Microtubule Organization during the Early Development of the Parthenogenetic Egg of the Hymenopteran *Muscidifurax uniraptor*

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The origin of the zygotic centrosome is an important step in developmental biology. It is generally thought that sperm at fertilization plays a central role in forming the functional centrosome which subsequently organizes the first mitotic spindle. However, this view is not applicable in the case of parthenogenetic eggs which develop without the sperm contribution. To clarify the problem of the origin of the zygotic centrosome during parthenogenetic development, we studied a hymenopteran, *Muscidifurax uniraptor.* Antitubulin antibody revealed that after activation several asters assembled in the egg cytoplasm. The number of asters varied in relation to the cell cycle. They became visible from anaphase of the first meiotic division and increased in number as meiosis progressed, reaching a maximum at the first mitosis. From anaphase–telophase of the first mitosis they decreased in number and were no longer found during the third mitotic division. To elucidate the nature of these asters we performed an ultrastructural study with transmission electron microscopy and immunofluorescence with antibodies against anti-γ-tubulin and CP190. In this way we showed the presence in these asters of centrosomal components and centrioles. Our observations suggest that the cytoplasm of *Muscidifurax* eggs contains a pool of inactive centrosomal precursor proteins becoming able to nucleate microtubules into well-defined asters containing centrioles after activation.   © 1998 Academic Press

INTRODUCTION

Microtubules, polymerized from a specialized microtubule-organizing-centers (MTOCs), play a key role in eukaryotic cellular organization. Indeed they are involved in cell shape and polarity determination, in organelle and vesicle transport, in chromosomes separation during mitosis and in the organization of the other cytoskeleton elements (Kalnins, 1992; Kellogg *et al.,* 1994). Centrosome is the main MTOC in animal cells and it generally consists of a pair of centrioles and amorphous material from which microtubules nucleate (Gould and Borisy, 1977). In spite of the functional importance of this organelle, several aspects of its behavior are not well understood.

During fertilization the centrosome organizes the first mitotic spindle that drives the mixing of parental chromatin and its correct distribution in two daughter cells. An intriguing question is how centrosome inheritance is monitored during fertilization. As a rule, except in the mouse (Maro *et al.,* 1985; Schatten *et al.,* 1986), the male gamete is essential for the formation of the functional zygotic

centrosome that is able to duplicate and organize the first mitotic spindle (see Schatten, 1994, for a review). An in vitro assay of microtubule nucleating centers using cell-free cytoplasmic extracts made from *Xenopus* eggs, showed that some antigens were present around the sperm centriole, whereas γ-tubulin and certain phosphorylated epitopes were associated with the male centrosome only after incubation of the sperm in egg extracts (Doxey *et al.,* 1994; Felix *et al.,* 1994; Stearns and Kirschner, 1994). These observations suggest that the ability to nucleate microtubules requires components of paternal and maternal origin. This is supported by the finding that the unfertilized frog egg has all components necessary to assemble over 1000 centrosomes (Gard *et al.,* 1990) and that injection of exogenous basal bodies triggers the assembly of stored maternal material into functional centrosomes (Heidemann and Kirschner, 1975).

Among insects, the *Drosophila* egg has centrosomal material associated with the meiotic apparatus (Riparbelli and Callaini, 1996). This material can nucleate microtubules, but is unable to duplicate and is lost at the end of meiosis.
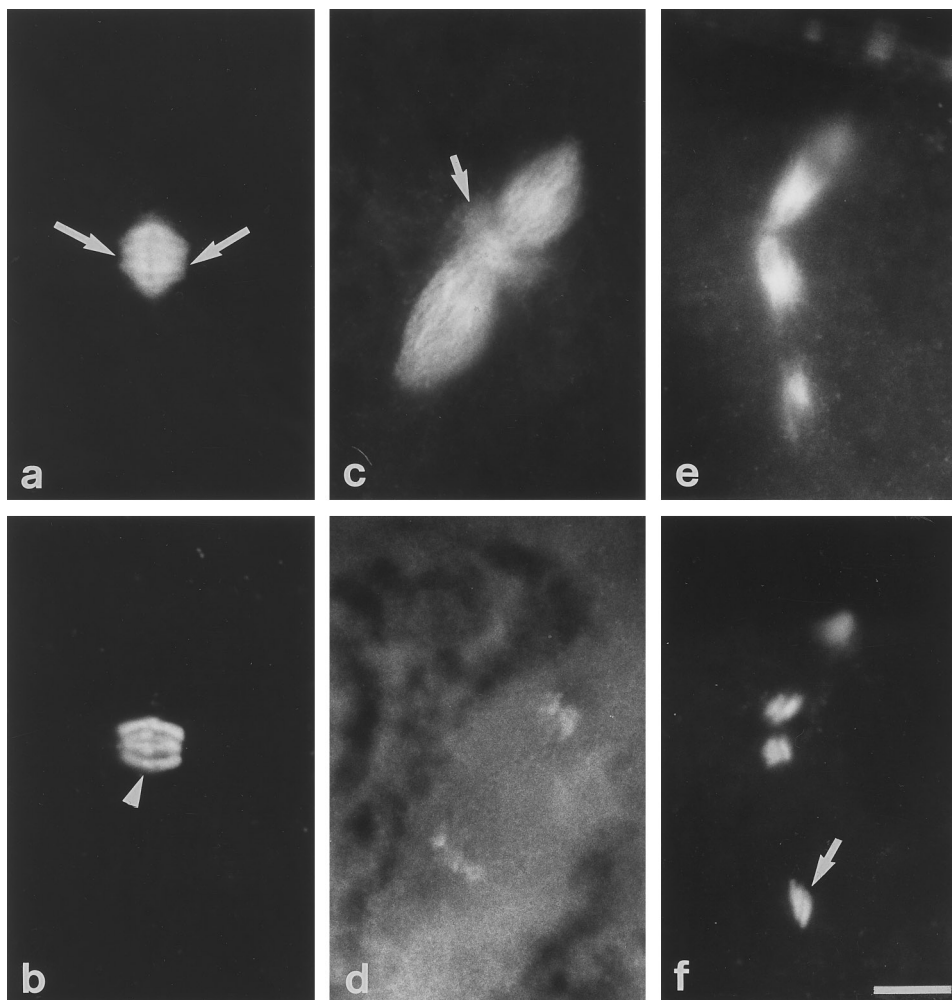
FIG. 1. Immunofluorescence detection of microtubules (a, c, e) and DNA (b, d, f) during female meiosis in *Muscidifurax uniraptor.* (a) After passage of the oocyte through the oviduct the meiosis resumes. The metaphase I spindle is barrel shaped with anastral poles (arrows); (b) homologous chromosomes are paired through chiasmata (arrowhead). (c) During metaphase II the meiotic apparatus is composed of two twin spindles aligned in tandem and separated by a monastral array of microtubules (arrow); (d) chromosomes are disposed in two distinct masses in the midzone of the spindles. (e) At anaphase II the twin spindles are thinner and hold four groups (f) of haploid complements; the putative female pronucleus (arrow) is found deeper than the other chromosome complements in the egg cytoplasm. Bar, 7.5 μm.

