Microtubule Solutions Display Nematic Liquid Crystalline Structure*

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We report a study of the spontaneous formation of ordered arrays of microtubules in solution. Form birefringence and anisotropic light-scattering appear rapidly and spontaneously when tubulin, initially present in homogeneous solution, self-assembles into microtubules. This phenomenon is reversible and occurs at protein concentrations of a few milligrams per ml, in the presence or absence of microtubule-associated proteins. Light and electron microscopic examination reveals that extensive regions of these birefringent solutions consist of nearly parallel microtubules. Measurement of the order parameter, S, yields a value of 0.81 ± 0.05, indicating a high degree of alignment. Comparison of these observations to qualitative predictions developed from the theory of Onsager (1949) leads to the conclusion that microtubules form a nematic liquid crystalline phase in vitro under ordinary conditions. Simultaneous spectrophotometric observation of turbidity (a measure of microtubule assembly) and birefringence shows that the parallel ordering lags only slightly behind assembly, thus demonstrating that much microtubule growth must occur by addition of tubulin to the ends of microtubules that are already aligned. These observations of anisotropy are important to the understanding of microtubule dynamics in vitro.

Microtubules in vitro are often assumed to exist as cylindrical rods, randomly dispersed in isotropic solution. Although it has long been known that microtubules can be aligned by flow (1–3) to produce intense form birefringence, the possibility that spontaneous ordering of these rod-like polymeric structures might occur has been little investigated. Such spontaneous mutual alignment of microtubules might be anticipated from theoretical considerations (4–7) and from the observed behavior of other rod-like particles that form liquid-crystalline domains (e.g. tobacco mosaic virus (8), DNA (9), hemoglobin S fibers (10), and "whiskers" of poly(tetrafluoroethylene) (11)). Spontaneous anisotropy is expected to affect such properties of microtubules as diffusion, viscosity, end-to-end annealing, formation of bundles, and biochemical reactivity.

Here we report a systematic qualitative study of the form birefringence that appears when tubulin, initially present at moderate concentration in homogeneous solution, self-assembles into microtubules. Observation by video-enhanced differential-interference-contrast microscopy and by electron microscopy reveals a well ordered nematic liquid crystalline array. Therefore, microtubules must be counted with the other rod-like liquid-crystal formers. Spectrophotometric observation of the kinetics of its formation shows that microtubule assembly and alignment occur nearly simultaneously. The effect is particularly apparent in solutions showing oscillating assembly/disassembly. Because of the very large length-to-diameter ratio of microtubules, it seems likely that the chief force leading to order is the entropic effect initially described by Onsager (4).

MATERIALS AND METHODS

Preparation of Proteins—Microtubule protein, isolated from bovine brain by three cycles of assembly and disassembly, and tubulin, purified from the microtubule protein by chromatography on phosphocellulose, were prepared, assayed, and stored as described by Correia et al. (12). Microtubule-associated proteins (MAPs) were eluted from the phosphocellulose column after the tubulin in PM buffer (0.1 M Pipes, 1 mM MgSO₄, 2 mM EGTA, 1 mM 1,4-dithioerythritol, pH 6.9) + 0.1 mM GTP + 0.75 mM NaCl. The pooled MAP-containing fractions were centrifuged for 30 min at 95,000 × g. MAPs were precipitated from the supernatant by addition of (NH₄)₂SO₄ to 55% of saturation at 0 °C, followed by centrifugation for 30 min at 95,000 × g. The pellet was resuspended in a minimum volume of PM buffer + 0.5 mM GTP + 0.1 mM phenylmethylsulfonyl fluoride, dialyzed 12 h against three changes of this buffer, then frozen drop-wise in liquid N₂ and stored at −70 °C. Immediately prior to use, protein solutions were centrifuged at 2,000 × g for 15 min to remove small amounts of aggregated protein, then exchanged into the buffer to be used by the rapid gel filtration method of Pencofsky (13).

