Centriole Distribution during Tripolar Mitosis in Chinese Hamster Ovary Cells

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ABSTRACT During bipolar mitosis a pair of centrioles is distributed to each cell but the activities of the two centrioles within the pair are not equivalent. The parent is normally surrounded by a cloud of pericentriolar material that serves as a microtubule-organizing center. The daughter does not become associated with pericentriolar material until it becomes a parent in the next cell cycle (Rieder, C. L., and G. G. Borisy, 1982, *Biol. Cell.*, 44:117–132). We asked whether the microtubule-organizing activity associated with a centriole was dependent on its becoming a parent. We induced multipolar mitosis in Chinese hamster ovary cells by treatment with 0.04 μg/ml colcemid for 4 h. After recovery from this colcemid block, the majority of cells divided into two, but 40% divided into three and 2% divided into four. The tripolar mitotic cells were examined by antitubulin immunofluorescence and by high voltage electron microscopy of serial thick (0.25-μm) sections. The electron microscope analysis showed that centriole number was conserved and that the centrioles were distributed among the three spindle poles, generally in a 2:1:1 or 2:2:0 pattern. The first pattern shows that centriole parenting is not prerequisite for association with pole function; the second pattern indicates that centrioles per se are not required at all. However, the frequency of midbody formation and successful division was higher when centrioles were present in the 2:1:1 pattern. We suggest that the centrioles may help the proper distribution and organization of the pericentriolar cloud, which is needed for the formation of a functional spindle pole.

During bipolar mitosis a centrosome is distributed to each daughter cell (1–3). The centrosome, in most animal cells, consists of a pair of centrioles surrounded by a cloud of fibrous substance designated the pericentriolar material (4–6).

Although centrioles have been studied extensively (7) it is only recently that the asymmetry of centrioles within the pair has been appreciated (5, 6). The pericentriolar cloud normally surrounds the parent and not the daughter centriole of a mitotic centrosome. The astral microtubules focus to the pericentriolar cloud (8) and therefore also to the parent centriole. In this sense, the parent centriole and surrounding cloud may be considered as the morphological expression of the spindle pole. The daughter centriole does not become associated with pericentriolar material and pole activity until the mitosis after it has become a parent in the next cell cycle (6).

Although it seems clear that centrioles are not absolutely required for mitosis (9–13), this does not mean they have no role in the division process where they normally are present. We wondered whether the pole-related activity of the daughter centriole depended upon its becoming a parent. Was it necessary that a centriole experience parenthood before it could become associated with the pericentriolar cloud or were these two events, normally associated in time, essentially independent of each other?

To answer this question, we referred back to two earlier investigations. Mazia et al. (14) investigated the multiplicity of mitotic centers and time course of their splitting and separation in sea urchin and sand dollar eggs. They assayed mitotic centers functionally in terms of the ability to serve as a pole in the division process. They used an agent, β-mercaptoethanol, to induce multipolar mitosis and inferred that the mitotic centers were essentially duplex in nature and that duplication of the centers followed one cell cycle after their splitting. Unfortunately, they did not carry out ultrastructural analyses and therefore could not identify the duplication and splitting of the centers with stages in the centriole cycle.

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The eggs of sea urchins and sand dollars are large and do not lend themselves to electron microscopic analysis. A report by Stubblefield (15) on induction of multipolar mitosis by colcemid treatment in Chinese hamster ovary cells (CHO)[1] prompted us to consider whether these tissue culture cells would be suitable for the analysis. Stubblefield's finding was that CHO cells treated with colcemid for several hours and then washed free of the drug frequently divided into three or four cells. We reasoned that, if centriole number were conserved during the treatment, multipolar mitosis might involve a splitting of parent and daughter centrioles from each other and provide a test of the independence of pole association and centriole parenting. In this study, we analyzed the number and distribution of centrioles during tripolar mitosis in CHO cells. We found that daughter centrioles may indeed split from their parents, acquire pericentriolar material, and participate in the formation of a mitotic pole. We also found that functional poles may form without the association of centrioles.

MATERIALS AND METHODS

CHO cells were grown as monolayers at 37°C under 5% CO_2, 95% air, in Ham's F10 medium (Gibco Laboratories, Grand Island, NY) supplemented with 15 mM HEPES buffer, pH 7.2, and 10% fetal calf serum.

Cell Synchronization: Cells were synchronized in mitosis as described previously (16). Cells were first accumulated to the G_1/S boundary or slowed in their progress through S-phase by the addition of 4 mM thymidine to the growth medium for 16 h. The cell monolayers were then washed free of thymidine and returned to culture medium for an additional 5 h. Culture dishes were shaken vigorously and the medium poured off to dislodge any round mitotic cells. Then, cells were incubated in medium with 0.04 μg/ml colcemid (Gibco Laboratories) for 4 h.

