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Characterization of a Nuclear Factor that Binds to AP1-Like Element in the Rat p53 Promoter During Liver Regeneration

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Abstract The transcription level of the rat p53 gene increases at 5–12 h in the regenerating liver after partial hepatectomy. It was previously reported that an activator protein 1 (AP1)-like element (-264–-284) mediated the induced transcription of the rat p53 gene during liver regeneration. In this study, we characterize the protein binding to the AP1-like element by various methods. Oligonucleotide competition assays showed that the binding protein did not require AP1 consensus sequence. Therefore, the binding protein is not an AP1 family protein. Zn^{2+} was required for maximum DNA-binding activity of the protein, suggesting that the binding protein contains zinc fingers. The binding protein was highly resistant to denaturant. Even 1.8 M urea did not eliminate the protein–DNA complexes. In addition, the binding protein was stable up to 55°C. The protein–DNA complexes were abolished in the presence of 0.6 M NaCl and higher. Protease clipping assay showed that the protein had a protease-resistant core DNA binding domain. These results provided new insights into the structure of the protein that binds to the AP1-like element of the p53 promoter during liver regeneration. J. Cell. Biochem. 80:124–132, 2000.

Key words: AP1-like element; characterization; liver regeneration; p53 promoter; partial hepatectomy; transcription regulation; zinc finger

p53 is a well-known tumor suppressor protein, and mutations in the p53 gene were commonly found to be associated with diverse types of human cancer [Hollstein et al., 1991]. It is a critical component of cellular mechanisms that respond to certain stresses to preserve genomic integrity by arresting cell-cycle progression or by inducing apoptosis. p53 performs a function as a checkpoint at the G1/S transition and arrests cell cycle at the G1 phase [Levine, 1997]. Recently, it was proposed that p53 is also a checkpoint at the G2/M transition [Cross et al., 1995]. The functions of p53 are achieved mainly by the transcriptional activation of its effector genes [Levine, 1997]. It binds to and transactivates the promoters containing a p53-responsive DNA sequence element [Weintraub et al., 1991; Funk et al., 1992; Kern et al., 1992; Schärer and Iggo, 1992]. Generally, p53 exists at a low concentration under normal physiologic condition. However, under certain stress conditions, such as DNA damage by ultraviolet irradiation or γ-irradiation, the expression level of p53 increases. In response to DNA damage, p53 induces cell-cycle arrest in the G1/S checkpoint and attempts to repair their DNA before it is replicated [Kastan et al., 1991; Kuerbitz et al., 1992; Lane, 1992; Nelson and Kastan, 1994]. Therefore, it eliminates premalignant cells that enter S phase inappropriately.

The adult liver is normally in a state of growth arrest [Bucher et al., 1983]. Prolifera-

Abbreviations used: AP1, activator protein 1; CRE, cAMP responsive element; DTT, 1,4-dithiothreitol; EDTA, ethylenediaminetetraacetate; EMSA, electrophoretic mobility shift assay; Hepes, N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid; NF1, nuclear factor 1; PF1, p53 factor 1; PF2, p53 factor 2; PMSF, phenylmethanesulfonyl fluoride; USF, upstream stimulating factor; YY1, ying yang 1.

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tion of hepatocytes can be induced by cell loss resulting from hepatocellular necrosis. In addition, removal of 70% of the liver weight elicits a partially synchronized proliferation of hepatocyte [Grisham, 1992]. After partial hepatectomy, the remaining lobes grow rapidly and restore the original mass of liver in 1–2 weeks. During liver regeneration, most hepatocytes undergo DNA synthesis. Studies on the alteration of gene expression during liver regeneration have led to the conclusion that liver regeneration can be defined as a reprogramming of gene expression with transient quantitative changes in the expression of proto-oncogenes such as *myc*, *fos*, and *ras* [Thompson et al., 1986]. The transcription level of the p53 gene also increases during liver regeneration [Thompson et al., 1986; Lee et al., 1998a].

