THE ROYAL SOCIETY

Published online 29 May 2002

Cytoskeleton and plant organogenesis

Benedikt Kost[1], Yi-Qun Bao[1] and Nam-Hai Chua[2*]

[1]*Laboratory of Plant Cell Biology, Institute of Molecular Biology, National University of Singapore, 1 Research Link, Singapore 117 604*

[2]*Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA*

The functions of microtubules and actin filaments during various processes that are essential for the growth, reproduction and survival of single plant cells have been well characterized. A large number of plant structural cytoskeletal or cytoskeleton-associated proteins, as well as genes encoding such proteins, have been identified. Although many of these genes and proteins have been partially characterized with respect to their functions, a coherent picture of how they interact to execute cytoskeletal functions in plant cells has yet to emerge. Cytoskeleton-controlled cellular processes are expected to play crucial roles during plant cell differentiation and organogenesis, but what exactly these roles are has only been investigated in a limited number of studies in the whole plant context. The intent of this review is to discuss the results of these studies in the light of what is known about the cellular functions of the plant cytoskeleton, and about the proteins and genes that are required for them. Directions are outlined for future work to advance our understanding of how the cytoskeleton contributes to plant organogenesis and development.

Keywords: actin; microtubules; cytoskeleton; development; organogenesis; plants

1. CYTOSKELETAL FUNCTIONS DURING CELLULAR PROCESSES REQUIRED FOR ORGANOGENESIS

The various functions of the cytoskeleton in plant cells have been well characterized, mostly based on studies employing a variety of single-cell model systems (Kost *et al.* 1999*b*). In addition to being required for basic cellular processes, such as nuclear division, organelle positioning, cytokinesis, membrane trafficking and cell expansion, the plant cytoskeleton plays key roles in cellular events that specifically are thought to be important for the development of multicellular organs. The rigid wall in which plant cells are enclosed renders the expansion of these cells essentially irreversible and prevents cellular motility in developing tissues. Stringent control of the direction of polar cell growth and of the positioning of cell division planes is therefore immensely important for the morphogenesis of plant organs. Microtubules and actin filaments (F-actin, microfilaments), the two key components of the plant cytoskeleton, are thought to have essential functions during these two processes (Smith 1999; Kropf *et al.* 1998). Furthermore, these cytoskeletal elements have also been shown to be required for the differentiation of characteristic secondary cell wall structures formed by vascular cells (Fukuda & Kobayashi 1989) and for cell-to-cell communication through plasmodesmata, which is likely to contribute significantly to the coordination of cellular behaviour during organogenesis (McLean *et al.* 1997).

Plant microtubules are organized into four different basic structures in the course of the cell cycle: the cortical interphase array, the pre-prophase band, the mitotic spindle and the phragmoplast (Goddard *et al.* 1994). As documented extensively in the literature, cortical interphase microtubules in expanding plant cells generally are strictly transversely arranged with respect to the main direction of cell growth (Cyr 1994; Cyr & Palevitz 1995; Hush & Overall 1996; Williamson 1991). Disruption of these transverse cortical microtubule arrays in plant cells has been shown to increase radial cell expansion at the expense of directional cell growth (e.g. Akashi *et al.* 1988; Baskin *et al.* 1994; Green 1980). In addition, hormones that regulate plant cell and organ growth have been demonstrated to alter the orientation of cortical microtubule arrays (Nick 1998; Shibaoka 1994). Hence, such arrays clearly have very important functions in determining the direction of the expansion of plant cells and organs. However, changes in microtubular orientation occasionally appear to occur only after alterations in the direction of cell and organ growth have been initiated (Blancaflor & Hasenstein 1995; Fischer & Schopfer 1998; Wenzel *et al.* 2000), indicating that other mechanisms may be involved in the control of cell and tissue expansion in these cases. Cortical microtubules in growing plants cells were observed to be co-aligned with recently deposited cellulose microfibrils in the cell wall (Ledbetter & Porter 1963; Vesk *et al.* 1996), and microtubule disruption was found to affect microfibril orientation (Green 1962; Hogetsu & Shibaoka 1978). These findings have led to the intriguing idea that cellulose synthase, an enzyme complex integrated into the plasma membrane, may be guided by cortical microtubules and lays down microfibrils in the cell wall in parallel to these cytoskeletal elements (Giddings & Staehelin 1991). However, the observation of regular arrangements of cell wall microfibrils in cells treated with

* Author for correspondence (chua@mail.rockefeller.edu).

One contribution of 12 to a Discussion Meeting Issue 'The plant cell: between genome and plant'.

drugs that disrupt microtubules, or showing complex microtubule organization, has caused some researchers to question this hypothesis (Emons & Mulder 2000; Sugimoto *et al.* 2000).

At the onset of mitosis, interphase microtubules rearrange and condense into a narrow cortical ring around the nucleus, the PPB. Although the PPB disappears long before cytokinesis occurs, its position invariably determines the future cell division plane by an unknown mechanism. The PPB dissolves as the mitotic spindle is formed, which mediates the distribution of the sister chromosomes to the two daughter cells during nuclear division. After anaphase, the microtubular cytoskeleton is reorganized into the phragmoplast, a unique structure found only in plant cells undergoing cytokinesis. The function of the phragmoplast is to organize the synthesis of a new cell wall at the correct position between the two newly formed daughter cells. The phragmoplast is assembled in the centre of the dividing cells and expands centrifugally as the new cell wall extends towards the cell cortex (for a review, see Goddard *et al.* 1994).

F-Actin structures are found in plants cells during all stages of the cell cycle. Actin filaments in typical interphase plant cells are randomly arranged in the cell cortex, form a network around the nucleus and extend through cytoplasmic strands (Kost *et al.* 1998; Seagull *et al.* 1987; Traas *et al.* 1987). It has been speculated that plant actin filaments may be involved in the transport of secretory vesicles to the plasma membrane (Dong *et al.* 2001*b*), as they are in yeast and animal cells (Doussau & Augustine 2000; Pruyne & Bretscher 2000). Facilitating the delivery of new cell wall and cell membrane material contained in these vesicles to the plasma membrane, and thereby promoting cell expansion, may represent a key function of the actin cytoskeleton in interphase plant cells. It has also been suggested that actin filaments play a role in the rearrangement of microtubule orientation (Fukuda & Kobayashi 1989; Hasezawa *et al.* 1998; Seagull 1990; Wernicke & Jung 1992), a key determinant of the direction of polar plant cell growth, as discussed above. Interestingly, filamentous actin structures were shown to control the establishment of growth polarity in zygotes of the brown alga *Fucus* by polarizing the distribution of unknown proteins in the plasma membrane and, subsequently, to organize localized secretion of unknown morphogenetic factors into the cell wall (Fowler & Quatrano 1997). Similar F-actin-dependent processes could be involved in the establishment of growth polarity also during the development of higher plants. In addition, F-actin structures were demonstrated in plasmodesmata (Blackman & Overall 1998; White *et al.* 1994), which are cytoplasmic channels between neighbouring cells in plant tissues. These F-actin structures appear to control, and possibly mediate, the selective transfer of macromolecules through plasmodesmata between cells, which could potentially constitute an important mechanism by which cells in a developing organ communicate with each other (Ding *et al.* 1996; McLean *et al.* 1997).

Interestingly, plant cells, in contrast to yeast and animal cells, appear to be able to undergo cell division in the presence of F-actin-depolymerizing drugs (Baluska *et al.* 2001; Wick 1991), although both the microtubular PPB and the phragmoplast have been shown to contain filamentous actin structures (Cleary *et al.* 1992; Kakimoto & Shibaoka 1987; Palevitz 1987; Smith 1999). However, pharmacological disruption of F-actin in dividing plant cells often results in the defective positioning of newly formed cell walls (Baluska *et al.* 2001; Mineyuki & Palevitz 1990; Palevitz & Hepler 1974; Wick 1991), indicating that filamentous actin structures have essential functions during this process.

Additional support for an important function of actin filaments in cell wall positioning during cytokinesis comes from several other observations. In some cell types, the cortical region that has been occupied by the microtubular PPB remains F-actin free throughout mitosis. It has been speculated that this ZAD may mark the future cell division plane after the disappearance of the PPB (Cleary *et al.* 1992; Smith 1999). Actin filaments have been found to connect the phragmoplast to the cortical ZAD (Lloyd & Traas 1988; Valster & Hepler 1997) and may control the direction of expansion of this structure. This is consistent with the recent observation of F-actin assembly at the edge of the expanding phragmoplast (Endle *et al.* 1998).

2. PLANT GENES ENCODING CYTOSKELETAL AND CYTOSKELETON-ASSOCIATED PROTEINS

A thorough understanding of how the cytoskeleton contributes to plant organogenesis requires detailed knowledge of the genes encoding cytoskeletal and cytoskeleton-associated proteins that are involved in this process. Cytoskeletal proteins are structural proteins that constitute microtubules and actin filaments, whereas cytoskeleton-associated proteins either regulate the organization of these cytoskeletal elements or are motor proteins required for their functions. Microtubules and F-actin are filamentous structures that form spontaneously under physiological conditions as a result of the polymerization of α- and β-tubulin heterodimers or of G-actin monomers, respectively. Both cytoskeletal structures are extremely dynamic and undergo constant assembly and disassembly at their ends (Chen *et al.* 2000; Joshi 1998; Margolis & Wilson 1998; Schoenenberger *et al.* 1999). Correspondingly, cells can reorganize their cytoskeleton very rapidly in response to environmental or developmental signals. In animal and yeast cells, numerous genes and their products have been shown to be involved in the control of cytoskeletal organization and in cytoskeletal functions (Andersen 2000; Ayscough 1998; Cooper & Schafer 2000; Walczak 2000). Our current knowledge of the regulation and the functions of the plant cytoskeleton is clearly less advanced. However, considerable efforts are being made to identify and functionally characterize plant genes that encode cytoskeletal or cytoskeleton-associated proteins.

