Microtubule organization in mouse oocytes after micro-injection of sea urchin sperm heads, midpieces and centrosomal complexes

SARA STEFFEN ZORAN, CALVIN R SIMERLY and GERALD SCHATTEN

Department of Zoology, University of Wisconsin-Madison, Madison, USA

The centrosome, typically composed of a centriole pair and the pericentriolar material, is responsible for microtubule nucleation, forming a bipolar spindle, determining cleavage axes, and cell locomotion. It is crucial that only one centrosome, or a single pair after replication, is present per cell. Evolutionarily, fertilization posed a particular problem because two cells, each with their own centrosome, would unite to form a single zygote. During gametogenesis, this problem is resolved by either eliminating a complete centrosome in one gamete (sperm or egg) while maintaining a complete centrosome in the other gamete. An alternative means would be to reduce the centrosome in each gamete by exactly half.

In this study we examined the experimental case where multiple centrosomes of differing origin co-exist in the same cytoplasm. Specifically, what are the effects of two dominant centrosomes co-existing in the same cytoplasm? Do the effects change as the cell cycle changes? Is there a temporal effect? To answer these questions, sea urchin sperm heads with intact centrosomes and distal centrioles were isolated and microinjected into metaphase II arrested mouse oocytes or pronucleate stage eggs. After different culture periods, some groups were treated with ethanol, cold, or nocodazole in order to view the cell at a particular stage in the cell cycle. Cells were then fixed, processed for immunocytochemistry, and analyzed by various microscopic methods. Results show that microinjected sperm heads induce different patterns of microtubule organization at or near the sperm head in a meiotic and mitotic cell but not in an interphase cell. Furthermore, the type of microtubule pattern changes over time. The microtubule organizing centers nucleating these microtubules are suggested to be of mouse and sea urchin origin initially and of mouse origin after an extended culture period. Finally, the imported sea urchin sperm chromatin does not undergo the decondensation/condensation cycle in tandem with the mouse oocyte chromatin. These results suggest that a supernumerary centrosome can function within a foreign, host cytoplasm and will follow the known "rules" concerning microtubule nucleation. This is only a temporary situation, however, and the extra centrosome will eventually degrade so as to return the cell to the normal number of centrosomes.

Key words: *centrosomal polyploidy, centrosome isolation, microinjection, microtubule nucleation, microtubule organizing centers.*

INTRODUCTION

The centrosome is a very complex and important organelle whose composition, replication and function are not completely understood. Mazia (1984) envisions the centrosome as a flexible, beaded string with the beads being potential sites for

Correspondence to: Sara S. Zoran, 317 Zoology Research Bldg., Zoology Department, University of Wisconsin-Madison, 1117 W. Johnson St., Madison, WI 53706, USA. Fax: (1-608) 262-7319

microtubule (MT) nucleation or microtubule nucleation centers (MTNCs). He suggests this string can be folded into different configurations, each leading to different three dimensional patterns of the MTNCs and thus a different microtubular structure. Recently, a new tubulin isoform, γ-tubulin, has been identified and localized to the centrosome (Oakley *et al*, 1990; Stearns *et al*, 1991; Zheng *et al*, 1991; Joshi *et al*, 1992; Palacios *et al*, 1993; Gueth-Hallonet *et al*, 1993). γ-Tubulin is thought to be crucial for MT nucleation and may be a fundamental component of Mazia's "beads". This, however, forces questions about the molecular nature of γ-tubulin binding proteins.

The traditional theory of centrosomal inheritance, as seen in many species including the sea urchin and frog, suggests that centrosomes degenerate during oocytogenesis while remaining intact and functional during spermatogenesis (Boveri, 1901). Exceptions, however, such as the mouse, were noted where the centrosomal inheritance appeared to be reversed. Schatten *et al* (1985; 1986; 1991) and Maro (1985) strengthened this observation by showing the existence of centrosomal material in mouse oocytes and the absence of centrosomal material in sperm. The inheritance pattern may, however, be one in which the composite centrosome is blended from both maternal and paternal sources as suggested by Holy and Schatten (1991). Both theories acknowledge that most cells cannot tolerate centrosomal polyploidy and gametes therefore have devised a means by which the normal centrosomal ploidy can be maintained at fertilization.

In an attempt to evaluate the response of a cell having more than one centrosome, we asked what would happen if two "dominant" centrosomes from two different sources were combined within the same cytoplasm. One might predict that if a gamete, which had already completed the partitioning of its centrosome and was given an additional centrosome, might try to selectively repress the extra centrosome. Attempts to answer this question were made by isolating sea urchin sperm heads with centriole-containing centrosomes and microinjecting them into metaphase II arrested mouse oocytes or pronucleate stage mouse eggs. Both of these gametes are believed to contribute the "dominant" centrosome at fertilization. We are specifically interested in the tolerance of the oocyte to a second centrosome, the activity of these centrosomes, the possible cell-cycle dependence of the activity, and the effect of time.

MATERIALS AND METHODS

Sea Urchin Sperm Head Isolation

Sperm heads with basal bodies in the centriolar fossa were prepared using a protocol from Maller *et al* (1976). Sperm from either *Lytechinus pictus* or *Strongylocentrotus purpuratus* were collected by 0.55 M KCl intracoelomic stimulation. The sperm were shed "dry" into Petri dishes on ice. Sperm were washed twice in 4°C Artificial Sea Water (ASW) and centrifuged at 1,500 g for 5 min. The pellet was resuspended in 4°C, 2.0 M sucrose with 10 mM $CaCl_2$ and 0.1% Triton X-100 and homogenized for 2 min on ice with a Wheaton homogenizer (pestle A). The homogenate was layered on a 4°C, 10 ml cushion of 1.7 M sucrose, 10 mM $CaCl_2$ and centrifuged at 10,000 g for 20 min. The pellet, which contained heads, residual tails and mitochondria, was rinsed 3 times by resuspending in cold 1.2 M sucrose and centrifuging at 1,500 g. The final pellet contained mostly sperm heads with intact basal bodies in the centriolar fossae and a residual amount of contaminants (membranous vesicles and axonemes). The final pellet was resuspended in a Microinjection Buffer which contained 20 mM NaCl, 115 mM KCl, 1 mM $MgCl_2$, and 10% Polyvinylpyrrolidone-40 (PVP) (w/v) pH 7.0 or was processed for Transmission Electron Microscopy (TEM).

