# Non-Spindle Microtubule Organizing Centers in Metaphase II–arrested Mouse Oocytes

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ABSTRACT A human autoantiserum (5051) directed against pericentriolar material (PCM) was used to study the distribution of microtubule-organizing centers (MTOCs) in the oocyte and during the first cell cycle of mouse development. In oocytes, the PCM was found not only at the poles of the barrel-shaped metaphase II spindle but also at many discrete loci around the cytoplasm near the cell cortex. The spindle poles were also composed of several PCM foci. In metaphase-arrested eggs only the PCM foci located near the chromosomes acted as MTOCs. However, after reduction of the critical concentration for tubulin polymerization by taxol, the cytoplasmic PCM foci were also found to be associated with nucleation of microtubules. After fertilization the cortical PCM foci remained in a peripheral position until the end of the S phase, when they appeared to migrate centrally towards the pronuclei. At prometaphase of the first mitotic division, numerous MTOCs were found around the two sets of chromosomes; these MTOCs then aligned to form two bands on either side of the metaphase plate of the first mitosis.

Microtubule organizing centers (MTOCs)[1] are cytoplasmic structures that form the poles of the mitotic spindle and organize the interphase cytoplasmic microtubule network in all animal cells. In most animal cells, the MTOC is composed of a pair of centrioles surrounded by an electron-dense material, the pericentriolar material (PCM). Centrioles and PCM constitute what is called the centrosome (for review see references 5, 9, 24, and 25). Within the centrosome, it is the PCM rather than the centriole that has the nucleating activity (12). Kinetochores are also MTOCs and can nucleate microtubules during mitosis (9, 41). The mechanisms by which centrosomes and kinetochores can promote microtubule growth around them have been analyzed recently in vitro (25–28) and in vivo (9). At least two mammalian cell types do not have centrioles in their MTOCs: the myotube, in which the MTOC consists of many PCM aggregates distributed around each nucleus (38), and the oocyte, in which the poles of the spindle are made up of bands of PCM with no centriole (7, 37). Whereas myotubes are a terminal stage of differentiation and do not undergo further division, in the egg numerous divisions will occur after fertilization, the first four or five in the absence of centrioles (36). The absence of centrioles in a cell line derived from a sterile mutant of *Drosophila melanogaster* has been also demonstrated (8).

In this paper we demonstrate by both immunofluorescence using an antiserum reactive with PCM (7), and by a microtubule nucleation assay in the presence of taxol, the existence in the mouse egg of MTOCs that are not associated with the meiotic spindle. These cytoplasmic MTOCs do not nucleate microtubules in the metaphase-arrested egg presumably because of the high critical concentration for tubulin polymerization that exists in the cytoplasm. In contrast, the critical concentration seems to be decreased near the chromosomes, where nucleation of microtubules occurs successfully around the PCM foci at the spindle poles. After fertilization (in interphase) all of the PCM foci become active as nucleating centers regardless of their spatial relationship to the chromosomes and subsequently participate in the formation of the poles of the spindle at the first mitotic division.

## MATERIALS AND METHODS

*Recovery and Fertilization of Oocytes:* A sperm suspension was prepared from the cauda epididymides of male HC-CFLP mice (Hacking and Churchill). Two epididymides were immersed in each 0.5-ml drop of Whittingham's medium (43) containing 30 mg/ml bovine serum albumin (BSA, Sigma

[1] *Abbreviations used in this paper*: HPEM, buffer of HEPES, PIPES, EGTA, and $MgCl_2$; MTOC, microtubule organizing center; PCM, pericentriolar material.

Chemical Co., Poole, U.K.) under liquid paraffin (BDH Chemicals Ltd., Poole, U.K.), which had been equilibrated overnight at 37°C in 5% $CO_2$ in air. The spermatozoa were released into suspension and left to capacitate for 1.5 h at 37°C.

Oocytes were recovered from 3–5-wk old (C57Bl.10 × CBA) $F_1$ or MFl mice after superovulation with 5 IU pregnant mares serum (Intervet) followed 48 h later by 5 IU human chorionic gonadotrophin (Intervet, Cambridge). The females were killed 12.5 h after the administration of human chorionic gonadotrophin and the ovulated oocytes were released from the oviducts into preequilibrated drops of Whittingham's medium containing 30 mg/ml BSA. 13.5 h after the administration of human chorionic gonadotrophin, the sperm suspension was mixed and diluted 1:9 in the drops containing the oocytes, giving a final sperm concentration of 1–2 × $10^6$/ml.

Eggs that were cultured for more than 4 h were removed from the spermatozoa and washed two or three times in preequilibrated medium 16 (42) containing 4 mg/ml BSA. The eggs were then cultured further in this medium until harvesting.

Unfertilized oocytes were treated in the same way, with the simple omission of the addition of the sperm suspension.

*Harvesting of Eggs and Oocytes for Analysis:* Oocytes were freed of their cumulus cells by brief exposure to 0.1 M hyaluronidase (Sigma Chemical Co.), and all oocytes and fertilized eggs were removed from their zonae pellucidae by brief exposure to acid Tyrode's solution (30) and then rinsed in medium 2 (11) containing 4 mg/ml BSA.

*Cell Fixation and Immunocytological Staining:* Cells were then placed in specially designed chambers as described by Maro et al. (21) except that the chambers were coated first with a solution of 0.1 mg/ml concanavalin A.

