ASTER FORMATION IN EGGS OF *XENOPUS LAEVIS*

Induction by Isolated Basal Bodies

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ABSTRACT

We have assayed various materials for their ability to induce aster formation by microinjection into unfertilized eggs of *Xenopus laevis*. We have found that purified basal bodies from *Chlamydomonas reinhardtii* and *Tetrahymena pyriformis* induce the formation of asters and irregular cleavage furrows within 1 h after injection. Other microtubule structures such as flagella, flagellar axonemes, cilia, and brain microtubules are completely ineffective at inducing asters or cleavage furrows in unfertilized eggs. When known amounts of sonicated *Tetrahymena* and *Chlamydomonas* preparations are injected into unfertilized eggs, 50% of the injected eggs show a furrowing response at approximately 3 cell equivalents for *Chlamydomonas* and 0.1 cell equivalent for *Tetrahymena*. These results are close to those expected if basal bodies were the effective astral-inducing agent in these cells. Other materials effective at inducing asters in unfertilized eggs, such as crude brain nuclei, sperm, and a particulate fraction from brain known to induce parthenogenesis in eggs of *Rana pipiens*, probably contain centrioles as the effective agent. Our experiments provide the first functional assay to indicate that centrioles play an active role in aster initiation.

None of the injected materials effective in unfertilized eggs produced any observable response in fully grown oocytes. Oocytes and eggs were found to have equal tubulin pools as judged by colchicine-binding activity. Therefore, the inability of oocytes to form asters cannot be due to a lack of an organizing center or to a lack of tubulin. Experiments in which D$_2$O was found to stimulate aster-like fibrous areas in eggs but not oocytes suggest that the inability of oocytes to form asters may be due to an inability of tubulin in oocytes to assemble.

Asters are radially symmetric structures composed of microtubules which assemble at the poles of most mitotic animal cells (19). Recent advances in our understanding of microtubule assembly in vitro (4, 14, 15, 30) have stimulated a reinvestigation of the regulation of aster assembly (31, 33). Aster assembly could be regulated on two distinct levels: the polymerization of tubulin into microtubules, and the localization and organization of this assembly process to form the aster structure. The study of aster formation in a living cell may therefore provide us with an understanding of how microtubule assembly and organization is controlled in vivo.
In experiments designed to determine the effect of the surrounding cytoplasm on nuclear structure and synthetic activity, Gurdon (10) injected nuclei into immature, fully grown oocytes and mature oocytes (unfertilized eggs) of *Xenopus laevis*. He observed that the nuclei injected into fully grown oocytes swell and synthesize RNA, while nuclei injected into mature oocytes condensed their chromosomes and formed multipolar spindles. The appearance of spindles in mature oocytes but not in immature oocytes in response to injected nuclei suggested that these cells differ in their ability to form asters. We have extended the work of Gurdon, using the *Xenopus* oocyte and egg system for two purposes. First, we have utilized microinjection in *Xenopus* eggs to assay materials for their ability to induce asters and we have tried to define the role of the centriole as such an inducing material. Second, we have made a preliminary attempt to understand what underlies the difference in the capacity of oocytes and eggs to form asters. Maller and Gerhart have also explored the injection of centriole-containing preparations and will report their results elsewhere. (J. Maller and J. Gerhart. Unpublished observations)

**MATERIALS AND METHODS**

**Injection Procedure**

Fully grown oocytes, stage 6 (6), were obtained by manual dissection from ovaries of *X. laevis* females. Small pieces of ovary were removed from the frog through a small incision in the lateral body wall. Unfertilized eggs were obtained by injecting females with human chorionic gonadotropin (Sigma Chemical Co., St. Louis, Mo.) as described by Gurdon (9). Eggs and oocytes were maintained before injection in Barth's saline (2) at pH 7.6. Eggs were allowed to remain in saline no longer than 1 h before injection. Just before injection, eggs were dejellied in 2% cysteine in 0.1 M Tris saline (2) at pH 7.6. Eggs were allowed to remain in Barth's saline immediately before injection. Basal bodies were isolated from *Xenopus* testes in 0.5 ml of Barth's saline in a glass tissue grinder. The homogenate was centrifuged at 150 x g for 3 min to remove large pieces of tissue which clogged the injection needle. Sperm were also suspended in Barth's saline without Ca ++ containing 0.5 mM EGTA. The number of sperm were determined by counting a dilution of the suspension on a Lang Bright-Line hemocytometer (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.).

