Trends in Plant Science

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Opinion

Gazing at Cell Wall Expansion under a Golden Light

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In plants, cell growth is constrained by a stiff cell wall, at least this is the way textbooks usually present it. Accordingly, many studies have focused on the elasticity and plasticity of the cell wall as prerequisites for expansion during growth. With their specific evolutionary history, cell wall composition, and environment, brown algae present a unique configuration offering a new perspective on the involvement of the cell wall, viewed as an inert material yet with intrinsic mechanical properties, in growth. In light of recent findings, we explore here how much of the functional relationship between cell wall chemistry and intrinsic mechanics on the one hand, and growth on the other hand, has been uncovered in brown algae.

Highlights

There is a current overwhelming paradigm of cell growth that promotes one main scenario: the intrinsic elasticity or plasticity of the cell wall controls growth.

In brown algae, which evolved independently from land plants and fungi, both the structure and chemical composition of the cell wall differ from their counterparts (i.e., cell wall of land plants, fungi, oomycetes, and other algae).

Beyond a complete inventory of cell wall components, their proportion and potential chemical modifications and interactions (covalent and/or electrostatic) with each other are still largely unknown, even in the most studied organisms, such as land plants.

Data on land plants and brown algae show that the propensity of the cell wall to grow does not systematically depend on its intrinsic mechanical properties.

Complexity and diversity of cell wall compositions and structures make preconceived transposition of cell wall growth mechanisms hazardous.

Cell Wall Expansion: Does the Known Matter Really Matter?

The most common paradigm of plant cell **growth** (see Glossary) involves the generation of tensile stress, mainly due to cell turgor, causing the cell wall to yield. In response to this tensile stress, cell volume increases due to the influx of water, and cell wall biosynthesis is activated, maintaining cell wall thickness and preventing disruption [1]. This increase in volume tends to attenuate turgor, but the ongoing re-establishment of the intracellular osmotic potential maintains the tensile stress. These dynamic processes lead to continuous growth, but only if the cell wall is able to yield. Many studies in land plants, fungi, and green and yellow-green algae have attempted to link the intrinsic chemical and mechanical (**elasticity** and **plasticity**, as assessed by short-term experiments) features of the cell wall to its potential for growth (a potentially long-term process). Seemingly intuitive, this relationship can be tested using current technologies that allow the acquisition of quantitative mechanical data. However, it remains plausible that cell wall growth does not necessarily involve cell wall resistance countering strong tensile stress, similar to two players pulling a rope in opposite directions, but instead may build on collaborative factors where tensile stress and **remodelling** factors work in concert to promote growth. In some cases, the regulation of the **intrinsic mechanical properties** of the cell wall may only be a potential third player, the role of which depends on its relative influence in the physical scrimmage. Determining the extent to which cell wall growth directly depends on the intrinsic features of the cell wall (viewed as an inert material that nevertheless has dynamic intrinsic properties) will benefit from widening the range of walled-organisms studied.

Uncoupling Cell Wall Growth from the Intrinsic Mechanical Properties of the Cell Wall

Growth implies an irreversible deformation of the cell wall and, thus, implicitly involves the plasticity of the material that comprises the cell wall. By definition, irreversibility is detected after the growth event has taken place. Hence, growth can be a two-step process, in which the cell wall yields according to the elastic nature of the material and this deformation is simultaneously made irreversible through consolidation of cell wall material [2]. Alternatively, growth can be a one-step process based on the plastic nature of the cell wall material, for which deformation

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 https://doi.org/10.1016/j.tplants.2018.10.013

itself is irreversible and deformation takes place only when the applied stress exceeds a given threshold (the 'yield threshold'). These two cases rely on the intrinsic mechanical properties of the cell wall taken as a physical material (Figure 1A) in which growth is made possible only when the mechanical properties of the cell wall are modified. A third mechanism is characterised by cell wall remodelling without modifying the intrinsic mechanical properties of the cell wall (Figure 1B). In this process, yielding is made possible, or is enhanced, due to modifications of the organisation of the cell wall material, and not necessarily of its actual chemical composition. These two mechanical properties [intrinsic mechanical properties (namely elasticity and/or plasticity) and remodelling] can theoretically be involved in cell wall growth in all organisms.

