Cytoskeleton and Integration of Cellular Function in Cells of Higher Plants

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Constraints and opportunities inherent in a cellular existence in which the living protoplast is surrounded by a more-or-less rigid wall have given rise to a number of uniquely botanical modes of cytoskeletal form and function. These are summarized here, with special reference to the concept of a structurally and functionally integrated cytoplasmic matrix.

The cell wall possesses at least some of the shape-maintaining properties that, in animal cells, are based on the cytoskeleton. A simple demonstration of this is the inability of a plant cell protoplast to withstand the forces of surface tension that it meets when it is removed from within its cell wall: it generally rounds up to a sphere, irrespective of its original shape.

The cell wall influences cytoskeletal functions in other, less obvious ways. For instance, because shared cell walls allow only slight adjustments to the relative positions of neighboring cells, plants must regulate very precisely the site and plane in which they divide and insert new walls. The mechanism is cytoskeletal, involving the preprophase band (PPB),[1] a specialized array of microtubules (MTs). The shape of the wall itself is largely determined by its directional yielding to hydrostatic turgor pressures, and in turn this geometry is related to the initial orientation of its cellulose microfibrils. MT arrays underlying the plasma membrane appear to participate in regulating wall microfibril orientation, providing an indirect cytoskeletal control over the shape that the cell will assume when it expands. Control of both wall insertion and wall shaping thus is at least partly mediated by MTs. Cells so large that diffusion is an insufficient means of transporting solutes along intracellular and intercellular pathways commonly develop, and in such cases intracellular motility based on actomyosin becomes prominent. Although presumably a modification of the system which, in animals, changes cell shape and drives cell migration, its function in large plant cells is primarily to distribute metabolites and organelles. Nevertheless, within the confines of the wall, the shape of the protoplast

can change with dramatic speed. Finally, cell walls normally allow only small solutes to reach the plasma membrane, so that cytoskeletal functions associated with bulk endocytosis are not conspicuous in plant cells.

Despite the specifically botanical context of these activities, plant cytoskeletal components share many features with their counterparts in animal cells. The major difference appears to be a lack of intermediate filaments. There is as yet no strong evidence that this category of cytoskeletal element occurs in plants, although few studies have been directed to the question. If they indeed are absent, it could be generalized that the plant cell wall has subsumed their roles, in addition to modifying the forms and functions of the remaining components of the cytoskeleton.

Microtubules

Plant tubulin (for recent reviews, see references 15 and 31) is not exactly like animal tubulin, though it does polymerize in vitro to make MTs (33, 34). Its drug sensitivity is different (15, 35, 36) and its β-subunit resembles that of *Physarum* on one-dimensional gels (29, 30, 33, 56). An antibody that is specific for tyrosylated α-tubulin (50) gives immunofluorescence reactions with all categories of MT array in higher plant cells (Fig. 1).

The MTs of meristematic plant cells pass through sequential changes in position and functioning (for immunofluorescence visualizations, see references 51–53). The interphase and preprophase arrays in the cell cortex are specifically oriented, but the polarizing influences underlying these orientations are of essentially unknown nature and origin. The succession of arrays convincingly demonstrates the ability of the plant cell cytoskeleton to develop a high degree of order, reflecting the polarity of the cell as a whole and often the polarity of the tissue or organ in which the cell lies. Until recently the existence of the cell wall has prevented fluorescent antibody localizations from being carried out on intact tissues, but this limitation has now been overcome through the use of polyethylene glycol embedding (Wick, S. M., and J. Duniec, unpublished data). Fig. 1 shows how this method enables the organization of the various types of MT to be examined in

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[1] *Abbreviations used in this paper:* CIPC, isopropyl *N*-(3-chlorophenyl)carbamate; MT, microtubule; MTOC, microtubule-organizing center; PPB, preprophase band.
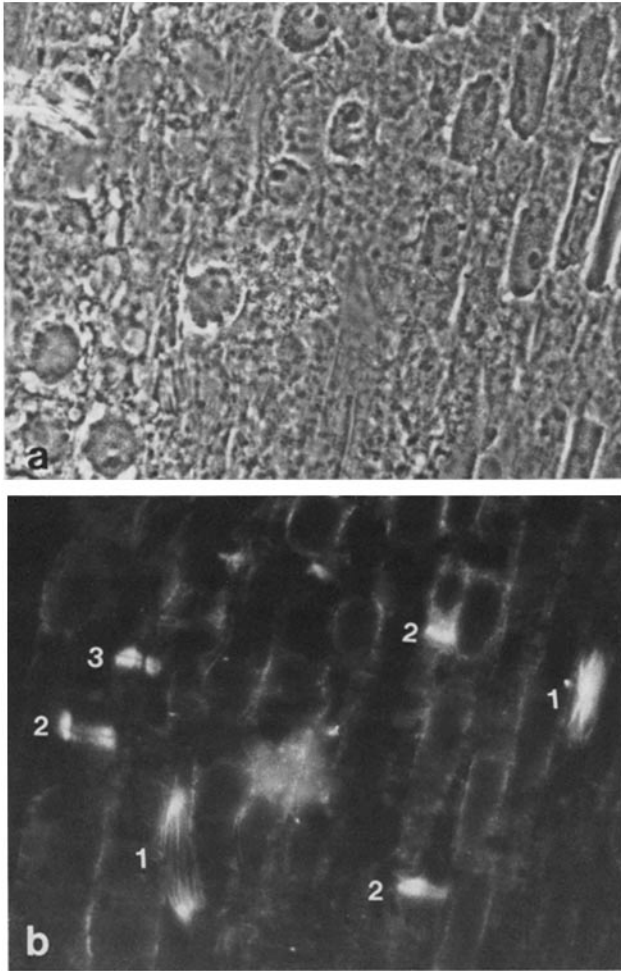
FIGURE 1   *Allium* root meristem embedded in polyethylene glycol 6000, sectioned, the medium removed, and the section processed for indirect immunofluorescence using rat monoclonal antitubulin, in phase-contrast (a) and fluorescence (b) views. Anaphase spindles (*1*); mature phragmoplasts (*2*); early phragmoplast (*3*). PPBs also react (not shown). × 540.

populations of cells that are developing in coordination in a meristem.