Basal bodies were also isolated from *Tetrahymena pyriformis* by the method of Rubin and Cunningham (21). The basal bodies obtained by this procedure were sedimented at 50,000 x g for 45 min and resuspended in Barth's saline immediately before injection. Basal bodies from chick and frog brain were also isolated from *Chlamydomonas reinhardtii*, strain C137 grown in 3/10 high salt medium in continuous light at 20°C (27), by the method of Snell et al. (25). The final discontinuous sucrose gradient purification step was omitted due to poor yield. The 35,000 x g pellet from the previous step was resuspended in Barth's saline immediately before injection. Basal bodies from *Escherichia coli* were also used for injection. A culture of *Chlamydomonas reinhardtii* as described was counted (Coulter Electronics Inc., Hialeah, Fla.) for cell density, and a 10-cc portion was then sonicated on a Branson Sonifier (Branson Instruments, Inc., Great Neck, N. Y.) attached by a short length of silicone tubing to a microneedle. This assemblage was filled with distilled water and mounted on a micromanipulator (Narashige Inst. Ltd., Tokyo, Japan). Microneedles were pulled freehand from 1.6 x 100-mm glass tubing. A needle point was then drawn from the pulled needle on a microforge and broken off to produce a 40-μm tip. The diameter of the barrel of the needle was measured and marked off into 1-mm sections with a marking pen for calibration of volume delivery. A small amount of air and then the sample were drawn into the needle. The air meniscus acted as a reference and allowed the delivery of a known volume into the cells. Fresh needles were routinely used for each sample or sample dilution to avoid contamination.

Eggs and oocytes for injection were placed in agar bottom dishes containing Barth’s saline at room temperature (22°C ± 1°C). Each egg or oocyte was injected with approximately 0.1 μl of material. Eggs and oocytes were injected in the vegetal hemisphere since this seemed to reduce cytolysis of unfertilized eggs. Immediately after injection, the eggs or oocytes were removed to another dish containing Barth’s saline and incubated, usually for 1 h before fixation.

**Preparation of Injected Materials**

Nuclei for injection were prepared from *Xenopus*, hamster, and chick brains by the method of Graham et al. (8). A subcellular preparation from chick and frog brain which sediments at 10,000 x g was prepared according to the method of Fraser (7). Sperm suspension for injection was obtained by homogenizing *Xenopus* testes in 0.5 ml of Barth’s saline in a glass tissue grinder. The homogenate was centrifuged at 150 x g for 3 min to remove large pieces of tissue which clogged the injection needle. Sperm were also suspended in Barth’s saline without Ca ++ containing 0.5 mM EGTA. The number of sperm were determined by counting a dilution of the suspension on a Lang Bright-Line hemocytometer (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.). Basal bodies were also isolated from *Chlamydomonas reinhardtii*, strain C137 grown in 3/10 high salt medium in continuous light at 20°C (27), by the method of Snell et al. (25). The final discontinuous sucrose gradient purification step was omitted due to poor yield. The 35,000 x g pellet from the previous step was resuspended in Barth’s saline immediately before injection. Basal bodies from *Tetrahymena pyriformis* by the method of Rubin and Cunningham (21). The basal bodies obtained by this procedure were sedimented at 50,000 x g for 45 min and resuspended in Barth’s saline immediately before injection. Crude preparations from *Chlamydomonas*, *Tetrahymena* and *Escherichia coli* were also used for injection. A culture of *Chlamydomonas reinhardtii* as described was counted (Coulter Electronics Inc., Hialeah, Fla.) for cell density, and a 10-cc portion was then sonicated on a Branson Sonifier (Branson Instruments, Inc., Great Neck, N. Y.) attached by a short length of silicone tubing to a microneedle. This assemblage was filled with distilled water and mounted on a micromanipulator (Narashige Ins. Ltd., Tokyo, Japan). Microneedles were pulled freehand from 1.6 x 100-mm glass tubing. A needle point was then drawn from the pulled needle on a microforge and broken off to produce a 40-μm tip. The diameter of the barrel of the needle was measured and marked off into 1-mm sections with a marking pen for calibration of volume delivery. A small amount of air and then the sample were drawn into the needle. The air meniscus acted as a reference and allowed the delivery of a known volume into the cells. Fresh needles were routinely used for each sample or sample dilution to avoid contamination.

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sucrose and sonicated for 2 min at the highest setting on the Branson Sonifier. Appropriate dilutions of this sonicate were prepared in Barth's saline immediately before injection. Purified pellicle fragments were prepared from the 1.7-1.8 M sucrose interface of the first sucrose gradient after substitution of sonication for the polytron treatment in the Rubin and Cunningham procedure which was resuspended in Barth's saline. Microscopic examination of this preparation showed it to be pellicle with no whole cells. A sonicate of E. coli was prepared by sedimenting 10 cc of an exponential culture at 5,000 x g for 20 min at 4°C, resuspending the pellet to 10^9 cells in Barth's saline and sonicating it on ice at the maximum setting of the Branson Sonifier for 1 min.

