The p115-interactive Proteins GM130 and Giantin Participate in Endoplasmic Reticulum-Golgi Traffic*

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The transport factor p115 is essential for endoplasmic reticulum (ER) to Golgi traffic. P115 interacts with two Golgi proteins, GM130 and giantin, suggesting that they might also participate in ER-Golgi traffic. Here, we show that peptides containing the GM130 or the giantin p115 binding domain and anti-GM130 and anti-giantin antibodies inhibit transport of vesicular stomatitis virus (VSV)-G protein to a mannosidase II-containing Golgi compartment. To determine whether p115, GM130, and giantin act together or sequentially during transport, we compared kinetics of traffic inhibition. Anti-p115, anti-GM130, and anti-giantin antibodies inhibited transport at temporally distinct steps, with the p115-requiring step before the GM130-requiring stage, and both preceding the giantin-requiring stage. Examination of the distribution of the arrested VSV-G protein showed that anti-p115 antibodies inhibited transport at the level of vesicular-tubular clusters, whereas anti-GM130 and anti-giantin antibodies inhibited after the VSV-G protein moved to the Golgi complex. Our results provide the first evidence that GM130 and giantin are required for the delivery of a cargo protein to the mannosidase II-containing Golgi compartment. These data are most consistent with a model where transport from the ER to the cis-medial-Golgi compartments requires the action of p115, GM130, and giantin in a sequential rather than coordinate mechanism.

Multiple proteins participate in the sorting and concentration of cargo at the endoplasmic reticulum (ER) exit sites, translocation of transport intermediates on microtubules, and the docking/fusion of transport intermediates to their target membranes (1). Proteins that regulate the docking and fusion events include the soluble Ras-related GTPases (Rab proteins) (2–6), NSF (the N-ethylmaleimide-sensitive fusion factor), and soluble NSF attachment proteins (SNAPs), and the transmembrane vesicular- and target-soluble NSF attachment protein receptors (SNAREs) (7–10). In mammalian cells, transport from the ER to Golgi requires the action of the Rab1 GTPase (2) and the SNAREs, rBet1 (11), syntaxin5 (12), Sec22b/ERS-24 (13), GOS-28/GS28 (14), and memrin (15). In addition, the peripheral associated membrane protein p115 is required for ER to Golgi transport at the level of vesicular tubular clusters (VTCs) downstream of the Rab1-requiring step and upstream of the Ca2+-dependent step (16). The recruitment of p115 to membranes is mediated by Rab1, and p115 can interact directly with a subset (memrin, rBet1, and syntaxin5) of the ER-Golgi SNAREs (17).

In addition to its VTC association, p115 directly interacts with two Golgi membrane proteins, GM130 and giantin (18, 19). GM130 is an extended rod-like protein (~130 nm) with coiled-coil domains, initially identified as a component of an insoluble Golgi matrix (20). GM130 is anchored by its C-terminal domain to the cytoplasmic face of the membrane through interaction with the myristoylated protein GRASP-65 (the Golgi reassembly stacking protein 65) (21, 22). The p115 binding domain of GM130 lies within its N-terminal 74 amino acids (23, 24). At the ultrastructural level, GM130 localizes predominantly to the cis-Golgi (20). This distribution partially overlaps with p115 labeling the cis-Golgi. However, p115 appears more concentrated on structures associated with the cis-most aspect of the cis-cisterna (18). The other p115 binding partner, giantin, is an integral component of the Golgi membrane that contains a large N-terminal cytoplasmic domain (>350 kDa) and a C-terminal membrane anchor domain (25–27). Giantin is predicted to form a segmented coiled-coil dimer rod of ~250 nm. The p115 binding domain has been mapped to the N-terminal 70 amino acids of giantin (24). Giantin has been localized to tubular-cisternal Golgi elements that might represent fenestrated connections between cisternal stacks (26). Such regions of the Golgi have been proposed to be specialized budding domains (28, 29). Confocal analysis indicates that giantin and p115 do not extensively colocalize in the Golgi region (30).

