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3 **A novel mechanism of bulk cytoplasmic transport**

4 **by cortical dynein in *Drosophila* ovary**

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7 Wen Lu, Margot Lakonishok, Anna S. Serpinskaya, Vladimir I. Gelfand*

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9 Department of Cell and Developmental Biology, Feinberg School of Medicine,
10 Northwestern University, Chicago, IL 60611

11 *Correspondence: vgelfand@northwestern.edu

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13 **Abstract**

14 Cytoplasmic dynein, a major minus-end directed microtubule motor, plays
15 essential roles in eukaryotic cells. *Drosophila* oocyte growth is mainly dependent on the
16 contribution of cytoplasmic contents from the interconnected sister cells, nurse cells. We
17 have previously shown that cytoplasmic dynein is required for *Drosophila* oocyte growth
18 and assumed that it simply transports cargoes along microtubule tracks from nurse cells
19 to the oocyte. Here we report that instead of transporting individual cargoes along
20 stationary microtubules into the oocyte, cortical dynein actively moves microtubules
21 within nurse cells and from nurse cells to the oocyte via the cytoplasmic bridges, the
22 ring canals. This robust microtubule movement is sufficient to drag even inert
23 cytoplasmic particles through the ring canals to the oocyte. Furthermore, replacing

24 dynein with a minus-end directed plant kinesin linked to the actin cortex is sufficient for
25 transporting organelles and cytoplasm to the oocyte and driving its growth. These
26 experiments show that cortical dynein performs bulk cytoplasmic transport by gliding
27 microtubules along the cell cortex and through the ring canals to the oocyte. We
28 propose that the dynein-driven microtubule flow could serve as a novel mode of fast
29 cytoplasmic transport.

Introduction

Microtubules perform many key cellular functions, such as cell division, migration, polarization/compartimentation, and intracellular long-range cargo transport. Cytoplasmic dynein (referred simply as dynein hereafter) is the major minus-end directed microtubule motor, and involved in numerous microtubule-based functions¹. In interphase cells, dynein is the main motor responsible for transporting various cargoes towards the microtubule minus-ends². In dividing cells, dynein functions at kinetochores, spindle poles, and at the cell cortex. Particularly, cortical dynein pulls astral microtubules and is therefore required for positioning the mitotic spindles^{3,4}.

The core dynein complex contains two copies of heavy chain (DHC), intermediate chain (DIC), intermediate light chain (DLIC) and three different dynein light chains (Roadblock, LC8/Cut up and Tctex/Dlc90F)^{2,5} (Figure. 1A). Dynein heavy chain contains a ring of six AAA+ domains, and ATP hydrolysis-induced conformational change results in dynein walking towards the minus-ends of microtubules⁶. The activity of dynein is regulated by the dynactin complex including the largest subunit p150^{Glued}/DCTN1⁷, and the Lis1-NudE complex⁸, as well as several activating adaptors, such as BICD2/BICDL1, Spindly and HOOK1/3^{2,5,9} (Figure. 1A).

Dynein has many essential functions during *Drosophila* oogenesis. First, it is required for germline cell division and oocyte specification^{10,11}. During mid-oogenesis, dynein is required for transport of mRNA ribonucleoproteins (RNPs) and organelles from nurse cells to the oocyte¹²⁻¹⁵. Within the oocyte, dynein transports and anchors the anterior and dorsal determinants that are critical for axis determination for future

embryos¹⁶⁻¹⁸. During vitellogenesis, dynein in the oocyte regulates endocytic uptake and maturation of yolk proteins from the neighboring somatic follicle cells¹⁹.

The *Drosophila* oocyte undergoes dramatic cell growth and polarization during oogenesis²⁰. Remarkably, the oocyte remains transcriptionally quiescent during most of the oogenesis. For its dramatic growth, the oocyte relies on interconnected sister cells, nurse cells, for providing mRNAs, proteins, and organelles through intercellular cytoplasmic bridges called ring canals^{20,21}. Previously, we showed that dynein heavy chain drives oocyte growth by supplying components to the growing oocyte¹⁵. Here, we study the mechanism of dynein-driven transport of cargoes from nurse cells to the oocyte. We find that microtubules, which had previously been considered as static tracks for dynein¹²⁻¹⁵, are robustly moved by dynein within the nurse cell cytoplasm, and more remarkably, from the nurse cell to the oocyte. We further demonstrate that this dynein-powered microtubule gliding creates cytoplasmic advection carrying cargoes in bulk to the oocyte, including neutral particles that do not interact with motors. Furthermore, we show that a chimeric gliding-only minus-end motor anchored to the cortex is sufficient to support transport of cargoes to the oocyte, and therefore to drive the oocyte growth. Finally, we describe here a novel mechanism for dynein-driven cytoplasmic transport: cortically anchored dynein drives microtubule gliding, and microtubules in turn move cytoplasmic contents in nurse cells and through the ring canals, driving oocyte growth. This provides a fast and efficient mode of bulk transport of a wide variety of components supplied by nurse cells to the oocyte.

Results

Dynein is required for oocyte growth

The growing *Drosophila* oocyte is transcriptionally silent and mostly relies on its interconnected sister nurse cells for mRNAs, proteins, and organelles^{20,21}. Dynein, the main minus-end directed microtubule motor in *Drosophila*, has been implicated in nurse cell-to-oocyte transport¹²⁻¹⁵.

Here we investigated the roles of the dynein complex and its regulators in oocyte growth by taking advantage of a germline-specific Gal4, maternal α tubulin-Gal4^[V37], that is expressed in germline cells after the completion of cell division and oocyte specification^{15,22}. This approach bypasses the requirement for dynein in early oogenesis. Knockdown of dynein by expressing either RNAi against dynein components (DHC, DLIC, Lis1) or a dominant negative construct of p150^{Glued}/DCTN1 (*p150^{Glued} Δ C*)¹² driven by this Gal4 line allowed for normal cell division and oocyte specification but caused complete arrest of oocyte growth (hereafter referred as the “small oocyte” phenotype) (Figure 1B-1D”; Figure 1—figure supplement 1A). In spite of the growth inhibition caused by dynein knockdown, we found that the oocyte marker, Orb (oo18 RNA-binding protein)²³ is properly concentrated in the early oocytes, emphasizing that our approach does not interfere with germline cell division or oocyte specification during early oogenesis. However, Orb is clearly dispersed from the small oocytes at later stages (stages 8-9) (Figure 1C”-1D” and 1E; Figure 1—figure supplement 1B), implying defects of nurse cell-to-oocyte transport. These data demonstrate that dynein core components and regulators are indeed essential for oocyte growth, likely via transporting cargoes into the oocyte.

As dynein activity relies on various cargo activating adaptors, we knocked down the *Drosophila* homologs of three main dynein cargo-specific adaptors, BicD, Spindly, and Hook^{9,24} by RNAi in the germ line. Among the three adaptors, knockdown of BicD inhibits the oocyte growth, while *Spindly-RNAi* and *hook-RNAi* do not cause obvious oocyte growth defects (Figure 1F).

This lack of oocyte growth phenotype in *Spindly-RNAi* and *hook-RNAi* animals is not due to low efficiency of the RNAi lines themselves, as knockdown of Spindly and Hook using these RNAi lines display typical loss-of-function mutant phenotypes of these adaptors. Maternal knockdown of Spindly (*mat atub[V37]>Spindly-RNAi*) caused all embryos to fail to hatch (N>200) and zygotic knockdown using a strong ubiquitous Gal4 (*Actin5C-Gal4*) led to 0% eclosion rate from *Spindly-RNAi* pupae (Figure 1—figure supplement 1C)²⁵. Hook is not required for fly viability or fertility, but is required for proper bristle formation²⁶, and *hook-RNAi* animals phenocopied the classic hooked-bristle phenotype observed in *hook* null allele (*hook¹¹*) (Figure 1—figure supplement 1D-1F')²⁶.

This set of data leads us to conclude that BicD is the most important dynein activating adaptor for *Drosophila* oocyte growth.

Dynein drives microtubule gliding in nurse cells

Having established that dynein and its associated proteins are required for the oocyte growth, we next examined dynein tracks, cytoplasmic microtubules. Microtubules are localized inside the intercellular cytoplasmic bridges, the ring canals (Figure 2—

figure supplement 1A-1A'''), consistent with previous reports¹²⁻¹⁵. However, when we examined the dynamics of microtubules in live samples using photoconversion²⁷⁻²⁹, we discovered that microtubules in the nurse cells are not stationary; they vigorously move and snake around in the nurse cells (Figure 2A-2A'; Figure 2-Video 1). Robust microtubule movement in nurse cells can also be seen with fluorescently labeled microtubule-associated proteins (MAPs), Ensconsin/MAP7 (EMTB)^{30,31}, Patronin/CAMSAP^{15,30}, and Jupiter^{15,27,28,30,32} (Figure 2-Videos 2, 3, and 4) as early as in stage 6 egg chambers. Even more remarkably, microtubules are seen moving from the nurse cell to the oocyte through the ring canal. This movement is most robust in stage 9 egg chambers and has an average velocity of ~140 nm/sec (Figure 2C-2E and 2H; Figure 2-Videos 1, 2, 3 and 4). Thus, microtubules are not just static tracks for dynein; instead, they actively move within the nurse cells and from the nurse cell to the oocyte.

When the *Drosophila* egg chambers reach stages 10B-11, nurse cells transfer all their cytoplasmic contents to the oocytes, the process called nurse cell dumping^{21,33}. We examined whether the microtubule movement in the ring canals and associated oocyte growth during mid-oogenesis is a result of an early slow form of nurse cell dumping. We first measured the nurse cell size between stage 8 to stage 10 and found that nurse cells still undergo dramatic growth during this phase (Figure 2—figure supplement 2A). This nurse cell growth from stage 8 to stage 10 is quite distinct from the dumping phase, when nurse cells squeeze their cytoplasm to the oocyte and thus quickly shrink³⁴.

Second, nurse cell dumping requires the activity of non-muscle myosin-II^{35,36}. We tested whether myosin-II is required for stage 9 microtubule movement and overall oocyte growth. We used a RNAi line against the myosin-II heavy chain Zipper (*zip-RNAi*) to knockdown myosin-II activity, and an antibody recognizing phosphorylated myosin-II regulatory light chain (p-MRLC, Ser19) as a readout of myosin-II activity^{36,37}. We found that that myosin-II activity is dramatically diminished in *zip-RNAi* egg chambers (Figure 2—figure supplement 2B-2C'), suggesting that the *zip-RNAi* does inhibit myosin-II efficiently. However, *zip-RNAi* shows no defects in oocyte growth or Orb concentration from early to mid-oogenesis (Figure 2—figure supplement 2D-2G). Additionally, we induced germline clones of a loss-of-function allele of *zip* (*zip*²)³⁸, and found that the majority of *zip*² mutant egg chambers with proper oocyte specification can develop to mid-oogenesis without obvious delay in oocyte growth (25 out of 28 egg chambers; Figure 2—figure supplement 2H-2K). This is consistent with previous reports showing that the myosin-II light chain *spaghetti squash* (*sqh*) mutants^{35,36,39} and the “dumpleless” mutants, such as chickadee, E2F, and RAP150B, develop normally to stages 9~10 without major oocyte growth defects⁴⁰⁻⁴². Thus, we conclude that the microtubule movement through the nurse cell-oocyte ring canals is not a part of myosin-II-driven nurse cell dumping; it truly represents a novel process of nurse cell-to-oocyte transport during mid-oogenesis.

