J Am Soc Nephrol 15: 2237–2239, 2004

Kidney-Specific Gene Targeting

PETER IGARASHI
Department of Internal Medicine and Division of Cell and Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas

Gene targeting in mice is a powerful tool for identifying the *in vivo* functions of proteins and for producing new animal models of human diseases. In the conventional approach, the gene encoding a protein of interest is disrupted by homologous recombination in embryonic stem (ES) cells. Targeted ES cells are injected into blastocysts to produce chimeric mice, which are then bred to produce knockout mice that are heterozygous or homozygous for the mutated gene. Phenotypic analysis of the knockout mice reveals whether the encoded protein plays important roles in murine development and physiology. Since the mutated gene is transmitted through the germline, the protein will be absent from all cells of homozygous mutant mice. Embryonic lethality may result if the protein is essential for the development of the embryo and may preclude the identification of important functions later in life. Early embryonic lethality or severe developmental abnormalities frequently prevent analysis of the functions of proteins in the kidney, an organ that arises relatively late in development. To circumvent these limitations, strategies have been devised to produce conditional gene knockouts in which gene targeting can be spatially and temporally regulated.

The approach that is most widely used for conditional gene targeting involves Cre/*loxP* recombination (1,2). Cre recombinase is an enzyme that is produced by bacteriophage *P1* and is not normally present in mammalian cells. Cre belongs to the integrase family of site-specific DNA recombinases and mediates recombination at 34-bp sequences, called *loxP*, without any requirement for accessory proteins or cofactors. If two *loxP* sites are inserted in the same orientation into the DNA flanking a sequence of interest, then Cre will mediate recombination between the *loxP* sites. The DNA segment between the two *loxP* sites will be excised, leaving behind a single *loxP* site in the original DNA (the excised segment containing the other *loxP* site is lost from the cell). Cre/*loxP* recombination, therefore, can be used to create deletions at any desired location in the genome.

To produce tissue-specific gene knockouts, two strains of mice are required. One strain expresses Cre recombinase under the control of the promoter of a tissue-specific gene. Typically, this strain is produced by conventional transgenic methods in which a DNA fragment containing the tissue-specific promoter linked to the coding region of Cre recombinase is microinjected into the pronuclei of fertilized mouse oocytes. After transfer into foster mothers, the progeny that express Cre recombinase in the desired pattern are identified. The second mouse strain contains two *loxP* sites flanking the DNA segment to be excised. Generally, the *loxP* sites are inserted by homologous recombination into introns flanking an essential exon(s) of the gene of interest, producing a so-called floxed gene. Since the *loxP* sites are short and located in introns, their presence usually does not affect gene expression and the mice have a wild-type phenotype, although this needs to be verified experimentally. Next, the strains are crossed to produce mice that are homozygous for the floxed gene and also carry the Cre transgene (Cre; flox/flox). Alternatively, mice carrying one floxed gene and one mutated gene can be used (Cre; flox/-). In either of these strains, Cre/*loxP* recombination will inactivate the gene, but only in the organs in which the tissue-specific promoter is active and in which Cre is expressed. In all other organs, Cre will not be produced and the expression of the gene will not be affected (Figure 1). Gene inactivation, therefore, will be tissue-specific.

In this issue of *JASN*, Rubera *et al.* describe the creation of transgenic mice that express Cre recombinase exclusively in the renal proximal tubule (3). To direct the expression of Cre, they used the promoter of the high-capacity (type 2) Na^+/glucose cotransporter gene (*Sglt2*), a gene that is only expressed in early proximal tubules. Transgenic mice carrying the *Sglt2* promoter linked to the Cre recombinase gene were produced by pronuclear microinjection. RT-PCR analysis showed that Sglt2-Cre mice expressed Cre only in the kidney and not in any other tissues examined. Within the kidney, Cre was only expressed in the proximal tubules. The Sglt2-Cre mice were then crossed with R26R mice carrying a *lacZ* reporter gene that is activated by Cre/*loxP* recombination. In the bitransgenic progeny, *lacZ* was only expressed in the proximal tubules, indicating proximal tubule-specific Cre/*loxP* recombination. Sglt2-Cre mice represent the first strain of transgenic mice that expresses Cre recombinase exclusively in renal proximal tubules, and they should be very useful for inactivating any gene of interest in this segment of the nephron. Sglt2-Cre mice are an important addition to the list of available kidney-specific Cre transgenic mice (14–18) (Table 1).

