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Selective Inhibition of Cytokinesis in Sea Urchin Embryos by Low Concentrations of Stypoldione, a Marine Natural Product That Reacts with Sulfhydryl Groups

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SUMMARY

Stypoldione is a marine natural product that inhibits cells division in marine embryos and in mammalian cell cultures. The mechanism responsible for the ability of the compound to inhibit cell division is not known. The compound was found in early studies to inhibit polymerization of tubulin into microtubules *in vitro*, which lead to the suggestion that inhibition of microtubule polymerization in cells might be responsible for the ability of the compound to inhibit cell division. More recently, stypoldione was found to react covalently with the sulfhydryl groups of a number of proteins including tubulin and with sulfhydryl groups of peptides and small molecules. Thus, stypoldione could potentially react with a large number of cellular targets. In the present study, we have examined the effects of stypoldione on the organization of microtubules and chromatin in cells, in relation to the ability of the compound to inhibit cell division. We used indirect immunofluorescence light microscopy of fixed and stained sea urchin embryos during the first and second divisions after fertilization, with stains specific for tubulin and DNA. We found that stypoldione exerted qualitatively different effects on cell division and microtubule organization and function at different concentrations. At the lowest effective concentrations, 5–10 μM, stypoldione selectively inhibited cytokinesis. Mitotic division occurred normally, usually with no discernible perturbation of microtubule organization or function, and cells became multinuclear. At somewhat higher concentrations, 20–40 μM, stypoldione blocked embryos before streak stage of the first division and, although microtubules were present, their organization was perturbed and they often formed unusual "spiral aster" arrays. At 80 μM and above, microtubules in blocked cells were largely absent. Thus, stypoldione uncouples cytokinesis from mitosis at the lowest effective concentrations and, although it can disrupt microtubules at relatively higher concentrations, it inhibits cell division at the lowest effective concentrations by a selective action on cytokinesis through a mechanism that does not appear to involve disassembly of microtubules.

Stypoldione is a natural product secreted by the marine brown alga *Stypopodium zonale*, which is toxic to reef-dwelling fish (1). Early studies revealed that the compound inhibited the first division of sea urchin embryos, with 50% inhibition occurring at 2.5 μM (2). Initial studies on the mechanism of action of stypoldione revealed that the compound inhibited microtubule polymerization *in vitro*, and it was suggested that stypoldione might inhibit cell division by inhibiting formation and function of the mitotic spindle (2, 3). However, further studies (4) revealed that mammalian cells incubated with high concentrations of stypoldione were not blocked in mitosis, as expected with a compound that acts selectively on microtubules, and also that stypoldione inhibited amino acid uptake and protein synthesis, cellular processes that are not dependent upon microtubule function.

Recently, we found that stypoldione reacts covalently with the sulfhydryl groups of tubulin and other proteins and also with the sulfhydryl groups of small thiol-containing compounds such as glutathione, thiophenol, and β-mercaptoethanol (5). Thus, the compound might act in cells by affecting a variety of cellular molecules in addition to tubulin. We undertook the present study to characterize the inhibition of cell division by stypoldione. Among our goals was to determine whether inhibition of cell division by stypoldione was caused by a perturbation of microtubule polymerization or organization. We also hoped to determine more precisely the stage of the cell cycle in which cells were blocked by the compound. We used indirect immunofluorescence light microscopy of fixed and stained embryos of the sea urchin *Strongylocentrotus purpuratus* to ad-

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ABBREVIATIONS: PABA, *p*-aminobenzoic acid; CFA, calcium-free artificial; PIPES, piperazine-*N*,*N*′-bis[2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*,*N*′-tetraacetic PBS, phosphate-buffered saline; DAPI, 4′,6-diamidino-2-phenylindole.

dress these question. Our results indicate that, at the lowest effective concentrations, stypoldione selectively inhibits cytokinesis while exerting no discernible effect on the organization or function of microtubules. Our data are consistent with the idea that, although stypoldione is sulfhydryl reactive and, thus, would be expected to affect many cellular targets, an especially sensitive target exists whose function is critical to cytokinesis.

Materials and Methods

Sea urchin gametes and embryos: drug incubations. Eggs and sperm were obtained from the sea urchin *S. purpuratus* by intracoelomic injection of 0.5 M KCl. Eggs were shed into ice-cold charcoal-filtered sea water, filtered through 150-μm mesh Nitex (Tetko, Inc, Elmsford, N.Y.) and washed by three cycles of settling and resuspension in fresh filtered sea water. Final resuspension was in filtered sea water containing 10 mM PABA. Sperm were collected undiluted and stored on ice.

Eggs were fertilized by adding freshly diluted sperm to a small volume of settled eggs. When fertilization envelops became visible by bright-field microscopy, approximately 20–30 sec after fertilization, embryos were diluted into CFA sea water (6) containing 10 mM PABA and were gently sedimented using a hand-operated centrifuge. Eggs were resuspended in the PABA-containing CFA sea water and resedimented twice more to remove excess sperm, resuspended in CFA sea water in the presence of PABA (0.5 ml of packed eggs to 10 ml of sea water), and filtered through 70-μm mesh Nitex into 40 ml of CFA sea water without PABA. The egg suspension was filtered twice more through 70-μm mesh Nitex, and 2-ml aliquots were pipetted into 5-ml wells of 12-well plastic cell culture dishes. Embryos were incubated at 15.5% without stirring.

