Journal of Cellular Biochemistry 88:1092–1100 (2003)

ARTICLES

Novel Protein RGPR-p117: The Gene Expression in Physiologic State and the Binding Activity to Regucalcin Gene Promoter Region in Rat Liver

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Abstract The binding activity of a novel regucalcin gene promoter region-related protein (RGPR-p117) to the TTGGC sequence of the rat regucalcin gene promoter region was investigated. The expression of RGPR-p117 mRNA was seen in the liver tissues of male and female rats. The sexual difference of this expression was not found. Liver RGPR-p117 mRNA expression was not changed with increasing age (1–50 weeks old), and its expression was not altered by fasting or refeeding. Nuclear factor I-A1 (NF1-A1) has been identified to be a transcription factor in stimulating the rat regucalcin gene promoter activity (Misawa and Yamaguchi [2002a] J Cell Biochem 84:795–802]. Recombinant nuclear factor I-A1 (NF1-A1) and RGPR-p117 proteins were used gel mobility shift assay. RGPR-p117 could not bind to TTGGC motif of the sequence between −525 and −504, which has been defined as a functional promoter element II-b. NF1-A1 was specifically bound to the II-b oligonucleotide. Moreover, RGPR-p117 was not bound to the II-b oligonucleotide in the presence of NF1-A1 or rat liver nuclear protein. The binding of NF1-A1 to the II-b oligonucleotide was not altered in the presence of RGPR-p117. This study demonstrates that RGPR-p117 mRNA, is expressed stably for physiologic change in rat liver, and that the recombinant protein does not directly bind to the TTGGC motif in rat regucalcin gene promoter. J. Cell. Biochem. 88: 1092–1100, 2003.

Key words: RGPR-p117; regucalcin; gene expression; nuclear factor I-A1; transcription factor; rat liver

Regucalcin plays a pivotal role as a regulatory protein in intracellular signaling system [Yamaguchi, 2000a,b; review]. Regucalcin is greatly expressed in the liver of rats [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993; Yamaguchi and Nakajima, 1999]. The rat regucalcin gene consists of seven exons and six introns, and several consensus regulatory elements exist upstream of the 5′-flanking region [Yamaguchi et al., 1996]. The gene is localized on the proximal end of rat chromosome Xq11.1-12 [Shimokawa et al., 1995]. The promoter characterization of the 5′-flanking region of the rat regucalcin gene and its transcriptional regulation by various signaling factors has been shown [Misawa and Yamaguchi, 2000a,b].

The promoter region of the rat regucalcin gene contains a nuclear factor I (NF1)-like motif $TTGGC(N)_6CC$ which is the nuclear factor binding site in rat liver [Misawa and Yamaguchi, 2000a,b]. More recently, it has been demonstrated that NF1-A1 is a transcriptional factor in stimulating the rat regucalcin gene promoter activity [Misawa and Yamaguchi, 2002a]. Moreover, an unknown protein has been found to bind to TTGGC motif using a yeast one-hybrid system [Misawa and Yamaguchi, 2001]. This protein was termed as a regucalcin gene promoter region-related protein (RGPR-p117). The cDNA coding for a human, rat, and mouse RGPR-p117 is determined [Misawa and Yamaguchi, 2001].

The entire rat RGPR-p117 cDNA consists of a 4,378 bp which contains an open reading frame

Abbreviations used: RGPR-p117, regucalcin gene promoter region-related protein; b(p), base(pair); NF1, nuclear factor I; GST, glutathione S-transferase; DIG, digoxigenin.

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Received 16 September 2002; Accepted 30 October 2002
DOI 10.1002/jcb.10437

(ORF) of 3,174 bp encoding a protein of 1,058 amino acid residues [Misawa and Yamaguchi, 2001]. The comparison of human RGPR-p117 cDNA sequence with the genomic sequence database indicates that the gene is consisted of at least 26 exons spanning approximately 41 kb and localized on human chromosome 1q25.2 [Misawa and Yamaguchi, 2001]. The cellular function of RGPR-p117 is unknown. PROSITE search gives that RGPR-p117 has a leucine zipper motif [$L(X)_6L(X)_6L(X)_6L$], and that its location is conserved among rat, mouse, and human [Landschulz et al., 1988; Bairoch et al., 1997; Misawa and Yamaguchi, 2001]. The leucine zipper pattern is present in many gene regulatory proteins [Clerc et al., 1988; Collum and Alt, 1990]. It is speculated that RGPR-p117 may have a role as a gene regulatory protein.