TEM Analysis

Analysis of the final pellet by TEM involved fixing the pellet in 2.5% glutaraldehyde in 10 mM PIPES and post-fixing in 1% osmium tetroxide followed by dehydration in a

graded ethanol series, rinsing in propylene oxide, and infiltration with Araldite resin. Silver and gold sections were cut with a glass knife on a Porter-Blum MT2 ultramicrotome and stained with 2% aqueous uranyl acetate and Reynolds lead citrate. The sections were viewed with a JEOL-100S TEM at 60 KV.

Mouse Sperm Head Isolation

Sperm from the caudal epididymis of an ICR outbred mouse (Sprague Dawley, Indianapolis, IN) were collected into M2 culture medium (Fulton and Whittingham, 1978). Sperm were homogenized on ice for 7 min and then subjected to sonication for 1.5 minutes to break the sperm at the head/midpiece junction. Tails were removed by repeated light centrifugations (200 g for 10 min) in a buffer containing 0.3 M gluconate, 0.3 M glycine, 2 mM MgCl, and 10 mM NaCl. The final pellet, which contained heads and residual tails, was resuspended in Microinjection Buffer.

Mouse Oocyte and Zygote Collection

ICR outbred mice were superovulated by injections with 7.5 IU PMSG followed 48 hr later by a 5.0 IU injection of hCG. Metaphase II arrested oocytes were collected from the ampullar oviduct of unmated mice beginning at 19 hr post hCG. Twenty to 27 hr post hCG, midstage pronucleate eggs were collected from the ampullar oviduct of mated mice. Cumulus cells were removed by 0.1% hyaluronidase treatment in HEPES buffered M2 culture medium (Fulton and Whittingham, 1978) and zona pellucida intact oocytes and eggs were placed in culture medium at 37° C.

Microinjection

Microinjection of sperm heads was performed by front-loading a beveled, 5.0 μm microinjection pipette with 1-5 heads or a small volume of centriole isolate. The zona pellucida was then punctured, cytoplasm was drawn up into the pipette and then expelled back into the oocyte along with the sperm head(s) or centriole isolate. After microinjection, oocytes and fertilized eggs were cultured for various lengths of time and treated as indicated in Table I. Parthenogenic activation of microinjected oocytes was achieved by a 7 min exposure to 7% ethanol in M2 culture medium (Cuthbertson, 1983). In some cases, oocytes spontaneously activated after being pricked by the microinjection pipette. These conditions were chosen so as to observe the effects of imported centrosomes and chromatin on a foreign cytoplasm at different stages, that is, meiosis, interphase, and mitosis, as well as to observe if the effects change with increased exposure to the mouse oocyte cytoplasm.

Oocyte Fixation and Immunolabelling

Shortly before fixation, the cells were denuded of their zona pellucida by a rapid treatment (10-30 sec) with M2 culture medium at pH 2.5, placed onto poly-L-lysine coated coverslips (Mazia *et al*, 1975) in calcium-free and protein-free M2 at pH 7.3, extracted with a microtubule stabilizing buffer, Buffer M (25% glycerol, 50 mM KCl, 0.1 mM EDTA, 1 mM EGTA, 0.5 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 50 mM imidazole hydrochloride, pH 6.7) (Bershadsky *et al*, 1978), 1% Triton X-100, and 10% methanol for 5 min and post-fixed in 100% cold methanol for 10 min. Cells were placed into phosphate buffered saline (PBS) overnight before beginning immunocytochemistry or electron microscopy protocols.

All groups were labelled with either a monoclonal antibody directed against β-tubulin (E7) from hybridoma culture medium, diluted 1:10 (Developmental Studies Hybridoma Bank) or affinity-purified rabbit-anti-tubulin IgG directed against α-tubulin at a concentration of 50 μg/ml for 50 min at 37° C. The cells were rinsed in PBS and labelled with a secondary antibody goat-anti-mouse IgG-FITC (fluorescein isothiocyanate) or goat-anti-rabbit IgG-FITC (both from Zymed Laboratories Inc.) or -TRITC (tetramethylrhodamine isothiocyanate; Sigma Chemical Co.) for 45 min at 37° C. In some cases, the cells were also labelled with an autoimmune antibody, SPJ, against human centrosomal antigen (diluted 1:100) (Balczon and West, 1991). The

TABLE I

Experimental design for microinjection of sperm heads into metaphase II arrested oocytes and pronucleate stage eggs.

