https://doi.org/10.1093/plphys/kiab584 PLANT PHYSIOLOGY 2022: 188: 675–679

Plant Physiology®

Editorial

The Plant Cell Atlas: focusing new technologies on the kingdom that nourishes the planet

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Introduction

Recent years have brought technological breakthroughs that provide a new perspective on the living cell. Single-cell RNA-sequencing (scRNA-seq) and other high-resolution omics can now routinely collect information on tens of thousands of different cells in a single experiment. Advances in live imaging offer unprecedented resolution into the dynamic function of organelles and cellular machines. Proximity labeling opens new avenues to interrogate protein interactions in live cells and inventory the proteome of cellular compartments in specific cell types. A common thread of these techniques and others we feature here is not only large-scale data collection but finer resolution of the plant cell and its compartments. In their scope and resolution, these new techniques offer a systems level approach to disentangle the cacophony of signals and processes of individual cell types into their functional units—the cell, the organelle, and the interactome of a single protein. This focus issue embarks on an early exploration of the application of these technologies to plant cell biology with 7 updates and 10 research articles. While many of these techniques were developed in animal systems, we turn attention to their application to plant-specific questions. How does the myriad of cell types in plants specialize their cell wall to give it specific properties? How do cells in growing tissues coordinate their division and shape to create functional organs? What are the complex ways in which plant cells respond to the environmental rhythms that rule their physiology, such as time of day, or constraints, such as mild drought? These are just some of the questions that arise in this issue that have potential impact on food security and sustainable energy production. They highlight a Plant Cell Atlas as both a compendium of data on plant cells and a coordinated effort to develop new technologies and pose new models of the cell that help us answer fundamental questions about plant biology.

Single-cell resolution of gene activity in developmental and environmental contexts

High-throughput scRNA-seq of plant cell protoplasts enables the deconstruction of tissue multicellularity so that genes associated with specific developmental processes or metabolic activities can be recognized. The Update review by Cuperus (2021) describes the exploitation of scRNA-seq and single nucleus (sn)-RNA-seq to identify subpopulations of cells with similar molecular signatures. The review anticipates that multi-species comparisons for homologous gene identification will be crucial to advance cell-type annotation as single cell/nuclei analyses expand to diverse species. Indeed, this focus issue includes an example of the use of gene orthogroup prediction to categorize cell clusters resolved by snRNA-seq of entire plants of the basal monocot Lesser Duckweed (*Lemna minuta*) (Abramson et al., 2022). To address challenges associated with single cell omics including the total number of cells/nuclei analyzed and the number of reads per cell/nucleus, Cuperus (2021) highlights advances in combinatorial multiplexing of transcriptome and epigenome readouts that may be essential for the statistical rigor necessary to appreciate *bona fide* stochasticity and other nuances of single-cell gene activity. Finally, the author posits that long read sequencing technologies may provide greater resolution of chromatin features, transcription factor occupancy, and DNA modifications in regions of over 10 kb. Long reads

Received December 01, 2021. Accepted December 03, 2021. Advance access publication December 22, 2021

can also provide spatial context to transcript isoform variation and plausibly meaningful RNA modifications (Parker et al., 2020).

scRNAseq studies of plants have focused on transcriptomes in a developmental context rather than on environmental effects, with rare exception (e.g., Jean-Baptiste et al., 2019; Wendrich et al., 2020). Two papers in this issue consider plants and their environmental interactions. Berrío et al. (2021) examine young differentiating leaves of Arabidopsis grown under well-watered and mild water deficit conditions. The mild drought used did not evoke an abscisic acid-mediated stress response, but was associated with patterns of conditional mRNA abundance. For example, epidermal cells had the greatest number of upregulated mRNAs, including a cellular wax biosynthetic gene that may provide protection from desiccation. Another study contrasted the root and shoot transcriptomes of Arabidopsis at the end of the day and night by scRNAseq to ask how the 24-h cycle influences cell subpopulations (Apelt et al., 2022). Mesophyll cells had more complex transcriptomes at the end of the day, reflective of active photosynthesis, and were enriched for mRNAs associated with starch degradation, a process that occurs during the night. By contrast, mRNAs encoding starch metabolism biosynthetic enzymes were enriched at the end of the night, likely in anticipation of photosynthesis after dawn.

From pretty images to quantitative, multimodal, three-dimensional imaging of plant cells, organelles, and molecules

Imaging technologies in biology are growing and diversifying at an exponential rate, from being able to image individual cells in 3D organs in non-invasive manners to capturing details of molecules at nearly atomic resolution. New approaches that integrate different imaging modalities (multimodal) are pushing the field of cell and developmental biology forward, For example, an image from one specimen can be collected across scales (from several microns or millimeters to nanometers) and/or correlated with multiple imaging methods (correlative microscopy).

