An efficient system for tissue-specific overexpression of transgenes in podocytes in vivo

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Abstract

Purpose: Although transgenic mice have been used extensively in studies on renal biology, the use of endogenous promoter fragments as transgene elements is often limited by low expression levels. In this study, we have constructed an improved floxed expression vector system in which transgene expression is controlled by a tissue-specific promoter. The system permits the continuous expression of transgenes in podocytes and facilitates the study of podocyte biology.

Methods: A 2,849-bp fragment of the CMV promoter was inserted adjacent to the enhanced green fluorescent protein (eGFP) gene in the naturally occurring loxP site of a CMV-driven expression vector. The construct was made flanked by loxP sites at each end to ensure the recombination in vivo. The construct was introduced into the genome of transgenic mice by pronuclear injection. A modified floxed expression vector was constructed in which the CMV promoter was exchanged for the CAGS promoter. The transgenic mice expressing eGFP under the control of the CAGS promoter were used to generate transgenic mice expressing a transactivator under the control of the CAGS promoter.

Results: The eGFP expression in transgenic mice carrying the floxed transgene was inducible and specific to podocytes. The expression of the transactivator and eGFP were inducible and specific to podocytes. The expression of the transactivator and eGFP were inducible and specific to podocytes.

Conclusions: This approach allows the continuous expression of transgenes in podocytes and facilitates the study of podocyte biology.

Our ability to produce mice that carry altered genomic loci or foreign transgenes has facilitated the study of many biological processes in vivo. Endogenous promoter fragments are commonly used to express transgenes in a tissue-specific and developmentally regulated fashion. However, the use of endogenous promoter fragments is often limited by low to intermediate transgene expression. Furthermore, endogenous promoter fragments may be silenced as a consequence of de- or transdifferentiation of the target cells. Promoter silencing also affects the use of established inducible transcriptional systems that rely on the continuous expression of a transactivator in the target tissue (e.g., tetracycline-responsive transactivator system). These limitations can be overcome by employing a bineic “floxed expression vector” system in which transgene expression under the control of a strong and potentially ubiquitous promoter is irreversibly activated by Cre-mediated excision of a stop-signal. Cre recombinase specifically recognizes the 34-bp loxP (locus of X-over of P1) site derived from the P1 genome and efficiently catalyzes reciprocal conservative DNA recombination between pairs of loxP sites. Over 100 different transgenic Cre-mouse lines have become available targeting multiple tissues.

The floxed expression vector strategy has been used in the past by several groups, albeit with various limitations. In some studies, the loxP sites were integrated into the open reading frame of the transgene. Others used endogenous tissue-specific promoter fragments to drive transgene expression. The most significant limitation arose from positional effects that silenced transgene expression in founder mice carrying the floxed transgene. This problem was overcome by introducing the floxed transgene into the genome of embryonic stem (ES) cells and screening multiple clones for transgene expression and successful Cre recombination in vitro. Subsequently, chimeric mice had to be generated from the identified ES clones to obtain transgenic mice carrying the floxed transgene.

In this study, we have constructed a floxed expression plasmid with two alternate ubiquitous strong promoter/enhancers (CMV and the chicken β-globin promoter with CMVIE enhancer, termed CAGS) (21). We show that transgenic founders with active high-level transgene expression can be rapidly identified by screening for transgene expression (i.e., β-galactosidase activity) in tail biopsies and subsequent crossing with a mouse line expressing Cre recombinase in the target tissue and testing for Cre recombination in doubly transgenic offspring. This approach should significantly facilitate transgenic experiments aimed at constitutively expressing transgenes in a tissue-specific fashion.

MATERIALS AND METHODS

Construction of pCMVfloX and pCAGSfloX. A 2,849-bp fragment was removed from pDNA3.1-E/Uni-lacZ (Invitrogen) by a restric-
The transgenes CMVflox-GFP and CAGGSflox-GFP were introduced using the manufacturer’s instructions (102-T, Viagen Biotech, Los Angeles, CA) or DirectPCR(tail) lysis reagent according to the manufacturer’s instructions. Genomic DNA was isolated from 3-wk-old mice using the DNeasy Tissue Kit (Qiagen, Valencia, CA). The transgenes CMVflox-eGFP and CAGSflox-eGFP were liberated from the plasmid vector backbone by digestion with PacI and XbaI restriction sites introduced to facilitate release of the subsequent gene of interest (STOP). The gene of interest (GOI) is cloned into a multiple cloning site (MCS; available restriction sites are indicated). The purified DNA fragments were microinjected into F2 hybrid eggs from (C57BL/6J x SJL/J) F1 parents (Jackson Labs, Bar Harbor, ME) as described (17). Two PC embryo (18) were bred with C57BL/6J wild-type mice (F3-F5) and Cloned into the MCS behind the floxed β-galactosidase cassette. The prokaryotic vector sequence was removed by digestion with PacI and XbaI restriction sites of the MCS behind the floxed β-galactosidase cassette. 

