

Strain- or Stress-Sensing in Mechanochemical Patterning by the Phytohormone Auxin

Jean-Daniel Julien, Alain Pumir, Arezki Boudaoud

▶ To cite this version:

Jean-Daniel Julien, Alain Pumir, Arezki Boudaoud. Strain- or Stress-Sensing in Mechanochemical Patterning by the Phytohormone Auxin. Bulletin of Mathematical Biology, 2019, 81 (8), pp.3342-3361. 10.1007/s11538-019-00600-5 . hal-02346830

HAL Id: hal-02346830 https://hal.science/hal-02346830

Submitted on 20 Jan 2024

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.

Noname manuscript No. (will be inserted by the editor)

Strain- or Stress-sensing in mechanochemical patterning by the phytohormone auxin

- $_3$ Jean-Daniel Julien^{1,2,3} · Alain Pumir² ·
- 4 Arezki Boudaoud¹

6 Received: date / Accepted: date

Abstract Both chemical and mechanical fields are known to play a major 7 role in morphogenesis. In plants, the phytohormone auxin and its directional 8 transport are essential for the formation of robust patterns of organs, such q as flowers or leaves, known as phyllotactic patterns. The transport of auxin 10 was recently shown to be affected by mechanical signals, and conversely, auxin 11 accumulation in incipient organs affects the mechanical properties of the cells. 12 The precise interaction between mechanical fields and auxin transport, how-13 ever, is poorly understood. In particular, it is unknown whether transport is 14 sensitive to the strain or to the stress exerted on a given cell. Here, we inves-15 tigate the nature of this coupling with the help of theoretical models. Namely, 16 we introduce the effects of either mechanical stress or mechanical strain in a 17 model of auxin transport, and compare the patterns predicted with available 18 experimental results, in which the tissue is perturbed by ablations, chemical 19 treatments, or genetic manipulations. We also study the robustness of the pat-20 terning mechanism to noise and investigate the effect of a shock that changes 21 abruptly its parameters. Although the model predictions with the two differ-22 ent feedbacks are often indistinguishable, the strain-feedback seems to better 23 agree with some of the experiments. The computational modeling approach 24 used here, which enables us to distinguish between several possible mechani-25 cal feedbacks, offers promising perspectives to elucidate the role of mechanics 26 in tissue development, and may help providing insight into the underlying 27

28 molecular mechanisms.

Keywords patterning · chemomechanical model · auxin transport · shoot
 apical meristem

¹Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, Lyon Cedex 07, France · ²Laboratoire de Physique, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, Lyon Cedex 07, France · ³Max-Planck Institute for Dynamics and Self-Organisation, Göttingen, D-37077, Germany

Julien et al.

31 1 Introduction

Understanding the formation of patterns in living organisms has long intrigued 32 scientists [1]. This phenomenon has been investigated in a wide variety of 33 species, such as zebrafish [23] or hydra [4]. We focus here on patterning in 34 plants. The highly regular positioning of the organs in plant shoots, called 35 phyllotaxis, relies on the patterns of the phytohormone auxin, whose local ac-36 cumulation is necessary for the emergence of new organs [24, 18]. Auxin efflux 37 is facilitated by the membrane-localized PIN FORMED1 (PIN1) protein [35, 38 51], which can be polarly (asymmetrically) distributed, inducing a directional 39 transport of auxin, and resulting in well-defined auxin patterns. This trans-40 port is essential for the development of the plant. Indeed, knocking down the 41 transporter activity, either genetically or chemically, leads to the absence of 42 organ primordia at the shoot tip. Organ development can be then rescued by 43 local application of exogenous auxin [34, 40, 41]. 44 Several models have been proposed to describe the interaction between 45

the hormone and its carrier in the context of the shoot apex, with the major assumption that the transporters allocation between the different sides of a cell is driven by auxin fluxes [49] or by auxin concentrations [48,22,44,47]. Here we focus on the second assumption, which has received more attention, because

⁵⁰ how the auxin concentration regulates the transporters remains unclear.

Since morphogenesis relies on changes in the structural elements of the or-51 ganism, biochemical patterns must also influence the mechanics of these struc-52 tural elements, in plants [16] and in animals [19,9,39]. Conversely, mechanics 53 can feedback on biochemical processes [16, 19, 20, 45], including gene expres-54 sion [11] and cell fate [3]. Plants are well suited to study the coupling between 55 biochemical and biophysical processes as hydrodynamic cell pressure generates 56 tremendous forces and results in a high tension in the polysaccharide-based 57 walls surrounding cells [13,16]. Recent progress in computational approaches 58 [8] and in the measurements of cell mechanics [28] has fostered a renewed 59 interest in the mechanics of plant morphogenesis. 60

Notably, at the shoot apex, organogenesis is associated with a decrease in 61 the stiffness of the cell wall [36] and likely with a reduced mechanical anisotropy 62 [46]. In the context of phyllotaxis, the coupling between mechanics and chem-63 istry was explored theoretically [33] and mechanics has been proposed to reg-64 ulate the transport of auxin in the shoot apical meristem [17]. A mechanical 65 feedback was postulated, that was capable of generating a local accumulation 66 of auxin [17]. Based on preferential localization of PIN1 in the regions of the 67 plasma membrane in contact with the wall with highest mechanical stress, this 68 mechanical feedback is supported by several experiments [17, 32, 7]. However, 69 whether PIN1 polarity is driven by the stress or by the strain of the cell wall 70 remains unclear. More generally, the issue of stress- or strain-sensing calls for 71 further investigation, especially in the context of plant development [31]. 72

Here we address this issue by investigating a model of mechanical feedback
on auxin transport, whose predictions can be compared with experimental
results. We also study the robustness of the pattern formation mechanism with

 $\mathbf{2}$

Stress- or strain-sensing in phyllotaxis

3

respect to noise and abrupt changes in some of the biochemical parameters.The predictions of the two mechanisms do not differ much. A feedback based

⁷⁸ on strain nonetheless leads to more realistic predictions, and to a patterning

⁷⁹ mechanism which is less sensitive to noisy inputs or sharp perturbations.

80 2 Results

81 2.1 A mechanochemical model of auxin patterning

We built a mechanochemical model of auxin transport and tissue mechanics 82 that incorporates the influence of auxin on the stiffness of the cell wall and 83 the mechanical feedback from the cell wall on auxin transport, as illustrated 84 in Fig. 1A. We briefly describe its main ingredients here, deferring further 85 details to the section ?Model formulation? below. We model a single cell layer, 86 accounting for the epidermis of the shoot apex. We simplify tissue topology and 87 assume the cells to form a regular hexagonal lattice, following [17], because we 88 aim at stereotypic simulation results that would ease the comparison between 89 strain- and stress-sensing mechanisms. The tissue is under global isotropic 90 tension from turgor pressure, the cellular inner pressure that is assumed to 91 be uniform. Each edge of the lattice is made of two contiguous cell walls 92 associated with either of the two neighboring cells. Each cell wall is modeled 93 94 by a linear spring, whose stiffness decreases with auxin concentration in the corresponding cell. This accounts for the positive effect of auxin on growth 95 in the shoot apex. Changes in auxin concentration are due to production, 96 degradation, diffusion and transport. We considered two hypotheses for the 97 mechanical feedback: PIN1 transporters are inserted preferentially facing the 98 cell walls that (i) undergo the highest elastic strain (elastic deformation) or 99 (ii) the highest stress (force). 100

We used two main observables: the polarity \mathcal{P} , defined as the ratio between 101 the highest and the lowest transporter concentrations along the cell edges, and 102 the membrane fraction \mathcal{F} , defined as the ratio between the number of trans-103 porters localized at the plasma membrane and the total amount of transporters 104 in a cell (Fig. 1B). Typical patterns obtained by solving the models numeri-105 cally are shown in Fig. 1C. In order to verify our simulations, we performed 106 an analytical linear stability analysis of the homogeneous state (supplemen-107 tary text and Fig. S1) and predicted wavelengths in agreement with numerical 108 results for a broad range of parameter values. 109

For a given set of parameters, wavelengths are larger for strain- than for stress-sensing (in agreement with linear stability analysis, see the supplementary note and Fig. S1). Because of this discrepancy in the wavelength, all analyses performed hereafter were also carried out on 4 other sets of parameters for the stress feedback, presented in the supplementary material (Fig. S2-6). These additional simulations largely support the conclusions presented here.