The functions of GM130 and giantin have been studied predominantly in a cell-free assay that is based on the observation that Golgi complex disassembles during mitosis and subsequently re-assembles during late telophase (31). In this assay, isolated Golgi membranes are treated with mitotic cytosols to generate Golgi fragments that under experimentally controlled conditions (in the presence of NSF, α- and γ-soluble NSF attachment proteins (SNAPs), and p115) re-assemble into cisternal elements and stacks (32). The interaction between p115 and GM130 appears functionally relevant in this assay since the addition of the N-terminal GM130 peptide that binds p115 inhibits cisternal regrowth and cisternal stacking (23, 33). Likewise, the addition of the p115 binding N-terminal giantin peptide inhibits cisternal regrowth and cisternal stacking (34), suggesting a functional interaction between p115 and giantin.
The requirement for both GM130 and giantin function in the assay is further supported by the ability of anti-GM130 or anti-giantin antibodies to block cisternal regrowth and subsequent cisternal stacking (33).

The role of GM130 and giantin in membrane transport has been largely unexplored, but their close relationship with p115 suggests that these proteins might also participate in ER-Golgi traffic. The involvement of GM130 in traffic was suggested by the finding that expression of a GM130 mutant lacking the p115 binding N terminus inhibited the surface delivery of VSV-G protein and resulted in the disappearance of Golgi cisternae with the concurrent accumulation of small vesicles in the Golgi region (35). The role of giantin in trafficking has not been previously analyzed.

We have examined the role of GM130 and giantin in ER-Golgi traffic by utilizing a VSV-G t5045 based semi-intact cell transport assay we previously used to document p115 requirement at the VTC stage of transport (16). Transport was inhibited by peptides corresponding to the p115 binding N-terminal domains of either GM130 or giantin and by anti-GM130 and anti-giantin antibodies. In all cases, the inhibition occurred before VSV-G protein delivery to the mannosidase II-containing medial/ trans-Golgi compartment. Both anti-GM130 and anti-giantin antibodies inhibited transport subsequently to the stage inhibited by anti-p115 antibodies. In agreement, morphological data showed that the anti-GM130 and the anti-giantin antibodies inhibit at the Golgi level at stages downstream from the VTC step inhibited by anti-p115 antibodies. Analysis of the kinetics of inhibition indicates that GM130 and giantin act at temporally different stages, with the GM130-requiring stage preceding the giantin-requiring stage. Our results document the novel requirement for GM130 and giantin in ER-Golgi transport and suggest that GM130 and giantin do not function simultaneously.

EXPERIMENTAL PROCEDURES

Antibodies—Rabbit polyclonal antibodies against p115 (36) and against GM130 (18) were affinity-purified. For affinity purification, lysates of E. coli expressing recombinant S-transferase fusion protein or GM130 were separated by SDS-PAGE and transferred to nitrocellulose. Nitrocellulose strips containing GST-p115 or GM130 were incubated with immune serum in phosphate-buffered saline, 5% fat-free dried milk, 0.1% Tween 20 for 3 h at room temperature. Bound antibodies were eluted with 0.1 M glycine, pH 3.0, neutralized with 1/10 volume 1 M phosphate buffer, pH 7.4, and dialyzed against 25 mm HEPES-KOH, pH 7.2. The antibodies were concentrated and then used in transport assays. Monoclonal anti-giantin G1/133 antibody has been described previously (25). Polyclonal antibodies against mannosidase II were kindly provided by Dr. Marilyn Farquhar (University of California at San Diego). Monoclonal antibodies against mannosidase II were from BabCO (Berkeley, CA). Monoclonal antibodies against VSV-G protein (P5D4) were kindly provided by Dr. Kathryn Howell (University of Colorado Health Sciences Center, Denver, CO). Goat anti-rat and anti-mouse antibodies conjugated with fluorescein isothiocyanate or rhodamine were purchased from Jackson ImmunoResearch (West Grove, PA).

