Roles of Two Homotetrameric Kinesins in Sea Urchin Embryonic Cell Division*

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To improve our understanding of the roles of microtubule cross-linking motors in mitosis, we analyzed two sea urchin embryonic kinesin-related proteins. It is striking to note that both of these proteins behave as homotetramers, but one behaves as a more compact molecule than the other. These observations suggest that these two presumptive motors could cross-link microtubules into bundles with different spacing. Both motors localize to mitotic spindles, and antibody microinjection experiments suggest that they have mitotic functions. Thus, one of these kinesin-related proteins may cross-link spindle microtubules into loose bundles that are “tightened” by the other.

Animal cell reproduction involves mitosis and cytokinesis, events that depend on the action of a bipolar protein machine, the mitotic spindle, which uses microtubules (MTs)$^1$ and MT-based motor proteins. A classic model system for studying mitosis and cytokinesis is the early echinoderm embryo where MTs and MT-based motor proteins are thought to position mitotic centrosomes, centrosomes dictate the positioning of the spindle, and the spindle in turn positions the cleavage plane (1). Antibody microinjection experiments are useful for probing the functions of MT-motors in sea urchin embryonic cell division (2); previously, the microinjection of pan-kinesin antibodies suggested that some kinesin motors, but apparently not conventional kinesin or heterotrimeric kinesin II, play important roles in mitosis and cell division (3–6).

One of these motors is a 110-kDa polypeptide, KRP$_{110}$, that reacts with an antibody, CHO1, to the mammalian mitotic motor, MKLP1 (3). MKLP1 is an anti-parallel MT-MT sliding motor that appears to be required for the organization of midzonal MTs and progression through mitosis and cytokinesis in several systems (7–12). The mechanism of how MKLP1 family members might function to cross-link microtubules and bundle them into the anti-parallel arrays required for cytokinesis is still unclear, because the size and subunit composition of the native holoenzyme is unclear.

The microinjection of the CHO1 antibody into sea urchin embryonic cells caused a prophase or metaphase arrest, suggesting that KRP$_{110}$ is a functional homologue of MKLP1 and is required for mitosis and cell division in this system (3). Work done in several systems suggests that interdigitating MTs in the spindle midzone are required to signal the proper progression of the contractile ring and completion of cytokinesis. However, in echinoderm cells, cleavage furrows can form, and subsequent cytokinesis can occur between two adjacent MT asters in the absence of such midzonal MTs (1). This raises the possibility that the MKLP1 homologue in echinoderm embryos, KRP$_{110}$, may be localized to the MT asters rather than the central spindle (13).

Another putative MT cross-linking motor that is also likely to participate in sea urchin embryonic mitosis is KRP$_{170}$, a member of the phylogenetically diverse bipolar bimC kinesin subfamily. Bipolar bimC kinesins are homotetramers that move slowly toward the plus-end of MTs (14–17). It has been proposed that bipolar kinesins serve to push spindle poles apart by cross-linking and sliding MTs in relation to one another, and thus play important roles in the maintenance and elongation of bipolar mitotic spindles (18, 19).

Here, we report the molecular characterization of native KRP$_{110}$ and KRP$_{170}$ which reveals that they are homotetrameric members of the MKLP1 and bipolar bimC kinesin families, respectively. Localization and functional studies of KRP$_{110}$ and KRP$_{170}$ suggest that they both play important roles in mitotic cell division in sea urchin embryos.

EXPERIMENTAL PROCEDURES

Materials—All chemicals used for studies described below were obtained from Sigma unless otherwise specified. Sea urchins Strongylocentrotus purpuratus were collected at Point Arena, CA. Lytechinus variegatus and Lytechinus pictus were obtained from Susan Decker and Marinus, Inc., respectively.

