Intracellular organelles mediate cytoplasmic pulling force for centrosome centration in the *Caenorhabditis elegans* early embryo

Kenji Kimura and Akatsuki Kimura[1]

Cell Architecture Laboratory, Center for Frontier Research, National Institute of Genetics, Mishima 411-8540, Japan

Edited by Judith Kimble, University of Wisconsin, Madison, WI, and approved November 23, 2010 (received for review September 9, 2010)

The centrosome is generally maintained at the center of the cell. In animal cells, centrosome centration is powered by the pulling force of microtubules, which is dependent on cytoplasmic dynein. However, it is unclear how dynein brings the centrosome to the cell center, i.e., which structure inside the cell functions as a substrate to anchor dynein. Here, we provide evidence that a population of dynein, which is located on intracellular organelles and is responsible for organelle transport toward the centrosome, generates the force required for centrosome centration in *Caenorhabditis elegans* embryos. By using the database of full-genome RNAi in *C. elegans*, we identified *dyrb-1*, a dynein light chain subunit, as a potential subunit involved in dynein anchoring for centrosome centration. DYRB-1 is required for organelle movement toward the minus end of the microtubules. The temporal correlation between centrosome centration and the net movement of organelle transport was found to be significant. Centrosome centration was impaired when Rab7 and RILP, which mediate the association between organelles and dynein in mammalian cells, were knocked down. These results indicate that minus end-directed transport of intracellular organelles along the microtubules is required for centrosome centration in *C. elegans* embryos. On the basis of this finding, we propose a model in which the reaction forces of organelle transport generated along microtubules act as a driving force that pulls the centrosomes toward the cell center. This is the first model, to our knowledge, providing a mechanical basis for cytoplasmic pulling force for centrosome centration.

endosome | lysosome | pronuclear migration | the centrosome-organelle mutual pulling model | yolk granule

The centrosome is a major microtubule-organizing center in animal cells. It is generally positioned in the cell center. The central positioning of the centrosome is critical for proper microtubule-dependent cellular activities such as cell division and organelle distribution (1–3). Centrosome centration is an active process in which centrosomes move toward the cell center. For example, after fertilization in many species, the centrosomes associated with the male pronucleus migrate from the cell periphery toward the cell center (4–6). Centrosome centration is achieved by forces that act through the microtubules (6–8). Actin filaments are reported to modulate the speed of centration, but they are not essential for this process (9, 10). Two microtubule-dependent forces, namely, the pushing force and pulling force, are thought to position the centrosome to the cell center. The pushing force is dependent on microtubule polymerization. The plus ends of the growing microtubules can push the cell cortex to move the centrosome away from the cortex (11). Centration of microtubule-organizing centers in fission yeast is driven by microtubule pushing forces (12, 13). In contrast, the major driving force for centrosome centration in animal cells is microtubule pulling forces through the action of cytoplasmic dynein, a minus end-directed motor protein complex (14, 15). The pulling force, but not the pushing force, accounts for the behavior of the centrosomes in *Caenorhabditis elegans* embryos (16). However, it is still unclear where cytoplasmic dynein is anchored inside the cell to pull the centrosomes toward the center (6, 17–20). In the present report, we refer to the structure as the centrosome centration anchor.

The centrosome centration anchor may be located at the cell cortex (17). Cytoplasmic dynein has been reported to function at the cortex in many cells (21–24). However, these cytoplasmic dyneins at the cortex may not be required for centrosome centration. In sand dollar eggs, centrosome centration occurs in a cortex-independent manner (25). In large amphibian eggs, the plus ends of microtubules do not reach the cell cortex in the traveling direction during centrosome centration (20). In *C. elegans*, molecules involved in anchoring dynein at the cell cortex are identified, but they are dispensable for centrosome centration. Gα proteins (GOA-1, GPA-16) and their regulators GPR-1 and GPR-2 are involved in anchoring dynein at the cortex (23, 24), and are required for the asymmetric displacement or rocking movements of the mitotic spindle. For centrosome centration, Gα/GPR are dispensable and have an inhibitory effect because the centrosomes reach the cell center within a shorter time in Gα/GPR-suppressed embryos (26–28). It should be noted here that Gα/GPR-dependent forces can modulate centrosome centration as knockdown of Gα/GPR regulators, LET-99 or casein kinase 1 (CSNK-1), positions the centrosomes at a posterior or anterior position, respectively (29, 30). The observations mentioned earlier collectively indicate that dyneins anchored at locations other than the cortex can sufficiently drive centrosome centration.

