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

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Abstract. SARI, a gene that has been isolated as a multicopy suppressor of the yeast ER-Golgi transport mutant secl2, 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 SARI is essential for cell growth. To test its function further, SARI has been placed under control of the GAL promoter and introduced into a haploid cell that had its chromosomal SARI 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 Sarlp 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, SECl2 (Nakano et al., 1988). In the process of cloning, two distinct genes were found to complement a secl2 ts mutation. Genetic analysis has shown that one of these two is the authentic SECl2 gene. Using an antibody raised against a lacZ-SECl2 fusion gene product, we have identified the SECl2 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 secl2 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 SARI, 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. SECl4 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). YPTI plays multiple roles in yeast cell structure. Analysis of null and dominant lethal mutations of YPTI 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 Ca²⁺ remedial phenotype (Schmitt et al., 1988). In this paper, we present a model that another GTP-binding protein, Sarlp, 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 SEI0 (F Δ[lac-pro] ara rpsL thi purF47::Tn5 [p80lacZΔM15]) (Emr et al., 1986) for cloning experiments and MVI193 (Δ[lac-proAB] thi supE6Δ[srl-reca] 306::Tn5[let]) F traD36 recA1 lacF lacZΔM15 (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 sec12-1 ura3-2 leu2 rpl1 his3), ANY1-7D (MATα ura3 leu2) (Nakano et al., 1988), and RDM15-5B (MATα sec62-1 ura3 leu2) were used.
ade2 pep4) (Deshaies and Schekman, 1987) were described previously. Strain ANY21 (MATa ade2 leu2 trpl his), which is isogenic to MBY10-7A, was obtained by marker rescue of sec2-4 with the SEC21 gene (Nakano et al., 1988). Strain JRY9 (MATa ade2 leu2 trpl his his) (a gift from Dr. J. Rine, California, Berkeley) was mated with YOT1 to obtain a diploid strain ANY102 (MATa/MATa ade2 leu2 trpl trpl his/his). ANY102 was transformed either with the disrupted sec2 DNA (pANY1-8, see Nakano et al., 1988) to give ANY104 (MATa/MATa SEC21::URA3 ade2 ade2 leu2 trpl trpl his/his) or with the disrupted sar1 (pMPMY2-1, see below) to yield ANY105 (MATa/MATa SAR1::URA3 ade2 ade2 leu2 trpl trpl his/his). ANY105 was transformed with SARI 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 ade2 ade2 leu2 trpl trpl his [SARI LEU2]) and ANY24 (MATa sar1::URA3 ade2 ade2 leu2 trpl trpl his [SARI LEU2]) were isolated. These haplotypes were further transformed with GALI-SARI plasmid (pANY2-18) using TRPI 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 GALL-SARI fusion gene on the plasmid pANY2-18 and the disrupted version of sar1 in the chromosome, were isolated and named ANY25 (MATa sar1::URA3 ade2 ade2 leu2 trpl trpl his [GALI-SARI TRPI]) and ANY26 (MATa sar1::URA3 ade2 ade2 leu2 trpl trpl his [GALI-SARI TRPI]). Strain YOT18 (MATa ade2 ade2 ade2 leu2 ade2/SARI LEU2) 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 YPD medium (2% polypeptone, 1% yeast extract, 2% dextrose) but did not affect the growth or repression of GALI in the presence of glucose. Cell density was monitored by OD600 using a spectrophotometer (Junior II; Coleman, Heywood, IL). One OD600 corresponded to ~5 × 106 cells/ml.

