Topic Introduction

Imaging Kidney Development

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INTRODUCTION

Development of the kidney involves interactions between several cell lineages and complex morphogenetic processes, such as branching of the ureteric bud (UB) to form the collecting duct system and condensation and differentiation of the mesenchymal progenitors to form the nephron epithelia. One of the advantages of the mouse kidney as an experimental system is that it can develop in culture, from the stage of initial branching of the UB (E11.5) for up to a week (although it achieves the size and degree of development of only an E13.5–E14.5 kidney in vivo). The availability of fluorescent proteins (FPs) has provided powerful tools for visualizing the morphogenesis of specific renal structures in organ cultures. Two categories of genetically modified mice that express FPs are useful for visualizing different cell lineages and developmental processes in these organ cultures: (1) transgenic mice that express a fluorescent reporter in the pattern of a specific gene; and (2) Cre reporter mice, which turn on an FP in cells with Cre recombinase activity (and their daughter cells), used in conjunction with cell type-specific Cre transgenic mice. Here, we describe some of the currently available Cre and FP transgenic lines that are useful for the study of kidney development.

RELATED INFORMATION

A protocol is available for **Dissection of Embryonic Mouse Kidney, Culture in Vitro, and Imaging of the Developing Organ** (Costantini et al. 2011).

KIDNEY DEVELOPMENT

Metanephric (adult) kidney development begins when the epithelial UB evaginates from the caudal end of the Wolffian duct (WD), at E10.5 in the mouse, and grows into the adjacent metanephric mesenchyme (MM). The UB branches repeatedly over the next several days and ultimately gives rise to the ureter, pelvis, calyces, and renal collecting ducts. At the same time, progenitor cells in the MM are converted to epithelial renal vesicles, which fold, elongate to form comma-shaped bodies (CSBs) and S-shaped bodies (SSBs), and then differentiate to generate the different segments of the nephron tubule (including proximal and distal tubules, loops of Henle, and glomerular podocytes). These processes depend on reciprocal induction in which the UB produces factors that promote and control nephrogenesis, while the MM produces factors that control UB branching morphogenesis. Both the UB and the MM derive from intermediate mesoderm, whereas another renal cell lineage, the stromal cells, derives from somitic mesoderm. Stromal cells are the precursors of the interstitial cells in mature kidneys but also play an inductive role in kidney development. The kidney also contains vascular cells, including the capillary loops of the glomeruli, and mesangial cells. Many reviews of kidney development are available (Saxen 1987; Carroll and McMahon 2003; Costantini 2006; Dressler 2006; Schedl 2007).

The genetic control of metanephric kidney development has been studied extensively, mostly in the mouse, and many genes that play important roles in different cell lineages have been identified, primarily through studies of knockout mice. Imaging the developing kidney is important not only for describing in detail the normal morphogenetic processes involved in organogenesis but also for understanding how genetic perturbations affect these processes.

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One of the advantages of the mouse kidney as an experimental system is that it can develop in culture, from the stage of UB evagination and initial branching (E11.0–E11.5), through the first eight to 10 rounds of UB branching (Watanabe and Costantini 2004). Because of limitations in current culture technology, development is slower in culture than in vivo, and the kidney takes ~4–5 d to develop to a size and complexity that it would reach in ~2–3 d in vivo. Nevertheless, many of the processes that occur in vivo also occur in organ culture, allowing them to be visualized through time-lapse imaging. These include UB branching, the formation and segmentation of nephrons, and the connection of nephrons to collecting ducts; however, the vascular component of the glomerulus does not form in cultured kidneys (Vaughan and Quaggin 2008). The standard conditions for kidney culture (see below), in which it is grown on a filter at the air-medium interface, cause the kidney to flatten, so that it grows mostly in two dimensions rather than in three dimensions. Although this is a disadvantage in that the three-dimensional (3D) organization of the kidney is largely lost, it is an advantage in that it makes imaging much easier. There are alternative culture conditions in which the kidney will grow in a more 3D pattern with less flattening (Giuliani et al. 2008; Rosines et al. 2010), but these have not been used as extensively. Another recently described culture system, in which kidneys are grown directly on glass, in a low volume of medium, allows more extensive growth than in the standard filter cultures (including development of a distinct cortex and medulla), although the kidneys also flatten in this system (Sebinger et al. 2010).

