The Pathogen-Actin Connection: A Platform for Defense Signaling in Plants

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Abstract

The cytoskeleton, a dynamic network of cytoplasmic polymers, plays a central role in numerous fundamental processes, such as development, reproduction, and cellular responses to biotic and abiotic stimuli. As a platform for innate immune responses in mammalian cells, the actin cytoskeleton is a central component in the organization and activation of host defenses, including signaling and cellular repair. In plants, our understanding of the genetic and biochemical responses in both pathogen and host that are required for virulence and resistance has grown enormously. Additional advances in live-cell imaging of cytoskeletal dynamics have markedly altered our view of actin turnover in plants. In this review, we outline current knowledge of host resistance following pathogen perception, both in terms of the genetic interactions that mediate defense signaling, as well as the biochemical and cellular processes that are required for defense signaling.

INTRODUCTION

Actin cytoskeleton: A dynamic, filamentous network of polymers constructed from a large pool of cytoplasmic actin monomers

The activation of innate immune signaling in plants results in not only the abrogation of pathogen growth but also signals a significant rearrangement in the genetics, the biochemistry, and the structure of the host cell(s). From the first stages of pathogen perception to the final activation of programmed cell death, host resources are allocated and redirected for the purpose of preventing infection, pathogen proliferation, and disease. It is estimated that during the process of disease resistance signaling, as many as 25% of the genes in a given cell display altered expression patterns following pathogen inoculation (5, 23, 138). At a biochemical level, studies have also shown induced changes in host protein localization, activation, and signaling, presumably, the function of which is to activate defense and resistance signaling (17, 93, 115). Thus, it is not surprising that the host cell has evolved a suite of sophisticated mechanisms to assist in the reallocation of resources to combat pathogen infection. For example, the endomembrane system has been implicated in the rapid delivery of host defense compounds, both at the early stages of infection as well as throughout the process of the resistance signaling (6, 21, 76, 77, 147). Unlike mammalian cells, plants must respond to biotic stress through what is often misunderstood as a static mechanism; the exception to this being the few defense processes that have been identified as being associated with vesicle trafficking. In recent years, studies on the dynamic movement of defense responses in plant cells have moved to the forefront, with an initial focus on the endocytosis of signal transducers following pathogen perception (3, 25, 43). However, unlike studies of mammalian innate immune signaling, few have been successful in defining the precise role of the central node in the dynamic organization of the host cell—the actin cytoskeleton. Responsible for processes ranging from development and reproduction to hormone signaling and organelle movement, the role of the actin cytoskeleton in plant defense signaling is an understudied component of the host-microbe interaction. In this review, we discuss our current understanding of the plant actin cytoskeleton, covering the genetic and biochemical regulation and recent advances in cell biology that will allow the analysis of host actin dynamics pre- and postpathogen infection.

INNATE IMMUNE SIGNALING IN PLANTS

Resistance signaling in response to pathogen infection requires the coordinated regulation of multiple nodes of the innate immune response (reviewed in 67). Often described as consisting of multiple layers, defense signaling in plants evokes a stepwise activation of inducible responses following pathogen perception and entry into host tissues. In the following section, we will briefly review three primary layers of resistance that must be overcome to not only promote pathogen virulence, but also represent the initial stages of the activation of innate immune signaling.

Structural Defenses

The architecture and basic structural features of plants present numerous hurdles that pathogens must overcome to gain entry (**Figure 1**). For example, the outer surface of the leaf is coated with a specialized cell wall known as the cuticle (100). Generated by a tightly interdigitated layer of epidermal cells, this waxy coating prevents dehydration and acts as a physical barrier inhibiting entry of harmful chemicals and microbes (139, 140). Epidermal cells are also responsible for the generation of trichomes, unicellular or multicellular structures whose formation and morphology are dependent upon the cytoskeleton (122). On the abaxial face of leaves, structures known as stomata—microscopic pores in the epidermal layer that function as points of gas and water exchange—dot the surface. Structurally, stomata comprise a pair of guard cells, which control the aperture of the stomate and respond to environmental and physiological stimuli. As

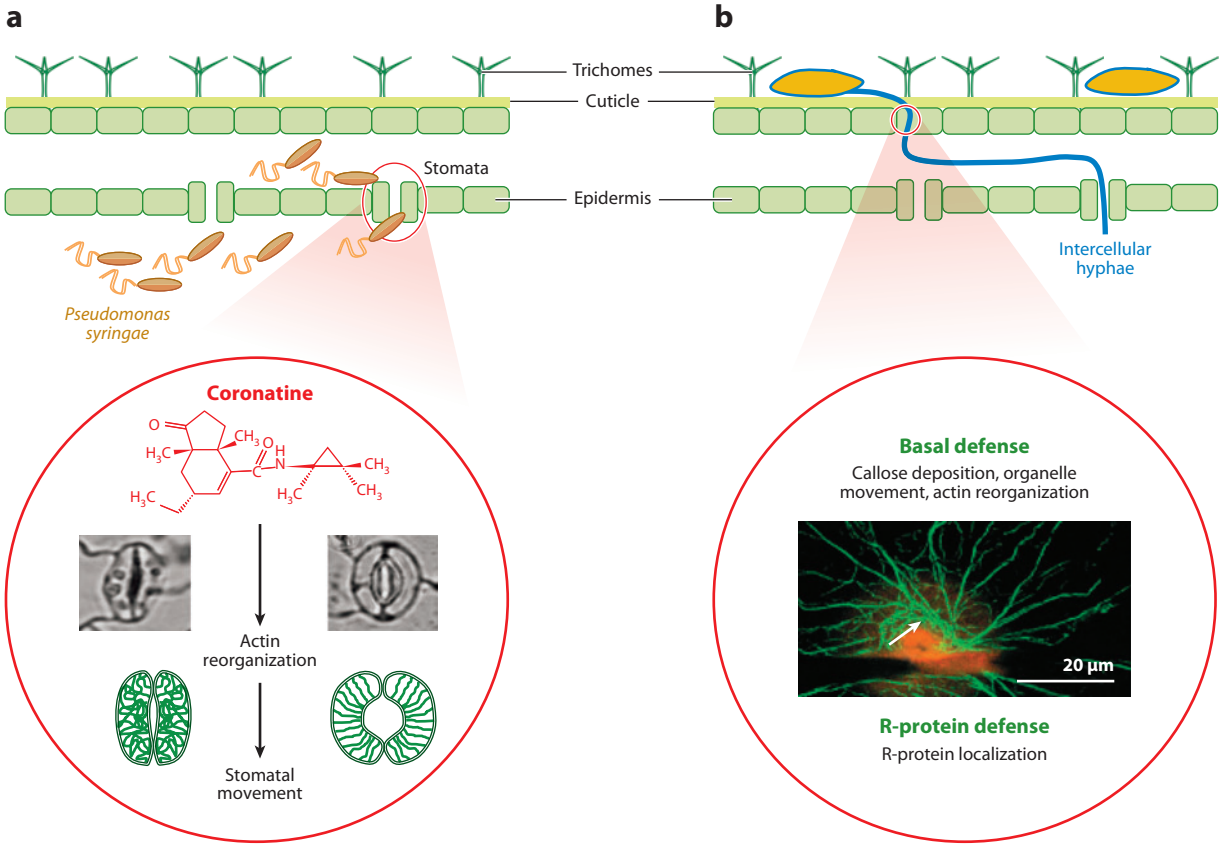

Figure 1

The plant actin cytoskeleton is associated with the activation of host resistance responses during pathogen infection. (*a*) A schematic cross section of a plant leaf, illustrating the structural barriers involved in defense: cuticle, upper and lower epidermis, trichomes, and stomata. In the case of *Pseudomonas syringae* pv. tomato infection, the pathogen-derived toxin coronatine stimulates the rapid opening of the plant stomate, facilitating pathogen entry. Inset illustrates the involvement of the actin cytoskeleton in guard cell shape changes during opening and closing. (*b*) A schematic cross section of leaf illustrating association with a fungal/oomycete pathogen. Inset illustrates several published examples of the host actin cytoskeleton-resistance association, including those in basal and resistance protein (R-protein)-mediated defenses and actin bundles focused at the site of fungal penetration [fluorescent image reproduced with permission from Opalski et al. (90)].

