The Coatamer-interacting Protein Dsl1p Is Required for Golgi-to-Endoplasmic Reticulum Retrieval in Yeast*

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Sec22p is an endoplasmic reticulum (ER)-Golgi v-SNARE protein whose retrieval from the Golgi compartment to the endoplasmic reticulum (ER) is mediated by COPI vesicles. Whether Sec22p exhibits its primary role at the ER or the Golgi apparatus is still a matter of debate. To determine the role of Sec22p in intracellular transport more precisely, we performed a synthetic lethality screen. We isolated mutant yeast strains in which SEC22 gene function, which in a wild type strain background is non-essential for cell viability, has become essential. In this way a novel temperature-sensitive mutant allele, dsl1-22, of the essential gene DSL1 was obtained. The dsl1-22 mutation causes severe defects in Golgi-to-ER retrieval of ER-resident SNARE proteins and integral membrane proteins harboring a C-terminal KKXX retrieval motif, as well as of the soluble ER protein BiP/Kar2p, which utilizes the HDEL receptor, Erg2p, for its recycling to the ER. DSL1 interacts genetically with mutations that affect components of the Golgi-to-ER recycling machinery, namely sec20-1, tip20-5, and COPII-encoding genes. Furthermore, we demonstrate that Dsl1p is a peripheral membrane protein, which in vitro specifically binds to coatamer, the major component of the protein coat of COPI vesicles.

Membrane-bound compartments in eukaryotic cells can fuse directly as shown for the endoplasmic reticulum (ER) and mitotic Golgi fragments as well as endosomal and lysosomal compartments (homotypic fusion; see Ref. 1). However, v-transport between distinct compartments mainly involves small coated vesicles whose formation from the donor membrane is mediated by proteinaceous coats, either COPI, COPII, or clathrin. After uncoating, vesicles fuse selectively with an acceptor membrane (heterotypic fusion; see Ref. 2). Both homotypic and heterotypic fusion events rely on specific attachment reactions to guarantee that only appropriate membranes can mix. The membrane attachment itself consists of two steps, tethering and docking, involving different sets of proteins (3, 4).

Tethering factors are peripherally membrane-associated protein complexes consisting of up to 10 different subunits, which share little sequence similarity.

The subsequent docking stage involves specific sets of membrane-anchored proteins, so-called SNARE proteins (SNARE is soluble NSF (for N-ethylmaleimide-sensitive fusion protein) attachment protein receptor) (5–7). SNAREs are inserted into the membrane either by a C-terminal transmembrane domain or through lipid moieties attached to C-terminal cysteine residues. In contrast to the tethering factors, all known SNARE proteins are members of either of three protein families: the syntaxins, the synaptobrevins or VAMPs, and the SNAP-25 family members. To induce membrane fusion, SNARE proteins from apposed membranes must interact in trans. The formation of a stable four-helix bundle may generate enough energy to promote mixing of the lipid bilayer (8–10).

Lipid mixing experiments using SNARE complexes reconstituted into lipid bilayer vesicles indicated that only cognate SNARE combinations are able to induce fusion (11). However, SNARE proteins are rather promiscuous when the formation of the tight SDS or heat-resistant SNARE complexes is analyzed (12, 13). Moreover, SNARE proteins can be part of more than one SNARE complex in vivo (14), and some SNARE proteins can functionally replace each other (15, 16). In vitro the synaptobrevin/VAMP homologs Snc1p and Snc2p in yeast can be replaced by two other members of the synaptobrevin family, the ER-Golgi SNARE Sec22p and the vacuolar SNARE Nyv1p (11). However, these SNAREs are unable to replace Snc1/2p in vivo (17), probably because they are retained in their specific compartments. Thus, the targeting of SNAREs to the right compartment is one way to increase the specificity of intracellular membrane attachment/fusion events.

We analyzed previously (18) the targeting of the ER-to-Golgi SNARE Sec22p and show that the correct targeting of Sec22p involves its recycling from the Golgi to the ER via COPI-coated vesicles. In this respect, Sec22p as well as Bos1p (19) behave like ER-resident proteins that carry a KKXX ER-retrieval signal (20). The coat of COPI vesicles in mammalian cells and yeast consists of seven subunits (α, β, β′, γ, δ, ε, and ζ-COP) and the small GTPase, ARF1 (21).

The observations made by Letourneur et al. (20) and Cosson et al. (22) that KXXX-tagged proteins require COPI components for retrieval from Golgi to the ER provided first evidence that COPI vesicles mediate this retrograde transport. The same is true not only for Sec22p but also for other yeast proteins that recycle from Golgi to ER, for example, Emp47p, a Golgi lectin-like protein; Erd2p, the HDEL receptor; Sed5p, a Golgi-localized syntaxin homolog; and Mnn1p, a glycosyltransferase (23–26). How Sec22p is sorted into COPI vesicles is currently unknown. Moreover, the function of Sec22p is not entirely understood. The SEC22 gene was first isolated by us as a multicopy suppressor of defects in the small GTPase Ypt1p.
involved in ER-to-Golgi transport (named SLY2; see Ref. 27). Later SLY2 was found to be identical to SEC22 (28) for which conditional mutant alleles had been identified by Novick et al. (29). Like several other SNARE proteins, Sec22p can be a component of more than just one SNARE complex. Its physical interaction with the SNARE proteins Sed5p, Bet1p, and other Golgi SNARE proteins argues for a role in anterograde traffic from ER-to-Golgi (6, 30, 31). Sec22p also co-precipitates with the ER proteins Ufe1p and Sec20p that function in retrograde Golgi-ER transport (24, 32, 33). We show that a new allele of \(\text{SEC}22\) was named \(\text{DSL}1\), with only slight effects on forward transport.


dsl1-22, isolated in our screen indeed affects Golgi-to-ER retrieval of several proteins with only slight effects on forward transport. \(\text{dsl}1-22\) interacts genetically with factors required for retrograde traffic, and \(\text{DSL}1\) binds coatamer. Taken together, our data provide strong evidence for a direct role of \(\text{DSL}1\) in Golgi-to-ER traffic.

