Cell Wall-Related Mechanisms underlying Plant Cell Expansion

Herman Höfte, Kris Vissenberg

Plant Cell Walls, Ed Anja Geitman,

Chapter 6

Cell Wall-Related Mechanisms underlying Plant Cell Expansion

Kris Vissenberg and Herman Höfte

Kris Vissenberg

http://orcid.org/0000-0003-0292-2095

Herman Höfte

https://orcid.org/0000-0002-5728-146X

Keywords :

Acid growth, auxin, cell expansion, diffuse growth, feedback regulation, history, pectin, tip growth, wall models, wall relaxation

Word count: 12522

Number of figures: 5

Boxes: 3

Abstract

A central question in plant biology is how plant cells can expand while maintaining a strong cell wall. Two main mechanisms have been proposed for irreversible wall expansion, intussusception and chemorheological growth. We discuss here the molecular nature of the cross-links and how their removal can contribute to growth of both tip-growing or diffusely-growing cells. We also discuss recent observations suggesting that at least in some cases processive intussusception may contribute to cell wall expansion in plants.

Introduction

The cells of prokaryotes, fungi, oomycetes, many protists, algae and plants are all surrounded by strong cell walls. A central question is how the walls of such cells manage to expand while resisting the force of the cell's turgor pressure and preventing rupture. As nicely reviewed by Dumais (Dumais 2013), different solutions for this conundrum can be found in nature, two of which allow irreversible cell wall expansion (see Box 1): (i) growth by processive intussusception, which refers to cell wall area increase driven by the insertion of cell wall elements in a regularly arranged monolayer, as for instance observed in prokaryotes, and (ii) chemorheological growth, which is defined as the chemically-mediated flow of polymeric materials under stress. Here, the deformation of the wall depends on the rate of chemical reactions leading to the breakage of load-bearing cross-links within the network, as observed in plant cell walls.

The current consensus Is that plant cell expansion is driven by the turgor pressure and limited by the extensibility of the cell wall, which relaxes through a chemorheological mechanism (see Box 1). This view has evolved over time. Early landmark publications (Nägeli 1864; Sachs 1874) proposed that primary cell walls grow by intussusception. An alternative view (Strasburger 1882) proposed that wall material is deposited through apposition onto the inner cell wall surface. Since such a mechanism on its own does not explain cell wall expansion, an additional process was needed to account for cell enlargement. During the same period there was the discovery of an ‘influence’, which moves from the site of light perception to the site of bending during phototropic bending of canary grass coleoptiles (Darwin 1880). This “influence” was later identified as auxin by Went (1928), which, together with observations by Cholodny (1926), led to the Cholodny-Went theory that states that during tropic responses, asymmetric distribution of auxin throughout the organ stimulates differential growth responses, which results in organ bending (Went and Thimann 1937). This in turn led to the formulation of the acid growth theory (Rayle and Cleland 1970), in which auxin triggers an acidification of the apoplast, promoting relaxation of the cell wall, supposedly through pH-sensitive cell wall hydrolytic activities (see Box 2). This was clearly in line with a chemorheological growth model, also adopted by Lockhart (1965), who formalized turgor-driven cell expansion, with the now famous formula $E = \varphi(P - Y)$, where E is the rate of extension, φ the plastic compliance, P the turgor pressure and Y the yield threshold and where Y drops and φ increases with decreasing pH. Finally, the identification of expansins, proteinaceous

agents responsible for acid-promoted relaxation of stretched cell wall preparations (McQueen-Mason et al. 1992), provided a biochemical correlate for φ and acid growth.

Box 1: Processive intussusception vs chemorheology

Processive intussusception is illustrated by the growth of the sacculus of gram-negative bacteria (Höltje 1998). This structure is a peptidoglycan monolayer with an extremely regular structure consisting of long glycan chains, which support circumferential stress, interconnected by short peptide chains, which support axial stress. Surface deformation is controlled by a protein complex that concomitantly removes peptide bonds, depolymerizes one template glycan chain and synthesizes three new glycan chains (Figure 1A). The regularity of the cell wall structure is such that the “shape” of the new material determines the deformation anisotropy of the cell wall (Figure 1B). The much less regular plant cell walls are thought to undergo chemorheological growth (Figure 1C), which differs from intussusception in that the deformation is dictated by the anisotropic relaxation (the anisotropy results from the anisotropy of both wall structure (Figure 1D) and the stresses that are exerted upon the wall (Figure 1E) upon the removal of load-bearing bonds. The newly inserted wall material is thought to be amorphous or sufficiently flexible to passively fill in the voids created by the removal of load-bearing cross-links, and thus does not contribute to orienting the deformation (Dumais 2013).

Box 2. Auxin-controlled cell expansion: an interplay between pH and cell wall modifications.

Since the Cholodny-Went theory (Went and Thimann 1937), we now know that during tropic and other growth responses (Estelle 1996; Ma and Li 2019; Fendrych et al. 2016), auxin levels are positively correlated with cell elongation and auxin is redistributed through the action of different auxin transport proteins to the cells in the zone that will elongate (Friml et al. 2002; Noh et al. 2001; Rakusová et al. 2011).

Curiously however, auxin can also elicit opposite growth responses, depending on the organ or even the process within the same organ. For instance, in line with Cholodny-Went (Du et al. 2020), lower auxin content or signaling in mutant Arabidopsis roots correlates with shorter epidermal cells and higher cell wall pH (Barbez et al. 2017). In contrast, in the same roots during the gravitropic bending response, higher auxin levels correlate with the *inhibition* of cell elongation at the lower side of the root (Band et al. 2012). Similarly, external auxin supply inhibits cell elongation (Růžička et al. 2007; Swarup et al. 2007) and promotes surface alkalinization in the root's elongation zone (Staal et al. 2011). Such opposite responses also occur within etiolated Arabidopsis hypocotyls, where auxin application within 20 min activates plasma membrane-localized H^+-ATPases leading to cell wall acidification and increased cell elongation (Fendrych et al. 2016), but where, during apical hook formation, auxin accumulation correlates with the inhibition of cell elongation at the future inner side of the hook (Liao et al. 2015). It

should be noted, however, that no matter the growth response to auxin, cell elongation rate inversely correlates with the cell wall pH in accordance with the acid growth theory.

It thus seems that auxin, depending on the context, can trigger either acidification or alkalinization of the cell wall. Lin et al. (2021) recently explained this dual action by showing that two antagonistically acting auxin signalling pathways converge on the apoplastic pH: the cell surface-located TRANSMEMBRANE KINASE1 (TMK1), which interacts with and mediates the phosphorylation of plasma membrane H^+-ATPases for apoplast acidification and the stronger intracellular canonical auxin signalling that promotes net cellular H^+ influx and apoplast alkalinization. The picture is more complex, however, since TMK1 can also mediate slower transcriptional responses. Indeed, Cao et al. (2019) showed that the differential growth response during apical hook formation also depends on TMK1, where local auxin accumulation in cells at the inner side of the hook, leads to cleavage of the C-terminus of TMK1, which then stabilizes IAA32 and IAA34 proteins that are specific regulators of auxin signaling (by repressing auxin response transcription factor (ARF) activity).

The study of apical hook formation provided additional insights into the link between auxin levels, cell wall pH and cell expansion. Indeed, Jonsson et al. (2021) observed that higher auxin levels at the inner side of the hook correlate with higher methylesterification levels of the pectin homogalacturonan (HG). Using mutants and transgenics, the authors show that auxin prevents HG de-methylesterification, but interestingly, that changes in HG methylesterification alter the auxin response domain

through a so far unidentified feedback loop. We will discuss below the potential relevance of pectin de-methylesterification for the control of cell elongation. The same group also provided evidence that xyloglucan (XG) is required to limit cell growth rates at the inner side of the hook, and that XG is required for the auxin maxima in the inner side cells by transcriptional regulation of auxin transporters through ARF2 (Aryal et al. 2020). Auxin therefore controls apical hook formation through changes in cell wall chemistry, and in turn, the cell wall provides mechanochemical feedback towards auxin.

Chemorheological cell wall expansion

As also discussed in chapters ULVSKOV and DOBLIN in this book, much of the literature is focused on the nature of the cross-links and on how they are broken to allow relaxation of the network. In the most advanced model, in terms of experimental validation and modeling, the load-bearing network of the primary cell wall consists of cellulose microfibrils, which make lateral contacts at a small number of locations (Zhang et al. 2021). Recent studies, using AFM (atomic force microscopy), cryo-focused ion beam milling and cryo-electron tomography (Nicolas et al. 2022), provided new insights into the cell wall architecture. These studies used the outer epidermal cell walls of onion bulbs as a model, which are up to 7 µm thick and consist of multiple lamellae, each ~40 nm thick. Each lamella consists of fields of aligned microfibrils, the overall orientation of which alternates in the successive lamellae between -45° and + 45° relative to the cell's long axis. The fibrils are surrounded by a reticulated matrix, referred to by the authors as « meshing », which consists primarily of homogalacturonan pectins. This meshing forms patches with no preferential orientation as well as segments in between the aligned fibers. Meshing was most abundant at the inner surface of the cell wall, and levels

abruptly dropped off around 1 μm into the wall. AFM combined with coarse grain modeling on walls before and after stretching, showed that tensile mechanics of the cell wall are dominated by complex movements of cellulose microfibrils (bundling, sliding), with no significant contribution from the matrix (Zhang et al. 2021). At least a subset of the lateral contact sides between microfibrils are thought to be sandwiched together by tightly-bound XG chains (at least in non-Commelinoid species) (see chapter DOBLIN for a description of the cell wall polysaccharides). The latter cross-links were referred to as “biomechanical hotspots” (Cosgrove 2018). The existence of a XG fraction closely associated with cellulose was shown by Pauly et al. (1999) and later by Vissenberg et al. (2005), who used an *in vivo* XG-endotransglycosylase (XET) assay, in which fluorescently labelled XG-oligosaccharides are covalently incorporated into existing XG by XET action. Upon removal of these incorporated XG-oligosaccharides by a XG-specific β-endoglucanase (XEG), a substantial portion of the XG remained in the cell wall, suggesting that they were in close contact with cellulose and hence protected against XEG enzyme action. The existence of such hybrid XG-cellulose structures, recalcitrant to XEG-treatment, was later confirmed by the discovery of a class of specialized fungal β-endoglucanases (members of the glycosyl hydrolase family 12, which is absent in plants) that specifically removes these structures while leaving the bulk of the “unprotected” XG intact (Park and Cosgrove 2012). If pathogenic fungi have evolved such highly specialized enzymes, one can expect that the structures that they target have some critical role in the cell wall’s architecture. Interestingly, Cosgrove and colleagues indeed showed that this class of β-endoglucanases caused a sustained deformation (creep) of isolated cell walls in an extensometer, which was not the case for XEGs (Park and Cosgrove 2012), such as those used by Vissenberg et al. (2005). In an elegant series of experiments using AFM, they could also show at the

nanoscale that these fungal enzymes promoted the relaxation of stressed walls by removing connections between individual microfibrils (Zhang et al. 2017). The same group also identified expansins as endogenous creep-promoting proteins, suggesting that they also disrupt these biomechanical hotspots, but through a poorly understood, non-enzymatic mechanism (McQueen-Mason et al. 1992; Wang et al. 2013). Interestingly, expansin activity requires low pH (≤ 5), consistent with the acid growth theory. Expansins also promote cell expansion of intact cells (see references in (Marowa et al. 2016)), as shown by ectopic administration on tomato apical meristems (Pien et al. 2001) or by overexpression in Arabidopsis stems and rosettes (Cho and Cosgrove 2000; Goh et al. 2014). An essential *in vivo* role for expansins in cell elongation was pinpointed by the demonstration that root hair growth was reduced in loss-of-function mutants for the root hair-specific expansin *OsEXPA17* in rice (ZhiMing et al. 2011) or in RNAi lines for *AtEXPA7* in Arabidopsis (Lin et al. 2011), that leaves and petioles were smaller and often twisted in antisense lines for *AtEXPA10* (Cho and Cosgrove 2000), that pollen tube growth was reduced in double *atexpa4/atexpb5* mutants and that the root meristem size was reduced in a mutant for *AtEXPA4* (Liu et al. 2021).

It should be noted that creep measurements in general are carried out on isolated and often heat-inactivated fragments from cucumber or Arabidopsis hypocotyls, mungbean epicotyls, oat coleoptiles or the above mentioned onion peels (Suslov and Vissenberg 2018). The cells in these tissues are unique in that cell expansion occurs uncoupled from cell wall deposition, perhaps as an adaptation allowing rapid growth. Indeed, the

outer epidermal cell walls of young cells are multilayered and very thick (> 1µm, up to 7 µm for onion cell walls) (Zhang et al. 2021; Suslov et al. 2009), in comparison to much thinner walls (35 to 50 nm) described for instance for anticlinal cell walls of pavement cells (Haas et al. 2020). A study using Arabidopsis hypocotyls showed that such thick walls are deposited during a slow growth phase, after which they undergo significant thinning during a rapid growth phase (Refrégier et al. 2004). The use of such tissues has allowed the assessment of the remodeling capacity of multilayered walls without having to worry about the replenishment of new cell wall material. The observation of the uncoupling of wall synthesis and cell elongation led Bonner to conclude in 1934 (Bonner 1934) “Increase of wall area due primarily to active intussusception of new material is therefore excluded, at least in the case of the Avena coleoptile.”. Subsequent studies using similar materials were often less careful in avoiding extrapolation to plant cell expansion in general.

Indeed, the problem is more complex for the majority of expanding cell walls, in which wall deposition and cell expansion are tightly coupled. This is illustrated by the anticlinal (perpendicular to the organ surface) walls of Arabidopsis epidermal pavement cells, which showed, on cryo-Scanning Electron Microscopy (SEM) sections, a thickness of 35 to 50 nm on each side of the middle lamella (Haas et al. 2020). Such walls can thus accommodate not more than a single cellulose layer (estimated to have a thickness of around 40 nm as determined by Field Emission Scanning Electron Microscopy (FESEM) and AFM in dried onion cell walls (Zhang et al. 2013) or a few cellulose layers, if we assume that these walls have a denser architecture than onion outer cell

walls. As discussed below, expansion of such walls cannot occur without a continuous insertion of new wall material.

Very few studies have addressed in plants how new cell wall material is inserted into the structure and how critical cross-links are formed. Ray (1967) fed oat coleoptile segments with Glucose-H^3 in the presence or absence of auxin and used electron microscope radio-autography to observe in which parts of the cell wall cellulose and matrix polysaccharides were incorporated. Consistent with the more recent observation of the activity of cellulose synthase complexes in the plasma membrane (McFarlane et al. 2014), they observed that cellulose was deposited close to the plasma membrane, whereas hemicellulose also penetrated into deeper cell layers. Anderson et al. (2012) used click chemistry upon feeding Arabidopsis seedlings with alkynylated fucose to show the incorporation of newly synthesized polymers, presumably fucose-containing rhamnogalacturonan I (RG-I), into the outer cell wall of root epidermis cells. They observed deposits of the polymer aligned with cellulose microfibrils and reorientation of these polymers associated with cell elongation consistent with the multinet growth theory by Roelofsen and Houwink (1953), the above mentioned AFM observations by Zhang et al (2017) and the results from coarse grain modeling (Zhang et al. 2021). More detailed observations were reported for the Charophyte *Chara corallina* (Boyer 2016). Charophytes are freshwater green algae, which descended from a common ancestor with land plants (Domozych et al. 2016). This proximity is reflected in a very similar composition of the cell walls (Popper et al. 2011). In Chara, calcium-pectate cross-links rather than XG-cellulose bonds appear to be the load-bearing structures of the cell wall

(Boyer 2016). A large body of evidence shows that these cell walls expand as a result of the insertion of pectate, which chelates calcium from load-bearing bonds, thus causing the relaxation of the cell wall and the formation of new load-bearing bonds. It should be noted that in this system, the insertion of new wall material is sufficient to promote wall expansion, which is reminiscent of intussusception (Figure 1B). The difference, however, is that in Dumais' view, the flexible pectin only adopts its structure by occupying the void created upon the turgor-driven relaxation of the cell wall network after removal of load-bearing cross-links (Dumais 2013) and hence does not orient the deformation like the insertion of new material in bacterial cell walls for instance.

In what follows we will first discuss the role of pectin metabolism in cell expansion and then compare models for cell wall expansion in tip-growing and diffusely growing cells.

Pectin structure-function relationships, the tip of the iceberg ?

As discussed in detail in chapter MOHNEN in this book, pectins are characterized by the presence of galacturonic acid (GalA) and constitute the most diverse family of cell wall polysaccharides in plants. Among those, HG, a linear polymer of α-1,4-linked GalA is, in many cell types of non-Commelinoid species, the most abundant cell wall polymer. For instance, it represents 20% of the cell wall dry mass in Arabidopsis leaf tissue (Zablackis et al. 1995) and even over 50% in the outer walls of the onion epidermis (Wilson et al. 2021). HGs are found as part of different block-copolymers (HG-RG-I, HG-xylogalacturonan, HG-RG-II the proteoglycan ARABINOXYLAN PECTIN

ARABINOGALACTAN PROTEIN1 (APAP1; (Tan et al. 2013)) and perhaps also as homogeneous independent HG polymers. The function of this structural modularity is not understood in most cases.

HGs are unique in the plant cell wall in that their charge patterns are precisely regulated. Indeed, they are thought to be secreted in a methylesterified form to become selectively de-esterified by cell wall-associated pectin methylesterases (PMEs) (see MOHNEN for a detailed discussion on HG methylesterification). This generates negatively charged residues in the polymer at pH values above the pKa of the polymer (which can range from 3.5 to 5 depending on its charge density (Ralet et al. 2011). Why does polymer charge matter that much? Firstly, anionic polymers, such as pectins but also glucuronated AGPs (Lopez-Hernandez et al. 2020), trap counterions and thus may affect the apoplastic pH, the proton motive force and ion currents across the plasma membrane (Grignon and Sentenac 1991; Sentenac and Grignon 1981), which control turgor and morphogenesis (Weisenseel and Kicherer 1981; Hoffmann et al. 2020). In addition, pectins, like many other polymers, form cross-linked networks that contain water in their interstices. The water retention capacity increases dramatically with increasing polymer charge, going from water-polymer ratios of 5:1 for uncharged polymers, up to 3000:1 for charged polymers (Osada and Gong 1993). To understand this, one should keep in mind that the volume of an ionized polymer gel is determined by its osmotic pressure, which is the sum of three forces: (i) the rubber elasticity, (ii) the polymer-polymer affinity and (iii) the counterion pressure (Tanaka 1981). The rubber elasticity derives from the elasticity of the individual polymer strands. The magnitude of

this pressure depends on the temperature, whereas the sign depends only on the volume of the gel (positive for a condensed gel, with polymer ends held close together or negative for a swollen gel, with polymer ends stretched far apart). The polymer-polymer affinity depends on the solubility of the polymers. Poor solubility will tend to draw polymers together creating a negative pressure. This pressure is temperature insensitive and decreases with increasing gel volume. The counterion pressure is the osmotic pressure resulting from counterions sequestered by the polymer's charges (Donnan effect). This pressure increases with temperature and is positive, since it tends to draw water into the polymer's interior and push its exterior parts away from one another. Changes in the “environment” (temperature, pH, ionic strength, calcium concentration, mechanical stresses, etc) of the gel will affect the three parameters in independent and often discontinuous ways. For instance, increasing the temperature will increase the osmotic pressure of the gel due to the increased rubber elasticity and counterion pressure. The resulting volume increase will lead to a decrease in polymer-polymer affinity, which, beyond a certain threshold, will again be counteracted by the negative pressure due to the stretching of the polymers. This leads to complex emergent behaviors, including discontinuous changes in volume (phase transitions) in response to even minute changes in the “environment” or the composition of the gel (Tanaka 1981) (Figure 2A, B). So it is easy to understand how even subtle chemical changes (e.g. de-methylesterification, de-acetylation, polymer cleavage) that affect one or more of these three parameters, can strongly affect the hydration capacity of the gel and hence that of the cell wall.