a mechanical feature regulating many of the processes associated with stomata function, the role of filamentous actin in regulating guard cell dynamics has been extensively investigated (79). Numerous studies have demonstrated that both internal (e.g., hormones, Ca^{2+}) and external (e.g., light, CO_2, bacterial pathogens) stimuli modify the actin cytoskeleton in guard cells and that these changes correlate with opening and closing. For example, a recent technical advance by Higaki et al. (46) demonstrates that the spatial orientation of actin filaments in guard cells of *Arabidopsis* changes dramatically and parallels the response to circadian rhythms. Previous work using pharmacological approaches demonstrated changes in actin filament organization during stomatal opening and closing (52). Specifically, using the actin inhibitor, cytochalasin D, it was demonstrated that actin filament disruption promotes light-induced stomatal opening (63). Conversely, latrunculin B promotes ABA-induced stomatal

PAMP: pathogen-associated molecular pattern

PTI: PAMP-triggered immunity

ETI: effector-triggered immunity

Pathogen effector: Secreted protein produced by pathogens whose function during the infection of hosts is to enhance the overall virulence capacity of the pathogen

closure (80), whereas phalloidin and jasplakinolide, which prevent actin depolymerization, inhibit closing (63, 80). In the recent study by Higaki and colleagues, it was found that not only do bundling and filament organization influence guard cell dynamics, but that the spatial arrangement of the filaments within the guard cells regulates aperture dynamics (46). In total, these studies suggest that filament disruption does not regulate stomatal opening, but rather the density and arrangement of the filaments into higher-order structures control guard cell shape changes.

Pathogen-Associated Molecular Pattern-Triggered Immunity

Once a pathogen has successfully navigated the physical barriers of the plant surface, the next challenge is to overcome one of the broadest and most ancient forms of resistance. As a basal defense process, PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI) (19) is a broad-based mechanism that is induced as a result of the perception of conserved PAMPs by their cognate pattern-recognition receptors (PRRs) (7, 26, 64, 126). Among the best-characterized PAMPs, chitin, flagellin, and lipopolysaccharide (LPS) have all been shown to elicit a highly effective level of defense responses (reviewed in 110). Following PAMP perception, amplification of the PTI response results in the activation of a mitogen-activated protein kinase (MAPK) signaling pathway, leading to an engagement of defense-signaling processes required for the abrogation of pathogen infection. As a conserved mechanism, with homologous processes present in both plants and animals, the activation of PTI requires perception, activation, and amplification to be fully effective (reviewed in 19, 67).

In plants, this involves perception of the PAMP by a cognate PRR, assembly and activation of the regulatory processes required for initiation of defense signaling, and finally, amplification of the signal and translation of this signal into the activation of resistance. In large part, many of these processes are regulated through the activity of the MAPK signaling cascade. MAPK signaling is a tightly regulated process that governs a host of developmental (73), reproductive (125), and stress processes (reviewed in 103). In mammalian cells, actin filament assembly is regulated by both p38 MAPK and phosphatidylinositol-3-kinase (PI-3-kinase) (60). In plants, a causal link has yet to be established between the activation of MAPK signaling and the regulation of the actin cytoskeleton; however, a number of studies have independently investigated functional and regulatory processes that potentially link MAPK signaling and cortical actin dynamics (44, 104, 105, 107).

Signaling cascades are often downregulated by removing the receptor and its ligand from the cell surface via receptor-mediated endocytosis. In yeasts and mammals, there is strong evidence that this requires coordinated actin assembly and disassembly at the plasma membrane (98). Indeed, evidence for plant PAMP receptors recycling by endocytosis that is actin-dependent has been obtained for the FLS2 receptor in *Arabidopsis* (102). Recent data also support the endocytic uptake of XA21 in rice, although this was not shown to be actin- or ligand-dependent (16). A related theme is that actin-mediated endocytosis of receptors is sometimes necessary for signaling from intracellular compartments. To our knowledge, this mechanism and its relationship to defense signaling have not been investigated in plants.

Effector-Triggered Immunity

As a specialized biochemical and genetic mode of resistance signaling against pathogen infection, effector-triggered immunity (ETI) (19) is often described as a countermeasure to pathogen suppression of PTI. In short, ETI relies on the recognition of pathogen-specific effector molecules or their activities to promote virulence. Regardless of the mechanism of delivery into their host and, moreover, independent of whether these are pathogens of plants or animals, pathogen effector molecules all share the common function of promoting

virulence through the manipulation of host processes specifically aimed at defending against pathogen infection, proliferation, and the elicitation of disease.

The concept of ETI was born out of the classical gene-for-gene hypothesis, which genetically describes the recognition of pathogens by plants through the interaction of pathogen-derived avirulence (*Avr*) genes and host-borne resistance (*R*) genes (30). Over the past several decades, our understanding of this resistance mechanism has evolved to encompass a suite of processes associated not only with resistance signaling, but also with basal plant processes such as development (95), transcription (58), and rearrangement of the actin cytoskeleton (129). Therefore, gene-for-gene resistance has morphed into what is now commonly referred to as the guard hypothesis (reviewed in 19). The guard hypothesis offers a broad view of defense signaling, illustrating the on-guard nature of the defense response. In short, plant defenses are not solely governed, nor regulated by, the transcriptional activation of resistance processes, but rather, abate these responses through the inactivation of key signaling processes. Numerous examples of the negative regulation of defense signaling in plants can be found throughout the literature (19, 24, 31, 55, 149).

At a more detailed level, the guard hypothesis aims to explain how multiple physiological responses in plants are hard wired into the signaling mechanisms that control not only resistance but also numerous physiological processes targeted by pathogens for virulence. For example, as described above, plants regulate the spatial orientation and organization of the actin cytoskeleton to regulate the opening and closing of stomata (45). By inference, it is likely that pathogens have evolved mechanisms to manipulate this organization to gain entry into the host plant. In support of this, it has been recently shown that the phytopathogen *Pseudomonas syringae* regulates stomatal aperture through the action of the toxin coronatine (**Figure 1*a***) (83). Through the manipulation of guard cell dynamics, it is feasible that the pathogen also impacts, either directly or indirectly, a multitude of processes, such as hormone signaling and osmotic responses to the environment, all of which are hard wired to the actin cytoskeleton.

