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# Identification and Functional Characterization of Yeast ζ-COP

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**Coatomer, the cytosolic protein complex, consists of seven subunits (α-, β-, β′-, γ-, δ-, ε-, and ζ-COP) and is involved in vesicle trafficking early in the secretory pathway in collaboration with a 20 kDa GTP-binding protein (ARF). In the present study, we have identified a yeast gene which encodes a protein having 39% amino acid sequence identity with bovine ζ-COP. This gene (*YZC1* for yeast zeta COP) is essential for vegetative growth and the growth defect of *Δyzc1* cells was restored by bovine ζ-COP cDNA. We isolated a temperature-sensitive mutant of *YZC1* (*yzc1$^{ts}$*) and examined its capacity for both the ER-to-Golgi transport and the double lysine motif (KKXX)-mediated retrograde transport from Golgi to ER. At non-permissive temperature, the *yzc1$^{ts}$* cells exhibited a weak defect in the anterograde transport, but a strong defect in the retrograde vesicle transport. We conclude that Yzc1p is a yeast homologue of mammalian ζ-COP and participates mainly in the Golgi-to-ER retrograde transport.**

**Key words: coatomer, COPI, double lysine motif, endoplasmic reticulum, vesicle transport.**

Intracellular protein transport between membrane-bound organelles of the exocytic and endocytic pathways is carried out through budding and fusion of several types of coated vesicles (*1-4*). Clathrin-coated vesicles are involved in receptor-mediated endocytosis and in the transport of lysosomal or vacuolar proteins from the *trans*-Golgi network (*5*). Non-clathrin coats, COPI (*6*) and COPII (*7*), are involved in vesicle transport early in the secretory pathway; COPI coats were first identified in mammalian cells and they are composed of α- (160 kDa), β- (110 kDa), β′- (102 kDa), γ- (98 kDa), δ- (61 kDa), ε- (35 kDa), and ζ-COP (20 kDa) subunits which form a cytosolic ≈800 kDa complex (coatomer). The coatomer is recruited to the vesicles from the cytosol by the action of a 20 kDa GTP-binding protein, ARF (*8-10*), which is thus involved in the transport from the ER to *cis*-Golgi and in intra-Golgi transport. The function of COPI is evolutionally conserved, since coatomer purified from yeast closely resembles that of mammals in its subunit composition; it is composed of peptides with the molecular weights of 150, 110, 105, 73, 35, and 25 kDa (*11*) and, furthermore, subunits of COPI coats have homologues in yeast; Ret1p, Sec21p, Sec26p, and Sec27p for α-, γ-, β-, and β′-COP, respectively (*11-13*). Yeast COPI components, Ret1p, Sec27p, and Sec21p have been shown to participate in the retrieval of the double lysine (KKXX)-containing membrane proteins from post-ER back to the ER in yeast cells (*12*). On the other hand, COPII proteins which were initially identified in *Saccharomyces cerevisiae* are composed of the Sec13p-Sec31p complex and the Sec23p-Sec24p complex. They are involved in the ER-to-Golgi vesicle transport when they are recruited to the ER by the activation of the small GTPase Sar1p (*7*). The possible involvement of COPI coats in both forward and retrograde transport, as well as the requirement for COPI and COPII coats in ER-to-Golgi transport still remains controversial.

As a step towards understanding the function of COPI in the vesicle budding in yeast, we tried to isolate genes encoding yeast homologues of smaller coatomer subunits. In the present study we have identified a gene *YZC1* encoding a homologue of ζ-COP. ζ-COP is the smallest coatomer subunit and has been shown to be required for the assembly of coated vesicles in mammalian cells (*14*). We show here that *YZC1* is essential for vegetative growth of yeast cells, and that its temperature-sensitive mutant exhibited a strong defect in the double lysine-dependent ER retrieval, but a weak defect in the anterograde transport.

## MATERIALS AND METHODS

*Antibodies*—Anti-α-factor antibodies and anti-Kar2p (BiP) antibodies were a gift from Masao Tokunaga of Kagoshima University. Anti-CPY (carboxypeptidase Y) or invertase antibodies were raised in rabbits by injecting CPY (Boehringer Mannheim GmbH) or invertase (Wako Chemicals) as an emulsion with complete adjuvant. CPY and invertase that had been treated with endoglycosidase H were conjugated to Sepharose 4B and used for selection of specific antibodies. Preparation of recombinant Yzc1p and generation of the antibodies were as follows: the coding region of *YZC1* was subcloned into pQE30 (QIAGEN) to generate a bacterial expression construct, with a 6×histidine tag fused to the N-terminus of Yzc1p at the DNA level. XL1-Blue *Escherichia coli* cells harboring this plasmid were grown overnight, then Yzc1p was induced by 1

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Abbreviations: ARF, ADP-ribosylation factor; COPs, coatomer proteins; GlcNAc, *N*-acetylglucosamine; IPTG, isopropyl-β-D-thiogalactopyranoside; VSV-G, vesicular stomatitis virus transmembrane glycoprotein.

mM IPTG. Yzc1p was recovered from inclusion bodies. The inclusion bodies were washed extensively with 150 mM NaCl containing 1% Triton X-100 and subjected to 15% SDS-PAGE. The major band of Yzc1p was excised from the SDS-PAGE gels, mixed with complete adjuvant and injected into rabbits. Anti-Yzc1p antibodies were affinity-purified from the sera using Yzc1p-conjugated Formyl-Cellulofine (Seikagaku Kogyo). Anti-Bmh1p antibodies were raised in rabbit by injecting *E. coli*-expressed and purified yeast Bmh1p (Iwahashi, J. *et al.*, in preparation).

