Evidence for the Involvement of Microtubules, ER, and Kinesin in the Cortical Rotation of Fertilized Frog Eggs

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Abstract. During the first cell cycle, the vegetal cortex of the fertilized frog egg is translocated over the cytoplasm. This process of cortical rotation creates regional cytoplasmic differences important in later development, and appears to involve an array of aligned microtubules that forms transiently beneath the vegetal cortex. We have investigated how these microtubules might be involved in generating movement by analyzing isolated cortices and sections of *Xenopus laevis* and *Rana pipiens* eggs. First, the polarity of the cortical microtubules was determined using the "hook" assay. Almost all microtubules had their plus ends pointing in the direction of cortical rotation. Secondly, the association of microtubules with other cytoplasmic elements was examined. Immunofluorescence revealed that cytokeratin filaments coalign with the microtubules. The timing of their appearance and their position on the cytoplasmic side of the microtubules suggested that they are not involved directly in generating movement. ER was visualized with the dye $DiIC_{16}(3)$ and by immunofluorescence with anti-BiP (Bole, D. G., L. M. Hendershot, and J. F. Kearney. 1986. *J. Cell Biol.* 102:1558–1566). One layer of ER was found closely underlying the plasma membrane at all times. An additional, deeper layer formed in association with the microtubules of the array. Antibodies to sea urchin kinesin (Ingold, A. L., S. A. Cohn, and J. M. Scholey. 1988. *J. Cell Biol.* 107:2657–2667) detected antigens associated with both the ER and microtubules. On immunoblots they recognized microtubule associated polypeptide(s) of ~115 kD from *Xenopus* eggs.

These observations are consistent with a role for kinesin in creating movement between the microtubules and ER, which leads in turn to the cortical rotation.

MICROTUBULE-DEPENDENT intracellular movements are involved in may important cellular processes, and have attracted much attention (see Gibbons, 1989 for an overview). Recent advances in this area have suggested that common elements may mediate movement between microtubules and other cellular components in such diverse processes as mitosis, fast axonal transport, and extension of tubular membrane networks (see Vale and Goldstein, 1990). We have been examining a microtubule dependent cytoplasmic movement that occurs in the fertilized frog egg. The movement comprises a translocation of the thin, outer egg cortex relative to the inner cytoplasm (Ancel and Vintemberger, 1948; Vincent et al., 1986), and has been known for many years because of its importance in specifying the embryonic axes (see Elinson, 1989 for review). During a period of 20–40 min (depending on species and temperature) in the second half of the first cell cycle, the entire cortex rotates relative to the cytoplasm by ~30° about a horizontal axis. This rotation is manifest in the eggs of some species by the appearance of a "grey crescent" on one side of the egg, where clear vegetal cortex is brought to overlie dark animal cytoplasm.

At the time of the cortical rotation an impressive array of aligned microtubules is found beneath the entire vegetal surface of the egg. The microtubules are oriented with the direction of movement and lie 1–3 μm beneath the plasma membrane (Elinson and Rowning, 1988; Ziscкind and Elinson, 1990). Given the sensitivity of the cortical rotation to various microtubule-depolymerizing agents (Manes et al., 1978; Scharf and Gerhart, 1983; Vincent et al., 1987) and evidence that the machinery for translocation is close to the vegetal surface of the egg (Manes and Elinson, 1980; Vincent et al., 1987; Elinson and Rowning, 1988), these microtubules are good candidates for a role in the generation of directed movement.

We have examined the organization of the vegetal cortex to understand how the microtubules of the array could be involved in moving the cortex. First we determined the polarity of the microtubules in the cortical array using the "hook" assay. Then we examined their relationship to other elements, in particular cytokeratin filaments and ER. A cytokeratin network is known to develop in the cortical region at about this time (Klymkowsky et al., 1987), and ER, also present in the egg cortex (Campanella and Andreuccetti, 1977) is known to interact with microtubules in other cells (Terasaki et al., 1986). In addition we used antibodies directed against the microtubule associated motor protein kinesin to look for related molecules that might be involved

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in force generation. Eggs from both *Xenopus laevis* and *Rana pipiens* were used. *Xenopus* eggs are smaller and more suitable for sectioning, whereas *Rana* eggs are more convenient to use for the isolation of pieces of cortex because the period of cortical rotation is longer, and because the grey crescent is clearly visible and provides an excellent natural indicator of the direction of rotation.

Materials and Methods

Collection of Eggs

Procedures for maintaining *Xenopus laevis*, induction of ovulation, insemination, and dejellying were exactly as described in Ziskind and Elinson (1990), and those for *Rana pipiens* as described in Elinson (1983). To compare experiments, all results are expressed on a normalized time scale (Scharf and Gerhart, 1983). Insemination is time 0 and first cleavage 1.0.

Determination of Microtubule Polarity

Microtubule polarity was determined by the "hook method" (Heidemann and McIntosh, 1980). Twice-cycled porcine tubulin was put through one further round of assembly/disassembly in 4/3 PB[1] (PB:0.5 M Pipes, 1 mM $MgCl_2$, 1 mM EDTA), centrifuged at 250,000 *g* for 3 h at 4°C and stored in liquid nitrogen. Strips of vegetal cortex were peeled from fertilized *Rana* eggs during the second half of the cell cycle in PHEM (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, 1 mM $MgCl_2$, pH 6.9; derived from Schliwa et al., 1981) using fine forceps. A tag of animal cortex marked either the sperm entry or grey crescent side. The cortices were equilibrated for 10–20 min in PBGD (PB with 20% glycerol, 2.5% DMSO), then incubated for 45 min at 37°C in 0.75 mg/ml tubulin in PBGD containing 0.1% Brij 58 and 1 mM GTP, rinsed in warm PBGD, and fixed in 2% glutaraldehyde in PB for 30–45 min (starting at 37°C). To facilitate further processing and maintain the cortices in known orientation they were attached to small strips of cellophane (membrane backing for slab dryer; Bio-Rad Laboratories, Oknard, GA) coated with Poly-L Lysine (0.1% solution; Sigma Chemical Co., St. Louis, MO).

Electron Microscopy

Untreated cortical pieces (peeled in P10EM: 100 mM Pipes, 10 mM EGTA, 1 mM $MgSO_4$, pH 6.9) were fixed for 30 min at room temperature with 2% glutaraldehyde in P10EM. EM processing was according to the protocol of McDonald (1984), using P10EM as the buffer for untreated cortices or PME (100 mM K-Pipes, 1 mM $MgCl_2$, 1 mM EDTA) for the hook assay.

Immunofluorescent Staining

Whole mount anti-tubulin immunofluorescence of methanol-fixed eggs was performed exactly as in Elinson and Rowning (1988). Cortical pieces, peeled in PHE (PHEM without $MgCl_2$), were fixed for 90 min or longer at −20°C in methanol containing 1% formaldehyde (from 37% formaldehyde solution), rehydrated to PBS and extracted with 0.25% Triton X-100 in PBS. Sectioning of methanol/formaldehyde-fixed eggs in polyester wax and staining of fixed cortices and sections were as in Houliston and Elinson (1991). For double staining, the two pairs of incubations were performed sequentially, usually the mouse monoclonal first and the rat second, using DTAF-goat anti-rat pre-absorbed against mouse immunoglobulins (Jackson Immunoresearch, West Grove, PA). In control experiments, either primary was omitted from the sequence. Cross-reaction and spillage through the filters was negligible except in the actin/tubulin combination (see Fig. 2, *j* and *k*).

Staining of unfixed cortices with anti-kinesin antibodies was performed at room temperature using PHE containing 10 μg/ml leupeptin for all incubation and washing steps. (Blocking step: 3 or 10 mg/ml BSA, 15 min. Primary antibody incubation: 30 min. DTAF-goat anti-mouse: 20 min. Washes: 10 min each.) The cortices were then fixed in methanol/formaldehyde and either rehydrated and mounted directly, or processed and stained with other antibodies (rat monoclonals followed by rhodamine anti-rat) as above. Using this protocol there was no cross-reaction between the antibodies or spillage through filters, as judged by omitting one or other primary antibody.

Specimens were mounted in 50% vol/vol glycerol/PBS pH 8.3 containing 1.5% *n*-propyl gallate and viewed on a Leitz Orthoplan microscope using A and N2 filters. In some experiments unfixed cortical pieces were extracted for 5 min with 0.5% Triton X-100 in PHE, then washed thoroughly in PHE.

