A dual role for actin and microtubule cytoskeleton in the transport of Golgi units from the nurse cells to the oocyte across Ring canals.

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Running Head: Golgi transport from nurse cells to oocyte.

Abbreviations Mts: microtubules; NC: nurse cell; RC: Ring canals;

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Axis specification during Drosophila embryonic development requires transfer of maternal components during oogenesis from nurse cells (NC) into the oocyte through cytoplasmic bridges. We found that the asymmetrical distribution of Golgi, between nurse cells and the oocyte, is sustained by an active transport process. We have characterized actin basket structures that asymmetrically cap the NC side of Ring canals (RC) connecting the oocyte. Our results suggest that these actin baskets structurally support transport mechanisms of RC transit. In addition, our tracking analysis indicates that Golgi units are actively transported to the oocyte rather than diffusing. We observed that RC transit is microtubules-based and mediated at least by Dynein. Finally, we show that actin networks may be involved in RC crossing through a Myosin II step process, as well as in dispatching Golgi units inside the oocyte subcompartment.
INTRODUCTION

Throughout the animal kingdom, germ cells commonly develop as part of syncitia in which cytoplasmic canals connect sister cells. Intercellular bridges represent specific, types of cellular contacts that allow the flow of cytoplasm to be shared among sister cells in order to synchronize their division, differentiation or hormone release (see for review Robinson and Cooley, 1996). These intercellular communications between adjacent cells are commonly found in the developing male and female germline of diverse species: in mammals' ovaries, in insects and mammalians spermatocytes as well as in plants', C.elegans', or mammals' somatic lineages (see for review Robinson and Cooley, 1996). The intercellular bridges in Drosophila oogenesis are better characterized and have a slightly different role than in the male germline: it allows the transfer of maternal components, including key mRNAs that encode axis-determining proteins, into the oocyte to support the subsequent development of the embryo. Additionally, oocyte growth also requires a supply of plasma membrane that is provided by the secretion pathway (Janusckhe et al., 2007). Thus, we were interested in understanding how membrane trafficking occurs and how it is regulated during Drosophila oogenesis.

A Drosophila egg chamber is composed of 16 germline cells encapsulated by somatically derived follicle cells (Brown and King 1964). This cyst of 16 germ cells is formed when cystocytes undergo four rounds of cell division without fully completing cytokinesis, giving rise to cells interconnected by cytoplasmic bridges. As development proceeds, these cytoplasmic connections are modified into stable ones, Ring Canals (RC). A RC is made up of two parts: a layer of circumferentially oriented F-actin-rich filaments that form the inner rim and a thickening of the plasma membrane originally derived from the arrested cleavage furrow forming the outer rim (Hime et al., 1996).
Among these 16 cells, only one differentiates into a mature oocyte while the other 15 become polyploid cells called Nurse cells (NC). The *Drosophila* oocyte is transcriptionally inactive throughout much of oogenesis, therefore the majority of nutrients (mRNA, proteins and organelles) required for its development are synthesized in the NC and transported into the oocyte through the RC in a slow process of cytoplasmic transfer (Clark *et al.*, 2007; Theurkauf and Hazelrigg, 1998). A key question concerns the mechanism by which this cytoplasmic transport is achieved. There is growing evidence for the importance of microtubules (MTs) cytoskeleton and its associated motor proteins, Kinesin (Brendza *et al.*, 2002; Januschke *et al.*, 2002) and Dynein (Cha *et al.*, 2002; Clark *et al.*, 2007, Januschke *et al.*, 2002) in active transport of organelles (Januschke *et al.*, 2007). Less is known concerning the role of the actin cytoskeleton and its associated proteins during this process. However, studies have shown the involvement of myosins in the transport of vesicles/organelles in vertebrates neurons (DePina and Langford, 1999), and the transient association of MyoVI with mitochondria during their RC transit in the *Drosophila* egg chamber (Bohrmann and Schill, 1997), suggesting a role for the actin network in organelle transport.

The mechanism of cellular organelle transport through RC remains poorly understood. It is not known for instance how the selection of what gets into the oocyte occurs or whether the secretion pathway sustains it by means of specific motors and cytoskeletal tracks. To address this issue, we focused on the regulation of transport of Golgi units from NC to the oocyte. In mammalian cells, Golgi is a discrete organelle that contains dozens of stacked Golgi cisternae linked together by tubules which form a single large structure capping the nucleus (Mellman and Warren, 2000). In *Drosophila*, the Golgi apparatus does not always exhibit a morphology of stacked cisternae. But, when stacks are present, they do not form a single copy organelle. Instead, they remain scattered throughout the cytoplasm (Kondylis and Rabouille, 2003; Herpers and Rabouille, 2004), an organization that is similar to that in yeast.
(Rossanese et al., 1999). Whatever the morphology of the Golgi apparatus, they are in proximity to tER sites. The resulting structure (one tER site and one Golgi complex) is called tER-Golgi unit (Kondylis and Rabouille, 2003).

In order to understand the regulation of cytoplasmic transport of Golgi to the oocyte through RC, we have analyzed the movement of particles expressing a Golgi marker, in living Drosophila egg chambers. We show that they are actively transported to the RC, where they accumulate before a subset transits through the cytoplasmic bridges at a much slower speed. Mechanisms of transport through RC seem to be structurally sustained by the presence of an asymmetric basket-like actin structure capping the NC side of RC. In addition, we show that MTs are required for the integrity of these baskets and that the transport towards and through RC is Dynein and MyoII-dependant.

