Immunolocalization of the Heterotrimeric Kinesin-Related Protein KRP\(_{85/95}\) in the Mitotic Apparatus of Sea Urchin Embryos

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We have used monoclonal antibodies to perform confocal light microscopic immunolocalization of KRP\(_{85/95}\), a heterotrimeric plus-end-directed microtubule motor protein, in dividing cells of sea urchin embryos. Embryos were stained during the first division cycle, and dissociated blastomeres were stained at the 32- to 64-cell stages. Double labeling of the dividing cells with anti-tubulin and anti-KRP\(_{85/95}\) showed a clear concentration of the motor protein in the mitotic apparatus; KRP\(_{85/95}\) appeared to associate with pericentriolar regions during prophase, with kinetochoore-to-pole microtubules during metaphase, and, in a striking fashion, with the spindle interzone during anaphase. KRP\(_{85/95}\) began to accumulate in the interzone immediately following chromosome separation and the area of concentration expanded with the lengthening of the interzonal region during anaphase. During telophase KRP\(_{85/95}\) appeared to disperse with the establishment of the cleavage furrow and did not concentrate in the midbody. KRP\(_{85/95}\) staining in the mitotic apparatus was punctate and detergent-sensitive, suggesting an association with membranous vesicles, but unlike kinesin, KRP\(_{85/95}\) did not appear to codistribute with calcequestrin-containing endoplasmic reticulum. Finally, KRP\(_{85/95}\) appears to be present in dividing blastomeres up to at least the blastula stage, but, unlike kinesin, it is not expressed in terminally differentiated, nonmitotic coelomocytes of the adult animal. These results suggest that the expression and targeting of KRP\(_{85/95}\) and kinesin differ and that KRP\(_{85/95}\) may play a role in vesicle transport during embryonic cell division. © 1995 Academic Press, Inc.

INTRODUCTION

A large body of evidence supports the idea that kinesin and members of the kinesin superfamily [kinesin-like proteins or KLPs] participate in intracellular membrane transport and cell division. Genetic and microinjection experiments support the hypothesis that kinesin itself drives organelle transport and does not play an essential role in mitosis [Saxton et al., 1991; Gho et al., 1992; Wright et al., 1991, 1993; Steinhardt et al., 1994]. Other candidate vesicle motors include the KLPs encoded by the Caenorhabditis elegans unc104 gene, the mouse KNF and KNF4 genes, and a neuron-specific form of kinesin [Otsuka et al., 1991; Azawa et al., 1992; Kondo et al., 1994; Niclas et al., 1994; Sekine et al., 1994]. Several KLPs appear to play essential roles in cell division, particularly with regard to the processes of spindle morphogenesis and dynamics [recently re-viewed by McIntosh, 1994; Pereira and Goldstein, 1993; Saunders, 1993; Sawin and Endow, 1993; Skoufias and Scholey, 1993]. For example, antibodies that react with multiple KLPs have implicated KLPs generally in spindle function [Sawin et al., 1992b; Hogan et al., 1993; Rodionov et al., 1993]. More specifically, members of the bimC subfamily of KLPs have been implicated in spindle pole separation and bipolar spindle assembly in a wide phylogenetic spectrum of organisms [Enos and Morris, 1990; Hoyt et al., 1992; Saunders and Hoyt, 1992; Roof et al., 1992; Hagan and Nagahida, 1990, 1992; Sawin et al., 1992a; O'Connell et al., 1993; Heck et al., 1993; Cole et al., 1994]. Two KLPs that appear essential for mitosis, namely CENP-E (Yen et al., 1992) and MKLP-1 [Nislow et al., 1992; Wright et al., 1993], have been localized to the interzone of anaphase spindles [Nislow et al., 1992] where they may mediate the sliding apart of antiparallel microtubules involved in the establish-
ment of the bipolar spindle and in spindle elongation during anaphase B. Other KLPs that appear to play important roles in chromosome segregation include KAR3, Ndc, and Nod (Meluh and Rose, 1990; Endow et al., 1990; Chandra et al., 1993; McDonald et al., 1990; Zhang et al., 1990; Theurkauf and Hawley, 1992).

