Evaluation of a New Tool for Exploring Podocyte Biology: Mouse *Nphs1* 5′ Flanking Region Drives LacZ Expression in Podocytes

MARCUS J. MOELLER,* IULIA A. KOVARI,* AND LAWRENCE B. HOLZMAN*†
*Division of Nephrology, Department of Internal Medicine, University of Michigan Medical School, and †Department of Veterans Affairs, Ann Arbor, Michigan.

Abstract. Study of podocyte biology has been hampered by limitations in available experimental models that both recapitulate the *in vivo* phenotypes of this cell and can be readily and specifically manipulated at the molecular level. Transgenic manipulation of the podocyte represents one approach that might circumvent these limitations. The purpose of this study was to identify a promoter-enhancer that would direct the expression of transgenes in a podocyte-specific manner. The nephrin (*Nphs1*) promoter was considered a good candidate for this purpose, because nephrin was thought to be expressed exclusively in podocytes. Two independent BAC clones that contained the murine *Nphs1* gene were identified. An 8.3-kb and a 5.4-kb fragment containing the 5′ flanking promoter sequence were identified and characterized. Two constructs were generated by placing a bacterial lacZ reporter with a nuclear localization signal under the control of these two DNA fragments. Mice transgenic for both constructs were generated. Using a chemiluminescence assay, β-galactosidase activity significantly above control was detected only in tissue homogenates of kidneys and brain of transgenic mice. In X-gal stained sections of transgenic adult kidneys, only podocyte nuclei expressed β-galactosidase. In adult brain examined by tissue sectioning, β-galactosidase activity was confined to a discrete area in the medulla. Identical patterns of β-galactosidase expression were observed in multiple transgenic founders, suggesting that the expression pattern observed was independent of the site of transgene integration. The developmental expression of β-galactosidase in transgenic embryos was also analyzed. Transgenes regulated by this promoter should be useful for studying the biology of gene products that regulate podocyte phenotype and function.

Diseases of the renal glomerulus that result in the nephrotic syndrome are important causes of morbidity and mortality that affect both adults and children. Unfortunately, the molecular mechanisms that govern development of the nephrotic syndrome are poorly understood (1,2). Glomerular visceral epithelial cells seem to play a central role in maintaining the selective filtration barrier of the renal glomerulus. These cells are also termed *podocytes* to describe the foot-like appearance of numerous interdigitating processes that arise from their cell bodies and cover glomerular capillary walls. Glomerular filtrate passes across the specialized intercellular junction—also termed the *slit diaphragm*—formed at the interface of these interdigitated foot processes (3).

In response to glomerular injury, podocytes undergo a dramatic change in morphology, termed *foot process effacement*, resulting in retraction and spreading of foot processes and alteration in their intercellular junctions (4). Foot process effacement is a fluid and reversible process that correlates closely with the development of proteinuria both in human disease and in experimental models (2,5). The cellular and molecular mechanisms that govern these changes in podocyte structure are incompletely defined, although recent progress has ignited significant interest in this area (6–8). Clearly, foot process effacement requires the interplay of multiple cellular events, including rearrangement of the structure of the cytoskeleton, spreading or dynamic cell adhesion of the foot process over the basement membrane, and disassembly or reassembly of the intercellular junction that comprises the slit diaphragm. Given the correlation between alterations in podocyte morphology and the occurrence of proteinuria, better understanding the molecular mechanisms that govern these changes should provide insight into the mechanisms of 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 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 (2,9). However, these cell lines do not reproduce the complex three-dimensional cyto-architecture of the podocyte *in situ* and lack typical foot processes that possess the specialized intracellular junction structures of this cell. These shortcomings limit the utility of these cell lines for

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Correspondence to Dr. Lawrence B. Holzman, University of Michigan Medical School, 1560 Medical Science Research Building II, Ann Arbor, MI 48109-0676. Phone: 734-764-3157; Fax: 734-763-0982; E-mail: lholzman@umich.edu
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studying the functional role of specific molecules in governing podocyte morphology and the maintenance of glomerular filter integrity.

Transgenic manipulation of the podocyte is one approach that might circumvent these limitations. The purpose of this study was to identify a promoter-enhancer that would direct the expression of transgenes in a podocyte-specific manner for later use in experiments aimed at manipulating the podocyte *in situ*. The murine nephrin (*Nphs1*) promoter was considered a good candidate for this purpose, because previous studies suggested that nephrin was expressed exclusively in podocytes (6,10,11).

Materials and Methods

Identification and Cloning of the Nphs1 Gene

A 0.5-kb mouse nephrin cDNA fragment (10) was used to identify two mouse 129-SvJ mouse genomic BAC clones (235 and 135 kb; Genome Systems Inc., St. Louis, MO). Upon restriction mapping, these clones were found to contain the entire *Nphs1* coding region and at least 60 kb of genomic sequence 5′ to the initiation codon.

