Molecular Cloning of a Novel Transcriptional Repressor Protein of the Rat Type 1 Vasoactive Intestinal Peptide Receptor Gene*

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This study demonstrates that the transcriptional repressor sequence of the rat vasoactive intestinal peptide receptor (VIPR) gene constitutes a 42-base pair core element that is the binding site for a nuclear protein. We showed that this element was able to confer transcriptional repression to a heterologous promoter and that deletion or point mutations within this element resulted in loss of transcriptional repression. Southwestern blot analysis indicated that the VIPR repressor element interacts specifically with a nuclear protein of about 72 kDa. By screening a rat lung expression library coupled with rapid amplification of cDNA ends polymerase chain reactions, we isolated a cDNA clone (designated as VIPR-RP) that contains an open reading frame of 656 amino acids. VIPR-RP is 78% identical to a previously characterized protein, differentiation-specific element-binding protein, which is a member of a family of proteins including components of the DNA replication factor C complex. However, VIPR-RP cDNA encodes for a much smaller protein than differentiation-specific element-binding protein because of a frameshift. VIPR-RP mRNA is expressed in multiple tissues, including lung, liver, brain, heart, kidney, spleen, and testis. VIPR-RP protein specifically interacts with the VIPR repressor element as demonstrated by gel shift assays. Transfection of VIP-RP expression vector into Cos cells resulted in transcriptional repression of a reporter construct containing multiple copies of the VIPR repressor element. These results indicate that VIPR-RP is a novel transcriptional repressor protein that regulates VIPR expression.

Transcriptional repression plays a critical role in regulating gene expression. Different repressors are known to function in a wide variety of biological settings (1–4). Repressors bind DNA and inhibit transcription by interference with 1) activator DNA binding, 2) the activity of DNA-bound activators, and 3) the general transcription machinery. Eukaryotic repressors that work by competitive DNA binding have been uncovered in a wide variety of biological settings (1–4). Repressors bind DNA and inhibit transcription by interference with 1) activator DNA binding, 2) the activity of DNA-bound activators, and 3) the general transcription machinery. Eukaryotic repressors that work by competitive DNA binding have been uncovered in a wide variety of biological settings (1–4).

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF059678.

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The abbreviations used are: VIP, vasoactive intestinal peptide; VIPR, VIP receptor; bp, base pair(s); PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; RAR, retinoid X receptor; RACE, rapid amplification of cDNA ends; DSEB, differentiation-specific element-binding protein; MHC, myosin heavy chain; RFC, replication factor C.

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cell lines were grown in F-12K and Dulbecco's modified Eagle's medium, respectively, supplemented with 10% fetal bovine serum. Cells were transfected with 5 μg of DNA by calcium phosphate precipitation (13). After 24 h incubation, cells were assayed for luciferase and CAT activities. Each construct was transfected using triplicate plates for each experiment and each construct was tested in at least three independent experiments. In all experiments, cells were co-transfected with 1 μg of TKCAT (thyminidine kinase promoter and CAT fusion gene) to monitor transfection efficiency. A negative control plasmid containing a promoterless luciferase gene (pPA3) and a positive control plasmid containing Rous sarcoma virus long terminal repeat promoter fused to luciferase were included in all experiments. Luciferase assays and CAT assays were performed as described previously (15).

**Ge Mobility Shift Assays**—Nuclear extract was prepared from cultured cell as described by Dignam (16). To generate the probe, an oligonucleotide containing DNA sequence between −815 and −773 bp of the VIP receptor promoter was synthesized. After annealing to the complementary strand, the double-stranded DNA was end labeled using [γ-32P]ATP and T4 polynucleotide kinase. 10,000 cpm of the probe was used in each reaction. Binding reactions were performed in a total volume of 20 μl containing 20 mM Tris, pH 7.5, 1 mM EDTA, 2 mM MgCl2, 50 mM NaCl, 20 mM dithiothreitol, 10% glycerol, and 1 μg of poly(dI-dC), with 15-μg nuclear extracts. Competitor DNA was mixed with the probe prior to receptor addition. Binding was for 30 min at room temperature. The anti-RXR antibody (1 μg) (Santa Cruz Biotechnology) was added, and the binding was continued on ice for an additional 30 min. The reactions were electrophoresed on 4% nondenaturing polyacrylamide gel, dried, and exposed to x-ray film for 3 h.