Unlike many genes transcribed by RNA polymerase II, the p53 promoter does not have remarkable TATA or CAAT boxes in its promoter region [Bienz-Tadmor et al., 1985; Lamb and Crawford, 1986]. However, the previous studies on the characterization of the p53 promoter showed that the p53 promoter has several potential transcription factor binding sites. These transcription factors include nuclear factor 1 (NF1) [Ginsberg et al., 1990; Furlong et al., 1996; Lee et al., 1998b; Lee et al., 2000], Myc/Max [Roy et al., 1994], upstream stimulating factor (USF) [Hale and Braithwaite, 1995], p53 factor 1 (PF1) [Ginsberg et al., 1990], and p53 factor 2 (PF2) [Hale and Braithwaite, 1995]. The binding DNA elements of them are well conserved across species. In addition to these elements, we found a cis-regulatory element required for the transcriptional induction of the p53 gene after partial hepatectomy [Lee et al., 1998a]. This element is located at -264–-284 and has the sequence of ACCCTGACTCTGCAAGTCCCC. This element contains the region that has high homology to activator protein 1 (AP1) consensus sequence. Therefore, the element was referred to as AP1-like element. It was also reported that a 39-kDa protein bound to the AP1-like element [Lee et al., 1998a]. In this study, to identify the binding protein to the element, we carried out oligonucleotide competition assay and characterized the binding protein by various biochemical methods. This study showed that the binding protein was not an AP1 family protein. In addition, the biochemical characterization of the binding factor provided new insights into the structure of the binding protein.

MATERIALS AND METHODS

Partial Hepatectomy and Preparation of Nuclear Extract

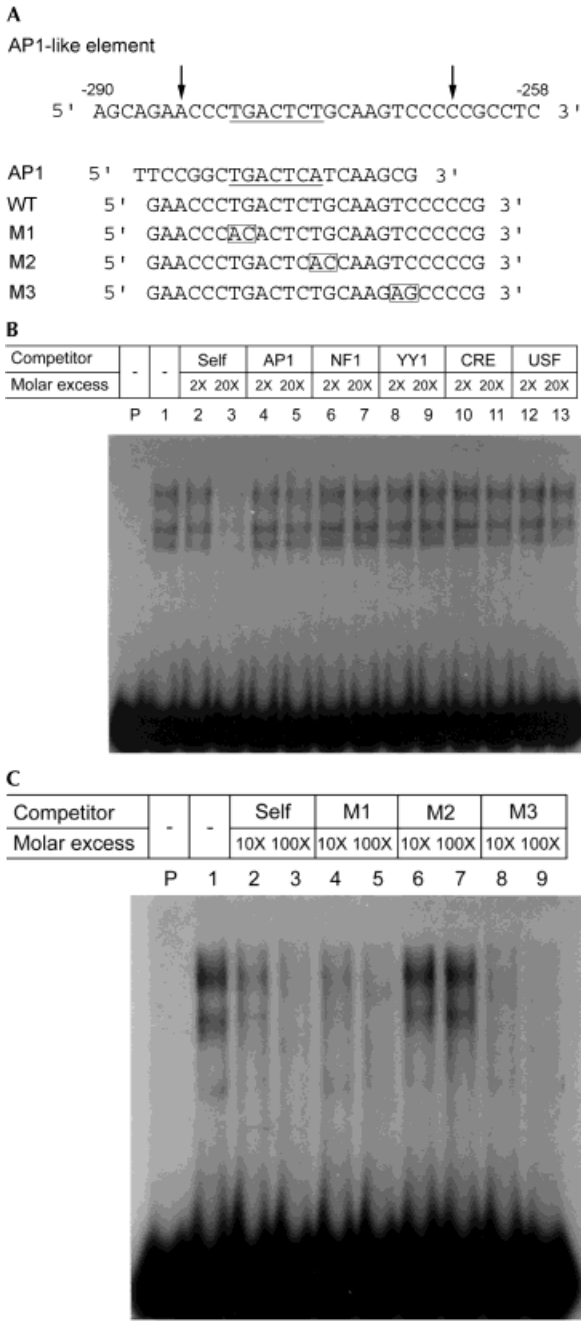
Female Wistar rats (Animal Breeding Center in Seoul National University) weighing 120–140 g were used for all experiments. The animals were kept in temperature-controlled rooms with 12-h alternating light and dark cycles. Partial hepatectomy was performed under ether anesthesia, with removal of the main lobes (67% of the liver was excised).