In general, the MTs of the interphase cortical arrays lie normal to the predominant axis of cell expansion, congruent with newly deposited cell wall microfibrils. The PPB is not necessarily oriented in the same direction as the interphase arrays. Many developmentally important categories of cell division involve precise shifts in orientation, the position of the PPB always predicting the site and plane of placement of the cell wall that is to be formed at telophase. Whatever its orientation, the PPB is physically linked to the nucleus, allowing the two to be retained as a complex when the surrounding cytoplasm is removed (Fig. 2) (51–53). The mitotic spindle appears to be the least controlled of all stages, at least in respect to its initial orientation. Its axis is usually approximately normal to that of the PPB site, but it can deviate significantly. Any initial misalignment is corrected in anaphase-telophase by a guidance mechanism that is sensitive to antiactin drugs (38) and centered on the PPB site, although the MTs of the PPB are no longer present. The phragmoplast is initiated as an independent MT system at the equatorial plane of the mitotic figure (which by then may or may not have become aligned). Its microtubule-organizing center

(MTOC) is planar and extends centrifugally toward the PPB site. The new cell wall appears as a progressively consolidating plate of membranes and contained wall material following the extending margin of the phragmoplast toward the parental walls. The eventual fusion of new and old walls approximately bisects the PPB site (for a review, see reference 14).

The phragmoplast is the only one of the successive arrays to have a distinct MTOC. The MTs that are generated there have their plus ends at the planar MTOC, and they extend in both directions normal to that plane (8). The spindle poles originate as aggregates of MT focal points at the nuclear surface (53). Manipulation of these MTOCs is possible with drugs such as isopropyl-*N*-(3-chlorophenyl)carbamate (CIPC), which apparently fragments the organizing capacity of mitotic MTOCs. Like its parent drug isopropyl *N*-phenylcarbamate (21), CIPC produces multipolar spindles (Fig. 3 *a*). Numerous nuclear fragments may be separated during anaphase, and phragmoplasts subsequently form between all adjacent ones, resulting in branched cell plates (Fig. 3 *b* and *c*). The drug aminopyrine (4-dimethylaminoantipyrine) also interferes with certain transitions in MT arrays, presumably by inhibiting the normal progression of MTOC activity: thus it causes formation of double-star configurations at late anaphase (37 and Fig. 3 *d* and *e*) and prevents radial expansion of the phragmoplast (Fig. 3*f*).

The site of origin of cortical arrays and their MTOCs is problematical. The available evidence shows a diversity of phenomena in mosses, ferns, and flowering plants.

MOSS: In vegetative cells of the moss *Sphagnum* the interphase cortical MTs appear to emerge from a diffuse zone between the telophase nuclei and the new cell wall, presumably taking up positions on selected cell faces by some form of self-assembly (44). Much more distinct MTOCs occur in meiocytes of mosses (1).

FERNS: Two studies of ferns have revealed foci consisting of electron-dense matrix material, vesicles or particles, and radiating MTs. They have been referred to as putative nucleating sites in *Azolla* root tip cells (for a review, see reference 13) and developing stomata (4) and as MTOCs in *Adiantum* stomata (10). They have a characteristic location close to, or at, the edges of the cell faces over which the MT array will form. They appear when both interphase and preprophase arrays are developing, and examination of cells that engage in successive rounds of division shows that they can reappear at the same edge in sequential cell cycles. This implies that there is a "memory" at such sites, just as the PPB site retains
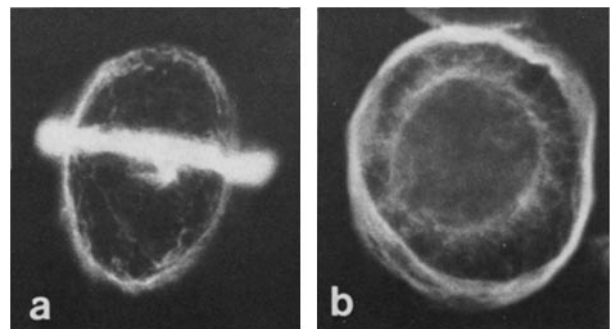

FIGURE 2   Linkages between the PPB and the nucleus before establishment of the mitotic spindle. Isolated PPB-nucleus complexes seen in side (a) and face (b) views. b shows MTs extending from PPB to nucleus. *Allium*. (a) × 1,500. (b) × 1,000.
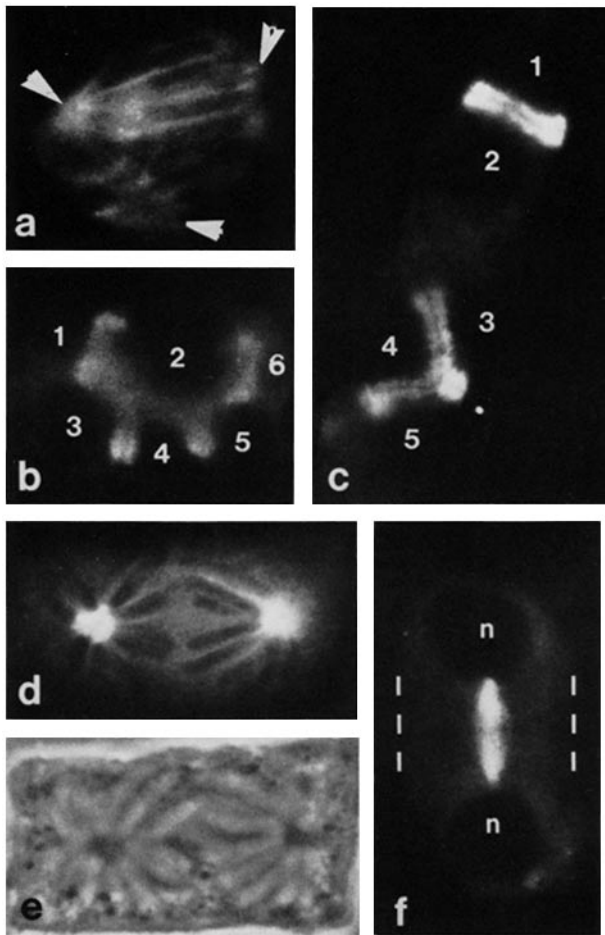
FIGURE 3   Drug treatments in *Allium* root tip cells: 1 mM CIPC induces multipolar prophase spindles (poles indicated by arrows in a); branched phragmoplasts (e.g., partitioning six nuclear fragments in b); or combinations of normal and branched phragmoplasts (e.g., separating five nuclei in c). (a) × 1,300. (b) × 1,250. (c) × 1,040. 2.3% aminopyrine induces star-shaped anaphases centered on tubulin-rich areas (d and e), and abnormally narrow phragmoplasts (f). At this stage the nuclei (n) are rounding off and the phragmoplast would normally have extended to the parental walls (bars). (d and e) × 1,300. (f) × 1,500.

