Taxol-requiring Mutant of Chinese Hamster Ovary Cells with Impaired Mitotic Spindle Assembly

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ABSTRACT In the accompanying paper (Cabral, F., 1982, *J. Cell. Biol.*, 97:22–29) we described the isolation and properties of taxol-requiring mutants of Chinese hamster ovary cells. We now show that at least one of these mutants, Tax-18, has an impaired ability to form a spindle apparatus. Immunofluorescence studies using antibodies to tubulin demonstrate that, when incubated in the absence of taxol, Tax-18 forms only a rudimentary spindle with few and shortened microtubules associated with the spindle poles. Furthermore, midbodies were not observed, consistent with an absence of cytokinesis. Essentially normal spindles and midbodies are seen in the presence of taxol. Electron microscopic examination indicates that centrioles and kinetochores are morphologically normal in the mutant strain. Pole-to-kinetochore microtubules were seen but interpolar microtubules were not. Taxol-deprived mutant cells stained with anti-centrosome serum show an elevated centriole content, indicating that the defect in Tax-18 does not affect centriole replication or prevent progression through the cell cyle. Although Tax-18 cells do not form a complete spindle in the absence of taxol, cytoplasmic microtubule assembly occurs in association with microtubule-organizing centers, and microtubules with apparently normal morphology exist throughout the cytoplasm. Observation of chromosome movement indicates that the defect in these cells occurs after prometaphase. These studies demonstrate that the formation of spindle microtubules requires cellular conditions that are different from those required for cytoplasmic microtubule formation. They further show that a normal spindle may be necessary for cytokinesis but not for progress of the cells through the cell cycle.

Mitosis has been the subject of many investigations during the last century. These studies have led to a detailed understanding of the morphological structures and mechanics of cell division (reviewed in references 19 and 33) but questions remain regarding the molecular components necessary to form these structures. In addition, very little is known about the factors that regulate entry into mitosis, centriole and chromosome movement, and cytokinesis. Some particularly insightful experiments, which have attempted to answer these questions, include the use of micromanipulation to rearrange chromosomes and organelles during mitosis (22), the use of UV microbeams to selectively destroy small areas of the mitotic apparatus (1, 2), studies of the action of mitotic inhibitors (8, 15, 31), and the development of a permeabilized model for studying chromosome movement and cell division (13).

We are attempting a genetic dissection of the factors that influence the ability of a cell to enter and progress through mitosis. Such an approach has been used by Hartwell (17) to study the control of cell division in yeast and more recently by Oakley and Morris (23) to show that nuclear division and movement is α- and β-tubulin dependent in *Aspergillus*. A number of investigators have used similar methods for studying mitosis in mammalian cells (10, 21). The mutants described in this and the accompanying paper (9) constitute an important new step in this approach. These mutants were selected for resistance to taxol, a drug recently shown to stabilize microtubules in vivo and in vitro (28, 29). In addition to being drug resistant, however, the cells were found to require taxol for cell division. In at least one case, Tax-18, the requirement for taxol is absolute. A number of experiments indicate that Tax-18 cells are unable to divide unless taxol is present (9). We now show that the defect in these cells affects their ability to assemble a mitotic spindle apparatus.

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MATERIALS AND METHODS

Antisera: Antibodies to bovine brain 6S tubulin were prepared in sheep and affinity purified according to the methods described in Fuller et al. (16). Human autoantibodies to centrosomes were found initially in a pooled human serum originating from >50 patients. The antiserum has been found to be specific for centrioles and pericentriolar material by immunoelectron microscopy (3).

Immunofluorescence: Cells were grown on 11 × 22-mm glass coverslips for immunofluorescence staining. For staining with antitubulin, cells were rinsed in PEM-PEG buffer (80 mM PIPES, 1 mM EGTA, 0.5 mM $MgCl_2$, with 4% polyethylene glycol 6,000) and permeabilized in PEM-PEG containing 0.5% Triton X-100 for 90 s. They were then rinsed again in PEM-PEG and fixed in 3% formaldehyde in PEM with 1% dimethyl sulfoxide for 30 min. Coverslips were then inverted cell-side-down over a 20-μl drop of antitubulin (100 μg/ml) and incubated for 60 min at 37°C. Following a 45-min wash in Dulbecco's phosphate-buffered saline (PBS), coverslips were again incubated at 37°C for 40 min in fluorescein isothiocyanate-conjugated anti-sheep immunoglobulin G obtained from immunized rabbits (Miles Laboratories, Elkhart, IN). After a 60-min wash in PBS, coverslips were mounted in 9:1 glycerol/PBS solution (pH 8.5–9.0) and observed in a Leitz orthoplan epifluorescence microscope equipped with a 100-W mercury lamp. For staining with anticentrosome, the serum was diluted 1:100 with PBS. The procedure for antitubulin was followed, except that the fixation and permeabilization steps were reversed.

Photographs were taken with the Plan 100× oil objective, with an NA of 1.25, on Kodak Tri-X-pan film at ASA 800–1000 and push-processed.

Colcemid Treatment: In some experiments, cells were treated with 0.06 μg/ml of colcemid (demecolcine) in media for 4 h.

Electron Microscopy: For electron microscopy, cells growing in plastic petri dishes (Lux Permanox) were fixed in situ with 3% glutaraldehyde in 0.1 M PIPES buffer, pH 6.9, and postfixed in 1% osmium tetroxide in PIPES buffer. After dehydration through an ethanol series, the cells were flat-embedded in Epon according to the method of Brinkley et al. (8). After polymerization for 24 h at 60°C, the culture dish was separated from the Epon wafer. Cells in the Epon wafer were examined with a phase-contrast microscope, and selected cells were marked, bored out of the disk, and glued to the tip of a blank Epon peg. Thin sections of the selected cells were cut on a Porter-Blum MT2B ultramicrotome with a diamond knife. Sections were picked up on collodion-coated, slotted grids and stained with alcoholic uranyl acetate followed by lead citrate. The sections were then examined and photographed in a JEOL 100CX electron microscope operated at 60 KV.

