

Review Article

Formins: Emerging Players in the Dynamic Plant Cell Cortex

Fatima Cvrčková

Department of Experimental Plant Biology, Faculty of Science, Charles University, Viničná 5, 128 43 Prague, Czech Republic

Correspondence should be addressed to Fatima Cvrčková; fatima@natur.cuni.cz

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Formins (FH2 proteins) are an evolutionarily conserved family of eukaryotic proteins, sharing the common FH2 domain. While they have been, until recently, understood mainly as actin nucleators, formins are also engaged in various additional aspects of cytoskeletal organization and signaling, including, but not limited to, the crosstalk between the actin and microtubule networks. A surprising diversity of domain organizations has been discovered among the FH2 proteins, and specific domain setups have been found in plants. Seed plants have two clades of formins, one of them (Class I) containing mostly transmembrane proteins, while members of the other one (Class II) may be anchored to membranes via a putative membrane-binding domain related to the PTEN antioncogene. Thus, plant formins present good candidates for possible mediators of coordination of the cortical actin and microtubule cytoskeletons, as well as their attachment to the plasma membrane, that is, aspects of cell cortex organization likely to be important for cell and tissue morphogenesis. Although experimental studies of plant formin function are hampered by the large number of formin genes and their functional redundancy, recent experimental work has already resulted in some remarkable insights into the function of FH2 proteins in plants.

1. Cortical Cytoskeleton in Plant Cell Growth, Morphogenesis and Differentiation

Plants possess two cytoskeletal systems shared by all eukaryotes, that is, the actin filament and microtubule networks, with a host of associated and regulatory proteins. Cytoskeletal networks in the cell cortex are crucial for the controlled remodeling of the plant cell wall, contributing thus substantially to cell growth and morphogenesis. In particular, polar cell growth, including both tip growth (encountered, e.g., in root hairs and pollen tubes) and nonisodiametric cell expansion (occurring as one-dimensional elongation, e.g., in the root elongation zone, as two-dimensional expansion e.g., in epidermal cells, or as localized expansion, e.g., during *Arabidopsis* trichome differentiation or shaping of epidermal pavement cell lobes) involves intricate co-ordination of cytoskeletal remodeling and membrane turnover (see e.g., [1, 2]). Polarized exocytosis, directed and/or restricted by cytoskeletal structures, may also take place without an increase in cell size, as documented by localized deposition of molecules, in particular proteins, to distinct regions of

the cell cortex or plasmalemma (comparable perhaps to the notorious example of apical versus basolateral polarity of metazoan epithelia). The same cell may exhibit several of these phenomena in the course of its life or simultaneously. For example, many cells deposit distinct proteins, such as, for example, PIN family auxin carriers, to their crosswalls while elongating (see [3–6]). Last but not least, cytokinesis, or cell division, may be viewed as a special case of “inward-oriented” cell growth with new cell wall material deposition oriented towards a specific intracellular compartment, the nascent cell plate.

Most attention has been so far devoted to cortical microtubules, which delimit areas where new cell wall material is inserted (reviewed in [7]). Local randomization of the cortical microtubule network is among the first observable events during transition from polar expansion to tip growth in lettuce trichoblasts [8], although this may be species specific, as no such phenomenon was observed in alfalfa (*Medicago*) [9]. Cortical microtubules were long believed to determine the direction of cellulose microfibrils of the primary cell wall, and therefore also of cell expansion, in elongating cells. However,

their disruption by the microtubule-depolymerizing herbicide oryzalin [10], or by the temperature-sensitive *mor1-1* mutation, which affects a microtubule-associated protein, results in loss of polarity and cell swelling while microfibrils remain ordered [11, 12]. Thus, microfibrils can organize in a microtubule-independent fashion, possibly by self-assembly driven by physical forces, while the role of microtubules may be, at least in some cases, restricted to determining microfibril length (see [7, 13]).

In plant tip-growing cells microtubules may control growth direction rather than growth itself, as microtubule disruption by oryzalin results in wavy root hair growth in *Arabidopsis* (see [14]), and depletion of tubulin by antisense RNA even induces ectopic root hair formation, branching, and occasional initiation of multiple hairs per bulge [15]. Also, in poppy pollen tubes, microtubules seem to be important for maintaining growth direction but not for growth as such [16], and extremely high concentrations of the microtubule-stabilizing drug taxol were required to inhibit tobacco pollen tube elongation [17].

It is becoming increasingly obvious that actin also plays an important part in plant cell morphogenesis, including polarized cell expansion. Specific *Arabidopsis* actin isoforms have been reported to participate in tip growth of root hairs, or at least to be abundantly expressed in tip-growing cell types [18–21], while others take part in diffuse cell growth during root elongation or callus expansion [19, 22, 23]. Interaction with actin may also contribute to the role of microtubules in expanding cells. In the thermosensitive *Arabidopsis* mutant *rsw6*, cortical microtubules are aligned within a cell but their position with respect to the root axis randomizes at the restrictive temperature, resulting in root swelling that can be prevented by LatB-induced actin depolymerization [24]. Moreover, LatB elicits swelling of *Arabidopsis* rhizodermis cells at high doses, and aggravates the effects of the *mor1-1* mutation at low concentration, indicating a crosstalk between the two cytoskeletal systems [25].