F-actin: filamentous actin

G-actin: globular or monomeric actin

THE ACTIN CYTOSKELETON

A complex and extensive actin cytoskeleton underpins a plethora of important cellular functions, including cell division and development (13, 27), cell pattern and positioning (66, 108), vesicle and organelle movements (74, 99), and signaling (106), as well as responses to biotic (40, 41, 90, 116) and abiotic stresses (56). This network of 5 to 7 nm diameter filaments (F-actin), as well as higher-order structures termed actin filament bundles or cables, is generated from a cytoplasmic pool of monomers or G-actin. In *Arabidopsis*, the concentration of G-actin is estimated to be greater than 50 μM (120, 121). Interestingly, less than 5% of a plant cell's G-actin pool is polymerized into filamentous actin (36, 118). This contrasts with a typical animal cell, where approximately 50% of total actin is in the monomer pool, and budding yeast, which has greater than 90% of total actin in polymeric form (65, 96). This huge pool of unpolymerized actin in plant cells reflects a potential for explosive polymerization because filament elongation rates are proportional to monomer concentration (96, 119, 120).

Monomeric actin polymerizes into filaments with a characteristic architecture and polarity (34, 88); polarity is conferred by the endogenous asymmetry of subunits that assemble in a head-to-tail fashion generating two intertwined, helical strands. Polarity is also dictated by a lag between assembly, nucleotide hydrolysis, and P_i release from subunits along the filament. The barbed or plus end of filaments is the preferred site for ATP-G-actin addition, whereas the pointed or minus end is the preferred site for loss of ADP-G-actin. The rate-limiting step for polymerization is the creation of trimeric actin seeds, or nucleation. Once seeds are formed, elongation proceeds rapidly and depends on the rate constant for

ABP: actin-binding protein

ADF: actin-depolymerizing factor

TIRFM: total internal reflection fluorescence microscopy

assembly and the G-actin concentration. At equilibrium in the test tube, the steady-state level of F-actin remains constant, but subunits treadmill through the filament by polymerizing at one end and depolymerizing at the opposite end. Although treadmilling has never been observed directly in vivo, it is a commonly held assumption that actin turnover within cells occurs by some variation of this behavior (97, 98). For plant cells, actin rearrangements are assumed to require precise control over actin turnover, but until recently the mechanisms were not well understood.

Regulation of Organization and Turnover by Actin-Binding Proteins

Because of the need to polymerize new actin filaments and to create unique structures in response to a multitude of intrinsic and extrinsic cues, it is not surprising that cells have greater than 100 actin-binding proteins (ABPs) that control actin polymerization and the generation of higher-order structures (97, 98). Elongation of actin filaments is regulated by the number and availability of filament plus ends, the size and activity of the monomer pool, and the nucleotide-loaded state of G-actin. In plants, the large G-actin pool is likely bound by profilin, adenylate cyclase-associated protein (CAP), and actin-depolymerizing factor (ADF) (15, 51).

Profilin is a small globular protein that forms a 1:1 complex with G-actin and is present in equimolar amounts with total actin in plant cells (15, 36, 136). Profilin suppresses spontaneous nucleation of actin, and the profilin-actin complex does not add onto filament minus ends (85). Therefore, when plus ends are capped, profilin is a sequestering protein that prevents assembly. When plus ends are uncapped, the profilin-actin complex shuttles actin onto filaments at rates similar to actin alone. Unlike orthologs from other organisms, plant profilin does not catalyze nucleotide exchange on actin and therefore cannot recharge the G-actin pool with ATP (71). In plants, this role is assumed by another monomer-binding protein, CAP (15, 27). The CAP-actin complex can add weakly onto uncapped plus ends, but probably passes recharged G-actin to profilin for assembly. Finally, ADF recycles actin monomers by severing and creating new filament ends (4, 61). ADF also has a preference for ADP-G-actin and may facilitate subunit loss from pointed ends (14, 101).

Actin dynamics are governed largely by the creation and availability of filament ends. The best-characterized filament end–binding protein in plants is heterodimeric capping protein (CP), which binds to plus ends with high affinity and dissociates slowly (48). CP is present at approximately 1:100 stoichiometry with total actin in plant cells (Jiménez López & Staiger, unpublished data); thus, we can assume that most filament ends in cells are capped. This hypothesis further predicts that new filaments would have to be generated by severing activity, nucleation, or uncapping. In plants, both villin/gelsolin and ADF isovariants are capable of severing or fragmenting filaments in vitro (61, 148).

Plant formins can use the pool of profilin-actin to nucleate filament formation and generate growing ends in vitro (48, 53, 85, 119, 142, 143). Whether formins, profilins, and CP coordinate filament dynamics according to their biochemical features awaits direct experimental evidence in living cells. However, a recent publication on a pollen formin (*At*FH5) provides compelling evidence for filament nucleation and generation of the subapical actin array in live pollen tubes (18).

ABPs also coordinate the generation and turnover of higher-order filament structures, such as cross-linked networks, filament bundles, and cables. In plants, fimbrins, villins, tandem LIM-domain containing proteins, and formins have all been shown to bundle actin filaments (11, 45, 91, 128). Time-lapse total internal reflection fluorescence microscopy (TIRFM) has been used to uncover the molecular mechanism of bundle formation in vitro (11). The *Arabidopsis* formin AFH1 nucleates new filaments from the side of preexisting filaments or bundles and zippers them together

into parallel and antiparallel bundles (84). Similarly, *Arabidopsis* VILLIN3 present on one filament captures a nearby filament and facilitates the zippering together of neighboring filaments (61). This catch and zipper mechanism has recently been observed in the cortical actin array of living epidermal cells of *Arabidopsis* and cells of the liverwort *Marchantia polymorpha* (29, 61). Several of the bundling proteins also appear to dampen dynamics by stabilizing filaments against depolymerization or by inhibiting the activity of ADF (49, 127, 128). For example, VILLIN1, a simple bundling protein of the villin/gelsolin family, interferes with ADF-mediated severing of individual filaments and prevents bundle disassembly (61).

Stochastic Dynamics of Single Actin Filaments in vivo

To understand at a molecular level how the actin cytoskeleton operates in cells, it is essential to have high-spatial and -temporal resolution images of individual actin filaments and their turnover. Recently, the textbook model for actin turnover by treadmilling has been challenged with direct observations of single actin filaments in living cells. Several groups have used state-of-the-art imaging methods and a new generation of fluorescent fusion protein reporters to visualize cytoskeletal dynamics in model genetic organisms: Pollard and colleagues used spinning-disk confocal microscopy on fission yeast cells undergoing cytokinesis (131), and Staiger and colleagues used variable-angle epifluorescence microscopy (VAEM) (70) to visualize dynamics of the cortical cytoskeleton in epidermal cells from dark-grown *Arabidopsis* hypocotyls (121). Rather than treadmilling, both groups provide evidence that single actin filaments or small filament bundles show a property termed stochastic dynamics.

