

UC San Diego

UC San Diego Previously Published Works

Title

Spatial Control of Cell Expansion by the Plant Cytoskeleton

Permalink

<https://escholarship.org/uc/item/5750v48c>

Journal

Annual Reviews of Cell and Developmental Biology, 21

Author

Smith, Laurie G

Publication Date

2005

Peer reviewed

Spatial Control of Cell Expansion by the Plant Cytoskeleton

Laurie G. Smith¹ and David G. Oppenheimer²

¹Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, California 92093-0116; email: lsmith@biomail.ucsd.edu

²Department of Botany and UF Genetics Institute, University of Florida, Gainesville, Florida 32611-8526; email: doppen@botany.ufl.edu

Annu. Rev. Cell Dev. Biol.
2005. 21:271-95

First published online as a
Review in Advance on
June 28, 2005

The *Annual Review of
Cell and Developmental
Biology* is online at
<http://cellbio.annualreviews.org>

doi: 10.1146/
annurev.cellbio.21.122303.114901

Copyright © 2005 by
Annual Reviews. All rights
reserved

1081-0706/05/1110-
0271\$20.00

Key Words

morphogenesis, actin, microtubules, tip growth, trichomes

Abstract

The cytoskeleton plays important roles in plant cell shape determination by influencing the patterns in which cell wall materials are deposited. Cortical microtubules are thought to orient the direction of cell expansion primarily via their influence on the deposition of cellulose into the wall, although the precise nature of the microtubule-cellulose relationship remains unclear. In both tip-growing and diffusely growing cell types, F-actin promotes growth and also contributes to the spatial regulation of growth. F-actin has been proposed to play a variety of roles in the regulation of secretion in expanding cells, but its functions in cell growth control are not well understood. Recent work highlighted in this review on the morphogenesis of selected cell types has yielded substantial new insights into mechanisms governing the dynamics and organization of cytoskeletal filaments in expanding plant cells and how microtubules and F-actin interact to direct patterns of cell growth. Nevertheless, many important questions remain to be answered.

Contents

INTRODUCTION.....	272
MICROTUBULES AND GROWTH DIRECTION: OLD AND NEW MODELS.....	272
ROLES FOR ACTIN IN CELL GROWTH AND ITS SPATIAL REGULATION	274
Tip Growth.....	274
Diffuse Growth.....	276
POLLEN TUBE GROWTH: A CASE IN POINT.....	277
TRICHOME MORPHOGENESIS .	278
Trichome Branch Initiation: Three Important Points	279
Trichome Branch Elongation	282
PAVEMENT CELL MORPHOGENESIS: BATTLE OF THE BULGES	285
Lobe Formation: A Collaboration Between Microtubules and F-Actin	285
Coordination of Microtubules and Actin by ROPs and RICs	287
CONCLUDING PERSPECTIVES .	288

INTRODUCTION

Plant cells exhibit a wide variety of shapes that make important contributions to cell function. A plant cell's shape is determined by its wall and is acquired during development according to the direction and pattern in which the wall extends as the cell expands under the force of turgor pressure. By directing the deposition of cell wall materials, the cytoskeleton plays a central role in the control of plant cell growth and its spatial regulation. Cellulose, the principle load-bearing structural element of expanding cell walls, is synthesized by enzyme complexes in the plasma membrane. The arrangement of cellulose microfibrils in the wall is a key determinant of cell expansion pattern and is clearly related to the arrangement of cortical microtubules in expanding

cells. Cell wall components other than cellulose and the related polymer callose are introduced into the wall via secretion. This includes a variety of carbohydrates that form cross-links between cellulose microfibrils, as well as enzymes that catalyze the breakage and reformation of these cross-links. Thus, spatial regulation of secretion by the cytoskeleton is crucial for determining patterns of wall extensibility and thus patterns of growth.

In this review, we discuss the roles of microtubules and actin filaments in plant cell growth and its spatial regulation, as well as a rapidly growing body of knowledge about how the dynamics and organization of both classes of filaments are controlled in expanding cells. Owing to the availability of several reviews on similar topics published in the last several years (e.g., Hepler et al. 2001, Smith 2003, Wasteneys & Galway 2003, Lloyd & Chan 2004, Mathur 2004) and to space limitations, this review does not comprehensively discuss all the relevant literature. Instead, we emphasize recent work leading to important new insights and ideas about regulation of the cytoskeleton and its contributions to patterning of plant cell growth. After a general discussion about the contributions of microtubules and actin, we discuss recent advances gained from studies on the morphogenesis of three cell types: pollen tubes, trichomes (epidermal hairs), and leaf epidermal pavement cells.

MICROTUBULES AND GROWTH DIRECTION: OLD AND NEW MODELS

The importance of both microtubules and cellulose in determining the direction of cell expansion has been clear for many years (reviewed in Baskin 2001, Wasteneys & Yang 2004, Lloyd & Chan 2004). The observation that the cellulose deposition pattern normally mirrors the pattern of cortical microtubules in expanding cells led to formulation of the cortical microtubule/cellulose microfibril co-alignment hypothesis, hereafter referred to as the co-alignment hypothesis, which states

Cellulose microfibril: a bundle of cellulose polymers each consisting of covalently linked glucose subunits

Microtubule: a 25-nm wide hollow tube that is a polymer of subunits consisting of α -tubulin/ β -tubulin heterodimers

that movement of cellulose synthase enzyme complexes in the plasma membrane is constrained by interactions with the cortical microtubules (Giddings & Staehelin 1991). The energy of cellulose polymerization supplies the force needed to move the enzyme complex through the membrane, and it only needs to be guided by direct or indirect interactions with the cortical microtubules.

Unfortunately, this straightforward co-alignment hypothesis is inconsistent with observations of continued synthesis of organized cellulose microfibrils following cortical microtubule disruption (reviewed in Baskin 2001, Sugimoto et al. 2003). In addition, the model fails to account for the inability of cortical microtubules to form ordered arrays when cellulose synthesis is inhibited (Fisher & Cyr 1998). To accommodate these observations, Baskin (2001) extended the co-alignment hypothesis with the templated incorporation model. In this model, bi-functional scaffold factors bind to existing microfibrils and newly synthesized microfibrils, facilitating local order. The model also posits the existence of integral membrane components linking the scaffold factors to the cortical microtubules. The persistence of a membrane-based scaffold following cortical microtubule depolymerization can explain how ordered deposition of nascent microfibrils can occur in the absence of cortical microtubule organization. Although the templated incorporation model goes a long way toward accounting for observations apparently contradicting the co-alignment hypothesis, it suffers from a lack of direct evidence as to either the scaffold or the membrane-based components that link the scaffold to cortical microtubules.

However, one of the predictions of the templated incorporation model is that mutants that fail to properly construct the putative membrane-based scaffold should exhibit normal cortical microtubule organization but show altered patterns of cellulose microfibril organization resulting in cell expansion defects. Recently, a mutant that displays this phenotype has been identified. The

fragile fiber 1 (fra1) mutation was identified in a screen for mutants that showed reduced mechanical strength of the inflorescence stem due to defects in interfascicular fiber cell differentiation (Zhong et al. 2002). In addition to weak inflorescences, *fra1* mutants showed moderate cell expansion defects that led to a general shortening of plant organs. Analysis of the cell wall composition of *fra1* mutants showed no significant difference compared with wild type, but examination of the cellulose microfibrils in fiber cell walls revealed that *fra1* mutants lack the densely packed, parallel arrangement of microfibrils observed in wild type. Instead, the cellulose microfibrils in *fra1* mutants were more loosely arranged and oriented in different directions. On the basis of the co-alignment hypothesis, one might expect that the cortical microtubules would show similar disorganization. Interestingly, the arrangement of cortical microtubules in *fra1* mutants is indistinguishable from wild type. Positional cloning of the *FRA1* gene showed that it encodes a member of the KIF4 family of kinesin motor proteins. Localization of the FRA1 protein to the cell cortex in expanding cells provides support for its role in microfibril orientation. If the templated incorporation model (Baskin 2001) is correct, then this gene product may be involved in organizing the membrane-based elements that link the putative scaffold to the cortical microtubules. Identification of the cargo of this kinesin will most likely provide important insight into its role in cellulose microfibril orientation.

Another contradiction with the co-alignment hypothesis arises from the study of the *microtubule organization1-1 (mor1-1)* mutant in *Arabidopsis* (Whittington et al. 2001). This temperature-sensitive mutant appears completely normal at permissive temperatures but rapidly loses cortical microtubule organization at restrictive temperatures, resulting in growth isotropy (loss of growth directionality). Surprisingly, mutant cells undergoing isotropic expansion still maintain transversely aligned cellulose microfibrils.

Actin filament (filamentous or F-actin):

an 8-nm wide, helical polymer of globular actin (G-actin) subunits

Dynamics (cytoskeletal/microtubule/actin dynamics):

refers to the turnover of filaments via lengthening (polymerization) and shortening (depolymerization)

Trichome: an epidermal hair; in *Arabidopsis*, trichomes have a branched morphology

Pavement cell: an unspecialized epidermal cell; in the leaves of most flowering plant species they have a lobed morphology

Motor protein: a protein that associates with a cytoskeletal filament and uses energy derived from ATP hydrolysis to move its cargo along the filament in a particular direction; kinesin motors move cargoes along microtubules; myosin motors move cargoes along actin filaments

FRA1: Fragile Fiber 1

Cortex: a thin shell of cytoplasm lining the inner surface of the plasma membrane

Isotropic expansion:

growth that is oriented uniformly in all directions

mor1: microtubule organization1

Tip growth:

an extremely polarized mode of plant cell growth exhibited by pollen tubes and root hairs in which wall extension and incorporation of new wall material occurs at a single site on the cell surface (the tip)

This led Wasteneys (2004) to propose the microfibril length regulation hypothesis, in which cortical microtubules participate in regulating the length of cellulose microfibrils rather than their alignment. As the major load-bearing structural element in plant cell walls, the arrangement of cellulose microfibrils is thought to resist expansion in the direction parallel to the orientation of the microfibrils much like a coil spring resists expansion in the radial direction. Loosening of the matrix polysaccharides linking adjacent microfibrils allows expansion perpendicular to the orientation of the microfibrils. But unless individual microfibrils extend for a considerable distance around the cell, loosening of the matrix polysaccharides would also allow adjacent microfibrils to slip past one another, resulting in radial expansion as well as elongation (**Figure 1**). The microfibril

length regulation hypothesis thus explains how radial expansion could occur in cells with transversely aligned microfibrils while still providing a role for cortical microtubules in guiding directional cell expansion. A test of this model would be to measure cellulose microfibril lengths in root cells from *mor1-1* mutants before and after growth at the restrictive temperature. Additionally, radial expansion of wild-type roots treated with short pulses of the cellulose synthase inhibitor, isoxaben, might provide additional support for this hypothesis.

ROLES FOR ACTIN IN CELL GROWTH AND ITS SPATIAL REGULATION

Tip Growth

Essential functions for F-actin in cell growth have long been recognized because of the growth-arresting effects of actin depolymerizing drugs (cytochalasins and latrunculins). The dependence of cell growth on F-actin was first recognized in tip-growing cells in which growth and extension of the cell wall is focused at a single site on the cell surface resulting in production of a cylindrical shape (**Figure 2a**). Thus, much work has been devoted to understanding how F-actin contributes to tip growth. Studies of F-actin organization in tip-growing cells have produced rather different results depending on the localization method being used, and no one method is ideal in every respect. However, as illustrated in **Figure 2a**, there is now widespread agreement that longitudinally oriented actin cables run along the length of tip-growing cells, and that a dense meshwork of fine actin filaments occupies an area near the tip but does not extend to the extreme apex (e.g., Miller et al. 1996, Kost et al. 1998, Miller et al. 1999, Ketelaar et al. 2002, Y.-S. Wang et al. 2004), although highly dynamic actin filaments have been reported to penetrate transiently into the apex (Fu et al. 2001). This fine F-actin network, occupying both the cell cortex and

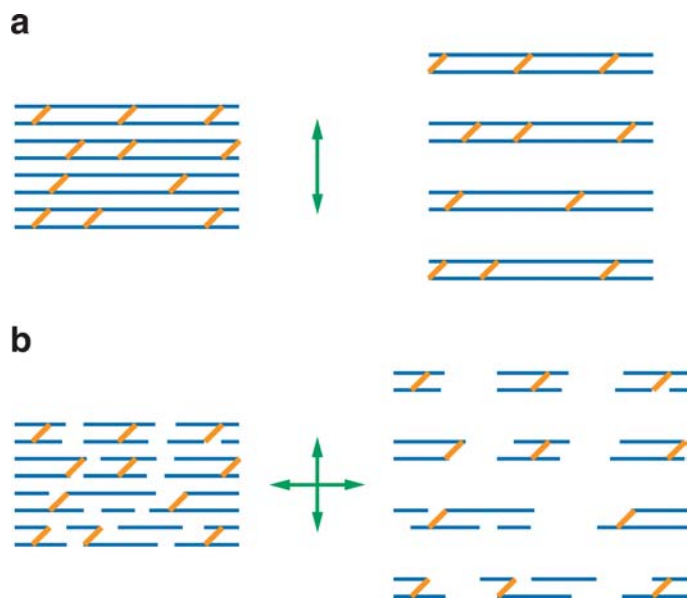


Figure 1

Microfibril length regulation model for control of anisotropic cell expansion proposed by Wasteneys (2004). (a) Long cellulose microfibrils (blue) are cross-linked by a given number of matrix polysaccharides (orange) that prevent sliding of adjacent microfibrils. Only expansion in the longitudinal direction is allowed (green arrows). (b) Short cellulose microfibrils cross-linked by the same number of matrix polysaccharides as in (a) cannot prevent slippage of some adjacent microfibrils. Expansion in the radial as well as the longitudinal direction is allowed.