Recovery from the Colcemid Block: All the operations were carried out at 37°C. Mitotic cells were collected by centrifugation in a tabletop centrifuge. The pellet of mitotic cells was washed once with fresh F10 medium to remove colcemid and centrifuged again. Then, the round mitotic cells were plated on concanavalin A–coated coverslips in tissue culture dishes containing Ham's F10 medium at 37°C. The preparation of concanavalin A–coated coverslips was performed according to Hanks et al. (17). The mitotic cells were allowed to recover from the mitotic block for ~25 min at 37°C in 5% CO_2, 95% air. Morphological changes during recovery were monitored by phase-contrast microscopy and recovery was terminated by fixation at different times. Round mitotic cells on concanavalin A–coated coverslips remained firmly attached during all the procedures needed for the electron microscopic or immunofluorescence studies.

Immunofluorescence Staining: Cells at different stages of mitosis were rinsed once and fixed with cold methanol for 6 min (18). After washing with phosphate-buffered saline, the coverslips were incubated with monoclonal antitubulin antibody raised against yeast tubulin (a generous gift from Dr. J. V. Kilmartin, Medical Research Council Laboratory of Molecular Biology, Cambridge, England [also see reference 19]) for 1 h at 37°C and then rinsed thoroughly with phosphate-buffered saline two or three times for 4 h. The coverslips were then incubated with fluorescein-labeled rabbit anti-rat IgG (Cappel Laboratories, Inc., Cochranville, PA) for 1 h at 37°C and washed in phosphate-buffered saline overnight. The coverslips were mounted with 1% N-propyl gallate, 90% glycerol, 10% Tris, pH 8.5, on microscope slides and observed by indirect immunofluorescence on a Zeiss Universal microscope equipped with epifluorescence optics (Carl Zeiss, Oberkochen, W. Germany).

Electron Microscopy: At the end of the recovery the cells attached to the coverslips were washed once in 60 mM PIPES, 25 mM HEPES, 10 mM EGTA, 2 mM $MgCl_2$, pH 6.9 (PHEM) (20), containing taxol at a concentration of 1 μg/ml. Some preparations were lysed with 0.1% Triton X-100 for 30 s in the PHEM buffer with taxol. The cells were then and fixed with 2.5% glutaraldehyde in PHEM buffer for 10 min at room temperature. Cells were washed overnight in PHEM buffer and postfixed in 1% O_sO_4 in the same buffer 30 min, then washed and stained for 90 min with 0.5% aqueous uranyl acetate. The cells were flat embedded in Epon-Araldite. The glass coverslip was removed from the embedded cells using dry ice. The tripolar mitoses were then relocated by light microscopy, circled with a diamond objective marker, excised and mounted on Epon pegs for trimming and sectioning. Serial sections 0.25 μm thick were collected and subsequently stained with uranyl magnesium acetate (7.5% at 40°C for 4 h) and lead citrate (23°C for 20 min). All sections were examined and photographed with the AEI-EM7 1 MeV Electron Microscope of the Madison High Voltage Electron Microscope Facility operated at 1 MeV and using an objective aperture of 30 μm. Dividing cells were reconstructed from micrographs of 0.25-μm sections traced onto separate transparent sheets of acetate as described in the review of Rieder (21).

RESULTS

Induction of Tripolar Mitosis

We first attempted to reproduce the results of Stubblefield (15). He reported that multipolar mitosis would result in CHO cells recovering from an extended colcemid block and that the frequency and degree of multipolarity increased as a function of time in colcemid. We were able to confirm these results and found up to six poles in spindles isolated from recovering cells (22). However, such a high degree of multipolarity resulted in irregular division patterns, characterized by blebbing and fragmentation. We desired to obtain a multipolar mitosis with normal cell morphology in which chromosome movement and spindle poles could be convincingly documented. Tetrapolar mitosis would have been ideal but was not obtained in sufficient frequency, therefore we settled for conditions that produced tripolar mitosis. This was considered sufficient for our purposes since the essential requirement of the experimental design was to split one mitotic center into two. Tetrapolar mitosis would achieve this for both mitotic centers but tripolar mitosis would do it for one.

The conditions settled on were to first pre-synchronize CHO cells with thymidine, then allow them to progress toward mitosis, then block them in mitosis for 4 h with 0.04 μg/ml colcemid, then wash out the drug and allow them to recover. Under these conditions, as shown by the phase-contrast micrographs of Fig. 1, cells frequently divided from one to three.

Immunofluorescence of Tripolar Mitosis

Phase-contrast microscopy permitted us to score living cells that could initiate cytokinesis. However earlier stages were

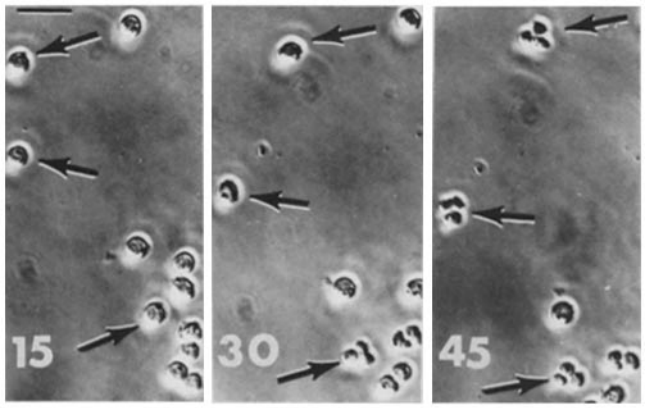


FIGURE 1 Phase-contrast micrographs of living CHO cells during recovery from a colcemid block (4-h treatment, concentration 0.04 μg/ml): 15 min after reversal; 30 min after reversal; and 45 min after reversal. Arrows indicate the cells that divided into three. Bar, 50 μm. × 150.

