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Golgi inheritance in small buds of Saccharomyces cerevisiae is linked to endoplasmic reticulum inheritance

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According to the cisternal maturation hypothesis, endoplasmic reticulum (ER)-derived membranes nucleate new Golgi cisternae. The yeast Saccharomyces cerevisiae offers a unique opportunity to test this idea because small buds contain both ER and Golgi structures early in the cell cycle. We previously predicted that mutants defective in ER inheritance also would show defects in Golgi inheritance. Surprisingly, studies of S. cerevisiae have not revealed the expected link between ER and Golgi inheritance. Here, we revisit this issue by generating mutant strains in which many of the small buds are devoid of detectable ER. These strains also show defects in the inheritance of both early and late Golgi cisternae. Strikingly, virtually all of the buds that lack ER also lack early Golgi cisternae. Our results fit with the idea that membranes exported from the ER coalesce with vesicles derived from existing Golgi compartments to generate new Golgi cisternae. This basic mechanism of Golgi inheritance may be conserved from yeast to vertebrate cells.

yeast | organelle | mitosis

An essential feature of eukaryotic cell division is the inheritance of organelles (1). In the case of the Golgi apparatus, studies of inheritance are providing insights into Golgi biogenesis (2). During vertebrate mitosis, the juxtanuclear Golgi ribbon breaks down in a process that is coordinated with the cell cycle machinery (2–4). It has been suggested that the Golgi fuses with the endoplasmic reticulum (ER) during mitosis and then reemerges as a separate organelle during cytokinesis (5). However, the preponderance of evidence now indicates that the mitotic Golgi consists mainly of small membrane fragments that are distinct from the ER (6–8). Yet Golgi reassembly during cytokinesis is inhibited by blocking ER export with a dominant-negative form of the Sar-1 GTPase (5, 9), possibly indicating a role for the ER in vertebrate Golgi inheritance.

Studies of other cell types also have revealed an involvement of the ER in Golgi inheritance. We found that in the yeast Pichia pastoris, Golgi stacks are found next to transitional ER (tER) sites, which contain nascent COPII transport vesicles (10, 11). As the cell grows, Golgi stacks form de novo in conjunction with newly formed tER sites (12). The implication is that tER sites in P. pastoris generate Golgi stacks. Similar results were obtained recently with the protozoan parasite Trypanosoma brucei (13). These data are consistent with the cisternal maturation hypothesis, which postulates that new Golgi cisternae are nucleated by membranes exported from the ER (14).

Does Golgi inheritance always require input from the ER? A promising organism for exploring this idea is the budding yeast Saccharomyces cerevisiae, which has been widely used to study organelle inheritance (15, 16). The ER and Golgi are among the first organelles to be found in the emerging bud (17–19). Here, we are focusing on this early stage of the cell cycle and are defining inheritance as the initial appearance of ER and Golgi structures in the bud. We predicted that the inheritance of Golgi cisternae would depend on the prior inheritance of ER membranes (19). However, the experimental results to date have offered no support for this model. In previous work, we labeled S. cerevisiae cells with the late (trans) Golgi marker Sec7p-GFP and then isolated mutants defective in Golgi inheritance (19). That screen yielded multiple alleles of CDC1, which encodes a putative phosphoesterase of unknown function (20). Additional experiments suggested that the actin cytoskeleton and the type V myosin Myo2p are important for late Golgi inheritance (19). By contrast, the inheritance of ER and early (cis) Golgi cisternae in cdc1 and myo2 mutant strains is normal. To explain these findings, we postulated that the primary function of Cdc1p and the actomyosin system in Golgi inheritance is the retention of Golgi cisternae in the bud. ER membranes present in the bud would give rise to early Golgi cisternae, which would mature into late Golgi cisternae while being retained in the bud. Thus, mutants defective in ER inheritance also should have defects in the inheritance of both early and late Golgi cisternae. It became possible to test this model when S. cerevisiae mutants defective in ER inheritance were identified (21–23). Surprisingly, Golgi inheritance in these mutants was found to be normal (21–23).

To take an in-depth look at this issue, we have now analyzed both existing and newly generated ER inheritance mutants. We found that small buds lacking detectable ER also lack early Golgi cisternae. These results demonstrate that Golgi inheritance in S. cerevisiae is indeed linked to ER inheritance.

