Golgi Structure Correlates with Transitional Endoplasmic Reticulum Organization in \textit{Pichia pastoris} and \textit{Saccharomyces cerevisiae}

Olivia W. Rossanese, Jon Soderholm, Brooke J. Bevis, Irina B. Sears, James O'Connor, Edward K. Williamson, and Benjamin S. Glick

Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, Illinois 60637

\textbf{Abstract.} Golgi stacks are often located near sites of "transitional ER" (tER), where COPII transport vesicles are produced. This juxtaposition may indicate that Golgi cisternae form at tER sites. To explore this idea, we examined two budding yeasts: \textit{Pichia pastoris}, which has coherent Golgi stacks, and \textit{Saccharomyces cerevisiae}, which has a dispersed Golgi. tER structures in the two yeasts were visualized using fusions between green fluorescent protein and COPII coat proteins. We also determined the localization of Sec12p, an ER membrane protein that initiates the COPII vesicle assembly pathway. In \textit{P. pastoris}, Golgi stacks are adjacent to discrete tER sites that contain COPII coat proteins as well as Sec12p. This arrangement of the tER-Golgi system is independent of microtubules. In \textit{S. cerevisiae}, COPII vesicles appear to be present throughout the cytoplasm and Sec12p is distributed throughout the ER, indicating that COPII vesicles bud from the entire ER network. We propose that \textit{P. pastoris} has discrete tER sites and therefore generates coherent Golgi stacks, whereas \textit{S. cerevisiae} has a delocalized tER and therefore generates a dispersed Golgi. These findings open the way for a molecular genetic analysis of tER sites.

Key words: apparatus, Golgi • endoplasmic reticulum • \textit{Pichia} • \textit{Saccharomyces} • microtubules
of Golgi-derived transport vesicles (Glick and Malhotra, 1998; Pelham, 1998).

Can the cisternal maturation model help explain why Golgi structure varies so dramatically between cell types (Mollenhauer and M orré, 1991)? If the Golgi is indeed an outgrowth of the tER, then some of the variation in Golgi structure may be due to differences in the tER organization. To test this idea, we examined two budding yeasts that display different Golgi morphologies. Pichia pastoris has stacked Golgi organelles (Gould et al., 1992; Glick, 1996). By contrast, the Saccharomyces cerevisiae Golgi rarely shows a stacked structure, but exists primarily as individual cisternae dispersed throughout the cytoplasm (Preuss et al., 1992). Here we demonstrate that the tER organization is also fundamentally different in the two yeasts. P. pastoris contains discrete tER sites. In S. cerevisiae, the entire tER network apparently functions as tER. We suggest that fixed tER sites in P. pastoris produce coherent Golgi stacks, whereas the delocalized tER in S. cerevisiae produces a dispersed Golgi. Thus, tER organization may be a key determinant of Golgi structure in budding yeasts.

**Materials and Methods**

**Strains and Plasmids**

Yeast strains and plasmids are listed in Table I. Experiments with S. cerevisiae were carried out using strain DBY1034 and derivatives thereof. DBY1034 was transformed with plasmid pOH to obtain expression of Och1p-HA. Strain DBY1034-S13G, in which the endogenous SEC13 gene has been replaced with SEC13-GFP, was constructed as follows. The URA3 cassette from pUC19-URA3 (B eden et al., 1994) was excised with HindIII, blunted, and inserted into the SspI site of pUC19 (Y anisch-Perron et al., 1985) to create pUC19-URA3 A. An 1,166-bp HindIII-HincII fragment spanning the 3’ portion of SEC13 was then amplified by PCR from S. cerevisiae genomic DNA and inserted into the corresponding sites in pUC19-URA3. The resulting plasmid was mutagenized using the QuikChange kit (Stratagene Inc.) to replace the SEC13 stop codon with a SnaBI site. The EGFP gene was excised from pEGFP-1 (Clontech) with BamHI and NotI, blunted, and inserted into this SnaBI site. The resulting construct was linearized at the unique BstEII site and integrated into the chromosomal SEC13 gene, yielding an intact SEC13-GFP fusion gene plus a 3’ fragment of authentic SEC13. A similar strategy was used to create strain DBY1034-S12m: a 1,328-bp BamHI fragment spanning the 3’ end of SEC12 was inserted into pUC19-ARG4; a c-myc epitope sequence was then inserted just upstream of the HDEL sequence, and the resulting construct was linearized with XhoI for integration into the SEC12 locus. To express myc-tagged S. cerevisiae Mnt1p (Chapman and Munro, 1996) in P. pastoris, the genomic gene was inserted into pYMT1BS (a gift of Sean Munro) and subcloned into pOOW3, a P. pastoris episomal vector that contains the GAP promoter. Plasmid pOA199 (Gould et al., 1992; Waterham et al., 1997; O. R. Rossanese, unpublished observations).

