The secretory membrane system in the *Drosophila* syncytial blastoderm embryo exists as functionally compartmentalized units around individual nuclei

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Drosophila melanogaster embryogenesis begins with 13 nuclear division cycles within a syncytium. This produces >6,000 nuclei that, during the next division cycle, become encased in plasma membrane in the process known as cellularization. In this study, we investigate how the secretory membrane system becomes equally apportioned among the thousands of syncytial nuclei in preparation for cellularization. Upon nuclear arrival at the cortex, the endoplasmic reticulum (ER) and Golgi were found to segregate among nuclei, with each nucleus becoming surrounded by a single ER/Golgi membrane system separate from adjacent ones. The nuclear-associated units of ER and Golgi across the syncytial blastoderm produced secretory products that were delivered to the plasma membrane in a spatially restricted fashion across the embryo. This occurred in the absence of plasma membrane boundaries between nuclei and was dependent on centrosome-derived microtubules. The emergence of secretory membranes that compartmentalized around individual nuclei in the syncytial blastoderm is likely to ensure that secretory organelles are equivalently partitioned among nuclei at cellularization and could play an important role in the establishment of localized gene and protein expression patterns within the early embryo.

Introduction

The precellularization stages of *Drosophila melanogaster* embryogenesis entail 13 rapid nuclear divisions within a common cytoplasm. The first nine of these nuclear divisions take place deep within the interior of the embryo to produce roughly 300–400 nuclei by the end of the ninth division. Development continues with the migration of these nuclei toward the periphery of the embryo. Once at the periphery, nuclei undergo four additional rounds of division (nuclear cycles 10–13) in the stage known as the syncytial blastoderm. Except for the pole cells located at the posterior end of the embryo, all nuclei in the syncytial blastoderm embryo occupy a common cytoplasm. The sharing of a common cytoplasm ceases when each nucleus becomes individually encased in plasma membrane during interphase of nuclear cycle 14. This cellularization event produces >6,000 primary epithelial cells (Foe and Alberts, 1983; Foe et al., 1993).

In most animal cell types, the organization of the ER and Golgi apparatus is intimately tied to the localization of the nucleus and astral microtubules derived from centrosomes. The ER extends off the nuclear envelope along microtubules as a tubule network (Terasaki et al., 1986; Waterman-Storer and Salmon, 1998), whereas the Golgi apparatus localizes adjacently to centrosomes or to ER export sites (Barr and Egerer, 2005). Interestingly, this arrangement of organelles is absent in the preblastoderm *Drosophila* embryo. There, maternally derived ER and Golgi membranes are localized distinctly at the embryo periphery (Ripoche et al., 1994; Bobinnec et al., 2003), whereas nuclei and centrosomes are found deep within the embryo interior (Freeman et al., 1986; Raff and Glover, 1989). A result of this arrangement is that when nuclei and centrosomes arrive at the embryo periphery, ER and Golgi membranes must somehow become segregated among the thousands of syncytial nuclei.

In this study, we address how the secretory membrane system partitions among nuclei in preparation for cellularization in the *Drosophila* syncytial blastoderm. Toward this end, we have used GFP-tagged ER, Golgi, and plasma membrane markers in a variety of biophysical-based experiments to examine membrane continuity and organellar dynamics in living

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Abbreviations used in this paper: BFA, brefeldin A; FLIP, fluorescence loss in photobleaching; GalT, galactosyltransferase; ROI, region of interest.

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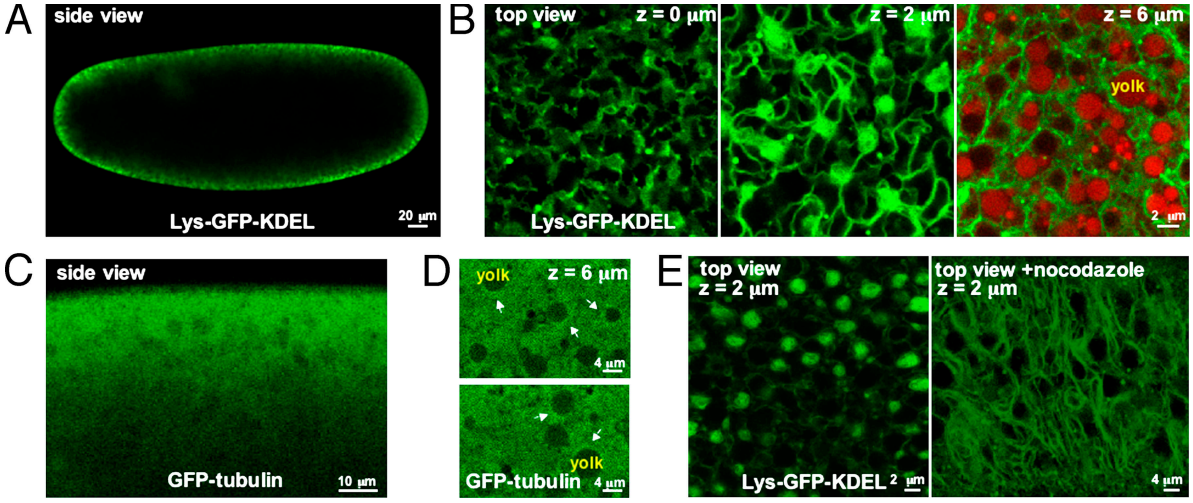

Figure 1. **ER organization before nuclear migration.** (A) The distribution of ER in a Lys-GFP-KDEL–expressing preblastoderm embryo. The ER was restricted to the periphery of the embryo during all nuclear divisions before cellularization (see Video 1, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). (B) Confocal sections of a Lys-GFP-KDEL–expressing preblastoderm embryo at z = 0, 2, and 6 μm beneath the plasma membrane. ER (green) forms an interconnected network of membrane sheets and accumulates in clusters directly beneath the plasma membrane (z = 0 and 2 μm) and forms a loose network of membrane tubules deeper into the periplasm (z = 6 μm), with yolk granules (red) filling the space between the tubules. (C) Confocal section of a GFP-tubulin–expressing embryo showing that tubulin is concentrated within 20 μm of the plasma membrane. (D) Confocal section of a GFP-tubulin–expressing embryo 6 μm beneath the plasma membrane. Filamentous, tubulin-rich structures surrounding spherical particles that excluded fluorescence can be seen (arrows). (E) ER membranes beneath the plasma membrane organize in tight clusters that are interconnected with membrane tubules (left). After nocodazole microinjection, ER clusters disappear and are replaced by long strands of ER (right).

Drosophila embryos. We report that distinct nuclear-associated secretory units of ER and Golgi emerge across the embryo in the absence of plasma membrane boundaries during the syncytial blastoderm stage. The secretory units are shown to mediate localized protein delivery to the plasma membrane and to require centrosome-derived astral microtubules for their maintenance. We discuss how this organization helps to equivalently partition ER and Golgi into daughter cells at cellularization, and we propose potential roles for it in the establishment and maintenance of localized gene and protein expression patterns within the early embryo.

Results

ER membrane organization during the preblastoderm stage and its dependence on cortical microtubules

To visualize ER in the early embryo, we used transgenic *Drosophila* embryos expressing the ER marker Lys-GFP-KDEL (Snapp et al., 2004), which contains an NH_2-terminal signal sequence (Snapp et al., 2004) and a COOH-terminal KDEL sequence (Munro and Pelham, 1987). Confocal imaging of live embryos expressing Lys-GFP-KDEL before nuclear arrival at the embryo periphery revealed that ER membranes were concentrated in a band at the cortex (Fig. 1 A and Video 1 for 3D image; available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). The membranes extended up to 30 μm beneath the plasma membrane, with no detectable pool in the embryo interior. Large, autofluorescent yolk granules (Fig. 1 B, red structures; z = 6 μm) filled the space between ER elements.