Although genes coding for these types of proteins have been cloned from different plant species, the largest number of such genes have been identified to date in *Arabidopsis thaliana*, a widely employed plant research model system whose genome has been completely sequenced. In *A. thaliana*, at least six α-tubulin (Kopczak *et al.* 1992), nine β-tubulin (Snustad *et al.* 1992) and eight actin genes (Meagher *et al.* 1999*a*) were shown to be expressed at different developmental stages. Also, two *A. thaliana* cDNAs have been cloned that code for γ-tubulins (Liu *et al.*

1994), tubulin isoforms believed to be essential for the nucleation stage of microtubule assembly (Canaday *et al.* 2000). In addition, many *A. thaliana* genes encoding homologues of known yeast or animal actin regulatory proteins have been cloned or identified, including genes encoding ADFs (nine genes; Dong *et al.* 2001*a*), profilins (five genes; Christensen *et al.* 1996; Huang *et al.* 1996), villins (four genes; Klahre *et al.* 2000), fimbrins (three genes; Kovar *et al.* 2000; McCurdy & Kim 1998; H. Christensen and N.-H. Chua, unpublished data), actin related protein 2 (Arp2) (one gene; Klahre & Chua 1999), capping proteins (one gene; Y.-Q. Bao and N.-H. Chua, unpublished data) and Rho-type small GTPase (at least eight genes; Xia *et al.* 1996; Li *et al.* 1998; Winge *et al.* 1997; Kost *et al.* 1999*a*). Although most of these genes and/or their products have been functionally characterized to some extent, a clear picture of how they interact to regulate actin organization *in vivo* and cell behaviour during organogenesis has yet to emerge. Plant actin filaments clearly have a number of unique functions that are different from those of F-actin structures in animal and yeast cells (see § 1). Therefore, plant cells are likely to express specific actin regulatory genes that cannot be isolated using the homology-based screens, which have resulted in the identification of most of the genes listed above. Some plant-specific genes encoding putative actin regulatory proteins have been isolated using a screen for *A. thaliana* cDNAs that alter cell morphology and growth when expressed in the fission yeast *Schizosaccharomyces pombe* (Xia *et al.* 1996; J. Mathur and N.-H. Chua, unpublished data). The cloning of additional such genes will be facilitated by recently developed techniques, which allow the purification of proteins that bind to monomeric or polymerized plant actin *in vitro* (Hu *et al.* 2000) and, possibly, by the characterization of plant developmental mutants with primary defects in the organization of the actin cytoskeleton (e.g. Gallagher & Smith 1999).

Plant MAPs that regulate the assembly and/or the disassembly of microtubules are expressed at low levels and appear to be quite different from their yeast and animal counterparts (Wick 2000). This has made the identification of such proteins, and of the genes that encode them, rather elusive. Only recently have the first genes encoding plant regulatory MAPs been cloned, either after the biochemical purification of their products based on *in vitro* binding to plant microtubules (Smertenko *et al.* 2000) or following the identification of a developmental mutant with primary defects in microtubule organization (Smith *et al.* 2001).

Cytoskeletal motor proteins, which have the ability to generate force by moving along microtubules (kinesin- or dynein-like proteins) or actin filaments (myosins) in an ATP-dependent manner, perform important functions in membrane trafficking, organelle positioning, cell division, cytoskeletal dynamics and polarized cell growth in yeast and animal systems (Fischer 2000; Reddy 2001; Titus & Gilbert 1999). Plant cells also seem to express a large number of such proteins, as illustrated by the fact that the *A. thaliana* genome contains at least 40 open reading frames with sequence homology to yeast or animal kinesins or myosins (Reddy 2001). Although quite a number of plant myosins and kinesin- or dynein-like proteins, as well as genes encoding such proteins, have been identified and preliminarily characterized (e.g. Barroso *et al.* 2000; Kao *et al.* 2000; Lee & Liu 2000; Moscatelli *et al.* 1995; Yamamoto *et al.* 1999); the function and the regulation of these proteins or genes is not yet known in detail. The *A. thaliana* '*zwichel*' mutant, which displays reduced trichome (leaf hair) branching, is defective in a gene that encodes a kinesin-like microtubule motor protein (Oppenheimer *et al.* 1997). This demonstrates that plant motor proteins can have important functions during cellular morphogenesis and, therefore, probably also during organogenesis.

Plant genes encoding cytoskeletal or cytoskeleton-associated proteins generally form extended families (see above), which are considerably larger than the corresponding gene families in other organisms. The members of these large plant-gene families typically display high sequence homology and partially overlapping expression patterns (Meagher *et al.* 1999*b*). Detailed analysis of the well-characterized *A. thaliana* actin, profilin and tubulin gene families has revealed that each of these families can be divided into two ancient classes of genes based on sequence homology and on expression pattern. The two different classes of genes in each family are predominantly expressed in vegetative or reproductive plant organs, respectively (Meagher *et al.* 1999*b*). Considering the extent of the sequence dissimilarity between the two classes of genes and the speed at which such differences are introduced during evolution, it has been estimated that the gene families split into vegetative and reproductive branches a few hundred million years ago, at about the same time that vascular land plants distinguished themselves from their algal ancestors (Meagher *et al.* 1999*b*). Early vascular plants formed the first true leaves, which were specialized for highly efficient photosynthesis and are thought to have derived from reproductive organs of earlier organisms. Altogether this may indicate that the development during evolution of new organs, the leaves, required the concomitant establishment of new groups of cytoskeletal genes (Meagher *et al.* 1999*b*). If this interpretation is correct, it lends further support to the notion that cytoskeletal elements play key roles during organogenesis.

Two possible explanations, which are not mutually exclusive, have been proposed for the observation that groups of highly similar cytoskeleton-related genes with partially overlapping expression pattern have been maintained in plants during evolution. First, multiple genes with similar coding sequences but different expression signals may allow enhanced flexibility in expression in response to developmental cues and environmental signals (McDowell *et al.* 1996). Second, it is conceivable that co-expression of non-identical but highly similar proteins can increase the functionality, the responsiveness and the stability of a system, a concept that has been termed 'isovariant dynamics' (Meagher *et al.* 1999*a*).

3. DIRECT ANALYSIS OF CYTOSKELETAL FUNCTIONS DURING ORGANOGENESIS

Although, as described above, the cellular functions of the plant cytoskeleton have been well characterized, and a significant number of plant genes that encode cytoskeletal or cytoskeleton-associated proteins have been identified, the role of the cytoskeleton during plant

organogenesis is not yet well understood. Only a limited number of studies that address this question directly have been published. In these studies, defects in organogenesis were analysed as exhibited by plants that: (i) were treated with cytoskeleton modifying drugs; (ii) contained mutations that caused apparent primary defects in cytoskeletal organization; (iii) carried T-DNA insertions in cytoskeletal or cytoskeleton-associated genes; or (iv) displayed altered expression levels of such genes after transformation with expression cassettes containing the corresponding cDNAs in the sense or in the antisense orientation.

(a) *Drug studies*

Baskin *et al.* (1994) have transplanted six-day-old *A. thaliana* seedlings onto a culture medium containing different concentrations of oryzalin or Taxol, drugs that depolymerize or stabilize microtubules, respectively. The effects of these drugs on the radial expansion of root tips, as well as on microtubule organization in cells of this organ, were investigated and found to increase in severity with rising drug concentrations. Effects on root elongation or on shoot development were not reported. At low concentrations (170 nM and 1 μM, respectively), oryzalin and Taxol had only minor effects on both radial expansion and microtubule organization. However, at a concentration of 1 μM, oryzalin almost completely disrupted the microtubular cytoskeleton in root cells and resulted in a pronounced radial swelling of the root tip in the elongation zone and in the apical cell division zone, which was, somewhat surprisingly, particularly dramatic in the latter region. Interestingly, Taxol at a concentration of 10 μM, which stimulated the formation of microtubules without altering their transverse alignment, also induced radial expansion of the root tip, although in this case the swelling was restricted to the elongation zone. These observations compelled the authors to conclude that the control of cell growth directionality in elongating root tips depends on microtubule dynamics rather than on microtubule alignment, and may be based on different mechanisms in distinct types of cells and tissues.

When *A. thaliana* seedlings were grown on a culture medium containing 1 mM latrunculin B, a drug that specifically binds to G-actin and prevents its polymerization, cells in all organs were effectively depleted of F-actin structures (Baluska *et al.* 2001). F-Actin-depleted *A. thaliana* seedlings were found to display severe dwarfism, apparently caused exclusively by a dramatic inhibition of polar cell growth. Effects of F-actin disruption on mitosis appeared to be restricted to minor defects in the positioning of cell division planes. Apart from their reduced size, the morphology of all organs formed by F-actin-free *A. thaliana* seedlings was observed to be essentially normal. Similar results were obtained when the actin cytoskeleton in developing rye seedlings and *Abies alba* × *Abies cephalonica* somatic embryos, as well as in rapidly growing rye coleoptiles and maize roots, were disrupted by treatment with latrunculin B. The authors of this study deduced from their observations that, during plant development, actin filaments are essential for rapid cell and organ growth, whereas mitosis and organogenesis can apparently occur in the absence of an intact actin cytoskeleton (Baluska *et al.* 2001; Wick 1991).

The pharmacological experiments described above have provided interesting insights into the role of the cytoskeleton during plant organogenesis. However, the observations made may have to be interpreted cautiously as they could have been influenced by uneven drug uptake into different cell or tissue types, by differential drug sensitivity of certain cytoskeletal structures and/or by possible non-specific effects of the drug treatments.

(b) *Developmental mutants with apparent primary defects in cytoskeletal organization or function*

Considering the large number of cytoskeletal and cytoskeleton-associated genes in plants, as well as the apparent importance of the cytoskeleton for plant organogenesis, one would predict the existence of many developmental plant mutants with primary defects in the organization or in the functions of microtubular or F-actin structures. Consistent with this expectation, the disruption of cytoskeleton-related genes has been demonstrated to cause developmental phenotypes in various other organisms (e.g. Gunsalus *et al.* 1995; Manseau *et al.* 1996; Takei *et al.* 1997).

