The Plant Cytoskeleton: Vacuoles and Cell Walls Make the Difference

Minireview

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The eukaryotic cytoskeleton is a dynamic filamentous network with various cellular and developmental functions. Plant cells display a singular architecture, necessitating a structurally and functionally unique cytoskeleton and plant specific control mechanisms.

Three different types of linear proteinaceous polymers comprise the cytoskeleton in animal cells: actin filaments, microtubules, and intermediate filaments. Whereas the presence of intermediate filaments in plant cells has not been unequivocally demonstrated, research concerned with the plant actin and microtubular cytoskeletons has progressed rapidly (Kost et al., 2001). Plant motor proteins (myosins, kinesins, dyneins) that move in a directional and energy-dependent manner along the cytoskeleton to translocate a variety of molecules or cellular structures have been identified but were functionally characterized only to a limited extent (Reddy, 2001). Here, we focus on current knowledge of the structure, regulation, and cellular or developmental functions of the plant cytoskeleton.

The Cytoskeleton in Nongrowing Interphase Plant Cells
Unlike animal cells, plant cells are enclosed in cell walls and generally contain large vacuoles that take up most of the cell volume. In vacuolated plant cells, the cytoplasm is restricted to thin layers in the cell cortex and around the nucleus, which are linked by transvacuolar cytoplasmic strands. Plasmodesmata, plasma membrane lined cytoplasmic channels in cell walls, interconnect adjacent plant cells (Figure 1a).

In interphase plant cells, actin filaments form randomly arranged networks in the cell cortex and around the nucleus, and extend through cytoplasmic strands. Filamentous actin structures are also found in association with plasmodesmata (Figure 1). In contrast to animal cells, where microtubules maintain intracellular organization and are required for membrane trafficking, actin filaments are mainly responsible for the positioning and the dynamic behavior of cell organelles in interphase plant cells. Organelles in these cells are constantly and rapidly moving along actin filaments in a myosin-dependent manner. This cytoplasmic streaming may be required to effectively distribute components throughout the thin cytoplasmic layers and strands. Actin disruption results in the disappearance of cytoplasmic strands and, consequently, in nuclear movement to the cell cortex.

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The actin cytoskeleton also mediates nuclear translocation to sites of pathogen attack and, at the onset of mitosis, to the center of the future cell division plane (see below). Actin structures associated with plasmodesmata are thought to control, and possibly mediate, intercellular transport of proteins and other macromolecules, which may play a key role in cell-to-cell communication (reviewed by Lloyd, 1991; Kost et al., 1999, 2001).

During interphase, plant microtubules are found predominantly in the cortical cytoplasm, where they are irregularly, obliquely, or longitudinally arranged in nongrowing cells (Figure 1a). In many cell types, sparse perinuclear microtubules radiate from the nucleus. Little is known about the functions of interphase microtubules in nongrowing plant cells. Although plant cells do not contain centrosomes, microtubule organizing centers (MTOCs) associated with the nucleus of animal cells, they do produce γ-tubulins, which are a major component of these structures. Plant microtubules, unlike their animal counterparts, appear to be nucleated in a decentralized manner at the nuclear envelope and possibly in the cell cortex (reviewed by Lloyd, 1991; Kost et al., 2001).

The Cytoskeleton during Polar Plant Cell Growth
Because plant cells are enclosed in cell walls, their polar growth is an essentially irreversible process that plays a key role during organ morphogenesis. Polar plant cell growth is largely controlled by cell wall properties and is therefore based on completely different mechanisms than the growth of animal cells.