γ-tubulin and CP190 antigens has not been found in mature sperm, but are recruited from the egg cytoplasm around the sperm centriole (Riparbelli *et al.,* 1997; Wilson *et al.,* 1997). The reconstitution of the functional centrosome therefore requires in the *Drosophila* zygote the contribution of both gametes.

Many insects species reproduce by parthenogenesis, a special mode of reproduction that does not requires the contribution of the male gamete. Eggs that develop without fertilization are useful for studying the assembly mechanisms of functional centrosomes from maternal components only. To clarify the reconstitution of a functional centrosome in naturally parthenogenetic eggs, we chose to use the hymenopteran *Muscidifurax uniraptor,* a thelyto-

kous pteromalid pupal parasite of synanthropic Diptera (Kogan and Legner, 1970). Our strain is peculiar because it is infected by bacteria of the genus *Wolbachia* which induce diploidization of the eggs. Restoration of diploidy in these eggs was presumably due to a segregation failure of the two sets of chromosomes in the first mitotic anaphase as reported in *Trichogramma* wasps (Stouthamer and Kazmer, 1994). We therefore obtain a large number of females which necessarily reproduce by parthenogenesis. Bacteria are thought to induce parthenogenesis, since their elimination with tetracycline or high temperatures leads to progeny that reproduce sexually (Stouthamer and Werren, 1993; Stouthamer *et al.,* 1993).

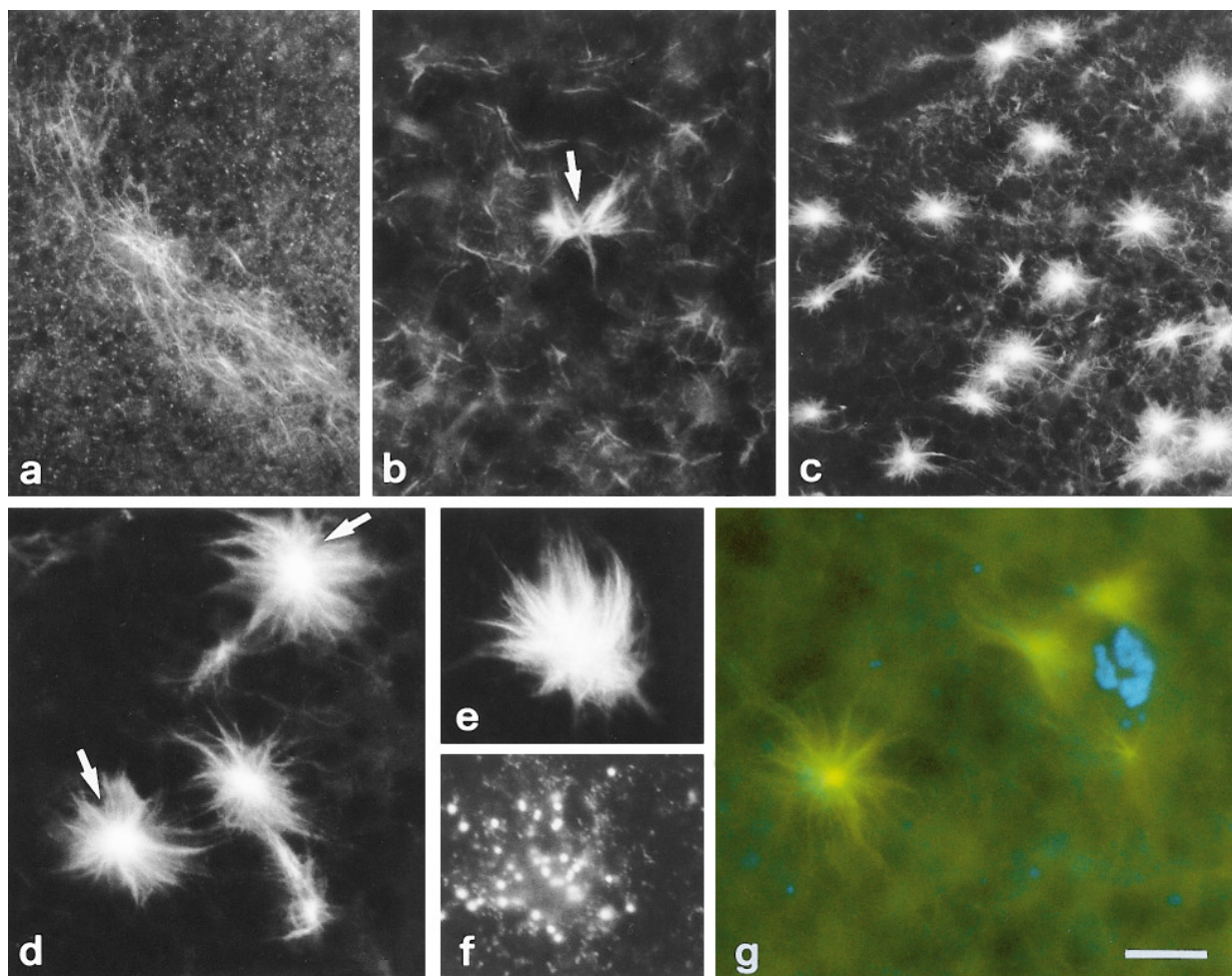In this study we showed the presence of many cyto-