The cells were then fixed in one of two says. (*a*) For tubulin staining with an anti-alpha tubulin monoclonal antibody (Amersham International, Amersham, U.K.) we used our standard fixation procedure (21): the cells were fixed for 30–45 min at 20°C with 3.7% formaldehyde (BDH Chemicals Ltd.) in phosphate-buffered saline (PBS), washed in PBS, then extracted for 10 min in 0.25% Triton X-100 (Sigma Chemical Co.) and washed in PBS.

(*b*) For optimal staining of PCM with the anti-PCM serum (7), cells were either extracted for 30 min in buffer M (25% glycerol, 50 mM KCl, 1 mM EGTA, 0.1 mM EDTA, 0.5 mM $MgCl_2$, 1 mM mercaptoethanol, 50 mM imidazole, pH 6.7; reference 3) containing 1% Triton X-100 for 30 min, washed in buffer M, and finally fixed for 30 min with 3.7% formaldehyde in buffer M, or extracted for 10 min in HPEM buffer (25 mM HEPES, 60 mM PIPES, 10 mM EGTA, 1 mM $MgCl_2$, pH 6.9; reference 35) containing 0.25% Triton X-100, washed in HPEM buffer and fixed for 30 min with 3.7% formaldehyde in HPEM buffer. By extracting the cells with a detergent before fixation, these procedures were able to reduce the cytoplasmic background to a level that allowed us to observe clearly the PCM foci.

Immunocytological staining was performed as described by Maro et al. (21) using fluorescein-labeled anti-human immunoglobulin antibodies or rhodamine-labeled anti-mouse immunoglobulin antibodies (affinity purified; gift of Dr. A. Goodall; Miles Laboratories Inc.; Cappel Laboratories; Institute Pasteur Production, Paris) as second layers. To stain chromosomes, fixed cells were incubated in Hoechst dye 33258 (5 μg/ml in PBS) for 20 min.

The anti-PCM serum used in our study has been characterized by immunofluorescence in a large variety of vertebrate cells and by immunoelectron microscopy in mouse cells (7): it stains antigen(s) associated mainly with the PCM and slightly with the spindle fibers and the mid-body. The molecular nature of the antigen(s) recognized by the anti-PCM serum has not been identified yet by immunoblotting in mammalian cell lines (Kirschner, M., personal communication) or in mouse oocytes (our unpublished observations using up to 800 oocytes per sample).

The HPEM fixation procedure was used to perform the double immunofluorescence experiments (PCM and tubulin). Because of the large size of the oocyte (70–80 μm in diam), which increases the background staining, and of the slight cross-reactivity of second layer antibodies the resolution was decreased (most fluorescein-labeled anti-human immunoglobulin antibodies tested could bind to mice immunoglobulins; the best one, a gift of Dr. A. Goodall, was used in these double labeling experiments).

*Photomicroscopy:* The coverslips were removed from the chambers and samples were mounted in Citifluor (City University, London) and viewed on a Leitz Ortholux II microscope with filter sets I2 for fluorescein-labeled reagents, and A for Hoechst dye. Photographs were taken on Kodak Tri-X film using a Leitz Vario-Orthomat photographic system.

*Drugs:* A stock solution of 12 mM taxol (lot T-4-112; gift of the National Institutes of Health, Bethesda, MD) in dimethylsulfoxide and a stock solution of 10 mM nocodazole (Aldrich Chemical Co., Ltd., Gillingham, U.K.) in dimethylsulfoxide were used in these experiments.

## RESULTS

### PCM Distribution in Oocytes

At the time of ovulation mouse oocytes are arrested at metaphase of the second meiotic division. The metaphase II meiotic spindle is located peripherally and parallel to the cell surface (Fig. 1, *a* and *b*). The spindle poles are composed of several foci of electron dense material with no associated centrioles (37). When such eggs were stained with the anti-PCM serum, we first confirmed the presence, at each pole of the spindle, of a broad band of fluorescence (7) within which aggregates can sometimes be observed (Fig. 1). We also observed numerous fluorescent foci dispersed throughout the cytoplasm of the egg, but mainly near the cell surface (Fig. 1). The staining pattern was similar when eggs with zonae pellucidae were used, the only difference being a higher background staining. A mean number of 10.7 ± 3.7 dispersed foci per cell was observed in a group of 223 oocytes; the distribution pattern is shown in Fig. 2.

### Spindle MTOCs Are Composed of PCM Foci

To investigate whether the broad bands of PCM at the poles of the meiotic spindle were composed of one or more aggregates of PCM, we used nocodazole, a drug that inhibits tubulin polymerization (14), to disassemble the spindle. When oocytes are incubated in 10 μM nocodazole for 4 to 6 h, the spindle is destroyed and the chromosomes are dispersed and redistributed around the egg cortex (21*a*). After such a treatment, bands of PCM material were no longer evident and only small PCM foci were observed in the oocytes (Fig. 3, *a* and *b*). The mean number of PCM foci in nocodazole-treated oocytes was increased by ~6 as compared with control oocytes (14.9 ± 4.4 vs. 9.4 ± 3.2; Fig. 4). This result suggests that, on the