Plasmids and DNA Manipulations

E. coli plasmids, pUC18 (Yaniach-Perron, 1985), pUC18 and pC19 (Vieira and Messing, 1987); yeast multicopy plasmid YEp35 (Boeck et al., 1979) and pSEY8 (Emr et al., 1986); yeast single-copy plasmids pSEYCS8 (Emr et al., 1986) and YCPG11 (Ohya, Ohya et al., 1986); and yeast integration plasmid Ylp5 (Botstein and Davis, 1982) have been described elsewhere. Isolation of SEC21 and SARI original clones, pSEC1230 and pSEC1210, respectively, which were done by Dr. M. Bernstein (University of California, Berkeley), has been described in a previous paper (Nakano et al., 1988). pSEC1210, pANY2-7, pANY2-10, pANY2-11, and pANY2-12 were derived from pSEY8, and pSEC1213, pANY2-9, and pANY2-13 were from pSEYCS8, whose inserts are shown in Fig. 1. pANY2-7 and pANY2-9 containing the Hind Ill-Sal fragment from pSEC1210 are also referred to as YEpSARI and YCPSARI, respectively. To construct the plasmid for SARI disruption, the unique Nde I site in pUC18 (NdeI) was cut. The ~80-base Bam HI-Eco RI fragment from YEpSARI was inserted into the multicloning sites of pUC18 (NdeI) (pANY2-15) and the ~50-base Bgl II-NdeI fragment was deleted from the insert. Ylp5 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 pAMY2-15 (pMPMY2-1). The 3.9-kb Bam HI-Eco RI fragment from pMPMY2-1 was used for the gene disruption experiment. The construction of GALI-SARI fusion plasmid, pANY2-18, is shown in Fig. 5, pUCGI containing promoter regions of GALI and GALIO was obtained from Y. Ohya.

DNA manipulations including restriction enzyme digestions, ligations, plasmid isolation, and E. coli transformations 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 GENE CLEAN 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(T) 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× Denhardt's, 100 μg/ml salmon sperm DNA, 0.5% SDS, 10 mM Na2HPO4, 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 with ~3 × 106 cpm/ml DNA probes, which were labeled with [α-32P]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 DNA Cloning of SARI

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 ~106 independent clones were obtained. Screening of the E. coli transfectants on nitrocellulose membranes, amplification of plasmids with chloramphenicol, replica plating, and immunobilization 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 with the same buffer at 42°C using ~106 cpm/ml labeled DNA fragment as a probe that was predicted to be internal to the second exon of the genomic SARI. 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 SARI probe. The plasmid containing the largest insert that hybridized with the probe was named pYN3-Y.

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 125I-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 for another 60 min. The membranes were washed four times with TBST four times for 5 min each, 0.5% Tween 20, and dried, and autoradiographed. In some experiments, alkaline phosphatase–conjugated anti-rabbit IgG antibody was used at a 1:5,000 dilution. 

Labeling of Cells and Immunoprecipitation

The intracellular transport of α-factor and carboxypeptidase Y (CPY) in the galactose-dependent sarl mutant was examined by pulse-chase experiments. Cells (ANY26) were inoculated at 2 × 10⁶/ml into MVGal or MVD medium containing 0.2% yeast extract and 100 μM (NH₄)₂SO₄, and grown at 30°C for 12 h. 3 × 10⁷ cells were harvested, washed with water, and suspended in 1.5 ml of MVGal or MVD with no sulfate. After preincubation for 5 min at 30°C, cells were pulse-labeled with 12.5 μCi/ml Tran35S-label (ICN Radiochemicals) at 30°C for 5 min and then chased by 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 was used at a 1:5,000 dilution. 

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, sec12ts, was transformed with a yeast genomic DNA library constructed on a multicopy plasmid YEpl3, 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 SARI.

To localize the suppression activity of the SARI 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, pSEVC58, 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 SARI

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 (boxed). Assuming that 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 SARI. A yeast cDNA library, pCY3, constructed by Miyajima et al. (1984) was used for screening. A ~320-base DNA fragment was prepared from the genomic SARI 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 ~10⁴ colonies screened, 12 positives were isolated. The inserts were confirmed for hybridization with the SARI 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, 346-977) 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 sec12-4 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 Vpt1 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 25.9% to 80% for the entire protein sequence.
The Journal of Cell Biology, Volume 109, 1989 2680

**Figure 1.** Restriction map of pSEC1210, the original isolate of SARI, and deletion analysis. (Top) The insert of pSEC1210 with relevant restriction sites. (Arrow) Position of the putative transcript of SARI, and the two solid bars denote the exons (see Fig. 2). The dashed line is derived from the vector, YEpl3. 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.

