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Force-driven polymerization and turgor-induced wall expansion

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Abstract

While many molecular players involved in growth control have been identified in the past decades, it is often unknown how they mechanistically act to induce specific shape changes during development. Plant morphogenesis results from the turgor-induced yielding of the extracellular, load-bearing cell wall. Its mechano-chemical equilibrium appears as a fundamental link between molecular growth regulation and the effective shape evolution of the tissue. Here, we focus on force-driven polymerization of the cell wall as a central process in growth control. We propose that mechanical forces facilitate the insertion of wall components, in particular pectins, a process that can be modulated through genetic regulation. We formalize this idea in a mathematical model, which we subsequently challenge with published experimental results.

Introduction

The link between gene regulation and growth, *i.e*. the irreversible spatial expansion of a cell or a tissue, is a major issue in plant and animal developmental biology. However, although many genetic regulators of morphogenesis have been identified, it is not clear how their activities precisely translate into chemical and physical changes within the cells and consequently into tissular morphogenesis. Here we will focus on the transduction between chemical and mechanical energies, which is at the heart of this multi-scale process.

The particularities of plant growth and morphogenesis find their origin within the structure of plant cells themselves, which can be considered as pressurized fluid droplets surrounded by a stiff cell wall. In order to grow, cells must expand their wall in an irreversible manner. Early work by *e.g*. Ray *et al* [1] provided evidence that wall expansion depends on chemical and metabolic regulation, as opposed to a situation where turgor would simply induce growth by breaking bonds between molecules. The current view is, that molecular networks that regulate morphogenesis in plants have to interfere with the chemical structure of their wall, making it yield to the internal turgor pressure at particular rates and in particular directions. To this end, the role of transcriptional and post-transcriptional regulators in growth control can be understood in terms of local wall remodeling.

From a thermodynamics perspective, plant morphogenesis can be seen as the result of an interplay between turgor-generated mechanical energy and the chemical reactions leading to cell wall expansion. Several conceptual frameworks have been developed to describe and analyze this complex process ([2] for review, see also: [3], [4]). In this context, Barbacci *et al* exposed a general thermodynamics framework to describe the coupling between mechanical and chemical

energies [5]. In the present opinion paper we take this approach further by showing how a specific biochemical and biomechanical process, force-driven polymerization, fits into this framework.

Up to now, the coupling between mechanics and growth is usually considered from a large scale perspective, individual walls being described as a continuous material, characterized by rheological properties (see Glossary) such as elasticity or viscosity [6-8]. Hereby, growth mechanisms are usually grasped through phenomenological equations similar to the ones depicting plasticity in non-living materials. These continuum mechanics-based approaches are very powerful as they benefit from existing simulation tools and concepts for the study of non-biological materials. Despite the usefulness of such models, however, their large-scale focus is also their limitation, for no attention is really paid to the underlying molecular mechanisms. As a result, molecular regulations are abstractly interpreted as modulations of rheological variables. Therefore it remains difficult to link experimentally measured transcriptional control or enzymatic activity to observed growth patterns. Moreover, useful and intuitive interpretations of underlying molecular functions such as repair mechanisms, self-assemblies, *etc*., are out of reach. We therefore need, in addition, more mechanistic models for growth, including the detailed molecular structure of the wall, explicitly linking molecular components to the overall rheological properties.

We therefore investigated if, from a simplified description of the molecular content of the wall, we could integrate a large-scale consecutive law of growing plant cells, which could then be used to characterize the function of individual molecular regulators.

We argue here, that mechanical forces generated by turgor could facilitate the insertion of wall

components. Such a coupling between mechanics and biochemistry, termed force-driven polymerization, has also been described for the assembly of actin bundles in animal systems [9]. Starting from known descriptions of cell wall structure (see [10] and references therein) we expose how force-driven polymerization could be central in scaling up molecular processes into viscoplastic behavior and ultimately growth of multicellular structures.

The cell wall is a fiber-reinforced hydrogel

The cell wall is composed of a network of rigid cellulose microfibrils embedded in a matrix, mostly composed of water, polysaccharides such as pectins and hemicelluloses, proteins and ions [11]. This matrix corresponds to a biphasic mixture between a porous solid phase and a liquid one [12]. Therefore, the cell wall as a whole can be considered as a fiber-reinforced hydrogel.

From a functional point of view, the wall performs two contrasting functions, as it has to resist turgor-induced constraints whereas simultaneously it should be able to expand. Because of their mechanical characteristics [13,14], the cellulose microfibrils tethered to each other by shorter hemicellulose chains are major actors in both of these functions (for reviews see: [10,15] [16]. The available experimental evidence suggests that cellulose microfibrils deposition is involved in growth inhibition rather than in growth promotion *per se*. Indeed, after drug-induced inhibition of cellulose deposition, cells can continue to grow during several tens of hours [17,18]. Similarly, growth also occurs in certain mutant backgrounds where cellulose synthesis is impaired [19,20]. These experimental clues suggest that growth of the cell wall relies on the expansion of the embedding matrix. In other words a scenario has emerged, where cell wall expansion is restricted by the synthesis of cellulose microfibrils and promoted by the synthesis of matrix material. In the following paragraphs we will discuss an important component of this matrix, *i.e*. pectin. As we

will see, there is increasing evidence that pectin could play an important role in growth control.

Pectin is an important structural component of the wall matrix

As indicated above, hemicelluloses such as xyloglucans have been classically considered as essential structural components, central to concepts of wall loosening and induction of growth. However, the recently produced Arabidopsis thaliana double mutant (*xxt1*/*xxt2*), showing a loss of xyloglucan synthesis, does not show major growth deficiencies [21,22], suggesting that the irreversible expansion of the cell wall does not rely, at least totally, on hemicellulose dynamics only. This has revived the interest in the role of pectins in growth control. Pectins form up to 35 % of the primary cell wall of dicots [11] - *i.e.* the wall surrounding young and fast growing cells. Their dynamics have been associated with growth control in several cell types, in particular tip-growing cells such as pollen tubes and root hairs, for review see [10] and references therein, see also *e.g.* [23], [24]. Moreover, AFM-based assays on *Arabidopsis thaliana*, demonstrated that pectin demethyl-esterification was required and tightly regulated during cell wall softening prior to aerial organ outgrowth [25], [26]. In addition, recent evidence indicates the existence of close interactions between cellulose and pectins which could function as tethers between the microfibrils [15,27]. Dwarf phenotypes in mutants where pectin synthesis or delivery is impaired further illustrate the importance of pectin production for plant growth [28,29]. Noteworthy, pectins have also been identified as important regulators of growth in more primitive plants such as the green algae [30,31].

