EDITORIAL: REFLECTIONS ON PLANT CELL CLASSICS

Auxin-Mediated Cell Cycle Activation during Early Lateral Root Initiation

Lateral roots emerge from deep within their parent root – from a minimum of three founder cells on either side of the xylem axis, named xylem pole pericycle cells. In the age before RNAseq and single cell RNA sequencing, even before the days where whole genome microarrays were de rigueur, the steps in lateral root primordia initiation and development had been elegantly anatomically described using microscopy (Malamy and Benfey, 1997). A handful of mutants had been identified in lateral root initiation, many implicating the hormone auxin. However, we were still left with a near blank slate when it came to an understanding of the molecular events that are required to start from three pericycle founder cells to result in a lateral root primordium with a completely autonomous stem cell niche which completely recapitulates that of a primary root tip. Furthermore, in young seedling roots, the staple organ of many root developmental biologists, a very small number of lateral roots initiate in a temporal sequence, with the oldest lateral roots near the root-hypocotyl junction, but the spacing between these events was poorly understood.

Many plant biologists are envious of the diversity and number of cell cultures represented in animal model organisms. Synchronized cell cultures were reported in Arabidopsis (Menges and Murray, 2002), but are not universally experimentally tractable. Then came The Plant Cell publication by Himanen et al. (2002). The authors experimented with N-1-naphthylphthalamic acid (NPA), an auxin transport inhibitor, and identified a concentration that was able to completely block lateral root initiation, but not root patterning. Then, a specific concentration of 1-NaphthaleneAcetic Acid (NAA), an auxin analog, was applied and voila! A synchronized set of xylem pole pericycle cells become “activated” – on either pole of the xylem. At the time, this both increased the number of lateral root primordia which could be experimentally assayed at a single time, and enabled identification of the cell cycle stages that xylem pole pericycle cells pass through at the time of “activation”.

Xylem pole pericycle cells that undergo the first asymmetric divisions first progress to the G2 phase as visualized by CYCA2;1 promoter activity. This was coincident with CYCB1;1 promoter activity. After 6 hours of induction, genes involved in the G2 to M transition CYCB1;1, CYCB2;1, CDKB1;1 and CDKB2;2 showed simultaneous induction. In addition, KRP2 gene expression was reduced upon induction and the authors functionally validated this observation in order to demonstrate that it is sufficient to block formative divisions in the xylem pole pericycle. This system set the framework for a number of transcriptome studies profiling lateral root initiation and development (Himanen et al., 2004; Vanneste et al., 2005; De Smet et al., 2007); followed by fluorescent activated cell sorting of pericycle cells coupled with microarray analysis (De Smet et al., 2008). Conversely, a lack of overlap between these datasets and another, demonstrated an independent “clock” that determines which cells will become competent to initiate lateral root primordia. (Moreno-Risueno et al., 2010).

I was a PhD student in 2002 when I read the Himanen et al. (2002) paper, and already deeply enamored with lateral root development. This study was so exciting to me as I could immediately envision its far-reaching implications once it had been married with transcriptomic approaches. It is definitely a paper to read for those who think about establishing a system that can allow one to explore their developmental program of interest, when their organ, tissue, or cell is hard to capture. Even in the age of single cell RNA sequencing, these tricks and tools of the trade can increase the pace at which we as scientists are able to advance biological knowledge.

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