Nuclear extracts were prepared from regenerating liver 9 h after partial hepatectomy by the method described previously [Lee et al., 1997]. Briefly, rat liver was homogenized in four volume of buffer A (10 mM Tris, pH 7.9, 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetate (EDTA), 1 mM spermidine, 1 mM 1,4-dithiothreitol (DTT), and 0.1 mM phenylmethanesulfonyl fluoride (PMSF)) containing 0.32 M sucrose. Homogenates were layered over buffer A containing 2 M sucrose and centrifuged for 50 min at 40,000*g*. The nuclei were suspended in lysis buffer (15 mM N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (Hepes), pH 7.9, 100 mM KCl, 3 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, and 0.1 mM PMSF), to which 0.1 volume of 4 M $(NH_4)_2SO_4$ was added and stirred at 4°C for 30 min. After being centrifuged for 1 h at 130,000*g*, 0.3 g of $(NH_4)_2SO_4$ per ml of supernatant was added and stirred for 30 min. Nuclear extract was centrifuged for 15 min at 16,000*g*, dissolved in dialysis buffer (20 mM Hepes, pH 7.9, 100 mM KCl, 20% glycerol, 2 mM $MgCl_2$, 1 mM DTT, 0.2 mM EDTA, and 0.5 mM PMSF), and dialyzed against dialysis buffer overnight at 4°C.

Oligonucleotide Competition Assay in Electrophoretic Mobility Shift Assay (EMSA)

The synthetic oligonucleotides containing a specific binding sequence (WT: 5′- GAACCCTGACTCTGCAAGTCCCCCG-3′, 5′-CGGGGGACTTGCAGAGTCAGGG TTC-3′) were annealed and labeled by using [γ-^{32}P]ATP and T4 polynucleotide kinase. Binding reactions were carried out as described previously [Lee et al., 1998b]. Binding reaction mixtures contained 3 μg of nuclear extract, radiolabeled WT oligonucleotide (30,000 cpm), 2 μg of poly(dI-dC),

and binding buffer (1×: 12 mM Hepes, pH 7.9, 60 mM KCl, 5 mM $MgCl_2$, 200 μM EDTA, 200 μM DTT, and 12% glycerol) in volume of 20 μl. For consensus oligonucleotide competition assay, double-stranded consensus oligonucleotide competitors [WT, AP1 consensus, NF1 consensus, ying yang 1 (YY1) consensus, cAMP responsive element (CRE), or USF consensus] were added to each reaction mixture indicated in Figure 1B. For mutant competition assay, double-stranded mutant oligonucleotides were added to the binding reaction mixtures indicated in Figure 1C. The sequences of the synthetic oligonucleotides are as follows. AP1 consensus, 5′-TTCCGGCTGACTCATCAAGCG-3′; NF1 consensus, 5′-TTTGGCACGGAGCCAAC-3′; YY1 consensus, 5′-AGCGGCCATCTTGGCTG-3′; CRE, 5′-TGCTGACGTCAAAC-3′; USF consensus, 5′-CCGGGCACGTGACCAC-3′; M1, 5′-GAACCCACACTCTGCAAGTCCCCCG-3′; M2, 5′-GAACCCTGACTCACCAAGTCCCCCG-3′; M3, 5′-GAACCCTGACTCTGCAAGAGCCCCG-3′. After the addition of end-labeled WT oligonucleotide (30,000 cpm), the mixtures were incubated at room temperature for 20 min. They were electrophoresed through a 6% polyacrylamide gel in the presence of 0.25× Tris-borate with EDTA (TBE). The gel was dried and subjected to autoradiography.



Treatment with Chelators

The divalent cation chelators used in EMSA were prepared as previously described [Hooft van Huijsduijnen et al., 1987]. The divalent cation chelators were added to the binding mixtures indicated in Figure 2A. Binding reaction mixtures contained 3 μg of nuclear extract, radiolabeled WT oligonucleotide (30,000 cpm), 2 μg of poly(dI-dC), and binding buffer in volume of 20 μl. After 20 min of binding reaction, the mixtures were

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Fig. 1. A: The sequence around the activator protein 1 (AP1)-like element and oligonucleotides competitors. The AP1-like motif is presented and AP1 consensus sequence is underlined. The protection boundaries in DNase I footprinting assay were indicated by arrows [Lee et al., 1998a]. The sequences of the oligonucleotides are also shown. AP1, AP1 consensus oligonucleotide; WT, wild type AP1-like oligonucleotide; M1, M2, and M3, Mutant type AP1-like oligonucleotides. The mutation positions are indicated by rectangles. **B:** Consensus oligonucleotides competition assay. Three micrograms of regenerating nuclear extract were assayed for protein binding to the radiolabeled WT oligonucleotide without competitor (**lane 1**) or in the presence of the competitor. **Lanes 2 and 3**: WT; **lanes 4 and 5**: AP1 consensus; **lanes 6 and 7**: nuclear factor 1 (NF1) consensus; **lanes 8 and 9**: ying yang 1 (YY1) consensus; **lanes 10 and 11**: cAMP responsive element (CRE); **lanes 12 and 13**, upstream stimulating factor (USF) consensus. P indicates electrophoretic mobility shift assay (EMSA) without nuclear extract. **C:** Mutant oligonucleotide competition assay. Three micrograms of regenerating nuclear extract were assayed for protein binding to the radiolabeled WT oligonucleotide without competitor (**lane 1**) or in the presence of the competitor. **Lanes 2 and 3**: WT; **lanes 4 and 5**: M1; **lanes 6 and 7**: M2; **lanes 8 and 9**: M3. P indicates EMSA without nuclear extract.