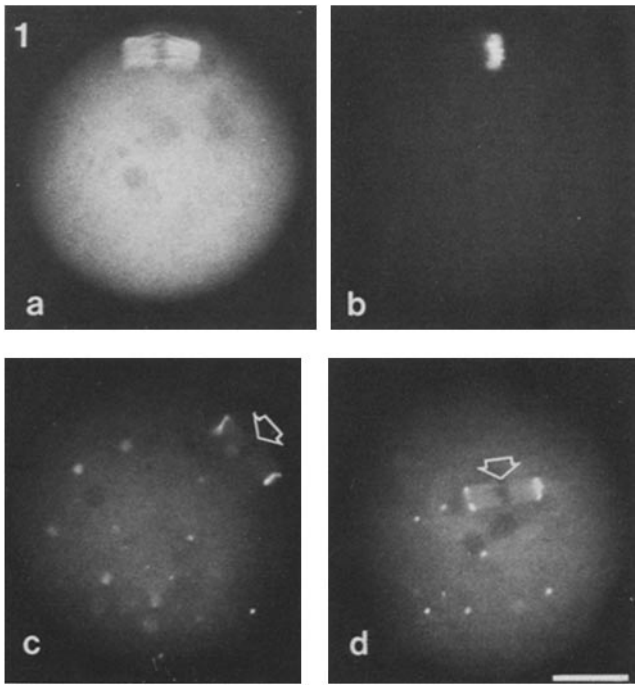



FIGURE 1 (*a* and *b*) Oocyte double stained for tubulin (*a*) and chromatin (*b*). (*c* and *d*) Distribution of PCM in mouse oocytes stained with the anti-PCM serum. Arrows point to the metaphase plate. Bar, 25 μm. × 400.

average, each spindle pole is composed of about three PCM foci that become dispersed on spindle dissolution.

## PCM Foci and Microtubule Nucleating Activity Are Associated

Unfertilized eggs were treated with taxol (33) to induce a small decrease in the critical concentration for tubulin polymerization. Such an approach should enable us to see if the PCM foci are associated with a microtubule nucleation activity. Wide ranges of taxol concentrations and incubation times were tested (Table I). At very low doses of taxol, we were able to see only large bundles of microtubules in the spindle area in some cells (Fig. 5*a*). For higher doses (and/or longer incubation times) cytoplasmic asters located near the cell surface were observed (Fig. 5, *b–f*). At high doses (and/or long incubation times) a network of free cytoplasmic microtubules masking first the cytoplasmic asters and then the spindle bundles was apparent (data not shown). In addition to qualitative changes in the microtubule arrays induced under different taxol conditions, we also observed quantitative differences. Thus, as the dose or duration of taxol incubation increased, the number of cytoplasmic asters also increased. This increase was paralleled by a corresponding increase in the number of PCM foci detected by the anti-PCM serum in the cytoplasm of taxol-treated cells (Fig. 3, *c* and *d* and Table II, Exp. 1). However, we did not observe as many asters as PCM foci. When the number of PCM foci and the number of asters were assessed in parallel experiments, we observed significant differences between these two distributions (Table II, Exp. 2). This discrepancy could have arisen because some PCM foci were not associated with a nucleating activity under the experimental conditions, or because several PCM foci were present inside a large aster, or because of the difficulty in counting smaller asters against the extensive cytoplasmic network of microtubules.

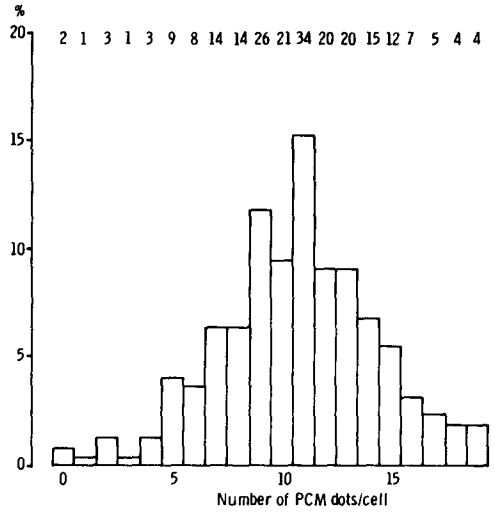



FIGURE 2 PCM foci distribution in mouse oocytes (20 h after the addition of human chorionic gonadotrophin, $n = 233$). The figures at the top indicate the actual number of oocytes within each class.

To resolve which of these alternatives is correct and to demonstrate that the PCM foci co-localize with the tubulin asters, we undertook some double staining experiments with the anti-PCM serum and anti-tubulin antibodies. It was possible to determine unequivocally that the PCM foci were located in the center of the microtubule asters (Fig. 6), and that, in some cases, two PCM foci were located inside a single aster (Fig. 6, *c–f*, arrowheads).

From these results we conclude that (*a*) PCM foci may be able to nucleate microtubules, but in metaphase-arrested eggs the critical concentration for tubulin polymerization in the cytoplasm is high, and only near the chromosomes is it low

