CBP and p300 are essential for renin cell identity and morphological integrity of the kidney

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Gomez RA, Pentz ES, Jin X, Cordaillat M, Sequeira Lopez ML. CBP and p300 are essential for renin cell identity and morphological integrity of the kidney. Am J Physiol Heart Circ Physiol 296: H1255–H1262, 2009. First published February 27, 2009; doi:10.1152/ajpheart.01266.2008.—The mechanisms that govern the identity of renin cells are not well understood. We and others have identified cAMP as an important pathway in the regulation of renin synthesis and release. Recently, experiments in cells from the renin lineage led us to propose that acquisition and maintenance of renin cell identity are mediated by cAMP and histone acetylation at the cAMP responsive element (CRE) of the renin gene. Ultimately, the transcriptional effects of cAMP depend on binding of the appropriate transcription factors to CRE. It has been suggested that access of transcription factors to this region of the promoter is facilitated by the coactivators CREB-binding protein (CBP) and p300, which possess histone acetyltransferase activity and may be, in turn, responsible for the remodeling of chromatin underlying expression of the renin gene. We hypothesized that CBP and p300 are essential for renin cell identity and morphological integrity of the kidney. Because mice homozygous for deletion of CBP or p300 die before kidney organogenesis begins, no data on kidney or juxtaglomerular cell development in these mice are available. Therefore, to define the role of these histone acetyltransferases in renin cell identity in vivo, we used a conditional deletion approach, in which floxed CBP and p300 mice were crossed with mice expressing cre recombinase in renin cells. Results show that the histone acetyltransferases CBP and p300 are necessary for maintenance of renin cell identity and structural integrity of the kidney.

cAMP; chromatin; homeostasis; juxtaglomerular cells

THE RENIN-ANGIOTENSIN SYSTEM is crucial in the regulation of blood pressure, fluid electrolyte homeostasis, and kidney growth (10, 16). In the adult mammalian kidney, renin is synthesized and released by the juxtaglomerular (JG) cells, a small group of granulated myoepithelioid cells located in the afferent arteriole at the entrance to the glomerulus. The availability of renin and, therefore, the control of homeostasis are achieved in great part by regulation of the number of cells that synthesize and secrete renin, rather than by upregulation of renin production in cells already committed to its synthesis (8, 9, 17). When homeostasis is threatened, cells that were capable of producing renin in early life, along the kidney vasculature, glomeruli, and interstitium, dedifferentiate, resume synthesis of renin, and reacquire the typical granulated endocrine phenotype (29). Thus the number of cells that synthesize renin is increased by dedifferentiation of smooth muscle cells that have retained the memory to reacquire the renin cell identity and, in so doing, reestablish blood pressure and fluid electrolyte homeostasis. Thus the ability to elicit this cellular memory and regain identity is fundamental to the survival of the organism when it is presented with physiological and pathological challenges. The mechanisms that govern the identity of renin cells are not well understood. We and others have shown that cAMP is a crucial pathway in the regulation of renin synthesis and release (3, 6, 7, 18, 23, 24, 27, 31). More recently, we demonstrated that the memory to reacquire the renin phenotype is maintained in long-term culture and that the identity of the renin cell is mediated by cAMP and histone acetylation at the CREB-responsive element (CRE) of the renin gene (27).

These experiments in vitro indicated that the cAMP pathway is a major regulatory pathway for the acquisition and maintenance of JG cell identity. However, the molecular mechanisms whereby cAMP may exert its effects on renin expression in vivo are not known. The transcriptional effects of cAMP are ultimately mediated by binding of the CRE-binding protein (CREB) to the CRE in the renin promoter. It has been suggested that access to this region of the promoter is facilitated by the coactivators CREB-binding protein (CBP) and p300 (28). CBP was originally identified as a coactivator of CREB. CBP acts as a bridging factor between CREB and the general transcription factor TFIIIB (4, 19). The CBP gene family contains one other member, p300. CBP and p300 bind to the phosphorylated form of CREB (2, 20). Recruitment of the transcriptional coactivators p300 and CBP may facilitate access of transcription factors to gene promoters and activate gene transcription. In addition to functioning as bridging molecules between transcription activators and the general transcriptional machinery, CBP and p300 have histone acetyltransferase (HAT) activity and very likely play a role in chromatin remodeling (12, 22, 32). Because acquisition of renin cell identity mediated by cAMP involves acetylation of histone H4 at the CRE of the renin promoter, we speculated that the HATs CBP and p300 are required for expression of the renin gene and maintenance of renin cell identity in vivo as well as in vitro. Because mice homozygous for deletion of CBP or p300 die before kidney organogenesis begins (approximately embryonic day 11), there are no data on kidney or JG cell development in these mice (34, 35). Fortunately, generation of "floxed" CBP and p300 mice has permitted the conditional deletion and analysis of the roles of these proteins in adult tissues (13, 15, 36).

