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Finding order in a bustling construction zone: quantitative imaging and analysis of cell wall assembly in plants

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Assembly of polysaccharide-based walls by plant cells involves the rapid synthesis, trafficking, and deposition of complex biopolymers, but how these events are controlled and coordinated to achieve a strong, resilient extracellular matrix has remained obscure for decades. Recent quantitative analyses of fluorescence microscopy data have revealed details of the trafficking and synthetic activity of cellulose synthases, and new methods for labeling matrix polymers have unveiled aspects of their regulated deposition in the wall. Detailed studies of the identity, architecture, activity, and trafficking of the proteins and protein complexes that synthesize wall polymers, combined with advances in image acquisition and analysis, will aid future efforts to dissect wall assembly.

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Introduction

Time-lapse videos of construction sites show frenetic activity, with workers, machines, and materials zipping everywhere. However, an ordered series of events underlies this apparent pandemonium. Similarly, the complex and dynamic walls of plant cells, which are initially built from scratch in only an hour during cytokinesis, seem to be assembled by a multitude of invisible and hasty workers, but in fact arise from an orchestrated series of tightly regulated cellular processes. Here, I explore how recent advances in quantitative imaging have enabled new discoveries concerning the enzymatic, trafficking, and apoplastic events that drive cell wall assembly, and discuss future research directions that promise to expand our knowledge of how plant cells construct their wondrous extracellular shells.

The walls of plant cells provide structural support, intercellular adhesion, and protection from environmental insults. The primary walls of growing cells must be flexible to facilitate growth [1], whereas secondary walls, made in certain cell types after growth cessation, provide rigidity and waterproofing [2]. Plant cell walls are composed of biopolymers, including cellulose, hemicelluloses, pectins, oligosaccharides, lignin, structural proteins, and enzymes, which function in a milieu of water and ions, including Ca^{2+} and H^{+}. They are typically organized into layers that are laid down sequentially during synthesis. Cellulose is made at the cell surface by protein complexes containing CELLULOSE SYNTHASEs (CESAs) [3], whereas matrix polysaccharides, such as pectins and hemicelluloses, are typically polymerized in the Golgi and delivered to the apoplast via exocytosis [4]. In *Arabidopsis thaliana*, CESA complexes (CSCs) contain three unique CESA isoforms (1, 3, and 6-like) for primary wall synthesis and three different isoforms (4, 7, and 8) for secondary wall synthesis [5]. Intracellular trafficking and signal transduction, including cytoskeletal networks and wall integrity signaling, are essential for proper wall assembly [4,6]. In the past few years, new experimental tools and quantitative image analysis have elucidated uncharted aspects of cell wall assembly in plants. Many mysteries remain, but some of these are solvable using the tools of quantitative cell biology. Due to space constraints, I do not discuss the post-delivery dynamics of wall polysaccharides, a topic that has been reviewed recently [1,7], and apologize to colleagues whose important work is not cited here.

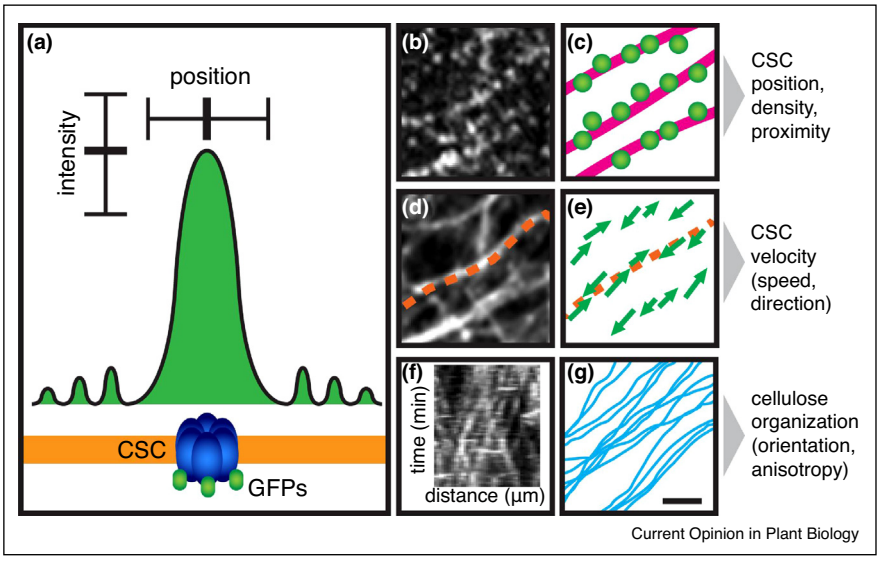
Quantitative imaging of cellulose synthases