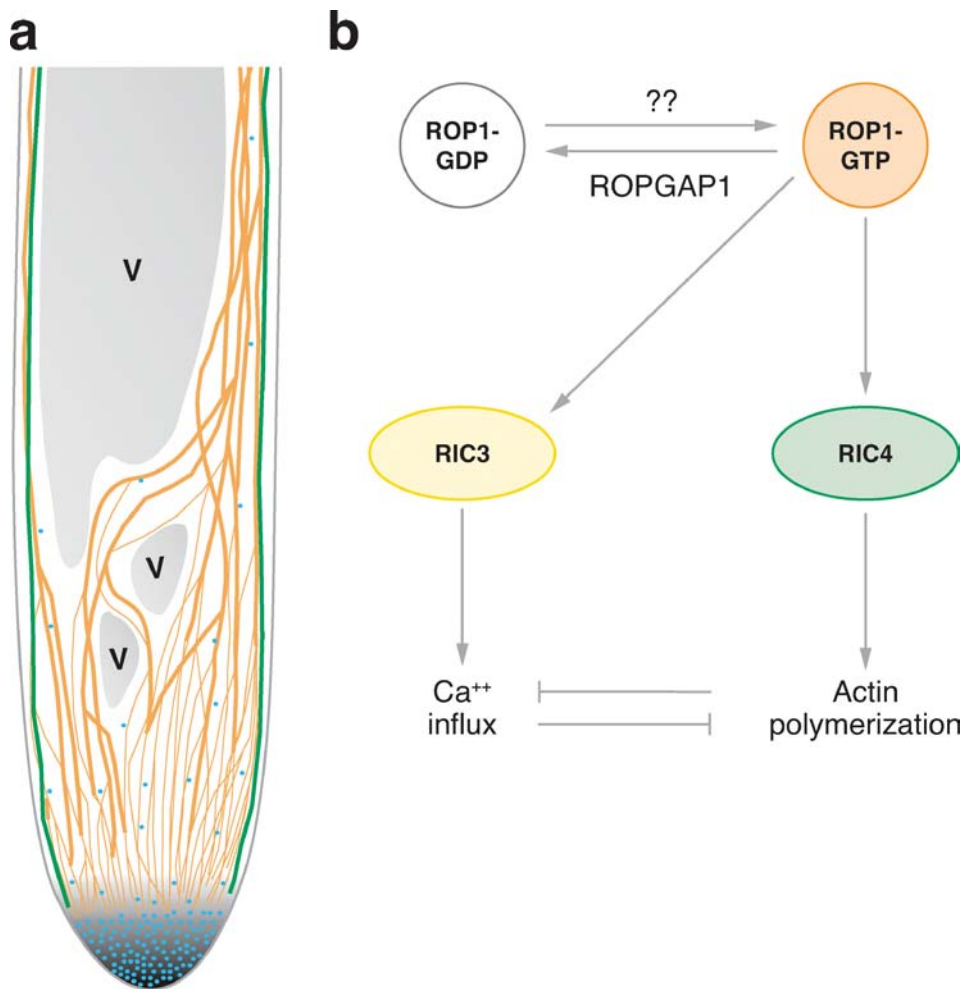


Figure 2

Cytoskeletal regulation of pollen tube growth. (a) Schematic, cross-sectional view of an elongating pollen tube. Cortical microtubules are shown in green, actin filaments and cables in orange (V, vacuole). Tip high cytoplasmic Ca^{2+} gradient is represented by gray shading in tip region with black representing the highest Ca^{2+} concentration. Blue dots represent vesicles (adapted from Ketelaar & Emons 2001). (b) Model schematically illustrating interactions occurring at the growing pollen tube tip between ROP1, RIC3, and RIC4 demonstrated by Gu et al. (2005).

cytoplasm, is often referred to as a subapical F-actin fringe or collar.

In tip-growing cells, myosins drive the movement of Golgi-derived vesicles and other cell components along longitudinally oriented actin cables toward the growth site via cytoplasmic streaming (Hepler et al. 2001). Thus, one important role for actin

in promoting tip growth is to drive the long-range movement of vesicles, ferrying the raw materials for growth (plasma membrane and cell wall materials) toward the tip. However, actin clearly plays additional roles important for growth and its spatial regulation in tip-growing cells. Treatment of growing pollen tubes and root hairs with low

Actin cable: a thick bundle of actin filaments

Golgi: a cellular compartment consisting of a stack of flattened, membrane-bounded sacs where proteins destined for secretion are modified and sorted as they pass through it on their way from the endoplasmic reticulum to the cell surface

Vesicle: a small membrane-bounded, spherical organelle inside which proteins and other materials are transported within the cell from one location to another

Cytoplasmic streaming: a directed, continuous flow of vesicles and other organelles that is driven by the actomyosin system in plant cells

Actin assembly/polymerization: elongation of an actin filament

Diffuse growth: the mode of growth exhibited by most plant cells in which extension of the wall and incorporation of new wall material is broadly distributed across the cell surface

ADF: actin-depolymerizing factor

concentrations of actin depolymerizing drugs can inhibit growth without blocking cytoplasmic streaming (Miller et al. 1999, Gibbon et al. 1999, Vidali et al. 2001). These treatments eliminate the subapical fine F-actin fringe without disrupting longitudinal F-actin cables and also result in loss of the vesicle-rich body of cytoplasm normally found at the apex. The sensitivity of subapical actin filaments to drug treatment suggests that they are more dynamic than the longitudinal F-actin cables. The effects of selective depletion of the subapical F-actin fringe show that it functions in some way to promote the accumulation and/or retention of vesicles at the growth site. Many ideas have been proposed to explain how subapical F-actin might accomplish these functions (reviewed in Geitmann & Emons 2000). A simple idea based on the observation that cytoplasmic streaming stops short of the apex and then reverses direction to produce a reverse fountain pattern of motility is that subapical F-actin traps vesicles, preventing them from leaving the tip region via cytoplasmic streaming, and actively transports them to the tip. This could be achieved via myosin-dependent transport through the actin mesh and/or via actin polymerization-driven propulsion of vesicles toward the apex. The subapical F-actin fringe may also serve as a barrier to prevent the loss of vesicles from the apex.

Interestingly, at concentrations even lower than those that inhibit tip growth in the presence of continued cytoplasmic streaming, actin depolymerizing drugs cause a slight depolarization of growth in the tip region, producing a swelling of the tip (e.g., Gibbon et al. 1999, Ketelaar et al. 2003). This observation indicates a role for subapical F-actin in fine-tuning the spatial distribution of growth in the tip region. One possible explanation for this finding is that subapical F-actin functions as a physical barrier to inhibit vesicle fusion in the subapical region. However, an alternative explanation is suggested by recent observations regarding the role of F-actin in Ca^{2+} channel regulation, to be discussed below in con-

nection with recent discoveries regarding the roles of ROP GTPases in regulation of pollen tube growth.

Diffuse Growth

Until relatively recently, studies on the role of F-actin in cell growth have focused mainly on tip growth. However, it is now clear from both pharmacological and genetic studies that F-actin also plays important roles in diffuse growth, the mode of growth exhibited by most plant cells, in which wall extension and incorporation of new wall material are distributed across the cell surface. Treatment with actin-depolymerizing drugs inhibits diffuse growth, causing an overall dwarfing effect (Thimann et al. 1992, Baluska et al. 2001). Moreover, although mutations knocking out individual actin isoforms have no obvious effects on diffuse growth, double mutants lacking both *ACTIN2* and *ACTIN7* function are severely growth-inhibited (Gilliland et al. 2002). Diffuse growth and tip growth have classically been viewed as mechanistically distinct growth processes, but it is now clear that they have much in common with regard to the regulation of F-actin dynamics and probably also with regard to the functions of F-actin in promotion of growth. For example, a variety of actin-binding proteins have similar roles in diffuse cell expansion and tip growth in vivo. Overexpression of actin-depolymerizing factor (ADF) disrupts cytoplasmic F-actin cables and reduces the expansion of diffusely growing cells and root hairs, whereas antisense inhibition of ADF produces an increase in the density of cytoplasmic F-actin and excess expansion of both diffusely growing cells and root hairs (Dong et al. 2001). RNAi-mediated downregulation of actin-interacting protein 1 (AIP1), which can modulate F-actin dynamics by capping actin filaments and enhancing the activity of ADF, causes excessive bundling of cytoplasmic F-actin and reduces the expansion of diffusely growing cells and root hairs alike (Ketelaar et al. 2004). Modulation of profilin levels achieved via overexpression

and gene knockout approaches also has similar effects on root hair elongation and diffuse cell expansion (Ramachandran et al. 2000, McKinney et al. 2001). These perturbations show that F-actin promotes both diffuse growth and tip growth. However, other lesions in the F-actin cytoskeleton reveal roles for F-actin in the spatial regulation of diffuse growth, as discussed below in connection with trichome and pavement cell morphogenesis. In diffusely growing cells, F-actin cables permeate the cytoplasm, promoting cytoplasmic streaming as in tip-growing cells and driving the motility of a variety of organelles. Several investigators have proposed that cytoplasmic F-actin is important for properly targeted delivery of Golgi-derived vesicles to diffusely growing cell walls, just as in tip-growing cells (Baskin & Bivens 1995, Miller et al. 1999, Dong et al. 2001).

POLLEN TUBE GROWTH: A CASE IN POINT

As discussed above, a variety of actin-binding proteins are implicated in regulation of actin dynamics in tip-growing cells such as pollen tubes, but there is little information at present regarding the specific roles of most of these proteins in the spatial regulation of growth in the tip region. However, members of a plant-specific family of Rho-related GTPases (called ROPs for Rho of plants) play key roles in the polarization of tip growth owing in part to their impact on F-actin dynamics. Analyses of ROP function in pollen tube growth have focused primarily on ROP1, but ROP3 and ROP5 are also expressed in growing pollen tubes and are nearly 100% identical to ROP1, so they are probably functionally equivalent (Gu et al. 2004). ROP1 is localized to the plasma membrane at the tips of elongating pollen tubes (Lin et al. 1996). Inhibition of ROP1 function results in loss of F-actin in the tip region (but not of longitudinal actin cables) and growth arrest (Lin & Yang 1997, Li et al. 1999). Conversely, overexpression of wild-type ROP1, or expression of a con-

stitutively active form of ROP1 (ROP1-CA), causes depolarization of growth, which is associated with excess/ectopic F-actin polymerization (Li et al. 1999). Analysis of cytoplasmic Ca^{2+} distribution in pollen tubes with inhibited or excess ROP1 function showed that ROP1 also promotes an influx of extracellular Ca^{2+} needed to maintain the tip-high Ca^{2+} gradient characteristic of tip-growing cells (Li et al. 1999), which is essential for tip growth and is thought to promote vesicle fusion at the growth site (reviewed in Hepler et al. 2001).

