**INTRODUCTION**

The tubulin superfamily comprises three types of evolutionarily related polypeptide chains: α, β and γ (Oakley and Oakley, 1989). The αβ-tubulin heterodimer constitutes the main component of microtubules. In contrast, γ-tubulin seems to be restricted to the mitotic poles: spindle pole bodies in *Aspergillus* (Oakley et al., 1990) and yeast (Horio et al., 1991), centrosomes in animal cells (Stearns et al., 1991; Zheng et al., 1991). In the yeast *Schizosaccharomyces* (Horio et al., 1991), γ-tubulin is also transiently present in a microtubule organizing centre responsible for the nucleation of cytoplasmic microtubules during the course of cytokinesis. In mammalian cells undergoing cytokinesis, the two daughter cells are linked by an intercellular cytoplasmic bridge for up to 4 hours after metaphase (Sanger et al., 1985). This organelle contains two microtubule bundles (McIntosh and Landis, 1971), which interdigitate by the microtubule plus ends, forming the midbody (Euteneuer and McIntosh, 1980). A faint staining of the midbody by rabbit polyclonal antibodies raised against human γ-tubulin has been reported, but the significance of this observation has been questioned (Zheng et al., 1991). Disruption of the gene coding for γ-tubulin, in both *Aspergillus* (Oakley et al., 1990) and *Schizosaccharomyces* (Horio et al., 1991), or micro-injection of γ-tubulin antibodies into mammalian cells prevented the nucleation of the mitotic spindle. These observations and the association of γ-tubulin with the minus end region of microtubules (Oakley, 1992) suggest that γ-tubulin plays a role in microtubule nucleation. Thus, the presence of γ-tubulin in the intercellular bridge linking daughter cells is potentially of interest in order to determine the origin of the microtubule skeleton characteristic of this organelle.

In mammalian cells, we show that γ-tubulin is associated with the microtubule bundles forming the midbody. In anaphase it is absent from the interzone located between the two separating sets of chromosomes and was observed to be associated with the minus ends of the microtubule bundles constituting the midbody in telophase. The maximum amount of γ-tubulin at the ends of the midbody is reached during the early part of cytokinesis. Gamma-tubulin becomes undetectable when the intercellular bridge elongates between the two separating cells. In agreement with these observations, micro-injection of γ-tubulin antibodies during the anaphase stage prevents the subsequent formation of the midbody between the two separating cells.

**SUMMARY**

Animal cells undergoing cytokinesis form an intercellular bridge containing two bundles of microtubules interdigitated at their plus ends, which constitute the midbody. Polyclonal antibodies raised against three specific amino acid sequences of γ-tubulin (EEFATEGGDREKV, NIQGEADPTDVHKSL and EYHAATRPDYISWGTQEQ) specifically stained the centrosome in interphase, the spindle poles in all stages of mitosis, and the extremities of the midbody in mammalian cells (*Potorous* human, Chinese hamster, mouse). This staining was prevented by the corresponding peptides, by Xenopus γ-tubulin, but was not modified by purified αβ-tubulin heterodimer. An identical staining was obtained with affinity-purified antibodies against the carboxyl-terminal amino acid sequence of human γ-tubulin. No γ-tubulin could be detected in the interzone during anaphase and early telophase. Material containing γ-tubulin first appeared in the two daughter cells on each side of the division plane in late telophase, and accumulated transiently at the minus ends of the two microtubule bundles constituting the midbody for one hour after metaphase. Micro-injection of γ-tubulin antibodies into anaphase cells prevented the subsequent formation of the microtubule bundles between the two daughter cells. In contrast with previous views, these observations suggest that the microtubules constituting the midbody may be nucleated on special microtubule organizing centres, active during late telophase only, and assembled on each side of the dividing plane between the daughter cells.

Key words: tubulin, microtubules, MTOC, microtubule organizing centre, mitosis, midbody, cytokinesis, micro-injection
MATERIALS AND METHODS

Peptide synthesis
Synthesized peptides (Fig. 1; Applied Biosystem 430 A, on 4-(oxy-methyl) phenylacetamido methyl resin) (Merrifield, 1963, 1964) have been purified by HPLC chromatography (Applied Biosystem 151, column Aquapore RP300, C8, 100 mm x 10 mm, 0.1% trifluoroacetic acid and elution by a linear gradient of 30% to 70% CH3CN in 0.1% trifluoroacetic acid at 3.5 ml/min for 40 min). Peptide purity has been checked by analytical HPLC (Column Aquapore RP 300, C8, 220 mm x 4.6 mm), and the mass of their protonated molecular ions has been determined (Fast Atom Bombardment, ZAB-HS double focusing with a VG analytical mass spectrometer, Manchester, UK): (Y)38-50 MH+ = 1615.5, (Y)323-338 MH+ = 1899.7, and 435-452 MH+ = 2155.