VAEM: variable-angle epifluorescence microscopy

In hypocotyl epidermal cells expressing the GFP-fABD2 reporter, actin filaments are arrayed in two overlapping populations (62, 121) (**Figure 2**). A collection of large, mostly axially-oriented actin filament cables is brighter, longer, less wavy, and longer-lived than the randomly oriented population of individual filaments. The former are also less sensitive to short treatments with low doses of latrunculin B, an agent that targets dynamic actin filaments

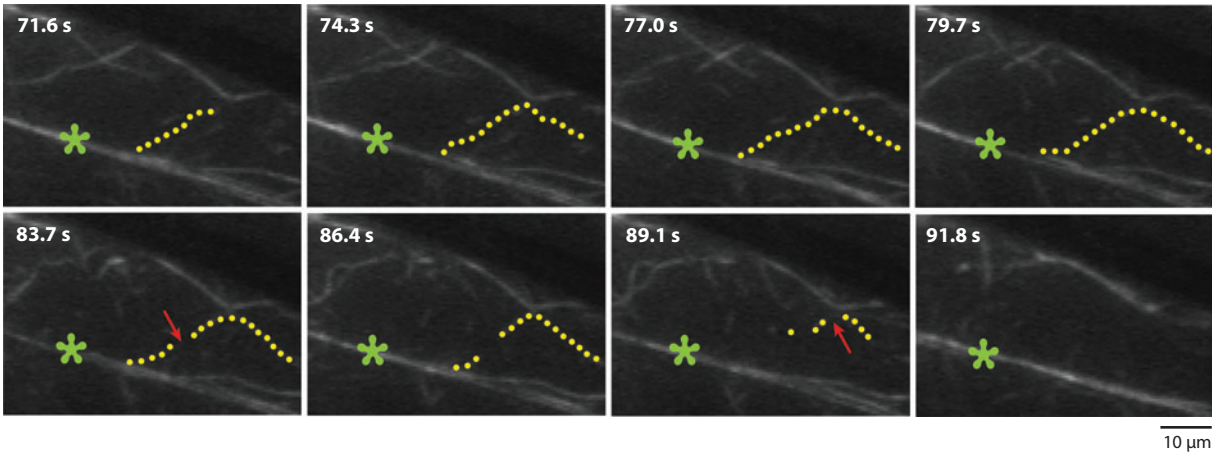

Figure 2

Time-lapse variable-angle epifluorescence microscopy (VAEM) allows real-time visualization of actin filament dynamics. VAEM was used to image the cortical actin cytoskeleton of etiolated *Arabidopsis thaliana* hypocotyl epidermal cells expressing GFP-fABD2 (121). Two populations of filaments exist in the cortical array, actin bundles and single actin filaments. A representative actin bundle remains stiff and bright throughout the time-lapse series (*green asterisk*), whereas a fainter single actin filament (*yellow dots*) grows and is then fragmented by prolific severing activity (individual breakage events shown by *red arrows*). This epidermal cell was imaged at approximately 1.5 s intervals for 20.2 s.

A MODEL FOR THE REGULATION OF ACTIN TURNOVER

The majority of actin in plant cells is present in a large pool of monomers bound to profilin (**Figure 3**). Profilin inhibits nucleation of filaments and prevents growth at filament minus ends ①. New filaments with available plus ends are generated by nucleation factors like formin or the Arp2/3 complex ②. Other filaments will grow from the end of fragments or from severed filaments with free plus ends ③. Once plus ends are created or made available by uncapping, filaments will elongate at rates proportional to the size of the monomer pool ③. Assembly rates can be further enhanced by processive formins, which use profilin-actin to elongate filaments while remaining attached to the plus end ②. Alternatively, a bursting mechanism, whereby filament fragments anneal onto existing uncapped ends, could also contribute to rapid elongation ④. Filament growth is terminated by capping proteins ⑤. Disassembly of filaments is mediated mainly by severing activity ⑤⑥, followed by capping and slow depolymerization ⑦. Monomers are recycled from capped fragments by the action of ADF in cooperation with CAP, which recharges actin subunits before passing them along to profilin ⑧. Single filaments also can be bundled and stabilized in higher-order structures ⑩ and this prevents their severing and disassembly by ADF ⑨.

by binding to monomeric actin and preventing its polymerization (36). In *Arabidopsis*, the single actin filaments are less bright, shorter, and more convoluted than filament bundles (121). Several parameters of single filament turnover were analyzed quantitatively to further reveal the mechanism of turnover. The growth of individual filaments is extremely rapid, nearly 2 $\mu m\ s^{-1}$, allowing filaments to span the width of a cell in about 10 s (**Figure 2**). Most filaments are short-lived (<30 s); their disappearance is not through loss of subunits from a filament end, as in the treadmilling model, but by prolific severing activity (**Figure 2**). Fragmentation of a typical filament with a length of 10 μm occurs at roughly 6 breaks min^{-1} (121). Newly created filament ends appear to be capped and therefore prevented from growing simultaneously with, or soon after, severing events. Further, new filaments are observed to originate de novo in the cytoplasm or from the side of preexisting filaments or bundles. These dynamic behaviors contribute to constant rearrangement in the cortical array and generate a randomly oriented network of filaments. A recent study shows that this overall behavior and actin dynamics are similar in various cell types, but the rates of assembly and severing are variable (117). Moreover, it was reported that growth might occur by a bursting mechanism or fragment annealing at filament ends (117), similar to that observed in yeast (89).

Based on the known biochemical features of the best-characterized ABPs from plants (reviewed above and in 11, 51, 119, 120, 128), we can propose a model for the regulation of stochastic dynamics and actin organization in plant cells (see sidebar, A Model for the Regulation of Actin Turnover and **Figure 3**). Features of this model are clearly testable using the power of reverse genetics in *Arabidopsis thaliana*. The rationale that underpins the development of the stochastic dynamic model is further elaborated in two recent review articles (11, 120).

Although drug studies and reverse genetics implicate the actin cytoskeleton in cell expansion (8, 33), the underlying mechanism is not very clear. Simple models suggest that actin filaments control the trafficking of secretory vesicles that deliver new wall materials during diffuse growth, similar to the well-established principles of tip growth in pollen tubes (18, 47). However, secretory vesicle trafficking during diffuse growth has never been observed directly, and it is difficult to reconcile the extremely dynamic and short-lived nature of individual filaments acting as tracks for vesicle movements or delivery at the plasma membrane. Moreover, the dynamic behavior of growth and disassembly is not quantitatively different in expanding versus nonexpanding hypocotyl epidermal cells (121). Nevertheless, filament density, the rate of change in convolutedness (a measure of filament waving) and the extent of bundling do indeed change during cessation of cell elongation (121; Henty et al., unpublished data), suggesting that overall actin organization rather than dynamics may play a role in secretion. Gutierrez et al. (38) propose that the motility

