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Progenitor programming in mammalian nephrogenesis

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Abstract

Nephrogenesis is dependent on the input of several transcriptional regulatory networks. However, the details of how these networks operate and converge to facilitate nephron progenitor specific programmes are largely unknown. To this end, recent studies have focused on identifying the precise regulatory mechanisms that modulate progenitor maintenance and induction. Continued focus on this area of research will help identify nephrogenic programmes which could be manipulated for therapeutic intervention of kidney disease.

Keywords

nephrogenesis; transcriptional networks; nephron progenitor programmes

The eloquent progression of nephrogenesis during embryonic kidney development requires a careful balance of nephron progenitor self-renewal and differentiation. This ensures a sufficient number of nephrons are formed to carry out their essential roles in waste filtration and body fluid homeostasis. In mammals this is a terminal process; no resident progenitors remain after fetal or early neo-natal stages. *De novo* nephron formation does not appear to be an option for the adult mammalian kidney, necessitating repair of existing nephrons following injury or disease. In this light, developing alternative, knowledge-based strategies to *de novo* nephrogenesis is an important therapeutic goal. As a first step, we need to develop a thorough understanding of the nephron progenitor population and the underlying regulatory programs governing its maintenance and nephron specific capabilities. Leveraging this knowledge base will spur the development of new strategies to treat the damaged and diseased kidney.

The mammalian kidney develops through reciprocal interactions of the ureteric epithelium with adjacent mesenchymal nephron progenitors. Signals from nephron progenitors support ureteric epithelial branching and the arborization of the urine transporting collecting duct network derived from this epithelium. In turn, the transition of multi-potent nephron progenitors into epithelial renal vesicles, the nephron precursor, requires signals from the ureteric bud. Over the last few decades, research efforts have uncovered a number of factors with integral roles in kidney development. In particular, the transcriptional regulators and

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associated components including: *Six1, Pax2, Hox11* paralogs, *Osr1, Sall1, Six2, Eya1* and *Wt1* are all expressed within the nephron progenitors, and the depletion of each from the murine kidney results in insufficient kidney development[1–8]. Loss of any factor other than *Six2* leads to a loss of nephron progenitors at the expense of nephron formation. In contrast, the entire nephrogenic mesenchyme of *Six2* mutants commits to nephron formation at the onset of kidney development, prematurely terminating the nephrogenic program with only a small number of renal vesicles in place[7,8]. Thus, *Six2* has a unique regulatory activity amongst these factors: promoting the self-renewal of the nephron progenitor population.

Self-renewal of nephron progenitors is normally opposed by Wnt signaling from the adjacent branching tips of the ureteric epithelium. Here, *Wnt9b* is expressed in a graded fashion with higher levels beneath the tips where induced mesenchyme cells first aggregate then epithelialize to generate renal vesicles, and at lower levels above the tip where the ureteric epithelium directly contacts the main body of the nephron progenitor pool[9]. Wnt9b-directed canonical Wnt signaling mediated by a β-catenin containing transcriptional complex induces renal vesicle formation[10]. Together, these genetic-based data highlight a complex regulatory network underpinning specification, maintenance, and commitment of nephron progenitors. What is not clear is how the transcriptional pathways direct these events.

The majority of functional studies have examined gene knockouts to infer function rather than directly addressing the transcriptional networks at play. A combination of *in vivo* and *in vitro* analysis has defined regulatory modules, uncovering some of the basic networks underpinning Six2 regulation[11]. However, a broader insight requires unbiased genome-scale methodology, integrating a full complement of the regulatory factors to take our understanding to a deeper, systems level. Combining advances in next generation sequencing with chromatin immunoprecipitation-mediated enrichment of transcriptional components at their target sites (ChIP-seq) has resulted in exciting new insights into critical control mechanisms regulating complex biological processes. Similarly, integrating ChIP-seq analysis with gene expression data in nephron progenitors is expected to lead to a new level of insight into transcriptional targets and modules of regulation, and to generate a clearer picture of how nephron progenitor status is programmed, maintained then lost on progenitor commitment to nephron fates.

