Endocrine (1995) 3, 543–547

Identification of two distinct Sp1- and RBF-1-like nuclear factors that bind to the upstream region of the human angiotensinogen promoter

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Angiotensinogen, the protein precursor of angiotensin II that is a crucial regulator of blood pressure and electrolyte balance, is constitutively produced by the liver. In the present study, we identified two nuclear factors that are possibly involved in maintaining the constitutive promoter activity of the human angiotensinogen gene. The 32 bp DNA region between −344 and −313 located in the 1.3 kb angiotensinogen upstream region (−1222 to +44) partially contributed to the maintenance of the efficient promoter activity in HepG2 cells. This segment was able to form the complexes with HepG2 nuclear extracts, which could be dissociated by competing recognition sequences that contain those of either Sp1 or RBF-1. An *in vivo* competition experiment demonstrated that the parental promoter activity is reduced about 65% by an RBF-1 competitor more effectively than by an Sp1 competitor. These results suggested that Sp1- and RBF-1-like factors play roles in maintaining the constitutively active angiotensinogen promoter.

Keywords: angiotensinogen; Sp1; RBF-1; transcription; hepatoma promoter

Introduction

The renin-angiotensin system plays a critically important role in the regulation of blood pressure, fluid volume balance and electrolyte homeostasis through the action of an octapeptide angiotensin II. Angiotensinogen, the unique precursor of angiotensin II, is constitutively produced by the liver and secreted into the circulation, where the successive proteolytic cleavages by renin and angiotensin-converting enzyme takes part in the peptide synthesis (Campbell, 1987). Genetic linkage analyses of the human angiotensinogen gene with high blood pressure previously proposed that the basal transcriptional mechanism of the angiotensinogen gene is involved in the pathogenesis of hypertension (Jeunemaitre *et al.*, 1992; Ward *et al.*, 1993). Furthermore, we have recently generated transgenic hypertensive mice by cross-mating separate lines of animals carrying either the human renin gene or human angiotensinogen gene (Fukamizu *et al.*, 1993), the latter of which is constitutively expressed in the transgenic liver (Takahashi *et al.*, 1991). At present, however, less information is available concerning the basal transcriptional machinery of the human angiotensinogen gene.

Previously, we demonstrated that the 1.3 kb flanking sequences are functional as a promoter in human hepatoma HepG2 cells (Fukamizu *et al.*, 1990). Although we re-examined its upstream DNA region using reconstituted promoter constructs (Fukamizu *et al.*, 1991), no exact *cis*-acting elements and *trans*-acting factors that are involved in maintaining the constitutive promoter activity of the human angiotensinogen gene have been reported. As a first step to understand the molecular regulation of the human angiotensinogen gene, the present study was designed to further define regulatory factors that participate in the control of its constitutive expression. For this purpose we have performed transient transfection experiments, in details, using the human angiotensinogen promoter with various length linked to chloramphenicol acetyltransferase (CAT) gene in HepG2 cells and identified the upstream DNA segment at nucleotide positions between −344 to −313 that partially contributes to the angiotensinogen promoter activity. A gel shift assay using this DNA region identified the formation of DNA/HepG2 nuclear factor complexes, which could be dissociated by competing recognition sequences that contain those of either Sp1, a widely expressed GC-box binding factor, or RBF-1, a novel GC-box binding factor regulating the human retinoblastoma (Rb) gene. Further *in vivo* competition experiments and gel shift assays suggested that Sp1- and RBF-1-like nuclear factors play roles in maintaining the constitutive promoter activity of the human angiotensinogen gene and that the gene is characterized as the second target for RBF-1 beside the Rb gene.

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Received 6 October 1994; accepted 17 February 1995

Results

Deletion analysis of the human angiotensinogen promoter

We have previously demonstrated that the upstream DNA region of the human angiotensinogen gene is able to direct the expression of CAT reporter gene in HepG2 cells (Fukamizu *et al.*, 1990). To identify an upstream DNA region responsible for the maintenance of the human angiotensinogen promoter activity, we constructed the CAT reporter plasmids containing various lengths of the 5′-flanking region of the human angiotensinogen gene into HepG2 cells. As shown in Figure 1, the deletion from nucleotide positions −516 to −345 slightly decreased the promoter activity to 83%. On the other hand, the 32 bp DNA deletion from −344 to −313 (DM8′cat) significantly reduced the promoter activity to 50.9% as compared with the