Construction of the Reporter Transgene

The β-galactosidase (β-gal) reporter plasmid used in this study was derived from pnlacF (12). pnlacF encodes *Escherichia coli* β-gal possessing a nuclear localization signal from simian virus fused to its amino terminus. A 14-kb *Hin*dIII cDNA fragment containing the 5′ nephrin coding region was cloned into puc18. To remove most of the nephrin coding region, a 5.5-kb *Sal*I fragment was released using a *Sal*I site 0.24-kb 3′ to the initiation codon and a *Sal*I site in the multiple cloning region of puc18. The resulting construct (Nphs1-PE-puc18) containing an 8.7-kb insert with 8.3 kb of the promoter-enhancer region 5′ of the initiation codon and 0.2 kb of the nephrin coding region was DNA sequenced using the GPS™-1 Genome Priming System (New England Biolabs, Beverly, MA) for random primer insertion and an automated DNA sequencer. An *Nco*I site was created at the initiation codon ATG by PCR-based mutagenesis and was verified by DNA sequencing (TGATG to CCATG). The 8.3-kb full-length nephrin promoter-enhancer was released using *Nco*I and *Hin*dIII. After blunt-ending the *Hin*dIII site (DNA Polymerase I Large [Klenow], Promega, Madison, WI), the 8.3-kb fragment was cloned into the *Sma*I and *Nco*I sites of the pnlacF reporter vector (p8.3N-nlacF). A second, shorter 5.4-kb fragment of the nephrin promoter-enhancer was subcloned into pnlacF using *Xba*I and *Nco*I (p5.4N-nlacF).

Sequence Analysis

The identified murine *Nphs1* 5′ flanking sequence (8298 bp) was compared with the 5′ flanking sequence of the human *NPHS1* gene (41.5 kb of cosmid clone R33502, Genbank accession number AC002133) using BLASTN 2.0.11 (default parameters) to identify conserved areas. With the use of the MatInspector V2.2 search engine and the TRANSFAC database (core sim = 1, matrix sim > 0.97; maximal stringency), potential transcription factor binding sites were identified in these conserved areas (13).

Generation of Transgenic Mice

The p8.3N- and p5.4N-nlacF construct (11.7 and 8.8 kb) were liberated from the plasmid vector backbone by digestion with *Kpn*I and *Hin*dIII, separated by agarose gel electrophoresis, isolated from the gel, and purified by Nuclespin columns (Clontech, Palo Alto, CA). The purified DNA fragment was microinjected into F2 hybrid eggs from (C57BL/6J X SJL/J) F1 parents at a concentration of 2 to 3 ng/μl (14). Eggs were transferred to day 0.5 postcoitus (dpc) pseudopregnant ICR females. Founder transgenic mice were mated to C57BL/6J or SJL/J wild-type mice. (C57BL/6J X SJL/J)F1 mice were obtained from The Jackson Laboratory (Bar Harbor, ME); ICR mice were obtained from Harlan (Indianapolis, IN). The University of Michigan Committee on Use and Care of Animals approved all procedures that used mice. All work was conducted in accordance with the principles and procedures outlined in the NIH Guidelines for the Care and Use of Experimental Animals.

Identification of Transgenic Mice

Transgenic mice were identified using a PCR strategy on DNA recovered from tail biopsies. The tails of 3-wk-old mice were lysed in TNES solution (10 mM Tris [pH 7.5], 400 mM NaCl, 100 mM ethylenediaminetetraacetate, 0.6% sodium dodecyl sulfate) containing 0.55 mg/ml proteinase K overnight at 55°C. The DNA was isolated using the DNeasy Tissue Kit (Quiagen, Valencia, CA). The transgene was identified by PCR (*Taq* DNA Polymerase, Promega) using the primers LacZ.fwd: TTC ACT GGC CGT CGT TTT ACA ACG TCG TGA and LacZ.rev: ATG TGA GCG AGT AAC AAC CCG TCG GAT TCT (200 ng of genomic DNA, 30 cycles, Tm = 60°C). The PCR product was detected on a standard 1% TAE-agarose (4.84 g Tris-Base, 1.142 ml glacial acetic acid, 0.744 g Na_2 EDTA-2H_2O to 1 L H_2O) gel as a 364-bp fragment.

β-Gal Assays

Assays of β-gal activity in tissue homogenates were performed using a chemiluminescence assay as described by Shaper *et al.* (15). Fresh tissues (100 mg) were dissected from transgenic and nontransgenic mice and were homogenized in 1 ml of lysis buffer containing 100 mM potassium phosphate (pH 7.8), 0.2% Nonidet P-40, 1 mM dithiothreitol, and 1 tablet/50 ml of Complete EDTA-free Protease Inhibitor Cocktail (#1836170, Roche Molecular Biochemicals, Indianapolis, IN). Homogenization was performed for 20 s on ice using a Micro Ultrasonic Cell Disrupter (Kontes, Vineland, NJ). After centrifugation at 12,500 × *g* for 10 min, the supernatants were heated at 48°C for 50 min to inactivate endogenous mammalian β-gal activity (16). After a second centrifugation at 12,500 × *g* for 5 min, the protein concentration in the supernatants was determined using the Bradford Protein Assay Reagent (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard. Aliquots of heat-inactivated lysate containing 10 mg of total protein was incubated for 60 min at 25°C with 300 μl of reaction buffer containing Galacto-Star (Tropix, Bedford, MA), 100 mM sodium phosphate (pH 7.5), 1 mM $MgCl_2$, and 5% Sapphire-II (Tropix). Light output was integrated in mV over 20 s using a 1251 luminometer (BioOrbit, London, UK). Assays of β-gal activity *in situ* were performed by perfusing freshly euthanized mice with ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.8) intracardially for 10 min (4.4 ml/min) and with 18% sucrose in PBS (pH 7.8) for 10 min (4.4 ml/min). Successful fixation was indicated by rapid blanching of the liver and kidneys and stiffening of the skeletal muscles.