Arabidopsis mutants in genes of the *DISTORTED* (*DIS*) class exhibit a syndrome that phenocopies the effects of anti-actin drugs LatB or CytD, characterized by deformed trichomes and misshapen epidermal pavement cells. This is accompanied by microtubule disorganization, and possibly stabilization, apparently secondary to disruption of actin [26, 27]. Four of the *DIS* genes code for subunits of the Arp2/3 actin nucleation complex [27–31]. Surprisingly, *Physcomitrella* mutants deficient in Arp2/3 subunits exhibit partial loss of cell polarity but also reduced tip growth, suggesting that the relatively insignificant role of this complex in tip growth may be specific to angiosperms [32, 33]. However, as we shall see below, in seed plants, Arp2/3 is apparently not the only actin-nucleating complex involved in localized cell expansion, albeit fine branched filaments nucleated by Arp2/3 are present in the cortex of diffusely growing tissue culture cells [34].

In tip-growing root hairs, actin filaments participate in root hair emergence at the bulge stage, and later form a fine dynamic network in the extending tip, merging into thicker cables in older parts of the hair. As the hair matures and ceases growing, cables extend into the area previously occupied by

the fine meshwork, suggesting a causal connection between tip growth and the presence of fine actin arrays [35–38]. Reversible disruption of the actin cytoskeleton by mild doses of inhibitors (LatB, CytD), sufficient to destroy the fine meshwork but not actin bundles, causes temporary tip swelling, either by mislocalized exocytosis or by perturbation of endocytosis. The former is more likely, as the pattern of internalization of the FM4-64 dye, which can serve as an indicator of endocytosis, does not change in root hairs temporarily depolarized by CytD [36]. At least part of the actin function in root hairs is myosin dependent, as documented by reduced root hair growth in *Arabidopsis* lacking one of the myosin XI isoforms [39].

Actin organization in pollen tubes resembles that in root hairs [40–42], with bundles of parallel filaments along the shank of the tube and a fine meshwork of randomly oriented short filaments in the tip region [43]. Like root hairs, pollen tubes of both angio- and gymnosperms respond to mild LatB treatment by tip swelling [44, 45]. However, swelling was observed also in lily pollen tubes treated by the actin-stabilizing drug jasplakinolide, which induced formation of short, thick actin cables throughout the tip region [46]. The effects of various cytoskeleton-perturbing treatments on tip-growing plant cells are summarized in Figure 1.

Localized secretion in nonexpanding cells also relies on cytoskeletal cues. Microtubules determine the position of secretory domains in *Arabidopsis* seed coat cells that deposit pectinaceous mucilage into their periplasm [47]. Also the localization of the PIN1 auxin efflux carrier at bottom-oriented crosswalls of root cortex cells is established in a microtubule-dependent manner already in the course of cytokinesis, while actin may be involved rather in endocytotic turnover of PIN auxin transporters [3, 4]. Localization of the AUX1 auxin influx carrier to the opposite cell poles is also actin-dependent [48]. Bundling of actin elicited by expression of a mouse talin-derived YFP fusion protein in cultured tobacco cells resulted in changes in cell division pattern consistent with perturbation of auxin transport, possibly due to altered localization of auxin transporters [49].

Cytokinesis in plant cells is intimately linked to cytoskeletal rearrangements as well. Prior to onset of mitosis, microtubules form a preprophase band that determines the position of the future cell plate (reviewed in [7]). Vigorous membrane turnover takes place at the adjacent plasmalemma, possibly associated with deposition of local markers that determine the future sites of cell plate fusion [50, 51]. After anaphase, microtubules reorganize into the phragmoplast that acts as scaffolding for assembly of the nascent cell plate by vesicle fusion. One of the alleles of the above discussed *MOR1* gene was previously identified as *gem1* based on a pollen cytokinesis defect [52]; detailed analyses revealed occasional occurrence of multinucleate cells and misaligned cell plates even in the *mor1-1* mutant originally believed to suffer only from a cell polarity defect [53, 54]. Microtubules, microfilaments and associated molecular motors (kinesins and myosins) all participate in phragmoplast function and cell plate formation [55–57].

The complex dynamics of the cortical cytoskeleton—closely interlinked with that of the plasmalemma,

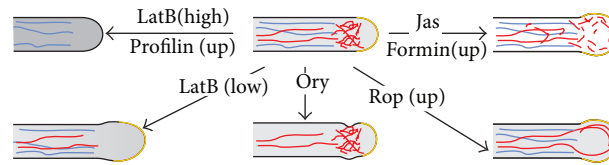


FIGURE 1: Schematic representation of the effects of perturbations of the actin (red) or tubulin (blue) networks in a tip-growing plant cell by pharmacological treatments or mutations, which reveal distinct roles for thick actin cables, fine cortical actin network, and microtubules. Ory: oryzalin, LatB: latrunculin B, and Jas: jasplakinolide. Growing portion of the cell surface is shown in yellow; shading indicates the levels of monomeric G-actin.

underlying cortical cytoplasm and the endomembrane system—is orchestrated by an intricate regulatory network including, among others, lipid-based and redox-based signalling pathways, as well as small GTPases of the Rop (Rho of plants) family with their co-factors (reviewed, e.g., in [6, 58–60]).