Recent work indicates that stimulation of F-actin assembly and extracellular Ca^{2+} influx are separate functions of ROP1 that are mediated by different RIC (ROP-interacting CRIB domain) effector proteins (Gu et al. 2005). Overexpression of either RIC3 or RIC4 causes ROP1-dependent delocalization of growth in pollen tubes via distinct mechanisms: RIC4 stimulates actin assembly at the tip, whereas RIC3 stimulates Ca^{2+} influx at the tip (Gu et al. 2005) (**Figure 2b**). Other than its CRIB domain, the sequence of RIC4 is novel, so it remains to be determined how it functions to stimulate actin assembly. One possibility is that it positively regulates the activity of formins, a class of actin-nucleating proteins recently implicated in actin nucleation in elongating pollen tubes (Cheung & Wu 2004). Another possibility is that RIC4 stimulates actin assembly by mediating ROP1-dependent downregulation of ADF. This possibility is suggested by recent evidence that the tobacco homolog of ROP1 (NrRAC1) inactivates ADF in elongating pollen tubes (Chen et al. 2003). In both plant and animal cells, Rho-family GTPases regulate production of reactive oxygen species (ROS) by NADPH oxidases (Gu et al. 2004), and NADPH oxidase-dependent ROS production has been shown to play an essential role in root hair elongation by stimulating an influx of extracellular Ca^{2+} at the tip (Foreman et al. 2003). Thus, RIC3 may stimulate Ca^{2+} influx at the tip by mediating ROP1 regulation of NADPH oxidases. This

GTPase: guanine nucleotide triphosphatase

ROP: Rho of plants

RIC: ROP-interacting CRIB domain protein

Actin nucleation: the initial assembly of actin monomers to form a seed for further actin polymerization; occurs inefficiently in living cells except in the presence of a nucleator such as a Arp2/3 complex

notion is consistent with the finding that plant NADPH oxidases lack the regulatory subunit that mediates Rac activation of mammalian NADPH oxidases (Gu et al. 2004).

Interestingly, although analysis of *ric3* and *ric4* loss-of-function mutants indicates that both RIC3 and RIC4 positively regulate tip growth, various observations demonstrate that these two proteins antagonize each other's functions in a manner that depends on their downstream effectors (F-actin and Ca^{2+}) (Gu et al. 2005). Thus, ROP1/RIC4-mediated stimulation of F-actin assembly at the tip negatively regulates Ca^{2+} influx, whereas ROP1/RIC3-mediated Ca^{2+} influx negatively regulates F-actin assembly at the tip (**Figure 2b**). Consistent with these findings, negative regulation by F-actin of inward Ca^{2+} channel activity has recently been demonstrated in pollen protoplasts and pollen tubes (Y.-F. Wang et al. 2004). In turn, RIC3-induced Ca^{2+} influx may suppress actin assembly at the tip by stimulating the Ca^{2+} -dependent actin depolymerizing activities of profilins, gelsolins, and/or perhaps villins as well (Kovar et al. 2000, Holdaway-Clarke & Hepler 2003, Huang et al. 2004). But how can mutual antagonism between RIC3 and RIC4 be reconciled with the ability of both of these proteins to positively regulate tip growth? One possibility is that RIC3/RIC4 antagonism is at least partially responsible for the temporal growth oscillations that have been observed in elongating pollen tubes and root hairs, in which pulses of Ca^{2+} influx and growth (reviewed in Hepler et al. 2001) alternate with pulses of F-actin polymerization (Fu et al. 2001). Consistent with this possibility, treatment of elongating pollen tubes with very low doses of actin depolymerizing drugs showed that their growth oscillations (and thus presumably pulses of Ca^{2+} influx) are F-actin dependent (Geitmann et al. 1996). Another possibility, not mutually exclusive with the first, is suggested by the observation mentioned above that pollen tubes and root hairs elongating in the presence of very low doses of actin depolymerizing drugs exhibit a slight

depolarization of growth in the tip region (tip swelling; Gibbon et al. 1999, Ketelaar et al. 2003). Subapical F-actin may negatively regulate Ca^{2+} influx in the subapical region, sharpening the Ca^{2+} gradient and thereby focusing vesicle fusion at the apex.

TRICHOME MORPHOGENESIS

The development of epidermal hairs (trichomes) in *Arabidopsis* has long been recognized as an excellent model for the study of cell shape control (Marks et al. 1991). *Arabidopsis* trichomes are single, epidermally derived cells that consist of three or four branches of equal length symmetrically arranged on top of a stalk. Their development begins with expansion of the trichome precursor as a domed cylinder out of the plane of the leaf blade, followed by two or three successive branching events. Further global expansion by diffuse growth (Hülkamp 2000) takes the 20- to 30- μm diameter incipient trichome to the final 300- to 500- μm tall mature trichome. The trichome's large size, distinctive shape, and dispensability makes them an ideal target for mutational studies of cell morphogenesis. Indeed, at least 20 genes have been identified that control trichome branching, and at least that many control the enlargement/branch elongation phase of trichome development (Mathur 2004). Because trichome development has been reviewed recently (Mathur 2004, Szymanski 2005), here we focus on new developments that link the cytoskeleton to specific events in trichome morphogenesis. The important role of the plant cytoskeleton in defining complex cell shapes was highlighted by the effects of actin and microtubule inhibitors on trichome development (Mathur et al. 1999, Szymanski et al. 1999, Mathur & Chua 2000). These studies initially defined separate roles for the microtubule and actin cytoskeletons in polar growth/branch initiation and extension growth, respectively. Genetic and molecular analyses support this view: Mutations in genes encoding microtubule-related proteins affect

branch number, and mutations in genes encoding actin polymerization regulators affect extension growth (reviewed in Mathur 2004). However, synergistic effects between mutations that affect branch number and those that affect branch elongation suggest that the same gene products are involved in both processes (Zhang et al. 2005a). In addition, recent work discussed below suggests that both cytoskeletal systems cooperate throughout trichome morphogenesis.

Trichome Branch Initiation: Three Important Points

Genetic studies have led to the identification of a variety of proteins involved in the initiation of trichome branches. The functional relationships between these proteins have been murky, but recent work discussed here suggests that Golgi stacks may be the key to understanding how the cytoskeleton directs trichome branch initiation.

Zwichel and *Angustifolia*: seemingly unlikely partners? The *zwichel* (*zwi*) mutant was first identified by Hülskamp et al. (1994) in a screen for mutants that affected trichome development. Mutations in *zwi* lead to trichomes with a reduced stalk and fewer than normal branches (Hülskamp et al. 1994, Folkers et al. 1997, Oppenheimer et al. 1997). In addition, one of the branches on *zwi* mutants often fails to elongate properly (Folkers et al. 1997, Oppenheimer et al. 1997, Luo & Oppenheimer 1999). Thus, this mutation provides the first evidence that branch initiation and elongation are related processes. Also, the site of branch initiation is altered in *zwi* mutants, resulting in reduced stalk height (Oppenheimer et al. 1997). This phenotype suggests that ZWI is involved in proper localization of a necessary branch initiation factor. The *ZWI* gene was cloned by T-DNA tagging (Oppenheimer et al. 1997) and shown to encode a novel kinesin previously identified in a screen for calmodulin-binding proteins [kinesin-like calmodulin-binding protein

(KCBP) (Reddy et al. 1996a,b)]. Hereafter, we refer to the *ZWI* product as KCBP. Although the *Arabidopsis* genome encodes 61 kinesins (Reddy & Day 2001), *ZWI* exists as a single copy gene. A unique feature of KCBP among plant kinesins is that its presumed cargo-binding tail contains a domain also found in unconventional myosins and talin (Oppenheimer et al. 1997, Reddy & Reddy 1999). This suggests that KCBP plays a role in one or more actin-dependent processes in addition to its function as a microtubule motor. The function of KCBP in trichome development has been recently reviewed (Reddy & Day 2000). Here, we focus on its interaction with AN.

Mutations in *ANGUSTIFOLIA* (*AN*) cause cell expansion defects that result in narrow cotyledons and leaves, bent and/or twisted seed pods, and reduced branching in trichomes (Rédei 1962, Hülskamp et al. 1994, Tsuge et al. 1996). At the cellular level, *an* mutants show altered microtubule organization in trichomes and leaf cells. The *AN* gene encodes a distantly related member of the C-terminal binding protein/brefeldin A-ADP ribosylated substrate (CtBP/BARS) family (Folkers et al. 2002, Kim et al. 2002). In animal cells, members of this family function as transcriptional co-repressors and as regulators of vesicle budding from Golgi stacks (De Matteis et al. 1994, Schaeper et al. 1998, Spano et al. 1999, Weigert et al. 1999, Nardini et al. 2003). The two functions are controlled by allosteric changes induced by the binding of the molecules NAD(H) and acyl-CoA. Binding of NAD(H) to CtBP/BARS induces a conformational change that allows it to function as a co-repressor, whereas binding to acyl-CoA induces an alternative conformation that activates the membrane fission function of the protein (Nardini et al. 2003). Currently, there is no direct evidence that AN possesses either of these functions. However, consistent with a role for AN as a transcriptional co-repressor are the observations that AN-GFP fusions are detected in the nucleus and that at least

ZWI: zwichel

KCBP: kinesin-like calmodulin-binding protein

AN:
ANGUSTIFOLIA

CtBP/BARS:
C-terminal binding protein/brefeldin A-ADP ribosylated substrate

GFP: green fluorescent protein

Trafficking (membrane/vesicle/golgi trafficking): directed movement of organelles within the cell, generally involving the cytoskeleton

XTH: xyloglucan endotransglucosylase/hydrolase

Exocytosis: fusion of secretory vesicles with the plasma membrane to release vesicle contents into the extracellular environment

eight genes are modestly (about three-fold) upregulated in *an* mutants (Kim et al. 2002). Moreover, the finding that AN-GFP fusions are also found in the cytoplasm and that AN and KCBP interact genetically and biochemically support a cytoplasmic role for AN in trichome branching (Folkers et al. 2002, Kim et al. 2002). In either case, AN's effect on microtubule organization is likely to be indirect.

Kinesin-13a: the golgi connection. The recent discovery of a Golgi-localized kinesin provides a key link between Golgi dynamics and localized cell expansion (Lu et al. 2005). The GhKinesin-13A protein was identified as a kinesin that was abundantly expressed during cotton fiber development. This kinesin has an internal motor domain and is most closely related to the mammalian mitotic centromere-associated kinesin (MCAK)/kinesin-13 subfamily. A role in trichome branching was uncovered by analysis of *Arabidopsis kinesin-13A* mutants; trichomes on the mutants had more branches than wild type. Co-immunolocalization of KINESIN-13A and Golgi markers showed that this kinesin is associated with Golgi stacks. Additional studies using GFP fusions to Golgi markers revealed association of some of the Golgi stacks with cortical microtubules. These observations were surprising because previous work had suggested that only the acto-myosin system is responsible for the intracellular transport of Golgi stacks (Nebenführ et al. 1999). However, Lu and co-workers (2005) also found that Golgi stacks were more clustered in trichomes and other cell types of *kinesin-13A* mutants than in wild type, supporting a role for KINESIN-13A in distribution of Golgi stacks at the cell periphery. Lu and co-workers (2005) present a model in which KINESIN-13A-associated Golgi stacks are transported from the perinuclear region along actin filaments to the cell cortex. The recent demonstration that the myosin XI MYA2 is required for trichome branch formation suggests that this myosin may be responsible for actin-based movement

of Golgi stacks to the cell periphery (Holweg & Nick 2004). According to the model of Lu et al. (2005), once Golgi come into contact with cortical microtubules, KINESIN-13A takes over transport of the Golgi stacks.

Golgi delivery model for trichome branch initiation. An intriguing model emerges from consideration of the work of Lu et al. (2005) on KINESIN-13A in relation to earlier work on ZWI and AN, which can explain the ZWI-AN interaction and the connection between Golgi trafficking and trichome branch initiation. As illustrated in **Figure 3**, this Golgi delivery model proposes that KINESIN-13A-directed transport of Golgi stacks along cortical microtubules brings them into contact with AN, which is localized to branch initiation sites by its interaction with KCBP. The postulated Golgi membrane fission-promoting activity of AN then facilitates delivery of Golgi-derived vesicles to the plasma membrane at branch initiation sites. The plant Golgi apparatus is not only the site of synthesis of matrix polysaccharides, but is also the site of post-translational modifications of the key cell wall-loosening enzyme, xyloglucan endotransglucosylase/hydrolase (XTH) (Campbell & Braam 1998, Rose et al. 2002). Localization of XTH activity at branch initiation sites in the cell wall could lead to localized weakening and bulge formation. In addition, once XTHs are released into the cell wall by exocytosis, they appear to be targeted to cellulose microfibrils, and this localization pattern relies on an intact cortical microtubule array (Vissenberg et al. 2005), providing another link between Golgi localization and cortical microtubule organization. Although increased activity of XTHs at trichome branch initiation sites has not yet been demonstrated, there is a precedent for this mechanism. A localized region of high XTH activity in root trichoblasts presages the site of bulge formation that signals root hair initiation (Vissenberg et al. 2001). In addition to XTHs, other cell wall-loosening enzymes are likely