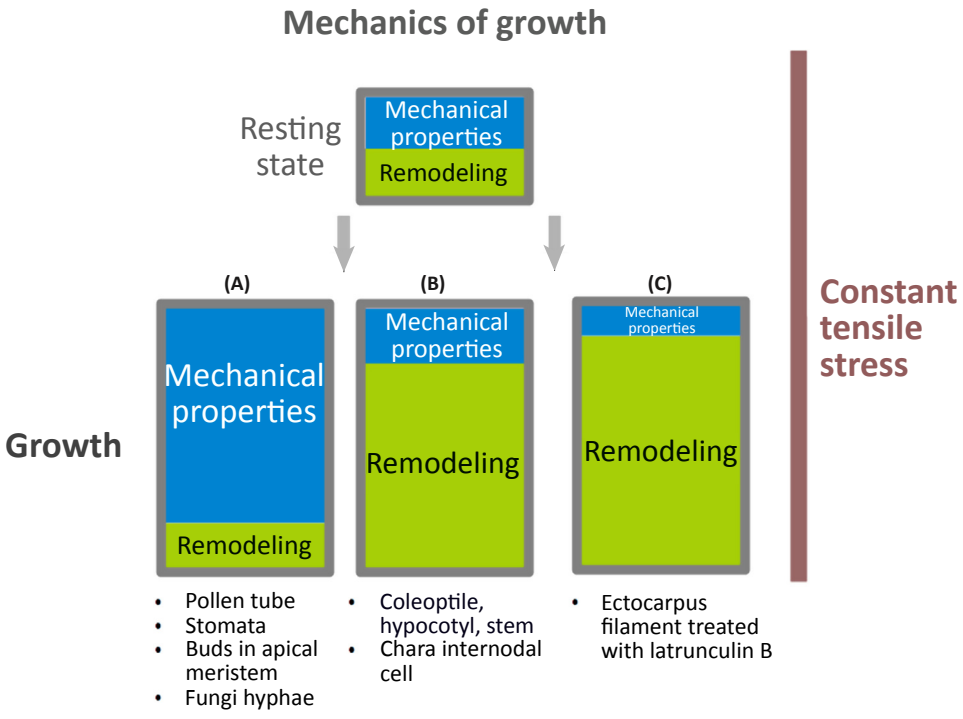

Figure 1. Cell Wall Mechanical Properties Involved in Cell Wall Expansion. Growth involves cell wall yielding, either in response to increased tensile stress (not considered here) and/or in response to an increase in the cell wall amenability to expand (shown here). The thick grey border represents the cell contour following cell wall growth. Coloured boxes represent the relative role of either the intrinsic mechanical properties (blue) or remodelling (green) in cell wall growth. The resting state is represented, by default, with boxes of equal areas. (A) Intrinsic mechanical properties are modified to allow cell growth. Among them, elasticity can promote growth due to the activity of enzymes [e.g., pectin-methylesterase (PME) inhibitors in the pollen tube in angiosperms, which maintains inactive PME and methyl-esterified pectins in the growing tip). Using nano- and microindentation techniques (see Table 1 in the main text), elasticity has been shown to be involved in the growth of many plant, algal, and fungal cells (see main text for references). However, the reliability of nano- and microindentation is questioned. The involvement of 'true' cell wall-intrinsic plasticity has been debated [52], because it is often confused with viscoelasticity. Analyses of indentation curves require more complicated models to infer quantitative data on the propensity of the cell wall to plasticity (hysteresis [68]). (B) Cell wall-remodelling factors (e.g., expansin and xyloglucan endotransglycosylase) displace the load-bearing bonds between components without modifying the overall chemical composition of the cell wall (e.g., expansins modify the bonds between cellulose and hemicellulose), thereby promoting growth. For example, in the green alga *Chara*, diffuse growth of the internodes relies on the cycling of distorted to nondistorted calcium–pectate complexes in new cell walls and calcium delivery to the cell membrane [14]. Dynamics in this cycle results in windows of increased cell wall elasticity and growth. (C) In the brown alga *Ectocarpus,* treatment with 1 μM latrunculin B resulted in an increase in growth whereby the cell increased its width significantly. Simultaneously, the cell lost its capacity to swell in response to a hypo-osmotic shock, meaning that its intrinsic elasticity (and potentially plasticity) was reduced (Charrier *et al.*, unpublished data).

Glossary

Elasticity: ability of a material to recover its initial dimensions after deformation (once the stress is released); also known as 'reversible deformability'.

Extensibility: capacity of the cell wall to grow through cell wall loosening (remodelling) in response to a stress (as defined by Cosgrove [9]).

Growth (or chemo-rheological expansion, as defined by [52]): increase in surface area, resulting from either enhanced stress or a modification of the cell wall propensity for deformation due either to an increase in elasticity or plasticity, or to cell wall remodelling.

Intrinsic mechanical properties: elasticity, viscoelasticity, or plasticity of a material; measurements of the intrinsic mechanical properties are performed either directly by intrusive equipment in contact with the biological material (e.g., nano- and/or microindentation), or indirectly by measuring strain on material undergoing external physical forces (creeping, stretching, or osmotic pressure).

Plasticity: the irreversible deformation of the cell wall. This process has a temporal dimension and, therefore, plasticity may be taken for viscoelasticity when the dynamics of viscosity are slow (i.e., much longer than observation time); also confusingly named 'irreversible elasticity' by some authors (e.g., [14]).

