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Characterization of the *Saccharomyces* Golgi Complex Through the Cell Cycle by Immunoelectron Microscopy

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The membrane compartments responsible for Golgi functions in wild-type *Saccharomyces cerevisiae* were identified and characterized by immunoelectron microscopy. Using improved fixation methods, Golgi compartments were identified by labeling with antibodies specific for α1–6 mannose linkages, the Sec7 protein, or the Ypt1 protein. The compartments labeled by each of these antibodies appear as disk-like structures that are apparently surrounded by small vesicles. Yeast Golgi typically are seen as single, isolated cisternae, generally not arranged into parallel stacks. The location of the Golgi structures was monitored by immunoelectron microscopy through the yeast cell cycle. Several Golgi compartments, apparently randomly distributed, were always observed in mother cells. During the initiation of new daughter cells, additional Golgi structures cluster just below the site of bud emergence. These Golgi enter daughter cells at an early stage, raising the possibility that much of the bud's growth might be due to secretory vesicles formed as well as consumed entirely within the daughter. During cytokinesis, the Golgi compartments are concentrated near the site of cell wall synthesis. Clustering of Golgi both at the site of bud formation and at the cell septum suggests that these organelles might be directed toward sites of rapid cell surface growth.

INTRODUCTION

Both genetic and biochemical studies of protein transport in *Saccharomyces cerevisiae* have proven fruitful for identifying and characterizing an increasing number of the components of the secretory pathway (for reviews, see Melançon *et al.*, 1991; Franzusoff, 1992; Pryer *et al.*, 1992; Rothman and Orci, 1992). Several gene products that reside and function within a single, subcellular compartment (such as the nuclear envelope, the endoplasmic reticulum, the Golgi complex, or the vacuole) or that direct transport between these compartments have been identified (cf. Orlean *et al.*, 1988; Wright *et al.*, 1988; Aris and Blobel, 1989; Rose *et al.*, 1989; Davis and Fink, 1990; see also reviews by Klionsky *et al.*, 1990; Roberts *et al.*, 1991; Franzusoff *et al.*, 1991b; and Jones, 1991). However, ultrastructural characterization of yeast organelles has lagged behind the genetic and biochemical investigations, and the distribution and segregation of many subcellular compartments has not been examined in detail.

We have begun to study the organelle systems in *S. cerevisiae* using immunolocalization of well-characterized marker proteins combined with improved cell fixation techniques. By localizing yeast proteins that are known to function within particular subcellular compartments, we are generating functional definitions of the *S. cerevisiae* organelles. This is necessary because of the limited morphological resemblance between some yeast organelles and those of higher cells, even though the proteins that carry out many (if not all) organelle

functions are well conserved. Further, the appearance of membranous compartments can vary with cell type and with progression through the mitotic cycle. Thus, a definition of intracellular structures should, where possible, be based on their functions, which are, of course, determined by the functions of their component proteins. This approach has additional important advantages; compartments with similar morphologies, such as vesicles, can be sorted into functional classes, and mutant strains with alterations that disrupt organelle structure can nevertheless be examined with confidence.

In this paper we address a specific example of the traditional difficulty with yeast ultrastructural analysis, the Golgi complex. Conventional electron microscopy preparations have revealed few structures corresponding closely to the elaborate Golgi complexes found in higher cells (Svoboda and Piedra, 1983; Svoboda and Necas, 1987), yet yeast clearly have Golgi functions, as summarized below. Animal cells typically contain one Golgi complex (Novikoff *et al.*, 1971; Rambourg *et al.*, 1981) that consists of several parallel and functionally distinct membranous sacs in which the oligosaccharides that decorate secretory proteins are successively extended and modified (reviewed in Kornfeld and Kornfeld 1985). Although stacked cisternal compartments (reminiscent of the mammalian Golgi complex) can be readily observed in other yeast species, such as *Schizosaccharomyces pombe* (Johnson *et al.*, 1982; Chappel and Warren, 1989), few of these distinctive structures have been described in *S. cerevisiae*.

Despite the absence of elaborate Golgi structures, genetic and biochemical analyses have established that the process of secretion in *S. cerevisiae*, including transport through the Golgi complex, parallels that of other eukaryotic cells (Novick *et al.*, 1980, 1981; reviewed in Schekman 1985; Franzusoff 1992; Rothman and Orci, 1992). Indeed, with careful sample preparation, stacked cisternae consisting of two to four membranous compartments can be found in *S. cerevisiae* cells (Moor and Muhlethaler, 1963; Svoboda and Piedra, 1983; Makarow, 1988), and similar compartments have been observed near the projections of cells responding to the mating pheromone, α-factor (Baba *et al.*, 1989). More definitive Golgi structures, reminiscent of those found in mammalian cells, are apparent in strains with mutations that disrupt traffic through the secretory pathway (Novick *et al.*, 1980, 1981; Svoboda and Necas, 1987). Cells with alterations in the *SEC7* or *SEC14* genes, defects that impair protein transport through the Golgi complex, accumulate cup-shaped organelles (termed Berkeley bodies) throughout the cytoplasm (Novick *et al.*, 1980). When conditional *sec7* mutant strains are incubated at the restrictive temperature and in medium with low levels of glucose, Golgi-like complexes consisting of 2–11 parallel cisternae become conspicuous (Novick *et al.*, 1981; Svoboda and Necas, 1987).

In a previous report, we used indirect immunofluorescence and immunoelectron microscopy techniques to localize proteins known to reside within the yeast endoplasmic reticulum (ER) and followed the distribution of that organelle through the cell cycle (Preuss *et al.*, 1991). Unambiguous characterization of the *S. cerevisiae* Golgi complex similarly requires markers specific for this compartment. Several useful antibody probes are presently available: antibodies specific for the α1–6 mannose residues added to secreted yeast proteins in the Golgi complex (Ballou, 1982) have proven useful for recognizing proteins that have gained access to the Golgi compartments (Esmon *et al.*, 1981; Baker *et al.*, 1988; Franzusoff and Schekman, 1989). Further, antibodies suitable for indirect immunofluorescence microscopy have been used to localize the Ypt1 (Segev *et al.*, 1988) and Sec7 proteins (Franzusoff *et al.*, 1991a), each of which are required for vesicular traffic from the ER and through the Golgi complex. Antibodies specific for both of these proteins label several patches within each mother cell, suggesting that the yeast Golgi complex consists of several discrete compartments. Antibodies that recognize the Kex2 protease, which processes pro-α-factor in a late Golgi compartment, stain, in immunofluorescence microscopy, ~80% of the structures visualized with anti-Sec7p antibodies (Franzusoff *et al.*, 1991a; Redding *et al.*, 1991). Similarly, antibodies directed against the Sec14 protein, a phosphatidylinositol-phosphatidylcholine transfer protein that is essential for yeast Golgi function, stain compartments labeled by anti-Kex2p antibodies (Cleves *et al.*, 1991).

In this report, we show that three of these antibodies (directed against Sec7p, Ypt1p, and α1–6 mannose residues) can be used to identify the yeast Golgi complex at the level of ultrastructure. With these reagents, we have characterized the structure, location, and distribution of the *S. cerevisiae* Golgi complex through the cell cycle.

MATERIALS AND METHODS

Yeast Strain and Growth Conditions

The haploid yeast strain DBY1034 (*MAT***a** *his4–539 lys2–801 ura3–52 gal2*) is a wild-type strain (except for auxotrophic markers) that is a direct descendant of strain S288C. It was grown in yeast extract-peptone (YEP) medium (Sherman *et al.*, 1986) containing 5% glucose at 30°C before preparation for the electron microscopy experiments described below.

Preparation and Purification of Primary Antibodies

Antibodies were raised in rabbits against a TrpE-Ypt1 fusion protein as described previously (Segev *et al.*, 1988), and were affinity purified by first passing the crude serum over a bacterial TrpE column and then over a column containing the TrpE-Ypt1p fusion protein. This purification was repeated twice, and the resulting antibodies bound tightly to the Ypt1 protein as judged by immunoblotting (Segev *et al.*, 1988). Antibodies that bound to the bacterial TrpE column were used as a control in some immunolocalization experiments.

Affinity-purified rabbit anti-Sec7p antibodies were described before (Franzusoff *et al.*, 1991a), and the specificity of the rabbit anti-α1–6

mannose antibody preparation has been demonstrated previously (Franzusoff and Schekman, 1989).

