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Cell Division and the Mitotic Spindle[1]

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The study of cell division spans the past full century. Lately, the field has blossomed, and exciting advances have been made, especially at the molecular and fine-structural levels. Yet as we commemorate the centennial of Flemming's discovery[2] of "indirect" cell division, or mitosis, many basic questions still remain unanswered or incompletely explained.

The first half-century of study on cell division is synthesized in Wilson's (2) classic treatise "The Cell in Development and Heredity."[3] While laying a solid foundation for the cytology of the dividing cell and the genetic and developmental significance of mitosis and meiosis, Wilson (Chapter IX) also directs our attention to an important viewpoint regarding the structural basis of cell function. Thus he quotes Brücke:

"We must therefore ascribe to living cells, beyond the molecular structure of the organic compounds that they contain, still another structure of different type of complication; and it is this which we call by the name of organization."

It is this aspect of the dividing cell, its organization, especially in its dynamic attributes, that I shall stress in this brief historical sketch. In particular, I shall focus on the organization of the ephemeral mitotic spindle, which emerges cyclically at each cell division. With it, the replicated, condensed chromosomes are separated and positioned for inclusion into the (two) daughter cells.

The Mitotic Spindle

As we entered the early 1950s, evidence pointed to two mechanisms of anaphase chromosome movement (summarized by Schrader [6]). Chromosomes were pulled toward the spindle poles, via their kinetochores,[4] by shortening of a traction fiber or the chromosomal spindle fiber. In addition, chromosomes were "pushed" apart by the pole-to-pole lengthening of the central spindle to which the chromosomal fibers were anchored. The notion of a musclelike contraction for poleward chromosome motion had been propounded by Flemming in 1879 (1) and earlier workers, and questioned by Wilson (2) as not being consistent with the "dynamic nature of the cytoplasmic fibrillae" observed in living cells. As to the dual mechanism, Ris (8), in working with living grasshopper spermatocytes, was able to inhibit the pole-to-pole elongation without affecting chromosome-to-pole movement by exposing the cells to a solution containing a few tenths of a percent chloral hydrate.

Yet the nagging doubt, expressed by Wilson (e.g., pages 178–198 in reference 2) and others regarding the physical nature of the "achromatic" fibrous machinery of the mitotic spindle, which was believed to be responsible for chromosome movement, had not abated. Rather, the problems were compounded by the late 1940s despite, and partly because of, the wealth of studies that had been made on carefully fixed and stained cells and by the deductions drawn from observations of living cell behavior (6). In that atmosphere it was first necessary to learn whether the mitotic figures seen in fixed cells in fact represented, in living cells, a physically integral body capable of moving chromosomes or exerting force enough to deform cell shape.

ISOLATION OF THE MITOTIC SPINDLE: In 1952, Mazia and Dan [9] succeeded in developing a method for the mass isolation of "mitotic apparatuses," thereby identifying the mitotic spindle, chromosomes, and asters as a coherent physical body separable from the rest of the cell (Fig. 1). Although there were earlier reports of expelling the spindle out of an intact cell (e.g., Foot and Strobell [10] from earthworm eggs), the pioneering work of Mazia and Dan finally opened the way for the mass isolation and characterization of the mitotic apparatus. That same year, Carlson (11), in an extensive micromanipulation study on living grasshopper neuroblasts, demonstrated the integrity and mechanical anisotropy of the metaphase spindle, as well as the "liquefaction" of the spindle mid-zone observed during anaphase.

[1] Dedicated to Professor Kenneth W. Cooper, University of California, Riverside, whose continued friendship and advice have added immensely to my work.

[2] Translated and reproduced in 1965. *J. Cell Biol.* 25(1; part 2):1–69. Flemming saw that the nucleus did not divide directly into two, but formed chromatin threads (hence mitosis). The condensed chromatin threads, or chromosomes, were moved apart and placed into two new cells by a transient, fibrillar achromatic apparatus, the "nuclear spindle" (1) formed from the hyaline kinoplasm.

[3] Wilson's work is complemented in the botanical realm by Sharp (3). Bělař (4) provides a thorough, thought-provoking examination of achromatic spindle components and varying patterns of mitosis in protists. Morgan (5) illustrates and raises penetrating questions regarding the role of cell division in embryonic development and gene expression.

[4] Chromosomes commonly possess a single spindle fiber attachment point, or kinetochore. Some chromosomes have, or behave as though they have, diffuse kinetochores along the length of their chromosomes (6, 7). They are called holokinetic chromosomes.

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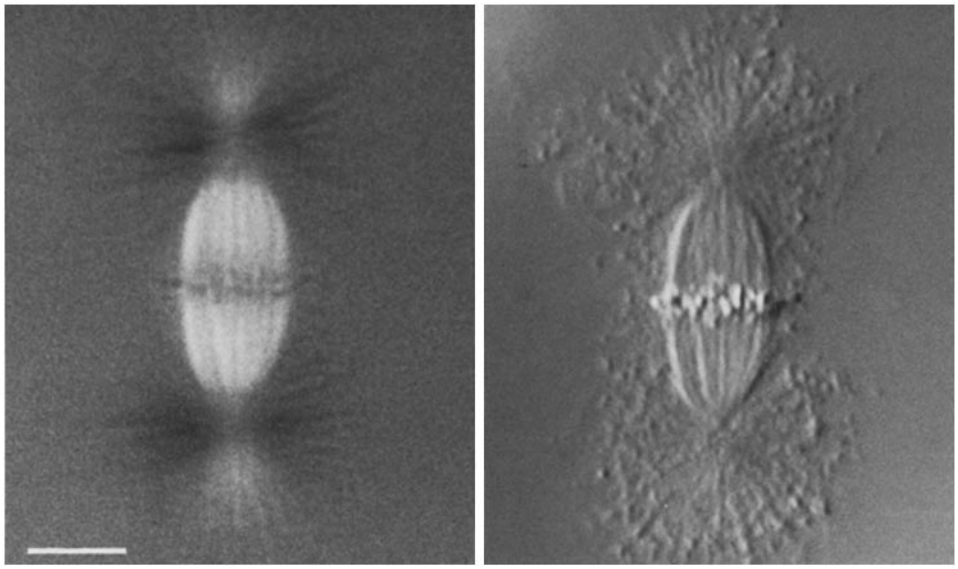

FIGURE 1 Mitotic spindle in metaphase isolated from the egg of a sea urchin, *Lytechinus variegatus*. (*Left*) Observed with a rectified polarizing microscope, spindle fibers and astral rays appear in light or dark contrast depending on their orientation. The weak birefringence (measuring a few nanometers in retardation) of the fibers produces the sharp contrast observed. Microtubular bundles are responsible for the (positive form) birefringence of the fibers. Chromosomes display little birefringence and appear as gray bodies at the equator of the spindle. (*Right*) The same spindle in Nomarsky differential interference contrast. Chromosomes show prominently. The microtubules in these clean spindles (isolated in a new medium devised by Salmon) depolymerize when exposed to *submicromolar* concentrations of calcium ions once glycerol is removed from the isolation medium. In these isolates, which lack vesicular components, the chromosomal fibers shorten as they are depolymerized by micromolar concentrations of calcium ions. Unpublished figures, courtesy of Dr. E. D. Salmon, University of North Carolina. Bar, 10 μm.

Many improvements were made on the basic isolation technique of Mazia and Dan. In particular, the work of Kane (12) that identified the pH and solute conditions (in effect, water activity) needed for mitotic apparatus isolation, helped shed light on the basic physicochemical parameters that delineated the functioning cytoplasm. On the other hand, early attempts at defining the chemical makeup of the mitotic spindle were less successful. In retrospect, that is not so surprising because the fibers of the spindle and aster are immersed in (and spun out from) the hyaline cytoplasm that permeates the cell. Large cytoplasmic granules are excluded from the spindle, but ribosomes and some membranes are not. Yolk and other granules also adhered to earlier isolates.

SPINDLE FIBERS IN VIVO: Whereas the isolated mitotic apparatus exhibited a physical coherence and clearly displayed spindle fibers, such fibers could have arisen by fixing or overstabilizing the cell, as was suggested by many investigators (see 6). Pollister (13), for example, argued that astral rays were not fibers in the living cell but rather were channels of flow of oriented molecules belonging to the hyaloplasm. Our own work, which paralleled that of Mazia and Dan, focused on the development of sensitive polarized light microscopy, with which we hoped to study directly in living cells the nature of the anisotropically arrayed molecules that made up the spindles and asters. From the 1930s to early 1950s, Schmidt (14), Hughes and Swann (15), Swann and Mitchison (16), Inoué and Dan (17), and Swann (18) had investigated how to optimize the performance of existing polarized light microscopes. They also showed that the mitotic spindle and astral rays in cleaving sea urchin eggs and cultured chick embryonic cells indeed displayed a longitudinal positive birefringence consonant with the presumed presence of molecules oriented parallel to the fiber axes. Each of the workers also noted the striking emergence, rise, fall, and disappearance of spindle birefringence (retardation) as a single cell progressed through prometaphase, metaphase, anaphase, and telophase. Each interpreted the observations in molecular terms, variously biased by the paradigm adopted.

By 1953, I was able to demonstrate clearly with the polarizing microscope (19) that "there is fibrous structure in living cells which in conformation is very close to what the cytologists have long observed in well-fixed preparations. There are continuous fibers, chromosomal fibers, and astral rays" (6). Coupled with Cleveland et al. (20) and Cooper's (21) earlier observations of fibrous structures in the spindles of certain other living cells, the issue of the "reality" of spindle fibers seemed to be settled.

Whereas these earlier studies vividly displayed some dynamic changes of spindle birefringence in living cells, changes which should reflect events taking place at the molecular level during cell division, a clearer outlook depended on better optical resolution and broader experience gained through observations and experimental manipulations of cells in division. With progressive improvements in the resolution and sensitivity of the polarizing microscope, culminating in the introduction of "rectified" optics (22), clearer images of individual spindle fibers were obtained, and the birefringence distribution within each fiber in a living cell could be clearly ascertained (Fig. 2). We could now seriously examine the nature of the spindle fibers and their roles in mitotic chromosome movement.

By the late 1950s to early 1960s, several dynamic attributes of the spindle fibers were discovered or confirmed.[5] *(a)* The birefringence of the spindle fiber could be reduced *reversibly* to an equilibrium value, or be abolished totally, by low temper-

[5] For other important approaches complementing the polarized light analysis, see later sections on spindle-associated movements and micromanipulation.

