Metabolic Inhibitors Block Anaphase A In Vivo

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Abstract. Anaphase in dividing guard mother cells of *Allium cepa* and stamen hair cells of *Tradescantia virginiana* consists almost entirely of chromosome-to-pole motion, or anaphase A. Little or no separation of the poles (anaphase B) occurs. Anaphase is reversibly blocked at any point by azide or dinitrophenol, with chromosome motion ceasing 1–10 min after application of the drugs. Motion can be stopped and restarted several times in the same cell. Prometaphase, metaphase, and cytoplasmic streaming are also arrested. Carbonyl cyanide *m*-chlorophenyl hydrazone also stops anaphase, but its effects are not reversible. Whereas the spindle collapses in the presence of colchicine, the chromosomes seem to "freeze" in place when cells are exposed to respiratory inhibitors. Electron microscope examination of dividing guard mother cells fixed during azide and dinitrophenol treatment reveals that spindle microtubules are still present. Our results show that chromosome-to-pole motion in these cells is sensitive to proton ionophores and electron transport inhibitors. They therefore disagree with recent reports that anaphase A does not require a continuous supply of energy. It is possible, however, that anaphase does not directly use ATP but instead depends on the energy of chemical and/or electrical gradients generated by cellular membranes.

WHILE no one doubts that chromosome separation during mitosis requires energy, there has been an ongoing controversy over the last 30 years about when this energy is made available, in what form, and for which processes it is needed. Early studies showing that various metabolic inhibitors have little or no effect after the onset of prophase led to the concept that cells become preloaded with a sufficient energy reservoir to carry them through the rest of mitosis and cytokinesis. Mazia (29), in summing up this work in 1961, referred to "points of no return" during mitosis as stages after which the cell is committed to progressing through the mitotic cycle.

Subsequent work by Epel (14) and Amoore (1, 2), however, indicated that the "energy reservoir" and "points of no return" concepts are not tenable (see also reference 30). For example, when sea urchin eggs are cultured in the presence of carbon monoxide they can be stopped at any stage of mitosis (14). Inhibition occurs when the ATP supply drops to 50% or less of the normal level. All stages of mitosis in pea roots including anaphase are sensitive to cyanide and oxygen deprivation. Amoore (1) reports, however, that inhibition is not due to reduced ATP levels but rather to an effect on a nonrespiratory ferrous complex. Regardless of the details, the conclusion that emerges from these studies is that there is no energy reservoir that is capable of carrying a cell through division.

In light of these studies, we were not surprised to find, during experiments on division plane determination in plants several years ago (36, 37), that anaphase motion is rapidly and reversibly inhibited by 2,4-dinitrophenol (DNP)[1] and sodium azide. These results were communicated in abstract form (34). Since that report, however, the possibility that anaphase uses stored energy has resurfaced. Using a permeabilized PtK_1 cells, Cande (8, 9) has reported that anaphase A (movement of chromosomes to the poles; 19) but not anaphase B (separation of the poles) is insensitive to the absence of ATP or the presence of ATPase inhibitors. It can thus be argued that anaphase A uses stored energy. The localization of creatine kinase in the spindle (21) and the movement of chromosomes in permeabilized cells in the presence of phosphoryl creatine (10) add credence to this conclusion. Further support comes from the work of Pickett-Heaps and Spurck (39) who found that in diatoms, metabolic inhibitors cause prometaphase chromosomes to quickly move to one pole or the other before stopping altogether.

The concept that anaphase A is driven by stored energy has received additional impetus from studies on pigment granule migration in chromatophores. Based on observations that the outward movement of granules requires energy whereas movement towards the cell center does not, Luby and Porter (24) have suggested that the organization of the cytoplasmic matrix (microtrabecular lattice) is a source of energy which, when liberated via the contraction of the matrix, moves the particles inward. Both McIntosh (31) and Cande (8) have used these findings in comparing anaphase A to the inward move-

1. *Abbreviations used in this paper:* CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; d'H_2O, deionized water; DNP, 2,4 dinitrophenol; GMC, guard mother cell; MT, microtubule; SHC, stamen hair cell.

ment of pigment granules. In addition, Pickett-Heaps et al. (40) have proposed that the collar material in dividing diatoms is an elastic matrix similar to the microtrabecular lattice that can move chromosomes poleward.

These ideas are attractive and may help resolve vexing questions concerning chromosome motion. However, it should be noted that considerable disagreement still exists concerning the role of the cytoplasmic matrix in the inward and outward movement of pigment granules (e.g., 12, 45). Furthermore, to the extent that any mitotic mechanism relies on the stored energy concept, we submit that it cannot be generally applied. We base this conclusion on the work reported here which shows that in two species of higher plants and in two cell types, anaphase A can be quickly and, depending on the inhibitor, reversibly blocked. Thus, anaphase A requires a continuous supply of energy in these cells. Part of this work appeared in the aforementioned abstract (34).

Materials and Methods

Two mitotic systems have been used in this study: (*a*) guard mother cells (GMCs) of *Allium cepa* L. (onion) and (*b*) stamen hair cells (SHCs) of *Tradescantia virginiana* L. (spiderwort). To prepare GMCs for analysis in the light microscope, paradermal slices from the cotyledons of 5-d-old seedlings were excised with a No. 11 scalpel blade and placed on microscope slides in deionized water (d'H_2O) or 0.01–0.05 M potassium phosphate buffer, pH 7.0, as previously described (36). The GMC, being the product of a highly asymmetric division in the epidermis, is easily recognized by its small size. Under simple culture conditions these cells divide longitudinally to form an immature guard cell pair which then differentiates into the stomatal complex (35). Mitotic events can be examined directly in GMCs using Nomarski differential interference contrast optics. While monitoring mitosis directly or with the aid of a video camera, respiratory inhibitors including DNP, sodium azide (both obtained from Sigma Chemical Co., St. Louis, MO), and carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) (Calbiochem-Behring Corp., La Jolla, CA; stock solution prepared in absolute ethanol) diluted in d'H_2O or phosphate buffer were perfused under the coverslip and subsequent chromosome motion was observed. After various times, the inhibitors were washed out by perfusion with water or buffer and the ability of the cells to resume anaphase was ascertained. In some experiments, inhibitor was replaced with the same solution containing colchicine or with colchicine alone (in d'H_2O or phosphate buffer). Observations were performed on a Zetopan microscope (C. Reichert, Vienna) and photographs taken with an automatic exposure system using Panatomic-X film. When a newvicon camera (model 65, Dage/MTI, Michigan City, IN) was used to facilitate observations, exposures were made directly from a video monitor using a 35-mm camera. In addition, video recordings were made on a ¾ inch time-lapse cassette unit (model VC-7505, Nippon Electric Co., Elk Grove, IL).