Experiment	Stage at Microinjection	Post Injection 37°C Culture Length (hrs)	Treatment	Number injected	Number with Sperm Heads (x sperm/cell)	Number with MT Organization	% of Cells with Sperm and MT organization
I	Metaphase II	1		21	18 (1.5)	8	44
II	Metaphase II	4		31	20 (2.4)	13	65
III	Metaphase II	3	90 minute cold treatment plus 2 minute recovery	6	4 (0.66)	2	50
IV	Metaphase II	1-4	Spontaneous activation	17	15 (1.25)	3	20
V	Metaphase II	4	Activation and culture for 1 hr	11	6 (1.7)	2	33
VI	Pronucleate	22 minutes		12	11 (1.5)	0	-
VII	Pronucleate	1	90 minute cold treatment plus 10 minute recovery	17	12 (1.2)	0	-
VIII	Pronucleate	11	10 m Nocodazole overnight. 30 minute recovery	14	5	2	40
IX	Pronucleate	Through mitosis (13 hrs)		9		0	-
X	Pronucleate	4	Overnight cold treatment plus recovery through mitosis	19 (4 completed mitosis)	15 (2)	0	-

secondary antibody for SPJ, biotinylated anti-human (Vector Laboratories, Inc.), was applied for 50 min at 37°C and the antigen was visualized by then applying 1.5 mg/ml streptavidin conjugated to FITC (Jackson Laboratory). Finally, the DNA was fluorescently labeled with 5 μg/ml DAPI (4,6-diamindino-2-phenylindole, Sigma Chemical Co.) for 15 min at room temperature.

Specimens were viewed with a Zeiss Axiophot microscope using a Plan Neofluar, 100x/1.30, oil immersion objective and appropriate filter sets for fluorescein, rhodamine and DAPI. Differential Interference Contrast (DIC) was also used. Photographs were taken using Kodak Tri-X black and white or Ektachrome color film.

RESULTS

Characterization of the Sperm Head Isolate

The enrichment procedure for the sperm centrosome involves decapitating the sperm, rinsing away the sperm tails and collecting the sperm heads with an intact centriole. Figure 1a is an electron micrograph of intact sperm before homogenization. The sperm head, midpiece, and tail are connected and a distal centriole is present within the centriolar fossa. Figure 1b shows sperm heads after breaking and removing the tails. Homogenization dislodges the midpiece from the sperm head and repeated washing removes it from the isolate. TEM analysis reveals sperm heads lacking acrosomal, nuclear, and plasma membranes and the midpiece mitochondrion while retaining a firmly condensed nucleus and attached distal basal body in the centriolar fossa. Some loose centrioles, presumably proximal centrioles were also occasionally seen. Very few contaminating axonemes were present.

Sperm Heads Microinjected into a Meiotic Cytoplasm

After metaphase II arrested oocytes were microinjected, they were cultured for either 1

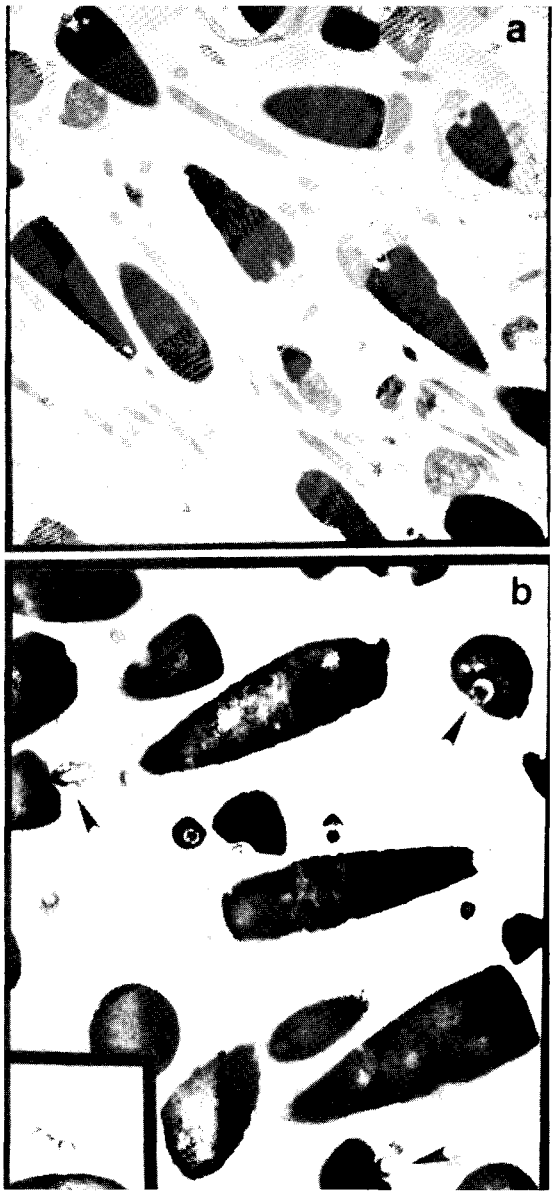

Fig 1. Sea urchin sperm head isolate characterization. (A) Whole sperm prior to homogenization: note the presence of chromatin, membranes, acrosomal vesicle, axonemes, midpiece mitochondrion, and basal bodies in the centriolar fossa (5,000x). (B) TEM of sperm heads with intact distal centrioles/basal bodies (arrowheads) and loose centrioles, possibly the proximal centriole (inset) after homogenization and detergent extraction. Midpieces, nuclear and plasma membranes, and axonemes are absent (8,500x).

or 4 hr at 37° C. Seventy-three percent of the microinjected oocytes in this group contained at least 1 sperm head as seen by DAPI staining. The remaining 27% served as sham-injection controls. Forty-four percent of the 1 hr culture and 65% of the 4 hr culture groups had some type of MT organization associated with the injected sperm head while the sham-injection controls had no such MT organization other than the normal cytasters.

In the 1 hr culture group, there was a distinct and repeatable MT pattern resembling nucleation of MTs from the sperm itself (Fig 2). This pattern will be referred to as a Type I aster-like pattern. This pattern has a MT focal point at or near the sperm head base, where the centrosome is located, and envelopes the sperm head. Although in some cases the focal point of the aster is removed from the sperm head base, it is possible that the centrosome and proximal centriole have dislodged from the centriolar fossa and migrated into the cytoplasm as occurs in normal sea urchin fertilization.