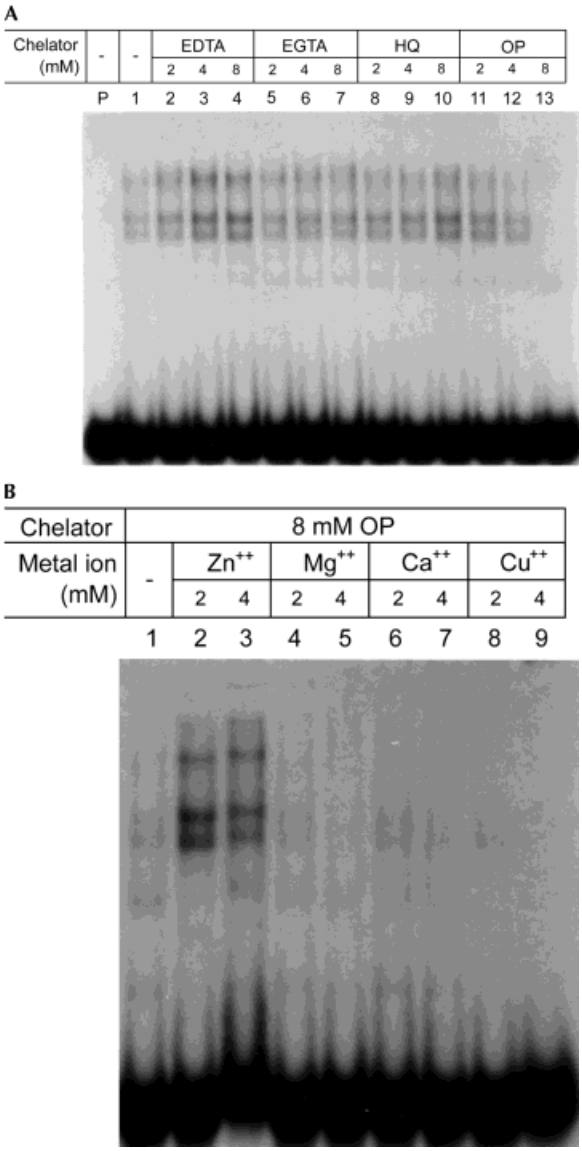

Fig. 2. **A:** Dependence of DNA–protein complex formation on divalent metal ions. Three micrograms of regenerating nuclear extract were assayed for protein binding to the radiolabeled WT oligonucleotide without chelator (**lane 1**) or in the presence of chelator. **Lanes 2, 3, and 4:** ethylenediaminetetraacetate (EDTA); **lanes 5, 6, and 7:** EGTA; **lanes 8, 9, and 10**: 8-hydroxyquinoline (HQ); **lanes 11, 12, and 13**: ortho-phenanthroline (OP). P indicates electrophoretic mobility shift assay (EMSA) without nuclear extract. **B:** Zn^{2+} requirement for DNA–protein complex formation. Three micrograms of regenerating nuclear extract were assayed for protein binding to the radiolabeled WT oligonucleotide in the presence of 8 mM ortho-phenanthroline. The reaction mixtures were supplemented with the indicated final concentrations of divalent cations. **Lanes 2 and 3**: Zn^{2+}; **lanes 4 and 5**: Mg^{2+}; **lanes 6 and 7**: Ca^{2+}; **lane 8 and 9**: Cu^{2+}.