Remodelling: defined here as the process by which the arrangement of the various cell wall components interacting with each other is modified. Remodelling does not change the net chemical composition of the cell wall and does not necessarily modify its intrinsic mechanical properties (e.g., modification of the position of hydrogen bonds without modifying their number), resulting in unchanged elasticity. It is promoted by molecular remodelling factors: expansin, xyloglucan endotransglucosylase/hydrolase, redox reactions (e.g., cross-linking bonds in fungal cell wall polysaccharides [53]) or finely tuned chemical cycles involving the interaction of calcium with polysaccharides (e.g., pectate

Experimentally, assessing the intrinsic mechanical properties of the cell wall is easier than deciphering the process by which the cell wall remodels. In particular, many available techniques can quantify cell wall elasticity, such as indentation using atomic force microscopy (AFM), or stretching [3,4] (Table 1). As a result, reports abound on the close relationship between growth and the intrinsic elasticity of the cell wall (e.g., recently in fungi [5]). Emergence and growth of buds in the *Arabidopsis* apical meristem have been correlated with an increase in elasticity [6], in a process similar to that occurring in the tip-growing pollen tube, in which elasticity continuously decreases from the tip to 20 μm behind it [7]. Similar observations have been reported in fungal hyphae [8], but far away from the growth zone. However, the technical flaws pertaining to AFM techniques (Table 1) recently highlighted by Cosgrove [9] raise *de facto* some issues about the role of intrinsic elasticity in growth demonstrated so far. At the cellular level, physical measurements of the ability of the cell wall to yield, which requires large cell wall surfaces (e.g., *Chara* and *Vaucheria* [10]), are rarely performed to confirm AFM data, especially in living cells. Nonetheless, in some cases, cellular expansion in response to hypo-osmotic treatments has confirmed the overlapping patterns of cell wall elasticity and cell growth [11].

When neither of the two intrinsic mechanical properties discussed above appear to be involved, and when growth is shown to require heat and/or living cells, cell wall-remodelling factors releasing the load-bearing bonds are introduced as necessary factors for the cell wall to yield (Figure 1B). The extent to which remodelling is separate from the intrinsic mechanical properties has been debated and likely depends on the cell, species, and growth mode (diffuse or localised, e.g., at the tip of an apical cell). Since the 1892 demonstration that the ascomycete *Peziza* hyphae bursts at the base of the apex where growth is slower and not at the tip where growth is higher [12], it has been clear that the most deformable positions do not necessarily correlate with actively growing zones. Similarly, **stiffness** does not correlate with slow-growing cells either. The inner layer of the cell wall of *Aspergillus* spores is extremely stiff (elastic modulus E up to 30 GPa [13]); nevertheless, this is where bud emergence takes place to initiate hyphal growth. Bamboo culms grow very fast via cell elongation at the base of internodes (cumulative growth rate of ~30 $mm.h^{-1}$), where secondary cell wall biosynthesis and lignification, initiated before the cessation of cell elongation, lead to stiff cell walls (E ~20 GPa [14]). This cell wall is 10 000 times stiffer than the cell wall of the pollen tube, which has an elongation rate 100 times slower (~300 $\mu m.h^{-1}$). Beyond these simple observations, experimental data have since demonstrated further this lack of correlation between the intrinsic mechanical properties and growth in land plant cell walls ([15], reviewed by [16]).

Brown algae are macroscopic, multicellular organisms displaying many differences from their land counterparts. Their ancestor likely diverged >1.6 million years ago (Mya) [17], a period during which three endosymbiotic events took place [18], leading to organisms with specific cellular and genomic features [19,20]. More importantly, their environment features mechanical properties completely different to those experienced on land. When immersed, most of their growing cells are permanently exposed to seawater moving at a density more than 1000 times greater than the air, generating forces similar to hurricane forces every few seconds [21]. Wave-swept animals develop stiff bodies to resist these forces, but seaweeds opted for a different strategy: their stiffness is ~100–1000 times lower than that of land plants, and they have high flexibility. In addition, due to periodic tides in their natural environment, brown algae are usually exposed to a large range of osmotic variations due to dehydration at one extreme of the range and to flooding with rainwater at the other. When immersed in either pure water or 2 M NaCl (corresponding to four times the concentration of seawater), cells of the brown alga *Ectocarpus* either expand by up to 70% or shrink down to 35% of their volume (corresponding to 40% of

distortion in green algae [14]). The term 'cell wall loosening' is used for remodelling processes resulting in growth.

Stiffness: the opposite of deformability (both elastic and plastic); assessed using Instron strain measurement techniques, indentation (atomic force microscopy), cell compression, stretching devices, and so on [3,4,39].