Dynein is known to glide microtubules *in vitro* and *in vivo*⁴³. Therefore, we asked whether dynein is the motor that moves microtubules in nurse cells. Knockdown of dynein using the *Dhc64C-RNAi* line results in a complete inhibition of microtubule movement within the nurse cells (Figure 2B-2B'; Figure 2-Video 5). The microtubules in

dynein-RNAi nurse cells are noticeably less curved than the control ones, implying no motors applying forces to them. More importantly, the motility of microtubules in the nurse cell-oocyte ring canals are dramatically reduced in *dynein-RNAi* (Figure 2-Video 6). In contrast, we found that the microtubule movement through the ring canal is not affected by myosin-II inhibition (Figure 2-Video 6). Altogether these data support the idea that dynein glides microtubules in nurse cells and through ring canals to the oocyte.

Microtubule flow carries cargoes to the oocyte

In addition to microtubule motility, we also observed synchronized movement of cargoes through nurse cell-to-oocyte ring canals in stage 9 egg chambers, at a rate similar to microtubule movement (Figure 2F-2H; Figure 2-Videos 7 and 8), indicating that microtubule-driven cytoplasmic advection occurs in the ring canals. Furthermore, like microtubule movement, bulk cargo movement transport through ring canals is dynein-dependent and myosin-II-independent (Figure 2-Video 9). This bulk cargo movement occurs at stage 9 when the oocyte undergoes the most dramatic growth (Figure 2I; Figure 2—figure supplement 1B-1C). This raises the possibility that, unlike the previously proposed canonical cargo transport (Figure 3A), dynein-powered microtubule gliding itself could create cytoplasmic advection carrying all types of cargoes from nurse cells to the oocyte to support the rapid oocyte growth (Figure 3B).

Therefore, to test this possibility, we simultaneously imaged microtubules and organelles (mitochondria or Golgi units) in the nurse cell-oocyte ring canals, and found that they move together with microtubules through the ring canal to the oocyte (Figure

3-Videos 1 and 2). This further suggests that mitochondria, Golgi units and probably other classes of cargoes could be carried through ring canals in bulk by microtubules gliding towards the oocyte (Figure 3B).

However, we cannot completely exclude the possibility that the motors attached to the cargoes walk on these moving microtubules and thus co-transport cargoes and microtubules through the ring canal. Thus, we decided to examine whether neutral particles that normally are not transported by motors along microtubules can be carried to the oocyte. We used Genetically Encoded Multimeric nanoparticles (GEMs) (Figure 3C), which self-assemble into ~40 nm fluorescent spheres⁴⁴. In *Drosophila* S2R+ cells, GEMs form bright compact particles and display mostly Brownian motion, which is very distinctive from typical movements of endogenous motor-driven organelles, such as lysosomes (Figure 3—figure supplement 1). However, when we expressed GEMs in *Drosophila* ovaries, we found that they move within nurse cells in a fast linear manner (Figure 3D-D'; Figure 3-Video 3). More importantly, GEMs move through the nurse cell-oocyte ring canal (Figure 3-Video 4) and concentrate in the oocytes (Figure 3F-3G). Knockdown of dynein dramatically diminishes GEM linear movements and eliminates GEM accumulation in the oocyte (Figure 3E-3K; Figure 3-Video 3). Altogether, we demonstrate that the direct dynein-cargo interaction is not necessary for nurse cell-to-oocyte transport, and neutral particles can be efficiently carried through the ring canals by dynein-driven microtubule movement.

Microtubule gliding delivers cargoes to oocytes

Next, we investigated whether cortical dynein glides microtubules in nurse cells and transports cargoes to the oocyte. We first examined dynein localization using a GFP-tagged Dlic transgenic line as well as antibodies against *Drosophila* dynein heavy chain and BicD. We found a clear cortical localization of dynein in the nurse cells after the soluble pool of dynein is extracted by detergent (Figure 4—figure supplement 1A-1F).

Then we tested whether a cortically anchored minus-end-directed motor is sufficient to drive oocyte growth. To constrain dynein activity to cell cortex, we replaced the endogenous dynein activating adaptor BicD with an ectopically expressed BicD that is recruited to the actin cortex by an actin-targeting motif, F-Tractin⁴⁵ (Figure 4—figure supplement 1G-1H'). Compared to *BicD-RNAi* in which no egg chambers developed passing stage 7, this cortically-recruited BicD construct allows >70% ovarioles to have egg chambers reaching mid-oogenesis with proper Orb concentration (Figure 4A-4D). In contrast, expression of a tdTomato-tagged F-Tractin alone⁴⁵ (not fused with BicD) did not rescue the oocyte growth defects in *BicD-RNAi* (Figure 4D), indicating that the rescue we observed with F-Tractin-BicD is not due to Gal4 activity dilution or F-Tractin overexpression.

To further test the idea that gliding microtubules drive nurse cell-to-oocyte transport, we created an artificial minus-end gliding-only motor. It contains a dimer motor region of a fast minus-end-directed plant kinesin-14, kin14Vib⁴⁶⁻⁴⁸ targeted to cell cortex with the F-Tractin probe (Figure 4—figure supplement 1G). This chimeric kinesin-14 motor, unlike dynein, cannot carry endogenous *Drosophila* cargoes, allowing us to test whether microtubule gliding alone is sufficient for transporting cargoes to the oocyte

and support the oocyte growth. With this chimeric gliding-only kinesin-14 motor, we found that oocyte growth is partially rescued (Figure 4E-4G and 4K). In addition to the oocyte size rescue, we also found that in more than 95% of the samples, the kinesin-14 motor is able to maintain Orb concentration in the oocyte of stage 8~9 egg chambers (Figure 4H-4J and 4L). The rescue of both oocyte growth and oocyte Orb concentration implies that the gliding-only motor restores the nurse cell-to-oocyte transport.

We directly examined mitochondria movement in the kinesin-14 rescued samples. We observed highly motile mitochondria in nurse cells, and synchronized mitochondria movement from the nurse cells to the oocyte, highly resembling mitochondrial flow in control ring canals (Figure 4-Video 1).

In summary, we use a minus-end gliding-only motor that cannot directly transport cargoes to distinguish the microtubule gliding function from the conventional cargo transport, and show that microtubule gliding by a motor attached to the nurse cell cortex is able to drive organelle movement and thus oocyte growth.

C-terminus of dynein light intermediate chain is sufficient for nurse cell cortical localization

Having established that cortically anchored dynein is the key to glide microtubules and deliver cargoes to the growing oocyte, we decided to investigate which part of the dynein complex is anchored to the nurse cell cortex. As we observed the cortical localization of Dlic-GFP in nurse cells (Figure 4—figure supplement 1B), we decided to make N-terminal and C-terminal truncations of Dlic (DlicNT and DlicCT;

Figure 5A) and examine their localizations in the germ line. The Dlic N-terminus carries a GTPase-like domain and is known to interact with dynein heavy chain via a patch of conserved aromatic residues⁴⁹. The C-terminal Dlic contains the effector-binding domain that interact with BICD, Spindly and Hook-family activating adaptors to form a stable processive dynein-dynactin complex²⁴. We tagged the DlicNT and DlicCT with an optogenetic system LOVTRAP⁵⁰, and in dark LOVTRAP probes bring the two Dlic truncations together (Figure 5A-5B). We found that, while DlicNT or DlicCT truncation alone does not rescue the oocyte defects caused by *Dlic-RNAi*, the DlicNT-DlicCT complex in dark is sufficient to resume the oocyte growth in *Dlic-RNAi* (Figure 5C). It indicates that both Dlic truncations are functional, and both are necessary for restoring the Dlic function in oocyte growth. We next examined the localization of DlicNT and DlicCT truncations in the germ line: DlicNT appears diffused in germline cytoplasm, whereas DlicCT shows a strong cortical localization in the nurse cells (Figure 5D-5E; Figure 5—figure supplement 1A).

Since the DlicCT is known to interact with dynein activating adaptors²⁴ and BicD is the most important activating adaptor for oocyte growth (Figure 1F), we then tested whether BicD is required for the localization of DlicCT to the nurse cell cortex. In *BicD-RNAi* background, DlicCT still localizes to the cortex and mostly evidently at the ring canal regions (Figure 5—figure supplement 1B), indicating that BicD is not essential for recruiting Dlic to the nurse cell cortex.

The dynactin complex includes a short actin-like filament composed of actin related proteins (Arp1 and Arp11) and β -actin that can potentially links dynein to the cell cortex. Previous studies in mitosis revealed that the dynactin complex facilitates dynein

localization at the cell cortex for spindle pulling and positioning^{51,52}. Therefore, we examined the DlicCT localization in the dominant negative p150^{Glued}/DCTN1 mutant (*p150^{Glued}ΔC*) and found that the DlicCT localization is still predominantly cortical with the inhibition of the dynactin complex (Figure 5—figure supplement 1C). On the other hand, Lis1 has been shown to localize at the oocyte cortex and it is required to recruit dynein to the oocyte cortex⁵³. However, we found that the cortical localization of DlicCT in nurse cells is not affected by *Lis1-RNAi* (Figure 5—figure supplement 1D).

Another potential linker could anchor dynein to the cell cortex is the actin-microtubule crosslinker, Short stop (Shot). Previously, we have shown that Shot is localized to the nurse cell cortex and knockdown of *shot* causes oocyte growth defects¹⁵. Therefore, we examined the DlicCT localization in the strongest *shot* loss-of-function mutant (*shot-RNAi* in *shot* heterozygote background), and found that DlicCT still remains cortically-associated (Figure 5—figure supplement 1E).

In conclusion, Dlic C-terminus could facilitate the dynein complex to localize to the nurse cell cortex, independent of the dynein activating components (BicD, dynactin and Lis1) and the actin-microtubule crosslinker Shot.

Finally, we propose that Dlic links the dynein heavy chain to the cell cortex and thus essential for microtubule gliding in nurse cells. Gliding microtubules create cytoplasmic advection within nurse cells and through the ring canals, bringing cytoplasmic contents from nurse cells to the growing oocyte (Figure 5F; Figure 5-Video 1). It remains to be determined which component(s) of the cortical network in nurse cells interact with Dlic and recruit the dynein complex to the cell cortex.

298

299 **Discussion**

300 As the main microtubule minus-end directed motor in animal cells, cytoplasmic
301 dynein is responsible for a wide variety of cellular functions, ranging from cell division to
302 intracellular transport. In *Drosophila* ovary, dynein plays an essential role in nurse cell-
303 to-oocyte transport of mRNAs and organelles¹²⁻¹⁵. This was logically attributed to the
304 conventional mode of dynein-driven transport: the motor attached to the cargo moves
305 on microtubule tracks located inside ring canals and carries cargoes to the oocyte
306 (Figure 3A).

