CHANGES IN THE ORGANIZATION OF TUBULIN DURING MEIOSIS IN THE EGGS OF THE SURF CLAM, *SPISULA SOLIDISSIMA*

RICHARD C. WEISENBERG

From the Department of Biology, Temple University, Philadelphia, Pennsylvania 19122 and The Marine Biological Laboratory, Woods Hole, Massachusetts 02545

ABSTRACT

Polymerized tubulin can be stabilized in Kane's spindle isolation medium (HGL solution), isolated by differential centrifugation and then assayed by colchicine binding activity. In the eggs of the surf clam, *Spisula solidissima*, the level of particulate tubulin undergoes a series of specific changes during first meiotic division. In either unactivated ("interphase") eggs or metaphase eggs the amount of particulate tubulin was about 13% of the total at 23°C. The amount of particulate tubulin decreased shortly after activation, reaching a minimum value at about 5 min, the time of nuclear membrane breakdown. The particulate tubulin concentration then rose, reaching a maximum at metaphase, and then decreased again during anaphase, reaching a minimum at first polar body formation. In HGL homogenates of unactivated eggs a structure is present which has been shown to contain the interphase particulate tubulin (IPT). This structure consists essentially of a 10-20 μ granular sphere attached to a membranous material which is probably part of the egg cortex. These particles are absent at the time of nuclear membrane breakdown, when the level of particulate tubulin is minimal and when the first signs of spindle formation are visible. Electron microscopy of these particles by negative staining indicates that they are composed of microtubules associated with a granular matrix which may be a polymorphic aggregate of tubulin.

INTRODUCTION

Microtubules are a major structural component of the mitotic spindle and, since they attach directly to the kinetochores, are clearly involved in the anaphase motility of chromosomes. In recent years a great deal of information has been accumulated concerning the structure and organization of tubules in the spindle (4, 6, 7, 11, 14). Very little is known, however, about the mechanism by which the spindle assembles, or about the mechanism by which it breaks down at the end of mitosis. Experiments of Inoué and others have established that microtubules are formed by a reversible polymerization of the microtubule subunit protein (tubulin), and that the formation of microtubules is inhibited by low temperatures and high pressures but is promoted by heavy water (8). These experiments used polarization microscopy to determine the amount of organized material in the spindle, but they did not determine directly the total polymer concentration, nor did they yield any direct determination of the monomer concentration.

Since measurements of polymer and monomer concentrations are essential for understanding any polymerization reaction (18), experiments were performed which would allow the amounts of
polymeric and monomeric tubulin to be measured during cell division. In the experiments to be reported here, microtubules were stabilized in Kane's spindle isolation medium (10), and the stabilized microtubules were separated from monomeric tubulin by differential centrifugation. The amount of tubulin in each fraction was assayed by colchicine binding activity.

**MATERIALS AND METHODS**

Live *Spisula solidissima* were obtained from the Snow Canning Co., Wildwood, N.J., the Wildwood Clam Co., Wildwood, N.J., or the Marine Biological Laboratory, Woods Hole, Mass. The ovaries were removed and the eggs washed at least three times in sea water. The eggs were activated by the method of Kane (10), by adding a volume of 0.52 m KCl equal to 7% of the initial volume of sea water, and were allowed to develop in this medium, usually at room temperature, until the desired time. The eggs were treated essentially by the method of Rebuhn and Sharpless (19) for isolation of the spindle. The eggs were washed once by gentle centrifugation in 1 m glycerol, 1 mm sodium phosphate at pH 8, and then the isolation medium was added to produce a final volume equal to ten times the packed egg volume. The isolation medium contained 1 m hexylene glycol (2-methyl-2,4-pentanediol), 0.01 m potassium phosphate at pH 6.2 (HGL solution). The eggs were broken by two passes through a hand homogenizer with a Teflon pestle (wall clearance 0.005-0.007 inch). Homogenization was performed at room temperature, then the homogenate was rapidly cooled to 0°C and was maintained at 0°C-4°C throughout the experiment.

Centrifugation of the homogenate was performed either in a Sorvall GLC-1 with an HL-4 rotor (for speeds up to 2500 rpm), or in a Sorvall RC2-B with an SS-34 rotor (for speeds between 5000 and 20,000 rpm), or in a Spinco Model L with a 40 rotor (for speeds to 40,000 rpm). Pellets either were washed once in HGL solution or, alternatively, centrifugation was performed through a cushion of 10% sucrose in HGL solution. Tubulin was extracted from the pellets by homogenization in 10 ml of 0.1 m KCl, 0.01 m potassium phosphate, 0.2 m guanosine triphosphate (GTP) at pH 7.0 (KPG solution). Supernatants and total homogenates were treated by diluting them 1:10 with KPG solution. The extracted samples were allowed to sit for 15 min, and then were clarified by centrifugation at 10,000 rpm for 10 min, and the supernatants were removed for colchicine binding determination.

