Granulation Rescue and Developmental Marking of Juxtaglomerular Cells Using “Piggy-BAC” Recombination of the Mouse *Ren* Locus

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Mice lacking a functional *Ren*-1*^d* gene exhibit a complete lack of renal juxtaglomerular cell granulation and atypical macula densa morphology. Transgenic mice carrying a 145-kilobase BAC clone encompassing the *Ren*-1*^d* and *Ren-2* loci were generated, characterized, and backcrossed with *Ren-1*^d/^d* mice. Homozygous *Ren*-1*^d-null* mice expressing the BAC clone exhibited complete restoration of normal renal structure. Homologous recombination in *Escherichia coli* was used to generate a modified version of the BAC clone, in which an IRES/geo cassette was inserted specifically into the *Ren-1*^d* gene. When introduced into the germline, the modified clone provided a marker for juxtaglomerular cell differentiation and β-geo was expressed appropriately in juxtaglomerular cells throughout development. Parallel backcross experiments onto the *Ren-1*^d-null* background demonstrated that the juxtaglomerular cells expressed the modified *Ren-1*^d* locus in the absence of regranulation. These data demonstrate that the nongranulated cells constitute *bona fide* juxtaglomerular cells despite their altered morphology, that overexpression of renin-2 cannot compensate for the loss of renin-1*^d*, and that primary structural differences between the two isoforms are responsible for the differences in granulation. The use of BAC modification as part of functional complementation studies illustrates the potential for in vivo molecular dissection of key physiological mechanisms.

Renin (EC 3.4.23.15), the catalyst of the angiotensin cascade, is predominantly produced in the juxtaglomerular (JG)1 cells of the kidney, where it is stored in electron-dense granules, and released following a number of physiological stimuli, which act to maintain blood pressure homeostasis (1, 2). Mouse strains vary in renin gene copy number (3, 4); CBACa contains the single ancestral gene *Ren-1*^d*, whereas 129/Sv contains *Ren-1*^d* and *Ren-2*, which are 97% homologous at the nucleotide level and are located 20 kb apart (5, 6). The three renin genes are differentially expressed in a range of tissues such as the submandibular gland (where *Ren-2* is highly expressed in the male) (7, 8).

Gene knockout studies on the mouse *Ren* locus have shown that *Ren-2-null* mice have no significant cardiovascular phenotype (9). Homozygous *Ren-1*^d* knockout mice, however, have nongranulated JG cells and altered macula densa cell number and morphology (10). These studies suggest that expression of *Ren-2* can partially compensate for the loss of *Ren-1*^d* with respect to maintaining arterial blood pressure but is unable to maintain normal juxtaglomerular apparatus structure.

During mouse embryogenesis, renin is first expressed in the developing vessels of the kidney around E14.5 (11). As the more distal vessels become differentiated, renin expression parallels the progression of vasculogenesis and is no longer observed in the proximal arteries. During post-natal renal maturation, renin expression becomes restricted to the distal portion of the afferent arterioles in the JG cells (12). Previous attempts to direct reporter gene expression under defined renin promoter sequences have had mixed success. Using a 4.6-kb *Ren-2* promoter fragment, T-antigen expression was restricted to JG cells in the adult kidney, but confounding effects of Tag expression may have led to apoptosis (13). Under the same promoter, the *Escherichia coli* LacZ gene was not expressed at all in adult kidney.2

To date no murine renin transgenic studies have completely replicated wild type levels of expression, presumably because key control elements were missing from the transgene or because expression was adversely affected by the insertion site (14–16). To achieve site-independent expression, large transgenes derived from PACs (17), BACs (18), and YACs (19) are now routinely used. Increased clone size makes modification by standard restriction-ligation protocols unfeasible because of a lack of unique restriction sites, but several homologous recombination strategies have now been devised for BAC modification (20–22).

We report the successful modification of a 145-kb BAC clone encompassing the entire mouse renin locus to engineer a reporter of renin transcription and to provide suitable reagents...
for functional rescue experiments. The use of the modified BAC, together with its parental BAC clone, in the phenotypic rescue of the Ren-1<sup>d</sup> knockout mouse demonstrates the critical role for renin-1 in determining JGA morphology and function. The strategy of BAC modification and functional complementation outlined illustrates the potential for in vivo molecular dissection of key physiological mechanisms.