The sea urchin egg and embryo system has proven to be a useful one for studying kinesin and KLPs in mitotic cells. Immunocytochemical localization suggests that kinesin itself is associated with microtubules and endoplasmic reticulum [ER]/vesicle membranes in the mitotic apparatus (MAs) of early seaurchin embryos. However, by the blastula stage, the MA association disappears in concert with the loss of membrane accumulation in the MA (Scholey et al., 1985; Wright et al., 1991). In terminally differentiated coelomocytes from adult animals kinesin appears associated with ER and endosomal membranes (Henson et al., 1992). The microinjection of SUK4, a function-blocking monoclonal antibody (Ingold et al., 1988), into early embryonic cells had no effect on mitosis (Wright et al., 1993) but it did appear to interfere with the delivery of vesicles to the cell surface during membrane resealing (Steinhardt et al., 1994). These results are consistent with the idea that kinesin is bound to vesicles and ER membranes (see also Skoufias et al., 1994) that are concentrated in the MA asters and transports them out along microtubule tracks towards the cell surface.

While antibody microinjection suggests that kinesin activity is not essential for mitosis in cells of early sea urchin embryos, some KLPs do appear to have important roles in mitosis in this system. The microinjection of antibodies against certain KLPs isolated from other species has been observed to disrupt spindle organization during mitosis in early sea urchin embryos (Wright et al., 1993). The best-characterized sea urchin egg KLP is KRP85/95, a plus-end-directed, heterotrimeric, kinesin-related microtubule motor protein recently purified from sea urchin eggs (Cole et al., 1992, 1993). KRP85/95 was initially identified as an AMP-PNP-enhanced, ATP-sensitive microtubule binding protein that reacts with pan kinesin peptide raised against a hyperconserved region of the kinesin superfamily motor domain (Cole et al., 1992). In addition to driving microtubule motility in vitro, KRP85/95 displays microtubule bundling activity (Cole et al., 1992, 1993). The KRP85/95 complex consists of 85- and 95-K kinesin-related polypeptides, plus an as yet uncharacterized 115-K subunit. Gene sequence comparisons indicate that close relatives of the 85- or 95-K subunits may be encoded by the mouse KIF3a gene (Aizawa et al., 1992; Kondo et al., 1994), the Drosophila KLP4 and KLP5 genes (Stewart et al., 1991), and the Chiromydomonas FLA10 gene (Walther et al., 1994). Sea urchin KRP85/95 is distinctive because of its multimeric nature and the fact that it is the first KLP to be isolated in a native form from its natural host cell.

As a first step towards the elucidation of the biological function of KRP85/95, we have performed immunocytochemical localization studies of KRP85/95 in dividing cells of sea urchin embryos. Using KRP85/95-specific monoclonal antibodies and confocal microscopy we demonstrate that this motor protein localizes to the mitotic apparatus of dividing cells, especially within the interzone region during anaphase. The staining is punctate and detergent-sensitive, suggesting that, like kinesin, KRP85/95 is associated with spindle vesicles. Interestingly, however, KRP85/95 and kinesin appear to associate with different regions of sea urchin embryonic spindles. Moreover, the spindle association of KRP85/95, but not kinesin, persists in blastula-stage embryos, and unlike kinesin, KRP85/95 levels decline with early embryogenesis and the complex cannot be detected in immunoblots of nonmitotic, terminally differentiated sea urchin coelomocytes. These results suggest that the expression and targeting of KRP85/95 and kinesin differ and that KRP85/95 may function as a vesicle motor potentially involved in the process of embryonic cell division.

