Pax2: a 'keep to the path' sign on Waddington's epigenetic landscape

Pax2: a '*keep to the path'* sign on Waddington's epigenetic landscape.

Abstract

Developing kidneys have Foxd1+ stromogenic stem cells and [Six2+, Pax2+] nephrogenic stem cells. Targeted Pax2 deletion converts the latter to the stromogenic path (or something very much like it), suggesting Pax2 normally represses an otherwise inevitable transition between sister lineages.

Main text

The classic abstract picture of cell differentiation is the epigenetic landscape sketched by Waddington (1957), who represented cells as marbles rolling down branching valleys (Fig 1a). At the branch points, different futures are equally accessible, though cells may be biased by signals that push them to the left or right fork. Many of these signals have been identified and understood. What has been much less explored is the biological mechanism represented by the valley walls, perhaps because Waddington's image suggests that jumping from one path to another is prevented by structures external to cells, instead of by cells' internal genetic networks (Kauffman, 1993). In this issue, Natalie Naiman and colleagues show that expression of Pax2 is critical for a specific type of renal stem cell to remain on its proper pathway instead of moving to a parallel one.

Mouse kidneys develop in a continuous process from E10.5 to early postnatal life, the organ growing outwards and laying down new excretory nephrons and collecting duct branches in outer parts as its older, inner parts mature. This continuous development depends on stem cells: there are epithelial stem cells in the tips of ureteric bud tree (collecting duct progenitor), and two types of mesenchymal stem cell populations nearby (Schreiner, 1902). One, called the 'cap' mesenchyme by Reinhoff (1922), directly surrounds ureteric bud tips, expresses Six2 and Pax2, and gives rise to nephrons (Kobayashi et al., 2008). The other, outside the cap, expresses Foxd1 and gives rise to interstitial cells and pericytes (Kobayashi et al., 2014) (Fig 1b).

Pax2 is expressed in the very early renal system, and Pax2-/- mutants fail to form kidneys: unfortunately, this phenotype that casts no light on the specific function of Pax2 in the cap mesenchyme. To circumvent this problem, Naiman et al. (2017: this issue) created compound heterozygotes in which one Pax2 allele ($Pax2^{del}$) was inactive due to severe truncation, and the other ($Pax2^{flox}$) was floxed. Introducing a *Six2-GFP-Cre* construct into the same mice resulted in deletion of Pax2 from the Six2-positive cap mesenchyme. In kidneys of these mice, the cap mesenchymes

formed apparently normally but were lost within about two days (except for a few in which Pax2 deletion failed). The tips of the ureteric bud tree were then surrounded directly by interstitial cells, which failed to support ureteric growth and therefore growth of the organ as a whole ceased. Examination of kidneys days later showed a continuing absence of cap mesenchymes and of nephrons. A small ureteric bud/ collecting duct tree was present, as were the FoxD1+ interstitial progenitors and their interstitial progeny (except, perhaps, for a specific type of outer medullary interstitial cell, development of which may therefore depend on nephrons).

Clearly, Pax2 is needed for the survival (but not formation) of the caps. What happens to the cap cells when they lose Pax2? Naiman et al. answered this question by including a *Rosa26-LacZ* Cre reporter in their mice, to label all cells that had expressed Six2-Cre so that their fates could be followed. The result was a surprise: the Pax2-bereft caps did not disappear: rather, they switched fate to become renal interstitium. In their new life, they expressed mainly (but not completely) normal markers of interstitial cell progenitors or markers of maturing cortical and medullary interstitium. These markers included Foxd1, the marker of the normal interstitial progenitor population. Careful analysis of gene expression showed that loss of Pax2 from Six2+ cap mesenchyme cells resulted in their briefly expressing both Foxd1 and Six2, before the Six2 disappeared along with cap cell morphology. Repeating the experiment with a tamoxifen-dependent Cre in the Six2-Cre construct, and using low doses of tamoxifen, allowed the experimenters to create mosaics of cells with or without Pax2 deletion within cap mesenchymes: the cells that had lost Pax2 left the nephrogenic cap mesenchyme population while the others remained, showing the decision to be cell-autonomous.

The overall implication of the team's results, which contain far more careful detail than can be represented in this preview, is that Pax2 is needed for cells to remain on the nephrogenic fate choice and that, without it, they cross over to the interstitial fate choice instead (Fig 1c). This interpretation depends on the Pax2-loss state being the normal interstitial path. Expression data in the paper are ambiguous on this: there are a few oddly-expressed transcripts but it is not clear whether these are just a transitory feature of converting cells, or a permanent difference. If full removal of Pax2 generates perfect conversion given enough time, then the Pax2 story really is about keeping cells determined to follow one path not another. If Pax2-deficient cells change to a subtly new state unlike anything in the embryo, then something more complicated is going on: Pax2 is still saying 'keep to the path' but without it cells may be getting lost, rather than transitioning to an alternative normal route across Waddington's famous landscape.

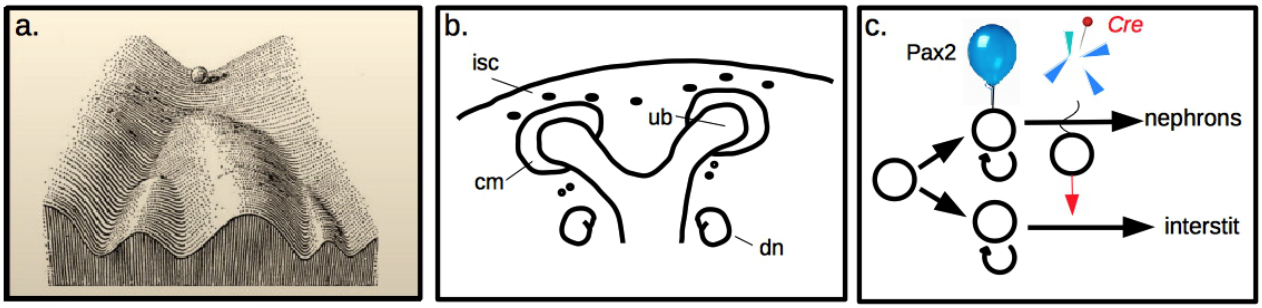

Figure 1: (a) Shows Waddington's own illustration of an epigenetic landscape, with the ball (cell) rolling down diverging valleys, the walls of which make a choice irrevocable once it has been made (Source: Waddington, 1957). (b) Illustrates the positions of the stem cell populations in developing kidney: the ureteric bud stem cells are (ub) at the tips of the tree. They are surrounded by cap mesenchyme (cm), which maintains itself and gives rise to developing nephrons (dn). Outside the caps are Foxd1+ stem cells for the interstitial population (isc). (c) Depicts the results of Naiman and colleagues; in normal development, an early progenitor gives rise to interstitium progenitors (bottom path) and nephrogenic progenitors (top path). The latter require Pax2 (the balloon) to maintain them in the top path and, if Pax2 function is lost (in the experiment via Six2-Cre), the cells fall to the bottom path and differentiate into interstitium.

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