Purification of KRP$_{110}$ and KRP$_{170}$—To monitor the purification of KRP$_{110}$ and KRP$_{170}$, we used immunoblotting with (a) CHO1 IgM and a peptide antibody to detect KRP$_{110}$ and (b) a rabbit polyclonal antibody to KRP$_{170}$ (15) and a mouse polyclonal antibody to the KRP$_{110}$ stalk/tail to detect KRP$_{110}$. Buffers used were as follows: (a) protease mixture (1 mM diithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM NaN$_3$, 20 μg/ml aprotinin, 100 μg/ml soybean trypsin inhibitor, 1 mg/ml p-tosyl-l-arginine methyl ester); (b) PME (85 mM K2PIPES, 15 mM KCl, 0.5 mM EDTA, 2.5 mM MgSO$_4$, 5 mM EGTA), pH 6.9; (c) PE (85 mM K2PIPES, 15 mM KCl, 0.5 mM EDTA, 2.5 mM MgSO$_4$, 5 mM EGTA), pH 6.9; (d) PME + high salt (PME, 300 mM KCl), pH 6.9; (e) low salt PIPE for HiTrap SP (50 mM PIPE, 0.5 mM EDTA, 2.5 mM MgSO$_4$, 1.0 mM diithiothreitol, 100 mM KCl), pH 6.9; (f) high salt PIPE for HiTrap SP (50 mM PIPE, 0.5 mM EDTA, 2.5 mM MgSO$_4$, 1.0 mM diithiothreitol, 1.0 mM KCl), pH 6.9; (g) sucrose gradient buffer (5 or 20%
Filtration fractions containing KRP110 or KRP170 were concentrated to an Omega membrane, 10-kDa cut off, Pall Filtron). The concentrated material was then centrifuged at 40,000 rpm for 30 min in a Ti50.2 rotor. The high salt eluate was collected after centrifuging at 40,000 rpm for 30 min in a Ti50.2 rotor. A second elution using 15 ml of high salt buffer (PME plus 300 mM KCl and 10 mM MgATP) was performed on ice for 4 h, and the supernatant (high salt eluate) was collected after centrifuging at 40,000 rpm for 30 min in a Ti50.2 rotor. The high salt eluate was concentrated down to 3–5 ml (15-ml spin concentrator, 50-kDa cut off, Millipore) and loaded onto a Superose 6 FPLC gel filtration column (AK16/70, 100-ml bed volume, Amersham Pharmacia Biotech). The peak fractions of KRP170 and KRP110 were pooled separately and diluted 1:1:1 (v/v/v) with a low saltPIPES buffer and water and loaded onto a 1 ml HiTrapSP column (Amersham Pharmacia Biotech). Bound proteins were eluted with a linear gradient of 100–500 mM KCl in PBE buffer over 30 min. Fractions containing either KRP110 or KRP170 were further concentrated to less than 200 μl using a Nanosep spin concentrator (0.5 ml, Omega membrane, 10-kDa cut off, Pall Filtron). The concentrated material was loaded onto 5-ml, 5–20% linear sucrose density gradients and centrifuged at 55,000 rpm for 9 h in a SW55 rotor. Alternatively, the gel filtration fractions containing KRP110 or KRP170 were concentrated to less than 2 ml, and exogenously polymerized bovine phosphocellulose-purified tubulin and 2 mM AMP-PNP were added to promote the binding of KRP110 to MTs. The bound material was pelleted by centrifugation and eluted differentially, first with 100 mM KCl and 10 mM MgATP in PBE, then followed by 300 mM KCl and 10 mM MgATP in PBE. The high salt eluate was loaded directly onto the sucrose density gradient and centrifuged as described above.

**Peptide Sequencing**—To microsequence KRP110 and KRP170 peptides, purified proteins were excised from polyacrylamide gels, digested with trypsin, and analyzed by liquid chromatography purification. Digests were analyzed by MALDI-TOF mass spectrometry, and selected peptides were sequenced using Edman degradation (16).

**Hydrodynamic Analysis**—The Stokes radii of KRP110 and KRP170 were determined by gel filtration chromatography in the presence of either high salt or low salt PBE buffers using a Superose 6 column calibrated with the following: ferritin (6.1 nm), thyroglobulin (8.5 nm), carbonic anhydrase (2.0 nm), cytochrome c (5.2 nm), aldolase (4.8 nm), myosin (19.9 nm), bovine serum albumin (4.3 nm), ovalbumin (4.4 nm), and lysozyme (1.4 nm).

