Centriole Disassembly In Vivo and Its Effect on Centrosome Structure and Function in Vertebrate Cells

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Abstract. Glutamylation is the major posttranslational modification of neuronal and axonemal tubulin and is restricted predominantly to centrioles in nonneuronal cells (Bobinnec, Y., M. Moudjou, J.P. Fouquet, E. Desbruyères, B. Eddé, and M. Bornens. 1998. Cell Motil. Cytoskel. 39:223–232). To investigate a possible relationship between the exceptional stability of centriole microtubules and the compartmentalization of glutamylated isoforms, we loaded HeLa cells with the monoclonal antibody GT335, which specifically reacts with polyglutamylated tubulin. The total disappearance of the centriole pair was observed after 12 h, as judged both by immunofluorescence labeling with specific antibodies and electron microscopic observation of cells after complete thick serial sectioning. Strikingly, we also observed a scattering of the pericentriolar material (PCM) within the cytoplasm and a parallel disappearance of the centrosome as a defined organelle. However, centriole disappearance was transient, as centrioles and discrete centrosomes ultimately reappeared in the cell population.

During the acentriolar period, a large proportion of monopolar half-spindles or of bipolar spindles with abnormal distribution of PCM and NuMA were observed. However, as judged by a quasinormal increase in cell number, these cells likely were not blocked in mitosis.

Our results suggest that a posttranslational modification of tubulin is critical for long-term stability of centriolar microtubules. They further demonstrate that in animal cells, centrioles are instrumental in organizing centrosomal components into a structurally stable organelle.

Key words: centrosome • centriole • tubulin polyglutamylation • mitosis • mitotic spindle

The centrosome was discovered at the start of modern cell biology over a century ago (Boveri, 1901), but it has remained largely resistant to molecular investigation (for recent reviews see Paoletti and Bornens, 1997; Stearns and Winey, 1997). Because of this, the two major properties of the centrosome, i.e., its capacity to reproduce by duplication and its ability to nucleate microtubules, are still poorly understood.

Within the centrosomes of animal cells, the centriole pair remains without a defined role. The only well-established function of the centriole is to act as a template for the growth of the primary or motile cilium axoneme (Rieder and Borisy, 1982). The absence of centrioles in the centrosome from other eukaryotic organisms has led to the dominant view that the centrosome pair is not relevant to centrosome activity. This view is also based on the fact that centrioles can be dispensable for spindle assembly, for example during the female meiosis in some species (Szöllösi et al., 1972; Calarco-Gilliam et al., 1983; Theurkauf and Hawley, 1992), or even in an established Droso phila cell line (Debec et al., 1982). However, several results support the alternative view. First, isolated centrosomes from somatic cells reveal that the centrosomal matrix tightly binds to the proximal wall and the proximal end of both centrioles and links them together (Bornens et al., 1987; Paintrand et al., 1992). Recent experiments suggest that NuMA redistribution at the onset of mitosis, which is essential for spindle pole stabilization (Gaglio et al., 1997), depends upon the correct segregation of pericentriolar material (PCM) between centriole pairs at the

1. Abbreviations used in this paper: IVEM, intermediate voltage electron microscopy; MAP, microtubule-associated protein; PCM, pericentriolar material.
onset of mitosis (Paoletti et al., 1997). Second, the ability of centrioles to replicate by orthogonal budding (Robbins et al., 1968; Kuriyama and Borisy, 1981; Kochanski and Borisy, 1990) has long been postulated to be essential for centrosome continuity. In agreement with this view, the reproductive capacity of centrosomes in sea urchin eggs depends upon the number of centrioles present (Sluder and Rieder, 1985a,b), and cells artificially deprived of centrioles by microsurgery cannot reform centrioles (Maniotis and Schliwa, 1991). Similar conclusions were drawn from experiments designed to identify the centrosome-associated activity responsible for triggering parthenogenetic development in Xenopus (Klotz et al., 1990).

However, centriole biogenesis other than by parental budding exists, for example, during differentiation of ciliated cells (Sorokin, 1968), at the beginning of development in parthenogenetic species (for a review see Beatty, 1967) or at the blastocyst stage of mouse embryo (Maro et al., 1985; Schatten et al., 1986). There are also numerous examples of so-called de novo assembly of centrioles in unicellular organisms, such as the amoeboflagellate Naegleria (Dingle and Fulton, 1966), or multicellular organisms like the fern Marsilea during spermatogenesis (Mizukami and Gall, 1966). The molecular basis of centriole generation in these cases as well as in the classical duplication pathway is still largely unknown.

Microtubules of centrioles are highly stable structures that resist all depolymerizing agents. This particular subset of microtubules shares with basal bodies and axonemes a large number of tubulin modifications, such as acetylation (Piperno and Fuller, 1985), detyrosination (Gundersen and Bulinski, 1986), and glutamylation (Eddé et al., 1990; Bobinnec et al., 1998). This latter modification consists in the formation of a lateral chain of glutamate units linked to a glutamate residue near the COOH terminus of both α- and β-tubulin.

In this paper, we investigate the relationship between glutamylation and centriole stability. Such an investigation was motivated by two sets of data. First, it has been demonstrated that the glutamate lateral chain acts as a regulator for the binding of microtubule-associated proteins (MAPs) and motors to microtubules (Boucher et al., 1994; Larcher et al., 1996). Second, antibodies directed against glutamylation of tubulin have been shown to block flagellar motility of reactivated sperm axonemes, probably through the inhibition of microtubule–dynein binding (Gagnon et al., 1996). The polyglutamate side chain is thus likely to be essential for the interaction of these highly stable microtubule subsets with stabilizing factors or with molecular motors.

We have introduced an antiglutamylated tubulin antibody into mammalian cells and checked for a possible effect on centrioles. We observed a complete disappearance of centrioles and the scattering of the associated pericentriolar material. This disappearance was transient, as centrioles reappeared and a centrosome reformed. During the acentrosomal phase, cells displayed no significant abnormalities of their microtubule network in interphase and apparently proceeded through mitosis with disorganized spindles. Our results confirm that centrioles act as organizers for pericentriolar material. Thus, the experimental system developed in this work provides a favorable situation to investigate the continuity of the centrosome as well as the function of centrioles during spindle pole assembly and mitotic progression.

Materials and Methods

Cell Culture and Cell Cycle Analysis

KE37 cell line was grown in RPMI 1640 medium containing 7% fetal calf serum, 2 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. HeLa cells were maintained in DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin, and streptomycin. Cells were grown in a humidified incubator with a 5% CO2 atmosphere at 37°C.