FIG. 2. Microtubule organization after egg activation as revealed by an antibody against β-tubulin. (a) Bundles of microtubules randomly oriented in the cytoplasm are visible as meiosis resumed. (b) Some microtubules converge to a central core (arrow). (c) At the first mitosis many asters of different sizes are present in the cytoplasm. (d) Higher magnification of the cytoplasmic asters shows that microtubules radiate from a central core (arrows). (e, f) Hoechst staining reveals that bacteria of the genus *Wolbachia* are associated with astral microtubules. (g) Double labeling for microtubules and DNA showing that some cytoplasmic asters interact with the zygotic condensing chromosomes. Bar, 7.5 μm in a, b, d, e, f, and g; 10 μm in c.

plasmic microtubule organizing centers, containing γ-tubulin and centrioles, in the parthenogenetic egg of *Muscidifurax*. These MTOCs, that nucleated microtubules in an aster-like fashion, increased in number after the resumption of meiosis and reduced dramatically from the second nuclear division cycle.

MATERIALS AND METHODS

Strain

The hymenopteran species used in this study was *Muscidifurax uniraptor* Kogan and Legner, a thelytokous pteromalid pupal parasite of synantropic Diptera. This strain carries microorganisms of the genus *Wolbachia* which cause diploidization of the eggs, from which develop females that reproduce parthenogenetically. Controls were performed by using a uninfected *Muscidifurax* strain: unfertilized haploid eggs give rise to male progeny. Wasps cultures were maintained on *Ceratitis capitata* Wied pupae at 24°C. Generation time under these conditions is approximately 25 days.

Egg Collection and Processing

Thelytokous females from 10 to 15 days old were placed in separate vials with one host each and allowed to oviposit. Just after oviposition the hosts were removed from vials and kept for a variable timespan to allow the *Muscidifurax* eggs to develop. Eggs were removed with fine needles by gently cracking the pupal case of the parasitized host in Grace medium.

Three different fixation protocols were used: (a) Some eggs were dechorionated in a 50% bleach solution and washed in distilled
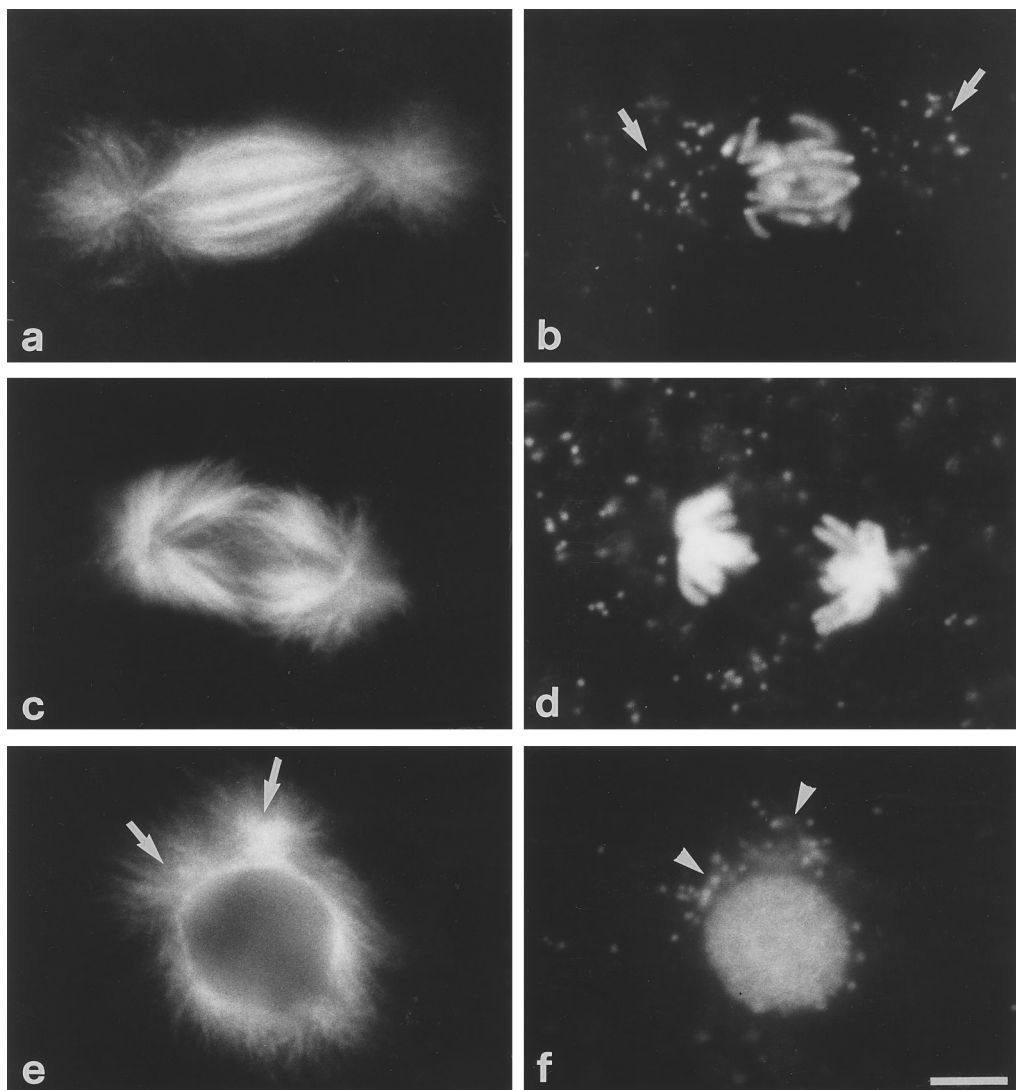
FIG. 3. Immunofluorescence staining with anti-tubulin antibody (a, c, e) and Hoechst dye (b, d, f) during early mitoses of *Muscidifurax* egg. (a, b) Anaphase A of the first zygotic division, *Wolbachia,* are clustered at the pole spindles (arrows). (c, d) Anaphase B of the third mitosis. (e, f) Interphase of the fourth nuclear division cycle. Microtubules form an envelope around the nucleus; two strongly labeled foci of microtubules, corresponding to centrosomes, are localized at a side of the nucleus (arrows). *Wolbachia* are associated with these two microtubule foci (arrowheads). Bar, 5 μm.

