The α Subunit of the *Saccharomyces cerevisiae* Oligosaccharyltransferase Complex Is Essential for Vegetative Growth of Yeast and Is Homologous to Mammalian Ribophorin I

Susana Silberstein, Paula G. Collins, Daniel J. Kelleher, Peter J. Rapiejko, and Reid Gilmore

Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01655-0103

Abstract. Oligosaccharyltransferase mediates the transfer of a preassembled high mannose oligosaccharide from a lipid-linked oligosaccharide donor to consensus glycosylation acceptor sites in newly synthesized proteins in the lumen of the rough endoplasmic reticulum. The *Saccharomyces cerevisiae* oligosaccharyltransferase is an oligomeric complex composed of six nonidentical subunits (α-ζ), two of which are glycoproteins (α and β). The β and δ subunits of the oligosaccharyltransferase are encoded by the *WBP1* and *SWP1* genes. Here we describe the functional characterization of the *OST1* gene that encodes the α subunit of the oligosaccharyltransferase. Protein sequence analysis revealed a significant sequence identity between the *Saccharomyces cerevisiae* Ost1 protein and ribophorin I, a previously identified subunit of the mammalian oligosaccharyltransferase. A disruption of the *OST1* locus was not tolerated in haploid yeast showing that expression of the Ost1 protein is essential for vegetative growth of yeast. An analysis of a series of conditional *ost1* mutants demonstrated that defects in the Ost1 protein cause pleiotropic underglycosylation of soluble and membrane-bound glycoproteins at both the permissive and restrictive growth temperatures. Microsomal membranes isolated from *ost1* mutant yeast show marked reductions in the in vitro transfer of high mannose oligosaccharide from exogenous lipid-linked oligosaccharide to a glycosylation site acceptor tripeptide. Microsomal membranes isolated from the *ost1* mutants contained elevated amounts of the Kar2 stress-response protein.

ASPARAGINE-linked glycosylation of proteins is a ubiquitous protein modification reaction in eukaryotic organisms that occurs in the lumen of the rough endoplasmic reticulum (Herscovics and Orlean, 1993; Kornfeld and Kornfeld, 1985). Addition of asparagine-linked carbohydrates to many glycoproteins is an obligatory event for folding and assembly of newly synthesized polypeptides (Helenius, 1994). The presence of oligosaccharides is often required for the efficient transport of individual glycoproteins through the secretory pathway (Guan et al., 1985; Riederer and Hinnen, 1991; Winther et al., 1991). Glycan groups contribute to the overall dynamic stability of proteins, in some cases rendering them more resistant to proteolysis in vivo (Barriocanal et al., 1986). Diverse biological roles for asparagine-linked oligosaccharides have been identified including serving as receptors for extracellular ligands, acting as protein-targeting signals, and modulating protein interaction and function (for review see Varki, 1993).

The lumenal enzyme oligosaccharyltransferase catalyzes the transfer of a preassembled high mannose oligosaccharide ($Glc_3Man_9GlcNAc_2$) from a lipid-linked oligosaccharide donor onto asparagine acceptor sites within the consensus sequon Asn-X-Ser/Thr, where X can be any amino acid except proline (Gavel and Von Heijne, 1990). Despite the evolutionary distance between mammals and yeast, the exact conservation of both the donor and acceptor substrates suggests that the oligosaccharyltransferase in these two diverse organisms should be structurally related. Mammalian and avian oligosaccharyltransferases have been purified as protein complexes consisting of ribophorin I (M_r = 66,000), ribophorin II (M_r = 63,000), and OST48 (M_r = 48,000) (Kelleher et al., 1992; Kumar et al., 1994). Ribophorins I and II are well characterized integral membrane glycoproteins of the rough endoplasmic reticulum (Kreibich et al., 1978; Marcantonio et al., 1982). Consistent with an active site located within the lumen of the endoplasmic reticulum, protein sequence analysis and protease accessibility studies indicate that the ribophorins and OST48 are type I integral

Please address all correspondence to Dr. Reid Gilmore, Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655-0103. Tel.: (508) 856-5894. Fax: (508) 856-6231.

The current address of Dr. Paula G. Collins is Division of Toxicology, Massachusetts Institute of Technology, Cambridge, MA 02139.

membrane proteins with large amino-terminal lumenal domains and shorter carboxy-terminal cytoplasmic domains (Crimaudo et al., 1987; Harnik-Ort et al., 1987; Marcantonio et al., 1982; Silberstein et al., 1992). Protein immunoblot experiments have shown that ribophorin I and II are expressed in all mammalian tissues tested (Marcantonio et al., 1982), and immunoreactive proteins of similar size were detected in avian and amphibian organisms but not in yeast (Crimaudo et al., 1987).

The homology between the mammalian and yeast oligosaccharyltransferase first became evident when canine OST48 protein was found to be 25% identical in sequence to Wbplp, a 45-kD integral membrane protein of *Saccharomyces cerevisiae* (Silberstein et al., 1992; te Heesen et al., 1991). Wbplp is essential for vegetative growth of yeast and is localized to the yeast endoplasmic reticulum (te Heesen et al., 1991). Phenotypic analysis of a yeast strain bearing a mutant allele of the *WBP1* gene has shown that the Wbplp is required for asparagine-linked glycosylation of proteins in vivo and for oligosaccharide transfer to acceptor peptides in vitro (te Heesen et al., 1992). A second gene (*SWP1*) encoding a 30-kD polypeptide was identified as an allele-specific high-copy suppressor of the *wbp1-2* mutant (te Heesen et al., 1993). Gene product depletion experiments indicate that Swplp is also required for oligosaccharyltransferase activity in yeast (te Heesen et al., 1993). Simultaneous overexpression of both Wbplp and Swplp does not increase the oligosaccharyltransferase activity of yeast microsomes (te Heesen et al., 1993), suggesting that additional subunits remain to be characterized. Indeed, purification of yeast oligosaccharyltransferase as a complex of six subunits (α-ζ) was recently reported (Kelleher and Gilmore, 1994). The β and δ subunits of the yeast oligosaccharyltransferase were shown to correspond to Wbplp and Swplp, respectively. Surprisingly, a protein sequence comparison revealed that the 30-kD Swpl protein is related to the carboxy terminal half of mammalian ribophorin II (Kelleher and Gilmore, 1994).

The finding that two of the yeast oligosaccharyltransferase subunits, Wbplp and Swplp, are homologous to the OST48 and ribophorin II subunits of the mammalian oligosaccharyltransferase, respectively, suggested that the yeast homologue of ribophorin I remained to be identified. Here, we report the isolation and characterization of the yeast *OST1* gene that encodes the α subunit of the yeast oligosaccharyltransferase complex. The Ost1 protein is homologous to mammalian ribophorin I and is shown to be an essential subunit of the yeast oligosaccharyltransferase.

Materials and Methods

Protein Purification and Peptide Sequencing

Yeast oligosaccharyltransferase (50–100 pmol), purified as described previously (Kelleher and Gilmore, 1994), was resolved into subunits by SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF)[1] membrane or onto a nitrocellulose sheet and stained with Ponceau S. The amino terminal sequence of excised bands corresponding to the 62-and 64-kD forms of the α subunit was determined by the Worcester Foundation for Experimental Biology Protein Chemistry facility. In situ proteolysis of the 64-kD glycoform, peptide purification by narrow-bore reverse phase HPLC, and the sequencing of two peptides were performed by the Harvard University Microchemistry Facility using previously described procedures (Aebersold et al., 1987).

Isolation and Sequencing of an OST1 Genomic Clone

PCR (Saiki et al., 1988) was used to amplify DNA encoding a portion of a 30-residue internal tryptic peptide (SFYNTVGIPYPEHVGMSEEQHLL WETNRLPL) derived from the α subunit of the yeast oligosaccharyltransferase. Two degenerate oligonucleotide primers (5'GTIGGNATHCCITAYCC and 5'GCTCTAGACKRTTIGTYTCCCA) were synthesized based on the underlined amino acid sequences. The eight nucleotides in bold-faced type in the antisense primer correspond to an XbaI site added as a linker sequence. PCR was performed in a 25-μl reaction volume with 125 pmol of each oligonucleotide primer, 0.4 U Taq DNA polymerase (Perkin Elmer Cetus Corp., Norwalk, CT) and 150 ng of *S. cerevisiae* genomic DNA. Yeast genomic DNA to be used as a PCR template and for Southern analysis was isolated as described previously (Hoffman and Winston, 1987). To amplify the 77-bp DNA fragment, 25 cycles of denaturation (94°C, 1 min), annealing (40°C, 1 min), and extension (72°C, 1.5 min), were carried out in an automatic heating/cooling cycler (Programmable Thermal Controller, MJ Research). The PCR product was recovered from an 8% polyacrylamide gel and cloned using TA Cloning System (Invitrogen, San Diego, CA) for DNA sequencing and to prepare hybridization probes.

Approximately 20,000 colonies bearing recombinant plasmids from a *S. cerevisiae* genomic library in YEp13 were screened by in situ colony hybridization (Sambrook et al., 1989) with a random hexamer ^{32}P-labeled hybridization probe prepared from the PCR product. Filters were hybridized overnight with the probe in 35% formamide, 5× SSC, 5× Denhardt's solution, 100 μg/ml of denatured salmon sperm DNA, 0.1% SDS at 42°C, washed in 2× SSC, 0.1% SDS at 55°C, and exposed for 4 h at −80°C (Sambrook et al., 1989).

EcoRI–SphI and SphI–XbaI fragments from a hybridization-positive clone designated as pOST1-1 were subcloned into pUC19 and M13mp18. DNA sequencing of both strands was by the dideoxy chain termination method (Sanger et al., 1977). DNA sequence analysis and protein sequence comparisons were done with the MacVector (IBI) and DNA Star Acid Align (AANW) software programs.