Fixation of Yeast Cells With Osmotic Support for Immunoelectron Microscopy

A 100-ml culture of exponentially growing cells (5×10^6 cells/ml) in YEP-glucose medium was harvested by filtration over a 0.45-μm nitrocellulose membrane; filtration was discontinued when the total volume in the filter apparatus was ~5 ml. To the cell suspension, still on the filter membrane, 25 ml of ice-cold fixative (40 mM potassium phosphate, pH 6.7, 1.25 M sorbitol, 3% formaldehyde [16% methanol free, EM grade; Polysciences, Delaware, MD] 0.5% glutaraldehyde [50%, EM grade; Polysciences], 1 mM $MgCl_2$, 1 mM $CaCl_2$) was added and mixed rapidly with the cells by pipetting the suspension several times. The cell suspension was then transferred to a cold 50 ml polypropylene centrifuge tube and incubated on ice for 30 min.

Method I: Preparation of Fixed Cells for Immunoelectron Microscopy

Fixed cells were spun at low speed in a clinical centrifuge and the pellet was resuspended in 25 ml 40 mM potassium phosphate buffer (pH 6.7) containing 0.75 M sorbitol. The cells were centrifuged again and washed successively three more times in the same buffer containing 0.50 M sorbitol, then 0.25 M sorbitol, and finally no sorbitol.

As described previously (van Tuinen and Riezman, 1987; Wright and Rine, 1989), the final pellet of fixed cells was resuspended in 25 ml 1% sodium metaperiodate, incubated for 15 min at room temperature, centrifuged again, and resuspended in 25 ml 50 mM ammonium chloride and incubated for 15 min at room temperature. The mixture was transferred to a glass test tube and the cells were dehydrated (on ice) by adding ice-cold ethanol to 30% (vol/vol); centrifuged (at 4°C) again, and washed by centrifugation (always maintaining 4°C) successively in 50, 70, 80, 90, and 95% ethanol and finally twice in 100% ethanol. After the last resuspension, 100% ethanol at room temperature was added, and a final centrifugation at room temperature was performed. The dehydrated cells then were infiltrated with L. R. White resin (Polysciences, Warrington, PA) and prepared for polymerization exactly as described by Wright and Rine (1989). The resin was polymerized by incubation at 47°C for 48 h. Thin sections measuring 80–90 nm (as determined by a gold interference color) were cut with a diamond knife and were mounted on 300 mesh nickel grids (Wright and Rine, 1989).

This procedure (Method I) was repeated on three different occasions. Thin sections from each of these preparations yielded identical results in immunolocalization experiments with anti-Ypt1p or anti-Sec7p antibodies. Localization with antibodies directed against α1–6 mannose–containing materials showed a noticable background (although staining of Golgi structures could readily be observed). This background was reduced by using the permeabilization procedure described in the next section.

Method II: Permeabilization of Fixed Cells and Extraction of Soluble Proteins for Immunoelectron Microscopy

Yeast ribosomes obscure membranous organelles and other cellular structures: they are abundant and have a very strong affinity for electron-dense stains. In an attempt to improve the resolution of the subcellular organelles, we fixed cells as described above and then processed them using an adaptation of methods described by Willingham (1983). As described below, we removed the cell walls, permeabilized the membranes with saponin, and attempted to digest the ribosomes with ribonuclease.

After the 30-min incubation in fixative at 0°C (described above), the cells were washed twice at room temperature in potassium phosphate buffer containing 1 M sorbitol and no fixative. Cell walls were removed by enzymatic digestion in 40 mM potassium phosphate, pH 6.7, 180 mM 2-mercaptoethanol, 20 μg/ml zymolyase (100T; ICN Immunobiologicals, Lisle, IL), 0.5% glusulase (Dupont Biotechnology Systems, Boston, MA), 1 M sorbitol. Incubation at 37°C was continued for ~30 min or until most of the cells were spherical and showed the reduced refractility characteristic of spheroplasts. After this incubation, the spheroplasts were washed twice at room temperature with 40 mM potassium phosphate, pH 6.7, 1 M sorbitol. The spheroplasts were centrifuged once more and treated with 13 μM sodium borohydride in 40 mM potassium phosphate, pH 6.7, 1 M sorbitol for 5 min at room temperature to reduce the residual formaldehyde and glutaraldehyde. The spheroplasts then were washed five times at room temperature in 40 mM potassium phosphate, pH 6.7, 1 M sorbitol.

To digest the ribosomes and extract soluble proteins, the spheroplasts were centrifuged and resuspended in 1 mg/ml boiled, pancreatic ribonuclease A (RNase, Sigma, St. Louis, MO), 0.1% saponin, 40 mM potassium phosphate, pH 6.7, with 1 M sorbitol; incubation was continued for 15 min at room temperature. The spheroplasts were transferred by centrifugation to a slightly hypertonic solution containing the same components, except with 1.25 M sorbitol and incubated for 30 min at room temperature. Finally, they were centrifuged and incubated again in 1 mg/ml RNase, 0.1% saponin, 40 mM potassium phosphate, pH 6.7, with 1 M sorbitol. The cells were washed four times in phosphate buffer with decreasing sorbitol as described above in Method I. Dehydration, infiltration, and embedding were performed exactly as in Method I. When the anti-α1–6 mannose antibodies were applied to cell sections prepared by this method (Method II), background staining was greatly reduced, without much loss of membrane-specific signals. Antigenicity of proteins (including Ypt1p and Sec7p) not as strongly associated with membranes was lost with this harsher procedure.

Preparation of Cells Stained With Potassium Permanganate

Enhanced contrast between membranous compartments and the surrounding cytosol was achieved by fixation with potassium permanganate (Kaiser and Schekman, 1990). Blocks of embedded cells were prepared for serial sectioning as described by Fahrenbach (1984). Sections 70–80 nm thick were transferred to carbon-coated, slotted grids (Ted Pella, Redding, CA), as described by Gay and Anderson (1954). These preparations are not suitable for immunolabeling.

Immunolabeling

Cell sections (produced by either Method I or Method II) were incubated with affinity-purified anti-Ypt1p antibodies diluted 1:5; anti-Sec7p diluted 1:20; or anti-α1–6 mannose diluted 1:80 000 in a solution (PBST) containing 140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.05% Tween 20 with 2% ovalbumin (Sigma). Gold-conjugated secondary antibodies were diluted as suggested by the manufacturer (BioCell, Cardiff, UK). All incubations were performed as described previously (Preuss *et al.*, 1991).

RESULTS

Identification of Yeast Golgi Complexes Using Immunoelectron Microscopy and Antibodies That Recognize α1–6 Mannose Residues

Secreted yeast proteins are post-translationally modified by N-linked "core" glycosylation within the ER, and, in subsequent secretory compartments, these core structures are elaborated with "outer" chains of polymannose residues. Outer chain glycosylation begins with the addition of linear chains of α1–6 mannose residues within the Golgi complex (Ballou, 1982). Anti-

bodies specific for these α1–6 mannose linkages can be generated easily (Ballou, 1982). Because these epitopes are present in particular abundance within the lumen of the Golgi complex, we labeled yeast sections with the anti-α1–6 mannose antiserum as a first step in characterizing the ultrastructure of this organelle.

Intense labeling of a characteristic subset of intracellular compartments was observed when the anti-α1–6 mannose antibodies were applied to thin sections (Figure 1). The images in Figure 1 were prepared with Method II (see above), the harsher of the two methods we used. Neither the nuclear envelope nor tubules of the endoplasmic reticulum were labeled (Figure 1A), while membranous compartments beyond the ER, such as the plasma membrane (Figure 1B) were stained heavily. The most prominent structures labeled typically consisted of a single, curved tubule (visible in both Figure 1, A and B). These organelles often were associated with smaller, apparently vesicular compartments, and were present in one to four copies per cell section. Because they were labeled heavily with the highly specific α1–6 mannose antibodies, these cisternae clearly represent compartments of the secretory pathway. Moreover, their striking morphology, which is distinct from the endoplasmic reticulum or other characterized yeast compartments, suggests they might indeed comprise the yeast Golgi complex. Occasionally we observed up to three of these structures in a parallel stack, but this configuration was atypical and is not shown here (see Figure 3 below).

