Studies on Cell Division in Mammalian Cells. VII. A Temperature-sensitive Cell Line Abnormal in Centriole Separation and Chromosome Movement

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ABSTRACT A temperature-sensitive Syrian hamster mutant cell line, ts-745, exhibiting novel mitotic events has been isolated. The cells show normal growth and mitosis at 33°C, the permissive temperature. At the nonpermissive temperature of 39°C, mitotic progression becomes aberrant. Metaphase cells and those cells still able to form a metaphase configuration continue through and complete normal cell division. However, cells exposed to 39°C for longer than 15 min can not form a normal metaphase spindle. Instead, the chromosomes are distributed in a spherical shell, with microtubules (MT) radiating to the chromosomes from four closely associated centrioles near the center of the cell.

The cells progress from the spherical monopolar state to other monopolar orientations conical in appearance with four centrioles in the apex region. Organized chromosome movement is present, from the spherical shell state to the asymmetrical orientations. Chromosomes remain in the metaphase configuration without chromatid separation. Prometaphase chromosome congression appears normal, as the chromosomes and MT form a stable monopolar spindle, but bipolar spindle formation is apparently blocked in a premetaphase state. When returned from 39° to 33°C, the defective phenotype is readily reversible.

At 39°C, the mitotic abnormality lasts 3-5 h, followed by reformation of a single nucleus and cell flattening in an interphase-like state. Subsequent cell cycle events appear to occur, as the cells duplicate chromosomes and initiate a second round of abnormal mitosis. Cell cycle traversion continues for at least 5 d in some cells despite abnormal mitosis resulting in cells accumulating several hundred chromosomes.

To achieve a more complete understanding of mitosis in mammalian cells, many studies on abnormal mitosis have been performed. The abnormality is often generated after the application of inhibitors or altered physical conditions that affect microtubule integrity, resulting in disruption of the mitotic spindle. While much information has been obtained from these studies, an obvious limitation is that changes in mitotic behavior generally result from indiscriminate microtubule dissociation and spindle dissolution. The treatments usually affect other cellular processes, resulting in difficulties in the analysis of specific mitotic events. An alternate approach to the study of mitosis is to use temperature-sensitive (ts) mutants in which discrete mitotic abnormalities presumably result from specific thermo-labile molecular species.

Previous papers in this series have reported the isolation and characterization of ts mutant Syrian hamster cell lines with defects in three distinct mitotic segments: prophase progression (13), metaphase block (12, 14), and post-metaphase chromosome movement (15). Several other mammalian cell mutants have been reported involving defects in cytokinesis (5, 10, 11) and both mitosis and cytokinesis (9). Here we report the isolation and characterization of a new mutant cell line, ts-745, with nonseparation of poles and apparently organized but novel chromosome behavior.

MATERIALS AND METHODS

HM-1 Syrian hamster cells (12, 13) were grown in Dulbecco's modified Eagle's medium (MEM) supplemented with 10% bovine calf serum (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) without antibiotics. Cell growth, mutagenesis, and mutant isolation procedures were done as described previously (12, 13, 15).

Cells cultured in 60-mm Falcon plastic dishes (Falcon Labware, Oxnard, CA)

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were maintained at the permissive temperature of 33°C according to experimental protocols. All experimental manipulations were performed in separate controlled environment rooms at 33.0° or 39.5° ± 0.1°C. A hemacytometer was used for counting cell number, two dishes for each point.

The mitotic index (MI) was determined by growing cells at 33°C until cultures were in log-phase growth. Half of the dishes were then switched to 39°C. At intervals, both supernatant and trypsinized cell layers were collected and centrifuged. Cells were resuspended in a 3:1 methanol:acetic acid fixative and kept at 0°C for 15 min. After a second centrifugation, the cells were dropped on cold slides, air-dried, and stained with 1% aceto-orcein. At least 500 cells per slide were examined to determine the MI which is expressed as the percentage of cells in mitosis.

For electron microscopic examination, cells were fixed at room temperature in a mixture of equal volumes of 2% glutaraldehyde in 0.1 M PIPES buffer at pH 7.0 and 1% aqueous OsO_4. The cells were rinsed and subsequently stained for 30 min in 0.5% uranyl acetate. After ethanol dehydration, the cells were infiltrated with Epon. The resin blocks were freed from the dish, and sections were cut parallel to the original dish surface.