The kidneys and brain were resected and incubated overnight in 30% sucrose in PBS (pH 7.8) on ice as cryoprotection. The tissues were embedded (Tissue-Tek, Miles Inc., Iowa City, IA) and 8- to 10-μm cryosections were cut. The sections were postfixed in 4% paraformaldehyde in PBS (pH 7.8) for 5 min and washed in PBS (pH 7.8). The samples were then incubated overnight at 30°C in a humid-

ified atmosphere in staining solution (1 mg/ml X-gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM $MgCl_2$ in PBS [pH 7.8]), washed with tap water, stained with Nuclear Fast Red Staining Solution (Vector Laboratories Inc., # H-3403, Burlingame, CA) for 1 min, washed with tap water for 10 min, and dehydrated through grades of ethanol and xylene. Newborn kidneys were resected and fixed for 25 min in 1.5% paraformaldehyde and 0.2% glutaraldehyde in PBS (pH 7.8) on ice with continuous agitation, washed thrice in PBS, and embedded, cut, and stained as described above. Whole mounts (up 13 dpc) were generated with timed pregnancies by mating heterozygous transgenic males with wild-type females. The yolk sack was recovered to extract DNA to identify transgenic embryos by PCR as described above. Whole mounts were fixed for 15 to 35 min (depending on gestational age) in 1.5% paraformaldehyde and 0.2% glutaraldehyde in PBS (pH 7.8) on ice with continuous agitation, washed thrice in PBS, and stained overnight at 30°C in X-gal staining solution (plus 0.02% Nonidet P-40 and 0.01% deoxycholate) as described above. Eleven dpc embryos were dehydrated in 100% methanol and cleared in benzyl benzoate: benzyl alcohol 2:1 (vol/vol).

Indirect Immunofluorescence

Indirect immunofluorescence was performed on 8-μm cryosections of kidneys from freshly euthanized transgenic or nontransgenic mice. Adult mice were intracardially perfused with ice-cold 4% paraformaldehyde in PBS (pH 7.4, 5 min) and washed with 18% sucrose in PBS, and their kidneys were frozen in liquid nitrogen immediately after resection. Sections were fixed with ice-cold acetone for 2 min, washed and blocked with 10% donkey serum, and incubated with the following antibodies: goat anti-β-gal polyclonal antibody (1:100 dilution, Biogenesis #4600-1409), Fluorescein (FITC)-conjugated AffiniPure Donkey Anti-Goat IgG (H+L) (1:100 dilution, Jackson Labs, #705-095-147), rabbit anti-WT-1 polyclonal antibody (1:100 dilution in 10% goat-serum, C-19, #sc-192, Santa Cruz) in 10% goat serum, and Cy3-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1:200 dilution, Jackson Labs, #111-165-144).

Results

Isolation, Molecular Cloning, and Sequence Analysis

An 8.3-kb fragment that included the 5′ untranslated region and the 5′ flanking region of the murine *Nphs1* gene was identified, cloned, and entirely sequenced as described in the Materials and Methods section. With the use of computer algorithms and visual inspection, this mouse sequence was aligned with the published human *NPHS1* genomic sequence (Figure 1). Like the human gene, no classical TATA box was found in the mouse gene (17). Eighteen conserved regions of 50 to 556 bp were identified. These conserved regions occurred in an array, the ordering of which was also conserved between the two species. Regions of conserved sequence extended to −7.3 kb in the mouse (+1 defined as the A at the translation initiation codon) and extended to −10.5 kb in the human gene. Within these conserved regions, consensus transcription factor binding sites were identified using the MatInspector search engine and the TRANSFAC database (Figure 1).

Generation of Transgenic Mice

The ability of the identified *Nphs1* 5′ flanking region to drive appropriate tissue-specific expression of a reporter gene was tested in mice. Two transgenes were created containing either 8.3 kb or 5.4 kb of the 5′ flanking region and the entire 5′ untranslated region of the *Nphs1* gene placed upstream to a bacterial lacZ gene encoding β-gal (Figure 2). This lacZ gene possessed a nuclear localization signal. Transgenic mice were generated by pronuclear injection, and founders were identified by PCR analysis of genomic DNA isolated from tail biopsies. Nine transgenic founders of 50 littermates (18%) were obtained after injection of the p8.3N-nlacF construct, and 24 founders of 84 littermates (29%) were obtained after injection of the p5.4-nlacF construct.

Tissue Screen for β-Gal Activity

Transgenic F_0 founders initially were examined at 6 to 8 wk postgestation using a sensitive chemiluminescence assay to detect β-gal activity in a survey of multiple tissues (Figure 3). Homogenates were examined from the tissues of three groups of animals. These groups included three independent adult F_0 transgenic founders obtained from each of the two transgenes (two female, one male for each transgene) and a group of three adult wild-type littermates. Endogenous β-gal activity was quenched by heating homogenates to 48°C before analysis. Remaining β-gal activity significantly above background was detected in kidneys and brains of founder mice transgenic for both transgenes but was not detected in other tissues. Indeed,

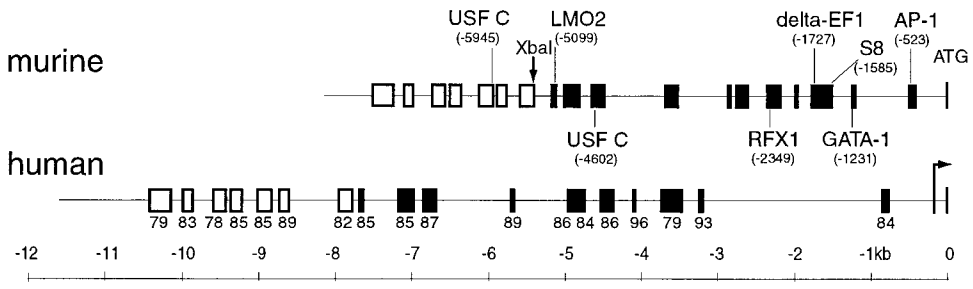

Figure 1. Analysis of the identified 8.3-kb 5′ flanking region of the murine *Nphs1* gene. Conserved regions between the murine and human genome are indicated as rectangles. The order of the conserved regions is preserved in both species, regions 5′ of the *Xba*I site (used to clone the p5.4-nlacF construct) are shown as white rectangles. The percentage sequence identity between murine and human conserved regions is indicated under each region. Potential transcription factor binding sites in the conserved regions of the murine genome and their nucleotide positions relative to the translation initiation site (ATG) are indicated. The +1 site of the human gene is marked with an arrow (17).