Generation of transgenic mice. The CMVflox-eGFP and CAGSflox-eGFP construct were liberated from the plasmid vector backbone by digestion with PacI, separated by agarose gel electrophoresis, and purified by Nucleospin columns (Clontech). The purified DNA fragments were microinjected into F2 hybrid eggs from (C57BL/6J x SJL/J) F1 parents (Jackson Labs, Bar Harbor, ME) as described (17). The CMVflox-eGFP cassette was released from pEGFP-N2 (Clontech, Palo Alto, CA) by a HindIII/XbaI digest and cloned into the respective sites of pCMVflox and pCAGGSflox, resulting in CMVflox-GFP and CAGGSflox-GFP. 

The Cre ORF was amplified by PCR using the primers Cre-NheI.fwd 5'-gtg cag tag gcc acc tgg tgg cag-3' and Cre-H3.rev 5'-gtg cag tag gcc acc ttc tgg cag-3'. In a second PCR, fragments A and B were merged using primers A.fwd and B.rev, digested with XmnI and ClaI and cloned into pCMVflox digested with XmnI and ClaI (plox). The amplified CAGS promoter PCR product was digested with XmnI and cloned into the unique AvrII site of plox. 

The transgene detection was performed on tail biopsies as described previously (17). Genomic DNA was isolated from tail biopsies of 3-wk-old mice using the DNasey Tissue Kit (Qiagen, Valencia, CA) or DirectPCR(tail) lysis reagent according to the manufacturer’s instructions (102-T, Viagen Biotech, Los Angeles, CA). The transgenes CMVflox-GFP and CAGGSflox-GFP were identified by PCR using the primers LacZ.fwd 5'-TTC ACT GCC CTT TTA ACA ACG TCG TGA-3' and LacZ.rev 5'-ATG TGA GCG AGT AAC ACG TCG TAT GCT TCT-3' (product length 364 bp). Transgene detection of the podocyte-specific Cre mouse line was performed as described (18). 

β-Galactosidase activity in tissue lysates or 5-mm tail biopsies were measured as described (17). In brief, mice were anesthetized and briefly perfused with ice-cold lysis buffer (100 mM potassium phosphate buffer, pH 7.8, 1 mM dithiothreitol, 0.2% NP-40, and protease inhibitors, Sigma-Aldrich, Taufkirchen, Germany). One hundred milligrams of each individual tissue were recovered, and gastrointestinal organs were carefully washed. Tissues were minced and sonicated in 1 ml lysis buffer on ice. After heat-inactivating endogenous β-galactosidase at 48°C for 50 min, lysates were centrifuged. The protein concentration in supernatants was measured, and 10 μg protein in total were added to a reaction buffer containing Galacto-Star (Tropix, Bedford, MA). Light output (V) was integrated over 10 s using a 1251 luminometer (Bioorbit, London, UK).

SDS-PAGE and immunoblotting was performed using a rabbit polyclonal anti-GFP antisera (8372, 1:5,000, Clontech) or a rabbit polyclonal anti-Cre antisera (69050–3; 1:10,000, Novagen, Madison, WI) as described (20). For immunofluorescent studies on cryosections, polyclonal anti-GFP antisera (kind gift of R. Wiggins, 1:200) (28) was used. Immunoelectron microscopic studies were performed using standard procedures.