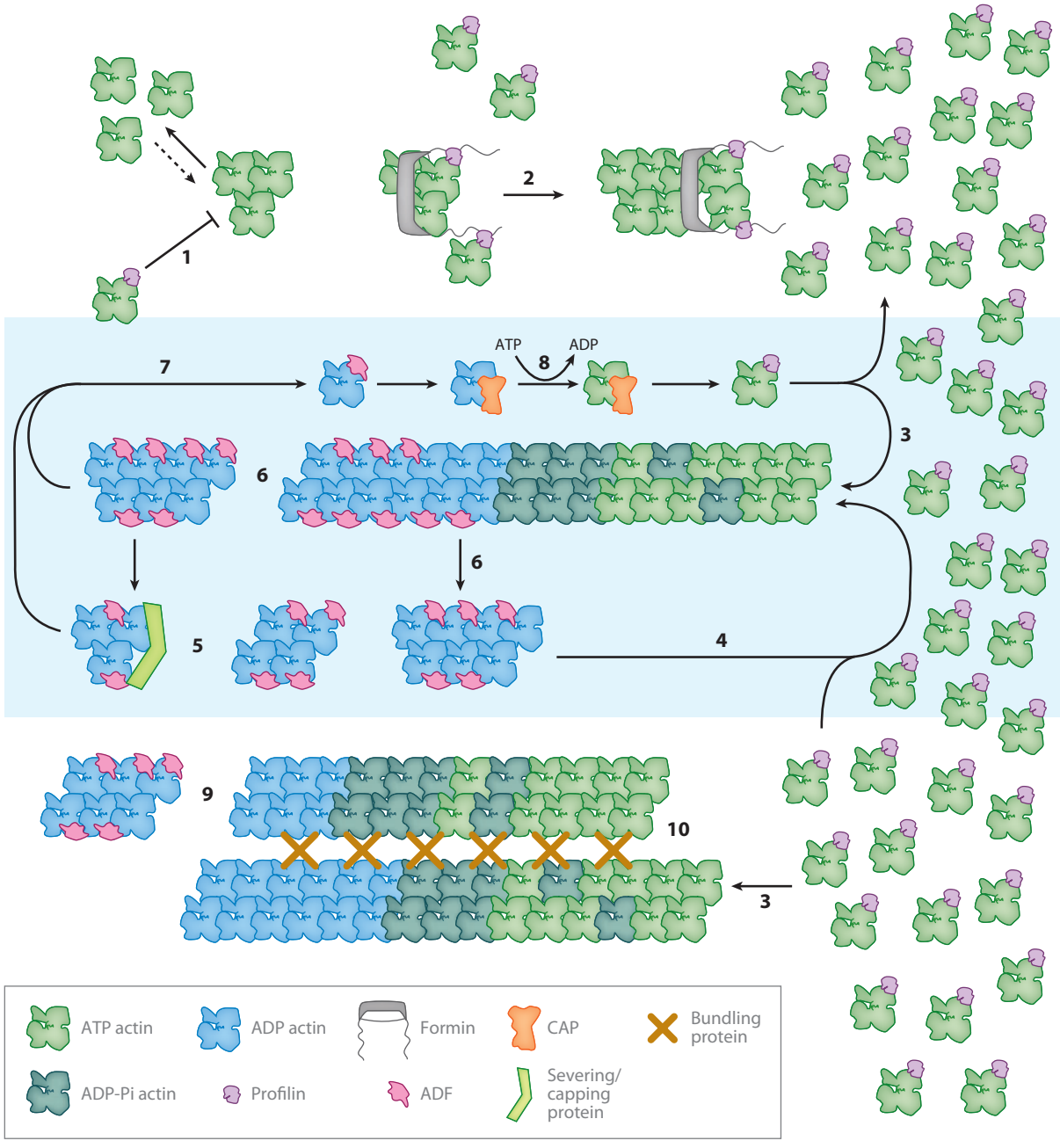

Figure 3

A model for the regulation of actin organization and turnover in plant cells. This figure displays a model for the coordination of actin dynamics and cytoskeletal organization in plant cells. The model is based on cellular abundance and biochemical properties of actin and the major actin-binding proteins. The blue box denotes the main reactions thought to be involved in the regulation of actin turnover by stochastic dynamics (11, 120). See sidebar, A Model for the Regulation of Actin Turnover for details.

and distribution of Golgi along actin filament cables in the cortical cytoplasm indirectly regulate the global distribution of microtubule-tethered, CesA-containing compartments that contribute to cellulose deposition. In such a model, single actin filaments could provide a constant source of building blocks for constructing and maintaining the actin filament cables that support organelle movements. An attractive alternative, but not mutually exclusive, model for the importance of single filament stochastic dynamics is that it represents a surveillance mechanism for responding to biotic and abiotic stimuli (121). In this model, constant turnover and rearrangement of single actin filaments would provide a pool of polymers to construct novel arrays at a moment's notice. It is easy to envision that changes to a single parameter (e.g., cessation or downregulation of severing activity) could lead to the stabilization of actin filaments and construction of a new filament array in response to locally altered conditions. In this regard, the observation that physical pressure applied to the cell wall of *Arabidopsis* epidermal cells, using a microneedle, results in rapid rearrangement of actin filament arrays is highly compelling evidence for this surveillance mechanism (41). The parallels with attempted penetration by fungi and oomycetes are considered further below.

PLANT INNATE IMMUNITY AND THE ACTIN CYTOSKELETON

As described above, the activation and regulation of innate immune signaling in plants requires the coordinated efforts of multiple nodes of resistance signaling. As a platform for defense-associated processes in plants, the actin cytoskeleton has demonstrable roles in many of these processes (reviewed in 123). For example, numerous fungal and oomycete species have an increased ability to invade plant tissues when the actin cytoskeleton is disrupted with cytochalasins, upon overexpression of ADF proteins, or through shutting down depolymerization activity (41, 54, 68, 86, 124). Cytologically, static images show that actin bundles focus on the site of nascent attack or penetration (**Figure 1*b***) (41, 90, 124), providing further evidence for the role of the actin cytoskeleton as a platform for defense signaling. In addition to a role in host-pathogen interactions, actin rearrangements have also been shown to occur during symbiotic interactions between plant cells and invading bacterial symbionts (144). In the following section, we summarize our current understanding of the role of the actin cytoskeleton as a platform for defense signaling.

Bacterial Pathogens of Mammalian Cells and the Host Actin Cytoskeleton

The role of the actin cytoskeleton in defense signaling has been best described through the characterization of effector proteins from bacterial pathogens and their action on mammalian host cells. Collectively, this work has defined not only the biochemical activity of these secreted molecules, but more precisely, has identified the host processes (and proteins) that are manipulated throughout the infection process. In this regard, the interaction between *Yersinia pestis* and macrophages has emerged as a model for characterizing the role of the actin cytoskeleton in innate immune signaling.

Regulating Actin Dynamics During Pathogen Invasion

Eukaryotic cells use a multitude of sophisticated mechanisms to actively generate or rearrange cytoskeletal arrays, and pathogens have found equally clever ways to hijack or usurp these pathways for their own purposes. For example, nucleation of new actin filament arrays at precise locations in mammalian cells powers cell crawling, protrusion of filopodia, and phagocytic uptake of bacteria and large objects, as well as vesicle motility. Actin nucleation by the Arp2/3 complex is enhanced by members of the WASP/Scar family of proteins, which serve as nucleation promoting factors (NPFs). Binding of an NPF to the Arp2/3-actin complex results in a conformational change that

allows the complex to mimic an actin seed. *Listeria* and *Shigella*, for example, express an NPF or a binding protein for host-cell NPF to recruit the polymerization machinery to the bacterial cell surface. This facilitates generation of an actin comet tail that the bacteria use for intracellular motility (98). As an additional regulatory mechanism, Rac, a monomeric Rho-GTPase, initiates a signaling cascade, which ultimately results in the phosphorylation, and thus deactivation, of ADF/cofilin. In short, the activation of several monomeric GTPases, belonging to the Rho-GTPase family, controls both actin nucleation and deactivation of actin depolymerization by the regulation of specific ABPs, such as ADFs, WASP/Scar, and formins (1, 94). Mechanistically, the catalytic domain of Rho-GTPases functions to hydrolyze the guanine nucleotide GTP to GDP. Rho-GTPases associate with downstream proteins via the switch I region when bound to GTP (133). Following nucleotide hydrolysis, the release of GDP from Rho-GTPases is a slow process but can be sped up by guanine-nucleotide-exchange factors (GEFs). Rho-GTPase can be sequestered in the GDP bound form by guanine-nucleotide-dissociation inhibitors (GDIs). Conversely, GTPase-activating proteins (GAPs) catalyze the hydrolysis of GTP to GDP, thus preventing GTPase association with downstream proteins.