MATERIEL AND METHODS

Fly stocks

w^{1118} was used as wild type, GalT (A. Debec, Institut Jacques Monod, Paris, France) as a Golgi marker (Januschke et al., 2007), Rab6-GFP (Januschke et al., 2002), Ph-GFP (L. Gervais Institut Jacques Monod, Paris, France) to visualize plasma membrane and tub-GFP-Dmn/CyO to visualize MTs. Overexpression of Dynamitin was performed as described previously in Januschke et al., 2002, with the following stocks: tub-gal4 / UAS-hDmn. Germline clones were generated by FRT/FLP-mediated recombination (Chou and Perrimon, 1992) by crossing y w sqh^{1} sn^{3} FRT101 /FM7 females to ovoD1, FRT101/Y ; hs-flipF38 males. 24h pulses of egg were allowed to develop to 2nd or early 3rd instar larvae before a 2h heat-shock at 37°C.
Immunostaining of whole mount egg chambers

Egg chambers were dissected in PBS-0.1% Triton X-100, and fixed in 4%PFA in PBS. Primary antibody staining was performed overnight at 4°C. To visualize the actin basket, egg chambers were fixed as described above and incubated 1h30 in a 1/20 Rhodamin-conjugated phalloidin (Molecular Probes) dilution and subsequently imaged on a spinning-disc confocal microscope. GFP-expressing strains were fixed 5min in 4% PFA if no additional antibody staining was required and imaged either on a spinning-disc confocal microscope or an Apotome system. Image J and Photoshop were used to process images.

Time-lapse imaging

In order to keep egg chambers alive during several hours, we used a POC chamber system (Leica). Living egg chambers were separated form each other directly on a coverslip in a 15µl drop of M3 medium supplemented with 2% FBS (Foetal Bovine serum, Sigma), 0.24µl insulin (Sigma), 0.01µg/ml Juvenil Hormon (Sigma), 50µg/ml penicillin/streptomycin solution (Sigma). To prevent evaporation, samples were covered by a special FoilCover (Leica) which is gas permeable CultFoil. Time-lapse video microscopy was performed with an inverted spinning-disk confocal microscope CSU 10 (Perkin Elmer) connected to a Coolsnap HQL camera (Photometrics) with 40x/1.25NA or 63x/1.4 NA objective lenses. In order to follow particles that moved along the Z axis, 3 Z-positions were selected at each time point, with a step size of 0.5um. Given that 4D visualization is not possible yet, trails of particles were generated by a Z projection. Speed of particles are shown as average speeds +/- standard error (S.E.).

Particle tracking in noisy biological environment
A dedicated program was developed to detect and track fluorescently labelled Golgi particles visualized in 3D with a spinning disk microscope. Spot detection was performed automatically by a multi resolution algorithm based on wavelet decomposition of the image, wavelet coefficients' correlation analysis and thresholding the output binary masks for each detected object (Olivo-Marin, 2002). Once particles have been detected in the image sequences, a Bayesian tracking algorithm is used to link them. Associations between existing tracks and detections at a given time were selected on the basis of the maximization of their kinetic likelihood. This likelihood was computed using the Interacting Multiple Model (Genovesio et al., 2006) estimator that makes an adaptive weighted mixture of three models of motion: Brownian motion, directed motion, and curvilinear motion. Since the weights of these models are automatically updated, changes in type of motion are automatically taken into account. Once all the tracks were determined, motions of particles in the ovocyte and the ring canal were characterized by their MSDs and velocities.

**Inhibitors' treatment.**

*Colchicine:* flies were fed with colchicines described in (Januschke et al., 2002).

*Latrunculin B:* directly after dissection in PBS+0.1% Triton-100X, egg chambers were incubated for 5 minutes in 150mM or 300mM Latrunculin B (Sigma) and fixed 20 minutes in 4%PFA. Stainings were subsequently performed as described in the *Immunostaining* section. For *live* imaging, the drug was diluted in M3 medium +hormones (see *Time lapse section*) and washed away after 5 minutes of incubation. For *live* imaging, we worked at a 150mM concentration to prevent chambers from blowing up as soon as the permeable membrane was in contact with them.
RESULTS

Golgi units are asymmetrically distributed within the Drosophila egg chamber.

Unlike mammalian cells in which Golgi units are found close to the nucleus, Drosophila egg chambers present Golgi units dispersed both throughout the NC and the oocyte cytoplasm (Herpers and Rabouille, 2004). Its correct distribution is essential for the proper development of the oocyte (Januschke et al., 2007; Coutelis and Ephrussi, 2007), but the process controlling the transport of Golgi units to the oocyte has not been addressed. To investigate the way the subcellular distribution of Golgi is controlled, we took advantage of a GFP-trap strain, GalT (Morin et al., 2001), in which a GFP-tag is inserted into a gene encoding UDP galactosyl:beta-N-acetylglucosamine beta-1,3-galactosyltransferase, a resident enzyme of Golgi stacks in mammals (Elhammer and Kornfeld 1984). In Drosophila, this protein co-purifies with Golgi fractions and colocalizes with Golgi markers (Janushcke et al., 2007). We chose to focus our analysis on stages 7 to 10A, before cytoplasmic streaming occurs (Gutzeit, 1986). During these developmental steps, transport of proteins and lipids to the plasma membrane are required for subsequent oocyte growth, presumably involving Golgi stacks and secretion vesicles to ensure the process.

