ELSEVIER

Breaking the WAVE complex: the point of *Arabidopsis* trichomes

Daniel B Szymanski

Actin filaments comprise an essential cytoskeletal array that organizes the cytoplasm during growth and cell division. In growing cells, actin filaments carry out many functions. Actin filaments position the endomembrane system and act as a substrate on which organelle motility occurs. Other actin-filament arrays appear to be more dynamic and to reorganize in response to growth signals and external cues. The diverse cellular functions of the actin cytoskeleton are mediated by actin-binding proteins that nucleate, destabilize, and bundle actin filaments. The distorted trichome morphology mutants provide a simple genetic system in which to study mechanisms of actin-dependent morphogenesis. Recent results from several groups indicate that 'distorted group' genes encode subunits of the actin-related protein (Arp)2/3 and WAVE complexes, and function in a cell morphogenesis pathway.

Addresses
Agronomy Department, Purdue University, Lilly Hall of Life Sciences, 915 West State Street, West Lafayette, Indiana 47907-2054, USA

Corresponding author: Szymanski, Daniel B (dszyman@purdue.edu)

Current Opinion in Plant Biology 2005, **8**:103–112

This review comes from a themed issue on
Growth and development
Edited by Liam Dolan and Michael Freeling

Available online 21st November 2004

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2004.11.004

Abbreviations

Abi-1	Abl interactor-1
ARP	actin-related protein
CRK	*CROOKED*
dis1	*distorted1*
G-actin	globular actin
GFP	green fluorescent protein
GRL	*GNARLED*
KLK	*KLUNKER*
Nap1	NCK-associated protein, $p125^{Nap1}$
PIR	*PIROGI*
RNAi	RNA interference
SCAR	suppressor of cAMP receptor defects
scd1	*stomatal cytokinesis-defective1*
Sra1	specifically RAC1-associated-1
WASP	Wiskott–Aldrich syndrome protein
WAVE	WASP family verprolin homologous protein

Introduction

In the battle of plants versus insects and pathogens, the leaf epidermis is the first line of defense in an unfriendly world. A tight seal among fields of pavement cells is essential. Although occasionally blind in their use of physical and chemical might [1], leaf trichomes can defend the plant against insect attack [2]. In the Brassicaceae, the defensive properties of unicellular trichomes are tied to their fierce shapes: multiple long pointed branches emanating from a central stalk. Trichome shape reflects the general growth mechanisms of the plant, because the pattern of trichome branching and trichome shape are highly useful predictors of evolutionary relationships [3].

In *Arabidopsis*, the genetics of epidermal cell shape control is a powerful model for those studying the mechanisms of cell and tissue morphogenesis. Forward and reverse genetic screens for epidermal morphology mutants have been very productive. Success stories in the areas of cell wall biogenesis [4], regulation of microtubule dynamics [5,6], and the role of phospholipid modification [7] as they pertain to morphogenesis have been reviewed. This review focuses primarily on the *Arabidopsis* 'distorted group' mutants and the clues they provide about actin filament nucleation and morphogenesis.

The actin cytoskeleton and organizing the cytoplasm

Actin filaments are directional polymers that preferentially add globular actin (G-actin) subunits to their barbed (plus) ends, and tend to lose subunits from their pointed (minus) ends. The spontaneous nucleation of actin filaments is slow; therefore, eukaryotic cells use several classes of actin-binding proteins to accelerate the nucleation process [8]. In non-plant systems, there are many examples in which the energy of actin polymerization participates directly in the motility and biogenesis of organelles [9,10]. Actin filaments are dynamic, and vary with respect to their growth rates, stability, and extent of bundling. Although *ACTIN* isoforms contribute to organ- and cell-type-specific functions of the actin cytoskeleton [11•,12,13], plant cells use diverse classes of actin-binding proteins to integrate signaling information with the reorganization of the actin cytoskeleton [14•].

Growing plant cells contain several types of actin arrays that are likely to carry out different functions in the cell. Heavily bundled actin filaments are commonly observed throughout the cytoplasm and in transvacuolar strands in growing plant cells. They provide a scaffold that positions the endoplasmic reticulum [15] and maintains conduits for long-distance organelle transport. Organelles such as chloroplasts [16•], peroxisomes [17], and Golgi stacks [18]

traffic on actin bundles. In highly vacuolated cells, efficient long-distance transport may control organelle inheritance [16•] or may be a means to distribute organelles to support local metabolic demands within the cell. Actin filaments mediate short-range recycling of endosomal compartments, such as those in which the auxin efflux carrier resides [19]. Plant cells also contain cortical actin filaments that are in close proximity to the plasma membrane. In many cell types, regions of active cell expansion correlate with the presence of fine networks of actin filaments; however, the *in-vivo* organization and function of cortical actin remains a mystery.