water. The dechorionated eggs were permeabilized in heptane. After 3 minutes an equal amount of cold methanol was added to the heptane, and the solution was gently shaken for 5 minutes. Then a drop of distilled water was added to the solution and the vitelline envelope, now detached from the egg membrane, was removed with fine needles. Devitellinized eggs were again fixed in acetone at −20°C for 5 minutes. (b) Some eggs were placed one at a time in a small drop (2-3 μl) of Grace medium on a glass slide. The slide was then put on a copper bar precooled in liquid nitrogen. The frozen eggs were cut longitudinally with a precooled razor blade and fixed in methanol at −20°C for 10 minutes. (c) Some eggs were placed three at a time in a small drop (2-3 μl) of Grace medium on a glass slide and gently squashed under a 20X20-mm coverslip. The slides were frozen on a copper bar precooled in liquid nitrogen

or directly in liquid nitrogen, and after removal of the coverslip with a razor blade, were immersed in methanol at −20°C for 10 minutes. The squashed samples adhered to the slides.

The devitellinized and cut eggs with envelopes were placed in a small drop of solution in Petri dishes covered with squares of Parafilm. The slides with squashed samples were immersed in washing solution and drops (4-5 μl) with antibodies were placed directly on the samples.

Fluorescence Microscopy

After fixation the samples were washed three times for 15 minutes in phosphate buffered saline (PBS) and incubated for 1 h in

PBS containing 0.1% bovine serum albumin (PBS/BSA) to block nonspecific staining. Microtubules were detected after overnight incubation at 4°C with either a monoclonal antibody against β-tubulin (Boehringer Mannheim, Indianapolis, IN; diluted 1:200), or with Yol1/34 (Harlan Sera Lab; England; diluted 1:100). The samples were then rinsed three times in PBS/BSA for 15 minutes and incubated for 1 hour in goat anti-mouse (Cappel, West Chester, PA), or in goat anti-rat (Harlan Sera Lab, England) antibodies both coupled with fluorescein (dilution 1:600). For double labeling the slides were incubated overnight at 4 C° with the polyclonal antiserum Rbcs1 raised against recombinant γ-tubulin of *Drosophila* (dilution 1:100). Centrosomal material was also detected with the anti-CP190 antibody (dilution 1:200) specific for the previously characterized CP190 antigen associated with the centrosome of *Drosophila* embryo (Frash *et al.,* 1986; Whitfield *et al.,* 1988, 1995). After rinsing in PBS the samples were treated with rhodamine-conjugated goat anti-rabbit IgG (Cappel, West Chester, PA; dilution 1:600) for 1 hr. To determine the exact stage of the embryos, the nuclei were stained by incubating for 3-4 minutes with 1 μg/ml Hoechst 33258 (Sigma, St. Louis, MO). The samples were rinsed again in PBS and mounted on glass microscope slides in 90% glycerol containing 2.5% *n*-propyl-gallate (Giloh and Sedat, 1982). Fluorescence observations were carried out with a Leitz Aristoplan microscope equipped with fluorescein, rhodamine and UV filters. Photomicrographs were taken with Kodak Tri-X 400 Pan and developed in Kodak HC110 developer for 7 minutes at 20°C. Confocal images were taken using a Leica TCS 4D laser scanning confocal microscope (Leica, Heidelberg, Germany). Digital images were printed on Kodak Ektachrome Elite 100 ASA film using a Polaroid CI-3000 digital palette.

Electron Microscopy

For transmission electron microscopy the eggs were fixed by pricking with fine needles in the trialdehyde solution of Kalt and
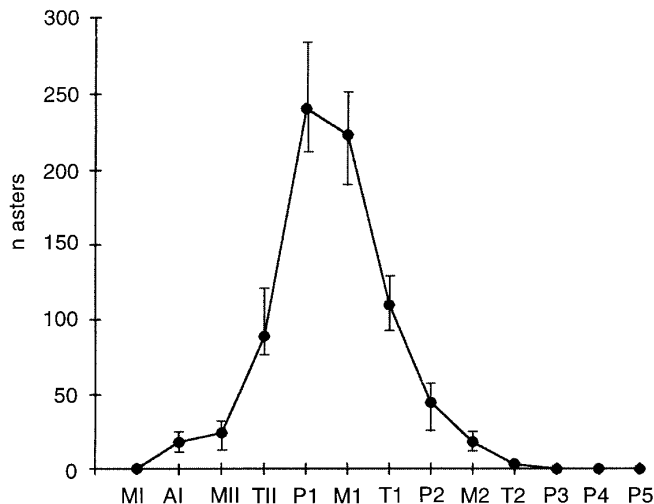
FIG. 5. Double labeling with antibodies against β-tubulin (a) and CP190 (b) showing the centrosomal antigens (arrows) at the center of cytoplasmic asters. Bar, 7.5 μm.