GST Fusion Constructs—GM130 fragment encoding amino acids 1 to 270 was generated with the polymerase chain reaction using the primer pair 5′-CCGGATCCGAAGGTCCGAGAAACAGG with 5′-GGTCTCA- GACCATGATTTCACCTGTCC and a template encoding rat GM130 cDNA in a pBluescript II vector (18). GST-GM130/1–270 was engineered by inserting the GM130 fragment into the BamHI-SalI restriction sites of the pGEX-5X-1 vector (Amersham Pharmacia Biotech). GST-giantin fragment (GST-GTN/1–261) containing coils I and II has been described previously (24). GST fusion protein expression and purification followed according to the manufacturer’s (Amersham Pharmacia Biotech) protocol.

Semi-intact Cell ER-Golgi Transport Assay—The ER to Golgi transport assay was performed as described previously (16, 37, 38). Briefly, NRK cells were grown on 10-cm Petri dishes (80–90% confluent) and infected with the temperature-sensitive strain of the vesicular stomatitis virus, t5045 VSV at 32 °C for 3–4 h (39). The cells were pulse-labeled with Tran35S-label (200 mCi/ml; ICN, Irvine, CA) at the restrictive temperature (42 °C) for 10 min, chased with complete medium for 5 min, and perfused by hypotonic swelling and scraping. Transport reactions were performed in a final total volume of 40 μl in a buffer containing 25 mm HEPES-KOH, pH 7.2, 75 mm potassium acetate, 2.5 mm Mg acetate, 5 mm NaCl, 1.8 mm CaCl2, 1 mm N-acetylglucosamine, ATP regeneration system (1 mm ATP, 5 mm creatine phosphate, and 0.2 μM of rabbit muscle creatine phosphokinase), 5 μl of rat liver cytosol, and 5 μl of semi-intact cells in 50 mm HEPES-KOH, pH 7.2, 90 mm potassium acetate. Transport was initiated by transferring cells to 32 °C. After 90 min of incubation, cells were pelleted, resuspended in appropriated buffer, and digested with endoglucosidase H (endo-H) as described previously (37). The samples were analyzed on 8% SDS-PAGE and by fluorography. Transport was quantitated using a GS-700 imaging densitometer (Bio-Rad). In some experiments, increasing concentrations of GST fusion peptides were added to a complete transport reaction mixture containing cytosol and incubated on ice for 30 min to allow the peptides to interact with cytosolic p115. The mixtures were then added to the semi-intact cells, and transport was initiated at 32 °C. In some experiments, increasing concentrations of antibodies were added to a complete transport reaction containing semi-intact cells, transport mixture, and cytosol and incubated on ice for 30 min to allow the antibodies to interact with cellular GM130 or giantin. Transport was then initiated at 32 °C. Kinetic staging experiments were performed as described previously (40, 41).

RESULTS

VSV-G Protein Transport Is Inhibited by the N-terminal Peptide of GM130 and the N-terminal Peptide of Giantin—To test whether the p115-interacting proteins GM130 and giantin participate in membrane traffic, we added recombinant GM130 or giantin peptide constructs to the semi-intact cell transport assay used previously to document p115 requirement during VSV-G protein transport (16). The peptide constructs were generated as GST fusion proteins and contained either the N-terminal 270 amino acids of GM130 (GST-GM130/1–270) or the first 261 amino acids of giantin (GST-GTN/1–261) fused to the C terminus of GST (Fig. 1A). We previously showed that each construct contains a domain sufficient and necessary for p115 binding (24). The fusion constructs and a control GST protein were expressed in bacteria and purified nearly to ho-
transport to the Golgi.

A. Active GST-GM130 and GST-giantin N-terminal fusion peptides. (GM130/1–987) and giantin (GTN/1–3259) compared with the mean of three separate experiments.