| Plasmid      | 5.8 kb | 100 bp | Copy number | sec12 suppression |
|--------------|--------|--------|-------------|-------------------|
| pSEC1210     |        |        | M           | +                 |
| pSEC1212     |        |        | M           | +                 |
| pSEC1213     |        |        | S           | +                 |
| pANY2-7 (YEpSAR1) |      |        | M           | +                 |
| pANY2-8 (YEpSAR1) |      |        | S           | +                 |
| pANY2-10     |        |        | M           | +                 |
| pANY2-13     |        |        | S           | -                 |
| pANY2-11     |        |        | M           | -                 |
| pANY2-12     |        |        | Bg          | -                 |

**Figure 2.** Nucleotide sequence of SARI 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 (GTAAAG ... TACAAA ... 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 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, 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 SARI**

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

AATAAT (1037–1042) after 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.
Table I. Comparison of Sar1p Sequence with Various GTP-Binding Proteins

| Species          | Gene Name | GTPase Active Site               | Guanine Recognition | Reference |
|------------------|-----------|----------------------------------|---------------------|-----------|
| S. cerevisiae    | SARI      | KLLFLGDLNAGKTTL                  | DLGQ                | VILGNKIDA  |
| S. cerevisiae    | YPT1      | KLLIGNSGVGVKSSLL                 | DTAGQ               |          |
| S. cerevisiae    | SEC4      | KLLIGDSVGVKSSLL                  | DTAGQ               |          |
| Mouse            | ypt1      | KLLIDGSGVGVKSSLL                 | DTAGQ               |          |
| Rat              | rasb      | KYYIIGDTVGKSSLL                  | DTAGQ               |          |
| S. cerevisiae    | RAS1      | KLVVVGGGYYGKSALT                 | DTAGQ               |          |
| S. cerevisiae    | RAS2      | KLVVVGGGYYGKSALT                 | DTAGQ               |          |
| D. discoideum    | ras       | KLVVVGGGYYGKSALT                 | DTAGQ               |          |
| Mouse            | v-ras-H   | KLVVVGGGYYGKSALT                 | DTAGQ               |          |
| Human            | c-ras-H   | KLVVVGGGYYGKSALT                 | DTAGQ               |          |
| S. cerevisiae    | RHO1      | KLVVIPDGACGKTCL                  | DTAGQ               |          |
| S. cerevisiae    | GPA1      | KLLLGAGESGKSTVL                  | DAGGQ               |          |
| Bovine           | Tα        | KLLLGAGESGKSTVL                  | DAGGQ               |          |
| S. cerevisiae    | GPA2      | KLLLGAGESGKSTVL                  | DAGGQ               |          |
| Rat              | Gαα       | RLLLGAGESGKSTIV                  | DAGGQ               |          |
| Rat              | Gi2α      | KLLLGAGESGKSTIV                  | DAGGQ               |          |
| Bovine           | Tα        | KLLLGAGESGKSTIV                  | DAGGQ               |          |
| E. coli          | EF-Tu     | NVGTVIHVDHGKTTL                  | DCQH                |          |
| S. cerevisiae    | EF-1α     | NVVIVGHDSGKSTTT                  | DAGH                |          |
| S. cerevisiae    | GSTI      | SLIFMVHDVAGKSTMG                 | DAGH                |          |
| E. coli          | era       | FIAIVGRNPVAGKSTVL                | DTQG                |          |
| E. coli          | lepA      | NFILAHIDHGKSTLS                  | DTQG                |          |

Motif Consensus*  |
|                  | GXXXG     | DXXG                             | NKKD                |
|                  | KAAAGXGGVGKSTVL | DTAGQ | AAGNKNXDL |

References: a, this study; b, Gallwitz et al., 1983; c, Salminen and Novick, 1987; d, Haarbruck et al., 1987; e, Touchot et al., 1987; f, DeFeo-Jones et al., 1983; Powers et al., 1984; Dhar et al., 1984; g, Fukui and Kaziro, 1985; h, Raymond 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; Dietz and Kurjan, 1987 (the same gene was called SCG1 in their paper); o, Nakafuku et al., 1988; p, Itoh et al., 1986; q, Yatsumi and Khorana, 1985; r, Tanabe et al., 1985; s, Arai et al., 1980; t, Nagata et al., 1984; u, Tavitian et al., 1985; v, Kikuchi et al., 1988; w, Ahn et al., 1986; x, 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).