The distorted window into the WAVE-ARP2/3 morphogenesis pathway

Arabidopsis trichomes usually form three sequential branches at very early developmental stages [20]. A vast majority of the cell volume is generated after branch initiation [21]. Experiments involving cytoskeleton inhibitors suggest that microtubules are required throughout trichome development, whereas an intact actin cytoskeleton is necessary to maintain polarized growth after branch initiation [22•]. A survey of recently cloned genes also suggests that trichome morphogenesis is regulated by sequential cellular activities. The dynamics of the endomembrane [23•,24•,25] and microtubule [26–28] systems are early determinants of polarity, whereas a subset of actin-based functions in the cell have a polarity maintenance function [29••–33••,34•]. Although there may be temporal and spatial separation in their functions, the endomembrane and cytoskeletal systems are likely to be integrated during polarized growth. Therefore, the molecular analysis of the 'distorted group' mutants presents a simplified, single-cell system to provide useful knowledge about actin-dependent morphogenesis *in vivo*.

Several recent papers indicate that the 'distorted group' genes encode subunits of two different complexes that directly regulate the actin cytoskeleton: the actin-related protein (ARP)2/3 complex that nucleates actin filaments and the WAVE complex that regulates the activity of ARP2/3. The Arp2/3 complex was originally purified from *Acanthamoeba* [35]. The intact complex is abundant in animal cells and contains seven subunits: the actin-related proteins ARP2 and ARP3, and the subunits ARPC1 (p40), ARPC2 (p34), ARPC3 (p21), ARPC4 (p20), and ARPC5 (p16). The *Arabidopsis* genome encodes homologs of each of the animal subunits, and several of the plant subunits are functionally interchangeable with their human and yeast homologs [30••–32••]. In an active state, purified Arp2/3 complex binds to the sides of existing actin filaments and nucleates new 'daughter' filaments [36]. The branched actin filament forms a characteristic 70° angle relative to the mother filament. The higher-order organization of Arp2/3-dependent actin filament networks depends on the balanced activity of additional actin-binding proteins [37]. In the presence of the actin-filament-bundling protein fascin, Arp2/3 can generate long bundles of aligned actin filaments. In the presence of proteins that cap the barbed end of actin filaments, Arp2/3 can generate highly branched actin filament networks that appear as a diffuse 'cloud' of fluorescence when visualized using a light microscope.

The phenotypes of ARP2/3 and WAVE complex subunit mutants

In non-plant cells, the energy of Arp2/3-dependent polymerization is harnessed directly to distort membranes. For example, cells that that crawl on a solid substrate *in vitro* use Arp2/3-dependent dendritic networks of actin filaments at the leading edge to drive plasma-membrane protrusion [38]. In budding yeast, the internalization of endocytic vesicles from the plasma membrane is Arp2/3-dependent [10] and has been implicated in the fusion of enlarged vacuoles [9]. In all non-plant organisms, the loss of Arp2/3 is lethal. For example, *Drosophila* embryos that lack the ArpC1 subunit fail to generate actin filaments that separate adjacent nuclei during cellularization [39]. In an RNA interference (RNAi) assay using substrate-attached insect cells, reduced Arp2/3 subunit function caused a dramatic loss of lamellipodia and a spikey cell morphology [40,41].

Surprisingly, *Arabidopsis* plants that harbor null mutations in ARP2/3 subunit genes have mild phenotypes: the plants are vigorous but have a modest reduction in shoot fresh weight, they have a normal seed set and overall plant architecture. The tip-growing cells of these mutants that have a strict growth requirement for the actin cytoskeleton, such as their pollen tubes and root hairs, do not have clear phenotypes. The obvious hypothesis is that ARP2/3 is not required for cell expansion and viability, but is involved in one of many pathways that lead to actin filament nucleation. However, the composition and activity of a plant ARP2/3 complex has not been analyzed. It is possible that plant ARP2/3 is unique in its ability to tolerate subunit loss or in the extent to which ARP2/3 subunits function independently of a complex. In order to determine conclusively if ARP2/3 is essential in plants, a thorough double-mutant analysis is needed. In addition, reagents are needed to assay the assembly status and activity of ARP2/3 in wild-type and mutant plants.

It is clear, however, that a variety of cell types within shoots have strict requirements for ARP2/3 during morphogenesis. Mutation of each of the *ARP2*, *ARP3*, *ARPC2*, and *ARPC5* subunit genes causes an array of phenotypes of equal severity. In the ARP2/3 subunit mutants, the length of etiolated hypocotyls and the size of epidermal cells are reduced relative to those of the wildtype [29••]. In both the hypocotyl [29••] and cotyledon epidermis [31••,33••], there are clear gaps between adjacent cells of these mutants. The phenotype is more severe in tissue-culture grown mutants, but occurs consistently in soil-