Hnf1beta and nephron segmentation

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Abstract

The nephron is the functional unit that executes the homeostatic roles of the kidney in vertebrates. Critical to this function is the physical arrangement of the glomerular blood filter attached to a tubular epithelium that is subdivided into specialized proximal and distal segments. During embryogenesis, nephron progenitors undergo a mesenchymal-epithelial transition (MET) and adopt different segment-specific cell fates along the proximo-distal axis of the nephron. The molecular basis of how these segments arise remains largely unknown. Recent studies using the zebrafish identified the Hnf1beta transcription factor (Hnf1b) as a major regulator of tubular segmentation. In Hnf1b-deficient zebrafish embryos, nephron progenitors fail to adopt the proximo-distal segmentation pattern of the nephron, yet still undergo MET. This observation suggests that the functional segmentation of renal tubular epithelial cells is independent of pathways that induce their epithelialization. Here we review this new role of Hnf1b for nephron segmentation during zebrafish and mouse kidney development.

Keywords

kidney; embryo; nephrogenesis; zebrafish; mouse; HNF1beta; transcription factor

Introduction

Nephrogenesis refers to the formation of the blood filtering tubules (nephrons) from mesenchymal progenitor cells. This process involves changes in cell morphology (mesenchymal to epithelial) and the adoption of distinct cell fates along the proximo-distal axis of the nephron. How is process is controlled is poorly understood, however recent studies in zebrafish have identified the Hnf1beta transcription factor (Hnf1b) as a major player [1]. This article reviews this new function of HNF1b during zebrafish as well as mouse nephrogenesis.

Nephrogenesis in mammals and zebrafish

Vertebrate kidney formation progresses through two or three stages, depending on the species, giving rise to the pronephric, mesonephric, and metanephric kidneys. Common to each is the nephron, comprising a glomerular blood filter attached to a tubular epithelium. The metanephric nephron can be broadly subdivided into proximal, intermediate, and distal segments that have the general functions of bulk filtrate reabsorption (proximal tubule), urine concentration (intermediate segments) and acid-base and salt 'fine-tuning' (distal nephron).

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By contrast, zebrafish pronephric nephrons are simpler, comprising a single glomerulus attached to two tubules that are subdivided into two proximal tubule segments and two distal tubule segments (Figure 1B)[2,3]. Whilst there are notable differences between the genesis of nephrons in the mammalian metanephros and the zebrafish pronephros, the overall process is very similar with mesenchymal nephron progenitors activating conserved transcriptional and signaling pathways and undergoing MET into segmented nephrons [2,4,5]. The nephrons of freshwater fish such as zebrafish, primarily function to reclaim filtered solutes, balance ions, and void water taken up from their freshwater environment. Evidence to date indicates that the two proximal tubule segments in zebrafish, with their well-developed apical brush borders and transporter expression profiles, are analogous to the proximal tubule of metanephric nephrons. The first distal segment ('distal early') shares a gene signature with the thick ascending limb segment in mammals, while the 'distal late' segment has molecular hallmarks of the distal convoluted segment in mammals [3].

During development of the mammalian metanephric kidney an outgrowth of the nephric duct called the ureteric bud (UB) interacts closely with a population of nephron progenitors and reciprocal communication coordinates progressive UB branching and nephron induction. Nephrogenesis is characterized by the epithelialization of nephron progenitors, close to the UB branches, which pass through stereotypical stages of maturation (pretubular aggregate, renal vesicle, comma-shaped body, and S-shaped body; SSB). The developing nephron fuses with the UB-derived collecting duct at the late renal vesicle stage [6,7]. The S-shaped body is subdivided into lower, mid, and upper limb compartments, with the glomerulus arising from the lower limb, the proximal tubule and loop of Henle most likely deriving from the mid limb, and the distal nephron from the upper limb (Figure 1A).

In the zebrafish, the pronephros arises from bilateral stripes of intermediate mesoderm that epithelializes into a simple two-nephron kidney (Figure 1B). Unlike in mammals, where the intermediate mesoderm differentiates into the nephric duct that migrates a significant distance to the cloaca, most of the zebrafish intermediate mesoderm differentiates *in situ* to form nephron progenitors. Because of this, the distal pronephros only migrates a short distance to the cloaca where it fuses [8]. The linear morphology of pronephric nephrons is a useful feature of the zebrafish model, as it means dynamic gene expression patterns [9,10] can be readily mapped during nephrogenesis and correlated to the final segmentation pattern of the nephron [2].