[1] *Abbreviations used in this paper:* CHO, Chinese hamster ovary; PCM, pericentriolar material; PC1, PC2, and PC3, clouds of PCM at poles 1, 2, and 3, respectively; PHEM, 60 mM PIPES, 25 mM HEPES, 10 mM EGTA, 2 mM $MgCl_2$, pH 6.9.

not accessible by this method since CHO cells round up when they divide, concealing the number of spindle poles. To examine earlier stages, particularly metaphase and early anaphase, when the cells were still round, we fixed the cells and examined them by antitubulin immunofluorescence to detect the distribution of microtubules. We also examined cleaving cells in late telophase. Fig. 2 shows examples of cells in early anaphase and in late telophase immediately after recovery was begun.

Of greater than 600 round cells scored (metaphase and early anaphase), 58% had a bipolar spindle. However, in 20% of these cells, a third microtubule-organizing center was observed, sometimes associated with one of the spindle poles by a few fibers. 40% of the mitotic cells had a tripolar spindle (Fig. 2) and 2% had a tetrapolar spindle. Of the tripolar cells, a proportion also contained a fourth microtubule-organizing center sometimes associated with one of the three poles.

Late telophase cells were scored for the presence of midbodies. They were all selected as tripolar mitoses by the presence of three cleavage furrows. Of 450 tripolar cells scored, 56% had three midbodies, 6% two midbodies, 23% one midbody, and 15% no midbodies at all. Presumably, cytokinesis in the tripolars without midbodies would abort and a single multinucleated cell would result. Cells with one midbody would be expected to ultimately divide into two. The salient conclusion from this examination was that tripolar mitosis could be reproducibly induced and frequently proceeded to completion.

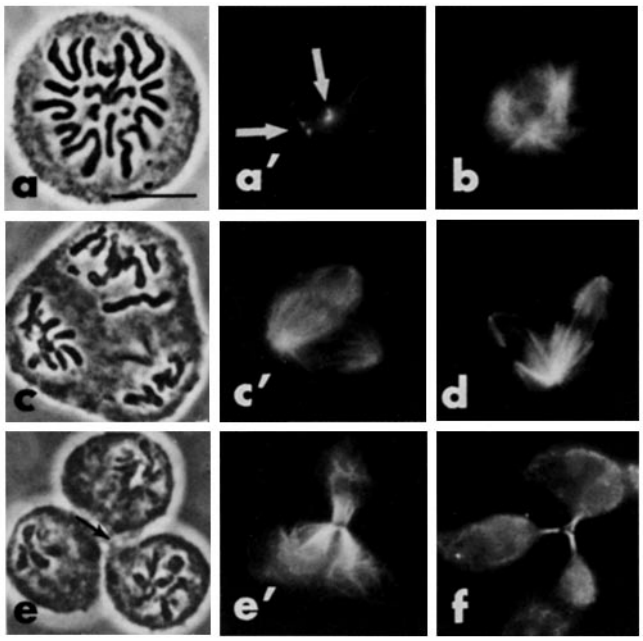


FIGURE 2 Indirect immunofluorescence staining of recovering CHO cells during tripolar division. Cells were washed free of colcemid, allowed to recover for varying times, fixed and stained with antitubulin antibodies. (*a* and *a'*). The same cell seen in phase and fluorescence at 5 min of recovery. Arrows point to separated pairs of centrioles. (*b*) Another cell after 15 min of recovery showing separation of mitotic centers and formation of asters. (*c* and *c'*) Tripolar anaphase cell in phase and fluorescence at 25 min of recovery (*d*) another tripolar anaphase cell. (*e* and *e'*) Tripolar late telophase cell in phase and fluorescence at 45 min of recovery showing three bundles of interzone fibers sharing a common focus. (*f*) Another tripolar telophase cell showing three distinct midbodies. Bar, 10 μm. × 1,450.

Electron Microscopic Analysis of Tripolar Mitosis

Having established the conditions for induction of tripolar mitosis, we were now prepared to evaluate whether the pole-related activity of a daughter centriole was independent of its parenting. For this analysis, we first had to determine whether centriole number was conserved during the tripolar mitosis and, second, to determine the distribution of centrioles at the spindle poles. Both of these objectives required a complete serial section analysis and 3-dimensional reconstruction of dividing cells at the electron microscopic level. Random sections or incomplete serial sections would not be adequate because supernumerary centrioles could escape undetected and a centriole at a pole would not then be identifiable as a daughter of a preexisting parent.

