Apolipoprotein B Gene Regulatory Factor-2 (BRF-2) Is Structurally and Immunologically Highly Related to Hepatitis B Virus X Associated Protein-1 (XAP-1)†

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ABSTRACT: Hepatic cell-specific expression of the human apolipoprotein B (apoB) gene is controlled by at least four *cis*-acting elements located between positions −128 and +122 [Chuang, S. S., & Das, H. K. (1996) *Biochem. Biophys. Res. Commun. 220*, 553−562]. The distal element (−128 to −85) appears to be liver specific because it shows positive activity in HepG2 cells and negative activity in HeLa cells. ApoB gene regulatory factor-2 (BRF-2) interacts with the sequence (−104 to −85). BRF-2 has been purified from rat liver nuclear extract, and its molecular weight has been determined to be ∼120 kDa [Zhuang et al. (1992) *Mol. Cell. Biol. 12*, 3183−3191]. In this paper we report the isolation of two isoforms of BRF-2 by further purification using high-performance liquid chromatography. Both isoforms produced a single ∼120-kDa band in sodium dodecyl sulfate polyacrylamide gel electrophoresis detected by silver stain. The amino acid sequences of two tryptic peptides derived from HPLC-purified heavier BRF-2 isoform were determined to be YLAIAPPIIK and ALYYLQIHPQELR. These two peptides were found to share 100% sequence homology with human hepatitis B virus X associated protein-1 (XAP-1) and monkey UV-damaged DNA-binding protein (UV-DDB). Anti-peptide antisera raised against two synthetic peptides of XAP-1 recognized a ∼120-kDa polypeptide band in both BRF-2 isoforms in a western blot analysis. By using apoB promoter fragments containing various internal deletions and a substitution mutation as templates for gel mobility shift assays, we identified the region between −104 and −85 as crucial for binding by the high-molecular weight form. In contrast, the lower molecular weight isoform bound to all apoB mutants tested. Anti-peptide 2 antiserum directed against XAP-1 was found to inhibit *in vitro* transcription of the apoB gene in rat liver nuclear extracts by 50%. These results suggest that BRF-2 and XAP-1 are structurally and immunologically highly related *trans*-activators of the apoB gene. We propose that BRF-2 exists both as a monomer (BRF-2M) and as a homooligomer, probably a homodimer (BRF-2D), in solution; oligomerization appears to be an essential step for imparting sequence-specificity to BRF-2 protein and thereby facilitating its role as a *trans*-activator of the apoB gene.

Apolipoprotein B-100 (apoB),[1] the sole protein component of low-density lipoprotein (LDL), is synthesized primarily in the human liver and acts as a ligand for the LDL receptor (Brunzell et al., 1984; Goldstein & Brown, 1977, 1982). This interaction mediates the removal of LDL from the circulation (Brunzell et al., 1984; Goldstein & Brown, 1977, 1982). Plasma levels of apoB and LDL cholesterol have a direct correlation with susceptibility to atherosclerosis (Havel & Kane, 1989; Kannel et al., 1979; Siervogel et al., 1980; Sniderman et al., 1980). Mutations in the regulatory regions that lower apoB gene transcription may diminish the plasma LDL cholesterol levels. Thus, the apoB gene plays an important role in determining plasma LDL cholesterol levels.

To better understand the regulation of apoB gene expression, a number of investigators have defined the *cis*-acting elements on the apoB promoter and the putative *trans*-acting factors which interact with these elements (Brooks et al., 1991; Carlsson & Bjursell, 1989; Carlsson et al., 1990; Das et al., 1988; Kardassis et al., 1990a,b, 1992; Metzger et al., 1990; Paulweber et al., 1991a−d; Paulweber & Levy-Wilson, 1991). Hepatic cell-specific expression of the apoB gene is regulated by at least two positive *cis*-acting elements located from −128 to −70 (Das et al., 1988); these elements are bound by several *trans*-acting protein factors (Carlsson et al., 1990; Das et al., 1988; Kardassis et al., 1990a,b, 1992; Metzger et al., 1990; Paulweber et al., 1991a−d; Paulweber & Levy-Wilson, 1991). The DNA sequence from positions −84 to −70 has a 10-fold positive effect in HepG2 cells

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[1] Abbreviations: ApoB, apolipoprotein B-100; LDL, low-density lipoprotein; BRF-2, apolipoprotein B gene regulatory factor-2; C/EBP, CCAAT/enhancer binding protein; HNF, hepatocyte nuclear factor; HBx, hepatitis B virus encoded X protein; XAP-1, hepatitis B virus X associated protein-1; CAT, chloramphenicol acetyltransferase; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; DTT, dithiothreitol; PIPES, piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography.

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and is indispensable for liver-specific expression of the apoB gene (Das et al., 1988). The distal element (−128 to −85) produces a 5-fold increase in positive activity in HepG2 cells in a transient transfection assay (Das et al., 1988). We have also identified two downstream regulatory elements located at (+20 to +40) and (+43 to +53) which have a 1.4-fold negative and 10-fold positive effect, respectively, on the transcription of the apoB gene in transient transfection assays using Hep G2 cells (Chuang et al., 1995). Together both of these elements (+20 to +40; +43 to +53) account for a 2-fold increase in the apoB transcription in Hep G2 cells. Recently, we reported the purification of apoB gene regulatory factor-2 (BRF-2) from rat liver nuclear extracts by a combination of DEAE cellulose, heparin agarose, and DNA sequence-specific affinity chromatography. BRF-2 interacts with an apoB gene promoter sequence (−104 to −85) (Zhuang et al., 1992). The molecular weight of BRF-2 has been determined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) to be ∼120 kDa (Zhuang et al., 1992). To determine the amino acid sequence of BRF-2, we further purified this ∼120-kDa protein by an additional step using high-performance liquid chromatography (HPLC).

In this paper we report that BRF-2 exists in two isoforms: we speculate that the two forms represent a monomer (BRF-2M) and a homo-oligomer (BRF-2D) with a subunit molecular weight of ∼120 kDa. The two forms were separated by high-performance liquid chromatography (HPLC), and both forms produced a single ∼120-kDa band on SDS−PAGE detected by silver stain. The amino acid sequence obtained from two tryptic peptides derived from the homo-oligomeric (BRF-2D) form showed 100% sequence homology with hepatitis B virus X (HBx) associated protein-1 (XAP-1) recently cloned from a human B cell cDNA library by Lee et al. (1995) and a putative UV-damaged DNA repair protein, UV-DDB (Takao et al., 1993). XAP-1 is the human homologue of a UV-damaged DNA-binding protein (UV-DDB) purified and cloned from a monkey kidney cell line (Abramic et al., 1991; Takao et al., 1993).

We also report that BRF-2 is structurally and immunologically highly related to XAP-1. Antisera raised against two hydrophilic synthetic peptides of XAP-1 recognized a ∼120-kDa BRF-2 polypeptide band from both isoforms in a western blot analysis. When genomic fragments of the apoB gene containing various internal deletions were used as templates in a gel mobility shift assay, the oligomeric form showed binding specificity to the sequence from −104 to −85. In contrast, the monomeric form bound to all fragments tested and thus did not exhibit any sequence specificity. Substitution mutation in the apoB gene sequence (−96 to −88) abolished the binding of the oligomeric form, whereas the monomer bound to the mutant DNA fragment in a gel mobility shift assay, confirming that the oligomer specifically recognizes the apoB sequence (−104 to −85).

