



Published in final edited form as:

Pediatr Nephrol. 2014 April ; 29(4): 553–564. doi:10.1007/s00467-013-2597-2.

Kidney Regeneration: Common Themes From the Embryo to the Adult

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Abstract

The vertebrate kidney has an inherent ability to regenerate following acute damage. Successful regeneration of the injured kidney requires the rapid replacement of damaged tubular epithelial cells and reconstitution of normal tubular function. Identifying the cells that participate in the regeneration process as well as the molecular mechanisms involved may reveal therapeutic targets for the treatment of kidney disease. Renal regeneration is associated with the expression of genetic pathways that are necessary for kidney organogenesis, suggesting that the regenerating tubular epithelium may be ‘reprogrammed’ to a less-differentiated, progenitor state. This review will highlight data from various vertebrate models supporting the hypothesis that nephrogenic genes are reactivated as part of the process of kidney regeneration following acute kidney injury (AKI). Emphasis will be placed on the reactivation of developmental pathways and how our understanding of the resulting regeneration process may be enhanced by lessons learned in the embryonic kidney.

Keywords

organogenesis; regeneration; acute kidney injury

Introduction

The paradigm that regenerative signaling events initiated in adult organs recapitulate the molecular pathways activated during embryonic development has been a long-held hypothesis [1, 2]. The rationale behind this hypothesis may not be immediately apparent, since the initial processes necessary for establishing organ fields during embryogenesis and those that compensate for tissue loss during regeneration are quite dissimilar. Establishing a developing organ field occurs via inductive signals and morphogenetic movements, while regeneration typically initiates with damage and cell death [3]. However, once the first regenerative steps have occurred, the next stages, which start with progenitor cell recruitment or establishment followed by proliferation and differentiation, rely on signaling cascades that also play a role in organ formation during development.

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The kidney is a key homeostatic regulator of electrolytes and water, and uses blood filtration as a means of waste removal. Despite performing these essential functions, the mammalian kidney possesses a limited regenerative potential. While localized lesions in the tubular epithelium of nephrons (the basic filtering units) can be repaired, entire nephrons cannot, resulting in a permanent deficit [4–6]. In contrast, the adult zebrafish kidney not only has the ability to repair injured tubular epithelium, but also the potential to generate nephrons *de novo* following severe injury through a process termed neo-nephrogenesis [7]. Although there is wide disparity in the renal regenerative potential found between different vertebrates, the reactivation of multiple embryonic signaling pathways during regeneration supports the hypothesis that these pathways play similar roles in both embryonic kidney formation and adult replacement of renal tissue [8–10]. In this review we will highlight the common features of kidney organogenesis and regeneration, as revealed from studies of several well-known molecular pathways (Figure 1).

Vertebrate Kidney Organogenesis

In adult human kidneys there are at least twenty-six different cell types [11]. However, all renal cells and their descendants arise during embryonic development from the intermediate mesoderm (IM), located between the paraxial mesoderm (PM) and lateral-plate mesoderm (LPM) [12]. Signals from these juxtaposed tissues define the boundaries of the emerging IM and induce the expression of the early-acting renal transcription factor genes *Pax2*, *Pax8*, *Lhx1*, and *Osr1* [13–15].

Vertebrates form up to three different types of kidney, with increasing complexity. In general, the nephrons of all kidney types are composed of a blood filter (glomerulus) and a renal tubule that is subdivided into proximal and distal segments. The first kidney type, the pronephros, forms in the upper trunk of the embryo and serves as the functional larval kidney in fish, amphibians and some reptiles [16, 17]. Because they contain only rudimentary nephric structures, the pronephroi of certain reptiles, birds, and mammals lack excretory capability [16]. However, regardless of pronephric activity, development of the subsequent kidney types is dependent on the initial formation of the pronephric (aka nephric) duct [15]. The next embryonic kidney to arise, the mesonephros, constitutes the adult kidney in fish and amphibians, and the functional fetal kidney of mammals. Mesonephric development begins when IM-derived mesenchymal cells near the nephric duct condense (pre-tubular aggregates) and epithelialize to form renal vesicles, the first step in nephron formation. Renal vesicles transition into S-shaped bodies and then finally into mesonephric nephrons [12]. Development of the final form of the kidney, the metanephros, which is the functional adult kidney in reptiles, birds, and mammals, begins with a posterior outgrowth of the nephric duct near the hind limb, called the ureteric bud [12]. Signals from the ureteric bud initiate the condensation of the metanephric mesenchyme into nephrons (via pre-tubular aggregate, renal vesicle, and S-shaped body transitions), while the metanephric mesenchyme reciprocally induces growth and branching of the ureteric bud. This process continues until the emergence of the adult kidney, the metanephros, which can contain approximately 1,000,000 nephrons per kidney in humans, along with a highly branched collecting duct system [12].

