



INSTITUT DE FRANCE
Académie des sciences

Comptes Rendus

Mécanique

Jan Traas and Olivier Hamant

Yves Couder: Putting mechanics back into the shoot apical meristem

Volume 348, issue 6-7 (2020), p. 679-684.

<https://doi.org/10.5802/crmeca.19>

Part of the Thematic Issue: Tribute to an exemplary man: Yves Couder

Guest editors: Martine Ben Amar (Paris Sciences & Lettres, LPENS, Paris, France),


Laurent Limat (Paris-Diderot University, CNRS, MSC, Paris, France),

Olivier Pouliquen (Aix-Marseille Université, CNRS, IUSTI, Marseille, France)

and Emmanuel Villermaux (Aix-Marseille Université, CNRS, Centrale Marseille, IRPHE, Marseille, France)

© Académie des sciences, Paris and the authors, 2020.

Some rights reserved.

 This article is licensed under the
CREATIVE COMMONS ATTRIBUTION 4.0 INTERNATIONAL LICENSE.
<http://creativecommons.org/licenses/by/4.0/>



*Les Comptes Rendus. Mécanique sont membres du
Centre Mersenne pour l'édition scientifique ouverte*
www.centre-mersenne.org



Tribute to an exemplary man: Yves Couder

Biology and Mechanics

Yves Couder: Putting mechanics back into the shoot apical meristem

Jan Traas*,^a and Olivier Hamant*,^a

^a Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRAE, F-69342, Lyon, France

E-mails: jan.traas@ens-lyon.fr (J. Traas), olivier.hamant@ens-lyon.fr (O. Hamant)

Abstract. In 2008, we published an article proposing that the microtubular cytoskeleton in plants use maximal tensile stress directions to guide organ growth [1]. Yves Couder was instrumental in that project. Here are some memories and prospects from this collaborative and interdisciplinary endeavor.

Keywords. Morphogenesis, Mechanical stress, Microtubules, Interdisciplinary research, Plant development.

A revisited question

Yves Couder played a central role in a collaborative project between 2005 and 2008, with colleagues in the US (Elliot Meyerowitz, Marcus Heisler), Sweden (Henrik Jönsson, Pawel Krupinski) and France (Arezki Boudaoud). Jan Traas had met Yves a number of years earlier, when he visited the lab at INRA in Versailles (France) to talk about phyllotaxis. Yves had, together with a PhD student, Stéphane Douady, developed a very original experimental system to study different phyllotactic patterns, based on magnetic drops floating in a bath with an electrical field [2]. Jan then already was very impressed by the way he was looking at biological problems based on holistic, much more conceptual approaches, combining theory with rigorous and highly creative experiments. It therefore seemed logical to invite him and his team to participate a couple of years later in a Human Frontier Science Program (HFSP) project aimed at understanding the role of mechanics in meristem function. At that time, the role of biophysics was largely ignored by mainstream plant developmental biologists (with some exception, e.g. [3, 4]). The few who had tried before, testing hypotheses on the role of physical forces in growing plant structures had been disappointed by technical difficulties and limitations. Overall the idea was that development was dictated by genes and that the physical components of cells and tissues were passively obeying orders issued by a rigid (“tightly controlled”) molecular program. Terms like “complex systems” or “emergent properties” were not really part of our vocabulary, developmental biology was largely a qualitative science and we only just had started to use mathematical, computational mecha-

* Corresponding authors.

nistic models with the group of Christophe Godin as a way to examine and formulate complex hypotheses [5] (for a discussion on engineering vs. systems view of the cell, see e.g. [6]).

From the initial visit to Yves' laboratory at the Ecole Normale Supérieure in Paris, it immediately became clear that he was the right person for this project. Indeed, his team was working on many different physical systems, with elegant and very original setups aimed at studying the dynamics diverse (macroscopical) systems including dunes, crack patterns in gels and ceramics [7], branching architectures [8] or microdroplets on vibrating oil baths [9]. Pioneering work on plants, in particular by Paul Green and colleagues, had suggested that patterns of strain and stress, generated by shape and growth, could be interpreted by the cells to control growth and cell differentiation. So far, however, it had been impossible to go much beyond the general and self-evident idea that physical forces are important in morphogenesis. We decided to re-examine this issue, exploiting new imaging technologies combined with genetics, micromechanics and modelling.

