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All's Well that Ends Well

Arresting Cell Proliferation in Leaves

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sufficient to cluster nodal molecules in the absence of NF186 (Zonta et al., 2008; but see Thaxton et al., 2011). This observation suggests that an early mechanism driven primarily by axon-glial interactions can also cluster molecules at nascent nodes in the CNS. This clustering can, however, occur in the absence of NF186 (Zonta et al., 2008) and gliomedin, which is not found at CNS nodes (Eshed et al., 2005), suggesting that there are important differences between the early modes of nascent node assembly in the PNS and CNS. The elegant experiments presented by Zhang et al. in *Neuron* have advanced our understanding of node formation in the PNS, and similar approaches that combine in vitro and in vivo manipulations with dynamic imaging of the various components of premyelinated and myelinated axons will also illuminate CNS node assembly and maintenance.

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All's Well that Ends Well: Arresting Cell Proliferation in Leaves

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The transition from cell proliferation to cell expansion is critical for determining leaf size. Andriankaja et al. (2012) demonstrate that in leaves of dicotyledonous plants, a basal proliferation zone is maintained for several days before abruptly disappearing, and that chloroplast differentiation is required to trigger the onset of cell expansion.

The final size and shape of plant leaves is under genetic control (Johnson and Lenhard, 2011). The genetic basis is evident from the uniformity of leaf size and shape within a given genotype, and, by contrast, the often large variation in leaf size among different genotypes, even when plants are grown in the same environment. Two cellular processes underlie leaf growth (Johnson and Lenhard, 2011): initially leaf cells proliferate, accumulating cytoplasmic mass, doubling in size and then dividing mitotically. Later on, after exiting the mitotic cycle, leaf cells grow by expansion, concomitant with a massive increase in the size of the central vacuole and often involving endoreduplication. The timing of the transition from proliferation to expansion is critical for setting final leaf size, as it determines how many cells form the "capital" for future expansion-driven growth (Poethig and Sussex, 1985). Indeed, many mutants affecting final leaf size appear to influence the timing of proliferation arrest (Mizukami and Fischer, 2000). Importantly, this arrest does not occur simultaneously throughout the leaf, but rather starts at the tip, and gradually moves to more basal cells (Donnelly et al., 1999). This process has led to the notion of a "proliferation-arrest front" that moves from the tip toward the base of the leaf. In fact, based on mutant and histological analyses, two successive arrest fronts have been proposed, with the first one terminating proliferation in most subepidermal cells excluding the vasculature and in epidermal pavement cells (i.e., ones not differentiating into trichomes and stomata), and the second one targeting specific cells like vascular or stomatal precursors that continue to proliferate for a longer time period (Nath et al., 2003; White, 2006).

In this issue of *Developmental Cell*, Andriankaja et al. subject this notion to closer scrutiny in *Arabidopsis thaliana* by quantifying the distribution of proliferating and expanding cells in the epidermis of the growing third leaf at daily intervals (Andriankaja et al., 2012). To do so, they develop an automated image-analysis algorithm that extracts cell shape parameters and uses these, based on an appropriate training data set, to classify cells as proliferating or expanding. After an initial phase when all cells are proliferating, Andriankaja et al. observe that expansion sets in at the very tip of the leaf. Over the next few days, the zone of proliferation at the base of the leaf blade actually increases in absolute terms both

in area and in cell number; however, relative to the total leaf area it decreases, because progressively more cells toward the leaf tip begin to expand. Proliferation then arrests abruptly throughout the basal region. As suggested before by the model of two successive arrest fronts, the exit from proliferation in stomatal precursors is delayed relative to that of epidermal pavement cells, yet spatially it follows a similar distal to proximal pattern.

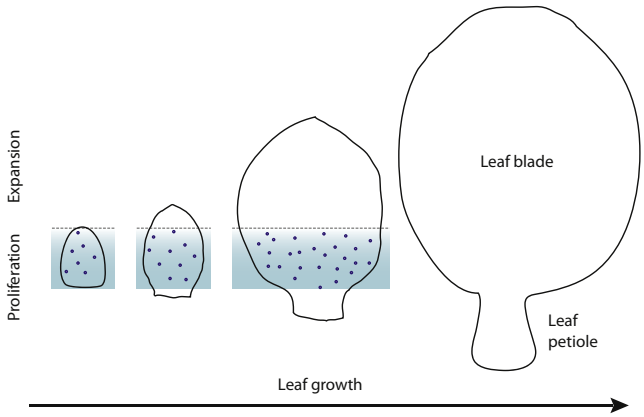

Figure 1. Cell Proliferation in Growing Leaves
Successively older leaves (left to right), showing regions containing proliferating cells (blue dots) and expanding cells. Blue shading illustrates a gradient of a hypothetical mobile growth factor emanating from the blade/petiole junction. Cells perceiving the signal continue to proliferate (below dashed line), while cells that are displaced distally beyond the reach of the signal begin to expand in response to retrograde signaling (above dashed line).