More recent studies have shown that the RGPR-p117 gene is present in various species, and that the RGPR-p117 mRNA expression in the liver is enhanced by the administration of $CaCl_2$ to rats in vivo [Misawa and Yamaguchi, 2002b]. In addition, the RGPR-p117 mRNA has been found to be expressed in the cloned rat hepatoma H4-II-E cells and the expression is stimulated by intracellular signaling factors [Misawa and Yamaguchi, 2002b], suggesting that RGPR-p117 has a regulatory role in cell function.

The present study was undertaken to clarify whether PGPR-p117 is involved in transcriptional regulation of rat regucalcin gene expression. We determined the expression of RGPR-p117 mRNA in the liver of rats with physiologic change and the binding of RGPR-p117 to TTGGC motif in the promoter region of rat regucalcin gene. We found that RGPR-p117 mRNA expression in the liver of rats was not altered by sexual difference, aging, and fasting; and that a recombinant RGPR-p117 did not directly bind to the TTGGC motif.

MATERIALS AND METHODS

Chemicals

Adenosine 5′-[γ-^{32}P]triphosphate ([γ-^{32}P]ATP; 111 TBq/mmol) was purchased from New England Nuclear (Boston, MA). Molecular size standards (0.28–6.6 kb RNA ladder) for electrophoresis of RNA were purchased from Promega (Madison, WI). pGEX-4T-1 fusion protein expression vector and glutathione-Sepharose 4B were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Digoxigenin (DIG) RNA labeling kit was obtained from Roche Molecular Biochemical, Inc. (Indianapolis, IN). Other reagents and chemicals used were of the highest grade of purity commercially available.

Animals

Male and female Wistar rats, purchased from Japan SLC, Inc. (Hamamatsu, Japan), were fed commercial laboratory chow (solid, Oriental Yeast Company Ltd., Tokyo, Japan) containing 57.5% carbohydrate, 1.1% calcium, and 1.1% phosphorus, and distilled water, ad libitum. Rats were killed by bleeding at appropriate ages, and the livers were perfused with 0.25 M sucrose solution, immediately removed and frozen at −80°C. Liver tissues were used to prepare RNA.

RNA Isolation

Total RNAs from rat liver were prepared as described [Chomczynski and Sacchi, 1987]. Liver tissues were homogenized in buffer solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl, 0.1 M 2-mercaptoethanol, and 2 M sodium acetate. Total RNAs were extracted by vigorous shaking in a mixture of phenol, chloroform, and isoamyl alcohol, and phases were separated by centrifugation at 10,000*g* for 20 min at 4°C. RNA located in the aqueous phase was precipitated with isopropanol at −20°C. RNA precipitates were pelleted by centrifugation, and the pellets were redissolved in 50 μl of DEPC-treated water.

Northern Blot Analysis

Twenty or forty micrograms of total RNAs extracted from liver tissues were electrophoresed in 1.2% agarose denaturing gels containing 2.2 M formaldehyde in MOPS buffer (pH 7, containing 20 mM 3-morpholinopropanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA) with 3 V/cm^3 for 3 h [Sambook et al., 1989]. The electrophoresed gels were transferred to nylon membranes by blotting. Northern blots were probed with DIG-labeled RGPR-p117 and G3PDH cRNA, respectively [Misawa and Yamaguchi, 2001]. The RGPR-p117 cRNA robe was corresponding to the position of 1,120–2,130 in rat RGPR-p117 cDNA antisense sequence [Misawa and Yamaguchi, 2001]. The blots were detected with alkaline phosphatase-conjugated anti-DIG antibody and a

chemiluminescent substrate according to the manufacturer's instructions (Roche). The X-ray films were exposed for 5 min. The size of mRNA was determined using an RNA ladder (Promega).

Expression and Purification of Recombinant GST Fusion Protein

A 3,234 bp (127–3,360) cDNA containing the coding region of RGPR-p117 was generated by PCR using a set of primers with the *Eco* RI site and *Xho* I site for 5′ and 3′, respectively. The DNA fragment was subcloned into the pGEX-4T-1 fusion protein expression vector (Amersham Pharmacia Biotech). The correct orientation, sequence, and in-frame of the fragment in the vector (GST-RGPR-p117) were confirmed by direct DNA sequencing. *Escherichia coli* carrying this plasmid was cultured at 30°C, and GST fusion protein was induced by addition of 0.1 mM isopropyl β-D-thiogalactopyranoside for 4 h. Bacteria were sonicated in phosphate-buffered saline containing 5 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride, and the GST fusion proteins were purified using glutathione-Sepharose 4B (Amersham Pharmacia Biotech) according to the manufacturer's instructions. The purified proteins were stocked at −80°C. Production of GST-NF1-A1 protein was described previously [Misawa and Yamaguchi, 2002a].

