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Actin Cytoskeleton in Plants: From Transport Networks to Signaling Networks

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ABSTRACT The plant actin cytoskeleton is characterized by a high diversity in regard to gene families, isoforms, and degree of polymerization. In addition to the most abundant F-actin assemblies like filaments and their bundles, G-actin obviously assembles in the form of actin oligomers composed of a few actin molecules which can be extensively cross-linked into complex dynamic meshworks. The role of the actomyosin complex as a force generating system — based on principles operating as in muscle cells — is clearly established for long-range mass transport in large algal cells and specialized cell types of higher plants. Extended F-actin networks, mainly composed of F-actin bundles, are the structural basis for this cytoplasmic streaming of high velocities On the other hand, evidence is accumulating that delicate meshworks built of short F-actin oligomers are critical for events occurring at the plasma membrane, e.g., actin interventions into activities of ion channels and hormone carriers, signaling pathways based on phospholipids, and exo- and endocytotic processes. These unique F-actin arrays, constructed by polymerization-depolymerization processes propelled via synergistic actions of actin-binding proteins such as profilin and actin depolymerizing factor (ADF)/cofilin are supposed to be engaged in diverse aspects of plant morphogenesis. Finally, rapid rearrangements of F-actin meshworks interconnecting endocellular membranes turn out to be especially important for perception-signaling purposes of plant cells, e.g., in association with guard cell movements, mechano- and gravity-sensing, plant host–pathogen interactions, and wound-healing. *Microsc. Res. Tech. 47:135–154, 1999.*

INTRODUCTION

Dynamic cytoskeletal networks of eukaryotic cells (e.g., Schliwa, 1986) are built by integrated homopolymers of tubulin dimers (microtubules, MTs), actin monomers (actin filaments, AF; filamentous actin generally, F-actin), and of vimentin or related proteins (intermediate filaments, IFs). In cooperation with diverse associated proteins (e.g., Mandelkow and Mandelkow, 1995, for MT-associated proteins; Vandekerckhove and Vankampernolle, 1992; and Pollard et al., 1994, for AF-associated proteins) and lipid molecules (Isenberg and Niggli, 1998), these cytoskeletal arrays play integral roles in the structural architecture of eukaryotic cells. Moreover, their integrated nature and inherent links with the nuclear skeleton underlie direct impacts on expression of the eukaryotic genome (Ingber, 1993; Ingber et al., 1994; Chicurel et al., 1998). The hunt for IFs in plants is, after the first promising results (Dawson et al., 1985; Miller et al., 1985; McNulty and Saunders, 1992), still an open race (Menzel, 1993) and it seems that, as with yeast cells, plant cells rely only on MTs and AFs.

Intracellular movements in plant cells have been well known since the 1950s (for historical overview, see Kamiya, 1986), when cytoplasmic filaments were observed by Jarosch (1958) in large algae cells. Despite this, research on AFs was hampered for a long time primarily due to two reasons: 1) the lack of muscles in plants, and 2) a lack of adequate methodological approaches for actin identification and visualization. When, however, nonmuscle actin was identified for the first time in connection with cytoplasmic streaming in slime mold *Physarum* (Alléra et al., 1971), research on plant actin and its associated proteins began anew (for reviews, compare Hepler and Palevitz, 1974; Staiger and Schliwa, 1987; Seagull, 1989; McCurdy and Williamson, 1991; Staiger and Lloyd, 1991; Shibaoka and Nagai, 1994; Davies et al., 1996). The progress in our knowledge of cytoplasmic streaming and plant actin resulted from investigations on giant cells of algae such as *Nitella*, *Chara*, *Bryopsis*, and *Acetabularia*, as well as specialized cells from higher plants having dynamic cytoplasm like pollen tubes and root or stamen hairs (for review, see Williamson, 1993). Meanwhile, the functional aspect of plant F-actin filaments, bundles, and networks, representing both architectural tool and intracellular transport device, has shifted from a rather static to a more dynamic view. Regulatory functions of the plant actin cytoskeleton start to emerge in the fields of algae morphogenesis (Menzel, 1996) and signaling pathways (Trewavas and Malhó, 1997; Thuleau et al., 1998), as they are well established for yeast and animal cells (Schmidt and Hall, 1998; Zigmond, 1996). This

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review aims to highlight this emerging shift from the classical field of intracellular transport device to new functions of actin associated especially with diverse signaling pathways of higher plants.

ACTIN AND ACTIN-ASSOCIATED MOLECULES

Actin occurs in plant cells in the form of monomers (G-actin), oligomers, or short AFs composed of few monomers and their meshworks, long AFs, and their more complex assemblies, represented by thick bundles and dense networks of F-actin. Plant actin was isolated either in the form of F-actin after phalloidin treatment (Abe and Davies, 1991) or G-actin originating from diverse sources (Vahey and Scordilis, 1980; Ma and Yen, 1989; Liu and Yen, 1992; Koropp and Volkmann, 1994; Andersland et al., 1994; Yen et al., 1995; Ren et al., 1997). However, except for the pioneering results from Staiger's group (Ren et al., 1997), detailed biochemical and structural characterizations of plant actin in vitro, as they have been reported for actin from animal sources (e.g., Kabsch and Vandekerckhove 1992), are still lacking. Nevertheless, it is known that the critical actin concentration for in vitro assembly of F-actin from maize pollen is 0.6 μM, depending on physiological conditions (Ren et al., 1997). These data are similar to those reported for nonplant actin, with 0.1 μM for the barbed and 0.5 μM for the pointed ends (Hatano 1994).

Intriguingly, plant actin gene families are much more abundant and diverse in comparison to other organisms. In *Arabidopsis*, for example, actin is encoded by 10 genes (McDowell et al., 1996). Eight isoforms have been detected in *Arabidopsis* (McDowell et al., 1996), and five in *Vicia faba* (Janβen et al., 1996). In comparison, only two nonmuscle isoforms, β and γ, are reported for vertebrates (Kabsch and Vandekerckhove, 1992). Recent preliminary experiments indicate that each of these plant actin isoforms are critical for successful viability of individual seedlings within a given plant population (discussed in McKinney and Meagher, 1998).

As of now, four plant actin binding proteins (ABPs) have been identified on a gene level, including myosins, profilin, ADF (actin depolymerizing factor), and fimbrin. Besides binding sites for actin, all of them possess binding capacities for several other molecules, especially the minor membrane phospholipid PIP_2. Since actin binding affinities depend on intracellular conditions, e.g., ionic strength, pH, ATP level, these binding proteins are especially suitable candidates for cytoskeleton-mediated interventions into various signaling processes. The molecular motor protein myosin (reviewed by Asada and Collings, 1997) was the first ABP characterized, although only using heterologous antibodies. Exploiting cDNA libraries from *Arabidopsis*, Knight and Kendrick-Jones (1993), Moepps et al. (1993), and Kinkema et al. (1994) identified several plant myosins showing conservation within their motor domains (Cope et al., 1996). On the basis of this conservation, plant myosins are grouped in the phylogenetic myosin tree separately as three unique plant classes (Cope et al., 1996). Recently, Williamson's group (Plazinski et al., 1997) described PCR primers amplifying gene fragments encoding part of the myosin head from a wide range of plant species.

Profilins are small, ubiquitous proteins that affect the dynamic behavior of AFs (e.g., Schlüter et al., 1997; Staiger et al., 1997) and have conserved structure throughout the eukaryotic kingdom (Fedorov et al., 1997). By their ability to sequester actin monomers in a 1:1 complex, profilin plays an important role in the actin organization. Valenta et al. (1991, 1992, 1993) discovered plant profilins in their search for plant allergens. Staiger et al. (1993) described the multigene family for maize profilin with at least five genes. Maize profilin isoforms have been shown to differ with respect to their biochemical properties (Gibbon et al., 1998), indicating diversity in function rather than redundancy. The three-dimensional structure of profilin (Thorn et al., 1997) is well conserved, although similarities in amino acid sequences are rather low (approximately 30%) in comparison to yeast and vertebrate profilins. Staiger and colleagues estimated binding capacities (average K_d values) of profilin for actin with 1–2 μM, for poly-L-prolin with 150–280 μM (Gibbon et al., 1998), and for phosphatidylinositol phosphates, especially PIP_2, with 24 μM (Drøbak et al., 1994). These binding characteristics, in addition to its plasma membrane location (von Witsch et al., 1998; c.f. Vidali and Helper, 1997) distinguish plant profilins as favorable candidates for putative communication between membranes and the cytoskeleton (Clarke et al., 1998; for nonplant profilin, cf. Machesky and Pollard, 1993).

Genes for ADF have been identified for lily (Kim et al., 1993), maize (Rozycka et al., 1995; Lopez et al., 1996), wheat (Danyluk et al., 1996), and *Arabidopsis* (Carlier et al., 1997). As with plant profilins, the amino acid sequences of plant ADFs share only approximately 30% of the overall homology with vertebrate ADFs. However, those sequences which possess the highest similarities represent the putative actin-binding and phosphorylation sites (Staiger et al., 1997). ADFs are stimulus-responsive actin cytoskeleton modulating proteins which increase the dynamism of AFs at the barbed ends (e.g., Carlier et al., 1997). Moreover, ADFs possess PIP_2 binding sites as well (Danyluk et al., 1996; Gungabissoon et al., 1998). Mutation of conserved tyrosine residues in maize ADF causes uncoupling of its F-actin and G-actin bindings (Jiang et al., 1997).

