

TECHNOLOGY REPORT

Podocyte-Specific Expression of Cre Recombinase in Transgenic Mice

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Summary: We report a transgenic mouse line that expresses Cre recombinase exclusively in podocytes. Twenty-four transgenic founders were generated in which Cre recombinase was placed under the regulation of a 2.5-kb fragment of the human NPHS2 promoter. Previously, this fragment was shown to drive beta-galactosidase (β -gal) expression exclusively in podocytes of transgenic mice. For analysis, founder mice were bred with ROSA26 mice, a reporter line that expresses β -gal in cells that undergo Cre recombination. Eight of 24 founder lines were found to express β -gal exclusively in the kidney. Histological analysis of the kidneys showed that β -gal expression was confined to podocytes. Cre recombination occurred during the capillary loop stage in glomerular development. No evidence for Cre recombination was detected in any of 14 other tissues examined. *genesis* 35:39–42, 2003.

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INTRODUCTION

Glomerular visceral epithelial cells of the kidney, termed podocytes, are necessary for the formation and integrity of the glomerular filtration barrier. Podocytes are increasingly recognized to play a central role in a variety of glomerular diseases such as diabetic nephropathy and focal and segmental glomerulosclerosis. Identification of mutated genes encoding podocyte-specific protein products that cause nephrotic syndrome and subsequent glomerulosclerosis has strengthened the hypothesis that the podocyte plays a central role in maintaining the filtration barrier of the glomerulus (Kestila *et al.*, 1998; Boute *et al.*, 2000). In addition to providing convincing evidence of the importance of the podocyte in maintaining filter integrity, the work by molecular geneticists has suggested that podocyte injury or loss is directly associated with the pathological process of glomerulosclerosis. Work by Kriz, Meyer, Wiggins, and others (Kriz *et al.*, 1998; Pagtalunan *et al.*, 1997; Kim *et al.*, 2001) investigating both human disease and experimental animal models has supported the contention that podocyte be-

havior plays a central role in determining outcome in glomerular disease.

The study of podocyte biology has been hampered by limitations in available experimental models that both recapitulate the complex in vivo phenotypes of this cell and that can be readily and specifically manipulated at the molecular level. Recent work has provided conditionally immortalized mouse cell lines that express molecular markers consistent with the in situ podocyte phenotype (Mundel *et al.*, 1997; Saleem *et al.*, 2002; Yaoita *et al.*, 2001). However, these cell lines do not reproduce the complex three-dimensional cyto-architecture and protein expression profile of the podocyte in situ. These shortcomings limit the utility of these cell lines for studying the functional role of specific molecules in governing podocyte morphology and the maintenance of glomerular filter integrity.

Genetic deletion of genes important in podocyte function may also result in an embryonic lethal phenotype that would preclude functional analysis in the mature podocyte. Eremina *et al.* (2002) used a 4.125-kb *Nphs1* promoter to obtain a mouse line that expresses Cre recombinase in podocytes. However, in this mouse Cre recombinase is also expressed in brain. To circumvent this problem, we sought to develop and characterize a mouse line expressing Cre recombinase in a truly podocyte-specific fashion.

We have recently identified and characterized a promoter fragment from the human NPHS2 gene that drives truly podocyte-specific transgene expression in mice (Moeller *et al.*, 2002). In this work, the identified NPHS2 promoter fragment was successfully used to create a

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p2.5P-Cre

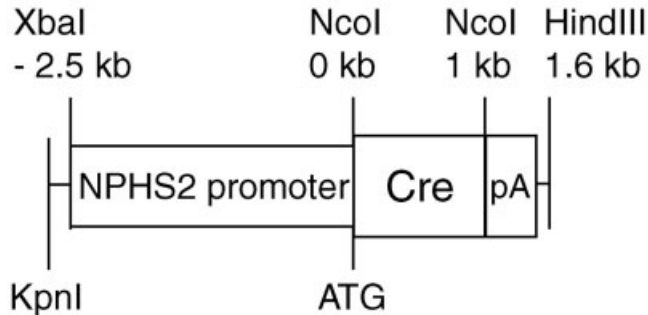


FIG. 1. Transgene construct. A 2.5-kb fragment of the human NPHS2 5' flanking region containing the entire 5' untranslated region was placed in front of a Cre recombinase cassette. The transgene was released for microinjection from prokaryotic vector sequence using KpnI and HindIII. Restriction sites are indicated with their position relative to the ATG, translation initiation codon; pA, murine protamine poly-adenylation signal.

transgenic mouse line that expresses Cre recombinase specifically in podocytes and not in other tissues.