Role of Hnf1b in nephron development

Hepatocyte nuclear factor-1 beta (*Hnf1b*) encodes a POU homeodomain transcription factor that is expressed in tubular portions of the nephron and the metanephric collecting duct system, starting at the renal vesicle stage [1,11–13]. In humans, mutations in *HNF1B* are a common cause of congenital anomalies of the kidney [14–16] and heterozygous mutations are associated with the 'renal cysts and diabetes' syndrome (RCAD) in young adults [17]. Despite obvious links to kidney development and function, defining the exact role of Hnf1b in the kidney has been hampered by the early embryonic lethality (E7.5) of the constitutive knock-out mouse, due to extra-embryonic visceral endoderm defects [18,19]. Various strategies have been employed to try to overcome this difficulty. Of these, transgenic overexpression of a dominant negative variant of *Hnf1b* in already formed, but still elongating, tubular segments leads to a polycystic kidney phenotype [20]. Further studies revealed that Hnf1b functions as a 'bookmarking factor' that is required after cell division to re-establish the expression of a number of genes associated with cystic kidney disease [21]. Utilization of tetraploid complementation experiments, which rescues the visceral endoderm defects while leaving the 'embryo proper' deficient in *Hnf1b*, demonstrated a requirement for *Hnf1b* in UB branching [12]. In addition, expression of *Wnt9b*, encoding a critical UB-

derived inducer of nephrogenesis, was absent in the UB tips of *Hnf1b*-deficient embryos [12]. While this study demonstrated the functional importance of Hnf1b in the UB, it precluded an assessment of its role during later stages of nephrogenesis.

In zebrafish there are two orthologues of *Hnf1b* (*hnf1ba* and *hnf1bb*) that are expressed in the intermediate mesoderm and developing pronephric tubules [1,22]. In *hnf1ba/b*-deficient zebrafish embryos (herein referred to as *hnf1b*-deficient), the pronephric tubules fail to adopt a proximo-distal segmentation pattern and mature markers of proximal and distal segment identity, such as solute transporter genes, are not expressed [1] This result provided the first evidence that Hnf1b factors play a role in establishing the tubular segmentation pattern of the nephron. This new function was confirmed in the mouse by two independent conditional knockout strategies utilizing the *Six2*-Cre and *Wnt4*-Cre drivers and floxed *Hnf1b* alleles [11,13]. Early nephrogenesis is relatively unaffected in these mutants, with polarized renal vesicles and comma-shaped bodies developing normally. However, morphological defects become apparent at the SSB stage with mutants failing to expand presumptive proximal tubule and loop of Henle precursors that are believed to be located in the mid-limb of the SSB. This region shows reduced proliferation and increased apoptosis compared to wild-type SSBs, providing one explanation for the altered SSB morphology in the mutants. Mid-limb markers are significantly reduced in *Hnf1b*-deficient embryos, including Notch components such as the *Dll1* ligand. Given that *Dll1* hypomorphs and *Notch2* nulls display proximal tubule defects that overlap with *Hnf1b* mutants, it is likely that disrupted Notch signaling plays a major role in the *Hnf1b*-deficient kidney phenotype [23]. In both zebrafish and mouse mutants, persistent expression of *Pax2* and *Lhx1* expression was noted, suggesting that *Hnf1b*-deficient tubular epithelial cells do not fully differentiate and retain the expression of genes that characterize earlier stages of nephrogenesis. Consistent with this, expression of mature proximal and distal tubule markers, which initiate later in nephrogenesis, is absent in *Hnf1b* mutant kidneys and the nephrons that ultimately form are comprised of dilated glomeruli connected to collecting ducts via short tubules. Taken together, the fish and mouse studies support a model in which Hnf1b acts during early nephrogenesis to regulate genes involved in establishing the maturation of all nephron tubular segments. The mammalian studies suggest that Hnf1b is not required for the initial patterning of the renal vesicle but instead acts during the transition to the SSB stage, when distinct tubular precursors are arising. In addition, Hnf1b may have a direct role in regulating the expansion and survival of tubular precursors, or alternatively the defects in proliferation and apoptosis seen in the mouse mutants (but not zebrafish) may be secondary to failed differentiation.

The zebrafish study revealed an unexpected role for Hnf1b in regulating the formation of podocytes, the highly specialized epithelial cells that contribute to the glomerular blood filter. *Hnf1b*-deficient zebrafish embryos display ectopic podocyte marker expression in regions of the intermediate mesoderm where the first proximal tubule segment would normally form. This result implicates Hnf1b factors in the developmental pathways that regulate the partitioning of podocyte and tubule fates from the intermediate mesoderm [1]. This function may not be conserved in mammals, as glomerular development appears largely unaffected in the conditional *Hnf1b* mutants [11,13]. During zebrafish pronephros formation, transcripts for the *hnf1b* genes are excluded from podocyte progenitors, suggesting that Hnf1b factors may act in proximal tubule progenitors to inhibit podocyte fate.