Genomic Disruption of OST1

The location of restriction sites and PCR primers used for construction of the plasmid pRS305R-L to disrupt the chromosomal *OST1* locus using the γ transformation procedure (Sikorski and Hieter, 1989) are shown in Fig. 1 *A*. A 547-bp MscI–HindIII fragment from pOST1-1 was subcloned into SmaI–HindIII digested pRS305 (Sikorski and Hieter, 1989), to generate pRS305R. A 320-bp HindIII–DraI fragment derived from pOST1-1 was generated by standard PCR methods using two primers (5'CCCAAGCTT-TAGCTCGGAACAAGACGCAAAC and 5'CTGACATTCCAACGTGC). The sense primer contained the underlined six nucleotide extension to generate a HindIII site. The 508-bp PCR product was digested with HindIII and DraI to obtain a 320-bp fragment which was ligated into plasmid pRS-305R that had been digested with XhoI, blunt-ended by filling in, and then digested with HindIII. The resulting construct (pRS305R-L) was linearized at the unique HindIII site that joins the *OST1* derived sequences and used to disrupt the genomic *OST1* locus in two diploid yeast strains (PRY238; *MATa/α, ura3-52/ura3-52, leu2-3,112/leu2-3,112, lys2-801/+, +/his4-619* and YPH274; *MATa/α, ura3-52/ura3-52, lys2-801/lys2-801, ade2-101/ade2-101, trp1-Δ1/trp1-Δ1, his3-Δ200/his3-Δ200, leu2-Δ1/leu2-Δ1*). PRY238 was obtained from P. Robbins (Massachusetts Institute of Technology, Cambridge, MA) (Orlean et al., 1988), YPH274 (Sikorski and Hieter, 1989) was obtained from the Amer. Type Culture Collection (Rockville, MD). Standard laboratory media were used for yeast growth and sporulation (Sherman et al., 1986). Yeast transformations were performed by a modification (Kuo and Campbell, 1983) of the LiOAc transformation procedure (Ito et al., 1983). From each genetic background, six independent diploid transformants were sporulated, asci were dissected and analyzed for spore viability, colony formation, and growth on synthetic complete media lacking leucine. A diploid strain generated by disruption of the *OST1* gene in PRY238 (RGY101; *MATa/α, ura3-52/ura3-52, leu2-3,112/leu2-3,112, lys2-801/+, +/his4-619, OST1/Δost1::LEU2*) was selected for the experiments shown in Table I.

1. *Abbreviations used in this paper*: CPY, carboxypeptidase Y; DPAP B, dipeptidyl aminopeptidase B; GST, glutathione-*S*-transferase; OST, oligosaccharyltransferase; PVDF, polyvinylidene difluoride.

Isolation of Temperature-Sensitive S. cerevisiae ost1 Mutants

A 2.5-kb EcoRI–XbaI fragment from pOST1-1 was subcloned into the yeast centromeric plasmids pRS316 and pRS317 (Sikorski and Boeke, 1991; Sikorski and Hieter, 1989), that were also digested with EcoRI and XbaI, to generate the plasmids designated pRS316-OST1 and pRS317-OST1. The diploid RGY101 was transformed with pRS316-OST1, and uracil prototrophs were selected, sporulated, and tetrads were dissected. Viable colonies were screened for growth on selective media and tested for mating type to obtain a haploid segregant with the genotype *MATa, ura3-52, leu2-3,112, lys2-801, Δost1::LEU2 [pRS316-OST1]* which we designate as RGY116. RGY116 was transformed with pRS317-OST1, lysine prototrophs were selected, and subsequently cured of the pRS316-OST1 plasmid by growth on synthetic complete media plates containing 5-fluoro-orotic acid (Boeke et al., 1987) to generate the recipient strain RGY117 (*MATa, ura3-52, leu2-3,112, lys2-801, Δost1::LEU2 [pRS317-OST1]*) for a plasmid-shuffle mutagenesis procedure (Sikorski and Boeke, 1991).

The plasmid pRS316-OST1 was used as a template for PCR performed under conditions that favor misincorporation of deoxynucleotides by Taq polymerase (Leung et al., 1989). Oligonucleotide primers complementary to plasmid sequences flanking the gene were designed to generate mutagenized PCR products consisting of the 2.5-kb EcoRI–XbaI segment containing the *OST1* gene flanked by 165 bp of 5′ and 256 bp of 3′ vector derived sequence. To enhance deoxynucleotide misincorporation, the concentration of one dNTP was reduced fivefold with respect to the other three dNTPs in each of four separate PCRs, in the presence of 1 mM Mg^{++} and 0.5 mM Mn^{++}. Each 100 μl PCR contained 250 pmol of each primer, 100 ng of plasmid DNA and 1 U of Taq DNA polymerase. After 30 cycles of DNA amplification, the reactions were pooled and the 2,891-bp product was gel purified. The PCR product was reamplified under standard conditions (1.5 mM $MgCl_2$, 0 mM $MnCl_2$, equimolar dNTPs at 200 μM) until μg quantities of DNA were obtained. The resulting DNA fragments were cotransformed with EcoRI–XbaI digested pRS316 into the *S. cerevisiae* strain RGY117 using a 10:1 ratio of PCR product to gapped plasmid. Transformants that repaired the gapped plasmid by homologous recombination (Ma et al., 1987) were selected at 25°C as Leu^+ Ura^+ prototrophs. Transformants that could lose the plasmid pRS317-OST1 bearing the wild-type gene were selected by replica plating onto synthetic minimal media containing DL-α-aminoadipate (Sikorski and Boeke, 1991) while simultaneously selecting for temperature sensitivity by incubation of replica plates at 25°C and 37°C. From ~900 transformants, twelve Leu^+ Ura^+ Lys^- colonies that could grow at 25°C but not at 37°C were isolated. Of these twelve colonies, eight were temperature-sensitive for growth when replica plated onto YPD plates. Plasmid DNA was prepared from the temperature-sensitive strains (Hoffman and Winston, 1987) and used to transform *E. coli* for amplification. The resulting plasmids were used to retransform the strain RGY117 and the plasmid-shuffle procedure was repeated to confirm that the temperature-sensitive phenotype was plasmid linked.

Radiolabeling and Immunoprecipitation of Glycoproteins

Before radiolabeling, yeasts were grown for 20 h at 25°C in synthetic minimal media supplemented with the appropriate amino acids until mid-log phase (0.8–1.6 OD at 600 nm). Cultures shifted to 37°C were preincubated for the indicated time before labeling. Cells were collected by centrifugation and resuspended at 5 A_{600}/ml in minimal medium prewarmed at the corresponding temperature. Cells were labeled for the indicated times with 50 μCi of [^{35}S]methionine (New England Nuclear, Boston, MA) per A_{600} units of cells. Labeling was terminated by the addition of NaN_3 to 10 mM. To deplete lipid-linked oligosaccharides, tunicamycin was added at a concentration of 10 μg/ml, 15 min before radiolabeling as indicated. Rapid lysis of cells with glass beads and immunoprecipitation of radiolabeled proteins with antibodies to carboxypeptidase Y (CPY) and dipeptidyl aminopeptidase B (DPAP B) were performed as described previously (Rothblatt and Schekman, 1989). Immunoprecipitated proteins were incubated for 20 min at 65°C in SDS-sample buffer and resolved on 8% SDS-polyacrylamide gels.

Membrane Isolation and Oligosaccharyltransferase Assay

Microsomal membranes were isolated from wild-type and *ost1* mutant yeast grown to mid log phase at 25°C in YPD medium using a scaled-down version of the procedure described previously (Kelleher and Gilmore, 1994). After centrifugation for 5 min at 2,000 *g* in a SS-34 rotor (Sorvall Instruments, Wilmington, DE) to remove unbroken cells and debris, the supernatant was collected, and cell membranes were pelleted by centrifugation at 120,000 *g* for 60 min in a Type 50 rotor (Beckman Instrs., Fullerton, CA). The membrane pellets were resuspended in 20 mM Tris-Cl pH 7.4, 1 mM DTT, 10% glycerol and protease inhibitor cocktail (Kelleher and Gilmore, 1994) and recentrifuged for 60 min at 120,000 *g*. Microsomal membranes obtained after the second centrifugation were resuspended in the same buffer and stored at −80°C. Oligosaccharyltransferase activity in digitonin extracts was assayed as described previously, using an iodinated tripeptide acceptor (N^{α}-Ac-Asn-[^{125}I]Tyr-Thr-NH_2) and bovine lipid-linked oligosaccharide as a donor (Kelleher and Gilmore, 1994; Kelleher et al., 1992). The protein concentration of the microsomal membranes was determined using the Protein Assay (BioRad Labs., Hercules, CA).

Preparation of Antibodies to Ost1p

The expression plasmid pGEX2T-Ost1 was constructed by cloning the 1,020-bp DraI–MscI fragment from pRS316-OST1 into the SmaI site of pGEX-2T (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). *E. coli* TG-1 cells transformed with this plasmid express amino acid residues 93 to 432 as a fusion protein with glutathione-*S*-transferase (GST). A culture of *E. coli* (100 ml with an A_{600} of 0.7–0.8) was induced to express the GST-Ost1p fusion during a 2-h incubation with 0.5 mM IPTG at 37°C. Inclusion bodies containing the fusion protein were isolated from the cells essentially as described (Sambrook et al., 1989). The washed inclusion bodies containing the fusion protein were solubilized in the presence of urea (Schloß et al., 1988) and purified using glutathione Sepharose 4B beads (Pharmacia LKB Biotechnology) as described (Smith and Johnson, 1988). The purified GST-Ost1 fusion protein was used to immunize rabbits at East Acres Biologicals.

Approximately 3.7 mg of the GST-Ost1 fusion protein was solubilized with 250 μl of 50 mM TEA, pH 7.5 containing 1% SDS. The sample was adjusted to 2.5 ml with 50 mM TEA, pH 7.5, before coupling to Affi-Gel-15 (BioRad Labs.) after the manufacturer's instructions to prepare an affinity column for immunoselection of antibodies to Ost1p. Ost1p specific antibodies were eluted from the Affi-Gel-15 affinity matrix using 100 mM triethylamine (pH 11.5) according to Harlow and Lane (1988).

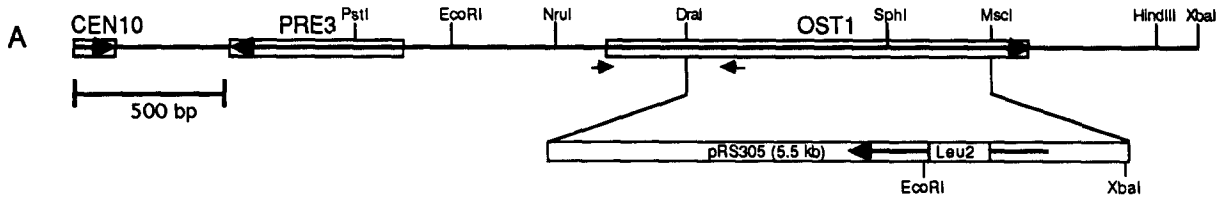
Protein Immunoblots and Endoglycosidase H Digestions

Proteins resolved by polyacrylamide gel electrophoresis in SDS were transferred to PVDF membranes. The membrane blots were probed with antiserum that recognize Ost1p, Wbp1p, or Kar2p. Peroxidase-labeled second antibodies were visualized using enhanced chemiluminescence (ECL Western blotting detection kit, Amersham Corp., Arlington Heights, IL). Endoglycosidase H was purchased from New England Biolabs (Beverly, MA); digestions were performed following the manufacturer's recommendations.

