

Two Gene Fragments that Direct Podocyte-Specific Expression in Transgenic Mice

MARCUS J. MOELLER,* SILJA K. SANDEN,* ABDULSALAM SOOFI,*
ROGER C. WIGGINS,* LAWRENCE B. HOLZMAN*[†]

*Division of Nephrology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan; and [†]Department of Veterans Affairs, Ann Arbor, Michigan.

Abstract. Transgenic manipulation of the glomerular visceral epithelial cell offers a powerful approach for studying the biology of this morphologically complex cell type. It has been previously demonstrated that an 8.3-kb and a 5.4-kb fragment of the murine *Nphs1* (nephrin) promoter-enhancer drives lacZ expression in podocytes, brain, and pancreas of transgenic mice, recapitulating the expression pattern of the endogenous nephrin gene. In this present study, two truly podocyte-specific promoters were identified that drive transgene expression in podocytes without expression in extrarenal tissues in adult or embryonic mice. A 1.25-kb fragment driving a lacZ reporter gene (p1.25N-nlacF) was derived from murine *Nphs1* promoter similar to a human NPHS1 promoter fragment previously reported. Transgenic mice were generated and beta-galactosidase (beta-gal) expression was analyzed. Four of twelve founder mice were found to express beta-gal in podocytes

(33% penetrance). Expression in brain and pancreas was absent in all animals, suggesting that nephrin expression in these organs might be driven by distinct cis-regulatory elements that can be removed to obtain podocyte-specific expression. A 2.5-kb fragment derived from the human NPHS2 (podocin) gene was designed in a similar fashion to drive lacZ expression in transgenic mice (p2.5P-nlacF). Twelve of twelve NPHS2 mouse founder lines expressed beta-gal exclusively in podocytes (100% penetrance). Beta-gal activity was not observed extrinsic to the kidney in p1.25N-nlacF or p2.5P-nlacF mouse embryos at gestational time points between 8.5 d post coitus and birth. In conclusion, the 2.5-kb NPHS2 promoter fragment may be useful for podocyte-specific transgenic expression when extrarenal expression of a transgene is problematic.

Podocytes are specialized glomerular epithelial cells of the kidney. Their interdigitating foot processes surround the glomerular capillary wall facing the urinary space. The specialized intercellular junction between podocyte foot processes, called the slit diaphragm, serves as an essential component of the glomerular filter. Most glomerulopathies are associated with podocyte dysfunction marked by foot process effacement, loss of the normal structure of the podocyte intercellular junction, and functional loss of glomerular permselectivity. Identification of mutations within 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. Positional cloning identified NPHS1 as the gene mutated in patients affected by Congenital Ne-

phrotic Syndrome of the Finnish type (1). Nephrin, the protein product encoded by NPHS1, is a member of the Ig-superfamily and is localized in podocyte foot processes at the slit diaphragm (1,2). Similarly, mutations in NPHS2 were found to cause autosomal recessive steroid-resistant nephrotic syndrome (3). Initial Northern blot analyses and *in situ* hybridization studies suggested that the protein product of NPHS2, termed podocin, is expressed in podocytes only (3). NPHS2 encodes an integral membrane protein with a predicted hairpin structure of unknown function.

Studies of podocytes have been limited by insufficient model systems. Available cell culture systems do not fully reproduce the complex cytoarchitecture or the gene expression profile of the podocyte *in vivo*. Transgenic animal models targeting the podocyte are new approaches that might overcome these limitations. We have previously reported two promoter constructs derived from the murine *Nphs1* gene that drive lacZ expression in podocytes of transgenic mice with >95% penetrance (4). Transgene expression driven by either 8.3-kb or 5.4-kb *Nphs1* promoter fragments essentially recapitulates the expression of the endogenous nephrin gene. Although these promoters drive podocyte-specific expression in the kidney, they also drive expression in pancreas and in structures of the developing and adult brain (reference 4; unpublished data). The purpose of this study was to identify a truly podocyte-specific promoter that might be useful for gain-of-function or conditional gene-ablation experiments in transgenic mice.

Dr. Moeller and Dr. Sanden contributed equally to this work.
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Correspondence to Dr. Lawrence B. Holzman, University of Michigan Medical School, 1560 MSRB II, Ann Arbor, Michigan 48109-0676. Phone: 734-764-3157; Fax: 734-763-0982; E-mail: lholzman@umich.edu

