Mechanical modeling and structural analysis of the primary plant cell wall
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Plant cell growth is a fundamental process during plant development whose spatial and temporal dynamics are controlled by the cell wall. Modeling mechanical aspects of cell growth therefore requires the integration of structural cell wall details with quantitative biophysical parameters. Recent advances in microscopic techniques and mechanical modeling have made significant contributions to the field of cell wall biomechanics. Live observation of cellulose microfibrils at high z-resolution now enables determining the dynamic orientation of these polymers in the different wall layers of growing cells. Mechanical modeling approaches have been developed to operate at the scale of individual molecules and will thus be able to exploit the availability of the high-resolution structural data. The combination of these techniques has the potential to make a significant and quantitative contribution to our understanding of plant growth and development.

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Introduction
Plant developmental processes such as growth and the generation of organ shape result from a combination of plant cell growth and differentiation, cell division and cell death [1]. During differentiation, plant cells increase in size from typically $10^2 \mu m^3$ (volume of a meristematic cell) to up to $10^4 \mu m^3$ (e.g. xylem vessel). This increase in cellular volume is accompanied by an increase in cellular surface, which requires the addition of building material in the form of cell wall polymers and membrane. While new cell wall material is incorporated, the existing material is deformed and stretched mechanically. The force for this deformation is supplied by the turgor pressure. However, the dynamics of the growth process as well as the resulting final cell size and cellular shape are controlled by the mechanical behavior of the cell wall. Therefore, quantifying the biophysical properties that govern the behavior of the primary plant cell wall under tensile stress has been a fundamental challenge for plant developmental studies. Understanding how molecular composition, intramolecular linkages, agents such as cell wall modifying enzymes, water content, ion concentration, and pH influence this mechanical behavior will help determining how these molecular players influence plant morphogenesis. These functional analyses will be crucial for the incorporation of the abundant data from genomic and proteomic studies [2–4] into a mechanistic framework of plant growth and development.

Visualization of the molecular geometry of the primary plant cell wall
The primary cell wall is often described as a fiber-reinforced composite material although this comparison has certain limitations. The fiber component is provided by the cellulose microfibrils, which because of their one-dimensional geometry and high tensile strength are able to confer anisotropy to the overall mechanical properties of the cell wall. An anisotropic cell wall is stiffer or more resistant to deformation in one particular direction resulting in preferential growth in the perpendicular direction. The microfibrils are linked to each other through hemi cellulose molecules, which bind the microfibrils into a network. This network in turn is embedded in a gel-like matrix formed mainly of pectin (and water). Anisotropic
behavior derives from preferential orientation of cellulose microfibrils [7], although other factors such as the degree of cross-linking [6] and crystallinity and the length of the individual microfibrils [8] are likely to influence their mechanical properties.

The orientation of an individual cellulose microfibril relative to the overall cell geometry is the result of two processes: the orientation in which the polymer has initially been deposited at the surface of the plasma membrane and any passive reorientation it may have undergone as a result of the deformation-induced convection within the matrix material. The mechanism of this passive reorientation can be illustrated by drawing diagonal lines on a large rubber band and observing their changing angles relative to the object while stretching the band. Depending on the cell type and plant species, populations of cellulose microfibrils have therefore been observed to be arranged randomly or highly organized. In some cases all microfibrils throughout the thickness of the wall are oriented approximately parallel to each other, in others sheets of parallel microfibrils display varying angles in different wall layers resulting in helicoidal or lamellar arrangements. Different arrangements can be observed in the different cells of the same organ such as the pith (parallel arrangement) and the epidermis (helicoidal arrangement) of Helianthus hypocotyls [9]. A truly parallel microfibril orientation throughout the wall is predicted to result in a strongly anisotropic distribution of mechanical properties of the wall segment, since tensile strength will be higher in the direction parallel to the net orientation of the microfibrils [7]. In a lamellar or helicoidal arrangement, the individual layers are strongly anisotropic, but the wall as a whole is less so or not at all. However, this layered packing is proposed to be mechanically very stable against stress in all directions in the plane of the wall [9]. In order to test these and other predictions, a mechanical model must be based on a realistic 3D structure of the wall geometry. To obtain these structural data from biological specimens, microscopical analysis of cellulose arrangement has to be performed at high resolution, ideally with the capability of visualizing individual microfibrils. A variety of methods have been employed to do so.