DNA Fragments for Gel Mobility Shift Assays

The radiolabeled probes used in the binding assays are shown in Figure 4. To obtain DNA fragment −710/+18 of the rat regucalcin gene, PCR was performed using pBluescript II SK^+ containing the 5.5 kb *Eco* RI–*Xho* I fragment of genomic λRCB2 [Yamaguchi et al., 1996] as the substrate using the pair of primer 5′-ACAGGTACCGAATTCCTGACTGATCTTT-3′ and 5′-ACACTCGAGGGTTGTAATGAC-TCCTGGC-3′. A PCR product was subcloned into TA vector, and sequenced. Fragment A was prepared by digesting −710/+18 TA vector with *Eco* RI and *Dra* I. A 22 bp double-stranded oligonucleotide probe corresponding to the promoter region [−525 to −504, termed as II-b, which contains a NF1-like motif TTGGC$(N)_6$CC] of the rat regucalcin gene was synthesized [Misawa and Yamaguchi, 2000a]. Fragment A and II-b double-stranded oligonucleotide were end-labeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase.

Preparation of Nuclear Extracts From Rat Liver

All steps were carried out at 4°C or on ice. Liver was rinsed twice with buffer A (10 mM HEPES/NaOH, pH 7.9, 0.35 M sucrose, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM dithiothreitol, 0.5 mM PMSF, 0.15 mM spermine, 0.5 mM spermidine, 2 μg/ml leupeptin, and 2 μg/ml aprotinin), resuspended in four volumes of buffer A, and homogenized by 15 strokes in a Potter/Elvehjem glass homogenizer with a teflon pestle. Nonidet P-40 was then added to the homogenates to a concentration of 0.1%, and homogenization was performed by five strokes in a Potter/Elvehjem glass homogenizer. The homogenates were filtered through two layers of cheesecloth and centrifuged at 1,000*g* for 10 min. The pelleted nuclei were washed three times with buffer A.

Nuclear extracts were prepared by a modification of the method of Dignam et al. [1983]. The nuclei were resuspended in three volumes of buffer B (10 mM HEPES/NaOH, pH 7.9, 1.5 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM dithiothreitol, 5% glycerol, and 1 mM PMSF), and then NaCl was added to the suspension to a concentration of 400 mM. After slowly mixing for 30 min at 4°C, the suspensions were centrifuged at 13,000*g* for 15 min. The supernatant was dialysed against 100 volumes of buffer C (20 mM HEPES/NaOH, pH 7.9, 75 mM NaCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, and 1 mM PMSF) for 3 h. The dialysate was then centrifuged at 13,000*g* for 15 min, divided into aliquots, and stored at −80°C. The protein concentration was determined by the method of Bradford [1976], using a kit from BioRad (Richmond, CA) and bovine serum albumin as a standard.

Gel Mobility Shift Assay

Gel mobility shift assays were carried out according to the method of Garner and Revzin [1981]. GST-fusion protein and rat liver nuclear extracts (3.0 μg of protein) were preincubated in 20 μl of binding buffer (10 mM Tris/HCl, pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 150 μg/ml poly(dI-dC)·(dI-dC), and 5% glycerol) for 15 min at 24°C. The labeled probes (0.015 pmol) were then added and incubated at 24°C for an additional 30 min. The reaction mixtures were loaded onto 4% nondenaturing polyacrylamide gels (acrylamide/

bisacrylamide ratio, 30:1) and electrophoresed at 10 V/cm for 90 min in 0.5 × TBE (45 mM Tris, 45 mM boric acid, and 1 mM EDTA). The gels were dried and analyzed by a Fujix bio-image analyzer BAS2000 (Fuji Photo Film Co. Ltd, Japan).

Sequence Analysis

DNA sequencing was performed in both directions on double-stranded plasmid DNA using an Applied Biosystems 373A automated DNA sequencer and Taq dye terminators.

RESULTS

Expression of RGPR-p117 mRNA in Liver Tissues With Physiologic Change

The expression of RGPR-p117 mRNA in the liver of male and female rats is shown in Figure 1. Rats 4 weeks old were used. Liver RGPR-p117 mRNA expression was not altered with sexual difference. In female rats, liver RGPR-p117 mRNA expression was not appreciably changed with increasing age (1, 3, 5, 10, or 50 week(s) old) (Fig. 2).

