A novel isoform of human Golgi complex-localized glycoprotein-1 (also known as E-selectin ligand-1, MG-160 and cysteine-rich fibroblast growth factor receptor) targets differential subcellular localization

Jongcheol Ahn, Maria Febbraio* and Roy L. Silverstein*‡

Department of Medicine, Division of Hematology and Medical Oncology, Weill Medical College of Cornell University, New York, NY 10021, USA
*Present address: Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA
†Author for correspondence (e-mail: silverr2@ccf.org)

Accepted 7 February 2005
Journal of Cell Science 118, 1725-1731 Published by The Company of Biologists 2005
doi:10.1242/jcs.02310

Summary

The initial step in trafficking of leukocytes through the vascular endothelium is mediated by an adhesive interaction between molecules of the selectin family and their cognate receptors. Previously, a putative murine E-selectin ligand-1 (ESL-1) was identified and found to be identical to Golgi complex-localized glycoprotein-1 (GLG1), also known as MG-160, and to a previously identified basic fibroblast growth factor (bFGF)-binding protein known as cysteine-rich FGF receptor (CFR). We report here a novel variant of the human GLG1 gene product that we call GLG2, cloned from a human monocyte cDNA library. GLG2 encodes a polypeptide identical to GLG1 except with a unique 24-amino-acid extension at the C-terminus of its cytoplasmic domain. Transfection of chimeric constructs into human embryonic kidney epithelial 293 cells revealed that the cytoplasmic domains of GLG1 and GLG2 targeted the expression of each chimeric protein differentially, GLG1 to the cell surface and GLG2 to the Golgi. Genetic analysis suggests that GLG1 and GLG2 are the products of a single gene, the mRNA of which can be processed by alternative splicing to generate two different transcripts encoding either GLG1 or GLG2. Northern blot analysis showed that the relative amounts of the mRNAs for either isoform differ in a cell- and species-specific manner. These data suggest that alternative splicing of the GLG1 gene transcript might regulate the function of its product.

Key words: Golgi complex-localized glycoprotein-1, E-selectin ligand-1, MG-160

Introduction

E-selectin is expressed on most cultured endothelial cells in response to stimulation by inflammatory cytokines and/or bacterial lipopolysaccharide (LPS) (Bevilacqua et al., 1987). It participates in trafficking of memory T cells in the skin (Picker et al., 1991) and transmigration of hematopoietic progenitor cells (Naiyer et al., 1999). Along with P-selectin, it also participates in the recruitment of neutrophils and monocytes at sites of inflammation and in hematopoiesis (Springer et al., 1994; Frenette et al., 1996). Several studies suggest that ligands for E-selectin may have signaling capacity. For example, engagement of E-selectin ligands enhances tyrosine phosphorylation and decreases Src activity in leukocytes (Soltesz et al., 1997), and human monocyte attachment to E-selectin results in upregulation of tissue factor procoagulant (Lo et al., 1995), inflammatory cytokines (Neumann et al., 1997) and the type B scavenger receptor CD36 (Huh et al., 1995). Although a specific leukocyte glycoprotein ligand for P-selectin, PSGL-1, has been well characterized and shown to participate both in adhesion and signaling (Nagata et al., 1993), characterization of E-selectin ligands remains less well understood.

A candidate murine E-selectin ligand (ESL-1) identified using a recombinant murine E-selectin/IgG fusion protein as an affinity probe (Steegmaier et al., 1995) was found by nucleic acid sequence analysis to be homologous to avian cysteine-rich FGF receptor (CFR) (Burrus et al., 1992) and to a membrane sialoglycoprotein of the medial cisternae of the rat Golgi complex, MG-160 (Gonatas et al., 1989). The gene encoding ESL-1/MG-160/CFR is now known as GLG1 (Golgi complex-localized glycoprotein-1) and has been mapped to human chromosome 16q22-q23 (Mourelatos et al., 1995) (GenBank accession #AC009153.10). The nomenclature GLG for the protein products of this gene will be used for the remainder of this manuscript.