Plasmid pA FB384, which encodes glutathione S-transferase fused to the NH2-terminal acidic domain of S. cerevisiae Sec7p (up to the Nael site in the coding sequence), was provided by A lex Francuzoff (U niversity of Colorado, Denver, CO).

**Nocodazole Treatment**

P. pastoris cultures were grown overnight at 30°C to an OD600 of 0.15 in 1% yeast extract, 2% peptone, 2% glucose, 20 mg/ml adenine sulfate, 20 mg/ml uracil, 50 mM sodium maleate, pH 5.5. Half of a culture then received nocodazole at a final concentration of 15 μg/ml, diluted from a 10 μg/ml stock solution in water-free dimethyl sulfoxide. As a control, the other half of the culture received only dimethyl sulfoxide. Incubation was continued for up to 2.5 h, and the cells were fixed for microscopy.

**Antibodies for Immunofluorescence**

A nti-HA monoclonal antibody (16B12; Berkeley A ntibody Co.), anti-myc monoclonal antibody (9E10; Boehringer Mannheim Biochemicals) and anti-green fluorescent protein (GFP) monoclonal antibody (a mixture from clones 7.1 and 13.1; Boehringer M annheim Biochemicals) were used at 5 μg/ml; in cells not expressing a tagged protein, each antibody gave only a faint background signal. The anti-GFP antibody was used to supplement the endogenous GFP signal, which is diminished by the immunofluorescence procedure. Anti–Pdi1p, the COOH-terminal 308 residues to glycoproteins transiting through the Golgi (Orlean, 1997; Pelham, 1998).

Trimble et al., 1991), these contaminating antibodies give a spurious label-
immunofluorescence, such antibodies primarily label early Golgi elements. Similarly, with P. pastoris, anti-α-1,6-mannose antibodies give a strong and relatively specific Golgi labeling. In several cases, we observed punctate staining with anti sera raised against various COPII proteins, but this staining was actually due to contaminating anti-α-1,6-mannose antibodies. This problem can be avoided by preincubating a diluted antibody either with fixed S. cerevisiae cells or with 0.5 mg/ml purified yeast mannan (M-7504, Sigma Chemical Co.).

Electron Microscopy

Thin-section electron microscopy was performed essentially as described (Kaiser and Schekman, 1990; Gould et al., 1992). In brief, a 50-ml culture of yeast cells in rich glucose medium was grown to an OD600 of ~0.5. The culture was concentrated to a volume of ~5 ml with a bottle-top vacuum filter, and 40 ml of ice-cold 50 mM KP0, pH 6.8, 1 mM MgCl2, 2% glutaraldehyde was added rapidly with swirling. A fixation time of 1 h on ice, the cells were washed repeatedly, and then resuspended in 0.75 ml 0.1% glutaraldehyde, and mixed for 1 h at room temperature. The cells were washed, and then resuspended in 0.75 ml 2% uranyl acetate and mixed for 1 h at room temperature. Finally, the cells were embedded in Spurr resin; 50 ml of yeast culture yielded enough cells for three BEEM capsules. The resin was polymerized for 2 d at 60 C. Sections were stained with uranyl acetate and lead citrate, and viewed on an electron microscope (100 CXI; JOEL U.S.A. Inc.).

For immunoelectron microscopy, we used a modification of previously published methods (Karger et al., 1992; Rieder et al., 1996). A 50-ml log-phase culture was concentrated to a volume of ~5 ml (see above) and fixed by adding 40 ml of 50 mM KP0, pH 6.8, 1 mM MgCl2, 4% formaldehyde, and 2% glutaraldehyde (aldehydes were EM grade from Ted Pella, Redding, CA.). A 1-h fixation at room temperature, the cells were washed twice with 20 ml PBS, pH 7.4, containing 0.5% 2-mercaptoethanol, 0.5 mM periodate was omitted because this compound may destroy certain antigens by reacting with amino acid side chains (Clamp and Hough, 1965). The washed cells were embedded in 100 μl of low-melting-temperature agarose, which was cut into cubic-millimeter blocks and infiltrated for 2 h at room temperature in 1.7 M sucrose, 25% polyvinylpyrrolidone K15 (Fluka Chemical Corp.) in PBS, prepared according to Tokuyasu (1989). Individual agarose blocks were then mounted on copper pins, frozen in liquid nitrogen, and cryosectioned at ~80 nm. Thawed cryosections were prepared and labeled as described (G riffiths, 1993; Karger et al., 1996), using a blocking buffer consisting of PBS, 20 mM glycine, 1% fish gelatin (Sigma Chemical Co.), and 10% fetal calf serum; the serum had been heat inactivated at 60 C for 1 h, and then centrifuged to remove complement. Dilutions were 1:10 for an affinity-purified polyclonal anti–HA antibody (a gift of Charles Zuker, University of California, San Diego, and Charles Charles Zuck, University of California, San Diego, and Charles). 1:50 for protein A–gold (10 nm; Goldmark Biologicals). A peroxidase the protein A–gold step, the sections were postfixed for 30 min in 1% glutaraldehyde in PBS, and then washed with distilled water and stained with 1.5% sodium thiosulfate in 2.5% polyvinyl alcohol (MW 15,000, ICN Biomedicals Inc.). The best results were obtained using relatively thick sections and a thin layer of stain. Electron micrographs were digitized and imported into Photoshop (A dobe Systems Inc.) to adjust brightness and contrast and to create composite images. For Figs. 3 and 7, the gold particles were darkened to improve visualization. Images were printed on an NP-1600 dye-sublimation printer (Codonics).