Immunolocalization of Proteins Required for Vesicular Transport Through the S. cerevisiae Golgi Complex

Antibodies that recognize yeast proteins functionally associated with the Golgi complex typically stain several punctate structures when viewed with indirect immunofluorescence microscopy (e.g., the *YPT1* product, Segev *et al.*, 1988, *SEC7*, Franzusoff *et al.*, 1991a; *KEX2*, Redding *et al.*, 1991; *SEC14*, Cleves *et al.*, 1991; and *RHO1*, McCaffrey *et al.*, 1991). In double-labeling experiments, some of these proteins, such as Kex2p and Sec7p (Franzusoff *et al.*, 1991a) and Kex2p and Sec14p (Cleves *et al.*, 1991), have been shown to co-localize. As many as 10 Kex2p staining structures per mother cell have been observed (Redding *et al.*, 1991), suggesting that yeast cells may contain multiple Golgi compartments. We tested antibodies directed against the Golgi proteins listed above to determine whether cell sections prepared for immunoelectron microscopy also could be labeled. When either the anti-Ypt1p antiserum (Segev *et al.*, 1988) or the anti-Sec7p antiserum (Franzusoff *et al.*, 1991a) were applied to sections from wild-type yeast cells, intensely stained intracellular compartments were observed (Figures 2 and 3). Unfortunately, in the other cases (Kex2p, Sec14p, Rho1p) we have not yet detected a measurable signal, perhaps because of the low abundance of the marker protein or to the loss of epitopes upon fixation.

The product of the yeast *YPT1* gene is a GTP-binding protein that facilitates vesicular traffic through the early portions of the secretory pathway and is located both in the cytosol and on the cytosolic surface of membranes (Segev *et al.*, 1988; Segev, 1991). A *YPT1* homologue (rab1) has been identified in mammals (Haubruck *et al.*, 1989), and anti-Ypt1 antibodies label the mammalian Golgi complex (Segev *et al.*, 1988). In yeast cells, the punctate, immunofluorescent staining pattern observed with the anti-Ypt1p antibodies is altered in cells carrying a mutation in the *SEC7* gene (Segev *et al.*, 1988). It had been shown previously that a *sec7* defect leads to the

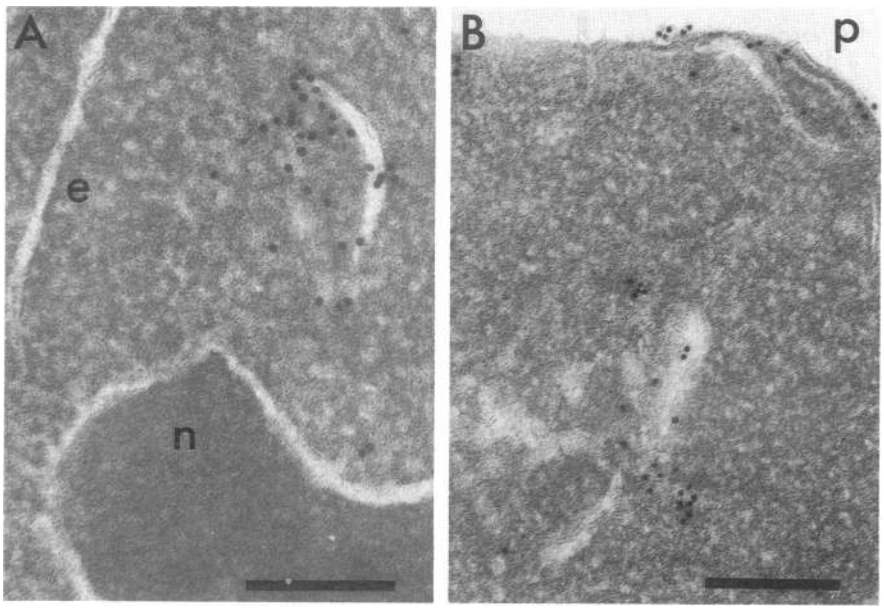

Figure 1. Electron micrograph of yeast Golgi compartments that are immunolabeled with anti-α1–6 mannose antibodies. The antibodies are not associated with the nuclear envelope (n) or with ER tubules (e, see A), but label the Golgi complex and all downstream secretory compartments, including the plasma membrane (p, see B). Bars, 0.2 μm.

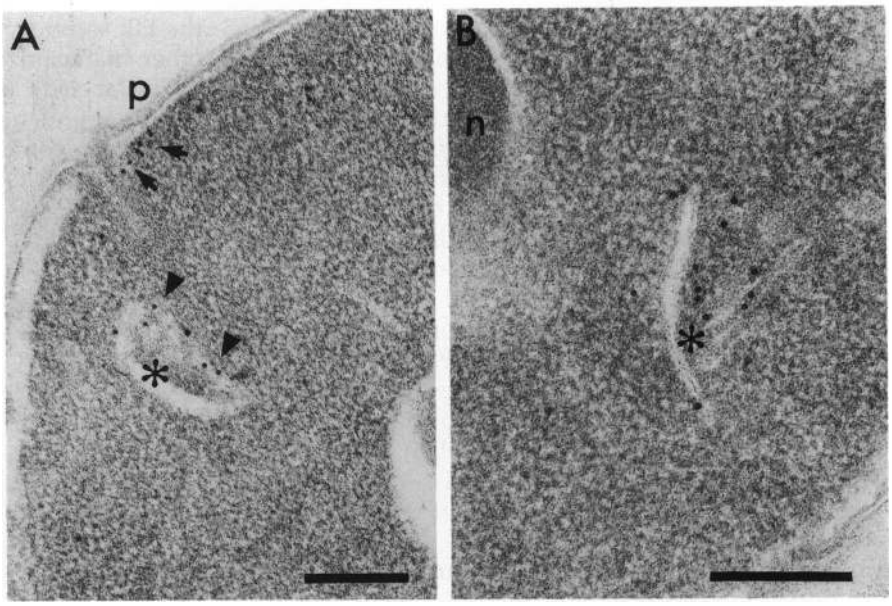

Figure 2. Immunogold labeling of yeast cell sections with anti-Ypt1p antibodies. Asterisks (*) indicate Golgi compartments that are associated with the Ypt1p. (A) the end of an ER tubule is labeled (arrows), as well as vesicles clustered nearby (arrowheads). n, nucleus; p, plasma membrane; bars, 0.2 μm.

accumulation of aberrant Golgi-like structures (Novick *et al.*, 1981).

As shown in Figure 2, when affinity-purified anti-Ypt1p antibodies were applied to cell sections prepared by Method I, compartments similar in morphology to those labeled by the anti-α1–6 mannose antibodies were seen. As before, the labeled structures typically consisted of isolated cisternae, but occasionally as many as three

