Inhibition of Cell Expansion by Rapid ABP1-Mediated Auxin Effect on Microtubules? A Critical Comment

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How do cortical microtubules shape plant cells? This has been an important question ever since the microtubular cytoskeleton was found to orientate the deposition of cellulose microfibrils in the primary cell wall and control long-term anisotropic cell expansion under isotropic turgor pressure (Green, 1962). In axial plant organs, longitudinal microtubule/microfibril arrays hamper expansion in length and favor expansion in girth, while transverse microtubule/microfibril arrays have the opposite effect (Baskin, 2001). By generating mechanical anisotropy in the cell wall, microtubule orientation controls the ratio of longitudinal versus circumferential cell expansion (the allometric ratio). A recent study by Chen et al. (2014) concludes that auxin inhibits cell growth by causing a rapid reorientation of microtubules from a transverse to a longitudinal orientation in cells of the submeristematic root zone and the elongation zone of the hypocotyl of Arabidopsis thaliana seedlings. This conclusion warrants attention because the cell expansion that drives auxin-mediated organ elongation is generally thought to be controlled by the regulated breaking of bonds between existing cell wall polymers by chemical means (wall loosening; Cosgrove, 2005).

It has been clear for more than 25 years that auxin does induce rapid changes in the orientation of microtubules in growing cells, either during straight growth (Bergfeld et al., 1988) or tropic bending (Nick et al., 1990). These studies form part of a group of investigations that show that numerous growth-affecting endogenous, environmental, and even artificial physical factors have very similar effects on microtubule orientation: during active cell expansion or related mechanical strains, microtubules are aligned against the direction of expansion, and they are aligned with it during the inhibition of expansion (Fischer and Schopfer, 1997). An important insight that emerges from this extensive evidence is that the type of reorientation elicited by a particular factor depends on its physiological context, thereby allowing auxin to induce either transverse or longitudinal microtubule orientations depending on whether elongation growth is promoted (as in shoot organs) or inhibited (as in roots).

Clearly, ordered microtubule reorientations require the input of directional information (Williamson, 1990). Auxin signaling as such cannot provide this information, but the directional growth responses produced by auxin can. This brings us to the crucial question: is the orientation of microtubules determined by the effector signal directly or by changes in growth? The answer given by Chen et al. (2014) comes as a surprise: the inhibition of cell expansion is mediated by the rapid AUXIN-BINDING PROTEIN1 (ABP1)-dependent action of auxin on microtubules. The authors imply that it is microtubular reorientation per se that is responsible for the sudden growth inhibition caused by auxin in roots and hypocotyls rather than any changes in cell wall structure. Related microtubule reorientations at the concave side of gravitropically curving roots are interpreted in a similar way.

Coming as an even bigger surprise, Chen and collaborators do not provide any evidence to support the claim made in their title, nor do they touch the obvious question of how microtubule reorientation from transverse to longitudinal might produce the growth inhibition elicited by auxin in the Arabidopsis root so quickly (Evans et al., 1994). Modification of the allometric ratio by changing the orientation of newly deposited cellulose microfibrils happens over hours and, therefore, is much too slow to account directly for the inhibition of cell expansion by auxin. Accompanying data on the growth changes produced by auxin in their experiments are not included in the report, nor are specifications of the investigated cell layers, despite the fact that the different cell layers show different responses to hormones (Ubeda-Tomás et al., 2012). Hence, critical questions regarding the quantitative relationship between microtubule reorientation and cell elongation remain unanswered. The gap between the experimental data presented and the far-reaching conclusions derived from them has been pointed out by Baskin (2015).

