Gos1p, a *Saccharomyces cerevisiae* SNARE protein involved in Golgi transport

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Abstract Specific transport between secretory compartments requires that vesicular carriers contain targeting proteins known as SNAREs. Ten v-SNAREs have been identified in the genome of the yeast *Saccharomyces cerevisiae* by sequence analysis. We report here the characterization of Gos1p, a v-SNARE localized to the Golgi compartment and likely homolog of the mammalian protein GOS-28/GS28. Gos1p is a type II membrane protein with characteristic SNARE sequence hallmarks and is functionally a SNARE protein. Gos1p was originally identified as a 28 kDa protein in an immunoprecipitate of the *cis*-Golgi t-SNARE Sed5p. This interaction between Sed5p and Gos1p is direct as demonstrated by in vitro binding with recombinant proteins. Deletion of *GOS1* results in viable haploids with modest growth and secretory defects. Close examination of the secretory phenotype of *GOS1*-disrupted cells suggests that Gos1p may play a role in multiple transport steps, specifically ER-Golgi and/or intra-Golgi transport.

Key words: Golgi transport; SNARE protein; Gos1p; *Saccharomyces cerevisiae*

1. Introduction

Sequestration of enzymatic activities into discrete compartments is a hallmark of the eukaryotic cell. The secretory pathway accomplishes this task by the vectorial transport of vesicular carriers from the point of entry into the secretory pathway, the endoplasmic reticulum, through the Golgi stacks, to various final destinations in the cell such as the plasma membrane. A picture of the molecular events involved in vesicular transport has emerged over the last few years [1–4]. Key amongst recent observations was the identification of a protein family responsible for targeted fusion of transport vesicles [5–9]. The original members of this increasing large family were identified as the membrane proteins responsible for recruiting the general transport factors *N*-ethylmaleimide sensitive factor (NSF) and soluble NSF attachment protein (SNAP) to their site of action, the membrane [5]. Based on these interactions, the protein family was termed SNAP receptors (SNAREs). Two classes of SNAREs, v- and t-SNAREs, have been identified, whose individual members are localized to distinct intracellular compartments. Cognate v- and t-SNAREs on opposing membranes pair a transport vesicle with its target membrane for fusion [10]. Recent experiments reconstituting SNAREs into liposomes have provided evidence that SNAREs themselves are sufficient to mediate lipid bilayer fusion [11].

The completed genome of *Saccharomyces cerevisiae* has so far revealed a total of 18 SNARE proteins readily identifiable by sequence analysis [12–14]. Seven of the 10 known v-SNAREs interact with the t-SNARE Sed5p in one fashion or another [12,15–18]. Immunoprecipitation of Sed5p under conditions of Sec18p inactivation resulted in the co-precipitation of six of these seven [19]. We report here the characterization of Gos1p, the final member of this complex. Gos1p directly interacts with Sed5p, is localized primarily to the Golgi complex, and likely functions in endoplasmic reticulum (ER)-Golgi and/or intra-Golgi transport.

2. Materials and methods

2.1. Strains and growth conditions

Yeast strains were routinely maintained in rich medium (YPD; 1% yeast extract, 2% peptone, 2% dextrose) or synthetic complete medium for plasmid maintenance (SCD; 0.67% yeast nitrogen base (Difco), 2% dextrose) with the appropriate nutritional supplements for auxotrophic requirements (Buffer Rad). Expression from the GAL1-10 promoter was induced by the addition of 2% galactose. The yeast strains W3031A, W3031B, RSY271 [20], MSY42 (MATa/α *ade2-1/ade2-1 leu2-3,112/leu2-3,112 ura3-1/ura3-1 trp1-1/trp1-1 his3-11,15/his3-11,15 can1-100/can1-100*, *GOS1::HIS3/GOS1*), MSY62 (MATα *ade2-1 leu2-3,112 ura3-1 trp1-1 his3-11,15 can1-100*, *GOS1::HIS3*), and JCY1 (MATα *ade2-1 leu2-3,112 ura3-1 trp1-1 his3-11,15 can1-100*, *GOS1::URA3*) were used in this study.

2.2. Plasmids and DNA methods

Standard DNA manipulations [21] were performed in *Escherichia coli* strain DH5α unless otherwise noted. Oligonucleotide sequences used in this report are available upon request. The bacterial expression plasmids pMS141 (Gos1pΔC-His_6), pJM22 (GST-Sed5p), pJM212 (GST-Gos1p), and pGEX-SEC17 (GST-Sec17p) were made by amplifying the respective coding regions by PCR and ligating into the pET3a (Novagen) based vector pMS/His [19] (pMS141), or pGEX-2T. All are full length with the exception of pMS141 which lacks the transmembrane domain and encodes amino acids 1–202 of Gos1p. The mammalian cell expression vector pJM192 (myc-Gos1p) was constructed in pcDNA3 (Invitrogen).

2.3. GOS1 disruption

Standard genetic procedures were performed according to Guthrie and Fink [22]. The *GOS1* locus was disrupted with the *HIS3* gene in the diploid strain W303. PCR fragments containing 5′ and 3′ noncoding regions of GOS1 interrupted with the HIS3 gene were assembled in pBluescript II^+ (Stratagene) to generate a disruption construct (pMS196). The diploid strain W303 was transformed with a linear fragment from pMS196 generating MSY42. Integration at the *GOS1* locus was confirmed by Southern blotting. MSY62 is an α-spore derived from MSY42.