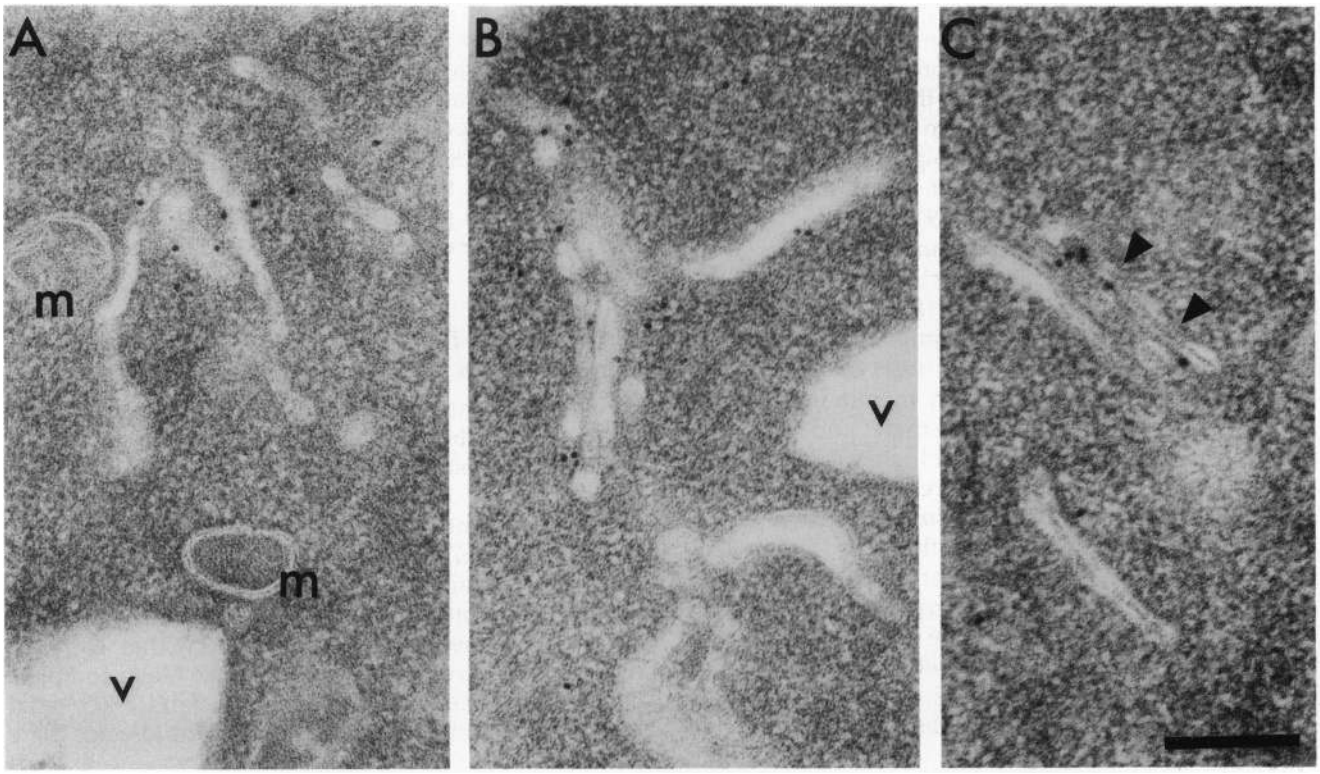

Figure 3. Immunogold labeling of yeast cell sections with anti-Sec7p antibodies. (A and B) several clustered cisternae are associated with Sec7p. (C) arrowheads indicate a parallel stack of labeled cisternae. m, mitochondrion; v, vacuole; Bar, 0.2 μm.

parallel cisternae were seen. In addition, the anti-Ypt1p antibodies were associated with small membranous compartments (measuring ~50 nm in diameter) that surround the cisternae (Figure 2A, arrowheads), and with the ends of some ER tubules (Figure 2A, arrows), although little, if any, localization along the length of ER tubules was observed. Association of the Ypt1 protein with the ER, as well as some localization near presumably vesicular compartments, is consistent with the role that this protein is thought to play in the transport of vesicles from the ER to the Golgi complex (Bacon *et al.*, 1989; Baker *et al.*, 1990; Segev, 1991). As a control, we tested antibodies that recognize the TrpE portion of a TrpE-Ypt1 fusion protein, but have no affinity for Ypt1 itself (see MATERIALS AND METHODS): these failed to label any structures in these sections.

Like the Ypt1 protein, the product of the *SEC7* gene is an essential protein that facilitates vesicular traffic through the secretory pathway (Franzusoff and Schekman, 1989, Franzusoff *et al.*, 1992). Strains with conditional mutations in the *SEC7* gene are defective in protein secretion and accumulate parallel stacks of as many as 11 Golgi-like cisternae (Novick *et al.*, 1981; Svoboda and Necas, 1987). The Sec7p has a predicted molecular mass of 227 kDa (Achstetter *et al.*, 1988) and associates with the cytosolic surface of the membranes of the Golgi complex (Franzusoff *et al.*, 1991a, 1992). Although Sec7p fractionates with both soluble and membrane-associated proteins, a large portion of this protein can be localized in nonmutant cells to discrete structures with immunofluorescence microscopy (Franzusoff *et al.*, 1991a). Finally, the pattern of Sec7p-associated structures is altered in a *sec14* mutant in which the appearance of the Golgi complex changes dramatically upon incubation at the nonpermissive temperature (Franzusoff *et al.*, 1991a).

Affinity-purified Sec7p-specific antibodies (Franzusoff *et al.*, 1991a) were used to label sections from wild-type yeast cells prepared by Method I (Figure 3). Compartments that were morphologically similar to those labeled with the anti-Ypt1p serum were observed. The *Sec7p* was found to be associated with membranous compartments that were generally single and only occasionally arranged into parallel stacks and were consistently surrounded by apparently vesicular compartments (Figure 3). In contrast, substantial staining of ER tubules was not observed. In this case (Figure 3, B and C) we have chosen images that show the relatively rare arrangement of parallel cisternae.

To summarize, in Figures 1–3, we show typical images of the membranous structures labeled by the three antibodies we selected on the basis of their affinity for Golgi proteins. In each case, we observed curved, "banana-shaped" cisternae that were usually single, often associated with vesicles nearby, but occasionally stacked together. These compartments were clearly distinct from the ER, secretory vesicles, vacuoles, mitochondria, and other characterized yeast organelles.

The labeled Golgi structures were also present in surprising abundance. As shown in Table 1, nearly every cell section contained at least one such compartment. Furthermore, the three antibodies label about the same number of Golgi compartments in each cell, and most of these structures (~90%) apparently consisted of a single cisterna (Table 1). Small differences in the fraction of Golgi compartments labeled by each antibody could conceivably reflect variation in the distribution of the protein markers. Alternatively, such differences could merely be the result of variation in the purity and affinity of these antibodies.

Visualization of Yeast Golgi Complexes With Permanganate Staining

Treatment of lightly fixed cells with potassium permanganate results in intense staining of cellular membranes while extracting much of the soluble protein. Although such preparations are generally not useful for immunolocalization experiments, they can reveal the morphological features of membranous compartments. Examination of permanganate-stained *S. cerevisiae* cells showed structures morphologically similar to the cisternae and vesicles labeled with the anti-Ypt1p, anti-Sec7p, and anti-α1–6 mannose antibodies. Moreover, both immunolabeling and permanganate-staining revealed Golgi structures at similar frequencies (Table 1). Figure 4 depicts two such compartments positioned between the nucleus and the cell surface. These Golgi structures appear to be distinct from the ER, which consists of long sheets that extend from the nuclear envelope and underlie the plasma membrane (Preuss *et al.*, 1991). Because the Golgi compartments stain heavily with potassium permanganate, we used this technique to examine the distribution of these compartments in more detail.

Serial Sections Define the Three-Dimensional Structure of the Yeast Golgi Complex

To examine further the structure of Golgi complex and to assess its subcellular distribution, serial sections of permanganate-stained yeast cells measuring 70–80 nm were prepared. The position and structure of the cisternal compartments similar to those labeled by the anti-Ypt1p, anti-Sec7p, and anti-α1–6 mannose antibodies were followed in 135 serial sections through portions of 12 cells. In total, we observed 74 organelles (counted after three-dimensional reconstruction) that were, in cross section, structurally similar to the Golgi complex. Because yeast cells measure ~5 μm (and ~65 serial sections are required to span a cell), on the average there are ~30 Golgi compartments in each yeast cell. Representative serial sections that span completely four such compartments are shown in Figure 5.

Table 1. Abundance of Golgi compartments

	Total Golgi	Total Golgi/section	Labeled Golgi	Labeled Golgi/section	% Golgi labeled	No. of cisternae/ Golgi complex (%)		
						1	2	3
Immunolabeled sections[a]								
anti-α1–6 mannose	109	2.2	92	1.8	84	86	13	1
anti-Ypt1p	140	2.8	96	1.9	69	86	13	1
anti-Sec7p	106	2.1	54	1.1	51	97	7	0
$KMnO_4$-stained sections[b]	310[c]	2.3	NA[d]	NA	NA	83	15	2[e]

[a] Compartments with a characteristic Golgi morphology were counted in thin sections from 50 cells.

[b] Golgi-like compartments visible in 135 serial sections, prior to serial reconstruction.

[c] After serial reconstruction, these structures were found to be portions of 74 Golgi compartments.

[d] NA, not applicable.

[e] After serial reconstruction, 60% of the Golgi compartments were found to consist of a single cisterna, 30% were comprised of two closely associated cisternae, and 10% were stacks of three cisternae.

After serial reconstruction, ~60% of the Golgi compartments were found to consist of a single membranous sac, but stacks of two (30%), and occasionally three (10%), cisternae were also observed. Small vesicles, measuring ~50 nm, were often near these compartments, and occasionally appeared to fuse with the membranous sacs (Figure 5I, arrowhead). The cisternae consistently measured 200–500 nm in length and therefore were easily distinguished from the ER tubules that emerged from the nuclear envelope (Preuss *et al.*, 1991) and varied dramatically in length from one section to the next. These cisternal compartments extended through 3–10 sections, with an average of 5 sections. Thus, the yeast Golgi structures are ~400 nm in diameter with a thickness of ~50 nm. They appear to be shaped much like a bowed, tapered disk (not unlike a frisbee): in cross section, such a disk will appear as a "banana-shaped" cisternal compartment.

