Adiponectin (Acrp30) is an insulin-sensitizing hormone produced and secreted exclusively by adipose tissue. Confocal fluorescence microscopy demonstrated the colocalization of adiponectin with the Golgi membrane markers p115, β-COP, and the trans-Golgi network marker, syntaxin 6. Treatment of cells with brefeldin A redistributed adiponectin to the endoplasmic reticulum where it colocalized with the chaperone protein BIP and inhibited secretion of adiponectin demonstrating a requirement for a functional Golgi apparatus for adiponectin release. Confocal fluorescence microscopy also demonstrated a colocalization of endogenous adiponectin with that of expressed GGA1myc (Golgi-localizing γ-adaptin ear homology ARF-binding protein) but with no significant overlap between adiponectin and the GGA2myc or GGA3myc isoforms. Consistent with confocal fluorescence microscopy, transmission electron microscopy demonstrated the colocalization of GGA1 with adiponectin. Although GGA1 did not directly interact with the adiponectin protein, the adiponectin enriched membrane compartments of adipocyte were precipitated by a GST-GGA1 cargo binding domain (VHS) fusion protein but not with a GST-GGA2 VHS or GST-GGA3 VHS fusion proteins. Moreover, co-expression of adiponectin with a GGA1 dominant-interfering mutant (GGA1-VHS domain) resulted in a marked inhibition of adiponectin secretion in both 3T3L1 adipocytes and HEK293 cells, whereas no inhibition was detected with the truncated mutants GGA2-VHSGAT or GGA3-VHSAGAT. Moreover, co-expression of wild type GGA1 with adiponectin enhanced secretion of adiponectin. Interestingly, lep tin secretion was unaffected by neither the wild type form or GGA1 mutant. Taken together these data demonstrate that the trafficking of adiponectin through its secretory pathway is dependent on GGA-coated vesicles.

Over the past several years many studies have documented that in addition to being a fat storage depot, adipocytes are a bona fide endocrine tissue that secrete several hormones that control insulin sensitivity and energy balance (1–3). In particular, adiponectin, also called adiponectin (3). The secreted protein consists of an amino-terminal collagen domain that localizes into the lumen of the endoplasmic reticulum (4, 31). The signal sequence that is cotranslationally removed as the protein translocates into the lumen of the endoplasmic reticulum (4, 31). The three-dimensional structure determined by x-ray analysis also revealed that adiponectin shares significant homology to tumor necrosis factor-α, another adipocyte-secreted hormone that is implicated in the induction of insulin resistance (32). However, in serum adiponectin circulates as two forms, a lower molecular weight trimer-dimer and a higher oligomeric molecular weight complex. Recent studies have demonstrated that disulfide bond formation through Cys-39 is essential for the assembly of the higher oligomeric complexes (33).

The GGA2 proteins (for Golgi localizing γ adaptin ear homology domain ARF-binding protein) are a family of ubiquitously expressed monomeric clathrin adaptors that mediate sorting at the trans-Golgi network (TGN) of specific cargo in an Arf-dependent manner. There are three different isoforms of GGA proteins termed GGA1, GGA2, and GGA3. These proteins have a modular structure consisting of an N-terminal VHS (Vps27, Hrs, STAM) domain, and Arf-binding GAT (GGA and TOM1) domain, a clathrin binding hinge region, and a COOH-terminal γ-adaptin “ear” (GAE) domain. The VHS domain binds acidic cluster-dileucine sorting signals present in the cytosolic domain of transmembrane vesicle proteins (34, 35) such as the mannose 6-phosphate receptor (36), sortilin (37), and β-secretase (38). The GAT domain binds to activated ADP-ribosylating factors (39–42), whereas adipocytes (4–7) and functions in vivo as an insulin sensitizer (8–10), reducing glucose production by the liver (11) and enhancing fatty acid oxidation in skeletal muscle (10) through the activation of two distinct receptor isoforms (12). These receptors mediate increased AMP-dependent kinase activation (13–15) and peroxisome proliferating activated receptor-α ligand activity (16).
the hinge region recruits clathrin (39). The GAE region has been shown to interact with accessory proteins including rabaptin-5 (43, 44), entho-
protin (45), γ-synergin (46), and with ubiquitin (47).

