Variable Pathways for Developmental Changes of Mitosis and Cytokinesis in *Physarum polycephalum*

Lilianna Solnica-Krezel, Timothy G. Burland, and William F. Dove

McArdle Laboratory for Cancer Research, Madison, Wisconsin 53706

Abstract. The development of a uninucleate ameba into a multinucleate, syncytial plasmodium in myxomycetes involves a change from the open, astral mitosis of the ameba to the intranuclear, anastral mitosis of the plasmodium, and the omission of cytokinesis from the cell cycle. We describe immunofluorescence microscopic studies of the amebal–plasmodial transition (APT) in *Physarum polycephalum*. We demonstrate that the reorganization of mitotic spindles commences in uninucleate cells after commitment to plasmodium formation, is completed by the binucleate stage, and occurs via different routes in individual developing cells. Most uninucleate developing cells formed mitotic spindles characteristic either of amebae or of plasmodia. However, chimeric mitotic figures exhibiting features of both amebal and plasmodial mitoses, and a novel star microtubular array were also observed. The loss of the ameba-specific α3-tubulin and the accumulation of the plasmodium-specific β2-tubulin isotypes during development were not sufficient to explain the changes in the organization of mitotic spindles.

The majority of uninucleate developing cells undergoing astral mitoses (amebal and chimeric) exhibited cytokinetic furrows, whereas cells with the anastral plasmodial mitosis exhibited no furrows. Thus, the transition from astral to anastral mitosis during the APT could be sufficient for the omission of cytokinesis from the cell cycle. However, astral mitosis may not ensure cytokinesis: some cells undergoing amebal or chimeric mitosis contained unilateral cytokinetic furrows or no furrow at all. These cells would, most probably, fail to divide.

We suggest that a uninucleate committed cell undergoing amebal or chimeric mitosis can either divide or else form a binucleate cell. In contrast, a uninucleate cell with a mitotic spindle of the plasmodial type gives rise only to a binucleate cells. Further, the decision to enter mitosis after commitment to the APT is independent of the developmental changes in the organization of the mitotic spindle and cytokinesis.

W E are studying the formation of the mitotic spindle and cytokinetic furrow in the acellular slime mold *Physarum polycephalum*. There are two vegetative phases in the life cycle of this organism, which display distinct types of cellular proliferation. In the uninucleate ameba, mitosis involves the disappearance of cytoplasmic microtubules and the duplication of the cytoplasmic microtubule organizing center (MTOC)[1] with its associated centrioles. During prophase, the daughter MTOCs migrate to opposite sides of the nucleus. When the nuclear membrane breaks down, these MTOCs organize a mitotic spindle with astral microtubules radiating from its poles. Amebal karyokinesis is followed by cytokinesis (Havercroft and Gull, 1983). This so-called open mitosis is typical of animal cells. In the syncytial plasmodium, all nuclei undergo mitosis synchronously. In contrast to the ameba, there are no detectable cytoplasmic microtubules and centrioles in the plasmodium. The mitotic spindle is organized by intranuclear MTOCs,

and astral microtubules are not observed at any stage of mitosis. The nuclear membrane remains largely intact throughout karyokinesis (Tanaka, 1973). This so-called closed mitosis is typical of fungi (Heath, 1980). In the plasmodium, cytokinesis is absent. Thus, when an ameba develops into a plasmodium, the open, astral mitosis must be substituted by intranuclear mitosis, and cytokinesis must be omitted from the cell cycle. Additionally, cytoplasmic MTOCs and cytoskeletal microtubules disappear (Gull et al., 1985; Solnica-Krezel et al., 1990). The reorganization of microtubules during this amebal–plasmodial transition (APT) is accompanied by changes in the repertoire of expressed tubulin isotypes. At least two tubulin isotypes, β1A and α3, disappear (Burland et al., 1984; Diggins and Dove, 1987; Sasse et al., 1987), whereas at least three, Nα1, Eα2, and β2, appear (Monteiro et al., 1987; Solnica-Krezel et al., 1988, 1990).

It has been suggested that the transition from open to closed mitosis occurs during one long cell cycle, in which a uninucleate developing cell becomes competent to develop into a plasmodium at a low cell density (commitment), and eventually forms a binucleate cell by means of a closed mito-

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1. *Abbreviations used in this paper*: APT, amebal-plasmodial transition; DAPI, diaminophenylindole; MTOC, microtubule organizing center.

sis without cytokinesis (Gull et al., 1985; Blindt et al., 1986; Bailey et al., 1987). However, the plasmodium-specific β2-tubulin isotype accumulates in developing cells usually only after the commitment event (Solnica-Krezel et al., 1990), but it can be incorporated into both astral and anastral mitotic spindles (Diggins-Gilicinski et al., 1989). Thus it is possible that committed cells can construct either astral or anastral mitotic spindles, and that the change from one type of mitosis to the other is gradual rather than abrupt.

Using apogamic strains of *P. polycephalum* in which amebae form plasmodia in clones (Cooke and Dee, 1974), and antibodies specific for developmentally regulated α3- and β2-tubulin isotypes, we have been addressing three questions concerned with the transition from amebal to plasmodial mitosis during the APT. First, at what stage of development is the amebal type of mitosis substituted by the plasmodial type? Second, are the changes in the organization of the mitotic spindles related to their content of tubulin isotypes? Third, is the omission of cytokinesis from the cell cycle during the APT coupled to the change in the type of karyokinesis?

Materials and Methods

Reagents

Except as follows, all were purchased from Sigma Chemical Co. (St. Louis, MO). Formaldehyde (EM grade) and Ladd-o-lac from LADD (Burlington, VT). Mouse mAb 6-11B-1 against acetylated α-tubulin was kindly provided by Dr. G. Piperno (The Rockefeller University, New York) (Piperno and Fuller, 1985). KMX-1 is a mouse IgG mAb recognizing all β-tubulin isotypes (Birkett et al., 1985). KMP-1 is a mouse IgM mAb specific for the *Physarum* α1-tubulin isotype (Walden et al., 1989). The β2-tubulin antibody is an affinity-purified polyclonal antibody raised in chickens against a β2-tubulin fusion protein and specifically recognizing the *Physarum* β2-tubulin isotype (Diggins-Gilicinski et al., 1989). For anti–mouse IgG, FITC-conjugated sheep antibody was obtained from Cappel Laboratories (Malvern, PA) and Sigma Chemical Co.; Texas Red-conjugated rabbit antibody was obtained from Cappel Laboratories; and goat antibody was obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Anti–mouse IgM fluorescein-conjugated goat antibody and anti–chicken Ig, FITC-conjugated rabbit antibody and Texas Red-conjugated goat antibody were obtained from Jackson ImmunoResearch Laboratories.

Cell Culture

The *P. polycephalum* strain used in this study was the apogamic mutant CL carrying a mutation at the *matA* mating type locus (Cooke and Dee, 1974). Amebae were cultured on liver infusion agar plates at 30°C, a temperature restrictive to plasmodial development, as described by Blindt et al. (1986). CL amebae were induced to develop into plasmodia as described by Solnica-Krezel et al. (1988). The proportion of cells committed to plasmodium formation was determined as in Youngman et al. (1977) and Solnica-Krezel et al. (1990). On average, 1,000 cells on five plates were assayed. Mean and SD reflect the variation between different assay plates in one experiment. Statistical analysis was performed using the *t* test; significance levels are given in the text.

Immunofluorescence Microscopy

Immunofluorescence of amebal and developing cells was performed as described by Solnica-Krezel et al. (1990). The stained coverslips were mounted in 1% wt/vol *p*-phenylenediamine in 90% vol/vol glycerol, 10% Tris-HCl, pH 7.5. Samples were observed with a Zeiss Axiophot microscope, using a total magnification of 1,000. Photomicrographs were taken with T-MAX, Ektachrome or Kodacolor Gold 400 ASA films.

Table I. β2-Tubulin in Mitotic Cells Developing into Plasmodia

Committed cells	Multinucleate cells	Mitotic cells	
		Total counted	β2-Positive
Mean ± SD %			%
0.0 ± 0.0	0.0 ± 0.0	206	0.0
0.0 ± 0.0	0.0 ± 0.0	787	0.0
0.1 ± 0.0	0.0 ± 0.0	574	0.0
0.4 ± 0.4	0.0 ± 0.0	351	0.3
0.6 ± 0.4	0.0 ± 0.0	103	0.0
4.6 ± 1.8	0.5 ± 0.2	102	6.8
6.5 ± 1.1	0.2 ± 0.1	398	6.2
11.9 ± 1.1	0.9 ± 0.9	641	22.3
14.6 ± 1.5	2.4 ± 0.9	121	23.1
16.4 ± 3.1	2.3 ± 0.1	773	20.4
18.0 ± 3.0	2.0 ± 0.6	86	29.8
18.2 ± 1.6	2.0 ± 0.8	514	32.3
20.7 ± 2.1	0.2 ± 0.1	213	23.5
30.8 ± 2.4	3.6 ± 1.1	85	30.6
34.6 ± 3.8	2.7 ± 0.2	282	46.8
36.9 ± 1.9	1.4 ± 0.1	171	38.6
40.6 ± 4.1	2.1 ± 0.6	114	32.0
44.9 ± 3.3	1.4 ± 0.4	634	34.9
46.6 ± 6.7	15.1	812	75.2
49.1 ± 1.6	17.0 ± 3.7	126	73.0
75.2 ± 0.9	32.6	61	67.2

Developing cultures at different stages were divided into two parts. One part was used to determine the percentage of cells committed to plasmodium formation by the replating assay. Cells from the other part of the culture were fixed, and subsequently stained with the KMX-1 antibody to visualize all microtubules, and with the antibody specific for β2-tubulin. Additionally, cells were stained with DAPI. To determine the percentage of multinucleate cells, an average of 1,000 cells on three slides were observed using phase-contrast optics and DAPI. The KMX-1 staining was used to screen for mitotic cells. Every mitotic spindle detected was then examined for staining with the β2-tubulin antibody.

