Abstract. Previous studies suggest that kinesin heavy chain (KHC) is associated with ER-derived membranes that accumulate in the mitotic apparatus in cells of early sea urchin embryos (Wright, B. D., J. H. Henson, K. P. Wedaman, P. J. Willy, J. N. Morand, and J. M. Scholey. 1991. J. Cell Biol. 113:817-833). Here, we report that the microinjection of KHC-specific antibodies into these cells has no effect on mitosis or ER membrane organization, even though one such antibody, SUK4, blocks kinesin-driven motility in vitro and in mammalian cells. Microinjected SUK4 was localized to early mitotic figures, suggesting that it is able to access kinesin in spindles. In contrast to KHC-specific antibodies, two antibodies that react with kinesin-like proteins (KLPs), namely CHO1 and HD, disrupted mitosis and prevented subsequent cell division. CHO1 is thought to exert this effect by blocking the activity of a 110-kD KLP. The relevant target of HD, which was raised against the KHC motor domain, is unknown; HD may disrupt mitosis by interfering with an essential spindle KLP but not with KHC itself, as preabsorption of HD with KHC did not alter its ability to block mitosis. These data indicate that some KLPs have essential mitotic functions in early sea urchin embryos but KHC itself does not.

Kinesin and kinesin-like proteins (KLPs)1 are microtubule (MT)-based motor proteins, some of which play important roles in cell division (Vallee et al., 1991; McIntosh and Pfarr, 1991; Sawin and Scholey, 1991). Genetic studies have been used to determine if the major polypeptide component of kinesin itself, the kinesin heavy chain (KHC), has an essential role in cell division. KHC mutations have no effect on mitosis in early Drosophila embryos (Saxton et al., 1991) but Caenorhabditis elegans embryos carrying a KHC mutant allele fail to correctly position mitotic spindles, resulting in misplaced cleavage planes and abnormal cell divisions (J. Plenefisch and E. Hedgecock, personal communication). Here, we report complementary studies of KHC function, involving the microinjection of sea urchin (SU) eggs with antibodies to KHC and KLPs.

SU eggs and embryos have proven to be a productive system for studying MT proteins in mitotic cells (reviewed in Bloom and Vallee, 1989; Wright and Scholey, 1992). KHC, in association with ER-derived membranes, is localized to the mitotic apparatus in early SU embryos (Scholey et al., 1985; Wright et al., 1991) indicating a potential role for this motor in mitosis. Here we show that cell division occurs normally in SU embryonic cells microinjected with KHC-specific antibodies, whereas two antibodies that react with KLPs essential for mammalian cell division block mitosis in these SU cells. Thus, we conclude that KHC, in contrast to some KLPs, is not essential for cell division in early SU embryos.

Materials and Methods

Materials

SUs Strongylocentrotus purpuratus and Lytechinus pictus were obtained from Marinus, Inc. (Venice, CA), whereas L. variegatus were obtained from Susan J. Decker (Hollywood, FL). Sea water was collected at the University of California Marine Biological Laboratory (Bodega Bay, CA). General reagents were from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. The dicarbocyanide dye DiIC16(3) (DiI) was obtained from Molecular Probes Inc. (Eugene, OR). Taxol was obtained from Calbiochem-Behring Corp. (La Jolla, CA). Mouse monoclonal CHO1 ascites was generously supplied by R. Kuriyama (University of Minnesota, Minneapolis, MN) and J. R. McIntosh and C. Nislow (University of Colorado, Boulder, CO). Rabbit polyclonal antisemur against bacterially expressed Drosophila kinesin head (HD) was a generous gift of V. Gelfand and V. Rodionov (University of California, San Francisco, CA). Isolated S. purpuratus metaphase mitotic spindles were a generous gift of R. Palazzo (Marine Biological Laboratory, Woods Hole, MA). Purified metaphase microsomal membranes were generously supplied by D. Skoufas (University of California, Davis, CA). Some of the SU kinesin and KLP fractions

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1. Abbreviations used in this paper: HD, Drosophila kinesin head antibody; KHC, kinesin heavy chain; KLP, kinesin-like protein; MT, microtubule; SU, sea urchin.
Antibody Preparation

Mouse monoclonal and affinity-purified rabbit polyclonal antibodies to SU KHC were as described previously (Ingold et al., 1988; Wright et al., 1991). CHO IgM (Nislow et al., 1990) was purified from ascites by hydroxyapatite chromatography and elution with a 0.05-0.75 M NaF gradient (Bukovsky and Kennett, 1987). HD IgG fractions were purified using Affigel protein A and the MAPs II antibody purification system (Bio-Rad Laboratories Inc., Richmond, CA). Antibodies in eluted fractions were identified on Coomassie blue-stained SDS–polyacrylamide gels and alkaline phosphatase–stained nitrocellulose blots using the appropriate secondary antibody (Fisher Scientific, Santa Clara, CA). Peak fractions were pooled and protein concentration measured using the Bradford microassay with a 3′-amid acid gels and either Coomassie blue stained or blotted to nitrocellulose membranes.