XAP-1 has been shown to associate with hepatitis B virus X protein (HBx), a known *trans*-activator of several viral and cellular genes (Lee et al., 1995). Further, the expression of XAP-1/UV-DDB is not restricted to skin cells, but is found also in hepatocytes and several other tissues, suggesting its involvement in an essential function other than solely in repair of UV-damaged DNA. Several proteins involved in DNA repair have recently been found to be components of the cellular transcriptional machinery (Schaeffer et al., 1993). Together, these observations prompted us to investigate the role of BRF-2 in the transcription of the apoB gene. We found that an anti-peptide antiserum directed against XAP-1 protein significantly reduced transcription of the apoB gene construct in an *in vitro* transcription assay. This result suggests an important role for BRF-2/XAP-1 in the *trans*-activation of the apoB gene. We also propose that oligomerization (probably dimerization) of the ∼120-kDa subunit of BRF-2 may impart sequence specificity and thus exert a regulatory role in the *trans*-activation of the apoB gene.

EXPERIMENTAL PROCEDURES

Materials. Frozen rat livers were purchased from Pel Freez (Rogers, AR). Heparin agarose, HEPES, PIPES, DTT, PMSF, benzamidine, leupeptin, aprotinin, pepstatin, anti-rabbit IgG antibody, and ExtrAvidin-peroxidase conjugate were purchased from Sigma Chemical Co. DEAE-cellulose (DE-52) was purchased from Whatman. [γ-^{32}P]ATP was a product of DuPont. Centricon-100 was a product of Amicon. DEAE-5PW HPLC column was purchased from TosoHaas. T4 polynucleotide kinase was obtained from New England Biolabs. Protein A column was purchased from Pierce (Rockford, IL). RNasin, AMV reverse transcriptase, and nucleotide triphosphates (NTPs) were bought from Promega. dNTPs were obtained from Boehringer Mannheim. Oligonucleotides were synthesized by Integrated DNA Technology (IDT, Coralville, IA). Sephacryl S300 was purchased from Pharmacia Biotech.

Purification of BRF-2. BRF-2 was purified from rat liver nuclei as described previously (Zhuang et al., 1992) by a combination of DEAE cellulose, heparin agarose, and DNA sequence-specific affinity chromatography with an additional step of HPLC. The protein eluted from the second affinity column was dialyzed against buffer M (10 mM Tris-HCl [pH 7.9], 0.2 mM EDTA, 1 mM DTT, 0.5 mM PMSF, and 20% glycerol) containing 50 mM KCl and was loaded onto a (7.5 mm × 7.5 cm) DEAE-5PW anion-exchange HPLC column equilibrated with buffer M containing 50 mM KCl. The column was washed for 10 min with buffer M containing 50 mM KCl at 30 mL/h and for an additional 10 min with buffer M containing 0.2 M KCl. The protein bound to the column was then step eluted with buffer M containing 0.4 M KCl. Fractions of 0.5 mL were collected and assayed for DNA-binding activity by gel mobility shift assay using a 44-bp double-stranded oligonucleotide probe containing the sequence from −128 to −85 of the apoB gene as described previously, (Das et al., 1988; Zhuang et al., 1992). The active fractions containing the activities of the oligomeric BRF-2D and the monomeric BRF-2M forms were pooled separately and dialyzed against buffer M containing 50 mM KCl for 2 h. The dialysates were concentrated by Centricon-100 to a volume of 100 μL, quickly frozen in liquid nitrogen, and stored at −80 °C. The purified homo-oligomeric protein BRF-2D from 20 batches (3000 rat livers) were pooled together, concentrated by Centricon-100 to a volume of 80 μL (5 μg), and used for amino acid sequence analysis.

SDS−PAGE. SDS−PAGE was performed on the concentrated samples by the method of Laemmli (1970). The proteins were run on a 6% separating and 4% stacking gel and stained by a silver staining kit purchased from Bio-Rad. Preparative SDS−8.5% PAGE was used to obtain BRF-2D for protein sequencing.

Trypsin Digestion and Protein Sequencing. The HPLC-purified BRF-2D was processed using the protocol of

Rosenfeld et al. (1992). Briefly, the SDS−PAGE gel was stained with Coomassie Blue. The ~120-kDa band was cut off from the gel and destained. After the gel was shrunk with 50% acetonitrile and 10 mM sodium borate, the gel was rehydrated with trypsin (1 μg)-containing solution (1% hydrogenated Triton X-100). The protein was digested for 24 h at 37 °C. After the digestion was completed, the peptides were eluted from the band and separated by reverse-phase HPLC. Several peptide peaks were analyzed by MALDI-TOF. Peaks containing a single peptide were analyzed by chemical sequencing using an Applied Biosystem model 475 peptide sequenator. These procedures were performed by Dr. Paul Matsudaira at the protein sequencing facility of Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, MA.

Gel Filtration on Sephacryl S300. Second affinity purified BRF-2 was passed through a S300 column (90 cm × 1.5 cm) at 4 °C. The column was equilibrated and eluted with buffer M containing 100 mM KCl. Eluted fractions from the column were assayed for BRF-2 activity by DNA binding and gel mobility shift. Positions of marker proteins eluted from the column were determined by monitoring OD_{280} of column fractions.

Preparation of Anti-Peptide Antisera of XAP-1. Anti-peptide 1 (amino acids 198−213) and anti-peptide 2 (amino acids 1113−1126) antisera directed against XAP-1 were raised in rabbits as described (Lee et al., 1995).

Purification of Anti-Peptide 1 and Anti-Peptide 2 Antisera. Rabbit anti-XAP-1 and preimmune sera were purified by using the ImmunoPure IgG purification kit. Sera were run through immobilized protein A columns, and bound IgG fractions were washed, eluted, and desalted. The affinity-purified antibody was proven to retain activity by immunoprecipitation of *in vitro* translated XAP-1 protein. Preimmune and immune sera contained the same amount of protein; protein concentrations of purified IgG were 7-fold lower than that of unpurified sera.

Western Blot Analysis. The purified proteins (BRF-2M, BRF-2D) obtained by HPLC were run on an SDS−6% PAGE gel and transblotted onto a nitrocellulose membrane overnight in the cold at a constant current of 100 mA. All subsequent steps were carried out with rocking at room temperature. The membrane was blocked for 2 h with 50% non-fat milk powder in TBST buffer (10 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.05% Tween-20). This was followed by incubation for 1 h with unpurified preimmune serum, anti-peptide 1 antiserum, or anti-peptide 2 antiserum (1:1000 dilution). After the blots were washed with TBST, the secondary antibody (anti-rabbit IgG biotin conjugate) and ExtrAvidin peroxidase conjugate were added consecutively at a dilution of 1:1000 and incubated for 1 h each. The blots were developed in the dark with diaminobenzidine and hydrogen peroxide, and the reaction was stopped by immersing in distilled water.