Most plant cells expand by diffuse growth driven by turgor pressure, elongating in all directions to some extent, but mainly along one axis. Cortical microtubules in such cells assume an essentially transverse (perpendicular to the main axis of the cell growth) orientation (Figure 1b). In different types of elongating plant cells, cortical microtubules were shown to be aligned in parallel with cell wall cellulose microfibrils. Pharmacological disruption of microtubules results in depolarized cell expansion and randomized deposition of cellulose microfibrils. In addition, the orientations of cortical microtubules and cellulose microfibrils are altered by the same plant growth regulators (auxin, gibberellins, ethylene) that control cell and tissue expansion. Transverse arrays of cortical microtubules clearly have key functions in the control of diffuse growth, although recent studies indicate that this process may occasionally occur in the absence of such arrays. One model predicts that cellulose synthase, a multiprotein enzyme complex integrated into the plasma membrane, may move along cortical microtubules and use them as templates for the parallel deposition of newly synthesized cellulose microfibrils in the cell wall. The transverse cellulose enforcements laid down as a consequence may restrict radial cell expansion and direct cell elongation perpendicular to the orientation of microtubules and microfibrils (reviewed by Lloyd, 1991).

Except for a slight increase in the number of longitudinally oriented actin filaments, the organization of the actin cytoskeleton is not significantly altered in cells expanding by diffuse growth (Figure 1b; Kost et al.,
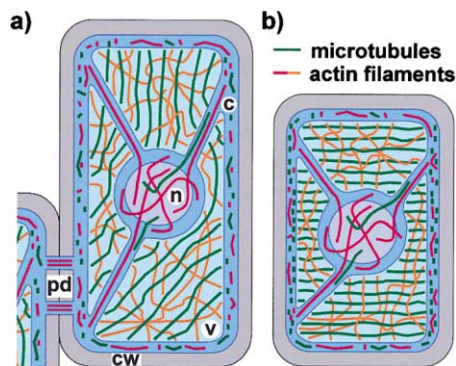
Figure 1. The Interphase Plant Cytoskeleton

Cytoskeletal structures in nongrowing cells (a) and in cells that elongate by diffuse growth (b). Unless stimulated by wounding or pathogen attack, fully expanded cells such as the one depicted in (a) are often free of cytoplasmic strands and contain nuclei located in the cell cortex. Cytoplasmic regions are indicated in dark blue, and in light blue when shown underlying the vacuole, or in purple when shown overlaying the nucleus. pd, plasmodesmata; c, cytoplasm; n, nucleus; v, vacuole; and cw, cell wall.
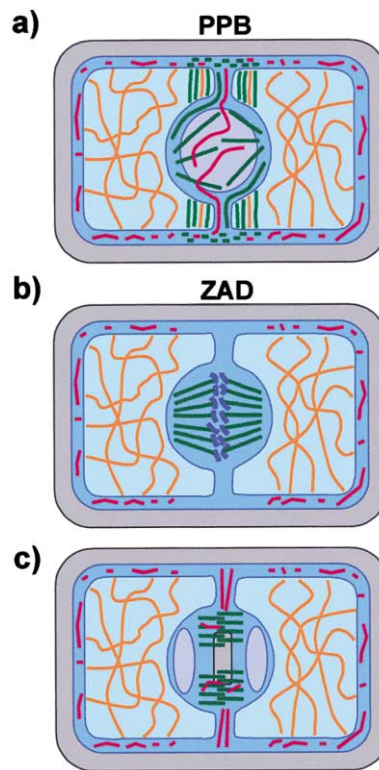

Figure 2. The Plant Cytoskeleton during Cell Division

Cytoskeletal structures during preprophase (a), metaphase (b), and cytokinesis (c). Meristematic cells do not contain large vacuoles, but divide using essentially the same mechanisms and cytoskeletal structures as vacuolated cells. The dark blue structures attached to microtubules in (b) represent chromosomes. PPB, preprophase band; ZAD, zone of actin depletion.

2001). However, pharmacological actin disruption partially inhibits this process. Actin filaments are required for the reorganization of microtubular structures in different cell types and may be involved in controlling the orientation of cortical microtubules during diffuse growth. In addition, actin-mediated cytoplasmic streaming distributes Golgi stacks throughout the cortical cytoplasm (Nebenfuehr et al., 2000), facilitating the delivery of Golgi derived secretory vesicles containing cell wall material to the cell surface. Although the increased presence of longitudinally oriented actin filaments may indicate preferential organelle transport to the two fast growing ends, secretory vesicles likely fuse with all areas of the plasma membrane during diffuse growth.