Microtubule Regrowth In Vivo

Microtubules were depolymerized by incubation of the cells with 5 μM nocodazole for 2 h on ice. Cells were washed thoroughly with fresh medium at 4°C to eliminate nocodazole before incubation at 37°C with prewarmed medium. Microtubules were allowed to grow for 2 or 15 min, and then cells were extracted in PHEM buffer (45 mM Pipes, 45 mM Hepes both adjusted to pH 6.9, 10 mM EGTA, 5 mM MgCl2, and 1 mM PMSF) containing 0.7% Triton X-100 and fixed by cold methanol.

Indirect Immunofluorescence Microscopy

Cells grown on coverslips were washed in PBS and routinely fixed in methanol −20°C for 7 min. For a better visualization of centrosomes by immunofluorescence, cells on coverslips were incubated with 5 μM nocodazole for 2 h on ice, extracted in PHEM buffer containing 0.7% Triton X-100 to remove soluble tubulin, and then fixed by cold methanol (−20°C) for 7 min. Visualization of microtubule cytoskeletons was enhanced by extraction of the cells in PHEM–Triton X-100 0.7% before cold methanol fixation.

After fixation, coverslips were washed in PBS containing 0.1% Tween 20. Primary antibodies diluted in PBS containing 3% BSA were added for 30 min at room temperature. Coverslips were then washed in PBS-Tween 0.1%. The same procedures were used for fluorescein- or rhodamine-labeled secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). Coverslips were dehydrated in ethanol and mounted in Citifluor (City University, London, UK). Centrosome preparations, obtained as described (Moudjou and Bornens, 1994), were centrifuged on coverslips and fixed in −20°C methanol for immunofluorescence procedure. Preparations were visualized on a Leica DMRD microscope (Leica, Inc., Heidelberg, Germany) and images recorded with a CCD camera (Hamamatsu, Japan).

Antibodies

GT355 is a monoclonal antibody raised against a synthetic glutamylated peptide and has been shown to react specifically with polyglutamylated α- and β-tubulin (Wolff et al., 1992). Anti-γ-tubulin polyclonal antibody was raised against bacterially expressed human γ-tubulin (Tassin et al., 1997). Antiacentrin polyclonal antibody 26-14.2 was generously given by Dr. J. Salisbury (Mayo Clinic, Rochester, MN) (Baron et al., 1992). Anti-Hsc70 and Anti-HsCen3 protein (Middendorp et al., 1997; Tassin et al., 1998).

Anti-NuMA serum is an autoimmune human antibody. Anti–α- and –β-tubulin monoclonal antibodies (clones DM1A and DM1B, respectively) were obtained from Amersham International (Little Chalfont, UK). Monoclonal antibody 1-6.1 specific for acetylated tubulin (Thompson et al., 1983) was a kind gift from Dr. L. Paturle-Lafanechère (Centre d’Etudes Nucleaires, Grenoble, France) (Paturle-Lafanechère et al., 1994). Purified nonimmune human IgGs were purchased from ICN Pharmaceuticals (Costa Mesa, CA). L3 rabbit serum specific for acetylated α-tubulin (Thompson et al., 1984) and monoclonal anti-NuMA antibody were purchased from ICN Pharmaceuticals (Costa Mesa, CA). L3 rabbit serum specific for deacetylated α-tubulin is a kind gift from Dr. L. Patatour-Lafanechère (Centre d’Etudes Nucleaires, Grenoble, France) (Patatour-Lafanechère et al., 1994).

Centrosomes were detected by rabbit serum No. 013 (Gosti et al., 1986). Polyclonal antibodies (clones DM1A and DM1B, respectively) were obtained from Amersham International (Little Chalfont, UK). Monoclonal antibody 1-6.1 specific for acetylated α-""
For microinjection and electroporation, pure 1-6.1 immunoglobulins were prepared by the caprylic acid method, and GT335 IgGs were affinity-purified on brain tubulin bound to a Hi-Trap column (Pharmacia Biotech, Piscataway, NJ). Purified GT335 IgGs, 1-6.1, and human immunoglobulins were used at 5 mg/ml for microinjection experiments. Immunoglobulins from the L3 serum were microinjected at 13 mg/ml. For microinjection and electropermeabilization, pure 1-6.1 immunoglobulins were concentrated at 10 mg/ml and dialyzed against electropermeabilization buffer containing 50 mM NaCl to avoid antibody precipitation. Final IgG concentration was brought to 6 mg/ml by addition of the cell suspension.

**Protein Analysis**

One-dimensional SDS-PAGE was performed according to Laemmli (1970) using 8% polyacrylamide gels. Transfer of proteins onto nitrocellulose membranes was performed as described (Towbin et al., 1979). After electrodtransfer, nitrocellulose sheets were stained with Ponceau red to register the location of proteins and saturated for 1 h in PBS-Tween 0.1%.

Protein content was determined according to Bradford (1976), using bovine serum albumin as a standard.

**Microinjection Experiments**

Cells were seeded onto glass coverslips for at least 2 d until 50% confluent. Coverslips were transferred into fresh medium before injection of immunoglobulins into the cytoplasm of interphase cells. Injection was carried out using an Eppendorf (Madison, WI) semiautomatic microinjection device coupled to the Zeiss (Thornwood, NY) Automated Injection System. Cell fixation (methanol) and immunofluorescence were routinely performed 3 and 24 h after injection. Injected immunoglobulins were detected with the corresponding secondary antibody. In parallel, detection of centrosomes was performed using a rabbit serum directed against pericentriolar material (serum No. 013; Gosti et al., 1986).

**Cell Electropermeabilization**

Cell electropermeabilization was performed using a PS-15 electropulsator (Jouan, Saint-Herblay, France). Electrodes are composed of two stainless steel plates placed 2 mm apart. Cell populations were pulsed in volumes as reduced as 50 µl, which allows the use of small amounts of antibodies even at large external antibody concentration. HeLa cells were harvested, transferred into low salt electropermeabilization buffer (10 mM Tris-HCl, pH 7.4, 250 mM sucrose, and 1 mM MgCl₂), centrifuged, and resuspended in the buffer containing the antibody at a final concentration of 5 × 10⁵ cells/ml. Final NaCl concentration should not exceed 30 mM. After a 5-min incubation at 4°C, 50 µl of the suspension was transferred between the electrodes. 10 square-wave electric pulses of 5 ms each were then immediately delivered at a voltage of 600 V/cm and a frequency of 1 Hz. Voltage can be adjusted to favor cell survival. Cells were then left for 5–10 min in a Petri dish at room temperature, pelleted to remove excess immunoglobulins, resuspended in fresh culture medium, and seeded in Petri dishes with or without glass coverslips. Under these conditions, cell viability is ~30%. Surviving cells spread out within the first 3 h, allowing us to eliminate floating dead cells by washing the Petri dishes. Centrosomes were detected with the 013 serum, and the percentage of cells displaying a centrosomal labeling was determined on at least 200 cells from randomly selected fields.