Alternatively, the centrosome centration anchor may be located throughout the cytoplasm. In this case, the pulling force per microtubule increases as the length of the microtubule increases because the longer the microtubule, the more contact with cytoplasmic dynein (6). This length-dependent pulling mechanism was initially proposed by Hamaguchi and Hiramoto in sand dollar eggs (25). In a computer simulation assuming that the centrosome centration anchors are located throughout the cytoplasm, the in vivo profile of centrosome centration in *C. elegans* was reproduced (16). However, we are aware of no reports that have elucidated the actual structure in the cytoplasm that anchors cytoplasmic dynein to pull the centrosomes (19).

The purpose of this study was to characterize the centrosome centration anchor. By using *C. elegans* embryos, we found that DYRB-1, which belongs to the dynein light chain/roadblock (LC7) family, is selectively involved in centration. Interestingly, DYRB-1 was also required for minus end-directed movement of organelles. Knockdown of genes involved in organelle movement revealed that minus end-directed transport of organelles is tightly

Author contributions: K.K. and A.K. designed research; K.K. performed research; K.K. and A.K. analyzed data; and K.K. and A.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: akkimura@lab.nig.ac.jp.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1013275108/-/DCSupplemental.

linked to centrosome centration. These results support the mutual pulling model in which organelles function as cytoplasmic anchors to generate pulling forces for centrosome centration.

Results

A Dynein Light Chain Subunit, DYRB-1, as a Molecule Selectively Involved in Centrosome Centration Anchor. We investigated male pronuclear migration in the *C. elegans* embryo as a model for studying the centrosome centration mechanism. After fertilization, the centrosomes and associated male pronucleus migrate from the cell periphery (i.e., near sperm-entry site) to the cell center (Fig. S1*A* and Movie S1) (4, 5). We first searched for genes specifically involved in centrosome centration anchor by using the PhenoBank database, in which each phenotype during early embryogenesis in *C. elegans* under full-genome RNAi (19,075 genes) is recorded and categorized (31). In this database, the genes involved in the anchor should be categorized into the P0 pronuclear centration and P0 pronuclear migration (male) defect groups. A total of 56 genes were found in these groups.

To exclude genes required for the motor activity of dynein and the elongation of microtubules, we examined whether the male and female pronuclei met in embryos defective for these genes. This pronuclear meeting occurs during centration and is also dependent on long microtubules and the dynein heavy chain subunit in *C. elegans* (DHC-1) (32, 33). Therefore, if the genes specifically required for the centrosome centration anchor are inhibited, the meeting of the male and female pronuclei occurs, but the nucleus–centrosome complex does not reach the cell center. We viewed all of the videos (N = 726) in PhenoBank showing the phenotypes of RNAi of the 56 genes and calculated the frequency of the videos displaying the expected phenotype (Table S1). For nine genes, more than 50% of the RNAi embryos showed the expected phenotype. We then checked the phenotype of RNAi-treated cells for the nine genes ourselves and confirmed the phenotype for eight genes (Table 1).

These eight genes included genes known to be dispensable for centration, such as *gpb-1* and *gpc-2*. Disruption of these genes inhibits centration via hyperactivation of the cortical pulling force (26–28). To exclude such genes, we performed RNAi of the eight genes in embryos with reduced cortical pulling force. To eliminate the cortical pulling force, *gpr-1* and *gpr-2* were knocked down by RNAi (Movie S2) (34, 35). Consistent with a previous report (27), the centration defect in *gpb-1* (RNAi) or *gpc-2* (RNAi) embryos was lost in *gpr-1/2* (RNAi) embryos, which indicates that *gpb-1* and *gpc-2* were not involved in centrosome centration anchor (Table 1). *let-754* and *lrg-1* were also eliminated as they showed similar phenotypes (Table 1). *dnc-1*, *dnc-2*, and *arp-1* were also eliminated in this stage of the screening for a different reason. These genes were eliminated because knockdown of these genes in the *gpr-1/2* (RNAi) embryos inhibited the meeting of pronuclei. We found that these embryos were defective in centrosome separation (Table 1 and Table S2). Without centrosome separation, elongation of astral microtubules is sterically hindered, and thus the pronuclear meeting was inhibited.

