Vol. 170, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1990 Pages 344-350

TISSUE AND CELL SPECIFIC EXPRESSION OF A RENIN PROMOTER-REPORTER GENE CONSTRUCT IN TRANSGENIC MICE

Curt D. Sigmund, Craig A. Jones, John R. Fabian,
John J. Mullins and Kenneth W. Gross

Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263

German Institute for High Blood Pressure Research, University of Heidelberg, Im Neuenheimer Feld 366, 6900 Heidelberg, Federal Republic of Germany

Received June 8, 1990

The tissue specific expression of a fusion transgene consisting of 4.6 kb of Ren-2 5' flanking sequence and the SV40 T antigen viral oncogene was analyzed by northern hybridization and immunocytochemistry. Among the 10 tissues examined, endogenous renin transcripts were identified in and restricted to kidney, submandibular gland, testes and ovary consistent with the expression pattern of the Ren-1^c gene. In addition to these tissues, significant levels of transgene mRNA were detectable in the brain. Expression of the transgene was restricted to juxtaglomerular cells in the kidney and the granular convoluted tubule cells of submandibular gland. These results suggest that 4.6 Kb of Ren-2 5' flanking sequence is sufficient to confer tissue and cell specific expression upon an exogenous reporter gene.

The three murine renin genes (Ren-1^c, Ren-1^d & Ren-2) are equivalently expressed in the kidney yet exhibit distinct expression profiles at a number of extra-renal sites (1-4). In order to localize the requisite cis-acting sequences conferring this differential expression we and others have studied expression of the individual renin genes in transgenic mice by examining intact renin genomic constructs (4-8). Although correct tissue-specific and hormonally responsive expression was reported, each transgene contained considerable amounts of 5' flanking, 3' flanking and intron sequences. Further refinement in the identification of the requisite controlling sequences necessitates analysis of constructs with progressively decreasing amounts of flanking or intron sequence. It has previously been reported that transgenic mice containing the proximal 2.5 Kb of Ren-2 5' flanking sequence fused to SV40 T antigen express the transgene in tissues irrespective of the normal pattern of renin expression (9). We have constructed transgenic mice containing a larger portion (4.6 Kb) of the Ren-2 5' flanking sequence fused to SV40 T antigen. This sequence was isolated from a genomic segment previously shown to exhibit proper tissue- and cell-specific renin expression (8). This allowed us to 1) demonstrate the fidelity of this 4.6

Abbreviations: JG, juxtaglomerular; SMG, submandibular gland; GCT, granular convoluted tubule.

0006-291X/90 $1.50

Kb 5' flanking sequence as a tissue- and cell-specific regulatory sequence, and 2) show these sequences can properly respond to the ontogenetic changes in the pattern of renin expression throughout fetal and postnatal kidney development. These ontogenetic changes are manifested by a shift in the renin expressing cell population from arterial smooth muscle cells to specialized JG cells during kidney development (10-13).

Materials and Methods

Construction of the fusion transgene and production of transgenic mice: This transgene contains 4.6 Kb of Ren-2 5' flanking sequence (-4600 to +6) cloned upstream of the SV40 T antigen structural gene. Transcription initiates at the major start site in the renin sequence (14) and translation initiates within T antigen. On northern blots the transgene mRNAs migrate as a doublet due to alternative splicing of the large and small T antigen introns. The T antigen gene was obtained from pCOP-TAG (15).

For microinjection, the transgene segment was excised, purified by agarose gel electrophoresis and prepared as previously described (16). Transgenic mice were produced by standard methods with approximately 100 copies of the transgene injected into the male pronucleus of fertilized one cell embryos derived from C57Bl/10Ros-$_p^d$ X C3H/HeRos (BCF_2). Methods for the production of transgenic mice have been described previously (15).

