Introduction

Most multicellular organisms use asymmetric cell division to generate cellular diversity. In *Caenorhabditis elegans*, the first cell division results in two daughters that are developmentally distinct and unequal in size. The size asymmetry is caused by precise positioning of the mitotic spindle toward the posterior of the cell. The spindle-pulling forces require the motor protein dynein and heterotrimeric G protein signaling. Interestingly, many of the factors implicated in asymmetric force generation (e.g., G\(_{o}\), G\(_{b}\), and dynein) are not obviously asymmetrically distributed (Gönczy et al., 1999; Gotta and Ahringer, 2001; Grill et al., 2001; Gotta et al., 2003; Srinivasan et al., 2003). In an effort to understand how asymmetric forces are generated, we developed a method to specifically measure processive dynein motor activity.

In yeast cells, microtubules (MTs) slide along the cortex in a dynein-dependent manner (Adames and Cooper, 2000). Therefore, we sought to quantify similar MT movements at the *C. elegans* cortex. In contrast to the simple MT arrays in yeast cells, *C. elegans* asters contain thousands of MTs that obscure the movements of individual polymers. To better resolve MT movements, we used a GFP-tagged EB1-like EBP-2 (end-binding protein-2; Srayko et al., 2005; Kozlowski et al., 2007) to develop an EB1 velocity assay (EVA). Because EB1 binds to the plus ends of growing MTs, the velocity of EB1 dots relative to the MT minus end represents the growth rate of an individual MT polymer. Furthermore, if the MT moves because of an external force, the EB1 particle will move with a velocity equal to the MT growth rate plus or minus the velocity caused by the external force. A previous study has shown that MT growth rates in the one-cell *C. elegans* embryo are consistent and reproducible between different samples in the same cell cycle stage and throughout the cytoplasm (Srayko et al., 2005). Therefore, we reasoned it should be possible to track EB1 to study the effects of dynein on MT gliding movements at the inner embryonic cortex.

Results and discussion

A population of dynein motors at the embryonic cortex propels MTs during anaphase

Most MTs that contact the cortex grow from centrosomes, where they are anchored at their minus ends and would not be...
expected to dislodge if pulled on by a motor. In a wild-type embryo, most MTs rapidly depolymerize (~1.4 s) after touching the cortex and do not grow or slide along the cortical surface (Kozlowski et al., 2007). Correspondingly, an important feature of EVA involves the creation of free, unanchored MTs that continue to grow and can slide along the cortex in response to external forces. We released MTs from the centrosome using a temperature-sensitive allele of mei-1 katanin, mei-1(c466). MEI-1 is normally female meiosis specific, but the c466 mutation causes katanin to persist into the first mitotic division in which it concentrates at centrosomes and chromatin, ectopically severing MTs (Fig. 1 A; Clandinin and Mains, 1993; Clark-Maguire and Mains, 1994; Srayko et al., 2000). Indeed, mei-1(c466) embryos exhibited many EB1 particles moving along the cortex (Video 1), indicating that MT plus ends that approach the cortex at shallow angles do not readily depolymerize. Thus, ectopic MT severing facilitated the tracking of EB1 particles along the inner embryonic cortex and allowed the detection of motor-dependent MT movements (Fig. 1, A and B). Additionally, because dynein motor activity is affected by applied load (Mallik et al., 2004), the katanin-generated free MTs allowed a direct comparison of cortical dynein motor activity under similar light-load conditions.

Dynein motors can exhibit varying walking speeds in vitro (Mallik et al., 2004), which raised the possibility that different areas of the one-cell cortex could modulate dynein speed to achieve asymmetric spindle-pulling forces. However, laser-based centrosome disintegration experiments suggested individual motor speeds need not be different between anterior and posterior cortices, and only the net force applied to the spindle poles appears to be asymmetric (Grill et al., 2003). As such, a higher number of force generators are predicted to be active at the posterior cortex (Grill et al., 2003). Using EVA, we sought to determine whether anterior–posterior asymmetry of force correlates with (a) differences in mean motor-dependent MT gliding velocity or (b) differences in the proportion of cortical MTs that exhibit dynein-dependent gliding.