*Yeast Strains and Media*—Yeast strains, SEY6210 (MAT*α leu2-3, 112 ura3-52 his3Δ200 trp1-Δ901 lys2-801 suc2-Δ9* GAL$^+$) and SEY6211 (MATa *leu2-3, 112 ura3-52 his3Δ200 trp1-Δ901 ade2-1 suc2-Δ9* GAL$^+$), were provided by Scott Emr (University of California, San Diego). Yeast cells were grown in rich medium consisting of 1% Bacto-Yeast extract (Difco), 2% Bacto-Peptone (Difco), and 2% glucose. Minimal medium containing 0.7% yeast nitrogen base w/o amino acids (Difco) and 2% glucose was supplemented with appropriate amino acids and used. 5-Fluoroorotic acid (FOA) medium contains 1% 5-FOA and 0.2% Drop-out mix (*15*).

*Gene Disruptions*—*ARF1* (*16*) and *VPS1* (*17*) genes were disrupted between their *Bgl*II-*Nco*I and *Xho*I-*Bam*HI sites by the *HIS3* fragment (1.8 kbp) and *LEU2* fragment (2.1 kbp), respectively. A *HIS3*-containing 1.8 kb *Bam*HI fragment derived from YIp1 was used for *YZC1* gene disruption.

*Plasmids*—A 2.3 kb DNA fragment of the *YZC1* gene amplified by the polymerase chain reaction (PCR) was cloned into the polylinker site of a shuttle vector pRS316 (CEN, *URA3*) to obtain pRYZT. pSYZT was constructed by cloning the *YZC1* fragment described above into pRS314 (CEN, *TRP1*). pDBZT was constructed by placing the open reading frame of bovine ζ-COP between *ADH1* promoter and *CYC1* terminator of pD$_2$ (*18*). The expression plasmids of invertase-Wbp1p fusion proteins (pFIW, pFIS) were constructed as follows: The fragment containing *ADH1* promoter and *CYC1* terminator was excised from pD$_2$ and inserted into YEplac195 (2$\mu$, *URA3*) at the *Sph*I and *Hin*dIII sites to generate pFo. An *Eco*RI-*Bam*HI fragment containing the entire coding region of *SUC2* (invertase) was prepared by PCR using pRB58 (*19*) as the template. An *Bam*HI-*Eco*RI fragment which encodes the C-terminal 111 amino acid residues of Wbp1p including the transmembrane domain was prepared by PCR using yeast genomic DNA as the template. Both fragments were ligated then cloned into the *Eco*RI site of pFo to generate pFIW. pFIS encoding invertase-Wbp1 fusion protein where the C-terminal double lysine motif (-K-K-T-N-COOH) was changed to -S-S-T-N-COOH was prepared by introducing the mutation according to the method of Kunkel (*20*).

*Screening of ts-Alleles of YZC1*—Low-fidelity PCR was performed for the 2.3 kb *YZC1* fragment using the primers described above and the PCR products were subcloned into pRS314. Separately, *E. coli* was transformed with pSYZT which had been treated with hydroxylamine for 24 h (*15*). *Δyzc1* strains harboring pRYZT (CEN, *URA3*, *YZC1*) were transformed with these two pRS314-based *YZC1*-libraries and incubated at 26°C. The colonies were grown on SD (−Trp) plates and they were replica-plated onto 5-FOA plates to lose the pRYZT plasmid. The viable cells were then examined for growth ability at 37°C to obtain *yzc1$^{ts}$* alleles.

*cDNA Cloning of Bovine ζ-COP*—Total RNA was extracted from bovine liver with Quick Prep total RNA extraction kit (Pharmacia Biotech) and was subjected to RT-PCR by using two pairs of primers consecutively to obtain the coding region of bovine ζ-COP cDNA. The coding region was subcloned into pD$_2$ and used for functional complementation in *Δyzc1* cells.