The *GOS1* gene was independently disrupted by the *URA3* gene. A fragment containing the *GOS1* ORF was amplified by PCR and li-

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gated into pUC19 (New England Biolabs) generating pUCGOS1. A fragment containing 133 bp upstream of the *GOS1* ATG start site through 60 bp from the translational stop site was removed from pUCGOS1 and replaced with a 1 kb *URA3* DNA fragment from YDp-U [23] (pUCGOS1::URA3). A linear fragment from pUCGOS1::URA3 was used to transform diploid W303. Uracil prototrophs were isolated and integration at the *GOS1* locus was confirmed by Southern blotting.

2.4. Sequence analysis

The multiple sequence alignment was performed as described [20]. The sequences are *S. cerevisiae* chromosome VIII ORF YHL031c, *Schizosaccharomyces pombe* ORF C4G8.10 (Z56276), *Arabidopsis thaliana* ORF (AC002387), *Cricetulus griseus* GOS-28 [24], *Rattus norvegicus* GS28 [25], and amino acids 1289–1521 of the predicted ORF F08F8.4 (U28991) from *Caenorhabditis elegans* chromosome III. This ORF is predicted to encode a 1741 amino acid protein translated from 28 exons. The region of similarity to Gos1p is encoded by exons 19–24. If exon 24 is translated past the proposed boundary by 35 bp, an additional 11 amino acids are created before an in frame stop codon is encountered. It is likely that this conceptual ORF combines the *GOS1* gene with another gene upstream.

2.5. Expression and purification of recombinant proteins

Recombinant, His_6-tagged Gos1p was purified from inclusion bodies in *E. coli* (BL21(DE3)) expressing pMS141 using nickel-NTA agarose according to the manufacturer's instructions. Full-length proteins (GST-Sed5p, GST-Gos1p and GST-Sec17p) were prepared using glutathione agarose beads. DH5α cells expressing the appropriate plasmid were grown at 37°C to an OD_{600} of ~0.8, then induced by the addition of IPTG to 1 mM for an additional 4 h. The induced cells were harvested by centrifugation and resuspended in breaking buffer A (25 mM HEPES-KOH pH 7.4, 300 mM KCl, 1 mM DTT, and protease inhibitors) followed by the addition of Triton X-100 to 4% (w/v). GST-Sec17p expressing cells were lysed in the absence of detergent. The cells were disrupted in a French press and the extract cleared by centrifugation.

The GST-Gos1p fusion protein was further purified and cleaved with thrombin. A 1.0 ml reaction containing 100 μl of GST-Gos1p extract, 60 μl of a 50% slurry of glutathione beads and 840 μl 'GST binding buffer' (25 mM HEPES-KOH pH 7.4, 100 mM KCl, 1 mM DTT, 0.5% (w/v) Triton X-100) was incubated at 4°C for 30 min on a rotating wheel. The beads were washed, resuspended in 100 μl of GST-binding buffer, and 10 units of thrombin (Sigma) was added for 1 h at room temperature. Thrombin was removed by benzamidine beads (Pharmacia) according to the manufacturer's instructions.

2.6. Analysis of CPY transport: CPY pulse/chase and steady-state immunoprecipitation

CPY immunoprecipitation was performed as described [26] with slight modifications. Lysed cells were preincubated with 50 μl of preclean agarose (Sigma) for 30 min at 4°C in Tween 20-IP/BSA buffer (50 mM Tris-HCl; pH 7.5, 0.1 mM EDTA, 150 mM NaCl, 0.5% Tween 20, 10 mg/ml of BSA). The beads were removed and the supernatant was incubated with affinity-purified anti-CPY antibodies (6 μg) overnight at 4°C with gentle shaking. Protein A-sepharose CL-4B (7 mg) was later added to the suspension and incubated for a additional 2 h at 4°C. The beads were collected by centrifugation, washed twice in Tween 20-urea buffer (100 mM Tris-HCl; pH 7.5, 200 mM NaCl, 2 M urea and 0.5% Tween 20), once in Tween 20-IP buffer and once in TBS. 50 μl of gel loading buffer (2×) was added, heated at 100°C for 3 min and the supernatant was loaded onto a 7.5% SDS-PAGE gel.

Steady-state CPY immunoprecipitations were performed essentially as described [27]. RSY271 (two cultures) or MSY62 were inoculated to 0.5 OD_{600}/ml and grown at 25°C and 30°C respectively for 5 h. Then one of the RSY271 (*sec18-1*) cultures was shifted to 37°C and all cultures continued to grow for an additional 1 h. 20 OD_{600} units were harvested by centrifugation, resuspended in 300 μl of 1% SDS and lysed in the presence of 0.3 g of acid-washed glass beads by two 45 s bursts on a vortexer. The extract was heated at 80°C for 5 min followed by the addition of 700 μl of IP buffer (10 mM Tris-HCl pH 7.4, 2 mM EDTA, 0.1% Triton X-100). The extract was cleared by centrifugation and CPY was immunoprecipitated with polyclonal CPY antiserum. Immune complexes were recovered with protein G agarose and were washed with IP buffer containing 0.1% SDS. The beads were finally resuspended in 50 μl of sample buffer. Twenty percent of each precipitate was loaded onto a 7.5% SDS-PAGE gel.