B. GST-N130/1–270 or GST-giantin/1–260 peptides. Results are presented as the percentage of VSV-G protein processed from the endo-H-sensitive to the endo-H-resistant form. Transport reaction with complete transport mixture and GST fusion peptides were expressed in bacteria and purified. Coomassie Blue-stained SDS-PAGE gel shows the size of the fusion peptides and their purity. C. ER to Golgi transport assay was performed in semi-intact NRK cells. Transport is measured as the percentage of VSV-G protein processed from the endo-H-sensitive to the endo-H-resistant form. Transport reaction with complete transport mixture (lane 2) is set as 100%, and transport with mixture without ATP (lane 1) is set as 0%. Reactions were preincubated in presence of GST (lane 3), GST-GM130/1–270 (lanes 4–6), or GST-giantin/1–260 (lanes 7–9). Transport of VSV-G protein was inhibited in the presence of GST-N130/1–270 or GST-giantin/1–260 peptides. Results are presented as the mean of three separate experiments ± S.E.

Fig. 1. The N-terminal peptides of GM130 and giantin inhibit VSV-G transport to the Golgi. A, structure of full-length GM130 (GM130/1–987) and giantin (GTN/1–3259) compared with the respective GST-GM130 and GST-giantin N-terminal fusion peptides. B, GST and GST fusion peptides were expressed in bacteria and purified. Coomassie Blue-stained SDS-PAGE gel shows the size of the fusion peptides and their purity. C, ER to Golgi transport assay was performed in semi-intact NRK cells. Transport is measured as the percentage of VSV-G protein processed from the endo-H-sensitive to the endo-H-resistant form. Transport reaction with complete transport mixture (lane 2) is set as 100%, and transport with mixture without ATP (lane 1) is set as 0%. Reactions were preincubated in presence of GST (lane 3), GST-GM130/1–270 (lanes 4–6), or GST-giantin/1–260 (lanes 7–9). Transport of VSV-G protein was inhibited in the presence of GST-N130/1–270 or GST-giantin/1–260 peptides. Results are presented as the mean of three separate experiments ± S.E.

mogeneity as judged by Coomassie Blue staining (Fig. 1B). The SDS-PAGE mobilities of the fusion constructs are appropriate for the specific composition of each peptide (GM130/1–270 is predicted to be 29,565 daltons, whereas GTN/1–3259 is predicted to be 30,144 daltons).

Increasing concentrations of GST-GM130/1–270 or GST-GTN/1–261 were added to cytosol and incubated on ice for 20 min to allow the peptides to bind to cytosolic p115. The mixtures were then used in the semi-intact cell transport assay. Transport was measured by following the processing of VSV-G protein from a core-glycosylated (endo-H-sensitive) ER form to a more mature (endo-H-resistant) form upon its arrival in a Golgi compartment containing mannosidase II. In agreement with previously published results (6, 14), when transport was measured with untreated cytosol, ~60% of VSV-G protein was processed to endo-H-resistant form, and this was set as 100% relative transport (Fig. 1C, lane 2). When transport was analyzed in the absence of ATP (with an ATP-depleting system), more than 90% of VSV-G protein remained endo-H-sensitive, and this was set as 0% processing (lane 1). The addition of 10 μM GST had negligible effect on transport (lane 3). In contrast, addition of increasing amounts of the GST-GM130/1–270 construct led to a dose-dependent inhibition of VSV-G protein transport (lanes 4–6). Adding the GST-GTN/1–261 construct to the transport assay was also inhibitory. At the lowest concentration tested (2 μM, lane 7), the fusion peptide had no effect on transport, but increasing the concentration to 6 or 8 μM led to a significant (~80%) inhibition of VSV-G protein-processing (lanes 8 and 9, respectively).

These data suggest that interactions between p115 and GM130 and between p115 and giantin are required for transport and that both GM130 and giantin function in ER-Golgi traffic. However, we have recently shown that the N-terminal domain of GM130 and the N-terminal domain of giantin interact with the same C-terminal acidic domain of p115 and compete for binding (24). Therefore, the addition of either the GST-GM130/1–270 or the GST-GTN/1–261 fusion peptide will prevent the interaction of p115 with both GM130 and giantin. Consequently, inhibition of transport by either peptide may not distinguish between GM130 alone, giantin alone, or both proteins as required for transport.