Time-lapse cinemicrography was performed on cells grown in a Rose chamber with a Zeiss microscope enclosed in a temperature-controlled chamber maintained at 39 ± 0.2°C. A Bolex 16-mm movie camera controlled by a Sage time-lapse system was used to photograph the cells.

Chromosomes were prepared as previously reported (6), using colchicine to accumulate c-metaphase (4) cells at 33°C. Since accumulation of c-metaphase-like figures characterized the behavior of ts-745 at 39°C (see Results), no colchicine was used at this temperature. Spindle and chromosome staining was achieved by a differential staining technique after preservation of the spindle structure (16), which stains dark blue against a light cytoplasmic background while chromosomes appear bright red.

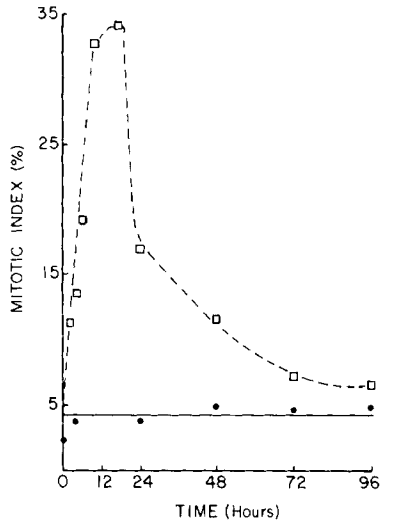

FIGURE 1 Mitotic index in ts-745 cells. (●) 33°C; (□) 39°C.

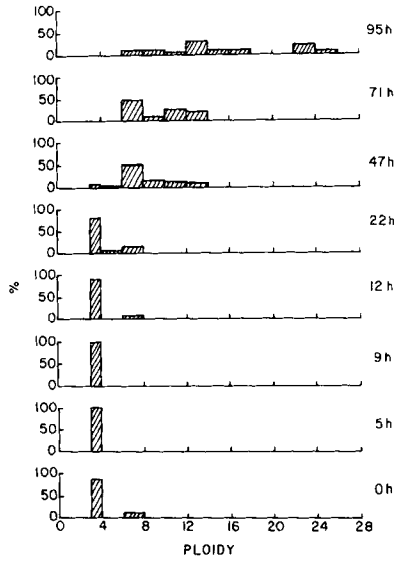

FIGURE 2 Increase in chromosome number in ts-745 cells at 39°C. When grown at 33°C, ts-745 cells contain about twice the Syrian hamster diploid chromosome number of 44.

RESULTS

The first indication of defective mitosis appeared when a ts-745 culture accumulated rounded cells at 39°C. As previously found with ts-546 (12) and ts-655 (13), increase in percentage of rounded cells was indicative of cells being delayed in mitosis. The mitotic index in ts-745 cultures was measured. Fig. 1 shows that the index increased almost immediately after the cultures were transferred to 39°C, reaching a peak of near 35% at 20 h and remaining higher than for 33°C-cells for the duration of the experimental period.

Growth curves of ts-745 cells showed only slight increase in cell number at 39°C for up to 5 d (data not shown). The cultures remained monolayer in appearance without the type of extensive detachment observed in ts-546 (12) and ts-655 (13). Cell size increased at 39°C (data not shown), indicating continued cell growth without completion of cell division.

Chromosome number of ts-745 cells maintained at 33°C was near-tetraploid (4N), higher than the near-diploid number of 45 (2N) for HM-1 parental cells. At 39°C, chromosome number in ts-745 increased continually, to the 20N range after 96 h (Fig. 2). The highest number counted after 96 h at 39°C was 576, or about 26N. It appeared likely that the chromosome number would continue to increase if the experiment were continued beyond 96 h. The chromosomes were found in metaphase configuration with both chromatids still joined together at the expected centromeric position.

Low-magnification (2.5 × objective and 10 × eye piece) time-lapse cinemicrography was performed to examine a large number of cells up to 4 d at 39°C (sequences not shown here). The cells were seen to round up but subsequently flattened out after 3 or 4 h and reattached to the substratum. These reattached cells were capable of later rounding up and flattening out again.

