A novel mechanism of bulk cytoplasmic transport by cortical dynein in *Drosophila* ovary

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Abstract

Cytoplasmic dynein, a major minus-end directed microtubule motor, plays essential roles in eukaryotic cells. *Drosophila* oocyte growth is mainly dependent on the contribution of cytoplasmic contents from the interconnected sister cells, nurse cells. We have previously shown that cytoplasmic dynein is required for *Drosophila* oocyte growth and assumed that it simply transports cargoes along microtubule tracks from nurse cells to the oocyte. Here we report that instead of transporting individual cargoes along stationary microtubules into the oocyte, cortical dynein actively moves microtubules within nurse cells and from nurse cells to the oocyte via the cytoplasmic bridges, the ring canals. This robust microtubule movement is sufficient to drag even inert cytoplasmic particles through the ring canals to the oocyte. Furthermore, replacing
dynein with a minus-end directed plant kinesin linked to the actin cortex is sufficient for
transporting organelles and cytoplasm to the oocyte and driving its growth. These
experiments show that cortical dynein performs bulk cytoplasmic transport by gliding
microtubules along the cell cortex and through the ring canals to the oocyte. We
propose that the dynein-driven microtubule flow could serve as a novel mode of fast
cytoplasmic transport.
Introduction

Microtubules perform many key cellular functions, such as cell division, migration, polarization compartmentation, 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 functions. In interphase cells, dynein is the main motor responsible for transporting various cargoes towards the microtubule minus-ends. 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.

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) (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 microtubules. The activity of dynein is regulated by the dynactin complex including the largest subunit p150Glued/DCTN1, and the Lis1-NudE complex, as well as several activating adaptors, such as BICD2/BICDL1, Spindly and HOOK1/3 (Figure 1A).

Dynein has many essential functions during Drosophila oogenesis. First, it is required for germline cell division and oocyte specification. During mid-oogenesis, dynein is required for transport of mRNA ribonucleoproteins (RNPs) and organelles from nurse cells to the oocyte. Within the oocyte, dynein transports and anchors the anterior and dorsal determinants that are critical for axis determination for future.
embryos. During vitellogenesis, dynein in the oocyte regulates endocytic uptake and maturation of yolk proteins from the neighboring somatic follicle cells.

The *Drosophila* oocyte undergoes dramatic cell growth and polarization during oogenesis. 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. Previously, we showed that dynein heavy chain drives oocyte growth by supplying components to the growing oocyte. 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 dynein, 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. Dynein, the main minus-end directed microtubule motor in *Drosophila*, has been implicated in nurse cell-to-oocyte transport.

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[, that is expressed in germline cells after the completion of cell division and oocyte specification. 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 p150Glued/DCTN1 (p150GluedΔC) 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) 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 \(^9\) 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 formation \(^26\), and *hook-RNAi* animals phenocopied the classic hooked-bristle phenotype observed in *hook* null allele (*hook\(^{1f}\)*) (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 reports\textsuperscript{12-15}. However, when we examined the dynamics of microtubules in live samples using photoconversion\textsuperscript{27-29}, 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)\textsuperscript{30,31}, Patronin/CAMSAP\textsuperscript{15,30}, and Jupiter\textsuperscript{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 \(\sim 140\) 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\textit{ Drosophila} egg chambers reach stages 10B-11, nurse cells transfer all their cytoplasmic contents to the oocytes, the process called nurse cell dumping\textsuperscript{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 shrink\textsuperscript{34}. 
Second, nurse cell dumping requires the activity of non-muscle myosin-II\textsuperscript{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 (\textit{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\textsuperscript{36,37}.