**TABLE I**

| Yeast strains | Genotype | Source |
|---------------|----------|--------|
| BSH-7C | MAT\(\alpha\), ura3, trp1, his3, suc2-39, bet-1 | This laboratory |
| MLY-100 | MAT\(\alpha\), ade2,  trp1, ade1::TRP1, containing pUFE315 (UFE1) | M. Lewis |
| MLY-101 | MAT\(\alpha\), ade2, trp1, ade1::TRP1, containing pUT1 (ufe1-1) | M. Lewis |
| MSUC-2D | MAT\(\alpha\), ura3, leu2, his3 | This laboratory |
| MSUC-3B | MAT\(\alpha\), ade2, ura3, leu2, his3 | This laboratory |
| MSUC-7C | MAT\(\alpha\), ade8, ura3, leu2, his3 | This laboratory |
| PC70 | MAT\(\alpha\), ura3, leu2, trp1, rot1-1 | P. Cosson |
| PC82 | Mata, ura3, leu2, his3, lys2, ste2::LEU2, STE2-WBP1::URA3, sec21-2 | P. Cosson |
| PC137 | MAT\(\alpha\), ura3, leu2, his3, lys2, ste2::LEU2, STE2-WBP1::URA3, sec21-2 | This laboratory |
| RH238-3A | MAT\(\alpha\), ura3, leu2, lys2, sec24, con27-1 | H. Riezman |
| RH270-2B | MAT\(\alpha\), ura3, leu2, lys2, sec24, bar1-1 | H. Riezman |
| SC23-3A | MAT\(\alpha\), ade2, ura3, his3, lys2, sec24, con27-1 | This laboratory |
| SHC2-12A | MAT\(\alpha\), ura3, his3, lys2, sec24-30, sec22-3 | This laboratory |
| SL1-2B | MAT\(\alpha\), leu2, ura3, lys2, sec24-30, sly1+ | This laboratory |
| SLA28-6C | MAT\(\alpha\), ade2, ade8, ura3, leu2, his3, trp1, sec22-1::HIS3 containing pHD2288 | This laboratory |
| STE2-4B | MAT\(\alpha\), ura3, leu2, his3, lys2, sec2-2::LEU2, STE2-WBP1::URA3, bar1-1 | This laboratory |
| S202P4-3-9A | MAT\(\alpha\), ura3, leu2, lys2, pep4::HIS3, sec20-1 | This laboratory |
| S21P4-9A | MAT\(\alpha\), ura3, leu3, pep4::HIS3, sec21-1 | This laboratory |
| S27P4-9C | MAT\(\alpha\), ura3, leu2, lys2, pep4::HIS3, sec27-1 | This laboratory |
| S32G-9A | MAT\(\alpha\), ura3, leu2, his3, sec32-1/bos1 | This laboratory |
| S39151 | MAT\(\alpha\), ade2, ade8, ura3, his3, lys2, sec2-2::HIS3 | This study |
| S39151-12D | MAT\(\alpha\), ade2, ade8, ura3, his3, lys2, sec2-2::HIS3 | This study |
| S39151-12D | (CEN6/ARS4, URA3, ADE8, SEC22) | This study |
| S5A | MAT\(\alpha\), ade2, ade8, ura3, leu2, his3, lys2, sec2-2::HIS3, containing pHDS228 | This study |
| TNY51 | MAT\(\alpha\), ura3, leu2, his3, lys2, ade2, sed5-1 | This laboratory |
| TNY140 | MAT\(\alpha\), ura3, leu2, his3, lys2, sec27-1 | This laboratory |
| Y21186 | MAT\(\alpha\), ura3, leu2, his3, lys2, sec27-1 | Eurosarf |
| YUA1-9C | MAT\(\alpha\), ade2, ade8, ura3, leu2, his3, lys2, sec2-2::HIS3 | This study |
| YUA3-1A | MAT\(\alpha\), ade2, ade8, ura3, leu2, his3, pep4::HIS3 | This study |
| YUA3-4B | MAT\(\alpha\), ade2, ade8, ura3, leu2, his3, pep4::HIS3, sec32-1 | This study |
| YUA11 | MAT\(\alpha\), ura3, leu2, his3, lys2, sec24, bar1-1, DSL1-2::His3-2myc::loxP-KanMX-loxP | This study |
| YUA41 | MAT\(\alpha\), ura3, leu2, his3, lys2, sec24, bar1-1, DSL1-2::His3-2myc::loxP-KanMX-loxP | This study |

**EXPERIMENTAL PROCEDURES**

Yeast Strains, Genetic Techniques, and Plasmids—Saccharomyces cerevisiae strains used are listed in Table I. Cells were grown in yeast extract/potato/dextrose or synthetic minimal medium containing galactose (2%) or glucose (2%) as carbon sources and supplemented as necessary with 20 mg/liter tryptophan, histidine, adenine, uracil, or 30 mg/liter leucine or lysine. To enhance the visualization of secreting colonies, plates with low adenine concentration (10 mg/liter adenine) were prepared. 5-FOA plates were prepared as synthetic minimal medium containing 0.1% 5-FOA. Yeast transformations were performed as described previously (44). Standard techniques were used for mating of haploid strains, complementation analysis, sporulation, and the analysis of tetrad (45). The assay to detect retention defects using Ste2-Wbp1p was described previously (20, 46). The analysis of synthetic lethal effects between the \(\text{dsl}1-22\) mutation and other \(\text{ER}-\text{Golgi}\) defects was performed with strains derived from the original mutant by three crosses to wild type strains or a \(\text{dsl}1-22\)-myc::\(\text{KanMX}\) strain derived from the original transformant by two crosses to wild type strains. When possible tetrad analysis was performed 2 or 3 days after placing diploid cells on potassium acetate plates. The viability of tetrad was determined by crosses to tester strains (complementation assays). The \(\text{dsl}1-22\)-myc::\(\text{KanMX}\) carrying spores were identified by their resistance to G418. 98% of the possible \(\text{dsl}1-22\), sec32-1, mutants, 80% of the possible \(\text{dsl}1-22\), bet1-1 double mutants, 64% of the \(\text{dsl}1-22\), sec22-3, 36% of the \(\text{dsl}1-22\),...
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**Table II**