As of June 2004, at least five genes have been successfully targeted using kidney-specific Cre/*loxP* recombination. Hoxb7-Cre mice (4) have been used to inactivate Sonic hedgehog (Shh) and the epithelial sodium channel (α-ENaC) in the ureteric

Correspondence to Dr. Peter Igarashi, Division of Nephrology, UT Southwestern, 5323 Harry Hines Blvd., MC8856, Dallas, TX 7539–8856. Phone: 214-648-2754; Fax: 214-648-2071; E-mail: peter.igarashi@utsouthwestern.edu

1046-6673/1509-2237
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000136298.09488.D7

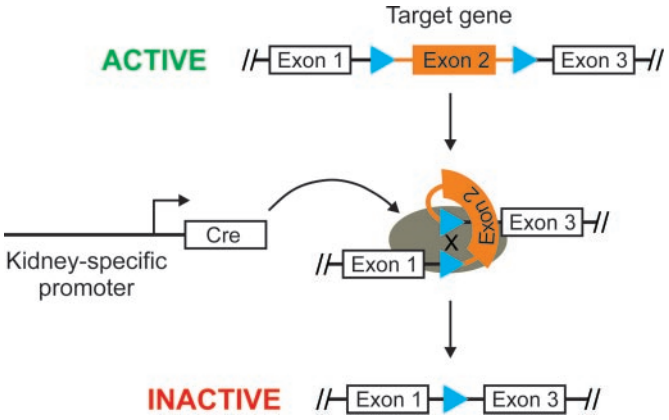

Figure 1. Kidney-Specific Cre/*loxP* Recombination. Cre recombinase is expressed under the control of a kidney-specific promoter (left) and two *loxP* sites (triangles) are inserted in the introns flanking an essential exon of a gene of interest (orange box). For simplicity, only one of two copies of the floxed gene is shown. In the kidney, Cre recombinase (brown) will be expressed and will excise the DNA sequence between the *loxP* sites, which will inactivate the gene. In all other tissues, Cre will not be expressed and the gene will remain active. Bent arrow indicates the transcription initiation site.

bud and renal collecting ducts. Inactivation of Sonic hedgehog produces hydronephrosis and hydroureter, and has revealed an important role for this protein in signaling from the urothelium to the surrounding mesenchyme (4). Collecting duct-specific inactivation of α-ENaC unexpectedly does not produce significant abnormalities in sodium or potassium balance, indicating the functional importance of this channel in more proximal nephron segments (5). Ksp-Cre mice that express Cre under the control of the Ksp-cadherin promoter (6) have been used to inactivate the ciliary protein KIF3A and the transcription factor HNF-1β in renal epithelial cells. Kidney-specific inactivation of either protein produces renal cysts, which supports the important roles of primary cilia and transcriptional regulation in the pathogenesis of polycystic kidney disease (7,8). Nephrin-Cre mice (9) have been used to inactivate vascular-endothelial growth factor (VEGF-A) in podocytes. Complete loss of VEGF-A prevents the formation of the glomerular filtration barrier, whereas incomplete loss produces a renal lesion that resembles human preeclampsia (10).

In addition to tissue-specific gene inactivation, Cre/*loxP* recombination can be used for cell lineage studies. Transgenic

Table 1. Kidney-specific Cre mouse strains

Promoter	Renal expression	Extrarenal expression	Gene knockouts	Ref.
γ-Glutamyl transpeptidase	Cortical tubules (incl. proximal tubules)	None	None*	(11)
Na^+/glucose cotransporter (SGLT2)	Proximal tubules	None	None	(3)
Aquaporin-2	Collecting ducts (principal cells)	Testis, vas deferens	None	(14)
Hox-B7	Collecting ducts, ureteric bud, Wolffian duct, ureter	Spinal cord, dorsal root ganglia	Epithelial Na^+ channel (α-ENaC) Sonic hedgehog	(5, 4)
Ksp-cadherin	Renal tubules, collecting ducts, ureteric bud, Wolffian duct, mesonephros	Müllerian duct	Kinesin-II (KIF3A subunit) Hepatocyte nuclear factor-1β	(6, 8, 7)
Tamm-Horsfall protein	Thick ascending limbs of loops of Henle	Testis, brain (low)	None	(15)
Nephrin	Glomeruli (podocytes)	Brain (low)	Vascular endothelial growth factor (VEGF-A)	(9)
Podocin	Glomeruli (podocytes)	None	None*	(16, 13)
Renin	Juxtaglomerular cells, afferent arterioles	Adrenal gland, testis, sympathetic ganglia, etc.	None*	(12)
Pax-2	Glomeruli, renal tubules, collecting ducts, nephric duct	Inner ear, brain	None*	(17)
Pax-8	Glomeruli, renal tubules, nephric duct, mesonephros	Inner ear, brain, thyroid, Müllerian duct	None*	(18)

Table lists the promoters used to express Cre recombinase in transgenic mice, the expression of Cre in the kidney and other tissues, and the genes that have been conditionally inactivated by Cre/*loxP* recombination (as of May 2004). * indicates mice that have been used for cell lineage studies.

mice expressing Cre from a cell-specific promoter are crossed with reporter mice (R26R, Z/EG, etc.) carrying a *lacZ*, EGFP, or other reporter gene that is activated by Cre/*loxP* recombination. In addition to the cells that express Cre, all their progeny will be genetically tagged by expression of the activated reporter gene, which allows their cell fates to be traced over time. Studies using this strategy have shown that fibroblasts can originate by epithelial-mesenchymal transition during renal fibrosis (11) and that renin-expressing cells can differentiate into non-renin-expressing smooth muscle, mesangial, and epithelial cells (12). Another interesting example that was published earlier this year in *JASN* showed that the crescents in experimental glomerulonephritis arise from podocytes as well as from parietal epithelial cells (13).