Therefore, to define the role of these HATs in renin cell identity, we used a conditional deletion approach in which floxed CBP and p300 animals were crossed with mice expressing cre recombinase (cre) in renin cells. Because renin cells are precursors for other cells in the nephron and renin is important in the regulation of kidney growth and development (29), we
also examined whether deletion of CBP and/or p300 affected kidney morphology. Results show that the HATs CBP and p300 are crucial in the maintenance of JG cells and the structural integrity of the kidney.

**MATERIAL AND METHODS**

*Generation of mice.* To delete the coactivators CBP and p300 in renin cells, we used three strains of animals: our Ren-cre mice (29), which express cre in renin cells, and mice with floxed alleles of the coactivators CBP [CBPfl/fl (13)] and p300 [p300flox (15)]. For deletion of CBP alone or p300 alone, homozygous floxed mice were bred to homozygous Ren-cre mice and the resulting double heterozygotes to produce the study animals. For deletion of CBP and p300 simultaneously, mice homozygous for CBPfllox and Ren-cre were bred to mice homozygous for p300flox (CBPfl/fl;p300flox/cre × CBPfl/fl;p300flox/cre) to produce animals heterozygous for CBPfllox, p300flox, and Ren-cre. Triple heterozygotes were bred to one another (CBPfl/fl;p300flox/cre × CBPfl/fl;p300flox/cre) to produce the study animals. The study animals represented numerous combinations of wild-type (WT) and floxed alleles of CBP and p300, as well as different dosages of Ren-cre. These mice have a mixed C57Bl/6 and 129/Sv genetic background. The renin gene is located on chromosome 1, and depending on the strain from which the mice are derived, they can have either one renin gene (Ren1c, as in the C57Bl/6 strain) or two renin genes (Ren2 and Ren1c) adjacent to one another (as in the 129/Sv strain). Thus any mice carrying a Ren-cre allele also have a Ren2 allele, since the targeting to produce this mouse was done in embryonic stem cells from the 129/Sv strain, which contains Ren1c and Ren2 (29). The mice in these studies each have two intact renin genes. The identity of the two renin genes in the mice of each genotype is shown in Table 1.

Standard PCR for genotyping was performed in an Eppendorf thermocycler using Taq polymerase (Promega, Madison, WI). The primers used to detect the deletion of CBP in genomic DNA (see Fig. 2A) are 5’ GCC GCT CCA AAT GGT GCA CT (forward) and 5’ GCT CTT GGA CGT TGG CTC ACC (reverse).

The study animals (2–2.5 mo of age) were weighed and then anesthetized with tribromoethanol (300 mg/kg) as previously described (29), and their kidneys were removed under anesthesia. Kidneys were weighed and preserved in RNA later solution (Ambion, Austin, TX) for RNA extraction or fixed for immunohistochemistry.

Housing and experimental use of the mice conformed to the “Guiding Principles in the Care and Use of Animals” approved by the Council of the American Physiological Society and with federal laws and regulations. All protocols have been reviewed and approved by the Animal Care and Use Committee of the University of Virginia. The University of Virginia is an Association for Assessment and Accreditation of Laboratory Animal Care-accredited institution.