Results

β2- and α3-Tubulin Isotypes as Developmental Markers

Plasmodium-specific β2-tubulin is incorporated into cytoplasmic microtubules and into mitotic spindles of both plasmodial and amebal types (Diggins-Gilicinski et al., 1989). During the APT, the relative frequencies of committed cells and cells positive for β2-tubulin indicate that β2-tubulin may be detectable only after the point of commitment to plasmodium formation (Solnica-Krezel et al., 1990). For the present studies, we wanted to test more rigorously whether, in mitotic cells, β2-tubulin is also detectable only after the commitment event. Therefore, we studied the temporal relationship between the accumulation of committed cells and β2-tubulin-positive mitotic spindles during the APT. Each of several developing cultures was assayed simultaneously for committed cells by the replating assay (Youngman et al., 1977), and for β2-tubulin-positive mitotic spindles and multinucleate cells by double-label indirect immunofluorescence microscopy (Solnica-Krezel et al., 1990). Fixed cells were stained with KMX-1 antibody, to detect all microtubules, and with the antibody specific for β2-tubulin (Diggins-

Gilicinski et al., 1989). Additionally, cells were stained with diamidinophenylindole (DAPI) to visualize DNA. During subsequent microscopic observation, the KMX-1 antibody pattern was used to screen for mitotic cells. Each mitotic spindle detected was then assayed for β2-tubulin staining. As before (Solnica-Krezel et al., 1990), mitotic figures containing β2-tubulin were not detected in cultures lacking committed cells (Table I). Moreover, β2-tubulin-positive mitotic spindles were generally not observed in cultures exhibiting <1% committed cells. These correlations are consistent with the hypothesis that most β2-tubulin-positive mitotic figures occur in committed cells. The proportion of β2-tubulin-positive mitotic figures increased as the proportion of committed cells increased and often exceeded the proportion of committed cells (Table I). However, the proportions of committed cells are probably underestimated, as large multi-nucleate cells often do not survive the replating procedure. The same cells are easily preserved by fixation and increase the observed proportion of mitotic spindles that contain β2-tubulin. Additionally, in late developing cultures, amebal

cells that have not started the process of plasmodium formation often undergo encystment (L. Solnica-Krezel, unpublished). Dormant cysts contribute to the uncommitted cell population but not to the mitotic fraction. One cannot rule out the possibility that some rare cells that are positive for β2-tubulin have not passed the commitment event (Solnica-Krezel et al., 1990). For this study, however, we assume that most, if not all, cells positive for β2-tubulin are committed to plasmodium formation.

α3-tubulin is an acetylated form of α1-tubulin (Green and Dove, 1984; Diggins and Dove, 1987; Sasse et al., 1987), specifically recognized by the mouse mAb 6-11B-1 (Piperno and Fuller, 1985). The localization of α3-tubulin to the MTOC region of mitotic and interphase amebae (Diggins and Dove, 1987; Sasse et al., 1987) makes it a useful marker for the rearrangement of mitotic spindles during the APT. The majority of β2-positive uninucleate cells and even a small fraction of binucleate and quadrinucleate cells contain detectable levels of this antigen (Solnica-Krezel et al., 1990).
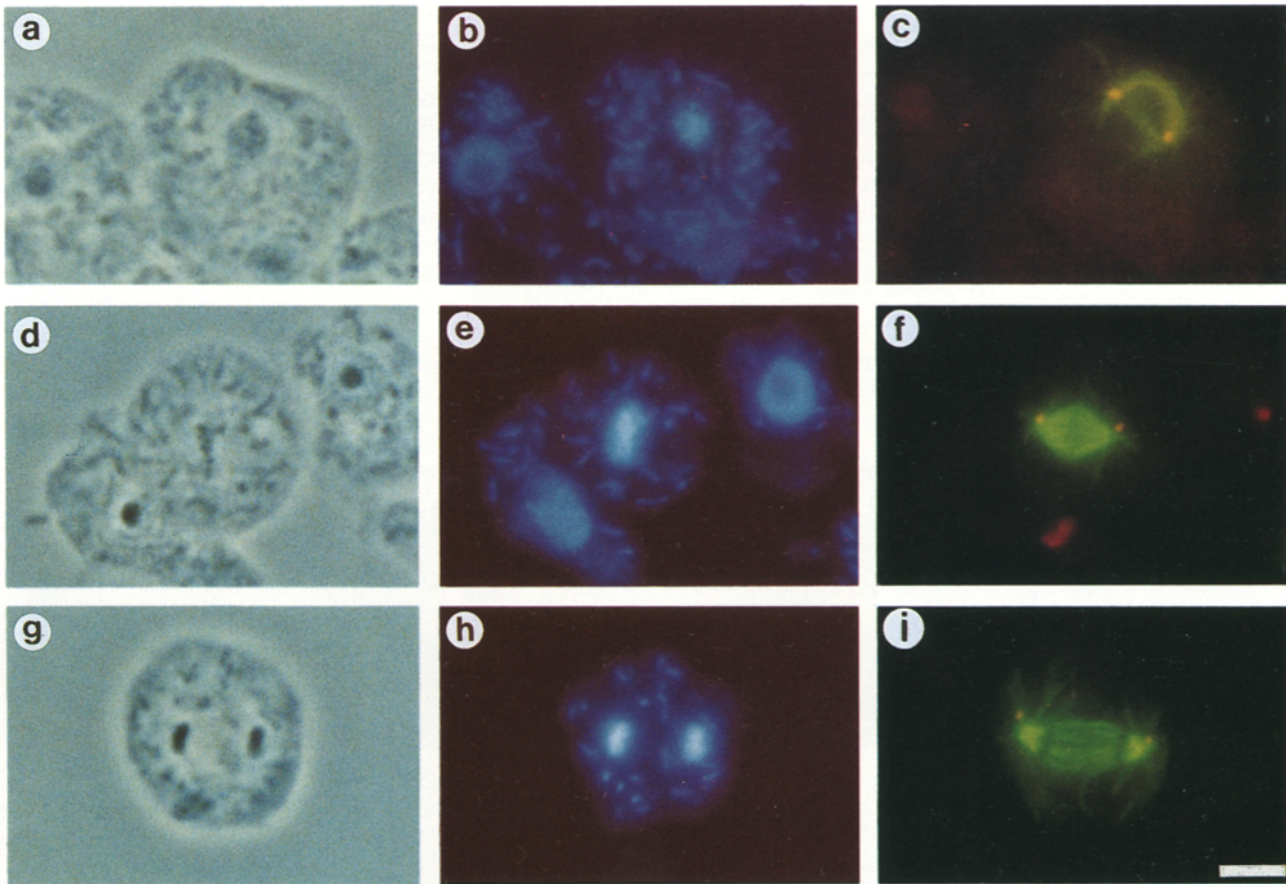

Figure 1. Immunofluorescent detection and localization of β2- and α3-tubulins in uninucleate cells undergoing the amebal type of mitosis. (*a, d,* and *g*) Phase-contrast images; (*b, e,* and *h*) DAPI images; condensed chromosomes are visible, additional staining represents mitochondria, ingested bacteria and other amebae. (*c, f,* and *i*) β2-tubulin pattern (*green*) obtained with secondary antibody conjugated to FITC and α3-tubulin pattern (*yellow* to *red*) obtained with secondary antibody conjugated to Texas Red. Yellow-to-red color variation results from the overlap of the patterns visualized with both FITC- and Texas Red–conjugated antibodies and from different levels of both developmentally regulated tubulin isotypes in individual cells. (*a–c*) Amebal mitotic prophase. α3-Tubulin is associated with the cytoplasmic MTOCs located at the opposite sides of the nucleus containing condensed chromosomes. (*d–f*) Amebal mitotic metaphase. α3-Tubulin is present at both astral spindle poles. Note two additional cells visible in the field which contain α3-tubulin and represent stages of development preceding expression of β2-tubulin. (*g–i*) Amebal telophase. Both mitotic poles radiate prominent astral microtubules and contain α3-tubulin. Bar, 2.5 μm.

Different Classes of Mitotic Spindles Formed in Transitional Cells

Using antibodies specific for α3- and β2-tubulins, we studied the organization of mitotic spindles in developing cells by double-label indirect immunofluorescence microscopy. These studies revealed that transitional cells can construct several morphologically distinct classes of mitotic spindles.

(a) Amebal Mitotic Figures. Fig. 1 shows some developing cells with β2-tubulin-containing mitotic spindles, which are otherwise indistinguishable from the spindles observed in amebal cells (Havercroft and Gull, 1983). During prophase, apparently extranuclear MTOCs stained with the α3-tubulin-specific antibody were located at opposite sides of the nucleus (Fig. 1, *a–c*). In metaphase (Fig. 1, *d–f*) through telophase (Fig. 1, *g–i*), α3-tubulin was located at both astral poles of the spindle.

(b) Plasmodial Mitotic Figures. Uninucleate β2-tubulin-positive cells also exhibited mitotic spindles of the plasmodial type (Fig. 2). In prophase, a separate set of microtubules not connected to α3-tubulin-positive MTOCs overlapped the condensed chromosomes, as described for plasmodial mitosis (Paul, 1986). However, beyond the apparently intranuclear microtubules, cytoplasmic MTOCs were also present in the majority of the uninucleate cells undergoing the plasmodial type of mitosis. Often the cytoplasmic MTOC behaved as a normal prophase amebal MTOC and duplicated; divided MTOCs migrated to opposite sides of the nucleus (Fig. 2, *a–c*). In some such mitoses, the divided MTOCs separated but one daughter MTOC appeared not to be directly associated with the nucleus (Fig. 2, *d–f*). Alternatively, the cytoplasmic MTOC did not seem to divide, it was either juxtaposed to the spindle (Fig. 2, *g–i*), or else not directly associated with the spindle (not shown). In metaphase through telophase, the mitotic spindle was anastral (Fig. 2, *l, o, r,* and *u*). Cytoplasmic MTOC(s) were either close to the nucleus (Fig. 2, *j–l, p–r, s–u*) or else distant from it (Fig. 2, *m–o*). Whenever detected, α3-tubulin appeared to be associated only with the cytoplasmic, additional MTOC(s), never with the anastral poles of the spindle.