**MATERIALS AND METHODS**

**Animals and Materials**

Sea urchins of the species Strongylocentrotus purpuratus were obtained from Martinus, Inc. (Long Beach, CA). The preparation and characterization of the mouse monoclonal antibodies to the 85- and 95-K subunits of the KRP85/95 complex and a mouse polyclonal anti-KRP85/95 antiserum were briefly described previously (Cole et al., 1993) and are described in detail below. An anti-sea urchin calasequestrin-like protein (SCS) antiserum was a generous gift of Drs. Benjamin Kaminer (Boston University Medical School) and David Begg (University of Alberta). Polyclonal anti-sea urchin tubulin antibody was purchased from Polysciences, Inc. (Fl. Washington, PA). The majority of other chemicals, antibodies, and reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

**Monoclonal Antibody Production**

KRP85/95 was purified from unfertilized sea urchin cytosol as described previously (Cole et al., 1992, 1993). Following the final sucrose density centrifugation step, from 200 ml of cytosol we typically obtained on the order of 50 µg of pure protein. Five bally/c female mice were each injected intraperitoneally [ip] with 20 µg of purified KRP85/95 mixed with 1 mg/ml mouse serum [Sigma] emulsified in RIBI adjuvant on Day 1. They were boosted on Days 26 and 53 with the same amount of KRP85/95 treated identically. Tail bleeds were tested by immunoblotting for anti-KRP85/95 reactivity on Day 102, and the mouse with the best titer was given a final ip booster injection of 20 µg KRP85/95 without adjuvant on Day 106 and 3 days later was sacrificed by cervical dislocation. B-cell hybridomas were obtained by routine procedures involving polyethylene glycol-induced fusion to mouse P3 myeloma cells. Cells were plated out at 1 × 10⁶ cells per well on a single 96-well plate in HAT media. Ten
days later culture supernatant was collected and screened for the presence of KRP_{85/95} reactivity. Seven of the "positive" wells still contained immunoreactive supernatants so cells from these wells were cloned by limiting dilution. After the first cloning, four cell lines yielded cloned hybrids that continued to secrete anti-KRP_{85/95}, and they were recloned to produce the stable lines K2.1, K2.2, K2.3, and K2.4 [Cole et al., 1993]. Ascitic fluid was prepared using each of these cell lines.

**Antibody Isotyping, Purification, and Characterization**

Antibody secreted into culture medium by each of the four cell lines was isotypic using Sigma Immunotype mouse monoclonal antibody isotyping kit following the manufacturer's instructions. All four IgGs were purified using protein A-agarose affinity chromatography with the MAPs-II monoclonal antibody purification system [Bio-Rad Laboratories, Inc., Richmond, CA] as described previously [Ingold et al., 1988]. IgG concentrations were estimated using absorbance at 280 nm, assuming a molar extinction coefficient of 1.4 for IgG.

**Handling of Sea Urchin Gametes**

Gametes were obtained by intracoelomic injection of 0.5 M KCl, with eggs collected and dejellied by washing in artificial sea water and sperm collected dry. Fertilization was performed in artificial sea water and the fertilization envelopes were stripped by brief treatment with 1 M urea (pH 8) followed by passage through an 80-μm Nitex mesh. The embryos were then washed into calcium-free sea water (formulated according to Burgess and Schroeder, 1977) for development at 12°C. For blastula-stage staining, the embryos were raised in artificial sea water and were not urea-stripped since they were used following hatching of the blastula from the fertilization envelope. Prior to fixation some embryos were extracted for 15 min in a microtubule stabilizing detergent lysis buffer (formulated according to Wright et al., 1991) consisting of 25 mM Pipes (pH 6.9), 1 mM MgSO4, 6 mM EGTA, 20% glycerol, 1% Nonidet P40 plus protease inhibitors (1 mM DTT, 1 mg/ml TAME, 100 μM PMSF, 100 μg/ml SBTI, 2 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin).