Vertebrate Kidney Regeneration

Regenerative responses show distinct patterns in different organs, and are usually related to the organs inherent rates of cellular turnover. Highly proliferative organs, such as the blood lineages, intestine, and skin contain a resident stem cell population serving as the source of replacement cells [18, 19]. In contrast, the mammalian kidney exhibits a low basal cellular turnover and does not appear to contain a well-defined stem cell population. However,

kidney damage can trigger varying degrees of regenerative responses depending on the organism and the extent of the damage [7].

Acute kidney injury (AKI) is defined as a rapid loss of kidney function as evidenced in mammals by elevated serum creatinine and/or blood urea nitrogen levels or a severe decrease in urinary output. It accounts for approximately 7% of all inpatient hospital admissions and is most commonly caused by ischemic injury, exposure to nephrotoxic agents and/or sepsis [20]. Renal replacement is the only approved therapy for the majority of the patients with severe AKI and there are no established treatments that have been proven to prevent renal injury or accelerate recovery following AKI.

At the cellular level, AKI-mediated damage results in depolarization of renal tubular cells and subsequent loss of function and cell death through apoptosis and necrosis [21–23]. The proximal straight tubule (S3 segment) is highly susceptible to ischemia and toxic insult leading to AKI [24]. Early cellular changes observed in the proximal straight tubule are loss of cell polarity and the brush border, disruption of tight and adherent junctions and redistribution of the Na⁺/K⁺ ATPase and integrins to the apical plasma membrane [25, 26].

Both the zebrafish pronephros (larval kidney) and mesonephros (adult kidney) have been successfully used as models to study AKI. Injection of the nephrotoxic agents, gentamicin and cisplatin into the circulatory system of zebrafish larvae, induces a decline of renal function and histological changes characteristic of mammalian AKI models [27–29]. In the adult zebrafish mesonephros, following gentamicin-mediated AKI, it was found that new nephrons formed after injury and were found to originate from cellular aggregates of potentially self-renewing progenitor cells in a process of regenerative neo-nephrogenesis [30].

In mammals, the renal epithelium has the capacity to self renew by symmetric division of differentiated cells that are indistinguishable from their progeny [6, 31]. However, the mechanism by which renal tubular epithelium regenerates following injury remains unclear. A large number of studies have used rodent models of ischemia-reperfusion, where epithelial cell death by necrosis is largely restricted to the S3 segment of the proximal tubule and occurs within the first 12–24 hours of injury. This is associated with renal tubular epithelial cell (RTEC) loss, denudation of areas of basement membrane, and loss of cell-cell junctions between surviving RTECs, resulting in flattened cellular morphology and the expression of mesenchymal markers such as *vimentin* [32–34]. Depending on the severity of the insult, regeneration of injured RTECs occurs over the next 1–7 days, as rapidly-dividing vimentin-positive cells undergo a mesenchymal-to-epithelial transition and eventually form functional, polarized epithelia.

Lineage tracing studies suggest that the bulk of regenerating RTECs are derived from intrinsic renal epithelium, and not through migration and differentiation of extrarenal or intrarenal stem cells [35]. The majority of these proliferating cells appear to be derived from slow-cycling, BrdU-retaining tubular epithelial cells, which are scattered throughout the proximal tubule and are indistinguishable from fully differentiated tubular epithelial cells prior to injury [36]. These findings are consistent with observations made in regenerating pancreas, liver and bronchial epithelial injury models [37–39], and suggests that the bulk of regeneration in these low-turnover epithelial organs occurs by dedifferentiation and proliferation of endogenous epithelial cells. Nonetheless, it is still possible that exist discrete populations of renal stem cells in the adult kidney, which are reactivated following certain types of injury. One such potential population has been found in the epithelial capsule that surrounds the glomerulus. A subset of these cells that co-express the stem cell markers, CD24 and CD133 are able to prevent renal damage and accelerate recovery time when

injected into mice with AKI [40]. The existence of adult renal stem cells in mammals and their role in kidney regeneration remains controversial and more lineage-tracing experiments are needed to resolve this issue [41–44].

In addition to the re-expression of mesenchymal markers, regenerating RTECs express genetic markers normally associated with pre-tubular aggregates and renal vesicles, including *Pax2*, *Wnt4*, *Lhx1* and components of the Notch and BMP signaling pathways [10, 45–47]. These genes are expressed within the first 24 hours following injury and cease to be transcribed once the cells undergo epithelial differentiation. The mechanism regulating reactivation of embryonic gene expression following AKI is unknown. In the following section, we will highlight several well-characterized pathways that are required for kidney organogenesis and are reactivated during regeneration, but by no means is this a complete list of conserved pathways (Table 1). While there is a lot of functional data for these pathways during kidney organogenesis, most of the reported regeneration data is based on reactivation of expression of conserved orthologs gene loci and functional studies are currently limited.