Flagella and flagellar axonemes from Chlamydomonas were isolated by the methods of Witman et al. (36). Cilia from Tetrahymena were isolated by the method of Watson and Hopkins (29). In all cases the isolated product was examined for purity in the light microscope and then was sedimented and resuspended in Barth's saline immediately before injection.

Microtubules were prepared from porcine brain by a modification (14) of the method of Shelanski et al. (24). Immediately before injection, they were resuspended in Barth's saline or Barth's saline without Ca++ containing 0.5 mM EGTA.

In some experiments sperm and basal bodies were also injected in the presence of 10^-4 M colchicine or 10^-7 M vinblastine sulfate (kind gift of Eli Lilly & Co., Indianapolis, Ind.).

Incubation of Eggs and Oocytes in D_2O

Double-strength Barth's saline was diluted 1:1 with D_2O (99.77%, Columbia Organic Chemicals Co., Columbia, S. C.). Unfertilized eggs and stage 6 oocytes obtained as described were allowed to incubate in this solution for 2 h. Samples were fixed at 1 and 2 h.

Histology

Eggs and oocytes were fixed in Perenyi's fluid (95% ethanol, 0.5% chromic acid, 10% nitric acid, 3:3:4) overnight, and then taken through a 70%, 90%, 100%, 100% ethanol series, 1 h for each change. The samples were cleared in two changes of xylene for 2 h and imbedded in Paraplast-plus wax (Sherwood Medical Industries, St. Louis, Mo.). 10-μm serial sections were cut and then stained with Meyers acid-alum hematoxylin and counterstained with 5% Chlorazol Black (Mattheson, Coleman & Bell, East Rutherford, N.J.) in 70% ethanol.

Colchicine Binding Assays

Tubulin pools were measured in soluble extracts of oocytes and eggs from the same three females by [^H]colchicine binding. Soluble extracts were prepared from replicate samples of 25 stage 6 oocytes and dejellied eggs in 0.1 M 2-[N-morpholino]ethane sulfonic acid (MES) buffer pH 6.4, 1 mM GTP according to the method of Heidemann et al. (11). These soluble extracts were incubated for 1 h at room temperature with [^H]colchicine (sp act 141 μCi/mmol, New England Nuclear, Boston, Mass.) so that the final colchicine concentration was 10^-4 M. Bound colchicine was then assayed by a modification of the column method of Wilson (35). A 15 X 0.6-cm column of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.) was used for 0.20-ml samples and eluted with 0.1 M MES buffer pH 6.4. Bound colchicine was determined by liquid scintillation counting of the void volume. [^H]Toluene was then added as an internal standard and recounted. From these data, the molar quantity of bound colchicine was calculated.

RESULTS

Aster Induction by Nuclear and Cytoplasmic Fractions of Brain

Crude nuclear preparations from mouse, chick, and hamster brain were injected into fully grown oocytes and unfertilized eggs of X. laevis. We observed, as had Gurdon (10), that unfertilized eggs developed asters while oocytes did not within 1 h after injection. In addition, unfertilized eggs but not oocytes formed irregular cleavage furrows in response to these injections. In order to determine whether the response of unfertilized eggs was due to the injected nuclei or contaminating material, brain nuclei were purified by sedimentation through 2.5 M sucrose and injected. When these purified nuclei were injected into oocytes and eggs, neither cell responded to form asters or cleavage furrows. This result suggested that some cytoplasmic factor and not the nucleus was responsible for the induction of cleavage furrows and asters in unfertilized eggs.

A particulate, cytoplasmic fraction of brain tissue known to induce artificial parthenogenesis in Rana pipiens (7, 23) showed the same activity as had crude nuclear preparations when injected into Xenopus eggs, further suggesting a cytoplasmic factor.

Characteristic Response of Unfertilized Eggs to Aster-inducing Materials

Injection of crude brain nuclei or the parthenogenetic agent caused similar responses in unfertilized eggs. This same response pattern has proven to be characteristic for unfertilized eggs and has not varied for any of the other effective materials injected. The response consisted of concurrent
formation of cleavage furrows, asters, and filamentous, aster-like structures.

The first observable response was the formation of irregular cleavage furrows (Fig. 1) which appeared as soon as 20 min after injection in some eggs and were nearly always present after 1 h. The number of furrows, which were most numerous in the animal hemisphere, increased in the 2nd h and then began to recede or were engulfed by material in the vegetal hemisphere. Most cleavage furrows did not transect the egg, and normal cleavage and division patterns were never observed.