The Golgi structures were always discrete; they were not contiguous with the ER or with other organelles. In addition, although the mammalian Golgi complex is near the nucleus as a consequence of its association with the microtubule organizing center (reviewed in Kreis, 1990), the yeast compartments were no closer to the yeast nucleus than they were to the cell wall.

Distribution of the Golgi Complex Through the Yeast Cell Cycle

During cell division, the Golgi, as well as other organelles, must be segregated faithfully to daughter cells. Because *S. cerevisiae* grows and divides asymmetrically, it is well suited to a genetic dissection of the molecular requirements for organelle movement (Yaffe, 1991). Previously, we demonstrated that ER tubules enter growing daughter cells well before other organelles such

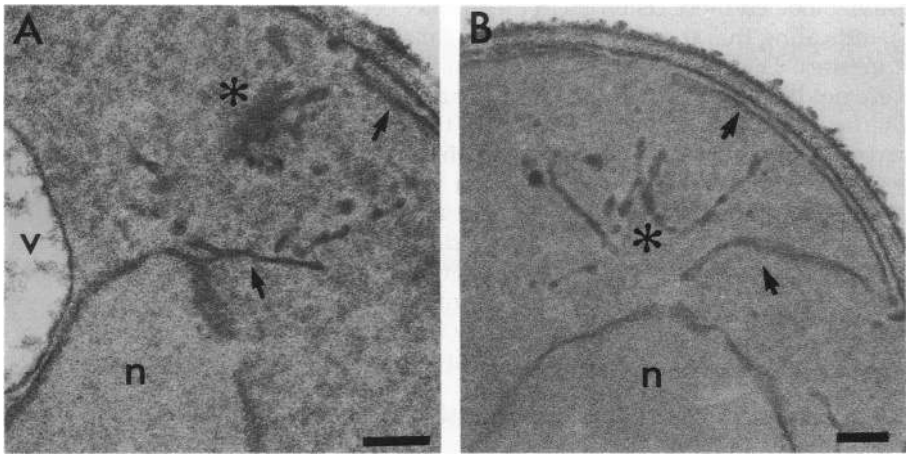

Figure 4. Sections of yeast cells stained with potassium permanganate. Asterisks (*) indicate the position of Golgi-like structures and arrows denote ER membranes that extend from the nuclear envelope or are associated with the cell periphery. n, Nucleus; v, vacuole; bars, 0.2 μm.

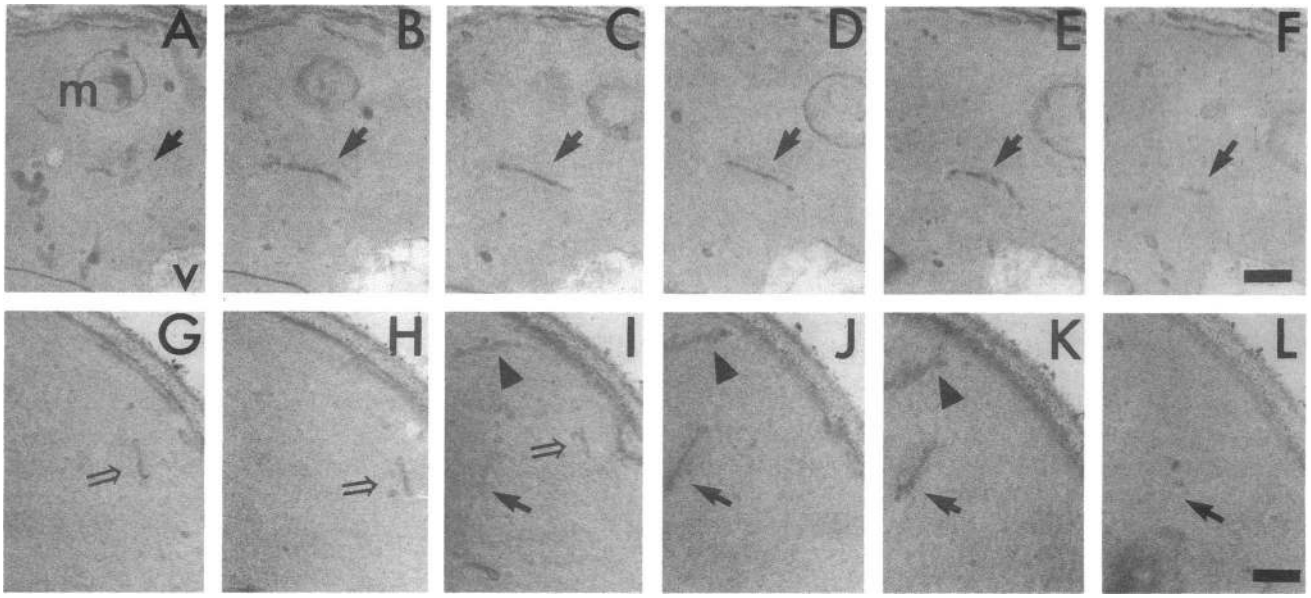

Figure 5. Serial sections (70–80 nm thick) of yeast cells stained with potassium permanganate. (A–F) six consecutive sections from one cell depict a compartment (arrows) like those typically labeled with antibodies to the Golgi proteins. (G–L) consecutive sections from another cell depict compartments as in A–F. The first (⇒) begins in G and extends through the next three sections. The second (arrowheads) and third (→) Golgi compartments begin in 5I and extend through, respectively, three or four sections. A small vesicle in proximity to a flat, cisternal compartment can be seen near the arrowhead in I. m, mitochondrion; v, vacuole; bars, 0.2 μm.

as the vacuole or mitochondrion (Preuss *et al.*, 1991). In addition, immunofluorescence studies have indicated that both the Ypt1 (Segev *et al.*, 1988) and Kex2 (Redding *et al.*, 1991) proteins appear to be concentrated in daughter cells early in the cell cycle. Using immunoelectron microscopy and the markers described above, we followed the segregation of the Golgi compartments throughout the yeast cell cycle.

Newly initiated yeast daughter cells (measuring ≤10% of the diameter of the mother) can be identified easily because they are filled with numerous 80–100 nm secretory vesicles (Matile *et al.*, 1969; Byers, 1981). Several flat cisternae are assembled in the mother cell, just below the base of the growing bud (Matile *et al.*, 1969). Antibodies specific for both the Sec7 and the Ypt1 proteins heavily labeled these cisternae in the mother cell (Figure 6), indicating that these compartments constitute Golgi structures. The secretory vesicles within the small buds were not labeled with the anti-Ypt1p or anti-Sec7p sera (Figure 6). A striking arrangement of the Golgi compartments between the nucleus and the nascent bud was always observed, both in random sections (>100 cases), such as those shown in Figure 6, A and B, as well as in several cells with nascent buds for which we have permanganate-stained serial sections. Labeling with anti-α1–6 mannose antibodies is uninformative in this case; indeed all the structures (secretory vesicles, plasma membrane as well as the putative Golgi) are heavily labeled, as one might expect.

When the daughter cells reach 10–25% of the mother cell diameter, few secretory vesicles are apparent, and those that are present appear to be in the process of fusion with the daughter cell's plasma membrane. By this point in the cell cycle, the Golgi compartments are entering the growing bud, as evidenced by labeling with the Ypt1p and Sec7p antibodies (Figure 7; note Golgi cisternae in the neck, A and B). Thus, the daughter cells inherit maternal Golgi compartments, as well as components of the ER (Preuss *et al.*, 1991), well before mitosis begins. As bud growth proceeds, these compartments assume a distribution similar to that observed in the mother cell.

At the end of the budding cycle, formation of a septum between the mother and daughter cells occurs. Although 80–100 nm secretory vesicles were rarely observed in the mother cell during much of the cell cycle, they were abundant at the site of septum formation (Figure 8A). Short, membranous cisternae typically cluster behind the vesicles at the septum, just as they do during the early stages of budding. These compartments were labeled with Ypt1p and Sec7p antibodies (Figure 8, B and C).

