Growth and death in the developing mammalian kidney: signals, receptors and conversations

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Summary
Because the kidney (metanephros) starts to function before completing development, its patterning and morphogenesis need to be closely integrated with its growth. This is achieved by blast cells at the kidney periphery generating new nephrons that link up to the extending collecting-duct arborisation, while earlier-formed and more internal nephrons are maturing and beginning to filter serum. This pattern of development requires that cell division and apoptosis be co-ordinated in the various kidney compartments (collecting-ducts, blast cells, metanephric mesenchyme, nephrons and vascular system). The underlying regulatory networks for cell proliferation are beginning to be unravelled, mainly through expression studies, mutation analysis and experimentation in vitro. This article summarises current knowledge of kidney growth and apoptosis, and analyses some of the 80 or so ligand–receptor pairings that seem to sustain development and growth. It also points to some unanswered questions, the most intriguing being what role does apoptosis play during normal kidney development?

Introduction
Growth has been something of a poor relation in the family of mechanisms responsible for embryogenesis, attracting far less attention than, for example, differentiation and morphogenesis. This is probably because its manifestations appear to be rather modest, i.e., an increase in size, usually due to cell proliferation, sometimes modulated by apoptosis. Indeed, the current interest in the topic is probably only due to the fact that the most common phenotype of a mutation in a regulatory gene is a growth abnormality that may be hypoplastic (e.g., small kidneys), hyperplastic (e.g., cystic nephrons) or neoplastic (e.g., Wilms' tumour).

The work of the past half century has, however, given a clear picture of how kidney development proceeds(1): a blastema of a few thousand metanephric mesenchyme (MM) cells forms in the caudal region of each intermediate mesoderm (~E10.5 in the mouse) and induces the ureteric bud to spur off the medial and adjacent Wolffian (or nephric) duct (E11). This bud invades the MM and initiates reciprocal induction (E11.5) that continues throughout development (and, in the case of the mouse, beyond birth). The MM induces the bud to bifurcate and form the collecting-duct system (Fig. 1a), while duct tips induce nearby MM to form small condensations. These condensations rapidly epithelialise (Fig. 1b) and fuse to the duct (Fig. 1c), with much of the remainder of the MM giving the stroma(2) and neuronal cells.(3) The primitive nephrons then elongate and fold to give S-shaped bodies, with each unfused “proximal” tip forming a cleft which is soon colonised by nearby capillaries to give the primitive glomeruli.

By about E13, the extending ducts have reached the periphery of the metanephros, and the cells of the accompanying MM caps spread out over the surface to give a rind of stem cells that both maintains duct development and is the source of new nephrons (Fig. 2). As time proceeds, existing, internal nephrons start to extend into the medulla forming the loop of Henle, mature and start functioning, while new, more peripheral nephrons continue to be induced by the ducts. Eventually, soon after birth in mice and ~6 weeks before birth in humans, the populations of blast and stromal cells disappear, leaving a stable, functioning kidney.

It should however be noted that this traditional description almost totally ignores the engine of cell proliferation that drives all of the superficially more interesting aspects of kidney growth has centred on nephron formation and duct arborisation, paying little attention to the generation of the cells that are needed to produce these new structures. It should however be pointed out that current interest in growth (or cell proliferation) derives not from any great paradigm shift, but from the fact that the most common phenotype of a mutation in a regulatory gene is a growth abnormality that may be hypoplastic (e.g., small kidneys), hyperplastic (e.g., cystic nephrons) or neoplastic (e.g., Wilms' tumour).

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development. Growth deserves more attention than it usually receives and the purpose of this article is to reconsider the normal story of kidney development, but from a growth standpoint. Little attention will be paid to other forms of growth such as the compensatory hypertrophy that occurs in one kidney after removal of the other, the epithelial growth that accompanies polycystic disease, and the neoplastic growth found in nephroblastomas.

While nephrons and the collecting-duct arborisation provide the major focus for any study of renal development, the kidney includes other important cell types such as the neurons and the endothelial cells of blood vessels, both of whose growth is necessary for normal kidney development. The approach adopted here is to start by summarising the core data on growth and apoptosis in the kidney, and then to consider in more detail what is known of the growth signal and receptor relationships that underpin them in each of the major compartments of the developing kidney.