The initial step in trafficking of leukocytes through the vascular endothelium is mediated by an adhesive interaction between molecules of the selectin family and their cognate receptors. Previously, a putative murine E-selectin ligand-1 (ESL-1) was identified and found to be identical to Golgi complex-localized glycoprotein-1 (GLG1), also known as MG-160, and to a previously identified basic fibroblast growth factor (bFGF)-binding protein known as cysteine-rich FGF receptor (CFR). We report here a novel variant of the human GLG1 gene product that we call GLG2, cloned from a human monocyte cDNA library. GLG2 encodes a polypeptide identical to GLG1 except with a unique 24-amino-acid extension at the C-terminus of its cytoplasmic domain. Transfection of chimeric constructs into human embryonic kidney epithelial 293 cells revealed that the cytoplasmic domains of GLG1 and GLG2 targeted the expression of each chimeric protein differentially, GLG1 to the cell surface and GLG2 to the Golgi. Genetic analysis suggests that GLG1 and GLG2 are the products of a single gene, the mRNA of which can be processed by alternative splicing to generate two different transcripts encoding either GLG1 or GLG2. Northern blot analysis showed that the relative amounts of the mRNAs for either isoform differ in a cell- and species-specific manner. These data suggest that alternative splicing of the GLG1 gene transcript might regulate the function of its product.

Key words: Golgi complex-localized glycoprotein-1, E-selectin ligand-1, MG-160

The function of GLG1 in the Golgi is not yet completely characterized. Previous studies (Zuber et al., 1997; Köhl et al., 2000) with bFGF-expressing Chinese Hamster Ovary (CHO) cells showed significant localization of bFGF to the Golgi, suggesting that GLG1 might have a chaperone function, perhaps playing a role in processing and targeting the growth factor in the cell. Similarly, a component of the latent transforming growth factor-β (TGF-β) complex has been shown to be identical to GLG1 (Olofsson et al., 1997). This complex is proposed to function in matrix association of TGF-β, perhaps by facilitating the proper physical orientation of the growth factor.
Because of our interest in the potential role of GLG1 as a monocyte signaling and adhesion receptor, we undertook an experimental approach to characterize the primary structure of human monocyte GLG1, postulating that there might be a unique feature of the monocyte protein that conferred function as an E-selectin receptor. Previous work had shown that, to function as an adhesion and/or signaling receptor for E-selectin, GLG1 protein must be expressed on the external plasma membrane and also undergo specific post-translational modification. Steegmaier et al. showed that GLG1 protein expression and N-glycosylation were necessary to generate a high-affinity binding site between E-selectin and the cells (Steegmaier et al., 1995). An apparent paradox in this field is that studies of CFR and MG-160 in transfected CHO cells, primary neuronal cells and PC12 cells seemed to suggest that GLG1 was localized primarily in the Golgi complex and not on the cell surface (Gonatas et al., 1989; Zuber et al., 1997). Recently, however, a variant of CFR-1 has been reported that is expressed on the cell surface of gastric and other carcinoma cells as well as on gastric mucosal cells in gastritis and gastric hyperplasia (Hensel et al., 2001). The variant, identified by a monoclonal antibody, appears to be related to the pattern of glycosylation of the protein.

In this manuscript, we now report the identification of a second isoform of the GLG gene product (termed GLG2), produced by alternate splicing of the GLG transcript. The variant protein encoded by the GLG2 mRNA contains a unique cytoplasmic domain with a C-terminal extension of 24 amino acids compared with GLG1. Using recombinant chimeric cDNA constructs, the putative cytoplasmic domain of the novel sequence was shown to be sufficient to target chimeric protein to the Golgi, whereas the cytoplasmic domain of GLG1 targeted chimeric protein to the cell-surface membrane. These data help to clarify the intracellular expression patterns of GLG1 and suggest that different functions of the GLG gene products (e.g. E-selectin adhesion/signaling ligand or a chaperon for FGF) might be regulated by mRNA splicing.

### Materials and Methods

#### Screening of cDNA and genomic libraries from human peripheral blood monocytes

One million phage plaques from a human monocyte cDNA library (Clontech) were screened by hybridization with a 3P-labeled, randomly primed probe from the full length of chicken CFR cDNA (graciously provided by B. Olwin, Purdue University). Hybridization was performed at 42°C in 6X SSC (1X SSC: 0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 50% formamide, 1% sodium dodecyl sulfate (SDS), 5X Denhardt’s solution and 100 μg/ml of denatured salmon sperm DNA. After a final wash in 0.1X SSC, 1% SDS at 42°C, the membranes were exposed to X-ray film at –70°C. A human genomic library from peripheral blood (Novagen) was also screened with a probe derived from the 3’ end of the cloned GLG2 cDNA, which contained the sequences encoding the extra 24 amino acids and 59 bp of its 3'-untranslated region (UTR). The probe was synthesized by PCR using GLG2-specific primers (refer to Fig. 1: primer 4 and primer 3). Membranes were then washed twice in 6X SSC (42°C for 30 minutes) and once in 1X SSC (42°C for 30 minutes) before the exposure.