We observed that, during the early phases of development, eventhough dispersed throughout NC and oocyte cytoplasm, Golgi units was present with a much higher density in the oocyte as shown in Figures 1B-D. Indeed, we observed that GalT-containing dots gradually accumulated in the oocyte as development progressed. First, at stage 6, the Golgi was found evenly distributed between NC and the oocyte (data not shown). But as soon as the nucleus migrated to the antero-posterior region of the oocyte (stage 7), the Golgi started to accumulate in the oocyte (Figure 1B). At stage 8 it was distributed throughout the entire
oocyte, although less abundantly at the anterior margin (Figure 1C). Finally, by stage 10, GaIT-containing-vesicles were uniformly scattered within the oocyte (Figure 1D). We interpreted the accumulation of the Golgi in the oocyte as an indication of active transport of Golgi into the oocyte against a concentration gradient. In Drosophila egg chamber, the Golgi is observed in close vicinity of ER-exit sites and forms functional units called “tER-Golgi units” (Kondylis and Rabouille, 2003; Herpers and Rabouille, 2004). In order to discriminate the transport we were following, either Golgi vesicles or tER-Golgi units, we performed a double labelling with a dCOG5-GFP transgene, which labels ER-exit-sites (Herpers and Rabouille, 2004), and a Lectin WGA, which distribution is similar to GaIT (Januschke et al., 2007). We observed that 45% of the WGA signal is associated with the dCOG5 labelling, either at the vicinity or inside RC (Supplemental Figure 1), indicating that whole tER-golgi units were transported into the oocyte. WGA signal that did not colocalize with dCOG5 may correspond to membrane vesicles that have left the tER-golgi units. In the following paper, we will use the term Golgi units when refering to tER-Golgi units.

Golgi units are transported towards and through the RC in a three step process.

To elucidate the Golgi units' transport mechanism from the NC to the oocyte, we studied their movement at both high magnification and time resolution in living egg chambers using a spinning disc confocal microscope. Speed mesure of Golgi units allowed us to demonstrate sequential events and distinct mechanisms of transport during RC approach and crossing. We used a computer program for automated 4D tracking of fluorescent particles, which allows the quantification of intracellular movements of vesicles relative to the mass center of the cell nucleus (see materiel and methods). Firstly, in the NC cytoplasm, we observed two categories of movements (Figure 2A): (1) “random” movements consisting of
sequences of discrete zigzag steps, previously described as Brownian movement (Doob, 1957); (2) linear and rapid movements interrupted from time to time by short stops or backward shifts, trajectories which would be compatible with directed transport along cytoskeleton tracks (Figure 2A, yellow arrow). Among these linear paths, we focused on a subset heading towards the oocyte and more specifically to the RC (Figure 2B, yellow arrow, Figures 2E left part of the panel; 2E’, “AT” label; Movie 1). We managed to track Golgi units moving towards RC and calculated an average velocity of 0.190µm/sec +/-0.024 during RC approach.

In front of the RC, no subsequent direct translocation into the oocyte was observed, instead, Golgi units paused and clustered at the RC entrance (Figure 2B, red bracket). This second step was characterized by short Brownian movements (Figure 2E’, “B” label). Then thirdly, a subset of Golgi units detached from lingering clusters and translocated through RC into the oocyte (Figures 2C, 2E”), suggesting that the transit through RC might be selective. Indeed, we scored only 17% of the Golgi units arriving at the RC transiting through them (see Movie 1: 8 particles crossed, out of 47 that were lingering outside the RC). Interestingly, speed calculation revealed a switch from fast motion during the approach (0.190µm/sec +/-0.024) to slower movement during RC transit (0.110µm/sec +/-0.026), suggesting that different molecular motors might be required for RC approach and transit. To further characterize RC crossing, trajectories and speed parameters for each Golgi units were calculated at each time point (Figure 2E-E”; Movie 2). It enabled us to distinguish two types of directional trajectories during RC transit depending on where Golgi units crossed (Figure 2E”). Transit in the middle of the RC exhibited straight trajectories (Figure 2E”, ”AT” label ) and a continuous motion (Figure 2F, blue line), which are features of active transport. On the other hand Golgi units crossing close to the canal rim displayed more complex trajectories of acceleration and short stops together (Figure 2F, pink line) with slight deflections from linear
(Figure 2E”, “AT+B” label), suggesting sequences of successive Brownian movements and
active motion. Finally, Golgi, units that managed to get into the oocyte left the RC vicinity
with a slow motion along straight trajectories (Figure 2D).

Altogether these data suggest that Golgi units are transported in a three step process
into the oocyte through RC, characterized by a switch from fast motion during approach, to a
slow movement during RC transit, interrupted in between by a pause at the RC entrance. In
addition, we pointed out a selective process that sort out Golgi units in front of RC.

A basket-like actin structure associated with RC.