Assembly and Photography—Microtubule assembly was initiated by introducing a solution of tubulin or microtubule protein, in PM buffer with additions as noted, at 0 °C, into a prefarwed cuvette. The internal temperature of the cuvette (measured with a small thermocouple thermometer) was held at 37 °C by means of a Cary cuvette holder and circulating water bath. Temperature was altered, when necessary, by changing water baths. Photographs of birefringence were obtained by placing the glass cuvette (2-mm optical path) between sheets of Polaroid (Edmund Scientific, Barrington, NJ) oriented with their axes of transmission at 90° ("crossed polars"), illuminating from behind with a quartz-halogen microscope illuminator ("Fiber-Lite," Dolan-Jenner Industries, Inc., Woburn, MA), and photographing with an ordinary camera on Kodak Panatomic X film. The cuvette was sufficiently strain-free as to appear dark when filled with buffer and photographed under these conditions. Photographs of scattering in the same cuvette were made by removing the Polaroids and illuminating the solution from the top, at 90° to the direction of observation.

Light Microscopy—Microtubule solutions were examined by means of video-enhanced differential interference contrast microscopy, following in essence the technique described by Schnapp (14). The apparatus consisted of a Zeiss Axiovert 35, mounted inside a large microscope.
37 °C air bath, and equipped with a 100-watt mercury arc, 100× Neofluar objective, 2.5× Optovar, and Dage Model 67 video camera. Background subtraction was performed by a Matrox MVP-AT video processor board, and the resulting images were tape-recorded in Super-VHS format (approximately 425 lines horizontal resolution). Photographs were taken directly from the video monitor with an exposure time of approximately 0.5 s. As employed, the microscope yielded images of (unresolved) single microtubules with contrast and clarity comparable to those shown, for instance, in Walker et al. (15).

Spectrophotometric Measurement—A Cary 118 spectrophotometer was outfitted with a device that allowed crossed polars and filters of an appropriate neutral density to be inserted rapidly (every 10–20 s) and alternately into the light path, so that changes in birefringence and turbidity could be recorded nearly simultaneously. Temperature was maintained at 37 or 2 °C with a thermostatted cell holder. Measurements were made at a wavelength of 420 nm instead of the usual 350 nm, because the Polaroids employed are nearly opaque at the shorter wavelength. Birefringence of the cuvettes was negligible, and the transmittance of the crossed polars alone was 3–4%. This value is shown as 0% transmittance in the figures.

To enable spectroscopometric study of microtubule assembly in thin chambers such as that used for microscopy, a special 140-µm thick spectrophotometric cell was constructed by fastening together cuvette-sized pieces of glass slide and coverslip with Parafilm cut to a pattern like that shown in Fig. 3a. This cell fit easily into the thermostatted cell holder of the Cary 118 spectrophotometer.

**RESULTS**

Fig. 1 shows light-scattering and apparent birefringence of a representative solution of microtubules that are undergoing assembly and disassembly under conditions commonly employed in vitro. Initially (Fig. 1a), the solution exhibits diffuse birefringence and homogeneous scattering. With time (Fig. 1, b and c), both the birefringence and scattering develop a striated appearance. The striations in scattering exhibit a pattern similar to that of the striations in birefringence, and both must result from inhomogeneities in the sample. Changes in the fine structure of the striations occur on the time scale of several minutes, giving the short-term impression of a nearly static solution. Cold-induced depolymerization of microtubules (Fig. 1d) is accompanied by a loss of both birefringence and turbidity. Qualitatively similar results are seen in solutions of microtubules assembled in either the presence or the absence of MAPs. The results are independent of whether assembly is carried out in a cuvette or a capillary tube and independent of whether the vessel is made of quartz, glass, or polycarbonate (with observation in glass). Although the striations disappear upon depolymerization, the sample does not pass through an intermediate stage of diffuse birefringence as it did during assembly (Fig. 1d). This observation indicates that the structures responsible for the striations, once formed, are as stable as the microtubules of which they are composed.