Methods

Strain Construction. All yeast strains were derivatives of JK9–3d (24). Fluorescent protein constructs were made by using the EGFP, enhanced yellow fluorescent protein (EYFP), and enhanced cyan fluorescent protein (ECFP) genes (BD Biosciences Clontech). In some cases, increased fluorescence was obtained by creating constructs with three tandem copies of EGFP, EYFP, or ECFP (19). Strains expressing SEC21 fused to a triple-EGFP or triple-EYFP cassette were created as described in ref. 19. Strains expressing SEC61-GFP or SEC35-GFP were generated by using a pop-in/pop-out procedure (19) to add a triple-EGFP cassette to the 3' end of the chromosomal gene. Strains expressing HMG1-GFP or HMG1-ECFP were created by amplifying the first 703 codons of HMG1 by PCR, fusing this gene fragment upstream of a single EGFP cassette or a triple-ECFP cassette, inserting the fusion gene into a derivative of the integrating vector YIplac204 (25) between the TP11 promoter and the CYC1 transcription terminator, and linearizing with Bsa361 for integration at the TRP1 locus.

The aux1Δ, myo4Δ, and seb2Δ mutations were introduced into JK9–3da by transformation with kanMX gene disruption cassettes (26). The seb1Δ mutation was generated with an analogous LEU2 gene disruption cassette.

Abbreviations: ER, endoplasmic reticulum; tER, transitional ER; DIC, differential interference contrast; ECFP, enhanced cyan fluorescent protein; EYFP, enhanced yellow fluorescent protein.

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Microscopy. Fluorescence and differential interference contrast (DIC) imaging were performed essentially as described in ref. 10. Cells that had been grown at 23°C and then fixed in formaldehyde and glutaraldehyde were flattened between a slide and coverslip, and 10 images were captured at 0.1-μm intervals along the z-axis to capture all of the fluorescence in the cells. Appropriate bandpass filters (Chroma Technology, Rockingham, VT) were used to visualize GFP, cyan and yellow fluorescent proteins, and Alexa 594 phalloidin. Each image was deblurred with OPENLAB software (Improvision, Coventry, U.K.) by using a 2D deconvolution algorithm. For projections, the relevant image planes were merged.

Identification of the sec8–599 Allele. To isolate ER inheritance mutants, the haploid strain BGY418, which contains the ER marker GFP-HDEL (26), was mutagenized to 13% survival with ethyl methanesulfonate. Temperature-sensitive clones were identified by using a rapid screening method (19). To screen for ER inheritance defects, the mutant clones were grown to log phase at room temperature in 96-well plates, which then were shifted to 37°C for 2 h. Aliquots of the cultures were viewed by DIC imaging to identify 20 small buds, each of which was examined by fluorescence microscopy for the presence of GFP-HDEL fluorescence in the bud.

One mutant that consistently showed buds with no visible fluorescence was chosen for further analysis. To identify the corresponding mutation, a backcrossed strain that retained the temperature sensitivity and ER inheritance phenotypes was transformed with a URA3-CEN genomic library (27), and transformants were selected for growth at 37°C. The complementing plasmid was isolated, and fragments of the genomic DNA insert were PCR amplified and tested for complementation ability. Full complementation was obtained with a DNA fragment spanning from ~500 bp upstream to 500 bp downstream of the SEC8 ORF. Sequencing of PCR-amplified genomic DNA from the parental and mutant strains identified the sec8–599 mutation.

This mutation was reintroduced into the parental J9K-3a strain as follows. A DNA fragment spanning from 755 bp upstream to 965 bp downstream of the sec8–599 mutation was PCR amplified from the originally isolated mutant strain, and cloned into YIpac211 (25) that had been digested with PvuII. The resulting construct was linearized with SpeI for integration at the endogenous SEC8 locus. The integrated plasmid was popped out by using 5-fluoroorotic acid, and the resulting colonies were tested for temperature sensitivity. The presence of the sec8–599 mutation in a candidate clone was confirmed by sequencing of PCR-amplified genomic DNA.

Quantitation of Organelle Inheritance. Yeast strains were grown at 23°C and then fixed with formaldehyde and glutaraldehyde (10). We measured organelle inheritance by using the previously described all-or-none method (19), in which a bud was scored simply for the presence or absence of detectable fluorescence from the marker protein. Buds were identified by using DIC micrographs and assigned to class 1–5 based on size. Each bud then was examined in merged DIC and fluorescence micrographs for the presence of the marker protein. For each of the graphs shown in Figs. 1, 2, and 4, at least two separate experiments were performed under identical conditions, and at least 40 small buds were analyzed for each bud size class.

