

The Reproduction of Centrosomes: Nuclear versus Cytoplasmic Controls

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Abstract. The tight coordination normally found between nuclear events and the doubling of centrosomes at each cell cycle suggests that nuclear activities may be part of the mechanism that controls the reproduction of centrosomes. To determine if this is the case, we used a micropipette to completely remove the nucleus from eggs of the sea urchin *Lytechinus variegatus* at prophase of the first mitosis, leaving only one centrosome in the cell. The subsequent behavior of this centrosome was then followed in vivo with the polarization microscope. In all cases the centrosome reproduced in a precise 1:2:4:8 fashion with a periodicity that was slightly slower than the centrosome cycle of control eggs. The cell cycle-related changes in centrosome morphology were identical to those of control eggs in that: (a) the astral birefringence varied cyclically to a normal extent, (b) the astral focus enlarged and then flattened during the telophase equiva-

lent, (c) cleavage furrows were initiated as the astral birefringence faded, and (d) daughter centrosomes separated before the increase in astral birefringence at the onset of each mitosis. To determine if centrioles also reproduced normally, enucleate eggs were followed in vivo until they contained eight centrosomes. They were then individually removed from the preparations, fixed, and embedded. Each egg was serially 0.25- μ m sectioned for observation with the high voltage electron microscope. We completely reconstructed 23 centrosomes in four eggs; all centrosomes contained two centrioles apiece. These results demonstrate that the subunits for complete centrosome assembly can be stockpiled ahead of time and that the properly controlled use of these subunits for centrosome reproduction does not require nuclear transcription or nuclear DNA synthesis at each cell cycle.

At the end of mitosis each daughter cell receives just one centrosome, and by the start of the next mitosis each daughter contains two and only two centrosomes. This reproduction of the centrosome requires the duplication of both the centriole pair (15) and its associated shell of osmiophilic material, known as the pericentriolar material, which serves as the centrosomal microtubule organizing center (MTOC)¹ (7). Not only must the number of centrosomes formed before each division be precisely controlled, but also centrosome reproduction must be properly coordinated with nuclear events.

The necessity for tight control of centrosome reproduction raises the important question of whether or not the nucleus regulates this process. This question has been given additional weight by reports that both the centrioles and the pericentriolar material may contain functionally important RNA (1, 2, 5, 9, 21, 24, 29). In addition, the temporal correlation between procentriole formation and the onset of DNA synthesis suggests that these events are causally related.

The interrelationship between nuclear activities and centrosome reproduction has not been clearly defined experimentally. Many workers (15, 22) have shown that inhibition of DNA synthesis by a variety of agents does not prevent the

formation or elongation of daughter centrioles. However, these agents arrested the cell cycle, and the cells did not enter mitosis. Consequently, these workers could not determine if repeated cycles of centriole duplication could occur without DNA synthesis. It is possible that the prerequisites for the observed centriolar duplication were completed before the block and were dependent upon the prior uninhibited round of DNA synthesis.

The importance of other nuclear activities, such as transcription, in centrosome reproduction has also been examined. Kuriyama and Borisy (15) enucleated cultured Chinese hamster ovary cells with cytochalasin B and examined the cytoplasts for centriole duplication and elongation. They found that existing procentrioles elongate, but new procentrioles do not form. They concluded that nuclear transcripts or "signals" from the nucleus were required at each cell cycle for centriole duplication. This conclusion is supported by reports showing that inhibition of nuclear transcription by actinomycin D arrests the cell cycle and blocks centrosome reproduction in cultured cells (4, 23).

However, all such studies of centrosome reproduction in cultured cells must deal with the fact that such cells need to grow before dividing. Since chemical or physical enucleation stops their cell cycle, the lack of centriole or centrosome reproduction could simply result from an arrest of the cell

1. *Abbreviation used in this paper:* MTOC, microtubule organizing center.

cycle when the cell is not prepared to duplicate these structures. A way of circumventing this problem might be to examine cells such as the sea urchin egg in which the necessary precursors for limited development are stockpiled in the cytoplasm.

Indeed, some very early work suggests that the nucleus is not required for duplication of asters in sea urchin eggs. In 1898 Ziegler (32) described a single fertilized sea urchin egg in which all the chromosomes remained in one blastomere at the first division. The aster in the enucleated daughter repeatedly doubled, and irregular cleavage furrows were formed. At about the same time Wilson and others (reviewed in reference 30) reported that cytasters induced by parthenogenetic treatments could double once in enucleate sea urchin egg fragments. Also, an excellent study by Lorch (16) in the early 1950's reported that the asters in enucleate sea urchin egg blastomeres could regularly multiply for up to 7 h before the blastomeres fragmented.

Contrary to the old work, recent studies on sea urchin eggs suggest that nuclear DNA synthesis is required for centrosome reproduction. Aphidicolin, a specific inhibitor of the alpha DNA polymerase, blocks nuclear DNA synthesis and prevents these eggs from entering mitosis as monitored by nuclear envelope breakdown. Although the first doubling of the sperm aster after fertilization occurred, subsequent doublings of the asters were not observed (3, 11, 19). The ability of centrioles to reproduce was not examined. In addition, the role of nuclear transcription in the reproduction of centrosomes in sea urchin eggs is unclear. When nuclear transcription is drastically reduced with actinomycin D, repeated cell division (and presumably centrosome reproduction) before gastrulation is not inhibited (8). However, a small but possibly significant amount of transcription occurs in these eggs.