Conserved Pathways in Kidney Development and Regeneration

1. Retinoic acid signaling

Retinoic acid (RA) is the active derivative of vitamin A, and is necessary for proper growth and development of the vertebrate embryo, including the kidney [48]. RA synthesis involves two oxidation steps: First, vitamin A is converted to retinaldehyde by alcohol dehydrogenases (ADHs), then retinaldehyde dehydrogenases (RALDH or ALDH) process the retinaldehyde to RA [49]. Degradation of RA is primarily mediated by the Cytochrome p450 enzymes (Cyp26 a1, b1 and c1) [50]. RA diffuses into the cell and binds to retinoic acid receptors (RARs α , β , and γ) in the nucleus, which heterodimerize with the retinoid X receptors (RXRs α , β , and γ) and recruit transcriptional co-activators on target genes [51, 52].

RA has been shown by several groups to be involved in the early events of kidney specification and induction of pronephric cell fates [53–59]. *In vitro* studies of *Xenopus laevis* ectoderm explants treated with activin A and RA resulted in induction of pronephric fates and implicated RA as a regulator of renal development [55]. RA has been shown to be one of the earliest signals in pronephros formation [53]. Defective RA signaling impairs pronephros development, prevents expression of the early pronephric genes *Lhx1* and *Pax8*, and reduces gene expression in proximal regions (nephrostomes and proximal tubule), suggesting that RA signaling regulates pronephric development by directly affecting pronephric progenitors during gastrulation [53]. Further, *raldh2*-deficient zebrafish embryos or those treated with compounds that block RA signaling have defects in pronephric formation characterized by fewer podocytes, shorter or absent proximal tubule segments and expanded distal tubules [58]. A gradient of RA is postulated to occur along the early field of nephron progenitors thereby determining whether a proximal (high RA) or distal (low or no RA) tubule fate is adopted [60].

Pronephric duct development is impaired in *Raldh2*^{-/-} mice suggesting that the role of RA signaling is evolutionary conserved in vertebrates during the early steps of kidney formation [53]. During metanephric development, reduced RA availability leads to multiple abnormalities. Depending on the extent of RA deficiency, the associated phenotypes include hydronephrosis, renal hypoplasia and horseshoe kidney [61–63]. These renal abnormalities are recapitulated in mice carrying genetic alterations in retinoid receptors [51, 52, 64]. In double mutant mice RAR $\alpha\beta\gamma$, kidneys show no nephrogenic zone at 18.5-dpc. The ureteric bud reaches the metanephric mesenchyme but branching morphogenesis is impaired

suggesting the requirement of both retinoic acid receptors in this process [64]. The observation that RA controls branching morphogenesis by regulating *Ret* expression in the ureteric bud and the observed phenotype in RAR α β 2 double mutant mice indicated that the renal malformation in these mice results from down-regulation of *Ret* signaling in the ureteric bud, ultimately causing impaired branching morphogenesis [65].

Retinoids have also been implicated in the process of repair and regeneration of the mammalian central nervous system [66–70], mammalian lungs [71–73], and zebrafish heart [74, 75]. In the kidney, retinoids have not been shown to augment the regenerative response but rather to ameliorate renal injury. In a nephritic rat model of glomerular damage induced by targeting mesangial cells, RA effectively limits damage, inducing a recovery in renal function and preserving glomerular structure [76]. Increased *Raldh2* expression in rats with podocyte damage suggests that endogenous RA production could play a role in the repair process [77]. Interestingly, administration of RA to these rats prevents puromycin aminonucleoside-induced proteinuria indicating the importance of RA in the repair process of the injured podocytes. In a rat model of potassium dichromate induced AKI, pre-treatment with RA prevents a decrease in glomerular function, reduces oxidative damage and accelerates recovery. This effect has been attributed to the prevention of cellular damage induced by oxidative stress and maintenance of epithelial integrity in the proximal tubule [78]. Together, these reports indicate a potential RA amelioration of renal damage although further studies are needed to elucidate the molecular mechanism involved.

2. Early transcription factors

The paired-box (Pax) gene family encodes transcription factors characterized by the presence of a paired domain that confers sequence-specific binding to DNA [79]. Two members of the family, *Pax2* and *Pax8*, are expressed in the developing mouse kidney [80–82]. In *Pax2*-null mice the nephric duct forms but does not fully migrate posteriorly and then degenerates. In addition, only a few mesonephric structures arise and there is a lack of metanephric kidney formation in *Pax2* mutants. *Pax8*-null mice exhibit no kidney defects [83, 84], however, *Pax2/Pax8* double mutants display a complete loss of all kidney structures [85]. Zebrafish embryos deficient in the homolog of *Pax2*, *pax2a*, and *pax8* lack pronephric tubules, although glomerular morphogenesis remains relatively unaffected [86].

The lack of metanephric development in *Pax2* null mouse embryos suggests that *Pax2* target genes may play a role in ureteric bud budding [84]. In support of this, *Pax2* is necessary for the expression of *Gdnf*, encoding an important secreted inducer of ureteric bud outgrowth [87, 88], in the metanephric mesenchyme [89]. Current evidence suggests that *Pax2* forms a complex with the co-regulators *Hox11* and *Eya1* to directly control the expression of *Gdnf* in the metanephric mesenchyme [90]. Exogenous replacement of *Gdnf* in *Pax2* null kidney organ cultures fails to restore ureteric bud formation [89] indicating that *Pax2* also regulates the response of the nephric duct to *Gdnf* [89]. Consistent with this, *Pax2* directly binds to the *Gdnf* receptor (*Ret*) promoter and expression of *Ret* is lost in *Pax2* null embryos [91].

