A “Molecular Toolbox” for the Nephrologist

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One of the many challenges facing researchers in the post-genomic era is the assignment of biologic functions to all the gene sequences now publicly available. In many centers, the mouse is the experimental model of choice for studying the genetics of human development and disease. The mouse is a powerful tool to study human biology because of the remarkable similarities of the genetic, cellular, and organ functions between these two species. Furthermore, the ability to manipulate the murine genome through targeted or random mutagenesis is unparalleled in other mammalian systems. Inactivation of genes in the germline has provided great insight into their function; however, the resulting phenotypes are often complex and may preclude analysis of the gene’s function in specific organs or tissues of interest. In part, investigators are able to overcome this problem by inactivating and/or overexpressing genes of interest in a cell-specific manner. This has required the identification and characterization of tissue-specific promoters, genetic elements that direct the expression of genes in specific cell types. Using this approach, great strides have been made in the areas of cardiac, lung, and gut development and disease (1–7). However, progress in the renal field has been slower due to the lack of useful tissue-specific promoters. In this issue of JASN, Moeller et al. (8) report the identification and characterization of two podocyte-specific promoters that promise to provide a catalyst to the study of gene function in the kidney.

The authors identified regulatory regions from two genes, nphs1 and NPHS2, which are responsible for congenital Finnish nephropathy (9) and autosomal recessive steroid-resistant nephrotic syndrome (10), respectively, and are able to direct the expression of beta-galactosidase specifically to glomerular visceral epithelial cells (podocytes). In transgenic mouse models, Holzman and his colleagues elegantly demonstrate that 1.25-kb of the proximal 5’ flanking region of the murine nphs1 gene and 2.5-kb of the human podocin (NPHS2) promoter contain all of the regulatory sequences required for podocyte-specific expression. Wong et al. (11) have previously shown that 1.25-kb of the human NPHS1 gene also provides podocyte-specific expression, whereas larger fragments that measure 8.3-kb, 5.4-kb, or 4.125-kb direct expression to neural subsets in the developing hindbrain in addition to podocytes (12,13). In their article, Moeller et al. (8) show that only 30% of the nphs1 founder lines express the transgene, suggesting that activity of the 1.25-kb nphs1 promoter is dependent on the site of chromosomal integration. In contrast, 100% of the NPHS2 transgenic founder lines express beta-galactosidase in podocytes. The property of integration-independence is obviously important when considering which promoter to choose in generating future transgenic lines.

To date, a handful of cell-specific promoters have been identified in the kidney; these include a 1542-bp fragment of the 5’ flanking region of the KAP gene (kidney androgen-regulated promoter) (14) and 346-bp of the gamma-Glutamyl Transpeptidase Type II promoter (15), which direct expression to the proximal tubule, 3.0-kb of the Tamm Horsfall Protein (THP) promoter, which directs expression to the thick ascending limb of the loop of Henle (TAL) and early distal convoluted tubules (16), 1.34-kb of the Ksp-cadherin promoter, which directs expression to the TAL and collecting ducts of the adult nephron and weakly in other cell types and to the ureteric bud, Wolfian duct, Mullerian duct, and developing tubules in the mesonephros and metanephros (17) while a 324-bp fragment limits expression to tubular epithelia of the developing kidney and GU tract (18), the HoxB7 promoter, which marks the ureteric bud and its derivatives (19), and 1.25-kb of the human NPHS1 and 8.3-kb, 5.4-kb, 4.125-kb, and 1.25-kb of the murine nphs1 promoters, which direct expression to podocytes (11–13). The present article adds NPHS2, another podocyte-specific promoter, to the list.

How will the identification of these tissue-specific regulatory sequences help nephrologists and researchers interested in kidney biology? The most obvious answer is the ability to express genes of interest in specific cell types within the kidney and look at the resulting phenotypes. For example, increased expression of numerous growth factors has been reported to occur in podocytes during glomerular injury (20,21). Using the promoters described by Moeller et al. (8) to direct the expression of these growth factors to podocytes, it will now be possible to test whether increased expression of these factors in podocytes underlies the pathogenesis of glomerular scarring or is simply a marker of disease (i.e., do the mice that overexpress a growth factor in podocytes develop glomerular scarring?) (Figure 1). Although this is a straightforward experiment, caution must be used in interpretation of any overexpression study. Most importantly, the relevance of overexpressing a
gene under the regulation of a heterologous promoter to sup-rphysiologic levels and/or in the presence of two normal copies of the endogenous gene must be established. In addition, one has to be aware of the developmental stage at which the promoter becomes active, as this may affect the phenotype.

