What can plants do for cell biology?

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ABSTRACT **Historically, cell biologists studied organisms that represented a reasonable sampling of life's diversity, whereas recently research has narrowed into a few model systems. As a result, the cells of plants have been relatively neglected. Here I choose three examples to illustrate how plants have been informative and could be even more so. Owing to their ease of imaging and genetic tractability, multicellular plant model systems provide a unique opportunity to address long-standing questions in cell biology.**

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INTRODUCTION

In the past century, research in cell biology uncovered an amazing and complex intracellular world. Fundamental discoveries were made in organisms throughout the tree of life, shedding light on and providing insights into cellular mechanisms. Early advances in cell biology stemmed from the ability to image tissues using both light and electron microscopy. Here plant cells, which tend to be large, had substantial impact. In the 1600s, trying to understand why cork floats, Robert Hooke examined a thin slice through the microscope and noticed empty chambers, which he termed *cells*, and thus can be said to have founded cell biology. Among other major discoveries made in plants are the nucleus (Brown, 1866) and microtubules (Ledbetter and Porter, 1963).

Foundational discoveries aside, one might suppose that plants differ too much from animals to be useful cell biological models. After all, plants have a simplified cytoskeleton, lacking intermediate filaments. Except for ferns and mosses, plants also lack flagella and microtubule organizing centers. Furthermore, plant cells are encased in a cell wall, an extracellular matrix that influences nearly every feature of the plant. This diversity, however, hides an underlying unity. Proteins and structures in plants and animals are not merely analogous but often homologous, and comparing them offers the chance to learn about the constraints guiding the evolution of both groups of organisms. This claim is verified by the undisputed value of research on yeast.

To illustrate the value, both achieved and potential, of plants, I have chosen three examples of cell biological problems in which significant advances have been made using plants. By choosing these examples, I do not imply that they are the best examples; rather, they are ones that I find interesting and that illustrate how research in plant cells could affect our understanding of cell biology across many taxa.

HOW ARE NONCENTROSOMAL MICROTUBULE ARRAYS ORGANIZED?

The lack of microtubule organizing centers is not unique to the plant lineage. In fact, cells throughout the eukaryotic tree, including differentiated animal cells such as muscle and neuronal cells, have noncentrosomal microtubule arrays. Yet how these arrays are established and maintained is largely unanswered.

Plants are an excellent system in which to study noncentrosomal microtubule arrays (Ehrhardt, 2008; Eren *et al.*, 2012). Interphase plant cells have a dynamic cortical array of microtubules that is built de novo after completion of cell division. Organization of the array is cell type specific. For example, rapidly expanding cells in the stem or root of the plant have highly ordered cortical arrays with microtubules aligned transverse to the long axis of the cell. As cells mature and begin to deposit secondary cell wall, the cortical array reorganizes. For example, in cells that are differentiating into vascular elements, microtubules form superbundles in areas of wall thickening (Ehrhardt, 2008). The ease of imaging microtubules in live plant cells and the wealth of genetic tools in both vascular and nonvascular plants has opened the door for a mechanistic understanding of how these arrays are generated and maintained (Ehrhardt, 2008; Eren *et al.*, 2012).

In particular, parallel microtubule self-organization in the plant cell cortex is one of the best model systems for investigating self-organization of cytoskeletal structures using a combination of experimental and modeling approaches. Recent studies demonstrated that after cell division or recovery from drug-induced microtubule disassembly, microtubule nucleation occurs randomly along the cell cortex. Microtubules become organized into a parallel array after some time, suggesting that array patterning is not dependent on the organization of nucleation sites but instead is an emergent

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Abbreviation used: CSI, cellulose synthase interacting.

property of the array (Ehrhardt, 2008). Microtubule nucleation occurs both along preexisting microtubules and at sites lacking any microtubules. After nucleation, the microtubule is severed from the nucleation site, and the polymer exhibits treadmilling (Ehrhardt, 2008). Organization into a parallel array likely results from favoring microtubule encounters that occur at shallow angles (<40°). When these encounters occur, the encountering microtubule reorients along the cortical microtubule, producing a bundle. In contrast, encounters that occur at large angles (>40°) result frequently in microtubule severing or depolymerization (Ehrhardt, 2008). Based on specific rules of interaction, assembly into parallel arrays can be readily modeled (Eren *et al.*, 2012).

Although these modeling efforts effectively describe generation of parallel arrays, several important questions remain. For instance, it is unclear how orientation is established after mitosis when there are no extant cortical microtubules. Further, cortical arrays can easily be reoriented; for example, blue light reorients the array from a predominantly transverse to a longitudinal orientation (Yuan *et al.*, 1994), and it is not clear how a desired orientation is specified. These questions are approachable, however, using live-cell imaging coupled with genetic mutants in microtubule-associated proteins and mathematical modeling. The mechanisms uncovered can be hypothesized to be similar to those acting on the noncentrosomal microtubule arrays of muscles and nerves.

HOW DOES THE CYTOSKELETON HELP TO PATTERN THE EXTRACELLULAR MATRIX?

Eukaryotes build a wide variety of extracellular structures, ranging from bone and shell in animals to the silica-based frustule in diatoms. Many of these structures are patterned over macroscopic scales. How organisms control this large-scale patterning of their extracellular matrices is an open question. Plant cells are encased in a complex extracellular matrix—the cell wall. With a variety of distinct cell types and known patterns of cell wall deposition, plant cells provide an excellent model system with which to dissect intracellular control in patterning of the extracellular matrix (Baskin and Gu, 2012).

