Transcriptional regulator RBP-J regulates the number and plasticity of renin cells

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Departments of 1 Pediatrics and 2 Biology, School of Medicine and Graduate School of Arts and Sciences, University of Virginia, Charlottesville, Virginia; 3 Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, New York; and 4 Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan

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Castellanos Rivera RM, Monteaudo MC, Pentz ES, Glenn ST, Gross KW, Carretero O, Sequeira-Lopez MLS, Gomez RA. Transcriptional regulator RBP-J regulates the number and plasticity of renin cells. Physiol Genomics 43: 1021–1028, 2011. First published July 12, 2011; doi:10.1152/physiolgenomics.00061.2011.—Renin-expressing cells are crucial in the control of blood pressure and fluid-electrolyte homeostasis. Notch receptors convey cell-cell signals that may regulate the renin cell phenotype. Because the common downstream effector for all Notch receptors is the transcription factor RBP-J, we used a conditional knockout approach to delete RBP-J in cells of the renin lineage. The resultant RBP-J conditional knockout (cKO) mice displayed a severe reduction in the number of renin-positive juxtaglomerular apparatuses (JGA) and a reduction in the total number of renin positive cells per JGA and along the afferent arterioles. This reduction in renin protein was accompanied by a decrease in renin mRNA expression, decreased circulating renin, and low blood pressure. To investigate whether deletion of RBP-J altered the ability of mice to increase the number of renin cells normally elicited by a physiological threat, we treated RBP-J cKO mice with captopril and sodium depletion for 10 days. The resultant treated RBP-J cKO mice had a 65% reduction in renin mRNA levels (compared with treated controls) and were unable to increase circulating renin. Although these mice attempted to increase the number of renin cells, the cells were unusually thin and had few granules and barely detectable amounts of immunoreactive renin. As a consequence, the cells were incapable of fully adopting the endocrine phenotype of a renin cell. We conclude that RBP-J is required to maintain basal renin expression and the ability of smooth muscle cells along the kidney vasculature to regain the renin phenotype, a fundamental mechanism to preserve homeostasis.

juxtaglomerular cells; cell identity; homeostasis; recruitment; conditional knockout; recombination signal binding protein for immunoglobulin kappa J region

IN THE ADULT MAMMALIAN KIDNEY renin is synthesized, stored, and released by juxtaglomerular (JG) cells, a specialized group of myoepithelioid granulated cells located in the afferent arteriole at the entrance to the glomerulus (6, 34). We have shown that renin precursor cells originate from the metanephric mesenchyme and give rise to JG cells, arteriolar smooth muscle cells (SMCs), mesangial cells, and a subset of proximal tubular cells (30, 31). In adult animals, when homeostasis is threatened (such as by dehydration or hypotension) there is an increase in the number of renin-expressing cells along the renal arterioles and in the glomeruli and interstitium, resembling the fetal pattern, a phenomenon called recruitment (5, 7). The process does not involve cell replication and/or migration (2), but it occurs by retransformation of arteriolar SMCs and mesangial cells into renin-expressing cells (30). We suggested that the ability of adult cells to synthesize renin does not occur randomly in any cell type but depends instead on the cell’s lineage. In other words, only cells that previously expressed renin have the memory and capability to re-express renin when homeostasis is challenged (30).

The events underlying the acquisition, maintenance, and reacquisition of the renin phenotype are intriguing. Different mechanisms seem to control the phenotype of renin-expressing cells (3, 14, 15, 17, 26, 29, 32). Interactions of renin cells with other cell types are likely to be crucial to promote, maintain, and regulate the expression of renin. In this regard, the Notch signaling pathway is very attractive to investigate because cell-cell signals conveyed by these receptors may be crucial in maintaining the renin cell phenotype. Interestingly, in vitro studies have shown that the DNA-binding protein RBP-J (Recombination signal Binding Protein for immunoglobulin kappa J region; CBF1 in mammals, Suppressor of Hairless in Drosophila, Lag-1 in Caenorhabditis elegans) within the renin promoter acts as a transcriptional repressor; however, the intracellular Notch 1 receptor can counteract this repressor function and activate the renin promoter in cooperation with the transcription factors HOXD10-PBX1b-PREP1 (19).

