Citron kinase controls a molecular network required for midbody formation in cytokinesis

Zuni I. Bassi, Morgane Audusseau, Maria Giovanna Riparbelli, Giuliano Callaini, and Pier Paolo D’Avino

Cytokinesis partitions cytoplasmic and genomic materials at the end of cell division. Failure in this process causes polyplody, which in turn can generate chromosomal instability, a hallmark of many cancers. Successful cytokinesis requires cooperative interaction between contractile ring and central spindle components, but how this cooperation is established is poorly understood. Here we show that Sticky (Sti), the *Drosophila* ortholog of the contractile ring component Citron kinase (CIT-K), interacts directly with two kinesins, Nebbish [the fly counterpart of human kinesin family member 14 (KIF14)] and Pavarotti [the *Drosophila* ortholog of human mitotic kinesin-like protein 1 (MKLP1)], and that in turn these kinesins interact with each other and with another central spindle protein, Fascetto [the fly ortholog of protein regulator of cytokinesis 1 (PRC1)]. Sti recruits Nebbish to the cleavage furrow, and both proteins are required for midbody formation and proper localization of Pavarotti and Fascetto. These functions require Sti kinase activity, indicating that Sti plays both structural and regulatory roles in midbody formation. Finally, we show that CIT-K’s role in midbody formation is conserved in human cells. Our findings indicate that CIT-K is likely to act at the top of the midbody-formation hierarchy by connecting and regulating a molecular network of contractile ring components and microtubule-associated proteins.

**Results**

N-Terminal Region of the Sti Coiled-Coil Domain Interacts with the Kinesins Pav and Neb. We previously identified a region in the Sti coiled-coil domain, dubbed “StiCC1,” which unexpectedly localized to the central spindle midzone (Fig. 1L and see Fig. S2) (17). This localization differs from that of endogenous Sti, which instead accumulates at the cleavage furrow (12, 17). As StiCC1 is not necessary for Sti localization to the cleavage furrow (17), we reasoned that StiCC1 localization to the midzone might reflect an interaction with one or more MAPs. To identify these MAPs, we tagged both StiCC1 and full-length Sti (StiFL) with two IgG binding motifs of protein A (PtA) and isolated proteins that interact with these two baits by affinity purification coupled with mass spectrometry (23). This approach led to the identification of two kinesins, Pav and Neb (Table S1). In vivo pull-down assay confirmed that PtA::StiCC1 formed a complex with both kinesins, suggesting that CIT-K was originally identified as a RhoA effector that could regulate myosin contractility by phosphorylating the myosin regulatory light chain (10, 11). However, CIT-K and its *Drosophila* ortholog, Sticky (Sti), are not required for furrowing (12–16), and evidence in both *Drosophila* and human cells has challenged the original model by showing that CIT-K is required for proper RhoA localization at the cleavage site during late cytokinesis, thus behaving more like a RhoA regulator than an effector (14, 17). Moreover, Sti is not required for myosin regulatory light chain phosphorylation and associates with actomyosin filaments to localize to the cleavage furrow (17). This indicates that CIT-K acts as an important regulator of late events during cytokinesis, but the molecular details are still missing. Here we show that Sti directly binds to two kinesins, Pavarotti (Pav, the *Drosophila* ortholog of human mitotic kinesin-like protein 1 (MKLP1), the other centralspindlin component) (18) and Nebbish (Neb, the fly counterpart of human KIF14) (19–21), which in turn interact with each other and with another central spindle component, Fascetto [Feo, the fly ortholog of protein regulator of cytokinesis 1 (PRC1)] (22). Furthermore, we show that Sti depletion affects MB formation and localization of Neb, Pav, and Feo, and that Sti kinase activity is necessary for these functions. We also found that Neb depletion phenocopied *stIr* RNAi MB defects. Finally, we report that CIT-K performs similar roles in human cells. Our findings, together with those of previous studies (14, 17), indicate that Sti/CIT-K acts as a key regulator of MB formation in late cytokinesis through its association with a network made up of contractile ring components (anillin, actin, myosin, and RhoA) and at least three microtubule-associated proteins (MAPs; Neb, Pav, and Feo) necessary for central spindle organization.