The antibodies used were anti-β-tubulin and anti-actin mouse mAbs from Amersham Chemical Co. (Arlington Heights, IL) (N.357 and N.350, both diluted 1/500), rat anti-α tubulin monoclonal YL1/2 (Kilmartin et al., 1982, gift of J. Kilmartin, Medical Research Council Laboratories, Cambridge, UK; diluted 1/1,000), mouse monoclonal anti-cytokeratin 1h5 (Klymkowsky et al., 1987, gift of M. Klymkowsky, University of Colorado, Boulder, CO; undiluted culture supernatant), rat monoclonal anti-BiP (Bole et al., 1986, gift of D. Bole, Howard Hughes Medical Institute, Ann Arbor, MI; culture supernatant diluted 1/5), rabbit polyclonal antibodies raised against *Drosophila* kinesin heavy chain (Saxton et al., 1988, gift of W. M. Saxton, Indiana University, Bloomington, IN; diluted 1/300), chicken kinesin heavy chain (gift of M. P. Sheetz, Washington University, St. Louis, MO; diluted 1/500), and bovine kinesin (gift of R. Vale, University of California, San Francisco, CA; diluted 1/300) and mouse mAbs SUK2, SUK4, and SUK5 against sea urchin kinesin heavy chains (Ingold et al., 1988, gift of J. Scholey, University of California, Davis, CA; used at 10 or 20 μg/ml). The same concentration of a mouse monoclonal antibody (IgG) raised against chick muscle Ca^{2+}-ATPase (CaS/Cl), Kaprielian and Fambrough, 1987, gift of Z. Kaprielian, Johns Hopkins University, Baltimore, MD) was used as a control.

Visualization of ER

The membrane staining dye $DiIC_{16}$ (3) has been used previously to visualize ER on sea urchin and ascidian egg cortices (Henson et al., 1989; Gualtieri and Sardet, 1989). Unfixed cortical pieces were transferred to a solution of $DiIC_{16}$(3) in PHE prepared freshly by dilution (1/200–1/500) of a 2.5 mg/ml stock in ethanol, then rinsed after 1–2 min and mounted in PHE.

Preparation of AMPPNP Microtubules from Xenopus eggs

Taxol-polymerized microtubules were prepared from *Xenopus* egg cytoplasm and incubated with the nonhydrolyzable ATP analogue adenylyl imidophosphate (AMPPNP) to associate putative kinesin molecules with the microtubules, using a modification of the method of Scholey et al. (1989*b*) for purification of kinesin from sea urchin eggs. Dejellied unfertilized eggs were washed in cold PMEG (0.1 M Pipes, 2.5 mM $MgSO_4$, 5 mM EGTA, 0.1 mM EDTA, 0.9 M glycerol, pH 6.9, with 0.1 mM PMSF, 1 μg/ml pepstatin, 1 mg/ml *p*-tame, 1 μg/ml leupeptin, and 0.5 mM DTT), left for 10 min on ice to depolymerize endogenous microtubules (Elinson, 1985) and homogenized in three times settled egg volume of PMEG. After a 30-s centrifugation at 1,000 *g* to remove most yolk, clarified cytoplasm from two further spins of 30 min at 55,000 *g* and 60 min at 150,000 *g* (both 4°C) was warmed to room temperature and incubated 20 min with 20 μM taxol 1 mM GTP to polymerize the tubulin. AMPPNP was then added to 2.5 mM and incubated for a further 10 min. The microtubules were spun through a 15% sucrose cushion (30 min, 55,000 *g*, 21°C), washed with PMEG resuspended, and boiled in SDS sample buffer. Similar samples of AMPPNP microtubules prepared from sea urchin egg cytoplasm were provided by J. Scholey.

Electrophoresis and Immunoblotting

Electrophoresis was performed using 7.5% SDS/polyacrylamide gels (Laemmli 1970) and the separated proteins transferred electrophoretically onto nitrocellulose (Towbin et al., 1979). Nonspecific sites were blocked with 3% milk powder in TBS (10 mM Tris-HCl pH 7.5, 150 mM NaCl), the blot then incubated overnight at 4°C with antibodies diluted in TBS/milk, washed with 0.05% Tween 20 in TBS, incubated 1 h with alkaline phosphatase-conjugated secondary antibodies (Jackson Immunochemicals) diluted in TBS/milk, washed in TBS/Tween, and bands visualized with 333 μg/ml nitroblue tetrazolium and 167 μg/ml 5-bromo-4-chloro-3-indolyl phosphate in 100 mM Tris/HCl, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5.

1. *Abbreviations used in this paper*: AMPPNP, adenylyl imidophosphate; PB, 0.5 M Pipes, 1 mM $MgCl_2$, 1 mM EDTA; PBGD, PB with 20% glycerol, 2.5% DMSO; PHEM, 60 mM Pipes, 25 mM Hepes, 10 mM EGTA, 1 mM $MgCl_2$, Ph 6.9; PIOEM, 100 mM Pipes, 10 mM EGTA, 1 mM $MgSO_4$, pH 6.9.

Results

Analysis of Microtubules on Isolated Vegetal Cortices

The cortex of the fertilized frog egg can be peeled off manually, an operation that becomes relatively easy during the second half of the first cell cycle when the cortical rotation is occurring. At this time the cytoplasm is firm (Elinson, 1983) and the vegetal microtubule array is present (Elinson and Rowning, 1988). We have exploited this situation to examine the organization of the frog egg vegetal cortex, since the use of cortical peels for microscopy avoids the problems of interference from the large volume of internal cytoplasm and yolk platelets. In most cases, all or most of the vegetal cortex and a small amount of animal cortex was removed (Fig. 1 *a*). *Rana* eggs were used for most experiments, since the cortices are easier to prepare and are more robust, but all staining patterns were confirmed using *Xenopus* eggs.

When cortical pieces were isolated during the second half of the first cell cycle, aligned microtubules were detected consistently by anti-tubulin immunofluorescence (Fig. 1 *b*). The alignment of the microtubules coincided with the direction of cortical rotation, as indicated by the position of the grey crescent in *Rana* eggs, irrespective of the direction in which the peel was performed. To check that all the microtubules of the array, not just a subset, were isolated with the cortex, we stained whole eggs from which pieces of cortex had been partially removed. In most cases, aligned microtubules were found exclusively in the cortex (Fig. 1 *c*). Occasionally, a few aligned microtubules remained on the underlying cytoplasm (Fig. 1 *d*). This is not unexpected since at least some of the microtubules in the array arise from the outward polymerization of microtubules from the cytoplasm and remain continuous with internal microtubules (Houliston and Elinson, 1991).

Transmission EM was used to examine the relationship of the cortical microtubules to other structures (Fig. 2, *a* and *b*). Isolated *Rana* cortices were usually 3–4 μm thick. As shown previously, they retain the plasma membrane, closely underlaid by a meshwork of filaments (including microfilaments: Gall et al., 1983) and some membranous elements. The microtubules were found in a distinct layer deeper in. Associated with them were many membranous structures (Fig. 2 *b*). Other organelles, presumably pigment granules and small yolk platelets, were also present throughout the specimens.

Polarity of the Cortical Microtubules

The "hook method" (Heidemann and McIntosh, 1980) was used to determine the polarity of the aligned microtubules. Exogenous tubulin added in certain conditions polymerizes as sheets from the edge of microtubule protofilaments, appearing in EM cross-section as hooks whose direction reflects the microtubule polarity. Isolated *Rana* cortices proved very convenient for this assay since the direction of cortical rotation was easily identified during the peeling operation by the position of the grey crescent. Furthermore, only mild detergent treatment was required to clear the surrounding membranous organelles in order to allow the exogenous tubulin access to the microtubules.

The vast majority of microtubules with clear hooks were determined to be oriented with their plus ends pointed towards the grey crescent. The hooks were anti-clockwise on cortices prepared on the grey crescent side of the egg and viewed looking toward the grey crescent (Fig. 2, *c* and *d*) and clockwise on cortices peeled and viewed towards the opposite (sperm entry) side of the egg (see Heidemann and McIntosh, 1980 for explanation of hook orientations). In total, 89% of the cortical microtubules examined had this orientation (Table I).

Relationship of Cytokeratin Filaments to Microtubules

In *Xenopus* eggs, an elaborate network of cytokeratin filaments develops after fertilization, beginning about half way through the first cell cycle (Klymkowsky et al., 1987). The network has a distinct "fishnet" appearance in the vegetal half of the egg and is localized strongly to the cortical region (Klymkowsky et al., 1987; Godsave et al., 1984; Gall et al., 1983). It has been proposed that this network may be involved in the cortical rotation since the filaments become