Recently, McCurdy and Kim (1998) cloned and sequenced two *Arabidopsis* genes encoding for fimbrin-like polypeptides of the spectrin family. One of these genes shows 40% similarity to nonplant fimbrins in general, while two tandem repeats have up to 74% homology within actin-binding sites. Spectrin is in the discussion without any available genetical evidences (de Ruijter and Emons, 1999). Preliminary data were published also on other actin-binding proteins, e.g., actin bundling protein P-135 isolated from pollen tubes of lily (Yokota et al., 1998), and F-actin binding annexins p34 and p35 of tomato (Calvert et al., 1996). Other actin-binding molecules are chitinase and osmotin-like protein of cultured potato cells (Takemoto et al., 1997), and sucrose synthase of maize (Winter et al., 1998).

IDENTIFICATION AND VISUALIZATION OF ACTIN FILAMENTS

Conventional electron microscopy (EM) combined with the heavy meromyosin decoration of F-actin was the first successful approach for ultrastructural identification of F-actin as critical structures supporting cytoplasmic streaming (Condeelis, 1974; Palevitz et al.,

1974). The fungal toxin phalloidin (Wulf et al., 1979) was the first breakthrough in our knowledge of tissue-specific distributions and functions of F-actin (e.g., Tiwari et al., 1984; Parthasarathy et al., 1985; Staiger and Schliwa, 1987).

By the early 1980s, Meagher's group provided the first complete nucleotide sequences of a soybean and maize actin gene families (Nagao et al., 1981; Shah et al., 1982, 1983), demonstrating high conservation among plant actins. Comparison of the deduced amino sequences showed homology to muscle and nonmuscle actins from animals. On the basis of these sequence homologies, two heterologous actin antibodies (the highly specific anti-chicken gizzard actin IgG, and a less specific anti-chicken gizzard actin IgM; for comparison see Koropp and Volkmann, 1994) are mainly in use for indirect immunofluorescence microscopy after high-pressure freeze-substitution (Ding et al., 1992; Roy et al., 1997), formaldehyde fixation (Baluška et al., 1997a; Blancaflor and Hasenstein, 1997; Vitha et al., 1997; Wasteneys et al., 1997), immunogold transmission EM (Lancelle et al., 1987; Lancelle and Hepler 1992; Miller et al., 1996), and scanning EM after silver enhancement (Reichelt et al., 1995). Critical evaluations of results obtained using affinity- and immunolabelings (Tang et al., 1989; He and Wetzstein, 1995; Walker and Sack, 1995; Vitha et al., submitted) demonstrate both advantages and disadvantages of these approaches. It is possible to argue that aldehyde fixation reduces antigenicity generally and destroys F-actin specifically, but this seems to be unjustified if we consider the general suitability of formaldehyde fixation for F-actin visualization in yeast and animal cells. Moreover, formaldehyde fixation does not preclude plant F-actin visualization (see Fig. 1), even using monoclonal actin antibodies (e.g., clone C4 from ICN), if other parameters of common techniques are optimized (Baluška et al., 1997a; Blancaflor and Hasenstein, 1997; Vitha et al., 1997; Wasteneys et al., 1997). Investigations of *Characean* internodal cells (Grolig et al., 1988) by perfusion technique (Tazawa, 1968) demonstrate similar results for aldehyde-fixed and unfixed material. At present, however, both approaches, affinity- and immunolabeling, cannot take into consideration the high diversity of plant actins (reviewed by Meagher, 1991), suggesting distinct functions for different plant actin isoforms (see also Outlook, below).

Finally, indirect evidence for plant actin functions is coming from diverse inhibitor experiments, primarily using different forms of the fungal toxin cytochalasin (for review, see Staiger and Schliwa, 1987). Often neglected is the unclear and complex mechanism of cytochalasin actions on the actin cytoskeleton of plant cells (for animal cells, see MacLean-Fletcher and Pollard, 1980; Cooper 1987). For instance, cytochalasins have a wide range of impacts on plant F-actin, including even increased actin polymerization and F-actin

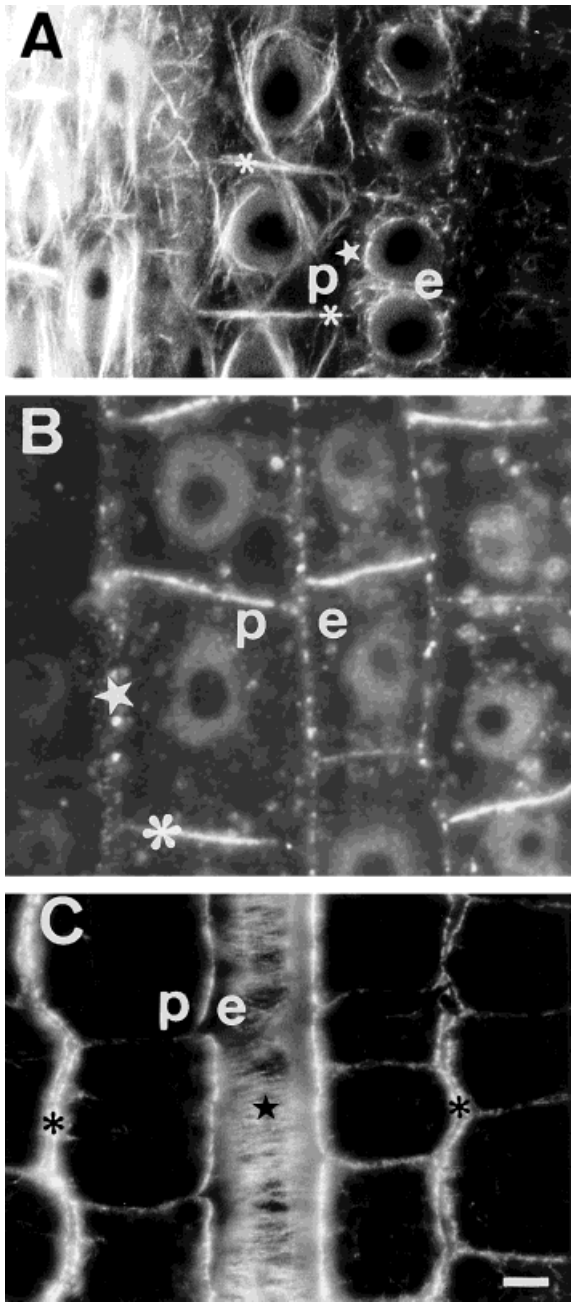

Fig. 1. Distributions of AFs (**A**), unconventional myosin VIII (**B**), and MTs (**C**) in postmitotic maize root cells traversing the transition root growth zone. AFs are organized in the form of perinuclear networks and bundles, the latter approaching actin-rich cross-walls (asterisks in **A**), while the side-walls are actin depleted (star in **A**). Note prominent labeling at the left side of **A** image where cells of the root stele are located, whereas the root cortex (the right side of image **A**) is rather actin-depleted. Unconventional myosin VIII associates preferentially with the cell periphery in the form of continuous labeling at cross-walls (asterisk in **B**) and distinct dots at side-walls (star in **B**). Immunogold EM investigation (data not shown) identified this labeling as myosin VIII associated with plasmodesmata and pit-fields. Image **C** shows distribution of MTs in comparable cells as shown in **A** and **B**. MTs are numerous at the cell cortex (so-called cortical MTs), especially under side-walls (black asterisks indicate cross-sections through these arrays, which are shown in their complete form in the center of image **C** as indicated with a black star). All three immunolocalizations have been done with the same technique, using Steedman's wax as the embedding medium; the only difference was in the first antibody (monoclonal anti-actin and anti-tubulin, polyclonal anti-myosin VIII; for methodological details see Baluška et al., 1997a); p indicates pericycle cell file, e indicates endodermis cell file. Bar = 5 μm for **A**, 3 μm for **B**, and 6 μm for **C**.

bundling (e.g., Williamson and Hurley, 1986; Palevitz, 1988; Lancelle and Hepler, 1988; Collings et al., 1995), impacts which are not expected for an F-actin disintegrating drug. Results obtained from experiments using cytochalasins clearly need confirmation with latrunculins, where the mode of action on the actin cytoskeleton is well described (Ayscough et al., 1997; Ayscough, 1998).

INTRACELLULAR INTERACTIONS: POLYSOMES, MICROTUBULES, PLASMA MEMBRANE

A unique population of AFs was shown to associate with polysomes of plant cells (Davies et al., 1991; Stankovic et al., 1993; Ito et al., 1994; Zak et al., 1997). However, the functional significance of these F-actin–polysome interactions needs further study. As shown for at least some plant cells, AFs are interacting with mRNAs, which confers unique subcellular localizations of these mRNAs, for instance, during formation of protein bodies (Muench et al., 1998). Both actin and tubulin were identified biochemically in the protein body fraction (Wu et al., 1998). In addition, plant AFs associate with such components of the protein translation machinery as elongation factor 1α (Clore et al., 1996; Wu et al., 1998). All of this indicates that the actin cytoskeleton of plant cells is closely linked to the protein translation machinery.

Several authors have shown that AFs colocalize with MTs in various plant cell types, for instance, in algal cells (Menzel and Schliwa, 1986), pollen tubes (Pierson et al., 1989; Lancelle and Hepler, 1991), and differentiating xylem cells (Fukuda and Kobayashi, 1989). Moreover, pharmacological treatments specifically affecting one cytoskeletal type had impacts on the other (Seagull, 1990; Wernicke and Jung, 1992; Chu et al., 1993; Kimura and Mizuta, 1994; Collings et al., 1996; Tominaga et al., 1997). It is clear that MTs and AFs interact also in both the preprophase band and phragmoplast during the plant cell cycle (e.g., Palevitz, 1987a,b; McCurdy and Gunning, 1990; Ding et al., 1991; Eleftheriou and Palevitz, 1992). Moreover, mutants specifically affected in MTs of spindles were shown to be additionally affected in the distribution of mitotic AFs (Staiger and Cande, 1991).

