



HAL
open science

Interaction between turgor pressure and plasmodesmata permeability

Valeria Hernández Hernández

► **To cite this version:**

Valeria Hernández Hernández. Interaction between turgor pressure and plasmodesmata permeability. Development Biology. Université de Lyon, 2019. English. NNT : 2019LYSEN076 . tel-02631574

HAL Id: tel-02631574

<https://theses.hal.science/tel-02631574v1>

Submitted on 27 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Numéro National de thèse: 2019LYSEN076

THESE DE DOCTORAT DE L'UNIVERSITE DE LYON

opérée par

l'Ecole Normale Supérieure de Lyon

École Doctorale No. 340

Biologie Moléculaire Intégrative et Cellulaire (BMIC)

Spécialité de doctorat: Biologie

Soutenue publiquement le 17/12/2019, par:

Valeria Hernández Hernández

Interactions between turgor pressure and plasmodesmata permeability

**Interactions entre la turgescence et la perméabilité des plasmodesmes au
cours du développement des plantes**

Kolb, Evelyne	Maître de conférences	UPMC	Rapporteuse
Leblanc-Fournier, Nathalie	Maître de conférences	UCA	Rapporteuse
Ingram, Gwyneth	Directrice de recherche	ENS Lyon	Examinatrice
Boursiac, Yann	Chargé de recherche	BPMP	Examinateur
Godin, Christophe	Directeur de recherche	ENS Lyon	Examinateur
Boudaoud, Arezki	Professeur ENS Lyon	ENS Lyon	Directeur de thèse

Abstract

Plant cells are surrounded by the rigid cell wall that precludes developmental processes that are central in animal development, like cell migration and tissue rearrangement. Instead, plant development relies mostly on cell division and expansion. The current paradigm assumes that cell expansion depends on the biomechanical properties of the cell wall and on the generation of turgor pressure. Plasmodesmata are membrane-lined channels that connect neighboring cells and allow free movement of molecules that are smaller than their diameter (i.e., permeability). It is known that their permeability changes during plant development and that these modifications can affect movement of solutes, such as sucrose. Because of these reasons, plasmodesmal permeability seems to be a good candidate for the regulation of turgor pressure during cell expansion, however, its contribution remains largely unexplored. In turn, previous studies suggest that plasmodesmata may respond to changes in turgor pressure. In this work we put forward the hypothesis that turgor pressure and plasmodesmal permeability may affect each other during plant development. We addressed this problem by, first, reviewing the experimental information that supports our hypothesis. This work allowed us to build and propose a network of interactions between different cellular and molecular factors that might mediate these feedbacks between turgor and plasmodesmata and to put forward testable predictions. Second, we generated a computational model to explore one direction of these interactions: the role of plasmodesmal permeability on turgor pressure regulation. Our model uses Lockhart's equations for irreversible cell expansion with addition of plasmodesmal-dependent fluxes of water and solutes. We used cotton fiber as a study model because it is a single cell without division that mostly increases in length. Furthermore, previous experimental studies in this system have correlated closure of plasmodesmata with peak values of turgor pressure. The results of our model suggest that plasmodesmal permeability is, indeed, a key factor in regulating turgor pressure. Moreover, we suggest that dynamical changes of plasmodesmal permeability are needed in order to recover turgor pressure behaviors that have been experimentally reported. Finally, we explored with our collaborators the potential contribution of plasmodesmal permeability in the evolution of complex multicellular plants using the "Dynamical Patterning Modules" (DPMs) framework. These ideas can be useful in understanding how plant body plans originated.

Résumé

Les cellules végétales sont entourées par une paroi cellulaire rigide, ce qui exclut des processus essentiels au développement animal, comme la migration cellulaire et le réarrangement des tissus. Au lieu de cela, le développement des plantes repose sur la division et l'expansion cellulaires. Le paradigme actuel suppose que l'expansion cellulaire dépend des propriétés biomécaniques de la paroi cellulaire et de la génération de la pression de turgescence. Les plasmodesmes sont des canaux membranaires qui relient des cellules voisines et permettent la libre circulation des molécules plus petites que leur diamètre (définissant leur perméabilité). Il est établi que la perméabilité des plasmodesmes change au cours du développement de la plante et que ces modifications peuvent affecter le mouvement des sucres. Pour cette raison, la perméabilité des plasmodesmes semble être un bon candidat pour la régulation de la pression de turgescence lors de l'expansion cellulaire, cependant, son rôle reste largement inexploré. Ainsi, des études antérieures suggèrent que les plasmodesmes peuvent répondre aux changements de pression de turgescence. Dans ce travail, nous avons avancé l'hypothèse selon laquelle la pression de turgescence et la perméabilité des plasmodesmes pourraient s'influencer au cours du développement de la plante. Nous avons abordé ce problème en mettant tout d'abord en avant un réseau d'interactions entre différents facteurs cellulaires et moléculaires susceptibles de médier ces rétroactions entre la turgescence et les plasmodesmes. Deuxièmement, nous avons généré un modèle informatique pour explorer une direction de ces interactions: le rôle de la perméabilité des plasmodesmes sur la régulation de la pression de turgescence. Notre modèle utilise les équations de Lockhart décrivant l'expansion cellulaire irréversible, auxquelles sont ajoutées les flux d'eau et de solutés à travers les plasmodesmes. Nous avons utilisé la fibre de coton comme système d'étude car il s'agit d'une cellule unique qui ne se divise pas et qui augmente généralement en longueur. De plus, des études expérimentales antérieures dans ce système ont montré une corrélation entre la fermeture de plasmodesmes et des valeurs maximales de la pression de turgescence. Les résultats de notre modèle suggèrent que la perméabilité des plasmodesmes est en effet un facteur clé dans la régulation de la turgescence et de la croissance de la fibre du coton. De plus, nous suggérons que des changements dynamiques de la perméabilité des plasmodesmes soient nécessaires pour récupérer les comportements de pression de turgescence qui ont été rapportés expérimentalement. Enfin, nous avons exploré avec nos collaborateurs l'apport potentiel de la perméabilité des plasmodesmes dans l'évolution des plantes multicellulaires complexes à l'aide du cadre des "modules de patterning dynamique" (DPM). Ces idées peuvent être utiles pour comprendre l'origine des plans d'organisation des plantes.

Contents

1	Introduction	7
1.1	The plant cell wall	8
1.1.1	Cell wall components	8
1.2	Cell expansion as a mechano-hydraulic process: the Lockhart model	10
1.2.1	Types of cell wall expansion	10
1.2.2	Cell wall loosening enzymes	11
1.2.3	Fluxes of water	11
1.3	Cotton fiber as a study system	13
1.3.1	The pattern of cotton fiber expansion	14
1.3.2	Biochemistry of the cell wall of cotton fiber during development	14
1.3.3	Aquaporins (AQPs)	14
1.3.4	Sources of solutes	15
1.3.5	Plasmodesmal permeability	15
1.4	General questions	17
2	Interplay between turgor pressure and plasmodesmata during plant development	19
3	A mechano-hydraulic model for the study of cotton fiber elongation	31
4	Discussion	61
4.1	Regulation of plasmodesmal permeability might have been critical for the evolution of complex multicellular plants	62
4.2	Turgor may affect plasmodesmal permeability	62
4.3	Plasmodesmal permeability may affect turgor-driven growth of cotton fiber cells	62
4.4	Removing the dynamics of plasmodesmal permeability may preclude non monotonic behavior of turgor pressure	63
4.5	General Conclusion	64
5	Appendix	71

1

Introduction

Growth of plant cells is based on a delicate balance between extension of the cell wall and the build up of turgor pressure. Understanding how turgor pressure is regulated is, thus, a central question for plant growth. Plasmodesmata channels are known for allowing free movement of molecules that are smaller than their diameter, such as sucrose and water. The diameter of plasmodesmata (also known as permeability) has been shown to vary during plant development. Given these peculiarities of plasmodesmata they seem to be good candidates for the regulation of turgor pressure changes during cell expansion, however, very few studies have addressed this question. We address this problem and extend it by asking if turgor pressure can, in turn, affect plasmodesmal permeability.

In this introduction, we will first present the plant cell wall and its main components. We will briefly describe Lockhart's model for cell expansion and try to relate its main parameters to cellular processes. Finally, we will introduce our study system, cotton fiber cells, and present the general questions. The topics discussed in this introduction are aimed at complimenting Chapter 2 and provide the reader with the necessary information for Chapter 3.

1.1 The plant cell wall

Plant cells are tied together by the cell wall, a mechanically strong structure. Hence, important processes in animal morphogenesis, such as tissue rearrangement and cell migration, are unavailable to plants. Instead, plant growth and development rely mostly on oriented cell division and anisotropic expansion of the cells [44].

The cell wall consists of a complex matrix of cellulose and two major groups of polysaccharides, pectins and hemicelluloses, linked together by covalent and non-covalent bonds [8]. It also contains proteins, enzymes and other polymers that modify the physical and chemical features of the cell wall [44]. It controls cell shape and allows high hydrostatic pressures to develop. As a physical material, the cell wall presents properties of both solids and liquids and is categorized as a viscoelastic material [44].

Plant cell walls are mainly categorized into two types: primary and secondary walls. **Primary cell walls** are formed after cell division and they are considered to be relatively unspecialized and similar in molecular composition across cell types. Their main components are cellulose microfibrils, hemicellulose and pectins. **Secondary walls** are frequently formed after cell expansion ceases and they are specialized structures that reflect the differentiated state of cells. They are often multilayered and contain more cellulose than primary cell walls and lignin.

1.1.1 Cell wall components

We will briefly describe in this section the main components of cell walls (Figure 1.1). **Cellulose** is a packed microfibril of linear chains of (1→4)-linked β -D-glucose. The current model suggests that parallel glucans link to cellulose fibrils to form a crystalline structure that is mechanically strong and resistant to enzymatic activity [8, 44]. **Hemicellulose** is a heterogeneous group of polysaccharides, being xyloglucan and arabinoxylan the most abundant types [8, 44]. Xyloglucans have short chains that contain xylose branches which, in turn, can be appended with galactose (Gal) and fucose (Fuc) residues. Arabinoxylan has a (1,4)-linked β -D-xylan backbone with arabinose branches [8]. **Pectins** are also a heterogeneous group of polysaccharides that contain acidic sugars like galacturonic acid and neutral sugars like rhamnose, galactose and arabinose [44]. The pectic polysaccharide homogalacturonan has a relatively simple structure of a (1→4)-linked polymer of α -D-glucuronic acid residues. The most abundant pectin, rhamnogalacturonan I (RGI) has a long backbone with alternating residues of galacturonic acid and rhamnose [8]. Rhamnogalacturonan II (RGII) has a complex structure with up to 11 different types of sugars as residues. **Callose** is a linear homopolymer of β -1,3-linked glucose with some β -1,6-branches [5, 51]. It is deposited at cell plates during cytokinesis and around plasmodesmata - membrane-lined channels that traverse the wall and create symplasmic continuity [3]. Accumulation of callose has been correlated to changes in the diameter of the plasmodesmal channels and in cell-to-cell movement of molecules [51]. Callose deposition can also be induced by wounding, abscisic acid, and pathogen infection [5].

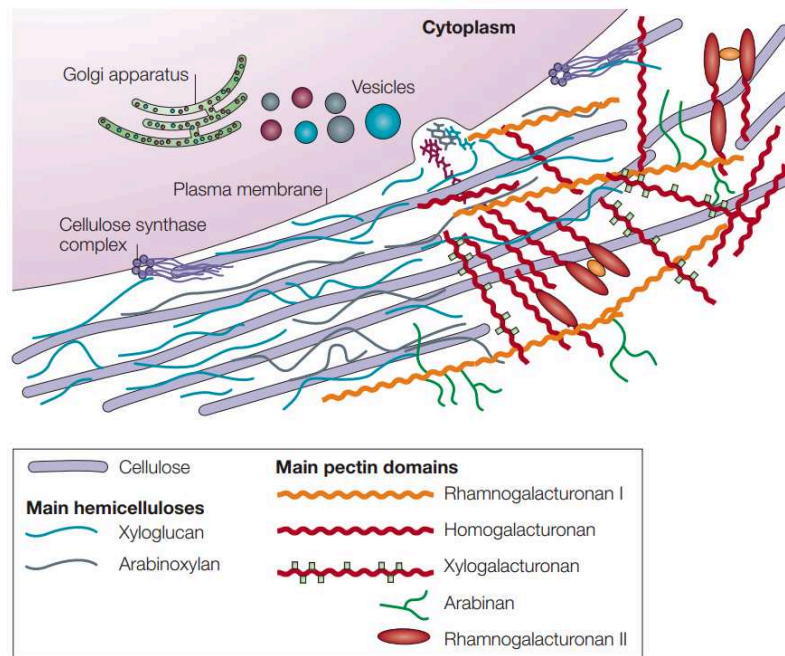


Figure 1.1: Schematic representation of the main structural components of the primary cell wall (taken from [8]).

1.2 Cell expansion as a mechano-hydraulic process: the Lockhart model

Turgor pressure (P) is the force that water molecules exert per unit surface of the plasma membrane. Turgor pressure and mechanical properties of the wall have been traditionally related to cell expansion. Relaxation of the wall stress is crucial because it reduces water potential of the cell which, in turn, drives water influx. Water uptake occurs passively following the difference in water potential $\Delta\Psi = \Psi_{outside} - \Psi_{inside}$, being $\Psi = P - \pi$, the difference between turgor and osmotic (π) pressures. Movement of water across the membrane depends on the permeability of plasma membrane, L , which is a function of the physical structure of the membrane and the movement of water by aquaporins, and the surface area A [44]. Changes in water volume are given by

$$\frac{dV}{dt} = AL\Delta\Psi$$

As water enters the cell, turgor pressure increases and when it surpasses the critical threshold, called yield turgor (Y), irreversible expansion of the cell wall occurs. This relationship is represented by:

$$\frac{dV}{dt} = \phi(P - Y)$$

with ϕ the extensibility of the cell wall, which can be defined as "the ability of the wall to irreversibly increase its surface area" [9]. Hence, cell expansion is a coordination between water uptake and yielding of the cell wall, a mechano-hydraulic process. These equations are known as Lockhart equations, and they describe growth of plant cells when pressure is constant, $\frac{dP}{dt} = 0$ [13].

1.2.1 Types of cell wall expansion

Cell walls are inhomogeneous in their molecular architecture and thickness whereby they do not yield to turgor pressure uniformly on the surface. Cell walls expand by polymer creep, a process in which microfibrils and associated polysaccharides slide to irreversibly expand the surface area of the wall [8]. It occurs when wall loosening mechanisms modify the molecular matrix of the wall and allow it to relax the mechanical stress created by turgor pressure and, therefore, yield to this force [8, 9]. The rate of wall loosening is related to the concept of wall extensibility ϕ , that we previously referred to. The spectrum of wall expansion types span from localized, as in tip-growing cells like pollen tubes, to diffuse, as in potato tube parenchyma [7]. **Tip expansion** occurs by apical cell wall synthesis and is commonly accompanied by parallel alignment of cortical microtubules with respect to the growth axis, zonation of organelles (mitochondria, Golgi and the endoplasmic reticulum) in the subapical region, and apical accumulation of vesicles (Figure 1.2). **Diffuse expansion** occurs when cell wall synthesis is distributed along the cell and cellulose microfibrils are randomly arranged, and it has no accumulation of vesicles nor organelles (Figure 1.2).

Although these have been commonly features associated to tip- and diffuse-growth, new discoveries suggest that this categorization might not be so clear-cut. Tip-growing trichomes of *Arabidopsis* had transversely aligned microtubules, as expected, but showed an apical region depleted from microtubules [26]. An actin meshwork operates in this microtubule-depleted region promoting diffuse growth and a wall thickness gradient that, according to model simulations, explains the tip bias to elongation and its rapid expansion [26]. This example suggests that the coordination of events to generate plant shapes might be more complex than usually assumed.

1.2.2 Cell wall loosening enzymes

Modifications of the wall that lead to stress relaxation can be caused by different types of signals such as hormones, e.g., auxin, and reactive oxygen species (ROS). Some candidates of wall loosening enzymes include: expansins, xyloglucan endotransglycolase/hydrolase, and endo-(1,4)- β -d-glucanase. We will focus on the activities of expansins and endo-(1,4)- β -d-glucanase because they have been studied in our model system, cotton fiber (subsection 1.3.2). Growing cell walls have a pH between 4.5 and 6 which typically activates expansins. Exogenous expansin application stimulates cellular growth and ectopic expression stimulates overall plant growth, while its gene silencing has the opposite effect [12, 36, 52]. Although the exact molecular action is unknown, expansins are believed to disrupt non-covalent bonds between cellulose microfibrils and between xyloglucan and cellulose [29]. The endo-(1,4)- β -d-glucanase activity has been correlated to fruit softening, abscission and growth. Their particular biochemical properties, substrate specificities and function in cell wall loosening are still unknown [8]. Potential substrates are cellulose and xyloglucan and indirect evidence suggests that they may loosen the wall by releasing xyloglucans from cellulose microfibrils [17, 34].

1.2.3 Fluxes of water

Movement of water molecules across the plasma membrane is made efficient through membrane water channel proteins, called aquaporins, and plasmodesmata. Based on sequence similarity **aquaporins** (AQPs) are divided into four subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), Nodulin26-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs) [22]. As indicated by the name, PIPs and TIPs localize at the plasma and tonoplast membranes, respectively. PIPs are further divided into PIP1 and PIP2, while TIPs fall into several classes (α TIPs, β TIPs, γ TIPs, etc). Evidence linking AQPs to water transport comes from heterologous expression of AQPs in *Xenopus laevis* oocytes system which results in a considerable increase in the osmotic water membrane permeability (P_f) [27]. P_f describes overall water movement in response to osmotic or turgor pressure gradients, ΔP and $\Delta\pi$, respectively. Additionally, it was found through mercury inhibition of AQPs that P_f of the membrane can be reduced by 70-80% in *Chara* and tonoplast vesicles from tobacco suspension cells [18, 28]. P_f and L permeability parameters give estimations of the membrane water transport capacity and are related by : $P_f = LRT/V$, with R, the universal gas constant; T, absolute temperature, and V, water volume [27].

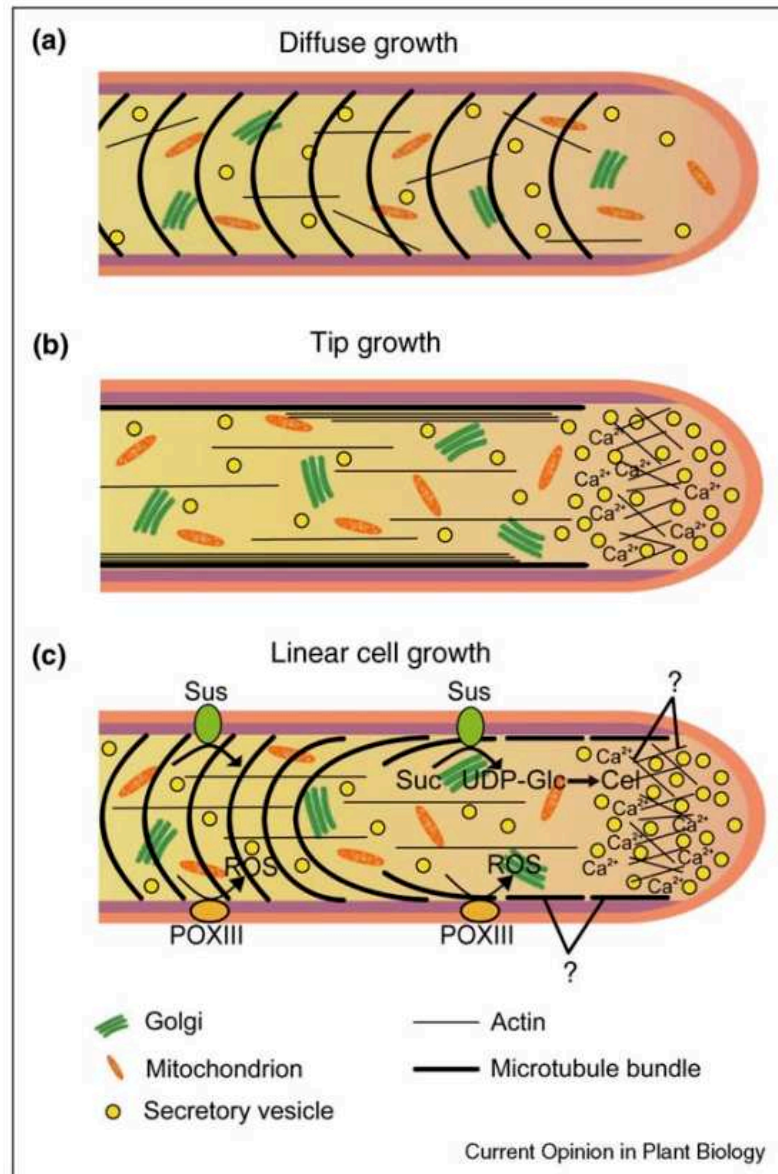


Figure 1.2: Schematic representation of the diffuse-, tip- and linear-growth types and the main processes that characterize them (taken from [38])

Contribution of AQPs and plasmodesmata to water transport capacity may be important for turgor pressure and cell expansion. Although the most used model for cell growth (i.e., Lockhart model) assumes that turgor drives expansion of the wall, how turgor pressure is built and the role of aquaporins and plasmodesmata in this process are problems that we are just starting to grasp. In *Arabidopsis* a decrease of turgor pressure is required to allow emergence of lateral root primordium [48]. This change seems to result from a reduction in aquaporin-dependent hydraulic conductivity induced by the phytohormone auxin [37]. A turgor pressure maximum has been linked to changes in the aperture of plasmodesmata during the fast expansion phase of cotton fiber cells [39]. These examples suggest that, indeed, aquaporins and plasmodesmata are important regulators of turgor pressure but we still ignore how they contribute to this process. Hence the importance of tackling these questions. We will discuss more about the potential role of aquaporins and plasmodesmata on turgor pressure and cell expansion in subsections 1.3.3 and 1.3.5.

1.3 Cotton fiber as a study system

Cotton fibers are outgrowths of single epidermal cells from the outer integument of ovules. In botanical terms, they are unbranched hairs or trichomes [1, 23]. They possess several attractive features for which they have been used as study systems for cell expansion and cell wall synthesis. The fiber cell mostly grows in length and it can be 1000- to 3000-times longer than its diameter [1]. Fiber expansion occurs over a long period of time without cell division, an advantage to study cell expansion processes in isolation [1, 46]. Fibers develop synchronously providing a relatively homogeneous population of cells. They present a temporal separation of primary and secondary wall synthesis and ovules are amenable to *in vitro* culture.

Most of the studies made have used *Gossypium hirsutum* species whose diameter ranges between 11 and 22 μm , and length between 2.2 and 3.0 cm [23]. Typically 25-30% of epidermal cells develop into mature cotton fibers, otherwise known as lints, whereas the rest of cells may develop into shorter fibers, called fuzz [38]. Cotton fiber development can be divided into four overlapping stages:

- **Initiation**, at the day of flower opening (called anthesis), fiber initials appear as spherical protrusions on the epidermal surface of the ovule
- **Fiber elongation**, starts right after fiber initials appear and continues until approximately 21 to 26 days after anthesis (DAA).
- **Secondary wall deposition**, is dominated by cellulose synthesis. This stage slightly overlaps with the previous one.
- **Maturation**, at approximately 45 to 60 DAA the fiber dies and dehydrates

Fiber cell growth is the result of the interactions of multiple cellular and molecules processes that include cell wall yielding, synthesis of wall materials, turgor pressure build up, accumulation of solutes, among others. We will describe in the following subsections some of these processes that have been studied in cotton fiber development.

1.3.1 The pattern of cotton fiber expansion

There is a lack of consensus about the type of cell expansion of cotton fibers inasmuch as experimental evidence supporting both diffuse- and tip-grow types has been reported. Genes implicated in vesicle coating and trafficking have been found to be highly expressed during expansion of cotton fibers, however, no apical vesicle accumulation has been observed [20, 46]. Subapical meshworks of actin filaments, which are commonly observed in tip-growing cells, seem to also be absent in cotton fibers [42]. While cortical microtubules are aligned parallel to the growth axis in tip-growing cells, in cotton fibers they appear transversely aligned [42]. Establishment of a calcium concentration at the tip, important to establish cell polarity, was identified in cell fibers and a peak influx rate was correlated to closure of plasmodesmata channels [45]. Based on the evidence aforementioned, Quin and Zhu (2011) put forward the hypothesis that cotton fibers experience a type of linear cell-growth in which cell wall synthesis occurs at the apical and distal regions, there is no zonation of organelles, and microtubules are aligned transversely (Figure 1.2).

1.3.2 Biochemistry of the cell wall of cotton fiber during development

Cell wall remodelling takes place during expansion of cotton fibers. Components of primary cell wall are mostly synthesized during the expansion stage and it is considered that the thickness of the cell wall remains constant for at least 12 DAA [30]. Measurements of mRNAs by reverse transcription qPCR analysis revealed that mRNA levels of expansins and endo-1,4- β -glucanase are high at the beginning of fiber development and decrease gradually as cell expansion stops [39, 43]. Synthesis of the primary cell wall is characterized by large amounts of xyloglucans which correlate with the rate of cell expansion, when the rate of expansion decreases xyloglucan amounts also decrease [43]. By 17-20 DAA secondary wall deposition starts and is characterized by a gradual increase in the level of cellulose content [30, 43]. At the end of the secondary wall synthesis stage, final thickness of the secondary cell wall can be 3 to 6 μm [16].

1.3.3 Aquaporins (AQPs)

Northern blot analysis and real-time qPCR demonstrated that AQPs of *G. hirsutum*, *GhPIP1-2* and *Gh γ TIP1* are expressed in cotton fiber cells during cell expansion having peak values at 5 DAA [25]. Another study found that four genes of *GhPIP2* are specifically expressed in cotton fibers during growth [24]. Knockdown expression of four *GhPIP2* genes by RNA interference (RNAi) reduced the final length of the fibers [24]. This down regulation did not affect density of fibers in the ovules nor the emergence

of fiber initials but the rate of expansion of the fiber was much slower compared to wild type (WT) fibers [24]. Ligon-lintless-1 (Li_1) and -2 (Li_2) mutants are monogenic dominant mutants that have shorter fiber cells. Identification of common fiber elongation related genes in fibers of (Li_1) and (Li_2) found that aquaporins were among the most significantly down-regulated genes [31]. Measurements of sap osmolarities of these mutants revealed that osmotic pressure of Li_1 fibers was significantly lower compared to WT fibers at 3-8 DAA [31]. Li_2 mutant fibers also had lower osmotic pressure than WT fibers at 3-5 DAA but higher than WT at 24 DAA [31]. These findings suggest that AQPs are important regulators of cotton fiber cell expansion. However, no measurements of turgor pressure were performed in these mutants as a way to test the relationship between AQPs and turgor.

1.3.4 Sources of solutes

To maintain expansion for long periods, the fiber cell has to compensate dilution of solutes that occurs as water enters into the cell. Soluble sugars, K^+ , and malic acid, account for $\sim 80\%$ of sap osmolarity [11]. Malate is synthesized in the cytoplasm through fixation of CO_2 by phosphoenolpyruvate carboxylase and sugars and K^+ are imported from the seed coat phloem [1, 21]. Patterns of expression of sucrose and K^+ membrane transporters - *GhSUT1* and *GhKT1* - were carried in *G. hirsutum* fibers. These analyzes found that both genes had low transcript levels at 6 DAA and increased to a maximum level at 10 DAA, whereupon *GhSUT1* remained relatively high up to 16 DAA while *GhKT1* decreased faster [39].

Another source of solutes that potentially contributes to avoid dilution is the Vacuolar Invertase 1 (VIN1). VIN1 is responsible for the hydrolyzation of sucrose into glucose and fructose and its activity has been linked to expanding tissues like sugar beet petioles [14]. In cotton fibers, its expression was found to be several times higher than in other tissues like roots, stems and leaves [50]. It was reported that VIN1 activity was higher at 5 and 10 DAA and lower at 20 DAA. Coincidentally, the concentration of glucose and fructose was higher at 10 DAA and by 15 DAA it lowered. When VIN1 expression was silenced by means of RNAi construct, VIN1 activity was reduced by 33% and this resulted in shorter fiber cells [50].

1.3.5 Plasmodesmal permeability

To examine the gating status of plasmodesmata channels, the fluorescent dye carboxy fluorescein (CF) was loaded into the shoot through cut ends. Confocal imaging revealed that CF moved from the phloem of the outer seed coat into the fiber cells between 2 and ~ 8 DAA [39]. At ~ 9 to 10 DAA CF failed to enter the fibers but this restriction lasted only ~ 5 days, as CF moved again into fiber cells at ~ 16 DAA [39]. Immunolocalizations and aniline blue staining of callose showed that callose was almost undetectable at 5 DAA, became evident at the base of the fiber at 12 DAA, and significantly reduced again at 20 DAA [40]. Expression pattern analysis of a fiber-specific β -1,3-glucanase, designated as *GhGluc1*, a protein responsible for callose degradation, showed correlation with callose levels and plasmodesmata opening. At 12 DAA, when plasmodesmata are closed, no expression of *GhGluc1* was detected but became evident at 20 DAA when immuno-gold particles of callose decreased and plasmodesmata re-open [39, 40]. The

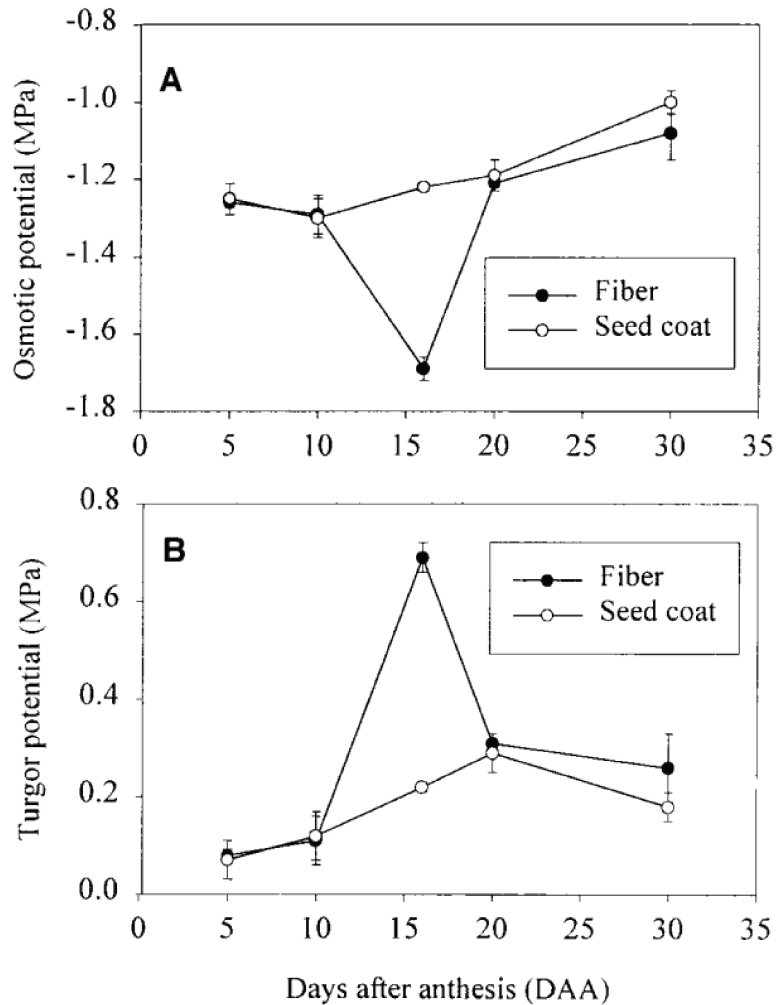


Figure 1.3: (A) Osmotic and (B) turgor potentials during expansion of cotton fiber cells (taken from [39]).

dynamical changes of plasmodesmal permeability and callose levels were found to correlate with the behavior of turgor and osmotic potentials. Between the fiber cell and seed coat cell these potentials were similar before 10 DAA, but at ~ 16 DAA peak values for both potentials were reported in cotton fiber cells with a subsequent decrease at 20 DAA [39]. The fact that high levels of sucrose and K^+ transporters correlate with peaks of turgor and osmotic potentials, has led to the suggestion that they might be important to avoid solute dilution during cotton fiber growth [39]. In this context, closure of plasmodesmata might be needed to avoid leakage of solutes and thus help generate the patterns of turgor and osmotic pressure that have been reported for fiber cells.

1.4 General questions

In this work, we aimed at studying if plasmodesmal permeability can regulate turgor pressure during cotton fiber expansion. We also addressed the question of whether turgor pressure can, in turn, affect plasmodesmal permeability, thus creating a feedback loop. We approached these questions by reviewing and integrating the information that could support our hypothesis (Chapter 2) and by the generating a computational model that allowed us to explore one direction of these interactions: the role of plasmodesmal permeability on turgor pressure (Chapter 3).

2

Interplay between turgor pressure and plasmodesmata during plant development

In the following review article, we put forward the hypothesis that plasmodesmal permeability and turgor pressure affect each other during plant development. I reviewed the available experimental evidence that supports this hypothesis, built a possible network of interactions between different types of factors (e.g., molecular, cellular, mechanical) that might mediate these feedbacks, and put forward some testable predictions. This article has been accepted for publication in the *Journal of Experimental Botany* (29th September 2019).



REVIEW PAPER

1.5 **Interplay between turgor pressure and plasmodesmata during plant development** 1.60

1.10 **Valeria Hernández-Hernández¹, Mariana Benítez² and Arezki Boudaoud^{1,*}** 1.65

¹ Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, Lyon, France

² Laboratorio Nacional de Ciencias de la Sostenibilidad, Instituto de Ecología & Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico City, Mexico

1.15 * Correspondence: arezki.boudaoud@ens-lyon.fr 1.70

Received 16 July 2019; Editorial decision 9 September 2019; Accepted 9 September 2019

1.20 Editor: Christine Raines, University of Essex, UK 1.75

Abstract

1.25 **Plasmodesmata traverse cell walls, generating connections between neighboring cells. They allow intercellular movement of molecules such as transcription factors, hormones, and sugars, and thus create a symplasmic continuity within a tissue. One important factor that determines plasmodesmal permeability is their aperture, which is regulated during developmental and physiological processes. Regulation of aperture has been shown to affect developmental events such as vascular differentiation in the root, initiation of lateral roots, or transition to flowering. Extensive research has unraveled molecular factors involved in the regulation of plasmodesmal permeability. Nevertheless, many plant developmental processes appear to involve feedbacks mediated by mechanical forces, raising the question of whether mechanical forces and plasmodesmal permeability affect each other. Here, we review experimental data on how one of these forces, turgor pressure, and plasmodesmal permeability may mutually influence each other during plant development, and we discuss the questions raised by these data. Addressing such questions will improve our knowledge of how cellular patterns emerge during development, shedding light on the evolution of complex multicellular plants.** 1.80
1.85
1.90

Keywords: Hydraulic conductivity, mechanical stress, permeability, plasmodesmata, turgor pressure.