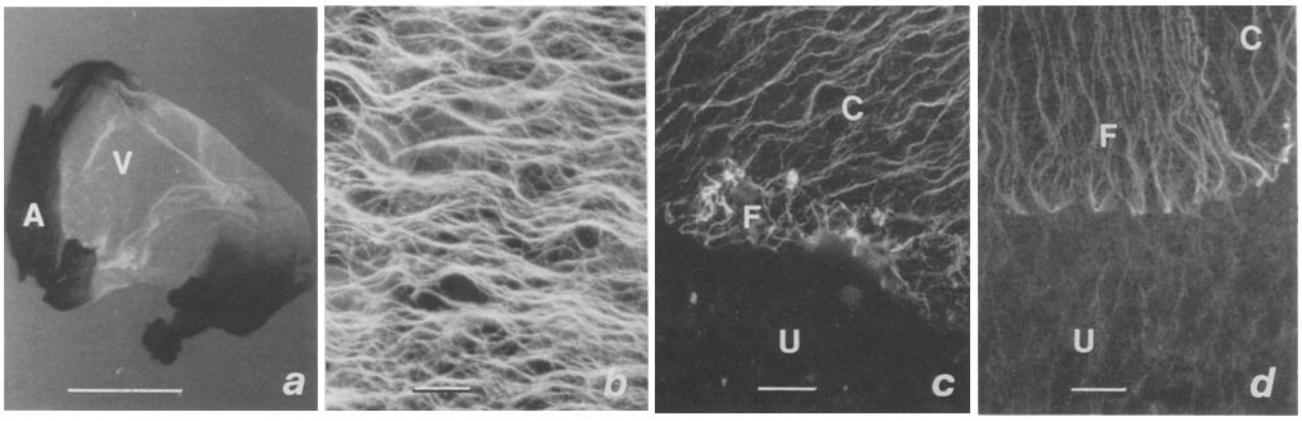

Figure 1. (*a*) Light microscope view of an isolated cortical piece from a *Rana pipiens* egg prepared during the second half of the first cell cycle. The animal cortex (*A*) is darkly pigmented whereas the vegetal cortex (*V*) is mostly white. (*b*) Anti-tubulin immunofluorescence of a similar piece of *Xenopus laevis* cortex. (*c* and *d*) Anti-tubulin immunofluorescence of whole *Rana* eggs from which pieces of cortex were partially removed. (*c*) In most specimens aligned microtubules were detected on the undisturbed cortex (*C*) and flap of peeled cortex (*F*) but not the underlying cytoplasm (*U*). (*d*) In a few cases sparse aligned microtubules were also found attached to the cytoplasm. Bars: (*a*) 1 mm; (*b–d*) 25 μm.

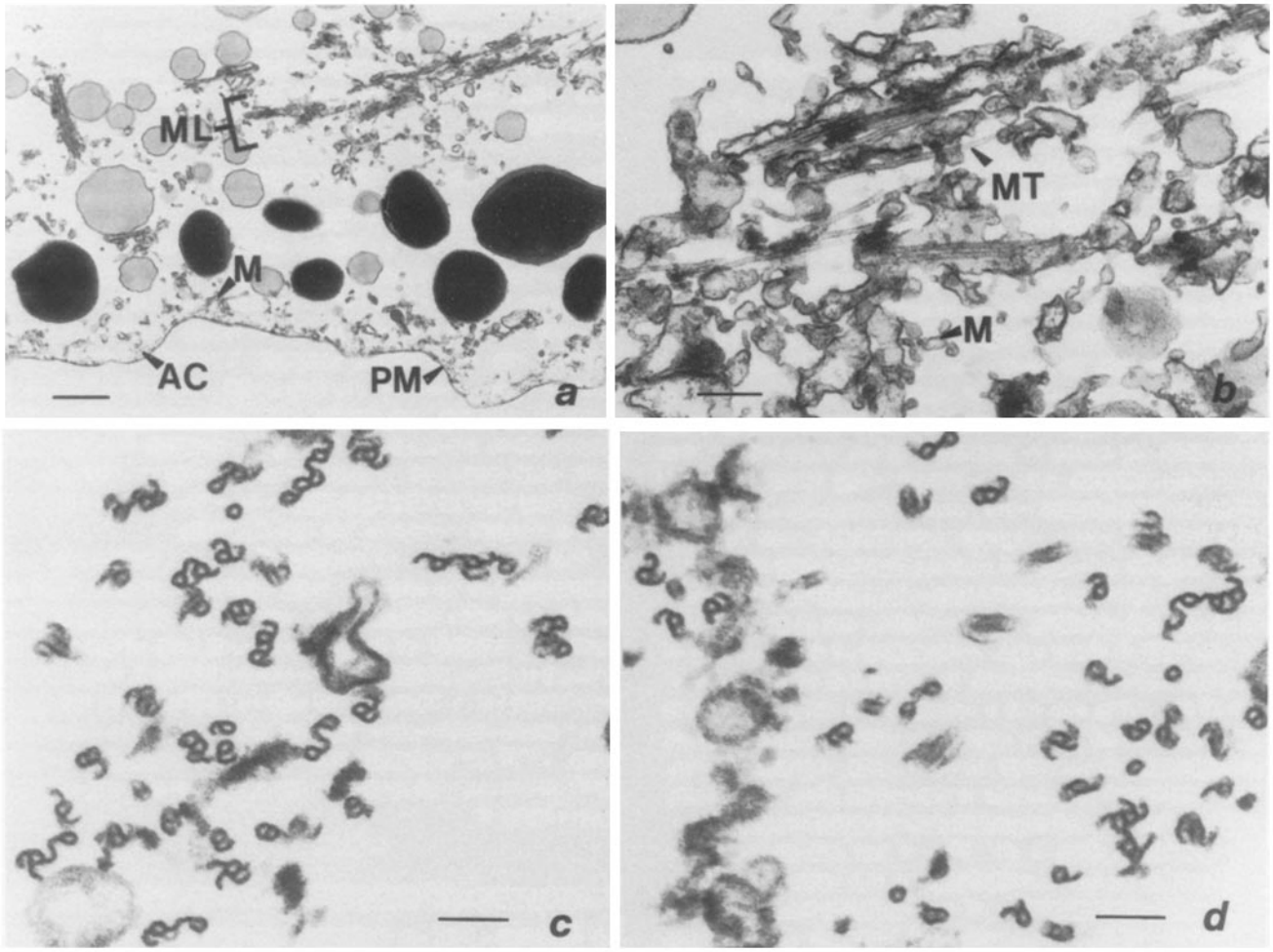

Figure 2. (*a* and *b*) Transmission EM of *Rana* cortices. (*a*) An actin-rich cortex (*AC*) lies close to the plasma membrane (*PM*), with a distinct microtubule-rich layer (*ML*) deeper in. Membranous organelles (*M*) are found in both layers. (*b*) The microtubules (*MT*) and membranes in the microtubule layer shown at higher magnification. (*c* and *d*) "Hooks" grown on cortical microtubules. Two regions from the same specimen. The images shown represent the view towards the grey crescent. Anti-clockwise hooks represent microtubules with their plus ends in this direction. Bars: (*a*) 1 μm; (*b–d*) 0.1 μm.

oriented in a common direction across the vegetal surface of the egg (Klymkowsky et al., 1987). We followed the timing, mode of appearance and location of cytokeratin filaments in the vegetal cortex, concentrating on their relationship with the microtubule array. For these experiments *Xenopus* eggs were used throughout since the reactivity of the cytokeratin antibody 1h5 (Klymkowsky et al., 1987) with *Rana* protein was weak. First we performed immunofluorescence on isolated cortical pieces. Like the microtubules, cytokeratin filaments were detected on cortices isolated at the time of the rotation. The pattern of cytokeratin staining was more variable than that of the microtubules, ranging from a complete network to only occasional strands or aggregates of protein. Double staining showed that, when present, the cytokeratin filaments did indeed coalign with the microtubules of the array (Fig. 3, *a* and *b*), suggesting an association, direct or indirect, between the two.

Immunofluorescence performed on polyester wax sections revealed asynchrony in the development of the filament network both within and between eggs, such that single eggs could show both punctate and continuous staining at times between times 0.35 and 0.65 (Fig. 3, *c–e*). Overall, the 1h5 network elaborated gradually, with more "cables" of fibers developing during the second half of the cell cycle. This behavior contrasts with that of the microtubules, which appear rapidly and synchronously in the vegetal cortex between times 0.45 and 0.55, and thin out late in the cell cycle (Elinson and Rowning, 1988; Houliston and Elinson, 1991). The

Table I. Polarity of Cortical Microtubules as Determined by the Hook Assay

Sample*	Number of microtubules with clear hooks	Number with plus end toward grey crescent
1	27	25 (93%)
2	101	97 (96%)
3	59	51 (86%)
Others	68	53 (78%)
Total	255	226 (89%)

* Samples 1, 2, and 3 were strips of cortex from eggs of three different frogs. The data from 8 other samples from these frogs, each having <20 microtubules with clear hooks, are combined.