In the present study, we demonstrate that adiponectin in the steady state is predominantly localized in a peri-nuclear compartment indis-
tinguishable from the Golgi apparatus/TGN. Consistent with the mor-
phological observations, secretion of adiponectin is severely inhibited by treatment of cells with brefeldin A (BFA). Furthermore a dominant-
interfering mutant of the GGA1 protein (GGA1 VHS GAT domain) was able to block both traffic of the GLUT4 glucose transporter to its insu-
lin-sensitive intracellular compartment and secretion of adiponectin but did not affect leptin secretion. In addition, confocal and transmis-
sion electron microscopy studies showed colocalization of adiponectin with the GGA1 isoform. These data suggest that adiponectin (but not leptin) secretion is dependent on GGA proteins and suggest that GGA adaptors participate in the regulation of selective adipokine trafficking in adipocytes.

EXPERIMENTAL PROCEDURES

Materials—Brefeldin A and cycloheximide were obtained from Sigma. Brefeldin A was prepared as a 5 mg/ml stock in methanol and used at a final concentration of 5 μg/ml. Cycloheximide was prepared as a 10 mg/ml stock and used at a final concentration of 10 μg/ml. Adi-
ponectin antibody was obtained as described previously (5). Antibodies for syntaxin 6, p115, and BIP were from BD Biosciences. Antibody for vesicular stomatitis virus-G (VSV-G) protein was obtained from Acu-
rate Biochemicals. Anti-myc and anti-GGA1 antibodies were from Santa Cruz Biotechnology. Biotin-conjugated anti-myc antibody was from Sigma. Antibody anti-GFP was from Roche Applied Science, Dul-
becco’s modified Eagle’s medium (DMEM), Opti-MEM I, fetal bovine serum, calf serum, and trypsin were from Invitrogen. Lipofectamine 2000 was obtained from invitrogen. Radioimmunoassay kit for adi-
ponectin was obtained from Linco Research (St. Charles, MO). ELISA kits for adiponectin and leptin were purchased from R&D Systems. The GGA1, GGA2, and GGA3 wild type constructs and eGFP-VHS-GAT domain were obtained from Dr. J. Bonifacino (National Institutes of Health). A plasmid encoding for the VSV-G protein wild type protein was a gift from Dr. M. McNiven (Mayo Clinic and Graduate School, Rochester, MN).

Generation of DNA Constructs—The GST-GGA1 VHS-GAT, GST-
GGA2 VHS-GAT, and GST-GGA3 VHS-GAT constructs were gener-
ated by PCR amplification of the VHS-GAT domains of the wild type forms of GGA1–3, followed by ligation into the GST-expressing vector pGEX4T (Amersham Biosciences).

Cell Culture and Transient Transfection—3T3L1 cells were obtained from the American Type Tissue Culture repository. Cells were cultured in DMEM supplemented with 25 mM glucose, 10% calf serum at 37 °C
with 5% CO2. The cells were differentiated into adipocytes with 1 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM isobutyl-1-methylkanthame as described previously (48). Differentiated adipocytes were electropo-
erated at 950 microfarads and 0.15 V, using a Gene Pulse II electroporator from Bio-Rad. Following electroporation cells were plated on collagen IV-treated coverslips and allowed to recover in DMEM supplemented with 10% Serum. HEK293 cells were cultured in minimal essential medium containing 10% FBS and penicillin/streptomycin at 37 °C with 5% CO2. Cells were plated in 24-well plates and transfected using Lipofectamine 2000 (Invitrogen) following the manufacturer’s instructions.