Antibody and MAPs fractions were run on replicate 7.5% SDS-polyacrylamide gels and the CHO1 antigen (Nislow et al., 1990) was purified from ascites by hydroxyapatite chromatography and elution with a 0.05-0.75 M NaF gradient (Bukovsky and Kennett, 1987). HD IgG fractions were purified using Affigel protein A and the MAPs II antibody purification system (Bio-Rad Laboratories Inc., Richmond, CA). For immunodepletion experiments, HD antibody was preabsorbed against SU kinesin (HD-KIN) by incubating 1.0 mg of protein–purified HD IgG first with a blot strip containing 0.1 mg of SU-KHC right at 4°C (to remove antibodies against denatured KHC), and then with 0.1 mg of native, sucrose gradient–purified kinesin (Cole et al., 1992; Skoufias et al., 1993), rocking overnight at 4°C (to remove antibodies against native KHC), and then spinning at 100,000 g for 1 h at 4°C to pellet antibody–antigen complexes. Immunoblot analysis showed that all of the antibody and over 97% of the anti-KHC antibodies had been precipitated. Antibody was similarly treated against a nonspecific control protein blot strip (BSA) and a buffer-only precipitation as a positive control (HD CON). Both HD and HD-KIN were immunodepleted against an excess of keratin by incubation at 4°C overnight prior to immunoblotting.

Sea Urchin Eggs and Extracts

SU gametes were obtained and stored by routine methods as previously described (Wright et al., 1991). High-speed supernatant of unfertilized S. purpuratus egg cytosol was prepared in PMEG buffer as described previously (Scholey et al., 1985). Cytosolic MTs were assembled by adding 10 μM Taxol and 1 mM Mg/GTP and pelleted in the presence of 2.5 mM AMP-5PNP. The pellets were washed with Mg-free buffer, nucleotide-sensitive MAPs were eluted with 10 mM Mg/ATP, and nonnucleotide sensitive MAPs were then eluted with high salt (0.6 M NaCl). These eluted MAPs were then fractionated by gel filtration over a 2.5 × 75-cm Biogel A1.5 M column equilibrated in PMEG containing either ATP or high salt, respectively. Fractions were assayed by SDS-PAGE and Western blotting with the appropriate primary antibody. Peak fractions containing KHC from the ATP MAPs and the CHOL antigen (Nislow et al., 1992) from the high-salt MAPs were pooled. Isolated mitotic spindles were prepared by R. Palazzo and coworkers (Rehbun and Palazzo, 1988), and ER-derived salt-stripped microsomal membranes (Oberdorfer et al., 1988) were prepared from metaphase embryos as previously described (Skoufias et al., 1993).

Immunoblotting

Antibody and MAPs fractions were run on replicate 7.5% SDS–polyacrylamide gels and either Coomassie blue stained or blotted to nitrocellulose (Schleicher & Schuell, Keene, NH) and probed with the above primary antibodies (SU4, 0.1 μg/ml; CHOL, 10 μg/ml; and HD, 2.5 μg/ml) and the appropriate HRP-conjugated secondary antibodies (anti-mouse Ig, 1:200 for IgMs, 1:1,000 for IgGs; and anti-rabbit Ig, 1:1,000) and developed using the ECL detection system (Amersham Corp., Arlington Heights, IL). Non–specific primary antibody controls (mouse monoclonal IgG MOPC 21, 0.1 μg/ml; mouse monoclonal IgM TEP 13, 10 μg/ml; non–preimmune protein A–purified rabbit IgG, 2.5 μg/ml) showed no specific reaction with the bands of interest.

