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The COG and COPI Complexes Interact to Control the Abundance of GEARs, a Subset of Golgi Integral Membrane Proteins[D]

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The conserved oligomeric Golgi (COG) complex is a soluble hetero-octamer associated with the cytoplasmic surface of the Golgi. Mammalian somatic cell mutants lacking the Cog1 (ldlB) or Cog2 (ldlC) subunits exhibit pleiotropic defects in Golgi-associated glycoprotein and glycolipid processing that suggest COG is involved in the localization, transport, and/or function of multiple Golgi processing proteins. We have identified a set of COG-sensitive, integral membrane Golgi proteins called GEARs (mannosidase II, GOS-28, GS15, GPP130, CASP, giantin, and golgin-84) whose abundances were reduced in the mutant cells and, in some cases, increased in COG-overexpressing cells. In the mutants, some GEARs were abnormally localized in the endoplasmic reticulum and were degraded by proteasomes. The distributions of the GEARs were altered by small interfering RNA depletion of ε-COP in wild-type cells under conditions in which COG-insensitive proteins were unaffected. Furthermore, synthetic phenotypes arose in mutants deficient in both ε-COP and either Cog1 or Cog2. COG and COPI may work in concert to ensure the proper retention or retrieval of a subset of proteins in the Golgi, and COG helps prevent the endoplasmic reticulum accumulation and degradation of some GEARs.

INTRODUCTION

Several multisubunit peripheral membrane protein complexes are thought to play key roles in controlling Golgi-associated membrane trafficking and glycoconjugate processing (Waters *et al*., 1991; Barlowe *et al*., 2002; Schekman and Orci, 1996; Sacher *et al*., 1998; Siniossoglou and Pelham, 2001; Conibear *et al*., 2003; Storrie and Nilsson 2002; Roth, 2002). One of these is the conserved oligomeric Golgi (COG) complex (Krieger *et al*., 1981; Kingsley *et al*., 1986; Podos *et al*., 1994; Wuestehube *et al*., 1996; VanRheenen *et al*., 1998, 1999; Walter *et al*., 1998; Chatterton *et al*., 1999; Kim *et al*., 1999, 2001; Spelbrink and Nothwehr, 1999; Whyte and Munro, 2001; Loh and Hong, 2002; Ram *et al*., 2002; Suvorova *et al*., 2002; Ungar *et al*., 2002; Farkas *et al*., 2003). Mutations in COG subunits (Cog1–8) have been shown to affect the structure and function of the Golgi in yeast, *Drosophila melanogaster* sperm, and mammalian somatic cells. Compromising COG function can cause defects in glycoconjugate synthesis, intracellular protein sorting, protein secretion and, in some cases, cell growth. For example, in mammalian recessive, null, Chinese hamster ovary (CHO) cell mutants called ldlB (Cog1-negative) and ldlC (Cog2-negative), multiple cisternae are dilated (Ungar *et al*., 2002), and there are pleiotropic defects in a number of *medial*- and *trans*-Golgi–associated reactions affecting virtually all N-linked, O-linked, and lipid-linked glycoconjugates (Kingsley *et al*., 1986). These glycoconjugate defects are somewhat reminiscent of those in brefeldin A-treated cells, in which the Golgi disintegrates and early Golgi processing enzymes are relocated to the endoplasmic reticulum (ER) (Doms *et al*., 1989; Perkel *et al*., 1989; van Echten *et al*., 1990; Sampath *et al*., 1992). The diversity and heterogeneity (e.g., partial N-linked sugar endoglycosidase H sensitivity) of these defects suggested that the COG mutations affect the regulation, compartmentalization, or activity of multiple Golgi glycosylation enzymes and/or their substrate transporters without substantially disrupting secretion or endocytosis (Kingsley *et al*., 1986; Reddy and Krieger, 1989). The activities of these proteins depend on their proper intra-Golgi localization and appropriate intralumenal environments (e.g., pH) (Harris and Waters, 1996; Skrincosky *et al*., 1997; Axelsson *et al*., 2001; Martínez-Menarguez *et al*., 2001; Mironov *et al*., 2001; Opat *et al*., 2001; Berger, 2002; Puri *et al*., 2002; Roth, 2002; Zerfaoui *et al*., 2002). Thus, COG might play a role directly or indirectly in resident Golgi proteins' transport to, retention at, or retrieval to appropriate sites, or otherwise determine the Golgi's structure and/or lumenal environment (Kingsley *et al*., 1986).

A direct role for COG in controlling anterograde or retrograde membrane trafficking was suggested by its ability to stimulate an in vitro intra-Golgi transport and glycosylation assay (Walter *et al*., 1998) and by genetic studies in yeast that have identified a large number of COG-interacting genes that encode proteins implicated in Golgi trafficking (VanRheenen *et al*., 1998, 1999; Kim *et al*., 1999, 2001; Spelbrink and Nothwehr, 1999; Whyte and Munro, 2001; Ram *et al*., 2002; Suvorova *et al*., 2002). Yeast COG has been proposed to function as a vesicle tethering factor in anterograde ER-to-Golgi trafficking (VanRheenen *et al*., 1998, 1999) or in COPI-

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mediated retrograde trafficking (Ram *et al.*, 2002; Suvorova *et al.*, 2002), or in cargo sorting during exit from the ER (Morsomme and Riezman, 2002; Morsomme *et al.*, 2003).

Here, we have identified seven COG mutation-sensitive Golgi resident integral membrane proteins (GEARs) among a wide variety of mammalian secretory pathway-related proteins. Their abundances were reduced in COG-deficient mutant cells and, in some cases, increased in COG-overexpressing cells. In the mutant cells, some GEARs were abnormally accumulated in or mislocalized to the ER and were degraded by proteasomes. Thus, COG selectively influences trafficking between the Golgi and the ER. Short interfering RNA (siRNA)-mediated ε-COP depletion established that COG and ε-COP, at least in part, contribute to similar functions necessary for normal Golgi organization (e.g., establishing the proper distribution of GEARs). The results, including the generation of synthetic phenotypes in cells deficient in ε-COP and either Cog1 or Cog2, raise the possibility that COG functions in concert with COPI to ensure the proper trafficking and localization of GEARs in the Golgi.

MATERIALS AND METHODS

Materials and Antibodies

Reagents and sources were as follows: ECL Plus detection kit (Amersham Biosciences, Piscataway, NJ); methionine- and cysteine-free Ham's F-12 medium (Invitrogen, Carlsbad, CA); Expre^{35}S^{35}S protein labeling mix (PerkinElmer Life Sciences, Boston, MA); MG132 and lactacystin (Calbiochem, San Diego, CA); and stock solutions (10 mM) prepared in dimethyl sulfoxide (DMSO), cycloheximide (Sigma-Aldrich, St. Louis, MO). The COG subunit expression plasmids (pLDLB-1 for Cog1, pLDLC-1 for Cog2, and pHM6-COG7 for hemagglutinin (HA)-tagged Cog7) used for establishing the COG-overexpressing cell lines were described previously (Podos *et al.*, 1994; Chatterton *et al.*, 1999; Ungar *et al.*, 2002).

Antibodies used for immunoblot (IB) and immunofluorescence (IF) studies were obtained from standard commercial sources and as gifts from generous individual investigators or generated by us (see below). Antibodies (and their dilutions) were as follows: rabbit polyclonal antibodies: affinity-purified anti-Cog1 antibody (IB, 1:2000; Ungar *et al.*, 2002), affinity-purified anti-Cog2 antibody (IB, 1:1000; Podos *et al.*, 1994; Ungar *et al.*, 2002), affinity-purified anti-Cog3 antibody (IB, 1:10,000; Suvorova *et al.*, 2001), anti-Cog4 antiserum (IB, 1:2000), affinity-purified anti-Cog5 antibody (IB, 1:500; Walter *et al.*, 1998), anti-Cog6 antiserum (IB, 1:1000), anti-Cog7 antiserum (IB, 1:2000), anti-Cog8 antiserum (IB, 1:2000), anti-mannosidase II antiserum (IB, 1:2000; IF, 1:1000; a gift from Kelley Moremen, University of Georgia, Athens, GA), anti-GM130 antibody (IF 1:150; from Nobuhiro Nakamura, Kanazawa University, Ishikawa, Japan), anti-ERp72 antibody (IF, 1:500; Stressgen Biotechnologies, San Diego, CA), anti-GPP130 antibody (IB, 1:1000; IF, 1:500; Covance), anti-giantin antibody (IB, 1:10,000; IF, 1:2000; Covance, Berkeley, CA), anti-CASP antiserum (IB, 1:1000; IF, 1:200; from Sean Munro, MRC, Cambridge, United Kingdom), anti-p115 antibody (IB, 1:400; from Gerry Waters, Princeton University, Princeton, NJ), anti-syntaxin 5 antibody (IB, 1:500; from James Rothman, Memorial Sloan-Kettering Cancer Center, New York, NY and William Balch, The Scripps Research Institute, La Jolla, CA), anti-α-COP antibody (IB, 1:1000; Affinity Bioreagents, Golden, CO), anti-β-COP antibody (IF, 1:200; Affinity Bioreagents), anti-rab6 antibody C-19 (IB, 1:250; Santa Cruz Biotechnology, Santa Cruz, CA), anti-ε-COP antiserum [#310] (IB, 1:5000; IF, 1:1500; Guo *et al.*, 1996), anti-Sec61β antibody (IF, 1:4000; Upstate Biotechnology, Lake Placid, NY); and murine monoclonal antibodies: anti-GM130 antibody (IB, 1:250; IF, 1:150; BD Biosciences, San Jose, CA), anti-Cog1/ldlBp antibody (IF, 1:100; BD Biosciences), anti-GOS-28 antibody (IB, 1:3000; IF, 1:1000; BD Biosciences), anti-GS15 antibody (IB, 1:250, IF; 1:150; BD Biosciences), anti-golgin-84 antibody (IB, 1:250, IF, 1:150; BD Biosciences), anti-membrin antibody (IB, 1:1000; IF, 1:250; Stressgen Biotechnologies), anti-β-COP antibody [maD] (IB, 1:1000; Sigma-Aldrich).

