Paraxial mesoderm contributes stromal cells to the developing kidney

Richard Guillaume [a], Michel Bressan [b], Doris Herzlinger [a,*]

[a] Weill Medical College of Cornell University, Department of Physiology and Biophysics, 1300 York Ave, New York, NY 10065, USA
[b] Cardiovascular Research Institute, University of California San Francisco, 4th Street, Box2711, San Francisco, CA 94158-2324, USA

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ABSTRACT

The development of most, if not all, tubular organs is dependent on signaling between epithelial and stromal progenitor populations. Most often, these lineages derive from different germ layers that are specified during gastrulation, well in advance of organ condensation. Thus, one of the first stages of organogenesis is the integration of distinct progenitor populations into a single embryonic rudiment. In contrast, the stromal and epithelial lineages controlling renal development are both believed to derive from the intermediate mesoderm and to be specified as the kidney develops. In this study we directly analyzed the lineage of renal epithelia and stroma in the developing chick embryo using two independent fate mapping techniques. Results of these experiments confirm the hypothesis that nephron epithelia derive from the intermediate mesoderm. Most importantly, we discovered that large populations of renal stroma originate in the paraxial mesoderm. Collectively, these studies suggest that the signals that subdivide mesoderm into intermediate and paraxial domains may play a role in specifying nephron epithelia and a renal stromal lineage. In addition, these fate mapping data indicate that renal development, like the development of all other tubular organs, is dependent on the integration of progenitors from different embryonic tissues into a single rudiment.

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The permanent kidney of birds and mammals develops from a rudiment composed of two tissues, the ureteric bud epithelium and the metanephric mesenchyme. In vitro tissue recombination experiments and more recently, genetic studies demonstrate that the metanephric mesenchyme secretes factors that support ureteric bud formation and growth into the renal collecting duct system (Grobstein, 1953; Pichel et al., 1996; Sainio et al., 1997; Sanchez et al., 1996; Schuchardt et al., 1996). The ureteric bud in turn, secretes factors that trigger six2+, cited1+ progenitors within the metanephric mesenchyme to differentiate into nephrons, the functional units of the kidney (Grobstein, 1953; Herzlinger et al., 1994; Carroll et al., 2005; Boyle et al., 2008; Kobayashi et al., 2008). The metanephric mesenchyme also contains foxd1+ and flk1+ progenitors that differentiate into the renal stroma and endothelia, respectively (Hatini et al., 1996; Humphreys et al., 2008; Shalaby et al., 1995; Tufro et al., 1999). In vitro experiments and gene ablation studies suggest that factors secreted by these stromal and endothelial progenitor cell populations are also required for renal morphogenesis (Bard, 1996; Hatini et al., 1996; Sariola et al., 1988a; Gao et al., 2005; Levinson et al., 2005; Quaggin et al., 1999). Thus, signaling between several distinct progenitor cell populations within the metanephric rudiment controls kidney development.

Despite the dependence of kidney development on interactions between ureteric bud epithelia, nephron, stromal and endothelial progenitor cell populations, the origins and the mechanisms that control the specification of these lineages remain poorly understood (Sariola and Sainio, 1998; Sariola et al., 2003). Using direct fate mapping protocols, we have shown that the progenitors that give rise to the nephric duct, the precursor of the ureteric bud and collecting tubules, derive from rostral intermediate mesoderm (Obara-Ishihara et al., 1999). Bmp signaling, transcriptional regulation by Pax2 and Pax8 and unknown factors secreted by the adjacent paraxial mesoderm are likely to play a role in specifying this renal progenitor cell population (Bouchard et al., 2002; Mauch et al., 2000; Obara-Ishihara et al., 1999).

The origins of the lineages comprising the metanephric mesenchyme remain less well understood. The metanephric mesenchyme is believed to be composed solely of cells derived from caudal intermediate mesoderm and a recent genetic fate mapping experiment performed in the developing mouse raises the possibility that all lineages present in the metanephric mesenchyme derive from Osr1+ progenitors (Saxen, 1987; Mugford et al., 2008). Osr1 is expressed in the intermediate mesoderm, however, this transcription factor is expressed in mesoderm prior its subdivision into paraxial and intermediate domains. Moreover, Osr1 is expressed in both the intermediate mesoderm and lateral plate (James and Schultheiss, 2003; Wilm et al., 2004). Finally, renal progenitors that derive from tissues other than the intermediate or lateral plate mesoderm may upregulate Osr1 as they enter the renal field. Thus, it remains unclear if the Osr1+ progenitor populations that generate nephron epithelial, stromal and endothelial progenitor populations all derive from the

* Corresponding author. Fax: +1 212 746 8434.
E-mail address: daherzli@med.cornell.edu (D. Herzlinger).

intermediate mesoderm (Mugford et al., 2008). For example, classic embryological studies utilizing chick-quail chimera suggest that the neural crest gives rise to renal stromal cells (Bronner-Fraser and Fraser, 1988; Le Douarin and Teillet, 1974).