The reader will soon notice that kidney development as viewed from the standpoint of growth has some rather odd but intriguing features and three are worth mentioning immediately. First, cell division is accompanied by apoptosis, but the developmental and evolutionary reasons for this remain unclear. Second, the growth of this relatively uncomplicated organ involves the expression of thirty or more signalling proteins involved in some 80 ligand–receptor pairings (see Fig. 4). Third, and at a slightly deeper level, the formation of a normal kidney requires that the growth of blast cells, ducts, nephrons, blood vessels and the other cell types be coordinated and no attention seems to have been paid to this most fascinating of problems.

**Growth and death**

It is convenient to view kidney growth as having three phases. The first involves the ureteric bud invading the MM, bifurcating several times and causing the tips of the ureteric bud. This expression is maintained as the MM forms small aggregates that epithelialise and differentiate into nephrons.\(^2\) (bar: 50 μm). Early aggregate formation. A stack of four confocal images (vertical separation ≈ 8 μm) near the surface of an E12.75 kidney showing a duct tip extending into a duct (MM). The duct tip is surrounded by a cap of NCAM-positive cells that have almost columnar morphology and, just proximal to it, is a small aggregate (≈ 8 cells) of MM cells (arrow) contained within the four optical sections. Note that the basal lamina is still present between the aggregate and the duct and there is no laminin staining on the outer surface of this small aggregate, which has yet to initiate epithelialisation (bar: 25 μm) [from Ref. 2]. A cross section of a collecting duct in an E12.75 kidney proximal to the tip and immediately adjacent to which are two early nephrogenic aggregates ≈ 10 cells apart. The cells in the aggregate on the right are starting to epithelialise and this is confirmed by the laminin staining (insert). Here, the laminin of the basal lamina between the duct and the aggregate has almost gone, while that at the periphery of the aggregate is as substantial as that of the duct. In contrast, the other aggregate (bottom of picture) has only a fine basal lamina on its outer surface while the basal lamina between the aggregate and duct is clearly being degraded. (bar: 25 μm) (From Ref. 2, reproduced with permission of the Journal of Anatomy.)

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**Figure 1.** Confocal microscope images of induced mouse metanephroi. Fluorescent staining: red, NCAM; green, laminin; blue: DNA (TO-PRO-3). [from Ref. 2] a: An E12.5 kidney with a ureteric bud that has bifurcated twice. NCAM is initially expressed by induced metanephric mesenchyme as it forms a cap of metanephric mesenchyme some 3–5 cells thick that surrounds the tips of the ureteric bud. This expression is maintained as the MM forms small aggregates that epithelialise and differentiate into nephrons.\(^2\) (bar: 50 μm). b: Early aggregate formation. A stack of four confocal images (vertical separation ≈ 8 μm) near the surface of an E12.75 kidney showing a duct tip extending into a duct (MM). The duct tip is surrounded by a cap of NCAM-positive cells that have almost columnar morphology and, just proximal to it, is a small aggregate (≈ 8 cells) of MM cells (arrow) contained within the four optical sections. Note that the basal lamina is still present between the aggregate and the duct and there is no laminin staining on the outer surface of this small aggregate, which has yet to initiate epithelialisation (bar: 25 μm) [from Ref. 2]. c: A cross section of a collecting duct in an E12.75 kidney proximal to the tip and immediately adjacent to which are two early nephrogenic aggregates ≈ 10 cells apart. The cells in the aggregate on the right are starting to epithelialise and this is confirmed by the laminin staining (insert). Here, the laminin of the basal lamina between the duct and the aggregate has almost gone, while that at the periphery of the aggregate is as substantial as that of the duct. In contrast, the other aggregate (bottom of picture) has only a fine basal lamina on its outer surface while the basal lamina between the aggregate and duct is clearly being degraded. (bar: 25 μm) (From Ref. 2, reproduced with permission of the Journal of Anatomy.)
of the balance between proliferation and apoptosis within the individual compartments of the kidney. These include the collecting ducts, the blast cells and their progeny (the nephrons, and the stromal and neuronal cells) and the vascular system. Lineage relationships are still unclear as it not yet known whether a single line of blast cells gives rise to the various cell types that surround the collecting duct system or whether the initial blastema contains their separate precursors.\(^4\)