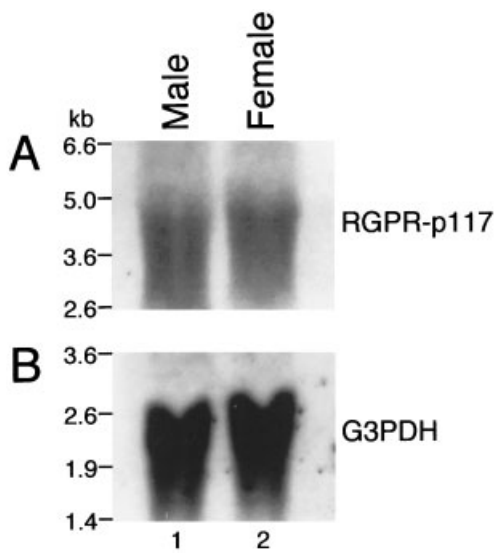


Fig. 1. The expression of RGPR-p117 mRNA in the liver of rats with sexual difference. Rats were killed by bleeding at 4 weeks old. The total RNAs extracted from rat liver were subjected to Northern blot analysis. Blots were hybridized with the probes of RGPR-p117 (**A**, 40 μg of total RNA) or G3PDH (**B**, 20 μg of total RNA) cRNA, respectively. The positions of the markers are indicated alongside the RNA lanes. The figure shows one of three experiments with separate rats. The densitometric data of liver RGPR-p117 mRNA levels in female rats showed 88.9 ± 6.5 (% for male rats; mean ± SEM of three rats). **Lane 1**: male rat liver; **lane 2**, female rat liver.

The effect of fasting or refeeding on RGPR-p117 mRNA expression in the liver of female rats (4 weeks old) is shown in Figure 3. Rats were fasted for overnight (16 h), and then were refed with dietary and 3 h later the animals were sacrificed by bleeding. Liver RGPR-p117 mRNA expression was not significantly changed by fasting or refeeding as compared with that of fed rats. The expression of RGPR-p117 mRNA in the liver of rats was stable for physiologic change (sexual difference, aging, and feeding).

Binding of a Recombinant GST-RGPR-p117 Protein to Regucalcin Gene Promoter Region

To determine whether the cloned RGPR-p117 can directly bind to rat regucalcin gene promoter region, we generated a recombinant RGPR-p117 as fusion protein with GST and subjected to gel mobility shift assay. As shown in Figure 5, GST-NF1-A1, which can specifically bind to TTGGC motif in rat regucalcin gene promoter region [Misawa and Yamaguchi, 2002a], was bound to radiolabeled II-b oligonucleotide as a single band or fragment A as double bands in a dose-dependent manner. Control GST protein did not bind to both II-b oligonucleotide or fragment A. When GST-RGPR-p117 protein (10–50 ng) with tenfold excess of GST-NF1-A1 was added in the binding mixture, the protein could not directly bind to both II-b oligonucleotide or fragment A. Similar result was obtained by using 1–5 ng of GST-RGPR-p117 protein (data not shown).

To examine the possibility of indirect binding of the RGPR-p117 protein to rat regucalcin gene promoter region, we performed gel mobility shift assay with GST-RGPR-p117 in the condition of coexistent with GST-NF1-A1 or rat liver nuclear extract. GST-RGPR-p117 protein (5 or 50 ng) was not bound in the presence of GST-NF1-A1 (5 ng) (Fig. 6). Also, GST-RGPR-p117 (2 or 20 ng) did not bind to these probes in the presence of rat liver nuclear extract (3 ng) (Fig. 7). The binding of GST-NF1-A1 to II-b oligonucleotide or fragment A was not altered in the presence of RGPR-p117 (Figs. 6 or 7).

In addition, the binding of nuclear extract (3 ng) to II-b oligonucleotide was not changed in the presence of GST protein (5 ng) in the reaction mixture of gel mobility shift assay (data not shown), indicating that the binding of nuclear proteins to the oligonucleotide does not interfere with GST. GST-RGPR-p117 protein