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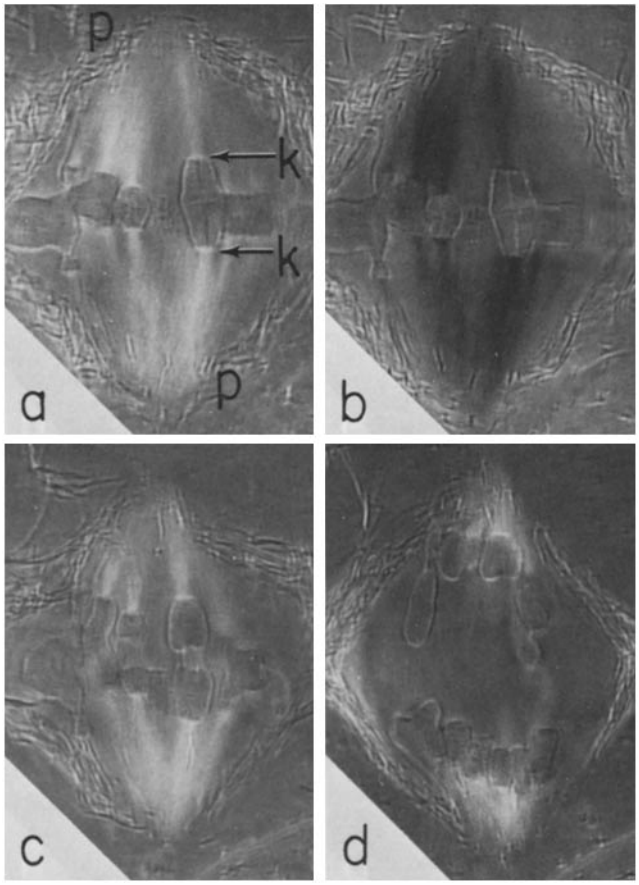

FIGURE 2 A *living* primary spermatocyte of *Pardalophora apiculata* (a grasshopper) as viewed with a rectified polarizing microscope (22). (*a*) Metaphase. Kinetochores of one bivalent are indicated by arrows (k) and polar regions by (p). Birefringent chromosomal spindle fibers run from each kinetochore toward a pole; the diffuse background birefringence of interpolar fibers is identified only with difficulty in the prints, but is readily measured. (*b*) Metaphase. The opposite compensator setting. (*c* and *d*) Anaphase. 22 and 36 min respectively, after *a*. From Nicklas (23). × 1,500.

ature (24) or with an antimitotic alkaloid, colchicine (25, 26). The morphological changes of the birefringent fibers (Fig. 3 *left*) suggested that the submicroscopic fibrils, from which the fibers resolvable with the light microscope were made up (18), were not simply coiling or becoming randomized but were, in fact, depolymerizing as the cells were cooled or treated with colchicine. (Also see important contributions by Beams and Evans [27], Östergren [28, 29], Pease [30], Wada [31], Gaulden and Carlson [32].) The fibrils repolymerized as the cells were rewarmed or the colchicine washed out. In other words, the fibers were not static structures, but rather existed in a dynamic state of flux. This strange capacity of reversible molecular assembly and disassembly, which was inducible by slight physiological perturbations, brought into line the seemingly paradoxical attributes of the achromatic spindle material. As emphasized by Östergren (29) and Wada (31) and puzzled over by Lewis (33), Wilson (2), Bělař (34), and others, there were indeed fibers made up of submicroscopic fibrils, yet the fibrils were made up of molecular subunits held together by labile bonds (Inoué [35, 36]). *(b)* The fibers were organized by "centers" (Boveri [37], Wilson [2], Wasserman [38]) such as centrioles (or equivalent structures), kinetochores, and, in typical plant cells, the cell plate material. As could be deduced from the higher birefringence adjacent to these centers and the temporal sequence of birefringent fiber growth in natural mitoses, as well as from the breakdown and regrowth behavior of the fiber which was microirradiated with a moderate dose UV-microbeam, the centers were capable of assembling or nucleating the fibrils from a preformed pool of unassembled subunits (36). *(c)* Depending on the activity of such centers and the physiological state of the cell, the spindle fibers could readily be built up, broken down, or reorganized. It appeared that the same molecules could enter one kind of fiber or another, depending on which center or polymerizing factor was active at that time (35, 36). *(d)* Chromosome movement ceased, and the chromosomes recoiled toward the metaphase plate when the chromosomal fiber birefringence was abolished in anaphase by cold. As shown in Fig. 3 *right*, poleward chromosome movement resumed in rewarmed cells after birefringent fibers had reappeared and reorganized into an anaphase configuration (36). (This paper also illustrates with many photographs, including excerpts from time-lapse motion pictures, changes in spindle-fiber birefringence that occur naturally in dividing plant and animal cells, as well as in experimentally modified cells.)

MICROTUBULES: In contemporary terms the birefringent submicroscopic fibrils of the spindle fibers and astral rays would be microtubules. By the early 1960s, electron microscopists had begun to describe mitotic microtubules, or "paired fibrils" (39–42); in 1963 "microtubule" still appeared in quotation marks (43). The equivalence of spindle fibrils and microtubules was therefore yet to be made.

By the mid-1960s, especially after the introduction of glutaraldehyde as a fixative for electron microscopy (44), mitotic and other microtubules were widely described and accepted as a basic cytoplasmic element as summarized by Porter (45). The lability and the reversible disassembling ability of the mitotic, and some cytoplasmic, microtubules (46–48) were shown to parallel the behavior of spindle fibrils deduced from their birefringence (49). Thus the ideas evolved that microtubules were the major structural element (fibrils) of the spindle fibers and astral rays,[6] and that the lability of the microtubules, in an equilibrium with a pool of their subunits, was responsible for the lability of spindle fibers (Inoué and Sato [49]).

The assembly of subunits into microtubules was seen to be mediated by hydrophobic bonds and to be entropy driven, as the assembly of tobacco mosaic virus A-protein (57), in the globular to fibrous transformation of actin (58), etc. The greater hydration predicted by this model for the subunits, as compared with the assembled microtubules, was consistent with the ability of D_2O, glycols etc., reversibly to increase the degree of spindle-fiber polymerization (49, 59). Low temperature and colchicine would both favor the disassembly state.

In the meantime, Taylor (60) succeeded in labeling colchicine with radioactive tritium and, in 1965 showed in an elegant study that the antimitotic action of colchicine was based on a tight but reversible, noncovalent binding of colchicine to a

[6] The quantitative correlation between the spindle fiber birefringence and microtubules has been questioned by some authors (e.g., 50–52), but has been affirmed after careful analysis by Sato et al. (53, also see 54 and 55). Marek ('56) reports that the amount of microtubules found by electron microscopy is only half of that expected from the birefringence in living grasshopper spermatocytes. However, even in careful studies such as Marek's, it is not unlikely that a significant fraction of the more sensitive microtubules have been lost by fixation (cf. 49 and 53).

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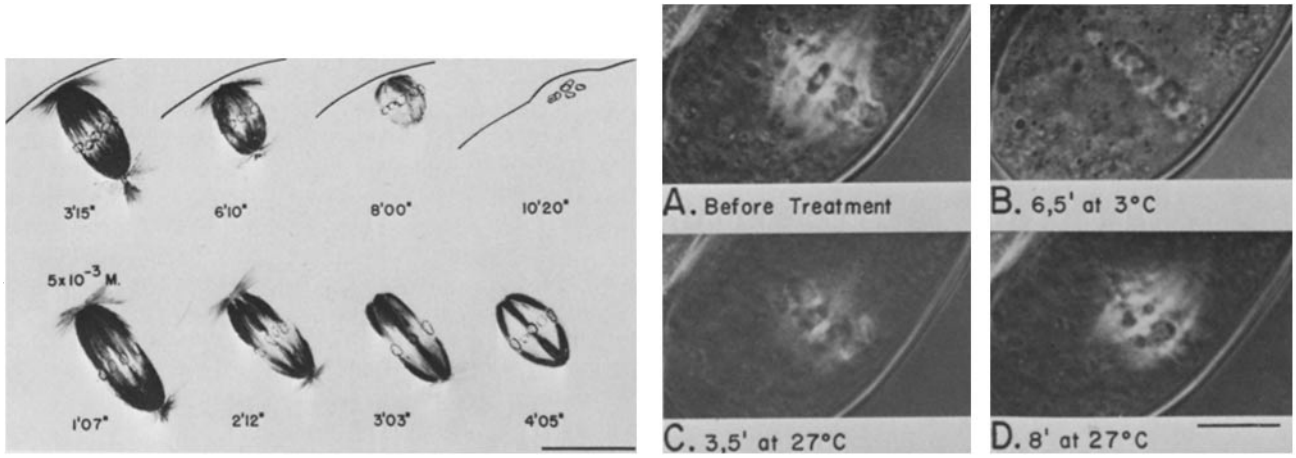

FIGURE 3 (*Left*) Effect of colchicine on the metaphase-arrested spindle in the oocyte of *Chaetopterus pergamentaceous* (a marine annelid worm). The changes in the position of the chromosomes and the morphology and birefringence of the spindle fibers were followed with a sensitive polarizing microscope. In 0.5 mM colchicine (*top*) the spindle fiber birefringence is lost in about 10 minutes. As the spindle decays, the chromosomal fibers shorten without thickening. The loss of birefringence indicates depolymerization of the microtubules making up the fibers. As the fibers shorten, the chromosomes and inner spindle pole are transported to the cell surface. The outer spindle pole is anchored to the cortical layer of the cell. At a higher concentration of colchicine (5 mM, *bottom*), the "continuous" (central) spindle fibers depolymerize and lose their birefringence first. Then the chromosomal fiber birefringence disappears, as the microtubules fall apart, before the fibers have shortened appreciably. The chromosomes are left "stranded." When colchicine is removed, the microtubules reassemble, and the elongating fibers transport the chromosomes and inner spindle pole away from the cell surface, eventually back to the metaphase configuration. Time in minutes (') and seconds (") after application of colchicine. From Inoué (25). Bar, 20 μm. (*Right*) Effect of chilling on pollen mother cell of *Lilium longiflorum* (an Easter lily) in early anaphase. The birefringence of the spindle fibers disappears in a few minutes as the microtubules depolymerize at 3°C. As the mitotic microtubules reassemble at 27°C, the birefringence returns rapidly. The chromosomes recommence anaphase movement in 8–10 min once the spindle organization has recovered. From Inoué (36). Bar, 20 μm.

critically small fraction (3–5%) of sites within the cell. Borisy and Taylor (61, 62) shortly thereafter isolated a colchicine-binding protein from extracts of sea urchin eggs and from isolated mitotic apparatuses. The binding-site protein showed a sedimentation constant of 6S and was identified by them to be the subunit protein of microtubules. An amusing sidelight of this study was that Borisy and Taylor found brain, which they chose as a control tissue expected to be free of dividing cells and hence of the (colchicine-binding, microtubular) spindle protein, to be a particularly rich source of the colchicine-binding protein.

Whereas the behavior of mitotic microtubules as seen in electron micrographs appeared to parallel the behavior of spindle-fiber birefringence as observed in living cells, the behavior of the in vitro, isolated mitotic apparatuses did not. Nor was the in vitro behavior of microtubules and their colchicine-binding 6S subunit, which was by that time isolated, characterized (63–67), and named tubulin (68) as similar as one would have liked to the behavior of microtubules in vivo. Isolated spindles were more stable in the cold than at room temperature, and they were insensitive to colchicine. Tubulin isolated from brain could be assembled into sheets and at times into microtubules, but only irreversibly so (66, 69).