a memory that guides the growth of the cell plate at cytokinesis, even though the MTs of the band disappear at prophase. In fact, because cell edges carry portions of PPB sites that are bisected at cytokinesis by fusion of the new cell plate with the parental walls (3), these two forms of memory could well be related.

FLOWERING PLANTS: The situation in flowering plants is different. Evidence for foci is scant, but they have been seen at cell edges in root tips of *Cyperus* and *Phleum* (12) and in stomata of *Zea* (9) and sugar cane (Busby, C. H., unpublished observation). Foci seen in *Phleum* stomata may be less localized (39). However, immunofluorescence studies of onion and other root tip cells have not given any evidence for foci located solely in restricted regions of the cell cortex. Rather, the reinstatement of new arrays seems to involve gradual appearance of MTs between the nucleus and all parts of the cortex and the appearance of randomly arranged MTs along cell faces during cytokinesis, followed by disappearance of the former and ordering of the latter (Wick, S., unpublished observation) (Fig. 4). A transition from random to more oriented arrays is also seen in regenerating protoplasts (48).

Reconstructions of the detailed features of cortical arrays (16, 46) indicate that MT interactions must be developmentally and/or functionally important. Certainly the distribution of foci is insufficient to account for all but the most gross aspects (e.g., foci tend to appear along edges lying normal to the predominant orientation of MTs in the developing array). MT-associated components could, however, assist in a self-ordering process, and their localization could account for observed subtleties such as one MT passing from one group to another across the intervening space.

It now seems probable that the conventional methods of preparing specimens for electron microscopy are not as good as freeze-substitution for preserving details of MT associations in plant cells. Figs. 5-8 illustrate this point, showing the general preservations of MTs and microfilaments, examples of extensive inter-MT bridging and the presence of fine fibrils lying parallel to MTs and occasionally bridged to them and to other filaments traversing the cytoplasmic matrix (Tiwari, S. C., unpublished). These features have also been seen in conventional preparations, e.g., as a herringbone pattern of inter-MT bridges (16), as other less striking instances of MT-MT and MT-membrane bridges (12, 46), and as fibrils lying parallel to MTs in a developing shoot meristem (17), guard cell protoplasts (7), root hairs (45, 46), and root tip cells (11, 19), where (as in fungal cells [23]) they have been tracked over short distances by serial sectioning (Fig. 9). The parallel fibrils seem to be markedly clearer after good freeze-substitution (Figs. 5 and 8; for *Tradescantia* stamen hair cells, Hepler, P. K., and Callaham, D., personal communication; for fungal cells, references 20 and 22-25). This association closely resembles that formed in vitro between MTs, actin, and microtubule-associated protein (MAP)-2 (41). As seen in Fig. 5, it appears in only a small proportion of the MTs, hence its possible role in determining the geometry of the arrays or as part of force-generating mechanisms that have been hypothesized in relation to MT-directed cellulose deposition (19) is conjectural. Decoration with heavy meromyosin (45) and fluorescence microscopy with rhodamine-labeled phalloin (22, 24) have shown that F-actin can lie parallel and close
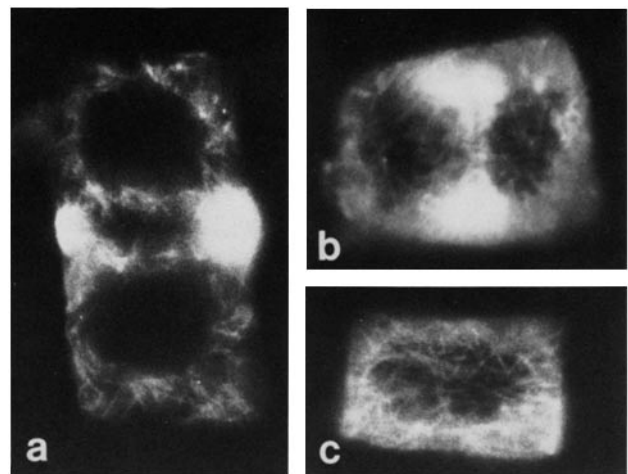