VSV-G Protein Transport Is Inhibited by Anti-GM130 Antibodies and Anti-giantin Antibodies—To further characterize the role of GM130 and giantin in transport, anti-GM130, and anti-giantin antibodies were added to the semi-intact cell assay. Increasing amounts of affinity-purified polyclonal anti-GM130 antibodies were added to transport mixtures containing cytosol. The mixtures were added to permeabilized cells, and the cells were incubated on ice for 30 min to allow the antibodies to bind cellular GM130. Cells were then shifted to 32 °C for 90 min. As shown in Fig. 2A, lanes 3–6, the addition of anti-GM130 antibodies inhibited transport of VSV-G protein in a dose-dependent manner. Transport was blocked 70% when 0.48 μg was present in the assay (lane 6). Preincubation of the antibodies with recombinant full-length GM130 immobilized on nitrocellulose strips efficiently neutralized the inhibitory effect (compare lanes 2 and 7). In contrast, the antibodies remained inhibitory when pre-incubated with recombinant full-length p115 immobilized on nitrocellulose strips (data not shown).

The addition of monoclonal antibodies directed against giantin also inhibited VSV-G protein transport. As shown in Fig. 2B, lanes 3–6, the addition of 0.1- 0.8 μg of anti-giantin antibodies inhibited relative transport of VSV-G protein to ~90%. Heat-inactivating the antibodies neutralized their inhibitory effect (compare lanes 2 and 7). Analogous results were obtained with affinity-purified polyclonal anti-giantin antibodies (data not shown). These data indicate that anti-GM130 and anti-giantin antibodies inhibit VSV-G protein transport and suggest that both GM130 and giantin participate in VSV-G protein movement from the ER to a mannosidase II-containing Golgi compartment.

Anti-GM130 Antibodies and Anti-giantin Antibodies Do Not Inhibit p115 Membrane Binding—We have shown previously that p115 binds to Golgi membranes by interacting with the N termini of GM130 and giantin (18, 24) and that p115 is required for ER to Golgi traffic of VSV-G protein (16). Therefore, the anti-GM130 and anti-giantin antibodies could inhibit VSV-G protein transport by preventing p115 membrane binding. To address this issue, transport mixture (without cytosol) was supplemented with increasing amounts of anti-GM130 or...
anti-giantin antibodies, then added to perforated NRK cells that have been washed with 0.5 M KCl to remove membrane-associated p115. The cells were preincubated on ice for 30 min to allow the antibodies to bind cellular GM130 or giantin. Cytosol was added as the source of p115, and the cells were incubated at 32 °C for 60 min to allow p115 binding. The amount of p115 recovered with the membranes was determined by Western blotting and was quantitated relative to the amount of p115 recovered with the membranes bound p115. Samples processed in an analogous manner but without cytosol do not contain detectable p115 (lane 2). As shown in lanes 3–6, the addition of the GST-GM130/1–270 peptide inhibited p115 binding to membranes. The relative binding was reduced to ~85% in the presence of 0.10 μM peptide, ~75% in presence of 0.17 μM peptide, ~50% in presence of 0.25 μM peptide, and 30% in presence of 0.33 μM peptide. Inhibition was specific since GST (0.50 μM) alone did not inhibit p115 binding to membranes (lane 7). These results indicate that the anti-GM130 and anti-giantin antibodies do not prevent p115 membrane interactions and directly inhibit the transport functions of GM130 and giantin.

Anti-p115, Anti-GM130, and Anti-Giantin Antibodies Inhibit VSV-G Protein Transport at Temporally Distinct Steps—The requirement for various transport factors during transport through the secretory pathway can be sequentially ordered by adding specific inhibitory reagents at different times during traffic and measuring the subsequent transport of VSV-G protein in the presence of the inhibitor. For factors that function early, the addition of inhibitors will block at early time points but not after the VSV-G protein has moved past the stage requiring the factor. For factors that act late, the addition of inhibitors will block at early and late time points, since VSV-G protein has not yet moved past the stage requiring the factor.