Cell Culture and Transfection

Wild-type CHO, ldlB, ldlC, ldlB[*COG1*], and ldlC[*COG2*] cells were maintained and incubated for experiments as described previously (Podos *et al.*, 1994; Chatterton *et al.*, 1999) at 37°C unless otherwise noted. Wild-type CHO and ldlB cells were transfected with the indicated expression plasmids by using FuGENE 6 (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. The transfected cells were selected in medium A (Ham's F-12 supplemented with 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin) containing 5% fetal bovine serum (medium B) and 0.3 mg/ml active Geneticin (Invitrogen) (medium C), and individual colonies were harvested and grown to mass culture. The relative levels of expression of the proteins of interest were determined using immunoblotting (see below), and those cell lines overexpressing the proteins were used in subsequent experiments.

Construction of the GS15 Expression Plasmid

The 780-base pair *Eco*RV/*Apa*I DNA fragment, containing the entire open reading frame for murine GS15, from the expressed sequence tag (EST) clone (IMAGE 4164150; Invitrogen) was cloned into the *Eco*RV/*Apa*I sites of pcDNA3.1 (Invitrogen) to construct the pGS15-1 plasmid.

Radioactive Labeling and Immunoprecipitation

Metabolic labeling of cells with Expre^{35}S^{35}S (a mixture of [^{35}S]methionine and [^{35}S]cysteine) in methionine- and cysteine-free medium A supplemented with 5% (vol/vol) dialyzed fetal bovine serum was performed as described previously (Kozarsky *et al.*, 1986).

Immunoprecipitation of GOS-28 from radioactive cell lysates by using monoclonal anti-GOS-28 antibody was carried out as described previously (Oka *et al.*, 1991).

Transfection of the siRNA Duplex for ε-COP

Hamster ε-COP was targeted with a siRNA duplex (sense, AGAUGAGGAUGCCACUCUCAC; antisense, GAGAGUGGCAUCCUCAUCUUG; Dharmacon Research, Lafayette, CO). Preparation and transfection of the siRNA duplex were performed as described previously (Novina *et al.*, 2002). A second hamster ε-COP siRNA duplex (sense, GAAGCUGCAAGAAGCCUACUA; antisense, GUAGGCUUCUUGCAGCUUCUC) was also used. On day 0, cells (~20,000–30,000 cells/well) were set in medium B in 12-well dishes. On day 1, cells were transfected with the siRNA duplex (0.1 μM) by using OligofectAMINE (Invitrogen) in medium B according to the manufacturer's instruction. After incubation at 34°C overnight, an equal volume of fresh medium was added, and cells were further incubated at the same temperature. At 48 h after transfection, cells were fixed with 2% (wt/vol) paraformaldehyde before immunofluorescence analysis.

Immunoblotting and Immunofluorescence Microscopy

Cells were grown at 37°C in medium B or medium C until nearly confluent, harvested, and solubilized by addition of buffer A (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol) with scraping, and heated at 95°C for 5–10 min. The lysates (20 μg of protein) were then fractionated by SDS-polyacrylamide (between 5 and 12%) gel electrophoresis, and the proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA) and immunoblotted with the indicated antibodies using the ECL Plus detection kit (Amersham Biosciences) according to the manufacturer's instructions.

Cells grown in medium B or medium C until subconfluent were fixed, stained, and analyzed by immunofluorescence microscopy as described previously (Ungar *et al.*, 2002). Images were obtained with an LSM510 confocal microscope Carl Zeiss, Thornwood, NY).

Preparation of Antibodies against Cog4, 6, 7, and 8

To prepare a recombinant fragment of the Cog6 protein, a segment of the COG6 cDNA, coding for amino acids 427–605, was cloned into the expression vector pQE31 (QIAGEN, Valencia, CA). The His-tagged recombinant protein was expressed in *Escherichia coli*, purified over a Ni^{2+}-NTA column (QIAGEN) and then dialyzed against phosphate-buffered saline buffer. To prepare Cog4, 7, and 8 antigens, a cDNA encoding each full-length protein was cloned into the expression vector pET28a (Novagen, Madison, WI). The proteins were expressed in *E. coli*, and the resulting inclusion bodies were solubilized in 6 M guanidinium hydrochloride. The solubilized proteins were loaded onto Ni^{2+}-NTA columns that were washed with 8 M urea buffers according to the manufacturer's instructions. Bound proteins were eluted by boiling in 1% SDS in the presence of 5 mM EDTA, and then dialyzed against PBS buffer.

Rabbits were injected with 1 mg of a purified antigen. Injections were performed once a month, and sera were collected every 2–4 wk starting at 6 wk after the first injection. Specificity of the antisera was tested by immunoblotting against both partially purified bovine brain COG complex and CHO cell lysates (Ungar *et al.*, 2002).

RESULTS

COG Sensitivity of a Set of Golgi-resident Proteins Called GEARs

To study the function of COG, we surveyed the effects of the COG mutations in ldlB and ldlC mutants on a diverse group of Golgi and ER-associated proteins. Wild-type CHO cells, the ldlB and ldlC mutants, and stable revertants of these mutants generated by transfection with expression plasmids

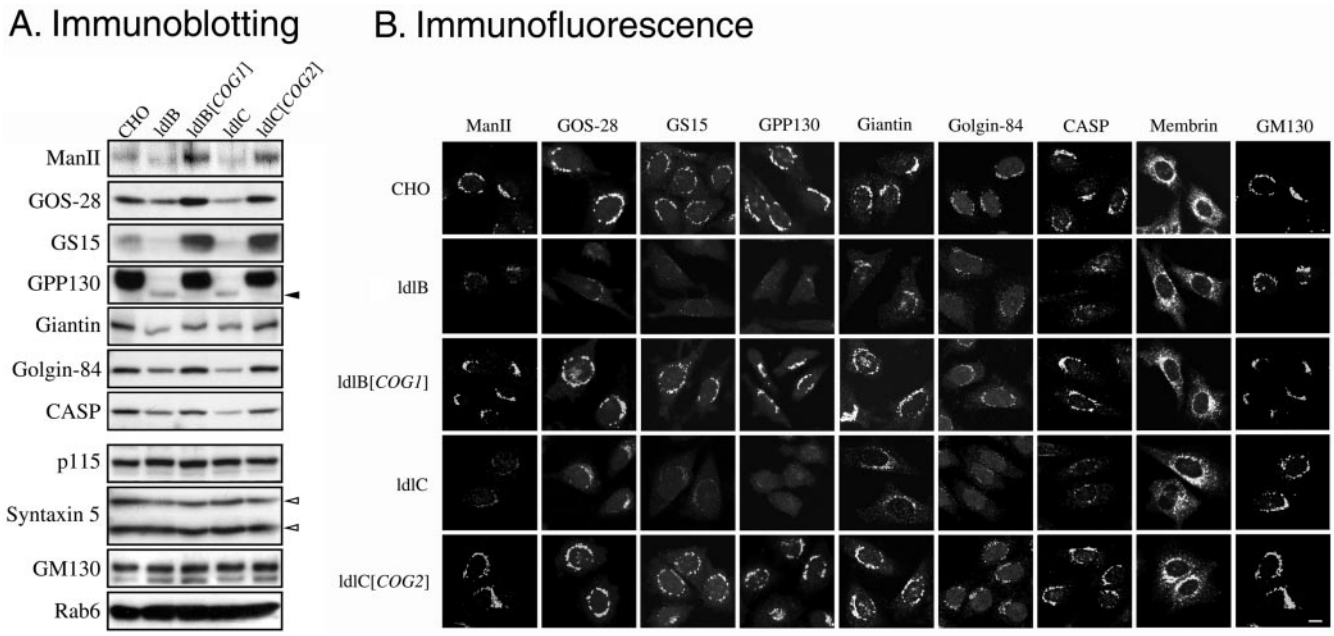


Figure 1. Immunoblotting and immunofluorescence localization of Golgi-associated proteins in wild-type CHO, mutant ldlB and ldlC, and transfected ldlB[*COG1*] and ldlC[*COG2*] cells. (A) Total cell lysates (20 μg of protein) were prepared from parental wild-type CHO, ldlB, ldlB[*COG1*] (ldlB cells transfected with COG1 cDNA, Cog1 overexpressed), ldlC and ldlC[*COG2*] (ldlC cells transfected with COG2 cDNA, Cog2 overexpressed) cells. Lysates were subjected to SDS-PAGE and immunoblotting by using antibodies to the indicated Golgi-associated proteins. Solid and open arrowheads indicated a 115-kDa band of GPP130 and two isoforms (42 and 35 kDa) of syntaxin-5, respectively. (B) The indicated cells (left) were grown at 37°C (except those stained with anti-CASP antibody, which were grown at 34°C), fixed with paraformaldehyde, and stained with antibodies to the indicated proteins. To permit comparison of signal intensities among the images in the same column, the images were collected with a fixed signal gain by confocal microscopy. Bar, 10 μm.

for the corresponding cDNAs (ldlB[*COG1*], ldlC[*COG2*]) were studied. We initially screened with a collection of antibodies by using immunoblotting and/or by immunofluorescence microscopy to assess their intracellular distributions and relative abundances.

