A Novel GTP-binding Protein, Sar1p, Is Involved in Transport from the Endoplasmic Reticulum to the Golgi Apparatus

Akihiko Nakano*‡ and Masaaki Muramatsu‡

*Department of Biology, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113; and ‡Department of Chemistry, National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

Abstract. *SAR1*, a gene that has been isolated as a multicopy suppressor of the yeast ER-Golgi transport mutant *sec12*, encodes a novel GTP-binding protein. Its nucleotide sequence predicts a 21-kD polypeptide that contains amino acid sequences highly homologous to GTP-binding domains of many *ras*-related proteins. Gene disruption experiments show that *SAR1* is essential for cell growth. To test its function further, *SAR1* has been placed under control of the *GAL1* promoter and introduced into a haploid cell that had its chromosomal *SAR1* copy disrupted. This mutant grows normally in galactose medium but arrests growth 12–15 h after transfer to glucose medium. At the same time, mutant cells accumulate ER precursor forms of a secretory pheromone, α-mating factor, and a vacuolar enzyme, carboxypeptidase Y. We propose that Sec12p and Sar1p collaborate in directing ER-Golgi protein transport.

In the secretory pathway, proteins that have been translocated across the membrane of ER are subject to further sorting and transport to their final destinations. The next step, transport from the ER to the Golgi apparatus, requires correct discrimination between proteins that should be retained in the ER and those to be transported to the Golgi. Recent studies have shown that anomalous folding or incomplete assembly of proteins in the ER prevents their transport to the Golgi, whereas some ER-localized proteins possess an ER retention signal (see Pfeffer and Rothman, 1987, for review).

To understand molecular mechanisms underlying such sorting processes, it is important to identify the cellular machinery functioning in this interorganellar transport. Genetics has been successfully applied to the secretory pathway in yeast *Saccharomyces cerevisiae*, and as many as 12 genes are now known to be required in the ER-Golgi transport (Novick et al., 1980; Newman and Ferro-Novick, 1987). We have recently cloned and analyzed one of these 12 genes, *SEC12* (Nakano et al., 1988). In the process of cloning, two distinct genes were found to complement a *sec12* ts mutation. Genetic analysis has shown that one of these two is the authentic *SEC12* gene. Using an antibody raised against a *lacZ-SEC12* fusion gene product, we have identified the *SEC12* gene product (Sec12p) as an integral-membrane glycoprotein. It resides in the ER and the Golgi, probably facing both the cytoplasmic and lumenal sides of the membranes.

In this study, we report that the second gene, which suppresses *sec12* temperature-sensitive (ts) mutation when its gene dosage is raised, encodes a novel GTP-binding protein and is itself involved in the ER-Golgi transport. We have named it *SAR1*, to represent a secretion-associated and *ras*-superfamily-related gene. In this context, two other *ras*-related genes have been reported to play roles in the yeast secretory pathway. *SEC4* is essential for the fusion of secretory vesicles with the plasma membrane, its product being associated with both of these membranes (Salminen and Novick, 1987; Goud et al., 1988). *YPT1* plays multiple roles in yeast cell structure. Analysis of null and dominant lethal mutations of *YPT1* first suggested a role in microtubule organization (Schmitt et al., 1986), but recent reports describe that a cold-sensitive allele shows a transport arrest mainly in the Golgi (Segev et al., 1988), whereas a ts allele accumulates ER and also shows a Ca^{2+} remedial phenotype (Schmitt et al., 1988). In this paper, we present a model that another GTP-binding protein, Sar1p, directly interacts with a membrane protein, Sec12p, on ER and/or Golgi membranes and regulates vesicular traffic between these two organelles.

Materials and Methods

Strains and Culture Conditions

Escherichia coli strains used in this study were SE10 (F⁻Δ[*lac-pro*] *ara rpsL thi pyrF*74::Tn*5* [φ80d*lacZ*ΔM15]) (Emr et al., 1986) for cloning experiments and MV1193 (Δ[*lac-proAB*] *thi supE* Δ[*srl-recA*] 306::Tn*10*[tet^r] F′ *traD*36 *proAB lacI^{q}Z* ΔM15) (provided by Dr. J. Vieira, Waksman Institute of Technology, State University of New Jersey) for nucleotide sequencing. Helper phage M13K07 for single-stranded DNA preparation (Vieira and Messing, 1987) was also from J. Vieira.

Yeast *sec* strains used for complementation tests are listed in Table II. Strains MBY10-7C (*MATα sec12-4 ura3 leu2 trp1 his*), ANY1-7D (*MATa ura3 leu2*) (Nakano et al., 1988), and RDM15-5B (*MATα sec61-2 ura3 leu2*

Dr. Muramatsu's present address is DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304.

ade2 pep4) (Deshaies and Schekman, 1987) were described previously. Strain ANY21 (*MATa ura3 leu2 trp1 his*), which is isogenic to MBY10-7A, was obtained by marker rescue of *sec12-4* with the *SEC12* gene (Nakano et al., 1988). Strain JRY9 (*MATα ura3 leu2 trp1 his3 his4*) (a gift from Dr. J. Rine, University of California, Berkeley) was mated with ANY21 to obtain a diploid strain ANY102 (*MATa/MATα ura3/ura3 leu2/leu2 trp1/trp1 his/his*). ANY102 was transformed either with the disrupted *sec12* (pANY1-8, see Nakano et al., 1988) to give ANY104 (*MATa/MATα SEC12/sec12::URA3 ura3/ura3 leu2/leu2 trp1/trp1 his/his*) or with the disrupted *sar1* (pMMY2-1, see below) to yield ANY105 (*MATa/MATα SAR1/sar1::URA3 ura3/ura3 leu2/leu2 trp1/trp1 his/his*). ANY105 was transformed with *SAR1* multicopy plasmid, pSEC1210, using *LEU2* as a marker and subjected to sporulation and tetrad dissection. Spores were screened for Ura^+ Leu^+ phenotype and two representative haploid strains ANY22 (*MATa sar1::URA3 ura3 leu2 trp1 his* p [*SAR1 LEU2*]) and ANY24 (*MATα sar1::URA3 ura3 leu2 trp1 his* p [*SAR1 LEU2*]) were isolated. These haploids were further transformed with *GAL1-SAR1* plasmid (pANY2-18) using *TRP1* as a marker and cultured in YPGal liquid medium at 30°C for 2 d to induce the plasmid loss of either pSEC1210 or pANY2-18. The cultures were streaked on YPGal plates and single colonies were screened for auxotrophic markers. Ura^+ Leu^- Trp^+ strains, which lost pSEC1210 but contained the *GAL1-SAR1* fusion gene on the plasmid pANY2-18 and the disrupted version of *sar1* in the chromosome, were isolated and named ANY25 (*MATa sar1::URA3 ura3 leu2 trp1 his* p[*GAL1-SAR1 TRP1*]) and ANY26 (*MATα sar1::URA3 ura3 leu2 trp1 his* p[*GAL1-SAR1 TRP1*]). Strain YOT18 (*MATa/MATα cmd1::URA3/cmd1::URA3 ura3/ura3 ade/ade +/leu2 +/his1 +/trp4 GAL2/GAL2* p[*GAL1-CMD1* $G418^r$]) was a gift from Dr. Y. Ohya (University of Tokyo) and used as a control of Gal-dependent growth.

Yeast strains were usually grown at 30°C in YP medium (2% polypeptone and 1% yeast extract) containing 2% glucose (YPD) or in Wickerham's minimal (MV) medium (Wickerham, 1946) containing 2% glucose and appropriate supplements (MVD). In labeling experiments, sulfate salts in MV media were replaced by chlorides. *sec* ts strains were cultured at 24°C and incubated at 37°C for restrictive experiments unless otherwise stated. For derepression or repression of the *GAL1* promoter, YP or MV medium was supplemented either with 5% galactose and 0.2% sucrose (YPGal and MVGal) or with 5% glucose (YPD and MVD) respectively. Although all strains used in this study except YOT18 contained the *gal2* mutation, which is defective in galactose permease activity (Tschopp et al., 1986), the *GAL1* promoter was efficiently derepressed in the presence of 5% galactose because of the leakiness of *gal2*. Addition of 0.2% sucrose helped increase the slow growth rate in galactose medium but did not affect the growth or repression of *GAL1* in the presence of glucose. Cell density was monitored by measuring OD_{650} using a spectrophotometer (Junior II; Coleman, Heywood, IL). One OD_{650} corresponded to $\sim 5 \times 10^7$ cells/ml.

Plasmids and DNA Manipulations

E. coli plasmids, pUC18 (Yanisch-Perron, 1985), pUC118 and pUC119 (Vieira and Messing, 1987); yeast multicopy plasmids YEp13 (Broach et al., 1979) and pSEY8 (Emr et al., 1986); yeast single-copy plasmids pSEYC58 (Emr et al., 1986) and YCpG11 (Ohya et al., 1986); and yeast integration plasmid YIp5 (Botstein and Davis, 1982) have been described elsewhere. Isolation of *SEC12* and *SAR1* original clones, pSEC1230 and pSEC1210, respectively, which was done by Dr. M. Bernstein (University of California, Berkeley), has been described in a previous paper (Nakano et al., 1988). pSEC1212, pANY2-7, pANY2-10, pANY2-11, and pANY2-12 were derived from pSEY8, and pSEC1213, pANY2-9, and pANY2-13 were from pSEYC58, whose inserts are shown in Fig. 1. pANY2-7 and pANY2-9 containing the Hind III-Sal I fragment from pSEC1210 are also referred to as YEpSAR1 and YCpSAR1, respectively. To construct the plasmid for *SAR1* disruption, the unique Nde I site in pUC18 was first destroyed by Nde I digestion and filling in with the Klenow fragment (pUC18 [$NdeI^-$]). The ~800-base Bam HI-Eco RI fragment from YEpSAR1 was inserted into the multicloning sites of pUC18 ($NdeI^-$) (pANY2-15) and the ~150-base Bgl II-Nde I fragment was deleted from the insert. YIp5 was digested with Bam HI and partially with Nde I, and the 3.1-kb fragment containing the whole *URA3* gene was purified. This fragment was inserted into the deleted part of the above plasmid pANY2-15 (pMMY2-1). The 3.9-kb Bam HI-Eco RI fragment from pMMY2-1 was used for the gene disruption experiment. The construction of *GAL1-SAR1* fusion plasmid, pANY2-18, is shown in Fig. 5. pUCG1 containing promoter regions of *GAL1* and *GAL10* was obtained from Y. Ohya.

DNA manipulations including restriction enzyme digestions, ligations, plasmid isolation, and *E. coli* transformation were carried out by the standard methods (suppliers' protocols; Maniatis et al., 1982). Yeast transformation was performed by the quick method using lithium thiocyanate (Keszenman-Pereyra and Hieda, 1988). Purification of DNA fragments from agarose gel pieces was performed using the GENECLEAN Kit (BIO101, La Jolla, CA). DNA nucleotide sequences were determined by the dideoxy method (Sanger et al., 1977) in combination with the deletion technique using exonuclease BAL31 (International Biotechnologies, Inc., New Haven, CT). Single-stranded DNA as templates of sequencing reactions was prepared according to Vieira and Messing (1987).

Southern and Northern Hybridization

Genomic DNA for Southern hybridization analysis was prepared as described (Payne and Schekman, 1985), digested with appropriate restriction enzymes, separated in an agarose gel, and transferred to a nitrocellulose membrane (Maniatis et al., 1982). For Northern hybridization experiments, total RNA was prepared as described by Bernstein et al. (1985) and subjected to oligo(dT) column chromatography (Collaborative Research, Inc., Lexington, MA) according to the supplier's protocol. Purified $poly(A)^+$ RNA was electrophoresed in an agarose gel containing 2.2 M formaldehyde and transferred to nitrocellulose in the same way as for Southern hybridization. In both Southern and Northern hybridizations, blotted nitrocellulose membranes were rinsed with 2× SSC, baked in vacuo at 80°C for 2 h, and prehybridized in 5× SSC, 2× Denhardts', 100 μg/ml salmon sperm DNA, 0.5% SDS, 10 mM Hepes-NaOH, pH 7.5, and 50% formamide (hybridization buffer) at 42°C for 4 h. DNA-DNA and DNA-RNA hybridization was achieved in the same hybridization buffer at 42°C for 15–20 h using $\sim 1 \times 10^6$ cpm/ml DNA probes, which were labeled with [α-^{32}P]dCTP by a random primer extension method using an oligolabeling kit (Pharmacia Fine Chemicals, Uppsala, Sweden). After hybridization, the membranes were washed once with 6× SSC/0.1% SDS at room temperature for 15 min, three times with 0.1× SSC/0.1% SDS at 50°C for 15 min each, and briefly with 0.1× SSC at room temperature, and then dried and autoradiographed.

Colony Hybridization and cDNA Cloning of SAR1

The yeast cDNA library, pcD-Y, was provided by Dr. A. Miyajima (DNAX Research Institute, Palo Alto, CA) (Miyajima et al., 1984). 1 μg DNA of this library was used to transform *E. coli* SE10 strain and $\sim 10^6$ independent clones were obtained. Seeding of the *E. coli* transformants on nitrocellulose membranes, amplification of plasmids with chloramphenicol, replica plating, and immobilization of DNA on the membranes were carried out as described (Maniatis et al., 1982). The membranes were washed three times with 3× SSC/0.1% SDS at 65°C for 1 h each and prehybridized in the above hybridization buffer at 42°C for 24 h. The hybridization was performed in the same buffer at 42°C for 21 h, using 1.3×10^6 cpm/ml ^{32}P-labeled DNA fragment as a probe that was predicted to be internal to the second exon of the genomic *SAR1*. The hybridized membranes were washed twice with 6× SSC/0.1% SDS at room temperature for 15 min each and twice with 0.1× SSC/0.1% SDS at 50°C for 1 h each, rinsed briefly with 0.1× SSC, and autoradiographed. Colonies that gave common positive signals in autoradiograms of duplicated membranes were retrieved from the master plates and subjected to the second screening. The candidate clones were purified, from which plasmids were isolated and analyzed by Southern blotting with the genomic *SAR1* probe. The plasmid containing the largest insert that hybridized with the probe was named pANY3-1.