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Materials and Methods

Plasmid Construction

The *Homo sapiens* chromosome 1 BAC clone RP11–545A16 (GenBank accession no. AL160286) containing the 5' flanking region of the human NPHS2 gene (3) was obtained from Roswell Park libraries (Oakland, CA). PCR oligonucleotide primers were designed to amplify 2.5-kb of the podocin promoter region 5' of the initiation codon (podocinPE.fwd: act cta gag ccc tcc tat tta gtc tct ctg cca cc; podocinPE.rev: tcc gcg ccc tcc tct cca tgg tca gag ctg). The amplified segment was sequenced (GenBank accession no. AF487463). An *Xba*I site at the 5' end and an *Nco*I site at the initiation codon (ATG) were created by PCR-based mutagenesis. The 2.5-kb podocin-promoter fragment was cloned into the *Xba*I and *Nco*I sites of the pnlacF reporter vector (5). Similarly, PCR oligonucleotide primers were designed to amplify 1.25-kb of the *Nphs1* (nephin-gene) promoter region 5' of the initiation codon (*Nphs1*PE.fwd: agg tct aga ggt gag agg ttt gta g; *Nphs1*PE.rev: act gtg gct tcc tta gct). The vector p5.4N-nlacF containing 5.4-kb of the *Nphs1* 5' flanking region was used as a template (4). A *Xba*I site at the 5' end was inserted into the primer *Nphs1*PE.fwd. A *Nco*I site was already present at the initiation codon (ATG) of the template p5.4N-nlacF. The 1.25-kb *Nphs1*-promoter fragment was cloned into the *Xba*I and *Nco*I sites of the pnlacF reporter vector (5). The full-length human podocin cDNA was amplified from IMAGE clone AI672038 using the primer podocin-HA.fwd (5' tga att cga gag gag ggc gcg gag c) containing an *Eco*RI-site and podocinHA.rev (5' tat taa gct tat ata aca tgg gag agt c) containing an *Hind*III-site. The resulting 1145-bp product was cloned in frame behind three hemagglutinin-tags previously inserted into pcDNA3.1 using *Eco*RI and *Hind*III. All constructs were DNA sequenced to assure amplification fidelity.

Generation of Transgenic Animals

Purified transgene constructs were microinjected into F2 hybrid mouse eggs from (C57BL/6 X SJL) F1 parents at a concentration of 2 to 3 ng/ μ l (6). Eggs were surgically transferred to 0.5 d post coitus (dpc) pseudopregnant ICR females (recipients). The transgenic founder animals were bred to C57BL/6J wild-type mice. (C57BL/6 X SJL) F1 mice and ICR mice were obtained from the Jackson Laboratory (Bar Harbor, ME). The University of Michigan Committee on Use and Care of Animals approved all procedures using mice. All work was conducted in accord with the principles and procedures outlined in the National Institutes of Health Guidelines for the Care and Use of Experimental Animals.

Transgene Detection by PCR

A PCR assay was established to identify transgenic mice as described previously (4). Transgenes were detected by PCR using the following primers: LacZ.fwd, 5' TTC ACT GGC CGT CGT TTT ACA ACG TCG TGA 3'; LacZ.rev, 5' ATG TGA GCG AGT AAC AAC CCG TCG GAT TCT 3'. The size of the expected amplification product was 364 bp.

Beta-Galactosidase Staining of Kidneys, Brain, and Embryos

Beta-galactosidase staining was performed on 8- μ m cryosections of adult and newborn kidneys from transgenic mice and nontransgenic littermates as described previously (4). Briefly, adult mice were perfused with 4% paraformaldehyde. Tissues were resected and frozen in liquid nitrogen. Newborn kidneys were fixed for 15 min in 4% paraformaldehyde. Eight-micrometer cryosections were cut and post-

fixed with 4% paraformaldehyde for 5 min. The sections were then incubated overnight at 30°C with X-gal staining solution (1 mg/ml X-gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM MgCl₂ in phosphate-buffered saline [PBS]), washed with PBS, dehydrated through grades of ethanol and xylene, and mounted. After beta-gal staining, slides were double-stained with WT1 for podocyte counts. Newborn kidney sections were double-stained with GLEPP1 for developmental analysis before the dehydration steps. Whole mounts (up to 13 dpc) were generated with timed pregnancies by mating heterozygous transgenic males with wild-type females (C57BL/6J). Yolk sack DNA was extracted to identify transgenic embryos as described above. Whole mounts were fixed for 15 to 35 min in 1.5% paraformaldehyde and stained overnight at 30°C in X-gal staining solution as described previously (4). Embryos were dehydrated in 100% methanol and cleared in benzyl benzoate: benzyl alcohol 2:1 (vol/vol). Eight-micrometer brain serial cryosections were cut, and every fifth section was stained for beta-gal.

Immunoperoxidase and Alkaline Phosphatase Histochemistry

After beta-galactosidase staining, the kidney sections were treated with Retrieve-All 1 (Signet Pathology Systems, Dedham, MA) for 2 h at 90°C. Immunoperoxidase and alkaline phosphatase staining was performed according to the Vectastain ABC and ABC-AP (Vector Laboratories, Burlingame, CA) kit's instructions. The sections were incubated with anti-GLEPP1 and anti-WT1 antibody. Sections were subsequently developed by immunoperoxidase using diaminobenzidine (DAB; Sigma, St. Louis, MO) or alkaline phosphate using the Vector Red substrate kit (Vector Laboratories).