Stypoldione was dissolved in 100% ethanol at 100 times higher concentration than the final concentration desired. Forty microliters of drug solution, or appropriate solvent without drug, was added to 1.96 ml of CFA sea water and mixed thoroughly. The entire 2-ml volume was added to the embryo suspension 30 min after fertilization, with gentle mixing, to produce the desired final drug concentration (or solvent control). Thus, the time of addition of stypoldione was 30 min in all samples.

At 15.5° and the incubation conditions used, pronuclear fusion was complete between 20 and 25 min after fertilization, streak stage occurred at approximately 60 min, and the first cytokinesis was complete at approximately 120 min. The stages of mitosis occurred approximately as described by Harris *et al.* (7). Interphase of the two-cell stage lasted approximately 15 min, with the second metaphase occurring between 140 and 150 min and the second cytokinesis taking place at 170 to 180 min. The spiral microtubule arrays between syngamy and streak stage of the first cell cycle described by Harris and co-workers (7–9) were not observed in untreated embryos, in agreement with the observations of Schroeder and Battaglia (10).

Stypoldione was added well after pronuclear fusion had been completed. In preliminary studies, we found that embryos incubated continuously with stypoldione beginning 30 min after fertilization which had not undergone first cleavage by 145 min, did not subsequently cleave for at least the next 90 min. This was sufficient time for untreated (control) embryos to develop to the eight-cell stage. Therefore, we report the effects of stypoldione at 145 min. The effects of each treatment were evaluated in 100–150 fixed and stained embryos, and charts were kept of the appearance of each cell as it progressed through the cell cycle. Micrographs were taken of typical embryos to illustrate the effects of various treatments.

Immunofluorescence microscopy. Preparation of embryos for immunofluorescence microscopy was carried out essentially by the method of Hollenbeck and Cande (11). At various times after drug addition, a concentrated suspension of embryos was gently pipetted onto polylysine-coated cover slips (540,000 molecular weight polylysine), and the sea water was replaced with extraction buffer (0.2–0.4% Triton X-100 in a PIPES buffer consisting of 50 mM PIPES, 6 mM $MgSO_4$, and 10 mM EGTA, pH 6.8). After extraction for 2 min, embryos were fixed for 10 min with 1%, w/v, formaldehyde and 0.1%, w/v, glutaraldehyde in PIPES buffer, rinsed three times in PBS (137 mM NaCl, 2.7 mM KCl, 8.0 mM sodium phosphate, 1.5 mM potassium phosphate, pH 7.5), and then treated with two 5-min changes of freshly dissolved sodium borohydride (4 mg/ml in PBS) to decrease aldehyde autoflorescence (12). Coverslips were next washed three times with PBS, followed by addition of 25–30 μl of anti-α-tubulin monoclonal antibody 1-2.3 in PBS containing 0.5% bovine serum albumin (13) and incubation at 37° for 60–75 min.

A solution of fluorescein- or rhodamine-labeled goat anti-mouse IgG (Cooper Biomedical, Malvern, PA) in PBS was mixed with an acetone powder of sea urchin eggs, incubated at room temperature for 15 min, and centrifuged for 10 min at 4° in an Eppendorf desktop centrifuge to remove any nonspecific binding activity present among the sea urchin egg proteins. When desired, DAPI was added to the second antibody solution, at a final concentration of 10 μg/ml, to stain chromatin. Eggs were incubated with 25–30 μl of secondary staining solution at 37° for 60–75 min. Coverslips were then mounted onto microscope slides previously wetted with 0.15 M *n*-propyl gallate in 0.1 M sodium borate, pH 8.0, and sealed with clear nail polish. Slides were stored at 4° in the dark until examined.

Embryos were viewed with a Zeiss Photomicroscope III using Plan-Neofluor 10×, 25×, and 40× objectives and were photographed using Kodak Technical Pan film at 25–400 ASA. All drugs and reagents were obtained from Sigma Chemical Company (St. Louis, MO) unless otherwise indicated.

Results

At 145 min after fertilization, untreated embryos typically had entered second mitosis. By immunofluorescence microscopy, the embryos were characterized by a prominent microtubule-free space between blastomeres, two sets of mitotic spindles, and condensed and organized chromosomes. An example of an untreated embryo in second anaphase, fixed 145 min after fertilization, is shown in Fig. 1, a and b. Partial extraction of the plasma membrane, cortical granules and soluble tubulin and sodium borohydride treatment after aldehyde fixation allowed clear visualization of the microtubules with little background fluorescence. The membranes separating the two blastomeres are not visible by immunofluorescence microscopy, but their presence could be inferred from the pattern of mitotic spindle microtubules. Untreated embryos, oriented with their spindles in the plane of focus, exhibit a "clear zone" between blastomeres that is free of microtubules. Such a clear zone is readily visible in the untreated embryo shown in Fig. 1. When viewed from this perspective, embryos at this stage also exhibit an oblong shape, due to the presence of two distinct blastomeres, rather than the spherical shape of embryos that have not undergone first cleavage.

