Wolpert's French Flag: what's the problem?

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Cell Wall Expansion: A Case Study of Biomechanical Process

Alexis Peaucelle

Abstract The mystery of plant biomechanical growth control lies in their ability to expand the cell wall without bursting. In this chapter, we will discuss pluralistic views on plant cell growth. We will try to show a multiples processes leading to growth and the redundant functions different components of the cell wall display in the growth process.

1 Basics of Plants Tissue Mechanics

1.1 Generalities

From the dawn of the humanity, the diversity of mechanical properties of the plant tissue was explored in tool-making, tissues, houses, furniture or cutlery. Even the discovery of artificial polymers did not entirely replace plants derived materials like linen or cotton.
In this chapter, we will focus only on the plant tissue mechanics and its link to growth: the interplay between organogenesis and mechanics of primary cell wall.
The main characteristic of plant cell is the presence of the cell wall, a rigid pectocellulose hydrogel encapsulating every single plant cell. Plant's cell wall forms a protective layer and provides structural support for the cell, generating unique properties of the plant tissue as well as strong constraints on the cell growth.

1.2 Basis of Plant Cell Wall Mechanics

The first thing you see in a plant tissue is the cell wall, as Robert Hook's historical description so remarkably demonstrated in Micrographia. This hydrogel, delimited by the membrane, surrounds the protoplast with its nucleus, mitochondria, plasters, and vacuole (fig. 1). The protoplasts exercise a pressure on the cell wall. A good metaphor is a bicycle tire and its tube. If you remove the pressure, cells collapse and the plant loses its shape. In some tissues, a process known as secondary cell wall thickening gives increases dramatically cell wall rigidity. In such tissues, turgor pressure is not required to maintain the organ shape. For more information read (Busse-Wicher, Grantham et al. 2016). Here we will focus on primary cell wall able to undertake expansion and growth.
Another metaphor that helps understanding the growth process in plants is the growing classroom: to expand a classroom in a brick building you need to extend the walls. For that, you will push on the walls and add new bricks or reshuffle the existing bricks in a less compact structure.
As with the tire metaphor, it helps understand the huge tension exerted on the cell wall, and the energy source needed to expand the cell wall. If the cell wall loses its integrity or the turgor pressure is too high, the cell bursts and the plant will collapses (fig. 2).

How does cell wall manage to expand without losing its integrity is an extraordinary biophysical puzzle that we will explore now.

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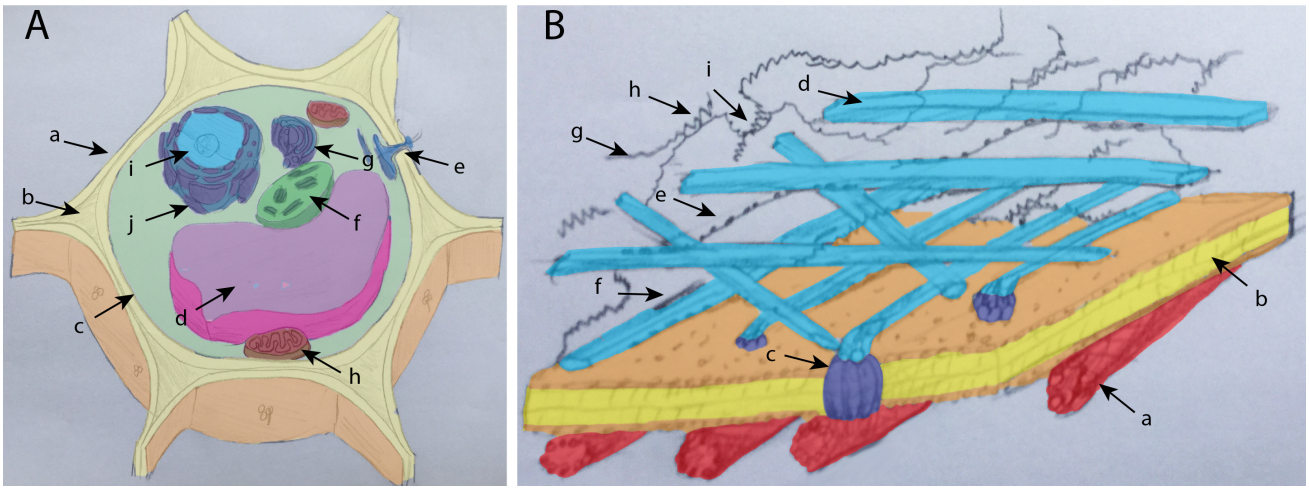