Figure 1 illustrates representative immunoblots (A) and immunofluorescence micrographs (B) from this survey with the results summarized in Table 1. Of the 32 proteins examined, seven were COG sensitive, in that they exhibited a significant reduction in their steady-state levels in the Cog1-negative ldlB and Cog2-negative ldlC mutants. They were mannosidase II (Man II), GPP130, CASP, giantin, golgin-84, GOS-28, and GS15. We refer to these COG-sensitive proteins as "GEARs." All seven GEARs are resident Golgi transmembrane proteins (Linstedt and Hauri, 1993; Nagahama *et al*., 1996; Subramaniam *et al*., 1996; Linstedt *et al*., 1997; Lowe *et al*., 1997; Hay *et al*., 1998; Xu *et al*., 1997; Bascom *et al*., 1999; Orci *et al*., 2000; Gillingham *et al*., 2002; Moremen, 2002) and are located virtually exclusively in a perinuclear Golgi ribbon-like distribution in wild-type cells (Figure 1B). In contrast, the COG-insensitive Golgi integral membrane proteins examined to date—membrin (Figure 1B), vti1a, syntaxin-5, and TGN38—could also be detected, at least in part, in distinct punctate structures. Other COG-insensitive proteins that were identified (Table 1; also see Figure 1) included Golgi-associated peripheral proteins p115 and GM130 (Figure 1B), the lipid-anchored GRASP55, the COPII coat protein mSec23, and the ER luminal proteins ERp72 and Bip. It is worth noting that several of the COG-insensitive proteins can form complexes with the COG-sensitive GEARs, e.g., p115 and GM130 with giantin (Sönnichsen *et al*., 1998), syntaxin-5 and mYkt6 with GOS-28 and GS15 (Shorter *et al*.,

Table 1. Summary of proteins tested in the COG mutants

	COG-sensitive proteins (GEARs)	COG-insensitive proteins
SNAREs	GOS-28 GS15	Membrin mYkt6 Syntaxin-5 Syntaxin-6 Vtila
Golgins	CASP Giantin Golgin-84	GRASP55 GM130 Golgin-97 Golgin-245
Small GTPases		Rab1 Rab6 Rab7[a] Rab8 Rab11 RalA
Others	Mannosidase II GPP130	Bip ERp72[a] mSec23[a] p115 RabGDI Sec8 Sec61α and β TRAPα TGN38[a]

[a] Determined by immunofluorescence only.

2002; Xu *et al.*, 2002), and rab1 with golgin-84 (Diao *et al.*, 2003; Satoh *et al.*, 2003).

The GEARs are members of several distinct classes of Golgi-associated proteins. Of the seven golgins tested (CASP, golgins-84, -97, -245, GM130, GRASP55, and giantin), only those three that are integral membrane proteins (CASP, golgin-84, and giantin) (Linstedt and Hauri, 1993; Bascom *et al.*, 1999; Gillingham *et al.*, 2002) are GEARs (Figure 1 and Table 1). Of the seven SNAREs tested, the only ones that seem to be exclusively Golgi localized, GOS-28 and GS15 (Nagahama *et al.*, 1996; Subramaniam *et al.*, 1996; Xu *et al.*, 1997; Orci *et al.*, 2000; Xu *et al.*, 2002) are GEARs. None of the small GTPases (e.g., rabs) examined are GEARs. It was somewhat surprising to find that only one of the GEAR genes, GOS-28, is orthologous to a yeast gene *GOS1* that genetically interacts with COG and this interaction is rather weak (Kim *et al.*, 1999), whereas several mammalian COG-insensitive proteins are orthologues of proteins encoded by genes that strongly interact with yeast COG, including the SNAREs Ykt6p (mYkt6) and Sed5p (syntaxin-5), Ypt1p (rab1), and Uso1p (p115).

The relatively low steady-state levels of the GEARs in the ldlB and ldlC mutants were due to the absence of the Cog1 or Cog2 subunits, because higher level expression was observed in the ldlB[*COG1*] and ldlC[*COG2*] transfectants (Figure 1). In the cases of GPP130, CASP, giantin and golgin-84, the levels in the transfectants were about the same as those in wild-type cells. Strikingly, the steady-state levels of GOS-28, GS15 and Man II were greater in the transfectants than in wild-type cells (also see Podos *et al.*, 1994). Because the levels of these three GEARs were reduced in the mutants and elevated in COG-overexpressing transfectants (see below), it seems likely that their abundances are directly influenced by COG function.

Only two of the seven GEARs are glycoproteins. One, GPP130, is a protein of unknown function (Linstedt *et al.*, 1997) whose localization in the Golgi is sensitive to changes in intralumenal pH (Linstedt *et al.*, 1997; Puri *et al.*, 2002). The other, Man II, is a glycosidase required for normal N-linked oligosaccharide processing (Moremen, 2002). In the ldlB and ldlC mutants, reductions in the amounts of Man II might contribute to the global defects observed in N-linked oligosaccharide synthesis (Kingsley *et al.*, 1986). As additional reagents for other glycoconjugate processing enzymes become available, it will be possible to determine whether some of them are also GEARs. As expected because of the global glycosylation defects in ldlB and ldlC cells (Kingsley *et al.*, 1986), the electrophoretic mobilities of the GPP130 and Man II glycoproteins in the mutants were increased, indicating abnormal glycosylation (Figure 1A). It is possible that some of the COG sensitivities of these proteins might be a secondary consequence of their abnormal glycosylation. However, similarities in the COG sensitivities of Man II to the unglycosylated GS15 and GOS-28 suggest that, at least in the case of Man II, such secondary effects do not account fully for the COG sensitivities.

Stabilities and Intracellular Distributions of the Golgi SNAREs GOS-28 and GS15 in ldlB and ldlC Mutants

To examine the mechanism(s) underlying the COG sensitivity of the GEARs, we focused on the unglycosylated SNAREs GOS-28 and GS15 so that any secondary effects due to changes in the GEARs' protein glycosylation would not confound the analysis. In the metabolic labeling, pulse-chase experiment shown in Figure 2A (top), newly synthesized GOS-28 in wild-type CHO cells was stable throughout the first 12 h of the chase, and there was only a small loss (~25%) of the protein between 12 and 24 h (Figure 2A, top). In the ldlB and ldlC mutants, the rate of GOS-28 synthesis (0-h chase) seemed to be only a little lower than that in the wild-type cells. However, the rates of loss of GOS-28 during the chase were substantially higher in the mutants than in the wild-type cells. Approximately 50% of the labeled protein was lost by 12 h of chase and ~10% remained at 24 h in the ldlB and ldlC mutants (half-lives of ~11–12 h in the mutants vs. >24 h in wild-type CHO cells). The abnormally rapid degradation of GOS-28 in the mutant cells was not observed in ldlB[*COG1*] transfectants (Figure 2A, bottom), indicating that the instability was a consequence of COG deficiency. Similar results were obtained when the stability of pulse-labeled GS15 was examined.

The mechanism underlying the rapid degradation of GOS-28 in the mutants was examined by determining with immunofluorescence confocal microscopy GOS-28's intracellular distribution in wild-type and mutant cells. To visualize the detailed distribution of GOS-28, which is present at lower levels in ldlB and ldlC cells, images of mutant cells were collected with higher signal gains than those of wild-type cells (Figures 2, 3, and 7). As shown in Figure 2B, in wild-type cells the classic perinuclear Golgi ribbon distribution of GOS-28 was observed as previously described (Subramaniam *et al.*, 1995), and did not overlap with the reticular distribution of the ER luminal protein ERp72 (Mazzarella *et al.*, 1990). A similar result was observed using coimmunofluorescence with the ER membrane protein Sec61β (Hartmann *et al.*, 1994). In contrast, in the ldlB and ldlC mutants, a significant fraction of GOS-28 could be seen in a reticular staining that was dispersed throughout the cytoplasm in a distribution consistent with it being mislocalized into the ER. The residual perinuclear Golgi-ribbon distribution of GOS-28 in the mutants is not seen as clearly as in Figures 1B or 3A, because of the relative positions of some of the confocal sections shown in Figure 2B (especially that for the ldlC cells). Indeed, this mislocalized protein colocalized with the ER markers ERp72 and Sec61β. Thus, we conclude that a substantial portion of the reticular pattern of GOS-28 staining in the mutants was due to its location in the ER. In the ldlB[*COG1*] and ldlC[*COG2*] cells, the distribution of GOS-28 was the same as in CHO cells, exclusively located in the Golgi region. The mislocalization of GS15 to the ERp72-positive reticular compartment in ldlB cells that overexpress this protein (ldlB[*GS15*] (Supplemental Figure 1, A and B) was similar to that seen for endogenous GOS-28 in ldlB cells (Figure 2B). Thus, these GEARs exhibited COG sensitivity of both their steady-state levels and intracellular localizations.