Immunization procedure
Peptides (5 mg) were coupled to 7 mg keyhole limpet haemocyanin (peptide 435-452 in 5 ml of 0.1 M NaHCO3, pH 8.4, by overnight incubation with 12 µl of 25% glutaraldehyde; and peptides (Y)38-50 and (Y)323-338 in 3 ml 0.16 M borate buffer, pH 8.7, with 0.6 ml of 24 mM bis-diazoo-O-tolidine for 4 h at 0°C, and dialysed for 24 hours against 0.15 M NaCl. Rabbits were repeatedly injected with 200-250 µg of peptide (≈ 0.5 mg of protein) in 50% complete Freund’s adjuvant (Calbiochem) and the titers of the sera were followed by fluorescence-labeling of PtK2 cells (Potoroid tridactylist).

Immunoblotting
PtK2 cells, resuspended in 10 mM PIPES (1,4-piperazine diethane sulfonic acid), pH 8.0, 50 mM EDTA (ethylenediamine tetraacetic acid), 0.1% Triton X-100, 0.25 M NaCl, 0.1 mM PMSF (phenylmethylsulfonyl fluoride), 1 µg/ml leupeptin, 50 µg/ml TLCK (Nα-tosyl-L-lysine chloro-methyl ketone), 100 µg/ml TPCK (N-tosyl-L-phenylalanine chloromethyl ketone), 1 µg/ml pepstatin, 1 µg/ml chymostatin, 1 mM diithiothreitol, were disrupted by sonication, and the extract was centrifuged for 5 min at 15,000 r.p.m. After denaturation, the proteins were submitted to SDS-PAGE (7.5% gel), electro-transferred to a nitrocellulose membrane (Hybond C. Amersham), and immunolabeled (Enhanced chemo-luminescence western blotting detection system, Amersham). Apparent molecular masses of the polypeptides labeled by the antibodies were determined by comparison with molecular mass markers: bovine carbonic anhydrase (31 kDa); ovalbumin (45 kDa); brain tubulin (50 kDa); bovine serum albumin (66 kDa) and phosphorylase b (97 kDa).

Immunofluorescence
Cells fixed for 60 min in 2% formaldehyde in PBS, were washed (3×5 min in PBS), incubated for 60 min in 2% glueine in PBS and washed. After 6 min in acetone (~20°C) and washing, cells were reacted for 90 min at 37°C with primary antibodies (in PBS with 25% fetal calf serum), washed (2×10 min with 0.05% Tween 20 in PBS and once in PBS). The staining was revealed with goat anti-rabbit or anti-rat antibodies (Nordic) in PBS with 40% fetal calf serum. Both simple labeling of γ-tubulin (with fluorescein or rhodamin-labeled secondary antibodies) and double labeling of γ- and α-tubulin (with fluorescein and rhodamin-labeled secondary antibodies, respectively, or the reverse combination) were performed. Nuclei and chromosomes were stained with 4′,6-diamidino-2-phenyindole (0.2 µg/ml; DAPI). Preparations were dried, mounted in Mowiol (Rodriguez and Deinhardt, 1960), sealed, and observed by epifluorescence with a Zeiss Axioshot equipped with 40× and 100× Plan-neofluar objectives (NA: 0.90 and 1.30, respectively), an Optovar varying from 1.25× to 2.5×, a 4× TV camera adaptor and a stabilized exciting beam. Images recorded in the linear dynamic range by a Nocticool camera (LH 4015 from Lhesa), were digitized (50-200 frames) with an image processing system (Sapphire from Quantel) and recalculated, without subtracting the fluorescence background, using a linear function (‘stretch’ program). Fluorescence intensities (‘luminance’ program) and organelle surfaces (‘threshold’ program) were determined in order to calculate integrated fluorescence values for each organelle. Cells grown on photoetched coverslips (Bellco Glass), fixed at different times after early anaphase, and processed for fluorescence staining, were used to determine the morphogenetic evolution of the midbody.