**Antibody Preparation**—The antibodies were affinity-purified using glutathione-agarose affinity chromatography, diluted 1:1 with Titermix (CytRx Corp.), and injected into mice for ascites preparation.

## RESULTS

**Molecular Analysis of sea urchin KRP110 and KRP170**—The amino acid sequences of the two presumptive mitotic motors, KRP110 and KRP170, were determined by microsequence analysis of cDNA clones (GenBank accession numbers AF292394 and AF292395) and analyzed using standard procedures to generate maps shown in Fig. 1, A and B. The cloned cDNAs are likely to encode the KRP110 and KRP170 polypeptides that are recognized by pan-kinase antibodies and co-purify with sea urchin egg MTs based on antibody reactivity (Fig. 1C). This idea was confirmed by a comparison of the deduced amino acid sequences with partial peptide sequences obtained from the corresponding MT-affinity-purified polypeptides (Fig. 1, A and B, and Fig. 2, D and E).

The deduced sequence of KRP110 predicts a tripartite 99-kDa, 870-amino acid residue polypeptide organized into motor (aa 1–450), stalk (aa 451–700), and tail (aa 701–870) domains. The 249-residue stalk contains a region (aa 550–693) that is predicted to form a 22-nm coiled-coil rod. There is an unusually high degree of sequence similarity (42–57% identity in the motor domain) to members of the MKLP1 family of kinesins, although KRP110 is unique in containing a proline-rich segment (aa 178–193) that may be a target for proline-directed protein kinase.

The deduced sequence of KRP170 predicts a tripartite 122-kDa, 1081-amino acid residue polypeptide organized into motor (aa 1–458), stalk (aa 459–800), and tail (aa 801–1081) domains. The 451-residue stalk contains a region (aa 556–772) that is predicted to contain three coiled-coil segments that form a 62-nm long rod. There is an unusually high degree of sequence identity (50–72% identity) in the motor region and bimC box (aa 934–946) with members of the bipolar bimC family of kinesins.

**Biochemical Analysis of Native KRP110 and KRP170**—Anti-KRP110 and KRP170 antibodies were used to probe for the presence of KRP110 and KRP170 in subcellular fractions prepared from sea urchin egg cytoskeletal extract (Fig. 2). We observed that KRP110 and KRP170 co-sedimented with MTs in the presence and absence of AMP-PNP or ATP (Fig. 1C). Both proteins were eluted from MT pellets by differential centrifugation in high ionic strength buffers and then subjected to gel filtration chromatography, ion exchange chromatography, or MT-affinity binding and sucrose density gradient centrifugation (Fig. 2, A–E). To investigate whether the high salt elution activated KRP 110. This region was subsequently used as a probe to screen out the rest of the 3′-end by conventional library screening. The 5′-end was identified by 5′-RACE (rapid amplification of cDNA ends).
conditions might dissociate a loosely bound accessory protein, we also performed hydrodynamic experiments under low salt conditions on clarified and filtered cytosol that was not subjected to prior fractionation steps (Table I).

From the fractionation data, it was clear that KRP<sub>110</sub> and KRP<sub>170</sub> behaved as two separate monodisperse peaks under high salt conditions, indicative of two distinct holoenzymes (Fig. 2A and Table I). Both proteins appeared to lack accessory subunits (Fig. 2B and C), as the only other polypeptides present in the KRP<sub>110</sub>- and KRP<sub>170</sub>-containing fractions were variable and highly substoichiometric to the major band (<0.1:1.0 mol/mol), with the exception of tubulin, which contaminated KRP<sub>170</sub> following the MT-affinity purification step (Fig. 2E).