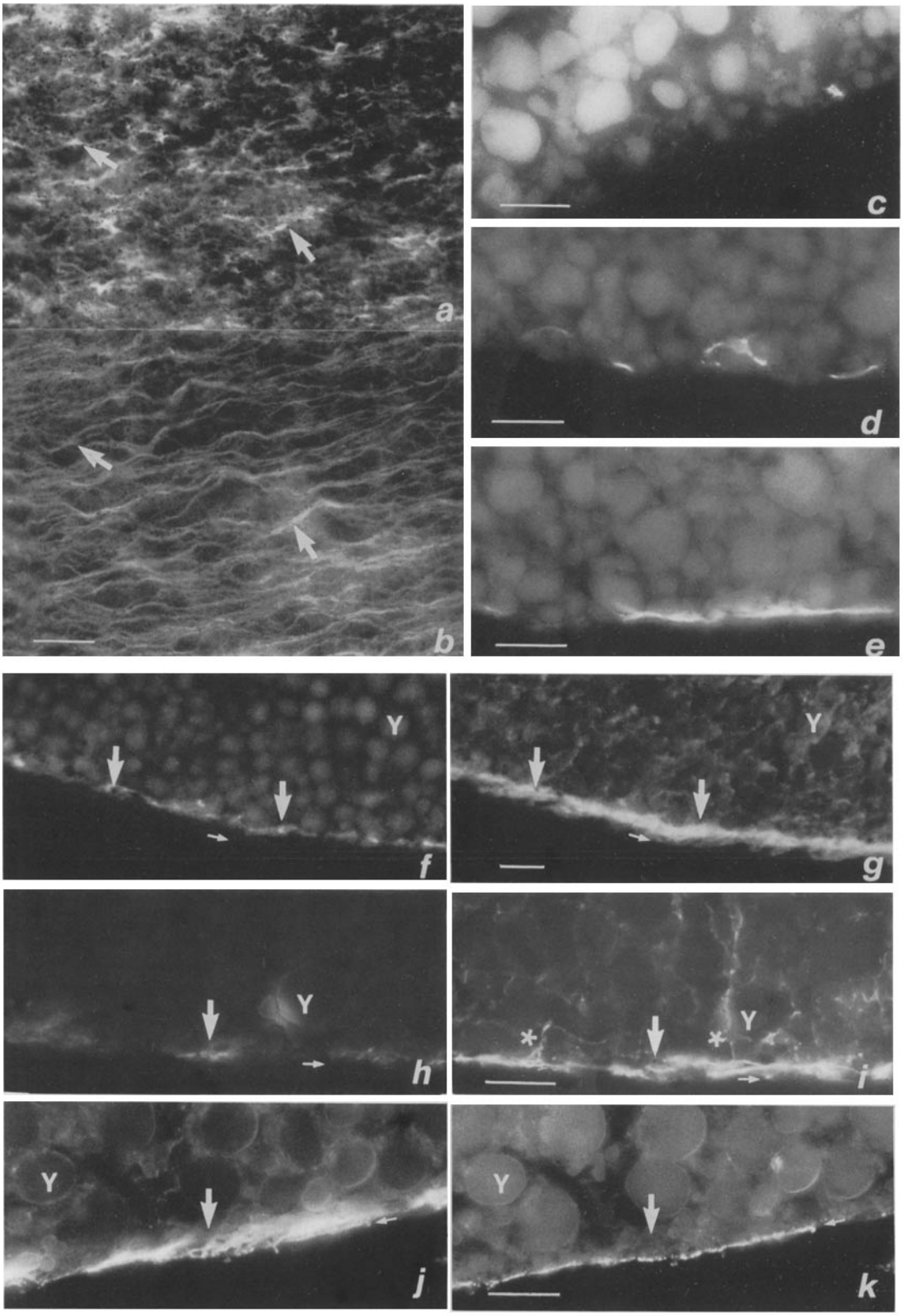

Figure 3. Distribution of cytokeratin filaments in the *Xenopus* egg cortex revealed by immunofluorescence with antibody 1h5. (*a*) Cortical isolate prepared during the second half of the cell cycle. (*b*) Same region stained for tubulin with antibody YL 1/2. Cytokeratin filaments and microtubules coalign. (*c*) Vegetal region of a section of an egg fixed at time 0.15. Only sparse cortical aggregates of cytokeratin protein are found. (*d* and *e*) Different vegetal regions of a section of an egg fixed at time 0.65. Cortical cytokeratin filaments are spreading, but the cover is not even at this time. (*f* and *g*) Double staining for cytokeratin (*f*) and tubulin (*g*) on a section of an egg fixed at time 0.7 reveals that the layer of cytokeratin filaments lies on the cytoplasmic side of the microtubule array (running parallel to the egg surface). (*h* and *i*) A vegetal region of a 0.65 egg section double stained for cytokeratin (*h*) and tubulin (*i*). Note that the microtubules in the array running parallel to the egg surface connect with ones in the cytoplasm (*stars*). (*j* and *k*) Double staining for tubulin (*j*) and actin (*k*) on a similar specimen shows that cortical actin lies external to the microtubules (some actin staining can be seen also in *j*). Pigment granules (*large arrows*), the plasma membrane (*small arrows*) and background fluorescence from yolk platelets (*Y*) can be used to align double stained pairs. Bars, 10 μm.

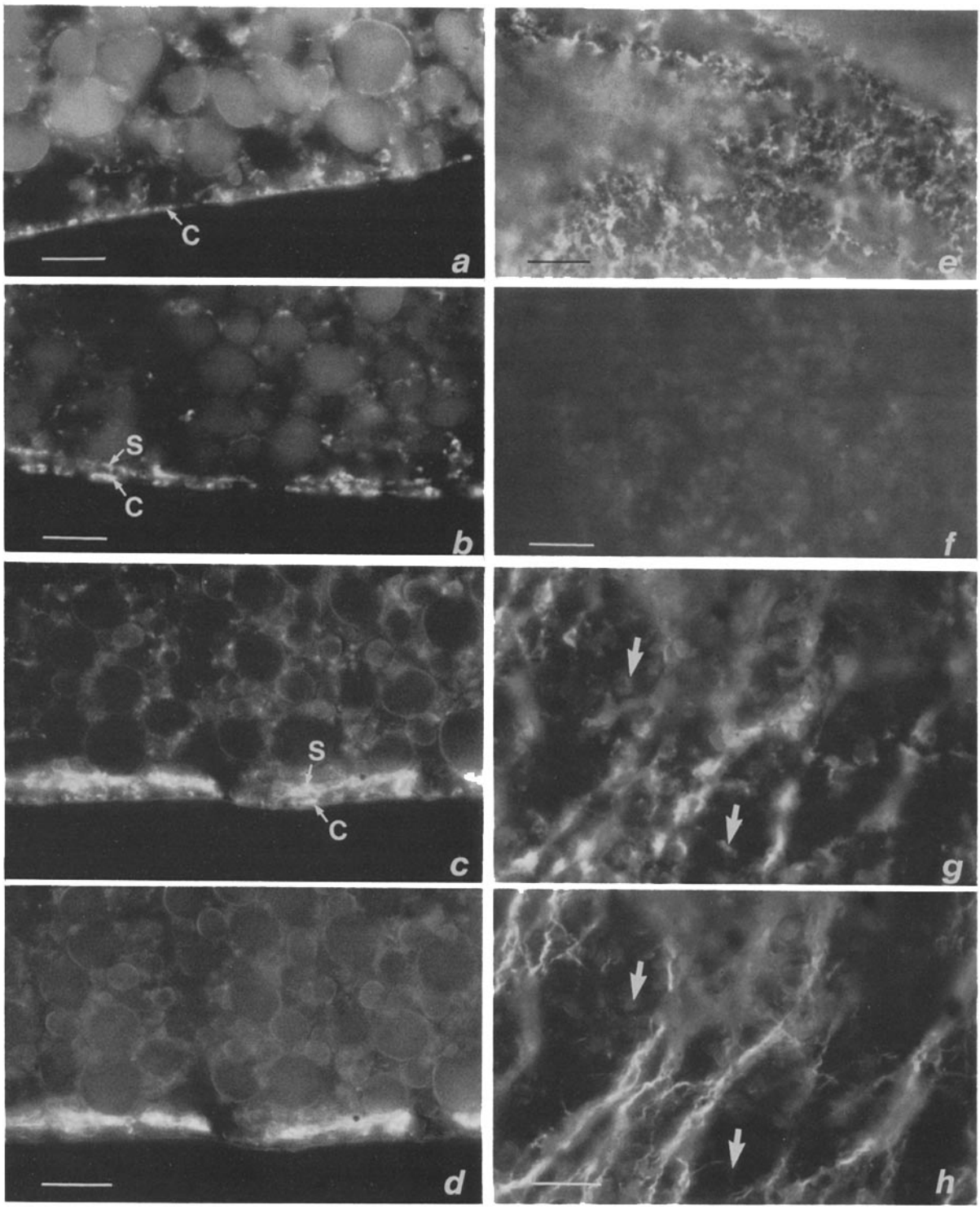

Figure 4. Distribution of ER as revealed by immunofluorescence with anti-BiP. (*a*) Vegetal region of a *Xenopus* egg section fixed at 0.3 reveals ER between the yolk platelets in the cytoplasm and in a distinct cortical layer (*C*). (*b*) At 0.7 ER in the subcortical region has become organized into a second layer (*S*). (*c* and *d*) Double staining with anti-BiP (*c*) and anti-β-tubulin (*d*) shows that microtubules codistribute with the subcortical ER but not with the outer cortical ER. (*e*) Anti-BiP staining on a *Xenopus* egg cortical isolate reveals a network-like arrangement of ER close to the plasma membrane and additional linear elements attached to the cytoplasmic side of the preparation. (*f*) The staining is abolished if cortices (this one from *Rana*) are pretreated with detergent. (*g* and *h*) Double staining with anti-BiP (*g*) and anti-β tubulin (*h*) shows ER both running with the microtubules and elsewhere (*arrows*). Note that this plane of focus shows the microtubule layer. More ER is found closer to the plasma membrane. Bars, 10 μm.