Electron microscopy
PtK2 cells were washed in 80 mM PIPES, pH 6.9, 5 mM EGTA, 1 mM MgCl2 (PEM), permeabilized for 5-15 s in PEM with 0.1-0.5% Triton X-100, washed (3 min in PEM), fixed (PEM with 1% glutaraldehyde, 30 min), and incubated with 2% glycine (2×30 min). Then, cells were treated for immunocytochemistry (immune serum from rabbit 75, 1/500, 37°C, 3 hours; gold-labeled secondary antibody, 37°C, 90 min), post-fixed with glutaraldehyde (2%, 14 hours) and processed for electron microscopy.

Fig. 1. Amino acid sequences used to prepare γ-tubulin polyclonal antibodies. Synthesized sequences are indicated by asterisks. The numbers correspond to human γ-tubulin. Asterisks indicate identical amino acid residues (Villasante et al., 1986; Wang et al., 1986; Oakley and Oakley, 1989; Zheng et al., 1991). Aspergillus γ-tubulin sequence 38-50, used to inject rabbit 38, showed 92% identity with human γ-tubulin, but 8 and 23% identity with α- and β-tubulin. Aspergillus γ-tubulin sequence 323-338, used to inject rabbit 70, showed 81% identity with human γ-tubulin, but 31 and 12% identity with α- and β-tubulin. Human γ-tubulin carboxyl-terminal amino acid sequence, used to inject rabbits 78 and 75 showed 9% identity with α- and β-tubulin. The underlined amino acids correspond to parts of the human γ-tubulin sequences most likely located at the surface of the molecule (Gaboriaud et al., 1987). A tyrosine residue (Y) has been added to the amino terminus of the oligopeptides 38-50 and 323-338 in order to allow their coupling to the carrier protein.
Micro-injections
Immunoglobulins (14 mg/ml) from the immune serum 70 (sequence 323-338) were purified with Protein A immobilized on agarose beads (Pierce) and centrifuged before micro-injection (Airfuge Beckman, 4°C, 5 min, 18 lbf/in²; 1 lbf/in² ~ 6.9 kPa, 75,000 r.p.m.). PtK₂ cells were micro-injected in early anaphase using a Zeiss inverted microscope (Axiovert with a 32× Achrostigmat phase-contrast objective, NA 0.40) equipped with a heating stage, a micromanipulator and a micro-injector Eppendorf (40-50 kPa for 0.3 s). After a further incubation of 30 min, cells were fixed and processed to label the microtubule cytoskeleton (YL1/2 rat monoclonal antibody; Kilmartin et al., 1982) and chromatin (DAPI).

RESULTS
Specificity of γ-tubulin antibodies
Polyclonal antibodies were prepared against three specific and distinct sequences of γ-tubulin (Fig. 1). All immune sera failed to detect pure sheep brain α- or β-tubulin after immunoblotting. Except for the immune serum raised against the sequence 38-50 (rabbit 38), all three other immune sera specifically revealed a polypeptide chain with an apparent molecular mass of 46 kDa in PtK₂ cell extracts (Fig. 2), in agreement with the reported value for human γ-tubulin (Zheng et al., 1991). However, the immune sera from rabbit 70 and rabbit 78 specifically labeled additional polypeptides (Fig. 2). The serum obtained from rabbit 75, against the carboxyl-terminal amino acid sequence of human γ-tubulin, showed the best specificity and stained a single polypeptide chain of 46 kDa.

Presence of γ-tubulin in the intercellular bridge
PtK₂ cells were stained by the immune serum against the carboxyl amino acid sequence of human γ-tubulin (rabbit 75) and the corresponding antibodies purified on a peptide-Sepharose affinity column. Staining labeled the centrosome during interphase and the spindle poles during all stages of mitosis, in agreement with the previously published localization (Stearns et al., 1991; Zheng et al., 1991). Furthermore, both serum- and affinity-purified antibodies labeled some, but not all, midbodies (Fig. 3A,G), in contrast with previous reports. The staining of the centrosome, spindle poles and midbody was specific. No staining was observed with the pre-immune serum (Fig. 3B) or when the immune serum was pre-incubated with the peptide 435-452 (Fig. 3C), or with Xenopus γ-tubulin (compare Fig. 3D and E). In contrast, the staining was not modified when the immune serum was pre-incubated with the carrier protein or with purified sheep brain αβ-tubulin (Fig. 3F).