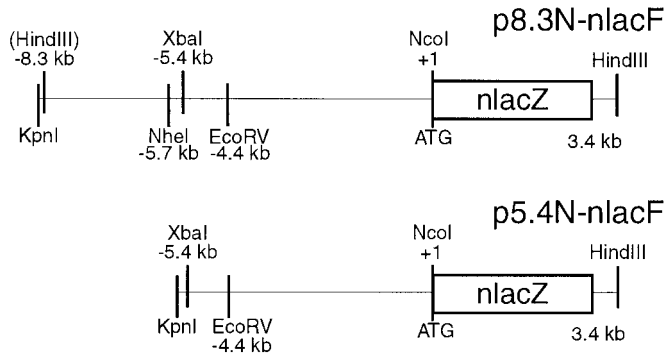

Figure 2. Map of the two reporter constructs used for the generation of transgenic mice containing 8.3 (p8.3-nlacF) and 5.4 kb (p5.4-nlacF) of the 5′ flanking region and the 5′ untranslated region of the murine *Nphs1* gene. The promoter constructs drive expression of an *Escherichia coli* lacZ reporter gene with a nuclear localization signal (nlacZ). Unique restriction sites and their nucleotide positions relative to the translation initiation site (ATG) are indicated. *Hin*dIII and *Kpn*I were used to release the constructs for microinjection.

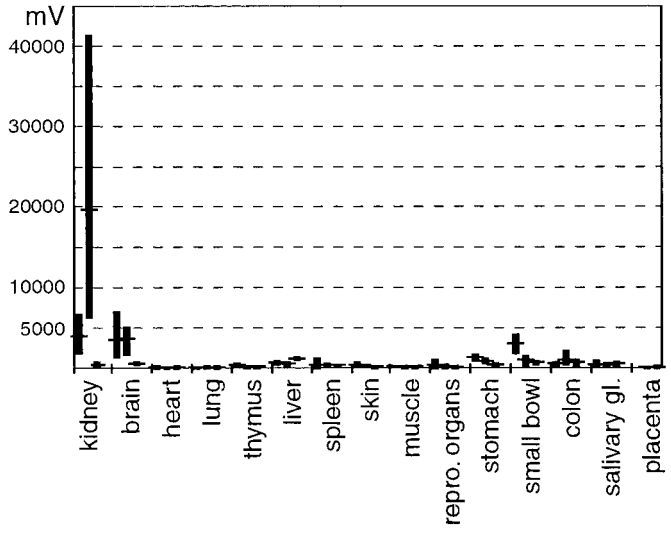

Figure 3. Tissue screen for β-galactosidase (β-gal) activity. Tissues from three transgenic founders of each construct were homogenized, and β-gal activity was measured in the heat-inactivated lysates using a chemiluminescence assay (first bar, p8.3-nlacF construct; second bar, p5.4-nlacF construct; third bar, wild type). Data are shown as means of mV of three independent experiments with the maximal and minimal value indicated by a vertical line. β-gal activity above background could be detected in the kidney and brain in both constructs. Kidney lysates from p5.4-nlacF founders contained higher β-gal activity than lysates from p8.3-nlacF founder animals. Activity in the brain was comparable in founders of both constructs. Elevated activity in the gut could be attributed to nonspecific endogenous β-gal activity (not shown).

when examined by X-gal staining of cryosections, 9 of 9 of p8.3N-nlacF founders examined and 19 of 20 of p5.4N-nlacF founders expressed β-gal in their kidneys (95 to 100% penetrance). Particularly elevated β-gal activity was measured in the kidneys of founders transgenic for p5.4N-nlacF compared with nontransgenic littermates. With the exception of small intestine, low activities were observed in all other tissues; these activities were not different than those of wild-type controls. Subsequent evaluation of sections of intestinal structures stained with X-gal did not demonstrate nuclear localization of β-gal activity in the small intestine or elsewhere in the gut (data not shown).

Expression of Nphs1-Driven Transgenes in Adult Kidney and Brain

Because β-gal activity was confined to adult kidney and brain in the initial screen, these tissues were examined microscopically for β-gal activity after X-gal staining of serial 8-μm cryosections. Nuclear lacZ staining was detected in all glomeruli in a characteristic peripheral distribution consistent with that of podocyte nuclei (Figure 4, A and B). This characteristic staining pattern was observed in all adult kidneys examined derived from multiple independent founders and carrying either transgene (6 of 6 of p8.3N-nlacF founders examined and 15 of 15 of p5.4N-nlacF founders). No β-gal activity was identified elsewhere within kidney or within associated adrenal gland or in kidneys of wild-type littermates. To confirm that *Nphs1* transgenes were indeed expressed in podocyte nuclei, sections of adult transgenic kidneys were double labeled with anti–β-gal and anti–WT-1 antibodies (Figure 4, C through E). Chosen as a marker because it is expressed solely in podocyte nuclei in adult kidney, WT-1 expression co-localized with that of bacterial β-gal (18). Therefore, both *Nphs1* transgenes are expressed like endogenous nephrin, in a podocyte-specific distribution in the adult kidney.