In plant defense signaling, the role of Rho-GTPases has been best characterized from studies investigating the activation of actin cytoskeletal dynamics, as well as in defense signaling during host-fungal interactions (reviewed in 12). As described above, the actin cytoskeleton is modified through the activation of a coordinated network involving Rho-GTPase family members and their respective effector proteins. As ubiquitous regulators of multiple processes in plants, our understanding of the role of Rho and ROP (Rho-GTPases of plants) (141) signaling centers around the regulation of processes associated with development and cellular morphogenesis, and extends into the cellular responses activated following biotic and abiotic stress (141).

Shutting Down Actin Dynamics

Human bacterial pathogens have evolved mechanisms to mimic the activity of actin remodeling enzymes and thereby subvert actin dynamics of host cells in order to increase virulence. The outcomes of pathogen-induced manipulation of the host cytoskeleton vary and include inhibition of phagocytosis (113), induction of internalization by nonphagocytic cells (81), hijacking of actin polymerization for bacterial motility (37), and alteration of actin homeostasis (78). In mammalian cells, the bacterium *Yersinia pestis* inhibits phagocytosis by macrophages via the deployment of four outer proteins, or Yops: YopH, YpkA, YopE, and YopT. With the exception of YopH, which targets components of the focal adhesion complex (28), *Yersinia* effectors are known to interact with the host GTPases RhoA, Rac, and Cdc42 (82, 132). YpkA mimics host Rho-GDI and inhibits nucleotide exchange. YopE has a domain with Rho-GAP activity, rendering Rho-GTPases inactive by facilitating hydrolysis of GTP, whereas the cysteine protease YopT specifically targets prenylated Rho-GTPases, cleaving and releasing them from the membrane. Once freed from the membrane, Rho-GTPases can no longer affect actin remodeling (114).

Promoting Actin Assembly and Pathogen Motility

In contrast to shutting down actin remodeling and the dynamic activation of actin-based defense responses, other bacterial pathogens have also evolved mechanisms to usurp actin dynamics in order to enhance virulence and facilitate infection of the host. One such example is *Salmonella* spp., which utilize a suite of type III secretion system (T3SS) effectors to alter the cytoskeleton for the purpose of invading intestinal epithelial cells (35). Through the action of a specialized class of effector proteins, the SPI-1 class (e.g., SipA, SipC, SopB, SopD, SopE, and SopE2), *Salmonella* induces a process known as membrane ruffling, allowing the

Type III secretion system (T3SS): Encompasses the structural apparatus through which bacterial effector proteins are secreted from the pathogen into the host. As a tightly regulated operon in the pathogen, the T3SS encodes both the machinery required to deliver pathogen effectors into the host during infection, as well as the pathogen effector molecules themselves

bacterium entry into the epithelial cell and ultimately the generation of a *Salmonella*-containing vacuole (SCV) (81). To accomplish this, secretion of the effector protein SipC initiates host-cell actin remodeling via its activities as an actin nucleation factor (42) and an actin bundling protein (87). In parallel, a second T3SS effector, SipA, enhances actin filament formation and stability by blocking ADF/cofilin binding to F-actin (81). ADF/cofilin activity and cycling between phosphorylated (inactive) and dephosphorylated (active) forms are necessary for efficient *Salmonella* entry into host cells (22). In plants, the role of ADFs in defense signaling was recently identified (129). Tian and colleagues demonstrate a role for *Arabidopsis* ADF4 as a key regulator in the host defense response to *P. syringae* expressing the cysteine protease effector AvrPphB. This work is described in further detail below.

As a second strategy to alter actin-based host-cell processes for the benefit of infection and disease, *Salmonella* spp. have evolved additional mechanisms to alter actin dynamics. Two effectors, SopE and SopE2, modulate actin dynamics by mimicking GEFs (92). SopE specifically targets host-cell Rac-1 and Cdc42, whereas SopE2 targets Cdc42. Once *Salmonella* has successfully entered the host cell, another effector, SptP, acts as a GAP in order to return the cytoskeleton to its normal state.

As mentioned above, *Listeria* expresses a cell surface–associated protein, ActA, to activate the Arp2/3 complex. This allows the pathogen to escape macrophage vacuoles, once phagocytosed, through rapid polymerization of actin filaments (145, 146). To accomplish this, the ActA effector relies on the function of two domains similar to the WASP homology 2 (WH2) domain that enable the binding of two actin monomers with high affinity. ActA binds to Arp2/3 in a similar fashion to WASP. However, unlike WASP, ActA activity does not require Rho-GTPases, yet instead, utilizes a proline-rich motif (PRM) that directly binds host-cell vasodilator-stimulated phosphoprotein (VASP), which in turn recruits profilin to the actin-nucleating complex (37). This machinery allows the polymerization and constant turnover of an actin filament comet tail that propels the bacterium through the host cytoplasm and perhaps facilitates escape from the host and infection of neighboring cells.

Shigella spp. also utilize an effector, IcsA, to promote actin-based comet tail motility; however, the mechanism by which IcsA operates is different from that described for ActA (37). IcsA does not mimic host-cell WASP family proteins, but instead recruits N-WASP to the surface of *Shigella*. In order to overcome the autoinhibition of N-WASP, *Shigella* recruits host Toca-1 (transducer of Cdc42-dependent actin assembly-1) via an unknown T3SS effector (75).

As illustrated above, invading pathogens have evolved mechanisms to not only modify actin cytoskeletal dynamics, but also to mimic the endogenous function of host ABPs for the purpose of host invasion for intracellular and intercellular motility. As another exciting example of this mechanism, a recent report on *Rickettsia* comet tail motility has identified a new mechanism for host-derived pathogen motility (39). Previously, it was thought that *Rickettsia* motility was driven by the bacterial effector RickA, which in concert with the host Arp2/3 complex, nucleates actin. This nucleation event, in turn, propels the pathogen within the host cell. In a recent study, it was determined that the bacterial protein Sca2 alone is sufficient for motility; however, this is accomplished through mimicking the function of eukaryotic formins, rather than the Arp2/3 complex, to effect polymerization of actin (39). Several other pathogenic bacteria generate actin filament arrays that are used for motility and infection, but use completely different strategies. For example, the *Vibrio* effector VopL assembles unbranched actin stress fibers in the absence of Arp2/3 (78). This unbranched actin formation is similar to that of formins and SPIRE, which, unlike Arp2/3, do not form branched actin filaments along the sides of existing actin filaments. VopL contains three WH2 domains and three PRMs that resemble domains found in formins, known to bind both profilin and actin-bound profilin. The

formation of unbranched actin stress fiber disrupts the homeostasis of cellular actin and leads to perturbation of epithelial cells in the intestines, resulting in an enterotoxic effect.