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although only in the presence of a cyclin-dependent kinase 1 (Cdk1) inhibitor (Fig. S1), thus suggesting that this ternary complex assembled preferentially after anaphase onset. Consistent with these findings, we found that Neb depletion prevented accumulation of StiCC1 at the midzone (Fig. S2). We could not test whether Pav was necessary for StiCC1 localization to the midzone because Pav depletion inhibits furrow ingression (24).

To assess whether StiCC1 could directly bind to Pav and Neb, we purified a StiCC1 fragment tagged with glutathione S-transferase (GST) from bacteria and tested its ability to pull down various radiolabeled Pav and Neb polypeptides synthesized by in vitro translation (Fig. 1B). These experiments indicated that StiCC1 interacted with the Neb C-terminal domain (Neb741–1121) and the Pav stalk domain (Pav461–685; Fig. 1A and B). MKLP1 and KIF14 are known to interact with another MAP, PRC1, in human cells (9, 25), but we found that StiCC1 could not pull down the Drosophila PRC1 ortholog, Feo (Fig. 1B), indicating that StiCC1 interacts specifically with Pav and Neb. Reciprocal pull-down experiments using GST::Neb fragments confirmed that Neb741–1121 interacted with StiCC1, whereas its N-terminal fragment, Neb1–118, was able to pull down both Feo and Pav461–685 (Fig. 1C). Moreover, GST::Pav461–685 could pull down StiCC1, and Pav461–685 interacted with both Feo and the N terminus of Neb (Fig. 1D). These results are consistent with our previous study, in which both Sti and Neb were identified as Feo-associated proteins in Drosophila cells (26). Two lines of evidence indicated that StiCC1 could form a ternary complex with Pav and Neb in vitro. First, GST::StiCC1 could simultaneously pull down both Neb741–1121 and Pav461–685 (Fig. 1E). Second, the binding of GST::StiCC1 to radiolabeled Pav461–685 was unaffected by the presence of increasing concentrations of GST::Neb741–1121, which binds to StiCC1 but not to Pav461–685 (Fig. 1F). Thus, Pav and Neb do not seem to compete for their binding to StiCC1. In sum, our findings indicate that Sti, Neb, Pav, and Feo form a complex in vivo in which the StiCC1 region binds to Pav and Neb, and in turn these two kinesins interact directly with each other and with Feo.

**Sti and Neb Colocalize to the Cleavage Furrow.** We then analyzed the localization of Sti, Neb, and Pav during cytokinesis. We used a cell line stably expressing a myelocytomatosis viral oncogene homolog (myc)-tagged Sti transgene (Sti::myc) that showed the same localization pattern as endogenous Sti and could fully rescue the cytokinesis defects caused by sti RNAi (17). Sti::myc and Neb::GFP displayed identical localization in HeLa cells (9). Importantly, the Neb antibody is specific because its signal disappeared after neb RNAi (Fig. S3) and Neb tagged with green fluorescent protein (GFP::Neb) displayed identical localization.
Consistent with our in vitro binding results, the Neb fragment that interacts with Sti (Neb741–1121) localized to the cleavage furrow, whereas the two Neb fragments that bind to Pav and Feo and lack the Sti-interacting region (Neb1–740 and Neb1–118) localized to the central spindle (Fig. 2D). Similar to Sti, GFP::Neb also appeared to only partially colocalize with Pav at the interface between the central spindle and the contractile ring in mid- and late telophase, whereas after formation of the intercellular bridge, Neb formed a buttonhole that completely encircled Pav (Fig. 2E). GFP::Neb also colocalized with Feo at the interface between the cleavage furrow and the central spindle, but Feo showed a wider distribution and disappeared after bridge formation (Fig. 2F).

**Sti Recruits Neb to the Cleavage Furrow and Is Necessary for Proper MB Formation.** It is unlikely that the interaction of StiCC1 with Neb and Pav might be important for Sti localization because this region is not required for cleavage furrow accumulation (17). Therefore, we analyzed MB formation and localization of Neb, Pav, and Feo after Sti depletion. In late cytokinesis, MBs stained for tubulin display a dark central region, which corresponds to tightly bundled midzone microtubules that are inaccessible to antitubulin antibodies because they are covered by MB ring proteins. This dark region was either completely absent or very thin and oblique in a significant number of Sti-depleted cells (34% vs. 10% in control cells), indicating that MBs did not form properly in the absence of Sti (Fig. 3A–E). Ultrastructural analysis by electron microscopy (EM) confirmed that the electron-dense MB matrix was scarce, disorganized, and fragmented in Sti-depleted cells (Fig. 3D). Interestingly, Neb failed to localize to the cleavage furrow in all sti RNAi cells (Fig. 3A), and Pav and Feo localization appeared diffuse in Sti-depleted cells with abnormal MBs (Fig. 3B and C). To investigate whether Sti kinase activity was necessary
for MB formation, we rescued depletion of endogenous Sti by expression of Myc-tagged wild-type Sti (StiWT), Myc-tagged kinase-dead Sti (StiKD), or Myc alone, as described (17). MB defects were rescued only by expression of StiWT (Fig. 3F).