In fact, a number of plant mutants with defective organogenesis has been shown to display alterations in the organization of the microtubular cytoskeleton. The development of homozygous *A. thaliana* mutants of the *pilz* class, which fail to form any of the four basic microtubular structures found in plant cells, are arrested at early stages during embryogenesis (Mayer *et al.* 1999). This may illustrate the fundamental importance of an intact microtubular cytoskeleton for plant organogenesis. *Arabidopsis thaliana fass*/*ton* mutants show more specific defects in microtubule organization. These mutants are unable to organize correctly arranged microtubular structures in the cell cortex (interphase arrays, PPBs), but form normal mitotic spindles and phragmoplasts. Consequently, they display defects in polarized cell growth and in cell wall positioning during cytokinesis. Quite strikingly, homozygous *fass*/*ton* mutants, although they remain highly stunted and show a severely abnormal overall morphology, are able to develop relatively well-defined organs that display clearly discernible apical–basal and radial pattering (Torres-Ruiz & Juergens 1994; Traas *et al.* 1995). Maize *tangled1* (*tan1*), *A. thaliana botero1* (*bot1*), and *A. thaliana spiral* (*spr1*, *spr2*) mutants (Hashimoto 2002) are defective in the organization of only one of the two cortical microtubular structures. In leaves of *tan1* plants, PPBs and mitotic cell walls are positioned incorrectly, which results in a highly irregular internal cellular organization. Amazingly, the morphology of *tan1* leaves remains essentially normal in spite of these defects (Cleary & Smith 1998). *bot1* mutants appear to be unable to align cortical interphase microtubules correctly and display reduced polar growth of all non-tip-growing cell types. Although all organs of *bot1* plants are considerably shorter than wild-type organs and show a clearly increased diameter, mutant plants undergo an otherwise remarkably unaffected ontogenesis and reproduce normally (Bichet *et al.* 2001). Plants carrying mutations in the *spr1* or *spr2* genes also appear to align cortical microtubules aberrantly, but the defect in these two mutants occurs predominantly in specific cell types, namely in subepidermal cortical cells either

in roots (*spr1*) or in hypocotyls, petioles and petals (*spr2*). The affected cell types in the spiral mutants show increased radial growth and remain shorter than wild-type cells, whereas cells in other tissues, including the epidermis, expand normally. This leads to tension between adjacent cell layers, which causes affected organs to develop helically arranged epidermal cell files and to grow in a skewed or twisted manner (Furutani *et al.* 2000; Hashimoto 2002).

Although all mutants described above are clearly defective in the organization of microtubular structures, it remains to be demonstrated that these defects are directly responsible for the mutant phenotypes. This will require the cloning of the genes that are disrupted in the different mutants and the characterization of the functions of the proteins they encode. The *tan1* gene has recently been cloned and the identification of the genes defective in the other mutants can be expected in the near future. It is encouraging to note that the protein encoded by the *tan1* gene, although it does not appear to share significant homology with any other known protein, has been demonstrated to bind to microtubules *in vitro* and to microtubular structures in dividing cells (Smith *et al.* 2001).

Assuming that the mutants described above in fact have primary defects in the microtubular cytoskeleton, a number of interesting conclusions can be drawn from their characterization. As exemplified by the *pilz* mutants, the disruption of genes that are required for the formation of mitotic spindles and phragmoplasts, structures that are essential for cell division to occur, apparently results in early embryo lethality. In contrast, mutations that exclusively affect the organization of cortical interphase arrays and/or PPBs, microtubular structures that are specifically required for the control of polar cell growth and cell wall positioning during cytokinesis, respectively, allow plant ontogenesis to proceed much further, although they cause striking defects in organ development. The observation that organogenesis and plant development can be surprisingly normal in the absence of correctly positioned PPBs (*tan1*), normal cortical interphase arrays (*bot1*, *spr1* and *spr2*) or even both of these structures (*fass*/*ton*) indicates that morphogenesis in plants is not governed by a cell-autonomous program that controls cell expansion and positioning of cell division planes via the regulation of cytoskeletal organization. Rather, during organogenesis plant cells appear to communicate with each other constantly, re-evaluate their position within developing organs and adjust their behaviour accordingly.

Interestingly, although plant developmental mutants with apparent primary defects in all basic microtubular structures have been identified, plant mutants with aberrant organogenesis caused by defects in the actin cytoskeleton have yet to be described. The unavailability of such mutants could indicate that the actin cytoskeleton is not essential for plant organogenesis, but this explanation appears doubtful in view of the various known functions of actin filaments in cellular processes that are required for the development of multicellular structures. It is more probable that the considerable redundancy in the large families of genes that encode actin and actin-associated proteins (see above) may minimize effects of mutations in these genes on F-actin organization and plant development.

(c) *Mutants with T-DNA insertions into genes encoding actin or actin-associated proteins*

Large collections of *A. thaliana* lines carrying random T-DNA or transposon insertions have been generated (Bouchez & Hoefte 1998; Krysan *et al.* 1999), which can be searched relatively easily using PCR-based techniques for mutants with insertions into known genes. This approach also allows the identification of lines with mutations in non-essential or redundant genes that are difficult to isolate using conventional mutant screens. The screening of T-DNA-mutagenized lines has resulted in the identification of *A. thaliana* lines with single insertions into three different actin genes that display distinct temporal and spatial expression patterns (Gilliland *et al.* 1998), as well as into the promoter of a profilin gene that appears to be expressed at moderate levels in all vegetative tissues (McKinney *et al.* 2001). Each of the insertions into the actin genes was located at a position at which it potentially disrupted gene expression, although this was not confirmed using molecular analysis. Quite strikingly, plants carrying homozygous insertions into any one of the three actin genes did not display detectable developmental defects and appeared to reproduce normally. However, the ability of these plants to compete with wild-type plants was markedly reduced, as indicated by the observation that the frequencies of all mutant alleles were significantly decreased already in the second generation derived from populations of heterozygous parent plants (Gilliland *et al.* 1998).

The T-DNA insertion into the profilin gene was shown to result in a 50% reduction of the expression of the affected gene, at both the transcript and protein levels, specifically in young seedlings. Correspondingly, morphological defects were detected exclusively during seedling development. The hypocotyls of mutant seedlings were found to be 1.5 to 2 times longer than those of wild-type seedlings and cells in mutant hypocotyls were abnormally elongated. It was speculated that the increased elongation of mutant hypocotyl cells and hypocotyls may have been caused by a stimulation of actin polymerization, which resulted from the observed reduction of profilin expression. However, F-actin organization in cells of mutant hypocotyls was not analysed. As compared with normal seedlings, mutant seedlings also displayed a reduced angle between cotyledon petioles and hypocotyls, which gave mutant cotyledons an unusually raised appearance, and produced more root hairs with an increased average length (McKinney *et al.* 2001).

Evidently, the developmental defects exhibited by each of the T-DNA insertion mutants described above were quite moderate. This may not be unexpected, considering the fact that close homologues of each of the mutated actin and profilins genes with essentially overlapping expression patterns are known to be present in the *A. thaliana* genome. To characterize further the developmental functions of the *A. thaliana* actin and profilin gene families, as well as of other families of cytoskeletal and cytoskeleton-associated genes, it will be necessary to generate lines with insertions in all the genes of these families that are expressed in particular developing organs at certain

developmental stages. However, it is conceivable that in many cases the development of such multi-knockout lines will be severely defective to an extent that precludes analysis of the functions of the affected gene family in detail.

(d) *Transgenic plants displaying altered expression levels of cytoskeletal or cytoskeleton-associated genes*

Our laboratory has generated transgenic *A. thaliana* plants that express cDNAs of members of different gene families that encode cytoskeletal or cytoskeleton-associated proteins in the sense or antisense orientation under the control of a constitutively active CaMV 35S promoter. Throughout ontogenesis, these plants displayed either reduced expression of several or all members of the targeted gene families, or increased expression of a representative family member. Using this approach, we have obtained plants that produced altered levels of cytoskeletal or cytoskeleton-associated proteins during the development of a variety of organs and displayed clear phenotypes, without being completely impaired in organogenesis. The characterization of these phenotypes has allowed us to draw a number of interesting conclusions concerning cytoskeletal functions during plant development.

(i) *Plants with altered levels of profilin or* ADF *gene expression*

In two complementary studies, we have employed the strategy described above to investigate the developmental functions of the *A. thaliana profilin* and *ADF* gene families (Dong *et al.* 2001*b*; Ramachandran *et al.* 2000). *Profilin* and *ADF* genes were demonstrated to be essential for the growth of yeast cells (Balasubramanian *et al.* 1994; Haarer *et al.* 1990; Iida *et al.* 1993; Moon *et al.* 1993) and for *Drosophila melanogaster* development (Gunsalus *et al.* 1995; Verheyen & Cooley 1994). The proteins encoded by these genes directly bind to actin and have been shown to be key elements of the regulatory network that controls F-actin organization in non-plant systems (Wear *et al.* 2000; Amann & Pollard 2000). *In vitro* studies have indicated that profilins, depending on the experimental conditions used, can either promote or inhibit F-actin formation, by lowering the critical G-actin concentration required for polymerization or by sequestering G-actin, respectively. In contrast, ADF was shown to stimulate F-actin depolymerization *in vitro*, by facilitating monomer release from F-actin and by severing actin filaments (Pollard *et al.* 2000). The results of studies concerned with the *in vivo* functions of profilins and ADFs were less conclusive (Ayscough 1998). Experimentally increased profilin or ADF protein levels, following protein microinjection or gene transfer, in different plant and animal single-cell systems, were described to have inconsistent effects on the actin cytoskeleton, including depolymerization, reorganization or *de novo* formation of F-actin structures (Aizawa *et al.* 1996; Cao *et al.* 1992; Finkel *et al.* 1994; Hussey *et al.* 1998; Nagaoka *et al.* 1995; Staiger *et al.* 1994).

The transgenic *A. thaliana* plants with altered levels of *ADF* or *profilin* gene expression that we have generated were fully fertile and indistinguishable from wild-type plants in many aspects of their development and morphology. However, they displayed a number of clear defects in organogenesis (Dong *et al.* 2001*b*; Ramachandran *et al.* 2000). Roots and root hairs of plants that produced a profilin protein (Pfn-1; Christensen *et al.* 1996; Huang *et al.* 1996) at about 20 times its normal level (Pfn1-O plants) were abnormally elongated. At the seedling stage, Pfn1-O roots and root hairs were determined to be roughly 1.5 times longer than those of wild-type plants. By contrast, the length of all organs of plants displaying a 50% reduction in the expression level of several (at least two) profilin isoforms, including Pfn-1 (Pfn-U plants), was very significantly reduced throughout ontogenesis. This effect was particularly obvious when the growth of etiolated (dark-grown) seedlings was analysed: etiolated Pfn-U hypocotyls only reached about 40% of the length of their wild-type counterparts. Detailed analysis of the cellular structure of etiolated Pfn-U hypocotyls using histological sections and light microscopy, as well as scanning electron microscopy, revealed that the average cell length was decreased roughly to the same extent as the length of the entire organ, indicating that reduced cell elongation fully accounted for the observed inhibition of hypocotyl growth. In addition, cells in Pfn-U hypocotyls were found to be clearly wider than corresponding wild-type cells and often displayed irregular shapes, which resulted in a notable disruption of tissue organization and in an uneven, rough epidermal surface. Interestingly, visualization of the actin cytoskeleton in etiolated Pfn-O and Pfn-U seedlings, after fixation using fluorescently labelled phalloidin as a probe for F-actin, did not reveal any detectable abnormalities. Rather unexpectedly, in addition to cell and organ growth, the timing of the induction of flowering was also affected in Pfn-U plants. As compared to wild-type plants, Pfn-U plants flowered 12 days earlier (average), after they had produced *ca*. 40% fewer rosette leaves (Ramachandran *et al.* 2000).