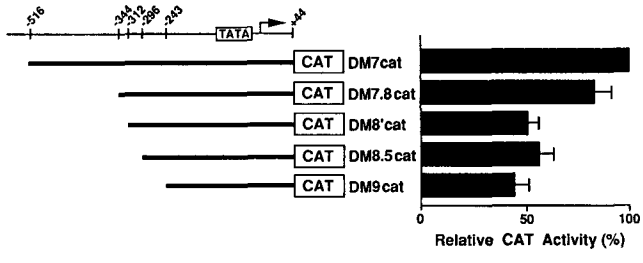

Figure 1 5′-Deletion analysis of DM7 fragment (−516 to +44) of the human angiotensinogen promoter. The TATA box is located at nucleotide positions −31 to −24 and the transcriptional start site is indicated by +1. Transfection was performed using 3 μg of reporter plasmid DNA/dish. Cells were harvested 36 h after transfection and aliquots of all extract containing equal amount of total protein (20 or 40 μg) were used for CAT assay. The CAT activity of DM7cat, which is nearly equal to that of the 1.3 kb human angiotensinogen promoter and SV40 enhancer/promoter, is designated as 100 and each value of CAT activity represents the mean ± SE for six independent experiments

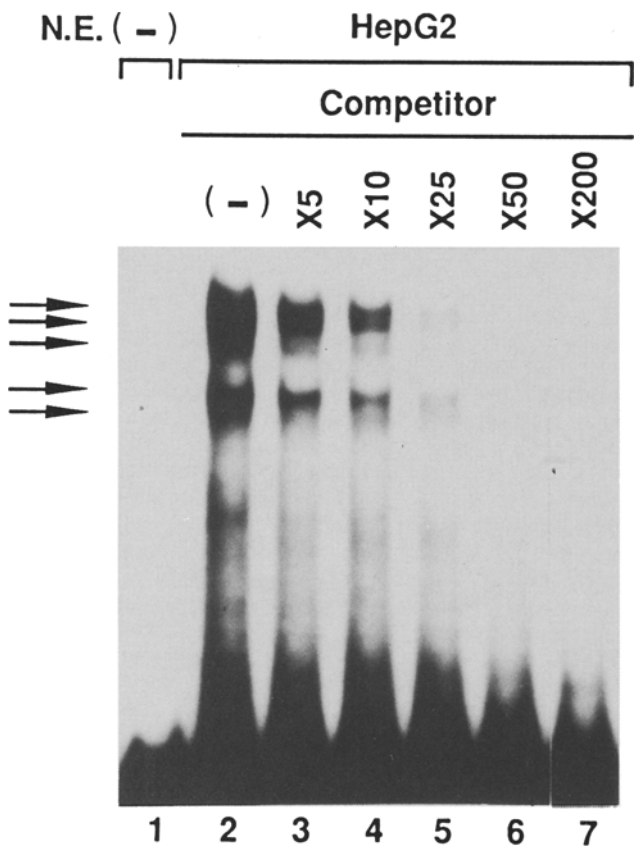

Figure 2 Identification of nuclear factors that bind to the 32 bp DNA element (−344 to −313). Gel shift and competition assays were performed with nuclear extracts from HepG2 cells (5 μg), except for lane 1, incubated with 1×10^4 c.p.m. (0.2 ng) of ^{32}P-labeled DNA probe. In competition assay, 5-, 10-, 25-, 50- and 200-fold molar excess of the unlabeled DNA element (lanes 3 to lane 7) were added to the reaction mixture