**EXPERIMENTAL PROCEDURES**

**BAC Characterization**—BAC clones were isolated from a commercial 129Sv mouse BAC library (Research Genetics Inc.) using the mouse Ren-2 cDNA probe. BAC clones were mapped as described previously (23) (see Fig. 1a). Southern blots of digested DNA were probed with PCR products generated using map pairs, D1Mit30 and D1Mit287 (24), which identify simple sequence length polymorphisms. Primers were obtained from Research Genetics Inc., and PCR reactions were as described (94 °C for 3 min; 25–30 cycles of 94 °C for 15 s, 55 °C for 2 min, 72 °C for 2 min; and then 72 °C for 7 min; Ref. 25).

**Specific BAC Modification of Ren-1<sup>d</sup>**—An IRES<sup>β</sup>-Geo cassette (26) modified to include the engrafted splicer acceptor and nuclear localization signal was excised from pKL53 (a gift from Dr. Austin Smith, CGR, University of Edinburgh) on a SalI fragment (27). The cassette was introduced into the unique SalI site of pR1K0 plasmid (10), so that β-Geo was flanked on each side by 3.5 kb of Ren-1<sup>d</sup> sequence, generating pR1SAIRES<sup>β</sup>-Geo (see Fig. 2). The suicide vector, pSV1.RecA, was modified by the introduction of a multiple cloning site cassette to yield pSV1.RecA60, and the incoming recombinant cassette from pR1SAIRES<sup>β</sup>-Geo was then inserted on an AscI/SalI fragment (28). The resulting suicide plasmid was electroporated into DH10B E. coli cells (10 ng of plasmid into 40 μl of electrocompetent cells; BIORAD electroporator: 2.5 volts, 25 μF, 250 C; Ref. 29) containing BACN10 and grown at 30 °C under tetracycline (12.5 μg/ml) and chloramphenicol (25 μg/ml) selection. Resistant bacteria were then incubated at 43 °C, and five BAC-specific co-integrants were identified, following alkaline lysis, by restriction digestion with EcoRI or PvuII and Southern blot hybridization with probes specific to exon 1 or exon 9, respectively. These co-integrants were grown on chloramphenicol plates containing 12 μg/ml fusaric acid, and a similar screening strategy was applied, using an additional probe internal to the reporter sequences. All restriction enzymes and DNA modification enzymes were purchased from Roche (Lewes, UK) and were used according to the manufacturer’s specifications. Antibiotics were purchased from Sigma-Aldrich.

**BAC Transgenic Preparation**—The BACs (wild type or modified) were linearized at the unique lox<sup>P</sup> site and purified by preparative pulse field gel electrophoresis, β-agarase treatment (New England Biolabs), and dialysis against injection buffer (10 mM Tris-HCl, pH 7.5, and 0.1 mM EDTA with 100 mM NaCl) (23). DNA at 1 μg/ml was microinjected using standard techniques.

Transgene-positive animals were identified by PvuII digestion of tail DNA, which was analyzed by Southern blot hybridization using a renin cDNA probe. We chose a Ren-1<sup>d</sup>-specific fragment of 10.6 kb and a Ren-1<sup>d</sup>-specific fragment of 7.9 kb in addition to the Ren-1<sup>c</sup> fragments at 7.5 and 2 kb (see Fig. 1b, lane 1). N10β-Geo transgene-positive animals yielded a triplet with the targeted Ren-1<sup>c</sup> fragment at 8.6 kb, the wild type Ren-2 fragment at 7.9 kb, and the Ren-1<sup>c</sup> fragment at 7.5 kb, as well as a β-geo-specific fragment of 6 kb (see Fig. 1b, lane 6).

In BACN10/Ren-1<sup>c</sup>-null crosses, the loss of the smaller 2-kb CBA-specific fragment and the gain of the Ren-1<sup>d</sup> 10.6-kb fragment was followed for genotyping, whereas for the modified BAC, the loss of the CBA-specific fragment and the addition of the 6-κb β-Geo-specific fragment were utilized. Copy number was determined by comparison of signal intensity with that of the endogenous 2-kb CBA-specific Ren-1<sup>c</sup>-specific fragment. Renin transcription was followed by Northern blot hybridization and was quantified by slot blot and phosphorimage analysis using total RNA (loading was controlled by hybridization with a U1 probe). Captopril was administered to male animals in their drinking water, at a concentration of 0.5 mg/ml for 10 days prior to sacrifice. All tissues were frozen directly on dry ice prior to RNA extraction and analysis.