| Plasmid name | Description | Source |
|--------------|-------------|--------|
| pHS28        | pRS316-SEC22-ADH8, URA3, CEN6/ARS4 | This laboratory |
| pBR177       | pGEX-TT-TIP20 | R. Peng |
| pTN159       | pUG36-SEC22, URA3, CEN6/ARS4 | This study |
| pUA18        | pRS315-sec22-3, LEU2, CEN6/ARS4 | This study |
| pUA20        | pRS315-SEC22, LEU2, CEN6/ARS4 | This study |
| pUA26        | pGEX-TT-SEC22Δe | This study |
| pUA30        | pGEX-TT-BOST1Δc | This study |
| pUA37        | pRS315-BOS1, LEU2, CEN6/ARS4 | This study |
| pUA39        | pRS315-UFE1, LEU2, CEN6/ARS4 | This study |
| pUA40        | pRS315-SED5, LEU2, CEN6/ARS4 | This study |
| pUA42        | pGEX-TT-SED5Δc | This study |
| pUA43        | pRS325-SED5, LEU2, 2µm | This study |
| pUA44        | pRS325-UFE1, LEU2, 2µm | This study |
| pUA45        | pRS325-BOS1, LEU2, 2µm | This study |
| pUA46        | pRS315-YKT6, LEU2, CEN6/ARS4 | This study |
| pUA73        | pRS315-DSL1, LEU2, CEN6/ARS4 | This study |
| pUA74        | pRS315-YNL260c, LEU2, CEN6/ARS4 | This study |
| pUA81        | pRS325-DSL1, LEU2, 2µm | This study |
| pUA86        | pRS315-dsl1-22, LEU2, CEN6/ARS4 | This study |
| pUA87        | pRS315-YNL260C-ATX1-YNL260c, LEU2, CEN6/ARS4 | This study |
| pUA93        | pGEX-TT-DSL1 | This study |
| pUA94        | pGEX-TT-dsl1-22 | This study |
| pUA101       | pEG-KT-DSL1 | This study |
| pUA102       | pEG-KT-dsl1-22 | This study |
| pUA114       | YEp13-YKT6, LEU2, 2µm | This study |
| pUHS2Myc     | EMBL accession number AJ132965 | A. De Antoni |
| pWE-Acyc     | CYC1-SEC22-myc-α, URA3, CEN6/ARS1 | W. Balliersiefen |

**sec5-1, and 38% of the dsl1-22, bos1 (sec31-1) double mutants could form colonies. The viability of dsl1-22 single mutants in these tetrads was higher than 90%. No double mutants were obtained when we tried to combine the dsl1-22 defect with the sec20-1, sec21-1, tip20-5, ret1-1, and ret1-1. An unexpected result was the very low viability of all dsl1-22 spores derived from a diploid heterozygous for dsl1-22 and sty1-1.**

Gemogenic tagging of the DSL1 gene was achieved as described by De Antoni and Gallwitz (47) using the oligonucleotides UA1 (5’-AAA CTG TAT ATG ACT ATA GGG AGG CCG GGA CAA CAG GAT GAT ATT GAT ATT ATT GAA ATT AGA GGC ACT CCT GTA GAT TCC CAC CAC CAT CAC CAC CAT CAC-3’) and UA3 (5’-GCG CCG ATT GAT ATT ATT GAA ATT AGA GGC ACT CCT GTA GAT TCC CAC CAC CAT CAC CAC CAT CAC CAC CAT CAC-3’), whereas the oligonucleotides UA1 and UA3 (5’-AGC TTT TAT TAC AAT GGG GAT TTT TAT CTT TTT TGG CAT GAC GAC GAA CTA ATC TCC CAC CAC CAT CAC CAC CAT CAC CAC CAT CAC-3’) were used for tagging the dsl1-22 mutant. Plasmids used in this work are listed in Table II.

**Synthetic Lethality Screen—**Mutants synthetically lethal with sec22Δ were isolated using the ade2/ade8, red/white sectoring system (48). The plasmid SL298-6C and SUA1-12D strains were red after transformation with pHS28 on selective plates but gave white sectors under conditions of Sec5 wild type, which suggests that the sec22Δ mutation is synthetically lethal with sec5Δ.

**Protein Extraction and Immunoblotting—**Western blotting analysis was performed as described by Boehm et al. (53). Aliquots (1 × 10^7 cells) of transformed cells were lysed in 2 M NaOH, 5% (w/v) ethanolamine, and proteins precipitated with 10% trichloroacetic acid, neutralized with 1.5 M Tris base, and dissolved in SDS sample buffer. Proteins were resolved on 12% SDS-PAGE.

**Purification of Recombinant Proteins and Affinity Binding Assay—**E. coli and S. cerevisiae strains expressing GST fusion proteins were lysed, and proteins were solubilized in lysis buffer (20 mM Hepes, pH 6.8, 150 mM KOAc, 5 mM Mg(OAc)_2, 1 mM dithiothreitol, 1% Triton X-100, protease inhibitor mix). GST fusion proteins were immobilized on glutathione-Sepharose 4B and washed 5 times with 10 volumes lysis buffer. Proteins bound to GST fusion proteins expressed in yeast were separated by SDS-PAGE and analyzed by immunoblotting. E. coli proteins immobilized on glutathione-Sepharose 4B were incubated at 4°C for 2 h with 100,000 × g supernatant of yeast cell lysate. The beads were washed five times, and proteins were separated by SDS-PAGE followed by immunoblot analysis.

**Subcellular and Sucrose Gradient Fractionation—**Yeast cells were harvested at mid-logarithmic phase. The cell pellet was washed twice with water and once with B88 (20 mM Hepes, pH 6.8, 250 mM sorbitol, 150 mM KOAc, 5 mM Mg(OAc)_2, resuspended in a minimal volume of B88 containing EDTA-free protease inhibitor mix (Roche Molecular Biochemicals), and pipetted into liquid nitrogen. Cells were ground up in a mortar. The cell powder was resolved in B88 (supplemented with EDTA-free protease inhibitor mix) and centrifuged twice at 500 × g for 5 min to remove cell debris, and the clear lysate was centrifuged at 15,000 × g for 15 min to obtain the P100 pellet. The S10 fraction was then subjected to centrifugation at 100,000 × g at 4°C for 1 h to obtain the P100 and S100. To investigate the membrane localization of Dsl1p, the supernatant of the cell lysate after a 500 × g centrifugation was divided into different portions that were treated for 30 min on ice with either 5 M urea, 1% Triton X-100, or 1 M NaCl. The 500 × g lysate was also subjected to sucrose density gradient centrifugation.

For fractionation experiments, lysates were loaded on sucrose density gradients (51) and spun at 4°C in a Beckman SW40 rotor at 37,000 rpm for 2.5 h. 1-ml fractions were taken, and the last fraction was adjusted to 1 ml with B88. Each fraction was mixed with 1 ml of SDS-PAGE sample buffer (8% urea, 50 mM Tris-HCl, pH 8.0, 2% SDS, 0.1 mg/ml bromophenol blue) and incubated at 50°C for 10 min prior to analysis by SDS-PAGE and immunoblotting.