Remarkably, *A. thaliana* plants with decreased levels of *ADF* gene expression (ADF-U) displayed similar developmental defects as plants that overexpressed a profilin isoform (Pfn1-O), and vice versa. ADF-U plants produced clearly reduced levels (less than one-third of wild-type levels) of two or more ADF isoforms, including AtADF1 (Dong *et al.* 2001*a*), whereas 30–40 times higher expression levels of one ADF isoform (At-ADF1) were detected in ADF1-O plants. The length of roots, root hairs, hypocotyls and cotyledons at the seedling stage, as well as of mature inflorescences, was clearly increased in ADF-U plants and significantly decreased in ADF1-O plants. Again, hypocotyls of etiolated seedlings were employed to characterize the growth defects in ADF-U and ADF1-O plants in detail. Etiolated ADF-U and ADF1-O hypocotyls reached 122% and 67%, respectively, of the length of wild-type hypocotyls 10 days after germination. Abnormal cell elongation was found to account fully for the observed effects on the length of hypocotyls of both types of transgenic seedlings. Whereas cells in ADF-U hypocotyls appeared essentially like wild-type cells, although they were somewhat wider and more elongated, ADF1-O hypocotyl cells often displayed not only a reduced length and an increased width, but also a highly irregular shape. These cellular defects resulted in a significant disruption of tissue organization and caused ADF1-O hypocotyls to develop a rough epidermal surface and a wavy morphology. Stable expression of a GFP–

mouse talin fusion protein, which allows non-invasive F-actin visualization in living plant cells (Kost *et al.* 1998, 2000), was employed to investigate effects on the organization of the actin cytoskeleton in cells of etiolated and light-grown ADF1-O and ADF-U hypocotyls. ADF1-O cells were found to be completely devoid of thick, longitudinally oriented actin cables, which were particularly prominent in rapidly elongating cells of etiolated wild-type hypocotyls, whereas the number of such actin cables was slightly increased in ADF-U cells. The disappearance of long actin cables was also observed in root hairs, trichomes and stomatal guard cells formed by ADF1-O plants. Affected root hairs and trichomes did not elongate normally (see also above) and showed morphological defects. In guard cells, F-actin disruption was found to induce stomatal closure. In addition to showing increased cell and organ growth, ADF-U plants flowered on average 14 days later than wild-type plants, after they had increased their number of rosette leaves by *ca*. 50% (Dong *et al.* 2001*b*).

The observations described above demonstrate that during *A. thaliana* organogenesis ADF proteins control cell and organ expansion by breaking down longitudinally oriented F-actin cables, which is in accordance with their molecular functions as predicted by *in vitro* studies (see § 3d(i)). In addition, our results indicate that ADF-mediated F-actin depolymerization may be involved in the control of stomatal closure in *A. thaliana*. We have recently found that ABA induces stomatal closure by inactivating a Rho-type small GTPase, which results in F-actin depolymerization (Lemichez *et al.* 2001). The hypothesis that ADF may act as an effector of the GTPase in this system is currently being tested in our laboratory. In animal cells, related Rho-type small GTPases in the activated state have been shown to stimulate a kinase (LIM (Lin-1, Isl-1, Mec-3 domain) kinase) which phosphorylates and inactivates ADF (Arber *et al.* 1998).

Because reduced expression of profilin genes had similar effects on *A. thaliana* development to ADF overexpression, and vice versa, we conclude that profilins and ADFs generally act in an antagonistic manner during plant organogenesis, which is in accordance with models for F-actin regulatory networks proposed in the literature (Wear *et al.* 2000; Amann & Pollard 2000). This implies that the capacity of profilins to promote actin polymerization is more important during most developmental processes in plants than their ability to bind and sequester actin monomers. However, particular profilin isoforms may not function according to this role in some cell types and tissues, as indicated by the observation that a reduction exclusively in *Pfn-1* expression, resulting from a T-DNA insertion into the promoter of this gene, stimulates the elongation of hypocotyls, presumably because it promotes F-actin polymerization in cells of this organ (McKinney *et al.* 2001; see § 3c). To further characterize the functions of ADF and profilin genes during plant organogenesis, it will be necessary to analyse the phenotypes of plants with altered expression levels of other individual *ADF* or *profilin* genes, as well as of different combinations of such genes. Interestingly, we did not detect clear effects of altered profilin expression levels on F-actin organization in transgenic *A. thaliana* tissues. This may indicate that, during plant development, profilins control dynamic properties of F-actin structures rather than the steady-state organization of the actin cytoskeleton. Alternatively, it is possible that effects of altered profilin expression on F-actin structures were not detected because of the use of phalloidin labelling for F-actin visualization, a technique that is difficult to apply to intact tissue (see § 4).

The analysis of plants with altered levels of *profilin* or *ADF* gene expression has provided further support to the notion that during plant development an intact actin cytoskeleton is needed for normal cell elongation. We speculate that filamentous actin structures, in particular the longitudinally oriented actin cables that appear to be the target of the activity of ADF proteins, may be required for the efficient delivery of secretory vesicles carrying new cell membrane and cell wall material to growth sites in cells within developing plant organs (Dong *et al.* 2001*b*). In contrast, we have not detected effects of altered *profilin* or *ADF* gene expression levels on cell division in developing *A. thaliana* organs, which is in accordance with previous observations indicating that filamentous actin structures are not essential for plant mitosis (Baluska *et al.* 2001; Wick 1991; see § 3a). It is possible, however, that certain F-actin structures, which may be required for cell division, were not affected by the increased or decreased levels of ADF or profilin proteins in the transgenic plants we have analysed. Interestingly, reduced expression of ADF or profilin genes resulted in delayed or early flowering, respectively, whereas ADF or profilin overexpression had no effect on the timing of this process. Although cell and organ growth were inhibited by ADF overexpression as well as by reduced profilin expression and were stimulated by profilin overexpression as well as by reduced ADF expression, only the reduced expression of *ADF* or *profilin* genes affected flowering. The effects of low ADF and profilin levels on the induction of flowering therefore do not seem to be a consequence of altered cell or organ growth. Rather, they may result directly from alterations in the organization or dynamic properties of the actin cytoskeleton. This is consistent with the finding that reduced expression of ADF and profilin proteins, which appear to have antagonistic effects on the actin cytoskeleton in plant cells, had opposite effects on the timing of flowering. Altogether these observations indicate a direct involvement of the actin cytoskeleton in the control of flowering. Possible functions of F-actin structures during the induction of flowering have not been reported before, although this process has been extensively studied at the molecular level (Koornneef 1997; Simpson *et al.* 1999). How exactly F-actin structures may contribute to the regulation of flowering remains to be determined. It is conceivable that F-actin-controlled or -mediated intercellular movement of signalling molecules through plasmodesmata may be required for the induction of flowering.

(ii) *Plants with reduced levels of α-tubulin gene expression*

As discussed above, α- and β-tubulin genes are present as large families in the genome of plants (Kopczak *et al.* 1992; Snustad *et al.* 1992), whereas there are no indications at present that plant regulatory microtubule-associated proteins are also encoded by multi-copy genes. We have generated transgenic *A. thaliana* plants that constitutively expressed a cDNA encoding α-tubulin 6 (Kopczak *et al.* 1992; Xia *et al.* 1996) in the antisense orientation. The transgenic lines we obtained (Tua-U

plants) displayed clearly but moderately reduced α-tubulin transcript and protein expression levels in all organs analysed. Using gene-specific probes, we have determined that the transcript levels of at least four different α-tubulin genes were reduced. The expression of β-tubulin genes, which do not share significant sequence homology with α-tubulin genes, was not affected (Bao *et al.* 2001).

A moderate reduction of α-tubulin gene expression did not detectably affect *A. thaliana* shoot organogenesis, but resulted in severely defective root development. Roots of Tua-U seedlings were indistinguishable from wild-type roots until 5 days after germination, when the elongation rate of wild-type roots reached a maximal level. At this stage, the elongation of Tua-U roots started to slow down and came to a complete halt about 2 days later. At the same time Tua-U root tips expanded radially, first in the elongation zone and later also in the apical cell division zone. Ultimately, the apical cell division zone showed the most dramatic radial expansion and the Tua-U root tips assumed a morphology that was highly reminiscent of the one displayed by *A. thaliana* roots grown on the microtubule-disrupting drug oryzalin (Baskin *et al.* 1994; Bao *et al.* 2001; see § 3a). Concomitant with the development of these defects in root growth and morphology, cells throughout the tip of Tua-U roots expanded abnormally, both longitudinally and radially, and developed highly irregular shapes. In addition, cells in the apical cell division zone were apparently impaired in cytokinesis, formed incomplete cell walls and became multi-nuclear. Careful investigation of aberrantly expanding Tua-U root tips also revealed defects in root hair initiation and morphogenesis, which included ectopic root hair formation in epidermal cell files that are normally root hair free, formation of multiple root hairs by single epidermal cells and root hair branching. Interestingly, before any of the developmental defects described above became apparent, Tua-U root tips 3 days after germination displayed an increased sensitivity to oryzalin and a diminished ability to respond to gravitropic stimuli.

Stable expression of a GFP–mouse MAP4 fusion protein, which has been shown to allow labelling of microtubules in living plant cells (Marc *et al.* 1998), was employed to observe microtubule organization in cells of developing Tua-U roots. Cortical microtubules, which showed a strict transverse orientation in rapidly elongating cells during the initial phase of normal root growth, were sparse and misaligned in abnormally elongating cells in root tips that displayed growth defects. In addition, typical mitotic microtubular arrays (PPBs, mitotic spindles and phragmoplasts), which were abundant in the apical cell division zone of normally elongating roots, could not be detected in tips of Tua-U roots after they had stopped elongating normally (Bao *et al.* 2001).