In the 4 hr culture group, there were three different MT patterns. One pattern was identical to that seen in the 1 hr culture group. A second pattern of "mini-spindles"

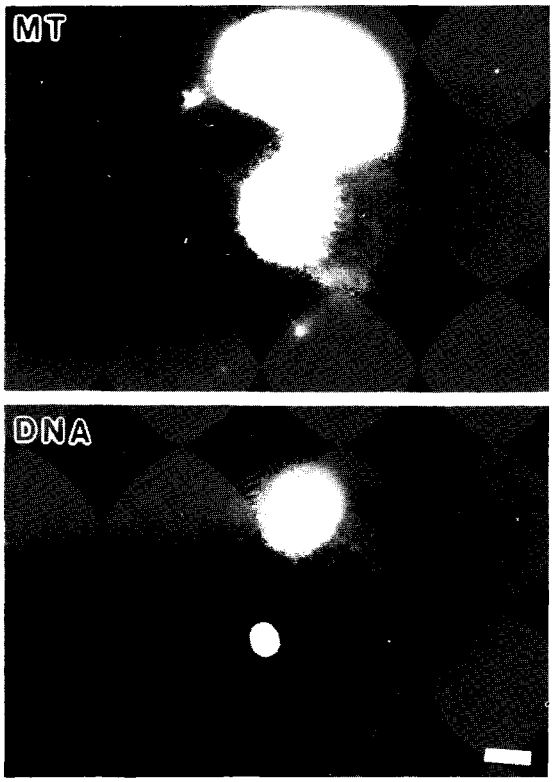

Fig 2. Metaphase II arrested oocytes microinjected with sperm heads and cultured for 1 hr. Oocytes in this group were extracted, fixed, and stained with a tubulin antibody (E7) and DAPI after microinjection and culturing. Forty-four percent of the oocytes with a microinjected sperm had a similar, aster-like, pattern of microtubules enveloping the sperm head. None of the sham-injected oocytes had any microtubule nucleation other than the normal cytasters. Bar = 7 μm.

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(Type II) was observed in at least 54% of the cases where MTs were organized near a sperm head (Fig 3A). These spindles were generally 2/3 the length of the meiotic spindle, bipolar, anastral, fusiform in shape, and symmetrically oriented about the sperm head. This suggests that the mini-spindles were not formed by random stabilization of cytaster MTs onto the sperm DNA. A third pattern (Type III, Fig 3B) resembled stabilization of cytaster MTs onto the sperm chromatin, possibly at the kinetechores. A cell with multiple injected sperm did not exhibit more than one type of MT pattern. Furthermore, none of these patterns were seen in sham-injected oocytes.

Activation of Microinjected Metaphase II Arrested Oocytes

To determine if the mini-spindles would progress through mitosis when the oocyte was activated, the oocytes were treated with a brief 7 min exposure to ethanol in the culture medium and cultured for an hour after the initial 4 hr culture period. Six of 11 injected oocytes contained sperm heads. Of the 6, 2 had MTs organized near the sperm head. Although in these two instances MT patterns similar to those mentioned previously appeared, in no case did the spindle change to a typical anaphase spindle (not shown). In 17 instances, the oocyte spontaneously activated after pricking by the microinjection pipette. Fifteen of these oocytes had sperm heads and 3/15 (20%) contained MTs organized at or near the sperm head.

Immunofluorescent Staining of the MTOCs

It is important to distinguish if the MTs are organized by mouse or sea urchin centrosomes or both and, therefore, immunocytochemistry was employed using mouse-specific centrosomal antibodies (SPJ). SPJ will recognize all mouse MTOCs (spindle and cytaster) but will not recognize sea urchin MTOCs. At the 4 hr culture period, mouse specific centrosomal antibody labelled some MTOCs which organized the "sperm asters" (Type I) and "mini-spindles"