To gain insight into the regulation of cytoplasmic transport from NC to the oocyte, we
analyzed the detailed structure of RC themselves. These bridges, interconnecting either NC or
linking NC with the oocyte (Figure 1A), are derived from the contractile rings of the
incomplete cytokinesis that characterizes cystocyte mitosis (Riparbelli and Callaini, 1995). To
label RC, we used rhodamine-conjugated phalloidin, which mainly stains the inner rim
(Tilney et al., 1996). These canals consist of a layer of circumferentially oriented actin
filaments, the inner rim and an outer region containing subcortical actin filaments forming a
crown radiating from the inner rim (Figure 3A). This outer domain is attached to a thickening
of the plasma membrane (Tilney et al., 1996; see review Robinson and Cooley, 1996) as
shown by a double labelling with actin and Pleckstrin Homology Domain of the
Phospholipase C fused to GFP (Ph-GFP) that specifically labels the plasma membrane
(Varnai and Balla, 1998) (Figures 3B,C). Under the standard staining protocol, cytoplasmic
actin filament bundles are weakly stained. However, we were able to detect actin cytoplasmic
structures composed of bundles of filaments that extended in the NC cytoplasm from the
external margin of the outer rim, which overall shape looked like a conical basket (Figures
3D, E) asymmetrically distributed on the NC side of the RC, connecting the oocyte with its
four neighboring NC. Such actin baskets were also observed on RC connecting adjacent NC (Figure 3F, F’), with the difference that they were present on both sides of the RC. The asymmetrical actin baskets were detected from stages 6-7, and gradually increased in size until stage 9-10, in term of length and number of filaments. By stage 10B, its identification was made difficult by the appearance of cytoplasmic actin filament bundles radiating from the plasma membrane, which prevented the nucleus to physically block RC during the dumping process (Guild et al., 1997). The identification of this actin basket between NC and the oocyte raised the possibility that this asymmetrical structure might have a function at the RC entrance.

*Actin network and MyoII are involved in the RC crossing efficiency and the Golgi redistribution within the egg chamber.*

In the *Drosophila* egg chamber, there are at least 3 sets of actin filaments in the NC: a subcortical layer associated with the cortical region of NC membrane, actin filaments lining the RC and a network of cytoplasmic actin bundles that extend in the cytoplasm (Guild et al., 1997). Since actin filaments are involved in the maintenance of the Golgi structure (see for review Egea et al. 2006), we asked whether the actin network could also be involved specifically in the transport of Golgi units from the NC to the oocyte in *Drosophila* egg chambers. We chose to focus on myosins, since they have been found to be involved in the transport and steady state localization of cytoplasmic particles or organelles in several systems (Mermall, et al. 1994, Bohrmann and Schill, 1997). At first, we observed the expression of a sqh-GFP construct in the RC (Figure 4A). The *spaghetti squash (sqh)* gene encodes the regulatory light chain of nonmuscle Myosin II without which Myosin is non functional (Karess et al. 1991). Then, to assess the requirement for Myosin II in the Golgi
units cytoplasmic transport, we induced homozygous germline clones of the hypomorphic mutation sqh\(^1\). In 14% (n=65) of sqh\(^1\) egg chambers, movements were detected: Brownian motion in 7% of the chambers and directional trajectories in the other 7% (Table 2). However present, rectilinear trajectories towards RC were slowed down by a factor 4 in a sqh\(^1\) mutant background (0.052\(\mu\)m/sec +/-0.010) compared to wt (0.190\(\mu\)m/sec +/-0.024) (Table1), suggesting a role of MyoII in the Golgi units transport to RC. Interestingly, at the RC vicinity, we noticed an abnormal lingering of the Golgi units along filaments of the actin baskets. Indeed, the transient pause observed in wt egg chambers lasted much longer in sqh mutants (data not shown). This observation gave us a first hint of the MyoII requirement during RC transit. Consistently, we also noticed an unusual accumulation of Golgi units clusters either in front of the RC (23%) (Figure 4B) or inside the RC (18%) (Figure 4C) (Table 2), which reinforced the hypothesis for which MyoII may be required during RC transit. However, transport of Golgi units to the oocyte did not seem to be completely abolished in the absence of MyoII. Indeed, the Golgi gradient was still present in sqh mutants (Figure 4B). In fact, speed calculation pointed out a 4 fold slower motion than in a wt context (0.023\(\mu\)m/sec +/-0.002 versus 0.110\(\mu\)m/sec +/-0.026 in wt). This remaining activity favors the hypothesis for which it is a complex of molecular motors that account for the transit process, rather than a single partner. Finally, we noticed as previously reported by Jordan and Karess, 1997, a delay in oocyte growth (Figure 4D compared to E, wt). In sqh background, the size of the oocyte was reduced in stage 10A egg chamber (borders cells contact the anterior margin, Figure 4D, E, yellow arrowhead). This phenotype may reflect a defect in membrane trafficking across RC. Altogether, these sets of data suggest that actin filaments network may contribute to RC transit through MyoII activity.

Next, we analyzed Golgi units trajectories in the presence of Latrunculin B (LatB), an inhibitor of actin filament assembly (Spector et al., 1983). In extreme cases ,in which no
cortical actin remained, treatment with LatB eliminated all particles' movements (data not shown). In less affected egg chambers with partial disassembly of the actin scaffold, some Golgi units still managed to reach the RC vicinity. However, they did not accumulate in front of the RC (Figures 4G, G’), which correlates well with the disappearance of the actin basket in 100% of the chambers (data not shown) and its putative role as a selective barrier. On the other hand, we observed in 4 out of 6 LatB-treated egg chambers a large cluster of Golgi units at the RC exit (Figures 4G, G’ arrow head) compared to wt (Figures 4F, F’). These observations suggest that an actin-dependent process might be involved in dispatching Golgi units to the oocyte sub-cellular compartments where they are required. This is also consistent with data from other systems implicating actin networks in aspects of vesicles sorting and distribution (Lantz et al., 1998 ; see for review Buss et al., 2002; Rogers and Gelfand, 2000).