Results

Isolation and Sequencing of the OST1 Gene

The α subunit of the yeast oligosaccharyltransferase is resolved into two major forms (62 and 64 kD) and one minor form (60 kD) by SDS gel electrophoresis (Kelleher and Gilmore, 1994). Amino terminal sequencing of the 62- and 64-kD polypeptides yielded a common sequence consistent with previous evidence indicating that these polypeptides are alternative glycoforms of a single protein (Kelleher and Gilmore, 1994). Two tryptic peptides derived from the 64-kD form were also sequenced. Coincidentally, the sequence of one tryptic peptide (AQYEPPATWENVDYKR) was identical to the mature amino terminal sequence. Two degenerate oligonucleotide primers were synthesized based on the sequence of a 30-residue tryptic peptide and were used to amplify a yeast genomic DNA template using PCR. DNA sequencing of a PCR product of the predicted size (77 bp)



B

```
GAATTCCATTCATGTTAACTTTCTGTGTGTACTTTGAAGTAGCAATTCTTATCGTAATTGTACACCGTCTCGCAACTTGTTTAAAATACT     90
GTTAAAGATTGCATTTCAATGCTTTTCCCTTTTTTGCCACCGGATTCCGTTGCGAGTGGAAAAGTGAAAATGACAAAATTCAAAAGAAAT    180
CTATCAATATAACAAAAACGCCAAATCATCAAAAATGATCGGTGTTGAAGAGCTTGGCACTCTTAAAGGCGGCTAGTTCATAATGCTATA    270
GGAATTGACTCAAGGAAACGGACTATGTCTTGTACTGAATACTGTCTTCATTTGCCCATAGAATGCATTAGTGTTACTCTTCTTCGCGAG    360
CAGGGTAAGTATGCGCGTAATGTTTTTTATTTTCTGAAAGGTTCAAAGTATGCAGAACAAATTAATGTTTGCTTTTTATTAAGCAAGCTA    450
CTTCTTTGACAAGTACCCGATTGCTTCTTTAGCTCGGAACAAGACGCAAACTACAAAATATAGGTGCTGAAAAAATGAGGCAGGTTTGGT    540
                                                                    M  R  Q  V  W

TCTCTTGGATTGTGGGATTGTTCCTATGTTTTTTCAACGTGTCTTCTGCTGCCCAATACGAGCCACCTGCGACTTGGGAGAATGTTGATT    630
6   F  S  W  I  V  G  L  F  L  C  F  F  N  V  S  S  A  A  Q  Y  E  P  P  A  T  W  E  N  V  D

ATAAGAGGACAATAGACGTGTCAAACGCTTATATTTCAGAAACAATCGAAATAACTATCAAAAACATAGCAAGCGAACCTGCGACTGAAT    720
36  Y  K  R  T  I  D  V  S  N  A  Y  I  S  E  T  I  E  I  T  I  K  N  I  A  S  E  P  A  T  E

ACTTCACAGCCTTTGAGAGTGGCATCTTCAGTAAAGTTTCCTTTTTTTCAGCCTATTTTACCAACGAGGCAACTTTTTTAAATAGCCAAT    810
66  Y  F  T  A  F  E  S  G  I  F  S  K  V  S  F  F  S  A  Y  F  T  N  E  A  T  F  L  N  S  Q

TACTTGCCAATTCGACTACAGCACCTGGTGACGATGGTGAAAGTGAAATTAGATACGGGATCATTCAATTTCCAAATGCAATTTCCCCTC    900
96  L  L  A  N  S  T  T  A  P  G  D  D  G  E  S  E  I  R  Y  G  I  I  Q  F  P  N  A  I  S  P

AGGAAGAAGTTTCTTTAGTGATTAAGAGCTTCTATAATACCGTAGGTATTCCTTATCCTGAGCACGTTGGAATGTCAGAAGAACAACACC    990
126 Q  E  E  V  S  L  V  I  K  S  F  Y  N  T  V  G  I  P  Y  P  E  H  V  G  M  S  E  E  Q  H

TATTGTGGGAAACGAACAGATTGCCGCTTTCTGCTTACGATACCAAGAAGGCCTCTTTTACGCTGATTGGTAGCTCATCATTTGAGGAGT   1080
156 L  L  W  E  T  N  R  L  P  L  S  A  Y  D  T  K  K  A  S  F  T  L  I  G  S  S  S  F  E  E

ACCACCCCCCAAATGACGAGAGTTTACTGGGAAAAGCTAATGGAAACTCTTTTGAGTTTGGGCCTTGGGAAGATATTCCGAGATTTTCTT   1170
186 Y  H  P  P  N  D  E  S  L  L  G  K  A  N  G  N  S  F  E  F  G  P  W  E  D  I  P  R  F  S

CAAACGAAACCTTAGCAATTGTTTATTCCCACAATGCCCCATTGAATCAGGTAGTGAATTTGAGAAGAGATATTTGGCTTTCTCATTGGG   1260
216 S  N  E  T  L  A  I  V  Y  S  H  N  A  P  L  N  Q  V  V  N  L  R  R  D  I  W  L  S  H  W

CTTCCACAATACAATTTGAGGAATATTATGAATTAACAAACAAAGCCGCAAAACTGTCTAAAGGATTTTCAAGATTAGAATTAATGAAAC   1350
246 A  S  T  I  Q  F  E  E  Y  Y  E  L  T  N  K  A  A  K  L  S  K  G  F  S  R  L  E  L  M  K

AGATTCAAACTCAGAATATGAGACAAACTCACTTTGTTACTGTCTTAGACATGCTCCTGCCTGAGGGAGCTACTGATCATTATTTCACTG   1440
276 Q  I  Q  T  Q  N  M  R  Q  T  H  F  V  T  V  L  D  M  L  L  P  E  G  A  T  D  H  Y  F  T

ATTTGGTTGGCCTTGTTTCCACGTCGCATGCAGAACGTGACCATTTCTTTATAAGACCAAGATTCCCAATCTTTGGAGGTTGGAACTACA   1530
306 D  L  V  G  L  V  S  T  S  H  A  E  R  D  H  F  F  I  R  P  R  F  P  I  F  G  G  W  N  Y

ATTTTACTGTCGGTTGGACTAATAAATTGTCCGATTTCTTGCATGTATCCTCTGGCTCAGACGAGAAATTCGTTGCTTCTATCCCAATTC   1620
336 N  F  T  V  G  W  T  N  K  L  S  D  F  L  H  V  S  S  G  S  D  E  K  F  V  A  S  I  P  I

TAAACGGCCCACCGGACACTGTATATGATAATGTTGAATTATCGGTATTTCTTCCGGAAGGGGCCGAAATATTCGATATTGATTCTCCAG   1710
366 L  N  G  P  P  D  T  V  Y  D  N  V  E  L  S  V  F  L  P  E  G  A  E  I  F  D  I  D  S  P

TCCCTTTTACAAATGTTTCTATAGAAACCCAGAAATCATACTTTGACCTAAATAAAGGTCACGTTAAATTAACTTTCAGTTACAGAAATT   1800
396 V  P  F  T  N  V  S  I  E  T  Q  K  S  Y  F  D  L  N  K  G  H  V  K  L  T  F  S  Y  R  N

TGATTAGTCAAGTTGCCAATGGCCAAGTCTTGATAAAGTACGACTACCCGAAAAGCTCTTTTTTCAAGAAGCCTCTGTCTATTGCTTGCT   1890
426 L  I  S  Q  V  A  N  G  Q  V  L  I  K  Y  D  Y  P  K  S  S  F  F  K  K  P  L  S  I  A  C

ATATTTTCACCGCACTAATGGGAGTTTTTGTCTTAAAAACTTTGAACATGAACGTAACTAACTGAGTTCACGTTTGAATGATATATACCT   1980
456 Y  I  F  T  A  L  M  G  V  F  V  L  K  T  L  N  M  N  V  T  N  *

TATGATGCAGAGCAATAAATTCAGTATTTAATTTTTTATAAGTTTTATAGATAAGAATTTTCCAATGAATAAAGGTATTTACAATCATTT   2070
CCTAGACATTCTCGTGGTCTTTTCTTTGCACAATCAATGGTCATAGTTTTTTTTGTTTAGTAGTTTAAGGACAGAAGAATAACAAAGATG   2160
TTCACGTTTTGAATGTTACCAGACATTCTCAGATTCATCCTTTAACCTAGCGACGCGCACAGGCTCCCAATCCTCTTCAGAAAGACCTTG   2250
TAAACGATAATATTCTTCTTTAATATCAAATTCCCTTTGATTCTTCCTTATTTTCAAGATATCCTCTTCATTAATTTCCTGAACTTTACG   2340
ATCGCCTTGCTCGTACTTGATCGCTGTAAAGCTTGACAACCAAAACGAACCCAATACTATTGTTGCACAAAATGGTAAACCAAAAAAAAG   2430
AAAGGGGTGCTTACTCAATGCTTTTTTGTATCGCCCCGCTAAAGATGCTTCATATACTAGCTGCTGTCTTCTAGA                  2505
```

Figure 1. Restriction endonuclease map, DNA sequence, and gene disruption of the *OST1* locus. (*A*) The *OST1* gene is located on the right arm of chromosome X adjacent to *PRE3* (gb X78991) and *CEN10*. The *OST1* locus was disrupted by replacement of the DraI–MscI DNA fragment with the yeast integrating plasmid pRS305 bearing the *LEU2* gene. The locations of two PCR primers used to amplify a portion of the *OST1* locus for construction of the gene disruption plasmid are designated by the arrows flanking the DraI site. Restriction sites used for constructions and mapping of the gene disruption are shown. (*B*) The nucleotide sequence of an EcoRI–XbaI genomic DNA fragment containing the *OST1* gene is shown together with the predicted amino acid sequence of Ost1p. Nucleotide residues are numbered on the right starting at the EcoRI site; amino acid residues are numbered on the left. The termination codon is indicated by an asterisk. Two tryptic peptides that were sequenced are underlined. The signal peptidase cleavage site is indicated by an arrow. The dashed line beneath residues 450-467 designates a predicted membrane-spanning segment detected by hydropathy analysis (Kyte and Doolittle, 1982). Four consensus sites for N-linked glycosylation are enclosed in boxes. These sequence data are available from the EMBL/GenBank/DDBJ under accession number Z46719.

confirmed the isolation of an authentic amplification product of the *OST1* gene.