BrdU incorporation assays show that there is considerable proliferation throughout the developing mouse kidney, and particularly in the blast cells at the rudiment periphery,\(^5\) a pattern that is maintained until beyond birth, when these blast cells decline in number and are eventually lost as the last nephrons form. This growth is modulated by apoptosis in three ways. First, it seems that the periphery of the early blastema is delimited by an envelope of apoptosis that gives integrity to the early kidney (Hannu Sariola, personal communication). Second, there is ongoing cell death in the induced MM that can be downregulated, with little evidence of developmental harm, by the addition of epidermal growth factor in vitro\(^6\) and in vivo.\(^5\) Third, there seems to be a burst of apoptosis in the stromal cells of the medulla towards the end of development,\(^6\) the space so created may facilitate the extension of the loops of Henle into the medullary area. In addition, the metanephric blastema dies if it fails to be induced by the ureteric bud, while presumptive neuronal cells undergo apoptosis in vitro in the absence of neurotrophin-3.\(^7\)

Quantitative data on growth and apoptotic rates in the developing rat kidney were published by Coles et al.\(^5\) who, using frozen sections and nuclear morphology (propidium iodide staining), counted dividing and dying cells. They found the mitotic rate in E14.5 kidneys to be about 3% and the apoptotic rate in the cortex also to be about 3%. This was a surprising result as the kidney is growing rapidly at this stage; with mitotic periods thought to be about 1 hour and apoptotic

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**Figure 2.** The growth of the ducts and the formation of peripheral blast cells. This stack of confocal images (separation ≈ 10 \(\mu\)m) shows Pax2-positive cells (presumptive stem cells) reaching the kidney periphery with the extending duct tips. **A:** Deep in the kidney, a duct extends towards the medial surface of the kidney; the basal part of a small duct (white arrow) is apparent. **B,C:** This small tubule (white arrow) leading to a more superficial T-shaped duct. **C,D,E:** The two domains of Pax2-positive MM cells surrounding the tips of this duct (an early Pax2-positive epithelialising aggregate is visible in D, black arrowhead). **F:** The surface of the kidney and two patches (white asterisks) of Pax2-positive cells overlying the caps surrounding the duct tips. (staining: Pax2 (green) and propidium iodide (red); bar: 50 \(\mu\)m. (From Ref. 2, reproduced with permission of the Journal of Anatomy.)
clearance times of about 1.5 hours, the net growth rate would be about 1%. This would lead to a kidney doubling time of well over two days. We are therefore re-examining mitosis and apoptosis in E12.5–E16.5 mouse kidneys using confocal microscopy (Fig. 3) on whole kidneys. Counts on \( \approx 75,000 \) nuclei in \( \approx 20 \) kidneys again have given a mitotic rate of about 3%, but an apoptotic rate of only about 0.3%, a much lower figure than the earlier one (J. Foley and J. Bard, unpublished data). The reasons for the difference is not immediately clear, but these preliminary data are certainly more in line with the macroscopic rates of increase in kidney mass.

**The human data**

Some insight into the mechanisms controlling kidney growth should, in principle, come from analysing human congenital kidney abnormalities, but relatively little has emerged so far. Potter \( ^8 \) in her classic book reviewed the results of more than 400,000 autopsies on humans of all ages and of 12,000 babies who died within a year of birth. Although these population were not random, it was clear that major congenital renal abnormalities are rare and fall into three main classes: in number (\( \approx 1:400 \), unilateral agenesis; 1: 4,800 bilateral agenesis), in size and in internal structure. Here, the most common cause was a defect in the collecting duct system that led to polycystic kidneys \( [1:100] \). Small kidneys with too few nephrons were rare, while a few particularly large kidneys were noted. Of these, the most interesting was 120 gm at birth (or 10 times the normal size), but still maintained a clear peripheral nephrogenic zone while not showing a Wilms’ tumour phenotype, the classic result to be expected when control of growth in the developing kidney is lost and there is uncontrolled proliferation of blast, stromal and epithelial cells. The genetic basis of this kidney is of course unknown, but it does demonstrate that regulation of growth is a normal part of development.

