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Cell wall mechanics: Some new twists

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Every plant cell is surrounded by a cell wall, a complex, dynamic system composed mainly of complex carbohydrates and proteins, that governs cell shape and size. These walls are known to be nonuniform in composition and structure across spatial scales, and the relationship between wall components and wall mechanics is not well understood. During cell expansion, the material properties of the wall, particularly the orientation of the main load-bearing wall component, cellulose microfibrils (CMFs), are thought to be a strong predictor of how much and in what direction cells expand. In "Cell twisting during desiccation reveals axial asymmetry in wall organization," Keynia et al. (1) add a twist to this model, using trichome branches as a model system to reveal some fascinating biophysics.

Trichomes, uniquely shaped, often branched, single cells on the surface of leaves are important for the plant to engage with its environment. Mature trichome branches frequently display a predominant left-handed twist after desiccation, an unexpected finding since the trichome branch geometry under turgor pressure is axisymmetric. To determine wall material properties and organization that might drive this chirality in trichome branch wall mechanics, Keynia et al. (1) developed a finite element model to replicate the observed branch behavior and concluded that direction and degree of the twist depended primarily on the orientation of CMFs. To then experimentally approximate CMF organization, they quantified orientation of cortical microtubules, a network that guides the trajectory of the cellulose biosynthetic machinery during biosynthesis, and discovered a similar predominant left-handedness in mature trichome branches. They finally propose a mechanism for how CMF orientation may shift during branch elongation, based on axial and bending stiffness along the branch, to explain the transition from an axisymmetric to chiral branch geometry with changing turgor pressure.

Inspired by the article by Keynia et al. (1), we asked the question, "What types of experimental and computational approaches are needed to bring our understanding of these systems to the next level?" This article provides two possible answers to this question: 1) the development of new experimental tools to enable visualization of carbohydrates within the cell wall, and 2) a wider adoption of population-based modeling techniques.

NEXT-LEVEL CELL WALL VISUALIZATION: CARBOHYDRATE-DIRECTED TOOLS

Keynia et al. (1) provide immensely helpful predictions of how wall components may be organized within this single-cell system. Examining cell wall carbohydrates experimentally, however, is not trivial, especially in living, growing cells; unlike proteins, carbohydrates are not genetically encoded, but synthesized by a fleet of enzymes, and thus cannot be simply tagged with fluorescent proteins for live-cell imaging. To understand how walls function in growth and morphogenesis, tools to observe specific wall structures and their dynamics are required.

Traditional approaches, such as Fourier transformation infrared spectroscopy, Raman spectroscopy, and most prominently carbohydrate-directed monoclonal antibodies (e.g. (2)) provide high spatial information of wall carbohydrates and wall structures (Fig. 1), but generally require destructive preparation and thus are very limited in their application to live-cell experimentation and the study of dynamic events in growth and morphogenesis.

In living cells, imaging of genetically tagged cellulose synthase (CESA) complexes has been successfully applied to gain insights into wall

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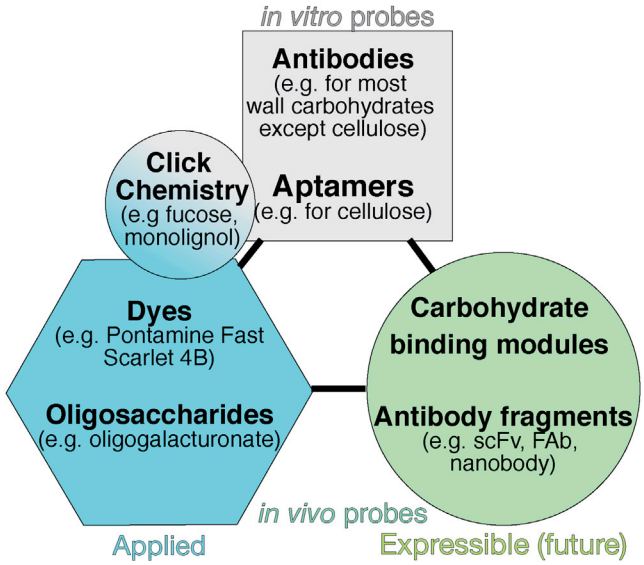

FIGURE 1 Overview of different in vitro and in vivo cell wall probes. To see this figure in color, go online.

structural dynamics. CESA complexes reside at the cell membrane where they synthesize cellulose; their trajectories allow inference for patterns of recent cellulose deposition (3). These complexes are positioned and guided by cortical microtubules that dictate patterns of cellulose deposition and orientation, a correlation that was used in Keynia et al. (1) to infer such patterns in trichomes. However, observations in tip growing cells reveal that CESA complexes are not always coupled tightly to microtubules (4), and it remains to be determined if this association may be more generally regulated in a cell-type and developmental-stage-specific manner.