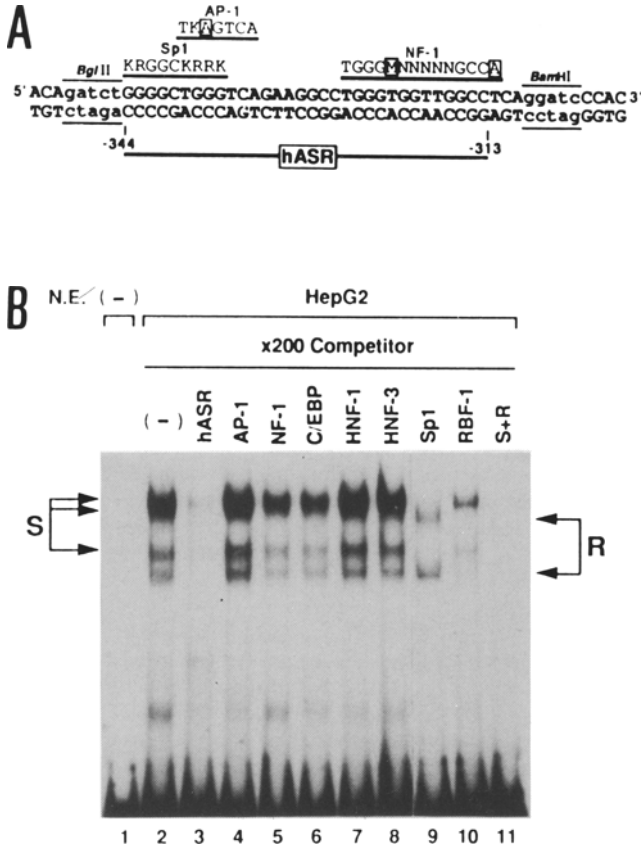

Figure 3 Gel shift and competition assays using the hASR and the recognition DNA fragments for the known transcriptional factors. (**A**) Nucleotide sequences of hASR fragment. Small letters indicate the restriction enzyme sites. Putative recognition sequences for known eukaryotic transcription factors, AP-1, Sp1 and NF-1 were compared with the sequences of the hASR element. Different sequences are shown by open squares, K, T/G; M, A/C; N, A/C/G/T; R, A/G. (**B**) Competition experiments of EMSA. Binding reactions using hASR fragment were performed as in the legend of Figure 2. In competition assay, 200-fold molar excess of unlabeled hASR fragment (lane 3), 200-fold molar excess of unlabeled various double-stranded oligonucleotides containing the binding sites for known transcription factors (lanes 4 to 11, AP-1, NF-1, C/EBP, HNF-1, HNF-3, Sp1, RBF-1), and 200-fold molar excess of unlabeled Sp1 and RBF-1 (total 400-fold molar, lane 12) were added to the reaction mixture. Arrows point to specific DNA-nuclear factor complexes S and R

parental plasmid, DM7cat, whose promoter activity was nearly equal to that found in the 1.3 kb 5'-flanking region-CAT hybrid construct as well as in the SV40 enhancer/promoter construct. Further deletions up to −243 did not give a significant change in CAT activity. These results suggested that the 32 bp DNA segment from −344 to −313 partially contributed to the maintenance of the efficient promoter activity of the human angiotensinogen gene.

Identification of transcription factors binding to the 32 bp DNA element

To identify nuclear factors that bind to the 32 bp DNA segment, we performed a gel shift assay with nuclear extracts prepared from HepG2 cells, using the end-labeled DNA fragment as a probe. Incubation of this probe with the nuclear extracts produced multiple DNA-nuclear factor binding complexes associated with the five major bands (Figure 2, lane 2). These complexes represented sequence-specific interaction between the labeled DNA probe and nuclear factors, since the binding competed with increasing molar excess of unlabeled DNA fragment (Figure 2, lanes 3 to 7).

Inspection of DNA sequences of the angiotensinogen upstream region revealed that the 32 bp segment contains the putative binding sites for AP-1, NF-1 and Sp1 (Figure 3A). To test whether their consensus sequences could prevent the observed complex formation, a competition experiment was carried out. The formation of first, second and fourth complexes, and of third and fifth complexes was partially inhibited by 200-fold molar excess of the oligonucleotides containing the binding sites for constitutively expressed GC-box binding factors Sp1 (complex S; Figure 3B, lane 9) and for RBF-1 (complex R; lane 10), respectively, but both elements when given together competed out these factors binding (lane 11). No other consensus sequences such as those of widely expressed transcription factors, AP-1 and NF-1, prevented these complex information (lanes 4 and 5, respectively). Furthermore, the various unlabeled oligonucleotides containing the recognition sites for typical liver-enriched transcription factors, C/EBP, HNG-1 and HNF-3 failed to inhibit the formation of complexes S and R (lanes 6 to 8). Such binding activities could also be detected with nuclear extracts prepared from HeLa, T98G and 3T3-L1 cells (data not shown). These results raised the possibility that the constitutive promoter activity of the human angiotensinogen gene might be regulated by Sp1- and RBF-1-like nuclear factors. Thus, we termed the DNA element located between −344 to −313 hASR, *h*uman *a*ngiotensinogen *S*p1/*R*BF-1 binding element.