RESULTS

Design of pCMVflox transgene. To derive a feasible system for inducible overexpression of transgenes, a floxed expression plasmid based on the univector plasmid fusion system (15) with two alternative promoter/enhancer elements was constructed (Fig. 1). The gene of interest (GOI) was cloned into a multiple cloning site (MCS) derived from pCDNA3.1(+) (Invitrogen) followed by a polyadenylation signal. In this study, GFP was used as GOI (reporter gene). A Kozak consensus sequence was engineered at the transcription initiation codon (ATG) of the eGFP cDNA. The open reading frame of the GOI was terminated by a stop-codon. The floxed expression plasmid was microinjected into F2 hybrid eggs from (C57BL/6J x SJL/J) F1 parents (Jackson Labs, Bar Harbor, ME) as described (17). Two PC embryo (18) were bred with C57BL/6J wild-type mice (F3-F5). 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 National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Genotyping. Transgene detection was performed on tail biopsies as described previously (17). Genomic DNA was isolated from tail biopsies of 3-wk-old mice using the DNasey Tissue Kit (Qiagen, Valencia, CA) or DirectPCR(tail) lysis reagent according to the manufacturer’s instructions (102-T, Viagen Biotech, Los Angeles, CA). The transgenes CMVflox-GFP and CAGGSflox-GFP were identified by PCR using the primers LacZ.fwd 5'-TTC ACT GCC CTT TTA ACA ACG TCG TGA-3' and LacZ.rev 5'-ATG TGA GCG AGT AAC ACG TCG TAT GCT TCT-3' (product length 364 bp). Transgene detection of the podocyte-specific Cre mouse line was performed as described (18).

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mid contained a floxed LacZ cassette coding for β-galactosidase placed between the MCS for the gene of interest and the promoter/enhancer (CMV or CAGS). The LacZ cassette was followed by a polyadenylation signal to allow expression exclusively of β-galactosidase and to prevent transcription of the following GOI (eGFP). The entire β-galactosidase (LacZ) cassette was flanked by modified loxP sites to enhance translation by decreasing secondary structure of the 5′-untranslated region. This modified loxH site remained preserved even after Cre-mediated excision of the interposed lacZ cassette (Fig. 1C). Two alternate promoter/enhancer elements were used to drive transgene expression in this study: CMV and CAGS (chicken β-actin promoter with CMVIE enhancer) (21).

Efficient recombination of pCMVfloxeGFP and pCAGSfloxeGFP in vitro. Before microinjection, the floxed expression plasmids were tested in vitro in COS7 cells (Fig. 2). Cells were transiently transfected with pCMVFlox-eGFP or a plasmid encoding Cre recombinase or both. Lysates were subjected to SDS-PAGE and immunoblotted for Cre recombinase and the reporter gene eGFP. Expression of eGFP was only detected in doubly transfected cells. Abundant β-galactosidase activity was detected in all lysates of pCMVFlox-eGFP-transfected cells using a chemiluminescent assay (Fig. 2A).

EGFP expression was also evaluated in transiently transfected cells by fluorescence microscopy (Fig. 2, B and C). In cells transfected with pCMVFlox-eGFP, low eGFP expression was detected in 1–5% of cells. In cells doubly transfected with pCMVFlox-eGFP and Cre recombinase, abundant eGFP expression was detected in >90% of cells. In summary, these experiments show that GOI expression (eGFP) can be efficiently activated by Cre recombination of pCMV in mammalian cells. In unrecombined pCMVflxed, GOI expression is almost entirely suppressed in the absence of Cre recombinase even when overexpressed in cultured COS7 cells. Similar results were obtained with pCAGSfloxeGFP (not shown).

Analysis of CMVFlox-eGFP and CAGSfloxeGFP transgenic mice. Transgenes were released from a prokaryotic vector sequence using two engineered PacI restriction sites (Fig. 1A) and submitted for pronuclear microinjection. Mice were genotyped at 3 wk of age by PCR using marker DNA obtained from tail biopsies. Seven CMVFlox-eGFP and 11 CAGSfloxeGFP transgenic founders were obtained.

Positional effects, arising from the site of transgene integration into the genome, may result in low or undetectable expression levels. To establish a simple system allowing rapid detection of F0 founder animals with active transgene expression, we measured expression of the floxed LacZ cassette as a reporter for transgene expression. Of note, in these singly transgenic mice the actual gene of interest (eGFP) remained silenced in the absence of Cre recombination.

It was reasoned that active β-galactosidase expression in a representative biopsy could be used as a marker for active transgene expression in the entire animal. To identify founder animals with active transgene expression, β-galactosidase activity was measured in tail biopsies (Tables 1 and 2) using a chemiluminescence assay as described previously (17). β-Galactosidase activity above background levels was detected in tail biopsies of 4/7 (57%) CMVFlox-eGFP founders. A lower proportion of β-galactosidase-expressing founders was identified in CAGSfloxeGFP mice (3/11; 27%).