Observations under higher magnification (25 × or 40 × objective) revealed greater details on mitotic progression. During the first 15 min after the cells were shifted to 39°C, some

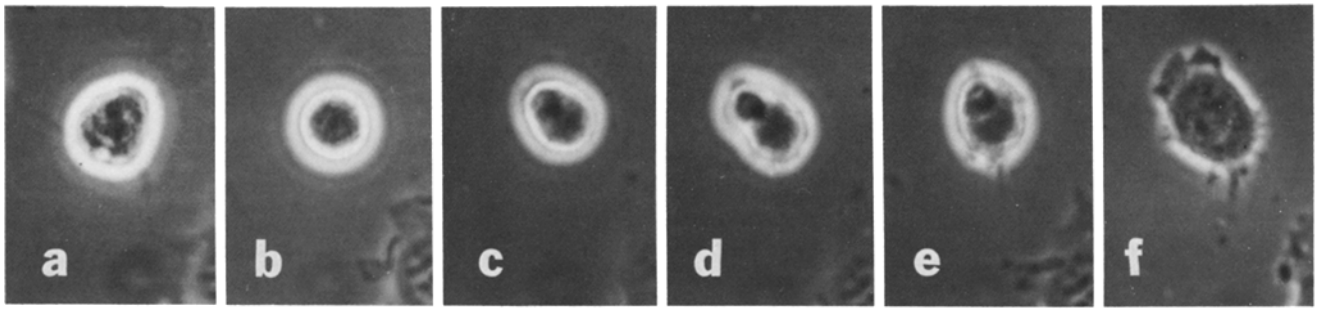

FIGURE 3 Time-lapse sequence of a ts-745 cell at 39°C for (*a*) 10, (*b*) 264, (c) 265, (*d*) 267, (*e*) 272, (*f*) 316 min. × 520.

mitotic cells were found in metaphase. These cells always proceeded through normal mitosis. The cells which failed to reach metaphase became defective. Fig. 3 shows a cell which initiated mitotis after 10 min at 39°C (Fig. 3 *a*). A few minutes later, the cell contained condensed chromosomes without formation of a metaphase plate. The cell remained in that state for over 3 h and retained the chromosomes which were distributed peripherally (Fig. 3 *b*) with a centrally oriented spindle, as seen with the spindle staining technique (not shown because the differential staining technique is not suitable for black-and-white printing). The chromosomes then organized into a ring-like state and rapidly moved to one side of the cell, forming a bulge at that side (Fig. 3 *c*). This state persisted for a few minutes (Fig. 3 *d* and *e*) followed by reformation of the nucleus (Fig. 3 *f*), and the cell became mononuclear and appeared interphase-like. After the incomplete mitosis, some cells traversed through the cell cycle with chromosome replication, increasing the chromosome number from near-tetraploid to

