Reporter gene recombination in juxtaglomerular granular and collecting duct cells by human renin promoter-Cre recombinase transgene

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Castrop, H., M. Oppermann, Y. Weiss, Y. Huang, D. Mizel, H. Lu, S. Germain, F. Schweda, F. Theilig, S. Bachmann, J. Briggs, A. Kurtz, and J. Schnermann. Reporter gene recombination in juxtaglomerular granular and collecting duct cells by human renin promoter-Cre recombinase transgene. Physiol Genomics 25: 277–285, 2006. First published January 17, 2006; doi:10.1152/physiolgenomics.00302.2005.—To assess the feasibility of using the renin promoter for expressing Cre recombinase in juxtaglomerular (JG) cells only, we generated five independent transgenic mouse lines (designated hRen-Cre) expressing Cre recombinase under control of a 12.2-kb human renin promoter. In the kidneys of adult mice Cre mRNA (RT-PCR) was found in the renal cortex, with Cre protein (immunohistochemistry) being localized in afferent arterioles and to a lower degree in interlobular arteries. Cre mRNA levels were regulated in a renin-typical fashion by changes in serum arginine, a hormonal stimulus of the renin-angiotensin system. The primary aim of the present study was to investigate whether Cre recombinase expression and enzyme activity driven by the renin promoter (Ren-Cre) is confined to JG cells so that it could be used for cell-specific excision of floxed targets. This aim was pursued by generating a mouse strain carrying a human renin promoter-Cre transgene (Ren-Cre mouse) and by crossing this line with the ROSA26-lacZ reporter strain in which the LacZ gene was placed under the control of a 12.2-kb fragment of the human renin promoter. Expression of β-galactosidase (β-gal) in a transgenic mouse in which the LacZ gene was placed under the control of a 12.2-kb fragment of the human renin promoter. Expression of β-galactosidase (β-gal) in a transgenic mouse in which the LacZ gene was placed under the control of a 12.2-kb fragment of the human renin promoter was recently shown to mirror the localization of expression of endogenous murine renin (6), suggesting that major regulatory sequences in the promoter responsible for the specific local activity of the promoter in vivo reside within this fragment. In the present experiments, we have therefore used this human renin promoter in a construct containing Cre recombinase to generate transgenic mice with JG cell-specific expression of the enzyme.

THE RENIN-ANGIOTENSIN SYSTEM plays a key role in the regulation of body fluid volume and arterial blood pressure. In general, the production and release of the proteolytic enzyme renin by juxtaglomerular (JG) cells is rate limiting for the generation of the bioactive end product angiotensin II. Thus an assessment of the response of the renin-angiotensin system to physiological and pathological challenges requires an understanding of the signaling mechanisms regulating the synthesis and secretion of renin. Investigations of the pathways affecting renin release by JG cells would be greatly aided by cell-specific deletions of individual signaling components. A possible first step to achieve such spatial control of gene expression in JG cells is the identification of a cell-specific promoter that can be utilized to express Cre recombinase in this cell type.

In the adult organism, renin is a rather specific marker of JG cells (29, 30). Although the enzyme can also be found in low levels in other locations, the renin promoter would therefore appear to be a good candidate to direct robust and selective expression of Cre recombinase to JG cells. Expression of β-galactosidase (β-gal) in a transgenic mouse in which the LacZ gene was placed under the control of a 12.2-kb fragment of the human renin promoter was recently shown to mirror the localization of expression of endogenous murine renin (6), suggesting that major regulatory sequences in the promoter responsible for the specific local activity of the promoter in vivo reside within this fragment. In the present experiments, we have therefore used this human renin promoter in a construct containing Cre recombinase to generate transgenic mice with JG cell-specific expression of the enzyme.

METHODS

Generation of transgenic mice. For the generation of transgenic mice expressing Cre recombinase under the control of the human renin promoter, a construct was designed consisting of a 12.2-kb...
The presence of the transgene in the genome were established. The presence of the transgene was confirmed by sequencing. One-cell embryos were harvested from superovulated female mice of the FVB strain. The purified construct DNA was then used as the backbone vector for pSG12000. For DNA microinjections, the NotI fragment consisting of a NotI/SalI linker, served as the backbone vector for pSG12000. DNA microinjections, the plasmid backbone of hRen-Cre was removed and the construct DNA consisting of MAR, human renin promoter, and Cre expression cassette was purified by gel electrophoresis, recovered and repurified with QiaEx II (Qiagen), and finally diluted to a concentration of 2.5 mg/μL. The correctness of the plasmid sequence was confirmed by sequencing. One-cell embryos were harvested from superovulated female mice of the FVB strain. The purified construct DNA was then used as the backbone vector for pSG12000.

