The diversity of cytoplasmic microtubules

Microtubules (MTs) were first identified by electron microscopy of flagella and cilia. Cytoplasmic MTs, however, were seen only after glutaraldehyde was introduced as a fixative (Sabatini et al., 1963). Most early studies of MTs were descriptive, listing where they were seen in different cells. Because all MTs looked alike, it was assumed that all were the same. Their functions were unknown, but because of their cellular locations it was assumed that MTs might be involved in cell motility or cell structure. Articles by Pickett-Heaps and Northcote (Pickett-Heaps and Northcote, 1966a; Pickett-Heaps and Northcote, 1966b), Tilney (Tilney, 1968), and Behnke and Forer (Behnke and Forer, 1967), in early volumes of *Journal of Cell Science*, were among the first to do more than just describe the presence of MTs. They started us towards our present ideas about MTs and raised questions that remain unanswered today.

Pickett-Heaps and Northcote deduced a potential function of specific preprophase MTs in wheat mitotic cells (Pickett-Heaps and Northcote, 1966a; Pickett-Heaps and Northcote, 1966b). Searching for cytoplasmic ultrastructural changes indicating that the cells were preparing to enter division, they saw nothing unusual at the ends of the cells where the spindle poles were going to form (Pickett-Heaps and Northcote, 1966a). In the plane of the future cell plate, however, they observed a ring of more than 150 MTs, three to four MTs deep, encircling the cells just under the cell wall. This 'preprophase band' of MTs disappeared as the spindle MTs began to appear and, in these symmetrically dividing cells, seemed to predict the cell plate position. To test this, Pickett-Heaps and Northcote studied the three successive asymmetric divisions of wheat stomatal complex cells, in which two of these divisions are perpendicular to the first and the positions of all future cell plates can be predicted from the positions of organelles in the adjacent cells (Pickett-Heaps and Northcote, 1966b). In every pre-prophase cell in the stomatal complex, they found a band of MTs near the cell wall where the cell plate subsequently forms. Because changed positions of the cell plates were reflected by changed positions of the preprophase bands, there could be little doubt that the pre-prophase band indicated the future cleavage plane.

The preprophase band has been studied a great deal since this first description – a Google search yields over 25,000 hits. We now know that actin filaments are present in the preprophase band (e.g. Mineyuki and Palevitz, 1990; Cleary et al., 1992), and that actin and myosin cause it to narrow after it forms initially (e.g. Eleftheriou and Palevitz, 1992; Granger and Cyr, 2001; Li et al., 2006), presumably by interacting with the MTs. Kinases also are involved (e.g. Katsuta and Shibaoka, 1992; Nogami and Mineyuki, 1999), but what causes the band to form at this position and what exactly it signifies still are not known.

Shortly after these articles on the preprophase band, Tilney (Tilney, 1968) described experiments on MTs in *Actinosphaerium* axopodia that were among the first to suggest that MTs function in both structural roles and force generation. Earlier, Tilney and Porter (Tilney and Porter, 1965) had shown that the thin, 250- to 300-μm long axopodia of *Actinosphaerium* contain an array of two intertwined 12-sided coils of evenly spaced MTs that are separated by 'flocculent' material. Each axopodial MT is straight and extends lengthwise along the axopod; some extend from end to end of the axopodial 'rod', but others end before the tip as the width narrows. Tilney (Tilney, 1968) assumed that all MTs have similar properties and thus he treated *Actinosphaerium* with colchicine, an agent known for its effects on spindle structure. Because colchicine caused depolymerization of axopodial MTs and retraction of the axopodia, and because both axopodia and MTs reformed after colchicine was removed (see Fig. 1), Tilney concluded that MTs are involved in both axopod maintenance and axopod formation, and thus that MTs have structural and force-producing roles (Tilney, 1968).