Given these uncertainties, we have examined with modern methods the ability of centrioles and the centrosomal MTOC to reproduce in enucleated sea urchin eggs. The major issue we sought to resolve was whether or not nuclear activities at each cell cycle are necessary for the control of centrosomal reproduction. In principle, the involvement of the nucleus in the mechanisms that control and execute the reproduction of centrosomes could be either direct or indirect. For example, the cell could require newly synthesized transcripts or their translational products at each cell cycle to assemble centrosomal structures. Such direct control of centrosome assembly at the genomic level could provide the cell with logical ways to coordinate centrosomal events with nuclear events and control the number of centrosomes formed. Alternatively, the nucleus might not be an integral or necessary part of the control mechanisms for centrosome reproduction. The cell could accumulate a nonlimiting pool of subunits for centrosome assembly. The time and number of centrosomes formed might entirely be under cytoplasmic control, as the early observations on enucleated sea urchin eggs suggest.

In this report we demonstrate that the repeated doubling of centrioles and centrosomal MTOCs occurs normally in enucleated sea urchin eggs. Thus, centrosome reproduction can be an entirely cytoplasmic phenomenon that does not require nuclear DNA synthesis or nuclear transcription at each cell cycle.

Materials and Methods

Living Material and Light Microscopy

Eggs from the sea urchin *Lytechinus variegatus* were obtained by intracoelomic injection of 0.5 M KCl, and the fertilization envelopes were mechanically removed just after insemination as described elsewhere (6, 28). Eggs were mounted in a microinjection chamber that was slightly modified from the design described by Kiehart (12) by using a single layer of Scotch double stick tape in place of mylar film. The region of the chamber holding the eggs was prewashed with 2-3% (wt/vol) protamine sulfate in distilled water (Sigma Chemical Co., St. Louis, MO; grade III from herring) to better anchor the eggs. Eggs were observed and photographed with a Zeiss ACM microscope (Carl Zeiss, Inc., Thornwood, NY) modified for polarization microscopy. Photographs were recorded on Kodak Plus X film that was developed in Kodak Microdol X.

Enucleations were performed using a Leitz micromanipulator in conjunction with the pressure injection system described by Hiramoto (10) and Kiehart (12). A small portion of the oil cap was expelled to mark the egg after the micropipette was inserted. The micropipette was then moved up against the prophase nucleus, and controlled suction was applied by backing off the screw feed on the syringe. After removal of the whole nucleus and one of the asters, the micropipette was withdrawn. For those few cases in which the nucleus fragmented, the eggs were immediately discarded. Video recordings of eggs *in vivo* were made with a Dage-MTI Inc. (Wabash, MI) 67M camera and a Gyr Products (Anaheim, CA) time lapse recorder. Measurements of astral spacing were made directly from the video monitor. Five replicate measurements of aster spacing were made for each time point.

Electron Microscopy

Selected enucleated eggs were followed for several hours until the original centrosome had undergone three rounds of reproduction (until eight asters were present). The egg was then dislodged by gentle lateral pressure with the shaft of the micropipette and was moved out of the holding step of the chamber, at which point it sank to the bottom of the preparation. Alternatively, the egg was dislodged by lateral pressure with the micropipette and then pulled out of the holding step of the chamber by grabbing it with the tip of the pipette and gentle suction. In either case, once at the bottom of the preparation, the egg was aspirated gently into a wide bore micropipette and transferred to the fixative. This portion of the procedure was performed under a dissecting microscope. Each egg was fixed, dehydrated, and infiltrated as described elsewhere (28). During processing for electron microscopy each egg was contained separately in a 1.5-ml plastic microcentrifuge tube (Bio-Rad Laboratories, Richmond, CA) whose tip was cut off and covered with a 50- μ m mesh nylon screen (TETKO Inc., Elmsford, NY). Each egg was embedded directly within the microcentrifuge tube which was removed from the embedment after polymerization. The methods used for the preparation and observation of serial 0.25- μ m sections have been described in detail elsewhere (25).

Results

Reproduction of Asters

Fertilized eggs were physically enucleated with a micropipette in early prophase of the first mitosis, when the astral birefringence was starting to increase. After insertion of the pipette, a small portion of the oil cap was expelled on the side of the egg furthest from the entry site to mark the egg. The tip of the pipette was then applied to the surface of the nucleus and controlled suction was applied. In over 90% of the cases, the nucleus deformed and smoothly entered the pipette as a single unit without any visible fragmentation. In the four to five remaining cases, the nucleus fragmented. This fragmentation was easily detected at the time, and those eggs were immediately discarded. In most cases, we removed one aster along with the nucleus. Eggs with two asters remaining after enucleation showed the same doubling of asters as those with only one aster.

To provide additional proof that we removed the nucleus completely, we transferred preparations containing enucleated eggs from the polarization microscope to a microscope equipped for differential interference contrast microscopy and relocated the enucleated eggs. Differential interference contrast microscopy is one of the best ways to detect fragments of nuclei in these optically clear eggs. Careful through focus observation of eggs judged to be enucleated on the polarization microscope revealed no evidence of residual nuclear fragments (Fig. 1). Yet more evidence for complete enucleation came from our serial 0.25- μ m section analysis (described later) which revealed no residual nuclear fragments after three cycles of centrosome reproduction.