We found that that myosin-II activity is dramatically diminished in \textit{zip-RNAi} egg chambers (Figure 2—figure supplement 2B-2C'), suggesting that the \textit{zip-RNAi} does inhibit myosin-II efficiently. However, \textit{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 \textit{zip} (\textit{zip}\textsuperscript{2})\textsuperscript{38}, and found that the majority of \textit{zip}\textsuperscript{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 \textit{spaghetti squash (sqh)} mutants\textsuperscript{35,36,39} and the “dumpless” mutants, such as chickadee, E2F, and RAP150B, develop normally to stages 9~10 without major oocyte growth defects\textsuperscript{40-42}. Thus, we conclude that the microtubule movement through the nurser 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 \textit{in vitro} and \textit{in vivo}\textsuperscript{43}. Therefore, we asked whether dynein is the motor that moves microtubules in nurse cells. Knockdown of dynein using the \textit{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 rung 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 spheres. 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-Tractin \(^{45}\) (Figure 4—figure supplement 1G-1H\(^t\)). 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 alone\(^{45}\) (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, kin14Vib \(^{46-48}\) 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 residues. 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 complex. We tagged the DlicNT and DlicCT with an optogenetic system LOVTRAP, 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 adaptors 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. Therefore, we examined the DlicCT localization in the dominant negative p150Glued/DCTN1 mutant (p150GluedΔC) 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 cortex. 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 defects. 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.
Discussion

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

In this study, we reveal a novel mechanism of bulk cargo transport by cytoplasmic dynein. First, we show that dynein core components and its regulatory cofactors are required for *Drosophila* oocyte growth (Figure 1). By imaging microtubules in live ovaries, we demonstrate that microtubules are actively moved by dynein from nurse cells to the growing oocytes (Figure 2). Furthermore, we use an artificial cargo that does not bind motors and show that direct dynein-cargo interaction is not necessary for the cargo movement in nurse cells or its transporting to the oocyte (Figure 3), supporting a “go-with-the-flow” mechanism driving cytoplasm transfer from nurse cells to the oocyte. To distinguish cytoplasmic advection from conventional cargo transport, we build a chimeric gliding-only motor by anchoring a minus-end plant kinesin, *kin14Vlb*, to the cortex, and find that this chimeric cortically-localized motor is sufficient to drive organelle transport and oocyte growth (Figure 4). Lastly, we identify that the C-terminus of Dlic is sufficient to target the dynein complex to the nurse cell cortex (Figure 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 oocyte \(^{12-15}\). 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 “dumpless” 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-filed 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 contain more plus-ends in the nurse cells and more minus-ends in the oocyte\(^{15}\). 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 adaptors. Recently, our lab reported that Spindly, a dynein activating adaptor that interacts with DlicCT and recruits dynein to the kinetochore in mitosis, anchors dynein to cortical actin in axons, thus pushing microtubules of the wrong polarity out of the axons in *Drosophila* neurons. 