The light transmitted through crossed polars could have its origin either in birefringence or in depolarization due to multiple scattering of light in the intervening solution. In order to control for the latter possibility, an experiment equivalent to that in Fig. 1 was carried out, but the crossed polars were rotated about the incident light beam and the resulting patterns observed. Fig. 2 shows that the pattern of light transmitted through the crossed polars changes with the angle. Patterns observed when the crossed polars are rotated by 90° (Fig. 2, a and e) are indistinguishable. Hence, birefringence must be the major cause of the observed transmission of light, although a small contribution from depolarization due to multiple scattering cannot be excluded. Fig. 2 also shows that substantial “dark” areas are present at any particular angle of the crossed polars. Such a dark area could represent either a nonbirefringent region of the solution, a region in which the direction of polarization of the incident light is parallel or perpendicular to the local optic axis, or an area in which the beam traverses regions of mutually canceling birefringence. Visual comparison of the photographs in Fig. 2 (verified by densitometric scanning, results not shown) shows that an area which appears dark at one angle of the polars appear bright at other angles. This finding implies that the solution is not a collection of ordered regions embedded in an isotropic surrounding, since such a specimen would be expected to have numerous nonbirefringent regions, dark at all angles. Rather, the solution appears to be composed of numerous abutting ordered domains with different orientations. This interpretation suggests that the inhomogeneities in scattering visible in Fig. 1 may result from regional variations in orientation as well as in density.

To investigate the structural basis for the observed birefringence on a microscopic scale, the chamber shown schematically in Fig. 3a was employed. The following controls were carried out to ascertain that this thin chamber did not exert gross effects on microtubule assembly. Fig. 3b shows that birefringence (similar to that in Fig. 1a) is clearly visible on a macroscopic scale in solutions of microtubule protein that have been introduced into the chamber at 0 °C and then allowed to polymerize at 37 °C. (Striations were seldom observed.) Fig. 3c shows that the rate and extent of assembly of microtubules in the microscope chamber, as monitored by turbidity, do not differ greatly from those in an ordinary cuvette. (The smaller “lag” in the thin cell might represent the effects of small differences in nucleation of microtubules, but most likely arises from the fact that the thin solution inevitably warms more quickly than the thick solution). It
appears, therefore, that microtubule assembly in the thin microscope chambers produces solutions that are very similar to those observed in cuvettes.

Fig. 4 shows microscopic views of a series of four microtubule solutions of increasing concentrations. Assembly was carried out under conditions closely resembling those in Figs. 1-3, and in the absence of MAPs so that long microtubules were formed. The critical concentration under these conditions is about 2.5 mg/ml. At the lowest concentration, alignment is not distinct (Fig. 4a). At a slightly higher concentration, however, mutually parallel orientation of microtubules becomes apparent (Fig. 4b). At still higher concentration (Fig. 4c) the effect becomes quite pronounced. The extent of oriented regions was large: one could survey more than a millimeter in any direction in the plane of the slide, and several tens of micrometers perpendicular to that plane, and see only a few nonaligned microtubules. These results confirm the idea that the observed birefringence is form birefringence caused by alignment of the microtubules. They also confirm the macroscopic observations that MAPs are not necessary to produce alignment. The concentration dependence apparent in Fig. 4 suggests strongly that excluded volume effects (see "Discussion") play a major role in producing alignment.

At concentrations higher than ~9 mg/ml, a second phenomenon becomes apparent: the linear elements in the microscopic field develop increased contrast and begin to appear wider (Fig. 4d). This effect is more clearly visible in Fig. 5, in a micrograph made at 27 °C. We attribute it to a side-to-side association of microtubules to form "cables." In the video recordings, microtubules could be seen to fuse over times of several minutes, by the slow movement of a "Y," at least three of which are visible in the figure. The mean number of microtubules per cable, estimated as described in the figure legend, was found to be 17 ± 5 in a typical sample, confirming the visual impression of apparent width and high contrast.

Fig. 6 shows the typical microscopic appearance of microtubules assembled from tubulin and MAPS. Although a few microtubules can be seen to have quasi-random orientation, the vast majority fell into a mutually parallel array. In fact, it appears (compare Fig. 4) that more uniform alignment is obtained in the absence of MAPs than in their presence. Cables like those of Fig. 5 have never been seen in MAP-containing solutions.
process, small flows that could occur during the warming process could not be completely excluded.

