Chromosome Motion and the Spindle Matrix

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During mitosis, three kinds of mitotic movement can be distinguished. Collectively they are responsible for chromosome separation. (For our present purposes, we will ignore several less common motions that are poorly understood, such as those of prophase chromosome movement.) Chromosome motion usually commences during prometaphase when the forming spindle invades the nucleoplasm. The consequent prometaphase interaction between microtubules (MTs)[1] and chromosomes generates the first type of motion, irregular chromosome oscillations directed initially at either pole that lead to the metaphase plate configuration. Anaphase usually involves two distinct phases (36): anaphase A moves the chromosomes to the pole, and during anaphase B, the spindle elongates. In this paper, we consider mainly prometaphase and anaphase A movements.

Once Inoué and collaborators (19) had shown that spindle fibers exist in living cells, cytologists could conceptualize the structural framework associated with mitotic movements. The advent of glutaraldehyde fixation made it possible to establish that these spindle fibers contain MTs. In most cells, a proportion of the MTs terminate in the kinetochores. This structural relationship was immediately correlated with other evidence suggesting that the kinetochores are the site of chromosome attachment to the spindle, where poleward forces are exerted upon the chromosomes. The deduction that these inserted MTs had been nucleated or polymerized by the kinetochore has received widespread support from biochemical (summarized in reference 30) and cytological observations, but recently there has been a reappraisal of this viewpoint. Furthermore, the attachment of MTs to the kinetochore suggests that they are mechanically functional in moving the kinetochore to the pole. Either or both of these conclusions are implicit in several models of mitosis. Although the issues are not yet decided, we believe that these two very influential conclusions are incorrect, and so current models of the spindle may be fundamentally flawed (30). Another serious shortcoming of all the well-known models is their inability to explain mitotic phenomena in all but the simplest terms; for example, the complex, erratic prometaphase activity of chromosomes has defied satisfactory explanation.

We have chosen two aspects of the many conceptual complexities of mitosis for discussion. The skeletal framework of the spindle is composed largely of MTs, and eventually one has to explain how this structure is assembled, organized, and then disassembled; our views on this aspect are set out elsewhere.[2] In the present paper we are concerned with the motility system that functions within the spindle. Whether spindle MTs are actively involved in generating chromosome motion remains unresolved, but we believe that MTs are passive in this context, and therefore we will discuss, with relevant evidence, a conceptual approach to explaining mitotic movement on this basis.

Kinetochore

The kinetochore is a key organelle in mitosis. We have maintained (27, 30, 39) that kinetochores do not significantly nucleate MTs, preferring the older idea that they attach to MTs originating from the poles (see also reference 35). During prometaphase, kinetochores in live cells exhibit vigorous pulling and pushing motions that arise in an unknown manner from a motile system functioning over a growing, skeletal set of polar MTs. We believe that during prometaphase MTs are soon "captured" by the kinetochore and come to terminate in it. The resultant spindle fibers (and perhaps their constituent MTs) may have to undergo considerable changes in length after their capture as some oscillating chromosomes move from near the pole to the metaphase plate (18; discussed in reference 30). Diatoms display a prometaphase behavior that is easier to understand; kinetochores appear to slide along MTs from one pole (without MTs terminating at the kinetochore), and from the analysis of cinematographical records and electron microscope data, bipolar attachment appears to be achieved when daughter kinetochores find and then associate with MTs from opposite poles (39). It is controversial whether prometaphase attachment in diatoms is representative of more conventional cells. We do not believe that diatoms have evolved a fundamentally different system for mitosis and contend that the differences between diatom spindles and other spindles may be only apparent.

[1] *Abbreviations used in this paper*: AP, away from pole; MTL, microtrabecular lattice; MTs, microtubules.

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"Spindle Matrix" and Chromosomal Motion

Although we believe the MTs play only a passive role, the MT skeleton is vital; numerous experiments (e.g., with anti-MT drugs such as colchicine and nocodazole) have shown that if the spindle MTs are disrupted motility ceases. The biochemical nature of the motility system remains unknown. Cytoplasmic constituents generally exhibit "saltatory" motion associated with cytoplasmic MTs (33); these and certain other examples of MT-based movement (12) are separable by various criteria from other motility involving actin. Such an MT-based motility system could be functional in the spindle. Many authors have suggested that actin does participate in chromosome movement, but the data supporting this contention is seriously suspect; we and many others believe that actin-based motile systems are not significantly involved in mitosis (see, for example references 19 and 30). If filamentous actin is eliminated as a major component of the spindle's motile machinery, few obvious alternatives remain. Evidence for the participation of dynein in the spindle is contradictory (30). Because the information on the biochemistry of MT-based motion is so sparse, it would be profitless to pursue a discussion of it here.