Despite the lack of proximo-distal segmentation in *hnf1b*-deficient zebrafish embryos, the mesenchyme-to-epithelial transition (MET) of tubule progenitors is relatively unaffected. Electron microscopy shows tubular epithelial cells in *hnf1b*-deficient animals with apical junctional complexes and a surrounding basement membrane. In support of this, the

expression of epithelial markers such as *cdh1*, *epcam,* and *laminin5* are maintained in *hnf1b*-deficient embryos [1]. No effect on MET was seen in the mouse studies either, however this was not unexpected as the timing of *Hnf1b* expression differs between zebrafish and mouse with transcripts appearing in the metanephros after the epithelialization of nephron progenitors into renal vesicles [11,13]. The zebrafish findings suggest that the patterning of nephron progenitors into distinct segments is independent of the MET that occurs during nephron development. In further support of this, genes that are critical for segment functionality, such as the $Na^+K^+ATPase$ subunit genes and solute transporters genes like *slc4a4* (proximal tubule), and *slc12a3* (distal late tubule), are expressed in zebrafish nephron progenitors prior to the onset of epithelial-associated genes such as *cdh1* and *epcam* (our observations and reference [10]).

Interactions between Pax2/8 and Hnf1b

If Hnf1b does not drive the MET of nephron progenitors then what factors do? Pax2 and the closely related Pax8 are among the earliest acting transcription factors in the intermediate mesoderm and likely candidates for inducing MET [24–28]. Both *in vivo* and *in vitro* studies have implicated Pax2/8 in the epithelialization of the intermediate mesoderm and metanephric nephron progenitors [29,30]. However, the downstream targets of Pax2/8 in this process are unclear. A role for Pax2/8 in directing the MET of zebrafish nephron progenitors is consistent with the observation that expression of *pax2a* and *pax8* persists in *hnf1b*-deficient zebrafish embryos. Interestingly, in *pax2a/8*-deficient embryos the intermediate mesoderm initially forms but fails to activate expression of *hnf1ba* (our observations). These results suggest a model in which Pax2a and Pax8 act upstream of the *Hnf1b* genes, resulting in the initiation of segment-specific gene expression programs, in addition to inducing MET through Hnf1b-independent pathways. Thus, we propose Pax2/8 control both tubular epithelialization and segment identity (Figure 1B).

Interactions between the Hnf1b and Iroquois transcription factors

Functional studies in zebrafish and *Xenopus* have implicated orthologues of the Iroquois (Irx) transcription factors *Irx1* and *Irx3* in the development of the pronephros [10,31,32]. In the mouse *Irx1*, *Irx2,* and *Irx3* are expressed in the mid-limb of SSBs, however a functional requirement of these factors for mammalian nephrogenesis has yet to be shown [11]. Chromatin-immunoprecipitation experiments demonstrated that Hnf1b is recruited to potential regulatory elements around the *Irx1* and Irx2 gene cluster [13]. Consistent with being direct targets of Hnf1b, expression of *Irx1* and *Irx2* are virtually undetectable in *Hnf1b* mutants. Similarly, there is a loss of *irx3b* expression in the pronephros of *hnf1b*-deficient zebrafish embryos [1]. Work in zebrafish implicates Irx3b in the morphogenesis of the first distal tubule segment although this role is poorly understood [10]. Whether the Irx genes directly regulate segment identity and/or maturation downstream of Hnf1b remains unclear and requires more study.

Conclusions and future directions

Recent discoveries using the zebrafish and mouse models have extended our knowledge of the multiple roles Hnf1b factors play during kidney development. In nephron progenitors, Hnf1b is required for the activation of segment-specific gene expression programs that are central to the function of the nephron. The direct targets of Hnf1b are beginning to be elucidated and while prior studies have focused predominantly on genes in the proximal tubule [33–38], it is now clear that the search needs to be broadened. The recent studies discussed here have identified new targets including key regulatory genes such as components of the Notch pathway and the Iroquois transcription factors that are expressed in

tubule precursors [11,13,23,31,32,39,40]. More work is needed to understand how these genes act downstream of Hnf1b to regulate the proximo-distal patterning of the nephron. If Hnf1b acts in all tubule segments then additional layers of regulation must exist to ensure that Hnf1b only activates targets appropriate to each segment. Solving this mystery will depend on further studies utilizing ChIP-Seq and the ability to examine Hnf1b binding to chromatin in epithelial cells from each segment. An additional challenge will be to understand how Hnf1b interacts with the pathways that initially establish the segmental pattern of the nephron. In zebrafish, retinoic acid signaling has been identified as a major regulator of proximo-distal patterning in the pronephros [2,3,10], whereas in mammals the Notch pathway is implicated in the establishment of proximal nephron fates [5,23,41–43]. How these pathways interface with the Hnf1b factors and whether they play a role in determining the targets that are available for activation in each segment remains to be determined.