To verify whether transgene expression in tail biopsies could be used to predict the activity of transgene expression in other organs or tissues, mice from four individual founder lines with active (Table 3, lines A–D) or from three lines with inactive transgene expression (see Table 3, lines E–G) in tail biopsies were randomly chosen. Lysates were prepared from 17 different tissues, and β-galactosidase activity was measured using the chemiluminescence assay to determine the activity of transgene expression (Table 3). Values under 10 represent absent β-galactosidase expression. Chemiluminescence data were reproducible within one log scale in repeat chemilumi-
Seven CMVflox-eGFP founder lines were mated to the podocyte-specific Cre-mouse line 2.5P-Cre. Doubly transgenic offspring were screened for constitutive expression of eGFP (gene of interest) in podocytes on cryosections of the kidney, indicating successful Cre recombination. Each line is either represented as + (positive eGFP expression in podocytes) or − (no eGFP expression), clustered according to β-galactosidase expression in tail biopsies or kidney lysates. Three lines expressed β-galactosidase in tail and kidney lysates and also expressed eGFP in podocytes, whereas two lines with no β-galactosidase expression in either tails or kidneys were also negative for eGFP expression in podocytes. Two lines could not be clearly segregated using this screening test.

In summary, most CMVflox-eGFP founder mice with inactive transgenes due to positional effects could be efficiently identified and excluded from further analysis by measuring transgene activity (β-galactosidase) in tail biopsies.

Transgene expression was also measured in a randomly chosen CAGSflox-eGFP line with active β-galactosidase expression in tail biopsies (CAGS A) and a line CAGS B with inactive β-galactosidase expression (Table 4). Even though higher expression levels were observed in various tissues of line CAGS A, overall expression levels were lower compared with CMVflox lines and less abundant. The CAGS promoter was particularly active in heart and the kidney. A more detailed analysis of CAGSflox-eGFP mice was subsequently not pursued because none of the three founder lines with active β-galactosidase expression in tail biopsies expressed the gene of interest in our target cells (podocytes) when bred with 2.5P-Cre mice.

Table 2. Correlation of β-galactosidase expression in tails and total kidney lysates with eGFP expression in podocytes

<table>
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<th>Founder Line</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tr>
<td>Kidney</td>
<td>28</td>
<td>26,791</td>
<td>353</td>
<td>3</td>
<td>778</td>
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<td>11</td>
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<tr>
<td>Hind brain</td>
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<td>eGFP expression in podocytes of doubly transgenic mice</td>
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Table 3. Transgene activity in tissues of CMVflox founder lines

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Primary chemoluminescent data (V) measured in various tissue lysates in individual pCMVflox-eGFP founder lines arranged according to β-galactosidase activity in tail biopsies. Background levels were subtracted. Values under 10 represent absent β-galactosidase expression. Lines with eGFP expression in podocytes in doubly transgenic offspring are indicated in the bottom line (+, positive; −, negative eGFP expression). In CMVflox-eGFP mice, β-galactosidase expression in tail biopsies correlated with β-galactosidase expression in the majority of tissues. Of note, light emission measurements cannot be compared with measurements reported in our previous studies because of minor modifications of the experimental protocol (17, 18).
2.5P-Cre that expresses Cre recombinase exclusively in podocytes of the kidney (18). Doubly transgenic offspring (CMV-flox-eGFP/P2.5-Cre) were perfusion fixed at 4–6 wk of age with 4% paraformaldehyde. The kidneys were recovered and cryoembedded. Sections (4 μm) were stained with polyclonal anti-GLEPP antiserum to visualize podocytes and examined by fluorescence microscopy (Fig. 3, A and A’). Robust eGFP expression, indicating successful Cre excision of the LacZ cassette, was reproducibly detected in podocytes in four of seven CMV-flox-eGFP founder lines (57%) and three of four tail biopsy positive lines (ppv = 75%, npv = 66%; summarized in Table 2). No eGFP expression was detected in singly transgenic littermates (CMVflox/eGFP) (Fig. 3, B and B’). Nonspecific fluorescence was seen arising from the internal elastic layers of small arteries in both doubly transgenic and control animals (Fig. 3, A’ and B’).