**Southern Blot Analysis**—Cell nuclear extracts (50 μg) were separated on a 10% polyacrylamide-SDS gel next to rainbow protein standards (Amersham Pharmacia Biotech). The protein was transferred onto nitrocellulose membrane, treated with decreasing concentrations of guanidine HCl (6, 3, 1.5, 0.75, 0.375, and 0.1875 M) and pre-bound in 10 mM NaPO4, pH 7.4, 5% nonfat milk, 150 mM NaCl, 1% bovine serum albumin, 2.5% PVP-40, 0.1% Triton X-100, at 4 °C for 1 h. Binding was performed at 4 °C overnight in a buffer containing 10 mM Tris, pH 7.5, 0.5% nonfat milk, 0.5% bovine serum albumin, 50 mM NaCl, 5 mM MgCl2, 1 mM EDTA, 2 mM dithiothreitol, 0.1% Triton X-100, 5% glycerol, and 10 μg/ml salmon sperm DNA. 106 cpm/ml of the above probe used for gel shift analysis was used. The membrane was washed twice for 30 min in the binding buffer without salmon sperm DNA at 4 °C, and exposed to x-ray films for 2 h.

**Expression Screening of agt11 Library**—A rat lung 5'-stretch plus cDNA agt11 library was purchased from CLONTECH. The library was screened using an end labeled double-stranded oligonucleotide containing DNA sequence between −773 and −815 bp of the VIP receptor promoter. An oligonucleotide containing point mutations within this region (see above) was used as a negative control. Approximately 106 plaque-forming units were screened using the protocol described by Singh et al. (17). Clones encoding sequence-specific DNA-binding proteins were identified and plaque-purified. Crude cell extracts from recombinant phage lysogens were prepared and translated in reticulocyte lysate using the method by Singh et al. (17) and were used in gel mobility shift assays to test the specific interaction between the fusion proteins and the DNA-binding sequence. The cDNA encoding the sequence-specific DNA binding was isolated by digesting phage DNA with EcoRI and was cloned into pGEM3Z plasmid vector for restriction mapping and DNA sequencing. DNA sequencing was performed on both strands using internal primers.

**Rapid Amplification of cDNA Ends (RACE)**—The full-length cDNA was obtained by 5' and 3' RACE reactions using a Marathon cDNA amplification kit (CLONTECH) according to manufacturer's instructions. Rat lung poly(A)+ RNA (CLONTECH) was used for cDNA synthesis. The 5' RACE gene-specific primer was: 5'-AGCTGCTTGGCGA-TGGGCTCATCA-3', and the 3' RACE gene-specific primer was: 5'-C-AAAAATCCACGACTGAGGCACGGTTC-3'. The PCR reactions included: 1) denaturing at 94 °C for 1 min; 2) 5 cycles of 94 °C for 30 s and 72 °C for 4 min; 3) 5 cycles or 94 °C for 30 s and 70 °C for 4 min; and 4) 25 cycles of 94 °C for 20 s and 68 °C for 4 min in a total volume of 50 μl. After the cycling was completed, 10 μl of the PCR products was analyzed on agarose gel. The PCR products were cloned into TA vector (Invitrogen) for further analysis. The entire coding region of the cDNA was amplified by PCR using the following primers: 5'-GGCTGGCATGGGA-CATTGGAATTCATTTTG-3' and 5'-TATGCGTCCAGTTTGTGACATAAAGTG-3'. The PCR reaction was performed in a total volume of 50 μl using the following program: 94 °C for 1 min, followed by 25 cycles of 94 °C for 30 s and 68 °C for 4 min. The PCR product was cloned into Eukaryotic TA vector (Invitrogen) for further analysis, and cotransfection experiments were conducted.

**Northern Blot Analysis**—A rat multiple tissue Northern blot was purchased from CLONTECH. Approximately 2 μg of poly(A)+ RNA/lane from eight different rat tissues was run on a denaturing formaldehyde 12% agarose gel, transferred to nylon membrane, and UV-cross-linked. The membrane was first hybridized to the full-length cDNA probe and then was stripped and rehybridized to a human β-actin cDNA control probe. Hybridization was performed at 60 °C for 1 h in ExpressHyb hybridization solution (CLONTECH). Washing was twice for 15 min at room temperature in 2× SSC, 0.05% SDS and twice for 15 min at 65 °C in 0.1% SSC, 0.1% SDS.