Pectins are produced in the Golgi and exocytosed within the cell wall. They are composed of four different polysaccharides: Homogalacturonan, Rhamnogalacturonan I, Rhamnogalacturonan II and Xylogalacturonan. Different pectin molecules correspond to various combinations of those

three residues covalently attached together. Hereby, homogalacturonans stand out, for they roughly account for 65% of all of them [32]. A fundamental feature of homogalacturonans is their ability to form multiple intermolecular chelation bonds in presence of calcium cations. These cations-dependent bonds are able to glue together pectin molecules, forming structures called “egg-boxes” (Fig.1). In a cation-rich environment, these adhesive interactions lead to the formation of a hydrogel [33]. In addition, pectins are able to interact with other cell wall components including cellulose and hemicellulose [34].

An interesting feature is that newly synthesized homogalacturonans cannot spontaneously form egg-box structures. Indeed, in order to trap divalent cations, the carboxyl groups of the homogalacturonan chains must be negatively charged. When synthesized, carboxyl residues are shielded by methyl groups, which must be removed to induce gelification. Therefore, two types of pectin molecules can be distinguished: (i) “inactive” methyl-esterifed M-pectin and (ii) “active”, demethyl-esterified pectin, able to aggregate into macromolecular assemblies. Demethyl-esterification is carried on within the cell wall by specialized enzymes, the pectin methyl-esterases (PMEs). In turn, PME-inhibitors (PMEIs) can modulate PME activity. This process is essential, for both the degree and the pattern of demethyl-esterification influence the mechanical/rheological properties of the constituted gel [35]. Accordingly, modifications in the levels of PME and PMEI expression significantly alter growth rates [36]. Note that other charged residues such as acetyl-esters [11] can also be present on homogalacturonans and their regulation could play a similar role. However, for the sake of simplicity we only consider methyl-ester groups here. Noteworthy, 66 PMEs and 69 PMEIs have been reported in the Arabidopsis genome, attesting for the importance of the process [33,37]. The PME/PMEI system appears as a key regulator of cell wall expansion:

- First, by regulating the amount of "active" demethyl-esterified pectins, within the matrix liquid phase, ready for insertion it influences its viscosity: The more active molecules available, the faster the expansion.
- Secondly, by removing more or less methyl groups on pectin molecules already stuck together, it regulates the strength of the bonds within the matrix solid phase. This can be interpreted as the tuning of the plastic yielding threshold of the cell wall.

In conclusion, in the previous paragraphs we have seen that the cell wall can be considered as a fiber reinforced hydrogel, which is put under tension by the osmotic pressure within the cells. To grow, the cells have to yield to this pressure by expanding their walls in an irreversible manner. We will next discuss the chemical and physical processes that are at the basis of this expansion, before indicating how molecular regulation can act to generate specific shapes of tissues and organs. To be able to go beyond qualitative descriptions of growth regulation, we will present a description in the form of a mathematical model. Finally we test this model using available quantitative data

Chemical equilibrium of the pectin hydrogel

If single molecules in solution can bind to each other, macromolecular assemblies are generated. Assuming a high enough initial concentration of those molecules, such a mechanism eventually leads to the formation of a hydrogel, *i.e.* the percolation of a macromolecular solid phase within a solution of single molecules. The pectin matrix corresponds to such a biphasic system [12]. When the cell is growing, new matrix material has to be added: new components are exocytosed within the liquid phase and components from the liquid phase have to be transferred into the solid one. Because the wall is a closed shell of constant thickness (*i.e.* with no border where an hypothetic

nucleation mechanism could take place), a way to add new components to the solid phase is by inserting them in between existing ones. Such a mechanism is sketched in Fig.1a and formalized in Eq.1, where N_b and M respectively depict one bond between two molecules in the solid phase and a free molecule in the liquid one.

$$N_b + M \rightleftarrows 2N_b \quad (1)$$

This chemical equilibrium between the liquid and solid phase of the gel can be formalized by standard concepts from thermodynamics. Indeed, phase equilibrium is reached once the *free* energy (we will drop the "*free*" for now on) difference ΔE ($= E_{bound} - E_{unbound}$) between the two sides of Eq.1 vanishes. This energy difference reads $\Delta E = E_b - k_B T \cdot ln\,(c/c_0)$ and encompasses two general classic features. First, the stabilization of molecules by the adhesive interactions between them is accounted for by the first term $E_b < 0$ (the negative sign meaning that adhesion stabilizes the system, *i.e.* lowers its energy). Second, the insertion of one molecule from the liquid phase into the solid one comes with the loss of some degree of freedom. This is expressed by the second term $-k_B T \cdot ln\,(c/c_0)$ a standard expression of mixing entropy, where $k_B T$ represents the thermal energy of the system (k_B and T respectively stand for the Boltzmann constant and the absolute temperature), c depicts the monomer concentration in the liquid phase and c_0 the water concentration in that same phase ($\simeq 55 mol \cdot L^{-1}$). In view of the above, the equilibrium condition $\Delta E = 0$ provides a value for a corresponding critical free molecule concentration (with $\beta = 1/k_B T$):

$$c_0^* = c_0 \cdot exp(\beta E_b) \quad (2)$$

In other terms, the actual concentration c of monomers in solution controls the chemistry of the

gel:

- If $c > c_0^* \Leftrightarrow \Delta E < 0$, monomers spontaneously insert into the solid phase, growth occurs.
- If $c < c_0^* \Leftrightarrow \Delta E > 0$, no spontaneous insertion, no growth occurs.

