Acta Bot. Neerl. 39(1), March 1990, p. 1–18

REVIEW

The plant cytoskeleton: its significance in plant development

J. DERKSEN, F. H. A. WILMS and E. S. PIERSON

Department of Experimental Botany, University of Nijmegen, Toernooiveld, NL-6525 ED Nijmegen, The Netherlands

CONTENTS

Introduction	1
Structure and composition of the cytoskeleton	1
General organization of the cytoskeleton	4
The cytoskeleton in cell morphogenesis and differentiation	7
The cytoskeleton in plant morphogenesis	12
Concluding remarks	12

Key-words: cytoskeleton, morphogenesis, cell differentiation.

INTRODUCTION

The cytoplasm of eukaryotic cells contains a three-dimensional network of filaments: the cytoskeleton. It connects the various organelles and other cytoplasmic elements of the cell with each other and with the plasma membrane and is involved in many dynamic processes in the cell, including cell division, morphogenesis, redistribution of surface components, endo- and exocytosis and the positioning of cytoplasmic elements (reviews: Dustin 1984; Lackie 1986; Bershadsky & Vasiliev 1988; plants: Traas 1989).

The three major constituents of this system are microtubules, microfilaments and intermediate filaments. They can be discriminated, based on their diameter and by means of immunocytochemistry.

The information concerning the cytoskeleton in plant cells seems scanty when compared to animal cells. However, as interest has been rapidly increasing, it will not be possible to do justice to all studies concerning the plant cytoskeleton. Literature prior to 1980 has been discussed extensively by Gunning & Hardham (1982), Hepler (1985) and Lloyd (1982). For a recent review on the biochemistry and genetics of plant cytoskeletal proteins see Fosket (1989).

As plant cells are immobile, a particular plant shape can only be obtained by directional cell division and cell expansion, i.e. by polar growth, or specific cell death. Thus, cell division and cell expansion within a tissue must occur in a co-ordinated way. Here we shall describe first structure and composition of the cytoskeleton of eukaryotic cells in general and following this we will discuss recent results on the organization of the cytoskeleton in higher, i.e. embryonic, plants with special reference to morphogenesis and cell differentiation.

STRUCTURE AND COMPOSITION OF THE CYTOSKELETON

Microtubules

Microtubules are tubular structures with an internal diameter of about 15 nm and an average external diameter of about 25 nm. They consist of two evolutionally related

proteins, α-tubulin and β-tubulin, each with a molecular weight of approximately 50 000 kD. The microtubular wall is generally made up of 13 protofilaments, but different numbers occur. Protofilaments consist of a chain of about 8 nm long, α-β tubulin dimers that are asymmetrical and are all oriented in one direction, resulting in polar protofilaments. The α-tubulin side is called the +side, the β-tubulin side the −side. In a microtubule all protofilaments have the same orientation and are shifted about 1 nm in respect to each other. Microtubules thus show a distinct polarity with dimers in a 10^0 left-handed helix (Dustin 1984).

Microtubules are no static elements but continuously assemble and disassemble. Two mechanisms have been proposed to explain these processes: treadmilling (Margolis & Wilson 1978) and dynamic instability (Mitchison & Kirschner 1984). The first model assumes a steady state with a continuous assembly at the +side and disassembly at the −side. Dynamic instability predicts the existence of a population of rapidly disassembling microtubules together with a population of slowly, eventually at both ends, growing microtubules. In mitotic spindles *in vivo*, microtubules appear to behave according to the dynamic instability model (Salmon *et al.* 1984). However, in the interphase cells of animals, part of the microtubules appeared to be much more stable. In such cases their dynamics have been described as a tempered mode of dynamic instability (e.g. Sammak & Borisy 1988). Tubulins of higher plants appear to be less conservative than in animal cells (Cleveland *et al.* 1980; Fosket 1989), which has been attributed to a different evolutionary pressure. This difference may result from the disappearance of cilia and flagella (Cavalier-Smith 1978), but may as well relate to the occurrence of a cell wall. This difference may also explain the relative insensitivity of plant microtubules to colchicine and their sensitivity to some herbicides (Bajer & Molè-Bajer 1986a). A variety of proteins has been found to associate with microtubules: the microtubule-associated proteins (MAPs) (review: Olmsted 1986, see also below). MAPs largely determine microtubule stability and its association with other cytoskeletal elements and organelles. Their activity appears to be regulated by a cascade of phosphorylating enzymes in which Ca^{2+}/calmodulin and cAMP may play an important role (Theurkauf & Vallee 1982; Larsson *et al.* 1985, Schulman *et al.* 1985).

Microtubules have been related to various types of movements: besides their well known role in movement of metaphase chromosomes and of cilia/flagella, they can be involved in the transport of vesicles and organelles, like in neurons and other animal cells (Dustin 1984; Bershadsky & Vasiliev 1988). Microtubules are probably also involved in the organization and dynamics of the endoplasmic reticulum (Chen & Lee 1988; Vale & Hotani 1988).

Microfilaments

The backbone protein of microfilaments is formed by actin which has a molecular weight of about 42 kD. In the filaments actin molecules are piled up in such a way as to form a double-stranded twisted rope. The major repeat distance is 38 nm, involving 13 molecules. Individual microfilaments have a diameter of 5–7 nm and show a distinct polarity. Microfilaments are also dynamic and both treadmilling and dynamic instability have been considered to occur. Microfilaments often occur in large bundles with a regular organization like in muscles and stress fibres, where they are very stable (reviews: Lackie 1986, Bershadsky & Vasiliev 1988). They can also form loose bundles, such as in many plant cells (review: Staiger & Schliwa 1986). The microfilaments that can be seen in electron microscopic preparations represent actin filaments, which have recently been confirmed

for plant cells with the use of immunological probes (Lancelle & Hepler 1989). Actin filaments can also form more or less dense networks, often associated with membranes. The different types of association of actin filaments and their stability depend on the various associated proteins from which numbers have now been identified (Lackie 1986; Bershadsky & Vasiliev 1988). As in microtubules many associated proteins are sensitive to Ca^{2+}/Ca^{2+}-calmodulin, which thus play an important regulatory role.

Actin is a regular component of plant cells (Condeelis 1974; Parthasarathy *et al.* 1985; Staiger & Schliwa 1987). Plasma-streaming depends on actin filaments. Also organelle movements and vesicle-mediated secretion often appear to be actin dependent (reviews in Jackson 1982; Kristen 1987; Staiger & Schliwa 1987). Also the organization of the endoplasmic reticulum has been thought to be actin dependent (Quader *et al.* 1987).

Intermediate filaments

Unlike microtubules and microfilaments, intermediate filaments are a family of heterogeneous, yet evolutionally related, proteins (reviews in Nagle 1988; Steiner & Roop 1988). In animals they are tissue specific, indicating a less conservative character to this system in the eukaryotic cell in general.

Intermediate filaments of plant cells were first described in carrot protoplasts (Powell *et al.* 1982). The diameter of individual intermediate filaments is 7–10 nm. Intermediate filaments form large bundles with a diameter of 50–100 nm. These bundles contain a number of different proteins, some of which show immunological homology with animal intermediate filaments. They co-localize with microtubules (Dawson *et al.* 1985; Hargreaves *et al.* 1989). Distinct distributions of plant intermediate filaments have recently been described by Goodbody *et al.* (1989). Recently, Wang & Yan (1988) have isolated a spectrin-like and an ankyrin-like protein from plant membranes. Maybe the spectrin-like protein is identical to part of the proteins detected at the plasma membrane by Goodbody *et al.* (1989).

Antibodies raised against extraction-resistant cell residues of fern spermatozoids (Marc *et al.* 1988) also co-localize with microtubules in immunofluorescent preparations, but do not show a filamental organization (Marc & Gunning 1988). They might detect intermediate filamental proteins, or perhaps MAPs.

The P-proteins in phloem cells form tubular structures (Cronshaw *et al.* 1973), that must also be regarded, by definition, as a plant-specific intermediate filament system.

Cytoskeleton-associated proteins

The various associated proteins of microtubules and microfilaments largely determine their organization and function. Numerous cytoskeleton-associated proteins are known to be present in animal systems (Lackie 1986; Bershadsky & Vasiliev 1988), but in higher plants few have been identified yet. They are summarized in Table 1 together with their possible functions.

In higher plants, the existence of an acto-myosin driven system has still not been proven, but the basis for such a concept has been set as myosin is clearly present.

Cytoplasmic microtubule-based motors, such as the dynein-like MAP-1C (Paschal & Vallee 1987) and kinesin (Vale et al. 1985) have not yet been established firmly in plants, but we expect them to be present in higher plants as well.

The latter may mediate the interactions between microtubules and the tubular endoplasmic reticulum (e.g. Vale & Hotani 1988). Directional movement may also depend on both actins and microtubules as shown for the alga *Bryopsis* (Menzel & Schliwa 1986).