We believe it more useful to compare the spindle with a cell type that displays very striking MT-based motility. This cell is the erythrophore (described elsewhere in this supplement), in which large numbers of pigment granules are transported radially inward and outward; synchronous behavior of numerous cells affects the color of the organism. The granules move over a stable cytoskeleton of MTs, and motion of the particles inward and outward are distinctively different. Motion inward, which is relatively rapid and of uniform velocity, has been described by Porter (31) as "resolute." In contrast, the particles disperse more slowly, exhibiting saltatory motion throughout. The motility system does not involve actin; it seems to be mediated by an elastic, contractile lattice, a differentiation of the cell's microtrabecular lattice (MTL). This portion of the MTL permeates the cytoskeleton of MTs and carries within it the pigment granules (reviewed by Porter et al. [32]).

Several early biologists noticed the similarity in biological activity between the double asters of mitotic cells and the single "aster" in these pigmented cells, speculating on whether the mechanism of the motility they display is similar. When formulating the concept of the MTL, Porter (31) soon realized its possible relevance in explaining chromosomal motion. He envisioned the spindle as consisting of two cytoplasts, organized by the two poles, whose margins would interact with each other and with the chromosomes during prometaphase and hold the chromosomes in a stable metaphase configuration. A surge of contractility in the MTL, synchronized with chromatid splitting, would pull the daughter chromosomes to opposite poles. Later, McIntosh (16) speculated further along these same lines and, in addition, attempted to explain anaphase elongation in terms of such expanding and contracting MTLs. However, although promising, this model like others is too simple to explain the full complexity of mitotic movement (see below). Two questions can now be raised. First, is there evidence that a structural matrix, the "spindle matrix," exists specifically for mitosis? Second, even if it is present, is there evidence that it is functional in moving chromosomes? In the diatom spindle, the answer to both questions is yes.

Role of a Spindle Matrix in Chromosomal Movement in Diatoms: Structural Evidence

In our initial ultrastructural investigations of diatoms, the mechanism of chromosomal attachment to the spindle was enigmatic. Although we initially had no reason to doubt the dogma prevailing at that time that the kinetochores should nucleate MTs and generate kinetochore fibers, we found that in several diatoms this was clearly not the case inasmuch as they did not have conventional kinetochore fibers. Instead, the chromosomes seemed to be attached to the surface of the rigid cylindrical "central spindle." This major structural component is composed of two half spindles whose constituent MTs interdigitate to form a central region of overlap (27). Other MTs, presumably of the same polarity as those in the adjacent half spindle, radiate from each pole into the region occupied by the chromosomes. These MTs appear to be vital to the achievement of chromosomal attachment, functioning in smaller diatoms (e.g., *Surirella* and *Pinnularia*) by providing the tracks that guide the chromosomes during prometaphase activity to their attachment site on the central spindle (21, 24). Some larger diatoms (e.g., *Nitzschia* and *Hantzschia*) do not have a large enough area on the central spindle to accommodate attachment of all the chromosomes. Consequently, prometaphase activity (22) leads to the creation of bundles with MTs ensheathing many of the stretched kinetochores and attaching them to the poles. Serial section tracking of the MTs in such bundles confirms that very few of these MTs end at the kinetochore (39).

In *Pinnularia* and *Surirella*, structural analysis revealed a spindle matrix, termed the "collar," that permeates the outer MTs of the central spindle and extends precisely from each pole to the nearby leading edge of the chromosomes (28, 29, 38). Here, then, is a structurally distinct spindle matrix that, if it is contractile, would be ideally positioned for generating chromosome motion to the pole, a reassuring observation inasmuch as MTs do not appear to be thus involved. The collar material is not as obvious in other diatoms, which have more discrete kinetochore bundles of MTs.