neb RNAi phenocopied Sti depletion, although the frequency of abnormal MBs (47%) was slightly higher than in Sti-depleted cells (Fig. 4A–E). Notably, the level of cytokinesis failure (i.e., increase in multinucleate cells) after neb RNAi, although significant (12.6% vs. 5% in controls; Fig. 4E), was considerably less than what was routinely observed after sti RNAi (45–59%) (12, 17), suggesting that Sti exerts additional functions in addition to recruiting Neb. Sti was able to accumulate at the cleavage furrow after neb RNAi (Fig. 4A, Bottom), probably because of MB disorganization. These results indicate that Sti is necessary for Neb recruitment to the cleavage site, but not vice versa. Pav and Feo localization also appeared diffuse after Neb depletion (Fig. 4B and C), and in some extreme cases, neb RNAi cells displayed very long and thin central spindles (Fig. 4B, Bottom), reminiscent of the phenotype observed after Feo inactivation (22). EM analysis confirmed that neb RNAi caused fragmentation, disorganization, and mispositioning of the MB matrix (Fig. 4D). Together, these data indicate that Sti recruits Neb to the cleavage site and then both proteins cooperate to regulate MB formation and proper localization of Pav and Feo to the spindle midzone. We cannot exclude that the improper localization of these two MAPs could simply be a consequence of general MB disorganization, but the
Citron Kinase Is Necessary for MB Formation in Human Cells. In human cells, CIT-K associates with KIF14, although it is not known whether this interaction is direct, and the two proteins are interdependent for their localization during cytokinesis (9). In turn, both KIF14 and MKLP1 are known to interact with PRC1 (9, 25). Thus, we investigated whether CIT-K could also directly interact with MKLP1. In vitro pull-down assays confirmed that the CIT-K region corresponding to StiCC1 interacted directly with the C-terminal region of MKLP1, but unlike in Drosophila, CIT-K bound to the MKLP1 tail region (aa 590–856; Fig. 5A). We then analyzed localization of CIT-K, KIF14, and MKLP1 in HeLa cells during cytokinesis. Consistent with previous findings (9), we found that KIF14 localized to the spindle midzone, but we also observed a clear KIF14 signal at the cortex, where this kinesin colocalized with CIT-K throughout cytokinesis (Fig. S4A). CIT-K also colocalized with MKLP1 at the interface between the cleavage furrow and the central spindle in mid- and late telophase (Fig. S4B), as already described for Pav and Sti (Fig. 2C). CIT-K depletion affected MB organization and localization of both MKLP1 and PRC1 (Fig. 5B) similarly to the phenotypes observed in fly cells. These defects were less frequent than in Drosophila but were nonetheless very significant (8.8% vs. 0.6% in controls; Fig. 5C) and represented about one third of the total number of multinucleate cells (27.2%; Fig. 5C). Thus, CIT-K’s role in MB formation is conserved in human cells.