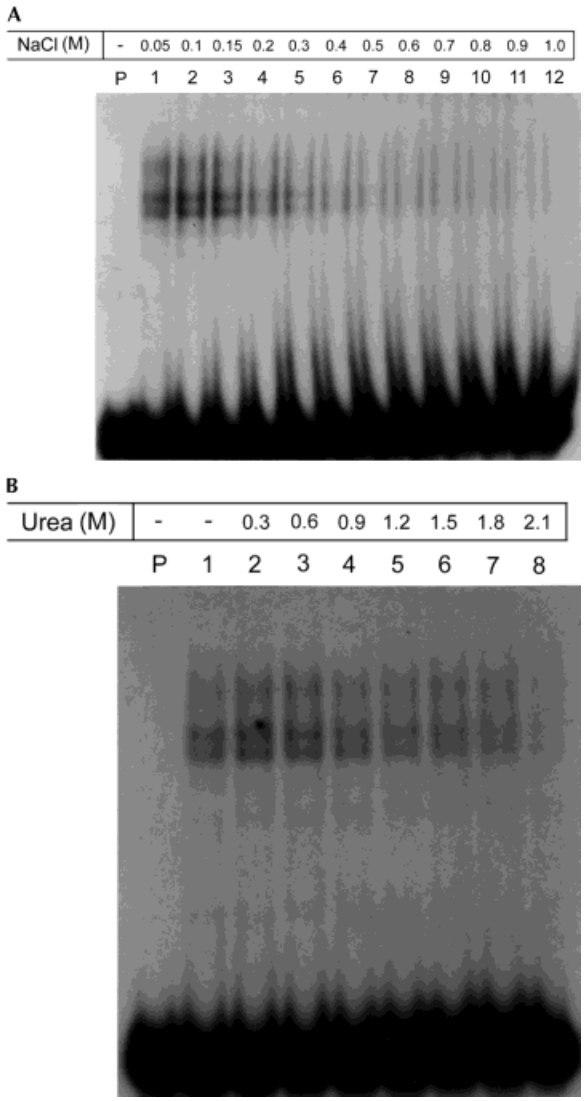

Fig. 3. **A:** Effects of ionic strength. Three micrograms of regenerating nuclear extract were assayed for protein binding to the radiolabeled WT oligonucleotide. The reaction mixtures were supplemented with the indicated final concentrations of NaCl. P indicates electrophoretic mobility shift assay (EMSA) without nuclear extract. **B:** Effects of urea. Three micrograms of regenerating nuclear extract were assayed for protein binding to the radiolabeled WT oligonucleotide. The reaction mixtures were supplemented with the indicated final concentrations of urea. P indicates EMSA without nuclear extract.

analyzed by EMSA. To identify the divalent cation required for protein binding, various divalent cations (Zn^{2+}, Mg^{2+}, Ca^{2+}, or Cu^{2+}) were added to the reaction mixtures in the presence of 8 mM ortho-phenanthroline. After 20 min of binding reaction, the samples were analyzed by EMSA.

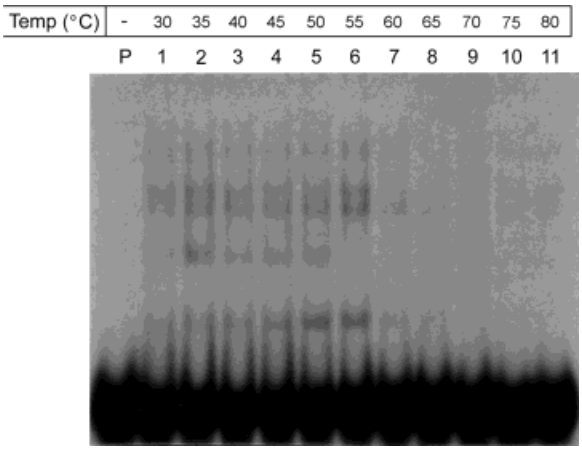

Fig. 4. Effects of temperature. Aliquots of regenerating liver nuclear extracts were incubated at the indicated temperature for 5 min then cooled on ice. Three micrograms of each regenerating nuclear extract were assayed for protein binding to the radiolabeled WT oligonucleotide. P indicates electrophoretic mobility shift assay (EMSA) without nuclear extract.

Ionic Strength, Urea, and Temperature Sensitivity Assays

To study the effects of ionic strength and urea on DNA–protein complex formation, NaCl and urea were added to the binding reaction mixtures indicated in Figure 3A and 3B, respectively. Binding reaction mixtures contained 3 μg of nuclear extract, radiolabeled WT oligonucleotide (30,000 cpm), 2 μg of poly(dI-dC), and binding buffer. After the binding reaction, the samples were analyzed by EMSA.

For the temperature sensitivity assay, aliquots of nuclear extract were incubated at the desired temperature indicated in Figure 4 for 5 min and placed on ice. The heat-treated nuclear extracts were used for binding reactions. Each binding reaction mixture contained 3 μg of nuclear extract, radiolabeled WT oligonucleotide (30,000 cpm), 2 μg of poly(dI-dC), and binding buffer. After the binding reaction, they were assayed by EMSA.

