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1 **Gazing at cell wall expansion under a golden light**

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10

11 **Keywords:** Brown algae, Cell wall, Mechanics, Evolution, Polysaccharides, Remodelling

12

13 **Abstract**

14

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

23

24 **Cell wall expansion: does the known matter really matter?**

25 The most common paradigm of plant cell growth involves the generation of tensile stress, mainly
26 due to cell turgor, causing the cell wall to yield. In response to this tensile stress, cell volume
27 increases due to the influx of water and cell wall biosynthesis is activated, maintaining cell wall
28 thickness and preventing disruption [1]. This increase in volume tends to attenuate turgor, but the
29 ongoing re-establishment of the intracellular osmotic potential maintains the tensile stress. These
30 dynamic processes lead to continuous growth – but only if the cell wall is able to yield. Many

31 studies in land plants, fungi, green and yellow-green algae have attempted to link the intrinsic
32 chemical and mechanical (elasticity, plasticity, as assessed by short-term experiments) features of
33 the cell wall to its potential for **growth** (a potentially long-term process). Seemingly intuitive, this
34 relationship can be tested using current technologies that allow the acquisition of quantitative
35 mechanical data. However, it remains plausible that cell wall growth does not necessarily involve
36 cell wall resistance countering strong tensile stress, like two players pulling a rope in opposite
37 directions, but instead may build on collaborative factors where tensile stress and **remodelling**
38 factors work in concert to promote growth. In some cases, the regulation of the intrinsic
39 mechanical properties of the cell wall may only be a potential third player, whose role depends on
40 its relative influence in the physical scrimmage. Determining the extent to which cell wall growth
41 directly depends on the intrinsic features of the cell wall – viewed as an inert material that
42 nevertheless has dynamic intrinsic properties – will benefit from widening the range of walled-
43 organisms studied.

44

45 **Uncoupling cell wall growth from the intrinsic mechanical properties of the cell wall**

46 Growth implies an irreversible deformation of the cell wall, and thus implicitly involves the
47 plasticity of the material that makes up the cell wall. By definition, irreversibility is detected after
48 the growth event has taken place. Hence, growth can be a two-step process in which the cell wall
49 yields according to the elastic nature of the material and this deformation is simultaneously made
50 irreversible through consolidation of cell wall material [2]. Or, growth can be a one-step process
51 based on the plastic nature of the cell wall material, for which deformation itself is irreversible
52 and deformation takes place only when the applied stress exceeds a given threshold (the ‘yield
53 threshold’). These two cases rely on the intrinsic mechanical properties of the cell wall taken as a
54 physical material (Figure 1A) in which growth is made possible only when the mechanical
55 properties of the cell wall are modified. A third mechanism is characterised by cell wall
56 remodelling without modifying the intrinsic mechanical properties of the cell wall (Figure 1B). In
57 this process, yielding is made possible – or is enhanced – due to modification in the organisation
58 of the cell wall material, and not necessarily in its actual chemical composition. These two
59 mechanical properties, *i.e.* (1) intrinsic mechanical properties (namely elasticity and/or plasticity)
60 and (2) remodelling can theoretically be involved in cell wall growth in all organisms.

61

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

77 When neither of the two intrinsic mechanical properties discussed above seem to be involved, and
78 when growth is shown to require heat and/or living cells, then cell wall remodelling factors
79 releasing the load-bearing bonds are introduced as necessary factors for the cell wall to yield
80 (Figure 1B). The extent to which remodelling is separate from the intrinsic mechanical properties
81 has been debated and most likely depends on the cell, species and growth mode (diffuse or
82 localised, e.g., at the tip of an apical cell). Since the 1892 demonstration that ascomycete *Peziza*
83 hyphae bursts at the base of the apex where growth is slower and not at the tip where growth is
84 higher [12], it has been clear that the most deformable positions do not necessarily correlate with
85 actively growing zones. Similarly, **stiffness** does not correlate with slow-growing cells either. The
86 inner layer of the cell wall of *Aspergillus* spores is extremely stiff (elastic modulus E up to 30
87 GPa; [13]); nevertheless, this is where bud emergence takes place to initiate hyphal growth.
88 Bamboo culms grow very fast via cell elongation at the base of internodes (cumulative growth
89 rate of $\sim 30 \text{ mm.h}^{-1}$), where secondary cell wall biosynthesis and lignification, initiated before the
90 cessation of cell elongation, lead to very stiff cell walls ($E \sim 20 \text{ GPa}$; [14]). This cell wall is
91 10,000 times stiffer than the cell wall of the pollen tube which has an elongation rate 100 times
92 slower ($\sim 300 \mu\text{m.h}^{-1}$). Beyond these simple observations, experimental data have since

93 demonstrated further this lack of correlation between the intrinsic mechanical properties and
94 growth in land plant cell walls ([15], reviewed by [16]).