This idea is nicely illustrated by studies of the mucilage that is released from the Arabidopsis seed coat upon seed imbibition. The released portion of the mucilage is a hydrogel consisting mainly of RG-I (Šola et al. 2019). Genetic studies showed that the removal of one or more galactose residues from a RG-I side chain by the β-galactosidase MUM2 is essential for mucilage release (Macquet et al. 2007). The authors provided evidence that galactoses in RG-I side chains, when oxidized by a putative galactose oxidase, encoded by the *RUBY* gene, are able to form hemi-acetal cross-links with neighboring sugars during dehydration (Šola et al. 2019). Mucilage swelling therefore appears to depend on preventing cross-linking through the enzymatic removal of galactose residues from RG-I side chains.

To understand the *in vivo* function of pectins, it should be kept in mind that their concentration in the cell wall is an order of magnitude higher than in the gels used in most *in vitro* physicochemical studies. Such concentrated gels can have tensile moduli close to those of plant cell walls (Zsivanovits et al. 2004), suggesting that, besides cellulose, pectins can significantly contribute to the mechanical properties of the cell wall. In addition, uni-axially oriented HG chains can form crystalline fibrils. This was shown in *vitro* using X- ray diffraction on oriented HG preparations of high Degree of Methylesterification (DM), gelified at low pH in the presence of sucrose, or calcium cross-linked low DM HG, where the two types of fibrils showed different crystal units (Palmer et al. 1947; Sterling 1957; Walkinshaw and Arnott 1981a, 1981b). Pectin fibrils were also shown to exist *in vivo* (Figure 3). For instance, electron microscopy and X-ray diffraction revealed predominantly axially oriented pectin fibrils in fresh collenchyma cell

walls of *Petasites vulgaris* petioles (Roelofsen and Kreger 1951) (Figure 3A). “Non-cellulosic fibrillar galacturonic acid” was also observed *in vivo,* pointing out from the cell wall into the extracellular medium of liquid plant cell cultures (Leppard et al. 1971) (Figure 3B), in the inner (cytoplasmic-facing) cell wall surface of these cells, or in root epidermal cell walls (Figure 3C). These structures with a diameter in the order of 20 nm, were initially christened “lignofibrils”, due to their weak UV absorption properties and Phloroglucinol-HCl staining. The UV absorption, however, could, besides lignin, also come from tyrosine-rich proteins such as polycationic extensin-related proteins, which can form polyelectrolyte complexes with pectate (Cannon et al. 2008; Valentin et al. 2010). This may go some way to explain why these fibres were reported to be largely resistant to pectinases (Leppard et al. 1971). Pectin fibrils were also observed in the lumen of poplar xylem fiber cells (Arend et al. 2008) (Figure 3D). More recently, super-resolution microscopy revealed uni-axially oriented HG in the anticlinal cell walls of pavement cells in the Arabidopsis epidermis (Haas et al. 2020) Figure 3E). The significance of this for cell expansion will be discussed below.

A last, so far poorly explored, aspect relevant for the regulation of cell expansion is how substitution and charge patterns of HGs may govern interactions with other polysaccharides or proteins. Well-studied examples for other polysaccharides are the periodic (glucuronic acid (GlcA), me-GlcA or acetyl) substitution patterns on xylan backbones that condition cellulose binding (Grantham et al. 2017) or, in the animal extracellular matrix, the sulfate substitution patterns of the glucoronoaminoglucan heparin that govern, often highly specific, interactions with proteins (Capila and Linhardt

2002). HGs can also be acetylated or xylosylated (at position 2 or 3) and can have random or blockwise charge patterns. In addition, specific repeated substitution patterns (such as $GalA_4MeAc$) could be inferred from the profiling of oligogalacturonides released from the cell wall by a fungal pectate lyase (Voxeur et al. 2019). Such patterns are the hallmarks of specific PME activities, which remain to be characterized.

PMEs are encoded by large gene families (66 members in Arabidopsis thaliana) and, in vascular plants, PME activity can be modulated by endogenous proteinaceous PME inhibitors (PMEIs), which are encoded by equally large families (71 in *A. thaliana*) (Wolf et al. 2009; Wang et al. 2013). In addition, demethylation exposes the HG to the activity of endogenous polygalacturonases (PGs) and pectate lyases (PLs), which require demethylated substrates (Hocq et al. 2017). The few plant PMEs that have been studied so far have neutral to basic pH optima and processive activities, generating blockwise charge patterns. At least some of these PMEs can loose processivity in certain conditions (low pH (Hocq et al. 2021) or in the presence of Ca^{2+} (Vincent et al. 2013)) thus generating non-clustered patterns. It will be interesting to see to what extent HG charge and substitution patterns can govern interactions with cell wall proteins. A recent study (Francoz et al. 2019) revealed that a specific PMEI (PMEI6) is required for the generation of a binding site for a cell wall peroxidase (PRX36), presumably by altering the activity of an unknown PME, at a specific location within the outer cell wall of the Arabidopsis seed coat. At this specific location, the peroxidase changes the cell wall properties generating brittle spots that selectively rupture upon hydration, facilitating the release of the seed mucilage. Many other cell wall proteins have basic

surfaces and bind to pectins as shown *in vitro* (Carpin et al. 2001; Jamet et al. 2008) and *in vivo* (Wang et al. 2015). This is expected to contribute not only to the nanoscale distribution of cell wall modifying activities, but also to the formation of polyelectrolyte complexes that may also locally impact the hydration, porosity and rheology of cell walls. For instance, the interaction with basic extensins induced the dehydration of pectin layers *in vitro* (Valentin et al. 2010). Similar effects can be expected from the interaction with other polycations such as endogenous polyamines (Messiaen and Van Cutsem 1999) or chitosan present in the walls of endophytic (Noorifar et al. 2021) or pathogenic fungi (Baker et al. 2007).

Tip growth

Tip growth is mainly studied in pollen tubes and root hairs. As such, there is much literature dealing with discoveries in both cell systems. It has been known since 1897 that growing pollen tubes contain moving granular protoplasm (Potter 1897). Years later, (Castle 1958) concluded that the apical regions of tip growing cells may have the highest relative elemental rates of elongation known. Following this, the oldest models of tip growth zone activities were developed by Green (1973), while in the same period electrophysiological experiments showed that calcium enters the growing pollen tube tip, with other positive ions, mainly protons, leaving at the more basal region (Weisenseel et al. 1975). The importance of calcium was backed up with the visualization of an internal Ca^{2+}-gradient in pollen tubes (Jaffe et al. 1975) and the observations that raising calcium in the tubes leads to growth arrest, but not of cell wall

buildup, resulting in a thicker cell wall (Picton and Steer 1983) while its deprivation or the use of a calcium-channel blocker results in localized swelling at the tube tip (Reiss and Herth 1985). Later on (Li et al. 1994) observed a periodically arranged pattern of esterified and unesterified pectin in pollen tubes, which was linked to growth velocity fluctuations by (Pierson et al. 1996). Further correlations between the oscillations of pollen tube growth, and those of calcium-ions and protons were described by (Pierson et al. 1996; Feijó et al. 1999), respectively. In the early years of the 21st century, localized cell wall remodeling by expansin and XTH in tip-growing root hairs was shown by Baluška et al. (2000) and Vissenberg et al. (2001), while the discovery that Rapid Alkalinization Factors (RALFs) regulate extracellular pH and growth of root hairs (Wu et al. 2007) opened up the findings that members of the *Catharanthus roseus* superfamily of receptor-like kinases (RLKs) control cell wall integrity at the pollen tube tip (Boisson-Dernier et al. 2009) and in root hairs (Duan et al. 2010). Only in 2018, Schoenaers et al. (2018) made a link between cell wall integrity sensing by one of these RLKs, ERULUS (ERU), and the oscillations of cell wall pectin methylesterification grade and the resulting growth of root hairs.

Like in Chara, pectin insertion thus also appears to drive the expansion of tip-growing pollen tubes and root hairs. In such cells, growth is restricted to the extreme apex and depends on the fusion of vesicles containing new cell wall material with the apical plasma membrane (Bove et al. 2008; Ketelaar et al. 2008; Balcerowicz et al. 2015). This results in polar growth that is highly responsive to local changes in the cell's environment (Schoenaers et al. 2017).

Valuable insights into growth mechanisms in these cells came from the observation of growth rate oscillations, which, as detailed below, are accompanied by oscillations with the same temporal or spatial frequency as a number of other cellular parameters (Kroeger and Geitmann 2012; Damineli et al. 2017). These include exocytosis rate, cell wall modification and thickness, Rho-like GTPase (ROP) activity, apical F-actin presence, cytoplasmic pH and calcium gradients, apoplastic pH and Reactive Oxygen Species (ROS) levels (Hepler et al. 2001; Hwang et al. 2010; Yan et al. 2009; Schoenaers et al. 2017). Such oscillations are also reflected in the periodic cell wall deposition patterns that were reported in tobacco pollen tubes (Geitmann et al. 1995; Derksen et al. 2011). McKenna et al. (2009) monitored PME-GFP levels in the cell wall of pollen tubes, as a marker for exocytosis. They concluded that exocytosis oscillates with the growth rate, where peaks in exocytosis precede periodic growth bursts. (A critical note here is that GFP is less fluorescent at lower pH (Kneen et al. 1998), hence PME-GFP fluorescence oscillations might also reflect apoplastic pH oscillations). Cell wall thickness was also shown to oscillate, also with its maxima preceding the growth rate maxima (Figure 4A). Interestingly, variations in the amount of deposited cell wall material correlated with the extent of the subsequent growth rate increases. This suggested that cell wall deposition determines the elongation rate of these cells (McKenna et al. 2009) (Figure 4B), in contrast to the above mentioned “biomechanical hotspot” model for diffusely growing cells, in which cell wall deposition somehow passively follows cell wall relaxation. A role for cytosolic calcium oscillations in the

control of oscillations in exocytosis, cell wall thickness and growth rate was explored in a model by (Kroeger et al. 2008).

The fusion of vesicles with the plasma membrane is controlled by members of the syntaxin family of soluble N-ethyl-maleimide sensitive factor attachment protein receptors (SNAREs; (Rodriguez-Furlán et al. 2016)), the exocyst complex (Hála et al. 2008) and in part by members of the plant ROP family. ROP activity oscillates in pollen tubes with the same frequency as tip growth oscillations (Hwang et al. 2010), and its apical location depends on the action of ROP1 ENHANCERs (RENs), proteins that are located sub-apically and help to remove ROPs from the plasma membrane using clathrin-mediated endocytosis (Li et al. 2018). ROPs interact with diverse effectors and mediate actin dynamics, microtubule organization (and thus vesicle trafficking, reviewed in (Nibau et al. 2006; Yalovsky et al. 2008), ROS production (Carol et al. 2005) and the formation of a tip-focused Ca^{2+} gradient (Li et al. 1999), and are therefore considered as 'hubs' for signal integration during tip growth.

The requirement of pectin deposition for proper tip growth was suggested by the observation that root hair growth was inhibited in mutants (for two distinct UDP-GalA biosynthetic enzymes) with reduced pectin levels (Pang et al. 2010) or in WT upon pectate lyase treatment (Park et al. 2011). Next to deposition, PME-driven pectin modification (see Box 2) is also critical for pollen tube and root hair growth. In pollen tubes, PME activity is essential for growth, as shown by the curved, irregularly shaped and stunted pollen tubes with reduced elongation rates upon inactivation of *PPME1 or*

VANGUARD1 (*VGD1*), which both encode pollen-specific PMEs (Tian et al. 2006; Jiang et al. 2005). The involvement of PME activity in proper tip growth of pollen tubes was modeled using Finite Element Modeling (FEM) and also experimentally validated (Fayant et al. 2010). In root hairs, genetic validation for a role of PME activity in elongation awaits the identification of *pme*-mutants with a root hair phenotype, but Schoenaers et al. (2018) were already able to show that inhibiting PME activity with a catechin extract also affected root hair growth. Interestingly PME activity also appears to oscillate in the tip of growing root hairs (Schoenaers et al. 2018) or pollen tubes (Rounds et al. 2011). This was shown by the oscillations in the staining intensity of propidium iodide, a dye that binds to anionic pectin. These oscillations may mirror oscillations in PME activity, which, given the high pH optimum of the bulk of PME activity (Hocq et al. 2017), is expected to be controlled by the oscillations in surface pH, reported for pollen tubes (Hepler et al. 2006) and root hairs (Monshausen et al. 2007) (Box 2).

In line with this, the activity of most other cell wall modifying enzymes is also highly pH-dependent. The activity of expansins is promoted in an acidic environment (McQueen-Mason et al. 1992), whereas recombinantly-produced XG endo-transglycosylase-hydrolases (XTHs) show widely diverging pH optima (Maris et al. 2009, 2011). Recombinant pectate lyases have alkaline pH optima (Ali et al. 2015), while polygalacturonases are more active at acidic pH (Hocq et al. 2017). In this respect, it needs to be mentioned that in alkaline conditions, PME activity on methylesterified pectin generates protons, and hence contributes to cell wall acidification (Moustacas et

al. 1991), in concert with plasma membrane-localised H^+-ATPases (AHAs). In pollen tubes, AHAs are specifically localized in the shank and they have been shown to be the drivers for cytosolic pH oscillations (Hoffmann et al. 2020). Indeed, mutants in AHAs show reduced H^+ and anion fluxes around the pollen tube tip, slower cytosolic pH oscillations, shallower cytosolic pH gradients and reduced growth rates. Root hair and pollen tube elongation therefore seem to be in line with the acid growth theory (Box 3) (Rayle and Cleland 1992), where cell wall pH oscillations cause periodic activation of PMEs at high pH in antiphase with the activation of expansins at low pH (Cosgrove 2000).

Based on the calcium-pectate exchange mechanism previously proposed for Chara, (Rojas et al. 2011) developed a computational model for pollen tube growth (Figure 4C). This model posits that cell wall deposition promotes cell elongation and that cell elongation inhibits cell wall deposition through a negative feedback loop. It also implies that cell expansion ceases when all available pectin is incorporated into the load-bearing cell wall layer and no new material is available. This model was able to predict pollen tube shape, trajectories of cell wall material at the growing surface and even growth oscillations, which occur above a certain elongation rate, where cell expansion outruns cell wall deposition. The model clearly indicates that in the case of tip-growing cells, wall expansion is limited by deposition and/or insertion of material. Evidently, turgor pressure provides the driving force for growth also in this model (Kroeger et al. 2011; Dumais 2021).

The calcium-pectate exchange model does not address the role in tip growth of cellulose-XG remodeling described in the biomechanical hotspot model. As discussed below, however, this cannot be ignored given the strong root hair growth phenotypes of mutants for cellulose or XG synthesis or expansins.

Cellulose deposition is essential for maintaining cell wall integrity as shown by the growth inhibition and/or rupture of root hairs treated with a cellulase or the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile (DCB) (Park et al. 2011). Mutant analysis and localization experiments suggest that proteins other than those involved in cellulose synthesis in primary walls (CESA1, CESA3 and CESA6) synthesize cellulose at the root hair tip (Endler and Persson 2011; Park et al. 2011). *CELLULOSE SYNTHASE LIKE (CSL)D2* and *CSLD3* (*KOJAK*) are likely candidates. Indeed their mutants produce abnormal root hairs that rupture early (Favery et al. 2001) or late (Bernal et al. 2008) during development or which show a swollen base, wavy growth or occasional branches. Evidence for a role of CSLD3 in cellulose synthesis came from the observation that a chimeric gene encoding CSLD3 with the catalytic domain of CESA6 complemented the *csld3/kjk* root hair phenotype (Galway et al. 2011, Park et al. 2011). In addition, recent data confirmed that CSLD3 is indeed a UDP-glucose-dependent (1-4)-β-glucan synthase that can be purified as higher-order complexes with similar ultrastructural features as those formed by CESA6 proteins (Yang et al. 2020). The fact that CSLDs are active where plasma membrane-associated cortical microtubules are generally absent (in the root hair tip or at the new cell plate), suggests that they contribute to the production of cellulose structures different from the long microfibrils

generated by microtubule-associated cellulose synthase complexes. It will be interesting to see whether the shorter disordered cellulose-like fibrils that can be observed in the tip of growing root hairs are produced by CLSD complexes (Akkerman et al. 2012; Newcomb and Bonnet 1965).

Xyloglucan is also essential for root hair growth. Indeed, mutants for XG 6-XYLOSYLTRANSFERASES (*XXTs*) (*xxt1/xxt2*, *xxt1/xxt2/xxt5)* lack detectable XG and have short root hairs with swollen bases (Cavalier et al. 2008; Zabotina et al. 2008). Disruption of the synthesis of a GalA-containing XG (in the *ROOT HAIR SPECIFIC8/ XYLOGLUCAN-SPECIFIC GALACTURONOSYLTRANSFERASE1* (*RHS8*/*XUT1*) mutant) results in shorter root hairs (Peña et al. 2012), which, however, do not rupture. The same is true for root hairs treated with a xyloglucanase (Park et al. 2011).

Expansins are also required for normal root hair growth as shown by the shorter root hair phenotypes of RNAi lines for *EXPANSIN A7* (*EXPA7*) in Arabidopsis (Lin et al. 2011), or an *OsEXPA17* mutant in rice (ZhiMing et al. 2011). In addition, application of expansin to growing root hairs triggered, at low concentrations, radial swelling of the tip and transient growth inhibition or, at higher concentrations, bursting (Cosgrove et al. 2002).

XTHs act on the same cellulose/XG network. Although XG endotransglycosylase activity (XET) was observed at the tip and in the shank of root hairs (Vissenberg et al.

2000, 2001, 2003), no *xth* mutants with a detectable root hair phenotype have been described so far, possibly due to redundancy.