LABILE MICROTUBULES: 1972 was a major turning point. Weisenberg (70) reported the in vitro reconstitution of *labile* microtubules from extracts of rat brain that contain a high concentration of tubulin. Unlike the earlier isolated spindles and reassembled microtubules, the new microtubules disassembled in the cold, and their assembly was inhibited by colchicine! In order to assemble such labile microtubules, it was important that the calcium ion concentration be kept low and that some magnesium ion and guanosine triphosphate (GTP) be present in the neutral (organic) buffer. These findings of Weisenberg's were rapidly confirmed (71, 72) and a new era in microtubule research had begun.

The labile microtubules which could now be assembled in vitro were reversibly disassembled by cold. Indeed, following Olmsted and Borisy (73), purification of tubulin and associated proteins have since been routinely accomplished by cold-warm recycling. Likewise, isolated and reconstituted microtubules were reversibly disassembled by hydrostatic pressure just as were the mitotic microtubules in intact dividing cells (74, 75). In vitro polymerized microtubules were in equilibrium with a pool of assembly-competent tubulin (76), and assembly was promoted by D_2O (77, 78). These properties of labile microtubules indeed seemed to parallel the behavior of mitotic microtubules in vivo.[7]

In detail, however, the assembly properties of the isolated tubulin, with or without accessory proteins,[8] still appeared to

[7] For a summary of the chemical and physicochemical properties of isolated microtubules and associated protein, see Haimo and Rosenbaum (this volume), the monograph by Dustin (79), records of two conferences (80, 81) and the following reviews (77, 82–86).

[8] Accessory proteins include high molecular-weight components "MAPS," presumably including dynein, lower molecular weight "tau's," and some with molecular weight not too different from tubulin (reviews, 86–88, and Haimo and Rosenbaum, this volume). Although they seem to affect the assembly and stability of microtubules, their role in mitosis does not seem very clear at this point. I will have little further to say about these components nor about the role of cyclic nucleotides and phosphorylation of tubulin although this is a field receiving much attention lately. (See especially [84], Haimo and Rosenbaum, this volume, and the references given at the end of the last footnote.)

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be not quite the same as in living cells. In vivo, colchicine and Colcemid depolymerized labile microtubules[9]; in vitro, they acted primarily to prevent assembly and did not seem to take apart preformed microtubules (63, 73, 83). In vivo, D_2O shifted the equilibrium toward more microtubules (49, 94), whereas in vitro, it primarily raised the rate of microtubule assembly but not the amount of microtubules in equilibrium with tubulin (77; but also see 95). In vivo, the net assembly reaction appeared to fit a simple equilibrium model TUBULIN ⇌ MICROTUBULES (assuming spindle-fiber birefringence to measure the concentration of its component, parallel aligned, microtubules) (35, 74, 94). In vitro, the tubulin (dimers) would be expected to enter and to leave at free ends of the microtubules and, as anticipated, the reaction was observed to take the form: MICROTUBULE + TUBULIN ⇌ LONGER MICROTUBULES (76). These differences may in part be accounted for by the fact that the microtubules in vivo appear to be constantly, and rather rapidly, turning over; they are in a dynamic equilibrium.

DYNAMIC EQUILIBRIUM: The concept of a steady-state, or dynamic, equilibrium had been postulated earlier for spindle fibers in living cells.[10] Also, a UV microbeam of appropriate dose[11] could induce an area of reduced birefringence ("arb") on a spindle fiber, and it was found that this marker traveled poleward in a metaphase crane-fly spermatocyte at ¼–½ μm/min, a velocity approximately equal to the anaphase poleward velocity of chromosomes at similar temperatures (Fig. 4) (50, 97). Likewise, in a (Nomarski) differential interference contrast microscope, "particles or states" barely resolvable with the light microscope were seen traversing poleward along chromosomal spindle fibers in *Haemanthus* endosperm cells and tissue culture cells (98, 99). These transport phenomena could be interpreted as reflecting a dynamic assembly-disassembly of kinetochore microtubules, the assembly occurring at the kinetochore and the disassembly at or near the spindle pole. The component tubulin molecule would then travel along the microtubule in a fashion similar to a link in a chain that is constantly being assembled at one end and disassembled at the other end. (To date, however, the mechanical properties of the arb are unknown, and there exists no tagging experiment that unequivocally shows a poleward flow of tubulin along mitotic microtubules in living cells.)

For a while, it appeared that the steady-state equilibrium of labile microtubules in vivo might explain the difference of their response to colchicine treatment, their thermodynamic properties, etc., as compared with the in vitro system. But even in vitro, the story has become more complicated. Microtubules are now known to be polarized and show a preferred end of growth (e.g., 100, 101, and Haimo and Rosenbaum, this volume). Additionally, Margolis and Wilson (102) have shown recently that "equilibrium" microtubules in vitro are also in a dynamic, steady state. Under equilibrium conditions, the net assembly of GTP-bound tubulin at one end of the microtubule is balanced by the net disassembly of guanosine diphosphate (GDP)-bound tubulin at the other end. Therefore, even at steady state in vitro, there is a net flow or "treadmilling" of tubulin along the microtubules; that rate can approach 1/10 μm/min in the presence of 10 mM adenosine triphosphate (ATP) (103; but also see 104 and the section on models).

CENTERS: In living cells, spindle fibrils, or mitotic microtubules, are assembled sequentially around centrioles or satellites, kinetochores, and in plant cell phragmoplasts, the cell plate (reviews in 87, 105–107). Pickett-Heaps (108) has called these structures collectively microtubule organizing centers (MTOCs). Indeed, when such centers are isolated from living cells they have the capacity, as shown in Fig. 5, to initiate the assembly of microtubules onto or around themselves in vitro (reviews 86, 88, Haimo and Rosenbaum, this volume).

In mitosis, microtubules would have to be properly assembled and dynamically anchored onto appropriate organizing centers in order to: form a functional bipolar spindle; proceed successfully through metakinesis and anaphase separation of chromosomes; and coordinate mitosis with cytokinesis. Thus the MTOC are somehow activated at the right time, location, and orientation (36). There are many studies on the structure and composition of centrioles and kinetochores (e.g., 88, 113, 114), but little is yet known of how the activity of these centers, and how the assembly capability of tubulin, are regulated. (See [110, 115–119] for suggestive results. For experimental dissection of centriole replication and cell division, see Mazia et al. [120] and Sluder [121]. The problem of *de novo* formation of centers is reviewed in [113; also see 122–124 and Haimo and Rosenbaum, this volume].) The concentration of assembly-competent tubulin can be altered by application of colcemid long before the cell enters mitosis (125), as might be expected if the activities of the centers primarily govern when and where assembly is to take place (36). On the other hand, the disassembly of microtubules in anaphase may be governed in part by removal of assembly-competent tubulin from the tubulin pool (126).

SPINDLE-ASSOCIATED MOVEMENTS: In parallel with the characterization of microtubules, the birefringent major linear elements of the spindle fibers, several other lines of approach were used to characterize the mitotic spindle and to explore the mechanisms of mitotic chromosome movements. The movements of chromosomes and particles in and around the mitotic figures were analyzed in living cells by bright-field and phase-contrast microscopy. As early as 1929, Bělař (34, 127) observed anisotropic "Brownian" motion in the anaphase spindle mid-region, the motion being decidedly greater parallel to the spindle axis than it was transverse to this direction. In the 1950s, through extensive frame-by-frame analysis of phase-contrast, time-lapse motion pictures, Bajer and Molè-Bajer (e.g., 128) followed the predominantly poleward expulsion of particles lying in the region between the chromosomes and the spindle poles. Östergren et al. (129) found that long arms of chromosomes were likewise transported poleward in prometaphase. The poleward flow of "particles or states" at this stage

[9] Behnke and Forer (89) summarize evidence for the presence of microtubules with varying degrees of stability in living cells. Others have pointed out that kinetochore microtubules are often more resistant to colchicine, cold, hydrostatic pressure etc., then the astral and interpolar or nonkinetochore microtubules (e.g., 25, 90–93).

[10] The term "dynamic equilibrium" used in some of my earlier papers (e.g., 49) referred to a labile, equilibrium assembly of subunit protein coupled with the dynamic nature of the fibers. The latter point is stressed in Wilson (2), Östergren (29), Wada (31), and Inoué (36). The interpretation that their fibrils were also in a steady state-flux with a dynamic through-flow of subunits was gradually developed over the years. The distinction between the two types of "dynamic" properties are clearly defined (in 96, page 6).

[11] The dose of UV used for microbeam irradiation is highly critical because the irradiation also produces a diffusible toxic product that abolishes spindle birefringence. Failure in critical adjustment of the dose (best accomplished by observing spindle birefringence change) probably accounts for the diversity of results reported in the literature.

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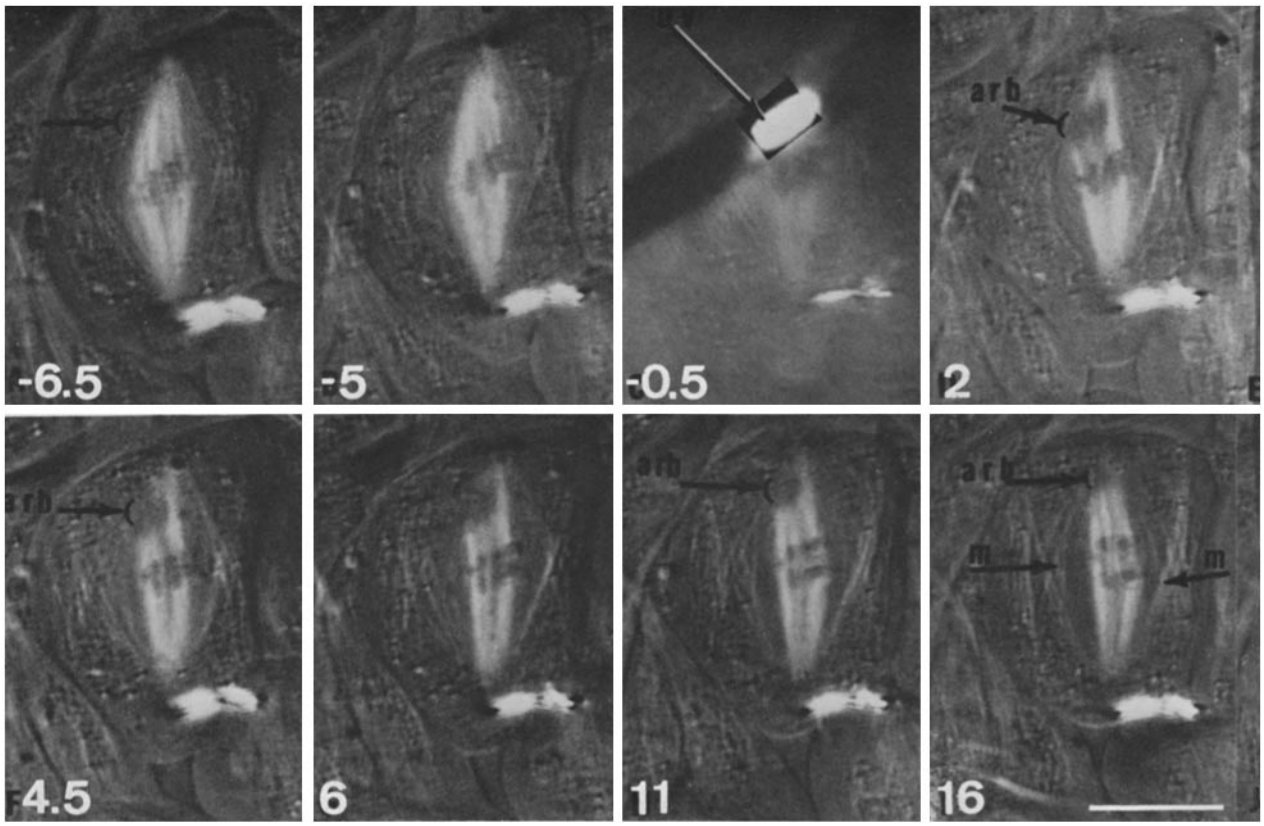