Immunofluorescence Microscopy

We modified previously described methods (Pringle et al., 1991) to enhance both the preservation and the visualization of intracellular structures. Improved preservation was achieved by rapidly digesting the cell wall with protease-free lytase in sorbitol-free wash buffer, and by post-fixing the cells in acetone. Improved visualization was achieved by adhering the cells directly to coverslips (Weiss et al., 1989; Hell and Stelzer, 1995) rather than to wells on a slide. A detailed protocol follows.

Fixation and Spheroplasting. A yeast culture is grown overnight in rich medium with good aeration to an OD600 of 0.25–1.0. 8 ml of this culture is pipetted onto a 150-ml bottle-top filter, and the liquid is removed by vacuum aspiration. Each well is washed three times with water, and 10 μl of spheroplast suspension is added. A fter a 30-min incubation in the dark, each well is washed eight times with PBS. The liquid is aspirated completely after the final wash. Each well then receives 5 μl of phenylene diamine-containing mounting medium (Pringle et al., 1991). The coverslip is inverted onto a slide and sealed with nail polish.

Microscopy. Samples were viewed on an A xiophot microscope equipped with a 100/Plan-A po 1.4 NA objective (Carl Zeiss, Inc.) and with bandpass filters for visualizing Hoechst dye, fluorescent/ Oregon green, and Texas red images. Images were captured with a Photometrics CCD camera using the Openlab software package (Improvement). For each field of cells, multiple images were recorded from focal planes spaced 0.2 μm apart; three to four of these images were deblurred using a simple deconvolution algorithm, and then combined to form a single projected image. Where indicated, the same cells were also photographed in differential interference contrast (DIC) mode at a single focal plane. Photoshop was used for colorization, adjustments of brightness and contrast, and creation of composite and merged images. Manipulations with the Openlab and Photoshop programs improved the quality of the images, but did not significantly alter their information content. Fluorescence and DIC images were printed on a Stylus Photo color inkjet printer (Epson E electronics). 150 μl of O ch1p-HA and Sec7p, digital images were examined with Photoshop. Cells that showed clear staining with both antibodies were used for quantitation. A spot of one color was scored as overlapping with a spot of the second color if the center of the first spot was contained within the second spot. To estimate the percentage of Golgi structures that are perinuclear in P. pastoris, the perinuclear and peripheral O ch1p-HA spots were counted in 50 cells that showed faint nuclear envelope staining (see Fig. 2 C). To estimate the average percentage of the cell area occupied by fluorescent spots of a given marker protein, 25 cells stained to reveal O ch1p-HA and Sec7p were analyzed using NIH Image (http://rsb.info.nih.gov/nih-image/): the total areas of the O ch1p-HA and Sec7p spots were summed and divided by twice the total areas of the cells.

Visualization of GFP Fusion Proteins in Intact Cells

Yeast cells expressing a GFP-labeled COPII protein were grown to log phase with good aeration. A 400-μl aliquot of the culture was then mixed rapidly with 400 μl of 100 mM KP0, pH 6.5, 2 mM MgCl2, 8% formaldehyde, 0.5% glutaraldehyde. A fixation time of 1 h in the dark, the cells were washed twice with 1 ml PBS, and then resuspended in 50 μl PBS. For viewing by fluorescence microscopy, 1–2 μl of the resuspended cells were spotted on a slide and spread with a coverslip.