Table 1. Microtubule (MT) and actin filament (AF) associated proteins that have been identified in the cytoplasm of cells of higher plants

Protein	Group	References	Possible role
MAPs	MT	Cyr & Palevitz (1989)	Microtubule bundling
Kinesin	MT	Moscatelli *et al.* (1988)	Force-generating, organelle transport,
Calmodulin	MT	Wick *et al.* (1985)	Ca^{2+}-binding protein, regulatory functions
Troponin	AF/MT	Lim *et al.* (1986)	Ca^{2+}-binding protein binds to tropomyosin, regulatory functions
Ankyrin	AF	Wang & Yan (1988)	Connecting actin to membrane proteins
Myosin	AF	Vahey *et al.* (1982) Parke *et al.* (1986) Yan *et al.* (1986) Grolig *et al.* (1988) Tang *et al.* (1989)	Organelle movement, force generation, intracellular movements
Spectrin	AF	Wang & Yan (1988)	Connecting actin to membrane proteins

The presence of ankyrin-like and spectrin-like proteins indicates that the associations of actin with the membranes may be similar to those in animal cells.

We expect that most cytoskeletal proteins of animal cells will be present somehow in plant cells.

GENERAL ORGANIZATION OF THE CYTOSKELETON

The techniques used

The organization of cytoskeletal elements has been studied using a number of techniques.

Electron microscopy. Classical electron microscopical (EM) techniques enable visualization of microtubules, microfilaments and intermediate filaments, but due to the conditions used, cytoskeletal elements, especially actin filaments, may become depolymerized during the procedure and fibrous elements may become only poorly contrasted as compared to the embedding medium, or are obscured by soluble components of the cytoplasm. Moreover, only small areas can be studied.

Fixation problems can be overcome by the use of cryo-techniques, (Hereward & Northcote 1973; Howard & Aist 1979; Emons & Derksen 1986; Lancelle *et al.* 1986; Craig & Staehelin 1988). The use of these techniques may be conditional for the use of immunological probes at the ultrastructural level (e.g. Lancelle & Hepler 1989).

Large surfaces allowing quantitative analyses can be obtained by using cleaving techniques (Traas 1984; Traas *et al.* 1985). Also sections of polyethylene glycol (PEG) embedded material (Wilms 1990) and whole mounts (Hawes 1985) may be useful. Larger cell parts can also be studied using serial sectioning, though this procedure is laborious.

Light microscopy. Cytoskeletal elements appear to be evolutionally conservative and antibodies raised against animal proteins, especially tubulin, could be used in plant cells. Immunofluorescent (IF) probes allow study of the spatial organization in entire cells (Lloyd *et al.* 1979; Wick *et al.* 1981) and in sections of PEG-embedded material (Hawes &

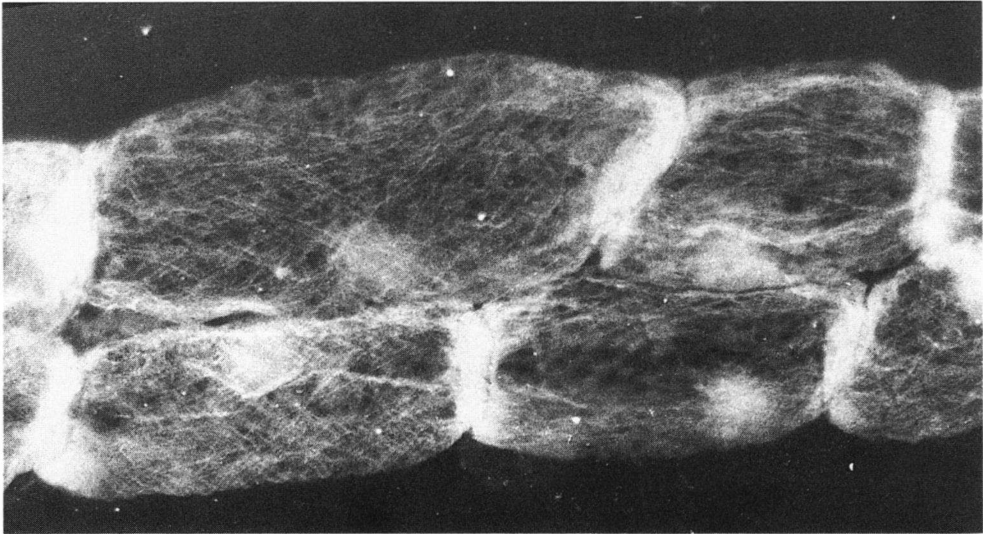

Fig. 1. Microtubules in root cortex cells of *Lepidium*. Immunofluorescence preparation according to Traas *et al.* (1984). In the left two cells the microtubules are helical, in the two cells on the right, the microtubules are almost parallel to the longitudinal axis of the cell. Magnification: × 1000.

Horne 1985). The introduction of fluorochrome conjugates of phalloidin (Wulf *et al.* 1979) allowed investigation of the three-dimensional organization of actin filaments, though antibodies have been used as well (McCurdy *et al.* 1988)).

The IF methods are extremely useful for large scale-studies, but for a detailed analysis EM techniques are required (e.g. Segaar 1990).

The use of advanced light microscopic techniques in plant cells such as confocal scanning laser microscopy (CSLM; e.g. Quader & Schnepf 1986) and video/computer enhanced image photography (e.g. Lichtscheidl & Weiss 1988) will contribute to a better understanding of the dynamics of the cytoskeleton and its interactions with other cellular structures.

General organization

Microtubules are present throughout the cytoplasm, but large arrays of parallel cortical microtubules are always present, and especially conspicuous in vacuolated cells (reviews in Gunning & Hardham 1982; Lloyd 1984; Traas 1989; see also Fig. 1). The cortical microtubules are interconnected and thought to form an almost uninterrupted helix throughout the cell (Lloyd 1984). Recently it could be shown that in protoplasts of *Nicotiana* about 50% of the cortical microtubules is regularly interconnected (H. Kengen & J. Derksen, unpublished data). If these interconnections were of a dynein-type such interconnected microtubules could not only withstand strong forces, but could even exert considerable forces on their environment.

At the surface of the nucleus microtubule organizing centres (MTOCs) are present that initiate microtubule assembly after cell division (e.g. Wick & Duniec 1983). They probably do not determine the organization of the cortical cytoskeleton, as nucleating sites are also present at the plasma membrane (Gunning *et al.* 1978) and in anucleate cytoplasts the microtubular skeleton may self-assemble into highly organized patterns (Bajer &

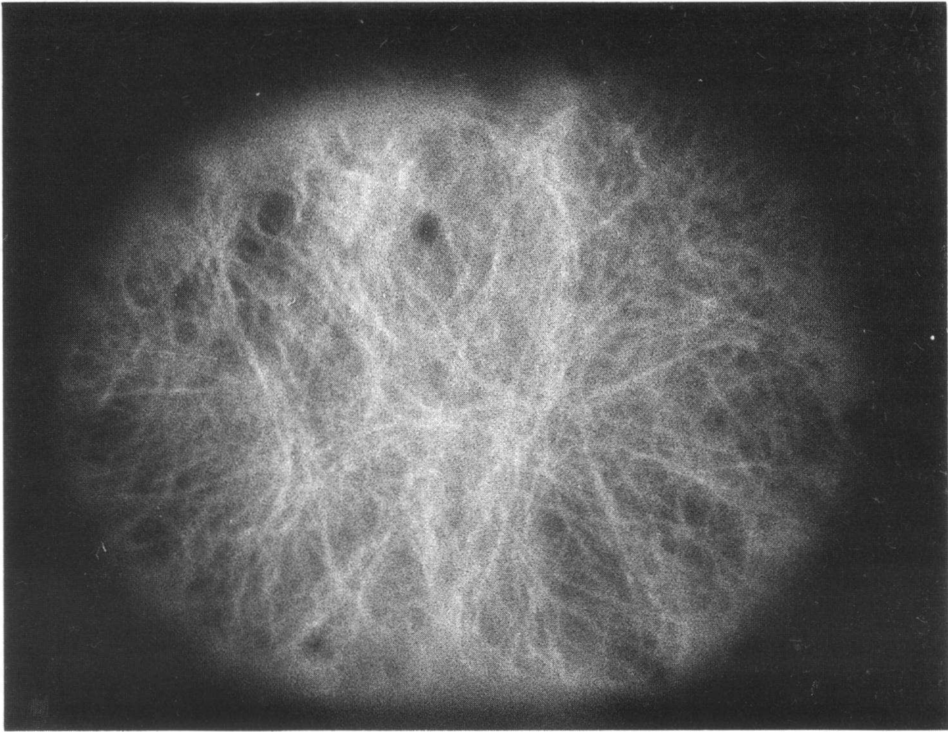

Fig. 2. Actin filaments in an anucleate subprotoplast of pollen tubes of *Nicotiana*. Immunofluorescence preparation with rhodamine-phalloidin as a probe, according to Rutten & Derksen (1990). Magnification × 8000. (Photograph: T. Rutten.)

