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Auxin inhibits expansion rate independently of cortical microtubules

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A recent publication announces that auin inhibits expansion by a mechanism based on the orientation of cortical microtubules. This is a textbook-revising claim, but as I argue here, a claim that is supported by neither the authors' data nor previous research, and is contradicted by a simple experiment.

Ironically, we do not know how auxin, the growth hormone, controls growth rate. The rate of expansion depends on the rates of two linked processes: water uptake into the symplast and the deformation of the cell wall. Even though, in principle, either could be limiting, the cell wall has been the focus of attention. The rate of cell wall deformation is usually alleged to be set by the rate of one of the following processes: proton efflux, formation in the cell wall of hydroxyl radicals, or secretion of cell wall polysaccharides. The favored process would then be adjusted by auxin. Which process does auxin in fact adjust? Strikingly we have no consensus for the answer.

Proton pumps, oxido-reductases, secretory machinery, and for that matter aquaporins, all are located in the cell's cortex, spotlighting this region as a key locus for growth rate control (Figure 1). Also present there are the cortical microtubules. This array, abutting the plasma membrane, is dense (many microtubules per micron) and dynamic (average half-life on the order of a minute). The array confounds cell biologists with its 'Look ma: no hands!' trick of organizing without an organizing center. Cortical microtubules have one agreed-on function: namely, to influence the direction in which cellulose microfibrils are deposited, an alignment that dictates the direction, but not the rate, of expansion. How these microtubules guide cellulose deposition is not clear. Nor is it clear whether the array has other functions, including ones that alter growth rate. Given that cortical microtubules pervade the cortex and bind hundreds of different proteins, multi-tasking is a reasonable, though as yet unproven, expectation.

With that background, readers can surmise that *Nature* editors heard the crack of a paradigm shattering in a recent paper by Chen *et al*. titled: 'Inhibition of cell expansion by rapid ABP1-mediated auxin effect on microtubules' [1].

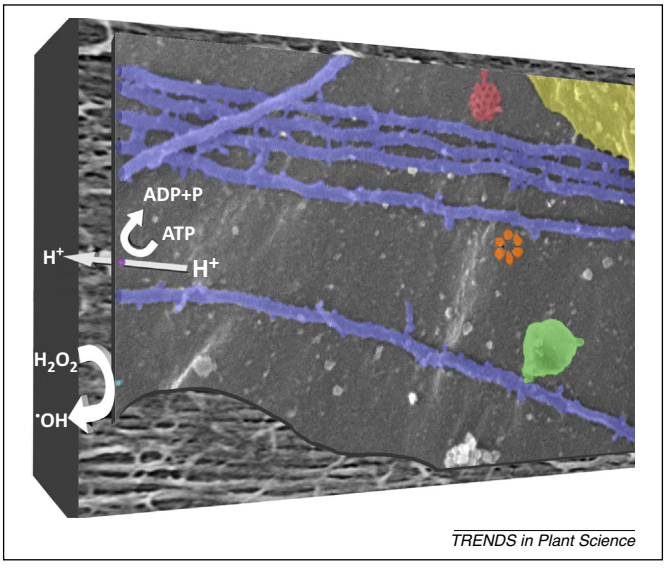

Figure 1. Spotlight on the cell cortex. Juxtaposed to the cell wall, the cortex, a region that includes the plasma membrane and ER (yellow), is a pivotal locus for governing production and behavior of the apoplast. The figure shows roughly a 1 μm x 1 μm patch, illustrating selected components and activities. The rate of expansion could be influenced by the rate of proton efflux mediated by the proton-ATPase (purple), of hydroxyl radical production mediated by a flow of electrons probably from an NADH oxidase (turquoise), or of vesicle secretion (green) and endocytosis (red). Any or all of these activities could be modulated by auxin to cause a changed expansion rate. For rapid adjustments, the cortex might entirely contain the responsible components (i.e., independent of the nucleus). The cortical microtubules (blue) guide the direction in which the cellulose synthase (orange) moves, thereby establishing the main alignment for cellulose microfibrils and the mechanical anisotropy of the cell wall. Microtubules are abundant in the cortex and might condition neighboring processes. The cutaway view through the membrane shows the cell wall at the same magnification as cell cortex.