Subsequent experiments have indicated that, in protrusions emanating from various cells, MTs exert an outward force that is resisted by actin and myosin (e.g. Solomon and Magedantz, 1981; Joshi et al., 1985; Madreperla and Adler, 1989; Ahmad et al., 2000). Other experiments (Mitchison et al., 2005) showed that, as spindles shrink after treatment with a MT-depolymerizing drug, spindle MTs become bent by forces from a spindle 'matrix'. Because *Actinosphaerium* MTs also bend during colchicine treatment (Tilney, 1968), similar 'matrix forces' might act in the axopods, and might involve actin and myosin, both of which interact with MTs in a variety of systems (e.g. Waterman-Storer and Salmon, 1997; Waterman-Storer et al., 2000; Yvon et al., 2001; Mandato and Bement, 2003; Rodriguez et al., 2003; Weber et al., 2004; Fabian et al., 2007). Indeed, in some longitudinal sections, the material between the axopodial MTs looks similar to arrowhead-labeled actin (Tilney and Porter, 1965).

How the double-coiled MT arrangement is generated is still not known. Tilney (Tilney, 1968) speculated that the cytoplasm might have some epigenetic 'memory', as it does in other systems (e.g. Beisson and Sonneborn, 1965) [discussed in Frankel (Frankel, 1989)], but, like the preprophase band, what causes this remarkable structure to form, and what the pattern signifies, remain enigmas.

When cytoplasmic MTs were being described, we knew nothing of their molecular components; as I have already mentioned, the prevailing assumption was that MTs are all the same. To see whether this was in fact the case, Behnke and I (Behnke and Forer, 1967) subjected MTs from crane-fly spermatids, rat sperm and rat tracheal cilia to various treatments, both in tissue and outside the cell. When whole cells were treated, cytoplasmic MTs responded differently from all axoneme MTs. Moreover, in the axoneme, the A-tubules responded differently from the B-tubules, and both were different from the two central MTs. There were similar differences when MTs were treated outside the cell, and when sections of cells were treated with pepsin, and there were differences along the lengths of the MTs (Behnke and Forer, 1967). We concluded that, despite their similar appearances, not all MTs are the same – that there can be 'intrinsic physical and/or chemical differences among the tubules themselves'.

There have been giant strides in our understanding of MT chemistry since then. We now know that all MTs have the same basic substructure of α-tubulin–β-tubulin heterodimers; that they exhibit 'dynamic instability' (e.g. Nogales and Wang, 2006); that motor proteins transport cargo along