To determine whether p115, GM130, and giantin act together or in a sequential fashion, we compared kinetics of traffic in the presence of inhibitory antibodies. Perforated NRK cells were supplemented with complete transport mixtures and incubated at 32 °C for different times (Δt) to allow transport. At each time point, one sample of cells was transferred to ice (control), another sample received anti-p115 antibodies (anti-p115), another sample received anti-GM130 antibodies (anti-GM130), and another sample received anti-giantin (anti-giantin) antibodies. The control samples were incubated on ice for a total of 90 min, whereas the other samples were incubated at 32 °C for a total of 90 min. As shown in Fig. 4, VSV-G transport from the ER to the medial/trans-Golgi compartments containing mannosidase II required a 20–30-min lag period at 32 °C, as previously published for control cells (40). Only after this time, processing of the VSV-G protein oligosaccharide chains can be detected. After 55 min of incubation at 32 °C, ~50% relative transport is observed, and maximal transport is obtained by 70 min. The amount of transport after a 90-min incubation at 32 °C (60% of VSV-G protein is processed at that point) is the maximal amount of processing that could be observed in all control samples and is set as 100% relative transport.

VSV-G protein transport was completely inhibited when anti-p115 antibodies were added at the beginning of incubation at 32 °C or after 5 or 10 min of incubation at 32 °C (Fig. 4). When the antibodies were added 15 min after the start of the 32 °C incubation, ~30% relative transport was observed. Almost 50% relative transport was seen when anti-p115 antibodies were added 30 min after the start of the 32 °C incubation, and >70% relative transport was detected when antibodies were added 45 min after the start of the 32 °C incubation. These results indi-
FIG. 3. p115 binding to membranes is not altered by anti-GM130 or anti-giantin antibodies. Semi-intact NRK cells were washed with 0.5 M KCl to remove membrane-associated p115. Cells were incubated with anti-GM130 or anti-giantin antibodies for 30 min at 4 °C. Cytosol was then added, and the cells were incubated for 60 min at 32 °C. Cells were recovered by centrifugation and rinsed, and the amount of p115 and calnexin was quantitated by densitometry after separation by SDS-PAGE and Western blot. Ratio of p115 to calnexin in control samples (lanes 1 in panel A and B) or without peptide is set as 100%. p115 was not detected in samples incubated in absence of cytosol (lanes 2, panels A, B, and C). A. p115 binding to membranes is not modified in the presence of increasing concentrations of anti-GM130 antibodies (lanes 3–6). Control antibody against mannosidase II (lane 7) does not influence p115 binding to membranes. B, increasing concentrations of anti-giantin antibodies (lanes 3–6) do not affect the amount of p115 bound to membranes. p115 was not detected in samples incubated without cells (lane 7). C, p115 binding to membranes decreased in the presence of increasing concentrations of the GST-GM130/1–270 peptide (lanes 3–6). GST does not inhibit p115 binding (lane 7). Results are presented as the mean of two separate experiments ± S.E. Representative immunoblots are shown.