The critical concentration c_0^* only depends on one parameter: the adhesive energy between molecules in the solid phase (E_b in Eq.2). In order to modulate this equilibrium the cell only has to tune the adhesive links between molecules. This can be achieved using enzymatic activities, *i.e.* through molecular regulation. However, another efficient way to do so is by pulling on those links (Fig.1), as detailed in the following paragraph.

Influence of mechanical forces on the chemical equilibrium

A tensile force (f) applied on the solid phase can ease the breaking of existing bonds, thus facilitating the insertion of soluble molecules. This shifts the chemical equilibrium towards polymerization — *i.e.* towards the right-hand side of Eq.1. In this case, the energy difference has to be lowered by the work $w = f \cdot a$ generated by the tensile force (f) over a distance a, characteristic of the deformation of loaded bonds: $\Delta E' = \Delta E - f \cdot a$. Consequently, the equilibrium condition $\Delta E' = 0$ yields, this time, a force-dependent expression for the equilibrium concentration c^*:

$$c^*(f) = c_0^* \cdot e^{-\beta \cdot f \cdot a} \quad (3)$$

This mechanism assures that tensile forces applied on a gel at equilibrium decrease its critical concentration. As a consequence molecules in solution are recruited into the solid phase, (Fig.1 &

2). This transduction mechanism is called force-induced polymerization and has been shown to play an important role in the dynamics of major load-bearing structures such as the actin cytoskeleton [38-41]. Note that this mechanism is by essence irreversible, for when an increased force is applied on a given configuration (black arrow on Fig.2a) and then removed (dashed black arrow on Fig.2a) the system does not go back to its original position.

Linking mechanics and biochemistry to geometry: spatial expansion of the pectin matrix under tensile force

One difference between the growth of living tissues and the plasticity of non-living materials is the mass increase of the former. Therefore, it seemed logical to seek for a growth law based on the equation of mass conservation of the solid phase of the matrix (see Box 1 for details). Formalizing the fact that the local expansion of the matrix is fuelled by the insertion mechanism previously exposed yields (combination of Eq. 2, 3 & B1-4):

$$R_g = K \cdot \delta c \cdot e^{\beta(f \cdot a - E_b)} \quad (4)$$

where the relative expansion rate (R_g) is expressed as a function of the tensile force (f), formalizing the out-of-equilibrium insertion reaction. $K > 0$ is a proportionality constant and δc represents the excess of free monomer in solution compared to the equilibrium concentration. Note that in an exhaustive approach the coefficient K should depend on the elastic energy stored in the solid phase. But in a turgid growing tissue this stored energy should be roughly constant and can be ignored for the sake of simplicity.

Reinforcing the gel: the cellulose microfibrils network steps in

Eq.4 depicts the expansion of a hydrogel under mechanical load, applied to the specific case of pectin within the cell wall. A corollary question concerns the integration of the cellulose microfibril network in this description.

The entanglement between the pectin-based matrix and the cellulose fibrils leads to a complex distribution of mechanical constraints at the molecular scale. An accurate mechanical analysis would require a precise structural description of the considered wall and intensive numerical simulations, similar to the ones produced by Kha *et al* [42] and Puri *et al* [43] in the case of cell wall models containing only cellulose and hemi-cellulose fibers. Such a study lies outside of the range of this article and we will only provide a qualitative analysis here.

In a wall containing both pectin and cellulose, the force (f) felt by a pectin strand in a given direction should in principle only be a fraction of the total force (f_{tot}) exerted on a unit volume of the wall. This fraction would depend, at the very least, on the relative concentration of cellulose fibers compared to pectin strands (r_{c-p}) and the rigidity of those fibers (Y_c): $f = f(f_{tot}, r_{c-p}, Y_c) \leq f_{tot}$.

Controlling both the angular distribution of cellulose fibers and their rigidity appears as a way to regulate the force exerted on pectin strands and consequently the expansion rate (R_g) as expressed in Eq.4. Following this idea, two stereotypical asymptotic cases can be evoked:

- All the cellulose fibers are oriented perpendicular to the expanding pectin strand (*c.f.* Fig. 1b-2). In this case, the cellulose fibers and the pectin strand can be considered in series and the whole force exerted on the wall is transmitted to the strand: $f = f_{tot}$.
- All the cellulose fibers are oriented in the same direction as the expanding pectin strand

(*c.f.* Fig 1b-3). In this case, the cellulose fibers and the pectin strand lie in parallel and the force felt by the pectin strand is smaller than the total one: $f \approx f_{tot}/\left(1 + r_{c-p} \cdot r_Y\right)$ where r_{c-p} and r_Y respectively account for the ratio of relative concentrations of pectin and cellulose and the rigidity ratio between a pectin strand and a cellulose fiber.

The qualitative point we want to make here is that enhancing the deposition of cellulose in a given direction should directly down-regulate the expansion rate of the matrix in this specific direction.

Finally, it is worth mentioning that a detailed thermodynamical description of such a fiber-reinforced hydrogel should not only include dissipation between matrix molecules but also between the fibers themselves and between the fibers and the matrix.

Experiments on Chara fit the force-driven polymerization model

We were not aware of quantitative experimental studies on higher plants focused on the relationship between molecular composition of the cell wall, turgor-generated forces and the morpho-dynamics of the whole tissue. Fortunately, such studies have been conducted on the green algae *Chara corallina* [44,45] and we confronted the results with our model (Fig.3).

In the case of isolated Chara cells, the mechanical force (f) exerted on the cell wall is proportional to the pressure differential (ΔP) between the inside and the outside of the wall. Combined with Eq.4, this yields an exponential relationship between the pressure differential exerted on a cell wall and its expansion rate. All other parameters kept constant, the ratio between two relative growth rates (R_g/R_g^0) as a function of the corresponding ratio of pressure differentials ($\Delta P/\Delta P_0$) reads:

$$\frac{R_g}{R_g^0} = exp\left(\mathrm{C}_1 \cdot \Delta P_0 \cdot \left(\frac{\Delta P}{\Delta P_0} - 1\right)\right) \quad (5)$$

see Fig.3a for a comparison between experimental points and the fitting function.