307 In this study, we reveal a novel mechanism of bulk cargo transport by
308 cytoplasmic dynein. First, we show that dynein core components and its regulatory
309 cofactors are required for *Drosophila* oocyte growth (Figure 1). By imaging microtubules
310 in live ovaries, we demonstrate that microtubules are actively moved by dynein from
311 nurse cells to the growing oocytes (Figure 2). Furthermore, we use an artificial cargo
312 that does not bind motors and show that direct dynein-cargo interaction is not necessary
313 for the cargo movement in nurse cells or its transporting to the oocyte (Figure 3),
314 supporting a “go-with-the-flow” mechanism driving cytoplasm transfer from nurse cells
315 to the oocyte. To distinguish cytoplasmic advection from conventional cargo transport,
316 we build a chimeric gliding-only motor by anchoring a minus-end plant kinesin,
317 kin14Vlb, to the cortex, and find that this chimeric cortically-localized motor is sufficient
318 to drive organelle transport and oocyte growth (Figure 4). Lastly, we identify that the C-
319 terminus of Dlic is sufficient to target the dynein complex to the nurse cell cortex (Figure
320 5). Therefore, we propose a novel mechanism of dynein for bulk cargo transport:

cortically anchored dynein glides microtubules in the nurse cells; in turn these gliding microtubules move cytoplasmic contents within the nurse cells and from the nurse cells to the oocyte through the ring canals to the growing oocyte (Figure 5F; Figure 5-Video 1).

A novel phase of nurse cell-to-oocyte transport

Previously, nurse cell-to-oocyte transport has been divided into two phases: the early slow selective phase and the late fast non-selective phase^{21,33}. The early phase is characterized by dynein-driven cargo transport along microtubules to the oocyte¹²⁻¹⁵. The late massive nurse cell-to-oocyte transport phase is known as nurse cell dumping, which occurs at late stage 10B to stage 11^{21,33}.

Here we report a new phase of cytoplasmic advection from nurse cells to the oocyte driven by microtubules that are transported by cortical dynein. This occurs between the two previously described phases, slow selective transport, and fast nurse dumping. As microtubules can drag adjacent contents in the viscous cytoplasm^{29,54}, we believe that this transport is non-selective. This conclusion is supported by the fact that the neutral particles (GEMs) are moved in the nurse cells and concentrated in the oocyte by dynein. Furthermore, we have found that a chimeric gliding-only motor unrelated to dynein that cannot directly transport cargoes along microtubules, plant kinesin-14, is able to rescue mitochondria transport from the nurse cell to the oocyte (Figure 4-Video 1). As we use a motor domain of a moss kinesin-14 that has no known homology with the motor proteins that interact the mitochondrial adaptor protein Milton (e.g., KHC and Myosin 10A)^{55,56}, we conclude that the advection created by

microtubule movement in the ring canals is not cargo-specific, and it is different from the early selective transport phase.

During the dumping phase, nurse cells “squeeze” all the cytoplasmic contents to the oocyte, which is caused by non-muscle myosin-II contraction^{34,36}, and associated with fast cytoplasmic streaming occurring in the oocyte mixing the dumped contents with the ooplasm^{21,54,57}. We reason that the dynein-driven microtubule flow we observed is distinct from nurse cell dumping: (1) it occurs prior to nurse cell dumping and ooplasmic streaming (stages 8~9 versus stages 10B-11); (2) the nurse cell size grows drastically during microtubule flow stages (Figure 2—figure supplement 2A), instead of fast shrinking associated with nurse cell dumping; (3) it requires cytoplasmic dynein, instead of myosin-II activity (Figure 2—figure supplement 2; Figure 2-Videos 6 and 9); (4) the “dumpleless” mutants develop normally without major oocyte growth defects to stages 9~10^{35,36,39-42}, which is noticeably different from the small oocyte phenotype we observed in dynein knockdown (Figure 1).

The dynein-driven microtubule flow occurs concurrently with the most rapid oocyte growth at stage 9 (Figure 2I). During stage 9, the oocyte can grow up to 10X within 6 hours (Figure 2—figure supplement 1B-1C). To achieve such a rapid growth, each of the four nurse cell-oocyte ring canals with a diameter of ~ 6.5 μm is estimated to have a flow of ~150 nm/sec throughout the whole time. This is consistent with our experimental measurements of the velocity of both microtubules and small spherical particles in bright-field images (Figure 2H). Noticeably, mitochondria move at a slightly lower velocity compared to microtubules and small spherical particles, which is

expected for organelles and large particles that are moved by viscous drag created by microtubule gliding (Figure 2H).

Microtubule movement in the nurse cells can be detected as early as in stage 6 egg chambers; however, the fast microtubule movement through the nurse cell-oocyte ring canals only starts in late stage 8 egg chambers, and becomes robust in stage 9 egg chambers. The stage-specific microtubule passage through the ring canals could be controlled by microtubule organization within the ring canals. In early oogenesis (stages 1-6), microtubules are nucleated from the oocyte and grow into the nurse cells with their plus-ends^{58,59}. Even at stages 7-8 the microtubules within the ring canals still contains more plus-ends in the nurse cells and more minus-ends in the oocyte¹⁵. This particular microtubule orientation allows efficient dynein-dependent conventional cargo transport to the oocyte (Figure 3A), but restricts dynein-dependent microtubule movement from nurse cells to the oocyte. Hence, these microtubules in the ring canals could basically function as a plug, and as a result microtubule flow through the ring canals is not often seen at these stages (Figure 2-Video 2). Massive microtubule reorganization occurs during mid-oogenesis (stages 7-8) and creates an anterior-posterior microtubule gradient in the oocyte with more microtubules minus-ends anchored at the anterior and lateral cortex^{30,58,60}. This microtubule reorganization in the oocyte could potentially disassemble the original “wrong” orientated microtubules in the nurse cell-oocyte ring canal, which facilitates the dynein-driven microtubule flow to the oocyte (Figure 2-Video 2; Figure 3B).

To summarize, the dynein-microtubule driven bulk cargo flow from nurse cells to the oocyte presents a novel phase between early selective transport and late non-

selective dumping, which is essential for *Drosophila* oocyte growth. The stage-specific microtubule flow through the ring canals suggests that the complete small oocyte we observed in the dynein mutants are probably caused by the combination of lack of direct transport along microtubules at early stages and absence of microtubule-driven cytoplasmic advection to the oocyte at stage 9.

Dynein anchorage at the cell cortex

In this study, we found that cortical dynein glides microtubules to create local cytoplasmic advection and move cargoes to the growing oocyte. Furthermore, we found that the C-terminus of dynein light intermediate chain (DlicCT) targets the dynein complex to the nurse cell cortex. DlicCT contains the effector-binding domain that interacts with multiple dynein activating adaptors²⁴. Recently, our lab reported that Spindly, a dynein activating adaptor that interacts with DlicCT²⁴ and recruits dynein to the kinetochore in mitosis²⁵, anchors dynein to cortical actin in axons, thus pushing microtubules of the wrong polarity out of the axons in *Drosophila* neurons⁶¹. However, knockdown of Spindly does not disrupt dynein-dependent oocyte growth (Figure 1F). Thus, it indicates that the ovary uses a different specific mechanism for dynein cortical targeting. We showed that cortically recruited BicD is able to rescue the oocyte growth arrest caused by *BicD-RNAi* (Figure 4A-4D), implying that BicD could contribute to the linkage between dynein and the cortex. Other studies also have suggested that the dynactin complex and Lis1 are involved in dynein cortical anchorage⁵¹⁻⁵³. Nevertheless, DlicCT cortical localization is unaffected after inhibition of BicD, dynactin/p150, and Lis1 (Figure 5—figure supplement 1), indicating Dlic uses a novel mechanism of anchoring

dynein to the cortex in nurse cells. Intriguingly, Dlic interacts with the *Drosophila* Par-3 homolog, Bazooka (Baz), and PIP5Kinase Skittles (SKTL) in the ovary⁶², while Baz and SKTL are known to localize at the nurse cell cortex^{62,63}. In vertebrates, dynein light intermediate chain 2 interacts with Par-3 and 14-3-3 ϵ and ζ to anchor the dynein complex at the cortex for regulating mitotic spindle orientation⁶⁴. Furthermore, NuMA and its *Drosophila* homology Mud link the dynein complex to the cortex via the interaction with membrane localized Gai, for pulling mitotic spindle poles in dividing cells⁶⁵. Recently, it has been shown that NuMA has a Hook domain and a CC1-Box-like Motif, both of which interact with the effector-binding domain of DLIC⁶⁶. Therefore, Baz/Par-3, SKTL, 14-3-3 ϵ and ζ , and NuMA/Mud are all potential linkers of Dlic to the cell cortex. However, loss of SKTL and Mud, mutants of 14-3-3 ϵ and ζ , or displacement of Baz from the cortex does not result in apparent oocyte growth defects, distinct from the dynein mutants^{62,63,67,68}. It is possible that these linkers are genetically redundant with each other to ensure the correct cortical localization of the dynein complex. Thus, disruption of one of the linkers does not cause the dynein complex to fall off from the cortex. Alternatively, Dlic is localized to the cortex independently of Par-3/Baz, SKTL, 14-3-3, and NuMA/Mud, by a new mechanism that awaits further studies.

Furthermore, we consistently observe cytoplasmic advection from the nurse cells to the oocyte in stage 9 egg chambers, suggesting a direction-controlling mechanism underlying the persistent oocyte growth. We speculate that the flow directionality could be attributed to different levels of dynein gliding activity. The dynein complex is strongly localized to the nurse cell cortex, but to a much lesser extent to the oocyte cortex (Figure 4—figure supplement 1). Consistent with the dynein localization, microtubules

are more cortically localized in the nurse cells than in the oocyte (Figure 2E). This differences in dynein localization and microtubule organization may result in a higher dynein-driven microtubule gliding activity in the nurse cells, and therefore creates the directional flow through the ring canals to the growing oocyte.

In addition, this directionality could be controlled by the gatekeeper protein Short stop (Shot). Recently, we demonstrated that Shot is asymmetrically localized at actin fibers of ring canals on the nurse cell side and controls the cargo transport direction between nurse cells and the oocyte¹⁵. Given the nature of Shot's microtubule-actin crosslinking activity, it could serve as an organizer of microtubules along the actin filaments of the ring canals on the nurse cell side and facilitate their transport towards the oocyte.

The “go-with-the-flow” mechanism of cytoplasmic transport

Here we report that the minus-end directed motor, cytoplasmic dynein, glides microtubules, and microtubules in turn drag the surrounding high viscous cytoplasm and transfer cytoplasmic contents from nurse cells to the oocyte. To our knowledge, this is the first report of microtubule gliding by cortical dynein driving cargo movement in interphase cells (the “go-with-the-flow” mechanism).

However, previously, we have demonstrated a similar mechanism for another major microtubule motor, conventional kinesin (kinesin-1). We have shown that kinesin-1 can slide microtubules against each other^{27,28,69-71}, and this microtubule sliding drives ooplasmic streaming, bulk circulation of the entire cytoplasm in late-stage oocytes that

is essential for localization of the posterior determinant, *osk*/Staufen RNPs^{29,54}. Thus, both major microtubule motors, plus-end directed kinesin-1 and minus-end directed dynein, in addition to the canonical mode of cargo transport along microtubules, can drive bulk transport of viscous cytoplasm. Yet kinesin-1 and dynein drive bulk movement in different manners: kinesin-1 drives microtubule sliding against each other, while dynein glides microtubules along the cortex; and for different purposes: kinesin-1 powers intracellular circulation, whereas dynein propels intercellular transport.