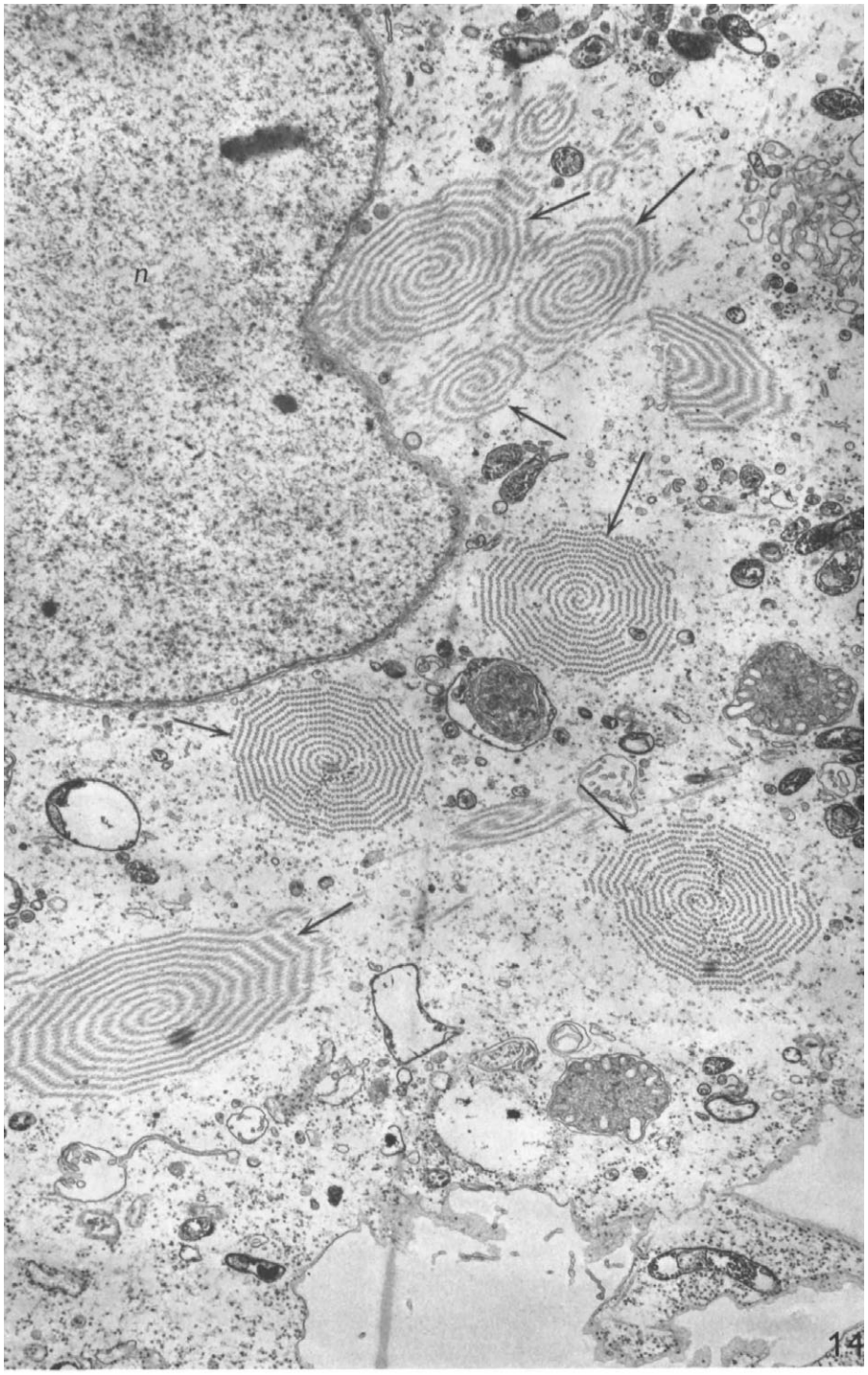

Fig. 1. Section cut through the medulla of an organism in the early stages of reformation of the axopodia. Situated around the nucleus (n) are twelve axonemes. These axonemes look normal with the exception that they are randomly orientated relative to each other. The arrows emphasize this by pointing along the plane of bilaterality induced in each axoneme by the overlap of the microtubules in each of the two interlocking coils (Tilney, 1968).

MTs and occasionally double as enzymes that depolymerize them (e.g. Helenius et al., 2006; Moores and Milligan, 2006; Howard and Hyman, 2007); that MTs can be severed by enzymes such as katanin (e.g. Baas et al., 2005); and that bacteria, for a long time thought to have no cytoskeleton, contain relatives of tubulin (e.g. FtsZ) (e.g. Gitai, 2007). But we also know that there are chemically unique tubulin subunits and different subunit isotypes, which confirms the earlier conclusion that there are chemical and physical differences between MTs. For example, MTs formed from different β-tubulin isotypes differ in their solubility (e.g. Verdier-Pinard et al., 2003) and their function in axonemes (e.g. Raff et al., 1997). The different α-tubulin isotypes and post-translational modifications confer different properties (e.g. Matsuyama et al., 2002), different positions within cytoplasmic MT arrays (e.g. Venkei et al., 2006) and specific localizations in cells (e.g. Walss-Bass et al., 2001).

The articles on MTs by Pickett-Heaps and Northcote (Pickett-Heaps and Northcote, 1966a; Pickett-Heaps and Northcote, 1966b), Behnke and Forer (Behnke and Forer, 1967) and Tilney (Tilney, 1968) published in the early issues of *Journal of Cell Science* were among the first to do more than just describe MTs; they provided experimental insights into various aspects of MTs and their behavior. Crucially, they gave impetus to further experimentation. We have come a long way since 1968, but we are still grappling with some questions raised then. For example, how do the different morphological arrangements of MTs arise, and what do these signify? And how do the different cytoskeletal systems – MTs, actin, intermediate filaments – interact? We have come to recognize that there is crosstalk between the different systems; we now need to understand the language and its grammar.