FIGURE 4   Reinstatement of interphase cortical arrays after mitosis in *Allium*. At late cytokinesis, when the phragmoplast has reached the parental walls, MTs appear between the nucleus and cell cortex (a) and lying randomly against cell faces (b). When the phragmoplast has disappeared, unorganized arrays can occasionally be found in the cortex (c), although establishment of a typically oriented array is apparently rapid. (a) × 1,560. (b) × 1,490. (c) × 1,370.
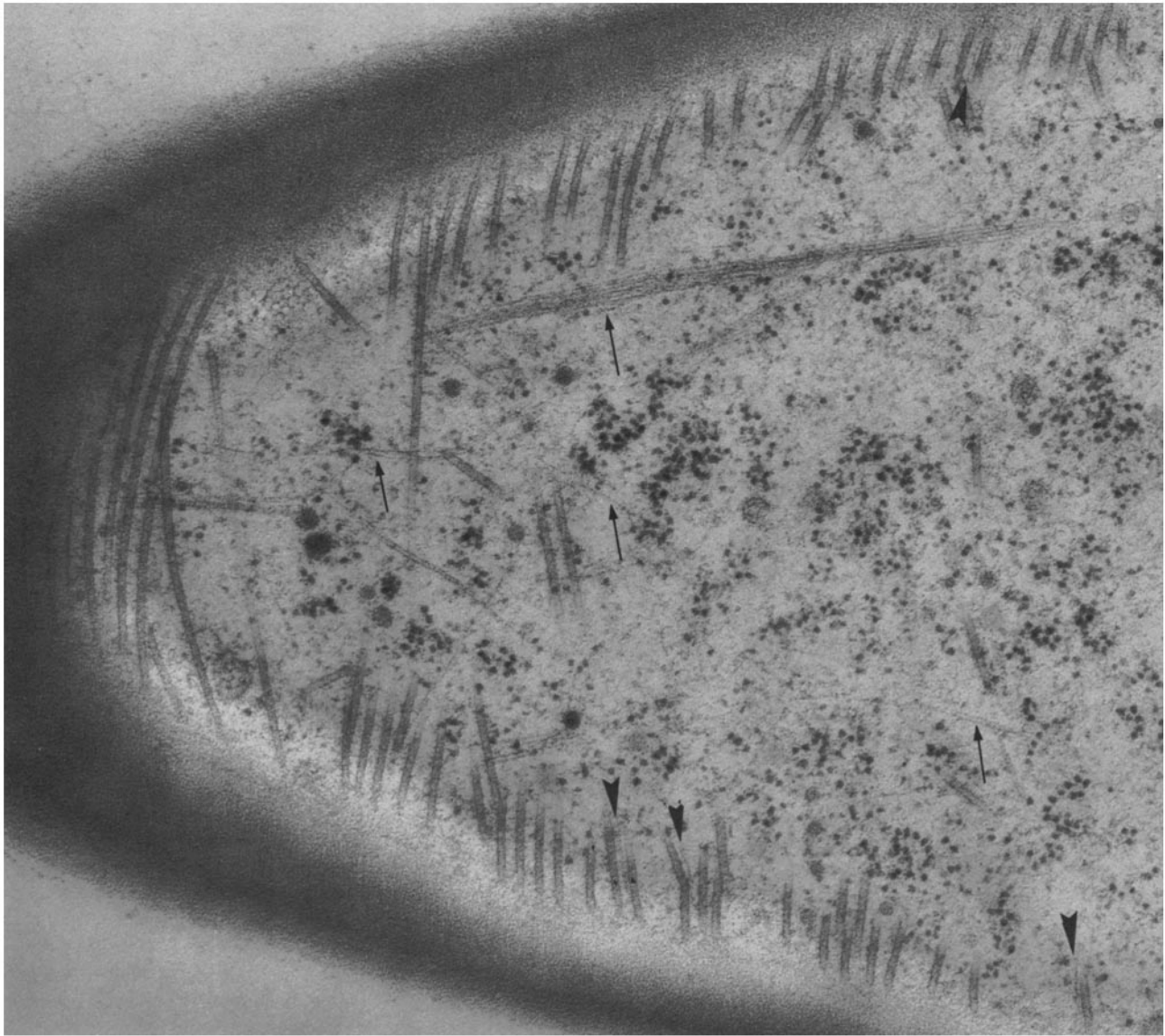
FIGURE 5 Oblique section of a young wheat leaf trichome frozen in cooled liquid propane and substituted in osmium tetroxide in acetone. Microfilaments occur singly or in bundles (arrows), and may be associated with MTs (arrowheads). × 67,000.
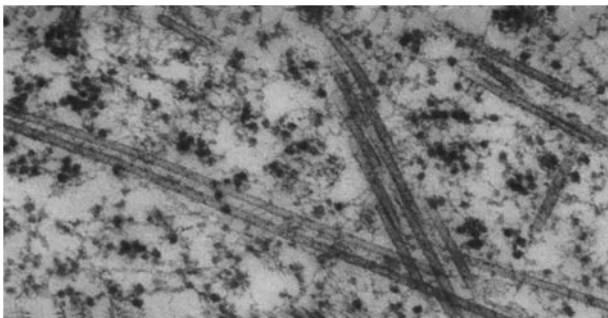

FIGURE 6 MTs in an epidermal cell of a *Datura* ovule, freeze-substituted as described in the legend to Fig. 5. The MTs are bridged to each other and apparently also to an extensive fibrillar reticulum in the ground substance. × 86,000.

to MTs in root hairs and fungal cells. MAP-2 has binding sites for both F-actin and tubulin (43). However, the only biochemical investigation of MAPs in higher plants concerns a calmodulin-like protein which coisolates with azuki bean

tubulin (32), and as yet there is no evidence for a botanical homologue of MAP-2. Immunofluorescence studies with antibodies to spinach calmodulin indicate a strong reaction with the phragmoplast region (Fig. 10), with the mitotic apparatus, and occasionally with PPB as well (Muto, S., and Wick, S. M., unpublished observation).

Microfilaments

Cytoplasmic microfilaments occur in plant cells as single filaments or as bundles (Fig. 5). Both categories have been identified as F-actin by meromyosin decoration (28, 45). Fig. 11 shows a relatively unordered array of bundles traversing vacuoles and in the peripheral cytoplasm of a stamen hair cell as visualized with NBD-phallacidin. Fig. 12 a and d shows the much more ordered system found in *Chara* as detected by immunofluorescence microscopy. As is the case with MTs, good freeze-substitution can give improved preservation of microfilaments in higher plant cells (Fig. 5) (for references on freeze-substitution of microfilaments in fungal hyphae, see references 23 and 24).