Discussion

Although it was proposed more than a decade ago that cytokinesis requires the coordinated action of contractile ring and central spindle proteins (2, 3), it is still unclear which contractile ring component or components might play a key role in this process. The results presented here, together with previous evidence that Sti/CIT-K associates with and controls the distribution of the contractile ring components actin, myosin, anillin, and RhoA (12, 14, 17), clearly establish that this kinase links contractile ring and central spindle components and that this role is important for MB formation in late cytokinesis (Fig. 6). Consistent with this model, we previously reported that Sti-depleted cells present cortical blebs at the cleavage site, which are likely to be caused by detachment of the contractile ring from the central spindle (12). Sti/CIT-K appears to play both structural and regulatory roles in MB formation. First, it can directly bind to Neb/KIF14 and Pav/MKLP1 and is required for their proper distribution during cytokinesis. Furthermore, our rescue experiments indicate that at least in flies, Sti kinase activity is necessary for its role in MB formation. Thus, we propose that CIT-K could phosphorylate one or more MAPs required for central spindle and MB assembly; the most obvious candidates are the three MAPs that form a complex with CIT-K: Neb/KIF14, Pav/MKLP1, and Feo/PRC1. Unfortunately, our various attempts to establish a kinase assay using Sti purified from Drosophila cells proved inconclusive. Mass spectrometry revealed that Sti is phosphorylated at multiple residues in its kinase domain, and it is likely that these post-translational modifications could regulate its kinase activity during cell division. Drosophila cells cannot be synchronized, preventing the purification of Sti specifically from cells in telophase, when this kinase should be fully active. Perhaps future attempts using CIT-K purified from telophase-synchronized human HeLa cells could lead to the identification of its targets during cytokinesis.

The multiprotein complex formed by Sti/CIT-K, Neb/KIF14, Pav/MKLP1, and Feo/PRC1 has been conserved during evolution, but the localizations of Neb and KIF14 are different: Neb localizes to the cleavage furrow, whereas KIF14 accumulates at both the cortex and spindle midzone (Fig. S4A). Furthermore, although CIT-K and KIF14 are interdependent for their localization during cytokinesis (9), in Drosophila Sti is necessary for Neb accumulation at the cleavage furrow, but not vice versa. These differences between human and fly cytokinines proteins are not new. For example, the Drosophila RhoGEF Pebble, which is responsible for RhoA activation at the cleavage site, localizes to the equatorial cortex and cleavage furrow (27), whereas its human homolog ECT2 accumulates at both the equatorial cortex and spindle midzone (28, 29). Why and when these differences originated during evolution is not known, but importantly, all these proteins have conserved similar functions in cytokinesis.

In conclusion, our results shed light on an important role of CIT-K indicating that in both Drosophila and human cells, this kinase controls a molecular network of MAPs involved in MB formation. This function of CIT-K could act in parallel and/or in concert with other proteins such as anillin to establish a robust
and cooperative interaction between contractile ring and central spindle components that is essential for MB formation and successful completion of cytokinesis. However, CIT-K is the only contractile ring component so far described to control MB localization of central spindle components, suggesting that this kinase could act at the top of the hierarchy controlling MB formation. Finally, as CIT-K, KIF14, MKL1P1, and PRC1 have all been found mutated in various cancers [catalogue of somatic mutations in cancer (COSMIC) database, www.sanger.ac.uk], our findings might have implications for the development of cancer therapies.

Materials and Methods

Drosophila cells were grown in serum-free medium supplemented with antibiotics. dsRNA production and RNAi treatments were performed as described (24). HeLa Kyoto cells were maintained in DMEM (Invitrogen) supplemented with 10% (vol/vol) FCS and antibiotics. CIT-K RNAi knockdown was performed using the siRNAs described in ref. 9.

Gateway technology (Invitrogen) was used in all cloning procedures, as described (24). For in vitro binding assays, [35S]methionine-labeled fragments were transcribed and translated in vitro using the TnT T7 Quick Coupled Transcription/Translation System (Promega), and GST-tagged proteins were expressed in bacteria, using the pDEST15 vector (Invitrogen).

More detailed description of expression vectors, immunostaining procedures, and transmission electron microscopy can be found in SI Materials and Methods.

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Supporting Information

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SI Materials and Methods

Affinity Purification and Mass Spectrometry. For protein A (PtA) purifications, ~10⁹ cells were harvested by centrifugation and frozen in liquid nitrogen. The cell pellet was then resuspended in 5 mL extraction buffer [50 mM Hepes at pH 7.5, 100 mM KAc, 50 mM KCl, 2 mM MgCl₂, 2 mM EGTA, 0.1% (vol/vol) Nonidet P-40, 5 mM DTT, 5% (vol/vol) glycerol, and Roche Complete Protease Inhibitors] and homogenized using a high-performance disperser (Fisher). Homogenates were agitated at 4°C for 30 min and clarified by centrifugation at 6,000 rpm in an SS34 rotor (Sorvall, Thermo Scientific). Next, 200 µL Dynabeads (Invitrogen) conjugated to rabbit IgG (Sigma) were added to the supernatants and incubated for 4 h under continuous agitation at 4°C. Beads were then washed five times for 10 min in 10 mL extraction buffer. Proteins were then eluted from beads with 0.5 M NH₄OH and 0.5 mM EDTA, concentrated, and analyzed by liquid chromatography-mass spectrometry (LC-MS)/MS. The MS/MS fragmentation data achieved were used to search the National Center for Biotechnology Information and Flybase databases, using the MASCOT search engine (www.matrixscience.com). Probability-based MASCOT scores were used to evaluate identifications. Only matches with P < 0.05 for random occurrence were considered significant.

