

# A Proximal Direct Repeat Motif Characterized as a Negative Regulatory Element in the Human Renin Gene

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**Abstract** The regulation of renin gene expression is thought to be fundamental to regulation of the total renin–angiotensin system. The human renin gene contains a direct repeat (DR) motif AGGGGTAC–AGGGCCA in the proximal region (–259/–245 bp), which contains similar sequence for nuclear receptor superfamily binding core motif, AGGTCA, and is the most similar to COUP-TFII consensus. The DR motif was evaluated as a functional *cis*-element with renal cortex and chorio-decidual cells by footprint assay, electromobility shift assay (EMSA) and reporter assay. The DR motif site was protected by footprint analysis with a clear hypersensitive and a minor hypersensitive region in good accordance with the DR of the consensus. One of the binding proteins was strongly suspected to be COUP-TFII-consensus-specific by EMSA. The DNA/protein complexes obtained with nuclear extract of renin producing cells could be completely blocked by homologous competitor and strongly blocked by the second-half mutant oligonucleotide of the DR motif but not by the first-half mutant oligonucleotide. Finally, the transcriptional activity of second-half mutant construct is slightly elevated and that first-half mutant construct is significantly stronger by twofold compared with wild type construct in reporter assay. These findings suggest that the DR motif site of the human renin gene functions as a negative regulatory element involved in a twofold repression of transcription and that member(s) of nucleic receptor superfamily bind the site and play important roles in the human renin gene expression with a possibility that one of the binding protein is COUP-TFII. *J. Cell. Biochem.* 102: 1043–1050, 2007. © 2007 Wiley-Liss, Inc.

**Key words:** renin; renin–angiotensin system; COUP-TFII; ARP-1; NR2F2; *cis*-element; *trans*-factor

The renin–angiotensin system (RAS) plays major roles in blood pressure regulation and electrolyte metabolism [Corvol et al., 1997] and in the pathophysiology of cardiovascular [Dzau, 2005], renal [Remuzzi et al., 2005] and metabolic conditions [Konoshita et al., 2006]. Recently the way for understanding of RAS has changed somehow with the discovery of new components, renin/prorenin receptor [Nguyen, 2006] and ACE2 [Danilczyk and Penninger, 2006]. However, as always, the rate-limiting step of the system is the conversion of angiotensinogen to angiotensin I cata-

lyzed by renin. Thus, the regulation of renin gene expression is thought to be fundamental to the regulation of the total system. Several human cell types have been used as models for renin-producing cells, one being human chorio-decidual cells [Pinet et al., 1988], as no human juxtaglomerular (JG) cell line currently exists, although a mouse line, As4.1, has been produced by transformation of JG cells with SV40 large T antigen [Sigmund et al., 1990]. Germain et al. [1998] showed that a 250 bp region located about 6 kb upstream from the transcription starting point of the human renin gene gave up to 57-fold higher transcription rates and is a distal enhancer with human chorio-decidual cells. On the other hand, Fuchs et al. [2003] recently identified a negative calcium response element (nCaRE) about 1.7 kb upstream. They also showed that 12 kb of 5' region of the human renin gene are needed for tissue-specific and regulated the human renin gene expression in transgenic mice [Fuchs et al., 2002]. These data

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Received 12 October 2006; Accepted 21 February 2007

