Advances in Plant Biology 2

Bo Liu *Editor*

The Plant Cytoskeleton

Springer

Advances in Plant Biology

Series Editor
J. Harada, Davis, California, USA

For other titles published in this series, go to
www.springer.com/series/8047

Bo Liu
Editor

The Plant Cytoskeleton

Springer

Editor
Bo Liu
Department of Plant Biology
University of California
1 Shields Avenue
Davis, CA 95616, USA
bliu@ucdavis.edu

ISBN 978-1-4419-0986-2 e-ISBN 978-1-4419-0987-9
DOI 10.1007/978-1-4419-0987-9
Springer New York Dordrecht Heidelberg London

Preface

This book is blessed with 14 wonderful chapters aimed at summarizing in part the current knowledge on the structure and function of plant microtubules and actin filaments. Since the initial discovery of microtubules in plant cells in 1963 and the visualization of green algal actin filaments in 1974, their dynamic behaviors and their roles in specific cellular functions have been embraced one after another. While plant cell biologists continue to be excited by the beautiful cytoskeletal network in plant cells, their understanding of the function behind the network has been greatly advanced by discoveries of novel proteins that interact with the network. Recent progress has benefited from technological advances in areas like live cell imaging, genetic screening, and the tools of genomics and proteomics.

In this book, the first six chapters visit the molecular basis of the plant cytoskeleton. Since the first plant genome was sequenced, the number of genes encoding cytoskeletal proteins has stunned us. From isolating actin and tubulin genes to uncovering those encoding myosins and kinesins, plant biologists have made tremendous progress in the past decade or so. Nineteen years ago when microtubule-translocating activities were first demonstrated in isolated phragmoplasts, no one could have predicted that the little Arabidopsis plant would have more microtubule-based motor kinesins than are encoded in the human genome. In the post-genomic era, new avenues have opened and are leading to explosive discoveries made by mining sequenced genomes and characterizing the functions of proteins encoded by novel cytoskeletal genes. Undoubtedly, characterizing the proteins that interact with the plant cytoskeletal network becomes a task that is integral for our understanding of plant evolution.

The second part of the book includes three chapters that cover how microtubules are arrayed during plant cell division. It is both puzzling and fascinating that plant cells are able to organize microtubules into arrays like the preprophase band, the bipolar spindle, and the phragmoplast in the absence of a structurally defined organizing center. Fortunately, the molecular mechanisms underlying the organization of these arrays are emerging. Again, advances made in this area will provide many clues regarding how land plants have evolved.

The book ends with a section devoted to connecting the cytoskeleton with plant growth and development. These five chapters summarize current knowledge on the mechanisms that regulate different patterns of cell growth as well as on how the

growth of whole organs is regulated by the cytoskeleton. Plants demonstrate remarkable patterns of growth, and their tissues and organs are built on cells of splendid shapes. How plant cells acquire their distinct shapes is an intriguing question. After their birth in the meristem, plant cells respond to internal and external cues that direct the acquisition of specific roles after differentiation. Once these cues are read, the cytoskeletal network is remodeled and it guides changes to the cell wall that influence cell shape. These changes, ultimately, determine the shape of the plant and they reflect adaptations to environmental conditions that plants have made over millions of years.

This book is not intended to cover every aspect of the plant cytoskeleton. Besides serving as a convenient reference, it is intended to generate enthusiasm amongst young scientists to join the efforts to dig out the root of the plant cytoskeleton. The plant cytoskeleton is no longer a subject reserved for cell biologists. Its impacts on plant growth and development can no longer be overstated. I hope that the chapters included in this book inspire additional in-depth studies focused not only on the cytoskeleton, but also on its links with developmental phenomena and plant responses to the environment.

Much of what we have learned about the plant cytoskeleton has been inspired by the careful observations of many pioneers in the field of plant cell biology. I am particularly indebted to my mentor Prof. Barry A. Palevitz who has been an inspirational source for me and others through his discoveries and visions.

Davis, CA Bo Liu

Contents

Contributors

Katherine L. Baldwin
Department of Genetics, University of Wisconsin – Madison, 425G Henry Mall, Madison, WI 53706, USA

Sherryl R. Bisgrove
Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6
sbisgrov@sfu.ca

David Bouchez
Institut Jean-Pierre Bourgin, Station de Génétique et Amélioration des Plantes, INRA Centre de Versailles, 78026, Versailles, Cedex, France
bouchez@versailles.inra.fr

Richard Cyr
Department of Biology, The Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802-5301, USA
rjc8@psu.edu

Irene S. Day
Department of Biology, Program in Molecular Plant Biology, Program in Cell and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA

Yann Duroc
Institut Jean-Pierre Bourgin, Station de Génétique et Amélioration des Plantes, INRA Centre de Versailles, 78026, Versailles, Cedex, France

Ying Fu
State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, People's Republic of China
yingfu@cau.edu.cn

Mitsuyasu Hasebe
National Institute for Basic Biology, 38 Nishigonaka, Myodaiji-cho, Okazaki, Japan

Takashi Hashimoto
Nara Institute of Science and Technology,
Graduate School of Biological Sciences, Ikoma, Nara 630-0192, Japan
hasimoto@bs.naist.jp

Chin-Min Kimmy Ho
Department of Plant Biology, University of California, Davis, CA 95616, USA

Takashi Hotta
Department of Plant Biology, University of California, Davis, CA 95616, USA

Shanjin Huang
Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Gengxiang Jia
Department of Genetics, University of Wisconsin – Madison, 425G Henry Mall, Madison, WI 53706, USA

Muthugapatti K. Kandasamy
Department of Genetics, Davison Life Sciences Building, University of Georgia, Athens, GA 30602, USA

Lori King
Department of Genetics, Davison Life Sciences Building, University of Georgia, Athens, GA 30602, USA

Yuh-Ru Julie Lee
Department of Plant Biology, University of California, Davis, CA 95616, USA

Bo Liu
Department of Plant Biology, University of California, 1 Shields Avenue, Davis, CA 95616, USA
bliu@ucdavis.edu

Clive Lloyd
John Innes Centre, Colney Lane, Norwich, UK

Jennelle L. Malcos
Department of Biology, The Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802-5301, USA

Patrick H. Masson
Department of Genetics, University of Wisconsin – Madison, 425G Henry Mall, Madison, WI 53706, USA
phmasson@wisc.edu

Richard B. Meagher
Department of Genetics, Davison Life Sciences Building, University of Georgia, Athens, GA 30602, USA
meagher@uga.edu

Takashi Murata
National Institute for Basic Biology, 38 Nishigonaka, Myodaiji-cho, Okazaki, Japan
tkmurata@nibb.ac.jp

Andreas Nebenführ
Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, 1414 Cumberland Ave, Knoxville, TN 37996-0840, USA
nebenfuehr@utk.edu

Eunsook Park
Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, 1414 Cumberland Ave, Knoxville, TN 37996-0840, USA

Martine Pastuglia
Institut Jean-Pierre Bourgin, Station de Génétique et Amélioration des Plantes, INRA Centre de Versailles, 78026, Versailles, Cedex, France