FIGURE 4 Ultraviolet (UV) microbeam irradiation of crane fly spermatocyte in late metaphase observed with a polarizing microscope. The bright patch (arrow) in the third frame shows the area to be irradiated by the heterochromatic UV microbeam. An area of reduced birefringence (arb) is induced in the spindle fibers by the microbeam irradiation. Note the gradual migration of the arb towards the upper spindle pole. Anaphase started 6 min after the irradiation. Mitochondrial sheath (m) surrounds the spindle. Time in minutes after irradiation. From Forer (97). Bar, 20 μm.

was mentioned earlier. In addition to poleward transport, time-lapse motion pictures at times displayed a striking lateral transport (130, 131) and compacting of nonkinetochore and phragmoplast microtubules (see [132] for grasshopper spermatocytes, [133] for *Haemanthus* endosperm cells). Bajer and Molè-Bajer (134) and Lambert and Bajer (135) found, by electron microscopy, that long stretches of microtubules are often "zipped" together in those cells (see later).

Mitochondria, yolk granules, and vesicles migrate radially toward and away from the spindle pole in a jerky, saltatory motion along astral rays (13, 136, 137). During prometaphase and metaphase, the centrospheres at the spindle poles can grow considerably in size, which perhaps reflects the transport and accumulation of vesicles into that region (but see 107). Similarly in telophase, small vesicles that are seemingly undergoing Brownian motion accumulate at the mid-region of a plant phragmoplast. There they fuse laterally to form the cell plate (138) by a process possibly reversing the pinching-off of vesicles from the Golgi body (Fig. 6). Whereas these movements have in common the transport of particles in a direction parallel to the lengths of microtubules, the nature of the transport mechanisms still remains to be solved (134, 137).

The whole spindle, as well as the nucleus, sometimes rock back and forth or spin around slowly. Such behavior is especially prominent in time-lapse motion pictures (e.g., 36, 139, and especially 140). Spindle-rocking is often accompanied by the "northern-lights" flickering of birefringence seen in the fibers, in and around the spindle. Some of these movements give the impression of being mediated by (microtubule) assembly-disassembly (36). Other aspects, such as the nuclear rotation, may be related to the revolution of the (actin-based?) polygonal cytoplasmic filaments studied in *Nitella* cytoplasm by Jarosch (141) and Kamiya (142; also see the variety of cytoplasmic movements described in 143, 144, and in Allen and Pollard in this volume).

In centrifuged living cells, particles accumulate along the centripetal side of the spindle, and spindle fibers and chromosomes are distorted. The pattern suggests a considerable mechanical integrity of the metaphase, but not anaphase, central spindle and of the chromosomal fibers which anchor the chromosomes to the poles (145, 146; summary in 6). Similarly, the premetaphase stretch of chromosomes, studied in detail by Hughes-Schrader (147, 148) in mantids and other insect spermatocytes, indicates, even before metaphase, a poleward force acting on the kinetochores of the unseparated sister chromatids.

These earlier analyses, and Östergren's classical studies on the paradoxical chromosome behavior during mitosis in *Luzula*,[12] reflect the dynamic mechanical behavior of spindle fibers (Östergren [29]). These intriguing mechanical properties

[12] In early metaphase of *Luzula purpurea* cells, the holokinetic chromosomes are interlocked in such a way that they could not undergo anaphase separation without breakage either of the chromosomes themsleves or of their kinetochore fibers. While the process of unlocking has not been seen in living cells, Östergren deduced from the abundance of normal anaphase figures in his fixed specimens that the kinetochore fibers must have been labile enough to be broken and reformed at the beginning of anaphase (29).

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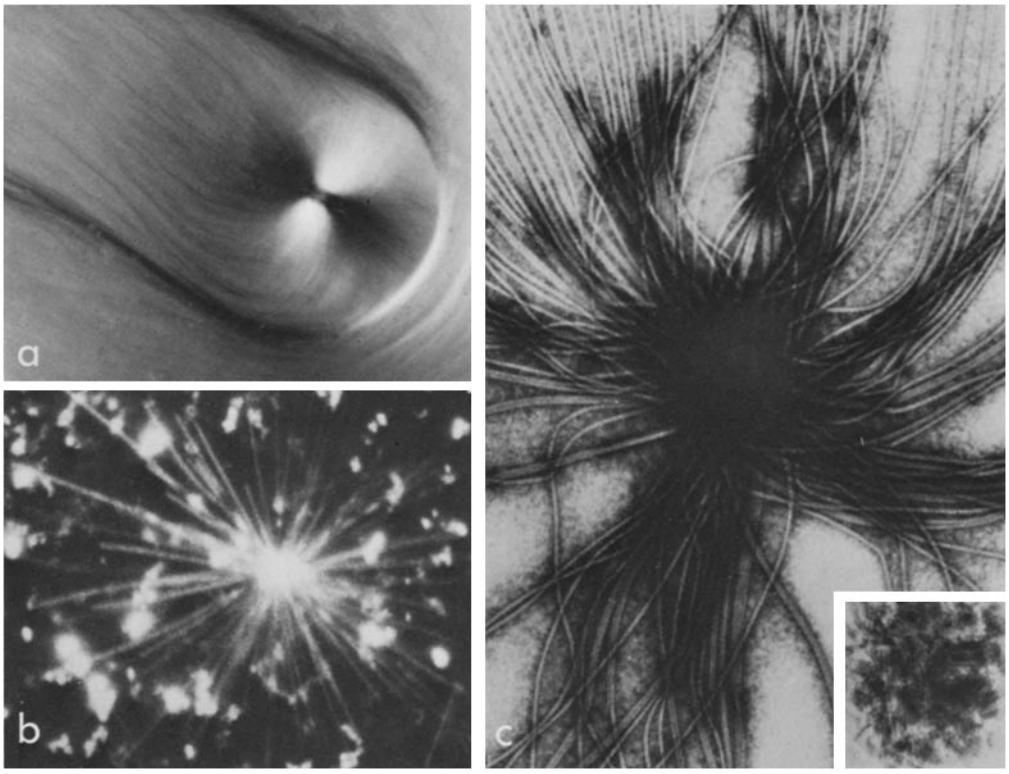

FIGURE 5 Artificial asters grown by addition of purified, heterologous tubulin onto isolated centrosomes. (*a*) Low power (~× 550) view in the polarizing microscope. Tubulin extracted from pig brain was polymerized onto a centrosome pressed out from a *Chaetopterus* oocyte in metaphase. The microtubules have grown to over 100 μm in length and show as long birefringent streamers. From Inoué and Kiehart (109). (*b*) Medium power (~× 2,200) view in dark field microscopy. Tubulin from chick brain was polymerized onto a centriolar complex isolated from HeLa cells blocked in "M-phase" with Colcemid. From Telzer and Rosenbaum (110). (*c*) Electron micrograph (~× 16,000) of a negatively stained preparation. Tubulin from pig brain was polymerized onto centrosomes isolated from CHO cells blocked with Colcemid. (*Inset*) The central region of the same micrograph printed lighter at a higher magnification (~× 30,000) to show the pair of centrioles. From Gould and Borisy (111). On isolated chromosomes, the kinetochores have similarly been shown to serve as microtubule-organizing centers (86, 111, 112).

of spindles have been investigated further by probing the interior of living cells with micromanipulation.

MICROMANIPULATION: The earlier micromanipulation studies of Chambers (149) and others were extended by Wada (150) and by Carlson (11) to analyze spindle structure. More recently, extensive and intricate manipulations of the chromosomes and spindle parts have become possible by use of the piezoelectric micromanipulator developed by Ellis (151). Thus Nicklas and co-workers (152, 153; review and interpretation in 23; also see 154) and Begg and Ellis (155, 156) were able to demonstrate the following: when a fine glass needle is inserted into a chromosome and gently tugged away from the spindle pole, the chromosome extends but the kinetochore-to-pole distance is virtually unchanged. Individual chromosomes (or chromosome pairs) can be swung about the spindle pole without disturbing other chromosomes (152, 155).

If the cell is already in anaphase, a chromosome can easily be pushed toward the spindle pole. That chromosome then waits until the other chromosomes catch up before it recommences its poleward travel. The chromosomes behave as though they were all being reeled in to the pole by individual fishing lines each attached to the kinetochore, but all sharing a common reel (155).

With the piezoelectric micromanipulator, Nicklas and Koch (157) detached individual chromosomes from their spindle fibers by tweaking the fiber near the kinetochore. Detached metaphase chromosomes reestablish a connection to the spindle; a kinetochore is drawn toward the pole it now faces. That may or may not be the pole to which it was originally joined!

The mechanical strength of a chromosomal spindle fiber was found to increase in parallel with its birefringence (156). Fiber strength increases in prophase as the fiber birefringence grows. Likewise, the mechanical integrity of the fiber disappears as fiber birefringence is eliminated by colchicine, and recovers as the birefringence returns during recovery from colchine treatment. Strangely, the fiber is also more stable, and chromosomes spontaneously detach and reorient less frequently, when the fiber is under tension (153).

These micromanipulation experiments directly confirmed and, to some extent, clarified the twin paradoxical properties of the spindle fibers, mechanical integrity and lability. Ellis and Begg (158) have prepared a comprehensive, thoughtful summary of the mechanical properties of the fibers connecting the kinetochore and the spindle pole, as revealed by micromanipulation studies.