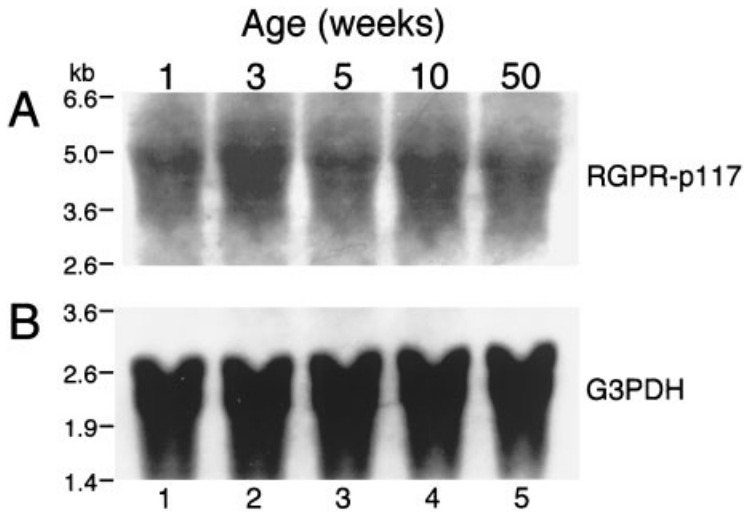


Fig. 2. The expression of RGPR-p117 mRNA in the liver of female rats with increasing age. Rats were killed by bleeding at 1, 3, 5, 10, or 50 week(s) old. The total RNAs extracted from rat liver were subjected to Northern blot analysis. Blots were hybridized with the probes of RGPR-p117 (**A**, 40 μg of total RNA) or G3PDH (**B**, 20 μg of total RNA) cRNA, respectively. The positions of the markers are indicated alongside the RNA lanes. The figure shows one of three experiments with separate rats. The densitometric data of liver RGPR-p117 mRNA levels at 3, 5, 10, or 50 weeks old rats showed 135.1 ± 10.7, 105.1 ± 9.8, 128.2 ± 15.5, and 92.5 ± 5.9 (% for the value of 1 week old rats; mean ± SEM of three rats), respectively. **Lane 1**: 1; **lane 2**, 3; **lane 3**, 5; **lane 4**, 10; **lane 5**, 50 week(s) old.

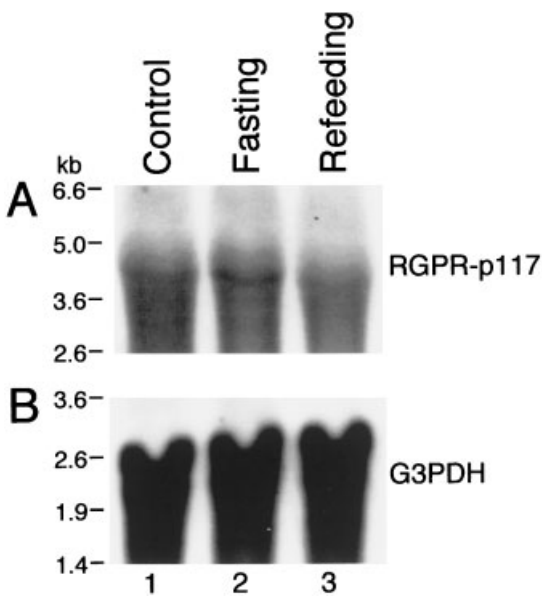
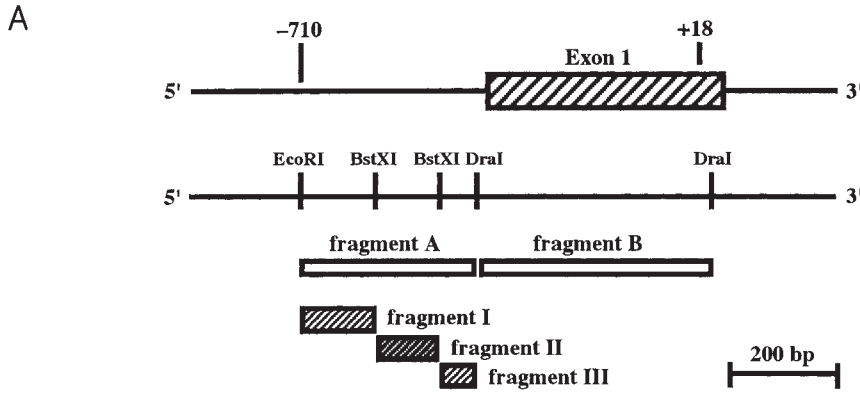


Fig. 3. Effect of fasting on RGPR-p117 mRNA expression in the liver of female rats. Animals (4 weeks old) were fasted for overnight (16 h), and then refed with dietary and 3 h later they were killed by bleeding. The total RNAs extracted from rat liver were subjected to Northern blot analysis. Blots were hybridized with the probes of RGPR-p117 (**A**, 40 μg of total RNA) or G3PDH (**B**, 20 μg of total RNA) cRNA, respectively. The positions of the markers are indicated alongside the RNA lanes. The figure shows one of three experiments with separate rats. The densitometric data of liver RGPR-p117 mRNA levels in fasting or refeeding rats showed 90.5 ± 7.1 and 83.7 ± 11.2 (% for the value from fed rats; mean ± SEM of three rats), respectively. **Lane 1**: control fed rats; **lane 2**, overnight-fasting; **lane 3**, refeeding after overnight-fasting.

was not degraded when it was assayed by SDS–PAGE. Also, the binding of GST-RGPR-p117 to II-b oligonucleotide was not seen in the presence of 1 mM ATP in the reaction mixture containing liver nuclear extract (3 ng) (data not shown). Moreover, GST-RGPR-p117 protein (50 ng) did not bind to II-b oligonucleotide with longer time (30 or 60 min) of incubation (data not shown).