AFs were indirectly implicated in supporting rearrangements of MTs in postmitotic plant cells. Late telophase MTs typically radiate from nuclear surfaces (Baluška et al., 1996a) and invade the cytoplasm under the plasma membrane as cortical MTs during the early G1 phase (Hasezawa et al., 1998). While distributions of these major cytoskeletal elements are different deeper in the cytoplasm, both AFs and MTs codistribute in the cortical portion of growing plant cells (McCurdy et al., 1988; Hasezawa et al., 1989; Jung and Wernicke, 1991; Baluška et al., 1997a). Pharmacological experiments suggest that fine arrays of cortical AFs support the transverse arrays of cortical MTs, which then determine the preferential cell growth polarity (Seagull, 1990; Takesue and Shibaoka, 1998). All these interactions between MTs and AFs are proposed to be mediated by actin- and/or tubulin-associated proteins (Nick, 1999).

In addition to polysomes and MTs, there are some examples of plant AFs interacting with the plasma membrane. Actually, these actin-plasma membrane interactions seem to be dependent on the presence and organization of cortical MTs (Collings et al., 1998). Intriguingly, the plasma membrane-associated actin (Sonesson and Widell, 1993, 1998; Kobayashi, 1996) is composed of unique actin isoforms (Janβen et al., 1996). One role of actin associated with the plasma membrane is the anchorage of AFs at the cell periphery. This concept finds support in studies showing protease-sensitive anchorages of F-actin bundles in mesophyll cells of *Vallisneria* (Masuda et al., 1991; Ryu et al., 1995).

Recently, other roles for actin plasma membrane interactions have emerged. Plasma membrane-associated actin was implicated in signaling pathways based on the phosphoinositol metabolism (Tan and Boss, 1992). Furthermore, interactions between the efflux carriers of the plant hormone auxin (e.g., Gälweiler et al., 1998; Müller et al., 1998) and F-actin at the plasma membrane (Cox and Muday, 1994; Butler et al., 1998) suggest that F-actin is involved in intercellular transport processes of this crucial plant hormone; however, not microtubules (Hasenstein et al., 1999). Intriguingly, cytochalasin D-mediated disintegration of AFs inhibits the basipetal auxin transport in zucchini hypocotyls (Butler et al., 1998). In fact, in most cells of root apices, actin is distributed in a polar fashion, with AF cables approaching actin-enriched cross-walls (Baluška et al., 1997a) which harbor the auxin efflux carrier *AtPIN2* (Müller et al., 1998). However, a small problem with this concept is that the above pattern of F-actin distribution is prominent in cells of the stele periphery, indicating a possible role in the acropetal auxin flow, but less so in cells of the epidermis and cortex (Baluška et al., 1997a), where the basipetal auxin flux occurs and where the efflux carrier *AtPIN2* was located (Müller et al., 1998).

INTRACELLULAR MOVEMENTS

Short-Range Transport: Directed and Saltatory Movements of Vesicles and Organelles

The plant cytoplasm is often extremely dynamic when motilities can reach speeds of several magnitudes higher (up to 100 μM per second, e.g., Wayne et al., 1990; Staves et al., 1995) than those recorded in other eukaryotic cells (Higashi-Fujime, 1991; Shimmen and Yokota, 1994). In addition, there is a whole range of different types of motilities in plant cells (e.g., Foissner et al., 1996). In contrast to animal cells, elements of plant endoplasmic reticulum (ER) and Golgi apparatus (GA) are not controlled by MTs and their associated motors, dynein and kinesin, but endocellular localization of ER and GA elements are fully under F-actin control. Early microscopical studies noted close spatial associations between ER membranes and bundles of AFs (e.g., Goosen-de Roo et al., 1983) and these interactions were confirmed later by more sophisticated high-pressure freezing-fixation followed by freeze-substitution (e.g., Lichtscheidl et al., 1990). Moreover, in vivo studies indicate that network-like distributions and mode of movements of ER elements resemble more distributions of F-actin networks, both at the cell cortex and deeper in the cytoplasm, while they are clearly different from parallel arrays of cortical MTs (Quader and Schnepf, 1989; Quader et al., 1987, 1989; Quader,

1990; Quader and Fast, 1990; Lichtscheidl and Url, 1990; Knebel et al., 1990). This F-actin–ER codistribution corresponds well to cytoskeletal drug studies clearly showing that actomyosin complexes, but not the MT cytoskeleton, are responsible for endocellular movements of ER elements in higher plant cells (Kachar and Reese, 1988; Quader et al., 1987, 1989; Knebel et al., 1990; Quader, 1990; Liebe and Menzel, 1995). The same feature is true also for the unicellular alga *Acetabularia* (Menzel, 1994a).

In addition to ER elements, GA stacks have also been shown to be moved via the actomyosin-based forces (Satiat-Jeunemaitre et al., 1996). This is in a sharp contrast to the situation in animal cells, where the MT cytoskeleton is primarily responsible for the endocellular distribution of GA stacks (Cole et al., 1996; Minin, 1997). Intriguingly, plant GA stacks are moving on F-actin/ER networks as visualized by GFP-sialyl transferase, which is targeted specifically to GA (Boevink et al., 1998). Cadherin- and catenin-like proteins, localized by heterologous antibodies, associate preferentially with the cell periphery, ER membranes, and actin bundles (Baluška et al., 1999). They could be considered as candidates of those factors which anchor AFs to endomembranes and participate in diverse membrane–membrane recognition and adhesion events. We suggest that they might also be involved, together with other cytoskeletal proteins and membranes (Abe et al., 1992), as well as noncytoskeletal plant-specific molecules like AGPs (Šamaj et al., 1998), in the building up of the recently introduced new structure of plant cells termed the "endomembrane sheath" (Reuzeau et al., 1997). These authors proposed that the endomembrane sheath, localized preferentially to surfaces of ER elements and specialized domains of the inner leaflet of the plasma membrane, structurally supports F-actin-based motilities and orchestrates diverse activities of plant cells.

Short-range movements are relatively poorly understood because vesicles and organelles perform only short courses in any direction and then, typically, change both speed and direction. Such motilities are presumed to be supported by short F-actin elements organized into dense meshworks which are, however, difficult to visualize as F-actin. For instance, this type of movement is typical for cells (e.g., root cap statocytes) and subcellular domains (e.g., tips of root hairs and pollen tubes, wound sites in *Chara* internodal cells) where F-actin could not be visualized as distinct filaments, bundles, or networks.

It is known that F-actin is essential for both tip-growth (e.g., Pierson and Cresti, 1992; Baluška et al., submitted) and wound-healing (Foissner, 1991; Foissner and Wasteneys, 1997; Foissner et al., 1996). These two situations (for details, see Tip-Growth: F-Actin Dependent Process and Wound Healing, below) represent examples of strictly targeted exocytosis based on F-actin tracks — as documented by pharmacological approaches — although individual AFs are not visible using the presently available visualization techniques (see Fig. 7 in Foissner and Wasteneys, 1997, for plaque-like F-actin meshwork). A striking feature is that these processes are extremely sensitive to low levels of cytochalasin and latrunculin, which still do not affect the long-range motility along more robust AFs or F-actin bundles (Chris Staiger, personal communication, for pollen tube growth; Miller et al., 1999, for root hair growth; Foissner and Wasteneys, 1997, for wound-healing).

A similar phenomenon, but on a larger scale, is characteristic for root cap statocytes, which are specialized as gravity-perceiving cells (for details, see Mechanosensing: Gravi-Orientation and Responses to Touch, below). These cells lack any distinct AFs (Baluška et al., 1997b; Blancaflor and Hasenstein, 1997) and display only diffuse actin fluorescence surrounding the plastid-based statoliths. F-actin oligomers are presumably organized into meshworks, as indicated by GFP-talin-transformed *Arabidopsis* seedlings (M. Jaideep, personal communication). Additionally, statoliths are enclosed in abundant plant myosin homologs (Wunsch and Volkmann, 1993; Baluška and Hasenstein, 1997) and perform continuous saltatory movements (Sack et al., 1986; Volkmann et al., 1999). Actomyosin-based forces might be implicated also in clustering of statoliths (Sack et al., 1986; Smith et al., 1997). Intriguingly, the same situation was reported also for vacuole-based statoliths of *Chara* rhizoids (Hejnowicz and Sievers, 1981; Bartnik and Sievers, 1988; Braun, 1996; Braun and Sievers, 1993; Sievers et al., 1996).