Cellulose microfibrils have a crystal structure and consequently they are birefringent [10]. This property has been exploited for decades in polarized light microscopy. Observing cell walls using a brightfield illumination pathway equipped with two polarizing filters oriented perpendicularly to each other, one placed before and another after the sample, allows visualizing microfibrils against the dark background of the other, non-birefringent cellular components [11,12]. The implementation of a liquid crystal (LC) compensator in the LC-Polscope [13] has significantly improved quantification and image acquisition and thus enabled rapid and high-resolution analysis.
of the bulk orientation and the abundance of the birefringent polymers. The interpretation of the data obtained from living cells is not without challenges; however, absolute quantification requires complementary techniques to determine parameters such as the thickness of the cell wall and the refractive index of the crystal [14*].

Polarizing light microscopy does not require any stains, which is advantageous for live cell imaging, but it has the drawback of being unspecific. Any birefringent structure in the light pathway will contribute to the signal and cellular geometry further influences the result. A more specific technique for microfibril detection has therefore been adapted to confocal laser scanning microscopy by using Congo red in combination with polarized laser light. This dye binds cellulose microfibrils and is birefringent itself [15]. Verbelen and coworkers used this technique to determine the net orientation vector of microfibrils within wall segments. Furthermore, they determined, at least semi-quantitatively, the degree of anisotropy of these segments by calculating the ratio between two fluorescence intensities measured in perpendicular orientation [16,17*].

A completely different principle for the determination of net microfibril orientation is based on the mechanical behavior of a fiber-reinforced composite material. When fractures or folds form in this type of heterogeneous material, they are oriented preferentially parallel to the vector of net fiber orientation [18]. This principle was exploited to determine cellulose orientation in pollen tubes [19*]. After chemical fixation, the large pectin moiety of the pollen tube cell wall was removed by enzymatic digestion and the remaining structure was critical point dried for observation in the scanning electron microscope. This process induced cracks in the cell wall, which were parallel to each other and oriented in a helix-shaped pattern around the tubular cell (Figure 2). From these artifacts, the principal orientation of cellulose was deduced.

Both the birefringence and the fracture approach have the drawback that they only provide information about the bulk properties of the cell wall, and not about the structure of individual layers. To visualize individual microfibrils, field emission scanning electron microscopy and atomic force microscopy have been employed successfully. However, both techniques are confined to observing surfaces and therefore the only microfibrils that are accessible to these high-resolution techniques are those located at the inner and outer faces of the wall. More centrally located wall layers remain hidden. Nevertheless, important information on the orientation of microfibrils upon initial deposition in different tissues and cell types was obtained using these techniques [20–25].

Insight into the central wall layers has been provided by transmission electron microscopy, since for this technique true cross-sections, or, even more informative, oblique sections through the wall are prepared [23,26,27]. However, the limitation of both transmission and scanning electron microscopic techniques has (until recently at least) been the fact that samples have to be fixed and are thus dead during observation. This has impeded direct observation of dynamic processes such as microfibril reorientation during cell growth. It is therefore very exciting that finally live observation of microfibrils, at 3D spatial resolution, seems to be within reach. Recently, Pontamine Fast Scarlet 4B, a dye that fluoresces preferentially in the presence of cellulose, has been used to determine microfibril orientation in the different wall layers of Arabidopsis root cells (Figure 3a) [28**]. Crucially, this dye proved to work on living and expanding tissues thus allowing for time lapse imaging. Anderson and coworkers were able to determine that the difference in orientation of cellulose between the inner and outer wall faces in root cells was likely caused by a continuous rotation of microfibrils from the transverse towards the longitudinal direction in the maturing layers of the wall during expansion (Figure 3b). The authors calculated that a passive reorientation of the fibers within the cell matrix suffices to explain the phenomenon. This provides new support for the mechanism of passive reorientation in multinet type walls [29] that seems to occur in the
expanding cells of the root. Interestingly, Anderson and coworkers also found that cellulose microfibrils seem to cluster and reorient as clusters and not necessarily individually. This finding raises questions about the dynamics of cross-linkage breaking and reforming before, during, and towards the termination of growth events.