Preparation of Rat Liver Nuclear Extract for in Vitro Transcription. Rat liver nuclear extracts for *in vitro* transcription assay were prepared according to the method of Gorski et al. (1986). All steps were carried out in the cold room at 4 °C, and all solutions contained PMSF (0.5 mM), pepstatin (1 μg/mL), benzamidine-HCl (1 mM), leupeptin (0.5 μg/mL), aprotinin (0.5 μg/mL), and (DTT) (1 mM). Fresh rat liver tissue (12−15 g) was washed with PBS, minced, and brought to 40 mL with homogenization buffer (10 mM HEPES [pH 7.6], 25 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1 mM EDTA, 2 M sucrose, and 10% glycerol). The tissue was homogenized with a Potter−Elvehjem homogenizer with five strokes of the pestle. The homogenate was diluted 2-fold with the homogenization buffer, loaded over 10-mL cushions of the homogenization buffer, and centrifuged at 25 000 rpm for 30 min in an SW28 rotor at 0 °C. The nuclear pellets obtained from 25 rat livers were suspended in a mixture of homogenization buffer and glycerol (9:1, v/v), layered over 10-mL cushions of the homogenization buffer, and centrifuged as described above. The clean nuclear pellets thus obtained were suspended in 20 mL of nuclear lysis buffer (10 mM HEPES [pH 7.6], 100 mM KCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol) and homogenized by gentle shearing using an all-glass Dounce homogenizer (tight-fit pestle). A portion was diluted 1:100 in 0.5% SDS, and the A_{260} was measured. The suspension was then diluted to 10−15 A_{260} units per mL, one-tenth volume of 4 M ammonium sulfate (pH 7.9) was added, and the mixture was rocked for 30 min. The lysed nuclei were then centrifuged at 40 000 rpm for 1 h at 0 °C in a Beckman 60 Ti rotor. Solid ammonium sulfate (0.3 g/mL) was added gradually to the supernatant, with stirring over an ice bath. The precipitated proteins were then sedimented by centrifugation at 40 000 rpm for 40 min. The pellets obtained were suspended in dialysis buffer (25 mM HEPES [pH 7.6], 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, and 10% glycerol) at 1 mL/200 A_{260} units and dialyzed twice for 2 h each against 250 mL of the same buffer. A precipitate that formed during dialysis was removed by centrifugation at 14 000 rpm for 10 min. The supernatant was divided into aliquots, frozen in liquid nitrogen, and stored at −80 °C.

Synthetic Oligonucleotides Used

BRF-2 WT: 5′-GGCTCAAAGAGAAGCCAGTGTA-GAAAAGCAAACAGGTCAGGCCC-3′

BRF-2LM5: 5′-GGCTCAAAGAGAAGCCAGTGTA-GAAAAGCAAAacttgacttCCC-3′

Plasmid Construction for in Vitro Transcription. Plasmid pKT-128B contains the sequence −128 to +122 of the apoB gene linked to the promoterless CAT structural gene in the pKT vector as described earlier (Das et al., 1988). Plasmid pSVCAT contains the SV40 early promoter sequences including two 72-bp and three 21-bp repeats inserted into the pKT polylinker (Das et al., 1988). Plasmid pKT-128LM5B was prepared by ligating the double-stranded LM5 oligonucleotide (−128 to −85) containing mutations from −96 to −88 with the *Sac*I−*Sma*I digested and agarose gel purified fragment of pKT-128B plasmid. The plasmids were grown under standard conditions and prepared by two CsCl density gradient centrifugation runs. Plasmids pKT-128B, pKT-128LM5B, and pSVCAT were linearized at the 5′ end by digesting with *Sac*I. Linearized plasmids were extracted with phenol and phenol:chloroform and precipitated with ethanol. Plasmid pSVCAT was used as an internal control for the *in vitro* transcription assay.

In Vitro Transcription Assay. An *in vitro* transcription assay was carried out in a total volume of 40 μL containing 1 μg each of linear template DNA (pKT-128B and pSVCAT) or (pKT-128LM5B and pSVCAT), 0.25 mM each of ATP, GTP, CTP, and UTP, 12.5 mM HEPES [pH 7.6], 50 mM KCl, 6 mM $MgCl_2$, 0.1 mM EDTA, 5% glycerol, 40 units

of RNasin, and 150−200 μg of rat liver nuclear protein. The reactions were carried out at 37 °C for 1 h and stopped by the addition of a stop buffer (100 mM Tris [pH 7.5], 12.5 mM EDTA, 150 mM NaCl, 1% SDS, and 10 μg of proteinase K). After an additional incubation at 37 °C for 1 h, the samples were extracted with phenol:chloroform and ethanol-precipitated overnight. The *in vitro* transcribed RNA was collected by centrifugation. Both pKT-128B and pSVCAT or pKT-128LM5B and pSVCAT were co-transcribed *in vitro*, and transcripts from both these plasmids were detected by primer extension (Stetler et al., 1982) of a labeled oligonucleotide corresponding to the RNA coding strand of the CAT gene sequence from 4956 to 4973 (5′-CGGTGGTATATCCAGTGA-3′). The primer was labeled at the 5′ end with [γ-^{32}P]ATP and polynucleotide kinase; 400 fmol (∼600 000 cpm) was added to the RNA pellet in 40 mM PIPES (pH 6.7), 1 mM EDTA, 0.2% SDS, and 0.4 M NaCl. Annealing was carried out at 37 °C for 5 h. After isopropanol precipitation, the pellet was dried and dissolved in a buffer containing 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 10 mM DTT, 75 mM KCl, 0.5 mM each of dATP, dCTP, dGTP, and dTTP, and 20 units of RNasin. Primer extension was carried out with 12 units of AMV reverse transcriptase at 42 °C for 2 h. The products were ethanol precipitated in the presence of 10 μg of tRNA as carrier. The pellets were dried and dissolved in 10 μL of a formamide dye containing 50 mM NaOH, bromophenol blue, and xylene cyanol and run on a 6% acrylamide−7 M urea gel. Radiolabeled *Hae*III-digested ϕX DNA was included in the gel as a size marker. The gels were dried and exposed to X-ray films in cassettes with intensifying screens at −80 °C overnight. The autoradiograms were quantitated by densitometry.

Gel Mobility Shift Assay. Nuclear protein−DNA binding reactions were carried out in 10 mM Tris-HCl buffer (pH 7.5), 50 mM KCl, 4 mM $MgCl_2$, and 5% glycerol. A typical reaction contained 40 000 cpm (∼0.25 ng) of 5′ end-labeled double-stranded oligonucleotide containing the apoB sequence (−128 to −85) (5′-gat cGG CTC AAA GAG AAG CCA GTG TAG AAA AGC AAA CAG GTC AGG CCC-3′), 1 μg of poly(dI-dC) as a nonspecific competitor DNA, and protein as indicated. After addition of protein, the reaction mix was incubated at 4 °C for 30 min. The samples were then separated on a 4% polyacrylamide gel in 0.25× TBE as described (Das et al., 1988; Zhuang et al., 1992). The apoB genomic fragments F128, F128LM5, F152C, F152D, and F152E, the latter three containing various internal deletions, were prepared by digesting the respective plasmid DNA with *Xba*I and *Sac*I as described previously (Zhuang et al., 1992). These apoB fragments were labeled at the 5′ ends by [γ-^{32}P]ATP and polynucleotide kinase and were used in gel mobility shift assays.

RESULTS

Earlier work from this laboratory had shown that a rat liver nuclear protein, designated BRF-2, binds to the upstream sequence element (−104 to −85) of the apoB gene (Zhuang et al., 1992). BRF-2 was purified by a combination of DEAE cellulose, heparin agarose, and DNA sequence-specific affinity chromatography (Zhuang et al., 1992). In order to ascertain the identity of BRF-2 and to understand its functional role in apoB gene transcription, we purified this protein in large quantities and determined its amino acid sequence.