Yves was very enthusiastic and spent several hours discussing both conceptual and technical issues related to the project with Jan. Just two days later, Yves phoned with the good news that his technical staff had designed a simple device to deform plant tissues. So, they spent several days trying to deform and squeeze the tiny meristems of *Arabidopsis* under a confocal microscope and it actually worked! The preliminary results were so promising that they formed one of the cornerstones of the proposal, that was finally accepted by HFSP, incidentally allowing Olivier Hamant and Alexis Peaucelle to join the team. This collaboration, involving two teams of physicists and two teams of biologists turned out to be one of the most successful and passionate in our careers. Also one of the most difficult ones, as we were coming from different disciplines, with different cultures. Physicists were from the start much more quantitative and precise in their approaches, the biologists much more qualitative and pragmatic when it came to dealing with the limitations of their experimental approaches. From the biologist's perspective, this angle was nevertheless particularly refreshing. In particular, addressing the physical aspects of morphogenesis seemed to bring us closer to causality, while opening many new research avenues. When we discussed the results we had obtained, looking at the effects of mechanical perturbations on the structural elements of the cells (the cytoskeleton), Yves suggested that they could be explained by a negative feedback loop, where cells would mechanically resist the main forces that acted on them (Figure 1A). Although the idea was attractive, some biologists in the collaborative network were somewhat skeptical. Invisible forces acting on cells? Why not simply propose that the cells were reacting to deformation? A passionate discussion followed by mail which lasted for several weeks. At a certain moment, Yves wrote a long message explaining why we definitely should not rule out physical forces (in the end arguing that planets do not turn around the sun because they always turn left!):

"In very general terms physics has built, over the centuries, abstract tools that have proven useful for the analysis of a large variety of natural phenomena. There was a time where biology and physics were both part of "natural philosophy". That the physics approach is insufficient to investigate the complexity of life is evident. To analyse it, biology has built new and powerful tools. However, living systems are also part of the physical world so that physics applies to them. The problem is rather on whether physics can help [to] solve biological riddles. This is what our project was about. In this regard some of the basic concepts of physics have to be accepted, otherwise the dialog becomes impossible. I agree that no one can see a stress in a living tissue. But no one has ever seen a stress in a piece of metal either. Yet the computation of stress fields turns out useful in designing e.g. aircrafts. Stresses are more abstract but not more complex than strains. Forces are more abstract than displacements, yet a simpler interpretation of the planetary motions is obtained in terms of forces than in terms of trajectories."

His input and the computational models subsequently made by our colleagues in Sweden further convinced us that the mechanical feedback Yves proposed, where cells would treat