**Modeling cell wall behavior at decreasing scale**

Mathematical and computational modeling allows establishing a framework of physical laws on the basis of which predictions are made that can then be tested experimentally [30,31]. The coincidence between prediction of a model and experimental data is not necessarily a proof of validity. However, a good model offers several possibilities of validation and, importantly, it can direct further experimentation.

In most models, the cell wall has been considered as a homogeneous, elastic or visco-elastic material or as a viscous fluid. Depending on the type of mechanics chosen, different laws apply for its behavior under stress. Many mechanical models describing plant cell wall behavior are based on the formalism initially established by Lockhart [32]. The equations formulated by Lockhart are based on the behavior of a viscous dashpot with a Bingham fluid (a viscoplastic material that behaves as a rigid body at low stresses but flows as a viscous fluid at high stress). Adaptations include the Augmented Growth Equations that account for changes in turgor pressure [6,33] and a generalization to three-dimensional deformation in tensor form [34,35]. The latter is able to explain organ bending phenomena such as those occurring during phototropism.

Although these models have contributed important insight into plant behavior and development, they generally simplify the biological reality by considering the cell wall to be a homogeneous material. In reality, the plant cell wall is of course a hierarchically built structure. It consists of multiple layers that may behave differently depending on their chemical composition and anisotropy. Furthermore, the cell wall material is composed of polymers whose individual mechanical behavior differs depending on chemical nature, spatial arrangement and length. Adding complexity, each of these polymers is linked to many others and the mechanical behavior of the links depends on their nature and the local conditions (e.g., pH and water contents). The mechanical deformation or stretching of the wall likely implies breaking and reformation of links, sliding of polymers against each other, slippage, uncoiling and stretching of individual molecules [36,37**]. Consequently, there are numerous degrees of complexity in the biological reality and choices need to be made when establishing a mechanical model.

How much detail does a model need? At which scale does a mechanical model of the cell wall need to operate in order to produce relevant information? No global answer to this question can be given, as the appropriateness of a model depends on the scientific question being asked. Treating the cell wall as a homogeneous material has worked fine for many approaches, especially those that explain behavior at tissue level. For example, elastic springs were used to model the anticlinal walls of the cells composing the shoot apical meristem (Figure 1c). This was sufficient to predict a change in the stress pattern upon ablation of a single cell [38]. In the experimental situation, microtubules in the neighboring cells reoriented as a consequence of the ablation. Their new orientation followed the stress lines predicted by the model which indicated its usefulness despite a certain degree of simplification. The model by Hamant and coworkers was established using finite element techniques, an approach that allows the subdivision of a complex structure into small elements (Figure 4).
strain at each location of the structure upon application of a load can then be calculated for each element and thus the behavior of the structure is determined in approximate manner. Finite element modeling is commonly used in engineering for modeling complex objects such as bridges and cars.

Representing the entire wall of a cell using one or few springs will not be adequate if the growth behavior of an individual cell is not uniform (or heterotropic [6]) (Figure 1d). The scale of the model needs to correspond to the scale of the biological question. An example for a finite element approach with subcellular resolution has been used successfully to model the deformation of a pollen tube under the locally applied load of a microindenter [39]. An analytical approach would have been difficult in this case because of the geometries of the tube and the loading situation. The results of the computational simulations allowed for the interpretation of experimental data that had not been trivial because of the complex situation [40–43].

Until recently, finite element modeling had only been applied to reproduce reversible deformations of the cell wall under transient load application [38,39,44]. However, this technique has great potential for modeling the irreversible deformation occurring during plant cell growth, since contrary to other modeling methods, it would be able to handle the complex geometries occurring in plant cells such as trichomes and pavement cells. In a recent study, a first attempt to exploit finite element modeling for heterotropic cellular growth has been adapted to polar growth processes (Figure 4) [45]. The simulations made detailed predictions for the mechanical profile of a tip growing cell. Intriguingly, these predictions corresponded nearly perfectly to the experimentally determined distribution of pectin molecules in the cell wall of the pollen tube, a cell that displays tip growth. This correlation confirmed earlier findings that had suggested an important role for the changing chemical configuration of pectins in determining the shape of this rapidly elongating cell [43].