Long-Range Mass Transport: Cytoplasmic Streaming

Many plant cells show dramatic intracellular movements known as cytoplasmic streaming (for reviews, cf. Kuroda, 1990; Shimmen and Yokota, 1994), especially giant (several centimeters in length) algae cells (*Nitella*, *Chara*, *Bryopsis*, *Acetabularia*) and tip-growing cells like rhizoids, root hairs, and pollen tubes. Environmental stimuli like light, temperature, mechanical pressure, and gravity can regulate at least some of these intracellular movements (Wayne et al., 1990; Staves et al., 1995; reviewed by Nagai, 1993). Streaming velocities in some plant cells can reach up to 100 μm per second (e.g., Wayne et al., 1990; Staves et al., 1995). In *Characean* internodal cells, the best-investigated plant system, thick F-actin bundles are localized at the interface between the peripheral stationary ectoplasm and the moveable endoplasm. This stationary ectoplasm is built up by cortical MTs and chloroplasts interconnected in the axial direction by numerous longitudinal F-actin cables. The latter function as tracks for transport of organelles (e.g., Williamson, 1993), mostly dictyosomes, mitochondria, ER membranes, and vesicles. The direction of cytoplasmic streaming is thought to be determined by the polarity of AFs. Nuclei are often moving at slower speeds or are even stable at their positions. In the most elegant experiments (for the original in vitro experiment, cf. Sheetz and Spudich, 1983), Shimmen and Yano (1984) demonstrated ATP-dependent movement of myosin-covered plastic beads and cell organelles along F-actin tracks of *Characean* cells in vitro. These authors also showed differences in movement velocities, depending on the source of myosins, indicating distinct effectiveness of the different myosin motor proteins (for reviews, see Higashi-Fujime, 1991; Shimmen and Yokota, 1994). In a similarly elegant experiment, Kohno et al. (1991) demonstrated that muscle F-actin can be moved in vitro in the presence of a crude extract obtained from pollen

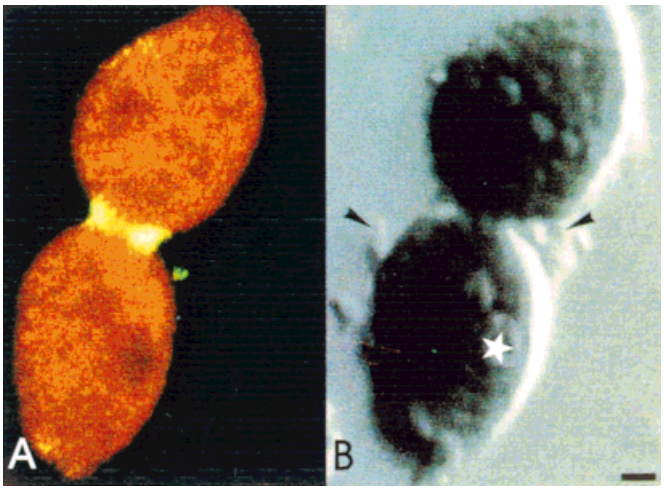

Fig. 2. Laserscan view of short-term fixed (15 minutes in buffered 2% paraformaldehyde/0.1% glutaraldehyde), isolated chloroplast in dividing stage from moss *Funaria*, (A) after labeling by monoclonal anti-actin antibody (ICN, clone 4), (**B**) corresponding differential interference contrast view; overlay of actin labeling (yellow) and chloroplast autofluorescence (red). Constriction of the dumbbell-shaped chloroplast is labeled by the actin antibody (**A**). Cytoplasmic fragments at the plastid envelope (arrowheads in **B**) show no labeling; starch grain (star). Similar results were obtained with chloroplasts from *Spinacia*, *Vicia*, and *Zea*. Micrograph courtesy of Dr. Martin Tewinkel. Bar: 1 μm.

tubes. Using heterologous antibodies, Grolig et al. (1988) detected two myosin-like polypeptides of 200 and 110 kDa, respectively, in *Chara*. Yokota and Shimmen (1994) purified a 170-kDa myosin from lily pollen tubes.

Cytoplasmic streaming and organellar movements in plants depend on phosphorylation-dephosphorylation processes and intracellular calcium concentrations (e.g., Tominaga et al., 1987). Significantly, all sequenced plant myosins belong to the category of myosin motors which are potentially inhibited by high calcium levels due to the release of their calmodulin-based light chains. This inhibition mechanism was confirmed for myosin isolated from pollen tubes of lily (Yokota et al., 1999). Calcium-mediated inhibition of this plant myosin has been shown to be associated with calmodulin dissociation from the myosin complex, providing the first biochemical information shedding light on calcium-mediated inhibition of cytoplasmic streaming in plant cells (e.g., Doree and Picard, 1980; Kohno and Shimmen, 1988; Williamson and Ashley, 1982). Calcium-dependent protein kinases (CDPKs) were also reported to colocalize with F-actin bundles (McCurdy and Williamson, 1991). These results suggest that long-range movements in plant cells occur principally on the basis of similar mechanisms (Kikuyama et al., 1996), as were well established for the actomyosin-based force generation in skeletal muscle. Nevertheless, important steps and critical molecules still await identification by dissecting the process on the basis of plant genes.

PLASTID DIVISION

For plastid division in higher plants (recent reviews Pyke, 1997; Osteryoung and Pyke, 1998), a clear correlation exists between cell size and plastid numbers. This correlation depends on cell type and stage of cellular differentiation (Leech and Pyke, 1988). In some plant cells, plastids are aligned by MTs running parallel to the longitudinal cell axis (Tewinkel and Volkmann, 1987). In the constricted neck of dividing plastids, filamentous structures were observed (for review, see Kuroiwa et al., 1998) associated with the outer plastid envelope, forming cytoplasmic plastid dividing (PD) rings. In addition, similar filamentous structures were described at the inner surface of the inner plastid envelope. With isolated chloroplasts, the outer PD ring shows clear actin labeling (Fig. 2; cf. also Hashimoto, 1986 and 1992). Recently, different genes have been identified in connection with plastid division (Pyke et al., 1994; Wakasugi et al., 1997; Oysteryoung et al., 1998; Strepp et al., 1998). Some of the related mutants show that plastid division is also under the control of an ancient tubulin gene, FtsZ, originally expressed in bacteria. Therefore, plastid division seems to be a highly complex process accomplished by cooperation between both major types of eukaryotic cytoskeleton. This complex situation clearly demonstrates that studies on downstream protein–protein interactions are an important complementary approach to investigations on the upstream genetic pathways.

CYTOMORPHOGENESIS AND GROWTH PROCESSES

Unicellular Algae

Due to their delicate and fascinating morphology, algae provide excellent material for morphogenetic studies. The single-cell *Acetabularia* (Hämmerling, 1955) and *Micrasterias* (Kiermayer, 1981) have been the preferred model systems for several decades. Apical morphogenesis in *Acetabularia* (Menzel, 1994b) is characterized by alternations between tip-growth and whorl branch initiation and is terminated by the cap formation, including development of cysts via nucleated cytoplasmic domains. Axially oriented cortical F-actin bundles are acting as tracks for long-range transport (up to some centimeters) which is bidirectional and highly complex in regard to the speed of moved organelles (Menzel, 1986). Rearrangement of axial F-actin at the tip into random networks occurs prior to branch

and cap formation. Moreover, at the arrival of nuclei in cap rays, F-actin rearranges and AF bundles encircle each disk of cap cytoplasm of the future cyst. The process of compartmentalization culminates in cyst formation, which starts with contraction of a cytokinetic F-actin ring around cytoplasmic domains. By lytic processes, the cyst walls bulge out and the actin ring contracts further. As a result of these forces, a unique cyst shape arises (Menzel, 1986). MTs and probably myosins belong to the molecular partners cooperating with F-actin in this process of intracellular pattern formation. In the end phase of cyst formation, F-actin forms cortical networks of randomly oriented short F-actin bundles.

Acetabularia morphogenesis is of special interest, insofar that the long-range transport in the stalk is intimately connected with polymerizing/depolymerizing processes and rearrangements of AFs at sites of pattern formation. This situation is in principal similar to that occurring in the slime mold *Physarum* (Brix et al., 1987) and amoebae (Stockem and Brix, 1994), where AF cables are connected to sites of actin polymerization at leading edges, showing extremely rapid polymerization-depolymerization circles. The particular situation in *Acetabularia*, however, is more complex in its morphogenetic end result.

With *Micrasterias*, the role of F-actin in radial morphogenesis is indicated by inhibitor experiments (Tippit and Pickett-Heaps, 1974; Ueda and Noguchi, 1988; Meindl, 1993). EM visualization of F-actin in the region of lobe formation, the most important site for morphogenesis, has failed so far (Meindl et al., 1992). One possibility is that the high-pressure freeze-substitution fixation does not reveal the category of F-actin meshworks composed of very short and dynamic F-actin oligomers. At least part of this labile and very dynamic population of F-actin was visualized using rhodamine-phalloidin in fixed cells (Ueda and Noguchi, 1988) as well as using FITC-phalloidin in vivo (Meindl et al., 1994). Interestingly, the cortical F-actin networks were found in both the growing and nongrowing cell portions (Meindl et al., 1994). The latter study also revealed another population of AFs ensheathing the nucleus during its migration.

Multicellular Algae

Induction of Cell Polarity in Fucoid Zygotes. Zygotes from fucoid brown algae *Fucus* and *Pelvetia* are an experimentally accessible system for accomplishing cytomorphogenesis and induction of multicellularity in dependency on environmental factors like light, attachment to substratum, and gravity (Jaffe, 1958; for comparison of three different morphogenetic systems of algae, see Menzel, 1996). Additionally, fucoid zygotes represent an excellent model system for studying the role of polarity and asymmetric cell division for the establishment of multicellularity (Kropf, 1994; Quatrano and Shaw, 1997; Bisgrove and Kropf, 1998; Alessa and Kropf, 1999). The first division of fertilized *Fucus* zygote is asymmetric, oriented transversely to the future growth, and produces rhizoid and thallus cells with different developmental fates. Cytochalasin treatment prevents the ability of activated zygotes to polarize and accomplish asymmetric division (Quatrano, 1973). Furthermore, drug treatment also disrupted endogenous currents associated with the polarization of the activated zygote (Brawley and Robinson, 1985). Later studies showed that the induction of zygote polarity is associated with rearrangements of the cortical actin cytoskeleton, forming a distinct plasma membrane associated actin cap at the prospective growth pole (Kropf et al., 1989; Kropf, 1994) to which the secretory apparatus is selectively targeted (Shaw and Quatrano, 1996; Hable and Kropf, 1998). The actin-associated plasma membrane generates a locally specialized domain where calcium channels accumulate, as indicated by external electrophysiological measurements and plasma membrane-associated calcium (Kropf, 1994; Love et al., 1997). Intriguingly, in situ hybridization studies revealed that actin mRNA is also asymmetrically distributed in activated zygotes, and this requires intact F-actin but not MTs (Bouget et al., 1996). The extracellular matrix, linked to the actin cytoskeleton via putative transmembrane linkers, plays a crucial role in the fixation of the formerly unstable growth axis (Kropf et al., 1988; Quatrano et al., 1991; Fowler and Quatrano, 1997).