RESULTS

Tax-18 Has Apparently Normal Cytoplasmic Microtubules

The discovery that Tax-18 requires taxol for cell division and our awareness of published data suggesting that taxol is a microtubule "stabilizing" agent led us to suspect that Tax-18 might be unable to form microtubules unless taxol were present to drive the tubulin equilibrium toward the polymerized state. To examine this possibility, mutant cells were cultured on glass coverslips in either the presence or absence of 0.2 μg/ml of taxol and viewed by indirect immunofluorescence using affinity-purified sheep antibodies to calf brain tubulin (16). The results shown in Fig. 1 indicate that Tax-18 cells are able to assemble cytoplasmic microtubules whether they are cultured in taxol (Fig. 1, *C–D*) or in its absence (Fig. 1, *E–F*). Interestingly, wild-type Chinese hamster ovary (CHO) cells treated with 0.2 μg/ml of taxol exhibit microtubule bundling (compare Fig. 1*A* with 1*B*), while Tax-18 cells do not (Fig. 1, *C–D*). This may reflect the increased resistance of Tax-18 to taxol.

Electron microscopic examination of mutant cells in interphase confirms the results seen by immunofluorescence. The cytoplasmic microtubules in Tax-18 have a normal morphology whether they are cultured with or without taxol. We thus conclude that the alteration in Tax-18 does not affect the ability of the cells to assemble normal-appearing cytoplasmic microtubules.

While we cannot be certain that these morphologically normal microtubules are functionally intact, time-lapse observation of mutant cells deprived of taxol suggests that at least one suspected microtubule-mediated function, saltatory motion, is normal in these cells.

Taxol Deprivation Impairs Spindle Formation

Although Tax-18 appears to be unimpaired in its ability to form cytoplasmic microtubules, an examination of the mitotic cells in the population indicated that the mutant is defective in its ability to form spindle microtubules. Fig. 2 shows a series of immunofluorescence photographs of wild-type and mutant cells in mitosis using antiserum to tubulin. Mutant cells cultured in 0.2 μg/ml of taxol (Fig. 2*B*) form a metaphase spindle apparatus similar in appearance to, but somewhat smaller than, spindles assembled by wild-type cells (Fig. 2*A*). In the presence of taxol, mutant spindles progress normally into anaphase (Fig. 2*C*) and telophase (Fig. 2*D*). When the mutant cells are deprived of taxol for 48 h, however, metaphase spindles are not found (Fig. 2, *E* and *F*). Centriole pair separation occurs and radial arrays of microtubules are found at each spindle pole, but a complete spindle apparatus does not form. Midbodies could be easily found in wild-type cultures and in mutant cultures grown in taxol (Fig. 2*D*) but were never found in mutant cultures deprived of taxol. Results similar to these were obtained using Tax-18 cells deprived of taxol for 24 h rather than the 48 h described in these experiments.

Tax-18 Cells Have Normal Appearing Centrioles and Distinct Kinetochores

One possible explanation for the inability of the mutant cells to form a metaphase spindle would be an impairment in the structure or nucleating capacity of the microtubule-organizing centers (MTOC). To explore this possibility, mutant cells in interphase and mitosis were fixed and processed for transmission electron microscopy. The results are summarized in Figs. 3 and 4. Mutant cells grown in the presence of taxol displayed all stages of mitosis and normal spindle ultrastructure. Some representative stages of mitosis are shown in Fig. 3, *A–F*. Mutant cells deprived of taxol displayed normal-appearing centrioles (Fig. 4*A*) with associated pericentriolar material. However, very few microtubules were seen associated with these centrioles (compare with Fig. 3*B*). Unlike the situation in colcemid-arrested cells (8), the centriole pairs were usually separated as in normal prometaphase. Interpolar microtubules were not detected either by indirect immunofluorescence or by electron microscopy and were presumed to be absent from the aberrant spindle. Otherwise, arrested mitosis in Tax-18 appeared very similar to prometaphase in normal cells.

Tax-18 mutants deprived of taxol displayed kinetochores with a fine structure characteristic of mammalian cells in prometaphase and metaphase stages of mitosis (7) and identical to that of kinetochores seen in mutant cells grown in taxol (Fig. 3). Many kinetochores contained a single electron-dense plate as is commonly seen in prometaphase or colcemid-arrested wild-type cells (7, 27). Fig. 3*E* shows an example of such a structure for taxol-supplemented Tax-18 cells. Some kinetochores in the taxol-deprived Tax-18 cells, however, clearly displayed double plates (Fig. 4*A*) forming a complete trilaminar disk characteristic of mature metaphase