Effects of Proteasome Inhibitors on the Stabilities of GOS-28 and GS15

The rapid degradation of GOS-28 and GS15 and their abnormal distribution in the ER raised the possibility that dislocation (retrotranslocation) and proteasomal degradation (Kostova and Wolf, 2003; McCracken and Brodsky, 2003) might be responsible for the instability of some GEARs in the mutants. Therefore, we added the proteasome inhibitors MG132 (25 μM) or lactacystin (25 μM) in DMSO to the chase media of a pulse-chase experiment to examine their effects on GOS-28 stability. Both inhibitors prevented the abnormally rapid degradation of GOS-28 that occurred in the mutants (Figure 2A, bottom). Neither inhibitor affected GOS-28's stability in wild-type CHO or ldlB[*COG1*] cells. Similar results were observed when the effects of these inhibitors on GS15 stability in the mutants were examined. The effects of these proteasome inhibitors on the steady-state levels of expression were confirmed by immunoblotting

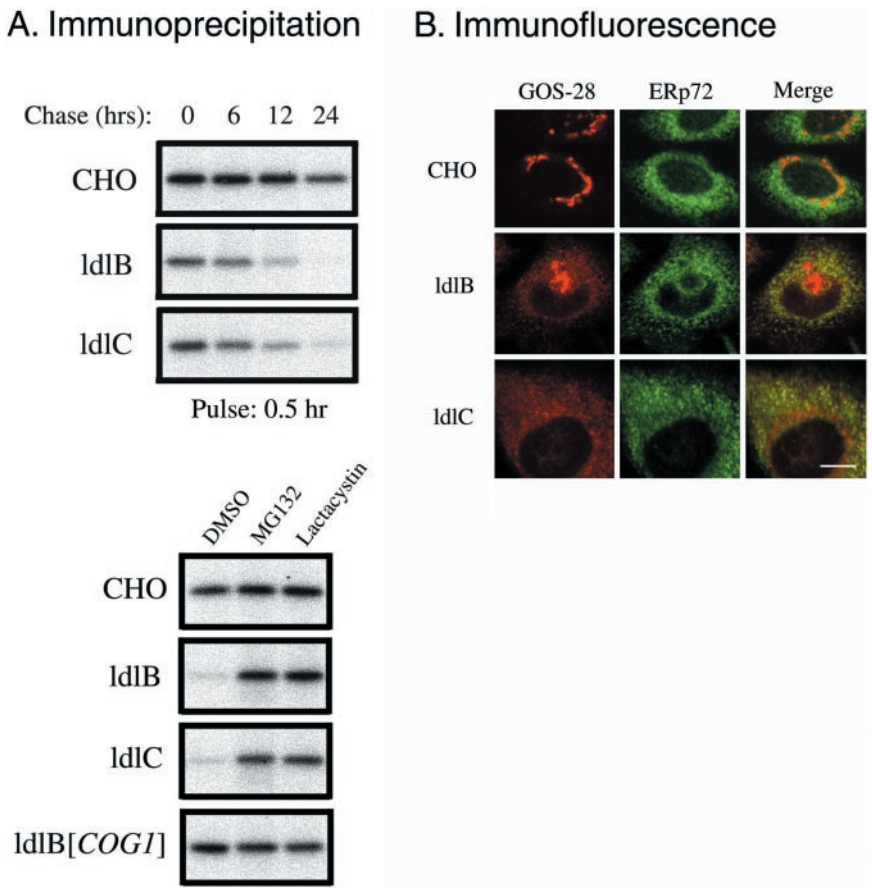


Figure 2. Stability and intracellular distributions of GOS-28 in wild-type CHO, ldlB, and ldlC cells. (A) Cells were metabolically labeled with [^{35}S]methionine/cysteine for 30 min (pulse), and then either chased for the indicated times in medium B containing unlabeled methionine and cysteine (top) or incubated for 24 h with the same medium plus 25 μM of the proteasome inhibitors MG132 or lactacystin (bottom). Cell lysates were subjected to immunoprecipitation with an anti-GOS-28 antibody. The immunoprecipitates were fractionated by SDS-PAGE, and the bands were visualized by autoradiography. (B) Cells were fixed and stained with antibodies to GOS-28 (red) and the ER marker ERp72 (green). To visualize the distribution of GOS-28 by using confocal microscopy, the images from ldlB and ldlC cells (weaker signals) were collected with higher signal gains than those from CHO cells. Note that because of the location of the section, some of the confocal images of the mutants do not show the perinuclear Golgi ribbon staining that is present (e.g., see Figure 1B). Bar, 10 μm.

analysis. The inhibitors restored the steady state levels of GOS-28 in the ldlB and ldlC mutants to those seen in the wild-type CHO and transfected control cells (Supplemental Figure 2A).

Prolonged incubation of cells with high concentrations of proteasome inhibitors can induce caspase activation (Drexler *et al*., 2000) and apoptosis-associated fragmentation of the Golgi (Mancini *et al*., 2000; Lane *et al*., 2002; Chiu *et al*., 2002), and thus might possibly indirectly alter the stability of the GEAR proteins. To ensure that the effects of these inhibitors were independent of apoptosis, we examined the dose dependence of lactacystin on the steady-state level of GOS-28 after only 4 h of incubation with the inhibitor. Lactacystin significantly increased the levels of GOS-28, even at concentrations as low as 1 μM (Supplemental Figure 2B). In control experiments, we measured activation of caspase-3 as a measure of apoptosis and observed no evidence that lactacystin-induced apoptosis at concentrations of 1–25 μM after incubations with the inhibitor for 4 h. This finding is consistent with the report that lactacystin induction of caspase-3 processing is relatively slow (~8 h) compared with its rapid inhibition of proteasomal activity (Kim *et al*., 2003). In contrast to the proteasome inhibitors, bafilomycin A (~10 nM), an inhibitor of lysosomal protein degradation (Yoshimori *et al*., 1991), did not significantly affect the stabilities of GOS-28 and GS15 in the ldlB and ldlC cells. Thus, even though we were unable to detect polyubiquitinated intermediates (Bonifacino and Weissman, 1998), the abnormal degradation of GOS-28 and GS15 in ldlB and ldlC mutants was apparently due to proteasome-mediated hydrolysis.

Effects of Proteasome Inhibitors on GOS-28 Accumulation in Reticular Structures

We next explored the relationship between the abnormal localization of GOS-28 in the ER and its proteasomal degradation in ldlB and ldlC mutants. In some cases, undegraded dislocated (retrotranslocated) proteins can be trapped in the cytoplasm by inhibiting proteasome activity (Wiertz *et al*., 1996a,b). In others, dislocation and proteasomal degradation are tightly coupled and treatment with proteasome inhibitors results in accumulation of the proteins within the ER (Johnston *et al*., 1998). We used immunofluorescence microscopy to determine the effects of lactacystin treatment (25 μM for 4 or 8 h) of wild-type and mutant cells on the intracellular distribution of GOS-28, along with the COG-insensitive Golgi marker GM130 (Nakamura *et al*., 1995).

Figure 3A compares confocal immunofluorescence images of lactacystin-treated and untreated cells. Because of the signal gain and the z-section used to collect the confocal signals, the relatively weak reticular patterns of GOS-28 staining in the untreated ldlB and ldlC mutants (Figure 2B) are difficult to see in these images. Even after incubation with lactacystin for 8 h, most of the GM130 signal in wild-type and mutant cells exhibited a perinuclear Golgi distribution (middle). There was some fragmentation of the GM130 staining pattern induced by lactacystin treatment; however, this was unlikely to be due to apoptosis, because some fragmentation was also observed at 4 h when there was no caspase-3 activation. The GOS-28 distribution (Figure 3A, left) in wild-type CHO cells was virtually identical to that of GM130 with or without lactacystin treatment,