Embryo Treatment and Antibody Microinjection

Embryos were wash after fertilized egg injection buffer (FIB: 150 mM potassium aspartate, 10 mM potassium phosphate, pH 7.3) or unfertilized egg injection buffer (UIB: same but pH 7.0) and concentreated by repeated (3×) dilution and reconcentration in either a 2-ml collodion bag apparatus (Schleicher & Schuell, Keene, NH) or in microcentrifuge concentrators (Ultraspun 30,000 mol wt-cutoff cellulose acetate filters; USA/Scientific Plastics, Ocala, FL) and finally filtered using 0.22-μm microcentrifuge filters (Ultrafree-MC; Amicon, Bedford, MA). Protein concentrations were measured with a micro-Bradford assay against gamma globulin standard (typically 10–20 μg/ml).

Microinjections of fertilized embryos were performed using methods modified from Kiehart (1982) and Hiramoto (1984). Briefly, washed and dejellied L. pictus or L. variegatus eggs were fertilized in 0.22-μm filtered sea water (MFSW) plus 10 mM PABA at the appropriate pH and temperature for each species (pH 8.1 at 14°C or pH 8.3 at 24°C, respectively; all results were identical in both species). Fertilized eggs were washed once in calcium-free sea water (CFSW) containing 5 mM EGTA and 10 mM PABA and laid into a temperature-controlled injection chamber to be heated to 25°C in a heating/cooling/pumping water bath. The reservoir was filled with MFSW/PABA and sealed with Wesson oil. Antibody and Wesson oil were layered into loading chambers, and single boluses of antibody and oil were first loaded into precalibrated glass microneedles. Between 10 and 20 either first or second interphase embryos were sequentially injected quantitatively with 1–10% of their own pronucleus volume and sealed with oil to mark the injected embryos. Only those embryos surviving the injection were scored (average survival rates were 75–80%) and of the survivors which those divided normally through three divisions were counted as normal.

Motility Assays

MT-motility assays for assessing antibody effects on kinesin activity were performed as previously described (Ingold et al., 1988). Peak Biogel-purified kinesin (~100 μg/ml) was absorbed to glass coverslips and then incubated with a fourfold excess of antibody (2 mg/ml; calculated to represent the maximal relative proportion of antibody to kinesin in injected eggs) before monitoring MT-gliding activity.

Immunostaining

To determine the intracellular localization of microinjected SUK4, injected cells were removed from the injection chamber at first metaphase and serially processed as previously described (Wright et al., 1991; Wright and Scholey, 1993) through detergent lysis and methanol fixation, and peroxidase-antiperoxidase–staining and mounting. To analyze the abnormal MT arrays in CHOL and HD-injected cells, embryos were similarly lysed and fixed, blocked with the appropriate unconjugated secondary antibody (1:10; Fisher Scientific, Santa Clara, CA), probed with monoclonal anti–β tubulin (M. Klymkowsky, Boulder, CO) and finally stained with rhodamine-conjugated anti–mouse IgG (1:20).

Microscopy, Recording, and Photography

As previously described (Terasaki and Jaffe, 1991), Dil-injected embryos were observed on a Zeiss Axiosplan microscope using a Planoapo 63× (NA 1.4) objective and imaged using a laser scanning confocal microscope (model 600; Bio-Rad Laboratories Inc., Richmond, CA) and recorded on an optical memory disk recorder (model 3031F; Panasonic). All other embryos were observed on a Zeiss IM-35 inverted microscope using a Plan 40× objective and imaged using a Variun video camera (model NC-67M; Dage-MTI, Inc., Michigan City, IN) on a timelapse video recorder (model AG-6050; Panasonic, Secaucus, NJ). Embryos were either photographed directly or their recorded images photographed from a high resolution monitor (model PVM-122; Sony) with a Plus-X Pan film (Eastman Kodak Co., Rochester, NY).

Results

Antibody Characterization by Immunoblotting

We here report the results of experiments that were performed using three antibodies to members of the kinesin superfAMILY. SUK4 is a monoclonal IgG that binds an epitope lying between amino acids 312 and 382 on the SU KHC (Wright et al., 1991), blocks SU egg kinesin-driven motility “in vitro” (Ingold et al., 1988), and interferes with radial
membrane dispersion in rabbit macrophages (Hollenbeck and Swanson, 1990). CHO1 is a mouse monoclonal IgM that reacts with a KLP that drives MT-MT sliding and is essential for mitosis in mammalian cells (Sellitto and Kuriyama, 1988; Nislow et al., 1990, 1992). Finally, HD is a rabbit polyclonal IgG fraction raised against bacterially expressed Drosophila KHC motor domain (Rodionov et al., 1991) that reacts with KHC plus a number of putative KLPs and blocks mitosis in mammalian cells (Rodionov, V. I., V. I. Gelfand, and G.G. Borisy, personal communication). For some experiments, HD was depleted of KHC antibodies by immunoprecipitation with sea urchin egg kinesin (HD-KIN).