**Electron Microscopy**

Cells were fixed at different times with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, for 30 min. The coverslip culture was then washed twice in cacodylate buffer and postfixed in 2% aqueous OsO₄ for 60 min at 4°C. After three washes in buffer, the cells were treated with 0.15% tannic acid (in buffer) for 1 min, washed once in buffer, and then washed twice in distilled water. Next, they were stained in 1% uranyl acetate (4°C; 60 min), washed in distilled water, dehydrated in a graded series of ethanol, and flat-embedded in Epon. Cells were then serially thick-sectioned (0.25 µm). Ribbons of sections were mounted in the center of formvar-coated slot grids, on which 25-nm colloidal gold had been lightly deposited to facilitate subsequent micrograph alignment. After staining in uranyl acetate and lead citrate, the sections were viewed and photographed on a Zeiss 910 electron microscope. Selected thick sections were reconstructed by intermediate voltage electron microscope (IVEM) tomography as detailed by McEwen et al. (1995) using an IVEM (model JEM 4000 FX; JEOL USA, Inc., Peabody, MA) equipped with a computer-controlled tilt/rotation specimen holder.

**Results**

**Centriole Microtubules Are Heavily Glutamyalted**

A previous investigation of the presence of glutamylated tubulin in nonneuronal cell lines demonstrated that this modification is mainly detected on centriole α- and β-tubulin, and to a much lesser extent on interphase and spindle β-tubulin (Bobinnec et al., 1998). When decorated with GT335, an mAb specific to glutamylated tubulin, isolated centrosomes from human KE37 cells were detected as small pairs of dots corresponding to centrioles (Fig. 1a), whereas a specific serum, which has been shown to stain pericentriolar material (Gosti et al., 1986), decorated bigger dots (Fig. 1b).

In brain, as in centriole preparations, GT335 mAb detected both α- and β-tubulin (Fig. 1c). α-Tubulin appeared more labeled than the β-subunit and was detected as a smear, suggesting that centrioles contained glutamyl chains longer than those of brain microtubules. As glutamylated α-tubulin isoforms are likely to represent >50% of the total tubulin in brain (Eddé et al., 1990; Au-debert et al., 1994), we conclude that glutamylation is a major modification of centriole α-tubulin.

**Microinjection of GT335 mAb Leads to the Loss of Centrosome Labeling**

We conducted a series of experiments in which we studied...
the effect of GT335 mAb microinjection on the structural and functional properties of glutamylated microtubules. In all microinjected cells, GT335 immunoglobulins (IgGs) are detected on cytoplasmic microtubules 3 h after microinjection and decorate the centrioles (Fig. 2 a). Immunostaining of the pericentriolar material with the 013 antibody confirmed a localization of the injected IgGs to the centrioles (Fig. 2 a’). By contrast, after 24 h most microinjected cells (67%, n = 452), identified by the staining of the cytoplasm, did not show a concentration of injected GT335 IgGs to the centrioles (Fig. 2 b). Double immunofluorescence labeling with the anticientrosome serum 013 (Gosti et al., 1986) showed that the cells lacking a labeling of the centrosomes by GT335 IgGs did not display any centrosomal staining either (Fig. 2 b’). All control cells, injected with purified IgGs from a nonimmune human serum (Fig. 2, c and c’), exhibited a labeling of the centrosome identical to that of the non-injected cells (Table I). This was also the case with IgGs directed against different post-translational modifications of tubulin present on HeLa centriole microtubules: L3, a rabbit serum directed against detyrosinated α-tubulin (Paturle-Lafancharé et al., 1994), and 1-6.1, a monoclonal antibody against acetylated α-tubulin (Thompson et al., 1984) (Fig. 2, d and d’). These results suggest a specific effect of GT335 mAb that leads to a loss of centrosomal labeling.

To further characterize the effect of GT335 IgGs on centrosomes, we set up a protocol that allowed us to load a large population of HeLa cells with an antibody.

**GT335 mAb Promote a Massive Disappearance of Centrioles and Centrosomes**

We combined previous electropermeabilization protocols (Teissié and Rols, 1988; Casabianca-Pignède et al., 1991) and improved the internalization of antibodies in HeLa cells. The resulting protocol (see Materials and Methods) is more efficient and uses less antibody than classical electroporation methods. As measured by introducing mock IgGs (which were detected with a fluorescent secondary antibody), electropermeabilization allowed us to load 80–90% of the cells. IgGs could be detected up to 1 wk after electropermeabilization (data not shown), with a progressive decrease in the intensity of the labeling. This suggests that the internalized IgGs are not rapidly degraded, but are rather diluted by successive division of the cells.

As a first step, we determined the optimal concentration of GT335 IgGs required to induce a loss of centrosome labeling. The proportion of cells that displayed a centrosome labeling was determined 24 h after loading by use of the specific anticientrosome antibody 013 (Fig. 3). As a control, cells were electropermeabilized in presence of human purified IgGs. In this case, the centrosomal labeling could be detected in all cells (Fig. 3 a). When electropermeabilized with GT335 antibody, immunofluorescence analysis (Fig. 3 b) showed that most of the cells had lost the centrosomal labeling. A maximum effect of GT335 IgGs (up to 80%) was reached at a concentration of 4 mg/ml

**Table I. Effect of Antibody Injection on Centrosome**

| Antibody injected | Hours after injection |
|-------------------|----------------------|
|                   | + 3 h                | + 24 h               |
|                   | Cells with centrosome (%) |
| GT335             | 194/194 (100)        | 150/452 (33)         |
| Human IgGs        | 458/458 (100)        | 337/337 (100)        |
| 1-6.1             | 109/111 (98)         | 177/178 (99)         |
| L3                | 171/171 (100)        | 210/210 (100)        |

Cells were microinjected with GT335 IgGs or control IgGs and fixed after 3 or 24 h. Cells were decorated by immunofluorescence with secondary antibody to detect the injected IgGs, and with anticientrosome antibody to determine the percentage of cells that presented a centrosomal labeling. These results were obtained from several independent experiments (percentages are shown in parentheses).
We thus chose to routinely use IgG concentrations of 6 mg/ml to ensure a better reproducibility of the experiments. Similar percentages of acentrosomal cells were scored using different anticentrosome antibodies from the laboratory, including an anti-\(\gamma\)-tubulin antibody.