*Pulse and Chase Experiments*—Cells (3.0 $A_{600}$ units) were cultured in 2 ml of SD medium lacking methionine for 2 h at 26 or 37°C then 4 MBq of [$^{35}$S]methionine was added and incubated either for 30 min (for 30-min pulse experiment) or 10 min (for pulse-chase experiment). After the pulse, 20 $\mu$l of 100× chase solution consisting of 0.4% methionine and 0.3% cysteine was added to the culture and incubation was continued for 30 min at 26 or 37°C. Alkaline lysis solution (7.2% NaOH and 7.4% $\beta$-mercaptoethanol) was added to the culture medium at 150 $\mu$l/ml and incubated for 10 min on ice. Then 130 $\mu$l of 60% TCA was added to the lysate to precipitate total proteins. The precipitates were suspended in 400 $\mu$l of TBES [50 mM Tris-HCl (pH 8.0), with 50 mM NaCl and 5 mM EDTA] containing 1% SDS and boiled for 10 min, followed by the addition of 1 ml of TBES containing 2% Triton X-100. Specific antibodies were added and incubated for 2 h at room temperature in the presence of Protein A-Sepharose. The immunoprecipitates were subjected to SDS-PAGE and the gels were analyzed with BioImage analyzer BAS2000. Pulse-chase of yeast cells harboring pFIW or pFIS was performed as described above except that immunoprecipitation with anti-invertase antibodies was repeated twice, followed by Endo Hf (NEB) digestion.

*Secretion of BiP from yzc1$^{ts}$ Cells*—Cells (3.0 $A_{600}$ units) were cultured in 2 ml of YPD medium containing 10 $\mu$g/ml BSA, 10 $\mu$g/ml $\alpha_2$-macroglobulin, 10% Trasylol, and 1 mM PMSF at semi-permissive temperature (30°C) for 6 h. The culture medium was precipitated with TCA, resolved by SDS-PAGE and then subjected to Western blotting with anti-BiP antibodies.

## RESULTS

*Identification of YZC1 Gene Encoding a Homologue of Mammalian ζ-COP*—To assess the *in vivo* function of low-molecular-weight components of COPI, we searched for a yeast gene homologous to the mammalian ζ-COP. A BLAST sequence homology search revealed an open reading frame (PIR Database, accession number P52521) that showed a significant homology to bovine ζ-COP (*14*). This protein sequence shared 39% amino acid identity with bovine ζ-COP (Fig. 1A) and showed a similar hydropathy profile to that of ζ-COP (Fig. 1B). Based on this and the experiment described below, we conclude that this gene codes for a homologue of ζ-COP and we designated it as *YZC1* (yeast zeta-COP).

Immunoprecipitation of the extract from [$^{35}$S]methionine-labeled *Δyzc1* cells harboring a Yzc1p-expression plasmid, pSYZT, with anti-Yzc1p antibodies gave a band of ≈25 kDa (Fig. 2), in good accordance with the reported molecular size of the smallest component of purified yeast coatomer complex (*11*). The antibodies also recognized bovine ζ-COP which was expressed in *Δyzc1* cells harboring pDBZT, although the recovery of the band was lower,

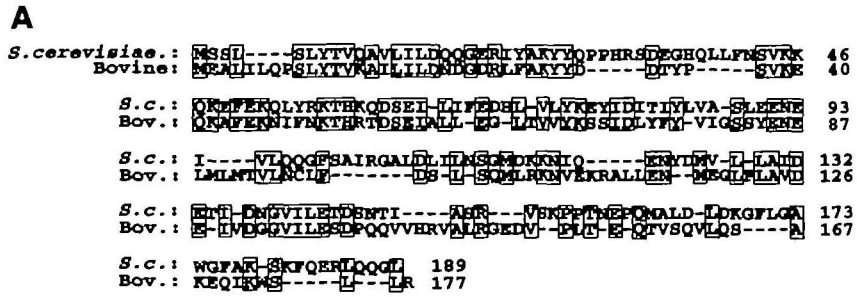
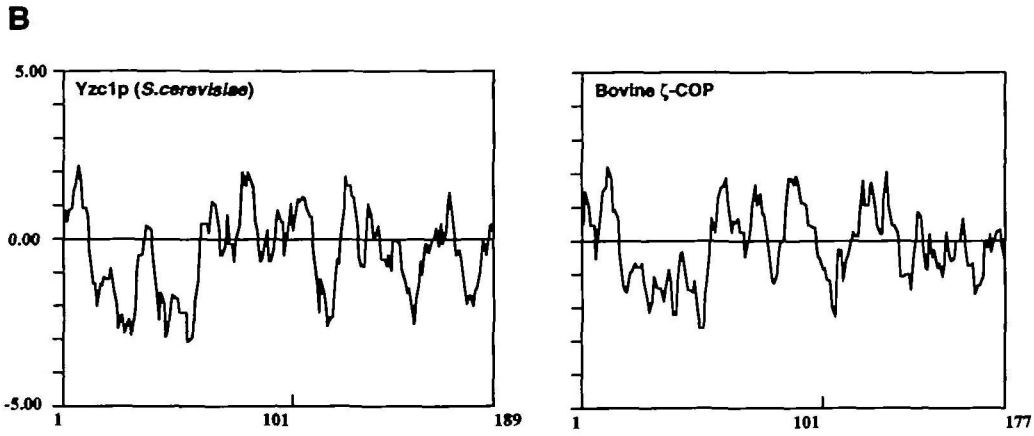



Fig. 1. **Homology between yeast Yzc1p and bovine ζ-COP.** (A) Sequence alignment of yeast Yzc1p with bovine ζ-COP. Yzc1p (PIR, accession number P52521) and bovine ζ-COP (accession number P35604) were aligned using DNASIS Software and matched amino acid residues were boxed. Total homology between the two amino acid sequences is 39%. (B) Hydropathy profiles of Yzc1p and bovine ζ-COP. The hydrophobicity was calculated by the method of Kyte and Doolittle with a window size of 8 amino acid residues.