The electron microscopic analysis was performed on selected cells that were identified as undergoing tripolar mitosis. They were flat-embedded and sectioned parallel to the plane of the substratum. To facilitate the effort of 3-dimensional reconstruction, we cut serial thick sections (0.25 μm) and examined them with a High Voltage Electron Microscope operated at 1 MeV. Cells were selected in metaphase, anaphase, and in late telophase where cytokinesis had progressed. Metaphase and anaphase cells permitted us to analyze centrioles at spindle poles at a time when their relation to spindle microtubules would be clearest. Cells in cytokinesis permitted us to analyze centriole distribution to daughter cells and their relation to midbody formation.

Centriole Number Is Conserved during Tripolar Mitosis

It was possible that the induction of additional poles was brought about by an experimentally induced proliferation of centrioles. Conceivably, tripolar cells would contain six centrioles and tetrapolar cells would contain eight centrioles instead of the normal four centrioles in a bipolar cell. Were this the case, then multipolar mitosis would not be useful for investigating the relationship between parent and daughter centrioles.

Complete serial section data and 3-dimensional reconstructions were obtained for 27 tripolar cells (Table I). In all cases a total of four centrioles was found. In addition, incomplete data were obtained for five other cells in which three centrioles were found. Therefore, in no case did we find evidence of induced centriole proliferation; rather, centriole number was conserved during tripolar mitosis.

Centriole Distribution during Tripolar Mitosis

To interpret the results on centriole distribution, it is helpful to first describe briefly some steps in the centriole cycle (see reference 23). Daughter cells formed by a cell division normally each receive a pair of orthogonally oriented centrioles. The daughter and parent centrioles in an orthogonal pair may be identified by the axis-intercept rule (6). The axis of the daughter centriole intercepts the parent centriole but the axis of the parent does not intercept the daughter. The two centrioles become disoriented and lose their orthogonal arrangment in late M or early G1 phase, and, after parenting new centrioles in late G1 or S phase, separate from each other in late G2 or M phase. The events of disorientation and separation, normally occuring in the next cell cycle, seem to proceed in the colcemid-arrested cells and thus may facilitate the phenomenon of multipolar mitosis.

TABLE I

Centriole Distribution in Tripolar Mitosis

Pattern*	Stage	No. of cases	Parent-daughter relation†			No. of midbodies			
			Orthogonal	Disoriented	Separated	0	1	2	3
	Metaphase	2	0	0	4	—	—	—	—
1	Anaphase	1	0	0	2	—	—	—	—
	Telophase	7	0	0	14	0	1	0	6
	Metaphase	4	6	0	2	—	—	—	—
0	Anaphase	2	4	0	0	—	—	—	—
	Telophase	11	6	6	10	3	5	0	3
Total	All	27	16	6	32	3	6	0	9

* Pattern 1 refers to distributions of centrioles in which the third pole contains 1 centriole, such as 2:1:1. Pattern 0 refers to distributions in which the third pole lacks centrioles, such as 2:2:0. See text for details.

† Parent-daughter relation is defined as follows: orthogonal, -parent, and daughter juxtaposed at a right angle; disoriented, -parent, and daughter are within 1 μm of each other but in no definite orientation; separated, -parent, and daughter are further apart than 1 μm and in no definite orientation. Since two centriole duplexes are present per cell, the number of duplexes analyzed is twice the number of cases.