The polarity of the F-actin filaments within bundles is uniform in the most highly ordered systems (28, 56), correlating with associated unidirectional cytoplasmic streaming toward the "barbed" ends. Nonuniform polarity in small bundles, correlating with bidirectional particle movement, also occurs (45). Mechanisms of force generation at microfilaments of F-actin in plant cells are discussed in references 27, 47, 54, and 55 and will not be considered here.

Little is known about the development of microfilament arrays in plant cells or about factors that regulate the association into bundles. Bundles in the peripheral cytoplasm of *Vallisneria* leaf cells evidently share the same polarity, as judged by the direction in which the chloroplasts move; if the bundles are broken down by cytochalasin treatment and allowed to regenerate, their polarity is once again uniform, though there is a 50% chance that it will not be in the same direction as before (26). Evidently, the cells regenerate and deploy a completely new array of bundles. By contrast, if existing bundles are destroyed locally, the exposed "pointed" and "barbed" ends are available as seeds on which regrowth can occur. A study of *Chara* (Williamson, R. E., U. Hurley, and J. Perkin, unpublished) shows that this type of regrowth in vivo is strictly polarized. The "barbed" end is preferred and regrows after a lag phase, while the "pointed" end remains essentially static for many hours. This plant system therefore has the same polarity of assembly as comparable bundles from animal sources (e.g., 40), and the relative inactivity of the nonpreferred end in vivo resembles that of the same end in in vitro preparations of animal F-actin held under physiological conditions (5). The regenerating bundles in *Chara* seem to become oriented passively by the continuing flow of endoplasm past the zone where regeneration is occurring. *De novo* formation of new bundles, which are less ordered in their growth orientation, also occurs as a minor component of the *Chara* system.

The bundles of F-actin in *Chara* cells lie at the interface between the mobile endoplasm and the stable ectoplasm. It has long been evident that the bundles participate in the spatial organization of the stable region in that they lie along files of chloroplasts (Fig. 12a and b). A recent finding that may also be relevant to higher plant cells is that fibrillar components that are immunologically related to components of the actin bundles have been detected elsewhere in the stable cortical cytoplasm by raising a library of monoclonal antibodies to components of the *Chara* cortex (Williamson, R. E., J. Perkin, and U. Hurley, unpublished). For example, antibody CC2 binds to the actin bundles and, in some cells, to fibrils lying between the chloroplasts and the plasma membrane that

are uninterrupted by the neutral line and oriented predominantly transverse to the main array of bundles (Fig. 12c and d). Whether the antibodies detect proteins that are identical to those in the actin bundles or ones that merely share an epitope remains to be seen. It will be important to see whether these fibrils occur in higher plant cells, where they could be associated with the cortical MT arrays and could aid in stabilizing the surface zone where the MTs must maintain their orientation even when cytoplasmic streaming is occurring in the vicinity.

Microtrabeculae

Early work on plant cells has indicated that an ordered cytomatrix exists in some situations (reviewed by Porter, K. R. [42], in this supplement). Direct support for the concept has come recently from detection of three-dimensional networks similar to the microtrabecular lattice in sections from which the embedding medium has been removed. This morphology has been seen in a variety of cell types by removing resin from epoxy sections and shadow-casting (49). Some freeze-substituted preparations also indicate fibrillar interconnections between MTs and the ground substance (Fig. 6). The polyethylene glycol technique also has revealed extensive interconnections between membrane systems of root tip cells. The familiar fixation image was restored when the sections
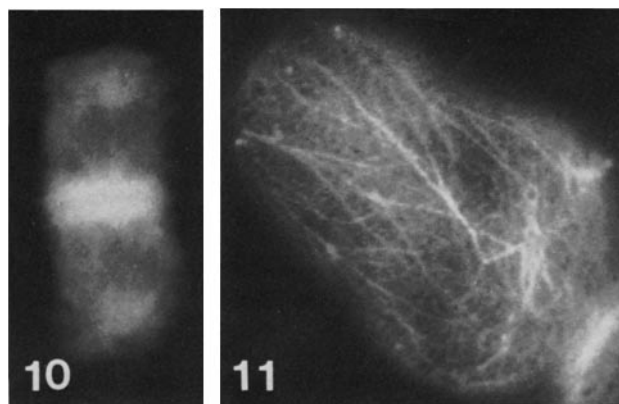

Figure 10   Indirect immunofluorescence indicates the presence of calmodulin in the phragmoplast. Its distribution is similar to that of tubulin. × 1,150.

Figure 11   *Tradescantia* stamen hair cell (mature) reacted with NBD-labeled phalloidin after fixation in paraformaldehyde showing a complex arrangement of F-actin strands ramifying through the cytoplasm. Video-enhanced fluorescence. × 770.
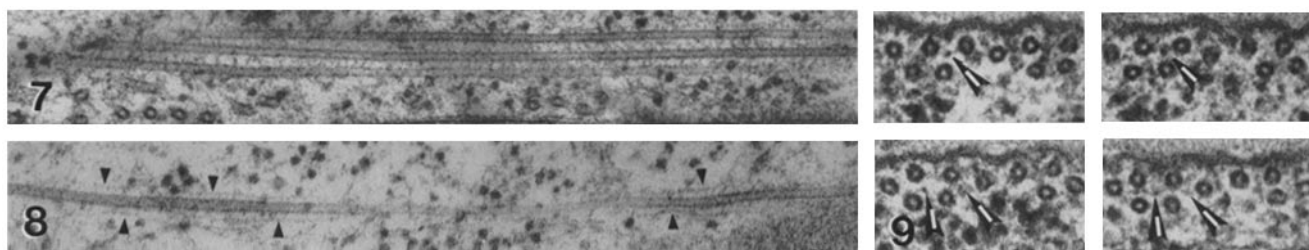

Figure 7   MTs in a *Datura* ovule cell show periodic cross-bridges linking them into a bundle. Freeze-substituted. × 72,000.