Reduced levels of α-tubulin proteins are expected to destabilize all microtubular structures known to be present in plant cells, namely cortical arrays, PPBs, mitotic spindles and phragmoplasts. Each of the Tua-U lines that we have obtained showed a relatively small reduction of α-tubulin gene expression, which in fact affected all these microtubular structures but did so exclusively in root tips. It appears likely that a more significant reduction of tubulin protein levels resulted in general defects in microtubule organization throughout ontogenesis, completely inhibited plant organogenesis and prevented the recovery of transgenic plants. Consistent with this hypothesis, '*pilz*' mutants with general defects in microtubule organization show early embryo lethality (Mayer *et al.* 1999; see § 3b). Roots of Tua-U plants developed visible defects at the tip only after they had reached maximal elongation rates, which indicates that only the most rapidly growing cells and tissues in these plants were sensitive to the moderate reduction of α-tubulin protein levels.

Detailed characterization of the developmental defects in Tua-U root tips has confirmed that the microtubular cytoskeleton is essential for plant organogenesis because it is required for normal cell division and cell expansion. Interestingly, meristematic cells in the apical cell division zone of Tua-U roots not only showed cytokinesis defects but also a higher competence for radial expansion than cells in the elongation zone. This may indicate that at the time at which root development started to become aberrant, meristematic cells, in contrast to cells in the elongation zone, had not undergone any cellular differentiations (e.g. cell wall modifications) that restricted their ability to grow. The observation of ectopic and excessive root hair formation by abnormally developing Tua-U roots indicates a role of microtubule destabilization in the initiation of root hair development. Apparently, microtubule destabilization resulting from reduced α-tubulin gene expression also interfered with gravitropic responses in Tua-U roots that were normally elongating at submaximal rates. This finding suggests that gravitropic reorientation of *A. thaliana* root growth may be based on a microtubule-dependent increase in the growth rate of cells on the side of the root that is facing in the opposite direction to the gravitational pull.

4. VISUALIZATION OF THE CYTOSKELETON IN DEVELOPING ORGANS

To investigate cytoskeletal functions during plant organogenesis, it is essential to have techniques that allow the visualization of the organization of the microtubular and the actin cytoskeleton in intact tissues of wild-type as well as of drug-treated, mutant and transgenic plants. Microtubules and actin filaments in plant cells can be labelled using immunofluorescence techniques. In addition, it is possible to stain plant F-actin structures using fluorescent derivatives of phalloidin and other phallotoxins, which are small peptides of fungal origin that specifically bind to F-actin (Cooper 1987). Various protocols based on antibody or phalloidin labelling have been successfully employed to visualize cytoskeletal structures in a variety of intact plant tissues (Baluska *et al.* 1992; Baskin *et al.* 1992; Blancaflor & Hasenstein 2000; Marc & Hackett 1989; Vitha *et al.* 1997; Wasteneys *et al.* 1997). Recently, a technique has been developed that permits simultaneous observation in intact *A. thaliana* roots of cortical microtubules and cell wall cellulose microfibrils (Sugimoto *et al.* 2000). This technique is highly valuable for the investigation of proposed interactions (see § 1) between these two structures in elongating plant cells. Although phallotoxin- and antibody-based methods for the visualization of actin filaments and microtubules in intact tissues have contributed significantly to our understanding of the cytoskeletal functions during plant organo-

genesis (see § 3), these techniques generally are labour intensive, tend to require considerable training and are not free of caveats. Their application involves fixation of the analysed tissue and/or membrane permeabilization, procedures that can significantly affect microtubules and, particularly, actin filaments in plant cells (Kost *et al.* 2000). Phallotoxins stabilize actin filaments by reducing the threshold G-actin concentration required for polymerization (Cooper 1987) and potentially alter the organization of the actin cytoskeleton even at the low concentrations typically used for F-actin visualization. To allow the penetration of phallotoxins and antibodies into intact plant tissues, cell walls need to be at least partially removed using enzymatic degradation or physical methods (sectioning, freeze fracturing). Because cytoskeletal structures and cell wall elements are likely to interact closely with each other (Giddings & Staehelin 1991; see § 1), cell wall removal is expected to have effects on cytoskeletal organization.

Microinjection of fluorescently labelled tubulin proteins or phalloidin can be applied to visualize microtubules or actin filaments, respectively, in living plant cells. This powerful technique allows *in vivo* analysis of the dynamic behaviour of cytoskeletal elements during various cellular processes and has been extensively used to characterize cytoskeletal rearrangements during cell division (Cleary *et al.* 1992; Endle *et al.* 1998; Zhang *et al.* 1990, 1993). Whereas microinjected fluorescent phalloidin at concentrations required to visualize certain F-actin structures may have toxic effects (Valster & Hepler 1997), fluorescent tubulin is an unparalleled marker for microtubules in living plant cells, which has been employed successfully to investigate transitions between different microtubular structures during the cell cycle, the dynamic turnover of microtubular structures in resting cells, and changes in microtubule organization induced by calcium signalling as well as by cold or drug treatment (Hepler & Hush 1996). Although polypeptides chemically linked to fluorescent tags are excellent markers for cytoskeletal elements, it is difficult to employ them for the analysis of the global organization of the cytoskeleton in developing plant organs because they need to be delivered into individual cells by microinjection.

As an alternative to the techniques described above, GFP fusion proteins were recently developed that allow reliable visualization of the *in vivo* organization of cytoskeletal structures in various types of plant cells. Expression of these fusion proteins can considerably facilitate the observation of cytoskeletal structures in developing organs of plant species that are amenable to genetic transformation. Stable expression of GFP fused to the F-actin-binding domain of mouse talin was shown to label the actin cytoskeleton in all organs of transgenic *A. thaliana* plants without affecting ontogenesis (Kost *et al.* 1998, 2000; Mathur *et al.* 1999). Similarly, GFP fused to *A. thaliana* α-tubulin 6 has been reported to stain microtubules non-invasively throughout stably transformed *A. thaliana* plants (Ueda *et al.* 1999). Microtubules in living plant cells (Marc *et al.* 1998; Mathur & Chua 2000) and tissues (Bao *et al.* 2001) can also be visualized using expression of a GFP–mouse MAP4 fusion protein. GFP fusion proteins were employed to characterize cytoskeletal defects in plants with altered levels of ADF or α-tubulin gene expression in our laboratory, as described in § 3d. The application of these proteins for microtubule and F-actin labelling is expected to contribute significantly to similar studies in the future.

5. CONCLUSIONS AND OUTLOOK

The detailed characterization of mutants with defects in organogenesis and in microtubule organization, of transgenic lines displaying reduced levels of α-tubulin gene expression and of plants treated with drugs that affect microtubules has established that the microtubular cytoskeleton is essential for cell division, cell expansion and organogenesis during plant development. Cell division and organogenesis are completely inhibited when the formation of normal mitotic spindles and/or phagmoplasts is blocked. Defects restricted to cortical microtubule structures affect organogenesis, but do not abolish this process. Abnormal interphase arrays cause defective cell and organ expansion, whereas the absence of correctly positioned PPBs results in aberrant positioning of new cell walls during mitosis and in irregular tissue organization. Quite strikingly, even when these two defects are combined, many aspects of plant organogenesis can still proceed remarkably normally. In general, the roles that microtubules play during organogenesis correspond very well to the cellular functions of these cytoskeletal elements as determined by analysis in single-cell systems.

The functions of the actin cytoskeleton during the development of plant organs are less well understood. Alterations of F-action organization in developing *A. thaliana* plants induced by latrunculin B treatment or by altered levels of the expression of actin regulatory proteins apparently interfered primarily with the extent of cell and organ elongation. Possibly, cell-to-cell communication thorough plasmodesmata during the induction of flowering was also affected. This is not consistent with observations in single-cell systems, which indicate essential functions of actin filaments during additional cellular processes that are thought to be required for organogenesis, such as the differentiation of vascular cells, the control of directional cell expansion (via the establishment of growth polarity, the reorientation of microtubules and the control of targeted secretion) and the correct positioning of cell walls during mitosis. The presence in plant genomes of large gene families encoding isoforms of actin and of numerous actin regulatory genes also points to a more prominent and complex role of filamentous actin structures during plant development. Evidently, latrunculin B treatment and altered levels of ADF or profilin expression have exclusively affected a subset of the F-actin structures in cells of developing *A. thaliana* organs, which were responsible for only some of the functions of the actin cytoskeleton during organogenesis. The considerable redundancy in the gene families that encode actin and actin-associated proteins appears to impede the isolation of developmental mutants with defects in these genes using traditional screens. The further characterization of F-actin functions during organogenesis will therefore depend on the analysis of the phenotypes of plants that contain multiple T-DNA/transposon insertions or sense/antisense cDNA expression constructs and are affected in expression levels of multiple genes encoding actin

or actin-associated proteins. Constantly growing collections of *A. thaliana* lines with random T-DNA (Bouchez & Hoefte 1998; Krysan *et al.* 1999) or transposon insertions, and new techniques for induced gene silencing (Smith *et al.* 2000), will facilitate the generation of such plants.

Even significant cytoskeletal defects do not completely abolish plant organogenesis, as long as they do not inhibit cell reproduction. Plants that show severe defects in the cytoskeletal control of cell expansion and/or mitotic cell wall positioning can still develop surprisingly well-formed organs. Plant morphogenesis therefore does not seem to be directed by a simple lineage-dependent, cell-autonomous program that determines the positioning of cell division planes and the direction of cell growth via the organization of the cytoskeleton. Rather, the plant cytoskeleton appears to act as an effector of a flexible system of unknown morphogenetic factors that control organ development. The identification and functional characterization of these factors is definitely an exciting challenge. Whatever the nature of these factors, one of the key functions they must have is the manipulation of cytoskeletal functions via the control of the activity of cytoskeleton-associated regulatory and motor proteins. The further characterization of the functions of such proteins, and of the regulatory network they constitute, is obviously also essential for a comprehensive understanding of how the plant cytoskeleton operates during organogenesis. This will require the identification of additional cytoskeleton-associated proteins, and of the genes that code for them, as well as the detailed functional analysis of a large number of such proteins and genes. In particular, it will be important to learn a great deal more about plant-specific actin regulatory proteins, regulatory plant MAPs and plant cytoskeletal motor proteins. With the recent completion of the sequencing of the *A. thaliana* genome, the development of large collections of tagged *A. thaliana* mutants and the availability of a number of new techniques that allow, as described above, (i) the purification of proteins, which bind to plant cytoskeletal elements *in vitro*, (ii) efficient downregulation of target gene families, (iii) easy and reliable visualization using GFP fusion proteins of cytoskeletal elements in intact plant tissues, as well as (iv) simultaneous observation of microtubules and microfibrils in such tissues, powerful tools are now at hand to address many of the open questions concerning the role of the cytoskeleton during plant development.