Understanding single-cell dynamics in the context of plant organ development is a major challenge in plant biology. This is especially true for organs that are not fully exposed and therefore, not easily imaged in microscopy. For example, most flowers are composed of concentric arrays of organs that are believed to derive from modified leaves; the reproductive organs (stamens and carpel) are completely covered by outer organs (sepals and petals) during development, which makes their analysis very difficult. Overcoming these difficulties, Silveira et al. (2021) present a new confocal time-lapse imaging platform to analyze cell growth dynamics in the context of stamen development in *Arabidopsis thaliana*. This approach provides novel quantitative information on cell elongation and division rates, tracking of different cell lineages, and timing of cell specification during the development of anther and filaments, opening exciting opportunities for similar non-invasive analysis of cell growth dynamics in other organs.

Imaging cell division and expansion in developing organs for a long period of time generates massive amounts of image data that are challenging to analyze in a meaningful manner. Several programs have been developed for automatically defining (segmenting) and tracking cells (Berg et al., 2019; Trullo et al., 2020), but many groups still prefer the human eye. Hernández-Herrera et al. (2021) developed a plugin for the popular image analysis package ImageJ to follow cell lineages in space and time via manual tracking but with a set of convenient tools that make the arduous process easier.

While growth patterns need to be tracked at a tissue level, the mechanisms that control growth take place at finer scales of resolution. To understand plant growth and dynamic changes in cells, more and better multiscale imaging methods are needed for integration of developmental cell patterns and subcellular dynamics in intact organs. Ovečka et al. (2022) discuss recent advances, current shortcomings, and future opportunities for multiscale plant imaging by light-sheet fluorescence microscopy and super-resolution microscopy.

Multimodal imaging can also help cover the "blind spots" of any individual imaging approach. For example, whereas most light-based microscopy approaches can image the dynamics of individual cells located not too far from the surface of a developing organ, other imaging approaches can capture three-dimensional information deep into large, intact organs. Along these lines, Duncan et al. (2021) discuss advances in the use of multimodal, three-dimensional, multiscale imaging to analyze the internal organization of plants. They show that the combination of improved sample preparation, three-dimensional imaging by X-ray microscopy, advanced cell-level image segmentation, and correlative scanning electron microscopy results in a powerful approach to image the internal cellular organization of a variety of plant structures, such as developing grass inflorescences and roots as well as developing floral primordia and nitrogen-fixing nodules from soybean.

Super-resolution microscopy approaches can reach accuracy in the 20–100 nm range in the lateral orientation (Huang et al., 2010; Komis et al., 2015; Sahl et al., 2017; Schubert, 2017; Shaw et al., 2019). However, as with most fluorescence-based imaging approaches, super-resolution microscopy is most commonly used to image targeted subcellular components or cellular processes using genetically encoded reporters or chemical probes, whereas untagged molecules, organelles, and processes go undetected. Weiner et al. (2022) discusses how electron microscopy offers unique opportunities for three-dimensional imaging of a wide range of untagged cells, organelles, and molecules. Improvements in sample preparation, imaging instrumentation, and image analysis are opening opportunities for imaging complete organs by serial block-face scanning electron microscopy all the way to resolving molecular machineries in their cellular context by cryo-electron tomography.

Sculpting the transcriptome: localizing transcripts in two- and three-dimensional space

Another modality that could be added to tissue imaging is the superimposition of transcriptome information. Spatial transcriptomics methods simultaneously map many transcripts directly to tissue sections, serving to both validate single-cell RNA-seq profiles and gain a fine-scale view of transcript localization on real tissue (Asp et al., 2020). Thus, they bridge the gap between cellular omics and in situ localization. Laureyns et al. (2021) report on one such technique, in situ sequencing (ISS), which uses ligation and cDNA synthesis to sequence mRNAs directly on a tissue section. The authors demonstrate the technique in maize tissues. With a different multimodal approach, Cox et al. (2021) propose to use spatial transcriptomics in combination with three-dimensional imaging techniques, such as light-sheet microscopy and X-ray microscopy, as discussed above, with spatial transcriptomics. This would lead to a "pseudo" three-dimensional transcriptome for tissues, moving spatial transcriptomics from flat art to a three-dimensional, sculpture-like view of gene expression. Their Update review also highlights various approaches to spatial transcriptomics, including microarray-like probe spots overlaid on slides and approaches that use semi-specific, sequential probe hybridization to barcode transcripts.