The earlier micromanipulation studies by Wada (150) are also interesting. Even though he acknowledged the labile attributes of the fibers, Wada (31) held firmly to his view that the nuclear membrane never breaks down during mitosis in higher eukaryotes. This view is contrary to the essentially universal observation that the nuclear envelope does break down during mitosis in such cells (see later for the many exceptions found in lower eukaryotic mitoses). Even so, the shape and volume of the spindle often do resemble those same attributes of the

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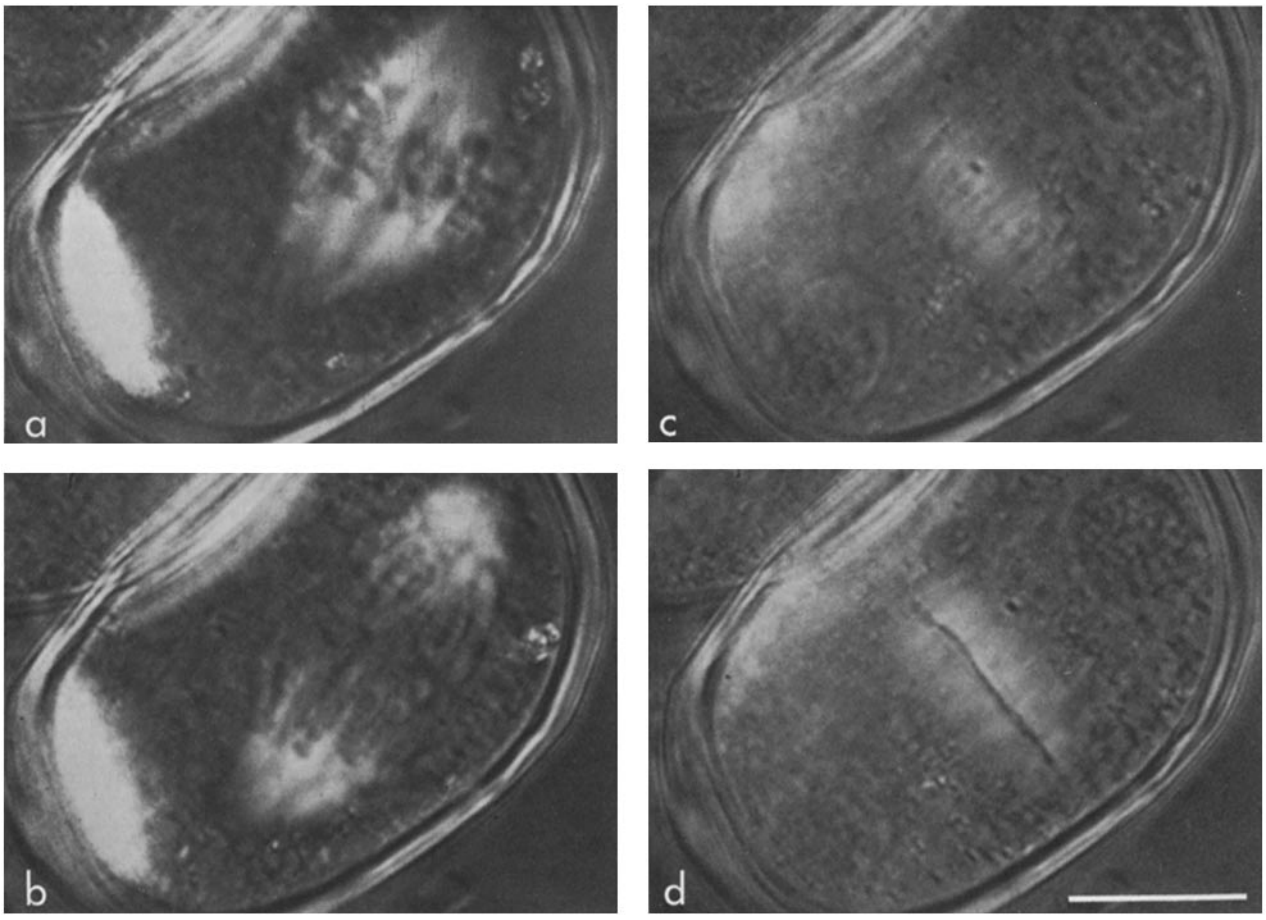

FIGURE 6 Pollen mother cell of *Lilium logiflorum*. Selected frames taken from a 16 mm time-lapse movie taken with a sensitive polarizing microscope. (*a*) Late metaphase. The chromosomes (dark gray) are still on the metaphase plate. They are not yet stretched but the strong birefringence (brightness) of the chromosomal fibers indicates that the cell is about to enter anaphase. (*b*) Mid-anaphase. The helical chromosomes, led by the birefringent chromosomal fibers, are just separating. The strong birefringence (signifying high concentration of microtubules) of the chromosomal fiber adjacent to the kinetochore persists from late metaphase to mid-anaphase. Notice weaker birefringence toward the poles and the absence of asters (cf., Figs. 1 and 11, and footnote 20). (*c*) Telophase. Chromosomes have formed the daughter nuclei. Between them the birefringence of the phragmoplast fibers is considerably stronger than was the mid-zone of the anaphase spindle (cf., *b*). Many new microtubules have been formed oriented parallel to the sparse microtubules that remained behind the separating chromosomes in late anaphase. Small vesicles are beginning to accumulate at the middle of the phragmoplast. (*d*) Cell plate formation. The vesicles have fused at the middle of the phragmoplast and have started to form the cell plate. The phragmoplast and the cell plate continue to grow laterally until the cell is completely divided. From Inoué (36). Bar, 20 μm.

nucleus before nuclear envelope breakdown. Nucleoplasm and the hyaline cytoplasm clearly must mix in establishing the spindle (e.g., see the extensive inclusion of ribosomes (?) amidst spindle microtubules in Fig. 7), but larger organelles[13] are excluded or expelled from the spindle region. In fact, the mitotic figure is frequently visible in living cells as a clear region from which most microscopically detectable granules are absent and which is outlined by mitochondria, yolk granules, etc. What accounts for this separation? This may be explained in part by the fact that the spindle is embedded in its own gel matrix. In addition, it may reflect another component that participates in mitotic cellular organization, the membranes.

MEMBRANES: Recently, increasing attention has been paid to the amounts of cytoplasmic membranes surrounding, although not completely enveloping, the spindle (e.g., Fig. 7, top right). As shown earlier by Porter and Machado (162) and more recently emphasized by Hepler (163), some lamellar or tubular cisternae also penetrate the spindle from the poles parallel to the chromosomal fibers. Harris has called attention to the many vesicles found in that region and especially the spindle-pole regions in sea urchin eggs (however, see 107). Are these, as Harris (164) postulates, calcium-sequestering or -releasing structures (in analogy with the sarcoplasmic reticulum in muscle cells)? Calcium seems to be accumulated within the vesicles or vesicular membranes (163, 165, 166). The distribution of vesicles and reticular membranes in and around the spindle, as well as the sensitivity of microtubules (70, 167), actin gels (168, 169), regulator-bound actomyosin (170, 171) etc. to micromolar concentrations of calcium ions, suggests some regulatory role for these membranes as discussed below.

CALCIUM: During the 1930s to 1950s, Heilbrunn sug-

[13] In some cells the nucleolus is not expelled from the spindle and is even regularly divided into two (e.g., 2, 6). Also see Cooper (161) for chromosome shaped "equatorial bodies" which retain the shape of chromosomes and remain on the metaphase plate as the chromosomes move poleward in anaphase.

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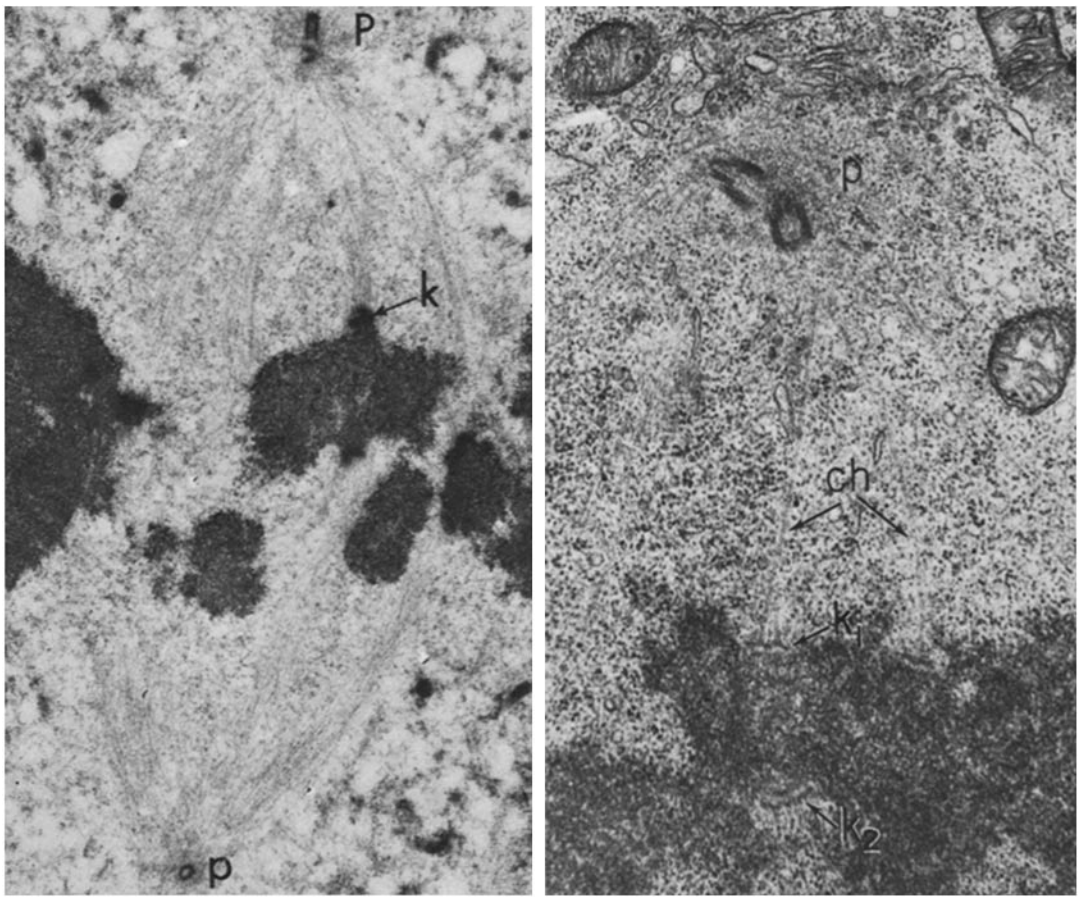

FIGURE 7 Electron micrographs. (*Left*) Thick (0.25 μm) section of a PtK_1 cell in early metaphase, observed at low power (~× 7,000) with a high voltage electron microscope. Both poles (p) of the spindle are clearly visible. Bundles of microtubules making up the chromosomal fibers run from the kinetochore (k) towards the spindle pole (cf., Fig. 2). The ribonucleoprotein stain employed in this preparation darkened the inner plate of the trilaminar kinetochore. From Rieder (159). (*Right*) Thin section of a rat kidney tubule cell in metaphase. The two kinetochores (k_1, k_2) of one chromosome clearly show the trilaminar structures. Chromosomal microtubles (ch) appear to terminate on the outer layer of the kinetochore. One pole (p) of the spindle is marked by a pair of centrioles. From Jokelainen (160). ~× 31,000.

gested a multifaceted physiological role for calcium ions (e.g., 172). While he was often scoffed at by his contemporaries, his now proven postulate regarding the sequestering and release of calcium and its role in the regulation of muscle contraction was prophetic (173). Heilbrunn further attributed to calcium ions the capacity to induce "mitotic gelation" in analogy with blood clotting. A calcium-activated ATPase was later found to be associated with the isolated mitotic apparatus (174). On the other hand, recent findings suggest that calcium ions solate, rather than gel, some of the components relevant to mitosis. In the presence of millimolar calcium, isolated microtubules depolymerize rapidly (70, 175). Purified microtubules reassociated with calmodulin, a calcium-binding protein similar to the muscle protein troponin C, rapidly depolymerize in the presence of calcium ions at even micromolar concentrations in vitro (167).