Photomorphogenetic Induction of Multicellularity in Characean Protonemata. Irradiation of the tip-growing *Characean* protonemata with blue or white light triggers photomorphogenetic induction of multicellularity (Hodick, 1993). Decreasing rates of tip-growth were associated with a morphogenetic switch to the diffuse cell growth, dismantling of the apical ER aggregation corresponding to the Spitzenkörper (Bartnik and Sievers, 1988), disappearance of the apical F-actin patch, and induction of mitotic divisions at the protonemata tip (Braun and Wasteneys, 1998). An intriguing feature of the first division is a prominent anterior spindle pole, having numerous long MTs extending into the F-actin-rich cell tip, whereas the posterior spindle pole is poorly developed. It can be proposed that this F-actin-rich apical cell cortex orients the mitotic spindle, as it is in the case of fucoid zygotes (see above), budding yeast (e.g., Drubin, 1991), and many other cell types and organisms (cf. the following section on mitosis in higher plants).

Higher Plants

Mitosis: F-Actin-Independent Process. Interphase cells of higher plants possess a well-developed, dense network of AFs typically radiating throughout the cytoplasm from surfaces of centrally positioned nuclei (Seagull et al., 1987; Traas et al., 1987; Schmit and Lambert, 1987; Lloyd and Traas, 1988; Baluška et al., 1997a). In addition to this dominant cytoplasmic F-actin network, there are also fainter cortical F-actin arrays which often co-align with cortical MTs and presumably associate with the plasma membrane (see also Intracellular Interactions: Polysomes, Microtubules, Plasma Membrane, above).

Early pharmacological studies showed that cytochalasins do not affect any mitotic phase of dividing plant cells (e.g., Palevitz, 1980; Schmit and Lambert, 1987; Mole-Bajer and Bajer, 1988; Mole-Bajer et al., 1988; Cho and Wick, 1990; Panteris et al., 1992). This characteristic feature has been confirmed by our preliminary results (Baluška, unpublished results) using the much more effective F-actin drug latrunculin (Ayscough, 1998). This new type of F-actin drugs causes complete depoly-

merization of F-actin in yeast (Ayscough et al., 1997), animal (Knoblich et al., 1997), lower plant (Gupta and Heath, 1997), and higher plant (Baluška and Volkmann, unpublished) cells. Meristematic maize root cells treated with saturated concentrations of latrunculin B, and obviously devoid of any F-actin, continue their mitotic cycles (Baluška, unpublished). Therefore, we can safely conclude that F-actin is not involved in the accomplishment of plant mitosis.

In accordance with the above experimental data, it is known that mitotic plant cells show depletion of F-actin from those cell periphery domains which are marked with the pre-prophase band (PPB), indicating the prospective division plane (Cleary et al., 1992; Liu and Palevitz, 1992; Cleary, 1995; Cleary and Mathesius, 1996; Baluška et al., 1997a). Depletion of F-actin during mitosis is typical also for deeper cytoplasmic regions within and around the mitotic spindle (Baluška et al., 1997a). However, distinct F-actin enriched cell periphery domains of mitotic plant cells (Baluška et al., submitted), although less accepted, appear to be relevant for organization of the cell cortex integrity and for keeping the mitotic spindle in a proper position. Experimental evidence for this concept came from cytochalasin D treatment of developing pollen of *Brassica* when the mitotic spindle was completely dislocated in F-actin depleted cells (Gervais et al., 1994). Similar effects of cytochalasin D on mitotic spindle positions were described also for carrot suspension cells (Lloyd and Traas, 1988). Mitotic dismantling of the cytoplasmic F-actin networks probably provides a structural basis for the cessation of cytoplasmic streaming and the exclusion of organelles from the spindle region in mitotic plant cells (Mineyuki et al., 1984; Mineyuki and Gunning, 1988).

Cytokinesis: F-Actin-Supported Process. In contrast to the above mitotic events, there are good indications that the actin cytoskeleton participates actively in some cytokinetic events during separation of daughter cells after the successful segregation of chromosomes into daughter nuclei. In contrast to all other eukaryotic cells, higher plant cells do not perform their cytokinesis via the actomyosin-driven cleavage, starting from the cell periphery, but typically perform MT-dependent (Palevitz, 1987b; Mole-Bajer and Bajer, 1988; Staiger and Cande, 1991; Panteris et al., 1992) assembly of the phragmoplast, a precursory structure of the new cell wall. This complex assemblage contains, besides MTs, also F-actin and abundant endomembranes. Phragmoplast forms in the center of postmitotic plant cells and then expand centrifugally. The phragmoplast initiates formation of the early cell plate by spatially targeted exocytosis, producing callosic deposits which soon transform into the cell plate, and then into the new cellulosic cell wall. This is the only known example, throughout the entire eukaryotic kingdom, where the plasma membrane is formed de novo by fusion of a unique population of GA-based vesicles containing phragmoplastin, a plant homolog of dynamin expressed only during plant cytokinesis (Gu and Verma, 1996, 1997).

F-actin accumulates selectively within the phragmoplast as soon as its early form appears, between the late anaphase – early telophase nuclei (Clayton and Lloyd, 1985; Gunning and Wick, 1985). This feature proved to be characteristic for all cell types from higher plants investigated up to now (Palevitz, 1987b; Kakimoto and Shibaoka, 1987; Zhang et al., 1993; Baluška et al., 1997a; Valster and Hepler, 1997), although the role of F-actin within phragmoplasts remains enigmatic. Microinjection of fluorescent phalloidin revealed that abundant AFs are newly assembled during phragmoplast formation (Schmit and Lambert, 1990). An intriguing finding is that most AFs located in the phragmoplast are of uniform polarity, facing the future cell division plane with their barbed ends (Kakimoto and Shibaoka, 1988). Therefore, it can be expected that the phragmoplast F-actin is essential for actomyosin-based vesicle accumulation within the cell division plane, which culminates in the assembly of the early cell plate. But there is serious reservation about this concept. Cytokinetic cells compromised or devoid of their F-actin, irrespective of whether this is achieved by drug treatment or microinjection of profilin (Valster et al., 1997), can still assemble phragmoplasts as well as cell plates, and can complete the plant cytokinesis. Nevertheless, phragmoplasts devoid of F-actin often show aberrant spatial distributions (Hepler and Palevitz, 1974; Palevitz, 1980; Gunning and Wick, 1985; Cho and Wick, 1990; Valster et al., 1997) and the frequency of phragmoplasts increases in proportion to spindles and PPBs (Hush and Overall, 1992), indicating that cytokinesis proceeds much slower without F-actin. We have applied effective concentrations of latrunculin B on meristematic cells of maize root apices and found that 20 minutes of latrunculin B treatment is powerful enough to induce twisting of existing phragmoplasts (Baluška and Volkmann, unpublished). Moreover, root apex cells of the *lilliputian* maize mutant (Dolfini et al., 1999), which are depleted of all F-actin in their dividing cells (Busti, Baluška, Dolfini, Volkmann, unpublished), show twisted phragmoplasts as well as chaotically laid cell plates and young cell walls. This indicates that F-actin within phragmoplasts is involved in the structural stabilization of an inherently unstable phragmoplast body and this appears to be relevant for the precise control over division planes of higher plant cells. Localization of unconventional myosin to the young cell plate (AT myosin VIII, Reichelt et al., 1999) additionally confirms that the actomyosin system is involved in stabilization of this unstable cell wall area. This view is supported by observations that F-actin elements of the phragmoplast are distributed not only in a parallel fashion, along with MTs, but some AFs obviously interlink adjacent MTs (Kakimoto and Shibaoka, 1988).

The most sensitive cytokinetic phase with respect to F-actin drugs is the finalization of the cell plate formation. Obviously, F-actin performs some essential roles in the final fusion of centrifugally expanding cell plates with mature parent cell walls at cortical domains predicted by the PPB (Mineyuki and Gunning, 1990; Valster et al., 1997). All available data suggest that these cortical domains of parent cell walls, organized via the PPB array during the previous prophase, selectively attract the leading edges of assembling cell plates. Interestingly, when dividing cells are microinjected with rather high fluorescent phalloidin, then unique F-actin patches localize to these PPB domains during cytokinesis (Valster and Hepler, 1997). Other experimental data suggest that putative signals left by PPBs use actin-based signals in order to finalize the

*TABLE 1. Effects of actomyosin drugs on number of transition zone cells and on final cell dimensions in maize root apices**

	Number of cells	Cell length	Cell width
Control	100% (24)	100% (169)	100% (45)
Latrunculin 2h	108%	85%	100%
Latrunculin 6h	188%	70%	89%
Latrunculin 2 + 12h	338%	36%	111%
BDM 100μM, 6h	125%	96%	80%
BDM 1mM, 6h	204%	85%	82%

*Number of cortical cells, expressed as a percentage from the control value, in the transition growth zone (Baluška et al. 1996c) in untreated maize roots and those exposed to latrunculin B (1μM) for 2h, 6h, and 2h followed by 12h of growth in latrunculin-free medium (F-actin does not recover even in this situation, data not shown). Progressive increase of cell numbers within the transition zone shows that the onset of cell elongation is F-actin dependent. Similar trend is obvious also in roots treated with 2,3 butanedione-monoxime (BDM) which is a general inhibitor of myosin ATPase. On the other hand, all actomyosin inhibitors inhibited cell elongation as evidence by decreased final cell lengths while effects on cell widths are less straightforward and there is even some stimulation of cell widths in the long-term absence of F-actin. Also data on cell lengths and widths are presented as percentages from control values. In control samples, numbers in brackets represent real values obtained for cortical maize root cells (cell lengths and widths are in micrometers). Values were obtained from 10 independent replicates and differences between replicates were insignificant.

plant cytokinesis within the plane predicted by the PBB (Giménez-Abián et al., 1998).