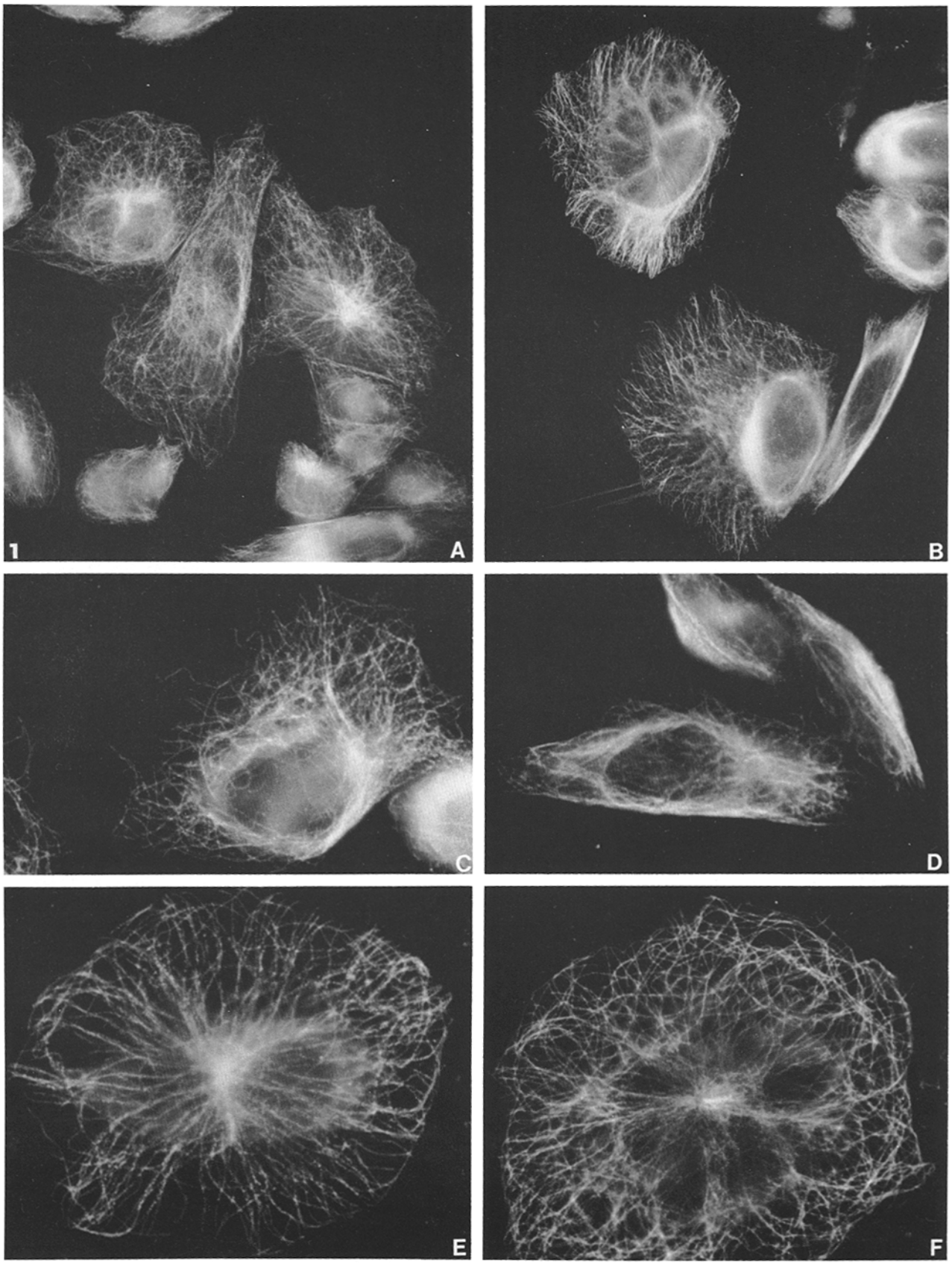

FIGURE 1 Tubulin immunofluorescence of CHO cells in interphase. (*A*) Wild-type (CHO); (*B*) wild-type cells grown in 0.2 μg/ml of taxol; (*C–D*) Tax-18 mutant grown in 0.2 μg/ml of taxol; (*E–F*) Tax-18 mutant grown without taxol. Note extensive microtubule complex and micronuclei.