LacZ Tissue Screen

Assays of beta-gal activity in tissue homogenates were performed using a chemiluminescence assay as described by Moeller *et al.* (4). Briefly, tissues were homogenized in lysis buffer, heat-inactivated to suppress endogenous beta-gal activity (48°C; 50 min), and incubated with reaction buffer (Tropix, Bedford, MA) for 1 h at 25°C. Light output was then measured in mV using a 1251 luminometer (BioOrbit, London, UK).

Cell Culture

COS7 cells (American Type Culture Collection, Rockville, MD) were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin, and streptomycin. Two $\times 10^5$ cells plated on a 35-mm tissue culture dish were transiently transfected with 1 μ g of the full-length podocin-HA expression plasmid using FuGene 6 (Roche, Mannheim, Germany) according to the manufacturer's protocol. After 24 h, cells were lysed in 300 μ l RIPA buffer (0.1% sodium dodecyl sulfate [SDS], 0.5% deoxycholate, 1% Triton X-100, 150 mM NaCl, protease inhibitor cocktail (Complete EDTA-free, Roche Diagnostics, Mannheim, Germany) as described previously (7).

Glomerular Isolation

Kidneys were obtained from adult C57 BL/6 mice. Glomeruli were enriched by sieving. The average purity of the glomerular preparations was approximately 60%. Glomeruli were lysed in RIPA buffer containing protease inhibitors (100 μ l glomeruli per ml extraction buffer).

Antibodies

Rabbit antibody to the COOH-terminal 17 amino acid residues of human and mouse podocin (100% sequence identity) was raised by immunizing rabbits (performed at Lampire Biological Laboratories Inc., Pipersville, PA) with a keyhole limpet hemocyanin-conjugated synthetic peptide (sequence N→C: SKPVEPLNPKKKDSPML). The peptide was synthesized by Research Genetics (Invitrogen Corp., Carlsbad, CA). Polyclonal podocin immune serum was diluted 1:100 in 1% bovine serum albumin in PBS for immunofluorescence and 1:2000 for Western blots in 1% skim milk in PBS. Monoclonal antibody against the rat GLEPP1 extracellular domain (1B4) (8) and FITC-conjugated goat anti-mouse IgG (ICN Biomedical Inc., Costa Mesa, CA) or Cy3-conjugated anti-rabbit IgG (1:200) (Jackson Laboratories, Westgrove, PA) were used for dual label immunofluorescence microscopy. The primary antibodies used for immunohistochemistry on beta-gal stained sections were anti-rabbit GLEPP1 and a commercially available rabbit polyclonal antibody to Wilms' tumor-1 protein (WT1, Santa Cruz Biotechnology, Santa Cruz, CA).

Indirect Immunofluorescence

Fresh frozen cryostat sections (4 μm) were fixed with ice-cold acetone blocked by incubation with 10% goat serum. They were then incubated with appropriate antibody as described previously (2). Sections were mounted with the ProLong Antifade Kit (Molecular Probes, Eugene, OR), viewed, and photographed.

Western Blot Analyses

For Western blot analysis, 30 μl of cell lysate or glomerular extract were separated under reducing conditions by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). As previously described (7), proteins were transferred to a nitrocellulose membrane (Schleicher & Schuell Inc., Keene, NH), blocked for 1 h in 4% skim milk in PBS, and incubated with the indicated antibody diluted in 1% skim milk in PBS. Blots were developed using ECL chemiluminescent reagent (Amersham, Arlington Heights, IL) and exposed (Biomax MR; Kodak, Cedex, France).

Results

Generation and Identification of Transgenic Mice

Two gene promoter fragments were chosen for analysis to identify a podocyte-specific promoter that drives renal limited transgene expression. Initially, a 1.25-kb fragment of the murine Nphs1 5' flanking region was selected for analysis; a similar construct derived from the human NPHS1 gene was reported previously to drive reporter gene expression only in podocytes (9). Additionally, a 2.5-kb fragment of the NPHS2 5' flanking region was selected because published work suggested that its gene product podocin is expressed in a podocyte-specific fashion (3). The Nphs1 transgene construct denoted p1.25N-nlacF was prepared by placing a bacterial lacZ reporter with a nuclear localization signal under the control of a mouse Nphs1 promoter. This promoter was comprised of 1.25-kb of flanking sequence 5' to the translation initiation codon and included the entire 5' UTR. The NPHS2 transgene denoted p2.5P-nlacF was prepared in a similar fashion. A map of the two transgenes is shown in Figure 1. Transgenic mice were generated by pronuclear injection. Twelve transgenic p1.25N-nlacF founders (F_0) out of 134 littermates (9%) and 18 transgenic p2.5P-nlacF founders of 88 littermates (20%) were iden-