To obtain an electron microscopic assessment of the internal structure of these solutions, 40-μl drops of a solution of tubulin (11 mg/ml) were suspended between two glass plates and warmed to 37 °C to induce assembly. The solutions exhibited visible birefringence, both before and after fixation, which was carried out by gently surrounding the drop with a warm solution of 2% glutaraldehyde, 1% tannic acid in 0.05 M sodium phosphate buffer, pH 7.0. After sectioning, electron microscopy of these samples yielded a visual impression that the axes of most microtubules in a given section were nearly parallel (Fig. 7). To obtain a quantitative measure of the degree of ordering, the nematic order parameter, S, was estimated as described in the legend to Fig. 7. Here, \( S = \frac{1}{2} <(3\cos^2\theta - 1) > \), where \(< >\) signifies the mean value of the quantity, and \( \theta \) is the angle between the axis of a given microtubule and the mean direction (the director or nematic axis) of the axes of all the microtubules (16). Values of \( S \) range from 0 for a randomly oriented sample to 1 for parallel rods. A value of 0.81 ± 0.05 was obtained for the microtubule sample, indicating a high degree of order (see "Discussion"). To measure the mean packing density, 429 cross-sections were counted in an area corresponding to 13.4 μm², giving 32 microtubules/μm², under the assumption that the microtubules are parallel. This number implies a concentration of about 8.6 mg/ml, which matches closely the density to be expected from the known mass concentration of microtubules in the sample. (Because the critical concentration for assembly is 2.5 mg/ml, the microtubules must account for 8.5 mg/ml.) The correspondence of the observed and calculated concentrations suggests that most if not all polymeric tubulin is present in the aligned array. The wide spacing of the microtubules in these samples contrasts sharply with the much closer packing characteristic of centrifugal pellets (17–21), demonstrating that alignment is not confined to regions of locally high density.

Two experiments were aimed at understanding whether convective movement within the solution might cause the macroscopic (millimeter scale) order seen in birefringence. First, to ensure movement, microtubules were assembled in a 2-mm thick cuvette with constant vigorous mechanical stirring. Intense birefringence was observed. When stirring was stopped, the striations were initially coarse, becoming progressively finer over a period of tens of minutes. Second, to minimize movement, microtubules were assembled in a cell that was specially jacketed to produce a bottom-to-top temperature gradient of 35–37 °C, and in another cell at constant temperature but in a sucrose density gradient of 0–5%. Solutions of microtubules assembled under either of these conditions exhibited intense but coarsely striated birefringence. Hence, bulk convective motion appears not to cause the birefringence that is observed under ordinary nonstabilized conditions, but the detailed macroscopic appearance of the solutions probably does result from a combination of subtle nucleating effects, thermal convection, and forces acting between microtubules.

Measurements of the rates of development of turbidity and of birefringence are shown in Fig. 8. Both in the presence and absence of MAPs, the initial development of birefringence lags only slightly behind the development of turbidity. This observation leads to the important conclusion that alignment of microtubules is nearly simultaneous with their elongation. A subsequent large increase in birefringence appears at longer times, as shown in the insets. Comparison with photographic results (Fig. 1) indicates that this later slow increase is related

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**FIG. 3. Chamber employed for microscopic study of solutions.**

(a) schematic diagram. A spacer of Parafilm (b, cross-hatched) was placed between a microscope slide (a) and a 22-mm square coverslip and sealed by gentle heating. The resulting chamber (c) was easily filled by application of a drop of solution at the left-hand opening of the coverslip and sealed by gentle heating. The resulting chamber (c) was about 140 μm thick, held approximately 16 μl, and could be filled with solutions. A subsequent large increase in birefringence appears at longer times, as shown in the insets. Comparison with photographic results (Fig. 1) indicates that this later slow increase is related to the development of turbidity.
FIG. 4. Representative video-enhanced DIC images of four MAP-free microtubule solutions, assembled and observed in the chamber described in Fig. 3, under the conditions described there. Concentrations in mg/ml: a, 7; b, 8; c, 9; d, 10.

However, under particular conditions (e.g. 12 mM Mg2+ and 2 mM GTP) microtubule solutions undergo dramatic oscillations in amount of polymeric tubulin (22, 25, 26) and the phenomenon of dynamic instability becomes apparent on a macroscopic scale. Fig. 9a demonstrates that under these conditions the birefringence oscillates, with a period approximating 2 min, producing nearly identical patterns in successive cycles of polymerization. This observation can be explained if one assumes that the microtubules still remaining at the minima of birefringence retain their orientation, providing templates for renewed directional growth during the next cycle of assembly. Fig. 9b shows that the oscillations in birefringence are tightly temporally coupled to those in turbidity, confirming the finding that alignment of microtubules occurs nearly simultaneously with their assembly. With time, the oscillations in light scattering and birefringence disappear, presumably because the polymerization-depolymerization cycles of individual microtubules become increasingly asynchronous (22, 26). Intense striations do not develop in these oscillating solutions.