**Protein Labeling, Immunoprecipitation, and Invertase Assay—**For detection of CPY processing cells were shifted to 37°C for indicated
times, pulse-labeled for 5 min with Tran35S-label (ICN) and chased for 30 min. The labeled proteins were immunoprecipitated using specific antibodies and separated by SDS-PAGE. After incubating the gel with Amplify (Amersham Pharmacia Biotech) for 45 min, the proteins were detected by exposing the gels to X-Omat AR (Eastman Kodak Co.) at −80 °C. Invertase activity staining was carried out as described previously (54).

Fluorescence and Electron Microscopy—Indirect immunofluorescence was performed as described by Schröder et al. (51) using rabbit polyclonal anti-Kar2p and monoclonal mouse c-Myc epitope (9E10) antibodies. Cy2TM-conjugated goat anti-rabbit or anti-mouse F(ab′)2 fragment (Jackson ImmunoResearch) served as secondary antibody. DNA was stained with 4',6-diamidino-2-phenylindole (DAPI). Cells expressing GFP fusion proteins were grown in SD medium at 25 °C to mid-log phase and placed onto a slide. A coverslip was added, and cells were examined immediately. DAPI staining was achieved after fixing cells in methanol at −20 °C for 10 min, washing with acetone at −20 °C, and washing three times with ice-cold PBS, pH 7.4. Confocal images were obtained with a TSC SP1 confocal laser-scanning microscope (Leica). For electron microscopy, yeast cells at mid-logarithmic phase were fixed and stained with permanganate to enhance visualization of membrane structures (54).

RESULTS
Identification of Mutants for Which SEC22 Is Essential—To find proteins that can substitute for Sec22p or to identify factors that prevent these proteins from functioning normally, we performed a synthetic lethality screen. Mutants inviable in the absence of SEC22 were isolated by using a colony sectoring assay (48). sec22A mutants that carry a functional SEC22 gene on the centromeric plasmid pHDS228 were mutagenized. In addition to SEC22 this plasmid contains the following two markers required for pyrimidine and purine biosynthesis: URA3 as selectable marker and ADE8, which can serve as a color marker in yeast strains carrying mutated versions of the ADE2 and ADE8 genes on the chromosomes. The ade8 mutation is epistatic to ade2 and prevents the formation of the red color typical for ade2 mutants. Therefore, cells expressing ADE8 from a plasmid are red, whereas those that lost the plasmid turn white. As expected, on rich media sec22A ade8, ade2, ura3 cells containing pHDS228 could form white sectors since neither SEC22, ADE8, nor URA3 are essential. After mutagenesis, we screened for non-sectoring colonies (for details see “Experimental Procedures”). To confirm that the non-sec- toring phenotype in fact reflects a positive selection for the absence of SEC22-carrying plasmid, all mutants were tested for their ability to lose the second plasmid-encoded marker, URA3. This test makes use of the drug 5-FOA (5-fluoroorotic acid), which is toxic to Ura− cells (55). In fact, most of the non-sectoring mutants were sensitive to 5-FOA and only these mutants were analyzed further. In addition to these two phenotypes, five mutants obtained in two independent screens were also temperature-sensitive for growth. Genetic analysis showed that the mutations are recessive and that the inability to lose the SEC22 gene is tightly linked to the growth defect at 37 °C (see Fig. 1A). They belong to three different complementation groups that we called “LSD1-2, -3” (lethal with SEC22 deletion).

We tried to clone the “LSD” genes from single copy or multicopy genomic libraries containing LEU2 as a selectable marker (27). To obtain complementing plasmids, we selected transformants on plates lacking leucine and looked for colonies with white sectors. The formation of white sectors indicated that the cells had again acquired the ability to lose the SEC22-carrying plasmid pHDS228. Those transformants, which had simply received an additional copy of SEC22 from the library, were identified by PCR and discarded. So far our attempts to isolate complementing plasmids from a single copy library were successful only for the “lsd1-1” mutant. The library plasmid that we obtained harbored three intact open reading frames.

Sequencing and subcloning showed that the presence of YNL258c alone was sufficient to suppress both the non-sec- toring phenotype and the temperature sensitivity of the lsd1-1 mutant. The open reading frame YNL258c, located on chromosome XIV, encodes an essential protein with a predicted molecular mass of 88 kDa with no similarity to other proteins in data bases (56).

The following observations confirmed that defects in YNL258c result in a SEC22-dependent phenotype as well as a conditional lethal phenotype. Cloning and sequencing of the lsd1-1 mutant allele revealed the presence of a stop codon at position 2173 of the 2265-base pair long reading frame. This would lead to a gene product, which is 30 residues shorter than the putative wild type protein. By using a PCR-based method described by De Antoni and Gallwitz (47), we replaced YNL258c either by a full-length or a shortened version, which were fused to sequences encoding a His6 epitope followed by two copies of a c-Myc tag. The KanMX cassette inserted downstream of the c-Myc-tagged YNL258c sequences served as a selectable marker that allows the transformants to grow in the presence of geneticin (G418). Temperature-sensitive transformants were obtained only when the C-terminally truncated version of YNL258c was introduced into wild type cells. Western blotting analysis showed that Ts− transformants in fact encode a shorter c-Myc-tagged YNL258c protein than cells.
expressing the full-length version (data not shown). Tetrad analysis also confirmed that these mutants need SEC22 for growth (see below). The same results were obtained when N-terminally tagged versions of YNL258c and its mutant variant expressed from a centromeric vector were used to complement the deletion of YNL258c. In summary, these data established that the deletion of 30 C-terminal triplets from the ORF YNL258c results in a conditional lethal phenotype. In cells carrying this mutation the otherwise non-essential SEC22 gene is rendered essential.

While this work was in progress Waters and co-workers (43) showed that mutations in YNL258c can make cells dependent on the SLY1-20 mutation. The mutants identified were accordingly named dsl1-1 to dsl1-7 (dependent on SLY1-20). SLY1-20 is a dominant mutation, which suppresses the defects in several yeast mutants affected in ER-to-Golgi transport (27, 37, 57–60). Accordingly, the mutant we obtained was renamed dsl1-22. Consistent with the results obtained by VanRheenen et al. (43), the temperature sensitivity of dsl1-22 is suppressed by the SLY1-20 mutation on a single copy plasmid (data not shown).