In this study we used direct fate mapping techniques to determine if renal stromal cells derive from the intermediate mesoderm and/or the neural crest (Cepko et al., 1998; Mikawa and Fischman, 1992; Obara-Ishihara et al., 1999). When *LacZ* was transferred into the intermediate mesoderm, few lineage tagged renal stromal cells were detected whereas large populations of lineage tagged nephron epithelia were observed. Similarly, few lineage tagged stromal cells were detected when the neural crest was fate mapped. Most importantly, large populations of lineage tagged renal stroma were detected when the paraxial mesoderm was fate mapped by either dye injection or gene transfer techniques. Specifically, lineage tagged cells deriving from the paraxial mesoderm were localized to *foxd1*+ zones of the developing kidney and exhibited morphological properties and protein expression patterns consistent with vascular smooth muscle, myofibroblasts, pericytes and mesangial cells.

Collectively, these data show that the paraxial mesoderm contributes renal cells to the developing kidney and provide some insight into why known derivatives of the paraxial mesoderm such as cartilage and muscle, are ectopically expressed in dysplastic renal tissues (Kakkar et al., 2006). In addition, our results suggest that renal morphogenesis is dependent on the integration of cells from both the intermediate and paraxial mesoderm into a single embryonic rudiment and raise the possibility that the specification of nephron epithelial and desmin+ renal stromal cells may be controlled by the same signals that subdivide mesoderm into intermediate and paraxial domains.

Methods

Fate mapping in ovo

White Leghorn fertilized eggs were incubated at 37.8 °C for 2 or 2.5 days prior to experimental manipulation. Embryos were exposed by making a blunt hole in the eggshell and staged according to the criteria of Hamburger and Hamilton (HH) (Hamberger and Hamilton, 1952). For short term fate mapping, ~ 10 nl of CM-DiI or DiO (Molecular Probes) at 1 µg/µl in ethanol/dimethylformamide, was injected into selected tissues of HH St 15–16 embryos using a microspritzer connected to a micropipette needle. Dye delivery involved rupture of ectoderm prior to penetration of the micropipette inside the mesoderm. Fluorescence was observed with a Nikon Eclipse TE200 equipped with a Super High Pressure Mercury Lamp and a C-SHG1 Power supply and observed under a rhodamine filter (G). Images were captured with a Hamamatzu camera controlled by Metamorph software.

For long-term fate mapping, 1–5 nl of a concentrated SNTZ retroviral stock (10^7–10^8 infectious virions/ml) was injected into
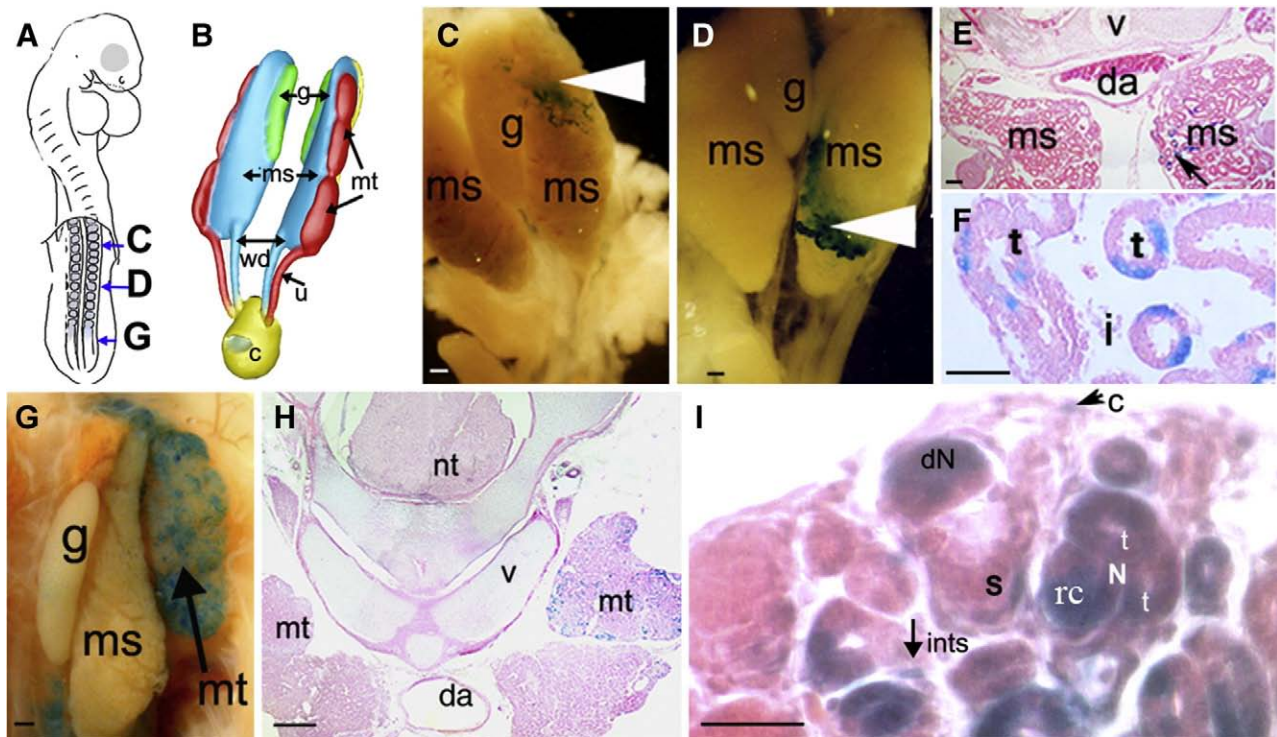