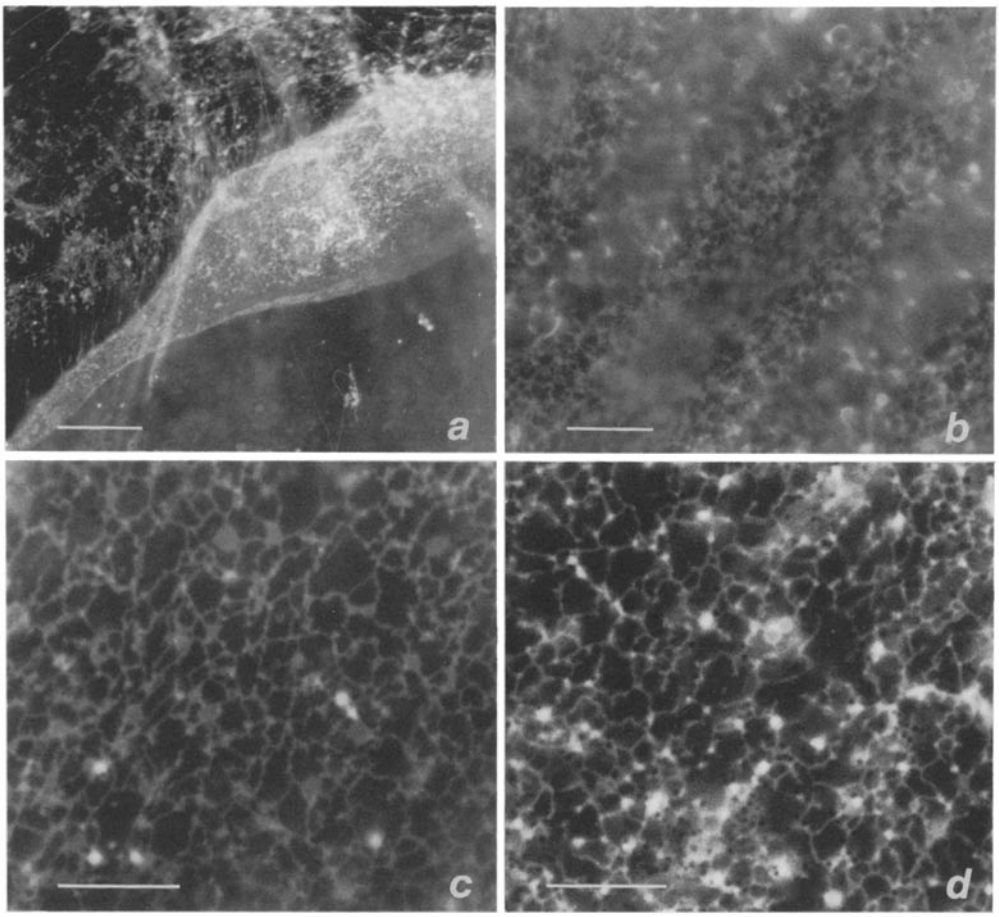

Figure 5. Visualization of ER on isolated cortices with $DiIC_{16}(3)$. (*a*) Loose tubular elements are seen connected to the edge of this piece of *Xenopus* egg cortex, prepared in the second half of the first cell cycle. (*b*) At higher magnification a clear network of ER is visible lying close to the plasma membrane in the plane of the cortex, while other, disorganized, membranes are found deeper in (out of this plane of focus). (*c* and *d*) Similar patterns are seen on cortical pieces from *Rana* eggs prepared either in the first (*c*) or second (*d*) half of the cell cycle. Bars (*a*) 25 μm; (*b–d*) 10 μm.

relative positions of microtubules and cytokeratin filaments were determined by double staining on sections. The cytokeratin filaments were found close to the boundary between the large yolk platelets of the vegetal cytoplasm and the microtubules of the array (Fig. 3, *f*, *g*, *h*, and *i*), regardless of their state of elaboration. The appearance of cytokeratin filaments at this position did not depend on the presence of the microtubule array, preceding it in some eggs.

We also stained sections with anti-actin antibodies. A layer of actin containing some microfilaments (Gall et al., 1983; see Elinson and Houliston, 1990, for review of other data concerning actin) is present throughout the first cell cycle and lies just below the plasma membrane (Fig. 3, *j* and *k*). In line with the observations of Gall et al. (1983), we found organized microfilaments only rarely in other areas of the cytoplasm.

Relationship of ER to Microtubules

EM studies (Grey et al., 1974; Campanella and Andreuccetti, 1977; Gall et al., 1983; Andreuccetti et al., 1984; this study) have demonstrated the presence of many membranous organelles including a cortical ER system in the cortical region of frog eggs. We examined the distribution of ER in the vegetal cortex during the first cycle using an mAb to BiP, a luminal protein of the ER (Bole et al., 1986; 1989). On sections of *Xenopus* eggs, abundant BiP staining was present throughout the cytoplasm. It also formed a distinct thin layer at the periphery of the cell, presumed to represent the cortical ER (Fig. 4, *a* and *b*). The staining consisted of irregular patches, probably due to collapse of the membrane system during fixation. After the onset of cortical movement a second, deeper, layer of ER developed in the subcortical region (Fig. 4 *b*). Double staining revealed that this was codistributed with the microtubules of the array (Fig. 4, *c* and *d*). Thus at least some of the membranous elements in each of the two layers seen in isolated cortices by EM (Fig. 2 *a*) comprise ER.

A clearer picture of the ER was obtained by staining pieces of isolated cortex (*Rana* or *Xenopus*) with anti-BIP. Vesicular-like patterns of antigen were found covering the cortex, sometimes forming a network (Fig. 4 *e*). The BiP staining was abolished by treatment of cortices with Triton X-100 before fixation (Fig. 4 *f*), as would be expected for specific staining of a luminal ER protein. During the second half of the cell cycle additional BiP staining in linear arrays was found, lying in a different plane of focus on the cytoplasmic face of the peel. Double staining revealed that this staining codistributed with the microtubules of the array (Fig. 4, *g* and *h*). It thus probably represents ER from the subcortical layer that comes away from the egg in association with the microtubules. The network-like layer closer to the egg surface was present both before and after the microtubules appeared (not shown).

In unfixed cortical pieces ER could be visualized with the lipophilic dye $DiIC_{16}(3)$ (Fig. 5). This revealed a network of cortical ER lying close to the plasma membrane, and strands of cytoplasmic ER that remained attached to the cortex on the cytoplasmic face (Fig. 5, *a* and *b*). The reticular

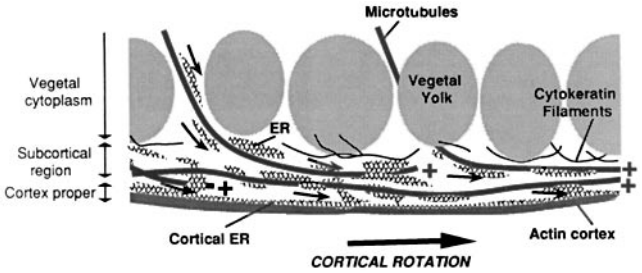

Figure 6. Diagram summarizing the relationship of the various elements in the frog egg vegetal cortex during the second half of the first cell cycle and our current hypothesis concerning the mechanism of cortical rotation. Microtubules growing out towards the cortex bend to have their plus ends (+) running in the direction of cortical rotation. Small arrows indicate that plus end–directed translocation of ER along microtubules using kinesin could be translated into movement of the entire cortex if subcortical and cortical ER become linked. We suggest that the vegetal yolk and the microtubules remain stationary, while the ER, cortical ER, and actin cortex move towards the future dorsal side of the egg.

nature of the cortical ER was comparable to that seen in sea urchin (Henson et al., 1988) and ascidian (Gualtieri and Sardet, 1989) eggs. Similar networks were seen in cortical pieces prepared at times both before and after the formation of the microtubule array (Fig. 5, *c* and *d*). The superficial position of the network and its presence at all times suggest that it corresponds to the outer layer of BiP staining. Unfortunately it was difficult to make out a pattern in the material attached to the cytoplasmic side of the preparations, corresponding to the microtubule-containing subcortical layer, since the form of the membranes was disrupted during isolation of the cortex.

Kinesin Staining in the Cortical Region

It has been proposed that a microtubule-associated motor molecule could be involved in translocating some element of the cortex or cytoplasm relative to the microtubules (Elinson and Rowning, 1988). Having established the arrangement of the major elements in the region of the vegetal cortex at the time of the cortical movement (summarized in Fig. 6), we addressed this possibility by staining with antibodies to kinesin. Kinesin is a plus end–directed microtubule motor (see Gibbons, 1989) so would be appropriate for translocation of the cortex relative to the microtubules, given their polarity.