The *OST1* gene was isolated from a yeast genomic library in the YEp13 vector by colony hybridization using the radiolabeled PCR product as a probe. Sequence analysis of a 2.5-kb EcoRI–XbaI restriction fragment from a hybridization-positive plasmid revealed an open reading frame encoding a protein of 476 amino acids (Ostlp) as well as 5′ and 3′ flanking sequences (Fig. 1 *B*). The predicted protein sequence contains perfect matches for both tryptic peptides derived from the α subunit of the yeast oligosaccharyltransferase. Hydropathy analysis revealed the presence of two hydrophobic protein segments near the extreme amino and carboxyl termini of Ostlp (Kyte and Doolittle, 1982). The amino-terminal hydrophobic segment resembles a cleavable signal sequence for initiating translocation across the endoplasmic reticulum (von Heijne, 1986). The predicted signal peptidase processing site (von Heijne, 1986) is located between residues Ala 22 and Ala 23 consistent with the amino terminal sequence data described above. The second hydrophobic segment located between residues 450 and 467 is predicted to function as a membrane-spanning segment. The arrangement of these two hydrophobic segments suggest that Ostlp is a type I integral membrane protein with the majority of the polypeptide located within the lumen of the endoplasmic reticulum, and with residues 468 to 476 located in the cytoplasm. Four consensus sites for asparagine-linked glycosylation are present in the mature sequence of Ostlp, consistent with endoglycosidase H digestion data showing that the two major glycoforms of Ostlp contain four and three N-linked oligosaccharides, respectively (Kelleher and Gilmore, 1994). Since asparagine-linked glycosylation only occurs on lumenally disposed consensus sequons, the presence of four such sites in the deduced protein sequence supports the predicted topology for Ostlp. The calculated molecular weight of 51,448 for mature Ostlp is in reasonable agreement with the M_r of 54 kD observed for the endoglycosidase H–digested α subunit of the oligosaccharyltransferase on SDS-polyacrylamide gels (Kelleher and Gilmore, 1994).

A search of DNA sequence databases using the BLASTN DNA sequence comparison algorithm (Altschul et al., 1990) revealed that the first 635 nucleotides of the *OST1* sequence shown here are 96% identical to the 640 nucleotides from the 5′ flanking sequence of the yeast *PRE3* gene (Enenkel et al., 1994). Although several discrepancies between the sequence reported here and the 5′ flanking sequence of the *PRE3* gene may represent strain differences, others appear to be errors in the latter sequence. The restriction endonuclease map and the chromosomal location of the *OST1* gene is shown in Fig. 1 *A*. DNA sequence alignment maps the *PRE3* gene 370 bp downstream from *CEN10* (Enenkel et al., 1994). Hence, the ATG codon of the *OST1* gene is located on the right arm of chromosome X, ~1.5 kb downstream from the centromere.

Ostlp Is Homologous to the Mammalian Ribophorin I

A search of protein sequence databases using the BLASTP protein sequence comparison algorithm (Altschul et al., 1990) disclosed a homology between Ostlp and ribophorin I (Fig. 2). The sequence of ribophorin I has been deduced from the rat and human cDNA clones (Crimaudo et al., 1987; Harnik-Ort et al., 1987). Because the two mammalian ribophorin I sequences are 97% identical, only the human sequence is shown here. Ribophorin I is an integral membrane glycoprotein of the rough endoplasmic reticulum (Kreibich et al., 1978; Marcantonio et al., 1982) that was recently identified as a subunit of the mammalian (Kelleher et al., 1992) and avian (Kumar et al., 1994) oligosaccharyltransferase. Protein sequence analysis, protease accessibility studies, and endoglycosidase H digestions have revealed that ribophorin I is a type I integral membrane glycoprotein with a large amino-terminal lumenal domain, a single membrane-spanning segment, and a 150–residue carboxy-terminal cytoplasmic domain (Crimaudo et al., 1987; Harnik-Ort et al., 1987; Marcantonio et al., 1982). The sequence similarity between Ostlp and human ribophorin I extends throughout the lumenal domains of both proteins (Fig. 2). Within the overlapping region of 474 amino acids, the overall sequence identity between the two proteins is 28%, whereas sequence similarity was estimated to be 58%. The consensus sites for asparagine-linked glycosylation are not conserved. The most striking difference between human ribophorin I and Ostlp is the abbreviated cytoplasmic domain in the yeast protein relative to mammalian ribophorin I. Recently, a relationship between ribophorin I and the 60/62-kD subunit of the oligosaccharyltransferase was proposed (Knauer and Lehle, 1994) based upon a six–amino acid alignment, after introduction of one gap, between human ribophorin I and the amino terminal 12 residues of the yeast 60/62-kD subunit.

OST1 Is Essential for Vegetative Growth of Yeast

To determine whether Ostlp is required for cell viability, the *OST1* locus in the diploid yeast strain PRY238 was disrupted using a γ transformation procedure (Sikorski and Hieter, 1989). A 1,020-bp segment between the DraI and MscI restriction sites in the *OST1* gene was replaced with the yeast integrating plasmid pRS305 bearing the *LEU2* gene (Fig. 1 *A*), thereby removing codons 93-432 from the *OST1* gene. Leucine prototrophs were selected and correct integration of pRS305 into the *OST1* locus was confirmed by Southern blots using a combination of restriction sites in pRS305 and the DNA sequences flanking the *OST1* gene (data not shown). Diploid strains heterozygous for the *OST1* gene disruption (e.g., RGY101) were sporulated and the tetrads dissected. For each tetrad dissected (Table I), a maximum of two viable colonies were produced, both of which were leucine auxotrophs. Identical results were obtained when spores were allowed to germinate at 25°C. Spores bearing the *OST1* gene disruption germinated and formed microcolonies of 4–8 cells (data not shown), indicating that the Ost1 protein is essential for the vegetative growth of yeast. Similar results were obtained when the *OST1* gene was disrupted in the diploid strain YPH274 (data not shown). The lethal phenotype of an *OST1* disruption could be rescued by transformation of RGY101 with a centromeric plasmid bearing an intact copy of the *OST1* gene (Table I). Viable Leu$^+$, Ura$^+$ colonies were obtained upon sporulation and dissection of tetrads from RGY116.

Isolation of Temperature-Sensitive ost1 Mutants

Conditional *ost1* mutants were generated to determine whether the essential in vivo function of the Ost1 protein was

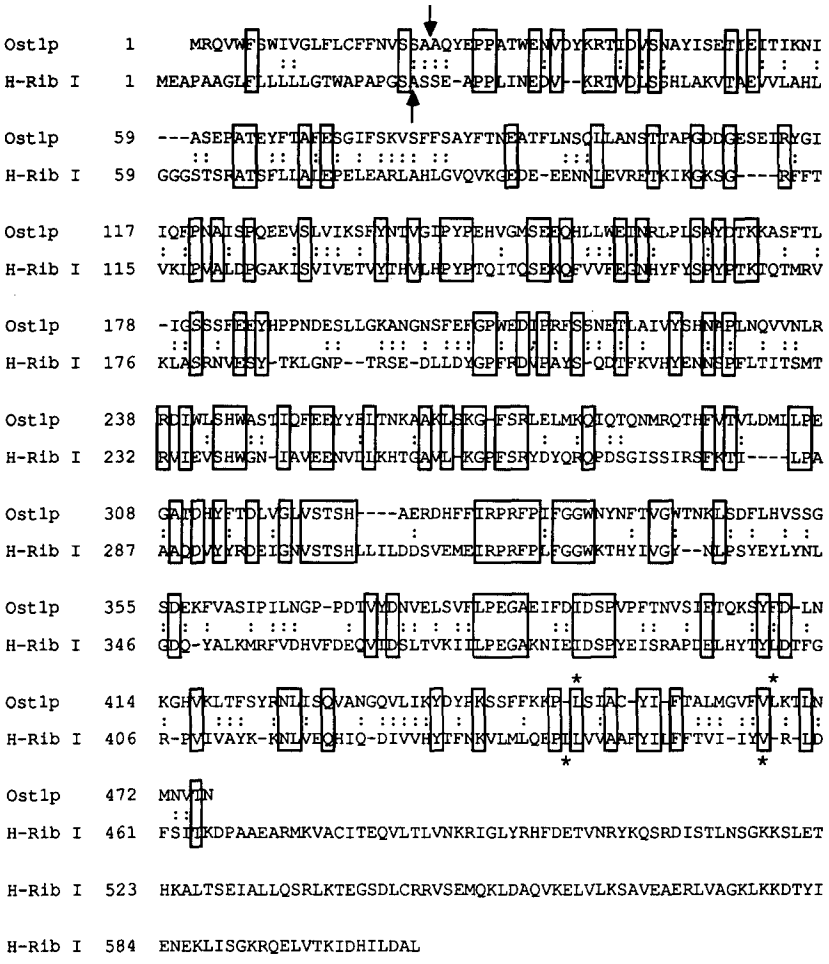


Figure 2. Sequence alignment between yeast Ostlp and human ribophorin I (H-Rib I). Identical amino acids are boxed. Conservative replacements are designated by colons using the following similarity rules: G=A=P=S; S=A=T; R=H=K; D=E=Q=N; M=I=L=V=F; F=W=Y. Gaps are indicated by dashes. Signal sequence cleavage sites for Ostlp and ribophorin I are indicated by arrows. The boundaries of the predicted membrane-spanning segments of Ostlp and human ribophorin I are indicated by asterisks.

directly related to asparagine-linked glycosylation of proteins. A haploid yeast strain bearing a chromosomal disruption of the *OST1* gene complemented by a plasmid borne copy of the wild-type *OST1* gene served as a recipient for a plasmid shuffle procedure (Sikorski and Boeke, 1991) wherein the plasmid bearing the wild-type *OST1* gene was replaced by a plasmid bearing a mutagenized *OST1* gene. The *OST1* gene was mutagenized by PCR amplification under conditions which enhance misincorporation of deoxyribonucleotides (Leung et al., 1989). Eight independent colonies were isolated that could grow at 25°C, but not at 37°C, and four of these were selected for further analysis. At the non-permissive temperature, the four *ost1* mutants arrested growth after 1–4 cell divisions (Fig. 3), unlike the strain bearing the wild-type control plasmid (RGY116). In liquid media at 25°C, doubling times similar to RGY116 were observed for *ost1-1* and *ost1-2*, whereas mutants *ost1-3* and *ost1-4* showed reduced growth rates at the permissive temperature (see legend to Fig. 3). Additionally, the *ost1-3* and *ost1-4* mutants form visible aggregates when grown in liquid culture at 25°C. Phase contrast microscopy indicates that cultures of *ost1-4* contain large clusters of 10–30 cells. Golgi defects that interfere with elongation of N-linked oligosaccharides (i.e., the *mnn* mutants) also cause this clumping phenotype, apparently due to defective separation of daughter and mother cells (Ballou et al., 1980).

ost1 Mutants Are Defective in Asparagine-linked Glycosylation In Vivo

In vivo synthesis of two yeast vacuolar glycoproteins, CPY and DPAP B, was examined in the four temperature-sensitive strains to determine whether the *ost1* mutants exhibit defects in asparagine-linked glycosylation. The soluble vacuolar protease CPY is synthesized as a proenzyme that acquires

Table I. Tetrad Analysis of OST1 Gene Disruption

Strain*	Relevant genotype and/or plasmid	Tetrads analyzed	Viable colonies per tetrad
PRY238	*OST1/OST1*	10	4
RGY101	*OST1/Δost1::LEU2*	24	2‡
RGY103	*OST1/Δost1::LEU2* [pRS316]	4	2‡
RGY116	*OST1/Δost1::LEU2* [pRS316-*OST1*]	25	3–4§

* Diploid strains were sporulated, tetrads dissected on YPD plates, and incubated for 4 d at 30°C. Colonies obtained were replica plated on selective medium to determine nutritional markers. PRY238 was the recipient for the *OST1* disruption to produce RGY101. The diploid RGY101 was transformed with the yeast centromeric vector pRS316 (*URA3*, *CEN6/ARS4*) that contained or lacked the *OST1* gene to obtain RGY103 and RGY116.