(c) Chimeric Mitotic Figures. Spindles with features of both amebal and plasmodial mitoses were observed in cells containing α3- and β2-tubulins (Fig. 3). These chimeric spindles could be readily distinguished from the anastral plasmodial mitotic spindles only at anaphase and telophase, when astral microtubules associated with the spindle poles were most clearly visible. Chimeric mitotic figures exhibited astral microtubules radiating from the poles, characteristic of the amebal type of mitosis. However, they also contained additional MTOC(s), characteristic of plasmodial mitosis. Most commonly α3-tubulin was associated with the additional MTOC(s), but not with the astral spindle poles, mimicking the α3-tubulin distribution in cells undergoing mitosis of the plasmodial type (Fig. 3, see also Fig. 6, *b, f, j,* and *n; c, g, k,* and *o;* and *d, h, l,* and *p*). Occasionally, α3-tubulin was associated with only one of two astral poles present in the cell (Fig. 5, *b, e, h,* and *k*); or with one pole of the astral spindle and an additional MTOC (Fig. 5, *c, f, i,* and *l*). Such an asymmetric distribution of α3-tubulin was never observed in the astral mitoses of amebae. Chimeric mitoses were rare, constituting no more than 3% of all mitotic spindles in developing cultures.

(d) Star Microtubular Arrays. Some transitional cells exhibited unusual microtubular arrays in which microtubules radiated in the form of a star from the center of the cell (Fig. 4). Two observations indicated that "stars" represented mitotic rather than interphase microtubular arrays. First, chromosomes were always condensed and located in the center of the microtubular array, mimicking a typical prophase DNA pattern (compare *a* and *b* of Figs. 1, 2, and 4). Second, star structures were usually associated with two α3-tubulin-containing structures, rather than one, indicating that the MTOC
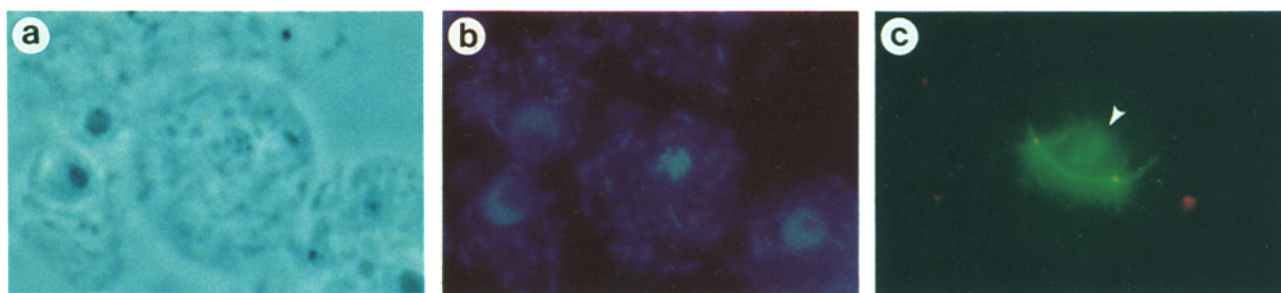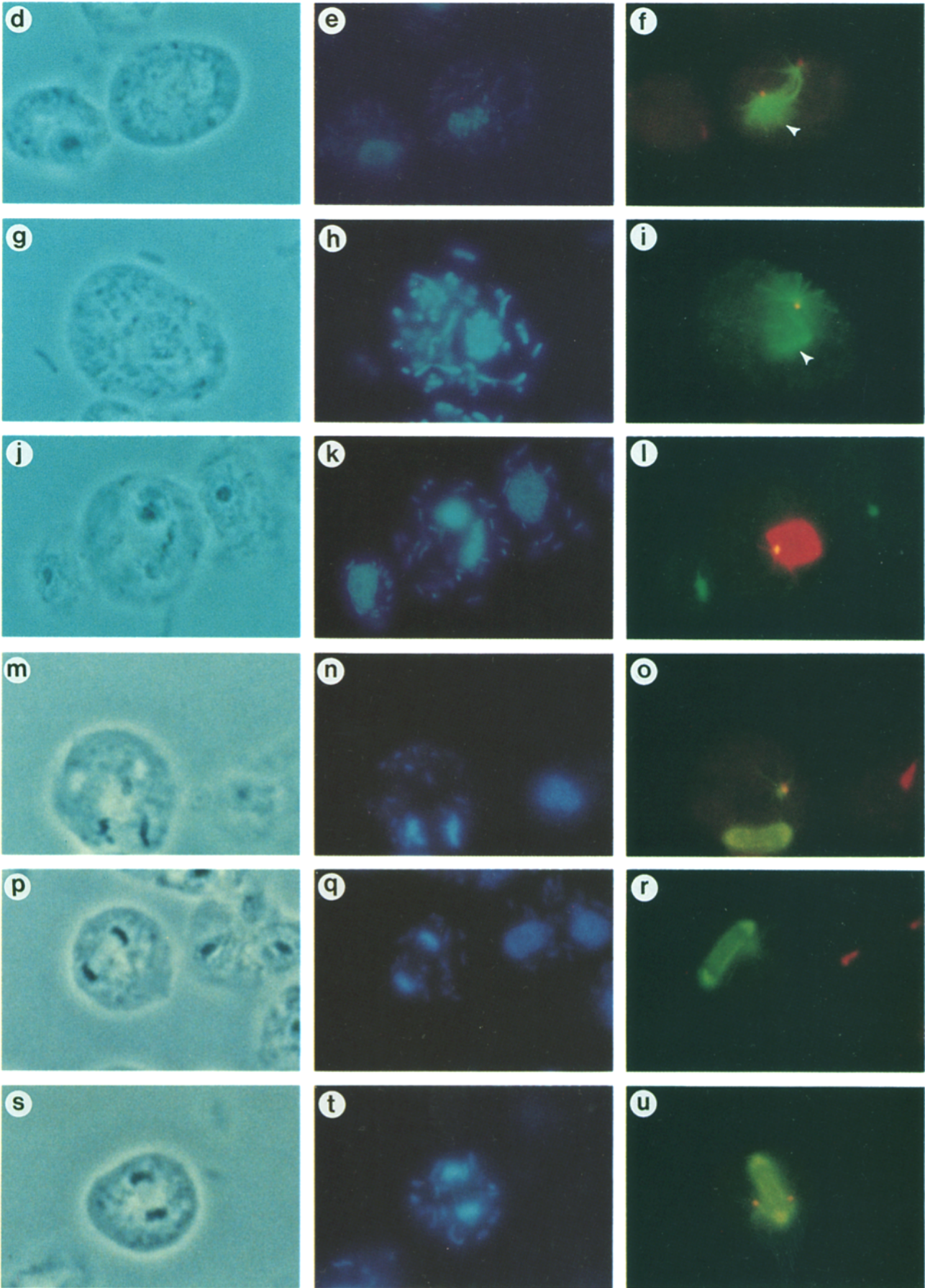

Figure 2. β2- and α3-tubulins in uninucleate developing cells undergoing plasmodial mitosis. (*a, d, g, j, m, p,* and *s*) Phase-contrast images; (*b, e, h, k, n, q,* and *t*) DAPI images; (*c, f, i, o, r,* and *u*) β2-tubulin patterns (*green*) and α3-tubulin patterns (*yellow* to *red*); (*l*) β2-tubulin pattern (*red*) and α3-tubulin pattern (*yellow* and *green*), in this case β2-tubulin was detected with secondary Texas Red–conjugated antibodies to chick Ig, whereas FITC conjugated antibodies to mouse IgG were used to visualize α3-tubulin. (*a–c*) Plasmodial mitotic prophase. One set of microtubules (*arrowhead*) overlaps the condensed chromosomes. Additionally, two apparently cytoplasmic MTOCs which contain α3-tubulin are located at opposite sides of the nucleus. (*d–f*) Another example of the plasmodial prophase. One set of microtubules (*arrowhead*) overlaps the condensed chromosomes. Of two α3-tubulin-containing MTOCs, one is juxtaposed to the nucleus and the other is distant from it. (*g–i*) Another example of plasmodial prophase. One set of microtubules (*arrowhead*) overlaps the condensed chromosomes. A single additional MTOC is juxtaposed to the nucleus. (*j–l*) Plasmodial metaphase in a cell that has ingested an ameba. A metaphase spindle that does not exhibit α3-tubulin is accompanied by an additional MTOC stained by the antibody specific for α3-tubulin. (*m–o*) Plasmodial anaphase/telophase. Note the anastral, α3-tubulin-negative spindle located at the cell periphery. A single, additional MTOC that contains α3-tubulin and is nucleating microtubules is distant from the spindle. (*p–r*) Plasmodial telophase. The anastral mitotic spindle is accompanied by a single additional MTOC. α3-Tubulin is absent from the cell undergoing mitosis but is detected in other cells present in the field. (*s–u*) Plasmodial telophase. The mitotic spindle exhibits anastral and α3-tubulin-negative poles. Two additional MTOCs containing α3-tubulin are located at opposite sides of the spindle. Magnification as in Fig. 1.
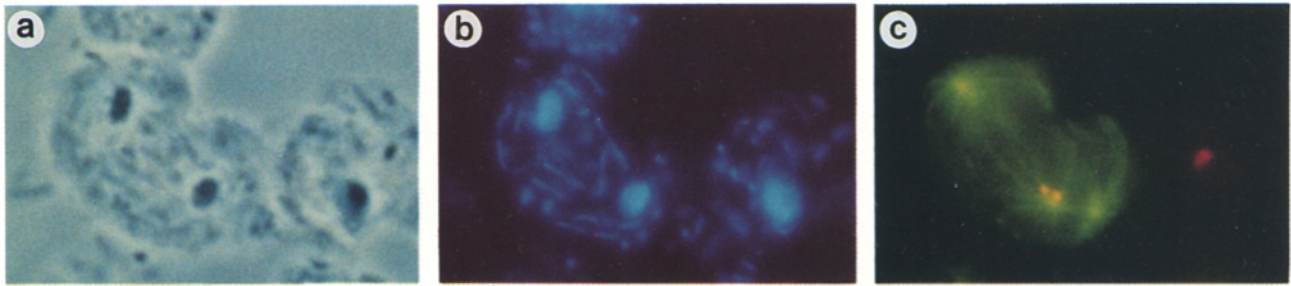
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Figure 3. β2- and α3-tubulins in a uninucleate developing cell undergoing the chimeric type of mitosis. (*a*) Phase-contrast image. Note that this cell has formed a unilateral cytokinetic furrow. (*b*) DAPI image. Only two chromosomal aggregations are present. (*c*) β2-tubulin pattern (*green*) and α3-tubulin pattern (*orange* to *red*). The mitotic poles radiate astral microtubules but do not contain detectable amounts of α3-tubulin. Two additional unseparated MTOCs containing α3-tubulin radiate short microtubules. Note that the additional MTOCs are asymmetrically localized in the cell and their position correlates with the missing cytokinetic furrow. Magnification as in Fig. 1.