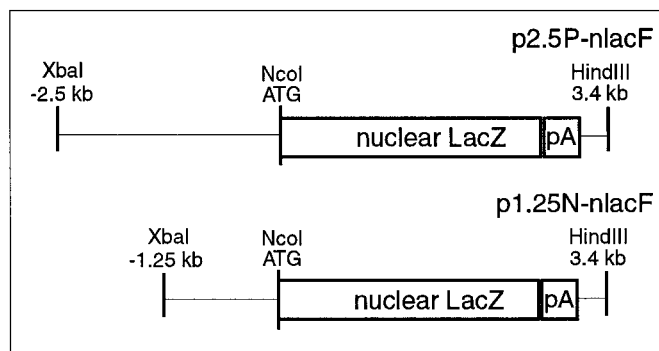


Figure 1. Map of reporter constructs used to generate transgenic mice. A 2.5-kb DNA fragment of the 5' flanking sequence of the human NPHS2 (podocin) gene containing the entire 5' untranslated region was amplified by PCR from the human BAC clone RPC1 11545 A16 (GenBank accession no. AF487463). This 2.5-kb fragment was cloned into the pnlacF reporter vector using *Xba*I and *Nco*I sites (p2.5P-nlacF) to drive expression of a LacZ cassette with a nuclear localization signal. The murine protamine poly-adenylation signal is indicated (pA). Similarly a 1.25-kb DNA fragment of the murine Nphs1 gene was PCR amplified and cloned into the pnlacF reporter (p1.25N-nlacF). The prokaryotic vector sequence was released using *Xba*I and *Hind*III prior to pronuclear microinjection.

tified by PCR analysis of genomic DNA isolated from tail biopsies.

Tissue Expression of Beta-Galactosidase in Transgenic p1.25N-nlacF and p2.5P-nlacF Mice

Tissue specificity of the two transgenes was examined using a sensitive chemiluminescence method in F_0 mice at 6 to 8 wk postgestation. Beta-gal activity was measured in a survey of tissue homogenates from transgenic founders and was compared with those from wild-type littermates. Beta-gal activity significantly above control was detected in kidneys of 4 of 12 p1.25N-nlacF transgenic founder lines tested (33% penetrance). In comparison, all of 12 p2.5N-nlacF transgenic founder lines tested expressed beta-gal above control in the kidney (100% penetrance). Beta-gal expression could not be detected in tissues other than kidney in either p1.25N-nlacF or p2.5P-nlacF mice (Figure 2).

Beta-Gal Expression in Kidney

Cryosections of kidneys from several transgenic founders (or their F_1 offspring) derived from both constructs were enzymatically stained overnight to detect beta-gal activity. Analysis of histology showed that nuclear lacZ staining was restricted to podocytes in kidneys of adult transgenic mice (Figure 3). To examine whether beta-gal expression was present in all glomerular podocytes, X-gal-stained sections were stained by immunohistochemistry for WT1. By this analysis, 85 to 100% of podocytes in each glomerulus stained positive for beta-gal (Figure 3B, inset). The percentage of podocytes per glomerulus that stained positive for beta-gal appeared to depend on the quality of perfusion fixation. Because nephrin expression had been noted previously in brain,