‡ Viable colonies were leu$^-$. Two microcolonies (<10 cells) were obtained per tetrad.

§ Viable leu$^+$ colonies were also ura$^+$.

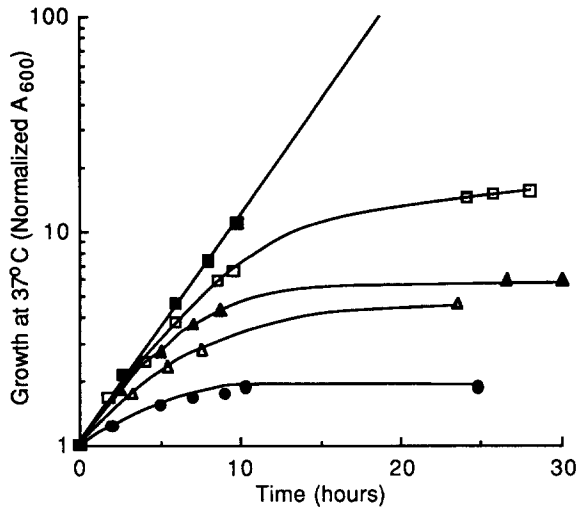


Figure 3. Growth of the *ostl* mutants at the restrictive temperature. Wild-type (RGY116) and *ostl* mutant strains were grown in YPD at 25°C until early logarithmic phase. At time 0, cultures were shifted to 37°C. As needed, cultures were diluted into fresh 37°C media to maintain an A_{600} of less than 0.8. The normalized increase in culture density (A_{600}) is shown for wild-type (■); *ostl-1* (□); *ostl-2* (▲); *ostl-3* (△); *ostl-4* (●) yeast strains. Doubling times in YPD at 25°C were as follows: wild-type (3.3 h), *ostl-1* (3.3 h), *ostl-2* (3.6 h), *ostl-3* (4.6 h), and *ostl-4* (7.1 h).

four core oligosaccharides in the endoplasmic reticulum (Stevens et al., 1982). The 67-kD pl form of proCPY is transported to the Golgi complex, where the core oligosaccharides are elongated by the addition of mannose residues to yield the 69-kD p2 form of proCPY. Upon arrival at the vacuole, the propeptide sequence is proteolytically removed to generate the mature 61-kD form of CPY. CPY is an ideal model glycoprotein for this analysis because intracellular transport of CPY is not completely inhibited when assembly of the lipid-linked oligosaccharide donor is blocked by tunicamycin treatment, indicating that defects in glycosylation should not prevent identification of CPY biosynthetic intermediates (Stevens et al., 1982; Winther et al., 1991).

CPY was immunoprecipitated from wild-type and *ostl* mutant yeast after radiolabeling for 1 h at 25°C or 37°C. As expected, the mature 61-kD vacuolar CPY was the predominant form produced by wild-type yeast at both temperatures (Fig. 4 *A*). A 51-kD polypeptide, that corresponds to unglycosylated vacuolar CPY, was detected when wild-type cells were treated with tunicamycin. The 59-kD polypeptide designated by the vertical arrow in the lanes derived from tunicamycin-treated yeast is an ER-arrested form of proCPY (Stevens et al., 1982). Transport of unglycosylated proCPY to the vacuole was reduced at 37°C, as observed by previous investigators (Winther et al., 1991). Multiple radiolabeled polypeptides were synthesized by each of the *ostl* mutants

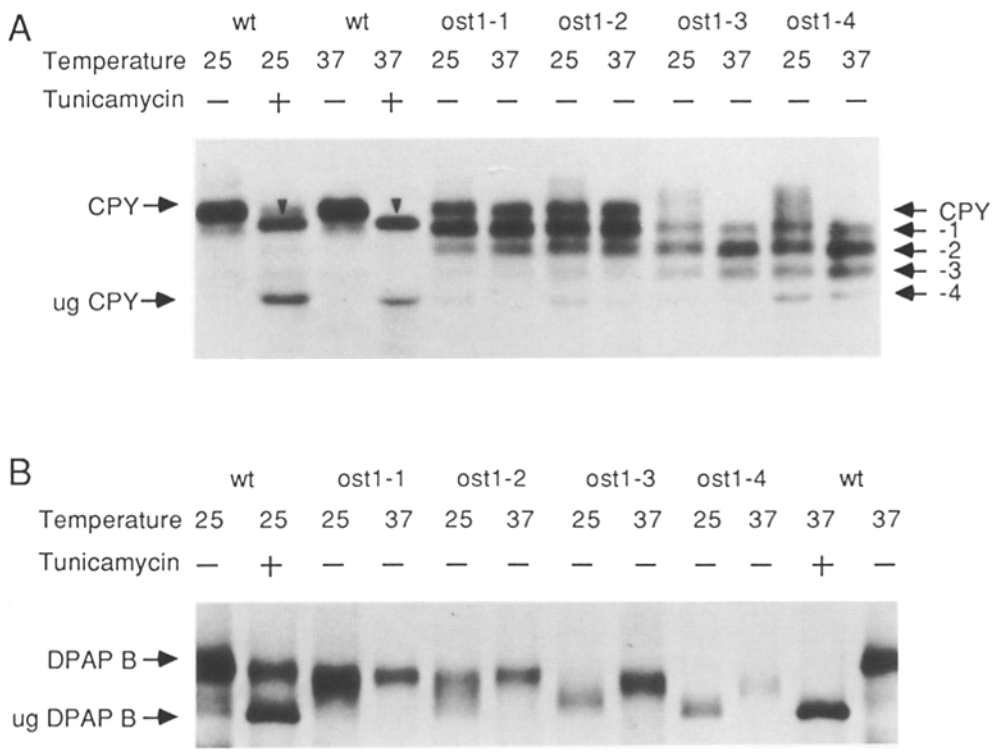


Figure 4. The *ostl* mutants are defective in core oligosaccharide transfer to CPY and DPAP B in vivo. Wild-type (RGY116) and *ostl* mutant cells grown in minimal media were maintained at 25°C or shifted to 37°C for 2 h before labeling with [^{35}S]methionine. As indicated, wild-type cells were incubated for 15 min with tunicamycin before labeling. (*A*) CPY immunoprecipitates from glass-bead extracts of cells labeled for 1 h with [^{35}S]methionine were resolved by PAGE in SDS. Fully glycosylated vacuolar CPY and unglycosylated vacuolar CPY (*ug* CPY) are designated by arrows. Vertical arrowheads in the tunicamycin-treated samples designate an ER-arrested form of proCPY (Stevens et al., 1982). Underglycosylated variants of CPY lacking between 1 and 4 asparagine-linked oligosaccharides are indicated by labeled arrows on the right side of the panel. (*B*) DPAP B immunoprecipitates from glass-bead extracts of cells labeled for 10 min with [^{35}S]methionine were resolved by PAGE in SDS. The migration position of the fully glycosylated and unglycosylated (*ug* DPAP B) forms of DPAP B are indicated by arrows. Underglycosylated forms of DPAP B show intermediate migration rates. Incomplete depletion of lipid-linked oligosaccharides by tunicamycin treatment at 25°C is responsible for the residual glycosylated DPAP B observed in the second lane.

at both the permissive and restrictive temperatures (Fig. 4 *A*). Based upon the number of evenly spaced bands that migrate between CPY from untreated and tunicamycin-treated wild-type yeast, we can conclude that the CPY variants labeled in the *ostl* mutant yeast contain between 0 and 4 N-linked oligosaccharides. Endoglycosidase H digestion of CPY immunoprecipitates from wild-type and *ostl* mutants yielded a major 51-kD product in each case, confirming that the primary difference between the CPY variants was the number of N-linked oligosaccharides (data not shown). Although low amounts of the p1 and p2 forms of proCPY were observed in immunoprecipitates from the *ostl-4* mutant after labeling at 25°C (Fig. 4 *A*), endoglycosidase H digestions showed that *ostl-4* cells do not accumulate unglycosylated 59 kD proCPY. The severity of the in vivo glycosylation defect at 25°C correlated with the growth defect displayed by the *ostl* mutants at 25°C; the mutants designated *ostl-3* and *ostl-4* showed the most dramatic pattern of underglycosylation of CPY. Likewise, the severity of the in vivo glycosylation defect at both temperatures correlated with the severity of the growth defect after cultures were shifted to 37°C.

In vivo glycosylation of the vacuolar membrane protein DPAP B by the wild-type and *ostl* mutant cells was also evaluated (Fig. 4 *B*). The antisera used for immunoprecipitation of DPAP B was raised against recombinant DPAP B expressed in *E. coli*, hence, the antibody recognizes protein rather than carbohydrate epitopes. In a previous study using this antisera, DPAP B was shown to be a type II integral membrane glycoprotein with eight consensus sites for N-linked glycosylation, six to seven of which, on average, are glycosylated in vivo (Roberts et al., 1989). The core glycosylated 110–113 kD ER form of DPAP B is converted to a mature 120-kD glycoprotein by further carbohydrate addition in the Golgi complex (Roberts et al., 1989). Unlike CPY, DPAP B does not undergo a proteolytic maturation reaction upon transport to the vacuole. The fully glycosylated DPAP B synthesized by wild-type cells during a 10-min labeling period at 25°C or 37°C (Fig. 4 *B*) corresponds to the Golgi form of DPAP B based upon pulse-chase studies reported by Roberts et al. (1989). A more rapidly migrating unglycosylated form of DPAP B (ug DPAP B) was detected in wild-type cells labeled in the presence of tunicamycin. DPAP B species that displayed intermediate mobilities, corresponding to different glycoforms of the protein, were synthesized by the *ostl* mutants at both the permissive and restrictive temperatures. As observed for CPY, the *ostl* mutants with the most severe growth defects displayed more extensive underglycosylation of DPAP B. Although the individual glycoforms of DPAP B were not well resolved, the protein synthesized by the *ostl-4* mutant at 25°C appears to contain, at the most, two N-linked oligosaccharides. Thus, underglycosylation of DPAP B appears to be more severe than underglycosylation of CPY at the permissive temperature. Surprisingly, DPAP B synthesized by the *ostl* mutants had, on average, more oligosaccharides at the restrictive temperature than at the permissive temperature.