**Genetic Interaction of dsl1-22 with Other Genes Whose Products Act in ER-Golgi Anterograde and Retrograde Transport**—In the process of cloning out sequences able to complement the dsl1-22 mutation, we also obtained clones from multicopy libraries. Among these clones were plasmids containing the YKT6 gene. YKT6 encodes a lipid-anchored member of the synaptobrevin family of SNARE proteins (61). This prompted us to test whether the overexpression of other SNARE-encoding genes has similar effects.

We found that, similar to the results obtained with YKT6, overexpression of SED5 allowed dsl1-22 mutants to tolerate the loss of SEC22. However, the overexpression of neither YKT6 nor SED5 was able to suppress the Ts phenotype of dsl1-22 mutants. Overexpression of the other SNARE-encoding genes specific for ER-Golgi transport, BET1, BOS1 or UFE1, was unable to suppress the non-sectoring phenotype of dsl1-22 mutants.

The approach, which led to the isolation of the dsl1-22, was based on the synthetic lethality of the dsl1-22 mutation when combined with the sec22 deletion. Therefore, we also addressed the question whether dsl1-22 is synthetically lethal with other defects in ER-to-Golgi transport. For this and all subsequent assays we used dsl1-22 mutants expressing SEC22 from its normal locus on chromosome XII: (i) a strain obtained by backcrossing cells derived from the original mutant (Fig. 1A) twice to wild type cells (SEC22), and (ii) a mutant in which we had introduced dsl1-22-ycm construct at the YNL258c locus (see above). The analysis of tetrads was greatly facilitated by the presence of the KanMX cassette closely linked to the dsl1-22-ycm allele which thus allowed us to identify the dsl1-22 mutants by their resistance to G418.

Viable double mutants were obtained when we combined the dsl1-22 defect with sec23-1, sec22-3, bet1-1, sed5-1, bos1 (sec31-1), and sec27-1 mutations. The first mutation leads to a block in anterograde ER-to-Golgi transport due to a defect in COPII assembly (62); bet1-1, sec22-3, and sed5-1 are mutations that affect genes encoding SNARE proteins involved in ER-Golgi transport, whereas SEC27 encodes a COPI component (63). The number of viable double mutants obtained differed to a great extent as determined by complementation assays and analyzing their resistance to G418 (for details see “Experimental Procedures”). The observation that sec22-3, dsl1-22 double mutants are viable whereas dsl1-22 mutants are inviable in the absence of SEC22 was confirmed by plasmid shuffling experiments using a dsl1-22 mutant and SEC22 or sec22-3 containing plasmids (data not shown). This finding illustrates that this assay is specific for certain alleles. Therefore, missing or weak genetic interactions mentioned above do not rule out that the gene products perform a related function. This may be true at least for BOS1 and DSL1 since all the bos1 (sec31-1), dsl1-22 double mutants that we obtained formed very small colonies. No double mutants were obtained when diploids heterozygous for the dsl1-22 and the sec22A, sec21-1, ret1-1, ret1-1, sly1°, sec20-1, or tip20-5 mutations were subjected to tetrad analysis. The sec21-1 (γ-COP), ret1-1 (α-COP), ret1-1 (δ-COP), sec20-1, and tip20-5 mutants primarily affect the retrograde transport from Golgi to ER, and defects in forward transport may be secondary (20, 22, 24, 32, 33). The strong genetic interaction between dsl1-22 and these mutations indicates that DSL1 may be required for Golgi-ER retrograde transport. The synthetic lethality of dsl1-22 and sly1° are consistent with the observation made by VanRheenen et al. (43) who isolated dsl1 mutants that depend on a dominant SLY1 mutation.

**The Dsl1p Mutant Shows Slight Defects in Forward Transport**—The dsl1-22 mutant cells gave rise to slightly smaller colonies than wild type cells even at room temperature. Accordingly, the growth rate of dsl1-22 mutant cells is slower when measured in liquid culture (Fig. 1B). Growth of dsl1-22 mutants stops completely 2 h after a shift to 37 °C. This Ts− phenotype allowed us to examine the function of Dsl1p in the secretory pathway at restrictive temperatures. First we analyzed the secretion of periplasmic invertase in wild type and dsl1-22 cells at different times after shifting cells to 37 °C. Measuring total invertase activity using intact and permeabilized cells (64) showed that the ratio of secreted to intracellular invertase does not change significantly up to 3 h after the shift to 37 °C (data not shown). To detect a possible glycosylation defect due to slower ER-to-Golgi transport in dsl1-22 mutants, intracellular and extracellular fractions of wild type and mutant cells were separated by non-denaturing PAGE. Invertase was visualized by an activity stain. As shown in Fig. 2A, dsl1-22 cells secrete partially underglycosylated invertase even at 25 °C. The shift to the restrictive temperature leads to some accumulation of the ER core-glycosylated form inside the cell. For comparison, at restrictive temperature the sec22-3 mutation also leads to the intracellular accumulation of core-glycosylated invertase and secretion of a small amount of underglycosylated enzyme. An incomplete block in anterograde transport also became evident when the maturation of the vacuolar protease CPY was analyzed in dsl1-22 cells (Fig. 2B). In pulse-chase experiments CPY appears first as a p1 precursor in the ER, is then modified to a larger form, p2, in the Golgi, and is transported to the vacuole where it is processed to its mature form (m) by proteolysis. As expected, sec22-3 mutant cells show a complete block in ER-to-Golgi transport 15 min after the shift to 37 °C. In this mutant only the ER form (p1) is visible consistent with a complete block in ER-to-Golgi transport. In dsl1-22 cells about half of CPY is still normally processed even 3.5 h after the shift to 37 °C. This corresponds to the results observed with other temperature-sensitive alleles of DSL1 (43).