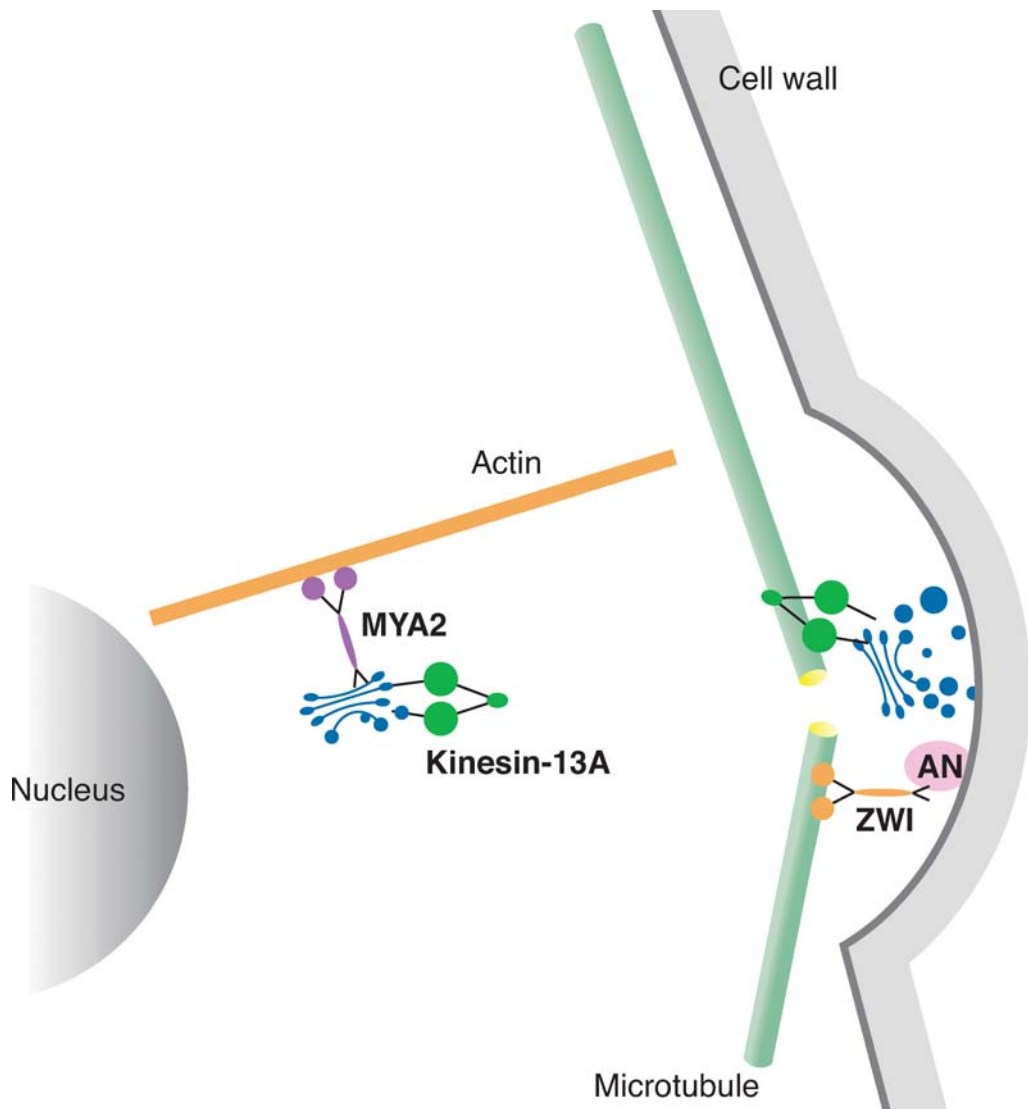


Figure 3

Golgi delivery model for trichome branch initiation. Golgi stacks (*blue*) are transported along actin filaments by the MYA2 myosin to the cell cortex where the Golgi-associated KINESIN-13A contacts cortical microtubules. KINESIN-13A transports Golgi stacks toward the minus ends of cortical microtubules (*yellow*). Localization of AN to the minus ends of microtubules is facilitated by its interaction with the minus-end-directed kinesin, ZWI. Interaction of AN with Golgi stacks promotes membrane fission and delivery of cell wall-loosening enzymes to the cell wall via exocytosis.

to be involved in the initial bulge formation as well.

The Golgi delivery model for trichome branch initiation is consistent with the phenotypes of several branching mutants. In *kinesin-*

13A mutants, Golgi stacks are transported to the cell cortex but apparently are not efficiently transported along the cortical microtubules because of the absence of KINESIN-13A. The resulting clustering of Golgi stacks

Arp2/3 complex (actin-related proteins 2 and 3):

a highly conserved complex of seven subunits, including two actin-related proteins (Arp2 and Arp3); functions in cells to nucleate actin polymerization in a temporally and spatially controlled manner

at random locations in the cell periphery could lead to cell wall loosening at ectopic sites owing to aberrant localization of XTH and other wall-loosening enzymes. Localized cell wall expansion could then set in motion the full process of branch initiation and expansion analogous to the initiation of leaf primordia by the localized application of the cell wall-loosening enzyme expansin (Fleming et al. 1997, Pien et al. 2001), thus producing an ectopic trichome branch. Also, according to this model, *an* mutants would have fewer branches than wild type because of the lack of the Golgi membrane fission-promoting activity. Similarly, lack of proper localization of AN in *zwi* mutants would lead to fewer branches as well. Even though AN and ZWI are single-copy genes in *Arabidopsis*, loss of either function does not completely block branching. This suggests that Golgi localization is unlikely to be the sole mechanism regulating branch initiation.

Trichome Branch Elongation

Role of the Arp2/3 complex. Drug treatments and mutations that interfere with actin polymerization demonstrate that F-actin plays a critical role in trichome branch elongation. Similar to the effects of near-complete depolymerization of F-actin by treatment with cytochalasin D or latrunculin B (Szymanski et al. 1999, Mathur et al. 1999), mutations disrupting four different subunits of the putative actin-nucleating Arp2/3 complex in *Arabidopsis* produce a distorted trichome phenotype characterized by a lack of trichome branch elongation, accompanied by swelling of trichome stalks (Le et al. 2003; Li et al. 2003; Mathur et al. 2003a,b; Saedler et al. 2004; El-Assal et al. 2004b). Because the volumes of distorted trichomes are similar to wild type, these findings indicate a critical role for Arp2/3-dependent actin polymerization in the spatial regulation of trichome growth (Szymanski 2005). Surprisingly, however, mutations disrupting the putative Arp2/3 complex have relatively subtle effects on the ap-

pearance of F-actin in expanding trichomes. In wild-type trichomes, longitudinally oriented F-actin cables extend throughout the length of elongating branches, whereas a fine network of cortical actin filaments (and microtubules) is aligned transversely to the branch axis (**Figure 4a**). In Arp2/3 complex subunit mutants, the most conspicuous difference in the F-actin cytoskeleton is a disorganization of cytoplasmic F-actin bundles (Le et al. 2003; Li et al. 2003; Mathur et al. 2003a,b; Saedler et al. 2004; El-Assal et al. 2004b). Thus, the putative Arp2/3 complex apparently influences actin dynamics in a manner that is vital for proper growth distribution in expanding trichomes, even though an extensive F-actin network can be maintained without it. Another surprising feature of Arp2/3 complex subunit mutants is that the shapes of other cell types are affected very little or not at all, even though the subunit genes are widely expressed. Why is the putative Arp2/3 complex so important for trichomes in particular? Compared to other leaf cell types, trichomes expand very rapidly, which might in part explain why trichomes are so sensitive to loss of Arp2/3 complex function. However, pollen tubes and root hairs also grow extremely rapidly and are affected very little by disruption of the putative Arp2/3 complex, even though their growth is clearly actin-dependent. Thus, although many proteins involved in the regulation of F-actin dynamics appear to have similar roles in diffusely growing and tip-growing cells, the putative Arp2/3 complex has a role in diffusely growing trichomes that is not critical for tip growth.

Several ideas have been proposed to explain the role of Arp2/3-dependent actin polymerization in trichome morphogenesis that are not mutually exclusive. If cytoplasmic F-actin bundles play important roles in targeted vesicle delivery to the cell surface, then disorganization of those bundles observed in Arp2/3 complex mutants could alter the spatial distribution of growth owing to vesicle mistargeting. The Golgi delivery model for

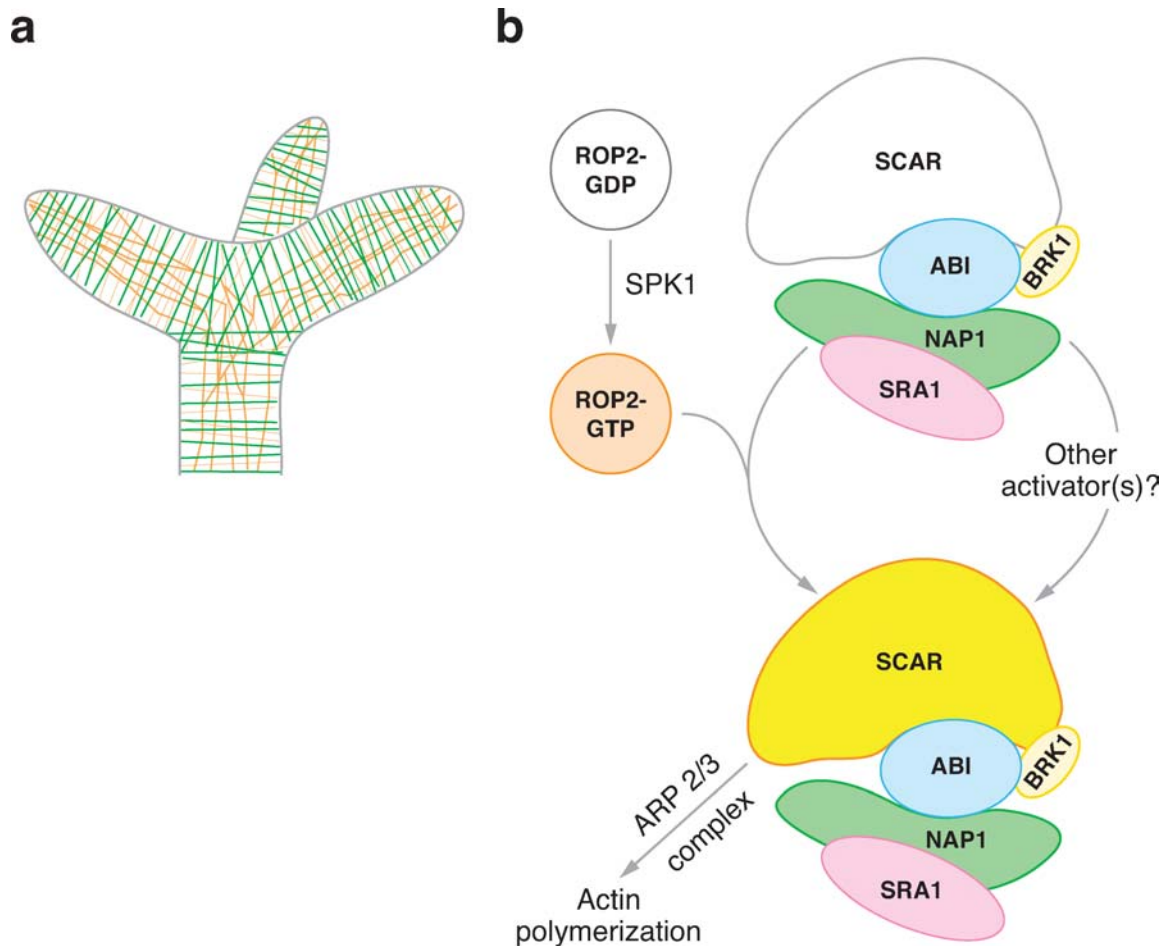


Figure 4

Cytoskeletal regulation of trichome morphogenesis. (a) Schematic illustration representing a two dimensional projection of the outer half of an expanding trichome showing transversely aligned microtubules (green) and fine actin filaments (orange) in the cell cortex, and longitudinally oriented actin cables (orange) in the central cytoplasm (based on data shown in Szymanski et al. 1999; Basu et al. 2005; S.N. Djakovic, M.P. Burke, M.J. Frank, L.G. Smith, manuscript submitted). (b) Model schematically illustrating activation of ARP2/3 complex–dependent actin polymerization in expanding trichomes by a plant Scar complex as proposed in the text. This pathway is also likely to contribute to actin polymerization in epidermal pavement cells. Note that there is controversy as to whether the WAVE complex remains intact upon Rac activation as shown here (Innocenti et al. 2004) or dissociates into two subcomplexes (Eden et al. 2002).

trichome branch initiation discussed above suggests a related possibility. If the distribution of Golgi stacks at the cell periphery is crucial for proper distribution of trichome growth, and if trafficking of Golgi stacks from the central cytoplasm to the cell periphery is actin-dependent (as shown for other cell

types; Nebenführ et al. 1999), then the disorganization of cytoplasmic actin bundles could alter the distribution of growth by disrupting Golgi trafficking. Consistent with this idea, aberrant motility and clustering of Golgi was observed in expanding trichomes of *Arabidopsis crooked* mutants lacking the ARPC5 subunit

Scar: suppressor of cAMP receptor

WAVE: WASP (Wiscott-Aldrich Syndrome protein) family verprolin-homologous protein

Sra1: specifically Rac-associated 1

Nap1: Nck-associated protein 1

Abi: Abelson-interacting protein

HSPC300: heat shock protein C300

of the putative *Arabidopsis* Arp2/3 complex (Mathur et al. 2003b). Another possibility stems from the observation that expanding trichomes of Arp2/3 complex subunit mutants show alterations in the organization of cortical microtubules as well as of F-actin bundles (Schwab et al. 2003, Saedler et al. 2004). Consistent with a variety of observations made in the past demonstrating interdependence between microtubule and F-actin organization, this observation suggests that aberrant growth patterns in mutant trichomes may be due at least in part to defects in microtubule organization, presumably leading to defects in the arrangement of cellulose microfibrils (Saedler et al. 2004). Another possibility is that potentially actin-dependent membrane trafficking events critical for growth (e.g., exocytosis, endocytosis, and release of vesicles from Golgi stacks) are facilitated by Arp2/3-dependent actin polymerization in expanding trichomes. If so, then disorganization of cytoplasmic actin bundles could be the result of membrane trafficking defects rather than being the primary cause of growth distortion in mutant trichomes (Szymanski 2005). Further insight into the role of Arp2/3-dependent actin polymerization in the spatial regulation of trichome expansion will likely emerge from identification of the intracellular sites where this polymerization occurs through localization of the putative Arp2/3 complex.