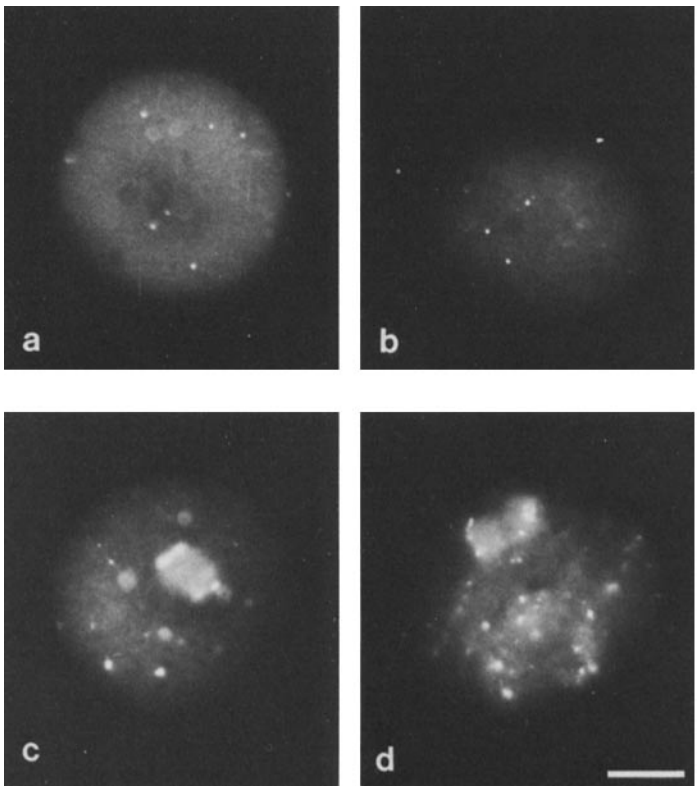



FIGURE 3 (*a* and *b*) Effect of nocodazole (10 μM for 4 h) on PCM foci distribution in mouse oocytes. The cells were stained with the anti-PCM serum. (*c* and *d*) Effect of taxol on PCM foci distribution in mouse oocytes. (*c*) 1.5 μM taxol for 5 min at 37°C; (*d*) 24 μM taxol for 5 min at 37°C. Bar, 25 μm. × 400.

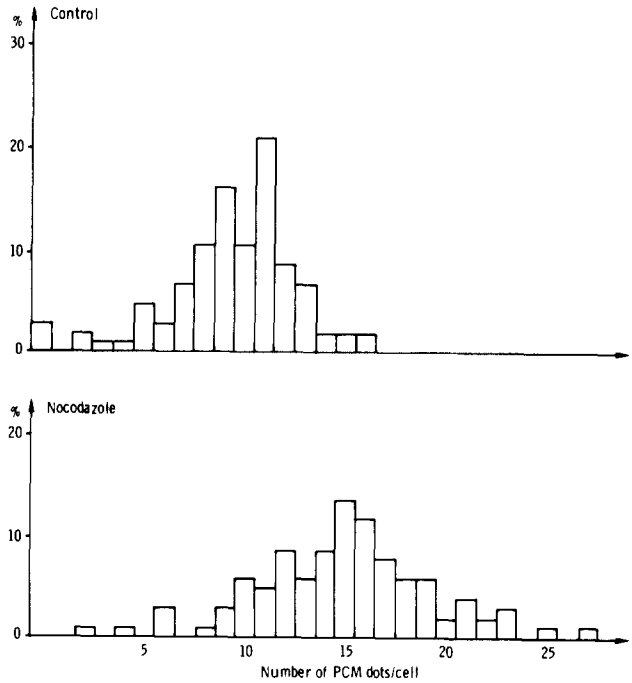



FIGURE 4 Effect of 10 μM nocodazole for 6 h on PCM foci distribution in mouse oocytes. Control (top histogram; $n = 105$) and treated (bottom histogram; $n = 103$) cells come from the same groups of oocytes, which were stained simultaneously.

TABLE I. *Effect of Taxol on Microtubule Nucleation in Oocytes*

| Taxol | Time at 37°C | Number of cells analyzed | Cells with spindle bundles | Number of cytoplasmic asters detected |
|---|---|---|---|---|
| *μM* | *min* | *%* | *mean ± SD* | |
| 0.4 | 1 | 16 | 12 | 0 |
| | 5 | 41 | 78 | 9.3 ± 3.3 |
| | 15 | 33 | 91 | 13.2 ± 4.3 |
| | 60 | 20 | 100 | 17.6 ± 4.2 |
| 1.5 | 1 | 6 | 100 | 0 |
| | 5 | 30 | 93 | 9.1 ± 3.2 |
| | 15 | 28 | 75 | 16.8 ± 5.1 |
| | 60 | 15 | 100 | 15.0 ± 5.6 |
| 3 | 1 | 39 | 79 | 0 |
| | 5 | 56 | 89 | 11.2 ± 2.3 |
| | 15 | 25 | 96 | 10.0 ± 1.8 |
| | 60 | 48 | 94 | 17.0 ± 4.8 |
| 6 | 1 | 29 | 86 | 13.5 ± 5.5 |
| | 5 | 47 | 85 | 13.4 ± 3.2* |
| | 15 | 19 | 68 | NS |
| | 60 | 23 | 78 | NS |
| 24 | 1 | 16 | 94 | NS |
| | 5 | 19 | 84 | NS |
| | 15 | 19 | 58* | NS |
| | 60 | 13 | 46* | NS |

NS, Not scorable; the cytoplasmic network of free microtubules is almost completely masking the asters.
* The cytoplasmic network of free microtubules is masking some spindle bundles or asters.

enough for nucleation to occur; and (*b*) exposure to taxol leads to an increase in the number of PCM foci and asters.

## *Fate of PCM Foci During the First Cell Cycle of Mouse Embryogenesis*

After fertilization, the second meiotic division resumes. The anaphase spindle rotates and the second polar body is extruded. Later pronuclear formation takes place and the two pronuclei migrate towards the center of the egg during the end of the G1 phase (15, 21). We found that throughout this period the cytoplasmic PCM foci remained peripheral, the only difference observed being a decrease in the intensity of the staining during G1. During S phase,the PCM foci were found in the cytoplasm at various distances from the plasma membrane, and by the end of the S phase the PCM foci surrounded the nuclei (Fig. 7, *e–h*). At prometaphase of the first cleavage division, the PCM foci were distributed around the two sets of haploid chromosomes (Fig. 7, *i* and *j*) and subsequently formed two bands on either side of the chromosomes at the poles of the metaphase and anaphase spindle (Fig. 7, *k–p*). Very rarely were PCM foci observed free in the cytoplasm during mitosis. After mitosis, two groups of PCM foci were observed in contact with the nuclear envelope of each daughter cell (Fig. 7, *q* and *r*).