A few types of highly specialized plant cells elongate by tip growth (pollen tubes, root hairs), a mechanism distinct from diffuse growth, or employ a combination of diffuse growth and other, unknown mechanisms to expand (trichomes). Current knowledge concerning cytoskeletal functions during the growth of these cell types has recently been reviewed (Kost et al., 1999).

The Cytoskeleton in Dividing Plant Cells

Cells in developing plant tissues generally divide anticlinally (division plane perpendicular to the organ surface) at a medial plane, and daughter cells elongate perpendicularly to the plane of cell division. Rare periclinal (parallel to the surface) or asymmetrical cell divisions in meristems and embryos result in the establishment of new cell layers and/or the formation of daughter cells with distinct developmental fates. Stringent control of the positioning of cell division planes, which is mediated by the cytoskeleton (see below, also reviewed by Lloyd, 1991; Kost et al., 1999), is therefore important for plant development.

At the onset of mitosis, cortical microtubules in most plant cells condense into a narrow ring around the nucleus, the preprophase band (PPB; Figure 2a), which determines the future cell division plane. Along with microtubules, cytoplasmic strands are moved into the cell division plane to create a cytoplasm-enriched domain at this location (Figure 2). PPB formation is accompanied by a dramatic increase in the density of perinucelar microtubules, which are later reorganized into the mitotic spindle. The PPB completely disappears during karyokinesis, which is mediated by a mitotic spindle whose poles, in the absence of centrosomes, are not as sharply focused as in animal cells and devoid of astral microtubules (Figure 2b). After anaphase, the spindle disintegrates and the phragmoplast develops, a microtubular structure involved in the generation of a cell wall, the "cell plate," between the two newly formed daughter nuclei during cytokinesis (Figure 2c). Initially, the phragmoplast is positioned in the center of the dividing cell and has the shape of a small cylinder. Its major components are two opposing sets of parallel microtubules that are aligned along the axis of the cylinder and overlap at a central plane, where cell plate formation is initiated. Cytoplasmic streaming is slow during cytokinesis, and Golgi stacks accumulate around the phragmoplast (Nebenfuehr et al., 2000). Cell wall materials are synthesized and delivered to the growing cell plate by targeted secretion. Available evidence suggests that Golgi-derived secretory vesicles are transported toward the growing cell plate by kinesin-like motor proteins along phragmoplast microtubules (Otegui et al., 2001), whose main function appears to be to funnel the massive flow of secretory

vesicles precisely to the edge of the growing cell plate. Other kinesin-like proteins have also been identified in the phragmoplast, which are not required for vesicle transport, but are required for the organization of this structure. As the cell plate matures, it expands centrifugally toward the parental cell wall, with which it eventually fuses. During later stages of cytokinesis, the microtubular phragmoplast assumes the shape of a torus, which remains associated with the edge of the expanding cell plate. The growing cell plate is often obliquely oriented with respect to the future cell division plane; however, actin-dependent corrective mechanisms ensure fusion with the parental cell wall at the predetermined position (see below).

In contrast to animal cells, in which a contractile actomyosin ring mediates cytokinesis, plant cells can divide in the presence of actin depolymerizing drugs, although actin disruption often results in defective cell plate positioning. The plant actin cytoskeleton controls the position of the nucleus (see above), around which the PPB is generally formed. During plant cell mitosis, F-actin structures are present in the cell cortex, PPB, and phragmoplast (Figure 2). The functions of these actin structures are unclear. Interestingly, after the disappearance of the PPB, the cortical region previously occupied by this structure generally remains actin free during later phases of cell division and has accordingly been named "zone of actin depletion (ZAD)." The ZAD was proposed to mark the position of the future cell division plane in the absence of a PPB. Actin filaments have been observed to link the edge of the expanding cell plate to the ZAD and are thought to guide this structure, possibly in a myosin-dependent manner, to the appropriate site of fusion with the parental cell wall.