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 anchorage. 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 ovary, while Baz and SKTL are known to localize at the nurse cell cortex. 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 orientation. Furthermore, NuMA and its *Drosophila* homology Mud link the dynein complex to the cortex via the interaction with membrane localized Gαi, for pulling mitotic spindle poles in dividing cells. 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 DLIC. 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. 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 oocyte. 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, 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. 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 cells, 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). 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 fashion (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); *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-GlD* (1-826 residues of DCTN1/p150 Glued, based on the *DCTN1/p150* mutation) (II, 16.1, from Dr. Thomas Hays, University of Minnesota); *UAS-Lis1-RNAi* (II, from Dr. Graydon Gonsalvez, Augusta University, targeting *Lis1 CDS* 1197-1217 nt, 5'-TAGCGTAGATCAAACAGTAAA-3'); *UAS-BicD-RNAi* (TRiP.GL00325, attP2, III, Bloomington *Drosophila* Stock Center #35405, targeting *BicD 3'UTR* 639-659 nt, 5'-ACGATTCTAGATGATGAA-3'); *UAS-Spindly-RNAi* (TRiP.HMS01283, attP2, III, Bloomington *Drosophila* Stock Center #34933, targeting *Spindly CDS* 1615-1635 nt, 5'-CAGGACGCGTGTATGAA-3'); *UAS-tdMaple3-αtub84B* (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)\textsuperscript{30,54,78}; \textit{Jupiter-GFP} (protein trap line ZCL2183, III)\textsuperscript{27,28,32}; \textit{UASp-LifeAct-TagRFP} (III, 68E, Bloomington \textit{Drosophila} Stock Center # 58714); \textit{UASp-F-Tractin-tdTomato} (II, Bloomington \textit{Drosophila} stock center #58989)\textsuperscript{45}; \textit{UAS-Zip-RNAi} (TRiP.GL00623, attP40, II, Bloomington \textit{Drosophila} Stock Center #37480, targeting \textit{Zip} 3'UTR 36-56 nt, 5'-CAGGAAGAAGGTGATGATGAA-3'); \textit{hs-FLP}\textsuperscript{[12]} (X, Bloomington \textit{Drosophila} Stock Center #1929); \textit{FRTG13 ubi-GFP.nls} (II, Bloomington \textit{Drosophila} Stock Center # 5826); \textit{FRTG13 zip\textsuperscript{2}/CyO} (Bloomington \textit{Drosophila} stock center # 8739); \textit{Sqh-GFP} (III, Bloomington \textit{Drosophila} stock center #57145); \textit{UASp-Mito-MoxMaple3} (II, and III)\textsuperscript{15}; \textit{ubi-GFP-Pav} (II, from Dr. David Glover, Caltech)\textsuperscript{79}; \textit{UASp-RFP-Golgi} (II, Bloomington \textit{Drosophila} Stock Center # 30908, aka \textit{UASp-GalT-RFP})\textsuperscript{80}; \textit{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); \textit{shot\textsuperscript{\Delta EGC}} (from Dr. Ferenc Jankovics, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences)\textsuperscript{81}; \textit{UAS-shot\textsuperscript{\textit{EGC}.RNAi} (in pWalium22 vector, inserted at attP2, III)\textsuperscript{15}}. The following fly stocks were generated in this study using either PhiC31-mediated integration or P-element-mediated transformation: \textit{UASp-Dlic-RNAi} (targeting \textit{Dlic} 3'UTR 401-421 nt, 5'-AGAAATTTAACAAAAAAAAAA-3', in pWalium22 vector, inserted at attP-9A (VK00005) 75A10 site, III, M5); \textit{UASp-GEM} (III, M1); \textit{UASp-F-Tractin-Myc-BicD} (II, M4); \textit{UASp-F-Tractin-GFP-Kin14Vlb} (II, M1); \textit{UASp-Myc-HA-DlicNT-LOV2} at attP14 (36A10, II, M2); \textit{UASp-Zdk1-DlicCT-sfGFP-Myc} at attP33 (50B6, II, M2).