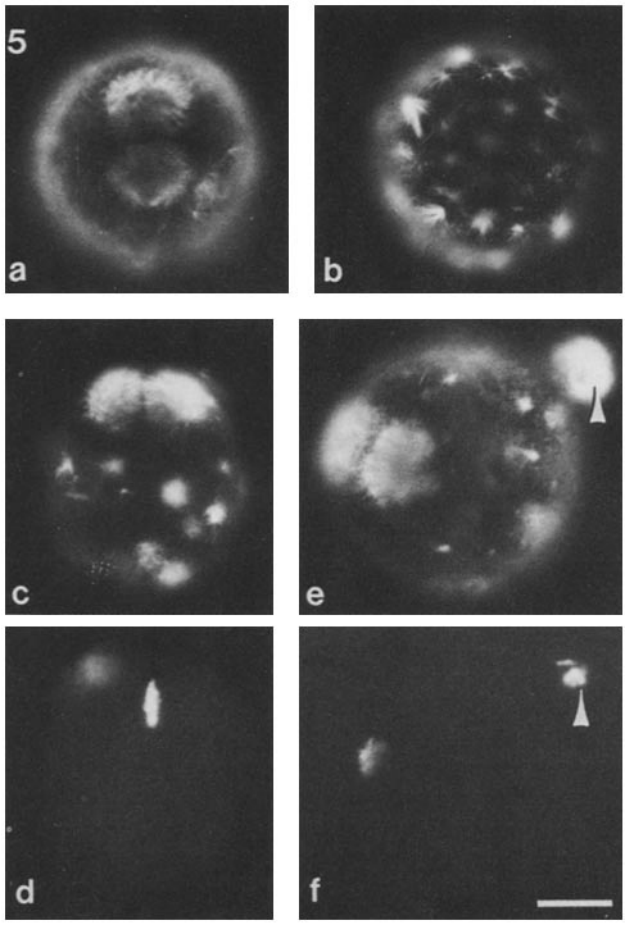



FIGURE 5 (*a* and *b*) Effect of taxol on microtubule distribution in mouse oocytes. (*a*) 1.5 μM taxol for 1 min at 37°C; (*b*) 6 μM taxol for 5 min at 37°C. (*c–f*) Oocytes treated with 6 μM taxol for 1 min at 37°C double stained for tubulin (*c* and *e*) and chromatin (*d* and *f*). Note the large microtubule bundles associated with the chromosomes. Arrowheads in *e* and *f* point to the first polar body. Bar, 25 μm. × 400.

## DISCUSSION

In this paper we describe the presence of subcortical cytoplasmic foci that react with an antiserum to PCM (7) and are not associated with the spindle in the meiotic oocyte. We

TABLE II. *Effect of Taxol on the Number of PCM Foci in Oocytes*

| Taxol 5 min at 37°C | Number of cells analyzed | Number of PCM foci per cell | Number of asters per cell |
|---|---|---|---|
| *μM* | *mean ± SD* | *mean ± SD* | |
| 0 | 223 | 10.7 ± 3.7 | |
| Exp. 1 | | | |
| 0.4 | 18 | 11.9 ± 4.0 | |
| 1.5 | 12 | 13.4 ± 3.4 | |
| 6.0 | 22 | 23.0 ± 8.6 | |
| 24.0 | 17 | 24.4 ± 6.1 | |
| Exp. 2 | | | |
| 1.5* | 20 | | 11.1 ± 2.3 |
| | 25 | 13.6 ± 3.3 | |
| 6.0‡ | 55 | | 12.6 ± 3.0§ |
| | 46 | 17.9 ± 4.6 | |

* $P < 0.01$ (*t* test).
‡ $P < 0.001$ (*t* test).
§ The cytoplasmic network of free microtubules is masking some asters.