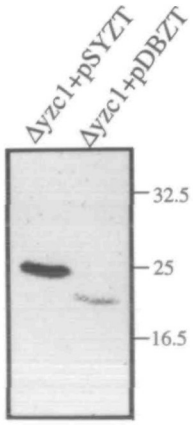




Fig. 2. **Immunoprecipitation of Yzc1p and bovine ζ-COP which were expressed in *Δyzc1* cells.** *Δyzc1* cells harboring pSYZT (CEN, *TRP1*, *YZC1*) or pDBZT (2μ, *TRP1*) harboring ζ-COP cDNA were labeled with [$^{35}$S]methionine at 30°C for 30 min. Immunoprecipitation was performed with anti-Yzc1p antibodies as described in "MATERIALS AND METHODS." Positions of the molecular size markers are indicated in the figure (in kDa).

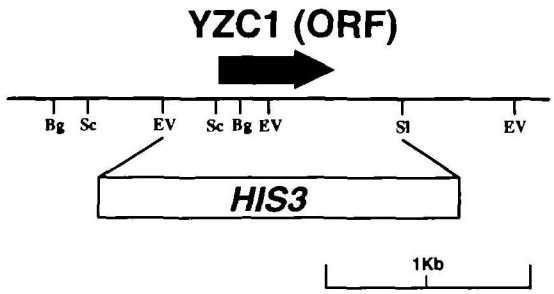



Fig. 3. **Restriction endonuclease map of *YZC1*.** The region used for *YZC1* disruption is shown. The bold arrow indicates the coding region of *YZC1*. The 1.2 kb *Eco*RV-*Sal*I fragment of *YZC1* was replaced with a 1.7 kb *Bam*HI fragment of *HIS3*. The abbreviations for restriction enzymes are: Bg, *Bgl*II; Sc, *Sac*I; EV, *Eco*RV; Sl, *Sal*I.

probably because of either the slower growth rate of the mutant cells or low cross-reactivity of the antibodies or both (Fig. 2).

*YZC1 Is Essential for Viability and Bovine ζ-COP Compensates for the Growth Defect of Δyzc1 Cells*—To examine whether *YZC1* gene is essential for vegetative growth, we disrupted the *YZC1* gene of haploid yeast strain (SEY6210) bearing pRYZT (CEN, *URA3*, *YZC1*) with *HIS3* at the region between the *Eco*RV and *Sal*I sites (Fig. 3). The gene replacement was verified by PCR using primers outside the replaced region (data not shown). Since the cells harboring the *URA3* gene metabolize 5-FOA to toxic material and thus cannot grow on the 5-FOA plate, only cells which had lost the plasmid carrying *URA3* marker can grow in the presence of 5-FOA. As shown in Fig. 4, the disruptant harboring pRYZT was unable to grow on a 5-FOA plate, indicating that the *YZC1* gene is essential. Furthermore, we disrupted one copy of the *YZC1* gene in the diploid strain (SEY6210×SEY6211) to generate *YZC1*/*Δyzc1*::*HIS3* and subjected it to tetrad analysis. Eight asci analyzed yielded a 2:2 segregation pattern of viable and non-viable spores and all the viable spores exhibited histidine auxotrophy (data not shown).

We then performed a plasmid shuffling experiment using 5-FOA plates to examine whether Yzc1p can be functionally replaced by mammalian ζ-COP. *Δyzc1* cells bearing pRYZT (CEN, *URA3*, *YZC1*) were transformed with

pDBYT (2$\mu$, *TRP1*) carrying bovine ζ-COP coding region placed between the *ADH1* promoter and *CYC1* teminator. *Δyzc1* cells harboring both plasmids could grow on a 5-FOA plate, although at a slower rate than wild-type cells (Fig. 4). In contrast, *Δyzc1* cells with pRYZT and $pD_2$ without any insert were unable to grow on a 5-FOA plate.

We found an ORF which lies downstream of *YZC1* in the opposite orientation and potentially encodes a protein with 1,039 amino acid residues. The above described gene replacement caused a deletion of the C-terminal 27 amino acid residues of this protein. Nevertheless, bovine ζ-COP cDNA compensated for the growth defect of the mutant. We thus speculate that this manipulation does not significantly affect the expression of the gene adjacent to *YZC1*.

We conclude that Yzc1p is a yeast functional homologue of mammalian ζ-COP and further that *YZC1* gene is essential for normal growth.

*Anterograde Protein Transport in yzc1$^{ts}$ Cells*—COPI coats are reported to be factors required for several steps of intracellular protein transport: in anterograde transport from ER-to-Golgi and between Golgi cisternae, in retrograde transport of membrane proteins from *cis*-Golgi back to the ER and in endosome function (*20*). However, the function of individual COPI components in various vesicle budding processes remains unclear; the finding that γ- and δ-COP do not bind to endosomes, whereas other subunits actually bind to them, suggests that COPI subunits may function in different processes in different subsets of functional complex (*21*).