DOI 10.1002/jcb.21341

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indicate the importance of the distal enhancers for tissue-specific and regulated human renin gene expression. Nevertheless, it is well known that proximal promoter regions of genes are indispensable for the regulation of the basic portion of gene expression (Fig. 1A). Recently, proximal promoters of mouse renin gene have been well investigated [Petrovic et al., 1996; Pan et al., 2001], whereas the human proximal promoter remains to be elucidated. Previously, we mapped six protein-binding sites in the proximal promoter region of the human renin gene (−336 to +16) by DNase I footprint assay with nuclear extract from human chorio-decidual cells or ischemic human renal cortex and designated these candidate *cis*-elements as footprint A to F temporarily [Borensztein et al., 1994; Germain et al., 1996; Konoshita et al., 1996] (Fig. 1B). Human renal cortex does not consist of homogeneous cells, but, at least, the presence of nuclear factors binding to the human renin promoter has been proven with this method. Searching for similar sequences with the footprint A to F among known consensus as *cis*-elements, we have hypothesized putative *cis*-elements: Ets motif like site (A: −29/−6) [Wasylyk et al., 1993], CRE (D: −234/−214) [Montminy et al., 1986], COUP-TFII (ARP-1) motif like direct repeat site (E: −259/−245) [Ldias and Karathanasis, 1991]. The candidate human renin CRE was proven to be a genuine CRE [Borensztein et al., 1994; Smith et al., 1994]. Initially, footprint B (−79/−62) was regarded as containing Pit-1 motif like sequence [Borensztein et al., 1994]. We demonstrated that the CRE and footprint B are implicated in the regulation of the human renin gene transcription by cAMP, but, at the same time demonstrated that the protein from chorionic-decidual cell binding to this region is distinct from Pit-1 [Germain et al., 1996]. A recent report indicates that this corresponding region of mouse *Ren-1<sup>c</sup>* is HOX–PBX recognition sequence although whether HOX and PBX proteins bind to the human proximal promoter element has not been examined [Pan et al., 2001]. Footprint C (−116 to −80) contains no known consensus sequence. Footprint F (F: −293/−272) contains an AGE3 like site but the corresponding factor has not been identified [Tamura et al., 1994]. Of these six genuine or putative *cis*-elements of the human renin gene, we compared the DNA/nuclear protein binding patterns between renin-producing cells and

non-renin-producing cells by electromobility shift assays (EMSA) and showed that the Footprint E, a direct repeat motif site has a obvious heterogeneity, suggesting that the site is involved in cell type specific renin production [Konoshita et al., 2004].

The aim of the present study was to characterize the direct repeat motif site (AGG-GGTAC–AGGGCCA, −259/−245 bp), which contains steroid receptor superfamily binding core motif, AGGTCA [Giguere, 1999] and is the most similar to the COUP-TFII consensus, as a functional *cis*-element of the human renin gene.

## MATERIALS AND METHODS

### Preparation of COUP-TFII (ARP-1) Containing Cellular Extract From COS-1 Cells, Nuclear Extract From Human Renal Cortex and Chorio-Decidual Cells

COUP-TFII (ARP-1) containing cellular extracts and nuclear extracts from human renal cortex and human chorio-decidual cells were prepared as described previously [Konoshita et al., 2004].

### DNase I Footprint Analysis

DNase I footprint analysis was performed as described previously [Konoshita et al., 1996]. Briefly; the −336 to +16 fragment of the renin gene was inserted in Bluescript. Primers were labeled with [ $\gamma$ -<sup>32</sup>P] ATP and T4 polynucleotide kinase before PCR amplification. Footprint analysis was performed in a 10  $\mu$ l reaction mixture. After 20 min of incubation at 4°C, 2  $\mu$ l of DNase I (Boehringer Mannheim, 10 IU/ $\mu$ l) was added, and digestion was allowed to proceed for 1 min at 20°C. The reaction mixture was incubated for 45 min at 42°C with stop solution for DNase inactivation.

### Electromobility Shift Assays (EMSA)

Table I summarizes the sequences of oligonucleotides used for EMSA. EMSA was performed as described previously [Konoshita et al., 2004]. Briefly, double-stranded oligonucleotides were synthesized (Applied Biosystems) and end-labeled with [ $\gamma$ -<sup>32</sup>P] ATP and T4 polynucleotide kinase. Binding reactions for COUP-TFII-REN were performed incubating COUP-TFII (ARP-1) transfected Cos-1 extract or each nuclear extract. These were incubated with labeled oligonucleotides with or without competitor oligonucleotides COUP-TFII-M1 mutated