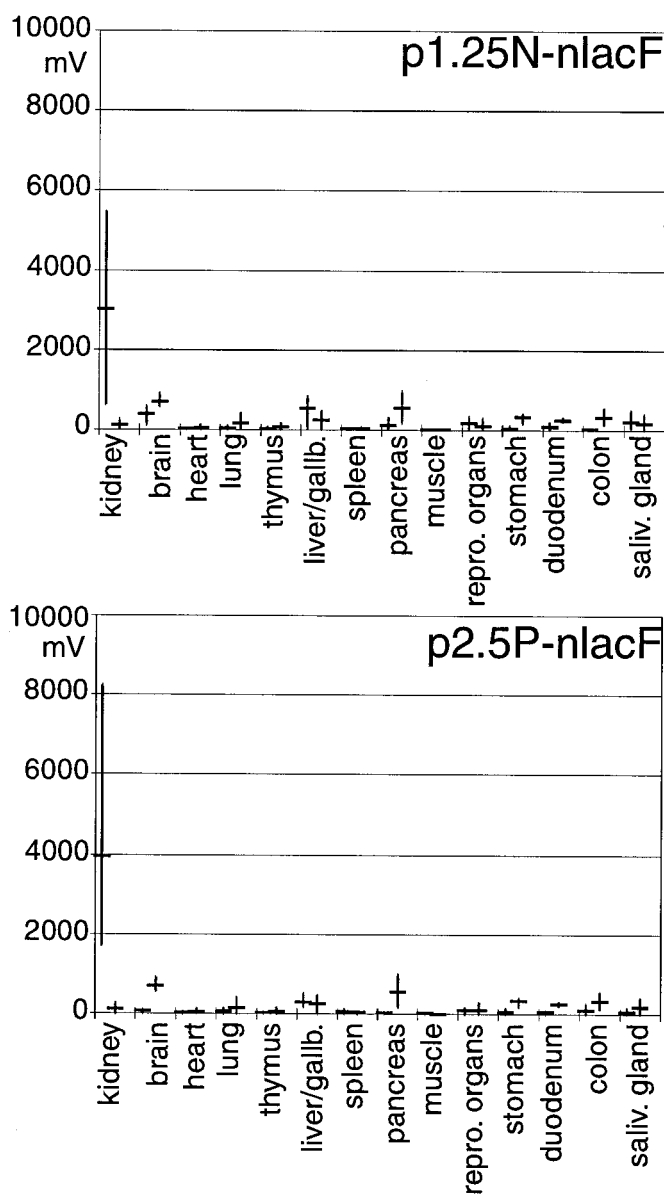


Figure 2. Tissue expression of beta-galactosidase (beta-gal) in transgenic and wild type mice. Tissues from three transgenic founders of each construct were homogenized, and beta-gal activity was measured in heat-inactivated lysates using a chemiluminescence assay (first bar, transgenic animals; second bar, wild type; crosshatch, mean). Data are shown as light output (mV) of three independent experiments with the maximal and minimal value indicated by a vertical line. Beta-gal activity above background was detected only in kidneys of both the p2.5P-nlacF and p1.25N-nlacF transgenic founders.

adult brain was serially sectioned and stained for beta-gal expression. Consistent with chemiluminescence analysis, no staining for beta-gal expression was detected in brain (data not shown). Expression of both transgenes was also evaluated in newborn kidneys because all developmental stages of metanephrogenesis can be evaluated simultaneously in these kidneys. In p1.25N-nlacF newborn mice, beta-gal expression was first detected during the capillary loop stage of glomerular

development. Similarly, beta-gal expression was observed during the capillary loop stage in p2.5P-nlacF newborn kidneys (Figure 3C). Endogenous GLEPP1 protein is first expressed in podocyte progenitor cells and precedes the expression of beta-gal driven by the 2.5-kb podocin promoter. No staining was seen elsewhere in the kidney of either p1.25N-nlacF or p2.5P-nlacF mice.

Beta-Galactosidase Expression during Embryonic Development

Whole-mount embryos from three transgenic founders of each construct were analyzed for beta-gal expression at time points between 7.5 dpc and 11.5 dpc. Wild-type littermates served as negative controls. Embryos older than 9.5 dpc were cleared before evaluation. At evaluated time points, no significant beta-gal staining was detected in any of the transgenic embryos of either construct (Figure 3, E through G). Homogenates from whole 13.5 dpc embryos and from corresponding placenta were used to confirm that no beta-gal expression was detected using the chemiluminescence assay.

Expression of Endogenous Podocin

To allow comparative studies of endogenous podocin expression with transgene expression driven by the 2.5-kb NPHS2 promoter fragment, a rabbit antiserum was raised against a 17-residue synthetic peptide encompassing the COOH-terminus of podocin. The podocin antibody specifically recognized a 53-kD band in lysates from COS7 cells transiently transfected with full length podocin-HA cDNA. This band was not present in COS7 cell lysates transiently transfected with the control vector pcDNA3.1-HA (Figure 4 D, middle panel). In mouse glomerular extract, the antiserum reacted with a major 39-kD and also with a faint 48-kD band (Figure 4D, middle panel). None of the described bands could be detected using preimmune serum or after preadsorption of the antiserum with unconjugated peptide (Figure 4D, left and right panels).

In immunofluorescence studies on adult mouse and rat kidney cryosections, the antibody reacted in a glomerulus-specific pattern. Rat kidney sections were double-labeled with anti-podocin and monoclonal anti-GLEPP1 antibody. Within the kidney, GLEPP1 is expressed solely in podocytes (8). Both antibodies co-localized within the glomerulus (Figure 4, A and B). Similarly, colocalization was observed with anti-podocin and anti-synaptopodin antibody (data not shown). No other structures within the kidney were detected by podocin antiserum. To determine the expression pattern of the endogenous podocin during development, cryosections of newborn kidneys from C57 BL/6 mice were stained with anti-podocin antibody. As shown in Figure 4C, podocin expression arises during capillary loop stage and persists in mature glomeruli. It can be concluded that the onset of NPHS2-driven transgene expression is similar to that of endogenous podocin expression in the developing kidney.