Regulation and Control of the Plant Cytoskeleton

Notwithstanding their conserved structures, actin filaments and microtubules form different cytoskeletal arrays with distinctive functions in plant and animal cells. It is therefore not surprising that clear differences in cytoskeletal control mechanisms also exist between the two cell types.

A large number of animal proteins bind actin and control its organization. Plant genes encoding numerous homologs of known animal actin binding proteins, including essential actin regulators such as ADF, profilin, and Arp2, have been cloned, and the functions of the proteins they encode are being investigated (reviewed by Kost et al., 2001). Interestingly, the completely sequenced *Arabidopsis* genome does not seem to contain homologs of genes encoding other key animal actin regulatory proteins, such as thymosin, integrin, and WASP. These proteins are either not required in plant cells or functionally supplanted by other as yet unidentified proteins.

Rho family small GTPases are important regulators of actin binding proteins and of actin organization. Animal cells express three subgroups of Rho family small GTPases (Rho, Rac, and Cdc42), each of which controls the formation of distinct actin structures that are not found in plant cells (stress fibers, dynamic cortical networks in lamellipodia, microspikes). The *Arabidopsis* genome contains 10 Rho related genes (*At-Rop* or *At-Rac*), with high sequence homology to each other and to animal Rac. Two members of this protein family have been shown to regulate actin organization in pollen tubes (Kost et al., 1999; Fu et al., 2001) and in guard cells (Lemichez et al., 2001). LIM kinases are key effectors of small GTPases in animal cells, which phosphorylate and inactivate ADF. These kinases do not have homologs in plants and are functionally replaced by calcium-dependent protein kinase (CDPK) (Allwood et al., 2001). Direct tyrosine-phosphorylation of actin by unidentified kinases has also been shown to be involved in plant actin regulation (Kameyama et al., 2000).

Most plant structural cytoskeletal and actin regulatory proteins are encoded by gene families much larger than the corresponding animal gene families. The *Arabidopsis* genome contains 6 α-tubulin, 9 β-tubulin, 8 actin, 9 ADF, and 5 profilin genes, along with many other large cytoskeletal gene families. Two different hypotheses, which are not mutually exclusive, have been proposed to explain the persistence of large groups of highly homologous cytoskeletal genes with partially overlapping expression patterns in plant genomes during evolution. Multiple genes encoding similar proteins under the control of different expression signals may enable plants to respond with increased flexibility to developmental cues and environmental signals. In addition, nonidentical but highly similar proteins that are simultaneously expressed in cells may allow greater functionality, responsiveness, and stability of the processes in which these proteins are involved. This concept has been named "isovariant dynamics" (Meagher et al., 1999).

The identification of plant "microtubule associated proteins (MAPs)," which directly bind to microtubules and control microtubular organization, has been rather elusive, because of their low expression levels and limited or no sequence homology to known animal MAPs. Only recently, the first genes encoding plant MAPs have been cloned, either after biochemical purification of the encoded protein (*NtMAP-65-1a*, Smertenko et al., 2000), or following the identification of mutants with apparent primary defects in microtubular organization (*tan1*, Smith et al., 2001; *mor1*, Whittington et al., 2001; *fra2/at-ktn1*, Burk et al., 2001). Only two of the four plant MAP genes cloned to date (*MOR1*, *AtKTN1*) show significant sequence homology to known animal MAP genes, and only *NtMAP-65-1a* is a member of a gene family. Initial functional characterization showed that NtMAP65-1a stimulates microtubule polymerization and binds to a subset of cortical interphase microtubules, the PPB, and overlapping microtubules in the center of the mitotic spindle and the phragmoplast. TAN1 associates specifically with mitotic microtubular arrays. MOR1 is a homolog of the TOGp-XMAP215 class of MAPs, which stimulate microtubule polymerization, and AtKTN1 is related to katanin, a microtubule-severing protein.

