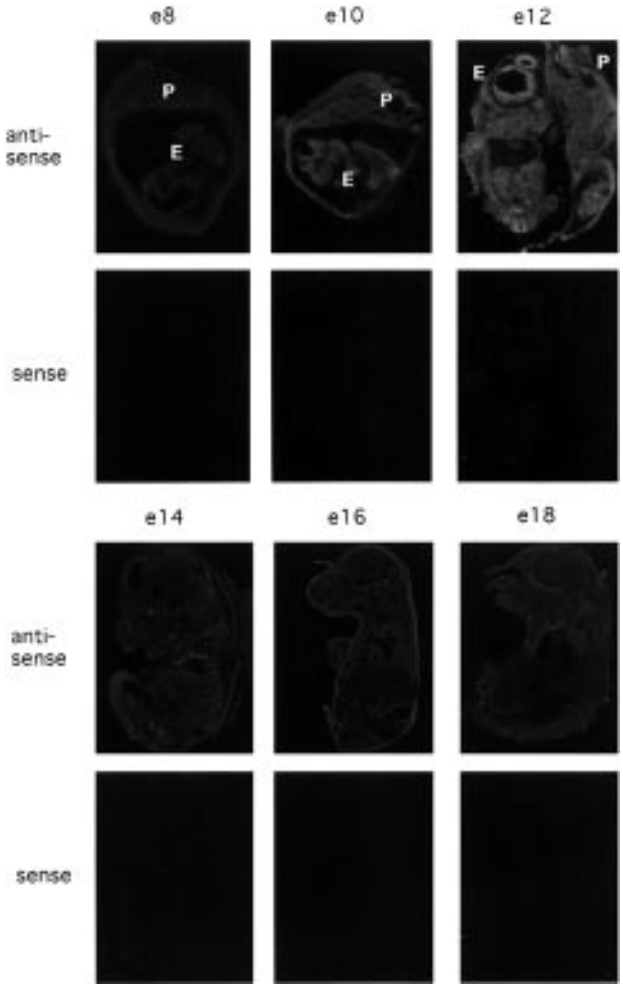


Figure 5. Distribution of mCBF mRNA in the developing conceptus. Sections through the whole conceptus (top panels) or through the fetus (lower panels) from day 8 to day 18 of gestation were hybridized to antisense or control sense strand mCBF riboprobes. Specific hybridization to mCBF RNA in the placenta (P) or embryo (E) is indicated by the bright regions in these dark field photomicrographs.

mCB2) indicates that these two forms of CBF can move between these two major cellular compartments. Since the transfected cell cultures were not synchronized, one possible explanation for these results is that mCBF1 and mCBF2 localization is cell cycle regulated.

Cell cycle alterations in protein localization might simply reflect the period just after the completion of M phase, when some mCBF1 is in the cytoplasm and has not yet translocated back into the reformed nucleus and some mCBF2 has been captured in the newly formed nucleus but not yet been transported back into the cytoplasm. However, co-expression of elevated levels of mCBF1 and mCBF2 resulted in nuclear mCBF2 and cytoplasmic mCBF1 in a high percentage of cells. Thus the mechanism of mCBF1 and mCBF2 redistribution in the cell cannot be explained solely by nuclear envelope breakdown and reassembly. Instead, mCBF1 and mCBF2 probably interact, either directly or through accessory factors, to regulate localization. Therefore, variations in the relative amounts of these two forms of CBF, or variations in mCBF post-translational modifications that may influence protein–protein interactions, may occur at specific stages of the cell cycle to regulate localization and function. Transient alterations in the cellular distributions of these proteins may have signficant effects, especially considering that the one known target gene for CBF, the *hsp70* gene, is serum inducible (1) and cell cycle regulated (21) and that the CBF binding protein p53 also undergoes a conformational change in response to serum (22). Furthermore, a truncated form of hCBF similar in length to mCBF2 is able to stimulate transcription from the *hsp70* gene promoter and to target E1a to this promoter (9), indicating that this form is capable of binding DNA.

Since mCBF1 and hCBF both contain the p53 and E1a interaction domain it is possible that mCBF2 also acts as a dominant interfering form of CBF, sequestering proteins in the cytoplasm that are needed for mCBF1-mediated transcriptional activation in the nucleus. Alternatively, mCBF1 and mCBF2 might share co-factors but act on different processes. For example, mCBF1 may interact with p53 in the nucleus to regulate transcription, whereas mCBF2 might cooperate with p53, which has been shown to bind to the 5.8S rRNA (23) to regulate protein synthesis in the cytoplasm. The accumulation of mCBF2 protein in the perinuclear region of the cytoplasm suggests that it may function in association with specific structures or compartments.

One possible means of identifying potential physiological effects of mCBF1 and mCBF2 is to correlate the chromosomal location of the *Cbf* gene with mapped mutations that cause abnormalities in the mouse. We have compared the interspecific map of chromosome 17 with a composite mouse linkage map that reports the location of many uncloned mouse mutations (compiled by M.T.Davisson, T.H.Roderick, A.L.Hillyard and D.P.Doolittle and provided from GBASE, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME). However, *Cbf* was found to map in a region of the composite map that lacks known mouse mutations (data not shown). The distal region of mouse chromosome 17 shares a region of homology with human chromosomes 18p and 2p (summarized in Fig. 3). In particular, *Tik* has been placed on human 2p22–p21. The tight linkage between *Tik* and *Cbf* in the mouse suggests that *Cbf* will also reside on human 2p.