Regulation of the Arp2/3 complex by the Scar/WAVE complex. Although many questions remain regarding the role of Arp2/3-dependent actin polymerization in the spatial regulation of trichome growth, rapid advances have been made recently in understanding its regulation. Although the plant Arp2/3 complex has not yet been purified or reconstituted for use in biochemical studies, it has long been known that efficient nucleation of F-actin in vitro by the mammalian or yeast Arp2/3 complex depends on the presence of an activator, such as a member of the WASP or Scar/WAVE family (Pollard & Borisy 2003). In mammalian cells, WAVE

proteins are regulated by the small GTPase Rac and the SH domain-containing adaptor protein, Nck (Higgs & Pollard 2001). Regulation of WAVE by Rac and Nck is mediated by their interaction with a five-protein complex consisting of WAVE, the Rac-binding protein Sra1, Nck-associated protein 1 (Nap1), Abelson-interacting protein (Abi)1 or Abi2, which is critical for complex assembly, and a small protein of unknown function called HSPC300 (Eden et al. 2002, Innocenti et al. 2004, Steffen et al. 2004, Gautreau et al. 2004). Although plants were thought initially not to have Arp2/3 complex activators of the WASP/Scar/WAVE family, a family of four *Arabidopsis* proteins distantly related to Scar/WAVE proteins has recently been shown to activate the mammalian Arp2/3 complex in vitro (Frank et al. 2004, Basu et al. 2005). *Arabidopsis distorted3/irregular trichome branch1* mutations disrupt one member of this family, *SCAR2*, producing trichome morphology defects and associated alterations in the F-actin cytoskeleton similar to (although less severe than) those observed in Arp2/3 complex subunit mutants (Basu et al. 2005, Zhang et al. 2005b). Thus, *SCAR2* is implicated to play an important role in activation of the Arp2/3 complex in expanding trichomes. However, since trichome morphology defects in *scar2* mutants are less severe than those of Arp2/3 complex subunit mutants, other activators must also be involved, perhaps including other members of the *Arabidopsis* SCAR family.

Homologs of Sra1, Nap1, Abi, and HSPC300 are also present in *Arabidopsis*; recent analyses of these proteins and the corresponding genes strongly suggest that, as in mammalian cells, these proteins form a complex that is essential for SCAR-mediated activation of the putative *Arabidopsis* Arp2/3 complex (**Figure 4b**). *Arabidopsis pirogi* and *gnarled* mutations affecting *SRA1* and *NAP1*, respectively, produce trichome morphology defects and alterations in the F-actin cytoskeleton of expanding trichomes similar to those observed in Arp2/3 complex subunit mutants

(Brembu et al. 2004, Basu et al. 2004, El-Assal et al. 2004a, Li et al. 2004, Zimmerman et al. 2004). HSPC300 is the mammalian homolog of *BRICK1* (*BRK1*), originally discovered because of its essential role in formation of localized cortical F-actin enrichments and epidermal pavement cell lobe formation in maize (Frank & Smith 2002). Like mutations in *SCAR2*, *NAP1*, and *SRA1*, mutations disrupting *Arabidopsis* *BRK1* result in trichome morphology defects and alterations in the F-actin cytoskeleton similar to those of Arp2/3 complex subunit mutants (L.G. Smith, unpublished observation; Szymanski 2005). Analyses of double mutants lacking both an Arp2/3 complex subunit and *NAP1*, *BRK1*, or *SCAR2* provide genetic evidence that all three of these putative SCAR complex components act in the same pathway with the putative Arp2/3 complex (Deeks et al. 2004; Basu et al. 2005; L.G. Smith, unpublished observation). Moreover, *Arabidopsis* *SRA1* and *NAP1* interact with each other in the yeast two-hybrid system (Basu et al. 2004, El Assal et al. 2004b), and *Arabidopsis* *BRK1* binds directly to the N-terminal Scar homology domains of *Arabidopsis* *SCAR1*, *SCAR2*, and *SCAR3* (Frank et al. 2004, Zhang et al. 2005b). Although no genetic evidence has yet been reported supporting the expectation that *Arabidopsis* homologs of Abi proteins also function to activate the putative Arp2/3 complex, one member of the family of four predicted Abi-related proteins in *Arabidopsis* has recently been shown to interact with the Scar homology domain of *Arabidopsis* *SCAR2* in the yeast two-hybrid system (Basu et al. 2005).

Given that the mammalian WAVE complex is activated by Rac (Eden et al. 2002, Innocenti et al. 2004, Steffen et al. 2004), an obvious question is whether ROPs function to activate the putative *Arabidopsis* SCAR complex. ROP2 is a member of the ROP family that is expressed in developing leaves and as discussed in detail below, it plays a critical role in the spatial regulation of epidermal pavement cell expansion owing in part to its ability to stimulate localized actin polymerization.

The idea that ROP2 is similarly involved in trichome morphogenesis is supported by the finding that expression of a constitutively active version of ROP2 causes a mild distorted trichome phenotype (Fu et al. 2002). Supporting the notion that ROP2 activates the putative *Arabidopsis* SCAR complex, ROP2 interacts with *Arabidopsis* *SRA1* (homologous to the Rac-binding component of the mammalian WAVE complex) in the yeast two-hybrid system (Basu et al. 2004). Interestingly, the *Arabidopsis* *SPIKE1* (*SPK1*) protein, which is required for the formation of trichome branches as well as for normal epidermal pavement cell morphogenesis, contains a domain found in a class of unconventional guanine nucleotide exchange factors that stimulate the GTPase activity of Rho family GTPases in animal cells (Qiu et al. 2002, Brugnera et al. 2002, Meller et al. 2002). Thus, *SPK1* may function to activate ROP2 in developing trichomes and pavement cells. Further work will be needed to establish whether, as illustrated in **Figure 4b**, ROP2 activates the putative *Arabidopsis* SCAR complex, as well as to elucidate the potential role of *SPK1* in that activation. In any case, other proteins are likely to be involved in regulation of the putative *Arabidopsis* SCAR complex. For example, the mammalian WAVE complex is activated by Nck as well as by Rac, and this activation is thought to involve binding of Nck to Nap1 (Eden et al. 2002). Thus, an as yet unidentified *Arabidopsis* Nck ortholog may interact with *NAP1* to activate the putative SCAR complex (**Figure 4b**).

PAVEMENT CELL MORPHOGENESIS: BATTLE OF THE BULGES

Lobe Formation: A Collaboration Between Microtubules and F-Actin

Unspecialized leaf epidermal cells (so-called pavement cells) are an interesting case study in cytoskeletal regulation of cell growth pattern. As illustrated in **Figure 5a** for *Arabidopsis*,

BRK1: BRICK1

WAVE complex: a complex of five proteins, including the Arp2/3 activator WAVE; functions in mammalian cells to regulate the activity and localization of WAVE

SPK1: SPIKE1

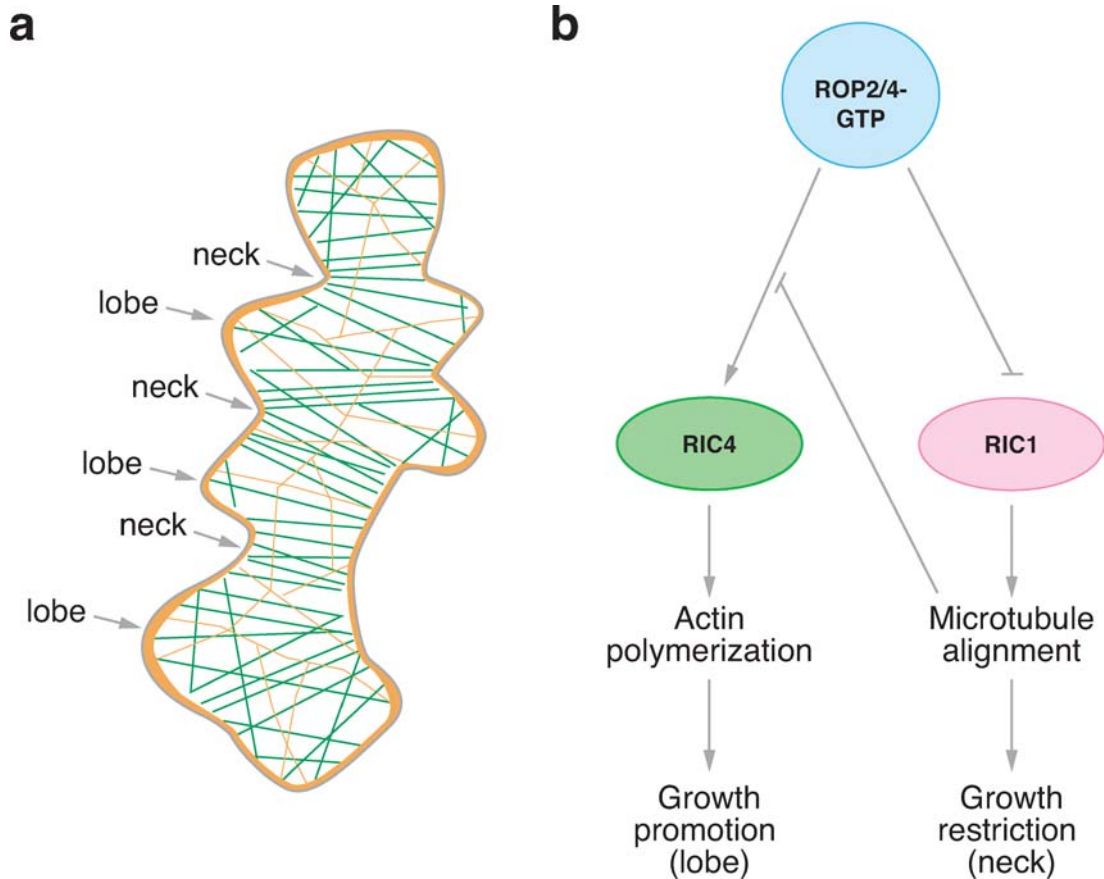


Figure 5

Cytoskeletal regulation of epidermal pavement cell morphogenesis. (a) Schematic illustration representing a two-dimensional projection of the outer half of an expanding pavement cell. Actin filaments are shown in orange, with cortical actin around the cell periphery and cytoplasmic actin cables permeating the cytoplasm; cortical microtubules are shown in green. For the sake of clarity, cortical actin on the outer face of the cell is not shown. (b) Model schematically illustrating regulation of actin polymerization and microtubule organization in expanding pavement cells by ROPs and two ROP-interacting RIC proteins (based on data presented in Fu et al. 2005).

epidermal pavement cells of most flowering plant species have lobed morphologies. The lobes of each pavement cell interdigitate with those of its nearest neighbors to form an interlocking cellular array. Thus, pavement cells not only acquire complex shapes, but they do so in a manner involving coordination of growth patterns between adjacent cells. Studies on the cytoskeletal basis of pavement cell morphogenesis have shown that microtubules are required for lobe formation and that they

tend to be organized into parallel bundles in areas of the cell periphery where lobes are not emerging (**Figure 5a**; reviewed in Smith 2003). Thus, microtubules have been thought to contribute to lobe formation by constraining cell expansion between lobes. Recent observations indicate that actin also plays a critical role in the spatial regulation of pavement cell growth. In expanding leaf epidermal pavement cells, localized accumulations of dense, fine cortical F-actin are found at sites of

lobe outgrowth in both maize and *Arabidopsis* (Figure 5a). In maize, *brk* mutations eliminate the formation of these localized F-actin enrichments and also eliminate the formation of lobes (Frank & Smith 2002, Frank et al. 2003). As discussed above, BRK1 is the plant homolog of a mammalian WAVE complex component and is thereby implicated as a regulator of Arp2/3 complex-dependent actin polymerization, which therefore appears to be essential for pavement cell lobe formation in maize.