Fig. 1: Cell wall structure:
(A) Schematic organisation of a plant cell: (a) cell wall (b) mid lamella (c) plasma membrane (d) vacuole (e) plasmodesmata (f) chloroplast (g) golgi apparatus (h) mitochondri (i) nucleus (j) endoplasmic reticulum
(B) Schematic structure of primary the cell wall: (a) microtubules (b) plasma membrane (c) Cellulose synthase complex (d) Cellulos microfibril (e) hemicellulose (f) xyloglucans (g) Pectin
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2 What is a Growth?

2.1 Definition

First, let us define growth as an irreversible extension of the cell. If we compare the plant tissue to an inert material (with no biological activity), it will be a plastic extension (e.g. plasticine). For a tissue to expand it requires the cell walls to expand through rearrangement of existing cell wall component or synthesis of new material.

To describe the process of growth we need to measure three parameters simultaneously (Boyer, Cavalieri et al. 1985)
- Turgor pressure: the force that is pulling the cell walls apart. This pressure originates from the highly concentrated water in the cytoplasm and the vesicles of the cell. The hydrophilic molecules in the cytoplasm attract water that flows freely in and out of the cells and from cells to cells through aquaporin's (pores in the plasma membrane) or the plasmodesmata (cell-to-cell cytoplasmic junction).
- Cell wall mechanics: how much energy is needed to expand the existing cell wall (elasticity) and how much it could reshuffle itself (plasticity)
- The synthesis of new material: exocytosis of new cell wall material and cell wall synthetizing enzymes.

To measure all these parameters simultaneously turns out to be very difficult and has never been