**Immunoblotting**

Gel samples of high speed supernatants of sea urchin eggs and coelomocytes and whole-cell homogenates of embryos [Wright et al., 1991] were run out on SDS-polyacrylamide gels [Laemmli, 1970] and then electrophoretically transferred to nitrocellulose filters [Towbin et al., 1979]. The filters were blocked with BLOTTO [Johnson et al., 1984] and incubated in the appropriate primary antibodies, followed by incubation in the appropriate alkaline phosphatase-conjugated secondary antibodies. Visualization of the alkaline phosphatase reaction product followed the method of Byers et al. [1987]. The concentrations of the primary antibodies were: anti-KRP_{85/95} and anti-KHC monoclonal mixtures are 5–10 μg/ml each; anti-KRP_{85/95} polyclonal antiserum is 1:250; and anti-β tubulin monoclonal is 1:10,000.

**Immunostaining**

Embryos at first division, between 8 and 9 hr after fertilization, or 24 hr after fertilization were allowed to settle onto poly-L-lysine-coated coverslips and then fixed by immersion in –20°C methanol plus 50 mM EGTA for at least 1 hr. After blocking with PBS plus 1% BSA and 2% goat serum the cells were stained with primary antibody. Labeling with anti-KRP_{85/95} involved a mixture of four monoclonals—K2.1 (against the 95-K subunit) and K2.2, K2.3 and K2.4 (against the 85-K subunit)—at a final total concentration of 20–200 μg/ml. Staining for sea urchin kinesin heavy chain (KHc) was performed using a mixture of domain-specific monoclonal antibodies, SUK 2, 4, and 5, at a total concentration of 50–200 μg/ml [Ingold et al., 1988; Wright et al., 1991]. The anti-calceinserotonin and anti-tubulin antisera were diluted 1:400. Following at least a 1-hr incubation in primary antibody the embryos were rinsed in PBS and then stained for an hour with the appropriate fluorophore-conjugated secondary antibody. Staining of chromosomes was accomplished by including 1 μg/ml of propidium iodide in the secondary antibody step. Coverslips containing embryos were mounted in an antiphoto bleach consisting of p-phenylene diamine in glycerol (pH 9).

Fluorescent specimens were viewed on a Zeiss Axioplan microscope using a planapo 63X [NA 1.4] objective and imaged using a laser scanning confocal microscope (Model 600; Bio-Rad Laboratories, Inc.). Frame-averaged and contrast-enhanced digitized images were displayed on a high resolution monitor for photorecording on 35-mm TMAX 100 or Ektachrome 100 film [Eastman Kodak Co., Rochester, NY].

**RESULTS**

**Characterization of Monoclonal Antibodies**

The four monoclonal IgGs (K2.1-K2.4), purified from ascitic fluid by protein-A-agarose affinity chromatography, react specifically with subunits of KRP_{85/95} and were used in the immunolocalization studies described here. These monoclonal antibodies were described briefly in a previous manuscript where we documented that the four IgGs reacted specifically with the 85- and 95-K subunits of the trimeric KRP_{85/95} complex in sea urchin egg cytosol. In MAPs eluted from AMP-FPN-MTs using ATP, and in preparations of highly purified KRP_{85/95}, but not with kinesin and other KLPs in these fractions [Cole et al., 1993]. We note here that the isotypes of the mAbs are: K2.1, IgG1; K2.2, IgG3; K2.3, IgG2a; and K2.4, IgG1. Based on immunoblotting experiments using 85- and 95-K motor domain.
KRP$_{85/95}$ Levels Decrease during Embryogenesis