95 Brown algae are macroscopic, multicellular organisms displaying many differences with their
96 land counterparts. Their ancestor likely diverged > 1.6 Mya [17], a period during which three
97 endosymbiotic events took place [18], leading to organisms with specific cellular and genomic
98 features [19,20]. More importantly here, their environment features mechanical properties
99 completely different to those experienced on land. When immersed, most of their growing cells
100 are permanently exposed to seawater moving at a density more than 1000 times greater than the
101 air, generating forces similar to hurricane-forces every few seconds [21]. Wave-swept animals
102 develop very stiff bodies to resist these forces, but seaweeds opted for a different strategy: their
103 stiffness is ~ 100 -1000 times lower than land plants, and they have high extensibility. In addition,
104 due to periodic tides in their natural environment, brown algae are usually exposed to a large
105 range of osmotic variations due to dehydration at one extreme of the range and to flooding with
106 rainwater at the other. When immersed in pure water or 2 M NaCl (corresponding to four times
107 the seawater concentration), cells of the brown alga *Ectocarpus* respectively expand by up to 70%
108 (in pure water) and shrink down to 35% of their volume (corresponding to 40% of their surface
109 area; unpublished personal data). In comparison, cells of the tomato shoot apical meristem expand
110 and shrink by about 9% in surface area [11].

111 Nevertheless, there is a disconnection between these intrinsic mechanical properties of the cell
112 wall and growth potential (Figure 1C). For example, in the apical cell of the filamentous brown
113 alga *Ectocarpus*, treatment with the actin-depolymerising drug latrunculin B promotes doubled
114 growth in width, but fully blocks cell swelling in the same axis after immersion in half-
115 concentrated seawater (unpublished personal data). This strongly suggests that in these conditions,
116 the underlying mechanics required for growth is distinct from the elasticity/plasticity involved in
117 rapid volume changes, regardless of the exact role of actin in this process. Similar cell wall
118 stiffening has been observed in the pollen tube in response to cytochalasin D, another actin-
119 destabilising drug [22], but the morphological effects are less pronounced and this result was
120 attributed to micro-indentation artefacts due to the dome shape. This explanation is excluded
121 when elasticity is measured from changes in cell volume and when deformability can be directly
122 measured in the plane of the cell wall, as performed in the case of *Ectocarpus*.

123

124 **Cell wall growth: demystifying polysaccharide chemistry**

125 Cell walls are a mixture of compounds whose relative organisation is still obscure, especially in
126 brown algae. At the chemical level, > 80% of brown algal cell wall is chemically different from
127 land plant cell walls (Table 2). As in land plants, polysaccharides are the main components, but
128 they are represented by large and rare cellulose microfibrils immersed in abundant alginates
129 (~40%) and sulphated fucans (~40%)([23], Figure 2). That results in cell walls with a much lower
130 degree of crystallinity compared to land plants, and altogether these major differences hinder any
131 reliable transposition between the two groups of organisms.

132 In the context of growth, a link between cell wall chemical composition and its propensity to
133 expand is intuitively natural. Fungal cell wall biosynthesis mutants are impaired in cell growth
134 [24] and the level of pectin methylesterification in angiosperm pollen tubes is directly
135 proportional to growth rate [25]. However, the role of alginates in growth, and especially of
136 mannuronans which are described as ‘soft’ components in *in vitro* studies [26], has no support
137 thus far. In the brown alga *Sargassum*, the position of new buds is not correlated with a specific
138 spatial pattern of alginates [27], and no correlation has been found between the active growth site
139 in the rhizoid of the embryo of the brown alga *Fucus* and the presence of soft or stiff alginates
140 [28].

141 In brown algae, can the polysaccharide composition control the intrinsic mechanical properties of
142 the cell wall, if not its expansion? ‘Soft’ mannuronan alginates have been shown to be
143 preferentially extracted from organs with flexible properties, whereas stiff guluronan alginates
144 [26], which form *in vitro* complexes with calcium as pectins do (Figure 2), have been extracted
145 from load-bearing organs exposed to drag forces (e.g., kelp stipes in environments exposed to
146 waves [29], and references therein). However, completely contrasting observations have also been
147 reported. Miller et al. [30] found that the highest levels of the stiff guluronans were measured in
148 the most mucilaginous and flexible seaweeds of their study, regardless of their age. This echoes
149 similar observations made in the *Arabidopsis* shoot apical meristem, where an increase in pectin
150 demethylesterification co-locates with an increase in elasticity [6], but stiffens the cell wall in the
151 shanks of the pollen tube [25]. Therefore, these examples illustrate that, in brown algae as in land
152 plants, the complexity of the mechanics of the cell wall, and moreover of growth cannot be
153 reduced to the presence or absence of a single, or even a handful of polysaccharides. Knowledge
154 of the complete interacting molecular network is the first step before translating chemical
155 composition into mechanics [31]. Even in land plants where most of the cell wall chemical
156 components have been identified and where there is a comprehensive set of positional patterns of

157 cell wall components (e.g., along the tip-growing pollen tube; [32]), the interactive network
158 remains vague and incomplete [33], preventing any simple, straightforward conclusion as to the
159 role of these compounds in growth. Other factors such as the degree of hydration, the ion
160 concentration or the rate of degradation of polysaccharides are alternative driving forces in cell
161 growth (as discussed in [34,35]).