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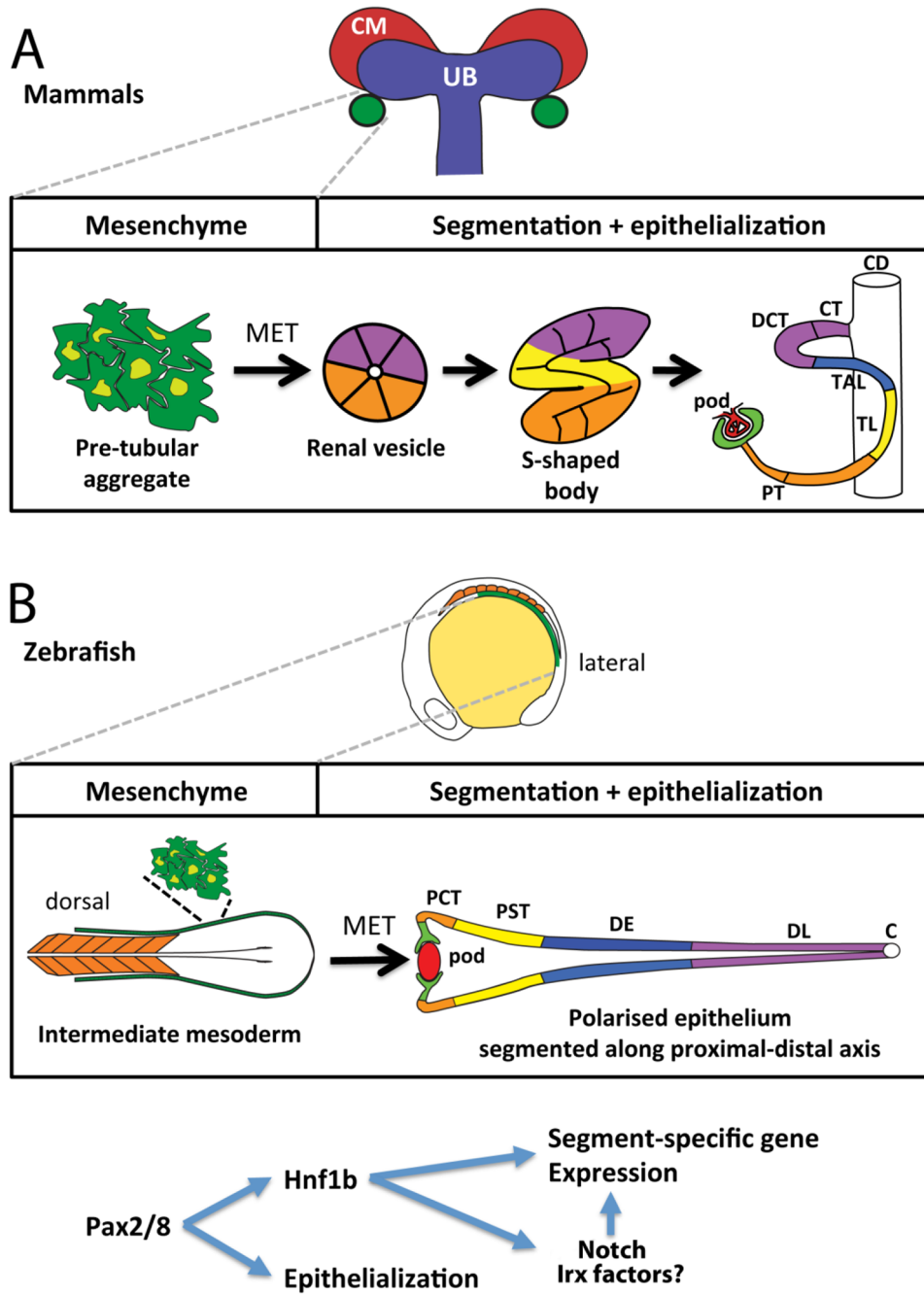
Figure 1.
A) Top panel shows the stages of mammalian metanephric nephron development from a pretubular aggregate to an early nephron. Bottom panel shows an overview of zebrafish pronephros formation from the intermediate mesoderm. **B**) Postulated genetic pathway during early nephrogenesis showing the hierarchical relationship between *Pax2/8*, *Hnf1b*, Notch pathway, and *Irx* genes and their proposed roles in directing the mesenchymal-to-epithelial transition (MET) of nephron progenitors and proximo-distal segmentation. Abbreviations: CM, cap mesenchyme; UB, ureteric bud; pod, podocytes; PT, proximal tubule; TL, thin limb; TAL, thick ascending limb; DCT, distal convoluted tubule; CT,

connecting tubule; CD, collecting duct. PCT, proximal convoluted tubule; PST, proximal straight tubule; DE, distal early tubule; DL, distal late tubule; C, Cloaca.