had duplicated in these cells. These putative MTOCs were most often localized in the center of the star, overlapping chromosomes (Fig. 4, *a–c*). Rarely, one of them was in the center and the other near the periphery of the cell (Fig. 4, *d–f*). The star arrays were detected neither in amebal cultures, nor in early developing cultures lacking committed and multinucleate cells (Table II). Stars were observed only in cultures in which committed and multinucleate cells were detected; their proportion increased as the percentage of committed and multinucleate cells in a developing culture increased. These observations are consistent with the notion that star structures are formed only in cells developing into plasmodia; they do not represent a particular configuration of amebal prophase. Two observations argued that the star configuration is distinct from the plasmodial prophase. First, a separate set of microtubules that overlap chromosomes but that are not connected to α3-tubulin-positive MTOCs, a characteristic of plasmodial prophase, was not observed in cells forming star arrays. Second, stars were observed in uninucleate cells exclusively, whereas plasmodial prophase was observed also in multinucleate cells.

(e) Mitotic Spindles in Multinucleate Cells. In cells with two or more nuclei, karyokinesis was synchronous and mitotic spindles were anastral. Thus, the transition from amebal to plasmodial mitosis is completed at the binucleate cell stage. α3-tubulin, associated with cytoplasmic MTOCs, was
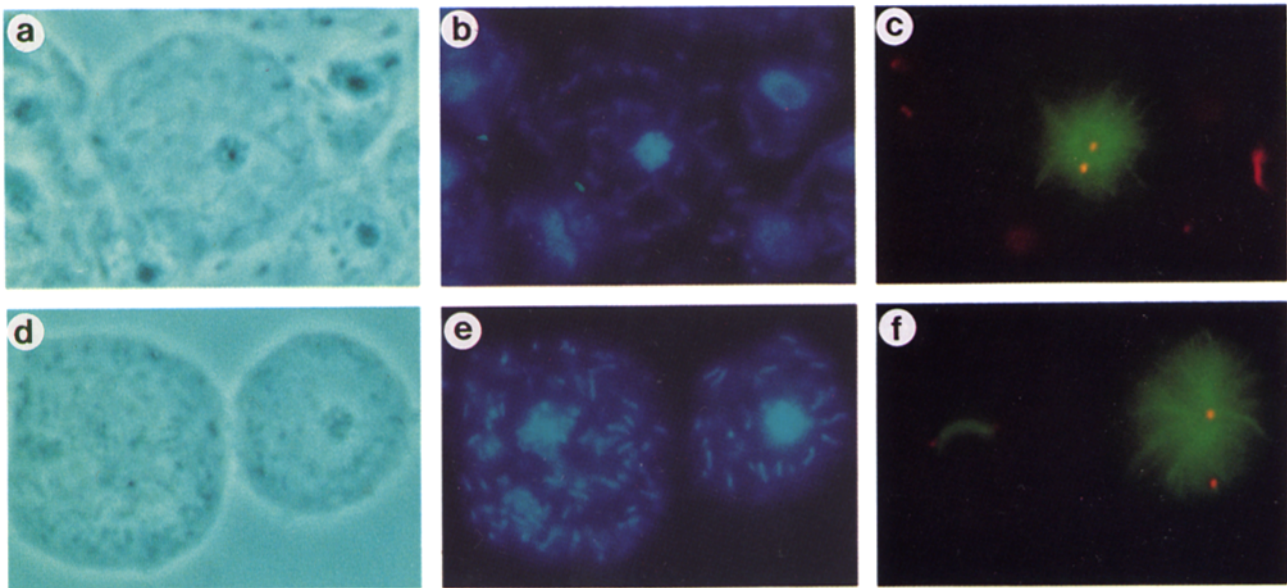

Figure 4. Immunofluorescent detection and localization of β2- and α3-tubulins in cells that have assembled star structures. (*a* and *d*) Phase-contrast images; (*b* and *e*) DAPI images; (*c* and *f*) β2-tubulin pattern (*green*) and α3-tubulin pattern (*orange* to *red*). (*a–c*) A cell with a typical star microtubular array. Note the round shape of the cell. β2-tubulin-containing microtubules radiate in the form of a star from the center of the cell, which contains condensed chromosomes. α3-tubulin is detected in two "dot-like" structures, presumably MTOCs, which are located near the center of the star array. (*d–f*) Comparison of a cell that formed a star microtubular array (*right*) with a cell that exhibits an early prophase of the amoebal type. Both cells have a round shape and condensed chromosomes. In the cell at the left, two MTOCs that contain α3-tubulin are migrating to opposite sides of the nucleus (compare with Fig. 1 *c*). In the cell at the right microtubules have formed a stellar array. α3-Tubulin is present in two presumptive MTOCs; one located in the center of the star array and the other at its periphery. Magnification as in Fig. 1.

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Table II. Kinetics of Accumulation of Star Structures during the APT

% Committed cells	% Multinucleate cells	Mitotic index	Mitotic cells	
			No. counted	% Stars
Mean ± SD	*Mean ± SD*	*Mean ± SD*		
0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.7	368	0.0
0.0 ± 0.0	0.0 ± 0.0	2.8 ± 0.9	1,627	0.0
0.0 ± 0.0	0.0 ± 0.0	2.7 ± 0.1	1,572	0.0
0.0 ± 0.0	0.0 ± 0.0	NA	1,071	0.0
0.1 ± 0.0	0.0 ± 0.0	2.7 ± 0.5	1,013	0.0
0.2 ± 0.2	0.0 ± 0.0	3.2 ± 1.3	265	0.0
0.6 ± 0.3	0.0 ± 0.0	2.0 ± 0.4	103	0.0
6.6 ± 0.6	0.2 ± 0.1	1.9 ± 0.3	522	0.4
11.9 ± 1.1	0.9 ± 0.9	NA	814	4.5
14.6 ± 1.5	2.4 ± 0.9	1.5 ± 0.7	118	2.5
16.4 ± 3.1	2.3 ± 0.1	2.7 ± 0.0	945	2.0
17.9 ± 2.7	2.2 ± 0.4	2.3 ± 0.3	191	7.5
18.2 ± 1.6	2.0 ± 0.8	NA	514	3.5
19.1 ± 5.2	1.2	NA	285	2.8
20.7 ± 2.1	0.2 ± 0.1	NA	614	2.4
38.8 ± 2.4	3.8 ± 1.5	2.4	261	9.2
40.6 ± 4.1	2.1 ± 0.6	NA	114	5.2
46.6 ± 6.6	15.0	NA	1,634	25.6
75.2 ± 0.9	32.6	3.7	208	14.1

Several developing cultures were assayed simultaneously for cells committed to plasmodium formation, by the replating assay, and for multinucleate cells, mitotic index and mitotic figures of the star type, by phase contrast and indirect immunofluorescence microscopy. See legend to Table I for methods used to determine the percentage of multinucleate cells. Cells stained with the KMX-1 antibody, which visualized all microtubules, were screened for mitotic cells. On average 1,500 cells on three separate slides were screened to establish the mitotic index. Every mitotic figure was examined to determine the percentage of star microtubular arrays. NA, not assayed.

occasionally detected in cells containing two or three mitotic spindles. In mitotic plasmodia with larger numbers of mitotic spindles, α3-tubulin was absent, even in cells that exhibited cytoplasmic MTOCs (not shown).

The Astral Mitotic Spindle May Be Necessary for Cytokinesis

Can a developing cell undergo more than one mitosis between the commencement of β2-tubulin synthesis and binucleate cell formation? If committed cells undergo cellular division, then some β2-tubulin-positive mitotic cells should exhibit cytokinetic furrows. We therefore studied phase contrast images of uninucleate β2-tubulin-positive cells that were in telophase.