**Molecular data**

For the kidney, more than 300 genes (signals, receptors, transcription factors, etc) have been identified in regulatory pathways, mainly on the basis of expression analysis. The *Kidney Development Database* \( ^9 \) holds details of when and in which compartment these genes are expressed, together with details of transgenic mice whose phenotype includes abnormal kidney development. Table 1 gives some of the signalling genes and transcription factors, mutations in which specifically affect kidney growth, but excludes mutated receptors (their phenotypes are in line with those of mutated ligands) and those genes whose role in kidney growth is likely to be secondary. Examples here are the retinoic acid receptors, \( ^{10} \) proteoglycans such as glypican-3 \( ^{11} \) that probably act as associate receptors, enzymes \( ^{12} \) that affect metabolism (e.g., Cox-2), integrins, \( ^{13,14} \) adhesion molecules such as cadherin-6 \( ^{15} \) and transcription factors such as Fox-d1, Hox genes, and Emx. \( ^{16–18} \) The list also excludes those genes whose effects are post embryonic (e.g., genes associated with polycystic kidney disease). \( ^{19} \)

The available transgenic data provide most of our current knowledge of gene function, but there is likely to be more to come. To highlight those genes that might activate the growth and apoptosis pathways, this review summarises the signal and receptor expression data from the kidney development database. \( ^{10} \) Essential features for each compartment can be shown in block diagrams (Fig. 4a–g): these show the signals from the other compartments that it can recognise on the basis of the receptors that it expresses. Asterisks mark signalling systems in which a mutation yields a growth abnormality.

Although these diagrams suggest that there are a very large number of molecular conversations among the compartments, the data do have limitations, the most important of which are as follows.

1. As some papers do not report expression data for every developmental period and compartment, it cannot be
assumed either that the tables are complete or that every signal and its receptor are present at the same time.  

2. The data give little indication as to the amount of signal and receptor present, and levels may sometimes be too low to activate signal transduction.  

3. Although the data mainly come from mice, some are from rats and humans and the different species do not necessarily use the same ligand–receptor pairs.  

4. Not all signals are long-range and some receptors may therefore not always be activated.  

5. The list is not complete: there are orphan receptors (c-ros42 and sFRP443) and ligands, (IGF529,30 and the BMPs20–22) although recent evidence44 suggests that MM has BMP receptors as the signal can rescue isolated and uninduced MM from apoptosis.  

6. Some signalling phenomena remain opaque: the transcription factor Foxd1 (BF2), only present in stromal cells, activates some unidentified signal that is needed for the correct formation and development of nephrogenic condensations.16  

The diagrams should therefore be seen as including both false positive and false negative data, and, when eventually corrected, may well give a clearer representation of how decisions are made by the developing kidney. Nevertheless, they provide a reasonable representation of what is currently known about the ligand–receptor pairs in the developing kidney, and the following section analyses this and the transgenic data in the context of growth and apoptosis.  

**General considerations**  
It has proven hard to elucidate the precise roles of all these ligand–receptor pairings; worse, it is quite likely that many are involved in more than one developmental event, and mutant analysis has only been able to resolve these problems in a few cases. Three classes of signals can however be identified.  

1. Signals that are permissive in the sense that the cells need them for normal growth, but whose presence does not drive any phenotypic change. Examples are probably transferrin45 and retinoic acid.(10)  

2. Signals that are redundant in the sense that their loss yields no phenotype because other signalling systems can compensate for them. HGF46 probably falls into this class as the need for this signal found in vitro is not demonstrated in the HGF knockout mouse.(47)  

3. Signals that direct specific growth activities, and whose loss stops that growth. A good example here is GDNF,(25–28) the signal that induces the growth of the ureteric bud.  

Although these classes are easy to distinguish formally, it is not always easy to assign a signal to one of them. Worse, it is not usually possible to disentangle how they activate proliferation, death, morphogenesis and differentiation; indeed, it is quite likely that these events cannot be activated separately. It is therefore clear that the following discussion can only be viewed as a first step in analysing how the growth of the kidney is controlled.  