Fig. 1. Nephron epithelia are preferentially labeled after *LacZ* transfer into the intermediate mesoderm. Chick embryos were injected with replication defective retrovirus encoding *LacZ* into the intermediate mesoderm on the right side between 50 and 55 h of incubation (HH St 15–16). Diagram of the embryo at this stage (A, reproduced with permission from Brenner-Anantharam et al., 2007) showing the axial levels of *LacZ* transfer for samples shown in C, D and G, which are whole mount views of isolated urogenital tracts fixed and processed for β-gal activity 12 days after gene transfer. The model in panel B illustrates the architecture of the avian urogenital tract at this stage (reproduced with permission from Brenner-Anantharam et al., 2007). The mesonephros (ms) lies ventral and medial to the metanephros (mt) and both kidney types are associated with outflow tubes, the wolffian duct (wd) and ureter (u), which empty into the cloaca (c). When *LacZ* was transferred into the intermediate mesoderm adjacent to somite 18 (C) or somite 23(D), β-gal+ cells (white arrows) were detected in the right mesonephros (ms) and gonad (g). Examination of H&E stained tissue sections demonstrate that lineage tagged cells are present in mesonephric nephrons (E, black arrow) and exhibit the polarized, cuboidal morphology typical of tubular epithelia (F, t). Tagged cells were not detected in the sparse mesonephric interstitium (i), the metanephros (mt), vertebrae (v), dorsal aorta (da), in any tissues on the left, un-injected side of the embryo or in control, sham injected embryos (data not shown). The metanephros (mt) was efficiently lineage tagged when *LacZ* was transferred into the intermediate mesoderm caudal to somite 28 (G–I). As can be seen in whole mounts of the upper urogenital tract (G) and low power H&E stained paraffin sections (H), the right metanephros (mt, arrow) exhibits intense β-gal staining whereas the gonads (g) and mesonephros (ms) lack lineage tagged cells. Tagged cells were not observed in the neural tube (nt), vertebrae (v) or dorsal aorta (da) or in the metanephros on the control side of the embryo. Examination of representative section at higher power (I) indicates that the vast majority of blue, β-gal+ cells are present in aggregates of nephrogenic mesenchyme in the developing nephrons (dN) and in more mature nephrons (N) including epithelia of the renal corpuscle (rc) and tubules (t). Although large populations of lineage tagged nephron epithelial were observed, few lineage tagged cells were detected in the intersitiium between the tubules (ints) or in the renal capsule (c, arrow) 100 µm − C, D and G and H Scale 50 µm − E, F and I.

selected tissues of the HH St 15–16 embryos as described by Mikawa and Fischman (Mikawa et al., 1991). The SNTZ retrovirus is replication defective and integrates a reporter, *LacZ*, into the genome of infected cells. Eggs were resealed with parafilm and incubated for stated times at 37.8 °C prior to embryo isolation and fixation. Embryos were fixed as whole mounts with 4% paraformaldehyde for 4–8 h and β-galactosidase enzyme activity was developed by histochemical techniques and photographed. Embryos were then embedded in paraffin and 10 μm thick serial sections prepared. Fate mapping experiments were quantified by analyzing the phenotype of all tagged renal cells in random sections from at least 3 different embryos. Results are expressed as a percentage of tagged cells expressing an epithelial phenotype, which was calculated by dividing the number of cells with a definitive epithelial phenotype by the total number of tagged cells counted (+/−standard error of the mean). A minimum of 100 tagged cells/section were analyzed.

Detection of proteins and mRNAs

Paraffin sections were dehydrated and processed for antigen retrieval as described by Boenisch (2007). Sections were incubated with a polyclonal antibody directed against desmin (Sigma Chemical Co.) and peroxidase-conjugated goat-anti rabbit IgG (Jackson Immunochemicals) was used to detect antibody binding. Control sections were incubated with secondary antibody only. *Foxd1* mRNA was detected by chromogenic in situ hybridization protocol (Henrique et al., 1995). A chicken *cfoxd1* cDNA fragment of 628 bp was produced by RT-PCR using primers designed from the published chicken *foxd1* (previously called *cBF-2*) cDNA sequence (GenBank accession number U47276.1) amplified from reversed transcribed total-RNA of 7 days old chicken embryos, according to the Guanidine Thiocyanate method for Total RNA extraction. The 628 bp fragment was cloned into PstBlue-1 (Novagen), linearized with BamHI and used with the SP-6 RNA transcriptase (Invitrogen) to produce a DIG-labeled antisense riboprobe (Boehringer labeling Kit) or linearized with HindIII and used with T7 to produce a sense riboprobe. Chick *Pax2* cDNA was provided by Dr Henrique and linearized with Xba1 to produce, with T3, an 800 bp antisense riboprobe.