162 As a result, attempts to piece together partial knowledge lead to complex scenarios, such as those
163 featured for pollen tube growth, where differential and often counter-intuitive gradients of factors
164 including calcium concentration and pectin-methylesterase enzyme (PME) activities, are squeezed
165 into a possible mechanism of tip growth [36,37]. However, the different biological contexts call
166 for putting all the cards back on the table. In brown algae, alginate stiffness is described as
167 depending directly on the calcium concentration, but this relationship degenerates when calcium
168 concentration is 10 times that of the seawater [38], a situation that can be reached locally *in muro*
169 in emerged thalli, especially in poro-elastic cell walls [32]. As for PME, recent studies suggest
170 that the control of methylesterification (including both PME activity and a PME inhibitor, PMEI)
171 is especially important for the fast growth of angiosperm pollen tubes, and less determinate in
172 gymnosperms in which the gradients of esterified pectins are less pronounced and PMEI is absent
173 [37]. Furthermore, studies of growth mechanisms in more basal green cells, such as in the
174 charophyte alga *Chara*, argue that the role of PME as described in the pollen tube may be limited
175 to the more recently evolved green plants [14]. This is just a sign of the diversity of mechanisms
176 that may be encountered in organisms whose phylogenetic position is distant to the most studied
177 plant models, and an indication that our understanding of their role in plant cell growth *lato sensu*
178 should mature with future evo-devo studies.

179
180 Interpretation of results becomes even more complex when cell wall polysaccharides of different
181 natures compensate each other. In brown algae, degradation of alginates leads to a stiffer cell wall
182 unable to expand in response to hypo-osmotic shock, suggesting that alginates are necessary to
183 ensure intrinsic cell wall elasticity (unpublished personal data). However, a closer look shows that
184 this decrease in elasticity is due to an over-accumulation of cellulose at the sub-cellular location
185 where growth takes place. The high stiffness of cellulose (E of up to 175 GPa; [39], compared
186 with alginate with value of E ~ a few kPa, [40] and pectin E of up to 1 MPa; [41]) easily accounts
187 for the observed decrease in cell wall extensibility. Similar cellulose accumulation occurred
188 during the over-growth of the apical cell in response to LatB treatment, showing that despite its

189 high stiffness, cellulose does not hinder growth. On the contrary, in plants, cellulose has also the
190 potential to promote growth [42]. This uncoupling between the role of cellulose in both the
191 intrinsic mechanical properties and cell wall expansion echoes the recent finding that growth and
192 cellulose biosynthesis are regulated by distinct pathways in the *Arabidopsis* hypocotyl [43].
193 Uncoupling metabolic activity from light-dependent circadian rhythms demonstrated that cell
194 wall biosynthesis is controlled by the former and growth by the latter. Furthermore, cellulose
195 synthases (GT2 family of glucosyl-transferases), as defined from sequence similarity, may not
196 synthesise only cellulose but instead produce mixed-linkage polysaccharides (MLGs) or even new
197 polysaccharides, such as arabinoglucan recently shown in the moss *Physcomitrella* [44]. These
198 results show that the links between cell growth and cellulose and/or cellulose synthase genes – as
199 a proxy for cellulose accumulation – are not direct. Clearly, there is a need to revisit the
200 assumption that the presence of stiff components in the cell wall prevents or mitigates its
201 expansion.

202 So, are polysaccharides more than just inert structural components subjected to the activities of
203 remodelling proteins during growth? Several distinct remodelling mechanisms have been
204 described in land plants, green algae and fungi. In *Chara*, the ongoing delivery of new cell wall
205 components modifies the dynamics of pectate-Ca²⁺ complexes formed *in muro* (the so-called
206 ‘pectate distortion’ mechanism [14]), thereby remodelling the cell wall. However, proteins are
207 central factors in most of the remodelling processes described so far. In land plants, the
208 xyloglucan-endo-transglycosylases-hydrolases (XTH) participate in cell wall expansion through
209 hemicellulose cutting and joining [45] and expansins modify hemicellulose-mediated bonds
210 between stiff cellulose fibres ([4] and subsequent papers). Any resulting gaps are filled with
211 freshly made or delivered material, allowing the overall expansion of the local cell wall. In fungi,
212 radical coupling catalysed by an oxidase occurs between the cell wall polymers
213 glucosaminoglycan and beta-glucan [12].