If we assume that the insertion of demethyl-esterified pectins is the molecular mechanism behind cell wall expansion, the growing rate is an affine function of the concentration of demethyl-esterified pectin molecules in the solute phase. Fig.3b depicts this behavior and qualitatively matches experimental evidence of this proportionality exposed on Fig.3 of [45]: putting Chara cells in a pectin-enriched solution increased their growing response to mechanical stress.

Another qualitative feature of Chara growth observed by Proseus *et al* and reproduced by our model is its dependency on temperature: Fig 3c depicts how the pressure-growth rate relationship is affected by a slight decrease of temperature (T), the shift from the plain curve to the dashed one has to be compared to the shift between the two curves exposed on Fig.13 of [44]. These qualitative agreements between our theoretical model and experimental data strengthen the idea that force-driven assembly mechanisms of the cell wall matrix play an important role in cellular growth.

Discussion, perspectives & conclusion

Our ambition was to demonstrate that a fundamental molecular mechanism (force-driven polymerization) applied to a key actor (pectin) can be at the heart of a complex integrated behavior such as morphogenesis.

Although force-driven insertion appears as an appealing mechanism to explain cell wall expansion at the molecular scale, it is worth noting that it might not be the only one possible. For

instance, Rojas *et al* [23] proposed a detailed study of pollen tube expansion using an apposition mechanism: Newly demethyl-esterified pectin molecules are not inserted into the solid phase but rather directly polymerized as a new layer next to the old one. This new layer modifies the mechanical properties of the load-bearing cell wall and induces its viscous relaxation. Such a mechanism does not exclude the model we propose here but is rather complementary: Rojas *et al* also describe a transduction mechanism between pectin chemistry and cell wall mechanics, focusing their attention on the chemical control of the process. This shift of perspective between their approach and ours (*i.e*. the question of causality between the chemical and the mechanical states of the cell wall) is a molecular version of the debate between Linthillac and Schöpfer that took place a few years back [46,47]: Is growth controlled by wall-loosening enzymes or by turgor-induced stresses ? Our point is that what matters is the resulting equilibrium between both ends which is formalized throught the critical concentration Eq.3.

Force-driven polymerization processes have been proposed to play a major part in several biological functions relying on active gel dynamics and mechano-chemical transduction, from actin filament formation and focal adhesion formation [9], [41], [48] (Kozlov et al) in animal cells to cell wall instability and cell division in bacteria [49]. In our opinion, force-driven polymerization processes could be as central in mechanobiology as Michaelis-Menten mechanisms in biochemistry.

Indeed, force-driven polymerization can be seen as a transduction mechanism between a mechanical force and a chemical reaction. From a theoretical perspective, in its full 3D version this mechanism provides a gateway to develop models that integrate quantitative geometrical variables and biochemical equations (Fig.4). In other words, it becomes possible to express molecular activities in terms of geometrical outputs at the level of entire tissues and organs in a

fully mechanistic manner. For this purpose, Eq.4 can be coupled to other differential equations describing the dynamics of enzymatically-regulated variables. For instance one could assume that the activation energy E_b in Eq.4 depends on the demethyl-esterification degree of pectin molecules and that pectin methyl esterases regulate its value. The influence of PME activity on growth by coupling Eq.4 could then be explored using an equation describing PME dynamics.

To assess the validity of the model a wide range of experiments can be considered. The first step would be to extend the quantitative analysis produced by Proseus and Boyer to cells and tissues from higher plants: quantitative tracking to follow cells and tissues submitted to various levels of turgor pressure could be a start. In complement, rescue assays could be tested to restore a normal growth phenotype to mutant or drug-treated plants by modulation of mechanical forces. Finally and more speculatively, the use and analysis of biomimetic systems such as artificial walls could also be very useful.

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Boxes

Trends box:

- Cellulose microfibrils have historically drained most of the attention of biomechanical studies of the cell wall. Recent results, however, suggest that the soft pectin matrix in which they are embedded might also play a significant role in the physico-chemical equilibrium of growing cells.
- At the molecular scale, biophysicists have shown how mechanical forces applied on molecular assemblies can modulate their chemical state and therefore initiate specific biological responses.
- At the tissular scale, numerical simulation tools borrowed from material sciences are increasingly used in developmental biology. Morphogenesis in plant tissues is particularly suited for this kind of approach.
- Bridging the gap between the molecular and the tissular scales is a major challenge in developmental biology, for it would help relating explicitly shape changes and specific molecular actors.

Glossary box:

Rheology: Study of the deformation of matter at a mesoscopic or macroscopic scale.

Viscosity: Rheological/mechanical property that relates the deformation rate of a material to its mechanical load. For a given loading force, the more viscous a material is the slower it will deform. Note that its inverse is called extensibility and is often used in growth modeling.

Thermal energy: The portion of the energy of a system due to the random relative microscopic movements of its components. Absolute temperature of the system is, by definition, a direct measure of this internal energy. For living systems which temperature is around 300 K the usual value of their thermal energy is: $\mathcal{E}_{th} \propto k_B T = 1/\beta \approx 4 \cdot 10^{-21}\mathrm{J}$, where $k_B = 1.38 \cdot 10^{-23} J \cdot K^{-1}$ and $T = 300\ K$ respectively stand for the Boltzmann constant and the absolute temperature.

Work of a force: Considering an object in motion and summited to a force, the work developed by this force corresponds to the scalar product between the force vector and the displacement vector.

Box 1: From matter conservation to force-induced expansion

The conservation equation of the number of bonds in the solid phase reads:

$$\frac{dn_b}{dt} = -n_b R_g + s_b \quad \text{(B1-1)}$$

The term of the left hand-side of Eq.B1-1 depicts the local time evolution of the bond density (noted n_b). The first term of the right hand-side is proportional to the relative expansion rate of the cell wall (R_g) and accounts for the fact that the matrix can expand. The minus sign in front of it insures that spatial expansion of the matrix ($R_g > 0$) tends to diminish the local bond density. Finally, the second term of the right hand-side (named the source term and denoted s_b) grasps the chemical insertion mechanism that transfers molecules from the liquid phase into the solid one when the two phases are out of equilibrium.