Based on the Stokes radius ($R_S$) of 11 nm and a sedimentation coefficient of 9.8 S obtained under high salt conditions (Table I), we estimated that the KRP<sub>110</sub> holoenzyme is a compact, asymmetric molecule with an axial ratio of <20 and a native molecular mass of 464 kDa, consistent with the hypothesis that KRP<sub>110</sub> is a homotetramer of four identical 110-kDa subunits (Table I). However, under low salt conditions, there was a major peak of KRP<sub>110</sub> for which the predicted molecular mass is somewhat higher (Table I). It is possible that this small difference in apparent molecular mass reflects variations due to experimental errors, but it is also consistent with the hypothesis that a 50-kDa polypeptide may associate with the holoenzyme under low salt, but not high salt, conditions.

Based on a $R_S$ of 16 nm and a sedimentation coefficient of 9.0 S, we estimated that the KRP<sub>170</sub> holoenzyme is a homotetramer with a native molecular weight of 610 kDa and a high axial ratio of 60–80, indicative of an elongated asymmetric shape. Under low salt conditions, the estimated molecular mass is elevated somewhat, but both estimates are consistent with a homotetramer (predicted molecular mass = 680 kDa).
KRP170 is thus likely to be a stable bipolar homotetramer like other members of the bipolar bimC kinesin family. The purification protocol was useful in providing enough pure material for protein microsequencing and for measuring the hydrodynamic properties of KRP110 and KRP170. Unfortunately, however, it has not so far proved feasible to obtain sufficient amounts of pure protein to examine the structure of either KRP110 or KRP170 by rotary shadow electron microscopy or to test their predicted ability to cross-link MTs into bundles.

Localization of KRP110 and KRP170 within Mitotic Spindles—Immunofluorescence microscopy revealed that KRP110 and KRP170 are associated with sea urchin embryonic mitotic spindles. KRP110 displayed a somewhat punctate staining pattern (Fig. 3a–d). During prophase, KRP110 was concentrated in a broad band that encircles the nucleus (Fig. 3a), and subsequently in metaphase it was concentrated throughout the entire mitotic apparatus (Fig. 3b). In unextracted anaphase cells, the protein was present in a broad band throughout the spindle interzone, between segregating sister chromatids (Fig. 3c). Following detergent lysis of anaphase cells, the KRP110 antibody staining of the interzone region was greatly enhanced, and clear staining of spindle poles became evident (Fig. 3c, inset). In the pre-extracted cells, the interzonal staining appeared to peak at the equatorial plane, which predicts the position of the future cleavage furrow. Faint but detectable punctate staining in the spindle asters was present in both extracted and unextracted anaphase spindles. During telophase, some KRP110 appeared to redistribute to the asters surrounding the reforming nuclei, but the staining of the spindle equator persisted as two intensely fluorescent bands located at the midbody (Fig. 3d, arrow and inset).

The anti-KRP170 antibody produced a fibrous, albeit punctate, staining pattern. During prophase, staining of the asters associated with separating centrosomes was observed (Fig. 3e). KRP170 appears to associate with both spindle fibers and spindle poles during metaphase (Fig. 3f) and at this stage we observed clear, albeit faint, staining of apparent interzonal MT bundles that cross the metaphase plate, similar to those that were stained with anti-CLP61F antibody in Drosophila embryos (18). The fibrous localization of KRP170 persisted through anaphase (Fig. 3g), but during early telophase, anti-KRP170 produced punctate perinuclear staining with no specific staining of the midzone (Fig. 3h).

Microinjection Studies of KRP110 and KRP170 Function—Previously, we showed that the microinjection of an anti-KRP110 antibody into one-cell sea urchin embryos leads to a prophase or metaphase arrest depending upon the time of injection (3). The timing of the prophase arrest corresponds to

**Table 1**

| Holoenzyme | Stokes radius | S value | Estimated native molecular mass | Axial ratio | Estimated composition | Schematics |
|------------|---------------|---------|---------------------------------|-------------|-----------------------|------------|
| KRP110     |               |         |                                 |             |                       |            |
| High salt  | 11.5          | 9.8     | 464                             | <20         | 4 × 110               |            |
| Low salt   | 11.8          | 10.7    | 510                             |             |                       |            |
| KRP170     |               |         |                                 |             |                       |            |
| High salt  | 16.5          | 9.0     | 610                             | 60–80       | 4 × 170               |            |
| Low salt   | 19.6          | 8.8     | 708                             |             |                       |            |