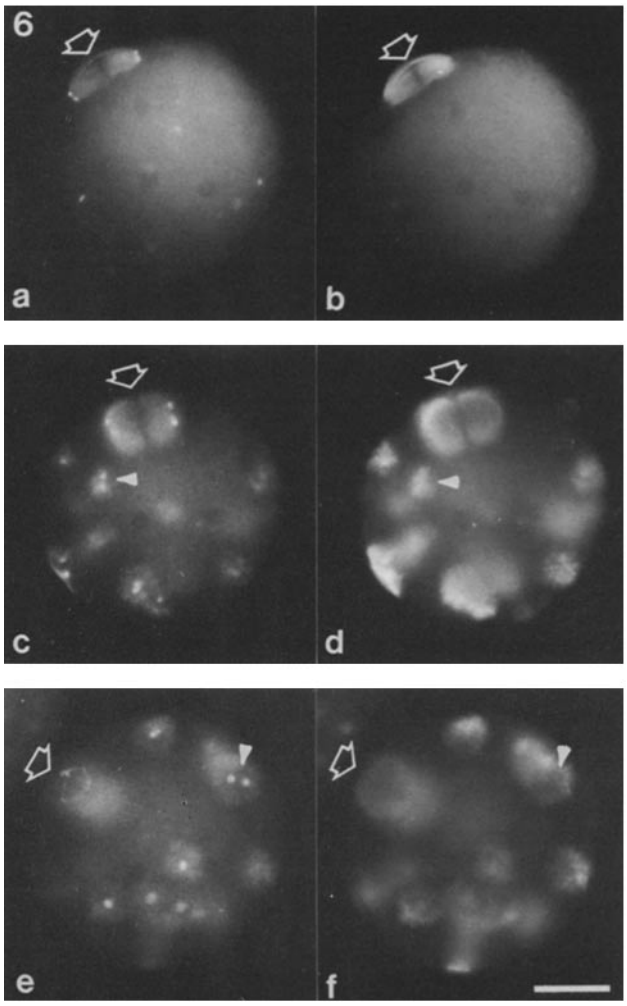



FIGURE 6 (*a* and *b*) Oocyte, double stained with the anti-PCM serum (*a*) and the anti-tubulin antibody (*b*). Arrows point to the metaphase plate. (*c–f*) Oocytes, treated with 6 μM taxol for 1 min at 37°C, double stained for PCM (*c* and *e*) and tubulin (*d* and *f*). Note the PCM foci in the center of the microtubule asters. Arrows point to the metaphase plate. Arrowheads point to asters that contain two PCM foci. Bar, 25 μm. × 400.

believe that they are foci of PCM with an associated MTOC activity for the following reasons: (*a*) They stain with an antibody that recognizes a determinant associated with MTOCs in many types of cells, including those at the meiotic and mitotic spindle poles in mouse oocytes and embryos (7; Maro, B., and S. J. Pickering, unpublished observation). (*b*) Immunoreactive PCM foci participate in the formation of the first mitotic spindle of the mouse embryo. (*c*) The staining intensity of these PCM foci decreases in telophase and G1, periods that correspond with a decrease in the nucleating capacity of the MTOCs (2, 18). (*d*) The poles of the meiotic spindle seem to be composed of identical structures, as shown by their apparent dispersion after nocodazole treatment; (*e*) the distribution and the number of these foci during the first cell cycle of mouse development corresponds with the distribution of microtubular asters (12.9 ± 3.5) described by Schatten et al. (33; see Fig. 8). (*f*) Taxol treatment of metaphase-arrested oocytes induces the formation of many asters centered on these foci.

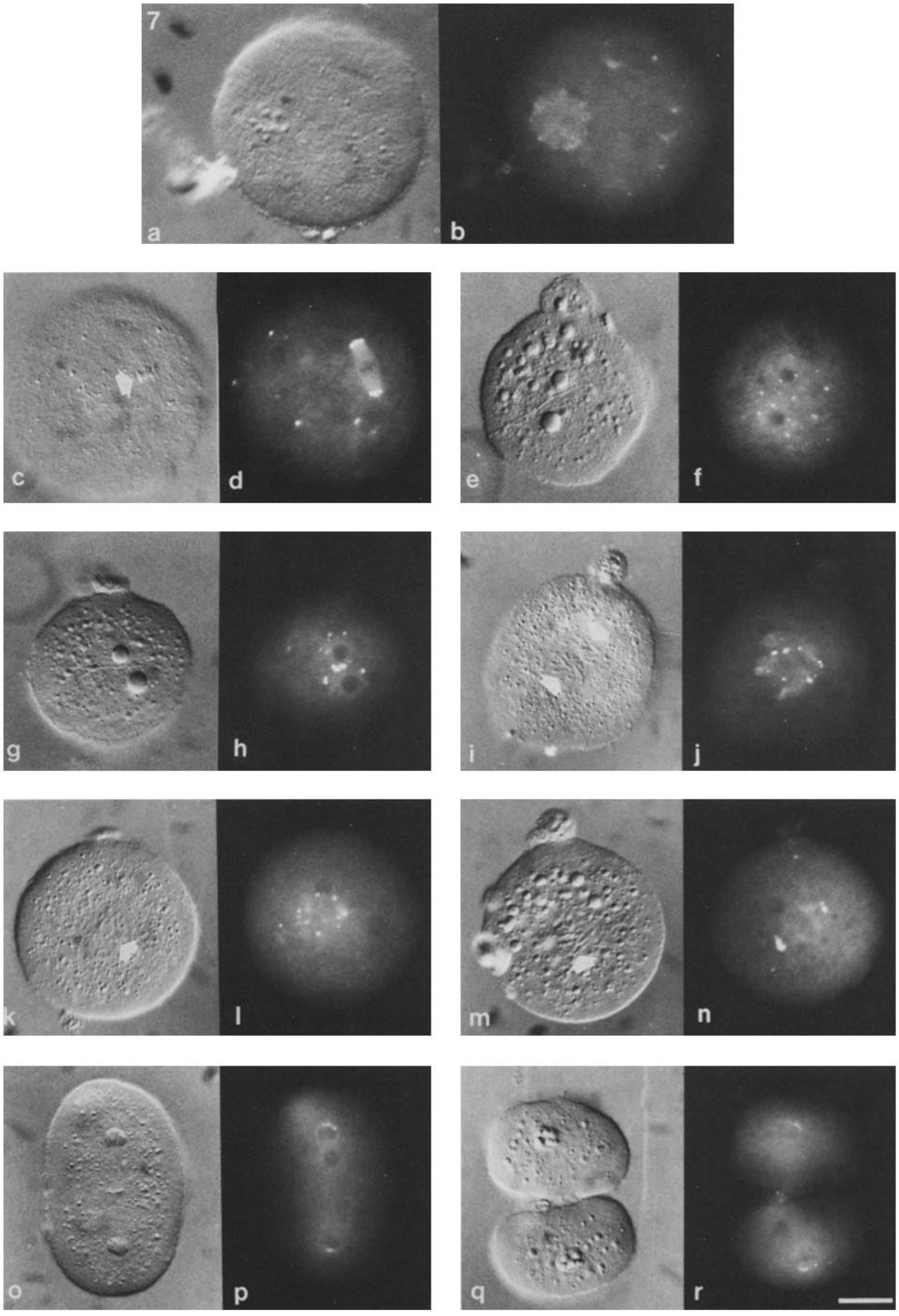
It could be argued that the subcortical location of the PCM foci we observed resulted from an artifactual dispersion during manipulation and fixation, from an original location at the spindle poles. However, we think that this is highly improbable for the following reasons: all the PCM foci observed during first mitosis remain associated with the spindle despite use of similar processing techniques; a range of extraction and fixation techniques has been tested and all gave the same results; the same pattern was observed in eggs from which the zonae were not removed; and taxol-induced asters were also observed dispersed around the cell cortex under very different processing conditions.