\textbf{Plasmid constructs.}
**pWalium22-Dlic3’UTR-shRNA.** The oligos of Dlic3’UTR-shRNA (agtAGAAATTTAACAAAAAATAaggatatattaagcataTTTTTTTTTTTGGTTAAATTTCTgc) were synthesized and inserted into the pWalium22 vector (*Drosophila* Genomics Resource Center, Stock Number #1473, 10XUAS) by *NheI(5’)/EcoRI(3’).

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

**pUASp-F-Tractin-Myc-BicD.** F-Tractin (the actin binding domain of rat Inositol trisphosphate 3-kinase A (ITPKA), residues 9-40, atgGGCATGGCCGACCCACCGGCGGGGCTGCAGCCCCGGTTGGAGCGG GCTCCGCGCCGGACGCTCGGGAGCTGCGCCTGCTCCTCGAA) was synthesized and inserted into pUASp by KpnI (5’)/SpeI (3’). Myc-BicD was amplified from pAC-FRB-GFP-BicD and inserted into pUASp vector by SpeI (5’)/XbaI (3’).

**pUASp-F-Tractin-GFP-Kin14VIb.** F-Tractin (the actin binding domain of rat Inositol trisphosphate 3-kinase A (ITPKA), residues 9-40, atgGGCATGGCCGACCCACCGGCGGGGCTGCAGCCCCGGTTGGAGCGG GCTCCGCGCCGGACGCTCGGGAGCTGCGCCTGCTCCTCGAA) was synthesized and inserted into pUASp by KpnI (5’)/SpeI(3’). EGFP and Kin14VIb were amplified from the vector of pET15b-EGFP-GCN4-kinesin14VIb (a gift from Dr. Gohta Goshima) by PCR and inserted into pUASp by Spel(5’)/NotI(3’) and Notl(5’)/Xbal (3’), respectively.
**pUASp-Myc-HA-DlicNT-LOV2-attB.** DlicNT (1-370 residues) and LOV2[WT] were amplified by PCR from pAC-Dlic-EGFP (a gift from Dr. Thomas Hays, University of Minnesota) and pTriEx-NTOM20-mVenus-LOV2[WT] (a gift from Dr. Klaus Hahn, University of North Carolina at Chapel Hill) and inserted into pMT.A vector by EcoRV(5’)/XhoI(3’) and XhoI(5’)/XbaI(3’), respectively. Myc-HA with linkers (GGSG) was synthesized and inserted into the pMT-A-DlicNT-LOV2[WT] by EcoRI(5’)/EcoRV(3’). Myc-HA-DlicNT-LOV2[WT] was subcloned from the pMT.A-Myc-HA-DlicNT-LOV2[WT] into the pUASp vector by KpnI(5’)/Xbal(3’), and attB site was subcloned from the pUASp-attB vector (Drosophila Genomics Resource Center/DGRC vector #1358) by AatII(5’)/AatII(3’) to create pUASp-Myc-HA-DlicNT-LOV2-attB.

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

**pcDNA3.1(+)—Myc-HA-DlicNT-LOV2.** Myc-HA-DlicNT-LOV2 was subcloned from pMT.A-Myc-HA-DlicNT-LOV2[WT] into pcDNA3.1(+) by KpnI(5’)/Xbal(3’).
pcDNA3.1(+) - Zdk1-DlicCT-sfGFP-Myc. Zdk1-DlicCT-sfGFP-Myc was amplified by PCR from pMT.A-Zdk1-DlicCT-sfGFP-Myc and inserted into pcDNA3.1(+) by HindIII(5′)/XbaI(3′).

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

Some samples were stained with rhodamine-conjugated phalloidin (0.2 µg/ml) and DAPI (1 µg/mL) for 1~2 h before mounting. Samples were imaged on a Nikon A1plus scanning confocal microscope with a GaAsP detector and a 20× 0.75 N.A. lens using Galvano scanning, a Nikon W1 spinning disk confocal microscope (Yokogawa CSU with pinhole size 50 µm) with Photometrics Prime 95B sCMOS Camera or Hamamatsu ORCA-Fusion Digital CMOS Camera and a 40 x 1.30 N.A. oil lens or a 40X 1.25 N.A.
silicone oil lens, or a Nikon Eclipse U2000 inverted stand with a Yokogawa CSU10 spinning disk confocal head with an Photometrics Evolve EMCCD camera and a 40× 1.30 N.A. oil lens, all controlled by Nikon Elements software. Z-stack images were acquired every 1 µm/step for whole ovariole imaging or 0.3~0.5 µm/step for individual egg chambers.

**Microtubule staining in Drosophila egg chambers.** Ovaries were dissected in 1X Brinkley Renaturing Buffer 80 (BRB80, 80 mM piperazine-N,N′-bis(2-ethanesulfonic acid) [PIPES]), 1 mM MgCl2, 1 mM EGTA, pH 6.8) and fixed in 8% EM-grade formaldehyde + 1X BRB80 + 0.1% Triton X-100 for 20 min on the rotator; briefly washed with 1X PBTA (1X PBS + 0.1% Triton X-100 + 0.2% BSA) five times and stained with FITC-conjugated β-tubulin antibody (ProteinTech, Cat# CL488-66240) 1:100 at 4C overnight; then samples were stained rhodamine-conjugated phalloidin and DAPI for 1 h before mounting. Samples were imaged using 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, controlled by Nikon Elements software. Images were acquired every 0.3 µm/step in z stacks and 3D deconvolved using Richardson-Lucy iterative algorithm provided by Nikon Elements.