We therefore examined the involvement of *YZC1* gene in protein transport in the secretory pathway. For this purpose we isolated a temperature-sensitive *yzc1* allelle. *yzc1$^{ts}$* cells exhibit a significant growth defect at 37°C (Fig. 5). DNA sequencing of this mutant revealed double mutations from Ser to Pro and from Leu to Val at codons 101 and 111, which are within nonconserved and conserved regions between bovine and yeast, respectively. We first examined transport of newly synthesized carboxypeptidase Y (CPY) in these mutant cells. As CPY traverses the secretory pathway it progresses from a core glycosylated ER form (p1) to an outer chain-glycosylated *medial*-Golgi form (p2) and eventually to proteolytically processed mature vacuolar form (m) (*22*). Mutant cells were labeled with [$^{35}$S]-methionine at 37°C for 30 min, then cells were lysed with alkali and subjected to immunoprecipitation. As shown in Fig. 6A, a significant amount of p1 and a smaller amount of

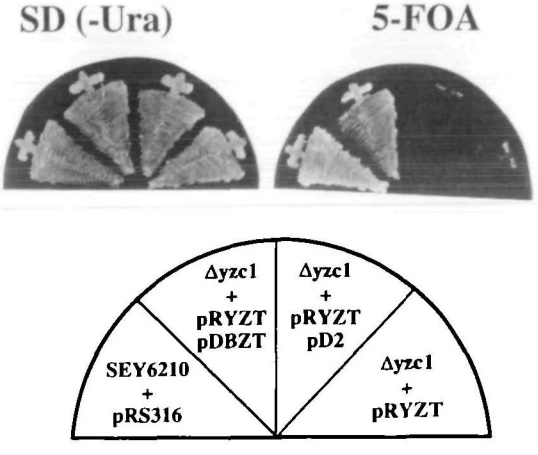



Fig. 4. ***YZC1* is an essential gene and the growth inability of *Δyzc1* is rescued by bovine ζ-COP cDNA.** Yeast strains harboring the indicated plasmids were streaked onto SD (−Ura) and 5-FOA plates and incubated at 30°C for 1 day and 3 days, respectively.

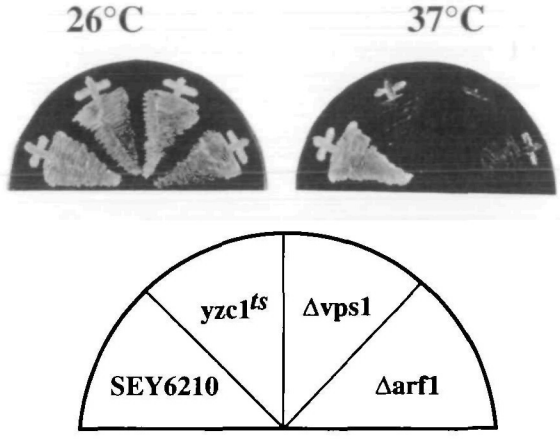



Fig. 5. **Temperature-sensitivity of *yzc1$^{ts}$*, *Δvps1*, and *Δarf1* strains.** Control and mutant cells were streaked onto YPD plates and incubated for 1 day at the indicated temperatures.

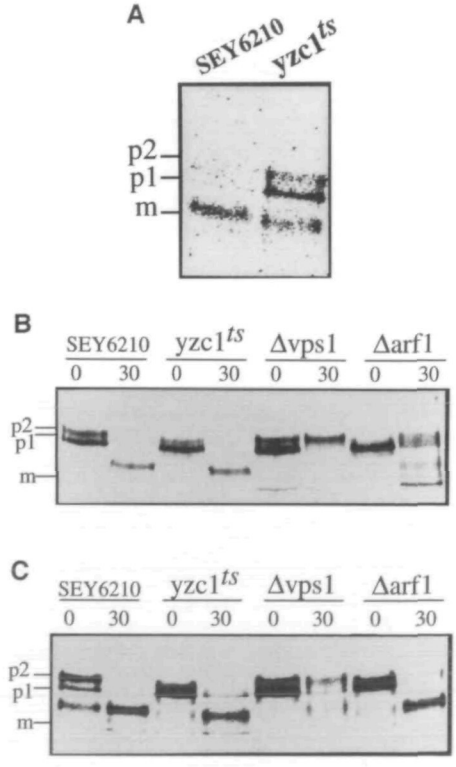



Fig. 6. **Intracellular transport of CYP in *yzc1$^{ts}$*, *Δvps1*, and *Δarf1* strains as revealed by immunoprecipitation.** (A) Equal amounts of cells were precultured at 37°C for 2 h and pulse-labeled with [$^{35}$S]methionine for 30 min. Cells were treated with alkaline lysis solution, then subjected to immunoprecipitation with anti-CPY antibodies. The positions of p1 (ER-modified), p2 (Golgi-modified), and mature (m) forms of CPY are indicated. (B) Cells were labeled with [$^{35}$S]methionine at 26°C for 10 min, then chased with cold methionine for 0 and 30 min. (C) Cells were precultured at 37°C for 2 h, pulse-labeled with [$^{35}$S]methionine for 10 min at 37°C and then chased with cold methionine at the same temperature for 0 or 30 min.