1.40 1.95

Introduction

1.45 Plasmodesmata are channels that traverse the cell walls and create symplasmic continuity. They have been shown to play a key role in the mobilization of different types of molecules such as hormones, sucrose, transcription factors, and viruses (De Storme and Geelen, 2014; Sager and Lee, 2014; Han and Kim, 2016). In opposition to animal development, which relies partly on cell migration to establish cellular patterns, plant cells are encased within the cell wall and therefore symplasmic connectivity seems pivotal to establishing cell types. Studies have proven that regulation of plasmodesmal permeability helps establish patterns of cell identity during plant development (De Storme and Geelen, 2014; Sager and Lee, 2014). Accordingly, it has been proposed that plasmodesmata may have had a key role in the evolution of multicellular forms in plants (Lucas and Lee, 2004; Benítez *et al.*, 2018). 1.100
1.105 One interesting feature of plasmodesmata is that their permeability can vary among different types of cells, organs, and throughout developmental stages. Changes in symplasmic connectivity have been correlated to changes in size of the plasmodesmal pore and in plasmodesmal number. Variations in symplasmic connectivity have been observed under a wide range of phenomena such as pathogen infection (Lee *et al.*, 1.110

1.55 © The Author(s) 2019. Published by Oxford University Press on behalf of the Society for Experimental Biology. 1.110
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

- 2011), mechanical stimulation (Jaffe *et al.*, 1985), transition to dormancy (Rinne and van der Schoot, 1998), flower initiation (Gisel *et al.*, 1999), the transition of leaves from sink to source (Oparka *et al.*, 1999), and as a response to manipulation of mechanical forces (Oparka and Prior, 1992). Interestingly, the interaction between mechanical forces and molecular processes is important for the development of plants (Hamant and Traas, 2009; Mouliá, 2013).
- Plant growth and morphogenesis involve cell expansion, which is driven by turgor pressure that, in turn, depends on the supply of solutes. The amount of solutes within the cell can vary as a result, for example, of their transport by membrane transporters, the enzymatic activity of proteins that synthesize *de novo* solute molecules, and intercellular transport through plasmodesmata. In this Review, we put forward the hypothesis that turgor pressure might also regulate plasmodesmal permeability based on previous observations that plasmodesmal permeability can respond to changes in turgor (Oparka and Prior, 1992) and that osmotic treatments are related to changes in the size of the plasmodesmal channel (Schulz, 1995). We discuss the evidence that suggests the existence of feedback interactions between turgor pressure and plasmodesmal permeability. Beforehand, we explain the concepts behind turgor pressure and briefly review the role of plasmodesmata. Finally, we discuss the potential relevance of these bidirectional interactions and identify some of the related open questions.
- ## 2.30 Turgor pressure in the cell
- Small molecules such as sucrose can move through plasmodesmata (Schulz, 1995) and patterns of unloading in sink tissues have been correlated with patterns of cell expansion in *Pisum sativum* stems (Schmalstig and Cosgrove, 1990). The amount of solutes such as sucrose, i.e. osmolytes, within the cell is important because they mix with water and thus tend to reduce the free energy of water, that is, the water potential (Ψ). Van't Hoff's equation may be used to estimate the osmotic potential (Ψ_{π}) for low concentrations of solutes, $\Psi_{\pi} = -RTc_s$, where R is the gas constant ($8.32 \text{ J mol}^{-1} \text{ K}^{-1}$), T is the temperature (given in kelvins), and c_s is the concentration of osmolytes per volume of water, i.e. the osmolarity expressed in moles of solutes per meter cubed of solution (Niklas and Spatz, 2012). Note that osmolarity differs from molarity, which corresponds to the concentration (in moles per meter cubed) before dissolution; for instance, sodium chloride with a molarity of 1 mol m^{-3} dissociates into Na^+ and Cl^- ions corresponding to an osmolarity of 2 mol m^{-3} , whereas the osmolarity of glucose in solution is equal to its molarity because there is no dissociation. The osmotic potential is negative because dissolution of solutes is thermodynamically more favorable than pure water ($\Psi=0$). The negative of Ψ_{π} is referred to as 'osmotic pressure'. As membranes are selectively permeable (i.e. they allow free movement of water, but not of all solutes), differences in concentration of solutes that do not move freely across the membrane generate a gradient in water potential that drives movement of water into or out of the cell. Two other factors also contribute to water potential: gravity potential (Ψ_g) and hydrodynamic pressure potential (Ψ_p):
- $$\Psi = \Psi_g + \Psi_p + \Psi_{\pi}$$
- Gravity causes water to move downward unless an opposite force acts. The effect of gravity potential depends on height, the density of water, and the acceleration of gravity (Niklas and Spatz, 2012). An upward vertical distance of 10 m is translated into an increase of 0.1 MPa in water potential. At the cellular scale, gravity is usually neglected because a vertical distance of 1 m can cause a change in the water potential of 0.01 MPa, which is small compared with the contribution of other factors to changes in water potential. The pressure potential is defined as the hydrodynamic pressure of a solution and is generally quantified as a deviation from the ambient pressure. When the hydrodynamic pressure is higher than atmospheric pressure it applies a force on the walls of the container, which is then 'pressurized' (Beauzamy *et al.*, 2014); such pressure is usually called 'turgor pressure'. Negative values (tensional force) of hydrodynamic pressure are achieved under very specific conditions, e.g. in the xylem (Niklas and Spatz, 2012).
- Turgor pressure is important for processes like cell expansion (Kroeger *et al.*, 2011), closure and opening of stomata (Buckley, 2005), and transport in the phloem (Münch, 1930; Niklas and Spatz, 2012); developmental changes in turgor have been proposed to be involved in lateral root emergence (Péret *et al.*, 2012; Lucas *et al.*, 2013; Vermeer *et al.*, 2014). Indeed, cellular elongation depends on the balance between turgor pressure and the mechanics of the cell wall. The cell wall is a rigid structure made of cellulose fibers embedded in a matrix of hemicellulose and pectin (Cosgrove, 2005). In normal conditions, the wall is stretched by the protoplast, creating mechanical stress in the load-bearing components of the cell wall that, in turn, compress the protoplast (Cosgrove, 1985; Schopfer, 2006). Turgor pressure is the hydrostatic internal force that acts in opposition to the compression by the wall. When the cell wall loosens (or relaxes) turgor drops and this, in turn, reduces the internal water potential, which then drives water influx and cell elongation (Schopfer, 2006).
- In a simple system, water will flow through the membrane from the compartment with the highest water potential towards that with the lowest water potential, tending to equilibrate this difference. In a plant cell, the plasma membrane is often idealized as a semi-permeable membrane, though it partially enables the movement of many osmolytes (Beauzamy *et al.*, 2014). Processes that regulate the internal concentration of osmolytes can also change the water potential. For example, the conversion of glucose into fructose and sucrose by vacuolar invertases (Wang *et al.*, 2010). Water absorption tends to dilute osmolytes within the cell and, if this were to continue indefinitely, osmotic and turgor pressures would fall to zero (Cosgrove, 1985; Schopfer, 2006). The synthesis, assimilation and movement of solutes are then important processes that prevent this situation and thus help maintain cell expansion for long periods.

Plasmodesmata and their permeability

In this section, we briefly describe the structure and function of a plasmodesma channel (Fig. 1). We then focus on questions such as how the size of the channel is regulated and why such regulation is important for turgor pressure. Our aim is not to provide a comprehensive discussion about plasmodesmal permeability regulation (see e.g. Benitez-Alfonso *et al.*, 2011; Faulkner and Maule, 2011; Burch-Smith and Zambryski, 2012; Knox and Benitez-Alfonso, 2014; Han and Kim, 2016) but only to draw attention to points relevant to the links between permeability and mechanical forces.

Plasmodesmata encase a tube of endoplasmic reticulum (ER), known as the desmotubule. Plasmodesmata are divided into two types, primary, which form during cytokinesis, and secondary, which form during cell expansion; they may be simple (a single tube), occur in pairs (two neighboring tubes), or be branched (multiple interconnected tubes) (Burch-Smith and Zambryski, 2012). Plasmodesmata may allow the movement of molecules between cells through their central cavity (the cytoplasmic sleeve around the desmotubule) or through the desmotubule (Barton *et al.*, 2011). Symplasmic connectivity has been related to changes in the diameter of the pore, which is related to the size exclusion limit (the size of the largest molecule that freely moves through the pore), to the type of plasmodesma, and to their density. For example, a decrease in green fluorescent protein (GFP) movement was observed in leaves that transitioned from sink to source that coincided with an increase in the formation of branched plasmodesmata (Oparka *et al.*, 1999). Regarding the size of the plasmodesmal channel, it is important to note that the movement of molecules that are small enough to pass through in

a passive way, called non-targeted transport, is differentiated from the targeted transport that may involve structural modifications of the channel or of the molecules in movement (Lucas *et al.*, 1995). The molecular size of fluorescent dyes has been used to study symplasmic transport and can be expressed in terms of molecular mass (in kDa) or physical size (in nm). 5(6)-Carboxyfluorescein (CF) is a dye whose molecular size (0.61 nm) is considered small enough for it to pass through the plasmodesmal pore (Wang and Fisher, 1994); it is confined to the phloem and its fluorescence pattern is similar to the distribution of [¹⁴C]sucrose (Grignon *et al.*, 1989). Furthermore, the molecular sizes of sucrose (0.47 nm) (Wang and Fisher, 1994; Terry *et al.*, 1995) and CF are comparable and therefore CF has been used as a fluorescent dye for live imaging of phloem unloading (Oparka *et al.*, 1994).

The observation of movement of fluorescent molecules small enough to pass through plasmodesmata has led to the discovery of symplasmic fields. These can be conceptualized as cells or tissues that are more connected among themselves than with surrounding cells, which implies a certain degree of discontinuity that can be incomplete and modified during development (Gisel *et al.*, 1999). For example, symplasmic fields were inferred in Arabidopsis from the patterns of movement of 1×-, 2×-, and 3×-GFP fusions during embryo development, which represented three molecular sizes (i.e. 27, 54, and 81 kDa) expressed under the SHOOT MERISTEMLESS (STM) promoter (Kim and Zambryski, 2005). At the early heart stage 1×-, and 2×-GFP could move throughout the whole embryo, but by the late heart stage 2×-GFP could not enter into the cotyledons (Kim *et al.*, 2005). Also at the late heart stage, 2×- but not 3×-GFP could move into the root tip, thereby suggesting the existence of a third symplasmic field (Kim and

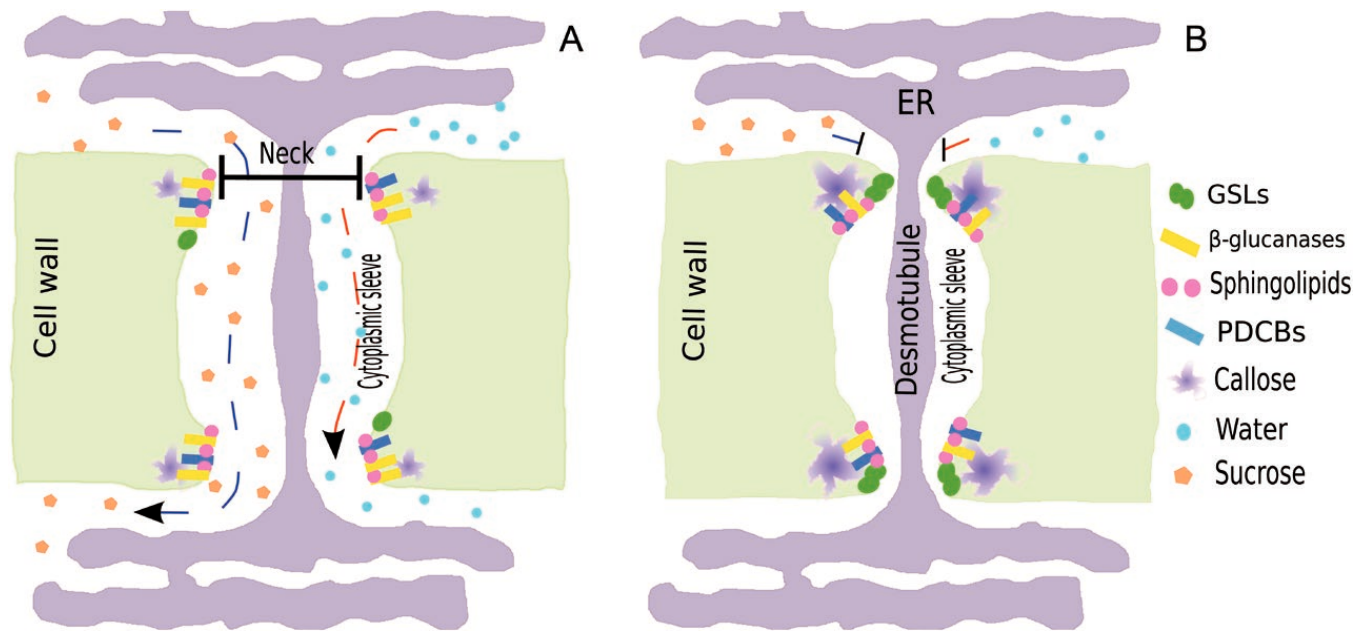


Fig. 1. Schematic representation of the plasmodesmal pore that connects two neighboring cells through their common cell wall. (A) As a result of a lower levels of callose at plasmodesmal necks, the pore is widened and molecules that do not exceed the size of the neck can freely pass. (B) Callose accumulates at plasmodesmal neck thus narrowing the channels and limiting free movement of molecules from one cell to its neighbor. GSL: GLUCAN SYNTHASE-LIKE; PDCB: PLASMODESMATA CALLOSE-BINDING PROTEIN.

Zambryski, 2005; Kim *et al.*, 2005). These experiments led to the conclusion that plants generate symplasmic fields that can spatiotemporally vary during development.

4.5 Plasmodesmal permeability has been related to levels of callose, a β -glucan that is incorporated into the cell wall at the plasmodesmal neck (Drake *et al.*, 1978; Guseman *et al.*, 2010; Vatén *et al.*, 2011; Benítez-Alfonso *et al.*, 2013; Han *et al.*, 2014). GLUCAN SYNTHASE-LIKE (GSL) proteins, also called Callose Synthases (CaS), have been established as the putative family for the synthesis of callose, while the degradation of callose was ascribed to plasmodesmal-localized β -1,3-glucanases (PdBGs) (Levy *et al.*, 2007; Guseman *et al.*, 2010). 4.10 Mutation of the callose synthase *GSL8* was linked to decreased callose levels at the cell plate and at plasmodesmata, defects in cell division, disruption of cellular and tissue patterning, formation of islands of excessive cell proliferation, and cell wall stubs (Chen *et al.*, 2009; Guseman *et al.*, 2010). 4.15 In the leaves of *Arabidopsis*, the *chor* mutant, a weak allele of *GSL8*, has abnormal stomatal clusters that are assumed to result from the leakage of cell-fate determinants from stomatal lineage cells towards surrounding epidermal cells due to higher plasmodesmal permeability (Guseman *et al.*, 2010). Conversely, a mutation of a *PdBG* gene would be expected to produce a less permeable plasmodesmal channel and reduce symplasmic movement of molecules. This was reported in *pdbg1,2* double mutants, which showed a decreased movement of GFP from the phloem to neighboring tissues in comparison with wild type *Arabidopsis* roots (Benítez-Alfonso *et al.*, 2013). Moreover, *pdbg1,2* double mutant presented formation of lateral root clusters, suggesting that lower permeability of the plasmodesmal channel impaired the specification of lateral root founder cells, probably by altering the movement of cell-specifying molecules (Benítez-Alfonso *et al.*, 2013). In agreement with the expected callose levels of these two mutants, the two studies reported lower (in the *GSL8* mutant) and higher callose levels (in *pdbg1,2* double mutant) by means of aniline blue staining and immunolocalizations (Guseman *et al.*, 2010; Benítez-Alfonso *et al.*, 2013). In summary, these findings support the idea that some members of the GSL and PdBG families of proteins regulate plasmodesmata opening by modulating callose levels. 4.40

4.45 What biophysical processes determine permeability of a plasmodesma? Two processes play a role in the movement of molecules in the symplasm: diffusion and advection (Squires and Quake, 2005; Verchot-Lubicz and Goldstein, 2009). On the one hand, diffusion is the net motion of molecules from regions of high to regions of low concentration. As a transport mechanism, diffusion becomes slower and slower as system size increases. Differences in concentration between neighboring cells drive diffusive movement of molecules through the plasmodesmata connecting the two cells. On the other hand, advection corresponds to molecule transport by coherent fluid movement, such as the active cytoplasmic movement known as ‘cytoplasmic streaming’ or bulk flow in phloem or in xylem, which is generally much more efficient for transport than diffusion (Squires and Quake, 2005; Verchot-Lubicz and Goldstein, 2009). Turgor pressure differences between neighboring cells cause a flow through the plasmodesmata that connect the two cells, driving advective transport of 4.55 4.58

molecules in the direction of the flow. Blake (1978) examined the contributions of fluid properties (viscosity varying with flow rate), of the desmotubule, and of the geometry of the cavity to plasmodesmal permeability and found that the diameter of the neck was the main determinant of permeability, suggesting that changes in neck opening are sufficient to modulate plasmodesma-dependent fluxes. More generally, it is expected that both advection and diffusion are involved in molecular movement through plasmodesmata, with relative weight that depends on the system and process of interest. For instance, phloem loading by sugars through plasmodesmata appears dominated by diffusion in trees and partially involves advection in herbs (Comtet *et al.*, 2017a, b). In the leaf epidermis, no pressure differences are expected between pavement cells, and so non-targeted transport of molecules through plasmodesmata is believed to be diffusion-driven (Crawford and Zambryski, 2001; Schönknecht *et al.*, 2008). In particular, Schönknecht and coworkers (2008) found that GFP movement in tobacco leaves depends exponentially on the inverse of temperature, like an Arrhenius function with a well-defined activation energy, leading them to ascribe movement to diffusion. Nevertheless, the existence of an activation energy means that the process is not simple diffusion—GFP movement would be driven by differences in concentrations between cells and would involve a limiting step (with a specific activation energy), which could be a change in GFP conformation before traversing the plasmodesma. Altogether, the movement of small molecules through the plasmodesma likely involves molecular diffusion and advection due to turgor pressure differences, whereas the movement of large molecules is likely limited by interactions with the channel. Note, however, that more complex processes might lead to transport through plasmodesmata, such as electroosmosis whereby ion movement is driven by electrostatic fields (Squires and Quake, 2005) due, for instance, to differences in membrane potential between neighboring cells. 4.60 4.65 4.70 4.75 4.80 4.85 4.90

4.95 Plasmodesmal permeability seems to be key to establishing patterns of cell types, which is a necessary process for the evolution of vascular tissues and complex multicellular plants. For example, ectopic expression of callose accumulation prevented intercellular movement of the transcription factor SHORT-ROOT and the microRNAs miR165/6 between stele and ground tissues (Vatén *et al.*, 2011). In light of the above, one could hypothesize that the role of plasmodesmal permeability in cell type specification and tissue development had important evolutionary significance. Plasmodesmata evolved several times in the plant lineage. Plasmodesmal permeability in some multicellular green algae, like *Bulbochaete hiloensis* (Fraser and Gunning, 1969), can also change during ontogeny yielding well-defined symplasmic fields (Raven, 1997; Cook and Graham, 1999; Kwiatkowska, 1999). This suggests, along with other lines of evidence, that plasmodesmata can fulfill similar functions in very diverse groups and that they might have enabled similar functions in early plant evolution (Benítez *et al.*, 2018). For instance, the specification of cell identities dependent on the creation of symplasmic fields may have led to the evolution of vascular tissues that are able to transport nutrients through long distances, which could have been 4.100 4.105 4.110 4.115 4.116

instrumental in the expansion of the plant lineage on land (Benítez *et al.*, 2018).

5.5 **A door closes: plasmodesmal permeability may help build turgor pressure**

5.10 The regulation of plasmodesmal permeability can affect the movement and accumulation of solutes and probably, by extension, osmotic pressure. It has been reported that a decrease of the plasmodesmal diameter at the neck is correlated with lower levels of import of ¹⁴C-labelled sucrose in pea root tips (Schulz, 1995). Due to the role of osmotic pressure in generating turgor, one could expect that plasmodesma-dependent movement of solutes affects turgor pressure and, by extension, cell expansion. Nevertheless, thorough exploration of the effects of plasmodesmal permeability on turgor pressure and cell expansion has not been carried out.

5.20 Cotton fibers provide an ideal system to test the role of plasmodesma-dependent effects on turgor pressure and cell elongation. Some experiments have correlated changes in plasmodesmal permeability, phloem unloading of CF, and cell elongation patterns in cotton fibers (Ruan *et al.*, 2001, 2004; Zhang *et al.*, 2017). A cotton fiber is a single trichome-like epidermal cell that elongates about 1000-fold from its initial size, without dividing. CF translocation via the phloem was observed at different times of cotton fiber elongation (Ruan *et al.*, 2001); CF was detected in the phloem of the seed coat of cotton and inside the fibers, except between 10 and 15 days after anthesis during which CF was reported absent from the fibers (Ruan *et al.*, 2001). These results led the authors to hypothesize that CF could no longer enter the fibers due to a closure of the plasmodesmal pore. To test this idea, the authors carried out aniline blue staining and immunolocalization, which showed accumulation of callose at the time of the supposed plasmodesmal closure, and they also reported an increase in expression of a fiber-specific β-1,3-glucanase at the time of re-opening (Ruan *et al.*, 2004). This time window also coincided with a higher expression of sucrose and potassium transporters that, together, resulted in larger values of osmotic and turgor pressures (Ruan *et al.*, 2001). The closure of plasmodesmata might be necessary for fast elongation of the fibers, by preventing leakage of osmolytes out of the cell and enabling turgor pressure buildup.

5.45 The hypothesis that plasmodesmal closure is necessary for the influx of water would be testable by using mutations or treatments that induce abnormal callose accumulation. Some evidence along these lines is provided by the consequences of changes in the composition of plasma membrane as explained hereafter. It has been shown that plasmodesmal membranes are enriched with detergent-insoluble membrane (DIM) components such as sterols and sphingolipids (Grison *et al.*, 2015). There are two proteins that are specifically localized at plasmodesmata, possibly due to their glycoposphatidylinositol anchors—callose-binding protein 1 (PDCB1) and β-1,3-glucanase (PdBG2)—whose mutations or misexpression affects callose levels and symplasmic movement of proteins (Levy *et al.*, 2007; Simpson *et al.*, 2009; Benitez-Alfonso *et al.*, 2013).

Moreover, these two proteins are known to specifically bind to DIMs of plasmodesmal membrane. It was recently observed that plasmodesmal permeability is altered when the overall composition of sterols is impaired (Grison *et al.*, 2015). In cotton, fiber length was reduced in plants with downregulation of a putative sterol carrier; these plants showed reduced expression of *GhPdBG3-2A/D*, a plasmodesmal β-1,3-glucanase that likely anchors to DIMs, and closed plasmodesmata from 5 to 25 days after anthesis, based on CF assay (Zhang *et al.*, 2017). These results point to the importance of plasmodesmal membranes for proper function of proteins that regulate callose levels. They also support the hypothesis that plasmodesmal permeability may be important for cell elongation, though further experiments would be needed to directly test this hypothesis, for example by manipulating expression of a *GSL* gene in fibers.

Turgor strikes back: pressure may induce changes in plasmodesmal permeability

In the previous section we discussed the evidence supporting the hypothesis that plasmodesmal closure is necessary to build turgor pressure during cell elongation. In this section we will present the experiments that suggest that turgor pressure can induce changes in the permeability of plasmodesmata.

The temporal and spatial variations of plasmodesmal permeability, during development and across tissues, raise the question of what factors can regulate the permeability of the channel. Olesen (1979) stained plasmodesmata in leaves of *Salsola kali* and in roots of *Epilobium hirsutum* and observed a ring-like structure at the entrance of the channel that led him to hypothesize that plasmodesmata served as ‘sphincters with a valve function’. Such a valve function could be mediated by deformation of the desmotubule (Blake, 1978), of the plasma membrane, or of the cell wall. Côté *et al.* (1987) measured the electric resistance between the vacuoles of two neighboring internodal cells in *Chara corallina*, and found that the resistance increased significantly when one of the two cells was bathed in a hyperosmotic solution, inducing a turgor pressure of about 0.24 MPa between the two cells. Because the electric conductance between cells is mostly dependent on symplasmic conductivity, they ascribed their observation to the difference in turgor pressure inducing gating of plasmodesmata. Oparka and Prior (1992) conducted a series of experiments with a pressure probe aimed at inducing pressure differentials between pairs of neighboring cells of tobacco trichomes. They punctured a cell to release turgor and also performed the reciprocal experiment by injecting a drop of oil inside to increase the pressure. To test whether there were any changes in the symplasmic connectivity after these changes of pressure, they injected Lucifer Yellow CH (LYCH) dye in the most apical cell of the trichome and observed its movement between cells. In control treatments, LYCH could move through the cells all the way down towards the base of the trichome. After puncturing one of the trichome cells, they measured turgor pressure of the apical and basal neighbors and found that these other cells also reduced their turgor. Furthermore, the pressure differentials

found correlated with LYCH movement, namely, LYCH could not enter the cell with the smallest turgor pressure, suggesting that plasmodesmata respond to turgor differentials. This phenomenon was recently modelled by Park *et al.* (2019) who hypothesized that fast closing of plasmodesmata would be due to higher turgor pressure on one side pressing the endoplasmic reticulum against the cell membrane; using plausible values of physical parameters, they recovered variations of relative permeability with pressure that are in semi-quantitative agreement with experiments.

When plants were treated with chemical solutions intended to induce plasmolysis, callose levels increased in oat coleoptiles (Drake *et al.*, 1978), plasmodesmal pores narrowed in pea root tips (Schulz, 1995), and symplasmic continuity was disrupted in *Egeria densa* leaves (Erwee and Goodwin, 1984). However, in the study performed by Schulz (1995), moderate treatments with mannitol that did not plasmolyse cells were shown to transiently increase the size of the plasmodesmal pore and enhance movement of sugars to osmotically stressed tissues. These findings suggest that osmotic treatments can induce two opposite responses on plasmodesmal permeability depending on the intensity of the stress. Schulz (1995) proposed that a transient enhancement of sugar import after a moderate osmotic stress was needed to lower the water potential to prevent plasmolysis. But when the stress is sufficient to plasmolyse the cell, plasmodesmata close, potentially preventing further cytoplasmic leakage. Hence, changes in turgor pressure can affect the permeability of plasmodesmal channels but the evidence suggests that the response is not linear. Further work is needed to fully understand plasmodesmal response to changes in turgor and determine what threshold values of turgor can close the plasmodesmal channel.

An analysis of the biochemical networks that regulate plasmodesmal permeability and turgor pressure reveals factors shared between these two processes. Reactive oxygen species (ROS) have been shown to play an important role in cell expansion. ROS production in the apoplast is linked to the loosening of cell walls through cleavage by hydroxyl radicals of polysaccharides such as pectins and homogalacturans (Fry *et al.*, 2001; Schopfer *et al.*, 2001), though ROS may also lead to cell-wall stiffening by creating new cross-links (Passardi *et al.*, 2004). Accordingly, ROS regulate turgor pressure through their effects on cell wall relaxation. In addition, ROS accumulation in roots of *Arabidopsis* and cucumber has been related to intracellular trafficking of aquaporins and a reduction in water transport, adding another layer to the relationship between ROS and turgor (Lee *et al.*, 2004; Boursiac *et al.*, 2008). ROS seem to also regulate plasmodesmal permeability as their accumulation is correlated to increases in callose deposition and plasmodesmal closure (Cui and Lee, 2016). CF movement was restricted after mechanical wounding and H₂O₂ spraying of *Arabidopsis* plants (Cui and Lee, 2016). Furthermore, ROS production seems to be downstream of mechanical signals. Mechanically wounded *Arabidopsis* plants that were pretreated with ascorbic acid—a reducing agent intended to block ROS production—did not show the mechanically induced plasmodesmal closure that was observed in non-pretreated plants (Cui and Lee, 2016).

The *Cals8* gene seems to mediate the response to mechanical wounding via extracellular ROS, as a *cals8* mutant was defective in both ROS- and mechanical wounding-induced plasmodesmal regulation (Cui and Lee, 2016). The production of ROS species, therefore, seems to be an important factor linking mechanical signals coming from cell expansion and wounding to responses like plasmodesmal permeability and turgor pressure regulation.

Ca²⁺ is a structural component of the cell wall and acts as a second messenger responding to environmental signals. Experimental evidence suggests that Ca²⁺ might play a role in the regulation of callose synthesis through its interactions with annexins (ANNs), calmodulins (CaM), and calreticulins. ANNs are membrane-bound proteins with GTPase activity dependent on Ca²⁺. They are expressed in elongating cotton fibers and were found to potentially interact with callose synthases (Andrawis *et al.*, 1993; Shin and Brown, 1999). Also a group of proteins that resemble plant and animal AANs were related to the activity of callose synthase *in vitro* in a Ca²⁺-dependent manner (Andrawis *et al.*, 1993). ANNs seem to respond to accumulation of ROS in *Arabidopsis* roots by regulating cytoplasmic Ca²⁺ through permeable channels, thus serving as a link between ROS and Ca²⁺ (Richards *et al.*, 2014). CaM are Ca²⁺ sensors that show conserved regulatory activity in eukaryotic cells. In root hairs of *Arabidopsis*, ROS and Ca²⁺ are involved in a positive feedback loop. First, ROS accumulation activates calcium influx (Foreman *et al.*, 2003) that, in turn, activates CaM, which then targets NADPH-dependent production of ROS (Harding *et al.*, 1997; Takeda *et al.*, 2008). In cotton fibers, overexpression of a cotton calmodulin, *GhCaM7*, leads to higher concentrations of ROS and promotes early fiber elongation, while exogenous H₂O₂ application in WT plants enhanced the expression of *GhCaM7* as well as early fiber elongation (Tang *et al.*, 2014). The calcium-binding protein calreticulin was found to be located in ER of plasmodesmal channels (Baluska *et al.*, 1999), there is a correlation between calreticulin and callose accumulation at plasmodesmata (Bilska and Sowinski, 2010), and calreticulin interacts with a glucosaminyl transferase-like (GnTL), which co-localizes with PDCB (Zalepa-King and Citovsky, 2013). This suggests that calreticulin may interact with enzymes that regulate callose levels at plasmodesmata.

Ca²⁺ might also close plasmodesmata in a callose-independent manner. This hypothesis stems from the observation that increases in cytoplasmic Ca²⁺ induced by microinjection of mastoparan in staminal hair cells of *Setcreasea purpurea* transiently close plasmodesmata within seconds, with plasmodesmata remaining closed for less than a minute (Tucker and Boss, 1996). Calreticulin might associate with acto-myosin and centrin at plasmodesmal necks to translate calcium fluctuations into transient contractions of the pore (Sager and Lee, 2014), which would constitute a callose-independent form of plasmodesmal permeability regulation.

Put together, experimental evidence discussed here suggests that the biochemical interactions that connect turgor pressure, plasmodesmal permeability, and cell elongation constitute a tightly coupled network (Fig. 2).

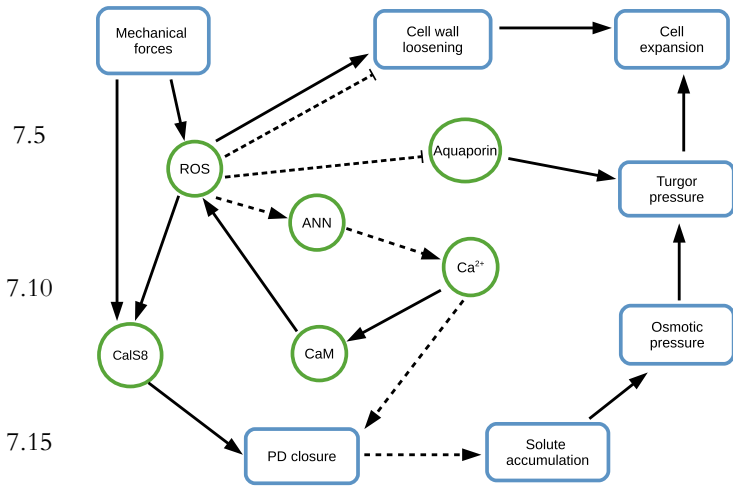


Fig. 2. Network of interactions between biochemical, mechanical factors, and processes that connect plasmodesmal permeability, turgor pressure, and cell wall expansion. Blue rectangles represent processes, and red ovals represent molecules. Arrows correspond to upregulation, T-ending lines to down-regulation; dotted arrows and lines indicate hypothetical interactions.

Questions and future directions

We have summarized available evidence suggesting that plasmodesmal permeability and turgor pressure are linked and put forward two hypotheses about how these two factors might interact. Namely, plasmodesmata permeability may contribute to osmolyte accumulation and hence turgor-driven cell expansion; turgor, in turn, can also affect plasmodesmal permeability, possibly through mechanically induced ROS accumulation and calcium signaling or directly by pushing the endoplasmic reticulum against the plasma membrane. However, there are many gaps to fill. In this section we discuss particular questions that need to be addressed.

Experimental data support the assumption that narrowing the neck of the pore is necessary for building turgor pressure (Ruan *et al.*, 2001, 2004); however, it is important to consider that symplasmic connectivity can also vary due to changes in plasmodesmal number, for instance by *de novo* formation of branched plasmodesmata (Oparka *et al.*, 1999), as observed during cotton fiber elongation (Ruan *et al.*, 2001). It is therefore necessary to investigate whether turgor is affected because of changes in diameter of simple plasmodesmata, formation of branched plasmodesmata, or a combination thereof. These possibilities also apply to the effects of turgor pressure on plasmodesmal permeability because loosening of the cell wall might be involved in the formation of branched plasmodesmata (Ehlers and van Bel, 2010). Using 3D reconstructions from transmission electron microscopy (Nicolas *et al.*, 2017), it is now possible to examine the physical structure of plasmodesmata and examine their nature, helping to clarify the aforementioned issues.

It would be interesting to test whether changes in turgor pressure induced by chemical treatments can alter the patterns of non-targeted symplasmic movement. Even considering the evidence provided by Schulz (1995) of osmotic treatments altering plasmodesmal diameter and sucrose unloading, actual

turgor pressure was not measured in this study to corroborate this link. This is particularly important because osmotic treatments can trigger many physiological responses in plants. It is then necessary to measure turgor pressure in osmotic treatments and verify whether those osmotic stress-induced values can alter callose deposition and symplasmic movement of molecules. These experiments can also be applied not only to the non-targeted but also to the targeted movement of transcription factors that are known to control the structure of plasmodesmata, e.g. KNOTTED1 (KN1) (Xu *et al.*, 2011). Such measurements of turgor pressure might be eased by indentation-based approaches that were recently developed (Beauzamy *et al.*, 2015; Weber *et al.*, 2015).

Osmotic stress can either increase or decrease permeability depending on intensity (Schulz, 1995), which means that the response of plasmodesmata to changes in turgor pressure may not be linear. In this context, it would be interesting to test how much turgor pressure needs to drop to activate the callose-dependent closure of plasmodesmata. Oparka and Prior (1992) found that a pressure differential of 300 kPa prevented symplasmic movement of LYCH, but it is unknown whether this response is mediated by deposition of callose at plasmodesmal necks. An approach combining chemical treatments, turgor measurements, the observation of symplasmic movement of fluorescent molecules, and mathematical or computational models can help elucidate this question. Computational models are particularly useful to provide mechanistic explanations in processes that are based on non-linear interactions and to elucidate the relative contribution of each of its components. As we propose in Fig. 2, there are many factors and processes connecting turgor and plasmodesmal permeability of different nature (e.g. mechanical, molecular), with different time responses (seconds, minutes, hours), and that seem to interact in non-linear ways, which could be integrated together in a computational model to put forward testable predictions. For example, a computational model that studies the changes in callose levels to turgor pressure could help find what type of function best describes this response and how this, in turn, could affect turgor.

Studies on the effects of turgor pressure on plasmodesmal permeability have been carried out with different systems. Cotton fibers would be appropriate to perform these studies in a single system, though it may not be the easiest choice because of its allopolyploidy and, until recently, because of the low efficiency of genetic transformation of cotton (Chen *et al.*, 2017). Nevertheless, the CRISPR/Cas9 system is becoming an efficient strategy for targeted mutagenesis of cotton and could help generate mutations of proteins that regulate callose levels to see how they affect cotton fiber elongation (Chen *et al.*, 2017; Gao *et al.*, 2017). An alternative system could be trichoblasts (and/or trichomes) of Arabidopsis, which are also single cells, and mutants of genes that code for proteins that regulate callose levels at plasmodesmata are readily available. To test the role of plasmodesmata in the elongation of trichoblasts, it would be necessary to follow the approaches of Ruan and coworkers (2001, 2004) for cotton fibers, evaluating the expression of *GSLs* and/or *PdGBs* and then testing the effects of mutations of these genes on root hair elongation.

Conclusions

Turgor pressure and plasmodesmal permeability can affect each other during plant development. Plasmodesmata control the content of osmolytes and water within the cell and turgor pressure can affect the levels of callose levels and the non-targeted movement of molecules. These interactions have important consequences for plant growth and development. Because plasmodesmal permeability helps establish patterns of cell types, for instance during differentiation of stomata or of vascular tissues, the study of how turgor pressure regulates plasmodesmal permeability can improve our understanding of how multicellular plants, and notably vascular plant, evolved. In this review, we have compiled the evidence that supports our hypothesis and we have identified some of the key questions that remain open in an attempt to bring attention to the subject.

Acknowledgements

This work was supported by CONACYT and by Institut Universitaire de France.

References

- Andrawis A, Solomon M, Delmer DP.** 1993. Cotton fiber annexins: a potential role in the regulation of callose synthase. *The Plant Journal* **3**, 763–772.
- Baluska F, Samaj J, Napier R, Volkmann D.** 1999. Maize calreticulin localizes preferentially to plasmodesmata in root apex. *The Plant Journal* **19**, 481–488.
- Barton DA, Cole L, Collings DA, Liu DY, Smith PM, Day DA, Overall RL.** 2011. Cell-to-cell transport via the lumen of the endoplasmic reticulum. *The Plant Journal* **66**, 806–817.
- Beauzamy L, Derr J, Boudaoud A.** 2015. Quantifying hydrostatic pressure in plant cells by using indentation with an atomic force microscope. *Biophysical Journal* **108**, 2448–2456.
- Beauzamy L, Nakayama N, Boudaoud A.** 2014. Flowers under pressure: ins and outs of turgor regulation in development. *Annals of Botany* **114**, 1517–1533.
- Benítez M, Hernández-Hernández V, Newman SA, Niklas KJ.** 2018. Dynamical patterning modules, biogeneric materials, and the evolution of multicellular plants. *Frontiers in Plant Sciences* **9**, 871.
- Benitez-Alfonso Y, Faulkner C, Pendle A, Miyashima S, Helariutta Y, Maule A.** 2013. Symplastic intercellular connectivity regulates lateral root patterning. *Developmental Cell* **26**, 136–147.
- Benitez-Alfonso Y, Jackson D, Maule A.** 2011. Redox regulation of intercellular transport. *Protoplasma* **248**, 131–140.
- Bilska A, Sowinski P.** 2010. Closure of plasmodesmata in maize (*Zea mays*) at low temperature: a new mechanism for inhibition of photosynthesis. *Annals of Botany* **106**, 675–686.
- Blake JR.** 1978. On the hydrodynamics of plasmodesmata. *Journal of Theoretical Biology* **74**, 33–47.
- Boursiac Y, Prak S, Boudet J, Postaire O, Luu DT, Tournaire-Roux C, Santoni V, Maurel C.** 2008. The response of *Arabidopsis* root water transport to a challenging environment implicates reactive oxygen species- and phosphorylation-dependent internalization of aquaporins. *Plant Signaling & Behavior* **3**, 1096–1098.
- Buckley TN.** 2005. The control of stomata by water balance. *New Phytologist* **168**, 275–292.
- Burch-Smith TM, Zambryski PC.** 2012. Plasmodesmata paradigm shift: regulation from within versus without. *Annual Review of Plant Biology* **63**, 239–260.