As previously mentioned, the morphology of the ER in most animal cell types is dependent on microtubules (Terasaki et al., 1986; Waterman-Storer and Salmon, 1998). In the *Drosophila* preblastoderm embryo, however, centrosomes are bound to nuclei deep within the embryo. Nevertheless, there are nonpolymerized and short polymerized forms of tubulin localized immediately beneath the plasma membrane to a depth of 10 μm (Karr and Alberts, 1986). These short microtubules are organized around spherical particles, which are likely cortical granules and/or yolk platelets (Karr and Alberts, 1986).

To investigate whether microtubules in the cortex of the preblastoderm embryo contributed to the observed organization of the ER, we examined the distribution of tubulin in GFP-tubulin–expressing embryos (see Materials and methods). Pronounced GFP-tubulin fluorescence was seen ~10–20 μm beneath the plasma membrane (Fig. 1 C) and included filamentous, tubulin-rich structures surrounding spherical particles that excluded fluorescence (Fig. 1 D, arrows), presumably representing yolk particles with associated microtubules as reported in other systems (Jaffe and Terasaki, 1994; Mehlmann et al., 1995; Terasaki et al., 1996). These observations led us to conclude that short microtubules were indeed present in the embryo cortex before nuclear migration.

We then examined whether these short microtubules played a role in ER organization in the preblastoderm embryo. Nocodazole (a microtubule-disrupting agent) was microinjected into preblastoderm embryos expressing Lys-GFP-KDEL, and changes in the distribution of Lys-GFP-KDEL were examined. Notably, in the region of the embryo in which nocodazole was injected, both the spherical cluster and tubule network patterns of the ER observed before nocodazole injection (Fig. 1 E, left) disappeared and were replaced by long, parallel strands of ER (Fig. 1 E, right). Injection of DMSO alone did not result in any obvious morphological change of ER clusters (unpublished data).

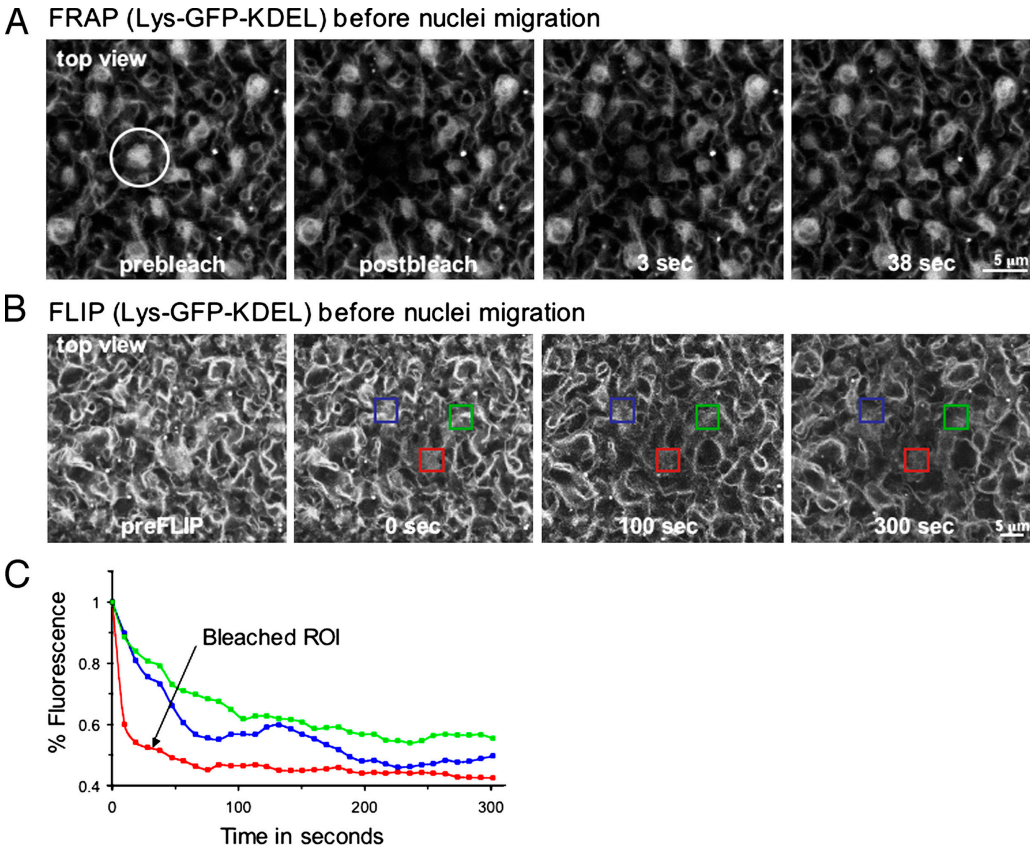

Figure 2. **ER membranes form an interconnected membrane network before nuclear migration.** (A) Lys-GFP-KDEL molecules freely diffuse within the ER lumen of the preblastoderm as determined by FRAP. A small ROI (outlined circle) was photobleached, and fluorescence recovery was monitored. Lys-GFP-KDEL fluorescence significantly recovered within 38 s of photobleaching. (B and C) FLIP of Lys-GFP-KDEL in the preblastoderm reveals the continuity of ER membranes. Repetitive photobleaching of a small ROI (red box) was performed. Fluorescence was depleted exponentially in distant regions (blue and green boxes) surrounding the bleached ROI, indicating that the ER membranes were interconnected.

These results indicated that cortical microtubules are necessary for the ER to become organized into spherical clusters and tubule networks during the preblastoderm stage.

ER membranes are interconnected before nuclear migration

We next asked whether ER membranes in the preblastoderm embryo existed as a single, interconnected system. To test this, we performed FRAP experiments in embryos expressing Lys-GFP-KDEL. A small region of ER at the embryo cortex was photobleached using a high intensity laser beam, abolishing GFP fluorescence in this area. Low power imaging was then used to assess fluorescence recovery into the photobleached region. As predicted for a continuous ER system, a rapid and complete recovery of Lys-GFP-KDEL fluorescence into the photobleached area was observed with no gross structural changes in ER membranes (Fig. 2 A).

Further evidence suggesting that the ER existed as a single, continuous membrane system was obtained from repetitive photobleaching experiments (fluorescence loss in photobleaching [FLIP]; Lippincott-Schwartz and Patterson, 2003). In these experiments, a region of the ER in preblastoderm embryos expressing Lys-GFP-KDEL was repeatedly photobleached, and fluorescence from distant areas was examined. If the ER exists as a continuous membrane system at this stage, fluorescence at distant areas should decrease during the FLIP protocol as a result of fluorescent molecules in these areas diffusing into the FLIP region of interest (ROI) and becoming photobleached. Consistent with this scenario, a 50% drop in Lys-GFP-KDEL fluorescence was observed from all ER areas in a 20-µm radius of distance from the FLIP ROI center within 5 min of photobleaching (Fig. 2 B, and see quantification in C). Hence, the ER behaves as a continuous system in preblastoderm embryos.

ER reorganization after nuclear migration and its dependence on astral microtubules

We next examined whether the morphology and continuity of the ER membrane changes upon nuclear migration to the embryo cortex, which occurs during interphase of nuclear cycle 10 (Foe and Alberts, 1983). In time-lapse sequences, no change in the total level of Lys-GFP-KDEL fluorescence occurred before or immediately after nuclear arrival (Video 2, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1), strongly suggesting that nuclei do not bring a significant amount of their own ER during this period. Nevertheless, the organization of already existing ER was significantly affected by the migrating nuclei (Fig. 3 A and Video 2). In particular, the spiral clusters of the ER membrane appeared to unwind as nuclei arrived at