p2 were accumulated, indicating that transport to the vacuole was not significantly affected. Western blotting of the *ts*-mutant cells which had been grown at 37°C for 3 h also revealed the accumulation of a small amount of p1 form (data not shown). We then performed a pulse-chase experiment at both permissive (26°C) and restrictive (37°C) temperatures (Fig. 6, B and C). In wild-type cells p1 and p2 forms of CPY were rapidly transported to the vacuole concomitantly with the processing to the mature form at both temperatures. *yzc1$^{ts}$* cells accumulated a small amount of p1 form of CPY after the chase period at 37°C but the accumulation was apparent at 26°C only after a longer exposure. In marked contrast, accumulation of p2 form was observed at nonpermissive temperature in *Δvps1* cells, in which the transport from *late*-Golgi to the vacuole was blocked (*23*). Species with intermediate mobility between p1 and p2 were detectable as a smear in *Δarf1* cells, confirming the report that heterogenous forms of invertase which were modified within *cis*-to-*late*-Golgi compartments were accumulated in *Δarf1* cells (*24*).

We then examined the intracellular transport of α-factor with anti-α-factor antibodies (Fig. 7). The carbohydrate modifications of α-factor precursor during transit through the secretory pathway are diagnostic for the localization of the enzyme: high mannose core, 1,6-mannose and 1,3-mannose modifications at the ER, *cis*, and *medial*-Golgi compartments, respectively (*25*). In wild-type cells, newly synthesized prepro-α-factor was rapidly transported to the *late*-Golgi compartment, processed there by Kex2p protease, and secreted from the cell.

Although accumulation of the precursor forms of α-factor was hardly observed in *yzc1$^{ts}$* cells, the core and α-1,6 mannose-modified forms were detectable at the end of the pulse reaction: a similar result was obtained in *Δarf1* cells. In *Δvps1* cells a significant accumulation of unprocessed, α-1,3 mannose-modified form was observed even after the chase period as a result of mis-localization of Kex2p protease (*26*). These results indicate that *yzc1$^{ts}$* cells exhibit a weak but significant defect in the ER-to-*cis*-Golgi transport, upstream of the step defective in *Δvps1* cells.

*yzc1$^{ts}$ Cells Are Defective in Retrieval of a Double Lysine-Tagged ER Membrane Protein from cis-Golgi Compartment*—It has been shown in yeast cells that α-, β′-, and γ-COP function in retrieval of the double lysine motif (KKXX)-tagged membrane proteins to the ER from post-ER compartment (*12*). We therefore examined the ER retention of a KKXX-tagged membrane protein in *yzc1$^{ts}$* cells. Wbp1p, a 45 kDa subunit of oligosaccharyltransferase complex, is a type I ER resident membrane protein which contains a cytoplasmic double lysine motif at the C-terminus (*27*). The region of Wbp1p including the transmembrane domain and the cytoplasmic tail was fused to the C-terminus of invertase to construct a chimeric membrane protein (inv-KK) at the cDNA level. As a control, we also constructed a mutated version, inv-SS, in

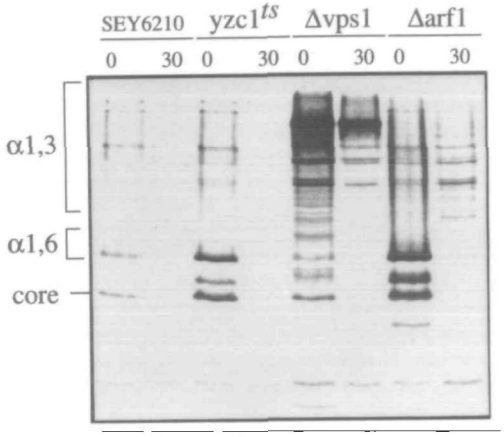



Fig. 7. **Intracellular transport of precursor of α-factor in *yzc1$^{ts}$*, *Δvps1*, and *Δarf1* strains.** The supernatant fractions obtained in the immunoprecipitation with anti-CPY antibodies in Fig. 6B were incubated with anti-pro α-factor antibodies. The immunoprecipitates were resolved by SDS-PAGE (15% gel). Other conditions are described in "MATERIALS AND METHODS." The positions of CPY with high mannose core (core), α-1,6-mannose (α1,6), and α-1,3-mannose (α1,3) modifications are indicated in the figure.