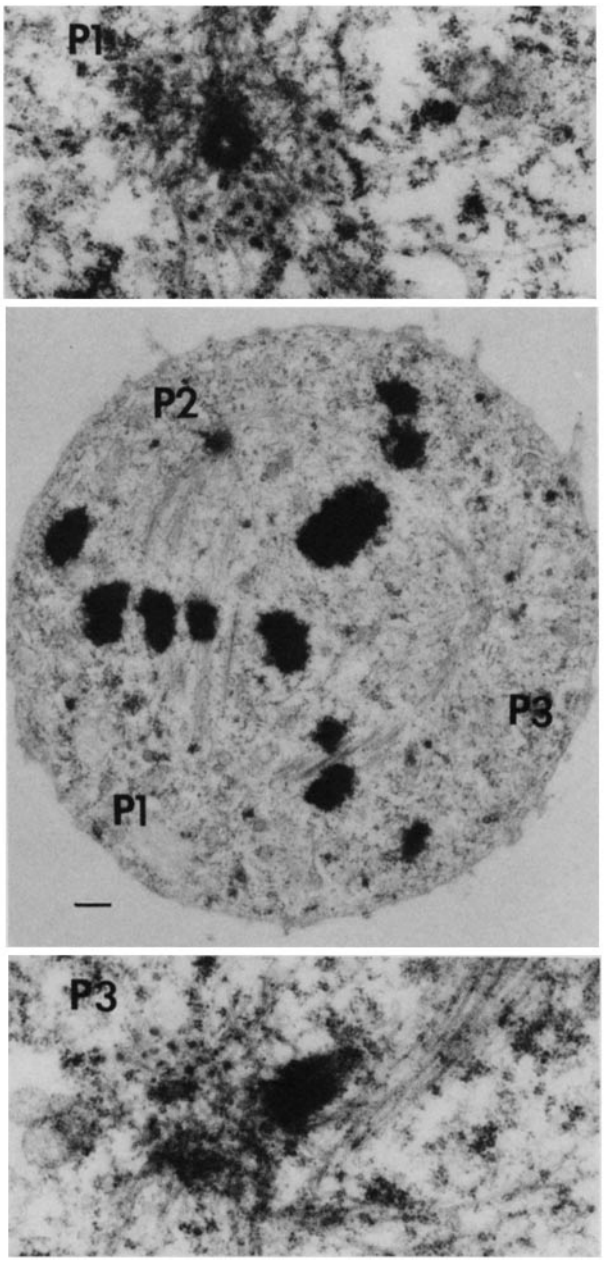


FIGURE 3 Electron micrograph of a mitotic CHO cell in metaphase with a tripolar spindle (0.25-μm section). The *middle* panel shows the three poles (*P1*, *P2*, and *P3*) with one centriole and PCM at *P2*.

METAPHASE: Several patterns of centriole distribution were observed, indicating some variability in the nature of the recovery process. Complete data were obtained for six metaphase cells. A tripolar metaphase cell is illustrated in Fig. 3. Each of the three poles was comprised of a single centriole and associated pericentriolar material (PCM), onto which the spindle microtubules were focussed. The clouds of PCM at pole 1 (P1) and pole 2 (P2) were large as evidenced by their presence in several serial sections, whereas the cloud at pole 3 (P3) was small. Virus-like particles were embedded in each pole. Virus-like particles are characteristically associated with the centrosomes of this cell line (24, 25) and may be taken as a marker for the PCM. The fourth centriole was located 3 μm from the centriole at P1 and a few microtubules were seen at one of its ends. The reconstruction of the cell is given in Fig. 4. It is evident that both pairs of parents and daughters have

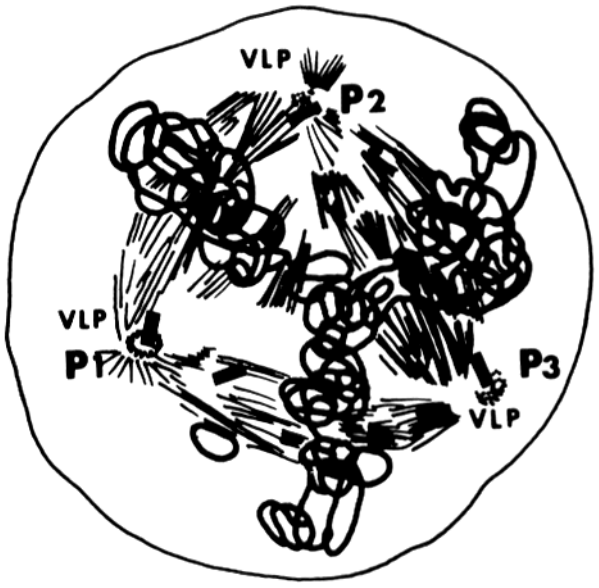
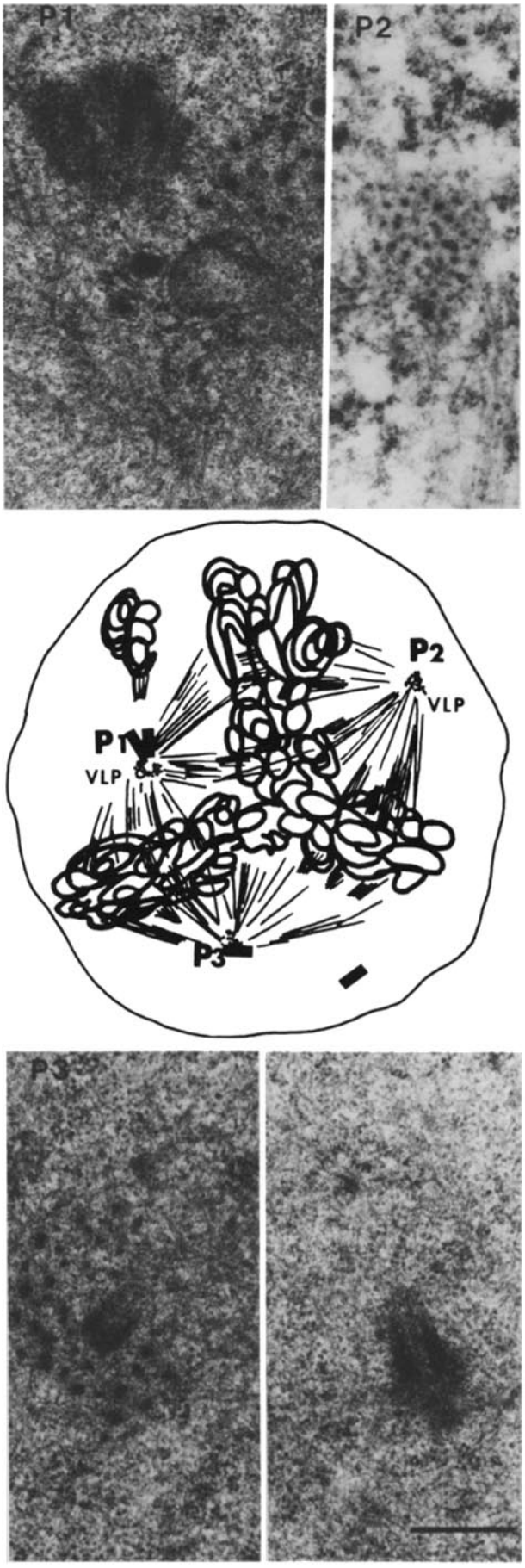


