

ARTICLE ADDENDUM

On growth and formins

Fatima Cvrčková, Denisa Oulehlová, and Viktor Žárský

Department of Experimental Plant Biology, Faculty of Sciences, Charles University, Prague, Czech Republic

ABSTRACT

Development of the plant aerial organs epidermis involves a complex interplay of cytoskeletal rearrangements, membrane trafficking-dependent cell surface expansion, and intra- and intercellular signaling, resulting in a pattern of perfectly interlocking pavement cells. While recent detailed *in vivo* observations convincingly identify microtubules rather than actin as key players at the early stages of development of pavement cell lobes in *Arabidopsis*, mutations affecting the actin-nucleating ARP2/3 complex are long known to reduce pavement cell lobing, suggesting a central role for actin. We have now shown that functional impairment of the *Arabidopsis* formin FH1 enhances both microtubule dynamics and pavement cell lobing. While formins are best known for their ability to nucleate actin, many members of this old gene family now emerge as direct or indirect regulators of the microtubule cytoskeleton, and our findings suggest that they might co-ordinate action of the two cytoskeletal systems during pavement cell morphogenesis.

Abbreviations: ABP1, auxin binding protein 1; ARP2/3, actin-related proteins 2 and 3; DAG, days after germination; FH1, formin homolog 1; FH2, formin homolog domain 2; FH17, formin homolog 17; ICR1, interactor of constitutively active ROPs 1; PIN, pin-formed; PTEN, phosphatase and tensin homolog; RIC, RHO-interacting CRIB; RHO, RAS homolog; ROP, RHO of plants; SMIFH2, small molecule inhibitor of FH2

ARTICLE HISTORY

Received 3 February 2016
Accepted 11 February 2016

KEYWORDS

Actin; cell growth; epidermal pavement cells; formins; FH2 proteins; microtubules

In the second edition of his influential monograph “On Growth and Form,” the great D’Arcy Thompson already included the notion of auxin-modulated local differences in plant cell wall extensibility as a pre-requisite of non-isotropic cell growth.^{1,p. 281} In the epidermis of above-ground plant organs, in particular cotyledons and leaves, cell wall expansion is precisely coordinated among neighboring cells, resulting in the development of a layer of intimately packed, interdigitating pavement cells whose shape often resembles jigsaw puzzle pieces (reviewed in refs. 2, 3). The concerted development of lobes and indentations (necks) in neighboring cells involves assembly of prominent microtubule bundles at the neck regions,⁴ and requires proper function of the actin cytoskeleton (or at least the ARP2/3 actin nucleation complex; summarized in ref. 5), myosins,⁶ the microtubule cytoskeleton including some microtubule-binding proteins,^{7,8} and the membrane trafficking apparatus.^{9,10}

Pavement cell morphogenesis is also controlled or modulated by a number of signaling pathways, involving those depending on RHO (ROP) GTPases and their effectors including RIC family and ICR1 proteins,^{11–13} and (not surprisingly) also auxin.^{14–16} However, the cytokinin response pathway, as well as some genes previously described in the context of sugar signaling (see ref. 3), brassinosteroid response,¹⁷ and plant defense¹⁸ have been also implicated. Part of the support for

auxin involvement comes, unfortunately, from the phenotype of the *Arabidopsis abp1-5* mutant which carries a point mutation in the *Auxin Binding Protein 1* gene and exhibits decreased pavement cell lobing.^{14,16} However, genuine null mutations in this gene have normal pavement cells,¹⁹ and the *abp1-5* line carries numerous additional mutations outside the *ABP1* locus, obviously contributing to its phenotype.²⁰ Nevertheless, pavement cell lobing is also altered in auxin biosynthesis mutants (reviewed in ref. 14), and the ROP-dependent pathway, mediated by ICR1 and the exocyst complex subunit SEC3, acts at least in part through modulation of membrane trafficking,¹² which important not only for cell expansion (see ref. 21) but also for the localization of PIN auxin transporters, also implicated in the regulation of pavement cell morphogenesis.^{14,15}