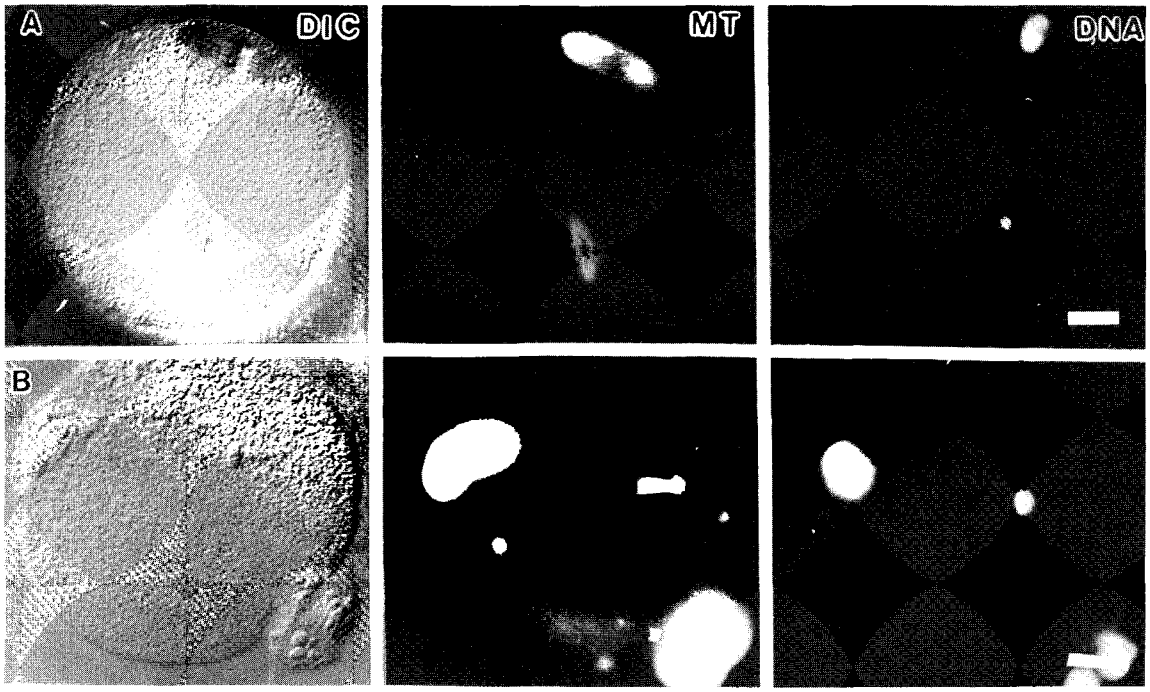

Fig 3. Metaphase II arrested oocytes microinjected with sperm heads and cultured for 4 hr. Oocytes in this group were extracted, fixed, and stained with E7 and DAPI. Three microtubule patterns were evident (A,B and that seen in Fig 2). One pattern (A), seen in 54% of the cases, was that of an anastral, fusiform, bipolar "mini-spindle". Bar = 6 μm. The third pattern, seen only once (B), appeared to be stabilization of mouse cytaster microtubules onto sperm chromatin. Bar = 6 μm.

(Type II) (Fig 4). In some cases, however, the MTOC region did not stain at all (14% of the mini-spindles and 44% of the asters; data not shown). Perhaps some MTs are organized by sea urchin MTOCs or by mouse MTOCs too small to be detected by SPJ. Since there is such a small amount of centrosomal antigen present in the sperm, this technique may not be sensitive enough to detect sea urchin specific centrosomes. Control experiments were performed in which sperm were stained with either mouse-specific SPJ or sea urchin-specific Ah6, a monoclonal antibody raised against *Drosophila* intermediate filament proteins but which stains sea urchin centrosomes in a manner identical to other centrosomal antibodies such as 5051 (Schatten *et al*, 1987). Sea urchin sperm showed negative staining with SPJ and positive staining at the centriolar fossa with Ah6 (data not shown).

Sperm Heads Microinjected into an Interphase Cytoplasm

Pronucleate stage mouse eggs were also microinjected with centrosome- containing sea urchin sperm and cultured for either 22 min or 2 hr. In no case did MTs nucleate at or near the sperm head (Fig 5A). Preliminary results show that microinjection of sperm heads into a mouse germinal vesicle (GV) oocyte also does not result in MT nucleation at or near the sperm head (data not shown).

In another series of experiments, microinjected pronucleate eggs were allowed to progress through mitosis to the 2-cell stage to observe the behavior of the sperm heads in another interphase cytoplasm. Three of the nine microinjected cells completed mitosis and no MT patterns formed at or near the sperm heads. Cytokinesis resulted in two normal-looking, equal-sized, blastomeres (Fig 5B). Therefore, mitosis, when it occurred, was apparently unaffected by the supernumerary, foreign sperm head.

Sperm Heads Microinjected into a Mitotic Cytoplasm

To view the eggs at mitosis and observe if MTs organized near sperm heads in another rapid-MT-turnover-cytoplasm, microinjected pronucleate eggs were cultured in 10 μM

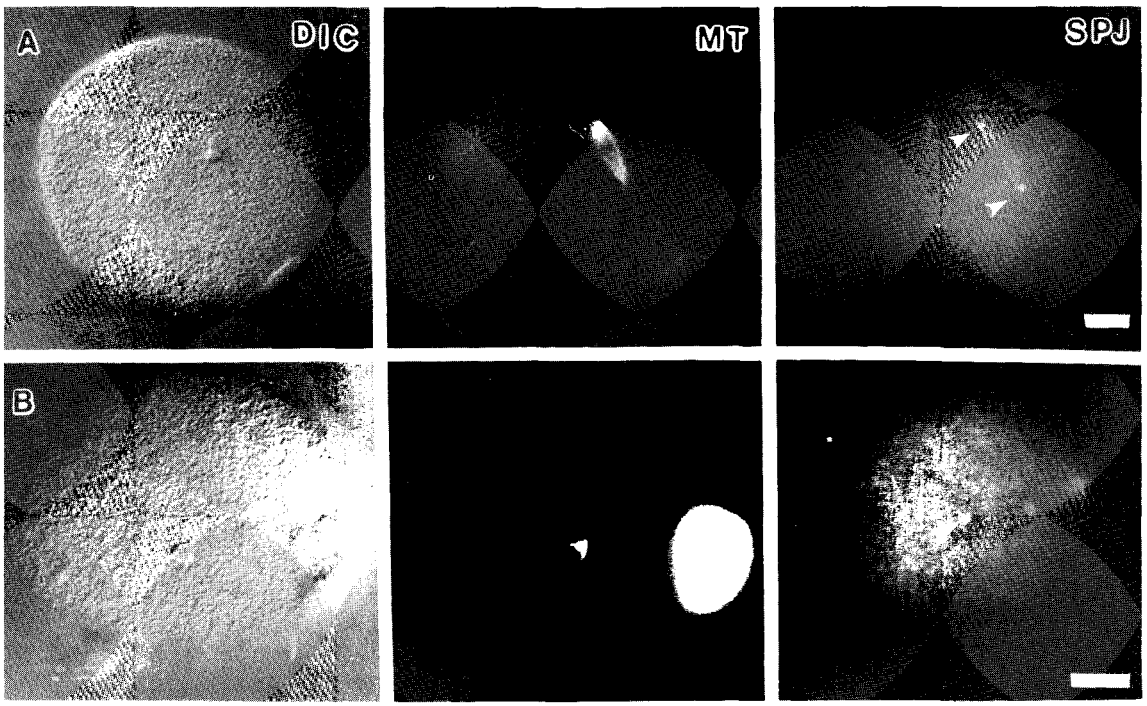

Fig 4. Metaphase II arrested oocytes microinjected with sperm heads and cultured for 4 hr before extraction, fixation, and triple-labelling of tubulin (E7), mouse centrosomes (SPJ), and DNA (DAPI). (A) A mini-spindle that stained with SPJ at both poles (arrowhead). The meiotic spindle poles also stained but are out of focus. Bar = 6 μm. (B) SPJ stained at one intense dot at the focal point of the aster (arrowhead). Bar = 7 μm.