On immunoblots (Fig. 1), SUK4 reacted only with the 130-kD KHC (B, lane 3) that was detected in egg cytosol (A, lane 5) in MTs precipitated from AMPPNP-treated cytosol (A, lane 6), in mitotic spindles (A, lane 7) but not in microsomes (A, lane 8). CHO1 reacted primarily with a 110-kD presumptive KLP (B, lane 6) that was greatly enriched in egg AMPPNP-MTs (A, lane 10) but could not be detected above background staining in cytosol or microsomes (A, lanes 9 and 12). A CHO1 signal was consistently detected in isolated spindles, but it was weak relative to AMPPNP-MTs (A, lane 11). HD recognized KHC (B, lane 7, and A, lane 14), but we saw no strong reaction with any of the KLPs that coprecipitated with MTs from AMPPNP-treated egg cytosol (A, lane 14; see Cole et al., 1992). The reaction of HD with KHC in cytosol and isolated spindles was weak (A, lanes 13 and 15), but prolonged exposure of the blots did reveal intense staining of KHC and weak staining of two additional polypeptides that are candidate KLPs in the spindle (A, lane 17). In addition, under conditions of prolonged exposure, a 160-kD microsomal polypeptide was stained intensely and a 130-kD polypeptide stained weakly (A, lane 18). Under identical conditions, HD that had been preadsorbed against SU egg kinesin (HD-KIN) displayed negligible reactivity with KHC and other polypeptides recognized by the unadsorbed HD (B, lane 9, and A, lanes 19-24); immunoblot titer indicated that over 97% of anti-SU KHC antibodies had been removed from HD-KIN as compared to HD (not shown).

Effects of Microinjection of SUK4, HD and CHO1 on Mitosis

SU zygotes were injected with SUK4, CHO1, or HD shortly after fertilization; \( \sim 10\% \) of the cell volume was injected with up to \( \sim 20 \) mg/ml of antibody (in the needle concentration), and the embryos were monitored for a period corresponding to at least the first three cell cycles in uninjected controls (Figs. 2 and 3; Table I). Injection of CHO1 at concentrations up to \( \sim 20 \) mg/ml during first interphase arrested cells in preprophase just before nuclear envelope breakdown, with the blocked cells remaining viable for up to 24 h (Fig. 2, A-C; Table I). These cells contained diastral MT arrays associated with the progressively enlarging nucleus that were obvious during through-focus observation of live cells using DIC microscopy. These MT arrays were difficult to reproduce photographically (Fig. 2 C) but were clearly stained with antitubulin for immuno-
Figure 2. Effects of antibody microinjection on mitosis in fertilized *Lytechinus pictus* eggs. Embryos are shown 2 h (A, D, and G) 4 h (B, E, and H) and 8 h (C, F, and I) after fertilization. In these cells, CHO1 (A–C) and HD (D–F) altered spindle morphogenesis and inhibited normal cell division, whereas SUK4 had no effect on cell division (G–I) throughout the cleavage stage of development. Bar, 25 μm.

Fluorescence in Figure 3 B. In agreement with the results of Nislow et al. (1990, 1992), in SU embryos injected after nuclear envelope breakdown during prophase or prometaphase, we observed metaphase arrest (Fig. 3 C). However, cells injected after anaphase onset completed the first cell division and arrested at second preprophase (not shown). Cells injected with decreasing antibody concentrations survived longer, but some of them slowly completed first division and arrested in the second cell cycle (not shown). When one blastomere of a two-cell embryo was injected with CHO1, the injected blastomere arrested in preprophase, whereas the uninjected control blastomere divided normally to form a diminutive blastula (not shown). Parallel injections of negative control IgM (TEPC 183) had no effect on cell division (Table I).