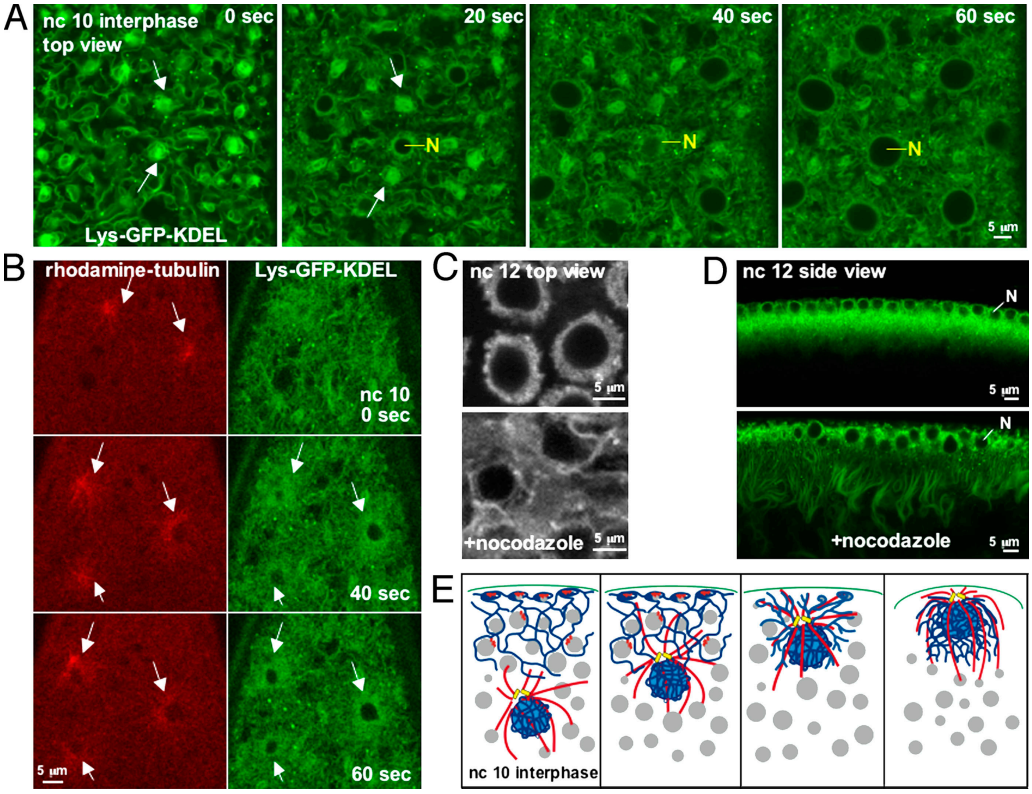

Figure 3. **Reorganization of ER membranes after nuclear migration is mediated by centrosomally derived microtubules.** (A) Time-lapse of nuclear migration in Lys-GFP-KDEL embryos. Once nuclei (N) reach the periphery, individual nuclei reorganize the maternal ER around themselves. The tight ER clusters (arrows) are sequestered to form individual ER units surrounding a given nuclei, and, by the end of migration, they have completely unwound (see Video 2, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). (B) Time-lapse images of nuclear migration in Lys-GFP-KDEL embryos injected with rhodamine-tubulin. Note that the concentration of ER (green) around nuclei correlates with the distribution of astral microtubules (red). (C and D) Confocal sections of a Lys-GFP-KDEL–expressing syncytial blastoderm embryo showing the tight association of ER with individual nuclei when viewed from the embryo surface (C, top) or at a cross section (D, top). This tight association is lost upon nocodazole microinjection (C and D, bottom). (E) A schematic diagram showing the movement of a nucleus to the surface of the embryo. Microtubules (red) help direct nuclei from the embryo interior toward the periphery during the interphase of cycle 10, at the same time displacing yolk granules (gray) into the interior. Interactions between centrosome-nucleated microtubules and the ER (blue) then lead to the recruitment of ER membranes around individual nuclei. Preexisting tight ER clusters unwind as their membranes are organized around each nucleus.

the cortex. Interestingly, the membrane from these clusters, together with other tubule ER elements, then reassembled around individual nuclei.

To test whether the observed changes in the ER during and after nuclear arrival were mediated by nuclear-associated microtubules, we imaged tubulin and ER simultaneously in Lys-GFP-KDEL–expressing embryos that were microinjected with rhodamine-labeled tubulin. Reorganization of the ER around an individual nuclei coincided with the appearance of nuclear-associated centrosomes and astral microtubules (seen as rhodamine-labeled microtubular arrays emanating from one side of the nucleus; Fig. 3 B). To test whether this required microtubules, we microinjected nocodazole, which caused microtubules to disassemble at the site of injection (unpublished data). ER membranes at the site of injection were no longer tightly organized around individual nuclei and appeared as a loose network (Fig. 3, C and D). DMSO injection alone resulted in no change in the morphology of nuclear-associated ER membranes (unpublished data). Hence, microtubules are important for the reorganization and maintenance of the ER around nuclei in the syncytial blastoderm embryo.

A model for how ER membranes reorganize as nuclei and their associated centrosomes migrate to the embryo cortex based on our results is depicted in Fig. 3 E. In this scheme, the existing tubulin at the cortex becomes incorporated into centrosome-derived astral microtubules as nuclei and their associated centrosomes migrate to the cortex. Growth of the astral microtubules recruits and retains ER membrane to areas specifically surrounding nuclei.

The ER membrane surrounding an individual nuclei in the syncytial blastoderm behaves as a distinct, isolated unit

Time-lapse images of a Lys-GFP-KDEL–expressing embryo during the syncytial blastoderm stage revealed the ER to be tightly organized around individual nuclei during interphase and mitosis (Fig. 4 A and Video 3, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). This is in accordance with earlier observations of ER dynamics during the syncytial mitoses (Bobinnec et al., 2003). Upon photobleaching a small area of ER adjacent to one nuclei, rapid recovery into this area occurred as a result of fluorescent molecules outside