EGFP expression was examined in kidneys of doubly transgenic newborns (CMVflox-eGFP/P2.5-Cre). Nephrogenesis proceeds in a telescoped fashion during the perinatal period, so all stages of glomerular development can be observed at birth. EGFP expression was activated during the capillary loop stage (Fig. 3, C and C’). This finding confirms our previous characterization of the podocyte-specific Cre mouse line 2.5P-Cre, where expression of Cre recombinase was activated during this late stage of glomerular development (18).

CMVflox-eGFP drives transgene expression in a mosaic fashion. In adult doubly transgenic CMVflox-eGFP/P2.5-Cre mice, eGFP was expressed only in a subpopulation of podocytes. The degree of mosaicism was highly variable between neighboring glomeruli in a single section. Numerous glomeruli were observed in which eGFP expression was abundant or entirely absent (not shown). Similarly, glomeruli with only a single podocyte expressing eGFP were observed (Fig. 4, A and A’). The percentage of eGFP expressing podocytes was determined in 50 glomeruli of each line by staining podocyte nuclei of kidneys of 2 different founder lines with WT-1 anti-serum. Overall, a similar percentage of podocytes was found to express eGFP in all four CMVflox-eGFP lines (30–
38%), indicating that mosaic transgene expression might be an inherent property of the floxed expression vector system.

Mosaic transgene expression in a subset of cells was also observed by immunoelectron microscopy in kidneys of doubly transgenic CMVflox-eGFP/P2.5-Cre newborns. While transgene expression was entirely absent in maturing podocytes of the early capillary loop stage, intense eGFP expression was seen in a subset of mature podocytes (Fig. 4, A and A'). Of note, eGFP expression was entirely absent in the remainder of podocytes, indicating that expression of the GOI is either on or off.

DISCUSSION

In the present study, an efficient bigenic system was developed aimed at driving high-level expression of transgenes in a tissue-specific fashion in transgenic mice. In this system, the experimental animals are the progeny of two different transgenic lines: one that carries a transgene of interest under the control of a ubiquitous strong promoter (pCMV) and another preexisting line that expresses Cre recombinase within the target tissue and specifies where and when transgene expression will be activated. Without expression of Cre, the GOI is not expressed within founder animals because it is placed behind a LacZ cassette flanked by modified lox sites (floxed) that can be recognized by Cre recombinase. In this study, the Cre line 2.5P-Cre was used because it was previously shown to mediate Cre recombination exclusively in podocytes with no evidence of mosaicism (18).

We suggest that by simply measuring reporter gene activity (β-galactosidase) in tail biopsies, one might increase the probability of identifying transgenic founders with active transgene expression in the tissue of interest. The design of this study builds on previous systems to express floxed transgenes in a tissue-specific fashion in vivo (16, 22). Lobe and co-workers (16) stably introduced a floxed transgene into the genome of ES cells and found that expression of the first reporter gene (β-galactosidase) in ES cell clones correlated with widespread expression of the transgene in embryos derived from these ES clones. In the present study, we found that the activity of the transgene in tail biopsies of transgenic founder mice similarly correlated with widespread transgene expression. However, the approach used in the present study does not require work with ES cells and subsequent generation of chimeric mice, significantly reducing the time to generate new lines.

Fig. 4. Mosaic expression of transgenes driven by the CMV promoter. A, A', and A'': as an inherent property of the CMV promoter, transgenes are expressed in a mosaic fashion in homogeneous cell populations in vivo. Similar to previous reports, ~25–50% of podocytes expressed the transgene eGFP. In the example shown, a single podocyte expresses eGFP within 1 glomerulus of a doubly transgenic CMVflox/eGFP × 2.5P-Cre animal. B: immunoelectron microscopy of doubly transgenic newborn kidneys visualizes the morphology of podocytes with and without transgene expression as well as their interaction with each other. During the capillary loop stage, no eGFP (GOI) is expressed. No morphological alterations could be observed in podocytes overexpressing eGFP. C: a single podocyte overexpressing the GOI (eGFP) is marked by multiple gold particles (anti-eGFP staining, arrow) next to a wild-type cell (arrowhead).
In contrast to published reporter lines, transgene activity is not ubiquitously active within an individual founder line. As shown in Table 3, transgene activity varies in individual tissues among different founder lines. For this reason, it may be advisable to measure transgene (β-galactosidase) activity within the target tissue and cross-breed several founder lines (e.g., Ref. 3) with the Cre line to check for Cre recombination and resulting expression levels. By this approach in this study, screening tails as well as the target tissue (i.e., kidney) for β-galactosidase activity positively predicted expression of the transgene in podocytes 100% of the time and negatively predicted the absence of transgene expression 80% of the time.