Edouard Pesquet
John Innes Centre, Colney Lane, Norwich, UK
and Umeå Plant Science Centre, Umeå University, Umeå 901 87, Sweden
Edouard.Pesquet@plantphys.umu.se

A.S.N. Reddy
Department of Biology, Program in Molecular Plant Biology, Program in Cell and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA
reddy@colostate.edu

Haiyun Ren
College of Life Science, Beijing Normal University, Beijing 100875, China
hren@bnu.edu.cn

Teruo Shimmen
Department of Life Science, Graduate School of Life Science, University of Hyogo, Harima Science Park City, Hyogo, 678-1297, Japan

Allison K. Strohm
Department of Genetics, University of Wisconsin – Madison, 425G Henry Mall, Madison, WI 53706, USA

Laura M. Vaughn
Department of Genetics, University of Wisconsin – Madison, 425G Henry Mall, Madison, WI 53706, USA

Julian C. Verdonk
Department of Genetics, University of Wisconsin – Madison, 425G Henry Mall, Madison, WI 53706, USA

Yun Xiang
School of Life Sciences, Lanzhou University, Lanzhou, Gansu 730000, China

Zhenbiao Yang
Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA

Etsuo Yokota
Department of Life Science, Graduate School of Life Science, University of Hyogo, Harima Science Park City, Hyogo, 678-1297, Japan
yokota@sci.u-hyogo.ac.jp

Robin E. Young
Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6

Part I
Molecular Basis of the Plant Cytoskeleton

Chapter 1
Actin Functions in the Cytoplasmic and Nuclear Compartments

Richard B. Meagher, Muthugapatti K. Kandasamy, and Lori King

1.1 Introduction

Eukaryotes share conserved roles for the actin cytoskeleton in cell polarity determination, cell division, vesicle transport, organelle movement, and newly described nuclear activities. In plants and animals, the number of genes encoding components of the actin system is expanded and individual genes are specialized to participate in directing multicellular development. With more than a dozen gene families encoding multiple actin and actin binding protein (ABP) variants, actin cytoskeletal proteins have a particularly high degree of combinatorial complexity in angiosperms, rivaling or exceeding that in mammals. A genome wide duplication event several hundred million years ago in a lycopod-like ancestor likely generated the two most divergent classes found within most families: the vegetative or constitutive protein variants and the reproductive protein variants. Subsequent gene duplication events allowed further subfunctionalization of the plant cytoskeletal gene and protein variant families. A major scientific focus of our research program and a theme throughout this review is the importance of dynamic interactions among ancient actin and ABP gene and protein family members resulting both from their differential regulation and from the differential protein-protein interactions of multiple protein variants.

This review will focus on recent genetic and molecular cell biological studies that have advanced our understanding of plant actin cytoskeletal dynamics and its role in various cellular functions including establishment of cell polarity, elongation of cells, signaling within cells, movement of cargo, and control of both genetic and epigenetic regulation. Divergent actin, actin depolymerizing factor (ADF/cofilins), profilin, and myosin variants have independent and often non-overlapping protein activities supporting different aspects of cell and organ development.

Recent studies have better resolved cell biological functions for the actin cytoskeleton. For instance, plant organelles tethered to actin filaments move independently

R.B. Meagher (✉)
Department of Genetics, Davison Life Sciences Building,
University of Georgia, Athens, GA 30602, USA
e-mail: meagher@uga.edu

B. Liu (ed.), *The Plant Cytoskeleton*, Advances in Plant Biology 2,
DOI 10.1007/978-1-4419-0987-9_1,

around plant cells on myosin motors in a relatively stationary cytoplasm. In some elongating plant cells, growth from the barbed end of actin filaments, filament severing, and changes in filament convolution are the dominant activities that reshape the cytoskeleton, undoubtedly affecting cargo movement. Actin and ADF variants have direct roles in glucose signaling, pathogen response, day-length control of flowering, cold response, gravity detection and response, drought stress signaling of guard cell closure, and control of the cell cycle.

This article will also address the nuclear functions of actin cytoskeletal proteins, proteins once thought to be purely cytoplasmic (e.g. actin, ADF, profilin, myosin). Conventional cytoplasmic actins and several ABPs are found at relatively high concentrations in plant nuclei with possible roles in nuclear cargo movement, transcription, regulation of chromatin structure, and epigenetic control of gene expression. Strictly nuclear components like the nuclear plant actin-related proteins (ARPs) and numerous actin-interacting proteins that function only in the nucleus such as Swi2/Snf2-related DNA dependent ATPases will be considered only tangentially and have been dealt to a significant extent in previous reviews [22, 96, 101, 103, 145]. To maintain focus on the roles of cytoplasmic and nuclear actins and their dynamics in the control of plant cell and organismal development, the functions of the plant ARP2/3 complex will not be addressed here, in spite of a new rich literature on this complex's role in plant growth and morphogenesis [11, 37, 77, 84, 133].

1.2 Evolutionary Origin and Phylogeny of Plant Actins and Actin Binding Proteins

Even in the compact Arabidopsis genome, the actin cytoskeletal genome is complex; it comprises more than 130 known genes divided into nearly a dozen gene families, each encoding between 3 and 21 proteins variants [95]. The estimated sizes of the actin and most ABP families in Arabidopsis are listed in Table 1.1. Since their common ancestry with the earliest land plants like moss ~600 MYA (million years ago), ancient genome-wide duplication events played a significant role in the evolution of this complex system of cytoskeletal genes in higher plants. In contrast to the higher plants, a brief survey of the literature and gene sequence databases suggests that the actins and many ABPs in the moss *Physcomitrella patens* are encoded by smaller or less divergent gene families or by gene singlets [5, 152, 154] (unpublished observations) as compared to Arabidopsis (Table 1.1).

In the history of Arabidopsis, at least three genome-wide duplication events occurred at an estimated 400, 150, and 40 MYA as summarized in Fig. 1.1a [14, 28, 51, 164]. Estimating the dates of these events and correlating them with plant morphological and gene family evolution is useful to understanding gene function. With the essentially complete genome level sequence data now available from Arabidopsis, Populus, Rice, Sorghum, Selaginella, and Physcomitrella and some green alga, we can expect even more precise dating of genome-wide and cytoskeletal gene duplication events in the near future.

Table 1.1 The plant actin cytoskeletal components are encoded primarily by gene families: Estimated family sizes in *Arabidopsis*[a]

Gene	# Members
Actin	8[b]
ADF/cofilin	11
Profilin	5
Formin	21
Annexin	9
ARPs (actin-related proteins)	8
Capping protein α and β subunits	2
CAP1	1
Myosins VIII	4
Myosins XI	13
Filamins	1
Fimbrins	5
Villins/gelsolins	5
Rho/Rop	11
Total	134

[a]Estimates of family size from TAIR and the literature [15, 29, 95, 114]
[b]Likely pseudogenes *ACT5* and *ACT9* are not included [93]

For more than 25 years, our laboratory has addressed the role of gene duplication in the evolution of actin cytoskeletal gene families [54, 95, 97, 102, 138]. Several of our independent estimates for the time of actin gene duplications reasonably agree with newer estimates of genome wide duplications (Fig. 1.1a), although we have not yet confirmed that the cytoskeletal gene duplication events and the genome duplication events were concurrent. One of the earliest gene duplication events (#1 in Fig. 1.1a) generated vegetative and/or nearly constitutive (V) and reproductive (R) classes of genes. We've argued that the fixation of these cytoskeletal paralogs from a genome-wide duplication was contingent upon the co-evolution of the first leaves as new structures derived from sterilized reproductive structures such as sporangia [107]. Novel or more restricted cytoskeletal functions in leaves may have provided the selective pressure for the subfunctionalization of these new classes of actins and ABPs. From this early event, approximately 350–500 MYA, we have proposed that all higher plants have separate vegetative and reproductive classes of actins and actin binding proteins such as Actin Depolymerizing Factors (ADF/cofilin) and profilins (PRF) (Fig. 1.1b–d). Genes in these separate classes are differentially regulated and their encoded protein variants are so divergent that they often do not fully complement mutants in the same complementary class of proteins [18, 64, 67, 104].