**dsl1-22 Cells Accumulate ER Membranes but Not Vesicles at Restrictive Temperature**—The morphology of wild type and dsl1-22 cells incubated at 25 or 37 °C was compared by electron microscopy. As shown by Kaiser and Schekman (36), mutants with defects in the budding reaction accumulate membranes, whereas mutants that exhibit defects in fusion of vesicles with target membranes accumulate vesicles. At 25 °C the morphology of dsl1-22 cells does not differ significantly from that of wild type cells grown at 37 °C (Fig. 3, A and B). Fig. 3C shows a representative micrograph of a dsl1-22 mutant cell after
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FIG. 3. Electron micrographs of wild type and dsl1-22 cells. Wild type cells (MSUC-3B) grown at 37 °C for 90 min were used as a control (A). Mutant cells (YUA1-9C) grown at 25 (B) or 37 °C for 90 min (C and D) were fixed with potassium permanganate to highlight membrane structures. Typical cells are shown for each condition. The arrowhead in D points to membranes emanating from the cortical ER (E), whereas arrows in C and D indicate sites of membrane accumulation at the nucleus (N), V, vacuole. Bars, 1 μm in A–C; 0.1 μm in D.

dsl1-22 and dsl1-22-myc mutants. This defect in BiP/Kar2p localization was also observed by immunofluorescence microscopy using an affinity-purified polyclonal anti-BiP/Kar2p antibody. In wild type cells BiP/Kar2p antibodies stain the nuclear periphery which is the characteristic ER staining in yeast (66). In contrast to the typical ER staining in wild type cells, we could observe a dot-like pattern in dsl1-22 cells even at permissive temperature (Fig. 4B), similar to “BiP bodies” observed in several ER-to-Golgi mutants at restrictive temperature (67).

To examine the defect in retrograde transport more specifically, we focused on the targeting of the SNARE protein Sec22p. As described previously (18, 46) a-factor fused to Sec22p through a Kex2p cleavage site, and a c-Myc epitope is a suitable tool for analyzing targeting of Sec22p. Several recycling mutants exhibit mislocalization of Sec22-a (18) resulting in cleavage by the late Golgi protease Kex2p. The removal of the a-factor reporter from Sec22p is easily detected by immunoblot analysis. Fig. 4C shows the steady state processing of Sec22-a in wild type, dsl1-22 and dsl1-22-myc strains incubated at 25 °C. About 75% of Sec22-a proteins was cleaved by Kex2p in mutant cells, whereas very little of the reporter was cleaved by Kex2p in wild type cells. Pre-shifting cells to 37 °C for 2 h did not result in more efficient cleavage (data not shown). It is unlikely that more efficient cleavage of Sec22-a in dsl1-22 is due to some Kex2p activity in the ER since mislocalization of a Sec22p-derived fusion protein was also obvious when we analyzed cells producing a GFP-tagged Sec22p protein (Fig. 4D). This fusion protein is fully functional since it is able to suppress the growth defect of sec22-3 mutants (data not shown). Moreover, GFP-Sec22p behaves like C-terminally tagged Sec22 proteins when analyzed in wild type and ufe1-1 mutant cells (Fig. 4D; see Ref. 18). In wild type cells fluorescence appeared as a ring around the nucleus which represents ER, whereas in dsl1-22 cells a punctuated staining was detectable, very likely representing Golgi structures (18). As with other recycling mutants, this defect already occurs at 25 °C (20). Taken together, both the efficient Kex2p processing of Sec22-a and the localization of GFP-Sec22 indicate that dsl1-22 mutants are defective in ER retention of Sec22p.

DSL1 Mutants Are Defective in the Retrieval of ER Proteins from the Golgi—The strong genetic interaction of the dsl1-22 defect with mutations affecting retrograde Golgi-to-ER transport and the incomplete block in anterograde transport when growth already had ceased indicated that the primary function of Dsl1p could be in the retrieval of proteins from the Golgi complex. Therefore, we employed different assays to compare retrograde transport in wild type and dsl1-22 cells.

Mutants affecting genes required in retrograde transport like SEC20 and SEC22 secrete large amounts of the soluble ER protein BiP/Kar2p (65). Fig. 4A shows that the same is true for incubation at the nonpermissive temperature for 90 min. Compared with wild type cells (Fig. 3A) dsl1-22 cells show a strong accumulation of membranes, which mainly emerge from the ER contiguous with the nuclear membrane (Fig. 3, C and D, arrow). Similar structures also originate from cortical endoplasmic reticulum close to the plasma membrane (Fig. 3D, arrowhead). No significant increase in the number of small vesicles was observed. Thus, dsl1-22 mutants very much resemble the coatomer mutant sec27-1 (63).

FIG. 2. dsl1-22 cells exhibit mild defects in anterograde ER-to-Golgi transport. A, fate of secreted invertase in dsl1-22 cells. Invertase synthesis was induced for 30 min in wild type (Dsl1-myc; YUA11), dsl1-22-myc (YUA41), and sec22-3 cells (SHC21-12A) either at 25 or 37 °C. Preincubation at 37 °C started 30 min before induction. Glycosylation level of the enzyme in intracellular (I) and extracellular fractions (E) was detected by an activity stain in non-denaturing gels. The position of highly glycosylated invertase (S) and ER core-glycosylated invertase (ER) is indicated. B, intracellular processing of carboxypeptidase Y in wild type (YUA11) and mutant cells (YUA41 and SHC21-12A). Cells were shifted to 37 °C for indicated times, pulse-labeled with [35S]methionine/cysteine for 5 min, and chased for 0 and 30 min. The cells were lysed; CPY was immunoprecipitated, and proteins were resolved by SDS-PAGE.

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To examine whether the dsl1-22 mutation also interferes with the ER retention of type I transmembrane proteins carrying the KKKK retrieval signal, we performed the Ste2-Wbp1-dependent mating assay described by Letourneur et al. (20). We introduced the dsl1-22-myc allele into a strain expressing a KKKK-tagged version of the α-factor receptor (Ste2-Wbp1p) instead of the wild type STE2 gene. Wild type cells of mating type a expressing only this receptor cannot mate with cells of mating type a since Ste2-Wbp1p is efficiently retained in the ER due to the KKXX sequence, Dsl1p contains no putative transmembrane domains. As type I transmembrane proteins like the v-SNARE Sec22p, as well as type I transmembrane proteins carrying a KKKK retrieval signal.

Subcellular Distribution of Dsl1p—According to its primary sequence, Dsl1p contains no putative transmembrane domains. Extracts from Dsl1-myc producing cells (YUA11) were used to examine a possible membrane association of Dsl1p. A 500 × g supernatant of cell lysate was treated either with buffer (B8), 5 mM urea, 1% Triton X-100, or 1 mM NaCl and subsequently centrifuged at 10,000 × g and 100,000 × g (Fig. 5). When incubated with buffer alone, no Dsl1-myc was detectable in the soluble fraction, whereas both urea and detergent treatment led to solubilization of Dsl1-myc. Less than 5% of the total amount of Dsl1-myc became soluble upon treatment with high salt suggesting that Dsl1p is a peripherally associated membrane protein. In contrast, the transmembrane protein Sec22p could only be solubilized by detergent. Experiments using a recently obtained Dsl1-specific serum gave identical results (data not shown).