Tradescantia stamen hairs were handled in much the same way as the *Allium* epidermal slices. However, because the hairs have a thin cuticle that impedes diffusion of drugs through the plasmalemma, they were pretreated for ~1 h at 20°C in a cutinase solution (0.1 mg/ml in 0.02 M Hepes–0.02 M KCl, pH 8.0) before mounting in 0.02 M Hepes–KCl, pH 7.0, in a glass slide chamber. The hairs were then examined on Reichert Zetopan or Photomicroscope III (Carl Zeiss, Inc., Oberkochen, Wuerttenberg, FRG) microscopes equipped with differential interference contrast optics. Again cells were allowed to enter anaphase under normal conditions. Thereafter, respiratory inhibitors diluted in 0.02 M Hepes–KCl, pH 7.0, were perfused through the chamber and the effect on anaphase movement was determined. Observations were again aided with a video camera and tape recordings were used to ascertain rates of anaphase motion.

Ultrastructural analyses were performed on control and treated GMCs of *Allium*. Whole seedlings or paradermal slices were treated with inhibitors for various periods of time and then fixed and flat-embedded for electron microscopy as previously described (36). Slices embedded in thin plastic wafers were examined in the light microscope and dividing GMCs identified. The cells were excised, thin sectioned, poststained in uranyl acetate and lead citrate, and viewed in an EM 10A electron microscope (Carl Zeiss, Inc.) at 60 kV.

Results

Anaphase Consists of Chromosome-to-Pole Motion, Or Anaphase A

Allium GMCs maintained in d'H_2O or dilute phosphate buffer readily continue division when observations are begun in late prophase or prometaphase. Cells remain in metaphase for a variable period of time before entering anaphase, which lasts for 10–20 min. Because of constraints imposed by the relatively small size of the cell (10–20 μm), the spindle occupies the maximum volume available by aligning more or less diagonally across the GMC (Fig. 1*A*). However, the future plane of the new cell wall is longitudinal, and to achieve this alignment, the daughter nuclei and forming cell plate undergo reorientation movements in late anaphase–early telophase (36, 37). While measurements of pole position and separation are difficult because the poles are initially placed near opposite corners of the cell and often in different planes of focus, it is nevertheless evident that the spindle during anaphase exhibits mostly chromosome-to-pole movement and comparatively little anaphase B. Indeed, significant pole separation would be difficult if not impossible because by metaphase or the beginning of anaphase the poles are usually abutted against the walls at opposite corners of the cell and are thus separated by nearly the maximum distance available. Subsequently during the initiation of spindle reorientation in late anaphase–early telophase, there may be a small reduction in the pole-to-pole distance as the daughter nuclei migrate along the side walls, but there is no increase. We conclude from these observations that anaphase in *Allium* GMCs is composed almost entirely of anaphase A.

The mitotic apparatus is usually not as confined in *Tradescantia* SHCs as it is in *Allium* GMCs (Figs. 1*B* and 2). The pole-to-pole separation may be 30–35 μm, with the end walls 50–60 mm apart. Nevertheless the motion displayed by the chromosomes is >90% anaphase A (Fig. 2). Despite the fact that the dimensions of the cell often permit a significant amount of pole separation, it does not occur. These observations show that the process we are examining in both cell types is anaphase A.

Respiratory Inhibitors Block Anaphase A

DNP and azide rapidly and reversibly inhibit anaphase motion. Cells were identified at metaphase or earlier and monitored until the onset of anaphase. The appropriate inhibitor was then perfused under the coverslip. A total of 31 cells were treated in this manner (16 GMCs and 15 SHCs). With GMCs, $1–3 \times 10^{-4}$ M DNP stops anaphase in 5–10 min (Fig. 3), while 2×10^{-3} M sodium azide acts more rapidly (1–3 min; Fig. 4). Anaphase can be halted early, soon after the chromosomes separate, or later as they approach the poles. With both agents, the chromosomes seem to "freeze" in place and become more refractile in appearance (Fig. 3*B*). Cells can also be arrested in prometaphase (or metaphase), but unlike the results reported for diatoms and animal cells (39, 40), the chromosomes do not move to the poles during treatment at this stage. Both DNP and azide also stop telophase reorientation and cytoplasmic streaming.

The results with *Tradescantia* SHCs are essentially the same. Under conditions in which the cuticle has been etched

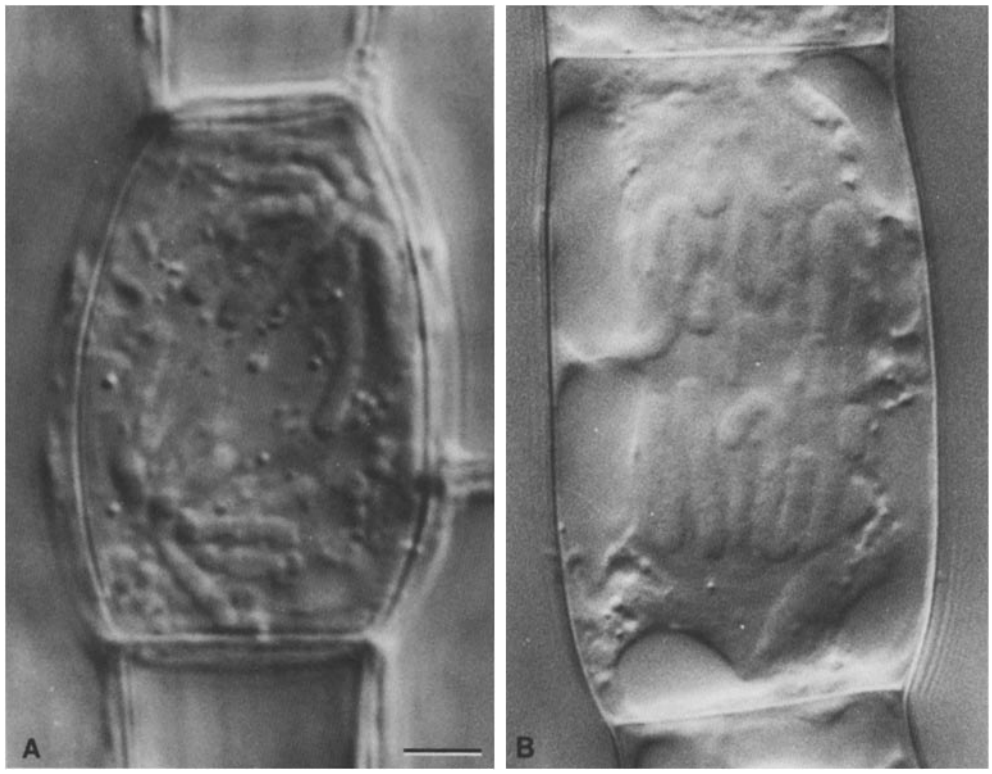

Figure 1. (*A*) A GMC of *Allium* in late anaphase. The spindle is obliquely oriented and daughter chromosome masses have separated toward opposite corners of the cell. (*B*) A *Tradescantia* SHC in mid to late anaphase. ~7 min after the metaphase–anaphase transition. Bar, 5 μm.