Reinforcing our belief that the collar is important structurally are findings in diatoms treated with colchicine. Fortunately, the central spindle is resistant to colchicine (and other forms of disruption). However, the other polar MTs along which the chromosomes slide at prometaphase are disassembled (25). When colchicine is applied to living cells of *Hantzschia* during prometaphase (shown in a film [23]), monopolar, oscillating chromosomes stop moving and are thereafter unable to attach to the spindle; at metaphase, as the peripheral bundles of MTs ensheathing the kinetochore in these larger diatoms are broken down, many bipolarly attached chromosomes release irregularly from either pole, springing slowly but elastically to the other pole. After 15–20 min, the metaphase spindle becomes severely disorganized, with irregular groups of paired chromatids clustered around either pole. The significance of these observations becomes apparent when similarly treated cells are examined under the electron microscope. The central spindle is essentially intact and other polar MTs are absent. Most importantly, the collar now appears as a flocculent matrix aggregated at each pole (Fig. 1 *a*); many kinetochores are attached to this material (Fig. 1 *b*; reference 25).

In summary, diatoms display a distinct spindle matrix

whose disposition suggests that it is involved in chromosomal motion; furthermore, chromosomes are apparently attached to this matrix by kinetochores, even when the MTs that form the structural basis for this attachment have been artificially removed.

Role of a Spindle Matrix in Chromosomal Movement in Diatoms: Functional Evidence

Evidence that the collar material is also involved functionally in mitosis has come from experiments with metabolic inhibitors (such as azide, cyanide, dinitrophenol, oligomycin, and others) that interfere with ATP formation (26; filmed results [23]). These inhibitors gave similar results and the effects were fully reversible, even after multiple exposures to the inhibitors. Several results were anticipated, for example, the rapid cessation of cytoplasmic movement and cell cleavage. However, more interesting is the behavior of treated prometaphase cells. Invariably, oscillating prometaphase chromosomes moved up to either pole before coming to rest with the cessation of the cytoplasmic activity elsewhere. When the drug was washed out, kinetochores were reactivated with a strong movement away from the pole; within 30 s their prometaphase behavior was reestablished, followed by attachment and a normal subsequent mitosis. This behavior suggested that movement to the pole is less sensitive to lack of ATP than movement away from the pole, and we designated these "P" and "AP" movement, respectively (30).

However, there is another important and subtle corollary. If oscillating prometaphase chromosomes move to the pole in the presence of metabolic inhibitors, they must have a physical and functional connection to that pole; without it, we would expect them to simply come to rest. Because the polar MTs do not terminate in the kinetochore at prometaphase (39), we conclude that the physical connection between kinetochore and pole must be a separate entity. We envisage this entity as stretching over the MTs and as sensitive to the cell's level of ATP. Clearly, the collar meets the criteria for this functional component.

Let us now make a functional comparison with the erythrophore. When the energy supply of erythrophores is lowered, they react by aggregating their pigment granules; apparently, the MTL contracts over the MT cytoskeleton (14). We have confirmed this behavior in erythrophores treated with metabolic inhibitors plus deoxyglucose to inhibit glycolysis. The phenomenon was not fully reversible in these preliminary experiments; when we washed out the inhibitors, saltatory motion resumed and partial pigment dispersion occurred, but dispersion was never complete. Nevertheless, second and even third exposures to these inhibitors caused repeated pigment aggregation. Although these cells are not as resilient toward metabolic inhibitors as diatoms, the behavior of granules in the erythrophore and that of monopolar prometaphase chromosomes in the diatom spindle have some obvious similarities. (It should be remembered that metaphase chromosomes cannot display this poleward motion because they are firmly attached to opposite poles.)