4 Julien et al.

116 2.2 Cell ablation induces radial polarity

When a single epidermal cell is laser-ablated at the shoot apex, the hypothesis 117 that the epidermis is in tension implies that the maximal stress orientation is 118 circumferential around the ablation [15]. The preferential localization of PIN1 119 transporters in the stretched walls would then makes the transport radial. A 120 model similar to ours was able to reproduce this observation with a stress-121 driven feedback [17]. Here, we specifically asked whether our model can, with 122 a strain-driven feedback mechanism, reproduce the experimental results. As 123 in [17], we modeled the ablated cell by removing auxin, its transporters, and 124 cell walls, and by preventing the insertion of transporters in the membranes 125 adjacent to ablated cells. Our results, see Figure 2, show a radial reorientation 126 of the transporters in neighboring cells, as observed in experiments [17]. It 127 thus appears that ablation experiments do not allow us to distinguish between 128 strain- and stress-feedback mechanisms. 129

¹³⁰ 2.3 Variations of polarity with turgor pressure

Experiments with osmotic treatments show that PIN1 polarity \mathcal{P} is affected by changes in turgor pressure [32]. By performing osmotic treatments, turgor pressure was varied by approximately a factor 2 in these experiments. Polarity was significantly smaller (-15%) with reduced turgor and slightly smaller (-5%) with enhanced turgor.

We simulated the effect of a gradual increase or decrease of turgor pressure by modulating tissue tension σ (see Eq.(1)) with respect to its reference value, σ_0 , and monitored the change in the polarity of the transporters with the two mechanical feedbacks. Our results, see Fig. 3, show that, irrespective of the feedback mechanism, polarity increases over a range $0.5 sigma_0 \lesssim \sigma \lesssim 2\sigma_0$, as observed experimentally.

The increase is sharper at small turgor pressure, consistent with experiments. At higher values of σ ($\sigma \approx 2\sigma_0$), the two models slightly disagree with experiments as they predict a slight increase in polarity while a small decrease is observed. It is unclear whether this is due to a shortcoming of the model or to a bias in experimental quantifications.

¹⁴⁷With the stress feedback, the sharp drop seen in Fig. 3 is due to a change ¹⁴⁸ in the overall generated pattern of auxin peaks, which is not relevant in the ¹⁴⁹ present context, and is followed by an increase in polarity; examples of tissues ¹⁵⁰ and PIN1 distributions before and after this transition are shown in Figure ¹⁵¹ S2.

¹⁵² 2.4 Global softening of the tissue increases polarity

¹⁵³ Cellulose is the stiffest polysaccharide in plant cell walls. Accordingly, impair-

¹⁵⁴ ing cellulose synthesis by chemical treatments leads to the softening of plant

Stress- or strain-sensing in phyllotaxis

tissues [42]. The consequence of such treatments on PIN1 localization was also

investigated in [17]. First, PIN1 polarity, \mathcal{P} , is amplified - the membrane con-

¹⁵⁷ centration of PIN1 increases where it is high before treatment and decreases

elsewhere. Second, the amount of PIN1 decreases in the cytosol, or equivalently

We simulated such treatments by decreasing the minimal stiffness, k_{min} , of

the cell walls (Fig. 4). The increase in polarity \mathcal{P} and in the membrane fraction

 $_{162}$ \mathcal{F} is reproduced with both feedbacks (Fig. 4). Therefore, these experiments

¹⁶³ do not allow us to discriminate between the two models of feedback.

¹⁶⁴ 2.5 Reducing auxin-driven softening disrupts polarity

It has been shown that de-methyl-esterification of the pectin homogalactur-165 onan by pectin methylesterases (PMEs) is necessary for auxin to soften the 166 cells [37]. Indeed, in plants overexpressing an inhibitor of PME activity, PME 167 INHIBITOR3, patterns of auxin accumulation are absent and organ primor-168 dia do no form. In addition, the application of auxin does not trigger any of 169 auxin response (no expression of the response reporter DR5), tissue softening, 170 or organ formation [7]. In these plant lines, polarity and membrane fraction 171 are both reduced. 172

To simulate such overexpression, we decreased the value of the parameter, 173 δk (see Eq.(3) in the method section), that controls the sensitivity of cell 174 wall stiffness to auxin. A vanishing δk means that auxin has no effect on wall 175 stiffness, and high values of δk imply that a small change in auxin level induces 176 a large change in stiffness. In parallel, we kept $k_{min} + \delta k$ constant so that the 177 reference stiffness in the absence of auxin remains unchanged. We measured 178 the polarity \mathcal{P} and the fraction \mathcal{F} of transporters in the cell walls with the 179 two types of feedbacks. 180

Fig. 5 shows that when δk decreases, the polarity converges towards 1, corresponding to a homogeneous state with no patterns. Thus the conclusion that the polarity is lost as a result of tissue stiffening is independent of the nature of the mechanical feedback. However, the variations of the membrane fraction \mathcal{F} depend on the precise feedback mechanism. When stiffening the tissue, \mathcal{F} decreases with the strain feedback, but remains approximately constant with the stress-feedback, as shown in Fig. 5.

The behavior of the model can be understood analytically. In the homoge-188 neous state, that is in the absence of auxin patterns as observed in the simula-189 tions when δk is decreased, the membrane fraction can be computed exactly, 190 as shown in the supplementary material. The calculations predict that \mathcal{F}^{stress} 191 remains constant, whereas \mathcal{F}^{strain} decreases when δk decreases. Note that it 192 is relevant to assume a homogeneous state of the system because naked meris-193 tems do not show auxin accumulation patterns. In view of these results, we 194 conclude that a strain feedback is more realistic to describe experiments where 195 PME activity is reduced. 196

¹⁵⁹ the membrane fraction \mathcal{F} increases.

6 Julien et	t al.
-------------	-------

¹⁹⁷ 2.6 Correlation between auxin level and polarity

Experiments showed that, at the initiation of a primordia, the concentration 198 of PIN1 transporters in the plasma membrane increases with the concentra-199 tion of auxin [18], likely independently of the increase in PIN1 transcription in 200 201 response to auxin. Comparison of auxin and PIN1 concentrations in our simulations show that with the strain-sensing mechanism, the model qualitatively 202 reproduces the experimental observations, whereas a stress-sensing mechanism 203 leads to the opposite behavior, see Fig. 6. Again, analytical calculations in the 204 limit of small auxin fluctuations around the homogeneous state confirm this 205 result, regardless of the choice of parameters (see supplementary material). 206 Accordingly, these results support a strain-sensing mechanism. 207

208 2.7 Robustness to noise

We now investigate the sensitivity of the pattern to noise. To this end, a random term, temporally and spatially uncorrelated, is added to the production rate of auxin or to the concentration of transporters, respectively s_a or P in Eq. (2). We measured the wavelength of the pattern and its dependence on the noise amplitude.

As shown by panel A in Fig. 7, the pattern is almost insensitive to the 214 effect of noise on the production of auxin. In contrast, see Fig. 7B, noise in the 215 concentration of transporters P alters significantly the pattern. With the stress 216 feedback, the wavelength is observed to increase by up to 200%, whereas the 217 increase in the wavelength of the pattern is only 20% with the strain feedback. 218 This difference in the sensitivity of the patterns to noise in the transporters, 219 PIN1, also discriminates between the two models. Although it is difficult to 220 assess the level of noise in plants, the striking robustness of phyllotaxis to 221 fluctuations in auxin levels [50] suggests that the feedback based on strain is 222 more plausible. 223

224 2.8 Robustness to sharp variations

Even though robustness to noise discriminates between the two models, noise is difficult to manipulate experimentally. Robustness can also be tested by applying a transient shock to the system, i.e. a sharp modification of the external parameters, and by observing whether the tissue returns to its pre-shock state. Experimentally, this is feasible by transient chemical perturbation, such as external auxin application or inhibition of auxin transport, as performed in [18].

We simulated such perturbations as follows. In a tissue at equilibrium, we modified the mean auxin level $\langle a \rangle$ (through the production rate s_a , as shown in Fig. 8A) or the concentration of transporters P, as shown in Fig. 8B, and let the system reach a new equilibrium. Then we reset the parameter to its

Stress- or strain-sensing in phyllotaxis

initial value and obtain a third equilibrium. We compared the wavelengths of
the first and third equilibria. Observing the same wavelength would mean that
the system exactly recovers its initial state.

Fig. 8 shows that large differences in wavelength can be observed between the first and the third equilibria, with both feedbacks. The strain feedback, however, is not as sensitive, compare the left and right columns in Fig. 8. This is qualitatively consistent with its lower sensitivity to noise, documented in

 $_{\rm 243}$ $\,$ the previous subsection.

244 3 Discussion

We developed here a mechanochemical model for auxin patterning in the plant shoot apical meristem. The central question, addressed with our model, is whether the insertion of the efflux facilitators (transporters) in the membrane depends on the strain of the cell walls or by the stress applied to them. To this end, we compared the predictions of the model with available experimental results.