When microinjected into sea urchin eggs, millimolar calcium chloride or EGTA-buffered micromolar concentrations of calcium ions depolymerize spindle microtubules locally and instantaneously. The process is so rapid that the progression of birefringence loss could not be followed under continuous observation (Kiehart [165]). The portion of the spindle whose microtubules are depolymerized is so limited that it shows as a discrete, sharply delineated patch from which the birefringence has disappeared (Fig. 8). This observation complements

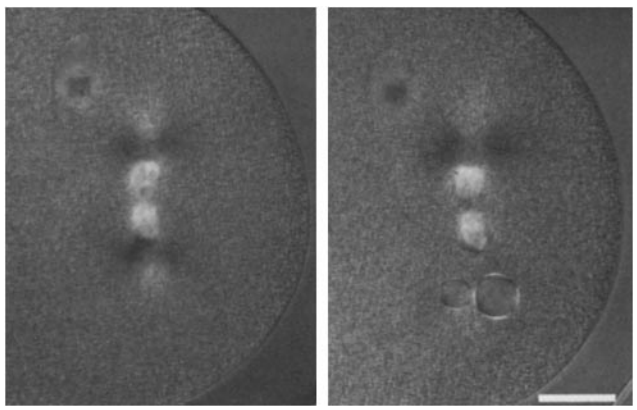

FIGURE 8 Microinjection of 1 mM $CaCl_2$ into *Asterias forbesi* egg at metaphase observed in polarized light. (*Left*) First injection at the upper left away from the spindle pole produced no effect on the spindle birefringence. (*Right*) Injection at the lower pole eliminated the birefringence of the aster and the tip of the spindle. Note the very sharp contour of the remaining spindle. When calcium-buffer solutions are injected, 5–10 μM equivalents of free Ca^{++} ions locally eliminate the spindle birefringence. Each pair of oil drops that had been used to cap the test solutions in the micropipette before the injection, indicates the volume of the test solution and the approximate site of injection. From Inoué and Kiehart (109). Bar, 30 μm.

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Rose's and Loewenstein's findings (176) that when calcium ions are microinjected into *Chironomus* salivary gland cells previously loaded with aequorin (a calcium-dependent, light-emitting protein), light is emitted only in that portion of the cell into which the calcium solution is directly applied. In other words, although it is a small, diffusible ion, calcium is sequestered so rapidly in the cytoplasm that the relatively high injected concentration is limited to the region of the cell that receives the microinjection directly.

The cytoplasmic membranes and mitochondria are likely candidates as calcium sequestrants. The calcium-dependent aequorin glow is no longer limited to the site of injection, but is spread out in the presence of respiratory poisons (176). Similarly, Sawada and Rebhun (177) have found that the birefringence of the spindle in some cells is abolished when the cell is exposed to respiratory poisons or uncouplers of oxidative phosphorylation. These agents, as well as caffeine, probably poison the calcium-pumping ATPase, making the cell membranes leaky to calcium ions and inducing the mitochondria and endoplasmic reticulum to dump their accumulated calcium ions. In caffeine-microinjected cells, there is a drop in spindle birefringence that is not sharply delineated, but diffuse, presumably because in contrast with calcium ions, caffeine is not sequestered by the cytoplasm and therefore diffuses normally (165).

Petzelt's calcium-dependent ATPase appears to be membrane bound (178). Membrane-delimited vesicles and cisternae are seen by electron microscopy to be concentrated at the spindle pole and in a sheath surrounding the spindle (163). Thus, as postulated by Harris (164), these membranes may well play an important role in calcium regulation of the cytoplasm, and they may do so in highly localized cell regions. Welsh et al. (179) observed, by fluorescent antibody staining, a higher concentration of calmodulin in the half-spindle. The anaphase location of calmodulin near the spindle poles, and the changes observed in their distribution during late anaphase, suggested to Marcum et al. (167) that calcium is an endogenous regulator of microtubule assembly through the activity of calmodulin (also, 180).

Models for Mitosis

We shall now consider some current models which have been proposed to account for the (anaphase) movement of chromosomes. The models have been reviewed (23, 134, 181) and extensively discussed (e.g., 79–81, 144, 182), except for the recent model that incorporates the treadmilling of tubulin along microtubules (183).

ACTIN AND MYOSIN: One of the oldest models for poleward movement of chromosomes invoked the contraction of a muscle-like fiber which linked the chromosome to the spindle pole (pages 178–184 in [2], pages 70–75 in [6]). From the earliest days, however, this model has been repeatedly questioned. Shortening chromosomal fibers generally do not get thicker; anaphase velocity is so much lower than the contraction velocity of skeletal muscle (of the order of 10 nm/s, in contrast with 100 μm/s for muscle); and the lability of the spindle fibers does not fit the properties of muscle.

For many years there was no reason to resurrect this model, although the counter arguments were not airtight. But recently the model has again gained some support. Actin and myosin, the two major proteins responsible for force production by muscle fibrils (review; e.g., 184, 185), were detected in the half-spindle regions of glycerinated cells. Fluorescent antibodies made against these proteins stained the spindle (186, 187), as did fluorescein-conjugated heavy meromyosin or subfragment-1, which carry the active ATPase sites of the myosin molecule (188–191). (However, the levels of immunofluorescent staining for actin and myosin are not greater in the spindle, according to the latest report from Aubin et al. [192].) In electron micrographs of glycerinated cells, some actin filaments were seen to terminate at or about the kinetochore and to run approximately parallel to microtubules in the half-spindle (e.g., 190, 193).

Whereas these observations on glycerinated cells are suggestive and have attracted considerable attention, the data in themselves do not imply a functional role for actomyosin or an actin system in the poleward movement of chromosomes. Like tubulin, actin is one of the major protein constituents of most cells (each at times amounting to several percent or more of the total cell protein). The spindle region in dividing cells excludes granular organelles such as mitochondria and yolk, so that, on this basis alone, one might expect to find a somewhat higher concentration of nonparticulate cytoplasmic constituents, including actin, in the spindle region of some cells (165, 194; also see Fig. 2 in 195 for a model demonstrating this point).

Two types of tests for the functional role of actomyosin and actin in mitosis have yielded negative results. The microinjection of an antibody against starfish-egg myosin (previously shown to suppress hydrolysis of ATP by egg myosin) prevented many successive cleavage divisions but did not interfere with mitosis in the same starfish eggs (Mabuchi and Okuno [196]). This is consistent with the strong evidence that cleavage is brought about by an actomyosin contractile ring (review, 197). In the eggs injected with anti-egg myosin (Fig. 9), birefringent spindles formed at regular intervals, chromosomes moved toward the spindle poles and spindles elongated normally in anaphase, and nuclear envelopes were reconstituted on schedule, despite the absence of eight or more cleavages (Kiehart [165]).

Likewise, in Cande's detergent-permeabilized tissue culture cells, cleavage was suppressed by the application of heavy meromyosin or subfragment-1, which had been treated with *N*-ethyl maleamide (198). These treated fractions of myosin bind to actin competitively and prevent the interaction of actin with normal myosin (199). In the permeabilized cells, cleavage was arrested, but anaphase movement was not affected by application of the modified myosin fragments. Chromosome movement, especially the part dependent on spindle pole-to-pole elongation was, however, reversibly inhibited by vanadate, a potent inhibitor of ciliary dynein ATPase (200). This ion showed little effect on cleavage in lysed cells, reinforcing the idea that different molecular mechanisms are operating in chromosome movement and cleavage.

In living cells, cleavage is suppressed, or regresses, when cells are bathed in solutions that affect actin gelation. Cytochalasin B and D are reported to weaken actin gels (201, 202), but not to affect mitosis when applied in concentrations adequate to suppress cleavage (e.g., 197). In contrast, colchicine and podophylotoxin, which prevent mitosis and even disassemble mitotic microtubules, do not affect cleavage once the cleavage message has been delivered from the spindle to the potential furrow (26, 203). Actin and myosin have been extracted from the cell cortex, and electron micrographs show a clear band of actin filaments in the cell cortex oriented circumferentially in the cleavage furrow (review, 196). Taken together, these data strongly support the role of an actomyosin system in cytoplas-

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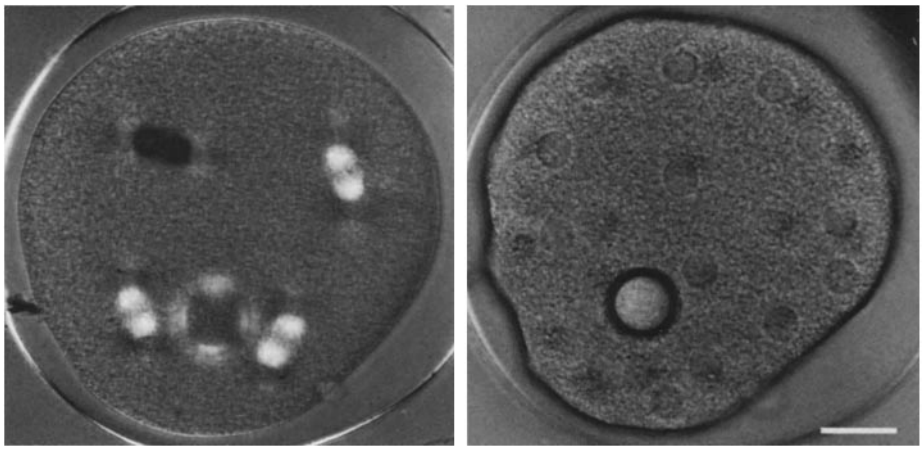

FIGURE 9 Egg of a starfish, *Asterias forbesi*, microinjected with an antibody made by Mabuchi and Okuno (196) against myosin of another species of starfish, *A. amurensis*. Cleavage is suppressed for up to nine divisions, but mitosis is unaffected. One nanogram of IgG, containing the antimyosin, was injected before first cleavage. (*Left*) Third division spindles seen in polarized light. (*Right*) Over 30 nuclei are visible 2.4 hours later. From Inoué and Kiehart (109). Bar, 30 μm.

mic cleavage, but do not favor the involvement of actin or an actomyosin system in mitotic chromosome movement.