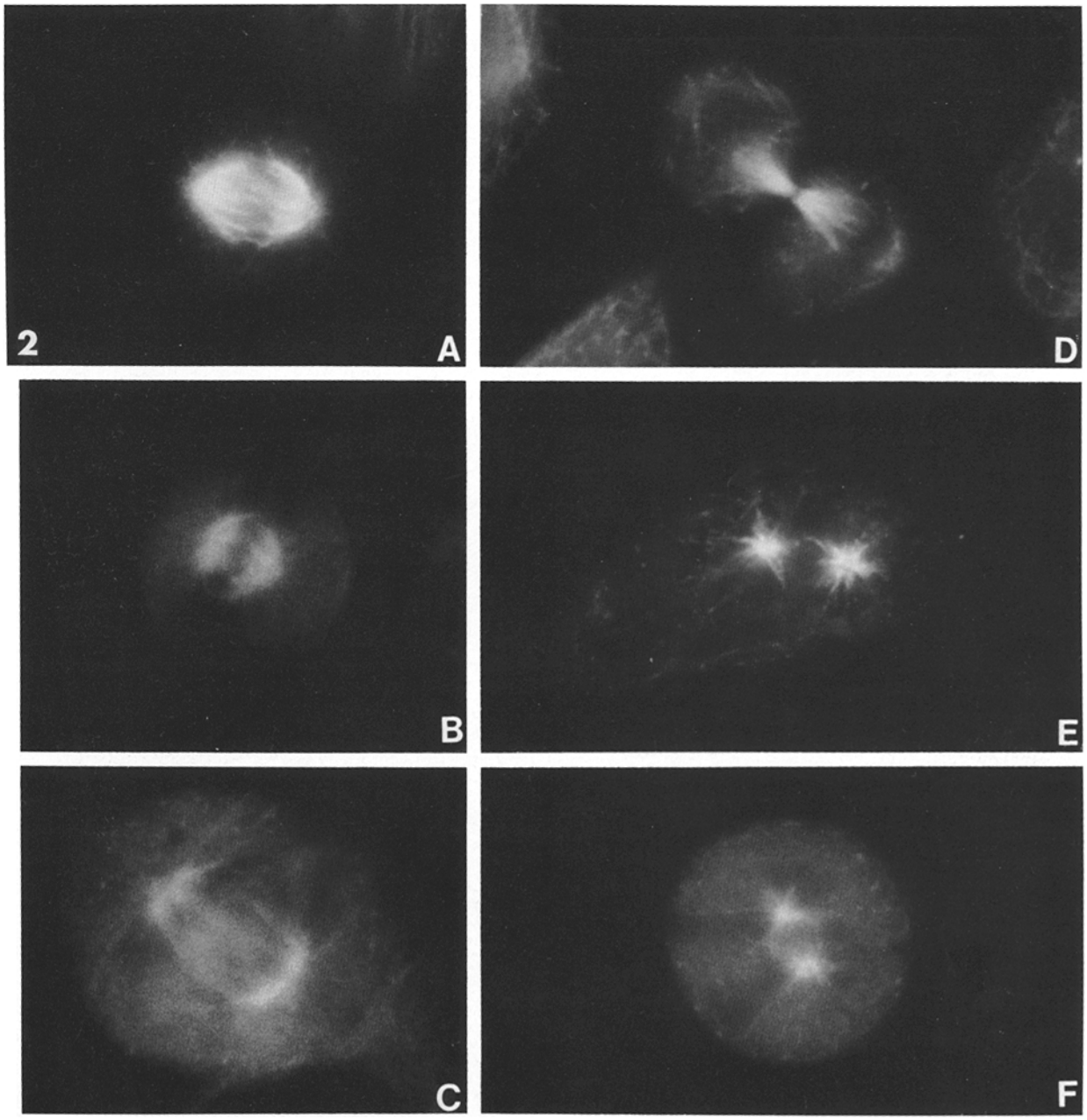

FIGURE 2 Tubulin immunofluorescence of CHO cells in mitosis. (*A*) Wild-type cells in metaphase; (*B*) Tax-18 plus taxol in metaphase, note smaller metaphase spindle; (*C*) Tax-18 plus taxol in anaphase; (*D*) Tax-18 plus taxol in telophase; (*E–F*) Tax-18 minus taxol for 48 h. Note radial array of microtubules at each pole but absence of complete metaphase spindle. Cultures referred to as "plus taxol" were grown continuously in 0.2 μg/ml of taxol. Examples of metaphase, anaphase, and telophase cells were not found in Tax-18 cultures deprived of taxol.

kinetochores (4, 27). This indicates that the kinetochores of taxol-deprived Tax-18 cells are capable of maturing into the metaphase structures. Overall, then, the electron microscopic images suggest that the structures of MTOC are normal in mutant cells. Subtle changes in these nucleating centers, however, cannot be excluded by these experiments.

Rudimentary Spindle Formed in Taxol-deprived Mutant Cells Contains Pole-to-Kinetochore but Not Interpolar Microtubules

Electron microscopic examination of the spindle region of mutant cells deprived of taxol for 48 h indicates the presence of pole-to-kinetochore microtubules (Fig. 4*B*). There appear to be fewer of these than in wild-type cells or in mutant cells supplemented with taxol. Typically, each kinetochore in a wild-type spindle contains 9–16 microtubules (32). The number of microtubules for mutant cells with taxol present in the growth medium falls within this range but the rudimentary spindle of Tax-18 cells deprived of taxol displayed only 4–6 microtubules per kinetochore (Fig. 4*B*, *inset*). Further experiments are required to determine whether or not this lowered number of kinetochore microtubules is a general feature of taxol-starved mutant cells and, if so, whether it reflects a decreased ability of the centrosome to nucleate these filaments or simply reflects the fact that there is an increased number

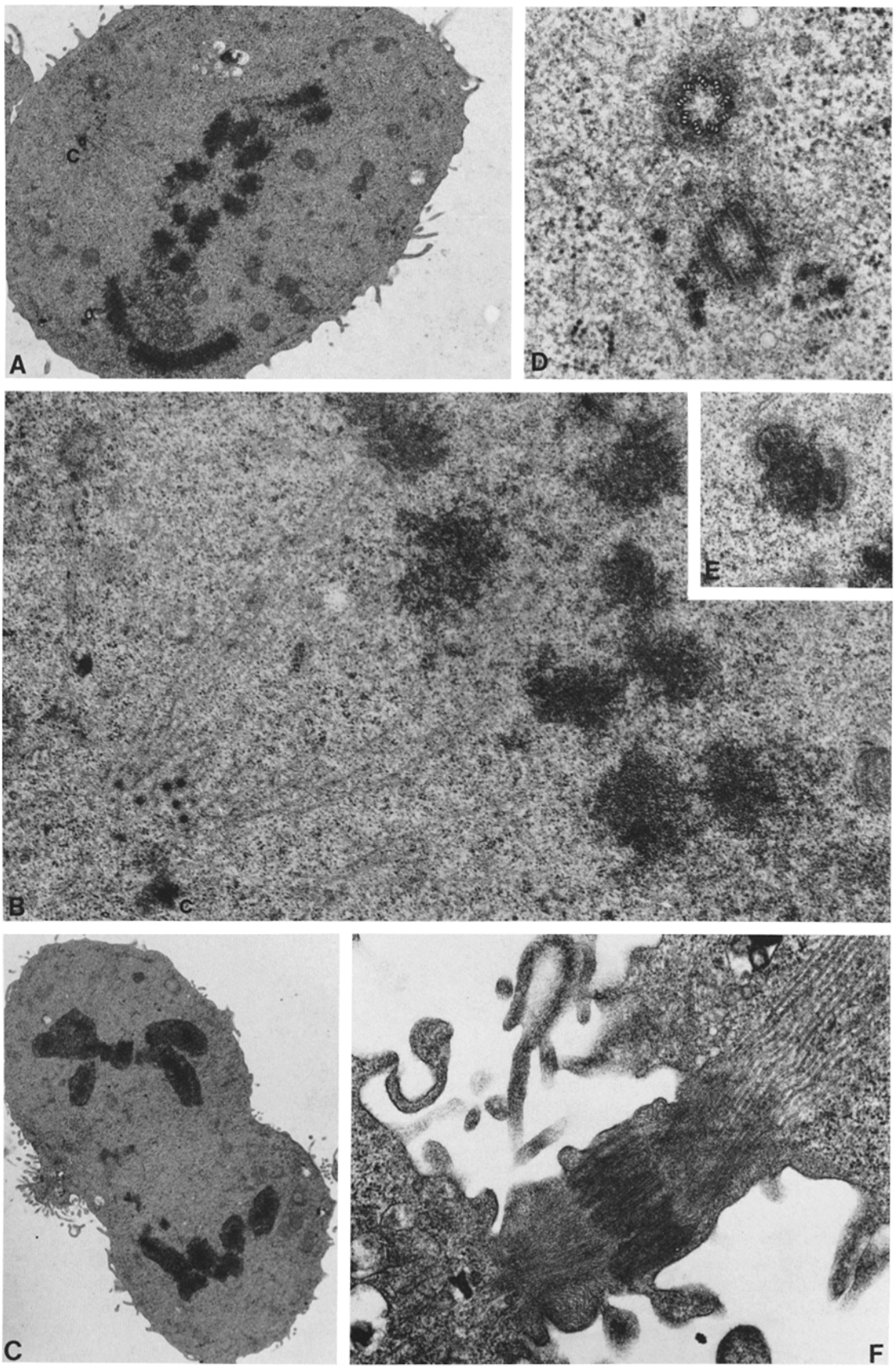

FIGURE 3 Spindle ultrastructure during mitosis in Tax-18 grown in 0.2 μg/ml of taxol. (*A*) Metaphase; (*B*) higher magnification of metaphase, note kinetochore-to-pole microtubules; One centriole pair (*c*) is in and the other centriole pair is out of the place of section. (*C*) telophase; (*D*) centriole pair; (*E*) prometaphase kinetochore exhibiting a single electron-dense plate; (*F*) midbody with microtubule array.