Low concentrations of stypoldione. One of the most interesting effects of stypoldione occurred at 5 μM stypoldione, slightly below the minimum concentration required to inhibit division of all treated embryos. In a typical experiment at this concentration, inhibition of cytokinesis had occurred in 65–75% of the embryos. Approximately one third of the inhibited embryos exhibited microtubule organization and chromosome distributions that were essentially indistinguishable from untreated embryos. This population of inhibited embryos had two sets of apparently normal spindles and a full array of astral microtubules and appeared to be at the same developmental stage as untreated embryos. The difference between these cells and

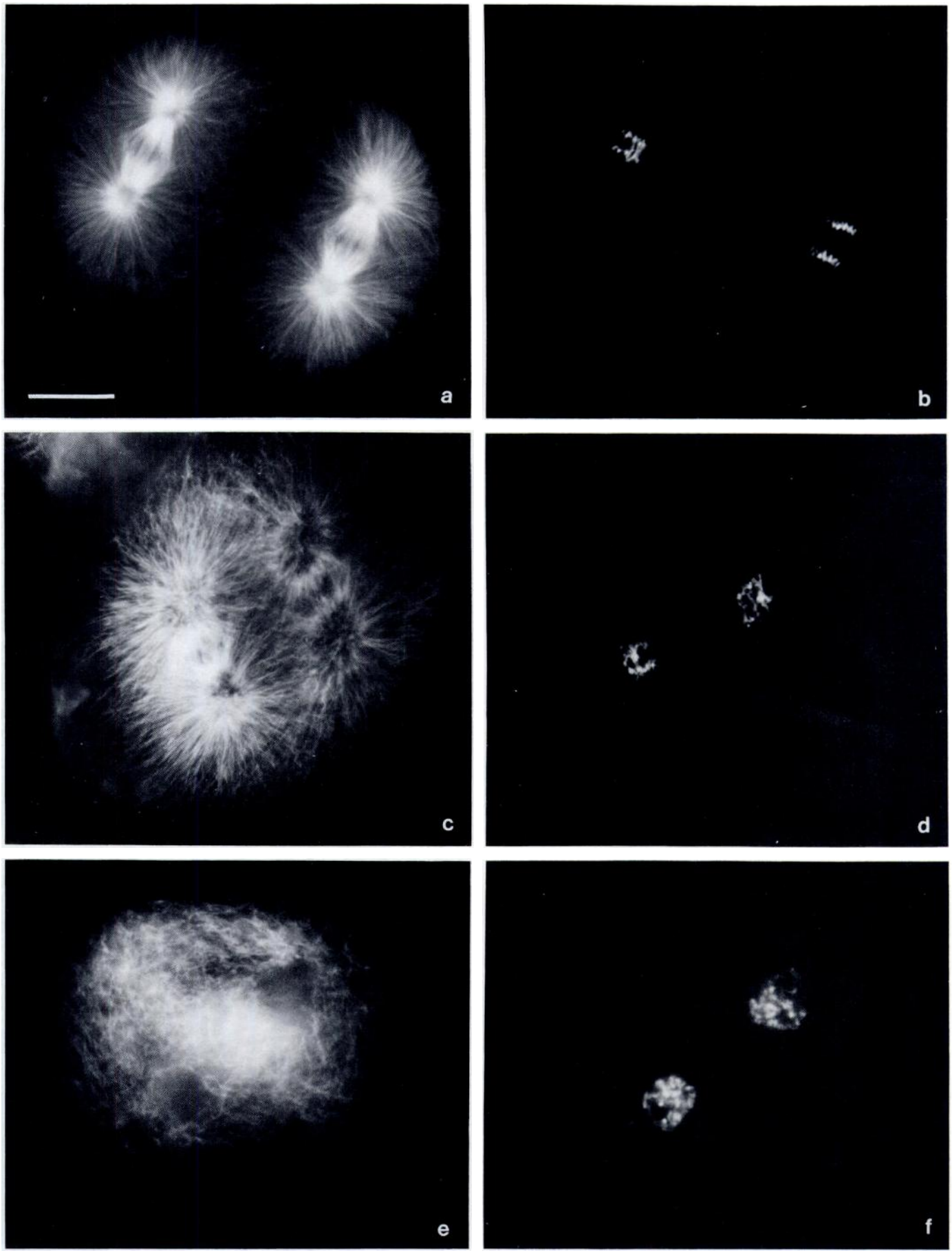

Fig. 1. Effect of low concentrations of stypolidone: evidence for a selective block of cytokinesis. Embryos were extracted and fixed 145 min after fertilization and stained with antitubulin (a, c, and e) or DAPI (b, d, and f). Stypoldione or solvent control was added 30 min after fertilization. a and b, an untreated control embryo showing two early anaphase spindles and chromosome beginning to move toward the poles. c and d, an embryo treated with 5 μM stypoldione. Cytokinesis had not taken place in this embryo, but two spindles and two sets of chromosomes are visible. e and f, an embryo treated with 10 μM stypoldione. Two nuclei as well as abundant microtubules are visible, but cytokinesis had not taken place. *Bar* = 20 μm.

control cells, however, was that cell cleavage had not occurred. Approximately one third of the uncleaved cells contained two nuclei and appeared to have completed the first mitotic division but had not undergone cytoplasmic division. These cells exhibited extensive arrays of microtubules and had not entered the second mitosis. The remaining uncleaved cells contained only one nucleus and appeared not to have undergone even one mitotic division, yet still had ample arrays of microtubules.

Fig. 1, c and d, shows an embryo that has been incubated with 5 μM stypoldione. The embryo had not undergone cytokinesis but its mitotic spindle function and developmental progression appeared to be unimpaired. Two mitotic spindles were present, and the microtubules were of normal length and abundance. The chromosomes, although appearing slightly disorganized, were condensed and appear to be moving toward their respective poles. The lack of a cleavage furrow or plasma membrane between the two blastomeres can be inferred from Fig. 1, because the spindles of each are in the plane of focus but there is no clear zone between them. Aster fibers from each spindle interdigitate extensively in the region where the clear zone between the two blastomeres would normally have been.