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References

Ahmad, F. J., Hughey, J., Wittmann, T., Hyman, A., Greaser, M. and Baas, P. W. (2000). Motor proteins regulate force interactions between microtubules and microfilaments in the axon. *Nat. Cell Biol.* **2**, 276-280.

Baas, P. W., Karabay, A. and Qiang, L. (2005). Microtubules cut and run. *Trends Cell Biol.* **15**, 518-524.

Behnke, O. and Forer, A. (1967). Evidence for four classes of microtubules in individual cells. *J. Cell Sci.* **2**, 169-192.

Beisson, J. and Sonneborn, T. M. (1965). Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. *Proc. Natl. Acad. Sci. USA* **53**, 275-282.

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

Eleftheriou, E. P. and Palevitz, B. A. (1992). The effect of cytochalasin D on preprophase band organization in root tip cells of *Allium*. *J. Cell Sci.* **103**, 989-998.

Fabian, L., Troscianczuk, J. and Forer, A. (2007). Calyculin A, an enhancer of myosin, speeds up anaphase chromosome movement. *Cell Chromosome* **6**, 1.

Frankel, J. (1989). *Pattern Formation. Ciliate Studies and Models*. New York, Oxford: Oxford University Press.

Gitai, Z. (2007). Diversification and specialization of the bacterial cytoskeleton. *Curr. Opin. Cell Biol.* **19**, 5-12.