Downloaded from on May 19, 2017

Rossanesre et al. Golgi and Transitional Endoplasmic Reticulum in Yeast

71
Results

Golgi Stacks in P. pastoris Are Closely Associated with tER Sites

Stacks of Golgi cisternae can be readily visualized in P. pastoris cells, with each stack containing about four cisternae (Fig. 1) (Gould et al., 1992; Glick, 1996). Previously published images of P. pastoris showed Golgi stacks near the nucleus (Gould et al., 1992; Rambourg et al., 1995; Glick, 1996). Golgi structures might form by growing out of the nuclear envelope, which constitutes a large fraction of the ER in budding yeasts (Preuss et al., 1991). Alternatively, P. pastoris Golgi stacks might be positioned near the nucleus by microtubule-dependent transport toward the centrosome, as occurs in vertebrate cells (Thyberg and Moskalewski, 1985; Kreis, 1990; Lippincott-Schwartz,

Figure 1. Thin-section electron microscopy of Golgi and ER structures in P. pastoris.
(A) A representative P. pastoris cell. Golgi stacks are found adjacent to ER membranes, including both the nuclear envelope and peripheral ER elements. (B) A representative P. pastoris cell after treatment with nocodazole for 2.5 h. The nucleus has failed to divide. However, Golgi morphology is unaffected by the drug treatment. This experiment was performed with strain PPY 1. Bars, 0.5 μm. G, Golgi stack; N, nucleus; ER, peripheral ER membranes; M, mitochondrion; V, vacuole.
fied immunofluorescence protocol that consistently yields high-quality images (see Materials and Methods). Because P. pastoris is closely related to S. cerevisiae (Higgins and Gregg, 1998), polyclonal antibodies raised against S. cerevisiae antigens often cross-react with the P. pastoris homologues. Alternatively, marker proteins were modified with epitope tags and visualized using specific monoclonal antibodies.

To visualize the general ER in P. pastoris, fixed cells were labeled with an antibody against protein disulfide isomerase (Pdi1p), a marker for the general ER (Fig. 2 D, red) (Sitia and Meldolesi, 1992; Nishikawa et al., 1994). The same cells were also incubated with Hoechst dye (Fig. 2 D, blue) to label DNA. The anti–Pdi1p antibody highlights the nuclear envelope as well as peripheral ER elements. This pattern resembles the ER distribution seen in S. cerevisiae (Rose et al., 1989; Preuss et al., 1991).

Fig. 2 A shows the S. cerevisiae Golgi. This organelle appears in immunofluorescence images as a set of punctate spots, with different Golgi markers often displaying only a partially overlapping localization (Segev et al., 1988; Franzusoff et al., 1991; Antebi and Fink, 1992; Lussier et al., 1995). In S. cerevisiae cells expressing an HA-tagged version of the early Golgi marker Och1p (Nakayama et al., 1992; Gaynor et al., 1994; Harris and Waters, 1996; Gaynor and Emr, 1997), an anti–HA monoclonal antibody (Fig. 2 A, green) reveals multiple spots of Och1p-HA labeling per cell. The same cells were also labeled with a polyclonal antibody (red) against Sec7p, a protein that is concentrated in late Golgi elements (Franzusoff et al., 1991). The two markers show no significant overlap (Fig. 2 A, merged; Table I).

Do early and late Golgi elements ever colocalize in
Saccharomyces cerevisiae

A Och1p-HA  Sec7p  Merged

Pichia pastoris

C Och1p-HA  Sec7p  Merged

D Pdi1p  DNA  Merged

Fig. 2. Immunofluorescence staining of Golgi and ER structures. (A) Golgi labeling in S. cerevisiae. Fixed cells of strain DBY1034/pOh were incubated with a monoclonal anti-HA antibody to label Och1p-HA, followed by Oregon green-conjugated anti-mouse antibody (green). The same cells were also incubated with a polyclonal antibody against Sec7p, followed by Texas red-conjugated anti-rabbit antibody (red). The merged image shows very little overlap between the two Golgi markers. (B) Golgi labeling in a sec14 mutant of S. cerevisiae. Strain CTY214 was grown at the permissive temperature of 23°C, and then shifted to the nonpermissive temperature of 37°C for 1 h before fixation. Antibody labeling was as in A. After the temperature shift, Sec7p-containing structures reorganize into large clusters, but the staining pattern of Och1p-HA is not significantly altered. Surprisingly, as illustrated by the cell on the right, budded sec14 cells often partition the Sec7p clusters into the bud. (C) Golgi labeling in P. pastoris. Fixed cells of strain PY212-OH were incubated with the same primary and secondary antibodies as in A. Och1p-HA staining overlaps strongly with Sec7p staining, as shown in the merged image. As shown in the merged image, Pdi1p is present in the nuclear envelope and peripheral ER structures. Bar, 2 μm.