Individual enucleated eggs were followed *in vivo* with a polarizing microscope to examine changes in astral morphology and to determine if the asters reproduced. We found in all 52 cases examined that the aster reproduced in a precise 1:2:4:8 fashion (Fig. 2, *b, f, j, and l*). In those preparations allowed to progress further, we found that the asters continued to regularly increase in number. However, the superposition of many asters in these eggs made precise counts difficult.

The birefringence and morphology of the asters in enucleate eggs varied cyclically as it does in normal eggs as they traverse the cell cycle (compare cycles of astral morphology shown in Fig. 2 with those of the control egg seen in Fig. 3). The eggs were enucleated in prophase when astral birefringence was weak (Fig. 2 *a*). The birefringence of the aster then increased to a maximum as if the egg cytoplasm had entered the mitosis portion of the cell cycle (Fig. 2 *b* vs. 3, *b-d*). Later, the aster became larger, adopted a more fibrous appearance, and then its birefringence precipitously dropped as if the egg were entering telophase (Fig. 2, *c-d* vs. 3, *e-f*). Immediately thereafter, the aster flattened (to a disc shape) as it would in a normal egg (20), and in time the two daughter asters became noticeable (Fig. 2 *e*). At second mitosis, astral birefringence increased as it would in a normal egg (Fig. 2 *f* vs. 3, *h-j*). Later the daughter asters enlarged and their birefringence precipitously dropped as the eggs entered what appeared to be a second telophase (Fig. 2, *g-h*). As in first telophase, the asters then flattened before the noticeable appearance of the new daughter asters (Fig. 2 *i*). In time, the astral birefringence increased at third mitosis (Fig. 2 *j*) and then precipitously faded as the egg entered

third telophase. Shortly thereafter, the egg initiated multiple furrows (Fig. 2 *k*) that later regressed. At fourth mitosis the egg contained eight asters (Fig. 2 *l*). In all cases, the time of visible aster duplication in enucleated eggs showed the same temporal coordination with cycles of astral birefringence as is found in normal eggs.

Cleavage furrows were initiated in enucleated eggs at second telophase but were weak. In all cases the furrows regressed before the egg entered the next mitosis. However, persistent furrows formed in a few eggs at later telophases. The weakness of furrows is not directly attributable to the slight flattening of these eggs in the preparations or to the coating of protamine sulfate on the glass surfaces, since control eggs in the same preparations cleaved in a normal fashion.

Daughter asters were not randomly distributed within the egg volume; they remained spatially associated with each other after each round of doubling (Fig. 2 *i*). As in normal eggs, the axis between the daughter pairs at the next mitosis was approximately at right angles to the axis between the parental asters (Fig. 2, *f-j* vs. 3, *d* and *j*). We could not determine with certainty if the third "division" asters established a polar orientation as they normally do. Since these eggs started with only one aster, they entered the third mitosis with four asters instead of eight as in normal cells. Also, the failure of cleavage furrows left the third division asters with a cytoplasmic volume that was larger and of different geometry than that normally found at this division. However, the aster pairs at the third mitosis were often tilted (Fig. 2 *j*). We could not be certain if this reflected a specific interaction of the asters with special portions of the cortex or alternatively a tendency of the asters to move as far apart as possible. In eggs that have tetrapolar spindles induced by mercaptoethanol, asters tend to establish an orthogonal arrangement (17, 27).

The cycles of astral birefringence and astral doubling were always longer and more variable in enucleate eggs than in controls. No one cycle seemed to be prolonged significantly more than any other. For this comparison we used the time to the first telophase and times between subsequent telophases (judged by the drop in astral birefringence and enlargement of the asters) because there were no nuclear events to be observed. The time from fertilization to the first telophase was \sim 31 min longer in the enucleated eggs than

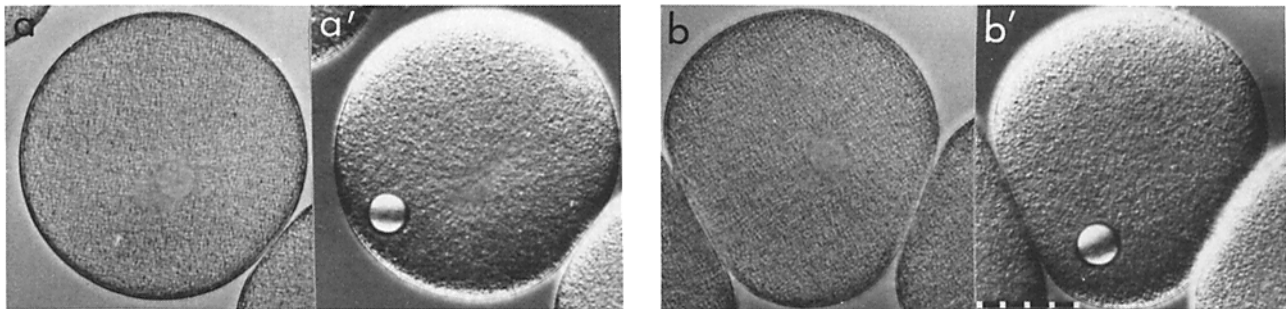


Figure 1. Demonstration of complete enucleation for two eggs. (*a* and *b*) The eggs as they appear under the polarization microscope shortly before enucleation. (*a'* and *b'*) The same eggs after enucleation as seen in the differential interference contrast microscope. The prominent sphere in each egg is the oil drop expelled from the micropipette to mark the egg. Careful through focus examination of enucleated eggs revealed no sign of nuclear fragments. The fibrous clear area to the right of the oil drop in *a'* is the single aster left from enucleation. 10 μ m per scale division.