**Specific compartments**  
**The initial blastema**  
The mechanism that establishes the metanephric blastema remains unknown, but the key molecular event that follows its demarcation within the intermediate mesoderm is the expression of the transcription factor WT1.39 In its absence both in vitro and in vivo, the blastema undergoes spontaneous apoptosis; in its presence, the MM synthesize GDNF which

**Table 1. Mouse mutations that affect the growth of the kidney**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP7</td>
<td>Signal</td>
<td>a: Severe hypoplasia few nephrons and few collecting ducts</td>
<td>a: 20,21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b: Normal growth but polycystic tubules</td>
<td>b: 22</td>
</tr>
<tr>
<td>FGF7 /-/-</td>
<td>Signal</td>
<td>a: Knockout: small kidneys</td>
<td>a: 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b: Overexpression: hyperplasia</td>
<td>b: 24</td>
</tr>
<tr>
<td>GDNF</td>
<td>Signal</td>
<td>No kidney development as the ureteric bud fails to grow</td>
<td>25, 28, 27, 28</td>
</tr>
<tr>
<td>IGF I</td>
<td>Signal</td>
<td>For both, hypoplasia in line with the rest of the embryo</td>
<td>29, 30</td>
</tr>
<tr>
<td>IGF II</td>
<td>Signal</td>
<td>(the effect of the IGFs on kidneys is probably non-specific)</td>
<td></td>
</tr>
<tr>
<td>PDGF-A</td>
<td>Signal</td>
<td>Small (80–90%) kidneys</td>
<td>31</td>
</tr>
<tr>
<td>PDGF-B</td>
<td>Signal</td>
<td>Few glomeruli, perhaps due to absence of mesangial cells</td>
<td>32, 33</td>
</tr>
<tr>
<td>Notch 2</td>
<td>Receptor</td>
<td>Glomeruli do not complete development</td>
<td>34, 35, 36</td>
</tr>
<tr>
<td>Bcl2</td>
<td>Mitochondrial regulation</td>
<td>Excess apoptosis during normal development and polycystic kidney results in hypoplasia</td>
<td>37, 38</td>
</tr>
<tr>
<td>WT1</td>
<td>Transcription factor</td>
<td>a: MM undergoes unrescuable apoptosis</td>
<td>a: 39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b: Podocyte abnormalities in Denys-Drash syndrome</td>
<td>b: 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c: Mutations can cause Wilms’ tumours in humans</td>
<td>c: 41</td>
</tr>
</tbody>
</table>
signals to the duct system, which in turn signals back to the MM. WT1 is responsible for the responding network that causes the MM to develop rather than die. WT1 clearly has a role in growth regulation as its loss can unleash apoptosis\(^{(39)}\) while mutations\(^{(40,41)}\) can lead to Wilms’ tumour (uncontrolled proliferation of blast, epithelial and stromal cells), although they can also result in the Denys-Drash syndrome\(^{(40)}\) characterised by abnormal podocyte development.

Figure 4. Signal–receptor interactions for the various compartments of the kidney (a: blast cells; b: ducts; c: early stages of nephrogenesis; d: early nephrons; e: glomeruli; f: endothelial cells; g: stroma). In each diagram, arrows towards the central compartment (yellow type) indicate receptors for which it has signals (yellow boxes). Arrows towards the signal boxes show their sources (white type). An asterisk indicates that a mutation has a renal phenotype (see text). A question mark means that the link is likely but not proven (see text). The signal–receptor expression data is accessible from the kidney development database.\(^{(9)}\)
We do not yet know which signal receptors are expressed by uninduced MM: the only one for which there yet seems to be good evidence is c-met, but its importance is unclear as the knockout of its ligand, HGF, yields no kidney phenotype. In addition, there is as yet no direct evidence that the receptors expressed by the induced MM (Fig. 4a) are not expressed before induction. The best evidence here comes from recent work on serum- and tissue-free MM induction showing that TGFβ, FGF2 and LIF can together enable rat MM to escape apoptosis and undergo tubulogenesis in vitro, while BMP7 can rescue mouse cells from apoptosis. Although these results are particularly important in the context of induction, they do not show how induction causes MM to switch from apoptosis to proliferation, or whether this switch is part of the nephron induction process.