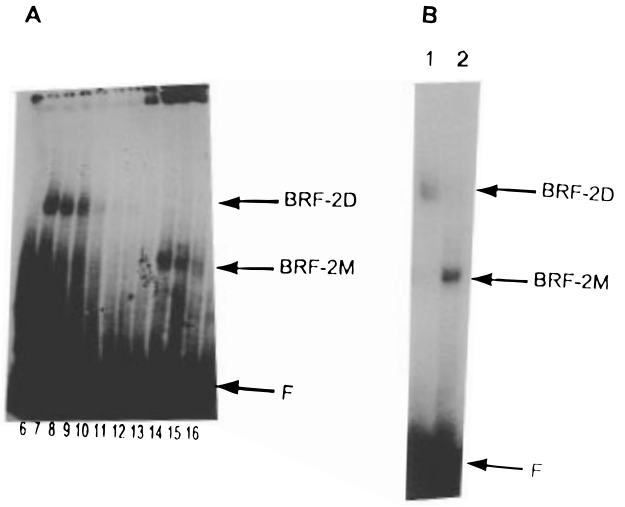

FIGURE 1: Purification of BRF-2M and BRF-2D by HPLC. (A) Elution profile of proteins fractionated by HPLC. Active BRF-2 fractions from two cycles of DNA sequence-specific affinity chromatography were pooled, dialyzed, and loaded onto an HPLC column as described in Experimental Procedures. A labeled 44-bp long double-stranded oligonucleotide (0.5 ng) containing the apoB sequence from −128 to −85 was used as the template for the DNA binding assay. A 2-μL portion of each eluant fraction was assayed for BRF-2 binding activity. F represents free probe. BRF-2M and BRF-2D indicate the positions of DNA−protein complexes corresponding to lower and higher molecular weight species of BRF-2, respectively. Numbers indicate fraction numbers. (B) Gel mobility shift assay of Centricon-100-concentrated BRF-2 fractions purified by HPLC. 2-μL (10 ng) portions of Centricon-100-concentrated low- and high-molecular-weight BRF-2 were used for DNA binding assays. The same 44-bp double-stranded oligonucleotide template described in A was used in this assay. F, BRF-2M, and BRF-2D indicate the positions of free probe and DNA−protein complexes with BRF-2M and BRF-2D, respectively.

Separation of Two BRF-2 Isoforms by HPLC. Rat liver nuclear extracts were prepared from frozen rat livers as described (Zhuang et al., 1992) and chromatographed sequentially through DEAE cellulose, heparin agarose, and two DNA sequence-specific affinity columns (Zhuang et al., 1992). The fractions from the second affinity column were assayed by gel mobility shift assay for DNA-binding activity using a 44-bp long double-stranded oligonucleotide containing the apoB sequence located between −128 and −85. The active fractions were pooled together and dialyzed against buffer M containing 50 mM KCl. The dialyzed sample was loaded onto a DEAE-5PW ion exchange HPLC column, and the bound proteins were step eluted with 0.4 M KCl. The elution profile of BRF-2 from the HPLC column was monitored using a gel mobility shift assay (Figure 1A). Part of the BRF-2 binding activity was eluted from the HPLC column in the earlier higher molecular weight fractions (fraction numbers 7−9). A second protein species of lower molecular weight was found to be eluted from the same HPLC column in the later fractions (fraction numbers 14 and 15). Both the higher and lower molecular weight species interacted with the apoB sequence (−128 to −85) and were designated as BRF-2D and BRF-2M, respectively. A part of BRF-2 was found to be degraded during HPLC purification, probably due to contaminating protease activity, and produced mobility shift corresponding to low-molecular-weight species (Figure 1A). Fractions containing BRF-2M and BRF-2D were pooled separately, dialyzed with buffer

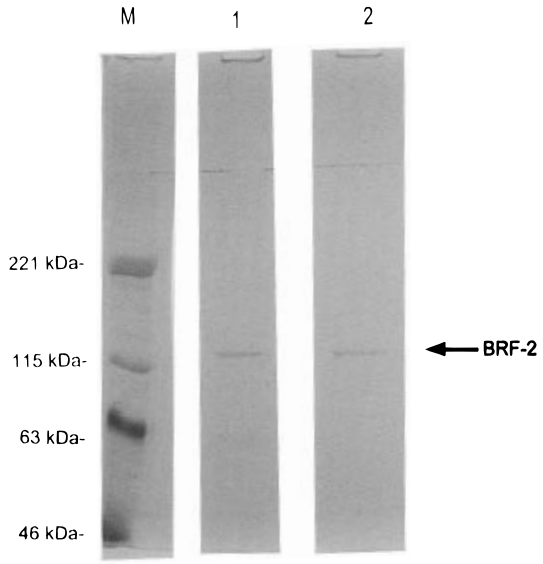

FIGURE 2: Analysis of HPLC-purified BRF-2M and BRF-2D by SDS−PAGE. Active BRF-2 fractions (75 ng) corresponding to BRF-2M and BRF-2D were separated by SDS−6% PAGE and visualized by silver staining. Lane M indicates protein molecular mass markers as follows: ovalbumin, 46 kDa; bovine serum albumin, 63 kDa; β-galactosidase, 115 kDa; myosin, 221 kDa. Lanes 1 and 2 show the profile of proteins present in the pooled fractions eluted from HPLC column corresponding to BRF-2M and BRF-2D isoforms and concentrated by Centricon-100, respectively. BRF-2 indicates the presence of a ~120-kDa protein band.

M containing 50 mM KCl, and concentrated by Centricon-100 for further characterization. The BRF-2M and BRF-2D fractions concentrated by Centricon-100 produced distinct bands in the gel mobility shift assay (Figure 1B). The low-molecular-weight DNA-binding activity present in the crude HPLC fractions was completely eliminated.

Both BRF-2M and BRF-2D Contain the Same Subunit of Molecular Weight ~120 kDa. To characterize the protein composition of BRF-2M and BRF-2D, the Centricon-100 concentrated fractions containing BRF-2M and BRF-2D activities were run on 6% SDS−PAGE alongside molecular weight markers. Both BRF-2M and BRF-2D yielded a single protein band that migrated at ~120 kDa as detected by silver stain (Figure 2, lanes 1 and 2). No lower molecular weight components were detected. These data suggest that both BRF-2 forms contain a subunit of molecular weight ~120 kDa. It was considered that BRF-2M and BRF-2D might represent two isoforms (i.e., monomer and homodimer) of BRF-2 or that they might be two different proteins able to recognize the same DNA sequence. HPLC-purified and Centricon-100-concentrated BRF-2D produced a single band in a native polyacrylamide gel visualized by a silver stain. BRF-2D recovered from native polyacrylamide gel by electroelution produced also a single ~120-kDa band in SDS−PAGE detected by silver stain and bound specifically with the apoB sequence (−128 to −85) (data not shown). Molecular characterization of BRF-2D electroeluted from a native polyacrylamide gel has also been reported previously by us (Zhuang et al., 1992).

BRF-2D Peptide Sequences Are Identical with Those of XAP-1 and UV-DDB. The amino acid sequences of two tryptic peptides of BRF-2D were determined as described. The sequences of these two peptides were found to be YLAIAPPIIK and ALYYLQIHPQELR. These sequences were subjected to a homology search using the BLAST network service at the National Center for Biotechnology Information. The search revealed 100% homology to corresponding sequences from a protein identified as hepatitis B virus X associated protein-1 (XAP-1) recently reported by Lee et al. (1995). XAP-1 in turn is a human homologue of UV-DDB, a protein possibly involved in the repair of UV-damaged DNA (Takao et al., 1993). The BRF-2 peptide YLAIAPPIIK corresponded to the amino acid sequence from position 245 to 254 of XAP-1 and UV-DDB (Lee et al., 1995). The peptide ALYYLQIHPQELR matched with the amino acid sequence from 515 to 527 of XAP-1 and UV-DDB (Lee et al., 1995). UV-DDB was purified and cloned from monkey kidney (CV-1) cells, and its molecular weight was determined to be 127 kDa (Abramic et al., 1991; Takao et al., 1993). The cDNA corresponding to XAP-1 was cloned from a human B lymphocyte library using the yeast two-hybrid system (Lee et al., 1995). The 4072 bp long cDNA contained an open reading frame (ORF) able to encode a protein of 1140 amino acids. The molecular weight of XAP-1 was estimated to be 127 kDa (Lee et al., 1995). As two peptide sequences of BRF-2 have 100% sequence homology with XAP-1 and UV-DDB and their molecular weights are similar, BRF-2 appears to be structurally highly related, if not identical, to XAP-1.