S. cerevisiae? In temperature-sensitive sec14 mutants of S. cerevisiae, multilamellar Golgi structures accumulate at the nonpermissive temperature (Novick et al., 1980; Rambourg et al., 1996); these structures are reminiscent of the Golgi stacks seen in higher eukaryotes. Therefore, we tested whether Och1p-HA and Sec7p colocalize in a sec14 mutant after incubation at the nonpermissive temperature of 37°C. The 37°C treatment causes redistribution of Sec7p into large clusters (Fig. 2B, red) (Franzusoff et al., 1991). However, the staining pattern of Och1p-HA is largely unchanged in the sec14 mutant (Fig. 2B, green), and there is still no significant overlap between Och1p-HA and Sec7p. It seems that S. cerevisiae is incapable of generating coherent Golgi stacks that contain both early and late Golgi proteins.

Fig. 2 C shows the P. pastoris Golgi. Using an integrating expression vector (Sears et al., 1998), we generated a P. pastoris strain that stably expresses HA-tagged S. cerevisiae Och1p. P. pastoris contains an α-1,6-mannosyltransferase activity like that ascribed to Och1p (Trimble et al., 1991; Nakanishi-Shindo et al., 1993), and expression of Och1p-HA in P. pastoris has no effect on growth or Golgi morphology (not shown). Och1p-HA localizes to two to six spots per P. pastoris cell (Fig. 2C, green). A subset (~45%) of the Och1p-HA spots clearly adjoin the nucleus (see Fig. 2C, legend). When P. pastoris cells are labeled with the antibody against S. cerevisiae Sec7p, two to six spots are again detected (Fig. 2C, red), presumably because this antibody reacts with the P. pastoris homologue of Sec7p. By con-
Golgi organization in P. pastoris is unaffected by microtubule disruption or cell cycle progression

P. pastoris resembles vertebrate cells in having stacked Golgi cisternae. We tested whether the two cell types are also similar with regard to Golgi dynamics. Pre-Golgi elements in vertebrate cells are transported along microtubules (Lippincott-Schwartz, 1998), but our electron microscopy data indicate that in P. pastoris, microtubules do not influence Golgi structure (see Fig. 1). This conclusion was confirmed at the immunofluorescence level. In untreated P. pastoris cells, microtubules are present and nuclei partition into daughter cells during mitosis (Fig. 4 A). Microtubule distribution shows no detectable relationship to Golgi distribution (Fig. 4 A). Nocodazole treatment depolymerizes microtubules and blocks nuclear migration, but the Golgi staining pattern is unaffected (Fig. 4 B). Moreover, the Golgi markers Sec7p and Och1p-HA still colocalize in nocodazole-treated P. pastoris cells (not shown).

To determine whether cell cycle progression alters Golgi organization in P. pastoris, as it does in vertebrate cells (Rabouille and Warren, 1997), we analyzed P. pastoris in the G1, S/G2, and M phases of the cell cycle. Immunofluorescence was used to quantify the average number of Golgi structures per cell. This number is similar in each phase of the cell cycle (Table IV). Thus, when observing P. pastoris under a variety of conditions, we consistently find that each cell contains a small number of distinct Golgi stacks.

### COPII Coat Proteins Show Different Distributions in S. cerevisiae and P. pastoris

The electron microscopy data suggest that P. pastoris contains...
tains discrete tER sites, whereas S. cerevisiae lacks such sites. To explore this idea further, we compared the localizations of COPII proteins in the two yeasts. GFP was fused to the COOH terminus of S. cerevisiae Sec13p, a coat protein that is incorporated at a late stage of COPII vesicle assembly (Pryer et al., 1993; Kuehn and Schekman, 1997). Replacement of the endogenous SEC13 gene with the SEC13-GFP gene has no detectable effect on cell growth, indicating that the Sec13p-GFP fusion can perform the essential function of Sec13p (Pryer et al., 1993). When S. cerevisiae cells expressing Sec13p-GFP are viewed by fluorescence microscopy, each cell contains ~30–50 tiny spots (shown in Fig. 5A, although this pattern is difficult to photograph accurately). The spots are distributed almost evenly throughout the cytoplasm, with some cells showing an apparent concentration of spots on the nuclear envelope. We surmise that these spots represent individual COPII vesicles. A vesicle is smaller than the resolution limit of light microscopy (Lacey, 1989), so the apparent size of the spots is probably misleading, but a single COPII vesicle contains many copies of Sec13p-GFP and hence should produce a detectable fluorescence signal. GFP was also fused to three other S. cerevisiae COPII coat proteins (Kuehn and Schekman, 1997): Sec23p, Sec24p, and Sec31p. All of these fusions are functional, and they all give the same fluorescence pattern as Sec13p-GFP (Fig. 5B and data not shown), indicating that we have visualized the normal distribution of COPII vesicles. These results strongly support the notion that COPII vesicles bud from the entire ER in S. cerevisiae.