We assayed embryos in late metaphase/anaphase because pulling forces are active at this time (Grill et al., 2001; Labbé et al., 2004; McCarthy Campbell et al., 2009). To measure EB1 movements caused by MT polymerization alone, we first measured the velocities of EB1 dots moving in a straight line away from the centrosome in the midplane of the embryo. We determined that <2% of EB1 dots emanating from the centrosomes (i.e., anchored MTs) exceeded 1.0 µm/s in control (empty vector) RNAi embryos (Fig. S1). Additionally, previous work showed dynein on the nuclear envelope can propel EB1 to move MTs (Fig. 1 A; Clandinin and Mains, 1993; Clark-Maguire and Mains, 1994; Srayko et al., 2000). Indeed, mei-1(c466) embryos exhibited many EB1 particles moving along the cortex (Video 1), indicating that MT plus ends that approach the cortex at shallow angles do not readily depolymerize. Thus, ectopic MT severing facilitated the tracking of EB1 particles along the inner embryonic cortex and allowed the detection of motor-dependent MT movements (Fig. 1, A and B). Additionally, because dynein motor activity is affected by applied load (Mallik et al., 2004), the katanin-generated free MTs allowed a direct comparison of cortical dynein motor activity under similar light-load conditions.

lin-5(RNAi) decreases the number of ddMTVs at the inner cortex

EVA detected EB1 movements at the inner cortex, but it is formally possible that long MTs with only their plus ends contacting the cortex could be propelled by motor proteins located in subcortical regions of the cytoplasm. To confirm that cortical located dynein is required for the ddMTVs in EVA, we depleted LIN-5. LIN-5 localizes dynein to the inner cortex and is also required for anaphase pulling forces (Srinivasan et al., 2003; Afshar et al., 2005; Nguyen-Ngoc et al., 2007). However, LIN-5 does not interfere with most other postmeiotic dynein-dependent processes that occur deeper in the cytoplasm, such as mitotic spindle assembly (Video 3; van der Voet et al., 2009). Therefore, we tested lin-5(RNAi) embryos to determine whether cortical ddMTVs were affected. lin-5(RNAi) significantly reduced the proportion of ddMTVs (Fig. 2 A). The clustering algorithm was unable to identify a population >1.0 µm/s in the lin-5(RNAi) dataset. However, a small number of ddMTVs were present above our cutoff value, which we estimated to represent 2.9% maximally (20/679; see Materials and methods), resulting in a significant decrease compared with control values (P = 3.5E−9; α = 0.01; Fig. 3 A). In agreement with previous work (Nguyen-Ngoc et al., 2007), our data are consistent with LIN-5 linking dynein to the cortex. This result also confirmed that EVA detects cortical ddMTVs specifically.

An anterior-like cortex has more ddMTVs than a posterior-like cortex

We next determined whether there were any differences in ddMTVs at the posterior versus anterior cortices. Because MTs at the cortex could conceivably span both the anterior and posterior domains in a wild-type embryo, we simplified our analysis by generating either an anterior- or posterior-like cortex on a whole embryo scale (via par-2(RNAi) or par-3(RNAi), respectively). Previous work showed that anaphase pulling forces on both spindle poles are anterior-like in par-2(RNAi) and...
Modulation of heterotrimeric G protein signaling does not significantly change the proportion of ddMTVs