Pax8 also contributes to collecting duct branching as the metanephric kidneys of double-heterozygous *Pax2/8* embryos exhibit decreased expression of *Wnt11*, a downstream target of *Gdnf*-*Ret* signaling [92]. Interestingly in these animals, the expression levels of *Gdnf* and *Ret* appear unaffected. While there are clearly many molecular details yet to be resolved, these results indicate that *Pax2* and *Pax8* have important roles both up- and down-stream of the GDNF-*Ret* pathway to regulate collecting duct branching.

Of the two members of the Pax family expressed during kidney organogenesis, *Pax2* has been studied in the greatest detail during kidney regeneration. In the metanephric kidney, *Pax2* expression is down-regulated during epithelial differentiation and maturation and is

not detected in the mature glomeruli and proximal tubules. One of the first molecular hallmarks of kidney regeneration is the reactivation of *Pax2*, as expression transiently reactivates in regenerating RTECs following acute tubular necrosis [93]. Using an ischemia-reperfusion model reactivation of *Pax2* in tubular cells after kidney injury was confirmed. BrdU/*Pax2* double-positive proliferating cells are regulated by activin A, and this regulation is critical for the processes of tubular cell growth and differentiation during regeneration [94]. In addition, numerous studies agree that *Pax2* protects tubular cells from apoptosis. Zhang *et al.* employed an *in-vitro* approach using rat renal proximal cells and reported that knocking down *pax2* increases cellular susceptibility to apoptosis [95]. Further evidence comes from the analysis of *Pax2* mutant mice [96]. The renal collecting ducts of heterozygous *Pax2*^{1^{Neu}} mice, when subject to unilateral ureteral obstruction (UUO), exhibit increased numbers of apoptotic cells when compared to wild-type mice [96]. Together, these data suggest an important role for *Pax2* in kidney regeneration by protecting renal epithelial cells from apoptosis initiated by injury.

Members of the LIM homeodomain (LIM-HD) family contain two LIM domains and a DNA binding homeodomain. *Lhx1* is the only family member to be expressed in all developing kidney types. Experiments in *Xenopus laevis* demonstrated that forced expression *Lhx1* increases the size of the pronephros. When *Lhx1* is overexpressed with *Pax2* or *Pax8* it induces the formation of ectopic pronephric tubules [97], consistent with an early and co-operative role for *Pax2*, *Pax8*, and *Lhx1a* in pronephros formation. In support of this, *Lhx1* knockdown in the *Xenopus laevis* results in an early failure of pronephros development, and data suggest that *Lhx1* might act as a competence factor necessary to establish the pronephric kidney field [98].

Homozygous null *Lhx1* mice, rarely live after birth and survivors lack head, gonads, and kidneys [99]. Homozygous null *Lhx1* mouse embryos also display disorganized intermediate mesoderm and are unable to properly form the nephric duct [100]. Using a floxed conditional *Lhx1* allele, it was found that *Lhx1* is required for the maintenance of the nephric duct and mesonephric tubules. In the developing metanephros, *Lhx1* is required for proper ureteric bud formation and patterning of renal vesicles during nephron formation [101]. Although molecular interactions between *Lhx1* and a downstream target genes have not yet been reported in the kidney, the Wnt signaling inhibitor, *Dkk1* was identified as a potential candidate [102].

The role of *Lhx1* during kidney regeneration has not yet been studied extensively in mammalian models of AKI, but reactivation of its expression has been reported in rats subject to ischemia-reperfusion injury [10]. In regenerating proximal tubules, *Lhx1* transcripts are observed 48 hours post-damage and persists for a period of 24 hours in the regenerating proximal tubules [10]. Following injury, *Pax2* and *Lhx1* are serially reactivated, supporting a regenerative process in which nephrogenic proteins are re-expressed in a sequence of events similar to those that occur during metanephric kidney nephrogenesis [103]. In adult zebrafish mesonephric regeneration, following gentamicin-mediated AKI, the new nephrons that formed were found to originate from cellular aggregates of potentially self-renewing progenitor cells that were marked by GFP driven by the *lhx1a* promoter [30]. Following injury these progenitors activate expression of the nephrogenic genes *wt1b* and *fgf8*, and undergo mesenchymal-to-epithelial transition into renal vesicles and new nephrons, supporting a role for *Lhx1* in the process of regenerative neo-nephrogenesis. Further studies in mammals are required to demonstrate the importance of *Lhx1* during metanephric kidney regeneration.

