Shaping the meristem by mechanical forces

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Abstract

A recent report shows that cells in the *Arabidopsis* apical meristem orientate their cortical microtubules along mechanical stress patterns generated during tissue morphogenesis. This in turn is expected to influence the mechanical properties of the cell via the modification of the cortical microtubule network and the cell wall. This feedback loop controlling the shape of the meristem may act in parallel with auxin signalling, which determines the site of organ primordium formation.

Introduction and context

The diversity of sizes and shapes exhibited by living organisms is an unlimited source of astonishment for the naturalist, and the origin of these shapes and sizes is a central question in developmental biology. Morphogenesis is studied from two different standpoints: the first considers the central role of genes and mobile signalling molecules (morphogens) that act on individual cells to modify basic features such as identities and growth characteristics [1]; the second, in an alternative physical perspective, considers how cells and tissues form structures with their own mechanical characteristics that are central for pattern formation [2,3].

These two views have been used to explain the patterning of new organs in the apex of plants (Figure 1) [4-6]. In plants, patterning and organogenesis are not restricted to embryogenesis, as is mostly the case for animals, they are ongoing processes throughout the life of the plant that take place within groups of undifferentiated and proliferating cells called meristems. The shoot apical meristem, located at the growing tips of the shoots, maintains at its centre a pool of stem cells, while at its flanks it initiates primordia of new lateral organs such as leaves or flowers in a predictable pattern [7]. This regular pattern, which is also recognizable along the shoot and referred to as phyllotaxis, has puzzled observers as far back as Pliny the Elder in ancient Roman times (reviewed in [5]). In the second half of the 19th century, Hofmeister suggested that new primordia arise regularly at the place of maximum space not occupied by the pre-existing primordia [5]. Ten years later, in 1878, Schwendener underlined the importance of contact pressure between neighbouring primordia for the formation of the phyllotaxis [5]. An alternative view was proposed by Schoute, who suggested that the positioning of new primordia was determined by the action of an inhibitor produced by older primordia, which locally prevents the emergence of a new primordium [5].

The importance of chemical signalling was recognized in recent years with the identification of the role of auxin gradients. Auxin is a small molecule that can both diffuse and be actively transported from cell to cell by a complex set of influx and efflux transporters [8,9]. Inactivation of one of the auxin efflux transporters, *PIN-FORMED1* (*PIN1*), leads to the absence of primordia initiation that can, however, be restored by local application of auxin [10-12]. These observations form the basis of a current model of phyllotaxis in which auxin is transported by the epidermis into the meristem, depleted from the vicinity of existing primordia, which act as sinks, and accumulated locally to induce the formation of new primordia [13]. This intuitive model was supported by computer modelling, which allowed several hypotheses to be

Figure 1. Spiral phyllotaxis in a daisy flower
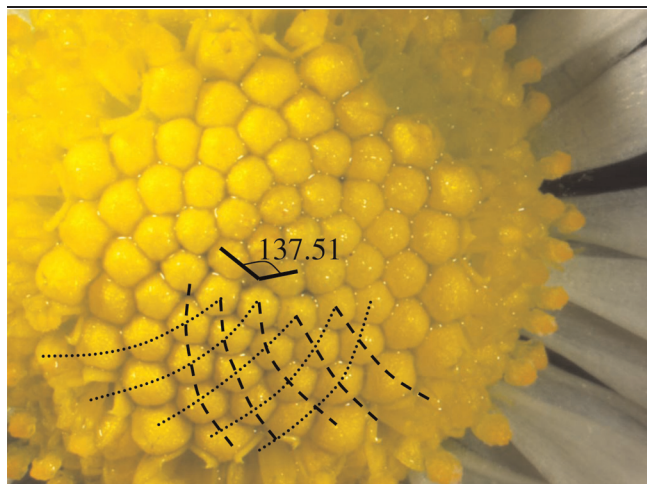
Spirals going in two directions are highlighted with dotted and dashed lines and the golden angle (137.51°) between successive primordia is shown.

tested, in particular, one proposing that the auxin distribution controls the polarity of PIN1 protein within the cell [14-16].

Now, in a recent paper, Hamant *et al.* [17] rehabilitate the role of mechanical forces in morphogenesis at the shoot apex by showing that tissue mechanics along with auxin signalling play a crucial role in the shaping of the apex.