Molè-Bajer 1986b). In protoplasts of the alga *Mougeotia* reorganization of the cortical microtubules takes place at the plasmalemma, independent from the nucleus (Galway & Hardham 1986).

Like microtubules, actin filaments are present in cortical arrays (Traas *et al.* 1987). The nucleus is anchored in a basket of actin filaments and large bundles connect the nucleus with the cells periphery (Derksen *et al.* 1986b). These bundles reflect plasma streaming (review: Staiger & Schliwa 1986). Their origin is uncertain, but in anucleate cytoplasts microfilaments may show self assembly (Rutten & Derksen 1989; see also Fig. 2).

Intermediate filaments in plant cells are probably present associated with the plasmamembrane and can be seen as patches that co-localize with microtubules in immunofluorescent preparations, also in those of mitotic spindles. Arrays of filament bundles seem to connect the nucleus with the cells periphery in interphase cells (Goodbody *et al.* 1989; Hargreaves *et al.* 1989).

Interactions between cytoskeletal elements

The various cytoskeletal elements form an integrated system. Microtubules and actin filaments may be cross-linked by MAPs or by actin-associated proteins (e.g. Bennett & Davis 1981; Griffith & Pollard 1982). In plant cells they often co-localize, and cross-links have been observed in both dry-cleaved preparations (Traas *et al.* 1985; Pierson *et al.* 1986) and sections of freeze-substituted material (Tiwari *et al.* 1984; Lancelle *et al.* 1987).

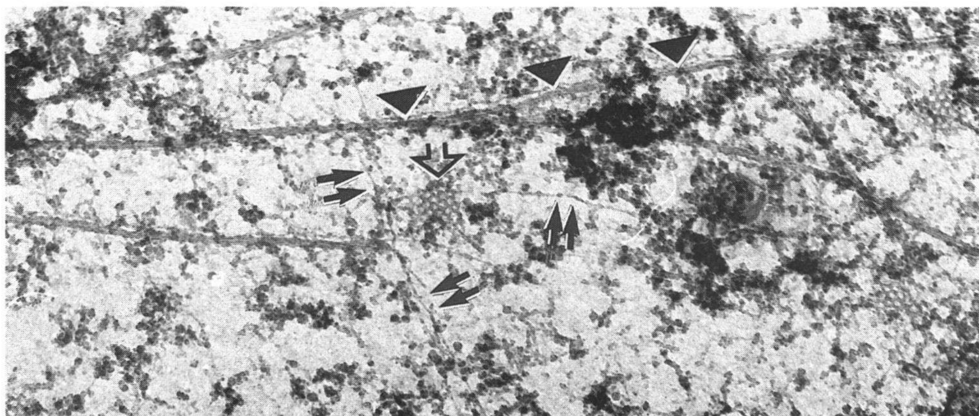

Fig. 3. Micrograph of a dry-cleaved preparation of a protoplast from a *Nicotiana* cell culture. Numerous putative microfilaments are present at the surface (↓↓). Often these filaments seem to be connected with coated pits (⇩), or to accompany microtubules (▼). Magnification: × 59 000. (Photograph: H. Kengen.)

Single microfilaments were co-aligned with microtubules over distances up to 1·46 μm in dry cleaved preparations of *Nicotiana* protoplasts (H. Kengen & J. Derksen, unpublished data); see also Fig. 3). However, doubt exists about the actual nature of these filaments (see below).

A clear co-localization over large parts of the cell has been observed in immunofluorescent preparations of pollen tubes (Raudaskoski *et al.* 1987; Pierson *et al.* 1989) and the distribution of the microtubules has been reported to depend, at least partly, on actin distribution (Derksen & Traas 1984).

Intermediate filaments of plant cells co-localize with microtubules in a patchy way (Goodbody *et al.* 1989). The present proteins may be at least partly identical to the filaments that are visible in dry-cleaved cells and that accompany microtubules and connect coated pits with microtubules (Emons & Traas 1986; Quader *et al.* 1986; H. Kengen & J. Derksen, unpublished data). The distribution of the larger bundles is independent from an intact actin skeleton, but depends on microtubules. They become dispersed after microtubule degradation (Goodbody *et al.* 1989).

THE CYTOSKELETON IN CELL MORPHOGENESIS AND DIFFERENTIATION

Determination of the division plane

The position of the nucleus may be largely determined by microtubules, as antimicrotubular drugs may cause disposition of the nucleus (Clayton & Lloyd 1984).

Prior to cell division, the nucleus will move towards the future division plane, which may be determined by microtubules radiating from the nucleus to the cells periphery and vice versa (Burgess 1970; Pickett-Heaps 1974). These microtubules may already be present during phragmosome formation and coincide in time and place with the formation of the pre-prophase bands (Venverloo & Libbenga 1987). However, a role of the preprophase bands in nuclear positioning is not unquestioned (Clayton & Lloyd 1984; Mineyuki *et al.* 1988). The fundamental regulatory mechanisms are unknown. Also, microfilaments are

present in the preprophase bands (McCurdy *et al.* 1988; Lloyd & Traas 1988). During metaphase the preprophase band microtubules disappear but the preprophase band actin filaments remain, and may help to guide the cytokinetic apparatus out along the predetermined path (Traas *et al.* 1987; Lloyd 1988). Both microtubules and actin filaments show distinct configurations during the meiotic divisions (see Lammeren *et al.* 1989; Traas *et al.* 1989; Bednara *et al.* 1990) and are probably involved in the co-ordination of the meiotic division process, but probably only microtubules are involved in the establishment of cell polarity (Traas *et al.* 1989).

Calmodulin is associated with the spindle and the phragmoplast, and not with the preprophase band (Gunning & Wick 1985), which indicates a regulatory role in cell division but not in determination of the division plane. The actual mechanisms underlying the changes in cytoskeletal organization prior to cell division are still basically unknown.

Hormonal control of the cytoskeletal organization

Little is known about the precise relationship between hormones and the cytoskeletal elements. Ethylene causes a re-orientation of cortical microtubules in epidermis cells of *Pisum sativum* and *Vigna radiata* (Steen & Chadwick 1981; Lang *et al.* 1982; Roberts *et al.* 1985) within a few hours. In cortex cells of tobacco explants, re-orientation of microtubules is accelerated after ethylene treatment (Wilms & Wolters-Arts 1989). Doonan *et al.* (1985) showed that high concentrations of benzylaminopurine (100 μM) can cause depolymerization of microtubules in tip-growing cells of *Physcomitrella*. Gibberellic acid causes rearrangement of cortical microtubules in epidermal cells of pea internodes (Akashi & Shibaoka 1987). It increases the number of transverse microtubules and it prevents depolymerization of microtubules by colchicine, cremart and low temperature (Mita & Shibaoka 1984). However, the effects of gibberellic acid are diverse: it may protect growth against cochicine inhibition but conversely, growth stimulation by gibberellic acid may be inhibited by colchicine. The differences however, may depend on the colchicine concentration used (Fragata 1974; for discussion see also Mita & Shibaoka 1984).

Hormones also may act indirectly on the organization of the cytoskeleton. Auxin treatment effects the direction of cell expansion and the orientation of the cortical microtubules in epidermal cells of maize coleoptiles (Bergfield *et al.* 1988). Since auxin induces ethylene production (Imaseki 1985), it may be concluded that the action of auxin on the organization of the cytoskeleton is indirect. However, the presence of auxin seems to be conditional for the development of a cortical microtubular skeleton in protoplasts of *Medicago* mesophyll cells (Meijer & Simmonds 1988). Hormones may act on cell polarity by changing the distribution of cation pumps and channels on the plasma-membrane (Saunders & Hepler 1981; Saunders 1986; review: Schnepf 1986). As Ca^{2+} may affect both microtubules and microfilaments (see above), such changes may also affect the organization of cytoskeletal elements.

Control of cell wall deposition

It is generally believed that the orientation of nascent cellulose microfibrils is controlled by cortical microtubules. Several models have been proposed to describe microtubular control of microfibril orientation (reviews: Robinson & Quader 1982; Heath & Seagull 1982). All models imply that microtubules connected to the plasma membrane, i.e. the cortical microtubules, would prevent free diffusion of cellulose microfibril synthesizing complexes in the membrane, leading to parallelism of microtubules and nascent microfibrils (see also: Herth 1985). Also the insertion of Golgi-vesicles with non-cellulosic wall material has

been thought to be under microtubular control (Goosen-de Roo 1973, see also Herth 1985).