Cell Elongation: F-Actin-Dependent Process. Plant cells leaving their mitotic cycles typically grow further by cytoplasmic growth. If they do not start a new round of mitotic cycle then, after some time, they typically initiate plant-specific cell growth which is based on the rapid vacuome formation. This unique mode of cell growth, found only in plants, is prominent, for instance, in roots where elongating cells of the maize root cortex increase their volume approximately four times within 2 hours (Baluška et al., 1996b).

There are preliminary experimental data suggesting that plant cell elongation is an F-actin-supported process. First of all, cytochalasin D and other treatments which partially deplete F-actin in plant cells invariably lead to inhibition of the rapid cell elongation both in shoots and roots (Thimann et al., 1992; Thimann and Biradivolu, 1994; Baluška et al., 1997a; Wang and Nick, 1998). We achieved a more detailed study on this aspect using latrunculin B, as a much more effective and specific F-actin drug, and the general inhibitor of myosin ATPase, 2,3 Butanedione-monoxime (BDM), the specificity of which was confirmed for diverse organisms (e.g., Herrman et al., 1992; May et al., 1998). Our data clearly show that the absence of F-actin decreases dramatically the final cell length in the maize root apices (Table 1). On the other hand, cell width is affected only slightly by removing all AFs. BDM at the effective concentration lowers both cell length and cell width (Table 1).

Intriguingly, maize root apices treated with cytochalasin D not only fail to perform efficient cell elongation but such cells typically accumulate within the transition zone (Baluška et al., 1996c, 1997a). The latter zone is interpolated between the apical meristem and elongation region and plays a crucial role in switching the cytoplasmic mode of cell growth into vacuome-based cell elongation (Baluška et al., 1997a). F-actin-devoid cells divide further and progress efficiently from the meristem into the transition zone (Baluška et al., 1996c), but then they fail to initiate the rapid cell elongation. These data have been confirmed using latrunculin B and BDM (Table 1). This indicates that one of the functions of the transition phase of plant cell development is to monitor its cellular F-actin level. Maize coleoptile cells show finer F-actin networks when elongating rapidly, whereas cells not elongating rapidly show a rather bundled F-actin system condensed into compact arrays (Waller and Nick, 1997). This indicates that especially fine and dynamic F-actin networks are critical for the execution of rapid cell elongation. However, this new topic needs further experimental studies in order to illuminate how precisely the actin cytoskeleton intervenes into the rapid cell elongation of plant cells.

Tip-Growth: F-Actin-Dependent Process. There are two higher plant cell types — pollen tubes and root hairs — which do not grow diffusely, but instead assemble and maintain unique cell periphery domains which selectively attract exocytotic vesicles preferentially to their growing tips, and this determines their characteristic tubular morphogenesis (Sievers and Schnepf, 1981). Early pharmacological experiments performed with pollen tubes (e.g., Franke et al., 1972) indicated that, in sharp contrast to MT-dependent polarity of diffuse growth, polarity of plant tip-growth is MT-independent and requires dynamic and intact F-actin meshworks (general review, Kropf et al., 1998; reviewed by Pierson and Cresti, 1992, for pollen tubes; Miller et al., 1997; and Baluška et al., submitted, for root hairs). MTs are organized similarly to AFs throughout the cytoplasm in the form of axial arrays (Franke et al., 1972; Pierson et al., 1986; Emons, 1987; Lloyd et al., 1987; Tiwari and Polito, 1988b; Pierson and Cresti, 1992).

An early model for the tip-growth of plant cells envisaged a dense network of fine AFs at growing tips which was proposed to maintain the dome-shaped architecture of rapidly growing turgid tips despite the absence of solid cellulose cell walls (Picton and Steer, 1982). In accordance with this model, early attempts using rhodamine-phalloidin visualized dense F-actin networks at tips of pollen tubes grown both in vivo and in vitro (Perdue and Parthasarathy, 1985; Pierson et al., 1986; Pierson, 1988; Tiwari and Polito, 1988b). However, subsequent high-pressure freeze-substituted EM studies on F-actin-rich tips reported just the opposite, F-actin depleted tips of pollen tubes (Lancelle et al., 1987; Lancelle and Hepler, 1992; Miller et al., 1996). The absence of a dense F-actin network at the very tip of pollen tubes growing in vivo was confirmed with GFP-talin fusion protein (Kost et al., 1998). Thus, only pollen tubes growing through stylar tissues seem to build dense F-actin caps, indicating that they might be involved in cell–cell interactions.

F-actin-depleted tips are reported also for tip-growing root hairs processed for conventional light microscopy (de Ruijter et al., 1998; Miller et al., 1999) and this feature was interpreted as a requirement for abundant exocytosis at this subcellular domain (de Ruijter and Emons, 1999). But in the latter case, the absence of F-actin-rich tips results from methodological problems. Permeabilization with L-α-lysophosphatidylcholine, used by the latter group, can be expected to deplete finer F-actin meshworks. In support of this notion, Steedman's wax-embedding technique (Baluška et al., 1992, 1997a,b), which allows omission of perme-

abilization, produces prominent F-actin caps at growing root hair tips. Also, the freeze-shattering technique (Wasteneyes et al., 1997), which is based on a mild permeabilization, reveals F-actin-rich tips in growing root hairs (Braun et al., 1999; Baluška et al., submitted). More importantly, these F-actin-rich root hair tips are almost identical to those obtained from in vivo observations using the GFP-talin-transformed *Arabidopsis* seedlings (Baluška et al., submitted; Kost et al., 1998). In accordance with prominent F-actin accumulations at tips of growing root hairs, such F-actin accumulations mark the outgrowth domain of trichoblasts during the root hair initiation (Baluška et al., submitted). Interestingly, conspicuous plasma membrane-associated F-actin-enriched domains are typical for the pollen grain germination when the site of pollen tube emergence is associated with the accumulation of AFs at the germinating aperture (Heslop-Harrison et al., 1986; Tiwari and Polito, 1988a; Heslop-Harrison and Heslop-Harrison, 1992).

The tip-growth of plant cells closely resembles the tip-growth of lower plant (Braun and Wasteneys, 1998) and fungal (Heath and Harold, 1992; Jackson and Heath, 1990, 1993; Heath, 1990) cells. For instance, the prospective area of side branch formation in the protonemata of mosses is marked by prominent accumulations of AFs (Quader and Schnepf, 1989). During the following transition to the tip-growth, this AF arrangement became loosened, but the apical dome still preserved a high amount of F-actin associated with the apical plasma membrane. Similarly, the formation of new buds in yeast also involves concentration of plasma membrane-associated F-actin at the exocytotic domain (Drubin, 1991), corresponding well to the situation during initiation of tip-growth by both pollen tubes and root hairs. In all these situations, the plasma membrane-associated actin cytoskeleton plays the dominant role in cytoarchitecture, and dynamic F-actin meshworks invariably define the targeting of exocytotic machineries. Several authors proposed that the plant tip-growth is completely different from the diffuse growth of plant cells and that it, in some aspects, resembles the movement of amoebae or fibroblasts (Lord and Sanders, 1992; Harold et al., 1996; Pickett-Heaps and Klein, 1998). These speculative ideas are fueled by the fact that tip growth is driven by actin-dependent assembly of cell periphery domains active in exocytosis (Gupta and Heath, 1997). In accordance with this concept, Rho GTPases determine F-actin-based localized cell growth both in yeast cells and plant pollen tubes (Lin et al., 1996; Li et al., 1998; Lin and Yang, 1997; Derksen et al., 1995).

CELL-TO-CELL INTERACTIONS: PLASMODESMATA AS ACTOMYOSIN-SUPPORTED CHANNELS

One of the most exciting recent developments in the study of plant actomyosin cytoskeleton concerns its putative involvement in the structural and functional organization of plant plasmodesmata. These permanent intercellular cytoplasmic channels are unique for walled plant cells and allow symplastic continuity throughout the plant body (for recent reviews see Mezitt and Lucas, 1996; Ghoshroy et al., 1997; Jackson and Hake, 1997; McLean et al., 1997; Ding, 1998; Kragler et al., 1998). Plasmodesmata are responsible for the supracellular, rather than multicellular, nature of higher plants (Lucas et al., 1993). Primary plasmodesmata are formed during plant cytokinesis when elements of cortical ER remain entrapped between fusing cytokinetic vesicles (Hepler, 1982). They are multimodular complex assemblies consisting of both membranes and numerous proteins from which, due to technical limitations (Kotlitzky et al., 1992; Turner et al., 1994), only a few have been identified up to now (Ehlers et al., 1996; Yahalom et al., 1998).