Analysis of Nucleic Acids: Genomic DNA was purified from tail biopsies (16) and was analyzed by dot blot hybridization to identify positive transgenic offspring. Blots were probed with a 1.8 Kb EcoRl - Sstl fragment derived from the 5' flanking sequence of Ren-2. Total RNA isolation and northern blot analysis were performed as previously described (17). Blots were probed for T antigen using an [α-^{32}P]GTP labeled T3 polymerase antisense T antigen transcript from the plasmid T3T7-TAG and renin using an [α-^{32}P]GTP labeled SP6 antisense renin transcript from the plasmid pSLM. S1 nuclease protection assays were performed as described in Field et al. (14). Immunocytochemistry and in situ hybridization were performed as described (13).

Results

Tissue specific expression: Northern hybridization of whole tissue RNAs revealed endogenous renin gene (Ren-1^c) expression in kidney, SMG , testes and ovary, all sites previously shown to contain abundant renin and renin mRNA (2,3,18,19), but not spleen, heart, skeletal muscle, liver, lung and brain, sites containing either little or no renin mRNA (2,4; Figure 1). Figure 1 further illustrates transgene expression in the same spectrum of tissues as Ren-1^c. Proper transcription initiation at the major start site in the renin 5' flanking region of the transgene (14) was confirmed by S1 nuclease protection (data not shown).

Significant level of transgene mRNA was also evident in the brain with low but detectable levels in heart and liver (but not spleen, lung or skeletal muscle). This is consistent with previous reports of low steady state levels of renin mRNA in these tissues (2,4,20).

In the SMG, the level of transgene derived RNA was greater in male than female transgenics (Figure 1, and data not shown). This result is consistent

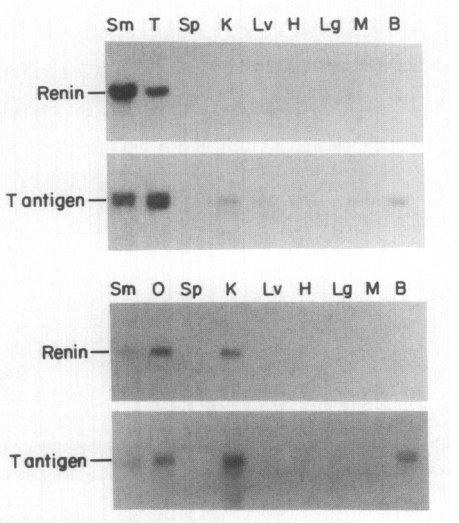

Figure 1. Tissue specific transgene expression.
20 μg total tissue RNAs (male, top panels; female, bottom panels) were electrophoresed in 1.5% formaldehyde-agarose gels, transferred to nitrocellulose and hybridized to either renin or T antigen probes as indicated. Sm, SMG; T, testes; Sp, spleen; K, kidney; Lv, liver; H, heart; Lg, lung; M, skeletal muscle; B, brain; O, ovary. All tissues were removed at 6 weeks of age. At this age severe T antigen-induced renal pathology develops which indirectly effects the levels of endogenous renin mRNA in this tissue (data not shown).

with the androgen inducible expression of renin RNA and protein previously characterized in the SMG of mice (18).

Cell specific expression: Figure 2, A-D, illustrates that the pattern of cells containing renin mRNA and immunoreactive T antigen are identical and colocalized in JG cells in serial sections of a 1 week old transgenic kidney. At this stage of development no T antigen immunoreactive cells were identified elsewhere in the kidney.

In the SMG, renin mRNA (Figure 2, E-F) and immunoreactive T antigen (Figure 2, G-H) were identified in GCT cells but not in the acinar cells. Correct cellular accumulation of T antigen was also evident in testicular interstitial Leydig cells (data not shown). No immunoreactive T antigen containing cells were evident in the lung or liver although a few widely scattered T antigen containing cells were occasionally observed in the heart.