To assay colchicine binding activity, 0.02 ml of a 10-6 m colchicine-H solution (a total of about 200,000 cpm) was added to each 10 ml fraction, and the fractions were then incubated at 37°C for 1 hr (2). The amount of bound colchicine was determined by a modification of the diethylaminoethyl (DEAE) filter assay of Weisengberg et al. (16). To each of the incubated samples was added 2 ml of a packed suspension of DEAE Sephadex A-50 medium, equilibrated with 0.01 m potassium phosphate at pH 7. All operations were carried out in 15 ml conical glass centrifuge tubes. The samples were stirred intermittently for 15 min to allow the proteins to bind to the Sephadex. The Sephadex was collected by gentle centrifugation and then washed four times in phosphate buffer (using about 13 ml for each wash) to remove unbound colchicine. After the final wash, the tubes were filled to 15 ml with Bray's scintillation medium (3) and the tubes were vigorously stirred to suspend the Sephadex. The Bray's medium was then transferred to a scintillation vial and counted. The Sephadex can be transferred with the Bray's since it does not seriously interfere with the counting efficiency.

For electron microscopy, particulate tubulin was partially purified by centrifugation through a cushion of 10% sucrose in HGL solution at 2500 rpm for 10 min. This procedure pelleted the particulate tubulin along with nuclei and some whole cells. The pellet was suspended in HGL solution, and a drop was placed on a 200 mesh copper grid coated with 0.25% Formvar. The drop was allowed to sit for 1 min and was then blotted off and replaced by a drop of 1% uranyl acetate, which was in turn blotted off. The grids were examined in a Philips Model 300 electron microscope operated at 60 kv.

All chemicals were reagent grade. Hexylene glycol was obtained from Eastman Kodak Co., Rochester, N.Y.; Tritiated colchicine was obtained from the Amersham/Searle Corp., Arlington Heights, Ill. at an original specific activity of 690 mCi/mnmole, labeled in ring methoxyl.

**RESULTS**

The ability of tubulin to bind colchicine has been demonstrated in a variety of systems, and colchicine binding activity has been used by several workers as an assay for tubulin (2, 9, 12). The isolated *Spisula* spindle is composed primarily of microtubules (14) and yields only a single major pair of equally stained bands after acrylamide gel electrophoresis in 8 m urea. This pair of bands migrates with purified calf brain tubulin. The specific colchicine binding activity of purified *Spisula* spindles is about ten times the activity of the nonspindle egg proteins. It is likely that tubulin is the major, and possibly the only, colchicine binding species detected in these experiments. Although not directly tested in these experiments, microtubule concentration in the iso-
lated spindle is assumed to be a fair representation of the concentration in the in vivo spindle (7). The assay used to detect tubulin-bound colchicine was a modification of the filter technique of Weisenberg et al. (16). The DEAE cellulose filters used previously tended to clog and gave inaccurate results. The DEAE Sephadex assay described here was reproducible within 5% in duplicate samples. The assay was linear in the range of protein concentrations used in these experiments, and the recovery of bound colchicine was about 75% of that determined by chromatography on G-25 Sephadex.

In the first series of experiments the amount of particulate tubulin in eggs homogenized in HGL before activation (unactivated eggs are assumed to be in interphase) was compared to the amount in eggs homogenized at 15 min after activation (this is the time of first meiotic metaphase). Little or no difference in the amount of particulate tubulin was observed in the 20,000 rpm pellets, although a significant increase was observed at metaphase in the 5000 rpm pellets (Table I). Note that the difference between the 5000 and 20,000 rpm pellets was recoverable by recentrifuging the 5000 rpm supernatant at 20,000 rpm, and that this pellet now has higher activity at interphase than at metaphase.

Although the total particulate tubulin (as determined by centrifugation at 20,000 rpm) is nearly the same at interphase as at metaphase, it is not constant between these two times (Figs. 1, 2, and 3). At all the centrifugation speeds used, a drop in pellet activity was observed within a few minutes after activation. The pellet activity reaches a minimum at about 5 min (prophase) and then rises, reaching a maximum at about 15 min. The observed increase in the pellet activity depends upon the centrifugation rate. At low speeds, the pellet activity at 5 min may be less than 25% of the activity observed at metaphase. At higher centrifugation rates, the difference between the maximum and minimum activities was generally much less, but a minimum was always observed at about 5 min after activation.