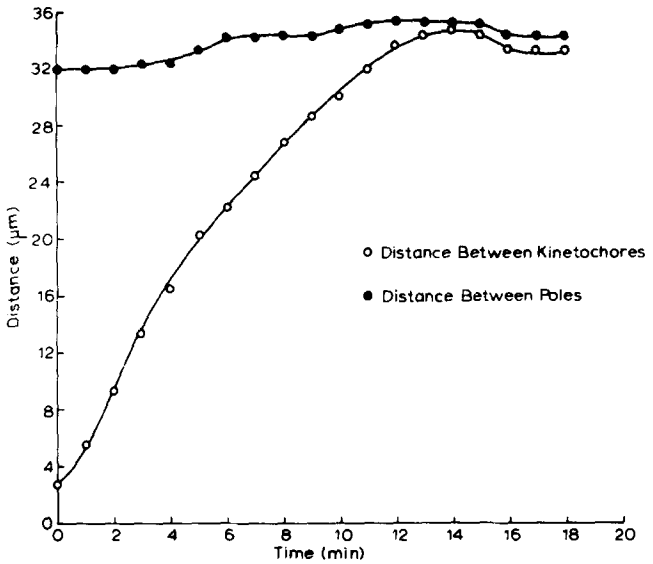

Figure 2. Chromosome motion and spindle pole separation in a *Tradescantia* SHC. The cell was incubated in 0.02 M Hepes–KCl, pH 7.0, and video recordings were initiated at the onset of anaphase. Measurements were then made from the recordings. Although kinetochore separation is dramatic, pole separation is relatively minor.

by preincubation in cutinase, DNP (5×10^{-4} M; Fig. 5) and azide (1×10^{-3} M; Figs. 6 and 7) stop chromosome motion in 1–3 min. As was found in GMCs, the drugs are effective at any point in anaphase (Fig. 5). In both cell types, chromosome motion resumes 2–10 min after the drugs are replaced with water or buffer (Figs. 3–7), although the SHC data show that the original rate of motion may not be attained in some cells (compare plots in Fig. 5). We have been able to stop and restart anaphase 2–3 times in the same cell in this manner (e.g., Fig. 4).

The effects of 5×10^{-6} or 10^{-5} M CCCP (in 1% ethanol) are somewhat different. Although this agent stops anaphase chromosome motion and cytoplasmic streaming in several minutes (3 GMCs and 7 SHCs were examined), its effects are not reversible at the concentrations used. Ethanol (1%) alone does not inhibit anaphase.

Respiratory Inhibitors Do Not Disrupt the Spindle

There has been considerable interest in the effect of metabolic inhibitors on microtubules (MTs), and some of these agents seem to directly inhibit MT polymerization or depolymerization (3–6, 13, 27, 28, 44). In addition, both DNP and CCCP are uncouplers of oxidative phosphorylation and, accordingly, may significantly alter the pH and/or Ca^{2+} milieu of the cytoplasm. Since both high pH and Ca^{2+} may destabilize MTs, it is possible that these agents achieve their effect on anaphase by disrupting spindle MTs in this manner as well. The following observations suggest that the inhibitors do not induce a collapse of the spindle. In cells treated with azide, DNP, or CCCP, the anaphase chromosomes quickly freeze in place. The spindle does not shrink, and for several minutes to one hour, the chromosomes remain essentially stationary (Figs. 5–7). Upon removal of DNP or azide, the chromosomes resume movement to the poles within 2 to 10 min (Figs. 3–7). Cells treated with 5×10^{-3} M colchicine exhibit very different behavior. Again anaphase motion is stopped, but the arrangement of the chromosomes on the spindle is drastically altered. They become scattered or clumped in the midzone as the spindle collapses. Cells treated first with DNP and subsequently with colchicine or colchicine plus DNP reveal, as predicted from the above observations, that the chromosomes first appear to freeze in place (Fig. 8, *B* and *D*). The spindle then shrinks after the colchicine is added (Fig. 8, *C* and *E*).