The actin, ADF/cofilin and profilin families and many of the ABP gene families listed in Table 1.1 encode ancient reproductive members that are most highly expressed in pollen and/or in pollen and ovules. In the Arabidopsis lineage, the third and most recent genome-wide duplication (24–65 MYA) is thought to have duplicated co-expressed, closely related pairs of actin and ABP genes in each family (Fig. 1.1a, #3) [58, 93, 98, 106, 107, 132]. The impact of this more recent event on

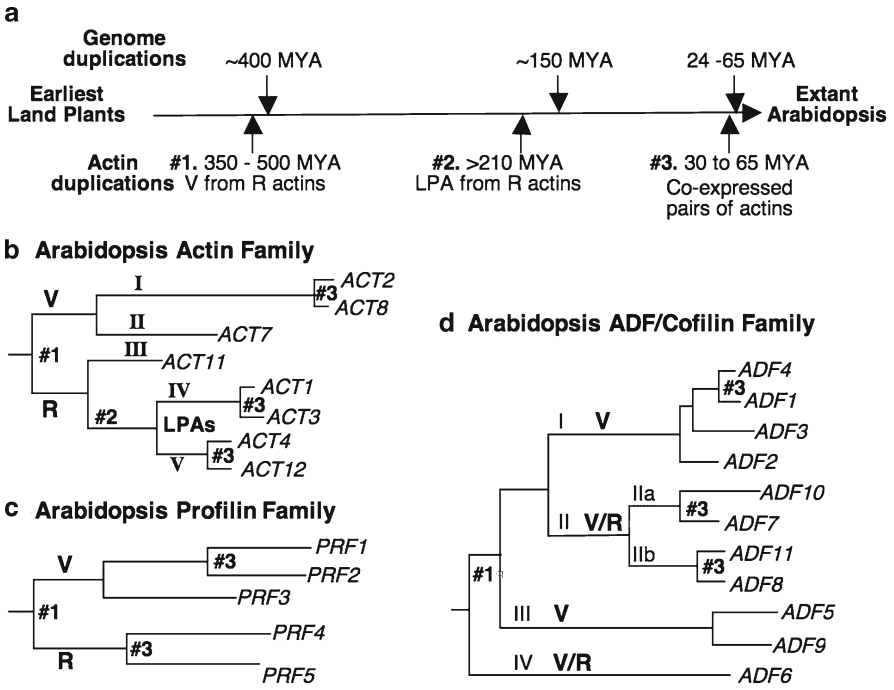


Fig. 1.1 Origin of cytoskeletal gene families by gene duplication. (**a**) Genome duplications vs. *ACTIN* gene duplications predicted in the history of Arabidopsis. Events #1 and #3 appear in the history of nearly all cytoskeletal gene families. Event #2, the origin of the late pollen actins (LPA), occurred in a shared ancestor separating dicots (e.g., Arabidopsis), monocots, and gnetales from gymnosperms. Dates of common ancestry in (**a**) were interpolated from published values [51, 100, 110, 161, 164]. (**b**–**d**) Three *Arabidopsis* cytoskeletal gene families, actin (**b**), profilin (**c**), and ADF/cofilin (**d**), which encode 8, 5, and 11 protein isovariants, respectively, are each shown in a neighbor-joining tree. Each family contains ancient subclasses that are differentially expressed in patterns defined as vegetative (all but pollen or ovules) and/or constitutive (all but pollen) (V) and reproductive (R). The duplication events numbered in (**a**) (#1, 2, 3) are used to label likely corresponding events in (**c**) and (**d**)

expression patterns and protein variant differences is less obvious and undoubtedly less significant.

Evidence for the second duplication comes from our analysis of the reproductive actin proteins; using an epitope-specific antibody that identifies the late pollen actins (LPAs), we demonstrated that an additional duplication event separated the LPAs from what is likely the basal reproductive actin more than 210 MYA (Fig. 1.1a) [66]. This event may also have separated the constitutively expressed vegetative actins (ACT2 and ACT8, Fig. 1.1b) from a more basal and highly regulated vegetative actin (ACT7, Fig. 1.1b). We are uncertain if the intermediate event(s) duplicating these actins was concurrent with the intermediate genome wide duplication described in Fig. 1.1a (*top*) or an endoduplication of an actin gene. Many of the other families of ABPs (Table 1.1) have interesting phylogenetic structures that need further quantification and dissection.

1.3 The Actin Cytoskeleton in Plant Cell Polarity and Elongation

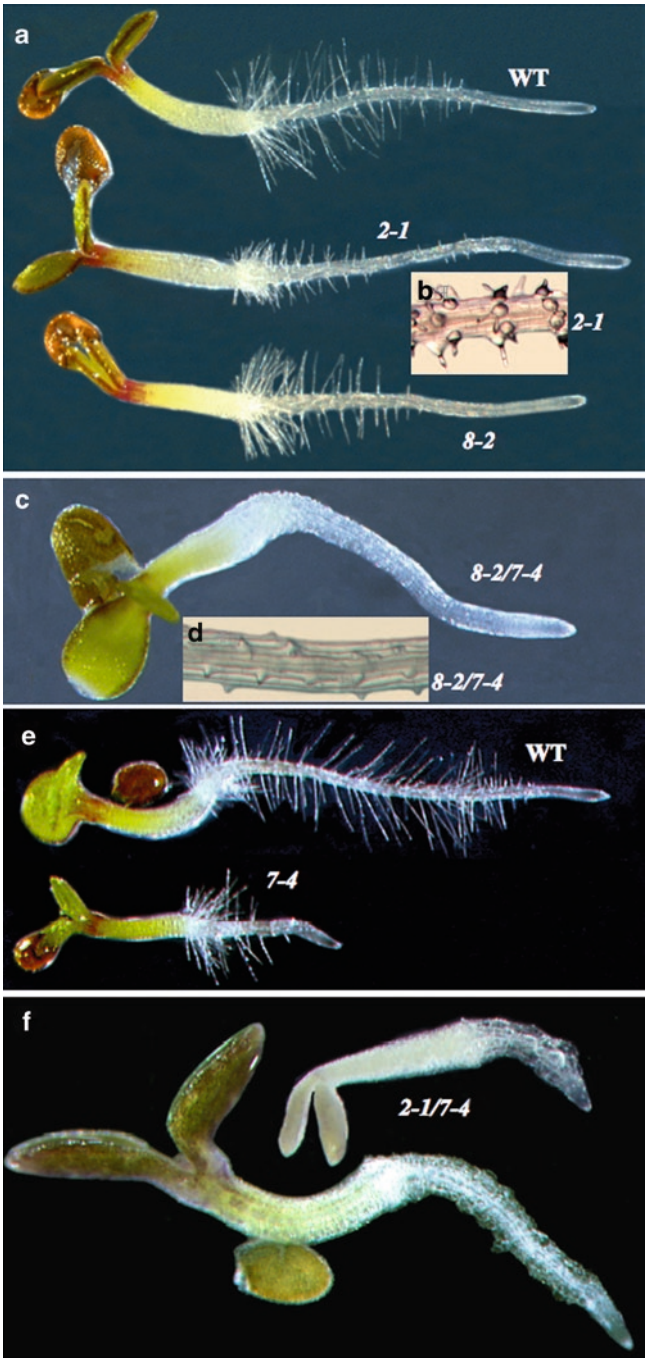
A combination of studies using fluorescent microscopy, histochemistry, and genetics demonstrate that actin and numerous ABPs are necessary for normal plant cell polarity and elongation [13, 19, 39, 52, 122, 154]. Actin filament nucleation, elongation, severing, turnover, and the dynamics among diverse cytoskeletal protein variants are thought to be at the heart of the process in plants, but only recently have scientists in the field begun to determine the importance of these components.