#### Construction of CD8/GLG chimeric plasmids

A cDNA for the extracellular domain of CD8 was obtained by Soil/EcoRV digestion of a plasmid pCD8Ext (Lee et al., 1996). The sequences encoding the transmembrane and cytoplasmic domains of GLG1 and GLG2 were amplified with Pfu DNA polymerase using the primers shown in Fig. 1. PCR products synthesized with primers 1 (5’-GGTTAATCTACATCTCTCTGGATGGG-3’) and 2 (5’-GGTTATCGATGGTAATCTCAGGTC-3’), and 2 (5’-CCCCGATCCAG-TAGAGCACAAGGAGTGT-3’), the 3'-untranslated region (UTR) of the sequences for the transmembrane and cytoplasmic domains of GLG1 or GLG2, respectively. The PCR products were digested with HpaI and BamHI, and then ligated into EcoRV and BamHI sites of the truncated CD8 plasmid. The chimeric cDNA inserts encoding the extracellular domain of CD8 linked to either GLG1 or GLG2 transmembrane and cytoplasmic domains were then re-cloned into the eukaryotic expression vector pcDNA3.1.

#### RT-PCR

Total and poly (A)+ RNA was purified from human peripheral blood monocytes. Primers 2 and 3 from the 3'-UTR of the GLG1 and GLG2 transcripts (Fig. 1) were used for the first-strand cDNA synthesis, respectively. The reaction mixture from the reverse transcription was used as a template for PCR (1 minute at 95°C, 1 minute at 50°C, and 1 minute at 72°C for 30 cycles). Isoform-specific PCR products were

---

**Fig. 1.** Sequence comparison of GLG1 and GLG2. The nucleotide and predicted amino acid sequences of the human monocyte-derived GLG2 are aligned with the published sequence of GLG1 (GenBank accession #NM012201). For convenience, the putative transmembrane and cytoplasmic domains and 3'-UTR of the transcripts for each isoform are shown. The first nucleotide here corresponds to nucleotide #3453 of the registered sequence. The sequences and the direction of the primers used in the present study were also included. The vertical arrow indicates a putative 5'-splicing site. Primers used in the present study were shown by arrows. The amino acid sequence in bold represents the 24-amino-acid extension of GLG2.

| Primer | Sequence |
|--------|----------|
| Primer 1 | h.GLG1: AAG GAC ACG AGG CTA CAA TAG ATT GCA CCA ATC AGC AGC ATC TGT ATA TGA TCC CTG ATC TGC GTG CTC TCG |
| Primer 2 | h.GLG2: AAC TAC ATT CTC TCT GTG ATC AGT GGG AGC ATC TGT ATA TTG TTC CTG |
| Primer 3 | h.GLG2: GTG TGG TCT CAG GAT GTG ACA GGC AGT CCA GCC tga cctttctgcacactc |
| Primer 4 | h.GLG2: AAG GAC AGG CTA CAA TAG ATT GCA CCA ATC AGC AGC ATC TGT ATA TGA TCC CTG ATC TGC GTG CTC TCG |
| Primer 5 | h.GLG2: AAC TAC ATT CTC TCT GTG ATC AGT GGG AGC ATC TGT ATA TTG TTC CTG |

**References:**

1. Lee, E.C., et al. (1996). Sci/EcoRV digestion of a plasmid pCD8Ext.
2. Gonzatas, et al. (1989). Proc. Natl. Acad. Sci. U.S.A. 86, 5238-5242.
3. Zuber, et al. (1997). J. Biol. Chem. 272, 2865-2870.
4. Steegmaier, et al. (1995). J. Biol. Chem. 270, 1726-1732.
5. Hensel, et al. (2001). J. Biol. Chem. 276, 31947-31954.
synthesized with primer 1 and primer 2 (GLG1) or primer 1 and 3 (GLG2), expecting 196 bp or 248 bp PCR products, including linker sequences.

Transfection of 293 cells and indirect immunofluorescence microscopy

Human embryonic kidney epithelial 293 cells (5×10^4 cells) were transfected using the Lipofectamine Reagent (Gibco-BRL) with pcDNA 3.1 containing the extracellular and transmembrane domains of CD8 (CD8Ex), the extracellular domain of CD8 linked to the transmembrane and cytoplasmic domains of GLG1 (CD8/GLG1), or the extracellular domain of CD8 linked to the transmembrane and cytoplasmic domains of GLG2 (CD8/GLG2). Clones resistant to G418 treatment were isolated and maintained in Dulbecco’s Modified Eagle’s Medium containing 10% fetal bovine serum. Transfectants grown on coverslips were fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS), permeabilized in 0.1% Triton X-100 in PBS and processed for two-color immunofluorescence microscopy. The coverslips were incubated with rabbit polyclonal antibody against human giantin as a Golgi marker (Berkeley Antibody Company), and then with FITC-conjugated murine monoclonal antibody against CD8 (Pharminen) and Rhodamine-conjugated goat anti-rabbit IgG (Molecular Probes). Giantin is a membrane-inserted component of cis and medial Golgi structure (Adam and Hauri, 1993). The polyclonal anti-giantin antibody was raised against the N-terminus of giantin, which is exposed on the luminal side of the cis and medial Golgi. For some experiments, cells were treated with 2 µg/ml of brefeldin A or with 5 µg/ml of cycloheximide before the immunofluorescence. Images were collected with a Zeiss LSM510 laser-scanning confocal microscope in the optical microscope core facility of Weill Medical College of Cornell University.