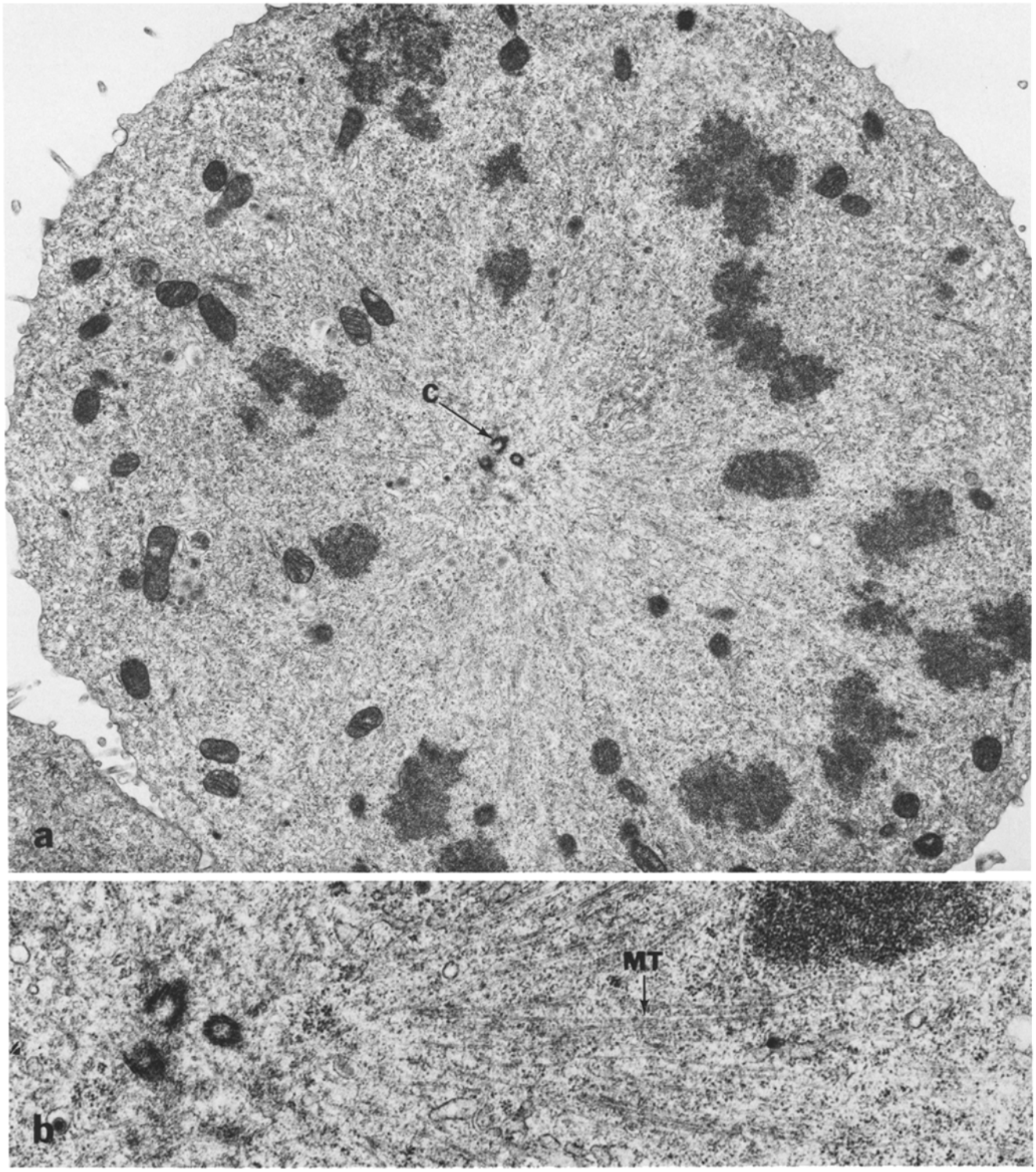

FIGURE 4 (*a*) Median section of a ts-745 cell incubated at 39°C for 4 h showing centrally located centrioles (C) and abundant centriole-chromosome microtubules. × 9,300. (*b*) Higher magnification of the centrioles and microtubules (*MT*). × 24,000.

near-octoploid, as reflected in Fig. 2. Cells appeared to be capable of progressing through several cell cycles culminating each in a defective mitosis and eventually accumulating several hundred or more chromosomes.

Cells in different stages of the abnormal mitotic process were examined under the electron microscope. Fig. 4*a* shows a medial section from a cell with a centrally oriented spindle which was c-metaphase-like (3) but containing abundant pole-to-chromosome microtubules (Fig. 4*a*). From the centrioles found in the center of the cell, MT radiated to the chromosomes distributed spherically. The central location shown under high magnification in Fig. 4*b* contained three centrioles but no pole-to-pole MT. A fourth centriole (not shown) was found in adjacent sections, demonstrating the presence of all four centrally positioned centrioles. The spherical chromosome distribution was confirmed by sections from the periphery (Fig. 5*a*) where chromosomes intermingled with ends of MT pointing toward the central location (Fig. 5*b*).

The centrally oriented spindle with spherical chromosome distribution was the first abnormal mitotic state exhibited by