A cassette encoding Cre recombinase was placed under the regulation of the 2.5-kb human NPHS2 promoter fragment that includes the entire 5' untranslated region of the NPHS2 (podocin) gene (Fig. 1) (GenBank accession AF487463). Twenty-four founder 2.5P-Cre mice were generated by pronuclear injection. For analysis, 2.5P-Cre founder mice were mated to homozygous ROSA26 mice, a well-characterized reporter line that expresses beta-galactosidase (β -gal) after Cre recombination (Soriano, 1999). Kidney homogenates of two doubly transgenic 2.5P-Cre/ROSA26 offspring age 4–8 weeks of each founder were screened for β -gal expression using a chemiluminescent assay. When crossed with ROSA26 mice, 8 of 24 (33%) founder lines expressed β -gal in kidney. Additional analysis of the homogenates of 14 other tissues derived from eight identified 2.5P-Cre mouse lines revealed no β -gal expression in other tissues (data not shown).

Subsequent characterization focused on one 2.5P-Cre founder line (#295). Figure 2 shows expression of β -gal in various tissues of three 2.5P-Cre(#295)/ROSA26 offspring compared to nontransgenic littermates. To assure that Cre recombination did not occur in brain tissue, the entire brain of 2.5P-Cre/ROSA26 offspring was sectioned serially and stained with X-gal. Again, no β -gal activity could be detected anywhere in the brain (data not shown). These findings are consistent with previous characterization of the 2.5-kb NPHS2 promoter fragment (Moeller *et al.*, 2002). Cryosections of kidneys from adult 2.5P-Cre/ROSA26 mice were examined histologically for β -gal expression. β -Gal expression was detected in all glomeruli (Fig. 3A). No β -gal expression was seen elsewhere in the kidney. β -Gal expression appeared to be present

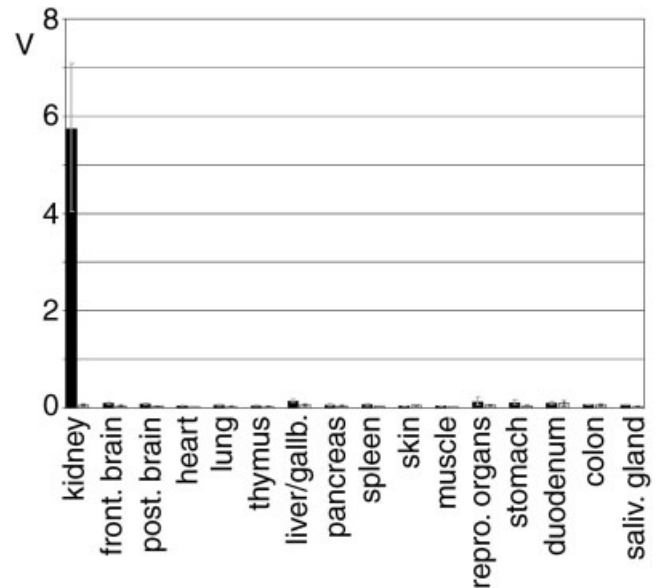


FIG. 2. Tissue screen for β -gal activity. Tissues from three doubly transgenic 2.5P-Cre/Rosa26 mice of founder line #295 and from three singly transgenic ROSA26 littermates were homogenized and β -gal activity was measured using a chemiluminescence assay (first bar in black 2.5P-Cre/ROSA26 mice; second bar in gray ROSA26 littermates). Data are shown as means of volts (V) of three independent experiments with the range indicated by a vertical line. β -Gal activity above background could be detected only in kidney lysates of doubly transgenic p2.5-Cre/ROSA26 mice. Of note, light emission measurements (in volts) cannot be compared to measurements reported in our previous studies (Moeller *et al.*, 2002) since a different luminometer was used in this study.

in all podocytes of all 2.5P-Cre/ROSA26 mice examined with no evidence for mosaicism as determined by GLEPP1 double-label immunohistochemistry (data not shown). However, ROSA26 mice express cytoplasmic β -gal after Cre excision, so that mosaicism or incomplete Cre excision in all podocytes cannot be entirely ruled out. Newborn 2.5P-Cre/ROSA26 kidneys were stained with X-gal to determine when Cre excision occurs during glomerular development. Nephrogenesis occurs in a telescoped manner in newborn kidney that allows the evaluation of all developmental stages in a single section. In newborn 2.5P-Cre/ROSA26 kidneys, β -gal expression arose in podocytes during late capillary loop stage of glomerular development and persisted in podocytes of mature glomeruli (Fig. 3B). Expression of Cre recombinase was also examined in doubly transgenic podocin-Cre/Rosa26 embryos as early as 8.5 dpc by staining whole embryos with X-gal and by assaying whole embryo lysates for β -gal activity using the chemiluminescence assay. β -Gal activity was not observed. These findings are also supported by our previous characterization of the 2.5-kb NPHS2 promoter fragment (Moeller *et al.*, 2002).