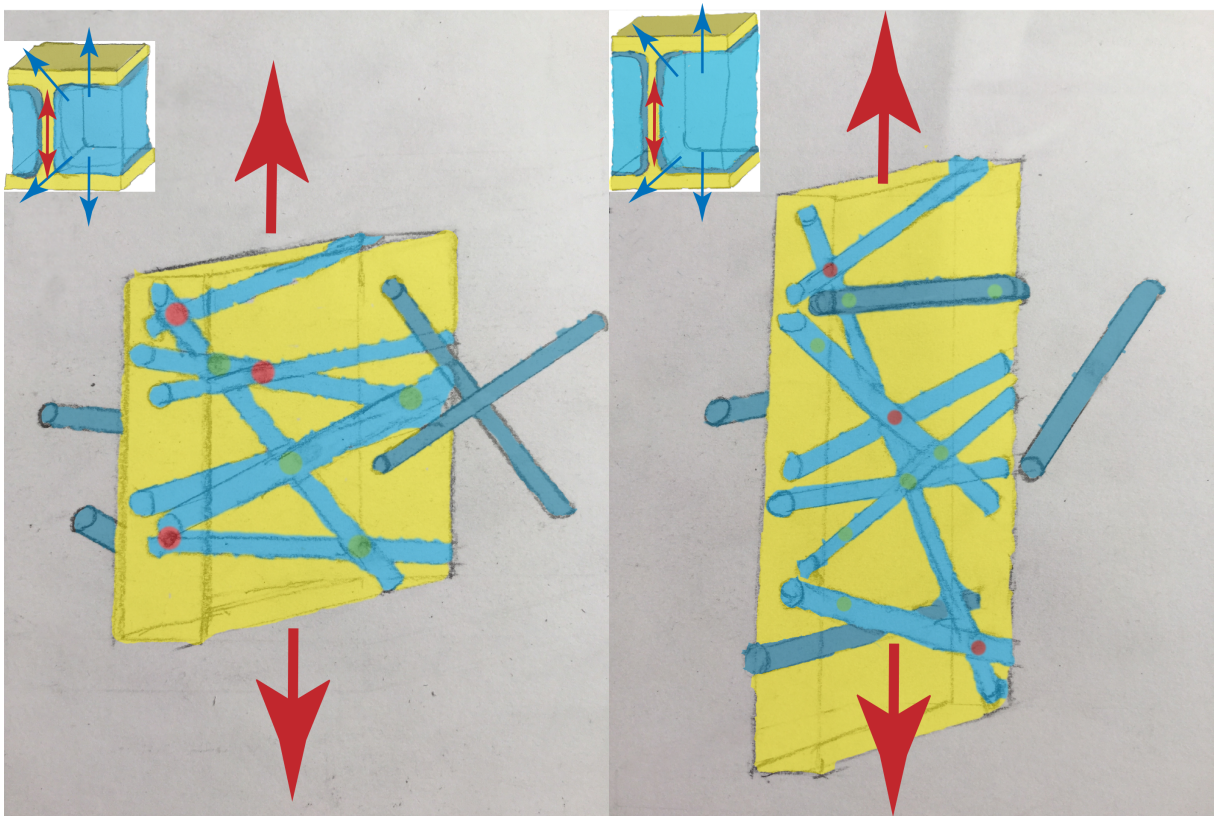

Fig. 2: The changes in cell wall during growth: Cell wall under the tension of the turgor pression (blue Arrow) will expend. Locale rearrangement of the cell wall along labile likes (in green) will permit rearrangement of the cell wall. Synthesis in scitu and exocytosis of new cell wall component (pupel elements) and the change in cell wall links to more rigid ones (Red) will prevent cell wall bursting.

achieved so far (with the exception of pollen tube). We will discuss all the technical challenges one by one in the following sections.

2.2 Is Growth Really a Mechanical Problem?

For long organogenesis was studied by tracking cells throughout the division, and was neglecting the cell expansion aspect of organogenesis. New organs correlate with new cell division patterns. For example, new organ formation in the meristem or later root is always associated with periclinal division in the deep layer of the tissue (Walles 1991). In some cases, the whole organogenesis could be described as series of organised symmetric or asymmetric divisions (Gunning, Hughes et al. 1978). This works have demonstrated how important is the cell division pattern for the organogenesis. In the early 90 a series of experiments increasing or decreasing cell division rate in elongating tissue showed that it had little or no effect on the organogenesis (Wyrzykowska, Pien et al. 2002). This brought back the old idea that cell mechanics more than the cell number controls the growth. However, cell division and cell expansion are linked; cell division is under the control of cell expansion. It is possible to predict cell division in the meristem by its increase in size and the mechanical stress it is under (A, Forero-Vargas et al. 2017). In other words, cell growth due to cell wall expansion prefigure the division pattern that is following the cell structure achieved by growth. Therefore, we could settle on the idea that growth and organogenesis in plants is cell wall expansion driven.

Yet, recently a study has shown that in the meristem the levels of cell wall synthesising enzyme are

cell division controlled (Yang, Schuster et al. 2016). Thus, there is mutual control: growth-associated changes in cell wall mechanics could be under the control of the cell division process itself.

Summary Cell division and growth are linked through the following feedback loop: cell wall mechanics controls cell expansion that control cell division which in turn affects cell wall mechanics.

3 The modelling and mathematical approach

The elements that control the cell wall expansion are clearly a complex process. To grasp a complex problem it is often helpful to propose a simple mathematical equation with minimum possible variables. This was most clearly stated by Lockhart in (Lockhart 1965) which propose a biophysical equation for the mechanical control of the growth of the cell wall.