Relationships between these molecular mechanisms and signaling pathways are still far from clear, including the relative contribution of the two main cytoskeletal systems at various stages of pavement cell morphogenesis. While microtubule bundles are generally acknowledged as restricting growth at the pavement cell indentations,^{3,4,13} a prominent role has been proposed for a fine actin meshwork assembling at expanding lobes, presumably driving lobe outgrowth in a manner reminiscent of tip growth or of leading edge advancement in metazoan cells.^{2,11} However, detailed *in vivo* time-lapse observations of fluorescent protein-labeled microtubules and microfilaments in

CONTACT Fatima Cvrčková ✉ fatima@natur.cuni.cz

Addendum to: Rosero A, Oulehlová D, Stillerová L, Schiebervová P, Grunt M, Žárský V, Cvrčková F. *Arabidopsis* FH1 formin affects cotyledon pavement cell shape by modulating cytoskeleton dynamics. *Plant Cell Physiol.*, in press; <http://dx.doi.org/10.1093/pcp/pcv209>

Armour WJ, Barton DA, Law AML, Overall RL. Differential growth in periclinal and anticlinal walls during lobe formation in *Arabidopsis* cotyledon pavement cells. *Plant Cell* 2015, 27:2484–2500; PMID: 26296967; <http://dx.doi.org/10.1105/tpc.114.126664>

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Arabidopsis cotyledon epidermis, recently reported by Armour et al.,²² convincingly identify establishment of microtubule bands at incipient indentation sites as the first detectable event of pavement cell shaping, taking place long before actual lobe outgrowth and prior to any noticeable actin enrichment in future lobes. Although actin apparently accumulates in growing lobes at advanced stages of expansion,¹¹ a recent study did not confirm the presumed lobe-specific localization of the ARP2/3 actin nucleation complex.²³ This is not at odds with the previously reported reduced pavement cell lobing in mutants defective in the function of the ARP2/3 complex⁵ or in other microfilament-dependent processes,^{6,22} since these mutants might suffer by partial impairment of actin- and myosin-dependent intracellular transport, resulting in reduced cell expansion within an existing pattern of lobes and indentations rather than an alteration of this pattern.² Even more likely, altered actin dynamics may affect microtubule organization,⁵ a phenomenon nowadays well documented.²⁴

We recently reported that impairment of formin function in *Arabidopsis* (either by mutations in the *FH1* locus or by pharmacological intervention) leads to increased complexity of

cotyledon pavement cell shape, associated with stabilization and bundling of microfilaments, reduced density of fine cortical actin meshwork, and increased dynamics of cortical microtubules.²⁵ Thus, formins, members of an evolutionarily ancient family of eukaryotic proteins possessing the FH2 domain whose dimer can nucleate actin, may play a key part in such actin-microtubule co-ordination. Indeed, formins often participate in microtubule organization in both metazoans and plants (reviewed in refs. 26, 27). Angiosperms have numerous formin paralogs that form clades termed Class I (usually with a transmembrane domain) and Class II (often with PTEN-like membrane binding domain). In *Arabidopsis*, FH1 is the most ubiquitously expressed Class I formin during vegetative development (see refs. 25, 27). This may explain the similarity of pavement cell shape changes caused by *fh1* loss-of-function mutation and by general inhibition of formin-dependent actin polymerization by treatment with the SMIFH2 compound.^{28,29}

In mature tobacco epidermal cells, heterologous FH1 specifically decorates plasmalemma regions free of cortical microtubules³⁰ but its natural localization during early pavement cell development awaits characterization. Most other Class I