Control experiments indicated that in amebal cultures, 95 ± 2% (n = 1,000; eight experiments) of telophase cells exhibited cytokinetic furrows. However, in developing cultures only 76 ± 9% of cells expressing β2-tubulin that formed astral (amebal and chimeric) mitotic spindles, displayed any signs of cytokinesis in telophase (n = 500, 13 experiments) (Figs. 5 and 6). No obvious differences were observed in the size or shape of astral microtubules between cells exhibiting and lacking furrows. A subset of β2-tubulin-positive cells showing signs of cytokinesis exhibited unusual unilateral cleavage furrows (21 ± 6%, n = 350, eight experiments) (Fig. 6). In contrast, dividing amebae practically never exhibited unilateral furrows (0.3 ± 0.4%, n = 250, two experiments). Cinematographic studies have shown that unilateral cleavage furrows are characteristic of cells that start cytokinesis but fail to complete it, eventually forming binucleate

Most likely, a uninucleate β2-positive cell undergoing astral mitosis can either produce two daughter cells, or else form one binucleate cell without furrowing or after developing a unilateral cytokinetic furrow. Cytokinetic furrows were never observed in β2-tubulin-positive cells undergoing the plasmodial type of mitosis (n = 500, 10 experiments) (Fig. 2, p–u), indicating that plasmodial mitoses in uninucleate cells usually lead to binucleate cell formation. Star microtubular arrays were never associated with cytokinetic furrows (n = 700, 27 experiments) (Fig. 4); the fate of the cells forming star arrays is unknown. These studies indicate that some developing cells undergo more than one mitosis between the initiation of β2-tubulin synthesis and binucleate cell formation. Further, the astral mitotic spindle may be necessary but is not sufficient for the formation of a normal cleavage furrow and completion of cytokinesis.

Cytoplasmic MTOCs Influence the Position of the Cytokinetic Furrow

The position of the cleavage furrow is determined by the position of the mitotic spindle or the spindle asters (Rappaport, 1989). We studied whether there is any correlation between the position of MTOCs in the transitional cells undergoing astral mitoses and the position of the cytokinetic furrow. A bilateral cytokinetic furrow was usually observed in cells undergoing typical amebal mitosis, in which α3-tubulin was associated with both poles of the astral spindle (Fig. 5, a, d, g and j). A typical cytokinetic furrow was also observed in cells exhibiting chimeric mitotic spindles in which α3-tubulin was associated with only one of the two astral poles of the spindle (Fig. 5, b, e, h, and k; c, f, i, and l). Notably, the cleavage furrow would have divided the cell into two daughters of unequal size. The larger prospective daughter contained either the mitotic pole associated with longer astral microtubules and with two α3-tubulin-positive dot-like structures (Fig. 5, b, e, h, and k), or an additional MTOC (Fig. 5, c, f, i, and l).

Unilateral furrows were most commonly observed in cells undergoing the chimeric type of mitosis (Fig. 6, b, f, j, and m; c, g, k, and o; see also Fig. 3). In such cases, the additional MTOCs with associated α3-tubulin staining were located on the side of the cell that lacked a furrow (93%, n = 40, 3 experiments). Unilateral furrows were occasionally observed in typical amebal mitoses (Fig. 6, a, e, i, and m). Fig. 6, d, h, l, and p shows another unusual example of asymmetric cytokinesis. This cell, undergoing chimeric mitosis, formed a cytokinetic furrow that is deeper on one side of the cell. In addition, the prospective daughter containing the additional α3-tubulin-positive MTOC is much bigger than its sibling. These results support the notion that an astral mitotic spindle is not sufficient for furrowing. The presence of additional MTOC(s) in a cell may affect the formation and position of the cytokinetic furrow.

Changes in Tubulin Composition and Organization of Mitotic Spindles during the APT

Are different types of mitotic spindles assembled in a defined sequence during consecutive mitoses preceding binucleate cell formation? We observed that cytokinetic furrows were exhibited by cells with astral (amebal and chimeric) spin-
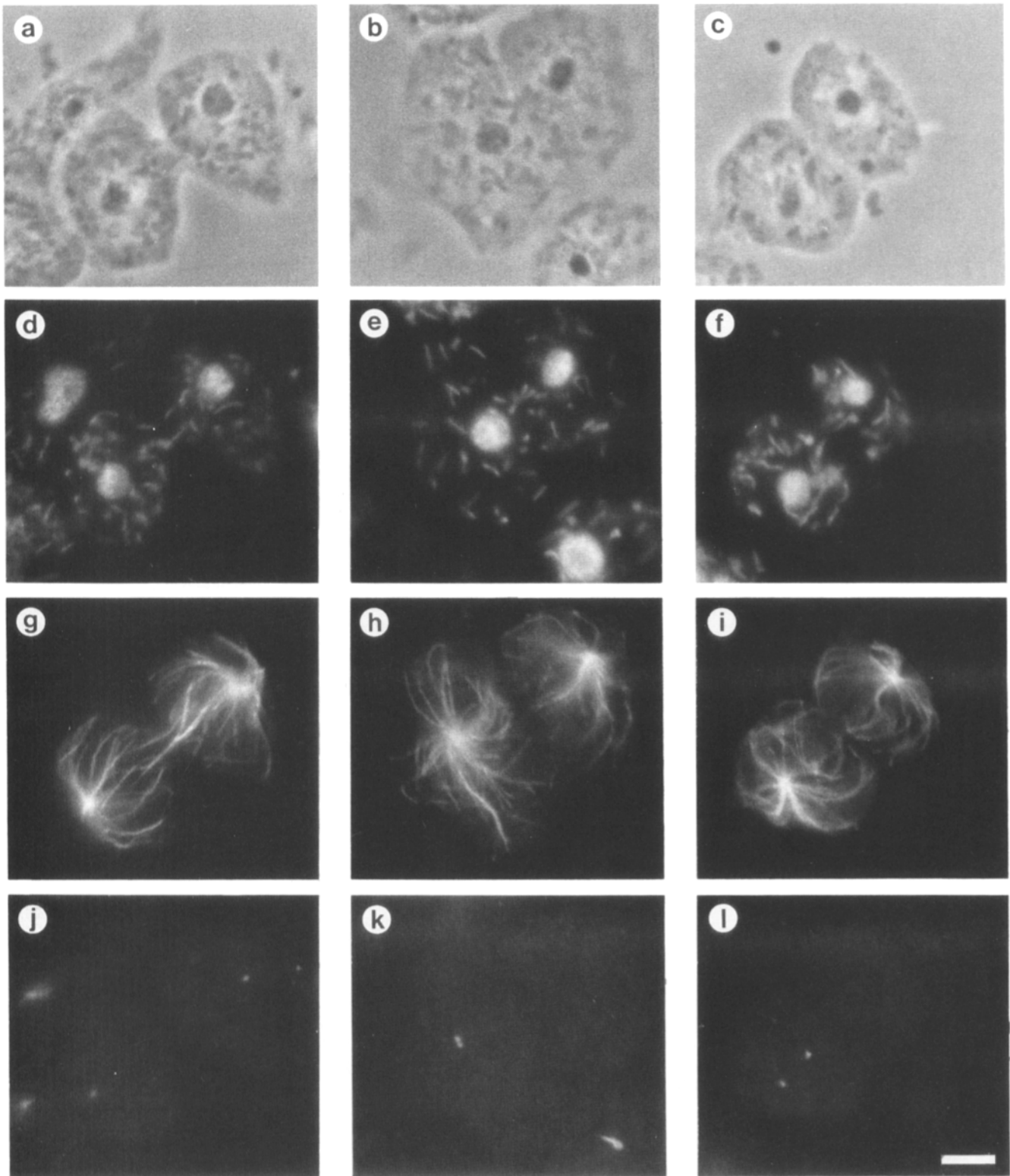
Figure 5. Immunofluorescent localization of spindle asters and additional MTOC(s) in uninucleate, developing cells at telophase of astral mitosis. (*a–c*) Phase-contrast images; (*d–f*) DAPI images; (*g–i*) β2-tubulin patterns; (*j–l*) α3-tubulin patterns. (*a, d, g,* and *j*) A normal cytokinetic furrow in a developing cell that assembled a mitotic spindle of the amebal type. Both mitotic poles are astral and exhibit α3-tubulin. (*b, e, h,* and *k*) A normal cytokinetic furrow in a developing cell undergoing mitosis of the chimeric type. One of the astral spindle poles is associated with a pair of α3-tubulin-positive MTOCs. Note that the prospective daughter containing these two MTOCs is bigger than the other daughter. (*c, f, i,* and *l*) A normal cytokinetic furrow in a developing cell that exhibits a mitotic spindle of the chimeric type. Both mitotic poles radiate astral microtubules. α3-Tubulin is detected at one of the poles and at the additional MTOC. Bar, 5 μm.
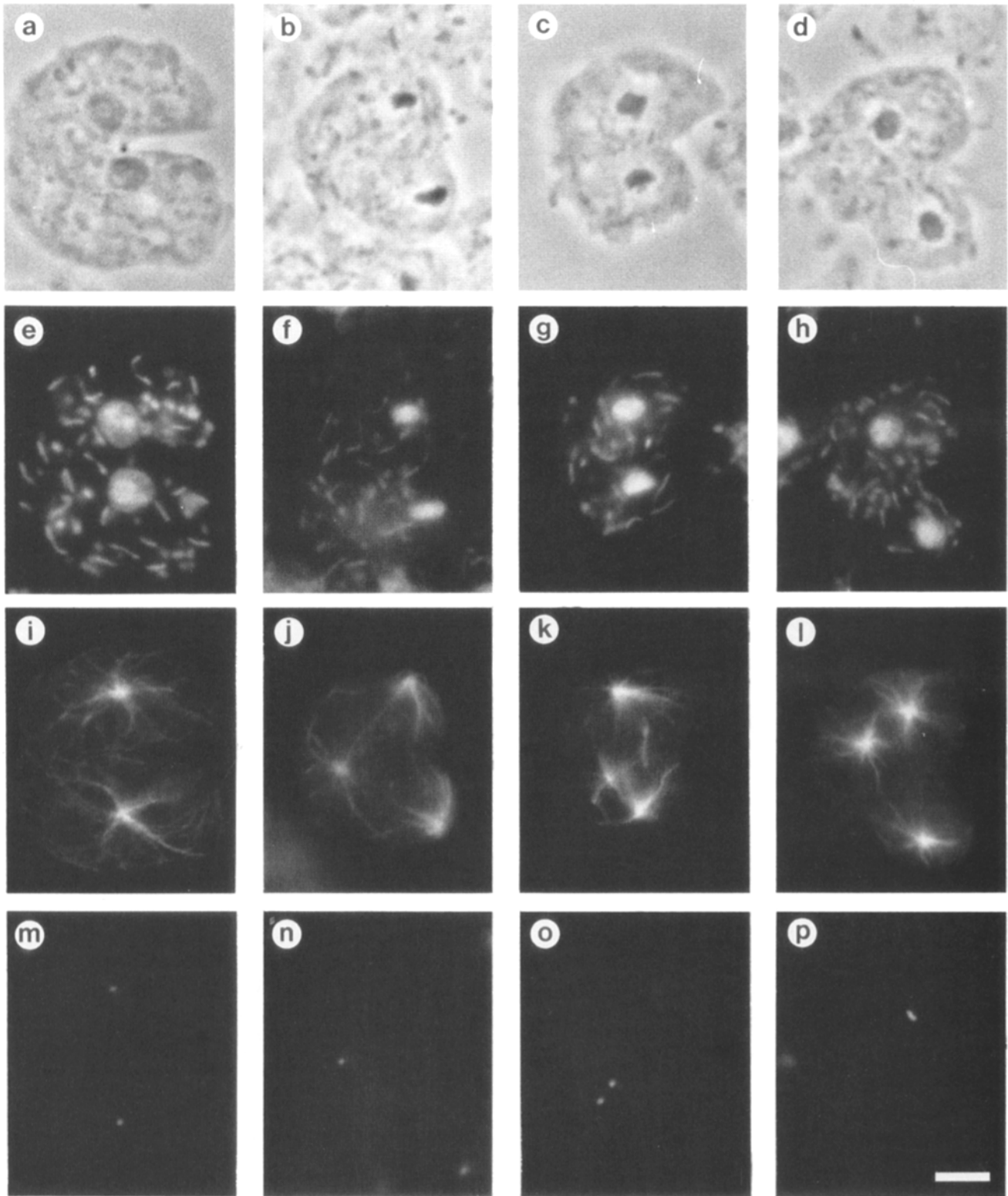
Figure 6. Immunofluorescent localization of spindle asters and additional MTOCs in uninucleate cells that formed unilateral cytokinetic furrows. (*a–d*) Phase-contrast images; (*e–h*) DAPI images; (*i–l*) β2-tubulin patterns; (*m–p*) α3-tubulin patterns. (*a, e, i,* and *m*) A unilateral cytokinetic furrow in a developing cell undergoing mitosis of the amebal type. Both spindle asters contain α3-tubulin. (*b, f, j,* and *n*) A unilateral cytokinetic furrow in a developing cell that formed a mitotic spindle of the chimeric type with one additional MTOC. The α3-tubulin-negative astral spindle is located close to the cell surface, which has started furrowing. An additional MTOC containing α3-tubulin is located in the equatorial region of the cell surface, opposite to the forming furrow. (*c, g, k,* and *o*) A unilateral cytokinetic furrow in a developing cell that formed a chimeric mitotic spindle with two additional MTOCs exhibiting α3-tubulin. Note that the cytokinetic furrow is missing on the side of the cell that is closer to additional MTOCs containing α3-tubulin. (*d, h, l,* and *p*) Asymmetric cytokinesis in a developing cell that formed a mitotic spindle of the chimeric type with one additional MTOC. Note that the prospective daughter that contains the additional MTOC is bigger than the other prospective daughter. Bar, 5 μm.