dyrb-1 was the only gene for which RNAi produced a centration defect but no meeting defect in more than 50% of the embryos, even under the *gpr-1/2* RNAi condition (Table 1). DYRB-1 in the *C. elegans* embryo was characterized in past studies, and its involvement in centrosome centration was mentioned (23, 36). *dyrb-1* (RNAi) embryos exhibited pronuclear meeting, but further centration was significantly impaired (Fig. 1*A* and Movie S3). The centrosomes in *dyrb-1* (RNAi) embryos fail to reach the cell center [i.e., 50% of egg length (EL)], but still reach approximately 40% of EL (Fig. 1*A*). We suspected that this partial centration in *dyrb-1* (RNAi) embryos was caused by the effect of pronuclear meeting. Pronuclear meeting is thought to be driven by pulling between male and female pronuclei through the function of dyneins anchored on the surface of pronuclei by a nuclear membrane protein ZYG-12 (32). When we eliminated the effect of pronuclear meeting by using a *zyg-12* mutant, the centrosome in *dyrb-1* (RNAi) embryos reach only 27% of EL (Fig. 1 *A* and *B*).

dyrb-1 encodes a light chain subunit of cytoplasmic dynein and thus may reasonably function with(in) dynein (23, 36). One role of dynein accessory subunits such as DYRB-1 is to mediate specific associations between cytoplasmic dynein and various cellular structures, such as intracellular organelles (37–40). Therefore, we suspected that DYRB-1 had a direct role in mediating the association between dynein and the centrosome centration anchor.

Intracellular Localization of GFP::DYRB-1. Previously, O'Rourke et al. and Couwenbergs et al. characterized DYRB-1 in *C. elegans* (23, 36). O'Rourke et al. focused on the repressive aspects of DYRB-1 in the regulation of DHC-1 activity (36). Because both DHC-1

Table 1. Effect on pronuclear meeting and centration of knockdown of the top nine genes identified in PhenoBank analysis as involved in centration

					Under *gpr-1/2* (RNAi)‖		
Rank*	Genotype	Meeting defect†	Centration defect‡	Score, %§	Meeting defect†	Centration defect‡	Score, %§
1	*dyrb-1*(RNAi)	0/9	9/9	100	2/13	13/13	85
2	*dnc-1*(RNAi)	2/7	7/7	71	8/12	12/12	33
2	*dnc-2*(RNAi)	2/6	6/6	67	4/9	7/9	33
3	*arp-1*(RNAi)	5/12	12/12	58	8/11	11/11	27
4	*gpb-1*(RNAi)	0/10	10/10	100	0/14	2/14	14
5	*let-754*(RNAi)	0/9	6/9	67	0/13	0/13	0
5	*gpc-2*(RNAi)	0/8	8/8	100	0/13	0/13	0
5	*lrg-1*(RNAi)	0/12	12/12	100	0/15	0/15	0
—	*csn-1*(RNAi)	0/14	4/14	29	—	—	—
—	WT¶	0/17	0/17	0	0/7	0/7	0
—	*dhc-1*(RNAi)	6/7	6/7	0	8/8	8/8	0

*Ranking of genes based on score [under the *gpr-1/2* (RNAi) condition].
†Number of embryos displaying pronuclear meeting defect divided by total number of embryos in each RNAi.
‡Number of embryos displaying centration defect divided by total number of embryos in each RNAi.
§Percentage of embryos displaying centration defect but not pronuclear meeting defect.
‖Knockdown of top eight genes performed under the *gpr-1/2* (RNAi) condition.
¶CAL0092 strain was used as WT.

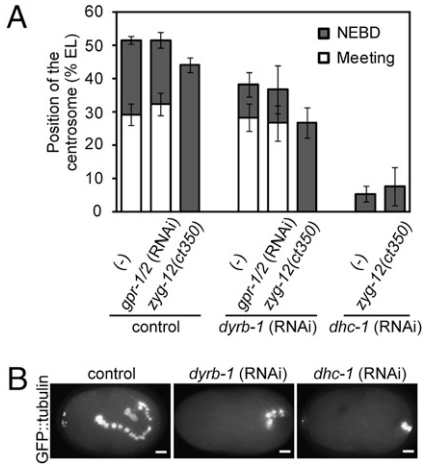

Fig. 1. DYRB-1 is required for centrosome centration. (*A*) Mean position of the centrosomes at pronuclear meeting (white bars) and nuclear envelope breakdown (NEBD, gray bars) under the indicated conditions are shown. In *dhc-1* (RNAi) embryos or the *zyg-12*(*ct350*) background at restrictive temperature, pronuclear meeting did not occur. Position is expressed as percentage of EL (posterior-most side of the egg, 0%). The average value ± SD is shown ($n \geq 5$). (*B*) Time-lapse images of control (untreated), *dyrb-1* (RNAi), and *dhc-1* (RNAi) embryos expressing GFP-tubulin under the *zyg-12*(*ct350*) background at restrictive temperature were projected onto single images to visualize the trajectory of the centrosomes movement until NEBD. (Scale bars, 5 μm.)

