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Review

Plant formins: Diverse isoforms and unique molecular mechanism

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ABSTRACT

The completed genome from the model plant *Arabidopsis thaliana* reveals the presence of a diverse multigene family of formin-like sequences, comprising more than 20 isoforms. This review highlights recent findings from biochemical, cell biological and reverse-genetic analyses of this family of actin nucleation factors. Important advances in understanding cellular function suggest major roles for plant formins during cytokinesis and cell expansion. Biochemical studies on a subset of plant formins emphasize the need to examine molecular mechanisms outside of mammalian and yeast systems. Notably, a combination of solution-based assays for actin dynamics and timelapse, single-filament imaging with TIRFM provide evidence for the first non-processive formin (AtFH1) in eukaryotes. Despite these advances it remains difficult to generate a consensus view of plant formin activities and cellular functions. One limitation to summarizing formin properties relates to the enormous variability in domain organization among the plant formins. Generating homology-based predictions that depend on conserved domains outside of the FH1 and FH2 will be virtually impossible for plant formins. A second major drawback is the lack of facile techniques for examining dynamics of individual actin filaments within live plant cells. This constraint makes it extremely difficult to bridge the gap between biochemical characterization of particular formin and its specific cellular function. There is promise, however, that recent technical advances in engineering appropriate fluorescent markers and new fluoresence imaging techniques will soon allow the direct visualization of cortical actin filament dynamics. The emergence of other model systems for studying actin cytoskeleton *in vivo*, such as the moss *Physcomitrella patens*, may also enhance our knowledge of plant formins.

1. General statement

Two years ago, we presented our latest data on *Arabidopsis* FORMIN1 (AtFH1). At the end of the talk a voice from the back of the room questioned "why so many formins in plants?"

Despite recent significant progress toward understanding the cellular and molecular functions of a subset of *Arabidopsis* formins, the large number and diversity of formin isoforms in plants complicates establishment of a consensus view of their mechanism of action. Perhaps such a consensus mechanism will never be applicable to the large plant formin family but, in any case, answering this question will require substantial further effort from the plant community. Although more than 20 formin isoforms within the genome of the model plant *Arabidopsis thaliana* provide an ample supply of targets [1–3], the powerful reverse-genetics approach readily available to plant researchers has revealed surprisingly few biological functions for plant formins [4]. This is likely due to overlapping expression patterns and functional redundancy among the formin isoforms. However, recent biochemical characterization of plant-specific formins including AtFH1, AtFH5, AtFH4 and AtFH8 has revealed unique features of these proteins compared to mammalian or yeast formins, including the first example of a non-processive formin [4–8]. This review focuses attention on the field of formin function by pointing out similarities and differences between plant and other eukaryotic formins.

2. Actin cytoskeleton in plants

Actin filaments are dynamic polymers that undergo assembly and disassembly simultaneously. In mammalian cells, actin cytoskeletal arrays provide a molecular framework for various cellular processes including cell morphogenesis, establishment of cell polarity and cell motility [9]. Powering these cellular functions often hinges on the ability of the actin cytoskeleton to produce forces as large as a few nanoNewtons near membranous structures [10]. These forces can be generated directly through polymerization of actin filaments in branched or bundled networks or through the activity of motor

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molecules, the myosins, propelling cargo or other filaments over actin-based tracks. Most models for cell motility hold that polymerization of a dendritic actin filament array at the leading edge is necessary for protrusion of the lamellipodium, whereas assembly of bundled filament arrays drives the formation of filopodia [11,12].

Besides the obvious fact that the cells in flowering plants are not motile, they are also encased by a thick cell wall that is in theory too stiff to be directly deformed by the actin cytoskeleton pushing on the plasma membrane [13]. Accordingly, many biologists take the view that actin filaments in plant cells, organized into prominent bundles and cables, mainly serve as passive tracks for myosin-based movement of organelles and endomembrane systems. Numerous reports document the actomyosin-dependent motility of mitochondria, chloroplasts, peroxisomes, and Golgi stacks (reviewed by [14–16]). Nevertheless, the actin cytoskeleton is an important contributor to plant cell growth and development. Indeed, pharmacological and genetic studies provide a wealth of evidence for actin's role in a variety of cellular processes such as guard cell shape changes, cell polarity establishment, polarized cell expansion, and cell division [17]. Clearly, actin filament cables alone cannot support all of these processes, and attention has therefore turned to studies of the organization and turnover of actin filaments near the plasma membrane. Unfortunately, a lack of imaging technologies with the high spatial and temporal resolution capable of revealing order within dense arrays or fast enough to capture extremely dynamic events have hindered rapid progress. Still several groups report fine arrays of filaments associated with sites of growth in epidermal pavement cells [18,19], and proposals for their function center on the regulation of secretory vesicle (or endocytic vesicle) traffic (reviewed by [17,20,21]. Our recent observations using timelapse variable-angle epifluorescence microscope imaging of the fluorescent actin-binding reporter GFP-fABD2 [22] in expanding epidermal cells of etiolated hypocotyl reveal extraordinary cortical actin filament dynamics, with assembly rates of 1.7 μm/s and most filament lifetimes <30 s due to prolific severing activity (Staiger et al., manuscript submitted). This phenomenal dynamic behavior of the cortical actin cytoskeleton is difficult to reconcile with models that center on vesicle trafficking events, but at least provide hope for linking observations of cytoskeleton turnover with growth parameters. Surprisingly, from this work and previous studies, there is little evidence for dendritic filament arrays at or near the plasma membrane of plant cells—although these may still escape attention if the filaments are well below the diffraction-limited observations of light microscopy. Another interesting and unique aspect of plant cells is the subtle defect in filament organization in mutant plants lacking a functional Arp2/3 complex (actin-promoting complex). Although homozygous mutant plants display defects in the expansion of trichomes, leaf epidermal pavement cells and some hypocotyl epidermal cells [23–25], the exact mechanism remains subject of intensive study. One possibility is that Arp2/3-dependent growth is associated with the behavior of vacuoles or other endomembrane compartments, rather than events at the plasma membrane. Subcellular localization of active Arp2/3 complex should help resolve these issues. The *distorted* class of trichome morphology mutants has also revealed that the Rac–WAVE–Arp2/3 pathway is conserved in plants and controls certain cell morphogenesis events [26,27]. Interestingly, the Brk1/HSPC300 subunit of the *Arabidopsis* WAVE complex is required to modulate the cellular concentration of the Arp2/3 activator SCAR [28–30].