The brain of an adult p5.4-nlacF F_0 founder was sectioned serially and was stained with X-gal. On microscopic examination, nuclear lacZ staining was detected in two regions of the medulla oblongata. Here, staining was observed in the nuclei within the area postrema at the caudal extremity of the fourth ventricle (Figure 4F). Staining was also observed in nuclei in an area of the medulla lateral to the midline and caudal to the fourth ventricle (Figure 4G). Isolated nuclei in the nucleus medialis of the cerebellum (2 to 20 nuclei/slide) also stained positive for X-gal (not shown). A similar pattern of nuclear localized β-gal activity was observed in two randomly sectioned brain specimens obtained from mice expressing each transgene. No X-gal staining was identified elsewhere in the brain or in the choroid plexus of these mice.

Expression of Transgenes during Embryonic Development

Whole-mount embryos were examined for β-gal activity after X-gal staining and tissue clearing. Embryos from four independent transgenic founders (embryos obtained from two independent founders for each transgene) were analyzed at 8.5 d postcoitus (dpc) and 11.5 dpc. Wild-type littermates served as negative controls. All transgenic embryos examined had similar tissue-specific expression patterns that were independent of the type of transgene expressed. At 8.5 dpc, β-gal activity was present in the neural tube in the region of the hindbrain in transgenic embryos (Figure 5, B and C). β-gal