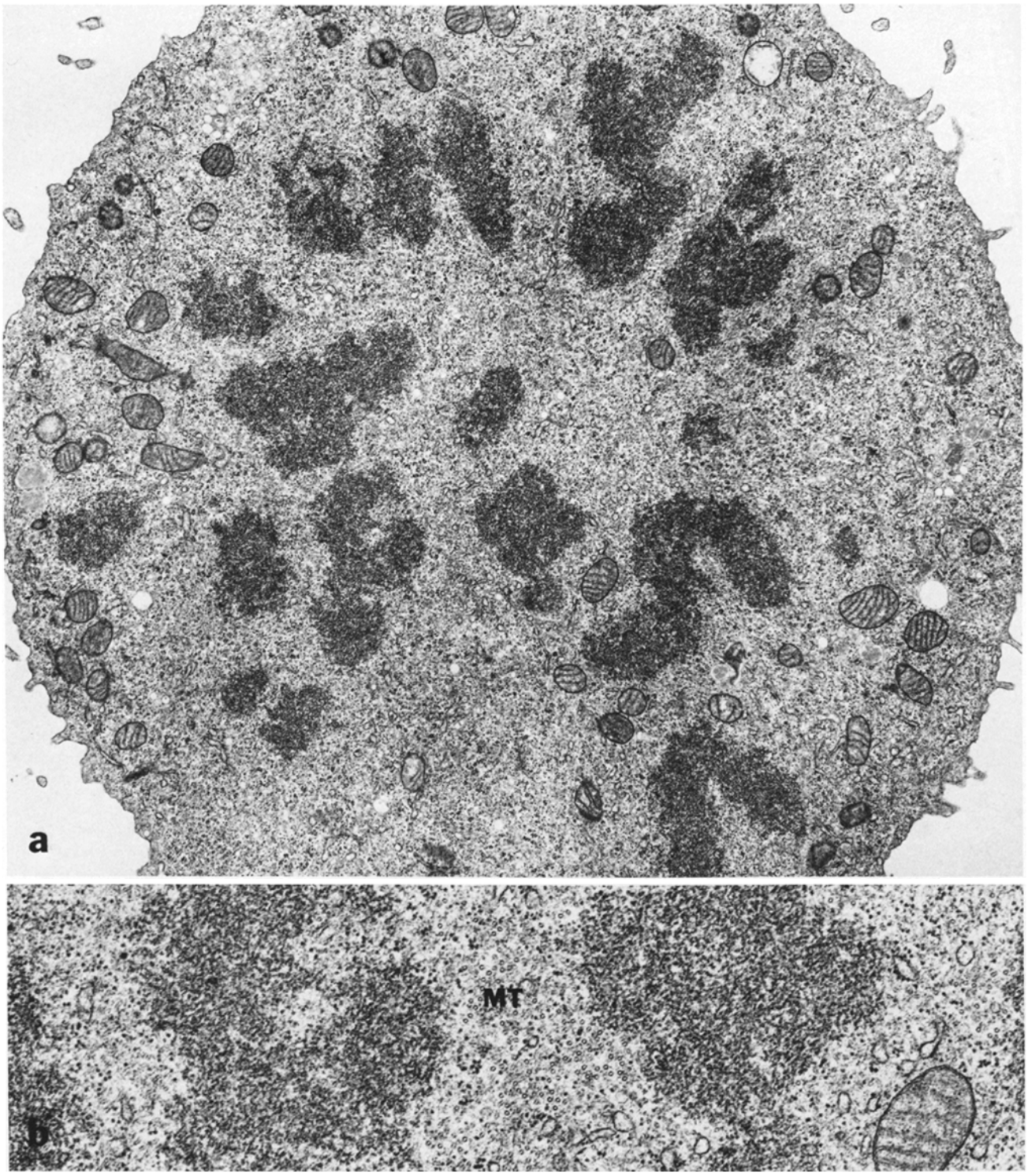

FIGURE 5 (*a*) Same cell as in Fig. 4 but sectioned nearer the periphery with chromosomes intermixed with microtubules pointing toward the centrally positioned centrioles. × 9,700. (*b*) Higher magnification of the microtubule cross sections (*MT*). × 27,000.

ts-745 cells at 39°C. The cells then progressed through several different chromosome and spindle orientations. Two types are shown in Figs. 6 and 7. One type had a cone-shaped monopolar spindle with chromosomes in the curved surface region, and MT forming the cone originated from the four centrally located centrioles, two of which were found in one section (Fig. 6) and the other two in adjacent sections (not shown). On the opposite side of the cell a bulge often formed which was devoid of visible spindle or chromosome material (Fig. 6). Another type, also monopolar, reversed the centriole and chromosome positions with centrioles near the cell periphery (Fig. 7). The bulge at the opposite side of the cell was also obvious (Fig. 7). Both conical monospindles contained an abundant amount of pole-to-chromosome MT (Fig. 6 and 7), but pole-to-pole MT were always absent. Similar to results obtained under the light microscope, chromosomes found under the electron microscope contain both chromatids still joined together in the centromeric region.