Results

Identification of GLG2

Analysis of two positive clones identified by screening a human monocyte cDNA library with a probe from the full-length chicken CFR cDNA revealed a novel 1.8 kb sequence highly homologous to human GLG1. In fact, the predicted sequences of the transmembrane and extracellular domains were identical (GenBank accession #NM012201; Fig. 1). The predicted sequence of the cytoplasmic domain encoded by the novel monocyte cDNA (GLG2) consists of 37 amino acids, the proximal 13 amino acids of which are identical to the entire GLG1 cytoplasmic domain, but the terminal 24 amino acids are a unique extension (Fig. 1, shown in bold). A cDNA sequence registered on GenBank (Accession #AK025457) contains a highly homologous sequence to GLG1 as well as GLG2-specific sequences.

Subcellular localization of CD8/GLG1 and CD8/GLG2 in stably transfected 293 cells

To determine whether the putative cytoplasmic domains of GLG1 and GLG2 direct expression to different cellular compartments, we constructed chimeric cDNAs encoding the extracellular domain of a T-cell-specific molecule, CD8, linked to the putative transmembrane and cytoplasmic domains of either GLG1 or GLG2 (CD8/GLG1 and CD8/GLG2). These chimeric polypeptides were expressed stably in human embryonic kidney epithelial 293 cells and their subcellular localization was analyzed by confocal immunofluorescence microscopy. The chimeric proteins were detected with a FITC-conjugated monoclonal antibody against the extracellular domain of CD8. A rabbit anti-giantin antibody was used to show the cisternae of the Golgi structure. As shown in Fig. 2 (panel a), the control construct containing the extracellular and transmembrane domains of CD8 (CD8Ex) was expressed on the plasma membrane. The green fluorescence did not overlap with the red fluorescence of the Golgi marker. The CD8/GLG1 chimera was also expressed on the plasma membrane (panel b) and medial Golgi structure (Adam and Hauri, 1993). The polyclonal anti-giantin antibody was raised against the N-terminus of giantin, which is exposed on the luminal side of the cis and medial Golgi. For some experiments, cells were treated with 2 µg/ml of brefeldin A or with 5 µg/ml of cycloheximide before the immunofluorescence. Images were collected with a Zeiss LSM510 laser-scanning confocal microscope in the optical microscope core facility of Weill Medical College of Cornell University.

Results

Identification of GLG2

Analysis of two positive clones identified by screening a human monocyte cDNA library with a probe from the full-length chicken CFR cDNA revealed a novel 1.8 kb sequence highly homologous to human GLG1. In fact, the predicted sequences of the transmembrane and extracellular domains were identical (GenBank accession #NM012201; Fig. 1). The predicted sequence of the cytoplasmic domain encoded by the novel monocyte cDNA (GLG2) consists of 37 amino acids, the proximal 13 amino acids of which are identical to the entire GLG1 cytoplasmic domain, but the terminal 24 amino acids are a unique extension (Fig. 1, shown in bold). A cDNA sequence registered on GenBank (Accession #AK025457) contains a highly homologous sequence to GLG1 as well as GLG2-specific sequences.

Subcellular localization of CD8/GLG1 and CD8/GLG2 in stably transfected 293 cells