214 Brown algal cell walls have been shown to contain proteins in significant amounts (>5% of the
215 cell wall biomass; [23,46]) and with a high diversity (> 900 different proteins secreted in brown
216 algae [47]). Interestingly, in brown algae, none of these proteins share similarity with expansin,
217 PME or even cellulase (Table 2; from genomic analysis; [48]). Domains of cell wall remodelling
218 proteins have been identified among secreted proteins (e.g. carbohydrate binding module CBM32
219 interacting with alginates; [47]) making them prime candidates for remodelling factors [49]. In
220 addition, families of secreted brown algal proteins are specific (e.g., alginate C5-epimerases) or

221 expanded (vanadium haloperoxidase, metalloproteinases) relative to those of land plants [47,50].
222 Finally, signalling proteins such as the Notch-Domain proteins, previously thought to be specific
223 to animal cells, are over-represented in brown algal cell walls [47]. Therefore, in light of recent
224 data, our current understanding, which still requires more knowledge on cell wall molecular
225 composition and organisation in dynamic conditions, is that brown algae developed a specific
226 secretome for cell wall remodelling.

227

228 **Concluding remarks and future prospects**

229 Work on non-conventional models phylogenetically distant from land plants gives the opportunity
230 to unveil the existence of alternative mechanisms of growth. In these organisms (and previously
231 noted in land plants and green algae [51]), the causal relationship between cell wall growth and
232 intrinsic cell wall mechanical properties, or cell wall growth and cell wall chemical composition,
233 are not obvious. Furthermore, the difference in growth strategies may also be related to the type of
234 organ (e.g., shoot apical meristem or pollen tube in land plants, internodes in green alga *Chara*),
235 its growth mode (respectively tip-growing or diffuse) or its growth dynamics.

236 The first results obtained in brown algae show that the distribution of cell wall polysaccharide
237 determinants is not easily linked to the cell growth pattern, and that the intrinsic mechanical
238 properties may not systematically correlate with growth potential. This leaves plenty of room for
239 alternative processes, including cell wall remodelling with no alteration of the intrinsic
240 mechanical properties. However, due to the very different composition and organisation of the
241 cell walls in green plants and brown algae, the molecular toolkits of the remodelling machinery
242 are likely fundamentally different. Beyond the potential conservation of molecular factors,
243 cellular and biomechanical studies carried out in brown algae will most likely lead to
244 breakthroughs in alternative mechanisms of cell wall remodelling.

245

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248

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250

251 **Glossary**

252

253 All related to the cell wall:

- 254 • **Elasticity**: refers to the ability of a material to recover its initial dimensions after
255 deformation (once the stress is released). Reversible deformability.
- 256 • **Extensibility** (as defined by D. Cosgrove): The capacity of the cell wall to grow through
257 cell wall loosening (remodelling) in response to a stress.
- 258 • **Growth** (or chemo-rheological expansion, as defined by [52]): The increase in surface
259 area, resulting from either enhanced stress or a modification of the cell wall propensity for
260 deformation due either to an increase in elasticity or plasticity, or to cell wall remodelling.
- 261 • **Intrinsic mechanical properties**: elasticity, visco-elasticity or plasticity of a material.
262 Measurements of the intrinsic mechanical properties are performed either directly by

263 intrusive equipment in contact with the biological material (e.g. nano-/micro-indentation),
264 or indirectly by measuring strain on material undergoing external physical forces
265 (creeping, stretching, osmotic pressure).

266 • **Plasticity:** refers to the irreversible deformation of the cell wall. This process has a
267 temporal dimension and, therefore, plasticity may be taken for visco-elasticity when the
268 dynamics of viscosity are very slow (*i.e.* much longer than observation time). Also
269 confusingly named “irreversible elasticity” by some authors (e.g., [14]).

270 • **Remodelling:** Defined here as the process by which the arrangement of the various cell
271 wall components interacting with each other is modified. Remodelling does not change the
272 net chemical composition of the cell wall and does not necessarily modify its intrinsic
273 mechanical properties, e.g., modification of the position of hydrogen bonds without
274 modifying their number, resulting in unchanged elasticity. It is promoted by molecular
275 remodelling factors: expansin, xyloglucan endotransglucosylase/hydrolase, redox
276 reactions (e.g., cross-linking bonds in fungal cell wall polysaccharides; [53]) or finely
277 tuned chemical cycles involving the interaction of calcium with polysaccharides (e.g.,
278 pectate distortion in green algae; [14]). The term ‘cell wall loosening’ is used for
279 remodelling processes resulting in growth.

280 • **Stiffness:** The opposite of deformability (both elastic and plastic). Assessed using Instron
281 strain measurement techniques, indentation (atomic force microscopy), cell compression,
282 stretching devices, etc. [3,4,39].