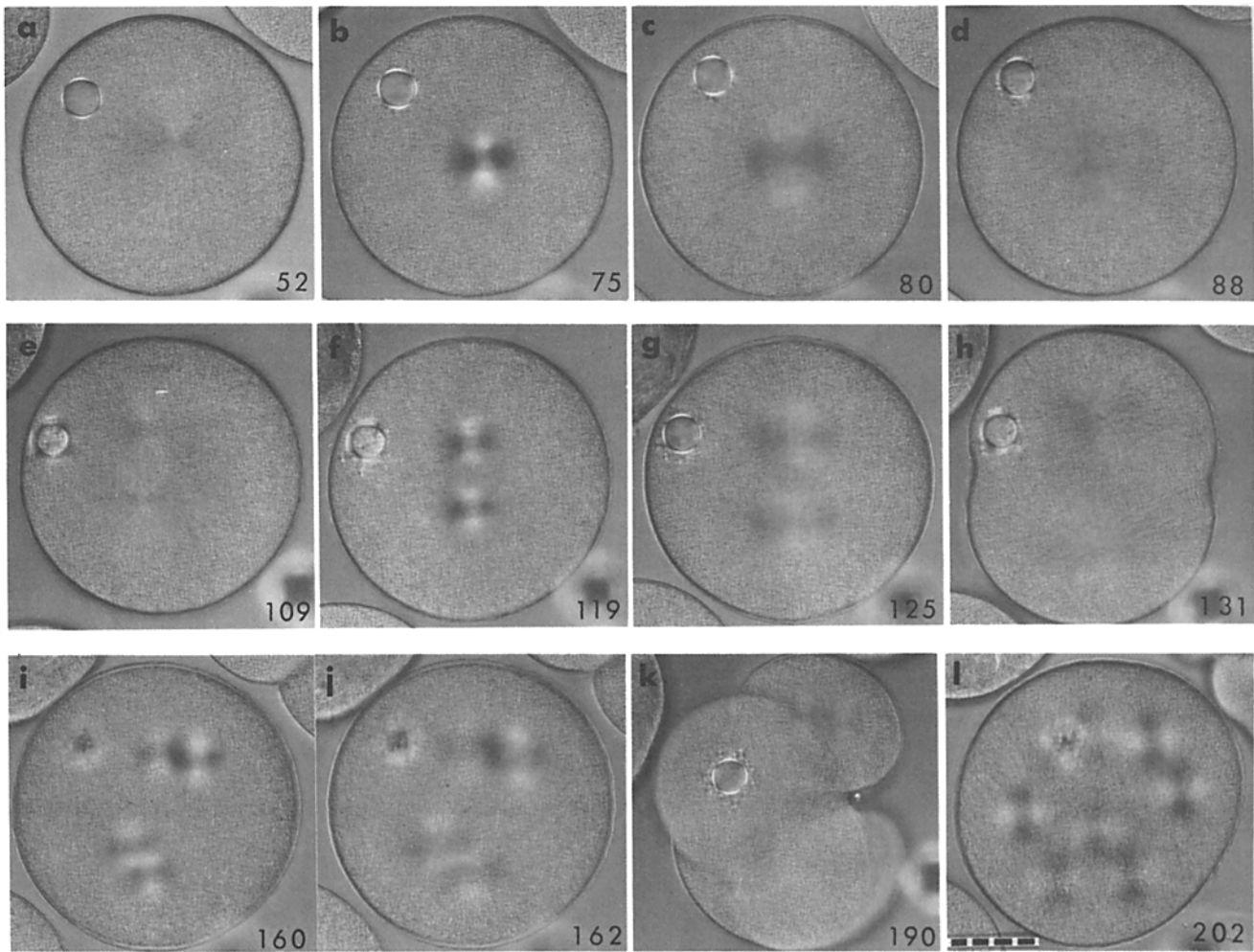


Figure 2. Reproduction of centrosomes in an enucleate egg. (a) Egg shortly after enucleation in prophase of first mitosis. The single remaining centrosome is centrally located and the oil drop expelled from the micropipette is seen in the upper left portion of the egg. (b) First mitosis. The birefringence of the aster increases markedly. (c–d) Telophase. The astral focus enlarges and its birefringence drops precipitously. (e) Second prophase. Two weakly birefringent daughter asters become visible. (f) Second mitosis. Astral birefringence has noticeably increased. (g–h) Second telophase. Astral birefringence decreases rapidly and the egg initiates a cleavage furrow that later regresses. (i–j) Third mitosis. Two centrosomes have reproduced to four. (k) Third telophase. Astral birefringence has faded and multiple furrows are initiated. These later regress. (l) Fourth mitosis. Four asters have reproduced to eight. Seven asters are visible at this plane of focus. The same egg is shown in all frames; minutes after fertilization are shown at lower right corner of each frame. Polarization microscopy $10\ \mu\text{m}$ per scale division.

in the controls (105 ± 26.7 vs. 76 ± 7.4 min, respectively). Telophase I to telophase II was ~ 38 min longer for the enucleates (77 ± 34.0 vs. 39 ± 3.9 min), and the time from telophase II to telophase III was ~ 24 min longer for the enucleated eggs (61 ± 23.4 vs. 37 ± 2.1 min). Since picking the exact time of the drop in astral birefringence was somewhat subjective, these times represent close approximations, with a maximum error of plus or minus 4 min.