ASSEMBLY-DISASSEMBLY OF MICROTUBULES: This model postulates that assembling microtubules, by their extension, push organelles apart, and slowly disassembling microtubules, by their shortening, pull organelles together. I conceived the model through the observation of *Chaetopterus* oocytes exposed to colchicine or cold. As the spindle-fiber material slowly depolymerized in these metaphase-arrested cells, the chromosomes and the inner spindle pole were transported toward the outer spindle pole, which is anchored to the cell cortex (Fig. 3). As the fibers reassembled upon removal of the depolymerizing agent, the chromosomes and inner pole were transported away from the outer spindle pole. Too high a dose of colchicine or overrapid chilling simply caused the spindle fibers to fall apart without appreciable displacement of chromosomes or pole (24, 25). This model at once seemed to explain the labile, yet cohesive, nature of the forces that held together the ephemeral fibrils of the spindle (36), as well as the slowness of chromosome movements.

The subsequent discovery of labile microtubules added credibility to the assembly-disassembly (or dynamic equilibrium) hypothesis (35, 49, 204), but its validity has been repeatedly questioned, presumably in part because the proposal is not intuitively compatible with macroscopic mechanics.[14] Nevertheless, Salmon (74) and Fuseler (126) performed experiments utilizing hydrostatic pressure and cold as microtubule depolymerizing agents, and confirmed that spindle shortening and chromosome movements are induced in living metaphase cells by the depolymerizing agents.[15] They also demonstrated a strict proportionality between the velocity of induced spindle-fiber shortening (and of natural anaphase movement) with the rate of microtubule depolymerization. Although slow depolymerization of microtubules induced a shortening of chromosomal fibers, the induced movement ceased altogether when the rate of microtubule depolymerization became too great, whether the depolymerization was induced by pressure, temperature, or colchicine (review, 96, 205). In Salmon's words (74), "Polymerization of microtubules does produce pushing force and, if controlled microtubule depolymerization does not actually produce pulling forces, at least it governs the velocity of chromosome-to-pole movement."

Whether or not it turns out that shortening microtubules can exert pulling forces in addition to the pushing forces generated by their growth, the dynamic anchorage of microtubules is essential for force transmission through the assembling and disassembling microtubules. In this context, dynein, the ATPase associated with ciliary and flagellar microtubules (206, 207) has lately received much attention. Cytoplasmic dynein has just been isolated and characterized (208), and its role in anaphase movement, at least in pole-to-pole elongation, finds much experimental and observational support, as discussed below.

THE SLIDING MODELS: In 1969 McIntosh et al. (209) proposed that mitotic chromosome movement was brought about by a combination of microtubule sliding (in analogy with muscle contraction and ciliary and flagellar beat) and microtubule assembly and disassembly. Though the details of this model soon needed to be revised (23, 210), it nevertheless struck a favorable chord with many investigators. The labile and dynamic attributes of the spindle fibers were ascribed to demonstrated properties of microtubules, and force production could be attributed to (dynein) cross bridges, whose ability to induce relative sliding of ciliary microtubules was soon to be established (211; review, 212).

While seeming to provide a rational model, some predictions of which were quite readily testable, the model failed to account for certain properties of some spindles. In studying mitosis in yeast and other lower eukaryotes,[16] Roos (213), Peterson and Ris (214), Heath (215) and others came upon connections between chromosome and pole (plaque) that consist of single microtubules! Some of the nonkinetochore microtubules that make up the central spindle appeared to span the whole distance between the spindle-pole structures, even as the spin-

[14] Contractile force production by a disassembling microtubule can be explained by viewing the labile microtubule as a cylindrical micelle, as explained in Inoué and Ritter (96).

[15] Salmon has now induced spindle-shortening and chromosome-to-pole movement in isolated metaphase spindles (of the type shown in Fig. 1), by depolymerizing the labile microtubules with micromolecular concentration of calcium ions.

[16] For mitosis in lower eukaryotes, which show many interesting and potentially instructive variations on mitosis and mitotic organelles, see (4, 20, 55, 215–218) and the excellent review by Kubai (219).

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dle elongated. Further, the central spindle microtubules appeared not to be in locations where they could interact with the "kinetochore" microtubules.[17] The single "kinetochore" microtubules appeared to shorten and to bring the "chromosomes" to the spindle pole independent of spindle pole-to-pole elongation.

In serial sections of cultured cells, Brinkley and Cartwright (221) did not find the number distribution of microtubule cross sections predicted by the McIntosh et al. model. On the other hand McIntosh et al. (222, 223) do report finding the distribution of microtubules appropriate for their model. Manton et al. (224) in an elegant study on mitosis and meiosis in a centric diatom, counted microtubule numbers in serial sections that are compatible with the overlapping of central spindle microtubules. In contrast, central spindles in some protozoa are known to elongate much more than twice their initial length, so sliding alone cannot account for spindle elongation; at least some spindle fiber growth is required (e.g., 55).

Despite these reservations, Tippit et al. (225) and McDonald et al. (226, 227) provide a most striking illustration of the overlapping microtubules in a configuration highly suggestive of interactions between oppositely polarized microtubules. In the mid-region of a diatom (*Melosira*) spindle, the cross sections of microtubules are arranged in a regular orthogonal array. And every other microtubule appeared to be connected to opposite spindle poles! In later anaphase, the overlap of the central spindle microtubules progressively decreased, although concomitantly there was growth of some microtubules (also see 228).

Although dynein has not yet been unambiguously demonstrated between opposing microtubules (summary regarding intertubule arms [229]), extensive periodic cross bridges have been seen in the metaphase (extranuclear) central spindle in a hypermastigote protozoan (Fig. 10). These observations, which might suggest the involvement of dynein-mediated sliding, are in fact fortified by two functional tests.

In isolated mitotic apparatuses, Sakai et al. (230) observed chromosome movement, which, while considerably slower than in living cells (but see improved movement reported in [231]), appeared to exhibit general features of in vivo anaphase movements. The movement which required a labile mitotic apparatus was prevented by excess tubulin in the medium and especially by vanadate ions and antibodies formed against dynein (review, 232).

These conclusions were complemented by observations in another type of cell model by Cande and Wolniak (200). In detergent-extracted rat-kangaroo cells in tissue culture, chromosome movement could be stopped by vanadate ions in the +5 oxidation state. After the chromosomes had stopped, they could be restarted by converting the vanadate to the inactive +4 state via the addition of norepinephrine. As shown by Gibbons et al. (233), the +5 vanadate is a potent inhibitor of ciliary and flagellar ATPase (however, see [234] for a lower vanadate sensitivity of cytoplasmic dynein).

Whereas these ongoing experiments require further confirmation,[18] the experiments on isolated spindles and extracted cell models strongly suggest the involvement of dynein in anaphase chromosome movement. It would seem quite likely that a dynein-mediated sliding mechanism is at least in part responsible for the pole-to-pole extension of the anaphase spindle. It is not as clear whether a dynein-mediated sliding is involved in the chromosome-to-pole movement. Perhaps dynein is a dynamic anchor for the kinetochore microtubules, but then we still must ask, how does microtubule disassembly govern the velocity of poleward chromosome movement?

FIGURE 10 Electron micrograph of central spindle in a hypermastigote protozoan *Barbulanympha* sp. Extensive cross bridges are seen in this micrograph, reinforced by superimposing two transparencies of the same image translated once along the microtubule axes. From Inoué and Ritter (96); also see Ritter et al. (55).

TREADMILLING: Recently, an alternative to the McIntosh et al. model, placing a greater emphasis on the role of microtubule assembly-disassembly was introduced by Margolis et al. (183). These authors found in vitro a "treadmilling" of tubulin through microtubules that were in an assembly steady state with soluble tubulin dimers in the presence of an adequate and continuous supply of GTP.[19] They postulate for dividing cells that all mitotic microtubules add microtubule subunits at the equatorial region of the spindle and at the kinetochores, and that the microtubules lose subunits at the spindle poles. They also propose that nonkinetochore microtubules, which overlap at the equator, slide by each other at a rate needed to keep the spindle poles separated. Kinetochore microtubules are thought to form a parallel linkage to the treadmilling interpolar microtubules (183). Depending on the relative rates of tubulin incorporation into the kinetochore and nonkinetochore microtubules and their rates of disassembly, one could increase or decrease spindle length as well as the distance between kinetochores and the spindle poles. Margolis and Wilson (103) report that the in vitro rate of microtubule treadmilling can come close to anaphase chromosome velocity. Because dynein

[17] In yeast, mitosis takes place within the nuclear envelope. The poles of the intranuclear spindle lack centrioles but are organized around spindle pole plaques on the nuclear envelope. The trilaminar kinetochore structure generally seen on chromosomes of higher eukaryotes has not been observed in yeast and chromatin appears to associate directly with microtubules (213). Ris and Witt believe that even in organisms with a trilaminar kinetochore, the kinetochore microtubules are directly linked with the centromeric chromatin (220).

[18] Both the Sakai et al. isolates (231), and the Cande cell models (198) have yet to be refined before the inference of these results is fully accepted. Both models run "downhill" rapidly, and to the best of my knowledge, vanadate and dynein antibody inhibition of mitosis has not yet been observed in living cells.

[19] Weisenberg (personal communication), Bergen and Borisy (235), and Karr and Purich (104) emphasize that treadmilling is not caused simply by assembly at one end of the microtubule and disassembly at the other. Rather, both assembly and disassembly take place at each end, but their rates differ in such a way that the *net* assembly at one end is greater than the *net* disassembly at the other end.

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has now been used successfully to "decorate" microtubules and to indicate their polarity (Haimo and Rosenbaum [236]; also [101, 237, and Haimo and Rosenbaum, this volume]), we should soon be able to learn whether the polarities of mitotic microtubules conform to those stipulated in the Margolis and Wilson or McIntosh et al. model.

OTHER PROPOSALS: Before we leave the models for anaphase chromosome movement, we should take special note of the work by the Bajers and co-workers who emphasize the lateral transport seen in spindles and the lateral interaction believed to take place between mitotic microtubules (summary, 131, 134, 238). Since the late 1940s, the Bajers have extensively analyzed chromosome and particle movements and spindle behavior directly in healthy, dividing plant and animal cells and in cells treated with a variety of antimitotic agents. Their analyses on electron micrographs of cells that had been followed and recorded up to the time of fixation with time-lapse cinematography, suggested the importance of the changing lateral association of microtubules seen within individual kinetochore fibers, as well as between kinetochores and nonkinetochores microtubules (134). In general, the presence of intrakinetochore fiber association correlated with cessation of chromosome movement, whereas association between kinetochore and nonkinetochore microtubules was evident whenever chromosomes were moving poleward (but also see [93]).