Of note, 2 out of 17 analyzed 2.5P-Cre/ROSA26 mice of founder line #295 did not express significant amounts of β -gal in the kidneys. Thus, penetrance of Cre expres-

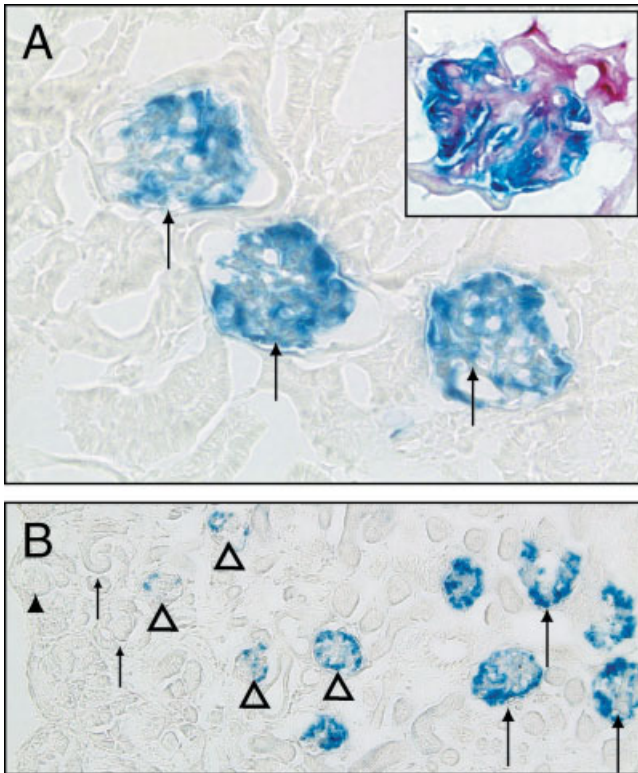


FIG. 3. A: Enzymatic X-gal staining of 4 μm cryosections of the kidney of adult mice doubly transgenic for 2.5P-Cre/Rosa26. Blue staining was seen in all glomeruli (arrows) indicating that Cre recombination—indicated by β -gal expression—occurs exclusively in podocytes in the kidney. Inset in **A** shows a glomerulus of a kidney cryosection of a p2.5P-Cre/Rosa26 transgenic mouse double-stained for β -gal (blue) and von Willebrand factor (red). No β -gal staining can be observed in endothelial cells of the glomerulus or the vascular pole. **B:** Cre recombination occurs during capillary loop stage in glomerular development. Cryosection of kidneys of doubly transgenic 2.5P-Cre/Rosa26 newborns were stained with X-gal. β -Gal expression arises during capillary loop stage of glomerular development in podocytes (empty arrowheads) and persists in podocytes of mature glomeruli (long arrows). β -Gal expression was not detected elsewhere in earlier forms of developing nephrons such as comma-shaped (full arrowhead) or S-shaped bodies (short arrows), nor elsewhere in the kidney.

sion is only 80–90%. A correlation with the animal's sex, generation, or age was not obvious. Genetic background heterogeneity might account for this observation. Currently, 2.5P-Cre mice are being backcrossed into a C57 BL/6J background.

It should be noted that F1 2.5P-Cre mice of founder line #295 were interbred to produce homozygous 2.5P-Cre offspring. At 10 weeks of age 2.5P-Cre (#295) homozygous mice displayed no overt pathology and were fertile, indicating that the integration of the transgene did not disrupt a critical endogenous gene.

The development of a mouse line expressing Cre recombinase in a truly podocyte-specific fashion should provide a valuable tool for examining gene function in the podocyte.

MATERIALS AND METHODS

Creation of 2.5P-Cre Mice

The lacZ cassette was removed from the vector p2.5P-nlacF used for the initial characterization of the 2.5-kb NPHS2 promoter fragment (Moeller *et al.*, 2002). The Cre ORF was PCR-amplified using the primers CreNcoI.fwd 5'aac cat ggg caa ttt act gac cgt aca cca3' and CreNcoI.rev 5'gtc cat ggc taa tcg cca tct tcc ag3' and inserted into the unique NcoI-site at the translation initiation codon of p2.5P-(nlacF). The entire 2.5P-Cre transgene was sequenced, liberated from prokaryotic vector sequence by HindIII and KpnI, and used for the generation of transgenic mice as described previously (Moeller *et al.*, 2000). 2.5P-Cre transgenic mice were detected by PCR as described previously (Cushman *et al.*, 2000).

β -Gal Assays

Assays of β -gal activity in tissue homogenates, X-gal stainings, and alkaline phosphatase histochemistry (rabbit polyclonal anti-vWF; Dako, Carpinteria, CA, 1:500 dilution) of cryosections of kidneys and brain were done as previously described (Moeller *et al.*, 2000) with the following modifications: an EG&G Berthold Autolumat LB953 luminometer was used to measure β -gal activity in tissues (acquisition time: 10 sec).

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