We note parenthetically that the timing of the cycles of astral birefringence for the enucleated egg seen in Fig. 2 does not appear to be significantly different from the corresponding cycles of the control egg shown in Fig. 3. The particular enucleated egg appearing in Fig. 2 cycled more rapidly than most other enucleates. On average, the timing of enucleated eggs is slower and more variable than the controls.

We used time lapse video recordings of six experiments to compare the initial spacing and subsequent movements of

daughter asters in enucleate and control eggs. We found that the initial spacing of the asters in enucleate eggs, when they first became visible at the start of mitosis, was generally several microns greater than that in control eggs at nuclear envelope breakdown. This is consistent with an observation from one of our previous studies showing that two asters without chromosomes have a greater spacing between them than two asters (in the same cell) that are engaged to chromosomes between them (Fig. 14 c of reference 26). During the progression of enucleate eggs through mitosis into the telophase there was a monotonic increase in aster separation at an average rate for all cycles of $1.6\ \mu\text{m}/\text{min}$, which was similar to the rate we observed in control eggs ($1.5\ \mu\text{m}/\text{min}$). The final maximum spacing between daughter asters at telophase was the same in both enucleate and control eggs ($30\ \mu\text{m}$).

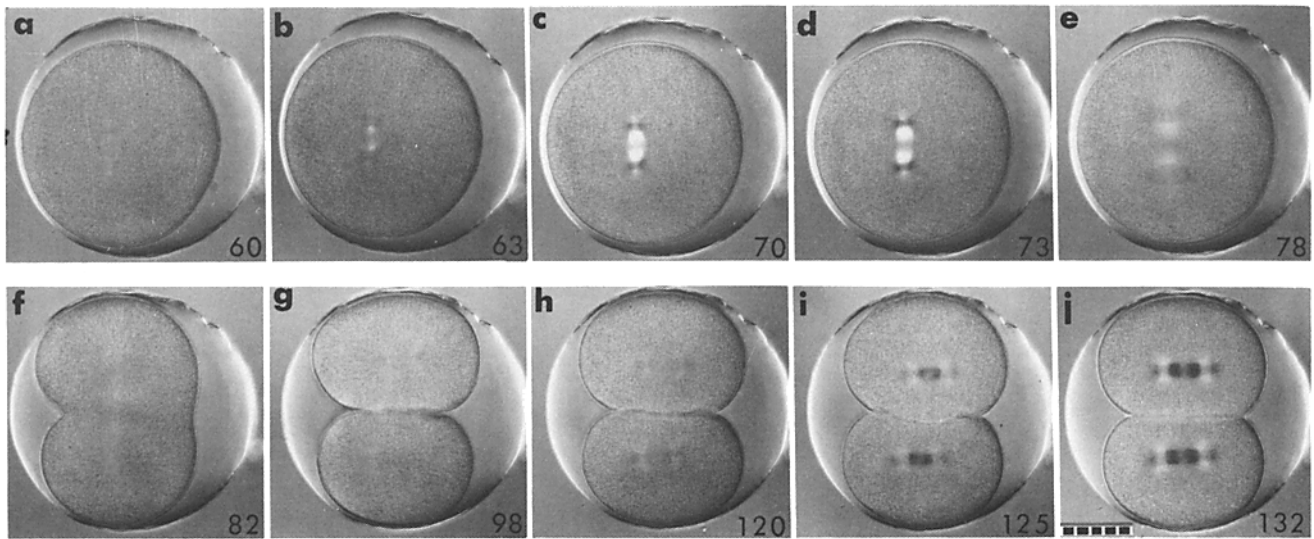


Figure 3. Cycles of astral birefringence in a normal fertilized egg from first to second mitosis. (a) Prophase, weakly birefringent asters on either side of nucleus. (b) Nuclear envelope breakdown; astral birefringence becomes stronger. (c) Metaphase. (d) Anaphase onset, astral birefringence at a maximum. (e) Telophase, astral focus enlarges and astral birefringence drops rapidly. (f) Cleavage in late telophase. (g) Early prophase, daughter asters are weakly birefringent. (h) Late prophase, astral birefringence increases. (i) Prometaphase, astral birefringence continues to increase. (j) Anaphase onset, astral birefringence greatest. Polarization microscopy. Minutes after fertilization shown in the lower corner of each frame. 10 μm per scale division.