As discussed by Hepler (1985) microtubular control of microfibril deposition seems to occur in elongating cylindrical cells, guard cells and during xylogenesis. Also in seed hairs a clear relationship seems to exist (Quader *et al.* 1986; 1987). Often such a relationship has been thought to be obligatory (e.g. Lloyd 1984).

In a number of cases, mainly in growing tip cells, a similar relationship cannot exist (pollen tubes: Derksen *et al.* 1985; root hairs: Emons 1982; Emons & Wolters-Arts 1983; Traas *et al.* 1984; Emons 1989; Traas & Derksen 1989). In *Raphanus* root hairs, after depolymerization of the microtubules by colchicine, a change in microfibril deposition occurred, but microfibrils were still deposited in ordered patterns (Emons *et al.* 1990). The effect of colchicine on microfibril deposition, or other drugs to the same effect, do not necessarily indicate a direct microtubular control as the entire cytoplasmic organization may be effected and thus indirectly will lead to changes in cell wall deposition. In the marine algae *Boergesenia forbesii* and *Valonia ventricosa* (Hayano *et al.* 1988) the orientation of microtubules and nascent microfibrils is clearly different. Also in mesophyll cells such a relationship between microtubules and microfibril deposition may be absent (Hahne & Hoffmann 1985).

A simultaneous change in orientation of microtubules and microfibrils has been observed in maize coleoptile segments after cessation of auxin supply and a microtubular control of microfibril orientation has been inferred (Bergfield *et al.* 1988). In cortex cells of tobacco explants, the organization of microtubules and microfibrils changes within the same time interval from transverse to parallel to the longitudinal cell axis (Wilms & Derksen 1988). However, elimination of microtubules by cremart or colchicine did not affect the change in microfibril orientation (Wilms & Wolters-Arts 1989). Thus, such simultaneous changes might be solely co-incidental and depend on common regulatory factors. The transverse orientation with respect to cell expansion of both microtubules and nascent cellulose microfibrils in elongating cells (Gunning & Hardham 1982), might also be co-incidental. Patterning of cellulose deposition may be more complicated as generally thought so far, and relations with microtubular patterns may need reconsideration in some instances (Emons *et al.* 1990; Wilms *et al.* 1990; Wilms & Kengen 1990).

Obviously, microtubule–microfibril interactions may be diverse and depend on cell differentiation. Various factors may be conditional, or may interfere otherwise. Such factors have been thought to be, e.g. Ca^{2+} (Quader *et al.* 1986), turgor pressure (Derksen 1986), electric fields (Preston 1988). It has also been proposed that microfibrillar organization would occur spontaneously by self-assembly or by the internal geometry of the cell wall (Roland *et al.* 1987). Particular wall textures have also been thought to arise from number and density of cellulose synthesizing complexes in the plasma membrane (Emons 1985) in combination with cell dimension and matrix substances (Emons 1986).

It should be pointed out that co-orientation itself it not to be taken as pre-requisite or evidence for a causal relationship between the orientation of nascent microfibrils and the cytoskeleton, nor can such a relationship be rejected based on the absence of co-orientation. If different orienting mechanisms exist, they may act as vectors, the vector-sum determining the orientation of the microfibrils.

Cytoskeleton and cell expansion

In expanding cells a central vacuole develops, leaving only a small layer of cytoplasm at the cell's periphery.

In stretching cells, microtubules generally appear to be oriented transverse to the direction of cell expansion (Gunning 1981; Gunning & Hardham 1982). In many cells, the microtubular skeleton shows helix-like configurations with different pitches (Lloyd 1983, 1984; Traas *et al.* 1985; Traas 1989; see also Fig. 1).

Traas *et al.* (1984) supposed that microtubules in expanding root cortex cells of *Raphanus* would be oriented transverse to the vector-sum of both axial and circumferential cell expansion, more or less as in stretching cells. The orientation of microtubules in the tip of tip-growing cells would be determined in a similar way (Traas *et al.* 1985; Emons 1989). The presence of randomly organized microtubules at non-expanding surfaces, of meristematic and cortex cells in these roots, may support this assumption (Derksen *et al.* 1986a).

It remains open whether these helical organizations result from cell expansion, or whether cell expansion is determined by these microtubules. The latter could occur indirectly, by the control of cellulose deposition, or directly by resisting turgor pressure. This might occur either by the rigidity of the microtubular skeleton or by active force generation involving microtubule-based motors.

Lloyd and coworkers assumed that the helical configurations would behave more or less like a spring, which becomes stretched out during cell expansion (Lloyd 1984; Roberts *et al.* 1985). Such a behaviour cannot be reconciled with the occurrence of helical patterns as observed in *Raphanus* and *Pisum* root cells (Traas *et al.* 1984; Hogetsu & Oshima 1986) and in *Avena* coleoptiles and mesocotyls, and *Pisum* epicotyls (Iwata & Hogetsu 1988). In these studies the changes from transverse to oblique or longitudinal occur mainly after elongation has ceased.

Both assumptions relate the microtubule orientation to cell expansion but fail to point out the exact mechanisms.

The first assumption requires a rather dynamic behaviour of microtubules, whereas the second one demands a more static, passive one, yet needs considerable reorganization of the microtubules to compensate for the loss in diameter of the helix during stretching.

In tissue explants of *Nicotiana*, the orientation of microtubules in the cells changes from transverse to longitudinal to the long cell axis immediately after explantation. This change occurs gradually without appreciable cell extension and has been related to dedifferentiation and a change in cell polarity. However, ethylene production by wounding might also play a role (Wilms & Derksen 1988). To explain the mechanism involved, Wilms & Derksen took into account the dynamic properties of the microtubules. They supposed that the change in orientation resulted from polarity determining factors in the cytoplasm, i.e. at the plasma membrane, during a dynamic phase of the microtubules after explantation.

The behaviour of the cortical actin filaments during cell elongation has not yet been described, but the organization of the endoplasmic bundles remain essentially the same (Derksen *et al.* 1985).

Differentiation

Cell differentiation is the event leading to cells with quantitatively or qualitatively different functions. In plants, differentiation is often accompanied by a local wall deposition. Here we will discuss a few examples, namely vascular elements, stomatal cells and statocytes.

The densities of microtubules increase gradually just before the initiation of wall thickening along the lateral walls of young sieve elements in root protophloem of wheat

(Eleftheriou 1987). Xylogenesis is one of the best studied cases with respect to the cytoskeletal organization during cell differentiation. In xylem cells, wall material is deposited locally in close relation with cytoskeletal elements (Goosen-de Roo 1973, Herth 1985). The formation of bands of microtubules before local wall deposition starts has also been reported for *Zinnia elegans* cells in culture, which differentiate into xylem elements (Falconer & Seagull 1988; Kobayashi *et al.* 1988). These cells have been extensively studied by Falconer & Seagull and Fukuda & Kobayashi, who recently reviewed this particular system (Fukuda & Kobayashi 1989).

The specific bands of microtubules at the sites of local wall deposition are reached via a characteristic sequence of actin and microtubule patterns. The initially more or less axial patterns of actin filaments disappear and large dots of actin are seen regularly distributed over the surface. Meanwhile the axial microtubules change their orientation to oblique, forming a network with the actin dots in the darns. This pattern changes gradually until transverse and oblique orientations are predominant. At the same time the microtubules form bundles. The actin dots are present exclusively between the microtubule bands and are finally located under the sites of cell wall deposition. Colchicine destroys the regular microtubule pattern and cell wall deposition (see also Herth 1985). Cytochalasin disrupts the regular pattern of actin filaments and also affects the microtubule pattern, like in pollen tubes (Derksen & Traas 1984). Microtubules and microfilaments appear to act in a co-ordinated way. In *Zinnia*, calmodulin is found between the regions of microtubule bundles and wall deposition (Dauwalder *et al.* 1986), which may indicate a role in the changes of the specific microtubule and actin filament patterns.

During stomatal development in grasses, asymmetric divisions of the guard mother cells take place, forming the guard cells and the subsidiary cells. Their function depends at least partly on the local deposition of wall material which finally results in the kidney-like shape of the guard cells. The organization of microtubules reflects the orientation of cellulose microfibrils in periclinal walls. In these cells a clear correlation between microtubules and cellulose microfibril orientation appears to be firmly established. Like in vascular elements, microtubule density increases prior to the deposition of the secondary cell wall (reviews: Palevitz 1982; Kristen 1986).

The initial, asymmetric, radial division of the mother cell is preceded by the asymmetric, radial organization of cortical microtubules and preprophase bands in the plane of the new cell wall. The radial arrays in both the pairs of guard cells and the pairs of subsidiary cells from mirror images (Cho & Wick 1989; Cleary & Hardham 1989; Mullinax & Palevitz 1989; Palevitz & Mullinax 1989, and references in these papers). During further development, the organization of the microtubules changes from radial to transverse in the subsidiary cells and from radial, over transverse and oblique, to axial in guard cells. In *Avena*, in the last stage of differentiation, both guard cells and subsidiary cells show axial microtubule patterns (Palevitz & Mullinax 1989). Thus, here too, a clear sequence in microtubule patterns occurs in a co-ordinated way in the different cells.