**In Vitro Transcription and Translation**—VIPR-RP was transcribed from T7 promoter and translated in reticulocyte lysate using transcription- and translation-coupled reticulocyte lysate system (Promega). A typical reaction contains 25 μl of rabbit reticulocyte lysate, 2 μl of reaction buffer, 20 mM amino acid mixture, 1 μg of DNA template, 40 units of ribonuclease inhibitor, and 10 units of T7 RNA polymerase in a total volume of 50 μl. The reactions were carried out at 30 °C for 1 h. 5 μl of the reaction was used in gel shift assays.

**RESULTS**

**A Lung Cell Nuclear Protein Interacts with a 42-bp Sequence in the VIPR 5'-Flanking Region**—Previous transfection studies in rat lung cells indicated that a potential transcriptional repressor sequence is present between −488 and −859 bp in the VIPR 5'-flanking region. To characterize this repressor sequence further, we first sought to determine whether nuclear proteins in lung cells interact with a specific sequence in this region. As shown in Fig. 1, when lung cell nuclear extracts were incubated with an oligonucleotide containing 42-bp VIPR 5'-flanking sequence between −773 and −815, a mobility shifted band was observed (Fig. 1, lane 2). This band was not competed by an oligonucleotide with unrelated sequences (Fig. 1, lane 3) but was competed by the unlabeled oligonucleotide of the same sequence (Fig. 1, lane 4). Because a sequence similar to the half-site for RXR (GGTGA) is present within this 42-bp sequence, an anti-RXR antibody was included in the reaction to...
test whether the mobility shifted band was a result of RXR interaction with this sequence. Fig. 1, lane 5 shows that addition of anti-RXR antibody has no effect on the mobility shifted band. These results indicate that a nuclear protein in lung cells specifically interacts with VIPR 5'9 sequence between 2773 and 2815 bp and that this protein is not RXR.

The GGTGA Motif Is Required for the Nuclear Protein Binding—The above results showed that RXR does not bind to the 42-bp sequence despite the presence of a half-site. To test whether the GGTGA motif is important for DNA-protein interaction, point mutations were made to change this sequence to tactg. As shown in Fig. 2, an oligonucleotide containing these mutations could not compete for binding of the lung nuclear protein with the wild-type sequence (Fig. 2, lanes 4 and 5). When the mutant oligonucleotide was used as the probe, no mobility shifted band was present (Fig. 2, lanes 7–10). These results indicate that the GGTGA motif is required for interaction of the lung cell nuclear protein with the 42-bp sequence.

Deletion of the 42-bp Sequence from VIPR Promoter Results in Loss of Transcriptional Repression—To test whether the 42-bp nuclear protein-binding site mediates transcriptional repression of the VIP receptor gene, this sequence was deleted from the VIPR-luciferase fusion construct. As shown in Fig. 3A, luciferase activity of the fusion construct containing 859 bp of the VIPR 5'9 sequence (–859LUC) is about 8-fold lower than that of –815LUC, whereas a construct containing deletion

FIG. 2. Mutations within the 42-bp VIPR 5'-flanking sequence abolished protein binding. Gel mobility shift assay using either the wild-type or the mutant 42-bp VIP receptor upstream sequence as the probe. Lanes 1 and 6, probe alone; lanes 2 and 7, probe + 15 μg of L2 cell extract; lanes 3 and 8, probe + 15 μg of L2 cell extract + 50-fold excess of unlabeled wild-type oligonucleotide; lanes 4 and 9, probe + 15 μg of L2 cell extract + 50-fold excess of unlabeled mutant oligonucleotide; lanes 5 and 10, probe + 15 μg of L2 cell extract + anti-RXR antibody. Lanes 1–5, wild-type probe; lanes 6–10, mutant probe. The arrowhead indicates the DNA-protein complex, and the arrow indicates the free probe.

FIG. 3. A, deletion of the 42-bp sequence from VIPR promoter resulted in loss of transcriptional repression. B, the 42-bp sequence confers transcriptional repression to a heterologous promoter. Lung cells were transiently transfected with indicated DNAs and assayed for luciferase reporter activity, represented as fold increase over a promoterless luciferase control pA3 (activity of the control is taken as 1) after normalizing to internal control CAT activity. Values represent means ± S.E.

FIG. 4. The VIPR repressor element detected a 72-kDa nuclear protein. Southwestern blot analysis. 50 μg of nuclear extracts was fractionated on 10% SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membrane, and probed with either the wild-type (WT) or mutant VIPR repressor element. Lane 1, L2 lung epithelial cells; lane 2, neuroblastoma cells; lane 3, pituitary tumor GH4 cells. The detected protein band is indicated by an arrow. The molecular mass marker is shown on the left side.