This “go-with-the-flow” mechanism is highly efficient for bulk cargo delivery, especially in large cells⁷², such as the oocyte. The dynein-driven cytoplasmic advection allows the oocyte to acquire cytoplasmic materials for its rapid growth. Interestingly, the ring canals have been observed in female germline cells of vertebrate organisms (e.g., human, rabbit, rat, hamster, mouse, chicken, and frog)⁷³. Particularly, it has been shown that cytoplasmic contents such as mitochondria and Golgi material are transferred to the mouse oocyte from interconnected cyst cells in a microtubule-dependent fashion⁷⁴ (Niu W. and Spradling AC. 2021. Mouse oocytes develop in cysts with the help of nurse cells. bioRxiv. doi: <https://doi.org/10.1101/2021.11.04.467284>). As dynein is highly conserved across species, it is important to have further studies to examine whether it plays a similar role in germline cytoplasmic transfer in higher organisms.

Materials and Methods

***Drosophila* strains.** Fly stocks and crosses were maintained on standard cornmeal food (Nutri-Fly® Bloomington Formulation, Genesee, Cat #: 66-121) supplemented with dry active yeast at room temperature (~24–25°C). The following fly stocks were used in this study: *mat atub-Gal4^[V37]* (III, Bloomington *Drosophila* Stock Center #7063); *Act5C-Gal4* (III, Bloomington *Drosophila* Stock Center #3954); *nos-Gal4-VP16* (III, from Dr. Edwin Ferguson, the University of Chicago ^{75,76}); *UAS-Dhc64C-RNAi* (line #1: TRiP.GL00543, attP40, II, Bloomington *Drosophila* Stock Center #36583, targeting *DHC64C* CDS 10044–10064 nt, 5'-TCGAGAGAAGATGAAGTCCAA-3'; line #2: TRiP.HMS01587, attP2, III, Bloomington *Drosophila* Stock Center #36698, targeting *DHC64C* CDS 1302–1322 nt, 5'-CCGAGACATTGTGAAGAAGAA-3') ^{28,77}; *UASp-G^{ΔC}* (1-826 residues of DCTN1/p150^{Glued}, based on the *DCTN1/p150¹* mutation) (II, 16.1, from Dr. Thomas Hays, University of Minnesota) ¹²; *UAS-Lis1-RNAi* (II, from Dr. Graydon Gonsalvez, Augusta University, targeting *Lis1* CDS 1197-1217 nt, 5'-TAGCGTAGATCAAACAGTAAA-3') ¹⁹; *UAS-BicD-RNAi* (TRiP.GL00325, attP2, III, Bloomington *Drosophila* Stock Center #35405, targeting *BicD* 3'UTR 639-659 nt, 5'-ACGATTCAGATAGATGATGAA-3'); *UAS-Spindly-RNAi* (TRiP.HMS01283, attP2, III, Bloomington *Drosophila* Stock Center #34933, targeting *Spindly* CDS 1615–1635 nt, 5'-CAGGACGCGGTTGATATCAAA-3') ⁶¹; *UAS-hook-RNAi* (TRiP.HMC05698, attP40, II, Bloomington *Drosophila* Stock Center #64663, targeting *HOOK* CDS 241-261 nt, 5'-TACGACTACTACAGCGACGTA-3'); *UAS-GFP-RNAi* (Bloomington *Drosophila* Stock Center #41551); *UASp-tdMaple3-atub84B* (II) ²⁹; *UASp-EMTB-3XTagRFP* (III) ³⁰; *mat atub67C-EMTB-3XGFP-sqh* 3'UTR (attP40, an unpublished gift from Yu-Chiun Wang lab, RIKEN Center for Biosystems Dynamics Research); *UASp-GFP-Patronin* (II) (from

Dr. Uri Abdu, Ben-Gurion University of the Negev)^{30,54,78}; *Jupiter-GFP* (protein trap line
 ZCL2183, III)^{27,28,32}; *UASp-LifeAct-TagRFP* (III, 68E, Bloomington *Drosophila* Stock
 Center # 58714); *UASp-F-Tractin-tdTomato* (II, Bloomington *Drosophila* stock center
 #58989)⁴⁵; *UAS-Zip-RNAi* (TRiP.GL00623, attP40, II, Bloomington *Drosophila* Stock
 Center #37480, targeting *Zip* 3'UTR 36-56 nt, 5'-CAGGAAGAAGGTGATGATGAA-3');
hs-FLP^[12] (X, Bloomington *Drosophila* Stock Center #1929); *FRTG13 ubi-GFP.nls* (II,
 Bloomington *Drosophila* Stock Center # 5826); *FRTG13 zip²/CyO* (Bloomington
Drosophila stock center # 8739); *Sqh-GFP* (III, Bloomington *Drosophila* stock center
 #57145); *UASp-Mito-MoxMaple3* (II, and III)¹⁵; *ubi-GFP-Pav* (II, from Dr. David Glover,
 Caltech)⁷⁹; *UASp-RFP-Golgi* (II, Bloomington *Drosophila* Stock Center # 30908, aka
UASp-GalT-RFP)⁸⁰; *pDlic-Dlic-GFP* (II, under the control of its native promoter, from Dr.
 Thomas Hays, University of Minnesota) (Neisch et al., submitted, reagent shared pre-
 publication); *shot^{ΔEGC}* (from Dr. Ferenc Jankovics, Institute of Genetics, Biological
 Research Centre of the Hungarian Academy of Sciences)⁸¹; *UAS-shot^{EGC}-RNAi* (in
 pWalium22 vector, inserted at attP2, III)¹⁵. The following fly stocks were generated in
 this study using either PhiC31-mediated integration or P-element-mediated
 transformation: *UASp-Dlic-RNAi* (targeting *Dlic* 3'UTR 401-421 nt, 5'-
 AGAAATTTAACAACAAAAAAAAA -3', in pWalium22 vector, inserted at attP-9A (VK00005)
 75A10 site, III, M5); *UASp-GEM* (III, M1); *UASp-F-Tractin-Myc-BicD* (II, M4); *UASp-F-*
Tractin-GFP-Kin14Vlb (II, M1); *UASp-Myc-HA-DlicNT-LOV2* at attP14 (36A10, II, M2);
UASp-Zdk1-DlicCT-sfGFP-Myc at attP33 (50B6, II, M2).

Plasmid constructs.

522 pWalium22-Dlic3'UTR-shRNA. The oligos of Dlic3'UTR-shRNA
523 (agtAGAAATTTAACAAAAAAAAAAtagttatattcaagcataTTTTTTTTTTGTAAATTTCTgc)
524 were synthesized and inserted into the pWalium22 vector (*Drosophila* Genomics
525 Resource Center, Stock Number #1473, 10XUAS)⁸² by NheI(5')/EcoRI(3').

526 pUASp-GEM. GEM (PfV-GS-Sapphire) was cut from pCDNA3.1-pCMV-PfV-GS-
527 Sapphire (Addgene plasmid # 116933; RRID:Addgene_116933)⁴⁴, and inserted into the
528 pUASp vector by NheI(5')/XbaI(3').

529 pUASp-F-Tractin-Myc-BicD. F-Tractin (the actin binding domain of rat Inositol
530 trisphosphate 3-kinase A (ITPKA), residues 9-40,
531 atgGGCATGGCGCGACACGGGGCGCGGGGCCCTGCAGCCCCGGGTTGGAGCGG
532 GCTCCGCGCCGGAGCGTCGGGGAGCTGCGCCTGCTCTTCGAA)^{45,83} was
533 synthesized and inserted into pUASp by KpnI (5')/SpeI (3'). Myc-BicD was amplified
534 from pAC-FRB-GFP-BicD⁷⁷ and inserted into pUASp vector by SpeI (5')/XbaI (3').

535 pUASp-F-Tractin-GFP-Kin14Vlb. F-Tractin (the actin binding domain of rat Inositol
536 trisphosphate 3-kinase A (ITPKA), residues 9-40,
537 atgGGCATGGCGCGACACGGGGCGCGGGGCCCTGCAGCCCCGGGTTGGAGCGG
538 GCTCCGCGCCGGAGCGTCGGGGAGCTGCGCCTGCTCTTCGAA)^{45,83} was
539 synthesized and inserted into pUASp by KpnI (5')/SpeI (3'). EGFP and Kin14Vlb were
540 amplified from the vector of pET15b-EGFP-GCN4-kinesin14Vlb (a gift from Dr. Gohta
541 Goshima)⁴⁶ by PCR and inserted into pUASp by SpeI(5')/NotI(3') and NotI(5')/XbaI (3'),
542 respectively.

543 pUASp-Myc-HA-DlicNT-LOV2-attB. DlicNT (1-370 residues) and LOV2[WT] were
544 amplified by PCR from pAC-Dlic-EGFP (a gift from Dr. Thomas Hays, University of
545 Minnesota)⁸⁴ and pTriEx-NTOM20-mVenus-LOV2[WT] (a gift from Dr. Klaus Hahn,
546 University of North Carolina at Chapel Hill)⁵⁰ and inserted into pMT.A vector by
547 EcoRV(5')/XhoI(3') and XhoI(5')/XbaI(3'), respectively. Myc-HA with linkers (GGSG)
548 was synthesized and inserted into the pMT.A-DlicNT-LOV2[WT] by
549 EcoRI(5')/EcoRV(3'). Myc-HA-DlicNT-LOV2[WT] was subcloned from the pMT.A-Myc-
550 HA-DlicNT-LOV2[WT] into the pUASp vector by KpnI(5')/XbaI(3'), and attB site was
551 subcloned from the pUASp-attB vector (*Drosophila* Genomics Resource Center/DGRC
552 vector #1358) by AatII(5')/AatII(3') to create pUASp-Myc-HA-DlicNT-LOV2-attB.

553 pUASp-Zdk1-DlicCT-sfGFP-Myc-attB. Zdk1 and C-terminus of Dlic (371-493 residues)
554 were amplified by PCR from pTriEx-mCherry-Zdk1 (a gift from Dr. Klaus Hahn,
555 University of North Carolina at Chapel Hill)⁵⁰ and pAC-Dlic-EGFP (a gift from Dr.
556 Thomas Hays, University of Minnesota)⁸⁴, and inserted into pMT.A by
557 SpeI(5')/EcoRI(3') and EcoRI(5')/EcoRV(3'), respectively. Superfolder GFP (sfGFP) with
558 a Myc tag was inserted into the pMT.A-Zdk1-DlicCT by EcoRV(5')/XbaI(3'). Zdk1-
559 DlicCT-sfGFP-myc was then subcloned into the pUASp-attB vector (*Drosophila*
560 Genomics Resource Center/DGRC vector #1358) by SpeI(5')/XbaI(3') to create pUASp-
561 Zdk1-DlicCT-sfGFP-Myc-attB.

562 pcDNA3.1(+)-Myc-HA-DlicNT-LOV2. Myc-HA-DlicNT-LOV2 was subcloned from pMT.A-
563 Myc-HA-DlicNT-LOV2[WT] into pcDNA3.1(+) by KpnI(5')/XbaI(3').

564 pcDNA3.1(+)-Zdk1-DLicCT-sfGFP-Myc. Zdk1-DlicCT-sfGFP-Myc was amplified by PCR
565 from pMT.A-Zdk1-DlicCT-sfGFP-Myc and inserted into pcDNA3.1(+) by
566 HindIII(5')/XbaI(3').