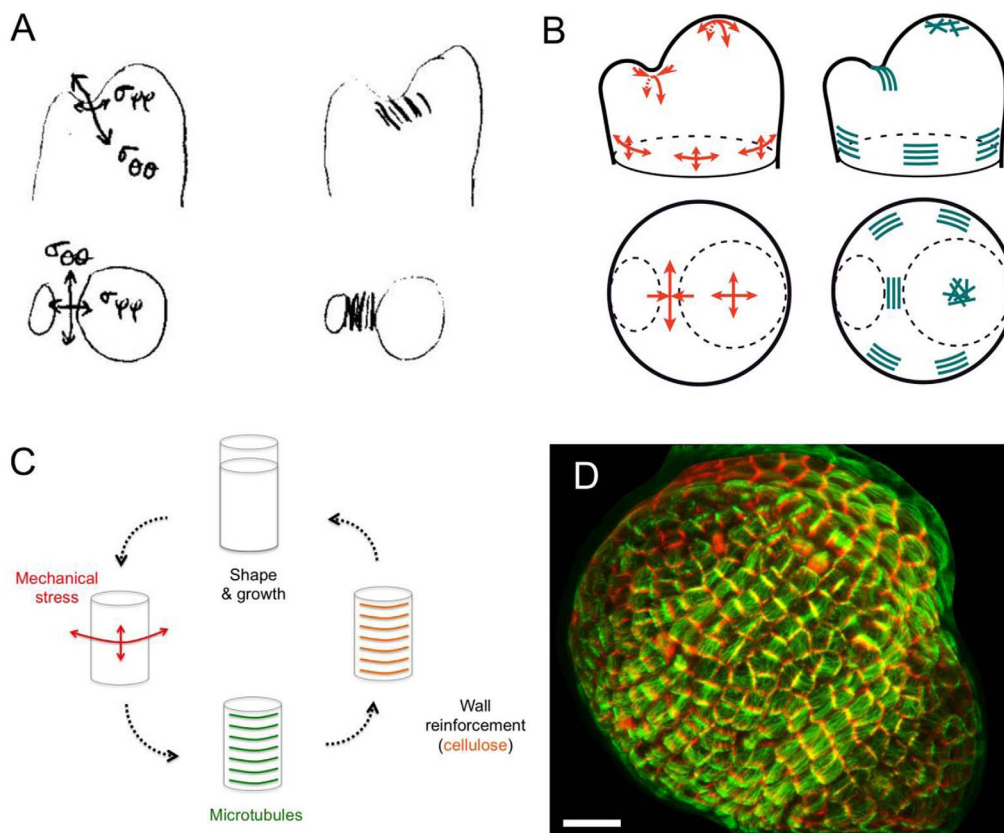


Figure 1. Matching stress and CMT pattern at the shoot apical meristem. A. Back of the envelope notes from Yves Couder. B. Original figure, reproduced with authorization from [1]. Stress pattern (left) and CMT orientations (right). C. Diagram showing the mechanical feedback on cortical microtubules for a (pressurized) cylindrical tissue. D. Cell contours (red) and CMT (green) orientations in the Arabidopsis shoot apical meristem. Scale bar: 10 μm . Panels B and D adapted with permission from [1].

forces as signals, could actually explain major shape changes in plants (Figure 1B–D, [1]). Yves even went on suggesting further work to explore the resemblance between meristematic cells treated with cytoskeleton destabilizing drugs and soap bubbles. We indeed found ourselves doing experiments which seemed completely trivial to us, but not at all to physicists. We thus finally showed that foams and plant cells, two apparently very different systems with very different dynamics shared a number of basic physical properties [10].

A result with many implications

As usual in science, our study on the cortical microtubule response to tensile stress required further confirmation to be fully established. We thus developed new mechanical perturbations, notably in the form of cell wall weakening with the cellulose synthase inhibitor isoxaben. In such conditions, we observed an hyper-alignment of CMTs in the shoot apical meristem, again matching the predicted stress pattern [11]. Over the years, we extended our study to other types of tissues, namely epidermal cells from cotyledons [12], sepals [13], hypocotyls and stems [14].

In parallel, other teams further confirmed this finding in these tissues but with different micromechanical tools, such as hypocotyl stretcher [15], and in other tissues, such as leaves [16] or immature seeds [17]. Note that earlier studies were performed in other species than *Arabidopsis thaliana*, like sunflower [18] or *Nitella* [19], with consistent predictions. We also used cell-cell adhesion mutants to reveal the tensile stress pattern: the orientation of the cracks in tissues being perpendicular to the maximal direction of the pulling force. This further confirmed a good match between tensile stress pattern and CMT orientation [14].

From this central feedback module, and because the key role of cellulose microfibrils in plant morphogenesis, we could derive important developmental implications. The examples below illustrate how this initial work with Yves turned out to be a major building block for our research. Using computational modeling and a mutant impaired in the response of CMTs to mechanical stress, we revealed that the shoot apical meristem actively maintained mechanical conflicts between cells growing at different speed, through the CMT response to mechanical stress: differential growth between adjacent cells trigger the alignment of CMTs, fueling growth anisotropy, which in turn, further amplifies differential growth. We believe that such a loop on growth heterogeneity primes organogenesis: a basal level of differential growth is always present at the shoot apical meristem, which can then be mobilized to trigger local outgrowth, e.g. upon local increase in the hormone auxin concentration [20]. In the sepal, such differential growth triggers a different response: cells around rapidly growing trichomes are resisting the induced tensile stress pattern by aligning their CMTs, and this mechanically isolates the fast-growing cell from the rest of the tissue, in the form of a predicted stiff ring around trichomes. We called this mechanism “mechanical shielding”. From our morphometric analysis, it seems that such a mechanism contributes to sepal shape reproducibility, because it would prevent fast growing cells from distorting the tissue [21].