**RT-PCR Analysis**—To distinguish between the transgene-derived and endogenous transcripts, a 156-bp RT-PCR product was generated using primers JJM 336 (AGAGGCCGGGATTGACAGT), and JMJ135 (AAGGTCGGATGGTGTTAT), which flank the exon 2-3 exon junction and bind with equal affinity to each transcript. The products generated from Ren-1<sup>c</sup>, Ren-1<sup>c</sup>, and Ren-2 are differentially digested using the restriction enzyme Sva3A. Briefly, 1 μg of total RNA was annealed with primer JMJ135 at 65 °C for 10 min and was then cooled rapidly on ice. Reverse transcriptase (AMV; Promega) was added in the presence of 1× RT buffer, 10 mM deoxynucleotides, and RNAsin, and the reaction was incubated for 10 min at 30 °C, 10 min at 37 °C, 50 min at 42 °C, and 2 min at 94 °C and was then transferred to ice. Following adjustment of the buffer (with PCR primers, fresh deoxynucleotides, and MgCl<sub>2</sub>) the PCR step was initiated (PCR conditions: 94 °C for 2 min; 25 cycles of 94 °C for 10 s, 61 °C for 30 s, 72 °C for 45 s; and 1 cycle 68 °C for 7 min). Products were purified using the Qiagen gel extraction kit (Qiagen) restriction digested with Sau3A according to manufacturers instructions and separated on a 12% nondenaturing acrylamide gel, stained by SYBR green I (Molecular Probes), and electroblotted onto positively charged nylon membrane (Roche Molecular Biochemicals). Following hybridization, using an exon 3-specific oligonucleotide (ACTGCGTTACGGTAGGTGTGAACGACCGCCGGAGTTAAGAG), and phosphorimage analysis, the following products were detected: a 156-bp product from Ren-1<sup>c</sup> (together with any undigested transgene-derived product), an 86-bp product from Ren-1<sup>c</sup>, and a major 56-bp product (plus minor bands of 130 and 82 bp depending on completion of digestion) from Ren-2 (see Fig. 3b). Histological and β-Galactosidase Analysis—Timed matings were set up (with E0.5 taken as the day of plug formation) and kidneys were removed from E14.5-day-old pups through to adult. One kidney was immersion fixed in 10% formalin or 4% paraformaldehyde for subsequent embedding in paraffin wax and preparation of 2–4 μm sections, which were stained with hematoxylin and eosin and examined by standard light microscopy. Immunocytochemistry on sequential sections was undertaken using anti-renin antibody (gift from Prof. Ingam: 1:10,000 dilution) and anti β-galactosidase antibody (ICN Biologicals; 1:600 dilution), respectively, which were visualized by streptavidin-biotin/peroxidase and developed by diaminobenzidine precipitation. To estimate the proportion of glomeruli with associated renin-positive JGA, which were also positive for β-galactosidase (and visa versa), 50 glomeruli were counted in each of three sections from...
BAC Modification and Renin Rescue

**Results**

Characterization of the Renin-encoding BAC Clones—The clone BACN10 contained both Ren-1<sup>d</sup> and Ren-2, located centrally, with approximately 45 kb of flanking sequences upstream and downstream of the structural genes (for restriction map see Fig. 1a). BACN10 was screened for sequence repeats, previously found to map at or near the renin locus (24), to ascertain whether or not the clone was chimeric. Sequences homologous to the map pairs D1Mit30 and D1Mit287 were identified, the former mapping 3–4 kb downstream of both Ren-1<sup>d</sup> and Ren-2, and the latter mapping within an 11-kb XhoI fragment upstream of Ren-2.

BAC Modification—The recombination cassette, designed to specifically replace exons 3 and 4 of Ren-1<sup>d</sup> with the IRESβ-Geo (26) reporter sequences, included homology arms identical to those used to generate the Ren-1<sup>d</sup> knockout mice (10), a splice acceptor to ensure that splicing did not occur around exons 3 and 4, and a nuclear localization signal to direct expression into the nucleus (Fig. 2). By using a suicide plasmid to introduce both the recombination cassette and RecA into the BAC host strain, DH10B, exposure of the BAC to RecA was shown to yield up to 0.1% of co-integrants incorporating the suicide plasmid, generated by switching to the nonpermissive temperature (43 °C), were screened by Southern blot hybridization using appropriate restriction enzymes and probes located outside the arms of homology.

Electron Microscopy—Samples were immersion fixed in 4% glutaraldehyde in cacodylate buffer at 4 °C. Tissues were processed and embedded in araldite blocks, and semi-thin sections were cut and stained in toluidine blue for orientation of specimen. Ultrathin sections were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate. Grids were viewed on a Phillips CM12 transmission electron microscope.