Although the finite element model by Fayant et al. [45] is able to reproduce morphogenetic phenomena at subcellular level, the cell wall is still treated as uniform material. Anisotropic behavior of the material can be accounted for in this model by varying the elastic modulus in the different directions, but cellulose is not yet treated as an individual structural component. Two recent, very different approaches have attempted to model cell wall behavior at the molecular level. A fiber-reinforced fluid model describes the cell as a pressurized axisymmetric viscous fluid sheet between rigid end plates while accounting for the anisotropic stresses arising from the reinforcement of the wall by microfibrils [46]. This is achieved by treating the cell wall as a fiber-reinforced composite, following approaches for nematic liquid crystals, textile fiber composites and the extensional flow of collagen gels. In this model the fibrous component consists of a single family of extensible fibers with a director field. A strain on the fluid sheet will cause
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the convection, stretching and reorientation of the fibers by the induced flow. On the other hand, the presence of the fibers induces a transverse isotropy within the fluid that is aligned with the director field. This approach can be extended to take into account variations in fiber orientation across the thickness of the wall and it has therefore the potential to be very useful for understanding growth behavior of individual plant cells. However, this model does not distinguish between the hemicellulose tethers that connect microfibrils and the pectin matrix that envelopes them. This kind of detail is accounted for in another recently developed software termed WallGen [47**]. The authors use a computational approach to build a fragment of virtual wall whose components have one-to-one spatial and mechanical correspondence with the cellulose microfibrils and hemicellulose links of a primary wall cellulose-hemicellulose network (Figures 1e, 4b).

The authors used finite element analysis to predict the mechanical properties of the entire fragment containing thousands of individual wall polymers. Importantly, this model allows for varying a number of input variables that can be manipulated experimentally. The effect of matrix components such as pectin is not accounted for in the present version of the model, but future versions will hopefully be produced once quantitative structural and mechanical data become available on which these can be based.

Conclusion

Plant development depends on the growth processes that occur in each individual cell composing the organism. How the cell wall controls both temporal and spatial dynamics of this process is determined by its mechanical behavior. The advances that have been achieved in both structural analysis and mechanical modeling, notably to operate at molecular scales and in living specimens, will help us direct our search for molecular players responsible for the mechanical phenomena that ultimately determine the plant phenotype.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest

● of outstanding interest


The study explores the power of the LC-PolScope for tracking dynamic structural changes in vivo by exploiting the birefringence of biomaterials. The authors point out that in order to obtain quantitative data, additional parameters regarding the geometry, composition, and refractive indices of the specimen have to be acquired using complementary methods.


Polarization confocal microscopy is used in combination with mechanical creep tests to elucidate the role of microfibril orientation for the mechanical behavior of the plant cell wall. The authors use onion epidermis as an experimental system and found that although net cellulose microfibril orientation defines the direction of growth, it does not determine the degree of growth anisotropy.


Polarized light has not been successful in determining microfibril angle in pollen tubes because of the low cellulose contents and a high number of birefringent organelles. Here, fracture mechanics is exploited to determine the mechanical anisotropy of the pollen tube cell wall.


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This review proposes how the insertion of new pectin material into a growing wall affects its mechanical properties by displacing calcium ions from existing polymers that are under tension due to the turgor pressure.


46. Finite element analysis was used to model apical growth in walled cells. The simulations predicted a spatial distribution of mechanical properties that corresponds exactly to that of changing pectin chemistry in the cell wall of pollen tubes.


This theoretical model of a growing cell represents the primary cell wall as a thin axisymmetric fiber-reinforced viscous sheet. The model shows how either dynamical changes in wall material properties or passive fiber reorientation may suppress cell elongation.


The software WallGen builds virtual cellulose-hemicellulose networks in which the mechanical elements are intended to have one-to-one spatial and mechanical correspondence with the cellulose microfibrils and hemicellulose chains in the biological structure.