We found that both stress and strain-based feedbacks lead to similar predic-251 tions when simulating cell ablation, turgor-induced changes in tissue tension, 252 or a global reduction of the stiffness of the cell walls, generally in agreement 253 with observations. Modeling PMEI-overexpressing plants allowed us to dis-254 criminate between the two models: Assuming that auxin effect on cell wall 255 stiffness is reduced in such plant lines, we found that only the strain-based 256 feedback directly accounted for observations. Interestingly, organogenesis is 257 abolished in these lines, showing that it is useful to study the system be-258 havior in the absence of patterns. We also compared patterns of auxin and 259 PIN1 concentrations, and show both numerically and analytically that only 260 a strain-sensing mechanism can explain their correlation, as observed in in-261 cipient primordia, whereas a stress-sensing mechanism leads to anti-correlated 262 patterns. Note that we assume the amount of transporters per cell to be con-263 stant, whereas experiments show that the application of auxin increases PIN1 264 concentration [18]. Although we cannot rule out that this effect compensates 265 for the observed anti-correlation with a stress-based feedback, a strain-sensing 266 mechanism seems more plausible. Finally, investigating the effect of noise, or 267 of a transient change in chemical parameters, on patterning strengthened our 268 conclusion: Patterns appear more robust with the strain-based feedback. 269 The model, however, partly failed to reproduce the observations concern-270

ing the effect of tissue tension on polarity. Irrespective of the feedback chosen, 271 we found that polarity increased with tension, whereas, experimentally, po-272 larity slightly decreases from isotonic to hypertonic conditions [32]. Again, a 273 possible explanation is that we assumed the amount of transporters per cell 274 to be constant, whereas PIN1 levels decrease in hypertonic conditions; other 275 processes might be triggered when plants react to such osmotic stress. Alter-276 natively, the slight decrease observed could be due to a small experimental 277 bias, and the difference between experiments and our simulations may not be 278

Julien et al.

very significant. In addition, the model yielded wavelengths that were smaller 279 for stress- than for strain-sensing. As we have found no explanation for this 280 difference, it is difficult to know whether the model is incomplete or whether 281 this result would also favor strain-sensing because auxin peaks are observed 282 to have a smaller spatial extent than inter-peak distance, which resembles 283 more simulated strain-sensing (Fig. 1C, right). Because of this difference in 284 wavelength, we also explored other values of parameters for the stress-based 285 feedback and found that all conclusions on the comparison with the strain-286 based feedback hold, except for the conclusions on robustness that are more 287 sensitive to parameter values (supplementary text, Figures S2-S7). 288

The idealized hexagonal geometry of the cells and the absence of tissue 289 growth are important limitations of the model. The agreement of the analyti-290 cal linear stability analysis with simulations (supplementary text, Figure S1) 291 makes it likely that cell topology has little effect on our conclusions. Nevethe-292 less, plants respond to mechanical stimuli by altering their growth rates [31]. 293 Increase in auxin levels induce cell growth [7], which induces tissue reorgani-294 zation, changes in mechanical stress [2], and ultimately feeds back on auxin 295 transport. Although these processes are important in development, mechanical 296 signals are quasi-instantaneous and mostly depend on the current state of the 297 tissue, so that we we do not expect them to significantly our main conclusions. 298

Altogether, our results favor a feedback based on strain, though many of 299 the experimental configurations are insensitive to whether the mechanical feed-300 back on auxin transport is provided by stress or strain. This raises the question 301 of the underlying molecular mechanisms. Many types of mechanosensors are 302 known [38]. In the case of PIN1, strain could shift the balance between en-303 docytosis and exocytosis, accounting for the strain-based feedback, because 304 osmotic stress affects cell trafficking, in particular through clathrin-mediated 305 endocytosis [53]. The contact between the plasma membrane and the cell wall 306 is needed for PIN1 polarity [5, 10], suggesting that the mechanical state of the 307 cell wall is relevant for polarity. However the role of the cell wall might just 308 be to reduce lateral diffusion of PIN1, helping to maintain the distribution of 309 PIN1 determined by cell trafficking [27]. 310

The question of strain- and stress-sensing was raised in the different, but 311 related, context of plant cortical microtubules orientation by mechanical cues, 312 and the combination of experimental and theoretical approaches suggests that 313 stress-sensing is more likely to be involved [15,6]. The same conclusion has 314 been reached concerning the actomyosin cortex in the drosophila wing disc 315 [25]. However, the case of isolated animal cells has been debated. Early ex-316 periments suggested force- [12] or deformation-sensing [43]. More recent ex-317 periments showed that deformation-sensing occurred at low force, while force-318 sensing occurred at high force [52], though other experiments showed that 319 cells could sense the stiffness of extracellular space [30], consistently with the 320 observation that cells can differentiate according to the stiffness of their envi-321 ronment [3]. Actually, mechanosensing occurs at different scales [38], so that 322 the mechanical variable sensed may depend on the sensing molecule and on 323

the specific function associated with sensing.

8

Stress- or strain-sensing in phyllotaxis

In order to disentangle the parameters involved in mechanosensing, it is necessary to combine experimental perturbations of cells or tissues with analytical and computational studies of their behaviors. This is now made possible by the improvement of micromechanical [28] and computational [8] approaches. In this spirit, the present work provides insight on how the interaction between biomechanical and biochemical fields may contribute to the robustness of morphogenesis.

332 Model formulation

Tissue mechanics. We model auxin transport through anticlinal cell walls in 333 the epidermis and thus we neglect the mechanical contribution of other cell 334 walls. We assume that the rest state of the tissue to be a regular hexagonal 335 tiling of the plane and we formulate the problem in terms of a vertex model 336 with periodic boundary conditions. The equilibrium positions of the vertices 337 are obtained by minimizing the mechanical energy of the N_C cells. The con-338 tribution to the mechanical energy of the cell wall common to adjacent cells i339 and j has a linear density 340

$$E_{i,j} = \frac{1}{2} k_{i,j} \varepsilon_{i,j}^2 \; ; \; \varepsilon_{i,j} = \frac{l_{i,j} - l^{(0)}}{l^{(0)}} \; ,$$

where $k_{i,j}$ is the stiffness of this wall, $l_{i,j}$ its length (with an equilibrium value equal to l^0), and $\varepsilon_{i,j}$ its strain. The stress in the anticlinal wall is then given

³⁴³ by the derivative of its energy with respect to strain:

$$S_{i,j} \equiv \frac{\partial E_{i,j}}{\partial \varepsilon_{i,j}}.$$

The forces resulting from turgor pressure and tissue curvature are accounted for by external stress with components σ_x , σ_y . The total energy of the tissue then takes the form

$$E = -\sigma_x L_y^{(0)} \left(L_x - L_x^{(0)} \right) - \sigma_y L_x^{(0)} \left(L_y - L_y^{(0)} \right) + \sum_{\langle i,j \rangle} l^{(0)} E_{i,j}$$
(1)

where L_x and L_y are the tissue dimensions along the x and y directions and $L_x^{(0)}$ and $L_y^{(0)}$ their values without external stress; the sum is over the pairs of neighboring cells $\langle i, j \rangle$. Here we considered only the case of isotropic stress, such that $\sigma_x = \sigma_y = \sigma$.

Auxin dynamics and coupling with the mechanics. We use the same assumptions as in previous studies [22,44]. Namely, we only model auxin concentrations in the cytosol. We also assume that PIN1 dynamics occurs with a time scale that is shorter than the time necessary for the transport of auxin through cell walls. The latter is therefore the limiting step. These assumptions allow

10

Julien et al.

us to reduce the model to only one chemical equation that describes the auxin concentration, a_i , in cell *i*:

$$\frac{da_{i}}{dt} = s_{a} - d_{a}a_{i} - \frac{P}{A} \sum_{\langle j \rangle_{i}} l_{i,j} \left(t\left(a_{i}\right) p_{i,j} - t\left(a_{j}\right) p_{j,i} \right) - \frac{D}{A} \sum_{\langle j \rangle_{i}} l_{i,j} \left(a_{i} - a_{j}\right), (2)$$

where s_a is auxin synthesis rate, d_a is auxin degradation rate by, and D is the diffusion coefficient. $\langle j \rangle_i$ is the set of indices of the 6 cells adjacent to cell i. We assume elastic strain to be small, so that the area of each cell is approximated by its rest area A. The total amount of PIN1 proteins per cell, $P \sum_{\langle j \rangle_i} l_{i,j}$, is assumed to be cell-independent; $p_{i,j}$ is the normalized linear concentration of

³⁶² PIN1 proteins localised at the membrane of cell *i* and facing cell *j* (this PIN1 ³⁶³ fraction is responsible for auxin efflux from cell *i* to cell *j*; *P* is used as a unit ³⁶⁵ of linear concentrations). The rate of auxin transport by PIN1 proteins, t(a), ³⁶⁶ has the following sigmoidal-dependence on auxin concentration:

$$t\left(a\right) = \frac{a^{H_{t}}}{K_{t}^{H_{t}} + a^{H_{t}}}$$

where K_t is a threshold in auxin concentration and H_t the Hill exponent.