**Live imaging of Drosophila egg chamber.** Young mated female adults were fed with dry active yeast for 16~18 hours and then dissected in Halocarbon oil 700 (Sigma-Aldrich, Cat# H8898) as previously described 15,29,30,54. Fluorescent samples were imaged using Nikon W1 spinning disk confocal microscope (Yokogawa CSU with pinhole size 50 µm) with Photometrics Prime 95B sCMOS Camera or Hamamatsu
ORCA-Fusion Digital CMOS Camera, and a 40 x 1.30 N.A. oil lens or a 40X 1.25 N.A. silicone oil lens, controlled by Nikon Elements software.

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

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

Induction of zip² germline clones. FRTG13 zip²/CyO virgin female flies were crossed with males carrying hs-flp¹²/y; FRTG13 ubi-GFP.nls/CyO. From the cross, young pupae at day 7 and day 8 AEL (after egg laying) were subjected to heat shock at 37 °C for 2 hours each day. Non CyO F1 females were collected 3-4 day after heat shock and fattened with dry active yeast overnight before dissection for fixation and Orb staining.

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

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-GFP) 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 coverslips. 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\textsuperscript{V37}/UASp-GEM and yw; UAS-Dhc64C-RNAi\textsuperscript{+}; mat atub-Gal4\textsuperscript{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, with a maximum particle jump distance of 3.2 μm/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. 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 ul extraction buffer (10 mM Tris-buffer, pH 7.4; 50 mM NaCl; 0.5 mM MgCl2); 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). 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).
**Statistical analysis.** The plots in figures show either percentage of phenotypes, or average values, as indicated in figure legends. Error bars represent 95% confidence intervals. N stands for number of samples examined in each assay, unless it is specified elsewhere in figure legends. Unpaired t tests with Welch's correction were performed in GraphPad Prism 8.0.2. P values and levels of significance are listed in figure legends.
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Declaration of interests

The authors declare no competing financial interests.

Data Availability and Code Availability statement

All data generated and analyzed during this study are included in this manuscript. This study did not generate computational code.
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Figures and figure legends

**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 p150Glued 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*\(^{V37}\)). 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 µm.

(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*\(^{V37}\)). Descriptions of Orb concentration and Orb dispersion are included in Figure 1—figure supplement 1B.
(F) Summary of oocyte growth phenotypes in RNAi lines against three listed dynein activating adaptors, BicD, Spindly and Hook (all with one copy of maternal αtub-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-αtub 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 µm. 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 µm. 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 µm. 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
nurse cell-oocyte ring canals (the dashed white boxes) are used to show the
cytoplasmic advection from the nurse cells to the oocyte. See also Figure 2-Video 7.

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

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

(H) Quantifications of the velocities of microtubules, small particles in bright-field images
(BF particles) and mitochondria in nurse cell-oocyte ring canals of stage 9 egg
chambers. The black bars on top of scattered plots stand for mean ± 95% confidence
intervals: microtubules (Jupiter-GFP) 139.7 ± 6.2 nm/sec (N=159); BF particles, 140.8 ±
6.4 nm/sec (N=143); mitochondria (Mito-MoxMaple3), 117.6 ± 5.7 nm/sec (N=147).

Unpaired t tests with Welch’s correction were performed in following groups:
microtubules and BF particles, p=0.8080 (n.s.); microtubules and mitochondria,
p<0.0001 (**); BF particles and mitochondria, p<0.0001 (**).