Canonical Notch cell-to-cell signaling plays a prominent role during development, imparting cell fate decisions in many tissues, and is involved in vasculogenesis and organogenesis in multicellular organisms (1a, 12, 28, 33). In vertebrates, Notch receptors belong to a receptor superfamily containing Notch 1 through 4. Notch signaling is activated by membrane anchored ligands on juxtaposed cells: the ligands are Delta-like and Serrate/Lag family members. Upon interaction with one of these ligands, two consecutive proteolytic cleavages liberate the intracellular Notch receptor domain (NIC), which translocates to the nucleus (1, 16). Once in the nucleus, NIC binds to RBP-J. NIC association replaces co-repressors from RBP-J and upregulates transcription (1). Thus, RBP-J is the main transcriptional effector of all Notch signaling. The specific role of the Notch pathway in the acquisition, maintenance, and plasticity of the renin phenotype has not been explored. In the present study, we used a conditional knockout approach to delete RBP-J in cells of the renin lineage to determine its role in the acquisition of renin expression during development and its subsequent maintenance during adult life. Furthermore, we investigated whether RBP-J is crucial in the plasticity of cells to respond to a homeostatic threat with a re-enactment of the
METHODS

Generation of mice with conditional deletion of RBP-J in renin cells. To study the role of RBP-J in renin cells we crossed Ren1\textsuperscript{dcre/+} mice (30) to RBP-J floxed (RBP-J\textsuperscript{fl/fl}) mice (kind gift of Dr. Tasuku Honjo) (10), which contain LoxP sites that flank exons 6–7 of the RBP-J gene. Cre-mediated recombination deletes exons 6 and 7 that code for the DNA binding domain of the RBP-J protein. To generate the conditional knockout (cKO) study mice (RBP-J\textsuperscript{fl/fl};Ren1\textsuperscript{dcre/+}), mice heterozygous for both RBP-J\textsuperscript{+/+} and Ren1\textsuperscript{dcre/+} were crossed to generate homozygous deletion of RBP-J. The control mice designated as RBP-J\textsuperscript{+/+} are heterozygous for Ren1\textsuperscript{dcre/+}. All procedures were performed following the National Institutes of Health guide for care and use of laboratory animals and were approved by the Animal Care and Use Committee of the University of Virginia.

Generation of Ren1\textsuperscript{Lys-P} mice with conditional deletion of RBP-J in renin cells. To label renin cells with deletion of RBP-J we bred RBP-J\textsuperscript{fl/fl} mice with Ren1\textsuperscript{Lys-P};RBP-J\textsuperscript{cKO} mice previously described (26). To generate the study animals (Ren1\textsuperscript{Lys-P};RBP-J\textsuperscript{cKO} RBP-J\textsuperscript{fl/fl};Ren1\textsuperscript{dcre/+}) mice were bred with RBP-J\textsuperscript{fl/fl};Ren1\textsuperscript{Lys-P} mice.

Cell isolation. Ren1\textsuperscript{Lys-P};RBP-J\textsuperscript{cKO} Ren1\textsuperscript{Lys-P};RBP-J\textsuperscript{fl/fl} mice were anesthetized with tribromoethanol as previously described (30) to minimize bleeding. The juxtaglomerular apparatus (JGA) index was calculated as the number of renin-positive JGA total number of glomeruli and expressed as a percentage. To determine the number of renin-expressing cells per section we counted the number of renin-positive cells in each JGA plus the number of renin-positive cells along the arterioles with visible glomeruli attached to them.