Tandler (1971) for 2 hr, rinsed in cacodylate buffer 0.1 *M,* pH 7.2, and postfixed in 1% osmium tetroxide for 2 hr. The samples were dehydrated in a graded series of alcohols and bulk-stained in 1% uranyl acetate for 1 hr. After treatment with propylene oxide the eggs were embedded in an Epon-Araldite mixture and polymerized at 60°C for 48 hr. Sections cut using an LKB Nova ultramicrotome and a diamond knife (Diatome Ltd., Switzerland) were collected on copper grids and stained with uranyl acetate and lead citrate. Sections were observed with a Philips CM10 electron microscope.

RESULTS

The Meiotic Apparatus

Meiosis resumes after the oocyte passes throughout the oviduct. Newly laid *Muscidifurax* eggs ($n = 25$) had a hexagonal-shaped metaphase I spindle aligned parallel to the egg surface. The spindle was anastral and had broad pole regions (Fig. 1a). Homologs were positioned between the metaphase plate and the spindle poles in a bilaterally symmetric manner (Fig. 1b). The meiotic apparatus was formed by parallel bundles of microtubules surrounding the exchange chromosomes that extended the whole length of the spindle. During anaphase ($n = 28$) the spindle poles gradually sharpened and the meiotic spindle changed orientation from a position parallel to the egg surface to a radial orientation. The second meiotic division, examined in 95 eggs (41 at metaphase, 28

FIG. 4. Cytoplasmic asters in *Muscidifurax* eggs at various stages of development. Abbreviations: MI, AI, metaphase and anaphase of the first meiosis; MII, TII, metaphase and telophase of the second meiosis; P1, M1, T1, prophase, metaphase, and telophase of the first mitosis; P2, M2, T2, prophase, metaphase, and telophase of the second mitosis; P3, P4, P5, prophase of the third, fourth, and fifth mitosis. Data are mean and range values of the astral numbers observed at each developmental stage.
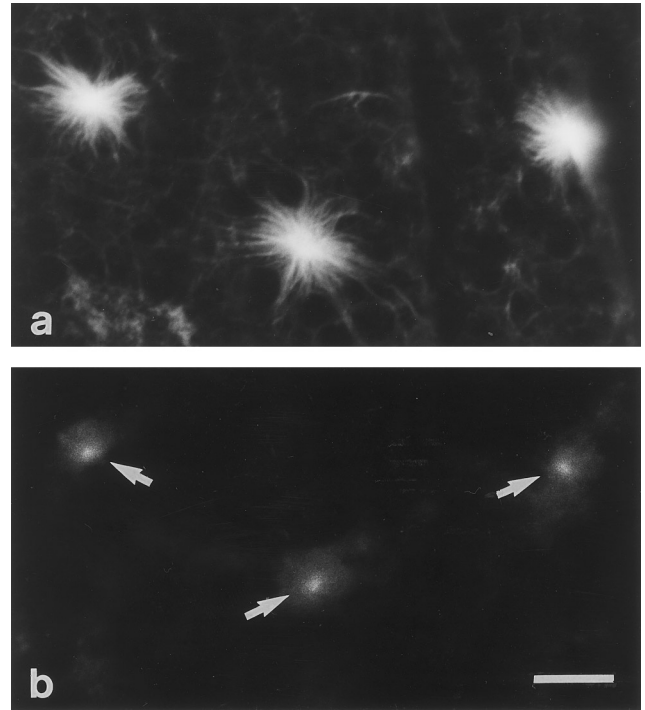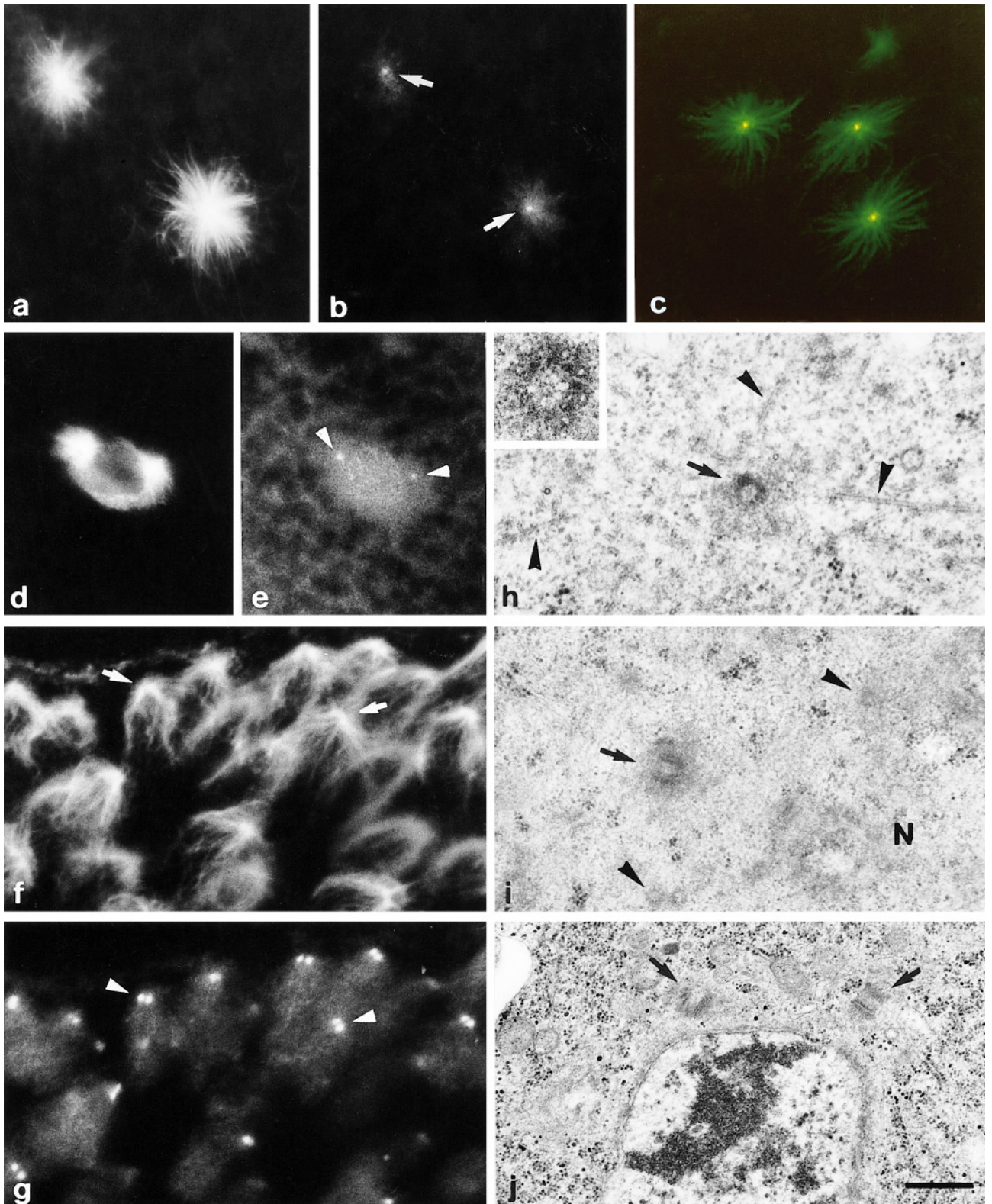
FIG. 6. Eggs stained for microtubules (a) and γ-tubulin (b) reveal that each aster is associated with a focus of γ-tubulin (arrows). (c) Overlay of confocal images of cytoplasmic asters (green) and γ-tubulin (yellow). A double labeling with antibodies against β-tubulin (d) and γ-tubulin (e) shows that the poles of the first mitotic spindle contain γ-tubulin spots (arrowheads). Immunofluorescence detection