We tested a variety of antibodies to kinesin: polyclonal antibodies against kinesin heavy chains from *Drosophila* embryos (gift of Dr. W. Saxton; Saxton et al., 1988), embryonic chicken kinesin heavy chains (gift of Dr. M. Sheetz) and bovine brain kinesin (gift of Dr. R. Vale), and mAbs against sea urchin kinesin (SUK2, SUK4, and SUK5: gift of Dr. J. Scholey; Ingold et al., 1988). The three polyclonal antibodies gave similar patterns of staining on isolated cortices. Along with a fairly uniform carpet-like staining, vesicular patches were found attached to the cortex and probably associated with the microtubules. Unfortunately, the level of nonspecific staining obtained with preimmune rabbit sera was high, giving spotty staining over the cortex and associated with many attached structures, especially yolk platelets. Although we suspected that the common elements stained were specific for kinesin, we did not pursue our studies with these antisera, concentrating instead on the sea urchin monoclonals. These were raised against native kinesin molecules (Ingold et al., 1988) and although they reacted only weakly with fixed material, staining of unfixed material followed by postfixation again gave a carpet-like pattern of staining close to the plasma membrane with additional lines and vesicular patches on the cytoplasmic face of the preparation (Fig. 7, *a–c*). This pattern was similar for all three antibodies. SUK2 (Fig. 7 *b*) or a mixture of SUK4 and SUK5 (Fig. 7 *c*) gave a stronger signal than SUK4 or SUK5 alone when used at the same concentrations (either 10 or 20 μg/ml). These three antibodies recognize distinct parts of the kinesin molecule (Wright et al., 1991), so the presence of all three antigens suggests that a molecule very similar to sea urchin kinesin is present.

As in previous studies (Pfister et al., 1989; Hollenbeck, 1989; Wright et al., 1991) anti-kinesin staining was greatly reduced by prior detergent treatment (Fig. 7 *d*), suggesting that the antigen detected was associated with membranes. Double staining with an anti-α tubulin antibody confirmed that some of the staining was associated with the microtubules of the vegetal array while some of the antigen also appeared to be independently associated with the cortex, mostly in a different plane of focus (Fig. 7, *e* and *f*). This was reminiscent of the staining obtained with anti-BiP (see Fig. 4), suggesting that the antigens recognized by the kinesin antibodies may be linked to ER. We double stained cortices with SUK2 and anti-BIP, and indeed there was a close correspondence between the distribution of the two (Fig. 7, *g* and *h*). As with the ER, some punctate kinesin staining was seen in the absence of microtubules during the first half of the cell cycle, in a layer close to the plasma membrane (Fig. 7 *k*).

Controls performed with an irrelevant mouse mAb (CaS/C1 against chick muscle Ca^{2+}-ATPase, Kaprielian and Fambrough, 1987) at the same concentration, or secondary antibody alone, showed only sparse dots, never recognizing microtubules or ER, and confirmed that cross-reaction between antibodies did not occur in double-stain experiments (Fig. 7, *i*, *j*, and *l*). Unfortunately staining with the SUK antibodies on sections was very weak and inconclusive, since fixed material had to be used, so the overall distribution of kinesin in the egg remains to be established.

Immunoblotting of Kinesin from Xenopus Egg Cytoplasm

Taxol-polymerized microtubules from *Xenopus* cytoplasm were prepared in the presence and absence of AMPPNP. All three SUK antibodies recognized a polypeptide of ~115 kD that only associated with the microtubules in the presence of AMPPNP (Fig. 8), confirming that these antibodies recognized a molecule very similar to sea urchin egg kinesin. In a previous study (Neighbors et al., 1988), antibodies raised against bovine and squid kinesins recognized proteins of ~115 and ~120 kD in similar preparations from *Xenopus* eggs.

Discussion

The Common Polarity of Microtubules In the Vegetal Cortex

The vast majority of microtubules in the vegetal cortical ar-

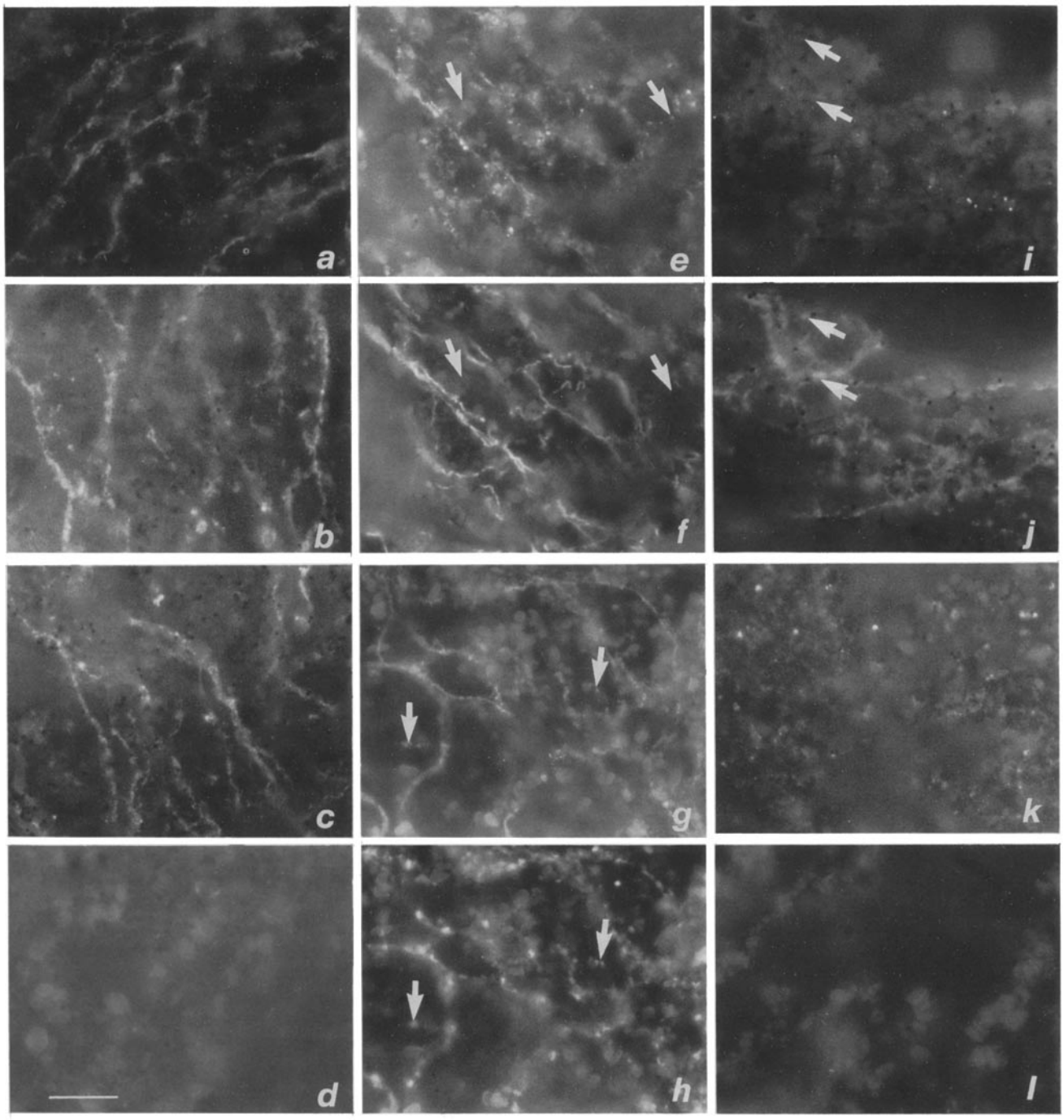

Figure 7. Immunofluorescent staining with mAbs to sea urchin kinesin on pieces of isolated cortex. (*a* and *b*) SUK2 reveals a layer of dots and additional linear elements on cortices peeled from *Xenopus* (*a*) or *Rana* (*b*) eggs during the second half of the cell cycle. (*c*) A similar pattern is revealed by a mixture of SUK4 and SUK5 on this *Rana* cortex (*d*) Staining with SUK2 on this *Rana* cortex was abolished by prior detergent extraction. (*e* and *f*) Double staining on *Rana* cortices with SUK2 (*e*) and anti-α-tubulin (*f*) shows that some kinesin staining lies along microtubules. Microtubule-independent staining is also seen (e.g., at arrows), mostly external to this plane of focus. (*g* and *h*) Double staining on *Rana* cortices with SUK2 (*g*) and anti-BiP (*h*) shows a close correspondence between the distributions of the two, both close to the plasma membrane and in the deeper, microtubule-containing, layer (e.g., at arrows in this photograph). (*i* and *j*) This is not seen when an irrelevant mAb (*i*) is used to double stain *Rana* cortices with anti-BiP (*j*), indicating that the SUK staining is specific, and that there is no cross-reaction between antibodies. (*k*) On *Rana* cortices prepared in the first half of the cell cycle, uniform punctate staining in the plane of the cortex is seen with SUK2. (*l*) When an irrelevant antibody was used on similar specimens, only sparse dots were found. Bar: (*a–l*) 10 μm.