Table III. Changes in the Tubulin Composition and Organization of Mitotic Spindles during the APT

Culture No.	Committed cells	Multinucleate cells	α3-tubulin + cells	β2-tubulin + cells	Mitotic index	No. MS
			mean ± SD			
1	0.0 ± 0.0	0.0 ± 0.0	99.4 ± 0.3	0.0 ± 0.0	4.5 ± 0.0	2,000
2	12.9 ± 2.3	0.8 ± 0.0	99.6	6.5 ± 0.4	NA	1,000
3	13.7 ± 0.6	0.0 ± 0.0	98.8 ± 0.3	1.0 ± 0.0	2.7 ± 0.4	1,500
4	33.2 ± 2.5	0.2 ± 0.1	98.8 ± 0.3	4.7 ± 0.4	2.7 ± 0.3	1,000
5	21.9 ± 2.1	1.7	94.0	13.9 ± 2.8	NA	1,000
6	44.4 ± 1.1	15.0 ± 0.0	75.7 ± 1.0	40.6 ± 5.8	2.5 ± 0.6	900
7	65.9 ± 4.2	29.2 ± 2.6	65.2	67.5 ± 2.8	NA	650
8	100.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	NA	600

Mitotic figures

Culture No.		Total A	S	PU	PM	U	β2-tubulin positive A	S	PU	PM	U
1	%	88 ± 1	0 ± 0	0 ± 0	0 ± 0	12 ± 1	–	–	–	–	–
	α3	100	–	–	–	100	–	–	–	–	–
	β2	0	–	–	–	0	–	–	–	–	–
2	%	92 ± 1	0 ± 0	0 ± 0	0 ± 0	8 ± 1	58	12	15	0	12
	α3	100	–	–	–	100	100	100	100	100	100
	β2	1	–	–	–	2	100	100	100	100	100
3	%	75 ± 9	2 ± 1	4 ± 1	0 ± 0	19 ± 7	52	6	22	0	20
	α3	100	100	100	–	100	100	100	100	–	94
	β2	13	83	94	–	100	100	100	100	–	100
4	%	77 ± 5	2 ± 1	3 ± 1	0 ± 0	19 ± 7	44	7	32	0	18
	α3	100	100	100	–	99	100	100	100	–	93
	β2	5	100	93	–	22	100	100	100	–	100
5	%	65 ± 0	5 ± 1	9 ± 0	0 ± 0	21 ± 2	40	8	29	1	22
	α3	99	100	84	–	95	90	100	83	50	81
	β2	17	74	100	–	43	100	100	100	100	100
6	%	35 ± 2	9 ± 1	24 ± 1	8 ± 2	24 ± 1	23	6	32	18	20
	α3	93	100	66	13	46	86	100	60	15	35
	β2	48	60	98	100	86	100	100	100	100	100
7	%	24 ± 1	20 ± 4	20 ± 3	9 ± 4	29 ± 4	22	11	32	11	24
	α3	98	95	73	41	83	90	94	80	31	74
	β2	80	80	100	100	89	100	100	100	100	100
8	%	0 ± 0	0 ± 0	0 ± 0	100 ± 0	0 ± 0	0	0	0	100	0
	α3	–	–	–	0	–	–	–	–	0	–
	β2	–	–	–	100	–	–	–	–	100	–

Each developing culture (Nos. 1–7) was divided into two parts. One part was assayed for committed cells as described in Materials and Methods. The other part was fixed, stained with DAPI, and three different combinations of antibodies: (1) KMP-1 antibody to stain all microtubules in the cell and 6-11B-1 antibody specific for α3-tubulin; (2) KMP-1 and antibody specific for β2-tubulin; and (3) the β2-tubulin antibody and the 6-11B-1 antibody. During microscopic observation, phase contrast and DAPI staining were used to establish the percentage of multinucleate cells. Antibody combinations 1 and 2 were used to determine the percentage of cells expressing α3- and β2-tubulins, respectively. On average, 1,000 cells on three separate slides were assayed. Standard deviations represent the variation between slides. Using combinations of antibodies 1 and 2, on average 1,500 cells on three separate slides were screehed for mitotic figures to establish the mitotic index (MI). Every mitotic spindle (MS) detected with KMP-1 antibody was assayed for staining with 6-11B-1 (combination 1), or β2-antibody (combination 2), and assigned according to its morphology to one of the following categories described in this paper: A, amebal; S, star; PU, plasmodial uninucleate; PM, plasmodial multinucleate; and U, unidentified. Data obtained using combinations of antibodies 1 and 2 were used to determine the mean frequency of different categories of mitotic spindles in the population. Antibody combination 3 was used to determine the frequency of different categories of mitotic spindles in developing cells expressing β2-tubulin. Additionally, every mitotic cell detected with the β2-tubulin antibody was assayed for the presence of α3-tubulin. To determine all the assayed parameters in developing plasmodia (culture 8), several multinucleate mitotic cells stained with the three combinations of antibodies were studied.