**The ureteric bud and ducting system**

The formation of the ducting system is characterised by bifurcation and growth, most of which occurs at duct tips, and, indeed, there is a case for claiming that bifurcation reflects a particular aspect of growth where mitosis is blocked at the bifurcation point but stimulated on either side. The signalling systems here are only partly known (Fig. 4b), but it is clear that GDNF, initially produced by the blast cells, is a key growth mediator: it is the one ligand whose absence or mutation will block duct formation and growth, although its role in branching remains to be clarified. Similar transgenic evidence demonstrates that FGF7, produced by stromal cells, plays a similar role, albeit a little later. Although HGF is expressed and can stimulate branching in vitro, the HGF knockout mouse develops normal kidneys so the significance of this signal remains unclear. It is not known whether apoptosis plays any role in duct morphogenesis, although it has been observed in duct epithelium.

**The blast cells**

Once induced, the Pax2- and WT1-positive blast cells form a cap of cells around the initial and all subsequent duct tips. As these tips extend, cap cells surround them and, when they reach the periphery (E13.5), spread over the surface of the primordium (Fig. 4). These blast cells, which are the source of future nephrons, show strong BrdU incorporation, but little is known of the growth stimuli to which they respond. One possibility is that this growth is intrinsic to the cells, but this seems unlikely as they have proven so difficult to grow in vitro. The more likely alternative is that this growth is a response to one or more of the many signals in their environment for which they have receptors. (Fig. 4a). Some, such as IGF I, IGF II, PDGF-A, PDGF-B and retinoic acid, seem relatively non-specific, but others, such as B61 and the FGFs may play a more regulatory role here.

A further complication is that there is some inhibition of blast cell proliferation towards the end of development which leads to the eventual cessation of nephron production. Likely elements of this inhibition are the transcription factor WT1 and genes within the WT2 complex which are located at the 11p13 and 11p15 locations, respectively. The evidence for this comes from an analysis of Wilms’ tumours: these masses of primitive kidney cells that can exceed a kilogram almost certainly derive from a single mutated cell that has lost its ability to regulate its own growth and to respond to any inhibitory signals. Some 10% of Wilms’ tumours have mutations in WT1 while there is loss of heterozygosity at 11p15 in about 40% of cases, usually in the maternal alleles. WT2 seems to be involved in those cases of the tumour associated with the Beckwith-Wiedemann syndrome, but we will need to identify the genes, mutations which are responsible for most of the other cases of Wilms’ tumour, before we properly understand growth regulation in the blast cells.

The amount and significance of apoptosis in induced MM remains unclear, but transgenic knockout observations show that it is at least minimised by the presence of Bcl-2. This gene plays a key role in downregulating apoptosis and, in its absence, there is excessive apoptosis that leads to the kidneys of newborn mice being less than half the size of controls and eventually showing polycystic kidney disease.

**Nephrogenic condensations and their epithelialisation**

This compartment includes all stages from the mesenchymal condensation to the epithelialised S-shape body. Developmental change is very rapid here and, even though the cells in this compartment carry a host of receptors (Fig. 4c), it is not yet clear whether early nephrogenesis is accompanied by a great deal of growth. One intriguing aspect of development here comes from the role of Foxd1 (originally known as BF2, Ref. 16), a transcription factor that is only expressed in the stromal cells. If this gene is knocked out, relatively few condensations form and these do not epithelialise but grow to a far larger size than normal. The developmental implications of this phenotype are still unclear, but suggest that rapid growth is inhibited to some extent in the early stages of nephrogenesis.

**Nephrons**

Nephron growth is unusual as it cannot occur at the ends (these are fixed by the glomerulus proximally and the collecting duct distally), and generates increases in length rather than diameter (apart from the broad proximal convoluted tubule, an effect that may be due to cell columnarisation). It also seems to be rapid although exact rates have yet to be established, with its middle region, in particular, lengthening considerably as it descends into the medulla to form the hairpin loop of Henle. The mechanism for this is unknown but an obvious possibility is a chemotactic stimulus from the pelvic region of the kidney.
that directs this descent. Growth also provides the motor for nephron differentiation, itself an extraordinary example of one-dimensional pattern formation. We know little of what is going on here, but it is quite possible that the signals responsible for the elongation (and pattern formation) of the nephron are generated within the closed epithelium rather than from some extra-nephron source.