- Chen X, Lu X, Shu N, Wang S, Wang J, Wang D, Guo L, Ye W.** 2017. Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. *Scientific Reports* **7**, 44304. 8.60
- Chen XY, Lin L, Lee E, Han X, Rim Y, Chu H, Kim SW, Sack F, Kim JY.** 2009. The *Arabidopsis* callose synthase gene *GSL8* is required for cytokinesis and cell patterning. *Plant Physiology* **150**, 105–113.
- Comtet J, Jensen KH, Turgeon R, Stroock AD, Hosoi AE.** 2017a. Passive phloem loading and long-distance transport in a synthetic tree-on-a-chip. *Nature Plants* **3**, 17032. 8.65
- Comtet J, Turgeon R, Stroock AD.** 2017b. Phloem loading through plasmodesmata: a biophysical analysis. *Plant Physiology* **175**, 904–915.
- Cook M, Graham L.** 1999. Evolution of plasmodesmata. In: Van Bel AJE, van Kesteren WJP, eds. *Plasmodesmata. Structure, function, role in cell communication*. Berlin, Heidelberg: Springer, 101–117. 8.70
- Cosgrove DJ.** 1985. Cell wall yield properties of growing tissue: evaluation by in vivo stress relaxation. *Plant Physiology* **78**, 347–356.
- Cosgrove DJ.** 2005. Growth of the plant cell wall. *Nature Reviews. Molecular Cell Biology* **6**, 850–861.
- Côté R, Thain JF, Fensom DS.** 1987. Increase in electrical resistance of plasmodesmata of *Chara* induced by an applied pressure gradient across nodes. *Canadian Journal of Botany* **65**, 509–511. 8.75
- Crawford KM, Zambryski PC.** 2001. Non-targeted and targeted protein movement through plasmodesmata in leaves in different developmental and physiological states. *Plant Physiology* **125**, 1802–1812.
- Cui W, Lee JY.** 2016. *Arabidopsis* callose synthases CalS1/8 regulate plasmodesmal permeability during stress. *Nature Plants* **2**, 16034. 8.80
- De Storme N, Geelen D.** 2014. Callose homeostasis at plasmodesmata: molecular regulators and developmental relevance. *Frontiers in Plant Science* **5**, 138.
- Drake GA, Carr DJ, Anderson WP.** 1978. Plasmolysis, plasmodesmata, and the electrical coupling of at coleoptile cells. *Journal of Experimental Botany* **29**, 1205–1214. 8.85
- Ehlers K, van Bel AJ.** 2010. Dynamics of plasmodesmal connectivity in successive interfaces of the cambial zone. *Planta* **231**, 371–385.
- Erwee MG, Goodwin PB.** 1984. Characterization of the *Egeria densa* leaf symplast: response to plasmolysis, deplasmolysis and to aromatic amino acids. *Protoplasma* **122**, 162–168.
- Faulkner C, Maule A.** 2011. Opportunities and successes in the search for plasmodesmal proteins. *Protoplasma* **248**, 27–38. 8.90
- Foreman J, Demidchik V, Bothwell JH, et al.** 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **422**, 442–446.
- Fraser TW, Gunning BE.** 1969. The ultrastructure of plasmodesmata in the filamentous green alga, *Bulbochaete hiloensis* (Nordst.) tiffany. *Planta* **88**, 244–254. 8.95
- Fry SC, Dumville JC, Miller JG.** 2001. Fingerprinting of polysaccharides attacked by hydroxyl radicals in vitro and in the cell walls of ripening pear fruit. *The Biochemical Journal* **357**, 729–737.
- Gao W, Long L, Tian X, Xu F, Liu J, Singh PK, Botella JR, Song C.** 2017. Genome editing in cotton with the CRISPR/Cas9 system. *Frontiers in Plant Sciences* **8**, 1364. 8.100
- Gisel A, Barella S, Hempel FD, Zambryski PC.** 1999. Temporal and spatial regulation of symplastic trafficking during development in *Arabidopsis thaliana* apices. *Development* **126**, 1879–1889.
- Grignon N, Touraine B, Durand M.** 1989. 6(5)Carboxyfluorescein as a tracer of phloem sap translocation. *American Journal of Botany* **76**, 871–877. 8.105
- Grisson MS, Brocard L, Fouillen L, et al.** 2015. Specific membrane lipid composition is important for plasmodesmata function in *Arabidopsis*. *The Plant Cell* **27**, 1228–1250.
- Guseman JM, Lee JS, Bogenschutz NL, Peterson KM, Virata RE, Xie B, Kanaoka MM, Hong Z, Torii KU.** 2010. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in *Arabidopsis chorua* (glucan synthase-like 8). *Development* **137**, 1731–1741. 8.110
- Hamant O, Traas J.** 2009. The mechanics behind plant development. *New Phytologist* **185**, 369–385.
- Han X, Hyun TK, Zhang M, Kumar R, Koh EJ, Kang BH, Lucas WJ, Kim JY.** 2014. Auxin-callose-mediated plasmodesmal gating is essential 8.115

- for tropic auxin gradient formation and signaling. *Developmental Cell* **28**, 132–146.
- Han X, Kim JY.** 2016. Integrating hormone- and micromolecule-mediated signaling with plasmodesmal communication. *Molecular Plant* **9**, 46–56.
- 9.5 **Harding SA, Oh SH, Roberts DM.** 1997. Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species. *The EMBO Journal* **16**, 1137–1144.
- Jaffe MJ, Huberman M, Johnson J, Telewski FW.** 1985. Thigmomorphogenesis: the induction of callose formation and ethylene evolution by mechanical perturbation in bean stems. *Physiologia Plantarum* **64**, 271–279.
- 9.10 **Kim I, Kobayashi K, Cho E, Zambryski PC.** 2005. Subdomains for transport via plasmodesmata corresponding to the apical-basal axis are established during *Arabidopsis* embryogenesis. *Proceedings of the National Academy of Sciences, USA* **102**, 11945–11950.
- Kim I, Zambryski PC.** 2005. Cell-to-cell communication via plasmodesmata during *Arabidopsis* embryogenesis. *Current Opinion in Plant Biology* **8**, 593–599.
- 9.15 **Knox JP, Benitez-Alfonso Y.** 2014. Roles and regulation of plant cell walls surrounding plasmodesmata. *Current Opinion in Plant Biology* **22**, 93–100.
- Kroeger JH, Zerzour R, Geitmann A.** 2011. Regulator or driving force? The role of turgor pressure in oscillatory plant cell growth. *PLoS One* **6**, e18549.
- 9.20 **Kwiatkowska M.** 1999. Plasmodesmal coupling and cell differentiation in algae. In: Van Bel AJE, van Kesteren WJP, eds. *Plasmodesmata. Structure, function, role in cell communication*. Berlin, Heidelberg: Springer, 205–224.
- Lee JY, Wang X, Cui W, et al.** 2011. A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in *Arabidopsis*. *The Plant Cell* **23**, 3353–3373.
- 9.25 **Lee SH, Singh AP, Chung GC.** 2004. Rapid accumulation of hydrogen peroxide in cucumber roots due to exposure to low temperature appears to mediate decreases in water transport. *Journal of Experimental Botany* **55**, 1733–1741.
- Levy A, Erlanger M, Rosenthal M, Epel BL.** 2007. A plasmodesmata-associated β -1,3-glucanase in *Arabidopsis*. *The Plant Journal* **49**, 669–682.
- 9.30 **Lucas M, Kenobi K, von Wangenheim D, et al.** 2013. Lateral root morphogenesis is dependent on the mechanical properties of the overlaying tissues. *Proceedings of the National Academy of Sciences, USA* **110**, 5229–5234.
- Lucas WJ, Bouché-Pillon S, Jackson DP, Nguyen L, Baker L, Ding B, Hake S.** 1995. Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* **270**, 1980–1983.
- 9.35 **Lucas WJ, Lee JY.** 2004. Plasmodesmata as a supracellular control network in plants. *Nature Reviews. Molecular Cell Biology* **5**, 712–726.
- Mouliat B.** 2013. Plant biomechanics and mechanobiology are convergent paths to flourishing interdisciplinary research. *Journal of Experimental Botany* **64**, 4617–4633.
- 9.40 **Münch E.** 1930. *Die Stoffbewegungen in der Pflanze*. Jena: Gustav Fischer.
- Nicolas WJ, Grison MS, Trépout S, Gaston A, Fouché M, Cordelières FP, Oparka K, Tilsner J, Brocard L, Bayer EM.** 2017. Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves. *Nature Plants* **3**, 17082.
- 9.45 **Niklas KJ, Spatz HC.** 2012. *Plant physics*. Chicago: University of Chicago Press, 71–103.
- Olesen P.** 1979. The neck constriction in plasmodesmata: evidence for a peripheral sphincter-like structure revealed by fixation with tannic acid. *Planta* **144**, 349–358.
- Oparka KJ, Duckett CM, Prior DAM, Fisher DB.** 1994. Real-time imaging of phloem unloading in the root tip of *Arabidopsis*. *The Plant Journal* **6**, 759–766.
- 9.50 **Oparka KJ, Prior DAM.** 1992. Direct evidence for pressure-generated closure of plasmodesmata. *The Plant Journal* **2**, 741–750.
- Oparka KJ, Roberts AG, Boevink P, Santa Cruz S, Roberts I, Pradel KS, Imlau A, Kotlizky G, Sauer N, Epel B.** 1999. Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell* **97**, 743–754.
- 9.55 **Park K, Knoblauch J, Oparka K, Jensen K.** 2019. Controlling intercellular flow through mechanosensitive plasmodesmata nanopores. *Nature Communications* **10**, 3564.
- 9.60 **Passardi F, Penel C, Dunand C.** 2004. Performing the paradoxical: how plant peroxidases modify the cell wall. *Trends in Plant Science* **9**, 534–540.
- Péret B, Li G, Zhao J, et al.** 2012. Auxin regulates aquaporin function to facilitate lateral root emergence. *Nature Cell Biology* **14**, 991–998.
- Raven JA.** 1997. Minireview: multiple origins of plasmodesmata. *European Journal of Phycology* **32**, 95–101.
- 9.65 **Richards SL, Laohavisit A, Mortimer JC, Shabala L, Swarbreck SM, Shabala S, Davies JM.** 2014. Annexin 1 regulates the H₂O₂-induced calcium signature in *Arabidopsis thaliana* roots. *The Plant Journal* **77**, 136–145.
- Rinne PL, van der Schoot C.** 1998. Symplasmic fields in the tunica of the shoot apical meristem coordinate morphogenetic events. *Development* **125**, 1477–1485.
- Ruan YL, Llewellyn DJ, Furbank RT.** 2001. The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and K⁺ transporters and expansin. *The Plant Cell* **13**, 47–60.
- 9.70 **Ruan YL, Xu SM, White R, Furbank RT.** 2004. Genotypic and developmental evidence for the role of plasmodesmal regulation in cotton fiber elongation mediated by callose turnover. *Plant Physiology* **136**, 4104–4113.
- Sager R, Lee JY.** 2014. Plasmodesmata in integrated cell signalling: insights from development and environmental signals and stresses. *Journal of Experimental Botany* **65**, 6337–6358.
- 9.75 **Schmalstig JG, Cosgrove DJ.** 1990. Coupling of solute transport and cell expansion in pea stems. *Plant Physiology* **94**, 1625–1633.
- Schönknecht G, Brown JE, Verchot-Lubicz J.** 2008. Plasmodesmata transport of GFP alone or fused to potato virus X TGBp1 is diffusion driven. *Protoplasma* **232**, 143–152.
- 9.80 **Schopfer P.** 2006. Biomechanics of plant growth. *American Journal of Botany* **93**, 1415–1425.
- Schopfer P, Plachy C, Frahy G.** 2001. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiology* **125**, 1591–1602.
- 9.85 **Schulz A.** 1995. Plasmodesmal widening accompanies the short-term increase in symplasmic phloem unloading in pea root tips under osmotic stress. *Protoplasma* **188**, 22–37.
- Shin H, Brown RM Jr.** 1999. GTPase activity and biochemical characterization of a recombinant cotton fiber annexin. *Plant Physiology* **119**, 925–934.
- 9.90 **Simpson C, Thomas C, Findlay K, Bayer E, Maule AJ.** 2009. An *Arabidopsis* GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. *The Plant Cell* **21**, 581–594.
- Squires TM, Quake SR.** 2005. Microfluidics: Fluid physics at the nanoliter scale. *Reviews of Modern Physics* **77**, 977.
- 9.95 **Takeda S, Gapper C, Kaya H, Bell E, Kuchitsu K, Dolan L.** 2008. Local positive feedback regulation determines cell shape in root hair cells. *Science* **319**, 1241–1244.
- Tang W, Tu L, Yang X, Tan J, Deng F, Hao J, Guo K, Lindsey K, Zhang X.** 2014. The calcium sensor GhCaM7 promotes cotton fiber elongation by modulating reactive oxygen species (ROS). *New Phytologist* **202**, 509–520.
- 9.100 **Terry BR, Matthews EK, Haseloff J.** 1995. Molecular characterisation of recombinant green fluorescent protein by fluorescence correlation microscopy. *Biochemical and Biophysical Research Communications* **217**, 21–27.
- Tucker EB, Boss WF.** 1996. Mastoparan-induced intracellular Ca²⁺ fluxes may regulate cell-to-cell communication in plants. *Plant Physiology* **111**, 459–467.
- 9.105 **Vatén A, Dettmer J, Wu S, et al.** 2011. Callose biosynthesis regulates symplasmic trafficking during root development. *Developmental Cell* **21**, 1144–1155.
- Verchot-Lubicz J, Goldstein RE.** 2009. Cytoplasmic streaming enables the distribution of molecules and vesicles in large plant cells. *Protoplasma* **240**, 99–107.
- 9.110 **Vermeer JE, von Wangenheim D, Barberon M, Lee Y, Stelzer EH, Maizel A, Geldner N.** 2014. A spatial accommodation by neighboring cells is required for organ initiation in *Arabidopsis*. *Science* **343**, 178–183.
- Wang L, Li XR, Lian H, Ni DA, He YK, Chen XY, Ruan YL.** 2010. Evidence that high activity of vacuolar invertase is required for cotton fiber
- 9.115
9.116

Page 10 of 10 | Hernández-Hernández *et al.*

and Arabidopsis root elongation through osmotic dependent and independent pathways, respectively. *Plant Physiology* **154**, 744–756.

Wang N, Fisher DB. 1994. The use of fluorescent tracers to characterize the post-phloem transport pathway in maternal tissues of developing wheat grains. *Plant Physiology* **104**, 17–27.

Weber A, Braybrook S, Huflejt M, Mosca G, Routier-Kierzkowska AL, Smith RS. 2015. Measuring the mechanical properties of plant cells by combining micro-indentation with osmotic treatments. *Journal of Experimental Botany*, **66**, 3229–3241.

Xu XM, Wang J, Xuan Z, Goldshmidt A, Borrill PG, Hariharan N, Kim JY, Jackson D. 2011. Chaperonins facilitate KNOTTED1 cell-to-cell trafficking and stem cell function. *Science* **333**, 1141–1144.

Zalepa-King L, Citovsky V. 2013. A plasmodesmal glycosyltransferase-like protein. *PLoS One* **8**, e58025.

Zhang Z, Ruan YL, Zhou N, Wang F, Guan X, Fang L, Shang X, Guo W, Zhu S, Zhang T. 2017. Suppressing a putative sterol carrier gene reduces plasmodesmal permeability and activates sucrose transporter genes during cotton fiber elongation. *The Plant Cell* **29**, 2027–2046.

10.5

10.10

10.15

10.20

10.25

10.30

10.35

10.40

10.45

10.50

10.55

10.58

10.60

10.65

10.70

10.75

10.80

10.85

10.90

10.95

10.100

10.105

10.110

10.115

10.116

3

A mechano-hydraulic model for the study of cotton fiber elongation

In the following chapter we generated a computational model that allowed us to study if plasmodesmal permeability can affect turgor pressure and growth of cotton fibers. Our model uses Lockhart's equations for irreversible plant expansion to which we added fluxes of water and solutes through plasmodesmata. I participated in the generation of the mathematical model, solved it numerically, and wrote the manuscript.

A mechano-hydraulic model for the study of cotton fiber elongation

Valeria Hernández-Hernández,
Annamaria Kiss,
Mariana Benitez,
Arezki Boudaoud

Abstract

Plant cell growth depends on turgor pressure which provides the energy for wall expansion. The regulation of turgor pressure depends on several factors such as the activity of vacuole invertases, aquaporins, the permeability of the plasma membrane, and the cell wall extensibility. Plasmodesmata are lined membrane channels that allow free movement of water and sugars and whose permeability varies during plant development. Experimental studies have correlated changes in the permeability of plasmodesmal channels to turgor pressure variations. Here we study the role of plasmodesmal permeability in the behavior of turgor pressure and on cotton fiber expansion. We used Lockhart's model for irreversible cell expansion of the cotton fiber with the addition of plasmodesmal-dependent movement of water and solutes. We performed a sensitivity analysis to changes in values of parameters and find that plasmodesmal permeability is among the most important factors for the overall behavior of the system and particularly to building up turgor pressure and expanding cotton fibers. Moreover, we find that non-monotonic behaviors of turgor pressure that have been reported previously in cotton fibers, can not be recovered without accounting for dynamic changes of the parameters used in this model. Our results suggest that changes in plasmodesmal permeability are important for the regulation of turgor pressure behaviors and the expansion of cotton fibers.

1 Introduction

Expansion of the plant cell depends on mechanical and hydraulic processes. Mechanical processes include the ability of the cell wall to increase in surface area, called wall extensibility. The hydraulic processes include the effect of aquaporins and the permeability of plasmodesmata. James Lockhart (20) developed a model that has become widely used in the study of the mechano-hydraulic processes behind irreversible plant expansion. In its original form Lockhart's did not account for plasmodesmata permeability. Plasmodesmata are intercellular channels that create symplasmic continuity. It has been shown that the diameter of the channel (i.e., permeability) varies during plant development which affects symplasmic movement of small molecules like sucrose (27). The idea that plasmodesmata can regulate fluxes of solutes and water has led to hypothesize that plasmodesmal permeability

may be important for building up turgor during cell expansion. As a consequence of this, studies have started to integrate the role of plasmodesmal permeability into models of plant cell expansion (5). This work aims at investigating the role of plasmodesmal permeability in the expansion of plant cells.

Cotton fibers are an ideal system to study the mechanisms behind cell expansion because they are single epidermal cells that mostly increase in length (4; 17). Fibers start growing at the day of anthesis until 20-26 days after anthesis (DAA) (4; 17). A study performed by Ruan and colleagues (24; 25), reported that plasmodesmata change permeability from open (0-9 DAA) to closed (10-15 DAA) to open (16 onwards) again during *Gossypium hirsutum* fiber growth. Another study also reported that plasmodesmata are open at 5 and 10 DAA and closed at 15, 20 and 25 DAA (30). Besides the dynamics of plasmodesmal permeability, Ruan and colleagues (2001) also found that turgor and osmotic pressures reached a maximum at around 15 DAA which correlated with closure of plasmodesmata. According to the authors, closure of plasmodesmata is necessary for turgor to behave in such a peculiar way in cotton fiber cell. Experimental observations reported that turgor pressure of fiber cells and of its adjacent seed neighbor are similar except for when plasmodesmata close (24). This observation was also demonstrated theoretically between groups of cells of the shoot apical meristem (SAM) in which low symplasmic permeability increased turgor pressure of the cells that, comparable to fiber cells, were growing (5). This evidence points at the regulation of plasmodesmal permeability as an important factor to regulate turgor pressure.

During fiber growth there is an increase in the expression level of sucrose and K^+ transporters (24), and of Vacuole Invertase 1 (VIN1) (29), all of which are processes that help accumulate solutes and increase osmotic pressure. It has been proposed that while VIN1 and solute transporters might help accumulate solutes within the fiber, closure of plasmodesmata helps avoid leakage of solutes and of water which results in turgor increases. (24). Then with the reopening of plasmodesmata channels, symplasmic fluxes between the fiber and its neighbor lower turgor pressure of the fiber and the pressure gradient is reduced (24; 5).

In the original Lockhart's model, the ability of the cell wall to grow is governed by the 'extensibility coefficient', ϕ , which is related to material properties of the wall (6). The hydraulic conductance coefficient L_r of the membrane is related to water influx as it describes how readily water can move across the membrane (6; 28). Water moves according to the osmotic pressure gradient, $\Delta\pi$, that is established between the cell and its neighbor. When turgor pressure is above a critical threshold (yield turgor pressure, Y), plastic extension of the cell wall occurs (26). Such wall extension drops turgor pressure which drives influx of water until turgor is restored. Plant cells are capable of maintaining growth for long periods through the action of processes that accumulate solutes and the enzymatic activity that allow wall extension. We take Lockhart's model and add to it fluxes of solutes and water through plasmodesmata in order to study the relative contribution of plasmodesmal permeability and its role in turgor pressure behavior and in growth of cotton fiber cells.

2 Model

In the following subsections we describe the model and its assumptions.

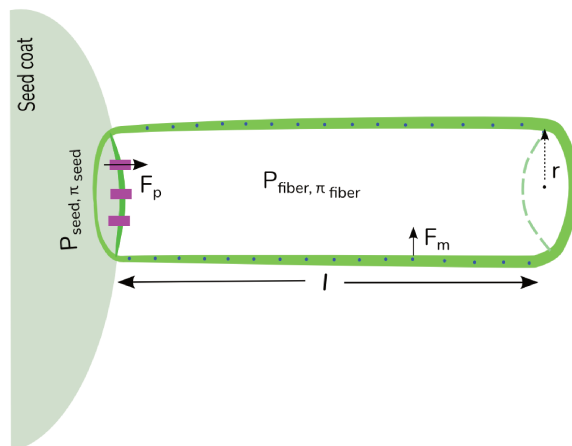


Figure 1: Schematic representation of the cotton fiber model using Lockhart equations with plasmodesmal permeability added. Purple rectangles represent plasmodesmata at the plasma membrane; blue dots represent water molecules. F_p : water fluxes through plasmodesmata channels; F_m : water fluxes through the plasma membrane; l : length of cell; r : radius of the cell.

2.1 Geometry

The main assumption regarding the geometry is cylindrical shape (of radius r and length l) and uniaxial growth of the cotton fiber, that is, the cylindrical fiber only grows in length.

2.2 Water uptake

Since water is incompressible and the fiber cell is supposed to be turgid (ie. plasma membrane is in contact with the cell wall) throughout the growth phase, the observed change in volume V of the cell is equal to the sum of all possible water fluxes occurring in the cell. We consider in our model that the total flux of water occurs through two pathways: the plasma membrane with flux F_m and plasmodesmata channels F_p :

$$\frac{dV}{dt} = F_m + F_p \quad (1)$$

Flux through the membrane is driven by the difference in water potential between the cell and the apoplast $\Delta\Psi = \Psi_{apoplast} - \Psi_{fiber}$, so that the lower Ψ in a compartment the more it favors movement of water towards this compartment,

$$F_m = LA_m\Delta\Psi, \quad (2)$$

with L , the hydraulic membrane conductivity, and A_m , the membrane surface through which water passes. Despite the fact that fiber cells are only connected to seed coat cells by the basal membrane, it is commonly held that water molecules can move through and along cell walls (28). We keep this assumption for the side walls of cotton fiber, and add that the area of the end walls is significantly smaller compared to the area of the side walls. Thus, the considered surface area for the membrane flux is the side of the cylinder, $A_m = 2\Pi r l = \frac{2V}{r}$, where V is the observed volume of the cell.

Water potential is determined by turgor (P) and osmotic (π) pressures, $\Psi = P - \pi$ (28). We assume that the epidermal seed cell that is adjacent to the fiber does not grow and therefore is in equilibrium with the apoplasmic space $\Psi_{apoplasm} \approx \Psi_{seed}$, which gives $\Delta\Psi = \Psi_{seed} - P_{fiber} + \pi_{fiber}$:

$$F_m = L \frac{2V}{r} (\Psi_{seed} + \pi_{fiber} - P_{fiber}) \quad (3)$$

When P_{fiber} is larger than P_{seed} , water flows out of the cell, and the inverse happens when π_{fiber} is larger than the osmotic pressure of the seed cell (13).

As pointed out in the introduction, water can also flow through plasmodesmata channels creating a symplasmic continuum between the fiber and seed cell next to it. Fluxes of water through plasmodesmata follow the gradient of turgor pressure between the two cell compartments,

$$F_p = \lambda A_p (P_{seed} - P_{fiber}) \quad (4)$$

with λ the conductivity per surface area of a plasmodesma and $A_p = \Pi r^2$ the surface area of the cylindrical cell's basis, where the fiber is in contact with the adjacent seed cell. This gives

$$F_p = \lambda \Pi r^2 (P_{seed} - P_{fiber}) \quad (5)$$

for the flux through a single plasmodesma.

Let us finally denote $L_r = \frac{2L}{r}$, the normalized hydraulic permeability of the plasma membrane (20; 6; 13), as well as $\mu = \lambda \Pi r^2$ the total plasmodesmal permeability, and put together the flux through the membrane and through plasmodesmata to get the following equation which accounts for the net change in water volume within the cotton fiber cell:

$$\frac{dV}{dt} = F = L_r V (\Psi_{seed} + \pi_{fiber} - P_{fiber}) + \mu (P_{seed} - P_{fiber}) \quad (6)$$

2.3 Solute uptake

Osmotic pressure is related to osmolarity or solute concentration $c = N/V$ of a solution, with N the number of solute molecules in the fiber. For non-dissociating substances like sucrose and relatively low concentrations, the osmotic pressure π_{fiber} can be estimated using Van't Hoff's equation (28):

$$\pi_{fiber} = c_{fiber} RT = \frac{NRT}{V} \quad (7)$$

where R is the gas constant, and T the absolute temperature.

The change in the number of solutes $\frac{dN}{dt}$ within the fiber depends on a source of solute molecules proportional to the volume of the cell, αV , and on fluxes of solutes through plasmodesmata channels. The volume of solute entering the fiber cell through plasmodesmata is $\mu(P_{seed} - P_{fiber})_+$. Note that the direction of the flux of solutes depends on the sign of ΔP : if the hydrostatic pressure in the seed is higher than in the fiber, solutes from the seed will enter into the fiber, otherwise solutes of the fiber will move out. These considerations can be formulated in the following equation:

$$\frac{dN}{dt} = \alpha V + \mu c_{seed}(P_{seed} - P_{fiber})_+ - \mu c_{fiber}(P_{fiber} - P_{seed})_+ \quad (8)$$

with $()_+$ denoting the positive part of the expression inside the brackets.

Using equation 7 to express N and the concentrations c_{seed} and c_{fiber} as a function of volume V and osmotic pressure, we obtain the following equation for changes in osmotic pressure of the cotton fiber cell:

$$\frac{d\pi_{fiber}}{dt} = \alpha - \frac{\pi_{fiber}}{V} \frac{dV}{dt} + \frac{\mu\pi_{seed}}{V} (P_{seed} - P_{fiber})_+ - \frac{\mu\pi_{fiber}}{V} (P_{fiber} - P_{seed})_+ \quad (9)$$

2.4 Growth and mechanics

According to the seminal work by Lockhart (20), the cell wall is under tensile stress that causes elastic stretching. When turgor pressure is above Y , the cell's length at rest l_0 (and the corresponding volume at rest $V_0 = \Pi r^2 l_0$) is changed. Therefore, the observed relative change in volume of the cylindrical cell is the sum of the irreversible (plastic growth) and reversible deformations (elastic stretching):

$$\frac{1}{V} \frac{dV}{dt} = \frac{1}{V_0} \frac{dV_0}{dt} + \frac{1}{\beta + P_{fiber}} \frac{dP_{fiber}}{dt} \quad (10)$$

with β a parameter related to the rigidity of the cell wall ($\beta = \frac{2Eh}{r}$, having E the Young modulus, and h the wall thickness).

Here we use the hypothesis of diffuse growth of the cotton fiber which means that irreversible growth occurs longitudinally along the cylindrical cell

$$\frac{1}{V_0} \frac{dV_0}{dt} = \phi(P_{fiber} - Y)_+ \quad (11)$$

Replacing this expression in equation 10 gives the following differential equation for turgor pressure of cotton fiber:

$$\frac{dP_{fiber}}{dt} = (\beta + P_{fiber}) \left(\frac{1}{V} \frac{dV}{dt} - \phi(P_{fiber} - Y)_+ \right) \quad (12)$$

In this context, the system of equations (6), (9) and (12) for variables V , π_{fiber} and P_{fiber} governs the behavior of cotton fiber by taking into account irreversible

growth, elastic stretching, water fluxes through the membrane and plasmodesmata, and solute fluxes through plasmodesmata. However, elastic equilibrium in plant cells occurs on a time scale of minutes while irreversible growth is on the order of hours. Given that we are interested in irreversible growth on a time scale of days, we neglect the elastic deformation on a first approximation:

$$\frac{1}{V} \frac{dV}{dt} = \frac{1}{V_0} \frac{dV_0}{dt} = \phi(P_{fiber} - Y)_+ \quad (13)$$

and the relation

$$(P_{fiber} - Y)_+ = \frac{1}{\phi V} \frac{dV}{dt} \quad (14)$$

becomes:

$$(P_{fiber} - Y)_+ = \frac{1}{\phi V} (L_r V (\Psi_{seed} + \pi_{fiber} - P_{fiber}) + \mu (P_{seed} - P_{fiber})) \quad (15)$$

When equation (6) is used, turgor pressure of the fiber, P_{fiber} , becomes a function of variables V and π_{fiber} :

$$P_{fiber} = \begin{cases} \frac{L_r(\Psi_{seed} + \pi_{fiber}) + \frac{\mu}{V} P_{seed}}{L_r + \frac{\mu}{V}}, & \text{if } \frac{L_r(\Psi_{seed} + \pi_{fiber}) + \frac{\mu}{V} P_{seed}}{L_r + \frac{\mu}{V}} \leq Y \\ \frac{L_r(\Psi_{seed} + \pi_{fiber}) + \frac{\mu}{V} P_{seed} + \phi Y}{L_r + \frac{\mu}{V} + \phi}, & \text{otherwise.} \end{cases} \quad (16)$$

In what follows we consider the system of two equations (6), (9) for variables V , π_{fiber} and the expression (16) for turgor pressure P_{fiber} .

2.5 Model parameters

2.5.1 Estimation of the source of solutes, α

Because we assumed that π_{fiber} is proportional to the concentration of solutes, the typical value of α was $\pi_{fiber} \cdot k$, where π_{fiber} was extracted from the data reported in (24) and k is the average value of growth rate estimated from four different curve fits. In order to perform these fits, we extracted experimental data of the length of cotton fiber cells reported in (8; 22; 2; 29). We used these lengths to calculate cell volume and fitted each set of data to the logistic growth equation:

$$f(x) = \frac{M}{1 + e^{-k(x-x_0)}} \quad (17)$$

where M is the maximum volume and x_0 is the midpoint of the curve. We then calculated and used the average value of k .

2.5.2 Estimation of plasmodesmal permeability, μ

For the estimation of plasmodesmal permeability, we followed the methodology of (12; 7) and first calculated the conductivity of a single plasmodesma using the Hagen-Poiseuille law modified to account for occlusion of plasmodesmata channels

by the desmotubule. We used the equation for annular flow to determine the volume of liquid that passes through a single plasmodesma channel as follows:

$$\lambda = \frac{\Pi}{8\eta\omega} \left[R_2^4 - R_1^4 - \frac{(R_2^2 - R_1^2)^2}{\log(R_2/R_1)} \right] \quad (18)$$

where R_1 and R_2 are the desmotubule and plasmodesma radii, respectively, and η is the viscosity of the cell sap, which we take to be a few times that of water as done by (16; 7). The value of λ , the conductivity of a plasmodesma, was then scaled to the cell level by multiplying λ by the surface area of the cell wall shared by the fiber and the contiguous epidermal cell, and D , the frequency of plasmodesmata. This estimation gave a value for μ , the plasmodesmal permeability of the fiber.

2.5.3 Estimation of water potential of the seed, Ψ_{seed}

The value of water potential of the adjacent seed cell was $\Psi_{seed} = P_{seed} - \pi_{seed}$, whose values, π_{seed} and P_{seed} , were extracted from (24).

2.5.4 V and π_{fiber} initial conditions

Measurements performed on fiber and epidermal cells of the seed coat show that at 0 DAA both cells have very similar values of osmotic pressure (24). Based on this observation, we took the same interval of values of π_{seed} for the initial condition of π_{fiber0} . We took cell length value at the start of the growth phase reported by (24), to calculate volume and fixed the value of the initial condition of volume V_0 to 1.88×10^{-4} . Therefore, the set of equations presented in the previous section for changes in volume, turgor and osmotic pressures has seven parameters plus one initial condition that varies.

2.5.5 Other parameters and extraction of experimental data

We reviewed the experimental literature in search for values that were measured in cotton fibers of *G. hirsutum*. When no values for our study system were found we used those reported for aerial organs of other species, or for other organs as last resource. To extract data from published works, we used the online software WebPlotDigitizer (version 4.2, accessed on March 2019).

2.6 Numerical resolution

All code was written in Python (version 3.6) and implemented in the Jupyter notebook environment (version 5.5.0). Numerical resolution of the differential equation system was made using the ODEINT function from the SciPy library. All graphs presented were generated with Matplotlib library.

In each simulation, we computed four ratios to test the relative contribution of each pathway/process to the changes in V , π_{fiber} , and P_{fiber} . A list of ratios computed in each simulation is presented. In this list, 't1', 't2', ..., 'tn' makes reference to the term number in equations 6 and 9 that represent different pathways/process for the changes in water volume and solute concentration.

- dV_{t1}/dV , contribution of water flow through plasma membrane over change in volume
- dV_{t2}/dV , contribution of water flow through plasmodesmata over change in volume
- VF/V_0 , final over initial volume
- $d\pi_{fiber}t_2/\alpha$, contribution of dilution by growth over contribution of source of solutes
- $d\pi_{fiber}t_3/\alpha$, contribution of plasmodesmata fluxes over contribution of source of solutes
- π_{max}/π_{min} , maximum value of π_{fiber} over its minimum value
- P_{max}/P_{min} , maximum value of P_{fiber} over its minimum value

2.7 Classification of types of behaviors

To evaluate the type of behaviors (monotonic, non-monotonic and constant) the system reached, we computed the percentages of increasing and decreasing changes of volume, osmotic and turgor pressures. In these computations, we disregarded the five initial time steps to allow stabilization of the system. For the rest of the growth time, we considered differences smaller than 1% from the total period as 'constant behaviors' to avoid counting numerical errors in the computation of these percentages.

To test whether a 1% threshold was suitable for the classification of the types of behaviors, we performed a second set of simulations using a value of $Th_2 = 1.49012 \times 10^{-8}$ as threshold for the classification of the types of behaviors. This value comes from the Python documentation (<https://docs.scipy.org/doc/scipy/reference/generated/scipy.integrate.odeint.html>, consulted on: 19/07/19) and is the default estimated local error of the y vector given in the ODEINT function.

As a second test, we compared the relative change of turgor pressure, $\frac{P_{final}-P_{min}}{P_{final}}$, of simulations where turgor pressure was classified as monotonic decreasing using the 1% threshold and to the relative change of experimental data of turgor potential reported by (8). We discuss the results of these two tests in the following section.

3 Results and Discussion

3.1 Parameter values

In Table 1 we present the complete set of parameters, their reference values and experimental studies from which they were extracted.