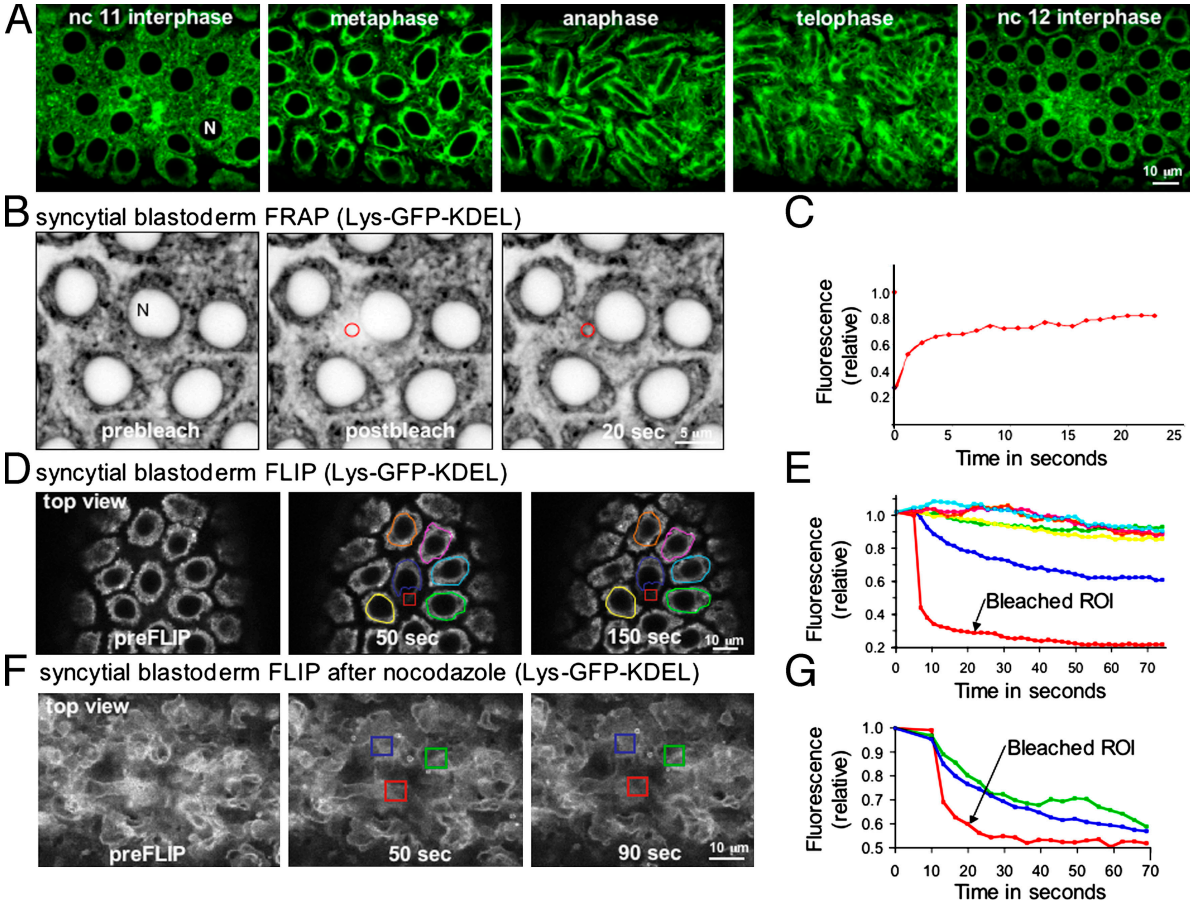

Figure 4. **Upon nuclear migration, the ER becomes compartmentalized around individual nuclei by a process that is dependent on astral microtubules.** (A) Top view of the ER during nuclear division 11. ER membranes surrounding individual nuclei (N) appeared as distinct units at the periphery during each interphase and through each nuclear division (see Video 3, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). (B and C) Lys-GFP-KDEL molecules freely diffuse within the ER lumen of the syncytial blastoderm as determined by FRAP. A small ROI (red circle) was photobleached, and fluorescence recovery was monitored. Lys-GFP-KDEL fluorescence significantly recovered within 20 s of photobleaching. Images shown in B were inverted. (D and E) Top view FLIP of Lys-GFP-KDEL in the syncytial blastoderm shows compartmentalization of ER membranes. Repetitive photobleaching of a small ROI (red box) adjacent to one nucleus was performed. Fluorescence was depleted exponentially in the region directly surrounding the bleached ROI (blue box), whereas the fluorescence of neighboring ER–nuclei systems (orange, magenta, blue, green, and yellow outlines) was minimally affected, indicating a loss in ER membrane continuity. (F and G) Top view FLIP of Lys-GFP-KDEL in the syncytial blastoderm after nocodazole microinjection results in the loss of ER compartmentalization. Embryos were injected with nocodazole as described in Materials and methods. Repetitive photobleaching of a small ROI (red box) was performed. Fluorescence was depleted exponentially in distant regions (blue and green boxes) surrounding the bleached ROI, indicating long-range diffusion of the Lys-GFP-KDEL molecules.

the bleached box redistributing into the bleached area (Fig. 4, B and C). The fluorescent redistribution appeared to occur primarily from nonbleached ER surrounding the one nuclei and not from ER surrounding other nuclei. This raised the possibility that ER membranes that surround individual nuclei at this developmental stage exist as segregated units that do not exchange components.

We investigated this possibility by performing FLIP in a small region of ER associated with one nucleus (Fig. 4 D). Notably, a 40% drop in fluorescence from the ER surrounding the nucleus adjacent to the bleached area occurred within 2–3 min of photobleaching (which is the duration of the examined interphase). Fluorescence in areas of ER that were associated with other nuclei was minimally affected during this period, with a 5% drop seen in areas extending 20 μm away from the FLIP ROI center (see Fig. 4 E for quantification). Given that the photobleaching experiment encompassed the whole interphase, these results suggested that the diffusion of Lys-GFP-KDEL molecules during this interval was restricted to areas of ER surrounding individual interphase nuclei. Hence, the ER around individual nuclei behaved as a compartmentalized system that did not exchange its components with ER surrounding other nuclei.

To determine whether this property of ER depended on astral microtubules, we performed a parallel FLIP experiment in Lys-GFP-KDEL–expressing syncytial blastoderm embryos microinjected with nocodazole. Upon repetitive photobleaching of a small region of ER, fluorescence in areas extending 20 μm away from the FLIP ROI center now showed a significant drop in fluorescence (~40%; Fig. 4, F and G) compared with the slight drop observed in untreated cells (i.e., ~5%; Fig. 4, B and C). Hence, microtubules were necessary for the ER to behave as isolated units surrounding individual nuclei.

It is known that the plasma membrane in the syncytial blastoderm partly invaginates around each nuclei (Karr and

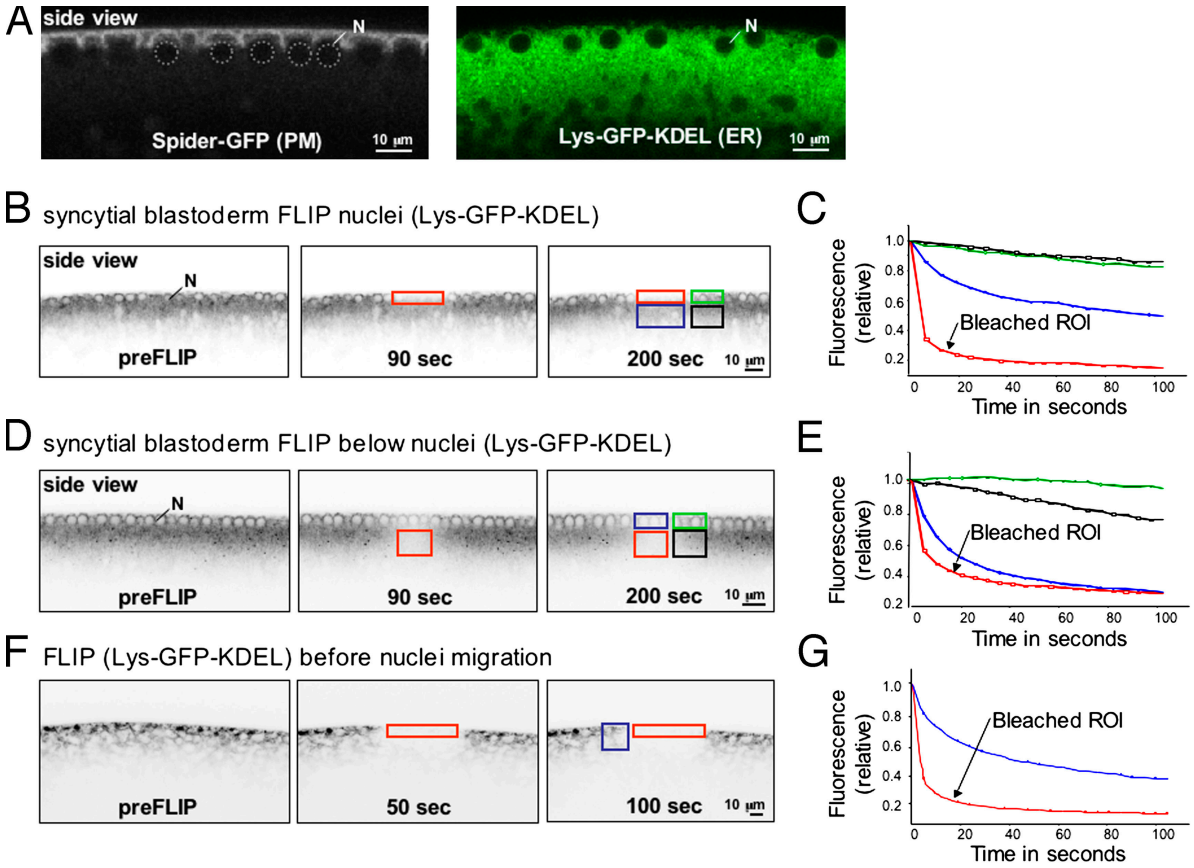

Figure 5. **Compartmentalization of ER around nuclei is not dependent on plasma membrane invaginations.** (A) Plasma membrane (PM) invaginations in the syncytial blastoderm during interphase were visualized with Spider-GFP (left; see Materials and methods). ER membranes visualized during the same nuclear cycle with Lys-GFP-KDEL extend much deeper than the plasma membrane (right). (B and C) Side view FLIP of Lys-GFP-KDEL over a row of nuclei (N). Repetitive photobleaching of a small ROI (red box) was performed. Fluorescence was depleted exponentially in the region directly below the bleached ROI (blue box), whereas neighboring regions (green and black boxes) were minimally affected. (D and E) Side view FLIP of Lys-GFP-KDEL below a row of nuclei. Repetitive photobleaching of a small ROI (red box) was performed. Fluorescence was depleted exponentially in the region directly above the bleached ROI (blue box), whereas neighboring regions (green and black boxes) were minimally affected. (F and G) Side view FLIP of Lys-GFP-KDEL in a preblastoderm embryo. Repetitive photobleaching of a ROI (red box) was performed. Fluorescence was depleted exponentially in the region directly adjacent to the bleached ROI (blue box), indicating long-range diffusion across the embryo periphery. Images shown in B, D, and F were inverted.