DISCUSSION

It has long been clear that the processes of protein secretion and membrane traffic in *S. cerevisiae* functionally resemble those of other eukaryotes; specifically, there is abundant evidence for yeast Golgi functions (cf. Melançon *et al.*, 1991; Franzusoff, 1992; Pryer *et al.*, 1992; Rothman and Orci, 1992). Indeed, proteins implicated in mammalian Golgi activities can also function in yeast

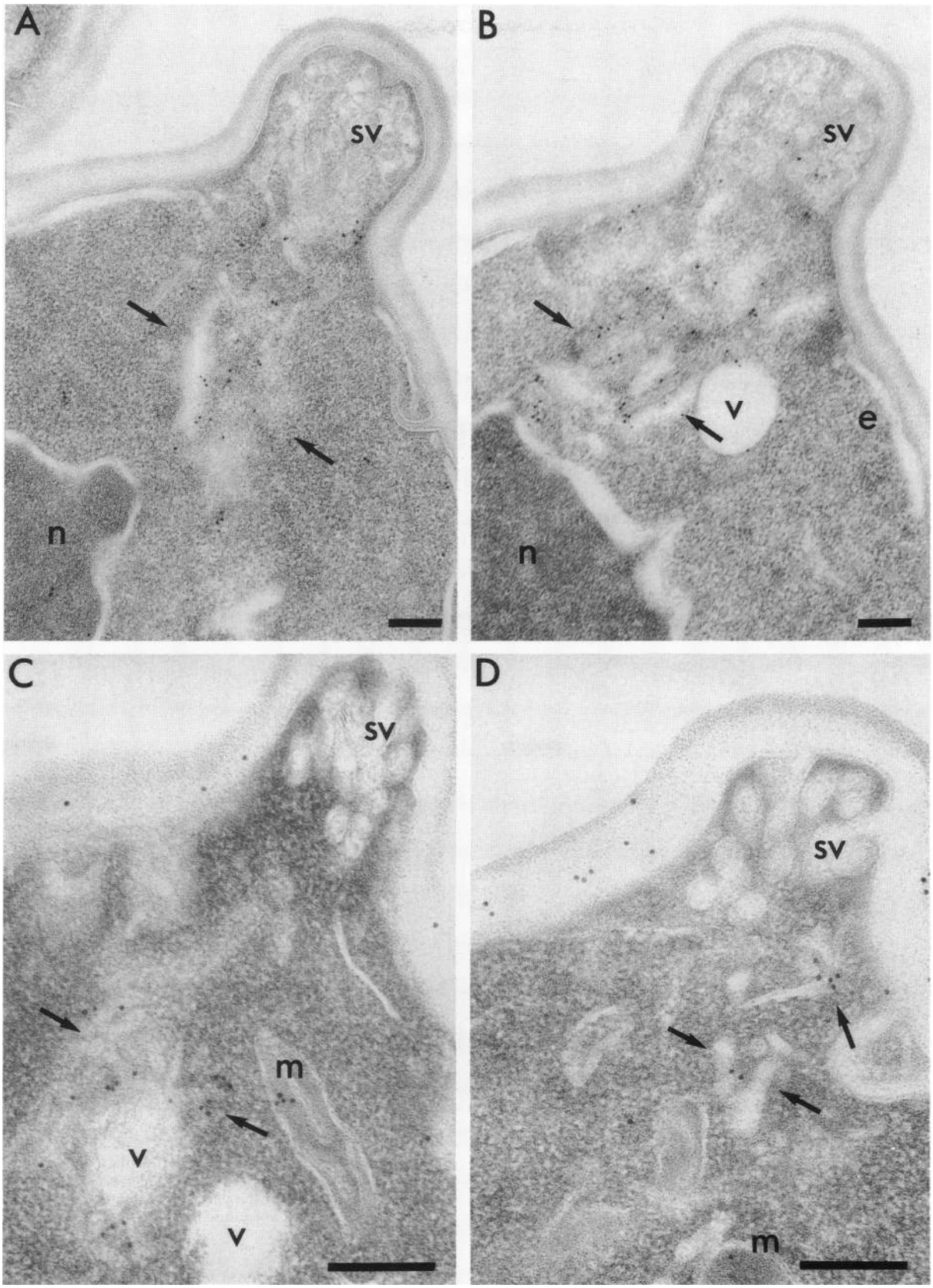

Figure 6. Distribution of Golgi complexes during bud emergence. Yeast cell sections were immunolabeled with anti-Ypt1p antibodies (A and B) or with anti-Sec7p antibodies (C and D). Arrows indicate clusters of membranous compartments that are labeled with the Golgi markers. Each panel shows a cell with a small bud that is filled with secretory vesicles (sv). The anti-Sec7p antibodies occasionally labeled cell walls, but the intracellular signal was apparently specific for the Golgi compartments (see also Figure 3). n, nucleus; sv, secretory vesicles; e, endoplasmic reticulum; v, vacuole; m, mitochondrion; bars, 0.2 μm.

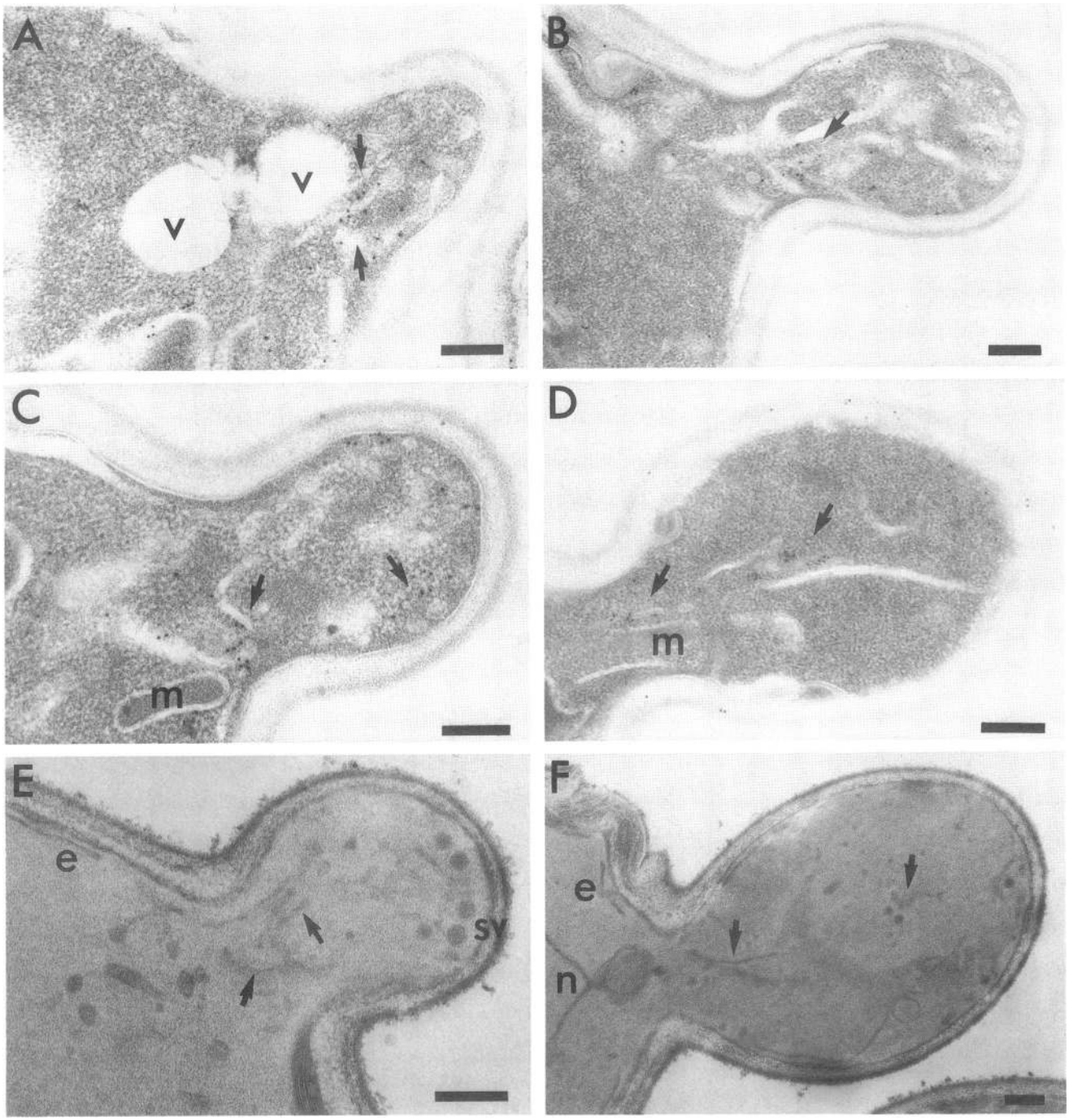

Figure 7. Distribution of Golgi complexes in small-budded yeast cells. Sections were immunolabeled with anti-Ypt1p antibodies (A–C), with anti-Sec7p antibodies (D), or were prepared from $KMnO_4$-stained cells (E and F). Arrows indicate Golgi compartments within the growing daughter cells. (A, C, and E) daughter cells that are slightly larger than those in Figure 6. (B, D, and F) an even later stage in bud growth. Although most of the secretory vesicles have disappeared from these cells, there are still a few near the plasma membrane (e.g., see the cells in A–C and in E). v, vacuole; m, mitochondrion; sv, secretory vesicles; n, nucleus; e, endoplasmic reticulum; bars 0.2 μm.