Statocytes of *Lepidium* show a distinct cytoplasmic organization. The nucleus is situated in the top of the cell, whereas in the distal part of the cell, amyloplasts and rough endoplasmic reticulum are present that are involved in graviperception (Volkmann & Sievers 1979). This typical organization is entirely actin dependent, as has been shown by Hensel (1985, 1987). Microtubules appear to be less involved but may yet contribute in stabilizing the distal endoplasmic reticulum (Hensel 1984). Thus, endoplasmic reticulum organization seems to depend on actin filaments and not on microtubules. Also the position of the nucleus seems to depend on actin filaments here, whereas in other systems it

depends mainly on microtubules, indicating the versatile, and perhaps interchangeable role of the various cytoskeletal elements. As a continuous transport of endoplasmic reticulum to the distal part seems to occur in statocytes (Hensel 1985), graviperception might also depend on a distortion of this transport. The polarity of the cell itself, however, is thought to depend on the cortical cytoskeleton, i.e. microtubules (Hensel 1985; 1987). Thus, cell polarity must be separated from graviperception, each involving a different part of the cytoskeleton.

THE CYTOSKELETON IN PLANT MORPHOGENESIS

In tobacco explants, cells change their polarity several times after explantation. Initially, the orientation of microtubules shifts from transverse to parallel to the longitudinal axis of the cell. In cells that do not divide anymore, the orientation of the cortical microtubules remains unchanged, but in three regions where cell divisions occur, the orientation of the cortical microtubules will become parallel to the different, future division planes. One of these regions shows meristematic properties with microtubules that are almost randomly distributed and with apparently randomly oriented divisions. Meristematic centres with cells having different microtubule orientations or even randomly organized microtubules that also do not show a preferential orientation of the division planes have also been observed in *Vinca* shoot apices (Sakaguchi *et al.* 1988; Lang Selker 1989) and *Hedera* tunica cells (Marc & Hacket 1989). These orientations become parallel to the division planes in regions with preferential division planes. However, in *Allium* guard mother cells the cortical microtubules are random before the first longitudinal division occurs (Mineyuki *et al.* 1989). As shown by Marc & Hacket (1988) in *Hedera* tunica cells, the microtubule pattern in the meristem may become aligned intercellularly. The microtubular pattern in the tunica cells may indicate the outline of the leaf primordia that will develop (Lang Selker 1989; Marc & Hacket 1989). The clear co-ordination between microtubule patterns between different cells may be, at least partly, hormone dependent, as gibberellic acid seems to promote the process of microtubule pattern formation in *Hedera* (Marc & Hacket 1989).

CONCLUDING REMARKS

The localization studies we have reviewed at present clearly show the involvement of the cytoskeleton in plant morphogenesis and cell differentiation. The cytoskeleton appears to be involved even in such a specific sensory function as graviperception.

The particular changes in organization may be used as an indicator for early cell differentiation. However, they cannot explain the actual mechanisms involved. Changes in cytoskeletal organization in different cells may occur in a highly co-ordinated way. Hormones probably partake in the co-ordination within tissues and organs, but other factors generally thought to affect plant morphogenesis may also be involved. Whether cell contacts are needed remains open, but at least cell differentiation of tracheids appears to be independent from direct cell contacts. The various types of cytoskeletal organization and the specific sequences that occur prove the versatile character of the different cytoskeletal elements. The actual composition of the various elements, their dynamics and polarity *in vivo* and their relation with other cellular structures and their mutual interactions probably are complex and still largely unknown. The interactions of the cytoskeleton with other structures are probably transient and therefore will be difficult to

determine from static images alone, the use of newly developed light microscopic techniques also appears to be very promising in plant cells.

However, the basis for a further understanding of the organization and function of the cytoskeleton is set, as a start has been made in the identification, detection and localization of intermediate filaments and the cytoskeleton-associated proteins. The latter may be of particular interest also for the still pending discussion on microtubular control of cellulose microfibril deposition, a control that must occur via an interaction with the plasmamembrane and that limits lateral diffusion of the cellulose synthesizing complexes.

As several groups are studying differential gene expression of cytoskeletal proteins and genetic studies to the function of the cytoskeleton are also en route, the near future promises to be exciting for those botanists interested in the cues of plant morphogenesis and plant cell differentiation.

ACKNOWLEDGEMENT

The authors are indebted to Professor M.M.A. Sassen for his critical support during the preparation of the manuscript.

REFERENCES

Akashi, T. & Shibaoka, H. (1987): Effects of giberellin on the arrangement and cold stability of cortical microtubules in epidermis cells of pea internodes. *Plant Cell Physiol.* **28:** 329–348.

Bajer, A.S. & Molè-Bajer, J. (1986a): Drugs with colchicine like effects that specifically disassemble plant but not animal microtubules. In: Soiter, D. (ed.): *Dynamic aspects of microtubule biology.* Ann. New York. Acad. Sci. **466:** 767–784.

— & — (1986b): Reorganization of microtubules in endosperm cells and cell fragments of the higher plant *Haemanthus in vivo. J. Cell Biol.* **102:** 263–281.

Bednara, J., Willemse, M.T.M. & Lammeren, V.A.A.M. (1990): Organization of the actin skeleton during megasporogenesis in *Gasteria verrucosa* (Mill.) H. Duval visualized with rhodamine-phalloidin. *Acta Bot. Neerl.* **39:** 43–48.

Bennett, & Davis, V. (1981): Erythrocyte ankyrin: immunoreactive analogues are associated with mitotic structures in cultured cells and with microtubules in brain. *Proc. Natl. Acad. Sci. USA* **78:** 7550–7554.

Bergfield, R., Speth, V. & Schopfer, P. (1988): Reorientation of microfibrils and microtubules at the outer epidermal wall of maize coleoptiles during auxin-mediated growth. *Bot. Acta* **101:** 57–67.

Bershadsky, A.D. & Vasiliev, J.M. (1988): Cytoskeleton. In: Siekievitz, P.H. (ed.): *Series on Cellular Organelles.* Plenum Press, New York, London.

Burgess, J. (1970): Interactions between microtubules and the nuclear envelope during mitosis in a ferm. *Protoplasma* **71:** 77–89.

Cavalier-Smith, T. (1978): The evolutionary origin and phylogeny of microtubules, mitoytic spindles and eukaryote flagella. *Biosystems* **10:** 93–114.

Chen, L.B. & Lee, C. (1988): Dynamic behaviour of endoplasmic reticulum in living cells. *Cell* **54:** 37–47.

Cho, S-O & Wick, S.M. (1989): Microtubule orientation during stomatal differentiation in grasses. *J. Cell Sci.* **92:** 581–594.

Clayton, L. & Lloyd, C.W. (1984): The relationship between the division plane and spindle geometry in *Allium* cells treated with CIPC and griseofulvin: an anti-tubulin study. *Eur. J. Cell Biol.* **34:** 248–253.

Cleary, A.L. & Hardham, A.R. (1989): Microtubule organization during development of stomatal complexes in *Lolium rigidum: Protoplasma* **149:** 67–81.

Cleveland, D.W., Lopata, M.A., MacDonald, R.J., Cowan, N.J., Rutter, W.J. & Kirschner, M.W. (1980): Number and evolutionary conservation of α- and β-tubulin using specific cloned cDNA probes. *Cell* **20:** 95–105.

Condeelis, J.S. (1974): The identification of F-actin in the pollen tube and protoplast of *Amarryllis belladonna. Expt. Cell Res.* **88:** 435–439.

Craig, S. & Staehelin, L.A. (1988): High pressure freezing of intact plant tissues. Evaluation and characterization of novel features of the endoplasmic reticulum and associated membrane systems. *Eur. J. Cell Biol.* **46:** 80–93.

Cronshaw, J., Gilder, J. & Stone, D. (1973): Fine structural studies in *Curcurbita, Cucumis* and *Nicotiana. J. Ultrastruct. Res.* **45:** 192–205.

Cyr, R.J. & Palevitz, B.A. (1989): Microtubule binding proteins from carrot. *Planta* **177:** 245–260.

Dauwalder, M.S., Roux, S.J. & Hardison, L. (1986): Distribution of calmodulin in pea seedlings: Immunocytochemical localization in plumules and root apices. *Planta* **168:** 461–470.

Dawson, P.J., Hulme, J.S. & Lloyd, C.W. (1985): Monoclonal antibody to intermediate filament antigen cross-reacts with higher plant cells. *J. Cell Biol.* **100:** 1793–1798.

Derksen, J., Pierson, E.S. & Traas, J.A. (1985): Microtubules in vegetative and generative cells of pollen tubes. *Eur. J. Cell Biol.* **38:** 142–148.