TABLE I. Alignment of Oligonucleotide Sequences Used in This Study

COUP-TFII CONSENSUS	AGGGGTCAN	AGGGNTCA
COUP-TFII-REN	-269TGCCTGCTCC <b>AGGGGTCAC</b>	<b>AGGGC-CAAGCCAGATAG</b> -233
COUP-TFII-M1	TGCCTGCTCC <b>AGGGGTCAC</b>	<u>GGTAC-CAAGCCAGATAG</u>
COUP-TFII-M2	TGCCTGCTCCAGG <u>AGATCC</u>	<b>AGGGC-CAAGCCAGATAG</b>

The direct repeat consensus sequence for COUP-TFII is indicated above the oligonucleotides in bold letters. N represents G, A, T or C. The sequences of all oligonucleotides used for EMSA or as competitors are shown. Top strands only are shown. Underlined and italicized letters in oligonucleotides indicate mutated sequences.

at the latter half of the consensus and COUP-TFII-M2 mutated at the first half of the consensus (Table I). To well characterize the direct repeat, the two oligonucleotides were newly designed completely different from those of our previous study [Konoshita et al., 2004].

#### Plasmid Constructions and Site-Directed Mutagenesis

The plasmid pGL<sub>2</sub>582+ was constructed as described previously [Germain et al., 1996]. The resulting plasmid pGL<sub>2</sub>582+ contained nucleotides (-582/+16) of the renin gene followed by the luciferase coding sequence. For site-directed mutagenesis, the *Hind*III fragment (-582/+16) was inserted into the polylinker of the M13mp18 vector (Boehringer Mannheim) to produce single-stranded DNA. COUP-TFII-M1 oligonucleotide replaced the latter half of the consensus, AGGG by GGTA (-249/-246) and COUP-TFII-M2 replaced the first half of the consensus GGTCA by AGATC (-255/-251). In vitro mutagenesis was performed with a MutaGene D kit (Bio-Rad), and the mutated sequences were confirmed by sequencing on both strands. The mutated fragments were then subcloned into pGL<sub>2</sub>basic creating plasmid pGL<sub>2</sub>582COUP-TFII-M1 in which the latter half of COUP-TFII consensus was mutated, and pGL<sub>2</sub>582COUP-TFII-M2 in which the first half of COUP-TFII consensus was mutated (Fig. 2). All constructions were verified by the dideoxy sequencing method (Sequenase Version 2.0 DNA Sequencing Kit, USB).

#### Cell Culture and Transfections and Reporter Assays

Primary and secondary cultures of human chorio-decidual cells were prepared as described previously [Pinet et al., 1988]. Transient DNA transfections of chorio-decidual cells were performed by calcium phosphate precipitation as described previously [Borenstein et al., 1994]. Transfected cells were washed

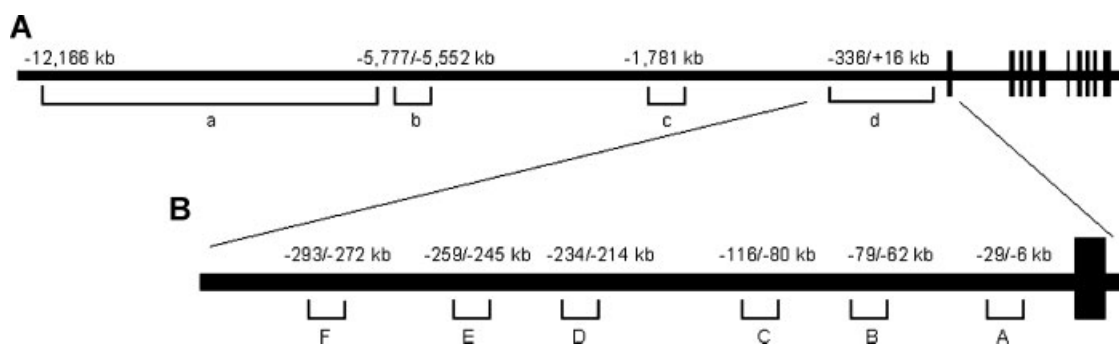
three times with PBS, lysed by adding 150  $\mu$ l of 1% Triton, 10 mM MgCl<sub>2</sub>, 1 mM EDTA, 25 mM Tris/phosphate, pH 7.8, 15% (w/v) glycerol, 1 mM dithiothreitol and 0.2 mM PMSF, and harvested by scraping. The lysates were centrifuged and the supernatant used immediately for the luciferase assay. Luciferase activity was estimated by measuring luminescence for 10 s with a Bio.Orbit 1250 luminometer, 30 s after addition of 50  $\mu$ l of luciferase reporter buffer (Promega). Quantitative determination of chloramphenicol acetyltransferase (CAT) was performed by a sandwich immunoassay (Boehringer Mannheim).