In parallel, the P. pastoris SEC13 gene was replaced with a SEC13-GFP fusion gene. The resulting fluorescence pattern is strikingly different from that seen in S. cerevisiae. Sec13p-GFP in P. pastoris is concentrated in only two to six large spots per cell (Fig. 6A). When examined by immunofluorescence microscopy, the Sec13p-GFP spots are adjacent to, but not quite overlapping, the Golgi

Table IV. The P. pastoris Golgi Retains Its Organization Throughout the Cell Cycle

<table>
<thead>
<tr>
<th>Phase of cell cycle</th>
<th>Average number of Och1p-HA spots per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3.8 ± 1.2</td>
</tr>
<tr>
<td>S/G2</td>
<td>4.1 ± 0.9</td>
</tr>
<tr>
<td>M</td>
<td>3.9 ± 1.3</td>
</tr>
</tbody>
</table>

An unsynchronized culture of P. pastoris strain PYY12-OH was processed for immunofluorescence as in Fig. 2C. Individual cells were assigned to a phase of the cell cycle based on morphology and nuclear distribution (Lew et al., 1997). 50 cells were examined for each phase of the cell cycle to determine the average number of Och1p-HA spots. The numbers listed refer to nucleated cells; buds lacking nuclei were excluded from the analysis. Standard deviations are indicated.

Figure 4. Immunofluorescence analysis of nocodazole-treated P. pastoris cells. (A) DNA, tubulin, and Sec7p distributions in untreated P. pastoris cells. Fixed cells of strain PPY 12 were incubated with Hoechst dye to stain DNA. Microtubules were visualized with a monoclonal anti-tubulin antibody followed by Oregon green-conjugated anti-mouse antibody. The Golgi marker Sec7p was visualized as in Fig. 2. (B) DNA, tubulin, and Sec7p distributions in P. pastoris cells treated with nocodazole for 2.5 h. Fixed cells were labeled as in A. As previously described for S. cerevisiae (Jacobs et al., 1988), microtubules are virtually undetectable within 1 h after nocodazole addition (not shown). Bar, 2 μm.

Figure 5. Visualization of GFP-labeled COPII coat proteins in intact S. cerevisiae cells. (A) Sec13p-GFP fluorescence in S. cerevisiae. Cells of strain DBY 1034-S13G were fixed and viewed directly. The same cells were imaged in DIC and fluorescence modes. (B) Sec23p-GFP fluorescence in S. cerevisiae strain DBY 1034-S23G. The experiment was performed as in A. Bar, 2 μm.
spots marked by the anti–Sec7p antibody (Fig. 6 B). This result suggests that Sec13p-GFP is localized to tER sites. Indeed, immunoelectron microscopy with an anti–GFP antibody revealed that Sec13p-GFP is present on tubulovesicular structures at the interface between ER membranes and Golgi stacks (Fig. 7; Table III). We conclude that COPII vesicle budding is restricted to discrete tER sites in P. pastoris.

**Sec12p Localizes to tER Sites in P. pastoris**

The earliest known player in the COPII assembly pathway is Sec12p, a membrane-bound guanine nucleotide exchange factor that recruits the small GTPase Sar1p to the ER membrane (Nakano et al., 1988; Nakano and Muramatsu, 1989; Barlowe and Schekman, 1993; Kuehn and Schekman, 1997). In previous studies of S. cerevisiae, Sec12p exhibited general ER staining (Nishikawa and Nakano, 1991, 1993). Our results confirm those earlier findings. S. cerevisiae Sec12p has traditionally been visualized in strains overexpressing this protein (Nishikawa and Nakano, 1991, 1993; Nishikawa et al., 1994). To eliminate possible ambiguities resulting from overexpression, we replaced the chromosomal SEC12 gene with a myc-tagged version. The resulting strain grows like the wild-type (not shown), implying that Sec12p-myc can functionally replace the essential wild-type protein (Nakano et al., 1988). Sec12p-myc (Fig. 8 A, green) colocalizes with Pdi1p (Fig. 8 A, red) in the nuclear envelope and in peripheral ER membranes. Although Sec12p-myc sometimes shows a discontinuous staining pattern, the fluorescence signal is relatively weak, and we have observed that the ER network often appears discontinuous when it is weakly stained (not shown). Hence, the combined data suggest that Sec12p-myc is present throughout the ER in S. cerevisiae.