BAC Rescue—A breeding strategy was designed to introduce one copy of the BAC transgene (wild type or modified) onto the Ren-1<sup>d</sup> knockout background. Briefly, an animal homozygous for the BAC (on a Ren-1<sup>d</sup> background) was crossed to an animal homozygous for Ren-1<sup>d</sup> knockout. The F1 animals were backcrossed to homozygous Ren-1<sup>d</sup> knockout animals, and 25% of the progeny were classed as hemizygous for BAC and homozygous for Ren-1<sup>d-null</sup>.

**Fig. 2.** a, the BAC modification strategy used to introduce β-Geo into Ren-1<sup>d</sup> of BAC N10. The β-Geo cassette, including the splice acceptor, nuclear localization IRES, and the SV40poly(A), replaced exons III and IV to give 3.5 kb of flanking homologous renin sequences (both 5’ and 3’) within the recombination fragment. Dotted lines indicate the sites where recombination can occur. The size of the wild type bands and the location of the 5’ and 3’ probes are shown (shaded boxes). E, EcoRI; P, PvuII. b, the resolved recombinant N10-β-Geo, with the IRESβ-Geo sequences (open bar) located within exons III and IV of the Ren-1 gene. c, the screening strategy used to identify N10β-Geo. Southern blot hybridization confirms the expected alteration to the Ren-1<sup>d</sup> banding pattern, whereas the Ren-2 pattern remains intact. d, long range restriction digestion to confirm homologous recombination. w, wild type BAC; m, modified BAC. N01 shows specific loss of the wild type 28-kb Ren-1<sup>d</sup> fragment and the gain of the 19- and 16-kb fragments; SmaI shows loss of the 25-kb fragment and the gain of the 20- and 9.8-kb fragments; the 100-kb wild type Sall fragment (which encompasses both genes) yields two smaller fragments of 53 and 47 kb, whereas the 25-kb Ren-1<sup>d</sup>-specific SfiI fragment increases by 7 kb in the modified clone.
utilizing rare-cutting restriction enzyme sites, which were either included within the β-Geo construct or distinguished between \( Ren-1^d \) and \( Ren-2 \) (Fig. 1 and 2d). Comparison of BACN10 and N10β-Geo confirmed the predicted changes to \( Ren-1^d \), whereas \( Ren-2 \) remained unaltered, indicating the specificity of the BAC modification.

**Derivation and Analysis of BAC Transgenic Lines**—Both BACN10 and the modified clone N10β-Geo were prepared for microinjection using Cre-lox linearization (23) and used to generate transgenic mice by pronuclear microinjection of fertilized C57B/6 × CBA/Ca F2 oocytes. Three lines, two carrying one copy and one carrying three copies of BACN10, were characterized in detail and Northern blot hybridization indicated renin expression in an appropriate range of tissues in all lines. RT-PCR confirmed that \( Ren-2 \) was the major renin transcript in the transgenic submandibular gland, a key indicator tissue for expression of the murine \( Ren \) locus.3

Kidney renin expression was not significantly different between transgenic and nontransgenic littermates, irrespective of transgene copy number (Fig. 3a). RT-PCR, however, clearly demonstrated the expression of \( Ren-1^d \) and \( Ren-2 \) (at levels analogous to those of the 129/Ola kidney) in addition to the endogenous \( Ren-1^d \) gene (Fig. 3), indicating that all three genes are subject to physiological feedback control. Treatment of transgenic and nontransgenic males with captopril, an inhibitor of angiotensin converting enzyme, increased kidney renin expression between 4- and 8-fold, all three genes being derepressed (Fig. 3a,c).

**Transgenic Lines Carrying the Modified BAC**—N10β-Geo was independently introduced into the mouse germline, and three founder transgenic animals were identified that had integrated either one (TGM(N10β-geo)1) or two (TGM(N10β-geo)2) complete copies of the modified BAC. To establish the developmental profile of β-galactosidase expression and to ascertain whether it was correctly restricted to the JG cells in adults, progeny were analyzed at the time points noted (Fig. 4a). The blue staining observed in progeny of the line TGM(N10β-geo) at E16.5 extended into the kidney from the renal artery, in progenitors of the interlobar arteries. By E17.5 staining branched into the developing arcuate artery, and by E18.5 staining was only observed in the arcuate and interlobar arteries. At 1 week of age (P7), expression in the large proximal arteries had ceased, with staining restricted to the afferent arterioles, and by P14 distinct foci of blue staining were observed, indicating that β-galactosidase expression had become restricted to a small subset of cells within the kidney (not shown). This staining pattern is consistent with the published developmental profile for renin (11, 12). Weaker staining was observed in equivalent samples taken from TGM(N10β-geo)1 (data not shown).