— (1986): Cytoskeletal control of cellulose microfibril deposition. In: Vian, B., Reiss, D. and Goldberg, R. (eds.): *Cell Walls 1986*. Proceedings of the 4th Cell Wall Meeting, Paris 1986. Groupe Paris, France.

— & Traas, J.A. (1984): Growth of tobacco pollen in vitro: effects of drugs interfering with the cytoskeleton. In: Willemse, M.T.M. & Went, J.L. v. (eds): *Proc. 8th Int. Symp. Sex. Reproduction in Seed Plants, Ferns and Mosses*. 64–67. Pudoc, Wageningen.

—, Jeucken, G., Traas, J.A. & van Lammeren, A.A.M. (1986a): The microtubular skeleton in differentiating root tips of *Raphanus sativus* L. *Acta Bot. Neerl.* **35:** 223–231.

—, Traas, J.A. & Oostendorp, T. (1986b): Distribution of actin filaments in differentiating cells of *Equisetum hyemale* root tips. *Plant Sci.* **43:** 77–81.

Doonan, J.H., Cove, D.J. & Lloyd, C.W. (1985): Immunofluorescence microscopy of microtubules in intact cell lineages of the moss *Physcomitrella patens*. Normal and CIPC-treated tip cells. *J. Cell Sci.* **75:** 131–148.

Dustin, P. (1984): *Microtubules*. Springer, Berlin.

Eleftheriou, E.P. (1987): Microtubules and cell wall development in differentiating protophloem sieve elements of *Triticum aestivum* L. *J. Cell Sci.* **87:** 595–607.

Emons, A.M.C. (1982): Microtubules do not control microfibril orientation in helicoidal wall. *Protoplasma* **113:** 85–87.

— (1985): Plasma-membrane rosettes in root hairs of *Equisetum hyemale*. *Planta* **163:** 350–359.

— (1986): A mathematical model for helicoidal cell wall deposition in cells with tip growth. In: Vian, B., Reiss, D. and Goldberg, R. (eds.): *Cell Walls 1986*. Proceedings of the 4th Cell Wall Meeting, Paris 1986. Groupe Paris, France.

— (1989): Helicoidal microfibril deposition in a tip-growing cell and microtubule alignment during tip morphogenesis: a dry-cleaving and freeze substitution study. *Can. J. Bot.* **67:** 2401–2408.

— & Derksen, J. (1986): Microfibrils, microtubules and microfilaments in the trichoblast of *Equisetum hyemale*. *Acta Bot. Neerl.* **35:** 311–320.

— & Traas, J.A. (1986): Coated pits and coated vesicles on the plasma membrane of plant cells. *Eur. J. Cell Biol.* **41:** 57–64.

— & Wolters-Arts, A.M.C. (1983): Cortical microtubules and microfibril deposition in the cell wall of root hairs of *Equisetum hyemale*. *Protoplasma* **117:** 68–81.

—, Wolters-Arts, A.M.C., Traas, J.A. & Derksen, J. (1990): The effect of colchicine on microtubules and microfibrils in root hairs. *Acta Bot. Neerl.* **39:** 19–27.

Falconer, M.M. & Seagull, R.W. (1988): Xylogenesis in tissue culture III: Continuing deposition during tracheary element development. *Protoplasma* **144:** 10–16.

Fosket, D.E. (1989): Cytoskeletal proteins and their genes in higher plants. In: A. Marcus (ed.): *The Biochemistry of Plants, Vol. 15: Molecular Biology*. 393–461. Academic Press, London.

Fragata, M. (1970): The mitotic apparatus: a possible site of action of giberellic acid. *Naturwissenschaften* **57:** 139–147.

Fukuda, H. & Kobayashi, H. (1989): Dynamic organization of the cytoskeleton during tracheary-element differentiation. *Develop. Growth Diff.* **31:** 9–16.

Galway, M.E. & Hardham, A.R. (1986): Microtubule reorganization, cell wall synthesis and establishment of the axis of elongation in regeneration protoplasts of the alga *Mougeotia*. *Protoplasma* **135:** 130–143.

Goodbody, K.C., Hargreaves, A.J. & Lloyd, C.W. (1989): On the distribution of microtubule-associated intermediate filaments antigens in plant suspension cells. *J. Cell Sci.* **93:** 427–438.

Goosen-de Roo, L. (1973): The relationship between cell organelles and cell wall thickenings in primary tracheary elements of the cucumber. I. Morphological aspects. *Acta Bot. Neerl.* **22:** 279–300.

Griffith, L.M. & Pollard, T.D. (1982): The interaction of actin filaments with microtubules and microtubule associated proteins. *J. Biol. Chem.* **257:** 9143–9151.

Grolig, F., Williamson, R.E., Parke, J., Miller, C.H. & Anderton, B.H. (1988): Myosin and Ca^{2+}-sensitive streaming in the alga *Chara*. Detection of two polypeptides reacting with a monoclonal anti-myosin and their localization in the streaming endoplasm. *Eur. J. Cell Biol.* **47:** 22–31.

Gunning, B.E.S. (1981): Microtubules and cytomorphogenesis in a developing organ: The root primordium of *Azolla pinata*. In: Kiermayer, O. (ed.): *Cytomorphogenesis in Plants*. Springer Verlag, New York.

—, Hardham, A.R. & Hughes, J.E. (1978): Evidences for initiation of microtubules in descrete regions of the cell cortex in *Azolla* root-tip cells, and an

hypothesis on the development of cortical arrays of microtubules. *Planta* **143:** 161–179.

— & Hardham, A.R. (1982): Microtubules. *Ann. Rev. Plant Physiol.* **33:** 651–698.

— & Wick, S.M. (1985): Preprophase bands, phragmoplasts and spatial control of cytokinesis. *J. Cell Sci.* Suppl. **2:** 157–179.

Hahne, G. & Hoffmann, F. (1985): Cortical microtubular lattices: absent from mature mesophyll and necessary for cell division? *Planta* **166:** 309–311.

Hargreaves, A.J., Dawson, P.J., Butcher, G.W., Larkins, A., Goodbody, K.C. & Lloyd, C.W. (1989): A monoclonal antibody raised against cytoplasmic fibrillar bundles from carrot cells, and its cross-reaction with animal intermediate filaments. *J. Cell Sci.* **92:** 371–378.

Hawes, C.R. (1985): Conventional and high voltage microscopy of the cytoskeleton and cytoplasmic matrix of carrot (*Daucus carota* L.) cells grown in suspension culture. *Eur. J. Cell Biol.* **38:** 201–210.

— & Horne, J.C. (1985): Polyethylene glycol embedding of plant tissues for transmission electron microscopy. *J. Micro.* **137:** 35–45.

Hayano, S., Itoh, T. & Brown, R.M. Jr (1988): Orientation of microtubules during regeneration of cell wall in selected giant marine algae. *Plant Cell Physiol.* **29:** 785–793.

Heath, I.B. & Seagull, R.W. (1982): Oriented cellulose fibrils and the cytoskeleton: a critical comparison of models. In: Lloyd, C.W. (ed.): *The Cytoskeleton in Plant Growth and Development.* Academic Press, London.

Hensel, W. (1984): A role of microtubules in the polarity of statocytes from roots of *Lepidium sativum* L. *Planta* **162:** 404–414.

— (1985): Cytochalasin B affects the structural polarity of statocytes from cress roots (*Lepidium sativum* L.). *Protoplasma* **129:** 178–187.

— (1987): Cytodifferentiation of polar plant cells: formation and turnover of endoplasmic reticulum in root statocytes. *Exp. Cell Res.* **172:** 377–384.

Hepler, P.K. (1985): The plant cytoskeleton. In: Robards, A.W. (ed.): *Botanical Microscopy 1985.* 233–262. Oxford University Press, Oxford.

Hereward, F.V. & Northcote, D.H. (1973): Fracture planes of the plasmalemma of some higher plants revealed by freeze-etch. *J. Cell Sci.* **13:** 621–635.

Herth, W. (1985): Plant cell wall formation. In: Robards, A.W. ed.: *Botanical Microscopy 1985.* 285–310. Oxford University Press, Oxford.

Hogetsu, T. & Oshima, Y. (1986): Immunofluorescence microscopy of microtubule arrangement in root cells of *Pisum sativum* L. var. Alaska. *Plant Cell Physiol.* **27:** 939–945.

Howard, R.J. & Aist, J.R. (1979): Hyphal tip cell ultrastructure of the fungus *Fusarium*: improved preservation by freeze-substitution. *J. Ultrastruct. Res.* **66:** 224–234.

Imaseki, H. (1985): Ethylene. In: Takahashi, N. (ed.): *Chemistry of Plant Hormones,* 249–264. CRC Press Inc. Boca Raton, Florida.