FIG. 4. Anti-p115, anti-GM130, and anti-giantin antibodies inhibit VSV-G protein transport at temporally distinct steps. Semi-intact NRK cells prepared as described under “Experimental Procedures” were incubated at 32 °C to initiate transport. At the indicated times (Δt), cells were transferred to ice (control), or supplemented with 0.4 μg of anti-p115 antibodies, 0.48 μg of anti-GM130 antibodies, or 0.8 μg of anti-giantin antibodies and incubated for a total of 90 min at 32 °C. Transport was measured as the relative percentage of VSV-G protein passed it by 45 min. A slightly shifted response curve was obtained with anti-GM130 antibodies (Fig. 4). Like anti-p115 antibodies, anti-GM130 antibodies completely inhibited transport when added at the beginning of incubation at 32 °C or after 5 or 10 min of incubation at 32 °C. However, when anti-GM130 antibodies were added 15 min after the start of the 32 °C incubation, only ~17% relative transport was observed, whereas ~30% relative transport was seen when anti-p115 antibodies were added at that time point. This result suggests that p115 is required before GM130, since the VSV-G protein passes the p115-inhibited stage faster than it passes the GM130-inhibited stage. The inhibition by anti-GM130 antibodies at later time points paralleled the inhibition by anti-p115 antibodies. Together, the data suggest that p115 acts independently of GM130 at an early stage (at ~15 min of transport) but that the two proteins might interact at a later event in transport (at ~30 min of transport).

A significantly different response curve was obtained with anti-giantin antibodies (Fig. 4). Unlike the anti-p115 and anti-GM130 antibodies, which showed only partial inhibition when added after 15 min of 32 °C incubation, anti-giantin antibodies remained completely inhibitory when added at that time point. Even when added 30 or 45 min after the start of the 32 °C incubation, at a time when the addition of anti-p115 or anti-GM130 antibodies had limited inhibitory effect, anti-giantin antibodies remained strongly inhibitory. For example, ~37% relative transport was obtained when anti-giantin antibodies were added after 45 min of incubation as compared with >75% relative transport when anti-p115 or anti-GM130 antibodies were added at the same time point. These data suggest that p115, GM130, and giantin function at sequential stages of transport, with the p115-requiring and the GM130-requiring stages preceding the giantin-requiring stage.

Anti-p115, Anti-GM130, and Anti-giantin Antibodies Inhibit VSV-G Protein Transport at Spatially Distinct Steps—The ad-
GM130 and Giantin Function in ER-Golgi Traffic

Fig. 5. Localization of anti-p115, anti-GM130, and anti-giantin antibodies in semi-intact cells. Semi-intact NRK cells were supplemented with anti-p115 (panel A), anti-GM130 (panel B), or anti-giantin (panel C) antibodies. After transport at 32°C for 90 min, cells were processed for double-label immunofluorescence to visualize the added antibodies and stained for mannosidase II. p115 antibody was detected in the Golgi region and in peripheral punctate structures (arrowheads), whereas anti-GM130 and anti-giantin antibodies were detected exclusively in the Golgi region. The semi-intact cells show normal Golgi pattern of mannosidase II staining.

Discussion

In this study, a semi-intact cell assay that measures biochemically or morphologically the movement of the VSV-G protein was used to assess GM130 and giantin function in ER-Golgi transport. Peptides derived from the p115 binding N-terminal domain of GM130 or the p115 binding N-terminal domain of giantin were potent transport inhibitors. In both cases, transport was arrested before the VSV-G protein acquiring endo-H resistance, indicating that the reporter protein was not processed by mannosidase II, a medial/trans Golgi enzyme. These results suggest that each peptide inhibited the corresponding p115 interaction and that p115-GM130 and p115-giantin interactions are required for VSV-G transport to the Golgi. We have recently mapped the GM130 binding domain and the giantin binding domain to the same C-terminal acidic domain of p115 and showed that GM130 and giantin compete for p115 binding. Our findings contradict those of Dirac-Svejstrup et al. (44) who show p115 binding simultaneously to both GM130 and giantin. The discrepancy is currently unexplained, but it should be noted that a trimeric GM130-p115-giantin complex has not been identified in vivo.