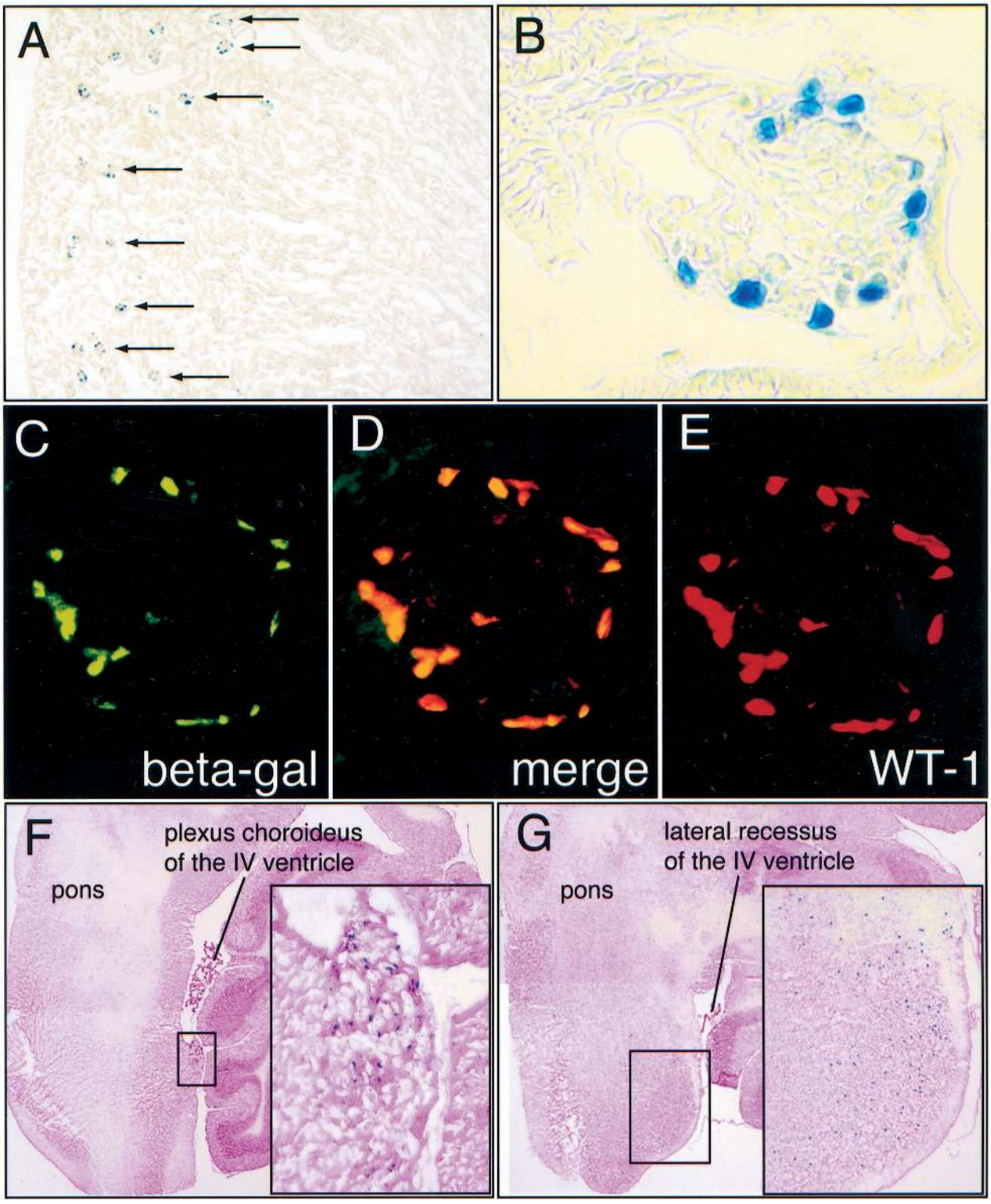

Figure 4. β-gal expression in the kidney and brain of adult transgenic mice. (A) β-gal *in situ* staining on an 8-μm cryosection of the kidney. β-gal is expressed exclusively in glomerular nuclei in the renal cortex in founder animals of both constructs (arrows). No β-gal activity can be detected elsewhere in the kidney. (B) Higher magnification of a single glomerulus of the kidney. β-gal expressing nuclei are distributed in a peripheral pattern in the glomerulus consistent with podocyte nuclei. (C through E) Cryosections of adult transgenic founder animals were double-stained with FITC-labeled antibacterial β-gal (C) and Cy3-labeled anti–WT-1, a marker specific for podocyte nuclei in adult kidneys (E). Superimposition of the two labels demonstrates that β-gal is expressed exclusively in podocyte nuclei expressing WT-1 (D). (F) Nuclear β-gal expression in the area postrema detected by *in situ* X-gal staining on serial cryosections of the brain of an p5.4-nlacF founder animal. The insert shows a higher magnification of the area postrema. The plexus choroideus is negative. (G) Nuclear β-gal expression could also be detected in nuclei of the medulla lateral to the midline and caudal to the fourth ventricle (insert shows higher magnification).

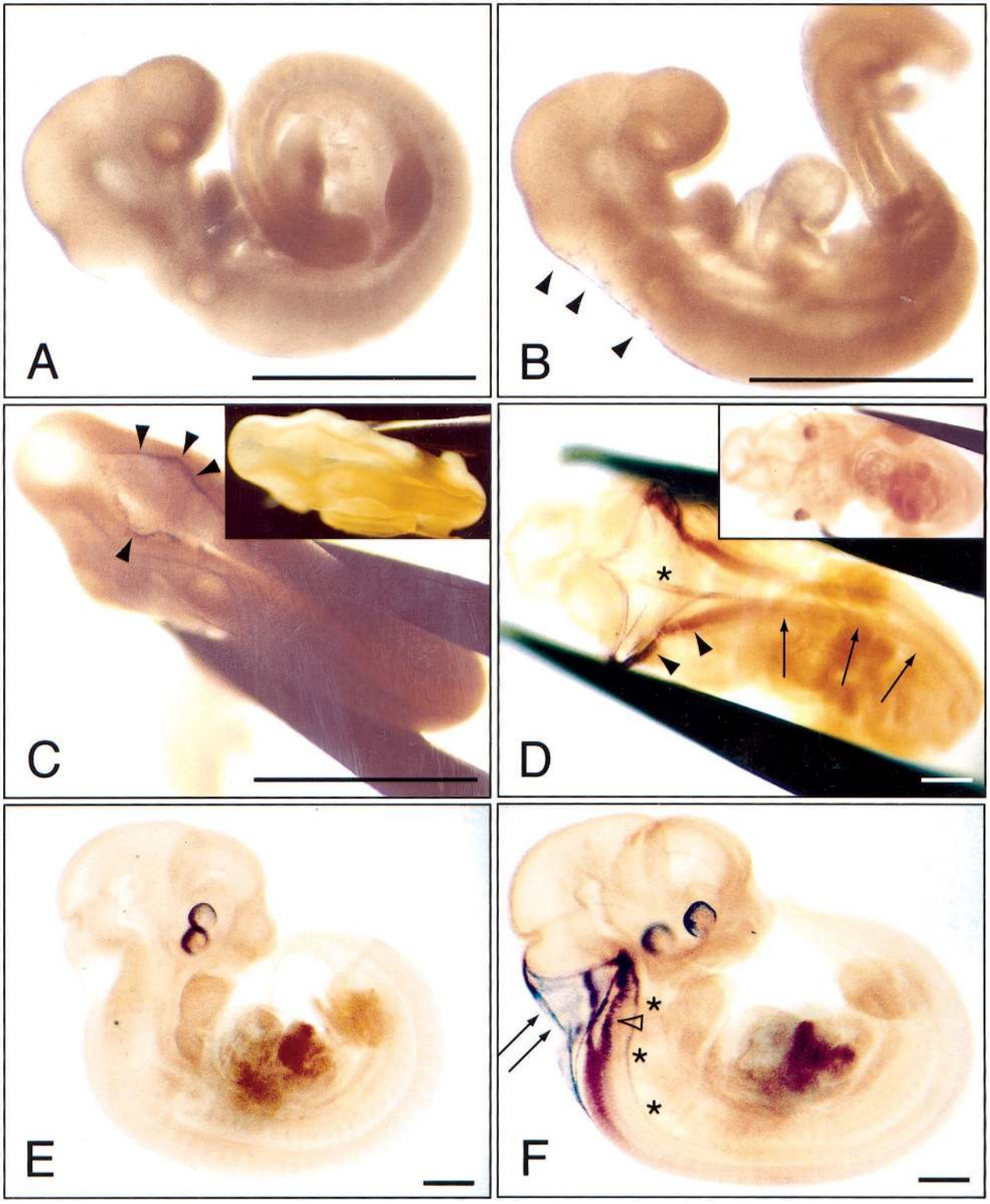

Figure 5. Embryonic expression of the transgenes in whole mounts at 8.5 and 11.5 days postcoitus (dpc). Transgenic embryos were obtained from timed pregnancies, fixed, and submitted to whole-mount X-gal staining overnight. Transgenic embryos were identified by PCR. A and the insert in C show 8.5 dpc wild-type littermate controls. At 8.5 dpc, β-gal activity is present in the region of the hindbrain and extends caudally along the first third of the neural tube (arrowheads in B). A dorsal view of the same 8.5 dpc embryo is shown in C (arrowheads identify β-gal expression). Embryos at 11.5 dpc were cleared before imaging (D through F). Wild-type littermates at 11.5 dpc are shown as controls in E and in the insert in D. At 11.5 dpc, β-gal is expressed in the met- and myelencephalic (arrowhead in F) region of the hindbrain. Arrows in F indicate faint β-gal expression in the roof of the fourth ventricle; stars indicate expression in the notochord. β-gal expression along the entire length of the neural tube can be seen on a dorsal view in D (arrows). In D, the star indicates the notochord; arrowheads point to the myelencephalon. Bar = 1 mm.