Given the many interlinked roles of the cytoskeletal networks in plant cell growth and cell morphogenesis, any protein affecting cytoskeletal function is likely to influence also these processes, and formins, a major family of evolutionarily conserved actin nucleators with a host of possible additional functions, present in most, if not all eukaryotes [61], should be no exception.

2. Formins as Cytoskeletal Organizers and Signaling Hubs

Formins, or FH2 proteins, are a large, ancient family of eukaryotic proteins sharing the evolutionarily conserved FH2 (formin homology 2) domain, usually, though not always, located in the C-terminal portion of the protein and preceded by a proline-rich FH1 domain [62–66]. The first member of the family was originally described on the basis of a mouse insertional mutation responsible for a limb deformity defect [67], and homologs were later found across vertebrates and yeasts [68–74]. Somewhat ironically, the originally described limb phenotype, which even rose to a brief fame of one of “poster cases” documenting that transgenesis as such may negatively affect animal welfare [75], turned out to be due not to disruption of the formin gene but to perturbed expression of a neighboring gene, *gremlin*, whose regulatory sequences overlap the formin locus [76]. While formin itself may also contribute to the limb phenotype through possible participation in modulating the secretion of extracellular regulatory peptides [77], it is noteworthy that involvement of formin genes in genome regions subjected to “large-scale” regulation involving multiple genes is, in at least one case, found also in plants (see discussion of AtFH5 below).

Until the end of the last millennium, yeast and metazoan FH2 proteins have been implicated in numerous cellular processes, often related to cytoskeletal, in particular microtubule-related activities (e.g., mitosis [74], cytoplasmic streaming [78], or cytokinesis [79]). However, they have been also localized to the cell nucleus [80, 81] and implicated in several signaling pathways whose ultimate outcome is the regulation of gene expression [82].

Metazoan FH2 proteins form a large gene family that can be classified into several distinct subfamilies [70, 83–85]. Among them, members of the best characterized Diaphanous clade (Diaphanous-related formins, or DRFs) exhibit a characteristic domain structure that is found also in fungal and *Dictyostelium* formins and thus believed to be ancient [86]. The DRFs contain a regulatory N-terminal region (GBD, sometimes also termed FH3 [87]) that can alternatively bind either to an autoinhibitory domain (DID) at the proteins’ C-end or to an activated (GTP-bound) small GTPase of the Rho clade, leading to Rho-dependent release of the autoinhibitory interaction and thereby to formin activation [88–90]. Rho-related small G-proteins, such as Rho *sensu stricto*, Rac and Cdc42 (Cft) of metazoans and fungi, or Rop of plants, are notorious for their role in the control of cell polarity, predominantly via regulating the actin cytoskeleton (reviewed e.g., in [61, 91–94]). However, not all functions of Diaphanous-like formins are exclusively actin-related; for instance, mammalian homologs localize to the mitotic spindle and contribute to the actin-microtubule crosstalk [95, 96]. As a rule, formins harbor the hallmark FH2 domain at or close to their C-end, preceded usually by the FH1 domain and by additional domains mediating regulatory interactions; for instance, the well-known DRFs have an additional conserved motif located C-terminally from the FH1/FH2 tandem [83, 84, 86, 97]. A remarkable exception from the usual domain order are the metazoan “inverted formins” (INFs) with the FH1 and FH2 domains located N-terminally and followed by a large C-terminal extension [98].

A decisive turn in the quest for the molecular mechanism of formin action was the discovery of an actin-nucleating ability in yeast formins [99, 100]. Subsequently, formins were recognized as a new class of actin nucleators, functioning by a mechanism independent of the Arp2/3 complex. Unlike the Arp2/3 complex, which associates with the pointed end of actin filaments, a dimer of formin’s FH2 domains acts as a processive or “leaky” cap at the barbed ends ([101]; for a review see, e.g., [64, 102–104]). While this cap may, at least for some formins, facilitate polymerization of G-actin subunits (whose supply is aided by the FH1 domain that acts as a “docking site” for profilin-actin complexes), other formins have been documented to function as “mere” capping proteins not engaged in actin polymerization (e.g., in the case of the fission of yeast *cdc12* protein, which acts a profilin-gated cap at the barbed end of actin filaments—[105]). The dimerization ability of FH2 domains raises also the question whether, and with what functional consequences,

could various members of the extensive formin family form heterodimers. Surprisingly little is known about this topic, and heterodimerization, (moreover mediated not by FH2 but by the GBD and DID motifs), was so far well documented only among closely related mammalian DRFs and between DRFs and INFs [106, 107].