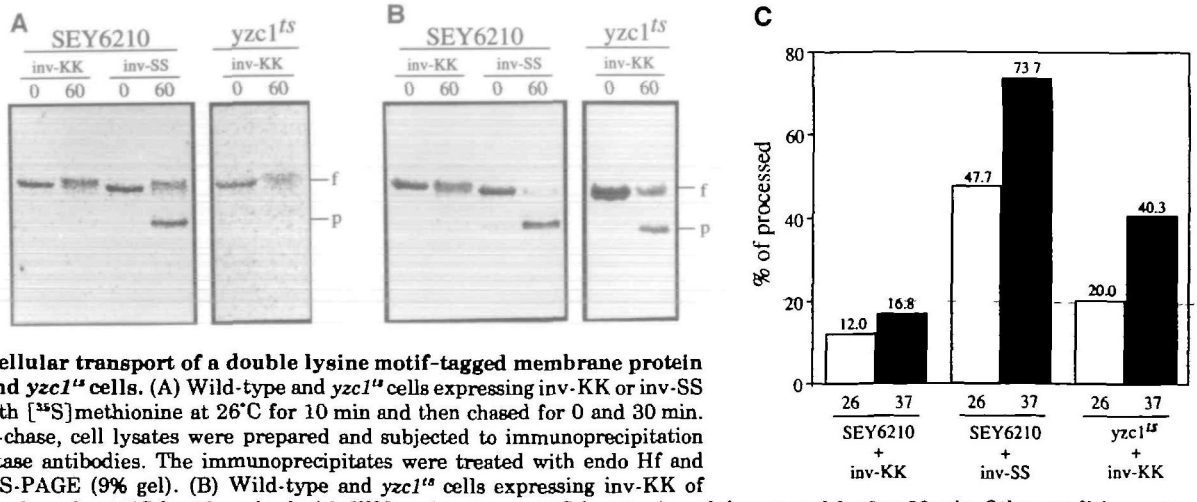



Fig. 8. **Intracellular transport of a double lysine motif-tagged membrane protein in wild-type and *yzc1$^{ts}$* cells.** (A) Wild-type and *yzc1$^{ts}$* cells expressing inv-KK or inv-SS were labeled with [$^{35}$S]methionine at 26°C for 10 min and then chased for 0 and 30 min. After the pulse-chase, cell lysates were prepared and subjected to immunoprecipitation with anti-invertase antibodies. The immunoprecipitates were treated with endo Hf and resolved by SDS-PAGE (9% gel). (B) Wild-type and *yzc1$^{ts}$* cells expressing inv-KK of inv-SS were precultured at 37°C for 2 h, pulsed with [$^{35}$S]methionine at 37°C for 10 min and then chased for 0 or 30 min. Other conditions were the same as in (A). The positions of the ER-form (f) and proteolytically processed form in the vacuole (p) of inv-KK or inv-SS are indicated in the figure. (C) Transport efficiency of inv-KK or inv-SS in wild-type and *yzc1$^{ts}$* cells. Efficiency of the processing after 60 min of chase was determined with the BioImage Analyzer. Percent processing indicates count (arbitrary unit) in p/count in (f+p).

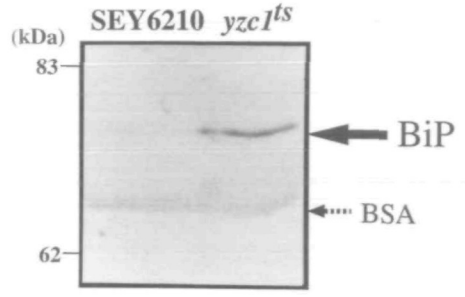



Fig. 9. ***yzc1ts* cells secrete BiP at a semi-permissive temperature.** Wild-type cells harboring pRS314 plasmid or *yzc1ts* cells were grown at 30°C for 6 h. The culture medium was subjected to SDS-PAGE followed by Western blotting with anti-BiP antibodies.

which the lysine residues of inv-KK (-KKTN-COOH) were changed to serine residues (-SSTN-COOH). If the fusion protein expressed in yeast cells escapes retention within the ER, it is transported to the vacuole, where it is processed by the Pep4p-dependent vacuolar protease cascade to produce a ≈56 kDa fragment (*28*). Therefore, the extent of processing of inv-KK is a good measure of the defect in the ER retention. Wild-type and *yzc1ts* cells expressing these chimeric proteins were subjected to a 10-min pulse with [$^{35}$S]methionine, then a 60-min chase at 26°C (Fig. 8A) or 37°C (Fig. 8B), followed by immunoprecipitation with anti-invertase antibodies. The immunoprecipitates were treated with endoglycosidase H and then subjected to SDS-PAGE. In wild-type cells the mobility of inv-KK was slightly decreased after the chase period; this was probably due to the fact that endo Hf cleaved the GlcNAc-GlcNAc bond of the high mannose type sugar chains, leaving GlcNAc-Asn in the substrate molecule. Nevertheless no cleavage of the protein was observed (a background processing of 12.0 and 16.8% was calculated at 26 and 37°C, respectively; Fig. 8C), indicating that inv-KK is efficiently retrieved from Golgi compartments to the ER in the wild-type cells. On the other hand, inv-SS underwent the cleavage at an efficiency of about 48% at 26°C and 74% at 37°C, confirming that the extent of proteolytic cleavage is a measure of the deficiency in the ER-retrieval. In *yzc1ts* cells, a significantly high proteolytic cleavage of inv-KK was observed at both permissive and nonpermissive temperatures: 20 and 40.3%, respectively (Fig. 8C). These results clearly indicate that Yzc1p is involved in the ER-retrieval of double lysine motif-tagged membrane proteins.