The comparative location of γ- and α-tubulin in the intercellular bridge has been followed in PtK₂ cells by double immunolabeling using the immune serum from rabbit 75 and a rat monoclonal antibody against α-tubulin (Kilmartin et al., 1982). In both cases, the fluorescent staining was symmetrical, in agreement with the ultrastructure of this organelle. However, the two staining patterns were different (Fig. 4). In early telophase, γ-tubulin was present over a large area located in each daughter cell. This area was coincident with most but not all the microtubule-rich region of the intercellular bridge: the zone devoid of detectable γ-tubulin was larger than the zone corresponding to the interdigitated midbody microtubules (Fig. 4, upper panel). Later, in typical midbody, the restriction of γ-tubulin labeling to the two minus regions of the microtubule bundles was more striking (Fig. 4, middle panel). This location of γ-tubulin is further supported by the observation that the maximum intensity of each peak of γ-tubulin did not coincide with the maximum intensity of α-tubulin labeling. The maxi-
mum intensity of γ-tubulin labeling corresponded to 76% and 52% of the maximum α-tubulin staining in early midbody (Fig. 4, upper panel) and in typical midbody (Fig. 4, middle panel), respectively. A relocation of γ-tubulin and/or a sliding and growth of midbody microtubules could account for these observations. In tripolar dividing cells, the three extremities of the midbody were labeled (Fig. 3H), in agreement with the overall shape of the midbody in these cells (Keryer et al., 1984). Thus, in all cases, the location of γ-tubulin in the intercellular bridge was preferentially restricted to the minus regions of the microtubule bundles. These observations were confirmed by electron microscopic immunolocalization of γ-tubulin using a secondary antibody labeled with gold particles (Fig. 5). No labeling was detected over most of the microtubule bundles (less than 1% of gold particles). Most of the gold particles (89%) were located in the two terminal minus regions of the microtubule bundles, both at and between the microtubule extremities. Moreover, 11% of gold particles were present in the outer region of the microtubule bundles, presumably revealing shorter microtubules diverging from the main bundles. This overall ultrastructural location of γ-tubulin in the minus region of microtubules is consistent with previous reports (Oakley et al., 1990; Horio et al., 1991; Stearns et al., 1991; Zheng et al., 1991). However, it is not possible to decide whether γ-tubulin is strictly located at the
Transient presence of \(\gamma\)-tubulin in the midbody

In PtK\(_2\) cells undergoing anaphase and early telophase, interzonal microtubule bundles labeled with \(\alpha\)-tubulin antibodies were present between the two masses of chromosomes (Fig. 6A and B, right), but the staining of \(\gamma\)-tubulin was negligible in the interzone (Fig. 6A and B, left, and Table 1). When cells progressed into telophase, interzonal microtubule bundles accumulated in the constriction plane (Fig. 6C, right), but did not seem to interdigitate, since there was no interruption of their staining by antibodies against \(\alpha\)-tubulin. The diffuse fluorescent staining that was observed with \(\gamma\)-tubulin antibodies on each side of the cleavage furrow increased 15 min after the end of metaphase (Fig. 6C, left) and its overall fluorescence was 2-4 times higher than the fluorescence detected over the centrosome (Table 1C). But most microtubule bundles did not extend from the division plane to these \(\gamma\)-tubulin-rich granules and no interruption of the microtubule staining was observed in the cleavage furrow (Fig. 6C, right). Then, microtubules interdigitated between the two daughter cells, as shown by the presence in the division plane of an area unstained by \(\alpha\)-tubulin antibodies (Fig. 6D, right). At this stage, they were apparently in contact with the granules stained by \(\gamma\)-tubulin antibodies, which increased in number (Fig. 6D, left), and constituted a large area of apparently filamentous structure (Fig. 6E-G, left). The spindle poles remained labeled by \(\gamma\)-tubulin antibodies and the fluorescent staining of \(\gamma\)-tubulin on the extremities of the midbody was twice that observed on the centrosomes (Table 1). When the intercellular bridge began to elongate between the two daughter cells, i.e. from 45-60 min after the end of metaphase, the areas stained by \(\gamma\)-tubulin antibodies were restricted to two small regions on each side of the midbody (Fig. 6H, left). Thus, \(\gamma\)-tubulin-rich material seemed to accumulate in the two daughter cells mostly independently from the interzonal microtubule bundles present in the cleavage furrow, and interacted with the microtubule bundles present in the intercellular bridge only when a typical midbody was assembled.