Needless to say, this work also echoes a number of studies in the animal kingdom. In particular, the CMT response to tensile stress appears quite homologous to that of actin cables to tensile stress. In particular, upon single stretching, actin cables form and align with the maximal direction of tensile stress [22]. Therefore, not only the initial work with Yves allowed to revisit a number of developmental questions in plants, it also generated new bridges with the development community working on animal systems.

A project opening new prospects

Yet, one essential element is still missing today. How could cells perceive the direction of mechanical stress? This is in fact a question that goes beyond plant biology, as most mechanoperception mechanisms actually relate to the perception of stress magnitude, not stress direction. Reports on animal systems suggest that actin could be a mechanosensor on its own. In particular, upon bending, actin becomes more branched and this affects the overall cortical network [23]. In a different mechanism, actin severing by cofilin depends on tension in the actin filament [24]. Because the actin filaments are extended structures, they convey and are sensitive to directional information. In that sense, they can behave as sensors of stress direction. Could CMTs share a similar function?

In vitro assays, using optical traps, demonstrate that microtubule polymerization is stimulated when they are pulled, whereas depolymerization occurs upon compression [25, 26]. Assuming that CMTs are indirectly connected to the cell wall, then tension in the wall may be transmitted to the microtubules, and polymerization of tensed microtubules would be favored. Such a bias would be sufficient to orient the network of CMTs in the cell, at least based on *in silico* studies [27]. In another scenario, rather comparable to the cofilin- and tension-dependent actin behavior, the microtubule severing katanin may be involved in the ability of CMTs to align with tensile stress.

The katanin mutant exhibits a slower CMT response to stress [20]. Furthermore, there is evidence that katanin preferentially targets defects in the microtubule lattice in *Drosophila* [28]. Therefore, one could speculate that tensed microtubules also exhibit different defect patterns than non-tensed ones, leading to their preferential alignment with maximal tensile stress direction. In addition to these speculations, two major black boxes in this framework remain.

First, the molecular factors of the CMT-cell wall continuum are still ill-described, and thus it is unclear how a cue from the cell wall may affect CMTs. There is evidence that CMTs are physically anchored to the plasma membrane (e.g. [29]). CMTs may be recruited to the plasma membrane through phospholipids, e.g. thanks to the interaction between phosphatidic acid and the microtubule bundling protein MAP65 [30]. Last, the CMT-cellulose machinery may contribute to the propagation of stress from the cell wall to the CMTs [31].

Second, even if cell wall cues could be transmitted to CMTs, these would rather be strains than stresses. How can cells sense stress direction through local strains? The idea that stress may induce damages to the cell wall and/or to the CMTs may provide a way to translate stress in some kind of code, in the CMT lattice. How this could work is still an open question. Alternatively, sensing stress may rather involve curvature than strain only. The example of actin bending illustrates how such local curvature can serve as a cue for cytoskeleton reorganization [23]. Alternatively, key cues may come for local curvature in the cell, and notably cell edges. There is evidence that such domains play important role in CMT organization [32, 33]. It remains to understand how the cell could sense such geometries, and translate them into cues for cytoskeleton organization.

Acknowledgements

We are grateful to Yves and our collaborators for many years of great scientific and personal interactions. The initial work was funded by the International Human Frontier Science Program Organization.