**Detection of Cre mRNA and murine renin mRNA.** Total RNA was isolated from various organs with TRIzol reagent (GIBCO-BRL Life Technologies). The cDNA was synthesized by Moloney murine leukemia virus reverse transcriptase (Superscript, Invitrogen). Remaining genomic DNA was digested with RNase-free DNase (Roche), and finally applied to sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a nitrocellulose membrane. The membrane was hybridized with radiolabeled cDNA probe to detect the presence of Cre and murine renin mRNA. The杂交信号 was visualized using a phosphorimager.

**RESULTS**

**Cre expression in adult kidney.** In the kidneys of adult mice, expression of Cre protein determined by immunohistochemistry was restricted to the renal cortex. As can be seen in Fig. 2, A and B, substantial Cre labeling was found in the afferent arterioles, consistent with the localization of endogenous renin in the adult. In addition to its expression in the juxtaglomerular apparatus, low Cre protein expression was also observed in interlobular arteries (Fig. 2C). No immunocytochemical signal for the presence of Cre was found in the renal medulla.

**Animal experiments.** All animal experiments were conducted according to National Institutes of Health guidelines for the care and use of animals in research. Animal studies were performed according to protocols examined and approved by the Animal Care and Use Committee of the National Institute of Diabetes and Digestive and Kidney Diseases. Male hRen-Cre mice aged 7–8 wk were subjected to the following treatments (n = 6 for each experimental group): 1) control group: mice were fed a standard rodent chow [0.4% NaCl (wt/wt)] and had free access to tap water; 2) salt-deficient group: mice were fed a low-salt diet [0.02% NaCl (wt/wt)] for 7 days and received tap water as drinking fluid; 3) salt-loaded group: mice were fed a high-salt diet [8% NaCl (wt/wt)] for 7 days and received tap water as drinking fluid; 4) water restriction group: mice were fed a normal salt diet [0.4% NaCl (wt/wt)] and the drinking bottle was removed for a period of 36 h; 5) isoproterenol group: a continuous infusion of isoproterenol (10 mg·kg⁻¹·day⁻¹) was administered for 48 h via subcutaneously implanted osmotic minipumps (Alzet) (13); mice were fed a standard rodent chow and had free access to tap water. After the respective treatments mice were anesthetized with isoflurane and euthanized by cervical dislocation. The kidneys were removed and frozen in liquid nitrogen. Organs were stored at −80°C before isolation of RNA as described above.

**Statistical analysis.** Multiple groups were analyzed with ANOVA followed by Bonferroni post test. A P value <0.05 was considered significant.
Furthermore, medullary Cre mRNA levels were \( \sim 200 \)-fold lower compared with the cortex and not significantly different from the negative control (PCR run without cDNA). Thus the basal activity of the human renin promoter is sufficient to cause transcription of the Cre recombinase transgene with relative JG cell specificity.