Auxin controls tissue mechanics by softening the cell walls (Fig.1). The

³⁶⁹ stiffness of the walls decreases with the amount of auxin in the cells:

$$k_{i,j}(a_i) = k_{min} + \delta k \frac{K_k^{H_k}}{K_k^{H_k} + a_i^{H_k}}$$
(3)

where k_{min} is the wall stiffness in the absence of auxin, δk the variations in stiffness, K_k the auxin threshold and H_k the Hill exponent.

The mechanical effects on the cell also affect auxin dynamics via its transport. The amount of PIN1 transporters in a cell membrane is affected by the strain or the stress, as illustrated in Fig. 1A. The normalized concentration, $p_{i,j}$, of PIN1 proteins localised at the plasma membrane of cell *i* and facing cell *j* reads

$$p_{i,j} = \frac{K_f^{H_f} \left(\left(\alpha + (1 - \alpha) \frac{k_{i,j}}{k_0} \right) \varepsilon_{i,j} \right)^{H_f}}{1 + \frac{\sum\limits_{\langle k \rangle_i} l_{i,k} K_f^{H_f} \left(\left(\alpha + (1 - \alpha) \frac{k_{i,k}}{k_0} \right) \varepsilon_{i,k} \right)^{H_f}}{\sum\limits_{\langle k \rangle_i} l_{i,k}}}$$

where $\alpha = 0$ for the stress feedback, $\alpha = 1$ for the strain feedback, and $\varepsilon_{i,j}$ and $k_{i,j}$ stand for wall strain and wall stiffness as described above. The parameter k_0 is set to k^* , the value of the stiffness in the homogeneous equilibrium state, so that the contributions of stress and strain are of the same order of magnitude in the homogeneous state. K_f sets the amplitude of the insertion function and H_f is the corresponding exponent.

Stress- or strain-sensing in phyllotaxis

11

³⁸³ Observables. Two observables are relevant for the comparison between our ³⁸⁴ simulations and experimental observations, see Fig. 1B [32,7]. The first is ³⁸⁵ polarity, defined as the ratio of the PIN1 concentrations on the most and least ³⁸⁶ enriched membrane of cell i:

$$\mathcal{P}_i = rac{p_i^{max}}{p_i^{min}} = rac{\max \left(p_{i,j}
ight)}{\min \left(q_{i,j}
ight)_i},$$

and the second is the ratio of plasma membrane localized PIN1 to the total
 amount in the cell:

$$\mathcal{F}_i = \frac{P_i^{wall}}{P_i^{in} + P_i^{wall}} = \frac{\sum_{\langle j \rangle_i} l_{i,j} p_{i,j}}{\sum_{\langle j \rangle_i} l_{i,j}}.$$

³⁸⁹ P does not appear in this equation because it was used to normalise $p_{i,j}$. In ³⁹⁰ practice we note \mathcal{P} and \mathcal{F} their distribution of the tissue.

Implementation. The model was programmed in C. The mechanical energy 1 391 is minimized thanks to the BFGS algorithm [26], implemented in the NLopt 392 library [21]. The differential equations for the auxin concentration 2 are solved 393 iteratively thanks to the GNU GSL library with a Runge-Kutta (2, 3) method 394 [14]. We assume that the typical time-scale to reach the mechanical equilibrium 395 is much smaller than the time scale for auxin regulation. Consequently, we com-396 pute the evolution of the system iteratively, by first computing the evolution 397 of the auxin concentration on a small time-step, and updating the configura-398 tion of the tissue by minimizing its mechanical energy. The time-step dt = 0.1399 for the evolution of auxin dynamics was chosen to limit the number of energy 400 minimization while generating the same results than smaller values. The code 401 is available on Github (github.com/JeanDanielJulien/auxinTransport). Sim-402 ulations results were analyzed and figures were made with Matlab. Table 1 403 summarizes the values of the parameters used in the present work. 404

405 Acknowledgements This work was supported by the DFG SFB 937 "Collective behavior

⁴⁰⁶ of soft and biological matter", project A9, to AP, and by the Human Frontier Science

⁴⁰⁷ Program and the ANR MechInMorph (ANR-12-BSV2-0023) to AB.

408 References

- Adler, I., Barabe, D., Jean, R.V.: A history of the study of phyllotaxis. Annals of botany
 80(3), 231–244 (1997)
- Alim, K.: Regulatory role of cell division rules on tissue growth heterogeneity. Frontiers
 in Plant Science 3 (2012)
- Bellas, E., Chen, C.S.: Forms, forces, and stem cell fate. Curr Opin Cell Biol **31C**,
 92–97 (2014)
- 4.15 4. Bode, H.R.: Axial Patterning in Hydra. Cold Spring Harbor Perspectives in Biology
 4.16 1(1), a000,463-a000,463 (2009)

12	Julien et al.

- 417 5. Boutté, Y., Crosnier, M.T., Carraro, N., Traas, J., Satiat-Jeunemaitre, B.: The plasma
 418 membrane recycling pathway and cell polarity in plants: studies on pin proteins. J Cell
 419 Sci 119(Pt 7), 1255–65 (2006)
- Bozorg, B., Krupinski, P., Jönsson, H.: Stress and strain provide positional and directional cues in development. PLoS Comput Biol 10(1), e1003,410 (2014)
- 422 7. Braybrook, S.A., Peaucelle, A.: Mechano-Chemical Aspects of Organ Formation in Ara423 bidopsis thaliana: The Relationship between Auxin and Pectin. PLoS ONE 8(3), e57,813
 424 (2013)
- Chickarmane, V., Roeder, A.H., Tarr, P.T., Cunha, A., Tobin, C., Meyerowitz, E.M.:
 Computational Morphodynamics: A Modeling Framework to Understand Plant Growth.
 Annual Review of Plant Biology 61(1), 65–87 (2010)
- 9. Davidson, L.A.: Embryo mechanics balancing force production with elastic resistance
 during morphogenesis. Curr Top Dev Biol 95, 215–41 (2011)
- 430 10. Feraru, E., Feraru, M.I., Kleine-Vehn, J., Martinière, A., Mouille, G., Vanneste, S.,
 431 Vernhettes, S., Runions, J., Friml, J.: Pin polarity maintenance by the cell wall in
 432 arabidopsis. Current biology : CB 21(4), 338–43 (2011)
- Fernández-Sánchez, M.E., Barbier, S., Whitehead, J., Béalle, G., Michel, A., Latorre-Ossa, H., Rey, C., Fouassier, L., Claperon, A., Brullé, L., Girard, E., Servant, N., Rio-Frio, T., Marie, H., Lesieur, S., Housset, C., Gennisson, J.L., Tanter, M., Ménager, C., Fre, S., Robine, S., Farge, E.: Mechanical induction of the tumorigenic -catenin pathway by tumour growth pressure. Nature 523(7558), 92–5 (2015)
- 438 12. Freyman, T.M., Yannas, I.V., Yokoo, R., Gibson, L.J.: Fibroblast contractile force is
 independent of the stiffness which resists the contraction. Exp Cell Res 272(2), 153–62
 (2002)
- 441 13. Geitmann, A., Ortega, J.K.: Mechanics and modeling of plant cell growth. Trends in
 442 Plant Science 14(9), 467–478 (2009)
- 443 14. Gough, B.: GNU Scientific Library Reference Manual Third Edition, 3rd edn. Network
 444 Theory Ltd. (2009)
- Hamant, O., Heisler, M.G., Jnsson, H., Krupinski, P., Uyttewaal, M., Bokov, P., Corson,
 F., Sahlin, P., Boudaoud, A., Meyerowitz, E.M., others: Developmental patterning by
 mechanical signals in Arabidopsis. science 322(5908), 1650–1655 (2008)
- 448 16. Hamant, O., Traas, J.: The mechanics behind plant development. New Phytologist
 449 185(2), 369–385 (2010)
- Heisler, M.G., Hamant, O., Krupinski, P., Uyttewaal, M., Ohno, C., Jnsson, H., Traas,
 J., Meyerowitz, E.M.: Alignment between PIN1 Polarity and Microtubule Orientation
 in the Shoot Apical Meristem Reveals a Tight Coupling between Morphogenesis and
 Auxin Transport. PLoS Biology 8(10), e1000,516 (2010)
- Heisler, M.G., Ohno, C., Das, P., Sieber, P., Reddy, G.V., Long, J.A., Meyerowitz, E.M.:
 Patterns of Auxin Transport and Gene Expression during Primordium Development
 Revealed by Live Imaging of the Arabidopsis Inflorescence Meristem. Current Biology
 15(21), 1899–1911 (2005)
- Howard, J., Grill, S.W., Bois, J.S.: Turing's next steps: the mechanochemical basis of
 morphogenesis. Nature Reviews Molecular Cell Biology 12(6), 400–6 (2011)
- Iskratsch, T., Wolfenson, H., Sheetz, M.P.: Appreciating force and shape the rise of mechanotransduction in cell biology. Nat Rev Mol Cell Biol (2014)
- 462 21. Johnson, S.G.: The NLopt nonlinear-optimization package (2010). URL
 463 https://nlopt.readthedocs.io/en/latest/
- 464 22. Jnsson, H., Heisler, M.G., Shapiro, B.E., Meyerowitz, E.M., Mjolsness, E.: An auxindriven polarized transport model for phyllotaxis. Proceedings of the National Academy
 of Sciences of the United States of America 103(5), 1633–1638 (2006)
- 467 23. Kondo, S.: The reaction-diffusion system: a mechanism for autonomous pattern forma-468 tion in the animal skin. Genes to Cells 7(6), 535–541 (2002)
- 469 24. Kuhlemeier, C., Reinhardt, D.: Auxin and phyllotaxis. Trends in plant science 6(5),
 470 187–189 (2001)
- 471 25. Legoff, L., Rouault, H., Lecuit, T.: A global pattern of mechanical stress polarizes cell
 472 divisions and cell shape in the growing drosophila wing disc. Development 140(19),
 473 4051-9 (2013)
- 474 26. Luksan, L.: New subroutines for large-scale optimization (2007). URL
 475 http://www.cs.cas.cz/luksan/subroutines.html