Table 1: List of parameters, ranges, and references consulted

Symbol, name	Range	References, notes
ϕ , cell wall extensibility	0.004-0.09 MPa ⁻¹ . h ⁻¹	Growing pea stems (6; 1)
L_r , normalized hydraulic conductivity of the cell membrane	18-18000 MPa ⁻¹ . h ⁻¹	4590 MPa ⁻¹ . h ⁻¹ for parenchyma cells of corn leaves (18); (21) (<i>Chara</i> algae, onion, pea)
Y , yield turgor pressure	0.06 - 0.2 MPa	Assuming $P_{fiber} > Y$, P_{fiber} varies from 0.07 0.26 MPa during fiber elongation (24)
P_{seed} , turgor pressure of seed cells	0.07 - 0.18 MPa	<i>G. hirsutum</i> (24)
π_{seed} , osmotic pressure of seed cells	0.99-1.29 MPa	<i>G. hirsutum</i> (24)
α , source of solutes	0.3-0.6 MPa. h ⁻¹	According to (24), π_{fiber} varies between 1.08 to 1.28 MPa, which was used as input in the estimation described in subsection 2.5.1
r , fiber radius	0.01- 0.02 mm	According to diameter values of <i>G. hirsutum</i> fiber cells (3; 17)
l , fiber length	21 - 30 mm	Final length of <i>G. hirsutum</i> (4; 17)
R_1 , plasmodesma radii	$1 - 3 \times 10^{-5}$ mm	Floral nectaries of <i>G. hirsutum</i> (10; 9)
R_2 , desmotubule radii	$9.2 \times 10^{-6} - 2.1 \times 10^{-5}$ mm	Floral nectaries of <i>G. hirsutum</i> (10; 9)
η , cell sap viscosity	$6 \times 10^{-13} - 1 \times 10^{-12}$ MPa.h	Phloem cells (15; 16)
ω , cell wall thickness	$2 \times 10^{-4} - 6 \times 10^{-3}$ mm	Wall thickness variation from the start to the end of <i>G. hirsutum</i> fiber growth (17; 14)
D , number of plasmodesmata per mm ²	$3 \times 10^6 - 13 \times 10^6$ mm ⁻²	Floral nectaries of <i>G. hirsutum</i> (10); tobacco trichomes (11)
μ , total plasmodesmal permeability	$3 \times 10^{-4} - 0.23$ mm ³ . MPa ⁻¹ . h ⁻¹	According to estimations described in subsection 2.5.2

To perform a sensitivity analysis of the model, we took x_{max} and x_{min} values from Table1 and calculated the geometric mean. We also added two more values, $x_{largest}$ and x_{lowest} , according to the following formulas:

$$\text{Geometric mean: } m = \sqrt{x_{max} \times x_{min}}$$

$$n = \frac{x_{max}}{x_{min}}$$

$$\text{Largest value of the range: } x_{largest} = x_{max} \times n$$

$$\text{Lowest value of the range: } x_{lowest} = \frac{x_{min}}{n}$$

giving a total of five values per parameter which are given in Table 2. We added a sixth value for μ ($\mu = 0$) which represents the case when plasmodesmata channels are closed. We obtained a total of $N = 468\,750$ simulations, in each one of which we computed the output ratios previously listed and made the classification of the type of behavior of pressures.

Table 2: Parameters and magnitudes explored in the sensitivity analysis

Symbol, name	Values
ϕ	2×10^{-4} , 4×10^{-3} , 0.019, 0.09, $2 \text{ MPa}^{-1} \cdot \text{h}^{-1}$
L_r	0.018, 18, 569, 18×10^3 , $18 \times 10^6 \text{ MPa}^{-1} \cdot \text{h}^{-1}$
Y	0.02, 0.06, 0.1, 0.2, 0.7 MPa
μ	0, 4×10^{-7} , 3×10^{-4} , 8×10^{-3} , 0.23, $176 \text{ mm}^3 \cdot \text{MPa}^{-1} \cdot \text{h}^{-1}$
α	0.1, 0.3, 0.5, 0.7, 1.6 MPa. h^{-1}
P_{seed}	0.023, 0.07, 0.11, 0.18, 0.54 MPa
π_{seed}	0.8, 1, 1.1, 1.3, 1.7 MPa

3.2 Effects of parameter changes in the behavior of the system

To study the effects that parameters had on the behavior of cotton fiber growth, we performed two cases of sensitivity analysis around middle values: (a) effect of one parameter on the value of ratios when all other parameters take their middle values; (b) effect of one parameter on ratios when all other parameters span their ranges. For the second case, (b), we plotted the mean and standard deviation (SD). The complete list of plots can be found in the Supplementary Material 1. According to these results, parameters can be classified in two groups: those that increase growth (α , L_r , ϕ and P_{seed}) and those that decrease it (μ , π_{seed} , and Y) (Figure 2).

It has been shown that over expression of molecules that contribute to the accumulation of solutes within cotton fibers, such as VIN1, can enhance the length of the fiber, probably by increasing the osmotic pressure (29). When more solutes are accumulated, water potential of the fiber lowers which drives water into the fiber that, in turn, increases turgor pressure. In our model, increases in the value of α result in larger π_{max}/π_{min} and P_{max}/P_{min} for case (a) which indicates that osmotic and turgor pressures have more changes during the simulation (Sup Mat 1.1). According to the value of VF/VI, the cell grows more with larger values of α , which seems to be in agreement with the experimental observations (Figure 2). Likewise, increasing the value of L_r results in larger cell volumes (Figure 2), which is consistent with experiments showing that overexpression of the plasma membrane aquaporin PIP2 in *G. hirsutum* results in longer fiber cells (19). As the wall extensibility, ϕ , becomes larger, water potential decreases creating the conditions for water import. This situation is expected to result in larger cell volume which is the result recovered in our simulations (Figure 2a).

Influx of water and solutes through plasmodesmata channels is favoured when $P_{seed} > P_{fiber}$. When the value of P_{seed} is increased we observed that $d\pi_{fiber}t_3/\alpha$, the efflux of water and solutes decreases and it becomes positive, indicating that as P_{seed} becomes larger, water and solute molecules move through plasmodesmata into the fiber cell (Sup. Material 1.1). As expected from this shift, we observe that the fiber attains larger volumes (Figure 2a). Increasing the yield turgor has the opposite effect on fiber growth (Figure 2b) which may be explained by the fact that a larger Y means that turgor pressure has to reach larger values to make the cell growth.

The effects observed for the changes in parameters on volume of the cotton fiber follow a similar trend between cases (a) and (b), however, in case (b) the changes in fiber volume seem to be much larger than in (a).

In the mechanistic model proposed by Ruan and colleagues (2001), plasmodesmata gating is necessary to increase turgor pressure in the cotton fiber. Computational results have also demonstrated that decreasing the symplasmic connectivity between groups of cells produces larger turgor values in cells that are growing (5). Another correlation between turgor pressure differentials and symplasmic isolation has also been reported in guard cells. These are specialized epidermal cells that can open and close to control gas exchanges between the plant and the environment. With the help of microinjections of the fluorescent dye Lucifer Yellow it was observed that dye movement from guard cells into the neighboring cells (and

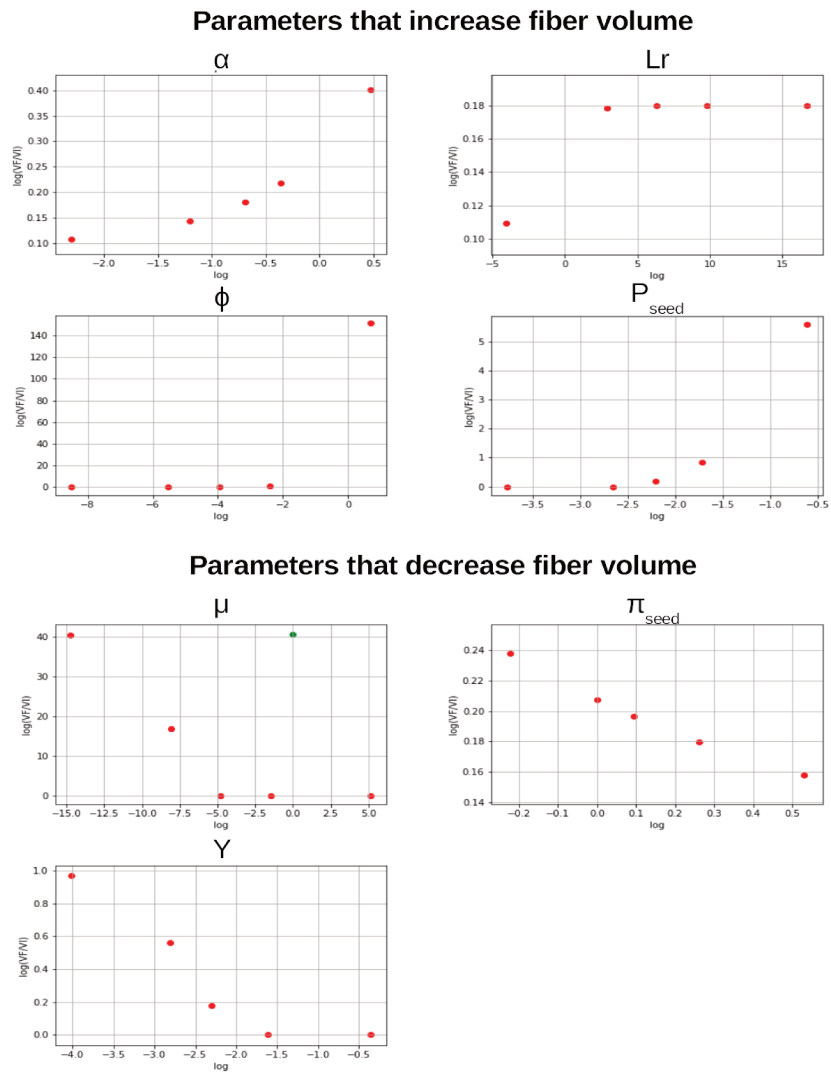


Figure 2: Effects of one parameter on the values of VF/VI when other parameters take their middle values. Panel (a): parameters that increase cell expansion; panel (b): parameters that decrease cell expansion. On top of each subplot is indicated the name of the parameter whose effect is plotted. Values in x- and y-axis are represented in logarithmic scale. For this reason, the smallest value of $\mu = 0$ (green dot) is shifted towards the right of the plot (instead of being placed at the beginning of it)

viceversa) was blocked, suggesting symplasmic isolation of guard cells (23). It is thought that this symplasmic isolation confers the necessary autonomy for guard cells to induce changes in turgor pressure that allow them to switch between the open-closed configurations. Our model recovered expected behaviors of smaller fiber volumes when increasing plasmodesmal permeability, μ (Figure 2b). These results support the idea that plasmodesmata closure allows building up turgor pressure.

Plasmodesmal permeability is regulated by the deposition of callose, a β -glucan of the cell wall. Ruan and colleagues (2004) correlated changes in plasmodesmal permeability to changes in callose levels. To this date, no mutants of the enzymes responsible for synthesizing nor degrading callose at plasmodesmata have been studied in cotton fibers. A mutant affected in sterol composition of the membrane shows an increase in callose levels at plasmodesmata sites and a decrease in plasmodesmal permeability, as suggested by observations of carboxy fluorescein diacetate (CFDA) movement (30). If we assume a linear relationship between plasmodesmata closure and turgor pressure we would expect that the mutants Zhang and colleagues study, had longer fibers. However, the authors of this study reported shorter fibers. Our work, and other theoretical studies (5), suggest that closure of plasmodesmata should increase the length of cotton fiber cells. It becomes therefore necessary to study how mutants that are known to affect callose levels and plasmodesmal permeability, such as GLUCAN SYNTHASE-LIKE 8 (GSL8), affect turgor pressure and fiber growth. Another interesting question to be experimentally addressed concerns the type of relationship between plasmodesmal permeability and turgor pressure (i.e., whether turgor pressure increases linearly with plasmodesmal closure).

3.3 Classification of types of behaviors

As expected, we obtained only constant and monotonic increasing behaviors for volume which represent 49.05% and 50.94% of the total simulations, respectively. Figure 3 shows the percentages of approximately Constant (C), Monotonic Increasing (MI), Monotonic Decreasing (MD), and Non-Monotonic (MN) behaviors for osmotic and turgor pressures. Except for one single case found for osmotic pressure, NM behavior was absent in our model which might suggest that, in order to obtain non-monotonic behaviors of osmotic and turgor pressures like those reported by Ruan and colleagues (2001), one has to add dynamical behaviors to the parameters. The values of parameters for the non monotonic case are: $\phi = 2$, $L - r = 0.018$, $Y = 0.02$, $\mu = 176$, $\alpha = 0.1$, $P_{seed} = 0.11$, $\pi_{seed} = 0.8$, $\pi_{fiber0} = 1.7$, note that, except for P_{seed} , all parameters and the initial condition of π_{fiber} take either x_{lowest} or $x_{largest}$. An example of each type of behavior obtained is shown in Figure 4.

We repeated the simulations excluding x_{lowest} and $x_{largest}$ from the intervals of all parameters. The three remainder values are closer to the biological data reported in the literature (Table1) and therefore compose the "biologically relevant interval" (BI). In these simulations we only obtained constant and monotonic increasing behaviors, whereas monotonic decreasing was lost for both pressures (Figure 3). Next, we obtained the frequency of pairs of behaviors of pressures which are shown as percentages in Table 3. The most frequent pair is when π and P behave as constant functions in both complete and biological intervals. We did not find any case where π is decreasing and P is constant nor π is monotonic decreasing and P is monotonic increasing. The simulations that used biological relevant values only

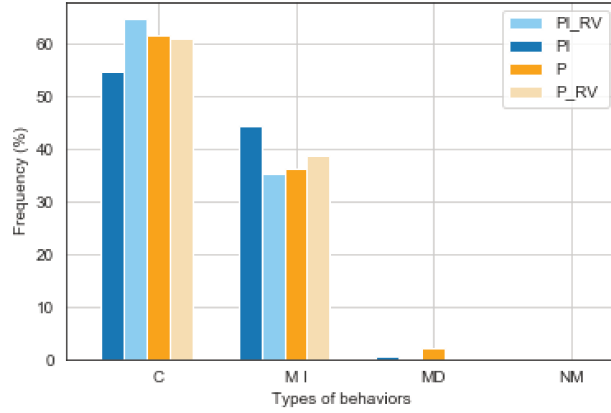


Figure 3: Classification of types of behaviors of osmotic and turgor pressures and their frequency in the total number of simulations, expressed as percentage. Dark colors are for simulations that used the complete interval of parameter values from Table 2 while light colors represent simulations that used the biological interval (BI). Constant (C), Monotonic Increasing (MI), Monotonic Decreasing (MD), and Non-Monotonic (MN) behaviors.

recovered three of these pairs of behaviors (Table 3).

Table 3: Pairs of types of behaviors of osmotic and turgor pressure

$\pi \backslash P$	Constant	Monotonic increasing	Monotonic decreasing
Constant	51.56% ; 61.11%	2.64% ; 3.63%	0.62% ; 0
Monotonic increasing	10.11% ; 0	33.55% ; 33.25%	0.81% ; 0
Monotonic decreasing	0 ; 0	0 ; 0	0.68% ; 0

Bold numbers are the percentages for simulations made using values from the BI interval

To try to understand why in some pairs P and π have different behaviors (e.g., π is constant and P is monotonic decreasing), we plotted the relative frequency per value per parameter and the distribution of the numerical values of the ratios computed in the simulations (Supplementary Material 2). These plots show that all pairs in which P behaves as monotonic decreasing have $\phi_{largest}$ as the most frequent value. The smallest value of the membrane permeability $L_{r,lowest}$ is associated to pairs in which osmotic pressure was classified as monotonic increasing. This explains why when we classified the types of behaviors using the values of the BI, only three

of the seven pairs of the complete interval are recovered. When both pressures are classified as monotonic increasing μ V0 and V2 are the most frequent values for both intervals. This result further supports the hypothesis that small permeabilities of plasmodesmata help increase the pressures of the cotton fiber cell.

When classifying the pressures using the second threshold, $Th_2 = 1.49012 \times 10^{-8}$, we obtained 0.71% and 2% of non monotonic behaviors for π_{fiber} and P_{fiber} , respectively. We used geometric means of the parameters that produce non monotonic turgor pressure to analyze the changes the pressure suffers. In Figure 5 we show the plots for V , π and P . Observe in 5b that turgor pressure first increases to a maximum value and then relaxes to a lower stable one. These changes in turgor pressure seem to be too small to be biologically relevant to the cotton fiber. We therefore estimated the difference between the maximum and the stable values of this simulation to be 3.511×10^{-5} MPa, which occurs in a time lapse of around 67.2 h (an average change of 5.2256×10^{-7} MPa per hour). As a way to compare if these changes in turgor pressure could have biological relevance, we used the data published by (8) and (24) for turgor pressure. We estimated the average change in turgor of experimental data to be around 1.2×10^{-3} MPa per hour. This value gives an average turgor change of around 0.0725 MPa for the 67.2 h time lapse. This is three orders of magnitude larger than the change observed in Figure 5. This suggests that non-monotonic behaviors found when using relative changes smaller than 1% are not biologically relevant for cotton fiber growth.

To test whether the 1% threshold is suitable to reveal biologically meaningful behaviors, we compared the relative change of monotonic decreasing turgor pressures to that of experimental data published by (8). From the total number of simulations (9968) in which turgor behaved as monotonic decreasing, the mean relative change is 1.26×10^{-6} and the maximum and minimum values are 0.0117 and 0, respectively. For experimental data we obtained a relative turgor change of 2.30. These results suggests using a 1% relative change as threshold may still be low to reflect biologically relevant behaviors. However, the fact that according to this threshold non-monotonic behaviors are absent in this system, supports the idea that using constant parameters for plasmodesmal permeability, the source of solutes, cell wall extensibility, etc, can not recover non monotonic behaviors of turgor and osmotic pressures (24). This idea leads to the hypothesis that in order to obtain non monotonic behavior that are comparable to those experimentally reported, dynamical parameters have to be added. According to what the experimental evidence suggests (24; 25; 30) and to what other computational models have found (5), we propose that adding dynamics to the plasmodesmal permeability of plasmodesmata might recover these type of behaviors.

4 Conclusions

We have put forward a basic model with constant parameters to explore the contribution of plasmodesmal permeability in the behavior of turgor and osmotic pressures during cotton fiber development. To address this question we used Lockhart's model with the addition of fluxes of water and solutes through plasmodesmata. We observe that increasing the permeability of plasmodesmal permeability negatively affects the growth of cotton fiber cells. This is in agreement to what other studies have found

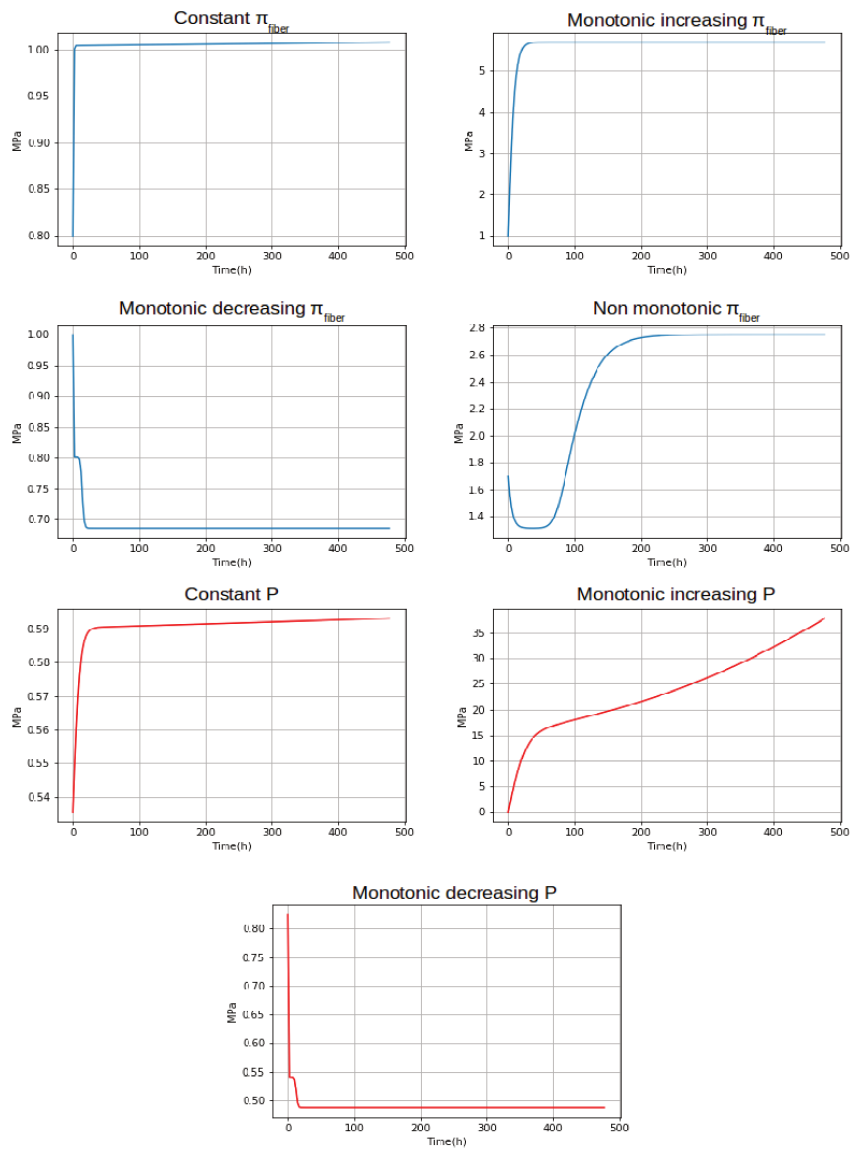


Figure 4: Curve examples of the type of behaviors of osmotic and turgor pressures obtained according to a 1% relative change threshold. The type of behavior is indicated as subtitle in each subplot.

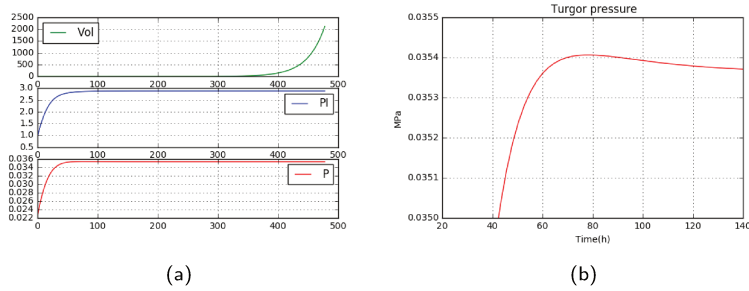


Figure 5: Example of turgor pressure classified as non-monotonic according to a 1.49012×10^{-8} threshold. Panel (a) curves for V , P_{fiber} , and π_{fiber} ; panel (b) zoom in of the region of interest of turgor pressure curve that shows an initial increase of turgor to a maximum value, followed by relaxation towards a lower one.

about the role of plasmodesmata channels on cotton fiber growth. Our model suggests that removing the dynamics of cellular processes that occur during growth of cotton fibers precludes the dynamical behaviors experimentally reported for turgor and osmotic pressures. Our results agree with the available experimental information and suggest that adding dynamical changes to plasmodesmal permeability might recover non monotonic behaviors of turgor pressure.

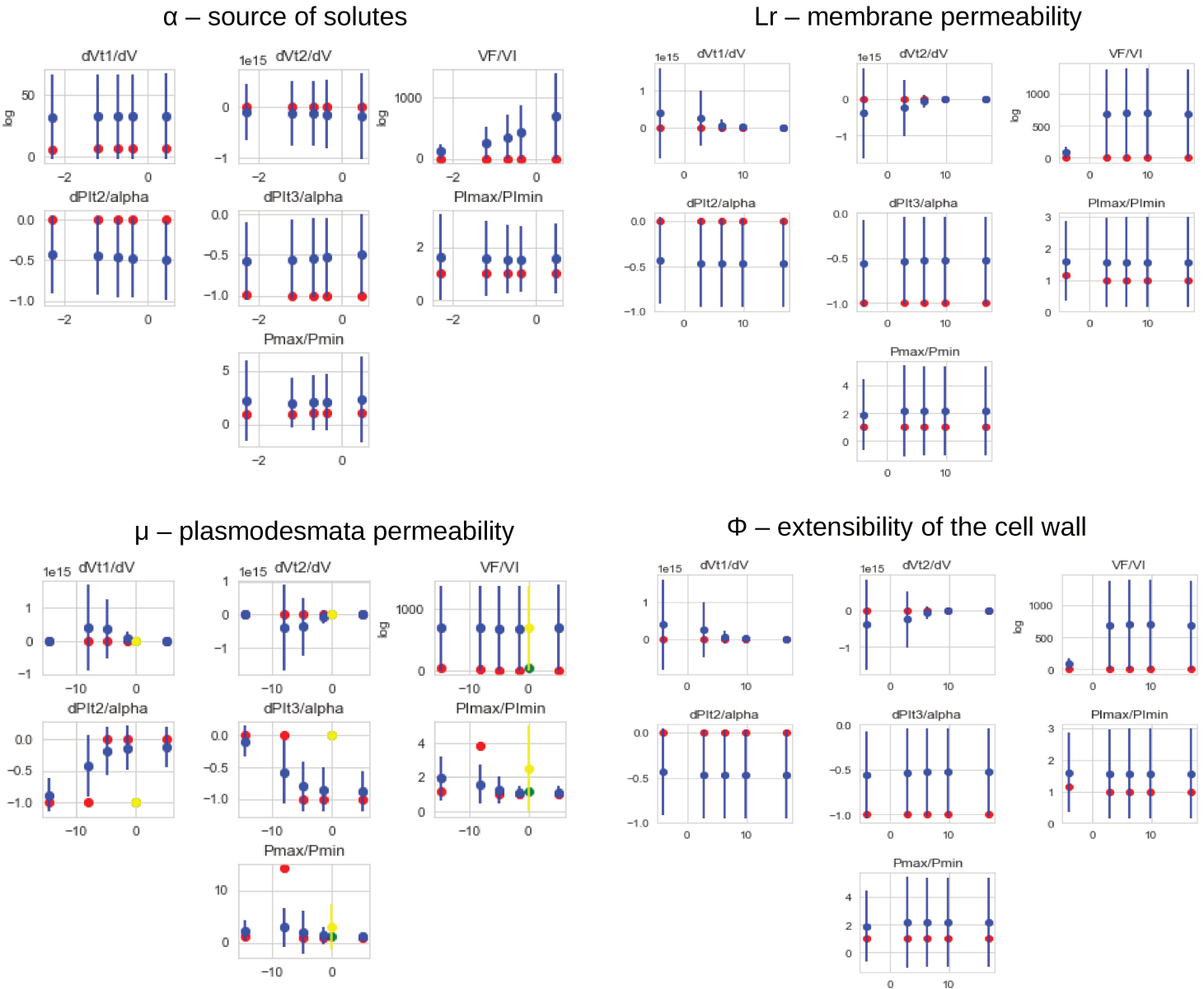
References

- [1] Sharifah Barlian Aidid and Hishashi Okamoto. Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *impatiens balsamina* to lead, cadmium and zinc. *Biometals*, 6(4):245–249, Dec 1993.
- [2] Wendy L. Applequist, Richard Cronn, and Jonathan F. Wendel. Comparative development of fiber in wild and cultivated cotton. *Evolution & Development*, 3(1):3–17, 2001.
- [3] Utku Avci, Sivakumar Pattathil, Bir Singh, Virginia L. Brown, Michael G. Hahn, and Candace H. Haigler. Cotton fiber cell walls of *gossypium hirsutum* and *gossypium barbadense* have differences related to loosely-bound xyloglucan. *PLOS ONE*, 8(2):1–13, 02 2013.
- [4] Amarjit S. Basra and C.P. Malik. Development of the cotton fiber. 89:65 – 113, 1984.
- [5] Ibrahim Cheddadi, Michel Génard, Nadia Bertin, and Christophe Godin. Coupling water fluxes with cell wall mechanics in a multicellular model of plant development. *PLOS Computational Biology*, 15(6):1–16, 06 2019.
- [6] Daniel J. Cosgrove. Cell wall yield properties of growing tissue. *Plant Physiology*, 78(2):347–356, 1985.
- [7] Valentin Couvreur, Marc Faget, Guillaume Lobet, Mathieu Javaux, François Chaumont, and Xavier Draye. Going with the flow: Multiscale insights into the composite nature of water transport in roots. *Plant Physiology*, 178(4):1689–1703, 2018.
- [8] Rajinder S. Dhindsa, Charles A. Beasley, and Irwin P. Ting. Osmoregulation in cotton fiber. *Plant Physiology*, 56(3):394–398, 1975.
- [9] K. Ehlers and Kollman R. Primary and secondary plasmodesmata: structure, origin, and functioning. *Protoplasma*, 216:1, 2001.
- [10] E. P. ELEFThERIOU and J. L. HALL. The Extrafloral Nectaries of Cotton: I. FINE STRUCTURE OF THE SECRETORY PAPILLAE. *Journal of Experimental Botany*, 34(2):103–119, 02 1983.
- [11] Christine Faulkner, Ozgur E. Akman, Karen Bell, Chris Jeffree, and Karl Oparka. Peeking into pit fields: A multiple twinning model of secondary plasmodesmata formation in tobacco. *The Plant Cell*, 20(6):1504–1518, 2008.
- [12] Kylie J. Foster and Stanley J. Miklavcic. A comprehensive biophysical model of ion and water transport in plant roots. i. clarifying the roles of endodermal barriers in the salt stress response. *Frontiers in Plant Science*, 8:1326, 2017.
- [13] Anja Geitmann and Joseph K E Ortega. Mechanics and modeling of plant cell growth. *Trends in Plant Science*, 14(9):467–478, 2009.
- [14] Candace Haigler, Lisette Betancur, Michael Stiff, and John Tuttle. Cotton fiber: a powerful single-cell model for cell wall and cellulose research. *Frontiers in Plant Science*, 3:104, 2012.

- [15] Kaare Jensen, Daniel Mullendore, Noel Michele Holbrook, Tomas Bohr, Michael Knoblauch, and Henrik Bruus. Modeling the hydrodynamics of phloem sieve plates. *Frontiers in Plant Science*, 3:151, 2012.
- [16] Kaare H. Jensen and Maciej A. Zwieniecki. Physical limits to leaf size in tall trees. *Phys. Rev. Lett.*, 110:018104, Jan 2013.
- [17] Hee Jin Kim and Barbara A. Triplett. Cotton fiber growth in planta and in vitro. models for plant cell elongation and cell wall biogenesis. *Plant Physiology*, 127(4):1361–1366, 2001.
- [18] Yangmin X. Kim and Ernst Steudle. Light and turgor affect the water permeability (aquaporins) of parenchyma cells in the midrib of leaves of *Zea mays*. *Journal of Experimental Botany*, 58(15-16):4119–4129, 12 2007.
- [19] Deng-Di Li, Xiang-Mei Ruan, Jie Zhang, Ya-Jie Wu, Xiu-Lan Wang, and Xue-Bao Li. Cotton plasma membrane intrinsic protein 2s (pip2s) selectively interact to regulate their water channel activities and are required for fibre development. *New Phytologist*, 199(3):695–707, 2013.
- [20] James A. Lockhart. An analysis of irreversible plant cell elongation. *Journal of Theoretical Biology*, 8(2):264 – 275, 1965.
- [21] Christophe Maurel. Aquaporins and water permeability of plant membranes. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48(1):399–429, 1997. PMID: 15012269.
- [22] Maureen C. Meinert and Deborah P. Delmer. Changes in biochemical composition of the cell wall of the cotton fiber during development. *Plant Physiology*, 59(6):1088–1097, 1977.
- [23] B.A. Palevitz and P.K Hepler. Changes in dye coupling of stomatal cells of allium and commelina demonstrated by microinjection of lucifer yellow. *Planta*, (164):473, 1985.
- [24] Yong-Ling Ruan, Danny J. Llewellyn, and Robert T. Furbank. The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and k⁺ transporters and expansin. *The Plant Cell*, 13(1):47–60, 2001.
- [25] Yong-ling Ruan, Shou-min Xu, Rosemary White, and Robert T Furbank. Genotypic and developmental evidence for the role of plasmodesmatal regulation in cotton fiber elongation mediated by callose turnover. *Plant physiology*, 136(4):4104–4113, 2004.
- [26] Peter Schopfer. Biomechanics of plant growth. *American Journal of Botany*, 93(10):1415–1425, 2006.
- [27] A. Schulz. Plasmodesmal widening accompanies the short-term increase in symplasmic phloem unloading in pea root tips under osmotic stress. *Protoplasma*, 188(1):22–37, 1995.
- [28] L. Taiz and E. Zeiger. *Plant Physiology*. Sunderland: SINAUER ASSOCIATES INC., U.S., 2nd edition, 2002.

- [29] Lu Wang, Xiao-Rong Li, Heng Lian, Di-An Ni, Yu-ke He, Xiao-Ya Chen, and Yong-Ling Ruan. Evidence that high activity of vacuolar invertase is required for cotton fiber and arabidopsis root elongation through osmotic dependent and independent pathways, respectively. *Plant Physiology*, 154(2):744–756, 2010.
- [30] Zhiyuan Zhang, Yong-Ling Ruan, Na Zhou, Fang Wang, Xueying Guan, Lei Fang, Xiaoguang Shang, Wangzhen Guo, Shuijin Zhu, and Tianzhen Zhang. Suppressing a putative sterol carrier gene reduces plasmodesmal permeability and activates sucrose transporter genes during cotton fiber elongation. *The Plant Cell*, 2017.