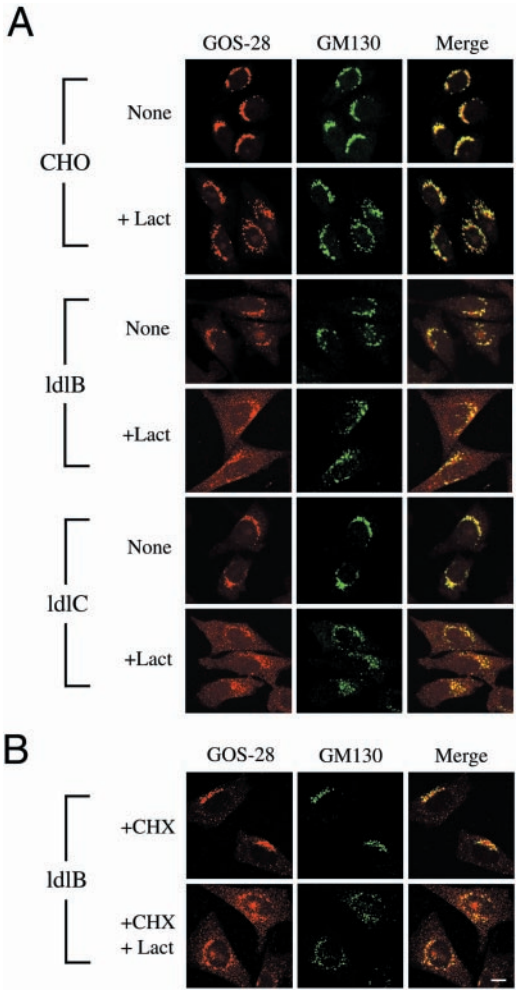


Figure 3. Immunofluorescence localization of GOS-28 and GM130 in CHO, ldlB, and ldlC cells treated without and with lactacystin. (A) Cells were incubated for 8 h without (none) or with (+Lact) 25 μM lactacystin, fixed, and stained with antibodies to GOS-28 (red) and GM130 (green). To visualize more clearly the distribution of GOS-28 by using confocal microscopy, the images from ldlB and ldlC cells (weaker signals) were collected with higher signal gains than those from CHO cells. However, a fixed signal gain was used to collect images from any given cell type when comparing the effects of incubation without or with lactacystin. (B) The ldlB cells were preincubated with cycloheximide (+CHX, 140 μg/ml) for 1 h and then further incubated for 8 h without or with (+Lact) 25 μM lactacystin in the presence of the cycloheximide. Bar, 10 μm.

whereas in both ldlB and ldlC cells lactacystin induced a significant increase in the ER-like reticular distribution of GOS-28. Coimmunofluorescence staining with antibodies to GOS-28 and the ER marker Sec61β established that a substantial amount of the lactacystin-induced increase in reticular staining was located in the ER. In wild-type and mutant cells, the distribution of the COG-insensitive Golgi SNARE membrin was, like GM130, not substantially affected by lactacystin treatment. To determine whether new protein synthesis was required for lactacystin-induced accumulation of these GEARs in the ER, we pretreated cells with 140 μg/ml cycloheximide for 1 h before adding 25 μM lactacystin and then incubated the cells for an additional 8 h. Protein synthesis was dramatically inhibited by this cycloheximide treatment (>90% inhibition after the 1-h preincubation and >95% inhibition by the end of the 8-h incubation). The intracellular distributions of GOS-28 and GM130 in wild-type cells with or without lactacystin treatment were not altered by the cycloheximide treatment. Figure 3B shows that inhibition of protein synthesis by cycloheximide in the ldlB cells also did not prevent lactacystin-induced accumulation of GOS-28 in the ER of these COG deficient cells, although the intensity of the ER staining was somewhat reduced by the cycloheximide treatment. Thus, transport of preexisting GOS-28 to the ER contributes in part to the lactacystin-induced accumulation.

Together with the stabilizing effects of lactacystin on GOS-28 and GS15, the results suggest that proteasomal hydrolysis accounts for the rapid degradation of these proteins and their reduced steady-state levels in the mutant cells and that inhibition of proteasomes blocks both GOS-28 degradation and its dislocation from the ER, but not its abnormal localization in the ER. Thus, it seems that abnormal ER localization of these GEARs, probably due at least in part to retrograde trafficking from the Golgi, contributes to their reduced steady-state levels.

Effects of Overexpressing Individual COG Subunits on the GEARs GOS-28 and GS15 and on the Other COG Subunits

In both wild-type and mutant cells, overexpression of the Cog1, Cog2, or HA-Cog7 COG subunits (ldlB[*COG1*], ldlC[*COG2*], CHO[*COG1*], CHO[*COG2*], or CHO[HA-*COG7*]) resulted in elevated steady-state levels of some of the GEARs, GOS-28, GS15, and Man II, relative to wild-type CHO cells (Figures 1A and 4). The levels of the COG-insensitive protein GM130 were not affected by the transfected COG genes (Figure 4, bottom). It seems likely that by mass action the excess Cog1, Cog 2, or Cog7 subunits induced the formation of a greater than normal steady-state concentration of COG complexes in the transfectants and thus resulted in a gain-of-function, or hypermorphic-like, response of some GEARs. We therefore used immunoblotting to compare the steady-state levels of the COG subunits in the wild-type, mutant, and transfectant cells. Figure 4 shows that in ldlB cells, in which there is no detectable Cog1, there was very little change in the levels of Cogs 2–4, whereas there was a substantial reduction in the amounts of Cogs 5–8. This is consistent with the proposal that Cogs 5–8 may form a subcomplex within the intact COG complex (Ungar *et al.*, 2002, also see Whyte and Munro, 2001). Strikingly, the patterns of protein levels for all of the COG subunits in the overexpressing transfectants were similar to those of GOS-28 and GS15: increased levels relative to untransfected wild-type CHO controls, with somewhat higher levels in the ldlB[*COG1*] and CHO[*COG1*] cells than in the CHO[*COG2*] and CHO[HA-*COG7*] cells. Thus, overexpression of any one of at least three of the COG subunits induces increased levels of the others, suggesting that the steady-state levels of the GEARs GOS-28, GS15, and Man II are proportional to the amounts of the COG complex (reduced in the mutants, increased in the transfectants).

Interactions between COG and COPI

Previous studies in yeast have suggested a functional and physical relationship between COG and the vesicle coat complex COPI (Kim *et al.*, 2001; Ram *et al.*, 2002; Suvorova *et al.*, 2002). Therefore, we determined the effects of the COG1 and COG2 mutations in the ldlB and ldlC mutants on the amounts and intracellular distributions of several COPI subunits. The amounts of the COPI α, β, and ϵ subunits in the mutants were unchanged relative to those in wild-type and

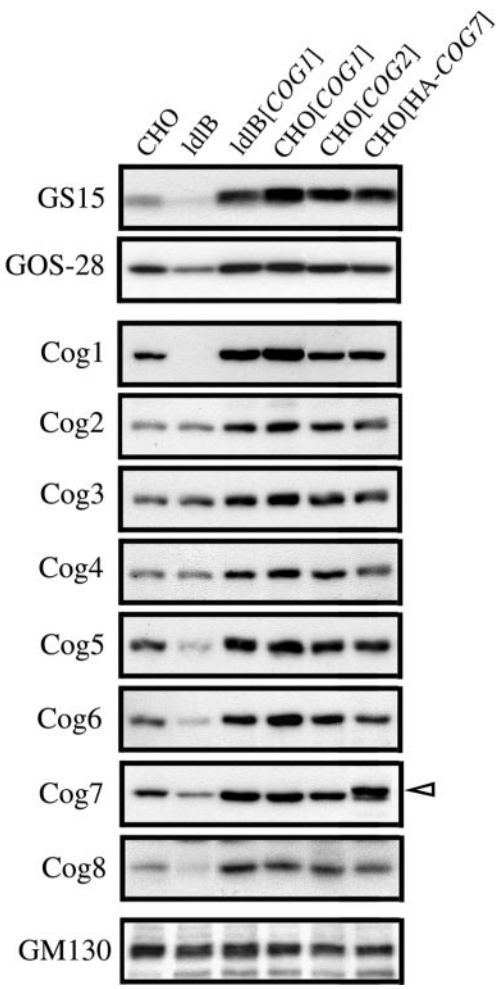


Figure 4. Steady-state levels of GOS-28, GS15, the subunits of COG and GM130 in untransfected and COG subunit-overexpressing CHO and ldlB cells. Total cell lysates from the CHO, ldlB, and ldlB[*COG1*] cells, as well as transfected CHO cells that overexpress exogenous Cog1, Cog2, or HA-tagged Cog7 (CHO[*COG1*], CHO[*COG2*] and CHO[HA-*COG7*]) were subjected to SDS-PAGE and immunoblotting. Open arrowhead indicates the HA-tagged Cog7 protein whose electrophoretic mobility is slightly less than that of the endogenous Cog7.

transfected cells (Figure 5A, similar results for β-COP were previously reported by Ungar *et al*., 2002). However, immunofluorescence microscopy (Figure 5B) showed that the distribution of the β subunit was altered in the mutants. In wild-type CHO and transfected cells, β-COP staining exhibited the typical, perinuclear Golgi pattern combined with only a relatively low-intensity, punctate signal seen distributed throughout the cytoplasm (also see Allan and Kreis, 1986). In the ldlB and ldlC mutants, the cytoplasmic β-COP staining increased, whereas the perinuclear staining decreased. Similar results were observed for ε-COP (Figures 6 and 7). Thus, the intracellular distribution of COPI is altered by mutations in COG1 and COG2.