Members of the heterotrimeric G protein pathway regulate cortical force generation and are implicated in the activation of dynein motors at the cortex. Simultaneous depletion of two Ga subunits (GOA-1 and GPA-16; Go) or the G protein activators GPR-1 and GPR-2 (hereafter referred to as GPR-1/2) severely reduce the rate and extent of anaphase spindle pole separation (Video 4; Colombo et al., 2003; Nguyen-Ngoc et al., 2007). Therefore, the proportion of ddMTVs was expected to decrease significantly when these factors were eliminated. However, we found no significant reduction in the proportion of ddMTVs upon RNAi of Go (9.73%, 66/681; P = 0.58), gpr-1/2 (10.96%, 62/568; P = 0.87), or lis-1, a dynein accessory protein implicated in posterior-like in par-3(RNAi) (Grill et al., 2001). We expected that ddMTVs in the par-2(RNAi) would be significantly decreased. However, we observed a significant increase in the number of ddMTVs in the par-3(RNAi) posteriorized cortex compared with control values (13.90%, 100/716; P = 0.043; α = 0.01; Figs. 2 C and 3 A). Surprisingly, these results indicated that proportionately more dynein motors were active in the anterior (where spindle-pulling forces are weaker) than in the posterior (where spindle-pulling forces are higher). In light of this result, we next tested components of the heterotrimeric G protein pathway for their effect on ddMTVs.
Figure 2. EVA analysis in various RNAi treatments. (A–H) The distribution of EB1 velocities >1.0 µm/s in RNAi-treated mei-1(ct46) embryos. The insets are the entire velocity distribution; the boxed areas within the insets are enlarged. The dotted lines are the computationally derived Gaussian population with a mean >1.0 µm/s. n = number of cortical EB1 trajectories. Each histogram and population analysis used pooled data (at least five embryos from at least two independent RNAi trials).
in cortically located anaphase spindle-pulling forces (13.38%, 120/903; \( P = 0.070 \) at \( \alpha = 0.01 \); Figs. 2, D–F, and 3 A; Videos 5, 6, and 7; Cockell et al., 2004). The \( \text{lis-1} \text{ (RNAi)} \) result was surprising because LIS-1 is required for a wide variety of dynein-dependent processes (Cockell et al., 2004). However, LIS-1 is implicated in affecting dynein motor activity under high load (McKenney et al., 2010), and EVA measures motor activity under low load, which might explain why no decrease in ddMTV proportion was observed in \( \text{lis-1} \text{ (RNAi)} \) embryos. We next tested whether ectopic activation of G protein signaling would increase the proportion of ddMTVs. \( \text{gpb-1} \text{ (RNAi)} \) embryos have reduced G\( \beta \) levels and exhibit an overall increase in cortical pulling forces during anaphase (Video 4; Gotta and Ahringer, 2001; Afshar et al., 2005). However, \( \text{gpb-1} \text{ (RNAi)} \) did not increase the proportion of ddMTVs compared with control values (10.55%, 62/589; \( P = 0.11 \); \( \text{gpr-1/2} \text{ (RNAi)} \), \( P = 0.85 \); \( \text{lis-1} \text{ (RNAi)} \), \( P = 5.0 \times 10^{-4} \); \( \text{nmy-2} \text{ (RNAi)} \), \( P = 0.012 \); \( \text{dnc-1} \text{ (RNAi)} \), \( P = 1.5 \times 10^{-4} \). The double asterisks indicate significance at \( \alpha = 0.01 \). Population analysis used pooled data (at least four embryos from at least two independent RNAi trials, except \( \text{L4440} \text{ (RNAi)} \) and \( \text{dnc-1} \text{ (RNAi)} \), which were from one RNAi trial).

To test whether cortical contractility affects ddMTVs, we depleted the nonmuscle myosin NMY-2. NMY-2 is required for polarity establishment and force generation in the embryo (Cuenca et al., 2003; Munro et al., 2004), possibly through the modulation of the elasticity of underlying substrates to which force generators like dynein could be linked (Kozlowski et al., 2007). As with the G protein activators, RNAi of \( \text{nmy-2} \) also failed to significantly alter the proportion of ddMTVs compared with control values (13.38%, 97/723; \( P = 0.087 \); \( \text{par-2} \text{ (RNAi)} \), \( P = 6.5 \times 10^{-4} \); \( \text{par-3} \text{ (RNAi)} \), \( P = 0.44 \); \( \text{gpa-1} \text{ (RNAi)} \), \( P = 4.6 \times 10^{-3} \); \( \text{gpr-1/2} \text{ (RNAi)} \), \( P = 0.11 \); \( \text{gpr-1/2} \text{ (RNAi)} \), \( P = 0.85 \); \( \text{lis-1} \text{ (RNAi)} \), \( P = 5.0 \times 10^{-4} \); \( \text{nmy-2} \text{ (RNAi)} \), \( P = 0.012 \); \( \text{dnc-1} \text{ (RNAi)} \), \( P = 1.5 \times 10^{-4} \)). Population analysis used pooled data (at least four embryos from at least two independent RNAi trials, except \( \text{L4440} \text{ (RNAi)} \) and \( \text{dnc-1} \text{ (RNAi)} \), which were from one RNAi trial).