283

284 **Tables**

285

286 Table 1: Techniques employed for the study of cell wall mechanics during expansion.

287 This table intends to illustrate the range of available techniques allowing the measurement of cell
288 mechanical properties. The list is not exhaustive. The “Parameters” column uses the author’s
289 terminology, but the exact definition of parameters may be subject to subtle variations between
290 authors. The “Reference” column mainly indicates reviews. The acquisition of accurate data of
291 cell wall mechanics during growth should be performed using a technique that can take
292 measurements i) on living organisms, ii) over a period of time in accordance with the dynamics of
293 growth, iii) at the precise position of the cell surface where growth takes place, whatever the scale,

294 iv) in the direction of expansion (mainly tangential position along the cell surface; z-axis is less
295 relevant); and that is v) adequate for 3D objects (e.g., AFM is sensitive to the orientation of the
296 contact plan, as in the dome of the pollen tube), vi) compatible with the mechanical properties of
297 the biological sample (e.g. biological materials, and especially the cell wall, do not behave as
298 linear elastic materials) and vii) able to measure the overall cell wall mechanical features, and not
299 only the superficial, outermost layer (e.g. nano-indentation). Literature cited: [3,9,16,39,54–61].
300

Underlying Mechanical basis	Scale	Technique	Parameters	On Living material (non destructive)	Benefit	Disadvantage	Reference	
Growth	Organ / tissue	Size measurement	Geometry	yes	Non intrusive; Cheap	Average of several tissues / cells	[58]	
	Cell	Size measurement	Geometry	yes	Automation possible	Tissue accessibility	[58]	
	Cell Wall	Marker displacement	Local strain	yes	Resolution < μm	Cells 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	[58]	
		Osmotic pressure shift	Elongation kinetics	yes	Mimics natural conditions	Low resolution	[16]	
		Resonance frequency (vibration)	Stiffness Damping coefficient	yes	High-throughput Non-destructive	Large scale, indirect	[61]	
		Pressure-block	Stress relaxation	yes	Precise control	Indirect	[9,57]	
	Cellular	Extensometer (instron)	Compressive modulus of elasticity	yes	Overall figure at the cell level	Requires precise cutting Low spatial resolution	[54]	
			Plastic compliance Creep	no	Wide range, in the plane of growth, both elasticity and plasticity		[9]	
		Micro-extensometer (ACME)	Elasticity Plasticity	yes	Microscale, 3D, automated, In the plane of growth Both elasticity and plasticity	Sophisticated equipment, Very recent	[56]	
		Creep measurement	Plastic yield stress	no		Stress-strain Not only CW properties	[9]	
		Micro-manipulation		yes		Artificial samples	[9]	
		Ball tonometry	Elasticity	yes	Overall figure at the 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	[58]
			Creep recovery					
		Microfluidics ("lab-on-a-chip")	Compression potential	yes	Continuous measurements with varying growth conditions Automation possible	Low spatial resolution Artificial environment	[3,59]	
		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	[9,57]		

Cellular force microscopy: indentation	Cell wall stiffness	yes	High resolution Relatively high forces (μN)	Complex equipment	[16]
Atomic force microscopy: micro-indentation	Stiffness, Elasticity, Plasticity, Adhesion	yes	High spatial resolution (μm scale) Surface mapping Outer and inner cell wall layers Possible in aquaous medium	Complex equipment In z-axis (not the growth plane) Sensitive to indentation angle Requires adherent sample	[3,16,38]
Atomic force microscopy: nano-indentation		yes	High spatial resolution (nm scale) Surface mapping, low force (nN) possible in aquaous medium	Complex equipment In z-axis Only outer cell wall layer Sensitive to indentation angle Requires adherent samples	[3,60]
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	[58]

303 Table 2: Cell wall components of land plants and brown algal cell walls

304 Table shows the nature and approximate abundance (% dry weight) of the different components of
 305 the cell wall in land plants (only primary cell wall; both dicotyledonous and monocotyledonous
 306 [33,62–64]) and in brown algae [46,65–67]. * Much higher abundance in Poales
 307 (monocotyledonous).

308

Class	Sub-class	Abundance	
		Land plants	Brown algae
Cellulose	No sub-class	15-33 %	1-8 %
Hemicelluloses	Homoxylans (X)		
	Arabinoxylans (AX)	~ 8 %	
	Glucuronoxylans (GX)		
	Glucuronoarabinoxylans (GAX)		
	Xyloglucans (XyG)	~ 20 %	
	Xyloglucuronans		Present
	Mannans (M)	Scarce	
	Glucomannans	Scarce	
	Galactomannans	Scarce	
	Galactoglucomannans	Scarce	
Glucuronomannans	Scarce		
Mixed-linkage-glucans (MLG)	Scarce*	Present	
Callose (β -1,3-glucans)	Potentially abundant	Present	
Pectins	Homogalacturonans (HG)	6-15 %	
	Rhamnogalacturonans I (RGI)	5-10 %	Present
	Rhamnogalacturonans II (RGII)	1-4 %	
	Apiogalacturonans	Scarce	
	Xylogalacturonans	Scarce	
Alginates	No sub-class		~ 40 %
Fuco-Containing Sulphated Polysaccharides (FCSP)	Fucans		
	Fucoglucuronans		
	Fucogalactans		~ 40 %
	Xylofucoglucuro-mannans		
Non-catalytic remodeling proteins	Uncharacterised FCSPs		
	Expansins	Present	
	YoaJ-like proteins		Present
Catalytic remodeling proteins	CBM32-containing proteins		Present
	Glucosidases	Present	
	Glucanases	Present	
	β -galactosidases	Present	
	Polygalacturonases (PGs)	Present	
	Pectate-lyases (PLs) and Pectase-lyase-like (PLLs)	Present	
	Xyloglucan EndoTransglycosidases (XETs)	Present	
	Xyloglucan endo-hydrolases (XEH)	Present	
	Xylosidases	Present	
	Pectin-Methyl-Esterases (PMEs)	Present	