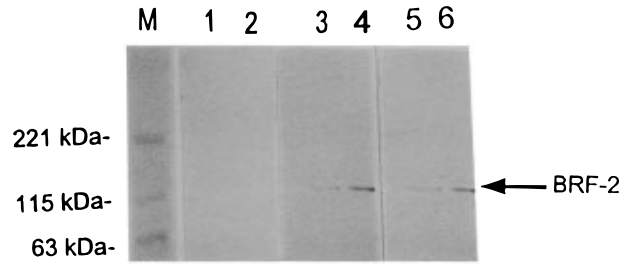

FIGURE 3: Western blot analysis of BRF-2M and BRF-2D using XAP-1 anti-peptide 1 and anti-peptide 2 antisera. 75 ng each of HPLC-purified and Centricon-100 concentrated BRF-2M (lanes 1, 3, and 5) and BRF-2D (lanes 2, 4, and 6) were run on SDS−6% PAGE and transblotted onto nitrocellulose membranes. Blots were probed with preimmune serum (lanes 1 and 2) or XAP-1 anti-peptide 1 antiserum (lanes 3 and 4) or XAP-1 anti-peptide 2 antiserum (lanes 5 and 6). Lane M indicates prestained protein molecular weight markers as follows: bovine serum albumin, 63 kDa; β-galactosidase, 115 kDa; myosin, 221 kDa. BRF-2 indicates the position of a ~120-kDa protein band recognized by anti-peptide 1 and anti-peptide 2 antisera.

Both BRF-2M and BRF-2D Are Recognized by XAP-1 Anti-Peptide Antisera. It has been shown previously (Lee et al., 1995) that anti-peptide 2 antiserum directed against XAP-1 immunoprecipitated a 127-kDa protein from HepG2 cell extracts. This suggested that XAP-1 is a 127-kDa protein, in agreement with the sequence data. In order to confirm the identity of BRF-2D with XAP-1, a western blot analysis was performed using HPLC-purified BRF-2M and BRF-2D (Figure 3). Both anti-peptide 1 (lanes 3 and 4) and anti-peptide 2 (lanes 5 and 6) antisera against XAP-1 recognized a single ~120-kDa protein band from both the BRF-2M (lanes 3 and 5) and BRF-2D (lanes 4 and 6) preparations. The preimmune sera did not recognize the ~120-kDa band (lanes 1 and 2). These data suggest that BRF-2M and BRF-2D share common epitopes and are immunologically highly related. Therefore we propose that BRF-2M and BRF-2D are two isoforms of the same protein; BRF-2M is presumably the monomer and BRF-2D is a homo-oligomer, most probably a homodimer.

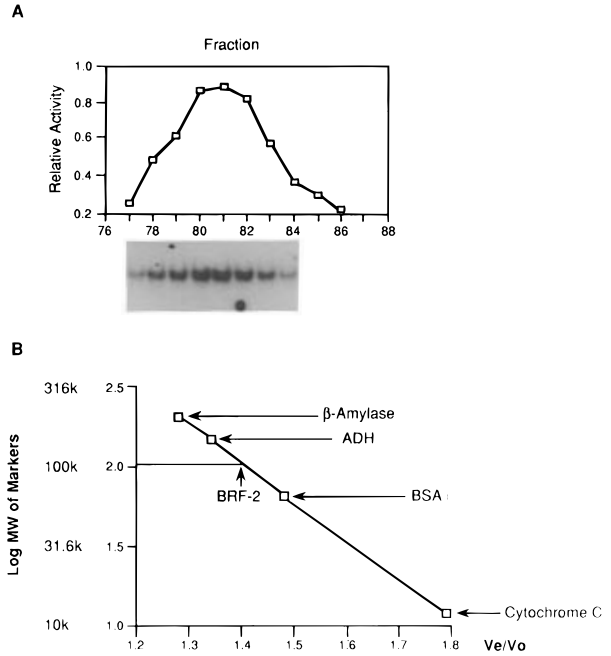

FIGURE 4: Determination of molecular weight of BRF-2M and BRF-2D by gel filtration. (A) BRF-2 prepared from the second cycle of DNA-specific affinity column was chromatographed on sephacryl S300 column as described under Experimental Procedures. Fractions were analyzed by DNA-binding and gel mobility shift assay using a 44-bp long double-stranded oligonucleotide containing the apoB sequence (−128 to −85). The regions of the autoradiogram containing the shifted bands are shown below panel A for fractions 77−84. Binding activity was determined by densitometric scanning of the autoradiograms of the mobility shift gls. (B) The column was calibrated by consecutive runs with the following molecular weight markers: β-amylase, 200 kDa; alcohol dehydrogenase (ADH), 150 kDa; BSA, 68 kDa; cytochrome *C*, 24 kDa. V_e is the elution volume of the protein, and V_o is the void volume of the column. Arrow indicates the activity peak of BRF-2.

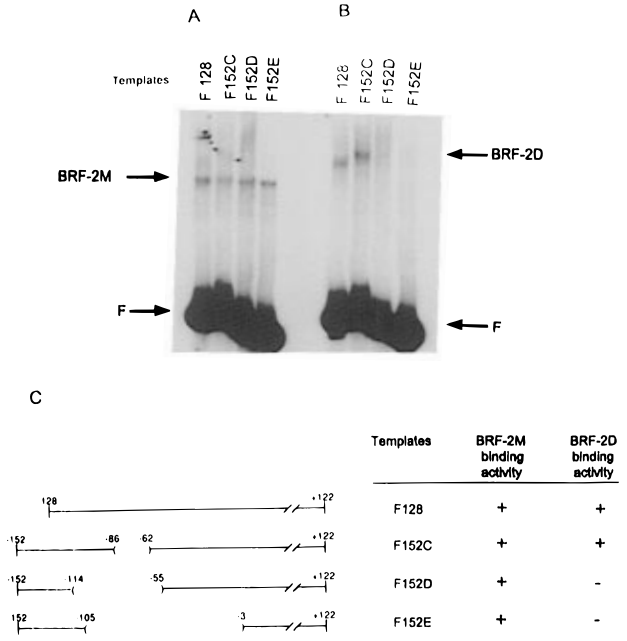

FIGURE 5: Gel mobility shift assays using wild-type and mutated apoB promoter fragments. Radiolabeled apoB promoter fragments F128 (−128 to +122), F152C, F152D, and F152E (1 ng = ∼20 000 cpm) were used as templates to detect the binding of HPLC-purified BRF-2M and BRF-2D activities. 2 μL (10 ng) each of HPLC column purified and Centricon-100 concentrated BRF-2M (panel A) and BRF-2D (panel B) were used in a DNA−protein binding assay. F, free labeled templates; BRF-2M and BRF-2D, positions of DNA−protein complexes with BRF-2M and BRF-2D, respectively. (Panel C) Schematic diagram showing the different apoB fragments used in the DNA-binding assays and a summary of BRF-2M and BRF-2D binding with different apoB promoter mutants. Mutations were produced by deletion of sequences from −85 to −63 (F152C), from −113 to −56 (F152D), and from −104 to −4 (F152E). The positions of nucleotides at the endpoints of each fragments are also marked. "+" and "−" symbols indicate the presence and absence of DNA−protein complex, respectively.