Functional analysis of hASR that interacts with nuclear factors

To test the functional importance of the hASR in the angiotensinogen promoter activity, the CAT constructs as

shown in Figure 4 were transiently introduced into HepG2 cells. The reporter plasmids, hASR(−)DM7 and hASRDM9, were constructed by internally deleting the hASR from DM7-cat by 5′ terminally adding hASR or DM9cat, respectively. The CAT activity directed from the former reporter gene was dramatically reduced to 52.7%, which were the levels similar to DM8′cat activity (Figure 1), and hASRDM9 was able to activate the DM9cat promotoer activity up to 82.5%, which were the similar levels to DM7.8cat activity (Figure 1). These reults suggested that Sp1- and RBF-1-like factors play functional roles in mediating the constitutive promoter activity of the human angiotensinogen gene.

We next performed *in vivo* competition experiments to confirm the functional roles of both factors as positive regulators. Eight tandem copies of the recognition sequences for Sp1 and RBF-1 were inserted into the pUC19 plasmid, named pc-Sp1 and pc-RBF-1, respectively. Each plasmid was cotransfected with DM7cat into HepG2 cells, and the cAT activities were analysed (Figure 5). The total amount of transfected DNA was made up to 6 μg by the addition of pUC19 DNA. The results obtained in these experiments showed that the CAT activity gradually decreased with increasing amounts of pc-Sp1 of pc-RBF-1 ($P<0.01$). Moreover, the competitive effect of pc-RBF-1 was greater than pc-Sp1 ($P<0.01$) at the concentration of 2 μg competitor plasmid. At this concentration of either pc-Sp1 or pc-RBF-1, however, the CAT activity derived from a reported plasmid C/EBP8DM12 constructed by fusing eight tandem copies of the C/EBP binding element to the minimal human angiotensinogen promoter (−32 to +44)-CAT hybrid gene (Fukamizu *et al.*, 1990) was not influenced in HepG2 cells (Figure 5). Therefore, these functional *in vivo* assays suggested that the hASR is important as the recognition sequences for constitutively active Sp1- and RBF-1-like nuclear factors, and that both factors could play roles in maintaining the promoter activity of the human angiotensinogen gene.

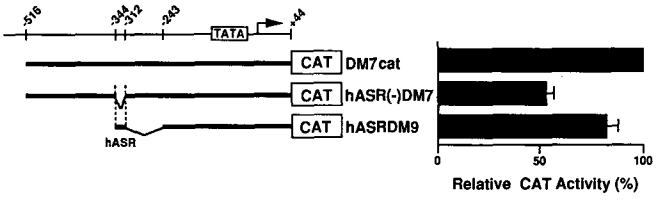

Figure 4 Effect of the hASR on the promoter activity. CAT assay was performed and relative CAT activities were determined as described in the legend of Figure 1. The value of CAT activity represents the mean±SE for six independent experiments

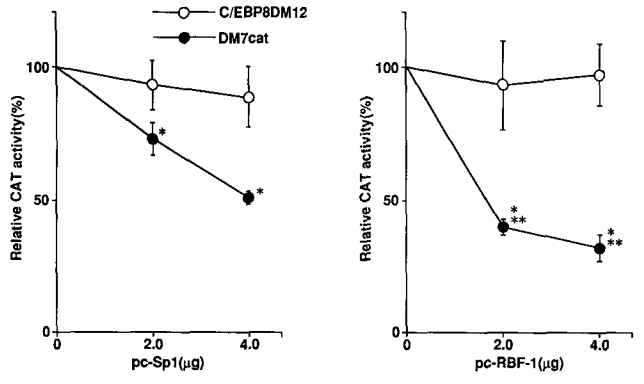

Figure 5 *In vivo* competition analysis of the hASR. Competitive plasmids containing eight tandem copies of the Sp1 fragment (pc-Sp1) or RBF-1 fragment (pc-RBF-1) were used in this experiment. The reporter plasmids of DM7cat (the angiotensinogen promoter-CAT chimeric construct; 2 μg) and C/EBPDM12 (the eight-tandem copies of the C/EBP binding element-CAT fusion construct; 2 μg), were transiently cotransfected with 2 or 4 μg of pc-Sp1 and pc-RBF-1, respectively, into HepG2 cells. Total amounts of DNA were adjusted to 6 μg by pUC19. CAT assay was performed as described in the legend of Figure 1. The CAT activity of DM7cat, cotransfected with 4 μg of pUC19, is designated as 100 and each value of CAT activity represents the mean±SE for five independent experiments. *$P<0.01$ *vs* the CAT activity of DM7cat cotransfected with 4 μg of pUC19. **$P<0.01$ *vs* the CAT activity of DM7cat cotransfected with pc-Sp1