Several considerations were employed when the floxed expression plasmid was constructed. The first lox site was modified (loxH) to allow its placement into the 5'-untranslated region of the promoter/enhancer CMV or pCAGS. Because of its palindromic structure, the loxP sequence might form a hairpin structure in transcribed mRNA that can decrease downstream gene expression if present in the 5'-leader (11, 25). However, not all 13 bp are essential for efficient recombination: in particular the first 4 bp (ATAA) of any of the 13-bp inverted repeats can tolerate some modification with little if any loss of recombinational proficiency or fidelity (24, 26). To minimize effects caused by the secondary structure, the hairpin of the first half of the first lox site was destabilized by introducing three recombinationally neutral alterations into the first inverted repeat (attaacctcata) (2).

Similar to previous studies employing floxed expression vectors with two genes, no overt leaky expression of the GOI was observed before Cre recombination in this study. This result is in agreement with a study by Lakso and coauthors (12) showing that a single polyadenylation signal effectivelly blocked expression of the subsequent GOI. Indeed, Grieshammer and coauthors (6) demonstrated that the intrinsic stop codon of the reporter gene LacZ was sufficient to entirely prevent expression of the subsequent GOI (diphtheria toxin).

A concern was that multiple copies of the floxed transgene might lead to undesired interchromosomal recombination events. However, transgenes are usually integrated into a single site as multiple copies oriented as “head-to-tail” tandem arrays (7, 10). Indeed, transgenic mouse lines bred as expected for unique single-site integration events in this study (data not shown). Lakso and coauthors (12) demonstrated that expression of the GOI was activated in 2 independent founder lines with 50 or more copies of the transgene. Indeed, Cre recombinase efficiently excised tandem arrays of a floxed transgene down to one single copy and one lox site (22). Cre recombinase strongly depends on the distance between two lox sites, and interchromosomal recombination events are rare (frequency $1 \times 10^{-7}$ to $5 \times 10^{-9}$) (23) and rarely lead to chromosomal loss (4). In summary, recombination events among an individual array of transgenes at one site are much more likely than recombination between transgenes located at different sites of the genome. Furthermore, it is unlikely that the floxed transgene is located on two different chromosomes in the F1 generation. We concluded that founder lines do not need to be screened for single-copy integrations of the transgene. Instead, we propose to functionally screen founder lines for transgene expression in tail biopsies or the target tissue (or both). In a subsequent screen, positive lines are mated to a line expressing Cre recombinase in the target tissue, and expression of the GOI (as an indication for Cre recombination) is analyzed in doubly transgenic offspring.

A surprising result of this study was that none of three CAGSflox-eGFP founder lines that tested positively in tail biopsies expressed eGFP in podocytes when mated to 2.5P-Cre mice. Previous studies indicated, that, besides abundant expression in other tissues, within the kidney the CAGS promoter may preferentially express transgenes in a podocyte-specific fashion (1, 9). In this study, the CMV promoter drove transgene expression with a higher penetrance and wider tissue distribution compared with the CAGS promoter.

As expected, expression levels mediated by the CMV promoter were higher compared with previously characterized endogenous promoter fragments derived from Nphs1 and NPHS2 (17, 19). However, transgene expression driven by the CMV promoter was only observed in a subset of cells within a seemingly homogenous cell population of podocytes. No mosaic expression of Cre recombinase was seen in podocyte-specific 2.5P-Cre mice employed in this study even after nine generations back-crossing into a C57/BL6 genetic background and cannot explain this finding (18). Positional effects leading to inefficient Cre recombination can lead to mosaicism or complete silencing of transgene expression in the target tissue (16). However, the degree of mosaicism was similar in all four founder CMVflox-eGFP lines in this study (~30%). Similar mosaic transgene expression has been observed by other investigators and may be an inherent property of the CMV promoter in transgenic mice (3, 27). This property may have served the cytomegalovirus to ensure the survival of its host. We observed by immunoelectron microscopy that mosaic transgene expression occurred in a binary fashion (either on or off). This property should allow one to observe individual cells expressing the GOI next to control cells that do not. Because the percentage of cells expressing the transgene varied significantly between individual glomeruli within the same kidney, it even should be possible to observe the effects of transgene expression in a graded fashion.

**REFERENCES**