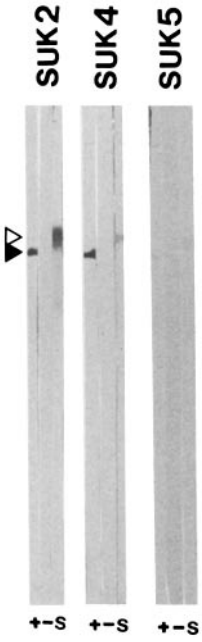

Figure 8. Antibodies to sea urchin kinesin used on a Western blot of samples containing microtubules prepared from *Xenopus* egg cytoplasm in the presence (+) and absence (−) of AMPPNP (equivalent amounts of protein loaded), and from sea urchin in the presence of AMPPNP (*S*). Each antibody recognized a *Xenopus* protein of 115–120 kD (*black triangle*, migration approximately the same as the 116 kD standard) specifically in the AMPPNP sample, as well as the 130-kD sea urchin polypeptide (*open triangle*).

ray in the frog egg are oriented with their plus ends pointed in the direction of cortical rotation, that is, toward the grey crescent or the future dorsal side of the egg. This finding fits well with our understanding of how the array forms. Antitubulin immunofluorescence performed on sections of *Xenopus* eggs shows that the array forms as microtubules growing outward from the cytoplasm turn to run beneath the egg surface in the direction of cortical rotation (Houliston and Elinson, 1991). In fertilized eggs, the rotation is usually directed away from the side of sperm entry, apparently because microtubules extending from the sperm aster are the first to reach the vegetal cortex, turning toward the vegetal pole. The sperm aster microtubules are centrosome nucleated so will have their plus ends growing out and reaching the cortex. Non–centrosome-nucleated microtubules also grow out from the inner cytoplasm to the cortex (Houliston and Elinson, 1991), and their plus ends will probably reach the cortex first too, because plus ends grow faster than minus ends. Differences in the origins of the microtubules between different regions of the cortical array may be reflected by local variation in the percentage of commonly oriented microtubules. Our sampling method did not address this possibility.

The aligned array of microtubules in the frog egg cortex represents a remarkable example of controlled microtubule polymerization. The layer of microtubules covers a huge area (>2 mm^2 in *Xenopus* and 4 mm^2 in *Rana*). The microtubules are aligned with predominantly common polarity and appear in a matter of minutes half way through the first cell cycle, then disappearing by the time of first cleavage (Elinson and Rowning, 1988).

Organization of the Cortical Region

In understanding the mechanism for the cortical rotation it is important to establish where the shear actually occurs. We have tried to build up a picture of the organization of the vegetal cortical region at this time. The cortex of the fertilized egg has been described previously as a dense felt-like layer 0.5–1 μm thick, containing polymerized and unpolymerized actin (Hebard and Herold, 1967; Grey et al., 1974; Gall et al., 1983). This layer, thought to be responsible for the contractile properties of the egg (see Sardet and Chang, 1987, for references and discussion) is present throughout the cell cycle. We have described a thin layer of cortical ER close to the plasma membrane, which, like the actin layer, is consistently removed when pieces of cortex are isolated. These components can be considered to be the "cortex proper". To distinguish this from the deeper elements in the cortical region (microtubules of the array, cytokeratin filaments, et cetera), we will henceforth refer to the latter as "subcortical" (see Fig. 6). It should be noted that pieces of cortex isolated manually from the egg contain both the cortex proper and most elements of the subcortical region.

In contrast to the cortex proper, the subcortical region changes in thickness and composition during the first cell cycle, as microtubules (Elinson and Rowning, 1988) and cytokeratin filaments (Klymkowksy et al., 1987; this study) appear there. The arrival of these two elements appears not to be closely coordinated, being distinct in both timing (either may arrive first) and position. The cytokeratin filaments elaborate gradually along the boundary between the yolky cytoplasm and the subcortical region, while the microtubules extend from the cytoplasm (Houliston and Elinson, 1991) to fill the subcortical zone relatively quickly.

We have shown recently that the microtubules of the array are linked to microtubules running through the vegetal cytoplasm throughout the period of the cortical rotation (Houliston and Elinson, 1991; Fig. 3 *i*). Furthermore it has been noted that during the cortical rotation, pigment granules lying less than 1 μm from the egg surface move with the outer cortex (Vincent et al., 1987), whereas some of those lying deeper (1–3 μm down, the depth of the microtubules) move with the cytoplasmic mass (Elinson and Rowning, 1988). Thus we can tentatively suggest that the shear occurs between the microtubules of the array and the cortex proper (see Fig. 6).

Possible Mechanisms for the Cortical Rotation

We examined the possibility that other elements of the subcortical region interact with the aligned microtubules to cause the rotation. Cytokeratin filaments initially seemed to be good candidates because of their cortical location, alignment and appearance during the first cell cycle. It seems unlikely, however, that they are involved directly since the protracted and asynchronous development of the cytokeratin network does not correlate with the period of cortical rotation (Vincent et al., 1986). The cytokeratin filament system continues to elaborate during subsequent cell cycles (Klymkowsky et al., 1987), so it may be involved with stopping the rotation process or fixing an altered arrangement of cytoplasm important for the localization of developmental information (see Elinson, 1989).

ER, on the other hand, is found both running with the microtubules in the subcortical region and in a distinct cortical layer external to the microtubules, as seen by both EM and immunofluorescence with anti-BiP. The codistribution of ER with microtubules on isolated cortices supports the idea that at least some of the subcortical ER is associated with microtubules. It thus seems possible that movement

is generated between microtubules and ER. The nature of the attachment of the subcortical layer of microtubules and membranes to the cortex proper is unclear at present. One possibility (shown in Fig. 6) is that the two layers of membrane become connected directly as ER fills the subcortical region and fuses with the layer of cortical ER. In this way force generated between the microtubules and the membranes surrounding them could be translated into cortical translocation. An analogous mechanism has been proposed to account for cytoplasmic streaming in Characean algae (Kachar and Reese, 1988), where it appear that ER linked to a sheet of endoplasm moves across polarized actin filaments attached to an outer layer. Alternatively, other elements may exist that stabilize an association between the cortex and ER.

Given the common polarity of the aligned microtubules, the idea that cortical rotation is the result of plus-end directed translocation of cortically attached ER using a microtubule "motor" molecule is attractive. We used antibodies to the plus end–directed motor kinesin to address this possibility. Fortunately, three antibodies raised against sea urchin kinesin (SUK2, 4 and 5, Ingold et al., 1988) recognized *Xenopus* egg kinesin. A previous study (Neighbors et al., 1988) detected a doublet of proteins of around the same size in *Xenopus* eggs with other anti-kinesin antibodies. The difference may be due to the presence of another protein related to kinesin in the egg, or reflect slight differences in the protein preparation methods or running conditions for the gels. SUK4 is known to bind specifically to the force transducing head domain of the kinesin heavy chain (Ingold et al., 1988; Scholey et al., 1989*a*), while SUK2 and SUK5 recognize epitopes on the stalk and tail portions of the molecules, respectively (Wright et al., 1991). Since all three antibodies gave similar staining patterns in the cortical region, it appears likely that a true frog egg kinesin is present. This is important because the head portion of the kinesin molecule can be present in minus-end directed microtubule motors (Walker et al., 1990; McDonald et al., 1990).

Whether kinesin is capable of translocating the frog egg cortex via its cortical ER remains to be seen. In vitro studies have shown that ER-like networks can translocate towards microtubule plus ends (Dabora and Sheetz, 1988) and can form by kinesin-driven microtubule movement (Vale and Hotani, 1988). SUK4 has been used to inhibit kinesin function in vitro (Ingold et al., 1988) and in vivo in macrophages (Hollenbeck and Swanson, 1990), so may be useful for testing the role of kinesin in the frog egg cortex. The possibility remains open that motor molecules of other types are involved in the cortical rotation, perhaps by generating sliding between two populations of microtubules with the same polarity, one attached to the cortex and the other to the cytoplasm.

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References

Ancel, P., and P. Vintemberger. 1948. Recherches sur le déterminisme de la symétrie bilatérale dans l'oeuf des amphibiens. *Bull. Biol. Fr. Belg. Suppl.* 31:1–182.

Andreuccetti, P., S. Denis-Donini, A. G. Burrini, and C. Campanella. 1984. Calcium ultrastructural localization in *Xenopus laevis* eggs following activation by pricking or by calcium ionophore A23187. *J. Exp. Zool.* 229:295–308.

Bole, D. G., L. M. Hendershot, and J. F. Kearney. 1986. Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. *J. Cell Biol.* 102:1558–1566.

Bole, D. G., R. Dowin, M. Doriaux, and J. D. Jamieson. 1989. Immunocytochemical localization of BiP to the rough endoplasmic reticulum: evidence for protein sorting by selective retention. *J. Histochem. Cytochem.* 37:1817–1823.