Table 1. Techniques Used for the Study of Cell Wall Mechanics during Expansion[a]

Underlying mechanical basis	Scale	Technique[b]	Parameters[c]	On living material (nondestructive)?	Benefit	Disadvantage	Refs[d]
Growth	Organ/tissue	Size measurement	Geometry	Yes	Non-intrusive; cheap	Average of several tissues/ cells	[54]
	Cell	Size measurement	Geometry	Yes	Automation possible	Tissue accessibility	[54]
	Cell wall	Marker displacement	Local strain	Yes	Resolution <μm	Cell adhesion required	[55]
Intrinsic mechanical properties (including elasticity and plasticity)	Tissue	Extensometer	Wall loosening	Yes	Long-lasting experiments; wide parameter range	Indirect; requires precise cutting; low spatial resolution; averaged data	[54]
		Osmotic pressure shift	Elongation kinetics	Yes	Mimics natural conditions	Low resolution	[16]
		Resonance frequency (vibration)	Stiffness; damping coefficient	Yes	High-throughput; nondestructive	Large scale, indirect	[56]
		Pressure block	Stress relaxation	Yes	Precise control	Indirect	[16,57]
	Cell	Extensometer (Instron)	Compressive modulus of elasticity	Yes	Overall figure at cell level	Requires precise cutting; low spatial resolution	[58]
			Plastic compliance; creep	No	Wide range, in plane of growth, both elasticity and plasticity		[69]
		Microextensometer (ACME)	Elasticity and plasticity	Yes	Microscale; 3D; automated, in plane of growth; both elasticity and plasticity	Sophisticated equipment, very recent	[59]
		Creep measurement	Plastic yield stress	No		Stress–strain; not only cell wall properties	[69]
		Micromanipulation	Elasticity	Yes		Artificial samples	[9]
		Ball tonometry		Yes	Overall figure at tissue level	Low spatial resolution	[39]
		Relaxation spectra	Stress relaxation	Yes	Wide parameter range	Requires data smoothing	[9]
		Mercury inflation	Multiaxial plastic extensibility	No		Intrusive; hazardous	[54]
			Creep recovery				
		Microfluidics ('lab-on-a-chip')	Compression potential	Yes	Continuous measurements with varying growth conditions; automation possible	Low spatial resolution; artificial environment	[3,60]

Table 1. (continued)

Underlying mechanical basis	Scale	Technique[b]	Parameters[c]	On living material (nondestructive)?	Benefit	Disadvantage	Refs[d]
		Inflation/deflation (osmotic changes)	Elastic modulus (linearity)	Yes	Easy to design	Approximate; mainly 2D only	[9]
	Cell wall	Extensometer (Instron)	Elastic compliance	No	Wide range; both elasticity and plasticity	Requires precise cutting; low spatial resolution	[57,69]
		Cellular force microscopy: indentation	Cell wall stiffness	Yes	High resolution; relatively high forces (μN)	Complex equipment	[16]
		AFM; microindentation	Stiffness, elasticity, plasticity, adhesion	Yes	High spatial resolution (μm scale); surface mapping; outer and inner cell wall layers; possible in aqueous medium	Complex equipment; in z-axis (not growth plane); sensitive to indentation angle; requires adherent sample	[3,16,38]
		AFM; nanoindentation		Yes	High spatial resolution (nm scale); surface mapping, low force (nN) possible in aqueous medium	Complex equipment; in z-axis; only outer cell wall layer; sensitive to indentation angle; requires adherent samples	[3,61]
		Dynamic nanoindentation (nanoDMA)	Viscoelasticity; storage/loss stiffness	Yes	High resolution (nanoscale); can be coupled to TEM and SEM	Requires sophisticated equipment	[9]
		Uniaxial stress	Mechanical anisotropy	No		Intrusive	[54]

[a]List of techniques is not exhaustive.
[b]Acquisition of accurate data of cell wall mechanics during growth should be performed using a technique that can take measurements: (i) on living organisms; (ii) over a period of time in accordance with the dynamics of growth; (iii) at the precise position of the cell surface where growth takes place, whatever the scale; (iv) in the direction of expansion (mainly tangential position along the cell surface; z-axis is less relevant); (v) that is adequate for 3D objects (e.g., AFM is sensitive to the orientation of the contact plan, as in the dome of the pollen tube); (vi) that is compatible with the mechanical properties of the biological sample (e.g., biological materials, and especially the cell wall, do not behave as linear elastic materials); and (vii) able to measure the overall cell wall mechanical features, and not only the superficial, outermost layer (e.g., nanoindentation).
[c]Parameters listed are based on the authors' terminology, but the exact definition of parameters may be subject to subtle variations between authors.
[d]Mainly reviews are cited.

their surface area), respectively (B. Charrier, 2017). In comparison, the surface area of cells of the tomato shoot apical meristem expand and shrink by ~9% [11].

Nevertheless, there is a disconnection between these intrinsic mechanical properties of the cell wall and growth potential (Figure 1C). For example, in the apical cell of the filamentous brown alga *Ectocarpus,* treatment with the actin-depolymerising drug latrunculin B promoted doubled growth in width, but fully blocked cell swelling in the same axis after immersion in half-concentrated seawater [55]. This suggests that, in these conditions, the underlying mechanics

required for growth is distinct from the elasticity and/or plasticity involved in rapid volume changes, regardless of the exact role of actin in this process. Similar cell wall stiffening has been observed in the pollen tube in response to cytochalasin D, another actin-destabilising drug [22], but the morphological effects are less pronounced and this result was attributed to micro-indentation artefacts due to the dome shape. This explanation is excluded when elasticity is measured from changes in cell volume and when deformability can be directly measured in the plane of the cell wall, as performed in the case of *Ectocarpus*.