To determine whether the putative cytoplasmic domains of GLG1 and GLG2 direct expression to different cellular compartments, we constructed chimeric cDNAs encoding the extracellular domain of a T-cell-specific molecule, CD8, linked to the putative transmembrane and cytoplasmic domains of either GLG1 or GLG2 (CD8/GLG1 and CD8/GLG2). These chimeric polypeptides were expressed stably in human embryonic kidney epithelial 293 cells and their subcellular localization was analyzed by confocal immunofluorescence microscopy. The chimeric proteins were detected with a FITC-conjugated monoclonal antibody against the extracellular domain of CD8. A rabbit anti-giantin antibody was used to show the cisternae of the Golgi structure. As shown in Fig. 2 (panel a), the control construct containing the extracellular and transmembrane domains of CD8 (CD8Ex) was expressed on the plasma membrane. The green fluorescence did not overlap with the red fluorescence of the Golgi marker. The CD8/GLG1 chimera was also expressed on the plasma membrane (panel b)
in a similar distribution to the control CD8Ex. By contrast, the CD8/GLG2 chimera (panel c) was expressed in a punctate intracellular manner with significant overlap (yellow) with the Golgi marker. Treatment of the cells with brefeldin A (panel d) to disrupt Golgi structure abolished the staining pattern of both giantin and CD8/GLG2. The staining pattern of CD8/GLG1 was not affected by the brefeldin A treatment (not shown). One hour after removal of brefeldin A, the staining patterns of both giantin and CD8/GLG2 recovered and resembled that of untreated cells (panel e). Even though some CD8+ endosome-like vesicles were still observed in (d) and (e), these data strongly suggest that CD8/GLG2 mainly localized on the Golgi structure. Treatment of the cells with cycloheximide to block new protein synthesis (panel f) decreased the amount of staining on the endosome-like vesicles, but increased the degree of overlapping staining of giantin and CD8/GLG2. These data suggest that the sequence of 24 amino acids unique to the cytoplasmic tail of GLG2 confers a localization signal sufficient for targeting to the Golgi.

GLG1 gene encodes two polypeptides GLG1 and GLG2

A single human GLG1 gene has been identified and localized to chromosome 11q22-q23 (Mourelatos et al., 1995). To determine the molecular basis underlying generation of the two forms of human GLG mRNA, a human genomic phage DNA library was screened with GLG2-specific probes generated by PCR using primers 4 and 3 (Fig. 1). A 10 kb genomic fragment was isolated and analyzed by Southern Blot using isoform-specific probes. The GLG1-specific probe was the PCR product from primers 5 and 6, corresponding to the 3′-UTR of the GLG1 mRNA. The GLG2-specific probe was the PCR product from primers 4 and 3, containing the 24 extra amino acids and 3′-UTR of the GLG2 mRNA. As shown in Fig. 3a (lanes 1 and 3), a single 3.8 kb EcoRI genomic fragment hybridized with both probes. Further digestion of the fragment with BamH1 generated two fragments of 2.9 and 0.9 kb. The smaller fragment hybridized only with the GLG1 probe (lane 2), whereas the larger fragment hybridized only with the GLG2 probe (lane 4). These data strongly suggest that GLG1 and GLG2 are products of a single gene. Nucleotide sequencing of the cDNA clones revealed consensus sequences for 5′ and 3′ splice sites immediately proximal to the translational stop codon for GLG1 and upstream of the codon for the initial amino acid of the unique extension of GLG2 (Fig. 3b). The guanidine nucleotide at the third position of the last codon of GLG1, encoding arginine, is included in the potential 5′ splice donor site, and the 3′ splice acceptor site contains the guanine nucleotide and the codons for the GLG2-specific amino acid sequence. Therefore, if the splicing site is silent, a transcript containing ‘intronic’ nucleotides between the splicing sites would be generated (Fig. 3c) to form the GLG1 3′-UTR. Since there is a stop codon immediately after the 5′ splice donor site, the GLG1 polypeptide with 1177 amino acid residues would be produced. If the splice site is active, the GLG1 stop codon and the intron are removed; the spliced transcript would therefore contain the downstream sequences encoding the additional 24-amino-acid sequence and GLG2 3′-UTR.

Human monocytes express transcripts for both GLG1 and GLG2

To characterize the expression of the two variant transcripts, we performed RT-PCR on human monocyte poly (A)+ RNA with the isoform-specific probes used in the analysis of GLG genomic structure. As shown in Fig. 4a, PCR products with the predicted size for both GLG1 and GLG2 were generated from monocyte mRNA. The nucleotide sequences for the PCR products were confirmed (data not shown). These data indicate that monocytes contain both transcripts and therefore that the cells are capable of generating both isoforms of GLG.

Cell-type specificity of alternative splicing for GLG1 and GLG2

We also investigated the relative amounts of transcript for GLG1 and GLG2 in several cancer cell lines. Northern blot analysis using a probe specific to GLG2 revealed three transcripts: 10 kb, 5 kb and 3.8 kb (Fig. 4b, right panel). By contrast, only the 10 kb and 5 kb transcripts hybridized with
the GLG1-specific probe. These data suggest the 3.8 kb transcript is the spliced variant encoding GLG2. The 10 kb species may be premature RNA. A probe derived from the 5' end of the cDNA also hybridized with all three transcripts (data not shown), suggesting that both transcripts have an identical 5' end. These data suggest that the amount of GLG1 and GLG2 mRNA might be regulated by the splicing activity on the 3' end of the transcript, such that the greater the activity, the greater the relative amount of GLG2. In HL60 leukemia, G361 melanoma and HeLa cell lines, the amounts of GLG1 and GLG2 transcript were approximately equal. In Molt 4 leukemia, K562 leukemia, SW480 colorectal carcinoma and A549 lung adenocarcinoma cell lines, there was significantly more GLG1. The significance of these differences remains to be determined.

