Cellular dynamics, a systems biology bottleneck

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Abstract

Developing a mechanistic understanding of plant growth regulation requires studying cell division and cell expansion in addition to molecular studies. Recent time-lapse confocal microscopy studies quantifying these processes in individual cells in growing organs in combination with multi-scale modelling provide profound new insights in the regulatory mechanisms involved.

Main Text

How plants regulate growth determines shape and size of organs underlying taxonomic grouping and horticultural value, but also their efficiency to utilize solar radiation, water and minerals to generate biomass. Society demands increased and more sustainable production of food and plant products, in a context of climate change, providing plant scientists with a major challenge. Because progress made by breeding alone is unable to meet this challenge, a change in the way plant science is performed, is required and new insights and technologies related to genome sequencing may form the basis. The number of sequenced plant genomes increases exponentially (https://www.ncbi.nlm.nih.gov/genome/browse#!/eukaryotes/Plants) , which has broadened the perspective of biologists from a hand full of components involved in specific processes to genome-wide. New technologies that facilitate genome-wide analyses, collectively indicated as "-omics techniques", involve most organizational levels in the plant, including genome, methylome, transcriptome, (phospho-)proteome, peptidome, metabolome and interactome. The need to valorize -omics knowledge in crops has prompted the need to quantify the phenotype of large numbers of mutant, natural or cultivated lines. Therefore, "phenomics", automated, high-throughput phenotyping is rapidly developing (https://www.plant-phenotyping.org). As multiple -omics approaches yield a better prediction of yield than genetics alone [1], we need to understand the connections between genome and growth across organizational levels.

Plant growth is determined by genotype and environment impinging on a complex of interacting networks at the molecular levels. Importantly, these networks ultimately converge on only two processes at the cellular level: cell division and expansion (Figure 1). Despite decades of studies, how these control growth and development is still poorly understood. It is therefore curious that the development of novel cellular analyses is not keeping up with the advances in -omics approaches.

Theoretically, organ growth is the integrated expansion of its cells (Figure 1). Cell division only subdivides cellular space generated by cell growth [2]. However, division provides the units for further growth and maintains their size homeostasis, thus it is crucial to understand how both processes are coordinated. Hypothetically, they could respond to growth regulatory signals by 4 distinct interactions: 1. Cell division is regulated and drives growth; 2. Cell growth is regulated and drives division; 3. Cell division and expansion are independently regulated 4. Coregulation of both processes. In plant tissues, growth of neighboring cells is subject to mechanical forces, providing an additional level of regulatory interaction between cells [3] (Figure 1).

Historically, a number of approaches have been used to study the cellular basis of plant growth regulation. The most widely used approach is determining the number and size of cells in mature organs, which has demonstrated that organ size is primarily determined by number of cells. Interestingly, perturbations that alter the number of cells in an organ are often compensated by an opposite change of their size. This "compensation" can be explained by cell division being downstream of cell growth. However, the inhibition of cell division needs to exceed a threshold before compensation is triggered and the increase in cell size is always less than the reduction in cell number [4], implying that additional feedback mechanisms must be involved that require analysis of the dynamics of cell division and expansion.
These dynamics have mostly been studied at a population level in linear organs, where cell division and expansion occur in a longitudinal gradient of cell division, expansion and maturation that drives organ growth. In organs like fruits and dicotyledonous leaves, proliferation and expansion phases are primarily separated over time, allowing cell division and expansion to be determined by the evolution of average cell number and size over time. A meta-analysis of published data obtained demonstrated that the size of leaves and roots across a broad range of species and treatments correlates with differences in cell production determined by the size of the zone of cell division, whereas differences in mature cell size were relatively limited [5]. This suggests that if spatio-temporal dynamics are taken into account, cell proliferation appears to drive organ growth. The cell length distribution in these linear systems reflects the balance between cell growth and cell division [2] and differences in the size of the zone where cells proliferate. However, division activity cannot be inferred from the cell length distribution alone. Indeed, the size of cells at mitosis can vary strongly even between adjacent cells, leading to larger cells in populations of proliferating cells [6] and dividing cells in zones of increasing cell size [7]. Therefore, the increasingly adapted approach to locate the proliferation zone boundary by the first cell that it is substantially larger than it's subtending neighbors [e.g. 8] is intrinsically flawed.