*yzc1ts Cells Secrete BiP at Semi-Permissive Temperature*—We next examined the effect of *yzc1* mutation on the HDEL-dependent retrieval of the ER luminal protein. HDEL-tagged proteins are recognized by the membrane-bound receptor in the post-ER compartments and are sorted to the retrograde transport pathway that retains them in the ER (*29*). *yzc1ts* cells were grown for 6 h at semi-permissive temperature, 30°C. As shown in Fig. 9, *yzc1ts* cells secreted Kar2p (BiP) into the medium, whereas wild-type cells did not. On the other hand, Bmh1p, a yeast cytoplasmic protein (*30*), was not detectable in the culture media of *yzc1ts* and wild-type cells, but was found within the cells, indicating that cell lysis did not occur under this condition (data not shown).

## DISCUSSION

Here we identified a yeast gene which encodes a protein sharing 39% amino acid identity with bovine ζ-COP and analyzed its function *in vivo*. It is essential for vegetative growth of cells and its function was partially substituted by expression of mammalian ζ-COP. Therefore, we designated this gene as *YZC1* after yeast zeta-COP.

ζ-COP was originally defined as the smallest 20 kDa subunit of coatomer proteins from bovine liver (*14*). Antibodies against ζ-COP inhibited both binding of the coatomer to Golgi membranes and the formation of COP-coated vesicles, resulting in the inhibition of transport of VSV-G protein within *cis*- and *medial*-Golgi compartments (*14*). Unlike other coatomer subunits, ζ-COP exhibits dual localization to coatomer and a free cytosolic pool and its interaction with coatomer is speculated to regulate coat assembly (*14*). It shares ≈30% amino acid identity with two small components of clathrin-associated protein complexes, AP17 and AP19 from rat brain (*31*). Yzc1p shares ≈30% sequence identity with Yaps1p and Yaps2p, which are putative yeast homologues of AP17 and AP19, respectively (*32*). These results may indicate that a common mechanism underlies the formation of clathrin- and COP-coated vesicles.

We isolated a *ts*-allelle of YZC1 and analyzed its capacity for both anterograde transport from the ER and retrograde transport from Golgi compartments. The *yzc1ts* cells exhibited a weak, but significant, defect in the anterograde transport at the ER-to-Golgi step. In marked contrast, we found that the double lysine motif-dependent ER-retrieval of inv-KK was significantly inhibited in *yzc1ts* at nonpermissive temperature and the extent of the defect was similar to those of *sec27-1* (β′-COP), *sec21-1*, and *sec21-2* cells, which are defective in retrieval of double lysine-tagged membrane proteins to the ER (*12*). These results suggest that Yzc1p plays an important role both in retrograde transport from Golgi to the ER and in the anterograde transport from the ER to Golgi compartments in cooperation with other COP subunits. The reason why ER-to-Golgi anterograde transport was only weakly inhibited in *yzc1ts* at nonpermissive temperature is unknown at present. It may be that COPI participates in both antero- and retrograde transport, although the present protocol was biased to select a *ts*-allelle having a pronounced defect in retrograde transport. Actually, *sec21-1* and *sec21-2* allelles show a significant difference in the defect of anterograde transport. However, it is equally possible that COPI components operate exclusively in retrograde Golgi-to-ER transport and a defect in recycling of the components required for anterograde vesicle transport (such as SNAREs) indirectly inhibits anterograde transport (*33*). Further work will be required to establish which is the case.

In *yzc1ts* mutant cells, a detectable amount of BiP was secreted into the culture medium. It has been reported that mutants that are defective in vesicle fusion (*sec17*, *sec18*, and *sec22* at a permissive temperature) and mutants that are defective in retrograde transport (*sec20*, *ufe1*, *rer1*, and *rer2*) secreted BiP, whereas budding mutants did not (*29*, *34*, *35*). Thus, *yzc1ts* behaves similarly to the retrograde transport mutants. We speculate that Yzc1p is involved in

the HDEL-dependent retrieval of the ER resident proteins and we are currently analyzing the effect of *yzc1* mutation on the sorting of the HDEL receptor. In this connection, Lewis and Pelhm (*34*) have recently reported that Sec21p ($\gamma$-COP) and Sec20p are involved primarily in the retrieval of the HDEL-receptor (Erd2p).

We have recently learned that Cosson *et al.* have identified *S. cerevisiae* genes *RET2* and *RET3* that complement *ret2-1* and *ret3-1* mutants which are defective in the KKXX-dependnent ER retrieval (*36*). *RET2* and *RET3* encode homologues of $\delta$- and $\zeta$-COPs, respectively, and are involved in ER retrieval of a double lysine-tagged membrane protein. In contrast to our present results, *ret3-1* allelle does not exhibit defects in anterograde transport.

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