Work done at Rockefeller University was supported by a DOE grant (DOE94ER20143) to N.H.C.

REFERENCES

Aizawa, H., Sutoh, K. & Yahara, I. 1996 Overexpression of cofilin stimulates bundling of actin filaments, membrane ruffling, and cell movement in *Dictyostelium*. *J. Cell Biol.* **132**, 335–344.

Akashi, T., Izumi, K., Nagano, E., Enomoto, M., Mizuno, K. & Shibaoka, H. 1988 Effects of propyzamide on tobacco cell microtubules *in vivo* and *in vitro*. *Plant Cell Physiol.* **29**, 1053–1062.

Amann, K. J. & Pollard, T. D. 2000 Cellular regulation of actin network assembly. *Curr. Biol.* **10**, R728–R730.

Andersen, S. S. 2000 Spindle assembly and the art of regulating microtubule dynamics by MAPs and Stathmin/Op18. *Trends Cell Biol.* **10**, 261–267.

Arber, S., Barbayannis, F. A., Hanser, H., Schneider, C., Stanyon, C. A., Bernard, O. & Caroni, P. 1998 Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. *Nature* **393**, 805–809.

Ayscough, K. R. 1998 *In vivo* functions of actin-binding proteins. *Curr. Opin. Cell Biol.* **10**, 102–111.

Balasubramanian, M. K., Hirani, B. R., Burke, J. D. & Gould, K. L. 1994 The *Schizosaccharomyces pombe cdc3+* gene encodes a profilin essential for cytokinesis. *J. Cell Biol.* **125**, 1289–1301.

Baluska, F., Parker, J. S. & Barlow, P. W. 1992 Specific pattern of cortical and endoplasmic microtubules associated with cell growth and tissue differentiation in roots of maize (*Zea mays* L.). *J. Cell Sci.* **103**, 191–200.

Baluska, F., Jasik, J., Edelmann, H. G., Salajova, T. & Volkmann, D. 2001 Latrunculin B-induced plant dwarfism: plant cell elongation is F-actin-dependent. *Dev. Biol.* **231**, 113–124.

Bao, Y.-Q., Kost, B. & Chua, N.-H. 2001 Reduced expression of α-tubulin genes in *Arabidopsis* specifically affects root growth and morphology, root hair development and root gravitropism. *Plant J.* **28**, 145–157.

Barroso, C., Chan, J., Allan, V., Doonan, J., Hussey, P. & Lloyd, C. 2000 Two kinesin-related proteins associated with the cold-stable cytoskeleton of carrot cells: characterization of a novel kinesin, DcKRP120-2. *Plant J.* **24**, 859–868.

Baskin, T. I., Busby, C. H., Fowke, L. C., Sammut, M. & Gubler, F. 1992 Improvements in immunostaining samples embedded in methacrylate: localization of microtubules and other antigenes throughout developing organs in plants of diverse taxa. *Planta* **187**, 405–413.

Baskin, T. I., Wilson, J. E., Cork, A. & Williamson, R. E. 1994 Morphology and microtubule organization in *Arabidopsis* roots exposed to oryzalin or taxol. *Plant Cell Physiol.* **35**, 935–942.

Bichet, A., Desnos, T., Turner, S., Grandjean, O. & Hofte, H. 2001 BOTERO1 is required for normal orientation of cortical microtubules and anisotropic cell expansion in *Arabidopsis*. *Plant J.* **25**, 137–148.

Blackman, L. M. & Overall, R. L. 1998 Immunolocalization of the cytoskeleton to plasmodesmata of *Chara corallina*. *Plant J.* **14**, 733–741.

Blancaflor, E. B. & Hasenstein, K. H. 1995 Time course of auxin sensitivity of cortical microtubule reorientation in maize roots. *Protoplasma* **185**, 72–82.

Blancaflor, E. B. & Hasenstein, K. H. 2000 Methods for detection and identification of F-actin in fixed and permeabilized plant tissues. In *Actin: a dynamic framework for multiple plant cell functions* (ed. C. J. Staiger, F. Baluska, D. Volkmann & P. W. Barlow), pp. 601–618. Dordrecht, The Netherlands: Kluwer.

Bouchez, D. & Hoefte, H. 1998 Functional genomics in plants. *Plant Physiol.* **118**, 725–732.

Canaday, J., Stoppin-Mellet, V., Mutterer, J., Lambert, A. M. & Schmit, A. C. 2000 Higher plant cells: gamma-tubulin and microtubule nucleation in the absence of centrosomes. *Microsc. Res. Tech.* **49**, 487–495.

Cao, L. G., Babcock, G. G., Rubenstein, P. A. & Wang, Y. L. 1992 Effects of profilin and profilactin on actin structure and function in living cells. *J. Cell Biol.* **117**, 1023–1029.

Chen, H., Bernstein, B. W. & Bamburg, J. R. 2000 Regulating actin-filament dynamics *in vivo*. *Trends Biochem. Sci.* **25**, 19–23.

Christensen, H. E. M., Ramachandran, S., Tan, C.-T., Surana, U., Dong, C.-H. & Chua, N.-H. 1996 *Arabidopsis* profilins are functionally similar to yeast profilins: identification of a vascular bundle-specific profilin and a pollen-specific profilin. *Plant J.* **10**, 269–279.

Cleary, A. L. & Smith, L. G. 1998 The *Tangled1* gene is

required for spatial control of cytoskeletal arrays associated with cell division during maize leaf development. *Plant Cell* **10**, 1875–1888.

Cleary, A. L., Gunning, B. E. S., Wasteneys, G. O. & Hepler, P. K. 1992 Microtubule and F-actin dynamics at the division site in living *Tradescantia* stamen hair cells. *J. Cell Sci.* **103**, 977–988.

Cooper, J. A. 1987 Effects of cytochalasin and phalloidin on actin. *J. Cell Biol.* **105**, 1473–1478.

Cooper, J. A. & Schafer, D. A. 2000 Control of actin assembly and disassembly at filament ends. *Curr. Opin. Cell Biol.* **12**, 97–103.

Cyr, R. J. 1994 Microtubules in plant morphogenesis: role of the cortical array. *A. Rev. Cell Biol.* **10**, 153–180.

Cyr, R. J. & Palevitz, B. A. 1995 Organization of cortical microtubules in plant cells. *Curr. Opin. Cell Biol.* **7**, 65–71.

Ding, B., Kwon, M. O. & Warnberg, L. 1996 Evidence that actin filaments are involved in controlling the permeability of plasmodesmata in tobacco mesophyll. *Plant J.* **10**, 157–164.

Dong, C.-H., Kost, B., Xia, G.-X. & Chua, N.-H. 2001*a* Molecular identification and characterization of the *Arabidopsis AtADF1, AtADF5* and *AtADF6* genes. *Plant Mol. Biol.* **45**, 517–527.

Dong, C.-H., Xia, G.-X., Hong, Y., Ramachandran, S., Kost, B. & Chua, N.-H. 2001*b* ADF proteins are involved in the control of flowering and regulate F-actin organization, cell expansion, and organ growth in *Arabidopsis*. *Plant Cell* **13**, 1–15.

Doussau, F. & Augustine, G. J. 2000 The actin cytoskeleton and neurotransmitter release: an overview. *Biochimie* **82**, 353–363.

Emons, A. M. C. & Mulder, B. M. 2000 How the deposition of cellulose microfibrils builds cell wall architecture. *Trends Plant Sci.* **5**, 35–40.

Endle, M.-C., Stoppin, V., Lambert, A.-M. & Schmit, A.-C. 1998 The growing cell plate of higher plants is a site of both actin assembly and vinculin-like antigen recruitment. *Eur. J. Cell Biol.* **77**, 10–18.

Finkel, T., Theriot, J. A., Dise, K. R., Tomaselli, G. F. & Goldschmidt-Clermont, P. J. 1994 Dynamic actin structures stabilized by profilin. *Proc. Natl Acad. Sci. USA* **91**, 1510–1514.

Fischer, J. A. 2000 Molecular motors and developmental asymmetry. *Curr. Opin. Genet. Dev.* **10**, 489–496.

Fischer, K. & Schopfer, P. 1998 Physical strain-mediated microtubule reorientation in the epidermis of gravitropically or phototropically stimulated maize coleoptiles. *Plant J.* **15**, 119–123.

Fowler, J. E. & Quatrano, R. S. 1997 Plant cell morphogenesis: plasma membrane interactions with the cytoskeleton and cell wall. *A. Rev. Cell Dev. Biol.* **13**, 697–743.

Fukuda, H. & Kobayashi, H. 1989 Dynamic organization of the cytoskeleton during tracheary-element differentiation. *Dev. Growth Differ.* **31**, 9–16.

Furutani, I., Watanabe, Y., Prieto, R., Masukawa, M., Suzuki, K., Naoi, K., Thitamadee, S., Shikanai, T. & Hashimoto, T. 2000 The *SPIRAL* genes are required for directional control of cell elongation in *Arabidopsis thaliana*. *Development* **127**, 4443–4453.

Gallagher, K. & Smith, L. G. 1999 *discordia* mutations specifically misorient asymmetric cell divisions during development of the maize leaf epidermis. *Development* **126**, 4623–4633.

Giddings, T. H. & Staehelin, L. A. 1991 Microtubule-mediated control of microfibril deposition: a re-examination of the hypothesis. In *The cytoskeletal basis of plant growth and form* (ed. C. W. Lloyd), pp. 85–99. London: Academic.

Gilliland, L. U., McKinney, E. C., Asmussen, M. A. & Meagher, R. B. 1998 Detection of deleterious genotypes in multigenerational studies. I. Disruptions in individual *Arabidopsis* actin genes. *Genetics* **149**, 717–725.