The presence of dense, fine F-actin networks at sites of lobe outgrowth in pavement cells presents an intriguing parallel with tip-growing cells, where a related actin configuration is observed near the growth site, as discussed above. In a further parallel with tip growth, two closely related members of the ROP GTPase family (ROP2 and ROP4) contribute to lobe outgrowth in part by stimulating localized F-actin assembly. Plasma membrane-localized ROP2-GFP is concentrated at sites of lobe outgrowth. Localized cortical F-actin accumulation and lobe outgrowth are both reduced in plants with impaired ROP2 and ROP4 function, although cytoplasmic F-actin density and organization is normal (Fu et al. 2002, 2005). Conversely, expression of a constitutively active version of ROP2 results in delocalization of cortical F-actin accumulation and causes growth to be uniformly distributed as well. Interestingly, ROP2 and ROP4 have also been implicated in polarization of actin polymerization and growth in tip-growing root hairs (Molendijk et al. 2001, Jones et al. 2002). Moreover, like ROP1 in pollen tubes, ROP2-dependent localization of F-actin polymerization in expanding pavement cells involves an interaction with RIC4 (this interaction is discussed below in more detail). Thus, polarization of diffuse growth in pavement cells is mechanistically related to that in tip-growing cells. However, whether the contribution of ROP-dependent F-actin polymerization to growth polarization is the same in pavement cells and tip-growing cells is unclear. As discussed

above, in pollen tubes exocytosis appears to be concentrated in the apical-most area of the tip where little or no F-actin is present. The subapical F-actin fringe, although important for vesicle delivery to and/or retention at the apex, has also been implicated in suppression of exocytosis in the subapical area (e.g., Ketelaar et al. 2003) (Figure 2a). In contrast, cortical F-actin is enriched at sites where exocytosis rates are presumably highest in expanding pavement cells, although patterns of exocytosis have not been directly examined. Although these findings may seem contradictory, there is evidence that cortical F-actin plays both inhibitory and stimulatory roles in exocytosis in neuroendocrine PC-12 cells (Lang et al. 2000). Thus, ROP-dependent actin polymerization may locally facilitate exocytosis in expanding pavement cells, and locally inhibit exocytosis in tip-growing cells.

In any case, the effects of ROP2 and ROP4 on pavement cell morphogenesis are not limited to their influence on F-actin polymerization. In plants with reduced *ROP2* and *ROP4* gene function, parallel bundles of transversely aligned microtubules are more broadly distributed throughout the cell cortex than they are in wild type (Fu et al. 2005). Conversely, expression of constitutively active ROP2 inhibits the formation of well-ordered arrays of cortical microtubules (Fu et al. 2002). Thus, ROP2 and ROP4 appear to have dual roles in promoting pavement cell lobe outgrowth: They locally activate F-actin polymerization at sites of lobe outgrowth and also suppress the formation of ordered arrays of transversely aligned cortical microtubule bundles in these areas.

Coordination of Microtubules and Actin by ROPs and RICs

As discussed above, the observed interaction between ROP2 and *Arabidopsis* SRA1 suggests that ROP2 can activate the putative SCAR complex. However, pavement cell lobe outgrowth and localized F-actin accumulation are reduced considerably less in Arp2/3

complex subunit mutants (Li et al. 2003) than they are in plants with reduced ROP2/4 function (Fu et al. 2002, 2005). Thus, in expanding pavement cells, activation of the putative SCAR complex apparently cannot be the only pathway through which ROP2 acts to stimulate F-actin assembly. Indeed, recent work analyzing interactions between ROP2 and CRIB-domain-containing RIC proteins has shown that, as for ROP1 in pollen tubes, ROP2/4 stimulates cortical F-actin assembly in expanding pavement cells via interaction with RIC4 (Fu et al. 2005). GFP-RIC4 is localized to sites of incipient lobe formation and lobe tips in young pavement cells, and this localization pattern is dependent upon ROP2/4 activity. Moreover, loss of RIC4 activity results in reduced accumulation of fine cortical F-actin and reduced outgrowth of lobes.

Interestingly, ROP2/4-mediated suppression of the formation of well-ordered cortical microtubule bundles also involves another CRIB-domain-containing protein, RIC1 (Fu et al. 2005). Similar to what was observed in plants with reduced ROP2/4 function, RIC1 overexpression causes transversely aligned microtubule bundles to form along the entire length of expanding pavement cells and reduces lobe outgrowth. Conversely, in *ric1* loss-of-function mutants, cortical microtubules are fewer, less bundled, and less well-ordered than they are in the neck regions of expanding wild-type pavement cells, and excess expansion of neck regions occurs. RIC1-GFP colocalizes with cortical microtubules; this localization is inhibited by expression of constitutively active ROP2, but is increased in mutants with reduced ROP2/4 function. Thus, RIC1 appears to mediate the formation of ordered arrays of transversely aligned cortical microtubules via a direct association with microtubules, and ROP2/4 activity inhibits this function of RIC1. RIC1 activity, in turn, suppresses cortical F-actin accumulation by inhibiting the interaction between ROP2 and RIC4. This effect of RIC1 is likely to be mediated by microtubules themselves because depolymerization of microtubules by oryzalin

treatment or by shifting *mor1-1* mutants to restrictive temperature enhances the RIC4-ROP2 interaction and increases cortical F-actin accumulation (Fu et al. 2005).

Together, these observations support the following model to explain the patterning of pavement cell growth via ROP-dependent activities of RIC1 and RIC4 (**Figure 5b**). Local enrichment of ROP2/4 activity at sites of lobe emergence promotes RIC4-dependent activation of cortical F-actin assembly and simultaneously suppresses RIC1-dependent formation of well-ordered cortical microtubule arrays in these areas. These effects of ROP2/4 cooperatively promote lobe outgrowth. Between sites of lobe emergence where ROP2/4 and RIC4 are less abundant, RIC1-dependent formation of transversely aligned cortical microtubule bundles can take place. Promotion of cortical microtubule alignment by RIC1 is self-reinforcing because the resulting microtubule arrays inhibit the ROP2/RIC4 interaction, further reducing the inhibition of RIC1 activity in neck regions. RIC1-dependent cortical microtubule arrays restrict cell expansion between lobes, amplifying the difference in growth rates between areas of the cell surface where lobes are emerging and neck regions between these lobes. This model goes a long way toward explaining cytoskeletal regulation of pavement cell growth pattern, but the question remains open as to what initially determines the sites where ROP2/4 will become enriched. Because growth patterns of adjacent cells must be coordinated, it seems likely that the initial localization of ROP2/4 enrichment sites depends on some form of cell-cell communication. Thus, important questions remain to be answered regarding the coordination of growth patterns among neighboring pavement cells.

CONCLUDING PERSPECTIVES

In recent years, dramatic advances have been made in our understanding of mechanisms regulating cytoskeletal dynamics and organization that are important for plant cell shape

determination. These advances have come from studies combining tools of genetics, genomics, molecular biology, cell biology, and biochemistry. However, much remains to be learned. For example, studies of the putative plant Arp2/3 complex have made it clear that the majority of F-actin in plant cells is nucleated in an Arp2/3-independent manner. Formins, which constitute a family of 21 predicted proteins in *Arabidopsis* (Deeks et al. 2002), are likely to serve as the primary F-actin nucleators in plant cells, but have only begun to be studied. A multitude of microtubule and actin-binding proteins are known to be important for cell growth and its spatial regulation in plants, but their precise roles remain to be elucidated. Another area still awaiting major breakthroughs is that of understanding how cytoskeletal filaments promote or spa-

tially regulate growth. More than a decade after the initial formulation of the cortical microtubule/cellulose microfibril co-alignment hypothesis, basic questions are still being asked about the microtubule/microfibril relationship, and the molecular nature of postulated linkages between microtubules and cellulose microfibrils remains a mystery. The precise mechanisms by which actin filaments promote and spatially regulate growth are also largely obscure, and there may be many of them. Elucidating the mechanisms by which cytoskeletal filaments control the spatial distribution of growth will most likely require innovative approaches employing tools not yet widely used to date, such as ultrastructural and biophysical analyses. Thus, many important challenges lie ahead in our progress toward understanding plant cell shape determination.

SUMMARY POINTS

1. The cytoskeleton plays key roles in the spatial regulation of plant cell growth primarily by influencing the pattern in which cell wall materials are deposited.
2. Microtubules are thought to control growth direction by influencing the pattern of cellulose deposition into the cell wall. A variety of models have been proposed to explain the precise nature of the microtubule/cellulose microfibril relationship.
3. Actin is essential for plant cell growth and also participates in the spatial regulation of growth, but the roles played by actin in these processes are only partially understood.
4. Polarization of pollen tube growth depends on spatially localized activities of ROP GTPases, which act through RIC effector proteins to stimulate actin polymerization and Ca^{2+} influx at the tip.
5. Trichome branch initiation is a microtubule- and actin-dependent process that may involve motor-driven transport of Golgi stacks to sites of branch initiation.
6. The putative actin-nucleating Arp2/3 complex is required for proper spatial distribution of trichome expansion and appears to be regulated by a complex of five proteins, including the Arp2/3 activator SCAR.
7. Lobe formation in epidermal pavement cells depends on the coordinated activities of microtubules and actin filaments, both of which are controlled by ROP GTPases. ROP-interacting RIC effector proteins promote the outgrowth of lobes by locally stimulating actin polymerization and restrict growth between lobes by stimulating microtubule alignment.

LITERATURE CITED

- Baluska F, Jasik J, Edelmann HG, Salajova T, Volkmann D. 2001. Latrunculin B-induced dwarfism: plant cell elongation is F-actin-dependent. *Dev. Biol.* 231:113–24
- Baskin TI. 2001. On the alignment of cellulose microfibrils by cortical microtubules: a review and a model. *Protoplasma* 215:150–71
- Baskin TI, Bivens NJ. 1995. Stimulation of radial expansion in *Arabidopsis* roots by inhibitors of actomyosin and vesicle secretion but not by various inhibitors of metabolism. *Planta* 197:514–21
- Basu D, El-Assal SE, Le J, Mallery EL, Szymanski DB. 2004. Interchangeable functions of *Arabidopsis* PIROGI and the human WAVE complex subunit SRA1 during leaf epidermal development. *Development* 131:4345–55
- Basu D, Le J, El-Assal SE, Huang S, Zhang C, et al. 2005. DISTORTED3/SCAR2 is a putative *Arabidopsis* WAVE complex subunit that activates the Arp2/3 complex and is required for epidermal morphogenesis. *Plant Cell* 17:502–24
- Brembu T, Winge P, Seem M, Bones AM. 2004. NAPP and PIRP encode subunits of a putative WAVE regulatory protein complex involved in plant cell morphogenesis. *Plant Cell* 16:2335–49
- Brugnera E, Haney L, Grimsley C, Lu M, Walk SF, et al. 2002. Unconventional Rac-GEF activity is mediated through the Dock180-ELMO complex. *Nat. Cell Biol.* 4:574–82
- Campbell P, Braam J. 1998. Co- and/or post-translational modifications are critical for TCH4 XET activity. *Plant J.* 15:553–61
- Chen CY, Cheung AY, Wu HM. 2003. Actin-depolymerizing factor mediates Rac/Rop GTPase-regulated pollen tube growth. *Plant Cell* 15:237–49
- Cheung AY, Wu HM. 2004. Overexpression of an *Arabidopsis* formin stimulates actin cable formation from pollen tube cell membrane. *Plant Cell* 16:257–69
- Deeks MJ, Hussey PJ, Davies B. 2002. Formins: intermediates in signal-transduction cascades that affect cytoskeletal reorganization. *Trends Plant Sci.* 7:492–98
- Deeks MJ, Kalariti D, Davies B, Malho R, Hussey PJ. 2004. *Arabidopsis* NAP1 is essential for Arp2/3-dependent trichome morphogenesis. *Curr. Biol.* 14:1410–14
- De Matteis MA, Di Girolamo M, Colanzi A, Pallas M, Di Tullio G, et al. 1994. Stimulation of endogenous ADP-ribosylation by brefeldin A. *Proc. Natl. Acad. Sci. USA* 91:1114–18
- Dong C-H, Xia G-X, Hong Y, Ramachandran S, Kost B, Chua N-H. 2001. ADF proteins are involved in the control of flowering and regulate F-actin organization, cell expansion, and organ growth in *Arabidopsis*. *Plant Cell* 13:1333–46
- Eden S, Rohtagi R, Podtelejnikov AV, Mann M, Kirschner M. 2002. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* 418:790–93
- El-Assal SE, Le J, Basu D, Mallery EL, Szymanski DB. 2004a. *Arabidopsis* GNARLED encodes a NAP125 homolog that positively regulates Arp2/3. *Curr. Biol.* 14:1405–9
- El-Assal SE, Le J, Basu D, Mallery EL, Szymanski DB. 2004b. DISTORTED2 encodes an ARPC2 subunit of the putative *Arabidopsis* ARP2/3 complex. *Plant J.* 38:526–38
- Fisher DD, Cyr RJ. 1998. Extending the microtubule/microfibril paradigm: cellulose synthesis is required for normal cortical microtubule alignment in elongating cells. *Plant Physiol.* 116:1043–51
- Fleming AJ, McQueen-Mason S, Mandel T, Kuhlemeier C. 1997. Induction of leaf primordia by the cell wall protein expansin. *Science* 276:1415–18
- Folkers U, Berger J, Hülskamp M. 1997. Cell morphogenesis of trichomes in *Arabidopsis*: differential control of primary and secondary branching by branch initiation regulators and cell growth. *Development* 124:3779–86