DISCUSSION

RGPR-p117 was found in the study for identification of transcriptional factors in rat regucalcin gene using a yeast one-hybrid system [Misawa and Yamaguchi, 2001]. The role of RGPR-p117 in the regulation of transcription activity in rat regucalcin gene has not been clarified, however. The expression of RGPR-p117 mRNA in the cloned rat hepatoma cells (H4-II-E) has been shown to be stimulated by various intracellular signaling factors [Misawa and Yamaguchi, 2002b]. Meanwhile, the expression of regucalcin mRNA in the liver of rats is sexual difference [Ueoka and Yamaguchi, 1998], and its expression is decreased by aging [Shimokawa and Yamaguchi, 1992] or fasting [Yamaguchi et al., 1995] in the liver of rats. However, hepatic RGPR-p117 expression was not appreciably altered by sexual difference,



B

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                                        HSF1
                                        HSF2                                    c-Myb
-580      -570      -560      -550      -540      -530      -520      -510      -500
 CCA GTTCACTGGT CTTTGGCCTA AAGTCCAAGC ATAGAATGTT CTTTGCCTGT GGGATTCAAG GCTCCCTGCC AAACTGGCCT
 GGT CAAGTGACCA GAAACCGGAT TTCAGGTTCG TATCTTACAA GAAACGGACA CCCTAAGTTC CGAGGGACGG TTTGACCGGA
                                        HSF1                            NF-1
                                        HSF2
            GATA-2
     -490      -480      -470      -460      -450
 CATGCAAGG AAGCAAGATA CAGCTAAGGG GGCTGAGTTC ATCCACTGCA GTGG
 GTACGTTCC TTCGTTCTAT GTCGATTCCC CCGACTCAAG TAGGTGACGT CACC
                                   HSF
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C

Oligonucleotide	Sequence	Position
NF1 consensus	5'-NNTTGGCNNNNNNCCNNN-3'	
II-b (NF1)	5'-CAGTTTGGCAGGGAGCCTTGAA-3'	-525 to -504 (antisense)

Fig. 4. The 5′-flanking region of the rat regucalcin gene and oligonucleotide sequences used in this study. **A**: DNA fragments of the rat regucalcin gene used in gel mobility shift assays. Each DNA fragment was produced from the 5′-flanking region of the rat regucalcin gene. Fragments A (*Eco* RI–*Dra* I) and B (*Dra* I–*Dra* I) were 330 and 437 bp. Fragments I (*Eco* RI–*Bst* XI), II (*Bst* XI–*Bst* XI), and III (*Bst* XI–*Dra* I) were 135, 124, and 71 bp, respectively. **B**: Nucleotide sequence from −582 to −446 bp (fragment II). Sequence data analysis was done with TRANSFAC database using TFSEARCH program with the threshold score of greater than 85.0 point. Potential binding sites for HSF1, HSF2, NF1, c-Myb, GATA-2, and HSF are marked by solid lines. **C**: Sequences of II-b oligonucleotide which contains a NF1-like motif $TTGGC(N)_6CC$.

aging, and fasting in rats. The expression of RGPR-p117 mRNA in the liver of rats was stable for physiologic change. RGPR-p117 was seemed to be not involved in the regulation of regucalcin gene expression in the liver of rats.

The promoter region of the rat regucalcin gene contains a nuclear factor I (NF1)-like motif $TTGGC(N)_6CC$ which is the nuclear factor binding site in rat liver [Misawa and Yamaguchi, 2000a,b]. NF1-A1 has been identified to be a transcriptional factor in stimulating the rat regucalcin gene promoter activity [Misawa and Yamaguchi, 2002a]. NF1-A1 can specifically bind to the II-b oligonucleotide or fragment A, which contains TTGGC motif in rat regucalcin promoter region using gel mobility shift assay [Misawa and Yamaguchi, 2002a]. GST-RGPR-p117 protein did not bind to the II-b oligonucleotide or fragment A, while GST-NF1-A1 bound to the TTGGC motif. Also, GST-RGPR-p117 protein did not bind to the II-b oligonucleotide in the presence of NF1-A1 or rat liver nuclear protein with various condition of binding incubation. Moreover, the binding of GST-NF1-A1 to the II-b oligonucleotide or fragment A was not altered in the presence of GST-RGPR-p117. These results suggest that recombinant RGPR-p117 protein cannot directly bind to the TTGGC motif in rat regucalcin gene promoter region using gel mobility shift assay.