	And PME-Inhibitors (PMEIs)		
	Pectin acetylsterases	Present	
	Xyloglucan acetylsterases	Present	
	Mannuronate-C5-Epimerases		Present
	Vanadate-dependant Halogenoperoxidases (vHPO)		Present
	GH88-familii proteins		Present
	Alginate-lyases		Present
	Pectin-lyase-fold Virulence factor domain proteins		Present
	Metalloproteinases and inhibitors (TIMP)-like proteins		Present
	Subtilisin-like serine proteases		Present
	CBM1-containing proteins		Present
Structural proteins	Arabinogalactan Proteins (AGPs)	Present	Present
	Prolin-Rich Proteins (PRPs)	Present	
	Hydroxyprolin-rich proteins (HPRPs) including Extensins	Present	
	Glycin-rich proteins (GRPs)	Present	Present
	Many uncharacterised CW proteins	Present	5-9 %
Phenolic compounds	Para-coumaryl acid	>2 %	
	Phlorotannins		Present

309

310

311 **Figure legends**

312

313 Figure 1: Cell wall mechanical properties involved in cell wall expansion

314 Growth involves cell wall yielding, either in response to increased tensile stress (not considered
315 here) and/or in response to an increase in the cell wall amenability to expand (shown here). The
316 thick grey border represents the cell contour following cell wall growth. Colour boxes represent
317 the relative part played by either the intrinsic mechanical properties (blue) or remodelling (green)
318 in cell wall growth. The resting state is represented, by default, with boxes of equal areas. (A)
319 Intrinsic mechanical properties are modified to allow cell growth. Among them, elasticity can
320 promote growth due to the activity of enzymes (e.g., pectin-methylesterase inhibitor in the pollen
321 tube in Angiosperms, which maintains inactive PME and methyl-esterified pectins in the growing
322 tip). Using nano- and micro-indentation techniques (Table 1), elasticity has been shown to be
323 involved in the growth of many plant, algal and fungal cells (see text for references). However,
324 the reliability of nano- and micro-indentation is questioned. The involvement of ‘true’ cell wall
325 intrinsic plasticity has been debated [52], because it is often confused with visco-elasticity.
326 Analyses of indentation curves require more complicated models to infer quantitative data on the

327 propensity of the cell wall to plasticity (hysteresis, [68]). (B) Cell wall remodelling factors (e.g.,
328 expansin, xyloglucan endo-transglycosylase) displace the load-bearing bonds between
329 components without modifying the overall chemical composition of the cell wall (e.g., expansins
330 modify the bonds between cellulose and hemicellulose), thereby promoting growth. For example,
331 in the green alga *Chara*, diffuse growth of the internodes relies on the cycling of distorted to non-
332 distorted calcium-pectate complexes in new cell walls and calcium delivery to the cell membrane
333 [14]. Dynamics in this cycle results in windows of increased cell wall elasticity and growth. (C)
334 In the brown alga *Ectocarpus*, a treatment with 1 μ M latrunculin B resulted in an increase in
335 growth whereby the cell increased its width significantly. Simultaneously, the cell lost its capacity
336 to swell in response to a hypo-osmotic shock, meaning that its intrinsic elasticity (and potentially
337 plasticity) was reduced (unpublished data from the authors).

338
339 Figure 2. Comparison of the cell wall chemical composition and structure in land plants and
340 brown algae.

341 Only the primary cell wall is considered. (A) In land plants (Angiosperms), the cell wall is mainly
342 composed of two networks: (i) cellulose microfibrils (MFs, both crystalline and non-crystalline
343 [62]) which are cross-linked by hemicelluloses chains (for simplicity only xyloglucans, XG, are
344 represented in the drawing) *via* hydrogen bonds, and (ii) pectin gel network. Pectins are
345 composed of several sub-structures: homogalacturonan (HG) and rhamnogalacturonan I and II
346 (RGI and II). Demethylesterified HGs are crosslinked by calcium ions and RGII are cross-linked
347 by borate. Extensins, which are structural proteins potentially cross-linking cellulose and pectins,
348 and arabinogalactan proteins (AGP) are also shown, although their detailed structure and
349 interaction are not certain [63,64]. For a detailed review on the composition of the cell wall of the
350 pollen tube, see [33]. (B) In brown algae, much less is known about the detailed composition and
351 structure of the cell wall compared with land plants. The model presented here is mainly based on
352 [47]. The cell wall is likely composed of at least two independent networks: (i) cellulose MFs
353 cross-linked with fucose-containing sulphated polysaccharides (FCSPs) and proteins, and (ii)
354 alginate gel networks cross-linked by phlorotannins. Cellulose MFs are ribbon-shaped and much
355 less abundant than in land plant cell walls (0-8% dry weight, Table 2). For simplicity, only
356 homofucans FCSPs are represented in the drawing. The identity and structure of putative cross-
357 linking proteins (in blue, including recently identified AGPs) and phlorotannins are speculative.
358 β -(1 \rightarrow 3)-glucans (callose) and β -(1 \rightarrow 3)-(1 \rightarrow 4)-glucans (mixed-linkage glucans, MLG, not