We assessed the effects of alterations in the expression of ε-COP on both COG and GEARs by using RNA interference (Elbashir *et al*., 2001) to knock down ε-COP expression in wild-type CHO cells.[1] Cells were transfected with a siRNA duplex specific for hamster ε-COP, maintained in culture for 46.5 h at 34°C and then either shifted to 39.5°C or kept at 34°C for 90 min before immunofluorescence analysis of the intracellular distribution of a variety of Golgi-associated proteins. Previous studies in yeast have suggested that ε-COP is not required at the normal growth temperature but is indispensable for cellular function at high temperature (Duden *et al*., 1998; Kimata *et al*., 1999). Figure 6A shows that there was a complete loss or major reduction in the amounts of detectable ε-COP protein in a subset of the siRNA-treated CHO cells at both 34 and 39.5°C (arrowheads). At 34°C, the ε-COP–deficient cells seemed to be viable and healthy, and we detected no changes in the intracellular distribution of GOS-28, GM130 (Figure 6, A and B), GS15, golgin-84 (Supplemental Figure 3, A and B), Man II, GPP130, membrin, vti1a, or β-COP. We also observed no changes in the intracellular distribution of COG (Cog1) in the ε-COP-depleted cells at 34°C. However, there was a significant reduction in the perinuclear staining of Cog1 in the ε-COP–depleted cells after incubation for 90 min at 39.5°C (Figure 6A). This was not due to a general breakdown of the structure of the Golgi apparatus, because there was no disruption in the normal perinuclear distribution of several other Golgi markers, including GM130 (Figure 6A; see below). Thus, the proper distribution of COG at 39.5°C requires ε-COP activity.

Interactions between COPI and GEARs

Ater incubation for 90 min at 39.5°C, those CHO cells in which ε-COP was depleted by introduction of the siRNA also lost their normal perinuclear Golgi distributions of the six GEARs examined (CASP distribution was not analyzed) (Figure 6B and Supplemental Figure 3). In contrast, there was no apparent effect of the siRNA on the distributions of GM130 (Figure 6A), membrin, or vti1a after incubation for 90 min at 39.5°C. One possible explanation for these observations is that the ε-COP activity is indispensable for COG function at 39.5°C. In ε-COP–depleted cells identified by alteration in the GOS-28 signal, the Golgi distribution of a different COPI subunit, β-COP, was unaffected after incubation at 39.5°C for 90 min (Figure 6C), even though COG and GEARs were affected. As an additional control, a second independent siRNA, corresponding to a different portion of the ε-COP coding sequence, was introduced into the cells, and its effects were similar to those of the first siRNA. Furthermore, the siRNA for a distinct gene, GFP (Novina *et al*., 2002), was introduced into the cells and had no effect on the intensity of staining or the distributions of GOS-28 or ε-COP, indicating that the effects of the ε-COP siRNA were ε-COP specific. Thus, under conditions where there is apparently some initial temperature-dependent loss of COPI function due to ε-COP-depletion, but not complete breakdown of the Golgi's structure or even dissociation of some COPI subunits, the COG-sensitive, but not-insensitive, proteins were mislocalized and possibly in some cases degraded. These findings suggest that COG and ε-COP, at least in part, contribute to similar functions necessary for normal

[1]We also examined the levels of Cog1 and Cog2 in the temperature-sensitive ε-COP mutant ldlF. In this mutant, a point mutation in ε-COP renders it unstable at the nonpermissive temperature of 39.5°C (Guo *et al*., 1994, 1996). At both the permissive temperature (34°C) and after 12 h at the nonpermissive temperature when the structure of the Golgi is disrupted (Guo *et al*., 1994), there were no obvious changes in the levels of Cog1 and Cog2 in ldlF cells, even though the distribution of these Cogs in ldlF at the nonpermissive temperature was dramatically altered concomitantly with other Golgi markers.

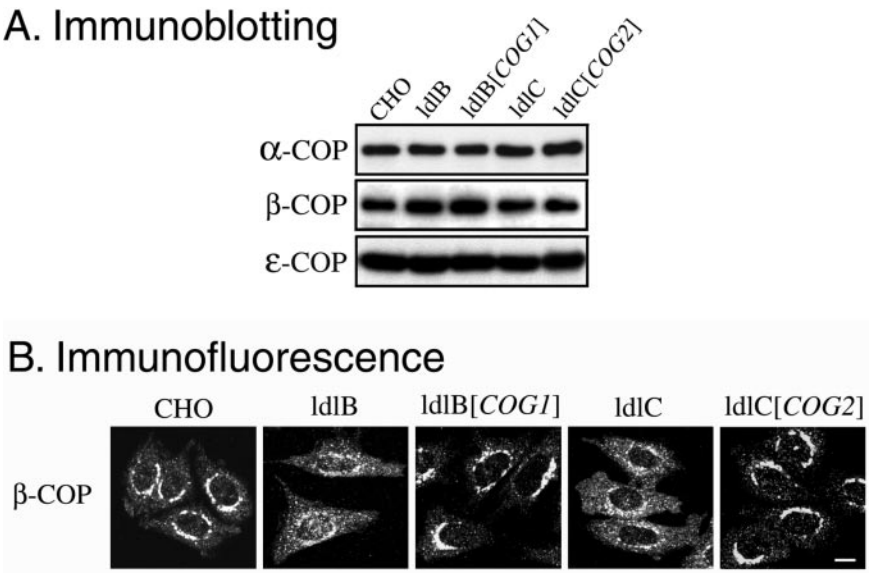


Figure 5. Immunoblotting and immunofluorescence localization of the α-, β-, and ε-COP subunits of COPI. (A) Lysates from the indicated cells were analyzed by immunoblotting with antibodies to α-, β-, or ε-COP. (B) The indicated cells were fixed and stained with an anti-β-COP antibody, and images were collected by confocal microscopy as described in Figure 1. Bar, 10 μm.

Golgi organization. They also suggest that ε-COP, presumably together with α and β′-COPs in a COPI subcomplex (Lowe and Kreis, 1995; Hoffman *et al*., 2003), is not required for the association of β-COP with the Golgi (Gomez *et al*., 2000).

Generation of Synthetic Phenotypic Defects by Simultaneously Disabling COG and COPI

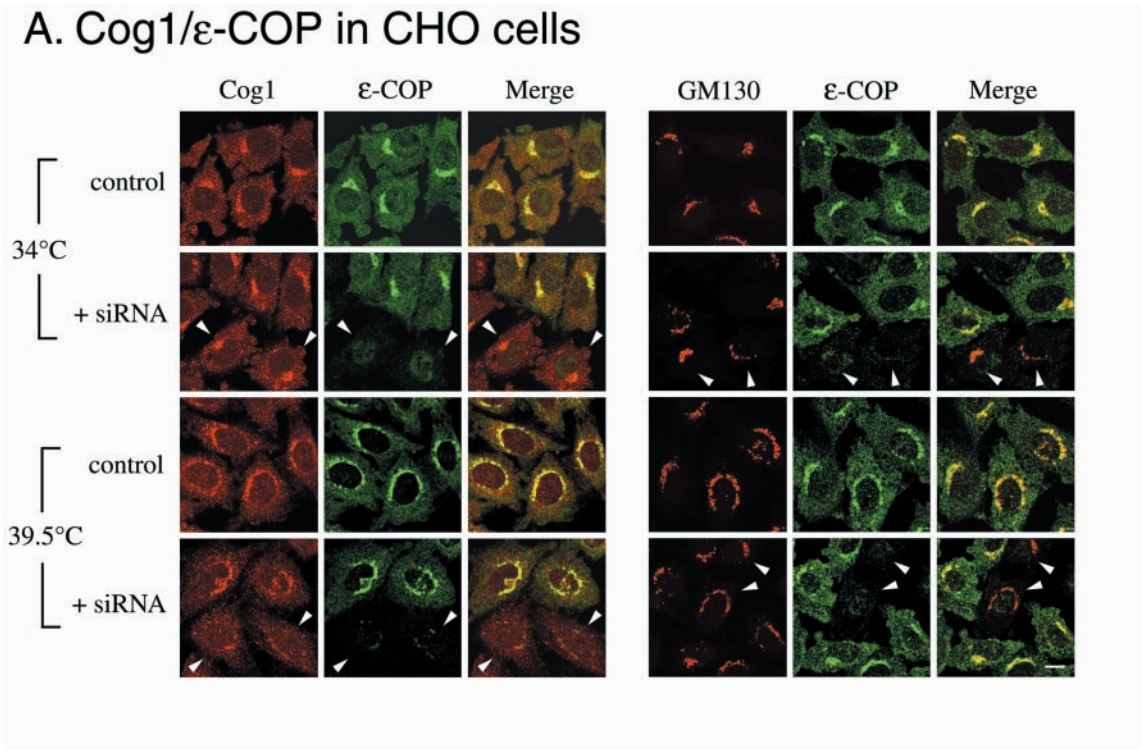
To explore the genetic interactions between COG and COPI in mammalian cells, we introduced the ε-COP siRNA into ldlB and ldlC mutants. Unlike the case with wild-type CHO or ldlB[*COG1*] cells, a substantial fraction of the mutants transfected with ε-COP siRNA exhibited abnormal morphologies when incubated at 34°C during the 2 d after transfection. Cell rounding and presumably cell death made it difficult to assess Golgi protein distributions in many of the transfectants. Immunofluorescence analysis of those ε-COP–depleted mutants that retained a reasonably well-spread morphology revealed various abnormalities in the distributions of Golgi markers, including an abnormally concentrated juxtanuclear distribution of both the COG-sensitive GOS-28 and the COG-insensitive GM130 (Figure 7). This suggested a substantial change in the structure (compaction) of at least portions of the Golgi under these conditions. We were unable to determine whether the abundances of these Golgi proteins were altered under these conditions. We also noted that in some of these cells there was a significant reduction in the reticular staining of GOS-28. This raises the possibility that ER accumulation of GOS-28 in the mutants might be dependent on the intracellular levels of ε-COP and that COPI function might contribute to the ER localization of GEARs in the COG-deficient mutants. However, the defects in the structure of the Golgi in these transiently transfected cells and the apparent toxicity of ε-COP depletion in COG-deficient mutants suggest that caution must be exercised in drawing conclusions about the role of COPI in the ER accumulation of GOS-28 in COG-deficient mutants. Nonetheless, the distinctive phenotypes of the ε-COP–depleted mutants were not observed in ε-COP-depleted ldlB[*COG1*] cells. The synthetic phenotypes (altered distribution of Golgi proteins at 34°C, apparent toxicity) generated by simultaneously disabling Cog1/Cog2 and ε-COP in CHO cells both suggest some redundancy in the functions of the COG and COPI complexes and are reminiscent of the synthetic lethality in yeast generated by combining specific mutations in COG and COPI (Kim *et al*., 2001; Ram *et al*., 2002; Suvorova *et al*., 2002).