1.3.1 Genetic Studies Demonstrate the Role of Actin and ABP Variants in Cell Polarity and Elongation

Recent studies in Arabidopsis have been essential in determining the role of particular actin and ABP variants in plant cell polarity and elongation. Considering that the small Arabidopsis genome encodes eight actins and more than 120 ABPs in several families (Table 1.1), the protein variant dynamics of different protein-protein interactions is likely to be quite complex [95, 99, 104, 106]. Genetic dissection has been an essential and fundamental tool in separating the different functions of gene family members, especially when combined with biochemical, immunochemical, and cell biological approaches.

Subclass I vegetative actins (ACT2 and ACT8) constitute about 60 and 40% of the total actin expressed in wild type shoot and root tissues, respectively. Yet single mutants in ACT2 or ACT8 (Fig. 1.1b) have wild type levels of total actin in shoots and roots because of the up-regulation of ACT7 [69]. However, these single mutants are still defective in root hair cell elongation and tip growth with the two mutants producing root hairs about ½ to ¾ the length of wild type, respectively, as shown in Fig. 1.2a, b. The *act2-1/act8-2* double mutant has approximately 90 and 80% of total actin levels in shoots and roots, respectively, again because of increased levels of ACT7. Trichoblast cells in the double mutant roots do not develop past the trichoblast initiation and bulge formation stage (Fig. 1.2c, d). Although somewhat dwarfed at early seedling stages, the aboveground organ structures in the adult plants are normal [69]. Expression of ACT2 or ACT8 variants from the ACT2 promoter can fully complement the dwarfing, root hair growth, and cell polarity phenotypes in the double mutant. Thus, although subclass I actins ACT2 and ACT8 are the most highly expressed actins and essential for root hair elongation, they may not be indispensable to most of plant development due to the up-regulation of ACT7.

Arabidopsis subclass II actin ACT7 makes up about 40 and 60% of the total actin in shoot and root tissues, respectively. ACT7 mutant roots have about 40% of the level of total actin found in wild type [69], because neither ACT2 or ACT8 is sufficiently upregulated to compensate for the loss of ACT7. Thus, ACT7 mutants have highly dwarfed roots (Fig. 1.2e), but also produce relatively normal, albeit slightly dwarfed, aboveground adult organs. The polarity of ACT7-deficient root cells is highly skewed resulting in severely disorganized root cell files, abnormal alignment of nuclei, and poor cell elongation [45, 69].


a
WT
2-1
b
2-1
8-2
c
8-2/7-4
d
8-2/7-4
e
WT
7-4
f
2-1/7-4

Double vegetative actin mutant plants lacking both subclass I ACT2 and subclass II ACT7 express only 15–25% of total normal actin protein levels. These double mutant plants are extremely small and have severely dwarfed organs and pleiotropic phenotypes, making apportionment of particular functions difficult [44, 69]. For example, root epidermal cells are bulbous and no longer recognizable as trichoblasts or atrichoblasts in these double mutants (Fig. 1.2f).

Single knockout mutants in 11 of the 17 Arabidopsis myosins had no discernable aboveground plant morphological phenotype under laboratory growth conditions, suggesting there is some functional redundancy among these genes for their aboveground activities. Knockout mutants in myosin *XI-K* or *XI-2* have defective root hair elongation similar to *ACT2* and *ACT8* mutants [121]. Comparisons of single and double myosin mutants suggest that myosin *XI-K*, *XI1*, and *XI-B* have partially redundant and additive functions essential to normal root hair elongation.

Microscopic and genetic studies suggest that other Arabidopsis ABP gene families like those encoding the profilins, ADF/cofilins, and formins also participate in cell elongation and root hair development [10]. However, with a few exceptions, genetic analysis of genes from these three families is too preliminary to draw any definite conclusions about their roles in cell elongation and/or polarity. An Arabidopsis regulatory mutant *prf1-1* expressing about ½ the normal levels of PROFILIN1 (PRF1, PFN1) produces long hypocotyls and hypocotyl cells approximately 1.5 times the length of wild type and extra root hairs [94]. The *prf1-1* mutant phenotypes may speak more to the regulation of profilin than what occurs in a profilin defective plant. Moreover, the two most closely related constitutive profilins, PRF1 and PRF2, have different poly(L) proline and actin binding properties and are differentially localized in plant cells [156]. We need a more extensive genetic analysis of profilin variants to further characterize their molecular and developmental activities. The moss *Physcomitrella patens* has three profilin genes. The simultaneous silencing of all three greatly reduced cell tip growth and resulted in dwarf plants. The expression of any moss or lilly profilin complements the phenotype [152]. Hence, in moss, profilin is essential for tip growth, but particular pofilin variants may not have distinct activities in tip growth.

Subclass II ADFs (Fig. 1.1d) are diverged from the other ADFs and thought to be involved in rapid cell elongation. In monocots like rice, subclass II variants are expressed in both pollen and roots, but in dicots gene duplication and subfunctionalization has further subdivided the subclass II variants into root hair-specific and pollen specific genes [88, 132]. Arabidopsis subclass IIb ADFs ADF8 and ADF11 are expressed exclusively in early trichoblast stage root cells and root hairs and are likely to play a role

Fig. 1.2 Protein variant-specific function of vegetative class actins in the control of root hair and root growth in *Arabidopsis*. (**a**–**d**) ACT2 and ACT8 control root hair growth. (**a**) 84 h-old wild-type (WT) and mutant (*act2-1*, *act8-2*) seedlings. (**b**) A Portion of *act2-1* mutant primary root depicting strong defects in root hair development. (**c**) 72 h-old *act2-1/act8-2* double mutant seeding showing a complete lack of root hair development. (**d**) An enlarged portion of the bald root of *act2-1/act8-2* double mutant. Note the bulges but no elongation of trichoblasts into root hairs. (**e**, **f**) ACT7 is involved in control of root growth. (**e**) 96 h-old wild-type and *act7-4* mutant seedlings. (**f**) 5-day-old *act2-1/act7-4* double mutant seedlings. Note the stunted roots with swollen epidermal cells and no root hairs

in root hair development [132] (Ruzicka and Meagher, unpublished data). By contrast, subclass IIb ADFs ADF7 and ADF10 are expressed exclusively in mature pollen and extending pollen tubes and are likely to be necessary for pollen tube growth [132].