*Microtubules inhibitors prevent Golgi transportation to the oocyte and disassemble the RC actin baskets.*

Given that microtubules (MTs) are required for subcellular localization of several mRNAs and Rab6 transport (Januschke et al., 2007 ; Clark et al., 2007 ; Theurkauf and Hazelrigg 1998), we next checked whether they could also be involved in the cytoplasmic transport of Golgi units. MTs are enriched at the RC entrance (Grieder and Hazelrigg, 2000 ; Moon and Hazelrigg, 2004; Clark et al., 2007; Mische et al., 2007). They concentrate and converge towards the cytoplasmic bridges (Figure 5A, B) and some extend through them (Figure 5B), suggesting that MTs might serve as tracks along which Golgi units could be towed.
To address this question, we first depolymerized MTs by a colchicine treatment (Janusckhe et al., 2002). We particularly focused on chambers in which the nucleus was mis-localized in the oocyte central region, a consequence of MTs depolymerization (Koch and Spitzer, 1982). This displacement served as an internal control (Figure 5D). In these egg chambers, Golgi units formed clusters that were scattered throughout NC and the oocyte cytoplasm (Figure 5D compared to untreated egg chamber (Figure 5C). In addition, colchicine treatment abolished Golgi units accumulation at the RC entrance as well as fast directional movements, indicating that transport of Golgi units towards RC along straight paths is MT dependent. Moreover, in colchicine-treated egg chambers, the gradient of Golgi units was not present anymore, instead Golgi units were equally distributed between the NC and the oocyte (Figure 5D), suggesting that MTs may also be required for active transport of Golgi units through RC. Eventhough no direct transport towards the RC occured, we observed that as soon as a cluster of Golgi units happened to be close to the RC, it was able to get into the oocyte with a different trajectory and velocity than in untreated conditions (Figures 5E-H) (0.160µm/sec +/-0.020 versus 0.194µm/sec +/-0.024 in wt (Movie 4). No pause or selection was observed. Instead, trajectories were straight, with a constant velocity as if clusters of Golgi units were dumped in the oocyte. Importantly, we also observed clusters leaving back the oocyte towards the NC (Figures 5E’-H’) (0.080 µm/sec +/-0.018 (n=7)) (Movie 5). They moved more slowly than those entering the oocyte, suggesting that an MT-independent transport mechanism towards the oocyte may still remain. Altogether, these records suggest that active transport was switched to free exchanges between both compartments, which would then imply that nothing maintain the gradient at the RC anymore. Indeed, a close look at the RC after colchicine treatment confirmed that the actin baskets were no longer present at the RC entrance (Figure 5J compared to 5I), suggesting that actin baskets may also play the role of physical barriers to prevent any outflow from the oocyte.
**Dynein is required for Golgi units transport from the NC to the oocyte.**

To obtain further evidence for MTs' involvement in RC transit, we sought to interfere with the MTs function by blocking Dynein, a minus-end-directed MTs' motor involved in the mRNA transport from the NC to the oocyte (Januschke et al. 2002; Tekotte and Davis, 2002; Duncan and Warrior, 2002; Bullock et al. 2006; Clark et al. 2007). Since null alleles of *dynein heavy-chain (dhc)* mutation compromise oocyte development (McGrail et al. 1995; McGrail et al. 1997), we chose to disrupt Dynein activity indirectly by overexpressing the dynactin subunit, Dynamitin (*Dmn*) (Burkhardt et al. 1997; Januschke et al. 2002; Duncan and Warrior, 2002). In these egg chambers, the Golgi units organized into big clusters (Figure 6B, C white arrow) compared to *wt* chambers (Figure 6A), which is consistent with a role for Dynein in Golgi subcellular organization. Firstly, we observed that the gradient of Golgi units between the NC and the oocyte was always conserved when Dynein activity was impaired (n=20) as well as the presence of the actin basket (Supplemental Figure 2). Secondly, in chambers in which movements were not totally abolished (10 out of 20 scored), we mainly observed Golgi units manifesting Brownian movements. Directional trajectories were detected in 15% of the chambers (Table 2) but the Golgi units velocity was substantially reduced, (0.114±0.033 µm/sec versus 0.190±0.024 in *wt*; Table 1). These results suggest that Dynein actively transports Golgi units towards RC.

Next, we had a closer look at RC transit in egg chambers in which Golgi units still exhibited directional movements (Figures 6C, C’; Movie 6). In the absence of functional Dynein, translocation through RC was approximately 4-fold slower than in control chambers (0.025±0.007 µm/sec versus 0.110±0.026 in *wt*). In extreme cases, either clusters accumulated in front of the RC (25%; Table 2) without crossing or did not managed to
disassemble as soon as RC transit started (10%; Table2) (Figures 6C, C’). Instead, they formed some sort of filaments that stretched out from one side of the canal to the other, before they reached the oocyte and retracted. These results suggest that Dynein participates in the active translocation of Golgi units through RC along MTs.

To visualize MTs in the vicinity of actin baskets, we took advantage of a transgenic strain expressing moderately a Dmn-GFP fusion protein that decorates the MTs without perturbing the transport (Januschke et al. 2002). Labeling of both MTs and actin networks revealed that MTs come very close to the actin baskets (Figures 6D-E”) and could be divided into at least 3 populations: (1) MTs running parallel to the actin filaments of the basket (see white arrowheads Figures 6D-D”); (2) MTs connecting either the filaments of actin baskets (see red arrowheads Figures 6D-D”) or the ring itself (Figures 6E-E”); (3) MTs coming directly from NC cytoplasm and passing through the RC as shown in Figure 5B (and mentioned by Grieder et al. 2000). Altogether, these observations suggest that each MTs' subgroup might be involved specifically in different steps of the Golgi transport from the NC to the oocyte.