Asters were always present in sections from furrowed eggs (Figs. 2, 3, 4). These asters were found predominantly in the animal hemisphere. The asters varied in size from 25 μm to 50 μm in diameter and always showed a radial arrangement of thin, straight filaments similar in appearance to normal asters in fertilized eggs. In eggs fixed at various times after injection, asters were first observed at the time the eggs first showed cleavage furrows and were present 2 h after injection. After 2 h, only aster-like areas, as described below, were seen.

Aster-like areas are filamentous yolk-free areas of injected eggs which closely resemble asters but lack radial symmetry at any level in the serial sections (Fig. 5). They were always observed along with asters in sections from furrowed eggs. It is possible that these aster-like areas may have been asters which lost some of their organization as an artifact of fixation since these fibrous, yolk-free areas were more commonly found toward the cell interior which is more slowly fixed.

None of these structures were observed in unfertilized eggs injected with Barth's saline or any of the ineffective materials to be described below (Fig. 6). Nor were these responses ever observed up to 6 h after injection in stage 6 oocytes injected with any materials tested (Fig. 7).

**Induction of Aster Formation by Isolated Basal Bodies**

It seemed likely that the aster-inducing agent was a centriole for the following reasons: (a) the active component was a particulate cytoplasmic structure, (b) it had some chemical properties similar to those of microtubules (7), and (c) it was found in sea urchin sperm heads but not tails (J. Maller and J. Gerhart, personal communication). We had also found that *Xenopus* sperm preparations were highly effective at inducing asters in *Xenopus* eggs.

Since centrioles have been shown to be identical in structure to basal bodies with which they interconvert (1, 34), we decided to assay basal bodies for inducing activity. Basal bodies were purified, according to recently published procedures, from *C. reinhardtii* (25) and *T. pyriformis* (21). These preparations consisted mostly of basal bodies with some amorphous materials and pieces of flagella as judged by electron microscopy. The basal bodies had the typical cylindrical structure, and in *Chlamydomonas* were generally found as pairs as described by Snell et al. (25). Both basal body preparations were highly effective at inducing asters and cleavage furrows, when assayed by injection into unfertilized eggs of *Xenopus*.

In order to show that basal bodies and not other microtubule-containing materials were the effective aster-inducing agent, various other microtubule structures were injected into unfertilized eggs and oocytes. Isolated flagella and flagellar axonemes from *Chlamydomonas* and cilia from *Tetrahymena* were totally ineffective at inducing asters. Microtubules from hog brain were also ineffective at inducing aster formation whether resuspended in Barth's saline or Barth's saline without Ca++ containing 0.5 mM EGTA. All preparations were ineffective in eggs from four females. A preparation of an *E. coli* cell sonicate, which should contain no microtubules or centrioles, was also completely ineffective in inducing asters. Injection of sperm or basal bodies in the presence of 10⁻⁴ M colchicine or 10⁻⁵ M vincristine eliminated aster and furrow formation. Injection of either chemical alone had no observable effect on eggs or oocytes (Table I).

The effectiveness of material from sperm, *Chlamydomonas*, and *Tetrahymena* was tested by injecting known numbers of sperm, sonicated *Chlamydomonas* and *Tetrahymena* into unfertilized eggs and then noting the response. Fig. 8 shows the percentage of eggs which manifested the furrowing response as a function of the titer of injected *Xenopus* sperm, *Chlamydomonas* sonicate, and *Tetrahymena* pellicle sonicate. The response for both sperm and *Chlamydomonas* fell to 50% at approximately 3 cell equivalents injected per egg. In contrast, the response for *Tetrahymena* fell to 50% at approximately 0.08 cell per egg and effective response is still observed at 0.01 cell per egg.
FIGURE 1 Irregular cleavage furrows in unfertilized eggs 1 h after injection of isolated basal bodies from *Tetrahymena*. 1 cm = 250 μm. × 70.

FIGURE 2 Section of an unfertilized egg fixed 1 h after injection with isolated *Chlamydomonas* basal bodies. Fibrous, yolk-free areas containing asters or aster-like structures are marked with arrows. 1 cm = 120 μm. × 25.
We have not been able to obtain direct microscopic evidence that the basal body nucleates aster formation. Electron microscopic studies have thus far been disappointing due to problems in fixation which are considerable for objects as large and as impermeable as frog eggs. However, some evidence that the injected material might actually serve as the organizing center for the aster came from experiments in which pieces of pellicle, which contain basal bodies (22), from *Tetrahymena* were injected into unfertilized eggs. Injected pellicle was effective at inducing aster formation in unfertilized eggs. When sections from these injected eggs were examined, induced asters contained large, deeply stained centers. We believe these centers to be pellicle because they stained a distinct color (blue-green) distinguishable from that of either yolk granules (purple) or filaments of the aster (black). The blue-green of these centers was identical to the color of isolated, fixed pellicle stained in the same manner. In addition, such centers were never present in asters induced by any other material but were always present in asters induced by pellicle preparations (Fig. 9).