Reproduction of Centrioles

Our light microscope observations show that the MTOC aspect of the centrosome can repeatedly reproduce normally in the absence of a nucleus. We additionally sought to determine if centrioles also reproduced, and if so, whether or not their reproduction was properly coordinated with the doubling of the centrosomal MTOC. Enucleated eggs were followed *in vivo* until the aster had doubled three times to form eight asters. Each egg was then removed from the preparation and prepared for electron microscopy. We serially sectioned each egg previously followed *in vivo* for observation with the high voltage electron microscope. This single cell correlative light and electron microscopic approach allowed us to know the prior history of each cell analyzed at the ultrastructural level. 23 asters in four eggs were completely reconstructed; all 23 contained just two centrioles apiece. The centrioles appeared normal in all cases. In 13 asters of two eggs, the centrioles were separated by distances ranging from 1 to 5 μm . In the other 10 asters the centrioles were close together in a roughly orthogonal arrangement. For a given egg, either all centrioles were close together in pairs or all the centrioles in each pair were slightly separated. This variability from egg to egg in centriole spacing may reflect differences in the point of the astral cycle at which the egg was fixed (see reference 20).

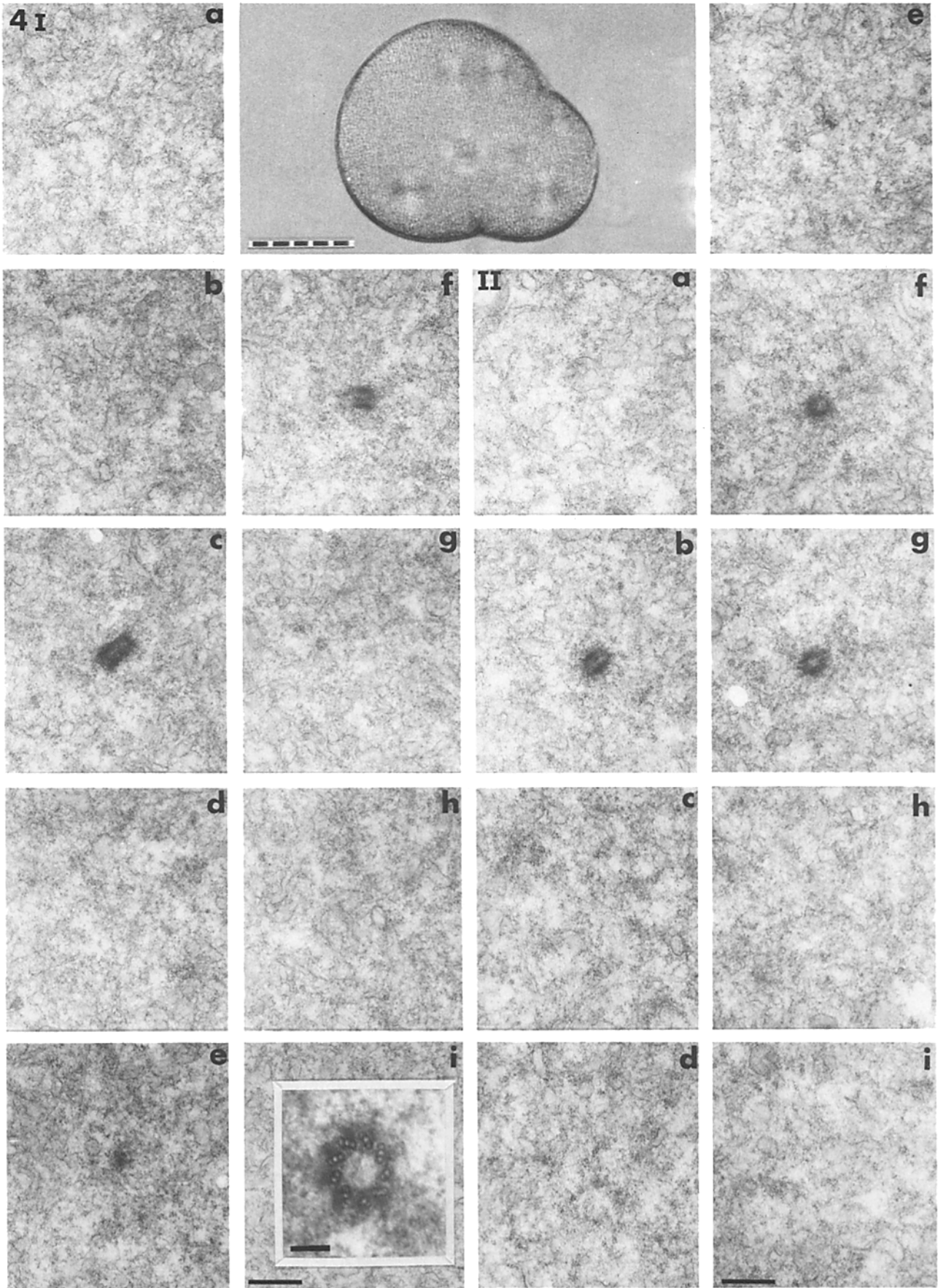
In Fig. 4, the particular egg whose centrosomes are shown in the serial sections is seen just before fixation in the inset at the top of the plate. This egg contained eight asters, the great granddaughters of the original aster remaining after enucleation. Only five asters are imaged at this plane of focus; the out of focus image of the oil drop is visible in the center of the egg. Serial sections through two centrosomes are shown in series I and II. The apparent lack of definition in these micrographs is due to the thickness of the sections and the consequent superposition of structures. All sections

were photographed at the same tilt angle and as a consequence, those centrioles cut obliquely do not have a textbook appearance. For this cell we completely reconstructed seven of the eight centrosomes and found two slightly separated centrioles in each. To demonstrate that the centrioles found in these centrosomes were structurally normal, we tilted the section shown in series I, frame f. This particular centriole is seen at higher magnification in the inset superimposed on series I, frame i (no centriolar structures were found in the section represented by frame i). Three of the triplet blades are cut obliquely due to the twist in the cylinder of centriolar microtubules.