Increasing the concentration of stypoldione from 5 to 10 μM increased, from 70 to 97%, the fraction of treated cells that did not undergo cytokinesis and also increasingly retarded progression through the cell cycle. Whereas 65% of embryos treated with 5 μM stypoldione had undergone first nuclear division, evidenced by the presence of two separate nuclei within one undivided cell, only 40–45% of embryos treated with 10 μM stypoldione had two nuclei. Moreover, the fraction of undivided cells that had entered second mitosis at 145 min was decreased from the 20–25% found at 5 μM, to 2–6% at 10 μM. Fig. 1, e and f, shows a binucleate embryo that had not progressed to second mitosis. Note that, although microtubules are abundant and appear organized around the nuclei, they do not appear as well defined or as well organized as those in Fig. 1, a and c. Cells with these characteristics were present in approximately 45% of cells treated with 5 μM stypoldione and 35% of those treated with 10 μM. At 20 μM and above, stypoldione inhibited both cytokinesis and first mitosis in most (>85%) treated embryos, and very few multinucleate embryos were visible.

Intermediate concentrations of stypoldione. Most embryos exposed to 20–80 μM stypoldione did not undergo either cytokinesis or first nuclear division (86% at 20 μM, 96% at 40 μM). Many such embryos exhibited dramatic microtubule whorls or "spiral asters" similar to those described by Harris and co-workers (7–9) and Schroeder and Battaglia (10) (Fig. 2). The characteristic spiral pattern was most apparent when viewed down the axis of the spiral (Fig. 2a). When viewed from near the equatorial plane, whorls appeared as parallel fibers. Different orientations of whorl patterns are shown in Fig. 2c. By focusing through the cells, we determined that the microtubule fibers comprising the whorls were located primarily near the cortex of the embryos.

DAPI staining provided strong evidence that the stypoldione-treated cells were blocked before streak stage. After pronuclear fusion, untreated embryos remained relatively unchanged in appearance until the beginning of streak stage. The microtubules in untreated cells were flocculent and thin in appearance with little apparent organization, similar to those observed in *L. variagatus* embryos by Hollenbeck and Cande (11). The appearance of the microtubules during this period in a typical control cell is shown in Fig. 3a, whereas Fig. 3b shows the DAPI-stained image of the same cell. The nucleus has two distinct sections, with the sperm chromatin visible as a small bright area within the more lightly stained egg chromatin that uniformly fills the remainder of the nucleus. This same nuclear staining pattern was observed in all untreated embryos after the completion of pronuclear fusion and did not change until the beginning of streak stage. The onset of streak stage was characterized by the apparent disappearance of the interphase mesh-like array of microtubules and formation of a highly ordered double aster, the streak diaster (Fig. 3c). The diaster appeared to be organized from two regions just outside the nuclear envelope, as described by Wilson (14). The organization of the chromatin at the onset of streak stage also changed, becoming granular in appearance (Fig. 3d). The distinct, brightly stained area within the interphase nucleus had disappeared at this time, and the nucleus exhibited a uniform punctate appearance.

Embryos treated with 20–40 μM stypoldione all had only one nucleus, and the chromatin was always stained with the pattern characteristic of untreated embryos after pronuclear fusion and before streak stage (Fig. 2, b and d). Therefore, these embryos appear to have been blocked soon after the addition of stypoldione, before the onset of streak stage. These results are consistent with the findings of White and Jacobs (4), who found that stypoldione blocked cells before prophase.

High concentrations of stypoldione. Most cells exposed to stypoldione at 80 μM or above lacked visible microtubules. In those embryos that did exhibit spiral asters, the microtubule fibers appeared vague or weakly stained, as though they were thinner or less antibody had bound. The nuclei of embryos incubated with high concentrations of stypoldione were indistinguishable from embryos exhibiting spiral asters, as described above.

Discussion

Low concentrations of stypoldione. Recently, stypoldione was found to react rapidly with the free sulfhydryl groups of tubulin and several proteins and also with the sulfhydryl groups of a number of small molecules such as β-mercaptoethanol and glutathione. It seems reasonable to assume that many of the biological actions of stypoldione are caused by reaction of the compound with sulfhydryl-containing cellular molecules. Further, one might expect a sulfhydryl-reactive compound to exert pleiotropic effects at all effective concentrations. Thus, it was surprising that, in the lowest effective concentration range (5–10 μM), stypoldione selectively blocked cytokinesis. This was true even while other cellular processes known to be sensitive to sulfhydryl perturbation, such as microtubule polymerization (15–18), continued normally. Stypoldione had no apparent effect on the size or apparent function of the spindle or mitotic asters at the lowest effective concentrations, although the microtubules of embryos that had been slowed in their progression through the cell cycle (e.g., Fig. 1e) did appear somewhat disorganized.

One reason for the apparent selectivity of stypoldione at low concentrations may be related to the solubility characteristics of the compound. Stypoldione is a hydrophobic molecule with low solubility in water. In the experiments described, we dissolved stypoldione in 100% ethanol and then diluted the ethanol solution into sea water. Under these conditions, the

