Journal of Supramolecular Structure 2:385–392 (1974)

AN INTRODUCTION TO MICROTUBULES

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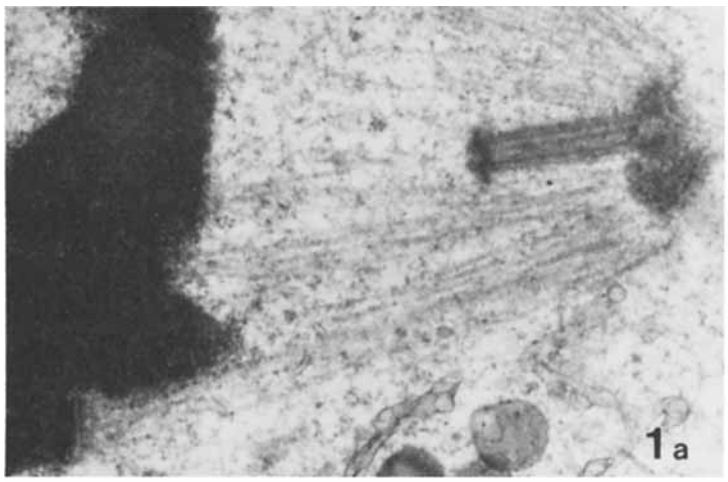
Microtubules are a class of proteinaceous, intracellular fibers found in a wide variety of organisms. They were originally defined by their characteristic morphology as seen in the electron microscope where they appear as tubes of 250 Å outside diameter with a 150 Å hole. They are unbranched and may run for many micrometers. Since the widespread use of glutaraldehyde as a fixative for electron microscopy, microtubules have been found in all eucaryotic cells examined. For example, they are the principal fibrous constituent of the mitotic spindle (Figs. 1a and b); they are present in cilia and flagella (Figs. 2a and b); they are incorporated into highly ordered arrays in numerous unicellular organisms (Figs. 3a and b); and they are a common component in the cytoplasm of many elongate cells (Figs. 4a and b).