Alberts, 1986; Miller et al., 1989). To address whether this could explain the restricted exchange of ER proteins between ER associated with different nuclei, we examined the depth of such plasma membrane invaginations using the plasma membrane marker Spider-GFP, a casein kinase I encoded by the gene *gilgamesh* that associates with the plasma membrane and secretory vesicles destined for the plasma membrane (see Materials and methods; Babu et al., 2002). In syncytial blastoderm embryos expressing Spider-GFP, plasma membrane invaginations were seen extending down ~5 μm from the embryo surface (Fig. 5 A, Spider-GFP). In contrast, ER membranes observed in Lys-GFP-KDEL–expressing embryos extended far deeper (~15 μm; Fig. 5 A, Lys-GFP-KDEL). Therefore, if ER compartmentalization was dependent on plasma membrane invaginations, no compartmentalization should exist at depths >~5 μm below the embryo surface.

To test for this, we performed a series of FLIP experiments in Lys-GFP-KDEL–expressing embryos in which regions directly over nuclei (where plasma membrane invaginations are present) or well below nuclei (where plasma membrane invaginations are absent) were repeatedly photobleached. When a region over nuclei was repeatedly photobleached (Fig. 5, B and C), 40% of fluorescence was lost from the ER extending well below the same nuclei (up to 20 μm below the embryo surface) compared with only 10% from the ER surrounding adjacent nuclei. When a region below nuclei (10–20 μm below the surface) was repeatedly photobleached (Fig. 5, D and E), 70% of fluorescence was lost from the ER above the nuclei, but only 10% was lost from the ER adjacent to the photobleached region below nuclei. Because the restricted diffusion of ER proteins to ER surrounding an individual nucleus occurred at all depths of the ER, we concluded that plasma membrane invaginations could not explain the observed compartmentalized character of ER around an individual nucleus.

We also performed FLIP experiments using Lys-GFP-KDEL in preblastoderm embryos whose ER had not yet become compartmentalized. The rate of fluorescence loss from regions lateral to the bleach area was significantly faster than that observed in the syncytial blastoderm embryo (Fig. 5, F and G), with a 60% drop in fluorescence (vs. 10% in the blastoderm) from a region of comparable distance from the bleached box within the same time period. Together, these data suggested that compartmentalization of ER in the embryo (1) only occurs after

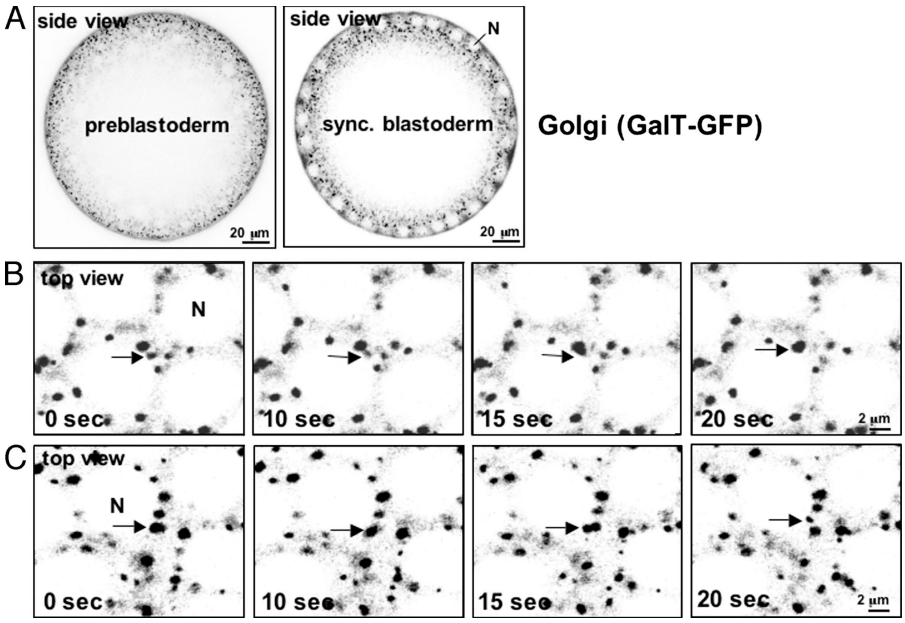

Figure 6. **Golgi distribution and dynamics.** (A) Golgi membranes labeled with GalT-GFP appeared as several thousand structures located at the periphery of the embryo (see Video 4, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). The images shown were inverted. (B and C) The dynamics of Golgi puncta were observed for 20 s during a syncytial interphase. Golgi puncta merged (B, follow arrow in sequence) and separated (C, follow arrow in sequence) in the immediate area around each nucleus (N; see Videos 5 and 6). The images shown were inverted.

nuclei have migrated to the cortex, (2) does not rely on plasma membrane invaginations, and (3) requires microtubules.

Organization of the Golgi apparatus in preblastoderm and syncytial blastoderm embryos

Given our observation that the ER reorganizes into compartmentalized, nuclear-associated units in the syncytial blastoderm, we asked whether other organelles that exchange components with the ER also exhibit such compartmentalization. The Golgi apparatus receives all soluble and membrane-bound cargo exported out of the ER and sorts these components either back to the ER or to the plasma membrane or endosomes (Lippincott-Schwartz et al., 2000). To gain insight into Golgi distribution and whether any organizational changes of the Golgi occur in the developing *Drosophila* embryo, we imaged transgenic embryos expressing galactosyltransferase (GalT)-GFP (GalT-GFP), a resident Golgi enzyme. The same transgenic lines have been used to look at Golgi structure in *Drosophila* ovaries (Snapp et al., 2004).

Before and after nuclear migration, the Golgi appeared as several thousand punctate structures located at the periphery of the embryo (Fig. 6 A), as previously reported (Ripoche et al., 1994; Stanley et al., 1997). The dispersed Golgi puncta were localized to areas of the embryo enriched in ER and, in time-lapse videos, exhibited a jostling motion throughout successive nuclear cycles (Video 4, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). The appearance of these dispersed Golgi puncta resembled that of Golgi puncta found in plants (Nebenfuhr and Staehelin, 2001), sea urchin embryos (Terasaki, 2000), and mammalian cells after treatment with nocodazole (Cole et al., 1996; Storrie et al., 1998). In the case of plant and nocodazole-treated mammalian cells, the Golgi puncta were shown to be localized next to ER exit sites (Cole et al., 1996). An advantage of this type of Golgi distribution is that it allows the Golgi both to receive ER-derived secretory cargo and to recycle proteins back to the ER in the absence of long-range vesicular transport (Cole et al., 1996). Therefore, a similar function might be served by the localization of Golgi puncta near ER in the *Drosophila* embryo.

To further understand the properties and behavior of the Golgi in the syncytial blastoderm, we asked whether the movement of Golgi puncta was restricted around individual nuclei. For this, we visualized puncta behavior in a syncytial blastoderm expressing GalT-GFP by confocal microscopy. Viewed from the embryo surface, Golgi structures were seen jostling around an individual nucleus but never moving between nuclei (Video 5, available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1). This could also be seen in images of embryos viewed from the side (Video 6). During their movement, Golgi structures often merged with other structures (Fig. 6 B, follow arrow in sequence), but merging only occurred between Golgi structures surrounding the same nuclei (Video 5). Finally, when a Golgi structure separated into two distinct structures, these two puncta always remained around the same nuclei (Fig. 6 C, follow arrow in sequence; and Video 5). These results indicated that the movements of Golgi puncta were restricted to areas surrounding a given nucleus.