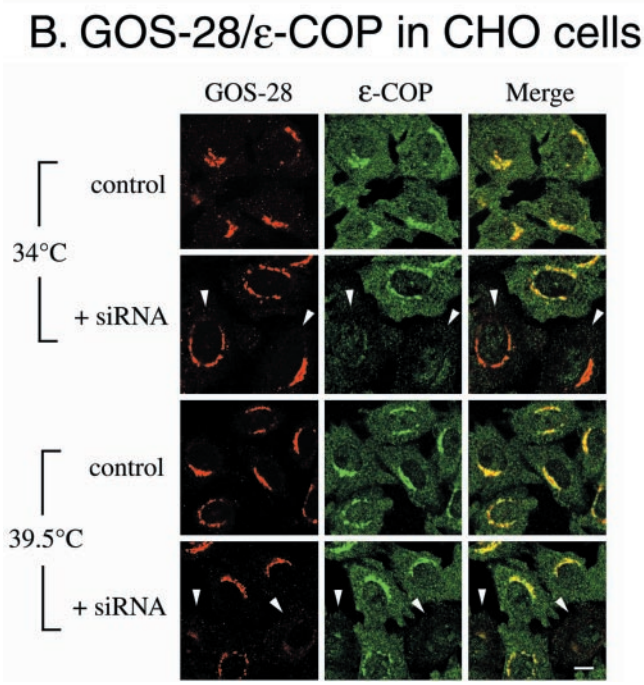
DISCUSSION

COG Controls the Abundance and Intracellular Distribution of a Distinct Set of Resident Golgi Proteins, the GEARs

The current study of the mammalian COG complex helps to further define the function of this peripheral Golgi-associated hetero-octameric protein complex. We have characterized the consequences of the loss of COG function on the steady-state levels and localization of a diverse set of 32 secretory pathway-related proteins that includes seven SNAREs, five rabs, seven golgins, and others. Seven of these exhibited abnormally low steady-state levels in the mammalian somatic cell mutants ldlB (Cog1 negative) and ldlC (Cog2 negative). We call these COG-sensitive proteins GEARs. The GEARs identified to date include two SNAREs (GOS-28 and GS15), three golgins (CASP, giantin, and golgin-84), a glycosidase (Man II), and GPP130, a phosphorylated glycoprotein of unknown function. These GEARs are all Golgi-resident type II transmembrane proteins that are normally virtually exclusively located in a perinuclear Golgi ribbon-like distribution. This is not the case for any of the COG-insensitive proteins examined in this study.

Although the structural features responsible for the COG sensitivity of the GEARs have not been defined, it is noteworthy that the three golgins among the GEARs (CASP, giantin, and golgin-84) all have highly conserved tyrosine and histidine residues within their transmembrane domains (Gillingham *et al*., 2002). These residues are functionally important in yeast CASP (Gillingham *et al*., 2002) and may contribute to their COG sensitivities. It seems likely that additional GEARs remain to be identified and that some of these will be involved in glycoconjugate processing (e.g., glycosidases, glycosyl transferases, and substrate transporters). Defects in their localization (Harris and Waters, 1996; Skrincosky *et al*., 1997; Axelsson *et al*., 2001; Martínez-Menarguez *et al*., 2001; Mironov *et al*., 2001; Opat *et al*., 2001;





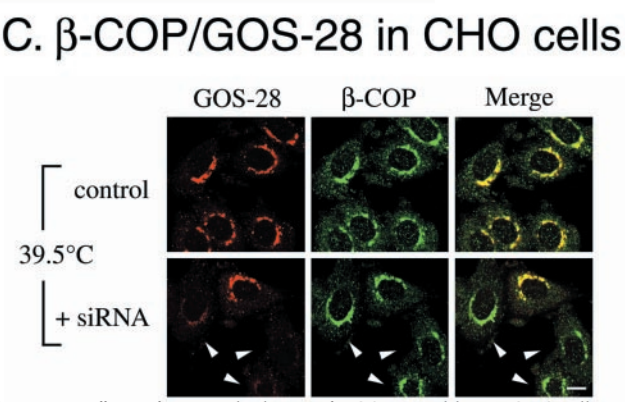


Figure 6. Effects of siRNA depletion of ε-COP in wild-type CHO cells on the immunofluorescence of Cog1, GOS-28, GM130, and β-COP. Wild-type CHO cells were transiently transfected without (control) or with (+siRNA) a 21-base pair siRNA duplex specific for hamster ε-COP. After a 46.5-h incubation at 34°C, the cells were further incubated for 90 min at either 34°C (where the loss of ε-COP had no apparent phenotype) or 39.5°C and then fixed and stained with antibodies to either ε-COP or β-COP (green) together with antibodies (red) to either Cog1, GM130, or GOS-28. Arrowheads indicate the cells in which there was a loss or major reduction in the signal intensity for either ε-COP (A and B) or GOS-28 (C), which indicated effective knockdown of ε-COP expression. Bars, 10 μm.

Berger, 2002; Puri *et al*., 2002; Roth, 2002; Zerfaoui *et al*., 2002) and steady-state levels could alter their activities and would provide an attractive explanation for the pleiotropic glycosylation defects observed in ldlB and ldlC cells (Kingsley *et al*., 1986).

We focused our detailed analysis of GEARs in the ldlB and ldlC mutants on the SNARE proteins GOS-28 and GS15, because they are not glycosylated. Thus, their COG sensitivities could not simply be consequences of changes in their structures due to the previously described glycoconjugate processing defects in these mutants. The steady-state levels of these SNAREs, as well as that of the GEAR Man II, were not only decreased in mutants with defective COG but also elevated in cells containing increased levels of COG. This suggests that COG controls the levels of at least some GEARs directly. In the mutants, but not in wild-type cells, GOS-28 and GS15 could be readily detected in the ER as well as the perinuclear Golgi ribbon, and they were abnormally rapidly degraded. This degradation was apparently a consequence of mislocalization/accumulation in the ER and directly coupled to dislocation/retrotranslocation from the ER and subsequent proteosomal degradation, because lacta-