Electron microscope examination of DNP- and azide-

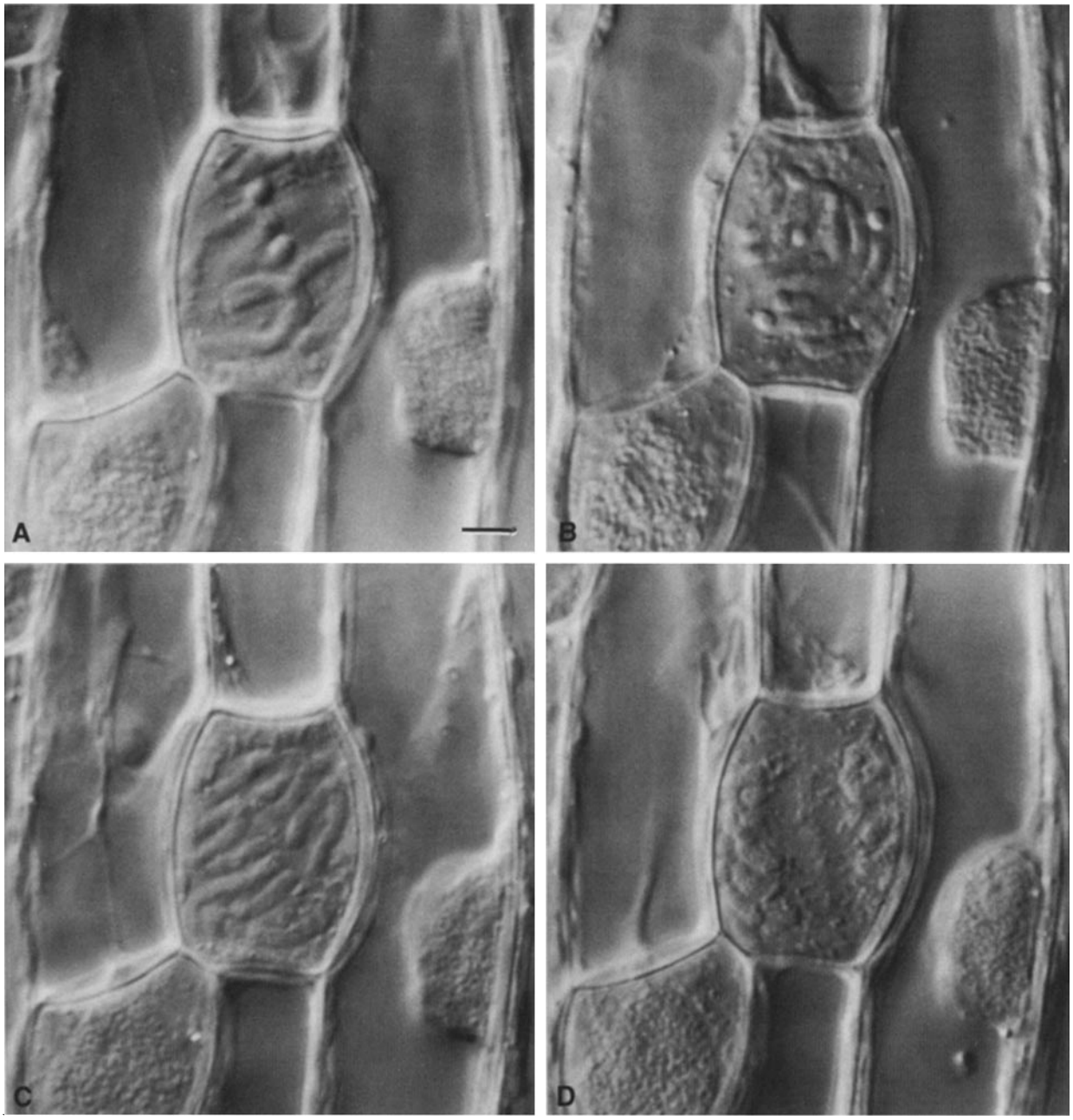

Figure 3. (*A–D*) DNP inhibition of anaphase and its reversal in a GMC. The cell was located while in metaphase (*A*) and monitored until the onset of anaphase, when 3×10^{-4} M DNP in d'H_2O was perfused under the coverslip. Within 5 min anaphase motion had ceased, as had most cytoplasmic streaming. *B* shows the cell ~15 min after the onset of DNP treatment. Note that the chromosomes have essentially frozen in place and have a more refractile appearance. The DNP was washed away with d'H_2O after 26 min. Anaphase motion then resumed, as did streaming. *C* shows the cell in late anaphase, ~20 min after the removal of DNP. The cell then began telophase (*D*), which is characterized by a migration of the daughter chromosome masses along the side walls and a shifting of the new oblique cell plate into the mid-longitudinal plane. Bar, 5 μm.

treated GMCs reveals that spindle MTs are present (Figs. 10 and 11). Whole seedlings treated for 1–3 h as well as epidermal slices exposed for 20–30 min were used. While a quantitative analysis has not been made, both treated (Figs. 10 and 11) and control (Fig. 9) cells show a similar complement of spindle MTs. Especially prominent in both treated and control GMCs are kinetochore MTs. In addition, the cortical MTs characteristic of differentiating guard cells are still present in DNP- and azide-treated material (not shown).

Discussion

Our results show that anaphase in GMCs and SHCs is blocked at any point by respiratory inhibitors. Because chromosome-to-pole motion comprises all or most of anaphase and little or no separation of the poles is evident, anaphase in these cells is equivalent to anaphase A of other cell types. It is especially noteworthy with DNP and azide that chromosome motion is brought to a standstill in 1–10 min, but then

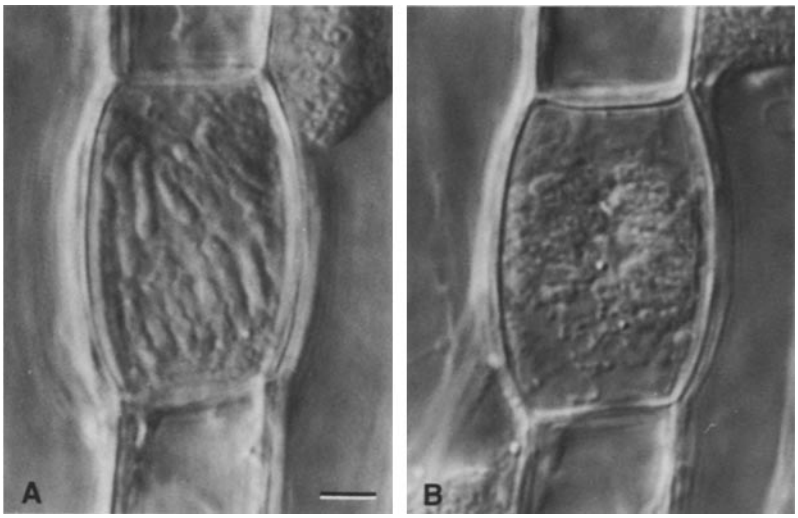

Figure 4. (*A* and *B*) A GMC treated with 2×10^{-3} M azide in d'H_2O in early anaphase. Chromosome motion ceased within 2 min of treatment. The azide was replaced with d'H_2O 15 min later, and anaphase motion resumed in less than 5 min. Azide was then reintroduced 7 min later and anaphase motion again ceased, at which time the cell was photographed (*A*). After 15 min, the azide was again replaced with d'H_2O and anaphase resumed within 5 min. *B*, taken 20 min later, shows that the cell completed anaphase as well as telophase reorientation. A cell plate is not evident as yet, however. Bar, 5 μm.

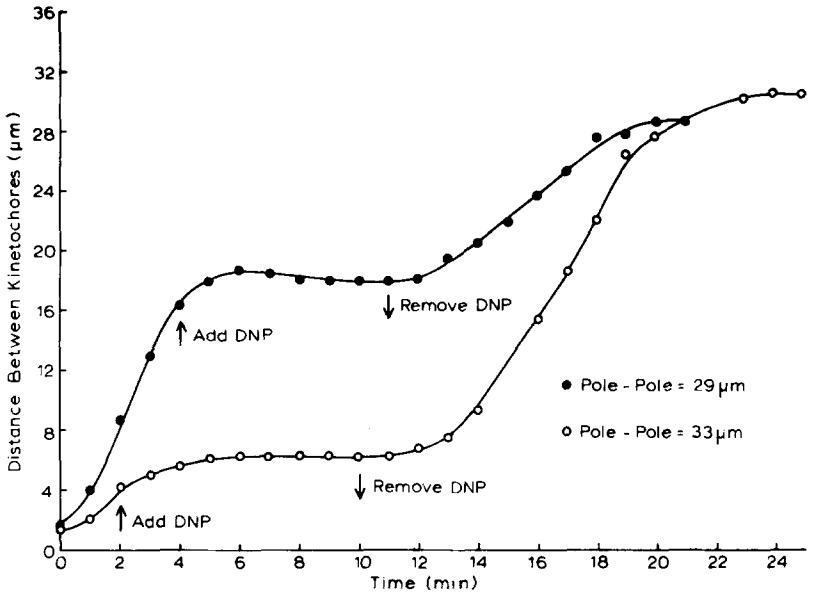

Figure 5. Effect of DNP on chromosome separation in two *Tradescantia* SHCs. DNP was introduced at the times indicated on the graphs. Chromosome motion ceased in both cells, but resumed after the DNP was removed. The final extent of kinetochore separation is similar in both cells. The initial distance between the poles in each cell at metaphase is indicated at the right.