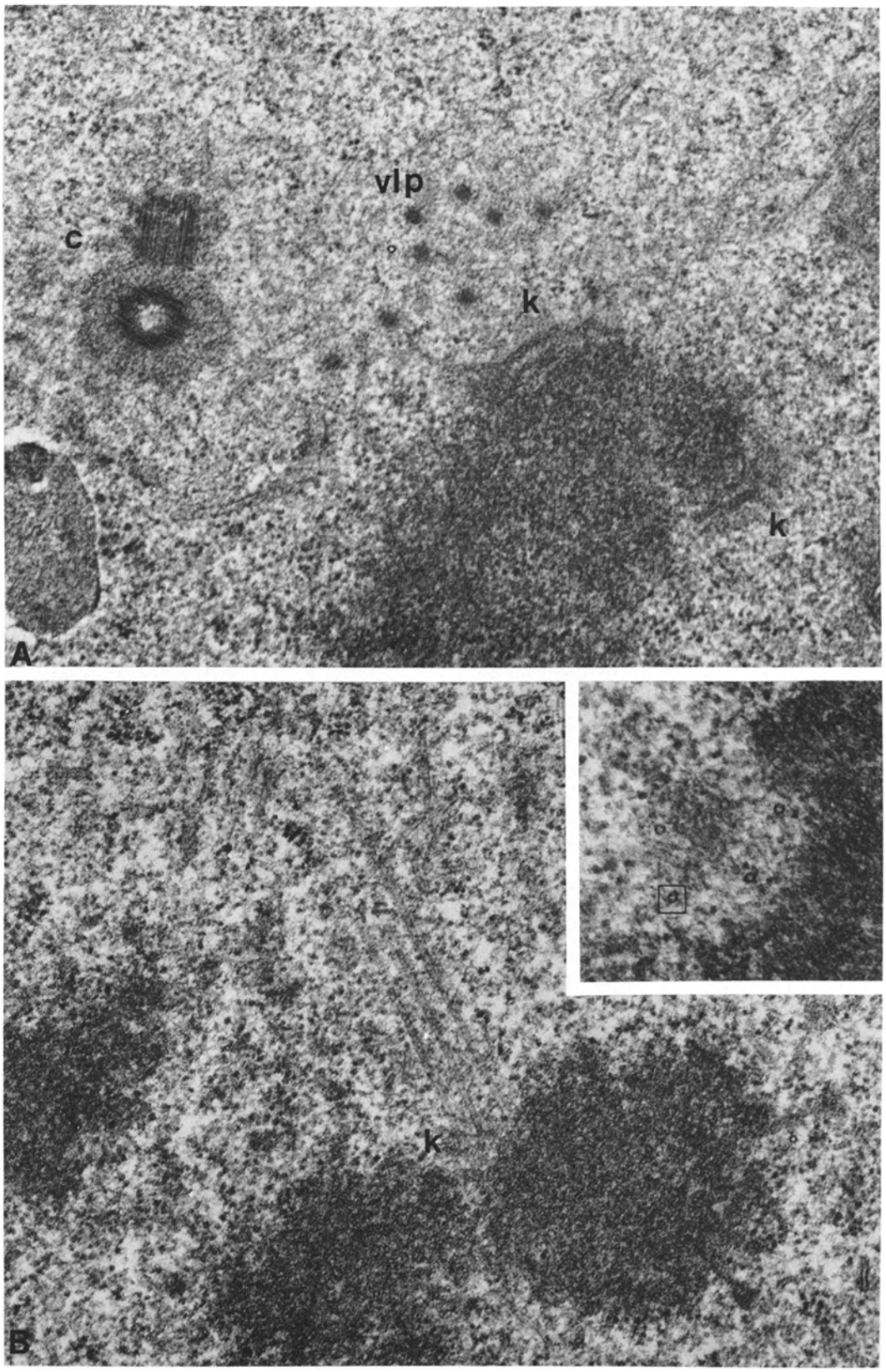

FIGURE 4 Spindle ultrastructure in Tax-18 mutant minus taxol. (*A*) Centriole pair (c) and chromosomes with kinetochores (*k*). Note viruslike particles (*vlp*) typical of CHO cells; (*B*) parallel array of kinetochore-to-pole microtubules. *Inset* shows kinetochore sectioned *en face*, revealing cross sections of several microtubules (the position of one such microtubule is indicated with a box).

of chromosomes competing for a constant number of microtubules. Interpolar microtubules were not observed although it is difficult to completely rule them out on the basis of thin-section analysis of mutant spindles. However, no traces of them were seen by immunofluorescence either.

Further evidence for the absence of interpolar microtubules

was derived by looking at the cold-stability of the microtubules in the spindle apparatus. Cells grown on coverslips were incubated at 0–4°C for up to 2 h before processing the cells for immunofluorescence with antibodies to tubulin. Metaphase spindles from Tax-18 cells grown in taxol were found to be missing microtubules from the midzone region, and only the half-spindles remained visible as described for other cold-treated cells (5). The rudimentary spindles of taxol-deprived mutant cells showed no change in their fluorescent image upon cold-treatment, consistent with the interpretation that the microtubules that exist in these spindle structures are of the pole-to-kinetochore and not of the interpolar or astral variety. Of course, this interpretation must be tempered by the realization that the existence of short interpolar-type microtubules could be missed by both the electron microscopic and immunofluorescence techniques.