## RESULTS

### Demonstration of the Sequence-Specific Binding of Nuclear Protein(s) from Human Renal Cortex to the Direct Repeat Motif Site of the Human Renin Gene Promoter (-259/-245) by DNase I Footprint Analysis and EMSA

To characterize the direct repeat motif site of the human renin gene promoter, which was designated as footprint E previously [Borenstein et al., 1994], a DNase I footprint analysis was performed with human renal cortex nuclear extracts. The direct repeat site (-259/-245) is protected by renal protein(s) (Fig. 3). As shown at the left side of the figure with nucleotides sequence, the protected region is in good accordance with the nuclear receptor family binding consensus (AGGTCA) [Giguere, 1999], especially with COUP-TFII consensus sequence (AGGGGTCAN-AGGGNTCA). The protected region showed a clear hypersensitive region and a minor hypersensitive region inside, which are also in good accordance with the direct repeat of the consensus, strongly indicating the binding of proteins in a sequence-specific manner of the nuclear receptor family.

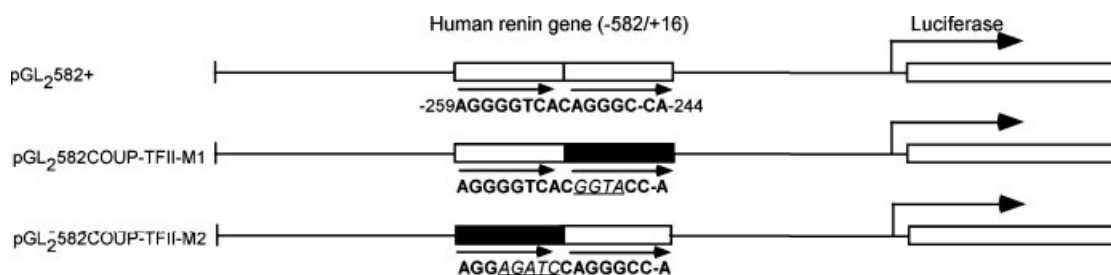
To more thoroughly characterize the sequence-specific binding of human renal cortex nuclear extracts to the direct repeat motif site of the



**Fig. 1.** Schematic representation of the human renin gene. **A:** A wide-ranged map of the human renin gene promoter. **a:** tissue-specific expression determining element, **b:** distal enhancer, **c:** negative calcium response element, and **d:** proximal promoter. Vertical bars represent exons of the human renin gene. **B:** A large scale map of the proximal promoter. **A:** Ets motif, **B:** HOX-PBX recognition sequence, **C:** no known consensus, **D:** CRE, **E:** direct repeat motif, and **F:** AGE3 like site.

