mutations until they lie on the verge of falling apart (Zeldovich et al., 2007). During evolution, it would be easy for a protein to end up with "hidden" destabilizing mutations that would make it unfold unless it possessed other, stabilizing features.

How often do proteins undergo parallel duplication and divergence? The reciprocal structural changes after Bub1 duplication make this a compelling example that should inspire systematic searches for other examples of what is likely to be a widespread phenomenon.

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How the Developing Mammalian Kidney Assembles Its Thousands of Nephrons: Fgfs as Stemness Signals

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In this issue of *Developmental Cell*, Barak et al. (2012) identify a critical role for Fgf9 and Fgf20 signaling in the nephron progenitors of the developing mammalian kidney. These Fgfs serve as survival and nephron-forming competence signals for purified Six2+ cells that represent the progenitors that normally go on to generate nephrons.

The mammalian metanephric kidney contains thousands of nephrons that represent the major functional units of the organ to control water and electrolyte homeostasis and blood pressure. During kidney development, induction of the nephron assembly process occurs each time the ureteric bud generates a new branch (Figure 1). Given such dynamic structural constraints, the progenitor cells that form the nephrons need to organize their renewal and differentiation in register with the ureteric branching process.

Barak and colleagues (2012) now demonstrate in this issue of *Developmental Cell* that Fgf9/20 signals are critical for nephrogenesis because their compound knockout reduces the number of nephron-forming progenitors and leads to premature expression of certain early nephron differentiation markers. While Fgf9 signals from the ureteric bud, the adjacent cap metanephric mesenchyme (CMM) expresses autocrine Fgf9, as well as Fgf20, to maintain pretubular cells (Figure 1). Moreover, in ex vivo cultures, Fgf9 and Bmp7 can together promote survival of the metanephric mesenchyme and maintain its competence for Wnt-dependent (Kispert et al., 1998; Stark et al., 1994) nephrogenesis. Together with Bmp7 (Dudley et al., 1999), Fgf9 can support survival of even purified CMM-derived Six2+ cells for at least 2 days, as judged by the capacity of these cells to form nephron structures in 3D organoid culture. These findings provide an important step not only toward identification of the mechanisms by which nephrons are formed from precursor cells, but also to how the stemness of nephrogenic progenitors is maintained. Thus, it is evident that Fgf signals are involved in maintenance of pretubular cells (see also Brown et al., 2011).

But where does the stem cell/progenitor cell niche in the developing kidney reside, what kind of cells does the niche generate, and how does it do so? Fate mapping of Cited1+, Six2+, and Wnt4+ cells within the CMM (Figure 1) indicated that the cells around each ureteric bud tip indeed generate the nephrons (Boyle et al., 2008; Shan et al., 2010). Does the whole CMM mesenchyme with the associated ureteric tip serve as the niche organizer unit? The *Fgf20* gene is expressed throughout the CMM and contributes to its survival with Fgf9. However the time-lapse analysis of Wnt4+ marked cells in cultured kidney

primordia revealed that the cells within the CMM are motile and slide along the ureteric bud toward its lateral and proximal regions where the pretubular aggregates (PTA) undergo mesenchyme-to-epithelium transition (MET) via Wnt4-, FGF8-, and Notch-dependent mechanisms (Figure 1; Boyle et al., 2011; Shan et al., 2010; Stark et al., 1994). During this process, the Fgf20/Six2+ cellular zone may become divided into subdomains that are characterized by certain sets of transcriptions factors (Figure 1; Mugford et al., 2009) reflecting that the cells in the inductive zone may indeed become specified step by step. It remains to be seen whether only certain cells in the Fgf9/20+ CMM undergo self-renewal while others serve as transit-amplifying cells so that each specific spatial cellular position in relation to the ureteric tip gradually primes the cells to acquire the potential to undergo MET. Given this more-detailed cell lineage map of the Fgf20+/Six2+/Cited1 CMM, pretubular cells should be examined to reveal which cells self-renew and which go on to generate nephrons during the ureteric bud branching process. Using such a detailed marker-based cell identity map, the subpopulations of the CMM could be purified and their molecular signature could be revealed in more detail to better understand the cellular and molecular dynamics of the control of stemness during nephrogenesis.