Stress- or strain-sensing in phyllotaxis

- 476 27. Martinière, A., Lavagi, I., Nageswaran, G., Rolfe, D.J., Maneta-Peyret, L., Luu, D.T.,
 477 Botchway, S.W., Webb, S.E.D., Mongrand, S., Maurel, C., Martin-Fernandez, M.L.,
 478 Kleine-Vehn, J., Friml, J., Moreau, P., Runions, J.: Cell wall constrains lateral diffusion
- of plant plasma-membrane proteins. P Natl Acad Sci Usa (2012)
- 28. Milani, P., Braybrook, S.A., Boudaoud, A.: Shrinking the hammer: micromechanical approaches to morphogenesis. J Exp Bot 64(15), 4651–62 (2013)
- 29. Milani, P., Gholamirad, M., Traas, J., Arnodo, A., Boudaoud, A., Argoul, F., Hamant,
 O.: In vivo analysis of local wall stiffness at the shoot apical meristem in Arabidopsis
 using atomic force microscopy: Measuring wall stiffness in meristems with AFM. The
 Plant Journal 67(6), 1116–1123 (2011)
- Mitrossilis, D., Fouchard, J., Pereira, D., Postic, F., Richert, A., Saint-Jean, M., Asnacios, A.: Real-time single-cell response to stiffness. Proceedings of the National Academy of Sciences of the United States of America (2010)
- 489 31. Moulia, B., Coutand, C., Julien, J.L.: Mechanosensitive control of plant growth: bearing
 490 the load, sensing, transducing, and responding. Frontiers in Plant Science 6 (2015)
- 32. Nakayama, N., Smith, R.S., Mandel, T., Robinson, S., Kimura, S., Boudaoud, A., Kuh lemeier, C.: Mechanical Regulation of Auxin-Mediated Growth. Current Biology 22(16),
 1468–1476 (2012)
- 494 33. Newell, A.C., Shipman, P.D., Sun, Z.: Phyllotaxis: Cooperation and competition be 495 tween mechanical and biochemical processes. Journal of Theoretical Biology 251(3),
 496 421-439 (2008)
- 497 34. Okada, K., Ueda, J., Komaki, M.K., Bell, C.J., Shimura, Y.: Requirement of the auxin
 498 polar transport system in early stages of Arabidopsis floral bud formation. The Plant
 499 Cell Online 3(7), 677–684 (1991)
- 35. Palme, K., Glweiler, L.: PIN-pointing the molecular basis of auxin transport. Current
 opinion in plant biology 2(5), 375–381 (1999)
- 36. Peaucelle, A., Braybrook, S.A., Guillou, L.L., Bron, E., Kuhlemeier, C., Höfte, H.:
 Pectin-induced changes in cell wall mechanics underlie organ initiation in arabidopsis.
 Current biology : CB (2011)
- 37. Peaucelle, A., Louvet, R., Johansen, J.N., Hfte, H., Laufs, P., Pelloux, J., Mouille, G.:
 Arabidopsis Phyllotaxis Is Controlled by the Methyl-Esterification Status of Cell-Wall
 Pectins. Current Biology 18(24), 1943–1948 (2008)
- 38. Pruitt, B.L., Dunn, A.R., Weis, W.I., Nelson, W.J.: Mechano-transduction: from molecules to tissues. Plos Biol 12(11), e1001,996 (2014)
- 39. Rauzi, M., Lenne, P.F.: Cortical forces in cell shape changes and tissue morphogenesis.
 Curr Top Dev Biol 95, 93-144 (2011)
- 40. Reinhardt, D., Mandel, T., Kuhlemeier, C.: Auxin regulates the initiation and radial
 position of plant lateral organs. The Plant Cell Online 12(4), 507–518 (2000)
- 41. Reinhardt, D., Pesce, E.R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M.,
 Traas, J., Friml, J., Kuhlemeier, C.: Regulation of phyllotaxis by polar auxin transport.
 Nature 426(6964), 255–260 (2003)
- 42. Ryden, P., Sugimoto-Shirasu, K., Smith, A.C., Findlay, K., Reiter, W.D., McCann,
 M.C.: Tensile properties of arabidopsis cell walls depend on both a xyloglucan crosslinked microfibrillar network and rhamnogalacturonan ii-borate complexes. Plant Physiol 132(2), 1033–40 (2003)
- 43. Saez, A., Buguin, A., Silberzan, P., Ladoux, B.: Is the mechanical activity of epithelial cells controlled by deformations or forces? Biophysical Journal **89**(6), L52–4 (2005)
- 44. Sahlin, P., Sderberg, B., Jnsson, H.: Regulated transport as a mechanism for pattern generation: Capabilities for phyllotaxis and beyond. Journal of Theoretical Biology
 258(1), 60-70 (2009)
- 45. Sampathkumar, A., Yan, A., Krupinski, P., Meyerowitz, E.M.: Physical Forces Regulate
 Plant Development and Morphogenesis. Current Biology 24(10), R475–R483 (2014)
- 46. Sassi, M., Ali, O., Boudon, F., Cloarec, G., Abad, U., Cellier, C., Chen, X., Gilles, B.,
- Milani, P., Friml, J., Vernoux, T., Godin, C., Hamant, O., Traas, J.: An Auxin-Mediated
 Shift toward Growth Isotropy Promotes Organ Formation at the Shoot Meristem in
 Arabidopsis. Current Biology 24(19), 2335–2342 (2014)
- 47. Smith, R.S., Bayer, E.M.: Auxin transport-feedback models of patterning in plants.
 Plant, Cell & Environment 32(9), 1258–1271 (2009)

14		Julien et al.

- 48. Smith, R.S., Guyomarc'h, S., Mandel, T., Reinhardt, D., Kuhlemeier, C., Prusinkiewicz,
 P.: A plausible model of phyllotaxis. Proceedings of the National Academy of Sciences
 of the United States of America 103(5), 1301–1306 (2006)
- 49. Stoma, S., Lucas, M., Chopard, J., Schaedel, M., Traas, J., Godin, C.: Flux-Based
 Transport Enhancement as a Plausible Unifying Mechanism for Auxin Transport in
 Meristem Development. PLoS Computational Biology 4(10), e1000,207 (2008)
- 50. Vernoux, T., Brunoud, G., Farcot, E., Morin, V., Van Den Daele, H., Legrand, J., Oliva,
 M., Das, P., Larrieu, A., Wells, D., Guédon, Y., Armitage, L., Picard, F., Guyomarc'h,
 S., Cellier, C., Parry, G., Koumproglou, R., Doonan, J.H., Estelle, M., Godin, C., Kepinski, S., Bennett, M., De Veylder, L., Traas, J.: The auxin signalling network translates
 dynamic input into robust patterning at the shoot apex. Molecular Systems Biology 7,
 508 (2011)
- 546 51. Vernoux, T., Kronenberger, J., Grandjean, O., Laufs, P., Traas, J.: PIN-FORMED 1
 regulates cell fate at the periphery of the shoot apical meristem. Development 127(23),
 5157-5165 (2000)
- 549 52. Yip, A.K., Iwasaki, K., Ursekar, C., Machiyama, H., Saxena, M., Chen, H., Harada, I.,
 550 Chiam, K.H., Sawada, Y.: Cellular response to substrate rigidity is governed by either
 551 stress or strain. Biophys J 104(1), 19–29 (2013)
- 53. Zwiewka, M., Nodzyński, T., Robert, S., Vanneste, S., Friml, J.: Osmotic stress modulates the balance between exocytosis and clathrin-mediated endocytosis in arabidopsis
 thaliana. Mol Plant 8(8), 1175–87 (2015)

Stress- or strain-sensing in phyllotaxis

15

Table 1 Values of the parameters used in the simulation: Parameters were estimated from previous models [22, 44, 17]. Stiffness dependence on auxin concentration was chosen to display 5-fold variations, in the range of realistic values [29]. The tension resulting from hydrostatic pressure was then chosen to yield a typical deformation of 4%, in the range estimated from osmotic treatments [32]. Finally, PIN1 insertion was tuned so that the fraction of transporters inserted in the membrane is around 0.7, similar to experimental estimates [32].