Next we performed subcellular fractionation studies using sucrose density gradients to compare the localization of Dsl1p with that of known Golgi- and ER-resident proteins. Cell lysates of strain YUA11 (DSL1-myc) were prepared and loaded on top of sucrose gradients, and fractions were collected after centrifugation as described under “Experimental Procedures.” Fig. 6, A and B, shows that Emp47p, a Golgi marker, the ER resident t-SNARE Ufe1p, as well as the ER-marker BiP/Kar2p display characteristic distributions (51, 24, 66). Like Ufe1p and BiP/Kar2p Dsl1-myc protein was detectable exclusively in the dense fractions when using the monoclonal anti-c-Myc anti-
The data represent average values from at least two experiments.

c-Myc to detect tagged Dsl1 protein expressed at wild type levels (C lysate (500 × g supernatant) was treated as indicated (see “Experimental Procedures”) and then centrifuged at 10,000 and 100,000 × g. The resulting pellet (P10 and P100) and supernatant (S100) fractions were resolved on a 12% polyacrylamide gel and immunoblotted with anti-Sec22p and anti-c-Myc antibody (9E10). In contrast to the integral membrane protein Sec22p, Dsl1-myc became soluble after incubation with 5 M urea.

FIG. 5. Dsl1p is a peripheral membrane protein. Logarithmically grown cells of YUA11 (DSL1-myc) were disrupted using glass beads. The lysate (500 × g supernatant) was treated as indicated (see “Experimental Procedures”) and then centrifuged at 10,000 and 100,000 × g. The resulting pellet (P10 and P100) and supernatant (S100) fractions were resolved on a 12% polyacrylamide gel and immunoblotted with anti-Sec22p and anti-c-Myc antibody (9E10). In contrast to the integral membrane protein Sec22p, Dsl1-myc became soluble after incubation with 5 M urea.

FIG. 6. Dsl1p cofractionates with ER markers in sucrose velocity gradients. Lysates of strains YUA11 (DSL1-myc) grown at 25 °C were loaded on 18–60% sucrose density gradients. After centrifugation, fractions were collected and subjected to Western blot analysis with antibodies directed against Emp47p, a Golgi marker (A, see Ref. 51), Ufe1p and BiP/Kar2p, two ER markers (B, see Refs. 24 and 66), and c-Myc to detect tagged Dsl1 protein expressed at wild type levels (C). The data represent average values from at least two experiments.

body 9E10 directed against the c-Myc epitope, presumably reflecting ER localization (Fig. 6C).

Dsl1p Interacts Physically with Coatomer—To get additional clues for the involvement of Dsl1p in retrograde and/or anterograde ER-to-Golgi transport, we investigated possible interactions of Dsl1p with proteins involved in these trafficking steps. First we tried to address this question by expressing Dsl1p tagged with glutathione S-transferase (GST) in yeast. The 100,000 × g supernatants from detergent-lysed yeast cells (YUA11) expressing GST or GST-Dsl1p were loaded on glutathione-Sepharose 4B to immobilize GST or GST-Dsl1p and associated proteins. After washing the beads to remove unbound proteins, antibodies were used to monitor the binding of several ER/Golgi proteins to Dsl1p. Anti-coatomer antibodies resulted in very strong signals (data not shown), whereas only weak signals were obtained with Emp47p-specific antibodies. These signals were specific for the Dsl1 part of the fusion protein since no binding was observed when lysates from GST-expressing cells were analyzed. The SNARE proteins Bet1p, Bos1p, Sec22p, and Sed5p as well as the COPII component Sec24p and the Rab-like GTPase Ypt1p were not retained on the affinity matrix in significant amounts.

To verify and extend these findings, we incubated extracts of detergent-lysed yeast cells with different GST fusion proteins purified from E. coli. In line with the results obtained with GST fusion proteins expressed in yeast, coatomer (COPI) showed strong binding to GST-Dsl1p. Notably, coatomer recruitment to GST-Dsl1p from E. coli takes place even at 4 °C (see “Experimental Procedures”), a temperature where enzymatic activities are low. As controls, GST, GST-Sed5p, GST-Bos1p, or GST-Sec22p were not able to recruit coatomer from cell lysates (Fig. 7). Very faint bands representing coatomer were seen when GST-Tip20p was loaded on glutathione-Sepharose 4B (Fig. 7B, lane 5). Dsl1p may mediate this indirect binding between GST-Tip20 and coatomer because Ito et al. (68) recently showed that Tip20p and Dsl1p interact in two-hybrid assays. However, so far we could not observe direct binding of Dsl1-myc to GST-Tip20p in vitro. In addition, Dsl1-myc did not bind to GST-Bos1p, GST-Sec22p, or GST-Sed5p (data not shown). Likewise, GST-Dsl1p was not able to bind Bet1p, Bos1p, Sec22p, Sed5p, Ypt1p, Sec24p, or Emp47p, suggesting that the weak binding of Emp47p mentioned above could be indirect via coatomer.

DISCUSSION

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DSL1

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that Dsl1p may play a role in Golgi-ER retrograde traffic. One could speculate that the need for Sec22p displayed by the dsl1-22 mutant may be due to the mislocalization of SNARE proteins that can functionally replace Sec22p. This is also indicated by the fact that the requirement for Sec22p at least at room temperature can be alleviated either by excess of Ykt6p or Sed5p, two other SNARE proteins. As discussed below, Sec22p as well as Bos1p are in fact mislocalized in dsl1-22 cells. Unexpectedly, SEC22 can be replaced in dsl1-22 mutants by the sec22-3 allele. This was surprising since the sec22-3 point mutation has stronger effects on the growth of certain strains than the deletion of SEC22 (sec22A cells that are not Ts− can become temperature-sensitive after introducing a sec22-3-containing plasmid).²

dsl1-22 Mutants Have a Strong ER Retention Defect—In dsl1-22 cells maturation of the vacuolar hydrolase CPY is only partially inhibited, similar to what has been described for the PCR-generated dsl1-5 and dsl1-6 mutants (43). Invertase secretion is almost normal in dsl1-22 mutants and a slight inhibition of anterograde transport is indicated by the accumulation of a small amount of core-glycosylated invertase. Electron microscopy analysis of mutant cells reveals a severe accumulation of membranes emerging from the ER after shift to non-permissive temperature. Similar structures were observed in a β′-COP mutant, sec27-1 (63). Since the morphology of dsl1-22 mutant cells is almost normal at 25°C, a temperature at which retrograde transport is already affected (see below), this EM phenotype at restrictive temperature is likely to be a more indirect effect due to perturbed forward transport.