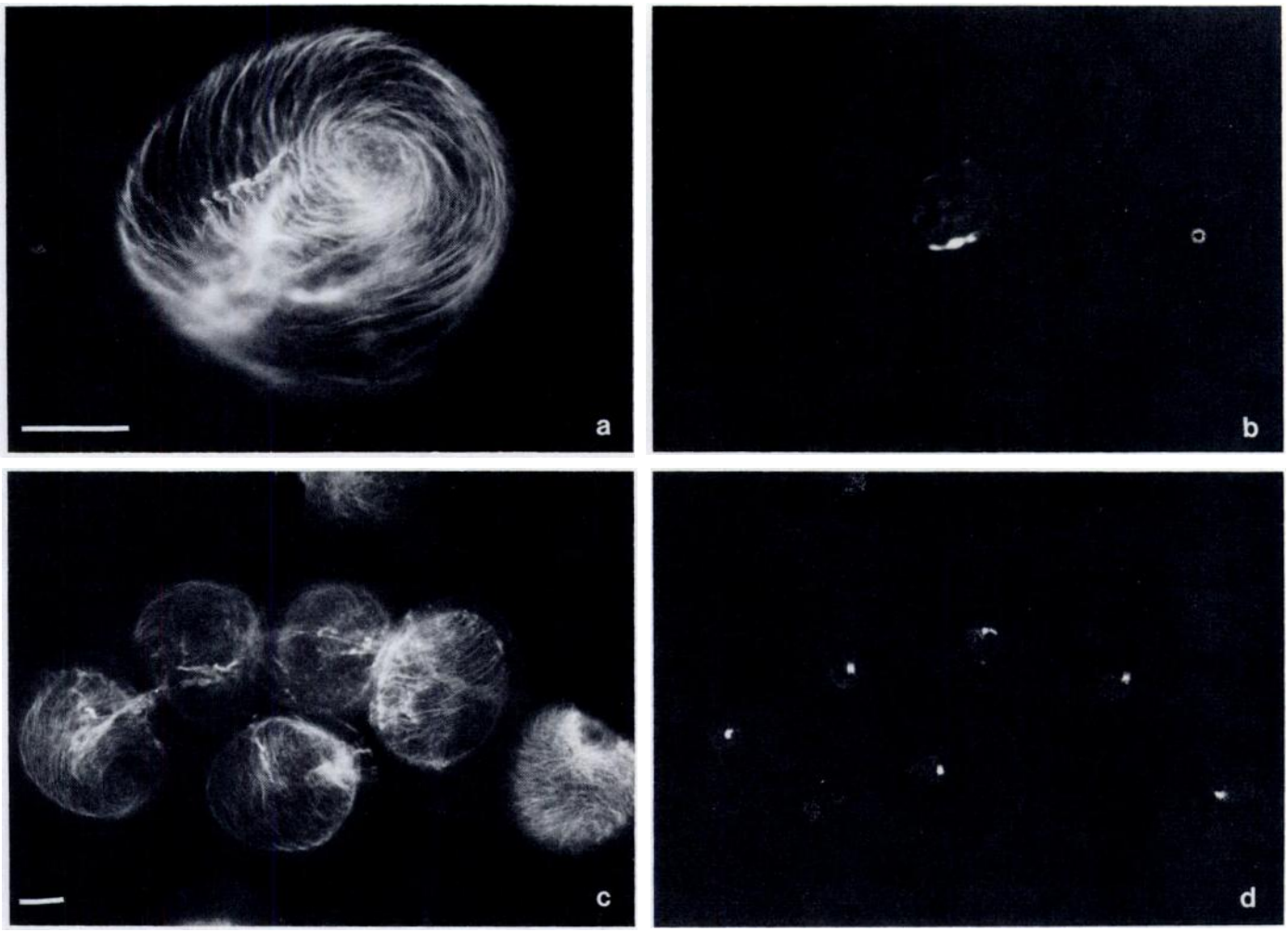

Fig. 2. Production of spiral asters by 40 μM stypoldione. Embryos treated with stypoldione were extracted and fixed 145 min after fertilization and stained with antitubulin (a and c) or DAPI (b and d). *Bars* = 20 μm.

compound precipitated within a few minutes. Thus, stypoldione might accumulate preferentially in hydrophobic cell structures such as the plasma membrane or other membrane-containing structures, with the remainder soon lost from the aqueous pools. In addition, potential cytoplasmic receptors for stypoldione would be protected from stypoldione by the high concentrations of glutathione present in the cytoplasm (1–5 mM) (19–22). Stypoldione reacts rapidly with glutathione (5). Thus, cytoplasmic receptors for stypoldione may be protected by a combination of the lipid partitioning of the compound, its short half-life in aqueous solution, and the high concentrations of glutathione in the cytoplasm.

One possible target for stypoldione is the contractile ring. Both actin and myosin are found in this structure, which forms adjacent to the plasma membrane at the cleavage furrow during cytokinesis and is essential for cytoplasmic division (23, 24). Compounds that react with sulfhydryl groups can inhibit the ATPase activity of myosin and the binding of myosin to actin (25, 26). Further, it has been shown that injection into cells of *N*-ethylmaleimide-modified heavy meromyosin or antibodies against myosin can block cytokinesis selectively (24, 27–28), producing multinucleate cells. Thus, due to its location near the plasma membrane, susceptibility to sulfhydryl reactive agents, and importance in cell cleavage, a direct effect of stypoldione on the contractile ring seems possible.

However, a more subtle and possibly more interesting effect of stypoldione might also be responsible for the apparent selectivity of the compound. A large body of evidence has accumulated to suggest that the signal determining the position and orientation of the cleavage furrow depends on the position and organization of the anaphase aster microtubules (see Ref. 29 for a recent review). The critical factor seems to be the interaction of the asters with the inner surface, or cortical region, of the plasma membrane. Thus, two general points of intereaction with the signal for cleavage furrow formation seem possible, disruption of the organization of the asters or interference with the effectiveness of the signal that the asters may deliver to the equatorial or polar region of the cell membrane. It seems that stypoldione can disrupt the organization of microtubules, as shown in Fig. 1e and also as evidenced in the production of spiral asters, and, therefore, an effect of stypoldione on the signal for furrow formation cannot be ruled out, especially at higher concentrations. However, it was clear that a significant proportion of the embryos that did not undergo cytokinesis did not exhibit overtly disorganized mitotic spindles and that the spindles appeared to segregate chromosomes normally. Thus, the functions of spindle microtubules did not appear to be disturbed.