The functions of microtubules have been investigated in a variety of systems. Comparative and experimental studies have implicated microtubules as causal agents in the development and maintenance of anisometric cell form. During differentiation of the lens of the chick eye, the epithelial cells elongate by a factor of about 4. At this time the cytoplasm of each cell contains about 100 microtubules running parallel to the developing long axis (1). When neural cells are cultured under appropriate conditions, they elongate and extend their processes for many micrometers. These slender protrusions contain numerous microtubules (2). The nuclei of many spermatids are elongate, and during spermiogenesis, nuclear shape change is associated with bundles of microtubules (3). The literature abounds with reports of microtubules found within cells changing their shapes, and several excellent reviews are available (4–6). There are experimental conditions that block microtubule formation or disrupt existing tubules: the plant alkaloids colchicine, podophyllotoxin, and vinblastin seem to be rather specific in their capacity to act reversibly at low concentrations to dissassemble microtubules (7, 8). High hydrostatic pressure (6,000 psi) or reduced temperature (about 2°C) have a similar, though less specific effect (9). These treatments have been applied to many elongate and elongating cells to provide a wealth of circumstantial evidence that assembling and assembled microtubules contribute to the definition of the cell shape.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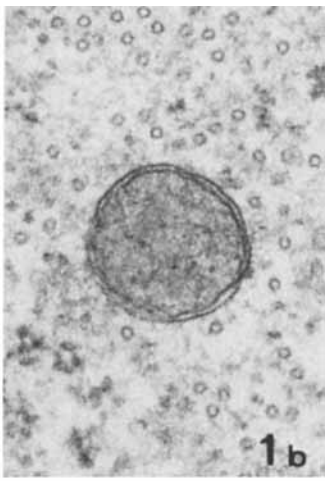
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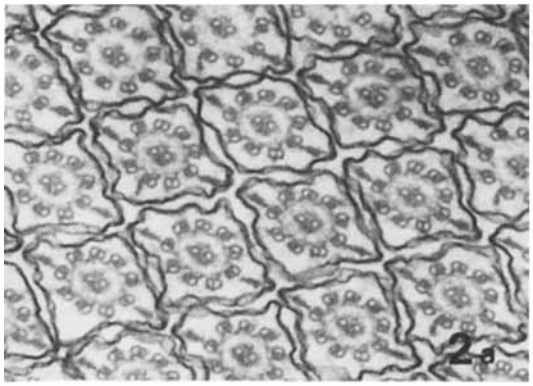
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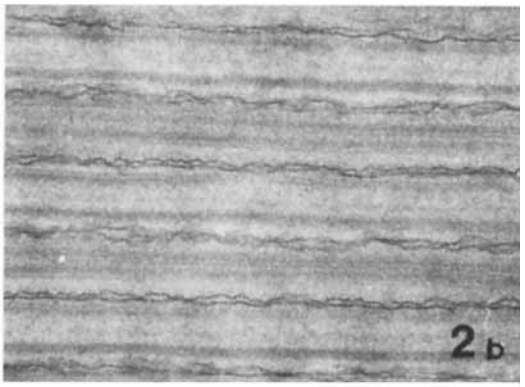
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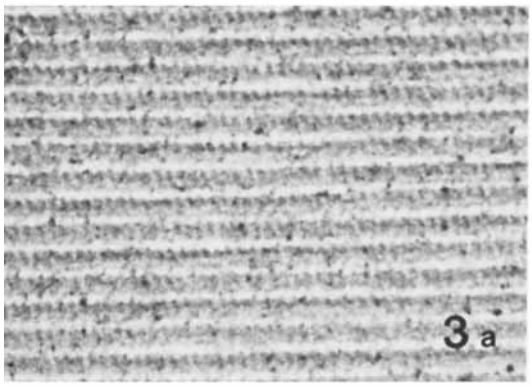
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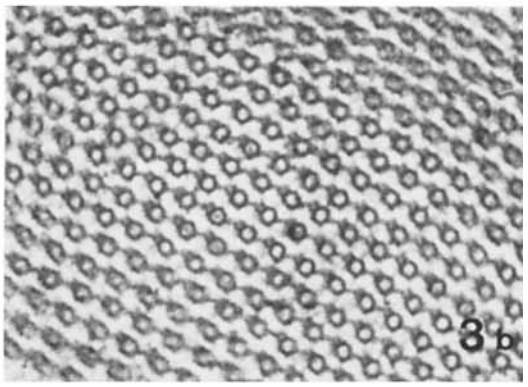
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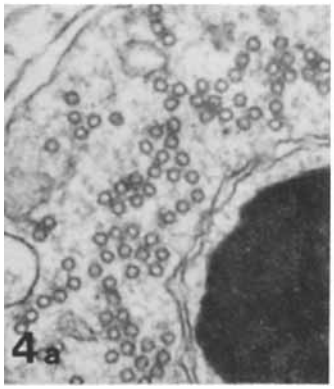
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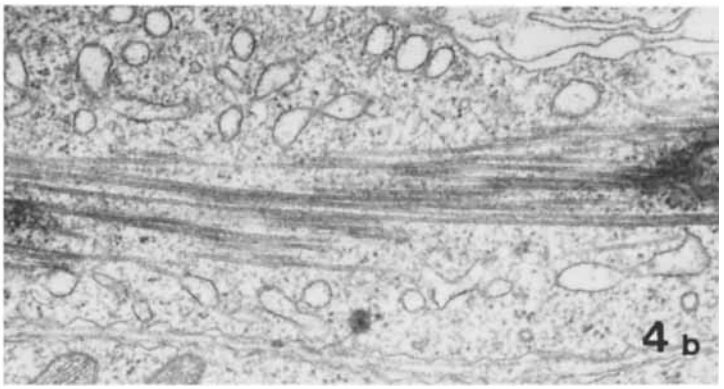
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Microtubules are also associated with some organelles which contribute to eucaryotic cell motility. Flagellar microtubules have been shown to slide over one another as the flagellum bends (10). Some of the flagellar tubules bind an ATPase called dynein which can form transient links between the tubules, probably contributing to the sheering forces that make the flagellar tubules slide (11–13). The motile axostyle found in certain protozoa is a paracrystalline array of cross-bridged microtubules that propagates waves of bending. Here an enzyme resembling dynein has been implicated in effecting relative motion of the tubules (14, 15). Tubules may thus be viewed as a framework upon which enzymes can be arranged so that their mechanochemical action can serve as an engine to generate cell motion. Less ordered arrays of microtubules are associated with other examples of cellular motion. The alignment and separation of the chromosomes on the mitotic spindle (16) and movement of granules in the arms of pigment-containing cells (17) are two well-described examples where colchicine treatment interferes with the normal physiology. Here the role of the tubules is not so well understood. Some investigators think that tubule growth or disassembly is the source of the motive force for chromosome and granule motion (18, 19), while others imagine that the tubules serve as a framework for mechanochemical enzymes (20, 21). Still others see the tubules as passive structural components and suggest that a completely different system, such as actomyosin, is the causal agent in motion (22). Studies on cytological effects of colchicine and vinblastin have implicated microtubules in processes of secretion (23, 24) and in the redistribution of cell surface molecules (25), but little is yet known about the direct involvement of tubules in these processes. The discovery of a colchicine binding protein in cell membranes (26) makes it difficult to interpret the observations available at this time.