The existence of multiple MTOCs in animal cells has been documented only in a tumor cell line, N115. These cells have many MTOCs, each composed of a single centriole surrounded by PCM, and can form bipolar mitotic spindles with unequal numbers of MTOCs at each pole (31). In contrast, spindle poles composed of an aggregate of PCM with no centriole have been observed during tripolar mitosis in Chinese hamster ovary cells (17).

The effect of nocodazole on the spindle poles is comparable to its effect on centrioles. In animal cells, nocodazole (or colcemid) leads to a splitting of the centrosome into two parts, both of which have a centriole cylinder (22, 40). The broad bands of PCM, which correspond to the linear centrosome of Mazia (23), can also be dissociated by the drug into smaller units that resemble the cytoplasmic MTOCs.

Taxol increases the number of PCM foci, a result that has also been observed in HeLa cells, where the drug induces the formation of multiple asters in mitotic cells. Each aster is formed around a small aggregate of PCM as visualized by an anti-PCM serum different from that used here (see reference 5, p. 121). Taxol could induce such a result either by fragmentation of the MTOCs or by concentration of previously dispersed PCM into visible foci. Taxol-induced asters that lack PCM have been described in PtK2 cells (10). However, in this electron microscopic study, difficulties in the identification of the PCM could occur in the absence of a specific marker and of serial sections.

In contrast to the mouse oocyte, in unfertilized oocytes of *Xenopus*, taxol does not induce the formation of multiple asters (13). But in *Xenopus* it is the sperm centrosome that is involved in the formation of the poles of the cleavage spindles, and the egg does not seem to contain a functional MTOC. In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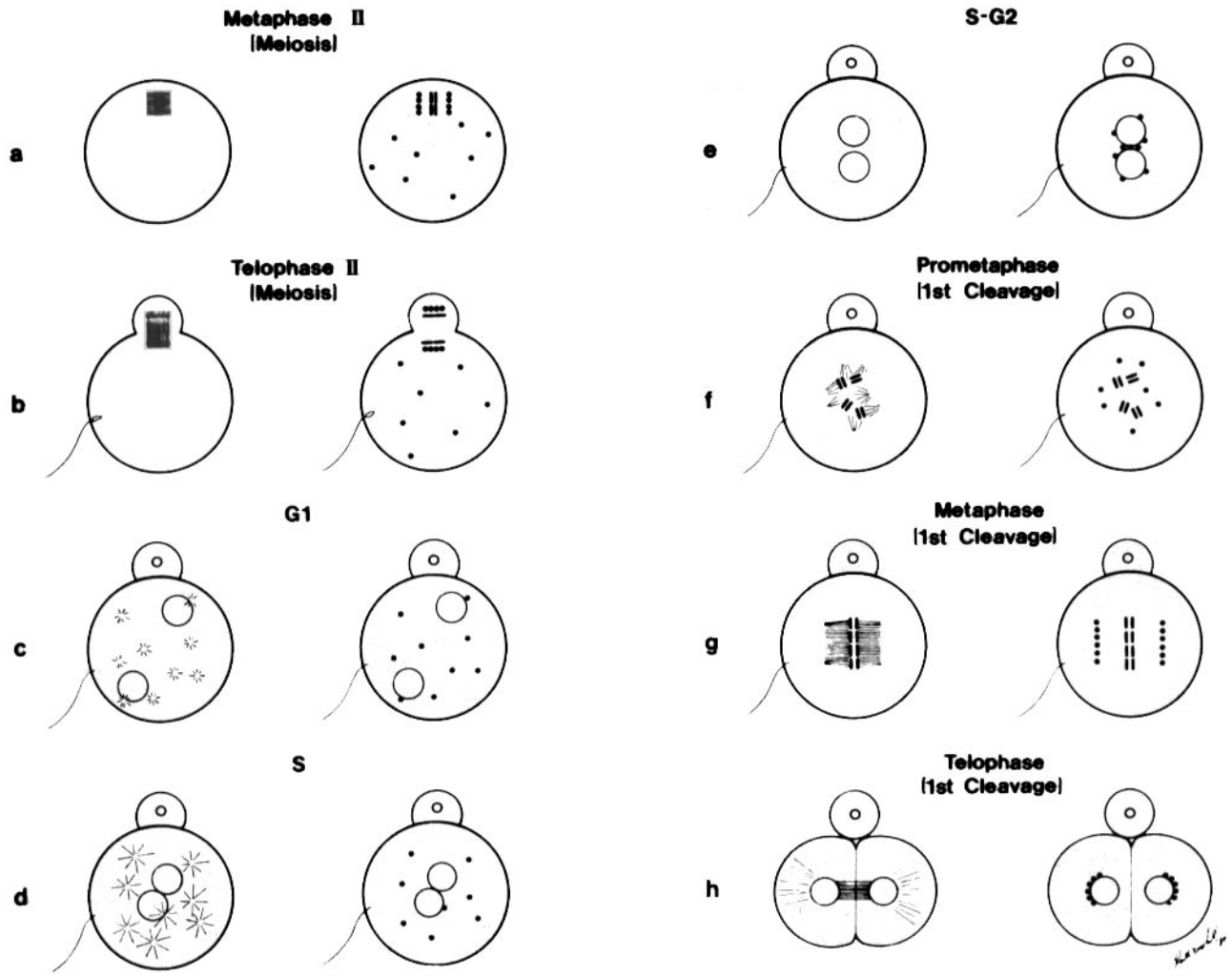