Besides direct actin nucleation, some formins may participate in the initiation of new actin filaments also indirectly, in particular via communication with other nucleation complexes. Spire, a metazoan actin-nucleating protein that is often associated with endosomal membranes, is known to interact directly with several formins (see [108]). Coordination between formins and the Arp2/3 complex may be mediated by common interactors, such as the IQGAP class of Rho GTPase activators that can, besides their function as G-protein cofactors, also directly bind both to the Arp2/3 activator WASP homologs and to Diaphanous-related formins in metazoans [109]. In *Drosophila*, the WASP family protein Wash mediates Rho-based regulation of both Arp2/3-dependent and Spire/formin-dependent actin nucleation [110]. Based on yeast and metazoan data, formins have been suggested to stimulate formation of actin bundles, while “classical” Arp2/3-driven actin nucleation promotes establishment of fine branched filament arrays [63]. Indeed, some formins, including the *Arabidopsis thaliana* AtFH1 protein (see below) are capable of bundling actin filaments side by side and/or crosslinking them [111–115].

In particular in the recent years, numerous reports suggesting that formins participate more or less directly in the organization of the yeast and metazoan microtubular cytoskeleton have appeared [96, 98, 116–119]; in several of these cases, direct binding between formins (in particular DRFs and INFs) and microtubules has been documented. Similar observations have been made also in plants ([120–123]; see also below). Some formins may be also involved in the coordination of the actin and intermediate filament systems (e.g., [124]).

While formins thus emerge as major regulators of diverse cytoskeletal functions, at least some of them may participate also in additional cellular processes less directly related to the cytoskeleton or cell morphogenesis, in particular in the nuclear events associated with gene expression. The *Caenorhabditis foxi-1* gene encodes a protein that contains both the FH2 domain and a DNA-binding zinc finger domain, and acts as a transcriptional regulator [125]. A family of formin-binding proteins (FBPs) participates in spliceosome assembly, raising the possibility that formins may be engaged also in pre-mRNA processing [126].

To summarize: besides some additional functions restricted only to certain members of the extensive gene family, FH2 proteins appear to play a part in numerous cytoskeleton-related processes, including those commonly occurring in the eukaryotic cell cortex. Indeed, cortical localization and/or association with membranes, including (but not limited to) the plasmalemma, has been documented for numerous paralogs in both yeasts and metazoans (see, e.g., [116, 127–131]), and formins have been implicated in the development of cortical structures such as cell-to-cell junctions [131] or filopodia [132, 133] in metazoan cells. It

can therefore be expected that formins might have analogous roles in cell cortex organization also in plant cells, whose rigid cell walls make the precise coordination between the cortical cytoskeleton and the endomembrane system even more important.

3. Formins in Plants: New Uses for an Old Domain

The first reports of FH2 proteins in plants date from 2000, when an *Arabidopsis thaliana* formin gene, AFH1 (later renamed AtFH1) has been cloned, and its product found to associate with membranes due to the presence of a N-terminal membrane insertion signal and a single transmembrane helix, features found also in additional putative genes predicted from the *Arabidopsis* genome sequence [134, 135]. After completion of *A. thaliana* genome sequencing [136], it became obvious that plant FH2 proteins form an extensive family (e.g., *Arabidopsis* has 21 paralogs, some of them possibly encoding multiple protein isoforms due to alternative splicing) with two distinct subfamilies, termed Class I and Class II, in flowering plants [104, 137, 138] (note that the current terminology of *Arabidopsis* formin genes was established in [138]). A third plant formin clade (Class III), related to Class I, has been found only in nonangiosperm “lower” plants such as the moss *Physcomitrella patens*, the lycophyte *Selaginella moellendorffii*, and some algae [84].

Each plant formin clade exhibits a characteristic domain layout (Figure 2; [84, 138]), although exceptions are common, reflecting possibly rapid evolution of multigene families with a great deal of degeneracy (“redundancy”) and resulting relaxation of evolutionary constraints. Indeed, especially within Class II, orthologs cannot be clearly assigned even between *A. thaliana* and *A. lyrata*, two closely related species that have separated mere five million years ago, while Class I appears to be better conserved. Nevertheless, no evidence of positive selection, that is, selection favoring diversification, was found in the FH2 domains of Class II formins, albeit it is still possible that positive selection operated on the N- and C-terminal portions of the proteins [139].

A typical representative of the angiosperm Class I formins (represented by 11 genes, AtFH1 to AtFH11, in *Arabidopsis*) has an N-terminal membrane insertion signal sequence, followed by a presumably extracellular proline-rich domain containing motifs reminiscent of some cell wall (glyco)proteins including extensins, known to participate in cell wall loosening, which is a prerequisite of cell growth [38, 140]. Downstream of these extracytoplasmic motifs is a single amphipathic transmembrane helix followed by a cytoplasmic portion with C-terminally located FH1 and FH2 domains. Parts of the protein between the transmembrane segment and the FH1-FH2 tandem are loosely conserved among some formin isoforms (see also [120] and the discussion of the GOE motif below).