Another critical oscillating parameter during root hair growth is the production of ROS in the apoplast. Indeed, mutations in *ROOT HAIR DEFECTIVE* (*RHD2*) encoding a plasma membrane NADPH oxidase, strongly decreased ROS accumulation and caused stunted roots with no or very short root hairs (Foreman et al. 2003). Different interpretations of this phenotype have been formulated. Foreman et al. (2003) proposed that extracellular ROS is required for the activation hyperpolarization-activated Ca^{2+}-channels in the plasma membrane, which form the cytoplasmic calcium gradient that is required for root hair growth and acts in the negative feedback regulation of ROP activity (Yan et al. 2009). Monshausen et al. (2007) proposed an alternative interpretation, in which the lack of root hairs in *rhd2* is the result of premature bursting due to reduced apoplastic ROS levels. In this view, cell wall expansion in root hairs reflects a balancing act between growth-promoting (e.g. acid growth) and growth-restricting activities, where apoplastic ROS promotes wall strengthening through oxidative cross-linking of cell wall extensins (Schnabelrauch et al. 1996; Velasquez et al. 2011). Support for this view comes from the observation that growth at higher pH, which slows down cell expansion, restored root hair growth in the *rhd2* mutant (Monshausen et al. 2007). The essential role for extensins (Velasquez et al. 2011; 2015a,b; Draeger et al. 2015; Møller et al. 2017) but also of other structural proteins such as PRP1, PRP3 (Bernhardt and Tierney 2000), PRPL1 (Boron et al. 2014) and

AGPs (Mollet et al. 2002; Marzec et al. 2015) in cell wall assembly of root hairs was shown by genetic and pharmacological studies.

Together, these observations show that understanding tip growth requires integration of the growth-promoting “calcium-pectate exchange” and “biomechanical hotspot” models but also growth-restricting activities involving oxidative cross-linking of cell wall proteins.

Given the importance and highly dynamic character of the cell wall at the growing root hair and pollen tube tip, the status of the cell wall must be constantly monitored to prevent (premature in case of the pollen tubes) bursting of the cell. This information on cell wall status must therefore feed back to the growth-controlling processes. RLKs of the *Catharanthus roseus* subfamily (CrRLK1Ls), such as FERONIA (FER) (Duan et al. 2010) and ERULUS (ERU) (Schoenaers et al. 2018), were suggested to function in the perception of cell wall status in root hairs by binding to specific cell wall ligands, and they are indeed related to growth control through activity changes of the apical plasma membrane-localized ROPs (Molendijk et al. 2001; Jones et al. 2002). Our current understanding states that ligand-bound RLKs activate ROPs, which then initiate NADPH-oxidase-dependent ROS production, leading to changes in calcium-channel activity and changes in the cell wall (Duan et al. 2010). It would be interesting to see which ligands serve as cell wall status indicators, and whether vesicle fusion and new cell wall deposition are controlled by RLK activation, especially in the light of the abovementioned calcium-pectate exchange model to explain tip growth, where RLKs could act in the control of wall deposition and expansion (Rojas et al. 2011; Dumais

2021). Schoenaers et al. (2018) have indeed shown that ERU is involved in the control of cell wall PME activity dynamics, since *eru* root hairs have elevated PME activity levels, reflected in increased de-methylesterified pectin abundance and in propidium iodide fluorescence intensity oscillations with a lower frequency and higher amplitude.

Diffuse cell expansion

In contrast to the extremely polarized expansion in tip growing cells, the growth of cells embedded in tissues is considered “diffuse”, i.e. not restricted to specific subcellular locations. The distinction between tip growth and diffuse growth, however, is not always that clear (Geitmann and Ortega 2009). Indeed, the “diffuse” growth pattern can be either homogeneous or non-homogeneous, referred to as “homotropic” and “heterotropic” respectively. In the latter case, local foci with increased growth rate, reminiscent of tip growth, create irregular deformation patterns, underlying the large variety of cell shapes that can be found in plants (Figure 5).

One well-studied example of heterotropic growth is that of the formation of the lobes of puzzle-shape pavement cells in the epidermis of Arabidopsis leaves or cotyledons (Figure 5A). In these cells, the appearance of inhomogeneities in cell wall deformation patterns depends, like in root hairs and pollen tubes, on ROP signaling modules, which not only promote the formation of specific membrane domains that locally organize the cytoskeleton and secretory events (Liu et al. 2021), but also other processes crucial for

growth, such as protein translation (through TARGET OF RAPAMYCIN (TOR) signaling upon activation by the plant hormone auxin) (Schepetilnikov et al. 2017).

This raises the question of how the position of these ROP domains is determined. A recent elegant study, also building upon data presented by Bidhendi et al. (2019), showed that stress patterns may provide initial positional cues (Belteton et al. 2021). They first used a 3D FEM model, elaborated from the observed geometry of a few epidermal cells, to show that, in developing pavement cells (Figure 5A), highest stress is found along the anticlinal (perpendicular to the surface) cell walls. Next, using time lapse imaging, the authors showed that the symmetry breaking event underlying lobe initiation was preceded by the local and transient formation of transfacial cortical microtubule bundles, facing both anticlinal and external periclinal (parallel to the surface) cell walls in the cell located at the convex site of the future lobe (Figure 5B). Using the 3D FEM model, the site of the transfacial microtubule accumulation could be predicted from the local maxima of the mechanical stress patterns, which are the resultant of the mechanical anisotropy of the walls of the cell and the anisotropy of the supracellular stress patterns within the organ (Box 1). It has been shown that cortical (i.e. right below the plasma membrane) microtubules align with maximum stress patterns through a so far unknown mechanism (Hamant et al. 2019). These microtubules in turn guide the trajectories of the cellulose synthase complexes, which move in the plasma membrane while depositing stiff microfibrils. The latter reinforce the cell wall along the stress patterns thus changing the anisotropy in wall mechanics and overall stress patterns and, as a result, the orientation of surface deformation (Hamant

et al. 2008; Mirabet et al. 2011). Consistent with this, also in pavement cells, the microtubule bundles facing the anticlinal walls co-localized with GFP-tagged cellulose synthase complexes. The authors proposed a model in which the cellulose microfibrils deposited in the anticlinal wall of the cell at the convex side of the future lobe, create a “patch of anisotropic wall”. The subsequent local anisotropic cell wall deformation, perpendicular to the microfibril orientation, would then drive lobe formation (Belteton et al. 2021).

This study provides a nice example of how mechanical stress patterns, predicted from a geometrical model, may provide initial positional cues for local cell wall deformation patterns, which may be sharpened and reinforced by ROP-mediated feedback signaling. The study, however, also points to inconsistencies in current models for diffuse cell expansion, in particular how turgor-driven cell wall relaxation can lead to lobe formation given that adjacent cells most likely have comparable turgor pressures. This is even more striking for the formation of furrows in cells in which cell adhesion was prevented (by treatment with low doses of pectinases, removing the middle lamella, or in mutants with reduced cell adhesion). These furrows consist of closely opposed walls from the same cell connected by a widened bulb at the apex, that grow into the cell (Verger et al. 2018; Belteton et al. 2021). The driving force for such ingrowths cannot be the turgor, but reflects an intrinsic capacity of the cell wall to increase its surface. A similar conclusion was drawn from the study of natural cell wall ingrowths observed in a number of cell types (Borowska-Wykret and Kwiatkowska 2018) (Figure 5C, D). The authors proposed a model in which ingrowth initiation is driven by the swelling of the

matrix between the oriented microfibrils in the anticlinal cell walls, selectively on the convex side of the lobe. Finally, turgor-driven cell wall expansion is also an unlikely mechanism for the formation of Rhizobium infection threads (Rae et al. 1992) or fungal infection structures that progress against the turgor pressure of the host cell (Huckelhoven 2007). Here again the driving force for cell wall deformation appears to come from the insertion and expansion of cell wall material.

A potential molecular mechanism for turgor-independent cell wall deformation was suggested by a recent study on Arabidopsis pavement cells (Haas et al. 2020). This study used super-resolution microscopy with anti-HG antibodies to reveal that HG, instead of forming an amorphous gel, can show a striking degree of organization, at least in anticlinal cell walls. Indeed, the epitopes for a subset of these antibodies (2F4, LM20 and LM19) formed filaments parallelling the cellulose microfibrils. Stacked parallel chains of HG are expected to form semi-crystalline fibrils, such as those that have been reported previously (Figure 3). Interestingly, other anti-HG antibodies (JIM7) did not reveal such filaments (Haas et al. 2020). This indicates that first, the observed filaments were not artifactual and second, different HG populations with a distinct mesoscale organisation must exist in the same cell wall. The study also showed a higher proportion of low/high DM HG in the wall on the convex side of the lobe. Given the above-mentioned increased distance between polymers in crystals of low DM HG relative to those of high DM HG, the authors proposed a mechanism in which HG de-methylesterification induces a phase transition causing the cell wall to swell. Since this occurred preferentially on one side of the cell wall, a lobe was expected to form. Indeed,

lobe formation could be simulated with a FEM model based on this mechanism. The causality between PME activity, the HG phase transition and lobe formation was suggested by the inhibition of all these events by the inducible overexpression of a PMEI in transgenic Arabidopsis. In addition, inducible overexpression of a PME promoted the accumulation of low DM HG on both sides of the wall, increased the distance between fibrils observed by SEM and promoted cell expansion while preventing lobe formation. The latter was expected given the lack of asymmetry in the connected opposing cell walls.

It should be noted that lobe formation occurs during the expansion of the entire cell. This requires differential expansion rates across an already expanding wall. If we assume that the expansion of the straight walls is also, at least in part, driven by pectin insertion and swelling, lobe formation could arise from enhanced activation of PME activity on the convex side or, perhaps more likely, from the inhibition of de-methylesterification on the concave side. It will therefore be interesting to see whether the local cell wall pH differs between the two opposing cell walls at the concave and convex sides, since it would affect the activities of PMEs and other growth regulators.

Together, the study of lobe and furrow formation suggests a turgor-independent driving force for cell wall deformation based on the conversion of chemical energy into mechanical energy associated with the de-methylesterification of HG.

New food for thought

In summary, three models for cell expansion are currently being proposed in different contexts: (i) the biomechanical hotspot model for acid growth in diffusely growing cells, (ii) the Ca^{2+}-pectate cross-link exchange model for Chara and tip-growing cells and (iii) the expanding pectin filament model for lobe formation in pavement cells. The question is whether these models explain cell expansion only in certain cell wall types or whether they are part of an integrated processing system in which overlapping, synergistic or antagonistic chemo-mechanical phenomena and control points might modulate cell wall expansion, with some aspects becoming dominant in some cell types/developmental stages/environmental conditions and others being completely absent in other instances. The above discussions showed that the latter possibility is the more likely one. Indeed, we discussed the requirement for XG-cellulose cross-link removal in tip-growing root hairs, in addition to Ca^{2+}-pectate cross-link exchange. Inversely, in diffusely growing cells, genetic evidence showed a key growth-promoting role for pectin de-methylesterification in a variety of contexts (Peaucelle et al. 2008, 2011, 2015; Andres-Robin et al. 2018; Haas et al. 2020; Wachsman et al. 2020; Jonsson et al. 2021; Stefanowicz et al. 2021), suggesting that Ca^{2+}-pectate exchange and/or pectin swelling also occurs in these cells, although more complex scenarios involving signaling or PME-dependent cell wall acidification impinging on cellulose-XG remodeling cannot be excluded. In pavement cells, FEM modeling showed that cell wall deformation driven by swelling pectins would create additional stresses in particular in external periclinal cell walls (Haas et al., 2020), and perhaps also between younger and older cell wall layers, which would require relaxation for instance through XG-cellulose cross-link removal.

Finally, pectin swelling upon de-methylesterification might also contribute to turgor-driven expansion. For instance, cell wall swelling was observed in Arabidopsis root epidermis cells upon auxin or brassinosteroid treatment (Elgass et al. 2009).

The study of tip growth showed the importance of the surface pH and ROS oscillations that contribute to expansion and cross-linking cycles leading to the formation of periodic cell wall deposition patterns. Similar processes could explain the deposition of layered cell walls of diffusely growing cells, although such oscillations would be more difficult to observe.

The observation of oriented HG chains and pectin fibrils raises the question how the HG chains can reach their uni-axial orientation. Is this through interaction with similarly oriented cellulose? Pectin-cellulose interactions have indeed been observed *in muro* using solid-state NMR on never-dried cell wall preparations (Wang et al. 2015). Also, fractionation studies revealed a pectin fraction strongly bound to the cellulosic residue. Interestingly, this fraction and not the chelator-extractable pectin fraction had disappeared in a seedling lethal mutant for the GalA transferase GAUT1 (Mohnen in this book), suggesting the existence of a specialized biosynthetic pathway. How pectin is bound to cellulose is not known, but it may be through xylan (Ralet et al. 2016), arabinan or galactan side chains (Zykwinska et al. 2005) of RG-I or even through covalent links that have been observed between HG and XG (Stratilová et al. 2020) or cellulose (Broxterman and Schols 2018) respectively. Alternative orientating mechanisms are not excluded, such as self-assembly through a nematic crystalline

phase (Sanchez et al. 2012; Saw et al. 2018) or perhaps through exocytosis by vesicular-tubular structures reminiscent of those observed in neurons or in suberizing plant cells (De Bellis et al. 2021).

Finally, the presence of uni-axially oriented HG filaments, the swelling of which contributes to the growth direction of single-layered anticlinal cell walls, corresponds more to a growth mechanism by intussusception than chemorheological growth. The above-mentioned statement of Bonner in 1934 (Bonner 1934) definitely cannot be extrapolated to all cell/tissue types, or species !

Acknowledgements

The authors acknowledge the financial support by the University of Antwerp (BOF-DOCPRO4) and the Research Foundation Flanders (FWO Post-doc fellowship) to KV and ANR project ANR-20-CE11-0008, HOMEOWALL to HH. The IJPB benefits from the support of Saclay Plant Sciences-SPS (ANR-17-EUR-0007).

References

Akkerman M, Franssen-Verheijen MAW, Immerzeel P, Den Hollander L, Schel JHN, Emons AMC (2012) Texture of cellulose microfibrils of root hair cell walls of *Arabidopsis thaliana*, *Medicago truncatula,* and *Vicia sativa*. *Journal of Microscopy* 247:60-67

Ali S, Søndergaard CR, Teixeira S, Pickersgill RW (2015) Structural insights into the loss of catalytic competence in pectate lyase activity at low pH. *FEBS Letters* 589:3242-3246

Anderson C T, Wallace IS, Somerville CR (2012) Metabolic click-labeling with a fucose analog reveals pectin delivery, architecture, and dynamics in Arabidopsis cell walls. *Proceedings of the National Academy of Sciences of the USA* 109:1329-1334

Andres-Robin A, Reymond MC, Dupire A, Battu V, Dubrulle N, Mouille G, Lefebvre V, Pelloux J, Boudaoud A, Traas J, Scutt CP, Monéger F (2018) Evidence for the regulation of gynoecium morphogenesis by *ETTIN* via cell wall dynamics. *Plant Physiology* 178:1222-1232

Arend M, Muninger M, Fromm J (2008) Unique occurrence of pectin-like fibrillar cell wall deposits in xylem fibres of poplar. *Plant Biology* 10-763-770

Aryal B, Jonsson K, Baral A, Sancho-Andres G, Routier-Kierzkowski A-L, Kierzkowski D, Bhalerao R (2020) Interplay between cell wall and auxin mediates the control of differential cell elongation during apical hook development. *Current Biology* 30:1733-1739

Baker LG, Specht CA, Donlin MJ, Lodge JK (2007) Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in Cryptococcus neoformans. *Eukaryotic Cell* 6:855-867

Balcerowicz D, Schoenaers S, Vissenberg K (2015) Cell fate determination and the switch from diffuse growth to planar polarity in Arabidopsis root epidermal cells. *Frontiers in Plant Science* 6:1–13

Baluška F, Salaj J, Mathur J, Braun M, Jasper F, Šamaj J, Chua NH, Barlow PW, Volkmann D (2000) Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-supported F-actin meshworks accumulated within expansin-enriched bulges. *Developmental Biology* 227: 618–632

Band LR, Wells DM, Larrieu A, Sun J, Middleton AM, French AP, Brunoud G, Mendocilla Sato E, Wilson MH, Péret B, Oliva M, Swarup R, Sairanen I, Parry G, Ljung K, Beeckman T, Garibaldi JM, Estelle M, Owen MR, Vissenberg K, Hodgman TC, Pridmore TP, King JR, Vernoux T, Bennett MJ (2012) Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. *Proceedings of the National Academy of Sciences of the USA* 109:4668-4673

Barbez E, Dünser K, Gaidora A, Lendl T, Busch W (2017) Auxin steers root cell expansion via apoplastic pH regulation in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the USA* 114:4884-4893

Belteton SA, Li W, Yanagisawa M, Hatam FA, Quinn MI, Szymanski MK, Marley MW, Turner JA, Szymanski DB (2021) Real-time conversion of tissue-scale mechanical forces into an interdigitated growth pattern. *Nature Plants* 7:989

Bernal AJ, Yoo C-M, Mutwil M, Jensen JK, Hou G, Blaukopf C, Sørensen I, Blancaflor EB, Scheller HV, Willats WGT (2008) Functional analysis of the cellulose synthase-like genes *CSLD1*, *CSLD2*, and *CSLD4* in tip-growing Arabidopsis cells. *Plant Physiology* 148:1238–1253

Bernhardt C, Tierney ML (2000) Expression of AtPRP3, a proline-rich structural cell wall protein from Arabidopsis, is regulated by cell-type-specific developmental pathways involved in root hair formation. *Plant Physiology* 122:705–714

Bidhendi AJ, Altartouri B, Gosselin FP, Geitmann A (2019) Mechanical stress initiates and sustains the morphogenesis of wavy leaf epidermal cells. *Cell Reports* 28:1237-1250

Bonner J (1934) Studies on the growth hormone of plants. V. The relation of cell elongation to cell wall formation. *Proceedings of the National Academy of Sciences of the USA* 20:393-397

Boisson-Dernier A, Roy S, Kritsas K, Grobei MA, Jaciubek M, Schroeder JI, Grossniklaus U (2009) Disruption of the pollen-expressed FERONIA homologs ANXUR1 and ANXUR2 triggers pollen tube discharge. *Development* 136:3279–3288

Boron AK, Orden JV, Markakis MN, Mouille G, Adriaensen D, Verbelen J-P, Höfte H, Vissenberg K (2014) Proline-rich protein-like PRPL1 controls elongation of root hairs in Arabidopsis thaliana. *Journal of Experimental Botany* 65:5485-5495

Borowska-Wykręt D, Kwiatkowska D (2018). Folding, Wrinkling, and Buckling in Plant Cell Walls. In *Plant Biomechanics*, eds Geitmann A, Gril J, 209-233. Springer, Cham.