Since centrioles were no longer decorated by GT335 IgGs in these cells, we investigated if these structures could still be detected with other anticentriole antibodies. To distinguish centrioles from the cytoplasmic microtubule network, cells were incubated with nocodazole at 4°C for 24 h (Fig. 3 c). We thus chose to routinely use IgG concentrations of 6 mg/ml to ensure a better reproducibility of the experiments. Similar percentages of acentrosomal cells were scored using different anticentrosome antibodies from the laboratory, including an anti-\(\gamma\)-tubulin antibody.

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for 2 h and further extracted with Triton X-100 to remove all soluble tubulin. Under these conditions, centrioles are the only microtubule structures resistant to nocodazole. We then carried out double immunofluorescence experiments with an antibody directed against centrin, an intracentriolar protein (Paoletti et al., 1996), and antitubulin antibody. Immunostaining of control cells (loaded with human IgGs) with anti-α-tubulin antibody showed two or four bright spots corresponding to centrioles, which colocalized with the anticentrin labeling. By contrast, double immunostaining with anti-α-tubulin and anticentrin antibodies failed to reveal such a typical centriole staining in 72% of the cells loaded with GT335 IgGs (not shown). In the same experiment, the use of an anti-PCM antibody showed an absence of centrosome labeling in 76% of the cells.

These results suggest that GT335 IgGs induce the complete disappearance of the centrioles and of the surrounding pericentriolar material. These conclusions were supported by electron microscopy analysis of complete serial section of GT335-loaded cells (see below).

**GT335 mAb-loaded Cells Have No Microtubules Nucleating Center**

Centrosomes are the main microtubule organizing centers of interphase cells. To investigate a possible disorganization of the microtubule cytoskeleton in acentrosomal cells, cells were detergent-extracted and processed for immunofluorescence 24 h after electroporation. Microtubules and centrosomes revealed no obvious differences in the organization of the interphasic microtubule network of centrosomal and acentrosomal cells, although a radial aster focused on the centrosome could be seen in some control cells and never in acentrosomal cells (Fig. 4). Interestingly, numerous detergent-extracted acentrosomal cells presented small foci of pericentriolar material scattered in the juxtanuclear region (Fig. 4 b').

To reveal nucleating capabilities of acentrosomal cells, we conducted microtubule regrowth experiments in cells after microtubule depolymerization. 24 h after electroporation, cells were incubated for 2 h with nocodazole, and microtubule regrowth from endogenous tubulin was allowed for 2 or 15 min. After 2 min, the formation of a small aster centered on the centrosome was observed in all control cells (Fig. 5 a). By contrast, no aster was found in the majority of the cells loaded with GT335 IgGs. Instead, very short microtubules were found disseminated within the cytoplasm (Fig. 5, b and c'). As expected, these cells presented no focused centrosomal labeling. Scattered pericentriolar material was detected in the juxtanuclear region, in the vicinity of growing microtubules (Fig. 5, c and c'), and the microtubule network appeared to be reconstituted when GT335 mAb-loaded cells were allowed to recover from nocodazole for 15 min (data not shown), suggesting that the discrete cytoplasmic nucleation sites observed in the cells, or spontaneous assembly, were sufficient for the reassembly of the microtubule network.

Together, the above-mentioned results imply that GT335 IgGs induce the disassembly of the centrioles, which in turn leads to the scattering of the pericentriolar material. Thus, the absence of centrioles does not seem to abolish

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**Figure 5.** Loaded cells have no functional microtubules nucleating centers. Control and GT335-loaded cells were treated with nocodazole and extensively washed to allow microtubule regrowth. Cells were further processed for double immunofluorescence with antitubulin (red) and anticentrosome (green) antibodies. Microtubules regrew as an aster focused on the centrosome in the control (a), whereas GT335 mAb-loaded cells displayed short individual microtubules disseminated in the cytoplasm (b). Pericentriolar material was detected by rabbit serum 013 close to the nucleus (c), where single microtubules were nucleated (c'). Bars, 10 μm.
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the capacity of pericentriolar material to organize and nucleate microtubules.

**Centrosome and Centriole Disappearance Is a Transient Phenomenon**

Next we determined the time course of the GT335 mAb effect on centrosome labeling. Cells were fixed every 12 h after loading with GT335 IgGs and analyzed by immunofluorescence with the 013 rabbit serum to decorate centrosomes. A representative experiment is shown in Fig. 6. A maximum effect of GT335 IgGs was reached 12 h after electroporation and maintained for 36 h. Strikingly, this effect was reversible, as centrosome labeling was progressively recovered in the whole cell population. 84 h after cell loading, almost all cells displayed a centrosomal labeling. Centrosomes were progressively detected anew within the cell population. At 84 h, almost all cells presented a centrosomal labeling.

![Figure 6](image)

**Electron Microscopy Analysis Confirms a Complete Loss of the Centrioles**

We further investigated by electron microscopy if any structure reminiscent of the centriole or centrosome could be seen. Complete cell reconstructions were obtained by serial thick sectioning (0.25 μm). Cells were prepared for electron microscopy 24 and 60 h after electroporation.

**Interphase Cells.** Of a total of 12 interphase cells analyzed at 24 h, 9 had no detectable centrioles or centriole-like structures. In the three others, two possessed a pair of normal centrioles, and one had a single centriole to which a procentriole was attached. In the latter case, the single centriolar cylinder appeared to be thinner and slightly kinked at the distal end. 60 h after treatment, we found centrioles in 8 out of 12 interphase cells. However, three cells contained one single centriole: one of these was normal, one was twice shorter than normal, and one showed a single microtubule triplet in its vicinity. Although obtained on a small number of cells, these data are in agreement with the percentage of acentriolar cells determined by immunofluorescence analysis of the same experiment (Table II).