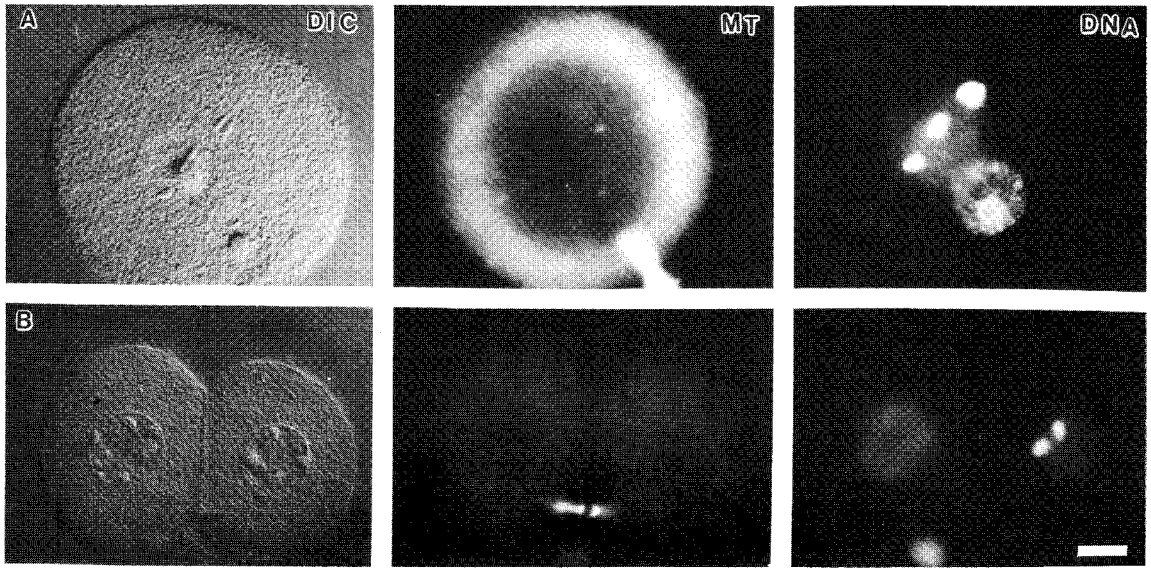

Fig 5. Microinjection of pronucleate eggs. (A) Pronucleate eggs were microinjected with sperm heads and cultured for 22 minutes before extraction, fixation, and labelling with E7 and DAPI. No MTs were organized about the sperm head. (B) Pronucleate eggs were microinjected with sperm heads and cultured until cytokinesis before extraction, fixation, and labelling with E7 and DAPI. The 2-cell embryo has normal appearing, equal sized blastomeres despite the presence of the supernumerary sperm. No MT organization was detected near the sperm head. Bar = 6 μm.

Nocodazole overnight and allowed to recover for 30 min. Figure 6 shows an egg treated in this manner. Of the 14 microinjected eggs, 5 received heads and 2 of these (40%) had an organized MT pattern near the sperm head. In some cases, a mitotic spindle formed around each pronucleus as is sometimes seen with nocodazole treated eggs (Schatten *et al*, 1991).

According to a recent paper by Gorbsky *et al* (1990), MTs in the mouse oocyte meiotic spindle turnover much more rapidly than those of the midbody at telophase and interphase. This suggested that perhaps there were indeed MT nucleation sites donated by the sperm but low monomeric tubulin population, resulting from stable MTs, resulted in no MT formation. To test this hypothesis, pronucleate stage microinjected eggs were subjected to a 90 min cold treatment to break down existing MTs and allowed to recover. Although 12/17 microinjected pronucleate eggs contained sperm heads, none formed any MT pattern (not shown). Conversely, 2/5 metaphase II oocytes had organized MTs associated with the sperm head (not shown). These results suggest that the lack of activity in the interphase cytoplasm is not due to the lack of availability of monomeric tubulin.

Microinjection of Mouse Sperm

In an attempt to decipher if these MT patterns were mouse cytaster MTs being stabilized by the sea urchin sperm chromatin or if there truly was centrosomal directed MT nucleation, we microinjected DNA from another source –mouse sperm– which does not appear to organize MTs *in vivo* and does not cross react with centrosomal antibodies (Schatten *et al*, 1986). Metaphase II arrested mouse oocytes were microinjected and cultured for 1 hr at 37° C. All 4 oocytes spontaneously activated. Only 1 of the 4 contained a sperm head and no MTs were associated with it. Also, the chromatin did not swell (data not shown).

Effects of the Mouse Cytoplasm on the Foreign Chromatin

It is interesting to note that the sea urchin sperm chromatin began to swell slightly after 1 hr of culture and even more after 4 hr of culture within the mouse meiotic cytoplasm.