Cell Wall Growth: Demystifying Polysaccharide Chemistry

Cell walls are a mixture of compounds the relative organisation of which remains obscure, especially in brown algae. At the chemical level, >80% of the brown algal cell wall is chemically different from land plant cell walls (Table 2). As in land plants, polysaccharides are the main components, but they are represented by large and rare cellulose microfibrils immersed in abundant alginates (~40%) and sulfated fucans (~40%) [23] (Figure 2). This results in cell walls with a lower degree of crystallinity compared with land plants and, together, these major differences hinder any reliable transposition between the two groups of organisms.

In the context of growth, a link between cell wall chemical composition and its propensity to expand is intuitively natural. Fungal cell wall biosynthesis mutants are impaired in cell growth [24] and the level of pectin methylesterification in angiosperm pollen tubes is directly proportional to growth rate [25]. However, the role of alginates in growth, especially of mannuronans, which are described as 'soft' components in *in vitro* studies [26], has no support thus far. In the brown alga *Sargassum*, the position of new buds is not correlated with a specific spatial pattern of alginates [27], and no correlation has been found between the active growth site in the rhizoid of the embryo of the brown alga *Fucus* and the presence of soft or stiff alginates [28].

In brown algae, can the polysaccharide composition control the intrinsic mechanical properties of the cell wall, if not its expansion? 'Soft' mannuronan alginates have been shown to be preferentially extracted from organs with flexible properties, whereas stiff guluronan alginates [26], which form *in vitro* complexes with calcium, as pectins do (Figure 2), have been extracted from load-bearing organs exposed to drag forces (e.g., kelp stipes in environments exposed to waves; [29] and references therein). However, completely contrasting observations have also been reported. Miller *et al.* [30] found that the highest levels of stiff guluronans were measured in the most mucilaginous and flexible seaweeds of their study, regardless of their age. This echoes similar observations made in the *Arabidopsis* shoot apical meristem, where an increase in pectin demethylesterification colocates with an increase in elasticity [6], but stiffens the cell wall in the shanks of the pollen tube [25]. Therefore, these examples illustrate that, in brown algae as in land plants, the complexity of the mechanics of the cell wall, and moreover of growth, cannot be reduced to the presence or absence of a single, or even a handful of polysaccharides. Knowledge of the complete interacting molecular network is the first step before translating chemical composition into mechanics [31]. Even in land plants, where most of the cell wall chemical components have been identified and where there is a comprehensive set of positional patterns of cell wall components (e.g., along the tip-growing pollen tube [32]), the interactive network remains vague and incomplete [33], preventing any simple, straightforward conclusion as to the role of these compounds in growth. Other factors, such as the degree of hydration, the ion concentration or the rate of degradation of polysaccharides, are alternative driving forces in cell growth (as discussed in [34,35]).

As a result, attempts to piece together partial knowledge lead to complex scenarios, such as those for pollen tube growth, where differential and often counter-intuitive gradients of factors,

Table 2. Cell Wall Components of the Cell Walls of Land Plants and Brown Algae[a]

Class	Subclass	Abundance	
		Land plants	Brown algae
Cellulose	No subclass	15–33%	1–8%
Hemicelluloses	Homoxylans (X)	~8%	n.d.
	Arabinoxylans (AX)		n.d.
	Glucuronoxylans (GX)		n.d.
	Glucuronoarabinoxylans (GAX)		n.d.
	Xyloglucans (XyG)	~20%	n.d.
	Xyloglucuronans		Present
	Mannans (M)	Scarce	n.d.
	Glucomannans	Scarce	n.d.
	Galactomannans	Scarce	n.d.
	Galactoglucomannans	Scarce	n.d.
	Glucuronomannans	Scarce	n.d.
	Mixed-linkage-glucans (MLG)	Scarce[b]	Present
	Callose (β-1,3-glucans)	Potentially abundant	Present
Pectins	Homogalacturonnans (HG)	6–15%	n.d.
	Rhamnogalacturonans I (RGI)	5–10%	Present
	Rhamnogalacturonans II (RGII)	1–4%	n.d.
	Apiogalacturonans	Scarce	n.d.
	Xylogalacturonans	Scarce	n.d.
Alginates	No sub-class	n.d.	~40%
Fucose-containing sulphated polysaccharides (FCSP)	Fucans	n.d.	~40%
	Fucoglucuronans	n.d.	
	Fucogalactans	n.d.	
	Xylofucoglucuro-mannans	n.d.	
	Uncharacterised FCSPs	n.d.	
Noncatalytic remodelling proteins	Expansins	Present	n.d.
	YoaJ-like proteins	n.d.	Present
	CBM32-containing proteins	n.d.	Present
Catalytic remodelling proteins	Glucosidases	Present	n.d.
	Glucanases	Present	n.d.
	β-Galactosidases	Present	n.d.
	Polygalacturonases (PGs)	Present	n.d.
	Pectate-lyases (PLs) and pectase-lyase-like (PLLs)	Present	n.d.
	Xyloglucan endotransglycosidases (XETs)	Present	n.d.
	Xyloglucan endo-hydrolases (XEH)	Present	n.d.
	Xylosidases	Present	n.d.
	Pectin methylesterases (PMEs) and PME inhibitors (PMEIs)	Present	n.d.
	Pectin acetylesterases	Present	n.d.