RNA isolation and qRTPCR analysis. Whole kidneys were cut sagitally and stored in RNA later (Ambion, Austin, TX) overnight at 4°C and then processed for RNA extraction or stored at −20°C until used. RNA extraction and cDNA generation by reverse transcription and quantitative real-time PCR using SYBR Green (Invitrogen, Eugene, OR) were performed as previously described (8). Renin mRNA expression was normalized to GAPDH expression. The changes in expression were determined by the ΔΔCT method and are reported as relative expression compared with control mice.

Analyses of plasma renin concentration and blood pressure measurement. Plasma renin concentration was determined as previously described (24, 25). Blood pressure (BP) was measured in conscious mice by a computerized tail-cuff method (CODA; Kent Scientific, Torrington, CT). Mice were acclimatized for 2 days, and the measurements taken on the third day were analyzed.

Recruitment study. To stimulate expression of renin, 2.5 mo old mice were treated with low-sodium diet (0.05%, Harlan, Madison, WI) plus captopril added to the drinking water (0.5 g/l) for 10 days. At the end of the treatment period, kidneys were harvested and processed for immunohistochemistry and histological analysis, and blood was collected to assess plasma renin concentration.

Statistics. Statistical significance was assessed by t-test and Mann-Whitney rank sum test using Sigma Plot 11.0.

Table 1. Deletion of RBP-J results in a significant decrease in the number of renin-expressing cells and renin mRNA levels in the kidney

<table>
<thead>
<tr>
<th>Denominations</th>
<th>Genotype</th>
<th>Age, mo</th>
<th>n</th>
<th>JGA index</th>
<th>Renin Cells in the JGA + aa/per Section</th>
<th>Relative Expression of Renin mRNA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBP-J\textsuperscript{+/+}</td>
<td>RBP-J\textsuperscript{+/+};Ren1\textsuperscript{dcre/+}</td>
<td>1</td>
<td>3</td>
<td>32.8 ± 0.9</td>
<td>264 ± 43</td>
<td>100</td>
</tr>
<tr>
<td>RBP-J cKO</td>
<td>RBP-J\textsuperscript{fl/fl};Ren1\textsuperscript{dcre/+}</td>
<td>1</td>
<td>7</td>
<td>9.6 ± 2.6†</td>
<td>53 ± 17.7†</td>
<td>34.3 ± 6*</td>
</tr>
<tr>
<td>RBP-J\textsuperscript{+/+}</td>
<td>RBP-J\textsuperscript{+/+};Ren1\textsuperscript{dcre/+}</td>
<td>4</td>
<td>5</td>
<td>44.2 ± 3.6</td>
<td>221 ± 42.6</td>
<td>100</td>
</tr>
<tr>
<td>RBP-J cKO</td>
<td>RBP-J\textsuperscript{fl/fl};Ren1\textsuperscript{dcre/+}</td>
<td>4</td>
<td>11</td>
<td>16.1 ± 2.1†</td>
<td>54 ± 10.4†</td>
<td>44.2 ± 12*</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, Number of mice. JGA, juxtaglomerular apparatus; aa, along the afferent arteriole. †P < 0.0005 and *P < 0.02 when compared with control RBP-J\textsuperscript{+/+} mice.
RESULTS