Class II formins (represented by AtFH12 to AtFH21 in *Arabidopsis*) lack membrane insertion signals and are supposed to be exclusively cytoplasmic. Their N-terminal portion usually contains a domain related to the mammalian

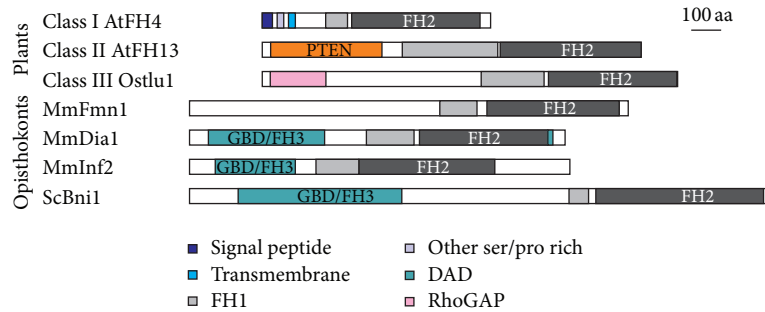


FIGURE 2: Domain layout of typical representatives of selected formin families from plants and opisthokonts (yeasts and metazoans). At—*Arabidopsis thaliana*, Ostlu : *Ostreococcus lucimarinus* (a prasinophyte alga), Mm : *Mus musculus*, and Sc : *Saccharomyces cerevisiae*. Modified from [83], for sequence details and accessions see there.

antioncogene PTEN (phosphatase and tensin homolog on chromosome ten, [141–143]). This domain is generally considered a lipid and protein phosphatase, acting preferentially on membrane phospholipids [141, 144]. However, while plants do have genuine enzymatically active PTEN homologs [145], the PTEN-like domain of plant Class II formins lacks critically important conserved arginine residue forming part of the phosphatase active site. This makes enzymatic activity extremely unlikely, and Class II PTEN-like domains have been proposed to mediate binding of the FH2 proteins to membranes [138].

Remarkably, Class III formins also carry a conserved domain that appears to have lost its original function due to a point mutation, namely, a RhoGAP-like domain, homologous to the GTPase-activating proteins associated with Rho clade small GTPases, but missing a critical conserved arginine finger motif. While this domain may have retained its ability to bind small GTPases, it is rather unlikely to be a genuine GTPase activator (unless it employs some alternative mechanism). Plant Class III formins would thus share the ability to bind Rho family members, common among GBD/FH3- carrying FH2 proteins such as the DRFs or fungal formins. The phylogenetic distribution of domain architectures among plant formins is compatible with a relatively simple evolutionary scenario where PTEN-like domains of Class II formins and RhoGAP-like domains (characteristic for Class III) have replaced the ancestral GBD/FH3 domains early in the plant lineage while maintaining a continuity of the Rho-FH2 and/or membrane-FH2 association. The subsequent acquisition of transmembrane domains in some formins (i.e., Class I), conspicuously coinciding with expansion of the formin family and also with the ascent of plants to dry land, was finally followed by loss of Class III formins in the lineage leading to angiosperms [84].

While all plant FH2 domains detected so far in genome databases can be unambiguously assigned to one of the three above-described classes (or only two classes in case of angiosperms), not all of them conform to the domain architecture typical for their class. Even in angiosperms, several Class I proteins (e.g., *Arabidopsis* AtFH7) lack the N-terminal membrane insertion sequences, and quite many

Class II formins deviate from the canonical PTEN-FH1-FH2 domain order. Out of the ten *A. thaliana* Class II formins, only four, AtFH13, AtFH14, AtFH18, and AtFH20, exhibit the canonical domain configuration [138]. However, aside of (non-FH2) domain losses and occasional internal duplications, plant formins as a rule do not contain additional conserved sequence motifs, with one remarkable exception of a *Physcomitrella patens* gene encoding a protein with a N-terminal Sec10-related domain and C-terminal FH1/FH2 domains [84, 146]. Sec10 is one of the eight subunits of the evolutionarily conserved exocyst complex responsible for exocytotic vesicle addressing towards distinct plasmalemma domains (but sometimes also other targets, such as, e.g., the nascent cell plate); its function is thus intimately interlinked with that of other cortical structures, including the cytoskeleton (see e.g., [6, 58, 147–149]). A direct link between a formin and a subunit of the exocyst may therefore be biologically meaningful. It is, however, not yet clear whether the suspected Sec10-formin fusion protein is expressed *in vivo* in the moss, or if the locus merely encodes alternative gene products whose expression may perhaps be somehow coordinated [146].

The large number of formin isoforms encoded by plant genomes raises several intriguing questions. First of all, it is far from clear how many distinct formin dimers can exist in plant cells; while very little is known about heterodimerization ability of formins (see above), this possibility cannot be excluded. Were the FH2 domains capable of free mutual interactions (and were they all coexpressed, which is obviously not the case), *Arabidopsis* would be able to generate up to 484 binary FH2 domain combinations (taking into account the presumed two distinct gene products for AtFH15 [138]). While the actual biologically relevant number is likely to be much lower, even if formin-formin interactions were restricted to homodimerization, there would still be at least 21 possible functional formin complexes. Since duplicated genes of identical function are likely to be eliminated by natural selection unless contributing to the fitness of the organism [150], surviving numerous formin varieties can be expected to differ functionally. Besides genuine functional specialization (subfunctionalization or neofunctionalization—[151]),

in sessile organisms, such as plants (and also yeasts—see [152]), the evolutionary advantage may be provided by “fine-tuning” that optimizes the protein function for specific intracellular or intraorganismal locations or environmental conditions.