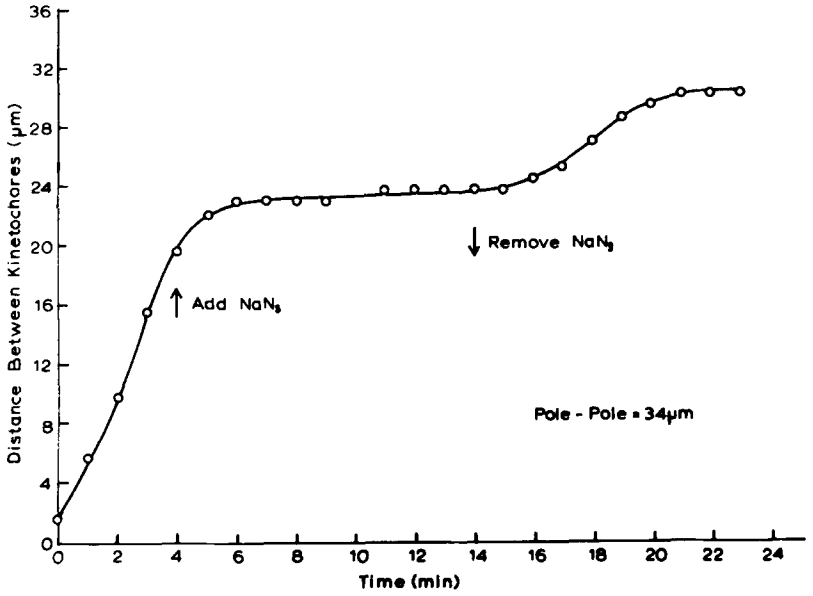

Figure 6. Effect of azide on chromosome motion in a *Tradescantia* SHC. Azide was added 4 min after the onset of anaphase and removed 10 min later. Note the rapid cessation of anaphase motion, as well as its resumption following the removal of the inhibitor.

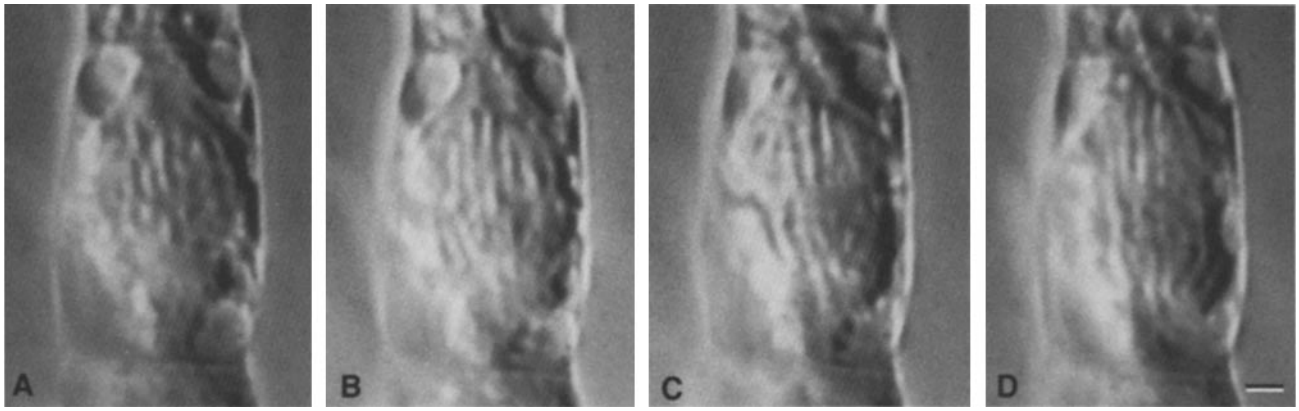

Figure 7. Micrographs made from the video tape used to plot Fig. 6. (*A*) 1 min before the addition of azide; (*B*) 2 min after the addition of azide; (*C*) 9 min after the addition of azide; (*D*) 5 min after the removal of azide. The position of the chromosomes in *B* and *C* is similar but motion has clearly resumed in *D*. Bar, 5 μm.