2.7. Immunofluorescence of Gos1p in HeLa cells

HeLa cells were grown on coverslips to 40% confluence and transfected with 1.5 μg *GOS1* DNA using SuperFect reagent (Qiagen) according to the manufacturer's instructions. In preparation for microscopy, the cells were washed twice with PBS and fixed with 4% paraformaldehyde for 15 min, then permeabilized with 0.1% Triton X-100 in PBS for 5 min, washed twice with PBS to remove detergent, and blocked with 0.2% gelatin (Bio-Rad) in PBS for 1 h. The monoclonal antibody 9E10 [28], directed against the *myc* tag of Gos1p, was diluted 1:1000 in gelatin/PBS (~5 μg/ml IgG). Affinity-purified rabbit polyclonal antibody to GOS-28 (0.2 mg/ml) [24] was diluted 1:2000 in gelatin/PBS. The permeabilized cells were incubated with both antibodies for 1 h, washed with gelatin/PBS and incubated with secondary antibodies (Texas red-X goat anti-rabbit IgG conjugate and Oregon Green 488 goat anti-mouse IgG conjugate, Molecular Probes), diluted 1:500 in gelatin/PBS, for 30 min. The coverslips were then washed and mounted with Gelvatol [29]. Cells were analyzed on a Zeiss Axioplan microscope equipped with a 63×/1.3 NA oil-immersion objective and fluorescein and Texas red filter sets.

2.8. In vitro binding reactions

GST-Sed5p was bound to beads in a reaction containing 100 μl GST-Sed5p bacterial extract (~50 μg GST-Sed5p), 200 μl of a 50% slurry of glutathione beads and 700 μl GST binding buffer. A control reaction contained 50 μl GST bacterial extract (~50 μg GST). The reactions were incubated at 4°C for 30 min on a rotating wheel, then pelleted by centrifugation. The beads were washed then resuspended in GST binding buffer for a final volume including the beads of 1 ml. The GST-Sed5p bound beads were aliquoted in 50 μl aliquots (~2.5 μg GST-Sed5p) and used in a binding reaction. Cleaved Gos1p was added to individual GST-Sed5p aliquots in increasing amount and GST binding buffer containing 0.5 mg/ml soybean trypsin inhibitor, 2 mM 4-(2-aminoethyl)benzenesulfonylfluoride] (AEBSF, Calbiochem), and 10 mM benzamidine was added to a final volume of 100 μl. The final concentrations of the reactants were ~350 nM GST-Sed5p, and 0 nM, 65 nM, 200 nM, 650 nM, 2 μM, and 6 μM Gos1p respectively. The samples were incubated at 25°C for 1 h, then the beads were washed with GST binding buffer and the bound proteins were eluted in 2×SDS sample buffer.

SNARE proteins in the salt-washed detergent extract were isolated by binding to GST-Sec17p beads. GST-Sec17p was bound to beads as described above, washed with GST binding buffer containing 250 mM KCl and aliquoted (~2.5 μg per reaction). GST-Sec17p beads or control glutathione beads without GST-Sec17p were incubated with ~350 μg of salt-washed detergent extract in a final reaction volume of 1.0 ml for 1 h at 4°C. The beads were washed and the bound proteins were eluted in 2×SDS sample buffer and resolved by SDS-PAGE.

3. Results

3.1. Gos1p is a yeast homolog of the SNARE protein GOS-28/GS28

Inactivation of Sec18p (yeast NSF) results in the accumulation of a distinct set of complexes containing the Golgi t-SNARE Sed5p [19]. Components of these complexes are the characterized ER-Golgi v-SNAREs Sec22p, Bet1p [30–32], Bos1p [33,34], and Ykt6p [20]. Additional proteins identified in this complex with sequence properties characteristic of v-SNAREs were proteins of 14 (p14) and 28 (p28) kDa. p14 was independently identified as Sft1p (suppressor of sed5 ts) by Banfield and colleagues [17] as a high copy number suppressor of a temperature sensitive allele of Sed5p. We recently reported the identity of the final component of this complex, p28, as the 25 kDa open reading frame YHL031c which we proposed be named Gos1p (Golgi SNARE) [20].

Analysis of the Gos1p primary sequence indicates a modest

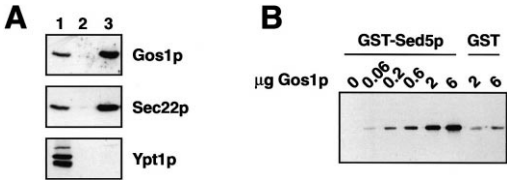

Fig. 1. Gos1p binds to GST-Sed5p and GST-Sec17p in vitro. A: Gos1p, present in a detergent extract, binds to GST-Sec17p. Lane 1: Approximately 3.5 μg of total yeast membrane extract. Glutathione agarose beads with (lane 3) or without (lane 2) ~2.5 μg GST-Sec17p were incubated with ~350 μg total yeast membrane extract, washed and eluted with sample buffer. Lanes 2 and 3 represent 20% of the total reaction volume. The samples were resolved by SDS-PAGE and immunoblotted with antibodies to Gos1p (1:2000), Sec22p (1:1000), Ypt1p (1:1000). B: Gos1p binds to Sed5p. Approximately 2.5 μg of GST-Sed5p was bound to glutathione agarose as described in Section 2. The indicated amounts of Gos1p were added to the GST-Sed5p beads and the amount of Gos1p bound was determined. The samples were resolved by SDS-PAGE, transferred to nitrocellulose and immunoblotted with an anti-Gos1p antibody.

similarity to the mammalian Golgi SNARE GOS-28/GS28. The overall sequence identity of Gos1p with GOS-28 is 27.6% and is spread throughout the entire sequence. A BLAST search using GOS-28 as the query sequence against the translated ORFs of the complete *S. cerevisiae* genome identified Gos1p as the only statistically significant match ($P < 6.7 \times 10^{-18}$). This observation suggests that Gos1p is the yeast homolog of GOS-28/GS28. Additional BLAST searches with Gos1p as the query sequence reveal potential homologs in fission yeast (*S. pombe*, 33% identity), plants (*A. thaliana*, 27% identity), and the nematode (*C. elegans*, 27% identity).