Over the past 12 years, CESAs have been intensively studied using live cell microscopy, enabled by fluorescent protein (FP)-tagged CESAs that rescue *cesa* mutations [8] and can also be transformed into wild type backgrounds without obvious detrimental effects [9]. Recently, FP-CESA imaging has expanded beyond *Arabidopsis*, with motile FP-CESA particles being observed and quantified in the grass *Brachypodium distachyon* [10] and the moss *Physcomitrella patens* [11]. Spinning disk confocal microscopy, which achieves fast, sensitive, and simultaneous full-field image acquisition, facilitates the quantification

of FP-CESA particle behavior. Micrographs of FP-CESA particles can be used to calculate the position and intensity of each particle, despite each CSC, at 25–35 nm in diameter, being a sub-diffraction object (Figure 1a). FP-CESA particle motility (Figure 1b–d) was first measured by kymograph analysis [8] (Figure 1), in which a line is traced along a track of FP-CESA trajectories, and pixels along this line from sequential timepoints are stacked to make a time versus distance image [8,11]. By measuring line slopes from the kymograph, particle speeds can be calculated, averaging ~200–300 nm/min for FP-CESA particles [8], although there is considerable variation in measured speeds, both within an individual dataset [8] and across studies [10,12]. If FP-CESA particle speed is inferred to equal the rate of cellulose synthesis, this variation might reflect speed variationin the extrusion of cellulose microfibrils which is hypothesized to provide the driving force for FP-CESA particle motility [8]. More recently, particle-tracking algorithms, such as those in Bitplane's Imaris package, have been used to simultaneously identify and track thousands of FP-CESA particles [9,13,14,15••]. The speeds of tracked particles match well with kymograph calculations. In both cases, particles moving faster than 600–650 nm/min are separated from the dataset, since these might represent intracellular FP-CESA-containing vesicles [16••]. Calculating directionality for tracked FP-CESA particle trajectories yields values for trajectory co-alignment and orientation relative to the growth axis in a given cell [13,14,15••], plus a directional bias measure called 'optical flow direction' [17], providing a snapshot of the patterned deposition of cellulose in the newest layer of the wall [9,18•] (Figure 1g). Algorithms that calculate the nanoscale 3D positions of fluorescent particles [19] might allow for quantification of subtle CSC behaviors, such as changes in speed or wobbling along the primary trajectory, that allow for insights into the catalytic mechanisms and microtubule associations of CESAs. On this front, super-resolution imaging, such as Structured Illumination Microscopy (SIM), has the potential to provide more precise positional information [20], although the acquisition time for SIM images is slower than that for spinning disk confocal microscopy.

Particle density can also be calculated from micrographs of FP-CESAs, using software or manual counting [9,15••]. However, FP-CESA particle density, which ranges from ~0.3 to 1.3 particles/μm^2 for primary wall CESAs [9,21,22] and is much higher for secondary wall CESAs

Figure 1



Quantitative parameters for FP-CESA particles. **(a)** 2D projection of a point spread function for a single CESA complex (CSC) tagged with multiple copies of Green Fluorescent Protein (GFP), with measurement uncertainties for its true position and intensity (bold lines) indicated by error bars. **(b)** Spinning disk confocal micrograph of GFP-CESA3 particles in part of a pavement cell of a 5-d-old cotyledon. **(c)** Schematic of FP-CESA particles (green) overlying cortical microtubules (magenta, not shown in micrograph). Density equals the number of particles per μm^2, and proximity can be calculated from the distances between the centroids of the particles. **(d)** Average projection of timelapse from which micrograph in (b) was extracted, showing tracks of particle trajectories. Orange dotted line represents a track from which a kymograph (f) can be derived. **(e)** Schematic of velocities (arrows) of FP-CESA particles in (c) imaged over time, showing speed (arrow length) and directionality (arrow orientation) for each particle. Different particles move at different speeds over the course of an imaging experiment. **(f)** Kymograph derived from the line trace in (d), where the absolute values of the slopes of the traces (distance/time) indicate particle speeds, and the signs of the slopes indicate particle direction along the line. **(g)** Cartoon of cellulose microfibrils (blue lines) synthesized by FP-CESA particles in (c), if each particle corresponds to a single CSC. Note that some microfibrils are closely aligned, and bundle in some places. The orientation relative to the growth axis and anisotropy (difference in degree of alignment along different axes) of cellulose can be calculated from images collected using Atomic Force Microscopy or Field Emission Scanning Electron Microscopy. Scale bar = 1 μm in (b–e, g).