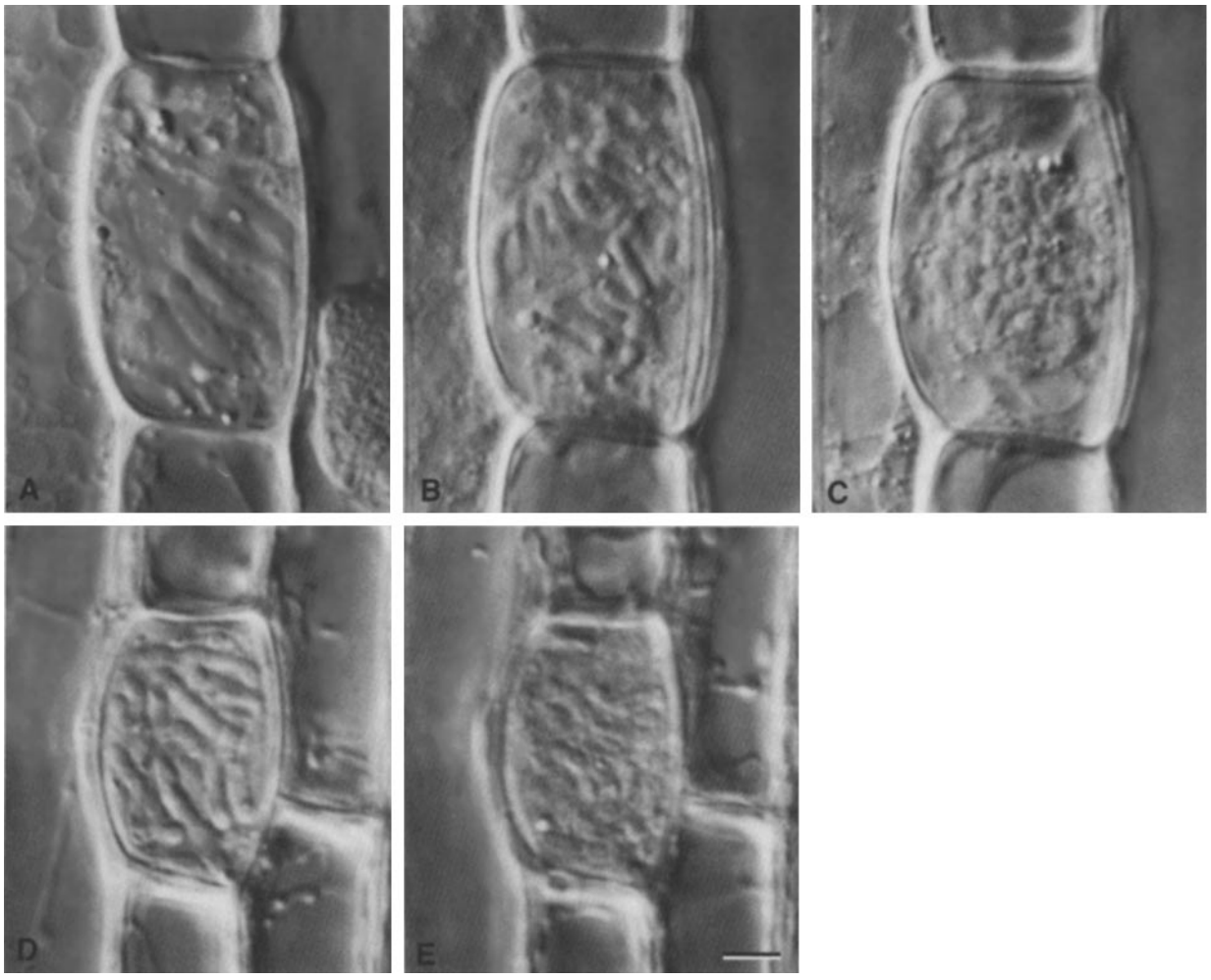

Figure 8. (*A–C*) A GMC monitored in metaphase (*A*). At the beginning of anaphase, 1×10^{-4} M DNP in d'H_2O was introduced under th coverslip. In *B*, the cell appears frozen in mid-anaphase 20 min after the addition of DNP. Note the refractile appearance of the chromosome The DNP was replaced with 5×10^{-3} M colchicine in d'H_2O 33 min later. The chromosomes then collapse into a central mass (*C*; taken 2 min after the introduction of colchicine). (*D* and *E*) A similar experiment in which a cell arrested in mid-anaphase (*D*) by 3×10^{-4} M DNP in dilute phosphate buffer was perfused with the same DNP solution containing 5×10^{-3} M colchicine. Again the chromosomes collapse into a central mass (*E*). Bar, 5 μm.

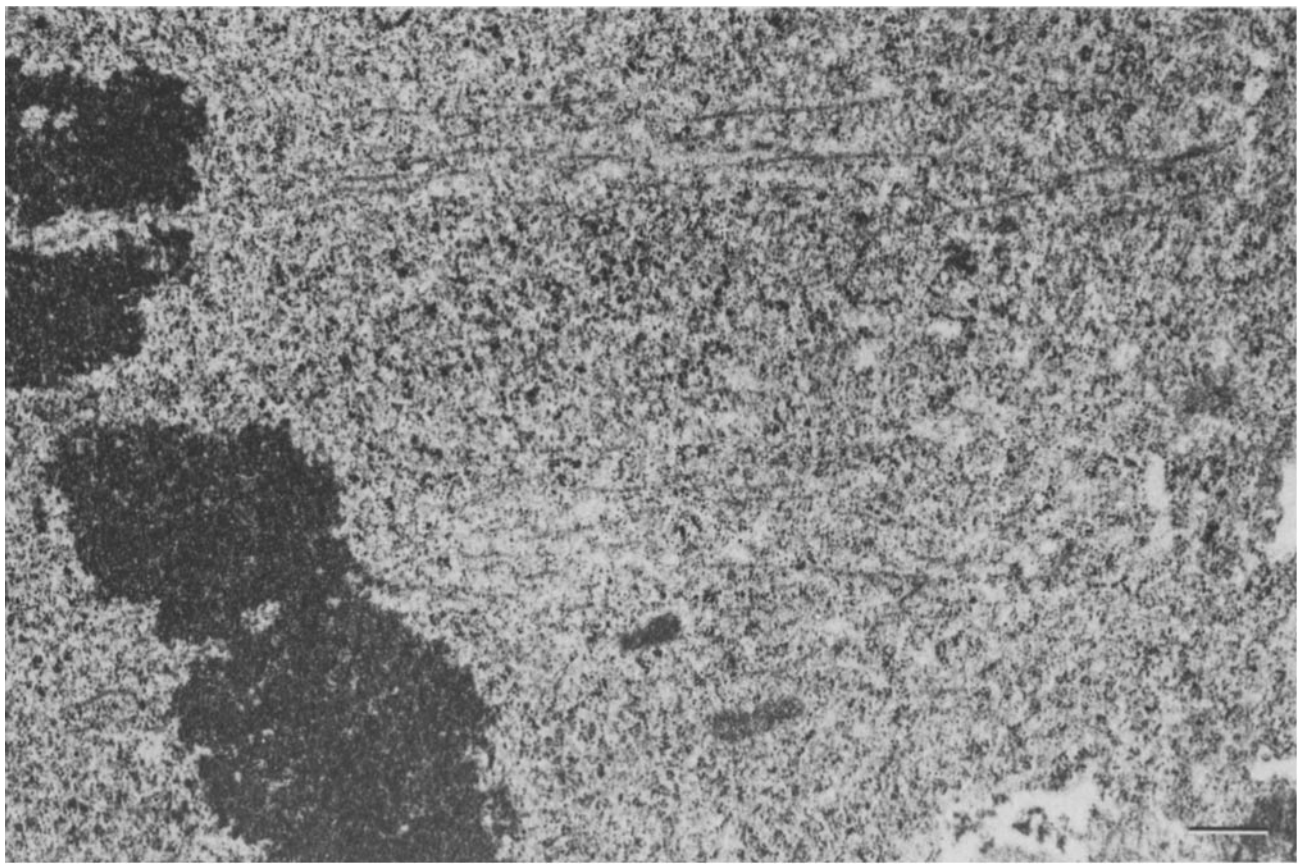

Figure 9. An electron micrograph of an untreated, dividing GMC. Kinetochore MTs are clearly present, but relatively few nonkinetochore MTs can be discerned. Bar, 0.5 μm.

continues once the inhibitor is washed out. Inhibition and reversal can be repeated 2–3 times in the same cell. Other stages of division are also arrested by these agents.

Sawada and Rebhun (44) reported that DNP and other uncouplers disrupt the spindle in dividing marine eggs and several studies have indicated that other metabolic inhibitors (rotenone, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone) have a direct inhibitory effect on the polymerization of MTs, including those of the spindle (3, 4, 27, 28, 32). We believe that the blockade of chromosomes motion in our cells caused by DNP, CCCP, and azide is not due to a collapse of the spindle, for the following reasons. First, when motion of the chromosomes is blocked, they simply freeze in place, and little if any shrinkage of the spindle is evident. The blocked anaphase configuration suggests that spindle fibers are still present and continue to maintain the position of the chromosomes. Second, spindles in which MTs have been destroyed by colchicine display a very different morphology than those blocked by metabolic inhibitors. Due to the breakdown of the spindle in colchicine, the chromosomes become scrambled or collapse into a central clump. Finally, electron microscope observations of DNP- and azide-treated cells reveal the presence of spindle MTs in numbers that appear similar to those seen in control cells fixed and embedded in the same manner.

Although a major disruption of the spindle is not observed after treatment with respiratory inhibitors, it is possible that certain MTs necessary for chromosome motion are affected by the presence of these agents. For example, since kinetochore MTs are more evident than nonkinetochore fibers in both control and treated GMCs (perhaps as a result of differential fixation), we may not be able to detect a preferential disruption of nonkinetochore MTs that could result from treatment with respiratory inhibitors. Such an effect must be considered because nonkinetochore MTs are more sensitive to several agents including cold and colchicine (22, 41–43). Furthermore, it is known that chromosome motion can be halted in the absence of nonkinetochore fibers, even though spindle length is maintained (22). Thus, although anaphase ceases without spindle shrinkage in our cells, we cannot rule out the possibility that DNP, azide, and CCCP block chromosome motion through a disruption of a subset of MTs such as those of the nonkinetochore fibers.