Figure 8   MT with associated parallel microfilamentous material (arrowheads) bridged to it in an *Agrostis* leaf epidermal cell. Freeze-substituted. × 77,000.

Figure 9   Four sections from a sequence in *Phleum* root tip in which MT-associated microfilaments could be tracked over short distances (e.g., arrowheads). Conventional processing. × 100,000.
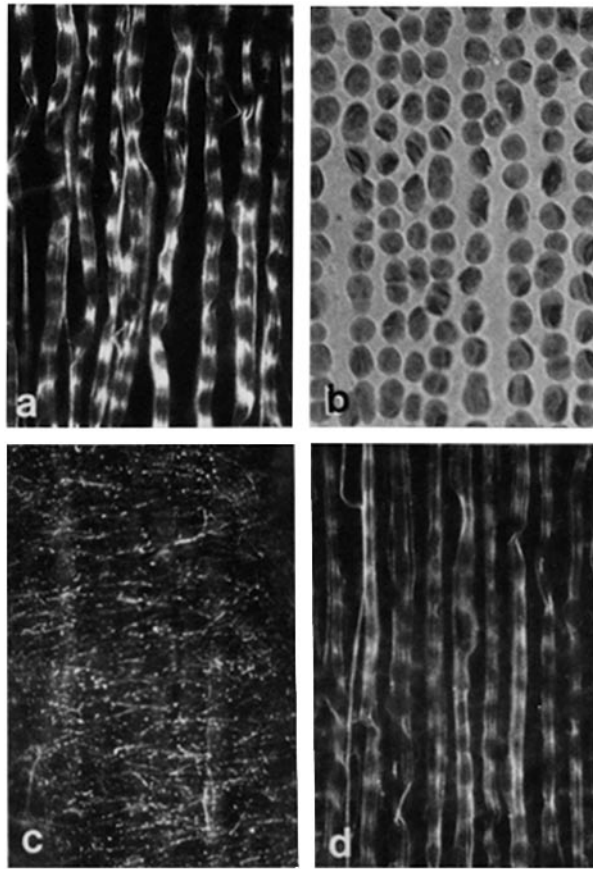
FIGURE 12 Fluorescence (a) and bright-field (b) micrographs of a *Chara* cell perfused with monoclonal antibody CC2. Groups of up to eight actin bundles occur beneath each file of chloroplasts. The overlying chloroplasts are nonfluorescent with the red-excluding barrier filter used but attenuate the bundle fluorescence relative to its intensity in the gaps between chloroplasts. Monoclonal antibody CC2 also reacts with fine granules and predominantly transverse filaments (in some but not all cells) lying between the chloroplasts and the plasma membrane (c). *d* shows the same area, but the focus is on the subcortical actin bundles. × 480.

were reembedded in epoxy resin (18). A. Scholler and J. Pickett-Heaps (personal communication) have also used polyethylene glycol embedding and high-voltage electron microscopy to visualize a microtrabecular lattice throughout the cytoplasm of cells in an alga, *Protosiphon*, and in onion root tips. Less distinct trabeculae have been observed in whole mounts of moss protonema cells (6). Combinations of immunocytology and polyethylene glycol embedding (Fig. 1) now offer prospects for seeing how MTs and microfilaments interact with microtrabeculae in the plant cell cytoskeleton.

General Consideration of Large-Scale Order in Plant Cell Cytoplasm

There are several locations in plant cells where a three-dimensionally stabilized matrix is likely to be especially important. Some have already received mention, for example the cell cortex, where MTs and associated components are not swept away by cytoplasmic streaming and are believed to enter into a functional interaction by which cellulose-synthetases lying in or on the plasma membrane receive spatial guidance.

Structural integration of PPBs and nuclei and of PPB sites

and phragmoplasts has also been mentioned, and it is now relevant to widen the context. The site at which a higher plant cell will divide is determined before mitosis, and in some cases before PPB formation, as evidenced by the appearance of a specifically placed raft of cytoplasm known as a phragmosome (14). Phragmosomes are best seen in vacuolated cells, but may also occur as differentiated but not readily distinguishable cytoplasm in less vacuolated cells. They retain their position in the cell over considerable periods. The nucleus undergoes mitosis in them, the phragmoplast and cell plate later extending within the phragmosomal cytoplasm toward the parental walls. In a number of reproductive cell types in which PPBs do not develop, the site of the future cell plate becomes marked by planar aggregations of mitochondria, lipid droplets, and, sometimes, plastids (14). Additional mechanisms exist in cells that have only one plastid, ensuring that plastid division is geared, both temporally and spatially, to nuclear division. The chloroplast moves (but only when the plane of cytokinesis is such as to require movement) so that the isthmus of its division is in the plane delimited by the PPB. Each daughter cell thus receives a daughter plastid (2). Clearly, the PPB is only one component of the cytoskeletal preparations for spatial control of cell division, which ramify from the cell surface to the nucleus and also involve other organelles. The structural basis of this three-dimensional organization and integrated cellular choreography is not understood.

Finally, nondividing plant cells are not to be viewed as collections of randomly arranged endoplasmic organelles within a more organized ectoplast. There are many examples, too numerous to review here, of specific placement of cell components in relation to (a) vectorial cell functions such as eccrine or granulocrine secretion, (b) polarized growth or other developmental events, and (c) metabolic exchanges among organelles. Together with the examples of specific deployment of cytoskeletal structures already given, these widespread phenomena demonstrate the capacity of the cytoplasmic matrix of plant cells to participate in structural ordering and functional interactions that may, indeed, be absent only in rapidly streaming endoplasm.