Results

Nephron epithelia are preferentially labeled after LacZ transfer into the intermediate mesoderm

The fate of cells deriving from the avian intermediate mesoderm was determined by transferring *LacZ* into this tissue between 50 and 55 h of incubation (HH St 15–16) and characterizing the differentiated phenotype of β-gal expressing cells 8–17 days after gene transfer. As would be predicted by classical embryological studies, *LacZ* transfer into the intermediate mesoderm at early stages of development resulted in the presence of β-gal expressing renal cells at later stages.

When *LacZ* was transferred into thoracic intermediate mesoderm from the axial levels of somites 15–28, β-gal tagged cells were detected in the mesonephros, a developmental intermediary excretory organ in birds as well as certain mammalian species including humans (Figs. 1C–F). The permanent kidney or the metanephros was labeled only when *LacZ* was transferred into the unsegmented mesoderm caudal and lateral to somite 28, approximating the position of the intermediate mesoderm (Figs. 1G–I). In all 6 embryos analyzed, β-gal + renal cells exhibited a cuboidal morphology and were within tubular structures. In addition, lineage tagged cells with the distribution and morphological properties consistent with podocytes were detected in the renal corpuscle (Fig. 1I). Some β-gal+ stromal cells were detected in the metanephros after *LacZ* was transferred into the caudal intermediate mesoderm (Fig. 1I). However, 87 +/− 9.8% of the

lineage tagged cells displayed the spatial distribution and morphological properties of nephron epithelia. Thus, *LacZ* was preferentially transferred into nephron progenitors when SNTZ retroviral injections were targeted to the intermediate mesoderm.

Lineage of renal stroma

The relative dearth of β-gal+ stromal cells observed after *LacZ* was transferred into the intermediate mesoderm may reflect the inability of the SNTZ retrovirus to infect stromal progenitors or transfer *LacZ* into this cell lineage. Alternatively, it is possible that renal stromal progenitors are located in tissues other than the intermediate mesoderm at early stages of embryonic development. To discriminate between these possibilities we tested whether renal stroma could be efficiently lineage tagged when other candidate embryonic tissues were fate mapped.
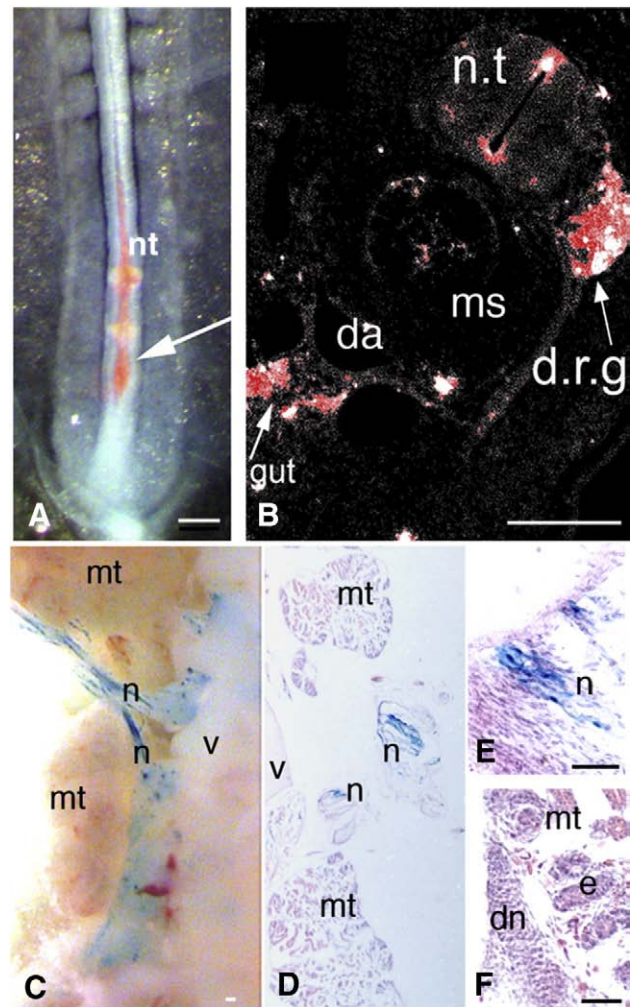

Fig. 2. The neural crest contributes few cells to the developing kidney CM-DiI, a fixation stable florescent dye, was injected into the neural tube at HH Stage 15–16 which is prior to caudal neural crest delamination. Embryos were photographed immediately (A) and incubated for an additional 5 days, fixed and vibratome sections prepared (B). Red, CM-DiI labeled cells were detected in the neural tube (nt), dorsal root ganglia (d.r.g), and around the gut and dorsal aorta (da). Few, if any CM-DiI tagged cells were observed in the developing mesonephros (ms). Similar results were obtained when retrovirus encoding *LacZ* was injected into the neural tube of St 15–16 embryos as described above, and embryos fixed and processed for β-gal activity 14 days later. Examination of whole mounts (C) and tissue sections (D–F) demonstrate that blue, *LacZ* expressing cells are present in peripheral nerve (n) including the large bundles that pass between the lobes of the metanephros (mt). Few, if any, lineage tagged cells were observed within the metanephros (mt) in either differentiating nephrons (dn) or more mature tubular epithelia (e). Bar: 50 μm.