Deletion of RBP-J results in a decreased number of renin cells. As illustrated in Table 1 and Fig. 1, RBP-J cKO mice at 1 and 4 mo of age have a significant decrease in the number of renin-positive JGAs when corrected for the total number of glomeruli (JGA index). At 4 mo of age, the percentage of glomeruli containing renin-positive JGAs in control mice was 44.2 ± 3.6, whereas in the RBP-J cKO mice the JGA index was decreased to 16.1 ± 2.1 (Table 1). In addition, RBP-J cKO mice had a severe decrease in the total number of renin-positive cells in the JGAs and along the afferent arterioles (control RBP-J+/+ mice: 221 ± 42.6 cells, RBP-J cKO mice: 54 ± 10.4 cells; Table 1). Similar findings were observed in 1 mo old mice (Table 1). The decrease in the number of cells expressing renin in the RBP-J cKO animals both at 4 and 1 mo of age was accompanied by a significant decrease in renin mRNA expression compared with controls. At 1 mo of age, kidneys from homozygous mutant mice had a relative renin mRNA expression of 34% with respect to control RBP-J+/+ mice (Table 1). At 4 mo of age, mutant mice showed a relative renin mRNA expression of 44% compared with controls (Table 1). Although the number of cells expressing renin was significantly diminished in 1 and 4 mo old RBP-J cKO mice, the number of renin-expressing cells was not affected early in ontogeny (not shown), suggesting that the decreased number of renin-expressing cells in the adult is not likely due to a decreased endowment of renin cell precursors. In addition, the renal size and morphology (including the renal vasculature) were not altered in the RBP-J cKO mice (not shown).

RBP-J is necessary to maintain the number of YFP-positive JG cells. To further investigate whether the decrease in renin mRNA was due to a decrease in the number of cells expressing the renin gene, we crossed our RBP-J cKO mice with mice that express yellow fluorescent protein (YFP) under the control of the Ren1c promoter (Ren1c-YFP). Expression of YFP therefore indicates cells actually transcribing renin mRNA. Using fluorescent activated cell sorting we quantified the number of YFP-positive cells in Ren1c-YFP;RBP-J+/+ and Ren1c-YFP;RBP-J cKO mice. Fig. 2. Deletion of RBP-J reduced the number of yellow fluorescent protein (YFP)-positive cells in Ren1c-YFP mice. The number of YFP-positive cells in Ren1c-YFP;RBP-J+/+ and Ren1c-YFP;RBP-J cKO mice was quantified by fluorescent activated cell sorting (FACS). The cKO kidneys had a reduced number (#) of YFP+ cells. The number of YFP-positive cells was corrected per 10^7 sorted cells from kidney cortices. Values are means ± SE, ***p < 0.0001.

Fig. 3. Systolic blood pressure (BP) measurement in control and RBP-J cKO mice. Values are means ± SE, P = 0.355.
mice. At 2 mo of age, deletion of RBP-J significantly reduced the number of YFP-positive cells (Fig. 2). Ren1c-YFP;RBP-J+/+ mice had 0.02% of YFP-positive cells per two kidneys, whereas the RBP-J cKO mice had 0.003% of YFP cells ($P < 0.0001$).

Effect of RBP-J deletion on arterial BP and circulating renin. Systolic BP in control 2.5 mo old mice was $105 \pm 8.8$ mmHg ($n = 8$) and in RBP-J cKO mice was $97 \pm 3.6$ mmHg ($n = 11$) (Fig. 3). Although RBP-J cKO mice had a slight tendency to reduced BP, due to the inherent variability of BP measurements the difference compared with control mice was not statistically significant ($P = 0.355$). We also observed that control mice had wider variation in their systolic BP (ranging from 76 to 153 mmHg) when compared with RBP-J cKO animals. On the other hand, RBP-J cKO mice had a limited variation in their systolic BP values (range: 83 to 116 mmHg), indicating that deletion of RBP-J limited the physiological BP variability. Under basal conditions, plasma renin concentration (PRC, ANG I $\mu$g·ml$^{-1}$·h$^{-1}$) was 20% lower in the RBP-J cKO (2.0 ± 0.7, $n = 24$) mice compared with control mice (2.5 ± 0.8, $n = 27$). This difference was not statistically significant.