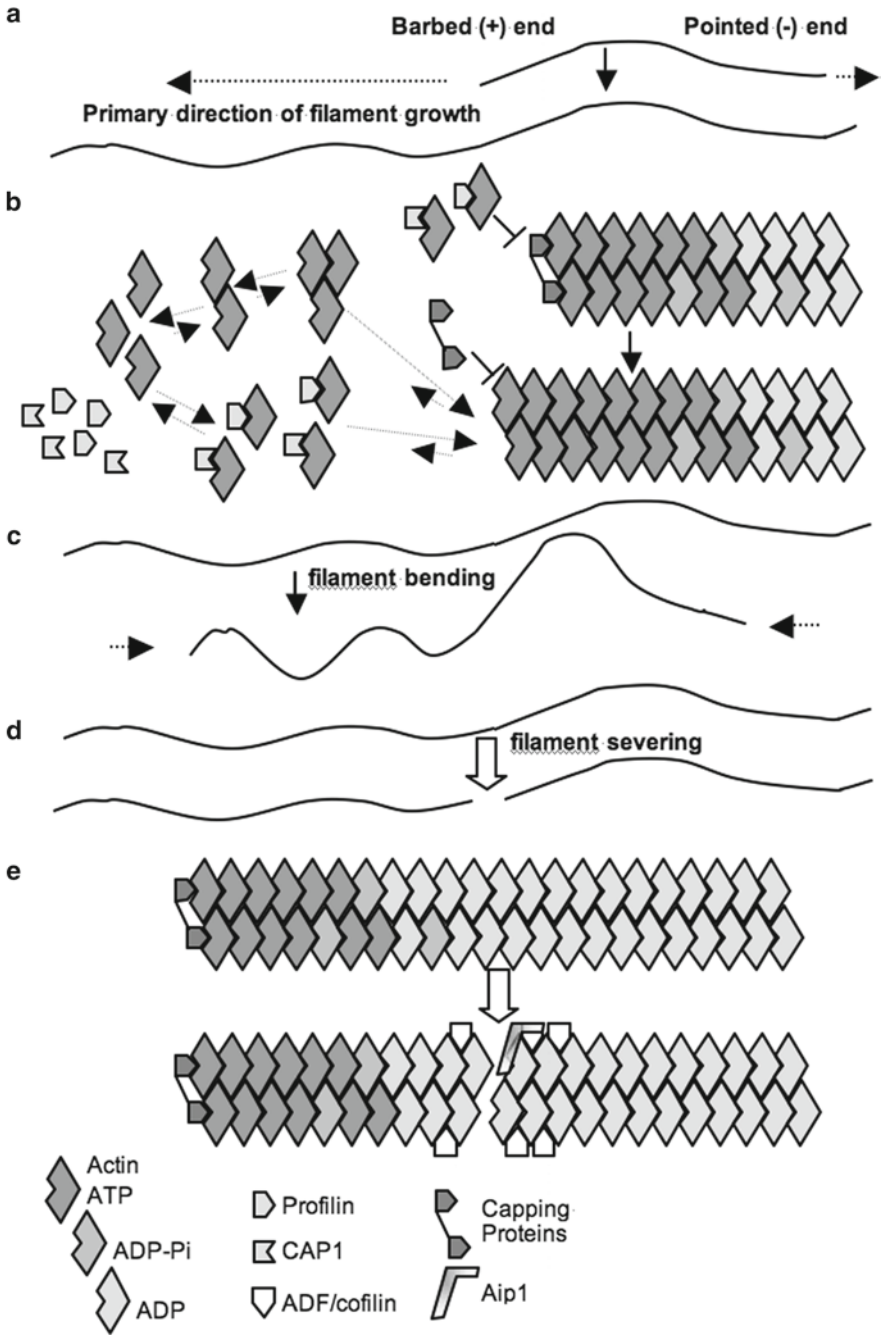
Formin, the membrane bound actin and profilin binding protein, is encoded by a family of 21 genes in Arabidopsis (Table 1.1). Expression of the N-terminal half of Arabidopsis formin FH4 (i.e., the two membrane anchor domains, the poly-L-proline profilin binding domain, and the first of two Formin Homology motifs FH1) fused to GFP produces a dominant negative loss of root hair phenotype [32]. These data strongly suggest that FH4 is a normal part of root hair cytoskeleton, and that the C-terminal domain is necessary for normal FH4 activity.

1.3.2 F-Actin Filament Dynamics and Cell Elongation

Recently, observations of single filament dynamics in living plant cells became possible with the development of novel fluorescent reagents and microscopic tools. Fluorescent live cell imaging shows that rapid movement of actin filaments is an important part of plant cell division, elongation, and differentiation [53]. Earlier studies used GFP-tagged talin to observe the organization of F-actin filaments in elongating live cells or to show the association of F-actin filaments with plastid stromules [75, 76, 82]. In addition, powerful reporters for imaging actin filaments in live plant cells were made from green fluorescent protein (GFP) fusions tagging either the N- or C-terminus or both of the second actin-binding domain from *Arabidopsis* FIMBRIN1 (fABD2) [139, 158]. As compared to the earlier TALIN GFP-mTn reporters, fABD2 reporter plants have fewer detrimental phenotypes [55, 74]. Living cells from transgenic plants expressing the doubly tagged *35S*:*GFP-ABD2-GFP* construct reveal brightly labeled single actin filaments and actin bundles [159]. Chris Staiger's laboratory (Purdue University) recently demonstrated they could follow the dynamics of single actin filaments in live plant cells by using both plants expressing *35S*:*GFP-ABD2-GFP* and time-lapse variable-angle epifluorescence microscopy (VAEM) for rapid sensitive imaging [143]. They focused their studies on the epidermal cells from the hook region of the hypocotyl, which are some of the most rapidly elongating cells in Arabidopsis.