The plant cell wall is fundamental to the structural integrity of the plant body and is predominantly composed of polysaccharides. Cellulose microfibrils are partly crystalline polymers of glucose held together by hydrogen bonding, which imparts high tensile strength. These glucose polymers are embedded in a pectin gel and further cross-linked by hemicelluloses and extracellular glycoproteins. As the longest and stiffest structures in the cell wall, the orientation of the cellulose microfibrils determines the direction of cell expansion.

Whereas the cell wall is a complex extracellular structure, its synthesis occurs from within the cell. Pectins, hemicelluloses, and extracellular proteins are delivered via exocytosis. In contrast, synthesis of cellulose microfibrils occurs by enzyme complexes residing directly on the plasma membrane. To effectively pattern the cell wall, delivery of cell wall components must be carefully orchestrated. Not surprisingly, the cortical microtubule cytoskeleton plays a fundamental role guiding cellulose synthase complexes at the plasma membrane (Baskin and Gu, 2012). This results in an ordered cellulose microfibril array, producing a cell wall that is pliable in one direction and controls the shape of the plant cell, which in many cases sums to and underlies the shape of developing tissues.

Based on the observation that cellulose microfibrils and cortical microtubules shared a common organization, several decades ago it was hypothesized that microtubules provide a guidance mechanism for the deposition of cellulose microfibrils in the cell wall (Ledbetter and Porter, 1963; Hepler and Newcomb, 1964). Only very recently, however, has a molecular picture of this guidance mechanism emerged. The protein cellulose synthase interacting protein (CSI) 1 interacts with cellulose synthase molecules at the plasma membrane and cortical microtubules (Gu *et al.*, 2010; Bringmann *et al.*, 2012; Li *et al.*, 2012; Mei *et al.*, 2012). Of importance, in mutants lacking CSI1 function, cellulose synthase complexes no longer track along microtubules (Bringmann *et al.*, 2012; Li *et al.*, 2012). In fact their trajectories are highly similar to cellulose synthase trajectories in the absence of microtubules. Further molecular insights into patterning cellulose deposition are sure to be forthcoming, particularly with respect to control of CSI1 function.

Plant secondary cell walls can be highly elaborate, with complex patterns. In particular, lignin deposition is often spatially restricted. Because lignin precursors are secreted, it has been suggested that exocytosis of lignin precursors or the lignin-remodeling enzymes is spatially regulated. It remains an open question, however, how exocytosis is spatially controlled. In some plant cells that exhibit highly polarized growth, spatial control of pectin secretion underlies polarized growth. The actin cytoskeleton is essential for polarized growth in plants, but the link with pectin secretion is unclear. It is likely that spatial regulation of exocytosis could be a general mechanism for patterning of the extracellular matrix.

IS THERE A COMMON MECHANISM FOR LINKING THE MITOTIC APPARATUS WITH PLACEMENT OF THE CELL DIVISION PLANE?

Placement of the plane of cell division underlies essential processes in all eukaryotes. Although the cell must divide such that the genetic material is properly segregated, this does not always occur in the geometric center of the cell. During development, asymmetric cell divisions often lead to daughter cells with different fates. How cells link positioning of the mitotic apparatus with cell division plane specification is an area of active investigation. In animal cells, there appear to be two redundant signals sent to the cell cortex to mark the cortical division site, one from the central spindle and another from the spindle asters (Fededa and Gerlich, 2012). However, there is a large degree of variability among cell types and organisms as to which is the primary signal (Fededa and Gerlich, 2012).

On the surface, cell division plane specification in plants appears to be quite distinct from the processes in animal cells or yeast. The majority of plant cells establish a cortical division site in prophase using an actin- and microtubule-based structure known as the preprophase band. Actin and microtubules in the preprophase band are disassembled during mitosis, but several proteins remain dynamically associated with the cortical division site, thereby marking where the new cell plate should be positioned (Rasmussen *et al.*, 2011). There is no analogous preprophase band in animal cells. Instead the central spindle and later the midbody of animal cells contain key molecules that signal between the mitotic spindle and the cortical division site (Fededa and Gerlich, 2012). In plants, the phragmoplast, which is analogous to the midbody (Otegui *et al.*, 2005), forms from the central spindle and builds the new cell plate to the cortical division site. Thus maintenance of the cell division site might have striking parallels between animals and plants.

In plants, due to the presence of the cell wall, placement of the cell division plane is critically important for subsequent cell shape and fate. Thus plants provide an excellent system in which to study division plane specification and maintenance. In the past decade, several key factors required for maintenance of the division plane have been identified (Rasmussen *et al.*, 2011). By studying how these proteins function and are regulated during cell division, a mechanistic understanding will emerge and may provide further parallels between plant and animal cells.

CONCLUSION

The cell wall is an example of a defining and distinguishing feature that might suggest that the cell biology of plants is fundamentally different from that of animals. Like other features, however, the cell wall poses many unique opportunities and facilitates cell biological studies because it enforces a simple and reproducible cell geometry. Because plants are multicellular and include well-established, highly manipulable model systems, they represent exciting experimental systems that, when exploited, will likely provide fundamental new insights into cell biology applicable throughout the eukaryotic tree of life.

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