**FIG. 2.** Partial purification of KRP110 and KRP170. A, immunoblots of gel filtration fractions 15–28 of high salt eluted MT-binding proteins probed with anti-KRP110 (top) and anti-KRP170 antibodies (bottom). B, fractions of KRP110 from a 5–20% sucrose density gradient on a silver-stained gel (top) and anti-KRP110 immunoblot (bottom). C, fractions of KRP170 from a 5–20% sucrose density gradient on a Coomassie Blue-stained gel (top) and an anti-KRP170 immunoblot (below). D and E, Coomassie Blue-stained gels of partially purified KRP110 (D) and KRP170 (E); identical samples were immobilized onto polyvinylidene membranes, and the excised bands (indicated by arrows) were sequenced.
a time when KRP_{110} displays a broad perinuclear distribution, whereas the metaphase arrest occurs just prior to the relocalization of KRP_{110} from the metaphase asters and half spindles to the spindle interzone (Fig. 3). In the present study, we used a similar approach to investigate the function of KRP_{170}.

When antibodies against the KRP_{170} stalk/tail region were injected into *L. pictus* blastomeres, 86% of the injected embryos were arrested in prophase at the one-cell or two-cell stage (n = 35) with swollen nuclei containing disc-shaped structures (Fig. 4 and Table II). This prophase arrest occurs at a time when KRP_{170} is associated with the prophase asters (Fig. 3E), and the arrest persists for more than 8 h before the cell dies. During a time period corresponding to the arrest of the injected cell, control embryos divided to form normal multicellular blastulae (Fig. 4, top panels). Immunofluorescence microscopy of injected embryos revealed that MT arrays appeared to emanate from two or sometimes four foci, which may correspond to separated ectopic microtubule-organizing centers associated with each nucleus (Fig. 4, bottom right; Table III). Curiously, we observed that approximately 14% of the anti-KRP_{170}-injected blastomeres arrested in anaphase rather than prophase (Table II).

In a complementary experiment, a dominant-negative fusion protein consisting of GST fused to the distal stalk/tail domains of KRP_{170} (GST_{170}Coil4Tail; Fig. 1B) was also microinjected into sea urchin embryos and produced identical mitotic defects to those observed using antibody inhibition (Table II). Thus, although the inhibition of KRP_{110} activity appears to induce a prophase or metaphase arrest (3), a loss of function of KRP_{170} appears to result in a prophase or anaphase arrest.

**DISCUSSION**

The data described here are consistent with the hypothesis that KRP_{110} and KRP_{170} are homotetrameric members of the MKLP1 and bipolar kinesin families, respectively, that are likely to play important roles in mitotic cell divisions by cross-linking spindle MTs into bundles (Fig. 5). Based on hydrodynamic data, KRP_{110} and KRP_{170} are

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**FIG. 3.** Localization of KRP_{110} and KRP_{170} within sea urchin embryonic mitotic spindles. **a**—**d**, confocal images of anti-KRP_{110} (yellow) and DAPI (blue)-stained sea urchin (*L. variegatus*) embryos fixed with cold methanol during the first cell division. **Inset c** is a pre-extracted embryo spindle and **inset d** is a close-up of the stained midbody (arrow). **e**—**h**, confocal images of anti-KRP_{170} (yellow)- and DAPI (blue)-stained sea urchin (*L. pictus*) embryos fixed with cold methanol during the 4–8-cell stage. **a** and **e**, prophase; **b** and **f**, metaphase; **c** and **g**, anaphase; and **d** and **h**, telophase.