Rate=m (Ψp-Y)
The growth rate (Rate) is proportional to the turgor pressure (Ψp) and extensibility (m) above the yield threshold (Y).
Behind this mathematical statement is the following idea. The pressure is associated with two parameters that describe cell wall mechanics: its capacity to expand irreversibly (m) and a threshold (Y). The existence of a threshold stands for the ability of plant tissue to halt the growth without stopping synthesis or by reducing to zero the turgor pressure.
Defining those parameters and estimating their numerical values is quite a challenge.
The extensibility 'm' is a complicated parameter to determine. In Lockhart, m stands for all the parameters that permits expansion: synthesis of new cell wall components and extension of the existing ones. Which of the two parts is important is a subject of debate that is polarising scientific community. In the creep experiments, which determine the cell wall remodelling under tensile stress, 'm' was often reduced to plastic deformation. As discussed above synthesis of cell wall component is also to be considered in irreversible expansion and is a component of the 'm' factor.
One other way of evaluating parameters is a computer simulation.
Since Lockhart publication, series of models describing organ growth was proposed. They are always faced with the geometrical problem and huge amount of unknown parameters e.g. thickness of the cell wall or synthesis rate. As for today successful models managed to describe growth in 2D.
Anja Geitmann (Parre and Geitmann 2005) was the first to propose a reliable model for pollen tube growth. The pollen tube is a cell presenting a very rapid tip growth; its goal is to project the sperm cell situated at the tip of the tube into the ovule and thus grows through the pistil. The most recent models are taking into account change of the local geometry of the cell wall over time. They are able to simulate the transient oscillatory growth in different pollen tube species observed in nature. In this work, the minimum number of parameters for the model was measured directly. To fit at best to the reality the "guessing" of the different parameters of the equation is associated with real measurement of cell wall elasticity, cell wall synthesis (exocytosis rate), turgor pressure, and measurement of the growth with high temporal resolution.

Summary modelling helps to test and evaluated the importance of different elements in growth. The most informative models are the simplest ones that could describe the observed growth, based on the minimal number of variables. The best studies also associate the evaluation of the parameters with in situ measurement.

4 Measuring the Turgor Pressure

Turgor pressure is a crucial parameter (Deri Tomos, Malone et al. 1989). Yet, measuring it is

technically challenging. Series of different methods have been proposed and used, but so far, there is only a handful of successful experiments on growing organs.
The first methods were based on finding the point at which the turgor pressure in the cell is balanced by the pressure in the mounting media. Above a threshold the turgor pressure is not acting on the cell wall, the cell is plasmolyzed. It is important that the osmolyte (the ion used in the media to compete with the cellular ionic concentration) cannot be internalised by the cell, and that in the same time the water can flow freely out of the tissue (Falk, Hertz et al. 1958, Nilsson, Hertz et al. 1958, Stadelmann 1984).
To observe when the ionic activity of the external solution matches the cell one classic method uses microscopy observation. This is a classical classroom experiment often performed on naturally coloured cells, as red onion or flower petals. The limitation is the field of view of the microscope. For a full tissue, one can use vibration to determine the plasmolysis point. This technique relayed on the fact that the vibration properties of the tissue are related to the rigidity of the tissue. The rigidity itself depends on the turgor pressure (Virgin 1955). The rigidity will drop with the pressure until plasmolysis is reached. At this point, the rigidity is not sensitive to plasmolysis and depends only on the cell wall elasticity.
Another approach is to measure the pressure directly by puncturing the cell with a tube. It works only in the big cells, but not in the very small cells important for the growing tissues (Green 1968, Green, Erickson et al. 1971, Büchner, Zimmermann et al. 1981) .
The most complete measurement was done on the root. In this study, the authors could not detect any differences in the turgor pressure along the elongating roots. This indicates that so far there is no evidence to support action of turgor pressure on the variability of growth rate observed within the organ.

Summary The tools available for measuring of the turgor pressure are not precise enough to measure single cell turgor pressure in the early stage of the organ formation. Especially in the model-plant, Arabidopsis Thaliana having particularly small cells.

5 Cell Wall Rheology

5.1 Definition

Rheology studies deformation and flow of matter; here we review rheology of the particular hydrogel, the cell wall. As any hydrogel, cell wall mechanical properties change with the amount of water it contains. Secondly, once dehydrated cell wall has irreversibly lost its original mechanical properties.
Mechanical properties of hydrogel depend on the ionic composition present in the solution. By the affinity to water ions influence water activity i.e. cell wall hydration. Monovalent ions intercalates into the gel affecting the distance between the polymers working as plasticizers. In contrast, the divalent ions could create bonds between charged of polymer mesh. For more details, look at the section on pectin. Thus, the mechanical properties of primary cell wall could be determined only on the fresh, intact cell wall with a protocol not changing the ion composition.
Now how do we actually measure the mechanical properties? By deforming the sample and by recording the force required to do so in time. Alternatively, by applying a constant force and recording the change in shape of the sample over time. Several parameters can be measured this way and it depends on the type of deformation observed: if the deformation is reversible, one measures elasticity (cell wall restores its original shape after the force has been removed). The time it takes to come back to its original shape is a measure of the viscoelasticity. Irreversible extension measures viscosity of the material (Braybrook, Hofte et al. 2012). To measure the change in shape indirectly one looks at the indentation depth or using fluorescent probes (Kim, Yi et al. 2015).