FIG. 5. Interaction of the phage lysogen protein with the VIPR repressor element. Gel mobility shift assay using VIPR repressor element as the probe. Lane 1, probe alone; lane 2, probe + 5 μg of lysogen extracts; lane 3, probe + 5 μg of lysogen extracts + 50-fold excess of unlabeled wild-type probe; lane 4, probe + 5 μg of lysogen extracts + 200-fold excess of unlabeled wild-type probe; lane 5, probe + 5 μg of lysogen extracts + 200-fold excess of unlabeled mutant probe. The arrowhead indicates the DNA-protein complex, and the arrow indicates the free probe.
The 42-bp Sequence Confers Transcriptional Repression to a Heterologous Promoter—

To test whether the 42-bp VIPR 5'-flanking sequence is able to repress transcription of a heterologous promoter, oligonucleotide containing either the wild-type or point mutation of this sequence was multimerized and cloned in front of a minimal thymidine kinase promoter linked to luciferase (TKLUC). Fig. 3B shows that the luciferase activity of the construct containing four copies of the wild-type sequence (4FTKLUC) is about 4-fold lower than that of TKLUC, whereas there is no significant difference in luciferase activity between the construct containing four copies of the mutant sequence (m4FTKLUC) and TKLUC. These results indicate that the 42-bp sequence is capable of conferring transcriptional repression to a heterologous promoter.

**Fig. 6—continued**

**Fig. 7. VIPR-RP mRNA expression in normal tissues.** Poly(A)^+ RNA from eight different rat tissues (indicated at the top of the figure) were used for Northern blot analysis. Molecular mass marker is shown on the left side. Exposure time was 24 h. Hybridization to VIPR-RP probe is indicated by an arrow.

VIPR-RP alignment to DSEB protein. The entire open reading frame for VIPR-RP is shown and aligned to amino acids 1–657 of DSEB protein. Regions of homology are shaded with the corresponding amino acid number shown on the right.

VIPR-RP and alignment to DSEB protein. The entire open reading frame for VIPR-RP is shown and aligned to amino acids 1–657 of DSEB protein. Regions of homology are shaded with the corresponding amino acid number shown on the left.

Fig. 6. A, nucleotide sequence of VIPR-RP cDNA. About 2.3 kilobase pairs of the VIPR-RP cDNA sequence is shown. The translation initiation and termination codons are underlined. B, amino acid sequence of VIPR-RP and alignment to DSEB protein.
Lane 1

Gel mobility shift assay using VIPR repressor element as the probe. Lane 1, probe alone; Lane 2, probe + 5 µl of in vitro translated VIPR-RP protein; Lanes 3–6, probe + 5 µl of in vitro translated VIPR-RP protein + 10-, 20-, 50-, or 200-fold excess of unlabeled wild-type (WT) probe, respectively; Lanes 7–10, probe + 5 µl of in vitro translated VIPR-RP protein + 10-, 20-, 50-, or 200-fold excess of unlabeled mutant (mut) probe, respectively; lane 11, probe + 5 µl of in vitro translated VIPR-RP protein + 200-fold excess of unlabeled Sp1 oligonucleotide; lane 12, probe + 5 µl of in vitro translated VIPR-RP protein + 200-fold excess of unlabeled AP2 oligonucleotide. The arrowhead indicates the DNA-protein complex, and the arrow indicates the free probe.

The VIPR Repressor Element Interacts with a 72-kDa Nuclear Protein—To characterize further the nuclear protein that interacts with the VIPR repressor element, Southwestern blot analysis was performed. Fig. 4 shows that the VIPR repressor element detected a protein of about 72 kDa from lung cell nuclear extracts (Fig. 4, lane 1). To determine whether this protein was specific to lung cells, nuclear extracts from a neuroblastoma and pituitary tumor cell lines were also included. As shown in Fig. 4 (lanes 2 and 3), the 72-kDa protein is also present in nuclear extracts from both cell lines. When an oligonucleotide containing point mutations within the repressor element was used to probe the same blot, no specific protein band was detected. These results indicate that the VIPR repressor elements interact specifically with a 72-kDa nuclear protein that is not only expressed in lung cells but also in other cell types.