**FIG. 4.** Inhibition of the KRP_{170} function in sea urchin embryo results in a prophase arrest. Microinjection of anti-KRP_{170} antibody. Differential interference contrast images of an affinity-purified anti-KRP_{170} stalk/tail antibody-injected embryo and a control-injected embryo. Control embryos divided normally, whereas anti-KRP_{170} antibody-injected embryos arrested in prophase with enlarged nuclei. The right-hand column shows tubulin staining of the same embryos shown in the left-hand and middle columns, post-injection and post-development, using indirect immunofluorescence.

**TABLE II**

| Injected reagent | Displaying mitotic arrest | % (n) |
|------------------|---------------------------|------|
| Anti-KRP_{170} stalk/tail antibody | 100 (35) |
| Non-specific mouse IgG | 0 (12) |
| R2.4 anti-kinesin II antibody | 0 (3) |
| GST-KRP_{170}Coil4Tail | 100 (5) |
| GST | 0 (2) |
| Anti-KRP_{110} (CHO1) | 100 (107) |

* Of these cells, 22.9% arrested during prophase (1-cell stage), 62% during prophase (2-cell stage), and 14.3% during anaphase (1-cell stage).

* Of these cells, 40% arrested during prophase (1-cell stage), 20% during prophase (2-cell stage), and 40% during anaphase (1-cell stage).

* From Wright et al. (3).

**TABLE III**

| MTOCs/blastomere | No. of blastomeres |
|-------------------|--------------------|
| 2                 | 2                  |
| 3                 | 4                  |
| 4                 | 2                  |

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thought to be homotramers, at least when partially purified by MT affinity binding and elution under high salt conditions. Because bipolar biric kinesins from Drosophila and yeast are bipolar, with motor domains at opposite ends of a central rod (15, 17), it is likely that KRP170 has a similar structure; hydrodynamic data obtained at both low and high ionic strength are consistent with this idea. Although our data are consistent with the notion that KRP110 consists of four 110-kDa polypeptides in the native protein complex, we cannot formally exclude the possibility that the native protein is a lower order multimer that contains undetected accessory polypeptides; but this seems unlikely in light of the fact that the contaminants detected in the preparations are variable and substoichiometric, and yet the protein behaves as a monodisperse peak during fractionation. Also, we cannot exclude the possibility that the difference in the predicted native molecular mass of KRP110 obtained under low salt conditions may be due to the presence of a small (50 kDa) accessory polypeptide that is dissociated from the complex under high salt conditions. Candidate associated polypeptides include Caenorhabditis elegans Cyk-4 (28) and polo kinase (7, 27), which are implicated in MKLP1 function in other systems, where they also play a role in central spindle formation and cytokinesis.

We speculate that KRP110 also has a bipolar organization, although further structural studies are needed to test this idea. A bipolar homotrameric structure would allow both KRP110 and KRP170 to bind MTs and cross-link them into bundles, but regardless of whether KRP110 is in fact bipolar, it is likely, based on hydrodynamic studies, to be a compact holoenzyme (axial ratio < 20) with a coiled-coil rod that is only 22 nm long. KRP170 is predicted to be more elongated (axial ratio of 60–80) with an α-helical rod ~62 nm in length; this would predict that KRP110 cross-links MTs into tighter, more-closely packed bundles than KRP170. Both KRP110 and KRP170 bind MTs in a nucleotide-insensitive fashion, but MT-cross-linking remains to be demonstrated. To test our hypothesis, it would be of interest to perform immunoelectronmicroscopic studies to visualize KRP110 and KRP170 cross-bridges within spindles. Such experiments are being initiated using the homologous proteins, PAV-KLP and KLP61F in Drosophila embryos, where we have already visualized KLP61F cross-bridges in situ (18).

An alternative hypothesis is that the bipolar structure of homotetrameric plus end-directed kinesins like KRP110 and KRP170 allows these motors to transport a cargo and localize it to the spindle interzone. Thus, rather than cross-linking and sliding anti-parallel MTs, these bipolar motors would move to the plus ends of MTs within MT bundles, concentrating in the region of overlap as a consequence of being “trapped” there (28). This would allow bipolar kinesins to localize regulatory molecules like the polo kinase and Cyk-4 to the interzone. Further work would be needed to address this hypothesis.