At 39°C, the fraction of mitotic cells which appeared abnormal increased rapidly. After the first 3 h at 39°C, virtually all the mitotic cells were in one of the abnormal states (Fig. 8). When the cells were returned to 33°C during this 3-h interval, the mitotic abnormality was rapidly replaced by normal mitosis (Fig. 8), indicating that the ts phenotype was readily reversed.

DISCUSSION

Our results showed that, when ts-745 cells were shifted from the permissive temperature (33°C) to the nonpermissive temperature (39°C), cell number ceased to increase. The cells initiated mitosis; however, mitotic cells remained in a suspended prometaphase state for 3–5 h. The abnormality was concluded by reformation of a single nucleus, followed by the cell flattened out on the dish surface in an interphase-like state. Subsequent cell cycle events appeared to occur, as the cells

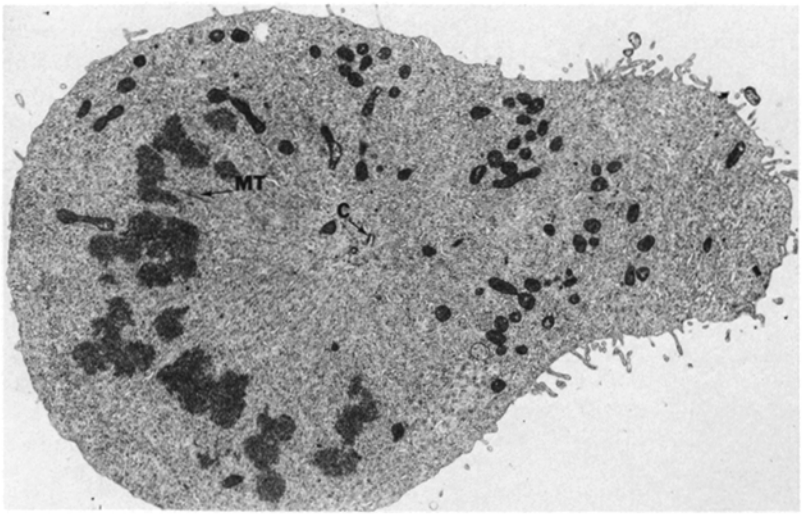

FIGURE 6 Monopolar spindle with cell bulge at the opposite end of the cell. Microtubules (*MT*) radiate from centrioles (*C*) near the center of the cell to the chromosomes near the periphery. × 5,800.

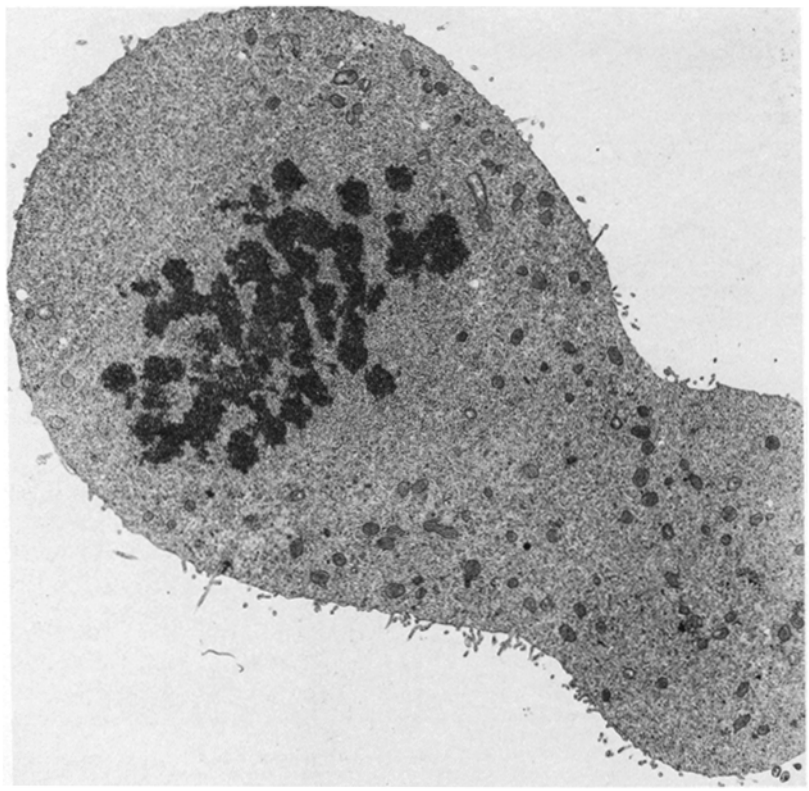

FIGURE 7 Monospindle and cell bulge at the opposite end. Centrioles were found in adjacent sections near the cell periphery at the end opposite the bulge. × 4,800.