Protein, mRNA expression analyses and experiments analyzing chick-quail chimeras raise the possibility that renal stroma derive from the neural crest (Bronner-Fraser and Fraser, 1988; Sainio et al., 1994; Sariola et al., 2003; Le Douarin and Teillet, 1974). Therefore we first asked whether the neural crest contributes cells to the developing kidney. Briefly, either the carbocyanine dye DiI or concentrated SNTZ retrovirus was injected directly into the caudal neural tube of at HH St 15 embryos prior to the onset of neural crest migration (Serbedzija et al., 1989). The differentiated phenotype of lineage tagged cells was analyzed at later stages of development (Fig. 2). This injection protocol resulted in large populations of tagged cells within the neural tube and well characterized neural crest derivatives including the dorsal root ganglion and peripheral nerve that transverses the kidney ($n = 16$). However, few lineage tagged cells were detected in either the mesonephros or metanephros suggesting that neural crest is not a major source of renal cells.

Paraxial mesoderm contributes cells to the developing kidney

We next tested whether the paraxial mesoderm was a source of renal stroma as it gives rise to a variety of stromal cell types including fibroblasts and myofibroblasts of the dermis and body wall, as well as pericytes and smooth muscle of the vasculature (Pouget et al., 2008; Pourquie, 2000). DiI was injected into the paraxial mesoderm of HH St 15–16 embryos, and the location of DiI tagged cells was evaluated both immediately after tagging and at later stages of development ($N = 12$, Figs. 3A–C). Observations made immediately following DiI injections demonstrated that the paraxial mesoderm was effectively labeled whereas DiI was not detectable in the nearby intermediate mesoderm (Fig. 3A). After a total of 8 days of incubation, large populations of fluorescent, lineage tagged cells were observed in known derivatives of the somites including the vertebrae, body wall and dermis (data not shown). Strikingly, labeled cells were also associated with both the meso- and metanephros. The relative position of paraxial mesoderm along the rostral to caudal embryonic axis was preserved along the rostral to caudal axis of the avian kidneys (Figs. 3A, B). Cells derived from the paraxial mesoderm rostral to somite 23 were detected in the mesonephric capsule and mesonephric renal corpuscles in a distribution consistent with mesangial cells (data not shown). Cells derived from paraxial mesoderm between the axial levels somite 23–28 were associated with the rostral and middle metanephric lobes, whereas paraxial mesoderm caudal to somite 28 was associated with the caudal metanephric lobe. Tagged metanephric cells were observed in the metanephric capsule, in the interlobular connective tissue and between the differentiating renal tubules (Fig. 3C). DiI labeled cells were difficult to detect after incubations longer than 8 days due presumably, to dilution of the dye by successive rounds of cell division. Therefore, we used an independent, long-term fate mapping protocol to further test the hypothesis that paraxial mesoderm contributes cells to the metanephros. The genomic lineage tag, *LacZ*, was transferred into the paraxial mesoderm caudal to the axial level of somite 25 in HH ST 15 chick embryos by replication defective retroviral infection and the distribution and morphology of β-gal+ cells analyzed after both an additional 24 h and 14 days of development (Figs. 3D–H). As expected, 24 h after gene transfer, the somites contained large numbers of β-gal positive cells (Fig. 3D). After longer incubations, large populations of lineage tagged cells were observed within well characterized derivatives of the paraxial mesoderm including the vertebrae, connective tissue, musculature of the body wall, the dermis (data not shown) as well as in renal tissues (Figs. 3E–H). Lineage
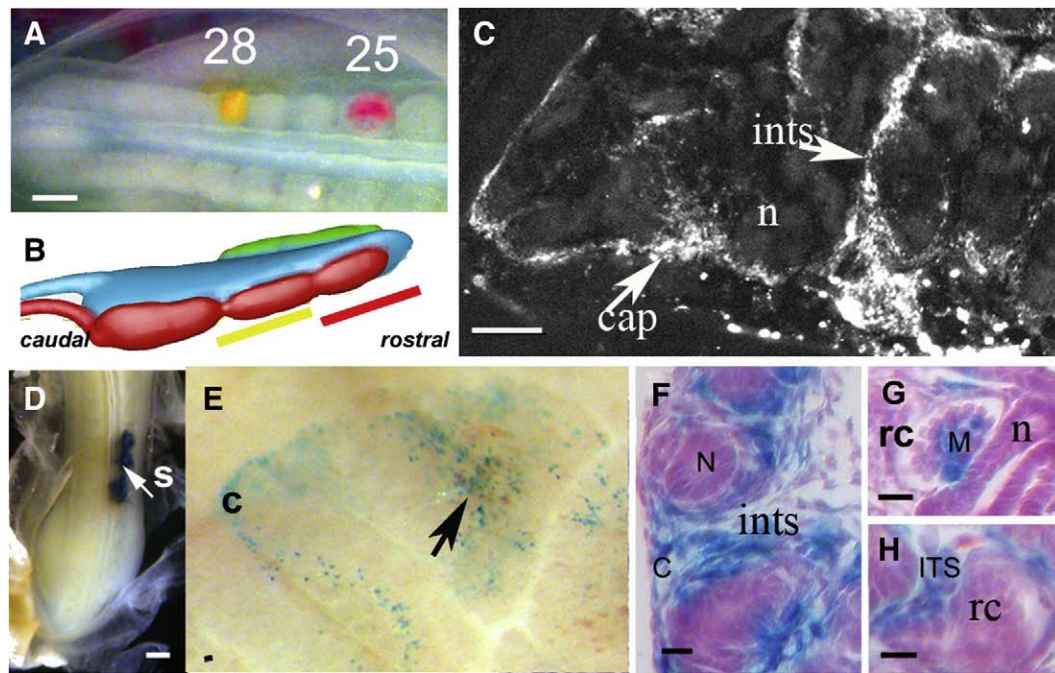