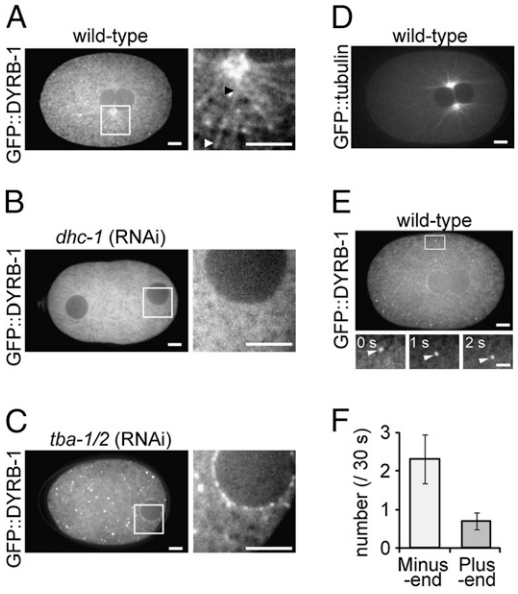

Fig. 2. Cytoplasmic localization of GFP::DYRB-1 during centrosome centration. Confocal fluorescence images of *C. elegans* embryos expressing GFP::DYRB-1 are shown (*A*–*C*). The boxed region in each panel is also shown magnified (*Right*). The arrowheads (black and white) in *A* indicate both ends of a filamentous signal of GFP::DYRB-1: (*B*) *dhc-1* (RNAi) and (*C*) *tba-1/2* (RNAi). (*D*) The pattern of astral microtubules revealed using a GFP::tubulin strain. (*E*) Tracking of DYRB-1 puncta. A fraction of DYRB-1 shows a punctate signal, a portion of which moves directionally as shown in the lower magnified images (bar, 2 μm). (*F*) The direction and frequency of fast (≥0.5 μm/s) and continuous (≥2 s) movements of DYRB-1 puncta within 30 s after pronuclear meeting ($n = 10$). The average number ± SEM is shown. (Scale bars, 5 μm except in *E*, *Lower*.)

and DYRB-1 positively contribute to centrosome centration, the role of DYRB-1 as a DHC-1 repressor should not function in the process of centrosome centration. Couwenbergs et al. focused on the function of the cortical fraction of DYRB-1, which acts with Gα and GPR proteins and regulates spindle positioning (23). Because incomplete centration was caused by *dyrb-1* RNAi even in the *gpr-1/2* loss-of-function background (23) (Fig. 1*A*), DYRB-1 has Gα/GPR-dependent and Gα/GPR-independent functions, and the latter is required for centrosome centration.

This Gα-independent function for centrosome centration is unlikely to be mediated by the cortical fraction of DYRB-1. If a cortical pulling force were responsible for centration, one would expect many microtubules to make contact with the anterior cortex. Visualization of microtubule plus ends during centrosome centration indicated that few microtubules reach the anterior cortex compared with those that reach the opposite direction (i.e., posterior) (Fig. S1 *B*–*E*). This observation suggests that pulling forces produced at the anterior cortex contributes little to centrosome centration.

To reexamine the localization of DYRB-1, we generated transgenic worms expressing GFP fused to DYRB-1 (GFP::DYRB-1). This construct partially rescued the defect in organelle transport (*DYRB-1 Is Required for the Minus End-Directed Movement of Organelles*) caused by RNAi targeting 3′-UTR of *dyrb-1* (Fig. S2). GFP::DYRB-1 was weakly localized throughout the cytoplasm, the cortex, and the spindle in early embryos (Fig. 2) (23, 36). We found filamentous signals of GFP::DYRB-1 that were similar to the pattern of astral microtubules (Fig. 2 *A* and *D*). Some GFP::DYRB-1 was observed in punctate form, which occasionally moved toward the centrosomes (Fig. 2 *E* and *F* and Table S3). The filamentous signal was not observed when DHC-1 or microtubules were impaired by RNAi-mediated knockdown of DHC-1 or α-tubulin (TBA-1/2), respectively (Fig. 2 *B* and *C*). The punctate signals were not observed in *dhc-1* (RNAi) embryos (Fig. 2*B*), or did not show apparent centrosome-directed movement in *tba-1/2* (RNAi) embryos (Table S3). These results indicate that cytoplasmic DYRB-1 moves along the astral microtubules in a dynein motor activity-dependent manner during centrosome centration.