Table 2. (continued)

Class	Subclass	Abundance	
		Land plants	Brown algae
	Xyloglucan acetylesterases	Present	n.d.
	Mannuronate-C5-epimerases	n.d.	Present
	Vanadate-dependant halogenoperoxidases (vHPO)	n.d.	Present
	GH88-familiy proteins	n.d.	Present
	Alginate lyases	n.d.	Present
	Pectin-lyase-fold virulence factor domain proteins	n.d.	Present
	Metalloproteinases and inhibitors (TIMP)-like proteins	n.d.	Present
	Subtilisin-like serine proteases	n.d.	Present
	CBM1-containing proteins	n.d.	Present
Structural proteins	Arabinogalactan proteins (AGPs)	Present	Present
	Prolin-rich proteins (PRPs)	Present	n.d.
	Hydroxyprolin-rich proteins (HPRPs), including extensins	Present	n.d.
	Glycin-rich proteins (GRPs)	Present	Present
	Many uncharacterised cell wall proteins	Present	5–9%
Phenolic compounds	Para-coumaryl acid	>2%	n.d.
	Phlorotannins	n.d.	Present

[a]This table shows the nature and approximate abundance (% dry weight) of the different components of the cell wall in land plants (only primary cell wall; both dicotyledonous and monocotyledonous [33,62–64]) and in brown algae [46,65–67]. Abbreviation: n.d., no data available.
[b]Higher abundance in Poales (monocotyledonous).

including calcium concentration and pectin-methylesterase enzyme (PME) activities, are squeezed into a possible mechanism of tip growth [36,37]. However, the different biological contexts call for putting all the cards back on the table. In brown algae, alginate stiffness is described as depending directly on the calcium concentration, but this relationship degenerates when the calcium concentration is ten times that of the seawater [38], a situation that can be reached locally *in muro* in emerged thalli, especially in poroelastic cell walls [32]. As for PME, recent studies suggest that the control of methylesterification (including both PME activity and a PME inhibitor, PMEI) is especially important for the fast growth of angiosperm pollen tubes, and less determinate in gymnosperms, in which the gradients of esterified pectins are less pronounced and PMEI is absent [37]. Furthermore, studies of growth mechanisms in more basal green cells, such as in the charophyte alga *Chara*, argue that the role of PME as described in the pollen tube may be limited to the more recently evolved green plants [14]. This is just a sign of the diversity of mechanisms that may be encountered in organisms the phylogenetic position of which is distant to the most studied plant models, and an indication that our understanding of their role in plant cell growth *lato sensu* should mature with future evo-devo studies.

The interpretation of results becomes even more complex when cell wall polysaccharides of different natures compensate each other. In brown algae, degradation of alginates leads to a stiffer cell wall unable to expand in response to hypo-osmotic shock, suggesting that alginates