(I) Measurement of oocyte sizes in egg chambers of different stages (mean ± 95%
confidence intervals): stage 4, 67.2 ± 4.9 µm² (N=57); stage 5, 139.0 ± 12.6 µm²
(N=58); stage 6, 230.5± 13.4 µm² (N=62); stage 7, 378.4 ± 25.1 µm² (N=38); stage 8,
1067.3 ± 120.0 µm² (N=41); stage 9, 5432.8 ± 771.8 µm² (N=69); stage 10A, 20105.0 ± 1930.0 µm² (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 µm. 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 ± 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-L) The values shown in the graphs are mean ± 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-L).
Figure 4

(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.
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).

Quantification of oocyte size in stage 8~9 egg chambers in listed genotypes. The values shown in the graph are mean ± 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 (** **).

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, 50µm.
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*[^16].

(D-E) DlicNT and DlicCT localizations in the germ line. The expression is driven by one copy of *maternal atub-Gal4*[^37]. 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*[^37] 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...
dynein heavy chain to the cell cortex, while the Dynactin/p150 complex, BicD and Lis1 are required for dynein activation. See also Figure 5-Video 1.
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 ± 95% confidence intervals). N=69.
(C) Ratio of either maximum oocyte size / minimum oocyte size (top) or ratio of 95% 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 ± 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 µm.

(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 αtub-Gal4V37.

(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, 50µm. Both samples carry one copy of maternal αtub-Gal4V37.

(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
GFP channel; (2) Orb staining becomes chunky and less evenly localized in the \textit{zip}^{2} homozgyous oocytes. Scale bars, 50 \textmu m.
Figure 3—figure supplement 1

Figure 3—figure supplement 1. GEMs and lysosomes in *Drosophila* S2R+ cells.
Single frame images (A-B) and temporal color-coded hyperstacks (A’-B’) of GEM particles (labeled with pAC-Gal4+pUASp-GEM) (A-A’) and lysosomes (labeled with pAC-LAMP1-GFP) (B-B’) in *Drosophila* S2R+ cells plated on Concanavalin-A coated coverslips. Scale bars, 10 µm.
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 µm.

(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[37]. Scale bars, 50 µm.
**Figure 5—figure supplement 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 (*GluedΔC*, C), *Lis1-RNAi* (D) and *shot-RNAi shot^{EGG+}* (E) egg chambers. Whole ovariole z-projections (30 ~ 40 µm) are used to show the DlicCT cortical localization. The numbers of ovarioles examined: Control, N=28; *BicD-RNAi*, N=39; *GluedΔC*, N=30; *Lis1-RNAi*, N=29; *shot-RNAi shot^{EGG+}*; N=33. All listed genotypes are with one copy of *maternal atub-Gal4^{V37}*. Scale bars, 50 µm.
**Videos**

**Figure 2-Video 1.** Microtubule movement (labeled with locally photoconverted tdMaple3-αtub, 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 μm. 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 μm for the whole egg chambers, and 20 μm 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 μm for the whole egg chamber, and 10 μm 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 µm. Related to Figure 2.

**Figure 2-Video 5.** No microtubule movement (labeled with locally photoconverted tdMaple3-αtub, red) in Dhc64C-RNAi nurse cells, compared to control. Scale bars, 20 µm. 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 µm. 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 µm. 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 µm. 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 µm. 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 µm in the whole egg chamber, and 10 µm 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 µm in the whole egg chamber, and 10 µm 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 µm. Related to Figure 3.

**Figure 3-Video 4.** GEM particles transport through a nurse cell-oocyte ring canal (labeled with F-Actin-tdTomato, magenta) in control. Scale bar, 10 µm. Related to Figure 3.
Figure 4-Video 1. Mitochondria movement (labeled with Mito-MoxMaple3, after global photoconversion) in the nurse cell-oocyte ring canals of control, Dlic-RNAi and Dlic-RNAi + F-Tractin-GFP-Kin14Vlb rescued samples. Ring canals are either labeled with GFP-Pav (in control and Dlic-RNAi samples) or F-Tractin-GFP-Kin14Vlb (in the Dlic-RNAi + F-Tractin-GFP-Kin14Vlb rescued sample), in magenta. Scale bars, 20 µm. Related to Figure 4.