Discussion

Our data demonstrate that 4.6 Kb of Ren-2 5' flanking sequence confers a tissue specific and cell specific expression profile on a reporter gene in transgenic mice. Thus, this 4.6 Kb 5' flanking sequence contains the regulatory sequences required to direct expression to the correct spectrum of tissues, as well as the correct subset of cells in the kidney, SMG and testes. Furthermore, our previous observation demonstrating correct temporal and spatial expression

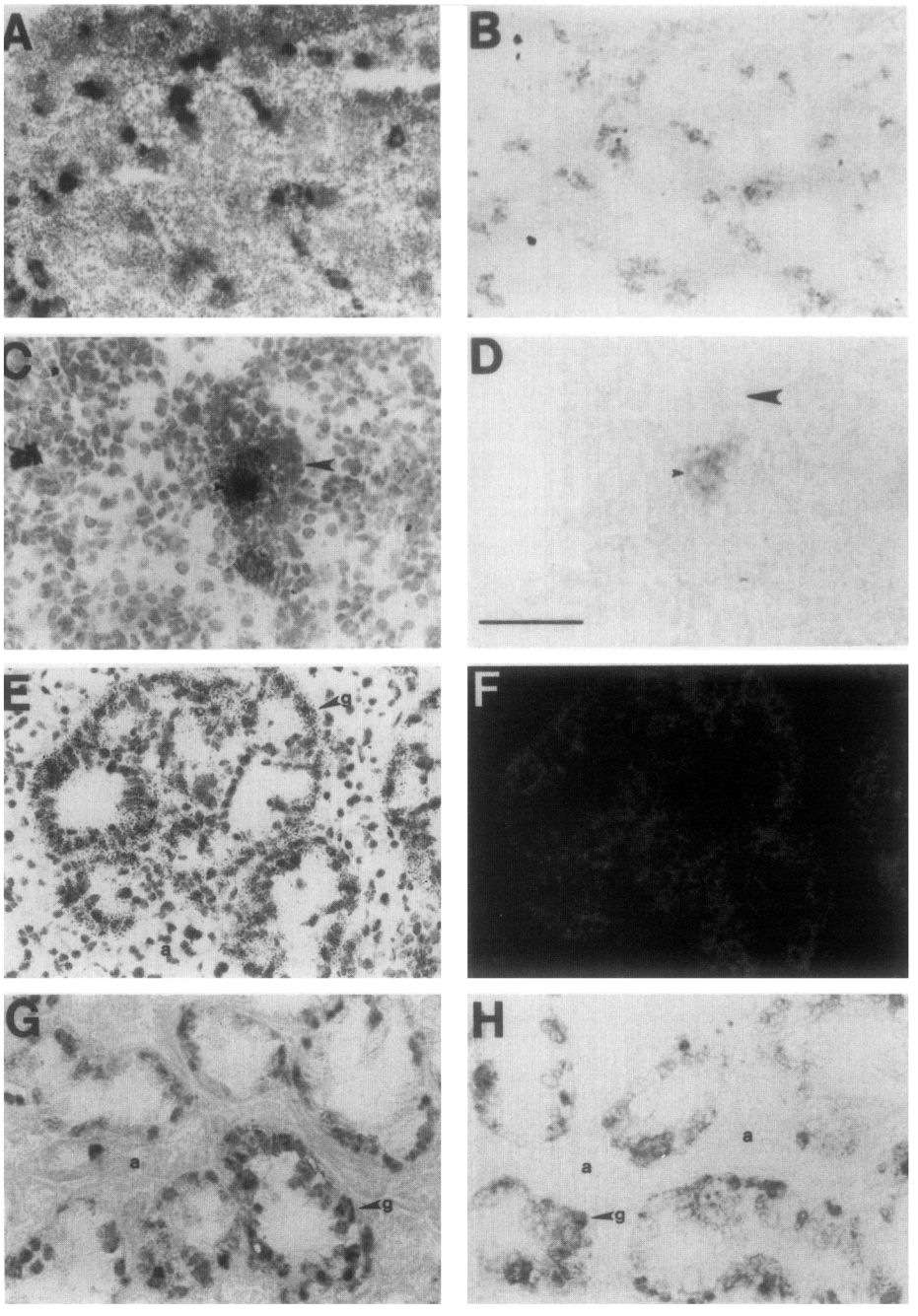

Figure 2. Cell specific transgene expression.