Tidicopy or single-copy plasmid. The details of this phenomenon were examined further. The Hind III-Sal I fragment of pSECl210 was subcloned into multicopy pSEY8 or single-copy pSEYC58 vector to yield SAP1 plasmids, which we hereafter refer to as YEpSARI or YCpSARI, respectively. SEC12 has three ts isolates, secI2-I, secI2-2, and secI2-4. These mutants were transformed with either YEpSARI or YCpSARI and cultured at the restrictive temperature 37°C. The two plasmids were both capable of suppressing each secI2 isolate; every transformant not only grew but also secreted invertase at 37°C, irrespective of the isolate or the copy number of SAP1 plasmid (see Table II). Since these secI2 mutants contain a wild-type copy of SAP1 gene in their chromosome, this observation indicates that duplication of SAP1 gene suppressed secI2 ts. To confirm that the duplication of SAP1 expression was in fact sufficient to suppress secI2, a Northern hybridization experiment was performed. (Poly A)5 RNA was prepared from secI2-4 strain and its transformants with YEpSARI or YCpSARI, an equivalent amount being electrophoresed in an agarose gel and transferred to a nitrocellulose membrane. The 320-base internal fragment of SAP1 was used as a probe to detect SAP1 message. The result is shown in Fig. 3. A single band of 1.750 bases was observed in the control (lane I). When the secI2 cells were transformed with the single-copy YCpSARI, the intensity of this band about doubled (lane 3). The multicopy YEpSARI 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 SARI

| sec     | Multi copy | Single copy | Strain              | Other genotype          | Reference or source of strain |
|---------|------------|-------------|---------------------|-------------------------|-------------------------------|
| sec1-1  |            |             | SEYS5016            | MATa ura3 leu2          | a                             |
| sec2-56 |            |             | ANS2-3A             | MATa ura3 leu2 his      | b                             |
| sec3-2  |            |             | NY412               | MATa ura3              | c                             |
| sec4-2  |            |             | ANS4-8C             | MATa ura3 leu2 his      | b                             |
| sec5-24 |            |             | ANS5-9D             | MATa ura3 leu2          | b                             |
| sec7-1  |            |             | SF821-8A            | MATa ura3 leu2 su2c2    | d                             |
| sec7-4  |            |             | SF911-13D           | MATa ura3 leu2 trpl his4| d                             |
| sec8-6  |            |             | ANS8-10C            | MATa ura3 leu2 his      | b                             |
| sec9-4  |            |             | ANS9-4C             | MATa ura3 trpl his      | b                             |
| sec10-2 |            |             | ANS10-12A           | MATa ura3 leu2 his      | b                             |
| sec11-7 | +          | +           | PBY3-9B             | MATa ura3 leu2 his4     | e                             |
| sec12-1 | +          | +           | ANY8-4D             | MATa ura3 leu2 trpl his | b                             |
| sec12-3 | +          | +           | ANY9-8A             | MATa ura3 leu2 trpl his | b                             |
| sec12-4 | +          | +           | MBY10-7A            | MATa ura3 leu2 trpl his | f                             |
| sec13-1 |            |             | MBY3-15A            | MATa ura3 leu2 his      | g                             |
| sec14-3 |            |             | ANS14-2C            | MATa ura3 leu2 his      | b                             |
| sec15-1 |            |             | ANS15-5B            | MATa ura3 leu2 trpl his | b                             |
| sec16-2 |            |             | MBY4-1A             | MATa ura3 leu2 trpl his | g                             |
| sec17-1 |            |             | MBY11-1D            | MATa ura3 leu2 trpl his | g                             |
| sec18-1 |            |             | MBY12-6D            | MATa ura3 leu2 trpl his | g                             |
| sec19-1 |            |             | ANS19-4A            | MATa ura3 leu2 his      | b                             |
| sec20-1 |            |             | MBY5-2A             | MATa ura3 trpl          | g                             |
| sec21-1 |            |             | MBY6-4D             | MATa ura3 leu2 trpl his | g                             |
| sec22-3 |            |             | MBY13-2D            | MATa ura3 trpl          | g                             |
| sec23-1 |            |             | MBY8-20C            | MATa ura3 leu2 trpl his | g                             |
| sec53-6 |            |             | MBY7-5C             | MATa ura3 leu2 trpl his | h                             |
| sec59-1 |            |             | SF604-9C            | MATa ura3 his4 su2c2    | d                             |
| sec61-1 |            |             | RDM7-4B             | MATa ura3 leu2 trpl his4| i                             |
| sec62-1 |            |             | RDM50-94C           | MATa 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, Bönh 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 SARI (data not shown). The level of SARI 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 YCP SAR1. Thus, as measured by the average RNA abundance, the duplication of SARI dosage suppressed sec12 ts phenotype.