Results and Discussion

Truncating the Sec3p Subunit of the Exocyst Produces Small Buds That Lack Detectable ER. The three previously described ER inheritance mutants carry deletions of either the AUX1 gene, which encodes a J-domain chaperone (21); the SEC3 gene, which encodes a subunit of the vesicle tethering complex known as the exocyst (22); or the MYO7 gene, which encodes a type V myosin (23). The sec3Δ allele is lethal in our genetic background (data not shown), but we were able to generate aux1Δ and myo4Δ strains. These strains were labeled with transmembrane ER markers, either Hmg1p-GFP or Sec61p-GFP (21, 22). Both markers are likely to be specific for the ER because (i) cells containing these markers showed no increase in cytosolic fluorescence; (ii) Hmg1p-CFP did not label the punctate structures that were labeled by the early Golgi marker Sec21p-YFP (see below and Fig. 3); and (iii) Sec61p is known to be excluded from ER-derived COPII vesicles (28). Consistent with the published reports, we saw reduced amounts of ER in buds of the aux1Δ and myo4Δ strains. When cells were photographed by using single-image planes, wild-type cells yielded a GFP fluorescence signal in nearly all of the small buds, whereas many buds of the aux1Δ and myo4Δ strains showed no visible fluorescence (Fig. 1A).

However, when aux1Δ and myo4Δ cells were photographed by using multiple-image planes, the projected z-stack images revealed that the buds usually had some fluorescence (Fig. 1A). We quantified ER inheritance by using the stringent criterion of whether a projected image showed any detectable fluorescence in the bud. By this measure, the aux1Δ and myo4Δ strains had no significant defect in ER inheritance (Fig. 1B).

To generate novel ER inheritance mutants that would be more suitable for our analysis, we performed a genetic screen by using a strain with a fluorescently labeled ER (19). This strain was mutagenized to generate 1,344 temperature-sensitive mutants, which were examined individually by fluorescence and DIC microscopy. One of the mutant strains showed a reproducible increase in the fraction of small buds devoid of detectable ER. The mutation was recessive, and it cosegregated with the temperature-sensitivity phenotype (data not shown). Transformation with a genomic library in a centromeric vector revealed that the mutant phenotypes could be complemented by the SEC8 gene. Sequencing the SEC8 locus in the mutant strain identified a C-to-T base change at position 1795 that changed codon 599 to a stop codon, thereby removing nearly half of the 1,065-amino acid Sec3p protein. We designated this allele sec8–599. Introducing the sec8–599 mutation into the parental strain recapitulated the temperature sensitivity and the ER inheritance defect, confirming that this mutation was responsible for the observed phenotypes.

Sec3p is part of the exocyst complex, which tethers post-Golgi transport carriers to the plasma membrane (29). We considered the possibility that the sec8–599 mutation might be affecting ER inheritance indirectly by disrupting the actin cytoskeleton, but actin organization was normal in sec8–599 cells (data not shown). A more direct role for Sec3p in ER inheritance seems likely because the exocyst interacts genetically and physically with the ER translocon (30–32) and probably mediates an early docking stage of ER inheritance (33, 34). As described above, other investigators reported that ER inheritance was compromised by deleting the exocyst subunit Sec3p (22). The sec8–599 mutation probably has a similar effect but has the advantage of being tolerated in our strain background.

When the sec8–599 strain was analyzed by using Hmg1p-GFP or Sec61p-GFP, the small buds frequently lacked detectable GFP fluorescence (Fig. 1A and B). This phenomenon was equally pronounced at 23°C and 37°C, so we performed all subsequent analyses at the permissive temperature of 23°C. The ER inheritance defect was particularly evident in the smallest class 1 and 2 buds (19), which ranged from ~0.8% to 3.6% of the mother cell volume. It is conceivable that some of these buds contained small amounts of ER that were below our threshold for detection. However, the markers that we used were bright enough in the mother cells to label structures resembling individual ER tubules, and there was a clear qualitative difference between buds that contained visible GFP fluorescence and those that did not. We therefore consider it likely that the buds scored as negative in our assay truly were devoid of ER.
Mutants Defective in ER Inheritance Are also Defective in Early Golgi Inheritance. To assess the inheritance of early Golgi cisternae in the sec8–599 strain, we fused GFP either to the Sec21p subunit of the COPI vesicle coat (19, 35) or to the Sec35p subunit of the intra-Golgi COG tethering complex (36). Sec21p-GFP and Sec35p-GFP are defined here as early Golgi markers because they colocalize with each other and with Golgi glycosyltransferases but exhibit only limited colocalization with Sec7p (19, 37). We quantified early Golgi inheritance in an all-or-none manner (19) by scoring buds as either containing or lacking at least one labeled cisterna. The sec8–599 strain showed a pronounced delay in early Golgi inheritance as determined by using either Sec21p-GFP or Sec35p-GFP (Fig. 2A). For comparison, the aux1Δ and myo4Δ strains showed normal inheritance of Sec35p-GFP (Fig. 2A). Moreover, we found previously that cdcl and myo2 strains exhibited normal inheritance of Sec21p-GFP (19). The sec8–599 strain is therefore, to our knowledge, the first identified mutant with a defect in early Golgi inheritance.