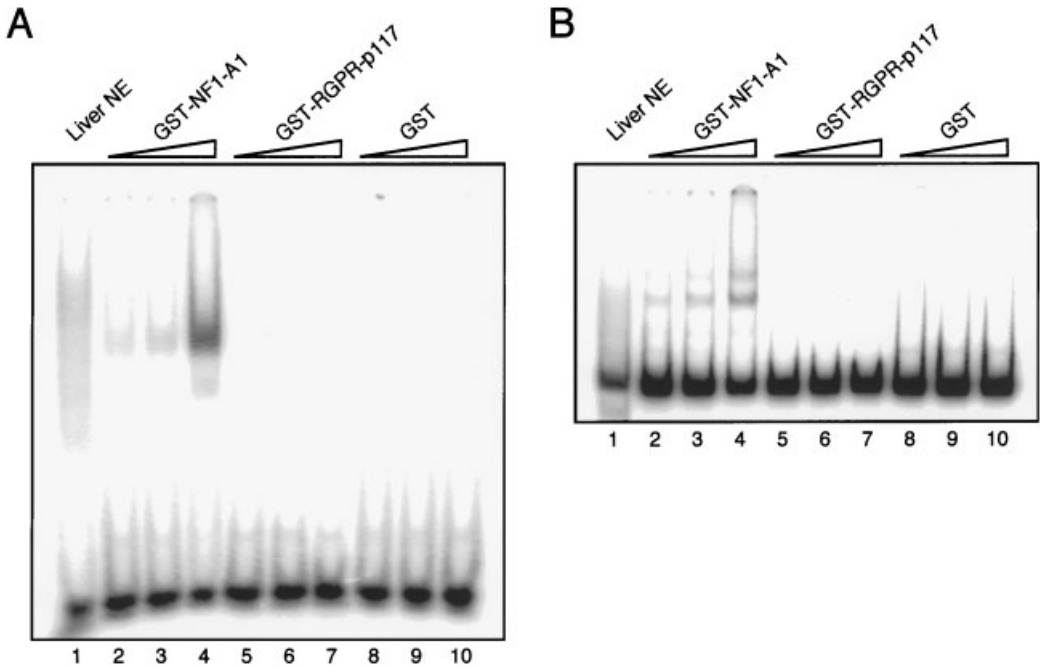

Fig. 5. Binding of GST-RGPR-p117 to II-b oligonucleotide or fragment A. Gel mobility shift assays were performed by using rat liver nuclear extract, recombinant GST-NF1-A1, GST-RGPR-p117, and GST protein with a radiolabeled II-b oligonucleotide (**A**) or fragment A (**B**) as a probe. In A or B, binding profile of rat liver nuclear extract (**lane 1**, 3 μg of protein), GST-NF1-A1 (**lanes 2–4**, 1, 2, or 5 ng of protein, respectively), GST-RGPR-p117 (**lanes 5–7**, 10, 20, or 50 ng of protein, respectively), or GST (**lanes 8–10**, 1, 2, or 5 ng of protein, respectively). The figure shows one of three experiments.

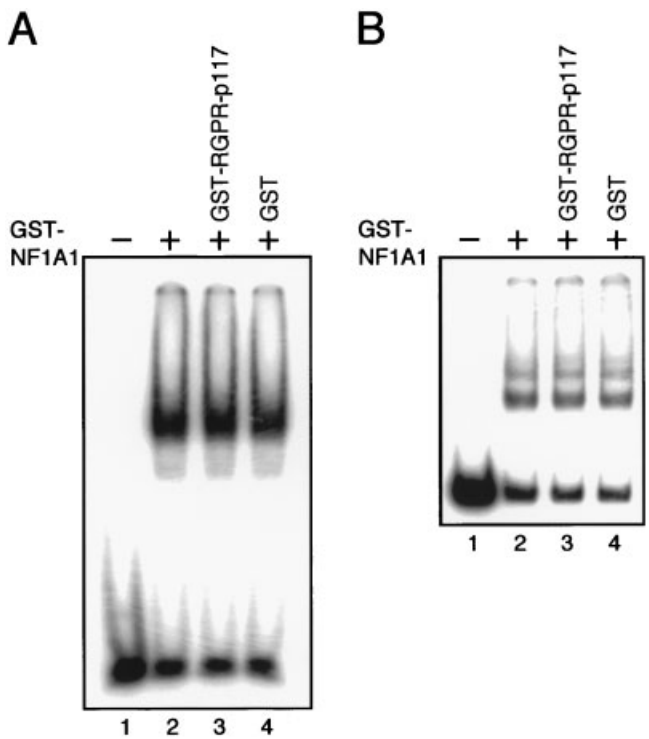

Fig. 6. Effect of GST-NF1-A1 on the binding of GST-RGPR-p117 to II-b oligonucleotide or fragment A. Gel mobility shift assays were performed using recombinant GST-NF1-A1, GST-RGPR-p117, and GST protein with a radiolabeled II-b oligonucleotide (**A**) or fragment A (**B**) as a probe. In A or B, binding profile of control (**lane 1**, without protein addition), GST-NF1-A1 (**lane 2**, 5 ng of protein), GST-NF1-A1 plus GST-RGPR-p117 (**lane 3**, 5 or 50 ng of protein, respectively), and GST-NF1-A1 plus GST (**lane 4**, 5 or 50 ng of protein, respectively). The figure shows one of three experiments.