Developmental immunoblots of embryos through late blastula indicate that while the levels of conventional KHC remain relatively constant, the levels of KRP$_{85/95}$ decrease [Figs. 1A and 1B]. In addition, KRP$_{85/95}$ subunits were not detected in immunoblots of nonmitotic and terminally differentiated sea urchin coelomocytes homogenates probed either with the KRP$_{85/95}$ monoclonal antibody mixture (Fig. 1C) or with a high concentration of a high titer anti-KRP$_{85/95}$ monoclonal antiserum which reacts with all three subunits of KRP$_{85/95}$ [Fig. 1D]. KRP$_{85/95}$ was also not detected in taxol-stabilized AMP-PNP microtubule preparations from coelomocytes prepared according to Cole et al., 1992 [data not shown]. KHC is expressed in coelomocytes [Fig. 1D; see also Wright et al., 1991, Henson et al., 1992], consistent with the notion that KHC is expressed ubiquitously, but KRP$_{85/95}$ expression may be restricted to early embryonic cells.

KRP$_{85/95}$ Localizes to the Mitotic Apparatus of Dividing Cells

Double labeling of KRP$_{85/95}$ and tubulin [Figs. 2 and 3] and of KRP$_{85/95}$ and chromosomes [Figs. 3a and 3b] in first division [Figs. 3a, 3b, and 3c] or 32- to 64-cell stage [Figs. 2 and 3d] embryos showed a definite concentration of KRP$_{85/95}$ in the MA. In prophase the punctate KRP$_{85/95}$ staining concentrated in the two pericentriolar regions adjacent to the swollen nuclear envelope which organize microtubules for the formation of the MA [Figs. 2a and 2b]. Note that there is some intranuclear KRP$_{85/95}$ staining at this stage, suggesting that the nuclear envelope has begun to lose its integrity [Fig. 2b]. By metaphase the KRP$_{85/95}$ labeling had concentrated in the half spindles defined by the kinetochores-to-pole microtubules, and often there appeared to be more KRP$_{85/95}$ labeling near the kinetochores and less labeling toward the poles [Figs. 2c, 2d, and 2e]. The most striking accumulation of KRP$_{85/95}$ labeling in the MA was apparent during anaphase A, the region of overlapping, anti-parallel microtubules located between the two groups of migrating chromosomes [Figs. 2c, 2d, 2e, 2f, 3c, and 3d]. The punctate KRP$_{85/95}$ staining was seen to accumulate in the interzone immediately following chromosome separation in anaphase A, and the area of concentration expanded with the lengthening of the interzonal region during anaphase B. KRP$_{85/95}$ staining of the interzone is particularly clear in embryos in which pseudocolored and contrast-enhanced digital images of the KRP$_{85/95}$ and tu-

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bulin patterns are superimposed (Figs. 3c and 3d). In some images, the anti-KRP<sub>85/95</sub>-stained elements appeared in linear arrays suggestive of binding to the interzone microtubules (Fig. 3d). During telophase the KRP<sub>85/95</sub> accumulation appeared to disperse following the establishment of the cleavage furrow, and there remained no concentration associated with the microtubules which compose the midbody (Figs. 2c and 2f). Interphase cells present in the blastula stage showed a punctate cytoplasmic KRP<sub>85/95</sub> staining pattern which did not appear to codistribute with microtubules (data not shown).

**Prefixion Detergent Extraction Eliminates KRP<sub>85/95</sub> Interzone Staining**

In an attempt to determine if the association between KRP<sub>85/95</sub> and the MA was dependent on detergent-sensitive structures, embryos undergoing mitosis were detergent-extracted under microtubule stabilizing conditions and then fixed and processed for KRP<sub>85/95</sub> and tubulin staining. Extracted first cleavage and 32- to 64-cell stage blastomeres showed a clear loss of KRP<sub>85/95</sub> label in the MA during all stages of mitosis (Fig. 4). KRP<sub>85/95</sub> did not appear to codistribute with microtubules as is the case for KHC in embryos extracted under identical conditions (Fig. 4, see also Wright et al., 1991).