Discussion

We have previously isolated the human angiotensinogen gene from the genomic DNA library and localized the promoter activity to the 1.3 kb 5′-flanking region of the gene (Fukamizu *et al.*, 1990). In addition, our transgenic studies demonstrated that these upstream DNA sequences function as a constitutively active promoter in the liver of transgenic mice (Takahashi *et al.*, 1991, 1992) and in the neuroectodermal tumor cells carrying the human adenovirus type 12 oncogenes (Fukamizu *et al.*, 1994). Although we found downstream enhancer elements of the human angiotensinogen gene (Nibu *et al.*, 1994a,b) and several groups identified multiple *cis*-acting elements in the rat and mouse angiotensinogen promoters (Brasier *et al.*, 1989; Congiu *et al.*, 1992; Zhao *et al.*, 1992; Tamura *et al.*, 1993), the exact *cis*-acting DNA elements involved in the constitutive promoter activity of the human angiotensinogen gene have still been uncharacterized.

In the present report, we suggested that two distinct nuclear factors, Sp1- and RBF-1-like nuclear factors, interact with a 32 bp DNA region (nucleotide positions −344 to −313; hASR) located in the upstream sequences of the human angiotensinogen gene to partially maintain its constitutive promoter activity. This conclusion results largely from two lines of evidence. First, the hASR was shown to partially contribute to the maintenance of the parental promoter activity by the deletion and *in vivo* competition CAT analyses. Second, the formation of the five shifted complexes derived from the DNA/HepG2 nuclear extracts (S and R) detected in EMSA when the hASR was used as the labeled probe was dissociated with both Sp1 and RBF-1 recognition sequences.

While the 5′ deletion of the 172 bp sequences (−516 to −345) (DM7.8cat) from the parental promoter DM7cat decreased in CAT activity no more than 17% in HepG2 cells, the significant reduction of the promoter activity to approximately 51% as compared with that of the parental one was first detectable in the deletion mutant DM8′cat lacking the only 32 bp hASR. This observation is consistent with the results from further mutation analysis that hASR(−)DM7 promoter internally removing hASR produces CAT activity (53%) similar to that from DM8′cat and that the CAT activity from hASRDM9 reconstituted in combination of hASR with DM9cat promoter with the lowest CAT activity (44%) was recovered to the levels (82.5%) found in DM7.8-cat. The sequential titration of either Sp1 or RBF-1 binding by means of the *in vivo* competition in HepG2 cells showed the differential effects on the reduction in the promoter activity at the lower concentration of competitors. This observation is likely to reflect the differential binding property of Sp1 and RBF-1 to hASR.

Of the five gel-shifted complexes generated when the hASR was incubated with HepG2 nuclear extracts, the three S complexes appear to contain Sp1 because this DNA binding activity competes with the Sp1 consensus GC box sequences. The S complexes most likely correspond to the 95 and 105 kDa Sp1 variants described previously (Briggs *et al.*, 1986; Kadonaga & Tjian, 1986; Jackson *et al.*, 1990). The appearance of such an Sp1 variant in gel shift assays has also been reported by other groups (Dawson *et al.*, 1988; Gustaf-

son & Kedes, 1989; Sartorelli *et al.*, 1990; Sakai *et al.*, 1991). These electrophoretic variants arise from differential phosphorylation of a single protein by a DNA-dependent protein kinase (Jackson *et al.*, 1990). Furthermore, multiple retarded bands in gel shift assays can also result from multimerization of a unique Sp1 protein species on top of a single DNA-bound Sp1 monomer (Pascal & Tjian, 1991). However, it remains to be determined whether Sp1 itself or closely related proteins bind to the hASR, since several novel factors which bind to GC or GT boxes have recently been identified by cDNA cloning (Hagen *et al.*, 1992; Imataka *et al.*, 1992; Kingsley & Winoto, 1991; Sogawa *et al.*, 1993).