567 **Immunostaining of *Drosophila* egg chambers.** A standard fixation and staining
568 protocol was previously described^{15,29,30,54,76}. Samples were stained with primary
569 antibody at 4°C overnight and with fluorophore-conjugated secondary antibody at room
570 temperature (24~25°C) for 4 h. Primary antibody used in this study: mouse monoclonal
571 anti-Orb antibody (Orb 4H8, Developmental Studies Hybridoma Bank, supernatant, 1:5);
572 rabbit phospho-MLC (pMLC 2/Ser19, 1:100, Cell Signaling, Cat# 3671); mouse
573 monoclonal anti-DHC antibody (2C11-2, Developmental Studies Hybridoma Bank,
574 concentrate, 1:50); mouse monoclonal anti-BicD antibody (anti-Bicaudal-D 4C2,
575 Developmental Studies Hybridoma Bank, supernatant, 1:5); mouse monoclonal anti-
576 Myc antibody (1-9E10.2, 1:100)⁸⁵. Secondary antibody used in this study: FITC-
577 conjugated or TRITC-conjugated anti-mouse secondary antibody (Jackson
578 ImmunoResearch Laboratories, Inc; Cat# 115-095-062 and Cat# 115-025-003) at 10
579 µg/ml; FITC-conjugated anti-rabbit secondary antibody (Jackson ImmunoResearch
580 Laboratories, Inc; Cat#111-095-003) at 10 µg/ml.

581 Some samples were stained with rhodamine-conjugated phalloidin (0.2 µg/ml) and DAPI
582 (1 µg/mL) for 1 ~2 h before mounting. Samples were imaged on a Nikon A1plus
583 scanning confocal microscope with a GaAsP detector and a 20× 0.75 N.A. lens using
584 Galvano scanning, a Nikon W1 spinning disk confocal microscope (Yokogawa CSU with
585 pinhole size 50 µm) with Photometrics Prime 95B sCMOS Camera or Hamamatsu
586 ORCA-Fusion Digital CMOS Camera and a 40 x 1.30 N.A. oil lens or a 40X 1.25 N.A.

587 silicone oil lens, or a Nikon Eclipse U2000 inverted stand with a Yokogawa CSU10
588 spinning disk confocal head with an Photometrics Evolve EMCCD camera and a 40x
589 1.30 N.A. oil lens, all controlled by Nikon Elements software. Z-stack.images were
590 acquired every 1 μm /step for whole ovariole imaging or 0.3~0.5 μm /step for individual
591 egg chambers.

592 **Microtubule staining in *Drosophila* egg chambers.** Ovaries were dissected in 1X
593 Brinkley Renaturing Buffer 80 (BRB80, 80 mM piperazine-N,N'-bis(2-ethanesulfonic
594 acid) [PIPES]), 1 mM MgCl_2 , 1 mM EGTA, pH 6.8) and fixed in 8% EM-grade
595 formaldehyde + 1X BRB80 + 0.1% Triton X-100 for 20 min on the rotator; briefly washed
596 with 1X PBTB (1X PBS + 0.1% Triton X-100 + 0.2% BSA) five times and stained with
597 FITC-conjugated β -tubulin antibody (ProteinTech, Cat# CL488-66240) 1:100 at 4C
598 overnight; then samples were stained rhodamine-conjugated phalloidin and DAPI for 1 h
599 before mounting. Samples were imaged using Nikon W1 spinning disk confocal
600 microscope (Yokogawa CSU with pinhole size 50 μm) with Photometrics Prime 95B
601 sCMOS Camera, and a 40X 1.25 N.A. silicone oil lens, controlled by Nikon Elements
602 software. Images were acquired every 0.3 μm /step in z stacks and 3D deconvolved
603 using Richardson-Lucy iterative algorithm provided by Nikon Elements.

604 **Live imaging of *Drosophila* egg chamber.** Young mated female adults were fed with
605 dry active yeast for 16~18 hours and then dissected in Halocarbon oil 700 (Sigma-
606 Aldrich, Cat# H8898) as previously described^{15,29,30,54}. Fluorescent samples were
607 imaged using Nikon W1 spinning disk confocal microscope (Yokogawa CSU with
608 pinhole size 50 μm) with Photometrics Prime 95B sCMOS Camera or Hamamatsu

609 ORCA-Fusion Digital CMOS Camera, and a 40 x 1.30 N.A. oil lens or a 40X 1.25 N.A.
610 silicone oil lens, controlled by Nikon Elements software.

611 **Photoconversion of tdMaple3-tubulin and Mito-MoxMaple3 in ovary.**

612 Photoconversions of tdMaple3-tubulin and Mito-MoxMaple3 were performed using
613 illumination from a Heliophor 89 North light in the epifluorescence pathway by a 405 nm
614 filter, either locally (for tdMaple3-tubulin) or globally (for Mito-MoxMaple3) through an
615 adjustable pinhole in the field diaphragm position for 10~20 s. Samples were imaged
616 either on a Nikon Eclipse U2000 inverted stand with a Yokogawa CSU10 spinning disk
617 confocal head with an Photometrics Evolve EMCCD camera and a 40x 1.30 N.A. oil
618 lens , or a Nikon W1 spinning disk confocal microscope (Yokogawa CSU with pinhole
619 size 50 µm) with Hamamatsu ORCA-Fusion Digital CMOS Camera and a 40X 1.25 N.A.
620 silicone oil lens, all controlled by Nikon Elements software.

621 **Induction of *zip*² germline clones.** *FRTG13 zip*²/*CyO* virgin female flies were crossed
622 with males carrying *hs-flp*^[12]/*y*; *FRTG13 ubi-GFP.nls/CyO*. From the cross, young pupae
623 at day 7 and day 8 AEL (after egg laying) were subjected to heat shock at 37 °C for 2
624 hours each day. Non CyO F1 females were collected 3-4 day after heat shock and
625 fattened with dry active yeast overnight before dissection for fixation and Orb staining.

626 **Extraction and fixation of ovary samples.** Ovaries were dissected and gently teased
627 apart in 1X BRB80. The dissected samples were extracted in 1X BRB80 + 1% Triton X-
628 100 for 20 min without agitation. After the extraction, the samples were fixed with 8%
629 EM-grade formaldehyde in 1X BRB80 + 0.1% Triton X-100 for 20min on rotator, washed
630 with 1X PBTB (1X PBS + 0.1% Triton X-100 + 0.2% BSA) five times before
631 immunostaining.

Measurement of velocity in the nurse cell-oocyte ring canals. Velocities of microtubules, small particles and mitochondria were measured based on kymographs generated along lines within the nurse cell-oocyte ring canals (10~15 μm line length, and ~5 μm line width) using the MultipleKymograph plugin in FIJI⁸⁶. Velocities were calculated on these kymographs using Kymograph (time space plot) Plugin for ImageJ, written by J. Rietdorf (FMI Basel) and A. Seitz (EMBL Heidelberg) (https://www.embl.de/eamnet/html/body_kymograph.html).

Measurement of nurse cell and oocyte size. Z stacks of triple color images of the ovarioles from *yw; ubi-GFP-Pav* stained with rhodamine conjugated-phalloidin and DAPI were acquired, and nurse cell area and oocyte area were specified (at the largest cross-section) and measured by manual polygon selection (area size) in FIJI⁸⁶.

S2R+ cell transfection and imaging acquisition. *Drosophila* S2R+ cells (DGRC Stock Number: 150) were transfected with 0.5 μg DNA (pAC-Gal4+pUASp-GEM, or pAC-LAMP1-GFP⁸⁷) in 12-well plate using Effecetene transfection kit (Qiagen, Cat. # / ID: 301425) and then were plated 48 hours after transfection on concanavalin A-coated glass coverslips⁸⁸. Cells were imaged using Nikon W1 spinning disk confocal microscope (Yokogawa CSU with pinhole size 50 μm) with Photometrics Prime 95B sCMOS Camera, and a 100 x 1.45 N.A. oil lens, controlled by Nikon Elements software.

Measurement of GEM particle movement in control and *dynein-RNAi*. Samples expressing GEM particles (*yw; mat atub-Gal4^[V37]/UASp-GEM* and *yw; UAS-Dhc64C-RNAi/+; mat atub-Gal4^[V37]/UASp-GEM*) were imaged on a Nikon W1 spinning disk confocal microscope (Yokogawa CSU with pinhole size 50 μm) with Photometrics Prime 95B sCMOS Camera and a 40X 1.25 N.A. silicone oil lens for 2 min at the frame rate of

every 2 sec, controlled by Nikon Elements software. Images were processed in Fiji and analyzed DiaTrack 3.04 Pro ⁸⁹, with a maximum particle jump distance of 3.2 $\mu\text{m/s}$.

HEK293T cell transfection and GFP-binder pulldown assay. HEK293T cells (American Type Culture Collection/ATCC, CRL-3216) were co-transfected with pcDNA3.1(+)-myc-HA-DlicNT-LOV2 and pcDNA3.1(+)-Zdk1-DlicCT-sfGFP-Myc by Calcium Phosphate transfection as previously described ⁹⁰. 40~48 hrs later cell extracts from transfected cells were made with red-only light on (no blue light, considered as “dark”): cells were rinsed twice with 1X PBS and scraped into 350 μl extraction buffer (10 mM Tris-buffer, pH 7.4; 50 mM NaCl; 0.5 mM MgCl_2); cracked in cell cracker and collected in 1.5 ml microtube. Triton X-100 was added to the extract to final concentration of 1%. The extract was centrifuged at 14k rpm for 15 min at 4°C (Eppendorf, 5804R). Supernatant was collected and divided equally in two parts. Both samples were mixed with 25 μl pre-washed GFP-binder agarose beads (Chromotek) ⁹¹. One sample was kept in aluminum foil for 2 hours at 4C (dark); another sample was illuminated first with blue light for 1 min and then full natural light for 2 hours at 4°C. Beads were washed twice and eluted with 40-50 μl 5x Sample Buffer. Initial cell extract and both eluted samples were separated by electrophoresis in 8% SDS-PAGE gel, and transferred to nitrocellulose membrane (Odyssey Nitrocellulose Membrane, LI-COR). Membrane was blotted with mouse monoclonal anti-Myc antibody (1-9E10.2, 1:1000), followed by HRP-conjugated goat-anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, Cat# 115-035-003, 1:10,000). The blot was developed on Odyssey phosphoimager (LI-COR).

677 **Statistical analysis.** The plots in figures show either percentage of phenotypes, or
678 average values, as indicated in figure legends. Error bars represent 95% confidence
679 intervals. N stands for number of samples examined in each assay, unless it is specified
680 elsewhere in figure legends. Unpaired t tests with Welch's correction were performed in
681 GraphPad Prism 8.0.2. P values and levels of significance are listed in figure legends.

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704 **Declaration of interests**

705 The authors declare no competing financial interests.

706 **Data Availability and Code Availability statement**

707 All data generated and analyzed during this study are included in this manuscript. This
708 study did not generate computational code.

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Figure 1

Figure 1. Dynein activity is required for *Drosophila* oocyte growth.

(A) A cartoon illustration of cytoplasmic dynein and its regulators. The dynein core complex is composed of dimers of dynein heavy chain (orange), dynein intermediate chain (gray), dynein light intermediate chain (blue), and three types of dynein light chains (green). Dynein activity is regulated by the dynactin complex (brown, with p150^{Glued} highlighted in red) and the Lis1-NudE complex (Lis1, yellow; NudE, magenta). A dynein activating adaptor, BicD (purple), is also shown to illustrate the linkage of the dynein complex with a cargo. To note: other cargo adaptors instead of BicD, such as Spindly, HOOK1/3, ninein/ninein-like (NINL), and RAB11 family-interacting protein 3, can be used for dynein activation and cargo recruitment (not shown)². BICDR1 and HOOK3 could recruit two dyneins for increased force and speed (not shown)².