Goddard, R. H., Wick, S. M., Silflow, C. D. & Snustad, D. P. 1994 Microtubule components of the plant cell cytoskeleton. *Plant Physiol.* **104**, 1–6.

Green, P. B. 1962 Mechanism of plant cell morphogenesis. *Science* **138**, 1404–1405.

Green, P. B. 1980 Organogenesis—a biophysical view. *A. Rev. Plant Physiol.* **31**, 51–82.

Gunsalus, K. C., Bonaccorsi, S., Williams, E., Verni, F., Gatti, M. & Goldberg, M. L. 1995 Mutations in *twinstar*, a *Drosophila* gene encoding a cofilin/ADF homolog, result in defects in centrosome migration and cytokinesis. *J. Cell Biol.* **131**, 1243–1259.

Haarer, B. K., Lillie, S. H., Adams, A. E., Magdolen, V., Bandlow, W. & Brown, S. S. 1990 Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. *J. Cell Biol.* **110**, 105–114.

Hasezawa, S., Sano, T. & Nagata, T. 1998 The role of microfilaments in the organization and orientation of microtubules during the cell cycle transition from M phase to G1 phase in tobacco BY-2 cells. *Protoplasma* **202**, 105–114.

Hashimoto, T. 2002 Molecular genetic analysis of left–right handedness in plants. *Phil. Trans. R. Soc. Lond.* B **357**, 799–808.

Hepler, P. K. & Hush, J. M. 1996 Behavior of microtubules in living plant cells. *Plant Physiol.* **112**, 455–461.

Hogetsu, T. & Shibaoka, H. 1978 Effects of colchicine on cell shape and on microfibril arrangement in the cell wall of *Closterium acerosum*. *Planta* **140**, 15–18.

Hu, S., Brady, S. R., Kovar, D. R., Staiger, C. J., Clark, G. B., Roux, S. J. & Muday, G. K. 2000 Technical advance: identification of plant actin-binding proteins by F-actin affinity chromatography. *Plant J.* **24**, 127–137.

Huang, S., McDowell, J. M., Weise, M. J. & Meagher, R. B. 1996 The *Arabidopsis profilin* gene family. Evidence for an ancient split between constitutive and pollen-specific *profilin* genes. *Plant Physiol.* **111**, 115–126.

Hush, J. M. & Overall, R. L. 1996 Cortical microtubule reorientation in higher plants: dynamics and regulation. *J. Microsc.* **181**, 129–139.

Hussey, P. J., Yuan, M., Calder, G., Khan, S. & Lloyd, C. W. 1998 Microinjection of pollen-specific actin-depolymerizing factor, ZmADF1, reorientates F-actin strands in *Tradescantia* stamen hair cells. *Plant J.* **14**, 353–357.

Iida, K., Moriyama, K., Matsumoto, S., Kawasaki, H., Nishida, E. & Yahara, I. 1993 Isolation of a yeast essential gene, *COF1*, that encodes a homolog of mammalian cofilin, a low-M_r actin-binding and depolymerizing protein. *Gene* **124**, 115–120.

Joshi, H. C. 1998 Microtubule dynamics in living cells. *Curr. Opin. Cell Biol.* **10**, 35–44.

Kakimoto, T. & Shibaoka, H. 1987 Actin filaments and microtubules in the preprophase band and phragmoplast of tobacco cells. *Protoplasma* **140**, 151–156.

Kao, Y. L., Deavours, B. E., Phelps, K. K., Walker, R. A. & Reddy, A. S. 2000 Bundling of microtubules by motor and tail domains of a kinesin-like calmodulin-binding protein from *Arabidopsis*: regulation by Ca^{2+}/calmodulin. *Biochem. Biophys. Res. Commun.* **267**, 201–217.

Klahre, U. & Chua, N. H. 1999 The *Arabidopsis* actin-related protein 2 (AtARP2) promoter directs expression in xylem precursor cells and pollen. *Plant Mol. Biol.* **41**, 65–73.

Klahre, U., Friederich, E., Kost, B., Louvard, D. & Chua, N. H. 2000 Villin-like actin-binding proteins are expressed ubiquitously in *Arabidopsis*. *Plant Physiol.* **122**, 35–47.

Koornneef, M. 1997 Plant development: timing when to flower. *Curr. Biol.* **7**, R651–R652.

Kopczak, S. D., Haas, N. A., Hussey, P. J., Silflow, C. D. & Snustad, D. P. 1992 The small genome of *Arabidopsis* contains at least six expressed alpha-tubulin genes. *Plant Cell* **4**, 539–547.

Kost, B., Spielhofer, P. & Chua, N.-H. 1998 A GFP-mouse talin fusion protein labels plant actin filaments *in vivo* and visualizes the actin cytoskeleton in growing pollen tubes. *Plant J.* **16**, 393–401.

Kost, B., Lemichez, E., Spielhofer, P., Hong, Y., Tolias, K., Carpenter, C. & Chua, N.-H. 1999*a* Rac homologs and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *J. Cell Biol.* **145**, 317–330.

Kost, B., Mathur, J. & Chua, N.-H. 1999*b* Cytoskeleton in plant development. *Curr. Opin. Plant Biol.* **2**, 462–470.

Kost, B., Spielhofer, P., Mathur, J., Dong, C.-H. & Chua, N.-H. 2000 Non-invasive F-actin visualization in living plant cells using a mouse talin fusion protein. In *Actin: a dynamic framework for multiple plant cell functions* (ed. C. Staiger, F. Baluska, D. Volkmann & P. Barlow), pp. 637–659. Dordrecht, The Netherlands: Kluwer.

Kovar, D. R., Staiger, C. J., Weaver, E. A. & McCurdy, D. W. 2000 AtFim1 is an actin filament crosslinking protein from *Arabidopsis thaliana*. *Plant J.* **24**, 625–636.

Kropf, D. L., Bisgrove, S. R. & Hable, W. E. 1998 Cytoskeletal control of polar growth in plant cells. *Curr. Opin. Cell Biol.* **10**, 117–122.

Krysan, P. J., Young, J. C. & Sussman, M. R. 1999 T-DNA as an insertional mutagen in *Arabidopsis*. *Plant Cell* **11**, 2283–2290.

Ledbetter, M. C. & Porter, K. R. 1963 A 'microtubule' in plant cell fine structure. *J. Cell Biol.* **19**, 239–250.

Lee, Y. R. J. & Liu, B. 2000 Identification of a phragmoplast-associated kinesin-related protein in higher plants. *Curr. Biol.* **10**, 797–800.

Lemichez, E., Wu, Y., Sanchez, J. P., Mettouchi, A., Mathur, J. & Chua, N. H. 2001 Inactivation of AtRac1 by abscisic acid is essential for stomatal closure. *Genes Dev.* **15**, 1808–1816.

Li, H., Wu, G., Ware, D., Davis, K. R. & Yang, Z. B. 1998 *Arabidopsis* Rho-related GTPases: differential gene expression in pollen and polar localization in fission yeast. *Plant Physiol.* **118**, 407–417.

Liu, B., Joshi, H. C., Wilson, T. J., Silflow, C. D., Palevitz, B. A. & Snustad, D. P. 1994 Gamma-tubulin in *Arabidopsis*: gene sequence, immunoblot, and immunofluorescence studies. *Plant Cell* **6**, 303–314.

Lloyd, C. W. & Traas, J. A. 1988 The role of F-actin in determining the division plane of carrot suspension cells. Drug studies. *Development* **102**, 211–221.

McCurdy, D. W. & Kim, M. 1998 Molecular cloning of a novel fimbrin-like cDNA from Arabidopsis thaliana. *Plant Mol. Biol.* **36**, 23–31.

McDowell, J. M., Huang, S. R., McKinney, E. C., An, Y. Q. & Meagher, R. B. 1996 Structure and evolution of the actin gene family in Arabidopsis thaliana. *Genetics* **142**, 587–602.

McKinney, E. C., Kandasamy, M. K. & Meagher, R. B. 2001 Small changes in the regulation of one Arabidopsis profilin isovariant, prf1, alter seedling development. *Plant Cell* **13**, 1179–1191.

McLean, B. G., Hempel, F. D. & Zambryski, P. C. 1997 Plant intercellular communication via plasmodesmata. *Plant Cell* **9**, 1043–1054.

Manseau, L., Calley, J. & Phan, H. 1996 Profilin is required for posterior patterning of the *Drosophila* oocyte. *Development* **122**, 2109–2116.

Marc, J. & Hackett, W. P. 1989 A new method for immunofluorescent localization of microtubules in surface cell layers: application to the shoot apical meristem of *Hedera*. *Protoplasma* **148**, 70–79.

Marc, J., Granger, C. L., Brincat, J., Fisher, D. D., Kao, T.-H., McCubbin, A. G. & Cyr, R. J. 1998 A *GFP-MAP4* reporter gene for visualizing cortical microtubule rearrangements in living epidermal cells. *Plant Cell* **10**, 1927–1939.

Margolis, R. L. & Wilson, L. 1998 Microtubule treadmilling: what goes around comes around. *Bioessays* **20**, 830–836.

Mathur, J. & Chua, N. H. 2000 Microtubule stabilization leads to growth reorientation in *Arabidopsis* trichomes. *Plant Cell* **12**, 465–477.

Mathur, J., Spielhofer, P., Kost, B. & Chua, N. H. 1999 The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in *Arabidopsis thaliana*. *Development* **126**, 5559–5568.

Mayer, U., Herzog, U., Berger, F., Inze, D. & Juergens, G. 1999 Mutations in the *PILZ* group genes disrupt the microtubule cytoskeleton and uncouple cell cycle progression from cell division in *Arabidopsis* embryo and endosperm. *Eur. J. Cell Biol.* **78**, 100–108.

Meagher, R. B., McKinney, E. C. & Kandasamy, M. K. 1999*a* Isovariant dynamics expand and buffer the responses of complex systems: the diverse plant actin gene family. *Plant Cell* **11**, 995–1005.

Meagher, R. B., McKinney, E. C. & Vitale, A. V. 1999*b* The evolution of new structures: clues from plant cytoskeletal genes. *Trends Genet.* **15**, 278–284.

Mineyuki, Y. & Palevitz, B. A. 1990 Relationship between preprophase band organization, F-actin and the division site in *Allium*. *J. Cell Sci.* **97**, 283–295.