In addition to Sp1, three of the proteins which are characterized in detail have been termed Sp2, Sp3 and Sp4. Sp1 binds with high affinity to GC and GT boxes (Kadonaga *et al.*, 1987), whereas Sp2 interacts preferentially with GT boxes. Sp3 and Sp4 bind with equal affinity to GC and GT boxes. Sp2 and Sp3 appear to be expressed ubiquitously, while the distribution of Sp4 is restricted to the brain (Hagen *et al.*, 1992). Interestingly, the hASR contains GT-box like sequence (positions −327 to −318). In view of the distribution of Sp1 family gene expression, we speculate that the observed multiple variants of the three S complexes in our gel shift assays may correspond to Sp1, Sp2, or Sp3 because the complex formation of S can also be detected in a wide variety of nuclear extracts including HeLa, T98G and 3T3-L1 cells (data not shown). This identification, however, remains purely speculative at the moment and will need to be verified using specific antibodies for each Sp1 family protein.

The formation of the second complex R, which is not inhibited even by 200-fold molar excess of Sp1 consensus GC sequences, suggests the existence of a non-Sp1-like nuclear factor with the ability to bind to the hASR. Recently, a nuclear factor (RBF-1) present in monkey kidney CV1 cells and in mouse embryonal carcinoma F9 cells was shown to interact with a DNA-binding site overlapping an already existing Sp1 recognition sequence in the 5′-flanking region of the human Rb gene (Sakai *et al.*, 1991). The complex formation of R was efficiently inhibited by the competitor oligonucleotides containing RBF-1 binding sites. Our present result suggests that, in addition to the human Rb gene, the human angiotensinogen gene could be a second target for RBF-1.

In conclusion, the experiments reported here suggested that the hASR in an important DNA element required for maintaining the constitutive promoter activity of the human angiotensinogen gene. The present characterization of Sp1 and RBF-1 that are possibly involved in the maintenance of the angiotensinogen promoter activity also implys that not only Sp1 family but also RBF-1 play critical roles in regulating the activity of many eukaryotic promoters and enhancers along with GC or GT boxes. In this respect, hARS of the human angiotensinogen gene will be an excellent model DNA element to precisely investigate the molecular relationship between Sp1 family and RBF-1 as to the regulatory mechanism of eukaryotic gene expression.

Materials and methods

Plasmid constructions

The human angiotensinogen promoter-chloramphenicol acetyltransferase (CAT) hybrid genes were constructed as follows: DM7cat (nucleotide positions −516 to +44) and DM9cat (−243 to +44) were constructed as described previously (Fukamizu *et al.*, 1991). A 389 bp (−344 to +44) *Sma*I/*Hind*III fragment, 357 bp (−312 to +44) *Eco*81I/*Hind*III fragment, and 341 bp (−296 to +44) *Bln*I/*Hind*III fragment were subcloned into the *Bgl*II/*Hind*III sites of pUCSV0CAT (Fukamizu *et al.*, 1991) to make DM7.8cat, DM8′cat and DM8.5cat, respectively. A 172 bp (−516 to −345) *Bgl*II/*Sma*I DNA fragment derived from DM7cat was inserted into the *Bgl*II site of DM8′cat to constract hASR(−)DM7. hASRDM9 was made by inserting the 32 bp *Sma*I/*Eco*81I DNA fragment (−344 to −313) derived from DM7cat into the *Bgl*II site of DM9cat. Competitive plasmids of pc-Sp1 and pc-RBF-1 were constructed by fusing the eight tanem copies of SP1 and RBF-1 binding elements into *Sma*I site of pUC19, respectively.

Cell culture, transient transfection and CAT assay

HepG2 cells were grown as previously described (Fukamizu *et al.*, 1990). Transient transfection of CsCl-purified DNA by the calcium phosphate precipitation and CAT assay were carried out essentially as described previously (Fukamizu *et al.*, 1990). The cells were harvested 36 h after transfection, cell extracts were prepared, and protein concentration was determined by the Bio-Rad protein assay kit. The results of conversion ratios of [^{14}C] chloramphenicol were measured by an image analyzer system (BAS2000; Fujix, Tokyo, Japan). All experiments were performed at least five times for each construct.

Preparation of nuclear extract

Nuclear extract from HepG2 cells were prepared according to a protocol of Dignam *et al.* (1983). The final protein concentration was 5 to 15 mg/ml.