Fig. 5, a and b shows an unseparated centriole pair from another egg we analyzed. Fig. 5 a shows a low power micrograph of one aster close to the oil drop. The centriole pair is seen in the upper central portion of the frame, and a portion of the oil drop is the clear area in the lower right corner of the frame. Fig. 5 b shows a higher magnification photograph of the centriole pair in this centrosome. Fig. 5, c-d shows serial sections of a slightly separated centriole pair from a second egg. The two centrioles are found at either end of a flattened mass of osmiophilic material that has the appearance of pericentriolar material. The appearance of this astral focus is the same as that described for asters at the early stages of splitting and separation in another species of sea urchin (20).

Discussion

The purpose of our study was to experimentally determine if DNA synthesis, nuclear transcripts, or signals from the nucleus at each cell cycle are a necessary part of the mechanism that controls the reproduction of centrosomes. We used sea urchin eggs because they do not need to grow between divisions as cultured cells do. Thus, removal of the nucleus



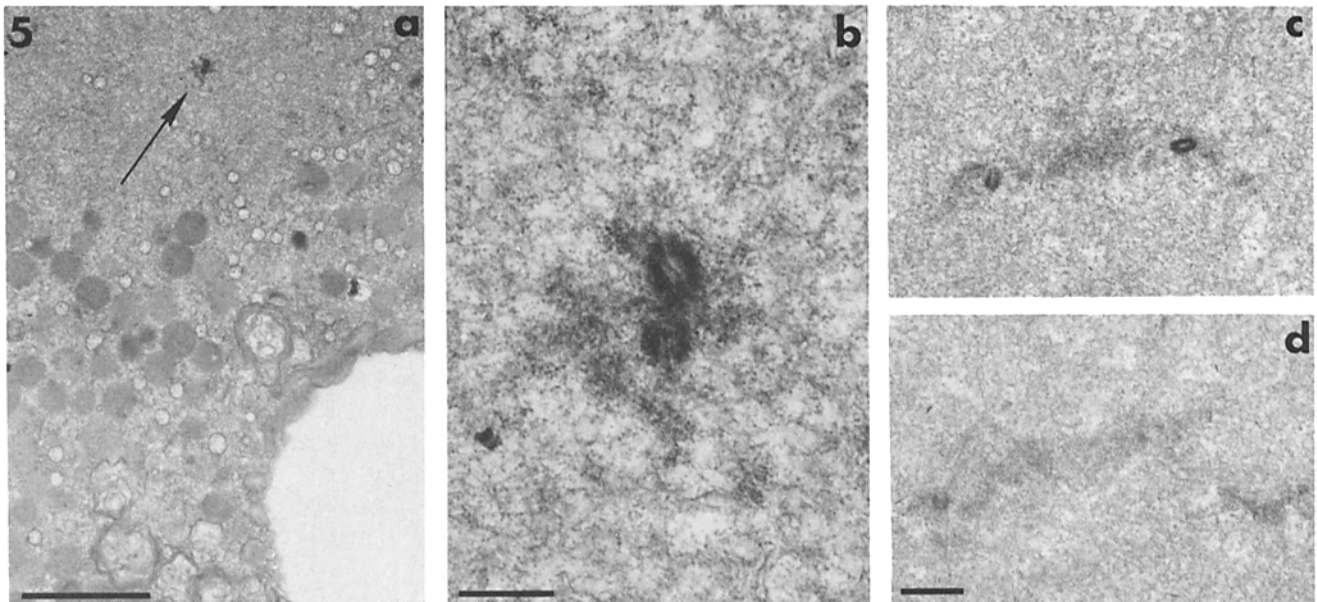


Figure 5. Centriole pairs in centrosomes from other enucleated eggs that contained eight centrosomes at the time of fixation. (a) Low magnification micrograph of centrosome near oil droplet. Centriole pair is indicated by arrow; a portion of the oil droplet is seen in the lower right corner of the frame. (b) Higher magnification view of the same centriole pair showing the osmiophilic pericentriolar material. (c-d) Two serial sections of one centrosome from another egg. Centrioles are found at ends of a flattened body of osmiophilic material. This centrosome is in the early stages of reproduction. Bars: (a) 5 μm ; (b) 0.5 μm ; (c and d) 1 μm .

from these eggs should not stop cell cycle events as a number of studies suggest (8, 14, 16, 30, 32). By physically enucleating the eggs, we avoided concerns about inhibitor studies that residual but possibly important nuclear activities continue. Furthermore, by following three or more rounds of centrosome reproduction, we avoided the criticism applicable to some inhibitor studies that the prerequisites for centrosome reproduction could have been fulfilled in the prior, uninhibited, cell cycle. Similarly, by following a minimum of three rounds of centrosome reproduction, we could be certain that we were looking at reproductive events rather than the subdivision of existing structures. This is important because conditions that prolong mitosis in sea urchin eggs allow existing centrosomal structures to split or subdivide without duplication (22, 27). In these situations, however, the centrosomes subdivide only once, and the progeny of the split (but not duplicated) centrosomes never visibly double before the next division. In our present study, every centrosome doubled before each mitosis.