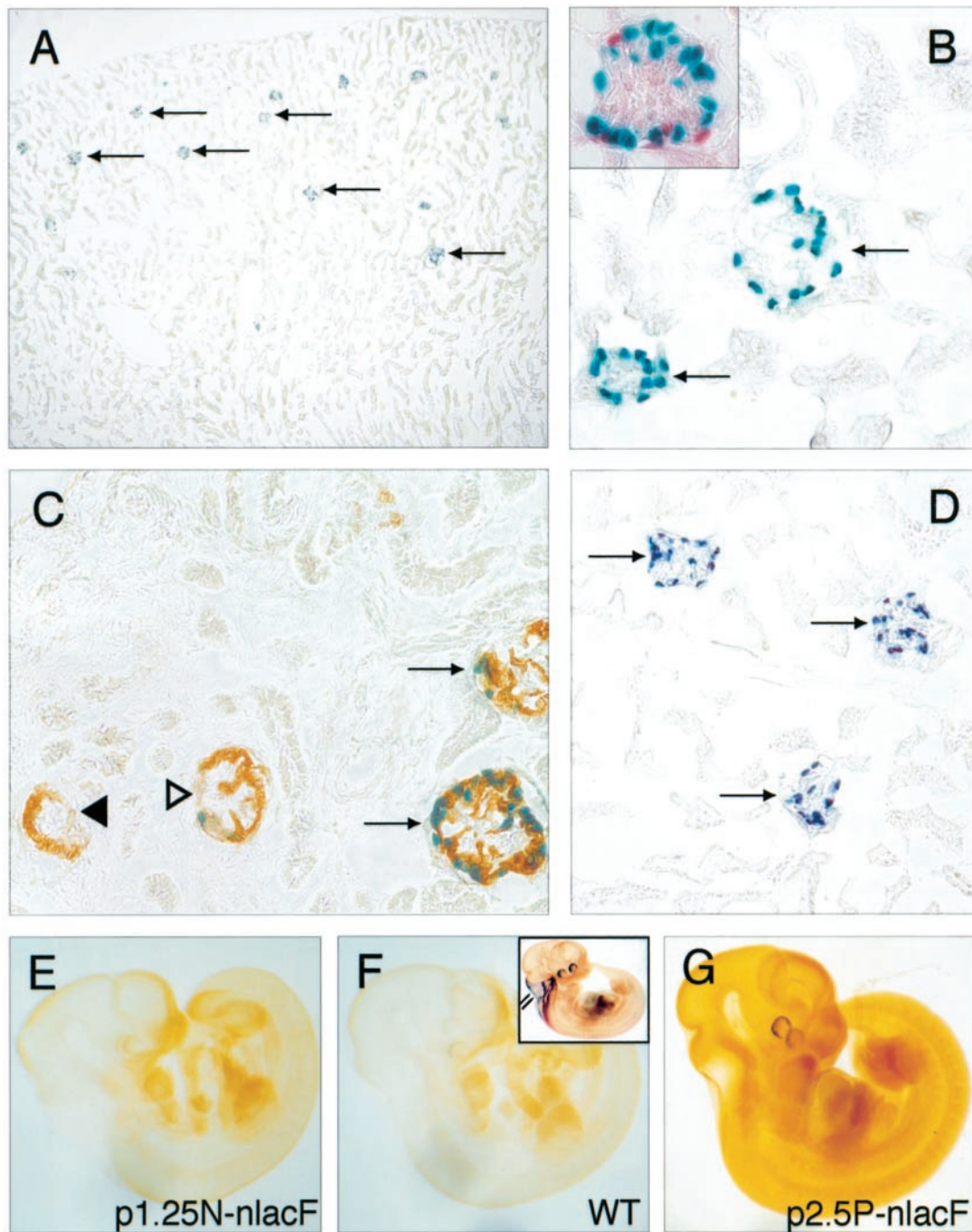


Figure 3. Analyses of beta-gal expression in kidneys of adult transgenic mice and during embryonic development. (A) Enzymatic beta-gal staining on 8- μ m cryosections of the kidney of p2.5P-nlacF transgenic mice. Beta-gal is expressed in glomerular nuclei in the renal cortex (arrows). No beta-gal activity can be detected elsewhere in the kidney. (B and D) Higher magnification of single glomeruli of p2.5P-nlacF (B) and p1.25N-nlacF (D) transgenic mice. Beta-gal-expressing nuclei are distributed in a peripheral pattern in the glomerulus consistent with podocyte nuclei (arrows). Inset in panel B shows a glomerulus of a kidney cryosection of a p2.5P-nlacF transgenic mouse double-stained for beta-gal (blue) and WT1 (red). This technique visualizes podocyte nuclei undetected by X-gal staining because blue X-gal staining masks red WT1 staining. (C) Cryosections of kidneys of p2.5P-nlacF transgenic newborns were double-stained with X-gal (blue) and GLEPP1 (brown). Beta-gal expression arises during capillary loop stage of glomerular development in podocyte nuclei (empty arrowhead) and persists in podocytes of mature glomeruli (arrows). In comparison, GLEPP1 expression arises at an earlier point during glomerular development (full arrowhead). Beta-gal expression was not detected elsewhere in earlier forms of developing nephrons or elsewhere in the kidney. (E through G) Transgenic embryos at 10.5 dpc were generated by timed pregnancies, fixed, and submitted to whole mount X-gal staining overnight. Transgenic embryos were identified by PCR (E, p1.25N-nlacF; G, p2.5P-nlacF); and wild-type littermates served as controls (F). Embryos were cleared before imaging. No beta-gal expression could be detected in multiple sets of embryos ranging from 8.5 to 13.5 dpc in age. Pigmentation of the eye in panel G is not due to beta-gal staining. For comparison, positive X-gal staining is shown in the inset of panel F in the hindbrain (arrows) and notochord of p5.4N-nlacF transgenic embryos.

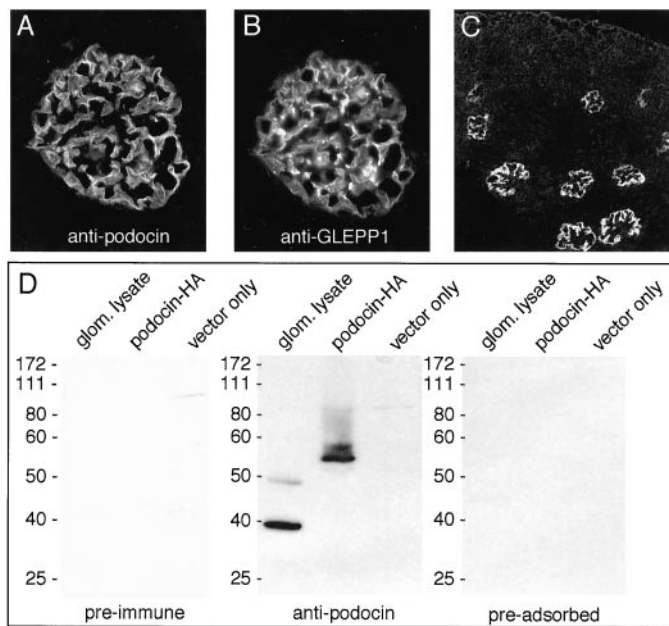