After metaphase I the pellet activity decreases again, reaching a minimum at about the end of anaphase I (25–30 min) (Fig. 4). The ratio of the metaphase maximum to the post anaphase minimum averaged 1.67 ± 0.15 (N = 3). This is nearly the same as the ratio of the interphase, or

### Table I

Relative Activities of Tubulin Fractions

| Fraction | Bound colchicine cpm |
|----------|----------------------|
| Interphase, 5000 rpm pellet | 10,600 |
| Metaphase, 5000 rpm pellet | 20,700 |
| Interphase, 20,000 rpm pellet of 5000 rpm supernatant | 21,900 |
| Metaphase, 20,000 rpm pellet of 5000 rpm supernatant | 14,100 |
| Total interphase pellets | 32,500 |
| Total metaphase pellets | 34,800 |
| Interphase final supernatant | 203,000 |
| Metaphase final supernatant | 196,000 |

Ratio of total particulate tubulin to soluble tubulin: 0.13 ± 0.03 (N = 14) at 23°-25°C.

Ratio of interphase soluble tubulin to metaphase soluble tubulin: 1.05 ± 0.18.

Ratio of interphase particulate tubulin to metaphase particulate tubulin:
- High speed centrifugation: 0.91 ± 0.17 (20,000 rpm or higher)
- Low speed centrifugation: 0.48 ± 0.11 (5000 rpm or less)

![Figure 1](image)

**Figure 1** Colchicine binding activity of 2500 rpm pellet. For each point, 0.8 ml of *Spisula* eggs was activated and homogenized as described in Materials and Methods. The homogenate was layered over 2 ml of 10% sucrose in HGL solution and centrifuged at 2000 rpm for 20 min; the resulting pellet was analyzed for colchicine binding activity.

268 *The Journal of Cell Biology* • Volume 54, 1972
metaphase, maximum to the prophase minimum, which was 1.55 ± 0.10 (N = 6) These values are for centrifugation rates of 20,000 rpm or higher. The three experiments performed, which determined the post anaphase pellet activities, all had approximately the same results as Fig 4; however, the size and position of the peaks observed varied somewhat. The peak of activity observed at 45–50 min probably corresponds to the formation of the second meiotic spindle. This peak was always considerably lower than the first metaphase peak. The small peak present between 25 and 35 min may result from the reformation of interphase particulate tubulin, although the reality of this peak has not been firmly established.

The total colchicine binding activity was relatively constant over the period studied, although there was some evidence of an increase in total tubulin beginning at about 30 min. No consistent changes were observed in the supernatant activity, but this was probably a result of the large error in the supernatant measurements. Since it was necessary to dilute the supernatant fraction tenfold to decrease the HGL concentration (which inhibits colchicine binding), the supernatant activity was relatively low and the error relatively high. The observed prophase minimum in particulate tubulin would typically result in less than a 5% increase in the supernatant activity, and this amount of change would be undetectable because of the error in the supernatant activity determination. In experiments in which a relatively large change in particulate activity occurred, correlation was possible between the particulate and soluble activities (Fig 5).

Microtubules are known to be depolymerized by low temperatures and, as shown in Fig. 5, the amount of interphase particulate tubulin is reduced at lower temperatures. Although the amount of particulate tubulin decreases at lower temperatures the amount of soluble tubulin increases, which indicates that a reversible equilibrium exists between the soluble and particulate fractions. The ratio of particulate to soluble tubulin varied from about 0.06 at 5°C to 0.6 at 30°C. It was not possible to accurately determine the effect of temperature upon the amount of tubulin in the metaphase spindle since temperature affects not only the size of the spindle but also the time course of the meiotic cycle. However, metaphase eggs transferred to 5°C or 10°C and held at that temperature for 10 min had nearly the same amount of particulate tubulin as

**Figure 2** Colchicine binding activity of 20,000 rpm pellet. For each point, 0.7 ml of eggs was homogenized, layered over 5 ml of 10% sucrose, and centrifuged at 20,000 rpm for 30 min.