359 shown in the drawing) have also been identified in brown algal cell wall (Table 2), but their
360 interactions with other components are unknown [65,66]. The cell wall of brown algae is also rich
361 in halogenated compounds (up to 19% dw), especially iodine species in the form of free ions (up
362 to 1.0% dw, i.e. 30,000-fold the concentration of the seawater) or included in halogenated
363 molecules (especially phlorotannins, [67]). All components are drawn to scale.

MECHANICS OF GROWTH

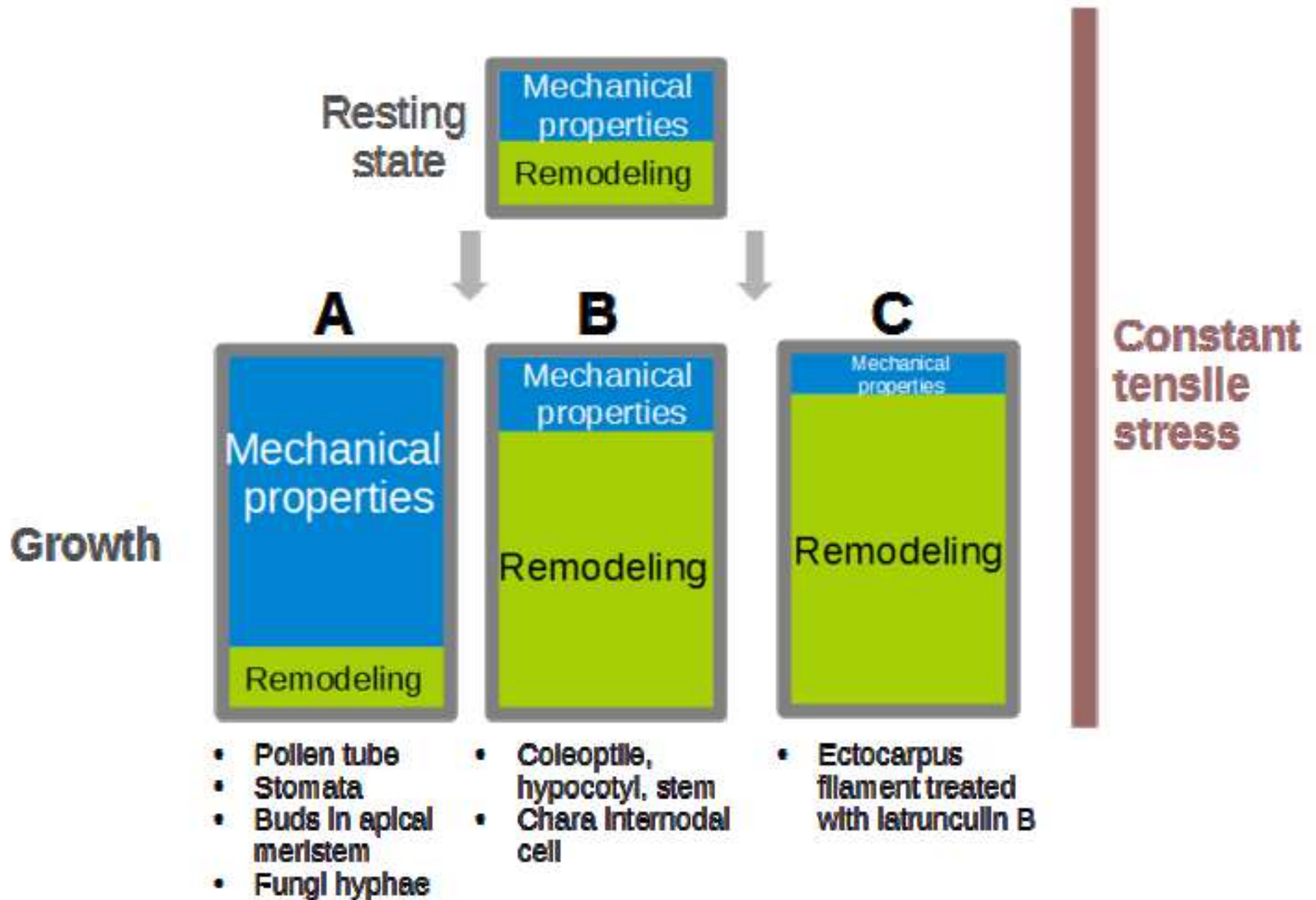
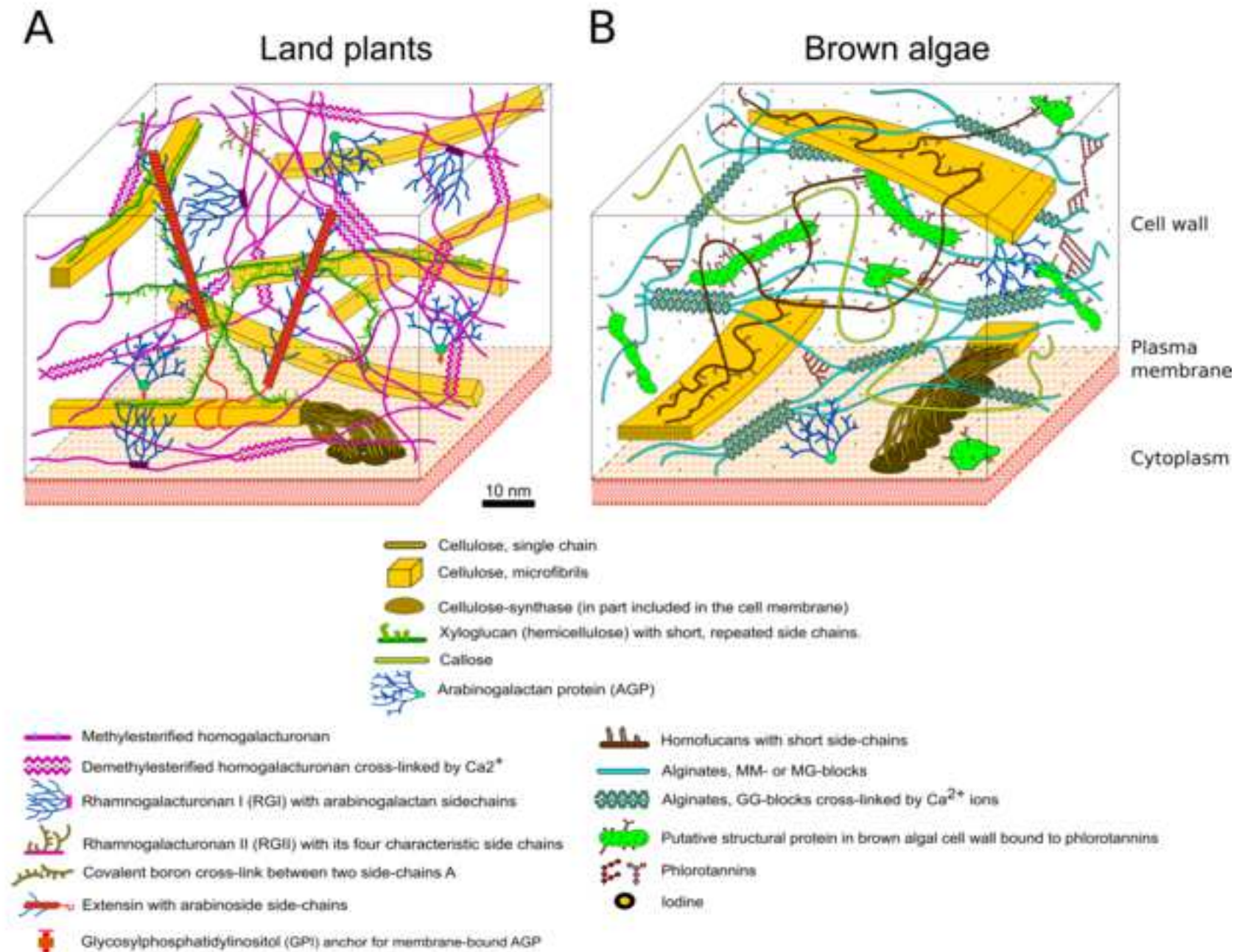


Figure 2





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Highlights

- There is a current overwhelming paradigm of cell growth that promotes one main scenario: intrinsic elasticity or plasticity of the cell wall control growth.
- In brown algae, which evolved independently from land plants and fungi, both the structure and the chemical composition of the cell wall differ from their counterparts.
- Beyond the complete inventory of cell wall components, their proportion and potential chemical modifications and interactions (covalent, 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 cell wall propensity to grow does not systematically depend on the intrinsic mechanical properties of the cell wall.
- Complexity and diversity of cell wall compositions and structures make preconceived transposition of cell wall growth mechanisms hazardous.

Outstanding questions

- What are the molecular bases of elasticity in brown algal cell wall, considering its specific composition?
- What is the molecular toolkit of cell wall remodelling in brown algae? Do proteins with similar functions as 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 process taking place during growth?
- Are current technical tools suitable to measure the relevant cell wall physical constants involved in growth ? Especially when several cell wall layers are involved ?
- Can cell wall mechanical properties measured at small time-scale be relevant for understanding processes occurring at long-time scale, typical of cell growth?
- To what extent can results obtained on model land plants be transposed to other species ? Which features should be common ? Chemical components, supramolecular structure and organization or intrinsic mechanical properties regardless of the chemical composition?