Immunoblotting Analysis

Rabbit antibody against prepro-α-factor was a gift from Dr. J. Rothblatt and Ms. A. Eun (University of California, Berkeley). Anti-carboxypeptidase Y antibody has been described (Stevens et al., 1982). Cell lysates were prepared by glass bead-homogenization in the presence of 1% SDS (Nakano et al., 1988), electrophoresed in SDS-polyacrylamide gels, and transferred to nitrocellulose membranes (Towbin et al., 1979). The blotted membranes were washed with water, briefly stained with Ponceau S to confirm the efficient transfer of proteins, and incubated in blocking buffer containing 1% nonfat dry milk in 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.05% Tween 20 (TBST) at room temperature for 60 min. Antibody was added to the blocking buffer at an appropriate dilution and the incubation continued

1. *Abbreviations used in this paper*: ARF, ADP-ribosylation factor; CPY, carboxypeptidase Y; MV, Wickerham's minimal medium; SRP, signal recognition particle; YP, 2% polypeptone, 1% yeast extract.

for another 60 min. The membranes were washed four times with TBST for 5 min each and incubated in the blocking buffer containing 0.1 μCi/ml ^{125}I-labeled Protein A (ICN Radiochemicals, Irvine, CA). After a 60-min incubation at room temperature, the membranes were again washed with TBST four times for 5 min each, dried, and autoradiographed. In some experiments, alkaline phosphatase–conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch Laboratories, Bar Harbor, ME) was used at a 1:5,000 dilution in place of [^{125}I]Protein A, and the immunoreactive proteins were visualized by a color reaction using nitro blue tetrazolium and 5-bromo-4-chloro-3-indoryl phosphate as substrates.

Labeling of Cells and Immunoprecipitation

The intracellular transport of α-factor and carboxypeptidase Y (CPY) in the galactose-dependent *sar1* mutant was examined by pulse-chase experiments. Cells (ANY26) were inoculated at 2 × 10^6/ml into MVGal or MVD medium containing 0.2% yeast extract and 100 μM $(NH_4)_2SO_4$, and grown at 30°C for 12 h. 3 × 10^7 cells were harvested, washed with water, and resuspended in 1.5 ml of MVGal or MVD with no sulfate. After preincubation for 5 min for 5 min at 30°C, cells were pulse-labeled with 12.5 μCi/ml Tran^{35}S-label (ICN Radiochemicals) at 30°C for 5 min and then chased by the addition of 15 μl of 300 mM $(NH_4)_2SO_4$, 25 mM cysteine, and 27 mM methionine. 240-μl aliquots were withdrawn at appropriate time points, mixed with 2.4 μl 1 M NaN_3 on ice, and subjected to preparation of cell lysates by glass-bead homogenization (Nakano et al., 1988). When the secreted mature α-factor was to be analyzed, the labeling medium contained 0.2 mg/ml ovalbumin. After sedimentation of the cells by a brief centrifugation, 200 μl of the medium was mixed with 5 μl of 20% SDS, boiled for 5 min, and made to 1 ml by the addition of 2% Triton X-100 in PBS. α-factor and CPY were immunoprecipitated from the lysates and the media as described (Nakano et al., 1988) and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.

Homology Search in the Protein Data Base

Proteins catalogued in the protein data base provided by the National Biomedical Research Foundation were screened for homology with the predicted amino acid sequence of Sarlp. The FASTP program (Lipman and Pearson, 1985) was obtained from Software Development Co., Ltd. (Tokyo, Japan). The SEQFP program (Wilbur and Lipman, 1983) in the Integrated Database and Extended Analysis System for Nucleic Acids and Proteins (IDEAS; M. Kanehisa) was available through the Laboratory of Molecular Biology, Institute of Medical Science, University of Tokyo.

Results

Isolation of SAR1

In a previous study (Nakano et al., 1988), we reported molecular cloning and analysis of the *SEC12* gene, which is required for the transport of secretory, plasma membrane, and vacuolar proteins from the ER to the Golgi. For the cloning, a temperature-sensitive mutant, *sec12-14*, was transformed with a yeast genomic DNA library constructed on a multicopy plasmid YEp13, and DNA clones that complemented the *sec12* ts mutation were selected. Interestingly, two distinct clones were obtained and named pSEC1210 and pSEC1230. As described in detail in the previous paper, pSEC1230 contained the authentic *SEC12* gene, and accordingly, the other clone pSEC1210 was assumed to contain a suppressor gene that conferred a Ts$^+$ phenotype to *sec12-4* only when overexpressed. This suppressor gene was designated *SAR1*.

To localize the suppression activity of the *SAR1* gene in the insert of pSEC1210, deletion analysis was performed. Various fragments from pSEC1210 were subcloned into a multicopy plasmid, pSEY8, or a single-copy CEN plasmid, pSEYC58, and introduced into *sec12-4*. Transformants were tested for growth at the restrictive temperature of *sec12-4*, 37°C (Fig. 1). Unexpectedly, large DNA fragments that suppressed *sec12-4* on a multicopy plasmid (Xho I-Sal I and Hind III-Sal I) did so on a single-copy plasmid as well, whereas the Bam HI-Eco RI fragment showed suppression activity only on a multicopy plasmid (see below). Smaller fragments (Xba I-Eco RI and Hind III-Bgl II) did not suppress *sec12-4* even on a multicopy plasmid. The 1.3-kb Hind III-Sal I fragment, the smallest piece of DNA that suppressed *sec12-4* on either a multicopy or single-copy plasmid, was subjected to nucleotide sequencing.

Nucleotide Sequence of SAR1

The DNA sequence of the Hind III-Sal I fragment was determined by the dideoxy method (Fig. 2). After a simple search for an open reading frame failed to find an appropriate initiator ATG codon, we recognized typical consensus sequences for RNA splicing, GTATGT and TACTAAC (*boxes*). Assuming that the GTATGT was the donor site of splicing and the first AG downstream the TACTAAC was the acceptor site, we found a complete open reading frame consisting of 573 bp (*bold face*). To confirm that the gene was in fact spliced, we isolated a cDNA clone of *SAR1*. A yeast cDNA library, pcD-Y, constructed by Miyajima et al. (1984) was used for screening. A ~320-base DNA fragment was prepared from the genomic *SAR1* clone by Eco RI digest followed by ~180-base upstream deletion by BAL31 nuclease and the second digest with Nde I. This fragment, which was predicted to be internal to the second exon, was labeled by random primer extension and used as a probe for colony hybridization. Among ~10^4 colonies screened, 12 positives were isolated. The inserts were confirmed for hybridization with the *SAR1* probe, and the largest one was sequenced.

The result was in complete agreement with our prediction from the genomic sequence; RNA splicing had occurred between GT (297–298, numbers correspond to nucleotide positions in Fig. 2) and AG (434–435). When spliced, two exons (269–296, 436–977) gave an open reading frame coding for a polypeptide of 190 amino acid residues. The calculated molecular weight was 21,450. We hereafter refer to this predicted protein as Sarlp. In the cDNA sequence, G (211) was the first nucleotide observed. AATAAT (1,037–1,042) seemed to function as a poly A addition site. The 5′-flanking region contained several TATA sequences (*double underline*). We assumed that one of the three TATAs upstream of the transcript (47–50, 63–66, 70–73) was used, but the downstream two TATAs (242–245, 254–257) may have functioned, though weakly, because the Bam HI-Eco RI fragment (187–1,020) suppressed *sec12* only when it was on a multicopy plasmid.

Sarlp Is a Novel Member of the GTP-binding Protein Superfamily

The predicted amino acid sequence of Sarlp was used to search for homologous proteins in the protein data bank from the National Biomedical Research Foundation. The FASTP algorithm (Lipman and Pearson, 1985) picked up yeast *ras*-related Yptl protein (Gallwitz et al., 1983) with 25.9% identity over 116 amino acids. The SEQFP algorithm (Wilbur and Lipman, 1983) also retrieved *ras*-family proteins, yeast *RAS1*, *RAS2*, human H-*ras*, K-*ras*, N-*ras*, and many others homologous to Sarlp, with amino acid identity ranging from

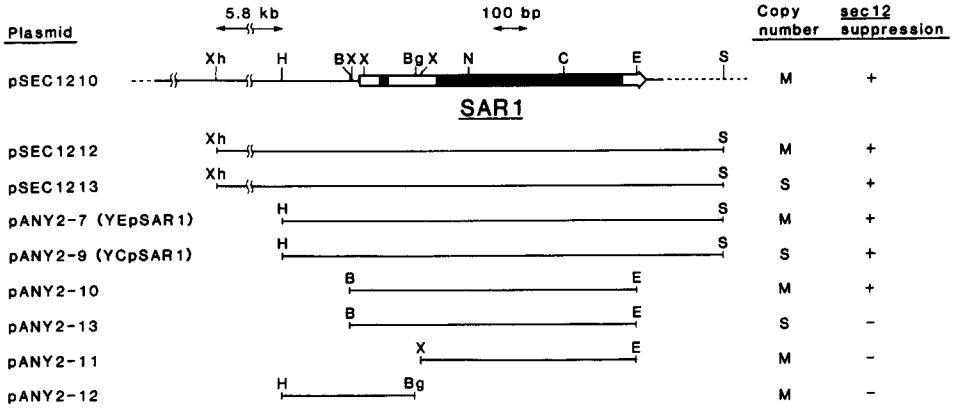

Figure 1. Restriction map of pSEC1210, the original isolate of *SAR1*, and deletion analysis. (*Top*) The insert of pSEC1210 with relevant restriction sites. (*Arrow*) Position of the putative transcript of *SAR1*, and the two solid bars denote the exons (see Fig. 2). The dashed line is derived from the vector, YEp13. Subclones are shown below. The copy number of the plasmid is indicated as *M* (multicopy) or *S* (single copy). The plasmids were introduced into a *sec12-4* strain, MBY10-7A, and their activities in suppressing *sec12-4* ts were examined by testing growth of the transformants at 37°C. Abbreviation of restriction enzymes are: *Xh*, Xho I; *H*, Hind III; *B*, Bam HI; *X*, Xba I; *Bg*, Bgl II; *N*, Nde I; *C*, Cla I; *E*, Eco RI; *S*, Sal I.

```
        10         20         30         40         50         60
GGATGGCACTTCTAAAATTCGCGCTCAACATGGCCGTATTGTACATTATATCGTTCTATC
        70         80         90        100        110        120
ATTATATCGTATACGCCCGCATTACCCGACAACTCCGTCTGCAACGCGTTGACCAGAAAA
       130        140        150        160        170        180
CTCGAACAAGAGATCGCATAAAAAACCAAAAGGAAACGAATTACTTGTCAAATAGTTATT
                BamHI XbaI 200        210        220        XbaI 240
GTAATGGATCCTCTAGAAAGGCAAACAGTAGATTTATTTCCTTCTTTTCTAGAAACATCA
                                  *
       250        260        270        280        290        300
TTATAACTAACAATATATAATTGGAATAATGGCTGGTTGGGATATTTTTGGTTGGTGTAT
                                  M  A  G  W  D  I  F  G  W  F
       310        320        330        340        350        360
GTTATCACCCTGTTCACGTTTTTCGGATACTTAGTTTTATTCAATGTGGTAAACATTGAA
        370       BglII      390  XbaI  400        410        420
TGTTTTCAGCTTAAGATCTATTTTTTTTTTTCTAGAAGAAATTGCGTCCTTTACTAACTT
       430        440        450        460        470        480
TATTTTACTGTACAGTCAGAGATGTGTTGGCTTCCCTTGGTCTGTGGAACAAACATGGTA
                  R  D  V  L  A  S  L  G  L  W  N  K  H  G  K
       490        500        510        520        NdeI       540
AACTACTTTTCTTGGGTTTGGATAATGCCGGTAAGACCACATTGCTACATATGTTAAAGA
  L  L  F  L  G  L  D  N  A  G  K  T  T  L  L  H  M  L  K  N
       550        560        570        580        590        600
ACGATAGATTGGCAACCTTACAACCAACATGGCATCCAACTTCTGAAGAACTGGCTATTG
  D  R  L  A  T  L  Q  P  T  W  H  P  T  S  E  E  L  A  I  G
       610        620        630        640        650        660
GTAACATTAAGTTTACAACTTTCGATTTGGGTGGTCATATTCAAGCTCGTCGTTTATGGA
  N  I  K  F  T  T  F  D  L  G  G  H  I  Q  A  R  R  L  W  K
       670        680        690        700        710        720
AGGATTATTTCCCAGAAGTTAATGGTATCGTCTTTTTAGTCGATGCTGCTGACCCTGAAA
  D  Y  F  P  E  V  N  G  I  V  F  L  V  D  A  A  D  P  E  R
       730        740        750        760        770        780
GATTTGATGAAGCACGTGTCGAATTAGATGCTTTATTCAACATTGCCGAATTGAAGGACG
  F  D  E  A  R  V  E  L  D  A  L  F  N  I  A  E  L  K  D  V
       790        800        ClaI       820        830        840
TTCCTTTTGTAATTCTTGGTAACAAGATCGATGCTCCAAACGCCGTTTCTGAAGCGGAGC
  P  F  V  I  L  G  N  K  I  D  A  P  N  A  V  S  E  A  E  L
       850        860        870        880        890        900
TACGTTCTGCTTTAGGATTATTGAATACCACTGGCTCTCAAAGAATTGAAGGTCAAAGAC
  R  S  A  L  G  L  L  N  T  T  G  S  Q  R  I  E  G  Q  R  P
       910        920        930        940        950        960
CAGTTGAAGTTTTCATGTGTTCCGTTGTTATGAGAAATGGTTATTTAGAGGCGTTCCAAT
  V  E  V  F  M  C  S  V  V  M  R  N  G  Y  L  E  A  F  Q  W
       970        980        990       1000       1010       1020
GGTTATCTCAATATATTTAATTGACGTATACATCTATACCCTTTATATGACATTCACATG
  L  S  Q  Y  I
EcoRI 1030       1040       1050       1060       1070       1080
AATTCAACAGCTTTGTAATAATCAACAAAATTGCATAGGATGCCACCTCATAACTTTTGA
                  ******
      1090
GCACAGCATTTCCGCGATC
```

Figure 2. Nucleotide sequence of *SAR1* and its deduced amino acid sequence. The genomic DNA sequence of the Hind III-Sal I fragment from pSEC1210 was determined. The consensus sequences of RNA splicing (GTATGT . . . TACTAAC . . . AG) are boxed. The nucleotide sequence of the cDNA clone (pANY3-1) was also determined, which started with G at position 211 and ended with AATAAT (1,037–1,042) before poly A (*asterisks*). The sequence between nucleotides 296 and 436 is missing in cDNA, yielding an open reading frame of 573 bp (bold face). The predicted amino acid sequence is shown in one-letter code. Upstream TATA sequences are indicated by double underlines.