FIGURE 4 Reconstruction of the cell presented in Fig. 3 from 40 consecutive serial sections, 0.25 μm thick (total specimen thickness 10 μm). From the reconstruction the spatial arrangement of the centrosomal components at the mitotic poles was determined. Kinetochore microtubules and pole-to-pole microtubules were traced. (▬) Centriole. *VLP*, virus-like particles. × 5,000.

One centriole was found at *P1* (*top* panel) two sections above this one. One centriole was found at *P3* (*bottom* panel) 11 sections below. The fourth centriole was found 3 μm from *P1*. Bar, 1 μm. *top* and *bottom*, × 26,000; *middle*, × 5,000.



disoriented and separated from each other, although the members of one pair have separated more than the other and only one daughter has become associated with a spindle pole. This cell demonstrates that a daughter centriole can acquire PCM and become associated with a pole even though it has not yet become a parent. It also shows that a centriole may be located away from a spindle pole.

A reconstruction of a tripolar metaphase cell illustrating a different pattern is shown in Fig. 5. In this cell a parent-daughter pair of centrioles was located at P1 with microtubules focussed on the PCM of the parent, as established by the axis-intercept rule. A single centriole with PCM was present at P3 but the third pole (P2), although marked by a large cloud of PCM-like material, embedding virus-like particles, lacked centrioles. The fourth centriole was found on the same side of the cell as P3, but 3.5 μm above the centriole at that pole. This cell illustrates that centrioles are not absolutely required to define a spindle pole.

Other metaphase cells fell into one of the above patterns, but, in one case, an orthogonal pair of centrioles was present at one pole but no centrioles were present at either of the other two poles. The remaining two centrioles were present as an orthogonal pair near the cell periphery. In yet another case, none of the three poles contained centrioles. The chromosomes were oriented in a tri-way metaphase plate toward PCM-like material embedding virus-like particles. The two centriolar duplexes were located near the periphery on opposite sides of the cell.

ANAPHASE: Complete serial section data were obtained for three cells in anaphase. The results for these cells (data not shown) were similar to that for metaphase cells. In one case, single centrioles were located at spindle poles and the fourth centriole was distant and apparently not associated with a pole. In the other two cases the four centrioles were distributed as orthogonal pairs to two of the three poles; the third pole contained PCM-like material but lacked centrioles.

TELOPHASE: Most of our data were obtained on telophase cells because the triway cleavage furrow provided an easy and certain indication of tripolarity. Sometimes the cleavage was completed and three fully separated daughter cells were formed. However, in some instances, the furrows were observed to regress resulting in a multinucleated cell or one furrow was completed while the other regressed resulting ultimately in a bipolar division. In all, 18 telophase cells were reconstructed.

Cells were scrutinized for midbody formation to determine whether there was any correlation between centriole distribution and successful cleavage. When three daughter cells were completely formed and linked to each other by a midbody, we found two patterns of centriole distribution. In the majority of cells examined, each daughter cell contained either one or two centrioles (Fig. 6). In the cell that contained the mother and daughter centrioles, these centrioles were always disoriented and almost always separated by several microm-

FIGURE 5 Reconstruction of another mitotic cell in metaphase (total specimen thickness, 8 μm). The upper left pole (*P1*) has a centriolar duplex and associated PCM; the upper right pole (*P2*) has PCM but lacks centrioles; the bottom pole (*P3*) has one centriole and a big cloud of PCM. The fourth centriole (*bottom right* panel) was detected on four consecutive sections; separated from *P3* by 3.5 μm. Bar, 0.5 μm. Reconstruction in the *middle* panel, × 5,000. *P1, P2, P3,* and *fourth* centriole, × 32,000.