REFERENCES

1. Brown, R. C., and B. E. Lemmon. 1983. Microtubule organization and morphogenesis in young spores of the moss *Tetraphis pellucida* Hedw. *Protoplasma.* 116:115–124.
2. Brown, R. C., and B. E. Lemmon. 1984. Plastid apportionment and preprophase microtubule bands in monoplastidic root meristem cells of *Isoetes* and *Selaginella. Protoplasma.* In press.
3. Busby, C. H., and B. E. S. Gunning. 1980. Observations on preprophase bands of microtubules in uniseriate hairs, stomatal complexes of sugar cane, and *Cyperus* root meristems. *Eur. J. Cell Biol.* 21:214–223.
4. Busby, C. H., and B. E. S. Gunning. 1984. Microtubules and morphogenesis in stomata of the water fern *Azolla*: an unusual mode of guard cell and pore development. *Protoplasma.* In press.
5. Coluccio, L. M., and L. G. Tilney. 1983. Under physiological conditions actin disassembles slowly from the nonpreferred end of an actin filament. *J. Cell Biol.* 97:1629–1634.
6. Cox, G., and B. E. Juniper. 1983. High voltage electron microscopy of whole, critical point dried cells—fine cytoskeletal elements in the moss *Bryum tenuisetum. Protoplasma.* 115:70–80.
7. Doohan, M. E., and B. A. Palevitz. 1980. Microtubules and coated vesicles in guard cell protoplasts of *Allium cepa* L. *Planta.* 149:389–401.
8. Euteneuer, U., W. T. Jackson, and J. R. McIntosh. 1982. Polarity of spindle microtubules in *Haemanthus* endosperm. *J. Cell Biol.* 94:644–653.
9. Galatis, B. 1982. The organization of microtubules in guard cell mother cells of *Zea mays. Can. J. Bot.* 60:1148–1166.
10. Galatis, B., P. Apostolakos, and Chr. Katsaros. 1983. Microtubules and their organizing centres in differentiating guard cells of *Adiantum capillus veneris. Protoplasma.* 115:176–192.
11. Gunning, B. E. S. 1979. Nature and development of microtubule arrays in cells of higher plants. *In* Proceedings of the 37th Annual Meeting of the Electron Microscope

Society of America. G. W. Bailey, editor. Claitor's Publishing Division, Baton Rouge, 172-175.

12. Gunning, B. E. S. 1981. Microtubules and cytomorphogenesis in a developing organ: the root primordium of *Azolla pinnata*. *Cell Biol. Monogr.* 8:301-326.

13. Gunning, B. E. S. 1982. The root of the water fern *Azolla*: cellular basis of development and multiple roles for cortical microtubules. *In* Developmental Order: Its Origin and Regulation. P. B. Green and S. Subtelney, editors. A. R. Liss, Inc., New York. 379-421.

14. Gunning, B. E. S. 1982. The cytokinetic apparatus: its development and spatial regulation. *In* The Cytoskeleton in Plant Growth and Development. C. W. Lloyd, editor. Academic Press, London. 229-293.

15. Gunning, B. E. S., and A. R. Hardham. 1982. Microtubules. *Annu. Rev. Plant Physiol.* 33:651-698.

16. Hardham, A. R., and B. E. S. Gunning. 1978. Structure of cortical microtubule arrays in plant cells. *J. Cell Biol.* 77:14-34.

17. Hardham, A. R., P. B. Green, and J. M. Lang. 1980. Reorganization of cortical microtubules and cellulose deposition during leaf formation in *Graptopetalum paraguayense. Planta.* 149:181-195.

18. Hawes, C. R., B. E. Juniper, and J. C. Horne. 1983. Electron microscopy of resin-free sections of plant cells. *Protoplasma.* 115:88-93.

19. Heath, I. B., and R. W. Seagull. 1982. Oriented cellulose microfibrils and the cytoskeleton—a critical comparison of models. *In* The Cytoskeleton in Plant Growth and Development. C. W. Lloyd, editor. Academic Press, London. 163-182.

20. Heath, M. C., and I. B. Heath. 1978. Structural studies of the development of infection structures of cowpea rust, *Uromyces phaseoli* var. *Vignae* 1. Nucleoli and Nuclei. *Can. J. Bot.* 56:648-661.

21. Hepler, P. K., and W. T. Jackson. 1969. Isopropyl *N*-phenylcarbamate affects spindle microtubule orientation in dividing endosperm cells of *Haemanthus katherinae* Baker. *J Cell Sci.* 5:727-743.

22. Hoch, H. C., and R. C. Staples. 1983. Relationship of F-actin with cytoplasmic microtubules in a filamentous fungus. *J. Cell Biol.* 97(2, Pt. 2):287a. (Abstr.)

23. Hoch, H. C., and R. C. Staples. 1983. Ultrastructural organization of the non-differentiated uredospore germling of *Uromyces phaseoli* variety *typica. Mycologia.* 75:795-824.

24. Hoch, H. C., and R. C. Staples. 1983. Visualization of actin *in situ* by rhodamine-conjugated phalloin in the fungus *Uromyces phaseoli. Eur. J. Cell Biol.* 32:52-58.

25. Howard, R. J. 1981. Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. *J. Cell Sci.* 48:89-103.

26. Ishigami, M., and R. Nagai. 1980. Motile apparatus in *Vallisneria* leaf cells. II. Effects of cytochalasin B and lead acetate on the rate and direction of streaming. *Cell Struct. Funct.* 5:13-20.

27. Jackson, W. T. 1982. Actomyosin. *In* The Cytoskeleton in Plant Growth and Development. C. W. Lloyd, editor. Academic Press, London. 3-30.

28. Kersey, Y. M., P. K. Hepler, B. A. Palevitz, and N. K. Wessells. 1976. Polarity of actin filaments in Characean algae. *Proc. Natl. Acad. Sci. USA.* 73:165-167.

29. Little, M., R. F. Ludueña, G. M. Langford, C. F. Asnes, and K. Farrell. 1981. Comparison of proteolytic cleavage patterns and β-tubulins from taxonomically distant species. *J. Mol. Biol.* 149:95-107.