In Vivo Pull-Down Assays. Six million cells stably expressing either PtA:StiCc1 (the N-terminal region of the Sti coiled-coil domain is dubbed “StiCc1”) or PtA alone were transfected with plasmids encoding GFP::Neb [Neb stands for Nebblish, the fly counterpart of human kinesin family member 14 (KIF14)] and Pav::Myc [Myc stands for Pavarotti, the Drosophila ortholog of human mitotic kinesin-like protein 1 (MKLP1)], and Myc stands for myelocytomatisis viral oncogene homolog] transgenes, using the FuGene HD transfection reagent (Promega) and incubated at 25°C for 24 h. Beads were then washed five times for 10 min in 10 mL extraction buffer. Proteins were then eluted from beads with 0.5 M NH₄OH and 0.5 mM EDTA, concentrated, and analyzed by liquid chromatography-mass spectrometry (LC-MS)/MS. The MS/MS fragmentation data achieved were used to search the National Center for Biotechnology Information and Flybase databases, using the MASCOT search engine (www.matrixscience.com). Probability-based MASCOT scores were used to evaluate identifications. Only matches with P < 0.05 for random occurrence were considered significant.

Molecular Biology, Cell Culture, DNA, dsRNA, and siRNA Transfections. Gateway technology (Invitrogen) was used in all cloning procedures as described (1). The destination vectors used for expression in Drosophila cultured cells were described (1–3). The pDEST15 vector (Invitrogen) was used for bacterial expression of GST-tagged proteins. Drosophila cDNAs were obtained from the Drosophila Genomics Resource Center. The MKLP1 cDNA was a gift of M. Mishima (University of Warwick, Warwick, United Kingdom). The CIT-K region corresponding to StiCC1, CIT-K (420–785) was amplified by PCR, using total RNA from HeLa Kyoto cells, the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen), and the following primers: forward, TACAGCAAGGCACTTGGGGATT, and reverse, CTCCAGTGTCTCCTTGTCAGC.

The Dmel strain of Schneider 2 (S2) cells (Invitrogen) was grown in serum-free medium supplemented with antibiotics. dsRNA production and RNAi treatments were performed as described previously (1). Primers used to generate sti dsRNAs were described (4); the primers used to generate the neb dsRNA were, forward, GCACCGGTATTTGACTGAGTGTGTTG, and reverse, ATCTTCATGCTTACGCGGAGTGTGAGT. DNA transfections and generation of fly stable cell lines were described (3).

HeLa Kyoto cells were maintained in DMEM (Invitrogen) supplemented with 10% FCS and antibiotics. CIT-K RNAi knockdown was performed using the siRNAs described in Grueneberg et al. (4) and Lipofectamine RNAiMax (Invitrogen), following the manufacturer’s instructions.

In Vitro Binding Assay. DNA fragments coding for Sti, Neb, Pav, and MKLP1 were generated by PCR and cloned into pDEST15 (Invitrogen) to express N-terminal GST-tagged polypeptides in Escherichia coli. The GST-tagged products were then purified using Glutathione Sepharose 4B (GE Healthcare), according to the manufacturer's instructions. [³⁵S]Methionine-labeled Sti, Neb, Pav, Fascetto, and CIT-K fragments were prepared from corresponding PCR products amplified using primers harboring a T7 promoter, and then transcribed and translated in vitro, using the TnT T7 Quick Coupled Transcription/Translation System (Promega) in the presence of [³⁵S]methionine (Perkin-Elmer). GST pull-down assays were carried out as described (5).