**RNA extraction and RT-PCR analysis.** Kidneys were homogenized in Tri-Reagent (Molecular Research Center, Cincinnati, OH), and total RNA was extracted according to the manufacturer’s directions. Contaminating DNA was removed using the DNA-free kit (Ambion). The cDNA was prepared from 2 µg of RNA with use of Maloney’s murine leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY) and an oligo(dT)15 primer according to the manufacturer’s directions. Quantitative real-time PCR using SYBR Green I (Invitrogen Molecular Probes, Eugene, OR) was performed in a DNA Engine Opticon 2 system thermocycler (M. J. Research, Waltham, MA). The following primers were used: renin [5’ACA GTA TCC CAA CAG GAG AGA ACA AG (forward) and 5’GCA CCC ACC ACC CAG ACA (reverse)] and GAPDH [5’AAC TTT GGC ATT GTG GAA GGG CTC (forward) and 5’ACC AGT GGA TGC AGG GAT GAT GTT (reverse)].

**Histological analysis and immunostaining.** Kidneys were fixed in Bouin’s solution and embedded in paraffin. Sections were cut and stained with hematoxylin-eosin, periodic-acid Schiff, and Masson’s trichrome for morphological assessment or processed for immunocytochemistry and examined by light microscopy. Immunocytochemistry for renin and α-smooth muscle actin (SMA) was performed as we previously described (30) using well-characterized and highly specific antibodies and a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). For identification of renin-expressing cells, a polyclonal goat anti-renin antibody (1:10,000 dilution; gift of Dr. T. Inagami) or a rabbit anti-mouse renin antibody (1:500 dilution) with similar specificity was used. For identification of smooth muscle cells, a monoclonal anti-α-SMA-specific antibody (isotype IgG2a, dilution 1:10,000; clone 1A4, Sigma, St. Louis, MO) was used. Immunostaining for CBP was performed using a rabbit polyclonal antibody (1:1,000 dilution; catalog no. ab28382, Abcam, Cambridge, MA).

The total number of JG areas stained positive for renin were counted in each kidney section and expressed as a percentage of the total number of glomeruli per section. In addition, the appearance and number of individual renin-positive cells within the JG areas were examined.

### RESULTS

**Deletion of CBP/p300 in renin cells results in renin cell loss.** To determine whether CBP and/or p300 are necessary for the maintenance of renin cell identity, we crossed floxed CBP and p300 mice with our Ren-cre mice to delete CBP and/or p300 in vivo in renin cells. We deleted each of these genes one at a time and then in various combinations. From the triple-heterozygous mating we obtained 111 animals. All the 27 possible genotypes were represented at the expected Mendelian frequencies, indicating that the viability of even the mice lacking CBP and p300 in the renin cell lineage was not affected.

Deletion of CBP and p300 did not affect somatic growth (not shown), whereas it had a marked effect on kidney growth (Fig. 1A). This is clearly illustrated by the significant reduction in kidney weight-to-body weight ratio correlated negatively with the number of copies of HAT genes removed by the recombinase (Fig. 1B).

Figure 2 shows the results for the homozygous deletion of CBP (with p300 kept intact) in JG cells. PCR of kidney cortex DNA shows the CBP deletion band in heterozygous and homozygous animals in the presence of Ren-cre (Fig. 2A). [Note that the “flox” band in the homozygous sample remains because of the presence in the cortex of cell types that are not of the renin cell lineage, and, consequently, CBPfllox is not deleted.] Immunostaining for CBP in WT kidney shows CBP (brown nuclei) in nearly all cells (Fig. 2B). As expected, in CBPfl/fl;Rencre/+ kidney, CBP is absent from the nuclei of JG cells (Fig. 2C). Contrary to other kidney structures, such as tubules in which the presence of CBP is obvious, CBP was removed by cre recombinase specifically in JG cells and arterioles (Fig. 2C). Immunostaining for renin in consecutive sections of CBPfl/fl;Rencre/+ kidneys shows no apparent alter-