To determine the gene-expression changes associated with these transitions in cellular behavior, the authors analyze the transcriptomes of growing leaves at the same time points assayed in the kinematic analysis. They observe that the greatest transcriptome shifts occur at the onset of cell expansion and at the time of the abrupt termination of proliferation. Genes required for cytoplasmic growth, ribosome biogenesis, and cell division are downregulated over the time course studied, whereas genes involved in cell wall formation and photosynthesis are upregulated. Although leaf greening parallels the onset of cell expansion both spatially and temporally, genes involved in the biosynthesis of the chlorophyll precursor Mg-protoporphyrin IX are prominently induced just before the onset of cell expansion. Chloroplast differentiation and nuclear gene expression are known to be tightly coordinated by signaling in both directions, and Mg-protoporphyrin IX has been implicated before as a candidate for a retrograde signal from the chloroplast to the nucleus (Nott et al., 2006), prompting the authors to ask whether chloroplast differentiation and retrograde signaling might be required for the onset of cell expansion. They tested this hypothesis by treating leaves with norflurazon, an herbicide that causes photo-oxidative damage to chloroplasts and thus disrupts retrograde signaling; indeed, norflurazon-treated leaves show a delayed onset of cell expansion at the tip. This effect is not due to norflurazon promoting proliferation, given that norflurazon treatment of younger leaves, before the normal onset of expansion, produces no effect. Thus, it appears that chloroplast differentiation and potentially retrograde signaling are required for the timely onset of cell expansion at the tip. Together, the authors' findings demonstrate a rather abrupt onset of cell expansion at the leaf tip and an abrupt termination of proliferation throughout the basal proliferative region; a similar, yet temporally delayed basipetal arrest of proliferation of stomata precursors; and most importantly, a requirement for chloroplast differentiation to trigger the onset of cell expansion.

The present study extends previous findings by the Tsukaya group quantifying the "movement" of the proliferation-arrest front (Kazama et al., 2010). The Tsukaya analysis indicated that the distance of the arrest front from the base of the leaf blade (i.e., the junction between the leaf blade and the petiole) stays constant for several days before proliferation ceases abruptly. Together, these results suggest that there is a corridor of proliferative competence of a largely fixed proximo-distal length that is anchored to the blade/petiole junction (Figure 1); young leaves fall entirely within this corridor, but as the first cells at the leaf tip are displaced out of the corridor by growth in more basal regions, their chloroplasts begin to differentiate and retrograde signaling triggers the onset of cell expansion and arrest of proliferation. After being maintained at a constant length for some days, the corridor of proliferative competence then breaks down abruptly. This model suggests that the increase in area and cell number in the proliferative region observed by Andriankaja et al. results largely from lateral growth of the leaf within the corridor, rather than a shift in the distal limit of the corridor away from the blade/petiole junction.

The notion of a basally anchored proliferation zone of fixed proximo-distal length in dicotyledonous leaves is reminiscent of leaf growth in monocotyledonous species, in which expanding cells are displaced distally from a basal growth zone, suggesting that leaf growth in these two lineages may not be so different after all. However, several important questions remain. First, what determines the proximo-distal length of the corridor? And second, what limits the time of corridor maintenance, and what determines the time of its abrupt disappearance? An attractive solution to the first question would be a gradient of a mobile growth factor emanating from the blade/petiole junction (Kazama et al., 2010); only cells up to a certain distance from this source would still perceive enough of the signal to maintain proliferation, while cells displaced distally would leave the range of the signal. Although no such signal has been identified, genetic analysis has uncovered transcription factors that appear to limit the proximo-distal length of the proliferation zone (Ichihashi et al., 2010; Nath et al., 2003). Concerning the second question, again relevant transcription factors have been found (e.g., Mizukami and Fischer, 2000), yet how their activity mechanistically influences the timing of the disappearance of the proliferation zone remains enigmatic at the moment. Both cell-autonomous mechanisms (e.g., accumulation of an inhibitor with each cell division to count cells in the leaf) and non-cell-autonomous mechanisms (e.g., dilution of a mobile growth factor to measure the overall size of the leaf) are conceivable, and more work will be

required to understand how leaves can know when to stop growing at the right time.

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