Iwata, K. & Hogetsu, T. (1988): Arrangement of cortical microtubules in *Avena* coleoptiles and mesocotyls and *Pisum* epicotyls. *Plant Cell Physiol.* **29:** 807–805.

Jackson, W.T. (1982): Actomyosin. In: Lloyd, C.W. (ed.): *The Cytoskeleton in Plant Growth and Development.* 3–29. Academic Press, London.

Kobayashi, H., Fukuda, H. & Shibaoka, H. (1988): Interrelation between the spatial disposition of actin filaments and microtubules during the differentiation of tracheary elements in cultured *Zinnia* cells. *Protoplasma* **143:** 29–37.

Kristen, U. (1986): General and molecular cytology: the cytoskeleton: microtubules. *Prog. Bot.* **48:** 1–22.

— (1987): General and molecular cytology: The cytoskeleton: Microfilaments. *Prog. Bot.* **49:** 1–12.

Lackie, J.M. (1986): *Cell Movement and Cell Behaviour.* Allen & Unwin. London.

Lammeren, van, A.A.M., Bednara, J. & Willemse, M.T.M. (1989): Organization of the actin skeleton during pollen development in *Gasteria verrucosa* (Mill.) H. Duval visualized with rhodamine-phalloidin. *Planta* **178:** 531–539.

Lancelle, S.A., Callaham, D.A. & Hepler, P.K. (1986): A method for rapid freeze fixation of plant cells. *Protoplasma* **31:** 153–165.

—, Cresti, M. & Hepler, P.K. (1987): Ultrastructure of the cytoskeleton in freeze-substituted pollen tubes of *Nicotiana alata. Protoplasma* **140:** 141–150.

— & Hepler, P.K. (1989): Immuno-gold labelling of actin on sections of freeze-substituted plant cells. *Protoplasma* **150:** 72–74.

Lang, J.M., Eisinger, W.R. & Green, P.B. (1982): Effects of ethylene on the orientation of microtubules and cellulose microfibrils of pea epicotyl cells with polylamellate cell walls. *Protoplasma* **110:** 5–14.

Lang Selker, J.M. (1989): Microtubule patterns in apical epidermis cells of *Vinca minor.* Submitted.

Larsson, R., Goldenring, J., Vallano, M. & Delorenzo, R. (1985): Identification of endogenous calmodulin dependent kinase and calmodulin binding proteins in cold stable microtubule preparations from rat brain. *J. Neurochem.* **44:** 1566–1574.

Lichtscheidl, I.K. & Weiss, D.G. (1988): Visualization of submicroscopic structures in the cytoplasm of *Allium cepa* inner epidermal cells by video-enhanced contrast light microscopy. *Eur. J. Cell Biol.* **46:** 376–382.

Lim, S.S., Hering, G.E. & Borisy, G.G. (1986): Widespread occurrence of anti-troponin T cross reactive

components in non-muscle cells. *J. Cell Sci.* **85:** 1–19.

Lloyd, C.W. (ed.) (1982): *The Cytoskeleton in Plant Growth and Development.* Academic Press, London.

— (1983): Helical microtubular arrays in onion root hairs. *Nature* **305:** 311–313.

— (1984): Towards a dynamic helical model for the influence of microtubules on wall patterns in plants. *Int. Rev. Cytol.* **86:** 1–35.

— (1988): Actin in plants. *J. Cell. Sci.* **90:** 185–188.

—, Slabas, A., Powell, A., MacDonald, G. & Bradley, R. (1979): Cytoplasmic microtubules of higher plant cells visualized with antitubulin. *Nature* **279:** 239–241.

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

Marc, J., Gunning, B.E.S., Hardham, A.R., Perkin, J.L. & Wick, S.M. (1988): Monoclonal antibodies to surface and cytoskeletal components of the spermatozoid of *Pteridium aquilinum. Protoplasma* **142:** 5–14.

— & — (1988): Monoclonal antibodies to a fern spermatozoid detect novel components of the mitotic and cytokinetic apparatus in higher plant cells. *Protoplasma* **142:** 15–24.

— & 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.

Margolis, R.L. & Wilson, L. (1978): Opposite end assembly and disassembly of microtubules at steady state *in vitro. Cell* **13:** 1–8.

McCurdy, D.W., Sammut, M. & Gunning, B.E.S. (1988): Immunofluorescent visualization of arrays of transverse cortical actin microfilaments in wheat root-tip cells. *Protoplasma* **147:** 204–206.

Menzel, D. & Shliwa, M. (1986): Motility in the siphonous green alga *Bryopsis.* II. Chloroplast movement requires organized arrays of both microtubules and actin filaments. *Eur. J. Cell Biol.* **40:** 286–295.

Meijer, E.G.M. & Simmonds, D.H. (1988): Interphase microtubule arrays in cultured mesophyll protoplasts of higher plants. *Physiol. Plant.* **72:** 511–517.

Mineyuki, Y., Wick, S.M. & Gunning, B.E.S. (1988): Preprophase bands of microtubules and the cell cycle: kinetics and experimental uncoupling of their formation from the nuclear cycle in onion root-tip cells. *Planta* **174:** 518–526.

—, Marc, J. & Palevitz, B.A. (1989): Development of the preprophase band from random cytoplasmic microtubules in guard mother cells of *Allium cepa* L. *Planta* **178:** 291–296.

Mita, T. & Shibaoka, H. (1984): Giberellin stabilizes microtubules in onion leaf sheath cells. *Protoplasma* **119:** 100–109.

Mitchison, T. & Kirschner, M. (1984): Dynamic instability of microtubule growth. *Nature* **312:** 237–242.

Moscatelli, A., Tiezzi, A., Vignani, R., Cai, G., Bartalesi, A. & Cresti, M. (1988): Presence of kinesin in tobacco pollen tube. In: Cresti, M., Gori, P. and Pacini, E. (eds): *Sexual Reproduction in Higher Plants.* 205–209. Proc. of the Tenth Int. Symp. on the sexual reproduction in higher plants. 30 May–4 June 1988. University of Siena, Italy. Springer Verlag, Berlin.

Mullinax, J.B. & Palevitz, B.A. (1989): Microtubule reorganization accompanying preprophase band formation in guard mother cells of *Avena sativa* L. *Protoplasma* **149:** 89–94.

Nagle, R.B. (1988): Intermediate filaments: a review of the basic biology. *Am. J. Surg. Pathol.* **12** Suppl. 1: 4–16.

Olmsted, J.B. (1986): Microtubule-associated proteins. *Ann. Rev. Cell Biol.* **2:** 421–457.

Palevitz, B.A. (1982): The stomatal complex as a model of cytoskeletal participation in cell differentiation. In: Lloyd C.W. (ed.): *The Cytoskeleton in Plant Growth and Development.* Academic Press, London.

— & Mullinax, B. (1989): Developmental changes in the arrangement of cortical microtubules in stomatal cells of Oat (*Avena sativa* L.) *Cell Motil. Cytoskel.* **13:** 170–180.

Parke, J., Miller, C.H. & Anderton, B.H. (1986): Higher plant myosin heavy-chain identified using a monoclonal antibody. *Eur. J. Cell Biol.* **41:** 9–13.

Parthasarathy, M.V., Perdue, T.D., Witztum, A. & Alvernaz, J. (1985): Actin network as a normal component of the cytoskeleton in many vascular plant cells. *Am. J. Bot.* **72:** 1318–1323.

Paschal, B.M. & Vallee, R.B. (1987): Retrograde transport by the microtubule associated protein MAP1C. *Nature* **330:** 181–183.

Pickett-Heaps, J.D. (1974): Plant microtubules. In: Robards, A.W. (ed.): *Dynamic Aspects of Plant Ultrastructure.* 219–255. McGraw-Hill, Maidenhead.

Pierson, E.S., Derksen, J. & Traas, J.A. (1986): Organization of microfilaments and microtubules in pollen tubes grown *in vitro* or *in vivo* in various angiosperms. *Eur. J. Cell Biol.* **41:** 14–18.

—, Kengen, H.M.P. & Derksen, J. (1989): Microtubules and actin filaments co-localize in pollen tubes of *Nicotiana tabacum* L. and *Lilium longiflorum* Thumb. *Protoplasma* **150:** 75–77.

Powell, A.J., Peace, G.W., Slabas, A.R. & Lloyd, C.W. (1982): The detergent-resistant cytoskeleton of higher plant protoplasts contains nucleus-associated fibrillar bundles in addition to microtubules. *J. Cell Sci.* **56:** 319–335.

Preston, R.D. (1988): Cellulose microfibril-orientating mechanisms in plant cell walls. *Planta* **174**: 67–74.

Quader, H. & Schnepf, E. (1986): Endoplasmic reticulum and cytoplasmic streaming: fluorescence microscopical observation in adaxial epidermis cells of onion bulb scales. *Protoplasma* **131**: 250–252.