### Table 1. Genotype designations of study animals

<table>
<thead>
<tr>
<th>Genotype (CBPfl/fl;p300flox/cre switch)</th>
<th>No. of Ren Genes</th>
<th>Identity of Ren Genes</th>
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<tr>
<td>CBPfl/fl;p300flox/cre × Rencre/+</td>
<td>2</td>
<td>Ren1c</td>
</tr>
<tr>
<td>CBPfl/fl;p300flox/cre × Rencre/r+</td>
<td>2</td>
<td>Ren2, Ren1c</td>
</tr>
<tr>
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<td>Ren2, Ren1c</td>
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<tr>
<td>CBPfl/fl;p300flox/cre × Rencre/cre</td>
<td>2</td>
<td>Ren2, Ren1c</td>
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ation in the distribution of renin in JG cells (Fig. 2C), and RT-PCR for renin confirmed that the levels of renin mRNA in the kidney cortex were not changed by the homozygous deletion of CBP in renin cells (not shown). Similar results were obtained when p300 was deleted, leaving its counterpart, the CBP gene, intact (see supplemental Fig. S1 in the online version of this article). These initial results suggested that CBP or p300 was not required for renin expression or, more likely, that CBP and p300 can compensate for one another when one of them is missing. To address this possibility, we generated mice in which both genes were deleted specifically in JG cells.

Figure 3 shows the results of renin immunocytochemistry and quantitative RT-PCR for renin mRNA in mice in which CBP and p300 genes were deleted simultaneously. As expected, in mice in which both genes were floxed but cre recombinase was not expressed (CBP<sup>fl/fl</sup>·p300<sup>fl/fl</sup>·Ren<sup>cre/+</sup>), there were no alterations in the distribution of renin immunostaining or kidney structure (Fig. 3A). The distribution of renin and the structure of the kidneys were indistinguishable between these animals and WT animals (not shown). Thus no deleterious effects were related to the floxed alleles, and these mice are shown for comparisons. In addition, deletion of two copies of p300 and one copy of CBP (CBP<sup>+/+</sup>·p300<sup>0/0</sup>·Ren<sup>cre/−</sup>) caused no abnormalities in renin distribution or kidney structure (Fig. 3B). Similar results were obtained when two copies of CBP and one copy of p300 (CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/−</sup>) were deleted (not shown), suggesting that one copy of either HAT is sufficient to maintain renin expression and specification of JG cells. Contrary to these findings, in double-homozygous (CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/−</sup>) mice, only an occasional renin-positive cell was present in each slide (Fig. 3C). Only 2% of JG areas were positive for renin in these animals. In addition, the number of renin-positive cells per JG area was reduced to 1.2 cells per JG area, whereas in the normal animals there are usually 4 cells per JG area. The few positive cells were not plump, as JG cells usually are, but were elongated and remarkably thin. The absence of renin cells was accompanied by a series of kidney abnormalities (see below). In addition, if homozygous deletion of the Ren<sup>1d</sup> gene was superimposed onto the four-copy deletion of both HATs (triple-homozygous mice, CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/cre</sup>), not only did renin cells disappear (renin-positive JG areas dropped to 0%), but the kidney abnormalities were significantly accentuated (Fig. 3D), as described below.

Quantitative RT-PCR for renin mRNA showed that homozygous deletion of both CBP and p300 resulted in a marked reduction in renin mRNA in CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/+</sup> kidneys and was further diminished in CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/cre</sup> kidneys (Fig. 3F).

Deletion of CBP/p300 in renin cells leads to kidney abnormalities. On gross inspection, the kidneys of double- and triple-homozygous mice hard to the cut and smaller than controls and had a fine granular and irregular surface with areas of cortical depressions that were subsequently found to correspond to bands of corticomedullary fibrosis in areas normally occupied by vascular structures.

As shown in Fig. 4, combined homozygous deletion of CBP and p300 (CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/−</sup>) caused significant alterations in kidney structure, including irregular kidney contour, disorganized corticomedullary junction, and cortical and medullary cysts (Fig. 4C). High-magnification views showed marked alterations in every compartment, including vascular, glomerular, and tubular structures (Fig. 5; also see supplemental Figs. S2–S4). A more severe phenotype was observed with the additional removal of the Ren<sup>1d</sup> gene in the triple-homozygous (CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/cre</sup>) group (Fig. 4D). These kidneys resembled the sponge kidneys, with numerous cysts throughout, particularly in the medulla and corticomedullary regions, which contained numerous glomerular and tubular cysts (Fig. 4D). Contrary to these findings, no abnormalities were found in control (CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>+/+</sup>) animals. In addition, no alterations in renin distribution or kidney architecture were found in CBP<sup>0/0</sup>·p300<sup>0/0</sup>·Ren<sup>cre/−</sup> mice, suggesting that one copy of either HAT is sufficient to maintain normal renin cell distribution and kidney structure (Fig. 4B).