Bove J, Vaillancourt B, Kroeger J, Hepler PK, Wiseman PW, Geitmann A (2008) Magnitude and direction of vesicle dynamics in growing pollen tubes using spatiotemporal image correlation spectroscopy and fluorescence recovery after photobleaching. *Plant Physiology* 147:1646–1658

Boyer JS (2016) Enzyme-less growth in Chara and terrestrial plants. *Frontiers in Plant Science* 7:866

Broxterman SE, Schols HA (2018) Interactions between pectin and cellulose in primary plant cell walls. *Carbohydrate Polymers* 192:263-272

Cannon MC, Terneus K, Hall Q, Tan L, Wang Y, Wegenhart BL, Chen L, Lamport DT, Chen Y, Kieliszewski MJ (2008) Self-assembly of the plant cell wall requires an extensin scaffold. *Proceedings of the Nationall Academy of Sciences of the USA* 105:2226–2231

Cao M, Chen R, Li P, Yu Y, Zheng R, Ge D, Zheng W, Wang X, Gu Y, Gelova Z, Friml J, Zhang H, Liu R, He J, Xu T (2019) TMK1-mediated auxin signalling regulates differential growth of the apical hook. *Nature* 568:240–243

Capila I, Linhardt RJ (2002) Heparin-protein interactions. *Angewandte Chemie International Edition* 41:391–412

Carol RJ, Takeda S, Linstead P, Durrant MC, Kakesova H, Derbyshire P, Drea S, Zarsky V, Dolan L (2005) A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. *Nature* 438:1013-1016)

Carpin S, Crévecoeur M, de Meyer M, Simon P, Greppin H, Penel C (2001) Identification of a Ca^{2+}-pectate binding site on an apoplastic peroxidase. *The Plant Cell* 13:511-520

Castle ES (1958) The topography of tip growth in a plant cell. *Journal of General Physiology* 41:913–925

Cavalier DM, Lerouxel O, Neumetzler L, Yamauchi K, Reinecke A, Freshour G, Zabotina OA, Hahn MG, Burgert I, Pauly M, Raikhel NV, Keegstra K (2008) Disrupting two Arabidopsis thaliana xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component. *The Plant Cell* 20:1519-1537

Cho H-T, Cosgrove DJ (2000) Altered expression of expansin modulates leaf growth and pedicel abscission in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* 97:9783-9788

Cholodny N (1926) Beiträge Zur Analyse Der Geotropischen Reaktion. *Jahrbücher für Wissenschaftliche Botanik* 65:447–459

Cosgrove DJ (2000) Loosening of plant cell walls by expansins. *Nature* 407:321-326

Cosgrove DJ (2018) Diffuse growth of plant cell wall. *Plant Physiology* 176:16-27

Cosgrove DJ, Anderson CT (2020) Plant cell growth: do pectins drive lobe formation in Arabidopsis pavement cells? *Current Biology* 30:R660-662

Cosgrove DJ, Li LC, Cho H-T, Hoffmann-Benning S, Moore RC, Blecker D (2002) The growing world of expansins. *Plant and Cell Physiology* 43:1436–1444

Damineli DSC, Portes MT, Feijó JA (2017) Oscillatory signatures underlie growth regimes in Arabidopsis pollen tubes: computational methods to estimate tip location, periodicity, and synchronization in growing Cells. *Journal of Experimental Botany* 68:3267-3281

Darwin CR (1880) The Power of Movement in Plants. London: Murray J.

De Bellis D, Kalmbach L, Marhavy P, Daraspe J, Geldner N, Barberon M (2022) Extracellular vesiculo-tubular structures associated with suberin deposition in plant cell walls. *Nature Communications* 13:1489

Derksen J, Janssen GJ, Wolters-Arts M, Lichtscheidl I, Adlassnig W, Ovecka M, Doris F, Steer M (2011) Wall architecture with high porosity is established at the tip and maintained in growing pollen tubes of Nicotiana tabacum. *The Plant Journal* 68:495–506

Domozych DS, Popper ZA, Sørensen I (2016) Charophytes: evolutionary giants and emerging model organisms. *Frontiers in Plant Science* 7:1–8

Draeger C, Fabrice TN, Gineau E, Mouille G, Kuhn BM, Moller I, Abdou MT, Frey B, Pauly M, Bacic A, Ringli C (2015) Arabidopsis leucine-rich repeat extensin (LRX) proteins modify cell wall composition and influence plant growth. *BMC Plant Biology* 15:1–11

Du M, Spalding ES, Gray WM (2020) Rapid auxin-mediated cell expansion. *Annual Review of Plant Biology* 71:379-402

Duan Q, Kita D, Li C, Cheung AY, Wu HM (2010) FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. *Proceedings of the National Academy of Sciences of the USA* 107:17821–17826

Dumais J (2013) Modes of deformation of walled cells. *The Journal of Experimental Botany* 64:4681-4695

Dumais J (2021) Mechanics and hydraulics of pollen tube growth. *New Phytologist* 232:549–1565

Elgass K, Caesar K, Schleifenbaum F, Stierhof YD, Meixner AJ, Harter K (2009) Novel application of fluorescence lifetime and fluorescence microscopy enables quantitative access to subcellular dynamics in plant cells. *PLoS One 4:e5716*

Endler A, Persson S (2011) Cellulose synthases and synthesis in Arabidopsis. *Molecular Plant* 4:199-211

Estelle M (1996) Plant tropisms: the ins and outs of auxin. *Current Biology* 6:1589-1591

Favery B, Ryan E, Foreman J, Linstead P, Boudonk K, Steer M, Shaw P, Dolan L (2001) KOJAK encodes a cellulose synthase-like protein required for root hair cell morphogenesis in *Arabidopsis*. *Genes and Development* 15:79-89

Fayant P, Girlanda O, Chebli Y, Aubin CEE, Villemure I, Geitmann A (2010) Finite Element Model of polar growth in pollen tubes. *The Plant Cell* 22:2579–2593

Fendrych M, Leung J, Friml J (2016) TIR1/AFB-Aux/IAA auxin perception mediates rapid cell wall acidification and growth of Arabidopsis hypocotyls. *Elife* 14:e19048

Feijó JA. Sainhas J, Hackett GR, Kunkel JG, Hepler PK (1999) Growing pollen tubes possess a constitutive alkaline band in the clear zone and a growth-dependent acidic tip. *Journal of Cell Biology* 144:483–496

Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 422:442-446

Francoz E, Ranocha P, Le Ru A, Martinez Y, Fourquaux I, Jauneau A, Dunand C, Burlat V (2019) Pectin Demethylesterification generates platforms that anchor peroxidases to remodel plant cell wall domains. *Developmental Cell* 48:261–276

Friml J, Benková E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K (2002) AtPIN4 mediates sink-driven auxin gradients and root patterning in Arabidopsis. *Cell* 108:661-673

Galway ME, Eng RC, Schiefelbein JW, Wasteneys GO (2011) Root hair-specific disruption of cellulose and xyloglucan in *AtCSLD3* mutants, and factors affecting the post-rupture resumption of mutant root hair growth. *Planta* 233:985–999

Geitmann A, Li YQ, Cresti M (1995) Ultrastructural immunolocalization of periodic pectin depositions in the cell wall of Nicotiana tabacum pollen tubes. *Protoplasma* 187:168–171

Geitmann A, Ortega JKE (2009) Mechanics and modeling of plant cell growth. *Trends in Plant Science* 14:467–478

Goh H-H, Sloan J, Malinowski R, Fleming A (2014) Variable expansin expression in Arabidopsis leads to different growth responses. *Journal of Plant Physiology* 171:329-339

Grantham NJ, Wurman-Rodrich J, Terrett OM, Lyczakowski JJ, Stott K, Luga D, Simmons TJ, Durand-Tardiff M, Brown SP, Dupree R, Busse-Wicher M, Dupree P (2017) An even pattern of xylan substitution is critical for interaction with cellulose in plant cell walls. *Nature Plants* 3:859-865

Green PB (1973) Morphogenesis of the cell and organ axis-biophysical models. *Brookhaven Symposium in Biology* 25:166–190.

Grignon C, Sentenac H (1991) pH and ionic conditions in the apoplast. *Annual Review of Plant Physiology and Plant Molecular Biology* 42:103-128

Haas KT, Wightmann R, Meyerowitz EM, Peaucelle A (2020) Pectin homogalacturonan nanofilament expansion drives morphogenesis in plant epidermal cells**.** *Science* 367:1003-1007

Hála M, Cole R, Synek L, Drdová E, Pečenková T, Nordheim A, Lamkemeyer T, Madlung J, Hochholdinger F, Fowler JE, Žárský V (2008) An exocyst complex functions in plant cell growth in Arabidopsis and Tobacco. *The Plant Cell* 20:1330–1345

Hamant O, Heisler MG, Jönsson H, Krupinski P, Uyttewaal M, Bokov P, Corson F, Sahlin P, Boudaoud A Meyerowitz EM, Couder Y, Traas J (2008) Developmental patterning by mechanical signals in *Arabidopsis*. *Science* 322:1650-1655

Hamant O, Inoue D, Bouchez D, Dumais J, Mjolsness E (2019) Are microtubules stress sensors? *Nature Communications* 10:2360

Hepler PK, Vidali L, Cheung AY (2001) Polarized growth in higher plants. *Annual Review of Cell and Developmental Biology* 17:159-187

Hepler PK, Lovy-Wheeler A, McKenna ST, Kunkel JG (2006) Ions and pollen tube growth. *Plant Cell Monographs* 3:47–69

Hocq L, Pelloux J, Lefebvre V (2017) Connecting homogalacturonan-type pectin remodeling to acid growth. *Trends in Plant Science* 22: 20–29

Hocq L, Habrylo O, Voxeur A, Pau-Roblot C, Safran J, Sénéchal F,Fournet F, Bassard S, Battu V, Demailly H, Tovar JC, Pilard S, Marcelo P, Savary BJ, Mercadante D, Njo MF, Beeckman T, Boudaoud A, Pelloux J, Lefèbvre V (2021) Arabidopsis AtPME2 has a pH-dependent processivity and control cell wall mechanical properties. *BioRXiv* **doi:** https://doi.org/10.1101/2021.03.03.433777

Höltje JV (1998) Growth of the stress-bearing and shape-maintaining murein sacculus of Escherichia coli. *Microbiology and Molecular Biology Reviews* 62:181-203

Hoffmann RD, Portes MT, Olsen LI, Damineli DSC, Hayashi M, Nunes CO, Pedersen JT, Lima PT, Campos C, Feijó JA, Palmgren M (2020) Plasmamembrane H^+-ATPases sustain pollen tube growth and fertilization. *Nature Communications* 11:2395

Hückelhoven R (2007) Cell wall-associated mechanisms of disease resistance and susceptibility. *Annual Review of Phytopathology* 45:101-127

Hwang JU, Wu G,Yan A, Lee YL, Grierson CS, Yang Z (2010) Pollen-tube tip growth requires a balance of lateral propagation and global inhibition of Rho-Family GTPase activity. *Journal of Cell Science* 123:340–350

Jaffe LA, Weisenseel MH, Jaffe LF (1975) Calcium accumulations within the growing tips of pollen tubes. *The Journal of Cell Biology* 67:488–492

Jamet E, Boudart G, Borderies G, Charmont S, Lafitte C, Rossignol M, Canut H, Pont-Lezica R (2008) Isolation of plant cell wall proteins. In *2D PAGE: Sample preparation and fractionation*, eds. Posch A. Series: Methods in Molecular Biology 425:187-201, Humana Press

Jiang L, Yang SL, Xie LF, Puah CS, Zhang XQ, Yang WC, Sundaresan V, Ye D (2005) VANGUARD1 encodes a pectin methylesterase that enhances pollen tube growth in the Arabidopsis style and transmitting tract. *The Plant Cell* 2005 17:584-596

Jones MA, Shen JJ, Fu Y, Li H, Yang Z, Grierson CS (2002) The Arabidopsis Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. *The Plant Cell* 14:763-776

Jonsson K, Lathe RS, Kierzkowksi D, Routier-Kierzskowski A-L, Hamant O, Bhalerao R (2021) Mechanochemical feedback mediates tissue bending required for seedling emergence. *Current Biology* 31:1154-1164

Ketelaar T, Galway ME, Mulder BM, Emons AMC (2008) Rates of exocytosis and endocytosis in Arabidopsis root hairs and pollen tubes. *Journal of Microscopy* 231:265–273

Kroeger J, Geitmann A (2012) The pollen tube paradigm revisited. *Current Opinion in Plant Biology* 15:618–624

Kneen M, Farinas J, Li Y, Verkman AS (1998) Green Fluorescent Protein as a noninvasive intracellular pH indicator. *Biophysical Journal* 74:1591-1599

Kroeger JH, Geitmann A, Grant M (2008) Model for calcium dependent oscillatory growth in pollen tubes. *Journal of Theoretical Biology* 253:363–374

Kroeger JH, Zerzour R, Geitmann A (2011) Regulator or driving force? The role of turgor pressure in oscillatory plant cell growth. *PLoS ONE* 6:e18549

Leppard GG, Colvin JR, Rose D, Martin SM (1971) Lignofibrils on the external cell wall surface of cultured plant cells. *Journal of Cell Biology* 50:63-80

Li H, Lin Y, Heath RM, Zhu MX, Yang Z (1999). Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. *The Plant Cell* 11:1731–1742

Li H, Luo N, Wang W, Liu Z, Chen J, Zhao L, Tan L, Wang C, Qin Y, Li C, Xu T, Yang Z (2018) The REN4 rheostat dynamically coordinates the apical and lateral domains of Arabidopsis pollen tubes. *Nature Communications* 9:2573

Li YQ, Chen F, Linskens HF, Cresti M (1994) Distribution of unesterified and esterified pectins in cell walls of pollen tubes of flowering plants. *Sexual Plant Reproduction* 7:145–152

Liao C-Y, Smet W, Brunoud G, Yoshida S, Vernoux T, Weijers D (2015) Reporters for sensitive and quantitative measurement of auxin response. *Nature Methods* 12:207-210

Lin C, Choi H-S, Cho H-T (2011) Root hair-specific EXPANSIN A7 is required for root hair elongation in *Arabidopsis*. *Molecular Cells* 31:393-397

Lin W, Zhou W, Tang W, Takahashi K, Pan X, Dai J, Ren H, Zhu X, Pan S, Zheng H, Gray WM, Xu T, Kinoshita T, Yang Z (2021) TMK-based cell-surface auxin signaling activates cell-wall acidification. *Nature* 599:278-282

Liu W, Xu L, Lin H, Cao J (2021) Two expansin genes, *AtEXPA4* and *AtEXPB5*, are redundantly required for pollen tube growth and *AtEXPA4* is involved in primary root elongation in *Arabidopsis thaliana. Genes* 12:249

Lockhart JA (1965) An analysis of irreversible plant cell elongation. *Journal of Theoretical Biology* 8:264–275

Lopez-Hernandez F, Tryfona T, Rizza A, Yu XL, Harris MOB, Webb AAR, Kotake T, Dupree P (2020) Calcium binding by arabinogalactan polysaccharides is important for normal plant development. *The Plant Cell* 32:3346-3369

Ma L, Li G (2019) Auxin-dependent cell elongation during the shade avoidance response. *Frontiers in Plant Science 10*:914

Macquet A, Ralet M-C, Loudet O, Kronenberger J, Mouille G, Marion-Poll A, North HM (2007) A naturally occurring mutation in an Arabidopsis accession affects a beta-D-galactosidase that increases the hydrophilic potential of rhamnogalacturonan I in seed mucilage. *The Plant Cell* 19:3990-4006.

Maris A, Suslov D, Fry SC, Verbelen J-P, Vissenberg K (2009) Enzymic characterization of two recombinant xyloglucan endotransglucosylase/hydrolase (XTH) proteins of Arabidopsis and their effect on root growth and cell wall extension. *Journal of Experimental Botany* 60:3959-3972

Maris A, Kaewthai N, Eklöf JM, Miller JG, Brumer H, Fry SC, Verbelen J-P, Vissenberg K (2011) Characterization of five recombinant xyloglucan endotransglucosylase/hydrolase (XTH) proteins of Arabidopsis reveals specific enzymatic properties. *Journal of Experimental Botany* 62:261-271

Marowa P, Ding A, Kong Y (2016) Expansins: roles in plant growth and potential applications in crop development. *Plant Cell Reports* 35:949-965

Marzec M, Szarejko I, Melzer M (2015) Arabinogalactan proteins are involved in root hair development in Barley. *Journal of Experimental Botany* 66:1245–1257

McFarlane HE, Doring A, Persson S (2014) The cell biology of cellulose synthesis. *Annual Review of Plant Biology* 65:69–94

McKenna ST, Kunkel JG, Bosch M, Rounds CM, Vidali L, Winship LJ, Hepler PK (2009) Exocytosis precedes and predicts the increase in growth in oscillating pollen tubes. *The Plant Cell* 21:3026–3040

McQueen-Mason S, Durachko DM, Cosgrove DJ (1992) Two endogenous proteins that induce cell wall extension in plants. *The Plant Cell* 4:1425–1433

Messiaen J, Van Cutsem P (1999) Polyamines and pectins. II. Modulation of pectic-signal transduction. *Planta* 208:247-256

Mirabet V, Das P, Boudaoud A, Hamant O (2011) the role of mechanical forces in plant morphogenesis. *Annual Review of Plant Biology* 62:365-385

Molendijk AJ, Bischoff F, Rajendrakumar CS, Friml J, Braun M, Gilroy S, Palme K (2001) Arabidopsis thaliana Rop GTPases are localized to tips of root hairs and control polar growth. *The EMBO Journal* 20:2779-2788

Møller SR, Yi X, Velásquez SM, Gille S, Hansen PML, Poulsen CP, Olsen CE, Rejzek M, Parsons H, Yang Z, Wandall HH, Clausen H, Field RA, Pauly M, Estevez JM, Harholt J, Ulvskov P, Petersen BL (2017) Identification and evolution of a plant cell wall specific glycoprotein glycosyl transferase, ExAD. *Scientific Reports* 7:1–16

Mollet JC, Kim S, Jauh GY, Lord EM (2002) Arabinogalactan proteins, pollen tube growth, and the reversible effects of Yariv phenylglycoside. *Protoplasma* 219:89–98

Monshausen GB, Bibikova TN, Messerli MA, Shi C, Gilroy S (2007) Oscillations in extracellular pH and reactive oxygen species modulate tip growth of Arabidopsis root hairs. *Proceedings of the National Academy of Sciences of the USA* 104:20996-21001

Moustacas AM, Nari J, Borell M, Noat G, Ricard J (1991) Pectin methylesterase: metal ions and plant cell wall extension. The role of metal ions in plant cell wall extension. *Journal of Biochemistry* 279:351–354

Nägeli C (1864) Über den inneren Bau der vegetabilischen Zellmembranen. Sitzungsberichte Der Königl, Bayer Akademie Der Wissenschaften Zu München 1:282–323

Newcomb EH, Bonnett HT (1965) Cytoplasmic microtubule and wall microfibril orientation in root hairs of radish. *Journal of Cell Biology* 27:575-589

Nibau C, Wu HM, Cheung AY (2006) RAC/ROP GTPases: 'hubs' for signal integration and diversification in plants. *Trends in Plant Science* 11:309-315

Nicolas WJ, Fassler F, Dutka P, Schur FKM, Jensen G, Meyerowitz E, Jensen G (2022) Cryo-electron tomography of the onion cell wall shows bimodally oriented cellulose fibers and reticulated homogalacturonan networks. *Current Biology* 32:1–15

Noh B, Murphy AS, Spalding EP (2001) Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *The Plant Cell* 13:2441-2454