Basal Defense and the Actin Cytoskeleton

PTI responses appear to be highly conserved, both in terms of activation and with respect to regulation, regardless of the perceived PAMP. For example, perception of the bacterial flagellin (i.e., flg22) (150) results in the activation of numerous cellular processes, including MAPK signaling, the induction of gene expression often associated with resistance, and the enhancement of systemic resistance signaling (112). Similar signaling has also been described in the case of chitin perception (reviewed in 134), as well as for the bacterial PAMP, elongation factor-Tu (EF-Tu) (72). The sum of these responses is sufficient to defend against bacterial, fungal, and oomycete pathogen infection and elicitation of disease. Of the basal defense responses associated with the onset of resistance, the deposition of defense compounds at the site of pathogen penetration/infection is one of the best characterized. Callose deposition results in the deposition of materials to the cell wall via vesicular trafficking; this also likely requires the function of the host actin cytoskeleton (10, 69). In total, these responses logically will require the activation (or inactivation) of several cytoskeletal regulators, such as Rop GTPase and profilin, both of which are recruited to sites of infection (109). Generally, actin rearrangements are thought to be indirect evidence for vectorial delivery of secretory vesicles; however, F-actin accumulation might also locally stabilize or activate defense machinery, recruit organelles to the plasma membrane, or facilitate wall deposition (40). One example of actin-independent mechanisms functioning in the delivery of defense components is that of PEN1, which still accumulates at the penetration site in the presence of cytochalasin E (130). In contrast, the ABC transporter, PEN3, requires an intact actin cytoskeleton to target sites of fungal attack (130). How exactly the cytoskeletal network responds to biotic stresses remains a mystery, but insight can be gained by characterizing the precise changes to actin dynamics during host-pathogen interactions. Moreover, through a better understanding of the regulatory processes required for the activation and deactivation of actin dynamics during pathogen invasion, insight into the manipulation of the host cytoskeleton by invading pathogens will be achieved.

R-protein: resistance protein

In Defense of Fungal and Oomycete Pathogens

The penetration and infection of plant tissues by obligate fungi and oomycetes is best illustrated as a two-step process, involving both pre- and postinvasive events (76). At the preinvasive stages of infection, pathogens must overcome the physical barriers to penetration (described above), including callose deposition. In addition, the activation of host exocytosis, which functions in large part in the delivery of antimicrobial compounds to the site of pathogen penetration, is also an integral part of abrogating the initial stages of infection. Once penetration has been achieved, the pathogen and host are next engaged in the postinvasive activation of virulence and resistance, often relying on the modulation of cellular processes through the activity of ETI, or in favor of the pathogen effector-triggered susceptibility (ETS). One of the many questions that remains unanswered in describing the activation of ETI is how resistance proteins (R-proteins) transduce defense signaling across a cell and, moreover, if R-protein relocalization is part of this process.

Plant Pathogen Effectors and the Actin Cytoskeleton

ETI occurs when a plant R-protein perceives the presence or action of a corresponding pathogen effector protein. With numerous similarities to PTI, the consequence of the R-protein-effector interaction is an enhanced PTI-like response, often typified by localized

cell death or the elicitation of the hypersensitive response (HR). In demonstrating the link between the action of the plant actin cytoskeleton and the elicitation of the HR, Wang et al. (135) first described the dynamic localization of a plant R-protein (i.e., RPW8.2) to the site of infection through the use of the host actin cytoskeleton (further detailed below). As noted above, actin organization and dynamics are controlled at many levels, both by ABP-actin interactions and by ABPs sensing the cellular environment. Moreover, the actin cytoskeleton is implicated in vesicle trafficking, organelle movements, cell wall deposition, and other processes that are likely to be altered during PTI. Thus, it is no surprise that plants have adapted the actin network as a platform for defense signaling (e.g., 40). As has been demonstrated for the case of mammalian pathogens, it is likely that plant pathogens have also evolved mechanisms to alter the dynamic reorganization of the actin cytoskeleton for the benefit of disrupting resistance signaling.

In a step toward defining the link between phytopathogenic bacteria and actin-dependent resistance in plants, Tian et al. (129) demonstrated for the first time that the normal function of the actin cytoskeleton is required for ETI by the R-protein RPS5 (RESISTANCE TO *PSEUDOMONAS SYRINGAE*-5). As one of the best-characterized R-Avr interactions, the activation of resistance through RPS5 requires the perception of the action of RPS5's cognate pathogen effector molecule, AvrPphB (137). AvrPphB is a cysteine protease delivered via the T3SS of *P. syringae*, and has been characterized for its ability to cleave the protein kinase PBS1 (114), leading to the activation of resistance signaling following pathogen infection in *Arabidopsis*. Interestingly, AvrPphB shares homology with YopT (described above), the cysteine protease from *Yersinia*, whose activity is responsible for shutting down actin cytoskeletal function. To link the function of AvrPphB, the activation of resistance via RPS5 and the modulation of actin cytoskeletal dynamics, Tian et al. (129) utilized a combination of genetics, biochemistry, and cell biology approaches to identify components of the actin machinery associated with resistance signaling in *Arabidopsis* following infection with the phytopathogen *P. syringae*. ADF4 was identified as an integral component of the RPS5-AvrPphB signaling cascade, thus establishing the actin cytoskeleton as a signaling platform for bacterial disease resistance signaling in plants. Further analysis of the molecular and biochemical mechanism(s) linking the function of the actin cytoskeleton and the action of AvrPphB will significantly advance our understanding of the pathogen-actin connection in plants. Moreover, with approximately 30 pathogen effector proteins delivered during *P. syringae* pv. tomato DC3000 infection of *Arabidopsis*, an opportunity exists to not only define the role of these virulence components in defense signaling, but to unravel the interplay between pathogen infection and the dynamic rearrangement of the host cytoskeleton.

Nematodes and Actin-Based Defenses

As described above, the actin cytoskeleton has been linked to the activation of defense signaling in plants; first through pharmacological approaches (86), and more recently, using a combination of genetic and biochemical experiments (129). This is a necessary component of responding to potential pathogens regardless of whether they are trying to gain entry into the cell, as in the case of fungal and oomycete species, or whether they colonize intercellular spaces and never invade the plant cell, as in the case of bacterial phytopathogens. Moreover, actin rearrangements occur in symbiotic microbe-plant interactions, suggesting that this is a universal response to potential invasion or colonization of plant tissues. Altogether, these studies demonstrate that actin turnover in plants is a tightly regulated and integral process in the activation of defense signaling and, moreover, is likely to be one of the consequences of PTI.

A recent study by Clément et al. (20) demonstrated that depolymerization of the actin cytoskeleton is essential for root-knot nematode infection of *Arabidopsis*. This study adds to the

growing evidence that a broad range of plant pathogens manipulates plant cytoskeletal dynamics for the benefit of altering cell structure to promote infection. Using gene expression analyses of *Meloidogyne incognita*–infected *Arabidopsis*, Clément and colleagues found that *ADF2* was specifically and differentially expressed between 14 and 21 days after infection. Further investigation into the possible role of *ADF2* in this interaction revealed that downregulation of *ADF2* transcripts in *Arabidopsis* tissues inhibited nematode feeding, likely through processes related to destabilization of the cortical actin network. These data are consistent, in principle, with previous studies (e.g., 68) that showed that regulation (i.e., promoting) of the depolymerizing activity in plants stimulates enhanced infectivity.