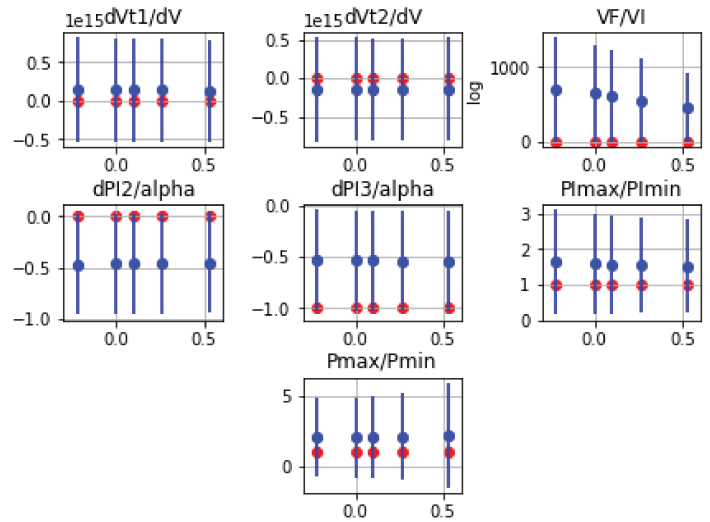
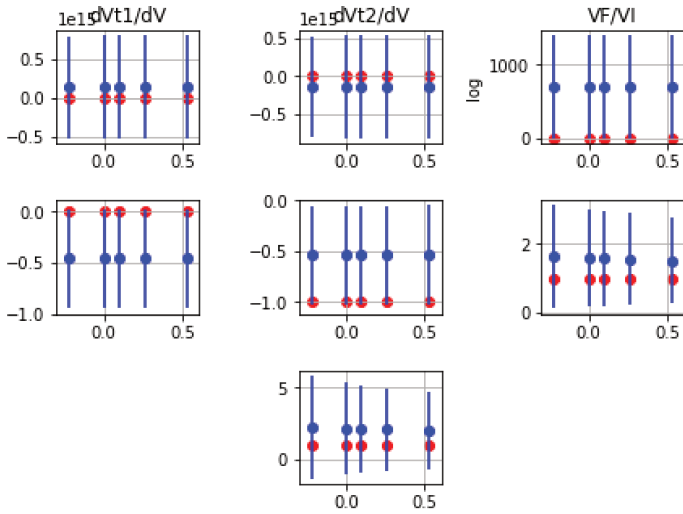
SENSITIVITY ANALYSIS AROUND MIDDLE VALUES Cases (a) and (b)



Sensitivity analysis around middle values a) effect of one parameter on ratios when other parameters take their middle values (red dot); b) mean (blue dot) and SD (blue line) of the effect of one parameter when all other parameters span their ranges. When ratios are represented in logarithmic scale it is indicated with a 'log' label on the y axis. Green and yellow dot/line μ plots correspond to the values of a) and b), respectively, when $\mu=0$. The values for all parameters on x-axis are expressed in logarithmic scale, which is why the values of $\mu=0$ are shifted towards the right (instead of being located first)

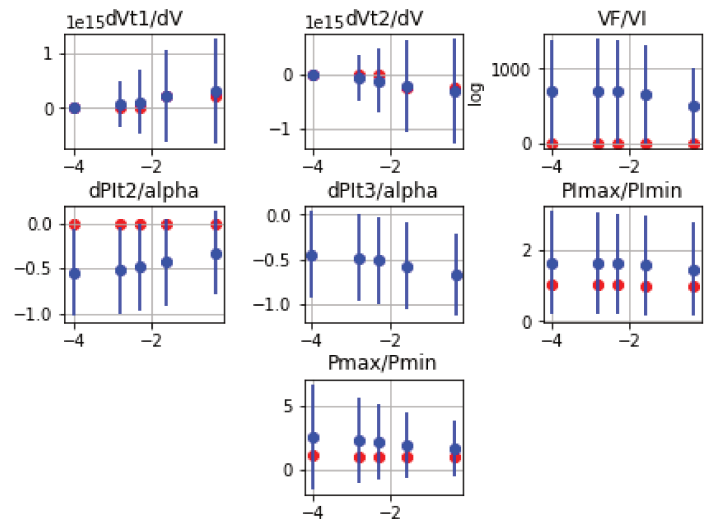
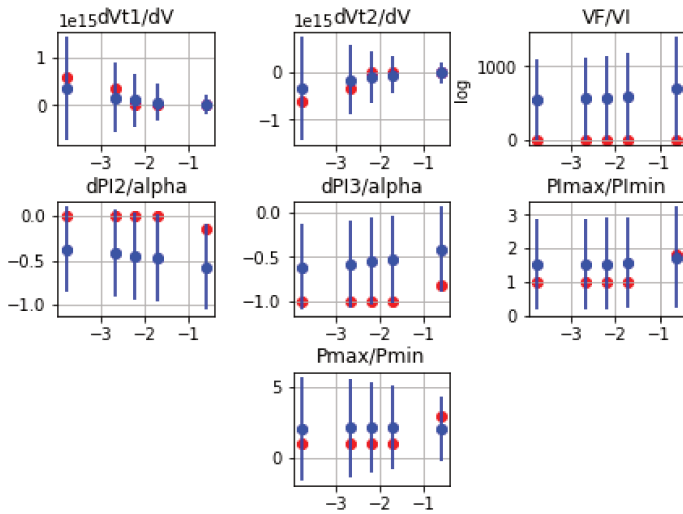
π fiber (initial) – osmotic pressure of the fiber

π seed – osmotic pressure of the seed



P seed – turgor pressure of the seed

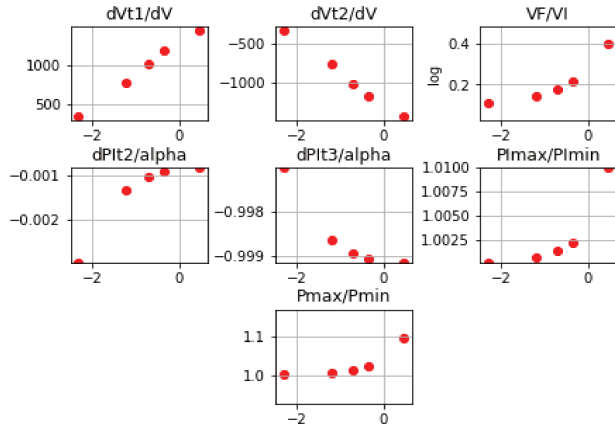
Y – yield turgor pressure



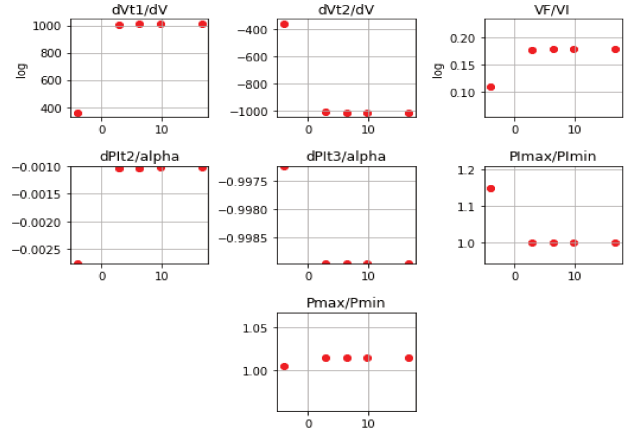
Sensitivity analysis around middle values a) effect of one parameter on ratios when other parameters take their middle values (red dot); b) mean (blue dot) and SD (blue line) of the effect of one parameter when all other parameters span their ranges. When ratios are represented in logarithmic scale it is indicated with a 'log' label on the y axis. The values for all parameters on x-axis are expressed in logarithmic scale

SENSITIVITY ANALYSIS AROUND MIDDLE VALUES Case (a)

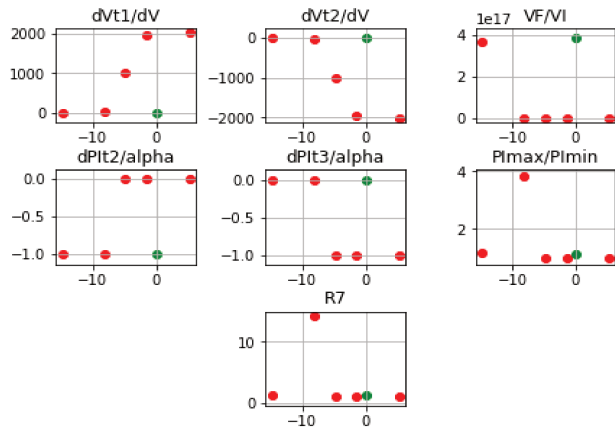
α – source of solutes



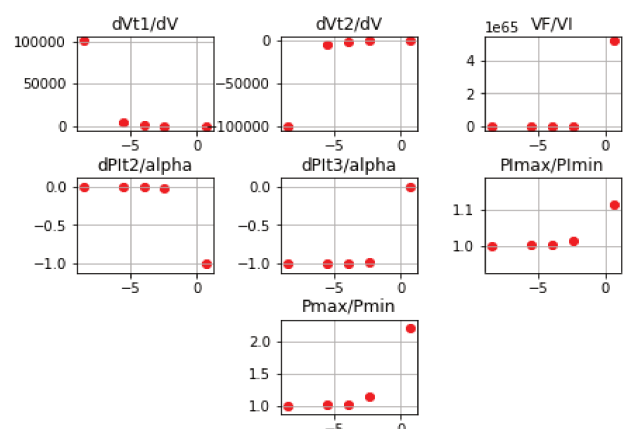
L_r – membrane permeability



μ – plasmodesmata permeability

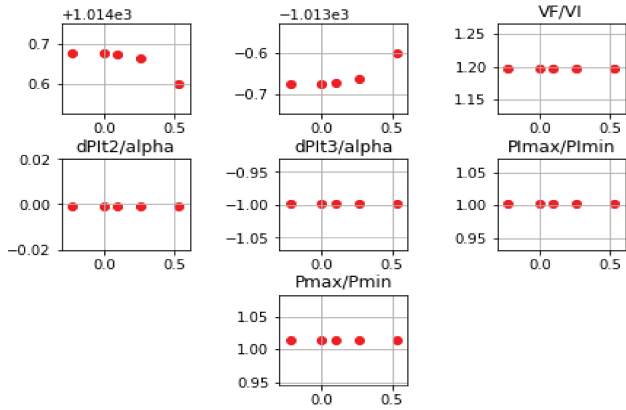


Φ – extensibility of the cell wall

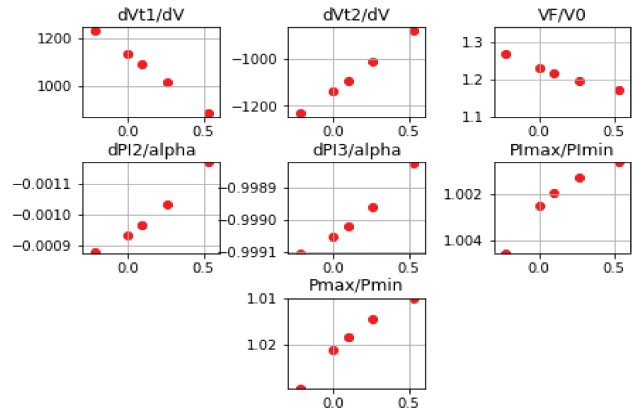


Effect of one parameter on ratios when other parameters take their middle values (red dot). When ratios are represented in logarithmic scale it is indicated with a 'log' label on the y axis. Green dots in μ plots represent the case when $\mu=0$. The values for all parameters on x-axis are expressed in logarithmic scale, which is why the values of $\mu=0$ are shifted towards the right (instead of being located first)

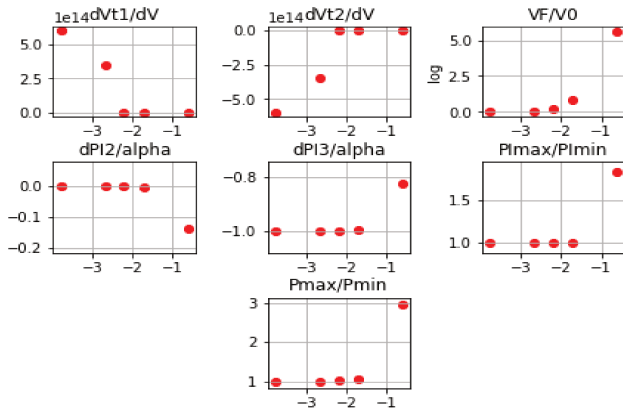
π fiber (initial) – osmotic pressure of the fiber



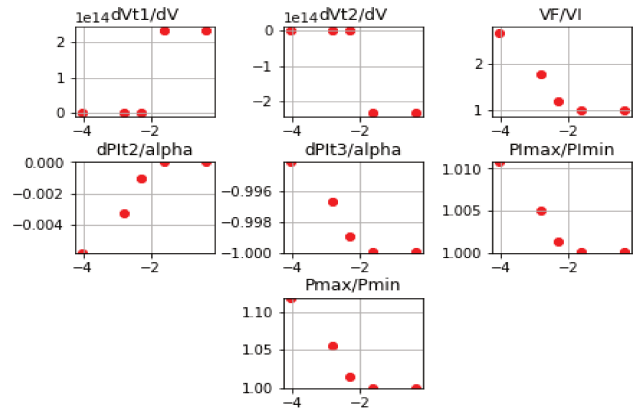
π seed – osmotic pressure of the seed cell



P seed – turgor pressure of the seed



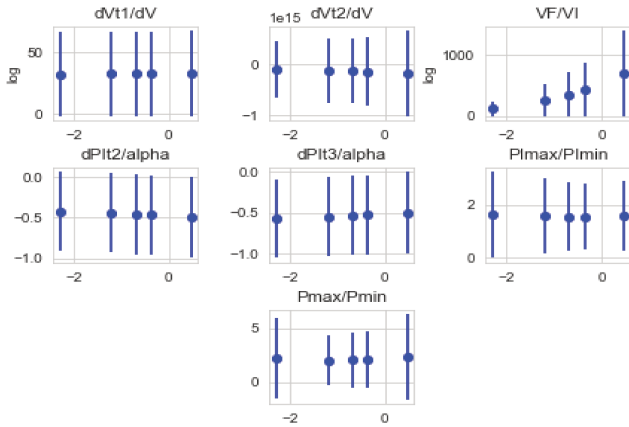
Y – yield turgor pressure



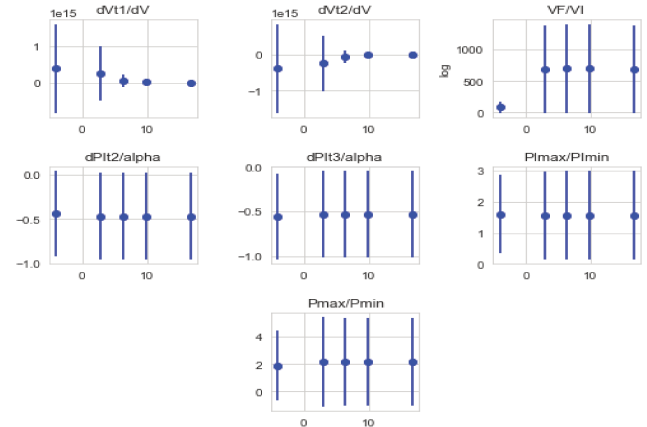
Effect of one parameter on ratios when other parameters take their middle values (red dot). When ratios are represented in logarithmic scale it is indicated with a 'log' label on the y axis. The values for all parameters on x-axis are expressed in logarithmic scale.

SENSITIVITY ANALYSIS AROUND MIDDLE VALUES Case (b)

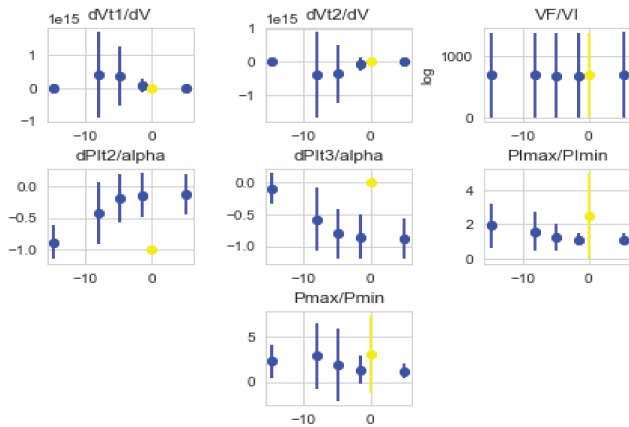
α – source of solutes



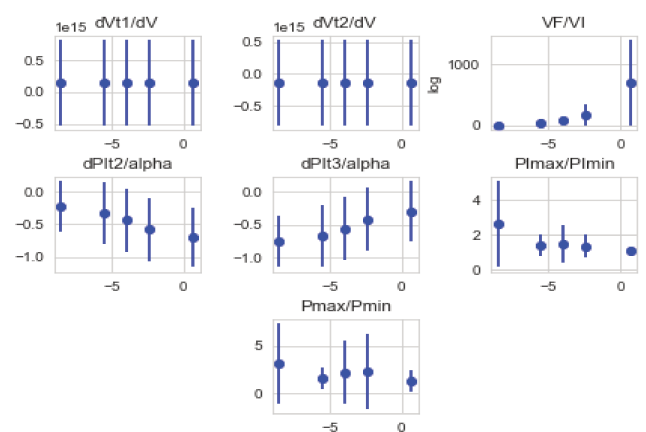
L_r – membrane permeability



μ – plasmodesmata permeability

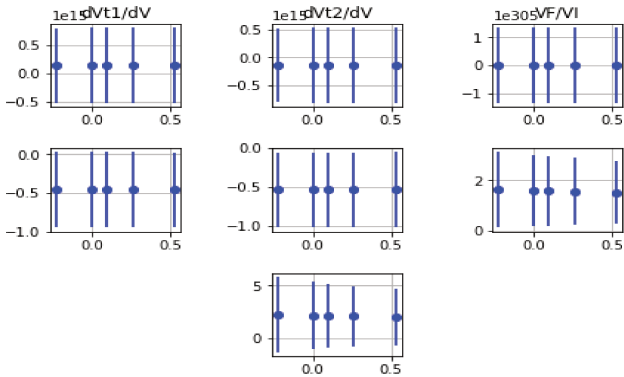


Φ – extensibility of the cell wall

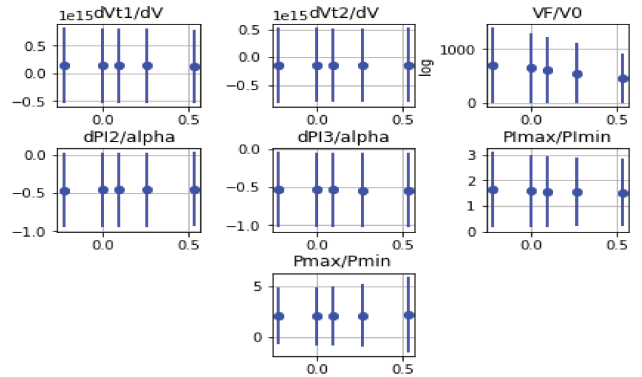


Mean (blue dot) and SD (blue line) of the effect of one parameter when all other parameters span their ranges. When ratios are represented in logarithmic scale it is indicated with a 'log' label on the y axis. Yellow dot/line in μ plots represent the case when $\mu=0$. The values for all parameters on x-axis are expressed in logarithmic scale, which is why the values of $\mu=0$ are shifted towards the right (instead of being located first).

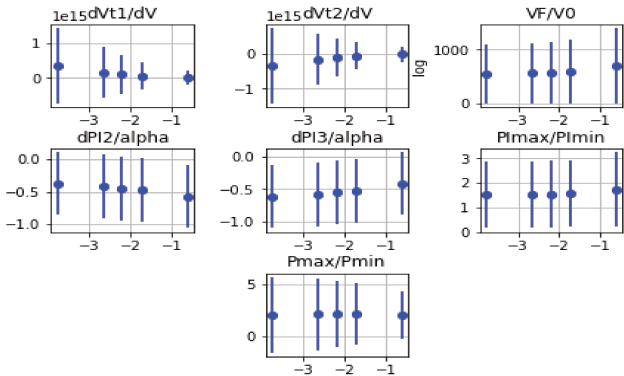
π fiber (initial) – osmotic pressure of the fiber



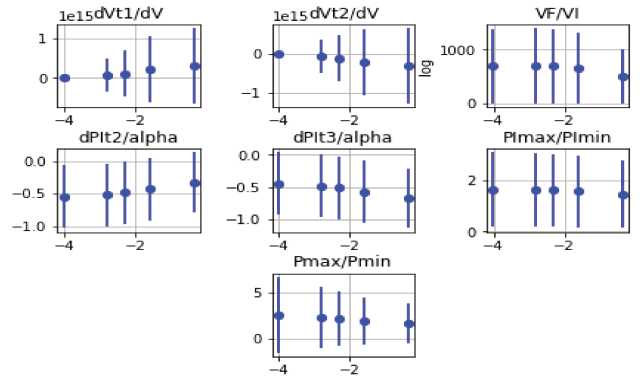
π seed – osmotic pressure of the seed cell



P seed – turgor pressure of the seed

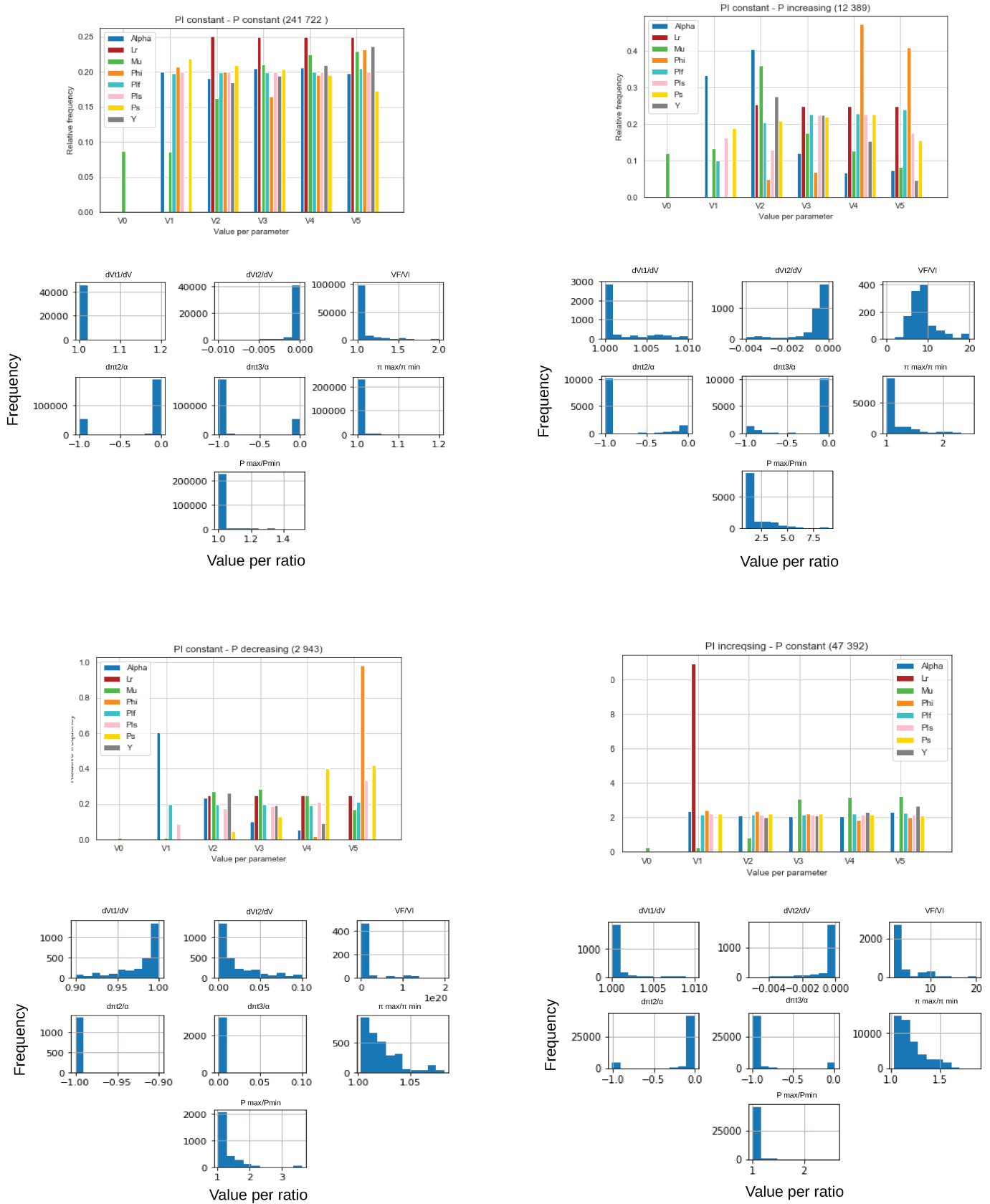


Y – yield turgor pressure



Mean (blue dot) and SD (blue line) of the effect of one parameter when all other parameters span their ranges. When ratios are represented in logarithmic scale it is indicated with a 'log' label on the y axis. The values for all parameters on x-axis are expressed in logarithmic scale

FREQUENCY OF PARAMETER VALUES AND OF RATIO VALUES FOR PAIRS OF BEHAVIORS -COMPLETE INTERVAL



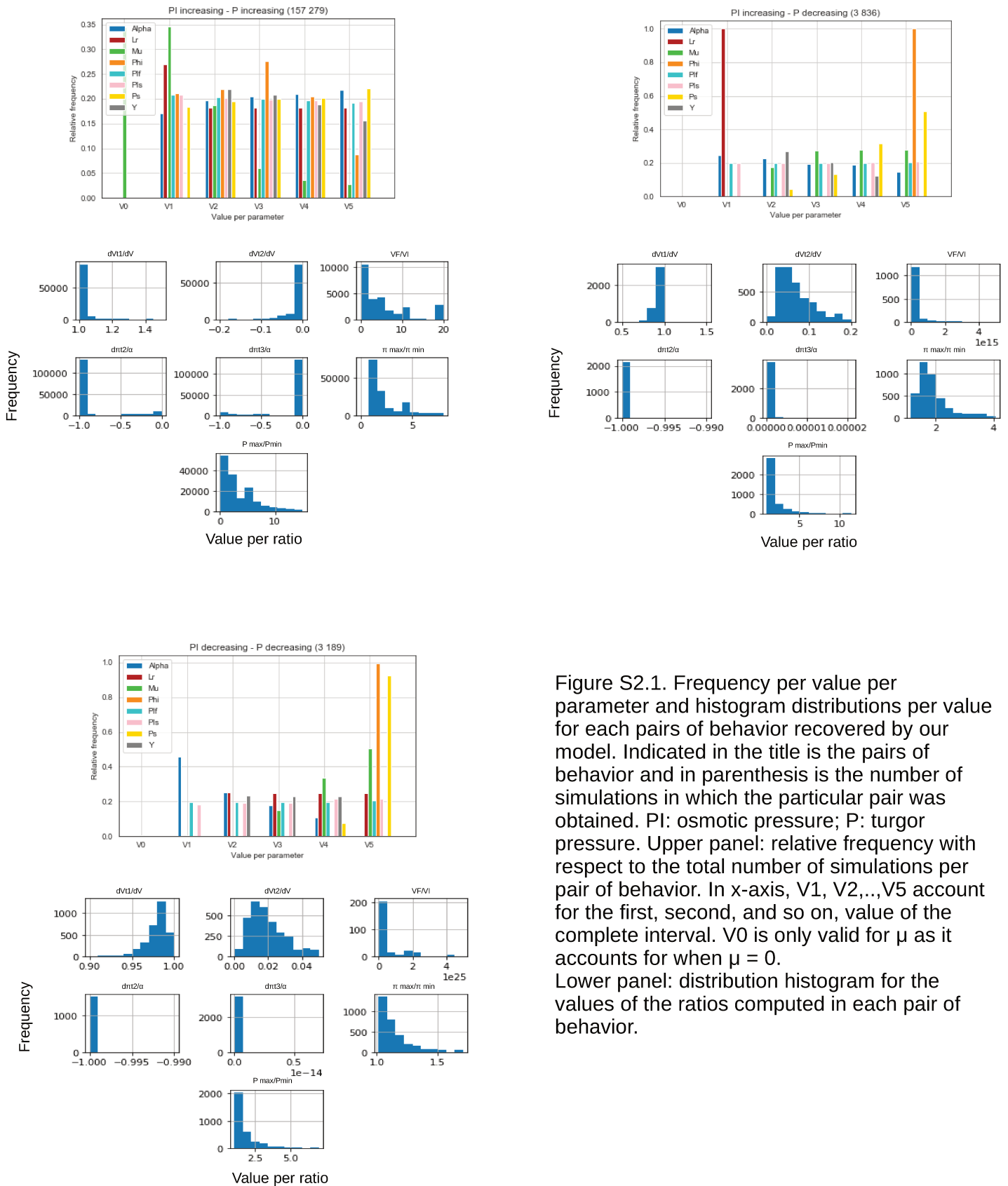


Figure S2.1. Frequency per value per parameter and histogram distributions per value for each pairs of behavior recovered by our model. Indicated in the title is the pairs of behavior and in parenthesis is the number of simulations in which the particular pair was obtained. PI: osmotic pressure; P: turgor pressure. Upper panel: relative frequency with respect to the total number of simulations per pair of behavior. In x-axis, V1, V2,...,V5 account for the first, second, and so on, value of the complete interval. V0 is only valid for μ as it accounts for when $\mu = 0$. Lower panel: distribution histogram for the values of the ratios computed in each pair of behavior.

FREQUENCY OF PARAMETER VALUES AND OF RATIO VALUES FOR PAIRS OF BEHAVIORS – BIOLOGICAL INTERVAL

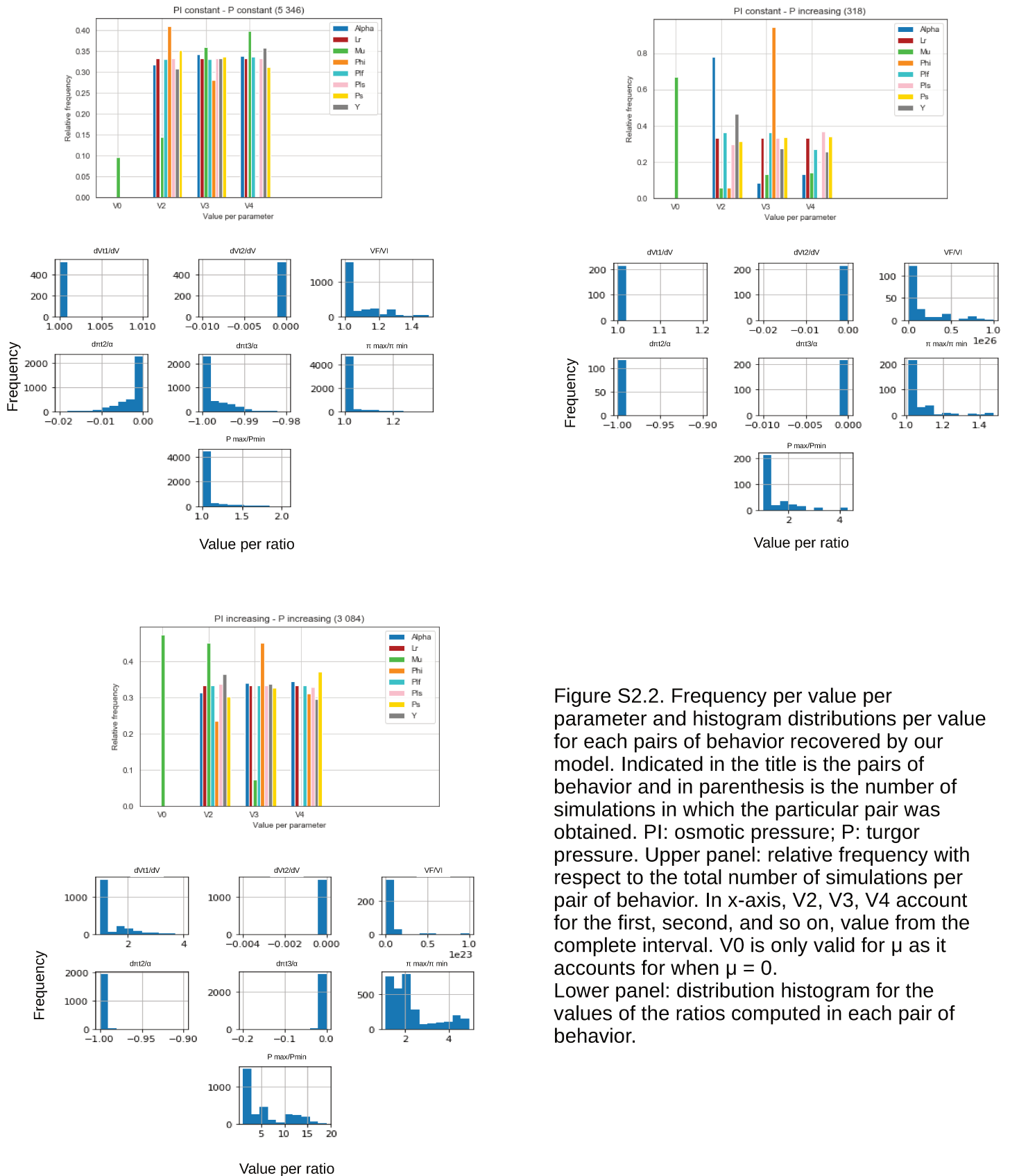


Figure S2.2. Frequency per value per parameter and histogram distributions per value for each pairs of behavior recovered by our model. Indicated in the title is the pairs of behavior and in parenthesis is the number of simulations in which the particular pair was obtained. PI: osmotic pressure; P: turgor pressure. Upper panel: relative frequency with respect to the total number of simulations per pair of behavior. In x-axis, V2, V3, V4 account for the first, second, and so on, value from the complete interval. V0 is only valid for μ as it accounts for when $\mu = 0$. Lower panel: distribution histogram for the values of the ratios computed in each pair of behavior.

4

Discussion

In this work we have put forward the hypothesis that plasmodesmal permeability and turgor pressure affect each other during plant development. We reviewed the experimental evidence that supports this hypothesis, built a network of interactions between different molecular and cellular factors that may link turgor and plasmodesmata, and proposed experimental predictions to be tested.

Using Lockhart's model for irreversible cell expansion of the plant cell with addition of solute and water fluxes through plasmodesmata, we studied one direction of these interactions: the role of plasmodesmal permeability on building up turgor pressure. We used growth of cotton fiber cells as a study system because it is a single cell with no division that mostly increases in length. Our results suggest that closure of plasmodesmata may contribute to building turgor pressure and that dynamical changes of their permeability are necessary to recover non monotonic behaviors that have been reported experimentally.

4.1 Regulation of plasmodesmal permeability might have been critical for the evolution of complex multicellular plants

Because plant cells are encased within the cell wall, common developmental processes in animals, like tissue rearrangement and cell migration, are unavailable to plants. For this reason, establishment of plant cell patterns mostly relies on movement of signaling molecules. In the context of the dynamical patterning modules (DPMs) framework [32, 47], we proposed the hypothesis that symplasmic transport through plasmodesmata might have enabled the evolution of vascular tissues by allowing movement of molecules that specify cell types between cells (see Appendix). We argue that plasmodesmal-dependent movement of molecules mobilizes biogeneric properties - i.e., passive diffusion, lateral inhibition, and reaction-diffusion - composing the spatially dependent differentiation (DIF) DPM of plants. The establishment of cell types with different functions drove the evolution of vascular tissues for the long-distance transport of water, hormones, and photoassimilates that could then enable the evolution of complex multicellular forms.

4.2 Turgor may affect plasmodesmal permeability

In our review we collected experimental information suggesting that turgor pressure may affect plasmodesmal permeability through the regulation of callose levels at the cell wall. However, it was recently proposed a different mechanism based on geometrical changes of the plasmodesmal channels induced by pressure gradients between neighboring cells. According to the model, a turgor pressure difference displaces the endoplasmic reticulum towards the cell wall which is resisted by the tethers that connect the DT to the plasma membrane. As a consequence of this, the geometry of the plasmodesmal channel is modified thus affecting permeability. The authors were able to reproduce reported changes in plasmodesmal permeability [35, 39] as a function of pressure gradients. This alternative mechanism may account for rapid changes in plasmodesmal permeability that have been observed under osmotic shock treatments [41], or when injected with Ca^+ [19] and which can not be explained by callose turnover. A caveat of this mechanism is that geometrical modifications of the channel may only apply to plasmodesmata that are formed during subsequent development as newly formed plasmodesmata seem to be depleted of spoke-like tethers [33].

4.3 Plasmodesmal permeability may affect turgor-driven growth of cotton fiber cells

In recent years, the regulation of plasmodesmal permeability has gained attention mainly due to its role in the movement of signaling molecules like transcription factors, hormones and in the spread of viruses. However, the permeability of plasmodesmata channels has also been shown to be important for the movement of small molecules like sucrose [41]. Non-targeted movement of sucrose is non-specific and has no energy costs, advantages which are utilized by plants to load/unload sugars to and from the

phloem. Inside the phloem the concentration of sugars help generate osmotic pressure gradients that create a bulk flow along the plant. We are starting to uncover the role of plasmodesmata in osmotically driven phloem transport [6, 10, 49], however the exploration of how these advantages may be utilize by plants to create pressure differentials at the cellular scale and how these might affect cell growth is still scarce [4].

Our model integrates the contributions of factors that have been experimentally observed in cotton fiber growth, like the hydraulic permeability of plasma membrane, a source of solutes, and plasmodesmal permeability. The results suggest that plasmodesmal permeability is one of the key factors that contributes to regulating turgor pressure. In particular, we found that increasing the permeability of plasmodesmata negatively impacts turgor pressure which in turn causes less growth of cotton fiber cells. These predictions are consistent with what experimental and theoretical studies have reported so far [4, 39, 40]

4.4 Removing the dynamics of plasmodesmal permeability may preclude non monotonic behavior of turgor pressure

Based on experimental observations, Ruan and coworkers [39, 40] proposed that transient closure of plasmodesmata is necessary to increase turgor pressure to a maximum after which the pressure decreases, coinciding with plasmodesmata re-opening. We removed all dynamical behaviors of parameters in our model as a first approximation and the results that stem from it suggest that the type of non monotonic turgor behavior described by Ruan and colleagues can not be recovered when all parameters are constant. We do not discard that adding dynamics to other parameters, for example the source of solutes, might retrieve experimental results. But the fact that other studies have found that modifications to plasmodesmal permeability affect the patterns of turgor pressure and growth of cotton fibers suggests that adding a dynamical behavior to plasmodesmal permeability to this model, might be sufficient to recover expected behaviors of turgor pressure.

More experimental studies on the role of plasmodesmal permeability on turgor pressure and cellular growth are needed to establish whether generalizations about the effects of plasmodesmal permability on turgor behavior and cell growth can be made. In their study, Ruan and coworkers (2001, 2004) reported a transitional closure of plasmodesmata channels during cotton fiber growth. However, in a more recent work performed on the same species (*G. hirsutum*) but different cultivar (accession W0) the authors observed that plasmodesmata of WT fibers were open at 5- and 10-DAA and closed at 15-, 20- and 25-DAA, without plasmodesmata re-opening. This difference in the behavior of plasmodesmal permeability in cotton fibers demands experimental studies to understand its cause. For example, a question to be addressed is whether these two different behaviors are proper of the cultivars of each study or they are caused by the different growth conditions used - greenhouse with partial control of

temperature [39] vs field [53].

According to the explanatory model proposed by Ruan and colleagues and our simulations, we would expect a decreased plasmodesmal permeability to result in longer cotton fibers, however this was not the case in the study of Zhang and colleagues (2017). A mutant affected in sterol composition of the membrane that reduces the expression of the PD-targeting β -1,3-glucanase (GhPdBG3-2A/D), had more callose accumulated at plasmodesmata and shorter fiber cells [53]. No turgor measurements were performed in these mutants. It would be interesting to corroborate these results using mutants of enzymes that have been shown to affect symplasmic movement of molecules. Mutants of Glucan Synthase-Like 8 (*GSL8*) and of plasmodesmata localized β -glucanase 1 *PDGB1*, are good candidates as they have been demonstrated in *Arabidopsis* to change plasmodesmal permeability as well as movement of fluorescent molecules [2, 15]. Measurements of turgor pressure and fiber length in overexpression and/or knockout mutations of these genes in the cotton fiber system will help clarify how plasmodesmal permeability affects turgor pressure. If the predictions of our model are correct, larger turgor pressures and fiber lengths should be observed when callose levels are higher and plasmodesmata are closed.

4.5 General Conclusion

This work integrated the available experimental observations that suggest that plasmodesmal permeability and turgor pressure feedback each other during plant development. We studied the effects of plasmodesmal permeability in the behavior of turgor pressure and growth of cotton fiber cells with the help of a computational model. Our results seem to be consistent with other studies about the effects of changes in plasmodesmal permeability on turgor pressure and allowed us to put forward testable hypothesis. However, more studies are needed to understand in more detail how these two factors affect each other during plant development.