Fig. 3. Paraxial mesoderm contributes cells to the metanephros. Representative HH Stage 15 embryo (A) injected with CM-DiI (red) into the paraxial mesoderm at the axial level of somite 25 and with DiO (yellow) at the axial level of somite 28. Embryos were fixed after an additional 7 days of incubation, and frozen sections were prepared (C). CM-DiI and DiO tagged cells were observed in known derivatives of the paraxial mesoderm such as the vertebrae and body wall connective tissue (data not shown) and in association with the metanephros. As shown in Panel B, tagged cells from the axial level of somite 25 (red line) were associated with the rostal metanephric lobes, whereas DiO tagged cells deriving from paraxial mesoderm at the axial level of 28 were detected in the middle and caudal metanephric lobes (yellow line). Examination of representative frozen section (C) demonstrates that fluorescent, lineage tagged cells (white arrows) can be detected in the metanephric capsule (cap) and in the interstitium (inst) between the tubules. Fate mapping experiments using retroviral mediated gene transfer techniques confirm this result (D–H). *LacZ* was transferred into the paraxial mesoderm caudal to somite 28 in HH Stage 16 embryo and 24 h later, β-gal+, lineage tagged cells were present in the somites (s, arrow, D). By 17 days, whole mount examination of the metanephros (E) reveals large numbers of tagged cells in a spotty distribution throughout the organ. Blue, lineage tagged cells were also observed in other derivatives of the paraxial mesoderm including the vertebra and body wall connective tissues (data not shown). Examination of metanephric tissue sections (F–H) indicates that blue, lineage tagged cells are present in the interstitium (ints) and in the renal capsule (c). In addition, cells within the renal corpuscle (rc) with a distribution consistent with the mesangium (m) were labeled. Tagged cells were not detected in the renal tubules (t) or developing nephrons (n). Scale bars: A, C, D and 50 μm; F–H, 10 μm.

tagged cells were located in the metanephric capsule, between the metanephric tubules and in the renal corpuscle with a distribution consistent with the mesangial cells (Figs. 3F–H).

To more precisely define the differentiated phenotype of renal cells derived from the paraxial mesoderm, we assayed them for the expression of markers differentially expressed by renal stromal and epithelial progenitors. As in the developing murine kidney, Foxd1+ defines avian renal stromal progenitors whereas Pax2 marks progenitors that give rise to epithelia of the nephrons and collecting system (Figs. 4A, B, (Dressler et al., 1990; Hatini et al., 1996). Lineage tagged cells derived from the paraxial mesoderm localized to Foxd1+-stromal zones of the renal parenchyma (4C). In contrast, Pax2+ or epithelial zones of the kidney were devoid of lineage tagged cells deriving from the paraxial mesoderm (Fig. 4D). Lineage tagged, β-gal+ cells present in the capsule, between tubules and within the renal corpuscle co-expressed desmin, an intermediate filament protein (Figs. 4E, F). Desmin is selectively expressed by striated muscle, visceral and vascular smooth muscle, myofibro-

Fig. 4. Renal cells originating in the paraxial mesoderm express foxd1 and desmin. In situ hybridization detection of Foxd1 (A) and Pax2 mRNA (B) in the avian metanephros on E7. The avian E7 rudiment is markedly similar to the E11.5 murine metanephric kidney rudiment with foxd1+ stromal progenitors (sp) at the periphery and more centrally located Pax2+ epithelial progenitors (np) that are aggregated around the ureteric bud (ub). In situ hybridization detection of Foxd1 (C), Pax2 (D) and immuncytochemical detection of desmin (E, F) on E14 kidney sections from embryos after LacZ transfer into the paraxial mesoderm at HH St15–16 (C–F). Lineage tagged β-gal + cells (C–F, blue) are present in the interstium (its) between the renal tubules that contains Foxd1+ cells (C; purple), but are excluded from aggregates of immature, Pax2+ epithelial progenitors (D; ep, purple). β-gal+ cells derived from the paraxial mesoderm between the renal tubules (E) and in the renal corpuscle express (F) desmin (brown reaction product), an intermediate filament protein indicative of stromal cells including myofibroblasts, vascular smooth muscle, pericytes and mesangial cells (m). Podocytes (p) are negative for the β-gal lineage marker and for the brown, desmin reaction product. Bars: A, 50 mm.

blasts, pericytes and mesangial cells, a modified smooth muscle population present in the renal corpuscle that plays a role in glomerular hemodynamics (Grupp et al., 1997; Holthofer et al., 1995; Lindahl et al., 1998). Conversely, few lineage tagged cells expressed Pax2, a marker of renal epithelial progenitors (Fig. 4D). Collectively, these results indicate that the paraxial mesoderm contributes cells to the developing kidney that exhibit morphological properties, gene expression patterns and a spatial distribution consistent with capsular myofibroblasts and myofibroblasts between the renal tubules, vascular smooth muscle, pericytes and mesangial cells.