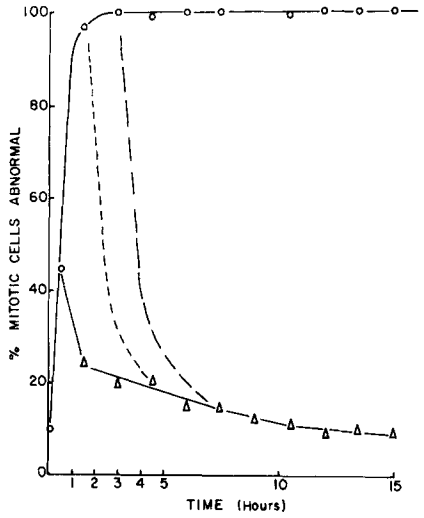

FIGURE 8 Increase in percent of abnormal mitotic ts-745 cells at 39°C (○), and reversal when returned to 33°C (△) at 0.5 h (—), 1.5 h (----), 3 h (- -).

were capable of chromosome duplication and initiating a second round of abnormal mitosis. Cell cycle traversion continued for at least 5 d in some of the cells despite abnormal mitosis resulting in cells accumulating several hundred chromosomes.

During the first 15 min after temperature shift to 39°C, some normal mitoses were still observed. Cells possessing metaphase plates invariably progressed through normal mitosis and cytokinesis. After the initial 15-min period, normal metaphase cells were rarely found. Mitotic cells exhibited a monopolar spindle in which all four centrioles were located in the center of the cell, with microtubules (MT) radiating from the centriolar region to the chromosomes arranged in a spherical shell located peripherally. An abundance of pole-to-chromosome MT was evident; however, pole-to-pole MT were not observed.

From the spherical monopolar state, the cells progressed to other types of monopolar orientations conical in appearance with all four centrioles in the apex region. Organized chromosome movement with the monopolar structure was present, from the first spherical shell state to the later asymmetrical orientations. Detailed examination of chromosome movement and displacement of other mitotic structures is currently in progress.

Throughout the abnormal mitotic process, chromosomes were found in the metaphase configuration without anaphase chromatid separation. The signals and mechanisms governing prometaphase chromosome congression appeared to be normal, as the metaphase chromosomes and MT formed a stable monopolar spindle. Formation of bipolar spindles was apparently blocked in a premetaphase state before metaphase plate formation. When cells at 39°C were returned to 33°C, the defective phenotype was readily reversed. In individual cells in abnormal mitosis at 39°C, change of temperature to 33°C resulted in progression from the spherical monopolar structure to pole separation and formation of a normal bipolar metaphase spindle (unpublished results).

The abnormal spherical spindle could result from failure of the centrioles to separate, or from the inability of pole-to-pole MT to assemble. Centriole separation and polar MT assembly could be intimately and causally related; however, no system is available for dissecting the two processes. Hopefully, further study on ts-745 will effect such dissection.

When mammalian cells are treated with a number of compounds which bind to tubulin and disrupt MT, "star metaphase" (3, 4, 8) bearing some resemblance to the sphere-metaphase exhibited by ts-745 cells at 39°C is found. The star metaphase configuration results only when relatively low concentrations of the compounds are present. At higher concentrations, virtually no mitotic MT structures are discernible, while the chromosomes still condense into the metaphase configuration. The existence of star metaphases suggests the presence of at least two types of microtubules, all sensitive to disruption by the inhibitors but in a concentration-dependent manner, with the pole-to-pole fibers more sensitive to the inhibitors than the kinetochore fibers. An alternative possibility is a greater abundance of kinetochore MT that requires a higher inhibitor concentration for complete disruption. Studies on spindle MT and in vitro MT polymerization revealed two types of MT, one cold stable and one cold sensitive (2). Ts-745 cells at the nonpermissive temperature enter the spherical metaphase state with complete absence of pole-to-pole MT but an apparently complete complement of chromosome MT. The all-or-none behavior of MT may allow identification of different types of mitotic MT.