human renin gene, electromobility shift assays (EMSA) were performed with COUP-TFII transfected Cos-1 cell extracts (COUP-TFII protein) and human renal cortex nuclear extracts with a labeled oligonucleotide (-269/-233) corresponding to the site of the human renin gene (COUP-TFII-REN). Especially, to best characterize the direct repetitive sequence of the consensus, besides the homologous competitor (COUP-TFII-REN), we synthesized two types of mutant competitors, a second-half mutant oligonucleotide (COUP-TFII-M1) and a first-half mutant oligonucleotide (COUP-TFII-M2). These two oligonucleotides were newly designed completely different from those of our previous study [Konoshita et al., 2004] to well characterize the direct repeat. As shown in the left panel of Figure 4, with COUP-TFII protein, one specific high-affinity DNA/protein complex was formed with the COUP-TFII

consensus like site of the human renin gene. The complex could be completely blocked by homologous competitor (COUP-TFII-REN) and strongly blocked by the second-half mutant oligonucleotide (COUP-TFII-M1) but not by the first-half mutant oligonucleotide (COUP-TFII-M2). With renal cortex nuclear extracts among non-specific complexes, consensus-specific DNA/protein complex showing the same migration and competition manner as COUP-TFII protein was observed, suggesting that the nuclear protein from renal cortex is COUP-TFII itself or other members of the nuclear receptor superfamily. Such DNA/protein complex was not observed with the nuclear extracts from non-renin producing JGE3 cells or HepG2 cells [Konoshita et al., 2004]. Before proceeding to functional evaluation of the consensus site, we had to characterize the nuclear extracts of model culture cells. As human chorio-decidual



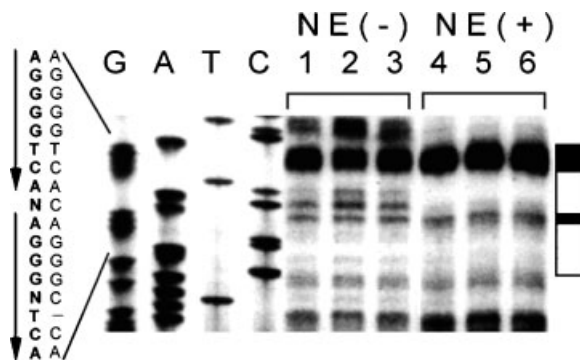
**Fig. 2.** Schematic representation of pGL<sub>2</sub>582+, pGL<sub>2</sub>582COUP-TFII-M1 and pGL<sub>2</sub>582COUP-TFII-M2. A 598 bp fragment of the human renin gene (-582/+16) was isolated by *Hind*III digestion of phrncAT06 derived from the plasmid subclone λHRn88 and subcloned into the *Hind*III site of pGL<sub>2</sub>basic. The resulting plasmid pGL<sub>2</sub>582+ contained nucleotides (-582/+16) of the renin gene followed by the luciferase coding sequence. The *Hind*III fragment (-582/+16) was inserted into the polylinker of the M13mp18 vector to produce

single-stranded DNA. In vitro mutagenesis was performed with a commercial kit and the mutated sequences were confirmed by sequencing on both strands. The mutated fragments were then subcloned into pGL<sub>2</sub>basic creating plasmid pGL<sub>2</sub>582+COUP-TFII-M1 in which the latter half of COUP-TFII consensus was mutated and pGL<sub>2</sub>582+COUP-TFII-M2 in which the first half of COUP-TFII consensus was mutated. Boxes represent repetitive consensus for COUP-TFII. Closed boxes represent the mutated parts of the repetitive consensus sequences.

cells are well known as a good model for human renin-producing cells, EMSA was performed with the nuclear extracts of these cells. As shown in the right panel of Figure 4, with nuclear extracts from chorio-decidual cells, consensus-specific DNA/protein complex showing the same migration and competition manner as COUP-TFII protein and renal cortex nuclear extracts was observed, suggesting that the nuclear protein from renal cortex is COUP-TFII itself or other members of the nuclear receptor superfamily.