Recently, it has been proposed that because depolymerization of MTs is ATP dependent, removal of ATP prevents MT breakdown (5, 6, 13). Because MTs must depolymerize in order for chromosomes to move to the poles in anaphase, it is possible that respiratory inhibitors prevent anaphase A motion by interfering with this depolymerization. We believe that this interpretation cannot explain our observations. We point out that in *Allium* GMCs arrested in mid-anaphase (or prometaphase/metaphase) by DNP, subsequent treatment with colchicine (plus or minus DNP) causes rapid collapse of the spindle without movement of the chromosomes to their respective poles. Thus, because spindle MTs are not stabilized by DNP, the energy requirement of anaphase A is probably not based on ATP-dependent MT depolymerization but instead may be due to more direct energy use by the mitotic

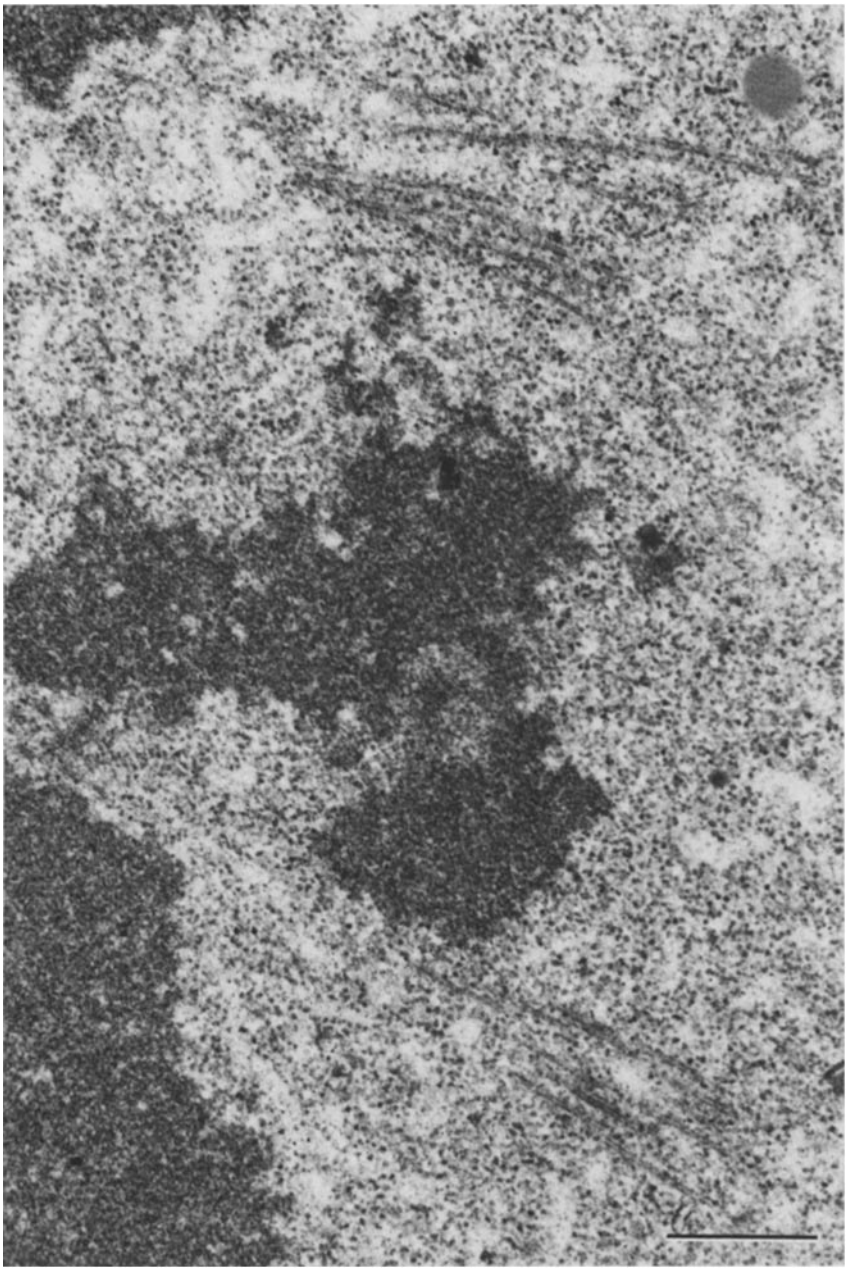

Figure 10. An electron micrograph of a dividing GMC. An epidermal slice containing the cell was incubated in 3×10^{-4} M DNP in dilute phosphate buffer, pH 7.0, for 30 min and then fixed and processed for electron microscopy. Numerous spindle microtubules are still present after DNP treatment. Bar, 0.5 μm.

motor. We cannot account for the discrepancy between our results and those of De Brabander et al. (13), Bershadsky and Gelfand (5, 6), and Pickett-Heaps et al. (40) who have reported that metabolic inhibitors prevent MT disruption by colchicine or nocodazole in animal cells. It is noteworthy, however, that DNP does not stabilize the spindle against colchicine treatment in another plant group (diatoms; 39).

The irreversibility of the CCCP effect distinguishes it from that of azide and DNP. We recognize that CCCP may lead to nonspecific disruption of motility via interaction with sulfhydryl groups (18). It is therefore important that agents such as azide and DNP whose effects are reversible are used in conjunction with CCCP studies. It is thus significant that other than reversibility, the effects of all three agents in our cells are similar. It is still possible, however, that CCCP acts on mitosis in a manner unrelated to respiratory inhibition.

The results presented here stand in variance with those recently published by Cande (8, 9). Using permeabilized PTK_1 cells in which it is possible to introduce a variety of agents that might not otherwise cross the plasmalemma, he has shown that removal of ATP, the presence of ATPase inhibitors, or the addition of nonhydrolyzable ATP analogs all fail to block anaphase A but stop the pole separation of anaphase B. While we cannot resolve the conflict between this work and our own, there are several points that deserve attention and that may help focus future experimentation. One obvious concern is that mammalian cells (PTK_1) and those of monocot plants (*Allium* and *Tradescantia*) may have different mechanisms of energizing chromosome motion. We note, for example, a number of conflicting reports on the effects of inhibitors on animal vs. plant cells during division (2, 14, 39, 40, 44). We fully acknowledge the wide evolutionary spread between these organisms, but nevertheless must recognize that certain characteristics are common to mitosis in all of