Figure 5-Video 1. Cortical dynein glides microtubules and transfers cytoplasm to the Drosophila oocyte. Related to Figure 5.
## Key Resources Table

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<td></td>
<td></td>
</tr>
<tr>
<td>UAS-F-Tractin-GFP-Kin14VIB</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UAS-Myc-HA-DlicNT-LOV2</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genetic reagent (Drosophila melanogaster)</td>
<td>UASp-Zdk1-DlicCT-sfGFP-Myc</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Tested negative for mycoplasma; authenticated by the vendor</td>
<td></td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
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</tr>
<tr>
<td>cell line (Drosophila melanogaster)</td>
<td>S2R+ cells</td>
<td>Drosophila Genomics Resource Center (DGRC)</td>
<td>Stock Number: 150; RRID:CVCL_Z831</td>
<td></td>
</tr>
<tr>
<td>cell line (homo sapiens)</td>
<td>HEK293T cells</td>
<td>ATCC; doi: 10.1096/fj.201800604R</td>
<td>CRL-3216</td>
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<tr>
<td>transfected construct (Drosophila melanogaster)</td>
<td>pcDNA3.1(+)-Myc-HA-DlicNT-LOV2</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Contains Drosophila Dlic residues 1-370 and LOVTRAP probe LOV2[WT]</td>
<td></td>
</tr>
<tr>
<td>transfected construct (Drosophila melanogaster)</td>
<td>pcDNA3.1(+)-Zdk1-DlicCT-sfGFP-Myc</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Contains LOVTRAP probe Zdk1 and Drosophila Dlic residues 371-493</td>
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</tr>
<tr>
<td>antibody</td>
<td>anti-Orb antibody (Mouse monoclonal)</td>
<td>Developmental Studies Hybridoma Bank (DSHB)</td>
<td>orb 4H8; RRID: AB_528418 (1:5) dilution</td>
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</tr>
<tr>
<td>antibody</td>
<td>FITC-conjugated anti-β tubulin antibody (Mouse monoclonal)</td>
<td>ProteinTech</td>
<td>Cat#: CL488-66240; RRID: AB_2883292 (1:100) dilution</td>
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<tr>
<td>antibody</td>
<td>anti-Drosophila dynein heavy chain Antibody (Mouse monoclonal)</td>
<td>Developmental Studies Hybridoma Bank (DSHB)</td>
<td>2C11-2; RRID: AB_2091523 (1:50) dilution</td>
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</tr>
<tr>
<td>antibody</td>
<td>anti-Myc antibody (Mouse monoclonal)</td>
<td>doi: 10.1126/science.1061086</td>
<td>Purified from Hybridoma cell line MYC 1-9E10.2 (RRID:CVCL_G671) (1:100) dilution</td>
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</tr>
<tr>
<td>antibody</td>
<td>Developmental Studies Hybridoma Bank (DSHB)</td>
<td>anti-Bicaudal-D 4C2; Antibody Registry ID: AB_528103</td>
<td>(1:5) dilution</td>
<td></td>
</tr>
<tr>
<td>----------</td>
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<td>---------------------------------------------------------</td>
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</tr>
<tr>
<td>antibody</td>
<td>anti-phospho-myosin light chain 2 (Ser19) (Rabbit polyclonal)</td>
<td>Cell Signaling</td>
<td>Cat# 3671; RRID: AB_330248</td>
<td>(1:100) dilution</td>
</tr>
<tr>
<td>antibody</td>
<td>Fluorescein (FITC) affiniPure anti-Mouse IgG (H+L) (Goat polyclonal)</td>
<td>Jackson ImmunoResearch</td>