In later stages, the staining of \(\gamma\)-tubulin remained restricted to two small dots (Fig. 6I, left), although the mid-
body itself was strongly labeled by α-tubulin antibodies (Fig. 6I, right). In the same cells, the intensity of the fluorescence labeling of γ-tubulin remained high at the centrosome level (Table 1). Eventually, from 45-60 min after the end of metaphase, as midbody microtubules elongated in the intercellular bridge (Fig. 6J, right), γ-tubulin was no longer detectable at the extremities of this organelle (Fig. 6J, left) although it remained present in the centrosome (Fig. 6J, left, arrowhead, and Table 1). Thus, γ-tubulin was present in the microtubule bundles constituting the midbody from 15 min to 45-60 min after the end of metaphase. But, as the intercellular bridge elongated between the two daughter cells, 45-60 min after the end of metaphase, γ-tubulin was no longer detectable, although a typical midbody was present for 3 hours after cytokinesis and γ-tubulin was detectable in the centriolar region.

Effects of γ-tubulin antibodies on the assembly of the midbody

γ-Tubulin present at the extremities of the microtubule bundles of the midbody could either be linked to already assembled microtubules, nucleate new microtubules, or interact with previously or newly assembled microtubules. Antibodies directed against the sequence 323-338 of γ-tubulin were injected into PtK2 cells undergoing anaphase. After a 30 min incubation, the microtubule cytoskeleton and chromatin were labeled in order to determine whether the injection prevented the formation of the midbody. All control non-injected cells showed a typical midbody 30 min after early anaphase, although the timing of the morphogenesis of this organelle differed somewhat from cell to cell (Schulze and Blose, 1984). The injected cells could be classified in two groups. In the first group, cells failed to progress further into mitosis (Table 2): their chromosomes remained condensed and the microtubule cytoskeleton was either blocked in early telophase or highly disturbed (Fig. 7A and B). In the other group, cells progressed towards cytokinesis (Table 2) exhibiting various stages of reconstruction nuclei (Fig. 7C and D). These distinct actions could reflect both the injection traumatism (16 % of the cells were blocked by buffer injection) and the uncertainty concerning the exact time of micro-injection, since anaphase is the most dynamic and shortest period of mitosis. Most of the cells progressing towards cytokinesis did not exhibit a midbody (Table 2; Fig. 7D), in contrast to cells micro-injected with buffer or γ-tubulin antibodies pre-incubated with the peptide 323-338 (10 mg/ml, 2 hours, 37°C). These observations suggest not only that γ-tubulin is transiently present at the extremities of the microtubule bundles of the midbody, but also that it plays a role in the formation of this organelle.

DISCUSSION

Using antibodies directed against γ-tubulin we demonstrate
that this protein is not restricted to the centrosome and the spindle poles in mammalian cells, but is also transiently present at the extremities of the microtubule bundles of the midbody during cytokinesis. It has been emphasized that the staining of a subcellular structure by antibodies raised against a specific protein sequence may be misleading, due to cross-reactivity of the antibodies with different but similar sequences present in unrelated proteins (Nigg et al., 1982). In order to avoid this problem we used four distinct polyclonal antibodies prepared against three different and specific epitopes of γ-tubulin, targeted against a peptide corresponding to the sequence 38-54 (Joshi et al., 1992), which overlaps one of the peptides (sequence 38-50) that we have used. In PtK2 cells the intensity of the fluorescent staining of γ-tubulin on the extremities of the midbody was equal to, or even higher than, that observed on the centrosomes in the same cells, but was lower in the midbodies of human and Chinese hamster cells. The transient location of γ-tubulin at the extremities of the midbody, the lower staining intensity observed in some cell types, and the variation of this labeling with the different sera, are all possibilities that could account for the difficulties of observing the presence of γ-tubulin in this organelle.