Sall proteins are a conserved family of transcription factors characterized by the presence of multiple double-zinc finger motifs [104]. These proteins play multiple roles during

embryonic development including organogenesis and cell fate determination [105]. Most Sall proteins are expressed during vertebrate kidney organogenesis but with the exception of Sall1 little is known about their role in kidney development [106–109]. In humans Sall1 mutations leads to an autosomal dominant disease characterized by multiple birth defects including renal hypoplasia [110]. The function of *sall* during metanephric kidney development has been mainly studied using *Sall1* knockout mice [111], however, mice carrying a mutant allele lacking the DNA binding domain, die during gestation and recapitulate the abnormalities found in humans with Townes-Brocks syndrome [112]. Homozygous mutant mice exhibit kidney agenesis as a result of failure in ureteric bud branching, and it is believed that Sall1 functions in the initial branching of the ureter by regulating canonical Wnt signaling in the ureteric bud cells. Wnt9b/ β -catenin signaling is reduced in the ureteric bud tip in the presence of Sall1, and this signaling event is required for proper branching [113].

Sall1 continues to be expressed in the adult metanephric kidney mainly in proximal tubule cells. Following ischemia-reperfusion injury approximately 90% of Sall1 positive cells proliferate and the levels of *sall1* mRNA increase significantly between 4 and 12 h post injury [114]. Based on previous reports of Sall1 being a marker of renal progenitors in the metanephric mesenchyme [115] and the asymmetric division of *sall1* expressing cells following injury, it has been proposed that Sall1 may be a marker of early proliferating renal progenitor cells necessary for replacing injured renal cells [114].

3. Bmp signaling

Representing the largest subfamily within the transforming growth factor beta superfamily, bone morphogenetic proteins (BMPs) are key inducers of the IM and emerging pronephros. Bmps emanating from the LPM and surrounding surface ectoderm form a gradient across the trunk mesoderm [14, 116]. Intermediate Bmp concentrations within the IM overcome repressive factors from the adjacent PM, allowing the expression of *Pax2*, *Lhx1*, and *Osr1* [14]. Conditional expression of a Bmp inhibitor in *Xenopus laevis* embryos after formation of the IM causes severe malformations of the pronephric duct and tubules, consistent with a later requirement for BMPs in the differentiation of these cell types [117].

Transcripts for *Bmps 2, 3, 4, 5, 6,* and *7* have been observed in the mouse metanephros during various stages of its development [118]. Most mammalian studies have focused on the contributions of Bmps 2, 4, and 7 to metanephric kidney development [119]. *Bmp7* null mice display a decreased number of metanephric mesenchymal condensates surrounding the ureteric bud and an overall reduction in branching morphogenesis [120]. Bmp7, acting together with Fgf2, maintains the responsiveness of the metanephric mesenchyme to inductive signals and prevents premature tubulogenesis [121]. Loss of Bmp7 has little effect on the expression of most nephrogenic genes, including *Pax2*, *Pax8*, *Wnt4*, and *Wt1* [122, 123]. However, the lack of *Bmp7* results in a drastic loss of mesenchymal progenitors expressing *Six2* [120]. A specific deletion of *Bmp7* in podocytes results in hypoplastic kidneys with normal glomeruli but a reduced number of proximal tubules due to impaired proliferation [124]. Taken together these findings indicate Bmp7 may play multiple roles in the metanephric kidney including an antagonist of ureteric bud branching, cap mesenchyme survival, and as a stimulator of proximal tubule growth.

Mice carrying a homozygous null mutation for *Bmp2* die before the formation of the IM [125]. *Bmp2* heterozygous null mice undergo normal nephrogenesis and generate nephrons in similar quantities to *wild type* mice [119, 125]. *Bmp4* homozygous null mutant mice also die around gastrulation before the initiation of kidney development [126, 127]. However, unlike *Bmp2*, a number of *Bmp4* heterozygous null mutants exhibit kidney cysts, cortical atrophy, and hydronephrosis when maintained on a C57BL/6 background [126]. Placing a

Bmp4-coated bead near a ureteric bud in explant culture stimulates the growth and elongation of the bud [128] whereas branching is inhibited [129–131]. The observed response of ureteric buds to exogenous Bmp4, reflects the ability of Bmp4 to interfere with the differentiation of the metanephric mesenchyme and promote elongation of the ureter within the metanephros [131]. Secreted Bmp antagonists, including Noggin, Gremlin, and USAG-1, also contribute to normal metanephric development [119, 132–135]. These act by direct binding to Bmp proteins and precluding their binding to specific cell surface receptors modulating BMP signaling.