Dyes for complex carbohydrates have also been applied as probes for wall structure, in both living and fixed tissue. To visualize cellulose fibrils at different stages of wall maturation, the cellulose-selective dye Pontamine Fast Scarlet 4B (S4B) has been imaged by confocal microscopy in living organisms (5). However, although simple to apply, as a rule, dyes often lack well-defined specificity, frequently limiting experimental interpretation. Recently developed tools, such as Alexa-Fluor-tagged oligosaccharides and click-chemistry (6,7), have advanced the field toward illuminating a small subset of carbohydrates in muro. Nontoxic click-chemistry reactions expand the use of this approach to image in vivo dynamics of certain wall components. Meanwhile, there is an apparent need for a more comprehensive set of highly specific probes for various wall carbohydrates.

The limitations in targeting and visualizing different wall carbohydrates delay probing interesting and important aspects of wall formation and maturation. For example, CESA complex containing vesicles frequently fuse along cortical microtubules (8). This observation, combined with other recent work (e.g., (9)), allows for the speculation that microtubules might play a broader role in establishing spatial wall organization, such as serving as a platform for secretion of other wall components, including complex carbohydrates and wall proteins. However, we cannot yet probe carbohydrate trafficking in vivo. Expressible probes specifically tagging different complex carbohydrates in vivo, perhaps based on small antibody fragments or carbohydrate-binding modules, may allow these questions to be addressed.

In summary, to obtain a deeper understanding of how wall components interact and organize to drive certain cell shapes and functions, a broader set of tools, both expressible and externally applicable, specific to various wall components as well as accompanying imaging techniques are needed to fill essential knowledge gaps and bring cell wall research to the next level.

NEXT-LEVEL MODELING: POPULATION-BASED MODELING

The accompanying paper by Keynia et al. (1) is a prime illustration of the value found in complementing experimental research with mathematical modeling. Mathematical models provide absolute control of all model parameters and are drastically cheaper than experiments (cost-per-specimen). Keynia et al. (1) used detailed models of the trichome to perform computational experiments that are not possible to perform using physical experiments. In the words of the genie from the film *Aladdin*, computational models provide "phenomenal cosmic power" at "itty-bitty" costs.

But interestingly, mathematical models are often applied in a fundamentally different manner than physical experiments. The most common experimental design for computational models is akin to a "case-study" experimental design scheme in which a few model parameters are varied, while the remainder are held entirely constant (10). Both physical and computational experiments typically consist of a relatively small set of "foreground" variables (those of primary interest) as well as a larger number of "background" variables (which are not of interest). Computational and experimental studies both tend to treat foreground variables in a very similar manner, using controlled experimental designs. For example, the sensitivity study of the accompanying paper used a trichome model consisting of 20 + parameters. In the sensitivity study, four foreground parameters were identified and varied

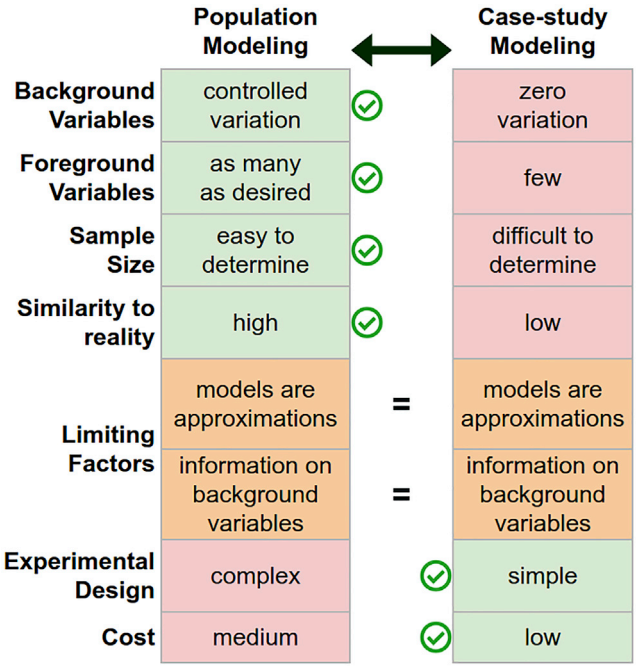

FIGURE 2 Comparisons between population modeling and case-study modeling across several important dimensions. To see this figure in color, go online.

using a full factorial design ($2 \times 2 \times 6 \times 24 = 576$ combinations). In the main body of the paper, two variables were varied to illustrate primary effects of length and fiber alignment.

In physical experiments, background variables vary naturally. However, the most common approach in computational studies is starkly different: zero variation in these variables (10). For example, the trichome model held all background variables constant throughout the study (1). When background variables are restricted in this way, we obtain tremendous focus, but the natural consequence is a narrowing of perspective and range of applicability. In other words, the "fix many, vary few" approach reduces apparent complexity, but it can also obscure interactions that may be relevant to the biological system being simulated. The case-study approach is sometimes justified by assuming that an "average" model will be representative of the population as a whole. Unfortunately, when complex models are used, average models often do not produce average behavior (11).