Noorifar N, Savoian MS, Ram A, Lukito Y, Hassing B, Weikert TW, Moerschbacher BM, Scott B (2021) Chitin deacetylases are required for *Epichloë festucae* endophytic cell wall remodeling during establishment of a mutualistic symbiotic interaction with *Lolium perenne*. *Molecular Plant Microbe Interactions* 34:1181-1192

Osada Y, Gong JP (1993) Stimuli-responsive polymer gels and their application to chemomechanical systems. *Progress in Polymer Science* 18:187-226

Palmer KJ, Merrill RC, Owens HS, Ballantyne M (1947) An X-ray diffraction investigation of pectinic and pectic acids. *Journal of Physical Chemistry* 51:710–720

Pang CY, Wang H, Pang Y, Xu C, Jiao Y, Qin YM, Western TL, Yu SX, Zhu YX (2010) Comparative proteomics indicates that biosynthesis of pectic precursors is important for cotton fiber and Arabidopsis root hair elongation. Molecular and Cellular Proteomics 9:2019-1033

Park S, Szumlanski AL, Gu FW, Guo F, Nielsen E (2011) A role for CSLD3 during cell-wall synthesis in apical plasma membranes of tip-growing root-hair cells. *Nature Cell Biology* 13:973–980

Park YB, Cosgrove DJ (2012) A revised architecture of primary cell walls based on biomechanical changes induced by substrate-specific endoglucanases. *Plant Physiology* 158:1933-1943

Pauly M, Albersheim P, Darvill A, York WS (1999) Molecular domains of the cellulose/xyloglucan network in the cell walls of higher plants. *The Plant Journal* 20:629-639

Peaucelle A, Louvet R, Johansen JN, Höfte H, Laufs P, Pelloux J, Mouille G (2008) Arabidopsis phyllotaxis is controlled by the methyl-esterification status of cell-wall pectins. *Current Biology* 18:1943–1948

Peaucelle A, Braybrook S, Guillou L, Bron E, Kuhlemeier C, Höfte H (2011). Pectin-induced changes in cell wall mechanics underlie organ initiation in Arabidopsis. *Current Biology* 21:1720-1726

Peaucelle A, Wightman R, Höfte H (2015) The control of growth symmetry breaking in the arabidopsis hypocotyl. *Current Biology* 25:1746–1752

Peña MJ, Kong Y, York WS, O'Neill MA. (2012) A galacturonic acid-containing xyloglucan is involved in Arabidopsis root hair tip growth. *The Plant Cell* 24:4511-4524

Picton JM, Steer MW (1983) Evidence for the role of Ca^{2+} ions in tip extension in pollen tubes. *Protoplasma* 115:11–17

Pien S, Wyrzykowska J, McQueen-Mason SJ (2001) local expression of expansin induces the entire process of leaf development and modifies cell shape. *Proceedings of the National Academy of Sciences of the USA* 98:11812-11817

Pierson ES, Miller DD, Callaham DA, Van Aken J, Hackett G, Hepler PK (1996) Tip-localized calcium entry fluctuates during pollen tube growth. *Developmental Biology* 174:160–173

Popper ZA, Michel G, Hervé C, Domozych DS, Willats WGT, Tuohy MG, Kloareg B, Stengel DB (2011) Evolution and diversity of plant cell walls: from algae to flowering plants. *Annual Review of Plant Biology* 62:567-590

Potter HB (1897) Streaming movements of the protoplasm in pollen of flowers. *Nature* 1446:248

Rakusová H, Gallego-Bartolomé J, Vanstraelen M, Robert HS, Alabadí D, Blázquez MA, Benková E, Friml J (2012) Polarization of PIN3-dependent auxin transport for hypocotyl gravitropic response in *Arabidopsis thaliana*. *The Plant Journal* 67:817-826

Rae AL, Bonfante-Pasolo P, Brewin NJ (1992) Structure and growth of infection threads in the legume symbiosis with *Rhizobium leguminosarum*. *The Plant Journal* 2:385-395

Ralet M-C, Dronnet V, Thibault J-F, Della Valle G, Popineau Y (2011) Enzymatcally and chemically de-esterified lime pectins: physico-chemical characterisation, polyelectrolyte behaviour and calcium binding properties. In *Plant polymer science : food and non-food applications*, eds. Renard D, D Valle G, Popineau Y, 55–62. Royal Society of Chemistry

Ralet M-C, Crépeau M-J, Vigouroux J, Tran J, Berger A, Sallé C, Granier F, Botran L, North H (2016) Xylans provide the structural driving force for mucilage adhesion to the Arabidopsis seed coat. *Plant Physiology* 171:165–178

Ray PM (1967) Radioautographic study of cell wall deposition in growing plant cells. *The Journal of Cell Biology* 35:659–674

Rayle DL, Cleland RE (1970) Enhancement of wall loosening and elongation by acid solutions. *Plant Physiology* 46:250–253

Rayle DL, Cleland RE (1992) The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiology* 99:1271-1274

Refrégier G, Pelletier S, Jaillard D, Höfte H (2004) Interaction between wall deposition and cell elongation in dark-grown hypocotyl cells in Arabidopsis. *Plant Physiology* 135:959-968

Reiss HD, Herth W (1985) Nifedipine-sensitive calcium channels are involved in polar growth of Lily pollen tubes. *Journal of Cell Science* 76:247–254

Rodriguez-Furlán C, Salinas-Grenet H, Sandoval O, Recabarren C, Arraño-Salinas P, Soto-Alvear S, Orellana A, Blanco-Herrera F (2016) The root hair specific SYP123 regulates the localization of cell wall components and contributes to Rizhobacterial priming of induced systemic resistance. *Frontiers in Plant Science* 7:1–14

Roelofsen PA, Houwink AL (1953) Architecture and growth of the primary cell wall in some plant hairs and in the phycomyces sporangiophore. *Acta Botanica Néerlandica* 2:218–125

Roelofsen PA, Kreger DR (1951) The sub-microscopic structure of pectin in collenchyma cell-walls. *Journal of Experimental Botany* 2:332-343

Rojas ER, Hotton S, Dumais J (2011) Chemically mediated mechanical expansion of the pollen tube cell wall. *Biophysical Journal* 101:1844-1853

Rounds CM, Lubeck E, Hepler PK, Winship LJ (2011) Propidium iodide competes with Ca^{2+} to label pectin in pollen tubes and Arabidopsis root hairs. *Plant Physiology* 157:175–187

Růžička K, Ljung K, Vanneste S, Podhorská R, Beeckman T, Friml J, Benková E (2007) Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. *The Plant Cell* 19:2197-2212

Sachs J (1874) *Lehrbuch der Botanik nach dem gegenwärtigen Stand der Wissenschaft*. Leipzig: Verlag von Wilhelm Engelmann.

Sanchez T, Chen DT, DeCamp SJ, Heymann M, Dogic Z (2012) Spontaneous motion in hierarchically assembled active matter. *Nature* 491:431-434.

Saw TB, Xi W, Ladoux B, Lim CT (2018) Biological tissues act as active nematic liquid crystals. *Advanced Materials* 30:1802579

Schepetilnikov M, Makarian J, Srour O, Geldreich A, Yang Z, Chicher J, Hammann P, Ryabova LA (2017) GTPase ROP2 binds and promotes activation of target of rapamycin, TOR, in response to auxin. *The EMBO Journal* 36:886–903

Schnabelrauch LS, Kieliszewski MJ, Upham BL, Alizedeh H, Lamport DTA (1996) Isolation of pI 4.6 extensin peroxidase from tomato cell suspension cultures and identification of Val-Tyr-Lys as putative intermolecular cross-link site. *The Plant Journal* 9:477–489

Schoenaers S, Balcerowicz D, Breen G, Hill K, Zdanio M, Mouille G, Holman TJ, Oh J, Wilson MH, Nikonorova N, Vu LD, De Smet I, Swarup R, De Vos WH, Pintelon I, Adriaensen D, Grierson C, Bennett MJ, Vissenberg K (2018) The auxin-regulated CrRLK1L kinase ERULUS controls cell wall composition during root hair tip growth. *Current Biology* 28:722-732

Schoenaers S, Balcerowicz D, Vissenberg K (2017) Molecular mechanisms regulating root hair tip growth: a comparison with pollen tubes. In *Pollen tip growth: from*

biophysical aspects to systems biology, eds Obermeyer G, Feijó JA, 167-243. Berlin, Heidelberg, New York: Springer

Sentenac H, Grignon C (1981) A model for predicting ionic equilibrium concentrations in cell walls. *Plant Physiology* 68:415-419

Šola K, Gilchrist EJ, Ropartz D, Wang L, Feussner I, Mansfield SD, Ralet MC, Haughn GW (2019) RUBY, a putative galactose oxidase, influences pectin properties and promotes cell-to-cell adhesion in the seed coat epidermis of Arabidopsis. *The Plant Cell* 31: 809–831

Staal M, De Cnodder T, Simon D, Vandenbussche F, van Der Straeten D, Verbelen J-P, Vissenberg K (2011) Apoplastic alkalinization is instrumental for the inhibition of cell elongation in the Arabidopsis root by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid. *Plant Physiology* 155:2049-2055

Stefanowicz K, Szymanska-Chargot M, Truman W, Walerowski P, Olszak M, Augustyniak A, Kosmala A, Zdunek A, Malinowski R (2021) *Plasmodiophora brassicae*-triggered cell enlargement and loss of cellular integrity in root systems are mediated by pectin demethylation. *Frontiers in Plant Science* 12:711838

Sterling C. (1957) Structure of oriented gels of calcium polyuronates. *Biochimica et Biophysica Acta* 26:186–197

Strasburger E (1882) *Ueber den Bau und das Wachstum der Zellhäute*. Jena: Verlag von Gustav Fischer

Stratilová B, Sestak S, Mravec J, Garajová S, Pakanová Z, Vadinová K, Kucerová D, Kozmon S, Schwerdt JG, Shirley N, Stratilová E, Hrmova M (2020) Another building block in the plant cell wall: Barley xyloglucan xyloglycosyl transferases link

covalently xyloglucan and anionic oligosaccharides derived from pectin. *The Plant Journal* 104:752-767

Suslov D, Vissenberg K (2018) Cell wall expansion as viewed by the creep method. In *Plant Biomechanics - From structure to function at multiple scales*, eds Geitmann A, Gril J, 305-320. Berlin Heidelberg New York: Springer Verlag

Suslov D, Verbelen J-P, Vissenberg K (2009) Onion epidermis as a new model to study the control of growth anisotropy in higher plants. *Journal of Experimental Botany* 60:4175–4187

Swarup R, Perry P, Hagenbeek D, van Der Straeten D, Beemster GTS, Sandberg G, Bhalerao R, Ljung K, Bennett MJ (2007) Ethylene upregulates auxin biosynthesis in Arabidopsis seedlings to enhance inhibition of root cell elongation. *The Plant Cell* 19:2186-2196

Tan L, Eberhard S, Pattathil S, Warder C, Glushka J, Yuan C, Hao Z, Zhu X, Avci U, Miller JS, Baldwin D, Pham C, Orlando R, Darvill A, Hahn MG, Kieliszewski MJ, Mohnen D (2013) An Arabidopsis cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan Protein. *The Plant Cell* 25:270–287

Tanaka T (1981) Gels. *Scientific American* 244:124–138

Tanaka T, Annaka M, Ilmain F, Ishii K, Kokufuta E, Suzuki A, Tokita M (1992) Phase transitions of gels. In *Mechanics of Swelling*, ed Karalis TK, 683-703. Berlin, Heidelberg: NATO ASI Series Springer

Valentin R, Cerclier C, Geneix N, Aguie-Beghin V, Gaillard C, Ralet MC, Cathala B (2010) Elaboration of extensin-pectin thin film model of primary plant cell wall. *Langmuir* 26:9891–9898

Tian G-B, Adams-Haduch JM, Bogdanovich T, Wanh H-N, Doi W (2011) PME1, an extended-spectrum β-lactamase identified in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* 55:2710-2713

Velasquez SM, Ricardi MM, Dorosz JG, Fernandez PV, Nadra AD, Pol-Fachin L, Egelund J, Gille S, Harholt J, Ciancia M, Verli H, Pauly M, Bacic A, Olsen CE, Ulvskov P, Petersen BL, Somerville C, Iusem ND, Estevez JM (2011) O-glycosylated cell wall proteins are essential in root hair growth. *Science* 332:1401–1403

Velasquez SM, Marzol E, Borassi C, Pol-Fachin L, Ricardi MM, Mangano S, Juarez SPD, Salgado Salter JD, Dorosz JG, Marcus SE, Knox JP, Dinneny JR, Iusem ND, Verli H, Estevez JM (2015a) Low sugar is not always good: impact of specific O-glycan defects on tip growth in Arabidopsis. *Plant Physiology* 168:808–813

Velasquez SM, Ricardi MM, Poulsen CP, Oikawa A, Dilokpimol A, Halim A, Mangano S, Juarez SPD, Marzol E, Salgado Salter JD, Dorosz JG, Borassi C, Möller SR, Buono R, Ohsawa Y, Matsuoka K, Otegui MS, Scheller HV, Geshi N, Petersen BL, Iusem ND, Estevez JM (2015b) Complex regulation of prolyl-4-hydroxylases impacts root hair expansion. *Molecular Plant* 8:734–746

Verger S, Long Y, Boudaoud A, Hamant O (2018) A tension-adhesion feedback loop in plant epidermis. *Elife* 7:e34460

Vincent RRR, Mansel BW, Kramer A, Kroy K, Williams MAK (2013) Micro-rheological behaviour and nonlinear rheology of networks assembled from polysaccharides from the plant cell wall. *New Journal of Physics* 15:35002

Vissenberg K, Martinez-Vilchez IM, Verbelen J-P, Miller JG, Fry SC (2000) In vivo colocalisation of xyloglucan endotransglycosylase activity and its donor substrate in the elongation zone of Arabidopsis roots. *The Plant Cell* 12:1229-1237

Vissenberg K, Fry SC, Verbelen J-P (2001) Root hair initiation is coupled to a highly localized increase of xyloglucan endotransglycosylase action in Arabidopsis roots. *Plant Physiology* 127:1125–1135

Vissenberg K, Van Sandt V, Fry SC, Verbelen J-P (2003) Xyloglucan endo-transglucosylase action is high in the root elongation zone and in the trichoblasts of all vascular plants from *Selaginella* to *Zea mays*. *Journal of Experimental Botany* 54:335-344

Vissenberg K, Fry SC, Pauly M, Höfte H, Verbelen J-P (2005) XTH acts at the microfibril-matrix interface during cell elongation. *Journal of Experimental Botany* 56:673-683

Voxeur A, Habrylo O, Guénin S, Miart F, Soulié M-C, Rihouey C, Pau-Roblot C, Domon J-M, Gutierrez L, Pelloux J, Mouille G, Fagard M, Höfte H, Vernhettes S (2019) Oligogalacturonide production upon Arabidopsis thaliana-Botrytis cinerea interaction. *Proceedings of the National Academy of Sciences of the USA* 116:19743-19752

Wachsman G, Zhang J, Moreno-Risueno MA, Anderson CT, Benfey PN (2020) Cell wall remodeling and vesicle trafficking mediate the root clock in *Arabidopsis*. *Science* 370:819-823

Wang M, Yuan D, Gao W, Li Y, Tan J, Zhang X (2013) A comparative genome analysis of PME and PMEI families reveals the evolution of pectin metabolism in plant cell walls. *PLoS ONE* 8:e72082

Walkinshaw MD, Arnott S (1981a) Conformations and interactions of pectins. *Journal of Molecular Biology* 153:1075–1085.

Walkinshaw MD, Arnott S (1981b) Conformations and interactions of pectins. *Journal of Molecular Biology* 153:1055–1073

Wang T, Park YB, Caporini MA, Rosay M, Zhong L, Cosgrove DJ, Hong M (2013) Sensitivity-enhanced solid-state NMR detection of expansin's target in plant cell walls. *Proceedings of the National Academy of Sciences of the USA* 110:16444-16449

Wang T, Park YB, Cosgrove DJ, Hong M (2015) Cellulose-pectin spatial contacts are inherent to never-dried Arabidopsis primary cell walls: evidence from solid-state nuclear magnetic resonance. *Plant Physiology* 168:871-884

Weisenseel M, Kicherer RM (1981) Ionic currents as control mechanism in cytomorphogenesis. In *Cytomorphogenesis in Plants*, ed. Kiermayer O. Cell Biology Monographs 8: 379-399. Springer.

Weisenseel M, Nuccitelli R, Jaffe LF (1975) Large electrical currents traverse growing pollen tubes. *Journal of Cell Biology* 66:556–567

Went FW (1928) Wuchsstoff und Wachstum. *Recueil des travaux botaniques néerlandais* 25:1–116

Went FW, Thimann KV (1937) *Phytohormones*. New York: Macmillan Press Ltd.

Wilson LA, Deligey F, Wang T, Cosgrove DJ (2021) Saccharide analysis of onion outer epidermal walls. *Biotechnology for Biofuels* 14:66

Wolf S, Mouille G, Pelloux J (2009) Homogalacturonan methyl-esterification and plant development. *Molecular Plant* 2:851–860

Wu J, Kurten EL, Monshausen G, Hummel GM, Gilroy S, Baldwin IT (2007) NaRALF, a peptide signal essential for the regulation of root hair tip apoplastic pH in Nicotiana attenuata, is required for root hair development and plant growth in native soils. *The Plant Journal* 52:877–890

Yalovsky S, Bloch D, Sorek N, Kost B (2008) Regulation of membrane trafficking, cytoskeleton dynamics, and cell polarity by ROP/RAC GTPases. *Plant Physiology* 147:1527-1543

Yan A, Xu G, Yang ZB (2009) Calcium participates in feedback regulation of the oscillating ROP1 Rho GTPase in pollen tubes. *Proceedings of the National Academy of Sciences of the USA* 106:22002–22007

Yang J, Bak G, Burgin T, Barnes WJ, Mayes HB, Peña MJ, Urbanowicz BR, Nielsen E (2020) Biochemical and genetic analysis identify CSLD3 as a beta-1,4-glucan synthase that functions during plant cell wall synthesis. *The Plant Cell* 32:1749–1767

Zablackis E, Huang J, Muller B, Darvill AG, Albersheim P (1995) Characterization of the cell-wall polysaccharides of Arabidopsis thaliana leaves. *Plant Physiology* 107:1129-1138

Zhang T, Mahgsoudy-Louyeh S, Tittmann B, Cosgrove DJ (2013) Visualization of the nanoscale pattern of recently-deposited cellulose microfibrils and matrix materials in never-dried primary walls of the onion epidermis. *Cellulose* 21:853–862

Zhang T, Vavylonis D, Durachko DM, Cosgrove DJ (2017) Nanoscale movements of cellulose microfibrils in primary cell walls. *Nature Plants* 3:17056

Zhang Y, Yu J, Wang X, Durachko DM, Zhang S, Cosgrove DJ (2021) Molecular insights into the complex mechanics of plant epidermal cell walls. *Science* 711:706–711

ZhiMing Y, Bo K, XiaoWei H, ShaoLei M, YouHuang B, WoNa D, Ming C, Hyung-Taeg C, Ping W (2011) Root hair-specific expansins modulate root hair elongation in rice. *The Plant Journal* 66:725-734

Zabotina OA, van de Ven WTG, Freshour G, Drakakaki G, Cavalier D, Mouille G, Hahn MG, Keegstra K, Raikhel H (2008) Arabidopsis XXT5 gene encodes a putative α-1,6-xylosyltransferase that is involved in xyloglucan biosynthesis. *The Plant Journal* 56:101-115

Zsivanovits G, MacDougall AJ, Smith AC, Ring SG (2004) Material properties of concentrated pectin networks. Carbohydrate Research 339:1317-1322

Zykwinska AW, Ralet MCJ, Garnier CD, Thibault J-FJ (2005) Evidence for in vitro binding of pectin side chains to cellulose. *Plant Physiology* 139:397–407

Figure legends

Figure 1: Models explaining (an)isotropic growth (adapted from Dumais (2013) with permission)

A) The three-for-one model of peptidoglycan wall assembly. The wall assembly machinery travels along one docking glycan strand, and depolymerizes the docking strand as it progresses forward. Meanwhile, it is synthesizing three new strands to replace it, resulting in a net growth of two glycan chains.