Structural Evidence for the Existence of a Spindle Matrix in Conventional Cells

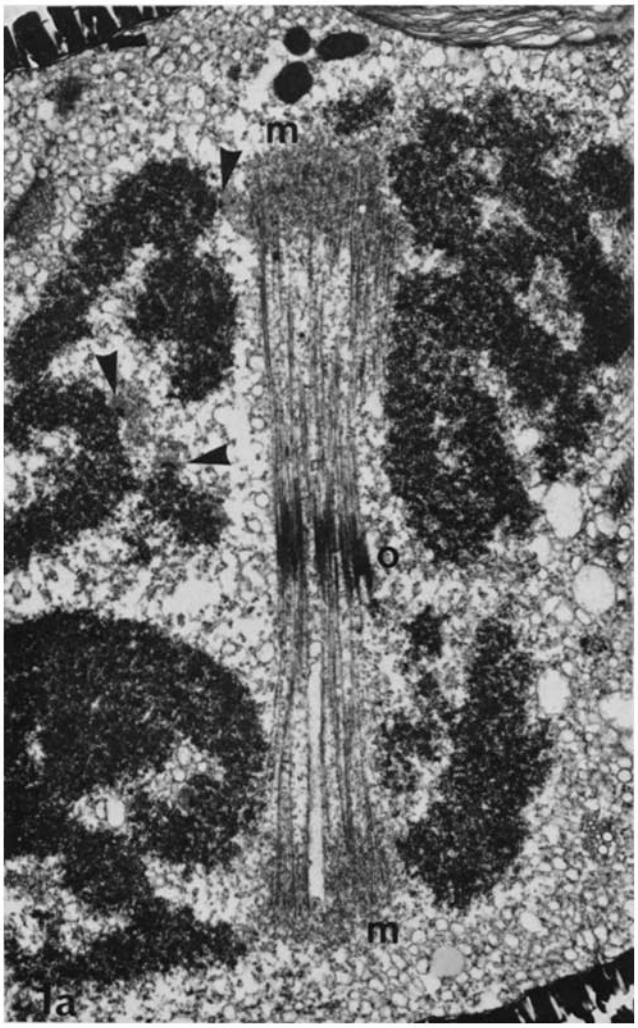
The features described above that make diatoms so suitable for experiments (their conspicuous, stable central spindle and kinetochores that function at prometaphase by sliding over MTs) renders their mitosis superficially different from that of more conventional cells and engenders the suspicion that diatoms may not be representative. The next step, therefore, was to determine the extent to which the above observations could be repeated in conventional spindles, and some preliminary results have been obtained (Spurck, T., et al., manuscript in preparation).

That the spindle matrix plays a role in the generation of chromosome movement in conventional spindles would be more firmly established if morphological evidence were available. Few investigations have been devoted to this end. Experiments in which tannic acid was used on the green alga *Oedogonium* revealed fine filaments attached to the kinetochores and interspersed with the MTs of kinetochore fiber; both filaments and MTs were found to be embedded in a matrix distinctly denser than the ground nucleoplasm (Fig. 3; reference 37). While it is attractive to equate this matrix with the collar, there is no direct evidence to support this interpretation. Conventional spindles sometimes seem to display a similar matrix but such judgments are subjective and many spindles do not (10). Kinetochore fibers subjected to cold shock are more evocative (34); however, it could be that the matrix thus revealed (Fig. 2) stabilizes the MTs and therefore may not represent a motile component. In summary, the morphological evidence for the existence of such a matrix is weak. However, when thin sectioned by conventional techniques, the erythrophore also demonstrates no component that could be associated with pigment movement. As Porter and his colleagues (32) have emphasized, special techniques for examining resinless cells or sections are necessary to reveal the MTL and the same may be true for the spindle matrix.

Evidence That a Spindle Matrix Plays a Functional Role in Chromosomal Movement in Conventional Cells

That components besides MTs (e.g., actin) are involved in the generation of chromosome movement is not a novel idea. Forer (9), after creating areas of reduced birefringence (presumably devoid of MTs) in kinetochore fibers, showed that the affected fibers could still move chromosomes poleward. He concluded that the motility system is separate from MTs, and he champions the idea that actin is a functional constituent of it. In general, however, the existence of a separate motility system is seldom discussed, and there is very little relevant evidence for it.