Determination of Molecular Weight of BRF-2M and BRF-2D by Gel Filtration. To confirm that BRF-2 exists as both monomer (BRF-2M) and homo-oligomer (BRF-2D), affinity-purified BRF-2 was chromatographed on a Sephacryl S300 gel filtration column and the molecular weight of the BRF-2 binding activity was determined by comparison with the elution of the standard proteins. The activity of BRF-2 in each column fraction was monitored by gel mobility shift assay using a labeled 44 bp long oligonucleotide containing the apoB sequence (−128 to −85). As shown in Figure 4A, BRF-2 activity was eluted in several fractions and corresponded to a single species in gel mobility shift assay. The peak BRF-2 activity corresponded to a molecular mass of ∼110 kDa (Figure 4B). We have frequently observed that BRF-2D appears to be quite unstable and converts rapidly to BRF-2M on storage. Therefore, it is possible that during gel filtration over several hours, the oligomer BRF-2D might have converted to monomer (BRF-2M). Consequently, BRF-2 was eluted solely as a monomer from the gel filtration column. Since it took approximately an hour to perform HPLC, two distinct forms of BRF-2 (BRF-2M, BRF-2D) were easily separated by HPLC method (Figure 1A).

Only the Oligomeric Isoform BRF-2D Interacts Specifically with the ApoB Promoter Region (−104 to −85). We showed previously that the binding site of BRF-2 was located between −104 and −85 in the apoB promoter (Zhuang et al., 1992). To determine if both BRF-2M and BRF-2D could recognize the same binding site (−104 to −85) of the apoB promoter, a series of labeled DNA fragments missing specific regions between −128 and +122 were used in a gel mobility shift assay. The radiolabeled fragment F128, containing the entire region from −128 to +122, as well as radiolabeled fragments F152C (containing a −85 to −63 internal deletion), F152D (containing a −113 to −56 internal deletion), and F152E (containing a −104 to −4 internal deletion) were all bound by BRF-2M (Figure 5A). This suggests that the binding site for BRF-2M is not located between −114 and −4. In contrast, BRF-2D was found to interact with only the F128 and F152C fragments; when fragments F152D and F152E were used as templates in the gel mobility shift assay, no portein−DNA complex was formed (Figure 5B). This suggests that the binding site for BRF-2D is located between −104 and −85. The binding of BRF-2M and BRF-2D with different apoB promoter mutants is summarized in Figure 5C. To better define the binding site of BRF-2M, another apoB promoter fragment, F152F (containing a −133 to −47 internal deletion), was used in a gel mobility shift assay, and it was found that BRF-2M formed a protein−DNA complex (data not shown). This result suggests that the monomeric form of BRF-2 (BRF-2M) is able to interact with all the apoB promoter fragments employed (with deletions spanning −133 to −4 of the promoter), whereas the oligomeric form BRF-2D interacts specifically with the apoB sequence located between −104 and −85. This result also

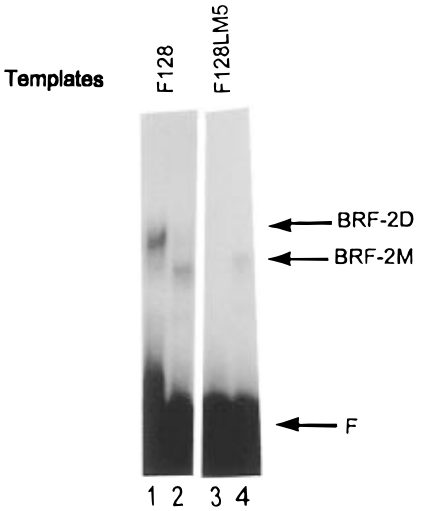

FIGURE 6: Gel mobility shift assay of BRF-2M and BRF-2D using wild-type and substitution mutant promoter fragments of the apoB gene. Radiolabeled apoB promoter fragments F128 (−128 to +122) and F128LM5 (1 ng = ∼20 000 cpm) containing apoB fragment (−128 to +122) with LM5 substitution mutations in the BRF-2 binding region (−96 to −88) were used as templates to detect the interaction of HPLC-purified BRF-2M and BRF-2D. 10 ng of HPLC column purified and Centricon-100-concentrated BRF-2D (lanes 1 and 3) and BRF-2M (lanes 2 and 4) were used in DNA−protein binding assay. F, free labeled template; BRF-2M and BRF-2D, positions of DNA−protein complexes with BRF-2M and BRF-2D, respectively.

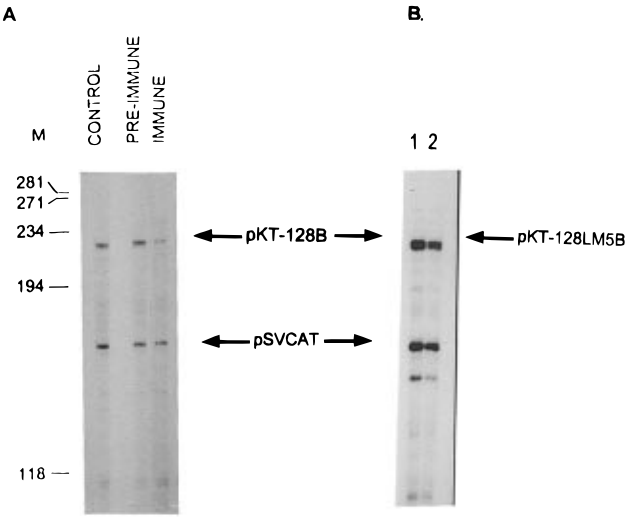

FIGURE 7: *In vitro* transcription assay of apoB/CAT (pKT-128B), pKT-128LM5B, and SV40/CAT (pSVCAT) constructs. (A) RNA was synthesized *in vitro* from 1 μg each of linearized pKT-128 and pSVCAT by using 200 μg of rat liver nuclear extracts in a total reaction volume of 40 μL. RNA was detected by primer extension of labeled oligonucleotide 5′-CGGTGGTATATCCAGTGA-3′ corresponding to the RNA coding strand of the CAT gene sequence from 4956 to 4973. Primer extension products were purified and analyzed by 6% acrylamide−7 M urea gel. pKT-128B and pSVCAT indicate the positions of 227- and 175-nucleotide long extension products from pKT-128B and pSVCAT plasmids respectively. M represents DNA molecular weight markers. Lane 1 contains *in vitro* transcription mixture preincubated with 8 μL of PBS. Lanes 2 and 3 contain *in vitro* transcription mixture preincubated with 8 μL each of purified preimmune or antipeptide 2 antiserum of XAP-1, respectively. Note the reduction in product from pKT-128B in extracts pre-treated with the XAP-1 antipeptide antiserum. (B) *In vitro* transcription of either pKT-128B and pSVCAT or pKT-128LM5 B and pSVCAT was done as described in A. Lane 1 contains pKT-128B (WT) and lane 2 contains pKT-128LM5B which has substitution mutations in the BRF-2 binding region (−96 and −88). pKT-128LM5B indicates the position of 227-nucleotide long extension product from the pKT-128LM5B plasmid. Note the reduction in product from pKT-128LM5B.

appears to suggest that the binding site for BRF-2M is not located between −128 and −85. Since both BRF-M and BRF-2D interacted with a double-stranded oligonucleotide containing the apoB sequence (−128 to −85) (Figure 1), this suggests that BRF-2M binds nonspecifically to any DNA sequence located between −128 and +122. Therefore, it appears that oligomerization of the ∼120−kDa subunit of BRF-2 imparts sequence-specificity. It is possible that this may facilitate its role in the transcriptional activation of the apoB gene.