The preceding results show that all four *ostl* mutants are defective in asparagine-linked glycosylation at both the restrictive and permissive temperatures. Yet, while relatively modest defects in glycosylation are lethal when cultures are shifted to 37°C (e.g., *ostl-1* and *ostl-2*), more severe glycosylation defects (e.g., *ostl-3* and *ostl-4*) are tolerated at 25°C.

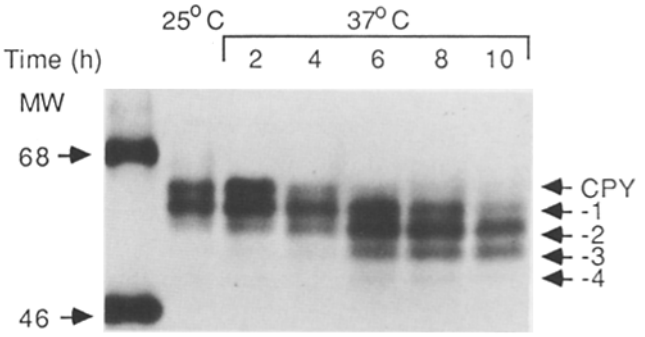


Figure 5. Extended incubation of *ostl-2* cells at 37°C results in reduced glycosylation of CPY. The *ostl-2* cells were either grown at 25°C or were grown for 2–10 h after shift to 37°C before a 1-h labeling period with [^{35}S]methionine. CPY immunoprecipitates from glass-bead extracts of labeled *ostl-2* cells were resolved by PAGE in SDS. Fully glycosylated vacuolar CPY and underglycosylated variants lacking between 1 and 4 asparagine-linked oligosaccharides are indicated by labeled arrows on the right side of the panel. Radiolabeled molecular weight (*MW*) markers are shown on the left.

The *ostl-2* mutant was chosen to address this apparent paradox because it shows modest defects in growth and in CPY glycosylation at the permissive temperature. CPY was radiolabeled at various time points after an *ostl-2* culture was shifted from 25°C to 37°C, and the immunoprecipitates were analyzed by SDS-gel electrophoresis (Fig. 5). Further reductions in glycosylation of CPY were observed at each successive time point. After 8 h of incubation at 37°C, the majority of the CPY glycoforms lack two or three Asn-linked oligosaccharides. The distribution of CPY glycoforms synthesized at the final time point was remarkably similar to those synthesized by the most defective mutants at 25°C (Fig. 4 *A*). Because the *ostl-2* cells cease to grow after incubation for 12 h at 37°C (Fig. 3), the glycoforms of CPY observed 10 h after the temperature shift may reflect the minimal levels of glycosylation that cells can tolerate at the restrictive temperature.

ostl Mutants Show Decreased Oligosaccharyltransferase Activity

Having established that mutations in the *OST1* gene do cause defects in asparagine-linked glycosylation, we next sought to determine whether the reduction in glycosylation was due to the presumed defect in the oligosaccharyltransferase. Microsomal membranes were prepared from cultures of wild-type and *ostl* mutant yeast grown at 25°C. The oligosaccharyltransferase activity of the digitonin-solubilized enzyme was assayed using dolichol-linked oligosaccharide isolated from bovine pancreas as the donor and the synthetic tripeptide N^{α}-Ac-Asn[^{125}I]Tyr-Thr-NH_2 as the oligosaccharide acceptor (Kelleher and Gilmore, 1994; Kelleher et al., 1992). The four mutant strains exhibited significantly reduced oligosaccharyltransferase activity relative to the wild-type strain (Fig. 6, *solid bars*). As the reduced oligosaccharyltransferase activity of the *ostl* mutants was observed in the presence of both exogenous donor and acceptor substrates, we conclude that the Ost1 protein is a bona fide subunit of the yeast oligosaccharyltransferase. Detergent extracts prepared from *ostl-1*

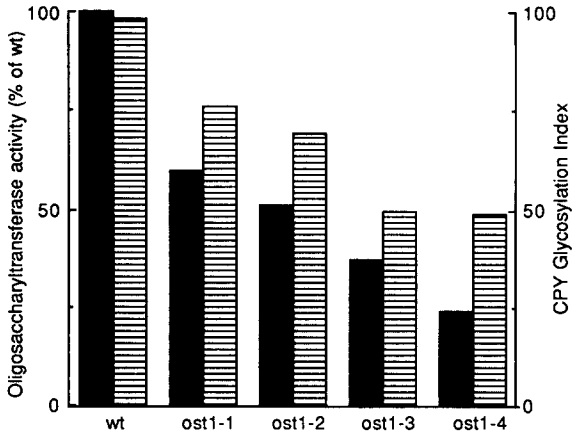


Figure 6. Oligosaccharyltransferase activity of the *ostl* mutants. Microsomal membranes isolated from the wild-type (RGY116) and *ostl* mutant yeast were assayed for in vitro oligosaccharyltransferase activity as described in the Materials and Methods. Activity values are the averages of two determinations. Solid bars represent the in vitro oligosaccharyltransferase activity expressed as a percentage of the wild-type strain, which had a specific activity of 0.5 pmol min^{-1} mg^{-1}. The CPY glycosylation index (*striped bars*) indicates the fraction of CPY glycosylation sequons that contain N-linked oligosaccharides in vivo at 25°C. The CPY glycosylation index was calculated from densitometric scanning of the fluorograph shown in Fig. 4 *A* using the following equation: CPY glycosylation index = $100\ (F_4 + 0.75 \times F_3 + 0.50 \times F_2 + 0.25 \times F_1)$, where F_1, F_2, F_3 and F_4 designate the fraction of CPY molecules that, respectively, contain one, two, three, or four N-linked oligosaccharides.

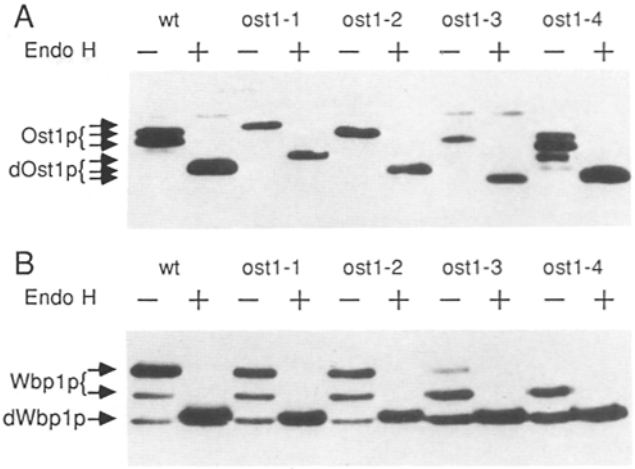


Figure 7. Glycosylation and expression of Ostlp and Wbplp. Membranes that were isolated from wild-type (RGY116) or *ostl* mutant yeast were incubated overnight in the presence or absence of endoglycosidase H. After resolution on a polyacrylamide gel in SDS, the proteins were transferred to PVDF membranes and probed with an affinity purified antibody specific for Ostlp (*A*) or an antiserum specific for Wbplp (*B*). Immunoreactive proteins were detected as described in the Materials and Methods. (*A*) The regions of the immunoblot that contain glycosylated (*Ostlp*) and endoglycosidase H–digested (*dOstlp*) forms of Ostlp are indicated by clusters of labeled arrows. Each gel lane for the Ostlp blot contained 47 μg of membrane protein except for the wild-type (73 μg) and the *ostl-3* mutant (83 μg). (*B*) Glycoforms of Wbplp are indicated by the arrow labeled Wbplp, while unglycosylated and endoglycosidase H–digested forms of Wbplp are indicated by the arrow labeled dWbplp. Each gel lane for the Wbplp blot contained 5 μg of membrane protein.

cells contained ~70% of the activity observed for extracts from wild-type cells, whereas only 25% of the wild-type activity was detected in extracts from *ostl-4* cells. In vivo use of the four glycosylation sequons in CPY at 25°C was quantified by scanning the autoradiogram shown in Fig. 4 *A*, and these data are expressed as the CPY glycosylation index (Fig. 6, *striped bars*). For each of the four mutants, the reduction in oligosaccharyltransferase activity correlated reasonably well with the CPY glycosylation index.

Expression of Ostlp, Wbplp, and Kar2p in the ostl Mutant Yeast

The membrane preparations that were assayed for oligosaccharyltransferase activity (Fig. 6) were subjected to protein immunoblot analysis using affinity-purified antibodies to Ostlp to determine whether the expression of OSTlp was altered in the *ostl* mutants (Fig. 7 *A*). Polyclonal rabbit antibodies were raised against Ostlp using a fusion protein consisting of codons 93-432 from Ostlp fused to glutathione-*S*-transferase. The mobility of Ostlp on SDS-polyacrylamide gels was altered in several of the *ostl* mutants as shown most readily by a comparison of the endoglycosidase H–digested samples (Fig. 7 *A*). Deglycosylated Ostlp from wild-type cells has a M_r of 54 kD, while Ostlp from the mutants had apparent M_r of 55 kD (*ostl-1*), 54 kD (*ostl-2*), 52 kD (*ostl-3*), and 53 kD (*ostl-4*). The alterations in the gel mobility of the mutant proteins are probably not caused by carboxy terminal extensions or truncations. Point mutations that introduce or eliminate charged amino acid residues can influence gel mobility by altering the quantity of protein-bound SDS. However, we have not sequenced the mutant alleles of *ostl*, so the origin of the altered gel mobility of Ostlp remains undefined. Ostlp from wild-type yeast migrates as a closely spaced glycoform doublet (Fig. 7 *A*). As shown previously by analysis of endoglycosidase H digestion intermediates, the 64-kD and 62-kD glycoforms of Ostlp contain four and three N-linked oligosaccharides (Kelleher and Gilmore, 1994). In contrast, a single predominant glycoform of Ostlp is present in membranes from the *ostl-1*, *ostl-2*, and *ostl-3* mutants. Examination of darker exposures of the protein immunoblot revealed the presence of four additional, more rapidly migrating forms of Ostlp in membranes isolated from *ostl-2* and *ostl-3* cells indicating that the predominant glycoform of Ostlp in these membranes contains four asparagine-linked oligosaccharides. Hence, Ostlp is hyperglycosylated in several *ostl* mutants relative to the wild-type control yeast. Additional experiments will be required to determine whether Ostlp synthesized by *ostl-1* cells contains three or four N-linked oligosaccharides. In contrast, membranes from *ostl-4* cells contain underglycosylated forms of Ostlp that contain between 1 and 4 N-linked oligosaccharides. Intensity differences in immunoreactivity were also observed when comparable amounts of membrane proteins were analyzed. Densitometric scanning of several different immunoblots indicates that membranes from the *ostl-3* mutant contain roughly 25–30% as much Ostlp as membranes from wild-type yeast. In contrast, membranes from the *ostl-1* and *ostl-2*