Functional omics: proteins, signaling, and the reconfiguration of auxin signaling

The Plant Cell Atlas also seeks to develop techniques that assay cellular processes, particularly those unique to plant cells (Plant Cell Atlas Consortium et al., 2021). Thus, systematic information on protein localization, protein–protein interactions, and the activity of signaling mechanisms at the cell level will be a critical component of the functional Plant Cell Atlas. Clark et al. (2021) review the prospects for single-cell proteomics in plants considering gains that have been made recently in animal systems. They discuss some of the specific challenges in adapting the techniques to plants, such as efficient cell isolation techniques.

Few proteins do their job alone, so the interactions between proteins in a cell or cellular compartment will be critical to deciphering function. Toward that aim, Bergmann and Mair (2022) provide a comprehensive overview of advances in proximity labeling, highlighting its potential to reveal cell-type specific protein–protein interactions as well as the proteomes of cellular compartments within specific cells (Bergmann and Mair, 2022). In particular, the ability of the technique to capture transient interactions holds promise for insights into trafficking and mechanisms that call on proteasomal degradation—two important features of plant cell signaling. Delving into one signaling mechanism, Ghusinga et al. (2021) take a modeling approach to G-protein signaling to demonstrate the feasibility of a hypothesis that, in plants, phosphorylation rather than nucleotide exchange is the key modulator of G-protein signaling. The difference in G-protein regulation would have important implications for signaling in many different cell types.

Plant signaling will be critically important in developing crops that optimize their response to environmental stresses for reliable food production. This often entails overriding complex regulatory mechanisms adapted to surviving in their original enviroments but not necessarily current agricultural conditions. Synthetic biology approaches have the potential to harness plant signal transduction to "reconfigure" the plant development so it can be tuned to specific environments, such as drought or higher heat. Brophy (2022) lays out the potential to use CRISPR and other genome engineering approaches to alter auxin signal transduction pathways in specific cells in plants (Brophy, 2022). The review argues that auxin—the plant hormone involved in so many developmental processes and environmental responses—is a prime target for such optimized engineering.

Constructing the complex and customizable cell wall

Finally, the cell wall is a quintessential feature that distinguishes plants from animals. At the birth of virtually every new plant cell, a phragmoplast forms in late cytokinesis and organizes the construction of a cell plate that will eventually form a wall between the two new daughter cells (Verma, 2001). Jawaid et al. (2021) use biophysical modeling to ask about the mechanics that control a critical moment in cell plate formation—when the tubular network formed by the fusion of post-Golgi-derived vesicles flattens out to give rise to a structure that resembles a slice of swiss cheese. Their model establishes the necessity for a spreading force, which the authors speculate is likely callose deposition—being at the right place at the right time.

During differentiation, plant cells can remodel their wall with an array of compounds that add durability, flexibility, water impermeability (Verma, 2001; Vogler et al., 2015). Particularly relevant to climate change mitigation, lignin adds strength to the cell wall and has the potential to sequester carbon more securely but also presents an obstacle to efficient biofuel generation (Carpita and McCann, 2020). Morel et al. (2021) present a new tool that features automated segmentation and quantification of lignin in intact tissues based on previously established staining techniques. They show how this approach has the potential to implicate new genes in lignin production, detecting changes in cell wall lignification in mutants that did not show lignin phenotypes in previous analyses.

Closing remarks

Many of society's most pressing problems, such as food security and climate change, amount to challenges in plant biology that must be tackled by a variety of approaches (Bailey-Serres et al., 2019). In turn, many of the fundamental questions in plant biology center on the function of

specialized cells and their compartments. This focus issue is a first foray into unifying a community-wide research effort—a Plant Cell Atlas—that aims to apply new technology to dissect the plant cell. We believe that adapting these technologies, as many of the authors above have done, and innovating new ones targeted specifically for plant cells, will lead to an acceleration of progress into basic questions in plant biology. Thus, new technologies will be critical in gaining novel insights into plant biology that can address pressing problems that threaten the health of our environment and our food supply.

Acknowledgments

We thank the Plant Cell Atlas Consortium for input and ideas on the PCA initiative, particularly David Ehrhardt, Nicholas Provart, and Diane Dickel for helpful discussions on the content of this issue. We also thank all the authors of this issue for their contributions.

Funding

K.D.B. is funded by the National Science Foundation grant 1934388 and the National Institutes of Health grant R35GM136362. M.S.O. is funded by National Science Foundation grants IOS-1840687 and MCB 2114603 J.B.S. is funded by the National Science Foundation grant IOS-2119820.

Conflict of interest statement. None declared.

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