We have recently taken advantage of such experimental analyses to identify the gene regulatory networks engaged by Six2 and canonical Wnt-directed transcriptional complexes. $Six2^+$ nephron progenitors were isolated from embryonic mouse kidneys and subjected to ChIP-seq either immediately (Six2 ChIP) or after treatment with a Wnt pathway agonist to induce β-catenin transcriptional engagement and epithelial commitment (β-catenin ChIP). While each factor was bound to an independent set of regulatory elements, a subset of genomic regions was directly engaged by both factors suggestive of overlapping regulatory functions. Potential targets included factors expressed within the nephron progenitors such as *Six2* and *Eya1*, as well as *Wnt4* and *Fgf8*, genes activated on induction of progenitors that are essential themselves for the transition to epithelial renal vesicles. Further analyses showed that Six2 likely engages in a complex with Lef/TCF factors, the DNA binding component of the β-catenin-dependent Wnt signaling transcriptional machinery, but that the

entry of β-catenin into this complex is restricted to newly induced and differentiating cells. These data suggest a model wherein Six2 action at these sites inhibits *Wnt4* and *Fgf8* expression in the nephron progenitors. Upon Wnt9b induction, β-catenin entry into the complex turns on the expression of *Wnt4, Fgf8*, and other targets, promoting commitment of these cells to a nephrogenic program (Figure 1)[12].

While our analyses have shed new light on the regulatory mechanisms that balance nephron progenitor self-renewal vs. differentiation, a host of transcriptional regulators have integral roles in kidney development and progenitor function. Future studies will employ a combination of ChIP-seq, expression analyses, biochemistry and *in vitro* and *in vivo* modeling to identify the regulatory modules employed by these factors. We expect to find independent regulatory networks utilized by each factor but hypothesize that a significant overlap will be identified with any combination of factors. The exploration of shared gene regulatory networks will undoubtedly uncover new mechanisms that help maintain nephron progenitor multi-potency. This knowledge will be critical to future research aimed at exploiting the potential of the nephron program for therapeutic intervention.

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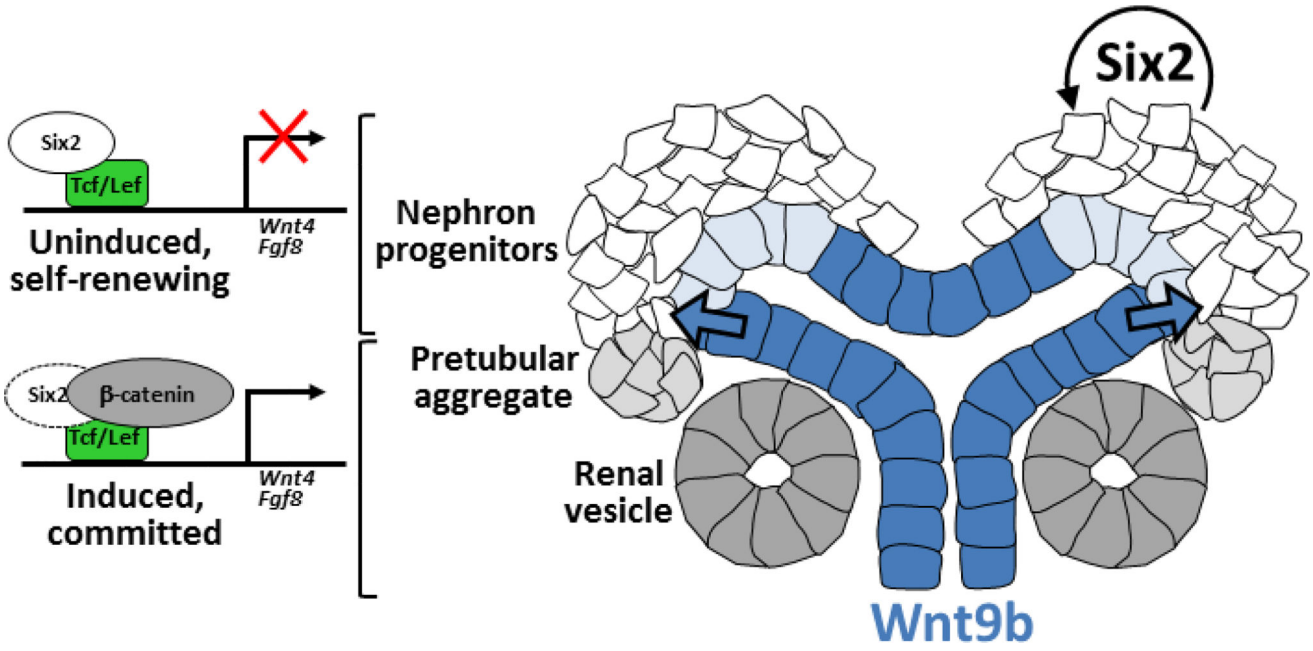

Figure 1.

Kidney development requires a balance of nephron progenitor self-renewal and induction. See text for further description.