**Figure 3** Colchicine binding activity of 88,000 rpm pellet. For each point, 0.7 ml of eggs was homogenized, layered over 2 ml of 10% sucrose in HGL solution, and centrifuged at 88,000 rpm for 30 min.
Post metaphase I particulate tubulin concentration. For each point, 0.7 ml of eggs was homogenized and centrifuged at 50,000 rpm for 20 min. The pellets were washed once in HGL solution, re-centrifuged, and the final pellets were assayed for colchicine binding activity.

unactivated eggs given the same temperature treatment.

The sedimentation data reported above established that about half of the total particulate tubulin in the unactivated eggs was pelleted at speeds as low as 2500 rpm. A particle sedimentable at this speed should be large enough to observe by light microscopy, and an examination of HGL homogenates was undertaken to establish if a specific structure could be shown to contain the interphase particulate tubulin (IPT). On the basis of several lines of evidence to be described below, a specific particle has been identified as the structure containing the IPT. Phase contrast micrographs of these particles are shown in Figs. 6–9. Although the exact structure of these particles is rather variable, they generally consist of a granular 10–20 μ sphere with a relatively clearer central region. Frequently, one side of the granular sphere is associated with a darker membranous structure. The granular sphere may alternatively be surrounded by a dark ring, which is presumably the membranous structure in a frontal view. From experiments in which eggs were observed while they were squashed under a cover slip, it appears that the membranous structure is derived from the egg cortex. In gently treated egg homogenates, the number of tubulin-containing structures (TCS) is the same as the number of nuclei, indicating that there is just one of these structures per cell.

Effect of temperature on the level of particulate and soluble tubulin. Unactivated eggs were incubated for 32 min at the indicated temperatures, washed in 1 M glycerol solution at the same temperature as the eggs, and then homogenized in HGL solution at room temperature. For each point, 2 ml of eggs were used. The homogenates were centrifuged at 50,000 rpm for 20 min, washed once by recentrifugation, and assayed for colchicine binding activity. For each temperature, 1.0 ml of the first supernatant was diluted with 10 ml of KPG solution and assayed for colchicine binding activity. The plotted supernatant activities are the activities of the 1.0 ml samples multiplied by the total supernatant volume (90 ml).——, supernatant; ——, pellet.

The morphology of the TCS can vary considerably; in particular, ovoid structures such as that of Fig. 10 are frequently observed. These are generally larger than the spherical particles and are most prevalent in eggs homogenized at higher temperatures. The ovoid particles are probably a larger version of the spherical particles.

The TCS is extremely fragile, and easily breaks up into fragments. The experiment shown in Table II indicates that vigorous shaking of an HGL homogenate causes colchicine binding activity to be transferred from the fraction sedimenting at 3000 rpm to the fraction sedimenting at 20,000 rpm. This change in the sedimentation properties of the tubulin corresponds to a loss of visible TCS.

The presence of tubulin in the TCS is indicated by several observations. (a) The TCS is stable in
HGL solution but is quickly destroyed by exposure to 0.1 M KCl. This behavior of the TCS is identical to that of the isolated spindle. Destruction of either the TCS or the spindle by KCl results in the solubilization of colchicine binding activity. (b) There is a direct correlation between the presence of TCS and the presence of colchicine binding activity in low speed pellets. In homogenates of eggs incubated at low temperatures there is very little particulate tubulin, and no TCS are visible in these homogenates. (c) No TCS are visible in homogenates of eggs incubated before homogenization in 10^-4 colchicine or 10^-4 vinblastine for 2 hr. (d) The behavior of the TCS in activated eggs correlates well with the behavior of the particulate tubulin. At 5 min after activation, when the level of particulate tubulin is minimal, TCS are no longer present in the egg homogenates. At this time the nuclear envelope has become blebbed before breaking down, and the first signs of spindle formation can be observed (Fig. 11). The spindle always forms in association with the nucleus, and, even at relatively late stages in the development of the spindle, it may have vesicles from the nuclear...
TABLE II

| Fraction                  | Centrifugation | Calchicine binding activity | Ratio TCS/Nuclei |
|---------------------------|---------------|-----------------------------|------------------|
| Gentle homogenization     | 5000 rpm      | 2730 rpm                    | 0.6              |
|                           | 20,000 rpm    | 5310                        |                  |
| Vigorous shaking          | 5000 rpm      | 1190                        |                  |
|                           | 20,000 rpm    | 7150                        |                  |

Centrifugation and homogenization were done as described in Materials and Methods, except that the 20,000 rpm pellet was prepared from the supernatant of the 5000 rpm centrifugation.

envelope attached to it (Fig. 12). These vesicles are not observed in the fully developed spindle (Fig. 13).