Bibliography

- [1] BASRA, A. S., AND MALIK, C. Development of the cotton fiber. 65 – 113.
- [2] BENITEZ-ALFONSO, Y., FAULKNER, C., PENDLE, A., MIYASHIMA, S., HELARIUTTA, Y., AND MAULE, A. Symplastic intercellular connectivity regulates lateral root patterning. *Developmental Cell* 26, 2 (2016/04/19 XXXX), 136–147.
- [3] BURCH-SMITH, T. M., AND ZAMBRYSKI, P. C. Plasmodesmata paradigm shift: Regulation from without versus within. *Annual Review of Plant Biology* 63, 1 (2012), 239–260. PMID: 22136566.
- [4] CHEDDADI, I., GÉNARD, M., BERTIN, N., AND GODIN, C. Coupling water fluxes with cell wall mechanics in a multicellular model of plant development. *PLOS Computational Biology* 15, 6 (06 2019), 1–16.
- [5] CHEN, X.-Y., AND KIM, J.-Y. Callose synthesis in higher plants. *Plant Signaling & Behavior* 4, 6 (2009), 489–492. PMID: 19816126.
- [6] COMTET, J., TURGEON, R., AND STROOCK, A. D. Phloem loading through plasmodesmata: A biophysical analysis. *Plant Physiology* 175, 2 (2017), 904–915.
- [7] COSGROVE, D. J. Expansive growth of plant cell walls. *Plant Physiology and Biochemistry* 38, 1 (2000), 109 – 124.
- [8] COSGROVE, D. J. Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology*, 6 (2005), 850–861.
- [9] COSGROVE, D. J. Plant cell wall extensibility: connecting plant cell growth with cell wall structure, mechanics, and the action of wall-modifying enzymes. *Journal of Experimental Botany* 67, 2 (11 2015), 463–476.
- [10] COUVREUR, V., FAGET, M., LOBET, G., JAVAUX, M., CHAUMONT, F., AND DRAYE, X. Going with the flow: Multiscale insights into the composite nature of water transport in roots. *Plant Physiology* 178, 4 (2018), 1689–1703.

- [11] DHINDSA, R. S., BEASLEY, C. A., AND TING, I. P. Osmoregulation in cotton fiber. *Plant Physiology* 56, 3 (1975), 394–398.
- [12] FLEMING, A. J., MCQUEEN-MASON, S., MANDEL, T., AND KUHLEMEIER, C. Induction of leaf primordia by the cell wall protein expansin. *Science* 276, 5317 (1997), 1415–1418.
- [13] GEITMANN, A., AND ORTEGA, J. K. E. Mechanics and modeling of plant cell growth. *Trends in Plant Science* 14, 9 (2009), 467–478.
- [14] GONZÁLEZ, M.-C., ROITSCH, T., AND CEJUDO, F. J. Circadian and developmental regulation of vacuolar invertase expression in petioles of sugar beet plants. *Planta* 222, 2 (Oct 2005), 386–395.
- [15] GUSEMAN, J. M., LEE, J. S., BOGENSCHUTZ, N. L., PETERSON, K. M., VIRATA, R. E., XIE, B., KANAOKA, M. M., HONG, Z., AND TORII, K. U. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in arabidopsis chorus (glucan synthase-like 8). *Development* 137, 10 (2010), 1731–1741.
- [16] HAIGLER, C., BETANCUR, L., STIFF, M., AND TUTTLE, J. Cotton fiber: a powerful single-cell model for cell wall and cellulose research. *Frontiers in Plant Science* 3 (2012), 104.
- [17] HAYASHI, T. Xyloglucans in the primary cell wall. *Annual Review of Plant Physiology and Plant Molecular Biology* 40 (1989), 139–168.
- [18] HENZLER, T., AND STEUDLE, E. Reversible closing of water channels in Chara internodes provides evidence for a composite transport model of the plasma membrane. *Journal of Experimental Botany* 46, 2 (02 1995), 199–209.
- [19] HOLDAWAY-CLARKE, T. L., WALKER, N. A., AND OVERALL, R. L. Measurement of the electrical resistance of plasmodesmata and membranes of corn suspension-culture cells. *Planta* 199, 4 (Aug 1996), 537–544.
- [20] HOVAV, R., UDALL, J. A., HOVAV, E., RAPP, R., FLAGEL, L., AND WENDEL, J. F. A majority of cotton genes are expressed in single-celled fiber. *Planta* 227, 2 (Jan 2008), 319–329.
- [21] IERSEL, M. W. V., AND OOSTERHUIS, D. M. Drought effects on the water relations of cotton fruits, bracts, and leaves during ontogeny. *Environmental and Experimental Botany* 36, 1 (1996), 51 – 59.
- [22] JOHANSON, U., KARLSSON, M., JOHANSSON, I., GUSTAVSSON, S., SJÖVALL, S., FRAYSSE, L., WEIG, A. R., AND KJELLBOM, P. The complete set of genes encoding major intrinsic proteins in arabidopsis provides a framework for a new nomenclature for major intrinsic proteins in plants. *Plant Physiology* 126, 4 (2001), 1358–1369.
- [23] KIM, H. J., AND TRIPLETT, B. A. Cotton fiber growth in planta and in vitro. models for plant cell elongation and cell wall biogenesis. *Plant Physiology* 127, 4 (2001), 1361–1366.

- [24] LI, D.-D., RUAN, X.-M., ZHANG, J., WU, Y.-J., WANG, X.-L., AND LI, X.-B. Cotton plasma membrane intrinsic protein 2s (pip2s) selectively interact to regulate their water channel activities and are required for fibre development. *New Phytologist* 199, 3 (2013), 695–707.
- [25] LIU, D., TU, L., WANG, L., LI, Y., ZHU, L., AND ZHANG, X. Characterization and expression of plasma and tonoplast membrane aquaporins in elongating cotton fibers. *Plant Cell Reports* 27, 8 (aug 2008), 1385–1394.
- [26] MAKOTO, Y., ANASTASIA S., D., SAMUEL A., B., EILEEN L., M., JOSEPH A., T., AND DANIEL B., S. Patterning mechanisms of cytoskeletal and cell wall systems during leaf trichome morphogenesis. *Nature Plants* 1 (2015), 15014.
- [27] MAUREL, C. Aquaporins and water permeability of plant membranes. *Annual Review of Plant Physiology and Plant Molecular Biology* 48, 1 (1997), 399–429. PMID: 15012269.
- [28] MAUREL, C., TACNET, F., GÜCLÜ, J., GUERN, J., AND RIPOCHE, P. Purified vesicles of tobacco cell vacuolar and plasma membranes exhibit dramatically different water permeability and water channel activity. *Proceedings of the National Academy of Sciences* 94, 13 (1997), 7103–7108.
- [29] MCQUEEN-MASON, S., AND COSGROVE, D. J. Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. *Proceedings of the National Academy of Sciences* 91, 14 (1994), 6574–6578.
- [30] MEINERT, M. C., AND DELMER, D. P. Changes in biochemical composition of the cell wall of the cotton fiber during development. *Plant Physiology* 59, 6 (1977), 1088–1097.
- [31] NAOUMKINA, M., THYSSEN, G. N., AND FANG, D. D. Rna-seq analysis of short fiber mutants ligon-lintless-1 (li 1) and – 2 (li 2) revealed important role of aquaporins in cotton (*Gossypium hirsutum* l.) fiber elongation. *BMC Plant Biology*, 15 (2015), 65.
- [32] NEWMAN, S. A., AND BHAT, R. Dynamical patterning modules: a pattern language for development and evolution of multicellular form. *International Journal of Developmental Biology*, 53 (2009), 693–705.
- [33] NICOLAS WJ, GRISON MS, T. S. G. A. F. M. C. F. O. K. T. J. B. L. B. E. Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves. *Nat Plants* 3 (2017), 17082.
- [34] OHMIYA, Y., SAMEJIMA, M., SHIROISHI, M., AMANO, Y., KANDA, T., SAKAI, F., AND HAYASHI, T. Evidence that endo-1,4- β -glucanases act on cellulose in suspension-cultured poplar cells. *The Plant Journal* 24, 2 (2000), 147–158.
- [35] OPARKA, K., AND PRIOR, D. Direct evidence for pressure-generated closure of plasmodesmata. *The Plant Journal* 2, 5 (1992), 741–750.

- [36] PIEN, S., WYRZYKOWSKA, J., MCQUEEN-MASON, S., SMART, C., AND FLEMING, A. Local expression of expansin induces the entire process of leaf development and modifies leaf shape. *Proceedings of the National Academy of Sciences* 98, 20 (2001), 11812–11817.
- [37] PÉRET, B., LI, G., ZHAO, J., BAND, L. R., VOSS, U., POSTAIRE, OLIVIER LUU, D.-T., DA INES, O., CASIMIRO, I., LUCAS, M., WELLS, D. M., LAZZERINI, L., NACRY, P., KING, J. R., JENSEN, O. E., SCHÄFFNER, A. R., MAUREL, C., AND J., B. M. Auxin regulates aquaporin function to facilitate lateral root emergence. *Nature Cell Biology* 14 (2012), 991–998.
- [38] QIN, Y.-M., AND ZHU, Y.-X. How cotton fibers elongate: a tale of linear cell-growth mode. *Current Opinion in Plant Biology* 14, 1 (2011), 106 – 111.
- [39] RUAN, Y.-L., LLEWELLYN, D. J., AND FURBANK, R. T. The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and k⁺ transporters and expansin. *The Plant Cell* 13, 1 (2001), 47–60.
- [40] RUAN, Y.-L., XU, S.-M., WHITE, R., AND FURBANK, R. T. Genotypic and developmental evidence for the role of plasmodesmatal regulation in cotton fiber elongation mediated by callose turnover. *Plant physiology* 136, 4 (2004), 4104–4113.
- [41] SCHULZ, A. Plasmodesmal widening accompanies the short-term increase in symplasmic phloem unloading in pea root tips under osmotic stress. *Protoplasma* 188, 1, 22–37.
- [42] SEAGULL, R. W. The effects of microtubule and microfilament disrupting agents on cytoskeletal arrays and wall deposition in developing cotton fibers. *Protoplasma* 159, 1 (Feb 1990), 44–59.
- [43] SHIMIZU, Y., AOTSUKA, S., HASEGAWA, O., KAWADA, T., SAKUNO, T., SAKAI, F., AND HAYASHI, T. Changes in Levels of mRNAs for Cell Wall-Related Enzymes in Growing Cotton Fiber Cells¹. *Plant and Cell Physiology* 38, 3 (01 1997), 375–378.
- [44] TAIZ, L., AND ZEIGER, E. *Plant Physiology*, 2nd ed. Sunderland: SINAUER ASSOCIATES INC., U.S., 2002.
- [45] TANG, W., TU, L., YANG, X., TAN, J., DENG, F., HAO, J., GUO, K., LINDSEY, K., AND ZHANG, X. The calcium sensor ghc^{am7} promotes cotton fiber elongation by modulating reactive oxygen species (ros) production. *New Phytologist* 202, 2 (2014), 509–520.
- [46] TIWARI, S. C., AND WILKINS, T. A. Cotton *Gossypium hirsutum* seed trichomes expand via diffuse growing mechanisms fiber. *Canadian Journal of Botany*, 73 (1995), 746 – 757.
- [47] VALERIA HERNÁNDEZ-HERNÁNDEZ, KARL J. NIKLAS, S. A. N., AND BENÍTEZ, M. Dynamical patterning modules in plant development and evolution. *International Journal of Developmental Biology*, 56 (2012), 661–674.

-
- [48] VERMEER, J. E. M., VON WANGENHEIM, D., BARBERON, M., LEE, Y., STELZER, E. H. K., MAIZEL, A., AND GELDNER, N. A spatial accommodation by neighboring cells is required for organ initiation in arabidopsis. *Science* 343, 6167 (2014), 178–183.
- [49] VOITSEKHOVSKAJA, O. V., KOROLEVA, O. A., BATASHEV, D. R., KNOP, C., TOMOS, A. D., GAMALEI, Y. V., HELDT, H.-W., AND LOHAUS, G. Phloem loading in two scrophulariaceae species. what can drive symplastic flow via plasmodesmata? *Plant Physiology* 140, 1 (2006), 383–395.
- [50] WANG, L., LI, X.-R., LIAN, H., NI, D.-A., HE, Y.-K., CHEN, X.-Y., AND RUAN, Y.-L. Evidence that high activity of vacuolar invertase is required for cotton fiber and arabidopsis root elongation through osmotic dependent and independent pathways, respectively. *Plant Physiology* 154, 2 (2010), 744–756.
- [51] ZAVALIEV, R., UEKI, S., EPEL, B. L., AND CITOVSKY, V. Biology of callose (β -1,3-glucan) turnover at plasmodesmata. *Protoplasma* 248, 1 (Jan 2011), 117–130.
- [52] ZENONI, S., REALE, L., TORNIELLI, G. B., LANFALONI, L., PORCEDDU, A., FERRARINI, A., MORETTI, C., ZAMBONI, A., SPEGHINI, A., FERRANTI, F., AND PEZZOTTI, M. Down-regulation of the petunia hybrida -expansin gene pexp1 reduces the amount of crystalline cellulose in cell walls and leads to phenotypic changes in petal limbs. *The Plant Cell* 16, 2 (2004), 295–308.
- [53] ZHANG, Z., RUAN, Y.-L., ZHOU, N., WANG, F., GUAN, X., FANG, L., SHANG, X., GUO, W., ZHU, S., AND ZHANG, T. Suppressing a putative sterol carrier gene reduces plasmodesmal permeability and activates sucrose transporter genes during cotton fiber elongation. *The Plant Cell* (2017).

5

Appendix

In this collaboration with Prof. Karl Niklas and Prof. Stuart Newman we put forward the hypothesis that plasmodesmal-dependent movement of molecules might have enabled the evolution of complex multicellular plants by mobilizing generic processes such as lateral inhibition, and diffusion. I particularly contributed to this article by writing the section "The spatially dependent differentiation (DIF) DPM and its role in the evolution of multicellular vascular plants and vascularization". This work was published in *Frontiers in Plant Science* (16th July 2018).



Dynamical Patterning Modules, Biogeneric Materials, and the Evolution of Multicellular Plants

Mariana Benítez¹, Valeria Hernández-Hernández², Stuart A. Newman³ and Karl J. Niklas^{4*}

¹ Centro de Ciencias de la Complejidad – Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico City, Mexico, ² Laboratoire de Reproduction et Développement des Plantes, Université de Lyon, École Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Centre National de la Recherche Scientifique, Institut National de la Recherche Agronomique, Lyon, France, ³ Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY, United States, ⁴ Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, United States

OPEN ACCESS

Edited by:

Verónica S. Di Stilio,
University of Washington,
United States

Reviewed by:

Daniel H. Chitwood,
Michigan State University,
United States
Olivier Hamant,
École Normale Supérieure de Lyon,
France

*Correspondence:

Karl J. Niklas
kjn2@cornell.edu

Specialty section:

This article was submitted to
Plant Evolution and Development,
a section of the journal
Frontiers in Plant Science

Received: 13 April 2018

Accepted: 04 June 2018

Published: 16 July 2018

Citation:

Benítez M, Hernández-Hernández V,
Newman SA and Niklas KJ (2018)
Dynamical Patterning Modules,
Biogeneric Materials,
and the Evolution of Multicellular
Plants. *Front. Plant Sci.* 9:871.
doi: 10.3389/fpls.2018.00871

Comparative analyses of developmental processes across a broad spectrum of organisms are required to fully understand the mechanisms responsible for the major evolutionary transitions among eukaryotic photosynthetic lineages (defined here as the polyphyletic algae and the monophyletic land plants). The concepts of dynamical patterning modules (DPMs) and biogeneric materials provide a framework for studying developmental processes in the context of such comparative analyses. In the context of multicellularity, DPMs are defined as sets of conserved gene products and molecular networks, in conjunction with the physical morphogenetic and patterning processes they mobilize. A biogeneric material is defined as mesoscale matter with predictable morphogenetic capabilities that arise from complex cellular conglomerates. Using these concepts, we outline some of the main events and transitions in plant evolution, and describe the DPMs and biogeneric properties associated with and responsible for these transitions. We identify four primary DPMs that played critical roles in the evolution of multicellularity (i.e., the DPMs responsible for cell-to-cell adhesion, identifying the future cell wall, cell differentiation, and cell polarity). Three important conclusions emerge from a broad phyletic comparison: (1) DPMs have been achieved in different ways, even within the same clade (e.g., phycoplastic cell division in the Chlorophyta and phragmoplastic cell division in the Streptophyta), (2) DPMs had their origins in the co-option of molecular species present in the unicellular ancestors of multicellular plants, and (3) symplastic transport mediated by intercellular connections, particularly plasmodesmata, was critical for the evolution of complex multicellularity in plants.

Keywords: plant evolution, plasmodesmata, algal evolution, convergent evolution, dynamical patterning modules

INTRODUCTION

The goal of this paper is to review the evolution of the multicellularity plant body plan within the conceptual framework of dynamic patterning modules (DPMs; Newman and Bhat, 2009; Newman et al., 2009; Newman, 2011), which provides a means of integrating physical and molecular-genetic aspects of developmental mechanisms. We have reviewed this topic previously (Hernández-Hernández et al., 2012; Niklas and Newman, 2013; Niklas, 2014). However, our focus here is on

the DPMs involved in the establishment of body plan polarity and cell-tissue differentiation. As in our previous treatments of the topic, a broad comparative approach is adopted here because multicellularity has evolved multiple times among the various eukaryotic photosynthetic lineages (Figure 1). The exact number of times it has evolved in large part depends on how multicellularity is defined. If multicellularity is regarded as any transient or permanent aggregation of cells, it is estimated to have evolved independently at least 25 times (Grosberg and Strathmann, 2007). If more rigorous criteria are applied, as for example the requirement for intercellular communication and cooperation, multicellularity has evolved multiple times in the Actinobacteria, Myxobacteria, and Cyanobacteria, at least three times in the fungi (chytrids, ascomycetes, and basidiomycetes), six times among the polyphyletic algae (twice each in the red, brown, and green algae), but only once in the Animalia (Niklas and Newman, 2013; Niklas, 2014).

Regardless of how multicellularity is defined or how many times it evolved, the repeated independent evolution of multicellularity evokes many important but as yet unresolved questions. For example, are the developmental and morphological motifs involved in the transformation of unicellular organisms into multicellular ones adaptations to the exigencies of life, the result of weak selection pressures, or the predictable consequences of physico-genetic laws and processes? Do sets of “master genes” for multicellularity exist among most or all eukaryotic clades? Indeed, given its ubiquity among pro- and eukaryotic lineages, has multicellularity truly

evolved independently among so many kinds of bacteria, fungi, algae, land plants, and animals, given the fact that all eukaryotes ultimately shared a last common ancestor?

The application of the framework of DPMs is particularly useful to address this last question because similar if not identical phenotypes can be achieved by the developmental mobilization of very dissimilar molecular systems or processes and because natural selection acts at the level of the phenotype and not at the level of the mechanisms that give rise to it. This dictum has been formalized by Newman and Bhat (2009), Newman et al. (2009), and Newman (2011) who have conceptualized the development and evolution of multicellular animals in the framework of DPMs each of which involves one or more sets of shared gene networks, their products, and the physical processes that relate to various types of matter. The importance of many of the physical processes involved in DPMs such as adhesion, cohesion, diffusion, activator–inhibitor dynamics, and viscoelasticity have long been recognized as important in development. Moreover, experimental research continues to demonstrate that the mechanical environment experienced by individual cells, tissues, and organs can alter gene expression patterns and thus cell fate specification (e.g., Swift et al., 2013).

Considering development and its evolution in terms of the DPM framework highlights the fact that the morphological motifs that are produced by physical processes evoked by specific molecules and pathways constitute a “pattern language” for configuring the basic body plans of multicellular animals and plants (Newman and Bhat, 2009; Newman et al., 2009; Hernández-Hernández et al., 2012; Niklas, 2014). These processes include mechanical forces resulting from the geometrical arrangements of mesoscale materials, irreversibility, the properties of network topologies and organization, and symmetry breaking. Importantly, many of the physical processes associated with DPMs are “generic” in that they are causally similar to the physical processes affecting the behavior of inorganic materials (Niklas, 1992; Niklas and Spatz, 2012). This congruence between the animate and inanimate world facilitated the rapid evolution of stereotypical generic morphologies once multicellularity was achieved in phylogenetically different groups of organisms, because there is ample evidence that some DPMs originated by means of the co-option of genes or gene regulatory networks (GRNs) present in ancestral unicellular organisms (Newman and Bhat, 2009; Newman et al., 2009).

Numerous examples of analogous DPMs operating across a broad spectrum of eukaryotic organisms can be given because of the many fundamental similarities existing among all eukaryotic cells (Wayne, 2009). For example, molecular pathways for the control of cell shape and polarity that evolved in unicellular organisms were mobilized by the novel protein Wnt in multicellular animals to mediate, via respective DPMs, lumen formation, and tissue elongation via convergent extension (reviewed in Newman, 2016b). Likewise, all eukaryotic cells have the capacity to produce extracellular (ergastic) polysaccharides and structural glycoproteins capable of self-assembly to create extracellular matrices containing interpenetrating polymeric networks of hydroxyproline-rich glycoproteins, e.g., collagen in animals and the extensin

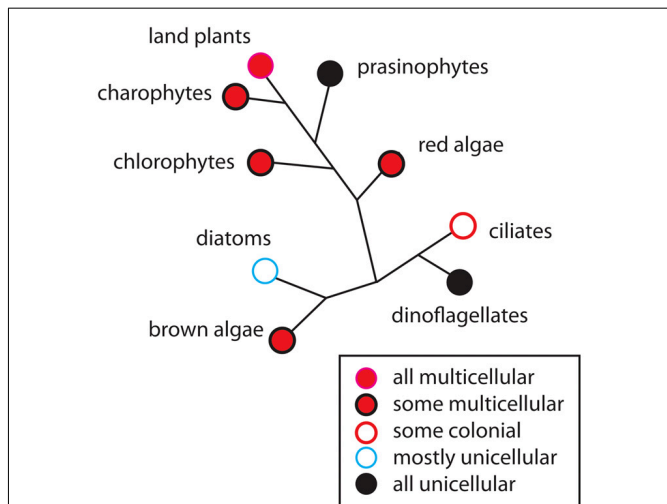


FIGURE 1 | The occurrence of multicellularity shown on a highly redacted and unrooted phylogenetic diagram of the major groups of photosynthetic eukaryotes. Although some groups are entirely unicellular or multicellular (e.g., prasinophytes and the land plants, respectively), most contain a mixture of body plans such as the unicellular and colonial body plans (e.g., diatoms), or a mixture of the unicellular, colonial, and multicellular body plans (e.g., brown algae). In general, early-divergent persistent lineages are dominated by unicellular species (e.g., prasinophytes in the green algal clade), whereas later-divergent lineages contain a mixture of body plans (e.g., chlorophytes and charophytes). Species-rich, late-divergent persistent lineages tend to be exclusively multicellular (e.g., the land plants and metazoans).

superfamily in numerous algal lineages and in the embryophytes (Ferris et al., 2001; Stanley et al., 2005) (**Figure 2**). These proteins manifest marked peptide periodicity, can form flexible rod-like molecules with repeat motifs (dominated by hydroxyproline) in helical configurations with arabinosyl/galactosyl side chains. This “superfamily” of intercellular adhesives operates among many unicellular organisms in gamete-to-gamete self-recognition and adhesion and the adhesion of cells to a substratum. It is very likely, therefore, that these “ancestral” adhesive capacities were co-opted to provide the cell-to-cell adhesives operating in many multicellular organisms, just as a wide array of microtubule-associated proteins in the algae, embryophytes, fungi, and metazoans (Gardiner, 2013) mediate related cell reshaping mechanisms utilized by DPMs in all these groups.

It is clear, however, that some of the DPMs operating in animals do not function in the various groups of algae, the land plants, and most fungi because of substantive differences among these lineages and clades (Meyerowitz, 2002; Newman and Niklas, 2018). Consider, for example, that the cells of animals are typically individually deformable and that during development they are free to move past one another in ways that permit differential adhesion, cortical tension, and other processes that permit the autonomous sorting and assembly of different tissues. In contrast, most plant and fungal cells possess a rigid cell wall that is firmly bound to the cell walls of adjoining cells. Likewise, plant signaling molecules acting as transcriptional modulators and determinants of tissue and cell fate can act intercellularly as well as intracellularly (see Cui et al., 2007; Urbanus et al., 2010; Garrett et al., 2012). This capacity, which is rare albeit not unknown in animal systems (Prochiantz, 2011), blurs the functional distinction of the GRNs affecting multi- versus single-cell differentiation.

Further, plant cell polarity involves the participation of PIN and PAN1 proteins in auxin polar and lateral transport and the regulation of metabolic fluxes by means of plasmodesmata. In contrast, animal cell polarity involves the participation of

integrin, cadherin, and PAR or CDC42 proteins (Geldner, 2009; Dettmer and Friml, 2011; Zhang et al., 2012). Further, cell, tissue, and organ polarity within the multicellular plant body is maintained by a complex phytohormone transport system that involves the differential and sometimes transient positioning of auxin transporters proteins (Dettmer and Friml, 2011; Zhang et al., 2012), the establishment of mechanical heterogeneities within the apoplastic infrastructure (Kutschera and Niklas, 2007; Geldner, 2009; Peaucelle et al., 2015; Galletti et al., 2016; Majda et al., 2017), and the regulation of metabolic fluxes by means of plasmodesmata. Although analogies can be drawn between the establishment and maintenance of cellular and tissue polar domains in animals and plants, the mechanisms by which polarity is achieved are very different. For example, tight junctions between the apical and basolateral plasma membrane domains in animal epithelial cells provide barriers preventing the intramembrane diffusion of proteins and other macromolecules (Shin et al., 2006), whereas in plants a variety of phenolic compounds are used to maintain tissue polarity domains (Alassimone et al., 2010). As a final example of the differences between DPMs among the eukaryotic lineages, consider the manner in which cell wall materials are delivered and deposited during cell division. The mechanics of this developmental process differs substantively among the desmids and among different filamentous ascomycetes (Hall et al., 2008; Seiler and Justa-Schuch, 2010). It even differs within the monophyletic Chlorobionta, i.e., phycoplasmic cell division in the Chlorophyta and phragmoplasmic cell division in the Streptophyta (see Graham et al., 2009; Niklas, 2014) (**Figure 3**).

Focusing on the distinctive physico-genetic morphogenetic modalities of plants, Hernández-Hernández et al. (2012) identified six DPMs involved in critical embryophyte developmental processes. These DPMs are (1) the production of intercellular adhesives (ADH), (2) the manner in which the future cell wall is formed and oriented (FCW), (3) the establishment of intercellular communication and spatial-dependent patterns of differentiation (DIF), (4) the establishment of axial and lateral polarity (POL), (5) the formation of lateral appendages or “buds” (BUD), and (6) the formation of lateral, leaf-like structures (LLS). Hernández-Hernández et al. (2012) discussed all six of these DPMs with an emphasis on the first four (i.e., ADH, FCW, DIF, and POL), because cell division, cell-to-cell adhesion, intercellular communication, and polarity are essential for achieving simple multicellularity across all clades and because these four DPMs operate in a pairwise manner in many multicellular algae and fungi as well as in the land plants (**Figure 4**). Here, we emphasize DIF and POL because these are essential for achieving complex multicellularity (define here as “the condition in which some cells are not in direct contact with the external environment”), and present new evidence that the evolution of plasmodesmata played a critical role in the evolution of cell, tissue, and organ differentiation and polarity. We also identify the characteristic molecules and molecular networks, and when possible, the physical processes they mobilize for each of the four key modules.

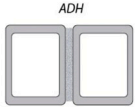
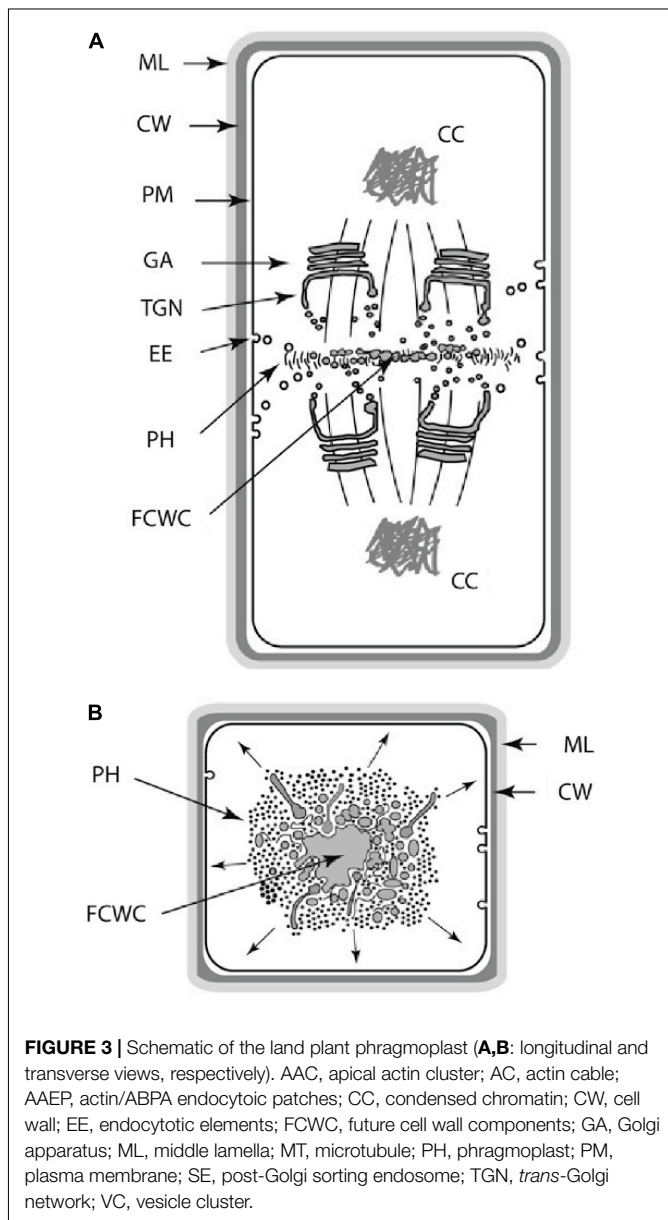
Lineage	Intercellular Adhesives	
Cyanobacteria	glycoproteins, lipopolysaccharides	Adhesion
Fungi	glycoprotein-based “glues”	
Rhodophytes	sulfated galactan polymers	
Phaeophytes	β -D-mannuronic and α -L-guluronic acid polymers	
Chlorophytes	hydroxyproline-rich glycoproteins	
Embryophytes	Ca-rhamnogalacturonic-dominated pectins	
Metazoa	type-1 transmembrane cadherin proteins	

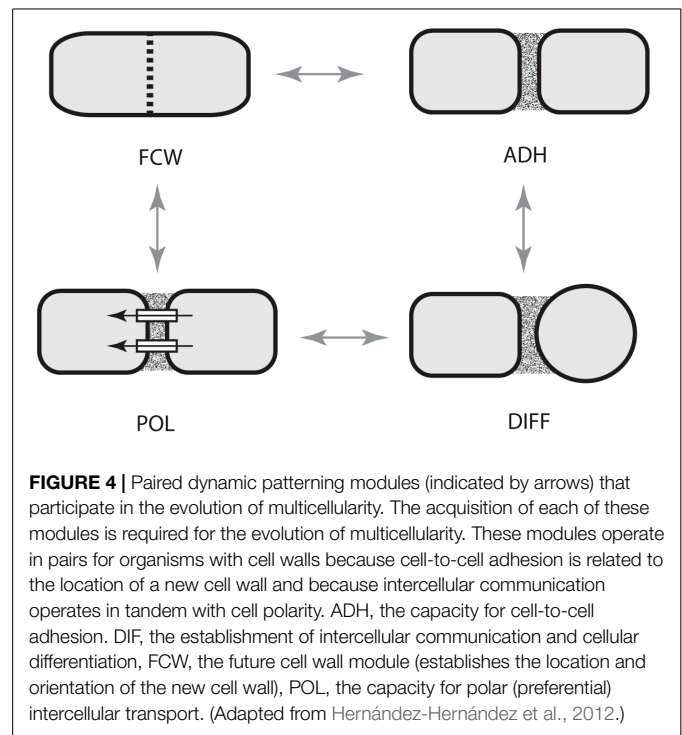
FIGURE 2 | The different adhesives utilized by the cell-to-cell adhesive (ADH) dynamic patterning module among some of the major multicellular lineages.



BIOGENERIC MATERIALS AND DYNAMICAL PATTERNING MODULES (DPMs)

Before proceeding to an exploration of the empirical evidence for the concepts that will be pursued, it is important to establish clear definitions for what is meant by biogenic materials and DPMs, particularly since these concepts may not be familiar to some researchers and because clarity in definitions is essential for clarity in thinking.

Like all matter, living matter manifests inherent morphogenetic properties, and characteristic morphological motifs that are in part expressions of inviolable physical laws and principles (Newman, 2017; see also Niklas and Spatz, 2012; Niklas, 2017). This idea is familiar to those who study the physical



sciences as is the notion that the operation of physical laws and principles is sensitive to scale (Niklas, 1994). On the macroscale, phenomena such as large-scale climate and oceanic systems generate fluidic patterns of various but discrete and recognizable morphologies. On the microscale, atoms and small molecules can be arranged and rearranged to form discrete molecules with well-defined chemical and physical properties. Living matter operates at an intermediate or *mesoscale*. Non-living mesoscale materials are familiar as solids, which can be amorphous or crystalline, and liquids, which can form vortices and waves. Living matter exhibits many of these generic physical properties (Green and Batterman, 2017).

Although all living cells have mesoscale properties in common (e.g., their cell membranes and cytoplasm are rheologically similar), we focus here exclusively on multicellular matter. For example, all tissues behave as viscoelastic materials (i.e., they behave as a combination of a liquid and a solid). However, the extent to which a material manifests viscoelasticity depends on the presence and quantity of its rigid components (Niklas, 1989, 1992). With few exceptions (e.g., bone and cartilage), most animal tissues are highly viscoelastic owing to an absence of rigid cell walls. In contrast, all plant and fungal tissues behave as deformable cellular solids because of the presence of rigid cell wall solids (e.g., cellulose and chitin) (Niklas, 1989, 1992). Animal, plant, and fungal tissues have shared generic properties, but they differ in the degree to which they respond to physical stresses. Thus, the subunits – cells – of animal tissues can be independently mobile and rearrange with respect to one another, particularly during development, when the tissues are more liquid-like than they are in the mature organism, whereas, with the exception of intrusive

growth, plant and fungal cells do not typically change their neighbors.

The generic physical properties of living matter lend predictability to the forms it can assume during development. Like non-living liquids, liquid crystals, and mixtures thereof, developing animal tissues can form immiscible layers, interior spaces, and undergo elongation (Forgacs and Newman, 2005). Like non-living deformable solids (undergoing, for example, accretion or melting), developing plant and fungal tissues can bud or branch (Fleury, 1999; Niklas and Spatz, 2012).

The liquid vs. solid nature of living tissues does not arise from the same subunit-subunit interactions that endow non-living materials with these properties. Instead of Brownian motion and the electronic weak attractive interactions among the molecular subunits of non-biological liquids, the cells in animal tissues move non-randomly by cytoskeletally generated forces and remain cohesive despite their translocation via transmembrane homophilic attachment proteins. In plant and fungal tissues, instead of the charge-based or covalent bonds of the atomic or molecular subunits of non-biological solids, the cells are cemented together by Ca^{2+} -rhamnogalacturonan-rich pectins, or members of the extensin superfamily of hydroxyproline-rich glycoproteins (Cannon et al., 2008; Lampion et al., 2011). For these and other reasons, the various viscoelastic and deformable solid materials that constitute living tissues have been termed “biogeneric” matter, in recognition of the predictability of their morphogenetic behavior and outcomes afforded by their generic properties, and the fact that these generic properties are dependent on evolved biological, rather than purely physical, effects (Newman, 2016a).

Another set of biogeneric properties that characterize living tissues, superimposed upon their identity as predominantly viscoelastic or non-deformable solid materials, is their *excitability*, that is, the ability to store energy and release it upon stimulation (Levine and Ben-Jacob, 2004; Sinha and Sridhar, 2015). Mechanically, chemically, and electrically excitable materials are not unknown in the non-living world (exemplified by loaded mousetraps, forest fires, and tunnel diodes) but they are uncommon. Multicellular systems are inevitably excitable, because their cellular subunits are biochemically, mechanically, and electrically active, the storage and controlled utilization of energy is intrinsic to all life (Lund, 1947).

During the development of multicellular organisms, communication among the cellular subunits can induce the spatiotemporal mobilization of mechanical, chemical, and electrical energy, leading to cellular pattern formation and morphogenesis. In animal embryos and organ primordia, this communication is generally short-range via extracellularly diffusible morphogens. However, mechanical and electrical fields can achieve nearly instantaneous long-range communication. In developing and remodeling plants and fungi, communication can be both intra- and intercellular and short and long-range. Like the biogeneric rheological and solid-state properties of animal, plant, and fungal tissues, the phenomena of excitability give rise to predictable morphological motifs – repetitive or fractal arrangements of ridges, appendages, venation patterns and cell types.