Immunofluorescence and Image Analysis—Transfected and intact adipocytes were washed in phosphate buffered saline (PBS) and treated

for 15 min with a solution containing 4% paraformaldehyde, 0.2% Triton X-100, and 0.4% bovine serum albumin (BSA). Cells were then incubated in blocking buffer containing 5% donkey serum (Sigma) and 1% BSA for 1 h at room temperature. Primary and secondary antibodies, as indicated in the figure legends to Figs. 1, 2, 4, and 7, were used at 1:100 dilution in blocking buffer. The cells were washed three times with PBS and mounted on glass slides with Vectashield (Vector Laboratories) and were imaged using a Zeiss LSM 510 META confocal microscope. Images were then imported to Adobe Photoshop (Adobe Systems, Inc.) for processing.

Temperature Block—After electroporation 3T3L1 adipocytes were placed overnight in DMEM containing 10% fetal bovine serum at either 16 or 19 °C. Following an overnight incubation cells were fixed and permeabi-
ized as indicated above and processed for immunofluorescence.

Pull-down Assay and Western Blot Analysis—For the pull-down
assay, whole cell extracts were prepared in HES buffer: 30 mM Hepes, pH 7.4, 1 mM EDTA, 250 mM sucrose) supplemented with 1 mM phen-
ethylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin. Cells were then homogenized in a Dounce homoge-
nizer (five times) and lysates centrifuged to 37,000 rpm for 10 min at 4 °C. Equal volumes of the supernatant were taken (1 mg of protein) and incubated with glutathione beads (GST or GST-GGA1 VHS GAT) for 2 h at 4 °C. Samples were centrifuged briefly (13,000 rpm for 1 min), and the beads were washed three times with PBS. Pelleted samples were eluted in 2× loading sample buffer. Samples were boiled for 5 min and loaded onto a SDS-PAGE. Samples transferred to a nitrocellulose membrane and immunoblotted with an antibody specific for adiponectin.

Radioimmunoassays and ELISA—Fully differentiated cells were trypsinized for 10 min and replated at equal densities into 12 well mul-
tidishes and were allowed to recover overnight in DMEM containing 10% FBS. Following an overnight incubation the medium was changed to DMEM without serum supplementation, and at different time inter-
vals an aliquot of medium was collected for radioimmunoassay follow-

ing the manufacturer’s instructions (Linco Research). Similarly, ELISA assays were performed on aliquots of medium taken 24–30 h following transfection. Prior to radioimmunoassay/ELISA floating cells were removed by centrifugation at 500 × g for 5 min and the supernatant used for the radioimmunoassay/ELISA quantification. In parallel an ali-
quot of the whole cell lysate was also quantified.

Transmission Electron Microscopy—HEK293 cells were collected by trypsinization (5 min) followed by inactivation of trypsin with 10% FBS medium. Cells were then centrifuged and washed twice with PBS. Pel-
leted cells were fixed in 2% paraformaldehyde, 0.2% glutaraldehyde for 24 h at 4 °C. Cells were dehydrated in ascending alcohol series (50–100%) 15 min each and infiltrated in LR White resin overnight. Samples were then cured at 50 °C for 48 h, sectioned, and mounted on nickel grids. Following aldehyde quenching with 50 mM glycine, for 30 min, samples were incubated in a blocking solution containing 5% donkey serum and 1% BSA for 1 h at room temperature. Immunolabeling with specific primary antibodies at 1:100 was done overnight at 4 °C. Samples were washed three times with PBS and secondary antibodies (goat anti-
rabbit IgG 25-nm gold and goat anti-mouse IgG 10-nm gold) were added at 1:50 for 2 h at room temperature. Samples were washed in PBS and water and visualized in a FEI CM100 microscope.

Statistical Analysis—One-way ANOVA was performed using SAS statistical software.