In the adult kidney of humans and rodents, *Bmp7* is expressed by podocytes and medullary tubular epithelial cells [136, 137]. Initially, two studies in rats subject to ischemia-reperfusion injury observed significant down-regulation of *Bmp7* transcript levels suggesting modulation of its expression may be important for kidney repair [138, 139]. Following these studies, numerous groups investigated the effect of administration of exogenous human recombinant BMP7 (hrBMP7) during the regeneration process and demonstrated its ability to preserve kidney function following acute ischemic injury [140–143]. In rats, hrBMP7 reduces the inflammatory response and decreases tubular cell apoptosis when administered before or after damage [142]. In addition to its function as a renal homeostasis factor, it has been suggested that Bmp7 has a role in preventing tubulointerstitial fibrosis by inhibiting interstitial infiltration and fibroblast transformation [141, 144]. Bmp7 may also reverse epithelial-mesenchymal transition (EMT) of tubular epithelial cells in response to injury by re-inducing expression of the epithelial cell adhesion molecule, E-cadherin [145, 146]. In distal and proximal tubular epithelial cells subject to injury, hrBMP7 enhances the repair of the tubular structures [146]. Insights into the mechanism of action for Bmp7 suggest that it acts by counteracting TGF- β -fibrotic effect (Zeisberg *et al.*, 2003b). The observation that Bmp7 has a reno-protective function during adult renal injury, suggests a conserved mechanism of action for Bmp7 in the processes of kidney organogenesis and regeneration. One question that remains to be elucidated is whether the antifibrotic effects of Bmp7 are specific for this Bmp or whether other Bmps have similar activities.

4. Wnt signaling

Wnt signaling can either proceed through canonical or non-canonical pathways, depending on the requirement for beta-catenin function (canonical pathway) [147]. During canonical signaling, Wnts bind a seven-pass transmembrane receptor, Frizzled, and an LDL receptor-related protein (Lrp) 5 or 6 co-receptor [148]. This complex recruits the scaffolding protein, Dishevelled, resulting in phosphorylation of the associated Lrp [148]. Upon activation, Lrp sequesters the Axin complex to the membrane, preventing Axin from degrading cytosolic beta-catenin. This permits beta-catenin to translocate to the nucleus where it binds the TCF/LEF family of transcription factors and activates target gene transcription [148]. Non-canonical Wnt pathways, including the planar cell polarity and calcium pathways, also transduce their signals through Dishevelled [147]. However, these pathways rely on the activation of downstream targets other than beta-catenin such as the GTPases Rho and Rac which in turn activate kinases that modulate cytoskeletal organization and transcription.

Work in *Xenopus laevis* has identified a key role for Wnt4 in the development of the pronephros. Morpholino knockdown of Wnt4 reduces glomerular and tubule formation [149], whereas injections of increasing amounts of *Wnt4* mRNA induce ectopic tubular structures [150]. Morpholino knockdown of the downstream Wnt/beta-catenin effector Lef1 in *Xenopus tropicalis* embryos also inhibited anterior tubulogenesis while decreasing the posterior expression of *Pax2* [151]. Similarly, injection of a dominant-negative form of beta-catenin resulted in a dose-dependent inhibition of pronephric tubule formation [152]. More

severe effects are seen with injection of a dominant-negative form of Lef which causes a loss of all pronephric structures [152].

Mice with homozygous null mutations in *Wnt4*, *Wnt7b*, *Wnt9b*, and *Wnt11* all display abnormal metanephric kidney development [153–156]. Mice lacking *Wnt4* show a complete lack of tubular development despite accumulation of cells at ureteric bud tips [155]. Those without *Wnt7b* show absence of the renal medulla, suggesting a role in establishing the corticomedullary axis [156]. Mice null for either *Wnt9b* or *Wnt11* display disruptions in branching morphogenesis [153, 154]. Loss of *Wnt9b* in mice prevents the expression of *Wnt4*, *Fgf8*, and *Pax8*, in the pretubular aggregates, resulting in decreased branching and tubulogenesis [153]. Therefore, *Wnt9b* likely functions upstream of *Wnt4* during metanephric development, although proper renal vesicle formation requires the expression of both genes [153]. Besides its role in nephron development, canonical Wnt signaling pathway is important during ureteric budding and branching morphogenesis [157, 158]. *Wnt11* expression in the ureteric epithelium reciprocally induces Ret/Gdnf signaling in the surrounding mesenchyme maintaining branching morphogenesis [154]. Therefore, input from both the canonical (*Wnt4*, *Wnt7b*, and *Wnt9*) and non-canonical (*Wnt11*) pathways control the development of the metanephric kidney.

Although *Wnt* expression is down-regulated in the adult kidney [159], current research suggests that Wnt/beta-catenin signaling pathway is reactivated during kidney regeneration. The initial observation of *Wnt4* reactivation was reported in a UUO model in rats [160], while *Wnt4* reactivation also was reported in the collecting duct epithelium and surrounding interstitial cells in a model of AKI induced by folic acid treatment [161]. The mRNA levels of *Wnt4* during the recovery phase of ischemia-reperfusion were transiently up-regulated in proximal tubular cells as early as 6 hours post injury and back to baseline levels by 24 hours post injury [47]. Increased levels of *Wnt4* correlated with increased protein levels of CyclinD1 and CyclinA implies that *Wnt4* might be promoting tubular cell proliferation via control of cyclins, and this ability of *Wnt4* to induce cell cycle progression in a renal epithelial cells is mediated by beta-catenin [47]. However, adding to the complexity of the role of *Wnt4* following injury, a recent study using a *Wnt4* reporter transgenic mouse line, following ischemia-reperfusion injury, finds *Wnt4* reactivation in interstitial myofibroblasts and not in tubular epithelium [162].