**Mitotic Cells.** We carried out the complete reconstruction of three mitotic cells fixed 24 h after antibody loading. One of these cells presented a bipolar spindle (Fig. 8, A–C). The poles were easily identified and contained pericentriolar material but no centrioles. The second cell showed a monopolar spindle with chromosomes arranged played a monopolar spindle, compared with 2% for control cells, whereas the other 42% displayed bipolar metaphase spindles (Fig. 7 A). Some bipolar spindles presented a centriole at one pole. Acentriolar spindles were often characterized by an asymmetry between the two half-spindles. Most poles appeared loosely organized, sometimes barrel-shaped at one pole (compare for example metaphase of Fig. 7, A and C, the latest still possessing one centriole at the leftmost pole). The pericentriolar material showed an unequal distribution at the acentriolar poles (Fig. 7 B). γ-Tubulin could be faintly detected at the acentriolar poles but appeared segregated in variable amount to each pole, just like the pericentriolar material (Fig. 7 C).

We further checked for the presence of NuMA, a protein involved in spindle pole stabilization (Gaglio et al., 1995). Double immunofluorescence with antipericentriolar material antibody showed that NuMA was indeed a component of the acentriolar spindle poles, but it did not show its usually sharp focused distribution (Fig. 7 D).

Taken together, these results indicate that the acentriolar spindle poles are organized by the same components as the centriole-containing poles, but these proteins appeared less focused. In spite of this, spindles could be functional, as anaphase and cytokinesis figures (Fig. 7 A) in which both daughter cells were devoid of centriole staining were observed.
around the centriolar pole (Fig. 8, D–F). In the third case, two sister cells still paired through a midbody were found, exhibiting no centriole in both cells (not shown). This latter observation supports our immunofluorescence data (see Fig. 7 A).

We further analyzed by electron microscopy mitotic cells 60 h after loading. For comparison, centriole pairs at both poles of a mitotic cell loaded with mock IgGs are presented in Fig. 9. On the six mitotic cells analyzed from a population loaded with GT335 mAb, one cell displayed a bipolar spindle with normal centriole pairs at the poles. Two cells showed well-organized bipolar spindles, but a careful examination revealed unusual organization of the spindle poles. Centriole-like structures, which resembled highly distorted incomplete cylinders, were present in both poles (one cell, see Fig. 10) or in one only of two mitotic poles (one cell, not shown). These centriole-like structures were not completely surrounded by the pericentriolar material of the pole, but rather were excluded from it.

Three other cells possessed a monopolar spindle. These monopolar spindles contained one or two distorted centriole-like structures embedded in pericentriolar material in their single poles (Fig. 11, A and B).

To further characterize these centriole-like structures, we performed three-dimensional IVEM tomography on two of them. Tomographic analysis revealed that indeed the walls of these “incomplete centrioles” were organized by microtubules (Fig. 11 C). However, these microtubules did not form triplets that are characteristic of normal centrioles. Individual microtubules were not parallel to each other and did not form a complete cylinder. Overall, the structure resembled a “partially disrupted” centriole.

Thus, we concluded that GT335 mAb leads to the disintegration of the centrioles and dispersion of the pericentriolar material.

### Acentriolar Cells and Mitotic Progression

By both immunofluorescence and electron microscopy, we obtained evidence that the cells are able to proceed through mitosis in the absence of centrioles. The unique specificity of this system, in contrast to an ablation approach (by laser irradiation, see Koonce et al., 1984, or by microsurgery, see Maniotis and Schliwa, 1991), is that the centrosome disappears only transiently. This raises the possibility that all centrosomal components are still present as scattered functional subcomplexes within the cell.

To characterize the behavior of acentriolar cells, the cell cycle distribution of GT335 mAb–loaded cells was analyzed by flow cytometry 24 h after electropermabilization. The data show a limited increase in G2/M cells for the highest concentrations in IgGs (Fig. 12 A). To extend these results, cells loaded with GT335 mAb were seeded at low density in Petri dishes, and the ability to form colonies was measured 4 d later. In this experiment, no significant differences were visible in terms of size and number of colonies between control and GT335 mAb–treated samples (Fig. 12 B).

Since we scored the acentriolar cells as ~70% in the whole population for both experiments, these results suggest that most cells proceed through mitosis in the absence of a centrosome and lead to the formation of colonies, whereas a small proportion of the cells presented a mitotic delay.

### Discussion

#### Centrosome Stability Depends upon Polyglutamylation of Centriole Tubulin

Glutamylation is the major posttranslational modification of neuronal and axonemal microtubules (Eddé et al., 1990; Fouquet et al., 1994). In other cell types, we previously found that this modification concerns primarily the centriole microtubules (Bobinnec et al., 1998). In this paper, we investigated the functional significance of this property.

Our data demonstrate that this modification, or at least the tubulin domain immediately proximal, is involved in centriole stability, as the introduction of a monoclonal antibody directed against glutamylated tubulin (GT335) leads to the disappearance of the whole centrosome structure. Other antibodies specific for other posttranslational modifications of tubulin have no effect, in particular L3 antibodies, which recognize a major centriolar epitope corresponding to the detyrosinated form of α-tubulin that localized very near the glutamylation site. However, whether the effect of GT335 mAb is manifested through its binding to the glutamylated site of α-tubulin, β-tubulin, or both is not known. Previous studies reported that the polyglutamate side chain mediates the binding of MAPs (Boucher et al., 1994) and motors (Larcher et al., 1996) to...
microtubules. Moreover, GT335 monoclonal antibody had been shown to inhibit the motility of reactivated sperm axonemes (Gagnon et al., 1996). Based on these data, we propose that GT335 antibody inhibits the binding of specific centriolar organizing proteins, either directly or by impairing tubulin polyglutamylation of centriole microtubules. Both effects might contribute.

Centriole disintegration could either be induced by loss of interactions between microtubule triplets or by the destabilization of the triplets themselves. Since we did not find, within the first 24 h, intermediate figures that could correspond to abnormal centriole structures (such as disconnected triplets), we believe that GT335 mAb leads to progressive destabilization of the doublet and triplet microtubules. This would fit with the observation that centriole destabilization by GT335 mAb is a rather slow process. No effect was observed 3 h after injection, and 12 h were necessary to reach a maximal effect, a time which is in the range of tubulin turnover in centrioles (Kochanski and Borisy, 1990). In this view, the exceptional stability of the centriole microtubules could be achieved by the regulated binding of stabilizing proteins through the polyglutamate

Figure 8. Cells were collected 24 h after electroporation and were processed for electron microscopy. (A) Overview of a bipolar spindle. (B and C) Magnification of the poles from the same section as in A. Centrioles were not visible at both poles. (D) Monopolar spindle with chromosomes displaying a rosette configuration. The center of this mitotic figure contained no centrioles, as shown after magnification of two adjacent sections, presented in E and F. Bars, 1 μm.