30. Little, M., R. F. Ludueña, R. Keenan, and C. F. Asnes. 1982. Tubulin evolution: two major types of α-tubulin. *J. Mol. Evol.* 19:80-86.

31. Lloyd, C. W., editor. 1982. The Cytoskeleton in Plant Growth and Development. Academic Press, London. 1-457.

32. Mizuno, K., and H. Shibaoka. 1982. Tubulin and tubulin-associated CDR-like protein from azuki bean epicotyls. *In* Biological Functions of Microtubules and Related Structures. H. Sakai, H. Mohri, and G. G. Borisy, editors. Academic Press, Inc., New York. 1-10.

33. Mizuno, K. 1984. In vitro assembly of microtubules from higher plant (*Phaseolus mungo*) tubulin. *Protoplasma.* In press.

34. Morejohn, L. C., and D. E. Fosket. 1982. Higher plant tubulin identified by self-assembly into microtubules in vitro. *Nature (Lond.)* 297:426-428.

35. Morejohn, L. C., T. E. Bureau, and D. E. Fosket. 1983. Oryzalin binds to plant tubulin and inhibits taxol-induced microtubule assembly in vitro. *J. Cell Biol.* 97(2, Pt. 2):211a. (Abstr.)

36. Morejohn, L. C., T. E. Bureau, L. P. Tocchi, and D. E. Fosket. 1983. Tubulins from different plant species bind colchicine differentially. *Plant Physiol. (Bethesda).* 72:30.

37. Östergren, G., A. Koopmans, and J. Reitalu. 1953. The occurrence of the amphiastral type of mitosis in higher plants and the influence of aminopyrin on mitosis. *Bot. Not.* 4:417-419.

38. Palevitz, B. A. 1980. Comparative effects of phalloidin and cytochalasin B on motility and morphogenesis in *Allium. Can. J. Bot.* 78:772-785.

39. Palevitz, B. A. 1981. Microtubules and possible microtubule nucleation centers in the cortex of stomatal cells as visualized by high voltage electron microscopy. *Protoplasma.* 107:115-125.

40. Pollard, T. D., and M. Mooseker. 1981. Direct measurement of actin polymerization rate constants by electron microscopy of actin filaments nucleated by isolated microvillus cores. *J. Cell Biol.* 88:654-659.

41. Pollard, T. D., S. C. Selden, and P. Maupin. 1984. Interaction of actin filaments with microtubules. *J. Cell Biol.* 99(1, Pt. 2):33s-37s.

42. Porter, K. R. 1984. The cytomatrix: a short history of its study. *J. Cell Biol.* 99(1, Pt. 2):3s-13s.

43. Sattilaro, R. F., W. L. Dentler, and E. Lecluyse. 1983. The effect of phosphorylation on the interaction of MAP-2 with F-actin and neurofilaments. *J. Cell Biol.* 97(2, Pt. 2):286a. (Abstr.)

44. Schnepf, E. 1984. Pre- and post-mitotic reorientation of microtubule arrays in young *Sphagnum* leaflets: transitional stages and initiation sites. *Protoplasma.* 120:100-112.

45. Seagull, R. W., and I. B. Heath. 1979. The effects of tannic acid on the in vivo preservation of microfilaments. *Eur. J. Cell Biol.* 20:184-188.

46. Seagull, R. W., and I. B. Heath. 1980. The organization of cortical microtubule arrays in the radish root hair. *Protoplasma.* 103:205-229.

47. Sheetz, M. P., and J. A. Spudich. 1983. Movement of myosin-coated fluorescent beads on actin cables *in vitro. Nature (Lond.).* 303:31-35.

48. Simmonds, D., G. Setterfield, and D. L. Brown. 1983. Microtubule organization in plasmolysed protoplasts before and during cell wall regeneration. *J. Cell Biol.* 97(2, Pt. 2):207a. (Abstr.)

49. Wardrop, A. B. 1983. Evidence for the possible presence of a microtrabecular lattice in plant cells. *Protoplasma.* 115:81-87.

50. Wehland, J., M. C. Willingham, and I. V. Sandoval. 1983. A rat monoclonal antibody reacting specifically with the tyrosylated form of alpha-tubulin. I. Biochemical characterization, effects on microtubule polymerization in vitro, and microtubule polymerization and organization in vivo. *J. Cell Biol.* 97:1467-1475.

51. Wick, S. M., R. W. Seagull, M. Osborn, K. Weber, and B. E. S. Gunning. 1981. Immunofluorescence microscopy of organized microtubule arrays in structurally stabilized meristematic plant cells. *J. Cell Biol.* 89:685-690.

52. Wick, S. M., and J. Duniec. 1983. Immunofluorescence microscopy of tubulin and microtubule arrays in plant cells. I. Preprophase band development and concomitant appearance of nuclear envelope-associated tubulin. *J. Cell Biol.* 97:235-243.

53. Wick, S. M., and J. Duniec. 1984. Immunofluorescence microscopy of tubulin and microtubule arrays in plant cells. II. Transition between the preprophase band and the mitotic spindle. *Protoplasma.* In press.

54. Williamson, R. E. 1975. Cytoplasmic streaming in *Chara*: a cell model activated by ATP and inhibited by cytochalasin B. *J. Cell Sci.* 17:655-668.

55. Williamson, R. E., and C. Ashley. 1982. Free Ca^{2+} and cytoplasmic streaming in the alga *Chara. Nature (Lond.).* 296:647-651.

56. Yadav, N. S., and P. Filner. 1983. Tubulin from cultured tobacco cells: isolation and identification based on similarities to brain tubulin. *Planta (Berl.).* 157:46-52.

57. Yamaguchi, Y., and R. Nagai. 1981. Motile apparatus in *Vallisneria* leaf cells. 1. Organization of microfilaments. *J. Cell Sci.* 48:193-205.