It was important to confirm that the link between defective ER inheritance and defective early Golgi inheritance is a general
phenomenon. For this purpose, we sought to generate an additional ER inheritance mutant. The exocyst reportedly interacts with the ER translocon Sec61β subunit, which is encoded in \textit{S. cerevisiae} by the functionally redundant genes \textit{SEB1} and \textit{SEB2} (38). \textit{seb1Δ seb2Δ} strains are viable, so we generated this double deletion in our strain background. The \textit{seb1Δ seb2Δ} strain showed an ER inheritance defect in class 1 and 2 buds (Fig. 2B). This defect was milder than the one seen with the \textit{sec8–599} mutation, suggesting that the exocyst might interact with other ER components in addition to Sec61β; however, the \textit{seb1Δ seb2Δ} phenotype was strong enough to use for our experiments. As expected, the \textit{seb1Δ seb2Δ} strain showed a delay in the inheritance of early Golgi cisternae (Fig. 2B).

\textbf{Golgi Inheritance Is Linked to ER Inheritance.} A caveat to these results is that the effects of the \textit{sec8–599} and \textit{seb1Δ seb2Δ} mutations on Golgi inheritance could be independent of the effects on ER inheritance. To rule out this possibility, we double-labeled the mutant strains with the ER marker Hmg1p-CFP and the early Golgi marker Sec21p-YFP and then examined ER and early Golgi inheritance in the same cells (Fig. 3). Because ER and Golgi inheritance defects are most pronounced in the smallest buds, the analysis was limited to buds that qualified as class 1 or 2. To determine whether early Golgi inheritance was associated with ER inheritance, we constructed a contingency table from the data (Table 1) and used the Fisher–Irwin exact test for independence (39). This statistical analysis indicated with a very high level of confidence \((p < 10^{-10})\) that early Golgi inheritance is linked to ER inheritance.

Strikingly, we found that buds lacking ER membranes almost always lacked early Golgi cisternae (Fig. 3 and Table 1). With the \textit{sec8–599} strain, 107 small buds lacked ER membranes, and 104 of these buds also lacked early Golgi cisternae. In the remaining three cases, ER membranes were present near the bud, and a
which detectable small Golgi subunits. Data are presented as mean ± SD and were analyzed using Student’s t-test. *p < 0.05. **p < 0.01. ***p < 0.001. 1. However, this discrepancy is not explained by the presence of Sec8p in the exocyst translocon (34). 2. Therefore, the effect of Sec8p on Golgi fusion is likely to be more complex than previously thought. 3. For example, Sec8p may act to facilitate the recruitment of other membrane proteins to the Golgi complex. 4. These results suggest that Sec8p plays a critical role in regulating Golgi function and that its loss leads to altered membrane trafficking and Golgi organization.

Table 1. Quantitation of ER inheritance and early Golgi inheritance in the same cells

<table>
<thead>
<tr>
<th></th>
<th>Small buds containing early Golgi</th>
<th>Small buds lacking early Golgi</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>sec8-599</td>
<td>20</td>
<td>26</td>
<td>46</td>
</tr>
<tr>
<td>Small buds lacking ER</td>
<td>3</td>
<td>104</td>
<td>107</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>130</td>
<td>153</td>
</tr>
<tr>
<td>seb1 seb2Δ</td>
<td>108</td>
<td>40</td>
<td>148</td>
</tr>
<tr>
<td>Small buds lacking ER</td>
<td>2</td>
<td>53</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>93</td>
<td>203</td>
</tr>
</tbody>
</table>

*Mutant strains were labeled with the ER marker Hmg1p-CFP and the early Golgi marker Sec21p-YFP. Small buds were defined as those that fell into class 1 or 2. We examined a total of 153 small buds for the sec8-599 strain and 202 small buds for the seb1 seb2Δ strain. The resulting data were analyzed by using the Fisher–Irwin exact test (one-tailed). The probability that early Golgi inheritance was independent of ER inheritance was calculated to be 9.6 × 10⁻⁶ for the sec8-599 strain and 1.0 × 10⁻⁶ for the seb1 seb2Δ strain.

single Golgi cisterna was located next to the ER and just beyond the bud neck (data not shown). Similar results were obtained with the seb1Δ seb2Δ strain: 55 small buds lacked ER membranes, and 53 of these buds also lacked early Golgi cisternae. By contrast, of the small buds that contained ER membranes, many also contained early Golgi cisternae (Table 1). A possible interpretation is that the sec8-599 and seb1Δ seb2Δ mutations cause a subset of the cells to be severely defective for early Golgi inheritance but only somewhat defective for ER inheritance. However, we prefer the simpler interpretation that early Golgi inheritance depends on ER inheritance.