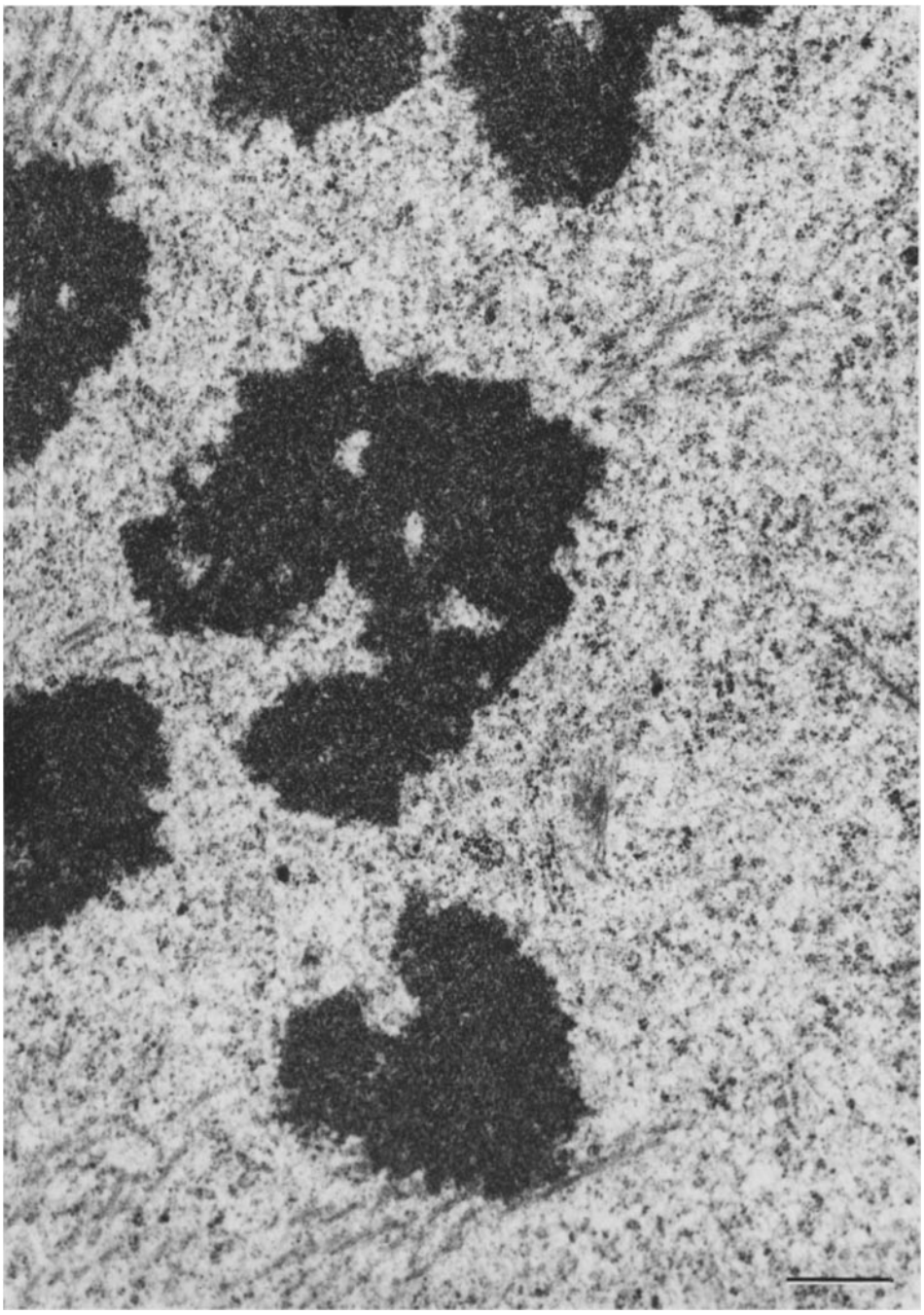

Figure 11. An electron micrograph of a dividing GMC treated for 20 min with 2×10^{-3} M sodium azide in d'H_2O in the manner described in the legend to Fig. 10. Numerous spindle microtubules are still present. Bar, 0.5 μm.

them. For example, the spindles of mammalian and plant cells are composed of MTs and associated membranous structures which probably contain common functional properties as well. Based on these and many other observations, there is no compelling reason to believe at this time that plant and animal cells use fundamentally different energy sources or mechanisms to move chromosomes.

Another major concern that can account for some of the different results stems from the artificial nature of permeabilized cell systems. We fully acknowledge the achievements that have been made with such systems, but nevertheless must point out that permeabilized cells exhibit only 30% of normal mitotic movement (8). Thus, 70% of control chromosome movement does not occur. It is reasonable to wonder whether the motion that remains after permeabilization is governed in ways not totally congruent with mitotic motion in vivo. We are concerned, for example, that the use of detergents, no matter how mild, may disproportionately alter the structure of spindle membranes and thereby destroy or greatly impair an important component in chromosome transport. It may be pertinent that the bulk of the ATPase activity that co-isolates with MTs in neuronal cells is primarily associated with membrane vesicles and not with the MTs per se (33). It is possible that the activity of membrane-associated ATPases in the mitotic apparatus (17, 38) is greatly impaired by the detergent required to prepare cell models.

A third area of concern simply relates to the different properties of the inhibitors used and the significance that

might be ascribed to their different actions. Cande's studies (8, 9) have focused quite specifically on the removal of ATP or the use of agents that block ATPase activity, whereas the work presented here bases its conclusions on the effects of proton ionophores and an inhibitor of electron transport. Perhaps we should consider the intriguing possibility that Cande's results (8, 9) and our own are not in direct conflict. We are reminded of the studies of Amoore (2) who, in comparing the responses of dividing sea urchin eggs and pea root cells to metabolic inhibitors, reached the conclusion that these agents do not inhibit chromosome motion directly by depleting ATP levels. If ATP is not required for chromosome motion, perhaps the inhibition caused by ionophores and azide indicates that anaphase A derives energy directly from another source, such as membrane potential or proton gradients, as does a bacterial flagellum (15, 16, 23, 26). We again refer to the importance that spindle membranes may have in the structure and function of the mitotic apparatus. It is increasingly evident that endomembranes (especially smooth endoplasmic reticulum) are prominent components of the mitotic apparatus and in some instances are associated specifically with kinetochore MTs (17). It is possible that changes in membrane potential or proton gradients developed across the spindle endoplasmic reticulum are coupled to kinetochore MTs and help move the chromosomes to the poles. There is evidence that sarcoplasmic reticulum vesicles can generate pH (25) and electrical potential (46) gradients, and proton-pumping ATPases have now been identified in a variety of intracellular compartments including secretory vesicles (7, 11, 20).

Much more experimentation is now needed to fully resolve these questions. With the use of intracellular microinjection, it is possible to avoid the permeabilization process and introduce inhibitors and large, nonpermeant probes directly into intact cells and thus bridge the gap between studies with cell models and those reported here. However, regardless of the particular mechanism that emerges to explain anaphase A, we believe that the process requires a continuous supply of energy in some form.

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Note Added in Proof: Recent experiments by one of us (B. A. Palevitz) using tubulin immunofluorescence show that spindle microtubules are lost in *Allium* root cells treated with colchicine for 30 min but remain in cells exposed to azide.

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