Staining with Masson’s trichrome revealed multiple fibrotic areas in kidneys of double-homozygous mice (Fig. 5B). The areas of fibrosis were more pronounced in the kidneys of triple-homozygous animals (Fig. 5C). The areas of fibrosis were prominent in the interstitium, surrounding dilated tubules and glomeruli. These areas of fibrosis corresponded to sites where blood vessels traverse the kidney and were interspersed with areas that were partially or totally spared from damage.
Immunostaining with an anti-α-SMA antibody (see supplemental Fig. S2) revealed the presence of “activated” myofibroblasts in the interstitium, in peritubular spaces, and heavy smooth muscle actin staining in periglomerular areas, which continue with the staining of the smooth muscle of arterioles (see supplemental Fig. S2, C and D). Intraglomerular expression of α-SMA was also observed in the mesangium. This feature was more prominent in the triple-homozygous mice (see supplemental Fig. S2, E and F). The arterioles were thinner in double- and triple-homozygous animals than in their WT counterparts that were not affected (see supplemental Fig. S2, E and F). The arterioles were thinner in double- and triple-homozygous animals than in their WT counterparts that were not affected (see supplemental Fig. S2, E and F). In addition, the vessels of double- and triple-homozygous animals were disorganized (see supplemental Fig. S2, C and D), resembling the vascular arrangement of an immature kidney. Double- and triple-homozygous animals showed an increase in cellularity and matrix in the adventitial layer of arteries resembling perivascular fibroplasia (see supplemental Figs. S3F and S4G).

A more detailed examination of periodic acid-Schiff-stained kidneys from double-homozygous (CBPfl/fl;Rencre/+ mice) is shown in supplemental Fig. S3. The kidney cortex shows hypercellular glomeruli (see supplemental Fig. S3, A and C), massive tubular dilation, and tubular casts (see supplemental Fig. S3, B, D, and E). The glomeruli are surrounded by many layers of epithelial and interstitial cells (see supplemental Fig. S3D). Arterioles are thin (see supplemental Fig. S3C, arrow) or contain multiple layers of capillary-like structures (see supplemental Fig. S3E). Some arterioles were affected by perivascular fibroplasia (see supplemental Fig. S3F, arrow). In addition to these alterations, there were numerous cystic glomeruli, with collapsed glomerular tufts, periglomerular and intraglomerular fibrosis, and hyperplasia of epithelial cells of Bowman’s capsule. Overall, these alterations were more accentuated in the triple-homozygous animals than in their double-homozygous counterparts.

Supplemental Fig. S4 shows some peculiar alterations in which the glomeruli were barely distinguishable from their adjacent structures that contained disorganized and tightly packed cells containing two or more nuclei, giving the appearance of neoplastic growth. Polynucleated cells are seen in and around tubular structures and in masses of interstitial cells in double-homozygous (see supplemental Fig. S4, A and E) and
triple-homozygous (see supplemental Fig. S4, B, C, D, and F) mice. Collapsed and cystic glomeruli, as well as arteries affected by fibroplasia, were observed in double- and triple-homozygous mice (see supplemental Fig. S4, E–G).

DISCUSSION

The present study shows that the HATs CBP and p300 are required for the maintenance of renin cells and the structural integrity of the kidney.