We then determined whether the activity of the human renin promoter as assessed by expression of Cre recombinase and the activity of the endogenous mouse renin promoter as assessed by expression of renin were modulated to a comparable extent by manipulations known to activate or suppress the endogenous renin system. As shown in Fig. 3, administration of a high-salt diet led to a suppression of renin and Cre mRNA levels to 48 \( \pm \) 5\% and 61 \( \pm \) 8\% of the respective control values (\( P = 0.01 \) and 0.02 vs. control, respectively). Conversely, salt restriction in combination with ACE inhibition stimulated the expression of both endogenous renin and Cre mRNA to 650 \( \pm \) 130\% and 980 \( \pm \) 320\% of control (\( P < 0.01 \) vs. control for both renin and Cre mRNA). For both maneuvers, the relative changes in Cre and mouse renin mRNA levels were not significantly different. Water deprivation for 36 h also led to a parallel stimulation of the expression of murine renin and Cre mRNA (to 470 \( \pm \) 111\% and 211 \( \pm \) 89\% of control,
respectively), although in this case the relative increase in Cre mRNA levels over control was significantly lower than that of murine renin mRNA \((P = 0.021\) vs. renin mRNA). To investigate the responsiveness of renin expression to an increase in intracellular cAMP, a known stimulator of renin promoter activity \((7, 11, 17)\), mice were infused with isoproterenol \((10 \, \text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1})\) via osmotic minipumps for 48 h. Chronic isoproterenol application stimulated endogenous renin mRNA expression to \(250 \pm 50\%\) of control \((P = 0.013\) vs. control). Cre mRNA levels were increased significantly to \(180 \pm 32\%\) \((P = 0.03\) vs. control), with relative increases being significantly lower compared with endogenous renin mRNA levels \((P = 0.01)\). Stimulation of Cre mRNA under all conditions was restricted to the renal cortex, whereas Cre mRNA in the renal medulla remained statistically undetectable. Together, the parallel and quantitatively comparable regulation of endogenous renin mRNA expression and Cre mRNA suggest that key regulatory elements in the 12.2-kb fragment of the human renin promoter are functional in the mouse model.

**Cre activity in adult mouse.** To track the activity of the human renin promoter throughout the ontogeny of the mouse, Cre activity was determined in offspring of crosses of hRen-Cre and ROSA26-lacZ or ROSA26-EGFP reporter strains. β-gal or EGFP signals are expected to be positive in cells derived from progenitors in which Cre-mediated recombination has occurred at any stage of development \((12, 28)\). In the

![Image of Cre activity in adult mouse](http://physiolgenomics.physiology.org/)
adult mouse, intense β-gal staining was found in the juxtaglomerular portion of the afferent arterioles and in interlobular arteries, thus overlapping with Cre protein expression (Fig. 4). Similar results were obtained for the EGFP reporter line (not shown). Some labeled cells were also found scattered throughout the glomerular mesangium (Fig. 4C). Unexpectedly, however, intense β-gal staining was consistently observed in the medulla of the adult kidney, with the most intense β-gal staining being present in medullary segments of collecting ducts (Fig. 4). Because Cre protein expression was undetectable in the medulla of the adult kidney, this observation indicates substantial medullary activity of the human renin promoter before adulthood. Costaining with an anti-aquaporin-2 antibody revealed colocalization of aquaporin-2 and β-gal activity in most, but not all, collecting duct cells (Fig. 4G). Cre-negative reporter offspring served as controls; no unspecific β-gal background staining was detected (Fig. 4, H and I). These studies indicate that Cre activity was sufficient to

Fig. 5. LacZ activity in extrarenal tissues of the adult mouse. β-gal-positive cells were detected in the brain (A), adrenal cortex (B), testis (C), large vessels of the liver (D; inset shows hepatic vessel), and aorta (E).
delete the floxed stop cassette of the reporter strain in collecting duct progenitor cells.

As shown in Fig. 5, extrarenal β-gal staining was restricted to the adrenal cortex, testis, aorta, single cerebral cells, and, at very low levels, large vessels in the liver. In all of these organs Cre mRNA expression in the adult mouse was very low compared with the kidney (not shown), suggesting activity of the human renin promoter confined to embryonic development. Thus the activity of the human renin promoter in the adult mouse is restricted to renin-typical expression sites, but as detected by Cre activity in reporter mice the human renin promoter is active in the renal collecting duct during kidney development and appears to become largely silent at this site in the adult kidney.

Cre activity during embryonic development. In view of the intense β-gal staining in the collecting ducts of the adult kidney despite the absence of Cre expression, we assessed β-gal staining as a surrogate marker of human renin promoter activity during embryonic development. Embryos were harvested at E12, E14, E16, and postnatal day 1, and the localization of β-gal staining in the reporter strain was determined. As shown in Fig. 6, in the embryonic kidney the human renin promoter is active in the developing collecting duct system including the branching tip of the collecting duct ampullae. In addition, β-gal-positive cells were also detected in large renal vessels (Fig. 6E). Intense activity of the human renin promoter was also observed in the adrenal gland cortex (Fig. 6F and Fig. 7 showing a cross section of the whole embryo at E14). In embryos from Cre-negative mice serving as controls, no considerable background staining was observed (not shown). These observations confirm our notion that the human renin promoter is active in the branching ureteric bud system during embryogenesis but turns largely silent during postnatal life.