contexts (e.g., Haubruck *et al.*, 1989). However, the degree of morphological similarity between the *S. cerevisiae* and mammalian secretory organelles (especially the Golgi compartments) has been in doubt. To define the ultrastructure of the yeast Golgi complex (or its functional equivalent), we immunolabeled yeast with antibodies directed against cytosolic, Golgi-associated proteins (Ypt1p and Sec7p), as well as against a

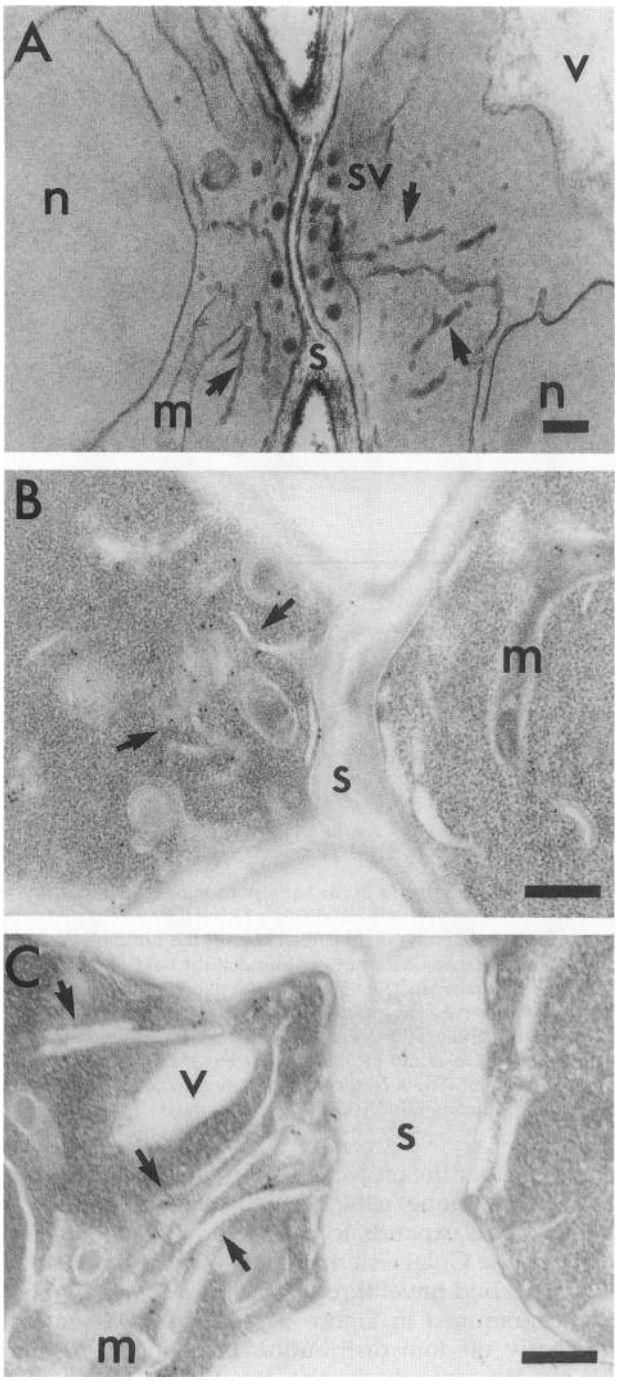

Figure 8. Distribution of Golgi complexes during cytokinesis. Sections of yeast cells stained with potassium permanganate (A) or immunolabeled with antibodies specific for Ypt1p (B) or Sec7p (C). The secretory vesicles (sv) that cluster near the cell septum (s) are apparent in A, and several Golgi cisternae are indicated with arrows (A). In B and C, the Ypt1 and Sec7 proteins are apparently associated with the cisternae (arrows). n, nucleus; sv, secretory vesicles; v, vacuole; m, mitochondrion; s, septum between mother and daughter cells; bars, 0.2 μm.

polysaccharide antigen (α1–6 mannose) that is present within the lumen of the Golgi and subsequent secretory compartments. This approach required no assumptions regarding the appearance of the Golgi complex.

The most significant result shown above is that each of the Golgi-related antigens were found associated with intracellular membranous compartments that were similar in appearance: in cross sections, they generally appeared as short, curved tubules. Morphologically comparable compartments were visualized with a simple permanganate–staining technique. While permanganate-stained sections were unsuitable for immunolocalization experiments, they were an excellent source of material for serial section analysis. With this technique we concluded that the yeast Golgi compartments number ~30 per cell and appear to be slightly concave disks measuring ~400 nm in diameter and 50 nm in thickness. Often vesicles similar in size to those generated during ER to Golgi transport (Kaiser and Schekman, 1990; Franzusoff *et al.*, 1992) apparently cluster around these Golgi cisternae. However, unlike the single Golgi complexes observed in animal cells (Novikoff *et al.*, 1971; Rambourg *et al.*, 1981) or the multi-copy compartments observed in other yeasts, such as *S. pombe* (Johnson *et al.*, 1982; Chappel and Warren, 1989), the *S. cerevisiae* compartments typically are not organized into parallel stacks of membranes. Clusters of three cisternae occur relatively infrequently (~10% of the Golgi bodies consist of such stacks), and larger stacks were not observed. These results are entirely consistent with previous immunofluorescent localization experiments (Segev *et al.*, 1988; Cleves *et al.*, 1991; Franzusoff *et al.*, 1991a; McCaffrey *et al.*, 1991; Redding *et al.*, 1991) that showed that yeast Golgi-associated antigens are dispersed to many locations within yeast cells.

Golgi Subcompartments and Intercisternal Transport

In mammalian cells, isolated Golgi cisterna are generally not observed (Novikoff *et al.*, 1971; Rambourg *et al.*, 1981), and the individual cisterna within a Golgi stack comprise functionally discrete subcompartments. The orderly progression of secretory proteins through these *cis, medial,* and *trans* subcompartments is well documented (reviewed in Kornfeld and Kornfeld, 1985). In the case of *Saccharomyces,* at least three functionally distinct Golgi compartments have been identified through biochemical analysis of secretion-defective yeast strains (Franzusoff and Schekman, 1989; Graham and Emr, 1991; Franzusoff, 1992).

Although it should be possible to distinguish the yeast Golgi subcompartments with the use of immunoelectron microscopy techniques, the antibody reagents described above cannot do so because they label a broad class of Golgi structures. Mannose residues in α1–6 linkages are added to secreted proteins in an "early" Golgi subcom-

partment (Baker *et al.*, 1988; Franzusoff and Schekman, 1989), and these epitopes are still present when the proteins reach their destination at the cell surface. Likewise the Ypt1 protein functions in transport from the ER to the Golgi apparatus (Schmitt *et al.*, 1988) or within early Golgi compartments (Segev *et al.*, 1988), and the Sec7 protein is required for transport through multiple Golgi subcompartments (Franzusoff and Schekman, 1989). Establishing the presence of physically distinct Golgi subcompartments in yeast cells thus awaits the availability of antibodies, suitable for immunoelectron microscopy experiments, that recognize antigens limited to discrete subcompartments.

The dispersed yeast Golgi cisternae might represent homogeneous compartments that contain all of the enzymes responsible Golgi functions. It seems more likely that they are each Golgi subcompartments, each carrying out a given subset of functions. We prefer the latter possibility because others have found that density gradient centrifugation of apparently intact yeast compartments can separate Golgi markers (Cunningham and Wickner, 1989; Cleves *et al.*, 1991). Further, this interpretation minimizes the differences between *S. cerevisiae* and other eukaryotes; according to this view, in *Saccharomyces* most Golgi subcompartments are simply not organized into stacks.