In addition to sequence similarity to GOS-28, Gos1p also contains predicted secondary structures characteristic of SNARE proteins. Coiled-coil structures are often found in SNARE proteins and Gos1p contains a region (amino acids 135–160) with a 60% propensity to from a coiled coil as predicted by the Lupas algorithm [35]. Hydropathy analysis by the method of Kyte and Doolittle [36] indicates that Gos1p has a carboxy-terminal membrane anchor characteristic of most synaptobrevin and syntaxin-like SNARE proteins, with the exception of Ykt6p and its homologs. Indeed, incubation of cellular membranes with 1 M NaCl, or 0.1 M Na_3CO_2, pH 11, demonstrates that Gos1p is tightly associated with membranes. Only the treatment that solubilizes membranes (1% Triton X-100) releases Gos1p from the membrane showing that it is has properties of an integral membrane protein (data not shown).

SNARE proteins interact with SNAP, either alone or when complexed to its cognate partner(s) in the case of v-SNAREs. Gos1p, likely complexed to a t-SNARE in the detergent extract, does bind to the yeast equivalent of α-SNAP, Sec17p (Fig. 1A). GST-Sec17p was bound to glutathione agarose and incubated with a salt-washed Triton X-100 extract of total yeast membranes. Fig. 1A shows that Gos1p is present in the detergent extract (lane 1) and is able to bind to the beads only when GST-Sec17p is present (lane 3 vs. lane 2). Sec22p, a well characterized v-SNARE, behaves in an identical manner. Conversely, the rab protein Ypt1p is found in the extract but does not bind to the beads with or without GST-Sec17p.

3.2. Gos1p associates directly with Sed5p

Since Gos1p was found in an immunoprecipitate with Sed5p, we wanted to determine if this interaction was the result of a direct association of Sed5p and Gos1p. To answer this question, we performed pairwise binding assays with recombinant proteins. Both full-length Sed5p and full-length Gos1p were expressed as glutathione *S*-transferase (GST) fusion proteins in *E. coli*. Both proteins were soluble, highly expressed and could be released from GST by thrombin cleavage and were therefore available as both fusion proteins and the free proteins. Full-length Gos1p binds, in a saturable manner, to GST-Sed5p (Fig. 1B). The reciprocal interaction is also possible (data not shown). The interactions are specific since neither Gos1p nor Sed5p binds appreciably to GST alone. These experiments demonstrate that Gos1p and Sed5p are capable of forming a direct association which is consistent with their co-immunoprecipitation with the Sed5p antibody.

3.3. Disruption of the GOS1 locus has minor effects on cell growth

In an effort to define the function of Gos1p, the *GOS1* gene was disrupted in the W303 diploid (*HIS3* gene in MSY42 and *URA3* in JCY1, Fig. 2). Sporulation and analysis of the resulting tetrads revealed that *GOS1* was not an essential gene. All four haploid progeny were viable at 25°C (Fig. 2B). Interestingly, when spores were germinated at 37°C, the disrupted spores failed to germinate (Fig. 2B, 37°C). This may indicate that Gos1p serves an essential function in germination, but is dispensable for vegetative growth. Haploids germinated at 25°C were capable of near wild-type growth at

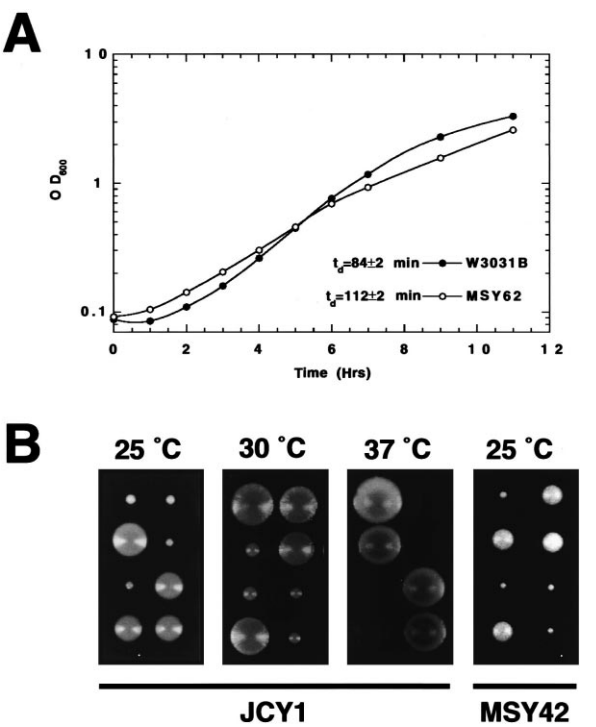

Fig. 2. Cells lacking the *GOS1* gene are viable. A: Growth of wild-type (W3031B) or *GOS1*Δ strain (MSY62) at 30°C in rich medium. Both strains were inoculated at ~0.1 OD_{600}/ml and sample taken at 1 h intervals. Each point represents three independent measurements. B: Tetrad analysis. The indicated diploid was sporulated and tetrads dissected. The resulting spores were germinated and grown at the indicated temperature.