Patterns of transmission of polarized light in these solutions varied with the angle of the crossed polars as in Fig. 2 (data not shown), indicating that the observed pattern results primarily from birefringence rather than from multiple scattering.
FIG. 5. Video-enhanced DIC image of tubulin (9.5 mg/ml) assembled for approximately 30 min at 27 °C and observed at 27 °C. The total length of all visible microtubules and their cables in the effective observation volumes was measured in a similar sample. The effective depth of field (1.2 ± 0.2 μm) was then taken to be the vertical distance within which the microtubules and cables were still discernible. The total measured length of discernible linear elements was then determined, by two observers, with the use of a digitizing tablet. The expected length of single microtubules was calculated from the total tubulin concentration less the critical concentration (spectrophotometrically measured to be 5.5 mg/ml under the conditions of observation) and the known number of subunits per unit length of microtubule. Dividing the expected length of microtubules by the total measured length of linear elements gave the mean number of microtubules in a cable.

FIG. 6. Representative video-enhanced DIC image of a MAP-containing microtubule solution. A mixture of tubulin (4 mg/ml) and MAPs (1 mg/ml) in PM buffer + 1 mM GTP was introduced into the chamber and assembled as described in the legend to Fig. 3, then observed at 37 °C. The plane of focus is approximately 3 μm from the coverslip.

FIG. 7. Representative electron micrograph of microtubules assembled and fixed in a hanging drop, as described in the text. Two polar angles determine each microtubule's orientation: ϕ, which lies in the plane of the micrograph, and ψ, measured with respect to an axis perpendicular to the plane. For a given microtubular cross-section, ϕ is approximated by measuring the angle between the long axis of the elliptical image and the horizontal, and ψ is calculated as the inverse tangent of the image length, minus the microtubule diameter, divided by the thickness of the section (approximately 0.05 μm). The arrow shows the projection onto the plane of the photograph of the nematic axis, which also made an angle of 22.6 ° with the perpendicular. It was determined as the mean orientation of the 77 microtubules in the sample. The cosine of the included angle between the nematic axis and a given microtubule is the dot product of unit vectors in the direction of each. (Note that error in the thickness of the section cancels when the dot product is determined. Effects of compression in cutting the section also tend to cancel.)

DISCUSSION

Figs. 1 and 2 show clearly that solutions of microtubules assembled in vitro become spontaneously birefringent. Because microtubules themselves do not possess substantial intrinsic birefringence (27), this striking effect must be the result of their organization into arrays that exhibit form birefringence. This conclusion is strongly reinforced by the microscopic observation (Figs. 3–7) of regions containing many thousands of microtubules with their long axes mutually parallel. Spectrophotometric experiments (Figs. 8 and 9), taken together with the whole cuvette observations, show that much alignment occurs nearly simultaneously with microtubule assembly. A second kinetic phase can also ensue, in which intensification of birefringence accompanies the development of visible macroscopic striations and stable or slightly decreasing turbidity. These are robust phenomena, observed under a wide range of conditions.

Microtubules Form a Nematic Liquid Crystal—Although bundles of aligned microtubules are frequently seen in cells, their spontaneous appearance in solution might be considered surprising in view of the absence of the spatial constraints of cellular geometry and in view of the absence or controlled presence of potential "bundling proteins" (18, 28, 29). The likeliest qualitative explanation for the overall phenomenon of alignment observed here is the increasing restriction of motion that microtubules experience as the solution becomes...
Liquid Crystalline Structure in Microtubule Solutions 1645