DYRB-1 Is Required for the Minus End-Directed Movement of Organelles. Because cytoplasmic DYRB-1 moves along microtubules, we investigated whether organelle movement along microtubules toward the minus ends occurs during centrosome centration and whether DYRB-1 is involved in the movement. We visualized the movements of early endosomes, yolk granules, or lysosomes by using strains expressing GFP::EEA-1 (FYVE*2) or GFP::VIT-2 or by exposing the worm to the lysosome marker reagent LysoTracker, respectively. In addition to the early endosomes as reported previously (41), yolk granules and lysosomes showed extensive movement toward the centrosomes during centrosome centration (Fig. 3).

These minus end-directed movements of organelles were significantly reduced in *dyrb-1* (RNAi) embryos (Fig. 3*G*). The result indicates a role of DYRB-1 in the cytoplasm during centrosome centration in mediating the interaction between DHC-1 and the organelles to move the organelles along microtubules toward the centrosomes. We did not detect colocalization of DYRB-1 with the organelles (e.g., lysosomes, RAB-5–positive vesicles). The number of motor proteins associated with intracellular vesicles is known to be small (42, 43) and thus hard to be detected by confocal microscopy.

We sought to determine whether the function of DYRB-1 in moving organelles is dependent on Gα/GPR. The minus end-directed movements of organelles were not impaired by RNAi-mediated knockdown of GPR-1/2 (Fig. 3*G*). Because centrosome centration and organelle movements were both mediated by a Gα/GPR-independent function of DYRB-1, we suspected causal relation between the two processes in a way that intracellular organelles associated with dynein serve as the centrosome centration anchor.

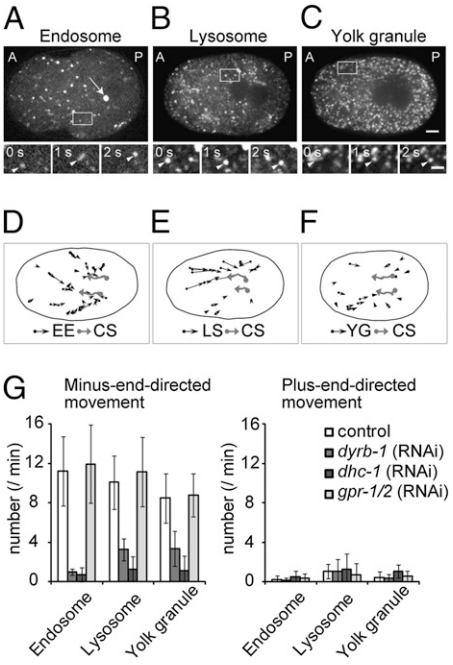

Fig. 3. Movements of intracellular organelles during centrosome centration. Confocal fluorescence images of (*A*) an embryo expressing both GFP::TBG-1 (arrow) and GFP::EEA-1(FYVE*2), (*B*) an embryo in which the lysosomes were stained with LysoTracker, and (*C*) an embryo expressing the yolk granule marker GFP::VIT-2. A, anterior; P, posterior. (Scale bar, 5 μm.) Three sequential fluorescence images of the boxed regions in *A*–*C* are magnified (bar, 2 μm) below each panel. The arrowhead marks a moving organelle. (*D*–*F*) A typical example of the movements of early endosomes (EE), lysosomes (LS), or yolk granules (YG; ≥0.5 μm/s, black arrows) for 2 min after pronuclear meeting is illustrated. Black ellipse represents the outline of the egg; gray arrows mark the movement of centrosomes (CS). (*G*) The number of each organelle that moved continuously for at least 2 s at a rate of at least 0.5 μm/s toward the minus end or plus end of the microtubules was counted for 2 min after pronuclear meeting in control, *dyrb-1* (RNAi), *dhc-1* (RNAi), and *gpr-1/2* (RNAi) embryos ($n \geq 5$). The average number ± SD of each organelle per 1 min is shown.