dles, but not by cells with plasmodial mitotic spindles and star microtubular arrays. This indicated that, in the sequence of mitoses after the commencement of β2-tubulin synthesis, an astral mitosis may preferentially be first, with a plasmodial mitosis or star array preferentially later. In such a case, and with the level of developmental synchrony in these experiments, astral β2-tubulin-positive mitoses should predominate in an early population of developing cells. In con-

trast, plasmodial mitoses and star-forming cells should be predominant in late developing cultures. We therefore studied the kinetics of accumulation of different types of mitotic spindles in the course of the APT. To determine whether the organization of the mitotic spindle is related to its tubulin composition, we also monitored the presence of α3- and β2-tubulins in the different types of mitotic spindles. After fixation, cells were stained with three different combinations of
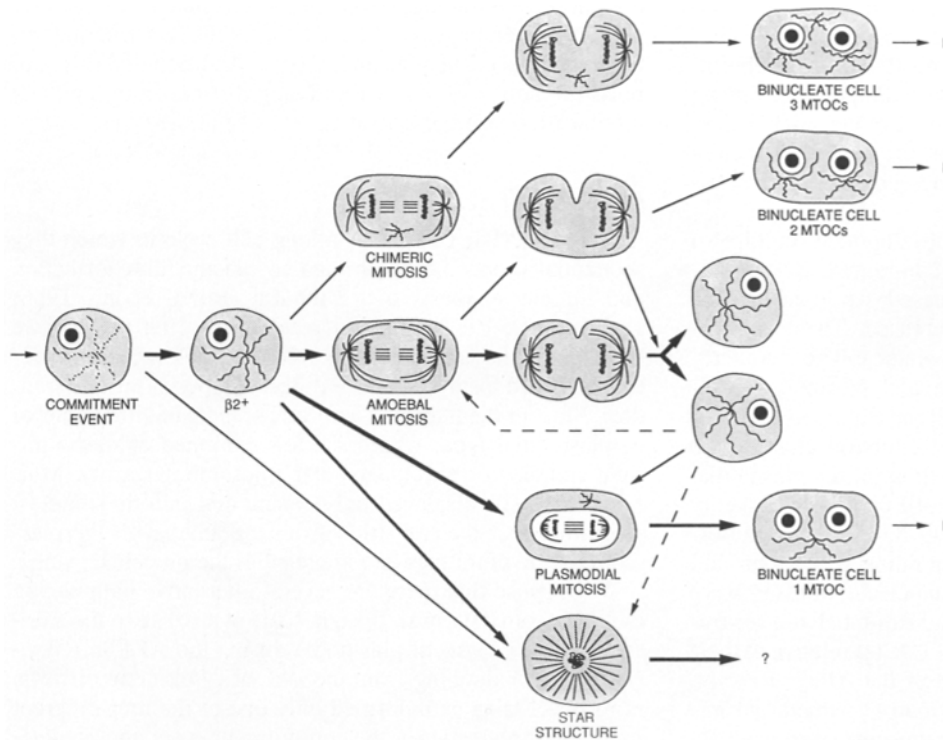
Figure 7. Variable pathways for the changes in mitosis and cytokinesis during the APT. The weights of arrows indicate the relative frequencies of specific events. After commitment, uninucleate cells express β2-tubulin. A uninucleate developing cell expressing β2-tubulin can undergo one of four different types of mitosis: amebal, plasmodial, chimeric, or star mitosis. Cells with amebal mitotic spindles usually form normal cytokinetic furrows and divide. The resulting daughter cells, upon entry into the next mitosis, could form one of four different mitotic spindles, but most likely would form spindles of the plasmodial type. A subset of cells undergoing the amebal type of mitosis develop unilateral cytokinetic furrows, fail to complete cytokinesis and most probably form binucleate cells. The resulting binucleate cells would contain two MTOCs. Some uninucleate developing cells undergo plasmodial mitosis. Plasmodial mitosis is not accompanied by cytokinesis and leads to binucleate cell formation. The resulting binucleate cell would contain one or two cytoplasmic MTOCs derived from the additional MTOCs present in the mitotic cell. Some developing cells form a star mitotic array. The fate of these cells is unknown. Rare uninucleate developing cells undergo mitosis of the chimeric type. Cells undergoing chimeric mitosis tend to form unilateral cytokinetic furrows and most probably fail to complete cytokinesis. The resulting binucleate cells would contain three or four MTOCs, with α3-tubulin present only in some of them.

primary antibodies: 1, KMP-1 to stain all microtubules and 6-11B-1, an antibody specific for α3-tubulin; 2, KMP-1 and the β2-tubulin antibody; and 3, the β2-tubulin and 6-11B-1 antibodies. All cells were stained with DAPI. Slides were screened microscopically for mitotic cells to establish the mitotic index, the proportion of mitotic cells that contained α3-tubulin, the proportion of mitotic cells that contained β2-tubulin, and the proportion of β2-tubulin-positive mitotic cells that contained α3-tubulin. For every combination of antibodies used, mitotic spindles were assigned according to their morphology to one of five classes: amebal (Fig. 1); plasmodial uninucleate (Fig. 2); star (Fig. 4); plasmodial multinucleate; and "unidentified". The unidentified category mainly contained spindles in metaphase, when the distinction between the amebal and plasmodial types of mitosis is most difficult. Mitotic spindles of the chimeric type could not be distinguished with all combinations of antibodies used. Therefore, they were included in the category of amebal spindles in these studies, mainly because of the presence of astral microtubules. In a given developing culture, the frequencies were established for different categories of mitotic spindles stained with the KMP-1 antibody and their α3- and β2-tubulin composition (combinations 1 and 2). The examination of samples stained with the β2- and 6-11B-1 antibodies (combination 3) provided the frequency of different categories of mitotic spindles in transitional β2-tubulin-positive cells of the same culture. Additionally, for each developing culture, the proportions of β2-tubulin-positive cells, α3-tu-

bulin-positive cells, committed and multinucleate cells were determined, to estimate the stage of development.

As shown in Table III, neither committed cells, β2-tubulin-positive cells nor multinucleate cells were detected early in development, and all cells contained α3-tubulin (culture 1). At this stage, only mitotic spindles of the amebal and unidentified classes were observed. All spindles contained the α3-tubulin epitope associated with both mitotic poles. This organization and tubulin composition of mitotic spindles is identical to that observed in amebal cultures.

Developing cultures containing 10–20% committed cells (cultures 2 and 3, Table III) exhibited low levels of β2-positive cells, few if any multinucleate cells, and almost 100% α3-tubulin-positive cells. In such cultures, mitotic spindles were also mainly of the amebal type. A very low proportion of mitotic spindles of the star and plasmodial–uninucleate categories were present. All mitotic cells observed contained α3-tubulin. A very low proportion of amebal, star, and unidentified mitotic figures contained β2-tubulin. However, practically all of the rare plasmodial mitoses were β2-tubulin-positive. In the population of cells stained with β2-tubulin antibody, spindles of the amebal type were most frequent.

In developing cultures containing 20–30% committed cells (cultures 4 and 5, Table III), low levels of multinucleate cells were detected, and the frequency of α3-tubulin-positive cells decreased. Amebal mitoses were still the major class in the population. However, the frequency of plasmodial uninucle-

ate and star mitoses increased relative to earlier stages of development. Practically all mitotic cells contained α3-tubulin, a significant fraction also being positive for β2-tubulin. Most plasmodial uninucleate mitoses were β2-positive. Among β2-tubulin-positive mitotic figures, those of the amebal class were still the most frequent. However, they were enriched in plasmodial uninucleate and in the α3-tubulin-negative mitoses.

In populations at later stages of development (cultures 6 and 7, Table III) the proportions of committed, β2-tubulin-positive and multinucleate cells increased, while the proportion of α3-tubulin-positive cells decreased. These cultures exhibited each category of mitotic spindles. The frequency of the star, plasmodial uninucleate and plasmodial multinucleate mitotic figures increased at the expense of amebal mitoses. Among spindles exhibiting β2-tubulin, those of the amebal type were less frequent than those of the plasmodial type. The tubulin composition of mitotic cells was markedly different from that observed in early developing cultures, with high proportions of those containing β2-tubulin and lacking α3-tubulin. Plasmodial uninucleate mitoses were uniformly positive for β2-tubulin, and exhibited a lower frequency of α3-tubulin in additional MTOC(s) relative to their counterparts from the earlier stages of the APT.

Finally, in cells containing more than two nuclei all mitotic spindles were of the plasmodial type and contained β2-tubulin (culture 8). α3-Tubulin was not detected in multinucleate mitotic plasmodia.

Two conclusions can be drawn from these studies. First, the changes in the organization and composition of mitotic spindles usually occurred in cells that were committed to plasmodium formation. Second, some committed cells can undergo sequentially amebal and then plasmodial mitosis, or form star array.

Stars and Plasmodial Mitoses Are Formed Preferentially at Distinct Stages of Uninucleate Cell Development

Cells representing more advanced stages of the APT generally are less likely to express α3-tubulin and more likely to express β2-tubulin relative to cells at earlier stages of development (Solnica-Krezel et al., 1990). This trend was observed for mitotic cells as well. Multinucleate, mitotic cells exhibited α3-tubulin with a significantly lower frequency than uninucleate mitotic cells (Table III). To test whether stars and plasmodial mitotic structures are formed at distinct stages of uninucleate cell development, we compared the α3- and β2-tubulin composition of a large number of uninucleate cells that formed these two microtubular arrays. Practically all cells that exhibited star mitotic figures were α3-tubulin-positive (99 ± 2%, mean ± SD; $n = 300$, 12 experiments). In contrast, significantly fewer (82 ± 17%) of the uninucleate cells that formed plasmodial mitotic spindles exhibited α3-tubulin ($n = 300$; 7 experiments; $P < 0.02$). Only 73 ± 22% of star structures exhibited β2-tubulin ($n = 400$, 14 experiments). However, significantly more and practically all of uninucleate cells that formed mitotic spindles of the plasmodial type contained β2-tubulin (98 ± 3%; $n = 400$; 9 experiments; $P < 0.001$). Thus, cells forming star structures were more often positive for α3-tubulin and less often positive for β2-tubulin than uninucleate cells assembling plasmodial mitotic spindles. This is consistent with the hypothesis that star mitotic figures are usually formed in uninucleate cells at earlier stages of the APT than cells constructing mitotic spindles of the plasmodial type. Additionally, this supports the notion of star arrays being distinct from the plasmodial prophase configuration.

Discussion

During the APT, cells enter a long cell cycle in which they sequentially become committed to plasmodium formation and initiate synthesis of β2-tubulin (Blindt et al., 1986; Bailey et al., 1987; Solnica-Krezel et al., 1990). Here, we have shown that uninucleate developing cells expressing β2-tubulin could form one of four different types of mitotic spindles. Most of them formed a mitotic spindle of either amebal or plasmodial type, whereas a few exhibited chimeric mitotic spindles or an unusual star microtubular array. Multinucleate cells displayed only plasmodial mitotic spindles, indicating that the transition from the amebal to the plasmodial type of mitosis is completed at the binucleate stage.