The Sequence AAAGCAAACA in the Element (−104 to −85) Is Important for Interaction with BRF-2D. It has been previously shown that the substitution mutant LM5 and other mutants within the apoB element (−104 to −85) abolished the binding of NF-BCB 1, NF-BCB 2, and NF-BCB 3 proteins and reduced apoB gene transcription in HepG2 cells 10-fold (Kardassis et al., 1992). This study also established the role of apoB sequence AAAGCAAACA in protein−DNA interaction and apoB gene transcription. We introduced LM5 mutation in the apoB fragment (−128 to +122) in the CAT construct pKT-128LM5B. ApoB fragment F128 (WT) and LM5 mutant fragment F128LM5 were used as templates in gel mobility shift assay with BRF-2M and BRF-2D. As shown in Figure 6, both BRF-2M and BRF-2D interacted with F128, but only the homo-oligomeric form BRF-2D failed to bind to F128LM5 (Figure 6). This result confirms the importance of the sequence AAAGCAAACA within the apoB promoter element (−104 to −85) for specific protein−DNA interaction by BRF-2D and also substantiates the conclusion that BRF-2M binds non-specifically to the apoB sequence (−104 to −85).

Preincubation with Anti-Peptide 2 Antiserum Inhibits in Vitro Transcription of the ApoB Gene. Kardassis et al. (1992) showed previously that LM5 mutation introduced into plasmid −268/+8 CAT (LM5CAT) reduced the transcription of the apoB gene 10-fold. It has also been reported previously that anti-peptide 2 antiserum could recognize native XAP-1 protein in HepG2 cell extracts and immunoprecipitate a 127-kDa protein (Lee et al., 1995). This prompted us to investigate whether anti-peptide 2 antiserum had an effect on *in vitro* transcription of the apoB gene. Rat liver nuclear extracts were used for *in vitro* transcription of plasmid pKT-128B, which contains sequence −128 to +122 of the apoB gene linked to the CAT gene. Plasmid pSVCAT was used as an internal control for *in vitro* transcription. Plasmids pKT-128B and pSVCAT were co-transcribed *in vitro* and transcripts from both plasmids were detected by primer extension of a labeled oligonucleotide corresponding to nucleotides 4956−4973 of the RNA coding strand of the CAT gene sequence. As shown in Figure 7A, 175- and 227-nucleotide long extension products were detected, corresponding to pSVCAT- and pKT-128B-derived products, respectively (lane 1). Preincubation of nuclear extracts with preimmune sera before the addition of template DNA and the start of the transcription reaction had no effect on the amount of transcription from either pKT-128B or pSVCAT (lane 2). In contrast, preincubation of rat liver nuclear extracts with anti-peptide 2 antiserum reduced the amount of transcription of pKT-128B by nearly 50%, whereas there

was no change in transcription of pSVCAT (lane 3). Introduction of LM5 mutation (pKT-128LM5B) also reduced apoB transcription *in vitro* by 50% (Figure 7B, lane 2). These data suggest that BRF-2/XAP-1 is a *trans*-activator of the apoB gene.

DISCUSSION

Background. We have shown previously that hepatic-cell-specific expression of the human apoB gene is determined by at least two *cis*-acting elements (−128 to −85) and (−84 to −70) (Das et al., 1988). The proximal element (−84 to −70) acts as a strongly positive element in HepG2 cells and as a mildly positive element in HeLa cells. Addition of the distal element (−128 to −85) produced 5-fold greater reporter CAT activity in HepG2 cells as compared to a construct containing the proximal positive element (−84 to −70); the distal element had a negative effect in HeLa cells. Previously, we reported the purification of a rat liver nuclear protein BRF-2 which specifically interacts with the apoB gene sequence (−104 to −85) (Zhuang et al., 1992). Kardassis et al. (1992) also fractionated three rat liver proteins (NF-BCB 1, 2, and 3) by heparin agarose column chromatography that bind to the apoB distal element (−128 to −85). Paulweber et al. (1991d) showed using gel mobility shift assays that *in vitro* synthesized HNF-3 from its cDNA clone also interacts with this element. These data suggest that several proteins may interact with the apoB distal element (−128 to −85) and may theoretically modulate the expression of the apoB gene under different physiological conditions.

BRF-2 and XAP-1 Are Highly Related Proteins. In order to characterize the interaction of BRF-2 with this distal element and with other transcription factors that may bind to the apoB gene promoter, we purified two isoforms of BRF-2 (designated BRF-2M, BRF-2D) to homogeneity from rat liver nuclear extracts and determined the amino acid sequence of two tryptic peptides derived from the heavier isoform (BRF-2D). Homology searches revealed that these two peptides had 100% sequence homology with the hepatitis B virus X associated protein-1 (XAP-1), cloned from human B lymphocytes and with a UV-damaged DNA-binding protein (UV-DDB) which was purified and cloned from a monkey kidney cell line (Abramic et al., 1991; Takao et al., 1993). XAP-1 appears to be the human homologue of UV-DDB. UV-DDB has a molecular weight of ∼127 kDa and binds strongly with UV-damaged DNA, reflecting its putative role as a DNA repair protein (Abramic et al., 1991). Recently, Hwang and Chu (1993) reported the purification from human placenta of a 125-kDa DNA-binding protein ('XPE-BF') that has high affinity for UV-irradiated DNA and shows striking biochemical similarity to the UV-DDB. Both UV-DDB and XPE-BF have been implicated in DNA damage repair following UV radiation (Abramic et al., 1991; Hwang & Chu, 1993). The protein is absent in cells from some subgroup E patients having the inherited disease xeroderma pigmentosum, a disease which is characterized by defective repair of DNA-damaged by UV radiation (Chu & Chang, 1988).

XAP-1 was cloned from a human B lymphocyte cDNA library using a yeast two-hybrid system (Lee et al., 1995). XAP-1 was found to associate with the HBV-encoded X protein (Lee et al., 1995), a *trans*-activator of several viral and cellular genes (Buendia, 1992; Rossner, 1992; Slagle et al., 1992) that reportedly interacts with cellular factors involved in transcription (Feitelson et al., 1993; Maguire et al., 1991; Wang et al., 1994). Therefore, the association of HBx protein with a putative cellular DNA repair protein (XAP-1/UV-DDB/XPE-BF) suggests a potential role for a DNA-repair protein in HBV gene transcription and, indirectly, in the development of human hepatocellular carcinoma (Lee et al., 1995).

BRF-2 Exists As a Monomer and a Homooligomer. In this paper we have presented evidence that BRF-2 exists as two isoforms in solution that we interpret to be monomeric and oligomeric forms. We have purified these two isoforms by HPLC, and both forms yielded a single protein band of ∼120 kDa by SDS−PAGE. Whereas it is possible that two isoforms represent different modification states of the protein, we prefer the oligomerization explanation. There is not agreement in the literature regarding the higher molecular weight forms represented by UV-DDB, reflecting perhaps the different sources of protein characterized. Abramic et al. (1991) purified UV-DDB from CV-1 cells and showed the molecular weight of the native protein deduced from gel filtration and native PAGE to be about 210 kDa whereas the denatured UV-DDB had a molecular weight of 126 kDa, suggesting that UV-DDB was present in solution as a homodimer. Hwang and Chu (1993) reported the purification of XPE binding factor (XPE-BF), which binds to DNA damaged by UV, cisplatin, or denaturation. The denatured XPE-BF protein migrated as a 125-kDa polypeptide on SDS−PAGE, and the native protein migrated primarily as a monomer on gel filtration and glycerol gradient sedimentation, although a minor portion of XPE-BF sedimented as a homodimeric form (Hwang & Chu, 1993). Keeney et al. (1993) purified a DNA damage binding protein (DDB) from HeLa cells and showed subsequently (Keeney et al., 1994) that microinjection of the purified DDB corrected the DNA repair defect in XP-E cells. The majority of the activity appeared to be a heterodimeric complex, composed of 124- and 41-kDa polypeptides (Keeney et al., 1993). This 41-kDa polypeptide has also been recently cloned and has been shown not to have any sequence homology with UV-DDB (Dualan et al., 1995). Purified UV-DDB, XPE-BF, and DDB bind UV-damaged DNA with much higher affinity than normal intact DNA, suggesting a possible role in DNA repair (Abramic et al., 1991; Hwang & Chu, 1993; Keeney et al., 1993). All three proteins appeared able to bind damaged DNA as either a monomer or dimeric form. It is likely that BRF-2, XAP-1, UV-DDB, DDB, and XPE-BF are the same protein.