<td>Cat# 115-095-062; RRID: AB_2338594</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>antibody</td>
<td>Rhodamine (TRITC)-affiniPure anti-Mouse IgG (H+L) (Goat polyclonal)</td>
<td>Jackson ImmunoResearch</td>
<td>Cat# 115-025-003; RRID: AB_2338478</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>antibody</td>
<td>fluorescein (FITC) affiniPure anti-Rabbit IgG (H+L) (Goat polyclonal)</td>
<td>Jackson ImmunoResearch</td>
<td>Cat# 111-095-003; RRID: AB_2337972</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>antibody</td>
<td>HRP-conjugated anti-mouse IgG (H+L) (Goat polyclonal)</td>
<td>Jackson ImmunoResearch</td>
<td>Cat# 115-035-003; RRID: AB_10015289</td>
<td>10 µg/ml</td>
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<tr>
<td>recombinant DNA reagent (Drosophila melanogaster)</td>
<td>pWalium22-Dlic3'UTR-shRNA</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Targeting Dlic 3’UTR 401-421 nt, 5’-AGAAATTTAACAAAAAAATTTCAAAACTTTGA-3’</td>
<td>DNA plasmid for generating transgenic flies</td>
</tr>
<tr>
<td>recombinant DNA reagent (Pyrococcus furiosus)</td>
<td>pUASp-GEM</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Subcloned from pCDNA3.1-pCMV-PfV-GS-Sapphire (Addgene plasmid # 116933; RRID:Addgene_116933)</td>
<td>DNA plasmid for generating transgenic flies</td>
</tr>
<tr>
<td>recombinant DNA reagent (Drosophila melanogaster)</td>
<td>pUASp-F-Tractin-Myc-BicD</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Includes the actin binding domain of rat Inositol trisphosphate 3-kinase A (ITPKA residues 9-40), Myc and Drosophila BicD</td>
<td>DNA plasmid for generating transgenic flies</td>
</tr>
<tr>
<td>recombinant DNA reagent (Physcomitrella patens)</td>
<td>pUASp-F-Tractin-GFP-Kin14Vlb</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Includes the actin binding domain of rat Inositol trisphosphate 3-kinase A (ITPKA residues 9-40), GFP and Physcomitrella patens kinesin14Vlb</td>
<td>DNA plasmid for generating transgenic flies</td>
</tr>
<tr>
<td>recombinant DNA reagent (Drosophila melanogaster)</td>
<td>pUASp-Myc-HA-DlicNT-LOV2-attB</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Contains Drosophila Dlic residues 1-370 and LOVTRAP probe LOV2[WT]</td>
<td>DNA plasmid for generating transgenic flies</td>
</tr>
<tr>
<td>recombinant DNA reagent (Drosophila melanogaster)</td>
<td>pUASp-Zdk1-DlicCT-sfGFP-Myc-attB</td>
<td>Generated in this study (available upon request, contact <a href="mailto:vgelfand@northwestern.edu">vgelfand@northwestern.edu</a>)</td>
<td>Contains LOVTRAP probe Zdk1 and Drosophila Dlic residues 371-493</td>
<td>DNA plasmid for generating transgenic flies</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>Rhodamine-labeled phalloidin</td>
<td>Thermo Fisher Scientific</td>
<td>Cat# R415</td>
<td>0.2 µg/ml</td>
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<tr>
<td>chemical compound, drug</td>
<td>DAPI</td>
<td>Sigma-Aldrich (MilliporeSigma)</td>
<td>D9542-1MG</td>
<td>1 µg/ml</td>
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<tr>
<td>chemical compound, drug</td>
<td>16% Paraformaldehyde de Aqueous Solution, EM Grade</td>
<td>Fisher Scientific (Electron Microscopy Science)</td>
<td>Cat# 50-980-487 (EMS 15710)</td>
<td>4% or 8% for fixation</td>
</tr>
</tbody>
</table>