Figure 9. Mitotic cell from a control population analyzed 60 h after loading with human nonimmune IgGs. This late prometaphase cell presented centriole pairs at both poles. (A–D) Adjacent sections of one pole. (E–H) Adjacent sections of the other pole. Bar, 500 nm.
side chain. The inhibition of these interactions by GT335 mAb would modify the dynamics of centriole microtubules and lead to their progressive disassembly and to the dispersal of the whole centrosome structure.

**Are Centriole Organizers Segregated to Acentriolar Daughter Cells?**

In mammalian cell lines, the generation of a new centriole is believed to arise from a structure associated with the preexisting centriole. As suggested earlier (Mazia et al., 1960; Sluder and Rieder, 1985a; Sluder et al., 1989), a non-tubulin structure could be responsible for the assembly of a new centriole. This conservative mechanism implies that the constitutive loss of the centriolar structure (Debec et al., 1982) or the microsurgical removal of the whole centrosome (Maniotis and Schliwa, 1991) would lead to the inability of the cell to generate a new centriole. In the present work, we observed the regeneration of centrioles after their complete disappearance, suggesting either that centriole organizers were preserved in transiently acentriolar cells, or that de novo centriole formation occurs in this system. We were not able to identify a structure that could correspond to such centriole organizers, but the fact that GT335 antibody acts through the destabilization of microtubules leads us to favor the hypothesis of the maintenance of such a structure. Previous studies reported that a procentriole appears on the parental centriole as a short cylinder that progressively elongates to reach its final size (Dippell, 1968; Gould, 1975; Kuriyama and Borisy, 1981). These observations suggest that centriole microtubules are gradually stabilized and linked to each other concomitantly to their elongation. In our system, we visualized abnormal centrioles 60 h after introduction of GT335 anti-

![Figure 10](image)

**Figure 10.** Metaphase spindles assemble in cells collected 60 h after loading with GT335 mAb but present partial centrioles at their poles. (A) Overview of the spindle. (B) Eight adjacent sections of the upper spindle pole. (C) 14 adjacent sections of the lower pole. Note in both cases the presence of several abnormal centrioles (arrows). They seemed excluded from the pericentriolar cloud and presented a weak staining of the walls. Pericentriolar material, however, was visible at both poles. Bars, 1 μm.
body. However, no intermediate structures were observed at earlier stages. We believe that these abnormal centrioles correspond to partially reassembled centrioles. Since we show that the GT335 antibody act in a dose-dependent manner, the progressive dilution of the antibody within the cell at each division might allow the progressive reassembly of the centrioles. 84 h after introduction of GT335 antibody, almost all cells presented a normal centrosome staining. This observation suggests that centriole organizers were segregated in all acentriolar daughter cells.

Do Acentriolar Cells Proceed through Mitosis?
The occurrence of acentriolar cells after loading with the GT335 antibody allowed us to address the question of the role of the centrioles in spindle formation. Our data suggest that acentriolar cells can proceed through mitosis, in agreement with other reports (Debec et al., 1982), even though many abnormal mitotic spindles were observed. We cannot exclude a contribution of GT335 antibody to disorganization of the spindle poles through an effect on spindle microtubules. However, the level of glutamylated tubulin in cytoplasmic microtubules is very low, whereas centriole tubulins are heavily modified (Bobinnec et al., 1998). When cells were fixed 3 h after injection of the GT335 antibody, some cells presented labeled metaphase spindles looking identical to noninjected cells. Abnormal spindles were indeed visualized 24 h after injection, a time where the effect of the antibody on centrioles was maximal. Moreover, spindle defects, such as loosely organized or barrel-shaped poles, were already reported in studies of acentriolar mitosis (Debec et al., 1982; Heald et al., 1997). We thus believe that spindle defects were not caused by the binding of GT335 to spindle microtubules but were rather the consequence of the absence of centrioles. Mitotic markers such as NuMA (Compton and Cleveland, 1994) were always detected at the poles, although in asymmetrical amounts. Pericentriolar material was found in various amounts at the spindle poles and was barely detectable in some cases. These observations suggest that the pathway of spindle assembly with or without centrioles involves indeed the same components (Merdes and Cleveland, 1997). In agreement with previous results that suggested that NuMA redistribution at the onset of mitosis depends upon the correct redistribution of PCM between centriole pairs (Paoletti et al., 1997), the present work indicates that in animal cells, when present, centrioles allow the correct segregation of the pericentriolar material and lead to more defined spindle poles.

Monopolar spindles observed presented partial centri-
significantly different. The distribution of the colonies according to their size did not appear different. For each colony size, the first bar (white) is for GT335 IgGs, and the second bar (black) is for control IgGs. The distribution of the colonies according to their size did not appear significantly different.

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**Figure 12.** Growth of GT335 mAb–loaded cells. (A) Flow cytometry analysis of cell cycles show an increase in G2/M phases for GT335 mAb–loaded cells after 24 h. N.E., nonelectropermeabilized cells; w/o Ab, cells electropermeabilized without antibody; 2–6 mg/ml, final antibody concentration for cell electropermeabilization. (B) Cells were plated at low density after loading with GT335 mAb, and the number of cells per colony was determined 5 d later. Bars represent the percent of colonies in the corresponding size. For each colony size, the first bar (white) is for GT335 IgGs, and the second bar (black) is for control IgGs. The distribution of the colonies according to their size did not appear significantly different.

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A Model for Centrosome Assembly

Two features of the GT335 mAb-induced disappearance of centrosome structure, and of the whole centrosome organelle as a consequence, are striking: First, it is a slow process. We believe that the observed time course for centrosome disappearance results from interference of the antibody with both the turnover of individual centrosomal components and the duplication cycle of the whole organelle. Centrioles could be more sensitive to destabilization by GT335 mAb during a limited period of the cell cycle. Indeed, important changes in centrosome organization and redistribution of the pericentriolar material are known to take place at the onset of mitosis (Rieder and Borisy, 1982; Vorobjev and Chentsov, 1982). We previously showed that concomitantly, centriole glutamylation is increased during mitosis (Bobinnec et al., 1998). Molecular (Lange and Gull, 1995) and structural differences (Paintrand et al., 1992; Doxsey et al., 1994) between mother and daughter centrioles could account for, or reflect, differential stability within the centriole pair. This could explain our observation of cells with a single centriole at 24 h as well as 60 h after loading.