References

- [1] O. Hamant, M. G. Heisler, H. Jonsson, P. Krupinski, M. Uyttewaal, P. Bokov, F. Corson, P. Sahlin, A. Boudaoud, E. M. Meyerowitz *et al.*, “Developmental patterning by mechanical signals in *Arabidopsis*”, *Science* **322** (2008), p. 1650-1655.
- [2] S. Douady, Y. Couder, “Phyllotaxis as a physical self-organized growth process”, *Phys. Rev. Lett.* **68** (1992), p. 2098-2101.
- [3] L. F. Hernandez, P. B. Green, “Transductions for the expression of structural pattern: analysis in sunflower”, *Plant Cell* **5** (1993), p. 1725-1738.
- [4] A. J. Fleming, “Induction of leaf primordia by the cell wall protein expansin”, *Science* **276** (1997), p. 1415-1418.
- [5] P. B. de Reuille, I. Bohn-Courseau, C. Godin, J. Traas, “A protocol to analyse cellular dynamics during plant development”, *Plant J. Cell Mol. Biol.* **44** (2005), p. 1045-1053.
- [6] D. J. Nicholson, “Is the cell really a machine?”, *J. Theor. Biol.* **477** (2019), p. 108-126.
- [7] Y. Couder, L. Pauchard, C. Allain, M. Adda-Bedia, S. Douady, “The leaf venation as formed in a tensorial field”, *Eur. Phys. J. B* **28** (2002), p. 135-138.
- [8] in *Branching in Nature: Dynamics and Morphogenesis of Branching Structures, from Cell to River Networks: Les Houches School, October 11–15, 1999* (V. Fleury, J.-F. Gouyet, M. Léonetti, eds.), Springer, EDP Sciences, Berlin, New York, Les Ulis, 2001.
- [9] S. Perrard, M. Labousse, M. Miskin, E. Fort, Y. Couder, “Self-organization into quantized eigenstates of a classical wave-driven particle”, *Nat. Commun.* **5** (2014), p. 3219.
- [10] F. Corson, O. Hamant, S. Bohn, J. Traas, A. Boudaoud, Y. Couder, “Turning a plant tissue into a living cell froth through isotropic growth”, *Proc. Natl Acad. Sci. USA* **106** (2009), p. 8453-8458.
- [11] M. G. Heisler, O. Hamant, P. Krupinski, M. Uyttewaal, C. Ohno, H. Jonsson, J. Traas, E. M. Meyerowitz, “Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport”, *PLoS Biol.* **8** (2010), article no. e1000516.