Likewise, given the limited duration and nephron production by Fgf/BMP-treated Six2+ cell cultures, it seems likely that additional factors remain to be identified, which may promote longer-term survival and more robust expansion of pretubular progenitors. The capacity to expand nephron-forming cells in large quantities so that they would retain their competence for nephrogenesis would enable better use of the kidney organ culture system to investigate nephrogenesis. Such knowledge would also be valuable to the analysis of how, e.g., in utero exposure to environmental stress can lead to reduced performance of the kidney in the adult via developmental programming. In addition, the opportunity to maintain embryonic renal stem cells in vitro would provide the opportunity to expand the cells to develop kidney cell replacement therapies.

In thinking about where to look for additional niche factors, it is worth noting the finding of Barak et al. (2012) that, other than the CMM where Fgf9/20 are expressed, the nephron-forming niche also involves ureteric bud-derived Fgf9 signaling (Figure 1). Thus, to reach a better understanding of the niche, the roles of ureteric bud epithelial, CMM, and the surrounding interstitial stromal cell (ISC) signals should be considered (Figure 1). The ISCs around the CMM also secrete critical regulators of kidney development such as retinoid acid. Other than the Fgfs, the CMM expresses Tgf-β family members such Gdnf and Bmps and certain Bmp antagonists, for example. In turn, Wnt9b in the ureteric bud also regulates renewal or differentiation of the pretubular cells in a *Six2*-dependent manner (Karner et al., 2011). Hence, like in certain other developmental model systems, we may expect that the control of the embryonic kidney niche involves other factors besides the Fgfs that may cooperate to regulate CMM cell fates concentration dependently. Based on the work of Barak et al. (2012), we can now place Fgf9, Fgf20, and Bmp7 in a recipe to test other candidate factors and characterize their contributions to the nephron-forming niche.

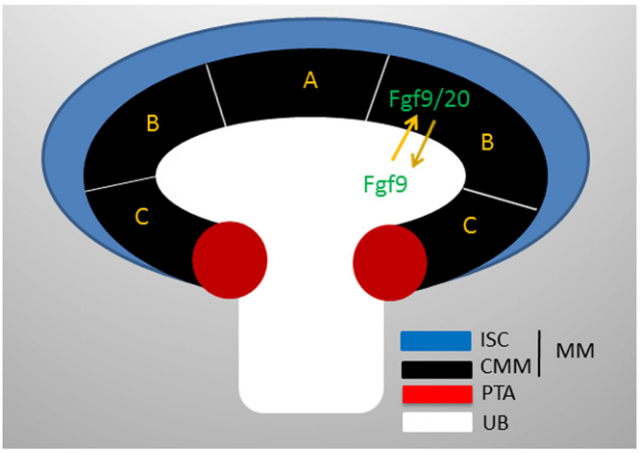

Figure 1. Schematic Representation of the Main Cell and Tissue Types that Construct the Kidney and Its Nephrons

The ureteric bud (UB) expresses Fgf9, which signals to the Six2+ CMM and promotes their survival and "stemness" for nephrogenesis with Fgf20. During epithelial UB growth and branching, Fgf9/20 throughout the CMM (in black) go on to maintain the potency of the CMM that becomes subdivided into certain territories, based in part by transcription factor expression profiles (segments A–C, Mugford et al., 2009). Wnt4, Fgf8, and Notch activity regulate formation of PTA from the CMM, a process that precedes the MET essential for subsequent nephron assembly. Barak et al. (2012) show that the nephrogenesis niche involves Fgf9/20 signaling. This is an important step to reveal how their action is coordinated by input from presumptive ISC, CMM, PTA, or UB-derived signals. The arrows depict the signaling taking place between the Fgfs and, in general, the dependence of the nephrogenesis niche on reciprocal cell signaling between epithelial and mesenchymal tissues.

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