Many more examples of kidney-specific gene targeting using Cre/*loxP* recombination will appear over the coming months. In addition, other site-specific DNA recombination systems utilizing FLP recombinase and ϕC31 integrase are gaining popularity in mice. Ligand-binding variants of Cre recombinase can be used to control the timing of Cre/*loxP* recombination and delay gene inactivation until adulthood. These methods will enable the creation of increasingly sophisticated mouse mutants that promise to deepen our understanding of kidney biology and disease.(14,15,16,17,18)

Acknowledgments

I apologize to those whose work was not included due to space constraints. Work from the author's laboratory is supported by grants from the NIH (DK-42921, DK-57328, DK-66535, and DK-67565), Texas Advanced Technology Program, and PKD Foundation.

References

1. Sauer B: Inducible gene targeting in mice using the Cre/*lox* system. *Methods* 14: 381–392, 1998
2. Nagy A: Cre recombinase: the universal reagent for genome tailoring. *Genesis* 26: 99–109, 2000
3. Rubera I, Poujeol C, Bertin G, Hasseine L, Counillon L, Poujeol P, Tauc M: Specific Cre/lox recombination in the mouse proximal tubule. *J Am Soc Nephrol* 15: 2050–2056, 2004
4. Yu J, Carroll TJ, McMahon AP: Sonic hedgehog regulates proliferation and differentiation of mesenchymal cells in the mouse metanephric kidney. *Development* 129: 5301–5312, 2002
5. Rubera I, Loffing J, Palmer LG, Frindt G, Fowler-Jaeger N, Sauter D, Carroll T, McMahon A, Hummler E, Rossier BC: Collecting duct-specific gene inactivation of αENaC in the mouse kidney does not impair sodium and potassium balance. *J Clin Invest* 112: 554–565, 2003
6. Shao X, Somlo S, Igarashi P: Epithelial-specific Cre/lox recombination in the developing kidney and genitourinary tract. *J Am Soc Nephrol* 13: 1837–1846, 2002
7. Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LSB, Somlo S, Igarashi P: Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc Natl Acad Sci USA* 100: 5286–5291, 2003
8. Gresh L, Fischer E, Reimann A, Tanguy M, Garbay S, Shao X, Hiesberger T, Fiette L, Igarashi P, Yaniv M, Pontoglio M: A transcriptional network in polycystic kidney disease. *EMBO J* 23: 1657–1668, 2004
9. Eremina V, Wong MA, Cui S, Schwartz L, Quaggin SE: Glomerular-specific gene excision in vivo. *J Am Soc Nephrol* 13: 788–793, 2002
10. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE: Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 111: 707–716, 2003
11. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG: Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* 110: 341–350, 2002
12. Sequeira Lopez ML, Pentz ES, Nomasa T, Smithies O, Gomez RA: Renin cells are precursors for multiple cell types that switch to the renin phenotype when homeostasis is threatened. *Dev Cell* 6: 719–728, 2004
13. Moeller MJ, Soofi A, Hartmann I, Le Hir M, Wiggins R, Kriz W, Holzman LB: Podocytes populate cellular crescents in a murine model of inflammatory glomerulonephritis. *J Am Soc Nephrol* 15: 61–67, 2004
14. Nelson RD, Stricklett P, Gustafson C, Stevens A, Ausiello D, Brown D, Kohan DE: Expression of an AQP2 Cre recombinase transgene in kidney and male reproductive system of transgenic mice. *Am J Physiol* 275: C216–226, 1998
15. Stricklett PK, Taylor D, Nelson RD, Kohan DE, Ohyama T, Groves AK: Thick ascending limb-specific expression of Cre recombinase. *Am J Physiol Renal Physiol* 285: F33–39, 2003
16. Moeller MJ, Sanden SK, Soofi A, Wiggins RC, Holzman LB: Podocyte-specific expression of Cre recombinase in transgenic mice. *Genesis* 35: 39–42, 2003
17. Ohyama T, Groves AK, Eckardt D, Theis M, Doring B, Speidel D, Willecke K, Ott T: Generation of Pax2-Cre mice by modification of a Pax2 bacterial artificial chromosome. *Genesis* 38: 195–199, 2004
18. Bouchard M, Souabni A, Busslinger M: Tissue-specific expression of Cre recombinase from the Pax8 locus. *Genesis* 38: 105–109, 2004

See related article, "Specific Cre/Lox Recombination in the Mouse Proximal Tubule," on pages 2050–2056.