Tax-18 Replicates Centrioles Even in the Absence of Cell Division

It was shown in the accompanying paper (9) that Tax-18 cells fail to divide in the absence of taxol. In spite of this lesion, the cells continue to synthesize protein and replicate their chromosomes, indicating that progression of these cells through the cell cycle is not prevented. We now show that another cell cycle-specific event, replication of centrioles, is also not prevented. Centrioles normally replicate during S phase (26), undergo a maturation process during G_2 phase (30), and then segregate into daughter cells during cell division, thereby maintaining a constant 1–2 pair of centrioles per CHO cell during the cell cycle. Using a human-derived serum, which contains antibodies capable of recognizing centrioles in a variety of cell types (3), we found that wild-type cells (Fig. 5*A*) and Tax-18 cells grown in the presence of 0.2 μg/ml of taxol (Fig. 5*B*) have the normal number of centrioles per cell. When Tax-18 is deprived of taxol, however, the cells accumulate multiple centrioles (Fig. 5*C*) consistent with the interpretation that these cells are able to progress through the cell cycle in the absence of cell division and carry out a number of normal cellular processes. The existence of multiple centrioles in these cells was also confirmed by electron microscopy.

Increased Centriole Content in Tax-18 Cells Correlates with an Increased Number of MTOC in Those Cells

The increased number of centrioles in taxol-deprived mutant cells does not necessarily mean that there is also an increased capacity of those cells to nucleate microtubule assembly. To test whether the additional centrioles in these cells were capable of acting as MTOC, the cells were first treated with colcemid to depolymerize their microtubules, the colcemid was then washed out, and the cells were allowed to recover for 15 min as described in previous studies (6, 24). The cells were then fixed, permeabilized, and stained by indirect immunofluorescence using antibodies to tubulin. Fig. 5, *D* and *E* show that, as expected, wild-type and Tax-18 cells grown in taxol have 1 or 2 MTOC per cell, correlating well with the 1–2 centriole pairs, which these cells possess (Fig. 5, *A* and *B*). Tax-18 cells deprived of taxol for 48 h, however, exhibit multiple MTOC per cell (Fig. 5*F*) at a time when they also exhibit multiple centriole pairs (Fig. 5*C*). These results suggest that the additional centrioles found in the taxol-deprived mutant cells are functional units capable of initiating microtubule assembly.

To quantitate the number of centrioles that these cells are able to accumulate, three investigators independently counted centrioles at random in immunofluorescently stained preparations of wild-type, Tax-18 plus 0.2 μg/ml of taxol, and Tax-18 deprived of taxol for 48 h. The results are summarized in Fig. 6. As expected, wild-type cells have 2–4 centrioles (1–2 pair) per cell (Fig. 6*A*). Tax-18 cells grown in taxol (Fig. 6*B*) have a centriole content similar to that of wild-type cells, but when these same cells are deprived of taxol for 48 h (Fig. 6*C*) their centriole content increases to a modal number of 8, and some cells have as many as 16 centrioles. These results indicate that Tax-18 cells are able to transverse 2–3 cell cycles in 48 h in the absence of cell division.

Taxol-starved Tax-18 Cells Continue to Exhibit Prometaphase Chromosome Movements

The presence of short microtubules apparently of the pole-to-kinetochore type in mitotic Tax-18 cells deprived of taxol led us to examine whether these residual microtubules might be effective in controlling chromosome movements in these cells. To explore this possibility, Tax-18 cells either supplemented with taxol or deprived of taxol for 24–48 h were examined under high magnification using phase-contrast video time-lapse photography. Mutant cells in the presence of taxol displayed normal CHO chromosome movements characteristic of prophase, prometaphase, metaphase, and anaphase. When the cells were examined in the absence of taxol, however, metaphase alignment and anaphase movement were absent. Instead, chromosomes exhibited an erratic prometaphase-like motion but never aligned into a metaphase plate. Membrane blebbing characteristic of telophase eventually occurred but, as previously noted (9), the blebbing was much more violent and prolonged. During this period, constriction of the cytoplasm was seen, suggesting that contractile ring assembly may occur in the mutant; but the final stages of cytokinesis, i.e., the cleaving of the cytoplasm to yield two separate cells, did not occur. At the end of this period, the chromosomes decondensed and nuclear membranes reformed as the cell entered G_1 phase.

DISCUSSION

In this paper we describe the morphological characterization of a novel mutant cell line. Tax-18 is a mutant derived from CHO cells selected for resistance to the microtubule-stabilizing drug taxol. In addition to being taxol-resistant, however, these cells exhibit the unique property of requiring taxol for cell division (9). Although these cells look normal and grow normally in the presence of low concentrations of taxol (0.1–0.3 μg/ml), they fail to divide when taxol is omitted from the culture medium. This failure to divide does not prevent them, however, from continuing to progress through the cell cycle. Thus, taxol-deprived mutant cells continue to synthesize protein and replicate chromosomes, thereby becoming much larger than their taxol-supplemented counterparts.

Given the fact that taxol is a microtubule-stabilizing drug (28, 29), we first hypothesized that Tax-18 might not be capable of forming microtubules unless taxol was present to stabilize them. Examination of the cells by indirect immunofluorescence using antibodies to tubulin, however, quickly