Our results do not address how the presence of ER in the bud might lead to the presence of early Golgi cisternae. It is possible that early Golgi cisternae arise in the mother cell and are captured by ER membranes in the bud. A more likely mechanism is that early Golgi cisternae arise in the bud by a process that requires export from the ER. Consistent with this idea, COPII coat proteins are found in very small buds (19).

If the bud acquires late Golgi cisternae primarily by the maturation of early Golgi cisternae, then preventing early Golgi inheritance also should prevent late Golgi inheritance. Indeed, when Sec7p-GFP was used as a marker (19), sec8-599 cells showed a pronounced delay in late Golgi inheritance (Fig. 4). Thus, all of our data are compatible with the hypothesis that ER-derived membranes in the bud nucleate the formation of early Golgi cisternae, which then mature into late Golgi cisternae (40).

Conclusions

This work extends earlier studies of ER and Golgi inheritance in *S. cerevisiae*. The first stage of ER inheritance seems to involve an exocyst–translocon interaction that tethers ER membranes to the tip of the emerging bud (33, 34). Sec6p and Seb1p/Seb2p might participate directly in this tethering reaction or might influence the tethering activities of other exocyst and translocon subunits. Aux1p and Myo4p likely act downstream of the initial tethering step to ensure that the growing bud contains a full complement of ER material (34). In previous characterization of ER inheritance mutants, no defects in Golgi inheritance were observed (21–23), but this apparent discrepancy can be explained by differences in the analysis methods and the properties of the mutant strains. Our experiments focused on buds that contained no detectable ER membranes. Such buds are abundant only in mutants with a compromised exocyst–translocon interaction, and even in those strains, only the smallest buds lack detectable ER. The previous studies of ER inheritance focused mainly on larger buds that showed a dramatic but incomplete reduction of ER membranes (see Fig. 1A). We infer that even a small amount of ER in the bud will promote Golgi inheritance.

Are Golgi cisternae in the bud derived exclusively from the ER? Under experimental conditions in which the entire vertebrate Golgi fuses with the ER, Golgi components subsequently can reemerge to form new cisternae (41). However, this mechanism is unlikely to operate during normal growth because the steady-state pool of Golgi components in the ER is probably small (42). Many peripheral Golgi proteins cycle through the

![Fig. 4. Late Golgi inheritance is delayed in the sec8-599 mutant. (A) Wild-type and sec8-599 cells were labeled with the late Golgi marker Sec7p-GFP. In these projected images, the arrowheads indicate typical small buds that either contain Golgi cisternae in the wild-type cells or lack Golgi cisternae in the sec8-599 cells. (Scale bar: 2 μm.) (B) The late Golgi inheritance defect in the sec8-599 strain was quantified as in Fig. 2.](image-url)
cytosol (43), and transmembrane Golgi proteins may travel in small vesicles, which diffuse through the cytosol quite rapidly (7). We favor the interpretation that ER-derived membranes fuse with Golgi-derived vesicles to form new cisternae, which recruit peripheral Golgi proteins from the cytosol (44). Thus, the biogenesis of Golgi cisternae would involve contributions both from the ER and from existing Golgi elements. Evidence for such a mechanism was provided by a study of Golgi inheritance in T. brucei (13).

The combined data suggest the following unified model for Golgi inheritance in all eukaryotes. COP II-dependent export from the ER generates a “platform” that nucleates the assembly of new Golgi cisternae. Such export can occur when ER membranes enter the bud of S. cerevisiae, when tER sites form in P. pastoris or T. brucei, or when vertebrate tER sites are reactivated during cytokinesis (9, 45). The ER-derived platform captures and fuses with vesicles derived from existing Golgi compartments, resulting in the formation of new Golgi cisternae. In this view, the reassembly of the vertebrate Golgi at the end of mitosis is mechanistically similar to the inheritance of Golgi cisternae in S. cerevisiae.

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