Evidence suggests that Wnt signaling from macrophages, contributes to kidney regeneration [163]. Several days after ischemia-reperfusion injury, surviving proximal tubule epithelial cells become Wnt responsive, and activated renal macrophages are a source of the ligand *Wnt7b*. Macrophage production of pro-survival factors could be key in the regeneration process possibly by preserving basement membrane integrity and preventing apoptotic cell death of RTECs, revealing the participation of non-tubular cells in the events that initiate tubular repair and regeneration after injury [163]. Even though the mechanistic details of Wnt-signaling activation remain to be elucidated, the Wnt-pathway appears to promote cell survival and enhance regeneration of the damaged tubule.

Conclusions

The reactivation of multiple embryonic signaling pathways during kidney regeneration has driven the hypothesis that these pathways play similar roles in both the specification and replacement of renal tissue (Figure 1, Table 1) [7–10]. As presented in this review, regeneration mediated cell proliferation and morphogenesis coincides with the reactivation of genes normally associated with kidney organogenesis, suggesting that regenerating tubular epithelium may be reprogrammed to a less-differentiated, multi-potent state. However, there is much yet to be elucidated regarding the mechanisms driving kidney

regeneration. The AKI studies to date are based mainly upon expression of reactivated genes and not how those genes influence regenerative events [8–10]. These nascent reactivation studies are similar to early embryonic expression studies. The goal of several large scale embryonic expression studies was to identify new genes with unique expression patterns during tissue and organ development, and from that initial starting point the mechanistic role of the newly identified genes was eventually deciphered [164–166]. In addition to deciphering the mechanistic role signaling pathways play during regeneration, understanding the temporal component of gene reactivation will also be important to determining how scientists can influence regenerative events. The multitude of published renal embryonic mechanistic studies are a good place to take cues from to further the understanding of the etiology of renal damage, identify new therapeutic treatments, and advance the cell replacement and tissue engineering fields.

Acknowledgments

Supported by grants from the National Institutes of Health, NAH: 2R01 DK069403, 2R01 HD053287, 1P30 DK079307; MPdC: 1R01 HL093057, 1P30 DK079341; NAH/MPdC 1RC4 DK090770; AJD: The Rutherford Foundation Trust and the Marsden Fund of New Zealand.

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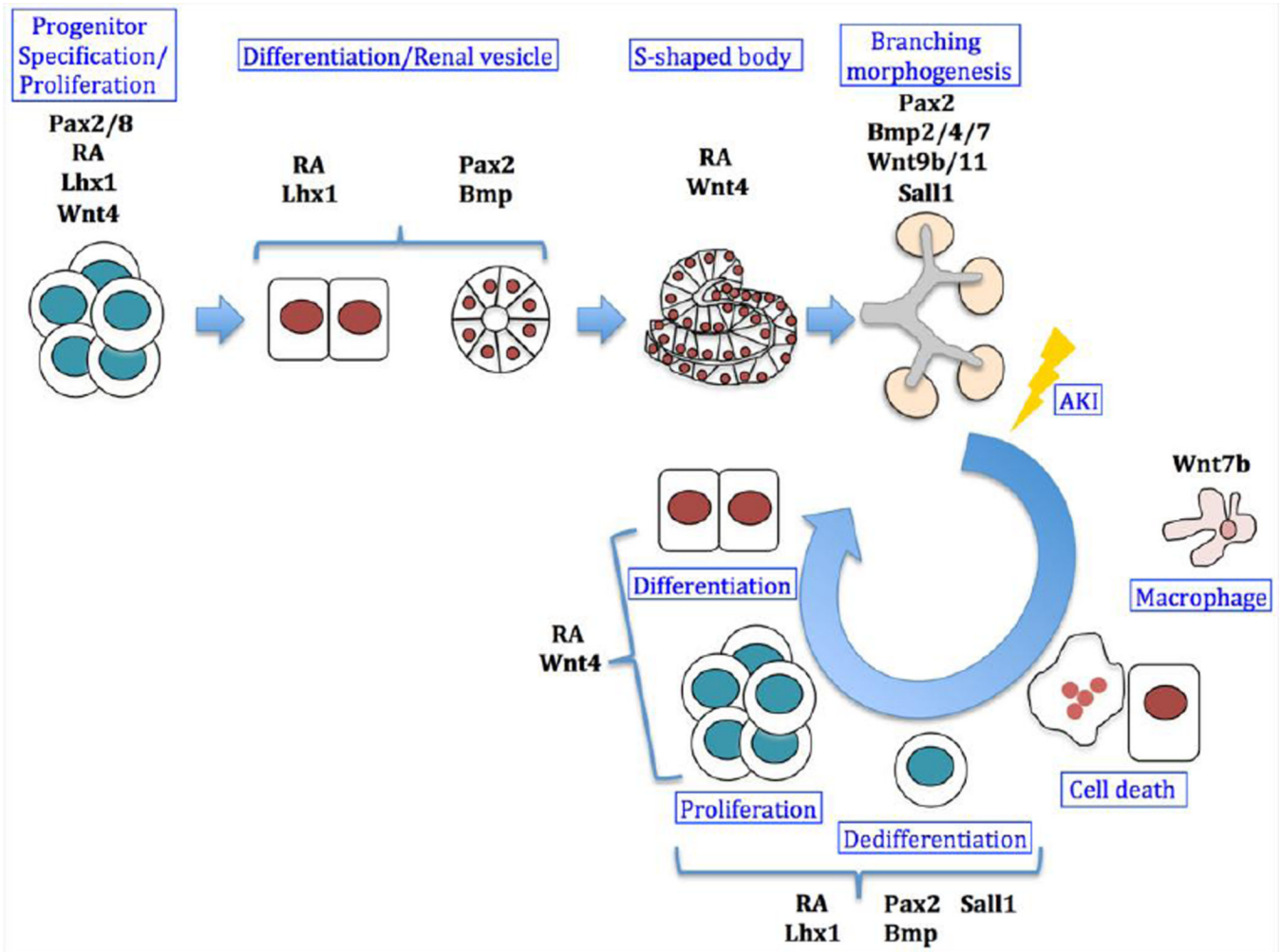