The weak inhibitory effect on forward transport appears to be a result of a strong defect in retrograde transport back to the ER. In dsl1-22 cells this block is already seen at permissive temperature, consistent with what has been seen with other recycling mutants (18, 20, 22). The dsl1-22 mutant allele affects the retrieval of recycling SNARE proteins, proteins sorted by their C-terminal KXXX motif, and the soluble ER protein BiP/Kar2p, whose recycling depends on the HDEL receptor Erd2p (65). How can retrograde transport defects have an effect on forward transport? Obviously, one possibility is that components of the vesicle budding and fusion machineries may become limiting due to their mislocalization. In addition, it is known that exit from the ER requires the proper folding of cargo molecules, and this in turn depends on chaperones like BiP/Kar2p or PDI (71, 72). These chaperones carry a C-terminal HDEL signal that mediates their retention in the ER. In dsl1-22 mutant cells, BiP/Kar2p and very likely PDI are not properly retained in the ER. Insufficient amounts of BiP/Kar2p and PDI in the ER could retard the exit of cargo molecules (71, 72).

The following results demonstrated that dsl1-22 cells are defective in Golgi-to-ER-retrieval of Sec22p. A GFP-tagged version of Sec22p localizes to the ER in wild type cells, whereas in dsl1-22 cells this block is already seen at permissive temperature, consistent with what has been seen with other recycling mutants (18, 20, 22). The dsl1-22 mutant allele affects the retrieval of recycling SNARE proteins, proteins sorted by their C-terminal KXXX motif, and the soluble ER protein BiP/Kar2p, whose recycling depends on the HDEL receptor Erd2p (65). How can retrograde transport defects have an effect on forward transport? Obviously, one possibility is that components of the vesicle budding and fusion machineries may become limiting due to their mislocalization. In addition, it is known that exit from the ER requires the proper folding of cargo molecules, and this in turn depends on chaperones like BiP/Kar2p or PDI (71, 72). These chaperones carry a C-terminal HDEL signal that mediates their retention in the ER. In dsl1-22 mutant cells, BiP/Kar2p and very likely PDI are not properly retained in the ER. Insufficient amounts of BiP/Kar2p and PDI in the ER could retard the exit of cargo molecules (71, 72).

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² T. Neumann, unpublished results.
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(67). These authors suggested that BiP bodies could be exit sites where leaving proteins accumulate in different mutant strains due to low efficiency of Golgi-to-ER retrieval. Some mutants even secrete Kar2p into the medium. Indeed, this phenomenon can be observed with dsl1-22 mutant cells. The level of Kar2p secretion by these cells is comparable to that of sec22-3, sec22A, sec20-1 cells (65).3

Besides mislocalization of SNARE proteins and of the luminal ER protein BiP/Kar2p, dsl1-22 cells exhibit also defects in retrieval of proteins sorted by their C-terminal KXXX motif. In this study we used Ste2-Wbp1p as a marker protein (20). Our results implicate Dsl1p in retrograde transport of dilysine-tagged proteins from the Golgi compartment to the ER. We also analyzed the localization of Emp47p, a Golgi protein carrying a variant of the dilysine-motif, KXXXX (51). Unlike Ste2-Wbp1p, the localization of Emp47p is unaffected in dsl1-22 cells. This is indicated by the results of gradient fractionation and immunofluorescence experiments (data not shown). In this respect, dsl1-22 mutants resemble ret1 (a-COP) mutants that also mislocalize KXXX-tagged proteins of the ER but not the KXXX-tagged Emp47p (23).

The Localization of Ds1p Is Still Unclear—Ds1p is a peripheral membrane protein that can be solubilized with 5 M urea and colocalizes with ER marker proteins in sucrose density gradients. Fractionation experiments were performed with a c-Myc-tagged Ds1p protein expressed at wild type levels. These results were later confirmed using antibodies raised against bacterially produced Ds1p protein. We also tried to determine the localization of Ds1p by immunofluorescence. Unfortunately, affinity purified polyclonal antibodies against Ds1p did not show any signal (data not shown).

Ds1p Interacts Strongly with Coatomer—As mentioned above, a recent systematic yeast two-hybrid study revealed direct interactions of Ds1p with Tip20p (68). Ds1p showed interactions with several other proteins. However, only in the case of Ds1p and Tip20p, this interaction was observed with Ds1p as bait as well as prey, i.e. both fusion orientations. This is consistent with the genetic data since the tip20Δ-5 defect is synthetically lethal in combination with dsl1Δ-22 (this study). The genetic as well as physical interaction between DSL1 and TIP20 and their gene products suggest that both proteins could be involved in the same transport step. Tip20p is able to bind to the cytosolic region of Sec20p (73). Together they form a complex with the SNARE proteins Ufe1p and Sec22p (32). This unconventional SNARE complex is involved in retrieval of dilysine-tagged proteins from Golgi to ER (33). In summary, Ds1p interacts directly with Tip20p (68), and the dsl1-22 mutation inhibits synthetic lethality in combination with sec22A, sec20-1, and tip20-5. Synthetic-lethal genetic interaction between mutations in sec22, sec20, tip20, as well as Ufe1 and mutations affecting cot jer subunits were established previously (69).

As expected, dsl1 mutants also exhibit genetic interactions with coatomer mutants (see Ref. 43; this study). Final evidence for Ds1p playing an important role in retrograde Golgi-ER traffic is our finding that Ds1p interacts physically with coatomer. Coatomer could be copurified with GST-Ds1p from yeast cells, and it could be recruited from yeast lysates to recombinant GST-Ds1p purified from E. coli. No additional factors present in the cell extracts were required for this interaction since purified coatomer can also bind to GST-Ds1p (data not shown). Interestingly, the C-terminal truncated mutant protein, Ds1l-22p, which leads to a defect in retrograde transport, is still able to bind all coatomer subunits with an affinity comparable to the full-length protein (data not shown). Thus the C terminus of Ds1l is not essential for binding of coatomer but perhaps could represent a binding region for other proteins involved in these transport steps.

Considering the fact that Ds1p binds coatomer as well as Tip20p, a component of the putative docking complex at the ER, we suggest that Ds1p is involved in a step between uncoding and docking. It will be important to determine whether Ds1p can bind both coatomer and Tip20p at the same time or whether the interaction is sequential.

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