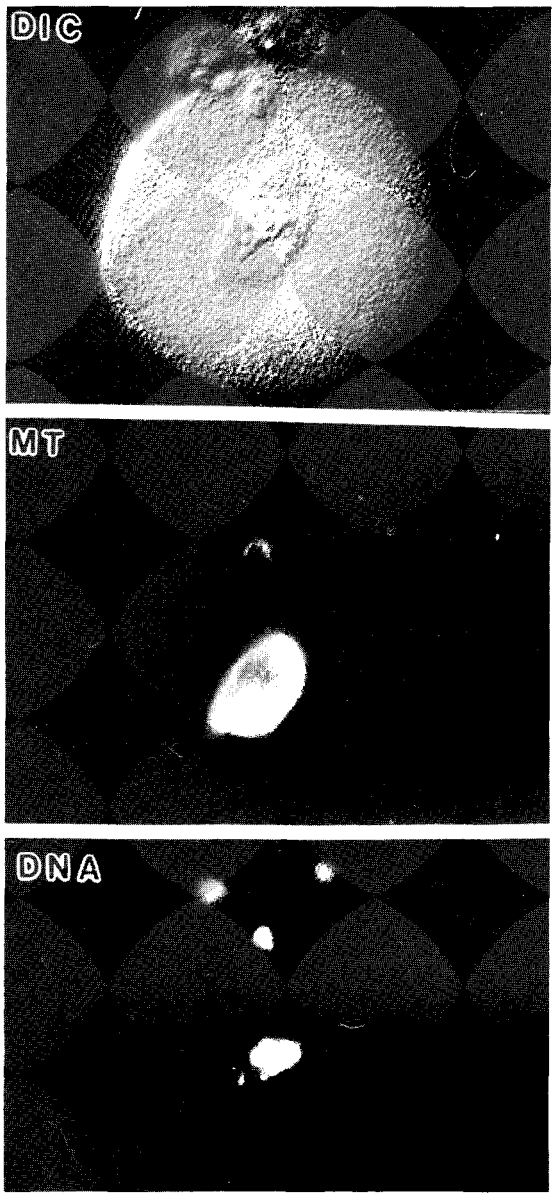

Fig 6. Microtubule formation from microinjected sperm heads in a mitotic cytoplasm. Pronucleate eggs were microinjected with sperm heads, cultured for 11 hr and then transferred to 10 μM Nocodazole overnight to block the cell at metaphase of first mitosis. Cells were then allowed a 30 minute recovery before extraction, fixation, and labelling with E7 and DAPI. MTs were seen to envelope the injected sperm head. Bar = 7 μm.

The head still remained intact but became more of a rounded cone. In no instance did the sperm chromatin swell to the typical round pronuclear shape. In an interphase cytoplasm, however, the sperm chromatin remained in an intact, unswollen, configuration for the duration of the experiment.

DISCUSSION

Demembranated, centrosome/centriole containing sea urchin sperm heads were microinjected into metaphase II arrested mouse oocytes or pronucleate stage eggs and cultured for various lengths of time. This allowed us to dissect out differences between meiotic, mitotic, and interphase cytoplasmic influences on the imported centrosome as well as to observe changes over time. It was expected that the centrosome may have some activity initially and that the centriole would still be intact. As exposure to this cytoplasm (that does not normally contain centrioles and supernumerary centrosomes) increases, however, it was expected that the centriole would degenerate, as suggested by Szöllösi *et al* (1986), and possibly also would its centrosomal activity.

Isolations

The sperm head isolation resulted in a preparation that had good structural preservation and low contamination. It is crucial, however, to establish if these isolated structures are still functional. Maller *et al* (1976) demonstrated that sperm treated in this manner appear to maintain a functional centrosome since they will induce cleavage furrows, spindle formation, and parthenogenesis after microinjection into *Xenopus laevis* oocytes. These effects appear to be solely attributable to the centrosome since disruption of the chromatin by vigorous agitation or salt and EDTA treatment resulted in a similar response. Very few contaminating axonemes were present in this preparation. Any MT formation from axonemes would likely have resulted in a biased, bidirectional MT pattern and would be clearly distinguishable from the aster-like patterns which were observed.

Oocytes Microinjected with Sperm Heads

Many meiotic or mitotic (but not interphase) mouse oocytes or eggs that have been microinjected with a sea urchin sperm head and centrosome are induced to form several different MT patterns in association with the sperm head. Upon microinjection and

subsequent incubation of oocytes, drastic differences were seen in the MT patterns around the microinjected sperm in a cell cycle dependant fashion. An average of 53% of the meiotic cytoplasms and 40% of the mitotic cytoplasms, but no interphase cytoplasms, supported MT nucleation. None of the sham-injected oocytes had any MT nucleation. The remaining oocytes which exhibited no activity could be explained if the sperm head lacked a centrosome or the centrosome was damaged during processing or microinjection.

Three types of patterns were seen; an aster-like pattern (Type I), a mini-spindle (Type II) and Type III which appeared to be a stabilization of mouse cytaster MTs onto the sperm chromatin. The differences in MT nucleating ability between a meiotic/mitotic cytoplasm and an interphase cytoplasm are not contributory to MT turnover rate or availability of monomeric tubulin as suggested by the cold treatment experiments. The differences may, however, reflect a change in the phosphorylation state of the centrosome. This suggestion is further supported by the results seen in microinjected oocytes that were at anaphase after activation (spontaneous or artificial). In these cases, MT patterns were seen in 20-33% of the cases (spontaneous and artificial activation, respectively) *versus* 44-60% (mitotic and meiotic, respectively) if the cell was in metaphase. This could be due to the dephosphorylation of the centrosome as it enters anaphase.

Increased exposure to the oocyte cytoplasm leads to a change in MT patterns. As the culture period after microinjection increased, there was a change in the type of MT pattern formed. Type I patterns only were seen in short culture periods. As the culture length increased, approximately half Type I and half Type II patterns were seen. This change in MT pattern could be reflective of a change in the centrosomal activity.

MTOCs within microinjected oocytes that are in anaphase or telophase have a decreased ability to organize MTs in the proximity of exogenous sperm heads, regardless of the mode of activation (spontaneous or by ethanol). After microinjection of sperm heads and a 4 hr culture, some metaphase II arrested oocytes were artificially activated with ethanol and fixed at telophase II. Some oocytes spontaneously activated from pricking by the microinjection pipette. Regardless of the activation method, an average of 26% of the sperm-head containing oocytes had MT organization (20% for spontaneous activation and 33% for artificial activation). These results, in addition to those at metaphase of either meiosis or mitosis and at interphase, suggest that the centrosomes are behaving in a cell-cycle dependent manner; MT nucleating activity being highest in metaphase, decreasing at telophase, and minimal at interphase. The cell cycle dependent presence of MTs suggests the foreign centrosomes may be regulated by the mouse oocyte cytoplasm –possibly by phosphorylation.