Last but not least, “redundancy” (or degeneracy) within the plant formin family is also a practical problem for researchers interested in the function of these proteins, since loss-of-function mutations can be, to an extent, compensated for by unaffected members of the gene family. This has also significantly hampered experimental studies of plant formins, making the gene family perhaps less attractive for researchers than it would deserve.

4. Functional Studies of Plant Formins

The majority of experimental work devoted to plant FH2 proteins has focused on members of the angiosperm Class I. This clade includes AtFH1, the first plant formin to be cloned [134], and also the most ubiquitously expressed (and thus presumably housekeeping) member of the formin family in *Arabidopsis thaliana* vegetative tissues according to publicly available microarray data [153]. The predicted membrane localization of AtFH1 was confirmed; this formin was also found to interact with FIP2 [134], a protein originally predicted to interact also with potassium membrane channels but later discovered to encode a putative E3 ubiquitin ligase [154]. Recently, it was demonstrated that AtFH1 preferentially localizes to membrane regions not occupied by microtubules, and that actin bundling elicited by AtFH1 overexpression depends on anchoring the formin within the cell wall and results in decreased organelle motility [155].

Also other members of the Class I formin family were found in the plasmalemma. Two closely related *Arabidopsis* formins from the Class I branch known as group Ie, AtFH4 and AtFH8, localize preferentially to plasmalemma adjacent to transversal cell walls in the rhizodermis cell files [156], that is, to actin-rich domains of the cell surface that exhibit intensive vesicle trafficking [157] and that are specifically enriched with auxin transporters. AtFH5 is localized in the cell plate in the course of cytokinesis [158]. AtFH6, another Class I formin, which is massively upregulated in expanding giant cells of nematode-induced galls, localizes to the cytoplasmic membrane in these cells [159]. Fluorescent protein-tagged AtFH6 was found at or around the nascent cell plate and newly developed cross-walls both in *Arabidopsis* seedlings and when heterologously expressed in cultured tobacco BY-2 cells [160].

Heterologous high-level expression of AtFH1 in tobacco pollen tubes [161] caused tube tip swelling with excessive formation of actin cables (see also Figure 1). Actin-bundling activity of AtFH1 [111, 112] might have contributed to the observed phenotype. Since AtFH1 is normally not expressed in pollen under normal circumstances, possible artifacts due to ectopic overexpression also cannot be excluded. However, overexpression of other plant formins such as AtFH8, another *Arabidopsis* formin that is normally expressed in root tissues and apparently lacks the bundling activity, elicits formation of abundant actin cables, partial depolarization and

branching in *Arabidopsis* root hairs [162], while expression of a nonfunctional derivative of AtFH8 suppresses root hair growth [156]. Even more remarkably, a heterologous (but not ectopic) overexpression of AtFH3, the main *Arabidopsis* Class I pollen formin, in tobacco pollen tubes also caused extensive actin bundling, followed by tube tip swelling, while inhibition of AtFH3 expression in *Arabidopsis* by RNAi led to partial inhibition of pollen tube growth [163]. Manipulation of the expression levels of closely related AtFH5, which is also expressed in pollen, and its tobacco homolog NtFH5, could shift the balance between longitudinal actin cables and the fine apical meshwork, with subsequent alterations in tube growth rate. In addition, inhibition of NtFH5 by RNAi often resulted in wavy pollen tube growth [164], suggesting that also microtubules might perhaps be compromised.

These observations are all consistent with a general requirement for finely balanced Class I formin-based actin nucleation in tip-growing cells of higher plants. Remarkably, disruption of the Arp2/3 actin nucleation pathway has only subtle effects in tip-growing cells while profoundly affecting other modes of cell expansion (see [1, 165] and above). However, in the moss *Physcomitrella patens*, Class I formins are dispensable for tip growth as such, since simultaneous RNAi silencing of all six moss Class I members only led to the reduction in cell growth and division, that is, “mere” quantitative growth defects without obvious morphogenetic phenotypes, in contrast to Class II formins which were found to be indispensable for maintaining cell polarity ([166], see also below).

Several plant Class I formins, including *Arabidopsis* AtFH1 [111], AtFH3 [163], AtFH4 [156], AtFH5 [158] and AtFH8 [162], have been demonstrated to nucleate actin *in vitro*. However, similar to their metazoan counterparts, plant Class I formins may have additional roles besides those directly related to actin organization and membrane anchoring. In particular, the Group Ie formin AtFH4 was demonstrated to bind microtubules both *in vitro* and *in vivo* via a conserved sequence motif that is characteristic for the Ie branch of Class I, the GOE domain, which is identical with the mutually related plant-specific domains of unknown function previously annotated as ProDom PD038281 and PD224441. AtFH4 also appears to be capable to mediate alignment of the endoplasmic reticulum along microtubules under certain circumstances [120].