Antibodies and Fluorescence Microscopy. The following antibodies were used in this study: mouse monoclonal anti-α-tubulin (clone DM1A, Sigma), chicken polyclonal anti-α-tubulin (Abcam, ab989984), mouse monoclonal anti-Myc (clone 9E10, Santa Cruz Biotechnology), rabbit polyclonal anti-Myc (Abcam, ab9106), rabbit polyclonal anti-Sticky (6), rabbit polyclonal anti-Pav (7), rabbit polyclonal anti-Neb (a kind gift of D. M. Glover and H. Okhura, University of Cambridge, Cambridge, United Kingdom), mouse monoclonal anti-CIT-K (BD Laboratories), rabbit polyclonal anti-KIF14 (Bethyl Laboratories), rabbit polyclonal anti-MKLP1 (clone sc-867, Santa Cruz Biotechnology), rabbit polyclonal anti-PRC1 (a kind gift of F. Barr, Oxford University, Oxford, United Kingdom) (4). Peroxidase and Alexa-fluor conjugated secondary antibodies were purchased from Jackson Laboratories and Invitrogen.

Dmel cells were grown on 22 x 22-mm coverslips (Menzel-Gläser) and fixed in PHEM [1.4 piperazinediethanesulfonic acid (PIPES), Hepes, EGTA, and MgCl₂] buffer (60 mM PIPES, 25 mM Hepes at pH 7, 10 mM EGTA, 4 mM MgCl₂, 3.7% formaldehyde) for 12 min. HeLa cells were also plated on coverslips but fixed in ice-cold methanol for 10 min. All cell types were then processed and visualized as described (5). ImageJ software was used to generate RGB fluorescence profiles.

Transmission Electron Microscopy. Cells were plated on coverslips and fixed overnight at 4°C in 2.5% glutaraldehyde in PBS, postfixed for 1 h in 1% OsO₄ in PBS, dehydrated in a graded series of alcohols embedded in Epon, and polymerized for 2 d at 60°C. Glass slides were separated from the resin after a short
immersion in liquid nitrogen. Sections were obtained with a LKB ultratome, stained with uranyl acetate and lead citrate, and observed and photographed with a Philips CM10 electron microscope at 80 kV.


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**Fig. S1.** StiCC1 forms a complex with Neb and Pav in vivo. S2 cells stably expressing either the StiCC1 fragment tagged with PtA or PtA alone were co-transfected with plasmids expressing GFP::Neb and Pav::Myc for 48 h and treated with the Cdk1 inhibitor RO-3306 2 h before collection, and then proteins were extracted and used in PtA pull-down assay. The extracts and pull downs were analyzed by Western blot to detect GFP, Myc, and PtA. The numbers on the left indicate the sizes, in kilodaltons, of the molecular mass marker.

**Fig. S2.** StiCC1 localization to the midzone requires Neb. Drosophila S2 cells stably expressing GFP::StiCC1 were treated with dsRNAs directed against kanamycin (control) or neb for 96 h and then fixed and stained to detect GFP, tubulin, and DNA (blue). Arrowhead marks GFP::StiCC1 localization to the spindle midzone. (Scale bars, 10 μm.)
**Fig. S3.** Analysis of Neb depletion in *Drosophila* S2 cells. (A) Western blot analysis of Neb protein levels in *Drosophila* S2 cells treated for 96 h with either kanamycin (kana, control) or neb dsRNAs. The arrows mark the bands corresponding to Neb and a nonspecific band used as loading control. The numbers on the left indicate the sizes in kilodaltons of the molecular mass marker. (B) *Drosophila* S2 cells were treated with dsRNAs directed against kanamycin (control) or neb for 96 h and then fixed and stained to detect Neb, tubulin, and DNA (blue). (Scale bars, 10 μm.)

**Fig. S4.** Localization patterns of CIT-K, KIF14, and MKLP1 during cytokinesis in HeLa cells. (A) HeLa (Kyoto) cells were fixed and stained to detect CIT-K, KIF14, and tubulin. (B) HeLa (Kyoto) cells were fixed and stained to detect CIT-K, MKLP1, and tubulin. The insets show a 2.5× magnification of the midbody. (Scale bars, 10 μm.)

**Table S1.** Identification of Neb and Pav by affinity purification and mass spectrometry

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<th>Bait and interactors</th>
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<th>Peptides</th>
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The two kinesins, Pav and Neb, identified from the PtA affinity purification assays are listed along with their respective MASCOT scores and number of peptides. Tumbleweed/RacGAP50C is the RacGAP centralspindlin component in *Drosophila*. The baits are indicated in bold font.