Molecular Cloning of the cDNA That Encodes for the 72-kDa Protein—To isolate the cDNA clones, a λgt11 cDNA expression library made from rat lung (CLONTECH) was screened using the end labeled 42-p VIP repressor element as the probe. About 106 plaques were screened according to Singh et al. (17) and Vinson et al. (18). One positive clone was obtained, and lysogens prepared from this phage clone were used in gel shift assays to test its specific binding to the VIPR repressor element. As shown in Fig. 5, when the extracts of the phage lysogen were incubated with the 42-bp VIPR repressor oligonucleotide, a mobility shifted band was observed (Fig. 5, lane 2). This band was competed out by unlabeled wild-type VIPR repressor oligonucleotide (Fig. 5, lanes 3 and 4), but not by the mutant oligonucleotide (Fig. 5, lane 5). These results indicate that the cDNA in this phage clone encodes a protein that exhibits sequence-specific DNA binding to the VIPR repressor element.

DNA sequencing showed that this clone contains an insert of 1 kilobase pair but does not contain either the 5′ or 3′ portion of the cDNA. RACE PCRs were performed to obtain the 5′ and 3′ ends of the cDNA, and the entire cDNA clone was obtained by PCR using primers at both ends of the cDNA (see “Materials and Methods”). This cDNA (designated VIPR-RP) contains an open reading frame of 656 amino acids (Fig. 6A) with a predicted molecular mass of 72 kDa.

Comparison of VIPR-RP protein sequence with sequences reported to GenBank revealed that VIPR-RP is 78% identical to differentiation-specific element-binding protein (DSEB) (19). The highest level of homology is between amino acids 367 and 493 (97%), corresponding to the DNA-binding domain of DSEB (Fig. 6B) (19). The sequence in this region also shares high identity to three bacterial ligases (20, 21). VIPR-RP sequence diverges from DSEB at amino acid 581 (Fig. 6B), where a single nucleotide deletion in VIPR-RP cDNA results in a frameshift and a termination codon at amino acid 657. VIPR-RP cDNA therefore encodes for a much smaller protein than DSEB, which contains 1131 amino acids.

The N-terminal portion of VIPR-RP also shares extremely high sequence identity with the human fetal β-myosin heavy chain (MHC)-binding factor (GenBank accession number X75917). As shown in Fig. 6C, the N-terminal 375 amino acids of VIPR-RP are almost identical to β-MHC-binding factor, except in three amino acids. The sequence homology ends at residue 376, and translation of β-MHC-binding factor terminates at amino acid 399, resulting in a much smaller protein (Fig. 6C).

Northern blot analysis of poly(A) RNA from several rat tissues was performed to determine the tissue distribution of VIPR-RP mRNA. A 4.6-kilobase pair mRNA band was observed in all tissues examined, including heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (Fig. 7). A smaller mRNA band was present only in testis (Fig. 7). These results indicate that VIPR-RP mRNA is widely expressed and that it has a testis-specific transcript.

VIPR-RP Exhibits Sequence-specific DNA Binding to the VIPR Repressor Element—Examination of the VIPR-RP sequence did not reveal any obvious homology with any DNA-binding motifs such as zinc fingers and homeodomains. To test whether VIPR-RP specifically interacts with the VIPR repressor element, VIPR-RP was transcribed and translated in vitro.

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2 E. Morkin, unpublished observation.
As shown in Fig. 8, when VIPR protein was incubated with the 42-bp VIPR repressor oligonucleotide, two mobility shifted bands were observed (Fig. 8, lane 2). The presence of the faster migrating band was likely to be the result of binding a partially transcribed and translated VIPR-RP protein to DNA. These bands were competed out by increasing amounts of the unlabelled wild-type VIPR repressor oligonucleotide (Fig. 8, lanes 3–6) but not by the mutant oligonucleotide (Fig. 8, lanes 7–10).

Unlabeled oligonucleotide containing consensus binding sites for transcription factor Sp1 or AP2 also did not compete for transcription of a heterologous promoter. This indicates that VIPR-RP exhibits sequence-specific DNA binding to the VIPR repressor element.

Transcriptional Repression by VIPR-RP—To assess the functional effect of VIPR-RP, VIPR-RP cDNA was inserted downstream of the cytomegalovirus promoter and co-transfected with reporter plasmids into Cos-7 cells. As shown in Fig. 9, cotransfection with the VIPR-RP expression vector resulted in 6-fold reduction in luciferase activity of the reporter plasmid containing four copies of the wild-type VIPR repressor element (Fig. 9, 4FTKLU C), whereas expression of VIPR-RP did not significantly affect luciferase activity of reporter plasmid containing four copies of the mutant VIPR repressor element (Fig. 9, m4FTKLU C) or the plasmid without the repressor element (Fig. 9, TKLU C). These results demonstrate that VIPR-RP acts as a sequence-specific transcriptional repressor in vivo.