Using these same promoters and conditional gene targeting strategies, it will also be possible to control the temporal, spatial, and/or level of gene expression precisely. Investigators will be able to “knock out” genes in specific renal cell types rather than from the germline, allowing the study of genes that might play important roles during earlier stages of development or in multiple organs. Several systems exist for generating conditional knockouts, including the Cre-loxP and flp-frt recombinase systems (22,23). To date, the Cre-loxP system has been most widely used in mammalian cells and tissues. Murine lines that demonstrate Cre-mediated excision from tubular epithelia and podocytes have already been reported (13,24). The general strategy for Cre-mediated excision is shown in Figure 2. Cre recombinase is a bacteriophage enzyme that causes site-specific recombination between loxP sites (34-bp DNA repeat sequences). Using 4.125-kb of the murine nphs1 and 1.34-kb of the ksp-cadherin promoter, investigators have already generated transgenic mice that express Cre-recombinase specifically in the podocyte or tubular epithelia, respectively. When these mice are bred with a transgenic strain that carries ‘loxP’ sites around a gene-of-interest, it will lead to the loss of the target gene ONLY from the cells that also express Cre-recombinase. In this manner, it will be possible to rescue early embryonic lethality that might occur when a gene is inactivated in the germline and to look at the function of removing 1 or 2 copies of a gene in specific cell types within

Figure 1. The podocyte-specific promoters reported in the manuscript by Moeller et al. (8) were used to drive the expression of beta-galactosidase in glomerular visceral epithelial cells. Upon lacZ staining, the transgenic podocytes turn blue. Investigators will be able to use these same promoters to direct expression of candidate “disease genes” to the podocyte to determine whether their overexpression leads to kidney disease.

Figure 2. General scheme for Cre-mediated DNA excision in vivo. A transgenic mouse line that expresses Cre-recombinase under the regulation of a podocyte-specific promoter is crossed to a reporter strain (the Z/EG mouse [27]). In cells where the Cre-recombinase is active (i.e., ONLY in podocytes), site-specific recombination occurs between the loxP sites (■) and leads to excision of the Beta-geo cassette and 3 poly-A signals, which functions as a STOP signal for transcription. After excision, the enhanced green fluorescent protein gene is transcribed and the offspring demonstrate green glowing podocytes. In other tissues where the promoter is inactive, no excision occurs and no fluorescence is visible. P, podocyte-specific; ■, loxP sites; EGFP, enhanced green fluorescent protein; Beta-geo, beta galactosidase and neomycin cassette.
the kidney. Furthermore, this strategy can also be used to activate the expression of reporter genes to perform lineage-tracing studies in vivo. Excision of a ‘STOP codon’ allows the expression of a cell marker such as enhanced green fluorescent protein (EGFP), which tags the cell at a specific time point and allows one to follow its fate during development and aging or in disease. The identification of truly podocyte-specific promoters as described in this issue of JASN should allow additional Cre-recombinase lines to be generated. Of note, the 4.125-kb nphs1 promoter used to generate the Cre-recombinase lines by Eremina et al. (13) also directs expression in a small subset of neural cells that derive from the first and second rhombi, which might interfere with the analyses of target genes that are also expressed there.

Perhaps of most value to adult nephrologists will be the generation of inducible knockout and overexpression systems in specific cell types within the kidney. Although several varieties exist, the tetracycline or estrogen-sensitive systems have both been successfully employed (25,26). In the first, it is possible to repress or activate the expression of a target gene when the transactivator tTA or ‘reverse’ rTA is bound to the Tet operator (tetO). Placing tTA or rTA under kidney specific control and crossing these transgenic lines with mice carrying Cre-recombinase or another gene of interest under the control of the TetO sequence, allows the precise control of target gene expression upon administration of tetracycline. Similarly, the administration of an estrogen agonist to transgenic mice that carry a fusion protein consisting of Cre-recombinase fused to an estrogen receptor results in activation of the Cre recombinase (26). Thus, the investigator will be able to choose the time of administration of these drugs to study gene function in the developing and/or adult kidney.

Although these promoters represent powerful new tools for studies in whole animals, they will also be valuable for in vitro applications. The identification of common regulatory elements between the human NPHS1, NPHS2, and mouse nphs1 genes should aid in the identification of novel and known transcription factors that are required for podocyte-specific expression as transcription factors bind to “consensus” sequences encoded by the DNA. Knowledge of these factors will lead to elucidation of the molecular pathways that promote differentiation and maintenance of a “healthy podocyte phenotype” and will help identify putative targets for therapy and treatment of patients with kidney disease. Interestingly, none of the knockouts of transcription factors that are known to be expressed in the podocyte have shown changes in nephrin mRNA expression. Although other obvious candidates such as WT1 exist, it is also possible that there will be novel factors required for its regulation.

In summary, characterization of the murine nephrin and podocin promoters adds two useful podocyte-specific promoters to the armamentarium of molecular tools for the nephrologist. Researchers will now be able to design elegant experiments and target gene expression in the kidney in both temporal and cell-specific manners.

References


See related article, “Two Gene Fragments that Direct Podocyte-Specific Expression in Transgenic Mice,” on pages 1561–1567.