Gel shift assy

Single-stranded oligonucleotides were annealed and the double-stranded DNA probes (100 ng) were end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. Unincorporated label was removed by gel filtration on a sephadex G-50 column. Five μg of nuclear extracts were incubated with 2 μg of poly (dI-dC) (Pharmacia) on ice for 5 min in the presence or absence of unlabeled competitor DNA and then incubated with the end-labeled oligonucleotide probe (approximately 1×10^4 c.p.m., 0.1 to 0.2 ng) at 20°C for 20 min. The binding reaction was carried out in a solution containing 10 mM Tris-HCl (pH 7.5), 30 mM KCl, 10% glycerol, 0.5 mM dithiothreitol and 0.5 mM phenylmethylsulfonylfluoride. The reaction mixture (20 μl) was directly loaded onto a 4% nondenaturing polyacrylamide gels containing 4% glycerol made in 0.5 × TBE that had been pre-electrophoresed for 20 min. After electrophoresis was performed at 130 V for 210 min or 225 min at 4°C, the gels were dried and autoradiographed with an intensifying screen.

Oligonucleotides

Oligonucleotide DNA was synthesized on a MillGen/Bioresearch Cyclone plus oligonucleotide DNA synthesizer, and purified on OPC columns (Applied Biosystems, Foster City, CA) as described by the manufacturer. Sources of the oligonucleotide sequences for competition analysis were as follows: the C/EBP binding site described by Landschulz *et al.* (1988); the HNF-1 binding site (nucleotide positions −103 to −74) of the rat β-fibrinogen promoter (Courtois *et al.*, 1987); the HNF-3 binding site (−111 to −85) of the mouse transthyretin promoter (Costa *et al.*, 1989); the RBF-1 binding site (Rb-Sp1-wt) of the Rb gene (Sakai *et al.*, 1991). Double-stranded oligonucleotides containing the recognition sites for transcription factors AP-1, NF-1 and Sp1 were purchased from Stratagene (GELSHIFT KIT La Jolla, CA).

The hASR fragment used in EMSA was generated from the DM7cat by the polymerase chain reaction (PCR) using a pair of oligonucleotide primers. The distal primer (−353 to −306), 5′-ACAgatctGGGGCTGGGTCAGAAG-3′, and the proximal primer (−306 to 353), 5′-GTGGgatccTGAGGC-CAACCACC-3′, were synthesized with a *Bgl*II restriction site (small letters) and a *Bam*HI site (small letter), respectively.

The PCR products were isolated from 5% polyacrylamide gels and used for EMSA.

Acknowledgements
We would like to thank Professor Ryou Katori (First Department of Internal Medicine, Kinki University) and Dr Masatsuga Horiuchi (Stanford University) for their continuous encouragement. We also thank Mr Masashi Sagara for his technical advise. This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan, the Japan Vascular Disease Research Foundation, the Naito Foundation, the Iwaki Foundation, the Ciba-Geigy Foundation (Japan) for the Promotion of Science, and Special Research Projects on Circulation Biosystems at the University of Tsukuba.