If the subcompartments of the yeast Golgi complex are indeed distributed throughout the cell, a mechanism (perhaps vesicular in nature) that can transport materials from one cisterna to another would be required. Because secretion in *S. cerevisiae* occurs rapidly (yeast proteins can be synthesized and secreted in <5 min; Novick *et al.*, 1981; Schauer *et al.*, 1985), all of the steps required for transport, including presumably vesicle formation, targeting, and fusion, must occur quickly and efficiently. The small size of the *S. cerevisiae* cell might mean that diffusion suffices to allow efficient transport, making extensive stacking of Golgi compartments unnecessary.

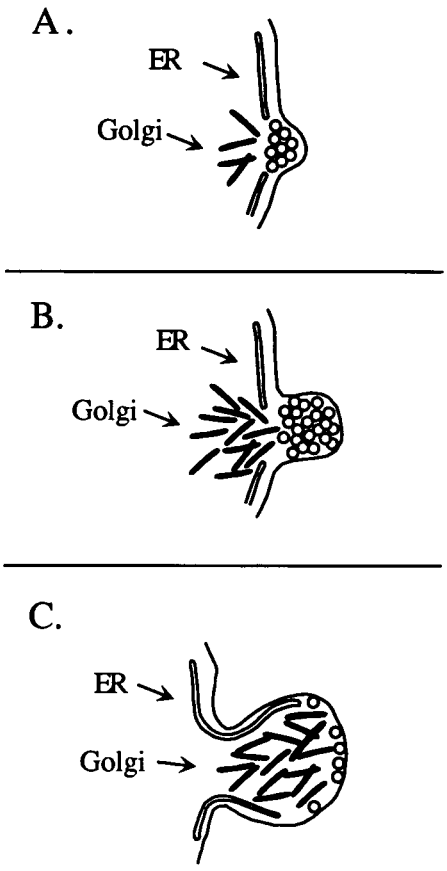

Figure 9. Order of events in the early portion of the budding cycle. (A) Initiation of bud growth. A cluster of secretory vesicles near the plasma membrane is the first visible event in the budding cycle. Invariably, membranous cisternae are found at the base of the growing bud. (B) Bud expansion. The volume of the daughter cell increases as more vesicles arrive. The number of cisternal compartments at the base of the daughter cell increases. (C) Acquisition of secretory compartments. The Golgi and ER membranes enter daughter cells when they are still small. The secretory vesicles are now less abundant and apparently are fusing rapidly with the cell surface.

Distribution of the Golgi Compartments Through the Cell Division Cycle

During the earliest stages of bud formation, the evolving buds contain numerous secretory vesicles of 80–100 nm in diameter (Matile *et al.*, 1969; Byers, 1981). These vesicles accumulate until the buds measure roughly 10% of the diameter of the mother cell, at which point they disappear, possibly because they begin to fuse rapidly with the growing plasma membrane of the bud. Concurrent with the presence of large numbers of secretory vesicles, Golgi compartments (as identified by their antigens) assemble near the bud site (diagrammed in Figure 9, A and B). As many as 20 clustered (although not neatly stacked) cisternae can be observed in the vicinity of the daughter cell, while additional Golgi compartments remain dispersed throughout the mother. Others have noted a similar arrangement of Golgi-like compartments near the projections of cells arrested with the mating pheromone, α-factor (Baba *et al.*, 1989).

As the bud expands to 10–25% of the mother cell diameter, the Golgi cisternae that were clustered at the base of the bud travel through the neck to the daughter cell (diagrammed in Figure 9C) and then assume an apparently random distribution throughout the bud. This result is entirely consistent with the unexpected, unusually strong immunofluorescent staining of young buds previously described with anti-Ypt1 antibodies (Segev *et al.*, 1988) or with anti-Kex2 antibodies (Redding *et al.*, 1991). The immunoelectron microscopy experiments presented here confirm that yeast Golgi compartments are indeed concentrated in the vicinity of and within small buds. The entry of Golgi cisternae can now be viewed as a landmark in bud development that coincides with a reduction in the number of visible secretory vesicles.

The distribution of Golgi compartments in the bud does not change markedly until cytokinesis. Upon formation of the cell septum, prominent clustering of Golgi compartments and 80–100 nm secretory vesicles was observed. The simplest interpretation of this clustering is that at this stage, as in the early bud, rapid deposition of cell surface materials, such as the plasma membrane, the wall and associated proteins is required.

In mammalian cells, vesicular traffic from the ER to the Golgi complex is arrested at the onset of mitosis (Warren, 1985). The mammalian nuclear envelope, ER, and Golgi apparatus are disrupted, forming vesicles that ultimately coalesce to reform the organelles after cytokinesis is complete (Robbins and Gonatas, 1964; Zeligs and Wollman, 1979; Warren, 1985; Lucocq *et al.*, 1987). Fragmentation of these organelles during mitosis might be required for their proper segregation into daughter cells. Alternatively, attachment of the mitotic machinery to chromosomes might require disassembly of the nuclear envelope, and disruption of the ER and Golgi complex could result merely from delays in vesicular transport.

In contrast, protein secretion continues in *S. cerevisiae* cells undergoing mitosis (Makarow, 1988). The nuclear envelope remains intact (Matile *et al.*, 1969; Byers, 1981), and the ER (30% of which is comprised of perinuclear membranes, Preuss *et al.*, 1991) appears to be undisturbed (Makarow, 1988; Preuss and Mulholland, unpublished observations). Makarow (1988) observed Golgi-like structures in yeast cells arrested with nocodazole at mitosis and found that some vesicularization of these Golgi-like structures was evident after a 2.5-h incubation. We did not notice strong differences in Golgi ultrastructure during mitosis. However, the two analyses may not be directly comparable, because cell culture conditions, sample preparation, and fixation techniques differed.

All eukaryotic cells face the problem of segregating subcellular organelles like the Golgi to the daughter cells, a process that, in mammalian cells, might require the fragmentation of the Golgi complex. Our experiments indicate that segregation of the *S. cerevisiae* Golgi is an orderly process that occurs at a precise point during the cell cycle as apparently preformed Golgi cisternae move through the neck between mother and bud. Similarly, the ER (Preuss *et al.*, 1991), mitochondria (Yaffe, 1991), and vacuoles (Weisman *et al.*, 1990) appear to enter daughter cells in an orderly fashion.

Implications for Bud Growth

The appearance of ER and Golgi structures in small buds implies that the developing daughter cells might have a functioning secretory pathway from an early stage in their growth. Because the bulk of protein secretion in yeast cells takes place from the surface of the growing bud, (Tkacz and Lampen, 1973; Field and Schekman, 1980), it now seems likely that much of daughter cell growth results from the activity of secretory organelles (ER, Golgi, and secretory vesicles) that are present within the bud, rather than from those organelles remaining in the mother. Instead of requiring transport of large numbers of secretory vesicles from the mother to the bud, our results suggest that the mRNAs that encode secreted proteins might diffuse from the mother cell into the bud where translation, translocation into the ER, and all subsequent events along the secretory pathway take place. In support of this view of secretion, we have rarely observed secretory vesicles in mother cells; those that do form appear mainly at the cell septum. While initiation of bud growth must, by definition, involve the secretory apparatus of the mother cell, *S. cerevisiae* might subsequently achieve its asymmetric growth by the segregation of early secretory organelles, such as the ER and Golgi complex, rather than secretory vesicles.

The mechanisms that move the ER and Golgi complex as well as the other membranous organelles into growing buds are as yet unknown. In vitro studies performed in other eukaryotic systems have implicated microtubules and the actin cytoskeleton in the partitioning of organelles (Adams and Pollard, 1986; Vale, 1987). In *S. cerevisiae*, the actin cytoskeleton might also play a pivotal role in organelle inheritance. It is asymmetrically organized throughout the growth of the daughter cell (Adams and Pringle, 1984; Kilmartin and Adams, 1984), and mutations that disrupt actin function also interrupt polarized cell growth (Novick and Botstein, 1985). The availability of marker proteins, high-resolution techniques, such as those described here, and a number of informative mutant strains should provide the means for identifying the components required for orderly and timely organelle segregation into the daughter cells of yeast.

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