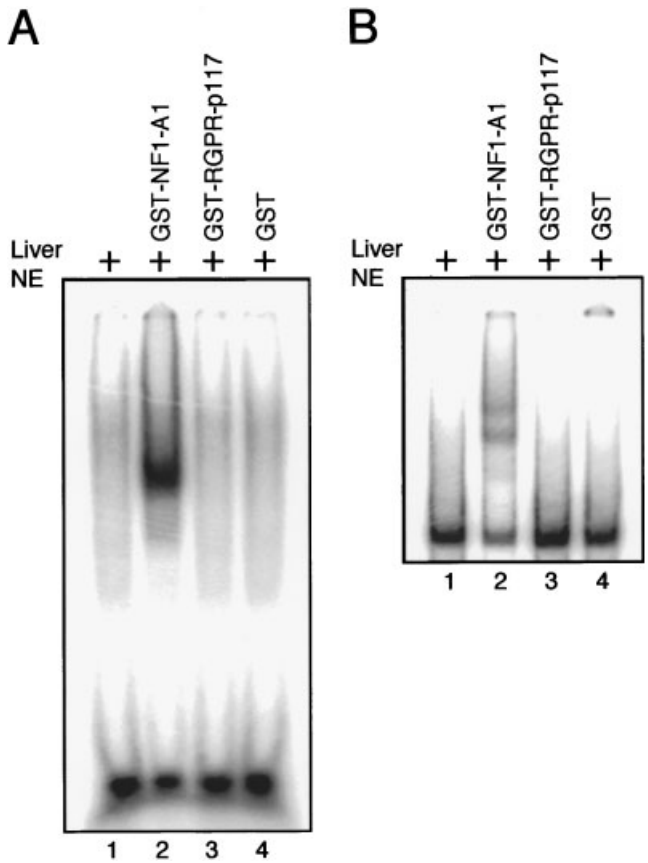

Fig. 7. Effect of rat liver nuclear extract on the binding of GST-RGPR-p117 to II-b oligonucleotide or fragment A. Gel mobility shift assays were performed using rat liver nuclear extract, recombinant GST-NF1-A1, GST-RGPR-p117, and GST protein with a radiolabeled II-b oligonucleotide (**A**) or fragment A (**B**) as a probe. In A or B, binding profile of rat liver nuclear extract (**lane 1**, 3 ng of protein), rat liver nuclear extract plus GST-NF1-A1 (**lane 2**, 3 or 2 ng of protein, respectively), rat liver nuclear extract plus GST-RGPR-p117 (**lane 3**, 3 or 20 ng of protein, respectively), and rat liver nuclear extract plus GST (**lane 4**, 3 or 20 ng of protein, respectively). The figure shows one of three experiments.

RGPR-p117 protein is shown to bind to the −525 to −504 bp sequence (CAGTTTGGCAGGGAGCCTTGAA, II-b oligonucleotide), which is rat regucalcin gene promoter region, in a yeast one-hybrid system [Misawa and Yamaguchi, 2001]. The gene of RGPR-p117 may be present in yeast [Misawa and Yamaguchi, 2002b]. In yeast, RGPR-p117 may be able to bind to the TTGGC motif of II-b oligonucleotide. In addition, there are several motifs concerning post-translational modification, such as casein kinase II phosphorylation, protein kinase C phosphorylation, *N*-myristoylation, *N*-glycosylation, and amidation in RGPR-p117 [Misawa and Yamaguchi, 2001]. Whether the modificated native RGPR-p117 binds to the TTGGC motif in the promoter region of regucalcin gene in liver nucleus remains to be elucidated.

RGPR-p117 has a leucine zipper motif which is conserved among RGPR-p117 in rat, mouse, and human [Misawa and Yamaguchi, 2001]. The leucine zipper pattern is present in many gene regulatory proteins [Clerc et al., 1988; Collum and Alt, 1990]. It is possible that RGPR-p117 has a role in the regulation of the nuclear function in rat liver. Further studies are needed to clarify a role of RGPR-p117 in cellular regulation.

In conclusion, it has been shown that RGPR-p117 mRNA is stably expressed for physiologic change, and that a recombinant RGPR-p117 is not directly bound to the TTGGC motif which is the nuclear factor binding site in rat liver using gel mobility shift assay in vitro.

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