Deletion of RBP-J in the renin cells impairs the recruitment response. To investigate whether deletion of RBP-J altered the ability of mice to elicit a recruitment response (an increase in the number of renin cells under physiological threat), we treated RBP-J cKO mice and controls (RBP-J+/+) with captopril and sodium depletion for 10 days. This treatment is known to elicit recruitment of renin cells in intact animals. Treated RBP-J cKO mice displayed an average relative expression of renin mRNA of 35% (range: 18–58%) when compared with control mice (Fig. 4C). As illustrated in Fig. 4, A and B, immunostaining for renin shows that in contrast to control mice, RBP-J cKO mice were unable to increase the number of renin-positive cells and to adopt the renin cell phenotype to the same extent as in the controls after treatment (Fig. 4B). RBP-J cKO mice had fewer and thinner renin-positive cells along the afferent arterioles (arrows) compared with control mice. Renin expression is shown in brown. C: renin mRNA in captopril-treated RBP-J cKO mice. Black bar, control ($n = 5$); gray bars, individual RBP-J cKO mice. The error bars indicate triplicate determinations of the same animal. Values are means ± SE, $P = 0.0006$. 

![Fig. 4](http://physiolgenomics.physiology.org/)
cells expressing renin with the resultant lack of elevation in circulating renin.

Interestingly, although the mean JGA index in treated RBP-J cKO mice was significantly reduced compared with control mice (RBP-J+/+; 55 ± 4, n = 5 and RBP-J cKO mice: 45 ± 2.2, n = 6; P < 0.02), the difference of only 10% indicated an attempt at compensation by the RBP-J cKO mice. However, the intensity of immunostaining was markedly different between the two genotypes. To illustrate this point, we quantified and scored the intensity of the staining of renin-positive JGAs from 1, low intensity to 4, high intensity (Fig. 6B). As shown in Fig. 6, treated RBP-J cKO mice had a significant decrease in the proportion of JGAs with the highest intensity scores (score 4) when compared with control mice (control RBP-J+/+ mice 49%, RBP-J cKO mice 26%; P < 0.02). This finding suggested that although the mutant renin cells attempt to compensate, most of the cells have a decreased amount of renin per cell, indicating that intracellular renin stores were depleted. To corroborate this, toluidine blue staining to detect renin granules showed that RBP-J cKO mice had a marked decrease in the number of granules per cell (Fig. 7). These results demonstrate the inability of individual renin cells to mount the appropriate physiological renin expression response when RBP-J is absent.

DISCUSSION

Role of RBP-J in renin-expressing cells. In the present study, we show that RBP-J is crucial for the maintenance and plasticity of the renin cell phenotype. The severe reduction in the number of JG cells in RBP-J cKO mice is accompanied by: 1) decreased renin mRNA levels, 2) decreased circulating renin, 3) lower blood pressure, and 4) marked impairment in
the ability of SMCs to re-express renin in response to a homeostatic threat.

The severe decrease in the number of JG cells in RBP-J cKO mice indicates that RBP-J is indispensable for the maintenance of the renin phenotype in the basal state. Significant progress has been made regarding some of the pathways that control renin expression (4, 8, 9, 13, 14, 17, 18, 20–23, 26, 29, 32); however, the role of RBP-J in maintaining renin expression in vivo has not been previously reported. Here, we show in vivo that RBP-J is an important factor in the expression of renin in unstressed adult mice. In vitro studies have shown that RBP-J can function either as a repressor or activator of transcription of the rat renin promoter; mutation of the RBP-J binding site in the renin gene resulted in an increase in renin promoter activity in vitro identifying the site as a repressor, whereas the presence of the intracellular receptors (Notch 1 and Notch 3) enhanced renin activation elucidating its activator ability (19). The present study suggests that RBP-J functions in vivo to maintain the number of renin-expressing cells in the kidney. The molecular mechanism and associated partners responsible for this effect remain to be determined.