(B) Summary of oocyte growth phenotypes in listed genetic background (all with one copy of *maternal atub-Gal4^{IV37}*). Classifications of oocyte growth phenotypes are included in Figure 1—figure supplement 1A. *Dhc64C-RNAi* (#1) is the RNAi line used for all *Dhc64C-RNAi* experiments in this study.

(C-D'') Phalloidin and Orb staining in control (C-C'') and *Dhc64C-RNAi* (D-D'') ovarioles. Oocytes and Orb staining are highlighted with either yellow arrowheads and brackets (C-D and C''-D''), or with yellow painting (C'-D'). Scale bars, 50 μ m.

(E) Summary of the Orb staining phenotypes in stage 8 (left) and stage 9 (right) egg chambers in listed genotypes (all with one copy of *maternal atub-Gal4^{IV37}*). Descriptions of Orb concentration and Orb dispersion are included in Figure 1—figure supplement 1B.

971 (F) Summary of oocyte growth phenotypes in *RNAi* lines against three listed dynein
972 activating adaptors, BicD, Spindly and Hook (all with one copy of *maternal atub-*
973 *Gal4*^[V37]).

Figure 2

Figure 2. Microtubule movement within nurse cells and from nurse cells to the oocyte.

(A-B') Microtubule movement labeled with photoconverted tdMaple3- α tub in control and *Dhc64C-RNAi* nurse cells. Photoconversion area is highlighted with a dotted purple circle and microtubules outside of the photoconversion zone are highlighted with purple arrowheads. Scale bars, 20 μ m. See also Figure 2-Video 1 and Figure 2-Video 5.

(C-C'') Microtubule movement is visualized by a 3XTagRFP-tagged microtubule binding domain of human Ensconsin/MAP7 (EMTB-3XTagRFP). Temporal color-coded hyperstacks are used to show the microtubule movement in a whole egg chamber (C) and zoom-in areas (the dashed orange boxes) within a nurse cell (C') and in a nurse cell-oocyte ring canal (C''). Scale bar, 50 μ m. See also Figure 2-Video 2.

(D-D'') microtubule movement labeled with a GFP-tagged microtubule minus-end binding protein Patronin. The ring canal is labeled with LifeAct-TagRFP. One microtubule moving through the ring canal is highlighted with orange arrowheads. Scale bars, 10 μ m. See also Figure 2-Video 3.

(E) Microtubule movement is visualized by a GFP protein trap line of an endogenous microtubule binding protein, Jupiter (Jupiter-GFP). The ring canal is labeled with F-Tractin-tdTomato. A kymograph of Jupiter-GFP in the nurse cell-oocyte ring canal (the dashed orange box) is used to show the microtubule movement from the nurse cell to the oocyte. See also Figure 2-Video 4.

(F) Cytoplasmic advection is visualized by bright-field imaging. The ring canals are labeled with a GFP-tagged myosin-II light chain, Sqh-GFP. Kymographs of the two

997 nurse cell-oocyte ring canals (the dashed white boxes) are used to show the
 998 cytoplasmic advection from the nurse cells to the oocyte. See also Figure 2-Video 7.

999 (G) Bulk movement of two types of cargoes, mitochondria (magenta, Mito-Moxmaple3,
 1000 without photoconversion) and Golgi units (RFP-Golgi, cyan), through the nurse cell-
 1001 oocyte ring canal, labeled with GFP-Pav (magenta). Kymographs of mitochondria and
 1002 Golgi units in the nurse cell-oocyte ring canal (the dashed orange box) is used to show
 1003 that both cargoes move at a similar speed through the ring canal to the oocyte. See also
 1004 Figure 2-Video 8.

1005 (E-G) The capped line on top of the kymograph indicates the ring canal region. Scale
 1006 bars, 50 μm .

1007 (H) Quantifications of the velocities of microtubules, small particles in bright-field images
 1008 (BF particles) and mitochondria in nurse cell-oocyte ring canals of stage 9 egg
 1009 chambers. The black bars on top of scattered plots stand for mean $\pm$ 95% confidence
 1010 intervals: microtubules (Jupiter-GFP) 139.7 ± 6.2 nm/sec (N=159); BF particles, $140.8 \pm$
 1011 6.4 nm/sec (N=143); mitochondria (Mito-MoxMaple3), 117.6 ± 5.7 nm/sec (N=147).
 1012 Unpaired t tests with Welch's correction were performed in following groups:
 1013 microtubules and BF particles, $p=0.8080$ (n.s.); microtubules and mitochondria,
 1014 $p<0.0001$ (****); BF particles and mitochondria, $p<0.0001$ (****).

1015 (I) Measurement of oocyte sizes in egg chambers of different stages (mean $\pm$ 95%
 1016 confidence intervals): stage 4, 67.2 ± 4.9 μm^2 (N=57); stage 5, 139.0 ± 12.6 μm^2
 1017 (N=58); stage 6, 230.5 ± 13.4 μm^2 (N=62); stage 7, 378.4 ± 25.1 μm^2 (N=38); stage 8,

1018 $1067.3 \pm 120.0 \mu\text{m}^2$ (N=41); stage 9, $5432.8 \pm 771.8 \mu\text{m}^2$ (N=69); stage 10A, $20105.0 \pm$
1019 $1930.0 \mu\text{m}^2$ (N=31).

Figure 3

Figure 3. Dynein-dependent GEM particle movement in the germ line.

(A-B) Cartoon illustrations of two possible mechanisms of dynein-dependent cargo transfer from the nurse cell to the oocyte.

(C) A schematic illustration of the GEM construct. 120 copies of a sapphire-tagged *Pyrococcus furiosus* Encapsulin scaffold protein self-assemble into a 40 nm particle.

(D-E') Temporal color-coded hyperstacks of GEM particles in control (D-D') and *Dhc64C-RNAi* (E-E'). Oo, the oocyte. Scale bars, 20 μ m. See also Figure 3-Video 3.

(F-G) Quantifications of total (F) and average (G) fluorescent intensities of GEM particles in control and *Dhc64C-RNAi*. The values shown in the graphs are mean $\pm$ 95% confidence intervals. (F) Control, N=22; *Dhc64C-RNAi*, N=27. Unpaired t test with Welch's correction between control and *Dhc64C-RNAi*: $p < 0.0001$ (****). (G) Control, N=22; *Dhc64C-RNAi*, N=27. Unpaired t test with Welch's correction between control and *Dhc64C-RNAi*: $p < 0.0001$ (****).

(H-K) Quantifications of velocities (H, J) and trajectories (I, K) of GEM movement in control and *Dhc64C-RNAi*. The number of particles tracked: control, N=7656; *Dhc64C-RNAi*, N=2083. (H-I) The values shown in the graphs are mean $\pm$ 95% confidence intervals. (H) Unpaired t test with Welch's correction between control and *Dhc64C-RNAi*: $p < 0.0001$ (****). (I) Unpaired t test with Welch's correction between control and *Dhc64C-RNAi*: $p < 0.0001$ (****). (J-K) Histograms of velocities (J) and trajectories (K) of GEM movement in control and *Dhc64C-RNAi* (the same set of data used in H-I).

Figure 4

Figure 4. A cortically anchored minus-end motor is sufficient to drive oocyte growth.

(A-C) Oocyte growth defect in *BicD-RNAi* is rescued by a cortically restricted BicD construct. (D) Summary of percentages of ovarioles with and without egg chamber(s) passing stage 7. Staging of *BicD-RNAi* egg chambers is determined by the nurse cell size.

(E-J) The defects of oocyte growth and Orb concentration in *Dlic-RNAi* are rescued by a cortically recruited plant kin14 (Kin14Vlb) construct. Phalloidin and DAPI staining (E-G) or Orb staining (H-J) in control (E, H), *Dlic-RNAi* (F, I) and *Dlic-RNAi* rescued by F-tractin-GFP-Kin14 (G, J).

(K) Quantification of oocyte size in stage 8~9 egg chambers in listed genotypes. The values shown in the graph are mean $\pm$ 95% confidence intervals. Control, N=40; *Dlic-RNAi*, N=49; *Dlic-RNAi* + *F-Tractin-Kin14*, N=33; *Dlic-RNAi* + *F-Tractin-tdTomato*, N=42. Unpaired t test with Welch's correction were performed in following groups: between control and *Dlic-RNAi*, $p < 0.0001$ (****); between Control and *Dlic-RNAi* + *F-Tractin-Kin14*, $p < 0.0001$ (****); between Control and *Dlic-RNAi* + *F-Tractin-tdTomato*, $p < 0.0001$ (****); between *Dlic-RNAi* and *Dlic-RNAi* + *F-Tractin-Kin14*, $p < 0.0001$ (****); between *Dlic-RNAi* and *Dlic-RNAi* + *F-Tractin-tdTomato*, $p = 0.2107$ (n.s.); between *Dlic-RNAi* + *F-Tractin-Kin14* and *Dlic-RNAi* + *F-Tractin-tdTomato*, $p < 0.0001$ (****).

(L) Summary of Orb staining phenotypes in stage 8 (left) and stage 9 (right) egg chambers in listed genotypes.

Oocytes are highlighted with either yellow arrowheads or yellow brackets. All listed genotypes carried one copy of *maternal atub-Gal4^{V37}*. Scale bars, 50 μ m.

Figure 5

Figure 5. The dynein complex is tethered to the nurse cell cortex via Dlic and glides microtubules to create cytoplasmic advection to the growing oocyte.

(A) A schematic illustration of *Drosophila* Dlic and Dlic truncations (DlicNT and DlicCT). C-terminus of DlicNT and N-terminus of DlicCT are tagged with the LOVTRAP probes, LOV2 and Zdk1, respectively. LOV2 interacts with Zdk11 in dark, and dissociates from Zdk1 in the presence of blue light.

(B) Zdk1-DlicCT interacts with DlicNT-LOV2 in dark. GFP-binder was used to pull down Zdk1-DlicCT in cell extracts from HEK293T cells expressing both DlicNT and DlicCT constructs, and anti-Myc antibody was used to probe for Dlic truncations. Lane 1: cell extract; Lane 2: GFP-binder pulldown sample in light; lane 3; GFP-binder pulldown sample in dark. The raw unedited blots can be found in Figure 5-source data 1: (1) the raw pulldown blot; (2) the labeled pulldown blot.

(C) Coexpression of DlicNT and DlicCT truncations reuses the oogenesis defects caused by *Dlic-RNAi*, while DlicNT truncation or DlicCT truncation alone fails to rescue. The expression is driven by one copy of *nos-Gal4*^[VP16].

(D-E) DlicNT and DlicCT localizations in the germ line. The expression is driven by one copy of *maternal atub-Gal4*^[V37]. A small Z-projection (~5 µm) is used to show the DlicNT localization, while a whole ovariole z-projection (~ 40 µm) is used to show the DlicCT cortical localization. To note: overexpression of DlicNT driven by *maternal atub-Gal4*^[V37] results in delayed oocyte growth. Scale bar, 50 µm.

(F) The model of cortically anchored dynein transferring cytoplasmic contents to the growing oocyte via gliding microtubules. Dynein light intermediate chain (Dlic) recruits

1088 dynein heavy chain to the cell cortex, while the Dynactin/p150 complex, BicD and Lis1
1089 are required for dynein activation. See also Figure 5-Video 1.

1090

1092 **Figure 1—figure supplement 1**

Figure 1—figure supplement 1. Summary of dynein-related RNAi phenotypes.