Our finding that sea urchin embryonic cells contain a member of the bipolar biric kinesins subfamily, KRP170, which is homotetrameric and is required for progression through mitosis, is consistent with work done in several other systems (29). Our localization data are consistent with the hypothesis that KRP170 associates generally with spindle MTs, and inhibiting the function of KRP170 leads to mitotic arrest. These results are consistent with the model that KRP170 may cross-link MTs into relatively loose bundles and slide them apart during the assembly and elongation of the mitotic spindle, consistent with data from Drosophila that implicate KLP61F in bipolar spindle maintenance and elongation (19). In Drosophila, KLP61F is sequestered in the nucleus during prophase and does not participate in the initial separation of the spindle poles, but only following nuclear envelope breakdown can KLP61F interact with MTs and exert forces that push apart the poles (18, 19). Our data on sea urchin embryonic cells is consistent with the hypothesis that in this system, KRP170 associates with cytoplasmic MTs where it interacts with MTs to help push apart the centrosomes during initial spindle assembly and anaphase spindle elongation, so that inhibiting its function leads to prophase or anaphase arrest (6).

Our characterization of KRP110 as a MKLP1 homologue that localizes to mitotic spindles extends our previous work showing that the microinjection of an antibody to mammalian MKLP1, CHO1, causes a mitotic arrest in sea urchin blastomeres (3). The injected antibody arrested mitosis at one of two distinct points, namely prophase and metaphase, depending upon the time of injection. KRP110 localizes to perinuclear regions during prophase and to spindles during metaphase, which are both regions of high MT density. In mammalian cells, Nisolow et al. (30) saw a similar metaphase arrest; but so corresponding prophase arrest was seen, perhaps because MKLP1 is localized to metaphase spindles but is sequestered in the nucleus at prophase, where it cannot interact with MTs. Thus, we propose that KRP110 functions to cross-link MTs into tight bundles and that inhibiting this process leads to a prophase or a metaphase arrest by interfering directly with spindle mechanics, by activating spindle assembly checkpoints, or by interfering with the localization of regulatory components of the spindle.

As in mammalian cells, the microinjection of the CHO1 antibody did not reveal any effects on later stages of mitosis or cytokinesis, suggesting that earlier mitotic defects may obscure any subsequent defects. However, the most striking localization pattern of KRP110 was a faint staining of the asters and an intense concentration in the spindle interzone and midbody during anaphase and telophase, respectively. In the interzone and midbody, the homotetrameric KRP110 motor could bind loosely organized, anti-parallel, midzonal MTs and cross-link them into the tight bundles that are thought to localize the components of the cleavage furrow and facilitate progression through cytokinesis (7–12). A role for KRP110 in the formation of tight midzonal MT bundles is consistent with the defects in midzonal MT organization seen in mammalian, Drosophila, and C. elegans cells when MKLP1 function is impaired. The low but detectable pool of KRP110 present in anaphase and telophase asters could be responsible for cross-linking anti-parallel
MTs emanating from two adjacent asters, thereby signaling the formation of the cleavage furrow between the two asters in the absence of an associated central spindle (1).

These data improve our understanding of the roles of MT-based motors in sea urchin embryonic mitotic cell divisions by suggesting that KRP$_{110}$ and KRP$_{170}$ are homotetramers that play important roles in mitosis. We hypothesize that the long KRP$_{170}$ motors cross-link MTs into relatively loose bundles and slide them apart during centrosome separation, whereas the compact KRP$_{110}$ motors organize MTs into more closely packed bundles that are required for progression through mitosis and completion of cytokinesis.

In sea urchin embryos, as in other systems (19), multiple mitotic motors are likely to function in a coordinated fashion. How KRP$_{110}$ and KRP$_{170}$ may work in concert with other mitotic motors, including kinesin C (25), KRP$_{180}$ (6), and cytoplasmic dynein are topics for future work.

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