Effects of D₂O on Oocytes and Eggs

Van Assel and Brachet (28) have reported that unfertilized eggs from *Xenopus* form cytasters when incubated in solutions of heavy water. We wished to determine whether the response of the unfertilized eggs to heavy water was similar to the response to injected basal bodies and whether D₂O could in fact induce asters in oocytes. When unfertilized eggs were incubated for 1 or 2 h in 50% D₂O, large numbers of fibrous areas were produced throughout the animal hemisphere. No effect was observed in oocytes treated in the same manner. The response to D₂O incubation, however, differed in a number of ways from the response of unfertilized eggs to injected basal bodies. First, cleavage furrows were not formed in D₂O-incubated eggs. In addition, very few of the fibrous areas in eggs incubated in D₂O showed any radial symmetry (Fig. 10). They appeared somewhat similar to the aster-like areas shown in Fig. 5. The fibrous areas induced by D₂O were more numerous and were often homogenously distributed in a connected array. In some cases they virtually eliminated yolk granules from the animal hemisphere (Fig. 11).

Tubulin Pools in Oocytes and Eggs

The size of the tubulin pools in soluble extracts of oocytes and eggs were measured by assaying for colchicine-binding activity since colchicine binding is highly specific for tubulin, and colchicine binds with approximately a one to one stoichiometry (5, 32).

Soluble extracts from 25 fully grown oocytes and 25 unfertilized eggs taken from the same three females were assayed for colchicine-binding activity as described in Methods. The results of the experiment are presented in Table II. Oocytes and eggs from the same female showed virtually identical colchicine-binding activity, and hence by this assay had identical pools of total free tubulin.
DISCUSSION

The mechanism of aster formation has elicited continuing interest despite the early and generally acknowledged conclusion that the aster forms under the influence of the centriole (34). Evidence for the role of the centriole has been based largely on its location in the center of the aster. The fundamental weakness of this evidence may account for the continuing interest in this problem. The location of the centriole is not in itself a test of its influence or role in aster formation, as has been emphasized recently by Pickett-Heaps (17, 18). He points out that there is a lack of a clear ultrastructural link between the centriole and astral microtubules and that there is accumulating evidence that many plant cells lack a centriole yet organize a mitotic apparatus. For these reasons, he postulates a passive role for the centriole, as being merely partitioned to daughter cells by the mitotic apparatus for later use as a basal body to organize flagellar growth.

Figure 4 Aster in section of an unfertilized egg injected with isolated Chlamydomonas basal bodies. 1 cm = 8 μm. × 400.

Figure 5 Aster-like, yolk-free area in a section of an unfertilized egg injected with isolated Chlamydomonas basal bodies. 1 cm = 12 μm. × 400.
The experiments reported here make use of microinjection in *Xenopus* eggs as an assay for components which initiate aster formation. Though there are some limitations imposed by the experimental system and the present purity of basal body preparations, the experiments indicate that basal bodies or centrioles isolated from two phylogenetically distinct sources are capable of initiating aster formation in a vertebrate egg. Moreover, preparations from *Tetrahymena*
TABLE I

| Aster-Forming Response of Oocytes and Unfertilized Eggs to Various Injected Materials |
|-----------------------------------------------|-----------------------------------------------|
|                                 | Unfertilized eggs | Oocytes |
| Crude brain nuclei                  | +                 | ~       |
| Purified brain nuclei                | ~                 | ~       |
| Parthenogenetic brain fraction      | ~                 | ~       |
| Xenopus sperm                       | +                 | ~       |
| Isolated basal bodies, Chlamydomonas| +                 | ~       |
| Isolated basal bodies, Tetrahymena   | +                 | ~       |
| Chlamydomonas flagella              | ~                 | ~       |
| Flagellar axonemes                  | ~                 | ~       |
| Tetrahymena cilia                   | ~                 | ~       |
| Hog brain microtubules              | ~                 | ~       |
| Tetrahymena pellicle                | +                 | ~       |
| E. coli sonicate                    | ~                 | ~       |
| Xenopus sperm + 10⁻⁶ M colchicine or 10⁻⁷ M vinblastine | ~       | ~       |
| Isolated basal bodies + 10⁻⁴ M colchicine or 10⁻⁷ M vinblastine | ~       | ~       |
| 10⁻⁴ M colchicine or 10⁻⁷ M vinblastine alone | ~       | ~       |