As mentioned above, the use of mutants in plant formin studies is complicated by the degeneracy of the extensive formin family. Indeed, very few phenotypes were described for plant FH2 protein mutants, even in case of Class I that, as a rule, contains relatively abundantly expressed proteins. Loss of AtFH5 leads to a cytokinesis delay during endosperm cellularization, consistent with cell plate localization of the AtFH5 protein; the tissue-specific defect is well explained by high levels of AtFH5 expression in the endosperm, and thus possibly its increased functional importance [158]. While the subsequent recovery of cytokinesis in AtFH5 mutants may be due to compensation by some other member(s) of the large formin family [137, 138], it is worth noting that in tobacco BY2 cells the actin polymerization inhibitor bistheonellide A caused only a temporary delay of cytokinesis but not its

permanent disruption; later stages of cell plate development, where major involvement of endocytosis is suspected, were affected more profoundly [57]. It is tempting to speculate that the relative importance of microfilaments and microtubules in cytokinesis may be opposite to that described for tip growth.

To complicate matters further, the *Arabidopsis* AtFH5 gene is involved in rather complex transcriptional regulation involving extensive chromatin modifications, somewhat reminiscent to the mammalian formin/gremlin gene tandem discussed above. Its expression is controlled by Polycomb-group proteins and the gene itself is maternally imprinted [167]. In our hands, young (less than two weeks old) seedlings of the reporter line used to characterize the endosperm-specific expression pattern [158] exhibited gene expression only in various root tissues and cotyledon vasculature. However, gene expression patterns in other tissues were highly variable and often changed in the course of development. In particular, true leaves exhibited only rare patches of gene expression in the vascular bundles, and even in the root tips gradual decline of reporter gene expression was observed (F. C. and Ann Sibyl Kuckuck, unpublished observations).

While loss of function mutants in genes from large, degenerate families are generally devoid of observable phenotypes due to compensation by their sibling loci, combination with mutations and/or pharmacological treatments affecting their targets (such as asymptomatic doses of cytoskeletal inhibitors in the case of formins) may be used to elicit observable phenotypes; this phenomenon is known as “synthetic lethality” or “synthetic toxicity” (see [168]). Indeed, seedling roots of *Arabidopsis* mutants lacking AtFH8 exhibited increased sensitivity towards the actin-depolymerizing drug LatB compared to wild-type plants [169].

Besides the already mentioned tobacco and moss studies [163, 166], there is not much data on Class I formins from other plant species. A tomato member of this family has been recently found as an interacting partner of a resistance protein involved in the response to fungal pathogens; however, silencing of the formin did not influence pathogen sensitivity [170], somewhat reminiscent of the already mentioned case of AtFH6 whose mutation did not affect nematode response, despite massive expression in nematode-induced galls [159].

Compared to Class I formins, even less is known about their Class II counterparts; this may be partly due to the combination of usually low expression levels and complex locus structures that make prediction of cDNA sequences and PCR-based cloning difficult. Actin nucleation and bundling have been demonstrated for rice FH5, a canonical Class II formin, which can also bind microtubules and whose mutation exhibits a pleiotropic phenotype including stunted growth and organ bending, suggestive of problems with cell expansion and/or phytohormone transport [122, 123]. *Arabidopsis* AtFH14 was also found to bind both actin and microtubules, and its loss led to mild defects in cell division and meiosis [121]. In the absence of GOE-related motifs, the mechanism of microtubule-binding must necessarily be different from that found in Class I formins. Very recently, another *Arabidopsis* Class II formin, AtFH19, was reported to nucleate actin *in vitro*. Remarkably, it competes with

AtFH1 in barbed-end binding and exhibits different kinetic properties, suggesting intriguing possibilities for modulation of actin dynamics by interaction of Class I and Class II formins [171].

The hypothesis that PTEN domains of typical Class II formins mediate binding to membranes (see above) has been recently confirmed for a *Physcomitrella patens* Class II formin [172]. Simultaneous RNAi knockdown of all moss Class II formins revealed that they are required for tip growth [166]. In angiosperms, phenotypes of Class II mutants are subtler. Besides the above-mentioned *Arabidopsis* and rice examples, loss of the outlier atypical *Arabidopsis* Class II formin AtFH12, which lacks the PTEN domain, caused only a minor decrease in the sensitivity of root growth towards LatB, raising the intriguing possibility that the PTEN-less Class II formin may somehow attenuate actin polymerization or destabilize actin filaments. Consistent with this hypothesis, mutants lacking AtFH12 also exhibited partial synthetic lethality with the fluorescent actin marker GFP-tagged mouse talin (GFP-mTalin), which is known to stabilize actin and induce actin-bundling, another example of the “synthetic lethality” phenomenon. Indeed, surviving plants carrying GFP-mTalin in the mutant background rapidly silenced the toxic transgene, and exhibited excessive and anomalous actin bundling in the still-expressing tissues ([139]; Figure 3).

Several Class II formins appear to respond to environmental cues; AtFH12 is induced by salt stress, but its loss does not affect salt sensitivity [139]. Although there are no data on the involvement of *Arabidopsis* Class II members in pathogen response, a typical representative of this clade was among genes dramatically repressed in phytoplasma-infected, diseased *Citrus aurantifolia* [173], suggesting that both Class I and Class II formins might be involved in the response to pathogens.