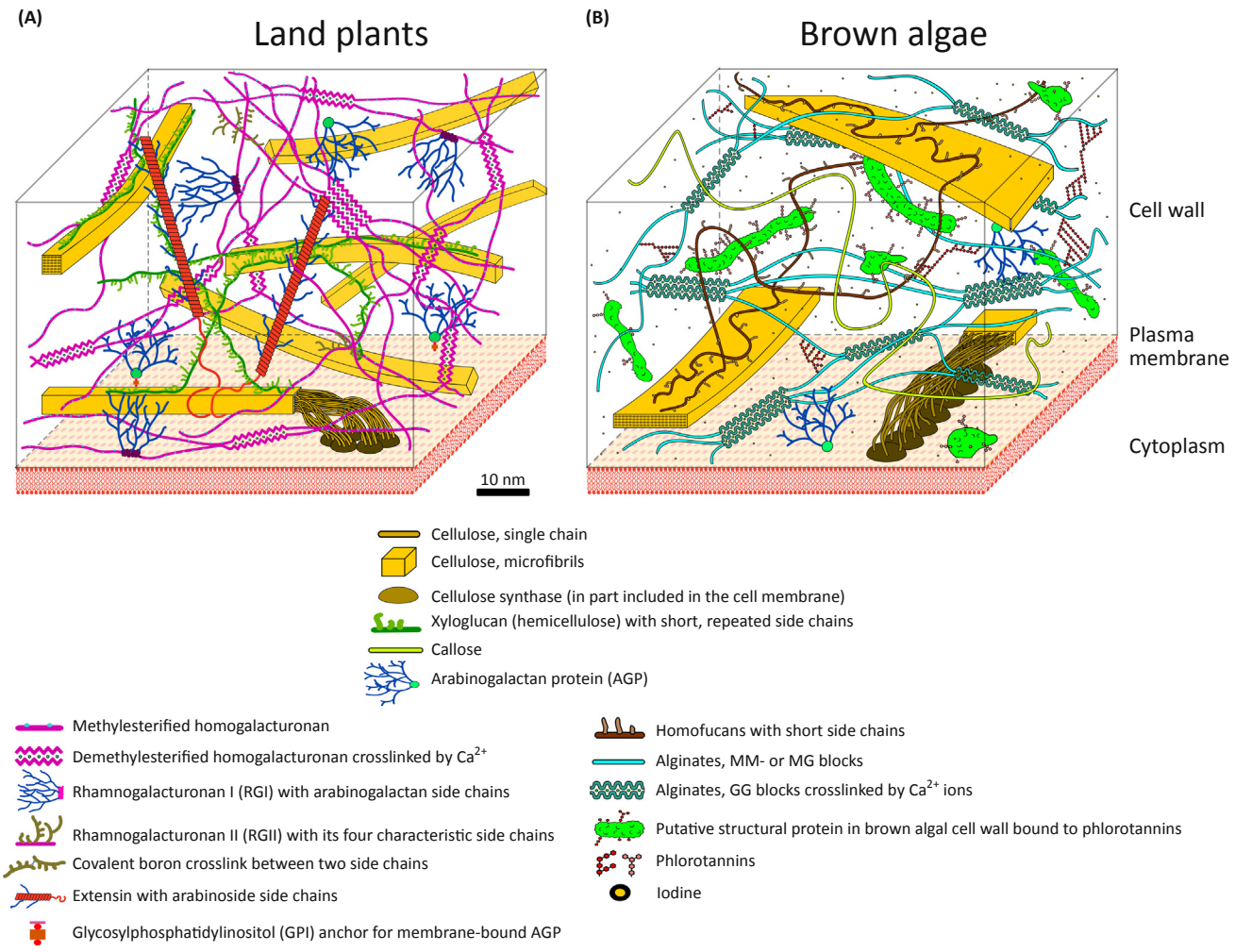

Figure 2. Comparison of the Cell Wall Chemical Composition and Structure in Land Plants and Brown Algae. Only the primary cell wall is considered. (A) In land plants (angiosperms), the cell wall mainly comprises two networks: (i) cellulose microfibrils (MFs, both crystalline and noncrystalline [62]), which are crosslinked by hemicelluloses chains (for simplicity only xyloglucans, XG, are represented in the drawing) via hydrogen bonds; and (ii) pectin gel network. Pectins comprise several substructures: homogalacturonan (HG) and rhamnogalacturonan I and II (RGI and II). Demethylesterified HGs are crosslinked by calcium ions and RGIIs are crosslinked by borate. Extensins, which are structural proteins potentially crosslinking cellulose and pectins, and arabinogalactan proteins (AGPs) are also shown, although their detailed structure and interaction are not certain [63,64]. For a detailed review of the composition of the cell wall of the pollen tube, see [33]. (B) In brown algae, less is known about the detailed composition and structure of the cell wall compared with land plants. The model presented here is mainly based on [46]. The cell wall likely comprises at least two independent networks: (i) cellulose MFs crosslinked with fucose-containing sulfated polysaccharides (FCSPs) and proteins; and (ii) alginate gel networks crosslinked by phlorotannins. Cellulose MFs are ribbon shaped and less abundant than in land plant cell walls (0–8% dry weight, see Table 2 in the main text). For simplicity, only homofucan FCSPs are represented here. The identity and structure of putative crosslinking proteins (in green or blue, including recently identified AGPs) and phlorotannins are speculative. β-(1 → 3)-glucans (callose) and β-(1 → 3)-(1 → 4)-glucans (mixed-linkage glucans, MLG, not shown) have also been identified in brown algal cell wall (see Table 2 in the main text), but their interactions with other components are unknown [65,66]. The cell wall of brown algae is also rich in halogenated compounds (up to 19% dry weight), especially iodine species in the form of free ions (up to 1.0% dry weight, i.e., 30 000-fold the concentration of seawater) or included in halogenated molecules (especially phlorotannins [67]). All components are drawn to scale.