B) Intussusception in a cell wall with ordered cell wall material. New material is deposited between existing material, separating former neighboring material and inducing cell wall growth in one direction during the process of intussusception. (Brown crosses and dotted lines depict the deposition of new cell wall material by synthase proteins/complexes, also in C)).

C) Chemorheological growth of a cell wall with randomly-organised cell wall material. New material becomes deposited and concomitantly chemical bonds between cell wall components are broken and reformed, leading to turgor-driven isotropic growth.

D) Anisotropic growth. Cell wall deformation is anisotropic when a cell wall with anisotropic material properties undergoes isotropic stress. (The thickness of the arrows reflect the level of stress in that direction, also in E))

E) Anisotropic growth. Cell wall deformation is anisotropic when an isotropic cell wall is suspect of anisotropic stress.

Figure 2: Phase separation in ionized polymer gels (Adapted from Tanaka et al. (1992)).

A) Large volume transitions (here in acrylamide gel beads) are triggered by subtle changes in the environment. From left to right : gradually increasing changes in environmental conditions lead to abrupt changes in the volume of the bead.

B) Large volume transition in a gel induced by minute changes in the hydrophobicity of the medium.

Figure 3: Visualization of fibrillar pectin.

A) Axially oriented pectin fibrils in fresh collenchyma cell walls of *Petasites vulgaris* petioles, as visualized by transmission electron microscopy (TEM). (Adapted from Roelofsen and Kreger (1951))

B) Non-cellulosic fibrillar galacturonic acid pointing out from the cell wall into the extracellular medium of liquid plant cell cultures, as visualized by TEM. (From Leppard et al. (1971), with permission)

C) Ropelike aggregate at the cell wall surface of *Phaseolus* culture cells, as visualized by TEM (From Leppard et al. (1971), with permission).

D) TEM images of pectin fibrils in the lumen of poplar xylem fiber cells. (From Arend et al. (2008), with permission)

E) Super-resolution microscopy showing uni-axially oriented HG in the anticlinal cell walls of pavement cells in the Arabidopsis epidermis. (From Haas et al. (2020)).

Figure 4: Calcium-pectate exchange model to explain tip growth.

A) Two DIC-images taken at different moments during oscillating pollen tube tip growth. The upper image shows a thick, and the lower a thin apical cell wall. (Images from McKenna et al. (2011), with permission)

B) Schematic representation of events happening at the tip during pollen tube tip growth. (1) Vesicles containing highly methylesterified pectin move towards the tip. The cell wall contains load-bearing calcium-pectate cross-links. (2) Methylesterified pectin is secreted into the inner cell wall layers, creating a periplasmic space, pushing the cell wall a bit forward and creating the thick cell wall appearance (see panel A, upper image). (3) PME activity de-methylesterifies the newly deposited pectin. (4) This de-methylesterified pectin competes for calcium-ions with the older de-methylesterified and load-bearing pectins and becomes integrated in the existing pectin matrix, emptying the periplasmic space and thinning the cell wall (see panel A, lower image). By dissociating the calcium-bonds and recreating new bonds with the young de-methylesterified pectin, the older pectins can yield under turgor pressure and are pushed aside, leading to cell wall growth. The cycle can now restart from (1). (Modified after McKenna et al. (2011), with permission)

C) More detailed view on the events explained in B) happening at the pollen tube tip (from Rojas et al. (2011), with permission). Load-bearing cross-links are altering positions in the wall, from the old pectin located more in the outer cell wall to the newly incorporated pectins more to the inner side of the wall. The

model is based on 3 key concepts: 1) cross-link dissociation allows turgor pressure to stretch (and thin) the cell wall, 2) newly deposited methylesterified and uncross-linked pectin serves as a reservoir of material that waits to be incorporated into the cross-linked network by dissociating existing cross-links, after being de-methylesterified by PMEs, and 3) cross-links reform at the same rate as they are broken, but in a relaxed state.

Figure 5: Lobe formation in pavement cells and ingrowth development in pine needles

A) Different stages of pavement cell morphogenesis. Rounded protodermal cells (top) grow and develop lobes (middle) generating cells with complex shapes (bottom). (Redrawn from Cosgrove and Anderson (2020), with permission)

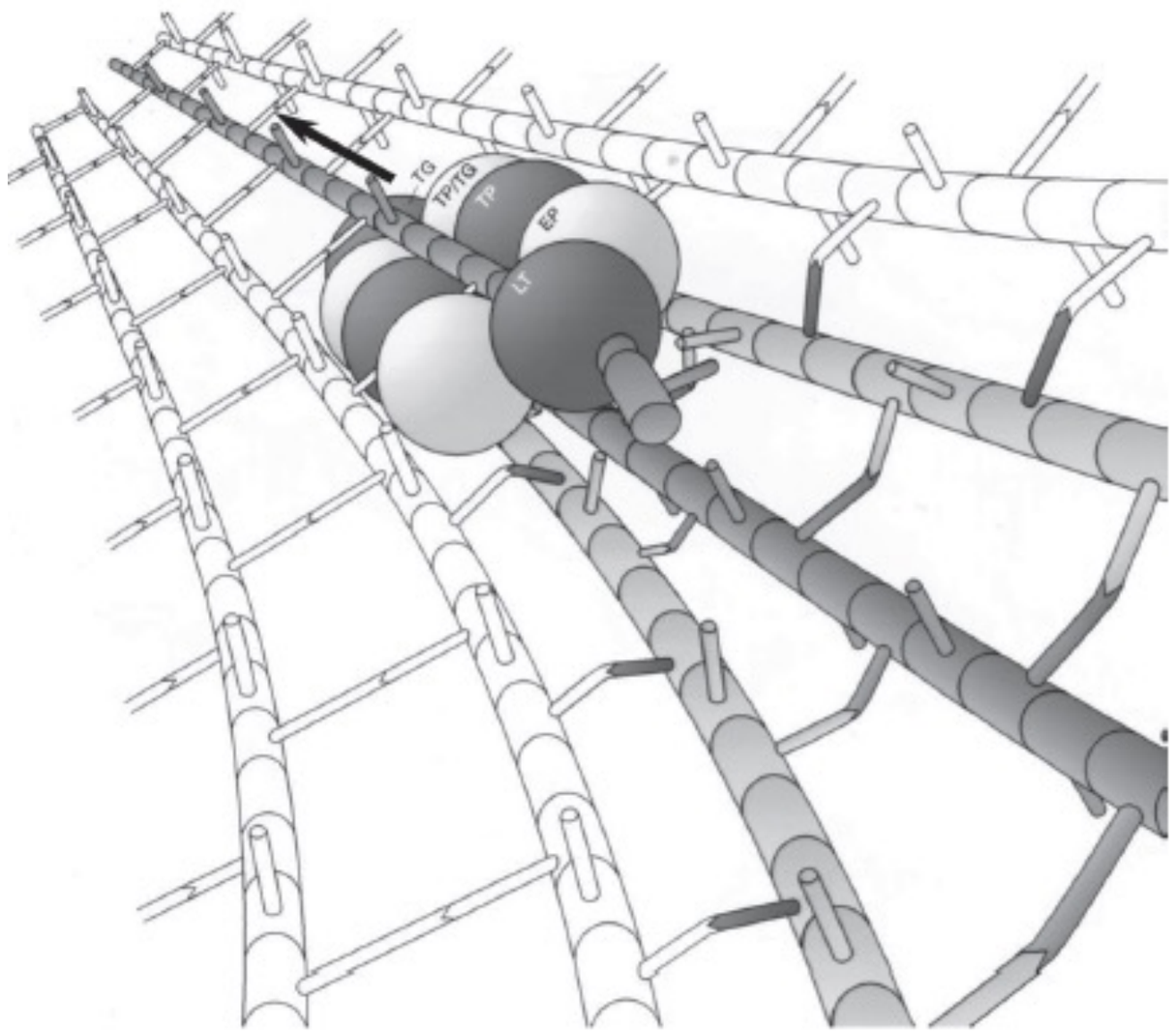
B) Involvement of cell wall components in the formation of lobes (structures not drawn to scale). Methylesterified HG forms vertical nanofibrils in the concave surfaces of the anticlinal cell walls of neck regions, interspersed with or connected to adjacent cellulose fibrils. The convex surfaces of the anticlinal walls of lobes are rich in de-methylesterified HG nanofibrils. (Redrawn from Cosgrove and Anderson (2020), with permission).

C) Pine needle mesophyll morphogenesis. a) Three adjacent mesophyll cells (C1-3) forming one mesophyll plate. In C1 one wall invagination (In) terminated by a loop (L) is labeled. b) Fragment of a needle cut along the vascular bundle (Vsc) with two exemplary mesophyll plates (MPl) spreading between the vascular bundle and the epidermis (Ep). A large gas space

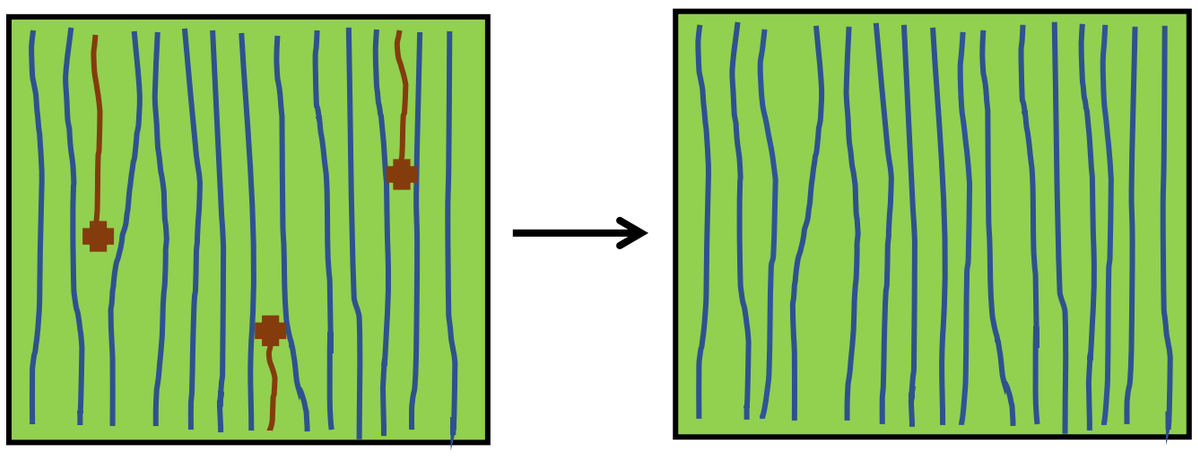
(GSp) is present between the plates. c) Ingrowth initiation in one of two contacting cell walls due to the swelling of the local wall thickening (B1 in c) is followed by the expansion of the ingrowth assisted by the stresses generated by the B1 layer (c', c"). (From Borowska-Wykret and Kwiatkowska (2018), with permission)

D) Jigsaw puzzle-shaped epidermal cells. (a, b) images showing optical paradermal sections of the leaf epidermis in Arabidopsis (a) and maize (b). Scale bars 20 μm. c) At the site of lobe (Lb) formation, cellulose microfibrils (blue/gray lines) form bundles in the anticlinal wall (AW) of cell 1 (C1), into which lumen the wall is bending. The cellulose microfibril bundles extend radiating into the outer (PWo) and inner periclinal walls (PWi) of C1. Pads (Pa) are formed at the junctions between the anticlinal and periclinal walls. d) Asymmetric swelling in one of the single walls comprising a compound wall fragment with fixed ends leads to the buckling of the wall (B1, A1, A2 in d). As a result, a lobe is initiated (d'). (From Borowska-Wykret and Kwiatkowska (2018), with permission).