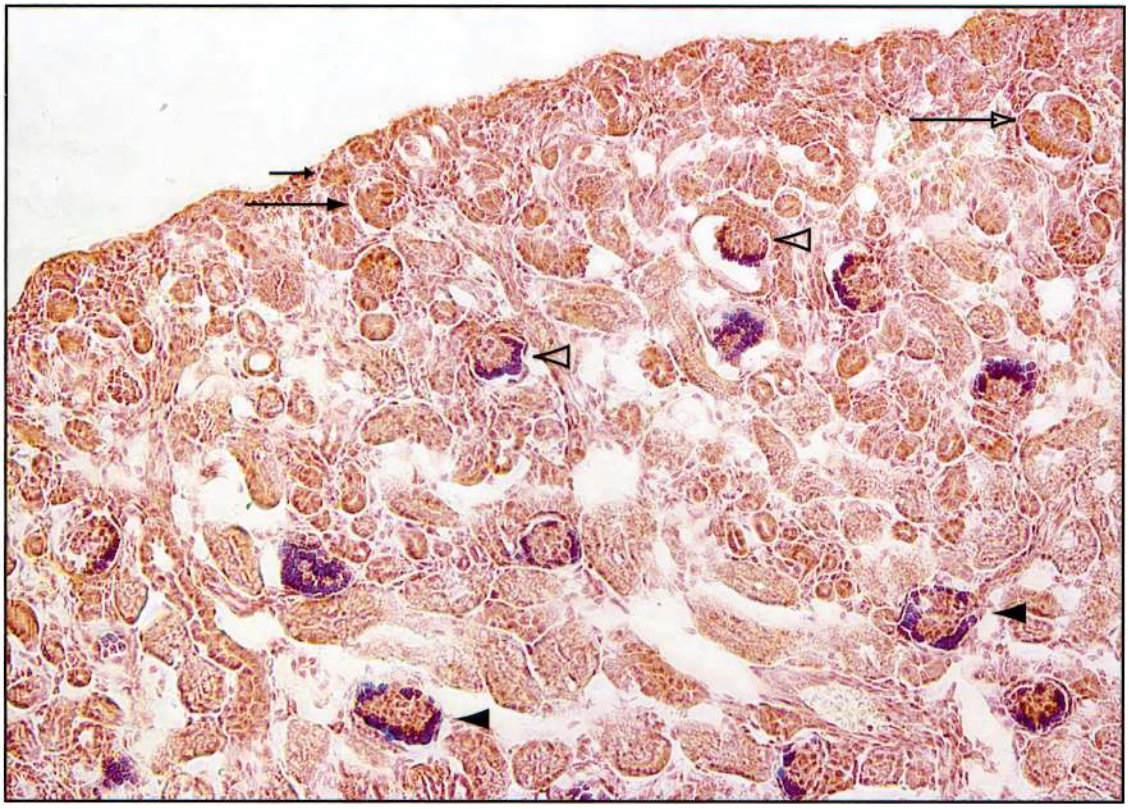

Figure 6. β-gal expression in the developing kidney. Eight-μm cryosections of kidneys of transgenic newborns were stained with X-gal and were counterstained with nuclear fast red. β-gal expression arises during early capillary loop stage of glomerular development in podocyte nuclei (open arrowheads) and persists in podocytes of mature glomeruli (arrowheads) in animals transgenic for both constructs. β-gal expression was not detected elsewhere in earlier forms of developing nephrons, such as pretubular condensation (short arrow), comma-shaped (arrow) and S-shaped bodies (arrow with open head), or elsewhere in the kidney.

activity extended caudally from the brain and could be detected in the cranial first third to one half of the neural tube. Among littermates, the extent and intensity of β-gal activity in the neural tube increased in a manner directly proportional to the degree of maturity. At 11.5 dpc, β-gal activity was again most prominent within the met- and myelencephalic part of the rhombencephalon, the neural tube structures of the hindbrain. At this time point, β-gal activity was now observed over the entire extent of the developing spinal cord (Figure 5, D and F), suggesting that neural tube *Nphs1* transgene expression extends caudally with developmental age. LacZ staining was also identified in the thin roof of the fourth ventricle (Figure 5F). Notably, β-gal activity could also be detected in the notochord of embryos at 11 dpc. β-gal activity was not detected elsewhere in embryos at 8.5 or 11.5 dpc.

Expression of the Transgene in the Neonatal Kidney

Nephrogenesis occurs in a telescoped manner in newborn kidney that allows the evaluation of all developmental stages in a single section. To determine when the *Nphs1* transgenes initiate expression of β-gal, newborn kidneys from several mice carrying either Nphs1 transgene were sectioned and examined after X-gal staining. As shown in Figure 6, nuclear localized β-gal activity was observed in early capillary loop stage glomeruli and in glomeruli in later developmental stages. β-gal activity was not observed in more primitive nephric structures, such as comma- and S-shaped figures, or elsewhere in these kidneys. These results demonstrate that both the 5.4-kb and 8.3-kb *Nphs1* transgenes drive lacZ expression in a pattern identical to that described for endogenous nephrin during metanephric development.

