Vesicular Traffic and Golgi Apparatus Dynamics During Mammalian Spermatogenesis: Implications for Acrosome Architecture

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ABSTRACT

Vesicular membrane trafficking during acrosome biogenesis in bull and rhesus monkey spermatogenesis differs from the somatic cell paradigm as imaged dynamically using the Golgi apparatus probes \( \beta\)-COP, giantin, Golgin-97, and Golgin-95/GM130. In particular, sorting and delivery of proteins seemed less precise during spermatogenesis. In early stages of spermiogenesis, many Golgi resident proteins and specific acrosomal markers were present in the acrosome. Trafficking in both round and elongating spermatids was similar to what has been described for somatic cells, as judged by the kinetics of Golgi protein incorporation into endoplasmic reticulum-like structures after brefeldin A treatment. These Golgi components were retrieved from the acrosome at later stages of differentiation and were completely devoid of immature spermatozoa. Our data suggest that active anterograde and retrograde vesicular transport trafficking pathways, involving both \( \beta\)-COP- and clathrin-coated vesicles, are involved in retrieving Golgi proteins missorted to the acrosome and in controlling the growth and shape of this organelle.

gametogenesis, sperm, spermatid, spermatogenesis, testes

INTRODUCTION

The acrosome is a secretory vesicle containing a number of hydrolytic enzymes that help the sperm penetrate the egg’s coats, and it is assembled only after the formation of the haploid spermatid [1–3]. However, the synthesis of many proteins involved in acrosome biogenesis, such as the proteolytic enzyme acrosin, starts at the pachytene spermatocyte stage and continues throughout the round and elongating spermatid stages [4–6]. In mammals, acrosome biogenesis begins with the fusion of proacrosomal granules synthesized by the Golgi apparatus in pachytene spermatocytes, similar to the formation of secretary granules in many other cell types [1, 7–9]. During the “Golgi phase,” the acrosomal vesicle attaches to the nuclear envelope and grows as a result of the constant arrival and fusion of Golgi-derived vesicles [1, 8, 10]. At later stages (cap and acrosome phase), the acrosomal vesicle flattens and spreads over the nucleus, covering up to two-thirds of its surface. During this process, acrosomal proteins condense and are packed in a paracrystalline structure called the acrosomal granule, which eventually forms the acrosomal matrix in mature sperm [4, 5, 11, 12].

During the early stages of spermiogenesis (Golgi and cap phase), there is a close relationship between the forming acrosomal vesicle and the Golgi apparatus. The Golgi is actively engaged in the formation of the acrosomal vesicle, producing and delivering the proteins and membranes needed for its enlargement and differentiation [3, 12, 13]. There are also a number of small coated vesicles (40–50 nm diameter) that may correspond to the coatamer-coated vesicles (COP), as described in somatic cells [14–16]. One of the components, \( \beta\)-COP, has been localized both in the Golgi apparatus and in the acrosomal membrane of rat spermatids, suggesting a role for COP-1 vesicles in membrane trafficking between these two organelles [17]. At later stages (acrosome and maturation phase), the Golgi apparatus migrates toward the cell pole opposite the nascent acrosome, and the acrosomal vesicle lies underneath the plasma membrane [8, 18, 19]. The molecular mechanisms involved in this cytoplasmic polarization are still unknown. Eventually, the Golgi apparatus remains in the cytoplasmic lobe and is discarded in the cytoplasmic droplet along with most of the other organelles [1, 20, 21].

The acrosome has a characteristic shape and size depending on the species. The shaping of this organelle does not seem to be related to changes in the cytoskeleton or cell-cell interactions. We have previously defined some of the molecular components present in the Golgi apparatus of rhesus monkey spermatids [19]. We hypothesized that either their presence or distribution in the molecular components involved in vesicular trafficking machinery may account for differences in the shaping and kinetics of acrosome biogenesis between mammals. Our data indicate that rhesus monkey and bovine spermatids share most of the Golgi

Received: 29 December 1999.
First decision: 21 January 2000.
Accepted: 15 February 2000.
components used by somatic cells. These components have similar spatial and temporal distribution in both species. Thus, acrosome shaping may involve other unidentified components or unique interactions between the cytoskeleton and the vesicular traffic machinery.

MATERIALS AND METHODS

Chemicals

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Brefeldin A was obtained from Epicentre Technologies (Madison, WI) and was stored as a stock solution of 1 mg/ml at −20°C. BODIPY FL C5-ceramide and NBD C6-ceramide were obtained from Molecular Probes (Eugene, OR) and were stored as a stock solution of 5 mM at −20°C.