A disruption of chromosomal SEC12 gene is lethal (Nakano et al., 1988). The effect of SARI overexpression on the lethality of a SEC12 deletion was tested. The multicopy SARI plasmid pSEC1210 or the SEC12 plasmid pSEC1230 was introduced into a heterozygous diploid in which one copy of chromosomal SEC12 was disrupted (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 SARI plasmid pSEC1210, none of the tetrads dissected gave more than two viable spores. About 20% of the tetrads contained the multicopy SARI plasmid, but all viable spores had the wild-type SEC12 and no sec12::URA3 haploid survived. Thus, overexpression of SARI was unable to replace the SEC12 function in the sec12 deletion mutant.

Next, suppression by SARI was examined with other sec mutations. 30 sec ts mutants, sec–sec23 (including two isolates of sec7 and three of sec2), sec53, sec59, sec61, and sec62, were transformed with either YEpSARI or YCP SAR1 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 YCP SAR1. In the presence of the multicopy YEpSARI, however, sec16 became viable at 37°C. sec6 was also weakly suppressed by the single-copy YCP SAR1; it grew at 33°C in the presence of either YEpSARI or YEp SAR1 but not in the absence of the plasmid (data not shown). Thus, SARI genetically interacts not only with sec12 but also with sec6, which is another sec mutant defective in ER-Golgi transport.

Construction of sar1 Mutants

To address the cellular function of SARI, we attempted to construct mutants. First, the null mutant of SARI was prepared by gene disruption. From the genomic SARI 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, was inserted into the deleted part of SARI. The resulting disrupted copy of SARI gene was excised out from the plasmid by Bam HI/Eco RI digestion, and introduced into a diploid strain which was
test disruption occurred at the SARI locus, a Southern hybridization experiment was performed. Chromosomal DNA prepared from the original diploid strain, the diploid transformant with the disrupted sarl, 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 SARI 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 SARI alleles was replaced by the disrupted copy. All viable progeny contained the wild-type gene (lanes 3–8). Thus the disruption of SARI 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 sarl did not initiate budding, as was also the case with SEC12 disruption.

Test of SARI Function in Secretory Pathway

Conditional lethal mutants of SARI were required for the functional analysis. One approach to obtain such a mutant was to place the SARI gene under the control of a regulatable promoter. To do this, a gene fusion of GALI and SARI was constructed. The procedures are summarized in Fig. 5. Briefly, the 1.2-kb Bam HI-Sal I fragment from YEpSARI, 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 GALI promoter, was inserted in the right orientation in front of the above SARI gene. To test if this fusion gene could express SARI 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 SARI gene in the plasmid was functional in suppressing sec12 in the presence of galactose but not in glucose.