As expected from in vitro and in vivo studies in other organisms, actin filaments in these epidermal cells elongate primarily from one end (Fig. 1.3) [143]. Growing

Fig. 1.3 Actin filament dynamics in elongating plant cells. (**a**) In rapidly elongating hypocotyl cells, actin filaments grow rapidly from the one end [143]. (**b**) Filament growth from the barbed end is modeled using a modification of the illustrations in Staiger and Blanchoin [141]. In Arabidopsis, ADENYLYL CYCLASE-ASSOCIATED PROTEIN1 (CAP1) may provide the primary filament capping activity to prevent filament elongation [21]. (**c**) F-actin filaments rapidly change their effective length by bending or unfolding, a property quantified as convolutedness, and undoubtedly involved in accurately repositioning bound cargo [143]. (**d**) Plant F-actin filaments are shortened primarily by severing. (**e**) The severing activity of actin filaments in (**d**) is modeled at the molecular level, with existing data suggesting that ADF/cofilin variants are the major effectors of severing in plants


a
Barbed (+) end
Pointed (-) end
Primary direction of filament growth
b
c
filament bending
d
filament severing
e
Actin
ATP
ADP-Pi
ADP
Profilin
CAP1
ADF/cofilin
Capping
Proteins
Aip1

filaments generally elongate at rates greater than 1 μm/s. The rate is consistent with levels of polymerizable actin monomer in the range of 50 μM and dose-dependent inhibition by the G-actin binding compound, Latrunculin B. As modeled in Fig. 1.3b, growth of the filaments probably occurs by the addition of ATP bound G-actin monomers to the barbed filament end [141].

We once thought that plants sequestered ATP-actin monomers as profilin-actin complexes that delivered actin subunits to the filament barbed ends. Plant profilin concentrations are relatively high, perhaps high enough to sequester most of the actin monomer pool. However, plant profilins lack the necessary activity to regenerate ATP-actin from ADP-actin, unlike animal and fungal profilins. Arabidopsis ADENYLYL CYCLASE-ASSOCIATED PROTEIN1 (CAP1) accelerates the rate of nucleotide exchange on actin monomers [21] and can therefore enhance F-actin filament growth from monomers in vitro. With a CAP1 to actin ratio of 1–3, CAP1 is an abundant protein in plants, and may play a significant role both complementing and augmenting the role of profilin as an actin monomer sequestering protein. Chaudhry et al. [21] propose a new model for actin filament turnover in plants in which CAP1, "serves as an intermediate between the severing/depolymerizing activity of ADF/cofilin and the assembly promoting function of profilin at filament barbed ends" (Fig. 1.3b).

We might have expected from work in non-plant systems that actin filament turnover in plants would proceed by rapid removal of monomers or small numbers of subunits from the ends of filaments [143]. Instead, Staiger et al. [143] observed in live elongating hypocotyl cells a remarkably high rate of filament severing, as summarized in Fig. 1.3d, e. In 5–10 s, they observed many single filaments cleaved at multiple locations. The lifetime for a typical filament was less than 30 s. Because the fragments can't be tracked unambiguously for more than several successive frames, the authors could not determine how the numerous small fragments generated by severing activity are further depolymerized to replenish the monomeric actin pool for subsequent re-polymerization. Staiger et al. predict a major role for ADF/cofilin in this F-actin severing and turnover from biochemical activities and its abundance in plants [142].

Another activity observed for actin filaments that contributes to their dynamic behavior is a rapid and significant filament bending and unbending as modeled in Fig. 1.3c [143]. Some filaments have a traced contour length twice their end-to-end length and some filaments changed their shape multiple times in a 30 s interval. The parameter of "*convolutedness*" was defined to describe the ratio of a filament's traced contour length to its end-to-end length as measured by the long side of an enclosing rectangle. In rapidly elongating hypocotyl cells, the average F-actin filament changes its *convolutedness* at a rate of about 8% per second. Thicker filaments, presumably actin bundles, did not significantly change their *convolutedness* or changed only slowly compared with single filaments. Imagining how the *convolutedness* of F-actin filaments might accommodate the rapid and accurate positioning and repositioning of organelles and other bound cargo within a growing cell is easy. Using the myosin inhibitor 3-Butanedione Monoxime (BDM), the authors provide preliminary evidence for myosin-dependent filament buckling and straightening.

The genetic tools to test this hypothesis directly are now in place. The Dolja laboratory has characterized a series of double and triple myosin XI mutants in Arabidopsis [121, 125] that may be useful in parallel microscopic studies to dissect the role of myosins in actin filament behavior.

1.3.3 Protein Variant Differences vs. Gene Regulation

Actin is a multifunctional protein encoded by moderately large and divergent gene families in vertebrates and angiosperms, with the best-studied actin families in flies, mice, humans, and Arabidopsis. The various actin protein variants produced in these organisms are most often expressed in a tissue-specific fashion that is conserved across distantly related species, suggesting that the presence of a particular variant in those cell types may be functionally relevant. However, only a handful of studies have attempted so far to explore in detail the relative importance of specific actin variants for various functions in any organism.

Genetic experiments by Fyrberg et al. [40] and Roper et al. [129] have demonstrated functional specialization among the classes of *Drosophila* actin variants. 88F is a gene encoding an adult muscle actin. Flies with a null 88F allele are flightless, a phenotype which can be rescued by a wild type copy of the 88F gene or suppressed by another flight muscle paralog, 79B. However, the flightless phenotype is not suppressed by larval muscle actin paralogs or any of the cytoplasmic actin paralogs, suggesting they have functional differences. Furthermore, the two highly conserved cytoplasmic actins (ACT5C and ACT42A) have only two amino acid differences, but Wagner et al. [155] have shown that only the regulated expression of ACT5C is important for fly development.

The mammalian genome encodes at least six distinct actin variants [50, 123] and the expression of these variants is spatially and temporally regulated throughout development and in the adult organism [149]. The variants are also often differentially distributed within a cell and some are specifically associated with certain subcellular structures such as mitochondria, costameres, neuromuscular junctions, and microvascular pericytes, implying that the cell recognizes different actin variants as functionally distinct entities [130]. As reviewed in Bulinski [17, 73], Karakozova et al. [73] have recently shown that the differential chemical modification (arginylation) of co-expressed, but differentially distributed, beta and gamma actin variants facilitate movement in non-muscle cells. Actin variant specific functions have been proposed for muscle contraction, cell migration, endo- and exocytosis and the maintenance of cell shape, but the specific functions for each of the actin variants during mammalian development likely remain unknown [149].

Arabidopsis researchers are also striving to establish whether the different actin genes and their encoded protein variants are specialized to perform a subset of the many essential actin functions in different organs and cell types. As detailed above, Arabidopsis actin is encoded by eight functional genes, grouped into ancient vegetative and reproductive classes with five subclasses based on phylogeny and expression

patterns (Fig. 1.1b). Several lines of evidence suggest that actin protein variants encoded by the two major classes (V & R, Fig. 1.1b) or the different subclasses (I, II, III, IV, V) of actin genes are functionally different.

First, promoter-*GUS* reporter gene expression studies [2, 3, 56, 57, 92, 107] and protein blots using actin class- or subclass-specific monoclonal antibodies [65, 66, 106] revealed the differential expression of actin variants during cell morphogenesis and organismal development. The vegetative actin genes are so named because they are specifically active in vegetative organs such as leaves, stems, roots and floral organs like sepals, petals, stigma, style and ovary wall, whereas the reproductive actins are predominantly active only in mature pollen and/or ovules, especially the embryo sac [106, 107].

During microsporogenesis, expression shifts from the vegetative actins to the reproductive actins. In microspore mother cells and microspores, actin expression is primarily vegetative, but once the microspore differentiates into mature pollen, actin expression changes so that all five reproductive actins are abundantly expressed [66]. Thus, upon germination the fast tip-growing pollen tubes are equipped with specific expression of reproductive-class actins.