Second, centrosome disappearance is accompanied by a scattering of the microtubules nucleating material in the cytoplasm and around the nucleus. Our data suggests that the focalization of the centrosomal material depends on centrioles. Also supporting such a role for centrioles, we noted that even abnormal centrioles, observed 60 h after introduction of GT335 mAb, were apparently able to concentrate material around them at the spindle poles (see Figs. 8 and 10). Such a role was also suggested in more physiological conditions. After the loss of centrioles during differentiation of myoblasts into myotubes, the pericentriolar material redistributes around the nucleus, where microtubules are nucleated (Tassin et al., 1985). All these data, as well as ultrastructural features of centrosome organization, suggest that some centrosomal components interact directly with the microtubule triplets of the centrioles.

A minimal model for centrosome assembly could propose that centrioles nucleate the assembly of the centrosomal matrix through two subsets of proteins, one able to bind to the wall, and another able to bind to the proximal end. For the first subset, progressive glutamylation of tubulin during centriole assembly would bring long-term stability to this edifice and trigger the lateral association of specific MAPs (Boucher et al., 1994; Larcher et al., 1996).

In the mean time, the nine triplets of stable and short centriole microtubules would provide at their proximal end a set of highly stable microtubule minus ends able to anchor a second subset of proteins, i.e., minus end–binding proteins, including γ-tubulin–containing complexes (Moritz et al., 1995; Zheng et al., 1995; Martin et al., 1998; Murphy et al., 1998; Tassin et al., 1998). These complexes could in turn trigger the progressive accumulation of centrosomal matrix components. The observation that microtubule-stabilizing drugs induce the redistribution of the centrosomal matrix from centrioles to the minus ends of free cytoplasmic microtubules supports this proposal (for a discussion of this aspect see Bornens, 1992; Paoletti et al., 1997). This model is admittedly highly hypothetical, since our knowl-
edge on centriole composition is still scarce. The recent isolation of the gene UN13 (which appears necessary for the assembly of basal bodies in *Chlamydomonas reinhardtii*) as a new tubulin gene, distinct from α-, β-, and γ-tubulins, might represent a new and important piece of information for understanding centriole assembly (Dutcher and Trabuco, 1998).

Whatever the molecular mechanisms controlling the assembly of centrioles, we can conclude from the present work that in animal cells, the centriole pair is a conspicuous part of the centrosome and plays a critical role for centrosome stability and reproduction. One of the important goals in the near future will be to understand how these roles are exerted.

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References

Audebert, S., A. Koulakoff, Y. Berwald-Netter, F. Gros, P. Denoulet, and B. Eddé. 1994. Developmental regulation of polyglutamylated α- and β-tubulin in mouse brain neurons. *J. Cell Sci.* 107:2313–2322.

Baron, A.T., T.M. Greenwood, C.W. Bazinet, and J.L. Salisbury. 1992. Centrin and an auto-antibody against pericentriolar material. *J. Cell Sci.* 100:118–126.

Birnboim, Y. 1991. Stability of antiricin antibodies introduced into DC-3F Chinese hamster cells by electropermeabilization. *J. Cell Biol.* 109:1545–1553.

Bobinnec, Y., M. Moudjou, J. Bersye, R. Maunoury, and M. Bornens. 1986. Identification of centrosomal proteins in a human lymphoblastic cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2545–2550.

Gould, R.R. 1975. The basal bodies of *Chlamydomonas reinhardtii*: formation from prosomal bodies, isolation, and partial characterization. *J. Cell Biol.* 65:65–74.

Gundersen, G.G., and J.C. Bulinski. 1986. Distribution of tyrosinated and non-tyrosylated α-tubulin during mitosis. *J. Cell Biol.* 102:1118–1126.

Heald, R., K. Tournitze, A. Habermann, E. Karsenti, and A. Hyman. 1997. Spindle assembly in Xenopus egg extracts: respective roles of centrosomes and microtubule self-organization. *J. Cell Biol.* 138:615–628.

Klotz, C., M.C. Dabauvaille, M. Paintrand, T. Weber, M. Bornens, and E. Karsenti. 1990. Pathogeneisis in *Xenopus* eggs requires centrosomal integrity. *J. Cell Biol.* 110:405–415.

Kochanski, R.S., and G.G. Borsy. 1990. Mode of centriole duplication and distribution. *J. Cell Biol.* 110:1599–1605.

Kooime, M.P.A., R.A. Clarke, D. Paoletti, and M.W. Berns. 1984. Laser irradiation of censnroles in new censinophils: evidence of centriole role in motility. *J. Cell Biol.* 98:1999–2010.

Kuriyama, R., and G.G. Borsy. 1981. Centriole cycle in Chinese hamster ovary cells as determined by whole-mount electron microscopy. *J. Cell Biol.* 91:814–821.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 76:383–388.

Beatty, R.A. 1987. Parthenogenesis in vertebrates. *In Fertilization. C.B. Metz* and A. Monroy, editors. Academic Press, New York. 413–440.

Bobinnec, Y., M. Moudjou, J.P. Fouquet, E. Desbruyères, B. Eddé, and M. Bornens. 1998. Glutamylation of centriole and cytoplasmic tubulin in proliferating non-neuronal cells. *Cell Motil. Cytoskel.* 39:223–232.

Bornens, M. 1992. Structure and function of isolated centrosomes. In *The Centrosome*. V.J. Kalnins, editor. Academic Press, San Diego. 1–43.

Bornens, M., M. Paintrand, J. Berge, M.C. Marty, and E. Karsenti. 1987. Structural and chemical characterization of isolated centrosomes. *Cell Motil. Cytoskel.* 10:238–254.

Boucher, D., J.C. Larcher, F. Gros, and P. Denoulet. 1994. Polyglutamylation of tubulin as a progressive regulator of intracellular interactions between the microtubule-associated protein Tau and tubulin. *Biochemistry.* 33:12471–12475.

Boveri, T. 1901. Über die Natur der Centrosomen. *Jena Z. Med. Naturw.* 281–283.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.

Calarco-Gilliam, P.D., M.C. Siebert, R. Hubble, T. Mitchison, and M. Kirschner. 1983. Centrosome development in early mouse embryos as defined by an auto-antibody against pericentriolar material. *J. Cell Biol.* 107:2313–2322.