Species specificity of the splicing activity is shown in Fig. 4c. Total RNA from murine RAW monocyte cells, African Green monkey kidney epithelial cells (COS-2) and human melanoma BOWES cells were analyzed with a probe from the 5' region of the cDNA that would hybridize to both GLG1 and GLG2 mRNA. Although cell lines from monkey and human showed three populations of mRNA at molecular weights of 10 kb, 5 kb and 3.8 kb (Fig. 4c, lane 1 and 2), the murine cells showed only a single transcript of about 9.5 kb (lane 3 and 4 for RAW cells, lane 6 for SP2/0 myeloma). Similar results were seen in northern analysis using total RNA from various murine tissues as well as from the CHO cell lines (lane 5). These data suggest that production of GLG2 may be species specific.

Discussion
This study demonstrates that the human GLG gene transcript exists as two isoforms, GLG1 and GLG2, resulting from alternative splicing of a single RNA. The two proteins encoded by these transcripts differ only in their C-terminal cytoplasmic domains, GLG2 having 24 additional amino acids extending from the highly conserved 13-amino-acid cytoplasmic domain of GLG1. Splicing activity varied among different tissues and cell lines such that some cells showed different ratios of GLG1 and GLG2 transcripts, but in no case was only one transcript expressed. Splicing was also species specific; we saw no evidence for the spliced transcript in rodent cells.

We hypothesized that the two cytoplasmic domains of GLG might target protein expression to different subcellular compartments and could serve different functions. Several unrelated functions have been assigned to GLG gene products that require different subcellular locations; for example, cell adhesion/E-selectin ligand function requires expression on the cell surface, whereas MG-160 is an integral membrane protein of the medial cisternae of the Golgi complex. Short sequences in the cytoplasmic tail of proteins, such as proteases of the trans-Golgi network (TGN), have been shown to be crucial for TGN localization. But the mechanism by which GLG1 is retained in the medial cisternae has not been elucidated. Gonatas et al. have shown that transfection of the full-length GLG1 cDNA into CHO cells resulted in expression confined to the Golgi medial cisternae with no cell-surface expression (Gonatas et al., 1998). They also showed that deletion of 1-9 residues of the 13-amino-acid, mostly basic, C-terminal domain, or substitution of the terminal basic amino acid arginine with alanine led to a small amount of surface
expression, generally less than 10% of the total protein. On the basis of these studies, they hypothesized that the C-terminal domain, specifically the arginine at position 1171, was involved in retention to the Golgi medial cisternae. They also suggested that intracellular cleavage of the C-terminal domain of GLG1/MG-160 is a mechanism for directing expression of GLG1 to the cell surface. A protease capable of this was not identified, nor was it determined whether the amount of cell-surface GLG1/ESL-1 on neutrophils could be accounted for by this mechanism.

All available antibodies to GLG react with either the luminal/extracellular domain or the 13-amino-acid C-terminal domain and thus cannot distinguish GLG1 and GLG2. Because we were unable to generate an antibody with specificity for the hydrophobic 24-amino-acid C-terminus unique to GLG2, we utilized recombinant hybrids consisting of the GLG transmembrane and C-terminal domains fused to the extracellular domains of the CD8 protein to study the intracellular localization of the two GLG isoforms. This strategy has been used successfully by others to study localization of other proteins, including the trans-Golgi protein TGN38 (Bos et al., 1993). Surprisingly, the chimeric molecule behaved very differently from that reported by Gonatas et al. for the intact GLG1/MG-160 (Gonatas et al., 1998). We found that the GLG1/CD8 hybrid was expressed on the cell surface of human 293 epithelial cells, with no overlapping staining with the Golgi marker protein giantin. This strongly suggests that the 13-amino-acid GLG1 cytoplasmic domain alone is not sufficient to direct expression of a peptide to the Golgi. This result is consistent with the mutagenesis studies reported by Gonatas et al. that showed that deletion of I-9 amino acids from the C-terminus was associated with a small amount of surface expression (Gonatas et al., 1998). However, the predominant expression site in all cases was the Golgi structure, even in the case of deletion of nearly the entire domain, suggesting that the C-terminal domain may not, in fact, be necessary for Golgi retention. It is possible that interaction of the luminal domain of GLG1 with other integral Golgi proteins or secretary proteins could be required for Golgi retention. This is supported by the observations that GLG1 can bind proteins with rather broad specificity (e.g. FGF, E-selectin and latent TGF-β complex). It also raises the possibility that GLG1 could lose the ability to be retained in the Golgi by modifying its ligand-binding capacity. Since GLG1 function as an E-selectin ligand requires specific fucosylation (Steegmaier et al., 1995), it is possible that regulated fucosylation of the luminal domain of GLG1 could lead to loss of affinity for a crucial Golgi luminal protein along with gain of affinity for E-selectin. Supporting this hypothesis is the recent detection of a novel CFR-1 antigenic variant expressed on the surface of several cancer cells (Hensel et al., 2001). The monoclonal antibody used to detect the variant was an IgM reactive with a unique, but undefined, carbohydrate epitope. Interestingly, mRNA for GLG was found in normal and malignant tissues, but antibody surface staining was only seen on the malignant cells, suggesting that the specific glycosylation variant is only on the cell surface. It is also possible that regulated expression of specific GLG1-binding partners could account for differential cell-surface expression. Proteolytic modification of the C-terminal domain could modulate the affinity of GLG1 for binding partners, resulting in some ‘leakage’ of the protein to the cell surface.