RESULTS

Intracellular Localization of Adiponectin in 3T3L1 Adipocytes—To characterize the intracellular localization of adiponectin, we examined

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the distribution of adiponectin with several intracellular markers. The endogenous adiponectin protein (Fig. 1, a, d, and g) was primarily localized in the perinuclear compartment as well as some scattered vesicle staining throughout the cytoplasm. The perinuclear distribution overlapped with Golgi markers p115 (Fig. 1, a–c), syntaxin 5 (Fig. 1, d–f), and β-COP (data not shown) and the TGN marker syntaxin 6 (Fig. 1, g–i).

We further confirmed the Golgi localization of adiponectin by using reduced temperature to block secretory protein trafficking. VSV-G protein is a membrane protein in the constitutive secretory pathway that accumulates in the endoplasmic reticulum at 16 °C and in the Golgi complex at 19 °C (49). Incubation of cells at 16 °C immediately following transfection resulted in a diffuse intracellular labeling of VSV-G protein, consistent with the endoplasmic reticulum distribution in these cells (Fig. 2A, panel a). At 19 °C, VSV-G protein displayed a predominant perinuclear distribution consistent with Golgi localization (Fig. 2, panel b). To ensure that the temperature block did not damage the cells and thereby resulted in an aberrant accumulation in the Golgi, following incubation at 19 °C the cells were shifted to 37 °C for 1 h (Fig. 2A, panel c). Under these conditions, the VSV-G protein was fully capable of exiting the Golgi and traffic to the plasma membrane. In parallel, at 19 °C VSV-G protein colocalized with endogenous adiponectin (Fig. 2B, panels d–f).

BFA is a fungal metabolite that inhibits guanylnucleotide exchange factors that act on class I Arf GTP-binding proteins (50). This inhibition results in a block of anterograde Golgi trafficking and thereby causes the collapse of the Golgi stacks back into the endoplasmic reticulum (51, 52). We next determined the effect of BFA on adiponectin secretion by radioimmunoassay (Fig. 3A). In control cells, there was a constitutive secretion of adiponectin into the medium that was relatively linear over the time course examined (Fig. 3A). Treatment with BFA completely blocked the release of adiponectin, whereas the protein synthesis inhibitor, cycloheximide, had no effect (Fig. 3A). Identical results were obtained when the incubation medium was assayed by immunoblotting (data not shown). Furthermore, cycloheximide treatment for 2 h did not alter the distribution of intracellular endogenous adiponectin in 3T3L1 adipocytes, which remained associated with the Golgi markers (Fig. 3B).

As a control for cycloheximide action, HEK293 cells were transfected with a vector coding for a green fluorescent protein. Immediately following transfection a subset of cells were treated with cycloheximide, which remained associated with the Golgi markers (Fig. 3B).

FIGURE 1. Localization of endogenous adiponectin in the Golgi/TGN. Fully differentiated adipocytes were fixed, permeabilized, and immunostained with a specific polyclonal antibody specific for adiponectin (panels a, d, and g) or with the cis-Golgi markers p115 (panel b) and syntaxin 5 (panel e), or with the trans-Golgi network marker syntaxin 6 (panel h). The merged images are shown in the right panels. The yellow color in the merged images (panels c, f, and i) indicates colocalization. Images were obtained using a Zeiss 510 META confocal microscope.