Our results show that input from the nucleus at each cell cycle is not mandatory for the repeated reproduction of the complete centrosome. Without a nucleus, centrioles and the centrosomal MTOC can repeatedly reproduce normally from a stored pool of subunits. In addition, the doubling of asters remains properly coordinated with the cyclical rise and fall of astral birefringence, and the cell maintains normal

control over the number of asters formed at each cycle. From these results we conclude that the mechanisms that control the assembly of the centrosomal subunits and the quantity of subunits that are used must be entirely cytoplasmic. This includes the use of both the proteins that make up the centrosomal structures and the RNAs that are thought to play important roles in the activity of the centrioles and the centrosomal MTOC (1, 2, 5, 9, 21, 22, 29).

In concluding that centrosome reproduction is independent of nuclear transcripts produced at each cell cycle, we do not imply that gene products are irrelevant for this process, since every cell component is directly or indirectly the product of nuclear transcripts. We simply show here that the gene products for centrosome reproduction can be stored as a pool and that their use can be entirely under cytoplasmic control.

At face value, our results are at variance with those of a recent study that shows that new procentrioles do not form in enucleated cultured cells (15). The simplest explanation for this discrepancy is that enucleation, inhibition of DNA synthesis, or inhibition of nuclear transcription stops the cell cycle in many cells (4, 15, 22, 23), and it presumably does not proceed to a point where complete centrosome reproduction is scheduled to occur. In this regard, it is clear that the sea urchin egg is a specialized cell. These eggs do not grow during the early cleavage stages, and many gene products are

Figure 4. Ultrastructural analysis of centriole reproduction in an enucleated egg. This egg was enucleated and followed until the single remaining centrosome had reproduced to eight. The egg was then fixed and serially 0.25- μm sectioned. Inset at top of plate, egg just before fixation. Only five of the eight centrosomes are visible in this plane of focus. (Sequence I) Complete serial reconstruction of one centrosome from egg shown in inset. Two slightly separated centrioles are found in this centrosome; seen in frames a and e-f. A higher magnification and tilted view of the centriole shown in frame f is seen in the inset superimposed on frame i. (Sequence II) Complete serial reconstruction of a second centrosome from same egg. This centrosome also contains two separated centrioles; seen in frames b and f and g. The remaining centrosomes all contain just two centrioles. (Inset) 10 μm per scale division. Electron micrographs: Bar, 0.5 μm . Bar (inset), 0.1 μm .

known to be stockpiled before fertilization. Thus, the key finding of our work is that the minimal, and essential, control mechanisms for centrosome reproduction can be entirely cytoplasmic.

In somatic cells, it is also conceivable that specialized interlocks exist between nuclear activities and the events of centrosome reproduction. Such mechanisms could help ensure the proper coordination of centrosome reproduction with other cell cycle events. The apparent dependence of centrosome reproduction on nuclear transcription in such cells may simply reflect the cell's need to replenish the cytoplasmic pool of centrosomal subunits at each cell cycle. It is also possible that the time of centrosome reproduction could be dictated by the stage-specific transcription of limiting numbers of key RNAs. If so, such limits operate in addition to the basic, minimal cytoplasmic control mechanisms that are at the core of centrosome reproduction.

Our results also vary from those of studies in which DNA synthesis in sea urchin eggs was specifically inhibited by Aphidicolin (3, 11, 19). Although the first round of centrosome doubling occurred at the time of pronuclear fusion, the eggs did not undergo nuclear envelope breakdown or show subsequent cycles of centrosome doubling. Since this finding is in contrast to our results with enucleated eggs, we have repeated these studies and found that centrosome duplication does repeatedly occur in the presence of Aphidicolin if the eggs are followed long enough (Sluder, G., and K. Lewis, manuscript submitted for publication). Our results are supported by those of Nagano et al. (18) which show that starfish zygotes will cleave to between 256 and 512 cells in the presence of Aphidicolin before they degenerate. The regular increase in cell number with time suggests that the eggs are not fragmenting but rather that centrosomes are reproducing normally.

Cell Cycle Considerations

Our observations that centrosome reproduction, microtubule nucleation at the centrosome, and cleavage furrow initiation can repeatedly cycle normally without a nucleus, add more items to the list of cell cycle events that seem to be controlled in the cytoplasm. For example, changes in cortical stiffness and the cytoplasm's ability to condense chromosomes have been observed to cycle in enucleated sea urchin eggs (14, 31). In enucleate *Xenopus* eggs, cyclical changes in the activity of maturation promoting factors and waves of surface contraction have been observed (reviewed in reference 13).

The cyclic nature of these events in the absence of a nucleus suggests that the mechanisms that drive the cell cycle are cytoplasmic not nuclear. Contrary to common practice, the cell cycle might best be defined in terms of cyclical changes in the cytoplasmic state rather than by changes in nuclear morphology or activity. Perhaps the substances required for progress through the cell cycle can be used in a properly controlled fashion from cytoplasmic pools if they have been stockpiled ahead of time. The experimentally observed requirement for nuclear activities in somatic cells may simply indicate that, for such cells, the substances required for the orderly progression through the cell cycle must be produced at or near the time they are needed. Thus, nuclear events may not drive the cell cycle but rather reflect the directions given by the changing cytoplasmic conditions.

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