Detection of renin lineage cell transdifferentiation to podocytes in the kidney glomerulus with dual lineage tracing

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Understanding of cellular transdifferentiation is limited by the technical inability to track multiple lineages *in vivo*. To overcome this we developed a new tool to simultaneously fate map two distinct cell types in the kidney, and genetically test whether cells of renin lineage (CoRL) can transdifferentiate to a podocyte fate. *Ren1cCreER/tdTomato/Nphs1-FLPo/FRT-EGFP* mice (CoRL-PODO mice) were generated by crossing *Ren1c-CreER/tdTomato* CoRL reporter mice with *Nphs1-FLPo/FRT-EGFP* podocyte reporter mice. Following tamoxifen administration in these animals, CoRL were labeled with red fluorescence (tdTomato) and co-localized with renin. Podocytes were labeled green (enhanced green fluorescent protein) and co-localized with nephrin. Following podocyte loss by nephrotoxic antibody and subsequent enalapril-enhanced partial replacement, tdTomato-EGFP-labeled CoRL were detected as yellow-colored cells in a subset of glomerular tufts, without the use of antibodies. Co-localization with podocin indicated that these cells are podocytes, derived from CoRL origin. Thus, our novel study shows that two distinct cell types can be simultaneously labeled in the mouse kidney and provide strong genetic evidence *in vivo* that lost podocytes can be replaced in part by CoRL.

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Site-specific recombinase (SSR) is an essential tool for lineage-tracing cells in mice.[1] DNA recombinases from the *P1* bacteriophage (Cre) and *Saccharomyces cerevisiae* (flippase, FLP), combined with cell-specific promoters and reporters silenced by *loxP*- or *FRT*-flanked STOP sequences, allow for permanent expression of either fluorescent or enzymatic reporters in specific cell populations.[2] Combined with ligand-dependent activation, such as the tamoxifen-responsive CreER^{T2} recombinase, SSR can be achieved within specific temporal periods.[3,4] This provides a noninvasive method of mapping cell fate throughout development or tracing cell lineages during disease and/or regeneration.

SSR techniques have been used extensively in the study of kidney regeneration, and have led to several significant findings regarding the ability of distinct cell populations to undergo repair.[4] Yet, despite these genetic advances, simultaneous lineage tracing of 2 distinct cell types has been a challenge, in part because the majority of SSR in the kidney utilizes only Cre-*loxP* recombination.[5,6] Expression of Cre recombinase, even under the control of distinct promoters and utilizing distinct reporters, cannot uniquely label 2 cell types. This has posed a barrier in our ability to observe transdifferentiation events, in which 1 cell type adopts the characteristics of another. The limited numbers of studies that have been performed that utilize Cre and FLP systems simultaneously, outside of the kidney, are intersectional or subtractive genetic fate mapping that do not allow for the continuous tracing of 2 distinct populations and require enzymatic or immunohistochemical staining of the β-gal reporter.[1,7]

Here, we have succeeded in simultaneously labeling 2 distinct kidney cell populations by combining Cre-*lox* and FLP-*FRT* recombination strategies in the same mouse, regardless of sex, with the use of directly observable fluorescent reporters that we are calling dual lineage tracing. We utilized this methodology to genetically demonstrate that cells of renin lineage (CoRL) transdifferentiate toward a podocyte fate following podocyte depletion.

RESULTS

Figure 1 shows a schema of our dual transgenic approach. Unlabeled CoRL activate the *Ren1c* promoter inducing CreER expression; however, CreER remains sequestered in the