Because the cell wall is constantly under tension, the spacing between molecules in the solid phase and therefore the bond density can be assumed constant over time (*i.e.* $dn_b/dt = 0$ in Eq.B1-1). This assumption implies that the two terms of the right hand-side of Eq.B1-1 compensate each other, leading to a direct relationship between the expansion rate of the cell wall and the pectin chemical insertion rate:

$$n_b R_g = s_b \quad \text{(B1-2)}$$

The proper derivation of chemical insertion rate (s_b) from thermodynamical

466 considerations lies outside the range of this opinion article but in a few words it can be
467 estimated as follows. It should be proportional to the density of insertion sites (n_b) and to
468 the excess of free molecules in solution compared to equilibrium: $\delta c = c - c^*$. It
469 therefore corresponds to a relaxation term when the pectin molecules in the two phases
470 are out of equilibrium. If the system is only slightly out of equilibrium, this relaxation
471 flux can be linearized with respect to the chemical potential difference between the two
472 pectin phases ($\partial \Delta \mathcal{E} / \partial c|_{c^*}$ in Eq.B1-3) leading to the following expression:

473 $$s_b \propto -\frac{\partial \Delta \mathcal{E}}{\partial c}\Big|_{c^*} \cdot \delta c \cdot n_b \quad \text{(B1-3)}$$

474 This yields the simple relationship between the relative spatial expansion rate of the wall
475 and the equilibrium concentration $c^*(f)$ that explicitly depends on the mechanical force f
476 as Eq.3 depicts:

477 $$R_g \propto \frac{1}{c^*(f)} \quad \text{(B1-4)}$$

Figures legends

Figure 1: Pectin strand dynamics & mechanical load distribution.

(a) Details on the matrix expansion mechanism. **(a-1)**: demethyl-esterified pectin chain at rest in equilibrium with DM pectin molecules in solution (light blue). **(a-2)**: When loading with a mild force the molecules constituting the chain are elastically deformed. Egg-box structures are undeformed and the corresponding bond strong. **(a-3)**: If the loading force is high enough (above a threshold value defined as $f_{th} = E_b/a$), egg-box structures are mechanically deformed, their corresponding strength is lowered. Consequently, they can be destabilized by close-by, demethyl-esterified pectin molecules in solution. **(a-4)**: Once the initial bond broken, the destabilizing molecule is inserted, increasing the total length of the chain. **(b) Influence of of cellulose microfibrils orientation**. **(b-1):** Structural sketch of a fiber reinforced hydrogel. The big purple bars represent cellulose microfibrils, the smaller orange one pectin strand composing the solid phase and the blue dots stand for the free pectin molecules in the liquid phase. We assume that cellulose fibrils are deposited by a preferred direction. Within this 2D representation, two 1D cases are evoked: **(b-2)**: The studied pectin strand is perpendicular to the cellulose microfibrils main direction. In that case, the two structure can be assume in series and the forces they experience are the same: $f_{tot\,y} = f_{c\,y} = f_y$ where those forces respectively correspond to the total force felt by the wall, the fraction felt by the cellulose fiber and the fraction felt by the pectin strand. **(b-3)**: The studied pectin strand is parallel to the main cellulose microfibrils direction. The total force exerted on the wall is distributed between both structures, this time in parallel: $f_{tot\,x} = f_{c\,x} + f_x$; the

499 fraction felt by the pectin strand is consequently lower than the total force: $f_x \leq f_{tot\,x}$.

Figure 2: Phase diagram of an hydrogel under tensile force.