Although these quantifications of the global/average dynamics of the entire population of cells provide a useful basis to understand the cellular basis of plant growth regulation, they fall short in situations where the activity of subpopulations of cells determine organ scale growth differences [e.g. 9, 10, 11]. Indeed, recent time-lapse confocal microscopy studies to determine dynamics of division and expansion of individual epidermal cells in *Arabidopsis* leaves revealed a large variation in the rates of cell expansion, cell cycle duration and the size at which cells divide [6]. This variability in cell size at division appears to be crucial to establish reproducible organ size and shapes [12]. These findings demonstrates that to unravel the complex interaction between cell growth and expansion in multicellular organs, simultaneous observation of the dynamics of (nearly) all cells in growing organs will be required. Moreover, to understand the molecular regulation of these cellular processes, this will need to be done in the context of perturbations of putative regulatory signals and interactions. Given the multitude of signaling pathways, impinging directly or indirectly on the regulation of cell growth and cell division, this

requires a high-throughput approach. Developing quantitative approaches to determine the dynamics of expansion and division of large populations of individual cells in growing organs is therefore urgently needed to obtain a mechanistic understanding of plant growth regulation, connecting molecular -omics approaches to phenomics-based growth observations. Progress in this direction depends on the development of new microscope systems that enable the 4D-analysis of large numbers of plant organs growing in controlled environmental conditions.

Like the development of bio-informatics tools over the last decades, new computational methods also need to be developed to integrate and visualize cellular dynamics and to translate data into mechanistic understanding of the regulatory mechanisms controlling cell division and expansion in growing plant organs. Cell-based multi-scale simulation modelling platforms have proven their value for testing if putative regulatory mechanisms can explain the experimental observations [13]. Fox et al. [6] showed that a dual control mechanism, in which spatio-temporal regulators act on both cell growth and division, can reproduce the complex dynamics of epidermal and sub-epidermal cells in a growing leaf. Only a limited set of growth parameters operating at the cell level were required that, in turn were regulated by a minimal system of spatio-temporal gradients. Candidate genes putatively encoding these factors were proposed. It is a significant finding that a limited model can explain most of the complexity in spatio-temporal cellular dynamics. However, ultimately all signaling pathways known to affect leaf development under optimal and limiting conditions, as well as details of molecular regulation of cell cycle and cell expansion, need to be integrated in such models to obtain a systems-wide understanding of the growth process. Generating such knowledge will be of scientific interest and provides a basis to engineer plants with superior growth characteristics under future climate conditions.

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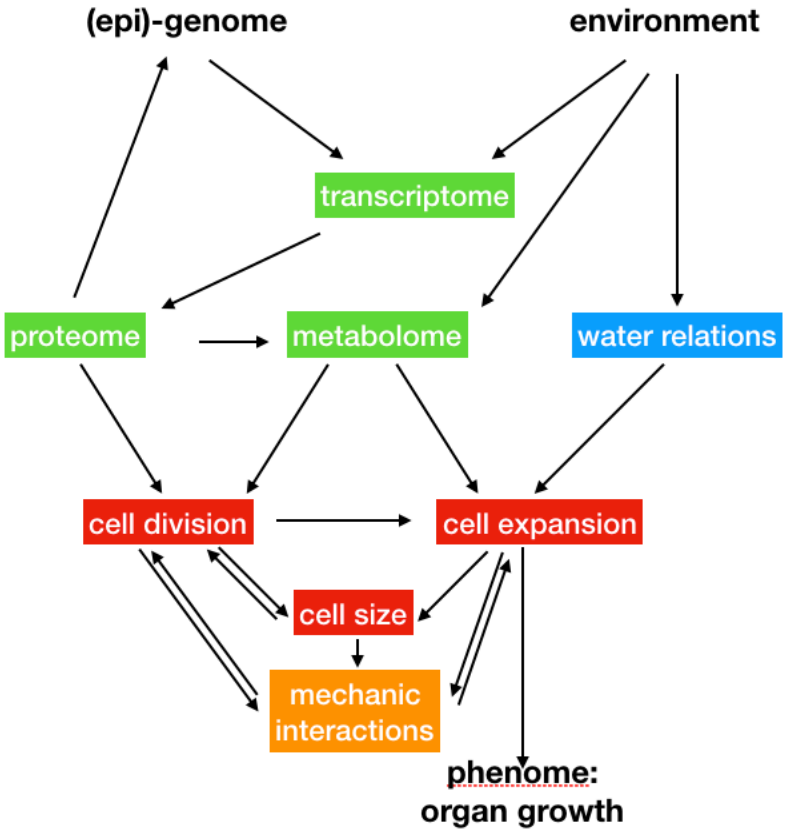

Figure 1.

The regulatory interactions connecting genetics and environmental conditions to organ level growth. Complexity is highest the molecular level (green), where current -omics technologies facilitate genome-wide studies of 10-thousands of components in parallel. In contrast, at the cellular level (blue) only two processes are involved, division and expansion, interacting via cell size. Ultimately, only expansion directly contributes to growth.