Role of the Cytoskeleton in Plant Development

Because of the presence of cell walls, cellular patterns in developing plant tissues are very rigid. It was previously thought that morphogenesis in plants may be governed by a lineage-dependent, cell-autonomous program, which simply controls cell expansion and cell plate positioning via cytoskeletal regulation. However, recent characterizations of mutants with defects in microtubular organization have indicated that plant cells in developing tissues must communicate with each other and respond to unknown positional cues (see below).

Although *Arabidopsis pilz* mutants, which are unable to form any of the four main plant microtubular structures (cortical interphase array, PPB, mitotic spindle, phragmoplast), display early embryo lethality (Mayer et al., 1999), plant mutants with microtubular defects that do not abolish cell division can develop surprisingly normally. *Arabidopsis fass/ton* plants fail to establish normal cortical interphase arrays and PPBs, and consequently display severe defects in polar cell growth and cell plate positioning (Traas et al., 1995). These mutant plants remain highly stunted and morphologically abnormal, but are able to form recognizable organs with a relatively well developed internal organization. Developing *tan1* maize leaves, in which PPBs and cell plates are mispositioned, show a highly irregular internal cellular organization, but an amazingly unaffected overall morphology (Smith et al., 2001). *Arabidopsis bot1* (Bichet et al., 2001), *spr* (Furutani et al., 2000), *lefty*, and *fra2/at-ktn1* (Burk et al., 2001) mutants display abnormalities exclusively in cortical interphase arrays in all or in some cell types, and show defects in the polar expansion of the affected cell types. Organs of these mutants are dwarfed with an enlarged circumference or twisted, but otherwise remarkably normal. Cell expansion and organogenesis are similarly affected under restrictive conditions in the temperature sensitive *Arabidopsis mor1* (Whittington et al., 2001) mutant, which also shows defects only in cortical interphase arrays. The *TAN1*, *MOR1*, and *AtKTN1* genes were found to encode MAPs (see above).

Because of the redundancy in the large gene families encoding actin and actin binding proteins, mutant analysis has not been very informative with regard to the developmental functions of the plant actin cytoskeleton. *Arabidopsis* plants with T-DNA insertions that disrupt single members of the actin or profilin gene families display no or only minor developmental defects (reviewed by Kost et al., 2001). Maize *lilliputian* is the only known plant mutant which shows aberrant development and irregular actin organization (Baluska et al., 2001). The defects in *lilliputian* plants appear to be restricted to irregular cell plate positioning in some cells and to reduced cell as well as organ elongation. Altered cell and organ growth were also the most obvious effects of the constitutive expression of ADF or profilin cDNA sequences in the sense or antisense orientation in transgenic *Arabidopsis* plants. Some of these plants also showed abnormal timing of flower induction, implicating the actin cytoskeleton in signaling events that control this process (Kost et al., 2001).

Outlook

A major question of future research on plant cytoskeleton is the identification of the molecular mechanisms involved in the initial establishment of cellular growth polarity. Single cell model systems (e.g., trichomes) may prove instrumental for the elucidation of these mechanisms. The branching of elongating trichome represents a developmentally controlled switch in growth polarity, which is affected in several *Arabidopsis* mutants. Recent studies have indicated a pivotal role of microtubules, which control growth polarity in yeast, in the initiation of trichome branching (reviewed by Kost et al., 1999). It is similarly important to determine how the different cytoskeletal structures implicated in cell plate position-

ing are functionally integrated. This will require the analysis of interactions between actin filaments and microtubules, which are likely to be essential not only for cell plate positioning, but also for other cytoskeletal functions. The cloning of the genes affected in mutants with defects in microtubular organization and/or trichome branching is expected to contribute significantly to the clarification of the questions listed above. *Arabidopsis* plants carrying insertions in several members of gene families encoding actin or actin regulatory proteins, or displaying altered expression of such genes due to genetic engineering, will help achieve a better understanding of the developmental functions of actin filaments. Another key area of future research is the characterization of the regulatory networks that reorganize cytoskeletons in response to signals. Recent technological advances, together with an increasing number of researchers engaging in plant cytoskeletal research, promise rapid progress of these investigations in the near future.

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