Antibodies

Antibodies against Golgin-97, Golgin-160, Golgin95/GM130, and giantin were prepared as described previously [22, 23]. Anti-Golgin and anti-giantin antibodies were used at dilutions of 1:200 and 1:500, respectively. The polyclonal antibody against perinuclear theca (pAb 427, dilution 1:200) was a gift from Dr. Richard Oko (Department of Anatomy and Cell Biology, Queens University, Kingston, ON, Canada). Monoclonal antibodies against human acrosin were a gift from Dr. Claudio Barros (Reproduction and Development Unit, Pontificical Catholic University of Chile, Santiago, Chile) and used in a dilution of 1:200. The antibodies against trans-Golgi network (TGN38), clathrin, and β-COP were purchased from Affinity Bioreagents (Golden, CO) and used according to the manufacturer's instructions. Goat antibody against mouse or rabbit IgG, conjugated with either tetramethylrhodamine B isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC), were obtained from Zymed Laboratories (San Francisco, CA).

Isolation and Culture of Spermatogenic Cells

Rhesus monkey testes were obtained from males undergoing necropsy for reasons unrelated to fertility. Bovine testes were obtained from a local slaughterhouse. Testes were dissected and transferred to a Petri dish filled with TALP-Hepes medium ([24]; modified Tyrode-lactate medium with pyruvate and albumin: 114 mM NaCl, 3.2 mM KCl, 2 mM CaCl2, 0.5 mM MgCl2, 25 mM NaHCO3, 0.4 mM NaH2PO4, 10 mM sodium lactate, 6.5 IU penicillin, 25 µg/ml gentamicin, 3 mg/ml fatty acid-free BSA, 0.2 mM pyruvate, buffered with 10 mM Hepes at pH 7.4) and minced with fine forceps as previously described [25]. The final cell suspension contained all the spermatogenic stages and was used for vital labeling, immunofluorescence, and colloidal gold labeling.

Cell Culture

Rhesus monkey 6308 cells were propagated in Dulbecco modified Eagle medium (D-MEM) supplemented with 10% fetal calf serum, 2 mM glutamine, and 0.3% gentamycin at 37°C in 5% CO2. When needed for immunofluorescence or vital labeling, the cells were grown onto glass coverslips.

Vital Labeling and Imaging of Spermatids and Cell Lines

The procedure for labeling the Golgi apparatus with BODIPY FL C5-ceramide or NBD C6-ceramide was essentially the same as described by Pagano et al. [26]. Spermatids attached to poly-L-lysine-coated coverslips or 6308 cells grown onto glass coverslips were incubated with BODIPY FL C5-ceramide at final concentration of 5 µM in D-MEM containing Hepes buffer instead of bicarbonate at 4°C for 30 min. The coverslips were then washed with TALP-HEPES and prepared for live epifluorescence microscopy. Labeling of cells with NBD C6-ceramide was carried out as described except that the cells were incubated at 4°C for 2 h.

Brefeldin A Treatment

Rhesus spermatids attached to poly-L-lysine-coated coverslips or 6308 cells grown onto glass coverslips were incubated in D-MEM containing brefeldin A for 30 min at 37°C with 5% CO2. The coverslips were then washed with TALP-HEPES and fixed for immunofluorescence. For drug recovery experiments, the coverslips were washed and incubated in D-MEM without brefeldin A for 30 min at 37°C and then fixed for immunofluorescence.

Immunofluorescence and Cell Imaging

Bull or rhesus monkey spermatogenic cells were attached to poly-L-lysine-coated coverslips in 1 ml of KMT medium (100 mM KCl, 2 mM MgCl2, 10 mM Tris-HCl, pH 7.0) and fixed for 1 h with 2% formaldehyde in 0.1 M PBS. The cells were permeabilized for 1 h in 1% Triton X-100 in PBS. Nonspecific antibody cross-reaction was blocked by a 1-h preincubation in 0.1 M PBS containing 2% BSA and 130 mM glycine. The coverslips were incubated with the primary antibody for 2 h and then for 1 h with either TRITC- or FITC-conjugated appropriate secondary antibodies. After the final wash with PBS containing 0.5% Tween 20, the DNA was labeled with 5 mg/ml of 4′,6′-diamino-2-phenylindole ( Molecular Probes) for 10 min. The coverslips were rinsed three times and mounted in a drop of Vectashield mounting medium (Vector Laboratories, Burlingame, CA). Coverslips were examined using either a Zeiss Axiohot or a Nikon Eclipse E1000 epifluorescence microscope and photographed using a chilled CCD camera (Princeton Instruments Inc., Trenton, NJ) operated by Metamorph software.