Fig. 8. Time course of development of turbidity and birefringence during assembly of microtubules at 37 °C. a, tubulin (4 mg/ml) + MAPs (1 mg/ml) in PM buffer + 1 mM GTP; b, tubulin (9 mg/ml) in PM buffer + 2 mM GTP. Axes of the insets show the same quantities as those of the main graphs, but on a longer time scale. Turbidity and birefringence were measured at 420 nm, rather than at 350 nm, because of spectral limitations of the polars employed. The relatively low precision of these measurements reflected in the "roughness" of the curves is due to slightly irreproducible placement of the polars as they were shifted in and out of the light beam of the spectrophotometer (see "Materials and Methods").

more crowded. To the extent that microtubules can be treated as rigid rods, the phenomenon can be understood on the basis of existing theory. Onsager (4) showed that, for a solution of uniform cylindrical rods that do not interact except by collision, there is a concentration, C*, above which the solution separates into two phases, one an isotropic solution and the other a nematic liquid crystal. As a rough approximation, $C^* = \left(\frac{16}{\pi}\right) \left(\frac{1}{L^3 D}\right)$, where L is the length of the rod, and D its diameter. For 10-μm microtubules, $C^*$ would approximate 4.6 mg/ml. (See Ref. 30 for a review of this treatment). Microtubules, of course, are not uniform (or even fixed) in length. They also interact electrostatically as well as by direct collision, and they possess some flexibility. The effects of these secondary characteristics on the formation of the anisotropic phase have been reviewed recently by Odijk (31). Both polydispersity (32-34) and electrostatic effects (4, 8, 35, 36) tend to lower the value of $C^*$. Flexibility, on the other hand, tends to raise $C^*$ (31, 37). Although the persistence length of microtubules has not been accurately measured, light micrographs of unfixed microtubules (cf. 15) show that their persistence length must be of the same order of magnitude as their overall length. They must therefore lie at the stiff end of the "semi-rigid" category of rod-like structures, where little effect of flexibility on the isotropic-nematic transition is expected. Considering all of these observations, it appears that the aligned regions we describe are nematic liquid crystals and that their formation can be understood qualitatively as an isotropic-nematic transition resulting chiefly from collisional interactions, but with possible contributions from other, less important, forces. This conclusion is reinforced by the fact that the measured value of the order parameter, $S = 0.81 \pm 0.05$, is in good agreement with the values of 0.78-0.79 that can be obtained numerically from Onsager's approach (32, 38).

Minimum Concentration for Alignment—The fact that alignment has been observed reliably at microtubule concentrations above 5.5 mg/ml in the absence of MAPs (i.e. a total concentration of 8 mg/ml minus the critical concentration for assembly of about 2.5 mg/ml characteristic of these condi-

In solutions, collisions between rods begin to become frequent when the concentration approximates 1/L. The mean microtubule in the solutions containing 4 mg/ml tubulin and 1 mg/ml MAPs is approximately 10 μm long (measurements by negative stain electron microscopy, data not shown), and the concentration corresponding to 1/L3 is about 3 x 10^{-7} mg/ml, about 3 orders of magnitude smaller than those investigated here. Hence, these are crowded solutions.

Fig. 9. Time course of oscillations in birefringence and turbidity. Solutions of tubulin at 15 mg/ml in PM buffer + 12 mM MgSO4 + 2 mM GTP were placed in a 2-mm cuvette at 37 °C. a, photographs of the cuvette between crossed polars, taken at intervals of 20 s after the first appearance of turbidity (approximately 3 min after initiation of polymerization). The time series is represented in an S-shaped montage. Not all parts of the cuvette oscillate strictly in phase (e.g. compare the upper left region of the cuvette with the lower center). b, record of oscillations in turbidity (-----) and birefringence (----), measured as in Fig. 8.
Liquid Crystalline Structure in Microtubule Solutions

...tions) and above 3.8 mg/ml in the presence of MAPs (where the critical concentration approaches 0.2 mg/ml) establish upper bounds for the values of C*. Below these concentrations (indeed, as low as 1 mg/ml of tubulin + MAPs and under favorable conditions), birefringence is occasionally seen, but we are uncertain whether alignment induced by convective processes may play a role in those observations. Attempts to determine a precise value for C* have been inconclusive. Sedimentation experiments, for instance, yielded values of the supernatant concentration that depended on time of sedimentation and on the total concentration of the solution being centrifuged. This outcome is not surprising in view of the fact that microtubules undergo continuing changes in their length distribution (23, 24, 39–41), so that there may not be a single value of C* in the equilibrium sense. Slow diffusional mobility, heterogeneity, and entanglement of the microtubules also complicate physical measurements in these solutions.