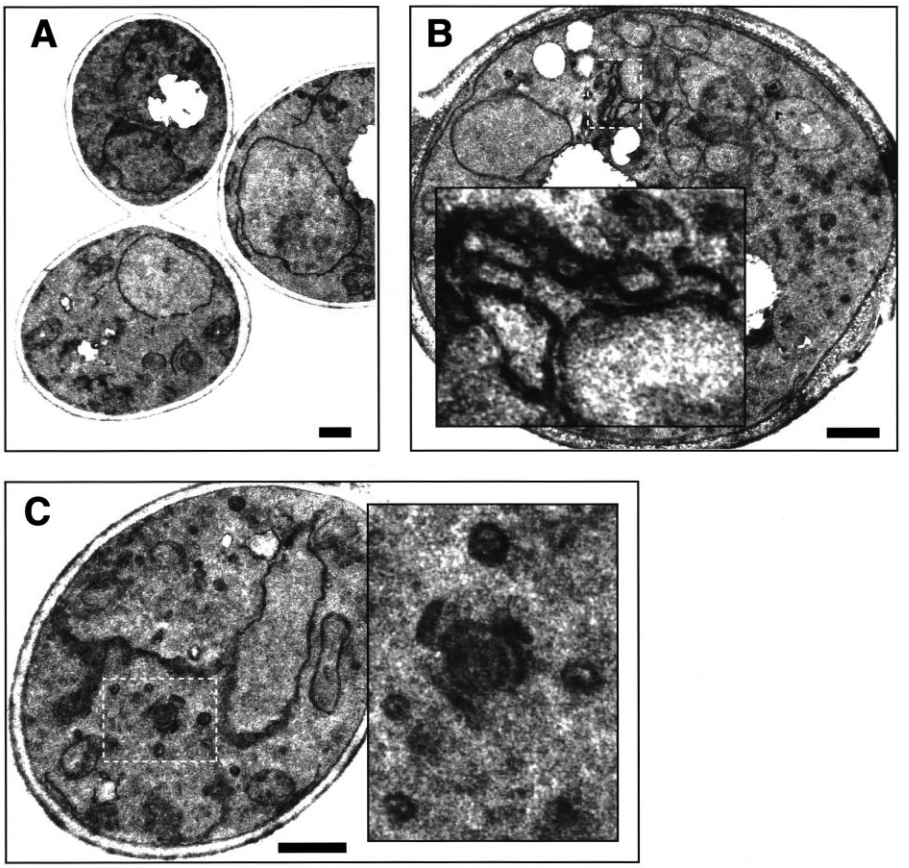

Fig. 3. Morphological analysis of *GOS1*Δ disrupted cells by electron microscopy. A: Wild-type cells (W3031B) were grown to OD_{600} ~1.0, stained with potassium permanganate and processed as previously described [20]. B and C: *GOS1*Δ cells (MSY62) were processed and in A. The area of the cell depicted in the insets of B and C are indicated by the dashed box. The bar represents 500 nm.

25°C in all media tested including raffinose, galactose, glucose, ethanol, glycerol, acetate, and glucose at 37°C (data not shown). A slight growth reduction of approximately 25% was observed in rich glucose medium at 25°C (doubling time of 84 min vs. 112 min in YPD, Fig. 2A).

3.4. Electron microscopic analysis of the GOS1 disruption

Consistent with the absence of a strong growth defect, many of the *GOS1*-disrupted cells appear morphologically normal; however, 10–20% of cells possessed a variety of aberrant structures (Fig. 3). This heterogeneity could depend on the visible section within a cell or might be inherent to distinct cell cycle stages or growth phases. Defects ranged from fragmentation of the vacuole, a common occurrence in secretory defects, to substantial accumulation of membranes in some cells (Fig. 3B, inset). These exaggerated membranes are similar in appearance to the ER membranes that accumulate in mutants that are defective in ER-Golgi transport [20,37]. Additionally, the darkly stained vesicular membranes seen in Fig. 3C (inset) are strikingly similar to those seen in the temperature sensitive mutant of *SFT1* [17]. Such structures have been postulated to be abnormal Golgi remnants.

3.5. Gos1p may be involved in both ER-Golgi and intra-Golgi transport

While growth appears relatively unaffected by disruption of the *GOS1* gene, subtle effects on secretory function may still be occurring. To assess secretory function, the export of two well characterized secretory markers, carboxypeptidase Y (CPY, product of the *PRC1* gene) and invertase (Suc2p), was analyzed. The externalization of invertase activity was compared in the wild-type strain W3031B and the isogenic *GOS1* disrupted strain MSY62. The absence of Gos1p had little effect on the appearance of external invertase activity (data not shown).

While the overall secretion of invertase was roughly normal, analysis of another well characterized secretory marker, the soluble vacuolar hydrolase CPY, produced a different result. CPY is translocated into the ER where it receives core oligosaccharide additions yielding the p1 form. These sugars are further processed along the secretory pathway [38]. Extension of the core sugars by the addition of α1,3-mannose residues in the *medial*-Golgi results in the p2CPY precursor form [39–41]. Finally, p2CPY is proteolytically processed in the vacuole resulting in the mature form of the enzyme. A pulse-chase examination of CPY processing revealed that the overall transit rate was slowed in the absence of Gos1p (Fig. 4A). The most apparent kinetic effect observed in the pulse-chase analysis is an increased lifetime of the p1CPY precursor. This effect indicates a reduction in the rate of either ER to Golgi transport or *cis* to *medial* intra-Golgi transport, or both. The reduction in the amount of mature CPY and the loss in total signal is readily explained by the secretion of the p2CPY intermediate (Fig. 4C, see below).