DISCUSSION

In this study, we have described the first live visualization of the transport of Golgi units through RC into the Drosophila oocyte. We carried out a detailed analysis with high-resolution time-lapse images and functional disruption approaches that provided new aspects required for transport from the NC to the oocyte. We have characterized an asymmetric basket-like actin structure capping the NC side of the four RC that connect the oocyte with its neighboring NC. We have shown that Golgi units are actively transported to the oocyte instead of diffusing. They move in a direct path towards the RC where they accumulate before a subset transits to the oocyte. We propose that the actin baskets structurally support the
Golgi units pause at the RC entrance. We show that partial loss of either Dynein or MyosinII activity reduces velocity of Golgi units, which is consistent with a MTs/actin-dependent transport from the NC to the oocyte.

**Identification of an asymmetric actin basket at the RC connecting the NC with the oocyte.**

We have characterized a new actin structure capping every RC present in the *Drosophila* egg chamber, which overall shape looks like a conical basket. These actin baskets are present on both sides of the RC connecting adjacent NC but are asymmetrically distributed on the NC side of the RC connecting the oocyte with its four neighboring NC. We showed that these actin baskets are sensitive to MTs depolymerisation and BFA treatment (E. Nicolas personal communication). It suggests that either MTs may serve as a scaffold that helps maintaining the basket structure or that Mts may sustain the addition of proteins or components that participate in the anchoring or maintenance of the actin baskets at the RC surface.

**Golgi units transport from NC to oocyte can be divided into three distinct steps.**

Altogether, our observations led us to propose the following working model for the NC to oocyte transport (Figures 7). (A) RC approach : Golgi units associated with Dynein motors' complex and MyoII are actively transported along MTs and actin filaments towards the RC. (B) Pause : At the RC entrance, Golgi units pause along the filaments of the actin baskets that decorate the NC side of the RC. We hypothesize that they might dissociate or/and associate with different motors and regulators allowing them to switch onto a second group of MTs that cross the canal. We propose that this step enables a specific selection of Golgi units that can get into the oocyte to prevent any occasional accidents such as direct crossing of organelles. However, the presence of MTs coming from the NC lumen and entering directly
into the RC suggests that some particles might be able to directly transit to the oocyte. These specific particles might be already hooked onto the “crossing” partners. (C) RC Transit: Once associated with the right partners, Golgi units transit through the RC. Our data show that Dynein and MyoII are required for RC crossing. (D) Distribution: Once at the RC exit side, vesicles may be transferred onto a third population of MTs in order to be distributed to the sub-compartments where they are required. The presence of large clusters of Golgi units at the RC exit upon LatB treatment supports the actin network role in the redistribution of secretory vesicles within the oocyte, which is consistent with other data showing functional interactions of actin and MTs networks (Lantz et al., 1998, Buss et al., 2002).

**Actin baskets participates in the transport mechanism through RC.**

The presence of actin baskets at the RC entrance led us to propose that active transport towards the oocyte might be structurally supported by these baskets for three reasons. (1) We observed, in living egg chambers, Golgi units organized in transient filament-like structures that colocalize with actin baskets. (2) Upon colchicine or LatB treatment, the absence of actin baskets correlates with no net accumulation of Golgi units in front of the RC and with bidirectional RC transit (colchicine treatment only). These two observations led us to speculate that actin baskets might “force” Golgi units to pause. This stop could provide the opportunity to change motor complex composition in order to modulate parameters of RC transit. Thus, actin baskets may function as platforms upon which specific Golgi units recruit specific motors and partners that ultimately direct them to the oocyte. (3) In sqh clones, Golgi units linger much longer on the actin basket filaments and, in extreme cases, cluster in front of the RC. (4) Finally, these baskets may also serve as physical barriers preventing outflow from the oocyte, thus assuring the maintenance of the gradient as shown by the observation of Golgi units getting out of the oocyte in colchicine-treated egg chambers.
Approach and RC transit are two specific processes.

This study provides several evidences for different transport mechanisms sustaining RC approach and RC transit. (1) Dynamics specificity. Vesicles arrive much more quickly (1.7 fold greater) at the RC than they do to cross it. The association to different motors and/or regulators could explain this difference (Bullock et al., 2006). (2) Trajectories specificity. Whereas Golgi units exhibit straight trajectories to reach the RC entrance, transit path characteristics depend on where the crossing occurs inside the RC. In the center, linear tracks correspond to active transport, while on the edges, Golgi units are constantly switching from Brownian motion to active transport. (3) Velocity specificity. Transport in NC is rapid. In contrast, Golgi units movement is significantly slower once they transit into the RC. This indicates that motor dependent movement is down-regulated as Golgi units cross the RC.

Transport of Golgi units is Dynein- and MyoII-mediated.