Actin-based Trafficking During Defense

As a structural component of the cellular architecture, the actin cytoskeleton is responsible for the organization of the cell during periods of growth, development, and responses to the environment. This includes, for example, the polar arrangement and distribution of individual vesicles and organelles during tip growth of root hairs and pollen tubes (32) as well as the focused trafficking of related cellular components to the site of pathogen penetration (135). Furthermore, depending upon the nature of the response (i.e., development versus abiotic or biotic stress), it is clear that the cell utilizes the actin cytoskeleton as a conduit for the movement of defense-associated resources and, moreover, that disruption of the actin cytoskeleton results in increased pathogen proliferation and infection, as well as host-cell death.

In the past decade, numerous studies have investigated and characterized the dynamic processes that mediate the movement and activation of rapid, localized defense signaling in plants. Much of this work has focused on the role of vesicular trafficking during innate immune signaling. Indeed, it is clear that the endomembrane system of plants is both a key regulator of defense signaling (43, 57, 111), as well as a target of pathogen virulence during the early stages of the infection process (9, 59, 111). To investigate the dynamic organization of the actin cytoskeleton during nonhost interactions, Takemoto et al. (124) demonstrated the focused delivery of defense components along the actin cytoskeleton. Taking advantage of the increased penetration response in the *Arabidopsis pen1-1* mutant (2), Takemoto and colleagues demonstrated that the actin cytoskeleton and microtubule network response was largely unaffected in the *pen1-1*, compared with wild-type *Arabidopsis*. In fact, actin filaments became bundled and focused at the site of pathogen penetration, just as observed in wild-type plants. However, a closer examination of the cortical actin array revealed an interesting response to later stages of the infection process—cell death. In a second series of experiments, the authors observed that there was an increase in the HR in the *pen1-1* mutant. Using this precise temporal and spatial marker, it was found that cell-to-cell communication utilizes the actin cytoskeleton as a shuttle for the focal accumulation of defense responses at the cell surface adjacent to dead or dying cells. Surprisingly, neighboring healthy cells were found to utilize the actin cytoskeletal network as a means to traffic defense materials (e.g., callose, organelles, etc.) to the anticlinal walls adjacent to the dead cell. Thus, the role of the actin network in plants extends beyond the activation of actin reorganization in defense-activated cells but also includes a preemptive role in protecting healthy, uninfected cells (50).

Following the theme of dynamic trafficking of defense signaling along the actin cytoskeleton, Wang et al. (135) demonstrated that the recruitment and subsequent activation of broad host range R-protein-mediated resistance also requires the function of the host actin cytoskeleton. In short, this work suggests that a functional actin cytoskeleton is required for broad-spectrum resistance to powdery mildew. During the activation of RPW8-mediated resistance in response to the powdery mildew pathogen *Golovinomyces cichoracearum*,

Arabidopsis utilizes the actin cytoskeleton to direct the localization of the R-protein RPW8.2 to the site of infection. Through a combination of pharmacological (i.e., cytochalasin E) and heterologous expression approaches, the authors found that pretreatment of plant tissues with an inhibitor of actin polymerization, or overexpression of an ADF, results in a significant reduction in RPW8.2 localization to the extrahaustorial membrane (EHM). As expected, the loss in RPW8.2 localization to the EHM coincides with an increased incidence of pathogen haustoria development. Taken together, these findings demonstrate that a functional actin cytoskeleton is both required for RPW8.2 localization, as well as in mediating the delivery of defense-associated molecules to the site of infection for the purposes of abrogating pathogen invasion. This study marks the first description of the relocalization of a plant R-protein to the site of pathogen invasion and, moreover, is the first to implicate the actin cytoskeleton as a conduit for this process.

FINAL THOUGHTS

The biochemical composition of plant cells represents a highly organized and dynamic machine, responsible for not only regulating inter- and intracellular signaling processes but also for coordinating the perception and activation of responses to external biotic stresses. As narrated above, the actin cytoskeleton plays a central role not only in the physical organization of the cell, but also in the dynamic (re)localization of organelles, proteins, and macromolecules in response to pathogen infection. As such, we posit that the actin network (ABPs and actin filaments) represents a critically understudied component of the pathogen defense response in plants. Moreover, as tracks for the assembly and movement of defense-signaling components following pathogen perception, the actin cytoskeleton logically represents a virulence target for pathogens. Indeed, this has been demonstrated in numerous examples of mammalian-pathogen interactions; however, in plants, the actin-pathogen connection is less well defined. Whether pathogens directly target the plant actin cytoskeleton via the action of secreted effector molecules, for example, or if the activity of these molecules disrupt the regulatory (i.e., GTPase) or structural (i.e., ABP) processes required for their assembly is not yet known. With only a few pathogen effector molecules extensively studied, it is likely that the targets of some will include components of the actin network.

SUMMARY POINTS

1. The actin cytoskeletons of both plant and animal cells play a fundamental role in a number of developmental and host responses to biotic stress.
2. Manipulation of the actin cytoskeleton by invading pathogens is a virulence mechanism that has evolved to shut down defense signaling via the redistribution of cellular components to the site of infection.
3. In plants, the actin cytoskeleton has been shown to rapidly redistribute to the site of microbial infection, both in symbiotic- and pathogen-associated interactions. The function of this redistribution, whether direct or indirect, is largely undefined.
4. New imaging technologies have enabled the in vivo investigation of the cellular processes associated with the dynamic organization of the host cytoskeleton. Advances in this area, especially related to the host-pathogen interface, are needed to fully understand how plants respond to pathogens, and moreover, how pathogens manipulate the host cell.

FUTURE ISSUES

1. What is the genetic and biochemical link between PTI, ETI, and the assembly or disassembly of the plant actin cytoskeleton, especially in host-microbe interactions with bacterial phytopathogens?
2. Are actin rearrangements a conserved mechanism for responding to symbiotic and pathogenic microbes, and is this necessary for secretion of defense machinery? Also, which vesicle trafficking events—secretion, endocytosis, or recycling—are essential for innate immunity?
3. Do plant pathogens directly or indirectly alter the dynamism of the host plant actin cytoskeleton, and is this part of PTI or ETI mechanisms?
4. Which specific pathogen T3SS effectors trigger or alter actin rearrangements during the infection process? Do these T3SS effectors bind to or modify plant actin filaments, ABPs, or regulators of actin dynamics (e.g., ROPs)?
5. To what extent does host defense trafficking utilize the actin cytoskeleton? Moreover, given the explosive nature of actin assembly in plants, is this a mechanism by which plants can quickly respond to and allocate resources to the site of pathogen infection?
6. From the standpoint of technology and tool development, it will be essential to develop new resources for the visualization, in vivo, of the host-pathogen interface, particularly through increasing the sensitivity of imaging the dynamics of the host cytoskeleton in various cell types responding to microbial attack.

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Contents

Errata

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