were much more effective than those from *Chlamydomonas* at eliciting a response from unfertilized eggs as judged by comparing the minimum titer required to produce furrowing in unfertilized eggs (Fig. 8). Both *Chlamydomonas* and sperm, a specialized cell known to be effective at initiating aster and cleavage formation at one copy per egg, produced a 50% response at about 3 cell equivalents injected. This result is close to the result of one effective unit per cell expected for the connected basal bodies of *Chlamydomonas* and for sperm. *Tetrahymena* preparations, on the other hand, produced a 50% response at one-tenth of a *Tetrahymena* cell injected per egg, indicating that there is far more than one effective unit in a *Tetrahymena* cell. This is expected for this ciliate organism which contains large numbers of basal bodies. The response of the egg to injected materials was somewhat less than expected. This could be due to incomplete disruption of the *Tetrahymena* pellicle, inactivation of basal bodies during preparation, leakage during injection, or injection of material into an area of the egg which may be unresponsive to aster or furrow formation.

It is difficult to rule out contaminating material as responsible for aster initiation. Both *Chlamydomonas* and *Tetrahymena* basal body purifications produced basal bodies as the major large component as judged by electron microscopy. Both procedures involve a number of centrifugation steps which should eliminate any soluble components. However, other microtubule structures such as flagella are still seen in the preparation. Nevertheless, when purified flagella, flagellar axonemes, cilia and hog brain microtubules as well as crude extract of *E. coli*, which contains no microtubules, are injected into *Xenopus* eggs, no asters are induced. The crude preparations which were effective at inducing aster formation, crude nuclei, the parthenogenetic, cytoplasmic fraction from brain, *Tetrahymena*, and *Chlamydomonas*, are likely to contain centrioles. It is known that the centriole occupies a position close to the nucleus in most nondividing cells (3) and might be a likely cytoplasmic contaminant of brain nuclei prepared in 0.25 M sucrose. Centrioles would also likely be removed from nuclei by sedimentation through 2.5 M sucrose as in the purified nuclear preparations which were ineffective in inducing asters. The activity of the parthenogenetic fraction from brain is also likely due to basal bodies or centrioles. Though Fraser proposed that the activity of this material might be due to microtubules (7), the properties of the factor more closely resemble those of centrioles. Fraser's active component sedimented to the 40-50% sucrose interface in a discontinuous sucrose gradient, the identical position at which basal bodies are found in the isolation procedure of Snell et al. (25). Moreover, Fraser's activity was cold stable, as is the structure of basal bodies (21), while the structure of isolated brain microtubules is not (15, 24, 30).

Due to the difficulty of fixation in large amphibian eggs, we have not yet been able to examine the asters in the electron microscope to see if each aster contains a centriole. The variable response of the egg cytoplasm and the difficulty in counting basal bodies have similarly precluded establishing a one to one correspondence between the number of asters formed and the number of basal bodies injected. However, we have found that in asters induced by *Tetrahymena* pellicle, portions of the pellicle are found in the center of the aster as shown in Fig. 9. We have also noted that sections of eggs into which an average of 10 sonicated *Chlamydomonas* was injected show only three to five asters while eggs into which 100 sonicated cells were injected show as many as 40 asters. However, the number of asters obtained with a large number of basal bodies varies, depending on the female, the injection, and other factors.

In addition to supporting the notion that the centriole or basal body plays an active role in initiating aster formation, our data allow other conclusions to be drawn. In the unfertilized...
Xenopus egg, where conditions are favorable for the assembly of a large number of asters, the addition of a basal body either injected or supplied by the sperm after fertilization is necessary and perhaps, along with tubulin, is sufficient to cause an aster to form. The ability of basal bodies isolated from ciliate and flagellate sources to induce asters, a function associated with centrioles, provides direct evidence for the interchangeable nature of these two morphologically identical structures. On the basis of these experiments, the type of structure organized by the basal body or centriole, whether an aster or flagellum appears to depend on the intracellular environment. The ability of basal bodies from primitive unicellular organisms to induce asters in vertebrate eggs suggests that the microtubule-organizing function of the centriole has been retained through evolution along with its highly conserved structure.