The GALI-SARI plasmid was then introduced into the cells which had their chromosomal SARI gene disrupted. To do this, a heterozygous diploid, SARI/sarl::URA3 (leu2/leu2 trpl/trpI), was first transformed with the original genomic SARI plasmid, pSEC1210 (containing LEU2). After sporulation and tetrad dissection, the haploid cells containing the disrupted copy of SARI in the chromosome and the wild-type gene on the plasmid were selected (Ura+ l_~u + Trp-). Representative haploids were further transformed with the above GALI-SARI 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 SARI plasmid (Ura+ Leu+ Trp+) and others dropped the GALI-SARI plasmid (Ura+ Leu+ Trp+). The former cells, which contained the functional SARI gene only on the GALI-SARI fusion plasmid, were the desired mutants.

homzygous for SEC12, SARI, 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 SARI disruption. To show that this disruption occurred at the SARI locus, a Southern hybridization experiment was performed. Chromosomal DNA prepared from the original diploid strain, the diploid transformant with the disrupted sarl, 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 SARI 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 SARI alleles was replaced by the disrupted copy. All viable progeny contained the wild-type gene (lanes 3–8). Thus the disruption of SARI 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 sarl did not initiate budding, as was also the case with SEC12 disruption.

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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 sarl, 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. 8A, the galactose-dependent sarl 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
presence of $\text{GALI-SARI}$ plasmid itself, because when the $\text{sarl}$ phenotype was complemented by the constitutive $\text{SARI}$ plasmid, $\text{pSEC1210}$, no such accumulation was observed (lanes 9 and 10).

The intracellular transport of $\alpha$-factor was further analyzed by a pulse-chase experiment. The $\text{sarl}$ 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 $\alpha$-factor. As shown in Fig. 8B, cells transported and secreted $\alpha$-factor normally in the control (+Gal). Early processing from prepro-$\alpha$ to ER- and Golgi-forms of pro-$\alpha$ was seen at 0 min chase, and appearance of extracellular mature $\alpha$-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 $\alpha$-factor was observed. The Golgi form did not accumulate significantly. The band migrating at $\sim$22 kD (asterisk), which may be unglycosylated pro-$\alpha$-factor, was not chased rapidly either in galactose or glucose incubation. Thus, both immunoblotting and pulse-chase experiments indicated that $\text{sarl}$ is defective in $\alpha$-factor secretion and accumulates the ER precursor species under the restrictive condition.

The $\text{sarl}$ mutant was also examined for intracellular transport of a vacuolar protein, CPY. The same samples as in Fig. 8A were analyzed by immunoblotting using anti-CPY antibody (Fig. 9A). When the $\text{sarl}$ cells were incubated in glucose to repress $\text{SARI}$ function, a novel precursor form of CPY accumulated (lanes 3–5) that comigrated with the ER form, pl, seen in $\text{sec12}$ and $\text{sec18}$ (lanes 6 and 7). The accumulation of the pl form was not detected when the mutant contained the constitutive $\text{SARI}$ gene (not shown). In this immunoblotting analysis of CPY, the accumulation of the ER form seems less severe than $\text{sec12}$ and $\text{sec18}$, unlike the case of $\alpha$-factor, although the reason is unclear. It may also be noticed that the proportion of p2 CPY increased when the $\text{sarl}$ 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 (see lane 2). Moreover, the same phenomenon was not observed when the wild-type cells were shifted from galactose to glucose (see lane 2). Thus, the transport of CPY was blocked in processing of CPY from the pl to p2 form. Only a little proportion of the labeled molecules seemed to have escaped the block, giving rise to the p2 form. The appearance of p2 form was not obvious in the same incubation. Thus, the transport of a vacuolar protein was also affected by the $\text{SARI}$ deficiency at the step of exit from the ER. It should be noted here that conversion of pl 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 Sarlp by $\text{GALI}$ 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 $\text{SARI}$ is required for the protein transport from the ER to the Golgi, like other $\text{SEC}$ genes involved in the ER-Golgi step.
Figure 6. Gal-dependent expression of SARI. The single-copy plasmid pANY2-18 containing the GALI-SARI 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 sarl mutant. The GALI-SARI plasmid pANY2-18 was introduced into a haploid strain with its chromosomal SARI gene disrupted (sarl::URA3), by using the plasmid exchange with pSEC1210 (for details see text). The resultant galactose-dependent sarl mutant (ANY25) was grown to late-log phase in YPGal medium. Cells were washed with sterile water, inoculated into either YPGal orYPD, and incubated at 30°C. The increase of cell density was monitored by measuring OD600 with a spectrophotometer (Coleman).