Membrane exchange between the Golgi and ER is compartmentalized to areas surrounding individual nuclei

Previous studies have shown that Golgi enzymes undergo constitutive cycling through the ER (Miles et al., 2001; Ward et al., 2001), a property that allows Golgi structures to continually modify their size and distribution within cells (Altan-Bonnet et al., 2004). Given this characteristic, we investigated whether Golgi protein exchange between the ER and Golgi was restricted to ER and Golgi structures surrounding a particular nucleus in the syncytial blastoderm.

We began by examining the rate of GalT-GFP exchange between Golgi puncta in embryos at the preblastoderm stage, in which the ER exists as a single continuous system and nuclei

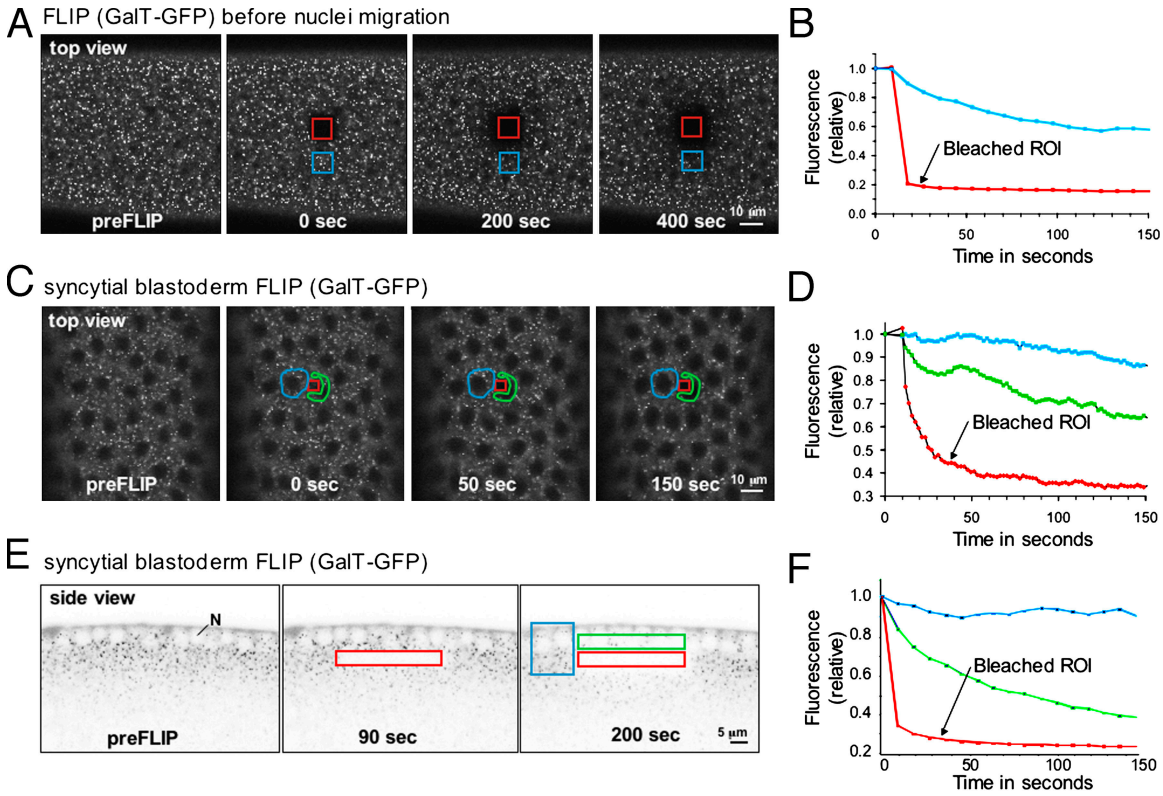

Figure 7. **Golgi enzymes recycle through an interconnected ER system before nuclear migration.** Recycling is restricted to individual ER–nuclei units after nuclear migration. (A and B) Top view FLIP of GalT-GFP in the preblastoderm shows recycling of Golgi enzymes through an interconnected ER membrane network. Repetitive photobleaching of a small ROI (red box) was performed. Fluorescence was depleted exponentially in distant regions (blue box) surrounding the bleached ROI, indicating long-range recycling of the enzymes through ER membranes. (C and D) Top view FLIP of GalT-GFP in the syncytial blastoderm shows compartmentalization of Golgi units. Repetitive photobleaching of a small ROI (red box) adjacent to one nucleus was performed. Fluorescence was depleted exponentially in the region directly surrounding the bleached ROI (green box), whereas the fluorescence of neighboring Golgi–nuclei systems (blue box) was minimally affected, indicating that the recycling of Golgi enzymes is now restricted to individual ER–nuclei units. (E and F) Side view FLIP of GalT-GFP below a row of nuclei (N). Repetitive photobleaching in a small ROI (red box) was performed. Fluorescence was depleted exponentially in the region directly above the bleached ROI (green box), whereas neighboring regions (blue box) were minimally affected. The images shown in E were inverted.

are deep within the embryo. Repetitive photobleaching of Golgi puncta in a preblastoderm embryo expressing GalT-GFP revealed a 40% drop in fluorescence from all areas in a 30-μm radius of distance from the FLIP ROI center within 3–5 min of photobleaching (Fig. 7 A; red box is the photobleached area, and blue box is the area being monitored; B shows quantification). As an individual Golgi puncta did not move laterally across the embryo for >30 μm (not depicted) and the extent of the lateral loss of Golgi fluorescence was similar to that observed in FLIP experiments with the ER marker (Fig. 2, B and C), the data were consistent with Golgi enzymes cycling through an interconnected ER membrane network.

We next examined GalT-GFP exchange between Golgi puncta during the syncytial interphases of nuclear cycles 11–12 after nuclei have migrated to the embryo cortex (Fig. 7, C and D). A ROI containing several Golgi puncta was repeatedly photobleached (Fig. 7 C, red outline), and fluorescence was monitored in the area adjacent to the bleached region (Fig. 7 C, green outline) and the areas surrounding a nearby nuclei (Fig. 7 C, blue outline). Notably, fluorescence loss was observed only from puncta in the immediate ER-nuclear unit that contained the area being photobleached, showing a 35% drop in fluorescence (Fig. 7 D, green curve) with a minimal effect on puncta in the area of surrounding ER-nuclear units, which showed only a 10% drop in fluorescence (Fig. 7 D, blue curve). This pattern of fluorescence loss was similar to that observed with the ER marker at this stage of the embryo (Fig. 4, D and E).

To address whether the compartmentalized exchange of GalT-GFP between Golgi puncta extended to deeper areas within the embryo, we repetitively photobleached an area located below several nuclei (Fig. 7 E, red box). Significant fluorescence loss was observed in Golgi puncta directly above the bleached region (Fig. 7 E, green box) but not from Golgi puncta lateral to the bleached region (Fig. 7 E, blue box). Quantification revealed a 60% loss of fluorescence occurring in the region of Golgi puncta above the bleached area compared with a 5–10% drop in the region lateral to the bleached area (Fig. 7 F). Thus, both the ER and Golgi behave as compartmentalized units surrounding individual nuclei in the syncytial blastoderm.