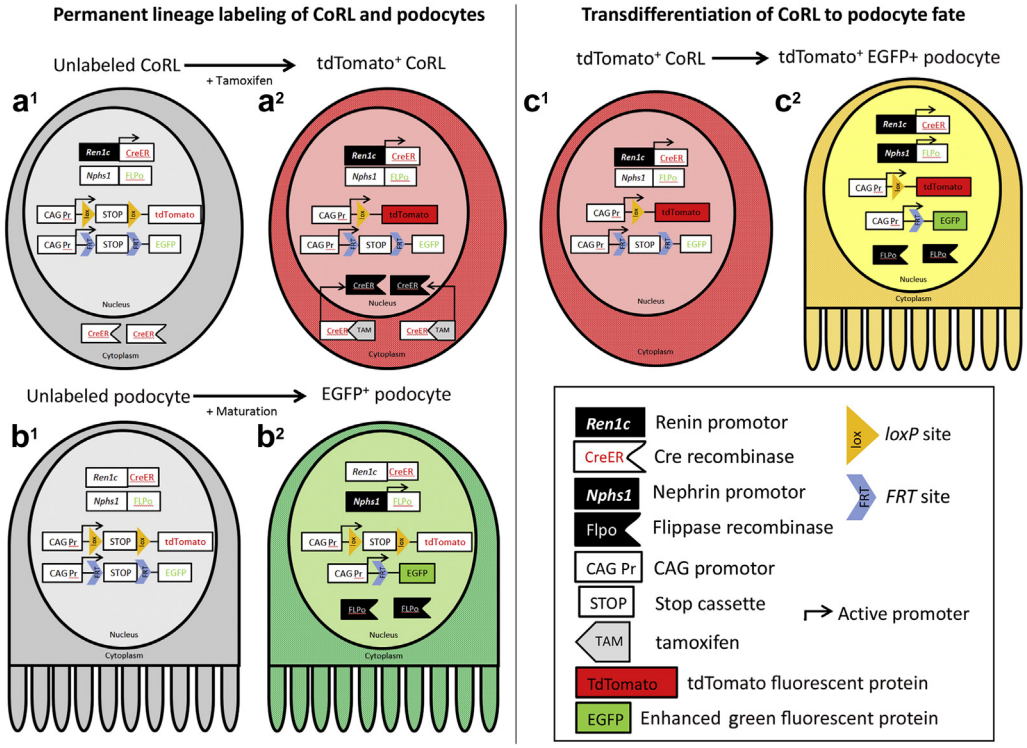

Figure 1 | Schema of dual lineage tracing transgenic approach. (**a**[1]) Unlabeled CoRL activate the *Ren1c* promoter (black box, white type) inducing CreER (white flag, red type). CreER remains sequestered in the cytoplasm and unable to bind *loxP*. (**a**[2]) Tamoxifen (gray flag, black type) binds to the ligand-binding domain of the Cre-estrogen receptor fusion protein, resulting in translocation to the nucleus (black flag, white type) and recombination of *loxP* sites (orange triangle, black type) and the intervening STOP cassette (white box, black type), inducing permanent tdTomato (red box, black type) expression. (**b**[1]) Immature unlabeled podocytes have not yet activated the *Nphs1* promoter (white box, black type). (**b**[2]) During podocyte maturation, the *Nphs1* promoter is activated (black box, white type), driving FLPase (black flag, white type) expression, which recombines the FLPase recognition targets (blue chevron, black type) and STOP cassette (white box, black type) to induce permanent enhanced green fluorescent protein (green box, black type) expression. (**c**[1]) tdTomato$^+$ CoRL have not yet activated the *Nphs1* promoter (white box, black type). (**c**[2]) Upon transdifferentiation to a podocyte fate, the *Nphs1* promoter is activated (black box, white type), driving FLPase (black flag, white type) expression; FLPase recombines the FRT sites (blue chevron, black type) and STOP cassette (white box, black type), inducing permanent enhanced green fluorescent protein (green box, black type), resulting in a yellow color.

cytoplasm and unable to bind *loxP* (Figure 1a[1]). Following tamoxifen, CreER translocates to the nucleus and recombines the *loxP* sites to remove the STOP cassette, thus inducing permanent tdTomato expression (Figure 1a[2]). As podocytes mature (Figure 1b[1]), the *Nphs1* promoter is activated to drive FLP expression, which recombines the FRT sites to remove the STOP cassette, thus inducing permanent EGFP expression (Figure 1b[2]). During transdifferentiation to a podocyte fate, tdTomato$^+$ CoRL (Figure 1c[1]) activate the *Nphs1* promoter, inducing FLP expression. FLP removes the *FRT*-flanked STOP cassette, inducing permanent EGFP expression and turning the originally red cell yellow (Figure 1c[2]).

Figure 2 shows validation of our dual lineage tracing approach. Following tamoxifen, young adult *Ren1cCreER/tdTomato/Nphs1-FLPo/FRT-EGFP* mice (herein called CoRL-PODO mice) expressed tdTomato specifically in cells in the juxta-glomerular compartment (JGC) (Figure 2a, arrow). As shown previously, tdTomato overlapped with 95% of renin$^+$ cells (Figure 2b, arrow). When CoRL-PODO mice were given the tamoxifen vehicle corn oil, tdTomato was not detected in the JGC (Figure 2c, arrow). Similarly, in mice lacking the *Ren1cCreER* transgene, tdTomato was not detected following tamoxifen administration (Figure 2d, arrow).

In the same mouse, EGFP (green) was detected within the glomerular tuft in a podocyte distribution pattern (Figure 2a) and overlapped with staining for the podocyte marker nephrin in 98% of EGFP$^+$ cells (Figure 2e, arrow). However, in CoRL-PODO mice lacking the *Nphs1-FLPo* transgene, EGFP was not detected (Figure 2f, arrow). There was no overlap between tdTomato and EGFP under normal nonstressed conditions (Figure 2a). These results show distinct inducible tdTomato labeling of CoRL in the JGC and constitutive EGFP labeling of podocytes in the glomerular tuft, in the same kidney. As this approach enabled simultaneous tracking of 2 cell types *in vivo*, we called it dual lineage tracing.

To genetically demonstrate that a subset of CoRL can replace lost podocytes in disease, experimental focal segmental glomerulosclerosis (FSGS), characterized by podocyte loss and subsequent partial replacement, was induced in CoRL-PODO mice with a nephrotoxic antibody.[8,9] Mice were treated with enalapril, which we have previously shown increases podocyte repletion.[8,9] Dual lineage tracing was performed to follow migration and transdifferentiation (Figure 3). At baseline,