High concentrations of stypoldione. At concentrations above approximately 20–40 μM, stypoldione exerted the wide-

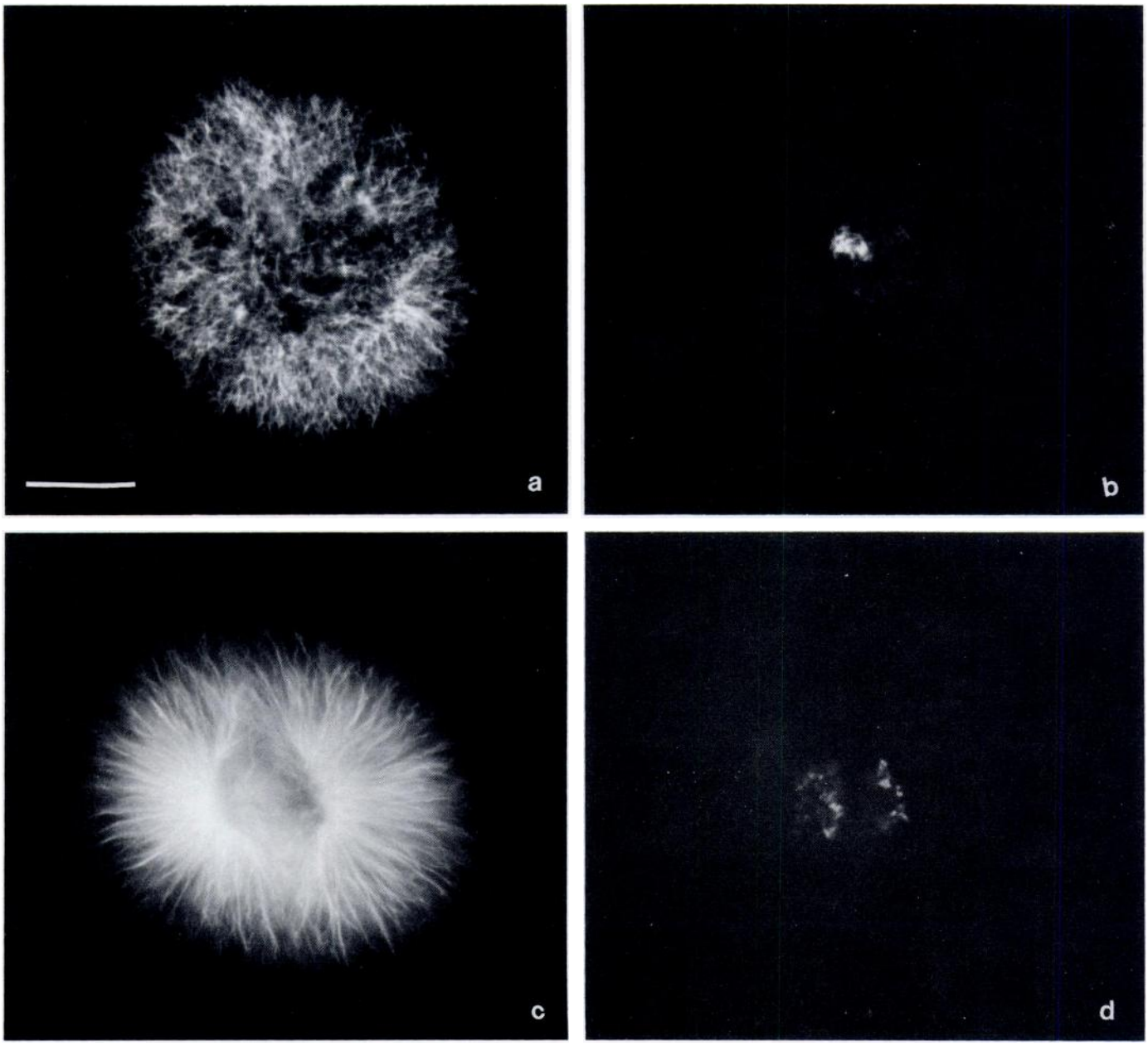

Fig. 3. An untreated embryo 40 min after fertilization (interphase) (a and b) and an untreated embryo 70 min after fertilization (streak stage) (c and d). The microtubules present in the interphase embryo show no definite organizing centers (a), and the chromatin is still segregated into a small brightly staining area, presumably constituting the sperm chromatin, and a diffusely staining area that fills the remainder of the nucleus (the egg chromatin) (b). The microtubules of the streak diaster (c), in contrast, show two distinct organizing centers, with long brightly stained microtubule fibers reaching to the cortex, while the sperm chromatin (d) has now become indistinguishable from the egg chromatin. The chromosomes seem to have condensed slightly and are distributed throughout the nucleus, giving the nucleus a punctate appearance. *Bar* = 20 μm.

spread effects expected of a compound with general sulfhydryl reactivity. Stypoldione caused increasingly dramatic effects upon the cytoskeleton and blocked the cell cycle well before the onset of mitosis. Embryos treated with 20–40 μM stypoldione almost always exhibited spiral asters (97% at 40 μM) and, by DAPI staining, always contained an easily distinguishable bright area in the nucleus, about the size of the sperm pronucleus, which we believe is the sperm chromatin. Because the sperm and egg chromatin in untreated embryos remained distinct and separate in the nucleus until the onset of streak stage, it would seem that treated embryos were blocked before streak stage.