Discussion

The present study was undertaken to identify a promoter-enhancer that would direct the expression of transgenes in a podocyte-specific manner for later use in experiments aimed at manipulating the podocyte *in situ*. The mouse *Nphs1* promoter was selected for investigation because previously published work suggested that this gene is expressed in a podocyte-specific pattern (10,19). Two constructs containing 8.3 and 5.4 kb of the murine *Nphs1* promoter-enhancer region and including the gene's complete 5′ untranslated region were found to recapitulate in an identical manner endogenous nephrin expression both in adult mice and in developing mouse embryos.

Nphs1-driven transgene expression was identified in the kidney and brain. In the kidney, the lacZ reporter transgene carrying a nuclear localization signal was expressed exclusively in podocyte nuclei. Expression of β-gal arose during the

early capillary loop stage of glomerular development mimicking the reported expression pattern of nephrin in the kidney (10).

β-gal activity was observed in developing transgenic mouse brain at 8 dpc in the region of the hindbrain and extended down the entire length of the neural tube during maturation. Strongest β-gal expression could be seen at 11.5 dpc in the met- and myelencephalic part of the rhombencephalon and in the roof of the fourth ventricle. During the execution of this study, it was reported by Putaala *et al.* (19) that the endogenous *Nphs1* gene is expressed in an identical distribution in wild-type mouse embryos at 11 dpc. Similarly, expression of β-gal expression in transgenic mice was observed in the lumbar area of the neural tube mimicking the distribution of the expression of the endogenous *Nphs1* gene (19). Previously, the expression of nephrin in adult brain had not been reported. The identification of β-gal activity in discrete nuclei of the adult medulla oblongata and cerebellum correlates appropriately with developmental expression in structures of the hindbrain.

The expression of nephrin in the central nervous system is an interesting finding and provides possible clues regarding the function of the protein. Because other members of the Ig superfamily of transmembrane proteins have been implicated as functionally important in neuronal growth and differentiation, it is plausible that nephrin, too, plays a similar role in development of the cerebellum, brainstem, and spinal cord (20,21). Indeed, it has recently been communicated that a subset of patients with the congenital nephrotic syndrome of the Finnish type, who carry mutations of the nephrin gene, exhibit discrete extrapyramidal signs (19).

It is likely that all *cis*-regulatory elements necessary for cell-specific expression in the mouse are present in the identified 8.3-kb portion of the 5′ flanking region of the *Nphs1* gene, because this region directed expression of the reporter transgene in a manner similar to the endogenous *Nphs1* gene. The high degree of sequence similarity between the human and murine 5′ flanking region is not surprising. However, there clearly are portions of this region that are distinctly more highly conserved. It is possible that there exist elements within these more highly conserved regions that are necessary to direct *Nphs1* gene expression. Lenkkeri *et al.* (17) reported the association between glomerular disease and deletions in a GA repeat sequence in the human *Nphs1* promoter. Of note, this GA repeat sequence was not identified in the murine *Nphs1* promoter, suggesting that the human GA repeat is not a functionally significant promoter element. Rather, this repeat may represent a polymorphism that cosegregates with the glomerular disease phenotype present in these patients.

The p5.4-nlacF reporter construct produced significantly higher expression of β-gal activity in the kidney compared with that of the full-length p8.3-nlacF construct. Expression levels in the brain, conversely, were similar in both constructs. This observation might be explained by the occurrence of multiple integrations of the transgene or by the effect of the site of transgene integration. However, because transgene expression in the brain does not vary between constructs, one might postulate that deletion of a binding element for a kidney-specific transcriptional repressor has resulted in increased β-gal expression in the shorter construct. Additional work is required to investigate these hypotheses.

It is important to note that the expression of both transgenes used in this study was characterized by a remarkably high penetrance. Moreover, the site of integration did not significantly influence expression of either transgene. No ectopic tissue expression was observed in this study. Such stable expression characteristics should prove to be valuable features when this promoter is used as a tool for the generation of transgenic mouse models.

The study of podocyte biology has been restricted by limitations imposed by available experimental models. Understanding the mechanisms that govern the unique podocyte cyto-architecture and the architecture of the specialized slit-diaphragm is central to the problem of glomerular pathophysiology. For this reason, experimental models of podocyte biology should preserve the physiologic three-dimensional *in situ* phenotype of these cells. To be useful, the ideal model should also be amenable to specific manipulations at the molecular level. The nephrin promoter constructs identified in this study provide unique reagents that will allow application of a transgenic approach for manipulating the podocyte *in situ*. In the kidney, the identified promoter directs expression solely in mature or maturing podocytes. Transgene expression arises late in nephrogenesis coinciding with the initiation of foot process formation. Therefore, future transgenes driven by this promoter should have little effect on early nephron developmental processes. In some circumstances, the use of *Nphs1*-driven transgenes to study the kidney may be limited by extrarenal expression in the region of the hindbrain and spinal cord. This may be particularly true during the gestational period, when aberrant expression of transgenes may result in embryonic lethal or severe developmental abnormalities that would preclude the study of mature kidneys. Ideally, identification of a region of the *Nphs1* promoter that drives podocyte-specific transgene expression would circumvent this problem. Short of this, the use of an inducible system in which the identified *Nphs1* promoter determines transgene expression should circumvent this problem.

In summary, the present study has identified the murine *Nphs1* promoter as a useful reagent that can drive transgene expression in a podocyte-specific pattern in the kidney. The use of this tool to express dominant negative proteins or to create conditional gene deletion models—each in podocyte-specific patterns—should provide important new approaches for studying the molecular basis of podocyte pathophysiology.

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