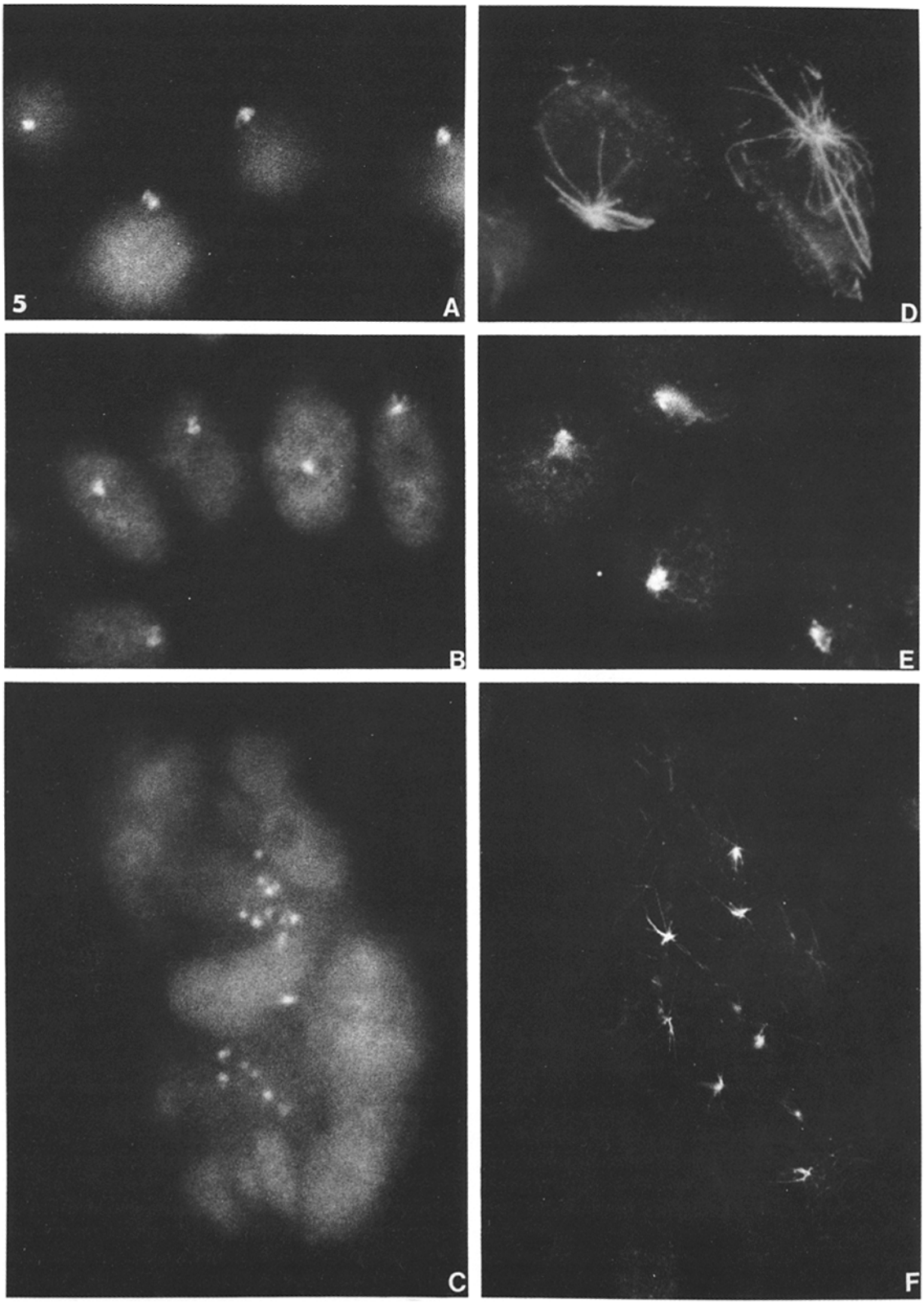

FIGURE 5 Centrosomes and MTOC in CHO wild-type and mutant cells. (*A*) Wild-type CHO cells stained with anticentrosome serum. Double appearance of centrosome is probably due to centriole pair; (*B*) Tax-18 mutant plus taxol showing centrosomes in each cell; (*C*) giant multinucleate cell in Tax-18 mutant minus taxol for 48 h. Note micronuclei lightly stained with the antiserum and multiple centrosomes (each single fluorescent spot probably represents one centriole); (*D–F*) cells were treated with 0.06 μg/ml of colcemid for 2 h to depolymerize microtubules. The colcemid was washed out and the cells allowed to recover for 15 min. Microtubule regrowth is shown by staining the recovering cells with antitubulin. (*D*) Short microtubules growing from centrosome in wild-type cells; (*E*) microtubule re-growth in four Tax-18 mutant cells plus taxol; (*F*) multiple MTOC observed in single giant Tax-18 mutant cell minus taxol.

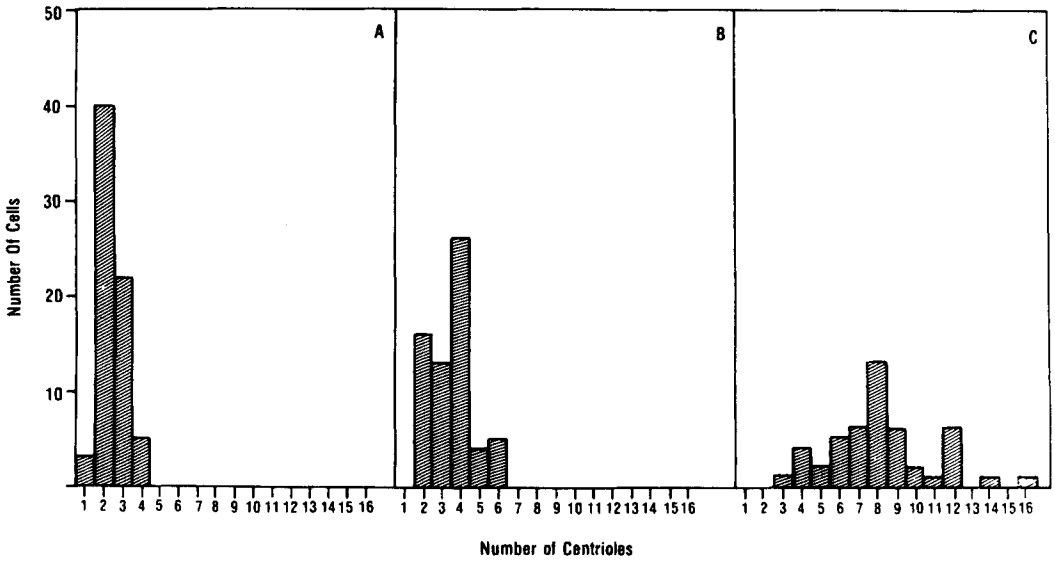

FIGURE 6 Histogram showing the frequency distribution of centrioles in wild-type (*A*), Tax-18 cells grown in 0.2 μg/ml taxol (*B*), and Tax-18 cells deprived of taxol for 48 h (*C*). Cells were grown on glass coverslips, fixed, and stained with anticentrosome serum. Centrioles were counted in cells picked at random and the frequency with which a given number of centrioles appears in these cells is plotted. Note the shift in the distribution toward higher numbers of centrioles per cell in Tax-18 cells deprived of taxol.

disproved this idea. Tax-18 cells are capable of assembling normal-appearing cytoplasmic microtubules. Although these microtubules look normal and the cells still exhibit saltatory motion, we cannot be certain that all cytoplasmic microtubule functions are intact.