FIGURE 8 Schematic representation of the distribution of microtubules (*left*, from reference 33) and MTOCs (*right*) during the first cell cycle of mouse embryogenesis.

*Haemantus* endosperm, taxol does induce the formation of multiple nucleating centers in prometaphase cells (29), but the distribution of the MTOC material has not been studied. The fact that taxol induces an increase in the number of PCM foci (and in their nucleating capacity) could explain the differences observed in sea urchin eggs between the effect of taxol and heavy water, both of which decrease the critical concentration for tubulin polymerization, where taxol induces the formation of many more asters than does heavy water (32).

During this study we observed an interesting effect of the chromosomes on microtubule nucleation in that the apparent critical concentration for tubulin polymerization was decreased near the chromosomes. A similar result has been observed in *Xenopus* eggs (16). In the latter study, centrosomes, which were injected into the cytoplasm of a metaphase-arrested egg, could not nucleate microtubules unless located near the chromosomes. However, after activation of the egg, all of the injected MTOCs throughout the egg could nucleate. Similarly, in the mouse egg, only spindle microtubules can be detected during meiosis (21*a*, 33, 39), but multiple cytoplasmic asters have been described in interphase just after fertilization (33). Although this effect of the condensed chromosomes on microtubule nucleation might be mediated through the kinetochores (8, 9, 41), in vitro a much higher concentration of tubulin is required by kinetochores to nucleate microtubules than is required by centrosomes (25, 27).

The PCM foci observed in these experiments are evidently located at the periphery of the germinal vesicle and are released into the cytoplasm when the germinal vesicle breaks down (6; see also Fig. 7, *a* and *b*). They become relocated adjacent to the nuclear envelope only after fertilization at the end of S phase and during G2. This association between MTOCs and the nuclear envelope is also a feature of the

FIGURE 7 Distribution of PCM in oocytes at the germinal vesicle breakdown stage (*a* and *b*), metaphase II oocytes (*c* and *d*), and fertilized mouse eggs (*e–r*) stained with the anti-PCM serum (*b, d, f, h, j, l, n, p,* and *r*). *a, c, e, g, i, k, m, o,* and *q* are the corresponding differential interference contrast images. (*a* and *b*) Germinal vesicle breakdown. (*c* and *d*) Metaphase II (meiosis). The arrow points to the metaphase plate. (*e* and *f*) Early S phase. (*g* and *h*) Late S–early G2 phase. (*i* and *j*) Prometaphase. Note the two haploid sets of chromosomes (arrows). (*k* and *l*) Prometaphase, the two sets of chromosomes are now mixed (arrow). (*m* and *n*) Metaphase. (*o* and *p*) Late anaphase. (*q* and *r*) Telophase. Bar, 25 μm. × 400.

centriolar MTOCs in animal cells (4, 18, 20).

The main question that remains to be answered is, What is the role of these non-spindle MTOCs? One possibility may be that, in order to mix the two haploid sets of chromosomes in such a big cell, multiple peripheral asters are necessary to "push" the pronuclei towards the center of the egg. Microtubules are indeed necessary for the migration of the pronuclei (21*a*, 33). But in bigger eggs such as sea urchin eggs, where microtubules are also involved in pronuclear migration, normal centriolar MTOCs are present. In the sea urchin the sperm aster, which is associated with the male pronucleus, migrates to the egg center and then the female nucleus migrates into the center of the sperm aster. Later the two pronuclei fuse (32). In the latter system, multiple nucleation sites for microtubules have been observed after a decrease in the critical concentration for tubulin polymerization with taxol or heavy water (32). Another possibility may be that these multiple MTOCs are necessary for the mixing of the chromosomes at the first mitotic division, since the two haploid sets of chromosomes are surrounded by numerous half-spindles, which later gather in two groups to form the metaphase spindle (44; Fig. 7). But approximation (which is the alignment of the two haploid sets of chromosomes on a common metaphase plate after dissolution of the pronuclear envelopes) is possible with only two centrosomes, as shown in nematodes (1). Finally, these multiple MTOCs (~16 per egg, including the spindle foci) could constitute a stock of MTOCs that will be used during the initial four or five cell cycles of development. Definitive centrioles are only observed at the 16-cell stage in a few cells (36) and at the blastocyst stage in most cells (19, 36). It is not until the blastocyst stage that new synthesis of PCM is observed (7). If all MTOCs within the oocyte were at the poles of the meiotic spindles, then formation of the two polar bodies would result in the loss of 75% of the MTOCs from the oocyte. Perhaps the dispersed pattern of most PCM foci is no more than a measure of cellular economy. Further investigations on these structures and their possible roles are underway.

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