$l^{(0)}$	σ	s_a	d_a	D	P	K_t	H_t	K_f	H_{f}	k_0	k_{min}	δk	K_k	H_k
1	1.4	1	0.5	5	100	2	1	30	3	30	10	40	2	2



Fig. 1 Modeling assumptions and observables: (A) Schematic of the model: the tissue consists of hexagonal cells. The auxin transporters are localized at the plasma membranes and are influenced by the mechanical status of the corresponding cells walls. The biochemical and mechanical effects are presented in two different boxes, and their interactions are represented by arrows. The tissue is stretched by the turgor pressure. PIN1 proteins facilitate auxin movement out of cells. The stiffness of each cell wall is a decreasing function of the auxin concentration in the cell. The amount of effective transporters increases with the stress or strain at the cell wall. (B) Schematic definition of the two observables: the triangles represents the PIN1 transporters, colored according to the definition of the observables. The polarity \mathcal{P} is the ratio between the two extremal concentrations in the cell walls of a cell. The fraction \mathcal{F} is the ratio between the number of effective transporters and the total number of transporters. (C) An example of pattern predicted by the model, with each feedback (left: stress; right:strain). The cells are colored according to their auxin concentration (gray levels), the cell walls according to the density of PIN1 transporters (blue-green levels).

16

Stress- or strain-sensing in phyllotaxis

17



Fig. 2 Radial polarity around a cell ablation: The red hexagon corresponds to the ablated cell, whose walls, auxin and transporters have been removed from the simulation. The transporters pointing toward this cell are also removed. The cells are colored according to their auxin concentration, the cell walls according to the concentration of transporters. The insertion of transporters is driven either by stress (A) or by strain (B). Auxin is not represented on the close-ups. Parameters for the strain feedback are indicated in Table 1. Parameters for the stress feedback are identical, except for $H_k = 1.06$, which was chosen to increase wavelength and better visualize the effect of an ablation on polarity (results are independent of the value of H_k).



Fig. 3 Dependence of polarity on turgor pressure: (A) Schematic of the simulations: starting from $\sigma^{(0)}$, the pressure is gradually increased or decreased, for pressures ranging from $0.1 \times \sigma^{(0)}$ and $10 \times \sigma^{(0)}$. (B) For each cell the polarity \mathcal{P} is measured. The curves represent the median value of \mathcal{P} and the shaded areas the interval between the 15th and 85th percentiles in a tissue of 3600 cells. The results are shown in red (left) for the stress-based feedback, in blue (right) for the strain-based feedback. The dashed lines show the range of pressure investigated experimentally [32] and the black crosses indicate the parameters for which tissues are shown in Fig. S8.



Fig. 4 Global softening of the tissue increases polarity: (A) Schematic of the simulations: starting from $k_{min}^{(0)}$, the minimal stiffness of the tissue is gradually decreased, ranging from $k_{min}^{(0)}$ to $k_{min}^{(0)}/10$. This corresponds to the stiffness at high levels of auxin. We quantified (B) the polarity \mathcal{P} and (C) the membrane fraction \mathcal{F} of transporters. The curves represent the median and the shaded areas the interval between the 15th and 85th percentiles in a tissue of 3600 cells. The results are shown in red (left) for the stress-based feedback, in blue (right) for the strain-based feedback.



Fig. 5 Reducing auxin effects on the cell wall disrupts polarity: (A) Schematic of the simulations: starting from $\delta k^{(0)}$, the amplitude of stiffness variations is gradually decreased, from $k_{min}^{(0)}$ to $0.3 \times k_{min}^{(0)}$. We quantified (B) the polarity \mathcal{P} and (C) the membrane fraction \mathcal{F} of transporters. The curves represent the median and the shaded areas the interval between the 15th and 85th percentiles in a tissue of 3600 cells. The results are shown in red (left) for the stress-based feedback, in blue (right) for the strain-based feedback.

21





Fig. 6 Correlation between auxin and PIN1 concentrations: Examples of pattern predicted by the model, with each feedback (A/B1: stress; A/B2: strain). The cells are colored according to their auxin concentration (gray levels, A1/2), or according to the density of PIN1 transporters, averaged over their walls (blue-green levels, B1/2).



Fig. 7 Robustness to noise: The auxin production rate s_a (A) or the PIN1 concentration P (B) is spatially and temporally random, with a uniform distribution centered around the value of these parameters without noise. The relative noise amplitude is half the ratio between the width of this interval and the average value of the variable. The wavelength is measured as the average distance between a peak and its nearest neighbor. The prediction of the model with the stress feedback is shown in red (left), while the one with the strain feedback is represented in blue (right). The curves represent the median and the shaded areas the interval between the 15th and 85th percentiles.



Fig. 8 Robustness to sharp variations: The auxin production rate s_a (A) or the PIN1 concentration P (B) is transiently modified over the entire tissue. Once the tissue reaches equilibrium, the original set of parameters is restored. The wavelength is measured at equilibrium before the shock and, and at equilibrium after reseting the original parameters. The wavelength before the shock is plotted in red for the stress feedback (left) and in blue for the strain feedback (right). The wavelength after the recovery is plotted in black. The curves represent the median, the shaded areas the interval between the 15th and 85th percentiles before the shock, and the hatched areas the same percentiles after the resetting.

Supplementary material Strain- or Stress-sensing in mechanochemical patterning by the phytohormone auxin

Jean-Daniel Julien Alain Pumir Arezki Boudaoud

Linear stability analysis

The analytical study of the system is performed in the case of isotropic external stress such that $\sigma_x = \sigma_y = \sigma$. The system is linearised by considering small variations of auxin around the homogeneous stable state $a^* + \delta_i$ where $a^* = s_a/d_a$. In the homogeneous stable state we can write the deformation of the walls, their stiffness and the rate of PIN1 insertion as:

$$\varepsilon^{*} = \frac{l^{*} - l_{0}}{l_{0}} = \frac{\sqrt{3}\sigma l_{0}}{2k(a^{*})},$$

$$k^{*} = k(a^{*}) = k_{min} + \Delta k \frac{K_{k}^{H_{k}}}{K_{k}^{H_{k}} + (a^{*})^{H_{k}}},$$

$$p^{*} = \frac{K_{f}^{H_{f}} \left[\left(\alpha + (1 - \alpha) \frac{k^{*}}{k_{0}} \right) \varepsilon_{i,j} \right]^{H_{f}}}{1 + K_{f}^{H_{f}} \left[\left(\alpha + (1 - \alpha) \frac{k^{*}}{k_{0}} \right) \varepsilon_{i,j} \right]^{H_{f}}},$$

We assume that the local variations in the mechanical tension are small compared with the variations of auxin. Consequently we can write:

$$\frac{l_{i,j} - l_{0}}{l_{0}} = \frac{\sqrt{3}\sigma l_{0}}{k(a_{i}) + k(a_{j})}$$

We then linearise the system:

$$\frac{d\delta_i}{dt} = -d_a\delta_i - \left[A - (1 - \alpha)B + 2(1 - \alpha)C\right]\sum_{\langle j \rangle_i} \left(\delta_i - \delta_j\right) + \frac{C}{N}\sum_{\langle j \rangle_i} \left[\sum_{\langle k \rangle_i} \left(\delta_i + \delta_k\right) - \sum_{\langle l \rangle_i} \left(\delta_j + \delta_l\right)\right] \;.$$