In plant biophysics, the majority of mechanical measurements are design to measure the growth capacity of the tissue, thus the measure of yet a different rheological property of the cell wall: the "creep".

5.2 Creep

Definition of the creep is inconsistent throughout literature. In general, creep refers to the growth capacity of the tissue. It could be thought as the 'm' factor in the Lockhart equation (Taiz 1984). If growth occurs mainly due to rearrangement of the cell wall network, it can be measured as the energy required to stretch the cell wall (Keegstra, Talmadge et al. 1973). Many components involved in loosening of the cell wall was characterised with this method. One of the founding fathers of this type of measurements is Paul Green. He worked on a giant cells from a single cell green algae (Cara) (Green 1976). He measured the relative importance of turgor pressure, elasticity and creep in growth of the cell wall, thanks to measurement of extension at a subcellular resolution and the extension capacity. He always kept a critical view on the creep measurement and inability to separate the part of rearrangement of the cell wall and cell wall synthesis (Green and Cummins 1974).
Recently, cell wall rearrangement during creep has been finally observed thanks to the use of atomic force microscopy (AFM). Demonstrating that cell wall rearrangement, at least in the epidermis, is associated with the elongation of the tissue and is reflected in the creep experiments (Zhang, Vavylonis et al. 2017).

5.3 Other Measurement of Rheology on the Cell Wall

Measurement of the elasticity, viscoelasticity and viscosity in the living tissue using nanoindenter has recently been developed. Surprisingly, elasticity, the reversible deformation of the cell wall, was correlated with growth and not viscosity or viscoelasticity (Peaucelle, Wightman et al. 2015). This is paradoxical, since elasticity is a reversible deformation, whereas growth is an irreversible process. It is also at first glance in opposition with the creep experiments that put cell wall remodelling at the heart of growth process. This may be explained if elasticity is correlated to the growth through the control of the cell wall synthesis and not cell wall remodelling. In other terms, the synthesis of new material is linked to the elasticity of the cell wall. This correlation was first demonstrated on the pollen tubes: locale changes in cell wall elasticity correlates with the position of exocytosis of cell wall component at the tip of a cell. This process was observed to involve cytoplasmic calcium signalling coupled with deformation sensitive calcium channels (Fayant, Girlanda et al. 2010).

Summary creep experiments measure directly the cell wall ability to rearrange in association to growth. Elasticity of the cell wall relates to the growth in a yet to determine manner that could be related to cell wall synthesis. Therefore, two independent growth processes may relate to different cell wall rheological properties.

6 Organogenesis and Polar Growth of Tissue: the Cell Wall Component Contribution

The turgor pressure that is driving the cell expansion is isotropic. If it was the only parameter controlling the growth, it could lead to homogenous elongation, thus a sphere. Then, plants will look like La gioconda-botero (fig. 3). Somehow, this isotropic force is transformed into an anisotropic orientated growth. Which component of the cell is responsible? A good candidate is a cellulose (Green 1980).

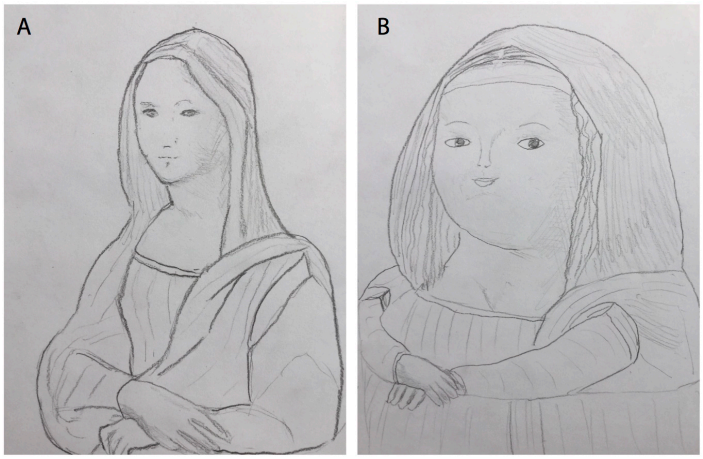

Fig. 3: Isotropic growth illustrated by (A) schematic representation of the Jocund Leonardo da Vinci and (B) the Jocund of Botero.