Figure 3. Relative proportions and mean velocities of the cortical ddMTV populations. (A) The proportion of EB1 velocities in the ddMTV population are shown. All RNAi was performed in a \( \text{mei-1} \text{ (ct46)} \) background. \( \text{dhc-1} \text{ (RNAi)} \) and \( \text{lin-5} \text{ (RNAi)} \) subpopulations >1.0 \( \mu \text{m/s} \) were not detected by the computer algorithm and were estimated (Materials and methods). (B) Mean velocity of the ddMTV subpopulation in micrometers per second. For \( \text{dhc-1} \text{ (RNAi)} \) and \( \text{lin-5} \text{ (RNAi)} \), the mean velocity was not measurable (Materials and methods). Error bars represent SEM. \( \text{par-2} \text{ (RNAi)} \), \( P = 6.5 \times 10^{-4} \); \( \text{par-3} \text{ (RNAi)} \), \( P = 0.44 \); \( \text{gpa-1} \text{ (RNAi)} \), \( P = 4.6 \times 10^{-3} \); \( \text{gpr-1/2} \text{ (RNAi)} \), \( P = 0.11 \); \( \text{par-1} \text{ (RNAi)} \), \( P = 5.0 \times 10^{-4} \); \( \text{nmy-2} \text{ (RNAi)} \), \( P = 0.012 \); \( \text{dnc-1} \text{ (RNAi)} \), \( P = 1.5 \times 10^{-4} \). The double asterisks indicate significance at \( \alpha = 0.01 \). Population analysis used pooled data (at least four embryos from at least two independent RNAi trials, except \( \text{L4440} \text{ (RNAi)} \) and \( \text{dnc-1} \text{ (RNAi)} \), which were from one RNAi trial).
pulling forces on the mitotic spindle while still maintaining a population of active cortical motors. Even though cortical dynein is capable of exerting force on individual MTs through lattice interactions, this behavior alone is not sufficient for the spindle-pulling force in anaphase.

To understand the potential function of MT gliding, we noted that ddMTVs were most abundant during pronuclear migration and centrosome/nuclear centration, before metaphase (29.5%, 69/235; Fig. 4 A). LIN-5, the only component other than dynein found to reduce ddMTVs, is also required for efficient pronuclear/centrosome centering (Park and Rose, 2008). Therefore, we predicted that any genes required for pronuclear/centrosome centering, but not anaphase, should perturb ddMTVs without affecting anaphase force. We tested dynactin, a protein implicated in increasing dynein motor processivity along the MT lattice (King and Schroer, 2000). In worm embryos, dnc-1(RNAi) causes pronuclear/centrosomal complex centration defects but centrosomes still separate around the male pronucleus, suggesting that some dynein-dependent processes are still functional (23/23 embryos, this study; Skop and White, 1998; O’Rourke et al., 2011). Furthermore, we found ddMTVs were significantly reduced in dnc-1(RNAi) during anaphase (10.7 vs. 6.1%; P = 1.5E $-$ 4) as well as pronuclear migration/centration (29.5 vs. 5.3%; P = 3.2E $-$ 4; Fig. 4 B) at $\alpha = 0.01$. Despite the observed centration defects, dnc-1(RNAi) embryos exhibited relatively dynamic centrosome movement during anaphase, with centrosomes separating just before cytokinesis at rates similar to control RNAi (Fig. 4, C and D; and Video 10). We conclude that dynactin is required for robust MT gliding at the cortex, and this behavior contributes to pronuclear/centrosome centering.

Figure 4. dnc-1(RNAi) reduces cortical MT gliding but not centrosome separation. (A) Cortical ddMTV proportions throughout the wild-type (WT) cell cycle. In the one-cell embryo, the proportion of ddMTVs increased during pronuclear migration/centration compared with anaphase (29.5%, 69/234; P = 2.1E $-$ 6). mei-1(ct46) embryos were used for pronuclear migration/centration and slow phase stages. L4440[RNAi] mei-1(ct46) embryos from Fig. 1 C were used for anaphase. (B) dnc-1(RNAi) reduces the proportion of ddMTVs during pronuclear migration/centration (5.3%, 21/397; P = 3.2E $-$ 4) and anaphase (6.1%, 63/1,040; P = 1.5E $-$ 4) compared with control. Significance at $\alpha = 0.01$ in A and B is indicated by double asterisks. Data in A and B were derived from at least four embryos pooled before population analysis from single RNAi trials (except pronuclear migration/centration dnc-1(RNAi) from two independent RNAi trials). (C) Both dnc-1(RNAi) and dhc-1(RNAi) embryos exhibit defects in centration [Video 10], but only dhc-1[RNAi] significantly reduced centrosome separation rates in anaphase. The box represents 53 ± 2 s before cytokinesis furrow ingression (t = 0). n = 4 embryos. (D) Mean rates of centrosome separation for the interval boxed in C are shown. SEM at 95% confidence is shown.
Two distinct mechanisms of dynein-dependent force generation at the cortex