Evidence for the localized delivery of secretory cargo to the plasma membrane from compartmentalized sources

Given the compartmentalized character of the ER and Golgi around individual syncytial nuclei, we wondered whether their secretory products were delivered to restricted areas of the

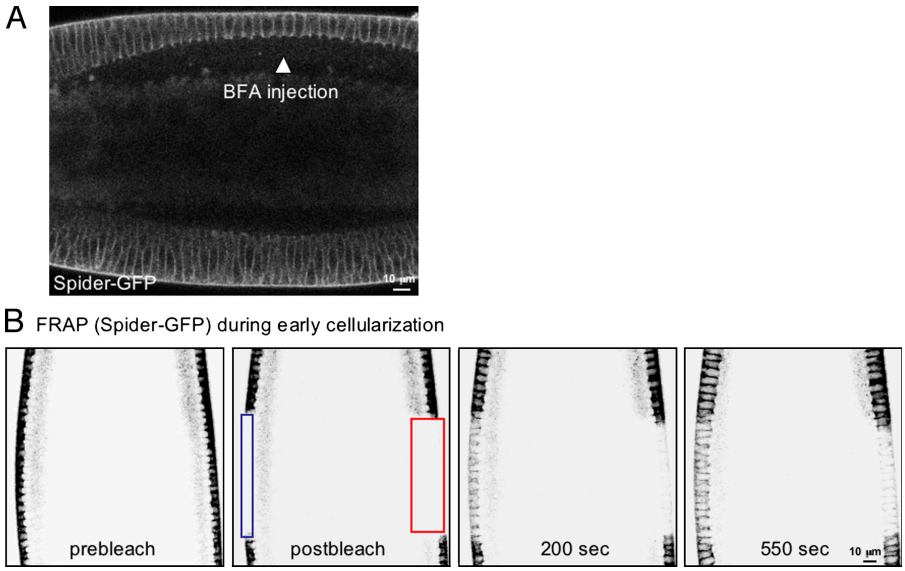

Figure 8. **Evidence that plasma membrane–bound material originates from localized secretory units around individual nuclei.** (A) Injection of BFA (arrowhead) in embryos expressing a plasma membrane marker (Spider-GFP; see Materials and methods) resulted in a dramatic slowdown of membrane invagination at the injection site, suggesting that BFA-induced impairment of secretory units at the injection site is not compensated for by adjacent secretory units. (B) Side view FRAP of a Spider-GFP–expressing embryo during early cellularization. ROIs encompassing plasma membrane–bound Spider-GFP (blue box) or both plasma membrane–bound and intracellular Spider-GFP pools (red box) were simultaneously photobleached, and fluorescence recovery was monitored. Spider-GFP fluorescence on the plasma membrane recovered very little in the case where the intracellular Spider-GFP pool was depleted (red box), indicating that secretion is taking place in a localized manner and not randomly through an extensive secretory system. The images shown were inverted.

plasma membrane near an individual nuclei. To test this possibility, we investigated whether blocking the secretory pathway in one area of the embryo resulted in the reduced delivery of secretory cargo to the plasma membrane only in that area or in other areas as well. To perturb the secretory pathway in these experiments, we used the drug brefeldin A (BFA), which blocks the transport of secretory cargo from the ER to the Golgi apparatus (Sciaky et al., 1997; Ward et al., 2001). To monitor secretory transport, we observed the invagination of the plasma membrane during cellularization, which is dependent on newly synthesized membrane moving through the secretory pathway (Sisson et al., 2000; Pelissier et al., 2003).

In embryos expressing Spider-GFP to label the plasma membrane, the injection of BFA resulted in a dramatic slowdown in membrane invagination during cellularization (Fig. 8 A). Notably, the slowing occurred only in the area of the embryo where BFA was injected, with sites far from the injection site invaginating their plasma membrane normally (i.e., up to 35 μm as found in wild-type embryos; unpublished data). Although the effect of BFA on plasma membrane invagination at cellularization has already been described (Sisson et al., 2000), the localized effect we observed is new. It suggested that delivery of material to the plasma membrane could not be compensated by surrounding secretory units and implied, therefore, that membrane insertion at the plasma membrane occurred in a localized manner in the embryo.

To further test for localized secretory membrane insertion at the plasma membrane, we performed FRAP in embryos expressing Spider-GFP during early cellularization. A region encompassing only the plasma membrane pool of Spider-GFP (Fig. 8 B, blue box) and a region spanning both plasma membrane and intracellular pools of Spider-GFP (Fig. 8 B, red box) were photobleached simultaneously. If membrane insertion at the plasma membrane occurred in a localized manner from an adjacent intracellular pool, the plasma membrane fluorescence associated with the red box (Fig. 8 B) should be unable to efficiently recover (as its adjacent intracellular pool was bleached), whereas the plasma membrane fluorescence associated with the blue box (Fig. 8 B) should recover (as its adjacent intracellular pool was not photobleached). Consistent with this prediction, we found that as cellularization progressed, a more complete and uniform recovery occurred in the region of the blue box (Fig. 8 B). Because the fluorescence in the blue box (Fig. 8 B) recovered simultaneously across all areas of the plasma membrane rather than initially from the edges of the bleach box, recovery was not caused by Spider-GFP diffusing laterally across the plasma membrane. Rather, the fluorescent pool responsible seemed to be derived from the area directly beneath the plasma membrane because when this area was bleached (as occurred in the red box; Fig. 8 B), much less plasma membrane fluorescence recovered.

Altogether, these findings suggested that the membrane carriers responsible for plasma membrane delivery of secretory products were derived from ER/Golgi membranes directly adjacent to the areas of insertion. Thus, all elements of the secretory pathway (ER, Golgi, and transport carriers) appeared to be compartmentalized around individual nuclei in the embryo cortex.

Discussion

The use of transgenic lines expressing GFP-tagged markers in the early embryo allowed us to follow and investigate the organizational and morphological changes in organelles of the

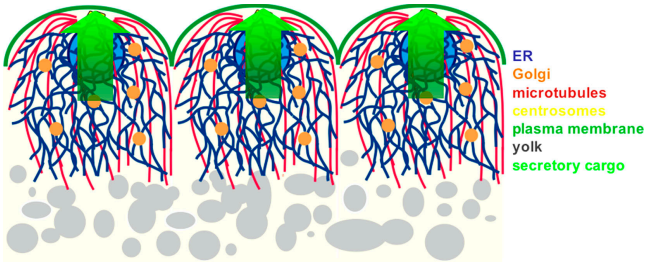

Figure 9. **Schematic model for restricted ER/Golgi secretory units in the *Drosophila* syncytial blastoderm.** ER and Golgi associate with individual nuclei, and this association is dependent on an intact microtubule network emanating from the centrosomes of each nucleus. Despite the absence of plasma membrane boundaries between nuclei, proteins within the ER lumen and Golgi membranes do not significantly exchange with molecules localized in the ER lumen and Golgi membranes surrounding adjacent nuclei. Instead, their movement is restricted to individual ER/Golgi units associated with individual nuclei. Secretory carriers moving from these ER/Golgi units to the plasma membrane likewise undergo restricted movement and insert into plasma membrane areas that are directly adjacent to the ER/Golgi system from which they were generated.

secretory membrane system during the precellularization and cellularization stages of *Drosophila* embryogenesis in real time. We found that before nuclear migration to the embryo cortex, the ER existed as a single, interconnected system through which proteins freely diffused. Upon nuclear arrival at the cortex, the ER and Golgi system became compartmentalized around individual nuclei. As a consequence, ER and Golgi resident proteins now moved only within the ER and Golgi associated with single nuclei. The carriers mediating the transport of secretory products to the plasma membrane also exhibited compartmentalized behavior, delivering their contents to the plasma membrane only in areas adjacent to the ER/Golgi system from which they were generated.

The organization and continuity of ER membranes has been extensively studied during oocyte maturation and early embryogenesis of other organisms, including starfish (Jaffe and Terasaki, 1994; Terasaki et al., 1996), sea urchin (Terasaki, 2000), mouse (Mehlmann et al., 1995), and frog (Terasaki et al., 2001). In these studies, ER membranes labeled by injecting either the ER lipophilic dye DiI or mRNA encoding for an ER lumenal protein marker (ssGFP-KDEL) were shown to exist as an interconnected network of membrane sheets and tubules in the cortex of the fertilized egg, as is the case in the fertilized *Drosophila* preblastoderm embryo. In addition, prominent ER clusters directly beneath the plasma membrane were described in the cortex of the mouse and frog embryos (Mehlmann et al., 1995; Terasaki et al., 2001). These were postulated to have a role in the generation of transient calcium waves or in the propagation of calcium oscillations. The ER clusters that we observed in the *Drosophila* preblastoderm embryo may have a similar role, which the recent study on the existence of distinct calcium microdomains during syncytial divisions (Parry et al., 2005) would support.