3. Introduction on the domain organization of plant formins

It is generally accepted that formin family members are defined by the presence of a formin homology-2 (FH2) domain. Based on this definition, the model plant *Arabidopsis thaliana* has at least 21 formin isoforms [3]. Each of these formins also contains a proline-rich, FH1 domain positioned upstream of the FH2 domain and sometimes separated by a variable sequence. The FH1 domain, which functions in binding to profilin and profilin–actin, is somewhat variable in terms of number of repeats and conservation of the proline-rich motif among the plant formins. This raises the possibility for isoform matching, different affinities for profilin, and/or variations in processive elongation rates [8]. *Arabidopsis* FORMIN isoforms are separated into two distinct phylogenetic subfamilies [1–3]. Class I is characterized by a putative signal peptide and predicted N-terminal transmembrane domain that probably targets the formin to or near the plasma membrane via the secretory pathway (Fig. 1 and [31–33]. In all class I formins, except AtFH7, a predicted transmembrane domain is localized in the N-terminal region of the protein [3,31]. Only limited data are currently available for class II formins. However, based on sequence prediction, class II formins seem to have a more diverse domain architecture that needs to be confirmed by full-length cDNAs sequences [3]. Nevertheless, an important difference regarding the domain organization between the plant formins and their amoebal, fungal and metazoan counterparts is the lack of the Rho GTPase-binding domain (GBD-GTPase binding domain/FH3), (Fig. 1). These regulatory domains partially overlap with the auto-inhibitory domain (DID) [34,35]. The current view of the regulation of the well-characterized mammalian mDIA1 is that the DID interacts with the diaphanous auto-regulatory domain (DAD) localized in the C-terminus of the protein in the auto-inhibited state and the binding of Rho GTPase to the GBD domain relieves this auto-inhibition. This model has been supported biochemically for mDIA1 [34,36]. This mechanism seems not to be conserved in *Arabidopsis* formins and understanding how plant formin activity is regulated remains an unresolved issue.

4. Physiological role of plant formins

Evidence for the plasma membrane localization of class I formins has been generated for at least five isoforms via the overexpression of fluorescent fusion proteins as well as, in a limited number of cases, by detection of the endogenous protein with specific antibodies

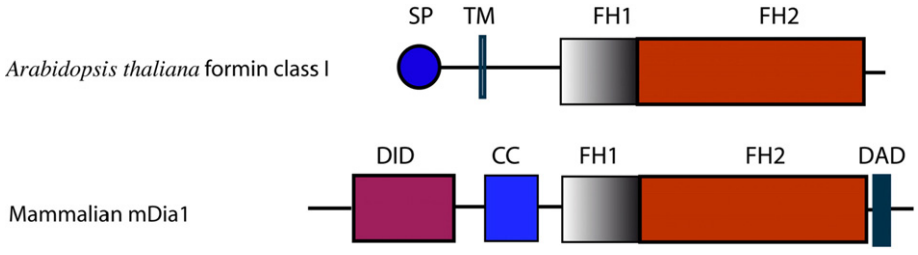

Fig. 1. Domain organization of a typical *Arabidopsis* class I formin compared to a diaphanous-like mammalian formin. Apart from the FH1 and FH2 domains, *Arabidopsis* class I shares no homology with the auto-inhibitory domain (DID) or diaphanous autoregulatory domain (DAD) of mDia1, a representative of mammalian formins. Further, the typical *Arabidopsis* class I formin has a putative signal peptide (Sp) and transmembrane domain (TM) at the N-terminus. Sp, signal peptide, TM, transmembrane domain, FH1, formin homology 1 domain, FH2, formin homology 2 domain, DAD, diaphanous autoregulatory domain, DID, auto-inhibitory domain, CC, predicted coiled-coil.