Cables—The forces underlying the formation of the cables of Fig. 5 is unclear. It is doubtful that they result from the action of trace amounts of bundling proteins, since they are never seen when MAPs are included deliberately in solution. We note that they may correspond to the columnar liquid crystals recently discussed in a theoretical context (42).

Kinetics—Two facts rule out the hypothesis that microtubules first assemble fully and then form a nematic array by subsequent diffusional processes. First, the microtubules of Figs. 4 and 6, when viewed in real time by video-enhanced differential interference contrast microscopy, show virtually no large-scale motion over times of many tens of minutes, a result that might be expected from excluded volume considerations. Second, despite this near total lack of movement, the delay between assembly and the associated increase in birefringence shown in Figs. 8 and 9 is very short. These facts thus lead to the conclusion that the majority of the assembly of microtubules in these solutions occurs by addition of tubulin to the ends of microtubules that are already in an aligned state.

Any straightforward kinetic mechanism by which this process could occur rapidly must involve initial elongation of newly nucleated microtubules in random directions. Partial orientation of the growing rods must then occur while they are still short enough to have reasonable rotational diffusion coefficients and a small tendency toward entanglement. It is difficult, though, to proceed beyond these simple interpretations without further experimental information. It is not clear, for instance, that excluded volume effects alone could produce substantial anisotropy without also slowing rotational diffusion. Additional aligning influences may have to be invoked. One such possibility is that dynamic instability (23, 24) plays a role, since any given microtubule can go through several shrinking and growing phases. A long unaligned microtubule that shrinks may become aligned while it is short (and has a large rotational diffusion coefficient), and subsequently regrow in an aligned state. This kind of process could account for the slow formation of the observed highly ordered, striated solutions. The observed sensitivity of the direction of bulk alignment to the direction of flow during filling of the cell (Fig. 3b) suggests also that minor convective disturbances or surface effects may act early in the process.

Energetic Coupling between Assembly and Alignment—It has been pointed out (6, 7, 43, 44) that assembly and orientational ordering are energetically coupled. Qualitative effects of this coupling produce a large decrease in the critical concentration of monomer and a sharp increase in the mean length of the rod-like polymers at total polymer concentrations above C*, as well as a tendency for the longer rods to be concentrated in the nematic phase. These predicted effects will be complicated by the fact that dynamic instability implies a lack of equilibrium. They remain to be measured in the microtubule system.

Significance for Other Studies—The results reported here have direct importance for the understanding of behavior of microtubules in vitro and indirect importance for understanding of cellular events. For instance, knowing how to produce (and how to avoid) nematic arrays should be useful in studies of bundling proteins and of microtubule-based motor molecules. Knowing that restriction of motion occurs at concentrations above C* allows one to avoid overly simple interpretation of hydrodynamic properties solely in terms of the dimensions of microtubules.

It has been observed that end-to-end annealing of microtubules in solution occurs with remarkable efficiency (40, 41), and one may speculate that participation in a nematic array provides them with some of the geometric orientation necessary for this process to occur. One may also speculate that the "overshoot" in turbidity that is commonly observed in the assembly of microtubules from pure tubulin (45, and references therein), may be caused by the same phenomena that bring about the striations in birefringence and scattering at late times. It is likely that diffusion of reacting molecules will be anisotropic in microtubule solutions. Finally we note that, in oscillating solutions, the nematic structure may help to coordinate the oscillations and to define their spatial patterns.

Because intracellular microtubules are usually anchored at one end, and because their dimensions are often comparable to the dimensions of the cell, it is doubtful whether many of the bundles and arrays seen in different cell types under different conditions have their origins entirely in the isotropic–nematic transition studied here. Nevertheless, the forces that act in solution do also apply in the cellular context, and it is reasonable to suppose that those forces affect such factors as the extent to which bundling proteins are actually required, the degree of ordering of a bundle, and the length-distribution of microtubules present within it, as well as the free monomer concentration in its vicinity.

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