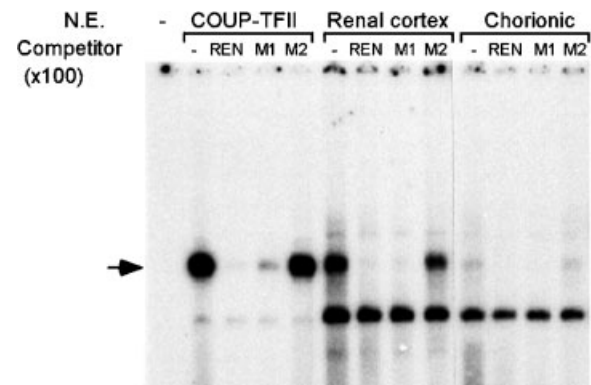
#### Demonstration of the Direct Repeat Motif Site in the Human Renin Gene Promoter as a Negative Regulatory Element

To characterize functionally the direct repeat motif site of the human renin gene which was demonstrated as a sequence-specific nuclear protein binding site, a series of chimeric genes was constructed in which the consensus site of the gene was mutated and fused to a luciferase reporter gene (Fig. 2). Two types of mutant were introduced to the site, of which each direct repeat consensus (AGGGGTCAN-AGGGNTCA) was replaced by non-specific nucleotides that were the same as the oligo-



**Fig. 3.** DNase I footprint analysis of the direct repeat motif site of the human renin gene (–259/–245). Footprint analysis was performed with human renal cortex nuclear extracts (NE). G, A, T, C represent the sequencing ladder. Lanes 1–3 represent the reaction performed in the absence of nuclear extracts (NE (–)) and decreasing amounts of DNase I (0.1, 0.05, and 0.025 U, respectively). Lanes 4–6 represent the reaction performed with nuclear extracts (NE (+)) with decreasing amounts of DNase I (0.1, 0.05, and 0.025 U, respectively). On the left of the figure, consensus sequences of direct repeat site and of the human renin gene are shown in bold and plain letters, respectively and two vertical arrows beside the consensus sequence represent the direct repeat of the consensus. On the right of the figure, the protected region of the direct repeat motif site and hypersensitive regions are represented as an open and a closed square, respectively.

nucleotides designed for EMSA (Fig. 2). The latter half of the consensus AGGG (–249/–246) of pGL<sub>2</sub>582+ was mutated to GGTA creating pGL<sub>2</sub>582+COUP-TFII-M1, whereas the first half of the consensus GGTC (–255/–251) was mutated to AGATC creating pGL<sub>2</sub>582+COUP-TFII-M2. These constructs were then transfected into human chorio-decidual cells which express the renin gene. Transfection efficiency was controlled by co-transfection with the pRSV-CAT plasmid. The results show that the promoter activity of pGL<sub>2</sub>582+COUP-TFII-M1 is slightly elevated and that pGL<sub>2</sub>582+COUP-TFII-M2 is significantly stronger compared with pGL<sub>2</sub>582+ (Fig. 5). The transcription activities of pGL<sub>2</sub>582+, pGL<sub>2</sub>582+COUP-TFII-M1 and pGL<sub>2</sub>582+COUP-TFII-M2 were 100 ± 14%, 110 ± 24% and 203 ± 41%, respectively. These results suggest that the direct repeat motif site of the human renin gene functions as a negative regulatory element involved in an about twofold repression of transcription. Furthermore, the activities are in good accordance with the results obtained by EMSA. These results



**Fig. 4.** Electromobility shift assay of COUP-TFII transfected Cos-1 extracts and nuclear extracts from renal cortex and chorio-decidual cells (3 µg protein in each reaction) with the direct repeat motif site of the human renin gene promoter (COUP-TFII-REN: –269/–233). A double-stranded labeled oligonucleotide containing the direct repeat motif site, COUP-TFII-REN (REN: –269TGCCTGCTCCAGGGGTCACAGGGCCAAGCCAGATAG-233), was used as a probe. Competitions were performed with a 100-fold molar excess of homologous DNA (COUP-TFII-REN), mutated oligonucleotide, COUP-TFII-M1 (M1: TGCCTGCTCC-AGGGGTCACGGTACCAAGCCAGATAG) or with mutated oligonucleotide COUP-TFII-M2 (M2: TGCCTGCTCCAGG-AGATCCAGGGCCAAGCCAGATAG). These two oligonucleotides were newly designed completely different from those of our previous study [Konoshita et al., 2004] to well characterize the direct repeat. Underlined and italicized letters in oligonucleotides indicate mutated sequence. The specific DNA/protein complexes are indicated by the arrow.