The constants A, B and C are defined as:

$$A = P \frac{l^*}{A^*} t'(a^*) p^* + D \frac{l^*}{A^*},$$

$$B = P \frac{l^*}{A^*} t(a^*) p^* \left(-\frac{H_f}{k^*} k'(a^*)\right),$$

$$C = P \frac{l^*}{A^*} t(a^*) (p^*)^2 \left(-\frac{H_f}{2k^*} k'(a^*)\right).$$

The system can be diagonalised by applying a Fourier transform $\delta_{\vec{k}} = \frac{1}{2\pi} \sum_{i} \delta_i e^{-i\vec{k} \cdot \vec{x}_i}$, where \vec{x}_i is the position of the cell *i*. Thus we obtain:

$$\dot{\delta_{\vec{k}}} = \left\{ -d_a - N \left[A - (1 - \alpha) B \right] \left(1 - S_{\vec{k}} \right) + NC \left[2 \left(S_{\vec{k}} - 1 \right) (1 - \alpha) + 1 - S_{\vec{k}}^2 \right] \right\} \delta_{\vec{k}}$$

$$\dot{\delta_{\vec{k}}} = \left\{ \left[-d_a - NA + N\left(1 - \alpha\right)B + N\left(2\alpha - 1\right)C \right] + \left[NA - N\left(1 - \alpha\right)B + 2N\left(1 - \alpha\right)C \right]S_{\vec{k}} - NCS_{\vec{k}}^2 \right\}\delta_{\vec{k}} \right\}$$

where $S_{\overrightarrow{k}} = \frac{1}{N} \sum_{p} e^{i \overrightarrow{k} \cdot \overrightarrow{e_{p}}}$ is the form factor of the tissue.

Then we can write the conditions of instability for the two models:

$$(1-\alpha)B + 2\alpha C > A,$$

$$N\frac{\left[\left(1-\alpha\right)B+2\alpha C-A\right]^{2}}{4C} > d_{a}$$
$$A+2\left(2-\alpha\right)C > \left(1-\alpha\right)B.$$

If these conditions are true, we can show that the most unstable wave vectors $\{\vec{k}_0\}$ fulfils

$$S_{\overrightarrow{k_0}} = \frac{A - (1 - \alpha)B + 2(1 - \alpha)C}{2C}$$

Note that $S_{\vec{k}}$ is a decreasing function of \vec{k} , and therefore an increasing function of the wavelength. Since $2C = p^*B < B$, the wavelength for the stress feedback ($\alpha = 0$) is always smaller than the wavelength for the strain feedback ($\alpha = 1$), for any set of parameters.

We compared these results with the wavelength measured in simulations for 2-fold changes in the value of each parameter, see Fig. S1. Overall, the linear stability analysis presented here provides a semi-quantitative explanation for the results of our numerical simulations.

Investigation of different parameters for the stress feedback

The most parsimonious set of parameters, presented in the main manuscript, leads to significanlty different wavelengths for the stress- and strain-sensing mechanisms. In order to evaluate the robustness of the results, we present here the same analysis with 4 different parameter changes of the stress feedback. We first tried modifying k_0 , the only parameter that distinguishes the two models. Decreasing k_0 increases the wavelength, but does not allow to reach wavelength of the strain-based feedback. Additionally, it increases the typical fraction of transporters inserted in the membrane p^* to 1, inconsistent with experimental observations. We present the results for $k_0 = k_{min}$, close to the wavelength saturation (panels 1 in all figures 2 to 7). We then tried to modify 3 parameters that are not involved in the definition of p^* , and which perturb neither the typical deformation of the cells ϵ^* nor their typical stiffness variations $\Delta k/k_{min}$. The three parameters values (panels 2: $H_k = 1.06$; panels 3: D = 17; panels 4: P = 30) were tuned by using the linear stability analysis to match the wavelength of the strain-based feedback. For figures 2 to 7, transporters concentration is regulated by stress. The curves all represent median values and the shaded areas the interval between the 15th and 85th percentiles in a tissue of 3600 cells. Parameters are the same as in Table 1 of the main manuscript, except for one parameter, modified in order to get comparable wavelengths for both feedbacks (1: $k_0 = k_{min}$; 2: $H_k = 1.06$; 3: D = 17; 4: P = 30).

The parameters sets presented here support the conclusion of the main manuscript. In particular, the qualitative differences between the stress-feedback and experiments, namely the absence of internalisation of transporters in simulated PMEI mutants (Fig. 4) and the anti-correlation of auxin and PIN1 patterns (Fig. 5), are valid for all other parameters investigated, consistent with the calculations in the limit of small fluctuations. Results regarding patterns robustness to noise (Fig. 6) and perturbations (Fig. 7) are mitigated, with very different sensitivities of the different parameters sets.



Figure 1: Dependence of the wavelength on each parameter of the model: The wavelength is measured for 2-fold changes in each parameter around the values given in Table 1 of the main text. Results are shown in red for the stress-based feedback, and in blue for the strain-based feedback. The black lines indicate the predictions from the linear stability analysis, in the two directions of the wave vector \vec{k} where the prediction is minimal and maximal. Note that these lines almost overlap, except for the smallest wavelengths.



Figure 2: Dependence of polarity on turgor pressure: Starting from $\sigma^{(0)}$, the pressure is gradually increased or decreased, for pressures ranging from $0.1 \times \sigma^{(0)}$ and $10 \times \sigma^{(0)}$. (1-4) Polarity of transporters for different parameters changes.



Figure 3: Global softening of the tissue increases polarity: Starting from $k_{min}^{(0)}$, the minimal stiffness of the tissue is gradually decreased, ranging from $k_{min}^{(0)}$ to $k_{min}^{(0)}/10$. (A1-4) Polarity and (B1-4) fraction of transporters for different parameters changes.



Figure 4: Reducing auxin effects on the cell wall disrupts polarity: Starting from $\delta k^{(0)}$, the amplitude of stiffness variations is gradually decreased, from $k_{min}^{(0)}$ to $0.3 \times k_{min}^{(0)}$. (A1-4) Polarity and (B1-4) fraction of transporters for different parameters changes.



Figure 5: Anticorrelation between auxin and PIN1 concentrations: Examples of pattern predicted by the model, with a stress feedback, for different parameters changes. The cells are colored according to their auxin concentration (gray levels, A1-4), or according to the density of PIN1 transporters, averaged over their walls (blue-green levels, B1-4).



Figure 6: **Robustness to noise:** The auxin production rate s_a (A) or the PIN1 concentration P (B) is spatially and temporally random, with a uniform distribution centered around the value of these parameters without noise. The relative noise amplitude is half the ratio between the width of this interval and the average value of the variable. The wavelength is measured as the average distance between a peak and its nearest neighbor, for different parameters changes (1-4).



Figure 7: Robustness to sharp variations: The auxin production rate s_a (A) or the PIN1 concentration P (B) is transiently modified over the entire tissue. Once the tissue reaches equilibrium, the original set of parameters is restored. The wavelength is measured at equilibrium before the shock and, and at equilibrium after reseting the original parameters. The wavelength before and after the shock is plotted in red and black respectively, for different parameters changes (1-4).

Change of pattern type with increased pressure for the stress-feedback

A sharp decrease of the median polarity \mathcal{P} was predicted with a stress-based feedback when tension is increased above $\sigma/\sigma_0 = 2.3$ (see Fig. 2C). This decrease is due to the change of pattern type. At low tension, the auxin peaks are limited to one or two cells. They become larger as tension increases. This enlargement corresponds to smaller auxin gradients between neighboring cells and accordingly to weaker polarities (see Fig. S8).

Dependence of the membrane fraction \mathcal{F} on δk

To simulate the effect of pectin methylesterase inhibition, we decrease the parameter δk , with the constraint that $k_{min} + \delta k$ remains constant. By doing so, the dependence of cell wall stiffness with auxin concentration is decreased but the stiffness in the absence of auxin remains the same. The simulations, presented in Fig. 3, show that with the two feedback mechanisms the polarity \mathcal{P} and the membrane fraction of PIN1 \mathcal{F} decrease until the auxin patterns vanish. After the disappearance of the pattern, the simulation results depend on the feedback mechanism. With a stress-based feedback, the fraction \mathcal{F} remains constant, whereas with a strain-based feedback it keeps decreasing. These different behaviors can be explained analytically. In the homogeneous state, the membrane fraction \mathcal{F} of transporters is:

$$\mathcal{F}^{stress} = \frac{K_f^{H_f} \left(\frac{k^*}{k_0} \varepsilon^*\right)^{H_f}}{1 + K_f^{H_f} \left(\frac{k^*}{k_0} \varepsilon^*\right)^{H_f}}$$
$$\mathcal{F}^{strain} = \frac{K_f^{H_f} \left(\varepsilon^*\right)^{H_f}}{1 + K_f^{H_f} \left(\varepsilon^*\right)^{H_f}}$$

where the star indicates the values in the homogeneous state. k^* is given by

$$k^* = k_{min} + \delta k \frac{K_k^{H_k}}{K_k^{H_k} + a^{*H_k}} = (k_{min} + \delta k) - \delta k \frac{a^{*H_k}}{K_k^{H_k} + a^{*H_k}}$$



Figure 8: Change in the overall pattern when tension increases: (A) Tissues are represented for $\sigma/\sigma_0 = 1.41$ (left) and $\sigma/\sigma_0 = 3.55$ (right). Each hexagon is a cell that is colored according to its auxin concentration. The two tissues correspond to the parameters marked by black crosses in Fig. 2B. (B) Probability distributions of the polarity \mathcal{P} computed on the respective tissues.