Since microtubules are associated with a wide variety of organisms and phenomena, it is no surprise that considerable research is being directed toward elucidating the details of their structure, biochemistry, and function. Structural studies under favorable conditions have revealed that the microtubule wall as seen in transverse view is composed of 13 subunits (27, 28). Longitudinal view of the tubule wall using a variety of negative stains shows strands of globular subunits called protofilaments which run parallel to the tubule axis. Optical diffraction (29) and computer-facilitated analysis (30) have shown that the microtubule is constructed from morphological units which appear as a two-lobed structure packed into a 40 X 51 Å unit cell (short dimension parallel to the microtubule axis). The morphological units are arranged in parallel rows (the protofilaments) and

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Fig. 1. a) Longitudinal thin section of a meiotic spindle from chicken testis. The dark-staining material at the left is the chromosomes. The spindle pole, containing a centriole and some amorphous, dark-staining material is to the right. X 25,000. b) Transverse thin section of a mitotic spindle from a cultured mammalian cell. The circular, membrane-bound structure is a mitochondrion. X 75,000.

Fig. 2. a) Transverse section of a cluster of cilia. Nine doublet microtubules surrounding two singlet tubules together with the associated densities and matrix contained within a single membrane constitute a cilium. This array is found on the comb-plates of the ctenophore, Mnemiopsis. X 50,000. b) Longitudinal section of the same structure.

Fig. 3. a) Longitudinal section of the "axostyle" of Saccinobaculus, a flagellated protozoan that lives in the hindgut of a wood-eating roach. The axostyle is a paracrystalline bundle of cross-bridged microtubules that propagates waves of bending. The line of sight for (a) is along the rows of tubules seen in (b). (X 100,000).

Fig. 4. a) Transverse section of a developing sperm cell from a chicken. The dark-staining circle is the chromatin in the sperm head. (X 75,000). b) Longitudinal section of the same structure. (X 25,000).

placed slightly out of register to make a 3-start helix (Fig. 5). Early results from x-ray diffraction suggested a different surface lattice for the tubule (31), but more recent x-ray studies are in agreement with the structure derived from negatively stained preparations (32).

Initial biochemical studies of microtubules were confined to the material available from cilia and flagella (33), because isolation of cytoplasmic microtubules proved difficult since there was no definition of the subunit molecule save its capacity to form tubules. In 1967 two groups independently reported the use of colchicine-binding activity in cell lysates as an assay for a specific and ubiquitous cytoplasmic protein that was available in quantity from tissues rich in microtubules (34, 35). Detailed study in these and other laboratories has confirmed that the colchicine binding protein is a subunit of microtubules. The protein, named tubulin, has been isolated from numerous sources and characterized with a variety of techniques. The biochemistry of tubulin has recently been reviewed by several experts in the field (36–38). The protein has a molecular weight of 110,000 daltons and a sedimentation velocity of 6S. It is dissociable in sodium dodecyl sulfate or other denaturing solvents into two dissimilar peptides called alpha and beta tubulin. These peptides probably correspond to the morphological units seen with negative staining in the electron microscope. There is circumstantial evidence to support the view that the 6 S molecule is made from one copy of alpha and one of beta tubulin (39), but there are conflicting views and evidence in this matter (40–42). The amino acid compositions of alpha and beta tubulin are similar but not identical, and an investigation of the primary structure of the first 24 amino acids at the N terminal end of the two peptides shows them to be sufficiently similar to support the suggestion that they arose from a common ancestor. Comparison of this same region of the tubulins from embryonic chick brain and from sea urchin sperm tails reveals a single amino acid substitution in the beta chains and no differences in the alpha, indicating a high degree of conservation over evolutionary time (43).