We also replaced P. pastoris SEC12 with a myc-tagged version. Once again the fluorescence signal is weak, but the pattern is clearly visible. In this case, the anti–myc antibody does not give a general ER staining, but instead labels several spots per cell (Fig. 8 B, green). Like Sec13p-GFP (see above), Sec12p-myc localizes to sites that are immediately adjacent to Sec7p-containing Golgi structures (Fig. 8 B). Thus, in P. pastoris, components at both early and late stages of the COPII assembly pathway are concentrated at tER sites.
Discussion

Why is the Golgi apparatus more photogenic in P. pastoris than in S. cerevisiae? These two yeasts are morphologically very similar; yet Golgi cisternae in P. pastoris are organized into stacks, whereas Golgi cisternae in S. cerevisiae are scattered throughout the cytoplasm. We propose the following hypothesis. In P. pastoris, COPII vesicles bud from fixed tER sites, and then fuse with one another to create new Golgi cisternae, which mature to yield polarized stacks (Glick and Malhotra, 1998). In S. cerevisiae, COPII vesicles bud throughout the ER, and therefore each Golgi cisterna forms at a different location (Fig. 9).

Our immunofluorescence data confirm that S. cerevisiae and P. pastoris have fundamentally different Golgi structures. A hallmark of the Golgi in S. cerevisiae is that various marker proteins often show distinct punctate distributions (Antebi and Fink, 1992; Chapman and Munro, 1994; Lussier et al., 1995). For example, the early Golgi protein Och1p-HA exhibits virtually no overlap with the late Golgi protein Sec7p. Although multilamellar Golgi structures are seen in temperature-sensitive sec7 and sec14 mutants of S. cerevisiae (Novick et al., 1980; Svodoba and Necas, 1987; Rambourg et al., 1993, 1996), we find that early and late Golgi markers still do not colocalize in sec14 mutant cells (Fig. 2), indicating that S. cerevisiae cannot make coherent Golgi stacks. With P. pastoris, on the other hand, Och1p-HA and Sec7p overlap almost completely (Fig. 2), as expected if each Golgi stack represents an ordered set of early, middle, and late cisternae.

Does this difference in Golgi structure correlate with a difference in ER organization? The ER of both yeasts comprises the nuclear envelope plus peripheral elements. However, in P. pastoris, vesicles can often be seen budding specifically from regions of the ER adjacent to Golgi stacks (Fig. 1) (Gould et al., 1992; Glick, 1996). Such vesiculating ER regions have not been seen in S. cerevisiae (Kuehn and Schekman, 1997). These observations suggested to us that P. pastoris contains discrete tER sites, whereas S. cerevisiae does not. To test this interpretation, we used COPII coat proteins as markers for the tER (Orci et al., 1999).
Rossanese et al. Golgi and Transitional Endoplasmic Reticulum in Yeast

Published April 5, 1999

et al., 1991; Kuge et al., 1994; Shaywitz et al., 1995; Paccaud et al., 1996; Tang et al., 1997). With S. cerevisiae, fusion of GFP to Sec13p or other COPII coat proteins revealed many small fluorescent spots that probably represent individual COPII vesicles (Fig. 5). These spots are found throughout the cytoplasm, consistent with the notion that COPII vesicles bud at random from the entire ER. By contrast, a Sec13p-GFP fusion in P. pastoris localizes to a small number of discrete regions (Fig. 6). These regions are immediately adjacent to Golgi stacks, and they contain tubulovesicular membranes (Fig. 7). Hence, we propose that Sec13p-GFP marks a compartment in P. pastoris that is analogous to the tER sites described previously in vertebrate cells (Palade, 1975; Bannykh and Balch, 1997).

How is Sec13p recruited to tER sites in P. pastoris? The assembly of COPII vesicles is an ordered process that begins with the action of Sec12p (Barlowe and Schekman, 1993; Kahn and Schekman, 1997). We compared the localization of epitope-tagged Sec12p in the two yeasts, consistent with previous reports (Nishikawa and Nakano, 1993; Nishikawa et al., 1994), Sec12p-myc in S. cerevisiae is distributed throughout the ER. In P. pastoris, on the other hand, Sec12p-myc is concentrated at tER sites (Fig. 8). This observation suggests a working model for the organization of tER and Golgi compartments in the two yeasts. We postulate that in P. pastoris, Sec12p is anchored at tER sites by unknown partner proteins that comprise a “tER scaffold.” Because Sec12p initiates the assembly of COPII vesicles, these vesicles bud exclusively from tER sites, and successive Golgi cisternae form at fixed locations to generate polarized stacks. This model assumes that tER sites are relatively stable entities, and, indeed, our studies of Sec13p-GFP dynamics in P. pastoris confirm that tER sites are long lived and slow moving (B.J. Bevis, unpublished observations). The situation is quite different in S. cerevisiae—this yeast apparently lacks a tER scaffold, so Sec12p is free to diffuse throughout the ER. As a result, COPII vesicles bud from the entire ER, and successive Golgi cisternae form at different locations, yielding a dispersed organelle (Fig. 9).