Granger, C. L. and Cyr, R. J. (2001). Use of abnormal preprophase bands to decipher division plane determination. *J. Cell Sci.* **114**, 599-607.
Helenius, J., Brouhard, G., Kalaidzidis, Y., Diez, S. and Howard, J. (2006). The depolymerizing kinesin MCAK uses lattice diffusion to rapidly target microtubule ends. *Nature* **441**, 115-119.
Howard, J. and Hyman, A. A. (2007). Microtubule polymerases and depolymerases. *Curr. Opin. Cell Biol.* **19**, 31-35.
Joshi, H. C., Chu, D., Buxbaum, R. E. and Heidemann, S. R. (1985). Tension and compression in the cytoskeleton of PC12 neurites. *J. Cell Biol.* **101**, 697-705.
Katsuka, J. and Shibaoka, H. (1992). Inhibition by kinase inhibitors of the development and the disappearance of the preprophase band of microtubules in tobacco BY-2 cells. *J. Cell Sci.* **103**, 397-405.
Li, C.-L., Chen, Z.-L. and Yuan, M. (2006). Actomyosin is involved in the organization of the microtubule preprophase band in *Arabidopsis* suspension cultured cells. *J. Integr. Plant Biol.* **48**, 53-61.
Madreperla, S. A. and Adler, R. (1989). Opposing microtubule- and actin-dependent forces in the development and maintenance of structural polarity in retinal photoreceptors. *Dev. Biol.* **131**, 149-160.
Mandato, C. A. and Bement, W. M. (2003). Actomyosin transports microtubules and microtubules control actomyosin recruitment during *Xenopus* oocyte wound healing. *Curr. Biol.* **13**, 1096-1105.
Matsuyama, A., Shimazu, T., Sumida, Y., Saito, A., Yoshimatsu, Y., Seigneurin-Berny, D., Osada, H., Komatsu, Y., Nishino, N., Khochbin, S. et al. (2002). *In vivo* destabilization of dynamic microtubules by HDAC6-mediated deacetylation. *EMBO J.* **21**, 6820-6831.
Mineyuki, Y. and Palevitz, B. A. (1990). Relationship between preprophase band organization, F-actin and the division site in *Allium*. *J. Cell Sci.* **97**, 283-295.
Mitchison T. J., Maddox, P., Gaetz, J., Groen, A., Shirasu, M., Desai, A., Salmon, E. D. and Kapoor, T. M. (2005). Roles of polymerisation dynamics, opposed motors, and a tensile element in governing the length of *Xenopus* extract meiotic spindles. *Mol. Biol. Cell* **16**, 3064-3076.
Moores, C. A. and Milligan, R. A. (2006). Lucky-13-microtubule depolymerisation by kinesin-13 motors. *J. Cell Sci.* **119**, 3905-3913.
Nogales, E. and Wang, H.-W. (2006). Structural intermediates in microtubule assembly and disassembly: how and why? *Curr. Opin. Cell Biol.* **18**, 179-184.
Nogami, A. and Mineyuki, Y. (1999). Loosening of preprophase band of microtubules in onion (*Allium cepa* L.) root tip cells by kinase inhibitors. *Cell Struct. Funct.* **24**, 419-424.
Pickett-Heaps, J. D. and Northcote, D. H. (1966a). Organization of microtubules and endoplasmic reticulum during mitosis and cytokinesis in wheat meristems. *J. Cell Sci.* **1**, 109-120.
Pickett-Heaps, J. D. and Northcote, D. H. (1966b). Cell division in the formation of the stomatal complex of the young leaves of wheat. *J. Cell Sci.* **1**, 121-128.
Raff, E. C., Fackenthal, J. D., Hutchens, J. A., Hoyle, H. D. and Turner, F. R. (1997). Microtubule architecture specified by a β-tubulin isoform. *Science* **275**, 70-73.
Rodriguez, O. C., Schaefer, A. W., Mandato, C. A., Forscher, P., Bement, W. M. and Waterman-Storer, C. M. (2003). Conserved microtubule-actin interactions in cell movement and morphogenesis. *Nat. Cell Biol.* **5**, 599-609.
Sabatini, D. D., Bensch, K. and Barnett, R. J. (1963). Cytochemistry and electron microscopy: the preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**, 19-58.
Solomon, F. and Magendantz, M. (1981). Cytochalasin separates microtubule disassembly from loss of asymmetric morphology. *J. Cell Biol.* **89**, 157-161.
Tilney, L. G. (1968). Studies on the microtubules in Heliozoa. IV. The effect of colchicine on the formation and maintenance of the axopodia and the redevelopment of pattern in *Actinosphaerium nucleofilum* (Barrett). *J. Cell Sci.* **3**, 549-562.
Tilney, L. G. and Porter, K. R. (1965). Studies on microtubules in Heliozoa. I. The fine structure of *Actinosphaerium nucleofilum* (Barrett), with particular reference to the axial rod structure. *Protoplasma* **60**, 317-344.
Venkei A., Gáspár, I., Tóth, G. and Szabad, J. (2006). α4-Tubulin is involved in rapid formation of long microtubules to push apart the daughter centrosomes during early *Drosophila* embryogenesis. *J. Cell Sci.* **119**, 3238-3248.
Verdier-Pinard, P., Wang, F., Martello, L., Burd, B., Orr, G. A. and Horwitz, S. B. (2003). Analysis of tubulin isotypes and mutations from taxol-resistant cells by combined isoelectricfocusing and mass spectrometry. *Biochemistry* **42**, 5349-5357.
Walss-Bass, C., Kreisberg, J. I. and Ludueña, R. F. (2001). Mechanism and localization of β_{II}-tubulin in the nuclei of cultured rat kidney mesangial cells. *Cell Motil. Cytoskeleton* **49**, 208-217.
Waterman-Storer, C. M. and Salmon, E. D. (1997). Actomyosin-based retrograde flow of microtubules in the lamella of migrating epithelial cells influences microtubule dynamic instability and turnover and is associated with microtubule breakage and treadmilling. *J. Cell Biol.* **139**, 417-434.
Waterman-Storer, C. M., Duey, D. Y., Weber, K. L., Keech, J., Cheney, R. E., Salmon, E. D. and Bement, W. M. (2000). Microtubules remodel actomyosin networks in *Xenopus* egg extracts via two mechanisms of F-actin transport. *J. Cell Biol.* **150**, 361-376.
Weber, K. L., Sokac, A. M., Cheney, R. E. and Bement, W. B. (2004). A microtubule-binding myosin required for nuclear anchoring and spindle assembly. *Nature* **431**, 325-329.
Yvon, A. C., Gross, D. J. and Wadsworth, P. (2001). Antagonistic forces generated by myosin II and cytoplasmic dynein regulate microtubule turnover, movement, and organization in interphase cells. *Proc. Natl. Acad. Sci. USA* **98**, 8656-8661.

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