18 to 23% over >130 residues. The homology of Sarlp to these *ras*-family proteins was not very high for the whole molecules but was remarkable if limited parts of the sequences were compared. Three highly homologous regions are shown in Table I in comparison with various GTP-binding proteins. The underlined bold face indicates that the amino acid residue coincides with the consensus sequence shown at the bottom of the table, and the bold face without underline means that the residue is similar to the consensus.

Recently, Dever et al. (1987) have proposed that GTP-binding domains of *ras*-related proteins are composed of three consensus motifs, GXXXXGK, DXXG, and NKXD, which are aligned in distinct spacings. The first and the second sequences are considered to constitute the active site for GTP hydrolysis and the third element is believed essential for the recognition of guanine base (de Vos et al., 1988; Lochrie and Simon, 1988). The conserved sequences in Sarlp and other GTP-binding proteins in Table I correspond exactly to these three sites. Spacing requirements (Dever et al., 1987), namely, 40–80 or 130–170 amino acid residues between the first and second elements and 40–80 residues between the second and third ones, are completely satisfied in Sarlp. According to the calculation by Dever et al. (1987), the probability of the three consensus sequences correctly spaced is at most 1/5,000. It thus is most probable that Sarlp is a novel member of the GTP-binding protein superfamily.

Suppression of sec12 by SAR1

As shown in Fig. 1, *SAR1* suppressed *sec12-4* either on a mul-

Table I. Comparison of Sar1p Sequence with Various GTP-Binding Proteins

		GTPase active Site		Guanine recognition	Reference
S. cerevisiae	*SAR1*	(25) KLLFLGLDNAGKTTLL	(73) DLGGH	(127) FVILGNKIDA	*a*
S. cerevisiae	*YPT1*	(10) KLLLIGNSGVGKSCLL	(63) DTAGQ	(116) KLLVGNKCDL	*b*
S. cerevisiae	*SEC4*	(22) KILLIGDSGVGKSCLL	(75) DTAGQ	(128) LLLVGNKSDM	*c*
Mouse	*ypt1*	(13) KLLLIGDSGVGKSCLL	(66) DTAGQ	(119) KLLVGNKCDL	*d*
Rat	*rab2*	(8) KYIIIGDTGVGKSCLL	(61) DTAGQ	(114) IMLIGNKSDL	*e*
S. cerevisiae	*RAS1*	(12) KIVVVGGGGVGKSALT	(64) DTAGQ	(118) VVVVGNKLDL	*f*
S. cerevisiae	*RAS2*	(12) KLVVVGGGGVGKSALT	(64) DTAGQ	(118) IVVVGNKSDL	*f*
S. pombe	*ras*	(10) KLVVVGDGGVGKSALT	(62) DTAGQ	(116) VVLVANKCDL	*g*
D. discoideum	*ras*	(5) KLVIVGGGGVGKSALT	(57) DTAGQ	(111) LILVGNKADL	*h*
Mouse	v-*ras*-H	(5) KLVVVGARGVGKSALT	(57) DTTGQ	(111) MVLVGNKCDL	*i*
Human	c-*ras*-H	(5) KLVVVGAGGVGKSALT	(57) DTAGQ	(111) MVLVGNKCDL	*j*
S. cerevisiae	*RHO1*	(12) KLVIVGDGACGKTCLL	(64) DTAGQ	(117) IILVGCKVDL	*k*
S. cerevisiae	*RHO2*	(9) KLVIIGDGACGKTSLL	(61) DTAGQ	(114) IVLVGLKKDL	*k*
Aplysia	*rho*	(7) KLVIVGDGACGKTCLL	(59) DTAGQ	(112) IILVGNKKDL	*l*
Monkey	*ral*	(16) KVIMVGSGGVGKSALT	(68) DTAGQ	(122) FLLVGNKSDL	*m*
S. cerevisiae	*GPA1*	(43) KLLLLGAGESGKSTVL	(319) DAGGQ	(383) FILFLNKIDL	*n*
S. cerevisiae	*GPA2*	(125) KVLLLGAGESGKSTVL	(296) DVGGQ	(359) VVLFLNKIDL	*o*
Rat	Gsα	(42) RLLLLGAGESGKSTIV	(223) DVGGQ	(287) VILFLNKQDL	*p*
Rat	Gi2α	(36) KLLLLGAGESGKSTIV	(201) DVGGQ	(265) IILFLNKKDL	*p*
Bovine	Tα	(31) KLLLLGAGESGKSTIV	(196) DVGGQ	(260) IVLFLNKKDL	*q*
S. cerevisiae	*ARF*	(19) RILMVGLDGAGKTTVL	(67) DVGGQ	(121) WLVFANKQDL	*r*
Bovine	*ARF*	(19) RILMVGLDAAGKTTIL	(67) DVGGQ	(121) LLVFANKQDL	*r*
E. coli	EF-Tu	(13) NVGTIGHVDHGKTTLT	(80) DCPGH	(130) IIVFLNKCDM	*s*
S. cerevisiae	EF-1α	(9) NVVVIGHVDSGKSTTT	(91) DAPGH	(148) LIVAVNKMDS	*t*
S. cerevisiae	*GST1*	(262) SLIFMGHVDAGKSTMG	(344) DAPGH	(401) MVVVVNKMDD	*u*
E. coli	*era*	(10) FIAIVGRPNVGKSTLL	(62) DTPGL	(119) VILAVNKVDN	*v*
E. coli	*lepA*	(6) NFSIIAHIDHGKSTLS	(77) DTPGH	(124) VVPVLNKIDL	*w*
Motif		GXXXXGK	DXXG	NKXD	
Consensus*		KAAAAGXGGVGKSTLL	DTAGQ	AAAAGNKXDL	

References: *a*, this study; *b*, Gallwitz et al., 1983; *c*, Salminen and Novick, 1987; *d*, Haubruck et al., 1987; *e*, Touchot et al., 1987 (they have also reported the sequence of rat *rabl* that is identical to that of mouse *ypt1*); *f*, DeFeo-Jones et al., 1983; Powers et al., 1984; Dhar et al., 1984; *g*, Fukui and Kaziro, 1985; *h*, Reymond et al., 1984; *i*, Dhar et al., 1982; *j*, Capon et al., 1983; Reddy, 1983; *k*, Madaule et al., 1987; *l*, Madaule and Axel, 1985; *m*, Chardin and Tavitian, 1986; *n*, Nakafuku et al., 1987; Dietzel and Kurjan, 1987 (the same gene was called *SCG1* in their paper); *o*, Nakafuku et al., 1988; *p*, Itoh et al., 1986; *q*, Yatsunami and Khorana, 1985; Tanabe et al., 1985; *r*, Sewell and Kahn, 1988; *s*, Arai et al., 1980; *t*, Nagata et al., 1984; Schirmaier and Philippsen, 1984; Cottrelle et al., 1985; *u*, Kikuchi et al., 1988; *v*, Ahnn et al., 1986; *w*, March and Inouye, 1985.
* Consensus means the most common amino acid residue in this table appearing more than ten times. A denotes hydrophobic aliphatic residues (V, I, or L).

ticopy or single-copy plasmid. The details of this phenomenon were examined further. The Hind III-Sal I fragment of pSEC1210 was subcloned into multicopy pSEY8 or single-copy pSEYC58 vector to yield *SAR1* plasmids, which we hereafter refer to as YEpSAR1 and YCpSAR1, respectively. *SEC12* has three ts isolates, *sec12-1*, *sec12-3*, and *sec12-4*. These mutants were transformed with either YEpSAR1 or YCpSAR1 and cultured at the restrictive temperature 37°C. The two plasmids were both capable of suppressing each *sec12* isolate; every transformant not only grew but also secreted invertase at 37°C, irrespective of the isolate or the copy number of *SAR1* plasmid (see Table II). Since these *sec12* mutants contain a wild-type copy of *SAR1* gene in their chromosome, this observation indicates that duplication of *SAR1* gene suppressed *sec12* ts. To confirm that the duplication of *SAR1* expression was in fact sufficient to suppress *sec12*, a Northern hybridization experiment was performed. (Poly A)$^+$ RNA was prepared from *sec12-4* strain and its transformants with YEpSAR1 or YCpSAR1, an equivalent amount being electrophoresed in an agarose gel and transferred to a nitrocellulose membrane. The 320-base internal fragment of *SAR1* was used as a probe to detect SAR1 message. The result is shown in Fig. 3. A single band of ~750 bases was observed in the control (lane *1*). When the *sec12* cells were transformed with the single-copy YCpSAR1, the intensity of this band about doubled (lane *3*). The multicopy YEpSAR1 further increased the level of this transcript and gave rise to new bands with lower mobility that were proba-

Table II. Complementation Test of Various sec Mutants by SAR1

sec	SAR1 Multi copy	SAR1 Single copy	Strain	Other genotype	Reference or source of strain
sec1-1	−	−	SEY5016	*MATα ura3 leu2*	*a*
sec2-56	−	−	ANS2-3A	*MATα ura3 leu2 his*	*b*
sec3-2	−	−	NY412	*MATa ura3*	*c*
sec4-2	−	−	ANS4-8C	*MATα ura3 leu2 his*	*b*
sec5-24	−	−	ANS5-9D	*MATα ura3 leu2*	*b*
sec6-4	−	−	ANS6-2D	*MATα ura3 leu2 trp1 his*	*b*
sec7-1	−	−	SF821-8A	*MATa ura3 leu2 suc2*	*d*
sec7-4	−	−	SF911-13D	*MATa ura3 leu2 trp1 his4*	*d*
sec8-6	−	−	ANS8-10C	*MATa ura3 leu2 his*	*b*
sec9-4	−	−	ANS9-4C	*MATa ura3 trp1 his*	*b*
sec10-2	−	−	ANS10-12A	*MATa ura3 leu2 his*	*b*
sec11-7	−	−	PBY3-9B	*MATα ura3 leu2 his4*	*e*
sec12-1	++	++	ANY8-4D	*MATα ura3 leu2 trp1 his*	*b*
sec12-3	++	++	ANY9-8A	*MATα ura3 leu2 trp1 his*	*b*
sec12-4	++	++	MBY10-7A	*MATa ura3 leu2 trp1 his*	*f*
sec13-1	−	−	MBY3-15A	*MATα ura3 leu2 his*	*g*
sec14-3	−	−	ANS14-2C	*MATα ura3 leu2 his*	*b*
sec15-1	−	−	ANS15-5B	*MATa ura3 leu2 trp1 his*	*b*
sec16-2	+	−	MBY4-1A	*MATa ura3 leu2 trp1 his*	*g*
sec17-1	−	−	MBY11-1D	*MATα ura3 leu2 trp1 his*	*g*
sec18-1	−	−	MBY12-6D	*MATα ura3 leu2 trp1 his*	*g*
sec19-1	−	−	ANS19-4A	*MATa ura3 leu2 his*	*b*
sec20-1	−	−	MBY5-2A	*MATa ura3 trp1*	*g*
sec21-1	−	−	MBY6-4D	*MATα ura3 leu2 trp1 his*	*g*
sec22-3	−	−	MBY13-2D	*MATa ura3 trp1*	*g*
sec23-1	−	−	MBY8-20C	*MATα ura3 leu2 trp1 his*	*g*
sec53-6	−	−	MBY7-5C	*MATα ura3 leu2 trp1 his*	*h*
sec59-1	−	−	SF604-9C	*MATα ura3 his4 suc2*	*d*
sec61-1	−	−	RDM7-4B	*MATα ura3 leu2 trp1 his4*	*i*
sec62-1	−	−	RDM50-94C	*MATα ura3 leu2 his4*	*j*

Reference or source: *a*, Emr, S. D. (California Institute of Technology); *b*, this study; *c*, Novick, P. (Yale University); *d*, Schekman, R., and C. Field (University of California, Berkeley); *e*, Böhni et al. (1988); *f*, Nakano et al. (1988); *g*, Bernstein, M. (University of California, Berkeley); *h*, Bernstein et al. (1985); *i*, Deshaies, R. J. (University of California, Berkeley); *j*, Deshaies, R. J. (1988).

bly artifacts due to read-through from vector promoters (lane 2). Vectors alone did not affect the expression level of *SAR1* (data not shown). The level of SAR1 transcript in these *sec12* transformants was quantified by densitometer scanning; the ratio of transformant:control was 8–10:1 for YEpSAR1 and 2–2.5:1 for YCpSAR1. Thus, as measured by the average RNA abundance, the duplication of *SAR1* dosage suppressed *sec12* ts phenotype.