We observed that transport of Golgi units towards and through the RC is Colchicine sensitive. It is reduced in NC and even more dramatically impaired during RC transit when Dynein, a minus–end directed motor, also known to associate with membranes of the trans-Golgi network (Matanis et al., 2002), is absent. These observations indicate a MTs-dependent mechanism for Golgi units transport to the oocyte. In addition, we highlighted the presence of at least three different groups of MTs relative to their localization at the actin basket and RC vicinity. We propose that they may be specifically involved in the different steps of RC approach and transit. Indeed, MacDougal et al., 2003 have suggested that accessory factors are required to specify the MTs subset along which Dynein-mediated transport occurs. We also identified Myosin II as a motor required for transport during RC approach as well as RC
transit. Surprisingly, the reduction of Sqh activity seems to have a stronger effect on Golgi units' velocity than on the impairment of Dynein activity. One can hypothesize that, in UAS-Dmn overexpressing egg chambers, the remaining activity of Dynein may be higher than what is left of Sqh function in sqh clones. Overall, our results show that both MTs and actin networks contribute to the regulation of Golgi units' transport, as we showed that disruption of either MTs or actin motors impaired Golgi units transport. Comparison of motility towards RC and during RC transit in both mutant backgrounds, suggests that at least, both Dynein and MyoII are required, given that the absence of a single one does not completely stop the traffic. It would be interesting to determine what their contribution is and how it is differentially regulated in order to better understand the cross talk between actin filaments and MTs. Investigating the role of cytoskeletal linkers such as Short stop, will be interesting, given that shot mutant egg chambers have a similar phenotype to Dynein associated proteins Egalitarian and Bicaudal B (Röper and Brown, 2003; Röper and Brown, 2004). In this study, we show that Golgi units as for mRNA such as grk (Clark et al. 2007), bcd (Mische et al., 2007) or hairy (Bullock et al. 2006), depend on Dynein for transport from NC to the oocyte. Interestingly, the average velocity of mRNA is faster than the transport of Golgi units (1.45µm/sec+/−0.087 (Clark et al. 2007) versus 0.190µm/sec +/-0.024, towards RC) and (0.25µm/sec+/−0.036 (Clark et al. 2007) versus 0.110µm/sec+/−0.026, through RC). This discrepancy of a factor 7 and 2 respectively, suggests that although a Dynein-based transport is conserved, their motor partners and/or regulators may differ. Indeed, recent observations suggest that (1) Dynein may be associated with motors of opposite polarity, like Kinesin I that acts as an antagonist of Dynein mediated-transport of Exu RNP complexes (Mische et al., 2007); (2) cooperativity of multiple motors can regulate force and velocity of motor complexes (Kural et al., 2005; Mallik et al., 2005; Levi et al., 2006). Thus, regulation of opposing motors may provide the means to control velocity of Golgi units during the different
steps of their journey to the oocyte. Further experiments will be required to characterize these specific partners.

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REFERENCES


FIGURE LEGENDS

Figure 1: Asymmetrical distribution of Golgi units between NC and the oocyte.
(A) Actin distribution at the plasma membrane cortex and in ring canals (RC) connecting adjacent nurse cells (NC) or between NC and the oocyte (Oo). (B-D) Golgi units labelled with galT-GFP gradually accumulate in the oocyte from stage 7 to stage 10 egg chambers. Scale bars: 38µm

Figure 2: Golgi units move in a direct path towards and across the oocyte.
(A-D) Trails of Golgi units were obtained by superimposing time-lapse snapshots taken every 5sec., during approximately 2min. (A,C) and 9min. (D). (A) In NC, Golgi units exhibited either Brownian movements (labelled as “B”) or directional trajectories (yellow arrow). (B) Trail of a Golgi unit approaching the RC (yellow arrow). Others linger at the RC front (red line). Blue stars point the RC rims. (C) Transit of Golgi units across the RC. (D) Golgi units leaving the RC. Scale bar: 9µm. n= number of egg chamber ; p=number of Golgi units.
(E-E”) Tracks of Golgi units while approaching and transiting across a RC were calculated by a computer program (see material and methods). (E) Trajectories of Golgi units were recorded during 15 min. Snapshots were taken at 6sec. intervals. Arrival, pausing and crossing are shown together with a different color for each tracked particle. GalT cortical accumulation (delineated in green) highlights the RC rims. Nurse cells (NC) Oocyte (Oo). (E’) A close- up of the RC entrance showing 4 trajectories selected in E. “AT” : Golgi units
actively transported towards the RC. “B”: Golgi units pausing at the RC entrance. They exhibited short back-and-forth movements, presumably Brownian movements. (E”) Close-up at the RC crossing. Time point calculation and trajectories analysis suggest that Golgi units are either actively transported “AT” or transit to the oocyte by successive Brownian and directed movements “AT+B”. (F) Graph of Golgi units' velocity during RC transit. Time point “O” corresponds to the RC entrance. Time point “10” corresponds to the RC exit. According to their location within the RC, Golgi units exhibited speed differences during RC transit. In the center (blue line) they crossed the RC with a constant speed, as for when close to the rim (pink line) they exhibited acceleration and deceleration phases.

**Figure 3: An actin-based structure capping RC.**

(A-C) RC are composed of an inner rim (In), which corresponds to bundles of actin filaments organized into a ring, and an outer rim (Ou) containing actin filaments that radiate from the inner rim. (C) This crown of actin filaments is embedded in a thickening of the plasma membrane as shown by the double-labelling with a membrane marker, PH-GFP. Whereas these actin baskets are asymmetrically present on the NC side of the four RC connecting the oocyte (D-E), they cap both sides of RC that connect neighbouring NC (F, F’). Ba: actin basket; In: inner rim; Ou: outer rim. Scale bar: 8µm. (G) Diagram of an actin basket–like structure capping a RC.