at anaphase, and 26 at telophase), led to the formation of twin spindles aligned in tandem and radially oriented with respect to the egg surface (Fig. 1c). The spindle poles were anastral, but a monastral array of microtubules was found between these spindles. The chromosomes were aligned in the equatorial region of the spindles (Fig. 1d). The twin spindles became thinner at anaphase II (Fig. 1e) and held four distinct haploid complements (Fig. 1f). At the end of meiosis only the spindle microtubules near the egg surface persisted and carried the haploid nuclei that eventually became the polar bodies (not shown). The putative zygotic pronucleus was found deeper than the other chromosomes in the egg cytoplasm.

Microtubule Organization after Egg Activation

As meiosis resumed a complex pattern of microtubule reorganization occurred. Starting from anaphase I, heterogeneous bundling of microtubules was observed, ranging from parallel bundles to partial clustering (Fig. 2a) and some short microtubules were seen to radiate from a central core to form tiny arrays scattered in the egg cytoplasm (Fig. 2b). As meiosis progressed the aster-like configurations increased in number. During the first mitosis the egg was filled with many asters of different size (Fig. 2c). The central region of the cytoplasmic asters was strongly stained with β-tubulin antibody (Fig. 2d). Since the distribution of the cytoplasmic asters was homogeneous throughout the egg cytoplasm, the organization of the microtubules in astral arrays seems to be an intrinsic property of the whole egg cytoplasm. Hoechst 33258 staining revealed that the infecting bacteria clustered around the cytoplasmic asters (Figs. 2e, f), suggesting close association with the astral microtubules.

In eggs ($n = 22$) at the beginning of the first cleavage division some cytoplasmic asters appeared to interact with the mass of condensing chromosomes of the zygote (Fig. 2g). At this stage, the microtubules associated with the zygotic nucleus had no apparent bipolar organization. Only one aster was subsequently found on either side of the chromatin mass and the microtubules acquired a distinct bipolar organization. The poles of the first mitotic spindle had clearly visible astral microtubules (Figs. 3a, b) in embryos at metaphase ($n = 31$) and anaphase ($n = 26$) stages and look very much like those observed in embryos during subsequent mitotic divisions ($n = 59$; Figs. 3c, d). Cytoplasmic bacteria accumulated towards the poles of the mitotic apparatus and formed large clusters aligned with the spindle axis, but not associated with interpolar spindle microtu-

bules (Figs. 3a, b). This association was more evident during the subsequent nuclear divisions (Figs. 3e, f). Bacteria were not found at the poles of spindles without astral microtubules, namely the meiotic apparatus (see Fig. 1). These observations agree with previous results in which *Wolbachia* were found at the spindle poles during the syncytial mitoses of the early *Drosophila* embryo (Callaini *et al.,* 1994).

Asters Were Transiently Present in the Ooplasm

The assembly of microtubules into aster-like configurations was only observed during early development, and seemed to follow a cell cycle pathway. To evaluate if specific changes in dynamic assembly of the cytoplasmic asters were really correlated with different cell cycle stages of the egg/embryo, we looked at the number of aster-like structures during meiotic progression and the early mitoses. Figure 4 shows the mean and the range of astral numbers found in 30 eggs/embryos for each meiotic/mitotic stage examined. Unfortunately, it was impossible to exactly score all cytoplasmic asters in these samples because of the difficulty of counting tiny asters against the extensive cytoplasmic network of microtubules during meiosis, and because some asters could escape detection when very numerous, as during the first mitosis. However, this analysis clearly shows that cytoplasmic asters become visible from anaphase of the first meiosis, when we counted 11 to 27 asters, and increased in number as meiosis progressed. During telophase of the second meiosis we found 79 to 127 asters. The number of cytoplasmic asters was higher at prophase of the first mitosis (we counted 226 to 281 asters). The number of asters decreased rapidly during the following anaphase and telophase, when we counted 87 to 132 asters. Cytoplasmic asters were rare during the second mitosis and were no longer visible during the third and subsequent nuclear divisions. The disappearance of cytoplasmic asters during the third mitosis is confirmed by the observation that the bacteria, previously clustered near the astral microtubules, dispersed in the cytoplasm.