**Interzonal KRP<sub>85/95</sub> Is Not Associated with the ER**

The detergent sensitivity of the KRP<sub>85/95</sub> staining pattern suggested an association with intracellular membrane-bound structures, therefore we double-labeled embryos for KRP<sub>85/95</sub> and SCS found in the ER lumen (Henson et al., 1989). We have previously demonstrated that KHC codistributes with SCS-containing ER membranes in sea urchin embryos and coelomocytes (Wright et al., 1991; Henson et al., 1992). The KRP<sub>85/95</sub>-labeled structures in the interzone appear not to be ER since these structures were not labeled by the anti-SCS antiserum, suggesting that KRP<sub>85/95</sub> binds to a different type of membrane in the interzone (Figs. 5a–5d). Both first cleavage and 32- to 64-cell stage embryos show a relatively small amount of KRP<sub>85/95</sub> and abundant ER within the vicinity of the MA asters (Figs. 3 and 5f see also Wright et al., 1991). Double labeling of KHC and SCS in 32- to 64-cell embryo shows a lack of accumulation of KHC in the interzone region (Figs. 5e and 5f). In general there appears to be a lack of ER accumulation in the interzone region of the anaphase MA as seen by SCS staining (Henson et al., 1989; Wright et al., 1991) and by labeling of the ER through microinjection of Dil [Terasaki and Jaffe, 1991; Wright et al., 1993].

**Immunolocalization of KRP<sub>85/95</sub> and KHC in Blastula Stage Embryos**

We have previously demonstrated that the association between KHC and MA does not persist through the blastula stage of development, so that KHC no longer accumulates in the MA of blastula stage blastomeres and that this loss of KHC staining correlates with a loss of ER accumulation in these same MAs [Wright et al., 1991]. When hatched blastula stage embryonic cells were stained for KRP<sub>85/95</sub>, KHC, and microtubules, KRP<sub>85/95</sub> appeared to associate with the MAs of these cells while KHC did not (Fig. 6). The KRP<sub>85/95</sub> labeling was punctate and codistributed with MA microtubules.

**DISCUSSION**

We have shown here that the heterotrimeric kinesin-related motor complex, KRP<sub>85/95</sub>, is localized in the mitotic spindle of early sea urchin embryonic cells. The motor is concentrated in pericentriolar regions during prophase, in the metaphase half spindles, and in the spindle interzone during anaphase. During telophase, KRP<sub>85/95</sub> staining becomes more diffuse as cytokinesis progresses, and there is no apparent association between KRP<sub>85/95</sub> and the midbody. The punctate vesicle-like staining pattern and the detergent sensitivity of spindle labeling suggest that KRP<sub>85/95</sub> is associated with membranous structures in the mitotic spindle and that it may function as a vesicle motor. Therefore our current working hypothesis is that both kinesin and KRP<sub>85/95</sub> move membrane-bounded vesicles toward the plus-ends of spindle microtubules, with kinesin moving membranous elements away from the centrosome along astral rays towards the cell surface and KRP<sub>85/95</sub> moving vesicles away from the centrosome along the overlapping interzonal microtubules of the central spindle.

It is striking to note that although KRP<sub>85/95</sub> and kinesin both appear to associate with membranous elements in the sea urchin MA, they appear to differ in terms of the nature of the associated membranes, as well as in their subcellular localization and developmental expression patterns. In early sea urchin blastomeres, kinesin is concentrated in the MA in association with ER, where it may be involved in the