- Folkers U, Kirik V, Schobinger U, Falk S, Krishnakumar S, et al. 2002. The cell morphogenesis gene *ANGUSTIFOLIA* encodes a CtBP/BARS-like protein and is involved in the control of the microtubule cytoskeleton. *EMBO J.* 21:1280–88
- Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, et al. 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 422:442–46
- Frank M, Egile C, Dyachok J, Djakovic S, Nolasco M, et al. 2004. Activation of Arp2/3 complex-dependent actin polymerization by plant proteins distantly related to Scar/WAVE. *Proc. Nat. Acad. Sci. USA* 101:16379–84
- Frank MJ, Cartwright HN, Smith LG. 2003. Three *Brick* genes have distinct functions in a common pathway promoting polarized cell division and cell morphogenesis in the maize leaf epidermis. *Development* 130:753–62
- Frank MJ, Smith LG. 2002. A small, novel protein highly conserved in plants and animals promotes the polarized growth and division of maize leaf epidermal cells. *Curr. Biol.* 12:849–53
- Fu Y, Gu Y, Zheng Z, Wasteneys G, Yang Z. 2005. *Arabidopsis* interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. *Cell* 120:687–700**
- Fu Y, Li H, Yang Z. 2002. The ROP2 GTPase controls the formation of cortical fine F-actin and the early phase of directional cell expansion during *Arabidopsis* organogenesis. *Plant Cell* 14:777–94
- Fu Y, Wu G, Yang Z. 2001. Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. *J. Cell Biol.* 152:1019–32
- Gautreau A, Ho HY, Steen H, Gygi SP, Kirschner MW. 2004. Purification and architecture of the ubiquitous WAVE complex. *Proc. Natl. Acad. Sci. USA* 101:4379–83
- Geitmann A, Emons AMC. 2000. The cytoskeleton in plant and fungal cell tip growth. *J. Microsc.* 198:218–45
- Geitmann A, Li YQ, Cresti M. 1996. The role of the cytoskeleton and dictyosome activity in the pulsatory growth of *Nicotiana tabacum* and *Petunia hybrida* pollen tubes. *Bot. Acta* 109:102–9
- Gibbon BC, Kovar DR, Staiger CJ. 1999. Latrunculin B has different effects on pollen germination and tube growth. *Plant Cell* 11:2349–63
- Giddings THJ, Staehelin LA. 1991. Microtubule-mediated control of microfibril deposition: a re-examination of the hypothesis. In *The Cytoskeletal Basis of Plant Growth and Form*, ed. CW Lloyd, pp. 85–99. New York: Academic
- Gilliland LU, Kandasamy MK, Pawloski LC, Meagher RB. 2002. Both vegetative and reproductive actin isoforms complement the stunted root hair phenotype of the *Arabidopsis act 2-1* mutation. *Plant Physiol.* 130:2199–209
- Gu Y, Fu Y, Dowd P, Li S, Vernoud V, et al. 2005. A Rho-family GTPase controls actin dynamics and tip growth via two counteracting downstream pathways in pollen tubes. *J. Cell Biol.* 169:127–38**
- Gu Y, Wang Z, Yang Z. 2004. ROP/RAC GTPase: an old new master regulator for plant signaling. *Curr. Opin. Plant Biol.* 7:527–36
- Hepler PK, Vidali L, Cheung AY. 2001. Polarized cell growth in higher plants. *Annu. Rev. Cell Dev. Biol.* 17:159–87
- Higgs HN, Pollard TD. 2001. Regulation of actin filament network formation through the Arp2/3 complex: activation by a diverse array of proteins. *Annu. Rev. Biochem.* 70:649–76
- Holdaway-Clarke TL, Hepler PK. 2003. Control of pollen tube growth: role of ion gradients and fluxes. *New Phytol.* 159:539–63

A groundbreaking study demonstrating that ROP GTPases interact with two different RIC effector proteins to promote formation of a lobed morphology in epidermal pavement cells.

An important paper showing that ROP GTPases interact with one RIC protein to promote actin assembly and another to promote influx of Ca²⁺ at the tip.

Interestingly, results in this paper suggest that actin-based processes are important for trichome branch initiation, contradicting results from earlier pharmacological studies using actin inhibitors.

- Holweg C, Nick P. 2004. *Arabidopsis* myosin XI mutant is defective in organelle movement and polar auxin transport. *Proc. Nat. Acad. Sci. USA* 101:10488–93
- Huang S, Blanchoin L, Chaudhry F, Franklin-Tong VE, Staiger CJ. 2004. A gelsolin-like protein from *Papaver rhoeas* pollen (PrABP80) stimulates calcium-regulated severing and depolymerization of actin filaments. *J. Biol. Chem.* 279:23364–75
- Hülkamp M. 2000. How plants split hairs. *Curr. Biol.* 10:R308–10
- Hülkamp M, Miséra S, Jürgens G. 1994. Genetic dissection of trichome cell development in *Arabidopsis*. *Cell* 76:555–66
- Innocenti M, Zucconi A, Disanza A, Frittoli E, Areces L, et al. 2004. Abi1 is essential for the formation and activation of a WAVE2 signaling complex mediating Rac-dependent actin remodeling. *Nat. Cell Biol.* 6:319–27
- Jones MA, Shen J-J, Fu Y, Li H, Yang Z, Grierson CS. 2002. The *Arabidopsis* Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. *Plant Cell* 14:763–76
- Ketelaar T, Allwood EG, Anthony R, Voigt B, Menzel D, Hussey PJ. 2004. The actin-interacting protein AIP1 is essential for actin organization and plant development. *Curr. Biol.* 14:145–49
- Ketelaar T, de Ruijter NCA, Emons AMC. 2003. Unstable F-actin specifies the area and microtubule direction of cell expansion in *Arabidopsis* root hairs. *Plant Cell* 15:285–92
- Ketelaar T, Emons AMC. 2001. The cytoskeleton in plant cell growth: lessons from root hairs. *New Phytol.* 152:409–18
- Ketelaar T, Faivre-Moskalenko C, Esseling JJ, de Ruijter NCA, Grierson CS, et al. 2002. Positioning of nuclei in *Arabidopsis* root hairs: an actin-regulated process of tip growth. *Plant Cell* 14:2941–55
- Kim GT, Shoda K, Tsuge T, Cho KH, Uchimiya H, et al. 2002. The *ANGUSTIFOLIA* gene of *Arabidopsis*, a plant CtBP gene, regulates leaf-cell expansion, the arrangement of cortical microtubules in leaf cells and expression of a gene involved in cell-wall formation. *EMBO J.* 21:1267–79
- Kost B, Spielhofer P, Chua NH. 1998. A GFP-mouse talin fusion protein labels plant actin filaments in vivo and visualizes the actin cytoskeleton in growing pollen tubes. *Plant J.* 16:393–401
- Kovar DR, Drøbak BK, Staiger CJ. 2000. Maize profilin isoforms are functionally distinct. *Plant Cell* 12:583–98
- Lang T, Wacker I, Wunderlich I, Rohrbach A, Giese G, et al. 2000. Role of actin cortex in the subplasmalemmal transport of secretory granules in PC-12 cells. *Biophys. J.* 78:2863–77
- Le J, El-Assal SE, Basu D, Saad ME, Szymanski DB. 2003. Requirements for *Arabidopsis* *ATARP2* and *ATARP3* during epidermal development. *Curr. Biol.* 13:1341–47
- Li H, Lin Y, Heath RM, Zhu MX, Yang Z. 1999. Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. *Plant Cell* 11:1731–42
- Li S, Blanchoin L, Yang Z, Lord EM. 2003. The putative *Arabidopsis* Arp2/3 complex controls leaf cell morphogenesis. *Plant Physiol.* 132:2034–44
- Li Y, Sorefan K, Hemmann G, Bevan MW. 2004. *Arabidopsis* NAP and PIR regulate actin-based cell morphogenesis and multiple developmental processes. *Plant Physiol.* 136:3616–27
- Lin Y, Wang Y, Zhu J-K, Yang Z. 1996. Localization of a Rho GTPase implies a role in tip growth and movement of the generative cell in pollen tubes. *Plant Cell* 8:293–303
- Lin Y, Yang Z. 1997. Inhibition of pollen tube elongation by microinjected anti-Rop1 antibodies suggests a crucial role for Rho-type GTPases in the control of tip growth. *Plant Cell* 9:1647–59

- Lloyd CW, Chan J. 2004. Microtubules and the shape of plants to come. *Nat. Rev. Mol. Cell Biol.* 5:13–22
- Lu L, Lee YR, Pan R, Maloof JN, Liu B. 2005. An internal motor kinesin is associated with the Golgi apparatus and plays a role in trichome morphogenesis in *Arabidopsis*. *Mol. Biol. Cell* 16:811–23**
- Luo D, Oppenheimer DG. 1999. Genetic control of trichome branch number in *Arabidopsis*: the roles of the *FURCA* loci. *Development* 126:5547–57
- Marks MD, Esch J, Herman P, Sivakumaran S, Oppenheimer D. 1991. A model for cell-type determination and differentiation in plants. In *Molecular Biology of Plant Development*, ed. G Jenkins, W Schuch, pp. 259–75. Cambridge, UK: Co. Biol. Ltd.
- Mathur J. 2004. Cell shape development in plants. *Trends Plant Sci.* 9:583–90
- Mathur J, Chua N-H. 2000. Microtubule stabilization leads to growth reorientation in *Arabidopsis* trichomes. *Plant Cell* 12:465–77
- Mathur J, Spielhofer P, Kost B, Chua N. 1999. The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in *Arabidopsis thaliana*. *Development* 126:5559–68
- Mathur J, Mathur N, Kernebeck B, Hülskamp M. 2003a. Mutations in actin-related proteins 2 and 3 affect cell shape development in *Arabidopsis*. *Plant Cell* 15:1632–45
- Mathur J, Mathur N, Kirik V, Kernebeck B, Srinivas BP, Hülskamp M. 2003b. *Arabidopsis* *CROOKED* encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. *Development* 130:3137–46
- McKinney EC, Kandasamy MK, Meagher RB. 2001. Small changes in the regulation of one *Arabidopsis* profilin isovariant, PRF1, alter seedling development. *Plant Cell* 13:1179–91
- Meller N, Irani-Tehrani M, Kiosses WB, Del Pozo MA, Schwartz MA. 2002. Zizimin1, a novel Cdc42 activator, reveals a new GEF domain for Rho proteins. *Nat. Cell Biol.* 4:639–47
- Miller DD, de Ruijter NCA, Bisseling T, Emons AMC. 1999. The role of actin in root hair morphogenesis: studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. *Plant J.* 17:141–54
- Miller DD, Lancelle SA, Hepler PK. 1996. Actin microfilaments do not form a dense meshwork in *Lilium longiflorum* pollen tube tips. *Protoplasma* 195:123–32
- Molendijk A, Bischoff F, Rajendrakumar CSV, Friml J, Braun M, et al. 2001. *Arabidopsis thaliana* Rop GTPases are localized to tips of root hairs and control polar growth. *EMBO J.* 20:2779–88
- Nardini M, Spano S, Cericola C, Pesce A, Massaro A, et al. 2003. CtBP/BARS: a dual-function protein involved in transcription co-repression and Golgi membrane fission. *EMBO J.* 22:3122–30
- Nebenführ A, Gallagher LA, Dunahay TG, Frohlick JA, Mazurkiewicz AM, et al. 1999. Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. *Plant Physiol.* 121:1127–42
- Oppenheimer DG, Pollock MA, Vacik J, Szymanski DB, Ericson B, et al. 1997. Essential role of a kinesin-like protein in *Arabidopsis* trichome morphogenesis. *Proc. Nat. Acad. Sci. USA* 94:6261–66
- Qiu JL, Jilk R, Marks MD, Szymanski DB. 2002. The *Arabidopsis* *SPIKE1* gene is required for normal cell shape control and tissue development. *Plant Cell* 14:101–18
- Pien S, Wyrzykowska J, McQueen-Mason S, Smart C, Fleming A. 2001. Local expression of expansin induces the entire process of leaf development and modifies leaf shape. *Proc. Nat. Acad. Sci. USA* 98:11812–17
- Pollard TD, Borisy GG. 2003. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112:453–65

This report provides direct evidence that microtubule-dependent Golgi transport is critical for trichome morphogenesis in *Arabidopsis*.