The origin of the microtubule bundles constituting the midbody is unclear. Numerous proteins co-localize at the level of this organelle. Some proteins have been reported to be present both in the centrosome and in the midbody (Bastmeyer and Russell, 1987; Tousson et al., 1991). In most cases, their role, if any, is still unknown (Tooze and Burke, 1987; Schollmeyer, 1988; He et al., 1991; Zhou et al., 1991). Microtubules constitute the major skeleton of the midbody (McIntosh and Landis, 1971). It has been suggested (McIntosh and Landis, 1971; McIntosh et al., 1975) that they could be remnants of interpolar microtubules (Fig. 8, left). Alternatively (Salmon et al., 1976), they could result from the transient nucleating activity of special microtubule organizing centres distinct from the centrosomes (Fig. 8, right). Both possibilities account for the orientation of the microtubules in the midbody, since their minus ends are directed towards the interzonal microtubule bundles localized at the level of the midbody (McIntosh and Landis, 1971). In this model, microtubules, nucleated at the two poles of the mitotic apparatus, are expected to leave the polar regions, then aggregate and interdigitate to constitute the midbody (Fig. 8, left). According to microtubule polarity (Euteneuer and McIntosh, 1981), γ-tubulin would be present at the minus ends of stable migrating microtubules during anaphase and telophase stages. We failed to detect γ-tubulin in the interchromosomal region in late anaphase and early telophase cells. The asynchronous migration of interzonal microtubules could perhaps prevent the detection of significant fluorescence over the background. However, no γ-tubulin was present on the extremities of the interzonal microtubules located in the cleavage furrow. Our observations support the second possibility (Fig. 8, right). In this model, the interzonal microtubule bundles would not be responsible for the formation of the midbody. The extremities of the interzonal microtubule bundles localized at the level of

### Table 1. Comparison of the fluorescence labeling of γ-tubulin in cells undergoing cytokinesis

<table>
<thead>
<tr>
<th>Stage of cytokinesis</th>
<th>Pictures in Fig. 6</th>
<th>Relative peak values*</th>
<th>Relative integrated values (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Centrosome Midbody‡</td>
<td>Centrosome Midbody‡</td>
</tr>
<tr>
<td>Anaphase</td>
<td>–</td>
<td>100 34 79</td>
<td>49 0 51</td>
</tr>
<tr>
<td>Early telophase</td>
<td>A</td>
<td>100 45 and 52 99</td>
<td>42 0 and 0 58</td>
</tr>
<tr>
<td>Telophase without midbody</td>
<td>–</td>
<td>100 71 and 77 80</td>
<td>14 30 and 36 9</td>
</tr>
<tr>
<td>Telophase with early midbody</td>
<td>C</td>
<td>100 98 and 93 83</td>
<td>12 48 and 30 11</td>
</tr>
<tr>
<td>Daughter cells with typical midbody</td>
<td>F</td>
<td>100 72 and 74 63</td>
<td>19 33 and 36 11</td>
</tr>
<tr>
<td>Daughter cells with elongated midbody</td>
<td>H</td>
<td>100 80 and 57 89</td>
<td>20 29 and 25 25</td>
</tr>
<tr>
<td>Daughter cells with highly elongated midbody</td>
<td>I</td>
<td>100 51 and 51 70</td>
<td>50 18 and 10 21</td>
</tr>
</tbody>
</table>

*The maximum value of fluorescence recorded in each cell was used as a reference, equal to 100, in order to recalculate the other maximal fluorescence values on the centrosomes and midbody extremities.
†The sum of the integrated fluorescence values obtained on the two centrosomes and the two extremities of the midbody was used as a reference, equal to 100, in order to recalculate the integrated fluorescence values corresponding to each of these organelles.
‡Interzonal region in anaphase, region located on each side of the interconnecting bridge in early telophase (A), and midbody thereafter.