The TCS can be partially purified by slow centrifugation through a 10% sucrose cushion, and their structure studied by electron microscopy. The most revealing observations have been made with whole mounts negatively stained with uranyl acetate. The TCS apparently fragment and flatten during drying of the grid, and a considerable amount of structural detail can be observed (Figs. 14–18).

Microtubules are present in the TCS and, as nearly as can be determined, they have the same structure as spindle microtubules. There are, however, differences in the organization of TCS microtubules and spindle microtubules. The TCS microtubules are much fewer in number and shorter than are the microtubules of the isolated spindle. Furthermore, the TCS microtubules have very little tendency to associate side to side in bundles, and cross-bridges are rarely observed between TCS microtubules. Bundles of tubules and cross-bridges are very common in meiotic spindles prepared and examined by the same procedures used with the TCS (Fig. 19).

Usually one or both ends of the microtubules of the TCS are embedded in a granular matrix of rather variable appearance. Most frequently, the matrix is composed of spherical particles about 200 Å in diameter which, in turn, have a beaded substructure. Besides the beaded spheres, the most commonly observed structures in the matrix are fibers about 100 Å in width. The 100 Å fibers are usually associated with both the tubules and the granular spheres (Figs. 14–18).

Although both ends or the centers of microtubules may be embedded in the matrix material, more often only one end is in contact with the matrix. The microtubules appear to radiate in random directions from an accumulation of matrix material. The exact point where a tubule ends within the matrix is usually difficult to locate precisely, and often the end of a tubule appears to be structurally continuous with the matrix (Figs. 15, 16).

**DISCUSSION**

Unactivated *Spioina* eggs have approximately the same amount of particulate tubulin as do eggs at metaphase I. This "interphase particulate tubulin" (IPT) is at least partially organized into a specific particle (the TCS) which is possibly associated with the egg cortex, and which breaks down just before formation of the meiotic spindle. Although other tubulin-containing components may be present in the unactivated eggs, the TCS is the only visible object large enough to account for the tubulin pelleted at low speed. This fraction accounts for only about half of the total particulate tubulin (Table I), but this is probably a result of fragmentation of TCS under the conditions used in these experiments (Table II). The TCS is composed of two major components, microtubules and a granular matrix. As compared to the meiotic spindle prepared and examined by the same procedures, the TCS contains many fewer microtubules. Since the spindle and the TCS occupy roughly the same volume, the total amount of tubules must be much less in the TCS than in the spindle. Yet the TCS has nearly the same colchicine binding activity as the spindle and, presumably, contains the same amount of tubulin. Therefore, some component of the TCS other than the microtubules must contain tubulin. It is likely that the granular matrix of the TCS is composed of tubulin in a different, or polymorphic, state of association.

The ability of tubulin to form different types of aggregates in vitro has been demonstrated with purified brain tubulin (17), and it is not unreasonable to expect different kinds of aggregates to exist.
normally in vivo. In the best studied example of a helical protein polymer, Tobacco Mosaic Virus (TMV), it has been shown that polymorphic aggregates exist, and that the helical aggregate is actually formed by the association of disc-shaped aggregates (5). It has been concluded from the study of TMV that rapid formation of a helical polymer is accomplished more readily by previous formation of an intermediate aggregate (the disc) than by direct addition of monomeric subunits into the helix. If a similar mechanism is true for the formation of microtubules, then the granular
Figures 14 and 15. Electron micrographs of isolated tubulin-containing structures negatively stained with uranyl acetate. Fig. 14, $\times 166,000$. Fig. 15, $\times 113,000$. 
Figures 16 and 17  Electron micrographs of isolated tubulin-containing structures negatively stained with uranyl acetate. Fig. 16, × 196,000. Fig. 17, × 75,000.
Figure 18  Electron micrograph of isolated tubulin-containing structure negatively stained with uranyl acetate. × 140,000.

Figure 19  A portion of a meiotic spindle, isolated in HGL solution and negatively stained with uranyl acetate. Microtubules are present in large bundles and no matrix material is observed. Apparent cross-bridges can be seen at a number of locations. × 55,000.
matrix of the TCS would be explained as an intermediate state in the assembly of the microtubule itself. The granular matrix of the TCS is a logical candidate for the “nucleating sites” or “organizing centers” for microtubule assembly which have been observed in a number of cells (15).