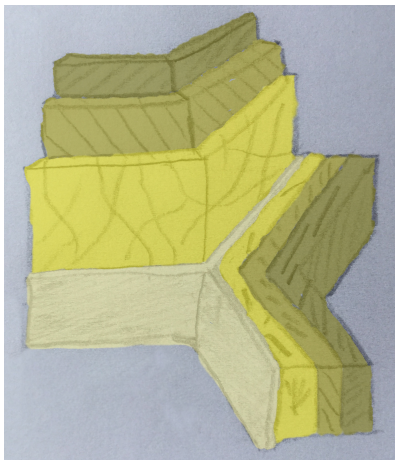

Fig. 4: Laminated cell wall schematic representation.

6.1 Cellulose

As you have red in chapter 1 on the history of the cell wall biochemistry, determining the structure of the cellulose was a long and difficult path. It took 30 years from the first chemical isolation of cellulose component to the determination of the polymer structure of the cellulose. From the start, cellulose was considered as the load-bearing component of the cell wall responsible for anisotropic growth.

The basic idea is that the bundles of cellulose fibrils build up an orientated network surrounding the cell and block the expansion in one direction. We could compare it to the metal rings around the barrel preventing it from opening up. Electron microscopy data and cell wall optical imaging support this theory. Series of brilliant imaging showed orientated microtubule not exactly perpendicular to the cell but organised in a sheets like the laminated wood structure (fig. 4). At the same time, Paul Green observed that in the giant algae cells the cellulose fibrils are orientated in a loser way, a network (Green 1960, Gertel and Green 1977). These two publications mark the point when scientific community divided into two camps. First one supports the laminated organisation of the cellulose and suggests that the orientation of the fibrils in a sheet is stable during growth. The loosening between the lamellas will lead to progressive reorientation of the whole sheet. In contrast, the network theory following Green observation suggest that the latest microtubules are deposited in an orientated way, but growth modify their orientation distorting the network. Here, only the latest synthesised cellulose fibrils control the anisotropic orientation (Marga, Grandbois et al. 2005). Recent observation of multi-network structure and its reorientation by Cosgrove (Zhang, Vavylonis et al. 2017) supports the concept of network.

In fact, both visions may be right: the multi-network structure was observed in the external cell wall of the epidermal cells, whereas the laminated structure mostly in the internal cell wall. Thus, the two concepts of the cell wall structure may simply relate to two different cell wall types.

The key role of cellulose in anisotropic growth was most strikingly demonstrated by the swelling phenotype of plants following chemical treatment affecting cellulose or microtubule synthesis as well as the phenotype of the mutation affecting cellulose synthase (botero figure? XX) (Ledbetter and Porter 1963, Heath 1974, Mueller and Brown 1982). The similarity of this phenotype with the effect of the inhibitor of the microtubule synthesis led to the idea that the orientation of microtubules is generated by the orientation of the cellulose (Heath 1974) . This concept is supported by the observations that the cellulose synthase and the microtubules are found in the close proximity. Commonly used model states that the cellulose synthase is polymerising the cellulose directly in the plasma membrane following the orientation of the microtubules and is supported by series of

microscopy observation. The microtubule orientation will then lead to a mechanical anisotropy in the cell wall and anisotropic expansion of the cells.
To complicate this picture, resent work (Peaucelle, Wightman et al. 2015) has shown that treatment affecting cellulose synthase or microtubule orientation also affect other component of the cell wall: the pectin methylation.