(A) A Cartoon illustration of oocyte growth phenotypes (oocytes are highlighted in magenta): (1) normal oocyte growth: all egg chambers in the single ovariole have normal-sized oocytes according to the stages; (2) complete small oocytes: oocyte growth is completely arrested throughout the stages, (3) partial small oocyte growth: some egg chambers display oocyte growth arrest, while others have normal-size oocytes in the same ovariole; (4) delayed oocyte growth: oocytes are smaller compared to wild-type at all stages.

(B) A cartoon illustration of Orb staining phenotypes (Orb staining is shown in green). The Orb staining in stage 8 and stage 9 egg chambers were characterized into either Orb concentrated in the oocyte (the average fluorescent intensity of Orb staining in the oocyte is >2x higher than in the nurse cells) and Orb dispersed in the oocyte (the average fluorescent intensity of Orb staining in the oocyte is <2x higher than in the nurse cells).

(C) Viability assay (from pupae to adults) in listed genotypes. As the *Act5C-Gal4* transgene is balanced with a TM6B (Tb) balancer, only non-Tb pupae were selected for this assay.

(D-F') Bristle phenotypes in control (D) and in *hook-RNAi* (E-F) male adults. Control and *hook-RNAi* are of the same genotypes as (C). Mild (E) and severe (F) hooked bristle phenotypes are seen in *Act5C>hook-RNAi* flies. (D'-E') Zoom-in images of bristles in the white dashed box in (D-E). Hooked bristles are highlighted with white arrowheads.

Figure 2-supplement 1

Figure 2—figure supplement 1. Microtubule in the ring canals and oocyte growth in different stages

(A) Tubulin staining in a control stage 9 egg chamber. Microtubules can be seen in all four ring canals connecting nurse cells with the oocyte (A'-A'''). Ring canals are labeled with rhodamine-conjugated phalloidin. Scale bar, 50 μm .

(B) The scattered plot of oocyte size in stage 9 egg chambers (black bars on top of the scatter plot, mean $\pm$ 95% confidence intervals). N=69.

- 1124 (C) Ratio of either maximum oocyte size / minimum oocyte size (top) or ratio of 95%
- 1125 percentile oocyte size / 5% percentile oocyte size (bottom) in listed stages.

Figure 2—figure supplement 2

Figure 2—figure supplement 2. Myosin-II activity is dispensable for oocyte growth during mid-oogenesis.

(A) Nurse cell size in stage 8 to stage 10 egg chambers (mean $\pm$ 95% confidence intervals). Stage 8, N=13; stage 9, N=23; stage 10, N=14.

(B-C') Phospho-myosin-II light chain (pMLC) antibody staining in control and in *zip-RNAi* samples. The pMLC staining between the nurse cells and the oocytes is abolished in *zip-RNAi* (C), compared to control (B, pointed with orange arrowheads). To note, the pMLC staining at the apical side of the somatic follicle cells is not affected by the germline knockdown of *zip-RNAi* and serves as an internal control of the antibody staining. Scale bars, 20 μ m.

(D-E) Summaries of oocyte growth phenotypes (D) and Orb staining phenotypes (E) in control and in *zip-RNAi*. Both control and *zip-RNAi* carry one copy of *maternal atub-Gal4^[V37]*.

(F-G) Characteristic Orb staining in control (F) and *zip-RNAi* (G) ovarioles. Oocytes are highlighted with either yellow arrowheads or yellow brackets. Scale bars, 50 μ m. Both samples carry one copy of *maternal atub-Gal4^[V37]*.

(H-K) Orb staining in *zip²* heterozygous (H-I) and homozygous (J-K, marked with the absence of nuclear-localized GFP signal) egg chambers. Among 46 *zip²* homozygous egg chambers examined, 28 egg chambers have proper oocyte specification (shown by Orb concentration). 25 out 28 *zip²* homozygous egg chambers display no major growth defects in mid-oogenesis. To note: (1) the Orb staining signal bleeds through to the

1148 GFP channel; (2) Orb staining becomes chunky and less evenly localized in the *zip*²
1149 homozygous oocytes. Scale bars, 50 μ m.

Figure 3—figure supplement 1

Figure 3—figure supplement 1. GEMs and lysosomes in *Drosophila* S2R+ cells.

1153 Single frame images (A-B) and temporal color-coded hyperstacks (A'-B') of GEM
1154 particles (labeled with pAC-Gal4+pUASp-GEM) (A-A') and lysosomes (labeled with
1155 pAC-LAMP1-GFP) (B-B') in *Drosophila* S2R+ cells plated on Concanavalin-A coated
1156 coverslips. Scale bars, 10 μ m.

Figure 4—figure supplement 1

Figure 4—figure supplement 1. The recruitment of dynein to nurse cell cortex.

(A-F) Dlic-GFP under its endogenous promoter (A-B), DHC antibody staining (C-D) and BicD antibody staining (E-F) in non-extracted (A, C, E) and extracted (B, D, F) samples. After extraction, Dlic-GFP, Dhc staining and BicD staining all show clear cortical localization in nurse cells. Scale bars, 20 μ m.

(G) A schematic illustration of the cortically recruited BicD and kin14 constructs.

(H-H') F-Tractin-Myc-BicD is predominantly localized to the cell cortex. The expression is driven by one copy of *maternal atub-Gal4^{V37}*. Scale bars, 50 μ m.

Figure 5—figuresupplement 1

Figure 5—figure supplement 1. Cortical localization of Dlic C-terminus is independent of BicD, Dynactin/p150, Lis1 and Shot.

DlicCT localizations in control (A), *BicD-RNAi* (B), Dynactin/p150 inhibited (*Glued* Δ C, C), *Lis1-RNAi* (D) and *shot-RNAi shot* ^{Δ EGC/+} (E) egg chambers. Whole ovariole z-projections (30 ~ 40 μ m) are used to show the DlicCT cortical localization. The numbers of ovarioles examined: Control, N=28; *BicD-RNAi*, N=39; *Glued* Δ C, N=30; *Lis1-RNAi*, N=29; *shot-RNAi shot* ^{Δ EGC/+}, N=33. All listed genotypes are with one copy of *maternal atub-Gal4*^[V37]. Scale bars, 50 μ m.

Videos

Figure 2-Video 1. Microtubule movement (labeled with locally photoconverted tdMaple3- α tub, red) in nurse cells and from the nurse cell to the oocyte via a ring canal (labeled with *ubi-GFP-Pav*). To note: the red signal observed in the oocyte was a combination of microtubule movement through the ring canal and soluble red tubulin incorporated into newly-formed microtubules in the oocyte. Scale bars, 20 μ m. Related to Figure 2.

Figure 2-Video 2. Microtubules labeled with fluorescently tagged human MAP7/Ensconsin microtubule binding domain (EMTB) move in nurse cells and through the nurse cell-oocyte ring canal in stage 9 egg chambers. Ring canals in EMTB-3XTagRFP are labeled with GFP-Pav in magenta. Comparison of EMTB-labeled microtubules in the ring canals at stage 7 and stage 9 is included at the end. Scale bars, 50 μ m for the whole egg chambers, and 20 μ m for the zoom-in ring canals, respectively. Related to Figure 2.

Figure 2-Video 3. Microtubules labeled with ectopically-expressed GFP-tagged minus-end binding protein, Patronin (GFP-Patronin) move in nurse cells and from the nurse cell to the oocyte through the ring canal (labeled with LifeAct-TagRFP in magenta). Scale bars, 50 μ m for the whole egg chamber, and 10 μ m for the zoom-in ring canals. Related to Figure 2.

Figure 2-Video 4. Microtubules labeled with a GFP-tagged endogenous microtubule-associated protein Jupiter (Jupiter-GFP) in nurse cells and in the nurse cell-oocyte ring canal (labeled with F-Tractin-tdTomato in magenta). Scale bar, 50 μ m. Related to Figure 2.

Figure 2-Video 5. No microtubule movement (labeled with locally photoconverted tdMaple3- α tub, red) in *Dhc64C-RNAi* nurse cells, compared to control. Scale bars, 20 μ m. Related to Figure 2.

Figure 2-Video 6. Microtubule movement (labeled with a GFP-tagged endogenous MAP, Jupiter-GFP) in nurse cell-oocyte ring canals (labeled with GFP-Pav) in control, *zip-RNAi* and *Dhc64C-RNAi*. Scale bars, 10 μ m. Related to Figure 2.

Figure 2-Video 7. Cytoplasmic advection from the nurse cells to the oocyte through ring canals (labeled with Sqh-GFP in magenta). Scale bar, 50 μ m. Related to Figure 2.

Figure 2-Video 8. Bulk movement of mitochondria (labeled with Mito-MoxMaple3, without photoconversion, magenta) and Golgi units (labeled with RFP-Golgi, cyan) through the nurse cell-to-oocyte ring canal (labeled with GFP-Pav, magenta) in a stage 9 egg chamber. Scale bars, 50 μ m. Related to Figure 2.

Figure 2-Video 9. Mitochondria movement (labeled with Mito-MoxMaple3, after global photoconversion, gray) in control, *zip-RNAi* and *Dhc64C-RNAi*. Ring canals are labeled with GFP-Pav (magenta). Scale bars, 50 μ m. Related to Figure 2.

Figure 3-Video 1. Mitochondria movement (labeled with Mito-MoxMaple3, without photoconversion, left) and microtubule movement (labeled with EMTB-3XTagRFP, right) in nurse cell-oocyte ring canals (labeled with GFP-Pav, left). Scale bars, 50 μ m in the whole egg chamber, and 10 μ m in zoom-in ring canal, respectively. Related to Figure 3.

Figure 3-Video 2. The movement of Golgi units (labeled with RFP-Golgi, left) and microtubules (labeled with Jupiter-GFP, right) in nurse cell-oocyte ring canals (labeled with F-Traction-tdTomato, left). Scale bars, 50 μ m in the whole egg chamber, and 10 μ m in zoom-in ring canal, respectively. Related to Figure 3.

Figure 3-Video 3. GEM particles in a control egg chamber and in a *Dhc64C-RNAi* egg chamber. Scale bars, 20 μ m. Related to Figure 3.

Figure 3-Video 4. GEM particles transport through a nurse cell-oocyte ring canal (labeled with F-Tractin-tdTomato, magenta) in control. Scale bar, 10 μ m. Related to Figure 3.

1240 **Figure 4-Video 1.** Mitochondria movement (labeled with Mito-MoxMaple3, after global
1241 photoconversion) in the nurse cell-oocyte ring canals of control, *Dlic-RNAi* and *Dlic-*
1242 *RNAi* + *F*-Tractin-GFP-Kin14Vlb rescued samples. Ring canals are either labeled with
1243 GFP-Pav (in control and *Dlic-RNAi* samples) or *F*-Tractin-GFP-Kin14Vlb (in the *Dlic-*
1244 *RNAi* + *F*-Tractin-GFP-Kin14Vlb rescued sample), in magenta. Scale bars, 20 μ m.
1245 Related to Figure 4.

1246

1247 **Figure 5-Video 1.** Cortical dynein glides microtubules and transfers cytoplasm to the
1248 *Drosophila* oocyte. Related to Figure 5.