Although the defect in Tax-18 does not seem to affect cytoplasmic microtubules, the spindle microtubules are clearly affected. In the absence of taxol, mutant cells are unable to assemble a metaphase spindle. Centriole pairs undergo separation at the onset of mitosis, and microtubules assemble in association with the spindle poles but the spindle apparatus is not able to progress beyond a prometaphase-appearing mitotic apparatus. Midbodies are also not seen, consistent with a lack of cell division. Electron microscopic examination confirms the presence of normal-looking cytoplasmic microtubules in interphase cells and the presence of spindle microtubules in mitotic cells. The spindle microtubules that are seen, however, are shorter and fewer in number. Most if not all of these residual spindle microtubules are of the pole-to-kinetochore variety; interpolar microtubules were not observed.

The inability of Tax-18 cells to form a metaphase spindle has at least two major consequences: (*a*) chromosomes are unable to segregate normally, leading to the formation of micronuclei, and (*b*) cytokinesis cannot occur. The first consequence is hardly surprising since there is ample evidence in the literature that microtubules participate in the orderly segregation of chromosomes (13, 14, 19). Our studies now provide compelling genetic evidence that microtubules are essential for this process. The second consequence is not so unanimously conceded in the literature. The contractile ring always bisects the spindle apparatus at the metaphase plate, and a number of studies using micromanipulation have shown that when the spindle is displaced the site of contractile ring formation is also displaced so that it again forms at the metaphase plate (14). Other experiments, however, have indicated that asters can direct cell division in the absence of a spindle (18, 25). The status of a contractile ring in the Tax-18 mutant remains uncertain. Although we have observed cytoplasmic constriction during time-lapse observation of mutant cells in the absence of taxol, we have not as yet demonstrated a contractile ring by electron microscopy. Thus, it is not yet possible to speculate on how the absence of a spindle in taxol-deprived mutant cells prevents cytokinesis. This should prove to be a fruitful area for further studies.

Based on the observation that chromosomes can undergo limited prometaphase movement in the Tax-18 mutants, we argue that a complete mitotic apparatus may not be necessary for prometaphase movements. Apparently, a few kinetochore-to-pole microtubules are sufficient to achieve movement, and interpolar microtubules may not be necessary for chromosome movements prior to metaphase.

It is also interesting that even though Tax-18 fails to divide in the absence of taxol the cells are still able to progress through the cell cycle. This was shown by the fact that the cells are capable of synthesizing proteins and are still able to perform cell-cycle-specific functions such as chromosome and centriole replication. A similar conclusion was drawn by observing the cells by time-lapse video photomicroscopy. Although the cells were clearly delayed in mitosis when taxol was absent, they were able to exit mitosis as evidenced by the cessation of chromosome and membrane motions, the decondensation of chromosomes, the reformation of nuclear membranes, and a flattening of the cell onto the culture dish. These results argue that the signals to exit mitosis and enter the G_1 phase of the cell cycle are not dependent upon the formation of an intact spindle, the orderly segregation of chromosomes, or division of the cytoplasm into daughter cells.

At present, we can only speculate about the lesion in Tax-18, which leads to the cellular characteristics described above. One possible explanation is that the mutant cells have a lower intracellular concentration of tubulin, which is sufficient for cytoplasmic but not for spindle microtubule assembly. Addition of taxol would then lower the critical concentration of tubulin necessary for assembly, thus allowing the spindle to form. However, two dimensional gel analysis of several inde-

pendently derived mutants with the Tax-18 phenotype has thus far failed to demonstrate any qualitative or quantitative alterations in either α- or β-tubulin. Also, Tax-18 differs from previously isolated tubulin mutants (11, 12) in several of its properties (9). While none of these arguments rules out a "silent" mutation in tubulin's being responsible for the Tax-18 phenotype, we are considering the possibility that Tax-18 lacks a (or has a defective) protein necessary for spindle but not for cytoplasmic microtubule assembly. This "spindle-specific" microtubule-associated protein (MAP) would bind to and stabilize the microtubules, thus acting as a natural cellular analogue of taxol. The absence of such a protein should render the microtubules more labile to microtubule-disrupting drugs as, indeed, appears to be the case (9). Cells with large amounts of this MAP would be sensitive to low amounts of taxol; cells with low amounts of this MAP (or its activity) would be resistant to higher levels of taxol. If the amount of the MAP was tightly regulated, then it is conceivable that a cell with lowered levels of the protein might require exogenous taxol to maintain a normal level of "microtubule stabilizing activity" in the cell. A variation on this scheme would postulate the existence of an intracellular enzyme or other modifying protein that "activates" a spindle-specific MAP (e.g., by phosphorylation). Such an enzyme would then be missing or defective in Tax-18. Mechanisms invoking inactivation of a spindle-specific protein by proteolysis or chemical modification are unlikely since the phenotype in Tax-18 behaves recessively in somatic cell hybridization experiments. A final possibility to explain the phenotype of Tax-18 would be the existence of a defective spindle-specific tubulin. Precedence for such a possibility has been provided by Kemphues et al. (20) who have demonstrated the existence of a testis-specific β-tubulin in *Drosophila*. At present we have no evidence to support or discount this explanation.

In summary, we have characterized a mutant defective in spindle but not cytoplasmic microtubule assembly. This result argues strongly that different cellular conditions (proteins ?) are required for spindle than are required for cytoplasmic microtubule assembly. In addition, the defect in these cells demonstrates that a mature spindle apparatus is required for the orderly segregation of chromosomes and for cell division but not for continued progression of cells through the cell cycle. Identification of the biochemical lesion in these cells should help us to define the proteins involved in spindle assembly. Further studies with Tax-18 and other mutant cells that have alterations in tubulin and are temperature sensitive for growth (11, 12) will help to further our knowledge of the role of microtubules in cellular physiology.

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