Immunohistochemistry using both renin and β-galactosidase antibodies, performed on sequential sections of adult kidney, indicated that β-galactosidase expression co-localized with renin (Fig. 4b). β-galactosidase showing nuclear localization and renin being detected in the cytoplasmic granules of JG cells. (Comparison of adjacent sections showed that 78.3% of glomeruli associated with renin positive JGA were also positive for β-galactosidase, whereas 85.7% of β-galactosidase positive JGA were positive for renin.) These results confirm that modification of the \( Ren-1^d \) gene did not compromise the developmental restriction of expression.

**BAC Rescue of JGA Morphology**—To test whether or not the phenotypic changes observed in \( Ren-1^d \)-null mice were due to the absence of the renin-1 protein, appropriate breeding strategies were devised to introduce either the wild type or modified BAC (using TGM(BACN10) or TGM(N10β-Geo) respectively) onto the \( Ren-1^d \) knockout background. Briefly, females homozygous for each BAC (on a \( Ren-1^d \) background; Fig. 1c, lanes 1 and 4, respectively) were crossed to animals homozygous for the \( Ren-1^d \) knockout on a 129/Ola background (Fig. 1b, lanes 2 and 5). The F1 progeny, which were obligate heterozygotes for \( Ren-1^d \) and \( Ren-1^d \)-null and hemizygous for the corresponding BAC, were backcrossed to homozygous \( Ren-1^d \) knockout animals, and the F2 progeny were screened by PvuII restriction digestion and Southern blot hybridization with a \( Ren-2 \) cDNA probe (Fig. 1). 25% of the progeny were classed as hemizygous for the BAC and homozygous for \( Ren-1^d \)-null (Fig. 5a, class d). These were compared with \( Ren-1^d \) knockout littermates lacking the respective BACs.

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**Fig. 3.** a, captopril induction of male kidney renin expression levels in TGM/BACN10/3 transgenics (with three copies of BACN10) versus non transgenic and 129/Ola controls. *, \( p < 0.05 \); **, \( p < 0.02 \). b, schematic representation of RT-PCR assay. Primers 211 and 135 produce a 156-bp product from all three renin gene transcripts, which are then differentially restriction digested with Sau3A as shown and are visualized using the exon 3-specific 44-mer. c, RT-PCR to show \( Ren-1^d \) and \( Ren-2 \) expression in kidney. Paired tracks (0.5 and 1 \( \mu \)g starting RNA, respectively) represent untreated and captopril-treated kidney samples from male 129/Ola, TGM/BACN10/3, and Tg-ve animals.
Histological analysis demonstrated hypertrophy and hyperplasia of the macula densa in homozygote knockout animals and littermates containing the modified BAC (Fig. 5b, panels 6 and 7, respectively). In the presence of wild type BACN10, however, the macula densa were indistinguishable from control 129/Ola samples (Fig. 5b, panels 8 and 5), being less columnar in shape.

Electron microscopy showed that 100% of JG cells in transgenic mice harboring BACN10 exhibited a clear granulated morphology (Fig. 5b, panel 4) with granules of different size visible throughout the cytoplasm. By contrast, no granules were observed in renal sections from knockout animals carrying N10β-Geo, indicating that the modified BAC, which is incapable of directing renin-1 synthesis, does not rescue the JG cell phenotype observed in the Ren-1<sup>-</sup>d-null mice (Fig. 5b, panels 2 and 3). The presence of an active Ren-1 gene is therefore both essential and sufficient for the normal morphology of the juxtaglomerular apparatus.

**DISCUSSION**

The isolation of a BAC clone spanning the renin locus, in conjunction with the successful application of BAC modification techniques, has provided valuable tools with which to investigate renin gene regulation and the minimum genetic requirements for normal JG and macula densa cell morphology. Kidney renin gene expression is under tight physiological feedback control, which can be derepressed by pharmacological treatment such as angiotensin converting enzyme inhibition (e.g. the administration of captopril). Low level expression of each renin genes were under tight physiological feedback control. Following captopril administration, expression of each renin gene was derepressed. A 45-kb human renin transgene including 25 kb of upstream sequences was reported to exhibit appropriate regulation of expression and secretion in transgenic mice (31), whereas more recently, 140- and 160-kb human renin PAC transgenes (containing 35 and 75 kb of upstream sequences, respectively) were expressed in the kidney at levels equivalent to the endogenous mouse renin gene and were up-regulated by captopril administration (32).