6.2 Pectin

Pectin form a fine meshed network surrounding all the other components of the cell wall. There are several chemically different components and we will focus on the homogalacturonan. This component is a polymer of galacturonan sugar, which presents a lateral carboxyl group, which can be methylated or not.
In the 1980-ties the 3D structure of the two polymers was predicted (Morris, Powell et al. 1982). The methylated pectin was predicted to form a very compact structure with proton stabilised interaction on the methylated carboxyl (Grant, Morris et al. 1973).
The demethylated pectin were anticipated to generate a more hydrated, less packed structure stabilised by calcium electronic interaction through demethylated carboxyl group. This model was named the egg box structure, where the stability of such conformation would be archived for at least 9 successive demethylated homogalacturonan. For long, the calcium bonds found in the demethylated pectin were thought to generate strong links in the cell wall and thus limiting cell wall remodelling and creep. They were suggested to slow down the growth. As for the methylated pectin interaction it was forgot except in food industry.
The vision of demethylated pectin linked to a rigid cell wall was first challenged when demethylation of the pectin was shown to lead to organ formation and reduction in cell wall elasticity in the meristem (Peaucelle, Braybrook et al. 2011).
Later on it was found that the anisotropic changes in the methylation are required for polarised elongation of the hypocotyl and associate with a reduction in the cell wall elasticity (Peaucelle, Wightman et al. 2015). This founding led to the proposal of a two-step process of an anisotropic elongation of the tissue. Antipodal changes in the cell wall elasticity due to changes in the pectin methylation led to a tenfold anisotropic elongation. This anisotropic growth is followed by the alignment of the microtubules and cellulose microfibers. Thus, the cellulose microfibers are needed for further anisotropic elongation that could achieve a 100 and even a 1000 fold anisotropic elongation.
Furthermore, these two components are interacting: the treatment affecting microtubule and microfibril orientation also affects the pectin methylation pattern (Peaucelle, Wightman et al. 2015).

Summary polar elongation is a two-step process: first, change in the pectin methylation leads to a change in the cell wall elasticity, followed by cell polarity (cell mechanical asymmetry). Microtubule reorients along the elongation axes leading to orientated cellulose synthesis. This generates a cell wall anisotropy.

6.3 Xyloglucans

Xyloglucans, a component of the hemicellulose, has focused a lot of attention since the strong interaction with the cellulose was described. Models predicted that reducing the amount of Xyloglucans could increase the creep by decreasing the cellulose microfibril cohesion and helping local rearrangement of the cell wall.
The enzymes that are controlling the structure of the Xyloglucans network were predicted. The genes coding for those proteins are expressed in a tight developmental pattern and are present in the places with strong elongation (Antosiewicz, Purugganan et al. 1997). Unfortunately, the multiple mutations

in those genes do not present an obvious growth defect phenotype. Do Xyloglucans have now function? Certainly not. We have seen that there are multiple mechanisms controlling the growth. It is likely that the absence of the Xyloglucans remodelling is compensated (Cosgrove 2016).

6.4 Expansins

Expansins form a family of the cell wall proteins. Their importance in growth was demonstrated when purified proteins were shown to accelerate the growth in some tissues(Fleming, McQueen-Mason et al. 1997). They are the only known proteins promoting the creep in vitro (Cosgrove 1998, Shieh and Cosgrove 1998). There are two isoforms present in a multi gene family in all the plant kingdom (Cosgrove 2015). The first one interacts with the cellulose and the second, in grasses, was shown to interact with glucuronoarabinoxylan, a grass specific carbohydrates.
Interestingly, only specific cells are sensitive to Expansins. This suggest that not all the cell walls could respond to Expansin demonstrating a multi-level control (McQueen-Mason and Cosgrove 1995).

Summary cell wall chemical components have redundant function in cell wall mechanical properties and growth. This chemistry is very dynamic and is under the control of complex signalling network that are still to be described. So far, we have seen only the tip part of the iceberg of this chemical complexity.

7 Input From Growth Measurement

Understanding plant cell wall mechanics and its link to cell wall chemistry is only one part of the problem. It is also important to focus on a detailed quantitative measurement of growth process itself in particular plant growth-induced motions.
Observation of the plant motion was at the heart of scientific debate for long: first reported in 400 BC it was already discussed in Hook's famous publication, which coined the word cell. The growth related motion, in particular circumnutation, fascinated Darwin (Physical Biology Mathieu Riviere, Julien Derre and Stephane Douady). The first movie of a growing plant dates from the end of the 19th cetury, yet quantification of the growth is still difficult event today because it occurs in three dimensions. Until now only the 2D gravity growth response have been fully described (Erickson 1976).
Those films reveal that plants adapted their shape to external stimulus such as light and gravity. These growth movements are named phototropism or gravitropism. There exist less known growth movement: ototropism also named proprioception (Bastien, Bohr et al. 2013). Proprioception means the plants can adapt shape to itself: plant is able to sense its own shape and control tissue growth in order stay straight. The shape of the Arabidopsis grown in microgravity at the international space station illustrates well the proprioception. Plants grown in space are almost identical to the control plants grown on earth (Link, Durst et al. 2003, Link, Busse et al. 2014) . Complete study of gravitropism have led to the same conclusion on earth-grown plants.
These exiting results reinforce the crucial importance of the feedback loop between growth mechanics and tissue structure not only at the subcellular level but also up to the whole organ and organism level.
Another fascinating thing about plant motion linked to proprioception is oscillatory movements revealing complex regulatory network of growth acting at different time scales. It also explains all the redundant functions and parallel growth processes we have discussed so far.