### Table 2. Effects of micro-injection of γ-tubulin antibodies into PtK2 cells during anaphase

<table>
<thead>
<tr>
<th>Cell phenotype 30 min after micro-injection of:</th>
<th>Buffer</th>
<th>Purified antibodies from rabbit 70</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–</td>
<td>– peptide + peptide</td>
</tr>
<tr>
<td>Total number of microinjected cells</td>
<td>42</td>
<td>124 42</td>
</tr>
<tr>
<td>Cells failing to proceed towards cytokinesis</td>
<td>7 (16%)</td>
<td>40 (32%) 10 (24%)</td>
</tr>
<tr>
<td>Cells progressing towards cytokinesis (A+B)</td>
<td>35 (84%)</td>
<td>84 (68%) 32 (76%)</td>
</tr>
<tr>
<td>A with midbody</td>
<td>29</td>
<td>28 22</td>
</tr>
<tr>
<td>B without midbody</td>
<td>6</td>
<td>56 10</td>
</tr>
<tr>
<td>B/(A+B)</td>
<td>17%</td>
<td>67% 31%</td>
</tr>
</tbody>
</table>
Fig. 6. Location of γ-tubulin during the morphogenesis of the intercellular bridge. The immune serum from rabbit 75 (1:1000) was used to detect γ-tubulin (left column), while microtubules (right column) were stained with a monoclonal antibody against α-tubulin (Kilmartin et al., 1982). Secondary antibodies were labeled with rhodamine and fluorescein, respectively. (A) Early telophase stages (chromosomes are still condensed and distinct). (B-F) Stages of cytokinesis showing the appearance of γ-tubulin on both sides of the cleavage furrow. The chromosomes are no longer distinguishable and form crescent daughter nuclei. (G-I) Successive stages of the elongation of the intercellular bridge. Gamma-tubulin is located to the minus extremities of the microtubule bundles. The midbody is
characterized by the interdigititation of the microtubules bundles and the subsequent absence of staining of α-tubulin (arrows). In (G) and (H), nuclei are grossly spherical, but their surface appears irregular. (J) Late stage of cytokinesis with elongated midbody devoid of γ-tubulin. The nuclei are indistinguishable from typical interphase nuclei. In all cases, centrosomes are stained by γ-tubulin antibodies. From anaphase to early cytokinesis, the two centrioles remain undistinguishable (A-H). In late stages of cytokinesis, the two centrioles (arrowheads) can be distinguished (IJ). Relative quantification of the fluorescence staining of γ-tubulin is given in Table 1 for (A,C,F,H, and I).
the division furrow in telophase are apparently not linked to the area stained by γ-tubulin antibodies; they would disassemble in agreement with their reported unstability (Salmon et al., 1976), while γ-tubulin would participate in the nucleation of the new microtubules forming the midbody (Alfa and Hyams, 1991; Oakley, 1992). This view accounts for several observations. First, there is a gradual accumulation and organization of γ-tubulin-containing material on each side of the division plane. Second, the interzonal microtubule bundles initially present between the two daughter cells do not interact with γ-tubulin-rich material. Third, γ-tubulin is present in the extremities of the

**Fig. 7.** Effects of micro-injections of γ-tubulin antibodies in anaphase cells. PtK2 cells, undergoing anaphase, were micro-injected with purified antibodies (immune serum from rabbit 70) and were fixed 30 min later. Left column: fluorescence labeling of the microtubule cytoskeleton (α-tubulin); right column: fluorescence labeling of DNA. All images have been recalculated with a non-linear function (histogram program from Quantel) in order to enhance the staining of the different parts of the microtubule cytoskeleton. (A and B) Cells prevented from proceeding towards cytokinesis. Chromosomes are still condensed. In (B) the spindle is multipolar and chromosomes form four distinct aggregates. (C) Cell reaching cytokinesis with partially decondensed chromosomes and a midbody that exhibits its most frequent appearance 30 min after early anaphase. (D) Cell reaching cytokinesis without a midbody. The two daughter cells do not possess a normal microtubule cytoskeleton, but the chromosomes are forming two typical reconstruction nuclei.
accumulate between the two daughter cells where they have been omitted. The first hypothesis is described in the left part of the figure. Microtubules with their nucleating material leave the centrosomes, could play a role during cytokinesis. In Schizosaccharomyces, cytoplasmic microtubules are nucleated from microtubule organizing centres located in the mitotic spindle. Their plus ends are directed towards the two daughter cells. These observations demonstrate that the subcellular location of γ-tubulin varies during the cell cycle, and suggest that γ-tubulin could be a universal element of all microtubule organizing centres.

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REFERENCES


Fig. 8. Origin of midbody microtubules. The nucleating material containing γ-tubulin is indicated by black dots; microtubules by straight lines and centrioles by open rectangles. Chromosomes are shown as solid triangles and centrosomes by open rectangles. Chromosomes have been omitted. The first hypothesis is described in the left part of the figure. Microtubules with their nucleating material leave the mitotic poles and interact in the interzonal region to form the midbody. The second hypothesis is described in the right part of the figure. During early cytokinesis, the interzonal microtubules accumulate between the two daughter cells where they disassemble. The nucleating material aggregates in the two daughter cells on each side of the division plane, and then nucleates new microtubules constituting the midbody.


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