Discussion

In this paper, we have presented evidence that a novel essential gene of yeast, SARI, encodes a GTP-binding protein and is involved in protein transport from the ER to the Golgi. Sequence analysis of SARI has shown that the gene codes for a 21-kDa 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 sarl mutant. This mutant, under a restrictive condition, accumulates the core-glycosylated ER-forms of proα-factor and proCPY. SARI 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 SARI

SARI 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 SARI plasmid, too. There is a single SARI locus in the chromo-
to immunoblotting using anti-prepro-a-factor antibody. The ER-accumulating ts mutants, MBY10-7C and MBY12-6D, were incubated for 18 h 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 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-a-factor antibody. The ER-accumulating ts mutants, MBY10-7C (sec2) and MBY12-6D (sec8), 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 Sarlp control, ANY26/pSEC1210 harboring both regulatory GAL/SAR/ plasmid (pANY2-18) and constitutive SAR/ plasmid (pSEC1210) was incubated for 18 h in MVGal or MVD lacking Trp and I.µu to keep both of the plasmids, respectively; pp, prepro-a-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 x 10^7 cells were harvested, washed, resuspended in sulfate-free medium, and pulse-labeled with 12.5 µCi/ml Tran35S-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 NaN3 on ice to terminate the reaction. Cell lysates were prepared and subjected to immunoprecipitation with anti-prepro-a-factor antibody (i). a-factor was also immunoprecipitated from medium at 5- and 10- min chase some, meaning that the duplication of SAR/ is enough to suppress the sec2 ts phenotype. In contrast to the ts mutants, the lethality of sec2 deletion mutant cannot be rescued even by the multicopy SAR/. These observations lead to an important notion that the suppression by SAR/ requires the sec2 gene product, even though it is partially defective. We suggest that this is indicative of a direct interaction between the two gene products, Sec1p and Sarlp. When the function of Sec1p is partly injured, elevation of Sarlp level may help keep the function of the complex normal. Cloning and structural analysis of the mutant sec2 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 sec15 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.

SAR/ Encodes a Novel GTP-binding Protein

The sequence analysis of SAR/ gene has revealed that it contains an intron. This is not a very frequent event for nuclear genes of Saccharomyces 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 in GTP-binding domains in a complete fashion, both in sequence similarity and in spacings between them. The first two elements, GXGXGXG 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 YPT/ family. This subgroup contains yeast YPT/ 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. 8A 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 p1 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; pl 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. 8B, and subjected to immunoprecipitation of CPY and SDS-PAGE.

The Journal of Cell Biology, Volume 109, 1989

Figure 9. sar1 accumulates ER-form of carboxypeptidase Y (CPY). (A) The same samples as in the experiment of Fig. 8A 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 p1 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; pl 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. 8B, and subjected to immunoprecipitation of CPY and SDS-PAGE.