Regulation of ACT7, one of the vegetative actins, is also specifically influenced by environmental and physiological cues, and this distinguishes ACT7 from the other vegetative actins ACT2 and ACT8. For instance, *ACT7* gene expression and ACT7 protein levels are strongly up-regulated in response to various plant hormones and pathogen attack, and its expression is essential for regeneration of callus on hormone-containing medium [65, 92]. Further, as mentioned earlier, ACT7 protein levels are up-regulated in *ACT2*-, *ACT8*, and *ACT2/ACT8*-defective plants.

Second, ectopic expression studies support the functional difference between the two classes of plant actin variants. To illustrate, when a reproductive actin, ACT1, is ectopically overexpressed in vegetative tissue, it severely disrupts the organization of the actin cytoskeleton which affects the development of the plant as a whole, resulting in abnormally dwarf plants with aberrant organs and cell types. However, when a vegetative actin, ACT2, is overexpressed to similarly high levels in vegetative tissue, it has only a mild effect on both actin organization and plant morphology [67]. Clearly, the vegetative and reproductive actin variants are functionally distinct. Given the functional distinction of the actin variants, we hypothesized that an associated paucity of the right class of interacting actin binding proteins (ABPs) for the reproductive class actin in the vegetative tissue might have affected actin dynamics and thereby the development of tissues and organs in the ACT1 misexpression plants.

Third, genetic characterization, complementation, and suppression studies of actin mutants further support the functional difference between different subclasses of actin variants. For instance, and as described above, single actin subclass I knockouts are affected mainly in root hair growth (Fig. 1.2a, b) [44, 69, 128]. Double mutant plants are completely devoid of any root hairs (Fig. 1.2c, d). However, ACT2 and ACT8 variants, but not ACT7, fully rescued the root hair growth defects of the double mutants [69]. The complete rescue of root hair growth by high levels of any of the two subclass 1 actins suggest that they are redundant, but again, the partial

rescue of root hair growth by the subclass 2 ACT7 shows that the ACT7 variant is functionally different from the other two vegetative actins.

The mutant phenotype, complementation, and suppression data for subclass 2 actin ACT7 are distinct from subclass I. ACT7 knockout mutants are drastically affected in root growth, epidermal cell specification, cell division, and root architecture (Fig. 1.2e) [45, 69], yet overexpression of almost any actin class or subclass variant (e.g., ACT1, ACT2, ACT8, or ACT7) from the ACT7 promoter can fully complement the root elongation and cell polarity phenotype of ACT7 knockout. These data suggest that the ACT7 variant has lost functions needed to fully complement ACT2 or ACT8, but other plant actin variants have all the functions contained in the ACT7 variant. To test this hypothesis [69], overexpressed the ACT8 variant from multiple actin regulatory sequences in an *act2-1 act7-4* double mutant background. The resulting plants had normal morphology. We conclude from these studies that differences in both regulation and sequence of actin paralogs are essential for normal plant development.

As is the case for actin (Fig. 1.1, Table 1.1), most of the ABPs in plants are also encoded by gene families and at least two of them, profilins and ADF/cofilins, exhibit phylogenetic grouping into vegetative (or nearly constitutive) and pollen-specific classes [68, 90, 132]. Like the actins, the protein variants reveal both class-specific functional and biochemical differences. For instance, the constitutively expressed profilins of maize have higher affinity for poly-L-proline, sequester more monomeric actin, and disrupt the actin cytoplasmic architecture in live cells more rapidly than pollen-specific profilin [78]. Like the two ADF/cofilin variants, UNC-60A and UNC-60B, encoded by *Caenorhabditis elegans unc-60*, the Arabidopsis reproductive (e.g. PRF4) and vegetative (e.g. PRF2) profilins bind differently to plant and vertebrate actins (C.J. Staiger, personal communication). In *C. elegans*, the most dramatic difference between UNC-60A and UNC-60B is that, at pH 7.0, UNC-60A binds more weakly to filamentous actin than UNC-60B and therefore has different depolymerizing activity [117, 118].

The class-specific activity of ADF/cofilins not only applies to their customary function in depolymerizing actin filaments, but also other novel functions in gene regulation and signaling. For example, in Arabidopsis, which encodes 11 ADFs, ADF4 is a component of the plant-signaling pathway that provides resistance against *Pseudomonas syringae* [148]. ADF9 regulates the expression of essential regulators of flowering time [18].

In addition to different protein variant functions, some of the *ADF* genes also exhibit distinct patterns of gene expression, being only active in specific cell types such as trichoblast and root hair cells (ADF8 and ADF11) and mature pollen and pollen tubes (ADF7 and ADF10) [132]. Thus, families of actins and ABPs in multicellular eukaryotes have functional diversity manifested through differences in gene regulation, amino acid sequence, and protein-protein interaction, all of which control normal multicellular development. Our understanding of the development of different tissues and organs and possibly the human diseases caused by mutations in different actin and ABP genes would be enhanced by further genetic proof of the functional necessity for the various actin and ABP variants (Table 1.1).

1.3.4 Protein–Protein Interactions Among Actin and ABP Families

The major classes of profilins and ADF/cofilins, in particular, show the tissue-specific expression and corresponding phylogenetic grouping into vegetative or constitutive and reproductive classes (Fig. 1.1). In recent ectopic coexpression studies, we have examined if there is a class-specific, preferential interaction between actin and ABP variants expressed in the two major plant tissues. For instance, in Arabidopsis, ectopic overexpression of a reproductive ACT1, but not overexpression of a vegetative ACT2, in vegetative tissues causes severe dwarfing of plants and abnormal actin cytoskeletal structures. We hypothesized that the misexpression of a pollen-specific ACT1 in vegetative cells adversely alters plant development by changing actin dynamics because of inappropriate or poor interaction with endogenous vegetative ABPs [67]. We tested this hypothesis by ectopically coexpressing reproductive profilin (PRF4) or ADF variants (e.g., ADF7) with ACT1 [64].

We found that coexpression of reproductive, but not vegetative, ABP variants considerably suppressed the ectopic ACT1 expression phenotypes, thus restoring wild-type stature and organ structure and normal actin cytoskeletal architecture in the double transgenic plants. Cells from vegetative tissue in these rescued plants contained high levels of both reproductive actin and reproductive ABPs. We conclude that in these cells the reproductive profilin or ADF properly interacts with ACT1, which compensates for the excess of reproductive ACT1 monomers and prevents formation of aberrant actin structures. These co-expression plants contain excessive amounts of actin, but it is organized into normal arrangements of filaments.

In plants misexpressing ACT1 alone or coexpressing ACT1 and a vegetative profilin or ADF variant, both actin filament organization and plant development were extremely abnormal. We hypothesize that aberrant actin filament structures and plant morphogenesis occurred because of the poor or inappropriate interaction of the endogenous or overexpressed vegetative ABPs with the misexpressed, excessive reproductive actin monomers. We conclude that actins and ABPs have evolved class-specific, protein–protein interactions that are essential to the normal actin cytoskeletal dynamics and plant development. Biochemical evidence for the differential binding of different classes of ABPs with the two major classes of plant actins would support the above model.