Figure 4. Characterization of a novel rabbit anti-podocin antibody. A polyclonal anti-podocin antiserum was raised in rabbits using a synthetic peptide of the last 17 amino acid residues of the COOH-terminus of podocin coupled to KLH. (A and B) Double-label immunofluorescence with rabbit anti-podocin (A) and mouse anti-GLEPP1 antiserum (B) on cryosections from adult rat kidney. Podocin specifically co-localizes with GLEPP1 in glomeruli. Other structures of the kidney did not stain. (C) Cryosection of newborn kidneys stained with rabbit polyclonal podocin antiserum. Podocin expression arises during the capillary loop stage of glomerular development and persists in mature glomeruli. No expression was detected in any other structure of the newborn kidney. Podocin expression was not detected in immature podocytes of comma- and S-shaped metanephric structures, and no specific labeling was found elsewhere in the kidney. (D) Glomerular lysates and lysates from COS7 cells transfected with HA-tagged full-length podocin cDNA or vector control were run on 10% SDS-PAGE, transferred to a nitrocellulose membrane, and immunoblotted with anti-podocin antiserum (middle panel), pre-immune serum (left panel), or with antiserum preadsorbed with the peptide used to immunize the rabbits (right panel). Podocin antiserum specifically recognizes a 39-kD and less intense 48-kD product in glomerular lysates. The reactivity could be specifically blocked by prior incubation of the antiserum with the peptide (right panel).

Discussion

The aim of this study was to identify a promoter that drives expression of transgenes selectively in podocytes. We expect that this approach will allow manipulation of podocytes *in situ*. An expression system with a podocyte-specific promoter can be used for experiments that express gain-of-function or dominant-negative proteins and can be used to create conditional gene deletion models.

Previously, we reported that transgenes could be expressed in podocytes of transgenic mice using an 8.3-kb and 5.4-kb fragment derived from the murine *Nphs1* promoter. However, these promoter fragments were found to also drive expression in the brain and pancreas, recapitulating the expression pattern

of the endogenous *nephrin* gene (4,10). To circumvent undesired phenotypes arising from extrarenal expression of transgenes driven by these promoters, we characterized two additional promoter fragments that drive expression specifically in podocytes without expression in any other adult or embryonic tissue.