Based on our results, we suggest there are at least two distinct mechanisms of dynein-dependent force generation at the cortex: one that moves MTs only through motor–lattice interactions (and is responsible for ddMTVs in EVA), and a second that generates spindle-pulling forces through a mechanism that does not use processive MT motor “walking” activity alone. In budding yeast cells, spindle positioning utilizes both lateral and end-on MT interactions with cortical force generators (Carminati and Stearns, 1997; Adames and Cooper, 2000). Interestingly, end-on MT interactions seem to be strongly correlated with spindle displacement (Carminati and Stearns, 1997). As in yeast, our data suggest two mechanistically distinct modes of MT pulling in *C. elegans*.

Because MTs predominantly polymerize from the centrosomes, it is likely that cortically located force generators interact with MT plus ends (Gönczy, 2002; Grill et al., 2003). Although it is difficult to detect MT end-on interactions in the *C. elegans* embryo, one study revealed the existence of cortical invaginations at the embryonic cortex during anaphase (Redemann et al., 2010). These invaginations are dependent on dynein and members of the G protein pathway and probably represent a major contribution to spindle-pulling forces in anaphase. However, MT gliding could contribute to residual spindle movements by pulling on MTs that contact the cortex at shallow angles. Our observations suggest that these MTs are rare in wild-type anaphase (because of geometrical constraints) but abundant during the first embryonic prophase when the pronuclear/centrosomal complex migrates to the center. We postulate that cortical MT gliding and end-on interactions as well as cytoplasmic pulling forces (Kimura and Kimura, 2011) could each contribute to centrosome/pronuclear centering.

The two modes of dynein–MT interactions proposed in Fig. 5 are probably not used exclusively, but rather, their relative proportions could be altered to regulate pulling force. Because GPR-1/2 is present at a higher level at the posterior cortex during metaphase/anaphase and is required for force asymmetry (Colombo et al., 2003; Gotta et al., 2003; Tsou et al., 2003), this could be a key factor that determines the different modes of MT pulling at the cortex. In specifying cortical polarity, the partitioning-defective proteins could also determine which pulling mechanism is used (i.e., via GPR-1/2 localization; Colombo et al., 2003; Gotta et al., 2003; Tsou et al., 2003). In this way, *par-2(RNAi)* would be expected to shift the balance away from MT end interactions to more lateral MT interactions, explaining our observed increase in the number of ddMTVs in these embryos.

In conclusion, we have shown that cortical dynein-dependent MT gliding behavior is not sufficient to generate significant pulling forces during anaphase in the one-cell embryo; rather, it is implicated in generating forces to help center the spindle in prophase. Our results fit well with computer simulations that suggest force generation by dynein motor activity along the MT lattice alone is probably insufficient (Grishchuk et al., 2005). Instead, the ability of a cortically localized factor to hold on to a depolymerizing MT could provide sufficient levels of force (Grishchuk et al., 2005). In support of this idea, normal MT dynamics are required for force generation and asymmetric spindle positioning in the embryo (Nguyen-Ngoc et al., 2007). Therefore, we suggest that the modulators of anaphase forces do not turn dynein motor activity on/off, per se, but rather, they might facilitate end-on interactions between MTs and dynein.
Materials and methods

RNAi by feeding

RNAi plates (nematode growth medium agar, 1.0 mM IPTG, and 25 mg/ml carbenicillin) were seeded with the desired RNAi feeding clone and cultured overnight at RT to express double-stranded RNA. Control RNAi was the L4440 RNAi feeding vector [Addgene, Inc.; A. Fire, Stanford University School of Medicine, Stanford, CA] lacking an insert. RNAi feeding clones used in this study are previously described in Kamoth et al. (2003) with the exception of gpa-16, which was cloned using the primers 5′-GCCCC-GGGGTCCTAAAGAAGGCGG-3′ and 5′-AAACTAGTTGGAATAGGGC-3′. Some genes exhibited phenotypes that were recognizable in the MAS94 L4 hermaphrodites were left for ≥36 h on the RNAi plates. 

Nmy-2(RNAi), a 1:1 mixture of the two cultures was seeded onto RNAi plates. 