Working Hypothesis: Minus End-Directed Transport of Organelles Generates Cytoplasmic Pulling Forces For Centrosome Centration. How can intracellular organelles be the centrosome centration anchors to produce forces for centrosome centration? Here, we propose a mutual pulling model in which the centrosomes and the organelles pull each other to appropriately position themselves (Fig. 4). A prevalent view of centrosome movement is that dynein is associated with a fixed structure, and upon dynein function, the centrosomes move toward the dynein (Fig. 4*A*, *Left*) (6). In contrast, for organelle movements, the centrosomes are fixed and thus the dynein and associated organelle move toward the centrosome (Fig. 4*A*, *Right*). Inside the cell, however, the centrosomes are not completely fixed and thus should somewhat move toward the dynein and organelle upon organelle movement. Although the movement of the centrosome aster should be small compared with that of a single organelle upon the mutual pulling, the rapid movement of many organelles toward centrosomes should generate significant amounts of force that can move the centrosomes (*Discussion*).

Correlation Between Movement of Early Endosomes and Centrosome Centration. If the movements of the organelles toward the centrosomes generate the pulling force for centration, organelle movements should correlate with centrosome movement. To examine the temporal correlation, we quantified the total movement of the centrosomes and early endosomes every 20 s

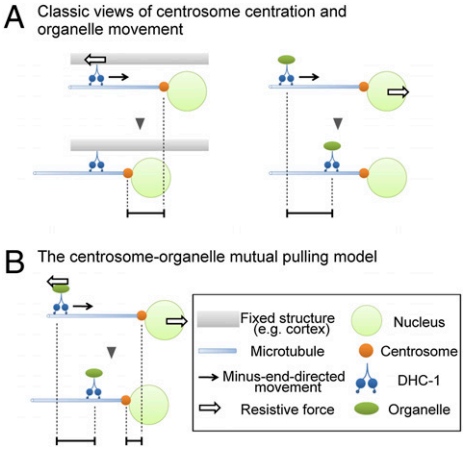

Fig. 4. The centrosome–organelle mutual pulling model for centrosome centration. (*A*) Classic views of centrosome centration and organelle movement in which either dynein (*Left*) or the centrosomes (*Right*) are fixed and the other side (centrosomes or organelles, respectively) moves. (*B*) The centrosome–organelle mutual pulling model. When dyneins slide along the microtubule, both dynein–organelle complexes and the centrosomes move toward each other. This mutual movement positions the centrosomes at the cell center.

from the time just after pronuclear meeting (Fig. 5*A*). Because the centrosomes move mainly along the anterior-posterior (AP) axis of the embryo, we focused on the AP axis component of the movements in the confocal section containing the centrosomes.

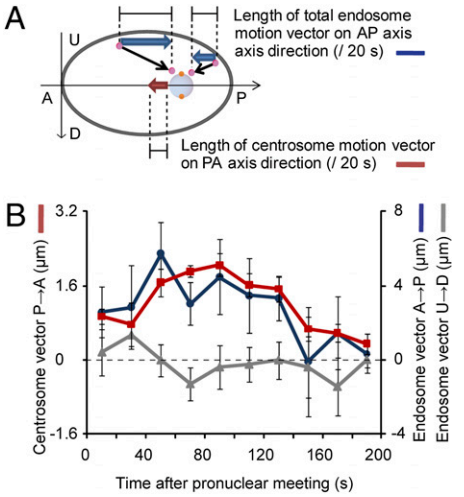

Fig. 5. The correlation between early endosome movement and centrosome centration. (*A*) Schematic of the measurement of the movement of early endosomes [GFP::EEA-1(FYVE*2), blue arrows] and centrosomes (GFP::TBG-1, red arrow) along the AP axis. The net movements of early endosomes in the anterior (A)-to-posterior (P) direction were calculated by subtracting the sum of P-to-A movements from the sum of A-to-P movements. Likewise, the net movements of early endosomes in the perpendicular direction to the AP axis [upside (U) – downside (D)] were calculated. (Gray ellipse, embryo; light blue circle, pronucleus; pink circle, early endosome; black arrow, movement of early endosome.) The length of movement of total early endosomes and the centrosomes was measured during a 200-s period just after pronuclear meeting. (*B*) The average length of movement of total early endosomes (blue line, AP axis component; gray line, perpendicular component) and centrosomes (red line, AP axis component) during each 20-s period on the equatorial plane is plotted ($n = 9$). Left *y* axis, the length of the centrosome movements (μm); right *y* axis, the length of total early endosome movements (μm); *x* axis, time of the measurement from after pronuclear meeting. Error bars represent SEM.