Campanella, C., and P. Andreuccetti. 1977. Ultrastructural observations on cortical endoplasmic reticulum and on residual cortical granules in the egg of *Xenopus laevis*. *Dev. Biol.* 56:1–10.

Dabora, S. L., and M. P. Sheetz. 1988. Microtubule dependent formation of a tubular vesicular network with characteristics of the endoplasmic reticulum from cultured cell extracts. *Cell.* 54:27–35.

Elinson, R. P. 1983. Cytoplasmic phases in the first cell cycle of the activated frog egg. *Dev. Biol.* 100:440–451.

Elinson, R. P. 1985. Changes in levels of polymeric tubulin associated with activation and dorsoventral polarization of the frog egg. *Dev. Biol.* 109:224–233.

Elinson, R. P. 1989. Microtubules and specification of the dorsoventral axis in frog embryos. *Bioessays.* 11:124–127.

Elinson, R. P., and B. Rowning. 1988. A transient array of parallel microtubules in frog eggs: potential tracks for a cytoplasmic rotation that specifies the dorso-ventral axis. *Dev. Biol.* 128:185–197.

Elinson, R. P., and E. Houliston. 1990. Cytoskeleton in *Xenopus* oocytes and eggs. *Sem. Cell Biol.* 1:349–357.

Gall, L., B. Picheral, and P. Gounon. 1983. Cytochemical evidence for the presence of intermediate filaments and microfilaments in the egg of *Xenopus laevis*. *Biol. Cell.* 47:331–342.

Gibbons, I. R. 1989. Microtubule based motility: an overview of a fast-moving field. *In* Cell Movement. Vol 1. The Dynein ATPases. F. D. Warner, P. Satir, and I. R. Gibbons, editors. Alan Liss, Inc., New York. 3–22.

Godsave, S. F., C. Lane, B. Anderton, and C. C. Wylie. 1984. Intermediate filaments in the *Xenopus* oocyte: the appearance and distribution of cytokeratin containing filaments. *J. Embryol. Exp. Morphol.* 83:157–167.

Grey, R. D., D. F. Wolf, and J. L. Hedrick. 1974. Formation and structure of the fertilization envelope in *Xenous laevis*. *Dev. Biol.* 36:44–61.

Gualtieri, R., and C. Sardet. 1989. The endoplasmic reticulum network in the ascidian egg: localization and calcium content. *Biol. Cell.* 65:301–304.

Hebard, C. N., and R. C. Herold. 1967. The ultrastructure of the cortical cytoplasm in the unfertilized egg and first cleavage zygote of *Xenopus laevis*. *Exp. Cell Res.* 46:553–570.

Heidemann, S. R., and J. R. McIntosh. 1980. Visualization of the structural polarity of microtubules. *Nature (Lond.).* 286:517–519.

Henson, J. H., D. A. Begg, S. M. Beaulieu, D. J. Fishkind, E. M. Bonder, M. Terasaki, D. Lebeche, and B. Kaminer. 1989. A calsequestrin-like protein in the endoplasmic reticulum of the sea urchin: localization and dynamics in the egg and first cell cycle embryo. *J. Cell Biol.* 109:149–161.

Hollenbeck, P. J. 1989. The distribution, abundance, and subcellular localization of kinesin. *J. Cell Biol.* 108:2335–2342.

Hollenbeck, P. J., and J. A. Swanson. 1990. Radial extension of macrophage tubular lysosomes supported by kinesin. *Nature (Lond.).* 346:864–866.

Houliston, E., and R. P. Elinson. 1991. Patterns of microtubule polymerization relating to cortical rotation in *Xenopus laevis* eggs. *Development.* 112:107–117.

Ingold, A. L., S. A. Cohn, and J. M. Scholey. 1988. Inhibition of kinesin-driven microtubule motility by monoclonal antibodies to kinesin heavy chains. *J. Cell Biol.* 107:2657–2667.

Kachar, B., and T. S. Reese. 1988. The mechanism of cytoplasmic streaming in characean algal cells: sliding of endoplasmic reticulum along actin filaments. *J. Cell. Biol.* 106:1545–1552.

Kaprielian, Z., and D. M. Fambrough. 1987. Expression of fast and slow isoforms of the Ca^{2+}-ATPase in developing chick skeletal muscle. *Dev. Biol.* 124:490–503.

Kilmartin, J. V., B. Wright, and C. Milstein. 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. *J. Cell Biol.* 93:576–582.

Klymkowsky, M. W., L. A. Maynell, and A. G. Polson. 1987. Polar asymmetry in the organization of the cortical cytokeratin system of *Xenopus laevis*

oocytes and embryos. *Development.* 100:543–557.
Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:11713–11720.
Manes, M. E., and R. P. Elinson. 1980. Ultra-violet light inhibits grey crescent formation on the frog egg. *Roux Arch. Dev. Biol.* 189:73–76.
Manes, M. E., R. P. Elinson, and F. D. Barbieri. 1978. Formation of the amphibian grey crescent: effects of colchicine and cytochalasin B. *Roux Arch. Dev. Biol.* 185:99–104.
McDonald, K. 1984. Osmium ferricyanide fixation improves microfilament preservation and membrane visualization in a variety of animal cell types. *J. Ultrastruct. Res.* 86:107–118.
McDonald, H. B., R. J. Stewart, and L. S. B. Goldstein. 1990. The kinesin-like *ncd* protein of *Drosophila* is a minus end-directed microtubule motor. *Cell.* 63:1159–1165.
Neighbors, B. W., R. C. Williams, and J. R. McIntosh. 1988. Localization of kinesin in cultured cells. *J. Cell Biol.* 106:1193–1204.
Pfister, K. K., M. C. Wagner, D. L. Stenoien, S. T. Brady, and G. S. Bloom. 1989. Monoclonal antibodies to kinesin heavy and light chains stain vesicle-like structures, but not microtubules in cultured cells. *J. Cell Biol.* 108:1453–1463.
Sardet, C., and P. Chang. 1987. The egg cortex: from maturation through fertilization. *Cell Differ.* 21:1–19.
Saxton, W. M., M. E. Porter, S. A. Cohn, J. M. Scholey, E. C. Raff, and J. R. McIntosh. 1988. *Drosophila* kinesin: Characterization of microtubule motility and ATPase. *Proc. Natl. Acad. Sci. USA.* 85:1109–1113.
Scharf, S. R., and J. C. Gerhart. 1983. Axis determination in eggs of *Xenopus laevis*: a critical period before first cleavage identified by the common effects of cold, pressure, and uv-irradiation. *Dev. Biol.* 99:75–87.
Schliwa, M., U. Euteneuer, J. C. Bulinsky, and J. G. Izant. 1981. Calcium lability of cytoplasmic microtubules and its modulation by microtubule associated proteins. *Proc. Natl. Acad. Sci. USA.* 78:1037–1041.
Scholey, J. M., J. Heuser, J. T. Yang, and L. S. B. Goldstein. 1989*a*. Identification of globular mechanochemical heads of kinesin. *Nature (Lond.).* 338:355–357.
Scholey, J. M., M. E. Porter, R. J. Lye, and J. R. McIntosh. 1989*b*. Cytoplasmic microtubule-associated motors. *In* The Cell Biology of Fertilization. H. Schatten, and G. Schatten, editors. Academic Press, Orlando, FL. 139–163.
Terasaki, M., L. B. Chen, and K. Fujiwara. 1986. Microtubules and the endoplasmic reticulum are highly interdependent structures. *J. Cell Biol.* 103:1557–1568.
Towbin, H., T. Staelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.
Vale, R. D., and H. Hotani. 1988. Formation of membrane networks in vitro by kinesin-driven microtubule movement. *J. Cell Biol.* 107:2233–2241.
Vale, R. D., and L. S. B. Goldstein. 1990. One motor, many tails: an expanding repertoire of force generating enzymes. *Cell.* 60:883–885.
Vincent, J.-P., G. F. Oster, and J. C. Gerhart. 1986. Kinematics of gray crescent formation in *Xenopus* eggs: the displacement of subcortical cytoplasm relative to the egg surface. *Dev. Biol.* 113:484–500.
Vincent, J.-P., S. R. Scharf, and J. C. Gerhart. 1987. Subcortical rotation in *Xenopus* eggs: a preliminary study of its mechanochemical basis. *Cell Motil. Cytoskel.* 8:143–154.
Walker, R. A., E. D. Salmon, and S. A. Endow. 1990. The *Drosophila claret* segregation protein is a minus-end directed motor molecule. *Nature (Lond.).* 347:780–782.
Wright, B. D., J. H. Henson, K. P. Wedman, P. J. Willy, J. N. Morand, and J. M. Scholey. 1991. Subcellular localization of sea urchin kinesin heavy chain: evidence for its association with membranes in the mitotic apparatus and in the cytoplasm. *J. Cell Biol.* 113:817–834.
Zisckind, N., and R. P. Elinson. 1990. Gravity and microtubules in dorsoventral polarization of the *Xenopus* egg. *Dev. Growth Differ.* 32:575–581.