The importance of these HATs in the development of the whole embryo has been previously demonstrated: systemic homozygous deletion of either HAT results in embryonic lethality. CBP homozygous mutants die at around embryonic day 10.5 as a result of massive hemorrhage caused by a defect in blood vessel formation. In addition, they show apparent developmental retardation, delayed hematopoiesis, defective neural tube closure, and defects in mesenchymal cells (34). Homozygous p300 mutants also show embryonic lethality, with death occurring between embryonic days 9 and 11.5. The causes of lethality appear to be multifactorial: the knockout mice display defects in neural tube closure, cell proliferation, and cardiac development (35). These animals presumably express normal levels of CBP, indicating that CBP and p300 have nonoverlapping functions, such that both coactivators are required, or that the total level of CBP and p300 is critical for normal development. Given that the metanephric kidney begins to form at around embryonic day 11, it had not been possible to study the effect of CBP and/or p300 on renin cell differentiation and/or kidney development. To circumvent this problem, we utilized a conditional knockout approach that takes advantage of mice that we previously generated that express cre recombinase in renin cells. We crossed these mice with mice in which the CBP and p300 genes were floxed (13, 15), thus enabling the deletion of one or both HATs, specifically in renin cells.

Homozygous deletion of either HAT while its counterpart is left intact did not result in alterations in renin gene expression or distribution of renin, which remained confined in the JG cells. These results suggested that, in JG cells, CBP and p300 can compensate for one another’s function. Nor did reduction of the gene dosage further by generation of mice with deletion of three of a total of four copies of the HATs (i.e., homozygous for CBP<sup>flox</sup> and heterozygous for p300<sup>flox</sup>, or vice versa) cause alterations in the distribution of renin, suggesting that, under basal conditions, in the unstressed animal a minimum of one copy of either HAT is sufficient to maintain expression of renin. Fig. 3. Combined homozygous deletion of CBP and p300 in cells from the renin lineage results in disappearance of renin cells, decreased renin expression, and structural abnormalities of the kidney. A: floxing of CBP and p300 per se does not cause alterations in renin distribution. B: preservation of 1 allele (either CBP, as in the case shown, or p300) is enough to maintain the presence of renin cells in a normal pattern. C: homozygous deletion of CBP and p300 results in disappearance of renin cells. D: triple homozygous animals have no renin cells and marked alterations in kidney structure. E: higher-magnification image of JG area in a double-homozygous kidney. JG area contained no renin (no brown cells). Staining for CBP in a consecutive section shows that CBP is deleted in the JG area (CBP-negative nuclei are unstained (blue); CBP-positive nuclei are brown). F: quantitative RT-PCR shows that renin mRNA is severely reduced in double-homozygous (CBP<sup>flox</sup>;p300<sup>flox</sup>;Ren<sup>cre/cre</sup>) kidneys and further diminished in triple-homozygous (CBP<sup>flox</sup>;p300<sup>flox</sup>;Ren<sup>voi/voi</sup>) kidneys. Values (means ± SE) are expressed relative to WT, which was set to 100.
the renin gene and the structural integrity of the kidney. Interestingly, in whole body knockout experiments, CBP/p300 double heterozygotes are invariably lethal, supporting the idea that critical developmental events are sensitive to the overall CBP/p300 gene dosage. In light of those findings, our data suggest the possibility of a heterogeneous cellular response: different cells may require different amounts of either HAT. In fact, incidence of lethality is increased in p300, but not CBP, heterozygotes compared with normal animals (11). Of interest in this regard is the finding that mutation of a single allele of CBP in humans causes Rubinstein-Taybi syndrome, characterized by mental retardation and cardiac, craniofacial, and skeletal malformations (28). Although double-heterozygous mice and mice missing up to three copies of the HATs did not have overt alterations in renin distribution, they did show a selected decrease in kidney weight. This suggests that deletion of these critical factors in the renin cell lineage affected general kidney growth without alterations in kidney morphology, indicating that alterations in kidney growth rate may precede the more serious structural alterations seen when the HAT copy number was further reduced. It will be interesting to test whether these animals are capable of responding to injury with the appropriate growth responses, as in models with experimental reduction in renal mass. In addition, it remains to be determined whether heterozygous animals will be capable of responding to physiological/pathological threats with an increase in the number of renin-expressing cells (recruitment) when more renin is needed to regain homeostasis.