We approached this question by performing experiments similar to our experiments on diatoms with metabolic inhibitors, using instead PtK_1 cells (Spurck, T., et al., manuscript in preparation). The effects again were found to be reversible. In vivo application of metabolic inhibitors rapidly causes a strong contraction of the cytoplasm around each pole of the mitotic spindle, a phenomenon not evident around the cell center of interphase cells. Quite quickly, spindle and cytoplasmic activity (incuding cleavage) ceases. At metaphase, the contraction around each pole apparently pulls them somewhat together. In prometaphase cells, many chromosomes soon cluster tightly around the poles, and some can move right up to the polar complex containing the centrioles; apparently, these chromosomes at the moment of application of the inhibitors do not yet possess a fully formed kinetochore fiber. This tight association is confirmed by electron micros-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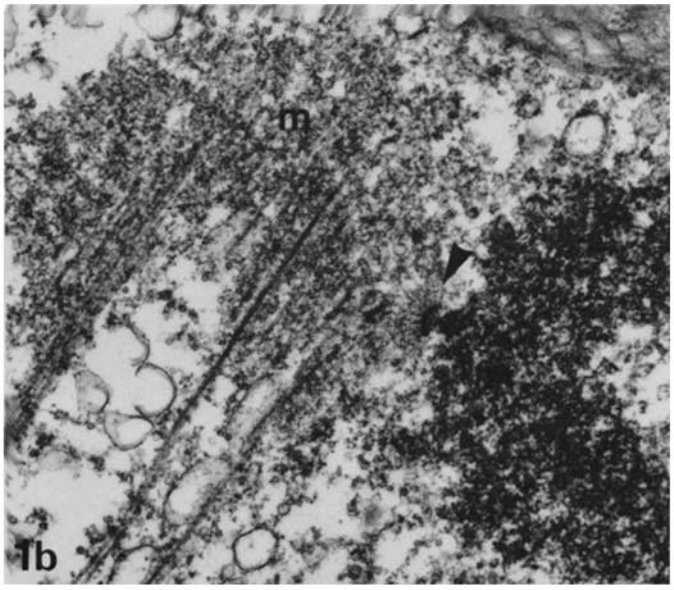
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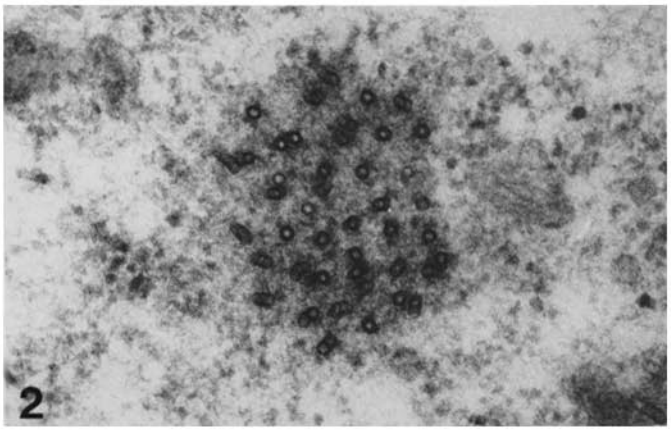
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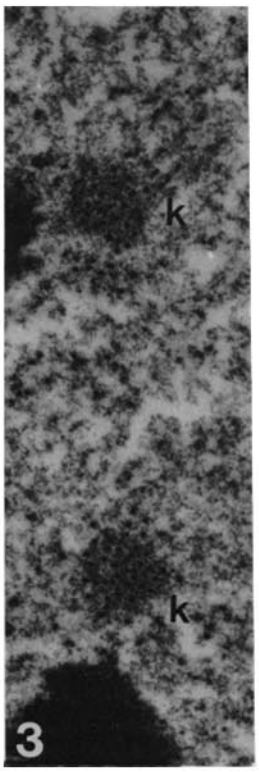
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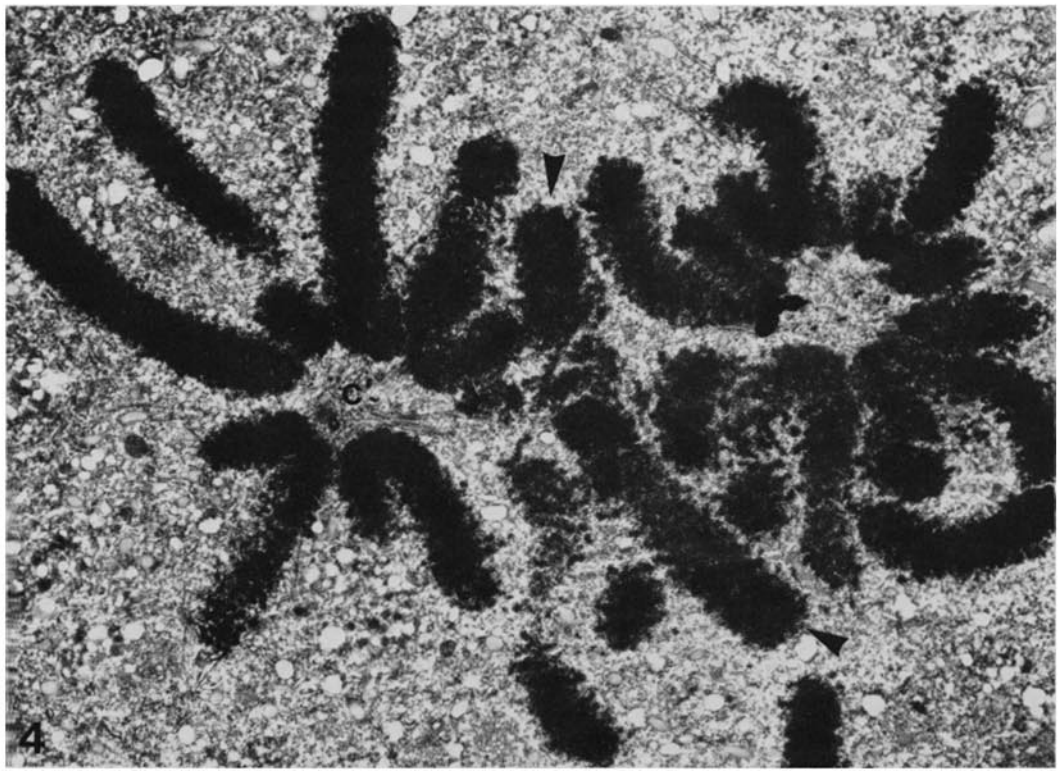
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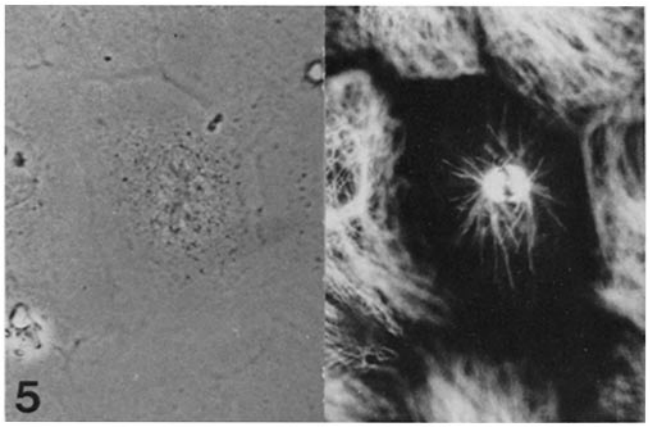