FIGURE 2. Adiponectin colocalizes with the vesicular stomatitis virus G protein. A, temperature block of the VSV-G protein. Fully differentiated adipocytes were electroporated with a construct coding for the vesicular stomatitis virus protein. After electroporation cells were incubated overnight at 16 °C (panel a), overnight at 19 °C (panel b), or incubated overnight at 19 °C and then placed at 37 °C for 1 h prior to analysis (panel c). Following the incubation cells were fixed, permeabilized, and immunostained with an antibody specific for VSV-G protein obtained from Acurate Biochemicals, Inc., as described under “Experimental Procedures.” Images were obtained using a Zeiss 510 META confocal microscope. B, colocalization of endogenous adiponectin and VSV-G protein. Fully differentiated adipocytes were electroporated with a construct coding for the vesicular stomatitis virus G protein. After electroporation cells were incubated overnight at 19 °C. The next day, cells were fixed, permeabilized, and immunostained with an antibody specific for VSV-G protein (panel d) or with an antibody specific for adiponectin (panel e) as described under “Experimental Procedures.” Panel f shows GFP VSV-G protein. The yellow color in the merged image (panel h) indicates colocalization. Images were obtained using a Zeiss 510 META confocal microscope. IF, immunofluorescence.

FIGURE 3. Secretion of adiponectin is inhibited by brefeldin A but not by cycloheximide. A, time course of adiponectin secretion. Fully differentiated adipocytes were replated in 12-well dishes with the same cell density and allowed to recover in DMEM with 10% FBS. 24 h later, medium was changed to DMEM without serum, in the absence of drug (No treatment), in the presence of 5 μg/ml brefeldin A, (+Brefeldin A), or in the presence of 10 μg/ml cycloheximide (+Cycloheximide). A small aliquot of medium was taken at the indicated times, spun for 5 min at 3000 rpm, and the supernatant collected to determine the amount of adiponectin by radioimmunoassay (RIA). B, Golgi preparation of 3T3L1 adipocytes. Differentiated adipocytes were left untreated or treated with 10 μg/ml cycloheximide (CHX) for 2 h at 37 °C. Following this time, cells were fractionated and the Golgi membranes purified as described under “Experimental Procedures.” Equal amounts of the total lysate or the purified Golgi fraction were loaded onto a SDS-PAGE and immunoblotted with the indicated specific antibodies. A representative blot of three independent experiments is shown. H, homogenate. C, cycloheximide inhibits the expression of the green fluorescent reporter gene AFR-1 GFP. HEK293 cells were transfected with a construct coding for a GFP-tagged AFR-1 protein. Immediately after transfection cycloheximide (+CHX) was added to group of cells, whereas a group of cells were left untreated (−CHX). Following the treatment with the drug, images of cells were taken on a NIKON TE2000 microscope on a fluorescein isothiocyanate (FITC) or brightfield setting.
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Adiponectin Colocalizes with GGA Isoforms—Since adiponectin was localized in the Golgi/TGN region at steady state in 3T3L1 adipocytes we postulated that adaptor proteins at the TGN could regulate adiponectin secretion in adipocytes. GGA proteins are molecular adapters that mediate sorting at the TGN of specific cargo vesicles and recruit clathrin in an Arf-dependent manner. Recent reports have documented that GGA adaptors facilitate transport of the glucose transporter GLUT4 to the insulin-sensitive compartment in adipocytes (53, 54). To investigate whether GGA coat adaptors are involved in adiponectin secretion, we first examined the colocalization of the GGA isoforms with endogenous adiponectin by confocal microscopy imaging. This was performed in adipocytes that expressed the wild type form of GGA1 myc (A), GGA2myc (B), or GGA3myc (C). Following an overnight incubation to allow for protein expression, cells were fixed, permeabilized, and stained as indicated under “Experimental Procedures” with an antibody specific for adiponectin (panels a and d) and an antibody for myc (panels b and c). When indicated a subset of cells was treated with 100 nM insulin for 30 min prior to fixation. Confocal imaging was performed in a Zeiss 510 META confocal microscope. The yellow color in the merged images (panels c and f) indicates colocalization. Shown are representative cells of three independent experiments.