Discussion

The metanephric mesenchyme gives rise to most renal cell types and until recently was believed to be composed of a homogeneous population of multipotent renal stem cells originating from the intermediate mesoderm (Saxen, 1987). However, mRNA and protein expression analyses raised the possibility that the metanephric mesenchyme is composed of different mesenchymal cell types and in vitro gene transfer lineage tracing experiments supported this hypothesis (Herzlinger et al., 1992; see review by Sariola et al., 2003). Recent genetic fate mapping studies performed in vivo clearly demonstrate that the metanephric mesenchyme contains at least 2 fate-restricted renal lineages (Boyle et al., 2008; Humphreys et al., 2008; Kobayashi et al., 2008). Specifically, six2+, cited-1+ mesenchyme gives rise to nephron epithelia but not the renal stroma whereas foxd1+ cells give rise to the renal stroma, but not the nephron epithelia (Boyle et al., 2008; Humphreys et al., 2008 ; Kobayashi et al., 2008). Although it is now clear that the metanephric mesenchyme contains distinct progenitor cell populations, when they are specified and what signals control this process remain poorly understood. Importantly, insight into these renal cell fate decisions will be essential for establishing novel stem cell based therapies to repair renal tissues which are subject to damage by common pathologies such as diabetes and hypertension.

A recent study demonstrates that nephron epithelia, endothelia, smooth muscle, mesangial cells and ureteral smooth muscle derive from Osr1+ progenitors present in the early embryo (Mugford et al., 2008). However, Osr1 is expressed in multiple tissues during embryonic development thus the exact location(s) of Osr1+ renal progenitors remains unclear (James and Schultheiss, 2003; Wilm et al., 2004). In addition, it is possible that Osr1+-nephron epithelial and Osr1+-stromal progenitors derive from different tissues. For example, classic embryological studies indicate that the nephron epithelia derive from the intermediate mesoderm whereas protein expression analyses and fate mapping studies analyzing chick-quail chimeras raise the possibility that renal stromal cells derive from the neural crest (Bronner-Fraser and Fraser, 1988; Sainio et al., 1994; Sariola et al., 1988b; Le Douarin and Teillet, 1974). In summary then, the mature kidney has multiple stromal cell types including fibroblasts, myofibroblasts, vascular smooth muscle and mesangial cells and it is possible that these cells derive from multiple progenitor cell populations present in different locations of the early embryo (Kaissling et al., 1996; Marxer-Meier et al., 1998).

In this report we performed direct fate mapping experiments to further analyze the renal cell types derived from the intermediate mesoderm and the neural crest. LacZ transfer into the intermediate mesoderm of the early chick embryo resulted in the presence of large populations of lineage tagged nephron epithelia at later stages of development. Few if any renal stromal cells were lineage tagged when the intermediate mesoderm was fate mapped. Similarly, few if any renal stromal cells were labeled when dye or retrovirus encoding LacZ was injected into the caudal neural crest, despite the presence of labeled cells in known neural crest derivatives such as the adrenal medulla and peripheral nerve.
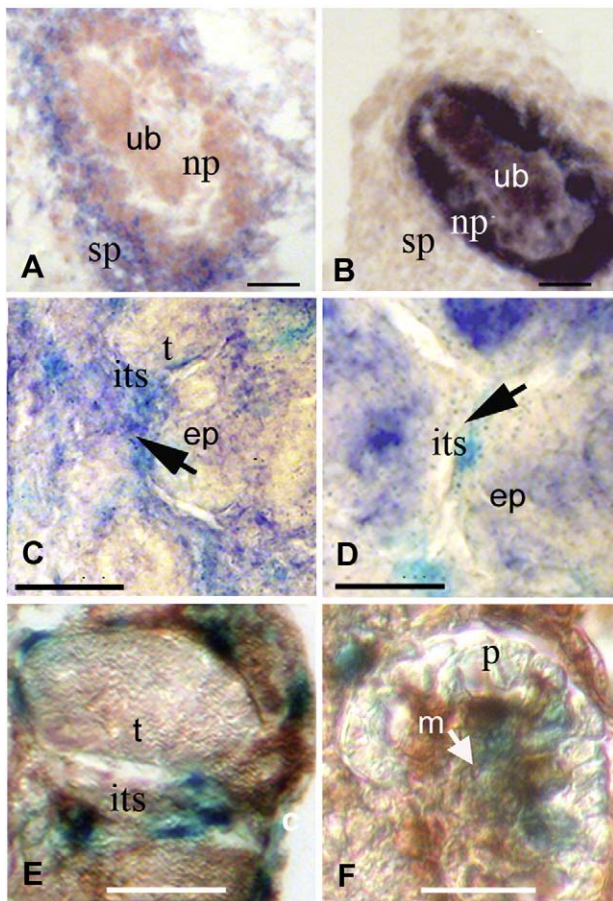
Although these data do not rule out the possibility that some renal stromal cells originate in the intermediate mesoderm and/or the neural crest, they strongly suggest that other tissues in the early embryo may also contribute stromal progenitors to the developing kidney. We hypothesized that renal stromal cells may derive from the paraxial mesoderm because this tissue generates the fibroblasts and myofibroblasts of the dermis and body wall (Brand-Saberi et al., 1996). Strikingly, when we fate mapped paraxial mesoderm in the early embryo by either dye injection or gene transfer lineage tracing techniques, large populations of lineage marked cells were detected within the kidney at later stages of development. Tagged cells deriving from the paraxial mesoderm were localized to *foxd1+* zones of the developing kidney indicative of a stromal phenotype and were labeled with antibodies to desmin. Thus, it is likely that the paraxial mesoderm gives rise to renal myofibroblasts, vascular smooth muscle, pericytes and mesangial cells (Hatini et al., 1996; Grupp et al., 1997; Holthofer et al., 1995; Lindahl et al., 1998). Few, if any renal epithelial cell types were lineage tagged when the paraxial mesoderm was fate mapped. These data definitively show that the paraxial mesoderm is a rich source of desmin+ renal cells including myofibroblasts, vascular smooth muscle, pericytes and mesangial cells.