- Ramachandran S, Christensen HEM, Ishimaru Y, Dong C-H, Chao-Ming W, et al. 2000. Profilin plays a role in cell elongation, cell shape maintenance, and flowering in *Arabidopsis*. *Plant Physiol.* 124:1637-47
- Reddy AS, Day IS. 2000. The role of the cytoskeleton and a molecular motor in trichome morphogenesis. *Trends Plant Sci.* 5:503-5
- Reddy AS, Day IS. 2001. Kinesins in the *Arabidopsis* genome: a comparative analysis among eukaryotes. *BMC Genomics* 2:2
- Reddy ASN, Narasimhulu SB, Safadi F, Golovkin M. 1996a. A plant kinesin heavy chain-like protein is a calmodulin-binding protein. *Plant J.* 10:9-21
- Reddy ASN, Safadi F, Narasimhulu SB, Golovkin M, Hu X. 1996b. A novel plant calmodulin-binding protein with a kinesin heavy chain motor domain. *J. Biol. Chem.* 271:7052-60
- Reddy VS, Reddy AS. 1999. A plant calmodulin-binding motor is part kinesin and part myosin. *Bioinformatics* 15:1055-57
- Rédei GP. 1962. Single locus heterosis. *Z. Vererbungsleh.* 93:164-70
- Rose JK, Braam J, Fry SC, Nishitani K. 2002. The XTH family of enzymes involved in xyloglucan endotransglucosylation and endohydrolysis: current perspectives and a new unifying nomenclature. *Plant Cell Physiol.* 43:1421-35
- Saedler R, Mathur N, Srinivas BP, Kernebeck B, Hülskamp M, Mathur J. 2004. Actin control over microtubules suggested by *DISTORTED2* encoding the *Arabidopsis* ARPC2 subunit homolog. *Plant Cell Physiol.* 45:813-22
- Schaeper U, Subramanian T, Lim L, Boyd JM, Chinnadurai G. 1998. Interaction between a cellular protein that binds to the C-terminal region of adenovirus E1A (CtBP) and a novel cellular protein is disrupted by E1A through a conserved PLDLs motif. *J. Biol. Chem.* 273:8549-52
- Schwab B, Mathur J, Saedler R, Schwarz H, Frey B, et al. 2003. Regulation of cell expansion by the *DISTORTED* genes in *Arabidopsis thaliana*: actin controls the spatial organization of microtubules. *Mol. Genet. Genomics* 269:350-60
- Smith LG. 2003. Cytoskeletal control of plant cell shape: getting the fine points. *Curr. Opin. Plant Biol.* 6:63-73
- Spano S, Silletta MG, Colanzi A, Alberti S, Fiucci G, et al. 1999. Molecular cloning and functional characterization of brefeldin A-ADP-ribosylated substrate. A novel protein involved in the maintenance of the Golgi structure. *J. Biol. Chem.* 274:17705-10
- Steffen A, Rottner K, Ehinger J, Innocenti M, Scita G, et al. 2004. Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. *EMBO J.* 23:749-59
- Sugimoto K, Himmelspach R, Williamson RE, Wasteneys GO. 2003. Mutation or drug-dependent microtubule disruption causes radial swelling without altering parallel cellulose microfibril deposition in *Arabidopsis* root cells. *Plant Cell* 15:1414-29
- Szymanski DB. 2005. Breaking the WAVE complex: the point of *Arabidopsis* trichomes. *Curr. Opin. Plant Biol.* 8:103-112**
- Szymanski DB, Marks MD, Wick SM. 1999. Organized F-actin is essential for normal trichome morphogenesis in *Arabidopsis*. *Plant Cell* 11:2331-47
- Thimann KV, Reese K, Nachmias VT. 1992. Actin and the elongation of plant cells. *Protoplasma* 171:153-66
- Tsuge T, Tsukaya H, Uchimiya H. 1996. Two independent and polarized processes of cell elongation regulate leaf blade expansion in *Arabidopsis thaliana* (L.) Heynh. *Development* 122:1589-600
- Vidali L, McKenna ST, Hepler PK. 2001. Actin polymerization is essential for pollen tube growth. *Mol. Biol. Cell* 12:2534-45

An in-depth review of the role of the putative Arp2/3 complex in *Arabidopsis* trichome morphogenesis and its regulation by a putative SCAR complex.

- Vissenberg K, Fry SC, Pauly M, Höfte H, Verbelen JP. 2005. XTH acts at the microfibril-matrix interface during cell elongation. *J. Exp. Bot.* 56:673–83
- Vissenberg K, Fry SC, Verbelen JP. 2001. Root hair initiation is coupled to a highly localized increase of xyloglucan endotransglycosylase action in *Arabidopsis* roots. *Plant Physiol.* 127:1125–35
- Wang Y-F, Fan L-M, Zhang W-Z, Zhang W, Wu W-H. 2004. Ca²⁺-permeable channels in the plasma membrane of *Arabidopsis* pollen are regulated by actin microfilaments. *Plant Physiol.* 136:3892–904
- Wang Y-S, Motes CM, Mohamalawari DR, Blancaflor EB. 2004. Green fluorescent protein fusions to *Arabidopsis* Fimbrin 1 for spatio-temporal imaging of F-actin dynamics in roots. *Cell Motil. Cytoskel.* 59:79–93
- Wasteneys GO. 2004. Progress in understanding the role of microtubules in plant cells. *Curr. Opin. Plant Biol.* 7:651–60**
- Wasteneys GO, Galway ME. 2003. Remodeling the cytoskeleton for growth and form: an overview with some new views. *Annu. Rev. Plant Biol.* 54:691–722
- Wasteneys GO, Yang Z. 2004. New views on the plant cytoskeleton. *Plant Physiol.* 136:3884–91
- Weigert R, Silletta MG, Spano S, Turacchio G, Cericola C, et al. 1999. CtBP/BARS induces fission of Golgi membranes by acylating lysophosphatidic acid. *Nature* 402:429–33
- Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, et al. 2001. MOR1 is essential for organizing cortical microtubules in plants. *Nature* 411:610–13
- Zhang X, Dyachok J, Krishnakumar S, Smith LG, Oppenheimer DG. 2005b. The *Arabidopsis* *IRREGULAR TRICHOME BRANCH 1 (ITB1)* gene encodes a plant homolog of the Arp2/3 complex activator Scar/WAVE that regulates actin and microtubule organization. *Plant Cell*. In press
- Zhang X, Grey PH, Krishnakumar S, Oppenheimer DG. 2005a. The *IRREGULAR TRICHOME BRANCH* loci regulate trichome elongation in *Arabidopsis*. *Plant Cell Physiol.* 17:1–13
- Zhong R, Burk DH, Morrison WH 3rd, Ye ZH. 2002. A kinesin-like protein is essential for oriented deposition of cellulose microfibrils and cell wall strength. *Plant Cell* 14:3101–17**
- Zimmermann I, Saedler R, Mutondo M, Hülskamp M. 2004. The *Arabidopsis* *GNARLED* gene encodes the NAP125 homolog and controls several actin-based cell shape changes. *Mol. Genet. Genomics* 272:290–96

The microfibril length regulation hypothesis presented in this review accounts for how radial expansion can occur in the *mor 1-1* mutant at restrictive temperature before changes in cellulose microfibril orientation.

This paper analyzes the *fra1* mutant in *Arabidopsis*, showing normal cell wall polymer composition and cortical microtubule organization but altered patterns of cellulose microfibril deposition.



Contents

Frontispiece <i>David D. Sabatini</i>	xiv
In Awе of Subcellular Complexity: 50 Years of Trespassing Boundaries Within the Cell <i>David D. Sabatini</i>	1
Mechanisms of Apoptosis Through Structural Biology <i>Nieng Yan and Yigong Shi</i>	35
Regulation of Protein Activities by Phosphoinositide Phosphates <i>Verena Niggli</i>	57
Principles of Lysosomal Membrane Digestion: Stimulation of Sphingolipid Degradation by Sphingolipid Activator Proteins and Anionic Lysosomal Lipids <i>Thomas Kolter and Konrad Sandhoff</i>	81
Cajal Bodies: A Long History of Discovery <i>Mario Cioce and Angus I. Lamond</i>	105
Assembly of Variant Histones into Chromatin <i>Steven Henikoff and Kami Ahmad</i>	133
Planar Cell Polarization: An Emerging Model Points in the Right Direction <i>Thomas J. Klein and Marek Mlodzik</i>	155
Molecular Mechanisms of Steroid Hormone Signaling in Plants <i>Grégory Vert, Jennifer L. Nemhauser, Niko Geldner, Fangxin Hong, and Joanne Chory</i>	177
Anisotropic Expansion of the Plant Cell Wall <i>Tobias I. Baskin</i>	203
RNA Transport and Local Control of Translation <i>Stefan Kindler, Huidong Wang, Dietmar Richter, and Henri Tiedge</i>	223

Rho GTPases: Biochemistry and Biology <i>Aron B. Jaffe and Alan Hall</i>	247
Spatial Control of Cell Expansion by the Plant Cytoskeleton <i>Laurie G. Smith and David G. Oppenheimer</i>	271
RNA Silencing Systems and Their Relevance to Plant Development <i>Frederick Meins, Jr., Azeddine Si-Ammour, and Todd Blevins</i>	297
Quorum Sensing: Cell-to-Cell Communication in Bacteria <i>Christopher M. Waters and Bonnie L. Bassler</i>	319
Pushing the Envelope: Structure, Function, and Dynamics of the Nuclear Periphery <i>Martin W. Hetzer, Tobias C. Walther, and Iain W. Mattaj</i>	347
Integrin Structure, Allostery, and Bidirectional Signaling <i>M.A. Arnaout, B. Mahalingam, and J.-P. Xiong</i>	381
Centrosomes in Cellular Regulation <i>Stephen Doxsey, Dannel McCollum, and William Theurkauf</i>	411
Endoplasmic Reticulum–Associated Degradation <i>Karin Römisch</i>	435
The Lymphatic Vasculature: Recent Progress and Paradigms <i>Guillermo Oliver and Kari Alitalo</i>	457
Regulation of Root Apical Meristem Development <i>Keni Jiang and Lewis J. Feldman</i>	485
Phagocytosis: At the Crossroads of Innate and Adaptive Immunity <i>Isabelle Futras and Michel Desjardins</i>	511
Protein Translocation by the Sec61/SecY Channel <i>Andrew R. Osborne, Tom A. Rapoport, and Bert van den Berg</i>	529
Retinotectal Mapping: New Insights from Molecular Genetics <i>Greg Lemke and Michael Reber</i>	551
In Vivo Imaging of Lymphocyte Trafficking <i>Cornelia Halin, J. Rodrigo Mora, Cenk Sumen, and Ulrich H. von Andrian</i>	581
Stem Cell Niche: Structure and Function <i>Linbeng Li and Ting Xie</i>	605
Docosahexaenoic Acid, Fatty Acid–Interacting Proteins, and Neuronal Function: Breastmilk and Fish Are Good for You <i>Joseph R. Marszalek and Harvey F. Lodish</i>	633
Specificity and Versatility in TGF- β Signaling Through Smads <i>Xin-Hua Feng and Rik Derynck</i>	659

The Great Escape: When Cancer Cells Hijack the Genes for
Chemotaxis and Motility
John Condeelis, Robert H. Singer, and Jeffrey E. Segall 695

INDEXES

Subject Index 719
Cumulative Index of Contributing Authors, Volumes 17–21 759
Cumulative Index of Chapter Titles, Volumes 17–21 762

ERRATA

An online log of corrections to *Annual Review of Cell and Developmental Biology* chapters may be found at <http://cellbio.annualreviews.org/errata.shtml>