In addition to culturing the whole kidney, it is possible to isolate the UB or the MM and grow them separately (Grobstein 1953, 1955; Qiao et al. 1999a). This is useful for isolating the processes of UB branching and nephrogenesis and for studying the secreted factors that are normally provided by the other tissue to promote these processes. Thus, the isolated UB will grow and branch without any mesenchyme if cultured in Matrigel (BD Biosciences) or collagen and supplied with appropriate growth factors (Qiao et al. 1999b, 2001), whereas the isolated MM will undergo nephrogenesis if cultured with either heterologous tissues (which fortuitously produce the necessary inducers) or with factors normally provided by the UB (Sariola 2002; Carroll and McMahon 2003; Dressler 2006).

Some of the questions that can be addressed by imaging cultured kidneys but cannot be answered as readily (or at all) by retrospective analysis of development in vivo are as follows:

- What is the normal sequence, relative timing, and pattern of UB branching morphogenesis and nephrogenesis?
- How do cell movements in the UB or nephron epithelia contribute to these processes?
- What is the fate of cells in different regions of the UB or the MM?
- How are these events affected by mutations or other genetic alterations that interfere with normal kidney development?
- How are these events altered by growth factors, drugs, or other agents?

TRANSGENIC MICE FOR IMAGING KIDNEY DEVELOPMENT

A certain amount of detail is visible in cultured live kidneys using bright-field or phase-contrast optics, but the utility of these images is limited, particularly at later stages of kidney development when the organ is more complex. Therefore, the availability of FPs has provided a great advantage for visualizing the morphogenesis of specific structures, such as UB branching, in organ cultures. There are two main categories of genetically modified mice that are useful for visualizing different cell lineages in renal organ cultures: (1) transgenic mice that express a fluorescent reporter in the pattern of a specific gene, and (2) Cre reporter mice, which turn on a fluorescent reporter in cells with Cre recombinase activity (and their daughter cells), used in conjunction with cell type-specific Cre transgenic mice.

Transgenic Mouse Strains Expressing Fluorescent Reporter Proteins

Several mouse lines that express green fluorescent protein (GFP) or other FPs in specific kidney lineages are listed in Table 1. For information on the functions of the many genetic markers mentioned in this chapter, whose promoters are used to drive transgenes, the reader is referred to recent review articles (Dressler 2009; Costantini and Kopan 2010). *Hoxb7/eGFP* (Srinivas et al. 1999), the first such strain, labels the WD and UB lineages and has been extremely useful for studying the rate and the pattern of WD, ureter, and UB morphogenesis (Fig. 1; Watanabe and Costantini 2004; Batourina et al. 2005).

Table 1. Transgenic mouse lines expressing fluorescent proteins in developing kidney

Gene/promoter	Fluorescent reporter	Expression sites in developing kidney	Reference
<i>Cdh16</i> (Ksp-cadherin)	GFP	UB, nephron tubules	Shao et al. 2002a
<i>Cxcl12</i>	GFP	Stroma, podocytes, arteries	Takabatake et al. 2009
<i>Foxd1</i>	GFP	Stroma	Levinson et al. 2005
<i>Hoxa11</i>	GFP	MM, CM, ureteral stroma	Nelson et al. 2008
<i>Hoxb7</i>	eGFP	WD, UB	Srinivas et al. 1999
<i>Hoxb7</i>	myr-Venus	WD, UB	Chi et al. 2009
<i>Ret</i>	GFP	WD, UB (tips)	Jain et al. 2006
<i>Sall1</i>	GFP	CM, CSB, SSB, renal tubules, podocytes	Takasato et al. 2004
<i>Six2</i>	eGFP/CreERT2	CM, early pretubular aggregates	Kobayashi et al. 2008

UB, ureteric bud; WD, Wolffian duct; CM, cap mesenchyme; CSB, comma-shaped bodies; SSB, S-shaped bodies.