Plasmodesmata allow free diffusion of proteins and mRNAs up to 1 kDa (Tucker, 1982; Goodwin, 1983). Newly available sophisticated experimental systems allowed the characterization of plasmodesmata as gateable cytoplasmic channels. For instance, proteins of the phloem sap, ranging in mass from 10–200 kDa, are transported rapidly into the neighboring cells after microinjection into mesophyll cells of *Cucurbita* cotyledons (Balachandran et al., 1997). Virtually nothing is known about transport mechanisms through plasmodesmata, but data are accumulating in favor of participation of cytoskeletal elements in this highly complex process. First of all, actin was localized to plasmodesmata, both to the neck regions as well as deeper in the canal, associated with the plasma membrane and the central ER element of plasmodesmata (White et al., 1994). Intriguingly, besides actin, plasmodesmata also contain myosins (Blackman and Overall, 1998; Radford and White, 1998; Reichelt et al., 1999), suggesting that actomyosin-based forces could be involved both in the structural and functional organization of plasmodesmata (for the hypothetical model, see Overall and Blackman, 1996). In support of this concept, disintegration of AFs via microinjection of profilin (Staiger et al., 1994) or cytochalasin D treatment resulted in a more open configuration of plasmodesmata, and these were then freely permeable up to 20 kDa, as shown by coinjection of fluorescent dextran particles of different sizes (Ding et al., 1996).

SENSING AND SIGNALING RELATED TO ENVIRONMENTAL FACTORS

Besides the role of the dynamic actin cytoskeleton in long-term processes such as cell growth and morphogenesis, as described above, recent experiments demonstrate the importance of plant actin cytoskeleton in short-term processes related to complex networks of signaling pathways which transform environmental stimuli into cellular signals and responses (Thuleau et al., 1998). Among these latter processes are chloroplast positioning, specialized movements of guard cells, responses towards gravity and touch, plant host–pathogen interactions, and wound-healing (for long-term morphogenetic processes under the control of environmental stimuli, especially light, cf. Induction of Cell Polarity in Fucoid Zygotes, Photomorphogenetic Induction of Multicellularity in *Characean* Protonemata (above) and Nick, 1999).

Chloroplast Positioning by Light

Light-induced movements of chloroplasts (Haupt, 1998), resulting in optimal positioning which allows the most effective use of light, was investigated in a wide variety of taxonomic groups and ecotypes. Perception of

light by blue and/or red light receptors is the first step in which a motor apparatus is connected in the stimulus–response chain. In this respect, the filamentous green alga *Mougeotia,* possessing one large chloroplast per cell, is the most comprehensively investigated species. Inhibitor experiments (Wagner et al., 1972), heavy mero-myosin decoration (Marchant, 1976; Klein et al., 1980), and rhodamine-phalloidin labeling (Mineyuki et al., 1995), all ascertained that the filamentous material observed with living material (Schönbohm, 1973a) is actin. Appearance and disappearance of thin AFs at the moving edge exactly correlate with the beginning and end of chloroplast rotation under the light regime (Mineyuki et al., 1995). Circular F-actin structures formed around the chloroplast accumulate at their endocellular destination and these structures disappeared in darkness (Kadota and Wada, 1992). Spatial changes in the organization of F-actin was suggested for chloroplast positioning in diverse plant systems (Cox et al., 1987; Grolig and Wagner, 1988; Menzel and Elsner-Menzel, 1989; Kadota and Wada, 1992; Dong et al., 1996). An intriguing possibility is that the actin cytoskeleton is responsible not only for moving of chloroplasts, but also for their clustering and anchoring at distinct subcellular domains, typically at the cell periphery (e.g., Witztum and Parthasarathy, 1985; Dong et al., 1998). Obviously, AFs not only provide tracks for intracellular movements, but they can also serve as an anchorage device.

What is unclear, however, is if pulling or propelling of chloroplasts is the process responsible for their positioning. In comparison to the actomyosin-driven cytoplasmic streaming, the speed of oriented chloroplast movements in the range of 10^{-2} μm per second is very slow (Grolig and Wagner, 1988). Myosin has not been detected so far in *Mougeotia*. Therefore, it is questionable if an actomyosin force is operating in this process. With respect to the symbiont hypothesis (e.g., Sitte, 1993), a propelling mechanism based on actin polymerization could become an attractive possibility, as was shown for intracellular movements of infectious pathogens like *Listeria* and *Shigella* in animal host cells (Cossart, 1995). This idea is in full agreement with AF elongation rates in vitro, counting approximately 10^{-2} μm per second at the barbed end (Bonder et al., 1983). Contradictory observations are, however, that AFs are extending from the leading edge of advancing chloroplasts (Schönbohm, 1973b). This situation calls for additional experiments investigating the polarity of AFs in strict correlation to the light regime and to the kinetic of chloroplast movements (Mineyuki et al., 1995; Haupt, 1998).

Multisensory Guard Cells: Stomatal Movements

At the interface between environment and plant body, guard cells are distinguished as a multisensory system (Assmann and Shimazaki, 1999) controlling gas exchange by their ability to open and close in relation to environmental conditions, e.g., light, water stress, and CO_2 concentration. Under water stress resulting in decreasing hydrostatic pressure, the stress hormone abscisic acid is synthesized and redistributed within the plant. As a consequence, K^+ and Cl^- ions as well as organic substances are released from guard cells, which is followed by closure of stomata apertures.

In several plant species, mature guard cells are characterized by radially oriented AFs (Cleary et al., 1993; Kim et al., 1995; Eun and Lee, 1997; Cleary and Mathesius, 1996; Apostolakos and Galatis, 1999). This organization of the actin cytoskeleton results from AF rearrangements during guard cell differentiation (Apostolakos and Galatis, 1999). Stabilization of AFs by phalloidin treatment inhibits stomatal closing in a concentration-dependent manner, whereas AFs fragmentation by cytochalasin D increases stomata opening but does not affect their closing (Kim et al., 1995). Under the action of light and abscisic acid, reorganization of AFs is closely related to aperture size. MTs colocalizing with AFs did not show any reorganizations (Eun and Lee, 1997). Experiments correlating size of stomatal aperture with K^+ inward channel activities under cytochalasin B/D and phalloidin treatments indicate that cytochalasins facilitate and phalloidin inhibits K^+ influx in guard cells, thus resulting in enhancement and inhibition of stomatal opening, respectively (Hwang et al., 1997). These data were confirmed by Liu and Luan (1998), indicating that F-actin may be involved in the osmosensing via targeting of inward K^+ channels for turgor regulation. These are the first examples of a putative regulatory role of the dynamic actin cytoskeleton on physiological processes of plant cells, via its impacts on ion channel activities within the plasma membrane.

Mechanosensing: Gravi-Orientation and Responses to Touch

Active orientation of growing plant cells and organs under the influence of gravity is highly important for ecophysiological adaptation (Hemmersbach et al., 1999). As for mechanosensing, the perception mechanism is accomplished in cells specialized for gravisensing (statocytes) when unconstrained sedimentation of dense intracellular particles (statoliths) is favored vs. a more general mechanism by strain exerted via the weight of the whole protoplast experienced at the plasma membrane (Sack, 1997). The unique lack of distinct AF networks and cables in root cap statocytes probably facilitates unconstrained sedimentation of their statoliths (Baluška and Hasenstein, 1997), and this is in agreement with results showing no effects of cytochalasin D on gravitropic bending of roots from different species (Staves et al., 1997). Interestingly, if statocytes of maize and barley were labeled for F-actin after their enzymatic release from root cap tissues, then distinct AF networks were found (White and Sack, 1990). This latter finding indicates that the actin cytoskeleton in root cap statocytes is capable of rapidly assembling a more robust system if these cells are treated with cell wall-digesting enzymes.

On the other hand, delicate meshworks of oligomeric AFs (M. Jaideep, personal communication) and myosin II (visualized by heterologous antibodies) have been localized in close proximity to statoliths in multicellular systems (Baluška and Hasenstein, 1997; Wunsch and Volkmann, 1993) and single cells (Sievers et al., 1996; Braun, 1996). This is probably the structural basis for continuous saltatory motions of statoliths even in their sedimented state (Hejnowicz and Sievers, 1981; Volkmann et al., 1999). Experiments under altered gravity conditions using cytochalasins showed that the position

of statoliths within statocytes depends on two forces, extracellular gravitational force and intracellular tensional forces provided by AFs (Volkmann et al., 1991; Buchen et al., 1993; Perbal et al., 1997; Volkmann et al., 1999). All this suggests that the stimulus transformation must occur close to the surface of statoliths (Volkmann and Tewinkel, 1996).

A perception mechanism has been proposed (Volkmann et al., 1999) according to which the dynamic actin cytoskeleton, built via dynamic and delicate F-actin meshworks of oligomeric AFs, is involved both in statoliths motion and gravity-sensing. Specifically, root cap statocytes might measure gravity-sensitive oriented statoliths motions on the background of F-actin supported randomized statoliths motions, and thereby perceive the gravity vector. Clustering of statoliths (Sack et al., 1986; Smith et al., 1997) by these dense networks composed of F-actin oligomers (M. Jaideep, personal communication) and putative myosins (Baluška and Hasenstein, 1997) may play an important role in this process. Interestingly, gravity influences not only delicate oligomeric F-actin meshworks and saltatory statoliths motions but also the long-range cytoplasmic streaming in large cells of *Characean* algae (Wayne et al., 1990; Staves et al., 1995). In this plant system, gravity perception is obviously achieved via interactions between F-actin supported cytoplasmic streaming acting as a gravity sensor and the adjacent plasma membrane acting as a gravity receptor (Staves, 1997; c.f. Ackers et al., 1994).