—, Deichgraber, G. & Schnepf, E. (1986): The cytoskeleton in *Cobaea* seed hair development: patterning during cell-wall differentiation. *Planta* **168**: 1–10.

—, Herth, W., Ryser, U. & Schnepf, E. (1987): Cytoskeletal elements in cotton seed hair development *in vitro*: their possible regulatory role in cell wall organization. *Protoplasma* **137**: 56–62.

—, Hoffmann, A. & Schnepf, E. (1987): Shape and movement of the endoplasmic reticulum in onion bulb epidermis cells: possible involvement of actin. *Eur. J. Cell Biol.* **44**: 17–26.

Raudaskoski, M., Astrom, K., Pertilla, K., Virtanen, I. & Louhelainen, J. (1987): Role of the microtubule cytoskeleton in pollen tubes: an immunohistochemical and ultrastructural approach. *Biol. Cell* **61**: 177–188.

Roberts, I.N., Lloyd, C.W. & Roberts, K. (1985): Ethylene-induced microtubule reorientation: mediation by helical arrays. *Planta* **164**: 439–447.

Robinson, D.G. & Quader, H. (1982): The microtubule–microfibril syndrome. In: Lloyd, C.W. (ed.): *The Cytoskeleton in Plant Growth and Development*. 109–126. Academic Press, London.

Roland, J.C., Reis, D., Vian, B., Satiat-Jeunemaitre, B. & Mosiniak, M. (1987): Morphogenesis of plant cell walls at the supramolecular level: internal geometry and versatility of helicoidal expression. *Protoplasma* **140**: 75–91.

Rutten, T. & Derksen, J. (1990): The actin skeleton in subprotoplasts of pollen tubes of *Nicotiana tabacum*. *Planta* (in press).

Sakaguchi, S., Hogetsu, T. & Hara, N. (1988): Arrangement of cortical microtubules in the shoot apex of *Vinca major* L. *Planta* **175**: 403–411.

Salmon, E.D., Leslie, R.J., Saxton, W.M., Karow, M.L. & McIntosh, J.R. (1984): Spindle microtubule dynamics in sea urchin embryo's: analysis using a fluoresceine labelled tubulin and measurement of fluorescence redistribution after laser photo bleaching. *J. Cell Biol.* **99**: 2165–2174.

Sammak, P.J. & Borisy, G.G. (1988): Direct observation of microtubule dynamics in living cells. *Nature* **332**: 724–726.

Saunders, M.J. (1986): Cytokinin activation and redistribution of plasma-membrane ion channels in *Funaria*. A vibrating-microelectrode and cytoskeleton-inhibitor study. *Planta* **167**: 402–409.

— & Hepler, P.K. (1981): Localization of membrane-associated calcium following cytokinin treatment in *Funaria* using chlorotetracycline. *Planta* **152**: 272–281.

Schnepf (1986): Cellular polarity. *Ann. Rev. Plant Physiol.* **37**: 23–47.

Schulman, H., Kuret, J., Jefferson, A.B., Nore, P.S. & Spitzer, K.H. (1985): Ca^{2+}/Calmodulin-dependent microtubule-associated protein kinase: broad substrate specificity and multi functional potential in diverse tissues. *Biochemistry* **24**: 5320–5327.

Segaar, P.J. (1990): The flagellar apparatus and temporary centriole-dissociated microtubule systems at the interphase-mitosis transition in the green alga *Gloeomonas kupfferei*: an example of the spatio-temporal flexibility of microtubule organizing centers. *Acta Bot. Neerl.* **39**: 29–42.

Staiger, C.J. & Schliwa, M. (1987): Actin localization and function in higher plants. *Protoplasma* **141**: 1–12.

Steen, D.A. & Chadwick, A.V. (1981): Ethylene effects in pea stem tissue, evidence of microtubule mediation. *Plant Physiol.* **67**: 460–466.

Steiner, P.M. & Roop, D.R. (1988): Molecular and cellular biology of intermediate filaments. *Ann. Rev. Biochem.* **57**: 593–625.

Tang, X., Hepler, P.K. & Scordilis, S.P. (1989): Immunochemical and immunocytochemical identification of a myosin heavy chain polypeptide in *Nicotiana* pollen tubes. *J. Cell Sci.* **92**: 569–574.

Theurkauf, W.E. & Vallee, R.B. (1982): Molecular characterization of the c-AMP dependent protein kinase bound to microtubule-associated protein 2. *J. Biochem. Chem.* **257**: 3284–3290.

Tiwari, S.C., Wick, S.M., Williamson, R.E. & Gunning, B.E.S. (1984): Cytoskeleton and integration of cellular function in cells of higher plants. *J. Cell Biol.* **99**: 63s–69s.

Traas, J.A. (1984): Visualization of the membrane bound cytoskeleton and coated pits of plant cells by means of dry cleaving. *Protoplasma* **119**: 212–218.

— (1989): The plasma membrane associated cytoskeleton. In: Larsson, C. and Moller, I.M. (eds.): *The Plant Plasma Membrane: Structure, Function and Molecular Biology*. Springer, Berlin.

—, Braat, P. & Derksen, J.W. (1984): Changes in microtubule arrays during the differentiation of cortical root cells of *Rahanus sativus*. *Eur. J. Cell Biol.* **34**: 229–238.

—, —, Emons, A.M.C. & Derksen, J.W.M. (1985): Microtubules in root hairs. *J. Cell Sci.* **76**: 303–320.

—, 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.

—, Burgain, S. & Dumas de Vaulx, R. (1989): The organization of the cytoskeleton during meiosis in

eggplant (*Solanum melongena* (L.)): microtubules and F-actin are both necessary for coordinated meiotic division. *J. Cell Sci.* **92:** 541–550.

— & Derksen, J. (1989): Microtubules and cellulose microfibrils in plant cells: simultaneous demonstration in dry cleave preparations. *Eur. J. Cell Biol.* **48:** 159–164.

Vahey, M., Titus, M., Trautwein, R. & Scordilis, S. (1982): Tomato actin and myosin: contractile proteins from higher land plant. *Cell Motil.* **2:** 131–147.

Vale, R.D., Rese, T.S. & Sheetz, M.P. (1985): Identification of a novel force generating protein, kinesin, involved in microtubule-based motility. *Cell* **40:** 559–569.

— & Hotani, H. (1988): Formation of membrane networks *in vitro* by kinesin-driven microtubule movement. *J. Cell Biol.* **107:** 2233–2241.

Venverloo, C.J. & Libbenga, K.R. (1987): Regulation of the plane of cell division in vacuolated cells I. The function of nuclear positioning and phragmosome formation. *J. Plant Physiol.* **131:** 267–284.

Volkmann, D. & Sievers, A. (1979): Graviperception in multicellular organs. In: Haupt, W. and Feinleib, M.E. (eds): *Encylopedia of Plant Physiology N.S. Vol. 7, Physiology of Movements.* 573–600. Springer, Berlin

Wang, Y.D. & Yan, L.P. (1988): The membrane proteins of leaf cell membrane of *Vicia faba. Kexue Tongbao (Science Bulletin)* **33:** 231–235.

Wick, S.M. & Duniec, J. (1983): Immunofluorescence microscopy of tubulin and microtubule arrays in plant cells. I. Preprophase bands develop and concomitant appearance of nuclear envelope-associated tubulin. *J. Cell Biol.* **97:** 235–243.

—, Seagull, R., Osborn, M., Weber, K. & Gunning, B. (1981): Immunofluorescence microscopy of organized microtubule arrays in structurally stabilized meristematic plant cells. *J. Cell Biol.* **89:** 685–690.

—, Muto, S. & Duniec, J. (1985): Double immunofluorescence labelling of calmodulin and tubulin in dividing plant cells. *Protoplasma* **126:** 198–206.

Wilms, F.H.A. & Derksen, J. (1988): Reorganization of cortical microtubules during cell differentiation in tobacco explants. *Protoplasma* **146:** 127–132.

—, Derksen, J. & Wolters-Arts, A.M.C. (1990): Orientation of cellulose microfibrils in cortex cells of tobacco explants. Effects of microtubule-depolymerizing drugs. Submitted.

— & Kengen, H.M.P. (1990): Simultaneous visualization of cytoskeletal elements and cellulose microfibrils in cortex cells of tobacco explants. *Acta Bot. Neerl.* **39,** 49–52.

Wulf, E., Deboben, A., Bautz, F.A., Faulstich, H. & Wieland, T. (1979): Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proc. Natl. Acad. Sci. USA* **76:** 4498–4501.

Yan, L.F., Wang, X.Z., Teng, X.Y., Ma, Y.Z. & Liu, G.Q. (1986): Actin and myosin in pollen and their role in the growth of pollen tubes. *Kexue Tongbao (Science Bulletin)* **31:** 267–272.