Dynamical patterning modules, defined above, are intrinsic to this “physico-genetic” account of the origin and evolution of multicellular life-forms (Newman, 2012). The DPM concept recognizes that the physical forces that shape tissues cannot be considered independently of the actual materials (cell collectivities and their molecules) that they act on. The activity of DPMs can be regulated in a given multicellular material (e.g., that characterizing the phylum to which a species belongs), leading to developmental transitions and phenotypic differences between members of a phylum. Insofar as the materials have biogeneric properties (as is the case with animal tissues, and to a large extent with plant and fungal tissues), specific DPMs will promote morphological outcomes familiar from the physics of non-living matter. In other cases, DPMs will mobilize physical forces to produce outcomes peculiar to varieties of living matter. Without exception, however, physics and genetics act together to effect morphological development.

The following sections will update earlier descriptions of DPMs in plant systems (Hernández-Hernández et al., 2012; Benítez and Hejatko, 2013; Niklas and Newman, 2013; Niklas et al., 2013; Niklas, 2014; Mora Van Cauwelaert et al., 2015) and attempt to assign evolutionary roles to them.

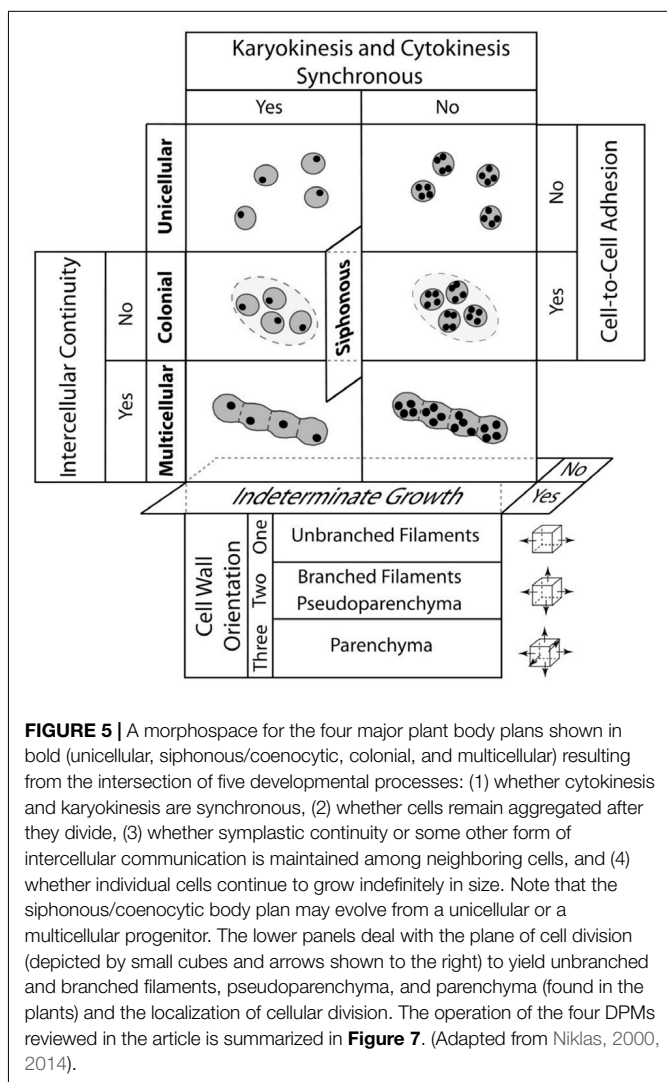
EVOLUTIONARY TRANSITIONS IN EUKARYOTIC PHOTOSYNTHETIC LINEAGES

The fossil record and contemporary molecular phylogenetic analyses indicate that the three major algal clades in which multicellularity evolved (i.e., the Streptophiles, Rhodophytes, and Chlorobionta) had independent evolutionary origins because of primary and secondary endosymbiotic events (reviewed by Kutschera and Niklas, 2005, 2008). Consequently, these three clades in tandem with the evolution of the land plants (from a green algal ancestor) can be viewed as independent “evolutionary experiments” that provide an opportunity to examine how the four DPMs (i.e., ADH, FCW, DIF, and POL) participated in achieving multicellularity in each case.

The significance of the four DPMs becomes apparent when they are placed in the context of a morphospace that identifies the major plant body plans and when their placement is juxtaposed with a series of phenotypic transformations predicted by multilevel selection theory for the evolutionary appearance of multicellularity regardless of the clade under consideration (Niklas, 2014). Following McGhee (1999), we define a morphospace as a depiction of all theoretically possible structural phenotypes within a specific group of organisms. The depiction is constructed using orthogonal axes, each of which represents a phenotypic character that has one or more character states, e.g., cellular aggregation: yes or no. The intersection of two or more such axes specifies a hypothetical or real phenotype defined by the variables or processes the participating and intersecting axes specify.

Niklas (2000, 2014) constructed such a morphospace for all photosynthetic eukaryotes using four characters, each of

which has two character states in the form of a question, i.e., (1) are cytokinesis and karyokinesis synchronous?; (2) do cells remain attached after cellular division?; (3) is symplastic or some other form of intercellular communication established and maintained among adjoining cells?; and (4) do individual cells continue to grow indefinitely in size? This simple morphospace identifies four plant body plans that can be either uninucleate or multinucleate, i.e., the unicellular, siphonous/coenocytic, colonial, and multicellular body plan (Figure 5). The different tissue constructs of the multicellular plant body can be identified also by adding a fifth axis that specifies the orientation of cell division with respect to the body axis. With the addition of this axis, three tissue constructs are identified, i.e., the unbranched filament (when cell division is restricted to one plane of reference), the branched filament and the pseudoparenchymatous tissue construct (when cell division occurs in two planes of reference and when branched filaments interweave, respectively), and the parenchymatous tissue construct (when cell division occurs in three planes of cell division).



The primary literature dealing with the algae (e.g., Graham et al., 2009) reveals that all four plant body plans (as well as the three tissue constructs) have evolved multiple times in the Stramenopiles, Rhodophytes, and Chlorobionta. This convergence reveals the evolutionary significance of the four DPMs that are the focus of our review, i.e., FCW, ADH, DIF, and POL (Figure 4). Specifically, ADH is required for the colonial and multicellular body plans; FCW is involved in whether cyto- and karyokinesis are synchronous and whether the tissue construction of a multicellular plant is filamentous (unbranched or branched) or parenchymatous, although how the FCW is determined remains problematic, even for the well studied land plants (Schaefer et al., 2017); and DIF and POL are required for intercellular cooperation and cellular specialization.

These four DPMs also help to identify evolutionary trends in the establishment of multicellularity predicted by multilevel selection theory (Folse and Roughgarden, 2010; Niklas and Newman, 2013; Niklas, 2014). This theory recognizes the unicellular organism as the ancestral state in each of the multicellular lineages or clades, and it identifies the colonial body plan as transitional to the multicellular body plan. Therefore, when multilevel selection theory is applied to the evolution of multicellularity, it identifies a “unicellular => colonial => multicellular” body plan transformation series (regardless of the type of organism) in which the participation of ADH, FCW, DIF, and POL are collectively required to establish and maintain a colonial body plan and to subsequently coordinate and specify the intercellular activities within an integrated multicellular body plan whose complexity exceeds simple dyadic interactions among conjoined cells, tissues, and organs.

The transformation series among the different genera and species of the volvocine algal lineage is consistent with the aforementioned multilevel selection theory’s predicted unicellular => colonial => multicellular transformation series (Bonner, 2000; Kirk, 2005; Herron and Michod, 2008; Niklas, 2014). The ancestral volvocine body plan undoubtedly possessed a unicellular organism that was morphologically and physiologically like *Chlamydomonas*. The transformation of this unicellular organism into a colonial organism is posited to have involved the modification of the ancestral cell wall into an extracellular adhesive matrix seen in the Tetrabaenaceae => Goniaceae => Volvocaceae transformation series (Kirk, 2005; see also Graham et al., 2009), which is consistent with what is known about the biochemistry of this ergastic material (Sumper and Hallmann, 1998). Subsequent evolutionary modifications exemplified by a hypothetical Goniaceae => Volvocaceae transformation series are predicted to have produced life-forms ranging from simple colonial aggregates (e.g., *Tetrabaena socialis*) to more colonies with asymmetric cell division, to multicellular organisms with a germ-soma division of labor (e.g., *Volvox carteri*) (Kirk, 2005; Herron and Michod, 2008). It is worth noting that in the case of multicellular volvocine algae, the cytoplasmic bridges that interconnect each cell to its neighbors have multiple functionalities. These bridges participate in the mechanics of a unique form of kinesin-driven inversion, and they provide avenues for the metabolic transport of nutrients to developing reproductive structures, called gonidia

(Hoops et al., 2000). In this sense, these bridges are analogous to land plant plasmodesmata, although their apertures are much wider than those of the latter (~200 nm in diameter; Green et al., 1981). Curiously, in some volvocines, these bridges are developmentally severed and thereby provide an interesting example of a multicellular-to-colonial transformation series.

DPMs AND BIOGENERIC PROPERTIES INVOLVED IN PLANT DEVELOPMENT AND EVOLUTIONARY TRANSITIONS

As noted previously, we proposed a set of DPMs associated with key plant developmental events and specified some of the physical and molecular components of these modules (Hernández-Hernández et al., 2012). After reviewing the phyletic distribution of the molecular elements of the DPMs, we hypothesized that these modules originated from the co-option of cell-molecular mechanisms related to single-cell functions in the unicellular ancestors of the major algal clades and the land plant lineages that mobilized, in the multicellular context, novel physical processes. One of our central conclusions is that once development is set into operation, much of it becomes self-organizing due to the mobilization of DPMs and biogenic properties. This view contrasts with the hypothesis that land plant diversification resulted mainly from the expansion of particular gene families (e.g., Vergara-Silva et al., 2000; Zažímalová et al., 2010). Certainly, while these molecules are central for plant development and, most probably also for plant evolution, we argued that the notion that diversification of certain gene families or molecular classes can be the main cause of morphological evolution is insufficient. Additionally, we suggested that the combination of different DPMs at different places and developmental stages may help understand the generation of the basic features of the multicellular plant body plan. We argued further that plant development has evolved into processes that occur in a physical medium that is dynamic over large scales, utilizing inherently multicellular systems of multifunctional hormones/morphogens/transcription factors that are unrestricted by cell boundaries in many of their functions. Under such conditions, the origin and mechanisms behind plant extraordinary plasticity becomes less enigmatic (Hernández-Hernández et al., 2012).

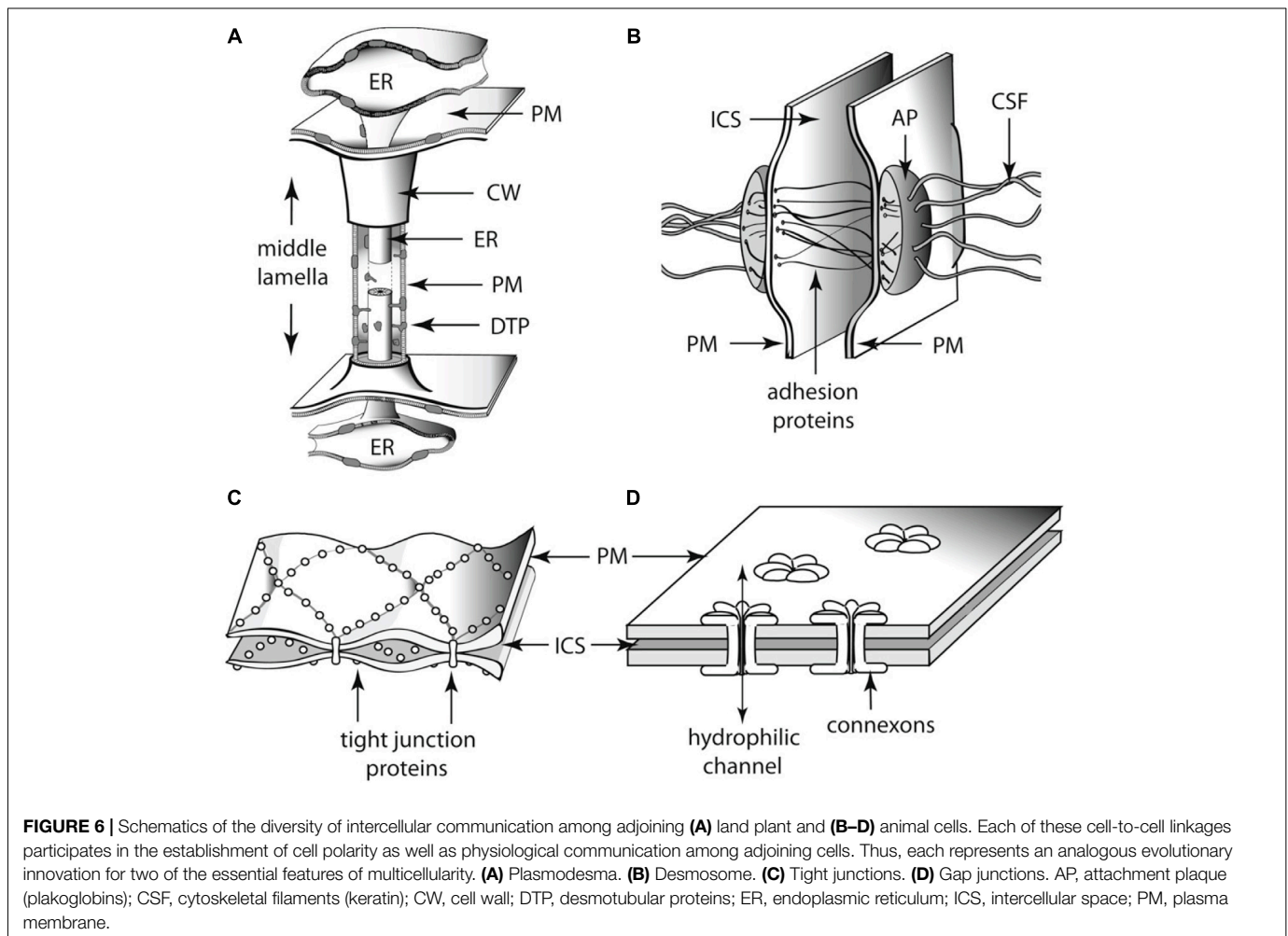
The Spatially Dependent Differentiation (DIF) DPM and its Role in the Evolution of Multicellular Plants and Vascularization

As groups of cells adhered to each other, some physical constraints were imposed on the transport of nutrients and signaling molecules. Multicellular aggregates eventually evolved a division of labor (Niklas, 2000; Kirk, 2005; Knoll, 2011) that required cell fate specification mechanisms (Niklas et al., 2014), and the ability of cells to coordinate their metabolism, patterns of cell growth, and the activity of molecular networks. In almost every multicellular aggregate two possible mechanisms for the exchange of nutrients and signaling molecules exist:

indirect and direct transport (Beaumont, 2009). The first case requires some cells to secrete nutrients to the external environment and other cells to take them up (Beaumont, 2009). In contrast, for direct cell-to-cell transport, the presence of transmembrane connections is required (Niklas, 2000; Knoll, 2011). During the course of evolution, intercellular connections evolved independently in multicellular lineages to respond to the biophysical challenges that multicellularity imposed (Figure 6). For example, animals have gap junctions, whereas the cells of plants and fungi, respectively, developed plasmodesmata and septa pores, respectively (Bloemendal and Kück, 2013). Here, we discuss the role of plasmodesmata-mediated transport in the coordination of cell type specification during plant development and how this could have been a prerequisite for the transitions from unicellular to multicellular (and from non-vascular to vascular) plants. We will also briefly review the phylogenetic data concerning the evolution of plasmodesmatal structure.

Given that plant cells are surrounded by a rigid cell wall, they rely on the transport of signaling molecules for the establishment of cell type patterns. As we discussed in previous work (Hernández-Hernández et al., 2012), the spatially dependent differentiation (DIF) DPM is composed of the plant intercellular channels, called plasmodesmata, and the biogenic properties they mobilize, viz., passive diffusion, lateral inhibition, and reaction-diffusion. Plants manifest a precise spatiotemporal control on the aperture (also called permeability) of plasmodesmata channels and can control the direction of plasmodesmata-mediated fluxes to create molecular concentration gradients (Sager and Lee, 2014). Using different techniques, Christensen et al. (2009) detected unidirectional transport of fluorescent probes from the basal epidermal cells into the apical cells of trichomes in the leaves of tobacco, *Nicotiana tabacum*. The probes were observed to move freely among trichome cells in both directions, but they were prevented from migrating in the opposite direction into subtending cells. Although the authors did not conclusively prove that this unidirectional flow depends on the aperture of plasmodesmata channels, they found that treatments with sodium azide, a metabolic inhibitor that alters plasmodesmata permeability, could reverse the direction of unidirectional flow from epidermal to trichome cells. A unidirectional transport of the photoconvertible dye Dendra2 was also observed in *Physcomitrella patens* (Kitagawa and Fujita, 2013), which indicated that this phenomenology is likely very ancient and thus of wide occurrence among the land plants. With the help of a controlled intercellular transport, plants can then modulate diffusion of signaling molecules in specific ways to generate or at least modulate patterns of cell type specification.

At the same time, the regulation of plasmodesmata permeability can generate morphogen gradients. Plasmodesmata aperture is regulated by the deposition and degradation of callose within the cell walls through which plasmodesmata pass (De Storme and Geelen, 2014). The turnover of callose is achieved by the participation of several families of proteins among which the GLUCAN SYNTHASE LIKE (GSL) proteins and β -glucanases, respectively, synthesize and degrade callose (Ruan et al., 2004; Guseman et al., 2010; De Storme and Geelen, 2014).



Further, genetic and chemical experiments have correlated the amount of callose at plasmodesmatal sites with the genetic expression of GSLs and β -glucanases, and the intercellular migration of molecules in several plant systems (Ruan et al., 2004; Guseman et al., 2010; Vatén et al., 2011; Benítez-Alfonso et al., 2013; Han et al., 2014). For example, in hypocotyls of *Arabidopsis* seedlings, it was demonstrated that the reduced callose deposition at plasmodesmata, resulting from an inducible knock down mutation of the *GLUCAN SYNTHASE LIKE 8* (*GSL8*) gene, had an enhanced diffusion of auxin (Han et al., 2014). Consequently, the loss of asymmetric auxin distribution prevented the differential cell elongation between the shaded and illuminated parts of the hypocotyl that is required for the phototropic response (Han et al., 2014). Based on these and other observations, Han et al. (2014) concluded that plasmodesmata closure is necessary to prevent auxin diffusion in *Arabidopsis* and to generate concentration gradients. In a similar way, it has been proposed that the main mechanism to establish auxin gradients in mosses such as *P. patens* is through plasmodesmata-mediated transport (Brunkard and Zambryski, 2017). Therefore, it seems likely that the regulation of plasmodesmata permeability has been key for land plants to establish concentration gradients of morphogens that coordinate developmental dynamics.

However, it is important to note that neither plasmodesmata nor multicellularity are required to achieve morphological complexity. This is evident from siphonous (coenocytic) algae such as the marine green alga *Caulerpa*. A recent intracellular transcriptomic atlas of this organism reveals that the acropetal transcript distribution conforms roughly to a transcription-to-translation pattern without the presence of internal cell walls (Ranjan et al., 2015; see also Menzel, 1996).

Cell type specification that depends on the intercellular transport of transcription factors is also accompanied by the closure of plasmodesmata to actuate a lateral inhibition mechanism. For example, the *chor* mutant of *Arabidopsis*, which encodes a putative *GSL8* protein, results in a significant increase in the number of stomatal lineage cells (Guseman et al., 2010). Further, in the epidermal cells of leaves, the expression of the *SPEECHLESS* (*SPCH*) transcription factor, which specifies the initiation of the stomatal lineage, is restricted to the meristemoid mother cells of stomata after asymmetric division (Pillitteri and Dong, 2013). The *gsl8* mutant has a lower amount of callose deposition resulting in the leakage of *SPCH* between epidermal cells that, in turn, results in abnormal stomata clusters (Guseman et al., 2010). By preventing the intercellular migration of *SPCH*, plasmodesmata inhibit the cells surrounding meristemoids to

differentiate into the stomata lineage and thus regulate the spacing of stomata in the epidermis of leaves. This demonstrates that the plasmodesmata aperture is necessary for the specification of cell identities by virtue of regulating lateral inhibition.

The non-cellular autonomous signaling mediated by symplasmic transport is a key mechanism to establish patterns of cell specification required for the development of vascular tissues. For example, in the root of *Arabidopsis* the transcription factor SHORT ROOT (SHR) moves from the stele into the cells within the quiescent center and the endodermis where it turns on the production of miRNA165/6 (Carlsbecker et al., 2010). The miRNA165/6 then moves back to the stele where it degrades the homeodomain leucine zipper PHABULOSA (PHB), which is necessary for the radial patterning of the xylem tissue and the pericycle (Carlsbecker et al., 2010). Mutations of the CALLOSE SYNTHASE GLUCAN LIKE 3/GLUCAN SYNTHASE LIKE 12 (CALS3/GSL12) gene, which the product of degrades callose, results in an increased callose deposition (Vatén et al., 2011). In these mutants, the signal of pSHR:SHR:GFP in the endodermis relative to that of the stele is decreased when compared with the wild type. This observation is consistent with the hypothesis that callose deposition prevents symplasmic transport (Vatén et al., 2011). Because of the downregulated symplastic transport, protoxylem cell identity was disrupted and metaxylem cells were ectopically expressed in the location of protoxylem cells (Vatén et al., 2011). In this manner, it is possible that plasmodesmata-mediated transport may have also driven the development of specialized cells and tissues by means of the spatiotemporal differential transport of nutrients.

Finally, it has become increasingly clear that the manner in which plasmodesmata are distributed within the multicellular plant body compartmentalizes this body into symplastic domains that can take on different functionalities by virtue of either sequestering aspects of metabolic activity, as for example during the dormancy of terminal tree buds (Tylewicz et al., 2018) or facilitating specific avenues of symplastic translocation, as for example the movement of mRNA within the phloem (Xoconostle-Cazares et al., 1999). When seen in this manner, the multicellular plant body plan is actually a continuous symplast incompletely partitioned by a continuous apoplast created by an infrastructure of perforated cell walls (Niklas and Kaplan, 1991).

Indeed, all the available evidence demonstrates the importance of plasmodesmata-mediated transport for plant development (Sager and Lee, 2014). Plasmodesmata seem to have appeared independently several times in the plant kingdom. Intercellular connections very similar to the plasmodesmata of land plants have been found in the multicellular species of the green, red, and brown algae (e.g., Cook et al., 1997, 1999; Raven, 1997). As in the case of the land plants, the plasmodesmata of the green alga *Bulbochaete hiloensis* are modulated during ontogeny in a manner that differentially limits intercellular transport and separates cellular domains into different functional identities (Fraser and Gunning, 1969; Kwiatkowska, 1999).

Some features of plasmodesmata seem to have evolved after the Chlorophyte–Streptophyte divergence. For example, there is some evidence that the encapsulation of the endoplasmic reticulum (ER) within the plasmodesmatal channel is unique to

the land plants. A close examination of plasmodesmata structure in the charophycean alga *Chara zeylanica* and in three putative early divergent bryophytes (the liverwort *Monoclea gottschei*, the hornwort *Notothylas orbicularis*, and the moss *Sphagnum fimbriatum*) reveals that in contrast to *C. zeylanica*, all three bryophytes have encased ER (Cook et al., 1997). The ER lumen serves as another pathway for intercellular transport making plasmodesmata transport more complex (Guenoune-Gelbart et al., 2008). The more complex plasmodesmata with internal ER of the land plants are present in some green algae, such as *Uronema* and *Aphanochaete* (Chlorophyceae) (Floyd et al., 1971; Stewart et al., 1973), and in some Laminariales brown algae (Marchant, 1976; Sideman and Scheirer, 1977). However, the movement of molecules through the lumen or the ER of these plasmodesmata has not been yet demonstrated for these algae. Based on these observations, it is reasonable to conclude that plasmodesmata lacking encased ER evolved first and that the encapsulation of ER is an evolutionarily derived feature that was present in the green algal ancestor of the land plants, well before bryophytes diverged (Lucas et al., 1993; Cook et al., 1997).

Given that the land plants are more complex than their algal ancestors because of the presence of specialized cell types and tissues for nutrient transport, we speculate that the increased complexity of multicellular plants is associated with the evolution of structurally complex encapsulated-ER plasmodesmata. This speculation emerges in part from a consideration of the limitations imposed by passive diffusion on the transport of metabolites and by the necessity of bypassing these limits as multicellularity resulted in larger and larger life-forms. Specifically, manipulation of Fick's second law of passive diffusion shows that the time it takes for the concentration of a non-electrolyte j initially absent from a cell's interior to reach one-half the concentration of j in the external ambient medium (denoted as $t_{0.5} - t_0$) is given by the formula

$$t_{0.5} - t_0 = \frac{V}{AP_j} \ln \frac{(c_0 - c_j)t_0}{(c_0 - c_j)t_{0.5}} = 0.693 \frac{V}{AP_j},$$

where V and A are the volume and the surface area of the cell, respectively, P_j is the permeability coefficient of j (a constant for any particular non-electrolyte), the expression $(c_0 - c_j)t_0$ is the initial difference between the external and internal concentrations of j at time zero, and the expression $(c_0 - c_j)t_{0.5}$ is the difference between the external and internal concentrations when the internal concentration of j reaches one-half that of the ambient medium (Niklas and Spatz, 2012). This formula shows that the time required for passive diffusion to provide essential metabolites to a cell increases in direct proportion to the volume of a cell. Beyond a certain surface area-to-volume limit, passive diffusion must be replaced by bulk flow, which is impossible within a unicellular non-aquatic organism. Consequently, the evolution of complex multicellularity requires intercellular bulk flow that necessitates some form of intercellular "porosity," e.g., phloem sieve plates. Likewise, intercellular transport systems require cell-type specialization, which has been shown to be positively correlated with genotypic and proteomic "complexity" (e.g., Niklas et al., 2014, 2018; Yruela et al., 2017).

Molecules that regulate plasmodesmata aperture and structure may have performed different functions in the ancestors of land plants. As previously noted, callose turnover is the main contributor to the regulating of plasmodesmata permeability. Although callose is widespread in the plant kingdom, its turnover regulated by plasmodesmata aperture has only been observed in the land plants (Scherp et al., 2001; Schuette et al., 2009). Thus, understanding the functionalities of plasmodesmata-localized proteins implicated in callose turnover could help elucidate the evolution of plasmodesmata structure and plasmodesmata-dependent transport. For example, glycosyl hydrolase 17 (GHL17) belongs to another family of proteins involved in callose degradation (Gaudioso-Pedraza and Benítez-Alfonso, 2014). A phylogenetic study using the sequences of GHL17 of fungi, algae, bryophytes, *Arabidopsis*, and monocots identifies a land plant specific clade characterized by plasmodesmata GHL17 localization (Gaudioso-Pedraza and Benítez-Alfonso, 2014). In contrast, the fungal and algal selected sequences appear to have diverged earlier than the land plant sequences, suggesting a more ancestral GHL17 origin (Gaudioso-Pedraza and Benítez-Alfonso, 2014). Other callose regulation proteins, such as the callose synthase (CalS) family, have been duplicated during the diversification of land plants (Drábková and Honys, 2017). Together, these findings indicate that plasmodesmata-localized proteins were already present in the land plant ancestor but that they played different roles.

As a consequence of plasmodesmata transport, plants can utilize the biogeneric properties of DPMs such as diffusion and lateral inhibition to specify cell identity and develop vascular tissues specialized in transporting nutrients over long distances. Without this capacity, plants would have not been able to generate the complex multicellular organisms that we know and that have become the major life form on earth. Despite the importance of plasmodesmata-mediated transport for plant development and diversification, little is known about their evolution. The reasons for this stem in part from the fact that plasmodesmata have structural characteristics that differ among different kinds of tissues as well as among the different plant lineages, and from the fact that the complete disruption of plant tissues is still challenging (Faulkner and Maule, 2011; Brunkard and Zambryski, 2017). However, some molecules that may be generically involved in the formation of plasmodesmata are now being postulated, as for example certain reticulons (Brunkard and Zambryski, 2017). It is likely that the advent of new methodologies that allow us to identify new plasmodesmata proteins will help elucidate the regulatory properties of plasmodesmata as well as the origins of these molecules and the genes encoding them in organisms that lack or that have less complex plasmodesmata.

MOLECULAR REGULATORY NETWORKS (MRNs): CO-OPTION, DRIFT AND PLANT EVOLUTIONARY TRANSITIONS

The notion of GRNs, recently referred to as molecular regulatory networks to include other types of molecules,

has allowed the fruitful exploration of the collective effect of genes and gene products in organismal development, although several other phenomena have been recently identified that call for a re-evaluation or update of current network modeling formalisms (e.g., the role of intrinsically disordered proteins in gene regulation, Niklas et al., 2015, 2018). Molecular regulatory networks integrate a set of nodes that can stand for genes, proteins, different types of RNA or other molecules, and a set of edges that correspond to the regulatory interactions among the elements represented by nodes. Multiple studies have aimed to study the dynamics of such networks, not only in plants, but also in animals, fungi and bacteria, mostly to test the idea that the steady states (attractors) of molecular regulatory networks correspond to specific cell types or cellular states (Kauffman, 1969; Thomas, 1991; Albert and Othmer, 2003; Alvarez-Buylla et al., 2007).

The picture emerging from theoretical and empirical studies is that molecular regulatory network steady states may indeed correspond to cell types or metabolic states, and that such different attractors can be present even in unicellular organisms that alternate between different phases or states in their life cycle (Quiñones-Valles et al., 2014; Mora Van Cauwelaert et al., 2015). However, the temporal coexistence of different cell types can only occur in multicellular organisms. Multiple studies have suggested that the molecular regulatory networks that underlie the specification of different cell-types in extant multicellular organisms may have been co-opted from multistable molecular regulatory networks, i.e., networks leading to more than one steady state, that were already present in their unicellular ancestors (Newman and Bhat, 2009; Mora Van Cauwelaert et al., 2015; Sebé-Pedrós et al., 2017). Indeed, mathematical and computational models have been used to perform proof-of-principle simulations that illustrate how single cells with multistable molecular regulatory networks can aggregate and couple via diverse communication mechanisms, giving rise to stereotypic and robust arrangements of cells with different identities (Furusawa and Kaneko, 2002; Mora Van Cauwelaert et al., 2015). This is a powerful idea, since this scenario requires no massive or abrupt genetic changes to explain one of the most major evolutionary transitions (Newman and Bhat, 2008, 2009; Niklas and Newman, 2013).

We have argued that some of the basic features of animal and plant body plans may have been generated by the cooption and differential spatiotemporal combination of DPMs (Newman and Bhat, 2008, 2009; Hernández-Hernández et al., 2012; Niklas and Newman, 2013; Niklas, 2014). However, DPMs are associated with molecules that are part of evolving regulatory networks such that DPM-related molecules and their regulatory interactions can change. As these networks evolve, DPMs may become canalized (*sensu* Waddington), this is, the patterns and shapes that were initially generated by generic physico-chemical processes mobilized by a few molecules can become somehow stabilized by the evolution of continuously more robust and intricate regulatory networks (Salazar-Ciudad et al., 2001). Molecular network evolution may also follow a trajectory characterized by

developmental system drift (DSD) (True and Haag, 2001), which suggests that genetic networks associated with phenotypes are both flexible and robust, and that differences between regulatory networks in related species arise by elimination or recruitment of new elements, erasing in this way traceable signals of common ancestry at the genetic level. DSD thus suggests that development of homologous traits in related species may not be mediated by homologous genetic factors (Müller and Newman, 1999; Rokas, 2006; Tsong et al., 2006; Kiontke et al., 2007; Nahmad and Lander, 2011; Sommer, 2012; Shbailat and Abouheif, 2013; Stolfi et al., 2014; Arias Del Angel et al., 2017). The mechanisms triggering divergence in the regulatory networks in DSD can involve both *cis*- and *trans*-regulatory changes, and the degree of change can vary from one system to another (Sommer, 2012; Stolfi et al., 2014).

We will now return to the DIF DPM example to illustrate some of these ideas.

All multicellular lineages with cellulosic cell walls appear to have evolved structures analogous to plasmodesmata. Indeed, as noted plasmodesmata evolved independently in different eukaryotic photosynthetic lineages and the molecules associated to their evolutionary origin are still unclear (Brunkard and Zambryski, 2017). However, some of the molecules that passively move through plasmodesmata and that are involved in the DIF DPM may have been co-opted from widely conserved molecular regulatory networks, some of which may predate plant multicellularity.

The case of auxin was briefly mentioned above. Indeed, currently available evidence shows that auxin biosynthesis was already present in the unicellular ancestors of multicellular eukaryotes (Beilby, 2016; Khasin et al., 2017; Ishizaki, 2017; Kato et al., 2017; among many other lines of evidence). However, this is not the case for the auxin transporters that have been identified and thoroughly studied in angiosperm model systems. It has thus been suggested that auxin initially moved only through plasmodesmata in a passive manner, contributing to multicellular organization through the formation of gradients and concentration patterns that could account for differential cellular behaviors and identities in vascular land plants, some algae, and bryophytes. If true, auxin transport seems to have been canalized and greatly potentialized by the evolution of complex molecular networks associated with its biosynthesis and transport. So much so, that in plants like *Arabidopsis* auxin local concentration is highly regulated and participates in diverse developmental processes and events under specific spatiotemporal conditions. Moreover, such tight regulation of auxin transport has enabled cellular and organ polarization, and has likely contributed to other evolutionary transitions, such as that to vascular plants. It also seems to be the case that extant networks associated with auxin biosynthesis and transport differ in particular elements and interactions, suggesting that the mechanisms of canalization have differed among plants or that some degree of developmental systems drift has occurred.

With regard to the DIF DPM, the role of molecular regulatory network cooption and further canalization is illustrated by the MYB-bHLH-WD40 protein complex. These plant proteins are

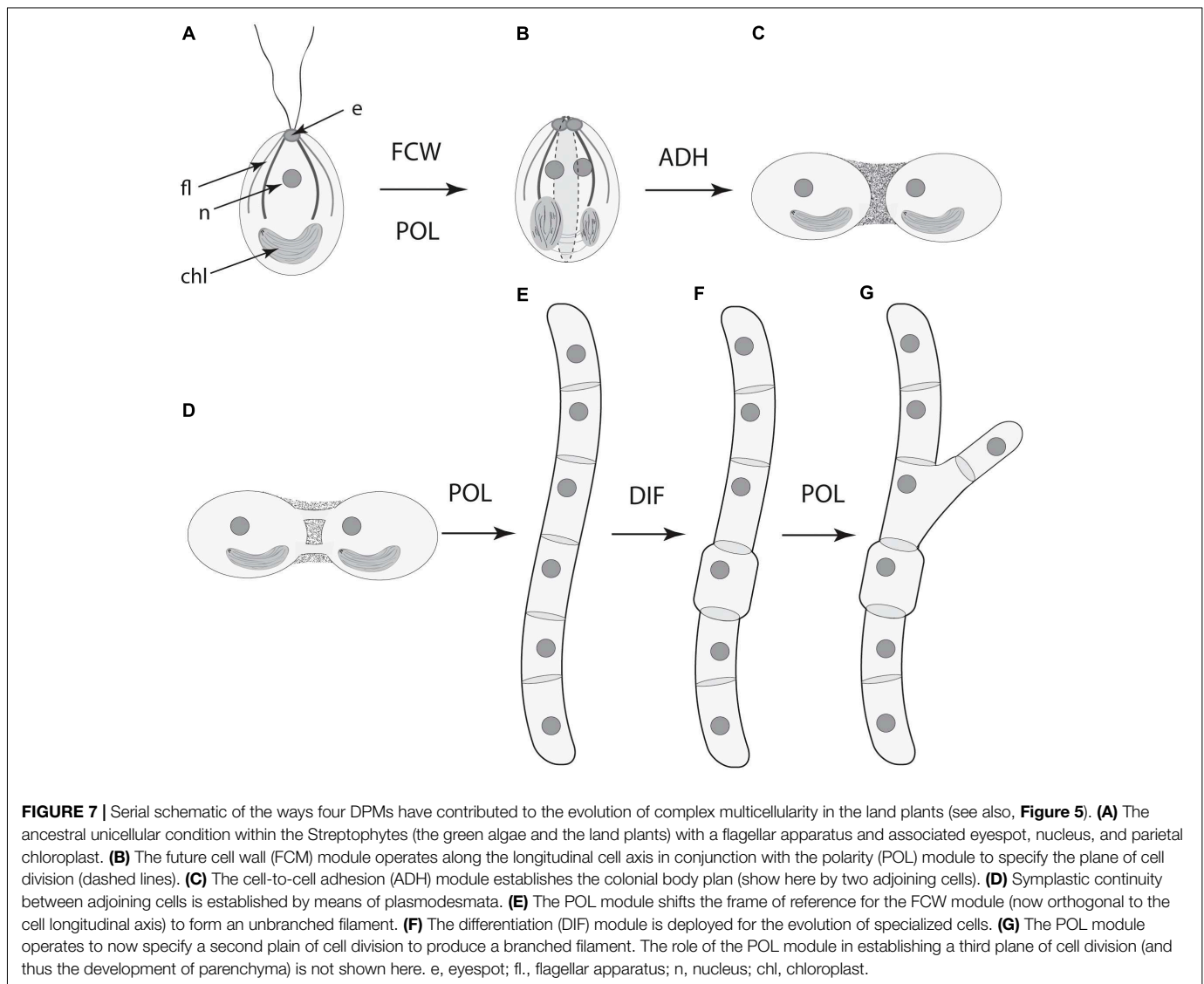
involved in complex networks that act in different plant organs and developmental stages, enabling the determination of diverse cell types, such as stomata, pavement cells, trichomes, and trichoblasts (Ramsay and Glover, 2005; Benítez et al., 2011; Torii, 2012; Horst et al., 2015; Breuninger et al., 2016). A central feature of this complex is that some of its components may move to neighboring cells through plasmodesmata, which gives rise to the coupling of otherwise intracellular networks and, concomitantly, the emergence of stereotypic cellular arrangements. Indeed, it is by the intercellular transport and mutual regulation of MYB, bHLH, and WD40 proteins that some of the well-known patterns of spaced-out stomata, trichomes, and aligned root hairs arise during plant development (Benítez et al., 2011; Torii, 2012; Horst et al., 2015). Interestingly, although the molecular regulatory networks in which these proteins take part seem to have come together in land plants, their key components appear to predate plant multicellularity (Ramsay and Glover, 2005). Consequently, some of the major events in the diversification of cellular types and functions have involved the co-option of ancient molecules, the presence of plasmodesmata, and the associated mobilization of certain DPMs, even if the MYB-bHLH-WD40 complex has drifted into regulatory systems that are currently species- or even organ-dependent.