Our fate mapping results are consistent with those performed in the mouse following the differentiated fate of *pax3+* progenitors. Specifically, large numbers of β-gal expressing renal stromal cells are detected in mice resulting from crosses between *Pax3*-Cre knockin and Cre-dependent reporter lines (Engleka et al., 2005). *Pax3* is expressed in both the paraxial mesoderm and neural crest; therefore the exact anatomical location of *pax3+* renal stromal progenitors cannot be assigned by this genetic fate mapping technique alone. However, *pax3* fate mapping data in the mouse combined with our anatomical fate mapping studies performed in the developing chick strongly support the hypothesis that the renal stromal cells derive from *pax3+* progenitors present in the paraxial mesoderm rather than the neural crest. Curiously, although we did not detect linage tagged renal epithelia when we transferred *LacZ* into the paraxial mesoderm, epithelial cell labeling was observed when the differentiated fate of *pax3* progenitors was analyzed in the mouse. Thus, *pax3* may also be expressed in a sub-set of the intermediate mesoderm or a sub-set of nephron epithelia may derive from *Pax3+* cells present in the paraxial mesoderm or neural crest in the developing mouse embryo. Alternatively, it is possible that the *pax3+* progenitors that give rise to nephron epithelia are present in the mesoderm prior to its commitment to an intermediate or paraxial fate.

Collectively our avian fate mapping data combined with the results of murine genetic fate mapping studies strongly suggest that the paraxial mesoderm gives rise to sizable populations of renal stroma. We show that cells derived from the paraxial mesoderm are present in *foxd1+* zones of the developing avian kidney and have previously shown that targeted mutation of *foxd1* in the mouse results in kidney patterning defects (Hatini et al., 1996). Preliminary somite ablation experiments we have performed in the developing chick support a role for the paraxial mesoderm in renal development. Specifically, when focal domains of caudal paraxial mesoderm were ablated from HH St 15 embryos, 60% of the operated embryos exhibited fused metanephroi. Kidney fusion across the midline is also observed in mouse lines with genetic defects in the renal stromal compartment (Hatini et al., 1996; Quaggin et al., 1999). However, the kidneys of these lines, including *Foxd1* and *Pod1* null mice, are also characterized by gross abnormalities in renal epithelial morhogenesis which were not observed in chick embryos after somite ablation. The low penetrance and mild nature of renal defects observed after somite ablation in the chick is due, most likely, to the limited amount of paraxial mesoderm that can be removed without compromising embryonic viability (Jungel-Waas et al., 1998). We will have to develop better techniques to ablate the paraxial mesoderm without compromising embryonic viability to further test the dependence of avian renal development on cells originating in the paraxial mesoderm.

In conclusion, our anatomical fate mapping studies combined with *pax3*-genetic fate mapping experiments in the mouse suggest that renal development is dependent on the recruitment of lineages derived from the intermediate and paraxial mesoderm into the metanephric anlagen. In addition, these data raise the possibility that the specification of nephron progenitors and at least one renal stromal lineage, progenitors that give rise to desmin+ smooth muscle, myofibroblasts, pericytes and mesangial cells, is controlled by the same signals that subdivide mesoderm into intermediate domains and paraxial domains. Thus, kidney morphogenesis, like the formation of all other tubular epithelial organs, is dependent on the morphogenetic processes that bring these distinct, interacting cell lineages together. Finally, since our data demonstrate that a renal stromal lineage derives from the paraxial mesoderm, a tissue known to differentiate into muscle, cartilage and bone, these results provide new insight into mouse mutants with skeletal and renal defects as well as the etiology of dysplastic kidneys characterized by tubules embedded in fibro-muscular or cartilaginous connective tissues (Kakkar et al., 2006; Lane and Birkenmeier, 1993; Nacke et al., 2000; Theiler, 1954; Watabe-Rudolph et al., 2002).

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