Aster formation seems closely linked to furrow formation in the cell cortex. The experiments of Rappaport (20) indicate that cleavage furrows are established by stimulation of the cell surface by asters. In our experiments the induction of asters was always accompanied by cleavage furrows, and vice-versa. Our finding that the furrowing response is eliminated by the microtubule poisons colchicine and vinblastine, which presumably act only on microtubules, points to dependence on asters in the furrowing response. The absence of a furrowing response in D_2O-stimulated eggs may be due to the nature of the D_2O-induced structures. Unlike Van Assel and Brachet (28), we failed to observe obvious radial symmetry in the fibrous structures induced by D_2O. Rather, our D_2O-stimulated eggs showed large areas of the animal hemisphere filled with fibrous material, which appear to contain microtubules in the electron microscope.

We were unable to induce asters or fibrous structure in oocytes by any of the means effective in unfertilized eggs of Xenopus. The inability of oocytes to form asters might arise in three ways: lack of an inducing center, an insufficient pool of tubulin subunits, or the lack of suitable cytoplasmic conditions or other unknown factors required for microtubule polymerization and aster assem-

**Figure 8** Effectiveness of injected sperm and sonicated *Chlamydomonas* and *Tetrahymena* preparations at inducing furrowing in unfertilized eggs. Unfertilized eggs were injected with 0.1 μl of various dilutions of sperm (○—○), *Chlamydomonas* (Δ—Δ), and *Tetrahymena* (□—□) as described in Methods. Furrowing response was judged 2 h after injection. Each data point is for a sample of 20 injected eggs.
FIGURE 9  Asters induced by preparations of *Tetrahymena* pellicle. Note densely stained pellicle fragments in the centers of the asters. 1 cm = 12 μm. × 400.

FIGURE 10  Fibrous, yolk-free areas induced by D₂O. Unfertilized eggs were incubated in a 50% D₂O solution for 2 h, fixed, and sectioned. 1 cm = 12 μm. × 400.

FIGURE 11  Low magnification of a D₂O-stimulated egg. 1 cm = 110 μm. × 25.
The homogeneous, fiber-filled animal hemisphere of D₂O-stimulated eggs (Fig. 11) could be due to a general nonlocalized polymerization of microtubules favored by D₂O. Since the oocyte shows no such apparent spontaneous assembly of tubulin upon D₂O stimulation, the tubulin in the oocyte may not be capable of polymerization. Experiments in progress are aimed at determining whether there is, in fact, a difference in the capacity of tubulin to assemble from oocytes and eggs.

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REFERENCES

1. ANDERSON, R. G. W., and R. M. BRENNER. 1971. The formation of basal bodies in the rhesus monkey oviduct. J. Cell Biol. 50:10-34.

2. BARTH, L. G., and L. J. BARTH. 1959. Differentiation of cells of Rana pipiens gastrula in unconditioned medium. J. Embryol. Exp. Morphol. 7:210-222.

3. BLOOM, W., and D. W. FAWCETT. 1968. A Textbook of Histology. W. B. Saunders Company, Philadelphia.

4. BORISY, G. G., and J. B. OLMSTEAD. 1972. Nucleated assembly of microtubules in porcine brain extracts. Science, (Wash. D. C.). 177:1196-1197.

5. BORISY, G. G., and E. W. TAYLOR. 1967. The mechanism of action of colchicine J. Cell Biol. 34:535-548.

6. DUMONT, J. N. 1972. Oogenesis in Xenopus laevis: stages of oocyte development in laboratory maintained animals. J. Morphol. 136:153-180.

7. FRASER, L. R. 1971. Physico-chemical properties of an agent that induces parthenogenesis in Rana pipiens eggs. J. Exp. Zool. 177:153-172.

8. GRAHAM, C. F., K. Arms, and J. B. Gurdon. 1966. The induction of DNA synthesis by frog egg cytoplasm. Dev. Biol. 14:349-381.

9. GURDON, J. B. 1967. African clawed frogs. In Methods in Developmental Biology. F. H. Wilt and N. K. Wessells, editors. T. Y. Crowell Co. New York. 75-84.

10. GURDON, J. B. 1968. Changes in somatic cell nuclei inserted into growing and maturing amphibian oocytes. J. Embryol. Exp. Morphol. 20:401-414.

11. HEIDEMANN, S. R., J. TOWSEND, and R. TOMPKINS. 1975. Synthesis of soluble protein in oocytes of Xenopus laevis. J. Exp. Zool. 191:253-260.

12. INOUÉ, S. 1964. Organization and functions of the mitotic spindle. In Primitive Motile Systems in Cell Biology, R. D. Allen and N. Kamiya editors. Academic Press, Inc., New York. 549-594.