Moon, A. L., Janmey, P. A., Louie, K. A. & Drubin, D. G. 1993 Cofilin is an essential component of the yeast cortical cytoskeleton. *J. Cell Biol.* **120**, 421–435.

Moscatelli, A., Del Casino, C., Lozzi, L., Cai, G., Scali, M., Tiezzi, A. & Cresti, M. 1995 High molecular weight polypeptides related to dynein heavy chains in *Nicotiana tabacum* pollen tubes. *J. Cell Sci.* **108**, 1117–1125.

Nagaoka, R., Kusano, K., Abe, H. & Obinata, T. 1995 Effects of cofilin on actin filamentous structures in cultured muscle cells. Intracellular regulation of cofilin action. *J. Cell Sci.* **108**, 581–593.

Nick, P. 1998 Signaling to the microtubular cytoskeleton in plants. *Int. Rev. Cytol.* **184**, 33–80.

Oppenheimer, D. G., Pollock, M. A., Vacik, J., Szymanski, D. B., Ericson, B., Feldmann, K. & Marks, M. D. 1997 Essential role of a kinesin-like protein in *Arabidopsis* trichome morphogenesis. *Proc. Natl Acad. Sci. USA* **94**, 6261–6266.

Palevitz, B. A. 1987 Actin in the preprophase of *Allium cepa*. *J. Cell Biol.* **104**, 1515–1519.

Palevitz, B. A. & Hepler, P. K. 1974 The control of the plane of division during stomatal differentiation in *Allium*. II. Drug studies. *Chromosoma* **46**, 327–341.

Pollard, T. D., Blanchoin, L. & Mullins, R. D. 2000 Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *A. Rev. Biophys. Biomol. Struct.* **29**, 545–576.

Pruyne, D. & Bretscher, A. 2000 Polarization of cell growth in yeast II. The role of the cortical actin cytoskeleton. *J. Cell Sci.* **113**, 571–585.

Ramachandran, S., Christensen, H. E. M., Ishimaru, Y., Dong, C. H., Wen, C. M., Cleary, A. L. & Chua, N. H. 2000 Profilin plays a role in cell elongation, cell shape maintenance, and flowering in *Arabidopsis*. *Plant Physiol.* **124**, 1637–1647.

Reddy, A. S. 2001 Molecular motors and their functions in plants. *Int. Rev. Cytol.* **204**, 97–178.

Schoenenberger, C. A., Steinmetz, M. O., Stoffler, D., Mandinova, A. & Aebi, U. 1999 Structure, assembly, and dynamics

of actin filaments *in situ* and *in vitro*. *Microsc. Res. Tech.* **47**, 38–50.
Seagull, R. W. 1990 The effects of microtubule and microfilament disrupting agents on cytoskeletal arrays and wall deposition in developing cotton fibers. *Protoplasma* **159**, 44–59.
Seagull, R. W., Falconer, M. M. & Weerdenburg, C. A. 1987 Microfilaments: dynamic arrays in higher plant cells. *J. Cell Biol.* **104**, 995–1004.
Shibaoka, H. 1994 Plant hormone-induced changes in the orientation of cortical microtubules. *A. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 527–544.
Simpson, G. G., Gendall, A. R. & Dean, C. 1999 When to switch to flowering. *A. Rev. Cell Dev. Biol.* **15**, 519–550.
Smertenko, A., Saleh, N., Igarashi, H., Mori, H., Hauser-Hahn, I., Jiang, C. J., Sonobe, S., Lloyd, C. W. & Hussey, P. J. 2000 A new class of microtubule-associated proteins in plants. *Nature Cell Biol.* **2**, 750–753.
Smith, L. G. 1999 Divide and conquer: cytokinesis in plant cells. *Curr. Opin. Plant Biol.* **2**, 447–453.
Smith, N. A., Singh, S. P., Wang, M. B., Stoutjesdijk, P. A., Green, A. G. & Waterhouse, P. M. 2000 Total silencing by intron-spliced hairpin RNAs. *Nature* **407**, 319–320.
Smith, L. G., Gerttula, S. M., Han, S. C. & Levy, J. 2001 Tangled1: a microtubule binding protein required for the spatial control of cytokinesis in maize. *J. Cell Biol.* **152**, 231–236.
Snustad, D. P., Haas, N. A., Kopczak, S. D. & Silflow, C. D. 1992 The small genome of *Arabidopsis* contains at least nine expressed beta-tubulin genes. *Plant Cell* **4**, 549–556.
Staiger, C. J., Yuan, M., Valenta, R., Shaw, P. J., Warn, R. M. & Lloyd, C. W. 1994 Microinjected profilin affects cytoplasmic streaming in plant cells by rapidly depolymerizing actin microfilaments. *Curr. Biol.* **4**, 215–219.
Sugimoto, K., Williamson, R. E. & Wasteneys, G. O. 2000 New techniques enable comparative analysis of microtubule orientation, wall texture, and growth rate in intact roots of *Arabidopsis*. *Plant Physiol.* **124**, 1493–1506.
Takei, Y., Kondo, S., Harada, A., Inomata, S., Noda, T. & Hirokawa, N. 1997 Delayed development of nervous system in mice homozygous for disrupted *microtubule-associated protein 1B* (*MAP1B*) gene. *J. Cell Biol.* **137**, 1615–1626.
Titus, M. A. & Gilbert, S. P. 1999 The diversity of molecular motors: an overview. *Cell Mol. Life Sci.* **56**, 181–183.
Torres-Ruiz, R. A. & Juergens, G. 1994 Mutations in the *Fass* gene uncouple pattern formation and morphogenesis in *Arabidopsis* development. *Development* **120**, 2967–2978.
Traas, J. A., Doonan, J. H., Rawlins, D. J., Shaw, P. J., Watts, J. & Lloyd, C. W. 1987 An actin network is present in the cytoplasm throughout the cell cycle of carrot cells and associates with the dividing nucleus. *J. Cell Biol.* **105**, 387–395.
Traas, J., Bellini, C., Nacry, P., Kronenberger, J., Bouchez, D. & Caboche, M. 1995 Normal differentiation patterns in plants lacking microtubular preprophase bands. *Nature* **375**, 676–677.
Ueda, K., Matsuyama, T. & Hashimoto, T. 1999 Visualization of microtubules in living cells of transgenic *Arabidopsis thaliana*. *Protoplasma* **206**, 201–206.
Valster, A. H. & Hepler, P. K. 1997 Caffeine inhibition of cytokinesis: effect on the phragmoplast cytoskeleton in living *Tradescantia* stamen hair cells. *Protoplasma* **196**, 155–166.
Verheyen, E. M. & Cooley, L. 1994 Profilin mutations disrupt multiple actin-dependent processes during *Drosophila* development. *Development* **120**, 717–728.
Vesk, P. A., Vesk, M. & Gunning, B. E. S. 1996 Field emission scanning electron microscopy of microtubule arrays in plant cells. *Protoplasma* **195**, 168–182.
Vitha, S., Baluska, F., Mews, M. & Volkmann, D. 1997 Immunofluorescence detection of F-actin on low melting point wax sections from plant tissues. *J. Histochem. Cytochem.* **45**, 89–95.
Walczak, C. E. 2000 Microtubule dynamics and tubulin interacting proteins. *Curr. Opin. Cell Biol.* **12**, 52–56.
Wasteneys, G. O., Willingale-Theune, J. & Menzel, D. 1997 Freeze shattering: a simple and effective method for permeabilizing higher plant cell walls. *J. Microsc.* **188**, 51–61.
Wear, M. A., Schafer, D. A. & Cooper, J. A. 2000 Actin dynamics: assembly and disassembly of actin networks. *Curr. Biol.* **10**, R891–R895.
Wenzel, C. L., Williamson, R. E. & Wasteneys, G. O. 2000 Gibberellin-induced changes in growth anisotropy precede gibberellin-dependent changes in cortical microtubule orientation in developing epidermal cells of barley leaves. Kinematic and cytological studies on a gibberellin-responsive dwarf mutant, M489. *Plant Physiol.* **124**, 813–822.
Wernicke, W. & Jung, G. 1992 Role of cytoskeleton in cell shaping of developing mesophyll of wheat (*Triticum aestivum* L.). *Eur. J. Cell Biol.* **57**, 88–94.
White, R. G., Badelt, K., Overall, R. L. & Vesk, M. 1994 Actin associated with plasmodesmata. *Protoplasma* **180**, 169–184.
Wick, S. M. 1991 Spatial aspects of cytokinesis in plant cells. *Curr. Opin. Cell Biol.* **3**, 253–260.
Wick, S. 2000 Plant microtubules meet their MAPs and mimics. *Nature Cell Biol.* **2**, E204–E206.
Williamson, R. E. 1991 Orientation of cortical microtubules in interphase plant cells. *Int. Rev. Cytol.* **129**, 135–206.
Winge, P., Brembu, T. & Bones, A. M. 1997 Cloning and characterization of Rac-like cDNAs from *Arabidopsis thaliana*. *Plant Mol. Biol.* **35**, 483–495.
Xia, G., Ramachandran, S., Hong, Y., Chan, Y.-S., Simanis, V. & Chua, N.-H. 1996 Identification of plant cytoskeletal, cell cycle-related and polarity-related proteins using *Schizosaccharomyces pombe*. *Plant J.* **10**, 761–769.
Yamamoto, K., Hamada, S. & Kashiyama, T. 1999 Myosins from plants. *Cell Mol. Life Sci.* **56**, 227–232.
Zhang, D., Wadsworth, P. & Hepler, P. K. 1990 Microtubule dynamics in living dividing plant cells: confocal imaging of microinjected fluorescent brain tubulin. *Proc. Natl Acad. Sci. USA* **87**, 8820–8824.
Zhang, D., Wadsworth, P. & Hepler, P. K. 1993 Dynamics of microfilaments are similar, but distinct from microtubules during cytokinesis in living, dividing plant cells. *Cell Motil. Cytoskeleton* **24**, 151–155.

GLOSSARY

ABA: abscisic acid
ADF: actin-depolymerizing factor
CaMV: cauliflower mosaic virus
CDNA: complementary DNA
GFP: green fluorescent protein
MAP: microtubule-associated protein
Pfn: profilin
PPB: pre-prophase band
ZAD: zone of actin depletion