A disruption of chromosomal *SEC12* gene is lethal (Nakano et al., 1988). The effect of *SAR1* overexpression on the lethality of a *SEC12* deletion was tested. The multicopy *SAR1* plasmid pSEC1210 or the *SEC12* plasmid pSEC1230 was introduced into a heterozygous diploid in which one copy of chromosomal *SEC12* was disrupted (*SEC12/sec12::URA3*). Transformants were sporulated and tetrads dissected. When the *SEC12* plasmid, pSEC1230, was provided as a control, about 40% of the tetrads harbored the plasmid and gave rise to complete four viable spores. However, in the case of *SAR1* plasmid pSEC1210, none of the tetrads dissected gave more than two viable spores. About 20% of the tetrads contained the multicopy *SAR1* plasmid, but all viable spores had the wild-type *SEC12* and no *sec12::URA3* haploid survived. Thus, overexpression of *SAR1* was unable to replace the *SEC12* function in the *sec12* deletion mutant.

Next, suppression by *SAR1* was examined with other *sec* mutations. 30 *sec* ts mutants, *sec–sec23* (including two isolates of *sec7* and three of *sec12*), *sec53*, *sec59*, *sec61*, and *sec62*, were transformed with either YEpSAR1 or YCpSAR1 and tested for growth at 37°C. As shown in Table II, none of these sec mutants, except *sec12*, grew at 37°C when they were transformed with the single-copy YCpSAR1. In the presence of the multicopy YEpSAR1, however, sec16 became viable at 37°C. *sec16* was also weakly suppressed by the single-copy YCpSAR1; it grew at 33°C in the presence of either YCpSAR1 or YEpSAR1 but not in the absence of the plasmid (data not shown). Thus, *SAR1* genetically interacts not only with *sec12* but also with *sec16*, which is another *sec* mutant defective in ER-Golgi transport.

Construction of sar1 Mutants

To address the cellular function of *SAR1*, we attempted to construct mutants. First, the null mutant of *SAR1* was prepared by gene disruption. From the genomic *SAR1* gene, a ~150-base Bgl II-Nde I fragment containing a part of the intron and a part of the second exon was deleted. The *URA3* gene, which was prepared as a 3.1-kb fragment from YIp5 by Bam HI/Nde I partial digestion, was inserted into the deleted part of *SAR1*. The resulting disrupted copy of *SAR1* gene was excised out from the plasmid by Bam HI/Eco RI digestion, and introduced into a diploid strain which was

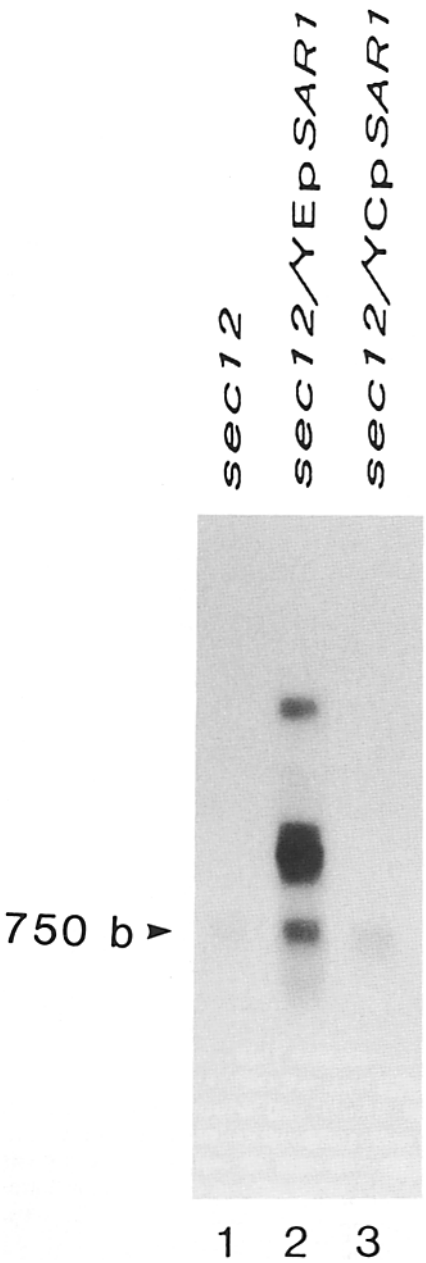

Figure 3. Expression of *SAR1* in the *sec12* mutant. Northern hybridization experiment was performed for *sec12-4* strain with or without *SAR1* plasmids. Cells of MBY10-7A (*sec12*), MBY10-7A/YEpSAR1, and MBY10-7A/YCpSAR1 were grown overnight at 24°C (without plasmid) or 37°C (with plasmid) in MVD medium. Total RNA was extracted from the log-phase cells and subjected to oligo(dT) column chromatography. $(Poly\ A)^+$ RNA was purified, ~7 μg of which (equivalent to 2×10^8 cells) was separated in an agarose gel and transferred to a nitrocellulose membrane. Hybridization was achieved under a stringent condition, using a DNA fragment internal to the second exon of *SAR1*, and the *SAR1* message (750 bases) was visualized by autoradiography. Slowly migrating species are probably artifacts due to promoters on the vector (pSEY8).

homozygous for *SEC12*, *SAR1*, and *ura3*. Representative transformants were subjected to sporulation. Among 55 tetrads dissected, 39 gave rise to two viable spores and 16 produced only one. None of these viable spores contained *URA3* gene, the marker of the *SAR1* disruption. To show that this disruption occurred at the *SAR1* locus, a Southern hybridization experiment was performed. Chromosomal DNA prepared from the original diploid strain, the diploid transformant with the disrupted *sar1*, and several surviving spores, was digested with Bam HI/Eco RI, resolved in an agarose gel, and transferred to a nitrocellulose membrane. Fig. 4 shows the result of hybridization using the 320-base *SAR1* internal fragment as a probe. The original diploid (lane *1*) showed the wild-type band only (0.8 kb), whereas the diploid transformant (lane *2*) gave rise to an additional band (3.9 kb), indicating that one of the chromosomal *SAR1* alleles was replaced by the disrupted copy. All viable progeny contained the wild-type gene (lanes *3–8*). Thus the disruption of *SAR1* in the chromosome was a lethal event. Another strategy of disruption, the insertion of *LEU2* gene into the Bgl II site, gave essentially the same result (not shown). A microscopic examination showed that the spores with the disrupted *sar1* did not initiate budding, as was also the case with *SEC12* disruption.

Test of SAR1 Function in Secretory Pathway

Conditional lethal mutants of *SAR1* were required for the functional analysis. One approach to obtain such a mutant was to place the *SAR1* gene under the control of a regulatable promoter. To do this, a gene fusion of *GAL1* and *SAR1* was constructed. The procedures are summarized in Fig. 5. Briefly, the 1.2-kb Bam HI-Sal I fragment from YEpSAR1, which contained ~80-base upstream sequence from ATG, the coding sequence including the intron, and the whole 3′-flanking region, was subcloned into a single-copy plasmid, YCpG11. The 0.8-kb Bam HI-Eco RI fragment from pUCG1, containing the *GAL1* promoter, was inserted in the right orientation in front of the above *SAR1* gene. To test if this fusion gene could express *SAR1* in a galactose-dependent manner, the plasmid was introduced into *sec12* and *SEC12* strains. The transformants were grown in MVGal medium (Wickerham's minimal medium plus 5% galactose and 0.2% sucrose) at 24°C and then streaked on YPGal (YP plus 5% galactose and 0.2% sucrose) or YPD (YP plus 2% glucose) plates and incubated at 24 or 33°C (Fig. 6). On YPD plates, *sec12* did not grow at 33°C. On YPGal plates, however, *sec12* grew at both 24 and 33°C. This indicates that the *SAR1* gene in the plasmid was functional in suppressing *sec12* ts in the presence of galactose but not in glucose.

The *GAL1-SAR1* plasmid was then introduced into the cells which had their chromosomal *SAR1* gene disrupted. To do this, a heterozygous diploid, *SAR1/sar1::URA3* (*leu2/leu2 trp1/trp1*), was first transformed with the original genomic *SAR1* plasmid, pSEC1210 (containing *LEU2*). After sporulation and tetrad dissection, the haploid cells containing the disrupted copy of *SAR1* in the chromosome and the wild-type gene on the plasmid were selected (Ura^+ Leu^+ Trp^-). Representative haploids were further transformed with the above *GAL1-SAR1* fusion plasmid containing *TRP1*. Trp^+ cells were selected and cultured in liquid YPGal medium for 2 d. Without any selective pressure during this liquid culture, some cells lost the genomic *SAR1* plasmid (Ura^+ Leu^- Trp^+) and others dropped the *GAL1-SAR1* plasmid (Ura^+ Leu^+ Trp^-). The former cells, which contained the functional *SAR1* gene only on the *GAL1-SAR1* fusion plasmid, were the desired mutants.

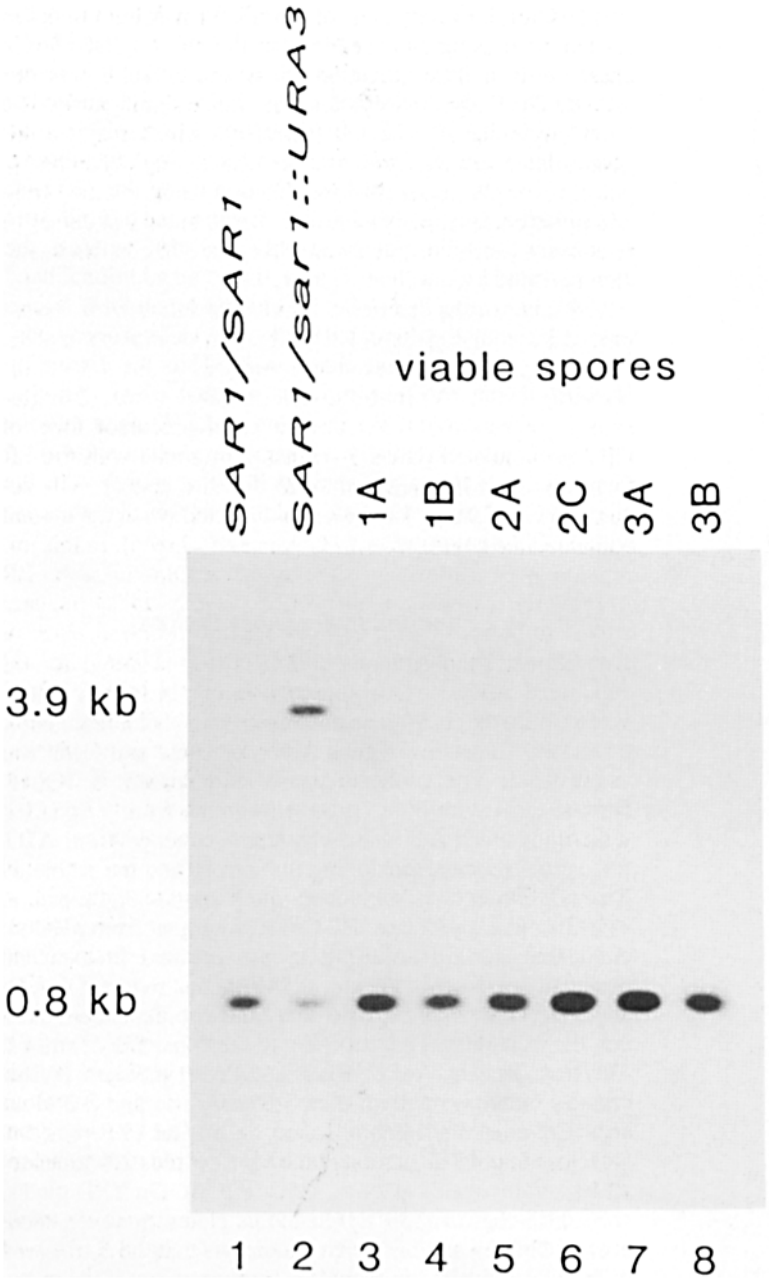

Figure 4. Disruption of *SAR1* is lethal. Genomic DNA was prepared from ANY102 (*SAR1/SAR1*), ANY105 (*SAR1/sar1::URA3*), and several viable spores obtained by sporulation and dissection of ANY105. After digestion with Bam HI and Eco RI, DNA was resolved in an agarose gel and transferred to a nitrocellulose. Hybridization was done with *SAR1* probe. *0.8 kb*, the band derived from the wild-type *SAR1*; *3.9 kb*, the band derived from *sar1::URA3*.