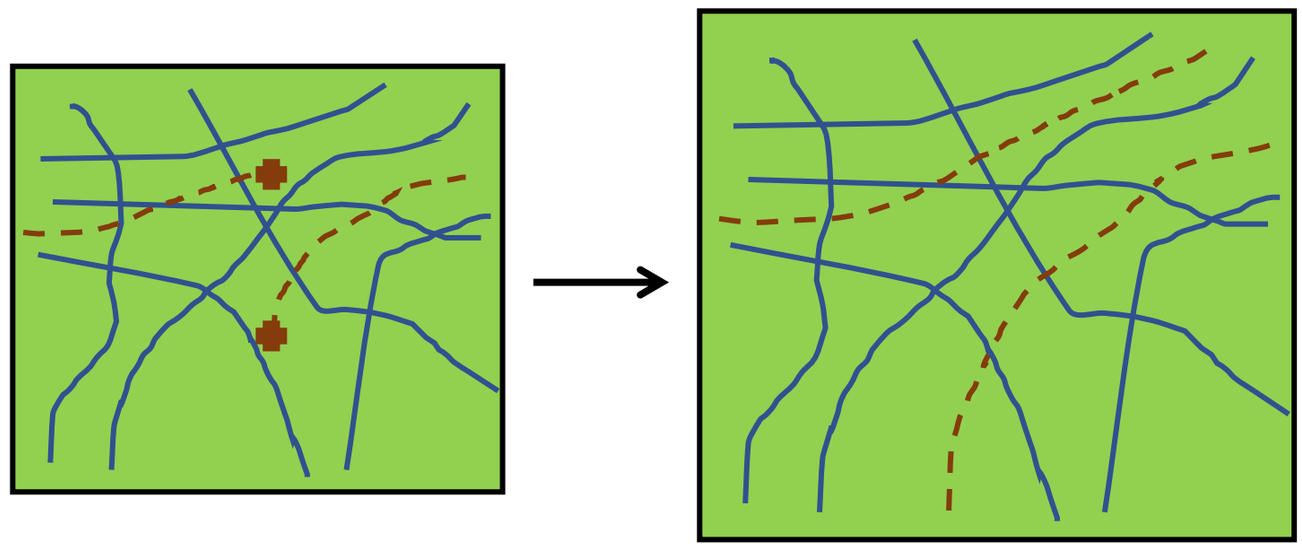
A



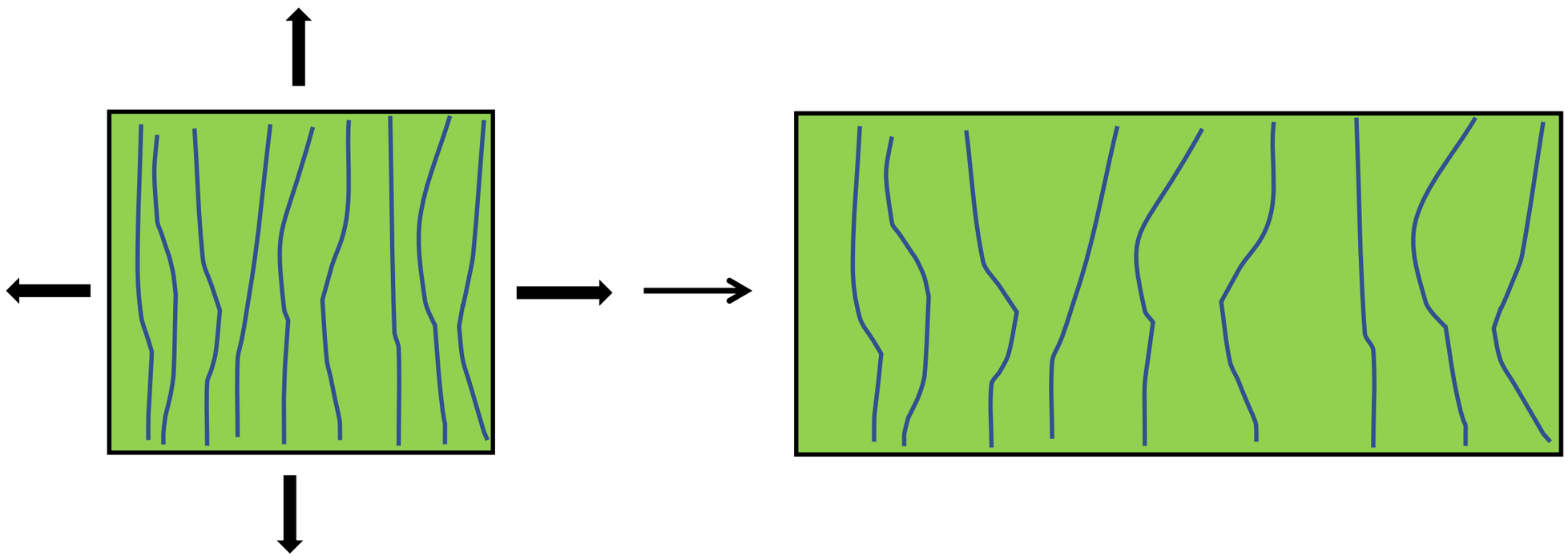
B **Processive intussusception**



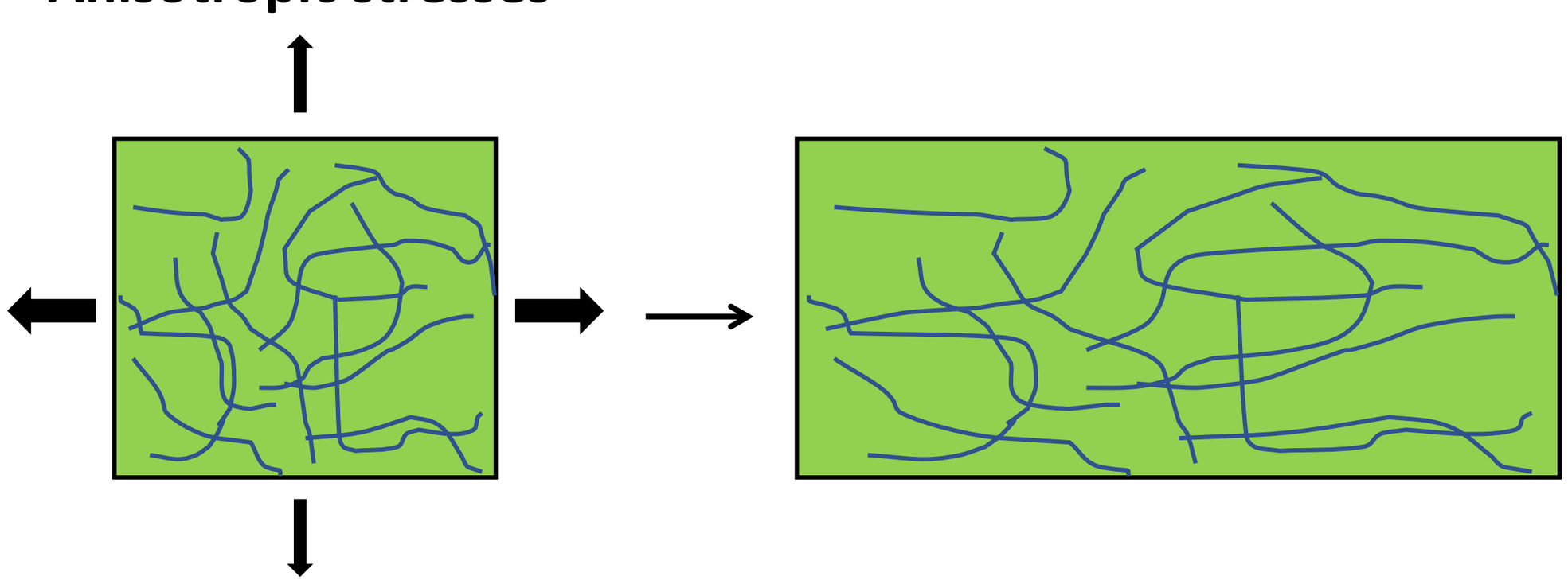
C **Chemorheological growth**



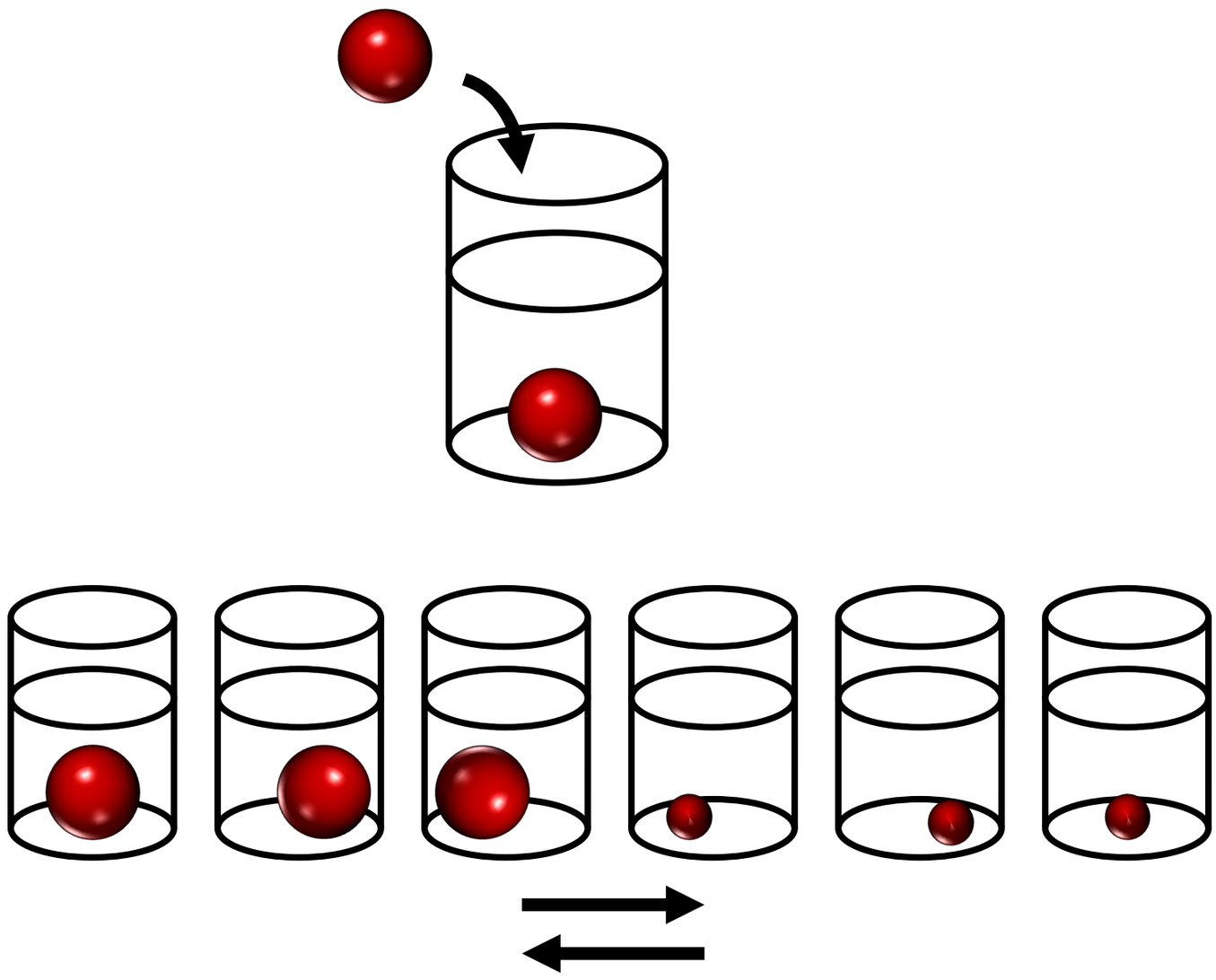
D **Anisotropic material properties**



E **Anisotropic stresses**



A



temperature, solvent composition, pH, ions
electric field, UV, light, specific molecules, or
chemicals

B

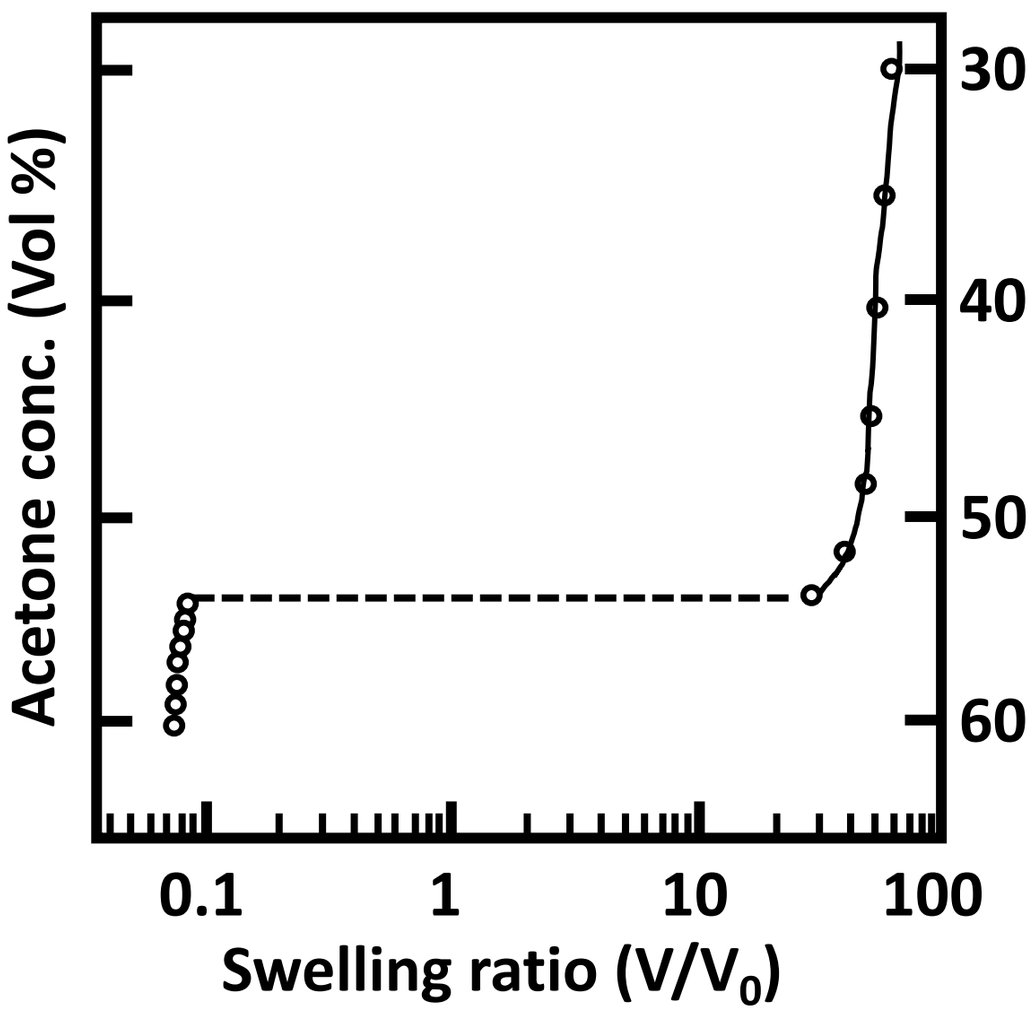


Figure 2

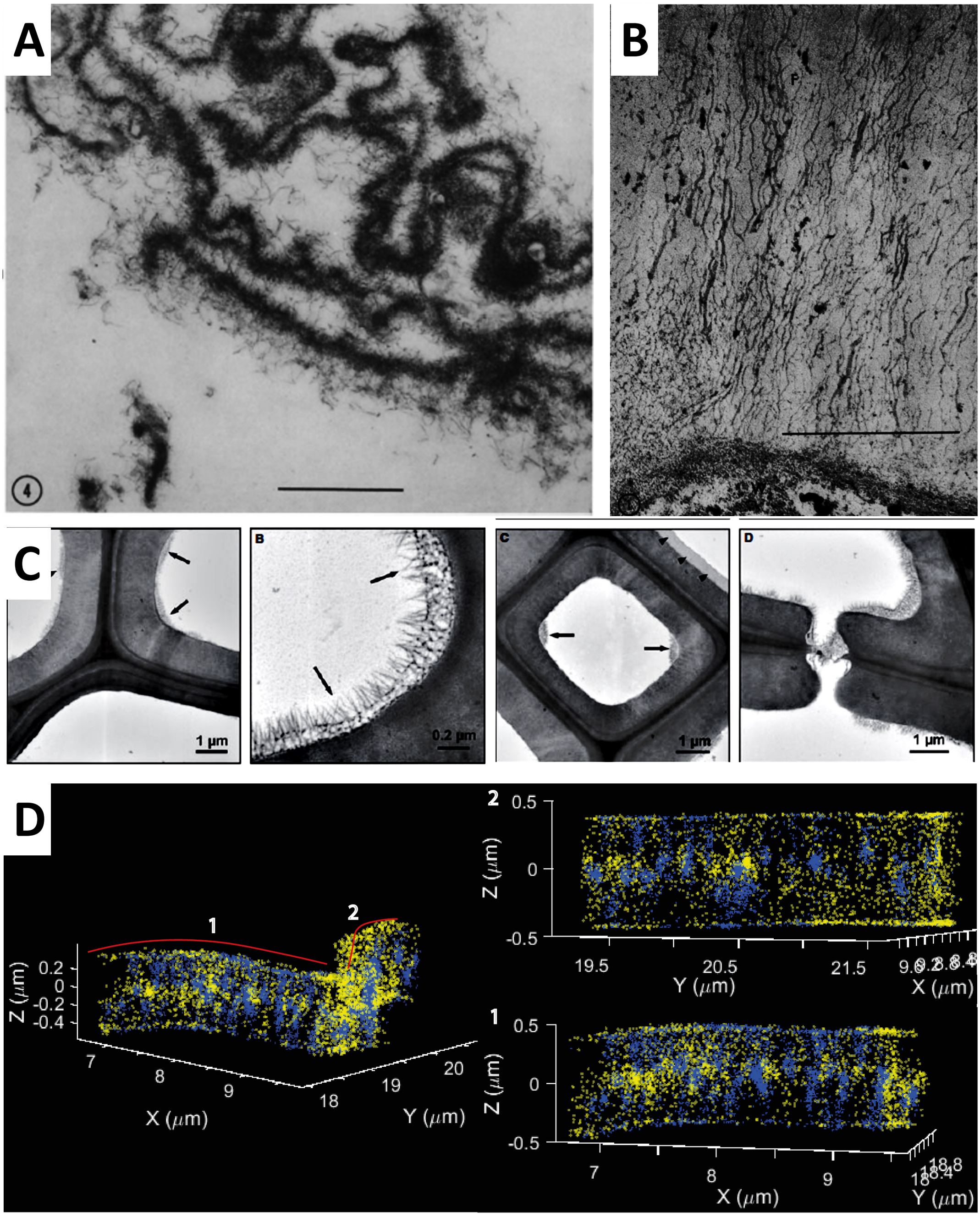
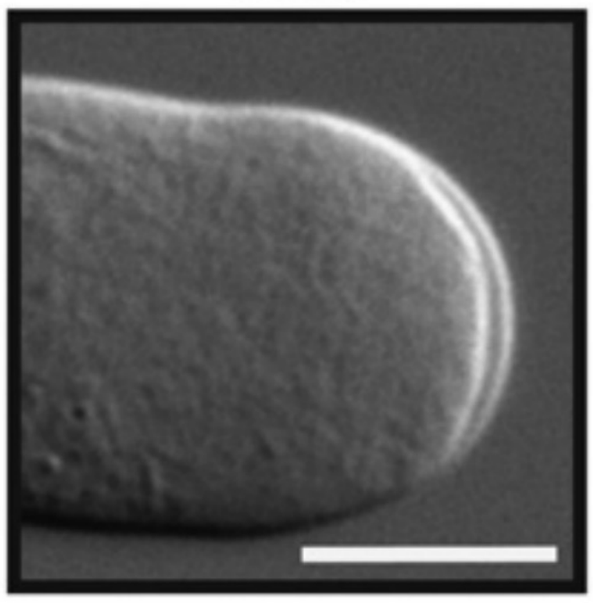

A
B
P
C
B
1 µm
0.2 µm
C
1 µm
D
1 µm
D
2
0.5
0
-0.5
Z (µm)
19.5
20.5
21.5
Y (µm)
X (µm)
1
2
0.2
0
-0.2
-0.4
Z (µm)
7
8
9
18
19
20
X (µm)
Y (µm)
1
0.5
0
-0.5
Z (µm)
7
8
9
X (µm)
Y (µm)