A-D, kidney sections from a 1 week old transgenic mouse hybridized with antisense renin probes (panels A&C) or immunocytochemically stained for T antigen (panels B&D). Sections A&B and C&D are serial. The large and small arrows in panels C&D show the positions of the glomerulus and juxtaglomerular apparatus, respectively.

E and F are bright field and dark field micrographs of a DBA/2Ros SMG hybridized *in situ* for renin mRNA. G and H are SMG from different transgenic individuals stained immunocytochemically for T antigen. a, acinar cells; g, GCT cells. The bar in D is 100 μm, the magnification of C-H are the same.

of the transgene in smooth muscle cells of the developing intra-renal arterial tree (13), when combined with the results presented above, strongly suggest these sequences confer upon the renin gene the regulatory information necessary for the proper ontogenetic changes characteristic of renin expression throughout kidney development.

These results stand in contrast to and are particularly interesting in light of a recent report describing inappropriate expression of a T antigen transgene containing only 2.5 Kb of Ren-2 5′ flanking sequence in which no transgene expression was evident in the SMG or kidney (9). These results in combination with those presented herein suggest that regulatory elements relevant to the control of GCT and JG expression are located between 2.5 and 4.6 Kb upstream of the transcription initiation site.

However, it has been reported that proper tissue specific and hormonally regulated expression was observed when the same proximal 2.5 Kb flanking region was integrated as part of a whole genomic construct (5). A potential explanation of these disparate findings would be offered by redundancy in regulatory elements controlling tissue specific expression of the renin gene. Sequences in both the 5′ and 3′ flanking regions (21-24) and in introns (25,26) have been shown to be important for tissue specific regulation of several genes. The case for redundancy in cis acting regulatory elements in the renin genes will need to be rigorously tested.

Conflicting reports have also been obtained from several laboratories employing transfection analysis using 5′ flanking renin sequences fused to the chloramphenicol acetyltransferase gene. For example, the identification of a negative regulatory element upstream of the mouse Ren-1 gene, and a cAMP responsive element upstream of both the Ren-1 and Ren-2 genes by transfection analysis in the noncognate cell lines, JEG and AtT-20, has been reported (27). Relative to Ren-2 these elements were reported within a 480 bp sequence approximately 345 bp upstream of the transcription start site. Similarly, no stimulation of transcription was evident when successive deletions were made in an analogous construct containing 449 bp of the 5′ proximal region of Ren-2 further suggesting the absence of negative regulatory elements (28).

In contrast, the proximal 2.5 Kb Ren-2 5′ flanking region, reported as conferring tissue specificity as part of genomic transgenes (5) failed to stimulate transcription in the noncognate cell lines commonly used for transfection studies unless coupled to a functional enhancer (29). These results led the authors to suggest that the lack of expression in these cells was due to the absence of necessary trans-acting factors needed to activate transcription. Importantly however, these same sequences failed to stimulate proper tissue specific transcription of a fusion gene construct in transgenic mice making it difficult to interpret the transfection results. The above results emphasize the

importance of testing sequences, identified as functional in noncognate cells, in whole animals.

Acknowledgments

We are grateful to Dr. Loren Field for his generous gift of rabbit anti-T antigen antibody and to Dr. Shimon Efrat for his gift of pT3T7-TAG. We would like to thank Frank Pacholec and Chuanzen Wu for injecting and implanting eggs, and Mary K. Ellsworth and Colleen Kane for their excellent technical assistance. This work was funded by NIH grant Nos. HL35792 and GM30248. C.D.S. is the recipient of NIH fellowship HL07963.

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