During the period studied, the speed of centrosome movement along the posterior-to-anterior direction was not constant. After the pronuclear meeting, the centrosomes initially moved toward the center at a relatively lower speed, and then the speed increased. As the centrosome neared the center, its speed slowed to near zero (Fig. 5*B*). When we summed the AP axis components of the endosome movements directed toward the posterior and subtracted the sum of those directed toward the anterior (*Materials and Methods*), the net movement gradually increased after the pronuclear meeting but then decreased to nearly zero as the centrosomes reached the cell center (Fig. 5*B*). This change in the net endosome movement is a result of the change in the direction of the movements as the centrosome moves. The correlation between centrosome movement and the net movements of early endosomes was statistically significant ($r = 0.81$; $P < 0.005$). As a negative control, we quantified the endosome movements along the axis perpendicular to the AP axis ("UD axis" in Fig. 5) and confirmed that the net movement along this axis was minimal and did not correlate with the centrosome movement along the AP axis ($r = 0.22$; $P > 0.5$). The strong temporal correlation between the net movement of endosomes and centrosome centration supports the idea that moving organelles drive centrosome centration.

***rilp-1* (C32A3.3), a *C. elegans* Counterpart of RILP, Is Required for Lysosome Transport and Timely Centrosome Centration.** To test whether minus end-directed organelle movements are required for centrosome centration, we attempted to decrease organelle movements by knocking down genes involved in the movements. In mammalian culture cells, the dynein–dynactin complex interacts with RILP, which in turn interact with Rab7, which associates with the surface membrane of late endocytic compartments (44–47). We checked whether this pathway is conserved in *C. elegans*. RNAi knockdown of *rab-7* and C32A3.3, the *C. elegans* putative homologue of RILP (48), significantly reduced the minus-end motility of lysosomes ($P < 0.005$; Fig. 6*A*). The result indicates that the molecular mechanism that anchors lysosomes to microtubules via dynein with RILP and Rab7 is conserved in *C. elegans*. Based on the primary sequence features and depletion phenotype, we named the C32A3.3 gene *rilp-1*, for encoding the *C. elegans* counterpart of RILP.

When minus end-directed movement is impaired in *rab-7* (RNAi) and *rilp-1* (RNAi) embryos, the movement of the centrosomes toward the center was significantly delayed ($P < 0.001$; Fig. 6*B*). As negative controls, we confirmed that other events that are dependent on microtubules and dynein were not delayed under these RNAi conditions. These control events included the fast phase of female pronuclear migration (33) ($P > 0.05$; Fig. 6*C*), the rocking movement of the spindle (24) ($P > 0.05$; Fig. 6*D*), and centrosome separation (14) (Table S2). Selective inhibition of organelle movement and centrosome centration was also observed upon inhibition of *rab-5* (Fig. 6). Rab5 regulates the early endocytic pathways and the motility of early endosomes on microtubules through the action of dynein (40, 49). These results indicate that *rab-7*, *rilp-1*, and *rab-5* do not affect microtubule- or dynein-dependent processes in general. Importantly, RNAi of these genes did not affect the rocking movement of the spindle (Fig. 6*D*), which was affected by *dyrb-1* RNAi through the regulation of Gα/GPR-dependent cortical pulling forces (Movie S3) (23). This finding suggests that the functions of *rab-7*, *rilp-1*, and *rab-5* are independent of the function of DYRB-1 in the cortical Gα/GPR pathway and supports the notion that the cytoplasmic fraction of DYRB-1 contributes to centrosome centration.

Discussion

In this study, we propose a model in which the centrosome and organelles pull each other to position the centrosome at the cell center. For centrosome centration, the force exerted upon each microtubule is proposed to be proportional to its length (6, 16, 18, 25), but no mechanical basis for this idea exists. Because the organelles are distributed equally throughout the cell, the resultant pulling force per microtubule will be proportional to the microtubule length. Our model thus provides the first mechanical basis, to our knowledge, for the microtubule length-dependent force for centrosome centration.