The role of the longer GLG2 C-terminal domain in targeting intracellular expression was also studied with a CD8/GLG2 chimeric protein. The expression pattern of the chimera was very different from that of CD8/GLG1. It showed a punctate intracellular perinuclear staining pattern that overlapped significantly with the staining of the Golgi marker. The perinuclear pattern was disrupted by brefeldin A treatment, suggesting that the subcellular organelle is the Golgi structure. The GLG2 C-terminal domain contains a YKGL sequence shown in the endopeptidase furin to be necessary for steady-state internalization and to participate with additional downstream acidic amino acid clusters in targeting the recycled endopeptidase to the Golgi (Voorhees et al., 1995). Although lack of staining of the plasma membrane with GLG2 strongly suggests that movement of the endosome-like molecules is towards the Golgi, it remains to be determined whether a similar mechanism for recycling of GLG2 holds.

In summary, splicing of the GLG message produces a longer cytoplasmic domain (GLG2) that alone may be sufficient to direct expression to the Golgi. The unspliced GLG transcript produces a shorter cytoplasmic domain (GLG1) that by itself may not be sufficient for Golgi retention. GLG1, however, may be predominantly retained in the Golgi, as shown by Gonatas et al. (1998), by as-yet-undefined mechanisms that could relate to proteolysis, fucosylation or ligand binding. Available data do not readily explain the mechanisms for surface expression of ESL-1 (Steegmaier et al., 1997), but it is interesting that the immuno-electron microscopy studies that most clearly showed surface localization of ESL-1 in neutrophil membrane microvilli were performed with murine cells, a species for which we found no evidence of GLG splicing. We speculate that expression of the unspliced GLG1 product coupled to specific cellular regulation of proteolysis, fucosylation or extra-Golgi ligand binding would generate surface expression. Thus, regulation of GLG mRNA splicing by cells might play a role in determining the amount of surface expression.

References

Adam, D. L. and Hauri, H.-P. (1993). Giantin, a novel conserved Golgi membrane protein containing a cytoplasmic domain of at least 350 kDa. Mol. Biol. Cell 4, 679-693.

Beverl, M. P., Pober, J. S., Mendrick, D. L., Cotran, R. S. and Gimbrone, M. A. (1987). Identification of an inducible endothelial-leukocyte adhesion molecule. Proc. Natl. Acad. Sci. USA 84, 9238-9243.

Bos, K., Wraight, C. and Stanley, K. K. (1993). TGN38 is maintained in the trans-Golgi network by a tyrosine-containing motif in the cytoplasmic domain. EMBO J. 12, 2219-2228.

Burrus, L. W., Zuber, M. E., Lueddeke, B. A. and Olwin, B. B. (1992). Identification of a cystein-rich receptor for fibroblast growth factor. Mol. Cell. Biol. 12, 5600-5609.

Frenette, P. S., Mayadas, T. N., Rayburn, H., Hynes, R. O. and Wagner, D. D. (1996). Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. Cell 84, 563-574.

Gonatas, J. O., Mezitis, S. G. E., Stieber, A., Fleisher, B. and Gonatas, N. K. (1989). MG-160. A novel sialoglycoprotein of the medial cisternae of the Golgi apparatus. J. Biol. Chem. 264, 646-653.