In this view, Golgi structure and positioning are strongly influenced by tER organization.

An important test of our model will be to alter tER organization in P. pastoris and ask whether Golgi structure is correspondingly affected. Such an experiment will reveal whether the existence of Golgi stacks in P. pastoris is due solely to the presence of fixed tER sites. The simplest view is that Golgi stacking is a kinetic phenomenon, with cisternal formation and maturation occurring too quickly for successive cisternae to diffuse away from one another. Alternatively, Golgi cisternae in P. pastoris might be held together by a cytoskeletal matrix (Mollenhauer and More, 1978; Cluett and Brown, 1992; Staehelin and Moore, 1995; Barr et al., 1997). These possibilities can be distinguished by generating P. pastoris mutants in which COPII proteins are delocalized, and then asking whether the loss of tER sites leads to Golgi dispersal. One promising approach focuses on determining whether P. pastoris Sec12p contains a tER localization signal that is recognized by specific partner proteins.

Although S. cerevisiae lacks discrete tER sites, this yeast can be used to explore the more general question of whether the Golgi is an outgrowth of the ER. Early in S-phase of the S. cerevisiae cell cycle, the small buds invariably contain both ER and Golgi structures (Segev et al., 1988; Redding et al., 1991; Preuss et al., 1991, 1992; O.W. Rossanese, unpublished observations). It is likely that the ER elements present in the emerging bud give rise to new Golgi cisternae. If so, mutants that fail to transport ER membranes into the bud should also lack Golgi cisternae in the bud. We are currently testing this prediction by characterizing S. cerevisiae mutants defective in Golgi inheritance.

A comparison of budding yeasts with other eukaryotes can indicate which aspects of the tER-Golgi system are cell type-specific. First, unlike many eukaryotes, S. cerevisiae contains neither stacked Golgi organelles nor discrete tER sites. It is unclear whether the absence of these structures in S. cerevisiae is adaptive, or whether it reflects a loss-of-function mutation during the evolution of this yeast. Second, tubular connections between Golgi stacks are present in vertebrate cells (Mironov et al., 1997), but have not been detected in P. pastoris, the fission yeast Schizosaccharomyces pombe (Chappell and Warren, 1989) or certain insect cells (Stanley et al., 1997). Third, the Golgi breaks down during mitosis in vertebrate cells (Rabouille and Warren, 1997; A charya et al., 1998), but not in higher plants (Driouich and Staehelin, 1997) or budding yeasts (Table IV) (Makarow, 1988).

Which aspects of the tER-Golgi system are universal? We propose that Golgi cisternae always form by the coalescence of tER-derived membranes. This relationship is particularly evident in cells that contain Golgi stacks immediately adjacent to tER sites (Whaley, 1975; Farquhar and Palade, 1981; Barr et al., 1996). We have now documented such an association in P. pastoris. A close apposition between tER sites and Golgi stacks is probably a general feature of cells that do not transport Golgi elements along cytoskeletal tracks. Consistent with this idea, microtubules have no influence on Golgi structure or positioning in P. pastoris (Figs. 1 and 4). However, in some cell types, nascent Golgi elements are transported away from tER sites, thereby obscuring the tER-Golgi connection. For example, in S. pombe, microtubules play a role in cisternal stacking and possibly in Golgi movement (Ayscough et al., 1993). In higher plants, Golgi structures are transported along actin filaments (Driouich and Staehelin, 1997; Boevink et al., 1998). The best-studied example of cytoskeleton-mediated Golgi movement is provided by vertebrate cells, which employ microtubules and associated motor proteins to generate a juxtanuclear Golgi ribbon (Lippincott-Schwartz, 1998; Burkhardt, 1998). As a consequence, tER sites and Golgi elements normally do not colocalize in vertebrate cells. Yet recent evidence suggests that nascent Golgi structures in vertebrate cells initially coalesce at tER sites (Presley et al., 1997; Scales et al., 1997; Rowe et al., 1998); in the presence of microtubule-depolymerizing agents, entire Golgi stacks are found next to tER sites (Cole et al., 1996; Storrie et al., 1998). Thus, when microtubules have been disrupted, the tER-Golgi system in a vertebrate cell resembles the tER-Golgi system in P. pastoris. It seems that in all eukaryotes, the Golgi can be viewed as a dynamic outgrowth of the tER.
References


Nakashima-Shindo, Y., K. Nakayama, A. Tanaka, Y. Toda, and Y. Jigami. 1993. Structure of the N-linked oligosaccharides that show the complete loss of α1,6-polymannose outer chain from ochl, och1 mnn1, and och1 mnn1 alg3 mutants of Saccharomyces cerevisiae. J. Biol. Chem. 268:26338–26345.