Tubulin in the 6 S form binds 1 mole of colchicine or podophyllotoxin per mole of protein. These two alkaloids compete for a single binding site (7). Vinblastin, which induces a nonphysiological aggregation of tubulin rather than inhibiting assembly, does not compete with the other drugs; the stoichiometry of its binding is currently in dispute (44, 45). The 6 S dimer also binds two molecules of GTP, one with high and one with low rate of exchange (45, 46). The vinblastin and GTP binding sites are not independent, and there is some evidence that the bound nucleotide is involved in the control of tubulin polymerization (45, 47).

Enzyme activity has been attributed to tubulin by several investigators. Tubulin has been implicated as both an ATPase and a protein kinase (48, 49). The evidence on hand (50), however, is most compatible with the view that tubulin is a structural protein which may bind enzymes but possesses enzyme activity itself only in the sense that hydrolysis of the terminal phosphate of GTP is associated with its polymerization (47). Preparations of isolated microtubules contain several proteins other than tubulin which apparently bind to microtubules (51). These molecules may account for the observations of ATPase and protein kinase activies in tubulin extracts.

Studies on the assembly and disassembly of tubulin in vivo have been conducted on a variety of systems. The most detailed investigations have been directed toward the mitotic apparatus of marine eggs using the relative retardation of perpendicular planes of polarized light observable as "birefringence" in the polarization microscope as an assay for ordered material. These results are difficult to interpret in molecular detail both because of the physics of birefringence and because of the possibility of indirect effects

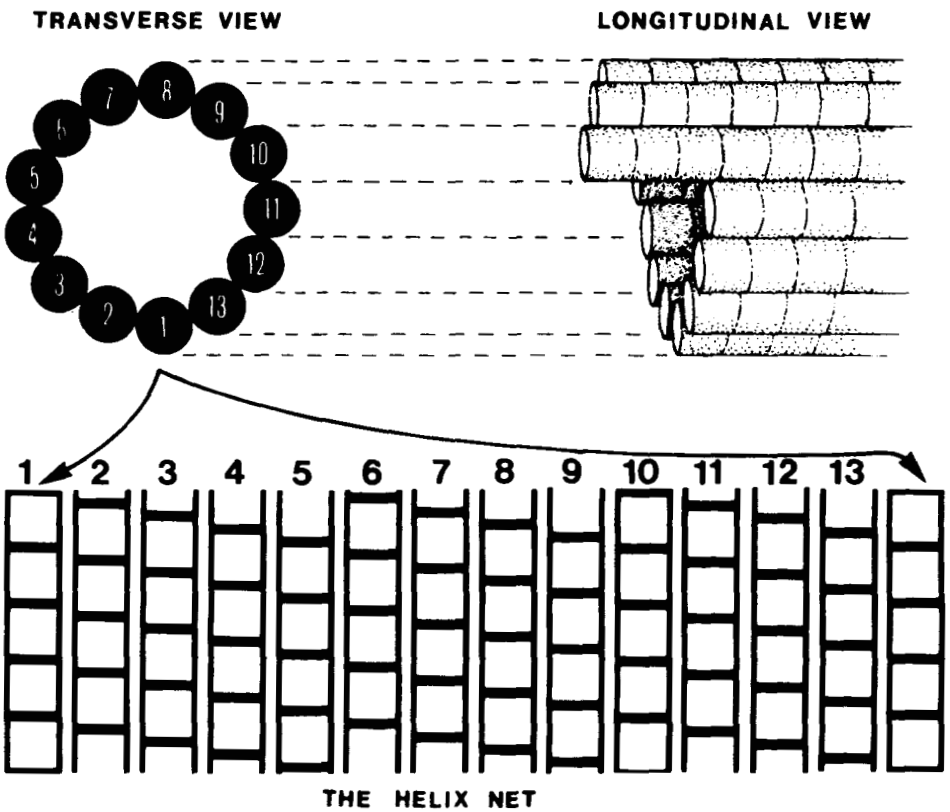

Fig. 5. Microtubule substructure. Transverse view shows the 13 protofilaments of the microtubule wall. The longitudinal view depicts the registration of the morphological units in adjacent protofilaments. In the helix net, the tubule wall is cut parallel to the tubule axis and rolled out flat to display the microtubule surface lattice. Information about the shape of the morphological units is presented by Erickson (this issue).