Formation of spiral asters. Spiral asters have been reported in the embryos of a number of species (7–10, 30, 31). In sea urchin embryos, spirals were reported in a small percentage of embryos treated with phenyl urethane and shaken just after formation of the fertilization membrane (30). This phenomenon seems to have been a rare occurrence, observed in embryos that did not complete a normal monaster cycle but that eventually divided normally. These early studies treated "bent aster fibers" as an interesting anomaly that was not necessarily a required part of normal development. In contrast, Harris and colleagues (7–9) reported that spiral aster formation was part of the normal sequence of events during the first cell cycle in *S. purpuratus*, beginning shortly after pronuclear fusion and persisting until the beginning of streak stage. Recently, Schroe-

der and Battaglia (10) observed spiral asters during the same period of the cell cycle but found that they formed only when embryos were exposed to temperatures above 16–18°. They also observed a correlation between "cytoplasmic rotation" and elevated temperatures during this same period. Both groups of investigators observed that formation of spiral asters did not necessarily prevent subsequent normal development.

Similar to the observations of Schroeder and Battaglia (10), we did not observe spiral asters in control embryos (incubated at 15.5°) at any time during the first cell cycle. This supports the idea that spiral asters do not occur normally in *S. purpuratus* embryos. In treated embryos, we could not determine precisely when during the first cell cycle the spiral asters formed, although we did observe spiral asters as early as 40 min after fertilization. The observation that embryos exhibiting spiral asters had completed pronuclear fusion but had not yet integrated their sperm and egg chromatin in the nucleus (e.g., Fig. 2b), indicates that embryos with spiral asters were blocked before the onset of streak stage. This is consistent with the timing noted by Harris and colleagues (7–9), Schroeder and Battaglia (10), and Painter (30) for the formation of spirals and also agrees well with the work of White and Jacobs (4), who demonstrated that stypoldione-treated cells under similar conditions had completed S phase but were blocked before the onset of prophase.

It is intriguing that spiral asters, whether formed by exposure to stypoldione or to high temperature, were always observed during the same period of the cell cycle. The time between syngamy and prophase has been shown in other embryos to be a critical one for the alignment and organization of the egg cytoplasm relative to the point of entry of the sperm (reviewed in Ref. 32). This alignment, establishing the dorsoventral axis, involves movement of pigment and yolk granules into specific regions of the cortex and endoplasm. Events associated with establishment of the dorsoventral axis in amphibian embryos are inhibited by drugs that depolymerize microtubules (33, 34), as is cytoplasmic rotation in *S. purpuratus* embryos (10). Thus, although spiral aster formation and cytoplasmic rotation in *S. purpuratus* embryos appear to be induced artificially, these phenomena may offer an interesting way to probe the inherent capability of the embryo to organize its cytoplasm, as well as a way to investigate functional relationships between cytoplasmic and cortical cytoskeletal elements that allow such movement to occur.

The mechanism by which stypoldione causes spiral asters is unknown. Spiral asters may result from blockade of the cell cycle at the stage at which they are likely to develop (between syngamy and streak stage). Perhaps more interestingly, formation of spiral asters might be the cause of cell cycle arrest. There is evidence that microtubules can exert forces upon cytoskeletal elements of the cortex (34, 35) and, to do so, the microtubules must associate in some way with these elements. If stypoldione (and perhaps high temperature) interferes with binding of microtubules to cortical elements, the linkage of the cortex to the cell interior might be eliminated, allowing formation of spiral asters and perhaps interfering in a critical manner with the transition into mitosis.