To verify if bacteria had some role in the formation of cytoplasmic asters during parthenogenetic activation, we also examined unfertilized eggs from a uninfected *Muscidifurax* strain. As a rule in Hymenopterans these haploid eggs give rise to male progeny. Aster-like configurations were found in the cytoplasm of these uninfected eggs, starting from anaphase of the first meiosis. In order to study the behavior of the cytoplasmic asters we scored their number from metaphase of the first meiosis until fourth mitosis in

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of microtubules (f) and γ-tubulin (g) reveals that the baskets of microtubules surrounding the blastodermic nuclei during cellularization (arrows) are associated with pairs of γ-tubulin spots (arrowheads). (h) Ultrastructural detection of centrioles at the center of cytoplasmic asters. Note the presence of electron dense material and microtubules (arrowheads) surrounding the centriole (arrow). Inset shows details of the centriolar structure. (i) Cross-section throughout an embryo at the beginning of the first cleavage division showing a centriole (arrow) near the nuclear region (N); arrowheads point nuclear pore complexes. (j) Cross-section of a cellularizing embryo at the same stage of that in f. Note the presence of centrioles (arrows) in the nucleating sites for the nuclear baskets of microtubules. Bar, 7.5 μm in a–g, 0.5 μm in h–j, and 0.2 μm in inset to h.

157 uninfected parthenogenetic eggs. To this purpose we examined 10-15 eggs/embryos for each meiotic/mitotic stage. This analysis (not shown) indicated that mean and range of the astral numbers was not different during early developmental stages between infected and uninfected parthenogenetic eggs. The presence of bacteria, therefore, has no influence on the formation of the astral arrays of microtubules.

Cytoplasmic Asters Contained Centrosomal Material and Centrioles

Immunofluorescence analysis of cytoplasmic asters revealed that they contained centrosomal material. We stained a sample of 194 *Muscidifurax* eggs at various stages of meiosis and early mitotic divisions with antibodies against CP190 ($n = 91$), a 190 kDa centrosome-associated protein in *Drosophila* (Frash *et al.,* 1986; Whitfield *et al.,* 1988, 1995) and γ-tubulin ($n = 103$). CP190 labeling was found in the center of the cytoplasmic microtubular foci (Figs. 5a, b). A double fluorescence showed that a bright γ-tubulin staining is also present in the center of cytoplasmic asters (Figs. 6a–c). γ-tubulin and CP190 were not detected in the cytoplasm of the *Muscidifurax* eggs before activation and were only found in association with the astral arrays of microtubules after meiosis resumed. To evaluate the localization of γ-tubulin following constitution of the zygotic centrosome, we examined early embryogenesis. γ-tubulin spots were detected in association with astral microtubules at the poles of the mitotic spindle at the beginning of the first cleavage division ($n = 27$; Figs. 6d, e) and through the following syncytial mitoses ($n = 53$; not shown). In cellularizing embryos ($n = 55$), bright foci of γ-tubulin were associated with the centrosomes that organized the microtubular baskets during nuclear elongation (Figs. 6f, g). CP190 (data not shown) was also found at the spindle poles in syncytial embryos ($n = 73$), and at the microtubular foci in cellularizing embryos ($n = 82$).

Cross-sections of 43 cytoplasmic asters from 19 eggs revealed that microtubules converged towards central foci containing electrondense material and centrioles (Fig. 6h). The ultrastructural organization of these cytoplasmic foci looks very much like the centrosomes that nucleated aster microtubules at the beginning of the first cleavage division ($n = 17$; Fig. 6i) and at various stages of the syncytial mitoses ($n = 21$; not shown). The same organization of the centrosome was found in embryos during nuclear elongation ($n = 25$; Fig. 6j). Ultrathin sections of the cytoplasmic asters showed that the centriole had a cartwheel structure with radial spokes, nine single microtubules, and a central tubule (Fig. 6h, inset). We cannot exclude the possibility that a second tubule was masked by the dense material that separated the peripheral tubules. If this is true the *Muscidifurax* centriole would be very similar to the centriole observed in the early *Drosophila* embryo (Callaini *et al.,* 1997). This ''immature'' morphology was unchanged when the centrioles were found at the poles of the mitotic spindles

in embryos at different developmental stages, from early syncytial mitoses to blastoderm cell formation.

DISCUSSION

Centrosome restoration must be precisely regulated during fertilization to avoid the formation of a cell with a double set of centrosomes. According to the hypothesis of Boveri (1901), the maternal centrosome is inactivated during oogenesis and the centrosome used in development is of paternal origin. Several findings support this point of view and in many organisms the sperm plays a prime role in centrosome formation at fertilization (see Schatten, 1994, for a review). However, during parthenogenetic development, the centrosome must be of maternal origin and egg cytoplasm must contain all the information necessary to form the zygotic centrosome. Early events of development have been studied in several artificially activated animal eggs, but surprisingly little is known about the natural process of parthenogenesis. As described here, the eggs of the hymenopteran *Muscidifurax uniraptor* have a number of characteristics that make them a suitable model for centrosome research during parthenogenetic development. First, *Muscidifurax* is easily maintained on pupae of several dipterans and the time of egg deposition is easily scored allowing for the acquisition of eggs at specific points in the meiotic and mitotic cell cycle. As a rule in insects the *Muscidifurax* oocytes are arrested at metaphase of meiosis I and parthenogenetic activation, occurring as the oocyte passes throughout the oviduct, induces resumption of meiosis and assembly of the first mitotic spindle. Finally, the infecting bacteria of the genus *Wolbachia* induce the diploidization of haploid eggs, resulting in lines producing only females. This makes the *Muscidifurax* eggs a unique natural model to study centrosome restoration during parthenogenetic development.