These cells grew normally in the presence of galactose (YPGal) but ceased growth when transferred to a glucose-containing medium (YPD, YP plus 5% glucose for liquid culture). A typical growth curve is shown in Fig. 7. The cells slowed down the growth ~12 h after the shift from YPGal to YPD medium, and completely stopped the increase of OD_{650} by 18 h. This growth arrest was reversible; the cells revived when they were washed and replenished with galactose, though the viability decreased after a long arrest in the presence of glucose (data not shown). The arrested cells did not show an apparent change in the population of budded cells, suggesting that there was no defect in a particular stage of the cell cycle.

Using this galactose-dependent mutant of *sar1*, the process of protein secretion was examined. In an experiment similar to the one in Fig. 7, aliquots of cell suspension were taken from the culture at appropriate time points, and cell lysates were prepared. The lysates were resolved in an SDS-gel, transferred to a nitrocellulose membrane, and subjected to immunoblotting using anti-prepro-α-factor antibody. As shown in Fig. 8 *A*, the galactose-dependent *sar1* mutant accumulated two species of α-factor precursor 12–18 h after the transfer from galactose to glucose medium (lanes *3–5*). These species exactly comigrated with core-glycosylated pro-α-factor, gp3 and gp2, which were seen in the ER-accumulating mutants *sec12* and *sec18* (lanes *6* and *7*). Accumulation of highly glycosylated precursor was not observed. Appearance of gp2 was exaggerated in longer incubation in glucose medium (lanes *4* and *5*), which might be a secondary effect. The accumulation of the ER-forms was not due to the

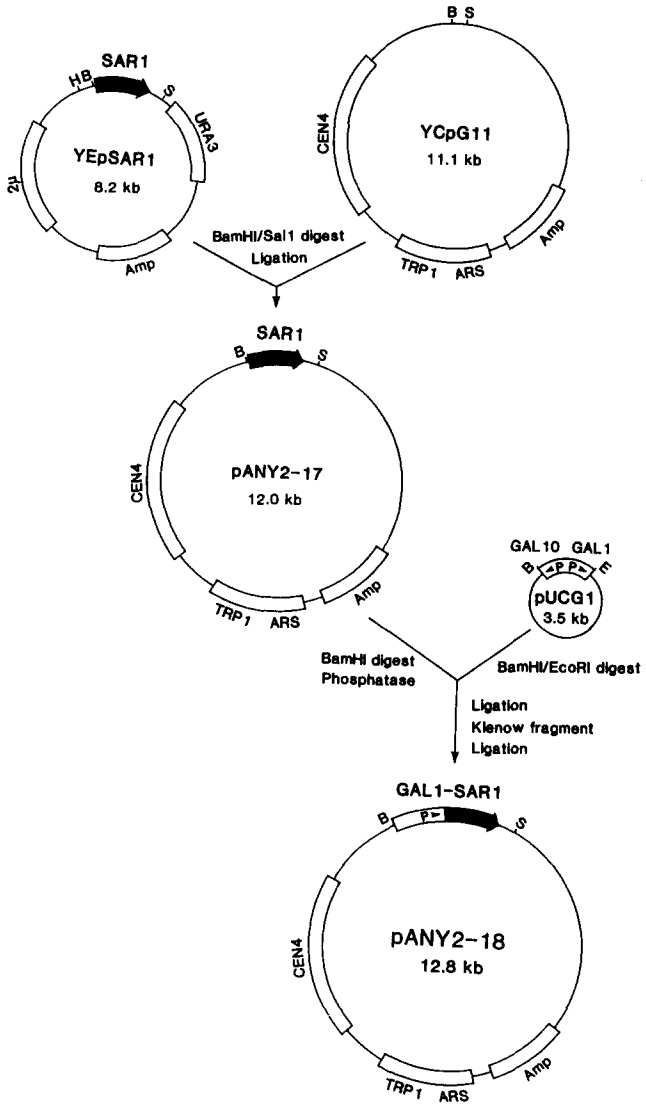

Figure 5. Construction of *GAL1-SAR1* gene fusion. Strategy for constructing a plasmid containing *GAL1-SAR1* fusion is shown. First, the Bam HI-Sal I fragment from YEpSAR1, which lacks the major promoter region of *SAR1*, was inserted into YCpG11 single-copy plasmid to yield pANY2-17. The Bam HI-Eco RI fragment from pUCG1 containing the promoter regions of both *GAL1* and *GAL10* was placed immediately in front of *SAR1* gene to produce the objective *GAL1-SAR1* fusion (pANY2-18). Abbreviations are: *H*, Hind III; *B*, Bam HI; *S*, Sal I; *E*, Eco RI.

presence of *GAL1-SAR1* plasmid itself, because when the *sar1* phenotype was complemented by the constitutive *SAR1* plasmid, pSEC1210, no such accumulation was observed (lanes *9* and *10*).

The intracellular transport of α-factor was further analyzed by a pulse-chase experiment. The *sar1* mutant cells were cultured in galactose or glucose for 12 h and pulse-labeled with ^{35}S for 5 min. After chase, cell lysates and media were subjected to immunoprecipitation of α-factor. As shown in Fig. 8 *B*, cells transported and secreted α-factor normally in the control (+Gal). Early processing from prepro-α to ER- and Golgi-forms of pro-α was seen at 0 min chase, and appearance of extracellular mature α-factor was almost complete by 10 min chase. In the restrictive incubation (+Glc), however, most of the ER form did not undergo further processing and remained in the cell even at 10 min chase; only a little secretion of mature α-factor was observed. The Golgi form did not accumulate significantly. The band migrating at ~22 kD (*asterisk*), which may be unglycosylated pro-α-factor, was not chased rapidly either in galactose or glucose incubation. Thus, both immunoblotting and pulse-chase experiments indicated that *sar1* is defective in α-factor secretion and accumulates the ER precursor species under the restrictive condition.

The *sar1* mutant was also examined for intracellular transport of a vacuolar protein, CPY. The same samples as in Fig. 8 *A* were analyzed by immunoblotting using anti-CPY antibody (Fig. 9 *A*). When the *sar1* cells were incubated in glucose to repress *SAR1* function, a novel precursor form of CPY accumulated (lanes *3–5*) that comigrated with the ER form, p1, seen in *sec12* and *sec18* (lanes *6* and *7*). The accumulation of the p1 form was not detected when the mutant contained the constitutive *SAR1* gene (not shown). In this immunoblotting analysis of CPY, the accumulation of the ER form seems less severe than *sec12* and *sec18*, unlike the case of α-factor, although the reason is unclear. It may also be noticed that the proportion of p2 CPY increased when the *sar1* cells were incubated in glucose medium (see lane *2*). However, the same phenomenon was observed when the wild-type cells were shifted from galactose to glucose (not shown) and probably is not related to the Sar1p deficiency. To exclude the possibility that gene repression by virtue of the *GAL1* promoter is itself responsible for the p1 accumulation in this *sar1* mutant, the effect of repression of another essential gene, *CMD1*, coding for yeast calmodulin, was examined. A strain containing the *GAL1-CMD1* fusion gene on a plasmid but having had its chromosomal copy disrupted was obtained from Dr. Y. Ohya. This strain, which also showed a Gal-dependent growth phenotype (Ohya and Anraku, 1989), was incubated in glucose medium for 18 h and intracellular CPY was analyzed. As shown in lanes *9* and *10* of Fig. 9 *A*, this Gal-dependent *cmd1* mutant did not exhibit any anomalous accumulation of CPY precursors 18 h after transfer from galactose to glucose, although the cells ceased growth before 12 h.

A pulse-chase analysis was also carried out on the transport of CPY. In a similar experiment as in Fig. 8 *B*, the *sar1* cells were incubated in galactose or glucose for 12 h, pulse-labeled with ^{35}S for 5 min, and chased (Fig. 9 *B*). In constrast to the control (+ Gal), *sar1* under the restrictive condition (+Glc) was blocked in processing of CPY from the p1 to the p2 form. Only a little proportion of the labeled molecules seemed to have escaped the block, giving rise to the mature CPY at 45 min (lane *12*). Appearance of p2 form was not obvious in the same incubation. Thus, the transport of a vacuolar protein was also affected by the *SAR1* deficiency at the step of exit from the ER. It should be noted here that conversion of p1 to p2 seems to be rate limiting even in the control, which is not the case with normally growing wild-type cells (see Payne et al., 1988). This might be due to the slower cell growth in galactose. Alternatively, the overproduction of Sar1p by *GAL1* promoter could have influenced the ER-Golgi transport somehow, although it did not affect the growth of wild-type cells significantly (data not shown).

Taken together, we conclude that intact *SAR1* is required for the protein transport from the ER to the Golgi, like other *SEC* genes involved in the ER-Golgi step.

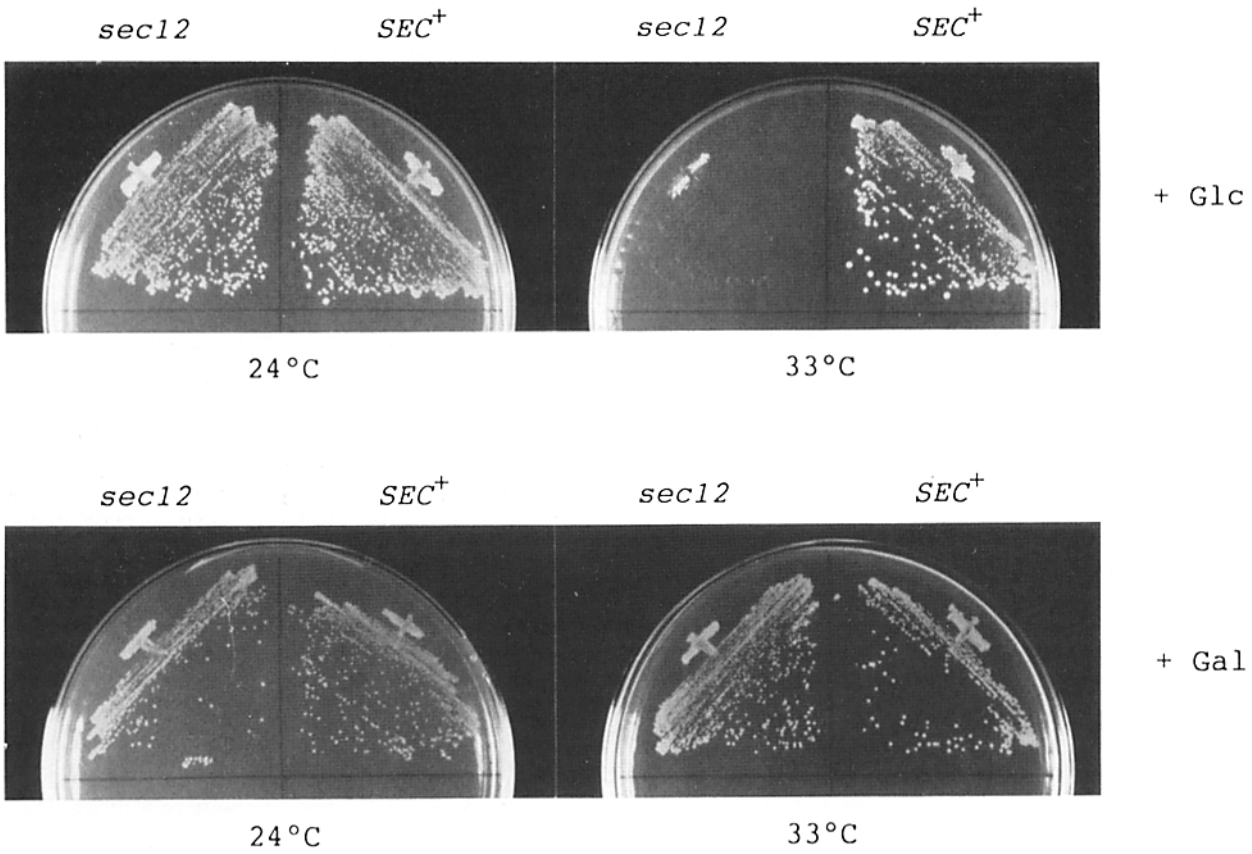

Figure 6. Gal-dependent expression of *SAR1*. The single-copy plasmid pANY2-18 containing the *GAL1-SAR1* fusion gene was introduced into MBY10-7A (*sec12-4*) and ANY21 (*SEC*$^+$). The cells were grown in a minimal medium containing galactose (MVGal), streaked out on YP plates containing either galactose or glucose, and incubated at 24 or 33°C. Note that the temperature sensitivity of *sec12* was suppressed by the plasmid in galactose.

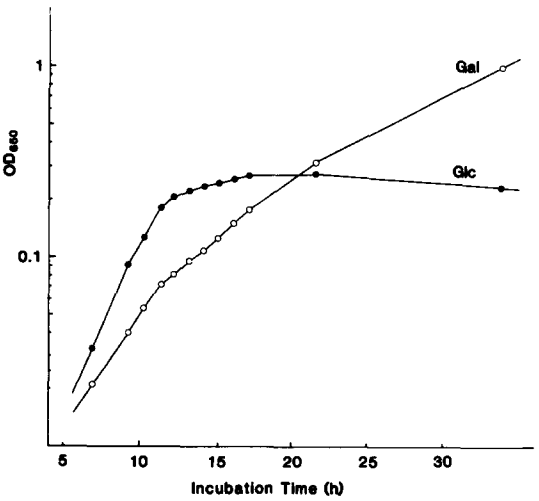

Figure 7. Growth of Gal-dependent *sar1* mutant. The *GAL1-SAR1* plasmid pANY2-18 was introduced into a haploid strain with its chromosomal *SAR1* gene disrupted (*sar1::URA3*), by using the plasmid exchange with pSEC1210 (for details see text). The resultant galactose-dependent *sar1* mutant (ANY25) was grown to late-log phase in YPGal medium. Cells were washed with sterile water, inoculated into either YPGal or YPD, and incubated at 30°C. The increase of cell density was monitored by measuring OD_{650} with a spectrophotometer (Coleman).