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**FIG. 2.** Anti-tubulin (a, c, e) and anti-KRP<sub>85/95</sub> (b, d, f) staining of prophase (a, b), metaphase (c, d), anaphase (e, d), and telophase (c, f) blastomeres from dissociated 32- to 64-cell stage embryos. In prophase (a, b) the labeled KRP<sub>85/95</sub> appears to concentrate in the two pericentriolar regions. In metaphase (right-hand cell in c, d) the KRP<sub>85/95</sub> staining has concentrated in the spindle in the region of the kinetochores-to-pole microtubules. During anaphase (left-hand cell in e, d), the spindle localization of KRP<sub>85/95</sub> seen in metaphase cells is replaced by a striking accumulation of KRP<sub>85/95</sub> in the interzone between the chromosomes (seen in negative image in d). During telophase (c, f) the KRP<sub>85/95</sub> labeling appears to disperse into the cytoplasm and is not concentrated in the midbody. Bar, 10 μm.
movement of ER out along astral ray microtubules and in the delivery of ER-derived vesicles for new cell surface membrane during a wound rescaling response (Henson et al., 1989; Wright et al., 1991, 1993; Steinhardt et al., 1994). On the other hand, the KRP_{85/95}-specific MA staining does not correspond to ER distribution, particularly with regard to the enrichment of KRP_{85/95} in the interzone, and therefore KRP_{85/95} and kinesin may associate with different classes of membranous elements. It is interesting to note that the KRP_{85/95} staining provides evidence for the existence of a class of spindle-associated vesicular components that is distinct from the ER. During development, the KRP_{85/95} staining of MAs persists through the blastula stage, in contrast to kinesin staining which loses its MA association (see also Wright et al., 1991). Furthermore, while KHC protein levels remain relatively constant, the levels of KRP_{85/95} appear to decrease over the course of early development. In terminally differentiated and nonmitotic sea urchin coelomocytes kinesin appears to associate with ER and endosomal membranes (Henson et al., 1992), while the expression of KRP_{85/95} is not detectable. One possible explanation of this expression pattern is that maternal KRP_{85/95} is ubiquitously expressed early in embryogenesis and then depleted, with later expression being either turned off or tissue-specific. Perhaps coelomocytes are a cell type which do not require KRP_{85/95} functional activity. Interestingly, Pesavento et al. (1994) have demonstrated that the Drosophila kinesin-like protein KLP68D, a close relative of the 85-K subunit of sea urchin KRP_{85/95}, is ubiquitously expressed early in embryonic development, disappears during celluarization, and then later reappears restricted to cells of nervous system lineage. Further experiments are needed to determine whether sea urchin KRP_{85/95} displays any tissue-specific expression in late-stage embryos and/or in the adult animal.

The fundamental differences between KRP_{85/95} and kinesin localization and expression reported here raise interesting questions concerning how these two plus-end-directed motors are differentially targeted within the MA and how their developmental expression patterns are regulated. It is also important to note the differential responses of KRP_{85/95} and kinesin to pre-fixation detergent extraction, which appears to substantially extract KRP_{85/95} from MAs, but leaves kinesin bound to MA microtubules in a nucleotide-sensitive fashion (see also Wright et al., 1991). This difference may reflect differences in microtubule binding properties of kinesin and KRP_{85/95}. The distribution of KRP_{85/95} during mitosis in early sea urchin embryonic cells is comparable with the localization of a number of other KLPs during mitosis in other organisms. For example, KRP_{85/95} localizes in the region of the centrosome during prophase and the kinetochore-to-pole microtubules during metaphase. Similarly, members of the bicM KLP subfamily have been localized to spindles, with an enrichment at the microtubule organizing centers during early mitosis (Hagan and Yanagida, 1992; Hoyt et al., 1992, Roof et al., 1992, Sawin et al., 1992a). During anaphase, KRP_{85/95} is noticeably enriched in the interzone between the segregating sister chromatids, and similar interzonal staining has been reported for the yeast cut7 protein (Hagan and Yanagida, 1992) and the mammalian KLPs, MKLP-1 (Nislow et al., 1992), and CENP-E (Yen et al., 1992). However, the mammalian KLPs appear to be distributed in a narrow array of linear elements in the middle of the interzone, while KRP_{85/95} staining is clearly punctate and is distributed throughout the interzone. In addition, unlike the two mammalian KLPs, we see no evidence of any association between KRP_{85/95} and the midbodies. Whereas most of these spindle-associated KLPs are thought to participate in MA assembly and dynamics, we speculate that KRP_{85/95} may move vesicles to the plus-ends of interzonal microtubules in order to deliver vesicles to the cleavage furrow region, thus supplying new membrane that is required to accommodate the increase in cell surface area that occurs as one cell divides into two. This would resemble vesicle delivery to the forming cell plate in the phragmoplast of higher plant cells.