FINAL REMARKS

Based on our review of the available evidence, we reach the following conclusions:

- Dynamical patterning modules, defined as sets of conserved gene products and molecular networks in conjunction with the physical morphogenetic and patterning processes they mobilize, have played ubiquitous and central roles in the evolution of multicellularity in the algae, land plants, fungi, as they have been shown to in metazoans (schematized in **Figure 7**).
- Four DPMs are critical in the context of multicellular evolution of plants and fungi: the DPM for the orientation of the future cell wall (FCW), the DPM for cell-to-cell adhesion (ADH), the DPM for polarity (POL), and the DPM for differentiation (DIF).
- Comparisons across the various fungal, algal, and land plant lineages indicate that these four DPMs have recruited different mechanisms and materials to achieve the same ends. For example, the materials used to achieve cell-to-cell adhesion differ dramatically among the various multicellular algal lineages and the land plants.
- These differences in the materials recruited by the various DPMs indicate that natural selection operates on morphological phenotypes and not on the components and mechanisms that produce them.

As exemplified in this study focusing on plant multicellularity, the DPM concept provides a valuable framework to further understand the processes behind multicellular development and evolution and can give rise to clear propositions that can in



turn be tested through comparative methods, mathematical and computational modeling, and experimental modification of parameters and biogenic properties. However, the DPM concept has not been fully integrated into the “standard model” of contemporary evolutionary developmental biology. Typically, “mechanism” is considered at the level of genes and gene networks, while morphology is handled descriptively, with adaptationist narratives where they pertain, and appeals to pleiotropy and its consequences when they do not (Minelli, 2018). This perspective is unsatisfactory as an explanatory framework for biological form in light of the unquestioned role of physical mechanisms of morphogenesis across all categories of multicellular (Forgacs and Newman, 2005; Niklas and Newman, 2013; Newman and Niklas, 2018) and increased recognition of the conservation of early-evolved architectural motifs despite drift in molecular mechanisms (True and Haag, 2001).

Such homoplasy is even more pervasive in plant than in animal systems, where, as we have described here and elsewhere,

there have been multiple routes to multicellularity rather than the single, classical cadherin-based, one in the metazoans (Niklas and Newman, 2013; Newman, 2016b). Moreover, the ability of the cyanobacteria, the land plants, and the brown algae to form plasmodesmata-like intercellular structures involves significantly different GRNs, gene products, and developmental processes. Yet, the result in each case is the same, i.e., intercellular adhesion, communication, and polarity.

Whereas in animal systems GRNs and DPMs act relatively independently of each other, with the former mainly specifying cell type identity and latter patterns and arrangements of cells (Newman et al., 2009), the molecular regulatory networks of plants and fungi act in a more integrated fashion, comprising both GRN- and DPM-type functions of metazoans. This is partly because transcription factors move more freely between cells in the non-metazoans. Moreover, since the physics embodied in DPMs often leads to predictable morphological outcomes, these modules have served as “simplification forces” in evolution, acting as major instructive cues that channel development in both

animals and plants. In contrast to animal GRNs, however, the mixed-nature plant molecular regulatory networks have been “complexification forces” in plant and fungal evolution, offering additional opportunities to use/modulate/bridge DPMs to generate an enhanced spectrum of morphological complexity.

The behaviors of developing tissues as excitable biogenic materials (liquids and liquid crystals in the case of animals, deformable cellular solids in the case of plants), are inescapable, as are the preferred morphological motifs generated by characteristic DPMs of these materials, whatever their molecular genetic underpinnings may be. Understanding these inherent properties is essential to mechanistic explanations of development and its transformations during the evolution of multicellular organisms (Newman, 2017). A challenge for future research is to determine how these modules recruit and integrate the ancillary processes required to achieve the morphological variety seen across the broad phylogenetic spectrum of multicellular plants and fungi.

REFERENCES

- Alassimone, J., Naseer, S., and Geldner, N. (2010). A developmental framework for endodermal differentiation and polarity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 5214–5219. doi: 10.1073/pnas.0910772107
- Albert, R., and Othmer, H. G. (2003). The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in *Drosophila melanogaster*. *J. Theor. Biol.* 223, 1–18. doi: 10.1016/S0022-5193(03)00035-3
- Alvarez-Buylla, E. R., Benítez, M., Dávila, E. B., Chaos, A., Espinosa-Soto, C., and Padilla-Longoria, P. (2007). Gene regulatory network models for plant development. *Curr. Opin. Plant Biol.* 10, 83–91. doi: 10.1016/j.pbi.2006.11.008
- Arias Del Angel, J. A., Escalante, A. E., Martínez-Castilla, L. P., and Benítez, M. (2017). An evo-devo perspective on multicellular development of Myxobacteria. *J. Exp. Zool. B Mol. Dev. Evol.* 328, 165–178. doi: 10.1002/jez.b.22727
- Baumont, N. (2009). Modelling the transport of nutrients in early animals. *Evol. Biol.* 36, 256–266. doi: 10.1007/s11692-008-9047-2
- Beilby, M. J. (2016). Multi-scale characean experimental system: from electrophysiology of membrane transporters to cell-to-cell connectivity, cytoplasmic streaming and auxin metabolism. *Front. Plant Sci.* 7:1052. doi: 10.3389/fpls.2016.01052
- Benítez, M., and Hejatko, J. (2013). Dynamics of cell-fate determination and patterning in the vascular bundles of *Arabidopsis thaliana*. *PLoS One* 8:e63108. doi: 10.1371/journal.pone.0063108
- Benítez, M., Monk, N. A., and Alvarez-Buylla, E. R. (2011). Epidermal patterning in *Arabidopsis*: models make a difference. *J. Exp. Zool. B Mol. Dev. Evol.* 316, 241–253. doi: 10.1002/jez.b.21398
- Benítez-Alfonso, Y., Faulkner, C., Pendle, A., Miyashima, S., Helariutta, Y., and Maule, A. (2013). Symplastic intercellular connectivity regulates lateral root patterning. *Dev. Cell* 26, 136–147. doi: 10.1016/j.devcel.2013.06.010
- Bloemendal, S., and Kück, U. (2013). Cell-to-cell communication in plants, animals, and fungi: a comparative review. *Naturwissenschaften* 100, 3–19. doi: 10.1007/s00114-012-0988
- Bonner, J. T. (2000). *First Signals: The Evolution of Multicellular Development*. Princeton, NJ: Princeton University Press.
- Breuninger, H., Thamm, A., Streubel, S., Sakayama, H., Nishiyama, T., and Dolan, L. (2016). Diversification of a transcription factor family led to the evolution of antagonistically acting genetic regulators of root hair growth. *Curr. Biol.* 26, 1622–1628. doi: 10.1016/j.cub.2016.04.060
- Brunkard, J. O., and Zambryski, P. C. (2017). Plasmodesmata enable multicellularity: new insights into their evolution, biogenesis, and functions in development and immunity. *Curr. Opin. Plant Biol.* 35, 76–83. doi: 10.1016/j.pbi.2016.11.007
- Cannon, M. C., Terneus, K., Hall, Q., Tan, L., Wang, Y., Wegenhart, B. L., et al. (2008). Self-assembly of the plant cell wall requires an extension scaffold. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2226–2231. doi: 10.1073/pnas.0711980105
- Carlsbecker, A., Lee, J. Y., Roberts, C. J., Dettmer, J., Lehesranta, S., Zhou, J., et al. (2010). Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465, 316–321. doi: 10.1038/nature08977
- Christensen, N. M., Faulkner, C., and Oparka, K. (2009). Evidence for unidirectional flow through plasmodesmata. *Plant Physiol.* 150, 96–104. doi: 10.1104/pp.109.137083
- Cook, M., Graham, L., Botha, C., and Lavin, C. (1997). Comparative ultrastructure of plasmodesmata of Chara and selected bryophytes: toward an elucidation of the evolutionary origin of plant plasmodesmata. *Am. J. Bot.* 84:1169. doi: 10.2307/2446040
- Cook, M., Graham, L., Van Bel, A. J. E., and van Kesteren, W. J. P. (1999). “Evolution of plasmodesmata,” in *Plasmodesmata: Structure, Function, Role in Cell Communication*, eds A. J. E. van Bel and W. J. P. Kesteren (Berlin: Springer).
- Cui, H., Levesque, M. P., Vernoux, T., Jung, J. W., Paquette, A. J., Gallagher, K. L., et al. (2007). An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. *Science* 316, 421–425. doi: 10.1126/science.1139531
- De Storme, N., and Geelen, D. (2014). Callose homeostasis at plasmodesmata: molecular regulators and developmental relevance. *Front. Plant Sci.* 5:138. doi: 10.3389/fpls.2014.00138
- Dettmer, J., and Friml, J. (2011). Cell polarity in plants: when two do the same, it is not the same. *Curr. Opin. Cell Biol.* 23, 686–696. doi: 10.1016/jceb.2011.09.006
- Drábková, L. Z., and Honys, D. (2017). Evolutionary history of callose synthases in terrestrial plants with emphasis on proteins involved in male gametophyte development. *PLoS One* 12:e0187331. doi: 10.1371/journal.pone.0187331
- Faulkner, C., and Maule, A. (2011). Opportunities and successes in the search for plasmodesmal proteins. *Protoplasmata* 248, 27–38. doi: 10.1007/s00709-010-0213-x
- Ferris, P. J., Woessner, J. P., Waffenschmidt, S., Kilz, S., Drees, J., and Goodenough, U. W. (2001). Glycosylated polyproline II rods with kinks as a structural motif in plant hydroxyproline-rich glycoproteins. *Biochemistry* 40, 2978–2987. doi: 10.1021/bi0023605
- Fleury, V. (1999). Un possible lien entre la croissance dendritique en physique et la morphogénèse des plantes. *C. R. Acad. Sci. Paris Ser. III Sci. Vie* 322, 725–734. doi: 10.1016/S0764-4469(00)80030-X
- Floyd, G. L., Stewart, K. D., and Mattox, K. R. (1971). Cytokinesis and plasmodesmata in Ulothrix. *J. Phycol.* 7, 306–309.
- Folse, H. J. Jr., and Roughgarden, J. (2010). What is an individual organism? A multilevel selection perspective. *Q. Rev. Biol.* 85, 447–472. doi: 10.1086/656905

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

Funding from the College of Agriculture and Life Sciences (to KN) and CONACyt (to VH-H) is gratefully acknowledged.

ACKNOWLEDGMENTS

The authors thank Drs. Verónica S. Di Stilio (University of Washington), Annette Becker (Justus-Liebig-Universität Gießen), and Natalia Pabón-Mora (Universidad de Antioquia Medellín) for inviting this contribution and reviewers for their perceptive and constructive comments.

- Forgacs, G., and Newman, S. A. (2005). *Biological Physics of the Developing Embryo*. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511755576
- Fraser, T. W., and Gunning, B. E. S. (1969). The ultrastructure of plasmodesmata in the filamentous green alga, *Bulbochaete hiloensis* (Nordst.) tiffany. *Planta* 88, 244–254. doi: 10.1007/BF00385067
- Furusawa, C., and Kaneko, K. (2002). Origin of multicellular organisms as an inevitable consequence of dynamical systems. *Anat. Rec.* 268, 327–342. doi: 10.1002/ar.10164
- Galletti, R., Verger, S., Hamant, O., and Ingram, G. C. (2016). Developing a ‘thick skin’: a paradoxical role for mechanical tension in maintaining epidermal integrity? *Development* 143, 3249–3258. doi: 10.1242/dev.132837
- Gardiner, J. (2013). The evolution and diversification of plant microtubule-associated proteins. *Plant J.* 75, 219–229. doi: 10.1111/tpj.12189
- Garrett, J. J., Meets, M. J., Blackshaw, M. T., Blackshaw, L. C., Hou, H., Styranko, D. M., et al. (2012). A novel, semi-dominant allele of MONOPTEROS provide insight into leaf initiation and vein pattern formation. *Planta* 236, 297–312. doi: 10.1007/s00425-012-1607-0
- Gaudio-Pedraza, R., and Benítez-Alfonso, Y. (2014). A phylogenetic approach to study the origin and evolution of plasmodesmata-localized glycosyl hydrolases family 17. *Front. Plant Sci.* 5:212. doi: 10.3389/fpls.2014.00212
- Geldner, N. (2009). Cell polarity in plants—A PARspective on PINs. *Curr. Opin. Plant Biol.* 12, 42–48. doi: 10.1016/j.pbi.2008.09.009
- Graham, L. E., Graham, J. M., and Wilcox, L. W. (2009). *Algae*, 2nd Edn. San Francisco, CA: Benjamin Cummings.
- Green, K. J., Viamontes, G. I., and Kirk, D. L. (1981). Mechanism of formation, ultrastructure and function of the cytoplasmic bridge system during morphogenesis in *Volvox*. *J. Cell Biol.* 91, 756–769. doi: 10.1083/jcb.91.3.756
- Green, S., and Batterman, R. (2017). Biology meets physics: reductionism and multi-scale modeling of morphogenesis. *Stud. Hist. Philos. Biol. Biomed. Sci.* 61, 20–34. doi: 10.1016/j.shpsc.2016.12.003
- Grosberg, R. K., and Strathmann, R. (2007). The evolution of multicellularity: a minor major transition? *Annu. Rev. Ecol. Evol. Syst.* 38, 621–654. doi: 10.1146/annurev.ecolsys.36.102403.114735
- Guenoune-Gelbart, D., Elbaum, M., Sagi, G., Levy, A., and Epel, B. L. (2008). *Tobacco mosaic virus* (TMV) replicase and movement protein function synergistically in facilitating TMV spread by lateral diffusion in the plasmodesmal desmotubule of *Nicotiana benthamiana*. *Mol. Plant Microbe Interact.* 21, 335–345. doi: 10.1094/MPMI-21-3-0335
- Guseman, J. M., Lee, J. S., Bogenschütz, N. L., Peterson, K. M., Virata, R. E., Xie, B., et al. (2010). Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in *Arabidopsis* CHORUS (*GLUCAN SYNTHASE-LIKE 8*). *Development* 137, 1731–1741. doi: 10.1242/dev.049197
- Hall, J. D., McCourt, R. M., and Delwiche, C. F. (2008). Patterns of cell division in the filamentous Desmidiaceae, close green algal relatives of land plants. *Am. J. Bot.* 95, 643–654. doi: 10.3732/ajb.2007210
- Han, X., Hyun, T. K., Zhang, M., Kumar, R., Koh, E. J., Kang, B. H., et al. (2014). Auxin-callose-mediated plasmodesmal gating is essential for tropic auxin gradient formation and signaling. *Dev. Cell* 28, 132–146. doi: 10.1016/j.devcel.2013.12.008
- Hernández-Hernández, V., Niklas, K. J., Newman, S. A., and Benítez, M. (2012). Dynamical patterning modules in plant development and evolution. *Int. J. Dev. Biol.* 56, 661–674. doi: 10.1387/ijdb.120027mb
- Herron, M. D., and Michod, R. E. (2008). Evolution of complexity in the volvocine algae: transitions in individuality through Darwin’s eye. *Evolution* 62, 436–451. doi: 10.1111/j.1558-5646.2007.00304.x
- Hoops, H. J., Nishii, I., and Kirk, D. L. (2000). “Cytoplasmic bridges in *Volvox* and its relatives,” in *Madame Curie Bioscience Database [Internet]* (Austin, TX: Landes Bioscience). Available at: <http://www.ncbi.nlm.nih.gov/books/NBK6424/>
- Horst, R. J., Fujita, H., Lee, J. S., Rychel, A. L., Garrick, J. M., Kawaguchi, M., et al. (2015). Molecular framework of a regulatory circuit initiating two-dimensional spatial patterning of stomatal lineage. *PLoS Genet.* 11:e1005374. doi: 10.1371/journal.pgen.1005374
- Ishizaki, K. (2017). Evolution of land plants: insights from molecular studies on basal lineages. *Biosci. Biotechnol. Biochem.* 81, 73–80. doi: 10.1080/09168451.2016.1224641
- Kato, H., Nishihama, R., Weijers, D., and Kohchi, T. (2017). Evolution of nuclear auxin signaling: lessons from genetic studies with basal land plants. *J. Exp. Bot.* 69, 291–301. doi: 10.1093/jxb/erx267
- Kauffman, S. A. (1969). Metabolic stability and epigenesis in randomly constructed genetic nets. *J. Theor. Biol.* 22, 437–467. doi: 10.1016/0022-5193(69)90015-0
- Khasin, M., Cahoon, R. R., Nickerson, K. W., and Riekhof, W. R. (2017). Molecular machinery of auxin synthesis, secretion, and perception in the unicellular chlorophyte alga *Chlorella sorokiniana* UTEX 1230. *bioRxiv* doi: 10.1101/172833 [Preprint].
- Kiontke, K., Barriere, A., Kolotuev, I., Podbilewicz, B., Sommer, R., Fitch, D. H., et al. (2007). Trends, stasis, and drift in the evolution of nematode vulva development. *Curr. Biol.* 17, 1925–1937. doi: 10.1016/j.cub.2007.10.061
- Kirk, D. L. (2005). A twelve-step program for evolving multicellularity and a division of labor. *Bioessays* 27, 299–310. doi: 10.1002/bies.20197
- Kitagawa, M., and Fujita, T. (2013). Quantitative imaging of directional transport through plasmodesmata in moss protonemata via single-cell photoconversion of Dendra2. *J. Plant Res.* 126, 577–585. doi: 10.1007/s10265-013-0547-5
- Knoll, A. (2011). The multiple origins of complex multicellularity. *Annu. Rev. Earth Planet. Sci.* 39, 217–239. doi: 10.1146/annurev.earth.031208.100209
- Kutschera, U., and Niklas, K. J. (2005). Endosymbiosis, cell evolution, and speciation. *Theory Biosci.* 124, 1–24. doi: 10.1016/j.thbio.2005.04.001
- Kutschera, U., and Niklas, K. J. (2007). The epidermal-growth-control theory of stem elongation: an old and new perspective. *Plant Physiol.* 164, 1395–1409. doi: 10.1016/j.jplph.2007.08.002
- Kutschera, U., and Niklas, K. J. (2008). Macroevolution via secondary endosymbiosis: a Neo-Goldschmidian view of unicellular hopeful monsters and Darwin’s primordial intermediate form. *Theory Biosci.* 127, 277–289. doi: 10.1007/s12064-008-0046-8
- Kwiatkowska, M. (1999). “Plasmodesmal coupling and cell differentiation in algae,” in *Plasmodesmata: Structure, Function, Role in Cell Communication*, eds A. J. E. Van Bel and W. J. P. van Kesteren (Berlin: Springer).
- Lampert, D. T. A., Kieliszewski, M. J., Chen, Y., and Cannon, M. C. (2011). Role of the extension superfamily in primary cell wall architecture. *Plant Physiol.* 156, 11–19. doi: 10.1104/pp.110.169011
- Levine, H., and Ben-Jacob, E. (2004). Physical schemata underlying biological pattern formation—examples, issues and strategies. *Phys. Biol.* 1, P14–P22. doi: 10.1088/1478-3967/1/2/P01
- Lucas, W. I., Ding, B., and Van der Schoot, C. (1993). Plasmodesmata and the supracellular nature of plants. *New Phytol.* 125, 435–476. doi: 10.1111/j.1469-8137.1993.tb03897.x
- Lund, E. J. (1947). *Bioelectric Fields and Growth*. Austin, TX: University of Texas Press.
- Majda, M., Groner, P., Sinton, I.-M., Vain, T., Milani, P., Krupinski, P., et al. (2017). Mechanochemical polarization of contiguous cell walls shapes plant pavement cells. *Dev. Cell* 43, 290–304. doi: 10.1016/j.devcel.2017.10.017
- Marchant, H. J. (1976). “Plasmodesmata in algae and fungi,” in *Intercellular Communication in Plants: Studies on Plasmodesmata*, eds B. E. S. Gunning and A. W. Robards (New York, NY: Springer), 59–78.
- McGhee, G. R. (1999). *Theoretical Morphology: The Concept and Its Applications*. New York, NY: Columbia University Press.
- Menzel, D. (1996). The role of the cytoskeleton in polarity and morphogenesis of algal cells. *Curr. Opin. Cell Biol.* 1, 38–42. doi: 10.1016/S0955-0674(96)80046-9
- Meyerowitz, E. M. (2002). Plants compared to animals: the broadest comparative study of development. *Science* 295, 1482–1485. doi: 10.1126/science.1066609
- Minelli, A. (2018). *Plant Evolutionary Developmental Biology: The Evolvability of the Phenotype*. Cambridge: Cambridge University Press. doi: 10.1017/9781139542364
- Mora Van Cauwelaert, E., Del Angel, A., Antonio, J., Benítez, M., and Azpeitia, E. M. (2015). Development of cell differentiation in the transition to multicellularity: a dynamical modeling approach. *Front. Microbiol.* 6:603. doi: 10.3389/fmicb.2015.00603
- Müller, G. B., and Newman, S. A. (1999). “Generation, integration, autonomy: three steps in the evolution in homology,” in *Homology*, Vol. 222, eds G. K. Bock and G. Cardew (Chichester: Wiley), 65–73.
- Nahmad, M., and Lander, A. D. (2011). Spatiotemporal mechanisms of morphogen gradient interpretation. *Curr. Opin. Genet. Dev.* 21, 726–731. doi: 10.1016/j.gde.2011.10.002

- Newman, S. A. (2011). The developmental specificity of physical mechanisms. *Ludus Vitalis* 19, 343–351.
- Newman, S. A. (2012). Physico-genetic determinants in the evolution of development. *Science* 338, 217–219. doi: 10.1126/science.1222003
- Newman, S. A. (2016a). 'Biogeneric' developmental processes: drivers of major transitions in animal evolution. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371:20150443. doi: 10.1098/rstb.2015.0443
- Newman, S. A. (2016b). "Multicellularity, the emergence of animal body plans, and the stabilizing role of the egg," in *Multicellularity: Origins and Evolution*, eds K. J. Niklas and S. A. Newman (Cambridge, MA: MIT Press), 225–246.
- Newman, S. A. (2017). "Inherency," in *Evolutionary Developmental Biology*, eds L. Nuño de la Rosa and G. B. Müller (Basel: Springer). doi: 10.1007/978-3-319-33038-9_78-1
- Newman, S. A., and Bhat, R. (2008). Dynamical patterning modules: physico-genetic determinants of morphological development and evolution. *Phys. Biol.* 5:15008. doi: 10.1088/1478-3975/5/1/015008
- Newman, S. A., and Bhat, R. (2009). Dynamical patterning modules: a "pattern language" for development and evolution of multicellular form. *Int. J. Dev. Biol.* 53, 693–705. doi: 10.1387/ijdb.072481sn
- Newman, S. A., Bhat, R., and Mezentseva, N. V. (2009). Cell state switching factors and dynamical patterning modules: complementary mediators of plasticity in development and evolution. *J. Biosci.* 34, 553–572. doi: 10.1007/s12038-009-0074-7
- Newman, S. A., and Niklas, K. J. (2018). "Dynamical patterning modules link genotypes to morphological phenotypes in multicellular evolution," in *Cells in Evolutionary Biology*, eds B. K. Hall and S. A. Moody (Boca Raton, FL: CRC Press), 235–266.
- Niklas, K. J. (1989). Mechanical behavior of plant tissues as inferred from the theory of pressurized cellular solids. *Am. J. Bot.* 76, 929–937. doi: 10.1002/j.1537-2197.1989.tb15071.x
- Niklas, K. J. (1992). *Plant Biomechanics*. Chicago, IL: University of Chicago Press.
- Niklas, K. J. (1994). *Plant Allometry*. Chicago, IL: University of Chicago Press.
- Niklas, K. J. (2000). The evolution of plant body plans — A biomechanical perspective. *Ann. Bot.* 85, 411–438. doi: 10.1006/anbo.1999.1100
- Niklas, K. J. (2014). The evolutionary-developmental origins of multicellularity. *Am. J. Bot.* 101, 6–25. doi: 10.3732/ajb.1300314
- Niklas, K. J. (2017). Size-dependent variation in plant form. *Curr. Biol.* 27, R853–R909. doi: 10.1016/j.cub.2017.02.007
- Niklas, K. J., Bondos, S. E., Dunker, A. K., and Newman, S. A. (2015). Rethinking gene regulatory network theory in light of alternative splicing, intrinsically disordered protein domains, and post-translational modifications. *Front. Cell Dev. Biol.* 3:8. doi: 10.3389/fcell.2015.00008
- Niklas, K. J., Cobb, E. D., and Crawford, D. R. (2013). The evo-devo of multicellular cells, tissues and organisms, and an alternative route to multicellularity. *Evol. Dev.* 15, 466–474. doi: 10.1111/ede.12055
- Niklas, K. J., Cobb, E. D., and Dunker, A. K. (2014). The number of cell types, information content, and the evolution of multicellularity. *Acta Soc. Bot. Pol.* 83, 337–347. doi: 10.5586/asbp.2014.034
- Niklas, K. J., Dunker, A. K., and Yruela, I. (2018). The evolutionary origins of cell type diversification and the role of intrinsically disordered proteins. *J. Exp. Bot.* 69, 1427–1446. doi: 10.1093/jxb/erx493
- Niklas, K. J., and Kaplan, D. R. (1991). "Biomechanics and the adaptive significance of multicellularity in plants," in *Proceedings of the Fourth International Congress of Systematics and Evolutionary Biology: The Unity of Evolutionary Biology*, ed. E. C. Dudley (Portland, OR: Dioscorides Press), 489–502.
- Niklas, K. J., and Newman, S. A. (2013). The origins of multicellular organisms. *Evol. Dev.* 15, 41–52. doi: 10.1111/ede.12013
- Niklas, K. J., and Spatz, H.-C. (2012). *Plant Physics*. London: University of Chicago Press. doi: 10.7208/chicago/9780226586342.001.0001
- Peaucelle, A., Wightman, R., and Höfte, H. (2015). The control of growth symmetry breaking in the *Arabidopsis* hypocotyl. *Curr. Biol.* 25, 1746–1752. doi: 10.1016/j.cub.2015.05.022
- Pillitteri, L. J., and Dong, J. (2013). Stomatal development in *Arabidopsis*. *Arabidopsis Book* 11:e0162. doi: 10.1199/tab.0162
- Prochiantz, A. (2011). Homeoprotein intercellular transfer, the hidden face of cell—Penetrating peptides. *Methods Mol. Biol.* 683, 249–257. doi: 10.1007/978-1-60761-919-2_18
- Quiñones-Valles, C., Sánchez-Osorio, I., and Martínez-Antonio, A. (2014). Dynamical modeling of the cell cycle and cell fate emergence in *Caulobacter crescentus*. *PLoS One* 9:e111116. doi: 10.1371/journal.pone.0111116
- Ramsay, N. A., and Glover, B. J. (2005). MYB–bHLH–WD40 protein complex and the evolution of cellular diversity. *Trends Plant Sci.* 10, 63–70. doi: 10.1016/j.tplants.2004.12.011
- Ranjan, A., Townsley, B. T., Ichihashi, Y., Sinha, N. R., and Chitwood, R. H. (2015). An intracellular transcriptomic atlas of the giant coenocyte *Caulerpa taxifolia*. *PLoS Genet.* 11:e1004900. doi: 10.1371/journal.pgen.1004900
- Raven, J. A. (1997). Miniview: multiple origins of plasmodesmata. *Eur. J. Phycol.* 32, 95–101. doi: 10.1080/09670269710001737009
- Rokas, A. (2006). Evolution: different paths to the same end. *Nature* 443, 401–402. doi: 10.1038/443401a
- Ruan, Y. L., Xu, S. M., White, R., and Furbank, R. T. (2004). Genotypic and developmental evidence for the role of plasmodesmatal regulation in cotton fiber elongation mediated by callose turnover. *Plant Physiol.* 136, 4104–4113. doi: 10.1104/pp.104.051540
- Sager, R., and Lee, J. Y. (2014). Plasmodesmata in integrated cell signalling: insights from development and environmental signals and stresses. *J. Exp. Bot.* 65, 6337–6358. doi: 10.1093/jxb/eru365
- Salazar-Ciudad, I., Newman, S. A., and Solé, R. (2001). Phenotypic and dynamical transitions in model genetic networks. I. Emergence of patterns and genotype-phenotype relationships. *Evol. Dev.* 3, 84–94. doi: 10.1046/j.1525-142x.2001.003002084.x
- Schaefer, E., Belcram, K., Uyttewaal, M., Duroc, Y., Goussot, M., Legland, D., et al. (2017). The preprophase band of microtubules controls the robustness of division orientation in plants. *Proc. Natl. Acad. Sci. U.S.A.* 356, 186–189. doi: 10.1126/science.aal3016
- Scherp, P., Grotha, R., and Kutschera, U. (2001). Occurrence and phylogenetic significance of cytokinesis-related callose in green algae, bryophytes, ferns and seed plants. *Plant Cell Rep.* 20, 143–149. doi: 10.1007/s002990000301
- Schuette, S., Wood, A. J., Geisler, M., Geisler-Lee, J., Ligrone, R., and Renzaglia, K. S. (2009). Novel localization of callose in the spores of *Physcomitrella patens* and phylogenomics of the callose synthase gene family. *Ann. Bot.* 103, 749–756. doi: 10.1093/aob/mcn268
- Sebé-Pedrós, A., Degnan, B. M., and Ruiz-Trillo, I. (2017). The origin of Metazoa: a unicellular perspective. *Nat. Rev. Genet.* 18, 498–512. doi: 10.1038/nrg.2017.21
- Seiler, S., and Justa-Schuch, D. (2010). Conserved components, but distinct mechanisms for the placement and assembly of the cell division machinery in unicellular and filamentous ascomycetes. *Mol. Microbiol.* 78, 1058–1076. doi: 10.1111/j.1365-2958.2010.07392.x
- Shbailat, S. J., and Abouheif, E. (2013). The wing-patterning network in the wingless castes of Myrmicine and Formicine ant species is a mix of evolutionarily labile and non-labile genes. *J. Exp. Zool. B Mol. Dev. Evol.* 320, 74–83. doi: 10.1002/jez.b.22482
- Shin, K., Fogg, V. C., and Margolis, B. (2006). Tight junctions and cell polarity. *Annu. Rev. Cell Dev. Biol.* 22, 207–235. doi: 10.1146/annurev.cellbio.22.010305.104219
- Sideman, E. J., and Scheirer, D. C. (1977). Some fine structural observations on developing and mature sieve elements in the brown alga *Laminaria saccharina*. *Am. J. Bot.* 64, 649–657. doi: 10.1002/j.1537-2197.1977.tb11905.x
- Sinha, S., and Sridhar, S. (2015). *Patterns in Excitable Media: Genesis, Dynamics, and Control*. Boca Raton, FL: CRC Press.
- Sommer, R. J. (2012). Evolution of regulatory networks: nematode vulva induction as an example of developmental systems drift. *Adv. Exp. Med. Biol.* 751, 79–91. doi: 10.1007/978-1-4614-3567-9_4
- Stanley, M. S., Perry, R. M., and Callow, J. A. (2005). Analysis of expressed sequence tags (ESTs) from the green alga *Ulva linza* (Chlorophyta). *J. Phycol.* 41, 1219–1226. doi: 10.1111/j.1529-8817.2005.00138.x
- Stewart, K. D., Mattox, K. R., and Floyd, G. L. (1973). Mitosis, cytokinesis, the distribution of plasmodesmata, and other cytological characteristics in the Ulotrichales, Ulvales, and Chaetophorales: phylogenetic and taxonomic considerations. *J. Phycol.* 9, 128–141. doi: 10.1111/j.1529-8817.1973.tb04068.x
- Stolfi, A., Lowe, E. K., Racioppi, C., Ristoratore, F., Brown, C. T., Swalla, B. J., et al. (2014). Divergent mechanisms regulate conserved cardiopharyngeal development and gene expression in distantly related ascidians. *eLife* 3:e03728. doi: 10.7554/eLife.03728

- Sumper, M., and Hallmann, A. (1998). Biochemistry of the extracellular matrix of *Volvox*. *Int. Rev. Cytol.* 180, 51–85. doi: 10.1016/S0074-7696(08)61770-2
- Swift, J., Ivanovska, I. L., Buxboim, A., Harada, T., Dingal, P. C., Pinter, J., et al. (2013). Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* 341:1240104. doi: 10.1126/science.1240104
- Thomas, R. (1991). Regulatory networks seen as asynchronous automata: a logical description. *J. Theor. Biol.* 153, 1–23. doi: 10.1016/S0022-5193(05)80350-9
- Torii, K. U. (2012). Two-dimensional spatial patterning in developmental systems. *Trends Cell Biol.* 22, 438–446. doi: 10.1016/j.tcb.2012.06.002
- True, J. R., and Haag, E. S. (2001). Developmental system drift and flexibility in evolutionary trajectories. *Evol. Dev.* 3, 109–119. doi: 10.1046/j.1525-142x.2001.003002109.x
- Tsong, A. E., Tuch, B. B., Li, H., and Johnson, A. D. (2006). Evolution of alternative transcriptional circuits with identical logic. *Nature* 443, 415–420. doi: 10.1038/nature05099
- Tylewicz, S., Petterle, A., Marttila, S., Miskolczy, P., Azeez, A., Singh, R. K., et al. (2018). Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. *Science* 360, 212–214. doi: 10.1126/science.aan8576
- Urbanus, S. L., Martinelli, A. P., Dinh, Q. D. P., Aizza, L. C. B., Dornelas, M. C., Angenot, G. C., et al. (2010). Intercellular transport of epidermis-expressed MADS domain transcription factors and their effect on plant morphology and floral transition. *Plant J.* 63, 60–72. doi: 10.1111/j.1365-313X.2010.04221.x
- Vatén, A., Dettmer, J., Wu, S., Stierhof, Y. D., Miyashima, S., Yadav, S. R., et al. (2011). Callose biosynthesis regulates symplastic trafficking during root development. *Dev. Cell* 21, 1144–1155. doi: 10.1016/j.devcel.2011.10.006
- Vergara-Silva, F., Martínez-Castilla, L., and Alvarez-Buylla, E. R. (2000). MADS-box genes: development and evolution of plant body plans. *J. Phycol.* 36, 803–812. doi: 10.1046/j.1529-8817.2000.03654.x
- Wayne, R. (2009). *Plant Cell Biology*. Amsterdam: Elsevier.
- Xoconostle-Cazares, B., Yu, X., Ruiz-Medrano, R., Wang, H. L., Monzer, J., Yoo, B.-C., et al. (1999). Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science* 283, 94–98. doi: 10.1126/science.283.5398.94
- Yruela, I., Oldfield, C. J., Niklas, K. J., and Dunker, A. K. (2017). Evidence for a strong correlation between transcription factor protein disorder and organismic complexity. *Genome Biol. Evol.* 9, 1248–1265. doi: 10.1093/gbe/evx073
- Zažímalová, E., Murphy, A. S., Yang, H., Hoyerová, K., and Hošek, P. (2010). Auxin transporters—why so many? *Cold Spring Harb. Perspect. Biol.* 2:a001552. doi: 10.1101/cshperspect.a001552
- Zhang, X., Facette, M., Humphries, J. A., Shen, Z., Park, Y., Sutimantanapi, D., et al. (2012). Identification of PAN2 by quantitative proteomics as a leucine-rich repeat-receptor-like kinase acting upstream of PAN1 to polarize cell division in maize. *Plant Cell* 24, 4577–4589. doi: 10.1105/tpc.112.104125

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Benítez, Hernández-Hernández, Newman and Niklas. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.