Microinjection of up to 22 mg/ml HD antibody blocked mitotic spindle assembly or caused spindle collapse, producing abnormal monastral MT arrays and inhibiting cell division (Figs. 2, D–F, 3 A, and Table I). After several hours, undivided HD-injected cells underwent waves of cortical contractions (Fig. 2 F) resulting in the initiation of multiple misplaced furrows and a failure to complete cytokinesis, coinciding with cycles of nuclear envelope breakdown and reassembly. Multiple DNA-containing structures, probably abnormal nuclei, were often observed (Figs. 2 F and 3 A, inset). The cortical contractions often caused blebbing of ab-
normal cytoplasmic lobes and eventually resulted in rupture of the plasma membrane and cell lysis. Decreasing antibody concentration or delaying injection until mitosis onset often resulted in abnormal first divisions followed by inhibition of the second division. Furthermore, injection into one blastomere of a two-cell embryo disrupted division of the injected cell but allowed formation of a small blastula from the control cell (not shown). Parallel injections of nonspecific control IgG had no significant effect on cell division (Table I). Generally, our results are similar to those reported for the microinjection of affinity-purified HD into cultured mammalian cells (Rodionov, V. I., V. I. Gelfand, and G. G. Borisy, personal communication).

The inhibition of mitosis and cell division by microinjected CHO1 and HD not only indicated that some members of the kinesin superfamily have essential roles in early SU embryonic mitoses but also served as positive controls for SUK4 injections. As SUK4 blocks kinesin-driven MT motility in vitro and membrane dispersion in vertebrate cells, it is likely to block kinesin function in SU eggs, yet the microinjection of up to 20 mg/ml SUK4 into first interphase embryos had no effect on mitosis and cell division (Fig. 2, G–I; Table I); SUK4-injected eggs divided normally and synchronously with uninjected and control antibody-injected cells (not shown), eventually forming normal blastulae (Fig. 2). Single-blastomere injections of two-cell embryos produced normal blastulae (not shown). Microinjection of negative control monoclonal IgG (MOPC 21) also had no effect on cell division and early development (Table I).

As observed with SUK4, all other KHC-specific antibodies tested, including affinity-purified polyclonal antibodies and mAbs (both IgGs and IgMs) had no effect on mitosis when microinjected into fertilized SU embryos (Table I). When mixtures of multiple monoclonal KHC antibodies were microinjected to immunoprecipitate kinesin in fertilized egg cytoplasm, we again observed normal cell divisions (Table I).

![Figure 3](image-url)  
**Figure 3.** Morphology of spindles in HD (A) and CHO1- (B and C) injected cells. First cell cycle *L. variegatus* embryos were injected before first mitosis (A and B) or during prophase (C) and then, at a time when uninjected control embryos had reached the 16–32 cell stage, were detergent lysed, fixed, and stained for tubulin. HD-injected embryos that had not divided, contained abnormal bundles of MTs (A) and several abnormal nuclei (revealed by Hoechst staining; A, inset). Cells injected with CHO1 before onset of mitosis did not divide and displayed diastral MT bundles (B). Injection of CHO1 after nuclear envelope breakdown but before metaphase resulted in a first metaphase block and the persistence of a single mitotic spindle (C). Bars, 20 μm.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Maximum amount injected*</th>
<th>% Dividing normally (n)</th>
<th>% Normal negative controls (n)</th>
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<tr>
<td></td>
<td>ng/egg mg/ml**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monoclonal anti-SUK</td>
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<td></td>
<td></td>
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<tr>
<td>Mixed Abs (both kinds)</td>
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<td>100.0 (27)</td>
<td></td>
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<tr>
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<td>Anti-MKLP-1</td>
<td>1.02 20</td>
<td>0.0 (107)</td>
</tr>
</tbody>
</table>

* L. variegatus and L. pictus embryos were injected with 1–10% of their cell volume of a 1–22 mg/ml antibody solution shortly after fertilization. Negative control antibodies (right-hand column) were identical concentrations and volumes of: MOPC 21 for monoclonal antikinesin, nonspecific rabbit IgG for polyclonal antibodies, and TEPC 183 for CHO1 IgM.

** "In the needle" concentration.

SUK, sea urchin kinesin; MKLP, mitotic kinesin-like protein; HD, *Drosophila* kinesin head; KIN, kinesin; CON, nonspecific control protein.
Injection of Immunodepleted HD Antibody

To determine whether the mitotic block induced by microinjection of HD antibody was due to inhibition of KHC function or cross-reaction of HD with some other protein that is essential for mitosis, we compared the effect of injecting SU zygotes with 20 mg/ml of either HD-KIN (HD depleted of KHC antibodies; see Fig. 1) or HD-CON (HD adsorbed with irrelevant protein and not depleted of KHC antibodies). The effect of these antibodies was indistinguishable from the effects of untreated HD, causing a complete mitotic block (Fig. 4; Table I; compare to Fig. 2F), suggesting that HD-induced blockage of cell division appears to be independent of the ability of HD to recognize KHC.