Sar1p 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 Sec12p in promoting protein transport from the ER to the Golgi. The phenotype of the Gal
dependent sarl strain supports this view. When SARl
is placed under control of the GAI promoter, its expression is
induced in the presence of galactose and repressed in the
presence of glucose. In fact, the GAI-SARl fusion gene sup-
presses sec12 ts growth in galactose but not in glucose. This
fusion gene has been introduced into a sarl deletion mutant
to yield a conditional lethal mutant that grows only in galac-
tose medium. When the culture is shifted from galactose
to glucose medium, division continues for a while, but eventu-
ally stops probably because the preexisting Sarlp is depleted
(Fig. 7). At this restrictive stage cells accumulate a secreto-
ry 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 ac-
cumulates in a core-glycosylated form (Fig. 9). Thus, sarl
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 sarl mutant is a terminal pheno-
type that is indirectly affected by the decreased viability of
the cells. However, another Gal-dependent mutant of cal-
modulin (cmdl), which shows almost the same growth pheno-
type 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 SARl plays a specific role in the ER-
Golgi transport. Isolation of other types of conditional mu-
tants, e.g., ts or cs alleles, will be helpful for the further anal-
ysis. 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 vesi-
cles, YPT7 has been shown to be involved in transport some-
where 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 nonhydrolyz-
able analogue of GTP, inhibits the transport of a viral glyco-
protein through the Golgi and from the ER to the Golgi, re-
spectively, 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 in-
hibition 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 (Con-
nolly and Gilmore, 1986, 1989; Römisch et al., 1989; Bern-
stein 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 pro-
teins mentioned above indeed play similar roles in secretory
pathway to G proteins, what kind of signals do they trans-
duce? What could be a regulatory function executed in a con-
stitutive secretion? In the case of Sarlp, the existence of a
putative partner, Sec12p, is reminiscent of receptor/G-
protein interactions on the plasma membrane. Although
there is no evidence that Sec12p is a receptor, it could trans-
mit as a transmembrane protein a signal from the lumenal
side to the cytoplasm. The signal might be the sorting infor-
mation 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 luminal signal
transduced by the Sec12p-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-bind-
ing 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 re-
lationships of bacterial EF-Tu have been extensively inves-
tigated with a focus on GDP-GTP allosterism by various kinds
of biochemical (see Kaziro, 1978) and physicochemical (Na-
kano et al., 1980; Jurnak, 1985) approaches. When the ter-
ary aminoacyl-tRNA-EF-Tu-GTP complex binds to the A
site of a ribosome, GTP is hydrolyzed and EF-Tu-GDP im-
mediately dissociates from the ribosome-aminoacyl-tRNA
complex. This dissociation enables the polypeptide elonga-
tion cycle to proceed and EF-Tu-GTP regenerated by the ac-
tion of EF-Ts brings another aminoacyl-tRNA to the ribosome.
Thus, the GTP hydrolysis is thought to drive the cycle of pep-
tide elongation reaction in an irreversible, unidirectional fash-
on (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 sug-
gested that a GTP-binding protein may recycle between do-
nor 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 Sec12p may recycle between
the ER and the Golgi membranes from the observation that
Sec12p molecules undergo slow but progressive modification
in the Golgi (Nakano et al., 1988). Like the polypeptide elon-
gation cycle, Sec12p-Sarlp-GTP may bring a vesicle contain-
ing a cargo of secretory proteins to the Golgi membrane.
Conceivably, somewhere in the processes of membrane fu-
sion 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 Sec12p 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
SARl suggests that a complex mechanism is operating in this
inter-compartmental transport step. The thorough under-
standing of the structure and function of the secretory ma-
chinery still remains as a challenging goal of our efforts.
The references include a wealth of scientific data and research findings. For instance, it mentions the cloning of a yeast gene encoding a protein required for secretion, work done by Botstein and Davis in 1982. The text also refers to the cloning of a yeast gene encoding the GDP domain of EF-Tu, done by Jurnak in 1985. Additionally, it highlights the work of Bourne in 1988, who investigated the role of GTPases in membrane traffic.

The text also references the work of other scientists, including Goud, Buhler, and Botstein, among others, who contributed to the understanding of signal recognition in yeast. The references are comprehensive, covering a wide range of topics from signal transduction to GTP binding proteins and their role in cellular processes.

The document provides a detailed account of the various research findings and the scientists involved in the field of cell biology. It is a valuable resource for anyone interested in the complexities of cellular processes and the role of GTP binding proteins in these processes.
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Nakano and Muramatu A Novel GTP-binding Protein in ER-Golgi Transport 2691