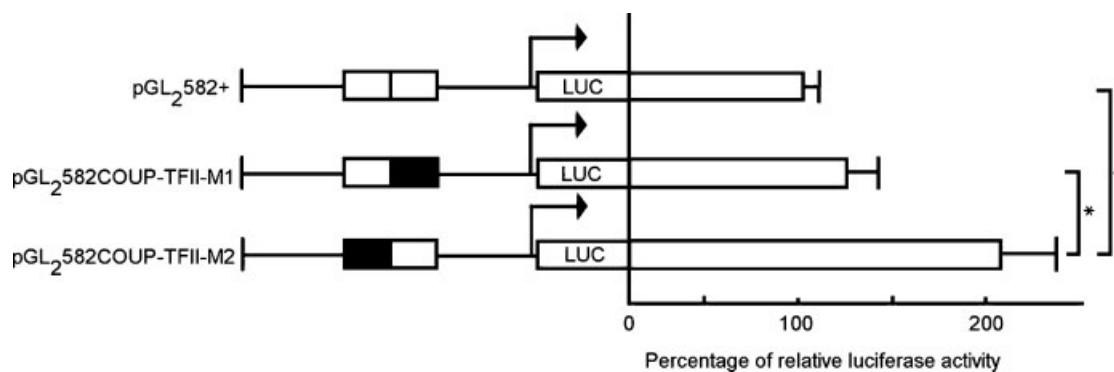
confirm that the sequence-specific binding has an important significance and indicated that the first half of the direct repeat is essential for the negative regulatory function.

## DISCUSSION

The main findings of the previous and present studies as summarized below strongly indicate that the direct repeat motif in the human renin gene is a negative regulatory element involved in an about 2-fold suppression in transcription: (a) The site is consensus specific with renin producing cell nuclear extracts [Konoshita et al., 1996, 2004]; (b) The site showed the protected region with two hypersensitive regions in good accordance with the direct repeat of the consensus by DNase I footprint analysis; (c) The DNA/protein complexes by EMSA is completely blocked by homologous competitor and strongly blocked by the second-half mutant oligonucleotide of the direct repeat motif but not by the first-half mutant oligonucleotide with renal cortex nuclear extract and chorio-decidual cell nuclear extract; (d) Deletion of the site showed an augmentation of transcript activity in a 5'-deletion mutant reporter assay by about 1.5-fold [Borensztein et al., 1994]; (e) Reporter assay showed that the mutation of the first portion of the binding region results in a significant increase of the transcriptional activity of the proximal promoter by 2-fold whereas the mutation of the second portion has only minimal effects. These observations are in good accordance with the results of the binding

assay. Taken together, our results suggest that it is the first half of the direct repeat that is essential for the protein binding and the negative regulatory function.

In the present study, the nuclear protein binding to the direct repeat of the human renin gene in a sequence-specific manner might be COUP-TFII itself. Chicken ovalbumin upstream promoter-transcription factors (COUP-TFs) are orphan members of the steroid/thyroid hormone receptor superfamily [Pereira et al., 2000], the official nomenclature of which are NR2F1 and NF2F2 for COUP-TFI and COUP-TFII, respectively [Giguere, 1999]. COUP-TFI was discovered as a transcription factor that bound a specific element, which regulates transcription of the ovalbumin gene [Wang et al., 1989]. Apolipoprotein regulating protein 1 (ARP-1) was cloned as a repressor for the apolipoprotein AI [Ladiaz and Karathanasis, 1991] and was proven to be identical with COUP-TFII, a homologue of COUP-TFI [Wang et al., 1991]. COUP-TFs are fundamentally regarded as negative regulators for a large number of genes, whereas they can also activate a growing number of gene promoters [Pereira et al., 2000]. COUP-TFs are thought to exist as homodimers and as heterodimers with RXR with consensus motifs essentially composed of AGGTCA core direct repeats. Consensus for ARP-1 (COUP-TFII) was first identified on the apolipoprotein AI gene promoter and characterized as AGGGGTCA–AGGGNTCA (TGANCCCT–NTGACCCCT) [Ladiaz and Karathanasis, 1991]. The human renin gene