Protease Clipping Assay

The binding reactions were carried out at room temperature for 20 min before treatment of protease. Indicated quantities of proteinase K or trypsin (Fig. 5A or 5B) were added to the binding reaction mixtures and the samples were incubated at room temperature for 10 min. After the protease reactions, the samples were immediately applied to polyacrylamide gel and analyzed.

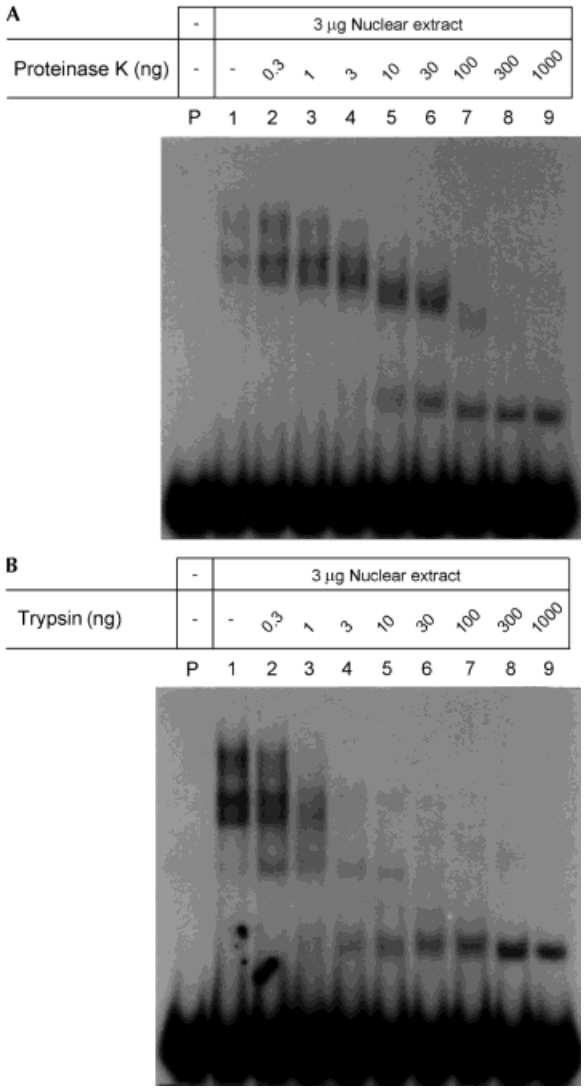

Fig. 5. Protease clipping assay. Reactions were set up as usual with 3 μg of regenerating liver nuclear extracts and the radiolabeled WT oligonucleotide. After binding reaction at room temperature for 20 min, proteinase K (**A**) or trypsin (**B**) was added. After further 10 min reaction, the samples were analyzed by electrophoretic mobility shift assay.

RESULTS

Oligonucleotide Competition Assay

It was previously reported that an AP1-like element in the rat p53 promoter was involved in the induction of the p53 gene during liver regeneration [Lee et al., 1998a]. This element is located at -264—-284 and contains the region that has high homology to AP1 consensus motif (AP1-like element in the p53 promoter, TGACTCT; AP1 consensus, TGACTCA; Fig. 1A). In consensus oligonucleotide competition

assay, the protein–DNA complexes were decreased by the addition of self competitor (Fig. 1B, lanes 2 and 3). However, AP1 consensus oligonucleotide, as well as other oligonucleotides, did not decrease the complexes (Fig. 1B, lanes 4–12). This result suggests that the binding protein to this element may not be an AP1 family protein.

To confirm this result, the mutants of the element were synthesized and a competition assay was carried out with them. AP1 consensus sequence has the inverted repeated sequence, TGACTCA. However, the AP1-like element in the p53 promoter has an incomplete inverted repeated sequence, TGACTCT. In M1 oligonucleotide, AP1 consensus sequence was disrupted (from TGACTCT to ACACTCT; Fig. 1A), and complete AP1 consensus sequence was restored in M2 oligonucleotide (from TGACTCT to TGACTCA; Fig. 1A). In M3 oligonucleotide, the bases at -274 and -273 were changed from TC to AG. These bases are located in the protected region of DNase I footprinting assay, but not included in AP1 consensus sequence (Fig. 1A). In competition assay, M1 oligonucleotide still competed with WT oligonucleotide, but M2 oligonucleotide did not (Fig. 1C, lanes 4–7). M3 oligonucleotide also reduced the WT–protein complex efficiently (Fig. 1C, lanes 8 and 9). These results revealed that AP1 consensus sequence was not required for protein binding to the element. Therefore, the binding protein to the motif is not an AP1 family protein. The result also suggests that thymine and guanine at -274 and -273 are essential for the maximum binding activity of the protein.