Goa-1(RNAi) and GPR-2(RNAi), as these proteins are 96% identical at the nucleotide level (Srinivasan et al., 2003). In the case of unc-119(ed3)abcIs3[pie-1-ebp-2–gfp;unc-119(+)] and nmy-2(RNAi), or ≥25 h (dnc-1(RNAi) and par-2(RNAi), see Text S7). Therefore, GPR-1/2 protein levels were not always obviously symmetric, which is consistent with previous studies (Colombo et al., 2003; Tsou et al., 2003). Therefore, GPR-1/2 protein levels were assessed directly via Western blotting performed on embryonic lysates from a cohort of gpr-2(RNAi) worms used in parallel for EVA (Fig. S3 B). R2B543 L4 hermaphrodites that contain a deletion in the efa-6 gene (genotype: efa-6(ok3533) IV; created by the elegans Gene Knockout Consortium; provided by the Caenorhabditis Genetics Center) were incubated for ≥24 h on mel26(RNAi) plates.

Imaging and temperature control protocol

Worms used for ex utero imaging were dissected in 340 mM osm egg buffer (188 mM NaCl, 48 mM KCl, 2 mM CaCl2, 2 mM MgCl2, and 25 mM Heps, pH 7.3) and mounted on a thin 2% agarose pad. Imaging was performed with an inverted microscope (IX81; Olympus; 60x, NA 1.42 oil objective) with a spinning-disc confocal head (CSU10; Yokogawa) modified with a condenser lens in the optical path (Quorum Technologies). All imaging was performed with EB1-GFP-wt (ORCA-R2; genotype: unc-119(ed3)abcIs3[pie-1-ebp-2–gfp;unc-119(+)]; Video 4) or transgenic worms expressing fluorescent markers PAR-2-GFP and PAR-6–mCherry (MAS1, genotype: tils153; ddsIs26 [par-2–gfp; rol-6(su1006); par-6;mCherry; unc-119(+)]; Fig. S3 A). For gpr-1(RNAi), the anaphase phenotype was not always obviously symmetric, which is consistent with previous studies (Colombo et al., 2003; Tsou et al., 2003). Therefore, GPR-1/2 protein levels were assessed directly via Western blotting performed on embryonic lysates from a cohort of gpr-2(RNAi) worms used in parallel for EVA (Fig. S3 B).

Tracking EB1-GFP and centrosomes

Image (National Institutes of Health) was used to track the movement of EB1 points, using the Image particle detector and tracker program (Salzarini and Kounoumtakos, 2005). TIFF image stacks were converted into 8-bit gray scales. Object size was limited to a 3-pixel radius, and the search area was restricted to a 10-pixel radius in the following frame. Threshold values were adjusted to maximize the number of EB1 particles detected in the first frame. The resulting tracks were filtered to show only those present for at least five frames (totaling 1.2 s or greater). All tracks were manually inspected to ensure that a single EB1 particle was followed. Excel 2008 (Microsoft) was used to calculate the velocity and create graphs. For growth rate measurements made in the embryo midplane, centrosomes were tracked manually with ImageJ and subtracted from the EB1 positions to correct for centrosomal movement. Centrosome positions for Fig. 4 were obtained by manually tracking x, y, and z positions using Meta Morph software. TH32 (genotype: unc-119(ed3) ruts32Il; ddsIs6) embryos were imaged with a spinning-disc confocal microscope as described in the previous section using z-stack (2-µm spacing; 7-s interval between stacks) or single-plane time-lapse acquisition.

The first visible sign of cytokinesis furrow ingestion was used as a reference (t = 0).

Statistical analysis

Resulting velocities for each set of RNAi embryos were imported into MATLAB software (MathWorks). Velocity data points from all embryos in a given RNAi trial were pooled before population analysis. The mmvn_toolkit was used to determine the mean velocity, variance, and proportion of normal and Gaussian populations in the entire velocity datasets. The mean velocity and variance of the Gaussian populations were found via the mmvn.fit(x,x) function (in which X represents the velocity dataset and k represents the number of normal populations/clusters that the program will search for). To detect a subpopulation having a mean value exceeding 1.0 µm/s, we increased the k value until a population >1.0 µm/s was detected by the clustering algorithm to a maximum of k + 5 subpopulations. In the dxh-1(RNAi) and lin-5(RNAi) datasets, no recognizable population of velocities was found >1.0 µm/s within the search parameters. Assuming that the remaining high velocity EB1 particles in these two trials have a normal distribution and the same mean ddMTV value as the control RNAi treatment (1.14 µm/s), then the proportion of velocities in this population can be estimated using the following equation: (bin values > 1.14) × 2 + (bin value of 1.12) = 2.575. Mean velocities of the ddMTV populations were compared with the control L4440(RNAi) value via a two-tailed Student’s t test assuming unequal variances at α = 0.01.