The paper reports that, in both roots and hypocotyls of *Arabidopsis thaliana*, auxin causes cortical microtubules to reorient from transverse to longitudinal, over the course of about an hour [1]. The reorientation requires ABP1 and certain other signaling components, and by tracking growing microtubule ends, the reorientation is shown to initiate within a minute or two of adding auxin.

As interesting as these results are concerning microtubule reorientation, they fail to substantiate the titular claim that microtubules drive growth inhibition. It could be the reverse: namely that auxin-mediated growth inhibition drives microtubules to reorient, a scenario that has experimental support [2,3]. These alternatives might have been distinguished by assaying microtubule orientation and expansion concomitantly but Chen *et al*. [1] present no expansion data whatsoever. Nevertheless, even were the kinetic relationship to be consistent with causality,

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microtubules and expansion could be no more than correlated responses to auxin and thus causally independent.

When a paradigm shifts, we are not only better able to explain something we also learn how the deposed explanation led us astray, as black-body radiation was misrepresented by assuming a continuous rather than a quantized distribution of energy. Cortical microtubules acting on growth direction rather than rate is accepted, in part, because the hypothesis that auxin changes expansion rate by changing microtubule orientation has been tested and refuted [4,5]. Reviewing dozens of papers on auxin and microtubule orientation, Shibaoka [6] stated: 'Since the short-term auxin-induced cell elongation does not involve auxin-induced cortical microtubule reorientation, it is unlikely that microtubule reorientation is involved in the tropic responses of plants.'

To be sure, many of those papers tested growth stimulation, where auxin treatment reorients microtubules from longitudinal to transverse, whereas Chen *et al*. [1] studied expansion inhibition, where microtubules go from transverse to longitudinal. But some tests used roots, where auxin inhibits expansion. For example, in root gravitropism, growth inhibition is correlated with a transverse-to-longitudinal microtubule reorientation but nevertheless the kinetics of gravitropism are scarcely affected by removing or stabilizing microtubules [7,8]. Granting microtubules a role in dictating expansion rate implies that the old paradigm was wrong. But how so? Chen *et al*. [1] cite none of this previous work and offer no explanation for their opposite conclusion.

If microtubules are to inhibit growth rate then there needs to be a mechanism. The canonical mechanism whereby microtubules influence expansion is through orienting cellulose microfibrils, but as mentioned above, this is widely understood to change the direction rather than the rate of expansion. Indeed, this classic role for microtubules was recently invoked to explain how auxin, again acting through ABP1, promotes primordium outgrowth at the shoot meristem by locally reorienting microtubules; expansion continues but in a new direction [9]. Chen *et al*. [1] propose no mechanism, an absence making their claim for a microtubule-mediated inhibition of expansion unpersuasive.

Logic and literature aside, the claim of Chen *et al*. [1] leads to a simple prediction: in the absence of microtubules, auxin should be powerless to stop growth. Curious, I checked with a quick experiment. I exposed 1-week-old *A. thaliana* seedlings to 170 nM indole acetic acid in the presence or absence of 1 μM oryzalin, a concentration that removes nearly all microtubules within ~60 min. Even in the absence of microtubules, auxin prevented the massive swelling seen over 24 hours on oryzalin, and strongly inhibited elongation rate within 15 min [10]. Contrary to the prediction, auxin is able to inhibit expansion in the virtual absence of microtubules.

That cortical microtubules preside over expansion direction rather than rate remains settled wisdom. Just as the circadian clock ramps up the photosynthetic apparatus before dawn to harvest early morning sunbeams, so too when development or physiology call for stopping growth, microtubules might reorient in anticipation to generate stable cell wall reinforcement. It is reasonable to expect growth and microtubule orientation to be linked by the tightest of correlations, and investigating how cells control microtubule orientation is valuable. But despite auxin being identified as the growth hormone for nearly a century, the mechanism whereby the hormone alters growth rate remains stubbornly elusive. Finally discovering that mechanism will require rigorously separating cause from correlation.

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