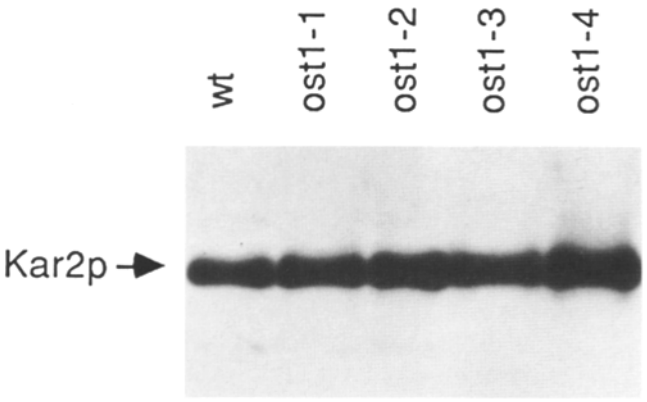


Figure 8. Induction of Kar2p expression in *ostl* mutants at the permissive temperature. Membranes isolated from wild-type or *ostl* mutant yeast were resolved on a polyacrylamide gel in SDS, transferred to a PVDF membrane, and probed with a rabbit polyclonal antibody to the Kar2 protein. Immunoreactive proteins were detected as described in the Materials and Methods. Each gel lane contained 12.5 μg of membrane protein.

mutants contain 70–90% of the Ostlp present in wild-type membranes. Membranes from the *ostl-4* strain do not show a reduction in the amount of the Ostlp.

Protein immunoblots of membranes isolated from wild-type and *ostl* mutant yeast were also probed with antisera to the Wbplp subunit of the yeast oligosaccharyltransferase complex (Fig. 7 *B*). The Wbpl protein sequence contains two sites for N-linked glycosylation (te Heesen et al., 1991). Endoglycosidase H digestion of the purified yeast oligosaccharyltransferase has shown that both sites are used in vivo (Kelleher and Gilmore, 1994). Although the predominant glycoform of Wbplp in the wild-type control strain does contain two oligosaccharides, less abundant glycoforms were also detected. Underglycosylated forms of Wbplp were readily apparent in membranes isolated from the *ostl-3* and *ostl-4* cells. Although membranes prepared from several *ostl* mutants show moderate reductions in the membrane content of Wbplp, as determined by densitometry, reductions in Wbplp content do not appear to correlate with reductions in oligosaccharyltransferase activity in the *ostl-3* and *ostl-4* cells.

The membrane preparations were also probed with an antibody to Kar2p (yeast BiP/grp78) to determine whether the unfolded protein-response pathway is induced in the *ostl* mutants at the permissive temperature (Fig. 8). Microsomes from the *ostl* mutants grown at 25°C contain 1.5–2-fold more Kar2 protein than the corresponding wild-type strain, indicating that Kar2p (BiP) expression is induced in these strains. The *ostl-4* mutant, which showed the most pronounced growth defect at 25°C, showed the most significant increase in Kar2p expression.

Discussion

Here, we have described the molecular cloning of *OST1*, the *S. cerevisiae* gene that encodes the 62/64-kD α subunit of the yeast oligosaccharyltransferase. The *OST1* gene was found to encode a 476–residue protein that shares significant sequence identity with ribophorin I, the 66-kD subunit of the mammalian oligosaccharyltransferase. Previous sequence comparisons had revealed that the Wbplp and the Swplp of *S. cerevisiae* are homologous to the OST48 and ribophorin II subunits of the canine oligosaccharyltransferase, respectively (Kelleher and Gilmore, 1994; Silberstein et al., 1992). Of the three yeast subunits sequenced to date, the Ostl protein has the highest degree of sequence identity to the corresponding mammalian subunit. Although structural similarities between the heterotrimeric canine oligosaccharyltransferase and the hexameric yeast oligosaccharyltransferase have been revealed by sequence comparisons, several noteworthy differences are also evident. Of the three homologue pairs so far identified, only the Wbplp-OST48 pair shows conservation of both sequence and protein size. Strikingly, ribophorin I contains a 150–residue cytoplasmic domain instead of the abbreviated cytoplasmic domain predicted for Ostlp. Although proteolytic digestion of the cytoplasmic domain of canine ribophorin I does not alter in vitro oligosaccharyltransferase activity (Kelleher and Gilmore, unpublished observations), a truncated form of ribophorin I that lacks the cytoplasmic domain was observed to be rapidly degraded in vivo (Tsao et al., 1992). Thus, this portion of ribophorin I may serve a function in vertebrate organisms that is dispensable in yeast, or is instead provided by one of the remaining uncharacterized subunits of the yeast oligosaccharyltransferase (γ, ϵ, or ζ).

As anticipated based upon its postulated function as a subunit of the oligosaccharyltransferase, expression of Ostlp was found to be essential for vegetative growth of yeast. The two genes (*WBP1* and *SWP1*) that encode the previously characterized subunits of the oligosaccharyltransferase are also essential for viability (te Heesen et al., 1992, 1993). Likewise, tunicamycin inhibition of lipid-linked oligosaccharide assembly is lethal in yeast (Barnes et al., 1984), as are mutations in the *ALG1* and *ALG2* genes that catalyze early steps in assembly of the lipid-linked oligosaccharide (Huffaker and Robbins, 1982; Jackson et al., 1993).

Yeast bearing mutations in the *OST1* gene were unable to grow at elevated temperatures and were defective in glycosylation of both soluble and membrane glycoproteins. Underglycosylation of newly synthesized proteins by the *ostl* mutants can be directly attributed to a defect in the oligosaccharyltransferase based upon the observed reduction in the glycosylation of an acceptor tripeptide by detergent extracts prepared from the *ostl* cells. These experiments provide in vivo confirmation for the biochemical identification of Ostlp as a subunit of the oligosaccharyltransferase (Kelleher and Gilmore, 1994). By extension, this study provides further support for the proposed role of ribophorin I as a subunit of the mammalian oligosaccharyltransferase (Kelleher et al., 1992) based upon the clearly defined homology between ribophorin I and Ostlp.

Notably, all four *ostl* mutants that were examined displayed defects in glycosylation at both the permissive and restrictive temperatures. Similar results were reported previously for the conditional *wbp1* mutants (te Heesen et al., 1992). Different explanations for the more pronounced growth defect observed at the elevated temperature should be considered. In the case of the *ostl-2* mutant, the glycosylation defect became considerably more severe at later time points after a shift to the restrictive temperature. This gradual decline in oligosaccharyltransferase function at the restrictive temperature would not be consistent with a tem-

perature-sensitive lesion in enzyme activity, but could be explained by reduced stability of newly synthesized Ostlp at the elevated temperature. A second explanation for the more pronounced growth defect at the restrictive temperature is provided by considering the role of N-linked oligosaccharides in protein folding. Inhibition of asparagine-linked glycosylation interferes with folding and intracellular transport of many, but not all, glycoproteins (Helenius, 1994). Malfolded proteins that accumulate upon tunicamycin treatment often contain aberrant disulfides, can become stably associated with the lumenal ER chaperone BiP (Kar2p), and are eventually degraded in the endoplasmic reticulum (Helenius, 1994). Protein folding is temperature dependent, hence, underglycosylation of proteins leads to more pronounced folding defects at higher temperatures. For example, in tunicamycin-treated cells the unglycosylated G protein of vesicular stomatitis virus accumulates in a Triton X-100 insoluble aggregate at 37°C, but not at 30°C (Gibson et al., 1979). Extended incubation of *ostl* cells at the restrictive temperature should result in the accumulation of multiple defective proteins, at least some of which will be required for cell viability.

Expression of the Kar2 protein was induced in the *ostl* mutants at the permissive temperature, consistent with the synthesis of folding-impaired proteins by the *ostl* mutants. Previous studies have shown that *KAR2* mRNA expression is induced by tunicamycin or 2-deoxy-glucose treatment of wild-type yeast (Rose et al., 1989), or by shifting *sec53* mutant strains of yeast to the restrictive temperature, thereby causing a block in assembly of the lipid-linked oligosaccharide (Rose et al., 1989). Consistent with this observation, *kar2 wbpl* double mutants show markedly reduced growth rates at the permissive temperature due to a synthetic interaction between these two gene products (te Heesen and Aebi, 1994).

Each of three glycoproteins examined (CPY, DPAP B and Wbplp) was underglycosylated by the *ostl* mutants. Glycosylation of the two integral membrane proteins appeared to be more severely reduced than glycosylation of CPY by mutations in *OSTl* at 25°C. Enhanced glycosylation of DPAP B at the restrictive temperature by the *ostl* mutants may be indicative of prolonged exposure of unfolded forms of DPAP B to the oligosaccharyltransferase. Hence, we speculate that folding of DPAP B is temperature-sensitive, and may be contingent upon acquisition of a minimum number of N-linked oligosaccharides at the restrictive temperature.

The protein immunoblot experiments suggest that the glycosylation defect in the two most severely impaired *ostl* mutants arise by diverse mechanisms. The reduced membrane content of Ostlp in membranes prepared from *ostl-3* cells correlated with the reduction in the in vitro oligosaccharyltransferase activity. The protein immunoblot experiments also showed hyperglycosylation of the α subunit (Ostlp) relative to that observed in wild-type yeast in two of four mutants analyzed (*ostl-2* and *ostl-3*). Malfolded proteins can be modified at glycosylation sequons that are not used, or are under-used in the wild-type protein resulting in hyperglycosylation of a folding-impaired protein (Bulleid et al., 1992; McGinnes and Morrison, 1994). We postulate that the basis for the reduced oligosaccharyltransferase activity in *ostl-3* is a defect in the assembly of the mutant protein into the oligosaccharyltransferase. Interestingly, the membrane content of the Wbplp subunit of the oligosaccharyltransferase was only slightly reduced in membranes prepared from *ostl-3* cells. Thus, the endoplasmic reticulum of *ostl-3* cells may contain both fully assembled active oligosaccharyltransferase complexes and inactive complexes that lack the α subunit. In contrast, the Ostl protein synthesized by the *ostl-4* mutant is underglycosylated, yet apparently stable. The latter observation would be expected if oligomeric assembly of the α subunit in the *ostl-4* cell results in a stable, yet catalytically impaired oligosaccharyltransferase.