If we decrease the value of δk from an initial value δk^0 with the constraint that $k_{min} + \delta k$ remains constant, equal to its initial value $k_{min}^0 + \delta k^0$, k^* is then:

$$k^* = k_{min}^0 + \delta k^0 - \delta k \frac{{a^*}^{H_k}}{K_k^{H_k} + {a^*}^{H_k}}$$

Thus k^* is a decreasing function of δk . The deformation of a regular hexagonal lattice of springs with rest length l_0 , stiffness k^* and under a tension σ is given by $\varepsilon^* = \frac{\sqrt{3}\sigma l_0}{2k^*}$.

In conclusion, the fractions are

$$\mathcal{F}^{stress} = \frac{K_f^{H_f} \left(\frac{\sqrt{3}\sigma l_0}{2k_0}\right)^{H_f}}{1 + K_f^{H_f} \left(\frac{\sqrt{3}\sigma l_0}{2k_0}\right)^{H_f}}$$
$$\mathcal{F}^{strain} = \frac{K_f^{H_f} \left(\frac{\sqrt{3}\sigma l_0}{2k^*}\right)^{H_f}}{1 + K_f^{H_f} \left(\frac{\sqrt{3}\sigma l_0}{2k^*}\right)^{H_f}}$$

These formulas show that \mathcal{F}^{stress} does not depend on δk whereas \mathcal{F}^{strain} actually decreases because k^* increases, The second behavior is consistent with available experimental results [?].

Correlation between auxin and transporters concentrations

We want to study the variation of the mean concentration of transporters in a cell's membrane, $\bar{p} = (\sum_{\langle j \rangle_i} l_{i,j} P p_{i,j}) / (\sum_{\langle j \rangle_i} l_{i,j})$, as a function of small fluctuations of auxin δ_i around the homogeneous equilibrium. Since we assume that the total concentration of transporters is the same in each cell, this is equivalent to

the variations of the fraction $\mathcal{F}_i = (\sum_{\langle j \rangle_i} l_{i,j} p_{i,j}) / (\sum_{\langle j \rangle_i} l_{i,j})$.

The change in fraction $\delta \mathcal{F}_i$ is given by

$$\delta \mathcal{F}_{i} = \delta \left[\frac{\sum_{\langle j \rangle_{i}} l_{i,j} p_{i,j}}{\sum_{\langle j \rangle_{i}} l_{i,j}} \right] = \frac{1}{N} \sum_{\langle j \rangle_{i}} \delta p_{i,j}.$$

Recall the expression for $p_{i,j}$:

$$p_{i,j} = \frac{K_f^{H_f} \left[\left(\alpha + (1-\alpha) \frac{k_{i,j}}{k_0} \right) \varepsilon_{i,j} \right]^{H_f}}{1 + \frac{\sum\limits_{\langle k \rangle_i} l_{i,k} K_f^{H_f} \left[\left(\alpha + (1-\alpha) \frac{k_{i,k}}{k_0} \right) \varepsilon_{i,k} \right]^{H_f}}{\sum\limits_{\langle k \rangle_i} l_{i,k}}.$$

We use the same assumption than for the linear stability analysis to write the fluctuations in strain and wall length as

$$\varepsilon_{i,j} = \frac{l_{i,j} - l_0}{l_0} = \frac{\sqrt{3\sigma l_0}}{k(a_i) + k(a_j)},$$

$$\delta\varepsilon_{i,j} = \varepsilon^* \left(\frac{-k'(a^*)}{2k^*}\right) \left(\delta_i + \delta_j\right),$$

$$l_{i,j} = l_0 \left(1 + \varepsilon_{i,j}\right),$$

$$\delta l_{i,j} = l_0 \delta\varepsilon_{i,j}.$$

Plugging these equations back in the PIN1 concentration, we obtain:

$$\delta p_{i,j} = p^* \frac{H_f \delta \varepsilon_{i,j}}{\varepsilon^*} - (p^*)^2 \frac{H_f \sum_{\langle k \rangle_i} \delta \varepsilon_{ik}}{N \varepsilon^*} - (p^*)^2 \frac{\sum_{\langle k \rangle_i} \delta l_{ik}}{N l^*} + (p^*)^2 \frac{\sum_{\langle k \rangle_i} \delta l_{i,k}}{N l^*} + (1 - \alpha) p^* \frac{H_f \delta k_{i,j}}{k^*} - (1 - \alpha) p^{*2} \frac{H_f \sum_{\langle k \rangle_i} \delta k_{ik}}{N k^*},$$

from which we get

$$\delta p_{i,j} = p^* H_f\left(\frac{-k'(a^*)}{2k^*}\right) (\delta_i + \delta_j) - (p^*)^2 H_f\left(\frac{-k'(a^*)}{2k^*}\right) \frac{1}{N} \sum_{\langle k \rangle_i} (\delta_i + \delta_k) \\ - (1-\alpha) p^* H_f\left(\frac{-k'(a^*)}{k^*}\right) \delta_i + (1-\alpha) p^{*2} H_f\left(\frac{-k'(a^*)}{k^*}\right) \delta_i.$$

Summing over the neighbours gives the change in fraction $\delta \mathcal{F}_i$:

$$\delta \mathcal{F}_{i} = \frac{1}{N} p^{*} H_{f} \left(\frac{-k' \left(a^{*}\right)}{2k^{*}} \right) \left(\left(2\alpha - 1\right) N \delta_{i} + \sum_{\langle j \rangle_{i}} \delta_{j} - p^{*} \sum_{\langle k \rangle_{i}} \left(\left(2\alpha - 1\right) \delta_{i} + \delta_{k}\right) \right)$$

We define the Fourier transform of $\delta \mathcal{F}$, $\Delta_{\overrightarrow{k}} = \frac{1}{2\pi} \sum_{i} \delta \mathcal{F}_{i} e^{-i \overrightarrow{k} \cdot \overrightarrow{x_{i}}}$, with the Fourier transform of the auxin fluctuations denoted as $\delta_{\overrightarrow{k}}$, and the form factor of the tissue $S_{\overrightarrow{k}} = \frac{1}{N} \sum_{p} e^{i \overrightarrow{k} \cdot \overrightarrow{e_{p}}}$.

$$\Delta_{\overrightarrow{k}} = \frac{1}{N} p^* H_f \left(\frac{-k'(a^*)}{2k^*} \right) \left[(2\alpha - 1) N \delta_{\overrightarrow{k}} + NS_{\overrightarrow{k}} \delta_{\overrightarrow{k}} - p^* \left((2\alpha - 1) N \delta_{\overrightarrow{k}} + NS_{\overrightarrow{k}} \delta_{\overrightarrow{k}} \right) \right]$$
$$\Delta_{\overrightarrow{k}} = p^* H_f \left(\frac{-k'(a^*)}{2k^*} \right) \left((2\alpha - 1) + S_{\overrightarrow{k}} \right) (1 - p^*) \delta_{\overrightarrow{k}}$$

We thus obtain the ratio between transporters and auxin fluctuations, for each feedback mechanism ($\alpha = 0$ corresponds to stress and $\alpha = 1$ to strain):

$$\begin{pmatrix} \Delta_{\overrightarrow{k}} \\ \overline{\delta_{\overrightarrow{k}}} \end{pmatrix}_{stress} = p^* H_f \left(\frac{-k'(a^*)}{2k^*} \right) \left(S_{\overrightarrow{k}} - 1 \right) (1 - p^*) < 0$$
$$\begin{pmatrix} \Delta_{\overrightarrow{k}} \\ \overline{\delta_{\overrightarrow{k}}} \end{pmatrix}_{strain} = p^* H_f \left(\frac{-k'(a^*)}{2k^*} \right) \left(S_{\overrightarrow{k}} + 1 \right) (1 - p^*) > 0$$

As a conclusion, fluctuations in auxin and PIN1 concentrations have the same sign for the strain feedback, and opposite signs for the stress feedback (we remind that k' < 0). This result supports a feedback from mechanical strain, since observations show that both auxin and PIN1 accumulate in incipient primordia. Simulations are in agreement with this result, see Figure 5.