when one is observing the results of external perturbations on a system as complex as a cell. Nonetheless, the studies by Inoué and co-workers (52)and more recently by Stephens (53) have provided strong evidence that the mitotic spindle exists in a dynamic equilibrium between an ordered and a disordered state of some subunit. Since tubules are the major fibrous component of the spindle, the results have been interpreted as pertinent to microtubule assembly in vivo. These studies suggest that cells contain a pool of unpolymerized tubulin which will add reversibly to the spindle under the influence of elevated temperature or a partial replacement of the exogenous water with D_2O. Cold treatments or dilute colchicine will reversibly dissolve the spindle. The results are consistent with the hypothesis that microtubules assemble as a result of noncovalent, solvent-dependent interactions between tubulin molecules. Disappearance and reappearance of spindle birefringence after temperature jumps conform to first order kinetics (54).

If a transient cell structure, such as the mitotic spindle, is assembled as a monomer-polymer equilibrium, the cellular mechanisms for control of that equilibrium are of immediate interest. In 1963 Taylor showed that protein synthesis could be inhibited

about 1 hour prior to spindle formation without influencing the capacity of a cell to divide (55). More recent studies have demonstrated that most of the tubulin made by a mammalian cell in culture is synthesized well before the spindle forms (56). We can conclude that transcriptional and translational controls are not sufficient to understand the regulation of tubule assembly in vivo. Some additional factors must govern the shift in the association equilibrium of the presynthesized tubulin pool so that the mitotic spindle forms at a time appropriate for its function.

Studies on the growth of various cellular structures made of microtubules reveal that tubule assembly is subject to spatial as well as temporal control. When the mitotic spindle forms in mammalian cells, the tubules do not form at random, but rather they grow from the region around the pair of centrioles found at the spindle pole of animal cells. Further, during the development of diverse multicellular organisms, the direction of the mitotic axis as well as the position and time of spindle formation is modulated by the organism. Thus the cellular system for control of tubulin assembly must govern time, place, and direction of microtubule formation.

Given the suggestive placement of the centriole at the pole of many spindles and at the base of the eucalyotic flagellum, it is tempting to infer that this organelle is in some way the seat of the microtubule control system. Although this view has been widely held, it is almost certainly insufficient, since there are no centrioles associated with spindles of higher plants, and chromosome motion in many lower plants and fungi takes place on spindles which possess a well-defined pole, but nothing resembling a centriole. Further, a variety of cytoplasmic microtubule arrays are not associated with a centriole. Porter has called attention to the cellular system which organizes tubulin polymerization, noting that the widespread association of tubules with cellular shape changes suggests that regulation of tubulin assembly may be significant for the control of cell form (4). The control system for tubulin assembly has properties which would allow it to serve as a source of pattern for cellular morphogenesis and thus as one of the intriguing factors that allow organisms to assume a shape other than the spherical one defined by DNA-dependent protein synthesis within a bag of membrane which tends to minimize its surface area. Pickett-Heaps has coined the term "microtubule organizing center (MTOC)" to include all kinds of cellular structures which might control microtubule assembly (57). All that these variegated structures have in common is an amorphous dark-staining appearance in the electron microscope. The substance of an MTOC has yet to receive a more informative appellation than "fuzz."

Given the importance of tubule assembly for cellular development, many people have tried to reassemble tubulin in vitro. Though several interesting model systems emerged (45, 58, 59), direct analysis of tubulin assembly awaited the discovery by Weisenberg of conditions which supported efficient polymerization in vitro (60). Since his paper in 1972, a considerable body of work has been done, and the fruits of much of that labor are described in the following papers. Characteristic of a new field, not all the results are in full agreement. Probably many of the differences are due to subtle but well-defined differences in procedure, such as the use of different sources of material, the transient presence of glycerol in some experiments but not others, a difference in buffer, the variation of a few tenths of a pH unit, or the difference in concentration of some important ion. It remains for further work to clarify the relationship between these in vitro systems and tubulin assembly in vivo. The important thing is that experiments can now be done which hold promise of significant discoveries about the molecular biology of cellular morphogenesis.

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