Cell lines grown onto coverslips were processed as described for spermatogenic cells.

Colloidal Gold Labeling and Electron Microscopy

Testicular cells were isolated, fixed in formaldehyde, permabilized with 0.1% Triton-X-100, and processed with anti-β-COP and anti-Golgin-95/GM130 antibodies as described for immunofluorescence, except that the fluorescently conjugated secondary antibodies were replaced by a 10-nm gold-conjugated goat anti-rabbit IgG (British BioCell, Cardiff, UK). Instead of being attached to microscopy coverslips, the cells were handled in suspension and washed by centrifugation in PBS with 1% BSA and 0.1% Triton X-100 between individual processing steps. The labeled cells were pelleted by centrifugation, fixed for transmission electron microscopy, and embedded in Epon 812 as described previously [25]. Ultrathin sections were cut on a Sorvall MT-5000 ultramicrotome (Ivan Sorvall, Norwalk, CT), stained by uranyl acetate and lead citrate, and examined in a Phillips EM 300 electron microscope.
FIG. 1. Distribution of Golgi proteins in a rhesus monkey cell line. Rhesus monkey 6308 cells were grown onto glass coverslips, fixed when they reached about 50% of confluence, and stained with antibodies against giantin (A), β-COP (B), Golgin-97 (C), Golgin-95 (D), and Golgin 160 (E). All the antibodies displayed a tubulelike appearance in the perinuclear region. This distribution pattern disappeared after brefeldin A treatment, as shown for giantin (F) and Golgin-97 (G).

RESULTS

Imaging the Golgi Apparatus in a Rhesus Monkey Cell Line

Giantin is a membrane protein that appeared to colocalize with β-COP-containing vesicles [27]. This protein displayed a tubulelike pattern around the nucleus in a nonhuman primate cell line derived from the mammary gland (Fig. 1A). Likewise, β-COP showed a perinuclear distribution and partially colocalized with giantin, although giantin had a wider distribution pattern (Fig. 1B). Golgin-97, Golgin-160, and Golgin-95/GM130 stained a compact perinuclear tubulelike structure very similar to the label obtained with giantin (Fig. 1, C, D, and E, respectively). However, the giantin-containing tubules appeared to protrude towards the plasma membrane and did not wrap around each other, as in the case of the Golgin proteins. Evidence supporting the localization of these antigens in the Golgi apparatus came from cells treated with the fungal metabolite brefeldin A. Our results confirm previous findings showing a rapid redistribution of Golgin-97 to the endoplasmic reticulum (ER) throughout the cell cytoplasm (Fig. 1G). Giantin undergoes a similar redistribution but with an increase in signal over the nucleus that may represent a preferential localization of the protein in the ER cisternae forming the nuclear envelope (Fig. 1F).

Intracellular Traffic in Mammalian Spermatids

In pachytene spermatocytes, giantin was present in a rounded structure formed by at least two crescent-shaped structures (Fig. 2A). These structures faced each other and surrounded additional central material with a dotted pattern. β-COP also showed a rounded shape formed by two crescent-shaped structures, which were probably the same as labeled by giantin. However, β-COP did not seem to label the central material as strongly as did giantin.

In round spermatids, both giantin and β-COP antibodies labeled a typical horseshoe-shaped Golgi apparatus, with the convex side facing the acrosomal vesicle (Fig. 2, B and B'). Giantin and β-COP appeared to colocalize in the spermatid Golgi apparatus, although whether they were present in the identical cisternae remains unresolved. As the acrosomal vesicle spread over the nucleus, the structure, position, and orientation of the Golgi apparatus was the same as in the earlier stage (Fig. 2, C and C'). The Golgi apparatus was located very close to the acrosomal vesicle, and at this resolution, it seemed that giantin and β-COP were present on the acrosomal vesicle. In early elongating spermatids, the Golgi apparatus migrated to the cell pole opposite the acrosome and was included in the cytoplasmic lobule (Fig. 2, D and D').