Direct involvement of GM130 and giantin in VSV-G protein transport was shown by using anti-GM130 and anti-giantin antibodies. Both antibodies were monospecific and recognized only their corresponding antigens (18, 25). Both reagents inhibited transport before VSV-G protein acquired endo-H resistance. Results from the Warren laboratory showing that anti-GM130 and anti-giantin prevent p115 binding to the corresponding antigen (19) led us to test whether our antibodies could also act in this manner. The inhibitory effects of our antibodies were not due to p115 displacement, since p115 membrane binding was unchanged in the presence of the antibodies. In our studies, inhibition of VSV-G protein transport by the antibodies appears direct and is most likely due to functional inactivation of GM130 or giantin. The anti-GM130 and the anti-giantin antibodies appear to inhibit events downstream of the p115-GM130 and the p115-giantin interactions. It is possible that the antibodies sterically hinder interactions of GM130 or giantin with other proteins required for traffic progression. Alternatively, the antibodies might prevent p115 dissociation from GM130 and giantin by preventing conformational changes and/or signaling events required for p115 dissociation. This would lead to the formation of stable complexes and could prevent subsequent events requiring unoccupied GM130 and/or giantin.

How are p115, GM130, and giantin involved in ER-Golgi traffic? A model that fits all the available data have the three proteins functioning at temporally and spatially different stages of transport. We propose that p115 first acts at the level of peripheral VTCs. This is supported by p115 localization to VTCs (18) and the arrest of cargo in peripheral VTCs in the absence of p115 (16). The VTC localization of p115 is the result of p115 recruitment by an activated Rab1 to COPII vesicles (17).
that subsequently form VTCs (45). The exact function of p115 at this stage is currently unknown. The yeast homologue of p115, Uso1p, tethers COP II vesicles to Golgi membranes (46–48), suggesting that p115 might also tether COP II vesicles. In COP II vesicles p115 interacts with specific ER-Golgi SNAREs, membrin, syntaxin5, and rbet1 (17), but the functional relevance of these interactions remains to be defined. Significantly, the requirement for p115 activity at the VTC stage of transport is independent of GM130 or giantin since VTCs lack GM130 and giantin (16, 18).

The second site of p115 action in our model is after VTC-derived transport intermediates move to the Golgi region. VTC-derived transport intermediates contain p115 (16), and we propose that p115 provides targeting specificity by interacting with GM130 localized on the cis side of the cis-Golgi. This tethering represents the first stage in a series of events that results in membrane fusion. Our anti-GM130 antibodies do not prevent the p115-GM130 interaction but inhibit transport, suggesting that GM130 participates in downstream events. The model also fits data showing that expression of a mutant GM130 lacking the p115 binding N-terminal domain results in the accumulation of tubulo-vesicular structures (possibly transport intermediates) in the Golgi region (35). A block in fusion of transport intermediates would result in such a phenotype.

The role of giantin in transport is more puzzling. Based on the ultrastructural localization of giantin to the Golgi rims (likely to be involved in COP I vesicle budding (28, 29)) and its presence in isolated COP I vesicles (19), a model has been proposed in which p115 would function to cross-bridge giantin in COP I vesicles to GM130 on Golgi membranes in a single tethering event (19). However, this model is not supported by our kinetic results. Although both anti-GM130 and anti-giantin antibodies block VSV-G protein transport before mannosidase II processing, staging experiments showed that the temporal site of inhibition by anti-giantin antibodies differs from the stage inhibited by anti-GM130 antibodies. Anti-giantin antibodies remained inhibitory at times when anti-GM130 antibodies no longer inhibited transport, suggesting that GM130 and giantin function at distinct steps of transport. We therefore propose a distinct model for p115, GM130, and giantin function in the Golgi, one based on dimeric interactions between p115 and GM130 and between p115 and giantin. We suggest that the p115-GM130 interactions are required for the anterograde delivery of the cargo into an early Golgi compartment and the p115-giantin interactions for the retrograde recycling of glycosyl transferases to that compartment from a later Golgi compartment. COP I vesicles have been shown to contain mannosidase II (49). Such a mechanism would functionally couple the delivery of cargo with the delivery of processing enzymes by utilizing a common molecule, p115, required for both events. This model would allow the coordination of the forward and the recycling pathways by utilizing p115 as a pivotal molecule regulating both stages. Additional analysis will be required to explore the exact molecular roles of p115, GM130, and giantin in the secretory pathway.
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