Recent genetic evidence for participation of cytoskeletal elements in gravi-sensing came from Masson's group (Sedbrook et al., 1999). In mutants showing altered response to gravity (*ARG1*), they found that the *ARG1* locus encodes for a 45-kDa protein containing a coiled-coil region homologous to coiled-coils found in cytoskeleton-interacting proteins. Additionally, possessing a stretch of 15–18 hydrophobic amino acids makes this protein a good candidate for forming a transmembrane helix. Interestingly, this *ARG1* is expressed in all plant organs investigated so far and the encoded protein is related to a conserved molecule involved in signal transduction in *C. elegans*. *ARG1,* therefore, might code for a phylogenetic very primitive part of signal transduction chains associated with the gravity-sensing process (for evolutionary considerations, cf. Barlow, 1995).

The anatomical basis for mechanosensing of some tendrils are sensitive areas (tactile blebs) in epidermis cells (Engelberth et al., 1995). These blebs are characterized by a unique arrangement of AFs forming a balloon-like structure which is linked to the AFs of the cytoplasm through central connecting AFs, whereas MTs form a ring-like structure. Membrane preparations revealed associations of actin and tubulin with ER membranes as well as with the plasma membrane. The specific architecture and composition of cell wall areas within the blebs and high abundance of membrane-associated calcium suggest a signal transduction chain (Engelberth et al., 1995) on the basis of a putative cell wall — plasma membrane — cytoskeleton continuum (for general information, see Wyatt and Carpita, 1993). Jasmonic acid and its derivatives are plant hormone substances controlling this mechanosensing-based response (Weiler, 1997). Formation of callose at the extracellular side of the plasma membrane during mechanical excitation indicates that the plant actin cytoskeleton communicates with the cell periphery especially when the plasma membrane associated synthase complexes support callose formation.

Plant Host–Pathogen Interactions

In the last few years it has becoming increasingly clear that the actin cytoskeleton plays critical roles in plant host–pathogen recognition events and in subsequent compatible or incompatible interactions (for general overviews of plant host–pathogen interactions, see, e.g., Hardham, 1992; Atkinson, 1993). During the resistance response of flax leaf cells to flax rust infection, dynamic reorganization of AFs occurred only during incompatible interactions when all cellular AFs became focused on penetration attempt sites of the rust fungus *Erysiphe* (Kobayashi et al., 1994). Interestingly, even uninfected cells showed reorganization of their AFs if neighboring cells accomplish hypersensitive cell death; again, all the AFs focused on necrotic cells. Subsequent experimental studies of this group confirmed that the actin cytoskeleton is actively involved in plant host–pathogen interactions when application of cytochalasins allowed even nonpathogens to succeed in penetration and infection of host cells (Kobayashi et al., 1997a,b). Arrangements of F-actin in host cells change dramatically after the first contacts of pathogens with surfaces of host organs, indicating that F-actin can act as a very sensitive sensor of both chemical and mechanical signals emanating from pathogens (Kobayashi et al., 1994; Xu et al., 1998; McLusky et al., 1999).

Local cytoplasmic aggregation represents one of the earliest resistance responses of potato protoplasts exposed to wall components of *Phytophthora infestans* and it was shown to be dependent on increased polymerization of AFs from a monomeric G-actin pool (Furuse et al., 1999; Takemoto et al., 1999). Moreover, cytochalasins were reported to inhibit hypersensitive cell death during potato–*Phytophthora* (Tomiyama et al., 1982) and barley–*Erisyphe* (Hazen and Bushnell, 1983) interactions. Similar F-actin-mediated plant host–pathogen interactions were also reported for cowpea and its rust fungus *Uromyces* (Škalamera et al., 1997). Here, the actin cytoskeleton seems to be related to callose synthesis in the resistant cultivar of cowpea (Škalamera and Heath, 1996; Škalamera et al., 1997). Interestingly, the actin cytoskeleton remained visible during later stages of cell death underlying the hypersensitive reaction, when all MTs had vanished, and this plant-unique form of programmed cell death was inhibited by cytochalasin E (Škalamera and Heath, 1998).

In conclusion, preliminary data clearly identify the actin cytoskeleton as a potentially crucial structure for host–pathogen recognition events, as well as for their developing into interactions where the plant host gains control over the pathogen. This complex process often culminates in hypersensitive death of selected cells and final host resistance.

Wound-Healing

Wounding induces a cascade of repair events resulting in the formation of a specific wound wall at the site of wounding in single cells and newly formed epidermal tissue in wounded organs. Foissner et al. (1996) investi-

gated cellular details by high resolution video microscopy, in combination with immunofluorescence microscopy, after wounding of isolated *Chara* internodal cells. Wounding occurred by hot spots of UV light ranging from 5–20 μm in diameter or mechanical puncturing of the cell. 1) The first step in this cascade is local inhibition of unidirectional vesicle movements (cytoplasmic streaming) in the wound area, which happens several seconds or minutes after the start of injury. Exocytotic vesicles then perform only oscillating saltatory motions and move much slower than in uninjured cells. This event is correlated with the disappearance of cortical F-actin bundles at the wound site. 2) Several minutes after injury saltatory movements of vesicles start and vesicle trajectories correspond to an irregular fine-meshed dense network of AFs. During this stage, wound wall material is selectively targeted, in an F-actin-dependent manner, to the wound site, which is sealed by numerous vesicle fusion/bursting events (Foissner and Wasteneys, 1997). 3) Several hours after wounding, unidirectional movements of vesicles regenerate along cortical actin bundles. The pattern of wound-induced vesicle motility corresponds well to the reorganization of the AF system from thick bundles to fine-meshed networks. These results clearly indicate that fine and dynamic meshworks of AFs are prerequisite for the spatially restricted exocytosis associated with the wound-healing in plant cells.

Injuring plant tissues and organs by cutting results, within less than 30 minutes, in formation of a fine cortical AF meshwork running parallel to the cut in cells adjacent to the wounded area (Goodbody and Lloyd, 1990). In the next stage, AFs are associated with the nucleus migrating toward the cell wall at the wounded area and F-actin-containing cytoplasmic strands line up from cell to cell parallel to the wound. This arrangement anticipates the future division plane in the cell layer adjacent to the wound, resulting finally in new epidermal tissue.

OUTLOOK

The importance of cytoskeletal networks for signaling pathways is widely accepted for animal cells (Zigmond, 1996; Carraway et al., 1998). Crucial in this respect are new techniques which are now available for investigations in the field of plant cell biology, e.g., the GFP-labeled talin being suitable for the in vivo visualization of AFs (Kost et al., 1998). As an F-actin binding molecule, GFP-talin visualizes even the shortest AFs composed of a few actin molecules which are not detectable by any other presently available cytological approach. From investigations with different cell lines, it is well known that these short oligomeric F-actin elements and their membrane-associated meshworks are crucial for signaling via, e.g., regulation of ion channel activities (Cantiello and Prat, 1996).

Specialized domains of the plant plasma membrane are enriched with dynamic AF meshworks and depleted with underlying cortical MTs (reviewed by Baluška et al., submitted). This interesting feature is characteristic especially for cytokinetic plant cells and for apices of tip-growing cells. Intriguingly, in tip-growing plant cells such as pollen tubes and root hairs, both growth polarity and cytomorphogenesis are F-actin-dependent. As dynamic F-actin has recently been discovered to direct the neuronal polarization during axon formation (Bradke and Dotti, 1999), the latter plant cell types might prove to be generally suitable for identification of those elusive protein–protein and protein–phospholipid interactions which drive F-actin-dependent polarity acquisition and maintenance in eukaryotic cells.

Components of the actin cytoskeleton might prove to be involved not only in the machinery for protein synthesis (Davies et al., 1991; Stankovic et al., 1993; for nonplant material cf. Bassel and Singer, 1997; Bassel et al., 1999) but also in the nucleo-cytoplasmic transport. For instance, actomyosin-based forces seem to be actively involved in transport through plasmodesmata and, due to some analogies between plasmodesmata and nuclear pores, this might turn out to be relevant also for nucleo-cytoplasmic trafficking. In fact, a recent report shows that the nucleus signal receptor importin α associates with the actin cytoskeleton of plant cells (Smith and Raikhel, 1998).

The excellent work of Meagher's group on actin molecular genetics calls for production of monospecific antibodies against actin isoforms and functionally specific actin epitopes, in spite of well-known difficulties with production of anti-actin antibodies (e.g., Gonsior et al., 1999). The availability of such antibodies is critical for comprehensive investigations on organ-, tissue-, and development-specific expression of plant actins. Furthermore, actin mutants can provide critical insight into functions for these isoforms.

As shown by Yen's and Staiger's groups, pollen seems to be the preferential material for isolation of actin and actin-associated proteins to perform in vitro experiments, e.g., concerning actin polymerization and depolymerization under defined physiological conditions, phosphorylation of actin, and its associated proteins, as well as actions of specific actin inhibitors.

Last, but not least, genetic identification of further plant actin-associated proteins as well as of proteins interlinking cytoskeletal elements to membrane and/or cell wall proteins is important for better understanding of actin involvements in the perception of environmental parameters and in execution of downstream signaling processes. Due to their sessile nature, plants have evolved extremely sensitive systems to sense and respond to the environment. Therefore, they might well prove to be a valuable biological system to study the involvement of the cytoskeleton in signal perception and transduction.

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