Little is yet known of how these microtubular organizations are controlled, nor whether there is or is not sliding of microtubules associated with the lateral interactions, but the Bajers alert us to the possible role of microtubule interactions that could play an important role in anaphase chromosome movement (238). In contrast, Thornburg (239) proposed that viscous coupling associated with *intra*microtubular conformational change might propel the microtubules with their attached chromosomes.

Coordination of Cytokinesis with Mitosis

Once the chromosomes are partitioned into two equivalent (or, in meiosis, nonequivalent) groups by mitosis, how are the daughter nuclei placed in the proper cytoplasmic environment? This question is not only important for the successful completion of cell division, but also for determining the future role of the nucleus, because it is the cytoplasm surrounding the nucleus, rather than the unequal division of the nucleus itself, that generally determines how a particular cell is to differentiate (2, page 1059), (also see [5, 240–242]).

In astral mitosis,[20] a close correlation has long been noted between the metaphase spindle axis and the cleavage plane. When a cell was left undisturbed, the cleavage furrow almost always started from the cell surface nearest the spindle and in a plane bisecting the pole-to-pole axis of the spindle (Fig. 11). In centrifuged eggs, the cleavage furrow would appear in a new location dictated by the displaced spindle (e.g., 145). In fact, the correlation was so universal that most postulates for cleavage-furrow induction ascribed a major role to the mitotic spindle (2, 105, 203, 245, 246). Not only was there present a spatial correlation between spindle axis and cleavage plane; in the late 1030s to 1040s, Katsuma Dan and his co-workers showed a striking geometrical relationship between the extending anaphase spindle and the progression of cleavage. In sea urchin and jelly fish eggs, small particulate markers, which were applied directly to the cell surface near the impending cleavage furrow, moved along the exact path predicted from the separation of the astral centers as the spindle elongated. Dan assumed that the cell cortex was connected to the astral centers by interdigitating, inextensible astral rays by which the elongating anaphase spindle drew in the cell cortex, thus forming the cleavage furrow (summary in 247). Whereas this hypothesis could also account for the many unexpected cleavage patterns found in eggs deformed into toroids (248), two sets of experiments negated Dan's hypothesis.

In 1953, Swann and Mitchison (26) applied high doses of colchicine (3 mM in seawater) to metaphase sea urchin eggs and showed that cleavage nevertheless proceeded after destruction of the birefringent asters and spindle, providing the chromosomes had progressed to mid-anaphase (also see 27). Further, in 1956, Hiramoto (249) managed to suck out the entire spindle and asters from a dividing sea urchin egg and showed that a cleavage furrow appeared in the expected location so long as the cell had reached metaphase before spindle extraction (further, detailed analysis in [250]). He thus eliminated the possibility that cleavage was mechanically effected through a noncolchicine-sensitive element of the spindle and aster. These experiments clearly showed that the late metaphase-to-anaphase spindle and asters were unnecessary for cleavage, but it was equally clear that at an earlier stage the mitotic figure did determine the cleavage plane.

If the spindle before metaphase was artificially reoriented, the cleavage furrow appeared perpendicular to and bisecting the spindle in its new position (summary in 105). During a brief critical period, the spindle could even initiate up to ten cleavage furrows in succession, if the spindle were squeezed along the length of a sand-dollar egg previously deformed into a cylinder (251)! Clearly, then, a message[21] must be sent from the spindle to the cell surface where the cortical layer contracts and produces the cleavage furrow[22] (but see 254 and 255).

While the furrow was normally localized where the two asters (whose foci lay at the two poles of a spindle) overlapped, two asters not joined by a spindle could also induce cleavage (203, 246). The message for cleavage induction therefore comes not directly from the spindle itself, but from the spindle poles or astral centers. The speed of the message, and the duration required for the cortex to respond, were determined by displacing the spindle (251). Interestingly, the message travels along the astral rays at about 6 μm/min, approximately the rate at which microtubules grow.

In anastral mitoses,[20] especially in cells of vascular plants, a large number of microtubules appears between the separating chromosomes in late anaphase (138, 256). The ellipsoidal bundle of microtubules, the phragmoplast, has long been thought to arise from central spindle fibers (e.g., Strasburger, 1888, in

[20] Astral mitosis: with asters (Figs. 1 and 11). Typically, but not always nor exclusively, found in animal cells. Anastral mitosis: without asters (Fig. 6). Typically found in, but not limited to, higher plant cells. See Dietz (243) for experimental dissociation of asters from the spindle pole in a living cell, and Aronson (244) for analysis of attractive forces between (astral) centers and nuclei.

[21] The notion of a cleavage-inducing message (substance X which acted through polar relaxation) that traveled along the astral rays was proposed by Swann (18) and Mitchison (252).

[22] Sadly, I must leave out a series of intriguing accounts on the search for the mechanism of cleavage itself. Many interesting experiments were performed and ingenious hypotheses constructed (excellent summaries in [203, 246, 251, 253]). For our present purpose, we proceed by accepting the finding that the "contraction" of a cortical actomysin system is responsible for cell cleavage see (e.g., Schroeder. [197]).

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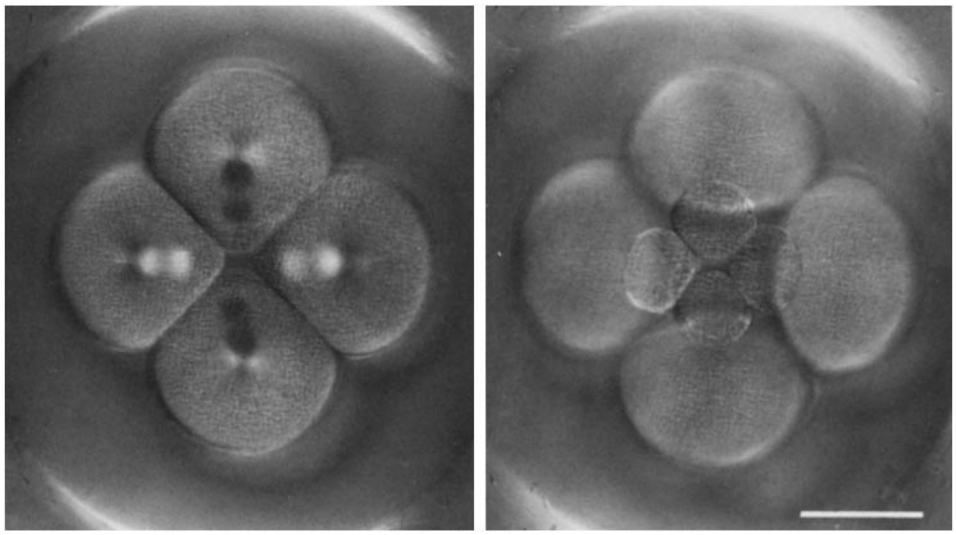

FIGURE 11 Fourth cleavage division in the egg of a sand dollar, *Echinarachnius parma*. The characteristically asymmetric cleavage of the eight-cell stage embryo is viewed from its vegetal pole in polarized light. (*Left*) Early anaphase. The positively birefringent spindles, and the asters at the center of each of the four cells in focus, stand out in bright or dark contrast. The spindles are tilted toward the observer at the vegetal pole (the middle of the picture). There, the astral birefringence is weak and the spindle fibers do not converge at the poles. (*Right*) Telophase. The four cells have cleaved perpendicular to the spindle axes and have given rise to four micromeres and four macromeres. Portions of the (birefringent) fertilization membrane show as bright crescents at the top and bottom of the pictures. From Inoué and Kiehart (109). Bar, 50 μm.

[2] page 160]). Time-lapse recording and direct observations with polarized light microscopy (Fig. 6) clearly showed the late anaphase waning of the central spindle fibrils (microtubules) and the dynamic waxing of the phragmoplast fibers (microtubular bundles), as well as the alignment of small "granules" (already observed by Becker [257] to be vesicular) at the midzone of the phragmoplast to form the cell plate (19, 133, 258; also see [259]). As summarized by Bajer and Molè-Bajer (134) and Hepler and Palevitz (260), these vesicles, which were postulated to be Golgi products (261, accumulate and fuse laterally in the midzone of the phragmoplast microtubule bundles to transform into the cell plate that divides the cell body into two. Cytokinesis thus takes place in the middle of the telophase spindle and insures the partition of the daughter nuclei into two cell bodies by a mechanism alternate to cleavage.[23]

In both astral and anastral mitoses, cytokinesis is coordinated with mitosis by an organized arrangement of mitotic microtubules and is not controlled directly by the chromatin or nuclei. In this respect also, the ephemeral achromatic fibers of the spindle and asters express the multifunctional, dynamic organization of the hyaline cytoplasm common to all cells.

Concluding Remarks

In this brief historical sketch, I have highlighted some of the research on the dynamic aspects and functions of the mitotic spindle that took place principally over the last quarter-century. This has been an exciting period in which the happy convergence of the morphological, physiological, and biochemical approaches, and the development and application of new methodologies, have led to major progress.

Although we are still searching for the exact molecules that move chromosomes in anaphase, we have learned much about the dynamic physiological behavior of microtubules, the ephemeral fibrils of the mitotic apparatus, and the hyaline kinoplasm of all cells.

The spin-off from these studies has improved our understanding of cell behavior in many unexpected directions: nerve and muscle growth, organogenesis, gametogenesis, secretory functions, phagocytosis, drug action, etc., (e.g., 79, 80). Thus, the major investments made by the investigators and the sponsors are bearing fruit, both for a better grasp of cell division and its regulation, and in the basic physiology of cytoplasmic organization applicable to an unexpectedly wide range of biomedical fields.

In so short a sketch, much interesting and important work could not be included, and my presentation is by no means balanced. Some important aspects of mitosis have not even been mentioned in this selective narrative. Fortunately, there are several excellent monographs and reviews that can remedy this situation. Several have been cited in the text, and the articles in the following references should provide a good introduction: for mitotic mechanisms and diversity (23, 134); for cell motility including mitosis and cytokinesis (81, 144); for mitotic microtubule assembly and its control (79, 86, 178); for the cell cycle and its regulation (265, 266); for mutants affecting mitosis (267, 268); and for an overview of mitosis and cell division (105, 269). Additionally, some earlier references, especially Wilson (2), Bělař (4, 34, 127), Wassermann (38), Gray (270), Hughes (139), and Schrader (6) contain much information and many ideas which sould be of contemporary and lasting value.

[23] Cytokinesis by cleavage and by phragmoplast formation are probably expressions of two extremes. It is quite possible that both contribute to cytokinesis in many cell types. Also note that the spindle is not always a single bipolar body, but may be made up of two or more smaller spindles arranged in parallel (e.g., 262, 263); also see (e.g. 264) for multipolar origin of a bipolar spindle.

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