13. INOUÉ, S., and H. SATO. 1967. Cell motility by labile association of molecules. J. Gen. Physiol. 50:259-288.

14. KIRSCHNER, M. W., R. C. WILLIAMS, M. WEINGARTEN, and J. C. GERHART. 1974. Microtubules from mammalian brain: some properties of their depolymerization products and a proposed mechanism of assembly and disassembly. Proc. Natl. Acad. Sci. U. S. A. 71:1159-1163.

15. OLMSTEAD, J. B., and G. G. BORISY. 1973. Microtubules. Annu. Rev. Biochem. 42:507-540.

16. OLMSTEAD, J. B., and G. G. BORISY. 1973. Characterization of microtubule assembly in porcine brain.
17. Pickett-Heaps, J. D. 1969. The evolution of the mitotic apparatus: an attempt at comparative ultrastructural cytology in dividing plant cells. *Cytobios*. 1:257–280.

18. Pickett-Heaps, J. D. 1972. Variation in mitosis and cytokinesis in plant cells. Its significance in the phylogeny and evolution of ultrastructural systems. *Cytobios*. 5:59–77.

19. Porter, K. R. 1966. Cytoplasmic microtubules and their functions. In *Principles of Biomolecular Organization*. G. E. W. Wolstenholme and M. O'Connor editors. J. A. Churchill Ltd., London. 308–345.

20. Rapaport, R. 1971. Cytokinesis in animal cells. *Int. Rev. Cytol.* 31:169–215.

21. Rubin, R. W., and W. P. Cunningham. 1973. Partial purification and phosphotungstate solubilization of basal bodies and kinetodesmal fibers from *Tetrahymena pyriformis*. *J. Cell Biol.* 57:601–612.

22. Satir, B., and J. L. Rosenbaum. 1965. The isolation and identification of kinetosome-rich fractions from *Tetrahymena pyriformis*. *J. Protozool.* 12:397–405.

23. Shaver, J. 1953. Studies on the initiation of cleavage in the frog egg. *J. Exp. Zool.* 122:169–192.

24. Shelanski, M. L., F. Gaskin, and C. R. Cantor. 1973. Microtubule assembly in the absence of added nucleotides. *Proc. Natl. Acad. Sci. U.S.A.* 70:765–768.

25. Snell, W. J., W. L. Dentler, L. T. Haimo, L. T. Binder, and J. L. Rosenbaum. 1974. Assembly of chick brain tubulin onto isolated basal bodies of *Chlamydomonas reinhardtii*. *Science* (Wash. D. C.). 185:357–359.

26. Stubblefield, E., and B. R. Brinkley. 1967. Architecture and function of the mammalian centriole. *Symp. Int. Soc. Cell Biol.* 6:175–218.

27. Sueoka, N., K. S. Chiang, and J. R. Kates. 1967. DNA replication in meiosis of *Chlamydomonas reinhardtii*: isotypic transfer experiments with a strain producing eight zoospores. *J. Mol. Biol.* 25:47–66.

28. Van Assel, P. S., and J. Brachet. 1966. Formation de cytasters dans les oeufs de bactriens sous l'action de l'eau lourde. *J. Embryol. Exp. Morphol.* 15:143–151.

29. Watson, M. R., and J. M. Hopkins. 1962. Isolated cilia from *Tetrahymena pyriformis*. *Exp. Cell Res.* 28:280–295.

30. Weisenberg, R. C. 1972. Microtubule formation in *vitro* in solution containing low calcium concentration. *Science* (Wash. D. C.). 177:1104–1106.

31. Weisenberg, R. C. 1973. Regulation of tubulin organization during meiosis. *Am. Zool.* 13:981–987.

32. Weisenberg, R. C., G. G. Borsy, and E. W. Taylor. 1968. The colchicine binding protein of mammalian brain and its relation to microtubules. *Biochemistry.* 7:4466–4479.

33. Weisenberg, R. C., and A. C. Rosenfeld. 1975. *In vitro* polymerization of microtubules into asters and spindles in homogenates of surf clam eggs. *J. Cell Biol.* 64:146–158.

34. Wilson, E. B. 1896. The Cell in Development and Inheritance. The MacMillan Company, New York.

35. Wilson, L. 1970. Properties of colchicine binding protein from chick embryo brain. Interaction with vinca alkaloids and podophyllotoxin. *Biochemistry.* 9:4999–5007.

36. Witman, G. B., K. Carlson, J. Berliner, and J. L. Rosenbaum. 1972. *Chlamydomonas* flagella. I. Isolation and electrophoretic analysis of microtubules, matrix, membranes and mastigonemes. *J. Cell Biol.* 54:507–539.