To test if the *GOS1* deletion also affects later transport steps we analyzed CPY at steady-state. Cells were lysed in the presence of SDS and all processing intermediates of CPY were immunoprecipitated with a polyclonal CPY antibody. *GOS1*-disrupted cells show a distinct steady-state accumulation of the p2CPY intermediate, with little p1CPY accumulation (Fig. 4B, lane 3). Such a p2CPY accumulation would not be expected, if the absence of Gos1p affects only early transport steps. For example, the *sec18-1* strain, which has an extremely rapid onset after shift to the restrictive temperature [39], accumulates p1CPY, and has no or little effect on p2CPY. At the permissive temperature (Fig. 4B, lane 1), the *sec18-1* strain shows little accumulation of p1CPY similar to wild-type controls (not shown). In contrast, the p2CPY accumulation in the *GOS1*-disrupted strain MSY62 indicates a role of Gos1p in later transport steps.

The strongest conclusions that can be drawn from these data are that Gos1p has a clear role in the early secretory pathway up to the *medial*-Golgi where the conversion of p1CPY to p2CPY occurs. Gos1p may also function in later intra-Golgi transport steps; however, it cannot be excluded that these effects might be a secondary consequence of the earlier defects.

3.6. Gos1p co-localizes with GOS-28 in HeLa cells

The absence of Gos1p results in minor processing defects to and within the Golgi apparatus. This information would suggest the function of Gos1p was also within the Golgi. As mentioned previously, Gos1p has a significant degree of homology with the mammalian intra-Golgi SNARE GOS-28/GS28, which also suggests an intra-Golgi function for Gos1p. Since the yeast Golgi is ill-defined morphologically, we chose to examine the localization of Gos1p in a mammalian cell system. To this end, *myc*-tagged Gos1p was transiently expressed in wild-type HeLa cells and its localization relative to endogenous GOS-28/GS28 investigated. Fig. 5A shows the previously described Golgi staining pattern of GOS-28/GS28 [24,25,42]. When the localization of Gos1p is determined in the same cells with the anti-*myc* antibody, a clear co-localization is revealed (Fig. 5B). A Golgi staining pattern is also observed when the distribution of Gos1p is followed with a polyclonal Gos1p antibody (data not shown). These results confirm that Gos1p is indeed localized identically to GOS-28/GS28 in the Golgi.

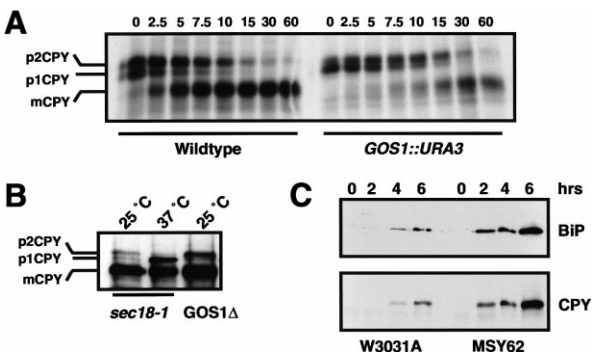

Fig. 4. Intracellular processing of carboxypeptidase Y in wild-type and *GOS1*Δ cells. A: JCY1 cells were pulse labeled with [^{35}S]methionine for 10 min and chased for the indicated times at 25°C. The cells were lysed, CPY immunoprecipitated, and proteins resolved by SDS-PAGE. B: CPY was immunoprecipitated from the *sec18-1* strain (RSY271) and the *GOS1*Δ strain (MSY62) as described in Section 2. The immunoprecipitates were resolved by SDS-PAGE on a 7.5% gel and immunobotted with a monoclonal CPY antibody (10A5-B5 1:1000, Molecular Probes). C: Wild-type cells (W3031A) or *GOS1*Δ cells (MSY62) were back diluted into fresh YPD medium to an OD_{600} of 0.1. The cells were grown at 30°C and 1 ml aliquots removed at the indicated time intervals. The cells were separated from the medium by centrifugation and proteins in the medium were precipitated by the addition of 10% TCA. The medium proteins were resolved by SDS-PAGE and immunoblotted with a polyclonal antiserum to Kar2p (BiP 1:1000) or the monoclonal CPY antibody (10A5-B5 1:1000).

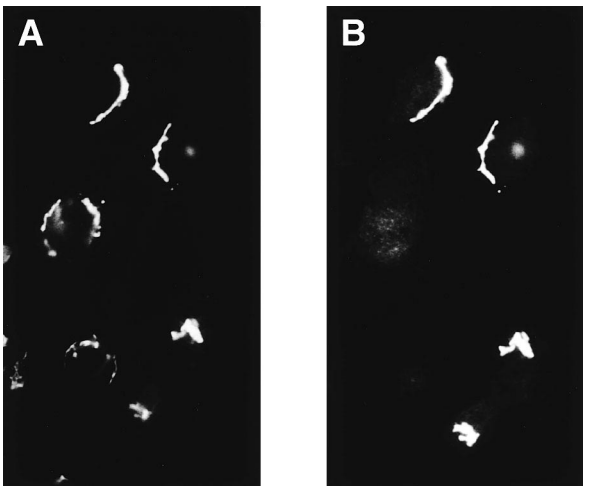

Fig. 5. Immunofluorescence of HeLa cells expressing yeast Gos1p. A: Transfected HeLa cells expressing mycGos1p immunodecorated with GOS-28 antisera [24]. B: Gos1p co-localized in the same field of cell with the 9E10 monoclonal anti-*myc* antibody.