Discussion

In this paper, we have presented evidence that a novel essential gene of yeast, *SAR1*, encodes a GTP-binding protein and is involved in protein transport from the ER to the Golgi. Sequence analysis of *SAR1* has shown that the gene codes for a 21-kD protein (Sarlp) containing amino acid sequences that are highly conserved among various GTP-binding proteins. The evidence that Sarlp is involved in ER-Golgi transport comes from the analysis of a conditional-lethal, galactose-dependent *sar1* mutant. This mutant, under a restrictive condition, accumulates the core-glycosylated ER-forms of pro-α-factor and proCPY. *SAR1* genetically interacts with *SEC12*, which is already known as an essential gene required for ER-Golgi transport; perhaps the interaction is between their protein products. Here we discuss these results and the roles of the two gene products in the first step of vesicular transport in the secretory pathway.

Interaction between SEC12 and SAR1

SAR1 has been isolated as a suppressor gene of a *sec12* ts mutation that confers a Ts$^+$ phenotype to the mutant when it is introduced on a multicopy plasmid. Interestingly, this suppression of the *sec12* ts mutation can be seen by a single-copy *SAR1* plasmid, too. There is a single *SAR1* locus in the chromo-

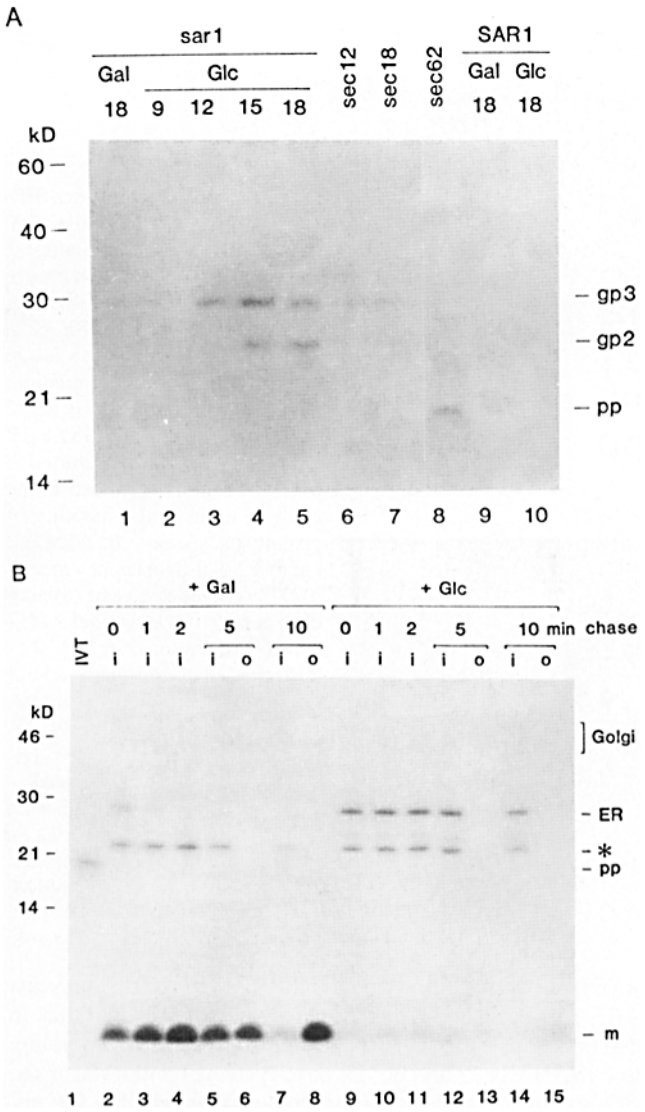

Figure 8. sarl accumulates ER-form of α-factor precursor. (*A*) Immunoblotting analysis. The Gal-dependent *sarl* mutant (ANY26) was incubated in YPGal (Gal) or YPD (Glc) medium as in the experiment in Fig. 7. At times indicated (9–18 h), aliquots were taken and lysates were prepared by glass bead-homogenization in SDS. The lysates were electrophoresed in a 12.5% SDS-gel and subjected to immunoblotting using anti-prepro-α-factor antibody. The ER-accumulating ts mutants, MBY10-7C (*secl2*) and MBY12-6D (*secl8*), and an ER-translocation mutant RDM50-94C (*sec62*) were grown in YPD at 24°C and, after 3 h additional incubation at 37°C, harvested to prepare lysates. As a *SARI*$^+$ control, ANY26/pSEC1210 harboring both regulatory *GAL1-SARI* plasmid (pANY2-18) and constitutive *SARI* plasmid (pSEC1210) was incubated for 18 h in MVGal or MVD lacking Trp and Leu to keep both of the plasmids, and intracellular α-factor was analyzed as above. *gp3* and *gp2*, pro-α-factor harboring three and two core oligosaccharide chains, respectively; *pp*, prepro-α-factor precursor. (*B*) Pulse-chase experiment. *sarl* cells (ANY26) were cultured in galactose or glucose medium with low sulfate at 30°C for 12 h. 2.5 × 10^7 cells were harvested, washed, resuspended in sulfate-free medium, and pulse-labeled with 12.5 μCi/ml Tran^{35}S-label for 5 min. Chase was started by the addition of excess sulfate, cysteine, and methionine. Aliquots were taken at times indicated and mixed with NaN_3 on ice to terminate the reaction. Cell lysates were prepared and subjected to immunoprecipitation with anti-prepro-α-factor antibody (*i*). α-factor was also immunoprecipitated from medium at 5- and 10- min chase (*o*). Fluorogram of a 15% SDS-gel is shown. ER-form (same as gp3 in *A*), highly glycosylated Golgi form, and mature (*m*) α-factor are indicated. Labeled prepro-α-factor (*pp*) was prepared by in vitro translation (*IVT*) (Hansen et al., 1986). The band migrating at ~22 kD (*) may be unglycosylated pro-α-factor.

some, meaning that the duplication of *SARI* is enough to suppress the *secl2* ts phenotype. In contrast to the ts mutants, the lethality of *secl2* deletion mutant cannot be rescued even by the multicopy *SARI*. These observations lead to an important notion that the suppression by *SARI* requires the *secl2* gene product, even though it is partially defective. We suggest that this is indicative of a direct interaction between the two gene products, Secl2p and Sarlp. When the function of Secl2p is partly injured, elevation of Sarlp level may help keep the function of the complex normal. Cloning and structural analysis of the mutant *secl2* genes will be necessary for further investigation of the suppression mechanism.

The suppression by gene duplication has also been reported in the case of the *SEC4* gene, which is required in a later step of secretion, fusion of secretory vesicles with the plasma membrane. *SEC4* suppresses *sec2*, *sec8*, and *secl5* ts mutations on a single-copy plasmid (Salminen and Novick, 1987). Sec4p is also shown to be a *ras*-related GTP-binding protein. We will come back to a view on the roles of these GTP-binding proteins in secretory pathway later.

SAR1 Encodes a Novel GTP-binding Protein

The sequence analysis of *SARI* gene has revealed that it contains an intron. This is not a very frequent event for nuclear genes of *Saccharomyus cerevisiae*, although we do not know at present what it means. The spliced message codes for a protein consisting of 190 amino acid residues, which shows significant homology to a wide variety of GTP-binding proteins.

As shown in Table I, Sarlp possesses three consensus elements of GTP-binding domains in a complete fashion, both in sequence similarity and in spacings between them. The first two elements, GXXXXGK and DXXG, are necessary for the binding of phosphate group and GTPase activity, whereas the third one is essential for the specific recognition of guanine base. Statistically, the probability that the three consensus sequences in correct spacings arise by chance is negligible (Dever et al., 1987). The remarkable sequence similarity in these elements strongly suggests that Sarlp is also a GTP-binding protein. A preliminary GTP-blotting experiment with a recombinant Sarlp expressed in *E. coli* has indicated that the protein in fact binds GTP (Nakano, A., unpublished results). Thus, we conclude that Sarlp is a novel member of GTP-binding protein superfamily.

In the large superfamily of GTP-binding proteins, which protein does Sarlp resemble best? We tentatively classify the proteins listed in Table I into seven subgroups as follows. (*a*) The *YPTI* family. This subgroup contains yeast *YPTI* and *SEC4* and mammalian *ypt* homologues. (*b*) Authentic *ras* proteins. (*c*) Eukaryotic *ras* homologues, *rho* and *ral*. (*d*) G protein α subunits. (*e*) ADP-ribosylation factors (ARF) and homologues. (*f*) Polypeptide chain elongation factors and yeast *GSTI*. (*g*) *E. coli ras* homologues. Several important features can be seen in a close comparison of these subgroups. First, most of them contain a glycine residue at posi-

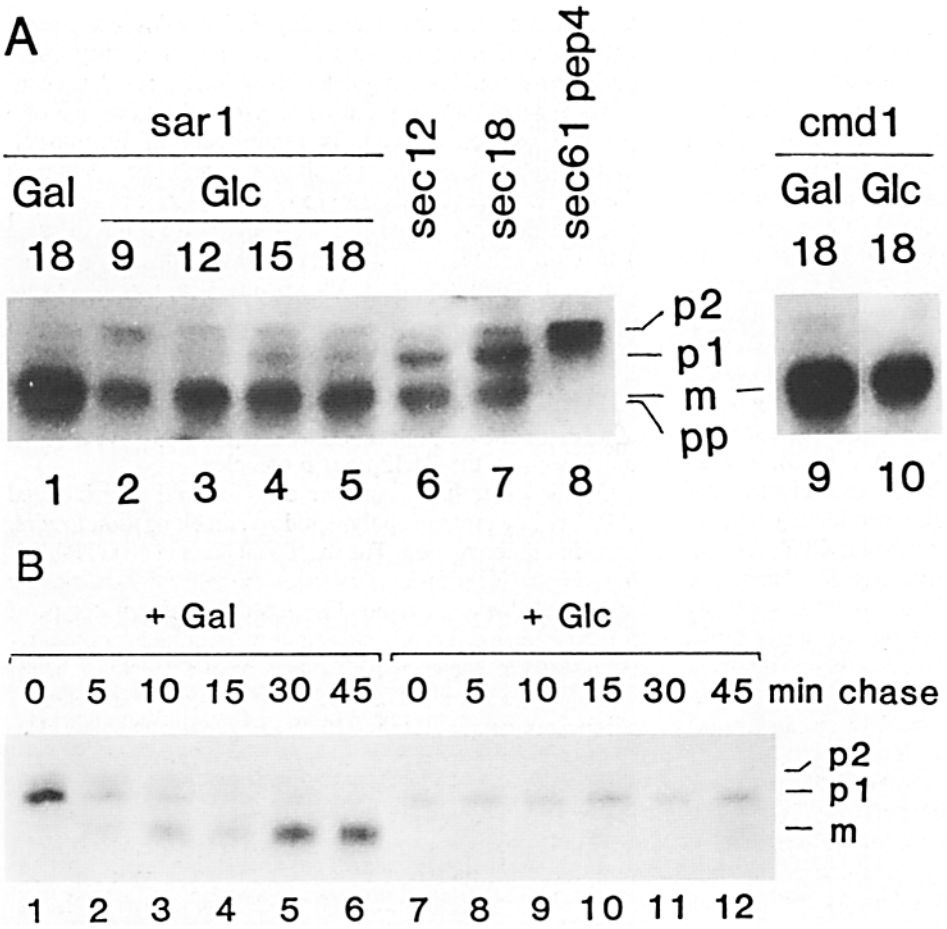

Figure 9. sar1 accumulates ER-form of carboxypeptidase Y (*CPY*). (*A*) The same samples as in the experiment of Fig. 8 *A* were subjected to an immunoblotting analysis of intracellular CPY. Note the appearance of p1 species in *sar1* cultured in glucose medium for 12–18 h, as well as in *sec12* and *sec18*. Strain RDM15-5B (*sec61 pep4*) was also cultured at 37°C for 3 h and included in the analysis to show the mobility of pp and p2 species. In addition, another Gal-dependent mutant YOT18 containing a yeast calmodulin gene (*CMD1*) under *GAL1* control was cultured in YPGal or YPD for 18 h and analyzed for CPY. *pp*, preproCPY; *p1* and *p2*, ER- and Golgi-forms of proCPY, respectively; *m*, mature CPY, (*B*) *sar1* (ANY26) cells incubated in galactose or glucose for 12 h were pulse-labeled and chased as in Fig. 8 *B*, and subjected to immunoprecipitation of CPY and SDS-PAGE.

tion 12 by the numbering of c-*ras*-H. This residue is believed to play a key role in GTPase activity (see Barbacid, 1987). Many oncogenic *ras* gene products have this residue substituted by arginine, valine, or something else; stay at the activated GTP-bound state longer because of the decreased GTPase activity; and hence exhibit a strong transforming activity. The *YPT1* family commonly contains a serine or threonine residue here instead of glycine, whereas elongation factors conserve valine. In *SAR1*, aspartic acid is used at this position. The family of ARFs also contains an aspartic acid at this position. Secondly, there are two typical patterns of the three motifs, *ras*-type G(G/A)GGVGK . . . DTAGQ . . . VGNKXD and G-protein-type GAGESGK . . . DVGGQ . . . FLNKXD. The sequences in Sarlp do not fit either of the two types. Thirdly, *ras*, *rho*, and *ral* proteins have a common sequence CAAX at the COOH terminus, where A denotes an aliphatic residue, and the proteins of *YPT1* family terminate with CC (not shown in Table II). Cysteine residues in the COOH-terminal region of these proteins are reported to function as a site for the addition of palmitic acid and polyisoprenoid, thus making the protein anchored in the membrane (Barbacid, 1987; Fujiyama and Tamanoi, 1986; Molenaar et al., 1988; Goud et al., 1988; Hancock et al., 1989). Sarlp does not have a cysteine residue near the COOH terminus. Lastly, as far as the size of the protein is concerned, 21-kD Sarlp is apparently closer to *ras*, *YPT1*, *rho*, and *ARF* families than to G proteins or elongation factors.