If the matrix is composed of tubulin, it is also possible that it is functioning as a storage form of tubulin rather than as an assembly intermediate. However, this would not explain the apparent continuity of the ends of the tubules with the matrix, although it may explain more readily the apparent decrease in particulate tubulin at prophase. The two possible mechanisms can be indicated as follows:

\[
\text{(a) monomer } \rightarrow \text{ matrix } \rightarrow \text{ tubule}
\]

\[
\text{(b) matrix } \rightarrow \text{ monomer } \rightarrow \text{ tubule}
\]

In mechanism (a) the formation of microtubules occurs via a direct transformation of matrix into tubule, with no change in the overall association of monomer. In mechanism (b) the formation of microtubules occurs as a result of an increase in the association constant for tubule formation, or as a result of a decrease in the association constant for matrix formation. Nothing in the existing data appears to eliminate either of these mechanisms.

Although the main evidence that the matrix is composed of tubulin is based upon the rarity of microtubules in the TCS, the properties of the TCS also suggest that this is true. The stability of the TCS in HGL solution, its breakdown at low temperatures, and its sensitivity to colchicine and vinblastine indicate that it has the same basic chemical characteristics as microtubules. It is also possible, however, that the few microtubules which are present are necessary for the structural integrity of the TCS. Clearly, much more work needs to be done to establish that the granular matrix is composed of tubulin and to determine its relationship to microtubule assembly and formation of the meiotic spindle.

This work was supported by grant GB-2406 from the National Science Foundation.

Received for publication 25 February 1972, and in revised form 28 March 1972.

REFERENCES

1. Allen, R. D. 1953. Fertilization and artificial activation in the egg of the surf clam Spisula solidissima. Biol. Bull. (Woods Hole) 105:213.

2. Borisy, G. G., and E. W. Taylor. 1967 The mechanism of action of colchicine. J. Cell Biol. 34:325.

3. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. 1: 279.

4. Brinkley, B. R., and J. Cartwright, Jr. 1971. Ultrastructural analysis of mitotic spindle elongation in mammalian cells in vitro. Direct microtubule counts J. Cell Biol. 50:416.

5. Butler, P. G. A., and A. Klug. 1971 Assembly of the particle of tobacco mosaic virus from RNA and discs of protein. Nat. New Biol. 229: 47.

6. Calarco, P. G., and D. Szollosi. 1971. Maturation of the mouse oocyte. Abstracts of the Eleventh Annual Meeting of The American Society for Cell Biology.

7. Cohen, W. D., and L. I. Rebhun. 1970. An estimate of the amount of microtubule protein in the isolated mitotic apparatus J. Cell Sci. 6: 159.

8. Ingoué, S., and H. Sato. 1967. Cell motility by labile association of molecules. The nature of the mitotic spindle fibers and their role in chromosome movement. J Gen. Physiol 50: 259.

9. James, A. K., and L. Austin. 1970. The binding in vitro of colchicine to axoplasmic proteins from chicken sciatic nerve. Biochem. J. 117: 773.

10. Kane, R. E. 1962. The mitotic apparatus isolation by controlled pH. J Cell Biol. 12:47.

11. McIntosh, J. R., and S. Landis. 1971. The distribution of spindle microtubules during mitosis in cultured human cells. J. Cell Biol. 49: 468.

12. Olmstead, J. B., K. Carlson, R. Kline, F. Riddle, and J. Rosenbaum. 1970. Isolation of microtubule protein from cultured mouse neuroblastoma cells. Proc. Natl. Acad. Sci. U. S. A. 65:129.

13. Rebhun, L. I., and T. K. Sharpless. 1964 Isolation of spindles from the surf clam Spisula solidissima. J. Cell Biol. 22:488.

14. Rebhun, L. I., and G. Sanderson. 1967 Ultrastructure and birefringence of the isolated mitotic apparatus of marine eggs. J. Cell Biol. 34:859.

15. Tilney, L. G., and J. Goddard. 1970. Nucleating sites for the assembly of cytoplasmic micro-
tubules in the ectodermal cells of the blastulae of Arbacia punctulata. J. Cell Biol. 46:564.

16. Weisenberg, R. C., G. G. Borisy, and E. W. Taylor. 1968. The colchicine binding protein of mammalian brain and its relation to microtubules. Biochemistry 7:4466.

17. Weisenberg, R. C., and S. N. Timasheff. 1970. Aggregation of microtubule subunit protein: The effects of divalent cations, colchicine and vinblastine. Biochemistry, 9:4110.

18. Winklmaier, D. 1971. A simple approach to the theory of cooperative aggregation of biological macromolecules. Arch. Biochem. Biophys. 147: 509.