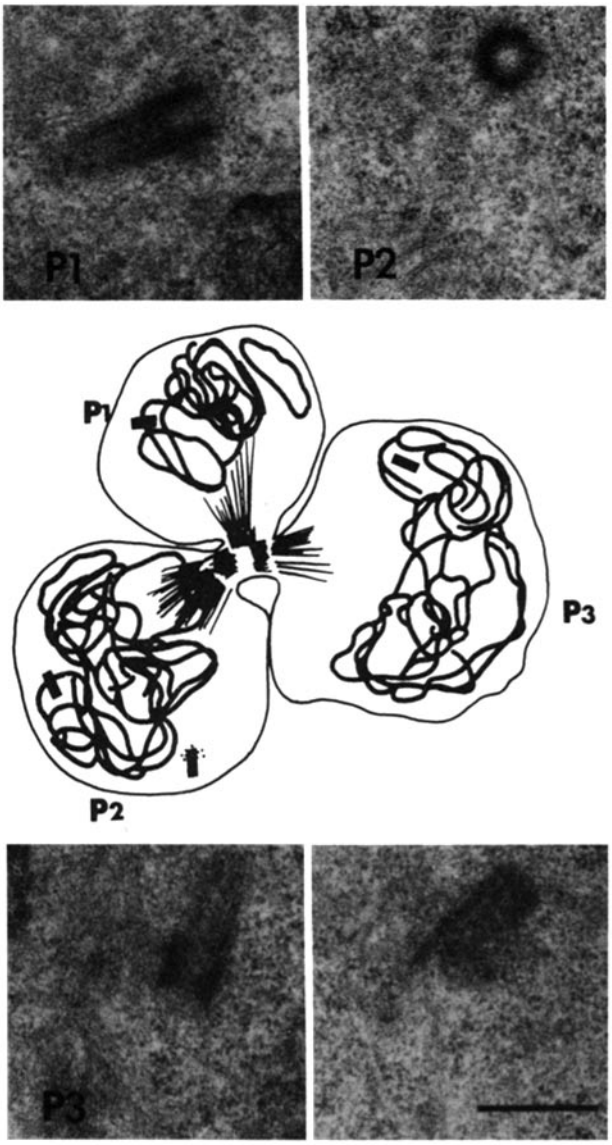


FIGURE 6 Reconstruction of a tripolar telophase cell showing almost complete cleavage and three midbodies (total specimen thickness, 10.25 μm). Two daughter cells (*P1* and *P3*, *top* panels) contained one centriole each. One daughter cell (*P2*) contained two centrioles (*bottom* panels) with one of the centrioles distant from the pole. Bar, 0.5 μm. Reconstruction, *middle* panel, × 5,000; *top* and *bottom* panels, × 32,000.

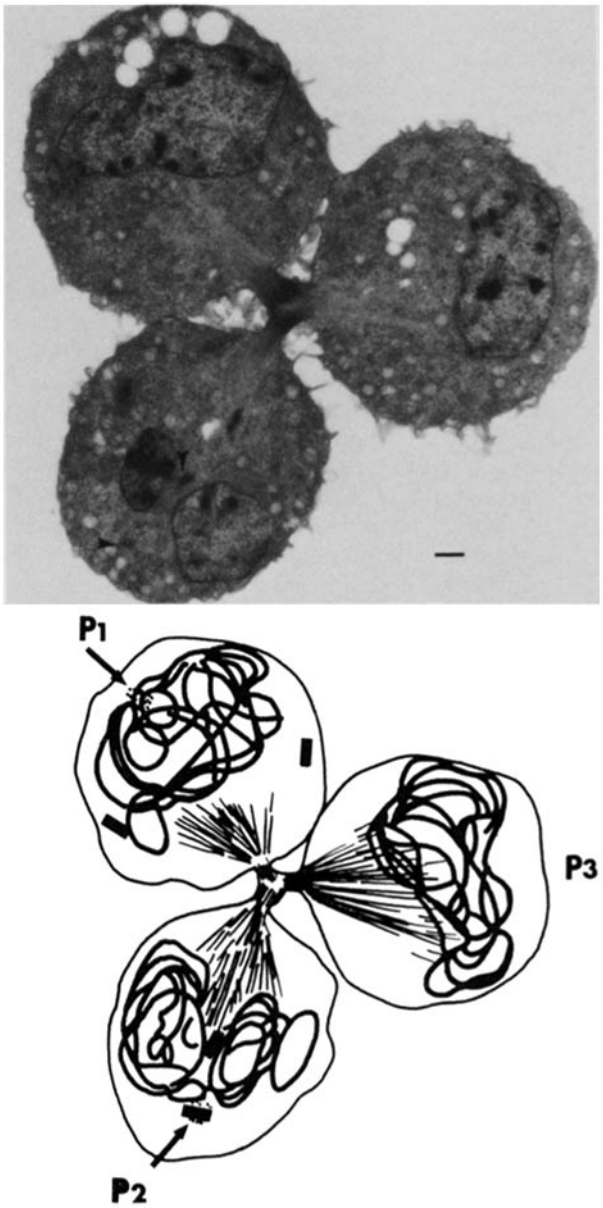


FIGURE 7 Low magnification electron micrograph and reconstruction of a cell completely dividing into three daughter cells. Centrioles were found only in two cells (*P1* and *P2*), the arrows point to the presumed poles. (▬) Centriole; virus-like particles (arrows). Bar, 1 μm. × 5,000.

eters. Therefore, these cells were equivalent to the metaphase cell of Fig. 3; that is, one centriole at each pole and one apparently not associated with a pole.

The other pattern of centriole distribution is shown in Fig. 7. Here, two daughter cells contained two centrioles well separated and the third cell lacked centrioles altogether. This pattern shows that centrioles are not required for midbody formation and cleavage.