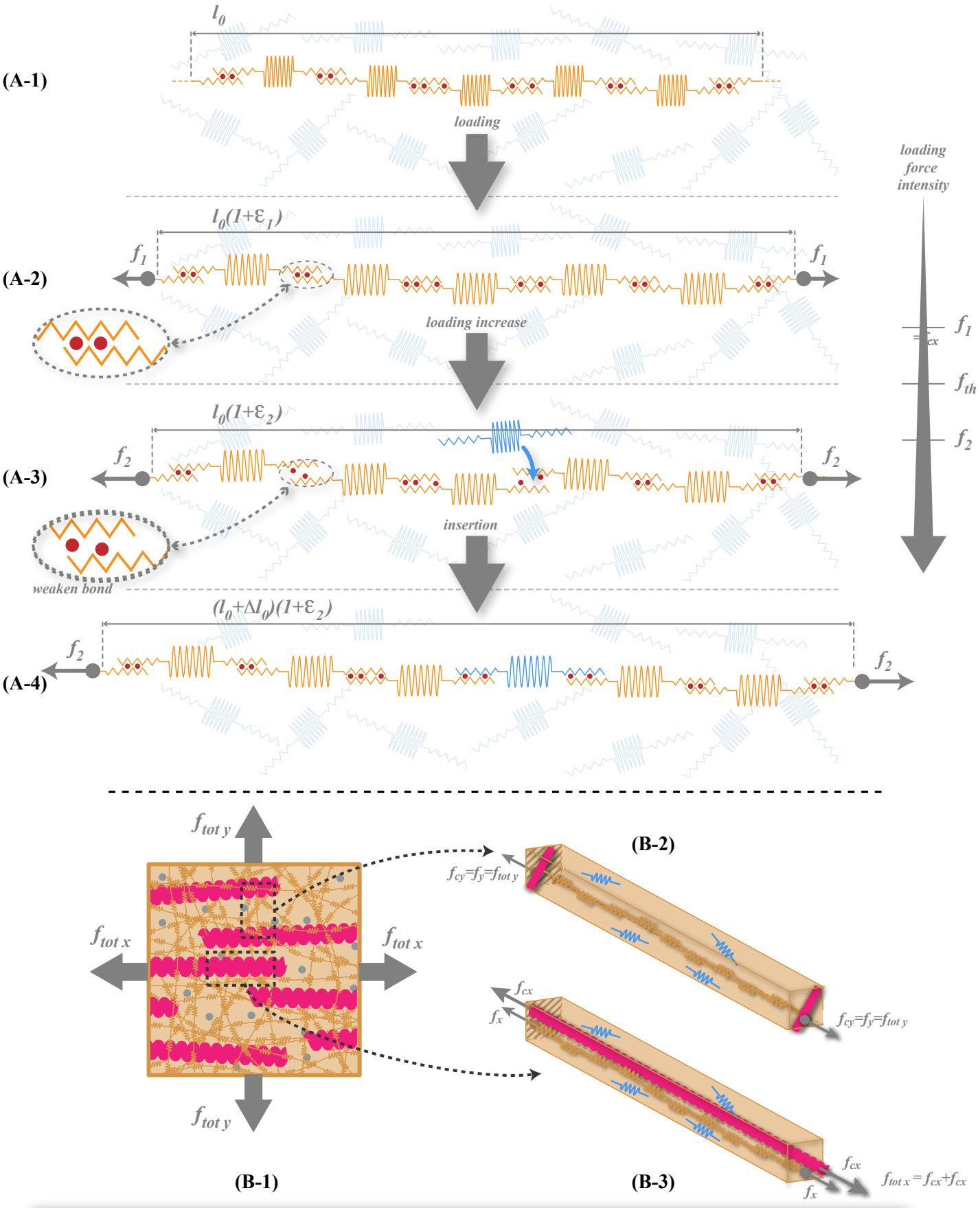
The abscise axis quantifies the work developed by the tensile force ($w = f \cdot a$) compared to the thermal energy available ($\beta = 1/k_B T$). The ordinate axis shows the normalize concentration of free active pectin molecules in the liquid phase. The orange curve is a visual representation of Eq.3 and therefore represents the chemical equilibrium between the solid and the liquid phase of the gel. Each point of the plane represents one configuration of the gel that corresponds to a specific couple "*concentration* / *force*", the points under the equilibrium curve correspond to gels that do not expand their solid phase whereas points above do. Sub-figures **(a)** & **(b)** show two simple putative growth scenarios: **(a)** From an initial non-growing state (black dot) we assume an increase of the mechanical load, that translates onto the phase plan as a rightward shift of the point describing the system state (gray dot). Being this time in the "growth zone", spontaneous insertion of monomers into the solid phase spontaneously happens. Note that from the initial black point it takes a minimal force (dashed part of the arrow) to get into the "growth zone", this can be interpreted as a force threshold f_{th}, depicted by the vertical dotted orange line. Finally the gray dashed arrow symbolizes the "out of equilibrium" driving force of the polymerization process that tends to diminish the concentration of free molecules in solution. Note that if the mechanical force goes back to its initial value (plain gray arrow), the system does not recover its initial state (black dot) but a new one (orange dot). **(b)** This time growth is initiated by a release of demethyl-esterified pectin in the liquid phase, again a threshold phenomenon is observed, this time in terms of concentration c_{th}, depicted by the horizontal dotted orange line.

Figure 3: Growth rate dependency on demethyl-esterified pectin concentration, turgor pressure and temperature; comparison between experimental data and model.

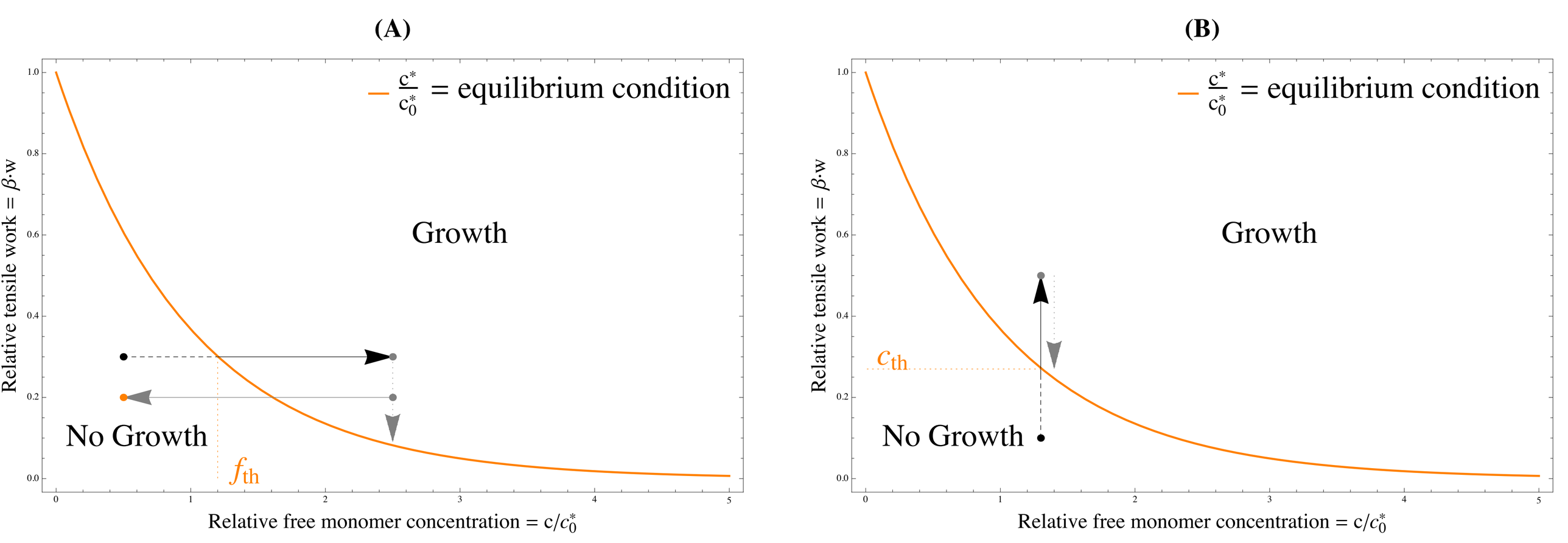
(a): Influence of turgor pressure on relative expansion rate. Comparison between experimental data and Eq.4. Dots represent experimental published data from (Proseus *et al*. 2000) and error bars the precision of our reading of these data. The dashed curve represents the best fit of these points by the exponential function exposed in Eq. 5: $R_g/R_g^0 = exp\big(C_1 \Delta P 0 \cdot (\Delta P/\Delta P_0 - 1)\big)$where C_1 is the fitting coefficient. ΔP_0 and $R_g^0 = R_g(\Delta P_0)$ are arbitrary values taken as references. **(b)&(c)**: Qualitative influence of various parameters on the growth behavior. **(b): Influence of an increase of free demethyl-esterified pectin concentration in the liquid phase (δc in Eq.4)**. As on sub-figure **(a)**, the curves depict the evolution of a normalized growth rate R_g/R_g^0 with respect to a normalized pressure differential ($\Delta P/\Delta P_0$) and mimick Fig.3B in[45]. The plain curve corresponds to the best-fit curve exposed on sub-figure **(a)**. The dashed curve is deduced from the plain one by a two-fold increase of the free demethyl-esterified pectin concentration (δc). **(c): Influence of the temperature**. This time the temperature dependency exposed in Eq.4 is investigated. $P_0, T_0$and $R_g^0 = R_g(\Delta P_0, T_0)$ are still arbitrary reference values. The plain curve corresponds to the best-fit curve exposed on sub-figure **(a)**. The dashed curve is deduced from the plain one by using a smaller value of the temperature similar to the drop of temperature studied by Proseus *et al*. in [44], see Fig.13-B.