3.7. Cells lacking Gos1p abnormally secrete BiP and CPY

Gos1p localizes to the Golgi apparatus in HeLa cells and manifests a minor defect in the late Golgi-localized processing of CPY, manifested as a modest accumulation of the p2CPY precursor. However, Gos1p was originally identified as an interacting partner of the *cis*-Golgi t-SNARE Sed5p, and has potential defects in ER-Golgi transport as well. One potential explanation of these two initially conflicting observations is that Gos1p is involved in directing anterograde vesicle to the Golgi apparatus from the ER and also participates in directing retrograde transport vesicle from a later Golgi compartment back to the CGN/*cis*-Golgi where Sed5p receives it. This latter role is similar to the function described for Sft1p, which was also found in the Sed5p immunoprecipitate. A temperature sensitive mutation in Sft1p also manifests defects in later Golgi enzyme processing. Hypersecretion of ER-resident proteins such as BiP (Kar2p) has been suggested to be an indirect measure of a defect in retrograde directed vesicles (ER retention defective, *erd* phenotype) [43]. *sft1-1* has an *erd* phenotype as does an allele of Sec22p (*sec22-3*), a v-SNARE known to be involved in ER-Golgi transport [17]. Recently, a more direct demonstration of Sec22p function in retrograde transport from the Golgi back to the ER was revealed as Sec22p can be immunoprecipitated with the ER-localized t-SNARE Ufe1p [44]. Since Gos1p is poised to be a retrograde v-SNARE similar to Sft1p, we determined if the *GOS1* deletion also showed an *erd* phenotype. Wild-type cells and Δ*GOS1* cells were back diluted into fresh medium and

followed over time. Proteins secreted into the medium were precipitated with TCA and resolved by SDS-PAGE. Fig. 4C shows that *GOS1*Δ cells secreted roughly five-fold more BiP than wild-type cells, confirming an *erd* phenotype. These samples were also assayed for secretion of CPY. Indeed the p2CPY intermediate was secreted well above wild-type levels.

4. Discussion

This work describes the characterization of the *S. cerevisiae* SNARE Gos1p. Gos1p is an integral membrane protein with a C-terminal membrane anchor, and a region with a strong propensity to form coiled-coils. Gos1p interacts directly and specifically with the Golgi t-SNARE Sed5p, and when complexed with Sed5p, binds Sec17p, the yeast SNAP homolog (Fig. 1).

Sporulation of tetrads carrying a single disruption of the *GOS1* locus produce viable progeny at 25°C. Interestingly, when spores were grown at 37°C, the disrupted spores fail to germinate (Fig. 2). Haploid *GOS1* disruptants germinated at 25°C displayed a modest growth reduction, manifested as ∼25% increase in generation time, abnormal membranous structure and vacuolar fragmentation (Fig. 3). While the absence of Gos1p marginally slowed cell growth, overexpression of Gos1p 10–20-fold, under the control of the strong galactose promoter, has no detectable effect on growth (data not shown). Close examination of the *GOS1*Δ cells show clear secretory defects. The processing of carboxypeptidase Y is slowed such that both the p1CPY and p2CPY intermediates accumulate. Additionally, high levels of the ER resident protein BiP are secreted, suggestive of an ER-retention defective (*erd*) phenotype (Fig. 4).

Gos1p likely participates in more than one transport step. Gos1p action in ER to Golgi transport or *cis* to *medial* intra-Golgi transport, or both, is indicated by the slowed kinetics of p1CPY processing. The increased secretion of BiP could also suggest a defect in retrograde transport from or within the Golgi. Evidence of a later role in the secretory pathway derives from a slight steady-state accumulation of p2CPY precursors and the secretion of p2CPY. However, while kinetic analysis does not discriminate between these two transport steps, the clear localization of Gos1p to the Golgi suggests that its role as a SNARE may be limited to transport within this organelle. Defects in later transport steps may be a consequence of an additional site of Gos1p action; however, secondary effects cannot be excluded. The secretion of p2CPY is somewhat puzzling but may be explained by a reduced steady state or a partial mislocalization of the CPY sorting receptor, Vps10p, which shuttles between the *trans*-Golgi and the prevacuolar/late endosomal compartment [45].

A v-SNARE functioning at multiple transport step is not without precedence [12,13,18]. In addition to the interaction between Gos1p and Sed5p, Gos1p might interact with other t-SNAREs. Another possibility would be that Gos1p in combination with other v-SNAREs may direct bi-directional ER-Golgi and intra-Golgi. Finally, it is possible that Gos1p-Sed5p interactions occur throughout the Golgi and that string proteins like Uso1p ensure sequential transport within the Golgi.