BRF-2/XAP-1 Is a trans-Activator of the ApoB Gene. Anti-peptide antisera directed against XAP-1 recognized a ∼120-kDa BRF-2 polypeptide by western blot analysis, indicating that BRF-2 and XAP-1 are structurally and immunologically highly related. Further, anti-peptide 2 antiserum, when pre-incubated with a rat liver nuclear extract, decreased the *in vitro* transcription of the human apoB gene by 50%, confirming that BRF-1/XAP-1 is the positive activator of the apoB gene. Mutations in the BRF-2 recognition sequence of the apoB promoter also reduced the *in vitro* transcription of the apoB gene 2-fold, but the same mutant reduced apoB gene transcription in transfected Hep G2 cells 10-fold (Kardassis et al., 1992). The difference between *in vivo* and *in vitro* data could be due to partial

loss of BRF-2 activity during the preparation of *in vitro* transcription extract.

To demonstrate the physical association of BRF-2/XAP-1 with anti-peptide antisera of XAP-1, we attempted supershift experiments but without success. There could be several reasons for this result. (1) The epitope recognized by the anti-peptide antisera may be hidden and inaccessible to antibody when BRF-2/XAP-1 is bound to DNA. (2) Complex formation between BRF-2/XAP-1 with anti-peptide antisera may mask the DNA-binding domain of the protein. (3) Complex formation between BRF-2/XAP-1 and DNA may change protein conformation and alter the antibody binding site. (4) Finally, the binding affinity between the antibody and BRF-2/XAP-1 complexed with DNA may be too weak to function in the supershift assay.

We also attempted gel mobility shift assays using XAP-1 protein synthesized *in vitro* in a coupled transcription/translation system. This experimental approach failed, perhaps because the amount of XAP-1 protein produced *in vitro* is actually very small. Additionally, protein modifications may be needed to produce functional BRF-2/XAP-1, and those modifications may not be present on *in vitro* synthesized proteins.

This study demonstrates the role of a putative DNA repair protein in the *trans*-activation of a liver-specific apoB gene. It remains to be determined if BRF-2/XAP-1 is able to *trans*-activate transcription of HBV genes. In addition to BRF-2/XAP-1, other proteins involved in DNA repair have recently been found to be the members of general transcription factors, e.g., the XPBC gene product has been identified as part of the basal transcription factor TFIIH, linking nucleotide excision repair and transcription (Drapkin et al., 1994; Schaeffer et al., 1993). More research will be necessary to understand how BRF-2/XAP-1 could be involved in the dual functions of DNA repair and gene transcription.

Association of XAP-1/UV-DDB with HBV-encoded HBx protein suggests that XAP-1 may participate in the repair of the incomplete HBV genome during replication. A deleterious effect of HBV on cellular DNA repair processes could contribute to the development of human hepatocellular carcinoma (Maguire et al., 1991). It is noteworthy that the expression of XAP-1/UV-DDB is not restricted only to skin fibroblasts but is expressed in a variety of tissues, including liver (Abramic et al., 1991). Expression of BRF-2/XAP-1 in liver cells and its participation in the *trans*-activation of the apoB gene indicate a potential involvement in the expression of other liver-specific genes.

Potential Mechanisms Controlling ApoB Gene Transcription and Damaged DNA Repair. Available evidence suggests that BRF-2/XAP-1 exists as two isoforms in solution, which we interpret to be a monomer and a homo-oligomer, probably a homodimer. The question remains whether both forms participate in transcription of the apoB gene. A gel mobility shift assay using a series of labeled DNA fragments missing specific regions between −128 and +122 and a fragment containing mutations from −96 to −88 showed that the BRF-2 homodimer recognized the apoB sequence (−104 to −85) specifically. In contrast, the BRF-2 monomer bound all the mutant apoB promoters equally well. This result suggests that the monomeric and dimeric forms of BRF-2/XAP-1 may have different roles. The homodimer may act as a specific *trans*-activator of the apoB gene. Since the monomer binds to DNA nonspecifically, it may participate in UV-damaged DNA repair. This is reminiscent of the observation that hepatocyte nuclear factors (HNF-1 and HNF-2/HNF-4) and C/EBP which participate in the transcription of several liver-specific genes also bind to the respective *cis*-acting elements as homodimers (Lamb & McKnight, 1991; Mendel & Crabtree, 1991; Sladek et al., 1990). Whereas HNF-1 binds to DNA as a homodimer, it exists in solution as a monomer (Mendel & Crabtree, 1991). HNF-2/HNF-4 binds specifically to DNA both as a monomer and a homodimer (Sladek et al., 1990; Tronche & Yaniv, 1992), whereas C/EBP recognizes specific DNA only as a dimer (Lamb & McKnight, 1991). Formation of homodimers by HNF-1, HNF-2/HNF-4, and C/EBP requires the presence of specific DNA (Lamb & McKnight, 1991; Mendel & Crabtree, 1991; Sladek et al., 1990). In contrast, BRF-2/XAP-1 does not require specific DNA for the formation of the homodimer. It is known that HNF-1, C/EBP, c-Jun, and other transcription factors form heterodimers within their respective family and modulate the binding affinity to *cis*-acting elements, thereby altering the *trans*-activation potential by those proteins (Chiu et al., 1988; Jones, 1990; Lamb & McKnight, 1991; Mendel & Crabtree, 1991; Tronche & Yaniv, 1992). Jun protein, for example, binds as a homodimer to the AP-1 binding site but with relatively low apparent affinity (Jones, 1990), whereas Jun−Fos heterodimers bind to the same site with high affinity. The difference in binding reflects differences in dimerization stability, with Jun-Fos dimers forming at least 500-fold more efficiently than Jun−Jun dimers (Chiu et al., 1988; Jones, 1990). Therefore, it is possible that BRF-2/XAP-1 may form heterodimers with unknown transcription factor(s) which could alter the DNA binding and transcriptional potentials of BRF-2/XAP-1 under different physiological conditions.

Several motifs, such as zinc fingers, leucine zippers, basic regions, and POU, and homeobox domains, have been implicated as important to the DNA-binding domains of the known transcription factors (Berg, 1990; Clerc et al., 1988; Herr & Cleary, 1995; Herr et al., 1988; Lamb & McKnight, 1991). The dimerization domains of several transcription factors have been identified to be leucine zipper, helix−turn−helix, and helix−loop−helix (Jones, 1990; Lamb & McKnight, 1993). Takao et al. (1993) searched for the presence of such DNA-binding and dimerization motifs in UV-DDB and could not locate any identifiable motif. In addition, they were not able to find similarities of UV-DDB to any other protein in the transcription factors database (Takao et al., 1993). This observation suggests the possibility that BRF-2/XAP-1/UV-DDB may belong to a new family of transcription factors. Identification of DNA-binding, dimerization, and *trans*-activation domains of BRF-2/XAP-1 will be necessary to elucidate the mechanism of *trans*-activation of the apoB gene by BRF-2/XAP-1.

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