Boscablanche-Pignède, M.R., L.M. Mir, J.B. Le Pecq, and A. Jacquemin-Sablon. 1991. Stability of anti-antiricin antibodies introduced into DC-3F Chinese hamster cells by electropermeabilization. *J. Cell Biol.* 107:285–297.

Compton, D.A., and D.W. Cleveland. 1994. NuMA, a nuclear protein involved in mitosis and nuclear rearrangement. *Curr. Opin. Cell Biol.* 6:343–346.

Debec, A., A. Szüllösi, and D. Szüllösi. 1982. A *Drosophila melanogaster* cell line lacking centrioles. *Biol. Cell.* 44:133–138.

Dingle, A.D., and C. Fulton. 1966. Development of the flagellar apparatus of *Naegleria*. *J. Cell Biol.* 31:43–54.

Dippoll, R. 1968. The development of basal bodies in *Paramecium*. *Proc. Nat. Acad. Sci. USA.* 51:461–468.

Dossey, S.P., P. Stein, L. Evans, P.D. Calarco, and M. Kirschner. 1994. Pericentrin, a highly conserved centrosome protein involved in microtubular organization. *Cell.* 76:639–650.

Dutcher, S.K., and E.C. Trabuco. 1998. The UN13 gene is required for assembly of basal bodies of *Chlamydomonas* and encodes α-tubulin, a new member of the tubulin superfamily. *Mol. Biol. Cell.* 9:1293–1308.

Eddé, B., J. Rossier, J.P. Le Caer, E. Desbruyères, F. Gros, and P. Denoulet. 1990. Posttranslational glutamylation of α-tubulin. *Science.* 247:83–85.

Fouquet, J.P., B. Eddé, M.L. Kann, A. Wolff, E. Desbruyères, and P. Denoulet. 1994. Differential distribution of glutamylated tubulin during spermatogenesis in mammalian testis. *Cell Motil. Cytoskel.* 27:49–58.

Gaglio, T., A. Saredi, and D.A. Compton. 1995. NuMA is required for the organization of microtubules into aster-like mitotic arrays. *J. Cell Biol.* 131:691–708.

Gaglio, T., M.A. Dionne, and D.A. Compton. 1997. Mitotic spindles poles are organized by structural and motor proteins in addition to centrosomes. *J. Cell Biol.* 138:1055–1066.

Gagnon, C., D. White, J. Cosson, P. Huitorel, B. Eddé, E. Desbruyères, L. Patutre-Lafanchère, L. Multigner, D. Job, and C. Cibert. 1996. The polyglutamylated lateral chain of α-tubulin plays a key role in flagellar motility. *J. Cell Sci.* 109:1545–1553.

Gost, F., M.C. Marty, J. Berge, R. Maunoury, and M. Bornens. 1986. Identification of centrosomal proteins in a human lymphoblastic cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2545–2550.
Paoletti, A., and M. Bornens. 1997. Organisation and functional regulation of the centrosome in animal cells. *Prog. Cell Cycle Res.* 3:285–299.
Paoletti, A., N. Giocanti, V. Favaudon, and M. Bornens. 1997. Pulse treatment of interphasic HeLa cells with nanomolar doses of docetaxel affects centrosome organization and leads to catastrophic exit of mitosis. *J. Cell Sci.* 110:2403–2415.
Patrulle-Lafanechère, L., M. Manier, N. Trigault, F. Prollet, H. Mazarguil, and D. Job. 1994. Accumulation of Δ2-tubulin, a major tubulin variant which cannot be tyrosinated, in neuronal tissues and in stable microtubule assembly. *J. Cell Sci.* 107:1529–1543.

Piperno, G., and M.T. Fuller. 1985. Monoclonal antibodies specific for an acetylated form of α-tubulin recognize the antigen in cilia and flagella from a variety of organisms. *J. Cell Biol.* 101:2085–2094.

Rieder, C.L., and G.G. Borisy. 1982. The centrosome cycle in PtK2 cells: asymmetric distribution and structural change in the pericentriolar material. *Biol. Cell.* 44:117–132.

Robbins, E., G. Jentzsch, and A. Micali. 1968. The centriole cycle in synchronized HeLa cells. *J. Cell Biol.* 36:329–339.

Schatten, H., G. Schatten, D. Mazia, R. Balczon, and C. Simerly. 1986. Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. *Proc. Natl. Acad. Sci. USA.* 83:105–109.

Sluder, G., and C.L. Rieder. 1985a. Centriole number and the reproductive capacity of spindle poles. *J. Cell Biol.* 100:887–896.

Sluder, G., and C.L. Rieder. 1985b. Experimental separation of pronuclei in fertilized sea urchin eggs: chromosomes do not organize a spindle in the absence of centrosomes. *J. Cell Biol.* 100:897–903.

Szöllözi, D., P.G. Callarco, and R.P. Donahue. 1972. Absence of centrioles in the first and second meiotic spindles mouse oocytes. *J. Cell Sci.* 11:521–541.

Tassin, A.M., C. Celati, M. Paintrand, and M. Bornens. 1997. Identification of an Spc110p-related protein in vertebrates. *J. Cell Sci.* 110:2533–2545.

Teissic, J., and M.P. Rols. 1988. Electropermeabilization and electrofusion of cells. In *Dynamics of Membrane Proteins and Cellular Energetics*. N. Latruffe, Y. Gaudemer, P. Vignais, and A. Azzi, editors. Springer Verlag, Berlin. 249–268.

Theurkauf, W.E., and R.S. Hawley. 1992. Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and effect of mutations in the *nod* Kinesin-like protein. *J. Cell Biol.* 116:1167–1180.

Thompson, W.C., J.J. Asai, and D.H. Carney. 1984. Heterogeneity among microtubules of the cytoplasmic microtubule complex detected by a monoclonal antibody to α-tubulin. *J. Cell Biol.* 98:1017–1025.

Wolff, A., B. de Néchaud, D. Chillet, H. Mazarguil, E. Desbruyères, S. Audibert, B. Eddé, F. Gros, and P. Denoulet. 1992. Distribution of glutamylated α- and β-tubulin in mouse tissues using a specific monoclonal antibody, GT335. *Eur. J. Cell Biol.* 59:425–432.

Zheng, Y., M.L. Wong, B. Alberts, and M. Mitchison. 1995. Nucleation of microtubule assembly by a γ-tubulin-containing ring complex. *Nature.* 378:578–583.