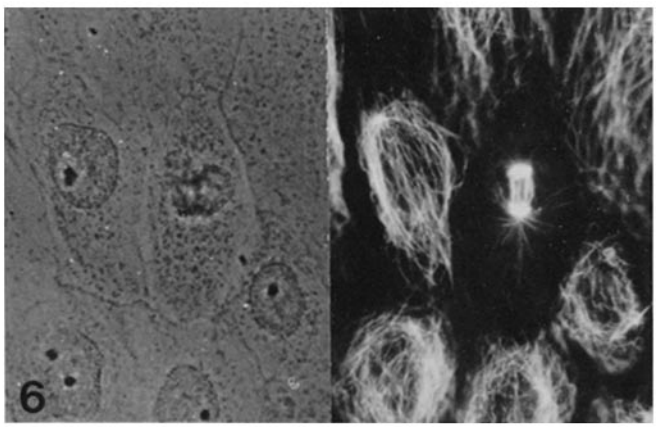

FIGURE 5 Cells treated as described in the legend to Fig. 4 for 10 min, fixed in methanol, and stained with monoclonal antibody against tubulin; photographed in phase and fluorescence. Neither the metaphase for adjacent interphase cells show evidence of MT breakdown induced by this treatment. × 680.

FIGURE 6 Cells treated as described in the legend to Fig. 5 and then with dinitrophenol/deoxyglucose plus nocodazole (0.2 μg/ml) for 10 min. Nocodazole at this concentration completely breaks down all spindle MTs and many in interphase control cells. However, pretreatment with dinitrophenol prevents most of this breakdown. In the early anaphase cell, kinetochore fibers specifically have been shortened (manuscript in preparation), whereas the interzonal fibers appear to be unaffected. × 680.