8 About the Regulatory Network

The next step is to explore regulatory networks involved in growth. The study of signalling network in plants is described in other chapters; here, we will briefly discuss two aspects. The auxin regulatory network is the most studied one. Auxin was isolated thanks to its capacity to promote growth. The growth induction capacity of auxin was rapidly associated to the acid form of the molecule. It was proposed that auxin promotes growth through acidification of the cell wall leading to cell wall rearrangement. This model was rapidly confirmed by the observation that expanding cell wall has low pH (Tepfer and Cleland 1979).

The most intriguing part of auxin growth network was obtained from the study in the meristem and the generation of the phyllotactic pattern. First, since the work of Stephane Douady and Yves Couder we know that the generation of the phyllotactic pattern requires a dynamic feedback loop between an inhibitory and activating signals in the meristem (Douady and Couder 1992). The isolation of the pin1 mutant and the arrival of fluorescent reporters revealed that auxin is the activator molecule necessary and sufficient for the induction of the organ formation (Okada, Ueda et al. 1991). The dynamics of the auxin transporter in the meristem depletes the areas surrounding the new organs thus acting as a inhibitory signal (de Reuille, Bohn-Courseau et al. 2006). The dynamics of the structure was suggested to be generated by the auxin concentration itself.

Recently, auxin was shown to be inducing the pectin demethylation in the primordia and that this change was necessary and sufficient for organ formation (Braybrook and Peaucelle 2013). Intriguingly, pectin demethylation was necessary and sufficient for auxin-induced growth, suggesting that the auxin acidity is not sufficient for organ growth and that the acidification of the cell wall commonly associated with growth could be instead attributed to the acidification by carboxyl group formed following pectin demethylation. Auxin polar transport regulation was also questioned; it could not simply be other auxin concentration as the changes in cell wall chemistry led to polar auxin transport destabilisation. In parallel cell ablation experiments in the meristem showed that, as microtubules, auxin polar transport was responding to mechanical stimulus (Hamant, Meyerowitz et al. 2011). These results suggest that the polar auxin transport is at least partially under the control of mechanical constraints arising from differential cell wall elasticity of the growing organ. This feedback loop is at the heart of organ formation. How exactly this feedback is generated is still to be discover; it could be chemical or mechanical signals.

How mechanical clues from the direct cellular environment could be synthetized and transduced to the cell is also an important future research area (Wolf, Hematy et al. 2012). One important component of this regulation is the transmembrane kinase receptor (Theseus and Feronia are the most studied ones). The extracellular domain of this protein could sense the chemical/mechanical stress of the cell wall and feedback through a kinase cascade to the nucleus and affect gene transcription. The beauty of the kinase-signalling cascade is its integrative capacity (for more information read the regulatory kinases in cell cycles in animals). If the plants regulatory system is as complicated as the one described in mammalian cell it could be decades before we managed to grasp all the subtlety of this regulatory network.

9 Conclusion and Perspective

Clearly, we are far from understanding the mechanics of plant growth. Yet in all direction, we are gaining new insight at an incredible pace. The precise description of several of the key element regulating growth puts the ground for the study of the regulatory network. Yet full part of the process is still invisible. Either it is because of the complexity or because we lack a crucial aspect, a complete understanding of the process is clearly out of our reach. If we come back to our original question - how cell wall is expanding without the cell bursting- we still do not have a satisfactory answer. New technology and thinking out of the box will certainly help to solve this puzzle.

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