DISCUSSION

In previous work we demonstrated the presence of a negatively acting element that regulates VIPR gene expression in lung cells (13, 15). In this report, we have restricted the biological activity of the negatively acting element to a 42-bp DNA sequence located between −773 and −815 bp that contributes importantly to transcriptional repression of the VIPR gene. We showed that deletion of this sequence results in loss of transcriptional repression and that this sequence can repress transcription of a heterologous promoter.

The VIPR repressor element interacts with a nuclear protein in lung cells as demonstrated by gel shift assays. Although a GGTTA motif similar to RXR-binding half-site is present within the repressor element, RXR does not appear to be the protein that binds to this element, for addition of anti-RXR antibody in gel shift assays did not change the pattern of the mobility shifted band. This motif, however, is essential for the nuclear protein-binding and -mediating transcriptional repression. Base substitutions within this motif resulted in loss of protein binding and transcriptional repression.

At least three categories of transcriptional regulation by negatively acting elements have been described (22): 1) repression via competition between repressors and activators for the same or overlapping binding sites, 2) neutralization of activators by interactions with a repressor protein, and 3) silencing through direct interaction of a repressor with the target site, regardless of distance from the promoter. The nuclear protein that interacts with the VIPR repressor element was characterized further by Southwestern blot analysis. The results showed that the repressor element detected a single protein of about 72 kDa in nuclear extracts of not only lung cells but also neuroblastoma and pituitary tumor cell lines. These results indicate that the VIPR-RP repressor element interacts with a ubiquitously expressed nuclear protein and that transcriptional repression is likely mediated by direct interaction of the repressor protein with this element.

Based on the specific interaction between VIPR repressor element and the 72-kDa nuclear protein, we isolated a cDNA clone that encodes for this protein (VIPR-RP). The deduced amino acid sequence of VIPR-RP shares high identity (78%) to DSEB (19) and the large subunit of murine activator 1 complex, Alp145 (23), which is also known as replication factor C (RFC) (24). DSEB and Alp145RFC are nearly identical in amino acid sequence, except for the differences in three amino acids. DSEB binds to an enhancer element in angiotensinogen gene promoter that mediates the irreversible induction of transcriptional activation during differentiation of 3T3-L1 adipoblasts to adipocytes (19). Alp145RFC is part of a heteropentameric protein complex essential for DNA replication (25). Interestingly, the N-terminal 375 amino acids of VIPR-RP are almost identical to the human β-MHC-binding factor, which has an unknown function. Our result also showed that the mRNA tissue distribution of VIPR-RP and DSEB is different. Although VIPR-RP mRNA is ubiquitously expressed, DSEB mRNA shows more selected expression pattern. DSEB mRNA was not detected in heart and skeletal muscle (19). These data indicate that although VIPR-RP is closely related to DSEB/RFC, it is a distinct gene.

Like DSEB and Alp145, VIPR-RP does not contain the well characterized DNA-binding motifs such as zinc fingers or homedomain. Sequence-specific binding of DSEB and Alp145 was demonstrated by gel shift experiments in which recombinant DSEB and Alp145 preferentially bind sequences within the promoter of angiotensinogen (19) or collagen IV (23), respectively. Our results showed that in vitro translated VIPR-RP protein binds to the VIPR repressor element specifically and that a GGTTA motif within the repressor element is essential for VIPR-RP binding. A similar motif GGTTA is present within the binding site for DSEB (19), but the functional importance of this motif has not been tested.

VIPR-RP differs from DSEB and Alp145 in that it encodes a much smaller protein. A single base deletion at amino acid 581 in VIPR-RP results in a frameshift and a termination codon at amino acid 657. As a consequence, the C-terminal 75 amino acids of VIP-RP show very little similarity to DSEB and Alp145. The precise functions of DSEB and Alp145 in DNA replication and transcriptional activation remain to be elucidated. DSEB was unable to transactivate reporter genes containing its binding sites when co-transfected into Cos-1 and NIH3T3 cells (19). Our results showed that VIPR-RP repressed transcription of a reporter plasmid containing four copies of its binding sequence when co-transfected into Cos-7 cells, suggesting that VIPR-RP not only binds to DNA but also mediates transcriptional repression. It is possible the C-terminal portion of VIPR-RP may possess functions that are distinct from DSEB and Alp145.

In summary, we have identified a negatively acting element in the VIPR promoter that is the binding site for a novel transcriptional repressor, VIPR-RP. Interaction of VIPR-RP with its target site results in transcriptional repression of the VIPR receptor gene.

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