The first promoter fragment analyzed in this study was derived from the murine *Nphs1* gene. A 1.25-kb human *NPHS1* promoter fragment was previously reported to be capable of driving expression in podocytes of transgenic mice (9). The results of this study suggested that expression in brain and pancreas might be driven by distinct *Nphs1* cis-regulatory elements that could be removed to obtain a podocyte-specific promoter. To test this hypothesis, a 1.25-kb promoter fragment derived from the murine *Nphs1* gene was used to generate transgenic mice. Indeed, deletion of a 4.15-kb region located immediately 5' of the 1.25-kb *Nphs1* promoter fragment resulted in selective abrogation of transgene expression in the brain and pancreas. Extrarenal transgene expression in the brain was also abrogated during embryonic development. The transcription initiation site of the murine *Nphs1* promoter has been mapped to 415 bp upstream of the translation initiation codon (Grunkemeyer JA and Kalluri R; GeneBank accession no. AF191090). Therefore, 835 bp of *Nphs1* 5' flanking sequence and 415 bp of 5' untranslated region are present in the p1.25N-nlacF construct used in this study. Interestingly, a 60-bp region that has previously been found to be 84% identical to a region in the human *NPHS1* promoter sequence is located immediately upstream of the +1 site of the murine *Nphs1* promoter. This region also contains an AP-1 binding site (100% core identity; 88% matrix similarity) (4). Whether this conserved region at the +1 site contains important cis-acting elements for expression specifically in podocytes remains to be investigated.

Truncating the murine *Nphs1* promoter to achieve podocyte-specific transgenic expression created potential disadvantages. Penetrance was reduced relative to other promoter constructs tested (33% versus >95%). Furthermore, the level of lacZ expression appeared to be reduced. Therefore the utility of the 1.25-kb *Nphs1* promoter for driving podocyte-specific expression might be limited.

The second promoter fragment tested in this study was derived from the human *NPHS2* gene. This promoter was selected for investigation because previously published work suggested that this gene is expressed in a podocyte-specific pattern (3). Two and one half kb of the human *NPHS2* promoter-enhancer region, including the entire 5' untranslated region, were found to express beta-galactosidase exclusively in podocytes. *NPHS2*-driven transgene expression arises during the capillary loop stage of glomerular development. These findings confirm previously published data demonstrating that podocin mRNA is expressed exclusively in podocytes and that earliest expression of podocin transcript arises in podocytes of late S-shaped bodies of fetal and neonatal human kidneys using *in situ* hybridization (3). It is possible that endogenous podocin is also expressed in other cells or tissues apart from the podocyte. However, careful examination of our p2.5P-nlacF transgenic mice, particularly in the brain, did not reveal any extrarenal expression of the reporter gene. Using a polyclonal

anti-podocin antibody, endogenous podocin expression was first detected during capillary loop stage. Of note, transgene expression driven by the 2.5-kb NPHS2 promoter fragment also arose during the capillary loop stage of glomerulogenesis.

The podocin antiserum used in this study selectively detects podocytes by indirect immunofluorescence of adult and newborn mouse kidneys. GLEPP1 and synaptopodin co-localized in normal mouse glomeruli. The molecular weight for podocin was predicted to be 42 kD (3). The podocin antiserum recognized a 39-kD band in mouse glomerular extract by SDS-PAGE under reducing conditions. This is in agreement with work published recently (11). The significance of a second product of 48 kD that is recognized by this antiserum remains unknown.

Both the Nphs1- and NPHS2-derived promoter constructs initiate transgene expression during the capillary loop stage of glomerulogenesis. Podocyte foot process formation and assembly of the specialized intercellular junction characterize the capillary loop stage. The synchronized regulation of expression of nephrin and podocin and the fact that the two proteins interact and co-localize at the slit diaphragm suggests that the two proteins serve a functionally related purpose during foot process formation (11,12). It is possible that nephrin and podocin expression is regulated by the same as yet unidentified transcription factor.

In conclusion, podocyte-specific transgene expression can be achieved by using a 1.25-kb Nphs1 promoter fragment. However, we have chosen to employ the 2.5-kb NPHS2 promoter fragment in additional experiments aimed at obtaining transgenic expression of proteins specifically in podocytes. This promoter construct drives podocyte-specific expression with higher penetrance than the 1.25-kb Nphs1 promoter. For this reason, the 2.5-kb NPHS2 promoter fragment was used to create a mouse line with podocyte-specific expression of Cre recombinase (Moeller MJ, Holzman LB, unpublished data). This and additional experiments will determine whether the 2.5-kb NPHS2 promoter fragment reliably drives transgene expression in podocytes at a level sufficient for intended effect.

Studies of podocyte biology remain limited due to lack of appropriate *in vivo* models to study molecular mechanisms. NPHS2 promoter-driven expression systems have good potential to achieve transgene expression in podocytes without incurring the detrimental effects of extrarenal expression during embryogenesis or adulthood.

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