To further confirm these studies, we next examined the localization of endogenous adiponectin and endogenous GGA1 in isolated mouse adipocytes and the localization of expressed adiponectin and GGA1-VHS-eGFP in HEK293 cells by transmission electron microscopy (Fig. 5). In isolated fat tissue, immunogold labeling of adiponectin (10-nm gold) and GGA1 (25-nm gold) indicated that these proteins were in close proximity (Fig. 5, A and B). Similar results were obtained in HEK293 cells expressing adiponectin (25-nm gold particles) and GGA1-VHS (10-nm gold particles), where both proteins were primarily confined to the perinuclear region (Fig. 5, C and D). Although we were unable to preserve a high degree of membrane morphology, higher magnification clearly demonstrated that the GGA1 positive gold particles were colocalized with the adiponectin-positive gold particles (Fig. 5, B and D).

Unfortunately, the lack of suitable GGA2- and GGA3-specific antibodies prevented us from examining the colocalization of these proteins with endogenous adiponectin in electron microscopy studies. To further characterize the interaction between adiponectin-containing vesicles and the different GGA isoforms, we designed biochemical and functional assays, which are detailed below.

To investigate whether recombinant GGA proteins could bind to adiponectin-containing vesicles in vitro, we next generated constructs expressing the cargo binding domain (VHS) and Arf binding domain (GAT) of either GGA1, GGA2, or GGA3 proteins fused in frame to GST (GST-GGA1VHS-GAT, GST-GGA2VHS-GAT, or GST-GGA3VHS-GAT) and performed in vitro pull down assays. Adiponectin vesicles

FIGURE 4. Colocalization of GGA proteins and endogenous adiponectin in adipocytes. Fully differentiated adipocytes were electroporated with a construct coding for the wild type form of GGA1 myc (A), GGA2myc (B), or GGA3myc (C). Following an overnight incubation to allow for protein expression, cells were fixed, permeabilized, and stained as indicated under “Experimental Procedures” with an antibody specific for adiponectin (panels a and d) and an antibody for myc (panels b and c). When indicated a subset of cells was treated with 100 nM insulin for 30 min prior to fixation. Confocal imaging was performed in a Zeiss 510 META confocal microscope. The yellow color in the merged images (panels c and f) indicates colocalization. Shown are representative cells of three independent experiments.

FIGURE 5. Colocalization of GGA proteins and adiponectin. A and B, isolated mouse adipocytes were fixed and processed for electron microscopy as indicated under “Experimental Procedures.” Immunolabeling was performed with an antibody against adiponectin and donkey anti-goat IgG 25-nm gold for GGA1. Samples were visualized in a FEI CM100 transmission electron microscope. The arrows indicate colocalization of the two proteins. C and D, HEK293 cells were cultured and cotransfected with a vector expressing adiponectin-myc and either the GGA1 VHS-GFP. 24 h following transfection cells were processed for transmission electron microscopy as described under “Experimental Procedures.” Immunolabeling was performed with an antibody against adiponectin and antibody against GFP (Roche Applied Science). Secondary antibodies were: goat anti rabbit IgG 10-nm gold for adiponectin and goat anti-mouse IgG 10-nm gold for GFP. Samples were visualized in a FEI CM100 transmission electron microscope. The arrows indicate colocalization of the two proteins.

GGA adaptors may be involved in the trafficking of adiponectin from Golgi/TGN compartments.
Expression of the full-length GGA1 protein had no significant effect on adiponectin-myc secretion in 3T3L1 adipocytes (Fig. 7B), whereas a small reproducible enhancement of adiponectin secretion was observed in HEK293 cells compared with cells transfected with adiponectin alone or cells transfected with adiponectin and an irrelevant protein, GLUT1 (Fig. 7C). In contrast, expression of the GGA1VHSGAT domain inhibited adiponectin secretion in both cell types. The inhibitory action of GGA1VHSGAT was specific, as the VHSGAT domains of GGA2 and GGA3 were without affect (Fig. 7, B and C). Moreover, the GGA1VHSGAT domain had no significant effect on the secretion of leptin (Fig. 7C, filled bars). Taken together, these data support a model in which adiponectin secretion is specifically dependent upon GGA1 function.