Fig. 4. Combined homozygous deletion of CBP and p300 in cells from the renin lineage results in severe alterations in renal morphology. Kidney structure was not affected in double-floxed mice in the absence of cre recombinase (A) or in mice with deletion of 3 of 4 alleles of HAT genes (B). Homozygous deletion of both CBP and p300, while one copy of the Ren1 gene remained intact (C), resulted in significant alterations in kidney structure, including irregular kidney contour, disorganized corticomedullary junction, and cortical and medullary cysts. D: more profound alterations, with cysts throughout the kidney, giving the appearance of a sponge kidney, in mice with triple-homozygous deletion that lack both HATs and the Ren1 gene.

Fig. 5. Combined deletion of CBP and p300 in cells of the renin lineage results in interstitial fibrosis. Cells were stained with Masson’s trichrome. A: no abnormalities in floxed animals devoid of cre recombinase. B: extensive fibrosis around glomeruli and vessels, as assessed by increased expression of collagen (blue), in double-homozygous (CBP^fl/fl;p300^fl/fl;Ren^cre/cy) mice. Depression in kidney surface (arrow) corresponds to a retracting fibrotic area. C: a more severe stage of renal fibrosis, with multiple cystic tubuli and glomeruli, in triple-homozygous (CBP^fl/fl;p300^fl/fl;Ren^cre/cre) mice.
As mentioned above, when both HATs where deleted simultaneously, there were marked abnormalities in the kidney characterized by a depletion of renin-expressing cells accompanied by an array of kidney abnormalities.

The depletion of renin cells emphasizes the crucial role of these proteins in the regulation of renin expression and agrees with our previous work in vitro demonstrating the crucial role of the cAMP pathway in renin cell identity that is regulated by acetylation of histone-4 at the CRE in the renin gene, the locus where CBP and p300 act to open the chromatin. On opening of the chromatin, transcription factors, such as Creb1 and/or their partners, have access and bind to CRE to initiate transcription of the renin gene. In addition, the present study agrees with the findings of Adams et al. (1) and Markus et al. (21), who generated mice with a deletion of the renin enhancer, a powerful DNA region that contains ≥11 transcription factor binding sites, including the CRE most likely affected by the lack of HATs in our study. Those investigators showed that deletion of the renin enhancer leads to marked reduction of renin expression in JG cells (1).

The depletion of renin cells in the present study could be due to downregulation of the expression of the renin gene and, therefore, suppression of renin synthesis. Mice with the double-homozygous deletion were found to have a decrease in renin mRNA and a virtual disappearance of renin immunoreactivity in JG cells or any other structure in the kidney, suggesting an effect on transcription, although an effect on mRNA stability and/or translation of the protein cannot be excluded and remains to be studied.

In CBPfl/fl;p300fl/fl;Rencre/+ mice, the number of renin-expressing cells is severely reduced to ~1–2% of the normal number. In CBPfl/fl;p300fl/fl;Ren+/+; CBPfl/fl;p300fl/fl;Rencre/+; and CBPfl/fl;p300fl/fl;Rencre/+ mice, however, renin expression was normal. As shown in Table 1, CBPfl/fl;p300fl/fl;Ren+/+ mice have two copies of Ren1; however, similar to the CBPfl/fl;p300fl/fl;Rencre/+ mice, CBPfl/fl;p300fl/fl;Ren+Rencre/− and CBPfl/fl;p300fl/fl;Rencre/+ mice have one copy of Ren2 and one copy of Ren1. That the difference in phenotype was due to the lack of HATS, and not to differences in the renin alleles present in the compared animals, is evidenced by the fact that whether CBPfl/fl;p300fl/fl;Rencre/+ mice (bearing the Ren1, Ren2 genotype) are compared with CBPfl/fl;p300fl/fl;Rencre/−+ or with CBPfl/fl;p300fl/fl;Rencre/+ (also Ren1, Ren2 genotype) animals, it is only in the CBPfl/fl;p300fl/fl;Rencre/+ animals that alterations in renin expression and aberrant kidney morphology are seen. In triple-homozygous animals, renin cells were completely absent and kidney abnormalities were more severe. These animals retained two copies of Ren2, which, under normal circumstances, would compensate for the lack of Ren1 and prevent the majority of kidney abnormalities (5, 25). However, when both of the acetylferases are missing, this compensation does not take place, resulting in lack of JG cells and severe kidney abnormalities. It is also possible that, in these animals, the two copies of cre recombinase may have ensured the removal of any remaining HAT, thus increasing the efficiency of HAT removal and explaining the additional effect.