1249

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
gene (<i>Drosophila melanogaster</i>)	BicD	doi: 10.7554/eLife.10140 ⁷⁷	CG6605	
gene (<i>Drosophila melanogaster</i>)	Dlic	Thomas Hays lab (University of Minnesota); doi:10.1091/mbc.E08-05-0483 ⁸⁴	CG1938	
gene (<i>Rattus norvegicus</i>)	Inositol trisphosphate 3-kinase A (ITPKA)	doi:10.1091/mbc.E09-01-0083 ⁸³ ; doi:10.1016/j.ydbio.2014.06.022 ⁴⁵	Gene ID: 81677	
gene (<i>Pyrococcus furiosus</i>)	PfV	Addgene plasmid # 116933; doi:10.1016/j.cell.2018.05.0 ⁴⁴	GenBank: AB214633.1; RRID:Addgene_116933	
gene (<i>Physcomitrella patens</i>)	kin14-VIb	Gohta Goshima lab; doi:10.1038/NPLANTS.2015.87 ⁴⁶		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>mat atub-Gal4[V37]</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 7063; FlyBase ID: FBti0016914	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>Act5C-Gal4</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 3954; FlyBase ID: FBti0012292	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>nos-Gal4-VP16</i>	Edwin Ferguson lab (University of Chicago); doi: 10.1016/s0960-9822(98)70091-0 ⁷⁵		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-Dhc64C-RNAi</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 36583; FlyBase ID: FBti0146459; TRiP.GL00543	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-Dhc64C-RNAi</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 36698; FlyBase ID: FBti0146710; TRiP.HMS01587	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-GIΔC</i>	Thomas Hays lab (University of Minnesota); doi:10.1091/mbc.E06-10-0959 ¹²	Line # 16.1	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-Lis1-RNAi</i>	Graydon Gonsalvez lab (Augusta University); doi:10.1534/genetics.115.180018 ¹⁹		

genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-BicD-RNAi</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 35405; FlyBase ID: FBti0144407; TRiP.GL00325	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-Spindly-RNAi</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 34933; FlyBase ID: FBti0144908; TRiP.HMS01283	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-hook-RNAi</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 64663; FlyBase ID: FBti0183911; TRiP.HMC05698	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-GFP-RNAi</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 41551; FlyBase ID: FBti0148872	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-tdMaple3-atub84B</i>	Our lab; doi:10.1073/pnas.1522424113 ²⁹		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-EMTB-3XTagRFP</i>	Our lab; doi:10.7554/eLife.54216 ³⁰		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>mat atub67C-EMTB-3XGFP-sqh 3'UTR</i>	Yu-Chiun Wang lab (RIKEN Center for Biosystems Dynamics Research) (an unpublished gift)		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-GFP-Patronin</i>	Uri Abdu lab (Ben Gurion University); doi:10.7554/eLife.54216 ³⁰ ; doi:10.1083/jcb.201709174 ⁵⁴ ; doi:10.1242/bio.039552 ⁷⁸ ; doi:10.1016/j.cub.2021.05.010 ¹⁵		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>Jupiter-GFP</i>	Yale GFP Protein Trap Database doi: 10.1073/pnas.261408198 ⁹² ; doi:10.1016/j.cub.2013.04.050 ²⁷ ; doi:10.1091/mbc.E14-10-1423 ²⁸ ; doi:10.1002/cm.20124 ³²	ZCL2183	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-LifeAct-TagRFP</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 58714; FlyBase ID: FBti0164964	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-F-Tractin-tdTomato</i>	Bloomington <i>Drosophila</i> stock center; doi:10.1016/j.ydbio.2014.06.022 ⁴⁵	BDSC: 58989; FlyBase ID: FBti0164816	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-Zip-RNAi</i>	Bloomington <i>Drosophila</i> Stock Center	BDSC: 37480; FlyBase ID: FBti0146538; TRiP.GL00623	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>hs-Flp^[12]; Sco/CyO</i>	Bloomington <i>Drosophila</i> stock center	BDSC: 1929; FlyBase ID: FBti0000784	

genetic reagent (<i>Drosophila melanogaster</i>)	<i>FRTG13 zip²/CyO</i>	Bloomington <i>Drosophila</i> stock center	BDSC: 8739; FlyBase ID: FBal0018863	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>FRTG13, Ubi-GFP.nls</i>	Bloomington <i>Drosophila</i> stock center	BDSC: 5826; FlyBase ID: FBti0001247 and FBti0016099	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>Sqh-GFP</i>	Bloomington <i>Drosophila</i> stock center	BDSC: 57145; FlyBase ID: FBti0150058	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-Mito-MoxMaple3</i>	Our lab; doi:10.1016/j.cub.2021.05.010 ¹⁵		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>ubi-GFP-Pav</i>	David Glover lab (Caltech); doi: 10.1242/jcs.115.4.725 ⁷⁹		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-RFP-Golgi</i>	Blomington stock center; doi:10.1038/s41598-017-05679-1 ⁸⁰ ; doi:10.1016/j.cub.2021.05.010 ¹⁵	BDSC: 30908; FlyBase ID: FBti0129915	
genetic reagent (<i>Drosophila melanogaster</i>)	<i>pDlic-Dlic-GFP</i>	Thomas Hays (University of Minnesota)		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>shotΔEGC</i>	Ferenc Jankovics, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences; doi:10.1242/jcs.193003 ⁸¹		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-shot-RNAi</i>	Our lab; doi:10.1016/j.cub.2021.05.010 ¹⁵		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-Dlic-RNAi</i>	Generated in this study (available upon request, contact vgelfand@northwestern.edu)		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-GEM</i>	Generated in this study (available upon request, contact vgelfand@northwestern.edu)		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-F-Tractin-Myc-BicD</i>	Generated in this study (available upon request, contact vgelfand@northwestern.edu)		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-F-Tractin-GFP-Kin14Vlb</i>	Generated in this study (available upon request, contact vgelfand@northwestern.edu)		
genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-Myc-HA-DlicNT-LOV2</i>	Generated in this study (available upon request, contact vgelfand@northwestern.edu)		

genetic reagent (<i>Drosophila melanogaster</i>)	<i>UASp-Zdk1-DlicCT-sfGFP-Myc</i>	Generated in this study (available upon request, contact vgelfand@northwestern.edu)		
cell line (<i>Drosophila melanogaster</i>)	S2R+ cells	<i>Drosophila</i> Genomics Resource Center (DGRC)	Stock Number: 150; RRID:CVCL_Z831	Tested negative for mycoplasma; authenticated by the vendor
cell line (<i>homo sapiens</i>)	HEK293T cells	ATCC; doi: 10.1096/fj.201800604R ⁹¹	CRL-3216	Tested negative for mycoplasma; authenticated by the vendor
transfected construct (<i>Drosophila melanogaster</i>)	pcDNA3.1(+)-Myc-HA-DlicNT-LOV2	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Contains <i>Drosophila</i> Dlic residues 1-370 and LOVTRAP probe LOV2[WT]	For transfection of HEK293T cells
transfected construct (<i>Drosophila melanogaster</i>)	pcDNA3.1(+)-Zdk1-DLicCT-sfGFP-Myc.	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Contains LOVTRAP probe Zdk1 and <i>Drosophila</i> Dlic residues 371-493	For transfection of HEK293T cells
antibody	anti-Orb antibody (Mouse monoclonal)	Developmental Studies Hybridoma Bank (DSHB)	orb 4H8; RRID: AB_528418	(1:5) dilution
antibody	FITC-conjugated anti- β tubulin antibody (Mouse monoclonal)	ProteinTech	Cat#: CL488-66240; RRID: AB_2883292	(1:100) dilution
antibody	anti-Drosophila dynein heavy chain Antibody (Mouse monoclonal)	Developmental Studies Hybridoma Bank (DSHB)	2C11-2; RRID: AB_2091523	(1:50) dilution
antibody	anti-Myc antibody (Mouse monoclonal)	doi: 10.1126/science.1061086 ⁸⁵	Purified from Hybridoma cell line MYC 1-9E10.2 (RRID:CVCL_G671)	(1:100) dilution

antibody	anti-BicD antibody (Mouse monoclonal)	Developmental Studies Hybridoma Bank (DSHB)	anti-Bicaudal-D 4C2; Antibody Registry ID: AB_528103	(1:5) dilution
antibody	anti-phospho-myosin light chain 2 (Ser19) (Rabbit polyclonal)	Cell Signaling	Cat# 3671; RRID: AB_330248	(1:100) dilution
antibody	Fluorescein (FITC) affiniPure anti-Mouse IgG (H+L) (Goat polyclonal)	Jackson ImmunoResearch	Cat# 115-095-062; RRID: AB_2338594	10 µg/ml
antibody	Rhodamine (TRITC)-affiniPure anti-Mouse IgG (H+L) (Goat polyclonal)	Jackson ImmunoResearch	Cat# 115-025-003; RRID: AB_2338478	10 µg/ml
antibody	Fluorescein (FITC) affiniPure anti-Rabbit IgG (H+L) (Goat polyclonal)	Jackson ImmunoResearch	Cat#111-095-003; RRID: AB_2337972	10 µg/ml
antibody	HRP-conjugated anti-mouse IgG (H+L) (Goat polyclonal)	Jackson ImmunoResearch	Cat# 115-035-003; RRID: AB_10015289	10 µg/ml
recombinant DNA reagent (<i>Drosophila melanogaster</i>)	pWalium22-Dlic3'UTR-shRNA	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Targeting <i>Dlic</i> 3'UTR 401-421 nt, 5'-AGAAATTTAACAACAAAAA-3'	DNA plasmid for generating transgenic flies

recombinant DNA reagent (<i>Pyrococcus furiosus</i>)	pUASp-GEM	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Subcloned from pCDNA3.1-pCMV-PfV-GS-Sapphire (Addgene plasmid # 116933; RRID:Addgene_116933)	DNA plasmid for generating transgenic flies
recombinant DNA reagent (<i>Drosophila melanogaster</i>)	pUASp-F-Tractin-Myc-BicD	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Includes the actin binding domain of rat Inositol trisphosphate 3-kinase A (ITPKA residues 9-40), Myc and <i>Drosophila</i> BicD	DNA plasmid for generating transgenic flies
recombinant DNA reagent (<i>Physcomitrella patens</i>)	pUASp-F-Tractin-GFP-Kin14Vlb	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Includes the actin binding domain of rat Inositol trisphosphate 3-kinase A (ITPKA residues 9-40), GFP and <i>Physcomitrella patens</i> kinesin14Vlb	DNA plasmid for generating transgenic flies
recombinant DNA reagent (<i>Drosophila melanogaster</i>)	pUASp-Myc-HA-DlicNT-LOV2-attB	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Contains <i>Drosophila</i> Dlic residues 1-370 and LOVTRAP probe LOV2[WT]	DNA plasmid for generating transgenic flies
recombinant DNA reagent (<i>Drosophila melanogaster</i>)	pUASp-Zdk1-DlicCT-sfGFP-Myc-attB	Generated in this study (available upon request, contact vgelfand@northwestern.edu)	Contains LOVTRAP probe Zdk1 and <i>Drosophila</i> Dlic residues 371-493	DNA plasmid for generating transgenic flies
chemical compound, drug	Rhodamine-labeled phalloidin	Thermo Fisher Scientific	Cat# R415	0.2 µg/ml
chemical compound, drug	DAPI	Sigma-Aldrich (MilliporeSigma)	D9542-1MG	1 µg/ml
chemical compound, drug	16% Paraformaldehyde Aqueous Solution, EM Grade	Fisher Scientific (Electron Microscopy Science)	Cat# 50-980-487 (EMS 15710)	4% or 8% for fixation

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