There is, to our knowledge, no evidence for any fast growth-controlling mechanisms involving microtubules. There is, on the other hand, ample evidence for a causal relationship between wall-relaxing processes (such as the secretion or activation of wall-loosening enzymes or the generation of reactive oxygen species) and the rapid regulation of cell growth by auxin (Cosgrove, 2005; Perrot-Rechenmann, 2010). Modification of the allometric ratio by changing the orientation
of newly deposited cellulose microfibrils appears much too slow (happening over hours) to account for the inhibition of cell expansion within minutes (Evans et al., 1994). Gravitropic bending of maize (Zea mays) roots has been shown to proceed normally even after the disassembly or immobilization of microtubules, and the inhibition of bending prohibits unilateral microtubule reorientation (Baluška et al., 1996). Chen et al. (2014) do not consider any of this evidence.

So, can the data presented by Chen and collaborators be explained without coming into conflict with previously published results? The answer to this question is straightforward and has long been accessible in the pertinent literature: the observed changes in microtubule pattern are trivial consequences of either growth inhibition or the auxin insensitivity of growth in abp1 (Tromas et al., 2009). Consider the following points.

First, mechanical forces can reorient microtubules. There is a large body of experimental evidence that indicates that microtubule reorientations in single cells or tissues can be induced by oriented mechanical forces causing oriented stresses and strains in the affected cell walls (Landrein and Hamant, 2013). For example, Fisher and Cyr (2000) subjected protoplasts, embedded in an elastic agarose matrix, to mechanically induced stretching. The originally randomly oriented microtubules responded to this treatment by aligning at right angles to the major tensile force vector. Similarly, growing coleoptile segments respond to mechanical bending by reorientating the microtubules of epidermal cells at right angles to the direction of tension (extended side) and parallel to the direction of compression (compressed side; Fischer and Schopfer, 1997).

Second, anisotropic cell growth mirrors patterns of stress and strain within the cell wall (Hamant and Traas, 2010). In biophysical terms, turgid plant cells can be regarded as pressurized vessels surrounded by an elastically stretched wall. Auxin-driven cell enlargement is brought about by changing the yielding properties of the wall and the resulting expansion in the direction of growth (Cosgrove, 2005).

Third, experiments with maize coleoptiles have shown that auxin, in addition to effecting growth-related microtubule reorientations, strongly promotes their responsivity to mechanical forces. The epidermal microtubules of auxin-deprived coleoptile segments barely respond to bending stresses but reorientate rapidly after a 1-h treatment with auxin (Fischer and Schopfer, 1997). Hence, cell wall strains generated by growth or applied stresses interact in orientating microtubules in a synergistic manner, pointing to a common signalling mechanism activated by changes in strain rate.

Summing up, there is well-founded evidence for the conclusion that the microtubule reorientations observed by Chen and collaborators occur as a result of changes in the physical strain pattern that underlies the auxin-induced changes in cell expansion. In agreement with established knowledge, the primary effect of auxin may be a rapid inhibition of cell wall loosening, mediated by the production of hydrogen peroxide (Ivanchenko et al., 2013). Based on these arguments and the weight of published evidence, we conclude that Chen and collaborators have inverted cause and effect.

Their observation that the inactivation of ABP1 (and downstream components of the ABP1 pathway) causes microtubules to become unresponsive to auxin and lose their transverse pattern in roots may be explained as trivial consequences of growth inhibition (Tromas et al., 2009). Serious questions now hang over the roles for ABP1 in auxin signaling and auxin-controlled development (Gao et al., 2015; Liu, 2015). However, this does not come as a complete surprise. Hayashi et al. (2008) previously showed that the auxin-induced growth inhibition of root and hypocotyl in Arabidopsis can be suppressed by α[2,4-dimethylphenyl-ethyl-2-oxo]-IAA (auxinole). This antagonist specifically competes with auxin at the TIR1-AUX/IAA-type receptor complexes and does not bind to ABP1, thereby suggesting that ABP1 does not act as a receptor in these pathways.

There are lessons here, not the least that literature from the pre-Arabidopsis era remains a relevant and valuable source of information.
concentration in tomato (Solanum lycopersicum) root tips while inhibiting root growth. Ann Bot (Lond) 112: 1107–1116