We thank Markus Aebi, Mark D. Rose, and Tom Stevens for providing antibodies that recognize Wbplp (M.A.), Kar2p (M.R.), CPY (T.S.), and DPAP B (T.S.). We thank Robert Lahue, Mark Rose, and Scott Emr for advice during the course of these experiments.

This work was supported by Public Health Services grant GM 43768 (R. Gilmore) and was initiated during the tenure of an Established Investigatorship of the American Heart Association.

Received for publication 18 October 1994 and in revised form 28 November 1994.

References

Aebersold, R. H., J. Leavitt, R. A. Saavedra, L. E. Hood, and S. B. H. Kent. 1987. Internal amino acid sequence analysis of proteins separated by one or two-dimensional gel electrophoresis after *in situ* protease digestion on nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 84:6970–6974.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.

Ballou, L., R. E. Cohen, and C. E. Ballou. 1980. *Saccharomyces cerevisiae* mutants that make mannoproteins with a truncated outer chain. *J. Biol. Chem.* 255:5986–5991.

Barnes, G., W. J. Hansen, C. L. Holcomb, and J. Rhine. 1984. Asparagine-linked glycosylation in *Saccharomyces cerevisiae*: genetic analysis of an early step. *Mol. Cell. Biol.* 4:2381–2388.

Barriocanal, J. G., J. S. Bonifacino, L. Yuan, and I. V. Sandoval. 1986. Biosynthesis, glycosylation, movement through the Golgi system, and transport to lysosomes by an N-linked carbohydrate-independent mechanism of three lysosomal integral membrane proteins. *J. Biol. Chem.* 261:16755–16763.

Boeke, J. D., J. Trueheart, G. Natsoulis, and G. R. Fink. 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* 154: 164–175.

Bulleid, N. J., R. S. Bassel-Duby, R. B. Freedman, J. F. Sambrook, and M.-J. H. Gething. 1992. Cell-free synthesis of enzymatically active tissue-type plasminogen activator. Protein folding determines the extent of N-linked glycosylation. *Biochem. J.* 286:275–280.

Crimaudo, C., M. Hortsch, H. Gausepohl, and D. I. Meyer. 1987. Human ribophorins I and II: the primary structure and membrane topology of two highly conserved endoplasmic reticulum-specific glycoproteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:75–82.

Enenkel, C., H. Lehmann, J. Kipper, R. Gückel, W. Hilt, and D. H. Wolf. 1994. *PRE3*, highly homologous to the human major histocompatibility complex-linked *LMP2* (*RING12*) gene, codes for a yeast proteasome subunit necessary for the peptidylglutamyl-peptide hydrolyzing activity. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 341:193–196.

Gavel, Y., and G. Von Heijne. 1990. Sequence differences between glycosylated and non-glycosylated Asn-X-Thr/Ser acceptor sites: implications for protein engineering. *Protein Eng.* 3:433–442.

Gibson, R., S. Schlesinger, and S. Kornfeld. 1979. The nonglycosylated glycoprotein of vesicular stomatitis virus is temperature-sensitive and undergoes intracellular aggregation at elevated temperatures. *J. Biol. Chem.* 254: 3600–3607.

Guan, J. L., C. E. Machamer, and J. K. Rose. 1985. Glycosylation allows cell surface transport of an anchored secretory protein. *Cell.* 42:489–496.

Harlow, E., and D. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. pp. 312–315.

Harnik-Ort, V., K. Prakash, E. Marcantonio, D. R. Colman, M. G. Rosenfeld, M. Adesnik, D. D. Sabatini, and G. Kreibich. 1987. Isolation and characterization of cDNA clones for rat ribophorin I: complete coding sequence and in vitro synthesis and insertion of the encoded product into endoplasmic reticulum membranes. *J. Cell Biol.* 104:855–863.

Helenius, A. 1994. How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. *Mol. Biol. Cell.* 5:253–265.

Herscovics, A., and P. Orlean. 1993. Glycoprotein biosynthesis in yeast. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 7:540–550.

Hoffman, C. S., and F. Winston. 1987. A ten-minute DNA preparation from

yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene(Amst.)*. 57:267–272.
Huffaker, T. C., and P. W. Robbins. 1982. Temperature sensitive yeast mutants deficient in asparagine-linked glycosylation. *J. Biol. Chem.* 257:3203–3210.
Ito, H., Y. Fukuda, K. Murada, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163–168.
Jackson, B. J., M. A. Kukuruzinska, and P. Robbins. 1993. Biosynthesis of asparagine-linked oligosaccharides in *Saccharomyces cerevisiae*: the *alg2* mutation. *Glycobiol.* 3:357–364.
Kelleher, D. J., and R. Gilmore. 1994. The *Saccharomyces cerevisiae* oligosaccharyltransferase is a protein complex composed of Wbp1p, Swp1p and four additional polypeptides. *J. Biol. Chem.* 269:12908–12917.
Kelleher, D. J., G. Kreibich, and R. Gilmore. 1992. Oligosaccharyltransferase activity is associated with a protein complex composed of ribophorins I and II and a 48 kD protein. *Cell.* 69:55–65.
Knauer, R., and L. Lehle. 1994. The N-oligosaccharyltransferase complex from yeast. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 344:83–86.
Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 54:631–664.
Kreibich, G., B. L. Ulrich, and D. D. Sabatini. 1978. Proteins of rough microsomal membranes related to ribosome binding I. Identification of ribophorins I and II, membrane proteins characteristic of rough microsomes. *J. Cell Biol.* 77:464–487.
Kumar, V., S. Heinemann, and J. Ozols. 1994. Purification and characterization of avian oligosaccharyltransferase. *J. Biol. Chem.* 269:13451–13457.
Kuo, C.-L., and J. L. Campbell. 1983. Cloning of *Saccharomyces cerevisiae* DNA replication genes: isolation of the *CDC8* gene and two genes that compensate for the *cdc8-1* mutation. *Mol. Cell. Biol.* 3:1730–1737.
Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydrophobic character of a protein. *J. Mol. Biol.* 157:105–132.
Leung, D. W., E. Chen, and D. V. Goeddel. 1989. A method for random mutagenesis of a defined DNA segment using a modified polymerase chain reaction. *Techniques.* 1:11–15.
Ma, H., S. Kunes, P. J. Schatz, and D. Botstein. 1987. Plasmid construction by homologous recombination. *Gene (Amst.).* 58:201-216.
Marcantonio, E. E., R. C. Grebenau, D. D. Sabatini, and G. Kreibich. 1982. Identification of ribophorins in rough microsomal membranes from different organs of several species. *Eur. J. Biochem.* 124:217–222.
McGinnes, L. W., and T. G. Morrison. 1994. The role of individual cysteine residues in the formation of the mature, antigenic HN protein of Newcastle disease virus. *Virology.* 200:470–483.
Orlean, P., C. Albright, and P. W. Robbins. 1988. Cloning and sequencing of the yeast gene for dolichol phosphate mannose synthase, an essential protein. *J. Biol. Chem.* 263:17499–17507.
Riederer, M. A., and A. Hinnen. 1991. Removal of N-glycosylation sites of the yeast acid phosphatase severely affects protein folding. *J. Bacteriol.* 173:3539–3546.
Roberts, C. J., G. Pohlig, J. H. Rothman, and T. H. Stevens. 1989. Structure, biosynthesis, and localization of dipeptidyl aminopeptidase B, an integral membrane glycoprotein of the yeast vacuole. *J. Cell Biol.* 108:1363–1373.
Rose, M. D., L. M. Misra, and J. P. Vogel. 1989. *KAR2*, a karyogamy gene, is the yeast homolog of the mammalian BiP/GRP78 gene. *Cell.* 57:1211–1221.
Rothblatt, J., and R. Schekman. 1989. A hitchhiker's guide to analysis of the secretory pathway in yeast. *Methods Cell Biol.* 32:3–36.
Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. DC).* 239:487–494.
Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.
Schloß, P., I. Hermans-Borgmeyer, H. Betz, and E. D. Gundelfinger. 1988. Neuronal acetylcholine receptors in *Drosophila*: the ARD protein is a component of a high-affinity α-bungarotoxin binding complex. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:2889–2894.
Sherman, F., G. R. Fink, and J. B. Hicks. 1986. Methods in Yeast Genetics: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sikorski, R. S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics.* 122:19–27.
Sikorski, R. S., and J. D. Boeke. 1991. In vitro mutagenesis and plasmid shuffling: from cloned genes to mutant yeast. *Methods Enzymol.* 194:302–318.
Silberstein, S., D. J. Kelleher, and R. Gilmore. 1992. The 48 kDa subunit of the mammalian oligosaccharyltransferase complex is homologous to the essential yeast protein WBP1. *J. Biol. Chem.* 267:23658–23663.
Smith, D. B., and K. S. Johnson. 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusion proteins with glutathione S-transferase. *Gene (Amst.).* 76:31–41.
Stevens, T., B. Esmon, and R. Schekman. 1982. Early stages in the yeast secretory pathway are required for transport of carboxypeptidase Y to the vacuole. *Cell.* 30:439–448.
te Heesen, S., and M. Aebi. 1994. The genetic interaction of *kar2* and *wbp1* mutations. Distinct functions of binding protein BiP and N-linked glycosylation in the processing pathway of secreted proteins in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 222:631–637.
te Heesen, S., R. Rauhut, R. Aebersold, J. Abelson, M. Aebi, and M. W. Clark. 1991. An essential 45 kDa yeast transmembrane protein reacts with anti-nuclear pore antibodies: purification of the protein, immunolocalization and cloning of the gene. *Eur. J. Cell Biol.* 56:8–18.
te Heesen, S., B. Janetzky, L. Lehle, and M. Aebi. 1992. The yeast WBP1 is essential for oligosaccharyltransferase activity in vivo and in vitro. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2071–2075.
te Heesen, S., R. Knauer, L. Lehle, and M. Aebi. 1993. Yeast Wbp1p and Swp1p form a protein complex essential for oligosaccharyl transferase activity. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:279–284.
Tsao, Y. S., N. E. Ivessa, M. Adesnik, D. D. Sabatini, and G. Kreibich. 1992. Carboxy terminally truncated forms of ribophorin I are degraded in pre-Golgi compartments by a calcium-dependent process. *J. Cell Biol.* 116:57–67.
Varki, A. 1993. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology.* 3:97–130.
von Heijne, G. 1986. A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* 14:4683–4690.
Winther, J. R., T. H. Stevens, and M. C. Kielland-Brandt. 1991. Yeast carboxypeptidase Y requires glycosylation for efficient intracellular transport, but not for vacuolar sorting, in vivo stability, or activity. *Eur. J. Biochem.* 197:681–689.