are necessary to ensure intrinsic cell wall elasticity [55]. However, a closer look shows that this decrease in elasticity is due to the overaccumulation of cellulose at the subcellular location where growth takes place. The high stiffness of cellulose (E of up to 175 GPa [39], compared with alginate with value of E of only a few kPa [40] and a pectin E of up to 1 MPa [41]) easily

accounts for the observed decrease in cell wall extensibility. Similar cellulose accumulation occurred during overgrowth of the apical cell in response to LatB treatment, showing that, despite its high stiffness, cellulose does not hinder growth. By contrast, in plants, cellulose also has the potential to promote growth [42]. This uncoupling between the role of cellulose in both the intrinsic mechanical properties and expansion of the cell wall echoes the recent finding that growth and cellulose biosynthesis are regulated by distinct pathways in the *Arabidopsis* hypocotyl [43]. Uncoupling metabolic activity from light-dependent circadian rhythms demonstrated that cell wall biosynthesis is controlled by the former and growth by the latter. Furthermore, cellulose synthases (GT2 family of glucosyl-transferases), as defined from sequence similarity, may not synthesise only cellulose but instead produce mixed-linkage polysaccharides (MLGs) or even new polysaccharides, such as arabinoglucan recently shown in the moss *Physcomitrella* [44]. These results show that the links between cell growth and cellulose and/or cellulose synthase genes (as a proxy for cellulose accumulation) are not direct. Clearly, there is a need to revisit the assumption that the presence of stiff components in the cell wall prevents or mitigates its expansion.

Thus, are polysaccharides more than just inert structural components subjected to the activities of remodelling proteins during growth? Several distinct remodelling mechanisms have been described in land plants, green algae, and fungi. In *Chara*, the ongoing delivery of new cell wall components modifies the dynamics of pectate–Ca^{2+} complexes formed *in muro* (the so-called 'pectate distortion' mechanism [14]), thereby remodelling the cell wall. However, proteins are central factors in most of the remodelling processes described so far. In land plants, the xyloglucan-endo-transglycosylase-hydrolases (XTH) participate in cell wall expansion through hemicellulose cutting and joining [45] and expansins modify hemicellulose-mediated bonds between stiff cellulose fibres ([4] and subsequent papers). Any resulting gaps are filled with freshly made or delivered material, allowing the overall expansion of the local cell wall. In fungi, radical coupling catalysed by an oxidase occurs between the cell wall polymers glucosaminoglycan and beta-glucan [12].

Brown algal cell walls contain proteins in significant amounts (>5% of the cell wall biomass [23,46]) and with a high diversity (>900 different proteins secreted in brown algae [47]). Interestingly, in brown algae, none of these proteins share similarity with expansin, PME or even cellulase (Table 2) (based on a genomic analysis [48]). Domains of cell wall-remodelling proteins have been identified among secreted proteins (e.g., carbohydrate binding module CBM32 interacting with alginates [47]), making them prime candidates for remodelling factors [49]. In addition, families of secreted brown algal proteins are specific (e.g., alginate C5-epimerases) or expanded (vanadium haloperoxidase or metalloproteinases) relative to those of land plants [47,50]. Finally, signalling proteins, such as the Notch-Domain proteins, previously thought to be specific to animal cells, are over-represented in brown algal cell walls [47]. Therefore, in light of recent data, our current understanding, which still requires more knowledge of cell wall molecular composition and organisation in dynamic conditions, is that brown algae developed a specific secretome for cell wall remodelling.

Concluding Remarks and Future Prospects

Work on nonconventional models phylogenetically distant from land plants gives the opportunity to unveil the existence of alternative mechanisms of growth. In these organisms (and previously noted in land plants and green algae [51]), the causal relationship between cell wall growth and intrinsic cell wall mechanical properties, or cell wall growth and cell wall chemical composition, are not obvious. Furthermore, the difference in growth strategies may also be related to the type of organ (e.g., shoot apical meristem or pollen tube in land plants, and

Outstanding Questions

What are the molecular bases of elasticity in the brown algal cell wall, considering its specific composition?

What is the molecular toolkit of cell wall remodelling in brown algae? Do proteins with functions similar to expansins exist?

How easily can distinct yet overlapping roles be considered for the cell wall in the lifespan of a cell? For example, can swelling in response to hypo-osmotic shock rely on mechanical properties or chemical components involved in the cell wall expansion processes taking place during growth?

Are current technical tools suitable to measure the relevant physical constants of the cell wall involved in growth, especially when several cell wall layers are involved?

Can cell wall mechanical properties measured on a small timescale be relevant for understanding processes occurring on a long timescale, typical of cell growth?

To what extent can results obtained from model land plants be transposed to other species? Which features should be common: chemical components, supramolecular structure, and organisation or intrinsic mechanical properties regardless of the chemical composition?

internodes in the green alga *Chara*), its growth mode (tip-growing or diffuse, respectively), or its growth dynamics.

The first results obtained in brown algae showed that the distribution of cell wall polysaccharide determinants is not easily linked to the cell growth pattern, and that the intrinsic mechanical properties may not systematically correlate with growth potential. This leaves plenty of room for alternative processes, including cell wall remodelling with no alteration of the intrinsic mechanical properties. However, due to the different composition and organisation of the cell walls in green plants and brown algae, the molecular toolkits of the remodelling machinery are likely fundamentally different. Beyond the potential conservation of molecular factors, cellular and biomechanical studies carried out in brown algae will most likely lead to breakthroughs in alternative mechanisms of cell wall remodelling (also see Outstanding Questions).

Acknowledgments

We thank Cécile Hervé for fruitful discussion about the cell wall composition.

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