Formation of spherical metaphase in ts-745 cells could also result from centrioles or materials associated with centrioles being abnormal. Another possibility is altered centriole activity leading to nonseparation. The force responsible for centriole separation remains to be determined, and such a force could be deficient in ts-745 cells at 39°C. Microinjection of various cellular components to reverse the mutant phenotype may identify the molecules required for generation of the force.

Mazia et al. (7) produced a monopolar spindle in fertilized sea urchin eggs by maneuvering the distribution of the poles to one per blastomere. In the subsequent mitosis, the single pole formed a monopole which was similar to a half-spindle. Monopolar half-spindles were occasionally observed in newt lung epithelium primary culture cells (1). Ts-745 provides a new type of monopole which is also available in large numbers and under well-defined conditions.

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REFERENCES

1. Bajer, A. S., DeBrabander, M., Molé-Bajer, J., DeMey, J., Paulaitis, S., Guvens, G. 1980. Mitosis: the mitotic aster, interzone, and functional autonomy of monopolar half-spindle. *In* Microtubules and Microtubule Inhibitors. M. DeBrabander and J. DeMey, editors. 399–425. Elsevier/North-Holland Press, New York.
2. Brinkley, B. R., and J. Cartwright, Jr. 1975. Cold-labile and cold-stable microtubules in the mitotic spindle of mammalian cells. *Ann. N.Y. Acad. Sci.* 253:428–439.
3. DeHarven, E. 1968. The centriole and the mitotic spindle. *In* Ultrastructure in Biological Systems. Vol. 3. A. J. Dalton and F. Hagnenan, editors. Academic Press, New York-London. 197–227.
4. Eigsti, O. J., and P. Dustin Jr. 1955. Colchicine in Agriculture, Medicine, Biology and Chemistry. The Iowa State College Press, Ames, Iowa.
5. Hatzfeld, J., and Buttin, G. 1975. Temperature-sensitive cell cycle mutants: a Chinese hamster cell line with a reversible block in cytokinesis. *Cell.* 5:123–129.
6. Kusano, T., R. Wang, R. Pollack, and H. Green. 1970. Human-mouse hybrid cell lines and susceptibility to poliovirus. II. Polio sensitivity and the chromosome constitution of the hybrids. *J. Virol.* 5:682–685.
7. Mazia, D., N. Paweletz, S. Greenfield, and F. Eva-Maria. 1981. Cooperation of kinetochores and pole in the establishment of monopolar mitotic apparatus. *Proc. Natl. Acad. Sci. U.S.A.* 78:377–381.
8. McGill, M., and B. R. Brinkley. 1972. Mitosis in human leukemic leukocytes during colcemid inhibition and recovery. *Cancer Res.* 32:746–755.
9. Shiomi, T., and K. Sato. 1976. A temperature-sensitive mutant defective in mitosis and cytokinesis. *Exp. Cell Res.* 100:297–302.
10. Smith, B. J., and N. M. Wigglesworth. 1972. A cell line which is temperature-sensitive for cytokinesis. *J. Cell. Physiol.* 80:253–259.
11. Thompson, L. H., and P. A. Lindl. 1976. A CHO-cell mutant with a defect in cytokinesis. *Somat. Cell Genet.* 2:387–400.
12. Wang, R. J. 1974. Temperature-sensitive mammalian cell line blocked in mitosis. *Nature (Lond.).* 248:76–78.
13. Wang, R. J. 1976. A novel temperature-sensitive mammalian cell line exhibiting defective prophase progression. *Cell.* 8:257–261.
14. Wang, R. J., and L. Yin. 1976. Further studies on a mutant mammalian cell line defective in mitosis. *Exp. Cell Res.* 101:331–336.
15. Wissinger, W., and R. J. Wang. 1978. Studies on cell division in mammalian cells. IV. A temperature-sensitive cell line defective in post-metaphase chromosome movement. *Exp. Cell Res.* 112:89–94.
16. Wissinger, W., and R. J. Wang. 1981. A differential staining technique for simultaneous visualization of mitotic spindle and chromosomes in mammalian cells. *Stain Tech.* 56:221–226.