One reason for this suggestion is that the potential for extranephrogenic control of growth is diminished since, as the S-shaped body differentiates to become the early nephron, many of the receptors previously expressed are downregulated (Fig. 4d). The remaining receptors mainly seem to be for the IGFs and FGFs, although we cannot be sure which of the latter family members are ligands for the nephrons as details of the isoforms of their receptors seem not to have been published.

There is no evidence to suggest unusual amounts of apoptosis in the developing nephron but cell death may regulate nephron morphogenesis indirectly: apoptosis seems to occur preferentially in the stroma immediately adjacent to the S-shaped body. Its inducing signal may derive from the early nephron while the resulting loss of cells either may help sculpt the nephron or may free space to facilitate the immigration of nerves and capillaries into the cleft of the S-shaped body that becomes the glomerulus. It should also be mentioned that, in the Bcl2−/− mouse, where apoptosis is fulminant, the tubules become cystic, although the mechanism for this is unclear.

**The glomerulus and endothelial cells**

The glomerulus is complicated as it includes the highly specialised podocytes that form from the proximal end of the early tubule, the endothelial cells that invade the early tubule and the mesangial and pericyte cells that colonise intercapillary space within the renal capsule. Examination of Fig. 4e shows that it is the target of a surprisingly large number of signals, most of whose roles are unclear. Although many of the events associated with the formation of the glomerulus are more concerned with differentiation than growth, the clear exception is the formation of the capillary plexus, and the key signal here is the expression of VEGF by the glomerular region and its receptor by the endothelial cells (Fig. 4e,f) which is almost certainly responsible for the growth and consequent invasion of capillaries into the cleft of the presumptive glomerulus. Later, during glomerular maturation, Angiopoietin1 which facilitates sprouting may become important. In addition, interactions between Delta1 (and perhaps Jagged1, although its site of expression may be mesangial or endothelial cells, Ref. 36) in the glomerular region and Notch2, its receptor, also expressed by the endothelial cells, seems necessary for normal glomerular development.

As these proteins are all membrane-bound, this interaction may play a role in stabilising the detailed geometry of the renal capsule rather than its growth. The status of Notch–Delta/Jag1 interactions between the nephrons and endothelial cells is still unclear: adult endothelial cells express Notch4, but the timing of its appearance seems not to have been reported.

The other signal that seems important in glomerulus morphogenesis is PDGF-B: this is made by endothelial cells in the glomerulus and is needed for mesangial differentiation. The PDGF-β receptor is expressed on early blast cells, but its expression by later cells seems not to have been examined; these links are therefore marked by queries in Figure 4f. In addition, there may eph-ephrin interactions in the developing vasculature: embryonic kidneys cultured in the anterior chambers of ROSA 26 mice are invaded by graft capillaries that stain for these proteins.

**Stroma**

The ligands for which the stroma has receptors are PDGF-A made by all stages of nephron development, Wnt4 made by early nephrons, and EGF and retinoic acid, neither of which seem to be synthesised within the metanephros (Fig. 4g). The effect of Wnt4 is likely to be local as it does not freely diffuse, while the effects of retinoic acid may not be cell-type-specific as all obvious derivatives of the MM carry the receptor. It thus seems that the most likely paracrine factor regulating stromal development is PDGF-A made by developing nephrons. Its significance is not however likely to be as great since those PDGF-A−/− mice that survive to birth and beyond have organs that are only 10–20% smaller than controls.

The role of the EGF receptor seems to be in apoptosis rather than growth as EGF treatment in vivo lowers apoptotic rates in developing rat kidneys. The significance of this role remains unclear as the EGF receptor knockout has no obvious kidney phenotype.

**Neuronal cells**

The final class of cells to be considered is the population of nerve cells that differentiates rather late in development under the influence of neurotrophin-3, a signal whose site of synthesis within the kidney is unknown. Relatively little is known about the origins of neuronal cells, which may derive from the stroma or from neural crest cells; the difficulty is that, even though peripheral nerve cells are thought to derive from neural crest cells, no early markers have been able to distinguish two distinct cell populations in the stroma. In vitro experiments have shown that presumptive neuronal cells (they express L1 and neurofilament) in cultured metanephric mesenchyme undergo apoptosis but can be rescued by Neurotrophin 3. The exact role of NT3 in normal kidney development remains unclear as the NT3 knockout mouse does not have an obvious kidney phenotype.