DISCUSSION

The aim of the present study was to assess the feasibility of using a 12.2-kb human renin promoter to induce JG cell-specific expression of Cre recombinase in a transgenic mouse line. The availability of such a model could facilitate the
exploration of signaling pathways in JG cells by permitting genetic modifications of specific targets in JG cells only. Our attempt was instigated by a previous study showing that this fragment of the human renin promoter caused expression of the LacZ reporter gene in a pattern similar to that of endogenous mouse renin (6). Our studies are in agreement with these results in that they show expression of the Cre recombinase transgene predominantly in afferent arterioles and to a lower degree in interlobular arteries of adult kidneys of five independent mouse lines. Differences in the activity of the human renin promoter in the five different transgenic mouse lines generated were marginal, confirming the efficiency of the MAR used in the transgene for the reduction of position effects (19) and rendering it unlikely that the expression pattern of Cre recombinase was substantially influenced by the integration site into the mouse genome. Thus these data confirm the previous conclusion that 12.2 kb of the human renin promoter is sufficient to target the expression of different transgenes to renin-typical sites (6). It appears that the regulatory sequences that might be present in introns or the 3'-untranslated region of the renin gene (1, 35) are not crucial to target the expression of the gene to its typical localization within the kidney. In earlier studies, constructs containing <5 kb of the human renin promoter failed to induce significant expression of luciferase or SV40 T-antigen transgenes in mice (26). On the other hand, 4 kb of mouse renin 5'-flanking sequence was shown to be adequate to direct the expression of green fluorescent protein (GFP) to JG cells (9).