The reason for the decrease in the number of renin-expressing cells is intriguing. It is unlikely that the deletion of RBP-J resulted in a diminished endowment of renin precursor cells because in our studies newborn mice do not show the striking decrease in the number of JG cells clearly found at one mo of age (not shown). It is possible that an early mosaic deletion of RBP-J may explain the lack of a noticeable phenotype at an early age. As the physiological demands of life and activity of the renin promoter accumulate, successive deletions occur, eventually affecting the phenotype, that become clearly manifest in the adult. Given that RBP-J has a binding site in the renin promoter, it is likely that the decreased number of renin-expressing cells is due to a direct effect of RBP-J on the renin gene. However, in silico analysis of the genes most prominently expressed in the renin cell (unpublished) indicates that >100 genes have predicted RBP-J binding sites in their promoters and could have potentially contributed to the observed findings. It could be argued that renin cells may undergo apoptosis and/or necrosis, diminishing the number of JG cells in the adult kidney. However, this possibility is unlikely because the number of apoptotic cells in the newborn and adult RBP-J cKO mice were not different from control animals (not shown). The most likely possibility is that lack of RBP-J results in decreased renin gene expression due to diminished RBP-J action on the renin promoter. Alternatively, deficiency of RBP-J could have resulted in a phenotypic switch of the renin cell altering its fate to another cell type with the attendant downregulation of renin expression. To answer this question, we are currently performing lineage-tracing studies in RBP-J cKO animals.

One of the most striking effects of the RBP-J deletion is the inability of mutant mice to elicit an appropriate recruitment response when their homeostasis is threatened. In response to sodium depletion and captopril treatment RBP-J cKO mice were unable to increase circulating renin. The lack of increase in circulating renin was due to the combination of several factors: first, in response to the homeostatic threat, RBP-J cKO mice were unable to increase the number of cells expressing renin, which normally occurs by retransforming vascular SMCs along the afferent arterioles into renin-expressing cells (5, 30, 35, 36). Thus, RBP-J may play a role in the reacquisition of the endocrine phenotype of arteriolar SMCs.

Although the JGA index for the treated RBP-J cKO mice was significantly different compared with treated control mice, this difference was only 10%. This, however, does not represent how markedly different the mutant mice were compared with the treated control mice. In fact, we observed that the remaining cells that did attempt to re-express renin along the kidney vasculature displayed striking morphological features: not only were they fewer but they were also very thin and contained barely detectable amounts of renin protein and very few granules. Although RBP-J cKO mice showed an inability to mount a satisfactory physiological recruitment response the fact that the JGA index increased (although not to control levels) suggests that the remaining renin cells attempted such compensation. However, the individual cells attempting such response had minimal amounts of renin and fewer granules indicating that the synthetic capacity of these cells was depleted. Thus, the inability to increase circulating renin was not only due to a
limited number of renin-expressing cells but also due to the inability of individual cells to produce enough renin when attempting a compensatory homeostatic response. We suspect that lack of RBP-J in renin cells impairs the synthetic machinery of renin cells, a defect that becomes more evident under physiological stress. As a consequence, the mutant cells are incapable of fully adopting the renin phenotype and are limited to the phenotype of a smooth muscle cell. Interestingly, when renin cells are placed in culture and are deprived of their normal intercellular interactions with other cell types, they stop synthesizing renin and adopt the phenotypic characteristics of smooth muscle and/or fibroblast cells. Thus, lack of the transducing mechanism conveying cell-cell interactions normally provided by RBP-J impairs the plasticity of cells to switch phenotype when confronted with a homeostatic challenge. Further studies will be conducted to determine the intracellular events whereby RBP-J controls the phenotypic endocrine switch of the renin cell.

In summary, we generated mice with deletion of RBP-J in cells of the renin lineage and showed in vivo that RBP-J is required to maintain renin expression and perhaps more importantly, RBP-J is necessary to maintain the memory of SMCs along the kidney vasculature to regain the renin phenotype, a fundamental mechanism to preserve homeostasis.

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GRANTS

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DISCLOSURES

No conflicts of interest (financial or otherwise) are declared by the authors.

REFERENCES