MTOCs responsible for nucleating these MT patterns may well be of dual origin. Centrosomal staining (and lack of staining) suggest some MTOCs are endogenous (mouse) and others are exogenous (sea urchin). Furthermore, the presence of an aster suggests these MTOCs are functional in their ability to nucleate MTs. The aster-like pattern is reminiscent of a sea urchin sperm aster seen during normal fertilization suggesting the MTOC is sea urchin in origin. On the other hand, mini-spindles (86% of which stain with SPJ) are reflective of a typical MT pattern seen in mouse meiotic oocytes, that is, a spindle. It is feasible that the mouse oocyte detected chromatin that was not incorporated into a meiotic spindle and moved to correct the "problem". It is interesting that the MT pattern changes from aster-like to mini-spindle as exposure to the cytoplasm increases. Also, SPJ staining is seen in 86% of the mini-spindles and only 44% of the aster-like patterns. From this we infer that SPJ-positive MTOCs (*i.e.*, mouse MTOCs) are responsible for most of the mini-spindle formation while SPJ-negative MTOCs (inferred sea urchin MTOCs) are responsible for most of the aster-like patterns. We suggest that the decrease in the percentage of aster-like patterns with the passage of time reflects a degradation or down regulation of sea urchin MTOCs over time.

Effects on the Exogenous Chromatin

The fate of the sperm chromatin varies in a manner dependent on the cytoplasmic state; decondensing slightly in a meiotic cytoplasm and not at all in an interphase cytoplasm. The chromatin will decondense slightly over time in a meiotic cytoplasm but will remain highly condensed and in a cone-shaped form in an interphase egg. Furthermore, the foreign sperm seems to be inert during mitosis because two, equal-sized blastomeres result after cytokinesis. It is interesting that the sperm DNA does not cycle with the mouse DNA; it is neither excluded from the cell nor incorporated into the nucleus. The sea urchin sperm may be so phylogenetically distant from the mouse oocyte that the sperm DNA cannot recognize cell cycle signals to decondense or condense. Furthermore, since two, equal-sized blastomeres resulted after cytokinesis, it appears that the extra centrosome has also become inert by this time. This further supports the theory that the sea urchin centrosome, while active initially, has degraded.

Other Microinjection Studies

In attempting to interpret these results, we turned to similar experiments such as those by Maller *et al* (1976), Heidemann and Kirschner (1975) and Karsenti *et al* (1984). All are similar in that they involve microinjection of a centriole/centrosome fraction (alone or associated with some type of nuclei) into a heterologous (*Xenopus laevis*) oocyte or egg. However, in no case was the host cell of mammalian origin and, furthermore, the number of centrosomes microinjected far outnumbered the amount injected in these experiments. It is also difficult to extrapolate from these experiments because each had slightly varying results using material from differing species. The results of Maller *et al* (1976) and Heidemann and Kirschner (1975) agree with each other and with ours in that only metaphase II oocytes microinjected with a centriole or basal body fraction, and not a purified nuclei fraction, would form asters. Karsenti *et al* (1984) claimed that it was only the activated oocytes and not the metaphase II oocytes that were capable of forming asters after microinjection with neuroblastoma centrosomes. Co-injection of centrosomes and karyoplast nuclei led to aster formation in activated oocytes and spindle formation in metaphase II arrested oocytes while injection of nuclei alone led to spindle formation in metaphase II oocytes only. They concluded that the centrosomes would induce MT polymerization in an interphase state –a state where the tubulin critical concentration was low–, but not in a meiotic state –where the critical concentration for tubulin is high– unless DNA was nearby to "influence" the centrosome behavior.

Implications

We suggest the following as possible interpretations of these results. It is possible that the MT patterns observed were caused by a stabilization of free MTs on the sperm chromatin or by some influential factor associated with the chromatin, as suggested by Karsenti *et al* (1984). This, however, does not exclude the possibility that the mouse oocyte, being able to tolerate only one centrosome, selectively degrades or down-regulates the supernumerary sea urchin sperm centrosome. It appears, however, that this mouse oocyte reaction is not immediate. Initially, the sperm centrosome is functional and follows the rules for centrosome activity, that is, its MT nucleating capacity is greatest at metaphase of meiosis and mitosis, decreases during anaphase and telophase, and is minimal at interphase. As exposure to the mouse cytoplasm increases, so too does the down-regulation or degradation of the sea urchin centrosome. This gradual degradation is manifested in two ways: a change in MT patterns from aster-like to mini-spindles and cytokinesis resulting in normal 2-cell embryos.

It is interesting that the centrosome of an invertebrate can be regulated by a mammalian cytoplasm, suggesting the conservation of crucial regulatory mechanisms. Another aspect of this study which should not be overlooked is that while the mouse oocyte is typically thought of as a static cell because it is arrested in metaphase

II of meiosis, it is still quite capable and efficient at monitoring and regulating centrosome function and number.

The results demonstrated herein provide additional evidence for centrosomal function in an "alternate *in vivo*" environment. This system provides a novel means of studying the centrosome in the environment of an intact cell where the number of functional centrosomes is known as opposed to an extract system where thousands of centrosomes or centrosomal components may be present.

ACKNOWLEDGEMENTS

This manuscript is dedicated to the memory of Luis Izquierdo. This work was supported by research grants from the NIH. The authors wish to thank Dr Kip Sluder for helpful advice with the centriole isolations and Drs Shirley Wright and Jon Holy for helpful discussions.

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