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References

[1] Rothman, J.E. (1994) Nature 372, 55–63.
[2] Rothman, J.E. (1996) Protein Sci. 5, 185–194.
[3] Bennett, M.K. (1994) Ann. NY Acad. Sci. 733, 256–265.
[4] Ferro-Novick, S. and Jahn, R. (1994) Nature 370, 191–193.
[5] Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) Nature 362, 318–324.
[6] Bennett, M.K. (1995) Curr. Opin. Cell Biol. 7, 581–586.
[7] Linial, M. (1997) J. Neurochem. 69, 1781–1792.
[8] Weimbs, T., Low, S.H., Chapin, S.J., Mostov, K.E., Bucher, P. and Hofmann, K. (1997) Proc. Natl. Acad. Sci. USA 94, 3046–3051.
[9] Terrian, D.M. and White, M.K. (1997) Eur. J. Cell Biol. 73, 198–204.
[10] Nichols, B.J., Ungermann, C., Pelham, H.R.B., Wickner, W.T. and Haas, A. (1997) Nature 387, 199–202.
[11] Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T.H. and Rothman, J.E. (1998) Cell 92, 759–772.
[12] Lupashin, V.V., Pokrovskaya, I.D., McNew, J.A. and Waters, M.G. (1997) Mol. Biol. Cell 8, 2659–2676.
[13] Holthuis, J.C.M., Nichols, B.J., Dhruvakumar, S. and Pelham, H.R.B. (1998) EMBO J. 17, 113–126.
[14] McNew, J.A., Hartmann, E. and Söllner, T.H. (1998) Chemtracts 11, 446–458.
[15] Stone, S., Sacher, M., Mao, Y., Carr, C., Lyons, P., Quinn, A.M. and Ferro-Novick, S. (1997) Mol. Biol. Cell 8, 1175–1181.
[16] Sacher, M., Stone, S. and Ferro-Novick, S. (1997) J. Biol. Chem. 272, 17134–17138.
[17] Banfield, D.K., Lewis, M.J. and Pelham, H.R. (1995) Nature 375, 806–809.
[18] Fischer von Mollard, G., Nothwehr, S.F. and Stevens, T.H. (1997) J. Cell Biol. 137, 1511–1524.
[19] Søgaard, M., Tani, K., Ye, R.R., Geromanos, S., Tempst, P., Kirchhausen, T., Rothman, J.E. and Söllner, T. (1994) Cell 78, 937–948.
[20] McNew, J.A., Søgaard, M., Ye, R.R., Machiko, S., Söllner, T. and Rothman, J.E. (1997) J. Biol. Chem. 272, 17776–17783.
[21] Sambrook, J., Fritsch, E.E. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[22] Guthrie, C. and Fink, G.R. (1991) in: Methods in Enzymology (Abelson, J.N. and Simon, M.I., Eds.), Vol. 194, Academic Press, New York.
[23] Berben, G., Dumont, J., Gilliquet, V., Bolle, P.-A. and Hilger, F. (1991) Yeast 7, 475–477.
[24] Nagahama, M., Orci, L., Ravazzola, M., Amherdt, M., Lacomis, L., Tempst, P., Rothman, J.E. and Söllner, T.H. (1996) J. Cell Biol. 133, 507–516.
[25] Subramaniam, V.N., Peter, F., Philp, R., Wong, S.H. and Hong, W. (1996) Science 272, 1161–1163.
[26] Gaynor, E.C., te Heesen, S., Graham, T.R., Aebi, M. and Emr, S.D. (1994) J. Cell Biol. 127, 653–665.
[27] Sorensen, S.O., van den Hazel, H.B., Kielland-Brandt, M.C. and Winther, J.R. (1994) Eur. J. Biochem. 220, 19–27.
[28] Evan, G.I., Lewis, G.K., Ramsay, G. and Bishop, J.M. (1985) Mol. Cell. Biol. 5, 3610–3616.
[29] Harlow, E. and Lane, D. (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[30] Newman, A.P., Shim, J. and Ferro-Novick, S. (1990) Mol. Cell. Biol. 10, 3405–3414.
[31] Rexach, M.F., Latterich, M. and Schekman, R.W. (1994) J. Cell Biol. 126, 1133–1148.

[32] Dascher, C., Ossig, R., Gallwitz, D. and Schmitt, H.D. (1991) Mol. Cell. Biol. 11, 872–885.
[33] Newman, A.P., Groesch, M.E. and Ferro-Novick, S. (1992) EMBO J. 11, 3609–3617.
[34] Shim, J., Newman, A.P. and Ferro-Novick, S. (1991) J. Cell Biol. 113, 55–64.
[35] Lupas, A., Van Dyke, M. and Stock, J. (1991) Science 252, 1162–1164.
[36] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[37] Duden, R., Hosobuchi, M., Hamamoto, S., Winey, M., Byers, B. and Schekman, R. (1994) J. Biol. Chem. 269, 24486–24495.
[38] Stevens, T., Esmon, B. and Schekman, R. (1982) Cell 30, 439–448.
[39] Graham, T.R. and Emr, S.D. (1991) J. Cell Biol. 114, 207–218.
[40] Graham, T.R., Seeger, M., Payne, G.S., MacKay, V.L. and Emr, S.D. (1994) J. Cell Biol. 127, 667–678.
[41] Graham, T.R. and Krasnov, V.A. (1995) Mol. Biol. Cell 6, 809–824.
[42] Subramaniam, V.N., Krijnse-Locker, J., Tang, B.L., Ericsson, M., Yusoff, A.R., Griffiths, G. and Hong, W. (1995) J. Cell Sci. 108, 2405–2414.
[43] Pelham, H.R., Banfield, D.K. and Lewis, M.J. (1995) Cold Spring Harbor Symp. Quant. Biol. 60, 105–111.
[44] Lewis, M.J., Rayner, J.C. and Pelham, H.R.B. (1997) EMBO J. 16, 3017–3024.
[45] Stack, J.H., Horazdovsky, B. and Emr, S.D. (1995) Annu. Rev. Cell Dev. Biol. 11, 1–33.