Interestingly, direct homology analysis with each subgroup has highlighted significant similarity of Sarlp to the *ARF* family. The amino acid identity between Sarlp and yeast ARF is 34.5% over 168 residues. They are also similar in that they conserve aspartic acid at the position corresponding to c-*ras*-H Gly-12 and do not have cysteine residues near the COOH terminus. However, we do not know whether it is appropriate to categorize Sarlp and ARFs into the same subgroup. The NH_2-terminal motif in ARFs (MGXXX[S/A]), which is considered to act as a myristylation signal (Sewell and Kahn, 1988), does not exist in Sarlp. Furthermore, the functional relationship between Sarlp and ARFs seems distant at present. The mammalian ARF has been shown necessary for ADP-ribosylation of Gs α subunit by cholera toxin (Kahn and Gilman, 1986), but no such reaction has been reported in yeast. The analysis of yeast *ARF* gene function will be important to address this problem. Again, we would like to define Sarlp as a novel type of member in the expanding *ras* superfamily. One should remember here that the glycine residue at position 12 by the numbering of c-*ras*-H is not essential for GTPase activity; EF-Tu turns into a potent GTPase when correctly targeted to the ribosome (see Kaziro, 1978). It should be also pointed out that neither lipid modification at the COOH terminus nor myristylation at the NH_2 terminus is prerequisite for binding to the membranes; Gs α subunits that are not acylated fulfill membrane association through the interaction with β and γ subunits (Lochrie and Simon, 1988).

Sarlp as a Protein Required for ER-Golgi Transport

We have shown that both *SEC12* and *SAR1* are essential for

cell growth. The putative interaction between the two gene products as predicted from genetic analysis suggests that Sarlp cooperates with Secl2p in promoting protein transport from the ER to the Golgi. The phenotype of the Gal-dependent *sar1* strain supports this view. When *SAR1* is placed under control of the *GAL1* promoter, its expression is induced in the presence of galactose and repressed in the presence of glucose. In fact, the *GAL1-SAR1* fusion gene suppresses *sec12* ts growth in galactose but not in glucose. This fusion gene has been introduced into a *sar1* deletion mutant to yield a conditional lethal mutant that grows only in galactose medium. When the culture is shifted from galactose to glucose medium, division continues for a while, but eventually stops probably because the preexisting Sarlp is depleted (Fig. 7). At this restrictive stage cells accumulate a secretory protein, α-mating factor, intracellularly (Fig. 8). The accumulating species comigrate with the core-glycosylated precursor forms that are seen in ER-accumulating mutants such as *sec12* and *sec18*. A vacuolar protein, CPY, also accumulates in a core-glycosylated form (Fig. 9). Thus, *sar1* shows a transport defect at a very similar point in secretory pathway to *sec12*. Formally, we cannot rule out a possibility that the defect seen in this *sar1* mutant is a terminal phenotype that is indirectly affected by the decreased viability of the cells. However, another Gal-dependent mutant of calmodulin (*cmd1*), which shows almost the same growth phenotype in glucose medium (Ohya and Anraku, 1989), does not exhibit any defect in the ER-Golgi transport (see Fig. 9). This supports the idea that *SAR1* plays a specific role in the ER-Golgi transport. Isolation of other types of conditional mutants, e.g., ts or cs alleles, will be helpful for the further analysis. As is also true for other ER-blocking *sec* mutants, the defect in ER-Golgi transport does not necessarily mean that Sarlp is involved only in this step. Subcellular localization of Sarlp will also help obtain further insights into the mode of action of this protein.

Role of a GTP-binding Protein in Intracellular Transport

Evidence is accumulating that there are several GTP-binding proteins functioning in protein secretion. In addition to *SEC4*, which is required in the exocytosis of secretory vesicles, *YPT1* has been shown to be involved in transport somewhere around the ER and/or Golgi (Segev et al., 1988; Schmitt et al., 1988). Melançon et al. (1987) and Beckers and Balch (1989) have shown that GTP-γS, a nonhydrolyzable analogue of GTP, inhibits the transport of a viral glycoprotein through the Golgi and from the ER to the Golgi, respectively, using mammalian cell-free systems, although the target GTP-binding protein(s) have not been identified. Baker et al. (1988) and Ruohola et al. (1988) have also reported inhibition by GTP-γS of ER-Golgi transport of α-factor in yeast cell-free systems. Studies on the protein translocation across the ER membranes have also revealed involvement of GTP-binding proteins including the signal recognition particle (SRP) receptor α subunit, and the 54K subunit of SRP (Connolly and Gilmore, 1986, 1989; Römisch et al., 1989; Bernstein et al., 1989).

A popular interpretation for the roles of these GTP-binding proteins is due to the analogy to the regulatory G proteins' function in transmembrane signaling across the plasma membrane (Gilman, 1987). If the GTP-binding proteins mentioned above indeed play similar roles in secretory pathway to G proteins, what kind of signals do they transduce? What could be a regulatory function executed in a constitutive secretion? In the case of Sarlp, the existence of a putative partner, Secl2p, is reminiscent of receptor/G-protein interactions on the plasma membrane. Although there is no evidence that Secl2p is a receptor, it could transmit as a transmembrane protein a signal from the lumenal side to the cytoplasm. The signal might be the sorting information in the lumen of the ER and/or Golgi and also could instruct the cytoplasmic machinery for budding, transport, or fusion of vesicles. Recent studies have suggested a close interaction of the ER network with microtubules (Dabora and Sheetz, 1988; Lee and Chen, 1988). Possibly, this and other cytoskeletal systems may be a target of a lumenal signal transduced by the Secl2p·Sarlp complex.

On the other hand, another class of well-characterized GTP-binding proteins, polypeptide chain elongation factors, provide a different view. The functional basis of all GTP-binding proteins is the strict conversion of their conformation by the ligand change between GTP and GDP. As a prototype of the GTP-binding protein superfamily, structure-function relationships of bacterial EF-Tu have been extensively investigated with a focus on GDP-GTP allosterism by various kinds of biochemical (see Kaziro, 1978) and physicochemical (Nakano et al., 1980; Jurnak, 1985) approaches. When the ternary aminoacyl-tRNA·EF-Tu·GTP complex binds to the A site of a ribosome, GTP is hydrolyzed and EF-Tu·GDP immediately dissociates from the ribosome-aminoacyl-tRNA complex. This dissociation enables the polypeptide elongation cycle to proceed and EF-Tu·GTP regenerated by the action of EF-Ts brings another aminoacyl-tRNA to the ribosome. Thus, the GTP hydrolysis is thought to drive the cycle of peptide elongation reaction in an irreversible, unidirectional fashion (Kaziro, 1978). By analogy, the role of the GTP-binding proteins in secretion may be to promote a cyclic reaction of protein transport unidirectionally. Bourne (1988) has suggested that a GTP-binding protein may recycle between donor and acceptor membrane compartments and direct the vectorial transport of proteins by using the energy of GTP. In the case of Sarlp, this recycling could involve membrane vesicles. We have speculated that Secl2p may recycle between the ER and the Golgi membranes from the observation that Secl2p molecules undergo slow but progressive modification in the Golgi (Nakano et al., 1988). Like the polypeptide elongation cycle, Secl2p·Sarlp·GTP may bring a vesicle containing a cargo of secretory proteins to the Golgi membrane. Conceivably, somewhere in the processes of membrane fusion and protein sorting, Sarlp would hydrolyze GTP and the release of Sarlp·GDP might enable the return of shuttling vesicles to the ER.

To address the roles of Secl2p and Sarlp in membrane traffic, cell-free ER-Golgi transport systems developed by Baker et al. (1988) and Ruohola et al. (1988) will be of great use. What is especially interesting to us is that these in vitro reactions are inhibited by GTP-γS. Whether the target protein is Sarlp or not should be tested. Our finding that another ER-Golgi *SEC* gene, *SEC16*, also shows genetic interaction with *SAR1* suggests that a complex mechanism is operating in this inter-compartmental transport step. The thorough understanding of the structure and function of the secretory machinery still remains as a challenging goal of our efforts.

We thank Jonathan Rothblatt and Arlene Eun for anti-prepro-α-factor antibody; Raymond Deshaies, Peter Novick, and Yoshikazu Ohya for strains and plasmids; Atsushi Miyajima for a cDNA library; Gregory Payne for suggestions in labeling α-factor; and Randy Schekman, Kunihiro Matsumoto, and Yasuhiro Anraku for helpful discussions and critical comments on the manuscript. We are also indebted to Mitchell Bernstein who isolated the original pSEC1210 and pSEC1230 clones and to Mirian Hasson who carried out the initial restriction analysis of these clones. Without their efforts, this work would not have started.

This work was supported in part by grants-in-aid from the Ministry of Education, Science, and Culture of Japan and by a research fund from Uehara Memorial Foundation.

Received for publication 21 June 1989 and in revised form 29 August 1989.

References

Ahnn, J., P. E. March, H. E. Takiff, and M. Inouye. 1986. A GTP-binding protein of *Escherichia coli* has homology to yeast RAS proteins. *Proc. Natl. Acad. Sci. USA.* 83:8849–8853.

Arai, K., B. F. C. Clark, L. Duffy, M. D. Jones, Y. Kaziro, R. A. Laursen, J. L'Italien, D. L. Miller, S. Nagarkatti, S. Nakamura, K. M. Nielsen, T. E. Petersen, K. Takahashi, and M. Wade. 1980. Primary structure of elongation factor Tu from *Escherichia coli. Proc. Natl. Acad. Sci. USA.* 77:1326–1330.

Baker, D., L. Hicke, M. Rexach, M. Schleyer, and R. Schekman. 1988. Reconstitution of *SEC* gene product-dependent intercompartmental protein transport. *Cell.* 54:335–344.

Barbacid, M. 1987. *ras* genes. *Annu. Rev. Biochem.* 56:779–827.

Beckers, C. J. M., and W. E. Balch. 1989. Calcium and GTP: essential components in vesicular trafficking between the endoplasmic reticulum and Golgi apparatus. *J. Cell Biol.* 108:1245–1256.

Bernstein, H. D., M. A. Poritz, K. Strub, P. J. Hoben, S. Brenner, and P. Walter. 1989. Model for signal sequence recognition from amino-acid sequence of 54K subunit of signal recognition particles. *Nature (Lond.).* 340:482–486.

Bernstein, M., W. Hoffmann, G. Ammerer, and R. Schekman. 1985. Characterization of a gene product (Sec53p) required for protein assembly in the yeast endoplasmic reticulum. *J. Cell Biol.* 101:2374–2382.

Böhni, P. C., R. J. Deshaies, and R. W. Schekman. 1988. *SEC11* is required for signal peptide processing and yeast cell growth. *J. Cell Biol.* 106: 1035–1042.

Botstein, D., and R. W. Davis. 1982. Principles and practices of recombinant DNA research with yeast. *In* Molecular Biology of the Yeast *Saccharomyces*: Metabolism and Gene Expression. J. N. Strathern, E. W. Jones, and J. R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 607–636.

Bourne, H. R. 1988. Do GTPases direct membrane traffic in secretion? *Cell.* 53:669–671.

Broach, J. R., J. N. Strathern, and J. B. Hicks. 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. *Gene (Amst.).* 8:121–133.

Capon, D. J., E. Y. Chen, A. E. Levinson, P. H. Seeburg, and D. V. Goeddel. 1983. Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. *Nature (Lond.).* 302:33–37.

Chardin, P., and A. Tavitian. 1986. The *ral* gene: a new *ras* related gene isolated by the use of a synthetic probe. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2203–2208.

Connolly, T., and R. Gilmore. 1986. Formation of a functional ribosome-membrane junction during translocation requires the participation of a GTP-binding protein. *J. Cell Biol.* 103:2253–2261.

Connolly, T., and R. Gilmore. 1989. The signal recognition particle receptor mediates the GTP-dependent displacement of SRP from the signal sequence of the nascent polypeptide. *Cell.* 57:599–610.

Cottrelle, P., D. Thiele, V. L. Price, S. Memet, J.-Y. Micouin, C. Marck, J.-M. Buhler, A. Sentanac, and P. Fromageot. 1985. Cloning, nucleotide sequence, and expression of one of two genes coding for yeast elongation factor 1α. *J. Biol. Chem.* 260:3090–3096.

Dabora, S. L., and M. P. Sheetz. 1988. The microtubule-dependent formation of a tubulovesicular network with characteristics of the ER from cultured cell extracts. *Cell.* 54:27–35.

DeFeo-Jones, D., E. M. Scolnick, R. Koller, and R. Dhar. 1983. *ras*-related gene sequences identified and isolated from *Saccharomyces cerevisiae. Nature (Lond.).* 306:707–709.

Deshaies, R. J. 1988. Genes required for protein translocation in the yeast endoplasmic reticulum. Ph. D. thesis. University of California, Berkeley.