To address the question of whether key regulatory elements for modulation of the activity of the human renin promoter were functional in the 12.2-kb fragment of the human renin promoter in the in vivo mouse model, we examined the effect of known inducers of renin expression on mRNA levels of Cre recombinase. We found that the activity of the human renin promoter as assessed by Cre expression was altered in parallel with the expression of the endogenous renin gene under a high-salt diet, during a low-salt diet in combination with ACE inhibition, during water restriction, and after isoproterenol infusion. This is in agreement with the earlier observation that the expression of the LacZ gene under control of the same human renin promoter was augmented in parallel to renin in mice treated with furosemide and a low-salt diet and in the clipped kidneys of mice with renal artery stenosis (6). In general, the expression of Cre recombinase and of mouse renin were regulated in parallel and to about the same extent. Nevertheless, mRNA levels of Cre recombinase during water restriction and isoproterenol administration were significantly lower compared with levels of endogenous renin mRNA. Both of these maneuvers are expected to increase cAMP levels in JG cells by activation of the sympathetic nervous system and by direct activation of β-receptors, respectively. It is conceivable that differences in steady-state levels of renin and Cre mRNA are a reflection of different effects of the cAMP/PKA pathway in stabilizing respective mRNAs. Although there is good evidence that cAMP prolongs the half-life of renin mRNA (1, 2), there is currently no reason to assume that the stability of Cre mRNA, as a readout of the activity of the human renin promoter, is similarly regulated. These data appear to support the functional relevance of the cAMP-responsive elements shown to be importantly involved in the regulation of the murine Ren1c promoter (11, 17) and—based on homology comparisons (14)—presumably also of the human renin promoter. Transgenic mice with mutations in presumed regulatory elements of the human renin promoter detected by in vitro studies therefore may prove to be valuable tools to investigate the function of the human renin promoter under in vivo conditions.
Although measurements of Cre expression in adult mice provide a measure of the persistence of the human renin promoter activity, this approach does not necessarily provide insights into renin promoter activity during development. Because renin expression shows a wider expression pattern in embryonic than adult kidneys, it is possible that Cre-mediated excision of floxed targets may take place in cells in which the renin promoter is transiently active during development; this recombination could then be transmitted to daughter cells in the same lineage (12, 28). Observations in offspring of crosses of the hRen-Cre transgenic mice with the ROSA26-lacZ reporter strain indicate that this is in fact the case. It is a striking finding of our study that robust and consistent β-gal staining was found in the renal medulla with a cellular localization in collecting ducts (Fig. 4). Because significant levels of Cre recombinase mRNA and Cre recombinase activity were not detectable in the renal medulla of adult mice, it is likely that recombination with excision of the floxed stop codon had taken place in embryonic life. Because the renin promoter-driven activation of Cre causes a recombination at the DNA level, all cells of the respective cell lineage are expected to be lacZ positive, even if the human renin promoter turns silent in postnatal life (12, 25). In fact, β-gal staining reflecting activity of the human renin promoter was noted in the developing collecting duct system of the embryonic kidney beginning as early as E12. Previous studies of renin-driven expression of reporter genes in transgenic mice have not reported measurable promoter activity in medullary tissue of either embryonic or adult kidneys. Nevertheless, GFP expression under control of a 4-kb renin promoter was found during embryogenesis in the Wolffian duct, the origin of the ureteric bud that gives rise to the collecting duct system (9). Similarly, a recent in vitro study showed the expression of renin in ureteral branches of kidney explants from E14 rat embryos (16). Activity of the human renin promoter in a similar localization in our studies could explain the β-gal staining found in the collecting duct system in later stages of kidney development. Furthermore, GFP expression under the control of a 4-kb mouse renin promoter was reported to be most pronounced in “cells with epithelial morphology,” although the localization of these cells was considered to be vascular (9). In another study in which GFP expression was under the control of the endogenous mouse Ren1d promoter the focus was exclusively on vascular expression, and no comment, positive or negative, can be found related to extravascular expression in the developing kidney (18). Similarly, in a recent study in which Cre expression was under the control of the endogenous Ren1d promoter, analysis of a cross with ROSA26 reporter mice revealed a similar pattern of Cre activity in the renal cortex including the renal vasculature and single mesangial cells (25). Again, the focus was on Cre expression in the renal cortex, and no data are given regarding medullary Cre activity (25). Although renin expression is absent or very low in the medulla of adult kidneys, renin expression was recently reported to be induced in the collecting ducts of rats under conditions of high circulating angiotensin II levels (20, 21). Renin mRNA and protein were also found to be expressed at low levels in the connecting tubule of mice during salt restriction (22, 23). The presence of renin mRNA in the medulla at least under some circumstances and our evidence for transient medullary activity of the renin promoter during development would add a mechanistic aspect to the notion that angiotensin II may be involved in kidney development and in particular in the morphogenesis of the branching ureteric bud (8). In agreement with this concept, renin-angiotensin system-deficient mouse models are consistently characterized by papillary atrophy and concentrating defects (5, 15, 32, 35). Utilization of a cryptic capacity for renin expression in derivatives of the collecting duct anlagen may be necessary for normal development of the renal medulla. The unexpectedly widespread activity of the human renin promoter driving Cre in the vasculature (9, 18) and, according to our data, in the developing collecting duct system of the embryonic kidney may hamper attempts to inactivate floxed targets specifically in JG granular cells. This issue might be circumvented by the use of inducible Cre transgenes under the control of the renin promoter. In this setting Cre expression would be induced in the adult mouse and, according to our data, might then be more restricted to JG granular cells.

Extrarenal expression of Cre recombinase reflecting the activity of the human renin promoter was at the detection limit in the adult mouse. However, in the embryo substantial activity of the human renin promoter was observed in the cortex of the adrenal gland, as has been described for embryonic mice (10, 25), sheep (34) and humans (3, 24). The activity of the human renin promoter in the embryonic brain and in cartilages seen in our transgenic model was also described previously for mice, rats, and humans (3, 24, 25, 27).

In summary, we generated transgenic mice expressing Cre recombinase under the control of a 12.2-kb fragment of the human renin promoter. The localization and regulation of this promoter fragment in vivo in the transgenic mouse model are highly similar to those of the endogenous renin gene, suggesting the presence of key regulatory elements within 12.2 kb of the 5′-flanking region of the human renin gene. Our data indicate further that the human renin promoter is active in the collecting duct system during early kidney development. Although this observation suggests a possible role of medullary renin expression in normal kidney development, it represents a complication for JG cell-specific expression of renin promoter driven transgenes. In general, the possibility of transmitting a Cre-mediated recombination along cell lineages emphasizes the usefulness of inducible transgenic systems.

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GRANTS

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