Major recent advances

To analyse the role of mechanical forces, Hamant *et al.* [17] concentrated on the cortical microtubule network. This network controls the deposition of cellulose microfibrils, the main structural components of the cell wall, by guiding the movement of the cellulose synthase complexes within the plasma membrane [18]. The orientation of the stiff microfibrils is expected to impose an anisotropy on the mechanical properties of the cell wall, which underlies anisotropic growth [19]. Starting in the early 1980s, Paul Green underlined the importance of physical forces during morphogenesis in a series of pioneering articles [2,20-22]. In particular, he showed that the cortical microtubules and the newly deposited cellulose microfibrils are essentially aligned and undergo a massive reorientation into a circumferential arrangement around developing leaves [23]. Hamant *et al.* [17] revisited this topic using modern cell biology tools and integrated multidisciplinary approaches. The authors visualized the orientation of the cortical microtubule network using microtubule-targeted green fluorescent protein combined with a powerful approach to image living meristems [24-27]. They revealed different

microtubule orientations depending on the meristem zone and a massive reorientation of the microtubules to align them along the boundary between the meristem and the emerging primordium (Figure 2). This orientation parallels that of the mechanical stress patterns as predicted by several models (at the tissue or cell level, including growth or not), suggesting that cortical microtubule arrays actively align along stress patterns. To confirm this, they ablated one or several cells or compressed growing meristems and observed a reorientation of the microtubules, in agreement with the new predicted stress pattern. Interestingly, the response to these treatments could vary within neighbouring cells, suggesting that each cell could perceive and react to the mechanical signal as shown before for isolated plant cells [28]. Hamant *et al.* [17] propose a new regulatory network by which cells sense and respond to mechanical signals by reorientating their microtubules and, presumably, the newly deposited cellulose microfibrils. This would be important for some aspects of morphogenesis at the shoot apex, such as the formation of the groove between the meristem and the primordium, but would have a limited effect on the growth and differentiation patterns. The latter aspect is supported by the observation that differential growth and cell differentiation were still observed when the microtubular network was disrupted by a drug [17,26]. Therefore, the mechanical feedback loop revealed here is likely to act in parallel

Figure 2. Stress patterns and cortical microtubule orientations in the apex of *Arabidopsis thaliana*
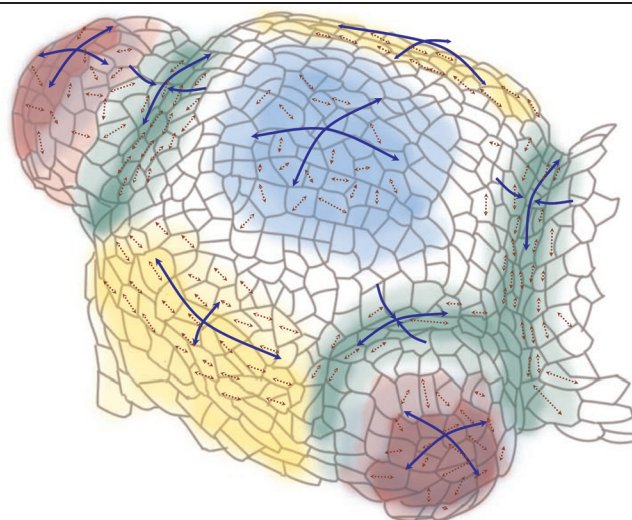
Thick, blue arrows indicate the modelled stress patterns whereas dark red arrows with dotted lines represent the orientations of the cortical microtubular arrays in different regions of the apex (light blue, centre of the meristem; purple, summit of a primordium; green, boundary domain between meristem and primordium; yellow, base of the meristem).

with the previously described auxin-mediated patterning mechanism.

Future directions

These observations raise a number of questions. Firstly, it still remains to be demonstrated whether, in the context of the meristem, the microfibrils are indeed deposited along the microtubules and, if so, whether a change in the deposition of the innermost cell wall layer is sufficient to explain the change in the growth direction of the cell. Secondly, what is the mechanism linking the mechanical stress patterns to the orientation of micro-tubules? Mechanoperception is an important emerging field at the interface between physics and biology, and innovative genetic screens are needed to dissect the signalling processes involved. Thirdly, to what extent do cell wall properties contribute to meristem function? Is the cell wall only a girdle limiting cell growth and whose anisotropy is mostly determined by the underlying microtubular network, or is there more to find out? Not surprisingly, the formation of a primordium, which involves local changes in growth rate and orientation, is associated with local modifications of cell wall proper-ties and the expression of cell wall-modifying agents [29,30]. For instance, local application of the cell wall-loosening proteins, expansins, is sufficient for precocious primordium formation at the expected position [31]. Primordia also can be formed, even at completely ectopic positions, when the methylesterification status of another cell wall component, pectin, is changed [29]. How such components integrate with the mechanical and auxin-dependant network awaits further multi-disciplinary research.

Abbreviations

PIN1, PIN-FORMED1.

Competing interests

The authors declare that they have no competing interests.

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