Deshaies, R. J., and R. Schekman. 1987. A yeast mutant defective at an early stage in import of secretory protein precursors into the endoplasmic reticulum. *J. Cell Biol.* 105:633–645.

Dever, T. E., M. J. Glynia, and W. C. Merrick. 1987. GTP-binding domain: three consensus sequence elements with distinct spacing. *Proc. Natl. Acad. Sci. USA.* 84:1814–1818.

de Vos, A. M., L. Tong, M. V. Milburn, P. M. Matias, J. Jancarik, S. Noguchi, S. Nishimura, K. Miura, E. Ohtsuka, and S.-H. Kim. 1988. Three-dimensional structure of an oncogene protein: catalytic domain of human c-H-*ras* p21. *Science (Wash. DC).* 239:888–893.

Dhar, R., R. W. Ellis, T. Y. Shih, S. Oroszlan, B. Shapiro, B. Maizel, D. Lowy, and E. Scolnick. 1982. Nucleotide sequence of the p21 transforming protein of Harvey murine sarcoma virus. *Science (Wash. DC).* 217:934–937.

Dhar, R., A. Nieto, R. Koller, D. DeFeo-Jones, and E. M. Scolnick. 1984. Nucleotide sequence of two *ras*st-related genes isolated from the yeast *Saccharomyces cerevisiae. Nucleic Acids Res.* 12:3611–3618.

Dietzel, C., and J. Kurjan. 1987. The yeast *SCG1* gene: a Gα-like protein implicated in the a- and α-factor response pathway. *Cell.* 50:1001–1010.

Emr, S. D., A. Vassarotti, J. Garrett, B. L. Geller, M. Takeda, and M. G. Douglas. 1986. The amino acid terminus of the yeast F_1-ATPase β-subunit precursor functions as a mitochondrial import signal. *J. Cell Biol.* 102:523–533.

Fujiyama, A., and F. Tamanoi. 1986. Processing and fatty acid acylation of RAS1 and RAS2 proteins in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA.* 83:1266–1270.

Fukui, Y., and Y. Kaziro. 1985. Molecular cloning and sequence analysis of a *ras* gene from *Schizosaccharomyces pombe. EMBO (Eur. Mol. Biol. Organ.) J.* 4:687–691.

Gallwitz, D., C. Donath, and C. Sander. 1983. A yeast gene encoding a protein homologous to the human c-*has/bas* proto-oncogene product. *Nature (Lond.).* 306:704–707.

Gilman, A. 1987. G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56:615–649.

Goud, B., A. Salminen, N. C. Walworth, and P. J. Novick. 1988. A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. *Cell.* 53:753–768.

Hancock, J. F., A. I. Magee, J. E. Childs, and C. J. Marshall. 1989. All *ras* proteins are polyisoprenylated but only some are palmitoylated. *Cell.* 57:1167–1177.

Hansen, W., P. D. Garcia, and P. Walter. 1986. In vitro protein translocation across the yeast endoplasmic reticulum: ATP-dependent post-translational translocation of the prepro-α-factor. *Cell.* 45:397–406.

Haubruck, H., C. Disela, P. Wagner, and D. Gallwitz. 1987. The *ras*-related *ypt* protein is an ubiquitous eukaryotic protein: isolation and sequence analysis of mouse cDNA clones highly homologous to the yeast *YPT1* gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4049–4053.

Itoh, H., T. Kozasa, S. Nagata, S. Nakamura, T. Katada, M. Ui, S. Iwai, E. Ohtsuka, H. Kawasaki, K. Suzuki, and Y. Kaziro. 1986. Molecular cloning and sequence determination of cDNAs for α subunits of the guanine nucleotide-binding proteins Gs, Gi, and Go from rat brain. *Proc. Natl. Acad. Sci. USA.* 83:3776–3780.

Jurnak, F. 1985. Structure of the GDP domain of EF-Tu and location of the amino acids homologous to *ras* oncogene proteins. *Science (Wash. DC).* 230:32–36.

Kahn, R. A., and A. G. Gilman. 1986. The protein cofactor necessary for ADP-ribosylation of Gs by cholera toxin is itself a GTP binding protein. *J. Biol. Chem.* 261:7906–7911.

Kaziro, Y. 1978. The role of guanosine 5'-triphosphate in polypeptide chain elongation. *Biochim. Biophys. Acta.* 505:95–127.

Keszenman-Pereyra, D., and K. Hieda. 1988. A colony procedure for transformation of *Saccharomyces cerevisiae. Curr. Genet.* 13:21–23.

Kikuchi, Y., H. Shimatake, and A. Kikuchi. 1988. A yeast gene required for the G_1-to-S transition encodes a protein containing an A-kinase target site and GTPase domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1175–1182.

Lee, C., and L. B. Chen. 1988. Dynamic behavior of endoplasmic reticulum in living cells. *Cell.* 54:37–46.

Lipman, D. J., and W. R. Pearson. 1985. Rapid and sensitive protein similarity searches. *Science (Wash. DC).* 227:1435–1441.

Lochrie, M. A., and M. I. Simon. 1988. G protein multiplicity in eukaryotic signal transduction systems. *Biochemistry.* 27:4957–4965.

Madaule, P., and R. Axel. 1985. A novel *ras*-related gene family. *Cell.* 41:31–40.

Madaule, P., R. Axel, and A. M. Myers. 1987. Characterization of two members of the *rho* gene family from the yeast *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA.* 84:779–783.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

March, P. E., and M. Inouye. 1985. GTP-binding membrane protein of *Escherichia coli* with sequence homology to initiation factor 2 and elongation factors Tu and G. *Proc. Natl. Acad. Sci. USA.* 28:7500–7504.

Melançon, P., B. S. Glick, V. Malhotra, P. J. Weidman, T. Serafini, M. L. Gleason, L. Orci, and J. E. Rothman. 1987. Involvement of GTP-binding "G" proteins in transport through the Golgi stack. *Cell.* 51:1053–1062.

Miyajima, A., N. Nakayama, I. Miyajima, N. Arai, H. Okayama, and K. Arai. 1984. Analysis of full-length cDNA clones carrying *GAL1* of *Saccharomyces cerevisiae*: a model system for cDNA expression. *Nucleic Acids Res.* 12:6397–6414.

Molenaar, C. M. T., R. Prange, and D. Gallwitz. 1988. A carboxyl-terminal cysteine residue is required for palmitic acid binding and biological activity of the *ras*-related yeast *YPT1* protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:971–976.

Nagata, S., K. Nagashima, Y. Tsunetsugu-Yokota, K. Fujimura, M. Miyazaki, and Y. Kaziro. 1984. Polypeptide chain elongation factor 1α (EF-1α) from yeast: nucleotide sequence of one of the two genes for EF-1α from *Saccharomyces cerevisiae*. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1825–1830.

Nakafuku, M., H. Itoh, S. Nakamura, and Y. Kaziro. 1987. Occurrence in *Saccharomyces cerevisiae* of a gene homologous to the cDNA coding for the α subunit of mammalian G proteins. *Proc. Natl. Acad. Sci. USA.* 84:2140–2144.

Nakafuku, M., T. Obara, K. Kaibuchi, I. Miyajima, A. Miyajima, H. Itoh, S. Nakamura, K. Arai, K. Matsumoto, and Y. Kaziro. 1988. Isolation of a second yeast *Saccharomyces cerevisiae* gene (*GPA2*) coding for guanine nucleotide-binding regulatory protein: studies on its structure and possible functions. *Proc. Natl. Acad. Sci. USA.* 85:1374–1378.

Nakano, A., T. Miyazawa, S. Nakamura, and Y. Kaziro. 1980. Proton nuclear magnetic resonance study on the roles of histidine residues in the binding of polypeptide chain elongation factor Tu from *Thermus thermophilus* with aminoacyl transfer ribonucleic acid and guanine nucleotides. *Biochemistry.* 19:2209–2215.

Nakano, A., D. Brada, and R. Schekman. 1988. A membrane glycoprotein, Sec12p, required for protein transport from the endoplasmic reticulum to the Golgi apparatus in yeast. *J. Cell Biol.* 107:851–863.

Newman, A. P., and S. Ferro-Novick. 1987. Characterization of new mutants in the early part of the yeast secretory pathway isolated by a [^{3}H]mannose suicide selection. *J. Cell Biol.* 105:1587–1594.

Novick, P., C. Field, and R. Schekman. 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell.* 21:205–215.

Ohya, Y., and Y. Anraku. 1989. A galactose-dependent *cmd1* mutant of *Saccharomyces cerevisiae*: involvement of calmodulin in nuclear division. *Curr. Genet.* 15:113–120.

Ohya, Y., S. Miyamoto, Y. Ohsumi, and Y. Anraku. 1986. Calcium-sensitive *cls4* mutant of *Saccharomyces cerevisiae* with a defect in bud formation. *J. Bacteriol.* 165:28–33.

Payne, G. S., and R. Schekman. 1985. A test of clathrin function in protein secretion and cell growth. *Science (Wash. DC).* 230:1009–1014.

Payne, G. S., D. Baker, E. van Tuinen, and R. Schekman. 1988. Protein transport to the vacuole and receptor-mediated endocytosis by clathrin heavy chain-deficient yeast. *J. Cell Biol.* 106:1453–1461.

Pfeffer, S. R., and J. E. Rothman. 1987. Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. *Annu. Rev. Biochem.* 56:829–852.

Powers, S., T. Kataoka, O. Fasano, M. Goldfarb, J. Strathern, J. Broach, and M. Wigler. 1984. Genes in S. cerevisiae encoding proteins with domains homologous to the mammalian *ras* proteins. *Cell.* 36:607–612.

Reddy, E. P. 1983. Nucleotide sequence analysis of the T24 human bladder carcinoma oncogene. *Science (Wash. DC).* 220:1061–1063.

Reymond, C. D., R. H. Gomer, M. C. Mehdy, and R. A. Firtel. 1984. Developmental regulation of a Dictyostelium gene encoding a protein homologous to mammalian *ras* protein. *Cell.* 39:141–148.

Römisch, K., J. Webb, J. Herz, S. Prehn, R. Frank, M. Vingron, and B. Dobberstein. 1989. Homology of 54K protein of signal-recognition particle, docking protein and two *E. coli* proteins with putative GTP-binding domains. *Nature (Lond.).* 340:478–482.

Ruohola, H., A. K. Kabcenell, and S. Ferro-Novick. 1988. Reconstitution of protein transport from the endoplasmic reticulum to the Golgi complex in yeast: the acceptor Golgi compartment is defective in the *sec23* mutant. *J. Cell Biol.* 107:1465–1476.

Salminen, A., and P. J. Novick. 1987. A *ras*-like protein is required for a post-Golgi event in yeast secretion. *Cell.* 49:527–538.

Sanger, F. S., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

Schirmaier, F., and P. Philippsen. 1984. Identification of two genes coding for the translation elongation factor EF-1α of *S. cerevisiae*. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:3311–3315.

Schmitt, H. D., M. Puzicha, and D. Gallwitz. 1988. Study of a temperature-sensitive mutant of the *ras*-related *YPT1* gene product in yeast suggests a role in the regulation of intracellular calcium. *Cell.* 53:635–647.

Schmitt, H. D., P. Wagner, E. Pfaff, and D. Gallwitz. 1986. The *ras*-related *YPT1* gene product in yeast: a GTP-binding protein that might be involved in microtubule organization. *Cell.* 47:401–412.

Segev, N., and D. Botstein. 1987. The *ras*-like yeast *YPT1* gene is itself essential for growth, sporulation, and starvation response. *Mol. Cell. Biol.* 7:2367–2377.

Segev, N., J. Mulholland, and D. Botstein. 1988. The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell.* 52:915–924.

Sewell, J. L., and R. A. Kahn. 1988. Sequences of the bovine and yeast ADP-ribosylation factor and comparison to other GTP-binding proteins. *Proc. Natl. Acad. Sci. USA.* 85:4620–4624.

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.

Tanabe, T., T. Nukada, Y. Nishikawa, K. Sugimoto, H. Suzuki, H. Takahashi, M. Noda, T. Haga, A. Ichiyama, K. Kangawa, N. Minamino, H. Matsuo, and S. Numa. 1985. Primary structure of the α-subunit of transducin and its relationship to *ras* proteins. *Nature (Lond.).* 315:242–245.

Touchot, N., P. Chardin, and A. Tavitian. 1987. Four additional members of the *ras* gene superfamily isolated by an oligonucleotide strategy: molecular cloning of YPT-related cDNAs from a rat brain library. *Proc. Natl. Acad. Sci. USA.* 84:8210–8214.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.

Tschopp, J. F., S. D. Emr, C. Field, and R. Schekman. 1986. *GAL2* codes for a membrane-bound subunit of the galactose permease in *Saccharomyces cerevisiae*. *J. Bacteriol.* 166:313–318.

Vieira, J., and J. Messing. 1987. Production of single-stranded plasmid DNA. *Methods Enzymol.* 153:3–11.

Wickerham, L. J. 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. *J. Bacteriol.* 52:293–301.

Wilbur, W. J., and D. J. Lipman. 1983. Rapid similarity searches of nucleic acid and protein data banks. *Proc. Natl. Acad. Sci. USA.* 80:726–730.

Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene (Amst.).* 33:103–110.

Yatsunami, K., and H. G. Khorana. 1985. GTPase of bovine rod outer segments: the amino acid sequence of the α subunit as derived from the cDNA sequence. *Proc. Natl. Acad. Sci. USA.* 82:4316–4320.