Because the ER exists as a continuous system in the *Drosophila* preblastoderm, the embryo must have a mechanism for partitioning this organelle among nuclei so that at cellularization, the newly formed cells have equivalent amounts of ER membrane. Our data suggest that this occurs by a microtubule-driven process that causes the ER to be divided up among the nuclei, resulting in each interphase nucleus becoming surrounded by a single ER membrane system that is separate from adjacent ones. This was demonstrated in FLIP experiments in which resident proteins of the ER and Golgi were seen to be rapidly circulating only within ER and Golgi membrane that associated with a particular nuclei during the four rounds of nuclear division at the periphery. Microtubule depolymerization by the microinjection of nocodazole resulted in the loss of ER and Golgi compartmentalization around a given nucleus, indicating that an intact microtubule network is essential to keep ER and Golgi structures close to individual nuclei in the syncytium. The significance of microtubules in the biogenesis and maintenance of the ER network has been unequivocally demonstrated (Terasaki et al., 1986; Waterman-Storer and Salmon, 1998), so our data reinforce the role of intact microtubules in organizing ER membranes around individual nuclei. Based on these data, we propose a model for the organization and distribution of distinct and separate ER and Golgi membranes with individual nuclei, as illustrated in Fig. 9.

The structural reorganization of the ER after nuclear migration reported in this study is likely to serve many important functions in the early embryo. One key function could be for cellularization. Membrane proteins are synthesized during the syncytial stages (Schweisguth et al., 1990; Hashimoto et al., 1991; Gay and Keith, 1992) as well as during cellularization (Burgess et al., 1997; Lecuit and Wieschaus, 2000). The partitioning of ER and Golgi upon nuclear migration ensures that equivalent amounts of ER and Golgi are packaged with nuclei in preparation for cellularization. During cellularization, such partitioning could additionally serve to facilitate plasma membrane synthesis by ensuring that it is homogenous across the embryo. Indeed, when we disrupted secretory trafficking locally by injecting BFA into a specific region of the embryo during cellularization, plasma membrane synthesis was inhibited only in this region, indicating that secretory vesicles do not randomly circulate in the embryo but must be locally produced near the plasma membrane.

Another function for the compartmentalization of ER and Golgi in the embryo could be to help establish and maintain localized gene and protein expression patterns (Blankenship and Wieschaus, 2001; Houchmandzadeh et al., 2002). Although zygotic transcription and protein synthesis increases dramatically during cellularization, transcription and protein synthesis has been reported to start as early as nuclear cycles 8–10 (Lamb and Laird, 1976; Edgar and Schubiger, 1986; Pritchard and Schubiger, 1996), resulting in highly localized expression patterns well before cellularization.

One simple and logical way that compartmentalization of the ER may affect gene expression patterning could be by the segregation of maternal mRNAs and proteins. In ascidians, some maternally loaded mRNAs have been shown to associate with the rough cortical ER and relocalize with the ER as it moves (Sardet et al., 2003; Prodon et al., 2005). Likewise, some maternal mRNAs in *Drosophila* have been shown to be anchored on ER membranes (Herpers and Rabouille, 2004).

Indeed, recent work has shown that *gurken* mRNA anchors to specific transitional ER–Golgi units in the *Drosophila* oocyte (Herpers and Rabouille, 2004), allowing specific sorting and secretion to take place. If maternally loaded mRNAs anchored on the ER become compartmentalized around individual nuclei upon nuclear migration, as our results would suggest, then these transcripts are likely to be locally expressed. Thus, an already existing polarity in the distribution of maternal material would not only be preserved but would also be further maintained during cortical divisions. To this end, ER and Golgi compartmentalization might provide a mechanism for spatially and temporally restricting maternally derived material during the early stages of *Drosophila* embryogenesis.

In summary, our data suggest that in the absence of plasma membrane boundaries surrounding nuclei but with the requirement of an intact microtubule network, the embryo is able to differentiate the secretory endomembrane system (i.e., ER and Golgi) into segregated nuclear-associated units. In a volume occupied by thousands of nuclei, this capacity for apportioning ER and Golgi among nuclei is likely to be vital for cellularization and for the establishment and maintenance of localized gene and protein expression patterns. The fact that many other organelles are organized by microtubules (i.e., endosomes, lysosomes, and mitochondria) further suggests that it is possible to have the functional equivalents of cells despite the complete absence of plasma membrane boundaries within a syncytium.

Materials and methods

Generation of *Drosophila* stocks
pUASp:Lys-GFP-KDEL and pUASp:GalT-GFP transgenic lines have been previously described (Snapp et al., 2004). The nanos-Gal4:VP16 driver (Van Doren et al., 1998) was used to express UASp transgenes in the early embryo.

Other *Drosophila* stocks
Spider-GFP flies were a gift from A. Debec (Universite Pierre et Marie Curie, Observatoire Oceanologique, Villefranche-sur-mer, France). They were generated using a "protein trap" methodology (Morin et al., 2001) and can be obtained from the European Drosophila Stock Center in Szeged (http://expbio.bio.u-szeged.hu/fly/). GFP-tubulin flies were a gift from A. Spradling (Carnegie Institution, Baltimore, MD).

Imaging of live embryos by laser confocal microscopy
Embryos were collected on apple juice–agar plates, dechorionated in 2% bleach, and placed flat or upright on a Lab-Tech chambered coverglass (Nunc). Chambers were then filled with *Drosophila* Ringer's solution (Tübingen and Düsseldorf). Confocal microscope images of live embryos were captured on an inverted microscope (510 Meta or ConfoCor-2; Carl Zeiss MicroImaging, Inc.) using the 488-nm line of an Ar laser with a 505–530 emission filter for GFP and a 543-nm HeNe laser line with a 560–615 emission filter for rhodamine. To image yolk autofluorescence, a two-photon laser (Chameleon; Coherent) at 820 nm was used with a 435–485 infrared emission filter. Images were captured with a C-Apochromat 1.2 NA 40× water immersion objective (Carl Zeiss MicroImaging, Inc.). Images were analyzed with Image software (W. Rasband, National Institutes of Health [NIH], Bethesda, MD) and Image Examiner software (Carl Zeiss MicroImaging, Inc.) and prepared by Adobe Photoshop 7.0.

Imaging of live embryos by confocal microscopy after microinjection
Embryos were collected for 30 min, aged on collection plates for 50 min, and dechorionated in 2% bleach. Dechorionated embryos were microinjected as previously described (Smith and DeLotto, 1994). Rhodamine-tubulin was obtained from Cytoskeleton, Inc. BFA (Sigma-Aldrich) was microinjected at 5 mg/ml in DMSO. For nocodazole injections, embryos were dechorionated, microinjected with nocodazole (Sigma-Aldrich) at 10 mg/ml in DMSO, mounted in chambers, and placed on ice for 10 min to depolymerize microtubules. Embryos were then imaged at 25°C.

Photobleaching and analysis
FRAP and FLIP were performed by photobleaching a small ROI and monitoring fluorescence recovery or loss over time as described previously (Lippincott-Schwartz and Patterson, 2003; Snapp et al., 2003). To create the fluorescence recovery or loss curves, the background-corrected fluorescence intensities were transformed into a 0–1 scale and were plotted using Microsoft Excel X.

Online supplemental material
Video 1 is a 3D rendering of ER membranes at the surface of a Lys-GFP-KDEL–expressing embryo. Video 2 shows nuclear migration during nuclear cycle 10 interphase in a Lys-GFP-KDEL–expressing embryo as viewed from the embryo surface. Video 3 shows divisions of ER membranes during cycles 10–13 in a Lys-GFP-KDEL–expressing embryo as viewed from the embryo surface. Video 4 shows the dynamics of Golgi puncta during cycles 11–13 at a cross section of a GalT-GFP–expressing embryo. Video 5 shows the movement of Golgi structures around individual syncytial interphase nuclei in a GalT-GFP–expressing embryo as viewed from the embryo surface. Video 6 shows the same as Video 5 at a cross section of the embryo. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200601156/DC1.

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