Hoxb7/GFP (and other FP transgenic lines) can be visualized in conjunction with bright-field or phase imaging to observe UB branching in the context of overall kidney growth. Transgenes such as this one can also be bred onto mutant backgrounds to study how different mutations affect these processes (e.g., Watanabe and Costantini 2004; Basson et al. 2006). Because enhanced green fluorescent protein (eGFP) is a cytoplasmic protein expressed in every UB cell, it does not readily allow the shapes and the movements of individual UB cells to be visualized. One solution to this limitation is to make chimeric embryos in which only a fraction of the cells carry the *Hoxb7*/eGFP transgene, so that labeled cells are often surrounded by unlabeled cells (Shakya et al. 2005b). Another approach is to express a myristoylated variant of GFP (myr-Venus), which labels the cell membrane, under the *Hoxb7* promoter (Chi et al. 2009). In live *Hoxb7*/myr-Venus kidneys, the outline of every cell is visible, allowing changes in cell shape and position to be observed. However, optimal imaging of the individual UB cells in these kidneys requires confocal microscopy, and four-dimensional movies have not yet been successfully generated.

Whereas *Hoxb7*/GFP labels the entire UB tree, *Ret*-GFP mice (in which GFP is targeted to the *Ret* locus) express GFP specifically at the tips of the UB (Jain et al. 2006). Ksp-cadherin (*Cdh16*)-GFP mice express GFP in both the UB and the nephron epithelia but not in the undifferentiated MM (Shao et al. 2002a). As *Cdh16*-Cre (Shao et al. 2002b) is expressed only in the UB trunks, not the tips, during early branching (Karner et al. 2009), it is likely that *Cdh16*-GFP also serves as a UB-trunk-specific marker.

Several mouse strains carry GFP under the regulation of *Six2* (Kobayashi et al. 2008) and express it specifically in the MM, the progenitor cells of the nephron lineage. In contrast, *Sall1*-GFP (Takasato et al. 2004) is expressed throughout the nephron lineage, from condensed mesenchyme, to CSBs and SSBs, and to renal tubules and podocytes. *Foxd1*/GFP mice express GFP specifically in the renal stroma (Levinson et al. 2005). Transgenic lines are available that express GFP (or Cre) in vascular cells (Constien et al. 2001; Koni et al. 2001; Motoike et al. 2003; Fraser et al. 2005); however, as the renal vasculature fails to develop in organ cultures (Vaughan and Quaggin 2008), these will be useful for live imaging only if conditions are found that permit vascular development.

Several additional strains are under development by a consortium (the GenitoUrinary Development Molecular Anatomy Project) sponsored by the National Institute of Diabetes and Digestive and Kidney

FIGURE 1. Time-lapse series from a cultured metanephric kidney. The branching ureteric bud is visualized by the expression of eGFP under the control of the *HoxB7* promoter (Srinivas et al. 1999). The surrounding metanephric mesenchyme is not labeled and, therefore, not visible. Time from start of culture is given in hours at the bottom left of each panel. Scale bar, 500 μ m.

Diseases (NIDDK) (McMahon et al. 2008) and will be listed at <http://www.GUDMAP.org> when available. Another potential source of new transgenic reporter strains is the Gene Expression Nervous System Atlas (GENSAT) (Gong et al. 2003) (<http://www.GENSAT.org>), a project to construct bacterial artificial chromosome (BAC) GFP reporter transgenic strains for many genes expressed in the nervous system, some of which are likely to also be expressed in the kidney. GENSAT strains are available via <http://www.MMRRC.org>.