Western blotting

Western blotting was performed to confirm the absence of GPR-1/2 in the gpr-2(RNAi) embryos as previously described (Hannak et al., 2002). In brief, L4440(RNAi) L4 hermaphrodites were plated with Escherichia coli expressing either gpr-2 or control L4440 double-stranded RNA at 25°C for 24 h. After incubation, 250 worms each from the gpr-2(RNAi) and L4440(RNAi) trials were picked into tubes with 1 ml M9 buffer (22 mM KH2PO4, 42 mM NaHPO4, 85 mM NaCl, and 1 mM MgSO4).

Worms were pelleted at 1,000 rpm for 1 min and washed 3x in M9, leaving a final volume of 50 µl. Freshly prepared 2x bleach solution (27.3 µl H2O, 5 µl of 10M NaOH, and 17.5 µl NaClO) was added to each tube and the embryos were lysed in a water bath sonicator for 10 min at RT followed by boiling at 95°C for 5 min. Samples were loaded into a 10% SDS-PAGE resolving gel. A prestained broad-range protein marker (7–175 kD; New England Biolabs, Inc.) was used to determine the relative mass of the proteins. After electrophoresis, the proteins were transferred to a Hi-band N nitrocellulose membrane. The membrane was blocked for 6 h (4°C) in TBST (200 mM Tris-HCl, pH 7.4, 500 mM NaCl, and 0.05% Tween 20) + 5% skim milk. The GPR-1/2 antibody (a gift from L. Rose, University of California, Davis, CA) and D1MA α-tubulin antibody were both used at 1:5,000 in TBST + 4% skim milk and incubated for 1 h at RT. Goat anti-rabbit and goat anti–mouse HRP-bound secondary antibodies (Bio-Rad Laboratories) were used at 1:5,000 in TBST + 4% skim milk. Antibody binding was detected via an ECL kit (SuperSignal West Pico; Thermo Fisher Scientific).
Online supplemental material

Fig. S1 shows EB1-GFP velocities from the centrosomes. Fig. S2 shows efo-6(ok3533) cortical EB1 measurements. Fig. S3 shows RNAi control treatment analysis. Video 1 shows the effect of katanin-mediated release of MTs from the centrosome. Video 2 shows a representative dhc-1(RNAi); mei-1(ct46); ebp-2–gfp one-cell C. elegans embryo. Video 3 shows a representative lin-3(RNAi); mei-1(ct46); ebp-2–gfp one-cell C. elegans embryo. Video 4 shows DIC videos of ebp-2–gfp C. elegans embryos to confirm RNAi phenotypes for a parallel-fed cohort of worms used for cortical EB1 measurements. Video 5 shows a representative gpa-1/gpl-16(RNAi); mei-1(ct46); ebp-2–gfp one-cell C. elegans embryo. Video 6 shows a representative gpr-1/2(RNAi); mei-1(ct46); ebp-2–gfp one-cell C. elegans embryo. Video 7 shows a representative lsa-1(RNAi); mei-1(ct46); ebp-2–gfp one-cell C. elegans embryo. Video 8 shows a representative gpb-1(RNAi); mei-1(ct46); ebp-2–gfp one-cell C. elegans embryo. Video 9 shows a representative nmy-2(RNAi); mei-1(ct46); ebp-2–gfp one-cell C. elegans embryo. Video 10 shows centrosome separation in wild-type, dhc-1(RNAi), or dhc-1(RNAi)-treated TH32 γ-tubulin–gfp; histone-gfp one-cell embryos. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201103128/DC1.

We thank Drs. Adriana Dawes and Leslie Rose for comments on the manuscript. This work was funded by grants from Canadian Institutes of Health Research (CIHR), Alberta Heritage Foundation for Medical Research (AHFMR), and Alberta Ingenuity (AI). M. Srayko was supported by scholar awards from AHFMR and CIHR, and E.M. Gusnowski was supported by awards from the Natural Sciences and Engineering Research Council and AI.

Submitted: 24 March 2011
Accepted: 5 July 2011

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