The lack of renin cells could also be due to an effect on the fate of renin cells. We previously showed that renin cells are precursors that differentiate into multiple cell types in the kidney, including arteriolar smooth muscle cells, mesangial cells, interstitial cells, and a small subset of tubular cells (29). It is possible that cells that express renin do so under the tonic influence of the cAMP/Creb/CBP/p300 pathway and that when cells are not capable of turning on the renin gene, they continue on a default pathway of differentiation toward the cell types into which renin cells normally differentiate, such as arteriolar smooth muscle cells, mesangial cells, or interstitial pericytes. We previously showed that when renin cells are placed in culture, after only 10 days they differentiate into other cell types, namely, smooth muscle cells (14), suggesting that the smooth muscle phenotype is their default pathway, unless the cells are stimulated to maintain their renin phenotype (27). This hypothesis is supported by our previous findings in vitro demonstrating that cAMP analogs, as well as histone deacetylase inhibitors, can maintain the renin phenotype for multiple passages in arteriolar smooth muscle cells derived from the renin lineage (27). The fate of CBPfl/fl-deficient renin cells will have to be investigated in detail. It is also possible that renin cells may undergo apoptosis and/or necrosis at an early age, diminishing the endowment of renin precursors in embryonic life. If CBP and p300 were crucial for the survival of cells of the renin lineage, they may also affect kidney development and explain in part some of the morphological alterations. These possibilities remain to be investigated. Because there are no other specific markers for JG cells, in the absence of renin-positive cells, it would be virtually impossible to determine whether apoptosis is a feature in these cells at the age studied. Therefore, a thorough cell fate study throughout development, together with markers of apoptosis, is needed to address both possibilities. Although unlikely, the possibility that loss of renin-expressing cells may be due to the alterations in kidney structure and/or blood pressure/hemodynamic alterations remains to be investigated.

In addition to the lack of renin cells, there were serious kidney abnormalities, including abnormal vasculature, poorly developed glomeruli, glomerular cysts, tubular dilation, fibrosis, and areas of undifferentiated tissue. Multiple developmental abnormalities in the kidneys have been previously shown in mice missing two copies of the Ren1 gene, suggesting that the presence of renin is necessary to maintain the integrity of the kidney tissue (33). However, some of the abnormalities observed in our HAT-deficient mice, including thinner glomerular arterioles, undifferentiated renal tissue, and abnormal Bowman’s capsule and mesangial areas, were not found in the renin-knockout animals. Because renin cells are precursors for other cell types, it is tempting to suggest that lack of renin cells and lack of Ren1 protein may be responsible for the alterations. It should be noted that ablation of renin cells also results in thinner vessels, contrary to the thicker renal vessels found in the renin deletion studies (26), suggesting the intriguing possibility that renin cells per se may have additional functions in arteriolar smooth muscle growth. Because Ren1 heterozygous animals have no kidney abnormalities and do not change the distribution of renin in the kidney, it is more likely that the alterations in the present study are due to the lack of renin cells, rather than the lack of renin per se. There is, however, a very plausible alternative: because the acetyltransferases are missing in all cells of the renin lineage from very early in development, that is, in all cells that expressed renin throughout development (such as renal arterioles, mesangial cells, and tubular cells), the alterations in kidney structure may also be
due to the loss of these key regulators in cells that expressed renin early in development. This possibility remains to be investigated by carefully performed timed deletion studies across the developmental history of the renin cells.

In summary, the present study shows that the HATs CBP and p300 are crucial in maintaining renin cell specification. Lack of these key regulators in early life in cells from the renin lineage leads to severe abnormalities in kidney morphology. Lack of these key regulators in early life in cells from the renin lineage leads to severe abnormalities in kidney morphology.

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