Both mCBF1 and mCBF2 mRNAs were found in all the tissues of the adult mouse that were examined, in the placenta, throughout the developing embryo from day 8 to day 18 of gestation and in actively growing 3T3 cells. Furthermore, no tissues could be detected in the embryo or adult that lacked mCBF mRNA. Thus mCBF may be active in most, if not all, cell types during fetal and placental development and in the adult. One difference that was consistently observed between mCBF1 and mCBF2 was a 5- to 10-fold greater level of mCBF1 compared with mCBF2 mRNA in each tissue. Since mCBF2 can apparently regulate mCBF1 translocation into the nucleus, it will be of interest to determine if the relative levels of mCBF2 and mCBF1 change under various physiological conditions.

The ability of hCBF to mediate transcriptional induction and repression of the growth-regulated *hsp70* gene by regulatory factors such as the adenovirus E1a and cellular p53 proteins suggests that mCBF1 and mCBF2 may be important components of cell growth regulation in the mouse. The finding that mCBF1 and mCBF2 are ubiquitously expressed during fetal development and in the adult further suggests that their actions will be found to be of general importance in the regulation of cell function.

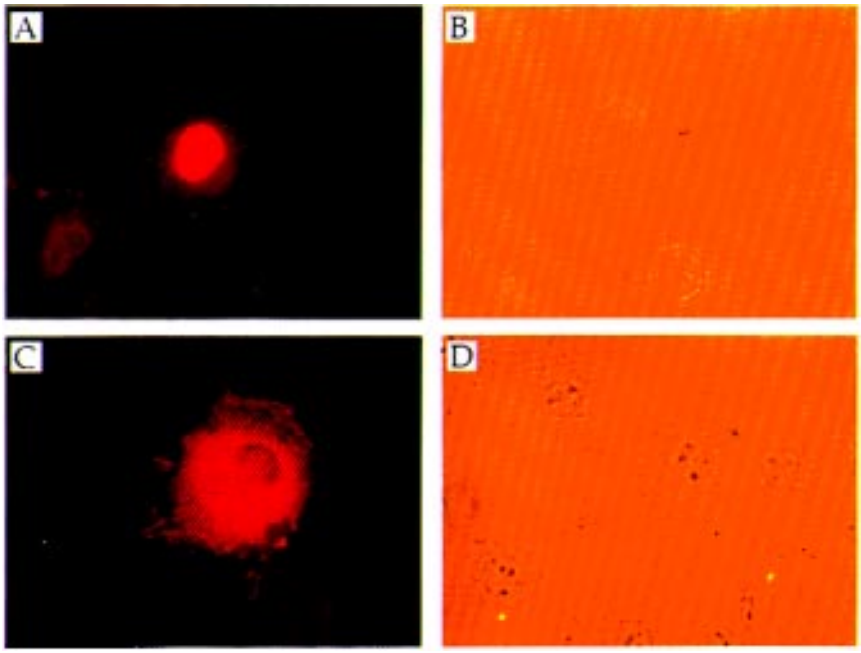


Figure 6. Cellular localization of mCBF1 and mCBF2 proteins. COS cells transfected with pMT2-mCBF1 (**A**, **B**) or pMT2-mCBF2 (**C**, **D**) were stained with rabbit anti-hCBF and Texas red-conjugated goat anti-rabbit secondary antibody. Left and right panels display the fluorescence and phase contrast images for each transfection. Intense staining is detected for mCBF1 in the nucleus of transfected cells; mCBF2 is detected in the cytoplasm in the perinuclear region.

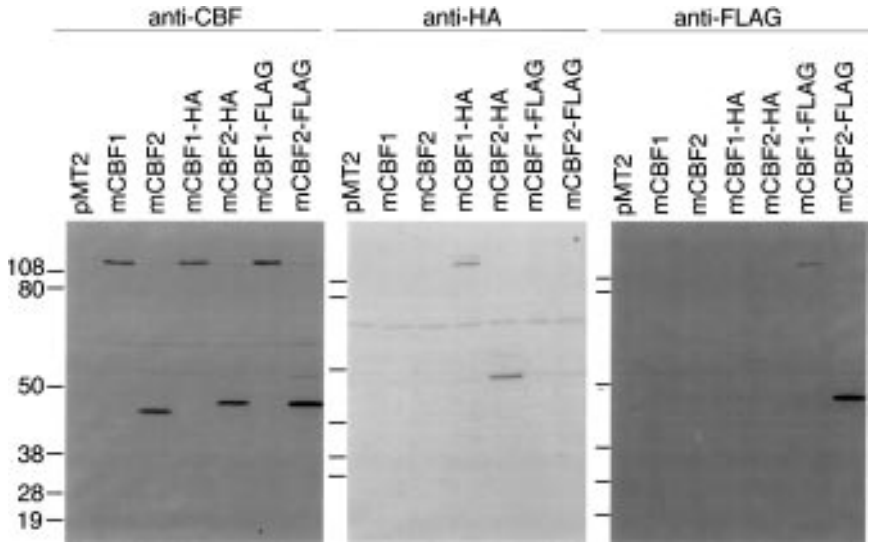


Figure 7. Expression of mCBF1 and mCBF2 proteins in transfected cells. COS cells were transfected with the pMT2 vector alone, pMT2-mCBF1 or pMT2-mCBF2 or with constructs that encode HA or FLAG epitope-tagged versions of mCBF1 and mCBF2. Cell extracts were analyzed by immunoblotting with a rabbit polyclonal antiserum prepared against hCBF (left) or with monoclonal antibodies against HA (center) or FLAG (right). The migration of marker proteins is indicated on the left side of each panel. As expected, mCBF1 and mCBF2 encode proteins of ~116 and 50 kDa, respectively.

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