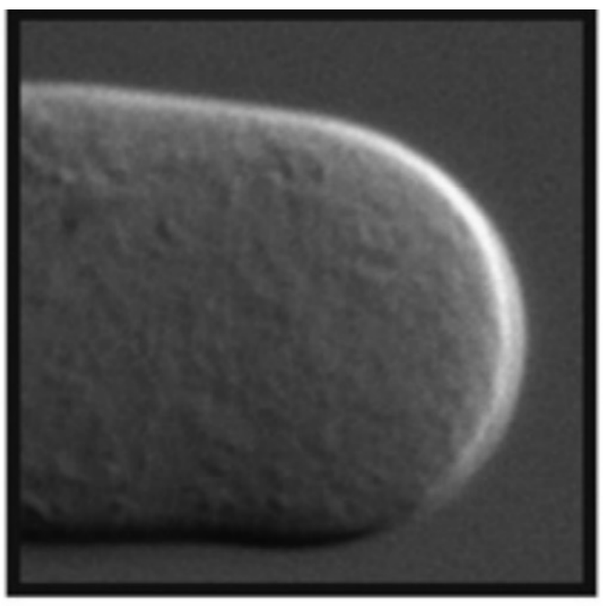
Figure 3

A

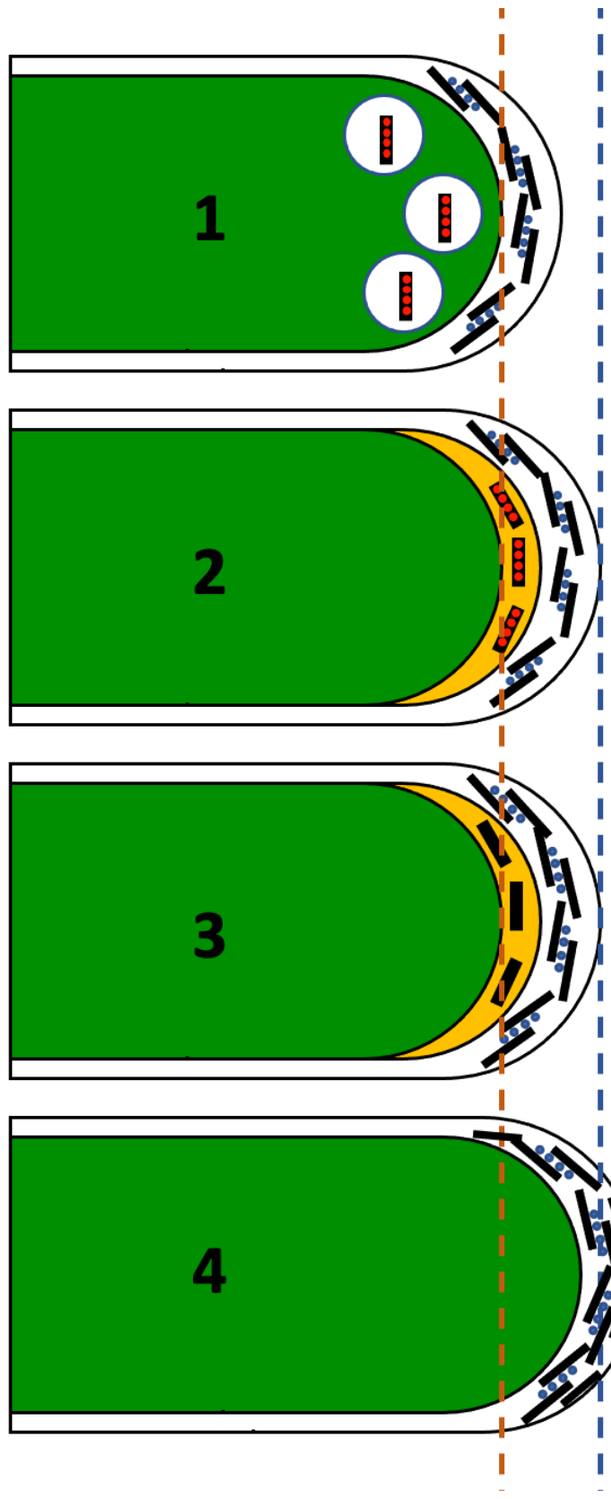
Thick

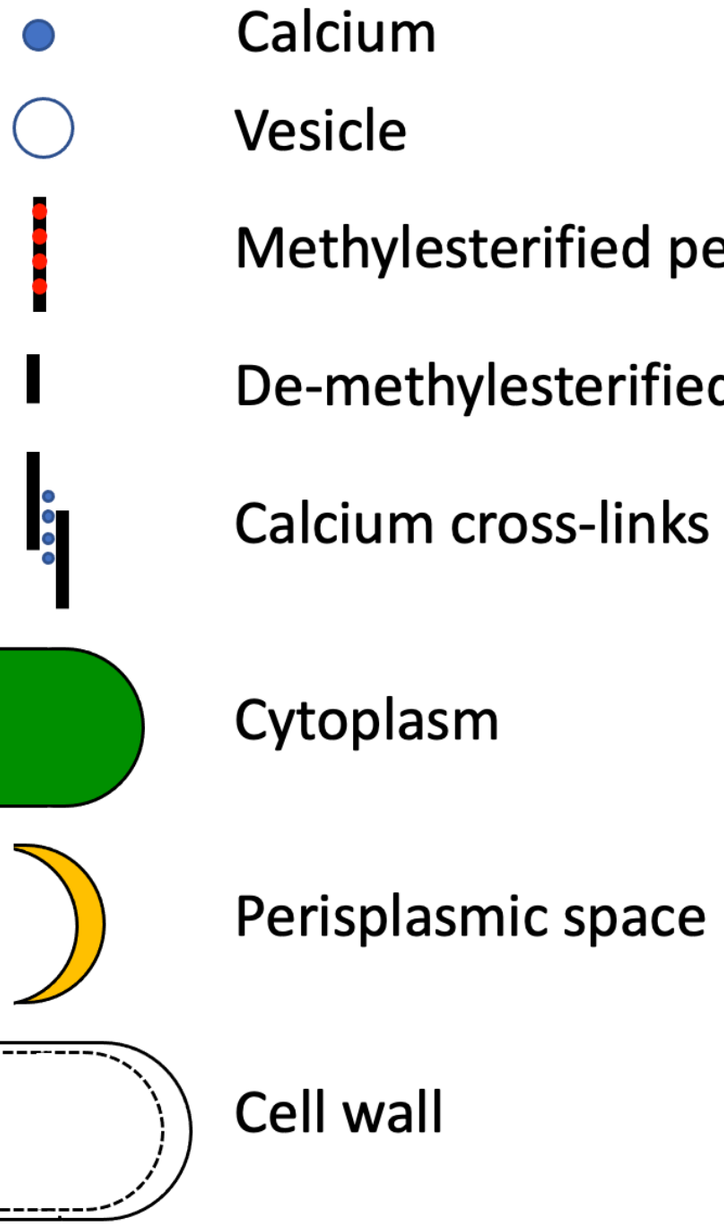


Thin



B





C

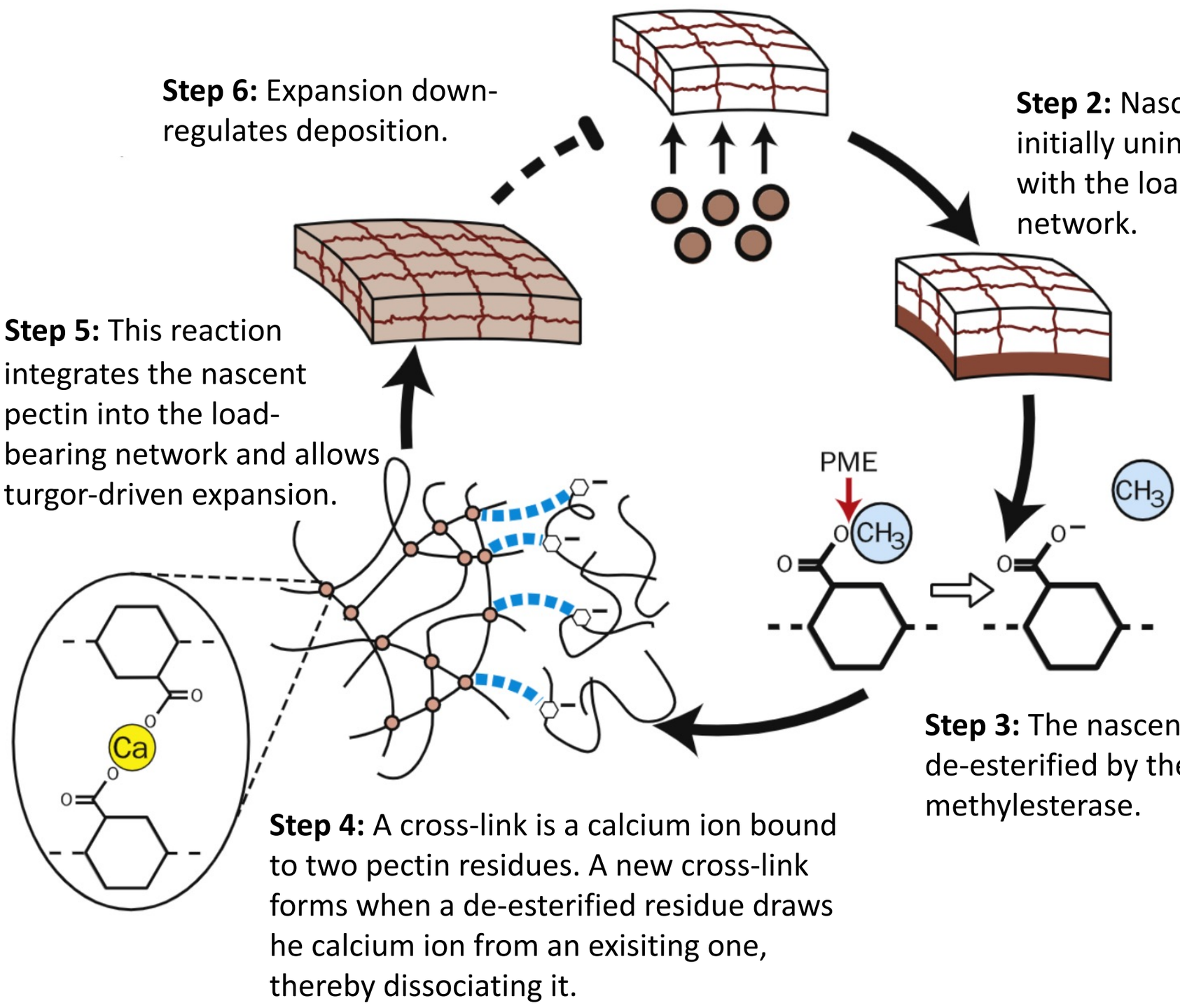
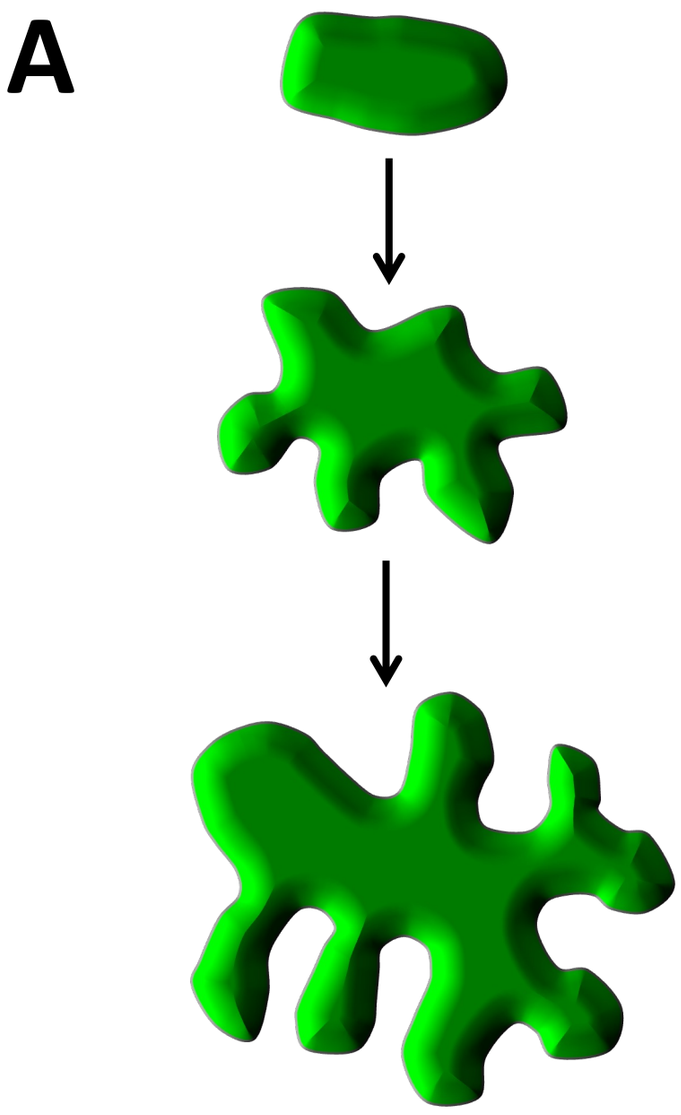
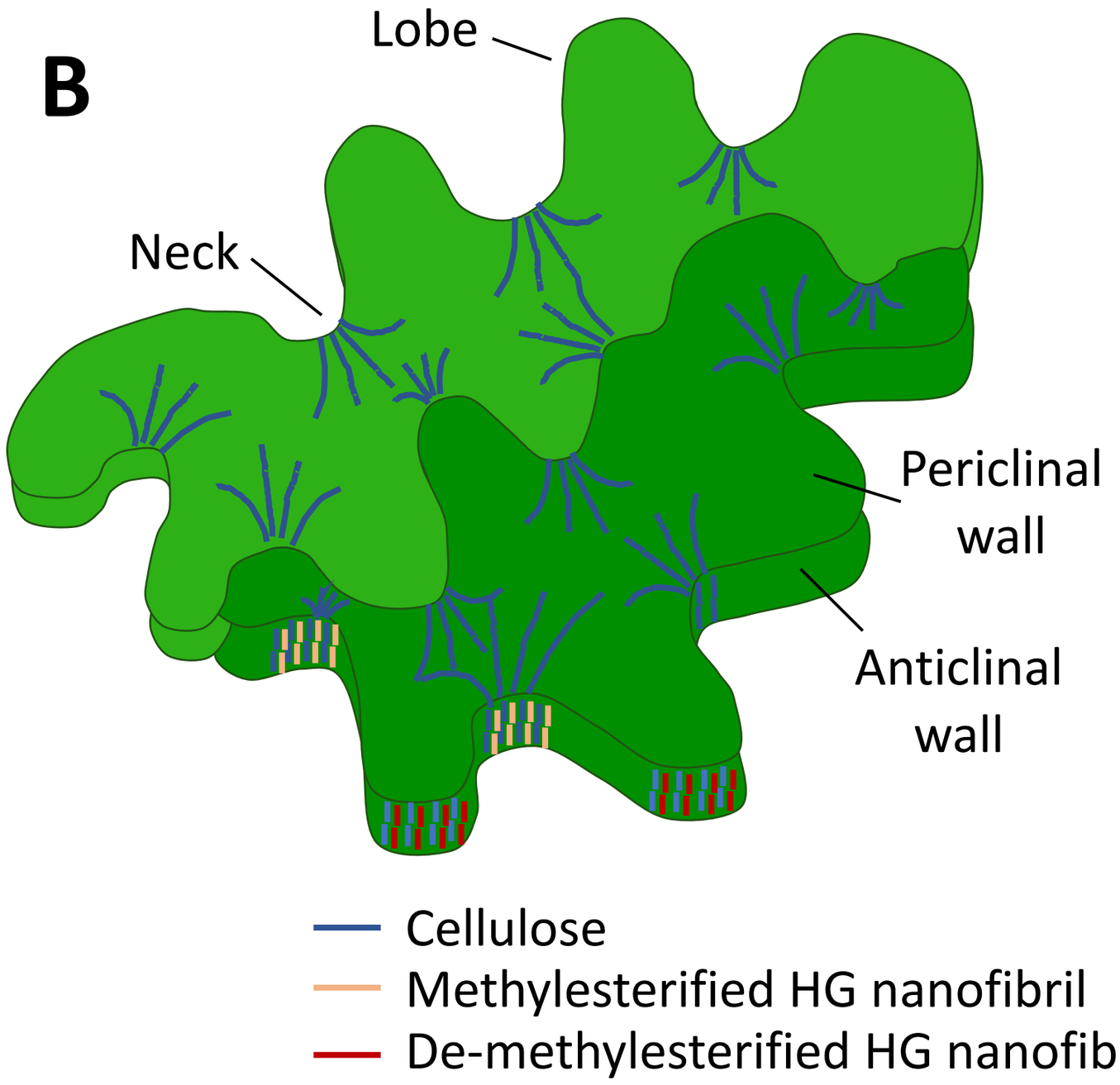
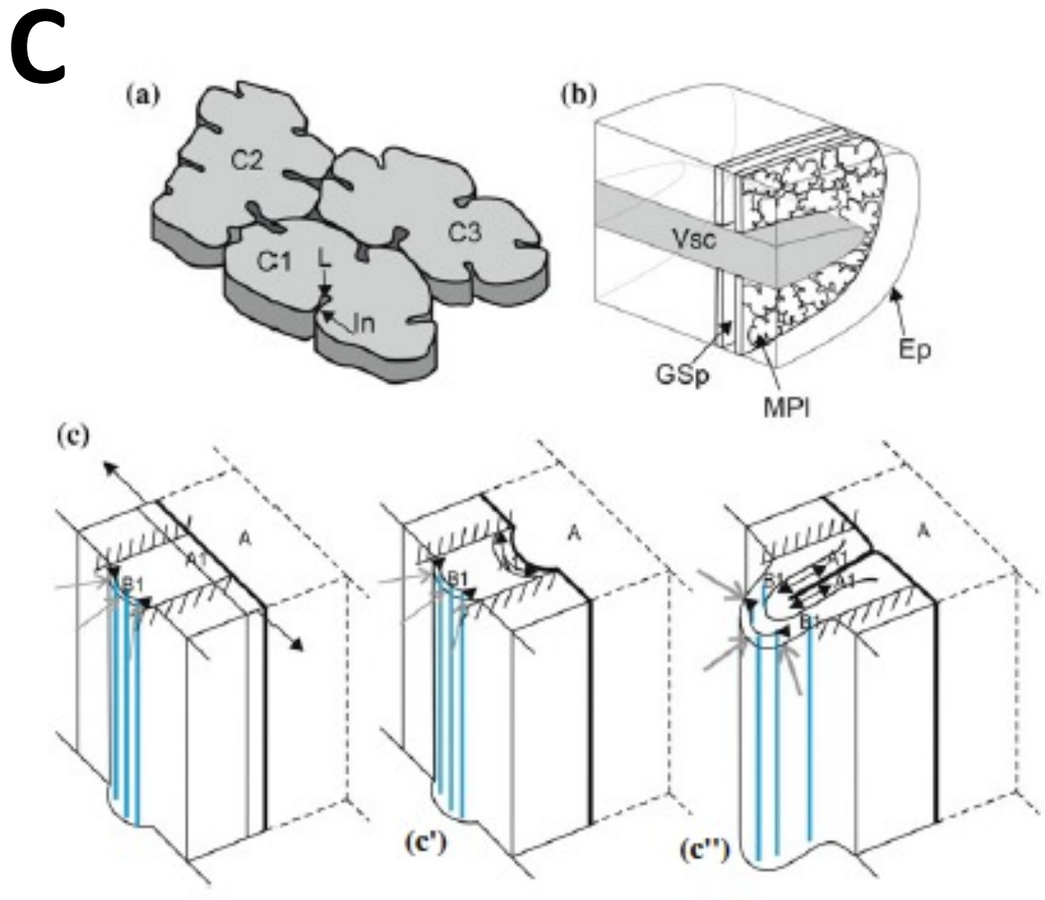


Figure 4


A


B
Lobe
Neck
Periclinal wall
Anticlinal wall
Cellulose
Methylesterified HG nanofibril
De-methylesterified HG nanofibril


C
(a)
C2
C3
C1
L
In
(b)
Vsc
GSp
MPl
Ep
(c)
A
A1
B1
(c')
(c'')

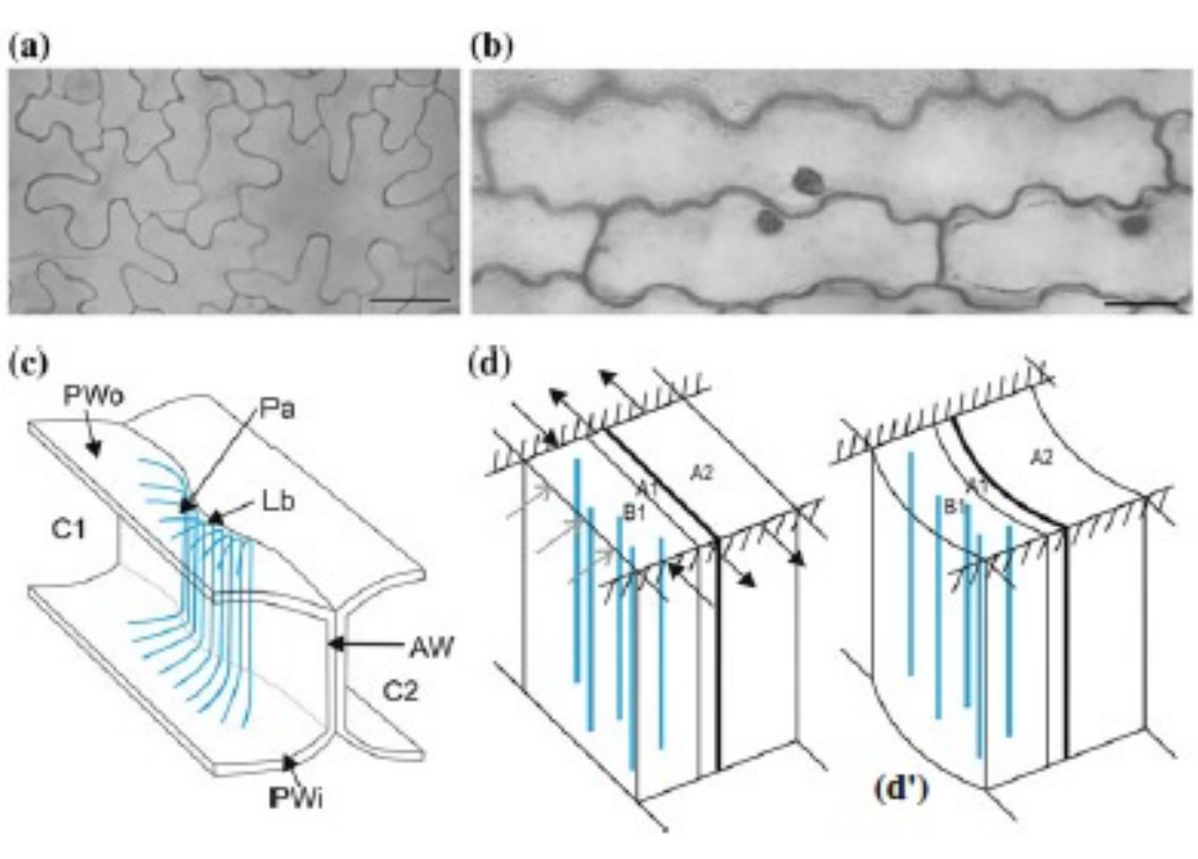

D
(a)
(b)
(c)
PWo
Pa
Lb
C1
AW
C2
PWi
(d)
A1
A2
B1
(d')

Figure 5