The introduction of β-Geo into the Ren-1<sup>d</sup> coding region located within BACN10 was accomplished using the BAC targeting strategy (20). BAC modification proved to be highly specific, albeit rare, because modification was limited entirely to Ren-1<sup>d</sup> even in the presence of the highly homologous Ren-2 sequences (97% at the DNA level). A similar experiment using a P1 clone (P1–1251, which only contains Ren-1<sup>d</sup>), produced a significantly higher number of correctly targeted clones (27, 28). A comparison of the two recombination experiments indicated a decreased recombination efficiency for the BAC clone, which may reflect plasmid size (100-kb P1 clone, compared with the 145-kb BAC) or sequence-specific effects.

Previous transgenic lines generated to direct reporter gene expression to the JG cells either failed to express the gene in the adult or displayed aberrant expression throughout the renal vasculature (8). In the present study, reporter expression from N10β-Geo did not compromise normal developmental restriction to the JG cells during kidney maturation, and immunohistochemistry confirmed that β-galactosidase expression coincided with renin expression in adult kidneys. In many transgenic studies β-galactosidase is silenced in adult tissues (33, 34). Clearly sequences present within the BAC clone are “immune” to any aberrant developmental repression of LacZ within the kidney, making these animals a potentially useful source for FACs sorted “renin”-expressing cells at any time during development.

Introduction of the modified and wild type BAC transgenes onto the Ren-1<sup>d</sup>-null background has confirmed that the presence of renin-1 is essential for granule formation and that the

**FIG. 4.** a, schematic and pictorial profile of whole mount β-galactosidase staining seen in kidneys taken from TGM/N10β-Geo2 embryos and pups, and nontransgenic littermates at times noted during development. b, immunohistochemistry of adult TGM/N10β-Geo2 transgenic kidney. β-Galactosidase (panel A) and renin (panel B) immunostaining in serial sections, showing nuclear (n) immunoperoxidase localization in JGA cells in the former (arrow), and diffuse cytoplasmic (c) immunoperoxidase staining in the latter (arrow). The glomerulus (g) is also labeled.
knockout renal phenotype is not due to an unrelated mutation introduced during gene targeting. Importantly, renin-1 appears to be essential for development of storage granules in the JG cell but is not essential for the development of these cells, because reporter expression in the absence of renin-1 indicates that the agranular cells have not dedifferentiated and are still bona fide JG cells. Recently, a Ren-1-null mouse was generated (35), and similar changes in kidney morphology to the Ren-1 knockout model were reported, consistent with our interpretation that expression of renin-1 is not causal in the differentiation of JG cells. During chronic hypotension the smooth muscle cells neighboring JG cells can be recruited and modified to a renin expressing phenotype by a process termed metaplastic differentiation (36). The genetic cues required for metaplastic differentiation remain unknown, but the BAC transgensics may prove useful in addressing the mechanisms behind metaplastic differentiation of smooth muscle cells and also the contribution of the macula densa to the tubuloglomerular feedback loop.

Renin-1 expression is necessary for normal macula densa morphology because only the wild type BACN10 was able to prevent hyperplasia and hypertrophy on the Ren-1 knockout background. Because renin is not expressed in macula densa cells, and there is no immunohistochemical evidence to suggest uptake of renin from the circulation, the effect of renin-1 on macula densa morphology may be mediated either by a direct signal from the JG cells or in response to altered cues from tubular fluid.

One of the primary biochemical differences between renin-1 and renin-2 is the lack of three potential N-linked glycosylation sites that are located in both renin-1c and renin-1d but not renin-2. Importantly, both the single rat and human renin genes also encode two of the three potential glycosylation sites. Because the presence of renin-1 is essential for granule formation, it is possible that renin glycosylation may be the signal for renin trafficking to dense granules in the JG cell, where it is stored until required. Using BAC modification to introduce subtle changes into the renin genes, we can now ascertain which epitopes within the protein are determinants of its maturation, transport, and physiological action in vivo. The general applicability of combining knockout, BAC modification, and complementation to dissect important physiological systems will add to the increasing arsenal of functional genomic approaches (37, 38) that are currently being integrated with molecular and physiological studies.

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