- [12] A. Sampathkumar, P. Krupinski, R. Wightman, P. Milani, A. Berquand, A. Boudaoud, O. Hamant, H. Jonsson, E. M. Meyerowitz, "Subcellular and supracellular mechanical stress prescribes cytoskeleton behavior in Arabidopsis cotyledon pavement cells", *eLife* **3** (2014), article no. e01967.
- [13] N. Hervieux, M. Dumond, A. Sapala, A.-L. Routier-Kierzkowska, D. Kierzkowski, A. H. K. Roeder, R. S. Smith, A. Boudaoud, O. Hamant, "A mechanical feedback restricts sepal growth and shape in Arabidopsis", *Curr. Biol.* **26** (2016), p. 1019-1028.
- [14] S. Verger, Y. Long, A. Boudaoud, O. Hamant, "A tension-adhesion feedback loop in plant epidermis", *eLife* **7** (2018), article no. e34460.
- [15] S. Robinson, C. Kuhlemeier, "Global compression reorients cortical microtubules in Arabidopsis hypocotyl epidermis and promotes growth", *Curr. Biol.* **28** (2018), no. 11, p. 1794-1802.e2.
- [16] E. Jacques, J.-P. Verbelen, K. Vissenberg, "Mechanical stress in Arabidopsis leaves orients microtubules in a "continuous" supracellular pattern", *BMC Plant Biol.* **13** (2013), p. 163.
- [17] A. Creff, L. Brocard, G. Ingram, "A mechanically sensitive cell layer regulates the physical properties of the Arabidopsis seed coat", *Nat. Commun.* **6** (2015), p. 6382.
- [18] Z. Hejnowicz, A. Rusin, T. Rusin, "Tensile tissue stress affects the orientation of cortical microtubules in the epidermis of sunflower hypocotyl", *J. Plant Growth Regul.* **19** (2000), p. 31-44.
- [19] P. Green, A. King, "A mechanism for the origin of specifically oriented textures in development with special reference to Nitella wall texture", *Aust. J. Biol. Sci.* **19** (1966), p. 421-437.
- [20] M. Uyttewaal, A. Burian, K. Alim, B. Landrein, D. Borowska-Wykret, A. Dedieu, A. Peaucelle, M. Ludynia, J. Traas, A. Boudaoud *et al.*, "Mechanical stress acts via katanin to amplify differences in growth rate between adjacent cells in Arabidopsis", *Cell* **149** (2012), p. 439-451.
- [21] N. Hervieux, S. Tsugawa, A. Fruleux, M. Dumond, A.-L. Routier-Kierzkowska, T. Komatsuzaki, A. Boudaoud, J. C. Larkin, R. S. Smith, C.-B. Li *et al.*, "Mechanical shielding of rapidly growing cells buffers growth heterogeneity and contributes to organ shape reproducibility", *Curr. Biol.* **27** (2017), p. 3468-3479.e4.
- [22] D. Riveline, E. Zamir, N. Q. Balaban, U. S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, A. D. Bershadsky, "Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism", *J. Cell Biol.* **153** (2001), p. 1175-1186.
- [23] V. I. Risca, E. B. Wang, O. Chaudhuri, J. J. Chia, P. L. Geissler, D. A. Fletcher, "Actin filament curvature biases branching direction", *Proc. Natl Acad. Sci. USA* **109** (2012), p. 2913-2918.
- [24] K. Hayakawa, H. Tatsumi, M. Sokabe, "Actin filaments function as a tension sensor by tension-dependent binding of cofilin to the filament", *J. Cell Biol.* **195** (2011), p. 721-727.
- [25] A. D. Franck, A. F. Powers, D. R. Gestaut, T. Gonen, T. N. Davis, C. L. Asbury, "Tension applied through the Dam1 complex promotes microtubule elongation providing a direct mechanism for length control in mitosis", *Nat. Cell Biol.* **9** (2007), p. 832-837.
- [26] A. Trushko, E. Schäffer, J. Howard, "The growth speed of microtubules with XMAP215-coated beads coupled to their ends is increased by tensile force", *Proc. Natl Acad. Sci. USA* **110** (2013), p. 14670-14675.
- [27] V. Mirabet, P. Krupinski, O. Hamant, E. M. Meyerowitz, H. Jönsson, A. Boudaoud, "The self-organization of plant microtubules inside the cell volume yields their cortical localization, stable alignment, and sensitivity to external cues", *PLoS Comput. Biol.* **14** (2018), article no. e1006011.
- [28] J. D. Díaz-Valencia, M. M. Morelli, M. Bailey, D. Zhang, D. J. Sharp, J. L. Ross, "Drosophila Katanin-60 Depolymerizes and Severs at Microtubule Defects", *Biophys. J.* **100** (2011), p. 2440-2449.
- [29] J. C. Ambrose, G. O. Wasteneys, "CLASP modulates microtubule-cortex interaction during self-organization of acentrosomal microtubules", *Mol. Biol. Cell* **19** (2008), p. 4730-4737.
- [30] Q. Zhang, W. Zhang, "Regulation of developmental and environmental signaling by interaction between microtubules and membranes in plant cells", *Protein Cell* **7** (2016), p. 81-88.
- [31] M. Bringmann, B. Landrein, C. Schudoma, O. Hamant, M.-T. Hauser, S. Persson, "Cracking the elusive alignment hypothesis: the microtubule-cellulose synthase nexus unraveled", *Trends Plant Sci.* **17** (2012), p. 666-674.
- [32] C. Ambrose, J. F. Allard, E. N. Cytrynbaum, G. O. Wasteneys, "A CLASP-modulated cell edge barrier mechanism drives cell-wide cortical microtubule organization in Arabidopsis", *Nat. Commun.* **2** (2011), p. 430.
- [33] C. Kirchhelle, D. Garcia-Gonzalez, N. G. Irani, A. Jérusalem, I. Moore, "Two mechanisms regulate directional cell growth in Arabidopsis lateral roots", *eLife* **8** (2019), article no. e47988.