Effects of SUK4 and HD on Kinesin-driven Motility in Vitro

We assessed the ability of the aforementioned antibodies, at molar ratios of antibody:kinesin similar to those used in the microinjection experiments, to block MT sliding over glass surfaces driven by SU egg kinesin (Ingold et al., 1988). In the presence of SUK4 or HD, the kinesin-driven MT gliding velocity was reduced to less than 3 and 54%, respectively, of the rates observed in the presence of buffer alone (100%) or control antibody (100%). In contrast, HD-KIN (depleted of KHC antibodies) did not significantly effect kinesin-driven motility in this assay (rate = 97% of control). CHOI, which does not recognize KHC on immunoblots (Fig. 1), also had no effect on kinesin-driven MT gliding.

Localization of Microinjected SUK4

To investigate whether the microinjected SUK4 was able to access KHC in the mitotic spindle, embryos injected with SUK4 (5-10% of 20 mg/ml antibody) were detergent-lysed, fixed, and the injected antibody was localized by PAP-staining (n = 51). We observed a clear association of SUK4 with mitotic spindles (Fig. 5A) where the antibody was presumably bound to ER membrane-associated KHC that is concentrated in these MAs (Henson et al., 1989; Wright et al., 1991; Terasaki and Jaffe, 1991). Uninjected control cells did not stain under these conditions (Fig. 5B).

Discussion

Microinjection of SU zygotes with the function-blocking mAb, SUK4, as well as several other KHC-specific antibodies, has no detectable effect on mitosis, ER distribution, or cell division. In contrast, CHOI, which reacts with a KLP essential for mitosis in mammalian cells (Nislow et al., 1992), potently inhibits progression through mitosis in early SU embryonic cells. Similarly, HD that reacts with the KHC motor domain as well as some presumptive KLPs (V. I. Gel-fand, personal communication) blocks normal mitotic spindle assembly and cell division in SU zygotes, whether or not it has been depleted of KHC antibodies (to the extent that it no longer interferes with kinesin activity in a motility assay). Thus, our results support the hypothesis that targets of CHOI and HD, but not KHC, have essential mitotic functions in SU as well as mammalian cells.

It is not unreasonable to propose that the SU egg 110-kD polypeptide recognized by CHOI is a plus-end-directed KLP that slides apart antiparallel MTs, like its mammalian counterpart (Nislow et al., 1992). Our CHOI microinjection results suggest that inhibiting the activity of this 110-kD protein before mitosis onset leads to the preprophase arrest of SU cells containing abnormal diastral MT arrays, suggesting that the 110-kD protein may normally cross-link antiparallel MTs and slide them apart during early spindle morphogenesis. However, CHOI-mediated inhibition of such MT-MT sliding apparently does not block spindle pole separation at this stage, which may be caused by astral “pulling” forces like those proposed to drive anaphase B (Hiramoto and...
Nakano, 1988). Injection of zygotes with CHO1 during prophase or prometaphase causes metaphase arrest of cells containing bipolar spindles, perhaps because these cells cannot undergo 110 kD-driven anaphase B (Nislow et al., 1992). Similarly, CHO1-injected mammalian cells were reported to arrest at metaphase but the arrest point was independent of the timing of CHO1 injection, and preprophase arrest was never observed (Nislow et al., 1990, 1992). Perhaps mammalian cells differ from SU eggs in containing a motor that can functionally replace the CHO1 antigen during early spindle assembly.

HD also appears to inhibit the activity of an antigen that is essential for normal spindle morphogenesis; the injection of HD before mitosis onset disrupts spindle assembly, whereas the injection of HD into cells that have already assembled their spindles induces spindle collapse and the formation of abnormal monastral MT arrays. The target of HD may therefore be a KLP that drives pole separation during spindle assembly, although we cannot rule out a protein unrelated to the kinesin superfamily. It is striking that kinesin-preadsorbed HD-KIN retains mitosis-disrupting activity but does not react strongly with any SU egg polypeptide on immunoblots, suggesting that the blotted filters contain undetectably low amounts of the antigen of interest, leaving us with no obvious candidate polypeptide to propose. However, the identification of this potentially interesting molecule is an important area for future investigation.