Subsequent to the identification and purification of sea urchin KRP_{85/95} (Cole et al., 1992, 1993), cDNAs encoding close relatives of its 85-K subunit have been characterized in other organisms, where they appear to play diverse roles. For example the mouse KIF3a and the Drosophila KLP68D proteins appear to serve as anterograde vesicle motors in neurons (Kondo, 1994, Pesavento et al., 1994), while the Chlamydomonas FLA10 gene product, KHP1, appears to be involved in flagellar assembly and maintenance (Walther et al., 1994). KIF4 is a mouse KLP which shares some interesting similarities with the sea urchin KRP_{85/95}. KIF4 is also a plus-end-directed vesicle motor that is expressed mostly in proliferative juvenile tissues, and it localizes to punctate elements in the MAs of dividing cells (Schiekne et al., 1994). This localization and developmental expression pattern have been cited as evidence that KIF4 has roles in both cell division and axonal transport (Schiekne et al., 1994).

In summary, the sea urchin KLP KRP_{85/95} has been localized to membranous elements in the MA of dividing sea urchin embryos. We therefore hypothesize that both KRP_{85/95} and kinesin function as vesicle motors which

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**FIG. 3.** KRP_{85/95} (a, b) and chromosome (inset) localization in first cleavage metaphase (a) and anaphase (b) embryos. KRP_{85/95} clearly concentrates in the metaphase spindle and in the anaphase interzone region between the migrating chromosomes. Pseudocolored and contrast-enhanced double imaging of KRP_{85/95} and tubulin in anaphase first cleavage embryos (c: red, tubulin, green, KRP_{85/95}) and a 32- to 64-cell stage blastomere (d: red, tubulin, blue, KRP_{85/95}). The punctate KRP_{85/95} staining concentrates in the interzone of the cells and some of the stained elements appear to align along microtubules (d). Bar in a, 10 μm (magnifications of a and b are equal); bar in c and d, 5 μm.
FIG. 4. Anti-tubulin [a, c] and anti-KRP<sub>dyn/93</sub> [b, d] staining of prefixation detergent-extracted 32- to 64-cell stage [a, b] and first cleavage [c, d] embryos. Anti-tubulin [e] and anti-sea urchin KHC [f] labeling of first cleavage embryo. Note that prefixation detergent extraction under microtubule stabilizing conditions appears to greatly diminish the MA and interzonal KRP<sub>165/93</sub> labeling [b, d], however, extraction results in the binding of KHC to MA microtubules [f]. Bars in a and c, 10 μm; magnifications of e-f are equal.
FIG. 5. (a–d) Staining of cal-sequestrin-containing ER (a, c) and KRP_{KHC} (b, d) in first cleavage (a, b) and 32- to 64-cell stage (c, d) embryos. Note that the anaphase stage cells (a, b, right-hand cell in c, d) in these embryos have concentrations of KRP_{KHC} staining in the interzone but no corresponding concentration of ER staining. (e, f) In 32- to 64-cell stage blastomeres double-labeled for KHC and the ER, there is no concentration of either staining pattern in the interzone of anaphase cells (right-hand cell in e, f). Bar, 10 μm; magnifications of c–f are equal.
transport different classes of membrane on microtubule tracks during embryonic cell division. While kinesin has been demonstrated to mediate the delivery of new membrane to the cell surface during wound resealing, the precise function of the KRP_{65,96} driven vesicle transport remains to be elucidated.

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