One limitation of the currently available strains is that nearly all of them express the same FP, GFP, so they cannot be used together to visualize different cell lineages. Ultimately, the use of transgenes expressing FPs with different emission spectra (e.g., cyan fluorescent protein [CFP], yellow fluorescent protein [YFP], and red fluorescent protein [RFP]) in different cell types in the kidney will allow the simultaneous imaging of multiple developmental processes.

Another way to express an FP in the developing kidney is to use a transgenic mouse expressing reverse tetracycline transactivator (rtTA) (Urlinger et al. 2000) together with a reporter strain, such as TetO-GFP-lacZ (Krestel et al. 2001). Administration of doxycycline allows the reporter gene to be turned on or off in a regulated manner. Hoxb7/rtTA mice (Shakya et al. 2005a) express rtTA in the WD and UB lineages, whereas Pax8-rtTA mice (Traykova-Brauch et al. 2008) express rtTA in the UB lineage and in the nephron tubules (but not in the MM, the CSBs, or the SSBs).

Cre Reporter and Cre Transgenic Mouse Lines

Another approach to image specific cell lineages in the developing kidney is to use transgenic lines expressing Cre recombinase (Igarashi 2004; Lantinga-van Leeuwen et al. 2006; Rubera et al. 2009) together with fluorescent Cre reporter lines. Cre reporter lines are generally designed to express an FP in a cell only after a transcriptional stop sequence preceding the gene has been excised by Cre recombinase. The FP reporter gene is inserted in a constitutively expressed locus, such as *Gt(ROSA)26Sor* (*ROSA26*), so that the reporter remains expressed in all the daughter cells, even if they stop expressing the gene driving Cre recombinase (Soriano 1999). Cre reporter lines that express GFP (Mao et al. 2001), YFP (Srinivas et al. 2001), CFP (Srinivas et al. 2001), tdRFP1 (Luche et al. 2007), and others have been generated in this way. Cre reporters have also been made using the ubiquitously expressed CAG promoter, with GFP (Novak et al. 2000) and the RFP DsRed3 (Vintersten et al. 2004).

A large number of Cre transgenic lines, which are useful for imaging the developing kidney, have been developed. These include lines with constitutive or inducible Cre activity. Both types of lines, when used with a Cre reporter, label most or all of the cells in which Cre recombinase is active (as well as all of their daughter cells) as the kidney develops. The advantage of inducible forms of Cre, such as CreERT2, is that the timing of recombinase activity can be controlled by the administration of a drug, such as tamoxifen or 4-OHT in the case of CreERT2. This makes inducible Cre transgenic lines particularly useful for genetic inducible fate mapping (Joyner and Zervas 2006) in which the cells that descend from a particular cell population at a specific time can be traced. Inducible Cre lines also offer the possibility of inducing a low level of recombination (i.e., in a subset of the cells that express the Cre transgene) by using a low dose of the inducing drug. This often makes it easier to visualize individual cells and to observe their movements or other behaviors. Several lines combine GFP and Cre or CreERT2 under control of the same promoter or locus; by crossing to a Cre reporter strain that expresses a reporter other than GFP, the cells expressing the transgene are labeled with GFP, and their descendants (which may or may not still express the GFP) are labeled with the second reporter (Kobayashi et al. 2008). This strategy has been used (although not yet in live cultures) to differentially label the MM cells with respect to their descendant cells in nephron epithelia, which no longer express *Six2* (Kobayashi et al. 2008). One potential limitation to keep in mind, however, is that there is generally a time lag between administration of the inducing drug and labeling of the cell by recombination.