An appealing alternative is population-based modeling (12), also known as simulated experiments (13). In this approach, foreground variables are controlled as usual, but background variables are randomly sampled from specified distributions, thus mimicking a physical experiment (14, see also Fig. 2). Another alternative is to use Latin hypercube sampling, which guarantees broad coverage of each model parameter (15). While case-study modeling produces clean trendlines (see for example, Fig. 4 in (1)), population modeling produces the same type of realistic scatter-plot data seen in real experiments. However, there is an additional difference. In experiments, we usually lack data on background variables due to cost constraints. But in population modeling, *complete information is available for each individual specimen*, therefore providing data sets of unparalleled richness and complexity.

Population modeling does require slightly more modeling effort and a more complex experimental design, but it provides a much more comprehensive perspective on the biological phenomena of interest. Fortunately, this work is becoming easier with the advent of many computational tools that support population-based modeling (16). For example, a population modeling study can be designed to study specific variables (just as in a traditional physical experiment), but the resulting data set can be used to statistically assess the influence of background variables. Alternatively, a sensitivity analysis can be performed on *all* model parameters simultaneously. The trichome study illustrates that sensitivity analyses can be effective in obtaining estimated ranges for variables when little or no experimental data are available (1,14).

The high level of focus provided by case-study modeling will always play an important role in the scientific process. But currently, population modeling is relatively uncommon. As seen in Fig. 2, population modeling has slightly higher design and operational costs, but in all other aspects, it provides distinct advantages over case-study modeling.

In conclusion, by increasing our reliance on population modeling, and developing methods for imaging cell wall carbohydrates, we will be able to obtain richer, more comprehensive insights, thus moving research on the plant cell wall to a higher level of understanding.

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REFERENCES

1. Keynia, S., T. C. Davis, J. A. Turner…, . 2022. Cell twisting during desiccation reveals axial asymmetry in wall organization. *Biophys. J* https://doi.org/10.1016/j.bpj.2022.02.013.

2. Pattathil, S., U. Avci, …, M. G. Hahn. 2010. A comprehensive toolkit of plant cell wall glycandirected monoclonal antibodies. *Plant Physiol.* 153:514–525.
3. Paredez, A. R., C. R. Somerville, and D. W. Ehrhardt. 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science.* 312:1491–1495.
4. Cai, G., C. Faleri, …, M. Cresti. 2011. Distribution of callose synthase, cellulose synthase, and sucrose synthase in tobacco pollen tube is controlled in dissimilar ways by actin filaments and microtubules. *Plant Physiol.* 155:1169–1190.
5. Anderson, C. T., A. Carroll, …, C. Somerville. 2010. Real-time imaging of cellulose reorientation during cell wall expansion in Arabidopsis roots. *Plant Physiol.* 152:787–796.
6. Mravec, J., S. K. Kračun, …, W. G. T. Willats. 2014. Tracking developmentally regulated post-synthetic processing of homogalacturonan and chitin using reciprocal oligosaccharide probes. *Development.* 141:4841–4850.
7. Anderson, C. T., I. S. Wallace, and C. R. Somerville. 2012. Metabolic click-labeling with a fucose analog reveals pectin delivery, architecture, and dynamics in Arabidopsis cell walls. *Proc. Natl. Acad. Sci. U S A.* 109:1329–1334.
8. Gutierrez, R., J. J. Lindeboom, …, D. W. Ehrhardt. 2009. Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nat. Cell Biol.* 11:797–806.
9. Kong, Z., M. Ioki, …, B. Liu. 2015. Kinesin-4 functions in vesicular transport on cortical microtubules and regulates cell wall mechanics during cell elongation in plants. *Mol. Plant.* 8:1011–1023.
10. Cook, D., M. Julias, and E. Nauman. 2014. Biological variability in biomechanical engineering research: significance and meta-analysis of current modeling practices. *J. Biomech.* 47:1241–1250.
11. Cook, D. D., and D. J. Robertson. 2016. The generic modeling fallacy: average biomechanical models often produce non-average results! *J. Biomech.* 49:3609–3615.
12. Fernandez, J., A. Dickinson, and P. Hunter. 2020. Population based approaches to computational musculoskeletal modelling. *Biomech. Model. Mechanobiol.* 19:1165–1168.
13. Winsberg, E. 2003. Simulated experiments: methodology for a virtual world. *Philos. Sci.* 70:105–125.
14. Robertson, D., M. Zañartu, and D. Cook. 2016. Comprehensive, population-based sensitivity analysis of a two-mass vocal fold model. *PLoS One.* 11:e0148309.
15. Melzner, M., F. Süß, and S. Dendorfer. 2021. The impact of anatomical uncertainties on the predictions of a musculoskeletal hand model–a sensitivity study. *Comput. Methods Biomech. Biomed. Eng.* 1–9. https://doi.org/10.1080/10255842.2021.1940974.
16. Mitra, E. D., and W. S. Hlavacek. 2019. Parameter estimation and uncertainty quantification for systems biology models. *Curr. Opin. Syst. Biol.* 1:9–18.