**Figure 4: Actin network is involved in RC crossing and in Golgi units' redistribution within the oocyte.**

(A) MyosinII is present in the RC inner rim. (B-C) Snapshots of sqh ovoD living egg chambers. Golgi units cluster at the RC entrance (B) or are clogged inside the RC (C) (D-E) The development of the oocyte is frequently delayed in sqh mutants (D), compared to wt (E):
the oocyte is much smaller in sqh mutant egg chambers, while the border cells have already reached the anterior margin (white arrowhead). n= number of egg chambers ; p=number of Golgi units. Scale bars : 6µm (A) and 38 µm (B, C, D-G’).

(F-G’) are snapshots of one time point of wt (F-F’) or LatB treated (G, G’) living egg chambers. (G-G’ arrowhead) Upon actin filaments depolymerization, Golgi units cluster at the RC exit. Scale bars : 6µm.

**Figure 5 : Alteration of Golgi units' transport towards and through RC upon MTs depletion.**

(A) MTs are visualized with a αtub84-GFP or (B) a Dmn-GFP construct (green). Phalloidin (red) labels actin at the RC and at the plasma membrane cortex. MTs converge towards RC. Some seem to extend through it. Arrowheads point to the RC hedges (A). Scale bar : 40µm. (C, D) MTs depletion upon colchicine treatment (D) induces the redistribution of Golgi units between NC and the oocyte as well as their clustering in big aggregates compared to untreated (C). Scale bar: 40µm. (E-H’) Successive snapshots taken at different time intervals in colchicine-treated living egg chambers. (E-H) Entrance: in absence of MTs, Golgi units lying at RC vicinity enter the oocyte with a straight trajectory and a constant and slow velocity, suggesting a switch from active transport to diffusion between NC and the oocyte. (E’-F’) Exit : unidirectional transport is abolished in absence of MTs network. Golgi units can now leave the oocyte. n: number of chambers ; p: number of particles. Scale bar: 6µm. (I-N) Fixed preparations of wt and colchicine-treated egg chambers. When the MTs' network is impaired, actin baskets are not present anymore. Scale bar : 6µm.

**Figure 6 : Dynein is required for RC crossing of Golgi units.**
Snapshot at one time-point of \( w t \) (A) and UAS-Dmn overexpressing (mimicking Dynein loss-of-function) living egg chambers. (A’-C’) are close ups of the above pictures. (B,B’) When Dynein function is impaired, Golgi units cluster (white arrowhead) and accumulate in front of RC. Most of them do not managed to cross. (C-C’) However, in extreme cases, some do cross as long stretches. Those stretches manage to get to the RC exit, but with a much slower motion than in \( w t \) chambers. Scale Bars : 10\( \mu \)m. \( n \): number of chambers ; \( p \): number of particles.

(D-E) : Distribution of MTs at the vicinity of the RC-capping actin-baskets. MTs are visualized with a Dmn-GFP construct (green) and actin by a phalloidin labelling (red). A sub population of MTs run parallel to the actin basket filaments (D-D” white arrowhead) while others connect directly to the inner rim (E-E”) or to actin filaments of the basket (D-D” red arrowhead).

**Figure 7 : Working model for Golgi units transport across RC.**

Cross- section of a RC (orange) is shown, capped by an actin basket (red). Left (green) is the NC side, and right (light orange) is the oocyte (Oo). (A) Approach: Black lines are MTs and red lines, filaments of the actin network. Green circles represent Golgi units, blue circles Dynein motor complex and pink circles Myosin II. These two motors contribute to Golgi units transport towards RC. (B) Pause: once at the RC entrance, Golgi units pause in order to associate with the right motors for transit. (C) Transit : Dynein and MyoII contribute to RC crossing, though MyoII to a lesser extend. (D) Distribution: Once in the oocyte, Golgi units' redistribution onto new Mts is actin network-mediated.

**Table 1 : Velocity of Golgi units transport in control, UAS-Dmn overexpressing and \( sqh \) living egg chambers.**
Rates are calculated from movies of living egg chambers of each genotype indicated at the top of the table. * n=number of egg chambers scored ; p = number of Golgi units scored.

Table 2 : Quantification of defects in control, UAS-Dmn overexpressing and sqh living egg chambers.

% of defects are calculated from movies of living egg chambers of each genotype indicated at the top of the table. Line 1 corresponds to the % of egg chambers in which rectilinear trajectories versus only Brownian motion are detected in NC. Line 2 corresponds to the % of chambers in which RC transit is slow down or does not occur, either because of Golgi unit clustered at the entrance, or blocked inside the canal.

*n= number of egg chambers scored
Figure 1

(A) Actin staining showing the region of interest (RC) and the notch (NC).
(B) Golgi staining at stage 7.
(C) Golgi staining at stage 8.
(D) Golgi staining at stage 10.
Figure 2

Towards the RC:
0.19 µm/sec ± 0.024 (n=14; p=35)

Through the RC:
0.11 µm/sec ± 0.026 (n=14; p=27)

Leaving the RC:
0.04 µm/sec ± 0.020 (n=2; p=2)
Towards: 0.052 μm/sec ± 0.010 (n=4; p=12)  
Through: 0.023 μm/sec ± 0.002 (n=2; p=13)
Colchicine

**Entrance:** 0.16μm/sec ±0.020 (n=3; p=11)  
**Exit:** 0.08μm/sec ±0.018 (n=1; p=7)
Table 1

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