We propose that there are several alternative pathways in the shift from the astral, open mitosis of amebae to the anastral, closed mitosis of plasmodia during the APT in *Physarum* (Fig. 7). Judging from the lack of cytokinetic furrows, most developing cells formed only one of the four different mitotic figures between the commitment event and binucleate cell formation. This supports the notion that these cells were committed to plasmodium formation, and is consistent with the cinematographic studies of the APT showing that most cells entering the long cell cycle give rise to binucleate cells (Bailey et al., 1987). A significant proportion of β2-tubulin-positive cells with astral mitotic spindles exhibited cytokinetic furrows and most probably divided. This indicated that some committed cells can undergo at least one cellular division, an idea supported by the cinematographic demonstration of developing cells that divided at the end of the long cell cycle (Bailey, 1989). The absence of cytokinetic furrows in plasmodial mitoses, their predomination and relative depletion in α3-tubulin at later stages of development implied that, in a developing cell, plasmodial mitosis usually follows amebal mitosis between the commitment and binucleate cell formation. Cells that exhibited the star microtubular array never demonstrated cytokinetic furrows and most probably can be formed, either at first or at second mitosis after the commitment. However, we do not know whether these cells give rise to binucleate cells or are arrested in development.

Model for Formation of Variable Mitotic Structures in Uninucleate Transitional Cells

We propose that, during the APT, the type of mitotic spindle formed in a uninucleate developing cell depends upon which morphogenetic factors are present when the cell enters mitosis. Further, the decision to enter mitosis after commitment does not depend on the changes in organization of the mitotic spindle. Thus, an amebal mitotic spindle would be formed in a cell at early stages after commitment, that has not accumulated factors sufficient for plasmodial spindle formation, but that still contains factors sufficient for duplication of MTOCs and the assembly of the amebal mitotic spindle. The star structure would be assembled when a developing cell enters mitosis possessing some but not all the factors neces-

sary for the formation of a plasmodial mitotic spindle; such a cell may have lost factors needed for organization of the amebal mitotic spindle. Chimeric karyokinesis would occur in cells representing intermediate stages of development that contain factors sufficient for the assembly of intranuclear mitotic spindle, but that still possess some factors specifying amebal karyokinesis. Specifically, chimeric mitosis would be a consequence of the closed mitotic spindle formation by the intranuclear MTOCs, followed by nuclear membrane breakdown later in mitosis and development of astral microtubules. This would explain the lack of α3-tubulin in the astral poles of the chimeric spindle, as the presence of the nuclear membrane during prophase would prevent cytoplasmic, α3-containing MTOC(s) from attaching their microtubules to chromosomes and from forming a multipolar spindle. Mitosis of the plasmodial type would occur in cells at later stages of development that acquired all the factors necessary for formation of intranuclear mitotic spindle and that had lost factors ensuring the nuclear membrane breakdown. Apparently, the ability of cytoplasmic MTOCs to duplicate, separate, and migrate to opposite sides of the nucleus is lost gradually, and was observed in some cells that formed chimeric or even plasmodial mitotic spindles.

There are two likely sources of heterogeneity in the composition of factors determining the morphology of the mitotic spindle in uninucleate, transitional cells. First, it is plausible that developmentally-regulated molecules, including those determining the type of karyokinesis, change levels heterogenously. Indeed, developing cells differ in the timing of loss and accumulation of α3- and β2-tubulin (Solnica-Krezel et al., 1990). Second, cells may enter mitosis at different stages of development, and consequently differ in their composition of developmentally regulated factors determining the type of karyokinesis. Indeed, star structures and plasmodial mitotic spindles were preferentially formed at distinct stages of uninucleate cell development. Additionally, intermitotic times exhibited by developing cells during the extended cell cycle can differ by up to 6 h, i.e., 20% of the length of the cycle (Bailey et al., 1987).

The involvement of three different types of mitoses in the formation of binucleate cells would explain differences observed previously in numbers of MTOCs in binucleate cells (Gull et al., 1985; Bailey et al., 1990; Solnica-Krezel et al., 1990) (see Fig. 7). In amebae, astral mitotic poles organize cytoplasmic microtubules upon completion of karyokinesis (Havercroft and Gull, 1983). Thus, binucleate cells resulting from amebal mitosis would have two MTOCs, both containing α3-tubulin. Intranuclear anastral mitotic poles vanish after karyokinesis and do not participate in the organization of an interphase cytoskeleton in plasmodia (Paul, 1986). Thus, binucleate cells resulting from plasmodial mitoses would exhibit one or two MTOCs, containing α3-tubulin or not, depending on the α3-tubulin composition of the additional MTOCs in the mitotic cell. It is reasonable to assume that both astral mitotic poles, and additional MTOC(s) of cells undergoing chimeric mitosis, would participate in the organization of the cytoskeleton upon completion of karyokinesis. Thus a binucleate cell resulting from chimeric mitosis would be expected to contain three to four MTOCs, two of which would not exhibit α3-tubulin. Such binucleate cells are present in developing cultures (Solnica-Krezel et al., 1990), and probably do not result from fusion between differ-

ent uninucleate cells, as such fusions are very rare (Bailey et al., 1987).

Changes in Tubulin Composition Are Not Sufficient for Reorganization of Mitotic Structures

α3-Tubulin disappears gradually from MTOCs during the APT (Solnica-Krezel et al., 1990). We have observed α3-tubulin in developing cells forming all four types of mitotic spindles. Thus, the disappearance of α3-tubulin is not a causative factor in the developmental changes of mitotic spindles. Further, detectable levels or accessibility of α3-tubulin appeared not to be necessary for the formation of the astral mitotic spindle. No correlation was observed between the loss of α3-tubulin and either the ability of amebal MTOCs to be duplicated during prophase, or the placement of cytoplasmic MTOC(s) relative to the nucleus in cells undergoing the plasmodial type of mitosis. These observations are consistent with earlier studies that demonstrated that detectable α3-tubulin in the MTOC was not necessary for its function in nucleating cytoskeletal microtubules (Solnica-Krezel et al., 1990). There are several possible explanations for the absence of the α3-tubulin at the poles of all plasmodial, most chimeric and rare amebal mitotic spindles. Most likely, these spindle poles are formed at a stage of the APT when de novo acetylation of α1-tubulin is absent. Thus, acetylated α3-tubulin would be associated only with the preexisting cytoplasmic additional MTOCs that retained the α3-tubulin epitope (Solnica-Krezel et al., 1990).

Plasmodium-specific β2-tubulin accumulates during the APT usually after the commitment and then participates in all microtubular structures detected in $P.$ polycephalum (Solnica-Krezel et al., 1990). Here, we have extended these observations by demonstrating the presence of β2-tubulin in chimeric and star mitotic structures. Thus, accumulation of β2-tubulin during the APT is not sufficient to induce the formation of a specific mitotic structure. However, the strong correlation between the presence of β2-tubulin and the plasmodial type of karyokinesis indicated that β2-tubulin might be necessary for morphogenesis of the intranuclear mitotic spindle.

Formation of Cytokinetic Furrows in Transitional Cells

Our studies indicated that an astral mitotic spindle may be necessary for cytokinesis in $P.$ polycephalum, as it is in animal cells (Rappaport, 1989). However, an astral mitotic spindle may not be sufficient for furrowing. The failure of a cell undergoing astral mitosis to develop cytokinetic furrow could be due to loss of other factors necessary for this process. This implies that factors determining the formation of the amebal mitotic spindle are regulated independently of factors executing cytokinesis.

The presence and position of additional MTOCs in mitotic cells can affect furrowing. Two lines of evidence indicated that the influence of additional MTOCs on furrowing is different from that of the astral spindle poles. First, the additional MTOCs observed in cells undergoing the plasmodial type of mitosis did not induce furrowing. Second, in cells undergoing chimeric mitosis, tripolar furrows were not observed even though, such furrows do form in *Physarum* cells that exhibit tripolar spindles (Bailey et al., 1990; L. Solnica-Krezel, unpublished). Thus, we hypothesize that the pres-

ence of additional MTOC(s) near the equatorial region of the cell inhibits furrowing. The inhibition of furrowing by physical obstructions in the cytoplasm between the metaphase plate and cell equator is interpreted as supporting the equatorial stimulation model of furrow formation (Rappaport, 1989; Salmon, 1989).

Biological Significance

The star structures and cytokinetic unilateral furrows we observed are also exhibited by cells undergoing heterothallic plasmodium development (Bailey, 1989; Bailey et al., 1990). Thus, the variable developmental pathways do not simply represent an idiosyncrasy of development resulting from the apogamic allele of matA.

The length of the cell cycle is profoundly modified during development of lower (Berger, 1989) and higher (O'Farrell et al., 1989) eukaryotes. With its extended transitional cell cycle, the APT provides an opportunity to study the relationship between development and cell cycle regulation. During the heterothallic APT in *Physarum*, fusions between mating amebae occur irrespective of their position in the cell cycle (Bailey et al., 1990). Our studies indicated that the decision to enter mitosis during the APT is independent of the changes in the organization of mitotic spindle and cytokinesis.

The observation of variable pathways for reorganization of mitotic spindles during the apogamic APT is consistent with previous demonstration that developing cells are heterogenous with respect to the timing of changes in expression of developmentally regulated tubulins, reorganization of interphase microtubules, and the order of physiological changes (Solnica-Krezel et al., 1990). We were able to detect these variable pathways only by examining large numbers of cells at multiple stages of development. We speculate that such variability is inherent to most developmental processes.

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