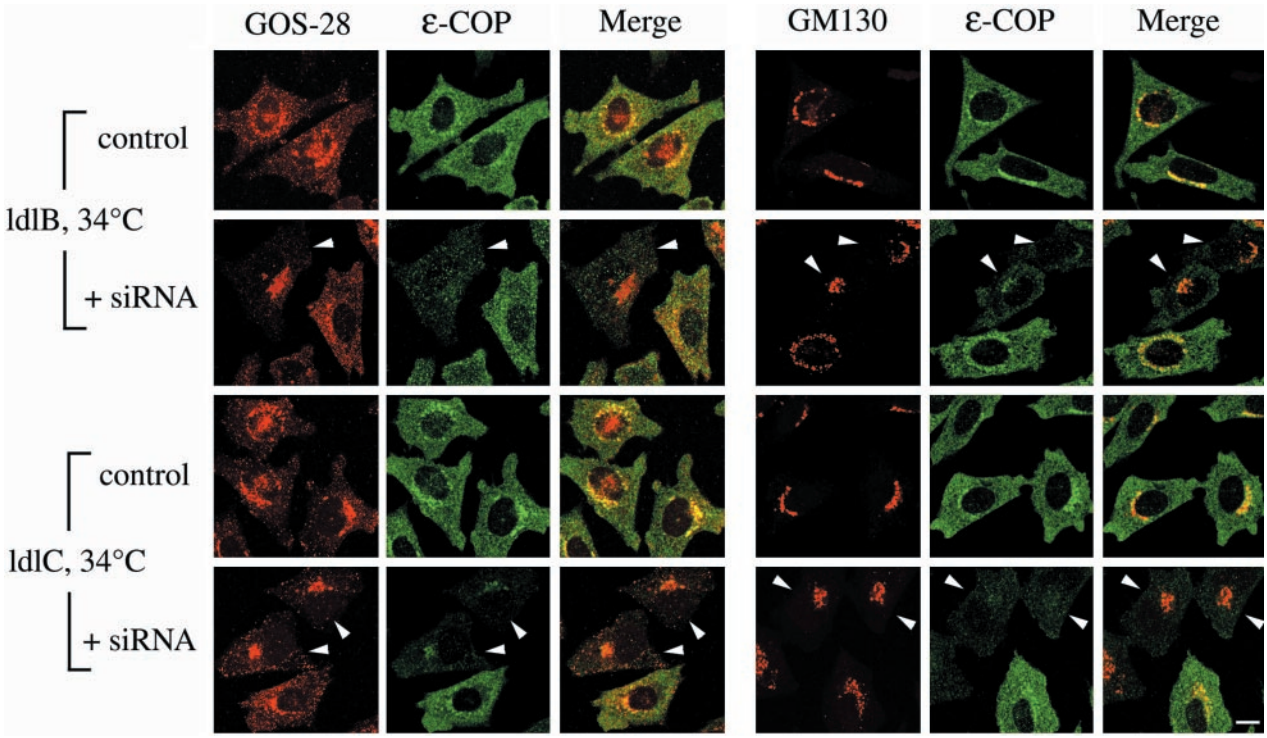


Figure 7. Effects of siRNA depletion of ϵ-COP in ldlB and ldlC cells on the immunofluorescence of GOS-28, GM130, and ϵ-COP. ldlB and ldlC mutants were transfected without (control) or with (+siRNA) ϵ-COP–specific siRNA. Cells were incubated at 34°C for 48 h and then fixed and stained with antibodies to either GOS-28 (red) or GM130 (red) and ϵ-COP (green). To facilitate visualization of the distribution of GOS-28 by using confocal microscopy, the images from ldlB and ldlC cells (weaker signals) were collected with relatively high signal gains. Arrowheads indicate cells in which there was a loss or reduction the signal intensity for ϵ-COP. Bar, 10 μm.

cystin inhibition of proteasomes blocked degradation and increased GOS-28's accumulation in the ER. A substantial amount of the lactacystin-induced accumulation of GOS-28 in the ER was independent of new protein synthesis. Thus, it is likely that transport from other compartments, most likely retrograde traffic from the Golgi, accounted for at least some of the lactacystin-induced accumulation of these GEARs in the ER.

These results suggest that COG might influence the distribution of these GEARs in several ways. First, COG might control intra-Golgi transport of the GEARs such that they are either directed by COG to their appropriate sites of residence or prevented by COG from inappropriate retrograde trafficking to the ER. A role for COG in directing GEAR transport to specific Golgi sites rather than blocking a transport step is supported by the observation that COG stimulates an in vitro intra-Golgi transport assay (Walter *et al.*, 1998). Second, COG might be required for efficient ER exit of some GEARs that are either newly synthesized (Morsomme and Riezman, 2002; Morsomme *et al.*, 2003) or constitutively recycling (Storrie *et al.*, 1998; Girod *et al.*, 1999; Zaal *et al.*, 1999; Miles *et al.*, 2001; Ward *et al.*, 2001; Stroud *et al.*, 2003). These processes involve Golgi-to-ER retrograde transport, which has been reported in numerous studies (Lippincott-Schwartz *et al.*, 1989; Letourneur *et al.*, 1994; Harris and Waters, 1996; Gaynor and Emr, 1997; Gaynor *et al.*, 1998; Glick and Malhotra, 1998; Pelham and Rothman, 2000; Todorow *et al.*, 2000; Opat *et al.*, 2001) of many normally Golgi-associated proteins, including the GEARs giantin and Man II (Storrie *et al.*, 1998; Girod *et al.*, 1999; Zaal *et al.*, 1999; Lippincott-Schwartz *et al.*, 2000; Seemann *et al.*, 2000; Storrie *et al.*, 2000; Miles *et al.*, 2001; Ward *et al.*, 2001; Shorter and Warren, 2002; Storrie and Nilsson 2002; Stroud *et al.*, 2003).

Relationship of COG with COPI and the Function of COG

COPI-coated transport vesicles have been proposed to play a key role in retrograde transport and the retrieval of Golgi resident proteins (Letourneur *et al.*, 1994; Gaynor and Emr, 1997; Gaynor *et al.*, 1998; Glick and Malhotra, 1998; Pelham and Rothman, 2000; Opat *et al.*, 2001; Duden, 2003). The differential effects on transport of blocking COPI activity by injection of anti-COPI antibodies have suggested that Golgi-to-ER retrograde transport can be either COPI dependent or COPI independent (Storrie *et al.*, 1998, 2000; Girod *et al.*, 1999; Storrie and Nilsson, 2002). A significant body of evidence supports the hypothesis that COG and COPI collaborate in controlling the correct localization and abundance of GEARs. In mammalian cells four GEARs (GOS-28, GS15, Man II, and giantin) can physically associate with COPI (Nagahama *et al.*, 1996; Sönnichsen *et al.*, 1998; Orci *et al.*, 2000; Martínez-Menarguez *et al.*, 2001; Xu *et al.*, 2002). Two other GEARs (GPP130 and golgin-84) are concentrated on vesicles and the lateral edges of the Golgi where COPI resides (Yuan *et al.*, 1987; Diao *et al.*, 2003; Satoh *et al.*, 2003). The kinetics of brefeldin A-induced dissociation from the Golgi of Cog2 and the COPI subunit β-COP are similar (Donaldson *et al.*, 1990; Podos *et al.*, 1994). In yeast, COG and COPI interact genetically and physically (Kim *et al.*, 2001; Ram *et al.*, 2002; Suvorova *et al.*, 2002).

Here, we report additional evidence for a functional relationship between mammalian COG and COPI. COPI was

partially dispersed throughout the cytoplasm in the ldlB and ldlC mutants, whereas COG's distribution was altered at 39.5°C in wild-type CHO cells in which ε-COP expression was suppressed by siRNA. Thus, the normal distribution of COPI was dependent on COG and that of COG was dependent on COPI. Furthermore, the GEARs were sensitive to alterations in COPI as well as being sensitive to mutations in COG. Disrupting intact COPI in wild-type CHO cells with ε-COP siRNA at 39.5°C altered the distributions of GEARs without affecting COG-insensitive proteins. Thus, although COG and COPI play distinct roles in membrane trafficking, they contribute, at least in part, to similar functions necessary for normal Golgi organization (e.g., establishing the proper distribution of GEARs). The changes in COG and GEARs induced by depleting cells of ε-COP were temperature sensitive (observed only after a temperature shift from 34 to 39.5°C). This is reminiscent of the previously reported temperature-sensitive growth of ε-COP null yeast mutants (Duden *et al*., 1998; Kimata *et al*., 1999). Finally, simultaneously disabling COG and COPI by introducing ε-COP siRNA into ldlB and ldlC mutants generated a synthetic phenotypic defect: disruption of normal Golgi morphology at 34°C. This indicates that COG and COPI may function in parallel to establish or maintain normal Golgi organization. In addition, the abnormal accumulation of GOS-28 in the ER of ldlB and ldlC mutants was in some cases reduced by ε-COP depletion. These findings raise the possibility that COPI vesicles might participate in the redistribution to the ER of at least one of the GEAR proteins when COG function is compromised. Despite the interactions between COG and COPI and their influence on the intracellular distribution of GEARs, it is possible that the retrograde movement of some GEARs to the ER in COG-deficient cells might be COPI independent, as in the systems studied by Girod *et al.* (1999). However, COPI-independent mechanisms may not play a major role in the ER accumulation of GEARs in COG-deficient cells.

The identification and characterization of GEARs and their relationships to COG and COPI provide additional insights into the function of COG. It is possible that COG influences the distributions and/or abundances of GEARs primarily by regulating their exit from the ER (Morsomme and Riezman, 2002; Morsomme *et al*., 2003). Thus, the ER accumulation of some GEARs in COG-deficient cells might be similar to the ER accumulation of several Golgi glycosylation proteins when ER exit is blocked by a dominant negative Sar1 protein (Aridor *et al*., 1995; Storrie *et al*., 1998; Girod *et al*., 1999; Seemann *et al*., 2000; Miles at el. 2001; Ward *et al*., 2001; Storrie and Nilsson 2002; Stroud *et al*., 2003). Another possibility is that COG influences the distributions and/or abundances of GEARs less directly by affecting the Golgi's structure and/or lumenal environment (Kingsley *et al*., 1986; Linstedt *et al*., 1997; Puri *et al*., 2002; Ungar *et al*., 2002). Because GEARs are normally exclusively Golgi localized, retrograde vesicular traffic (COPI dependent and/or independent) is probably required for their mislocalization in COG mutant cells. It seems likely that a major function of COG is to work in concert with COPI to control retrograde trafficking (retrieval), thereby ensuring the proper intra-Golgi distribution and abundance of the GEARs. Thus, in the absence of normal COG function in the mutants, some GEARs are abnormally transported by retrograde traffic from the Golgi to the ER. The effects of COG on retrograde trafficking and proteasomal degradation of some GEARs may provide insights into the broader issue of how the steady-state levels of Golgi-resident proteins are controlled. The GEAR/COG/COPI system provides new tools to study both this relatively unexplored area of membrane cell biology as well as other aspects of the structure and function of the Golgi apparatus.

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