The extent of our current understanding concerning plant formins is therefore far behind the thesaurus of knowledge available on formins of fungi and metazoa, reflecting clearly the more than dozen years delay. Plant formin research has now reached the phase when any new observations spawn a host of questions to be addressed. Undoubtedly, major advances can be expected with the progress of *in vivo* imaging techniques such as the VAEM microscopy, allowing observations of individual microtubules or microfilaments *in vivo*, that has only recently been applied in first plant studies [174].

5. Conclusions

The progress in plant formin studies has been the topic, and mostly main focus, of several reviews in the last decade [104, 137, 175, 176]; however, these were either brief updates restricted by journal space, or focused on specific aspects of formin biology or biochemistry. Here I attempted to provide an exhaustive account of published work on plant formins in the broader context of current understanding of FH2 protein roles in general, and of the structure and function of the plant cell cortex.

Although phenotypes of plant formin mutants are rarely dramatic due to the degeneracy of the extensive plant formin

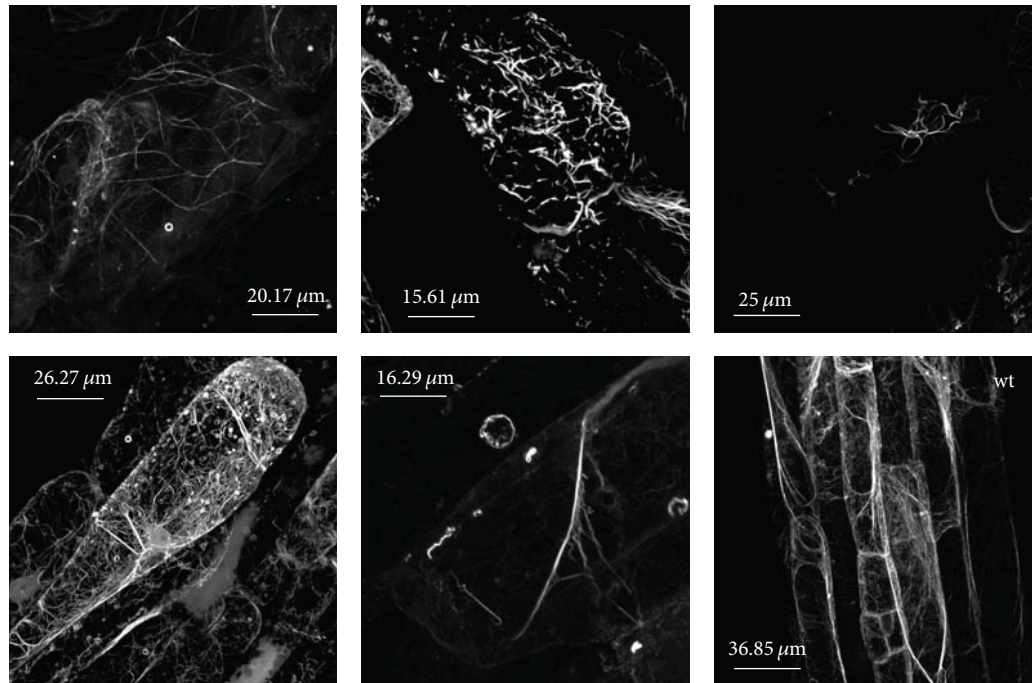


FIGURE 3: Confocal microscopy images of fragmented and malformed cortical actin cytoskeleton observed in the roots of 4-to-7-days-old *Arabidopsis thaliana* seedlings expressing the moderately toxic actin marker, GFP-tagged mouse talin, in a genetic background lacking the Class II outlier formin AtFH12. Corresponding tissue from a wild-type (wt) isogenic seedling is shown in the right bottom corner. Modified from [138]; confocal images obtained by the author.

families and resulting mutual replaceability of products of multiple genes, the emerging picture suggests that FH2 proteins participate in multiple cellular processes crucial especially for precise control of cell morphogenesis, including various modes of cell expansion.

Remarkably, formins appear to be not only important regulators of the actin cytoskeleton, but also prime candidates for mediating the co-ordination between microfilaments and microtubules also in plants, despite using mechanisms of microtubule interaction different from those of their metazoan counterparts.

The unique plant-specific domain structure of plant Class I formins enables these proteins to act as direct membrane-crossing linkers between the cytoskeleton in the cortical cytoplasm and the cell wall; however, this does not exclude the possibility that formins may also associate with intracellular membrane compartments such as the ER.

In summary, it becomes increasingly clear that plant formins are far from copying the tasks of their opisthokont counterparts; on the contrary, they operate in plant-specific contexts to accomplish plant-specific function, documenting thereby the extreme versatility of evolutionarily ancient protein domains and domain combinations such as, for example, the FH1-FH2 tandem or the PTEN domain.

Acknowledgments

While the author did her best to provide a complete coverage of reports devoted to plant FH2 proteins, the introductory

sections of this paper are intended rather as a collection of relevant examples illustrating the workings of the plant cell cortex and the scope of functions of FH2 proteins in nonplant organisms. She therefore apologizes to all authors whose work was not included for the sake of readability and focus of the present review. The author thanks Michal Grunt for critical reading of this paper. The work on formins in the author's laboratory is currently supported by the GACR P305/10/0433 and MSM 0021620858 Projects.

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