Assignment of the gene encoding renin binding protein (*Renbp*) to rat chromosome Xq37 by in situ hybridization and radiation hybrid mapping

H. Zürcher,^{a,b} P. Van Vooren,^c J. Szpirer,^c C. Szpirer,^c and R. Kreutz^{a,b}

Departments of ^aClinical Pharmacology and ^bMedicine IV Nephrology, Benjamin Franklin Hospital, Freie Universität Berlin (Germany)

^cDepartment of Molecular Biology, Université Libre de Bruxelles (Belgium)

Rationale and significance

The renin-angiotensin system (RAS) plays a crucial role in the regulation of blood pressure (Laragh, 1995). Within the RAS, the physiological relevance of renin binding protein (RnBP) has been controversial for several years (Schmitz et al., 2000). Originally, RnBP was identified as a protein in the kidney that was capable of binding renin in renal homogenates giving rise to a complex designated high molecular weight renin (Takahashi et al., 1983). It was subsequently proposed that RnBP might act as an endogenous cellular renin inhibitor. Recent studies demonstrated that human RnBP is the enzyme N-acetyl-D-glucosamine (GlcNAc) 2-epimerase (Takahashi et al., 1999). Moreover, gene-targeting experiments in the mouse excluded a specific interaction between RnBP and renin in the kidney or in the circulating RAS of the mouse (Schmitz et al., 2000). So far, the gene encoding RnBP has been mapped on chromosome X in different mammals: Xq28 in human (van den Ouweland et al., 1994), X 29.53 cM in mouse (<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19703>) and X

in canine (Stoy et al., 1998). As mapping data for the rat homolog *Renbp* is still lacking, it is unclear whether this gene is located in blood pressure quantitative trait loci (QTL) termed *BP/SP-2* and *SS-X* on rat chromosome X. *BP/SP2* has been identified in an intercross between the stroke prone spontaneously hypertensive rat (SHRSP) and the normotensive Wistar-Kyoto (WKY) rat (Hilbert et al., 1991) and *SS-X* has been characterized in the Sabra hypertension-prone rat (Yagil et al., 1999). We therefore set out to test the chromosome location of *Renbp* by performing chromosomal mapping analysis in the rat using fluorescence in situ hybridization (FISH) and radiation hybrid (RH) mapping.

Materials and methods

Fluorescence in situ hybridization

A 1092-bp rat *Renbp* cDNA fragment spanning a region between position 140 and 1232 of the reported mRNA sequence (Inoue et al., 1991; GenBank accession no. D10233) was generated by reverse transcription PCR as reported (Kreutz et al., 1997) using the following oligonucleotides: 5'-CCTTGGCCGCGATGGCAGGTATATG-3' and 5'-GGGGCGGGCCC-AAGGCGTTGGAGTAG-3'. The FISH experiments were done as previously described (Laes et al., 1998).

Probe name: Renbp

Probe type: cDNA

Proof of authenticity: Amplifiable with specific primers and sequencing.

Gene reference: Inoue et al. (1991); GenBank accession no. D10233

Radiation hybrid mapping

50 ng of genomic DNA from the rat-hamster radiation hybrid panel (Research Genetics, <http://www.resgen.com>) were amplified with a set of primers specific for a segment of the rat *Renbp* gene in exon 5. PCR was performed on all 106 clones of the RH-panel (Steen et al., 1999; Watanabe et al., 1999) and analyzed by gel electrophoresis. The data vector obtained for *Renbp* was: 00001 00100 00000 00000 00101 00000 00000 00000 00001

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Corresponding author: Reinhold Kreutz, MD
Department of Clinical Pharmacology, Benjamin Franklin Hospital
Freie Universität Berlin, Hindenburgdamm 30, D-12200 Berlin (Germany)
telephone: +49-30-8445-2280; fax: +49-30-8445-4482
e-mail: kreutz@medizin.fu-berlin.de

Fig. 1. Regional localization of *Renbp* on rat chromosome X. Portion of a rat cell metaphase showing the signals generated by the probe at Xq37. The chromosomes were DAPI-counterstained and the banding and probe signal images were captured and treated with the ISIS imaging system (MetaSystems, Althussheim, Germany).

Fig. 2. RH-mapping of *Renbp*. The mapping position of *Renbp* is indicated by a thick horizontal bar, the corresponding microsatellite marker *DXWox3* is underlined. The thick vertical bars on the right indicate the QTL intervals of *BP/SP-2* (a QTL previously identified in a cross between SHRSP $\times$ WKY) and *SS-X* (a QTL previously identified in the Sabra rat model); and the dotted horizontal lines indicate the borders of the confidence intervals, respectively. Genetic distances in centiRay (cR) to the closest markers around *Renbp* are shown on the left (not drawn to scale).

10011 01001 01010 01100 00011 00100 01000 01010 01100 00000 00001 01100 0. The results of presence or absence of PCR amplification were entered into the web page of the Otsuka GEN Research Institute (<http://ratmap.ims.u-tokyo.ac.jp/>). The mapping position was determined using MultiMap at the same web page (Matise et al., 1994).

Primer names: Primer sequences:
Renbp-S: 5'-ACCGCCTGGCAAGAAATGTG-3'
Renbp-AS: 5'-CACAGTTCATTCATGGCCATGGTGTGA-3'
PCR conditions: 94 °C 15 s, 60 °C 60 s, 72 °C 60 s (30 cycles)

Proof of authenticity: Amplification with the primers generated a specific band at the expected size of 111 bp of *Renbp* exon 5, which was confirmed by sequencing.

Results

RH mapping with the vector from exon 5 and an additional independent vector obtained by PCR amplification of a sequence from intron 9 (data not shown) revealed linkage to both RNO1 (maximum lod score 9.0) and RNOX (maximum lod score 9.4). FISH analysis, however, showed that *Renbp* is located on chromosome X at Xq37 (Fig. 1). This result was confirmed by somatic cell hybrid analysis (data not shown). Chromosomal placement on the X chromosome by RH mapping confirmed the telomeric localization of *Renbp* close to marker *DXWox3* (Fig. 2). Placement for *Renbp* was next to *DXWox3*, 26.4 cR away from the closest marker *D19Mit1* (*D19Mit1* represents a marker previously localized on rat chromosome 19, but reassigned to chromosome X (Bihoreau et al., 1997) on one side and 28.4 cR away from flanking marker *DXRat22* on the other side. Construction of a comparative map integrating the previously reported confidence intervals for the placement of the blood pressure QTL *BP/SP-2* between markers *DXMgh5* and *DXMit4* (SHRSP $\times$ WKY intercross; Hilbert et al., 1991) and *SS-X* between markers *DXRat4* and *DXMgh10* (Sabra rat model, Yagil et al., 1999) revealed that *Renbp* falls outside the BP QTL regions on chromosome X (Fig. 2).

Mapping data for Renbp

Most precise location: Xq37

Number of cells examined: 13

Number of cells with specific signal: 1 (3), 2 (9), 3 (1) chromatids per cell.

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