Gonatas, J. O., Chen, Y.-J., Stieber, A., Mourelatos, Z. and Gonatas, N. K. (1998). Truncations of the C-terminal cytoplasmic domain of MG160, a medial Golgi sialoglycoprotein, result in its partial transport to the plasma membrane and filopodia. J. Cell Sci. 111, 249-260.

Hensel, F., Brändlein, S., Eck, M., Schmidt, K., Krenn, V., Kloetzer, A., Bachi, A., Mann, M., Müller-Hermelink, H. K. and Vollmers, H. P. (2001). A novel proliferation-associated variant of CFR-1 defined by a human monoclonal antibody. Lab. Invest. 81, 1097-1107.
Huh, H. Y., Lo, S. K., Yesner, L. M. and Silverstein, R. L. (1995). CD36 induction on human monocytes upon adhesion to tumor necrosis factor-activated endothelial cells. J. Biol. Chem. 270, 6267-6271.

Köhl, R., Antoine, M., Olwin, B. B., Dickson, C. and Kiefer, P. (2000). Cysteine-rich fibroblast growth factor alters secretion and intracellular routing of fibroblast growth factor 3. J. Biol. Chem. 275, 15741-15748.

Lee, S. Y., Park, C. G. and Choi, Y. (1996). T cell receptor-dependent cell death of T cell hybridomas mediated by the CD30 cytoplasmic domain in association with tumor necrosis factor receptor-associated factors. J. Exp. Med. 183, 669-674.

Lo, S. K., Cheung, A., Zheng, Q. and Silverstein, R. L. (1995). Induction of tissue factor on monocytes by adhesion to endothelial cells. J. Immunol. 154, 4768-4777.

Mourelatos, Z., Gonatas, J. O., Nycum, L. M., Gonatas, N. K. and Biegel, J. A. (1995). Assignment of the GLG1 gene for MG-160, a fibroblast growth factor and E-selectin binding membrane sialoglycoprotein of the Golgi apparatus, to chromosome 16q22-q23 by fluorescence in situ hybridization. Genomics 28, 354-355.

Nagata, K., Tsuji, T., Todoroki, N., Katagiri, Y., Tanoue, K., Yamazaki, H., Hanai, N. and Irimura, T. (1993). Activated platelets induce superoxide anion release by monocytes and neutrophils through P-selectin (CD62). J. Immunol. 151, 3267-3273.

Naiyer, A. J., Jo, D.-Y., Ahn, J., Mohle, R., Peichev, M., Lam, G., Silverstein, R. L., Moore, M. and Rafii, S. (1999). Stromal derived factor-1 induced chemokinesis of cord blood CD34+ cells (long-term culture-initiating cells) through endothelial cells is mediated by E-selectin. Blood 94, 4011-4019.

Neumann, F. J., Marx, N., Gawaz, M., Brand, K., Ott, L., Rokitta, C., Sticherling, C., Meinl, C., May, A. and Schömig, A. (1997). Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. Circulation 95, 2387-2394.

Olofsson, A., Hellman, U., Ten Dijck, P., Grimsby, S., Ichijo, H., Moren, A., Miyazono, K. and Helsin, C.-H. (1997). Latent transforming growth factor-β complex in Chinese hamster ovary cells contains the multifunctional cystein-rich fibroblast growth factor receptor, also termed E-selectin-ligand or MG-160. Biochem. J. 324, 427-434.

Picker, L. J., Kishimoto, T. K., Smith, C. W., Warnock, R. A. and Butcher, E. C. (1991). ELAM-1 is an adhesion molecule for skin-homing T-cells. Nature 349, 796-799.

Soltesz, S. A., Powers, E. A., Geng, J.-G. and Fisher, C. (1997). Adhesion of HT-29 colon carcinoma cells to E-selectin results in increased tyrosine phosphorylation and decreased activity of c-src. Int. J. Cancer 71, 645-653.

Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76, 301-314.

Steegmaier, M., Levinovitz, A., Isenmann, S., Borges, E., Lenter, M., Kocher, H. P., Kleuser, B. and Vestweber, D. (1995). The E-selectin ligand ESL-1 is a variant of a receptor for fibroblast growth factor. Nature 373, 615-620.

Soltesz, S. A., Powers, E. A., Geng, J.-G. and Fisher, C. (1997). Adhesion of HT-29 colon carcinoma cells to E-selectin results in increased tyrosine phosphorylation and decreased activity of c-src. Int. J. Cancer 71, 645-653.

Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76, 301-314.

Steegmaier, M., Levinovitz, A., Isenmann, S., Borges, E., Lenter, M., Kocher, H. P., Kleuser, B. and Vestweber, D. (1995). The E-selectin ligand ESL-1 is a variant of a receptor for fibroblast growth factor. Nature 373, 615-620.