To confirm the specificity of the labeling, rhesus monkey
FIG. 2. Distribution of Giantin and β-COP in rhesus monkey spermatocytes and spermatids. Rhesus monkey spermatogenic cells were attached to poly-L-lysine-coated coverslips and fixed in 2% formaldehyde for 1 h. Following permeabilization with 1% Triton X-100, the Golgi apparatus was visualized by immunofluorescence using antibodies against giantin (green; A–D) or the vesicular protein β-COP (red; A’–D’). The Golgi apparatus in secondary spermatocytes is composed of two half-circles facing each other through their concave sides (A and A’). This arrangement produces a rounded shape with some material inside, clearly seen in those cells stained with anti-giantin antibody but not with anti-β-COP (A’). In spermatids, both giantin and β-COP display a horseshoe-like appearance, with the concave side facing the acrosome (B and B’). At this stage, it seems that both proteins are also located on the acrosome (B and B’). This distribution is maintained during stages 6–7, when the acrosomal vesicle has already started to flatten and spread over the nucleus (C and C’). At later stages of differentiation, the Golgi apparatus migrates towards the opposite pole of the cell, and giantin and β-COP are no longer detected on the acrosome (D and D’). Bar = 5 μm.

spermatids were treated with 7.5 μM brefeldin A. The effect of the drug was tested at two differentiation stages: Golgi phase (Fig. 3, A and C) and cap phase (Fig. 3, B and D) spermatids. Both cell types showed a rapid redistribution of giantin throughout the cytoplasm after brefeldin A treatment. Restoration of Golgi and vesicular staining was observed 90 min after removal of brefeldin A (data not shown). The effects of brefeldin A on spermatids were therefore in accordance with what has been described for somatic cells.

In the bovine round spermatids, the Golgi had a crescent-like shape as visualized with anti-Golgin-97 and anti-Golgin-95/GM130 antibodies (Fig. 4, A and B). In elongating spermatids, besides staining the Golgi itself, both antibodies gave a strong signal extending along the acrosomal vesicle (Fig. 4, C and D). This acrosomal localization is similar to the distribution pattern of the proteins forming the perinuclear theca, a cytoskeletal structure implicated in acrosomal biogenesis (Fig. 4E) [28]. Testicular spermatozoa did not show any labeling with either Golgi-97 or Golgin-95/GM130 (Fig. 4, F and G).

Immunogold electron microscopy of bull spermatids showed that Golgin-95/GM130 localized in a vesicle-rich
FIG. 3. Effect of brefeldin A in rhesus monkey spermatids. Rhesus monkey spermatids were attached to poly-L-lysine-coated glass coverslips and incubated with brefeldin A (5 mg/ml) in D-MEM for 30 min at 37°C. The cells were then fixed, permeabilized, and stained for giantin. Two different stages of differentiation are depicted. At stages 3–4 (A and C) and stages 6–7 (B and D), giantin is distributed throughout the cell (C and D), with no typical horseshoe-like pattern near the acrosomal vesicle (A and B; compare with Fig. 3, B and C). Because brefeldin A disrupts ER-to-Golgi traffic, these results are interpreted as giantin being incorporated in the spermatid ER. Bar = 5 μm.

FIG. 4. Imaging the Golgi apparatus and acrosome in bull spermatids. Bull spermatids were mounted onto poly-L-lysine-coated coverslips, fixed, and stained with antibodies against acrosin (red) and Golgin-95 (A and C) or Golgin-97 (B and D). At the round spermatid stage (A and B), both Golgin-95/GM130 and Golgin-97 display a similar distribution in the Golgi apparatus. In elongating spermatids (C and D), both antibodies label both the acrosome vesicle and the Golgi apparatus, localized on the other side of the cell. This label is on the acrosome because it shows the same pattern as an antibody against the perinuclear theca (green, E). In both round and elongating spermatids, the Golgi shows a horseshoe-shaped structure that does not change in elongating spermatids (A and C). Neither Golgin-95 nor Golgin-97 label a fully differentiated testicular sperm (F and G). Bar = 5 μm.
FIG. 5. Localization of Golgin-95 and β-COP in bull spermatids by immunogold electron microscopy. Golgin-95 (A–C) and β-COP (D–H) were immunolocalized by electron microscopy using 5-nm colloidal gold particles. Golgin-95 shows a strong label over the Golgi apparatus (A) and a weaker label on the acrosomal membrane (B and C). β-COP shows a weak label on the acrosomal membrane (D and E) and a stronger label in cytoplasmic vesicles near the acrosomal vesicle (G). β-COP is also localized at the rim of Golgi stacks (E, arrowhead). The Golgi apparatus in round spermatids appears associated with the axoneme (A and E, arrow). Neither β-COP (F) nor Golgin-95 (B) labeled the acrosomal granule (asterisk) or any other structure in testicular sperm (H). The label was specific because no gold particles were found in other spermatid organelles, such as the nucleus or mitochondria.