Zn^{2+} Is Required for Protein Binding to AP1-Like Element

Many transcription factors require the presence of metal ions for maximum DNA-binding activity. Therefore, the binding activity of the protein was measured in the presence of various metal ion chelators such as EDTA, EGTA, 8-hydroxyquinoline, and ortho-phenanthroline. The binding activity of the protein was reduced in the presence of 8 mM of orthophenanthroline, but other chelators did not show this effect (Fig 2A).

To determine which divalent cation is required for maximum DNA-binding activity of the protein, the ability of Zn^{2+}, Mg^{2+}, Ca^{2+}, or Cu^{2+} to restore complex formation was examined in the presence of 8 mM orthophenanthroline (Fig. 2B). As a result, Zn^{2+} restored the formation of the protein–DNA complexes (Fig. 2B, lanes 2 and 3). Other divalent cations were unable to restore the formations of the complexes.

Effects of Ionic Strength, Urea, and Temperature

To determine the sensitivity of the binding protein to ionic strength or urea, the indicated quantity of NaCl or urea was added to the binding reaction mixture (Fig. 3A and 3B). There was a significant decrease in the formation of the protein–DNA complexes in the presence of low concentrations of NaCl, and protein–DNA complexes were completely abolished at 0.6 M NaCl and higher (Fig. 3A). The binding protein was highly resistant to urea. Even 1.8 M of urea did not eliminate the protein–DNA complexes (Fig. 3B).

To test the heat sensitivity of the binding protein, aliquots of the nuclear extracts were heated at the indicated temperature for 5 min before assaying for DNA-binding activity (Fig. 4). In this assay, two bands migrating faster than three major bands were identified. We expected these faster-migrating bands to be heat-proteolyzed peptide–DNA complexes, because they could also be detected in the protease clipping assay (Fig. 5A and 5B). The DNA–protein complexes were comparatively stable at high temperature, but stability decreased over 60°C (Fig. 4).

Effects of Protease

Many transcription factors have a structurally distinct DNA-binding domain. The DNA-binding domain was traditionally identified as a protease-resistant "core" polypeptide [Hooft van Huijsduijnen et al., 1987]. To determine whether the binding protein has a protease-resistant DNA-binding domain, proteases such as trypsin and proteinase K were added to the binding mixtures (Fig. 5A and 5B). As a result, a faster-migrating complex was detected. The three protein–DNA complexes were reduced to a band with increasing concentrations of proteases. The limited digest was stable over a wide range of proteinase K or trypsin concentration. Trypsin or proteinase K treatment showed similar migrating patterns in EMSA. However, a partially digested intermediate peptide–DNA complex was detected at the in-

termediate concentrations of trypsin. Although proteinase K is a nonspecific endopeptidase, trypsin is a serine protease of narrow specificity. Therefore, digestion of the binding protein with trypsin at intermediate concentrations may make a clearer band of the partial digestion product than with proteinase K.

DISCUSSION

Liver regeneration is an ideal model system to study the mechanisms of gene expression that control cell proliferation. After partial hepatectomy, the remaining intact lobes rapidly grow and the growth ceases in about 7 days when the original organ mass is regained [Grisham, 1992]. At the early stage of liver regeneration, transcription of the p53 gene increases [Thompson et al., 1986; Lee et al., 1998a]. A 39-kDa protein binds to the AP1-like element and induces the transcription of the p53 gene [Lee et al., 1998]. In this study, the 39-kDa binding protein was characterized by various methods.

Oligonucleotide competition assay showed that the binding protein did not recognize the AP1 consensus sequence and bound to the sequence that was not compatible with the AP1 consensus motif. In Figure 1B, the AP1 consensus oligonucleotide did not reduce the protein–DNA complexes. In addition, the competition assay with the mutant oligonucleotides showed that the AP1 consensus sequence was not required for protein binding to this motif (Fig. 1C). On the contrary, thymine and guanine at the position of -274 and -273 were required, which were not contained in the AP1 consensus sequence (Fig. 1C, lanes 8 and 9).