The macroevolution of organs and tissues in higher plants and animals may have been contingent upon the expansion of numerous cytoskeletal gene families encoding interacting proteins [98, 104, 107]. Once gene family members evolve compartmentalized expression, protein variants are free to evolve new interactions with partners that may be incompatible with protein networks in other compartments. Ancient classes of actin and actin-binding proteins, which elaborate intercellular structures influencing organismal development, are clear examples of such coevolving networks. The above described ectopic expression and suppression data provide evidence for the co-evolution of organ-specific protein-protein interactions. Understanding the contingent relationships between the evolution of organ-specific protein variant networks and organ origination may prove key to explaining multicellular development.

1.3.5 *Organelle Streaming and Vesicle Movement Within a Relatively Stationary Cytoplasm*

The beauty of chloroplasts spinning around in plant and algal cells has lured many plant cell biologists into the field. Although debated for more than 50 years, the most widely accepted model for this activity says that organelles, including chloroplasts, mitochondria, peroxisomes, and some golgi stacks, are carried around the cell in a streaming cytoplasm [62, 63, 140]. An alternative model states that forces within the cytoplasm act directly on organelles and power their movement. This flow of organelles itself generates the force necessary to carry along some adjacent cytoplasm, giving the illusion of cytoplasmic streaming.

Supporting the latter model is the observation that actin filaments and bundles are tightly bound to many organelles, suggesting organelles are individually tethered to and moved along the cellular actin/myosin system. For example, a basket of F-actin surrounds chloroplasts, with single actin filaments and bundles often extending from these baskets into the surrounding cytoplasm [71, 72]. Chloroplasts are attached to the cytoskeleton by a nuclear-encoded chloroplast outer membrane protein CHUP1 that interacts with actin and profilin [136]. Until recently, however, tests for the above described models for organelle movement around plant cells were too complex to interpret, and a deep understanding of cytoskeletal-organelle dynamics remained elusive. Using digital microscopy to analyze the independent movement of large numbers of fluorescently tagged organelles in single cells is beginning to help to clarify these processes [113].

Valerian Dolja and his colleagues at Oregon State recently demonstrated that many individual organelles move independently, different organelles move at different rates, and occasionally some organelles move in opposite directions from the bulk of organelles [6, 7, 121, 125, 135]. They concluded that organelles move independently in a relatively stationary cytoplasm. The illusion of a fully streaming cytoplasm is created by the predominant movement of organelles in one direction around the cell periphery. Therefore, neither of the older models appears to be strictly correct.

Because organelles and their surrounding cytoplasm are rich in actin filaments and myosin and because many studies support the inhibition or enhancement of organelle movement by actin inhibitors like cytochalasin, phalloidin, and latrunculin, the theory that actin and myosin motors power organelle movement has seldom been in doubt [20, 38, 52, 153, 160]. The most definitive data demonstrating the role of actin/myosin motors in organelle movement come from molecular genetic analysis in the Arabidopsis system in Dolja's laboratory and in Andreas Nebenfuhr's laboratory at the University of Tennesee.

Arabidopsis encodes 17 myosins, falling into two ancient classes, named VIII and XI to distinguish them from myosins in other kingdoms [127]. Knockout mutants in myosin *XI-K* or *XI-2*, but not other myosins, showed reduced transport of Golgi stacks, peroxisomes, and mitochondria [121]. Figure 1.4a illustrates the movement of a Golgi stack in mutant cytoplasm compared to a Golgi stack in wild type cytoplasm. Conclusions drawn from these studies were made by summing

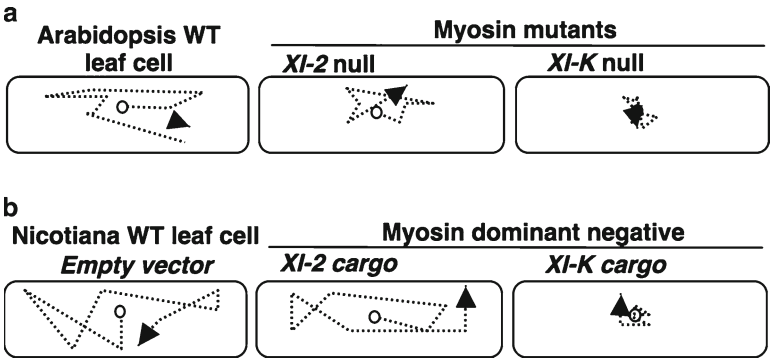


Fig. 1.4 Myosin defects disrupt organelle trafficking. (**a**) This illustration shows the restricted path of Golgi vesicle transport observed in two myosin null mutants deficient in XI-2 and XI-K as compared to wild-type *Arabidopsis* Columbia leaves. Golgi location in a leaf vein cell was monitored at 2 s intervals and plotted relative to a common origin [121]. The origin is shown with a *circle* and the end point with an *arrowhead*. Thousands of such individual golgi, mitochondria, and peroxisomes were monitored in different studies to demonstrate the role of different myosins. (**b**) Restricted path of Golgi vesicle transport in *Nicotiana benthamiana* leaf cells in the presence of transgene expressing the *N. benthamiana* myosin *XI-K-cargo* domain as compared to plants expressing the *XI-2-cargo* domain or and empty vector [7]. *N. benthamiana* is a close relative of tobacco

similar quantitative observations on tens of thousands of individual Golgi. Comparisons of single and double mutants suggest that myosin *XI-K*, *XI-1*, *XI-2*, and *XI-B* have partially redundant and additive functions essential to organelle movement and normal root hair elongation. Double knockout mutants showed less than 10% of the mean organelle velocity of wild type [7, 125]. These data suggest that individual myosin variants are necessary for organelle transport. Molecular genetic studies are beginning to define the specificity of individual myosin globular tail domains in transport [86, 87].

To test if these data are specific to the Arabidopsis system, further experiments were done in a distant angiosperm, *Nicotiana benthamiana.* As illustrated for one Golgi stack in Fig. 1.4b, the authors show that, indeed, over expression of the cargo-carrying tail domain of XI-K myosin in *N. benthamiana* produces a dominant-negative phenotype by reducing the transport of populations of Golgi, peroxisomes, and mitochondria [7].

In a process requiring class VIII myosins, plant virally encoded Heat shock protein 70 (Hsp70) proteins are localized to plasmodesmatal complexes through the ER and endocytic vesicles. Using overexpression of the tail (cargo) domain of class VIII myosins, but not the motor domain, to generate dominant negative phenotypes [6], show that a few Arabidopsis VIII myosins (e.g., VIII-1, VIII-2, VIII-B) participate in this process, but based on mutant analysis, XI-class myosins are not involved.

Specialized actin filaments are attached to and bundled around chloroplasts [61, 71] and probably determine the chloroplast's movement in the cytoplasm and orientation to the light. Drug treatments confirm a role for actin/myosin motors, but not tubulin, in chloroplast movement with response to light intensity [163]. Blue light plays the most significant role in chloroplast relocation through the photoreceptors Phototropin1 and 2 [80, 81]. However, none of the above mentioned