Distribution of Post-Golgi and Sorting Compartments in Rhesus Monkey Spermatids

In Golgi stage round spermatids, TGN38 was concentrated at the apical pole cell over the acrosomal vesicle (Fig.
spermatids (Fig. 6B). In elongating spermatids, the label of clathrin followed the plasma membrane. In round spermatids, clathrin was present in a dotted pattern over the acrosome and was present around the sperm tail. Bar = 5 μm.

6A). At this stage, TGN38 was in close association with the Golgi apparatus, as shown by a partial colocalization with β-COP. However, TGN38 had a wider distribution pattern than did β-COP vesicles (Fig. 6A). In elongating spermatids, the compartments labeled by TGN38 and β-COP appeared completely distinct, without any visible overlap (Fig. 6C). At this stage, the acrosomal vesicle did not contain β-COP or TGN38. We never detected surface localization of TGN38 in round or elongating spermatids.

Clathrin is another protein involved in post-Golgi trafficking and recycling of proteins between the TGN and plasma membrane. In round spermatids, clathrin was present in a region overlying the acrosome with great intensity and was found as scattered dots around the nucleus and near the cell surface (Fig. 6B). However, clathrin was not in the region occupied by the Golgi apparatus at this stage. In elongating spermatids, the label of clathrin followed the shape of the acrosomal vesicle, avoiding the region of the acrosomal granule (Fig. 6D). In addition, the protein was also found in a dotted pattern over the acrosomal vesicle and in large aggregates of vesicles around the spermatid tail (Fig. 6D) but could not be detected in testicular sperm (data not shown).

Live Imaging of the Golgi Apparatus During Spermatogenesis

The Golgi apparatus is an organelle that contains a particularly high concentration of phosphoinositidios and sphingolipids. Fluorescent lipid probes such as BODIPY FL C₅-ceramide or NBD C₆-ceramide can easily enter into the cell and then be metabolized in the Golgi apparatus and incorporated into its membrane system. These probes gave a pattern very similar to the one described for giantin and Golgin-97 in live 6308 cells (Fig. 7, A and B). To visualize the Golgi apparatus in live rhesus monkey spermatids, we used the lipid probe BODIPY FL C₅-ceramide. This probe gave a clear and bright signal in round spermatids, labeling a horseshoe-shaped Golgi apparatus with the convex side facing the acrosomal vesicle (Fig. 7C). In cap phase spermatids, BODIPY FL C₅-ceramide-labeled Golgi faced the acrosomal vesicle, which at this stage attached to the nucleus and started to expand (Fig. 7D). In early elongating spermatids (cap phase), the Golgi apparatus was at the opposite pole of the acrosome (Fig. 7D). In contrast to β-COP and giantin, the fluorescent lipid probes never labeled the acrosomal vesicle. The inserts show the phase contrast image of each cell.

DISCUSSION

Spermatogenesis is a complex process that involves nuclear remodeling, cytoplasmic movements and the formation of new and unique organelles [1, 10]. The haploid round spermatid becomes polarized in the first stages of differentiation, as evidenced by the asymmetric distribution of its organelles [10, 29, 30]. Plasma membrane domains that will eventually perform specialized functions during fertilization are also generated at this stage [31]. However, little is known of the protein sorting and mechanisms involved in the formation of the acrosome and the different sperm head domains.

In this work, we have shown that four Golgi proteins, giantin, β-COP, Golgin-97, and Golgin-95/GM130, are present in primary pachytenes and round spermatids. In round spermatids, these proteins are localized in a horseshoe-shaped Golgi apparatus and in membranes surrounding the acrosomal vesicle. In elongating spermatids, giantin, β-COP, Golgin-97, and Golgin-95/GM130 are no longer found in the acrosome, suggesting that they may have been retrieved into the Golgi. At the final stages of differentiation, these proteins are shed in the discarded cytoplasmic droplet [19]. In somatic cells, Golgin-95/GM130 interacts with GRASP65 to be correctly targeted to the Golgi apparatus [32]. GRASP65 is a membrane-associated protein involved in the reassembly of Golgi stacks in a cell-free system [32, 33]. In mammalian spermatids, both GRASP65 and Golgin-95/GM130 may keep this interaction in order to bind to the acrosomal membrane. The localization of this complex in the acrosome may allow the proper targeting of β-COP vesicles to this organelle.