Table I summarizes the data obtained for the 27 cells completely analyzed. The cells have been placed into two principal categories depending upon the number of centrioles at the third pole, which in all cases was either 1 or 0. Category 1 cells have either a 2:1:1 distribution of centrioles or a 1:1:1 distribution with the fourth centriole not participating. 40% of the tripolar cells analyzed showed centriole distributions of category 1. All stages of mitosis were represented. Category 0 cells, so named because at least one pole lacked centrioles, comprised the balance of the sample and was more heterogeneous. Centriole distributions observed in this category were 1:1:0 with two centrioles not participating, 2:0:0 with two not participating, 2:1:0 with one not participating and 2:2:0. A noteworthy feature of the data is that six of seven telophase cells in category 1 contained three midbodies and completed cleavage, whereas only three of eleven telophase cells in category 0 contained three midbodies and completed cleavage.

DISCUSSION

The objective of this study was to test the independence of centriole parenting and pole association. Although a daughter centriole normally does not become associated with pole activity until the mitosis after it becomes a parent (4–6, 23),

this correlation in time does not necessarily imply a dependency relation.

To test this dependency relation, it was necessary to separate the daughter centriole from its parent and provide it an opportunity to associate with a pole. Induction of tripolar mitosis by colcemid treatment met these requirements. Under conditions of constant centriole number, parent and daughter centrioles disoriented and separated from each other and in many instances, the daughter centrioles were found at poles. These results are consistent with a previous study of Kuriyama (26), which showed that the continuous presence of colcemid did not inhibit centriole disorientation. In fact, each of the disoriented centrioles subsequently nucleated the formation of daughters, which then, afterwards, partially elongated. Thus, some steps of the centriole cycle continued in the presence of colcemid, even though microtubule and spindle formation was blocked. Further evidence that some cell cycle events occur in the presence of colcemid was provided by Kuriyama's observations (26) that reformation of nuclei and change in cell shape occurred and that the cells went through cycles of nuclear breakdown and reformation.

Therefore, we interpret the induction of multipolar mitosis by colcemid treatment as follows. Colcemid blocks mitosis by preventing spindle formation. However, parent-daughter centriole disorientation, which normally occurs in early G1 (23), is not blocked. This may correspond to the "splitting" event referred to by Mazia et al. (14) in their analysis of mitotic centers. After removal of colcemid, microtubule formation is permitted. Separation of the parent and daughter centrioles (now disoriented), which normally does not occur until late G2 or M phase of the next cell cycle, occurs now because the cellular milieu (M phase) is appropriate. A consequence of the separation of parent and daughter centrioles is that the daughter centriole has the opportunity to serve precociously at a pole. Whether it has the capacity to do so was a separate question. Our results demonstrate that indeed the daughter centriole does have the capacity to serve at a pole. It is not required to first become a parent.

Our results also raise questions about the nature of poles. In ~60% of the tripolar cells analyzed, one or more poles lacked centrioles altogether. This result demonstrates that centrioles are not required for mitosis. What then is a pole? A pole may be defined functionally in terms of two criteria: (*a*) A pole is where the chromosomes move to in anaphase. (*b*) Two poles define an axis that is the normal to the plane of cleavage, bisecting the line connecting the poles. Note that this definition says nothing about the morphological expression of a pole.

An accumulation of studies (9–13) has indicated that centrioles are not required for pole functions, and attention has shifted to the material that normally surrounds the centrioles, the PCM (11, 12, 16, 25, 27). Perhaps a cloud of PCM is the essential component of a pole. But the problem remains of how a bipolar cell normally produces only two of these units. We wish to suggest that an interaction may exist between the centrioles and the cloud of PCM. PCM may have the capacity to self-aggregate and serve as poles in the absence of centrioles, but may require some auxiliary information to ensure two-ness. Centrioles are not absolutely required to establish two-ness and a variety of pole structures in cells have been described, leading Pickett-Heaps (28) to introduce the more generic term for pole structures of microtubule-organizing center. However, since the replication of centrioles is precisely determined, they may provide one mechanism of ensuring or at least facilitating two-ness and bipolar mitosis.

The data on telophase cells and completion of cleavage may indicate another aspect of the role of centrioles. Cells with the 2:1:1 distribution of centrioles contained three midbodies and completed cleavage in six of seven cases analyzed, whereas in cells lacking a centriole at one pole, only three of eleven cases contained three midbodies. As mentioned previously, one aspect of pole function is to cooperate with another pole to accomplish cleavage. We note here that the process of cleavage has two components. One is furrowing and the second is termination of cell union. Furrowing apparently can be elicited by poles whether or not centrioles are present at them. Termination may be different. If a midbody is not present, the cleavage furrow regresses and division is aborted. Our results indicate that the presence of a centriole at a pole is correlated with a higher frequency of midbody formation and successful division.

It should be noted, however, that our results do not necessarily conflict with the prevailing view (28) that centrioles do not contribute essentially to mitosis but rather are passively distributed to the daughter cells by the spindle mechanism. It may be that successful cleavage depends upon a critical amount of pericentriolar material at the pole and that association of a centriole with a pole increases with the amount of pericentriolar material. The precise role that centrioles serve in the proper distribution and organization of the pericentriolar cloud remains a problem for future investigation.

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