Table 2 contains a list of Cre transgenic strains that are useful for labeling different cell lineages in the developing kidney in organ culture. These include lines expressing Cre or CreERT2 in the intermediate mesoderm, WD, UB, MM, nascent nephrons, podocytes, and stromal cells. Several Cre lines label different segments within the more differentiated nephron in adult kidneys (e.g., proximal tubules, loop of Henle, distal tubules, and podocytes). However, only those that have been reported to express Cre activity in fetal kidneys are listed in Table 2. Additional lines that express in adult kidneys, and may be useful in cultured fetal kidneys, have been listed in several articles (Igarashi 2004; Lantinga-van Leeuwen et al. 2006; Rubera et al. 2009). Another source of information about Cre lines is *Cre-X-Mice: A Database of Cre Transgenic Lines* (<http://nagy.mshri.on.ca/cre/index.php>). This database is still being

Table 2. Transgenic mouse lines expressing Cre recombinase in developing kidney

Gene/promoter	Cre type	Expression of Cre reporter in (developing) kidney	Reference
Bmp 7	Cre	WD, UB, MM, nephrons	Oxburgh et al. 2004
Cdh16	Cre	WD, UB (trunks only, before E15.5)	Shao et al. 2002b; Karner et al. 2009
(Ksp-cadherin)			
Cdh16	CreERT2	(Not examined in fetal kidney)	Lantinga-van Leeuwen et al. 2006; Patel et al. 2008
(Ksp-cadherin)			
Cited1	CreERT2	CM (after E13.5), nephrons	Boyle et al. 2008
Hoxb7	Cre-IRES-GFP	WD, UB	Zhao et al. 2004
Hoxb7	Cre	WD, UB	Yu et al. 2002
Nestin	Cre	IM, MM, nephrons	Tronche et al. 1999; Chen et al. 2006
Nphs1 (Nephrin)	Cre	Podocytes	Eremina et al. 2002
Nphs2 (Podocin)	Cre	Podocytes	Moeller et al. 2003
Osr1	Cre-ERT2	IM, most cell types (if induced early), MM and nephrons (if induced after 10.5)	Mugford et al. 2008
Osr2	Cre	SSB, PT, glomeruli	Lan et al. 2007
Pax2	Cre	WD, UB	Pedersen et al. 2005
Pax2	Cre	WD, UB, MM, nephrons	Ohyama and Groves 2004
Pax3	Cre	MM, nephrons, stroma	Engleka et al. 2005
Pax8	Cre	IM, WD, ureter, nephron tubules, glomeruli	Bouchard et al. 2004
Rarb	Cre	IM, MM, nephrons, cortical stroma, ureter mesenchyme	Kobayashi et al. 2005
Ren1	Cre	UB	Castrop et al. 2006
Six2	Cre	MM, nephrons	Kuure et al. 2007
Six2	Tet-off-eGFP-Cre	MM, nephrons	Kobayashi et al. 2008
Six2	eGFP-CreERT2	MM, nephrons	Kobayashi et al. 2008
Six2	CreERT2	MM, nephrons	Kobayashi et al. 2008

UB, ureteric bud; WD, Wolffian duct; CM, cap mesenchyme; IM, intermediate mesoderm; MM, metanephric mesenchyme; SSB, S-shaped bodies; PT, proximal tubules.

Refer to original references for details of expression pattern. Many lines with reported adult expression that may also express in fetal kidney are not listed.

developed, and it will list new Cre lines as they are developed in the future. One can also look up Cre lines on the *Mouse Genome Informatics* website (<http://www.informatics.jax.org/>).

As most or all of the available lines express Cre recombinase (as opposed to Fip recombinase), it is not yet possible to use two different Cre lines simultaneously to label different structures in different colors. For example, mice with a Hoxb7-Cre, a Cited1-Cre, and a YFP reporter strain will express YFP in both the UB and nephron lineages. However, it is feasible to use an RFP Cre reporter strain with Cited1-Cre, together with Hoxb7-GFP transgenic mice, to generate kidneys in which the UB is GFP⁺ and the nephrons are RFP⁺.

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