copy (Fig. 4). Occasionally, a prometaphase chromosome will belatedly (i.e., several minutes after the drug has affected the cell) move quite rapidly from the periphery of the spindle to one spindle pole along a linear track. Because MT depolymerization is inhibited in these ATP-depleted cells (Fig. 5; reference 6–8), we believe that the most reasonable explanation of this behavior is that these kinetochores slide, as they do in diatoms, along stabilized polar MTs and that their motion is generated by a system structurally separate from the MTs that link the kinetochore to the pole. (We have not, however, been able to entirely eliminate the possibility that MT disassembly occurs along with movement at these chromosomes; we consider it most unlikely in view of the stability accorded MTs during ATP depletion). A connection to the pole along the polar MTs is probably necessary; if the motility were generated by a localized motor (e.g., at the kinetochore) one would not expect this to function all the way to the pole in these experiments. In contrast, Bajer (2) claims that chromosomes do not slide along MTs during prometaphase, because kinetochores often have many MTs inserted in them. It is indeed difficult to imagine how sliding is operative if one believes that this insertion represents the actual site of attachment of chromosomes to the motile apparatus, defined as the kinetochore MTs. Bajer's argument does not simplify the problem of explaining metakinesis, because one still has to account for the changes in the lengths of these MTs necessary to accommodate metakinesis (18), a serious problem whatever model one prefers; we have discussed[2] a mechanism that attempts to account for these changes. Our recent results (manuscripts in preparation) suggest that Bajer's conclusions are not valid during the initial prometaphase interaction of kinetochores and MTs.

This response of mammalian cells to metabolic inhibitors is clearly analogous to that of the diatom spindle and the pigment-transporting system of the erythrophore. However, the situation is complicated in conventional spindles because numerous MTs soon terminate in the kinetochores during later prometaphase. In cells treated with dinitrophenol, chromosomes with well-established kinetochore fibers (e.g., during metaphase) do not move to the pole—apparently because these fibers cannot depolymerize and shorten. The astral contraction discussed above cannot be ascribed to the metabolic inhibitors merely causing depolymerization of MTs. We have confirmed (6–8) that metabolic inhibitors stabilize MTs (Fig. 5). If cells are treated with dinitrophenol and later with nocodazole, cytoplasmic MTs still do not break down (7, 8),

FIGURE 1 Metaphase spindle of the diatom *Hantzschia amphioxys* treated with colchicine (0.1% wt/vol) for 15 min before fixation. (*a*) Many double chromosomes have detached from one pole and moved to the other; a few remain central. The collar matrix (*m*) is aggregated at each pole. Three kinetochores are visible (arrows), two on central unattached chromosomes and one at the pole (*o*, overlap of central spindle). (*b*) Detail of serial section of cell shown in *a* showing a kinetochore (arrow) attached to the collar matrix at the pole. (*a*) × 5,100; (*b*) × 19,000.

FIGURE 2 Transverse thick section of a metaphase kinetochore bundle in a cold-shock PtK_1 cell; the MTs are embedded in a matrix. From Rieder (34). × 60,000.

FIGURE 3 Metaphase spindle of the green alga *Oedogonium cardiacum*; transverse section showing the dense matrix of two kinetochore bundles (*k*) of MTs. From Schibler and Pickett-Heaps (37). × 26,600.

FIGURE 4 Early prometaphase PtK_1 cell treated for 5 min with 10^{-3} M dinitrophenol (10^{-3} M deoxyglucose). These chromosomes have rapidly and very tightly clustered around each pole (*c*, centriole). A few chromosomes (e.g., between the two arrows) have already achieved bipolar attachment and have well-developed kinetochore bundles; the others apparently have slid along astral MTs. The effects of this treatment are fully reversible. × 4,800.

nor do most spindle MTs. Energy appears to be necessary for the disassembly of these MTs, even when nocodazole is present (Fig. 6). However, in contrast, the half spindles do shorten further under these conditions (paper in preparation), but kinetochore fibers always remain present. One interpretation of these results is as follows. The aster contains a radially contractile entity connected to kinetochores and extended over but separate from the MTs. When the cell's energy supply is depleted, this entity contracts, pulling chromosomes with no or poorly formed kinetochore fibers poleward. Although most MTs are stabilized under such ATP-depleted conditions, the compression engendered in fully formed kinetochore fibers by astral contraction renders the MTs in and associated with the fibers more susceptible to depolymerization than other (e.g., astral and interzonal) MTs, as is indicated by their reaction to nocodazole. The role of ATP in mitosis thus may be quite different from the role that appears intuitively likely. We believe that ATP is not necessary to generate the force that moves chromosomes to the pole during anaphase A; we suggest that the motility apparatus in the aster in fact contracts elastically when depleted of ATP (as demonstrated during prometaphase). However, we believe that ATP is needed for normal anaphase—for depolymerizing MTs of the kinetochore fiber (disassembly being modulated by the kinetochore) and permitting the contractile apparatus to function. Cande (4) states that ATP is not needed for anaphase A (the converse of the conclusion drawn from previous similar experiments [3, 5]), but his experiments did not address the role of ATP in MT depolymerization. Elucidation of this role may be a central to our understanding of how anaphase is accomplished,[2] particularly if MT depolymerization at the kinetochore is rate limiting for the movement. We find that in vivo anaphase A ceases in the presence of metabolic inhibitors (20) and we suspect that this inhibition of movement occurs because the kinetochore fibers cannot shorten, even though the motile apparatus is pulling the chromosomes poleward.