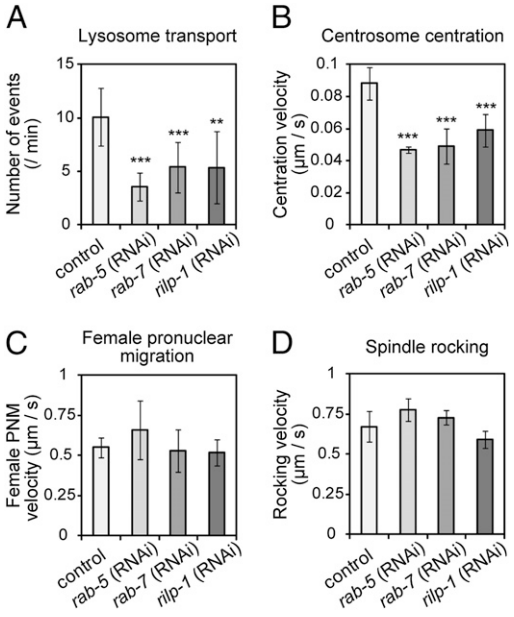

Fig. 6. Centrosome centration was delayed when organelle movement was impaired. (*A*) The average number ± SD of lysosomes that moved continuously for at least 2 s at a rate of at least 0.5 μm/s during the 60 s after pronuclear meeting in WT, *rab-5* (RNAi), *rab-7* (RNAi), and *rilp-1* (RNAi) embryos ($n \geq 7$). (*B*) Average velocity ± SD (μm/s) of centrosome centration ($n \geq 7$). (*C*) Average velocity ± SD (μm/s) of female pronuclear migration during the fast phase ($n \geq 8$). (*D*) Average velocity ± SD (μm/s) of the maximum swing of the posterior spindle pole at metaphase ($n \geq 4$). A statistically significant difference from control is indicated by asterisks (***$P < 0.001$, **$P < 0.01$).

In theory, movements of small organelles can produce sufficient force to move the nucleus-centrosome complex for centration. If we assume that the cytoplasm is a viscous fluid for which the Stokes law is valid, the drag force scales with the radius of the objects and its velocity (6, 50). In the *C. elegans* embryo, the pronuclear radius is approximately 5 μm, which is 10-fold larger than the radius of the organelles (e.g., approximately 0.5 μm for early endosomes). In contrast, the average velocity of organelle movement is approximately 1 μm/s, which is actually approximately 10 times faster than that of the nucleus–centrosome complex (0.1 μm/s) during centrosome centration. Therefore, if the Stokes law is valid for the movement of the nucleus–centrosome complex, fast movement of only one small organelle is sufficient to move the complex. The cytoplasm might not behave as an ideal viscous fluid, but this estimation suggests that organelle movements can exert sufficient forces to drive the centration of the nucleus-centrosome complex. Daniels et al. successfully estimated the viscosity in early *C. elegans* embryos (51), suggesting that the cytoplasm behaves as a viscous fluid. Further biophysical studies on the properties of cytoplasm are required for accurate estimation of the force balance under the centrosome centration.

In most cells, the centrosome is centrally located (8). Because cytoplasmic dyneins are required for the centration in many organisms (14, 15, 18), the mutual pulling model can be applied to these cells in general. Characterization of the relationships be-

tween organelle transport and the centration of the centrosomes and other structures in other cells is thus an interesting topic for future study. In the proposed mutual pulling model, we do not have to assume that a specific anchoring structure specialized for centrosome centration exists. Even if the centrosomes and organelles are randomly positioned, self-organization inside the cell will lead to the positioning of the centrosome at the center and the organelles around the centrosome. Centrosome centration is a fundamental feature of animal cells that is important for equal cell division. Our mutual pulling model provides a simple and robust mechanism for centrosome centration and thus the basis for organelle positioning inside the cell.

Materials and Methods

Strains and Manipulation of *C. elegans*. *C. elegans* strains were maintained using standard techniques (52). RNAi was performed by injecting dsRNAs as described previously (53). Details of the strains and the procedure of RNAi experiments are described in *SI Materials and Methods*.

Microscopy, Quantification, and Statistical Analyses. Image acquisition, quantification of the movements of organelles and centrosomes, and statistical analyses were performed as described in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We are grateful to the members of our laboratory for discussion and J. Ahringer (University of Cambridge, Cambridge, United Kingdom), H. Kagoshima, Y. Kohara, N. Kurata, K. Noguchi (National Institute of Genetics, Mishima, Japan), S. Onami (RIKEN, Yokohama, Japan), G. Seydoux (Johns Hopkins University, Baltimore), A. Sugimoto, M. Terasawa (RIKEN, Kobe, Japan), and T. Uemura (Kyoto University, Kyoto, Japan) for providing clones, strains, and technical advice. Strains were also provided by the *Caenorhabditis* Genetics Center, funded by the National Institutes of Health. We appreciate P. Gönczy (École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland), I. Tolic-Nørrelykke (Max-Planck-Institute, Dresden, Germany), M. Ginzberg and M. Wühr (Harvard Medical School, Boston) for reading the manuscript, and S. Bullock (Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom), T. Schüpbach, and E. Wieschaus (Princeton University, Princeton, NJ) for advice. K.K. is a National Institute of Genetics postdoctoral fellow. This study was supported by a grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by the Transdisciplinary Research Integration Center of the Research Organization of Information and Systems, Japan.

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