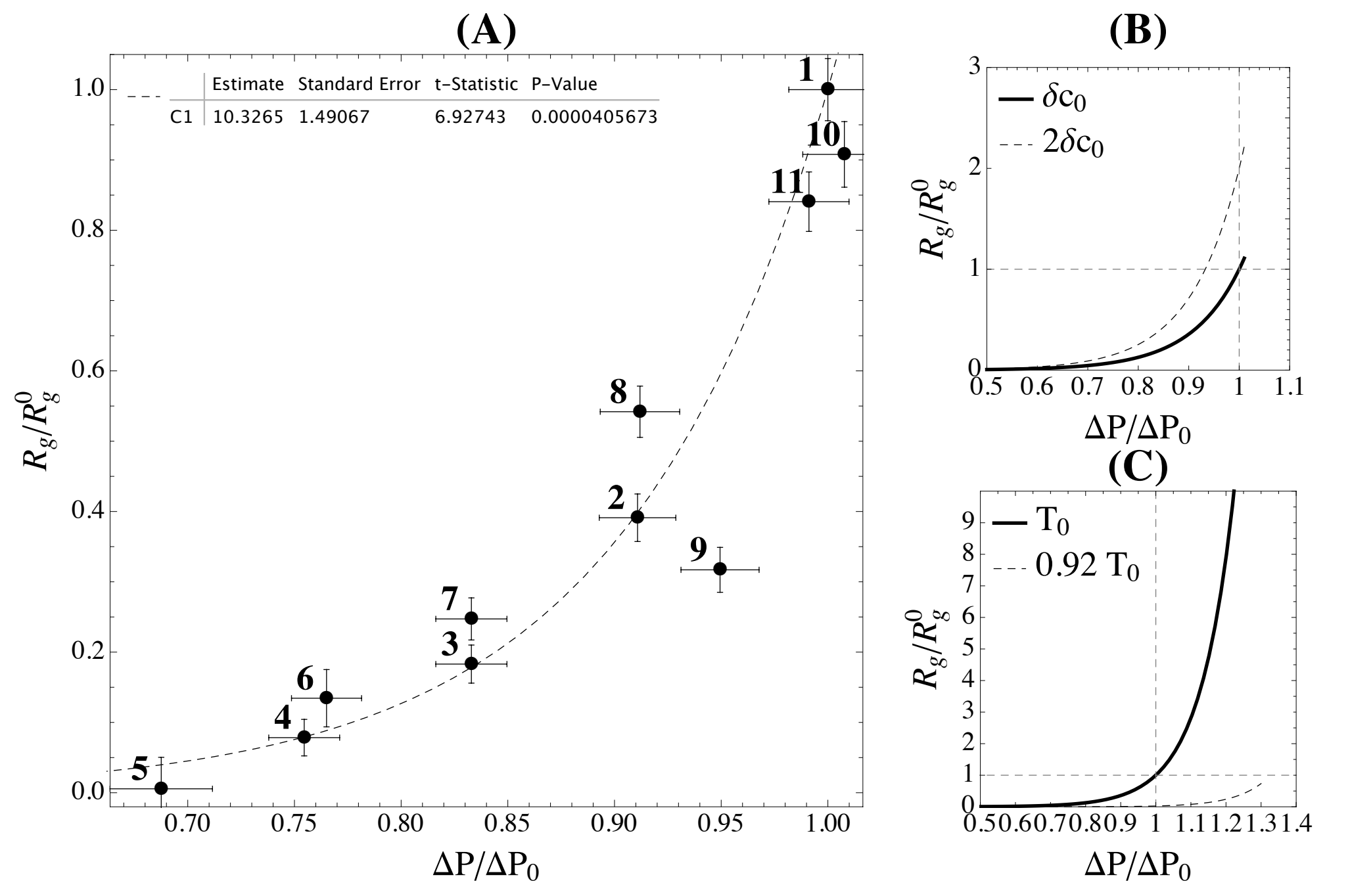
Figure 4: Transducing chemical & mechanical inputs into morphological outputs