The shape of the Golgi apparatus varies among different cell types and is cell cycle dependent [16, 34]. Biogenesis and maintenance of this organelle is dependent on protein transport through the cisternae [16]. Using the fungal metabolite brefeldin A, we showed here that there is an active ER-to-Golgi traffic in round and elongating spermatids. The kinetics of giantin redistribution to the ER in the presence of the brefeldin A and the dose required to achieve this effect are comparable to those found in somatic cells [35–37]. Thus, it seems that the formation of β-COP-coated vesicles and anterograde/retrograde trafficking pathways are similar in both systems.

Formation of the mammalian acrosome is a very slow process when compared with the biogenesis of other secretory vesicles, such as insulin granules [9]. Spermiogenesis,
FIG. 7. Live imaging of the Golgi apparatus in rhesus monkey spermatids. NBD C₆-ceramide (A) and BIODIPY FL-C₅-ceramide (B) are fluorescent lipids that specifically stain the Golgi apparatus in rhesus 6308 cells. Live rhesus monkey spermatids are labeled with the fluorescent lipid BIODIPY FL-C₅-ceramide. The plate depicts early spermatids in stages 3–4 (C), 5–7 (D), and 9–10 (E). Fluorescence is observed in a horseshoe-shaped Golgi apparatus (C and D) near the acrosomal vesicle (E, arrow). The Golgi apparatus faces the acrosomal vesicle at a later stage of differentiation and then migrates towards the opposite pole of the cell, as already described using other Golgi markers (C–E). Insert shows the phase contrast image. Bar = 5 μm.

FIG. 8. Role of vesicular trafficking in acrosome biogenesis. In early round spermatids (stages 1–4; A), there is an anterograde Golgi-to-acrosome vesicular traffic. Incoming vesicles bind preferentially to the border of the acrosomal vesicle, thus increasing the surface area attached to the nucleus as the acrosome expands and flattens. At this stage, few clathrin (red) and β-COP (green) vesicles are budding from the acrosomal membrane. Clathrin coats are also found in vesicles budding from the TGN, and β-COP is also detected at the rims of the Golgi cisternae. At later stages (stages 5–8; B), the budding rate of clathrin- and β-COP-coated vesicles from the acrosomal increases. At this time, the anterograde vesicle flow begins to decrease, and the Golgi apparatus starts to migrate towards the opposite pole of the cell.

The process of spermatid differentiation, may take from 14 days in mice up to 23 days in humans [38]. Acrosome formation accounts for about 50% of the total time required, which may be due to either slow vesicular traffic or slow interaction kinetics between the different docking/fusion machinery components. It is not known when or how acrosome components are sorted out from proteins with other destinations. During spermiogenesis, there are a number of lysosomal proteins detected in the acrosomal vesicle that are retrieved subsequently throughout differentiation [39, 40]. Clathrin- and probably also β-COP1-coated vesicles may participate in the retrieval of missorted proteins from the acrosome. The acrosome of mature sperm contains both unique acrosomal enzymes and common enzymes of lysosomal origin [41]. Many of these enzymes may have no functional role in fertilization, and their presence in the acrosome may merely reflect the low efficiency of the spermatid sorting machinery. This process may be similar to the processing and maturation of secretory granules in endocrine cells [42, 43]. Besides having a role in the retrieval of misrouted proteins from the acrosomal vesicle, vesicular trafficking may also have a profound influence in the modeling and shaping of the acrosome. During stages 4–7 of spermatogenesis, the acrosomal vesicle flattens and spreads around the nucleus, finally covering up
to two-thirds of its surface at stages 10–12 in the mouse or the equivalent stages in other mammals [1, 30, 44]. In addition, the mechanism by which the species-specific final shape of the acrosome is reached is not known [1, 30, 44]. We propose that acrosome flattening and spreading over the nucleus may be the result of vesicle fusion at the edges of the acrosome coupled with membrane retrieval at the center. This appears to be the case in early rhesus monkey round spermatids. According to this model (Fig. 8), there is a net Golgi-to-acrosome membrane flux during the first stages of differentiation (Fig. 8A) that is responsible for the initial growth of the organelle. Over time, Golgi-to-acrosome traffic begins to decline, and there is a concomitant increase in the opposite acrosome-to-Golgi pathway. The spermatid constantly adjusts both trafficking routes until the acrosome has flattened and the Golgi apparatus has migrated towards the opposite pole of the cell (Fig. 8B).

ACKNOWLEDGMENT

We thank Michael Webb for the preparation of ultrathin sections.

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