References

Brasier, A.R., Tate, J.E., Ron, D. & Habener, J.F. (1989). *Mol. Endocrinol.*, **3,** 1022–1034.
Briggs, M.R., Kadonaga, J.T., Bell, S.P. & Tjian, R. (1986). *Science*, **234,** 47–52.
Campbell, D.J. (1987). *J. Clin. Invest.*, **79,** 1–6.
Congiu, M., Clouston, W.M., Fernley, R.T. & Richards, R.I. (1992). *J. Mol. Endocrinol.*, **9,** 19–29.
Costa, R.H., Grayson, D.R. & Darnell, J.E. Jr. (1989). *Mol. Cell. Biol.*, **9,** 1415–1425.
Courtois, G., Morgan, J.G., Campbell, L.A., Fourel, G. & Crabtree, G.R. (1987). *Science*, **238,** 688–692.
Dawson, P.A., Hofmann, S.L., van der Wethuysen, D.R., Sudhof, T.C., Brown, M.S. & Goldstein, J.L. (1988). *J. Biol. Chem.*, **263,** 3372–3279.
Dignam, J.D., Lebovitz, R.M. & Roeder, R.G. (1983). *Nucleic Acids Res.*, **11,** 1475–1489.
Fukamizu, A., Takahashi, S., Seo, M.S., Tada, M., Tanimoto K., Uehara, S. & Murakami, K. (1990). *J. Biol. Chem.*, **265,** 7576–7582.
Fukamizu A., Tanimoto, K., Uehara, S., Seo, M.S., Handa, S., Sagara, M., Takahashi, S., Imai, T. & Murakami, K. (1991). *Biochim. Biomed. Acta,* **50,** 659–663.
Fukamizu A., Sugimura, K., Takimoto, E., Sugiyama, F., Seo, M.-S., Takahashi, S., Hatae, T., Kajiwara, N., Yagami, K. & Murakami, K. (1993). *J. Biol. Chem.*, **268,** 11617–11621.
Fukamizu, A., Sagara, M., Sugiyama, F., Horiguchi, H., Kamma, H., Hatae, T., Ogata, T., Yagami, K. & Murakami, K. (1994). *J. Biol. Chem.*, **269,** 31252–31258.
Gustafson, T.A. & Kedes, L.H. (1989). *Mol. Cell. Biol.*, **9,** 3269–3283.
Hagen, G., Müller, S., Beato, M. & Suske, G. (1992). *Nucleic Acids Res.*, **20,** 5519–5525.
Imataka, H., Sogawa, K., Yasumoto, K., Kikuchi, Y., Sasno, K., Kobayashi, A., Hayami, M. & Fuijii-Kuriyama, Y. (1992). *EMBO J.*, **11,** 3663–3671.
Jackson, S.P., MacDonald, J.J., Lees-Miller, S. & Tjian, R. (1990). *Cell*, **63,** 155–165.
Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y.V., Lifton, R.P., Williams, C.S., Charru, A., Hunt, S.C., Hopkins, P.N., Williams, R.R., Lalouel, J.-M. & Corvol, P. (1992). *Cell*, **71,** 169–180.
Kadonaga, J.T. & Tjian, R. (1986). *Proc. Natl. Acad. Sci. USA*, **83,** 5889–5893.
Kadonaga, J.T., Carner, K.R., Masiatz, F.R. & Tjian, R. (1987). *Cell*, **51,** 1079–1090.
Kingsley, C. & Winoto, A. (1992). *Mol. Cell. Biol.*, **12,** 4251–4261.
Landschulz, W.H., Johnson, P.F., Adashi, E.Y., Graves, B.J. & McKnight, S.L. (1988). *Genes Dev.*, **2,** 786–800.
Nibu, Y., Takahashi, S., Tanimoto, K., Murakami, K. & Fukamizu, A. (1994a). *J. Biol. Chem.*, **269,** 28598–28605.
Nibu, Y., Tanimoto, K., Takahashi, S., Ono, H., Murakami, K. & Fukamizu, A. (1994b). *Biochem. Biophys. Res. Commun.*, **205,** 1102–1108.
Pascal, E. & Tjian, R. (1991). *Genes Dev.*, **5,** 1646–1656.
Sakai, T., Ohtani, N., McGee, T.L., Robbins, P.D. & Dryja, T.P. (1991). *Nature*, **353,** 83–86.
Sartorelli, V., Webster, K.A. & Kedes, L. (1990). *Genes Dev.*, **4,** 1811–1822.
Sogawa, K., Imataka, H., Yamasaki, Y., Kusume, H., Abe, H. & Fujii-Kuriyama, Y. (1993). *Nucleic Acids Res.*, **21,** 1527–1532.
Takahashi, S., Fukamizu, A., Hasegawa, T., Yokoyama, M., Nomura, T., Katsuki, M. & Murakami, K. (1991). *Biochem. Biophys. Res. Commun.*, **180,** 1103–1109.
Takahahsi, S., Fukamizu, A., Sugiyama, F., Kajiwara, N., Yagami, K. & Murakami, K. (1992). *J. Vet. Med. Sci.*, **54,** 367–369.
Tamura, K., Tanimoto, K., Ishii, M., Murakami, K. & Fukamizu, A. (1993). *J. Biol. Chem.*, **268,** 15024–15032.
Ward, K., Hata, A., Jeunemaitre, X., Helin, C., Nelson, L., Namikawa, C., Farrington, P.F., Ogasawara, M., Suzumori, K., Tomoda, S., Berrebi, S., Sasaki, M., Corvol, P., Lifton, R.P. & Lalouel, J.-M. (1993). *Nature Genet.*, **4,** 59–61.
Zhao, Y.Y., Gu, W., Siddiqui, M.A.Q. & Kumar, A. (1992). *Cell. Mol. Biol.*, **38,** 71–80.