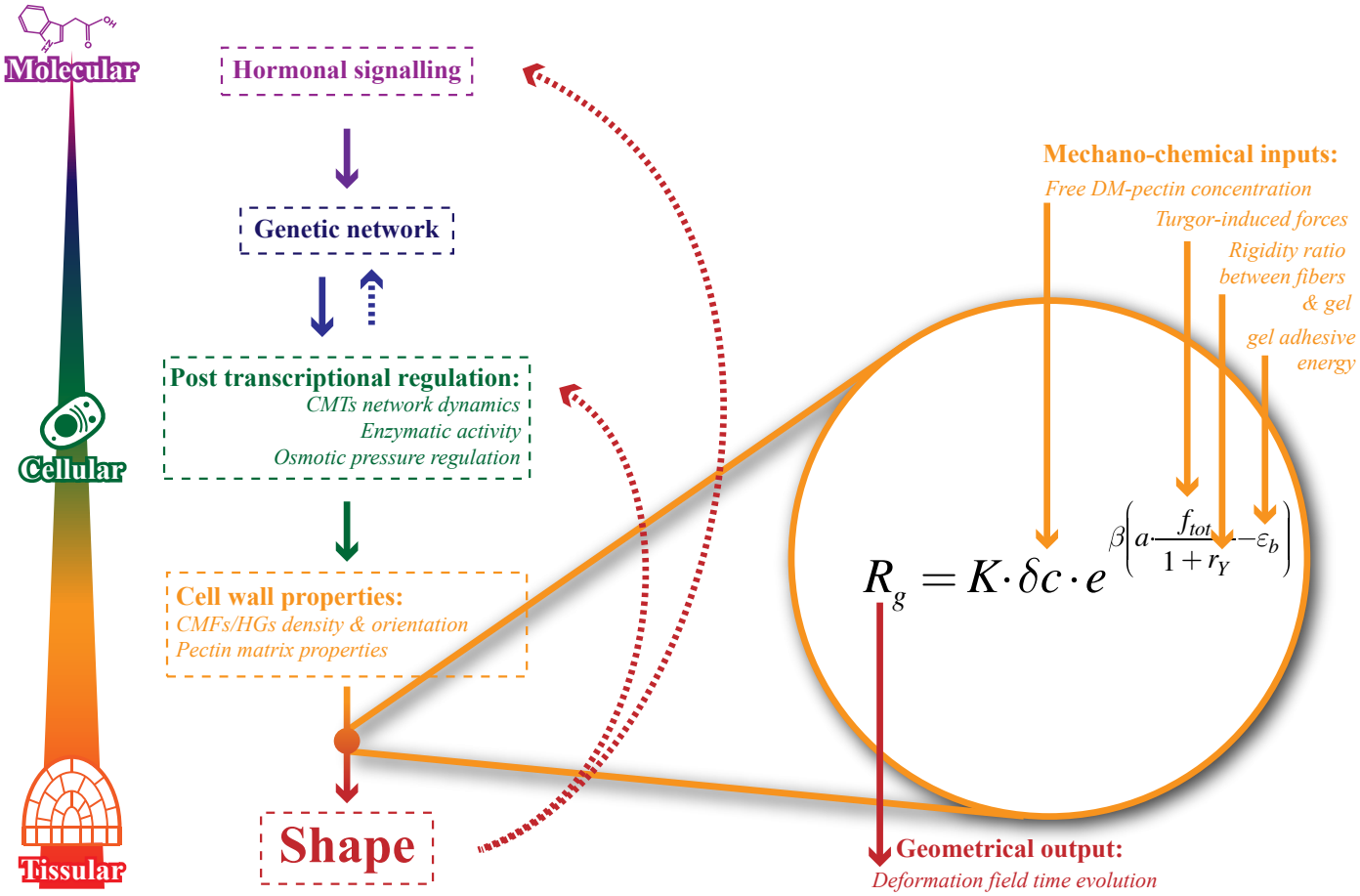
The morphological evolution of the tissue is quantified by its relative growth rate R_g. The growth law that relates it to turgor-induced mechanical forces and to the cell wall mechano-chemical properties is a direct consequence of the cell wall molecular organization. Its expression exposed here corresponds to the combination of Eqs.4 & 5. From a functional perspective the growth law can be seen as the final step of the integrative chain between gene expression and shape evolution. It also appears as the step where biochemical properties (quantified by scalar variables, *i.e.* « simple » numbers) are combined with mechanical and geometrical properties (quantified by oriented variables, namely vectors and matrices).


(A-1)
l_0
loading
(A-2)
$l_0(1+\varepsilon_1)$
f_1
f_1
loading increase
(A-3)
$l_0(1+\varepsilon_2)$
f_2
f_2
insertion
weaken bond
(A-4)
$(l_0+\Delta l_0)(1+\varepsilon_2)$
f_2
f_2
loading force intensity
f_1
f_{cx}
f_{th}
f_2
(B-1)
$f_{tot\,y}$
$f_{tot\,x}$
$f_{tot\,x}$
$f_{tot\,y}$
(B-2)
$f_{cy}=f_y=f_{tot\,y}$
$f_{cy}=f_y=f_{tot\,y}$
(B-3)
f_{cx}
f_x
f_{cx}
f_x
$f_{tot\,x}=f_{cx}+f_{cx}$
Ca^{2+} incorporated in an egg-box
DM pectin molecule in the liquid phase
or
freshly added DM pectin molecule in the solid phase (stretched)
DM pectin molecule in the solid phase (unstretched)
DM pectin molecule in the solid phase (stretched)
Cellulose microfibril and hemicellulose network
f Loading force on a matrix strand
f_c Loading force on a microfibril
f_{tot} Loading force on a whole cell wall

Key Figure


(A)
(B)
$\frac{c^*}{c_0^*}$ = equilibrium condition
Growth
No Growth
f_{th}
c_{th}
Relative tensile work = $\beta \cdot w$
Relative free monomer concentration = c/c_0^*
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
4
5


(A)
Estimate Standard Error t-Statistic P-Value
C1 10.3265 1.49067 6.92743 0.0000405673
1
10
11
8
2
9
7
3
6
4
5
R_g/R_g^0
$\Delta P/\Delta P_0$
(B)
δc_0
$2\delta c_0$
R_g/R_g^0
$\Delta P/\Delta P_0$
(C)
T_0
0.92 T_0
R_g/R_g^0
$\Delta P/\Delta P_0$


Molecular
Cellular
Tissular
Hormonal signalling
Genetic network
Post transcriptional regulation:
CMTs network dynamics
Enzymatic activity
Osmotic pressure regulation
Cell wall properties:
CMFs/HGs density & orientation
Pectin matrix properties
Shape
Mechano-chemical inputs:
Free DM-pectin concentration
Turgor-induced forces
Rigidity ratio between fibers & gel
gel adhesive energy
$R_g = K \cdot \delta c \cdot e^{\beta\left(a \cdot \frac{f_{tot}}{1+r_Y} - \varepsilon_b\right)}$
Geometrical output:
Deformation field time evolution