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References

1. Gerwick, W. H., and W. Fenical. Ichthyotoxic and cytotoxic metabolites of the tropical brown alga *Stypopodium zonale* (Lamouroux) Papenfus. *J. Org. Chem.* **46**:22–27 (1981).
2. Jacobs, R. S., S. White, and L. Wilson. Selective compounds derived from marine organisms: effects on cell division in fertilized sea urchin eggs. *FEBS Proc. Meet.* **40**:26–29 (1981).
3. O'Brien, E. T., R. S. Jacobs, and L. Wilson. Inhibition of bovine brain microtubule assembly in vitro by stypoldione. *Mol. Pharmacol.* **24**:493–499 (1983).
4. White, S. J., and R. S. Jacobs. Effect of stypoldione on cell progression, DNA and protein synthesis, and cell division in cultured sea urchin embryos. *Mol. Pharmacol.* **24**:500–508 (1983).
5. O'Brien, E. T., D. J. Asai, A. Groweiss, B. H. Lipshutz, W. Fenical, R. S. Jacobs, and L. Wilson. Mechanism of action of the marine natural product stypoldione: evidence for reaction with sulfhydryl groups. *J. Med. Chem.* **29**:1851–1855 (1986).
6. Balczon, R., and G. Schatten. Microtubule-containing detergent-extracted cytoskeletons in sea urchin eggs from fertilization through cell division: antitubulin immunofluorescence microscopy. *Cell Motil.* **3**:213–226 (1983).
7. Harris, P., M. Osborn, and K. Weber. Distribution of tubulin-containing structures in the egg of the sea urchin *Strongylocentrotus purpuratus* from fertilization through first cleavage. *J. Cell. Biol.* **84**:668–679 (1980).
8. Harris, P. A spiral fiber system in fertilized sea urchin eggs. *Dev. Biol.* **68**:525–532 (1979).
9. Harris, P., M. Osborn, and K. Weber. A spiral array of microtubules in the fertilized sea urchin egg cortex examined by indirect immunofluorescence and electron microscopy. *Exp. Cell Res.* **126**:227–236 (1980).
10. Schroeder, T. E., and D. E. Battaglia. "Spiral asters" and cytoplasmic rotation in sea urchin eggs: induction in *strongylocentrotus purpuratus* eggs by elevated temperature. *J. Cell. Biol.* **100**:1056–1062 (1985).
11. Hollenbeck, P. J., and W. Z. Cande. Microtubule distribution and reorganization in the first cell cycle of fertilized eggs of *Lytechinus pictus*. *Eur. J. Cell Biol.* **37**:140–148 (1985).
12. Weber, K., P. C. Rathke, and M. Osborn. Cytoplasmic microtubular images in glutaraldehyde-fixed tissue culture cells by electron microscopy and by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. USA* **75**:1820–1824 (1978).
13. Asai, D. J., C. J. Brokaw, W. C. Thompson, and L. Wilson. Two different monoclonal antibodies to *alpha*-tubulin inhibit the bending of reactivated sea urcin spermatozoa. *Cell Motil.* **2**:599–614 (1982).
14. Wilson, E. B. *Fertilization and karokinesis in the Ovum*. Macmillan and Co., New York (1985).
15. Kuriyama, R., and H. Sakai. Role of tubulin sulfhydryl groups in polymerization of microtubules. *J. Biochem.* **76**:651–654 (1974).
16. Mellon, M. G., and L. I. Rebhun. Sulfhydryls and the *in vitro* polymerization of tubulin. *J. Cell Biol.* **70**:226–238 (1976).
17. Ikeda, Y., and M. Steiner. Sulfydryls of platelet tubulin: their role in polymerization and colchicine binding. *Biochemistry* **17**:3454–3459 (1978).
18. Luduena, R. F., and M. C. Roach. Interaction of tubulin with drugs and alkylating agents. 1. Alkylation of tubulin by iodoacetamide and *N,N'*-ethylenebis (iodoacetamide). *Biochemistry* **20**:4437–4444 (1981).
19. Rapkine, L. Chemical processes of cellular division. *Compt. Rend.* **191**:871–874 (1930).
20. Mazia, D., and K. Dan. The isolation and biochemical characterization of the mitotic apparatus of dividing cells. *Proc. Natl. Acad. Sci. USA* **38**:826–838 (1952).
21. Ii, I., and H. Sakai. Glutathione reductase in the sea urchin egg. *Biochem. Biophys. Acta* **350**:151–161 (1974).
22. Fahey, R. C., S. D. Mikolajczyk, G. P. Meier, D. Epel, and E. J. Carroll, Jr. The glutathione thiol-disulfide status in the sea urchin egg during fertilization and the first cell division cycle. *Biochem. Biophys. Acta* **437**:445–453 (1976).
23. Schroeder, T. E. The contractile ring. I. Fine structure of dividing mammalian (HeLa) cells and the effects of cytochalasin B. Z. Zellforsch. **109**:431–449 (1970).
24. Mabuchi, I., and M. Okuno. The effect of myosin antibody on the division of starfish blastomeres. *J. Cell Biol.* **74**:251–263 (1970).
25. Sekine, T., and W. W. Keilley. The enzymatic properties of *N*-ethylmaleimide modified myosin. *Biochem. Biophys. Acta* **81**:336–345 (1964).
26. Silverman, R., E. Eisenberg, and W. W. Kielley. Interaction of SH_1-blocked HMM with actin and ATP. *Nature New Biol.* **240**:207–208 (1972).
27. Meeusen, R. L., J. Bennett, and W. Z. Cande. Effect of microinjected *N*-ethylmaleimide modified heavy meromyosin on cell division in amphibian eggs. *J. Cell Biol.* **86**:858–865 (1980).
28. Kiehart, D. P., I. Mabuchi, and S. Inoue. Evidence that myosin does not contribute to force production in chromosome movement. *J. Cell Biol.* **94**:165–178 (1982).
29. Rappaport, R. Establishment of the mechanisms of cytokinesis in animal cells. *Int. Rev. Cytol.* **105**:245–281 (1986).
30. Painter, T. S. Contributions to the study of cell mechanics I. Spiral asters. *J. Exp. Zool.* **20**:509–527 (1916).
31. Wilson, E. B. *The cell in Development and Heredity*, 3rd. Ed. MacMillan and Co., New York (1928).
32. Gerhart, J. C. Mechanisms regulating pattern formation in the amphibian

egg and early embryo, *in Biological Regulation and Development* 2 (R. Goldberger, ed.). *Plenum Press*, New York, 113–316 (1980).
33. Manes, M. E., R. P. Elinson, and F. D. Barberi. Formation of the amphibian grey crescent: effects of colchicine and cytochalasin B. *Wilhelm Roux's Arch. Dev. Biol.* **185**:99–104 (1978).
34. Ubbels, G. A., K. Hara, C. H. Koster, and M. W. Kirschner. Evidence for a functional role of the cytoskeleton in determination of the dorsoventral axis in *Xenopus laevis* eggs. *J. Embryol. Exp. Morph.* **77**:15–37 (1983).
35. Schroeder, T. W. Physical Interactions between asters and the cortex of echinoderm eggs. Belle W. Baruch Symp. **15**:69–89 (1985).