We identified the divalent cation required for maximum DNA-binding activity of the protein. In the treatments of chelators, the protein–DNA complexes were reduced by the addition of 8 mM ortho-phenanthroline (Fig. 2A). Ortho-phenanthroline is an efficient chelator of Zn^{2+}, Mn^{2+}, Cu^{2+}, and Fe^{2+}, and has been frequently used at mM concentrations to demonstrate the presence of Zn^{2+} in the catalytic center of Zn^{2+} metallo-enzymes. Although the other chelators have affinities for metal ions that overlap those of ortho-phenanthroline, they did not reduce the complex formation. A possible explanation for the reduced sensitivity of the complex formation in the presence of other chelators is that, unlike ortho-phenanthroline, other chelators are unable to chelate metal ions that are already complexed internally with the active center of protein [Hooft van Huijsduijnen et al., 1987]. DNA-binding ability of the protein was recovered by Zn^{2+} (Fig. 2A and 2B). DNA-binding proteins that require Zn^{2+} include SP1 transcription factor (SP1) [Kadonaga et al., 1987], YY1 [Hariharan et al., 1991; Seto et al., 1991], and Transcription factor IIIA (TFIIIA) [Ginsberg et al., 1984; Miller et al., 1985]. These proteins interact with DNA via their DNA-binding domains that contain one or more zinc fingers. Therefore, it is likely that the binding protein to the AP1-like element contains such zinc fingers. However, AP1 family proteins have basic leucine zipper domains and do not contain zinc fingers. Therefore, this result also serves to confirm that the binding protein is not an AP1 family protein.

The binding protein was resistant to denaturant. Urea (1.8 M) did not completely abolish DNA-binding activity of the protein. DNA–protein complexes were comparatively stable up to 55°C. Although low concentrations of NaCl reduced the level of the complexes, residual DNA-binding activity of the protein remained at 0.5 M NaCl. The binding protein also has a protease-resistant core polypeptide. The polypeptide was remarkably stable over a wide range of protease. The intensity of the faster-migrating band increased with increasing amount of protease, suggesting that the faster-migrating band was a proteolyzed product of the binding protein (Fig. 5A and 5B).

Three different DNA–protein complexes were detected in EMSA with radiolabeled WT oligonucleotide. However, the complexes seem to contain the same DNA binding protein. First, the three bands in EMSA showed almost the same sensitivity to urea, NaCl, or temperature (Fig. 3A, 3B, and 4). Second, only the 39-kDa protein was detected in southwestern blotting assay as a binding protein to AP1-like element [Lee et al., 1998]. Third, in the protease clipping assay (Fig. 5A and 5B), one protease-resistant core polypeptide was detected after the protease treatment. Therefore, it is likely that the 39-kDa protein may bind to the AP1-like element as multimeric forms with itself or other proteins, which produce the three complexes. These multimers may be dissociated in the course of the protease treatment and a DNA-binding core remained after the protease treatment.

Previously, the AP1-like element was also elucidated as a cis-regulatory element in the induction of the mouse p53 gene by phorbol ester and referred to as PF1 [Ginsberg et al., 1990]. Interestingly, PF1 element in the mouse p53 promoter did not compete with AP1 consensus oligonucleotide, and the purified AP1 did not recognize the PF1 element. However, in the cotransfection with c-*jun* expression vector, PF1 responded to the expression of c-*jun* and activated the mouse p53 promoter. Therefore, as an explanation for these phenomena, it was proposed that PF1 might be an alternative complex of Jun and Fos-related factor or that the binding protein might be activated by Jun [Ginsberg et al., 1990]. However, it is not clear whether PF1 is the same protein as the 39-kDa protein binding to the AP1-like element in the rat p53 promoter during liver regeneration. Recently, it was reported that AP1 was a critical factor that regulates the human p53 transcription [Kirch et al., 1999]. However, another nuclear factor, not AP1, is involved in the induction of the rat p53 gene after partial hepatectomy or in the induction of the mouse p53 gene by phorbol ester [Ginsberg et al., 1990]. Thus, it is possible that the transcription of the p53 gene may be regulated by various mechanisms depending on the transcription activation signal for the p53 gene.

In summary, we characterized the protein binding to the AP1-like element in the p53 promoter during liver regeneration. Although the AP1-like element contains the region that has high homology to AP1 consensus motif, another factor, not AP1, binds to the AP1-like element. This protein may have zinc finger motifs in the DNA-binding domain. In addition, the binding protein has structurally distinct DNA-binding domain. The sensitivity to urea, NaCl, or temperature was also determined. These biochemical properties provided new insights into the structure of the 39-kDa binding protein and should be useful in attempts to verify the identity of the purified preparations of the protein.

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