CHO1- and HD-injected SU eggs disrupted in mitosis also fail to divide, but the mechanism of inhibition of cytokinesis appears to differ in the two situations. CHO1-arrested cells display no evidence of cell cycle progression; perhaps these cells cannot transit checkpoints required for progression into cytokinesis. If such checkpoints associated with spindle assembly exist, they do not block cell cycle progression of HD-injected cells, which proceed through coordinated cycles of nuclear assembly disassembly and contraction relaxation throughout the entire cortex (rather than only at the equatorial region). The failure to form a normal equatorial contractile ring could result from HD directly inhibiting the transport of the signal that localizes the cleavage furrow.

Figure 5. Intracellular localization of SUK4 IgG after its microinjection into fertilized SU eggs. *L. pictus* embryos, previously injected with SUK4 (A) or uninjected controls (B), were allowed to develop to first metaphase, detergent lysed, fixed, and then probed with anti-mouse 2° and 3° PAP antibodies and developed. Following antibody injection, both specimens were processed identically. Bar, 25 μm.

Figure 6. Effects of SUK4 antibody microinjection on ER organization. Nonspecific control (A) or SUK4 (B) IgG was coinjected with DiI dissolved in Wesson oil into recently fertilized *L. pictus* eggs. Embryos were then allowed to develop for a time period corresponding to the attainment of second telophase/cytokinesis in control embryos and imaged by laser scanning confocal microscopy to reveal organization of ER membranes. Bright spot at left of A is a remnant of the DiI-containing oil drop. Bar, 10 μm.
branes that accumulate in MAs of early SU embryos are has no role in early SU embryos, being stockpiled for use published results). For example, one of these clones is kinesin peptide antibodies and cDNA cloning in this system drawn from a study of KHC mutants in Drosophila (Saxton et al., 1991). In contrast, a C. elegans KHC mutant demonstrated a defect in spindle positioning during the first two divisions, resulting in misplaced cleavage furrow planes (J. Plenefisch and E. Hedgecock, personal communication). Our results do not rule out the possibility that KHC performs a nonessential function in mitosis, cell division, or ER membrane transport in early SU embryos, with the function of KHC being redundant to that of a KLP. In this context, we note that several KLPs have been identified using pan-kinesin peptide antibodies and cDNA cloning in this system (Cole et al., 1992; Wedaman, K. P., and J. M. Scholey, unpublished results). For example, one of these clones is predicted to encode the SU homologue of the putative plus-end-directed membrane motor, unc-104 (Hall and Hedgecock, 1991). It seems plausible, therefore, that KHC and unc-104 might perform redundant functions in early SU embryos, controlling the distribution and organization of ER membranes by moving them toward the plus ends of MT tracks, although at present such ideas are speculative. Another plausible explanation of our results is that kinesin has no role in early SU embryos, being stockpiled for use later on in development. For example, we hypothesize that kinesin-driven membrane transport is not directly involved in mitosis, but KHC is bound to membranes that are stored in early mitotic spindles and is responsible for the outward dispersion of these membranes along MT tracks into the cytoplasm and toward the cell surface during later embryonic development. Such a model is consistent with immunofluorescence microscopy showing that the ER membranes that accumulate in MAs of early SU embryos are dispersed into the cytoplasm of cells of the blastula-stage embryo (Wright et al., 1991). To test such a model will require the development of methods for reliably inactivating KHC at later stages of embryogenesis; however, our preliminary studies suggest that this would be technically very difficult to accomplish using our current antibody microinjection techniques. Although immunolocalization experiments are consistent with the idea that KHC performs a role in transporting membranes along MT tracks in SU cells (Wright et al., 1991; Henson et al., 1992), the precise function of kinesin-driven membrane transport in this system remains unknown. The work described here lends support to the hypothesis that some members of the kinesin superfamily, such as the 110-kD polypeptide recognized by CHO1, have essential mitotic functions in cells of early SU embryos, but the activity of KHC is not essential for mitosis, cell division, or ER membrane distribution in these cells.

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Note Added in Proof: Our attention was recently drawn to the work of Sluder, G., F. J. Miller, and K. Spanjian (1986. J. Exp. Zool. 238:325-336) on the role of spindle MT polymerization in regulating sea urchin embryonic cell division, which suggests that the effects of colchicine described by Yoneda and Schroeder (1984) were probably not due solely to MT depolymerization.

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