**Fig. 5.** Normalized luciferase activity of different renin promoter/luciferase constructs transfected into chorio-decidual cells. Luciferase activity was normalized to co-transfected RSV-CAT activity and the values were expressed as relative percentage to that of pGL<sub>2</sub>582. Each value represents the result of three independent transfection experiments performed in triplicate flasks. Levels of significance were calculated by Student's *t*-

test. Groups that are significantly different from each other are indicated by asterisks (pGL<sub>2</sub>582 vs. pGL<sub>2</sub>582COUP-TFII-M1,  $P=0.28$ ; pGL<sub>2</sub>582COUP-TFII-M1 vs. pGL<sub>2</sub>582COUP-TFII-M2,  $P<0.0001$ ; pGL<sub>2</sub>582 vs. pGL<sub>2</sub>582COUP-TFII-M2,  $P<0.0001$ ). Boxes represent repetitive consensus. Closed boxes represent the mutated parts of the repetitive consensus sequences.

AGGGGTCAC–AGGGCCA (–259/–245) possesses a strong similarity with this motif. COUP-TFII is expressed in the salivary gland, atrium of the heart, mesonephros and kidney [Pereira et al., 1995]. Some important roles are suggested in the regulation of steroidogenesis in human adrenal cortex and its disorders [Suzuki et al., 2000; Shibata et al., 2001]. COUP-TFII null mutant mice die in utero due to defects in angiogenesis, vascular remodeling and heart development, indicating the important role for this sequence in the correct development of the vascular system [Pereira et al., 1999]. Considering these characteristics of COUP-TFII, the sequence-specific repression of the human renin gene via the COUP-TFII motif like site might play important roles in cardiovascular-renal development and regulation.

The murine *Ren-1<sup>α</sup>* homologous promoter site of the human COUP-TFII motif site (–259/–245) is thought to be located at about –700 bp from the transcription starting point [Burt et al., 1989]. The sequence of the corresponding site is AGAGGTTGCGGGCCCA and is considerably different from the human sequence AGGGGTCACAGGGCCA, especially with respect to the fact that no AGGTCA core direct repeat is found, so that the corresponding site does not seem to function as a *cis*-element for COUP-TFII. On the other hand, recent reports revealed the pivotal functions of nuclear transcription factors on murine renin gene promoter, consisting of LXR $\alpha$  [Tamura et al., 2000], retinoic acid [Shi et al., 2001], vitamin D3 [Li et al., 2002] and Ear2 [Liu et al., 2003]. The latter three ligands share the common *cis*-element containing AGGTCA motif at about –2,000 bp. It is possible that the direct repeat motif site (–259/–245) of the human renin gene that contains similar sequence of AGGTCA repeat is also involved in these other members of nuclear receptor superfamily and that competitive occupation of the site or competitive forming of homo- or hetero-dimers play some roles in the gene expression.

In conclusion, these findings suggest that the direct repeat motif site of the human renin gene functions as a negative regulatory element involved in a twofold suppression of transcription and that some corresponding nucleic receptors might play important roles in the human renin gene expression. Further elucidation would be necessary to clarify the role of the DR by the use of other human renin-producing

cells, Calu6 cells [Lang et al., 1995], and experiments with a mutated DR element within a longer promoter fragment.

#### ACKNOWLEDGMENTS

We are grateful to Mr. John S. Gelblum for a critical reading of the manuscript and to Miss Youko Hayashida for secretarial assistance.

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