Figure 1. Molecular pathways involved in kidney development and regeneration

A simplified scheme of kidney development (top row) and acute kidney injury (AKI) induced regeneration (circle). During development, RA drives renal progenitor cells to undergo specification followed by proliferation events while expressing the early pre-tubular markers *Pax2/8*, *Lhx1* and *Wnt4*. Later, RA induces expression of *Lhx1* which has a role in the patterning of the renal vesicles together with *Pax2*, and *Bmp* proteins which induce mesenchyme polarization. The formation of the S-shaped bodies coincides with activation of the Wnt pathway, which is essential for the proximo-distal patterning of the nephron together with RA signaling. Branching of the ureteric bud within the metanephric mesenchyme is regulated by *Pax2* and *Wnt9b/11* among other factors. *Bmp* signaling has a role in regulating branching, both positively (*Bmp7*) and negatively (*Bmp4* and *Bmp2*). Following tubular damage induced by AKI, epithelial cells are lost and the basement membrane is denuded. *Wnt7b* might act as a pro-survival signal from macrophages that preserves basement membrane integrity. During the regeneration process, the surviving epithelial cells undergo dedifferentiation and reactivation of nephrogenic markers such as *Pax2*, *Bmps*, and *Lhx1*. Proliferation and differentiation of these cells is possibly mediated by RA and *Wnt4*, providing the source of replacement cells necessary for restoring a functional tubule

Table 1

Summary of the pathways and genes with roles in kidney development and regeneration following injury.

Pathway	Role in development	Role in regeneration	References
Retinoids	Pronephros: specification, tubule morphogenesis, segmentation of the tubule Metanephros: branching morphogenesis	Pro-survival	Development: [53–59, 64, 65, 167] Regeneration: [76, 168]
Pax (Pax2/Pax8)	Pronephros: specification of the pronephric lineage Metanephros: ureteric budding, branching morphogenesis	Anti-apoptotic Pro-survival	Development: [84–86, 92] Regeneration: [95, 96, 169]
Lhx1	Pronephros: specification of the pronephric lineage Mesonephros: maintenance of nephric duct and tubules Metanephros: formation of ureteric bud and renal vesicles patterning	Unknown in mammals Reactivated maker of stem cells during repair following AKI in adult zebrafish	Development: [98, 101, 170, 171] Regeneration: [10, 30]
Sall1	Expressed in pronephros and mesonephros Metanephros: uretheric bud branching	Increased expression in mice following ischemia-reperfusion injury. Increased proliferation of Sall1-positive cells	Development: [107, 109, 112, 113] Regeneration: [114]
Bmp	Pronephros: patterning of the intermediate mesoderm Metanephros: patterning (Bmp7), regulation of branching morphogenesis (Bmp2/Bmp4/Bmp7).	Anti-apoptotic, reduction of immune response, prevention of tubulointerstitial fibrosis	Development: [14, 116, 117, 122–124, 128, 131, 172] Regeneration: [140–142, 145, 146]
Wnt	Pronephros: patterning of the intermediate mesoderm, tubulogenesis (Wnt4/Wnt11) Metanephros: mesenchyme-to-epithelium transition (Wnt4), tubulogenesis (Wnt4), cortico-medullar morphogenesis (Wnt7b), branching morphogenesis (Wnt9b/Wnt11)	Reactivated during repair following AKI (Wnt4/Wnt7b) Anti-apoptotic (Wnt7b)	Development: [149, 150] [153–156] Regeneration: [47, 160, 162, 163]