In this study we report that parthenogenetic activation of the *Muscidifurax* eggs leads to the resumption of meiosis and to the formation of several astral arrays of microtubules. These asters reached a maximum during the first mitosis, drastically decreased during the second mitosis, and were no longer visible at the third nuclear cycle. A large number of cytoplasmic asters, some of which take part in the organization of the first mitotic spindle, is also observed in the mouse zygote, where the centrosome is of maternal origin (Maro *et al.,* 1985; Schatten *et al.,* 1985, 1986, 1991). In *Muscidifurax* also some of the cytoplasmic asters are presumably involved in the formation of the first mitotic apparatus. The rapid disappearance of these asters once the first zygotic spindle is formed could be due to the fact that the asters are not stabilized by the interaction with the kinetochore or chromatin (Karsenti *et al.,* 1984).

The presence of γ-tubulin and CP190 antigens in the cytoplasmic asters of the parthenogenetic *Muscidifurax* egg seems to eliminate the possibility that the cytoplasmic asters were simply aggregation of microtubules, and suggests that they polymerize from discrete cytoplasmic

MTOCs. Indeed, γ-tubulin is a highly conserved molecule, localized to MTOCs and it is involved in microtubules nucleation (Oakley *et al.,* 1990; Horio *et al.,* 1991; Stearns *et al.,* 1991; Zheng *et al.,* 1991; Joshi *et al.,* 1992). Ultrastructural observations revealed that the cytoplasmic asters found in the *Muscidifurax* egg also contained centrioles and pericentriolar material from which microtubules nucleate. De novo formation of centrioles has been already reported in sea urchin eggs (Dirksen, 1961; Kuriyama and Borisy, 1983; Kallenbach, 1985) and lysates of *Spisula solidissima* oocytes (Palazzo *et al.,* 1992) after artificial activation, but the mechanism of their assembly is not clear. It has been proposed that pericentriolar material is involved in de novo centriole nucleation and in its location (Mazia, 1984; Gueth-Hallonet *et al.,* 1993). This material could also determine the assembly of pro-centrioles during each cell cycle (Kurijama and Borisy, 1981). γ-tubulin has been found both in the pericentriolar material and inside the proximal end of the centriolar cylinder of mammalian centriole and could play a role in centriolar duplication, perhaps acting as template for growth of the centriolar microtubules (Fuller *et al.,* 1995).

The observations that the meiotic apparatus is anastral and that a large number of asters assembled shortly after activation, suggest that in the *Muscidifurax* egg the cytoplasmic asters do not originate from the replication of a preexisting centrosome, but rather from a pool of cytoplasmic components that organized in well defined MTOCs after activation. This agrees with reports showing that *Xenopus* embryos contain sufficient components to assemble at least 1000-2000 centrosomes (Gard *et al.,* 1990). Centrosomal material has also been observed in unfertilized sea urchin and starfish eggs in which the maternal centrosome is lost during oogenesis and the functional zygotic centrosome is of paternal origin (Sluder *et al.,* 1989).

The mechanism by which the microtubule nucleating material becomes functional with egg activation is an intriguing question. It is known that activation of the egg, both during fertilization and parthenogenesis, is triggered by an increase in pH and internal concentrations of calcium ions (Steinhardt *et al.,* 1974; Cuthbertson and Cobbold, 1985; Swann, 1990; Kline and Kline, 1992; Vincent *et al.,* 1992). These conditions could also determine a change in microtubule polymerization properties, as suggested by the formation of astral arrays of microtubules in unfertilized sea urchin eggs after the raising of cytoplasmic pH to 8.5 (Harris and Clason, 1992; Schatten *et al.,* 1992) or incubation in hypertonic sea water (Kallenbach and Mazia, 1982; Kallenbach, 1985). It has been suggested that ovulation and *in vitro* hydration induce the increase of intracellular pH in *Drosophila* that triggers resumption of meiosis and microtubule reorganization (Mahowald *et al.,* 1983; Theurkauf *et al.,* 1992; Foe *et al.,* 1993; Page and Orr-Weaver, 1997). In *Muscidifurax* also the permeability of the egg envelopes could lead to hydration of the oocyte and the increase in intracellular pH could release the meiotic arrest and trigger the reorganization of the microtubular network. Phosphorylation of centrosomal components and accumulation of per-

icentriolar material are reported to be important mechanisms in the microtubule nucleating activity of centrosomes in cell-free extracts of *Xenopus* eggs (Ohta *et al.,* 1993). The observation that the bundles of microtubules progressively organize into well defined asters in newly laid *Muscidifurax* egg may indicate an initial phosphorylation of the microtubule associated proteins which control microtubule dynamics. In unfertilized sea urchin eggs the increasing concentration of agents that induce cytaster formation, as well as temperature and duration of treatment, determine a progress in microtubule organization from a loose network and spirals to astral arrays, and indicate that microtubule interactions are crucial for aster formation (Harris and Clason, 1992). In this system, movement of material related to centrosome proteins was observed along the astral microtubules in a minus-end direction. Several findings indicate that microtubules, together with cytoplasmic motor proteins, play a role in transporting intracellular components (Vale, 1987; Schroer, 1991; Waterman-Storer *et al.,* 1993). In *Muscidifurax* eggs the microtubule of the asters may aggregate and concentrate the centrosomal material dispersed in the cytoplasm. This may be confirmed by the observation that the dimensions of cytoplasmic asters increased in time, reflecting a gradual accumulation of materials for microtubule nucleating activity.

ACKNOWLEDGMENTS

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