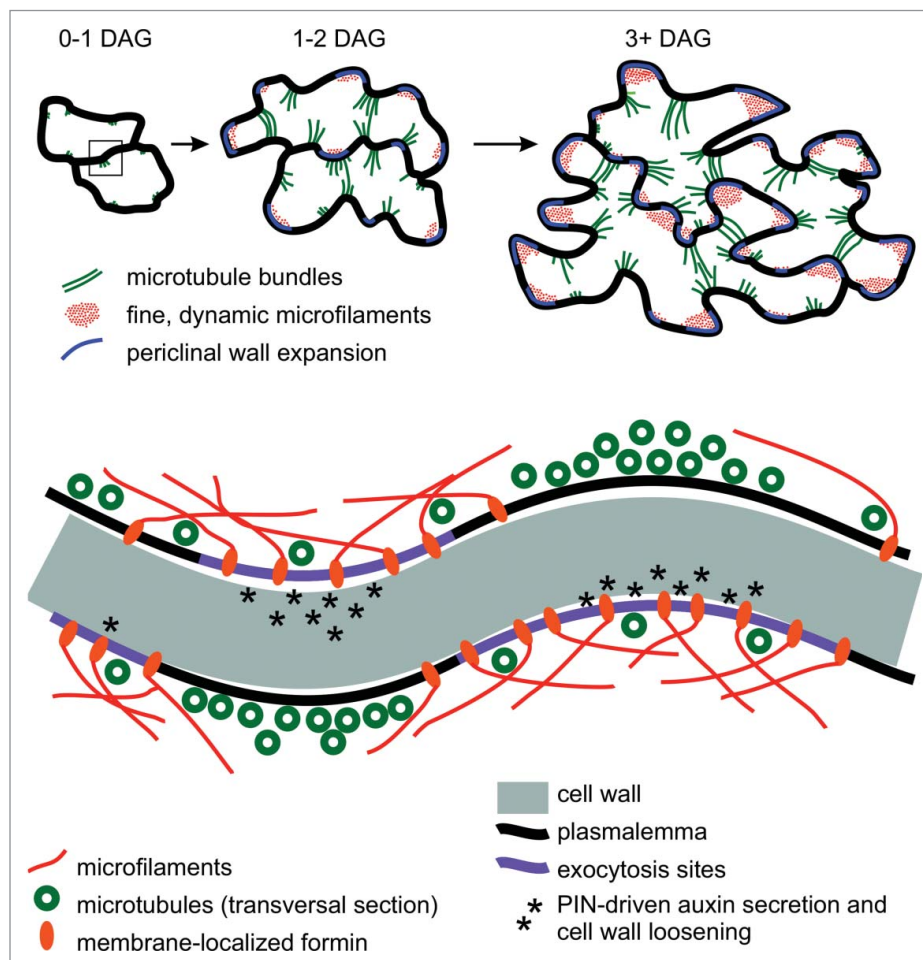


Figure 1. A model of the structural aspects of pavement cell shape specification. Top: temporal sequence of cytoskeletal rearrangements²² during early stages of pavement cell development.¹¹ Bottom: close-up of a periclinal section of the interface of two neighboring cells at an early stage of pattern establishment (box in the top part of the figure). Membrane-anchored formins may contribute to the focusing of microtubule bundles to future indentations by restricting lateral mobility of cortical microtubules through nucleation of membrane-attached microfilaments, promoting assembly of cortically anchored actin cables.²⁵ Unlike ARP2/3-nucleated actin arrays, these cables might form only a sparse meshwork, sufficient to prevent microtubule bundling but not interfering with exocytosis (compare ref. 21). At later stages, ARP2/3-mediated actin nucleation might take place on the existing formin-generated actin filaments, aiding actomyosin-driven vesicle delivery to expanding areas of the cell cortex. DAG - days after germination.

formins are also inserted into membranes; additional plant formins either possess membrane-binding domains (Class II, see above), or at least may bind other membrane-associated proteins; this might perhaps include heterodimerization with membrane-bound formins via the conserved FH2 domain. Thus, formins are emerging as plausible candidates for co-ordinating cytoskeletal and membrane dynamics at the cell cortex.²⁷ Intriguingly, the Arabidopsis *abp1-5* line carries several mutations inactivating the *FH17* gene, encoding a yet uncharacterized Class II formin.²⁰ It remains to be seen if this locus contributes to the *abp1-5* pavement cell lobing defect that was originally attributed to impairment of auxin signaling.

Regardless of the above-outlined open questions (and many others bound to emerge from future research), results of the recent studies can be summarized (Fig. 1), updating previously published models of pavement cell morphogenesis,^{3,11} and advancing thereby our understanding of what the classic has termed “the heterogenous chemistry of the cell”^{1,p. 400} - or, in today’s language, the complex interplay of structural and regulatory components resulting in precise specification and coordination of the plant cell morphogenesis required for formation of functional tissues and organs.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Amparo Rosero for the many inspiring ideas shared during her time in our laboratory, and Ministry of Education, Youth and Sports of the Czech Republic for support from the NPUI LO1417 project.

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