**DISCUSSION**

Adiponectin increases energy expenditure and decreases circulating glucose and fatty acid levels without a reduction in food intake (9, 10, 30). Based upon the central role that adiponectin plays in the control of metabolism, energy homeostasis, and insulin sensitivity, we elected to study the secretory membrane trafficking pathways that are responsible for the release of this adipokine from adipocytes. Confocal microscopy studies in 3T3L1 adipocytes showed that adiponectin was mostly distributed to the perinuclear region, with some punctuate staining throughout the cytoplasm. The adiponectin perinuclear distribution stained positive for markers of the Golgi/TGN, such as p115 and syntaxin 6, whereas no colocalization was detected with endosomal markers or endoplasmic reticulum markers (data not shown). In addition, temperature blocks at 19 °C showed colocalization of endogenous adiponectin with the VSV-G protein, which at this temperature is trapped specifically in the TGN. Thus, our data support the hypothesis that a substantial amount of adiponectin in the steady state is located in the Golgi/TGN. Another study had previously reported that adiponectin-containing transport vesicles partially overlap with the endoplasmic reticulum marker GRP94 (56). Although we do not know the basis for these differences, consistent with adiponectin transport through the Golgi complex, brefeldin A completely blocked adiponectin secretion, indicating that a functional Golgi apparatus is required for adiponectin secretion.

It is well established that traffic through the Golgi requires the recruitment of coat proteins on Golgi membranes, through a process regulated by members of the ADP-ribosylation factors (Arf) family of small GTP-binding proteins (39, 42, 58). These factors recruit adaptor molecules that in turn recruit clathrin to the nascent vesicles. To examine what other elements are required for the trafficking of adiponectin-containing vesicles, we examined the role of the coat adaptor GGA proteins. Recently it has been reported that these proteins mediate the sorting of GLUT4-containing vesicles into the insulin-responsive storage compartment in 3T3L1 adipocytes (53, 54).

To examine whether this interaction between GGA and adiponectin occurs in vivo, we transfected fully differentiated adipocytes with either GGA1myc, GGA2myc, or GGA3myc constructs and analyzed by confocal microscopy whether endogenous adiponectin colocalized with these GGA proteins. Our results showed that expressed GGA proteins exhibited a substantial degree of colocalization with that of endogenous adiponectin in differentiated 3T3L1 cells. Transmission electron microscopy studies also confirmed localization of GGA1 adaptor proteins with adiponectin in mouse adipocytes.

Structural analysis of GGA proteins has showed differences in the VHS domains of GGA1 and GGA2, with GGA2 having a more flexible and unwound e6 helix end (55), and this can contribute to the recogni-
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In addition to the ability of GGA1 to associate with adiponectin-containing compartments, expression of the dominant-interfering GGA1 mutant that contains the vesicle binding domain VHS-GAT inhibited adiponectin secretion. This again was specific for GGA1, as the same domains in GGA2 and GGA3 were without affect. GGA1 function was also specific for adiponectin secretion as another adipokine, leptin, was not affected by expression of the dominant-interfering GGA1 mutant. Interestingly, we also found that expression of full-length wild type GGA1 protein enhanced adiponectin release in HEK293 cells but not in differentiated 3T3L1 adipocytes, which suggests that the amount of GGA1 protein in HEK293 cells may be rate-limiting for adiponectin secretion in these cells.

In summary, our microscopy biochemical and functional data strongly suggest that GGA coat adaptors regulate selective cargo formation at the TGN of adipocytes. While intracellular traffic and secretion of adiponectin is mediated by GGA1-coated vesicles, leptin secretion is independent of GGA adaptors. Further studies will now be needed to determine whether GGA-mediated adiponectin secretion is a direct plasma membrane secretory pathway, independent of the recycling endosome system, and to identify other protein components that participate in the generation of adiponectin-containing vesicles.