Some Significant Problems with the Simple MTL Model of Spindle Behavior

There are some serious unresolved problems with the simple MTL model proposed by Porter (31) and later by McIntosh (16). It does not account for the individualistic behavior of chromosomes during prometaphase. In marked contrast to the movements displayed by chromosomes (except in anaphase), pigment granules in erythrophores behave synchronously. The saltations of the granules during dispersion are trivial in comparison to the coherence of the major phases of pigment movement. Few workers (2, 15, 18) seem to appreciate the importance and subtlety of the erratic oscillations of prometaphase, which can send individual chromosomes asynchronously toward either pole. The mechanism generating these oscillations is unknown; the MTL, if solely involved, would have to generate large-scale pushing and pulling movements simultaneously, often confined to narrow sectors of the spindle, since adjacent chromosomes can be translocated in opposite directions. Other organelles in the spindle display both saltatory motion (33) and directed transport (1, 11); in diatoms, mitochondria, for example, oscillate along linear tracks (presumably polar MTs) in and out of the spindle (39). Thus, concurrent, bidirectional, and very localized transport is not confined to kinetochores.

If kinetochores are actively motile organelles (30), perhaps their interaction with MTs complicates their interaction with a spindle matrix. It is not clear yet whether AP movement is necessarily generated by the second kinetochore of the pair, perhaps interacting again with polar MTs (30); the microbeam irradiation experiments of Izutsu (13) and McNeill and Berns (17) indicate that the second kinetochore is necessary. (In these experiments, when one kinetochore is inactivated, a double chromosome will then move steadily to the pole that the other faces.) Likewise, single kinetochores at anaphase generally exhibit steady poleward movement, although Bajer (2) reports that even this motion may be irregular. Furthermore, when chromosomes are grouped around a monopolar spindle, they continuously oscillate (2), and so generation of AP movement presumably does not need the presence of the second pole. This activity of chromosomes is not what would be expected of chromosomes interacting with the edges of one or two unit MTLs.

Synchronous anaphase movement of chromosomes obviously resembles the behavior of granules in the erythrophore, as Porter (31) and McIntosh (16) have emphasized. However, even this comparison does not hold under close scrutiny. We refer again to the experiments of Izutsu (13) and McNeill and Berns (17) who demonstrate the immediate and steady movement of a chromosome poleward during metaphase (or even prometaphase) when one of its two kinetochores is inactivated. This movement suggests that chromosomes, even during metaphase, must in fact be continuously subjected to tension directed toward both poles and that this pulling force is capable of moving individual chromosomes, even when nearby chromosomes are stationary. Thus, anaphase is not explicable in terms of a simple wave of contractility in the MTL that coincides with chromatid splitting. One can avoid this problem by suggesting that sectors in the MTL develop individuality, a complex concept that contradicts the assumption that the MTL is a single entity functioning coherently. In contrast to the erythrophore, the motility system of the spindle paradoxically is capable of generating linear large-scale movement in both directions within a very short time interval and with very localized spatial discrimination.

In summary, although we feel that the concept of the involvement of the spindle matrix in chromosomal movement is the most useful available at present, the complexities of spindle behavior demand a considerably more sophisticated and complex model than that suggested by a simple comparison of the erythrophore with the aster. Although these complexities must not be ignored, they need not detract from the usefulness of the general principle in sharpening our perception of the complexities of mitotic movement. Anaphase is the simplest manifestation of a cytoplasmic system whose complexities will not be understood until we also understand those fascinating and superficially disordered events that precede and ultimately make possible the perfection of anaphase.

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