**Discussion**

Members of almost every signal family, with the notable exception of the hedgehogs, seem to have a role in kidney
development, and it is proving hard to elucidate their separate roles in growth, apoptosis and differentiation, as well as the extent to which they can compensate for one another. We cannot even tell whether, when receptors for a particular signal are expressed by more than one compartment, signal transduction has the same effect in each.

The transgenic (Table 1) and expression data (Fig. 4) show that, of the large numbers of signals present within the developing kidney, relatively few have direct, non-redundant effects on growth in individual compartments (FGF7,(23,24) GDNF,(25,28) Notch2,(34,36) PDGF-B,(32,33) VEGF, Ref. 60), while a few others have a more disseminated effect (IGF I/II,(23,30) retinoic acid, Ref. 10). In the case of these latter signals, however, it is still not obvious whether they directly affect more than one compartment, or whether an effect on a single compartment has downstream effects on others. It is nevertheless clear that a subset of the signals present in the developing kidney have clear roles in stimulating growth and development. The situation for the remainder will doubtless become clear in due course.

Our understanding of apoptosis is less clear. While the need for growth is self-evident, that for apoptosis remains opaque. In most cases of pronounced apoptosis in mouse development, the anatomical reasons are obvious (e.g., digit delineation,(67) elimination of unwanted neuronal (68) and neural crest cells, (69) and cavity formation, Ref. 70). There does not, however, seem to be any obvious need for the large amounts of apoptosis reported in the developing kidney. Apoptosis does of course occur in metanephric mesenchyme that remains uninduced (e.g., by the ureteric bud) and in neuronal cells that are not exposed to Neurotrophin 3,(2,7) but that remain uninduced (e.g., by the ureteric bud) and in neuronal cells that are not exposed to Neurotrophin 3,(2,7) but these events can be viewed as the clearing of unwanted cells when development does not proceed normally.

The main roles adduced for apoptosis in normal kidney development are loss of cells adjacent to the forming glomeruli which might aid nephron morphogenesis,(6) and the later loss of medullary stroma(3) which could create space for the descent of loops of Henle. Neither would account for the considerable amount of apoptosis reported throughout the kidney during normal development (E13.5–16.5), even if the rates are less than originally thought. The simplest explanation for cell death in the kidney is that it reflects leakage of cells through the protective barrier controlled by the Bcl2 pathway. If so, apoptotic rates in the kidney should be much the same as in other developing tissues, but comparisons seem not to have been made.

This review has focused on the relationship between growth and signalling as the kidney develops, touching on how signal transduction activates transcription factors such as WT1 but not considering the pathways linking induction to proliferation and apoptosis. Although this area will clearly be of major importance over the next few years, relatively little is currently known about how specific gene expression in the kidney initiates these activities. One reason is that it hard to investigate these events directly in kidneys, so progress mainly depends on analysing molecular behaviour in cell lines and genomic data. The current emphasis is on the proteins with which WT1 and genes within the WT2 locus interact and their importance in Wilms’ tumour and Beckwith-Wiedemann syndrome,(71–73) Such work is giving tantalising pieces of information, but there are not yet enough of them to integrate into networks.

Perhaps it is best to end this review not with a summary of what we know, but with a list of questions, answers to which would clarify our understanding of how growth and apoptosis are mobilised throughout kidney development, and how they regulate kidney morphogenesis. Obvious examples are as follows.

- Which growth factors are specific and which are non-specific?
- Why are there so many growth factors present?
- How is the growth of the different compartments coordinated?
- What is the role of each of the many FGFs expressed(23,45,49,54,55) in the developing kidney?
- What are the genes whose mutations account for neoplastic growth in those Wilms’ tumours where WT1 and WT2(40,56,57) are normal?
- What are the signal transduction pathways that activate cell proliferation and apoptosis? Does each compartment use the same pathway?
- What roles does apoptosis play in normal kidney development?

References


